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Discovery of 3*H*-imidazo[4,5-*c*]quinolin-4(5*H*)-ones as potent and selective dipeptidyl peptidase IV (DPP-4) inhibitors: Use of a carboxylate prodrug to improve bioavailability

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ABSTRACT

We have previously reported a novel series of 3*H*-imidazo[4,5-*c*]quinolin-4(5*H*)-ones with potent dipeptidyl peptidase IV (DPP-4) inhibitory activity. However, these compounds showed poor oral absorption. We attempted in this study esterification of the carboxylic acid moiety to improve the compounds **1**–**4** plasma concentrations. Our efforts yielded **10h** with a 5-methyl-2-oxo-1,3-dioxol-4-yl methyl ester as an S9/plasma-cleavable functionality. Compound **10h** showed significantly high oral absorption and potent DPP-4 inhibition in vivo and decreased Zucker fatty rats glucose levels in the oral glucose tolerance test. Optimization of the ester moiety revealed that rapid conversion to the carboxyl form in both liver S9 fractions and serum was important for prodrugs not to be detected in the plasma after oral administration. In particular, lability in the serum was found to be an important characteristic. Through our investigation, we were able to develop a novel efficient synthetic method for construction of 3*H*-imidazo[4,5-*c*]quinolin-4(5*H*)-ones using intramolecular radical cyclization.

1. Introduction

Diabetes is a major metabolic disorder that affects approximately 382 million people worldwide (2013 statistics), and this number is predicted to increase to 592 million by 2035.[1] Forty-two percent of people treated for type 2 diabetes mellitus (T2DM), which is the most common form of diabetes, do not reach their blood glucose goals, thereby risking organ damage, blindness and even death.[2] Currently used antidiabetic agents, such as peroxisome proliferator-activated receptor γ (PPARγ) agonists, sulphonylurea derivatives, biguanides, and α-glucosidase inhibitors can produce beneficial effects in patients with T2DM by effectively enhancing insulin secretion and/or decreasing glucose absorption. However, these agents are often associated with undesired side effects, including hypoglycemia, weight gain, gastrointestinal disorders, and lactic acidosis. There remain therefore important unmet medical needs in the treatment of T2DM.

Glucagon-like peptide 1 (GLP-1) is an incretin hormone secreted from the intestine in response to glucose absorption after meal ingestion. GLP-1 stimulates insulin biosynthesis and thus contributes to maintenance of postprandial glycemic control.[3,4] However, active GLP-1 (7-36 amide) is rapidly degraded by dipeptidyl peptidase IV (DPP-4), a serine protease, to give inactive GLP-1 (9-36 amide) in vivo, which limits its ability to normalize blood glucose levels.[5–8] Thus, inhibition of DPP-4 would help maintain appropriate levels of active GLP-1 in plasma, which in turn would stimulate insulin secretion. In fact, development of DPP-4 inhibitors is emerging as a promising approach for the treatment of patients with T2DM with low risk of hypoglycemia.[9,10] Clinical proof-of-concept studies have shown that DPP-4 inhibitors are more efficient and safer than conventional antidiabetic agents.[11,12] Based on these findings, a number of DPP-4 inhibitors, including sitagliptin,[13] vildagliptin,[14] saxagliptin,[15] alogliptin,[16] and linagliptin (Fig. 1)[17] have already been approved for the treatment of T2DM.

Recently, we have reported a novel series of 3*H*-imidazo[4,5-*c*]quinolin-4(5*H*)-ones as DPP-4 inhibitors (Fig. 1).[18,19] In this series, compounds **1**–**4** with a carboxyl group at the 8- or 7-position showed more potent DPP-4 inhibitory activity in vitro than marketed DPP-4 inhibitors with excellent selectivity against various DPP-4 homologues (Table 1). However, these compounds had poor

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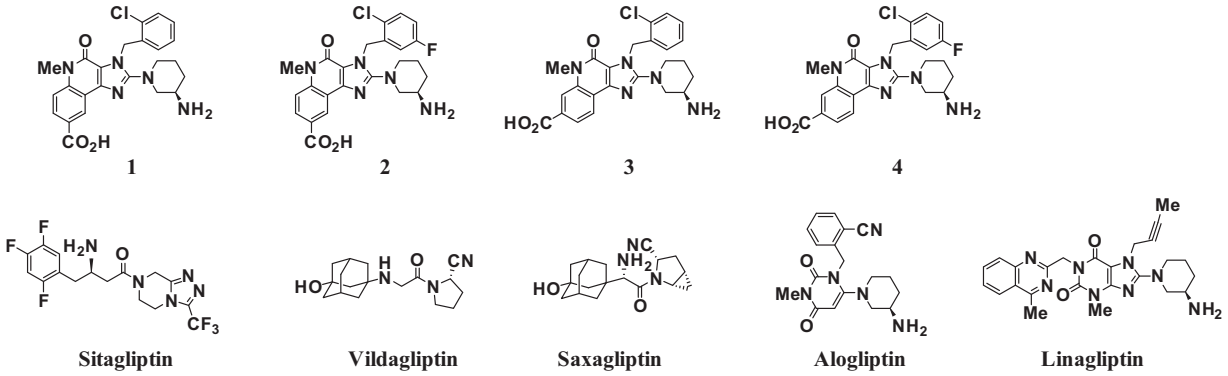


Figure 1. 3*H*-Imidazo [4,5-*c*]quinolin-4(5*H*)-ones (**1**–**4**) and representative marketed DPP-4 inhibitors.

oral absorption because of low membrane permeability caused by the formation of a zwitterion between the carboxyl group and the amino group.[20] In fact, a pharmacokinetic (PK) study in rats showed that compounds **1** and **4** had bioavailability (BA) values of <0.1% and 2.7%, respectively. Therefore, we had to consider other strategies to improve these compounds bioavailability. In this study, we describe a rational ester introduction to **1**–**4** to construct prodrugs that would allow improvement of these compounds plasma concentrations with focus on in vitro metabolic characterization. We also present a novel efficient synthetic method for the preparation of 3*H*-imidazo[4,5-*c*]quinolin-4(5*H*)-ones using intramolecular radical cyclization. Our investigation led to **10h** as an orally active DPP-4 inhibitor with a 5-methyl-2-oxo-1,3-dioxol-4-yl methyl (Dox) ester.[21,22]

2. Chemistry

The prodrug esters **10a**–**10l** were prepared as shown in Scheme 1. Compounds **9a**–**9l** were synthesized by alkylation with various alkyl halides using the previously reported intermediates **5**, **6**, **7**, or **8**.[19] Removal of the *tert*-butoxy carbonyl group yielded the prodrugs **10a**–**10l**. Compound **9a** was thought to be a key intermediate because the orally active DPP-4 inhibitor **10h** was synthesized via **9a**. Although preparation of **9a** from diphenyl cyanocarbonimidate has already been reported,[18,19] the proposed method not only requires 8 steps, but provides an unsatisfactory total yield of 19.9%. An efficient synthetic route to **9a** was therefore necessary. First we designed a new synthetic route for preparation of 3*H*-imidazo[4,5-*c*]quinolin-4(5*H*)-ones from **12** using intramolecular radical cyclization via the diazonium salt **11** (Scheme 2).[23–25] The methyl 4-methylaminobenzoate was converted to **13** in quantitative yield by alkylation with chloroacetyl chloride (Scheme 3). Replacement of one phenoxy group in the diphenyl cyanocarbonimidate with (*R*)-*tert*-butoxycarbonylaminopiperidine, followed by treatment with 2-chlorobenzylamine at 80 °C provided **14** in high yield. N-Alkylation of **14** with **13** at 40 °C afforded a mixture of **15** and **12**, and subsequent heating at 60 °C to promote cyclization gave **12** (one pot) in 75% yield. Next, we attempted intramolecular radical cyclization of **12** (Scheme 4).

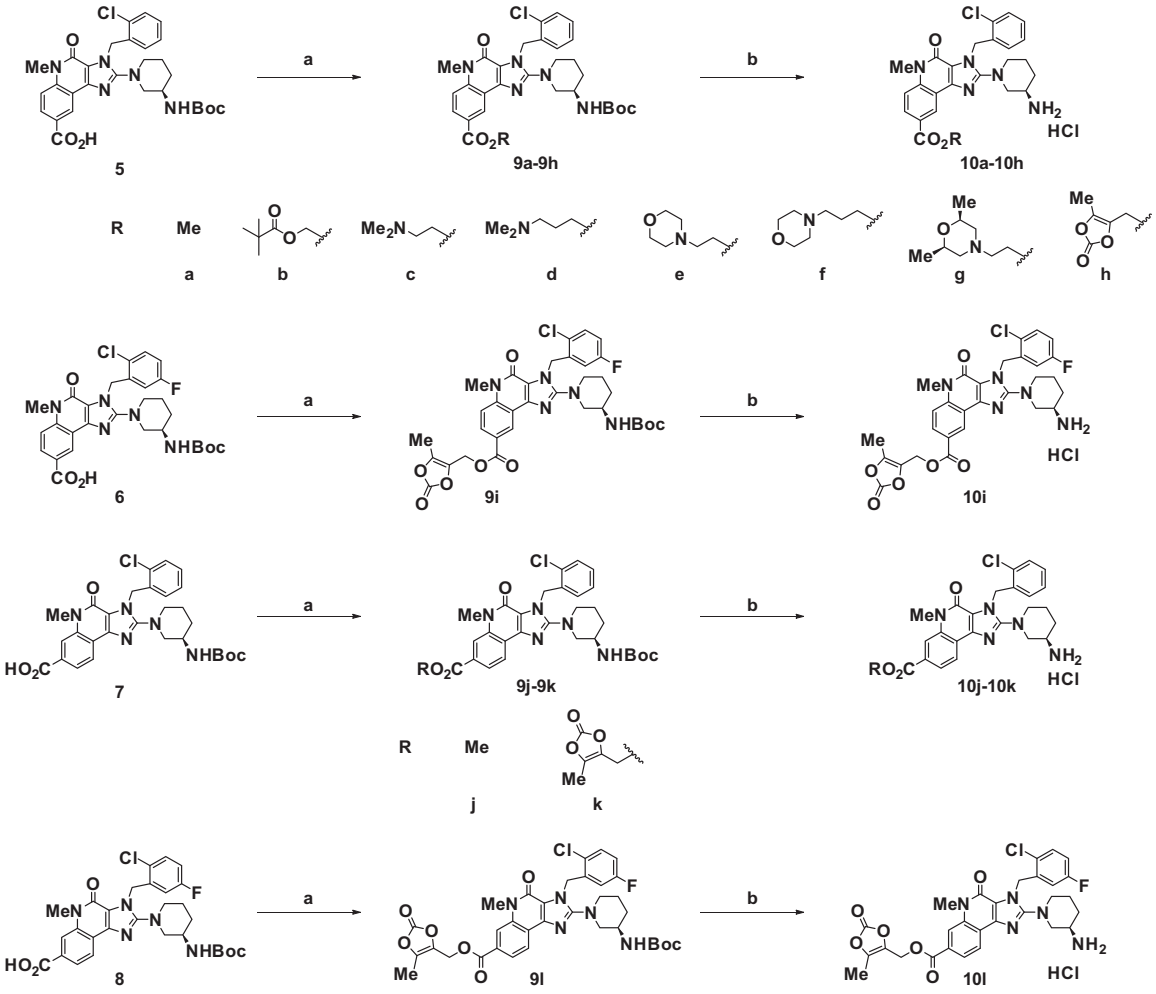
Table 1
Compounds **1**–**4** inhibitory activity for DPP-4 and DPP-4 homologues (IC_{50} nM)

	DPP-4	FAPα	DPP-2	DPP-8	DPP-9
1	5.8	>100,000	>100,000	>100,000	>100,000
2	4.8	>100,000	>100,000	>100,000	>100,000
3	1.6	20,400	>10,000	>100,000	>100,000
4	0.48	30,600	>10,000	>100,000	>100,000

