Bioorganic & Medicinal Chemistry 19 (2011) 4482–4498

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Discovery of potent, selective, and orally bioavailable quinoline-based dipeptidyl peptidase IV inhibitors targeting Lys554

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ARTICLE INFO

Article history:
Received 11 April 2011
Revised 8 June 2011
Accepted 10 June 2011
Available online 7 July 2011

Keywords:
Dipeptidyl peptidase IV
Quinoline-based inhibitors
Type 2 diabetes

ABSTRACT

Dipeptidyl peptidase IV (DPP-4) inhibition is a validated therapeutic option for type 2 diabetes, exhibiting multiple antidiabetic effects with little or no risk of hypoglycemia. In our studies involving non-covalent DPP-4 inhibitors, a novel series of quinoline-based inhibitors were designed based on the co-crystal structure of isoquinolone **2** in complex with DPP-4 to target the side chain of Lys554. Synthesis and evaluation of designed compounds revealed 1-[3-(aminomethyl)-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl]piperazine-2,5-dione (**1**) as a potent, selective, and orally active DPP-4 inhibitor (IC_{50} = 1.3 nM) with long-lasting ex vivo activity in dogs and excellent antihyperglycemic effects in rats. A docking study of compound **1** revealed a hydrogen-bonding interaction with the side chain of Lys554, suggesting this residue as a potential target site useful for enhancing DPP-4 inhibition.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a rapidly increasing metabolic disease in the human population. Prolongation of the pathological conditions may cause serious and often mortal complications, such as nephropathy, neuropathy, retinopathy and atherosclerosis. Because T2DM currently affects more than 230 million people worldwide and is projected to affect 366 million individuals by 2030,[1–3] a variety of therapeutic options have been proposed to treat this disorder. Recently, glucagon-like peptide-1 (GLP-1) therapy has been investigated as a potential option for T2DM treatment.[4,5] GLP-1 is a 30-amino acid peptide hormone secreted from the gastrointestinal tract in response to food intake. The active form of GLP-1 (GLP-1[7-36]amide) plays an important role in glucose-dependent insulin biosynthesis and secretion from pancreatic β-cells. Furthermore, GLP-1[7-36]amide exhibits multiple antidiabetic effects in mammals, such as inhibiting of glucagon secretion, slowing gastric emptying, reducing appetite, and stimulating β-cell regeneration and differentiation. However, the GLP-1[7-36]amide is rapidly inactivated by dipeptidyl peptidase-IV (DPP-4, EC 3.4.14.5) and has a half-life of less than 1 min. DPP-4, a ubiquitously distributed serine protease that cleaves the peptide bond at the penultimate residue of the amino terminus position, converts GLP-1[7-36]amide to inactive GLP-1[9-36]amide.[6–8] DPP-4 inhibition prevents rapid GLP-1 inactivation and potentiates circulating levels of active GLP-1, improving the strict glucose-dependent secretion of insulin with little or no risk of serious hypoglycemia. Therefore, DPP-4 inhibitors are optimal therapeutic options to treat T2DM.[9–13] Numerous studies have identified and confirmed a number of inhibitors for their clinical antidiabetic effects.[5–8] Although many reported DPP-4 inhibitors, including vildagliptin[14–17] and saxagliptin,[18] are thought to covalently interact with the Ser630 residue in the S1 pocket,[19,20] sitagliptin[21] and alogliptin[22] are believed to achieve their therapeutic effects even without a covalent ligand–enzyme interaction.

Our efforts focus on the design of novel non-covalent DPP-4 inhibitors, extensively using structural information derived from DPP-4 structures co-crystallized with small molecules originating from different chemical classes. Notably, we previously identified

Abbreviations: DPP-4, dipeptidyl peptidase IV; T2DM, type 2 diabetes mellitus; GLP-1, glucagon-like peptide 1; SAR, structure–activity relationship; SBDD, structure-based drug design; Boc, *tert*-butoxy carbonyl; TBS, *tert*-butyldimethylsilyl; Cbz, benzyloxycarbonyl; ADME, absorption, distribution, metabolism and elimination; *F*, Bioavailability; CL_{total}, plasma clearance; V_{dss}, distribution volume.

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doi:10.1016/j.bmc.2011.06.032

a structurally novel isoquinolone DPP-4 inhibitor **2** (Fig. 1).[23,24] Co-crystallographic studies of compound **2** revealed a unique binding mode whereby the inhibitor interacts with the catalytic His740 and Ser630 mediated by water molecules (Fig. 2). This indicates that compound **2**, unlike previously published covalent inhibitors,[12,18,25–31] can make non-covalent interactions with DPP-4 catalytic residues. Our studies also revealed that compound **2** utilizes binding sites in three directions. The 2- and 3-substituents of compound **2** mimic the P2-site of known ligands and are located in the large hydrophobic S2 pocket of the enzyme. The 2-isobutyl group is positioned to allow a CH–π interaction with the aromatic ring of Phe357. The primary amino group at the 3-position interacts with residues Glu205, Glu206 and Tyr662, the recognition site for the charged N-terminal end of peptide substrates. The 4-pendant phenyl group extends into the hydrophobic S1 pocket. However, this substituent only partially fills the S1 pocket, the bottom of which lies deeper with the carbon–carbon distances of 3.9–4.4 Å than the *meta*- or *para*-carbon of the 4-phenyl ring (Fig. 2B). Therefore, a *meta*- or *para*-substitution onto the 4-phenyl ring would most likely enhance the affinity to the S1 pocket. Interestingly, the 6-substituent is positioned in the deep cleft of S1' pocket. This cleft is lined by hydrophobic residues Tyr547, Trp629, Gly632 and Val546, and terminated by hydrophilic Lys554 and Asp545 residues. The 6-substituent of compound **2** forms not only a hydrophobic interaction with Tyr547 but also hydrogen-bonding interactions with Lys554 and the backbone carbonyl group of Trp629. The hydrogen-bonding interaction with Lys554 may be applicable in the novel design of the inhibitors although none have been reported to date. Therefore, based on the X-ray co-crystal structure of compound **2**, we developed a pharmacophore model

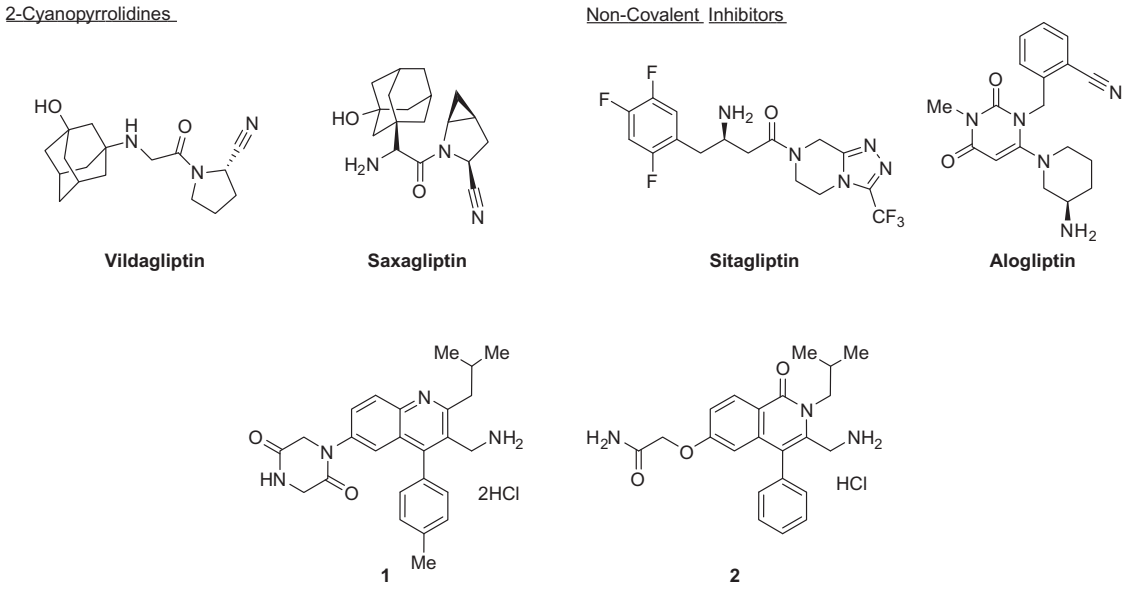


Figure 1. Structure of reported DPP-4 inhibitors (vildagliptin, saxagliptin, sitagliptin, and alogliptin), compounds **1** and **2**.

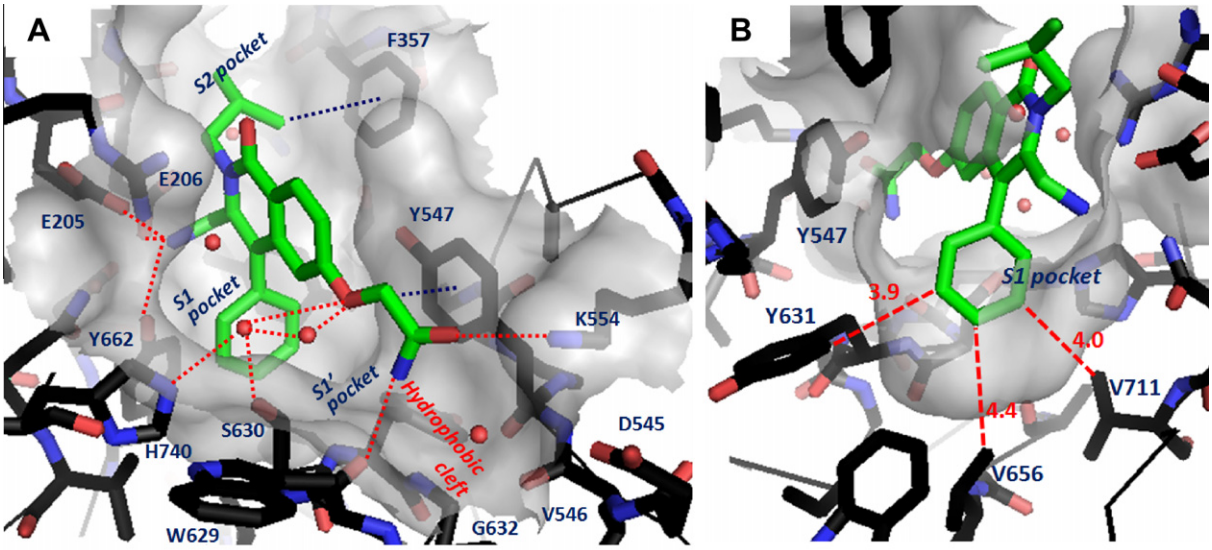


Figure 2. Compound **2** in complex with human DPP-4. (A) The amino acids (black carbons, blue texts) in the binding sites of compound **2** (green carbons) are indicated. Major interactions are depicted as dotted lines (hydrophobic, blue; hydrophilic, red). (B) The S1 binding site is indicated in cross-sectional view. Distances between carbon atoms of the 2-phenyl group and the representative hydrophobic residues lining the S1 pocket are shown as red dotted lines. Values listed are the carbon–carbon distances in angstroms.

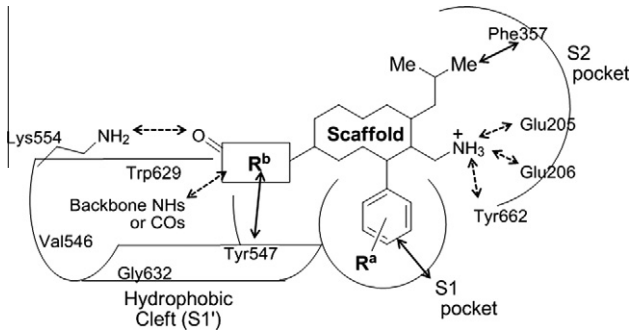


Figure 3. Proposed pharmacophore model based on the co-crystal structure of compound **2**. R^a and R^b are additional substituents to potentiate the affinity. Expected interactions are indicated as arrows (solid, hydrophobic; dashed, hydrophilic).

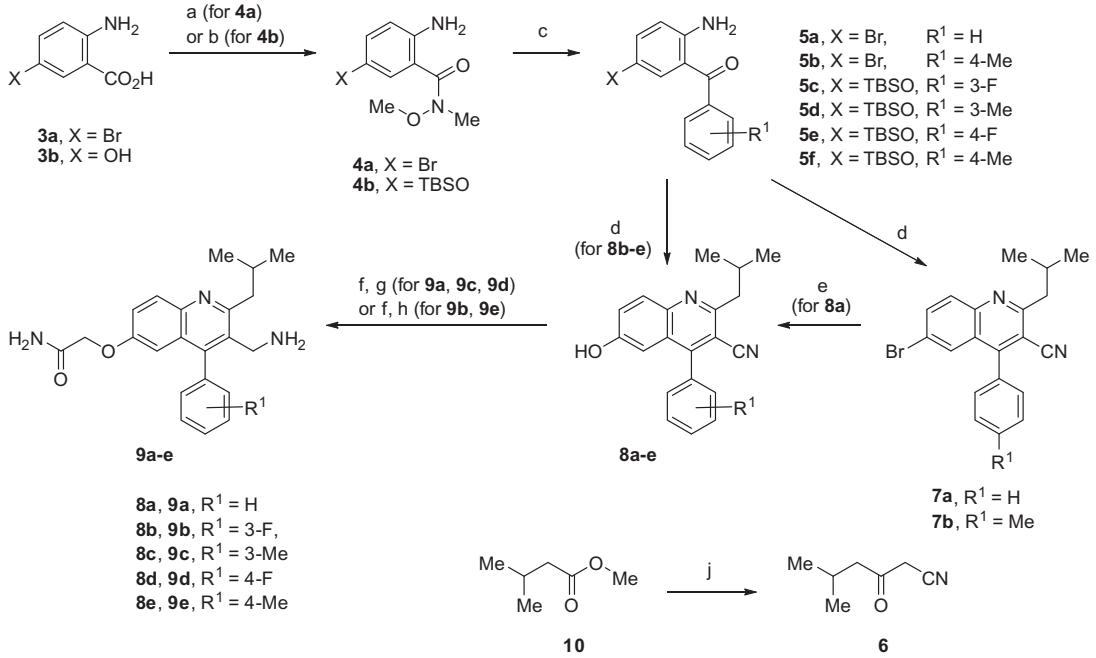
for DPP-4 inhibitor involving its unique interaction with Lys554 (Fig. 3). In this model, the isobutyl group, the amino methyl group, and the pendant phenyl group make similar interactions to those of compound **2** with Phe357, the recognition site for the amino moiety, and the S1 pocket, respectively. The scaffold orients all of these substituents into the appropriate positions. We hypothesized that the introduction of R^a and R^b substituents into this model would improve the affinity to the enzyme. R^a on the pendant phenyl would provide a better fit to the bottom of the S1 pocket. R^b extending toward the hydrophobic cleft would make hydrophobic interactions with the hydrophobic residues, such as Tyr547 and Trp629 and make hydrogen-bonding interactions with the backbone atoms of the enzyme and the side chain of Lys554, which was utilized by compound **2**.

Here, we describe a drug design strategy utilizing structure-based drug discovery to explore the optimal DPP-4 active site pocket occupancy for enhanced potency. We focused on the optimization of quinoline derivatives with an alternative scaffold to the isoquinolone and achieved significant improvement of inhibition, resulting in the discovery of compound **1**, dihydrochloride of 1-[3-(aminomethyl)-4-(4-methylphenyl)-2-(2-methylpropyl) quinolin-6-yl]piperazine-2,5-dione, as a lead compound of a novel class of DPP-4 inhibitors. Results from a docking study of compound **1** suggest the usefulness of our pharmacophore model involving the interaction with Lys554.

