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Discovery of a 3-Pyridylacetic Acid Derivative (TAK-100) as a Potent, Selective and Orally Active Dipeptidyl Peptidase IV (DPP-4) Inhibitor[†]

Yasufumi Miyamoto,[‡] Yoshihiro Banno,[‡] Tohru Yamashita,[‡] Tatsuhiko Fujimoto,[‡] Satoru Oi,[‡] Yusuke Moritoh,[‡] Tomoko Asakawa,[‡] Osamu Kataoka,[‡] Hiroaki Yashiro,[‡] Koji Takeuchi,[‡] Nobuhiro Suzuki,[‡] Koji Ikedo,[‡] Takuo Kosaka,[‡] Shigetoshi Tsubotani,[‡] Akiyoshi Tani,[‡] Masako Sasaki,[‡] Miyuki Funami,[‡] Michiko Amano,[‡] Yoshio Yamamoto,[‡] Kathleen Aertgeerts,[§] Jason Yano,[§] and Hironobu Maezaki*[,‡]

[‡]*Pharmaceutical Research Division, Takeda Pharmaceutical Company Limited, 17-85, Jusohonmachi 2-Chome, Yodogawa-ku, Osaka 532-8686, Japan, and* [§]*Takeda San Diego, Inc., 10410, Science Center Drive, San Diego, California 92121, United States*

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Inhibition of dipeptidyl peptidase IV (DPP-4) is an exciting new approach for the treatment of diabetes. To date there has been no DPP-4 chemotype possessing a carboxy group that has progressed into clinical trials. Originating from the discovery of the structurally novel quinoline derivative **1**, we designed novel pyridine derivatives containing a carboxy group. In our design, the carboxy group interacted with the targeted amino acid residues around the catalytic region and thereby increased the inhibitory activity. After further optimization, we identified a hydrate of [5-(aminomethyl)-6-(2,2-dimethylpropyl)-2-ethyl-4-(4-methylphenyl)pyridin-3-yl]acetic acid (**30c**) as a potent and selective DPP-4 inhibitor. The desired interactions with the critical active-site residues, such as a salt-bridge interaction with Arg125, were confirmed by X-ray cocrystal structure analysis. In addition, compound **30c** showed a desired preclinical safety profile, and it was encoded as TAK-100.

Introduction

Inhibition of dipeptidyl peptidase IV (DPP-4[a]) is an exciting new approach for the treatment of diabetes. DPP-4 is a serine protease that is ubiquitously distributed as both a membrane-bound protein and a soluble protein in plasma.[1] It mediates the activities of regulatory peptides by cleaving dipeptides from the N-terminus of glucagon-like peptide-1 [7-36] amide (GLP-1[7-36]-NH_2) and glucose-dependent insulinotropic polypeptide [1-42] (GIP[1-42]) to yield inactive GLP-1[9-36]-NH_2 peptide and GIP[3-42], respectively.[2] Consequently, inhibition of DPP-4 is rapidly emerging as a novel therapeutic approach for the treatment of type 2 diabetes. Extensive research efforts from both academia and industry have yielded potent DPP-4 inhibitors.[3] Indeed, these inhibitors have shown antidiabetic activities in both animals and humans, and clinical proof of concept has already been established with DPP-4 inhibitors. Among these, (2*R*)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine (MK-0431, sitagliptin),[4,5] which is the first approved DPP-4 inhibitor, has been prescribed in the US since 2006. 1-[[(3-Hydroxy-1-adamantyl)amino]acetyl]-2-cyano-(*S*)-pyrrolidine (LAF-237, vildagliptin) was approved in Europe in 2008,[6] and (1*S*,3*S*,5*S*)-2-[(2*S*)-2-amino-2-(3-hydroxytricyclo[3.3.1.13,7]-dec-1-yl)acetyl]-2-azabicyclo[3.1.0]hexane-3-carbonitrile (BMS-477118, saxagliptin)[7] has been approved and on the market in the US and Europe for several years. 2-({6-[(3*R*)-3-Aminopiperidin-1-yl]-3-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl}methyl)benzonitrile (SYR-322, alogliptin) was approved in Japan in 2010.[8]

We previously discovered a structurally novel quinoline derivative **1**[9] that showed potent DPP-4 inhibitor activity (Figure 1).

A docking study of the DPP-4 protein and compound **1** was conducted, and the result is shown in Figure 2. This study showed that the primary amino group at the 3-position of the quinoline ring can interact with the Glu-motif (Glu205-Glu206) of the enzyme. The pendant phenyl group can effectively occupy the S1 pocket, and the isobutyl group can have a hydrophobic interaction with Phe357. In addition, the 2 carbonyl groups on the piperazine-2,6-dione substituent can form hydrogen bonds with Lys554 and Tyr631. This hydrogen bond formation with Lys554 indicates that the piperazine-2,6-dione can be replaced by a more appropriate substituent bearing a carboxy group. The resulting salt-bridge interaction between the carboxy group and Lys554 could then serve to increase the DPP-4 inhibitory activity. Although a number of DPP-4 inhibitors have been designed and their X-ray cocrystal structures obtained,[3−8,10] there are currently no reports of DPP-4 inhibitors targeting and achieving an interaction with the Lys554 of the enzyme. As described in Figure 3, some quinoline derivatives **17a**−**d** possessing a carboxy group and with an appropriate spacer were designed and synthesized, and subsequent evaluations of these derivatives showed that they had potent DPP-4 inhibitory activities. Thus, the compounds **17a**−**d** afforded us the novel and important information that the incorporation of a carboxy group is an effective and useful way to increase the inhibition of DPP-4.

[†]PDB ID codes: 3O9V (compound **27c**) and 3O95 (compound **30c**).

*Corresponding author. Tel: +81-6-6300-6263. Fax: +81-6-6300-6306. E-mail: Maezaki_Hironobu@takeda.co.jp.

[a]Abbreviations: DPP-4, dipeptidyl peptidase IV; SBDD, structure-based drug design; SAR, structure−activity relationship.

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Compounds **17a−d** are characterized by the presence of a carboxy group as a substituent. However, only a few studies have reported DPP-4 inhibitors bearing a carboxy group,[10] and none of the reported molecules progressed to human clinical trials. Therefore, the discovery of DPP-4 inhibitors with a carboxy group would provide an interesting new finding in this field. As described above, the docking study of compound **1** provided us the information regarding a pharmacophore that contained a salt-bridge interaction with the Glu-motif, a hydrophobic interaction with the S1 pocket, and a hydrophobic interaction with Phe357; this pharmacophore is marked in red dotted lines in Figure 2. The structure of this pharmacophore was consistent with what we had obtained from our X-ray cocrystal studies of small molecules with the DPP-4 enzyme and from the results of our docking studies. Therefore, we utilized this pharmacophore in the subsequent design of our pyridine derivatives. When the pyridine derivatives were designed, we employed our new finding that a carboxy group could increase the inhibitory activity by interacting with amino acid residues, especially Lys554. Although the docking study of compound **1** showed that Lys554 is a suitable target for increasing the inhibitory activity of the drug, Lys554 was widely separated from the pyridine ring. Therefore, we focused on other amino acid residues that could more easily interact with the carboxy group, for example, Arg125, His740, Ser630 and Tyr547 (Figure 4). These amino acid residues were located so close to the quinoline ring that the quinoline derivatives could not effectively utilize these interactions. In contrast, the newly designed pyridine derivatives would be able to interact with these residues effectively.

Herein, we describe our approach that yielded a novel and highly potent DPP-4 inhibitor, a hydrate of [5-(aminomethyl)-6-(2,2-dimethylpropyl)-2-ethyl-4-(4-methylphenyl)pyridin-3-yl]acetic acid (TAK-100). This approach involved 3 major steps: (1) introduction of a carboxy group to interact with Lys554 by structure-based drug design (SBDD) analysis; (2) design of novel pyridine derivatives by preserving the fundamental pharmacophore of quinolines; and (3) optimization of the pyridine-based DPP-4 inhibitors.

Chemistry

The quinoline ring was constructed by the condensation of an *o*-amino benzophenone and a β-ketonitrile as described in Scheme 1. The *o*-amino benzophenone **5** was prepared from commercially available anthranilic acid **2** in 3 steps: The prepared compound **5** was condensed with the β-ketonitrile **11A**, which was prepared from the compound **10A**,[11,12] to yield the quinoline derivative **6**. The bromo group of **6** was then converted to a hydroxy group by performing palladium-catalyzed etheration followed by acidolysis of the resulting *tert*-butyl ether to yield **7**. The cyano group of **7** was hydrogenated using a Raney-nickel (Ni)/H_2 system, and the resulting amino group was protected with a *tert*-butoxycarbonyl (Boc) group to afford **8**. The obtained compound **8** was converted to the desired intermediate trifluoromethane sulfonate **9** with good yields.

The quinoline derivatives possessing a carboxy group **17a−d** were synthesized as described in Scheme 2. Ethyl acrylate and acrolein were introduced to the compounds **6** and **9** by Heck reaction with palladium catalyst to obtain **12** and **13**, respectively. The aldehyde **13** was reduced to the corresponding alcohol. After simultaneous hydrogenation of the cyano group and olefinic double bond using the Raney-Ni/H_2 system, the amino group was protected with a Boc group, and the hydroxy group was converted to a cyano group to obtain **14**. Horner−Wadsworth−Emmons reaction of **13** yielded compound **15**. 5-Cyano-1-pentyne was introduced to compound **9** by Sonogashira coupling with a copper catalyst

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

Figure 3. Design of novel quinoline derivatives possessing a carboxy group.

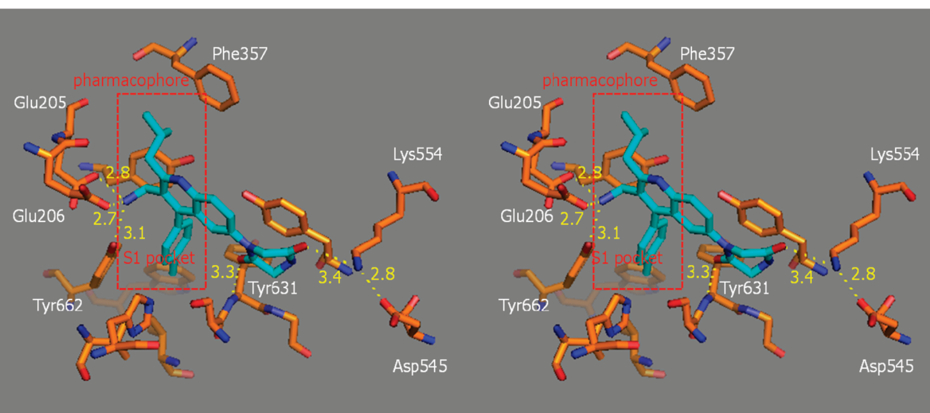

Figure 2. Docking study of DPP-4 protein and compound **1** (blue carbon). The amino acids (orange carbons, white text) and binding sites (red text) are indicated. The important pharmacophore is indicated by the square of red dotted lines. The hydrogen bonds are shown as yellow dotted lines.

Figure 4. Design for reducing the conjugation of the molecule.

Scheme 1. Synthesis of Compounds **6** and **9**[a]

[a] Reagents and conditions: (a) triphosgene, THF, reflux, 2 h; (b) *N*,*O*-dimethyl hydroxylamine hydrochloride, Et_3N, EtOH, reflux, 2 h; (c) 4-Me-C_6H_4−MgBr, THF, Et_2O, 0 °C, 30 min; (d) **11A**, MsOH, toluene, reflux, Dean−Stark, 17 h; (e) (1) *tert*-BuOH, *tert*-BuONa, racemic BINAP, $Pd(OAc)_2$, toluene, 70 °C, 15 h; (2) TFA, THF, rt, 4 h; (f) (1) H_2, Raney-Ni, 25% NH_3, MeOH, 70 °C, 8 h; (2) $(Boc)_2O$, THF, rt, 3 h; (g) NaH, *N*-phenyl bis(trifluoromethanesulfonimide), DMF, rt, 30 min; (h) NaH, MeCN, THF, reflux, 15 h.

to obtain the nitrile **16**. The quinolin-6-ylpropenylate **12** was successively hydrogenated, hydrolyzed, and deprotected to obtain the quinolin-6-ylpropanoic acid **17a**. Alkali hydrolysis of the nitriles **14** and **16** followed by Boc-deprotection yielded the quinolin-6-ylbutanoic acid **17b** and the quinolin-6-ylhexanoic acid **17d**, respectively. Hydrogenation of **15** by using Raney-nickel (Ni)/H_2 in the DMA system yielded the corresponding quinolin-6-ylpentanoate, and subsequent hydrogenation of the cyano group by using Raney-Ni/H_2 in the EtOH system, Boc-protection, hydrolysis of ester, and Boc-deprotection yielded the quinolin-6-ylpentanoic acid **17c**.

The nicotinic acid derivatives **27a−i** were synthesized as described in Scheme 3. The pyridine ring was constructed using modified Hantzsch's pyridine synthesis.[13] Commercially available esters **10A−D** were converted to *β*-ketonitriles **11A−D**.[11,12] From **11A−D**, the benzylidene derivatives **18A−D** were prepared using Knoevenagel condensation with *p*-tolualdehyde. Condensation of Meldrum's acid **20** with acid chlorides yielded the corresponding acyl-Meldrum's acids **21A−E**. The obtained **21A−E** were reacted with *tert*-butanol and subsequently with ammonia to obtain aminoacrylates **22C−G**. The aminoacrylate **22B** was prepared from *β*-ketoester **19A**. The aminoacrylates **22A−G** were condensed with the benzylidene derivatives **18A−D** in acetic acid (AcOH) to obtain dihydropyridines **23a−j**. The dihydropyridines **23a−j** were oxidized with cerium ammonium nitrate (CAN) to obtain pyridine derivatives **24a−j**. A cyano group of **24a−j** was reduced to an aminomethyl group by hydrogenation with Raney-Ni/H_2 to obtain **25a−j**. After Boc-protection of **25a−c**, the obtained **26a−c** were hydrolyzed with sodium hydroxide and then deprotected with hydrochloride to obtain compounds **27a−c**. Acidolysis of *tert*-butyl esters **25d−i** yielded the desired compounds **27d−i**.

3-Pyridylacetic acids **30a−c**, 3-pyridylpropenoic acid **32**, and 3-pyridylpropanoic acid **33** were synthesized as described in Scheme 4. A methoxycarbonyl group of compounds **25c**, **25d**, and **25j**[14] was converted to a hydroxymethyl group with diisobutylaluminum hydride (DIBAL-H), and then an amino group of the obtained compounds was protected with the Boc group to obtain **28a−c** in good yield. In contrast, the DIBAL-H reduction after the *N*-Boc-protection provided only low yields of the product (data not shown), which was because of

Scheme 2. Synthesis of Compounds **17a−d**[a]

[a] Reagents and conditions: (a) $PdCl_2(PPh_3)_2$, ethyl acrylate, Et_3N, DMF, 70 °C, 6 h; (b) acrolein, $Pd(OAc)_2$, $BnEt_3NCl$, DMF, 70 °C, 20 h; (c) $NaBH_4$, THF, MeOH, 0 °C, 10 min; (d) H_2, Raney-Ni, 25% NH_3, MeOH, 70 °C, 3 h; (e) $(Boc)_2O$, THF, rt, 1−3 h; (f) MsCl, Et_3N, DMF, 0 °C, 30 min; (g) KCN, DMSO, 60 °C, 1.5 h; (h) NaH, $(EtO)_2P(O)CH_2COOEt$, THF, rt, 10 min; (i) 5-cyano-1-pentyne, $PdCl_2(PPh_3)_2$, Et_3N, CuI, THF, 80 °C, 10 min; (j) H_2, Pd/C, EtOH, THF, rt, 2−3.5 h; (k) 1 or 2 M NaOH, THF, rt, 40 min−4 h; (l) 4 M HCl in AcOEt or dioxane, rt, 20 min−4 h; (m) 2 M NaOH, EtOH, reflux, 6−13 h; (n) (1) H_2, Raney-Ni, 25% NH_3, DMA, rt, 4 h; (2) H_2, Raney-Ni, 25% NH_3, MeOH, rt, 3 h.

the instability of the *N*-Boc group under DIBAL-H reduction. After mesylation of the hydroxy group of **28a−c**, a cyano group was introduced by treating trimethylsilyl cyanide (TMSCN) and tetrabutylammonium fluoride (TBAF) in good yield. Finally, the cyano group of **29a−c** was hydrolyzed to a carboxy group under acidic conditions, and the subsequent *N*-deprotection yielded the desired 3-pyridylacetic acids **30a−c**. The obtained **28a** was oxidized to the corresponding aldehyde and subsequently converted to an α,β-unsaturated ester **31** by the Horner−Wadsworth−Emmons reaction. Alkali hydrolysis and deprotection yielded 3-pyridylpropenoic acid **32**. The alkali hydrolysis and deprotection for compound **32** after hydrogenation of **31** yielded 3-pyridylpropanoic acid **33**.