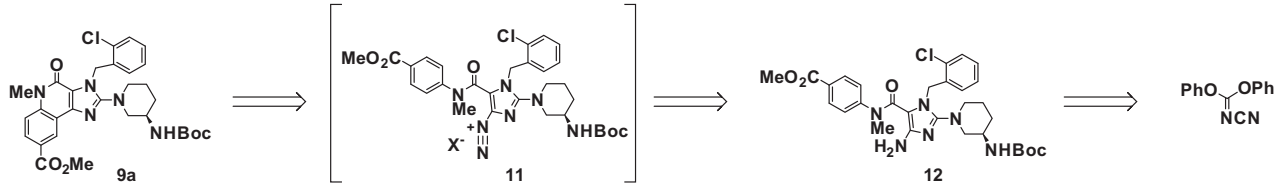
All of the substrate **12** was consumed following treatment with sodium nitrite in AcOH at room temperature to give the diazonium salt **11**. Compound **9a** was obtained in 51% yield by adding Cu to the reaction mixture (entry 1). When CuCl (I) was used as an additive instead of Cu, no difference in the yield of **9a** was seen, although reaction rate was slower (entry 2). The additive $CuCl_2$ (II) gave lower yield than Cu or CuCl (I) (entry 3). No change in the yield of **9a** was observed at 80 °C using sodium nitrite and Cu (entry 4). Using isoamyl nitrile as a diazotization reagent slightly reduced the yield of **9a** compared to sodium nitrite in AcOH (entry 5). As for solvent effect, no diazonium salt **11** was detected in toluene, and **9a** was produced at room temperature without addition of Cu (entry 6). While AcOH helped stabilize the diazonium salt **11**, toluene did not have such effect. It was thus believed that thermal homolysis of the diazonium salt intermediate[26] in toluene led to generation of a radical and subsequently cyclization reaction to give **9a**. Furthermore, reaction in toluene resulted in fewer side-products compared to AcOH. We next examined the effect of temperature on the reaction yield using toluene as a solvent. Carrying out the reaction at 80 °C significantly improved the yield of **9a** (entry 7). Using isoamyl nitrite, we sought to optimize the reaction solvent at 80 °C without use of additive. Although carrying out the reaction in THF gave **9a** in a yield similar to that obtained in toluene, the use of xylene, pyridine or acetonitrile gave unsatisfactory result (entries 8–11). Among the test-solvents, 1,4-dioxane provided the best yield of **9a** (entry 12). Using intramolecular radical cyclization, we could develop a novel and efficient 4 steps synthetic method for construction of 3*H*-imidazo[4,5-*c*]quinolin-4(5*H*)-ones, including the key intermediate **9a** (52% total yield).

3. Results and discussion

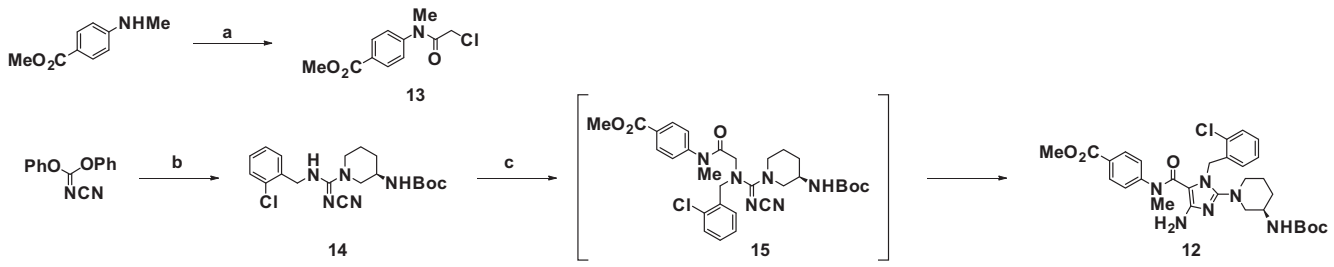
Although compound **1**–**4** had excellent clearance in human and rat microsomes (<0.01/<0.01 mL/min/mg protein, respectively), they showed poor oral absorption. To overcome this problem, we considered the use of prodrugs. Initially, we used **1** and **3**, both of which have a carboxyl group at a different position, as representative active metabolites and evaluated the metabolic properties of the methyl esters **10a** and **10j** in vitro (Table 2). Compound **10a** was rapidly metabolized in the rat liver S9 fractions and showed moderate serum stability. On the other hand, **10j** was stable in the rat liver S9 fractions, but labile in serum. In each case, only the active metabolite **1** or **3** was generated, and no other byproducts were obtained. Although **10a** and **10j** had different metabolic properties, they were expected not to remain in the plasma after oral administration in rats. While **1** and **3** membrane permeability, as determined by the parallel artificial membrane permeability assay (PAMPA; pH 5.0/7.4, 10^{-6} cm/s), was poor (<0.1/<0.1,



Scheme 1. Preparation of the prodrugs **10a**–**10l**. Reagents and conditions: (a) R-halide, K_2CO_3, DMF, 34%-quant.; (b) HCl, 1,4-dioxane, 70%-quant.

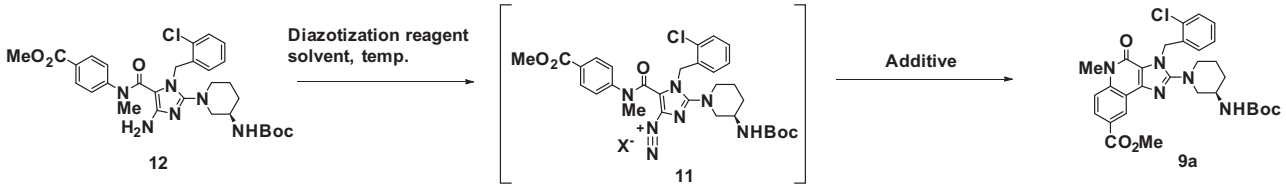


Scheme 2. New synthetic route for the preparation of 3*H*-imidazo[4,5-*c*]quinolin-4(5*H*)-ones by intramolecular radical cyclization.



Scheme 3. Synthesis of **12**. Reagents and conditions: (a) chloroacetyl chloride (1.2 equiv), pyridine (1.0 equiv), THF, 0 °C, quant.; (b) (*R*)-3-*tert*-butoxycarbonylaminopiperidine (1.0 equiv), ^{i}PrOH then 2-chlorobenzylamine (1.5 equiv), 80 °C, 95%; (c) **13** (1.1 equiv), Cs_2CO_3 (2.0 equiv), MeCN, 40 °C then 60 °C, 75%.

respectively), that of **10a** and **10j** was markedly increased by esterification, exhibiting high PAMPA values of 34.1/47.4 and 36.2/41.4, respectively. Next, the oral absorption and plasma concentration of both compounds **1** and **3** and their respective prodrugs **10a** and **10j** were evaluated in rats (Fig. 2). The BA value for **1** after oral administration of **10a** was only 2.4%. Contrary to our expectation, much



Entry	Diazotization reagent (equiv.)	Additive (equiv.)	Solvent	Temp.	Time (h)	Yield (%)
1	$NaNO_2$ (3.0)	Cu (1.0)	AcOH	rt	5	51
2	$NaNO_2$ (3.0)	CuCl (1.0)	AcOH	rt	26	43
3	$NaNO_2$ (3.0)	$CuCl_2$ (1.0)	AcOH	rt	26	29
4	$NaNO_2$ (3.0)	Cu (1.0)	AcOH	80 °C	3	53
5	isoamyl nitrite (2.0)	Cu (1.0)	AcOH	rt	1.5	36
6	isoamyl nitrite (3.0)	none	toluene	rt	22	33[a]
7	isoamyl nitrite (5.0)	none	toluene	80 °C	3	71
8	isoamyl nitrite (5.0)	none	xylene	80 °C	3	59
9	isoamyl nitrite (5.0)	none	pyridine	80 °C	3	34
10	isoamyl nitrite (5.0)	none	acetonitrile	80 °C	3	51
11	isoamyl nitrite (5.0)	none	THF	80 °C	3	64
12	isoamyl nitrite (5.0)	none	1,4-dioxane	80 °C	3	73

[a] The yield was determined by HPLC using external standard method after quenching the reaction

Scheme 4. Intramolecular radical cyclization of **12**.

Table 2
Compounds **10a** and **10j** membrane permeability and metabolic properties in the rat (r-) and human (h-) sera and liver S9 fractions

Prodrug	Active metabolite	Remaining ratio[a] (%)				PAMPA Pe (10^{-6} cm/s) pH 5.0/7.4
		r-liver S9	r-serum	h-liver S9	h-serum	
10a	1	<0.5	72	>99	80	34.1/47.4
10j	3	70	8.4	>99	92	36.2/41.4

[a] Compounds were incubated at 37 °C for 30 min.

of **10a** remained in the plasma with an area under the plasma curve (AUC) ratio 6.3-fold that of **1**. Oral administration of **10j** resulted in excellent plasma concentration of compound **3** with a BA value of 90%. Only a small amount of the prodrug **10j** was detected in the plasma, and no **10j** remained in the plasma as indicated by a concentration below the limit of quantification (LOQ; 1.0 ng/mL) six hours later, as indicated by a plasma AUC ratio 0.1-fold that of **3**. Highly lipophilic prodrugs with an amino group are inappropriate for plasma exposure, because they may induce inhibition of hERG channel, mechanism-based inhibition of cytochrome P450, drug-induced phospholipidosis, and hepatotoxicity in humans.[27] We considered that in addition to rapid metabolism in the serum, adequate transformation in liver S9 fractions is crucial in achieving a prodrug rapid clearance.

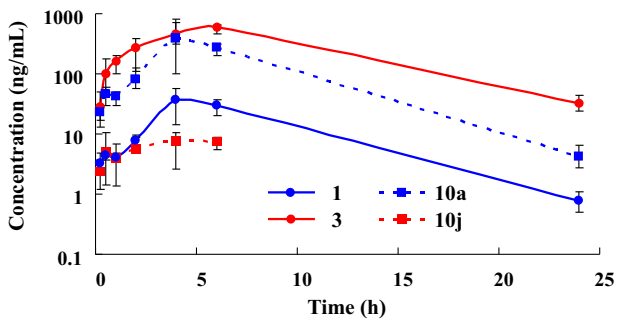


Figure 2. Time-dependent concentrations of **1** and **10a** after administration of **10a**, and **3** and **10j** after administration of **10j** in the plasma of rats (10 mg/kg).

As high quantities of **10a** and **10j** were predicted to remain in the plasma after oral administration in humans, we sought a prodrug that can rapidly be transformed into a carboxyl form both in the human serum and liver S9 fractions. Using the active metabolite **1**, various esters were synthesized and evaluated for their metabolic properties in vitro (Table 3). Although **10b** with a pivaloyloxymethyl (POM) ester[28] was rapidly metabolized in human liver S9 fractions, it showed insufficient conversion in human serum. As alkyl amino alkyl esters are reported to be labile in human plasma, we considered the use of the dimethyl amino ethyl ester[29] **10c** and the dimethyl amino propyl ester **10d**. Compounds **10c** and **10d** were completely converted to the active metabolite **1** in human serum. However, these esters were hardly metabolized in human liver S9 fractions and had poor membrane permeability. Based on these findings, we prepared a series of morpholino alkyl esters. Although compound **10e** with a morpholino ethyl ester[30] was sufficiently converted to **1** in human serum, it was unsatisfactorily transformed in human liver S9 fractions and had low membrane permeability. Both compounds **10f**[31] and **10g** were converted to the carboxyl form in human serum. Compound **10g** was not only metabolized to a greater extent than **10e** in human liver S9 fractions, but also had good membrane permeability. However, **1** was scarcely detected in human liver S9 fractions, and other byproducts were obtained. In conclusion, no alkyl amino alkyl ester or morpholino alkyl ester with the desired profile could be isolated. It is reported that the 5-methyl-2-oxo-1,3-dioxol-4-yl methyl (Dox) ester is labile in human plasma.[32] Accordingly, **10h**, which has a Dox ester, was labile in human serum and liver S9 fractions and had good membrane permeability. Compound **10h** was therefore expected to have sufficient oral absorption with good release of the active metabolite **1**. Furthermore, no **10h** was

Table 3
Ester membrane permeability and metabolic properties in the human (h-) and rat (r-) serum and liver S9 fractions

entry	Prodrug	Active metabolite	Remaining ratio[a] (%)				PAMPA Pe(10^{-6} cm/s) pH 5.0/7.4
			h-liver S9	h-serum	r-liver S9	r-serum	
1	**10b**	1	<0.5	73	<0.5	0.6	6.5/6.3
2	**10c**	1	>99	<0.5	>99	97	<0.1/0.6
3	**10d**	1	>99	<0.5	>99	38	<0.1/<0.1
4	**10e**	1	37	6.8	>99	50	0.9/7.1
5	**10f**	1	48	7.0	78	76	<0.1/3.4
6	**10g**	1	22	<0.5	30	92	10.1/18.0
7	**10h**	1	7.8	<0.5	<0.5	<0.5	10.3/12.1
8	**10i**	1	3.5	<0.5	1.0	<0.5	11.0/8.4
9	**10k**	3	17	<0.5	1.0	<0.5	12.5/14.2
10	**10l**	3	13	<0.5	3.6	<0.5	15.8/15.9

[a] Compounds were incubated at 37 °C for 30 min.