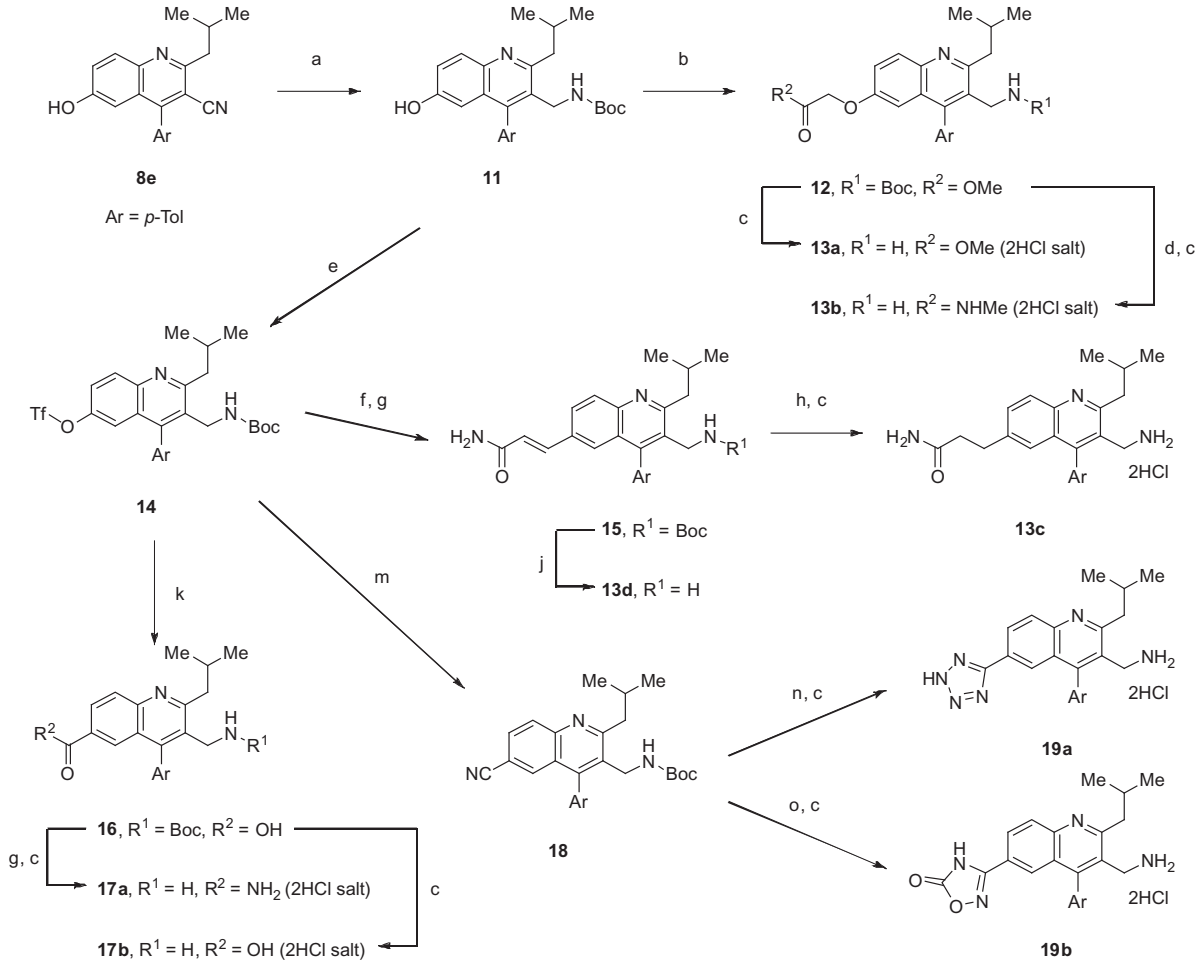
2. Chemistry

DPP-4 inhibitors discussed in this report were derived from quinoline-3-carbonitriles synthesized by a modified Friedlander reaction (Scheme 1).[32,33] Weinreb amide **4a** was prepared using the method described in a previous report.[34,35] Anthranilic acid **3b** was protected with TBS and Cbz (for OH and NH_2, respectively) and coupled with *N*,*O*-dimethylhydroxylamine followed by deprotection of Cbz to yield compound **4b**. Amide **4** was reacted with substituted or unsubstituted aryl magnesium bromides, which were commercially available or prepared from their corresponding aryl bromides, to afford 2-aminobenzophenone **5**. The methanesulfonic acid-catalyzed Friedlander reaction between compound **5** and 3-ketonitrile **6**, which was synthesized from ester **10** according to a previously described method,[36] yielded quinoline-3-nitriles **7** and **8**. Compound **8a** was prepared from compound **7a** by palladium-catalyzed etherification[37] and acidic cleavage of the *tert*-butyl moiety. The hydroxy group of 6-hydroxyquinoline **8** was alkylated, followed by hydrogenation of the 3-cyano group using Raney cobalt or Raney nickel as a catalyst to produce 3-aminomethylquinoline **9**.

Catalytic hydrogenation of the 3-cyano group of quinoline-3-carbonitrile **8e** and subsequent Boc protection provided intermediate **11** (Scheme 2). Intermediate **11** was alkylated with 2-bromoacetate



Scheme 1. Synthesis of compounds **9a–e**. Reagents and conditions: (a) (1) bis(trichloromethy1) carbonate, THF, reflux, 4 h; (2) *N*,*O*-dimethylhydroxylamine hydrochloride, Et_3N, EtOH, 75 °C, 17 h; (b) (1) Cbz-Cl, $NaHCO_3$, Et_2O, water, room temperature, 0.5 h; (2) *N*,*O*-dimethylhydroxylamine hydrochloride, EDC, Et_3N, DMF, room temperature, 2.5 h; (3) TBSCl, imidazole, DMF, room temperature, 14 h; (4) H_2, Pd/C, EtOH, THF, room temperature, 20 h; (c) R^1-C_6H_4-MgBr, THF, Et_2O, 0 °C–room temperature, 0.5–1 h; (d) **6**, MsOH, toluene, reflux, 6–60 h, Dean-Stark; (e) (1) *tert*-BuOH, $Pd(OAc)_2$, *rac*-BINAP, *tert*-BuONa, toluene, 90 °C, 1 h; (2) TFA, THF, room temperature, 1 h; (f) $ClCH_2CONH_2$, K_2CO_3, DMF, 60–80 °C, 4–96 h; (g) H_2, Raney-Co, 25% NH_3, MeOH, THF, 70–85 °C, 6–12 h; (h) H_2, Raney-Ni, 25% NH_3, MeOH, THF, room temperature, 7–9 h; (j) NaH, MeCN, THF, 70 °C, 15 h.



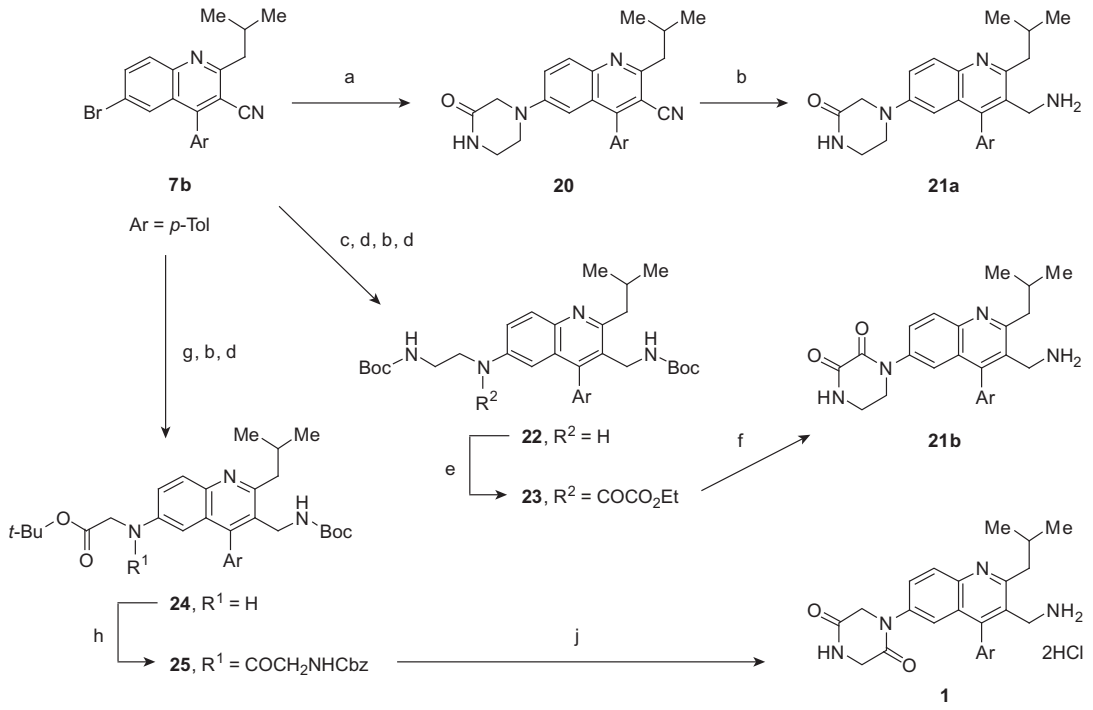
Scheme 2. Synthesis of compounds **13a–d**, **17a**, **17b**, **19a** and **19b**. Reagents and conditions: (a) (1) H_2, Raney-Co, 25% NH_3, MeOH, 90 °C, 8 h; (2) $(Boc)_2O$, THF, room temperature, 60 h; (b) $BrCH_2CO_2Me$, K_2CO_3, DMF, 2.5 h; (c) 4 M HCl/(1,4-dioxane or EtOAc), room temperature, 1–3 h; (d) (1) 1 M NaOH, MeOH, THF, room temperature, 1.5 h; (2) 1 M $MeNH_2$/THF, HOBt, EDC, DMF, room temperature, 15 h; (e) *N*-Phenyl bis(trifluoromethanesulfonimide), NaH, DMF, 0 °C, 0.5 h; (f) (1) ethyl acrylate, $PdCl_2(PPh_3)_2$, Et_3N, DMF, 70 °C, 20 h; (2) 1 M NaOH, EtOH, THF, room temperature, 17 h; (g) HOBt·NH_3, EDC, DMF, room temperature, 2–17 h; (h) H_2, Pd/C, EtOH, THF, room temperature, 3.5 h; (j) (1) 4 M HCl/EtOAc, room temperature, 1.5 h; (2) 10% K_2CO_3; (k) (1) CO, $Pd(OAc)_2$, dppf, Et_3N, MeOH, THF, 100 °C, 3 h, sealed tube; (2) 1 M NaOH, MeOH, THF, 60 °C, 1 h; (m) $Zn(CN)_2$, $Pd(PPh_3)_4$, NMP, 80 °C, 2 h; (n) NaN_3, NH_4Cl, DMSO, 70 °C, 48 h; (o) (1) H_2NOH·HCl, *tert*-BuOK, EtOH, 70 °C, 6 h; (2) CDI, EtOAc, THF, reflux, 3 h.

and converted to ester **13a** and amide **13b**. Intermediate **11** was subjected to a sulfonylation using *N*-phenyl bis(trifluoromethanesulfonimide) and sodium hydride to give triflate **14**, which was used for a variety of palladium-catalyzed coupling reactions, such as the Heck reaction, carbonylation and cyanation.[38,39] The Heck reaction using ethyl acrylate followed by two-step conversion of the ester to an amide provided acrylamide derivative **15**. The olefin moiety of **15** was hydrogenated, and deprotection resulted in propionamide **13c**. Acrylamide **13d** was obtained by *N*-Boc deprotection of **15**. Methoxycarbonylation of triflate **14** was followed by alkali hydrolysis of the resulting ester to afford carboxylic acid **16**. Carboxylic acid **16** was then converted to amide **17a** by amidation followed by deprotection, whereas *N*-Boc deprotection of **16** yielded amino acid **17b**. Bioisosteric replacement of the carboxy group of compound **16** was achieved through nitrile **18**, which was prepared by cyanation of triflate **14**. Nitrile **18** was reacted with sodium azide to form a tetrazole ring[40] and then converted to compound **19a**. Reacting nitrile **18** with hydroxylamine yielded the corresponding amidoxime, which was treated with 1,1′-carbonyldiimidazole to form a 1,2,4-oxadiazolone ring.[41] *N*-Boc deprotection of the 1,2,4-oxadiazolone derivative resulted in compound **19b**.

Introduction of a piperazinone group to the 6-position was achieved through palladium-catalyzed amination[42] of quinoline-3-carbonitrile **7b** (Scheme 3). Piperazin-2-one was efficiently reacted with **7b** by using cesium carbonate as base. The resulting 6-substituted quinoline **20** was reduced by hydrogenation to compound **21a**. Piperazin-2,3-dione and piperazin-2,5-dione ring were formed by amination and subsequent N-acylation as follows. Ethylenediamine was coupled with compound **7b** and protected with a Boc group. Conversion of the 3-cyano group to the corresponding Boc-aminomethyl group yielded **22**, which was acylated with ethyl chloro(oxo)acetate to give **23**. Construction of the piperazin-2,3-dione ring system could be achieved by acidic Boc deprotection of **23** followed by neutralization to yield compound **21b**. Glycine ester was introduced to **7b**, after which the 3-cyano group was converted to a Boc-aminomethyl group to yield **24**. Glycine analogue **24** was acylated with the acid chloride of Cbz-glycine to produce compound **25**. Cbz deprotection resulted in subsequent cyclization to the piperazin-2,5-dione ring, and subsequent Boc deprotection afforded compound **1**.

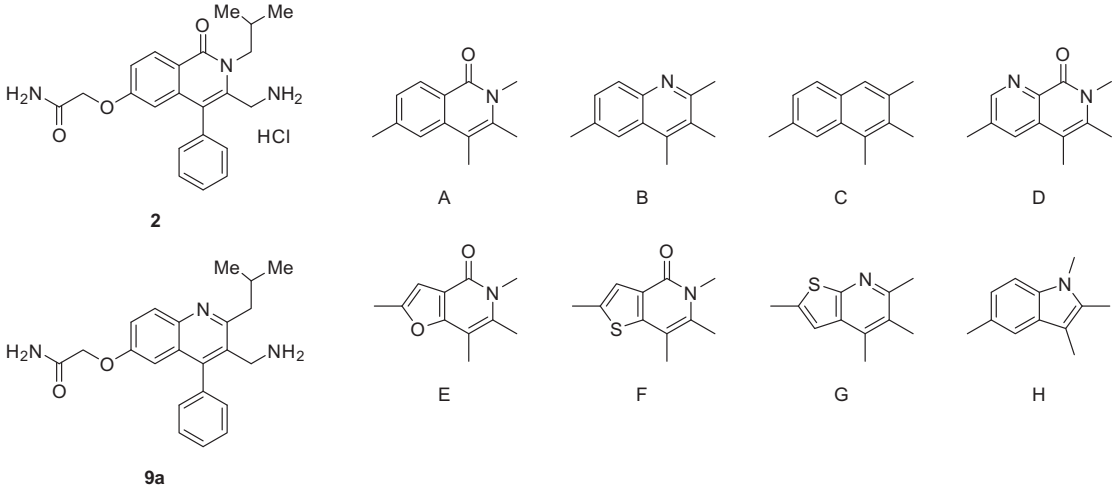
3. Results and discussion

The quinoline derivatives described above were evaluated their ability to inhibit human DPP-4, and the results are shown in Tables 2 and 3 as IC_{50} values with 95% confidence limits (shown in parentheses) that were calculated from the concentration–response curves generated by GraphPad Prism.



Scheme 3. Synthesis of compounds **21a**, **21b** and **1**. Reagents and conditions: (a) piperazin-2-one, $Pd(OAc)_2$, *rac*-BINAP, Cs_2CO_3, 1,4-dioxane, 80 °C, 24 h; (b) H_2, Raney-Ni, 25% NH_3, MeOH, THF, room temperature, 6 h; (c) ethylenediamine, $Pd(OAc)_2$, *rac*-BINAP, *tert*-BuONa, toluene, 80 °C, 2 h; (d) $(Boc)_2O$, THF, room temperature, 0.5–1 h; (e) ethyl chloro(oxo)acetate, satd $NaHCO_3$, EtOAc, room temperature, 0.5 h; (f) (1) TFA, room temperature, 0.5–1 h; (2) 10% K_2CO_3; (g) (1) *tert*-butyl glycinate, $Pd(OAc)_2$, *rac*-BINAP, *tert*-BuONa, toluene, 85 °C, 4 h; (h) Cbz-$NHCH_2COCl$, DMAP, pyridine, THF, room temperature, 1 h; (j) (1) H_2, Pd/C, EtOH, THF, room temperature, 24 h; (2) 4 M HCl/1,4-dioxane or EtOAc, room temperature, 1 h.

Table 1
Designed scaffolds and results of GOLD scoring

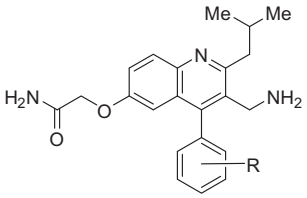


Scaffold[a]	GoldScore[b]	Ext_HB	Ext_vdW	Int_Torsion	Int_vdW
A	73.8	24.3	64.6	−11.8	−3.3
B	78.4	24.6	62.1	−8.0	−0.3
C	79.7	21.7	64.4	−5.3	−1.1
D	76.6	23.0	61.7	−6.4	−1.7
E	69.7	23.5	62.9	−12.6	−4.1
F	69.0	24.2	58.5	−10.1	−3.7
G	70.1	21.6	60.5	−9.4	−2.5
H	68.4	22.4	56.5	−10.1	−0.5

[a] Experimental IC_{50} values of inhibitory activity against human DPP-4 of compound **2** (scaffold A) and **9a** (scaffold B) were 47 (42–53) and 34 (31–37) nM, respectively, where the IC_{50} values are means of triplicate measurements. IC_{50} values and 95% confidence limits (shown in parentheses) were calculated from the concentration–response curves generated by GraphPad Prism.

[b] GoldScore consists of protein–ligand hydrogen bond energy (Ext_HB), protein–ligand van der Waals energy (Ext_vdW), ligand torsional strain energy (Int_Torsion) and ligand internal van der Waals energy (Int_vdW).

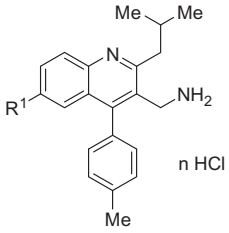
Table 2
SAR summary for the 4-position of quinoline derivatives

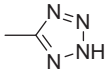
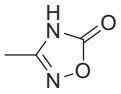
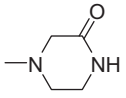
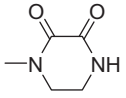
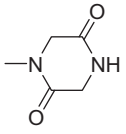


Compound	R	DPP-4 IC_{50}[a] (nM)
9a	H	34 (31–37)
9b	3-F	49 (41–59)
9c	3-CH_3	57 (50–66)
9d	4-F	26 (24–28)
9e	4-CH_3	4.2 (3.5–5.0)

[a] IC_{50} values are means of triplicate measurements. IC_{50} values and 95% confidence limits (shown in parentheses) were calculated from the concentration–response curves generated by GraphPad Prism.