Results and Discussion

The synthesized compounds were evaluated for their in vitro inhibitory activity against human DPP-4, and this activity was described in terms of IC_{50} values. We tested the enzyme selectivity against DPP-2, DPP-8, and DPP-9, since the inhibition of DPP-2[15] has been shown to result in the apoptosis of quiescent T-cells[16] and safety studies using a DPP-8/9 selective inhibitor suggest that the inhibition of both DPP-8 and DPP-9 is associated with profound toxicity in rats and dogs.[17]

The Structure−Activity Relationships (SARs) of Quinoline Derivatives. First, we investigated the effect of the introduction of a carboxy group at the 6-position of the quinoline ring, and the SAR summary of this investigation is shown in Table 1. Compounds **17a−d** showed potent DPP-4 inhibitory activities, and these results verified our hypothesis that the introduction of a carboxy group increased the DPP-4 inhibitory activity. Compound **17a**, which possessed 2 carbon atoms between the quinoline ring and the carboxy group, showed weaker activity. On the other hand compound **17d**, which possessed 5 carbon atoms between the quinoline ring and the carboxy group, showed the most potent activity. The docking study of compound **17d** with DPP-4 protein was performed to explain the activity, and the result is shown in Figure 5. Compound **17d** has similar binding mode to those shown by compound **1**. For instance, the primary amino group at the 3-position of the quinoline ring can interact with the Glu-motif of the enzyme, and the pendant phenyl group can effectively occupy the S1 pocket. Furthermore, in our design, the carboxy group forms a hydrogen bond with Lys554. If the length of the linkage is two carbon atoms, the carboxy group can interact with Lys554 only in an extended form. However, if the length is five carbon atoms, multiple conformations are obtained, and the carboxy groups can interact with Lys554 by partially curling their alkyl chains. The potent inhibitory activity is

Scheme 3. Synthesis of Compounds **27a–i**[a]

compound	R^1	R^2	R^3	yield 23	24	25	26	27
a	Pr	Me	Me	39%	89%	67%	70%	80%
b	Bu	Me	Me	24%	65%	68%	89%	66%
c	*i*-Bu	Me	Me	52%	82%	95%	93%	57%
d	neopentyl	Me	*tert*-Bu	53%	96%	92%	-	71%
e	neopentyl	Et	*tert*-Bu	38%	43%	74%	-	90%
f	neopentyl	Pr	*tert*-Bu	62%	59%	74%	-	90%
g	neopentyl	*i*-Pr	*tert*-Bu	30%	51%	51%	-	88%
h	neopentyl	*i*-Bu	*tert*-Bu	22%	49%	89%	-	93%
i	neopentyl	Bn	*tert*-Bu	69%	-	15%	-	91%

[a] Reagents and conditions: (a) NaH, MeCN, THF, reflux, 8–15 h; (b) *p*-tolualdehyde, piperidine, AcOH, toluene, reflux, Dean–Stark, 5–8 h; (c) 25% NH_3, MeOH, rt, 15 h; (d) R^2COCl, CH_2Cl_2, rt; (e) (1) *tert*-BuOH, toluene, reflux, 6 h; (2) 25% NH_3, MeOH, rt, 12 h; (f) AcOH, 80 °C, 1 h; (g) CAN, H_2O, acetone, rt, 30 min; (h) H_2, Raney-Ni, 25% NH_3, MeOH, 40 °C, 6 h; (i) $(Boc)_2O$, THF, rt, 1 h; (j) TFA, rt, 4 h; (k) (1) 1 M NaOH, THF, MeOH, rt, 15–72 h; (2) 4 M HCl in AcOEt or dioxane, 2 h.

well explained by two effects. One is the increase in hydrophobic interaction between the alkyl chain and DPP-4 protein, and the other is the flexible conformation of the alkyl chain that contributes to shift the carboxy group to an appropriate position near Lys554. Therefore, analysis of compounds **17a–d** yielded the novel and important finding that a carboxy group is an effective and useful option to increase the inhibition of DPP-4. On the basis of these results, we designed novel pyridine derivatives that contained both a carboxy group and the core pharmacophore. In the design, as shown above, Lys554 was widely separated from the pyridine ring. Therefore, for interaction with the carboxy group, we focused on other amino acid residues located near the pyridine ring, e.g., Arg125, His740, Ser630, and Tyr547.

The SARs of Pyridine Derivatives. The contribution to the activity of a carbon spacer between the pyridine ring and a carboxy group is shown in Table 2. Notably, the initially designed compounds shown in Figure 3 possessed potent DPP-4 inhibitory activities that validated our rational design. The 3-pyridylacetic acid **30a** showed potent DPP-4 inhibitory activity. The DPP-4 inhibitory activities of compounds **32** and **33**, which had a longer chain, were lower. These results indicate that the carboxy group of compounds **27c** and **30a** could interact with some of the amino acid residues near the 3-position of the pyridine ring, e.g., Arg125, Ser630, and Tyr547, as we had envisioned while designing these molecules. Longer ethylene linkage chains were found to be less appropriate for the interaction with the target residues. The X-ray structure of compound **27c** in a complex

Scheme 4. Synthesis of Compounds **30a**−**c**, **32** and **33**[a]

25c (R^1=isobutyl, R^2=Me, R^3=Me)
25d (R^1=neopentyl, R^2=Me, R^3=*tert*-Bu)
25j (R^1=neopentyl, R^2=Et, R^3=Me)

28a (R^1=isobutyl, R^2=Me) : 75%
28b (R^1=neopentyl, R^2=Me) : 80%
28c (R^1=neopentyl, R^2=Et) : 80%

29a (R^1=isobutyl, R^2=Me) : 89%
29b (R^1=neopentyl, R^2=Me) : 93%
29c (R^1=neopentyl, R^2=Et) : 92%

c (for **30a, b**) or d (for **30c**)

30a (R^1=isobutyl, R^2=Me; 2HCl salt) : 92%
30b (R^1=neopentyl, R^2=Me; 2HCl salt) : 75%
30c (R^1=neopentyl, R^2=Et; hydrate) : 77%

e (from **28a**) 62% → **31**; f, g 84% → **32** (2HCl); h, f, g 37% → **33** (2HCl)

[a] Reagents and conditions: (a) (1) DIBAL-H, toluene, −78 °C, 0.5−2 h; (2) $(Boc)_2O$, THF, rt, 1 h; (b) (1) MsCl, Et_3N, THF, 0 °C, 30 min; (2) TMSCN, TBAF, THF, rt, 1 h; (c) (1) 6 M HCl, reflux, 20−24 h; (2) $(Boc)_2O$, AcOEt, 8 M NaOH, rt, 1−17 h; (3) 4 M HCl in dioxane, rt, 2−3 h; (d) (1) 6 M HCl, reflux, 24 h; (2) $(Boc)_2O$, AcOEt, 8 M NaOH, rt, 1 h; (3) 6 M HCl, rt, 3 h; (4) 1 M NaOH, 5 °C, 1 h; (e) (1) MnO_2, THF, rt, 19 h; (2) $(EtO)_2P(O)CH_2CO_2Et$, NaH, THF, rt, 45 min; (f) 1 M NaOH, THF, 60 °C, 12 h; (g) 4 M HCl, dioxane, rt, 15 min; (h) H_2 (0.5 MPa), Pd/C, EtOH, 1 h.

Table 1. SAR Summary for the Quinoline Derivatives

17a-d (2HCl) **1** (2HCl)

compound	*n*	DPP-4 IC_{50} (nM)[a]
17a	2	23 (20−25)
17b	3	8.7 (7.8−9.6)
17c	4	9.6 (8.3−11)
17d	5	6.6 (5.9−7.4)
1		1.1 (1.1−1.2)

[a] Inhibitory activity against human DPP-4. IC_{50} values are shown as means with 95% confidence intervals (n = 3). IC_{50} values and 95% confidence intervals were calculated from the concentration−response curves generated by GraphPad Prism and are shown in parentheses.

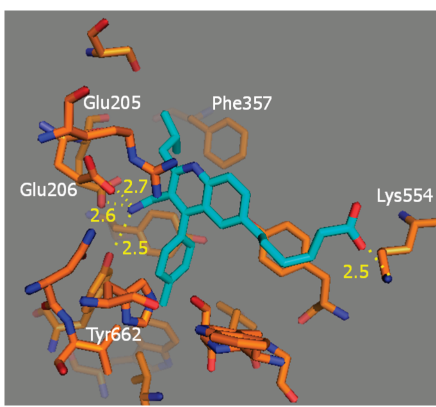

Figure 5. A docking study of DPP-4 protein and compound **17d** (blue carbons). The amino acids (orange carbons, white text) are indicated. The hydrogen bonds are shown as yellow dotted lines.

with human DPP-4, as shown in Figure 6, also establishes our design rationale. This structure showed that the important pharmacophore was preserved, and the primary amino group at the 5-position of the pyridine ring interacts with the Glu-motif and Tyr662. The pendant phenyl group and the isobutyl group can show interactions similar to those observed with compound **1**. Meanwhile, the methyl group at the 2-position of the pyridine ring has no important contribution to the inhibitory activity. The carboxy group at the 3-position of the pyridine ring interacts with Tyr547, which is one of our targeted amino acid residues near the pyridine ring. Compound **27c** could show potent activity by interacting with Tyr547, which was a feature that the quinoline derivatives lacked. The 3-pyridylacetic acid derivative **30a** also might interact with the targeted amino acid residues effectively. Additionally, the 3-pyridylacetic acid derivative **30a** showed remarkable selectivity for the related proteases.

Because the nicotinic acid and 3-pyridylacetic acid derivatives showed almost the same activity, we selected the nicotinic acids as our lead compounds based on the fact that

Table 2. SAR Summary for the 3-Position of the Pyridine Derivatives[a]

compound	R	DPP-4 IC_{50} (nM)[a]	DPP-2 IC_{50} (nM)[b]	DPP-8 IC_{50} (nM)[c]	DPP-9 IC_{50} (nM)[d]
27c	COOH	18 (17–19)	5600 (5100–6200)	11000 (8000–15000)	30000 (21000–41000)
30a	CH_2COOH	20 (19–22)	50000 (45000–56000)	>30000	>30000
33	CH_2CH_2COOH	39 (35–43)	1100 (970–1300)	>30000	>30000
32	*–CH=CH–C(=O)OH	66 (59–75)	NT[e]	NT[e]	NT[e]

[a] Superscript *a* in column heading refers to Table 1. Superscript *b*−*d* in column heading indicate inhibitory activity against rat DPP-2, human DPP-8, and human DPP-9. IC_{50} values are shown as means with 95% confidence intervals ($n = 3$). IC_{50} values and 95% confidence intervals were calculated from the concentration−response curves generated by GraphPad Prism and are shown in parentheses. Superscript *e*: NT, not tested.

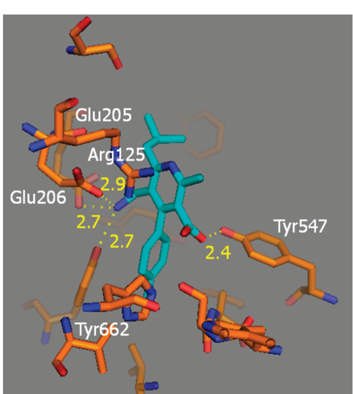

Figure 6. X-ray structure of compound **27c** (blue carbons) in a complex with human DPP-4. The amino acids (orange carbons, white text) are indicated. The hydrogen bonds are shown as yellow dotted lines.

they were more suitable for structural conversions from a synthetic point of view. In our previous study, the *p*-tolyl group was found to be one of the most effective substituents at the 4-position of the pyridine ring. Actually, in the X-ray structure of compound **27c** in a complex with human DPP-4 (Figure 6), the pendant phenyl group extends into the S1 pocket and shows a hydrophobic interaction. Specifically, the methyl group of the *p*-tolyl group fits well in the small hydrophobic hole at the bottom of the S1 pocket. Therefore, we investigated the SARs of the nicotinic acid derivatives at the 2- and 6-positions of the pyridine ring.

The in vitro activities of compounds **27a**, **27b**, and **27d**, which bore varieties of alkyl groups at the 6-position of the pyridine ring, were evaluated and are shown in Table 3. As described above, because the 6-substituent can show a hydrophobic interaction with Phe357, the effect of hydrophobic substituent was mainly examined at the 6-position. The DPP-4 inhibitory activity of the sterically bulky analogue **27d** was same as that of **27c**. The DPP-4 inhibitory activities of compounds **27a** and **27b**, which possessed a linear alkyl chain, were 3 times lower than that of **27c**. These results indicate that the bulky alkyl chain shows more potent DPP-4 inhibitory activity. These results were explained by an interaction of the bulky hydrophobic group with the side chain of Phe357. Meanwhile, the substituents at the 6-position of the pyridine ring did not influence the selectivity for the related proteases. The neopentyl group was selected as the best substituent at the 6-position of the pyridine ring, and, next, we focused on the substituents at the 2-position of the pyridine ring.

The in vitro activities of compounds **27e**−**i** bearing varieties of alkyl groups at the 2-position of the pyridine ring were investigated, and the results are shown in Table 4. Selectivity for the related proteases DPP-2, DPP-8, and DPP-9 are also described in Table 4. Except in the case of compound **27g**, as the substituent at the 2-position of the pyridine became bulkier (Me < Et < Pr < *i*-Bu < Bn), the DPP-4 inhibitory activities increased. The DPP-4 inhibitory activity of compound **27g**, which possessed an isopropyl group, was lower than that of **27d**. This indicates that a branched-alkyl chain directly attached to the pyridine ring decreases the DPP-4 inhibitory activity and that an appropriate methylene linker between the pyridine ring and the bulky group could be necessary for potent inhibitory activity. Although compound **27i** having a benzyl group showed the most potent DPP-4 inhibitory activity, it showed poor selectivity for DPP-2. Concerning the substituent at the 2-position of pyridine ring, the presence of a bulkier substituent increased DPP-4 inhibitory activity and concurrently decreased the selectivity for DPP-2. In view of both activity and selectivity, we selected a methyl group and an ethyl

Table 3. SAR Summary for the 6-Position of the Pyridine Derivatives

compd	R	IC$_{50}$ (nM)			
		DPP-4[a]	DPP-2[b]	DPP-8[c]	DPP-9[d]
27a	Pr	57 (51−63)	NT[e]	NT	NT
27b	Bu	58 (49−69)	11000 (9300−13000)	NT	NT
27d	neopentyl	18 (17−19)	10000 (8700−12000)	12000 (8200−17000)	12000 (9800−16000)

[a] Refers to Table 1. [b] Refers to Table 2. [c] Refers to Table 2. [d] Refers to Table 2. [e] NT: Not tested.