Table 4
Pharmacokinetic parameters of the active metabolites **1**–**4** after iv administration in rats

Active metabolite	Dose (mg/kg)	AUC (ng·h/mL)	CL (mL/min/kg)	V_d (L/kg)	$T_{1/2}$ (h)
1	1.5	188	132	2.9	1.0
2	1	75.3	222	7.1	1.3
3	3	276	181	4.5	0.6
4	1	99.9	167	31.5	8.3

expected to remain in human plasma. When the Dox ester was introduced to the active metabolites **2**–**4**, the obtained prodrugs **10i**, **10k**, and **10l** exhibited properties similar to those of **10h** and were readily converted to the corresponding active metabolites. Additionally, **10h**, **10i**, **10k**, and **10l** metabolic profiles in rat liver S9 fractions were similar to those in human fractions. Compounds **10h**, **10i**, **10k**, and **10l** with a Dox ester were then considered for further evaluation.

To determine plasma concentrations of the prodrugs and the corresponding active metabolites, we performed PK studies in rats. First we examined the PK profiles of the active metabolites **1**–**4** after intravenous (iv) administration (Table 4). Compounds **1**–**4** had very high clearance values exceeding liver blood flow, and **3** showed a short half-life of 0.6 h. Better exposure (AUC) and long half-life were seen with **1** and **4**, respectively. We also determined plasma concentration of the active metabolites **1**–**4** after oral administration of their prodrugs in rats (Table 5). Remarkably, the plasma concentration of **1** after oral administration of **10h** significantly improved with a BA value of 35%. Furthermore and as expected, no **10h** remained in the plasma as indicated by a concentration below the limit of quantification (LOQ: 1.0 ng/mL) at all sampling points. On the other hand, the prodrugs **10i**, **10k** and **10l** gave unsatisfactory results. The BA values of **2**, **3** and **4** after oral administration of **10i**, **10k** and **10l** were 11.7%, 4.0%, 4.8%, respectively. No marked prodrug effect on oral absorption was observed. Because the solubility of **10h** at pH 2.5 was markedly better than that of the other prodrugs, **1** might show the best oral absorption. In humans, **1** is expected to show sufficient plasma concentration after oral administration of **10h**, and no **10h** is expected to remain in the plasma, because **10h** metabolic properties in human are similar to those in rats. Thus, **10h** was selected for further evaluation, including assessment of its in vivo inhibitory activity for DPP-4 and its effect on plasma glucose level in oral glucose tolerance test (OGTT).

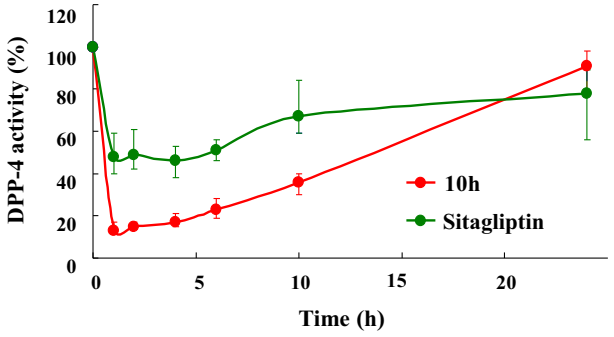


Figure 3. Time-dependent plasma DPP-4 activity after oral administration of **10h** (3 mg/kg) and sitagliptin (3 mg/kg) in SD rats.

To assess the potency of single oral administration of **10h** (3 mg/kg), DPP-4 activity in SD rats was evaluated over a time-period of 0–24 h (Fig. 3). Compound **10h** showed potent DPP-4 inhibition with 87% and 64% inhibition of plasma DPP-4 activity at 1 and 10 h, respectively. This effect was superior to that of sitagliptin at the same dose (3 mg/kg). For OGTT (Fig. 4), ZF rats aged 11 weeks were fasted overnight and were orally administered either the vehicle or **10h** at different doses (0.3 or 1 mg/kg). After one hour ($t = 0$), the rats were orally administered glucose (2 g/kg), and plasma glucose levels were measured against time. Compound **10h** showed a dose-dependent reduction in blood glucose levels (Fig. 4), and a significant reduction of glucose levels was observed at 1 mg/kg against glucose tolerance. These findings suggest that the therapeutic effect of **10h** is mediated via increase in GLP-1 level following inhibition of DPP-4. Thus, compound **10h** is a promising agent for T2DM.

4. Conclusion

In summary, to identify an orally active 3*H*-imidazo[4,5-*c*]quinolin-4(5*H*)-one as a DPP-4 inhibitor, we pursued a prodrug strategy

Table 5
In vitro activity of DPP-4 and pharmacokinetic parameters after oral administration in rats and solubility of the prodrugs **10h**, **10i**, **10k**, and **10l**

Prodrug	h-DPP-4 IC_{50} (nM)	Active metabolite	Dose (mg/kg)	C_{max} (ng/mL)	T_{max} (h)	AUC (ng·hr/mL)	BA (%)	Solubility (mg/mL) pH 7.4/5.5/2.5
10h	6.0	1	20	94.1	2	728	35	<0.001/0.003/0.210
10i	4.7	2	10	14.8	2	72.7	11.7	<0.001/0.002/0.025
10k	1.4	3	20	81.7	0.25	61.9	4.0	0.002/0.002/0.054
10l	3.4	4	10	nd[a]	nd[a]	43.9	4.8	<0.001/0.001/0.047

[a] Not determined.

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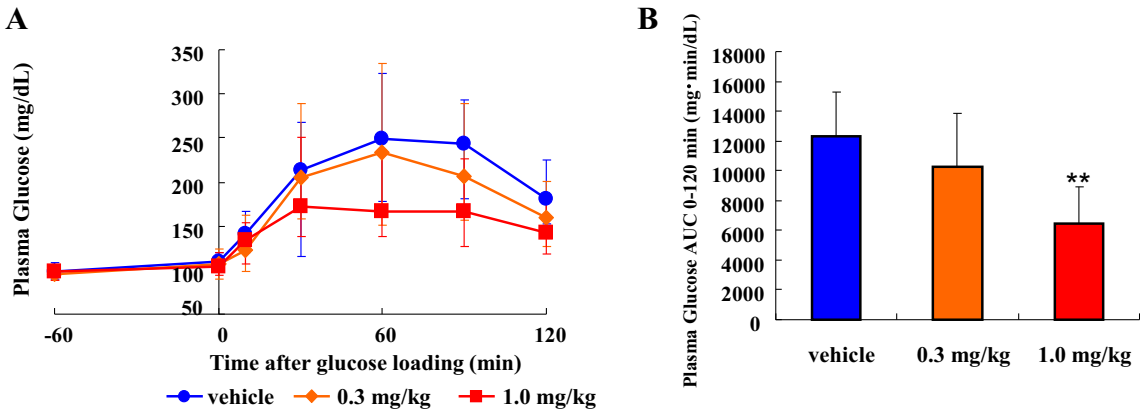

Figure 4. (A) Effect of **10h** on plasma glucose concentration in OGTT in male Zucker fatty rats. (B) Changes in AUC of plasma glucose from 0 min to 120 min. Each value represents the mean and SEM, $n = 10$ rats in each group. $^{**}P < 0.0125$ versus vehicle control.

using **1**-**4** as active metabolites. Optimization of the ester moiety showed that for the ester not to remain in the plasma after oral administration, rapid metabolism into the carboxyl form in both liver S9 fractions and serum was important. In particular, lability in the serum was found to be crucial. We were able to identify **10h** having a 5-methyl-2-oxo-1,3-dioxol-4-yl methyl ester in the C-8 carboxylate of **1** as a prodrug with good oral absorption and efficient release of **1** in rats. Compound **10h** exhibited potent DPP-4 inhibitory activity and potent antidiabetic effect in vivo. Through our investigation, we have developed a novel efficient synthetic method for construction of 3*H*-imidazo[4,5-*c*]quinolin-4(5*H*)-ones using intramolecular radical cyclization. The potent and orally active DPP-4 inhibitor **10h** is expected to be potentially useful for the treatment of T2DM.

5. Experimental section

5.1. Chemistry

5.1.1. Generals

^{1}H NMR and ^{13}C NMR spectra were recorded on a JEOL JNM-LA300 spectrometer and a Brucker AVANCE 400 spectrometer in the stated solvents using tetramethylsilane as an internal standard. Chemical shift (d) are expressed in parts per million. IR spectra were recorded on a JEOL JIR-SPX60 spectrometer as ATR. High resolution MS spectra were recorded on a Thermo Fisher Scientific LTQ orbitrap Discovery MS equipment. Elemental analysis was conducted at Sumitomo Analytical Center Inc. Reactions were followed by TLC on silica gel 60 F254 using precoated TLC plates (E. Merck). Column chromatography was carried out on a Yamazen W-prep system using prepacked silica gel or amino silica gel or performed on silica gel 60 (230–400 or 70–230 mesh, Merck). Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. All solvents were of the commercially available grade. Reactions requiring anhydrous conditions were performed under nitrogen atmosphere.

Compounds **1**, **2**, **3**, **4**, **5**, **7**, **9a**, **9j**, **10**a and **10j** were synthesized as previously reported.[18,19]

5.1.2. 2-{(3*R*)-3-[(*tert*-Butoxycarbonyl)amino]piperidin-1-yl}-3-(2-chloro-5-fluorobenzyl)-5-methyl-4-oxo-4,5-dihydro-3*H*-imidazo[4,5-*c*]quinoline-8-carboxylic acid (6)

A mixture of methyl 2-{(3*R*)-3-[(*tert*-butoxycarbonyl) amino] piperidin-1-yl}-5-methyl-4-oxo-4,5-dihydro-3*H*-imidazo[4,5-c] quinoline-8-carboxylate[19] (312.5 mg, 0.686 mmol), K_2CO_3 (284 mg, 2.05 mmol), and 2-chloro-5-fluorobenzyl bromide (185 μL, 1.37 mmol) in DMF (3 mL) was stirred at 60 °C for 3 h. After cooling to room temperature, the reaction mixture was quenched with saturated NH_4Cl aqueous solution, and extracted with EtOAc. The organic layer was washed with saturated NH_4Cl aqueous solution twice and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica-gel column chromatography to give methyl 2-{(3*R*)-3-[(*tert*-butoxycarbonyl)amino]piperidin-1-yl}-3-(2-chloro-5-fluorobenzyl)-5-methyl-4-oxo-4,5-dihydro-3*H*-imidazo[4,5-*c*]quinoline-8-carboxylate (306.0 mg, yield 75%) as a white powder. A mixture of above-product (216.5 mg, 0.362 mmol) and 1M NaOH (1 mL) aqueous solution in THF (1 mL) and MeOH (1 mL) was stirred at room temperature for 6 h. The mixture was acidified with 5% $KHSO_4$ aqueous solution and extracted with $CHCl_3$. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure to give **6** (165.7 mg, yield 78%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.65 (d, $J = 2.0$ Hz, 1H), 8.04 (dd, $J = 2.0$, 9.0 Hz, 1H), 7.64 (d, $J = 9.0$ Hz, 1H), 7.56–7.52 (m, 1H), 7.18–7.13 (m, 1H), 6.88 (d, $J = 7.7$ Hz, 1H), 6.59 (dd, $J = 2.5$, 9.3 Hz, 1H), 5.48 (d, $J = 18.0$ Hz, 1H), 5.42 (d, $J = 18.0$ Hz, 1H), 3.62 (s, 3H), 3.48–3.44 (m, 1H), 3.38–3.27 (m, 2H), 2.89–2.84 (m, 1H), 2.75–2.70 (m, 1H), 1.79–1.71 (m, 2H), 1.61–1.58 (m, 1H), 1.38–1.32 (m, 10H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.9, 161.0 (d, ^1J(C, F) = 243 Hz), 158.0, 154.7, 154.0, 141.5, 140.0, 137.7 (d, ^3J(C, F) = 7.2 Hz), 131.0 (d, ^3J(C, F) = 7.9 Hz), 128.7, 126.2 (d, ^4J(C, F) = 3.0 Hz), 124.1, 123.5, 118.8, 116.0, 115.7 (d, ^2J(C, F) = 23.0 Hz), 115.7, 114.0 (d, ^2J(C, F) = 25.0 Hz), 79.1, 54.9, 50.2, 46.5, 46.3,305, 28.9, 28.1, 23.2; HRMS (ESI) $[M+H]^+$ calcd for $C_{29}H_{32}O_5N_5ClF$ 584.2071, found 584.2068; IR (ATR): 1700, 1652, 1648, 1569, 1533, 1500, 1469, 1429, 1388, 1365, 1309, 1267, 1241, 1222, 1164, 1112 cm^{-1}.