Table 3
Inhibitory activities of quinoline derivatives



Compound	R^1	*n*	DPP-4 IC_{50}[a] (nM)
13a	$OCH_2CO_2CH_3$	2	84 (79–90)
13b	$OCH_2CONHCH_3$	2	52 (46–59)
13c	$CH_2CH_2CONH_2$	2	7.5 (6.4–8.7)
13d	(*E*)-CH=CHCONH$_2$	0	1.8 (1.7–1.9)
17a	$CONH_2$	2	55 (52–57)
17b	CO_2H	2	100 (86–120)
19a		2	9.2 (8.8–9.7)
19b		2	11 (10–12)
21a		0	2.2 (2.1–2.4)
21b		0	2.7 (2.4–3.1)
1		2	1.3 (1.1–1.5)

[a] IC_{50} values are means of triplicate measurements. IC_{50} values and 95% confidence limits (shown in parentheses) were calculated from the concentration–response curves generated by GraphPad Prism.

First, we explored an alternative central scaffold to isoquinolone **2** (IC_{50} = 47 (42–53) nM, *n* = 3) to enhance DPP-4 inhibitory activity. As shown in Table 1, 6 new core scaffolds B–H that can preserve the 2-, 3-, 4-, and 6-substituents of compound **2** in similar positions were designed. Among them, we decided to investigate the quinoline scaffold B based on the synthetic feasibility for structural modification. This strategy was supported by the docking studies of designed compounds using GOLD. In these studies, the binding affinities of the compounds were estimated as a GoldScore, which consists of four components: Ext_HB, Ext_vdW, Int_Torsion, and Int_vdW, and the results are listed in Table 1. The GoldScore of compounds incorporating scaffolds A–D are more than three points higher than that of E–H. Among them, the quinoline scaffold B was predicted to exhibit the highest level of affinity for the enzyme. Further elemental comparison between the quinoline B and isoquinolone A shows that the sum of their Ext_HB and Ext_vdW scores are comparable, assuming a similar potential to interact with the enzyme. However, the sum of Int_Torsion and Int_vdW scores of the quinoline was estimated to be higher than that of the isoquinolone, which predicts lower conformational stability of the isoquinolone scaffold. This instability of the isoquinolone is probably explained by the steric repulsion between the 1-carbonyl group and 2-isobutyl group, and therefore, quinolines are expected to have an advantage over corresponding isoquinolones in adopting a preferable conformation for interacting with the enzyme. Accordingly, quinoline analogue **9a** was synthesized and evaluated for DPP-4 inhibitory activity. Quinoline **9a** exhibited similar inhibitory activity to isoquinolone **2** as shown in Table 2, and we identified the quinoline as a potential alternative scaffold to the isoquinolone.

Next, the structure–activity relationships of 3-aminomethyl-4-aryl-2-isobutylquinolines were investigated. As described in Figure 2B, the X-ray co-crystal structure of compound **2** suggests that introduction of a small hydrophobic substituent onto the *meta*- or *para*-position of the 4-phenyl ring should achieve a better fit into the pocket.

We applied this hypothesis to the quinolines, and the potency-enhancing effects of fluoro or methyl substitution onto the 4-phenyl ring were evaluated and are described in Table 2. *para*-Substituted analogues (**9d**, **9e**) exhibited similar or higher potency compared to the lead compound **9a**, whereas *meta*-substituted analogues (**9b**, **9c**) showed decreased potency. Particularly, compound **9e**, with a *p*-methyl group on the pendant phenyl, exhibited more than eightfold increase over **9a**, and was found to inhibit DPP-4 with nanomolar potency. Surprisingly, **9e** exhibited sixfold more potent inhibition than *p*-fluoro analogue **9d**, suggesting that the fluoro group was too small to reach the bottom of the S1 pocket. These results clearly indicate that the *p*-tolyl group at the 4-position of the quinoline ring should be appropriate both in size and orientation to occupy the S1 pocket, which leads to potentiation of the activity.

A hydrophilic substituent at the 6-position of 2-isobutyl-4-(4-methylphenyl)quinoline analogue was investigated, as shown in Table 3. Inhibitory activity was significantly influenced by the nature of the 6-substituent. Compounds bearing methoxycarbonyl or *N*-methylcarbamoyl groups at the terminal end of the 6-substituent (**13a** and **13b**, respectively) exhibited a significant decrease in potency by more than 12-fold compared to the unsubstituted amide **9e**. Deletion of the linker moiety at the 6-position of **9e** resulted in 13- and 20-fold potency loss for compound **17a** and its corresponding carboxylic acid **17b**, respectively. These results clearly indicate the importance of both the approach and interaction of the 6-substituent with the side chain of Lys554. Propionamide **13c**, which has a methylene linker showed a 1.8-fold decrease in potency when compared to **9e** which has an ether linker. This is likely due to the lack of hydrogen bonding to the enzyme via water molecules observed in compound **2** (Fig. 2A). However, the potency loss was compensated in acrylamide **13d** by conversion to a *trans*-olefin linker that can form a π–π stacking interaction with the aromatic ring of Tyr547 in the hydrophobic

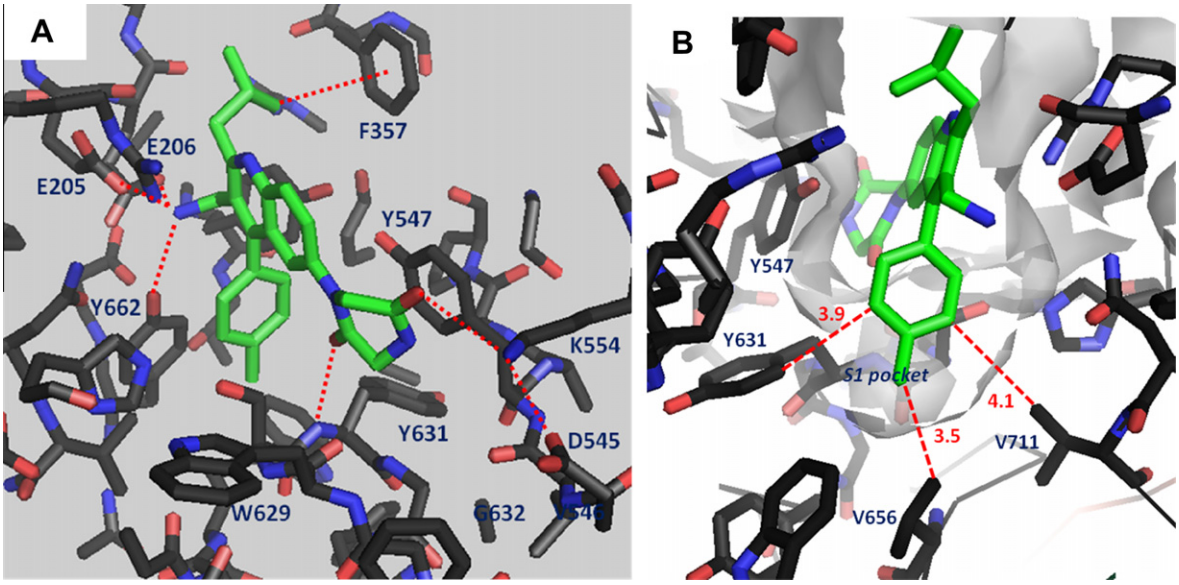


Figure 4. Docking study between DPP-4 protein and compound **1**. (A) The amino acids (black carbons, blue texts) in the binding sites of compound **1** (green carbons) are indicated. Major interactions are depicted as red dotted lines. (B) The S1 binding site is indicated in cross-sectional view. Distances between carbon atoms of the 2-*p*-tolyl group and the representative hydrophobic residues lining the S1 pocket are shown as red dotted lines. Values listed are the carbon–carbon distances in angstroms.

cleft. Surprisingly, bioisosteric replacement of the carboxylic acid **17b** to tetrazole **19a** or oxadiazole **19b** resulted in more than ninefold potency enhancement. These results suggested a contribution of the hydrophobic interaction between the substituents at the 6-position and the hydrophobic cleft of the enzyme. Thus, we focused on a variety of heterocyclic substituents at the 6-position which could make interactions with both hydrophilic Lys554 and hydrophobic residues in the target cleft.

Compound **21a**, which has both a hydrogen-bonding moiety and a lipophilic moiety in the 6-substituent, showed a fivefold increase in potency relative to **19b**. The effect of an additional oxo group was dependent on the position of the oxo group on the piperazinone substituent. Piperazin-2,3-dione derivative **21b** is similar in potency to **21a**. However, the corresponding piperazin-2,5-dione derivative **1** exhibited a potency-enhancing effect and was found to be the most active inhibitor of this series, with an IC_{50} value of 1.3 nM.

To gain further insight into the binding mode of the quinoline derivatives, a docking study between compound **1** and DPP-4 was conducted (Fig. 4). The primary amino group at the 3-position of the quinoline ring can make hydrogen bonds with the Glu-motif (Glu205-Glu206) and Tyr662 whereas the 2-isobutyl group can make a hydrophobic interaction with Phe357. The methyl group of the *p*-tolyl group at the 4-position of the quinoline ring fits well in the small hydrophobic hole at the bottom of the S1 pocket enabling it to maximize hydrophobic interactions with surrounding residues (Fig. 4B). The isoquinolone derivative with phenyl group **2** can likely not occupy this region as efficiently (Fig. 2B). This result explains the significant potency-enhancing effects of the introduction of methyl group, as shown in Table 2. The piperazin-2,5-dione ring at the 6-position of the quinoline scaffold can be positioned to allow a hydrophobic interaction with Tyr547. Notably, the docking study indicated that the 2-oxo group on the piperazin-2,5-dione substituent can accept a hydrogen bond from Lys554. The 5-oxo group can form an additional hydrogen bond with the backbone NH of Tyr631. This backbone hydrogen-bonding can explain the observed potency increase of compound **1** compared to compounds **21a** and **21b**. Therefore, compound **1** is assumed to achieve excellent activity by taking advantage of the desirable piperazin-2,5-dione substituent by forming hydrophobic interactions and 2 hydrogen-bonding interactions, including one with Lys554.

Table 4
Selected pharmacokinetic profiles of compound **1**[a,b]

Species	V_{dss} (mL/kg)	CL_{total} (mL/h/kg)	AUC_{po} (μg·h/mL)	*F* (%)
Rat	10,712 (1148)	2819 (390)	28.5 (2.3)	8.0 (1.2)
Dog	1490 (406)	352 (73)	1409 (126)	49.2 (8.0)

[a] 1.0 mg/kg oral dose and 0.1 mg/kg intravenous.
[b] Values are means and standard deviations of three experiments.

Enzyme selectivity of compound **1** was evaluated and resulted in excellent selectivity for DPP-4 over closely related peptidases, DPP-2 (IC_{50} = 20 μM), DPP-8 (IC_{50} >60 μM), and DPP-9 (IC_{50} >60 μM). The ADME profiles of compound **1** revealed that it is highly soluble in water (>5 mg/mL at pH 6.8) and moderately or highly stable in liver microsomes (human, dog, rat: 16, 2.0, 96 μL/min/mg, respectively). Pharmacokinetic properties are represented in Table 4. Compound **1** exhibited moderate or high bioavailability in rats and dogs.

An ex vivo study in dogs revealed that compound **1** could exhibit potent and long-lasting inhibition at low dosage (Fig. 5). In this study, administration of compound **1** in dogs at a dosage of 0.5 mg/kg resulted in more than 50% inhibition of the plasma DPP-4 activity for 24 h. In an oral glucose tolerance test in female Wistar fatty rats, the compound dose-dependently elevated plasma insulin levels and exhibited a potent plasma glucose lowering effect with a minimum effective dose of 1 mg/kg, po (p <0.025 vs control, Williams test). On the basis of the results of the ex vivo and the in vivo studies, compound **1** is hypothesized to be a new type of antidiabetic agent.

4. Conclusions

Using the structure-based drug design starting from the isoquinolone **2** scaffold, a novel class of potent DPP-4 inhibitors with a quinoline scaffold has been discovered. The representative dihydrochloride of 1-[3-(aminomethyl)-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl]piperazine-2,5-dione (**1**) showed

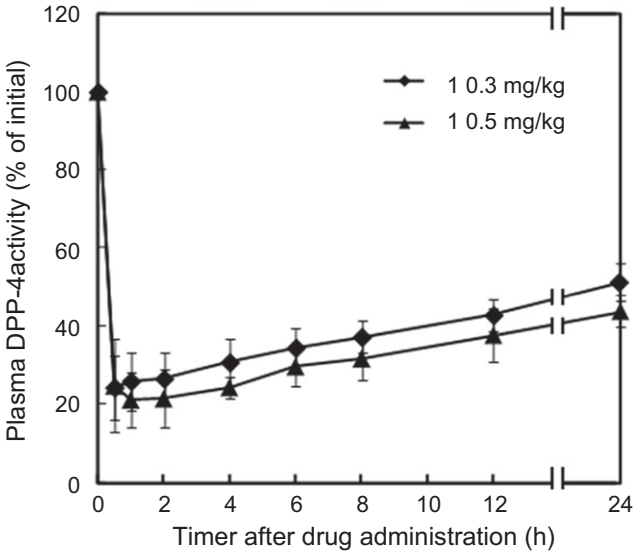


Figure 5. Ex vivo study of compound **1** in beagle dogs. Compound **1** was formulated in a 0.5% suspension of methylcellulose at 0.3 mg/mL. Five dogs (28–42 months old) were orally administered a suspension of compound at doses of 0.3 and 0.5 mg/kg. Blood samples were collected from the cephalic vein before (0 h) and 0.5, 1, 2, 4, 6, 8, 12, and 24 h after dosing. Plasma sample was prepared from each blood sample and the residual DPP-4 activity was measured by using a method similar to that described in Section 5.

excellent DPP-4 inhibition in dog plasma for more than 24 h and potent glucose lowering effects in rats. These results suggest the potential for once-daily treatment in T2DM patients. A docking study of compound **1** to DPP-4 suggested that an efficient fit into the binding site and a hydrogen bond to Lys554 are important in enhancing the inhibitory activity. Therefore, our results are useful for designing novel, potent non-covalent DPP-4 inhibitors.

5. Experimental section

5.1. Chemistry

^{1}H NMR spectra were recorded on a Varian Gemini-200 (200 MHz) or a Varian Ultra-300 (300 MHz) instrument in $CDCl_3$, DMSO-d_6 or CD_3OD solutions. Low-resolution mass spectra (MS) were determined using a Waters Liquid Chromatography-Mass Spectrometer. High-resolution mass spectra (HRMS) experiments were carried out by Takeda Analytical Laboratories Ltd. All MS experiments were performed using electrospray ionization (ESI) in positive or negative ion mode. Elemental analyses were also obtained from Takeda Analytical Laboratories Ltd. The purity of final products was assessed by HPLC analyses using Shimadzu UFLC L-column 2 ODS (3.0 × 50 mm, 2 μm). Elution was conducted at 1.2 mL/min using a gradient of 5–90% solvent B in solvent A (solvent A was 0.1% trifluoroacetic acid (TFA) in water, and solvent B was 0.1% TFA in acetonitrile). Final purity (λ 220 and 254 nm) is noted with the analytical data for each compound. Melting points were determined using a Yanagimoto melting point apparatus or a Büchi melting point apparatus B-545 and were uncorrected. All commercial chemicals and solvents are reagent grade and were used without further purification. Amounts of Raney nickel and Raney cobalt are theoretical values calculated from their weights in water. Column chromatography was performed on Silica Gel 60 (0.063–0.200 or 0.040–0.063 mm, E. Merck) or NH silica gel (Chromatorex® NH, 100–200 mesh, Fuji Silysia) using the solvent systems indicated. Yields are unoptimized.

5.1.1. 2-Amino-5-bromo-*N*-methoxy-*N*-methylbenzamide (4a)

5.1.1.1. Step A. To a solution of 25 g (116 mmol) of 2-amino-5-bromobenzoic acid (**3a**) in 350 mL of THF was added 24.4 g (82 mmol) of bis(trichloromethyl) carbonate was added, and the resulting suspension was stirred at reflux temperature for 4 h. The reaction mixture was poured onto ice, and the precipitate was collected by filtration, washed with methanol and dried to afford 27.1 g (96%) of 6-bromo-2*H*-3,1-benzoxazine-2,4(1*H*)-dione as a white powder. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.19 (d, J = 8.4 Hz, 1H), 7.96–8.02 (m, 1H), 8.09 (d, J = 2.6 Hz, 1H).

5.1.1.2. Step B. A mixture of 16.4 g (168 mmol) of *N,O*-dimethylhydroxylamine hydrochloride and 13.4 mL (168 mmol) of triethylamine in 70 mL of ethanol was stirred at room temperature for 30 min. To the suspension was added 27.1 g (112 mmol) of 6-bromo-2*H*-3,1-benzoxazine-2,4(1*H*)-dione, and the resulting mixture was stirred at 75 °C for 17 h. The reaction mixture was cooled and filtered. The filtrate was concentrated in vacuo, and the residue was neutralized with saturated aqueous sodium bicarbonate solution and extracted with ethyl acetate. The extract was washed sequentially with saturated aqueous sodium bicarbonate solution and brine, dried over anhydrous magnesium sulfate and concentrated in vacuo. Purification by column chromatography (silica gel, eluting at a gradient of 50–80% ethyl acetate in hexanes) was followed by recrystallization from diisopropyl ether/hexanes to afford 21.8 g (72%) of the title compound as pale orange crystals: mp 78–81 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.34 (s, 3H), 3.59 (s, 3H), 4.67 (br s, 2H), 6.64 (d, J = 8.4 Hz, 1H), 7.27 (dd, J = 8.4, 2.4 Hz, 1H), 7.50 (d, J = 2.4 Hz, 1H). MS m/z 198 $(M-N(Me)OMe)^+$.