Table 4. SAR Summary for the 2-Position of the Pyridine Derivatives

compd	R	IC$_{50}$ (nM)			
		DPP-4[a]	DPP-2[b]	DPP-8[c]	DPP-9[d]
27e	Et	5.7 (5.1−6.4)	4000 (3400−4500)	820 (560−1200)	2400 (1700−3300)
27f	Pr	4.4 (4.0−4.8)	2000 (1700−2300)	630 (470−840)	1600 (1300−2000)
27g	*i*-Pr	19.0 (17−21)	1600 (1500−1800)	2300 (1700−3000)	11000 (5400−21000)
27h	*i*-Bu	4.6 (4.2−5.0)	1500 (1200−1700)	420 (340−520)	3000 (2300−3800)
27i	Bn	2.5 (2.3−2.7)	250 (210−310)	2000 (1600−2600)	5800 (3900−8600)

[a] Refers to Table 1. [b] Refers to Table 2. [c] Refers to Table 2. [d] Refers to Table 2.

Table 5. DPP-4 Activity and Selectivity of Compounds **30b** and **30c**

compd	R	salt	additive	IC$_{50}$ (nM)			
				DPP-4[a]	DPP-2[b]	DPP-8[c]	DPP-9[d]
30b	Me	2 HCl	none	10 (9.6−11)	NT[e]	> 100000	> 100000
30c (TAK-100)	Et	none	H_2O	5.3 (5.1−5.4)	> 100000	> 100000	60000 (48000−79000)

[a] Refers to Table 1. [b] Refers to Table 2. [c] Refers to Table 2. [d] Refers to Table 2. [e] Refers to Table 2.

group as the substituents at the 2-position of the pyridine ring, and we investigated the activities of the corresponding 3-pyridylacetic acids.

The in vitro activities and selectivity of the selected corresponding 3-pyridylacetic acids are shown in Table 5. Between the two compounds, the DPP-4 inhibitory activity of 3-pyridylacetic acid **30b** was lower than that of **30c**. Remarkably, the 3-pyridylacetic acid **30c** showed significant selectivity for the related proteases. Meanwhile, compound **30b** offered no advantage over **30c** in terms of activity and selectivity. Considering its potent DPP-4 inhibitory activity and good selectivity, compound **30c** was selected for further evaluations.

The X-ray structure of **30c** in a complex with human DPP-4 (Figure 7) was obtained at a resolution of 2.85 Å with well-defined electron density for the compound. The primary amine group interacts with the Glu-motif, which is located on an α-helix that protrudes from the *β*-propeller domain

into the active site and usually recognizes the charged N-terminal end of peptide substrates. The *p*-tolyl group occupies the well-defined and hydrophobic S1 pocket. The neopentyl group shows a hydrophobic interaction with Phe357. The carboxy group interacts with the catalytic residues Ser630 and His740. In addition, it forms a salt-bridge interaction with Arg125 to yield a tight binding with the enzyme. This X-ray structure established our design rationale that some amino acid residues such as Arg125, His740, and Tyr547 would be located near the pyridine ring and the interaction with these residues could increase DPP-4 inhibitory activity.

The ADME properties of **30c** were evaluated and revealed to be excellent, as shown in Table 6. Compound **30c** exhibited good metabolic stabilities and also showed very high solubility. The bioavailabilities in both rats and dogs are high, and the $t_{1/2(\mathrm{oral})}$ values are very large, which indicated potent and long-lasting effects in in vivo studies. Although compound **30c** was metabolized into the glucuronide of compound **30c** in rats and dogs, the compositional analysis of the plasma after single oral administration to rats and dogs revealed that compound **30c** was the main component and only a small amount of glucuronide was detected in the plasma. In an ex vivo study, compound **30c** quickly inhibited DPP-4 activity in plasma almost completely within 1 h after oral administration at the dose of 0.5 mg/kg in dogs (Figure 8). Moreover, approximately 60% and 30% inhibition of DPP-4 activity was maintained for 12 and 24 h, respectively. Compound **30c** was assessed for its ability to improve glucose tolerance in female Wistar fatty rats. Administration of single oral doses reduced the blood glucose excursion in an oral glucose tolerance test (OGTT) in a dose-dependent manner from 0.1 mg/kg when administered 60 min before an oral glucose challenge (1 g/kg) as shown in Figure 9. These ex vivo and in vivo studies in dogs raised expectations of reliable efficacy and therapeutic duration in clinical studies.

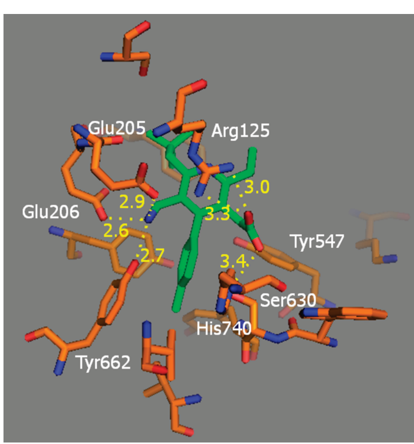

Figure 7. X-ray structure of compound **30c** (green carbons) in a complex with human DPP-4. The amino acids (orange carbons, white text) are indicated. The hydrogen bonds are shown as yellow dotted lines.

Conclusion

We optimized the lead compound 1 by using structure-based drug design (SBDD) to obtain a novel DPP-4 inhibitor for the treatment of the type 2 diabetes. We designed the introduction of a carboxy group that could increase the inhibitory activity by interacting with Lys554, which none of the previously reported DPP-4 inhibitors interacted with. Some quinoline derivatives possessing a carboxy group actually showed potent DPP-4 inhibitory activity, and the carboxy group was shown to interact with Lys554 in a docking study. Furthermore, the introduction of a carboxy group was indicated to yield DPP-4 inhibitors with a novel design. After assessing more than 50 DPP-4 X-ray cocrystal structures with small molecules originating from different chemical classes and by performing docking studies, we found an important pharmacophore for DPP-4 inhibitory activity. This pharmacophore includes a salt-bridge interaction with the Glu-motif, a hydrophobic interaction with the S1 pocket and a hydrophobic interaction with Phe357. On the basis of the information obtained by analysis of this pharmacophore, we preserved the fundamental substituents on the quinoline ring and removed the benzene ring of the quinoline. Furthermore, on the basis of the results of the docking study, we positioned the carboxy group at an appropriate site to interact with the target amino acid residues that were located near the pyridine ring, such as Arg125, His740, Ser630 and Tyr547. Actually, the designed pyridine derivatives showed potent DPP-4 inhibitory activities

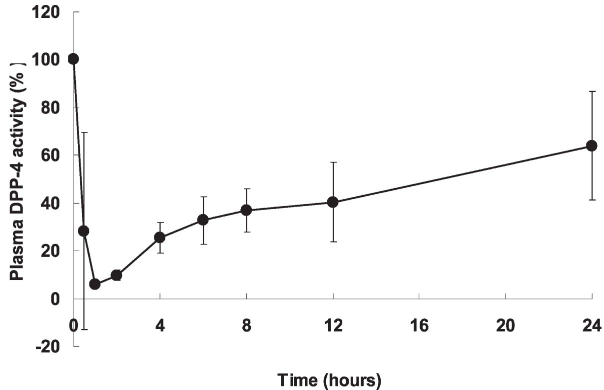

Figure 8. Ex vivo study of compound **30c** in dogs. Compound **30c** was dissolved in a 0.5% suspension of methylcellulose. Five dogs (age, 28−42 months) were orally administered the suspension at the dose of 0.5 mg/kg. Blood samples were collected from the cephalic vein before (0 h) and 0.5, 1, 2, 4, 6, 8, and 24 h after dosing. Plasma sample was prepared from each of the blood samples, and the residual DPP-4 activity was measured by a method similar to that described in the Experimental Section.

Table 6. ADME Profiles of **30c**

pharmacokinetic profile[a]				metabolic stability remaining ratio[c] (%)			solubility (μg/mL) (37 °C, 2 h)	
$t_{1/2(\mathrm{oral})}$ (h)		BA[b] (%)						
rat	dog	rat	dog	rat	dog	human	JP1[d]	JP2[e]
5.74 ± 2.6	4.97 ± 2.0	53.3 ± 12.8	88.0 ± 21.7	100.4	96.8	100.0	> 1000	930

[a] Rats and dogs were administered the drug intravenously at 0.1 mg/kg and orally at 1.0 mg/kg (rat: n = 3; dog: n = 4). [b] BA: bioavailability. [c] Metabolic stability in hepatic microsome (rat, dog, and human). [d] First fluid for the disintegration test described in the Japanese Pharmacopoeia 15th edition (pH 1.2). [e] Second fluid for the disintegration test described in the Japanese Pharmacopoeia 15th edition (pH 6.8).

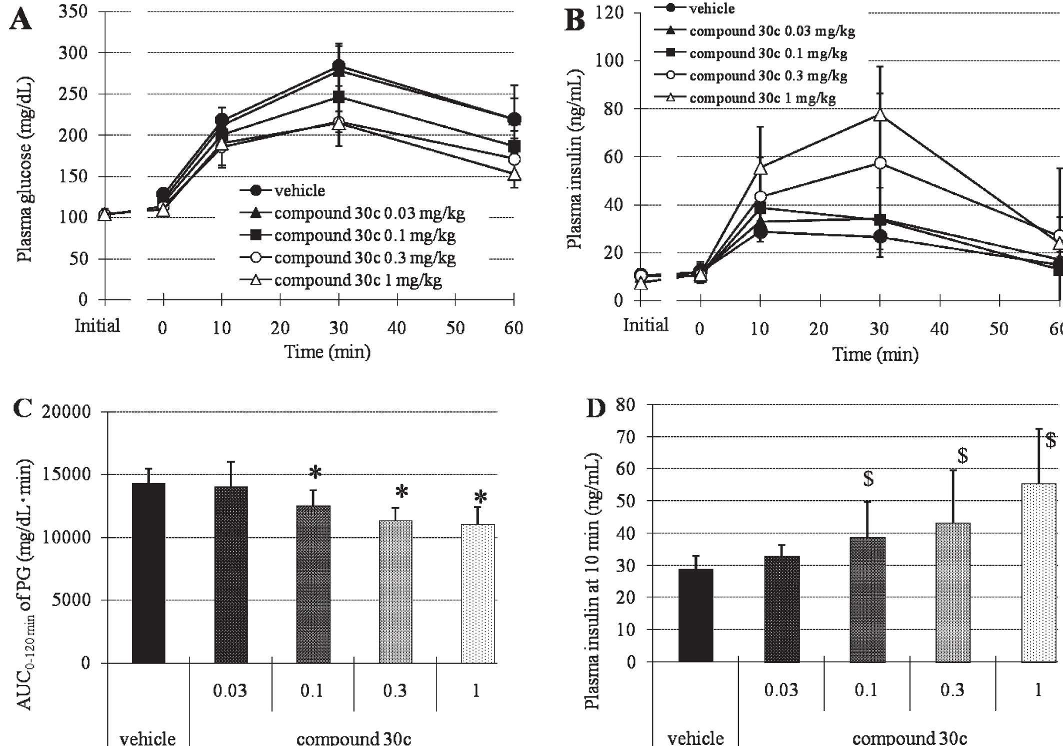

Figure 9. Effects of **30c** on plasma glucose (A), plasma immunoreactive insulin (IRI) (B), baseline (0 min)-adjusted $AUC_{0-60min}$ of plasma glucose levels (C), and plasma IRI levels at 10 min after the oral glucose load (D) in glucose tolerance test of female Wistar fatty rats. These studies were conducted using monohydrate of compound **30c**. Doses are normalized to reflect the amount of free base administered. Values are the mean and SD (n = 6): (*) $p \leq 0.025$ vs control by one-tailed Williams test; ($) $p \leq 0.025$ vs control by one-tailed Shirley−Williams test.

that were linked to the discovery of novel pyridine derivatives possessing a carboxy group as potent DPP-4 inhibitors. Finally, after further optimization, we identified a hydrate of [5-(aminomethyl)-6-(2,2-dimethylpropyl)-2-ethyl-4-(4-methylphenyl)pyridin-3-yl]acetic acid (**30c**) as a potent and selective DPP-4 inhibitor. The interaction between the carboxy group of **30c** and the target amino acid residues was confirmed by the X-ray cocrystal structure, and, especially, the carboxy group showed a salt-bridge interaction with Arg125. Finally, compound **30c** showed a desired preclinical safety profile.

Experimental Section

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 doublets; brs, broad singlet; brd, 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. The purities of all compounds tested in biological systems were assessed as being > 95% using analytical HPLC. The HPLC analyses were performed using a Shimadzu UFLC instrument. Elution was done with a gradient of 5−90% solvent B in solvent A (solvent A was 0.1% TFA in water, and solvent B was 0.1% TFA in acetonitrile) through an L-column 2 ODS (3.0 × 50 mm, 2 μm) column at 1.2 mL min^{-1}. Area % purity was measured at 220 and 254 nm.

6-Bromo-2*H*-3,1-benzoxazine-2,4(1*H*)-dione (3). A mixture of 2-amino-5-bromobenzoic acid **2** (25 g, 116 mmol) and triphosgene (34.4 g, 116 mmol) in THF (500 mL) was stirred at 70 °C for 2 h. The reaction mixture was cooled to room temperature and poured into water. The precipitate was collected by filtration and washed with MeOH to give **3** (27.1 g, 97%) as a white powder. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.19 (1H, d, J = 8.4 Hz), 7.96−8.02 (1H, m), 8.09 (1H, d, J = 2.6 Hz).

2-Amino-5-bromo-*N*-methoxy-*N*-methylbenzamide (4). A mixture of triethylamine (22.3 mL, 160 mmol) and *N*,*O*-dimethyl hydroxylamine hydrochloride (15.6 g, 160 mmol) in EtOH (100 mL) was stirred at room temperature for 30 min. To the mixture was added **3** (27 g, 112 mmol), and the mixture was refluxed for 2 h. The reaction mixture was poured into water and extracted with AcOEt, and the mixture was washed with brine. The organic layer was separated, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/AcOEt = 50/50) to give **4** (21.4 g, 74%) as a yellow powder. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.34 (3H, s), 3.59 (3H, s), 4.67 (2H, brs), 6.59 (1H, d, J = 8.4 Hz), 7.26 (2H, dd, J = 2.6, 8.8 Hz), 7.50 (1H, d, J = 2.4 Hz).

(2-Amino-5-bromophenyl)(4-methylphenyl)methanone (5). To a solution of **4** (5.0 g, 19.3 mmol) in diethyl ether (50 mL) and THF (10 mL) was added 1 M *p*-tolyl magnesium bromide THF solution (60 mL, 60 mmol) dropwise at 0 °C, and the mixture was stirred for 30 min. The reaction mixture was quenched with water and extracted with AcOEt. The organic layer was washed with brine, separated, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/AcOEt = 75/25) to give **5** (3.54 g, 63.2%) as a yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.44 (3H, s), 5.98 (2H, brs), 6.64 (1H, d, J = 8.9 Hz),

7.28 (2H, d, J = 7.9 Hz), 7.35 (1H, dd, J = 2.3, 8.9 Hz), 7.55−7.60 (3H, m).

6-Bromo-4-(4-methylphenyl)-2-(2-methylpropyl)quinoline-3-carbonitrile (6). A mixture of **5** (3.4 g, 11.7 mmol), **11A** (1.76 g, 14.1 mmol), methanesulfonic acid (1.13 g, 11.7 mmol) and toluene (200 mL) was heated under reflux for 17 h using a Dean−Stark trap. The reaction mixture was allowed to cool to room temperature, washed with saturated $NaHCO_3$ solution, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/AcOEt = 5/1) to give **6** (2.72 g, 61.2%) as colorless crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.05 (6H, d, J = 6.8 Hz), 2.30−2.50 (1H, m), 2.50 (3H, s), 3.10 (2H, d, J = 7.4 Hz), 7.34 (2H, d, J = 8.2 Hz), 7.42 (2H, d, J = 8.2 Hz), 7.80−7.90 (2H, m), 7.99 (1H, d, J = 9.0 Hz). mp 168.9−169.1 °C.