5.1.3. 2-{(3*R*)-3-[(*tert*-Butoxycarbonyl)amino]piperidin-1-yl}-3-(2-chloro-5-fluorobenzyl)-5-methyl-4-oxo-4,5-dihydro-3*H*-imidazo[4,5-*c*]quinoline-7-carboxylic acid (8)

A mixture of methyl 2-{(3*R*)-3-[(*tert*-butoxycarbonyl)amino] piperidin-1-yl}-3-(5-fluoro-2-methylbenzyl)-5-methyl-4-oxo-4, 5-dihydro-3*H*-imidazo[4,5-*c*]quinoline-7-carboxylate[19] (195 mg, 0.326 mmol) and 1M NaOH (2 mL) aqueous solution in THF (2 mL) and MeOH (2 mL) was stirred at 50 °C for 4 h. The mixture was acidified with 5% $KHSO_4$ aqueous solution and extracted with $CHCl_3$. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure to give **8** (171.7 mg, yield 90%) as a white amorphous. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.18 (d, $J = 8.1$ Hz, 1H), 8.06 (d, $J = 1.1$ Hz, 1H), 7.88 (dd, $J = 1.1$, 8.1 Hz, 1H), 7.56–7.53 (m, 1H), 7.18–7.13 (m, 1H), 6.93 (d, $J = 7.7$ Hz, 1H), 6.59 (dd, $J = 2.5$, 9.4 Hz, 1H), 5.52 (d, $J = 17.6$ Hz, 1H), 5.46 (d, $J = 17.6$ Hz, 1H), 3.66 (s, 3H), 3.48–3.25 (m, 3H), 2.87–2.82 (m, 1H), 2.76–2.71 (m, 1H), 1.79–1.69 (m,

2H), 1.60–1.57 (m, 1H), 1.40–1.35 (m, 1H), 1.33 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.3, 161.2 (d, ^1J(C, F) = 243 Hz), 158.2, 154.9, 154.3, 141.0, 137.9 (d, ^3J(C, F) = 7.4 Hz), 137.0, 131.2 (d, ^3J(C, F) = 8.3 Hz), 130.3, 126.4 (d, ^4J(C, F) = 2.8 Hz), 122.9, 122.3, 120.1, 119.9, 116.6, 116.0 (d, ^2J(C, F) = 23.0 Hz), 114.3 (d, ^2J(C, F) = 25.0 Hz), 77.9, 55.1, 50.5, 46.7, 46.6, 29.7, 28.8, 28.4, 23.4; HRMS (ESI) $[M+H]^+$ calcd for $C_{29}H_{32}O_5N_5ClF$ 584.2071, found 584.2071; IR (ATR): 1712, 1660, 1646, 1623, 1508, 1473, 1456, 1423, 1400, 1365, 1349, 1313, 1290, 1251, 1240, 1220, 1149, 1122, 1112, 1068, 1049, 1027 cm^{-1}.

5.1.4. [(2,2-Dimethylpropanoyl)oxy]methyl 2-{(3*R*)-3-[(*tert*-butoxycarbonyl)amino]piperidin-1-yl}-3-(2-chlorobenzyl)-5-methyl-4-oxo-4,5-dihydro-3*H*-imidazo[4,5-*c*]quinoline-8-carboxylate (9b)

A mixture of **5** (217.3 mg, 0.384 mmol), K_2CO_3 (159.2 mg, 1.15 mmol), and chloromethyl pivalate (112 μL, 0.766 mmol) in DMF (2 mL) was stirred at room temperature for 12 h. The reaction mixture was quenched with saturated NH_4Cl aqueous solution, and extracted with EtOAc. The organic layer was washed with saturated NH_4Cl aqueous solution twice and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica-gel column chromatography to give **9b** (89.0 mg, yield 34%) as a white amorphous. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.99 (d, J = 2.1 Hz, 1H), 8.17 (dd, J = 2.1, 9.0 Hz, 1H), 7.45 (d, J = 9.0 Hz, 1H), 7.40–7.38 (m, 1H), 7.18–7.09 (m, 2H), 6.66 (d, J = 7.1 Hz, 1H), 6.04 (s, 2H), 5.76 (d, J = 16.8 Hz, 1H), 5.63 (d, J = 16.8 Hz, 1H), 5.12 (br s, 1H), 3.79 (br s, 1H), 3.74 (s, 3H), 3.45 (dd, J = 3.1, 12.3 Hz, 1H), 3.16–3.08 (m, 3H), 1.79–1.61 (m, 4H), 1.41 (s, 9H), 1.22 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.2, 164.7, 158.5, 155.0, 142.1, 140.9, 134.9, 131.9, 129.5, 129.4, 128.6, 127.1, 126.5, 125.4, 125.4, 122.7, 119.5, 116.8, 114.8, 79.9, 79.2, 55.3, 51.4, 46.4, 46.0, 31.5, 29.2, 28.3, 26.8, 22.6; HRMS (ESI) $[M+H]^+$ calcd for $C_{35}H_{43}O_7N_5Cl$ 680.2846, found 680.2845; IR (ATR): 1733, 1716, 1683, 1652, 1558, 1540, 1508, 1473, 1457, 1363, 1270, 1218, 1157, 1078, 1033 cm^{-1}.

5.1.5. 2-(Dimethylamino)ethyl 2-{(3*R*)-3-[(*tert*-butoxycarbonyl)amino]piperidin-1-yl}-3-(2-chlorobenzyl)-5-methyl-4-oxo-4,5-dihydro-3*H*-imidazo[4,5-*c*]quinoline-8-carboxylate (9c)

Compound **9c** was prepared from **5** in a manner similar to that described for compound **9b** with a yield of 85% as a white amorphous. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.93 (d, J = 2.0 Hz, 1H), 8.15 (dd, J = 2.0, 9.0 Hz, 1H), 7.43 (d, J = 9.0 Hz, 1H), 7.40–7.37 (m, 1H), 7.20–7.09 (m, 2H), 6.66 (d, J = 7.3 Hz, 1H), 5.76 (d, J = 16.8 Hz, 1H), 5.61 (d, J = 16.8 Hz, 1H), 5.14–5.11 (m, 1H), 4.51 (t, J = 5.7 Hz, 2H), 3.81 (br s, 1H), 3.73 (s, 3H), 3.42 (dd, J = 3.3, 12.5 Hz, 1H), 3.15–3.06 (m, 3H), 2.82 (t, J = 5.7 Hz, 2H), 2.40 (s, 6H), 1.80–1.58 (m, 3H), 1.41 (m, 10H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.1, 158.5, 155.1, 155.0, 142.3, 140.5, 134.9, 131.9, 129.5, 129.2, 128.6, 127.1, 126.5, 124.9, 124.0, 119.5, 116.8, 114.7, 79.2, 62.9, 57.8, 55.2, 51.4, 46.4, 46.0, 45.8, 29.5, 29.2; HRMS (ESI) $[M+H]^+$ calcd for $C_{33}H_{42}O_5N_6Cl$ 637.2900, found 637.2892; IR (ATR): 1712, 1652, 1569, 1506, 1457, 1388, 1363, 1311, 1272, 1234, 1166, 1112, 1066, 1049 cm^{-1}.

5.1.6. 3-(Dimethylamino)propyl 2-{(3*R*)-3-[(*tert*-butoxycarbonyl)amino]piperidin-1-yl}-3-(2-chlorobenzyl)-5-methyl-4-oxo-4,5-dihydro-3*H*-imidazo[4,5-*c*]quinoline-8-carboxylate (9d)

Compound **9d** was prepared from **5** in a manner similar to that described for compound **9b** with a yield of 70% as a white amorphous. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.94 (d, J = 2.1 Hz, 1H), 8.16 (dd, J = 2.1, 9.0 Hz, 1H), 7.45 (d, J = 9.0 Hz, 1H), 7.41 (dd, J = 1.1, 7.9 Hz, 1H), 7.22–7.18 (m, 1H), 7.15–7.11 (m, 1H), 6.68 (d, J = 7.4 Hz, 1H), 5.79 (d, J = 17.2 Hz, 1H), 5.65 (d, J = 17.2 Hz, 1H), 5.13 (br s, 1H), 4.44 (t, J = 6.4 Hz, 2H), 3.82 (br s, 1H), 3.76 (s, 3H), 3.45 (dd, J = 3.2, 12.3 Hz, 1H), 3.17–3.08 (m, 3H), 2.54 (t, J = 7.2 Hz, 2H), 2.33 (s, 6H), 2.08–2.01 (m, 2H), 1.79–1.72 (m, 2H), 1.62–1.60 (m, 2H), 1.43 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.0, 158.3, 154.9, 154.9, 142.2, 140.3, 134.9, 131.8, 129.4, 129.0, 128.5, 127.1, 126.4, 124.6, 124.1, 119.3, 116.7, 114.6, 79.1, 63.3, 56.2, 55.2, 51.3, 46.3, 46.0, 45.4, 29.5, 29.1, 28.3, 27.0, 22.1; HRMS (ESI) $[M+H]^+$ calcd for $C_{34}H_{44}O_5N_6Cl$ 651.3056, found 651.3051; IR (ATR): 1716, 1654, 1508, 1457, 1272, 1220, 1166, 1112, 1033 cm^{-1}.

5.1.7. 2-(Morpholin-4-yl)ethyl 2-{(3*R*)-3-[(*tert*-butoxycarbonyl)amino]piperidin-1-yl}-3-(2-chlorobenzyl)-5-methyl-4-oxo-4,5-dihydro-3*H*-imidazo[4,5-*c*]quinoline-8-carboxylate (9e)

Compound **9e** was prepared from **5** in a manner similar to that described for compound **9b** with a yield of 73% as a white amorphous. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.94 (d, J = 2.0 Hz, 1H), 8.15 (dd, J = 2.0, 8.9 Hz, 1H), 7.46 (d, J = 8.9 Hz, 1H), 7.41 (dd, J = 1.1, 7.9 Hz, 1H), 7.20–7.18 (m, 1H), 7.15–7.11 (m, 1H), 6.68 (d, J = 7.4 Hz, 1H), 5.78 (d, J = 17.2 Hz, 1H), 5.65 (d, J = 17.2 Hz, 1H), 5.12–5.10 (m, 1H), 4.55 (br s, 2H), 3.80–3.75 (m, 6H), 3.72–3.68 (m, 1H), 3.46 (dd, J = 3.2, 12.3 Hz, 1H), 3.15–3.08 (m, 3H), 2.88 (m, 2H), 2.66–2.57 (m, 5H), 1.82–1.72 (m, 2H), 1.62–1.60 (m, 2H), 1.43 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.0, 158.4, 155.0, 155.0, 142.2, 140.4, 134.9, 131.9, 129.5, 129.1, 128.5, 127.1, 126.5, 124.8, 123.9, 119.5, 116.8, 114.7, 79.2, 66.9, 62.2, 57.1, 55.2, 53.8, 51.4, 46.4, 46.0, 29.5, 29.2, 28.3, 22.2; HRMS (ESI) $[M+H]^+$ calcd for $C_{35}H_{44}O_6N_6Cl$ 679.3005, found 679.3003; IR (ATR): 1708, 1691, 1654, 1569, 1498, 1465, 1430, 1388, 1351, 1309, 1274, 1218, 1164, 1116, 1066, 1039 cm^{-1}.