5.1.2. 2-Amino-5-{[*tert*-butyl(dimethyl)silyl]oxy}-*N*-methoxy-*N*-methylbenzamide (4b)

5.1.2.1. Step A. A solution of 52 mL (0.36 mmol) of benzyl chloroformate in 200 mL of diethyl ether was added dropwise with a vigorous stirring to a mixture of 50 g (0.33 mmol) of 2-amino-5-hydroxybenzoic acid (**3b**), 109 g (1.3 mol) of sodium bicarbonate, 200 mL of diethyl ether and 200 mL of water at room temperature. The mixture was stirred at room temperature for 30 min, and then neutralized with concentrated hydrochloric acid. The reaction mixture was extracted with ethyl acetate, and the extract was washed with brine, dried over anhydrous magnesium sulfate. Concentration in vacuo was followed by crystallization from diisopropyl ether–hexanes to afford 92 g (98%) of 2-{[(benzyloxy)carbonyl]amino}-5-hydroxybenzoic acid as a white powder, which was used in the next step without further purification. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 5.13 (s, 2H), 7.00 (dd, J = 9.0, 3.0 Hz, 1H), 7.33–7.41 (m, 6H), 8.00 (d, J = 9.0 Hz, 1H), 9.50 (br, 1H), 10.32 (s, 1H).

5.1.2.2. Step B. To a mixture of 91 g (0.32 mol) of 2-{[(benzyloxy)carbonyl]amino}-5-hydroxybenzoic acid and 36 g (0.36 mol) of *N,O*-dimethyl hydroxylamine hydrochloride in 500 mL of DMF were sequentially added 55 mL (0.36 mol) of triethylamine and 70 g (0.36 mol) of EDC at room temperature, and the resulting mixture was stirred for 2.5 h at room temperature. After quenching with 1 M hydrochloric acid, the mixture was extracted with ethyl acetate. The extract was washed with brine, dried over anhydrous magnesium sulfate, and concentrated in vacuo. Purification by column chromatography (silica gel, eluting sequentially with 1:3 ethyl acetate/hexanes, 1:1 ethyl acetate/hexanes, and ethyl acetate) gave a brown oil. A mixture of the obtained brown oil, 48 g (0.32 mol) of *tert*-butyl(chloro)dimethylsilane and 22 g (0.32 mol) of imidazole in 500 mL of DMF was stirred at room temperature for 14 h. Water was added and the mixture was extracted with ethyl acetate. The extract was washed sequentially with 1 M hydrochloric acid and brine, dried over anhydrous magnesium sulfate, and concentrated in vacuo. Purification by column chromatography (silica gel, 1:3 ethyl

acetate/hexanes) gave 85 g (59%) of 2-{[(benzyloxy)carbonyl]amino}-5-{[*tert*-butyl(dimethyl)silyl]oxy}benzoic acid as a white powder. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 0.28 (s, 6H), 1.08 (s, 9H), 3.45 (s, 3H), 3.62 (s, 3H), 5.28 (s, 2H), 7.02 (dd, J = 8.8, 2.6 Hz, 1H), 7.06 (d, J = 2.6 Hz, 1H), 7.44–7.51 (m, 5H), 8.04 (d, J = 8.8 Hz, 1H), 8.45 (br, 1H).

5.1.2.3. Step C. A mixture of 85 g (0.19 mol) of 2-{[(benzyloxy)carbonyl]amino}-5-{[*tert*-butyl(dimethyl)silyl]oxy}benzoic acid, 2.5 g of 10% Pd/C, 250 mL of ethanol and 250 mL of THF was stirred under atmospheric hydrogen at room temperature for 20 h. The reaction mixture was filtered, and the filtrate was concentrated in vacuo to afford 40 g (69%) of the title compound as a white powder. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 0.25 (s, 6H), 1.07 (s, 9H), 3.45 (s, 3H), 3.68 (s, 3H), 6.70 (d, J = 8.8 Hz, 1H), 6.84 (dd, J = 8.8, 3.0 Hz, 1H), 6.97 (d, J = 3.0 Hz, 1H).

5.1.3. (2-Amino-5-bromophenyl)(phenyl)methanone (5a)

To a solution of 5.0 g (19 mmol) of 2-amino-5-bromo-*N*-methoxy-*N*-methylbenzamide (**4a**) in 50 mL of diethyl ether was added 24 mL (48 mmol) of 2 M phenylmagnesium bromide in THF dropwise at 0 °C. The resulting mixture was stirred at 0 °C for 15 min. After quenching with saturated aqueous ammonium chloride solution, the reaction mixture was extracted with ethyl acetate. The extract was washed with brine, dried over anhydrous magnesium sulfate and concentrated in vacuo. Purification by column chromatography (silica gel, eluting at a gradient of 5–25% ethyl acetate in hexanes) afforded 3.21 g (60%) of the title compound as yellow crystals. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 6.08 (br s, 2H), 6.65 (d, J = 8.8 Hz, 1H), 7.26–7.66 (m, 7H).

5.1.4. (2-Amino-5-bromophenyl)(4-methylphenyl)methanone (5b)

The title compound (3.5 g) was prepared from 5.0 g (19.3 mmol) of 2-amino-5-bromo-*N*-methoxy-*N*-methylbenzamide (**4a**) and 60 mL (60 mmol) of 1 M *p*-tolylmagnesium bromide in THF using a procedure similar to that described for the synthesis of compound **5a** as yellow crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.44 (s, 3H), 5.98 (br s, 2H), 6.64 (d, J = 8.9 Hz, 1H), 7.28 (d, J = 7.9 Hz, 2H), 7.35 (dd, J = 8.9, 2.3 Hz, 1H), 7.55–7.60 (m, 3H). MS m/z 290, 292 $(M+H)^+$.

5.1.5. (2-Amino-5-{[*tert*-butyl(dimethyl)silyl]oxy}phenyl)(3-fluorophenyl)methanone (5c)

The title compound (1.2 g) was obtained from 2.5 g (8.1 mmol) of 2-amino-5-{[*tert*-butyl(dimethyl)silyl]oxy}-*N*-methoxy-*N*-methylbenzamide (**4b**) and 3-fluorophenylmagnesium bromide prepared from 5.6 g (32 mmol) of *m*-bromofluorobenzene using a procedure similar to that described for the synthesis of compound **5a** as a yellow syrup. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 0.09 (s, 6H), 0.92 (s, 9H), 5.75 (br s, 2H), 6.66 (d, J = 8.4 Hz, 1H), 6.80–6.95 (m, 2H), 7.15–7.25 (m, 1H), 7.30–7.50 (m, 3H). MS m/z 346 $(M+H)^+$.

5.1.6. (2-Amino-5-{[*tert*-butyl(dimethyl)silyl]oxy}phenyl)(3-methylphenyl)methanone (5d)

The title compound (1.5 g) was prepared from 2.0 g (6.4 mmol) of 2-amino-5-{[*tert*-butyl(dimethyl)silyl]oxy}-*N*-methoxy-*N*-methylbenzamide (**4b**) and 30 mL (30 mmol) of 1 M *m*-tolylmagnesium bromide in THF using a procedure similar to that described for the synthesis of compound **5a** as a crude brown syrup. MS m/z 342 $(M+H)^+$.

5.1.7. (2-Amino-5-{[*tert*-butyl(dimethyl)silyl]oxy}phenyl)(4-fluorophenyl)methanone (5e)

The title compound (1.3 g) was obtained from 2.5 g (8.1 mmol) of 2-amino-5-{[*tert*-butyl(dimethyl)silyl]oxy}-*N*-methoxy-*N*-methylbenzamide (**4b**) and 4-fluorophenylmagnesium bromide prepared from 5.6 g (32 mmol) of *p*-bromofluorobenzene using a procedure similar to that described for the synthesis of compound **5a** as an orange syrup. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 0.10 (s, 6H), 0.93 (s, 9H), 5.63 (br s, 2H), 6.65 (d, J = 9.2 Hz, 1H), 6.80–6.95 (m, 2H), 7.05–7.20 (m, 2H), 7.60–7.75 (m, 2H).

5.1.8. (2-Amino-5-{[*tert*-butyl(dimethyl)silyl]oxy}phenyl)(4-methylphenyl)methanone (5f)

The title compound (1.4 g) was prepared from 4.6 g (14.5 mmol) of 2-amino-5-{[*tert*-butyl(dimethyl)silyl]oxy}-*N*-methoxy-*N*-methylbenzamide (**4b**) and 44 mL (44 mmol) of 1 M *p*-tolylmagnesium bromide in THF using a procedure similar to that described for the synthesis of compound **5a** as a yellow syrup. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 0.10 (s, 6H), 0.93 (s, 9H), 2.43 (s, 3H), 5.58 (br s, 2H), 6.64 (d, J = 8.8 Hz, 1H), 6.70–6.75 (m, 2H), 7.00–7.10 (m, 2H), 7.25 (d, J = 8.8 Hz, 2H), 7.55–7.65 (m, 2H). MS m/z 342 $(M+H)^+$.

5.1.9. 5-Methyl-3-oxohexanenitrile (6)

To a suspension of 30.2 g (666 mmol) of sodium hydride (60% in oil) in 300 mL of THF was added a mixture of 50 mL (333 mmol) of methyl 3-methylbutanoate **10** and 39 mL (666 mmol) of acetonitrile dropwise at 70 °C, and the resulting mixture was stirred at 70 °C for 15 h. The reaction mixture was cooled and carefully quenched with ice water. The aqueous layer was washed with hexanes, acidified with concentrated hydrochloric acid and extracted with ethyl acetate. The extract was washed with brine, dried over anhydrous magnesium sulfate and concentrated in vacuo to give 41 g (98%) of the title compound as a pale yellow oil, which was used in the next step without further purifications. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.96 (d, J = 6.6 Hz, 6H), 2.11–2.28 (m, 1H), 2.50 (d, J = 6.8 Hz, 2H), 3.42 (s, 2H).

5.1.10. 6-Bromo-2-(2-methylpropyl)-4-phenylquinoline-3-carbonitrile (7a)

A mixture of 4.2 g (15.2 mmol) of (2-amino-5-bromophenyl)(phenyl)methanone (**5a**), 2.28 g (18.2 mmol) of 5-methyl-3-oxohexanenitrile (**6**), methanesulfonic acid (1.46 g, 15.2 mmol) and 200 mL of toluene was heated to reflux for 6 h using a Dean-Stark trap. The reaction mixture was allowed to cool to room temperature, washed sequentially with saturated aqueous sodium bicarbonate solution and brine, dried over anhydrous magnesium sulfate and concentrated in vacuo. Purification by column chromatography (silica gel, eluting at a gradient of 5–20% ethyl acetate in hexanes) was followed by recrystallization from ethyl acetate/hexanes to afford 4.85 g (88%) of the title compound as colorless crystals: mp 136–137 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.06 (d, J = 6.6 Hz, 6H), 2.25–2.55 (m, 1H), 3.11 (d, J = 7.3 Hz, 2H), 7.40–7.50 (m, 2H), 7.55–7.65 (m, 3H), 7.79 (d, J = 2.2 Hz, 1H), 7.87 (dd, J = 8.4, 2.2 Hz, 1H), 8.80 (d, J = 8.4 Hz, 1H). MS m/z 365, 367 $(M+H)^+$.

5.1.11. 6-Bromo-4-(4-methylphenyl)-2-(2-methylpropyl)quinoline-3-carbonitrile (7b)

The title compound (6.6 g) was prepared from 8.7 g (30 mmol) of (2-amino-5-bromophenyl)(4-methylphenyl)methanone (**5b**) and 4.5 g (36 mmol) of 5-methyl-3-oxohexanenitrile (**6**) using a procedure similar to that described for the synthesis of compound **7a** as pale orange crystals: mp 168–169 °C. ^{1}H NMR (200 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). MS m/z 379, 381 $(M+H)^+$.

5.1.12. 6-Hydroxy-2-(2-methylpropyl)-4-phenylquinoline-3-carbonitrile (8a)

5.1.12.1. Step A. To 20 mL of toluene under nitrogen atmosphere were added 0.51 g (0.82 mmol) of *rac*-BINAP and 0.062 g

(0.27 mmol) of palladium acetate. After being stirred at 40 °C for 1 h, 2.0 g (5.5 mmol) of 6-bromo-2-(2-methylpropyl)-4-phenylquinoline-3-carbonitrile (**7a**), 1.1 mL (11 mmol) of *tert*-butanol and 0.79 g (8.2 mmol) of sodium *tert*-butoxide were added. The resulting mixture was stirred at 90 °C for 1 h. The reaction mixture was cooled to room temperature and filtered through Celite. The filtrate was washed with brine, dried over anhydrous magnesium sulfate and concentrated in vacuo. Purification by column chromatography (silica gel, eluting at a gradient of 5–20% ethyl acetate in hexanes) was followed by recrystallization from ethyl acetate/hexanes to afford 1.8 g (92%) of 6-*tert*-butoxy-2-(2-methylpropyl)-4-phenylquinoline-3-carbonitrile as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.06 (d, J = 6.6 Hz, 6H), 1.33 (s, 9H), 2.30–2.50 (m, 1H), 3.10 (d, J = 7.2 Hz, 2H), 7.14 (d, J = 2.5 Hz, 1H), 7.40–7.65 (m, 6H), 8.03 (d, J = 9.1 Hz, 1H). MS m/z 369 $(M+H)^+$.

5.1.12.2. Step B. A mixture of 1.0 g (2.8 mmol) of 6-*tert*-butoxy-2-(2-methylpropyl)-4-phenylquinoline-3-carbonitrile, 10 mL of TFA and 2 mL of THF was stirred at room temperature for 1 h. The reaction mixture was poured into water and extracted with ethyl acetate. The extract was washed with brine, dried over anhydrous magnesium sulfate and concentrated in vacuo. Recrystallization from diisopropyl ether/hexanes afforded 0.81 g (95%) of the title compound as a yellow powder. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.10 (d, J = 6.6 Hz, 6H), 2.28–2.43 (m, 1H), 3.29 (d, J = 7.5 Hz, 2H), 7.13 (d, J = 2.4 Hz, 1H), 7.45–7.51 (m, 2H), 7.62–7.70 (m, 3H), 7.73 (dd, J = 9.3, 2.4 Hz, 1H), 8.32 (br s, 1H), 8.57 (d, J = 9.3 Hz, 1H).

5.1.13. 4-(3-Fluorophenyl)-6-hydroxy-2-(2-methylpropyl)quinoline-3-carbonitrile (8b)

The title compound (0.83 g) was prepared from 1.2 g (3.4 mmol) of (2-amino-5-{[*tert*-butyl(dimethyl)silyl]oxy}phenyl)(3-fluorophenyl)methanone (**5c**) and 0.64 g (5.1 mmol) of 5-methyl-3-oxohexanenitrile (**6**) using a procedure similar to that described for the synthesis of compound **7a** as an off-white powder: mp 273–276 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.05 (d, J = 6.6 Hz, 6H), 2.25–2.45 (m, 1H), 3.08 (d, J = 7.4 Hz, 2H), 5.46 (br s, 1H), 6.68 (d, J = 2.8 Hz, 1H), 7.10–7.35 (m, 3H), 7.48 (dd, J = 9.2, 2.8 Hz, 1H), 7.50–7.65 (m, 1H), 8.05 (d, J = 9.2 Hz, 1H). MS m/z 321 $(M+H)^+$.

5.1.14. 6-Hydroxy-4-(3-methylphenyl)-2-(2-methylpropyl)quinoline-3-carbonitrile (8c)

The title compound (0.05 g) was prepared from 1.35 g (4.0 mmol) of (2-amino-5-{[*tert*-butyl(dimethyl)silyl]oxy}phenyl)(3-methylphenyl)methanone (**5d**) and 0.64 g (5.1 mmol) of 5-methyl-3-oxohexanenitrile (**6**) using a procedure similar to that described for the synthesis of compound **7a** as an off-white powder: mp 275 °C (decomp). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 0.99 (d, J = 6.6 Hz, 6H), 2.20–2.35 (m, 1H), 2.43 (s, 3H), 2.96 (d, J = 7.3 Hz, 2H), 6.81 (d, J = 2.6 Hz, 1H), 7.25–7.60 (m, 5H), 7.97 (d, J = 9.2 Hz, 1H), 10.20 (s, 1H).

5.1.15. 4-(4-Fluorophenyl)-6-hydroxy-2-(2-methylpropyl)quinoline-3-carbonitrile (8d)

The title compound (0.88 g) was prepared from 1.4 g (4.0 mmol) of (2-amino-5-{[*tert*-butyl(dimethyl)silyl]oxy}phenyl)(4-fluorophenyl)methanone (**5e**) and 0.76 g (6.0 mmol) of 5-methyl-3-oxohexanenitrile (**6**) using a procedure similar to that described for the synthesis of compound **7a** as an off-white powder: mp 247–249 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.04 (d, J = 6.6 Hz, 6H), 2.15–2.45 (m, 1H), 3.07 (d, J = 7.2 Hz, 2H), 5.87 (br s, 1H), 6.89 (d, J = 2.6 Hz, 1H), 7.10–7.50 (m, 5H), 8.04 (d, J = 9.2 Hz, 1H). MS m/z 321 $(M+H)^+$.