6-Hydroxy-4-(4-methylphenyl)-2-(2-methylpropyl)quinoline-3-carbonitrile (7). A mixture of racemic BINAP (1.0 g, 1.6 mmol) and palladium acetate (121 mg, 0.54 mmol) in toluene (200 mL) was stirred at 50 °C for 30 min under argon atmosphere. The reaction mixture was cooled to room temperature, and then **6** (20.5 g, 54 mmol), *tert*-BuOH (10.5 mL, 110 mmol) and sodium *tert*-butoxide (7.7 g, 80 mmol) were added. The mixture was stirred at 70 °C for 15 h under argon atmosphere. The reaction mixture was allowed to cool to room temperature, washed with 1 M HCl, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/AcOEt = 85/15) to give 6-*tert*-butoxy-4-(4-methylphenyl)-2-(2-methylpropyl)quinoline-3-carbonitrile (19.9 g, 99%) as colorless crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.06 (6H, d, J = 6.6 Hz), 1.34 (9H, s), 2.31−2.44 (1H, m), 2.49 (3H, s), 3.09 (2H, d, J = 7.2 Hz), 7.20 (1H, d, J = 2.1 Hz), 7.33−7.36 (2H, m), 7.37−7.40 (2H, m), 7.48 (1H, dd, J = 2.1, 9.0 Hz), 8.02 (1H, d, J = 9.0 Hz). A mixture of 6-*tert*-butoxy-4-(4-methylphenyl)-2-(2-methylpropyl)quinoline-3-carbonitrile (19.9 g, 54 mmol), trifluoroacetic acid (50 mL) and THF (10 mL) was stirred at room temperature for 4 h. The reaction mixture was concentrated under reduced pressure. The residue was partitioned between AcOEt and water. The organic layer was washed with brine, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was crystallized from diisopropyl ether to give **7** (10.4 g, 61%) as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.03 (6H, d, J = 6.6 Hz), 2.20−2.45 (1H, m), 2.46 (3H, s), 3.06 (2H, d, J = 7.3 Hz), 6.28 (1H, brs), 6.97 (1H, d, J = 2.9 Hz), 7.25−7.40 (4H, m), 7.41 (1H, dd, J = 2.9, 9.2 Hz), 8.02 (1H, d, J = 9.2 Hz). mp 247.7−249.1 °C.

***tert*-Butyl {[6-Hydroxy-4-(4-methylphenyl)-2-(2-methylpropyl)-quinolin-3-yl]methyl}carbamate (8).** A mixture of **7** (10 g, 33 mmol), 25% NH_3 solution (10 mL), Raney-Ni (10 mL), THF (50 mL) and MeOH (50 mL) was stirred at 70 °C under H_2 atmosphere (0.5 MPa) for 8 h and filtered through a Celite pad. The filtrate was partitioned between AcOEt and water. The organic layer was separated, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was dissolved in THF (100 mL), and to the solution was added $(Boc)_2O$ (7.8 g, 36 mmol). After stirring at room temperature for 3 h, the mixture was concentrated under reduced pressure. The residue was crystallized from diisopropyl ether to give **8** (7.5 g, 54%) as a pale yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.97 (6H, d, J = 6.6 Hz), 1.40 (9H, s), 2.22−2.27 (2H, m), 2.43 (3H, s), 2.91 (2H, d, J = 7.2 Hz), 4.27 (2H, s), 6.63 (1H, d, J = 3.0 Hz), 7.08 (1H, dd, J = 3.0, 9.0 Hz), 7.09 (1H, br), 7.21−7.29 (3H, m), 7.89 (1H, d, J = 9.0 Hz).

3-{[(*tert*-Butoxycarbonyl)amino]methyl}-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl Trifluoromethanesulfonate (9). To a solution of **8** (1.5 g, 3.6 mmol) in DMF (10 mL) was added sodium hydride (60% in oil) (0.21 g, 5.4 mmol) at 0 °C, and the mixture was stirred for 10 min. To the reaction mixture was added *N*-phenyl-bis(trifluoromethanesulfonimide) (1.9 g, 5.4 mmol), and the mixture was stirred at room temperature for 30 min. The mixture was partitioned between AcOEt and water. The organic layer was separated, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was crystallized from diisopropyl ether to give **9** (1.5 g, 75%) as a pale yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.01 (6H, d, J = 6.6 Hz), 1.41 (9H, s), 2.33−2.420 (1H, m), 2.49 (3H, s), 2.97−3.01 (2H, m), 4.33 (2H, s), 7.12 (2H, d, J = 8.0 Hz), 7.25 (1H, d, J = 3.0 Hz), 7.36 (2H, d, J = 8.0 Hz), 7.52 (1H, dd, J = 3.0, 7.4 Hz), 8.13 (1H, d, J = 7.4 Hz).

5-Methyl-3-oxohexanenitrile (11A). Compound **11A** was prepared by procedures described in the references.[11,12] 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 **10A** (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 **11A** (41 g, 98%) as a pale yellow oil. Compound **11A** 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).

3-Oxohexanenitrile (11B). A suspension of sodium hydride (60% in oil) (18 g, 0.44 mol) in THF (160 mL) was stirred at 70 °C, and a solution of methyl butyrate (**10B**) (26 mL, 0.22 mol) and acetonitrile (23 mL, 0.44 mol) in THF (50 mL) was added dropwise. The mixture was stirred at 70 °C for 8 h. Water was added to the reaction mixture and filtered. The filtrate was washed with hexane and hexane−diethyl ether (1:1). The aqueous layer was separated, acidified with concentrated HCl and extracted with AcOEt. The extract was washed with brine and dried over anhydrous $MgSO_4$. The solvent was evaporated to give **11B** (20 g, 82%) as a pale yellow oil. ^{1}H NMR (300 MHz, CD_3OD) δ: 0.95 (3H, t, J = 7.2 Hz), 1.60−1. 72 (2H, m), 2.59 (2H, t, J = 7.2 Hz), 3.50 (2H, s).

3-Oxoheptanenitrile (11C). Compound **11C** was prepared in a manner similar to that described for **11A** in quantitative yield (crude). ^{1}H NMR (300 MHz, $CDCl_3$) δ:0.92 (3H, t, J = 7.3 Hz), 1.31−1.33 (2H, m), 1.58−1.62 (2H, m), 2.60−2.64 (2H, m), 3.46 (2H, s).

5,5-Dimethyl-3-oxohexanenitrile (11D). Compounds **11D** was prepared in a manner similar to that described for **11A** in quantitative yield (crude). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.05 (9H, s), 2.49 (2H, s), 3.43 (2H, s).

Ethyl (2*E*)-3-[3-{[(*tert*-Butoxycarbonyl)amino]methyl}-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl]prop-2-enoate (12). To a solution of **9** (1.5 g, 2.8 mmol) in DMF (10 mL) were added ethyl acrylate (0.65 mL, 6.0 mmol), triethylamine (4.2 mL, 30 mmol) and $Pd(PPh_3)_2Cl_2$ (21 mg, 0.030 mmol), and the resulting mixture was stirred at 70 °C for 6 h. The reaction mixture was cooled to room temperature, and water was added. The mixture was extracted with AcOEt. The organic layer was separated, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by basic silica gel column chromatography (AcOEt) to give **12** (0.87 g, 62%) as a pale yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.05 (6H, d, J = 6.6 Hz), 1.32 (3H, t, J = 7.2 Hz), 1.41 (9H, s), 2.34−2.39 (1H, m), 2.51 (3H, s), 4.25 (2H, q, J = 7.2 Hz), 4.33−4.36 (4H, m), 6.41 (1H, d, J = 15.9 Hz), 7.12 (2H, d, J = 7.8 Hz), 7.36 (2H, d, J = 7.8 Hz), 7.41 (1H, s), 7.63 (1H, d, J = 15.9 Hz), 7.89−7.91 (1H, m), 8.13−8.14 (1H, m).

4-(4-Methylphenyl)-2-(2-methylpropyl)-6-[(1*E*)-3-oxoprop-1-en-1-yl]quinoline-3-carbonitrile (13). To a solution of **6** (5 g, 13.2 mmol) in DMF (40 mL) were added acrolein (2.22 g, 6.0 mmol), triethylamine (5.5 mL, 39.6 mmol), benzyltriethylammonium chloride (3 g, 13.2 mmol) and palladium acetate (150 mg, 0.66 mmol), and the resulting mixture was stirred at 70 °C for 3 h. To the reaction mixture were added acrolein (2.22 g, 6.0 mmol) and palladium acetate (150 mg, 0.66 mmol), and the resulting

mixture was stirred at 70 °C for 17 h. The reaction mixture was cooled to room temperature, and water was added. The mixture was extracted with AcOEt. The organic layer was separated, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/AcOEt = 75/25) to give **13** (3.26 g, 70%) as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.07 (1H, d, J = 6.0 Hz), 2.36−2.47 (1H, m), 2.52 (3H, s), 3.14 (2H, d, J = 9.0 Hz), 6.74 (1H, dd, J = 9.0, 15.0 Hz), 7.37 (2H, d, J = 9.0 Hz), 7.44 (2H, d, J = 9.0 Hz), 7.52 (1H, d, J = 15.0 Hz), 7.84 (1H, d, J = 3.0 Hz), 8.01 (1H, d, J = 9.0 Hz), 8.06 (1H, d, J = 9.0 Hz), 9.71 (1H, d, J = 9.0 Hz).

***tert*-Butyl {[6-(5-Cyanopropyl)-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-3-yl]methyl}carbamate (14).** To a solution of **13** (4.04 g, 11.4 mmol) in THF (30 mL) and MeOH (30 mL) was added sodium borohydride (216 mg, 5.69 mmol) at 0 °C, and the mixture was stirred for 10 min. The reaction mixture was quenched with saturated ammonium chloride solution and 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 = 60/40) to give a yellow powder. The obtained yellow powder was dissolved in THF (20 mL) and MeOH (60 mL), and then 25% NH_3 solution (5 mL) and Raney-Ni (10 mL) were added. The mixture was stirred at 70 °C under H_2 atmosphere (0.5 MPa) for 3 h and filtered through a Celite pad. The filtrate was partitioned between AcOEt and water. The organic layer was separated, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was dissolved in THF (100 mL), and to the solution was added $(Boc)_2O$ (2.28 g, 10.4 mmol). After stirring at room temperature for 3 h, the reaction mixture was concentrated under reduced pressure. The residue was crystallized from hexane−diisopropyl ether to give *tert*-butyl {[6-(3-hydroxypropyl)-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-3-yl]methyl}carbamate (2.85 g, 54%) as a pale yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.02 (6H, d, J = 6.0 Hz), 1.41 (9H, s), 1.79−1.89 (2H, m), 2.29−2.41 (1H, m), 2.48 (3H, s), 2.72 (2H, t, J = 6.0 Hz), 2.95 (2H, d, J = 6.0 Hz), 3.64 (2H, q, J = 6.0 Hz), 4.24−4.34 (3H, m), 7.09 (1H, brs), 7.12 (2H, d, J = 9.0 Hz), 7.33 (2H, d, J = 9.0 Hz), 7.52 (1H, dd, J = 3.0, 9.0 Hz), 7.99 (1H, d, J = 9.0 Hz). To a solution of *tert*-butyl {[6-(3-hydroxypropyl)-4-(4-methylphenyl)-2-(2-methylpropyl)-quinolin-3-yl]methyl}carbamate (400 mg, 0.865 mmol) and triethylamine (175 mg, 1.73 mmol) in THF (15 mL) was added methanesulfonyl chloride (150 mg, 1.30 mmol) at 0 °C, and the resulting solution was stirred at 0 °C for 30 min. The reaction mixture was quenched with saturated $NaHCO_3$ solution and extracted with AcOEt. The organic layer was separated, washed with brine, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give a brown oil. The brown oil was dissolved in DMSO (10 mL), and potassium cyanide (380 mg, 5.8 mmol) was added. The mixture was stirred at 60 °C for 1.5 h. The reaction mixture was diluted with AcOEt and washed with saturated $NaHCO_3$ solution and brine, sequentially. The organic layer was separated, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/AcOEt = 70/30) to give **14** (370 mg, 90.6% for two steps) as a yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.03 (6H, d, J = 9.0 Hz), 1.42 (9H, s), 1.89−1.98 (2H, m), 2.28 (2H, t, J = 6.0 Hz), 2.32−2.43 (1H, m), 2.49 (3H, m), 2.49 (3H, s), 2.79 (2H, t, J = 6.0 Hz), 2.96 (2H, d, J = 6.0 Hz), 4.26−4.36 (3H, m), 7.09 (1H, d, J = 3.0 Hz), 7.12 (2H, d, J = 9.0 Hz), 7.34 (2H, d, J = 6.0 Hz), 7.48 (1H, dd, J = 3.0, 9.0 Hz), 8.02 (1H, d, J = 9.0 Hz).

Ethyl (2*E*,4*E*)-5-[3-Cyano-4-(4-methylphenyl)-2-(2-methylpropyl)-quinolin-6-yl]penta-2,4-dienoate (15). To a solution of ethyl diethylphosphonoacetate (1.90 g, 10.2 mmol) in THF (50 mL) was added sodium hydride (60% in oil) (408 mg, 10.2 mmol) at 0 °C, and the resulting mixture was stirred for 10 min. To the reaction mixture was added **13**, and the mixture was stirred at room temperature for 10 min. The reaction mixture was diluted with a mixture of THF and AcOEt, and the mixture was washed with 2 M NaOH solution, 1 M HCl, saturated $NaHCO_3$ solution and brine, sequentially. The organic layer was separated, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was crystallized from diisopropyl ether to give **15** (2.71 g, 75%) as a yellow powder. The mother liquor was concentrated under reduced pressure and purified by silica gel column chromatography (hexane/AcOEt = 50/50) to give **15** (320 mg, 9%) as a yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.06 (6H, d, J = 6.0 Hz), 1.31 (3H, t, J = 6.0 Hz), 2.33−2.45 (1H, m), 2.51 (3H, s), 3.12 (2H, d, J = 9.0 Hz), 4.23 (2 H, q, J = 6.0 Hz), 6.03 (1H, d, J = 15.0 Hz), 6.90−6.92 (2H, m), 7.35−7.45 (5H, m), 7.63 (1H, d, J = 3.0 Hz), 7.99 (1H, dd, J = 3.0, 9.0 Hz), 8.10 (1H, d, J = 9.0 Hz).

***tert*-Butyl {[6-(5-Cyanopentyl)-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-3-yl]methyl}carbamate (16).** To a mixture of **9** (3.03 g, 6.13 mmol), copper iodide (176 mg, 0.92 mmol), triethylamine (10 mL) and hex-5-ynenitrile (2.86 g, 30.7 mmol) was added $Pd(PPh_3)_2Cl_2$ (21 mg, 0.030 mmol), and the resulting mixture was stirred at 80 °C for 10 min under argon atmosphere. The reaction mixture was cooled to room temperature and quenched with saturated ammonium chloride solution. The mixture was extracted with AcOEt. The organic layer was separated, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give a black powder. The black powder was recrystallized from AcOEt and hexane to give a white powder (2.56 g). A mixture of the obtained white powder (2.56 g), 10% Pd/C (50% wet, 550 mg), THF (35 mL) and EtOH (200 mL) was stirred at room temperature under H_2 atmosphere for 3.5 h. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure to give **16** (2.37 g, 92%) as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.02 (6H, d, J = 6.0 Hz), 1.41 (9H, s), 1.56−1.69 (6H, m), 2.29 (2H, t, J = 6.0 Hz), 2.32−2.39 (1H, m), 2.49 (3H, s), 2.64 (2H, t, J = 6.0 Hz), 2.95 (2H, d, J = 6.0 Hz), 4.24−4.36 (3H, m), 7.05 (1H, d, J = 3.0 Hz), 7.13 (2H, d, J = 9.0 Hz), 7.34 (2H, d, J = 6.0 Hz), 7.49 (1H, d, J = 9.0 Hz), 7.99 (1H, d, J = 9.0 Hz).