5.1.8. 3-(Morpholin-4-yl)propyl 2-{(3*R*)-3-[(*tert*-butoxycarbonyl)amino]piperidin-1-yl}-3-(2-chlorobenzyl)-5-methyl-4-oxo-4,5-dihydro-3*H*-imidazo[4,5-*c*]quinoline-8-carboxylate (9f)

Compound **9f** was prepared from **5** in a manner similar to that described for compound **9b** with a yield of 86% as a white amorphous. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.92 (d, J = 2.0 Hz, 1H), 8.15 (dd, J = 2.0, 8.9 Hz, 1H), 7.45 (d, J = 8.9 Hz, 1H), 7.42–7.40 (m, 1H), 7.22–7.18 (m, 1H), 7.15–7.11 (m, 1H), 6.68 (d, J = 7.5 Hz, 1H), 5.77 (d, J = 17.2 Hz, 1H), 5.64 (d, J = 17.2 Hz, 1H), 5.13–5.11 (m, 1H), 4.56 (t, J = 6.4 Hz, 2H), 3.79 (br s, 5H), 3.75 (s, 3H), 3.46 (dd, J = 3.2, 12.3 Hz, 1H), 3.16–3.07 (m, 3H), 2.59 (br s, 6H), 2.10 (br s, 2H), 1.81–1.71 (m, 2H), 1.61–1.59 (m, 2H), 1.42 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.1, 158.4, 155.0, 155.0, 142.2, 140.4, 134.9, 131.9, 129.5, 129.0, 128.5, 127.1, 126.5, 124.6, 124.0, 119.4, 116.7, 114.7, 79.1, 66.7, 63.3, 55.5, 55.2, 53.6, 51.4, 46.3, 46.0, 29.5, 29.2, 28.3, 25.8, 22.2; HRMS (ESI) $[M+H]^+$ calcd for $C_{36}H_{46}O_6N_6Cl$ 693.3167, found 693.3155; IR (ATR): 1716, 1652, 1558, 1506, 1457, 1363, 1272, 1220, 1166, 1112, 1066 cm^{-1}.

5.1.9. 2-[(2*R*,6*S*)-2,6-Dimethylmorpholin-4-yl]ethyl 2-{(3*R*)-3-[(*tert*-butoxycarbonyl)amino]piperidin-1-yl}-3-(2-chlorobenzyl)-5-methyl-4-oxo-4,5-dihydro-3*H*-imidazo[4,5-*c*]quinoline-8-carboxylate (9g)

Compound **9g** was prepared from **5** in a manner similar to that described for compound **9b** with a yield of 49% as a white amorphous. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.92 (d, J = 2.0 Hz, 1H), 8.15 (dd, J = 2.0, 9.0 Hz, 1H), 7.45 (d, J = 9.0 Hz, 1H), 7.40 (d, J = 7.9 Hz, 1H), 7.21–7.17 (m, 1H), 7.14–7.10 (m, 1H), 6.67 (d, J = 7.4 Hz, 1H), 5.77 (d, J = 17.2 Hz, 1H), 5.63 (d, J = 17.2 Hz, 1H), 5.12–5.10 (m, 1H), 4.53 (m, 2H), 3.81–3.75 (m, 6H), 3.44 (dd, J = 3.0, 12.3 Hz, 1H), 3.14–3.07 (m, 3H), 2.90–2.82 (m, 4H), 1.94 (t, J = 6.6 Hz, 2H), 1.79–1.71 (m, 2H), 1.61–1.59 (m, 2H), 1.42 (s, 9H), 1.17 (d, J = 6.3 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.0,

158.5, 155.1, 155.0, 142.3, 140.5, 134.9, 132.0, 129.6, 129.2, 128.6, 127.2, 126.5, 124.8, 123.9, 119.6, 116.8, 114.8, 79.3, 62.2, 59.6, 56.7, 55.3, 51.5, 46.4, 46.0, 31.5, 29.2, 28.4, 22.6, 22.2, 19.1; HRMS (ESI) $[M+H]^+$ calcd for $C_{37}H_{48}O_6N_6Cl$ 707.3318, found 707.3308; IR (ATR): 1716, 1654, 1569, 1558, 1506, 1457, 1363, 1309, 1272, 1236, 1220, 1166, 1114, 1066 cm^{-1}.

5.1.10. (5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 2-{(3*R*)-3-[(*tert*-butoxycarbonyl)amino]piperidin-1-yl}-3-(2-chlorobenzyl)-5-methyl-4-oxo-4,5-dihydro-3*H*-imidazo[4,5-*c*]quinoline-8-carboxylate (9h)

Compound **9h** was prepared from **5** in a manner similar to that described for compound **9b** with a yield of 96% as a white amorphous. ^1H NMR (400 MHz, $CDCl_3$) δ 8.96 (br s, 1H), 8.15 (dd, J = 2.1, 8.9 Hz, 1H), 7.46 (d, J = 8.9 Hz, 1H), 7.41 (dd, J = 1.0, 7.9 Hz, 1H), 7.22–7.18 (m, 1H), 7.15–7.11 (m, 1H), 6.69 (d, J = 7.5 Hz, 1H), 5.77 (d, J = 17.2 Hz, 1H), 5.64 (d, J = 17.2 Hz, 1H), 5.14 (s, 2H), 5.11 (br s, 1H), 3.82 (br s, 1H), 3.75 (s, 3H), 3.47 (dd, J = 3.3, 12.3 Hz, 1H), 3.16–3.09 (m, 3H), 2.28 (s, 3H), 1.81–1.72 (m, 2H), 1.61–1.60 (m, 2H), 1.43 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.1, 158.1, 154.9, 154.0, 152.1, 141.3, 140.6, 140.5, 135.2, 133.7, 131.2, 129.4, 128.9, 128.8, 127.7, 126.9, 123.5, 122.3, 119.0, 116.2, 116.0, 77.9, 55.1, 54.7, 50.6, 46.7, 46.2, 29.9, 29.0, 28.4, 23.5, 9.1; HRMS (ESI) $[M+H]^+$ calcd for $C_{34}H_{37}O_8N_5Cl$ 678.2325, found 678.2325; IR (ATR): 1822, 1716, 1673, 1654, 1506, 1434, 1394, 1315, 1270, 1216, 1160, 1105, 1093, 1066, 1049 cm^{-1}.

5.1.11. (5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 2-{(3*R*)-3-[(*tert*-butoxycarbonyl)amino]piperidin-1-yl}-3-(2-chloro-5-fluorobenzyl)-5-methyl-4-oxo-4,5-dihydro-3*H*-imidazo[4,5-*c*]quinoline-8-carboxylate (9i)

Compound **9i** was prepared from **6** in a manner similar to that described for compound **9b** with a yield of 87% as a white amorphous. ^1H NMR (300 MHz, $CDCl_3$) δ 8.95 (d, J = 2.2 Hz, 1H), 8.16 (dd, J = 2.2, 9.0 Hz, 1H), 7.48 (d, J = 9.0 Hz, 1H), 7.40–7.36 (m, 1H), 6.95–6.88 (m, 1H), 6.44–6.42 (m, 1H), 5.70 (d, J = 17.2 Hz, 1H), 5.60 (d, J = 17.2 Hz, 1H), 5.14 (s, 2H), 5.10 (br s, 1H), 3.81 (br s, 1H), 3.76 (s, 3H), 3.48 (dd, J = 2.9, 12.1 Hz, 1H), 3.12–3.06 (m, 3H), 2.28 (s, 3H), 1.85–1.75 (m, 3H), 1.42 (s, 9H), 1.27–1.15 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.2, 162.3 (d, ^1J(C, F) = 245 Hz), 159.2, 155.7, 155.7, 152.9, 143.0, 141.6, 141.0, 138.0 (d, ^3J(C, F) = 7.1 Hz), 134.4, 131.6 (d, ^3J(C, F) = 8.2 Hz), 130.1, 127.5 (d, ^4J(C, F) = 3.2 Hz), 125.9, 123.5, 120.1, 117.5, 116.4 (d, ^2J(C, F) = 22.8 Hz), 115.6, 114.6 (d, ^2J(C, F) = 24.5 Hz), 80.3, 56.2, 54.9, 52.1, 47.1, 46.9, 30.3, 30.0, 29.0, 23.2, 10.2; HRMS (ESI) $[M+H]^+$ calcd for $C_{34}H_{36}O_8N_5ClF$ 696.2231, found 696.2231; IR (ATR): 1818, 1716, 1652, 1569, 1506, 1473, 1386, 1305, 1272, 1218, 1166, 1091, 1049 cm^{-1}.

5.1.12. (5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 2-{(3*R*)-3-[(*tert*-butoxycarbonyl)amino]piperidin-1-yl}-3-(2-chlorobenzyl)-5-methyl-4-oxo-4,5-dihydro-3*H*-imidazo[4,5-*c*]quinoline-7-carboxylate (9k)

Compound **9k** was prepared from **7** in a manner similar to that described for compound **9b** with a yield of 73% as a white amorphous. ^1H NMR (400 MHz, $CDCl_3$) δ 8.35 (d, J = 8.2 Hz, 1H), 8.13 (d, J = 1.1 Hz, 1H), 7.96 (dd, J = 1.1, 8.2 Hz, 1H), 7.41 (dd, J = 1.0, 7.9 Hz, 1H), 7.22–7.19 (m, 1H), 7.15–7.11 (m, 1H), 6.69 (br d, J = 7.5 Hz, 1H), 6.00 (br s, 1H), 5.78 (d, J = 17.2 Hz, 1H), 5.65 (d, J = 17.4 Hz, 1H), 5.15 (s, 2H), 3.81 (br s, 1H), 3.80 (s, 3H), 3.44 (dd, J = 3.3, 12.6 Hz, 1H), 3.25–3.21 (m, 1H), 3.08 (m, 2H), 2.28 (s, 3H), 1.77–1.72 (m, 3H), 1.56–1.51 (m, 1H), 1.46 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.4, 159.0, 155.8, 155.5, 152.7, 141.6, 141.0, 137.8, 135.4, 134.1, 132.5, 130.2, 129.2, 128.7, 127.8, 127.1, 123.6, 123.5, 121.6, 121.4, 117.2, 79.7, 55.4, 55.1, 52.1, 47.0, 46.4, 30.1, 29.8, 29.1, 22.2, 10.1; HRMS (ESI) $[M+H]^+$ calcd for $C_{34}H_{37}O_8N_5Cl$ 678.2325, found 678.2325; IR (ATR): 1824, 1716, 1652, 1558, 1508, 1473, 1307, 1216, 1166, 1097, 1049, 1033 cm^{-1}.