5.1.16. 6-Hydroxy-4-(4-methylphenyl)-2-(2-methylpropyl)quinoline-3-carbonitrile (8e)

The title compound (7.2 g) was prepared from 8.5 g (25 mmol) of (2-amino-5-{[*tert*-butyl(dimethyl)silyl]oxy}phenyl)(4-methylphenyl)methanone (**5f**) and 3.8 g (30 mmol) of 5-methyl-3-oxohexanenitrile (**6**) using a procedure similar to that described for the synthesis of compound **7a** as pale yellow crystals: mp 247 °C (decomp). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.03 (d, J = 6.6 Hz, 6H), 2.20–2.45 (m, 1H), 2.46 (s, 3H), 3.06 (d, J = 7.3 Hz, 2H), 6.28 (d, J = 2.9 Hz, 1H), 7.25–7.40 (m, 4H), 7.41 (dd, J = 9.2, 2.9 Hz, 1H), 8.02 (d, J = 9.2 Hz, 1H). MS m/z 317 $(M+H)^+$.

5.1.17. 2-{[3-(Aminomethyl)-2-(2-methylpropyl)-4-phenylquinolin-6-yl]oxy}acetamide (9a)

5.1.17.1. Step A. A mixture of 0.76 g (2.5 mmol) of 6-hydroxy-2-(2-methylpropyl)-4-phenylquinoline-3-carbonitrile (**8a**), 0.36 g (3.8 mmol) of 2-chloroacetamaide and 0.53 g (3.8 mmol) of potassium carbonate in 10 mL of DMF was stirred at 60 °C for 8 h. The reaction mixture was poured into water and extracted with ethyl acetate. The extract was washed with 1 M hydrochloric acid, dried over anhydrous magnesium sulfate and concentrated in vacuo. Purification by column chromatography (silica gel, eluting at a gradient of 30–100% ethyl acetate in hexanes) to give 0.71 g (79%) of 2-{[3-cyano-2-(2-methylpropyl)-4-phenylquinolin-6-yl]oxy}acetamide as a white powder. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.05 (d, J = 6.6 Hz, 6H), 2.32–2.43 (m, 1H), 3.09 (d, J = 7.2 Hz, 2H), 4.40 (s, 2H), 5.75 (br s, 1H), 6.51 (br s, 1H), 6.92 (d, J = 3.0 Hz, 1H), 7.41–7.48 (m, 2H), 7.49 (dd, J = 9.0, 3.0 Hz, 1H), 7.57–7.64 (m, 3H), 8.09 (d, J = 9.0 Hz, 1H).

5.1.17.2. Step B. A mixture of 0.65 g (1.8 mmol) of 2-{[3-cyano-2-(2-methylpropyl)-4-phenylquinolin-6-yl]oxy}acetamide, ca. 10 g of Raney cobalt, 5 mL of 25% aqueous ammonia, 60 mL of methanol and 20 mL of THF was stirred under hydrogen atmosphere at 0.5 MPa for 7 h at 70 °C. The reaction mixture was cooled to room temperature and filtered. The filtrate was poured into 5% aqueous potassium carbonate solution and extracted with ethyl acetate. The extract was washed with brine, dried over anhydrous magnesium sulfate and concentrated in vacuo. Purification by column chromatography (silica gel, eluting at a gradient of 0–30% methanol in ethyl acetate) to give 0.43 g (66%) of the title compound as a white powder: mp 139–141 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.04 (d, J = 6.6 Hz, 6H), 1.24 (br s, 2H), 2.32–2.44 (m, 1H), 3.00 (d, J = 7.2 Hz, 2H), 3.78 (s, 2H), 4.33 (s, 2H), 5.88 (br s, 1H), 5.55 (br s, 1H), 6.56 (d, J = 2.7 Hz, 1H), 7.25–7.28 (m, 2H), 7.30 (dd, J = 9.3, 2.7 Hz, 1H), 7.49–7.58 (m, 3H), 8.02 (d, J = 9.3 Hz, 1H). Anal. Calcd for $C_{22}H_{25}N_3O_2$: C, 72.70; H, 6.93; N, 11.56. Found: C, 72.35; H, 6.80; N, 11.28. HPLC purity: 96.69% (λ 220 nm), 95.60% (λ 254 nm).

5.1.18. 2-{[3-(Aminomethyl)-4-(3-fluorophenyl)-2-(2-methylpropyl)quinolin-6-yl]oxy}acetamide (9b)

5.1.18.1. Step A. 2-{[3-Cyano-4-(3-fluorophenyl)-2-(2-methylpropyl)quinolin-6-yl]oxy}acetamide (0.38 g) was prepared from 0.40 g (1.25 mmol) of 4-(3-fluorophenyl)-6-hydroxy-2-(2-methylpropyl)quinoline-3-carbonitrile (**8b**) using a procedure similar to that described as step A for the synthesis of compound **9a** as an off-white powder: mp 186–193 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.06 (d, J = 6.6 Hz, 6H), 2.30–2.50 (m, 1H), 3.10 (d, J = 7.3 Hz, 2H), 4.43 (s, 2H), 5.74 (br s, 1H), 6.52 (br s, 1H), 6.88 (d, J = 2.8 Hz, 1H), 7.10–7.25 (m, 2H), 7.25–7.40 (m, 1H), 7.52 (dd, J = 9.2, 2.8 Hz, 1H), 7.55–7.65 (m, 1H), 8.11 (d, J = 9.2 Hz, 1H). MS m/z 378 $(M+H)^+$.

5.1.18.2. Step B. A mixture of 0.35 g (0.93 mmol) of 2-{[3-cyano-4-(3-fluorophenyl)-2-(2-methylpropyl)quinolin-6-yl]oxy}acetamide, ca. 4 g of Raney nickel, 4 mL of 25% aqueous ammonia, 50 mL of methanol and 10 mL of THF was stirred under hydrogen atmosphere at 0.5 MPa for 9 h at room temperature. The reaction mixture was filtered, and the filtrate was concentrated in vacuo. Purification by column chromatography (silica gel, eluting at a gra-

dient of 0–20% methanol in ethyl acetate) was followed by recrystallization from aqueous ethanol to give 0.27 g (76%) of the title compound as colorless crystals: mp 106–108 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.04 (d, J = 6.6 Hz, 6H), 1.51 (br s, 2H), 2.25–2.50 (m, 1H), 3.00 (d, J = 7.2 Hz, 2H), 3.78 (s, 2H), 4.36 (s, 2H), 5.62 (br s, 1H), 6.53 (d, J = 2.8 Hz, 1H), 6.55 (br s, 1H), 6.90–7.30 (m, 3H), 7.34 (dd, J = 9.2, 2.8 Hz, 1H), 7.45–7.60 (m, 1H), 8.03 (d, J = 9.2 Hz, 1H). MS m/z 382 $(M+H)^+$. Anal. Calcd for $C_{22}H_{24}FN_3O_2 \cdot H_2O$: C, 66.15; H, 6.56; N, 10.52. Found: C, 65.92; H, 6.43; N, 10.31. HPLC purity: 98.31% (λ 220 nm), 97.68% (λ 254 nm).

5.1.19. 2-{[3-(Aminomethyl)-4-(3-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl]oxy}acetamide (9c)

5.1.19.1. Step A. 2-{[3-Cyano-4-(3-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl]oxy}acetamide (0.21 g) was prepared from 0.25 g (0.79 mmol) of 6-hydroxy-4-(3-methylphenyl)-2-(2-methylpropyl)quinoline-3-carbonitrile (**8c**) using a procedure similar to that described as step A for the synthesis of compound **9a** as an off-white powder: mp 123–125 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.05 (d, J = 6.8 Hz, 6H), 2.25–2.45 (m, 1H), 2.48 (s, 3H), 3.10 (d, J = 7.2 Hz, 2H), 4.41 (s, 2H), 5.64 (br s, 1H), 6.53 (br s, 1H), 6.93 (d, J = 2.8 Hz, 1H), 7.15–7.30 (m, 2H), 7.30–7.55 (m, 3H), 8.09 (d, J = 9.2 Hz, 1H). MS m/z 374 $(M+H)^+$.

5.1.19.2. Step B. The title compound (50 mg) was prepared from 0.18 g (0.48 mmol) of 2-{[3-cyano-4-(3-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl]oxy}acetamide using a procedure similar to that described as step B for the synthesis of compound **9a** as a white powder: mp 98–101 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.04 (d, J = 6.6 Hz, 6H), 1.60 (br s, 2H), 2.30–2.45 (m, 1H), 2.45 (s, 3H), 2.99 (d, J = 7.4 Hz, 2H), 3.78 (br s, 2H), 4.34 (s, 2H), 5.63 (br s, 1H), 6.57 (d, J = 2.6 Hz, 1H), 6.60 (br s, 1H), 7.05 (d, J = 7.2 Hz, 1H), 7.25–7.40 (m, 1H), 7.32 (dd, J = 9.2, 2.6 Hz, 2H), 7.40–7.50 (m, 1H), 8.01 (d, J = 9.2 Hz, 1H). MS m/z 378 $(M+H)^+$; HRMS (ESI) calcd for $C_{23}H_{27}N_3O_2$ $(M+H)^+$ m/z 378.2176, found m/z 378.2151. Anal. Calcd for $C_{23}H_{27}N_3O_2 \cdot 2H_2O$: C, 66.81; H, 7.56; N, 10.16. Found: C, 66.73; H, 7.05; N, 9.93. HPLC purity: 95.87% (λ 220 nm), 95.11% (λ 254 nm).

5.1.20. 2-{[3-(Aminomethyl)-4-(4-fluorophenyl)-2-(2-methylpropyl)quinolin-6-yl]oxy}acetamide (9d)

5.1.20.1. Step A. 2-{[3-Cyano-4-(4-fluorophenyl)-2-(2-methylpropyl)quinolin-6-yl]oxy}acetamide (0.93 g) was prepared from 0.84 g (2.6 mmol) of 4-(4-fluorophenyl)-6-hydroxy-2-(2-methylpropyl)quinoline-3-carbonitrile (**8d**) using a procedure similar to that described as step A for the synthesis of compound **9a** as pale yellow crystals: mp 153–154 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.05 (d, J = 6.6 Hz, 6H), 2.30–2.50 (m, 1H), 3.10 (d, J = 7.4 Hz, 2H), 4.43 (s, 2H), 5.68 (br s, 1H), 6.51 (br s, 1H), 6.90 (d, J = 2.9 Hz, 1H), 7.25–7.40 (m, 2H), 7.40–7.50 (m, 2H), 7.52 (dd, J = 9.2, 2.9 Hz, 1H), 8.11 (d, J = 9.2 Hz, 1H). MS m/z 378 $(M+H)^+$.

5.1.20.2. Step B. The title compound (0.42 g) was prepared from 0.85 g (2.2 mmol) of 2-{[3-cyano-4-(4-fluorophenyl)-2-(2-methylpropyl)quinolin-6-yl]oxy}acetamide using a procedure similar to that described as step B for the synthesis of compound **9a** as colorless crystals: mp 142 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.04 (d, J = 6.6 Hz, 6H), 1.54 (br s, 2H), 2.25–2.50 (m, 1H), 2.99 (d, J = 7.2 Hz, 2H), 3.77 (s, 2H), 4.36 (s, 2H), 5.63 (br s, 1H), 6.54 (br s, 1H), 6.54 (d, J = 2.8 Hz, 1H), 7.20–7.30 (m, 4H), 7.33 (dd, J = 9.2, 2.8 Hz, 1H), 8.03 (d, J = 9.2 Hz, 1H). MS m/z 378 $(M+H)^+$. Anal. Calcd for $C_{22}H_{24}FN_3O_2 \cdot 0.75H_2O$: C, 66.90; H, 6.51; N, 10.64. Found: C, 66.56; H, 6.61; N, 10.43. HPLC purity: 99.54% (λ 220 nm), 99.39% (λ 254 nm).

5.1.21. 2-{[3-(Aminomethyl)-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl]oxy}acetamide (9e)

5.1.21.1. Step A. 2-{[3-Cyano-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl]oxy}acetamide (0.37 g) was prepared from 0.40 g (1.26 mmol) of 6-hydroxy-4-(4-methylphenyl)-2-(2-methylpropyl)quinoline-3-carbonitrile (**8e**) using a procedure similar to that described as step A for the synthesis of compound **9a** as a white powder: mp 184–186 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.05 (d, J = 6.6 Hz, 6H), 2.30–2.50 (m, 1H), 2.51 (s, 3H), 3.04 (d, J = 7.3 Hz, 2H), 4.42 (s, 2H), 5.71 (br s, 1H), 6.53 (br s, 1H), 6.96 (d, J = 2.9 Hz, 1H), 7.25–7.45 (m, 4H), 7.50 (dd, J = 9.2, 2.9 Hz, 1H), 8.08 (d, J = 9.2 Hz, 1H). MS m/z 374 $(M+H)^+$.

5.1.21.2. Step B. The title compound (0.24 g) was prepared from 0.33 g (0.88 mmol) of 2-{[3-cyano-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl]oxy}acetamide using a procedure similar to that described as step B for the synthesis of compound **9b** as a white powder: mp 175–177 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.04 (d, J = 6.6 Hz, 6H), 1.60 (br s, 2H), 2.25–2.45 (m, 1H), 2.50 (s, 3H), 2.99 (d, J = 7.3 Hz, 2H), 3.78 (br s, 2H), 4.34 (s, 2H), 5.68 (br s, 1H), 6.58 (br s, 1H), 6.59 (d, J = 2.9 Hz, 1H), 7.15 (d, J = 8.1 Hz, 2H), 7.25–7.40 (m, 3H), 8.01 (d, J = 9.2 Hz, 1H). MS m/z 378 $(M+H)^+$. Anal. Calcd for $C_{23}H_{27}N_3O_2 \cdot 1.25H_2O$: C, 69.06; H, 7.43; N, 10.51. Found: C, 69.27; H, 7.50; N, 10.11. HPLC purity: 99.10% (λ 220 nm), 98.84% (λ 254 nm).

5.1.22. *tert*-Butyl {[6-hydroxy-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-3-yl]methyl}carbamate (11)

A mixture of 27.4 g (86.6 mmol) of 6-hydroxy-4-(4-methylphenyl)-2-(2-methylpropyl)quinoline-3-carbonitrile (**8e**), ca. 101 g of Raney cobalt, 50 mL of 25% aqueous ammonia, 300 mL of methanol and 150 mL of THF was stirred under hydrogen atmosphere at 0.5 MPa for 8 h at 90 °C. The reaction mixture was cooled to room temperature and filtered. The filtrate was poured into brine and extracted with ethyl acetate. The extract was dried over anhydrous magnesium sulfate and concentrated in vacuo. The residue was dissolved in 500 mL of THF and then 22.7 g (104 mmol) of di-*tert*-butyl dicarbonate was added. The resulting mixture was stirred at room temperature for 96 h, and concentrated in vacuo. The residual solid was triturated with diisopropyl ether to afford 30.2 g (83%) of the title compound as an off-white powder. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 0.97 (d, J = 6.6 Hz, 6H), 1.40 (s, 9H), 2.15–2.30 (m, 1H), 2.42 (s, 3H), 2.92 (d, J = 7.3 Hz, 2H), 4.25–4.35 (m, 3H), 6.67 (d, J = 2.5 Hz, 1H), 7.08 (d, J = 7.3 Hz, 2H), 7.20–7.30 (m, 3H), 7.88 (d, J = 8.9 Hz, 1H). MS m/z 421 $(M+H)^+$.

5.1.23. Methyl {[3-{[(*tert*-butoxycarbonyl)amino]methyl}-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl]oxy}acetate (12)

A mixture of 1.5 g (3.6 mmol) of *tert*-butyl {[6-hydroxy-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-3-yl]methyl}carbamate (**11**), 0.55 g (4.0 mmol) of methyl 2-bromoacetate and 0.92 g (6.0 mmol) of potassium carbonate in 10 mL of DMF was stirred at room temperature for 2.5 h. After quenching with water, the reaction mixture was extracted with ethyl acetate. The extract was washed with brine, dried over anhydrous magnesium sulfate and concentrated in vacuo. The residual solid was triturated with diisopropyl ether to afford 1.86 g (94%) of the title compound as a white powder. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 0.99 (d, J = 6.6 Hz, 6H), 1.35 (s, 9H), 2.26–2.32 (m, 1H), 2.43 (s, 3H), 3.09–3.10 (m, 2H), 3.10 (s, 3H), 4.12 (s, 2H), 4.77 (s, 2H), 6.54 (s, 1H), 7.12 (s, 1H), 7.24 (d, J = 7.8 Hz, 2H), 7.40 (d, J = 7.8 Hz, 2H), 7.75–7.77 (m, 1H), 8.48–8.51 (m, 1H).