3-[3-(Aminomethyl)-4-(4-methylphenyl)-2-(2-methylpropyl)-quinolin-6-yl]propanoic Acid Dihydrochloride (17a). A mixture of **12** (12.57 g, 25 mmol), 10% Pd/C (50% wet, 6.1 g), THF (150 mL) and EtOH (150 mL) was stirred at room temperature under H_2 atmosphere for 2 h. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was crystallized from THF and diisopropyl ether to give ethyl 3-[3-{[(*tert*-butoxycarbonyl)amino]methyl}-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl]propanoate (11.59 g, 92%) as a white powder. The obtained white powder was dissolved in THF (100 mL) and EtOH (100 mL), and 1 M NaOH solution (45 mL) was added. The mixture was stirred at room temperature for 4 h. The reaction mixture was neutralized with 1 M HCl and extracted with AcOEt. The organic layer was separated, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was crystallized from AcOEt and diisopropyl ether to give 3-[3-{[(*tert*-butoxycarbonyl)-amino]methyl}-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl]propanoic acid as a white powder (10.21 g, 93%). The obtained white powder (0.47 g, 0.99 mmol) was added to 4 M HCl in 1,4-dioxane (5 mL). The mixture was stirred at room temperature for 1 h. After evaporation of the solvent, the residue was dissolved in EtOH (5 mL). To the solution was added propylene oxide, and the resulting mixture was stirred at room temperature for 10 h. The reaction mixture was concentrated under reduced pressure, and the residue was crystallized from AcOEt to give **17a** (210 mg, 57%) as a pale yellow powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.01 (6H, d, J = 6.8 Hz), 2.33−2.42 (1H, m), 2.46 (3H, s), 2.85 (2H, t, J = 7.3 Hz), 2.96 (2H, d, J = 6.8 Hz), 3.93 (2H, s), 7.06 (1H, d, J = 1.3 Hz), 7.27 (2H, d, J = 7.7 Hz), 7.43 (1H, d, J = 7.9 Hz), 7.66 (1H, dd, J = 8.6, 1.8 Hz), 7.95 (1H, d, J = 8.7 Hz). LC/MS m/z 377.2 (M + H). HPLC

purity 97.61% (220 nm), 98.45% (254 nm). ESI-HRMS calcd for $C_{24}H_{28}N_2O_2$ *m*/*z* 377.2224 (M + H), found 377.2206 (M + H).

4-[3-(Aminomethyl)-4-(4-methylphenyl)-2-(2-methylpropyl)-quinolin-6-yl]butanoic acid Dihydrochloride (17b). A mixture of **14** (3.10 g, 6.57 mmol), 2 M NaOH solution (16.5 mL) and EtOH (60 mL) was refluxed for 13 h. The mixture was acidified with 6 M HCl and extracted with AcOEt. The organic layer was separated, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was crystallized from diisopropyl ether to give a yellow powder (2.68 g, 83%). The yellow powder (400 mg, 0.815 mmol) was dissolved in 1,4-dioxane (5 mL) and added to 4 M HCl in 1,4-dioxane (10 mL). The mixture was stirred at room temperature for 20 min. The reaction mixture was concentrated under reduced pressure to give **17b** (347 mg, 99%) as a pale yellow powder. ^1H NMR (300 MHz, CD_3OD) δ: 1.14 (6H, d, J = 6.0 Hz), 1.83−1.93 (2H, m), 2.28 (2H, t, J = 6.0 Hz), 2.30−2.36 (1H, m), 2.54 (3H, s), 2.82 (2H, t, J = 6.0 Hz), 4.33 (2H, brs), 7.35−7.44 (3H, m), 7.58 (2H, d, J = 9.0 Hz), 8.11 (1H, d, J = 9.0 Hz), 8.25−8.33 (1H, m). LC/MS *m*/*z* 391.3 (M + H). HPLC purity 100% (220 nm), 100% (254 nm). ESI-HRMS calcd for $C_{25}H_{30}N_2O_2$ *m*/*z* 391.2380 (M + H), found 391.2368 (M + H).

5-[3-(Aminomethyl)-4-(4-methylphenyl)-2-(2-methylpropyl)-quinolin-6-yl]pentanoic Acid Dihydrochloride (17c). Compound **15** (1.51 g, 3.55 mmoL) was dissolved in *N*,*N*-dimethyl acetamide (DMA) (150 mL), and 25% NH_3 solution (5 mL) and Raney-Ni (4 mL) were added. The mixture was stirred at room temperature under H_2 atmosphere (0.5 MPa) for 4 h and filtered through a Celite pad. The filtrate was partitioned between AcOEt and water. The organic layer was separated, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/AcOEt = 70/30) to give ethyl 5-[3-cyano-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl]pentanoate (840 mg, 55%) as a yellow oil. The obtained ethyl 5-[3-cyano-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl]pentanoate (840 mg, 1.96 mmol) was dissolved in MeOH (150 mL), and 25% NH_3 solution (5 mL) and Raney-Ni (5 mL) were added. The mixture was stirred at room temperature under H_2 atmosphere (0.5 MPa) for 3 h and filtered through a Celite pad. To the filtrate was partitioned between AcOEt and water. The organic layer was separated, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (AcOEt/MeOH = 90/10) to give ethyl 5-[3-(aminomethyl)-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl]pentanoate (815 mg, 97%) as a yellow oil. The obtained ethyl 5-[3-(aminomethyl)-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl]pentanoate (815 mg, 1.9 mmol) was dissolved in THF (100 mL), and to the mixture was added $(Boc)_2O$ (2.28 g, 10.4 mmol). After stirring at room temperature for 3 h, the reaction mixture was concentrated under reduced pressure. The residue was crystallized from hexane−diisopropyl ether to give ethyl 5-[3-{[(*tert*-butoxycarbonyl)amino]methyl}-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl]pentanoate (940 mg, 95%) as a pale yellow powder. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.03 (6H, d, J = 6.0 Hz), 1.22 (2H, t, J = 6.0 Hz), 1.20−1.28 (2H, m), 1.55 (2H, brs), 1.59−1.62 (2H, m), 2.27 (2H, t, J = 6.0 Hz), 2.33−2.39 (1H, m), 2.49 (3H, s), 2.60−2.66 (2H, m), 3.00 (2H, d, J = 9.0 Hz), 3.77 (2H, brs), 4.06−4.16 (2H, m), 7.03 (1H, brs), 7.17 (2H, d, J = 9.0 Hz), 7.34 (2H, d, J = 9.0 Hz), 7.47 (1H, dd, J = 3.0, 9.0 Hz), 7.97 (1H, d, J = 9.0 Hz). Ethyl 5-[3-{[(*tert*-butoxycarbonyl)amino]methyl}-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl]pentanoate (940 mg, 1.81 mmol) was dissolved in THF (30 mL) and EtOH (20 mL), and 2 M NaOH solution (8.2 mL) was added. The mixture was stirred at room temperature for 40 min. The reaction mixture was neutralized with 6 M HCl and extracted with AcOEt. The organic layer was separated, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure to give 5-[3-{[(*tert*-butoxycarbonyl)-amino]methyl}-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl]pentanoic acid as a colorless amorphous. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.03 (6H, d, J = 6.0 Hz), 1.41 (9H, s), 1.59−1.67 (4H, m), 2.27−2.37 (3H, m), 2.48 (3H, s), 2.61−2.69 (2H, m), 2.99−3.06 (2H, m), 4.30 (2H, brs), 7.08 (1H, d, J = 3.0 Hz), 7.13 (2H, d, J = 9.0 Hz), 7.34 (2H, d, J = 6.0 Hz), 7.53 (1H, dd, J = 3.0, 9.0 Hz), 8.10 (1H, brs). The obtained 5-[3-{[(*tert*-butoxycarbonyl)amino]methyl}-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl]pentanoic acid was dissolved in 1,4-dioxane (10 mL), and 4 M HCl in 1,4-dioxane (10 mL) was added. The mixture was stirred at room temperature for 3 h. The reaction mixture was concentrated under reduced pressure to give **17c** (578 mg, 69% for two steps) as a pale yellow powder. ^1H NMR (300 MHz, CD_3OD) δ: 1.11−1.15 (6H, m), 1.50−1.69 (4H, m), 2.27 (2H, t, J = 6.0 Hz), 2.31−2.36 (1H, m), 2.55 (3H, s), 2.80 (2H, t, J = 6.0 Hz), 3.32−3.34 (2H, m), 4.33 (2H, brs), 7.39 (2H, d, J = 9.0 Hz), 7.41 (1H, s), 7.58 (2H, d, J = 6.0 Hz), 8.10 (1H, dd, J = 3.0, 9.0 Hz), 8.28 (1H, d, J = 9.0 Hz). LC/MS *m*/*z* 405.3 (M + H). HPLC purity 100% (220 nm), 100% (254 nm). ESI-HRMS calcd for $C_{26}H_{32}N_2O_2$ *m*/*z* 405.2537 (M + H), found 405.2523 (M + H).

6-[3-(Aminomethyl)-4-(4-methylphenyl)-2-(2-methylpropyl)-quinolin-6-yl]hexanoic Acid Dihydrochloride (17d). A mixture of **16** (2.37 g, 4.74 mmol), 2 M NaOH solution (12 mL) and EtOH (40 mL) was refluxed for 6 h. The mixture was acidified with 6 M HCl and extracted with AcOEt. The organic layer was separated, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was crystallized from diisopropyl ether to give 6-[3-{[(*tert*-butoxycarbonyl)amino]methyl}-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl]hexanoic acid as a white powder (2.37 g, 96%). ^1H NMR (300 MHz, $CDCl_3$) δ: 1.13 (6H, d, J = 6.0 Hz), 1.40 (9H, s), 1.45−1.98 (6H, m), 2.30 (2H, t, J = 6.0 Hz), 2.34−2.43 (1H, m), 2.52 (3H, s), 2.69 (2H, d, J = 6.0 Hz), 3.38−3.57 (2H, m), 4.30−4.48 (2H, m), 7.10−7.20 (3H, m), 7.43 (2H, J = 6.0 Hz), 7.69−7.78 (1H, m), 8.85−8.99 (1H, m). A mixture of 6-[3-{[(*tert*-butoxycarbonyl)amino]methyl}-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl]hexanoic acid (310 mg, 0.598 mmol), 4 M HCl in 1,4-dioxane (5 mL) and 1,4-dioxane (10 mL) was stirred at room temperature for 4 h. The reaction mixture was concentrated under reduced pressure to give **17d** (294 mg, 99%) as a pale yellow powder. ^1H NMR (300 MHz, CD_3OD) δ: 1.14 (6H, d, J = 6.0 Hz), 1.27−1.38 (2H, m), 1.51−1.70 (4H, m), 2.24 (2H, t, J = 6.0 Hz), 2.27−2.35 (1H, m), 2.55 (3H, s), 2.79 (2H, t, J = 6.0 Hz), 3.32−3.34 (2H, m), 4.33 (2H, brs), 7.36−7.43 (3H, m), 7.58 (2H, d, J = 6.0 Hz), 8.10 (1H, dd, J = 3.0, 9.0 Hz), 8.29 (1H, d, J = 9.0 Hz). LC/MS *m*/*z* 419.3 (M + H). HPLC purity 99.35% (220 nm), 99.32% (254 nm). ESI-HRMS calcd for $C_{27}H_{34}N_2O_2$ *m*/*z* 419.2693 (M + H), found 419.2684 (M + H).

5-Methyl-2-[(4-methylphenyl)methylidene]-3-oxohexanenitrile (18B). A mixture of **11B** (60 g, 500 mmol), *p*-tolualdehyde (58.2 mL, 500 mmol), piperidine (5 mL, 50 mmol), AcOH (5.7 mL, 100 mmol) and toluene (300 mL) was heated under reflux for 8 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 **18B** (197 g, crude) as a brown oil. The obtained oil was used for the next reaction without further purifications.

5-Methyl-2-[(4-methylphenyl)methylidene]-3-oxohexanenitrile (18A). Compound **18A** was prepared in a manner similar to that described for **18B** in quantitative yield (crude). ^1H 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).

2-(3,3-Dimethylbutanoyl)-3-(4-methylphenyl)acrylonitrile (18D). Compound **18D** was prepared in a manner similar to that described for **18B** in 75% yield. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.10 (9H, s), 2.44 (3H, s), 2.81 (2H, s), 7.31 (2H, d, J = 8.1 Hz), 7.92 (2H, d, J = 8.3 Hz), 8.13 (1H, s). mp 76.4−78.0 °C.

5-(1-Hydroxy-2-methylpropylidene)-2,2-dimethyl-1,3-dioxane-4,6-dione (21C). To an ice-cooled solution of Meldrum's acid

(**20**) (14.41 g, 0.10 mol) and pyridine (16.2 mL, 0.2 mol) in dichloromethane (100 mL) was added dropwise 2-methylpropanoyl chloride (13.4 mL, 0.11 mol). The obtained mixture was stirred under ice-cooling for 2 h. The reaction mixture was poured into 0.5 M HCl and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure to give crude **21C** (21.4 g, quantitative) as a brown oil. The crude **21C** was used for the next reaction without further purifications. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.25 (6H, d, J = 6.3 Hz), 1.74 (6H, s), 4.05−4.14 (1H, m).

Compounds **21A**, **B**, **D** and **E** were prepared in a manner similar to that described for **21C**.

5-(1-Hydroxybutylidene)-2,2-dimethyl-1,3-dioxane-4,6-dione (21B). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.03 (3H, t, J = 8.9 Hz), 1.66−1.81 (2H, m), 1.74 (6H, s), 3.04−3.10 (2H, m).

5-(1-Hydroxy-3-methylbutylidene)-2,2-dimethyl-1,3-dioxane-4,6-dione (21D). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.01 (6H, d, J = 6.8 Hz), 1.73 (6H, s), 2.09−2.22 (1H, m), 2.99 (2H, d, J = 6.3 Hz).

5-(1-Hydroxy-2-phenylethylidene)-2,2-dimethyl-1,3-dioxane-4,6-dione (21E). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.72 (9H, s), 4.43 (2H, s), 7.02−7.42 (5H, m), 15.33 (1H, brs).

***tert*-Butyl (2*E*)-3-Aminobut-2-enoate (22B).** A mixture of *tert*-butyl acetoacetate **19A** (253 g, 1.94 mol), 25% ammonia solution (500 mL) and MeOH (500 mL) was stirred at room temperature for 15 h. The reaction mixture was extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure to give crude **22B** (253 g, quantitative) as a brown oil. Compound **22B** was used for the next reaction without further purifications. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.47 (9H, s), 1.87 (3H, s), 4.46 (1H, s).

***tert*-Butyl (2*E*)-3-Amino-4-methylpent-2-enoate (22E).** Compound **21C** (22.7 g, 122 mmol) was added to toluene (100 mL) and *tert*-butyl alcohol (11.2 g, 150 mmol) and stirred under reflux for 6 h. The reaction mixture was cooled to room temperature. The mixture was extracted with AcOEt. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure to give a brown oil (9.31 g). The obtained brown oil was added to MeOH (100 mL) and 25% ammonia solution (100 mL), and stirred at room temperature for 12 h. The reaction mixture was concentrated under reduced pressure and extracted with AcOEt and water. The organic layer was washed with brine, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give **22E** (9.26 g, 41%) as a brown oil. ^{1}H NMR ($CDCl_3$) δ:1.14 (6H, d, J = 6.8 Hz), 1.48 (9H, s), 2.18−2.37 (1H, m), 4.49 (1H, s).

Compounds **22C**, **22D**, **22F** and **22G** were prepared in a manner similar to that described for **22E**.

***tert*-Butyl (2*E*)-3-Aminopent-2-enoate (22C).** ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.13 (3H, t, J = 7.6 Hz), 1.47 (9H, s), 2.13 (2H, q, J = 7.6 Hz), 4.48 (1H, s).

***tert*-Butyl (2*E*)-3-Aminohex-2-enoate (22D).** ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.95 (3H, t, J = 7.3 Hz), 1.47 (9H, s), 1.49−1.68 (2H, m), 1.92−2.17 (2H, m), 4.47 (1H, s).