5.1.13. (5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 2-{(3*R*)-3-[(*tert*-butoxycarbonyl)amino]piperidin-1-yl}-3-(2-chloro-5-fluorobenzyl)-5-methyl-4-oxo-4,5-dihydro-3*H*-imidazo[4,5-*c*]quinoline-7-carboxylate (9l)

Compound **9l** was prepared from **8** in a manner similar to that described for compound **9b** with a quantitative yield as a white amorphous. ^1H NMR (400 MHz, $CDCl_3$) δ 8.38 (d, J = 8.2 Hz, 1H), 8.14 (d, J = 1.2 Hz, 1H), 7.99 (dd, J = 1.2, 8.2 Hz, 1H), 7.40–7.36 (m, 1H), 6.95–6.90 (m, 1H), 6.45–6.42 (m, 1H), 5.90 (s, 1H), 5.71 (d, J = 17.0 Hz, 1H), 5.62 (d, J = 17.0 Hz, 1H), 5.15 (s, 2H), 3.82 (br s, 1H), 3.81 (s, 3H), 3.47 (dd, J = 3.2, 12.4 Hz, 1H), 3.21–3.10 (m, 3H), 2.28 (s, 3H), 1.84–1.56 (m, 4H), 1.46 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.5, 162.3 (d, ^1J(C, F) = 245 Hz), 159.0, 155.8, 155.6, 152.7, 141.9, 141.1, 137.9, 137.9 (d, ^3J(C, F) = 6.8 Hz), 134.1, 131.6 (d, ^3J(C, F) = 8.2 Hz), 129.0, 127.4 (d, ^4J(C, F) = 2.7 Hz), 123.8, 123.7, 121.6, 121.2, 117.3, 116.4 (d, ^2J(C, F) = 22.8 Hz), 114.5 (d, ^2J(C, F) = 24.6 Hz), 79.8, 55.7, 55.2, 52.2, 47.0, 46.6, 32.2, 29.9, 29.1, 23.3, 10.1; HRMS (ESI) $[M+H]^+$ calcd for $C_{34}H_{36}O_8N_5ClF$ 696.2231, found 696.2238; IR (ATR): 1824, 1716, 1652, 1558, 1540, 1508, 1473, 1457, 1363, 1307, 1216, 1164, 1103, 1049, 1033 cm^{-1}.

5.1.14. [(2,2-Dimethylpropanoyl)oxy]methyl 2-[(3*R*)-3-aminopiperidin-1-yl]-3-(2-chlorobenzyl)-5-methyl-4-oxo-4,5-dihydro-3*H*-imidazo[4,5-*c*]quinoline-8-carboxylate hydrochloride (10b)

To a solution of **9b** (80.0 mg, 0.118 mmol) in 1,4-dioxane (2 mL) was added 4N HCl-1,4-dioxane (2 mL). The reaction mixture was stirred at room temperature for 2 h and concentrated under reduced pressure to give **10b** (76.3 mg, quantitative yield) as a white amorphous. ^1H NMR (400 MHz, DMSO-d_6) δ 8.68 (d, J = 2.1 Hz, 1H), 8.41 (br s, 3H), 8.07 (dd, J = 2.1, 8.9 Hz, 1H), 7.70 (d, J = 8.9 Hz, 1H), 7.50 (d, J = 7.9 Hz, 1H), 7.32–7.28 (m, 1H), 7.23–7.19 (m, 1H), 6.69 (d, J = 7.3 Hz, 1H), 6.01 (s, 2H), 5.61 (d, J = 17.2 Hz, 1H), 5.55 (d, J = 17.2 Hz, 1H), 3.73–3.65 (m, 1H), 3.63 (s, 3H), 3.49–3.45 (m, 1H), 3.33–3.23 (m, 1H), 3.10–3.07 (m, 1H), 2.89–2.82 (m, 1H), 1.97–1.96 (m, 1H), 1.78–1.76 (m, 1H), 1.62–1.48 (m, 2H), 1.16 (s, 9H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 176.5, 164.1, 157.6, 154.0, 140.7, 140.6, 134.9, 130.9, 129.4, 129.0, 128.9, 127.6, 126.9, 123.7, 121.8, 119.1, 116.3, 115.8, 80.0, 59.2, 52.1, 50.9, 46.3, 46.1, 29.1, 27.2, 26.5, 22.0; HRMS (ESI) $[M+H]^+$ calcd for $C_{30}H_{35}O_5N_5Cl$ 580.2319, found 580.2312; IR (ATR): 1704, 1672, 1616, 1594, 1508, 1442, 1375, 1317, 1245, 1218, 1114, 1049 cm^{-1}.

5.1.15. 2-(Dimethylamino)ethyl 2-[(3*R*)-3-aminopiperidin-1-yl]-3-(2-chlorobenzyl)-5-methyl-4-oxo-4,5-dihydro-3*H*-imidazo[4,5-*c*]quinoline-8-carboxylate dihydrochloride (10c)

Compound **10c** was prepared from **9c** in a manner similar to that described for compound **10b** with a yield of 98% as a white amorphous. ^1H NMR (400 MHz, DMSO-d_6) δ 10.98 (s, 1H), 8.78 (d, J = 1.8 Hz, 1H), 8.51 (br s, 3H), 8.23 (dd, J = 1.8, 8.8 Hz, 1H), 7.69 (d, J = 8.8 Hz, 1H), 7.51 (d, J = 7.8 Hz, 1H), 7.32–7.28 (m,1H), 7.24–7.20 (m, 1H), 6.73 (d, J = 7.5 Hz, 1H), 5.64 (d, J = 17.2 Hz, 1H), 5.58 (d, J = 17.2 Hz, 1H), 4.63 (br s, 2H), 3.75–3.72 (m, 1H), 3.64 (s, 3H), 3.61–3.50 (m, 3H), 3.37–3.27 (m, 2H), 3.12–3.09 (m, 1H), 2.88 (s, 6H), 1.97 (m, 1H), 1.75 (m, 1H), 1.64–1.62 (m, 1H), 1.51–1.48 (m, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 165.0, 157.1, 153.9, 140.4, 140.1, 134.8, 130.9, 129.4, 129.4, 129.0, 127.7, 127.0, 123.9, 122.7, 118.9, 116.0, 115.3, 59.4, 54.8, 52.1, 50.8, 46.5, 46.0, 42.5, 29.1, 27.1, 21.9; HRMS (ESI) $[M+H]^+$ calcd for $C_{28}H_{34}O_3N_6Cl$ 537.2375, found 537.2372; IR (ATR): 1718, 1670, 1627, 1608, 1592, 1558, 1515, 1457, 1446, 1371, 1321, 1276, 1261, 1241, 1218, 1126, 1114, 1066, 1051, 1039 cm^{-1}.

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5.1.16. 3-(Dimethylamino)propyl 2-[(3*R*)-3-aminopiperidin-1-yl]-3-(2-chlorobenzyl)-5-methyl-4-oxo-4,5-dihydro-3*H*-imidazo[4,5-*c*]quinoline-8-carboxylate dihydrochloride (10d)

Compound **10d** was prepared from **9d** in a manner similar to that described for compound **10b** with a quantitative yield as a white amorphous. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.88 (br s, 1H), 8.72 (d, J = 2.2 Hz, 1H), 8.52 (br s, 3H), 8.10 (dd, J = 2.2, 9.0 Hz, 1H), 7.65 (d, J = 9.0 Hz, 1H), 7.51 (dd, J = 1.1, 7.9 Hz, 1H), 7.32–7.27 (m, 1H), 7.24–7.19 (m, 1H), 6.72 (d, J = 6.8 Hz, 1H), 5.63 (d, J = 17.2 Hz, 1H), 5.55 (d, J = 17.2 Hz, 1H), 4.41 (t, J = 6.2 Hz, 2H), 3.77–3.73 (m, 1H), 3.63 (s, 3H), 3.33–3.21 (m, 4H), 3.11–3.07 (m, 1H), 2.90–2.83 (m, 1H), 2.78 (s, 6H), 2.24–2.19 (m, 2H), 1.97 (m, 1H), 1.75 (m, 1H), 1.65–1.61 (m, 1H), 1.51 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.5, 157.5, 154.2, 140.6, 140.5, 135.1, 131.1, 129.6, 129.2, 129.2, 127.9, 127.1, 123.7, 123.2, 119.2, 116.2, 115.8, 62.3, 53.8, 52.3, 51.1, 46.6, 46.3, 42.2, 29.3, 27.4, 23.7, 22.2; HRMS (ESI) $[M+H]^+$ calcd for $C_{29}H_{36}O_3N_6Cl$ 551.2532, found 551.2531; IR (ATR): 3355, 1697, 1670, 1652, 1616, 1508, 1465, 1442, 1386, 1322, 1274, 1241, 1218, 1108, 1052, 1039 cm^{-1}.

5.1.17. 2-(Morpholin-4-yl)ethyl 2-[(3*R*)-3-aminopiperidin-1-yl]-3-(2-chlorobenzyl)-5-methyl-4-oxo-4,5-dihydro-3*H*-imidazo[4,5-*c*]quinoline-8-carboxylate dihydrochloride (10e)

Compound **10e** was prepared from **9e** in a manner similar to that described for compound **10b** with a quantitative yield as a white amorphous. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.77 (br s, 1H), 8.76 (d, J = 2.0 Hz, 1H), 8.47 (br s, 3H), 8.21 (dd, J = 2.0, 9.0 Hz, 1H), 7.67 (d, J = 9.0 Hz, 1H), 7.51 (dd, J = 1.3, 7.9 Hz, 1H), 7.32–7.28 (m, 1H), 7.25–7.20 (m, 1H), 6.73 (d, J = 6.4 Hz, 1H), 5.64 (d, J = 17.2 Hz, 1H), 5.57 (d, J = 17.2 Hz, 1H), 4.75–4.74 (m, 2H), 4.00–3.88 (m, 4H), 3.75–3.64 (m, 6H), 3.54–3.50 (m, 2H), 3.32–3.28 (m, 4H), 3.08 (m, 1H), 2.91–2.85 (m, 1H), 1.97 (m, 1H), 1.76 (m, 1H), 1.61–1.52 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.1, 157.1, 154.0, 140.6, 139.8, 135.0, 131.1, 129.6, 129.5, 129.2, 127.9, 127.2, 124.1, 122.9, 119.0, 116.2, 115.3, 63.4, 59.4, 54.6, 52.3, 51.5, 51.0, 46.8, 46.2, 29.4, 27.3, 22.1; HRMS (ESI) $[M+H]^+$ calcd for $C_{30}H_{36}O_4N_6Cl$ 579.2481, found 579.2464; IR (ATR): 1718, 1668, 1592, 1521, 1508, 1473, 1317, 1251, 1130, 1110, 1049, 1033 cm^{-1}.

5.1.18. 3-(Morpholin-4-yl)propyl 2-[(3*R*)-3-aminopiperidin-1-yl]-3-(2-chlorobenzyl)-5-methyl-4-oxo-4,5-dihydro-3*H*-imidazo[4,5-*c*]quinoline-8-carboxylate dihydrochloride (10f)

Compound **10f** was prepared from **9f** in a manner similar to that described for compound **10b** with a quantitative yield as a white amorphous. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.51 (br s, 1H), 8.72 (d, J = 2.1 Hz, 1H), 8.47 (br s, 3H), 8.11 (dd, J = 2.1, 8.9 Hz, 1H), 7.67 (d, J = 8.9 Hz, 1H), 7.51 (dd, J = 0.9, 7.9 Hz, 1H), 7.32–7.28 (m, 1H), 7.24–7.20 (m, 1H), 6.71 (d, J = 7.4 Hz, 1H), 5.63 (d, J = 17.2 Hz, 1H), 5.57 (d, J = 17.2 Hz, 1H), 4.20 (t, J = 6.4 Hz, 2H), 3.97–3.93 (m, 2H), 3.86–3.82 (m, 2H), 3.75–3.69 (m, 1H), 3.64 (s, 3H), 3.47–3.44 (m, 2H), 3.37–3.26 (m, 4H), 3.12–3.04 (m, 3H), 2.31–2.24 (m, 2H), 1.98–1.96 (m, 1H), 1.78–1.75 (m, 1H), 1.64–1.51 (m, 1H), 1.50–1.48 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.4, 156.6, 153.9, 140.4, 139.2, 134.9, 131.1, 129.6, 129.3, 129.2, 127.9, 127.2, 123.9, 123.3, 118.7, 116.1, 114.8, 63.4, 62.5, 53.4, 52.3, 51.1, 51.0, 46.8, 46.2, 29.4, 27.2, 22.9, 22.1; HRMS (ESI) $[M+H]^+$ calcd for $C_{31}H_{38}O_4N_6Cl$ 593.2643, found 593.2637; IR (ATR): 1704, 1670, 1635, 1596, 1508, 1457, 1442, 1322, 1263, 1218, 1106, 1051 cm^{-1}.