5.1.24. Methyl {[3-(aminomethyl)-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl]oxy}acetate dihydrochloride (13a)

To a solution of 0.15 g (0.3 mmol) of methyl {[3-{[(*tert*-butoxycarbonyl)amino]methyl}-4-(4-methylphenyl)-2-(2-methylpropyl) quinolin-6-yl]oxy}acetate (**12**) in 5 mL of ethyl acetate was added 5 mL of 4 M hydrogen chloride in ethyl acetate at room temperature. The resulting mixture was stirred at room temperature for 1.5 h, and then concentrated in vacuo. The residue was crystallized from diisopropyl ether to afford 0.13 g (92%) of the title compound as a yellow powder. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.00 (d, J = 7.2 Hz, 6H), 2.24–2.32 (m, 1H), 2.46 (s, 3H), 3.25 (s, 2H), 3.59 (s, 3H), 3.93–3.99 (m, 2H), 4.75 (s, 2H), 6.49 (s, 1H), 7.33 (d, J = 7.5 Hz, 2H), 7.45 (d, J = 7.5 Hz, 2H), 7.72–7.75 (m, 1H), 8.39–8.42 (m, 1H), 8.56 (br s, 3H). HRMS (ESI) calcd for $C_{24}H_{28}N_2O_3$ (M+H)$^+$ m/z 393.2173, found m/z 393.2155. HPLC purity: 98.26% (λ 220 nm), 98.12% (λ 254 nm).

5.1.25. 2-{[3-(Aminomethyl)-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl]oxy}-*N*-methylacetamide dihydrochloride (13b)

5.1.25.1. Step A. A mixture of 1.6 g (3.2 mmol) of methyl {[3-{[(*tert*-butoxycarbonyl)amino]methyl}-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl]oxy}acetate (**12**), 10 mL of 1 M aqueous sodium hydroxide solution, 5 mL of methanol and 5 mL of THF was stirred at room temperature for 1.5 h. The reaction mixture was neutralized with 1 M hydrochloric acid and extracted with ethyl acetate. The extract was washed with brine, dried over anhydrous magnesium sulfate and concentrated in vacuo. The residual solid was triturated with diisopropyl ether to afford 1.32 g (85%) of {[3-{[(*tert*-butoxycarbonyl)amino]methyl}-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl]oxy}acetic acid as a white powder. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.05 (d, J = 6.6 Hz, 6H), 1.40 (s, 9H), 2.29–2.31 (m, 1H), 2.48 (s, 3H), 3.36 (s, 2H), 4.53 (s, 2H), 4.89 (br, 1H), 6.71 (s, 1H), 7.12–7.13 (m, 2H), 7.27–7.40 (m, 3H), 8.57 (d, J = 8.1 Hz, 1H).

5.1.25.2. Step B. To a mixture of 0.48 g (1.0 mmol) of {[3-{[(*tert*-butoxycarbonyl)amino]methyl}-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl]oxy}acetic acid, 2 mL of 1 M methylamine in THF and 3 mL of DMF were added 0.16 g (1.2 mmol) of HOBt and 0.23 g (1.2 mmol) of EDC. The resulting mixture was stirred at room temperature for 15 h. The reaction mixture was diluted with ethyl acetate, washed sequentially with 1 M hydrochloric acid and brine, dried over anhydrous magnesium sulfate and concentrated in vacuo. Purification by column chromatography (silica gel, 1:1 ethyl acetate/hexanes) to give 0.27 g (55%) of *tert*-butyl ({6-[2-(methylamino)-2-oxoethoxy]-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-3-yl}methyl)carbamate as a pale yellow solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.00 (d, J = 6.6 Hz, 6H), 1.41 (s, 9H), 2.30–2.33 (m, 1H), 2.49 (s, 3H), 2.88–2.98 (m, 5H), 4.28–4.34 (m, 4H), 6.58–6.59 (m, 2H), 6.60 (br, 1H), 7.07 (d, J = 8.1 Hz, 2H), 7.32 (d, J = 8.1 Hz, 2H), 7.33–7.34 (m, 1H), 8.02 (d, J = 9.0 Hz, 1H).

5.1.25.3. Step C. To a solution of 0.26 g (0.53 mmol) of *tert*-butyl ({6-[2-(methylamino)-2-oxoethoxy]-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-3-yl}methyl)carbamate in 5 mL of ethyl acetate was added 5 mL of 4 M hydrogen chloride in ethyl acetate at room temperature. The resulting mixture was stirred at room temperature for 3 h, and then concentrated in vacuo. The residue was crystallized from diisopropyl ether to afford 0.20 g (79%) of the title compound as a pale yellow powder. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 0.99 (d, J = 6.6 Hz, 6H), 2.26–2.30 (m, 1H), 2.46 (s, 3H), 2.58 (s, 3H), 3.18 (s, 2H), 3.97 (s, 2H), 4.42 (s, 2H), 6.64 (s, 1H), 7.32 (d, J = 8.4 Hz, 2H), 7.44 (d, J = 8.4 Hz, 2H), 7.72–7.73 (m, 1H), 8.07–8.08 (m, 1H), 8.41 (br s, 3H). HRMS (ESI) calcd for $C_{24}H_{29}N_3O_2$ (M+H)$^+$ m/z 392.2333, found m/z 392.2316. HPLC purity: 99.08% (λ 220 nm), 99.05% (λ 254 nm).

5.1.26. 3-{[(*tert*-Butoxycarbonyl)amino]methyl}-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl trifluoromethanesulfonate (14)

To an ice-cooled mixture of 1.0 g (2.4 mmol) of *tert*-butyl {[6-hydroxy-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-3-yl] methyl}carbamate (**11**) and 1.28 g (3.6 mmol) of *N*-phenyl bis(trifluoromethanesulfonimide) in 15 mL of DMF was added 0.14 g (3.6 mmol) of sodium hydride (60% in oil). The resulting mixture was stirred at 0 °C for 30 min. After quenching with water, the reaction mixture was extracted with ethyl acetate. The extract was washed with brine, dried over anhydrous magnesium sulfate and concentrated in vacuo. Purification by column chromatography (silica gel, eluting at a gradient of 5–20% ethyl acetate in hexanes) to give 1.30 g (99%) of the title compound as a white powder: mp 194–196 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.04 (d, J = 6.7 Hz, 6H), 1.42 (s, 9H), 2.30–2.45 (m, 1H), 2.49 (s, 3H), 2.99 (d, J = 7.2 Hz, 2H), 4.33 (br s, 3H), 7.12 (d, J = 2.8 Hz, 2H), 7.22 (d, J = 2.8 Hz, 1H), 7.36 (d, J = 8.2 Hz, 2H), 7.53 (dd, J = 9.2, 2.8 Hz, 1H), 8.14 (d, J = 9.2 Hz, 1H). MS m/z 553 (M+H)$^+$.

5.1.27. *tert*-Butyl ({6-[(1*E*)-3-amino-3-oxoprop-1-en-1-yl]-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-3-yl}methyl)carbamate (15)

5.1.27.1. Step A. A mixture of 1.48 g (2.78 mmol) of 3-{[(*tert*-butoxycarbonyl)amino]methyl}-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl trifluoromethanesulfonate (**14**), 0.65 mL (6.0 mmol) of ethyl acrylate, 0.021 mg (0.03 mmol) of bis(triphenylphosphine)palladium(II) dichloride and 4.2 mL (30 mmol) of triethylamine in 10 mL of DMF was stirred under nitrogen atmosphere at 70 °C for 20 h. The reaction mixture was partitioned between ethyl acetate and water, and the organic layer was washed with brine, dried over anhydrous magnesium sulfate and concentrated in vacuo. Purification by column chromatography (silica gel, eluting at a gradient of 0–25% ethyl acetate in hexanes) gave 0.87 g of crude solid, which was used directly in the next step. To a solution of the obtained crude solid in 10 mL of ethanol and 10 mL of THF was added 10 mL of 1 M aqueous sodium hydroxide solution at room temperature, and the resulting mixture was stirred at 60 °C for 4 h. The reaction mixture was cooled to room temperature, neutralized with 1 M hydrochloric acid, and extracted with ethyl acetate. The extract was washed with brine, dried over anhydrous magnesium sulfate and concentrated in vacuo. The residual solid was triturated with diisopropyl ether to give 0.41 g (70%) of (2*E*)-3-[3-{[(*tert*-butoxycarbonyl)amino]methyl}-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl]prop-2-enoic acid as a white powder. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.13 (d, J = 6.0 Hz, 6H), 1.41 (s, 9H), 2.36–2.39 (m, 1H), 2.52 (s, 3H), 3.15 (s, 2H), 4.35 (s, 2H), 4.35 (br, 1H), 6.43 (d, J = 15.6 Hz, 1H), 7.14 (d, J = 8.1 Hz, 2H), 7.26 (s, 1H), 7.28 (d, J = 8.1 Hz, 2H), 7.45 (s, 1H), 7.70 (d, J = 15.6 Hz, 1H), 7.93 (s, 1H).

5.1.27.2. Step B. A mixture of 0.30 g (0.63 mmol) of (2*E*)-3-[3-{[(*tert*-butoxycarbonyl)amino]methyl}-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl]prop-2-enoic acid, 0.18 g (1.2 mmol) of HOBt ammonium salt and 0.23 g (1.2 mmol) of EDC in 5 mL of DMF was stirred at room temperature for 17 h. The reaction mixture was partitioned between ethyl acetate and water. The organic layer was washed with brine, dried over anhydrous magnesium sulfate and concentrated in vacuo. Purification by column chromatography (silica gel, eluting at a gradient of 45–100% ethyl acetate in hexanes) gave 0.25 g (84%) of the title compound as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.03 (d, J = 6.6 Hz, 6H), 1.41 (s, 9H), 2.35–2.39 (m, 1H), 2.50 (s, 3H), 2.97 (d, J = 7.2 Hz, 2H),

4.32 (s, 3H), 5.49 (br, 2H), 6.42 (d, J = 15.6 Hz, 1H), 7.11 (d, J = 3.1 Hz, 2H), 7.33–7.37 (m, 3H), 7.58 (d, J = 15.6 Hz, 1H), 7.82 (dd, J = 8.7, 2.1 Hz, 1H), 8.04 (d, J = 8.7 Hz, 1H).

5.1.28. 3-[3-(Aminomethyl)-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl]propanamide dihydrochloride (13c)

5.1.28.1. Step A. A mixture of 0.25 g (0.53 mmol) of *tert*-butyl ({6-[(1*E*)-3-amino-3-oxoprop-1-en-1-yl]-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-3-yl}methyl)carbamate (**15**), 0.25 g of 10% Pd/C, 5 mL of ethanol and 5 mL of THF was stirred under atmospheric hydrogen at room temperature for 3.5 h. The reaction mixture was filtered, and the filtrate was concentrated in vacuo to afford 0.20 g (81%) of *tert*-butyl {[6-(3-amino-3-oxopropyl)-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-3-yl]methyl}carbamate as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.03 (d, J = 6.6 Hz, 6H), 1.41 (s, 9H), 2.31–2.39 (m, 1H), 2.46 (t, J = 7.8 Hz, 2H), 2.49 (s, 3H), 3.00 (t, J = 7.8 Hz, 2H), 3.00–3.03 (m, 2H), 4.31 (s, 2H), 4.34 (br, 1H), 5.28 (br s, 1H), 5.40 (br s, 1H), 7.12 (d, J = 8.1 Hz, 2H), 7.12–7.15 (m, 1H), 7.35 (d, J = 5.1 Hz, 2H), 7.55–7.61 (m, 1H), 8.17 (br, 1H).

5.1.28.2. Step B. To a solution of 0.15 g (0.32 mmol) of *tert*-butyl {[6-(3-amino-3-oxopropyl)-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-3-yl]methyl}carbamate in 5 mL of ethyl acetate was added 5 mL of 4 M hydrogen chloride in ethyl acetate at room temperature. The resulting mixture was stirred at room temperature for 1.5 h, and then concentrated in vacuo. The residue was crystallized from diisopropyl ether to afford 0.14 g (96%) of the title compound as a yellow powder. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 0.84 (d, J = 6.3 Hz, 6H), 2.11–2.17 (m, 1H), 2.15 (t, J = 7.2 Hz, 2H), 2.36 (s, 3H), 2.71 (t, J = 7.2 Hz, 2H), 3.09 (s, 2H), 3.82–3.88 (m, 2H), 6.57 (br, 1H), 7.03 (s, 1H), 7.09 (br, 1H), 7.20 (d, J = 7.8 Hz, 2H), 7.29 (d, J = 7.8 Hz, 2H), 7.75–7.76 (m, 1H), 8.28–8.33 (m, 1H), 8.32 (br s, 3H). HRMS (ESI) calcd for $C_{24}H_{29}N_3O$ $(M+H)^+$ m/z 376.2383, found m/z 376.2366. HPLC purity: 99.10% (λ 220 nm), 99.50% (λ 254 nm).

5.1.29. (2*E*)-3-[3-(Aminomethyl)-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl]prop-2-enamide (13d)

5.1.29.1. Step A. To a solution of 0.126 g (0.28 mmol) of *tert*-butyl ({6-[(1*E*)-3-amino-3-oxoprop-1-en-1-yl]-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-3-yl}methyl)carbamate (**15**) in 5 mL of ethyl acetate was added 5 mL of 4 M hydrogen chloride in ethyl acetate at room temperature. The resulting mixture was stirred at room temperature for 2 h, and then concentrated in vacuo. The residue was crystallized from diisopropyl ether to afford 0.094 g (93%) of (2*E*)-3-[3-(aminomethyl)-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl]prop-2-enamide dihydrochloride as a pale yellow powder.

5.1.29.2. Step B. A mixture of 0.22 g (0.50 mmol) of (2*E*)-3-[3-(aminomethyl)-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl]prop-2-enamide dihydrochloride and 100 mL of 10% aqueous potassium carbonate solution was stirred at room temperature for 5 min. The mixture was partitioned between ethyl acetate and water. The organic layer was washed with brine, dried over anhydrous magnesium sulfate and concentrated in vacuo. The residual solid was recrystallized from aqueous ethanol to afford 0.16 g (89%) of the title compound as a white powder. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.04 (d, J = 6.6 Hz, 6H), 1.26 (br s, 2H), 2.33–2.44 (m, 1H), 2.50 (s, 3H), 3.02 (d, J = 6.9 Hz, 2H), 3.82 (s, 2H), 5.51 (br s, 2H), 6.42(d, J = 15.6 Hz, 1H), 7.16 (d, J = 8.1 Hz, 2H), 7.34–7.36 (m, 3H), 7.59 (d, J = 8.1 Hz, 1H), 7.81 (dd, J = 8.7, 1.8 Hz, 1H), 8.03 (d, J = 8.7 Hz, 1H). MS m/z 374 $(M+H)^+$. Anal. Calcd for $C_{24}H_{27}N_3O{\cdot}2H_2O$: C, 70.39; H, 7.63; N, 10.26. Found: C, 70.36; H, 7.77; N, 10.25. HPLC purity: 96.28% (λ 220 nm), 95.83% (λ 254 nm).

5.1.30. 3-{[(*tert*-Butoxycarbonyl)amino]methyl}-4-(4-methylphenyl)-2-(2-methylpropyl)quinoline-6-carboxylic acid (16)

5.1.30.1. Step A. A mixture of 12.2 g (22 mmol) of 3-{[(*tert*-butoxycarbonyl)amino]methyl}-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl trifluoromethanesulfonate (**14**), 30 mL of methanol, 0.25 g (1.1 mmol) of palladium acetate, 0.61 g (1.1 mmol) of 1,1'-bis(diphenylphosphino)ferrocene and 3.4 mL (24 mmol) of triethylamine in 50 mL of THF was stirred under carbonoxide atmosphere at 0.5 MPa for 3 h at 100 °C. After being cooled, the reaction mixture was poured into water and extracted with ethyl acetate. The extract was washed with brine, dried over anhydrous magnesium sulfate and concentrated in vacuo. Purification by column chromatography (silica gel, eluting at a gradient of 10–40% ethyl acetate in hexanes) gave 6.8 g (67%) of methyl 3-{[(*tert*-butoxycarbonyl)amino]methyl}-4-(4-methylphenyl)-2-(2-methylpropyl)quinoline-6-carboxylate as a white powder: mp 192–194 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.04 (d, J = 6.3 Hz, 6H), 1.42 (s, 9H), 2.34–2.37 (m, 1H), 2.49 (s, 3H), 2.99 (d, J = 7.2 Hz, 2H), 3.87 (s, 3H), 4.32 (br s, 3H), 7.13 (d, J = 7.8 Hz, 2H), 7.35 (d, J = 7.8 Hz, 2H), 8.06–8.10 (m, 2H), 8.23 (dd, J = 8.7, 2.1 Hz, 1H).

5.1.30.2. Step B. To a suspension of 6.5 g (14 mmol) of methyl 3-{[(*tert*-butoxycarbonyl)amino]methyl}-4-(4-methylphenyl)-2-(2-methylpropyl)quinoline-6-carboxylate in 20 mL of methanol and 20 mL of THF was added 28 mL of 1 M aqueous sodium hydroxide solution, and the resulting mixture was stirred at 60 °C for 1 h. The reaction mixture was pured into water, acidified with 1 M hydrochloric acid and extracted with ethyl acetate. The extract was washed with brine, dried over anhydrous magnesium sulfate and concentrated in vacuo. Recrystallization of the residual solid from THF/diisopropyl ether afforded the title compound as a white powder: mp 276–277 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.05 (d, J = 6.6 Hz, 6H), 1.41 (s, 9H), 2.30–2.49 (m, 4H), 3.18 (br s, 2H), 4.35 (d, J = 5.1 Hz, 2H), 4.49 (br s, 1H),7.15 (d, J = 7.5 Hz, 2H), 7.33–7.38 (m, 3H), 8.19 (s, 1H), 8.29–8.41 (m, 2H). Anal. Calcd for $C_{27}H_{32}N_2O_4{\cdot}0.5H_2O$: C, 70.87; H, 7.27; N, 6.12. Found: C, 70.54; H, 7.00; N, 5.94.