***tert*-Butyl (2*E*)-3-Amino-4-methylpent-2-enoate (22F).** ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.95 (6H, d, J = 7.2 Hz), 1.47 (9H, s), 1.86−1.95 (3H, m), 4.44 (1H, s).

***tert*-Butyl (2*E*)-3-Amino-4-phenylbut-2-enoate (22G).** ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.47 (9H, s), 3.43 (2H, s), 4.58 (1H, s), 7.02−7.38 (5H, m).

Methyl 5-Cyano-2-methyl-4-(4-methylphenyl)-6-propyl-1,4-dihydropyridine-3-carboxylate (23a). A mixture of **18B** (197 g, crude), **22A** (commercially available) (57.5 g, 500 mmol) and AcOH (500 mL) was stirred at 80 °C for 30 min. The reaction mixture was ice-cooled and the precipitated crystals were collected by filtration, washed with 75% aqueous ethanol and dried to afford **23a** (60 g, 39%) as a brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.96 (3H, t, J = 7.4 Hz), 1.54−1.66 (2H, m), 2.30 (3H, s), 2.32−2.41 (2H, m), 2.35 (3H, s), 3.58 (3H, s), 4.56 (1H, s), 5.80 (1H, s), 7.09 (2H, d, J = 8.1 Hz), 7.13 (2H, d, J = 8.1 Hz).

Methyl 6-Butyl-5-cyano-2-methyl-4-(4-methylphenyl)-1,4-dihydropyridine-3-carboxylate (23b). Compound **23b** was prepared in a manner similar to that described for **23a** in 24% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.92 (3H, t, J = 7.3 Hz), 1.30−1.42 (2H, m), 1.49−1.60 (2H, m), 2.30 (3H, s), 2.34−2.39 (2H, m), 2.35 (3H, s), 3.58 (3H, s), 4.56 (1H, s), 5.77 (1H, s), 7.07−7.14 (4H, m).

***tert*-Butyl 5-Cyano-6-(2,2-dimethylpropyl)-2-methyl-4-(4-methylphenyl)-1,4-dihydropyridine-3-carboxylate (23d).** Compound **23d** was prepared in a manner similar to that described for **23a** in 53% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.01 (9H, s), 1.29 (9H, s), 2.17 (1H, d, J = 13.9 Hz), 2.30 (3H, s), 2.32 (3H, s), 2.34 (1H, d, J = 13.9 Hz), 4.52 (1H, s), 5.43 (1H, brs), 7.06−7.10 (2H, m), 7.11−7.15 (2H, m).

Methyl 5-Cyano-2-methyl-4-(4-methylphenyl)-6-propylpyridine-3-carboxylate (24a). To an ice-cooled solution of **23a** (152 g, 0.40 mol) in acetone (500 mL) was added dropwise a solution of CAN (550 g, 1 mol) in water (300 mL). The obtained mixture was stirred under ice-cooling for 1 h and concentrated under reduced pressure. The residue was partitioned between AcOEt and water, and the organic layer was washed sequentially with saturated $NaHCO_3$ solution and brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was crystallized from hexane to afford **24a** (134 g, 89%) as a white powder. ^{1}H NMR ($CDCl_3$) δ:1.05 (3H, t, J = 7.4 Hz), 1.79−1.91 (2H, m), 2.41 (3H, s), 2.62 (3H, s), 3.02−3.07 (2H, m), 3.60 (3H, s), 7.23−7.29 (4H, m).

Methyl 6-Butyl-5-cyano-2-methyl-4-(4-methylphenyl)pyridine-3-carboxylate (24b). Compound **24b** was prepared in a manner similar to that described for **24a** in 65% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.97 (3H, t, J = 7.3 Hz), 1.40−1.52 (2H, m), 1.74−1.84 (2H, m), 2.41 (3H, s), 2.62 (3H, s), 3.04−3.09 (2H, m), 3.60 (3H, s), 7.23−7.29 (4H, m).

Methyl 5-Cyano-2-methyl-4-(4-methylphenyl)-6-(2-methylpropyl)pyridine-3-carboxylate (24c). Compound **24c** was prepared in a manner similar to that described for **24a** in 82% yield. ^{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.

***tert*-Butyl 5-Cyano-6-(2,2-dimethylpropyl)-2-methyl-4-(4-methylphenyl)pyridine-3-carboxylate (24d).** Compound **24d** was prepared in a manner similar to that described for **24a** in 96% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.06 (9H, s), 1.26 (9H, s), 2.41 (3H, s), 2.64 (3H, s), 2.99 (2H, s), 7.21−7.31 (4H, m).

***tert*-Butyl 5-Cyano-6-(2,2-dimethylpropyl)-2-ethyl-4-(4-methylphenyl)pyridine-3-carboxylate (24e).** Compound **24e** was prepared in a manner similar to that described for **24a** in 42% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.07 (9H, s), 1.25 (9H, s), 1.30−1.38 (3H, m), 2.41 (3H, s), 2.89 (2H, q, J = 7.5 Hz), 3.01 (2H, s), 7.26 (4H, s).

***tert*-Butyl 5-Cyano-6-(2,2-dimethylpropyl)-4-(4-methylphenyl)-2-propylpyridine-3-carboxylate (24f).** Compound **24f** was prepared in a manner similar to that described for **24a** in 59% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.98 (3H, t, J = 7.3 Hz), 1.02 (9H, s), 1.14 (2H, brs), 1.14 (9H, s), 1.73−1.86 (2H, m), 2.39 (3H, s), 2.72−2.77 (2H, m), 2.87 (2H, s), 3.68 (2H, s), 7.13 (2H, d, J = 8.1 Hz), 7.21 (2H, d, J = 8.1 Hz).

***tert*-Butyl 5-Cyano-6-(2,2-dimethylpropyl)-2-(1-methylethyl)-4-(4-methylphenyl)pyridine-3-carboxylate (24g).** Compound **24g** was prepared in a manner similar to that described for **24a** in 51% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.06 (9H, s), 1.11 (6H, d, J = 6.6 Hz), 1.25 (9H, s), 2.40 (3H, s), 3.00 (2H, s), 3.14−3.23 (1H, m), 7.07 (2H, d, J = 7.2 Hz), 7.31 (2H, d, J = 7.2 Hz).

***tert*-Butyl 5-Cyano-6-(2,2-dimethylpropyl)-4-(4-methylphenyl)-2-(2-methylpropyl)pyridine-3-carboxylate (24h).** Compound **24h** was prepared in a manner similar to that described for **24a** in 49% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.95 (6H, d, J = 6.6 Hz), 1.06 (9H, s), 1.24 (9H, s), 2.22−2.35 (1H, m), 2.40 (3H, s), 2.76 (2H, d, J = 7.2 Hz), 3.00 (2H, s), 7.19−7.35 (4H, m).

***tert*-Butyl 2-Benzyl-5-cyano-6-(2,2-dimethylpropyl)-4-(4-methylphenyl)pyridine-3-carboxylate (24i).** Compound **24i** was prepared in a manner similar to that described for **24a**. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.00 (9H, s), 1.13 (9H, s), 1.24 (9H, s), 2.40 (3H, s), 2.76 (2H, d, J = 7.2 Hz), 2.99 (2H, s), 4.26 (2H, s), 7.12−7.40 (9H, m).

Methyl 5-(Aminomethyl)-2-methyl-4-(4-methylphenyl)-6-propylpyridine-3-carboxylate (25a). A mixture of **24a** (22 g, 71.3 mmol), Raney-Ni (22 mL), 25% NH_3 solution (22 mL), THF (100 mL) and MeOH (100 mL) was stirred at room temperature 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 **25a** (15 g, 67%) as pale yellow crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.02 (3H, t, J = 7.3 Hz), 1.64−1.85 (2H, m), 2.37 (3H, s), 2.53 (3H, s), 2.93−3.05 (2H, m), 3.47 (3H, s), 3.82 (2H, d, J = 5.5 Hz), 7.19 (2H, d, J = 8.1 Hz), 7.31 (2H, d, J = 8.1 Hz), 8.38 (3H, s).

Methyl 5-(Aminomethyl)-6-butyl-2-methyl-4-(4-methylphenyl)-pyridine-3-carboxylate (25b). Compound **25b** was prepared in a manner similar to that described for **25a** in 68% yield as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.95 (3H, t, J = 7.3 Hz), 1.35−1.54 (2H, m), 1.61−1.81 (2H, m), 2.37 (3H, s), 2.53 (3H, s), 2.94−3.09 (2H, m), 3.47 (3H, s), 3.82 (2H, d, J = 5.5 Hz), 7.19 (2H, d, J = 8.1 Hz), 7.30 (2H, d, J = 8.1 Hz), 8.38 (3H, s).

Methyl 5-(Aminomethyl)-2-methyl-4-(4-methylphenyl)-6-(2-methylpropyl)pyridine-3-carboxylate (25c). Compound **25c** was prepared in a manner similar to that described for **25a** in 95% yield as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.98 (6H, d, J = 6.6 Hz), 1.39 (2H, brs), 2.15−2.30 (1H, m), 2.39 (3H, s), 2.53 (3H, s), 2.80 (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.

***tert*-Butyl 5-(Aminomethyl)-6-(2,2-dimethylpropyl)-2-methyl-4-(4-methylphenyl)pyridine-3-carboxylate (25d).** Compound **25d** was prepared in a manner similar to that described for **25a** in 92% yield as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.02 (9H, s), 1.19 (9H, s), 2.40 (3H, s), 2.54 (3H, s), 2.86 (2H, s), 3.68 (2H, s), 7.13 (2H, d, J = 8.1 Hz), 7.22 (2H, d, J = 7.9 Hz).

***tert*-Butyl 5-(Aminomethyl)-6-(2,2-dimethylpropyl)-2-ethyl-4-(4-methylphenyl)pyridine-3-carboxylate (25e).** Compound **25e** was prepared in a manner similar to that described for **25a** in 74% yield as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.98 (3H, t, J = 7.3 Hz), 1.02 (9H, s), 1.14 (2H, brs), 1.14 (9H, s), 1.73−1.86 (2H, m), 2.39 (3H, s), 2.72−2.77 (2H, m), 2.87 (2H, s), 3.68 (2H, s), 7.13 (2H, d, J = 8.1 Hz), 7.21 (2H, d, J = 8.1 Hz).

***tert*-Butyl 5-(Aminomethyl)-6-(2,2-dimethylpropyl)-4-(4-methylphenyl)-2-propylpyridine-3-carboxylate (25f).** Compound **25f** was prepared in a manner similar to that described for **25a** in 74% yield as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.98 (3H, t, J = 7.3 Hz), 1.02 (9H, s), 1.14 (2H, brs), 1.14 (9H, s), 1.73−1.86 (2H, m), 2.39 (3H, s), 2.72−2.77 (2H, m), 2.87 (2H, s), 3.68 (2H, s), 7.13 (2H, d, J = 8.1 Hz), 7.21 (2H, d, J = 8.1 Hz).

***tert*-Butyl 5-(Aminomethyl)-6-(2,2-dimethylpropyl)-2-(1-methylethyl)-4-(4-methylphenyl)pyridine-3-carboxylate (25g).** Compound **25g** was prepared in a manner similar to that described for **25a** in 51% yield as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.04 (9H, s), 1.18 (9H, s), 1.30 (6H, d, J = 6.9 Hz), 1.32 (2H, brs), 2.39 (3H, s), 2.85 (2H, s), 3.04−3.13 (1H, m), 3.66 (2H, s), 7.13 (2H, d, J = 8.0 Hz), 7.20 (2H, d, J = 8.0 Hz).

***tert*-Butyl 5-(Aminomethyl)-6-(2,2-dimethylpropyl)-4-(4-methylphenyl)-2-(2-methylpropyl)pyridine-3-carboxylate (25h).** Compound **25h** was prepared in a manner similar to that described for **25a** in 89% yield as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.93 (6H, d, J = 6.6 Hz), 1.02 (9H, s), 1.17 (9H, s), 1.24 (2H, brs), 2.22−2.31 (1H, m), 2.39 (3H, s), 2.66 (2H, d, J = 7.5 Hz), 2.87 (2H, s), 3.68 (2H, s), 7.13 (2H, d, J = 8.0 Hz), 7.21 (2H, d, J = 8.0 Hz).

***tert*-Butyl 5-(Aminomethyl)-2-benzyl-6-(2,2-dimethylpropyl)-4-(4-methylphenyl)pyridine-3-carboxylate (25i).** Compound **25i** was prepared in a manner similar to that described for **25a** in 15% yield as a white powder (2 steps from compound **23i**). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.96 (9H, s), 1.07 (9H, s), 2.39 (3H, s), 2.85 (2H, s), 3.67 (2H, s), 4.18 (2H, s), 7.11−7.32 (9H, m).

Methyl 5-{[(*tert*-Butoxycarbonyl)amino]methyl}-2-methyl-4-(4-methylphenyl)-6-propylpyridine-3-carboxylate (26a). Compound **25a** (13 g, 41.6 mmol) was dissolved in THF (100 mL), and to the mixture was added $(Boc)_2O$ (9.8 g, 45 mmol). After stirring at room temperature for 3 h, the mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/AcOEt = 50/50) to give **26a** (12 g, 70%) as a pale yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.03 (3H, t, J = 7.4 Hz), 1.39 (9H, s), 1.72−1.79 (2H, m), 2.38 (3H, s), 2.53 (3H, s), 2.84−2.90 (2H, m), 3.49 (3H, s), 4.15 (2H, d, J = 5.1 Hz), 4.25 (1H, s), 7.05 (2H, d, J = 8.1 Hz), 7.20 (2H, d, J = 8.1 Hz).

Methyl 5-{[(*tert*-Butoxycarbonyl)amino]methyl}-6-butyl-2-methyl-4-(4-methylphenyl)pyridine-3-carboxylate (26b). Compound **26b** was prepared in a manner similar to that described for **26a** in 60% yield as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.95 (3H, t, J = 7.2 Hz), 1.39 (9H, s), 1.41−1.52 (2H, m), 1.63−1.77 (2H, m), 2.38 (3H, s), 2.53 (3H, s), 2.83−2.94 (2H, m), 3.49 (3H, s), 4.14 (2H, d, J = 4.9 Hz), 4.25 (1H, brs), 7.05 (2H, d, J = 8.0 Hz), 7.20 (2H, d, J = 8.0 Hz).

Methyl 5-{[(*tert*-Butoxycarbonyl)amino]methyl}-2-methyl-4-(4-methylphenyl)-6-(2-methylpropyl)pyridine-3-carboxylate (26c). Compound **26c** was prepared in a manner similar to that described for **26a** in 93% yield as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.97 (6H, d, J = 6.8 Hz), 1.39 (9H, s), 2.10−2.30 (1H, m), 2.39 (3H, s), 2.54 (3H, s), 2.78 (2H, d, J = 7.2 Hz), 3.50 (3H, s), 4.15 (2H, d, J = 4.9 Hz), 4.24 (1H, t, J = 4.9 Hz), 7.06 (2H, d, J = 7.9 Hz), 7.20 (2H, d, J = 7.9 Hz).

5-(Aminomethyl)-2-methyl-4-(4-methylphenyl)-6-propylpyridine-3-carboxylic Acid Dihydrochloride (27a). To a solution of **26a** (2 g, 4.8 mmol) in MeOH (10 mL) and THF (10 mL) was added 1 M NaOH solution (10 mL). The mixture was stirred at room temperature for 3 days. The reaction mixture was neutralized with 0.5 M HCl and extracted with AcOEt. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure to give a white powder. The obtained white powder was dissolved in 1,4-dioxane (4 mL) and added to 4 M HCl in 1,4-dioxane (4 mL, 16 mmol). The mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated under reduced pressure and crystallized from diisopropyl ether to give **27a** (1.7 g, 80%) as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.02 (3H, t, J = 7.4 Hz), 1.69−1.82 (2H, m), 2.37 (3H, s), 2.62 (3H, s), 3.01−3.07 (2H, m), 3.82 (2H, d, J = 5.3 Hz), 7.24 (2H, d, J = 8.1 Hz), 7.31 (2H, d, J = 8.1 Hz), 8.41 (3H, s). LC/MS m/z 299.2 (M + H). HPLC purity 98.07% (220 nm), 100% (254 nm). ESI-HRMS calcd for $C_{18}H_{22}N_2O_2$ m/z 299.1754 (M + H), found 299.1742 (M + H).