5.1.19. 2-[(2*R*,6*S*)-2,6-Dimethylmorpholin-4-yl]ethyl 2-[(3*R*)-3-aminopiperidin-1-yl]-3-(2-chlorobenzyl)-5-methyl-4-oxo-4,5-dihydro-3*H*-imidazo[4,5-*c*]quinoline-8-carboxylate dihydrochloride (10g)

Compound **10g** was prepared from **9g** in a manner similar to that described for compound **10b** with a quantitative yield as a white amorphous. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.87 (br s, 1H), 8.72 (d, J = 2.2 Hz, 1H), 8.41 (br s, 3H), 8.21 (dd, J = 2.2, 9.0 Hz, 1H), 7.71 (d, J = 9.0 Hz, 1H), 7.51 (dd, J = 1.3, 7.9 Hz, 1H), 7.32–7.19 (m, 2H), 6.70 (d, J = 6.8 Hz, 1H), 5.65 (d, J = 17.2 Hz, 1H), 5.58 (d, J = 17.2 Hz, 1H), 4.74 (m, 2H), 4.08–4.04 (m, 3H), 3.69–3.55 (m, 7H), 3.37–3.21 (m, 2H), 3.10–3.05 (m, 1H), 2.89–2.75 (m, 3H), 1.95 (m, 1H), 1.76 (m, 1H), 1.63–1.48 (m, 2H), 1.15 (d, J = 6.2 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.2, 157.7, 154.2, 140.8, 140.6, 135.2, 131.1, 129.6, 129.4, 129.2, 127.9, 127.1, 123.8, 122.9, 119.2, 116.2, 115.9, 68.6, 59.2, 55.5, 54.7, 52.3, 51.2, 46.5, 46.3, 29.3, 27.4, 22.1, 18.5; HRMS (ESI) $[M+H]^+$ calcd for $C_{32}H_{40}O_4N_6Cl$ 607.2794, found 607.2794; IR (ATR): 1718, 1673, 1652, 1635, 1558, 1540, 1508, 1457, 1324, 1272, 1220, 1132, 1060, 1033 cm^{-1}.

5.1.20. (5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 2-[(3*R*)-3-aminopiperidin-1-yl]-3-(2-chlorobenzyl)-5-methyl-4-oxo-4,5-dihydro-3*H*-imidazo[4,5-*c*]quinoline-8-carboxylate hydrochloride (10h)

Compound **10h** was prepared from **9h** in a manner similar to that described for compound **10b** with a quantitative yield as a white amorphous. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.67 (d, J = 1.8 Hz, 1H), 8.40 (br s, 3H), 8.07 (dd, J = 1.8, 9.0 Hz, 1H), 7.68 (d, J = 9.0 Hz, 1H), 7.50 (d, J = 7.7 Hz, 1H), 7.32–7.27 (m, 1H), 7.23–7.19 (m, 1H), 6.69 (d, J = 7.3 Hz, 1H), 5.60 (d, J = 17.4 Hz, 1H), 5.53 (d, J = 17.4 Hz, 1H), 5.29 (s, 2H), 3.73–3.69 (m, 1H), 3.62 (s, 3H), 3.41–3.23 (m, 2H), 3.08–3.05 (m, 1H), 2.87–2.81 (m, 1H), 2.30 (s, 3H), 1.97–1.89 (m, 1H), 1.75 (m, 1H), 1.62–1.51 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.1, 157.8, 154.2, 152.1, 141.0, 140.7, 140.6, 135.1, 133.7, 131.1, 129.6, 129.1, 129.1, 127.8, 127.1, 123.6, 122.6, 119.3, 116.3, 116.0, 54.7, 52.3, 51.1, 46.4, 46.3, 29.3, 27.4, 22.2, 9.2; HRMS (ESI) $[M+H]^+$ calcd for $C_{29}H_{29}O_6N_5Cl$ 578.1801, found 578.1782; IR (ATR): 1830, 1716, 1670, 1592, 1558, 1540, 1508, 1473, 1230, 1130, 1033 cm^{-1}.

5.1.21. (5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 2-[(3*R*)-3-aminopiperidin-1-yl]-3-(2-chloro-5-fluorobenzyl)-5-methyl-4-oxo-4,5-dihydro-3*H*-imidazo[4,5-*c*]quinoline-8-carboxylate hydrochloride (10i)

Compound **10i** was prepared from **9i** in a manner similar to that described for compound **10b** with a quantitative yield as a white amorphous. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.68 (d, J = 2.1 Hz, 1H), 8.39 (br s, 3H), 8.08 (dd, J = 2.1, 8.9 Hz, 1H), 7.69 (d, J = 8.9 Hz, 1H), 7.59–7.55 (m, 1H), 7.21–7.16 (m, 1H), 6.65–6.62 (m, 1H), 5.58 (d, J = 17.4 Hz, 1H), 5.52 (d, J = 17.4 Hz, 1H), 5.29 (s, 2H), 3.70–3.67 (m, 1H), 3.64 (s, 3H), 3.49–3.48 (m, 1H), 3.29–3.24 (m, 1H), 3.15–3.08 (m, 1H), 2.91–2.86 (m, 1H), 2.24 (s, 3H), 1.97 (m, 1H), 1.81–1.79 (m, 1H), 1.64–1.51 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.0, 161.2 (d, ^1J(C, F) = 243 Hz), 157.8, 154.2, 152.1, 141.1, 140.7, 140.6, 137.7 (d, ^3J(C, F) = 7.4 Hz), 133.6, 131.3 (d, ^3J(C, F) = 8.6 Hz), 129.1, 126.4 (d, ^4J(C, F) = 2.6 Hz), 123.6, 122.6, 119.3, 116.3, 116.3 (d, ^2J(C, F) = 24.7 Hz), 116.2, 114.4 (d, ^2J(C, F) = 24.6 Hz), 54.7, 52.3, 51.2, 46.5, 46.3, 29.3, 27.4, 22.1, 9.1; HRMS (ESI) $[M+H]^+$ calcd for $C_{29}H_{28}O_6N_5ClF$ 596.1707, found 596.1708; IR (ATR): 1816, 1716, 1668, 1592, 1558, 1540, 1521, 1508, 1457, 1429, 1373, 1303, 1255, 1224, 1191, 1110, 1049, 1033 cm^{-1}.

5.1.22. (5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 2-[(3*R*)-3-aminopiperidin-1-yl]-3-(2-chlorobenzyl)-5-methyl-4-oxo-4,5-dihydro-3*H*-imidazo[4,5-*c*]quinoline-7-carboxylate hydrochloride (10k)

Compound **10k** was prepared from **9k** in a manner similar to that described for compound **10b** with a yield of 95% as a white amorphous. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.42 (br s, 3H), 8.25 (d, J = 8.3 Hz, 1H), 8.06 (d, J = 1.3 Hz, 1H), 7.91 (dd, J = 1.3, 8.3 Hz,

1H), 7.50 (dd, J = 1.1, 7.7 Hz, 1H), 7.32–7.27 (m, 1H), 7.24–7.21 (m, 1H), 6.71 (d, J = 6.6 Hz, 1H), 5.62 (d, J = 17.4 Hz, 1H), 5.54 (d, J = 17.4 Hz, 1H), 5.28 (s, 2H), 3.69 (br s, 1H), 3.66 (s, 3H), 3.27–3.21 (m, 2H), 3.03 (m, 1H), 2.82–2.79 (m, 1H), 2.24 (s, 3H), 1.97 (m, 1H), 1.90 (m, 1H), 1.74–1.55 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.3, 157.7, 154.1, 152.1, 140.7, 140.1, 137.1, 135.1, 133.6, 131.1, 129.5, 129.1, 128.3, 127.9, 127.1, 122.9, 122.7, 120.5, 120.2, 116.7, 55.0, 52.3, 51.1, 46.5, 46.4, 29.0, 27.4, 22.2, 9.2; HRMS (ESI) $[M+H]^+$ calcd for $C_{29}H_{29}O_6N_5Cl$ 578.1801, found 578.1786; IR (ATR):1810, 1733, 1681, 1621, 1560, 1508, 1473, 1448, 1309, 1224, 1122, 1052, 1033 cm^{-1}.

5.1.23. (5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 2-[(3*R*)-3-aminopiperidin-1-yl]-3-(2-chloro-5-fluorobenzyl)-5-methyl-4-oxo-4,5-dihydro-3*H*-imidazo[4,5-*c*]quinoline-7-carboxylate hydrochloride (10l)

Compound **10l** was prepared from **9l** in a manner similar to that described for compound **10b** with a yield of 70% as a white amorphous. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.31 (br s, 3H), 8.26 (d, J = 8.2 Hz, 1H), 8.09 (d, J = 1.3 Hz, 1H), 7.93 (dd, J = 1.3, 8.2 Hz, 1H), 7.59–7.56 (m, 1H), 7.22–7.17 (m, 1H), 6.68–6.63 (m, 1H), 5.60 (d, J = 17.6 Hz, 1H), 5.55 (d, J = 17.6 Hz, 1H), 5.28 (s, 2H), 3.68 (s, 3H), 3.59–3.47 (m, 1H), 3.38–3.22 (m, 2H), 3.10–3.07 (m, 1H), 2.90–2.85 (m, 1H), 2.24 (s, 3H), 1.97–1.96 (m, 1H), 1.79–1.78 (m, 1H), 1.61–1.53 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.1, 161.0 (d, ^1J(C, F) = 243 Hz), 157.5, 154.0, 151.9, 140.4, 140.0, 137.5 (d, ^3J(C, F) = 8.2 Hz), 136.9, 133.3, 131.1 (d, ^3J(C, F) = 9.9 Hz), 128.2, 126.2 (d, ^4J(C, F) = 4.9 Hz), 122.7, 122.6, 122.5, 120.3, 116.5, 115.9 (d, ^2J(C, F) = 23.6 Hz), 114.3 (d, ^2J(C, F) = 25.6 Hz), 54.8, 52.0, 50.9, 46.4, 46.1, 28.8, 27.1, 21.8, 8.9; HRMS (ESI) $[M+H]^+$ calcd for $C_{29}H_{28}O_6N_5ClF$ 596.1707, found 596.1707; IR (ATR): 1810, 1718, 1683, 1635, 1508, 1473, 1307, 1218, 1112, 1051, 1033, 1008 cm^{-1}.

5.1.24. Methyl 4-[{[4-amino-2-{(3*R*)-3-[(*tert*-butoxycarbonyl)amino]piperidin-1-yl}-1-(2-chlorobenzyl)-1*H*-imidazol-5-yl]carbonyl}(methyl)amino]benzoate (12)

A mixture of **14** (3.72 g, 9.50 mmol), **13** (2.53 g, 10.47 mmol) and cesium carbonate (6.19 g, 19.00 mmol) in MeCN (10 mL) was stirred at 40 °C for 4 h, and stirred at 60 °C for 3 h. After cooling to room temperature, the mixture was filtered through a Celite pad. The filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give **12** (4.27 g, yield 75%) as a pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.86 (d, J = 8.8 Hz, 2H), 7.39–7.37 (m, 1H), 7.25–7.21 (m, 2H), 6.91 (d, J = 6.3 Hz, 1H), 6.77 (d, J = 8.8 Hz, 2H), 5.03 (br s, 1H), 4.87 (s, 2H), 4.12 (s, 2H), 3.90 (s, 3H), 3.76–3.73 (m, 1H), 3.29 (s, 3H), 3.27–3.25 (m, 1H), 2.97–2.94 (m, 3H), 1.73–1.56 (m, 4H), 1.41 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.0, 163.0, 154.8, 148.8, 134.4, 133.2, 130.2, 130.0, 129.4, 129.3, 128.9, 126.8, 126.4, 125.5, 122.6, 103.8, 78.8, 54.8, 51.8, 51.2, 47.0, 45.7, 36.5, 29.3, 28.1, 22.1; HRMS (ESI) $[M+H]^+$ calcd for $C_{30}H_{38}ClN_6O_5$ 597.2587, found 597.2579; IR (ATR): 1705, 1600, 1512, 1473, 1435, 1404, 1346, 1311, 1276, 1246, 1165, 1107, 1049, 1014, 991 cm^{-1}.