5.1.31. 3-(Aminomethyl)-4-(4-methylphenyl)-2-(2-methylpropyl)quinoline-6-carboxamide dihydrochloride (17a)

5.1.31.1. Step A. A mixture of 0.22 g (0.5 mmol) of 3-{[(*tert*-butoxycarbonyl)amino]methyl}-4-(4-methylphenyl)-2-(2-methylpropyl)quinoline-6-carboxylic acid (**16**), 0.16 g (1.0 mmol) of HOBt ammonium salt and 0.20 g (1.0 mmol) of EDC in 10 mL of DMF was stirred at room temperature for 2 h. The reaction mixture was partitioned between ethyl acetate and water. The organic layer was washed with brine, dried over anhydrous magnesium sulfate and concentrated in vacuo. The residue was crystallized from ethyl acetate/diisopropyl ether to give 0.12 g (55%) of *tert*-butyl {[6-carbamoyl-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-3-yl]methyl} carbamate as a white powder: mp 206–208 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.02 (d, J = 6.3 Hz, 6H), 1.41 (s, 9H), 2.29–2.48 (m, 1H), 2.52 (s, 3H), 3.49 (d, J = 6.6 Hz, 2H), 4.37 (d, J = 5.1 Hz, 2H), 5.26 (br s, 1H), 5.84 (br s, 1H), 7.23 (d, J = 7.8 Hz, 2H), 7.39 (d, J = 7.8 Hz, 2H), 8.08 (br s, 2H), 8.55 (d, J = 9.0 Hz, 1H), 8.79 (d, J = 9.0 Hz, 1H).

5.1.31.2. Step B. A mixture of 90 mg (0.2 mmol) of *tert*-butyl {[6-carbamoyl-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-3-yl]methyl}carbamate and 5 mL of 4 M hydrogen chloride in 1,4-dioxane was stirred at room temperature for 1 h. The reaction mixture was concentrated in vacuo, and the residual solid was triturated with ethyl acetate/diisopropyl ether to afford 70 mg (88%) of the title compound as a white powder: mp 194–196 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.03 (d, J = 6.3 Hz, 6H), 2.32–2.49

(m, 4H), 3.17 (br s, 2H), 3.99 (d, J = 6.3 Hz, 2H), 7.36 (d, J = 7.8 Hz, 2H), 7.47 (d, J = 7.8 Hz, 2H), 7.59 (br s, 1H), 7.89 (s, 1H), 8.21–8.33 (m, 3H), 8.42 (br s, 3H). HRMS (ESI) calcd for $C_{22}H_{25}N_3O$ $(M+H)^+$ m/z 348.2070, found m/z 348.2044. HPLC purity: 98.61% (λ 220 nm), 98.20% (λ 254 nm).

5.1.32. 3-(Aminomethyl)-4-(4-methylphenyl)-2-(2-methylpropyl)quinoline-6-carboxylic acid dihydrochloride (17b)

A mixture of 0.22 g (0.5 mmol) of 3-{[(*tert*-butoxycarbonyl)amino]methyl}-4-(4-methylphenyl)-2-(2-methylpropyl)quinoline-6-carboxylic acid (**16**) and 5 mL of 4 M hydrogen chloride in 1,4-dioxane was stirred at room temperature for 1 h. Concentration in vacuo was followed by trituration with ethyl acetate to afford 0.20 g (95%) of the title compound as a white powder: mp 284–286 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.04 (d, J = 6.3 Hz, 6H), 2.28–2.44 (m, 1H), 2.49 (s, 3H), 3.27 (d, J = 6.9 Hz, 2H), 4.02 (d, J = 5.4 Hz, 2H), 7.41 (d, J = 8.0 Hz, 2H), 7.49 (d, J = 8.0 Hz, 2H), 8.00 (d, J = 1.5 Hz, 1H), 8.34–8.45 (m, 2H), 8.62 (br s, 3H). HRMS (ESI) calcd for $C_{22}H_{24}N_2O_2$ $(M+H)^+$ m/z 349.1911, found m/z 349.1876; calcd for $C_{22}H_{24}N_2O_2$ $(M-H)^-$ m/z 347.1765, found m/z 347.1771. Anal. Calcd for $C_{22}H_{24}N_2O_2{\cdot}2HCl{\cdot}0.45H_2O$: C, 61.53; H, 6.31; N, 6.52. Found: C, 61.89; H, 6.48; N, 6.14. HPLC purity: 99.74% (λ 220 nm), 99.57% (λ 254 nm).

5.1.33. *tert*-Butyl {[6-cyano-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-3-yl]methyl}carbamate (18)

To a mixture of 3.87 g (7.0 mmol) of 3-{[(*tert*-butoxycarbonyl)amino]methyl}-4-(4-methylphenyl)-2-(2-methylpropyl) quinolin-6-yl trifluoromethanesulfonate (**14**) and 0.49 g (4.2 mmol) of zinc cyanide in 40 mL of NMP under argon atmosphere was added 0.40 g (0.35 mmol) of tetrakis(triphenylphospine)palladium(0). The resulting mixture was stirred at 80 °C for 2 h. The reaction mixture was poured into water and extracted with ethyl acetate. The extract was washed with brine, dried over anhydrous magnesium sulfate and concentrated in vacuo. Purification by column chromatography (silica gel, eluting at a gradient of 0–25% ethyl acetate in hexanes) gave 2.18 g (73%) of the title compound as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.03 (d, J = 6.6 Hz, 6H), 1.42 (s, 9H), 2.34–2.47 (m, 1H), 2.50 (s, 3H), 3.00 (d, J = 7.2 Hz, 2H), 4.34 (br s, 3H), 7.11 (d, J = 7.8 Hz, 2H), 7.37 (d, J = 7.8 Hz, 2H), 7.72 (d, J = 1.8 Hz, 1H), 7.77 (dd, J = 8.7, 1.8 Hz, 1H), 8.12 (d, J = 8.7 Hz, 1H). Anal. Calcd for $C_{27}H_{31}N_3O_2{\cdot}0.1H_2O$: C, 75.18; H, 7.29; N, 9.74. Found: C, 74.96; H, 7.45; N, 9.53.

5.1.34. 1-[4-(4-Methylphenyl)-2-(2-methylpropyl)-6-(2*H*-tetrazol-5-yl)quinolin-3-yl]methanamine dihydrochloride (19a)

5.1.34.1. Step A. A mixture of 0.60 g (1.4 mmol) of *tert*-butyl {[6-cyano-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-3-yl]methyl}carbamate (**18**), 0.18 g (2.8 mmol) of sodium azide and 0.30 g (5.6 mmol) of ammonium chloride in 10 mL of DMSO was stirred at 70 °C for 48 h. The reaction mixture was partitioned between ethyl acetate and 0.1 M hydrochloric acid. The organic layer was washed with brine, dried over anhydrous magnesium sulfate and concentrated in vacuo. The residue was crystallized from ethyl acetate/hexanes to afford 0.38 g (57%) of *tert*-butyl {[4-(4-methylphenyl)-2-(2-methylpropyl)-6-(2*H*-tetrazol-5-yl)quinolin-3-yl]methyl}carbamate as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.00 (d, J = 6.4 Hz, 6H), 1.39 (s, 9H), 2.25–2.45 (m, 1H), 2.47 (s, 3H), 2.86 (d, J = 7.0 Hz, 2H), 4.07 (d, J = 4.3 Hz, 2H), 7.09 (t, J = 4.3 Hz, 1H), 7.35 (d, J = 8.1 Hz, 2H), 7.41 (d, J = 8.1 Hz, 2H), 8.05 (d, J = 1.8 Hz, 1H), 8.18 (d, J = 8.7 Hz, 1H), 8.28 (dd, J = 8.7, 1.8 Hz, 1H).

5.1.34.2. Step B. A mixture of 0.17 g (0.36 mmol) of *tert*-butyl {[4-(4-methylphenyl)-2-(2-methylpropyl)-6-(2*H*-tetrazol-5-yl)quinolin-3-yl]methyl}carbamate and 5 mL of 4 M hydrogen chloride in 1,4-dioxane was stirred at room temperature for 2 h. The reaction mixture was concentrated in vacuo. Recrystallization from methanol/diisopropyl ether gave 0.14 g (89%) of the title compound as a pale yellow powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.05 (d, J = 6.8 Hz, 6H), 2.30–2.50 (m, 1H), 2.49 (s, 3H), 3.08 (d, J = 7.0 Hz, 2H), 4.00 (d, J = 5.5 Hz, 2H), 7.38 (d, J = 7.8 Hz, 2H), 7.49 (d, J = 7.8 Hz, 2H), 8.08 (d, J = 1.5 Hz, 1H), 8.29 (d, J = 8.8 Hz, 1H), 8.33 (br s, 3H), 8.43 (dd, J = 8.8, 1.5 Hz, 1H). MS m/z 373 $(M+H)^+$; HRMS (ESI) calcd for $C_{22}H_{24}N_6$ $(M+H)^+$ m/z 373.2135, found m/z 373.2115. HPLC purity: 99.44% (λ 220 nm), 99.42% (λ 254 nm).

5.1.35. 3-[3-(Aminomethyl)-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl]-1,2,4-oxadiazol-5(4*H*)-one dihydrochloride (19b)

5.1.35.1. Step A. To a suspension of 3.0 g (7.0 mmol) of *tert*-butyl {[6-cyano-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-3-yl]methyl}carbamate (**18**) and 0.73 g (11 mmol) of hydroxylamine hydrochloride in 75 mL of ethanol was added 1.18 g (11 mmol) of potassium *tert*-butoxide. The resulting mixture was stirred at 70 °C for 6 h, cooled to room temperature and filtered. The filtrate was concentrated in vacuo, and the residue was partitioned between ethyl acetate and water. The organic layer was washed with brine, dried over anhydrous magnesium sulfate and concentrated in vacuo. The residual solid was triturated with dichloromethane/ethyl acetate to afford 2.38 g (74%) of *tert*-butyl {[6-(hydroxycarbamimidoyl)-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-3-yl]methyl}carbamate as an off-white powder: mp 224–225 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.03 (d, J = 6.6 Hz, 6H), 1.43 (s, 9H), 2.25–2.45 (m, 1H), 2.47 (s, 3H), 2.96 (d, J = 7.2 Hz, 2H), 4.29 (d, J = 4.7 Hz, 2H), 4.49 (t, J = 4.7 Hz, 1H), 4.75 (br s, 2H), 7.12 (d, J = 7.5 Hz, 2H), 7.33 (d, J = 7.7 Hz, 2H), 7.40–7.50 (m, 1H), 7.80–7.95 (m, 1H), 8.01 (d, J = 8.7 Hz, 1H). MS m/z 463 $(M+H)^+$.

5.1.35.2. Step B. To a solution of 0.25 g (0.54 mmol) of *tert*-butyl {[6-(hydroxycarbamimidoyl)-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-3-yl]methyl}carbamate in 10 mL of THF and 10 mL of ethyl acetate was added 0.26 g (1.62 mmol) of 1,1'-carbonyldiimidazole, and the resulting mixture was stirred at reflux for 3 h. The reaction mixture was poured into 50 mL of 0.1 M aqueous citric acid solution and extracted with 2:1 ethyl acetate/THF. The extract was washed with brine, dried over anhydrous magnesium sulfate and concentrated in vacuo. The residual solid was crystallized from ethyl acetate/hexanes to afford 0.24 g (90%) of *tert*-butyl {[4-(4-methylphenyl)-2-(2-methylpropyl)-6-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)quinolin-3-yl]methyl}carbamate as an white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.99 (d, J = 6.6 Hz, 6H), 1.38 (s, 9H), 2.30–2.50 (m, 1H), 2.45 (s, 3H), 2.85 (d, J = 6.8 Hz, 2H), 4.05 (d, J = 4.2 Hz, 2H), 7.08 (t, J = 4.2 Hz, 1H), 7.31 (d, J = 8.0 Hz, 2H), 7.38 (d, J = 8.0 Hz, 2H), 7.81 (d, J = 1.9 Hz, 1H), 8.03 (dd, J = 8.8, 1.9 Hz, 1H), 8.14 (d, J = 8.8 Hz, 1H), 13.21 (br s, 1H).

5.1.35.3. Step C. A mixture of 0.20 g (0.41 mmol) of *tert*-butyl {[4-(4-methylphenyl)-2-(2-methylpropyl)-6-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)quinolin-3-yl]methyl}carbamate and 5 mL of 4 M hydrogen chloride in 1,4-dioxane was stirred at room temperature for 3 h. Concentration in vivo was followed by recrystallization from methanol/diisopropyl ether to afford 0.14 g (74%) of the title compound as pale yellow crystals. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.04 (d, J = 6.6 Hz, 6H), 2.30–2.45 (m, 1H), 2.49 (s, 3H), 3.08 (d, J = 7.2 Hz, 2H), 3.97 (d, J = 5.3 Hz, 2H), 7.34 (d, J = 7.9 Hz, 2H), 7.46 (d, J = 7.9 Hz, 2H), 7.83 (d, J = 1.8 Hz, 1H), 8.16 (dd, J = 8.9, 1.9 Hz, 1H), 8.25 (d, J = 8.9 Hz, 1H), 8.36 (br s, 3H), 13.33 (br s, 1H). HRMS (ESI) calcd for $C_{23}H_{24}N_4O_2$ $(M+H)^+$ m/z 389.1972, found m/z 389.1956. HPLC purity: 99.96% (λ 220 nm), 99.92% (λ 254 nm).

5.1.36. 4-(4-Methylphenyl)-2-(2-methylpropyl)-6-(3-oxopiperazin-1-yl)quinoline-3-carbonitrile (20)

To 30 mL of 1,4-dioxane under nitrogen atmosphere were added 0.12 g (0.19 mmol) of *rac*-BINAP and 0.014 g (0.063 mmol) of palladium acetate. After being stirred at 40 °C for 30 min, 0.50 g (1.25 mmol) of 6-bromo-4-(4-methylphenyl)-2-(2-methylpropyl)quinoline-3-carbonitrile (**7b**), 0.25 g (2.5 mmol) of piperazin-2-one and 0.57 g (1.75 mmol) of cesium carbonate were added. The resulting mixture was stirred at 80 °C for 24 h. The reaction mixture was cooled to room temperature, diluted with ethyl acetate and filtered through Celite. The filtrate was washed sequentially with water and brine, dried over anhydrous magnesium sulfate and concentrated in vacuo. Purification by column chromatography (silica gel, eluting at a gradient of 0–5% methanol in ethyl acetate) afforded 0.35 g (71%) of the title compound as a yellow powder: mp 237–238 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.04 (d, J = 6.6 Hz, 6H), 2.25–2.45 (m, 1H), 2.50 (s, 3H), 3.07 (d, J = 7.4 Hz, 2H), 3.45–3.60 (m, 4H), 3.81 (s, 2H), 6.22 (s, 1H), 6.83 (d, J = 2.8 Hz, 1H), 7.30–7.45 (m, 4H), 7.53 (dd, J = 9.4, 2.7 Hz, 1H), 8.04 (d, J = 9.4 Hz, 1H). MS m/z 399 $(M+H)^+$.

5.1.37. 4-[3-(Aminomethyl)-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl]piperazin-2-one (21a)

The title compound (0.18 g) was prepared from 0.32 g (0.80 mmol) of 4-(4-methylphenyl)-2-(2-methylpropyl)-6-(3-oxopiperazin-1-yl)quinoline-3-carbonitrile (**20**) using a procedure similar to that described as step B for the synthesis of compound **9b** as a pale yellow powder: mp 155–157 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.03 (d, J = 6.6 Hz, 6H), 1.55 (br s, 2H), 2.25–2.40 (m, 1H), 2.49 (s, 3H), 2.97 (d, J = 7.2 Hz, 2H), 3.35–3.45 (m, 2H), 3.45–3.55 (m, 2H), 3.72 (s, 2H), 3.75 (s, 2H), 6.21 (d, J = 10.9 Hz, 1H), 6.50 (d, J = 2.8 Hz, 1H), 7.15 (d, J = 7.8 Hz, 2H), 7.33 (d, J = 7.8 Hz, 2H), 7.37 (dd, J = 9.2, 2.8 Hz, 1H), 7.99 (d, J = 9.2 Hz, 1H). MS m/z 403 $(M+H)^+$; HRMS (ESI) calcd for $C_{25}H_{30}N_4O$ $(M+H)^+$ m/z 403.2492, found m/z 403.2478. HPLC purity: 97.72% (λ 220 nm), 95.11% (λ 254 nm).

5.1.38. *tert*-Butyl {[6-({2-[(*tert*-butoxycarbonyl)amino]ethyl}amino)-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-3-yl]methyl}carbamate (22)

5.1.38.1. Step A. 6-[(2-Aminoethyl)amino]-4-(4-methylphenyl)-2-(2-methylpropyl)quinoline-3-carbonitrile (2.2 g) was prepared using a procedure similar to that described for the synthesis of the synthesis of compound **8a** from 1.0 g (2.6 mmol) of 6-bromo-4-(4-methylphenyl)-2-(2-methylpropyl)quinoline-3-carbonitrile (**7b**) instead of compound **7a** and 0.44 mL (6.6 mmol) of ethylenediamine instead of *tert*-butanol as a crude yellow syrup, which was used directly in the next step.