5-(Aminomethyl)-6-butyl-2-methyl-4-(4-methylphenyl)pyridine-3-carboxylic Acid Dihydrochloride (27b). Compound **27b** was prepared in a manner similar to that described for **27a** in 66% yield as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.95 (3H, t, J = 7.4 Hz), 1.39−1.49 (2H, m), 1.65−1.75 (2H, m), 2.37 (3H, s), 2.61 (3H, s), 3.03−3.08 (2H, m), 3.81 (2H, d, J = 5.3 Hz), 7.24 (2H, d, J = 8.1 Hz), 7.31 (2H, d, J = 8.1 Hz), 8.40 (3H, s). LC/MS m/z 313.2 (M + H). HPLC purity 100% (220 nm), 100% (254 nm). ESI-HRMS calcd for $C_{19}H_{24}N_2O_2$ m/z 313.1911 (M + H), found 313.1896 (M + H).

5-(Aminomethyl)-2-methyl-4-(4-methylphenyl)-6-(2-methylpropyl)pyridine-3-carboxylic Acid Dihydrochloride (27c). Compound **27c** was prepared in a manner similar to that described for **27a** in 57% yield as a white powder. ^{1}H NMR (300 MHz,

DMSO-d_6) δ: 0.97 (6 H, d, J = 6.59 Hz), 2.12−2.29 (1 H, m), 2.37 (3 H, s), 2.56 (3 H, s), 2.89 (2 H, s), 3.81 (2 H, d, J = 4.33 Hz), 7.23 (2 H, d, J = 7.72 Hz), 7.30 (2 H, d, J = 7.91 Hz), 8.23 (3 H, s). Anal. Calcd for $C_{19}H_{24}N_2O_2 \cdot 2HCl \cdot 2H_2O$: C, 54.16; H, 7.18; N, 6.65. Found: C, 54.57; H, 7.31; N, 6.12. LC/MS m/z 313.2 (M + H). HPLC purity 100% (220 nm), 100% (254 nm).

5-(Aminomethyl)-6-(2,2-dimethylpropyl)-2-methyl-4-(4-methylphenyl)pyridine-3-carboxylic Acid Dihydrochloride (27d). A mixture of **25d** (0.4 g, 1 mmol) in TFA (4 mL) was stirred at room temperature for 4 h. The reaction mixture was concentrated under reduced pressure. The residue was dissolved in 1,4-dioxane (4 mL) and added to 4 M HCl in 1,4-dioxane (4 mL, 16 mmol). The mixture was concentrated under reduced pressure to give a white powder. The white powder was recrystallized from distilled water and acetonitrile to give **27d** (281 mg, 71%) 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, brs). LC/MS m/z 327.2 (M + H). HPLC purity 95.34% (220 nm), 94.55% (254 nm). ESI-HRMS calculated for $C_{20}H_{26}N_2O_2$ m/z 327.2067 (M + H), found 327.2054 (M + H).

5-(Aminomethyl)-6-(2,2-dimethylpropyl)-2-ethyl-4-(4-methylphenyl)pyridine-3-carboxylic Acid Dihydrochloride (27e). Compound **27e** was prepared in a manner similar to that described for **27d** in 90% yield as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.02 (9H, s), 1.26 (3H, t, J = 7.5 Hz), 2.37 (3H, s), 2.78 (2H, q, J = 7.5 Hz), 2.92 (2H, s), 3.83 (2H, d, J = 5.4 Hz), 7.21 (2H, d, J = 8.0 Hz), 7.29 (2H, d, J = 8.0 Hz), 8.13 (3H, brs). LC/MS m/z 341.2 (M + H). HPLC purity 96.18% (220 nm), 96.73% (254 nm). ESI-HRMS calcd for $C_{21}H_{28}N_2O_2$ m/z 341.2224 (M + H), found 341.2213 (M + H).

5-(Aminomethyl)-6-(2,2-dimethylpropyl)-4-(4-methylphenyl)-2-propylpyridine-3-carboxylic Acid Dihydrochloride (27f). Compound **27f** was prepared in a manner similar to that described for **27d** in 90% yield as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.93 (3H, t, J = 7.3 Hz), 1.02 (9H, s), 1.69−1.81 (2H, m), 2.37 (3H, s), 2.74−2.79 (2H, m), 2.94 (2H, brs), 3.84 (2H, d, J = 5.1 Hz), 7.22 (2H, d, J = 8.0 Hz), 7.29 (2H, d, J = 8.0 Hz), 8.14 (3H, brs). LC/MS m/z 355.2 (M + H). HPLC purity 100% (220 nm), 100% (254 nm). ESI-HRMS calcd for $C_{22}H_{30}N_2O_2$ m/z 355.2380 (M + H), found 355.2365 (M + H).

5-(Aminomethyl)-6-(2,2-dimethylpropyl)-2-(1-methylethyl)-4-(4-methylphenyl)pyridine-3-carboxylic Acid Dihydrochloride (27g). Compound **27g** was prepared in a manner similar to that described for **27d** in 88% yield as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.04 (9H, s), 1.25 (6H, d, J = 6.6 Hz), 2.36 (3H, s), 2.90 (2H, s), 3.03−3.13 (1H, m), 3.81 (2H, d, J = 5.4 Hz), 7.22 (2H, d, J = 8.2 Hz), 7.28 (2H, d, J = 8.2 Hz), 8.18 (3H, brs). LC/MS m/z 355.2 (M + H). HPLC purity 95.90% (220 nm), 98.54% (254 nm). ESI-HRMS calcd for $C_{22}H_{30}N_2O_2$ m/z 355.2380 (M + H), found 355.2361 (M + H).

5-(Aminomethyl)-6-(2,2-dimethylpropyl)-4-(4-methylphenyl)-2-(2-methylpropyl)pyridine-3-carboxylic Acid Dihydrochloride (27h). Compound **27h** was prepared in a manner similar to that described for **27d** in 93% yield as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.89 (6H, d, J = 6.6 Hz), 1.02 (9H, s), 2.18−2.31 (1H, m), 2.37 (3H, s), 2.66 (2H, d, J = 7.2 Hz), 2.91 (2H, s), 3.84 (2H, d, J = 5.1 Hz), 7.21 (2H, d, J = 8.1 Hz), 7.29 (2H, d, J = 8.1 Hz), 8.08 (3H, brs). LC/MS m/z 369.2 (M + H). HPLC purity 100% (220 nm), 100% (254 nm). ESI-HRMS calcd for $C_{23}H_{32}N_2O_2$ m/z 369.2527 (M + H), found 369.2527 (M + H).

5-(Aminomethyl)-2-benzyl-6-(2,2-dimethylpropyl)-4-(4-methylphenyl)pyridine-3-carboxylic Acid Dihydrochloride (27i). Compound **27i** was prepared in a manner similar to that described for **27d** in 91% yield as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.95 (9H, s), 2.37 (3H, s), 2.89 (2H, s), 3.82 (2H, d, J = 5.4 Hz), 4.14 (2H, s), 7.18−7.31 (9H, m), 8.17 (3H, brs). LC/MS m/z 403.2 (M + H). HPLC purity 99.85% (220 nm), 99.73% (254 nm). ESI-HRMS calcd for $C_{26}H_{30}N_2O_2$ m/z 403.2380 (M + H), found 403.2369 (M + H).

***tert*-Butyl {[5-(Hydroxymethyl)-6-methyl-4-(4-methylphenyl)-2-(2-methylpropyl)pyridin-3-yl]methyl}carbamate (28a).** A solution of **25c** (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 **28a** (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, brs), 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.

***tert*-Butyl {[2-(2,2-Dimethylpropyl)-5-(hydroxymethyl)-6-methyl-4-(4-methylphenyl)pyridin-3-yl]methyl}carbamate (28b).** Compound **28b** was prepared in a manner similar to that described for **28a** in 80% yield as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.01 (9H, s), 1.29 (1H, t, J = 5.7 Hz), 1.37 (9H, s), 2.42 (3H, s), 2.68 (3H, s), 2.84 (2H, s), 4.10 (2H, d, J = 4.9 Hz), 4.10−4.25 (1H, m), 4.36 (2H, d, J = 5.7 Hz), 7.05 (2H, d, J = 8.1 Hz), 7.26 (2H, d, J = 8.1 Hz). LC/MS m/z 413.2 (M + H). Mp 168.4−168.6 °C.

***tert*-Butyl {[2-(2,2-Dimethylpropyl)-6-ethyl-5-(hydroxymethyl)-4-(4-methylphenyl)pyridin-3-yl]methyl}carbamate (28c).** Compound **28c** was prepared in a manner similar to that described for **28a** in 80% yield as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.02 (9H, s), 1.28 (1H, t, J = 5.8 Hz), 1.35 (3H, t, J = 7.5 Hz), 1.37 (9H, s), 2.41 (3H, s), 2.85 (2H, s), 2.97 (2H, q, J = 7.5 Hz), 4.09 (2H, brd, J = 5.1 Hz), 4.15−4.20 (1H, m), 4.36 (2H, d, J = 5.8 Hz), 7.06 (2H, d, J = 8.1 Hz), 7.25 (2H, d, J = 8.1 Hz). LC/MS m/z 427 (M + H). mp 138.5−138.9 °C.

***tert*-Butyl {[5-(Cyanomethyl)-6-methyl-4-(4-methylphenyl)-2-(2-methylpropyl)pyridin-3-yl]methyl}carbamate (29a).** To an ice-cooled mixture of **28a** (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 **29a** (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.

***tert*-Butyl {[5-(Cyanomethyl)-2-(2,2-dimethylpropyl)-6-methyl-4-(4-methylphenyl)pyridin-3-yl]methyl}carbamate (29b).** Compound **29b** was prepared in a manner similar to that described for **29a** in 93% yield as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.02 (9H, s), 1.37 (9H, s), 2.43 (3H, s), 2.65 (3H, s),

2.85 (2H, s), 3.30 (2H, s), 4.11 (2H, d, J = 4.7 Hz), 4.15−4.20 (1H, m), 7.05 (2H, d, J = 7.91 Hz), 7.30 (2H, d, J = 7.9 Hz). LC/MS m/z 422.1 (M + H).

***tert*-Butyl {[5-(Cyanomethyl)-2-(2,2-dimethylpropyl)-6-ethyl-4-(4-methylphenyl)pyridin-3-yl]methyl}carbamate (29c).** Compound **29c** was prepared in a manner similar to that described for **29a** in 92% yield as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.03 (9H, s), 1.37 (9H, s), 1.38 (3H, t, J = 7.4 Hz), 2.43 (3H, s), 2.86 (3H, s), 2.90 (2H, q, J = 7.4 Hz), 3.32 (2H, s), 4.10 (2H, brd, J = 4.9 Hz), 4.10−4.25 (1H, m), 7.05 (2H, d, J = 7.9 Hz), 7.30 (2H, d, J = 7.9 Hz). LC/MS m/z 436.3 (M + H).

[5-(Aminomethyl)-2-methyl-4-(4-methylphenyl)-6-(2-methylpropyl)pyridin-3-yl]acetic Acid Dihydrochloride (30a). Compound **29a** (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 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 [5-{[(*tert*-butoxycarbonyl)amino]methyl}-2-methyl-4-(4-methylphenyl)-6-(2-methylpropyl)pyridin-3-yl]acetic acid (14.0 g, 92%) as a white powder. A mixture of [5-{[(*tert*-butoxycarbonyl)amino]-methyl}-2-methyl-4-(4-methylphenyl)-6-(2-methylpropyl)pyridin-3-yl]acetic acid (0.050 g, 0.11 mmol) and 4 M HCl 1,4-dioxane solution (5 mL) was stirred at room temperature for 2 h. The reaction mixture was concentrated under reduced pressure, and the residue was triturated with diisopropyl ether to afford **30a** (48 mg, 100%) as a pale yellow powder. ^{1}H NMR (300 MHz, CD_3OD) δ: 1.10 (6H, d, J = 6.4 Hz), 2.09−2.25 (1H, m), 2.48 (3H, s), 2.84 (3H, s), 3.10 (2H, d, J = 7.4 Hz), 3.60 (2H, s), 4.09 (2H, s), 7.20 (2H, d, J = 7.9 Hz), 7.49 (2H, d, J = 7.7 Hz). Anal. Calcd for $C_{20}H_{26}N_2O_2 \cdot 2HCl \cdot 2H_2O$: C, 55.17; H, 7.41; N, 6.43. Found: C, 55.47; H, 7.29; N, 5.85. mp 203 °C. LC/MS m/z 327.2 (M + H). HPLC purity 100% (220 nm), 99.25% (254 nm).

[5-(Aminomethyl)-6-(2,2-dimethylpropyl)-2-methyl-4-(4-methylphenyl)pyridin-3-yl]acetic Acid Dihydrochloride (30b). Compound **29b** (15.5 g, 36.8 mmol) was suspended in 6 M HCl (200 mL), and the suspension was stirred at 90 °C for 24 h. The reaction mixture was allowed to cool to room temperature and washed with Et_2O. The aqueous layer was alkalified (pH 9) with 4 M NaOH solution, and THF (100 mL) and $(Boc)_2O$ (8.45 mL, 36.8 mmol) were added. The mixture was stirred at room temperature for 17 h. The reaction mixture was neutralized with 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 [5-{[(*tert*-butoxycarbonyl)amino]methyl}-6-(2,2-dimethylpropyl)-2-methyl-4-(4-methylphenyl)pyridin-3-yl]acetic acid (13 g, 80%) as a white powder. A mixture of [5-{[(*tert*-butoxycarbonyl)-amino]methyl}-6-(2,2-dimethylpropyl)-2-methyl-4-(4-methylphenyl)pyridin-3-yl]acetic acid (0.628 g, 1.43 mmol) and 4 M HCl 1,4-dioxane solution (4 mL) was stirred at room temperature for 3 h. The reaction mixture was concentrated under reduced pressure, and the residue was triturated with diisopropyl ether to afford **30b** (557 mg, 94.2%) as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.03 (9H, s), 2.41 (3H, s), 2.73 (3H, brs), 3.19 (2H, brs), 3.35−3.45 (2H, m), 3.75−3.90 (2H, m), 7.16 (2H, d, J = 7.4 Hz), 7.38 (2H, d, J = 7.4 Hz), 8.16 (3 h, brs). Anal. Calcd for $C_{21}H_{28}N_2O_2 \cdot 2HCl \cdot 2H_2O$: C, 58.47; H, 7.48; N, 6.49. Found: C, 58.33; H, 7.80; N, 5.80. mp 157.0−163.7 °C. LC/MS m/z 341.0 (M + H). HPLC purity 98.41% (220 nm), 97.99% (254 nm).