5.1.38.2. Step B. The obtained crude syrup was dissolved in 100 mL of THF, and then 0.70 g (3.2 mmol) of di-*tert*-butyl dicarbonate was added. The resulting mixture was stirred at room temperature for 1 h and concentrated in vacuo. Purification by column chromatography (silica gel, eluting at a gradient of 20–40% ethyl acetate in hexanes) afforded 0.85 g (70%) of *tert*-butyl (2-{[3-cyano-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl]amino}ethyl)carbamate as a yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.03 (d, J = 6.6 Hz, 6H), 1.43 (s, 9H), 2.25–2.40 (m, 1H), 2.48 (s, 3H), 3.03 (d, J = 7.4 Hz, 2H), 3.15 (q, J = 5.5 Hz, 2H), 3.36 (q, J = 5.5 Hz, 2H), 4.57 (br, 1H), 4.74 (br, 1H), 6.49 (d, J = 2.5 Hz, 1H), 7.18 (dd, J = 9.1, 2.5 Hz, 1H), 7.30–7.40 (m, 4H), 7.89 (d, J = 9.1 Hz, 1H). MS m/z 459 $(M+H)^+$.

5.1.38.3. Step C. A mixture of 0.80 g (1.7 mmol) of *tert*-butyl (2-{[3-cyano-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl]amino}ethyl)carbamate, ca. 2 g of Raney nickel, 5 mL of 25% aqueous ammonia, 50 mL of methanol and 10 mL of THF was stirred under hydrogen atmosphere at 0.5 MPa for 6 h at room temperature. The reaction mixture was filtered, and the filtrate was concentrated in vacuo. The residue was dissolved in 50 mL of THF, and then 0.57 g (2.6 mmol) of di-*tert*-butyl dicarbonate was added. The resulting mixture was stirred at room temperature for 30 min and concentrated in vacuo. Purification by column chromatography (silica gel, eluting at a gradient of 20–50% ethyl acetate in hexanes) afforded 0.75 g (77%) of the title compound as a yellow powder: mp 57 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.01 (d, J = 6.6 Hz, 6H), 1.41 (s, 9H), 1.43 (s, 9H), 2.20–2.40 (m, 1H), 2.46 (s, 3H), 2.89 (d, J = 7.2 Hz, 2H), 3.10 (q, J = 5.3 Hz, 2H), 3.31 (q, J = 5.3 Hz, 2H), 4.20–4.25 (m, 3H), 4.31 (t, J = 3.6 Hz, 1H), 4.72 (br s, 1H), 6.17 (d, J = 2.5 Hz, 1H), 7.05 (dd, J = 9.0, 2.5 Hz, 1H), 7.11 (d, J = 7.8 Hz, 2H), 7.30 (d, J = 7.8 Hz, 2H), 7.85 (d, J = 9.0 Hz, 1H). MS m/z 563 $(M+H)^+$.

5.1.39. Ethyl ({2-[(*tert*-butoxycarbonyl)amino]ethyl}[3-{[(*tert*-butoxycarbonyl)amino]methyl}-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl]amino)(oxo)acetate (23)

To a vigorously stirred mixture of 0.50 g (0.89 mmol) of *tert*-butyl {[6-({2-[(*tert*-butoxycarbonyl)amino]ethyl}amino)-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-3-yl]methyl}carbamate (**22**), 50 mL of ethyl acetate and 50 mL of saturated aqueous sodium bicarbonate solution was added 0.30 g (2.2 mmol) of ethyl chloro(oxo)acetate at room temperature. After being stirred vigorously for 30 min, the organic layer was separated, washed with brine, dried over anhydrous magnesium sulfate and concentrated in vacuo. Purification by column chromatography (silica gel, eluting at a gradient of 20–40% ethyl acetate in hexanes) afforded 0.53 g (90%) of the title compound as a white powder: mp 110–114 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.93 (t, J = 7.1 Hz, 3H), 1.03 (d, J = 6.6 Hz, 6H), 1.33 (s, 9H), 1.42 (s, 9H), 2.30–2.45 (m, 1H), 2.48 (s, 3H), 2.98 (d, J = 7.4 Hz, 2H), 3.31 (q, J = 6.0 Hz, 2H), 3.85–3.90 (m, 2H), 3.90 (q, J = 7.1 Hz, 2H), 4.30 (br s, 3H), 4.75 (t, J = 4.7 Hz, 1H), 7.08 (d, J = 7.9 Hz, 2H), 7.14 (d, J = 2.1 Hz, 1H), 7.34 (d, J = 7.9 Hz, 2H), 7.56 (dd, J = 8.9, 2.1 Hz, 1H), 8.09 (d, J = 8.9 Hz, 1H). MS m/z 663 $(M+H)^+$.

5.1.40. 1-[3-(Aminomethyl)-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl]piperazine-2,3-dione (21b)

A mixture of 0.50 g (0.75 mmol) of ethyl ({2-[(*tert*-butoxycarbonyl)amino]ethyl}[3-{[(*tert*-butoxycarbonyl)amino]methyl}-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl]amino)(oxo)acetate (**23**) and 3 mL of TFA was stirred at room temperature for 30 min. The reaction mixture was concentrated in vacuo, and the residue was partitioned between ethyl acetate and 10% aqueous potassium carbonate solution. The organic layer was dried over anhydrous potassium carbonate, and concentrated in vacuo. Purification by column chromatography (silica gel, eluting at a gradient of 0–20% methanol in ethyl acetate) afforded 0.21 g (65%) of the title compound as a pale yellow powder: mp 229–232 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.02 (d, J = 6.6 Hz, 6H), 1.66 (br s, 2H), 2.30–2.45 (m, 1H), 2.46 (s, 3H), 3.01 (d, J = 7.2 Hz, 2H), 3.55–3.65 (m, 2H), 3.80 (s, 2H), 3.85–3.90 (m, 2H), 7.16 (d, J = 8.0 Hz, 2H), 7.18 (d, J = 2.3 Hz, 1H), 7.32 (d, J = 8.0 Hz, 2H), 7.57 (dd, J = 9.0, 2.3 Hz, 1H), 8.06 (d, J = 9.0 Hz, 1H), 8.08 (br s, 1H). MS m/z 417 $(M+H)^+$; HRMS (ESI) calcd for $C_{25}H_{28}N_4O_2$ $(M+H)^+$ m/z 417.2285, found m/z 417.2271. HPLC purity: 99.67% (λ 220 nm), 99.42% (λ 254 nm).

5.1.41. *tert*-Butyl *N*-[3-{[(*tert*-butoxycarbonyl)amino]methyl}-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl]glycinate (24)

5.1.41.1. Step A. *tert*-Butyl *N*-[3-cyano-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl]glycinate (2.0 g) was prepared using a procedure similar to that described for the synthesis of compound **8a** from 2.0 g (5.3 mmol) of 6-bromo-4-(4-methyl-

phenyl)-2-(2-methylpropyl)quinoline-3-carbonitrile (**7b**) instead of compound **7a** and 1.52 g (11.6 mmol) of *tert*-butyl glycinate intead of *tert*-butanol as yellow crystals: mp 128–129 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.03 (d, J = 6.6 Hz, 6H), 1.44 (s, 9H), 2.25–2.40 (m, 1H), 2.49 (s, 3H), 3.04 (d, J = 7.2 Hz, 2H), 3.71 (d, J = 5.2 Hz, 2H), 4.81 (t, J = 5.2 Hz, 1H), 6.45 (d, J = 2.6 Hz, 1H), 7.22 (dd, J = 9.0, 2.6 Hz, 1H), 7.30–7.40 (m, 4H), 7.92 (d, J = 9.0 Hz, 1H). MS m/z 430 $(M+H)^+$.

5.1.41.2. Step B. The title compound (0.96 g) was prepared from 1.4 g (3.3 mmol) of *tert*-butyl *N*-[3-cyano-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl]glycinate using a procedure similar to that described as step C for the synthesis of compound **22** as pale yellow crystals: mp 181–185 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.01 (d, J = 6.6 Hz, 6H), 1.41 (s, 9H), 1.44 (s, 9H), 2.20–2.40 (m, 1H), 2.48 (s, 3H), 2.89 (d, J = 7.4 Hz, 2H), 3.65 (d, J = 5.3 Hz, 2H), 4.22 (d, J = 4.9 Hz, 2H), 4.30 (br, 1H), 4.42 (t, J = 4.9 Hz, 1H), 6.14 (d, J = 2.5 Hz, 1H), 7.08 (dd, J = 9.0, 2.5 Hz, 1H), 7.11 (d, J = 8.0 Hz, 2H), 7.31 (d, J = 8.0 Hz, 2H), 7.87 (d, J = 9.0 Hz, 1H). MS m/z 534 $(M+H)^+$.

5.1.42. *tert*-Butyl *N*-[(benzyloxy)carbonyl]glycyl-*N*-[3-{[(*tert*-butoxycarbonyl)amino]methyl}-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl]glycinate (25)

To an ice-cooled mixture of 0.78 g (3.75 mmol) of *N*-[(benzyloxy)carbonyl]glycine, 0.1 mL of DMF and 20 mL of THF was added 0.33 mL (3.7 mmol) of oxalyl chloride in a dropwise manner. The resulting mixture was stirred at 0 °C for 1 h, and added dropwise to a mixture of 0.40 g (0.75 mmol) of *tert*-butyl *N*-[3-{[(*tert*-butoxycarbonyl)amino]methyl}-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl]glycinate (**24**) and 0.3 mL of pyridine in 100 mL of THF at room temperature. After being stirred at room temperature for 1 h, 10 mg (0.08 mmol) of DMAP was added, and the resulting mixture was stirred at room temperature for 60 h. The reaction mixture was partitioned between ethyl acetate and water. The organic layer was washed sequentially with water, saturated aqueous sodium bicarbonate solution and brine, dried over anhydrous magnesium sulfate and concentrated in vacuo. Purification by column chromatography (silica gel, eluting at a gradient of 25–50% ethyl acetate in hexanes) afforded 0.47 g (87%) of the title compound as a white powder: mp 81–83 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.04 (d, J = 6.8 Hz, 6H), 1.37 (s, 9H), 1.42 (s, 9H), 2.25–2.45 (m, 1H), 2.46 (s, 3H), 3.00 (d, J = 7.4 Hz, 2H), 3.69 (d, J = 4.3 Hz, 2H), 4.22 (s, 2H), 4.32 (br, 3H), 5.04 (s, 2H), 5.60 (t, J = 4.3 Hz, 1H), 7.11 (d, J = 7.9 Hz, 2H), 7.25–7.40 (m, 8H), 7.60 (dd, J = 8.9, 2.1 Hz, 1H), 8.11 (d, J = 8.9 Hz, 1H). MS m/z 725 $(M+H)^+$.

5.1.43. 1-[3-(Aminomethyl)-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl]piperazine-2,5-dione dihydrochloride (1)

5.1.43.1. Step A. A mixture of 0.45 g (0.62 mmol) of *tert*-butyl *N*-[(benzyloxy)carbonyl]glycyl-*N*-[3-{[(*tert*-butoxycarbonyl)amino]methyl}-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-6-yl]glycinate (**25**) and 40 mg of 10% Pd/C in 100 mL of ethanol was stirred under atmospheric hydrogen at room temperature for 24 h. The reaction mixture was filtered through Celite, and the filtrate was concentrated in vacuo. The residue was dissolved in 10 mL of ethanol, and the resulting solution was stirred at reflux for 17 h. The reaction mixture was concentrated in vacuo, and the residue was purified by column chromatography (silica gel, eluting at a gradient of 0–10% methanol in ethyl acetate) followed by crystallization from ethyl acetate to afford 0.16 g (44%) of *tert*-butyl {[6-(2,5-dioxopiperazin-1-yl)-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-3-yl]methyl}carbamate as pale yellow crystals: mp 128–129 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.02 (d, J = 6.6 Hz, 6H), 1.42 (s, 9H), 2.25–2.45 (m, 1H), 2.48 (s, 3H), 2.98 (d, J = 7.2 Hz, 2H), 4.16 (s, 2H), 4.28 (s, 2H), 4.30 (br s, 3H), 6.13 (s, 1H), 7.12 (d, J = 7.8 Hz, 2H), 7.14 (d, J = 2.3 Hz, 1H), 7.34 (d, J = 7.8 Hz, 2H), 7.62 (dd, J = 9.0, 2.3 Hz, 1H), 8.11 (d, J = 9.0 Hz, 1H). MS m/z 517 $(M+H)^+$.

5.1.43.2. Step B. A mixture of 0.14 g (0.27 mmol) of *tert*-butyl {[6-(2,5-dioxopiperazin-1-yl)-4-(4-methylphenyl)-2-(2-methylpropyl)quinolin-3-yl]methyl}carbamate and 5 mL of 4 M hydrogen chloride in 1,4-dioxane was stirred at room temperature for 1 h. The reaction mixture was concentrated in vacuo. Recrystallization from aqueous ethanol gave 0.11 g (83%) of the title compound as a pale yellow powder: mp 250 °C (decomp). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.03 (d, J = 6.6 Hz, 6H), 2.25–2.40 (m, 1H), 2.46 (s, 3H), 3.13 (d, J = 7.0 Hz, 2H), 3.92 (s, 2H), 3.98 (d, J = 5.5 Hz, 2H), 4.21 (s, 2H), 7.23 (d, J = 1.9 Hz, 1H), 7.34 (d, J = 7.9 Hz, 2H), 7.45 (d, J = 7.9 Hz, 2H), 7.88 (d, J = 8.8 Hz, 1H), 8.24 (dd, J = 8.8, 1.9 Hz, 1H), 8.30 (br s, 1H), 8.35 (br s, 3H). MS m/z 417 $(M+H)^+$; HRMS (ESI) calcd for $C_{25}H_{28}N_4O_2$ $(M+H)^+$ m/z 417.2285, found m/z 417.2272. Anal. Calcd for $C_{25}H_{28}N_4O_2 \cdot 2HCl$: C, 61.35; H, 6.18; N, 11.45; Cl, 14.49. Found: C, 61.11; H, 6.22; N, 11.33; Cl, 14.44. HPLC purity: 99.90% (λ 220 nm), 99.93% (λ 254 nm).

5.2. Theoretical calculation

Docking studies were performed using GOLD software version 3.0 (Cambridge Crystallographic Data Centre, www.ccdc.cam.ac.uk), based on the co-crystal structure model of DPP-4 in complex with compound **2**. After the automatic docking into DPP-4 using GOLD, the conformations of the substituents in the docked compounds were aligned to the corresponding moiety of compound **2** and then energy-minimized at the MMFF94s force field using MOE 2005.06 (Chemical Computing Group Inc., www.chemcomp.com).

5.3. Biology

5.3.1. 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 M Tris–HCl pH 7.5, 0.25% bovine serum albumin, 0.125% CHAPS) and mixed with 20 μL of human DPP-4 fraction. After the mixture was incubated at room temperature for 15 min, the reaction was initiated by adding 100 μL of 1 mM 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.[43] Compounds (1 μL) dissolved in DMSO was mixed with 29 μL of distilled water, 10 μL of 1 M 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 mM 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 M 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 mM of Gly-Pro-pNA·Tos for DPP-8 or 4 mM of Gly-Pro-pNA·Tos for DPP-9 and run at 37 °C for 90 min.

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

5.3.2. Effects of single administration of compound 1 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 five groups based on plasma glucose levels and body weights (6 rats in each group). Plasma glucose 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 **1** at doses of 0.03, 0.1, 0.3, and 1 mg/kg as free base form of compound **1**. One hour later, all animals were 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 **1**). 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). Plasma glucose 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).

5.4. Determination of ADME and pharmacokinetic profiles

5.4.1. Solubility

Compound **1** was added to the second fluid for the disintegration test regulated in the Japanese Pharmacopoeia 15th edition (pH 6.8). After incubation, precipitates were separated by filtration. The thermodynamic solubility was determined by HPLC analysis of the filtrate.

5.4.2. Microsomal stability

In vitro oxidative metabolic studies of compound **1** were carried out using hepatic microsomes obtained from rats, dogs, monkeys, and humans. The incubation mixtures were prepared under ice-cold conditions by adding the microsomes (0.2 mg protein/mL), and the solution of compound **1** (1 μM) 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 60 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 the solution of compound **1**. After the samples were mixed and centrifuged, the supernatant fractions were subjected to high performance liquid chromatography with UV detector.

5.4.3. Pharmacokinetic profile in rats and dogs

Compound **1** 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 M ammonium acetate and centrifuged again. The compound concentrations in the supernatant were measured by LC/MS/MS.

Acknowledgments

The authors thank Drs. Yu Momose, Junichi Sakamoto, Hiroshi Nara, Yuji Ishihara, Kaneyoshi Kato, Shigenori Ohkawa, Takashi Sohda and Akio Miyake for helpful discussions; Dr. Yukihiro Ikeda for measuring solubility; and Dr. Akihiko Sumita for measuring metabolic stabilities. We also thank ALS, which is supported by the Director, Office of Science, Office of Basic Energy Sciences, Materials Sciences Division of the U.S. Department of Energy under contract DE-AC03-76SF00098 at Lawrence Berkeley National Laboratory.

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