[5-(Aminomethyl)-6-(2,2-dimethylpropyl)-2-ethyl-4-(4-methylphenyl)pyridin-3-yl]acetic acid hydrate (30c). Compound **29c** (1.9 g, 4.3 mmol) was suspended in 6 M HCl (100 mL), and the suspension was stirred at 90 °C for 24 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 AcOEt (200 mL) and $(Boc)_2O$ (1.5 mL, 6.5 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 [5-{[(*tert*-butoxycarbonyl)amino]methyl}-6-(2,2-dimethylpropyl)-2-ethyl-4-(4-methylphenyl)pyridin-3-yl]acetic acid (1.8 g, yield 94%) as a white powder. ^{1}H NMR (300 MHz, CD_3OD) δ: 1.04 (9H, s), 1.24−1.47 (12H, m), 2.31−2.43 (3H, m), 2.67−2.94 (4H, m), 3.19−3.49 (2H, m), 3.93−4.16 (2H, m), 4.28 (1H, s), 6.85−7.36 (4H, m). The obtained [5-{[(*tert*-butoxycarbonyl)amino]methyl}-6-(2,2-dimethylpropyl)-2-ethyl-4-(4-methylphenyl)pyridin-3-yl]-acetic acid (0.14 g, 0.31 mmol) was suspended in 6 M HCl (5 mL), and the suspension was stirred at room temperature for 3 h. The reaction mixture was concentrated under reduced pressure, and the residual solid was triturated with diisopropyl ether to afford [5-(aminomethyl)-6-(2,2-dimethylpropyl)-2-ethyl-4-(4-methylphenyl)pyridin-3-yl]acetic acid dihydrochloride (0.13 g, 99%) as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.03 (9H, s), 1.27 (3H, t, J = 7.4 Hz), 2.41 (3H,s), 2.90−3.25 (4H, m), 3.43 (2H, brs), 3.82 (2H, brs), 7.17 (2H, d, J = 7.7 Hz), 7.37 (2H, d, J = 7.7 Hz), 8.27 (3H, brs). LC/MS m/z 355.2 (M + H). Anal. Calcd for $C_{22}H_{30}N_2O_2 \cdot 2HCl \cdot 0.5H_2O$: C, 60.55; H, 7.62; N, 6.42. Found: C, 68.68; H, 7.58; N, 6.40. mp 224.5−225.8 °C. [5-(Aminomethyl)-6-(2,2-dimethylpropyl)-2-ethyl-4-(4-methylphenyl)pyridin-3-yl]acetic acid dihydrochloride (1.0 g, 2.3 mmol) was dissolved in water (2 mL), and the solution was neutralized with 1 M NaOH solution under ice-cooling. The obtained suspension was stirred at 5 °C for 1 h, and the resulting crystals were collected by filtration. The obtained crystals were washed with cold water and dried to afford **30c** (0.69 g, 83%) as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.00 (9H, s), 1.20 (3H, t, J = 7.2 Hz), 2.36 (3H, s), 2.65 (2H, d, J = 7.2 Hz), 2.79 (2H, s), 3.16 (2H, s), 3.42 (2H, s), 7.05 (2H, d, J = 7.8 Hz), 7.23 (2H, d, J = 7.8 Hz). Anal. Calcd for $C_{22}H_{30}N_2O_2 \cdot H_2O$: C, 69.20; H, 8.60; N, 11.96. Found: C, 69.23; H, 8.54; N, 11.53. mp 186 °C. LC/MS m/z 355.2 (M + H). HPLC purity 98.24% (220 nm), 97.95% (254 nm).

Ethyl (2*E*)-3-[5-{[(*tert*-Butoxycarbonyl)amino]methyl}-2-methyl-4-(4-methylphenyl)-6-(2-methylpropyl)pyridin-3-yl]prop-2-enoate (31). To a solution of **28a** (1.95 g, 4.9 mmol) in THF (50 mL) was added MnO_2 (4.9 g, 56 mmol) and stirred at room temperature for 19 h. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford *tert*-butyl {[5-formyl-6-methyl-4-(4-methylphenyl)-2-(2-methylpropyl)pyridin-3-yl]methyl}carbamate (1.25 g, 65%) as yellow crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.98 (6H, d, J = 6.6 Hz), 1.39 (9H, s), 2.21−2.35 (1H, m), 2.43 (3H, s), 2.79 (3H, s), 2.82 (2H, d, J = 7.2 Hz), 4.15 (2H, d, J = 4.9 Hz), 4.38 (1H, brs), 7.10 (2H, d, J = 8.1 Hz), 7.29 (2H, d, J = 8.1 Hz), 9.71 (1H, s). To a solution of ethyl (diethoxyphosphoryl)acetate (0.033 g, 1.5 mmol) in THF (10 mL) was added sodium hydride (60% in oil) (60 mg, 1.5 mmol) at 0 °C, and the mixture was stirred for 20 min. To the reaction mixture was added a solution of *tert*-butyl {[5-formyl-6-methyl-4-(4-methylphenyl)-2-(2-methylpropyl)pyridin-3-yl]methyl}carbamate (0.38 g, 0.98 mmol) in THF (5 mL). The mixture was stirred at room temperature for 45 min. The residue was partitioned between AcOEt and water. The organic layer 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 = 50/50) to afford **31** (0.44 g, 96%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.98 (6H, d, J = 6.6 Hz), 1.23 (3H, t, J = 7.2 Hz), 1.39

(9H, s), 2.16−2.27 (1H, m), 2.40 (3H, s), 2.64 (3H, s), 2.77 (2H, d, J = 7.4 Hz), 4.08−4.17 (4H, m), 4.21 (1H, brs), 5.76 (1H, d, J = 16.4 Hz), 6.95 (2H, d, J = 8.1 Hz), 7.23 (2H, d, J = 8.1 Hz), 7.37 (1H, d, J = 16.4 Hz).

(2*E*)-3-[5-(Aminomethyl)-2-methyl-4-(4-methylphenyl)-6-(2-methylpropyl)pyridin-3-yl]prop-2-enoic Acid Dihydrochloride (32). To a solution of **31** (320 mg, 0.687 mmol) in THF (10 mL) was added 1 M NaOH solution (3.4 mL). The mixture was stirred at 60 °C for 12 h. The reaction mixture was neutralized with 0.5 M HCl and extracted with AcOEt. The organic layer 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 = 25/75) to afford (2*E*)-3-[5-{[(*tert*-butoxycarbonyl)amino]methyl}-2-methyl-4-(4-methylphenyl)-6-(2-methylpropyl)pyridin-3-yl]prop-2-enoic acid (280 mg, 93%) as a white powder. The obtained (2*E*)-3-[5-{[(*tert*-butoxycarbonyl)amino]methyl}-2-methyl-4-(4-methylphenyl)-6-(2-methylpropyl)pyridin-3-yl]prop-2-enoic acid (90.3 mg, 0.206 mmol) was dissolved in 4 M HCl in 1,4-dioxane (4 mL, 16 mmol). The mixture was stirred at room temperature for 15 min. The reaction mixture was concentrated under reduced pressure and crystallized from diisopropyl ether to give **32** (76.6 mg, 90%) as a white powder. ^1H NMR (300 MHz, CD_3OD) δ: 1.10 (6H, d, J = 6.6 Hz), 2.12−2.27 (1H, m), 2.46 (3H, brs), 2.84 (3H, s), 3.05 (2H, d, J = 7.5 Hz), 4.13 (2H, s), 5.98 (1H, d, J = 16.3 Hz), 7.20 (2H, d, J = 8.0 Hz), 7.25 (1H, d, J = 16.3 Hz), 7.46 (2H, d, J = 8.0 Hz). LC/MS m/z 339.2 (M + H). HPLC purity 98.66% (220 nm), 98.95% (254 nm). ESI-HRMS calcd for $C_{21}H_{26}N_2O_2$ m/z 339.2067 (M + H), found 339.2060 (M + H).

3-[5-(Aminomethyl)-2-methyl-4-(4-methylphenyl)-6-(2-methylpropyl)pyridin-3-yl]propanoic Acid Dihydrochloride (33). A mixture of **31** (700 mg, 1.5 mmol), 10% Pd/C (50% wet, 160 mg) and EtOH (15 mL) was stirred at room temperature under H_2 atmosphere for 1 h. The mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/AcOEt = 50/50) to afford ethyl 3-[5-{[(*tert*-butoxycarbonyl)amino]methyl}-2-methyl-4-(4-methylphenyl)-6-(2-methylpropyl)pyridin-3-yl]-propanoate (480 mg, 68%) as a white powder. ^1H NMR (300 MHz, $CDCl_3$) δ: 0.96 (6H, d, J = 6.6 Hz), 1.18 (3H, t, J = 7.2 Hz), 1.38 (9H, s), 2.11−2.30 (3H, m), 2.40 (3H, s), 2.57 (3H, s), 2.62−2.68 (2H, m), 2.72 (2H, d, J = 7.4 Hz), 3.96−4.07 (4H, m), 4.18 (1H, brs), 6.98 (2H, d, J = 7.91), 7.24 (2H, d, J = 7.9 Hz). Compound **33** was prepared from ethyl 3-[5-{[(*tert*-butoxycarbonyl)amino]methyl}-2-methyl-4-(4-methylphenyl)-6-(2-methylpropyl)pyridin-3-yl]propanoate in a manner similar to that described for **32** in 55% yield as a white powder. ^1H NMR (300 MHz, CD_3OD) δ: 1.09 (6H, d, J = 6.6 Hz), 2.09−2.22 (1H, m), 2.30−2.38 (2H, m), 2.48 (3H, s), 2.80−2.88 (2H, m), 2.90 (3H, s), 3.05 (2H, d, J = 7.5 Hz), 4.05 (2H, s), 7.26 (2H, d, J = 7.9 Hz), 7.51 (2H, d, J = 8.1 Hz). LC/MS m/z 341.2 (M + H). HPLC purity 99.18% (220 nm), 100% (254 nm). ESI-HRMS calcd for $C_{21}H_{28}N_2O_2$ m/z 341.2224 (M + H), found 341.2215 (M + H).

DPP-4 + 27c or 30c Crystal Structure Determination. The cDNA encoding human DPP-4 was isolated by PCR from spleen cDNA (Clontech), and the extracellular domain (residues 39−766) was cloned into a modified pFastBacHTb vector (Invitrogen). The final construct contains a baculovirus gp67 signal peptide followed by a His6 tag fused to the coding sequence corresponding to residues 39−766 of DPP-4. Recombinant baculovirus was generated by transposition using the Bac-to-Bac system (Gibco-BRL). Large-scale production of recombinant protein was performed by infection of *Trichoplusia ni* (Hi5) insect cells (Gibco-BRL) for 48 h in 5 L Wave Bioreactors (Wave Biotech). The secreted glycosylated recombinant protein was isolated from the cell culture medium by diafiltration using cross-flow ultrafiltration followed by passage over a nickel chelate resin (Binding buffer: 25 mM Tris, pH 7.9; 400 mM NaCl). The column was washed overnight (0.2 mL/min) with 50 mM K_2HPO_4 pH 7.9; 400 mM NaCl; 20 mM imidazole-HCl and 0.25 mM TCEP followed by 5 column volumes (1 mL/min) of 50 mM Tris HCl pH 7.9; 400 mM NaCl and 0.25 mM TCEP. Protein bound was eluted with 4 column volumes of 50 mM Tris-HCl, pH 7.9, 400 mM NaCl, 200 mM imidazole-HCl and 0.25 mM TCEP. To remove oligomeric forms, the sample was further purified over a size exclusion column (BioSep SEC S3000, 300 × 21.2 mm, Phenomenex) equilibrated with 25 mM Tris pH 7.6; 150 mM NaCl; 0.25 M TCEP and 1 mM EDTA. Wild-type DPP-4 in free form was concentrated to ~14 mg/mL and crystallized at 4 °C. The reservoir solution was 20% PEG MME 2000, 100 mM bicine (pH 8.0−8.5). Thick plate shaped crystals appeared in about 5 days that grew to about 0.5 mm in longest dimension and varying width and thickness. All protein−inhibitor complexes were obtained by soaking preformed DPP-4 crystals in a solution containing 1 mM of compound of interest. Crystals were then cryoprotected with ethylene glycol and flash frozen in liquid nitrogen. X-ray diffraction data were collected at Advanced Light Source (ALS) beamlines 5.0.2 and 5.0.3, and processed using the program HKL2000.[18] The structures of DPP-4 inhibitor complexes were determined by molecular replacement using MOLREP, utilizing the previously determined coordinates of DPP-4 with accession code 1R9M.[19,20] Subsequent structure refinement and model building were performed utilizing REFMAC and XtalView.[20,21] Bound inhibitors were clearly visible in the electron density maps. Data-collection and model refinement statistics are summarized in Table 7.

Table 7. Data Collection and Model Refinement Statistics[a]

	27c	30c
PDI code	3O9V	3O95
Data Collection		
space group	P21	P21
unit cell lengths (Å)	122.2;122.9;144.4	121.7;123.3;144.4
unit cell angles (deg)	91;114.6;90.0	90;114.8;90.0
resolution (Å)	2.75	2.85
observations	317,105	291,608
unique	100,525	89,585
completeness (%)	98.9 (98.6)	99.3 (92.4)
I/σ_I	13.4 (2.2)	10.7 (1.9)
R_{sym} (%)	0.087 (0.514)	0.106 (0.510)
Model Refinement		
reflections (work/free)	100346/5026	89524/4489
R_{factor} (work/free %)	20.33/26.15	18.70/24.85
protein molecules per ASU	4	4
solvent molecules	451	487
mean B value (Å^2)	50.7	49.1
rmsd ideal bond lengths (Å)	0.009	0.009
rmsd ideal bond angles (deg)	1.20	1.24

[a] $R_{sym} = \sum|I - \langle I\rangle|/\sum I$, where I is the integrated intensity for a reflection. $R_{factor} = \sum|F_P - F_c|/\sum F_P$, where F_p and F_c are the observed and calculated structure factor amplitudes, while R_{free} is calculated on 5% of the data excluded from refinement. Values in parentheses are for the highest resolution shell.

Docking Study in DPP-4. The X-ray crystal structure of DPP-4 complexed with 2-{[3-(aminomethyl)-2-(2-methylpropyl)-1-oxo-4-phenyl-1,2-dihydroisoquinolin-6-yl]oxy}acetamide was utilized in the docking calculations. The compounds were docked into DPP-4 using Gold 3.0.[22] After the automatic docking, the conformations of the substituents in the docked compounds were aligned to the corresponding moiety of 2-{[3-(aminomethyl)-2-(2-methylpropyl)-1-oxo-4-phenyl-1,2-dihydroisoquinolin-6-yl]-oxy}acetamide and then energy-minimized at the MMFF94s force field using MOE 2005.06.[23]

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.[24] 1 μL 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.

Incubation of Compound 30c with Hepatic Microsomes. In vitro oxidative metabolic studies of compound **30c** were carried out using hepatic microsomes obtained from rats, dogs and humans. The incubation mixtures were prepared under ice-cold conditions by adding the microsomes (0.2 μg protein/mL), and compound **30c** solution (1 μmol/L) at the final concentrations indicated. The reactions were initiated by adding the solution containing NADPH to the incubation mixtures. Incubations were conducted at 37 °C for 20 min and terminated by adding the ice-cold acetonitrile. The zero-time incubations which served as the controls were terminated by adding the ice-cold acetonitrile before adding compound **30c** solution. After the samples were mixed and centrifuged, the supernatant fractions were subjected to high performance liquid chromatography with UV detector.

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.

Pharmacokinetic Profile in Rats and Dogs. Compound **30c** 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.

Effects of Single Administration of Compound 30c on Glucose Tolerance in Female Wistar Fatty Rats. Female Wistar fatty rats were obtained from Takeda Rabics, Ltd. Each animal was fed a commercial diet (CE-2) and tap water ad libitum. At the age of 13 weeks, the rats were fasted overnight and divided into 5 groups based on PG levels and body weights (6 rats in each group). PG levels and plasma insulin levels at the grouping point were used as the data of initial point. Each group was orally administered vehicle (0.5% methylcellulose) or compound **30c** at doses of 0.03, 0.1, 0.3, and 1 mg/kg as free base form of compound **30c**. One hour later, all animals received an oral glucose load (1 g/kg). Blood samples were collected from tail vein at 0, 10, 30, and 60 min after the glucose load (time 0; 40 min after administration of compound **30c**). Heparin/EDTA was used as anticoagulant, and 100 μM DPP-4 inhibitor (Linco Research Inc.) as final concentration was added to the blood sample for measurement of active GLP-1 concentration in plasma (0 and 10 min). PG level was determined by an enzyme assay method (L-type Glucose 2; Wako Pure Chemical Ind., Ltd.). Plasma insulin level was determined by ELISA kit (Morinaga, Japan).

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Supporting Information Available: Experimental procedure for compound **25j**. This material is available free of charge via the Internet at http://pubs.acs.org.

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