5.1.25. Methyl 4-[(chloroacetyl)(methyl)amino]benzoate (13)

To a solution of chloroacetyl chloride (1.23 g, 10.89 mmol) in THF (5 mL) were added methyl 4-methylaminobenzoate (1.50 g, 9.08 mmol) and pyridine (718 mg, 9.08 mmol) in THF (10 mL) solution dropwise at 0 °C, and the mixture was stirred at 0 °C for 3 h. The reaction mixture was quenched with saturated NH_4Cl aqueous solution, extracted with EtOAc, washed with saturated $NaHCO_3$ aqueous solution, brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica-gel column chromatography to give **13** (2.19 g, quant.) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (d, J = 8.5 Hz, 2H), 7.32 (d, J = 8.5 Hz, 2H), 3.92 (s, 3H), 3.85 (br s, 2H), 3.32 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.8, 165.7, 146.5, 131.1, 129.8, 126.7, 52.2. 41.2. 37.6; HRMS (ESI) $[M+H]^+$ calcd for $C_{11}H_{13}O_3NCl$ 242.0578, found 242.0585; IR (ATR): 1716, 1674, 1600, 1504, 1435, 1408, 1373, 1257, 1188, 1172, 1095, 1045, 1014, 952 cm^{-1}.

5.1.26. *tert*-Butyl {(3*R*)-1-[*N*-(2-chlorobenzyl)-*N*′-cyanocarbamimidoyl]piperidin-3-yl}carbamate (14)

To solution of (*R*)-3-*tert*-butoxycarbonylaminopipiridine (1.63 g, 8.14 mmol) in *i*PrOH (60 mL) was added diphenyl cyanocarbonimidate (2.00 g, 8.14 mmol) at 0 °C. The mixture was stirred at room temperature for 1 h. To the reaction mixture was added 2-chlorobenzyl amine (1.73 g, 12.22 mmol) at rt, and the mixture was stirred at 80 °C for 2 h. After cooling to room temperature, the reaction mixture was concentrated in vacuo. The residue was diluted with 5% potassium hydrogensulfate aqueous solution, and extracted with AcOEt, washed with 5% potassium hydrogensulfate aqueous solution, 1N NaOH aqueous solution twice, brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica-gel column chromatography to give **14** (3.04 g, yield 95%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.46–7.44 (m, 1H), 7.38–7.36 (m, 1H), 7.28–7.25 (m, 2H), 5.78 (br s, 1H), 4.66 (s, 1H), 4.65 (s, 1H), 3.75–3.70 (m, 1H), 3.62–3.57 (m, 2H), 3.45 (br s, 1H), 3.27 (br s, 1H), 1.90–1.88 (m, 1H), 1.78–1.74 (m, 2H), 1.63–1.59 (m, 2H), 1.41 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.3, 155.5, 134.8, 133.3, 129.9, 129.4, 129.0, 126.9, 117.5, 79.8, 51.3, 47.4, 46.8, 45.0, 29.6, 28.1, 22.9; HRMS (ESI) $[M+H]^+$ calcd for $C_{19}H_{27}O_2N_5Cl$ 392.1848, found 392.1853; IR (ATR): 2164, 1685, 1570, 1531, 1438, 1388, 1365, 1311, 1284, 1242, 1165, 1080, 1049, 1022, 948 cm^{-1}.

5.1.27. Methyl 2-{(3*R*)-3-[(*tert*-butoxycarbonyl)amino]piperidin-1-yl}-3-(2-chlorobenzyl)-5-methyl-4-oxo-4,5-dihydro-3*H*-imidazo[4,5-*c*]quinoline-8-carboxylate (9a)

A mixture of **12** (119 mg, 0.199 mmol), isoamylnitrite (0.134 mL, 1.00 mmol) in 1–4 dioxane (2 mL) was stirred at 80 °C for 3 h. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give **9a** (84.7 mg, yield 73%) as a pale yellow amorphous. Mp 186–188 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.95 (d, J = 1.8 Hz, 1H), 8.18 (dd, J = 2.1, 9.0 Hz, 1H), 7.47 (d, J = 11.6 Hz, 1H), 7.42 (d, J = 7.5 Hz, 1H), 7.27–7.12 (m, 2H), 6.70 (d, J = 6.9 Hz, 1H), 5.78 (d, J = 17.4 Hz, 1H), 5.63 (d, J = 17.4 Hz, 1H), 5.40–5.37 (m, 1H), 3.98 (s, 3H), 3.84 (br s, 1H), 3.76 (s, 3H), 3.48–3.31 (m, 1H), 3.24–3.18 (m, 1H), 3.10 (m, 2H), 1.78 (m, 4H), 1.44 (s, 9H); HRMS (ESI) $[M+H]^+$ calcd for $C_{30}H_{35}O_5N_5Cl$ 580.2321, found 580.2314; IR (ATR): 1718, 1689, 1652, 1569, 1500, 1446, 1429, 1388, 1353, 1313, 1274, 1234, 1174, 1124, 1110, 1066, 1039 cm^{-1}; Anal. Calcd for $C_{30}H_{34}ClN_5O_5$: C, 62.12; H, 5.91; N, 12.09, found: C, 62.11; H, 5.88; N, 12.07.

5.2. Biological experiments

5.2.1. PD test in SD rats

SD rats (male, 11 weeks old) were fasted for 24 h prior to predose blood sampling (−0.5 h) and kept fasted until the end of blood sampling performed 12 h after dosing. The rats received the test-compound (or test-drug) solution orally by gavage. Blood samples were collected 0.5 h before (−0.5 h), and 1, 2, 4, 6, 10 and 24 h after dosing (0 h). Time-course changes in plasma DPP-4 activity after administration of **10h** (3 mg/kg) or sitagliptin (3 mg/kg) were determined. DPP-4 activity in plasma samples was measured by the following procedure. Plasma dilution buffer (5 μL containing 80 mmol/L $MgCl_2$, 140 mmol/L NaCl, 25 mmol/L HEPES, and 1% (w/v%) BAS, pH 7.8) was placed into each well of a 96-well plate

and a 96 well Half Area Black with Clear Flat Bottom 3881 (Corning Incorporated), and 5 μL of each plasma sample or distilled water (for measurement of background reaction) was added to each individual well, mixed and left to stand for at least 5 min. To initiate the reaction, 10 μL of 100 μmol/L Gly-Pro-MCA solution diluted with substrate dilution buffer (140 mmol/L NaCl, 25 mmol/L HEPES, 1% (w/v%) BAS, pH 7.8) was added to each well containing a plasma sample or distilled water. Twenty minutes later, 5% acetic acid was added to stop the reaction. Fluorescence intensity in RFUs (excitation wavelength 360 nm/emission wavelength 460 nm) was continuously monitored using a fluorescence microplate reader (SpectraMax® Gemini EM). Next, 20 μL of standard AMC solution (1.28. 2.52, 5, 10, 20, 40 μmol/L; diluted with plasma dilution buffer, substrate dilution buffer, rat plasma and DMSO) was placed into each of the wells with no plasma sample or distilled water, and fluorescence intensity in RFUs (excitation wavelength 360 nm/emission wavelength 460 nm) of the standard AMC solution was measured to construct a calibration curve of RFU against AMC per well. The curve was constructed on an arithmetic scale using unweighed linear regression in SoftMax®Pro version 4.3.1 (Molecular Devices Corporation), within the fluorescence microplate reader. The rates of AMC production in the samples and background wells were calculated using the constructed calibration curve. The net rate of AMC production (nmol/min) in a given sample well was determined by subtraction of mean background rate from the rate for the sample well. DPP-4 activity (mU/mL) per plasma unit was calculated by dividing the rate of AMC production by the volume of plasma sample used for the measurement. Duplicate measurements were performed for the standard AMC solution and for each plasma sample.

5.2.2. Oral glucose tolerance test (OGTT) in Zucker fatty rats

Zucker fatty rats (male, 11 weeks) were fasted overnight before each OGTT. Glucose (0.2 g/mL D-glucose solution) was orally loaded by gavage in a volume of 10 mL/kg (2 g of D-glucose/kg). Each concentration of **10h** (0.3, 1.0 mg/kg) suspension or 0.5% Methyl cellulose (MC) solution used as a control was administrated once 1 h prior to glucose loading. Blood samples for determination of blood glucose concentration were taken just before administration of the test-substance or 0.5% MC solution (−1 h relative to the time of glucose loading), just before glucose loading (taken as 0 min), and 10, 30, 60, 120 min after glucose loading. Blood glucose concentration was measured for all the blood samples collected. A 10 μL blood sample taken from the tail vein of each rat was immediately mixed with 100 μL of 0.62 mol/L perchloric acid solution. Potassium carbonate solution (50 μL, 0.37 mol/L) was then added and mixed. The deproteinized samples were stored in a refrigerator set at 4 °C until measurement of blood glucose concentration. Blood glucose concentration was measured using a commercially available kit, Glucose CII test Wako (Wako pure Chemical Industries, Ltd). The deproteinized samples were centrifuged at 2000 rpm for 10 min, and 15 μL of the supernatant of each test sample and glucose standard solution (100, 250, 500, 750 mg/dL) were added to 96-well plates, followed by addition of 200 μL of a coloring reagent to each well and mixing. Color reaction was allowed to develop for 18–35 min at room temperature, and absorbance was measured using a microplate reader (SpectraMax®190) at a main wavelength of 505 nm and a sub-wavelength of 700 nm. A linear calibration curve was constructed from differences in absorbance (A_{505}–A_{700}, *Y*-axis) of the glucose standard solution (mg/dL, *X*-axis), using a software provided with the microplate reader (SoftMax® Pro version 4.3.1). Glucose concentration of each test sample was determined by interpolation of its absorbance (A_{505}–A_{700}) on the standard curve. Samples and standard solution glucose concentrations were determined in duplicate.

5.3. Metabolic stability test

5.3.1. Materials

Human serum was purchased from COSMO BIO Co., Ltd (Tokyo, Japan). Pooled liver S9 fractions from male Sprague–Dawley (SD) rats, male beagle dogs and humans were purchased from Xenotech, LLC (Lenexa, KS). Rat intestinal S9 fraction prepared without protease inhibitor was purchased from Biopredic international (Rennes, France). β-NADPH was purchased from Oriental Yeast Co., Ltd (Tokyo, Japan).

5.3.2. Hydrolysis experiments

Hydrolysis experiments were performed in serum and in tissue S9 fractions from rats, dogs and humans. Test-compounds were incubated at 37 °C for 30 min in 100 μL of serum or in an S9 reaction mixture consisting of 50 mM potassium phosphate buffer (pH7.4), 3 mM NADPH and 1 mg protein/mL S9 fraction. The final concentration of each test-compound was set to 1 μM. After preincubation at 37 °C for 5 min, the hydrolysis reaction was initiated by addition of the test-compound dissolved in acetonitrile–water (1:1, v/v) and terminated by addition of 300 μL of ice-cold acetonitrile. The reaction mixture was then centrifuged at 4500 rpm for 10 min to remove precipitated proteins, and the supernatant was filtrated using a 0.45 μm 96-well filter plate (Varian Inc., Palo Alto, CA). The filtrate was next injected onto a high-performance liquid chromatography/tandem mass spectrometry (HPLC/MS/MS) system.

5.3.3. Analytical procedure

The concentrations of test-compounds and their sample hydrolysates were measured using an HPLC-MS/MS system consisting of a TSQ 7000 mass spectrometer (Thermo Fisher Scientific Inc., Waltham, MA) with a Shimadzu 10A series HPLC system (Shimadzu Corporation, Kyoto, Japan). Chromatography was performed using a Symmetry C18 column (5 mm particle size, 2.1 × 150 mm, Waters Corporation, Milford MA) warmed to 40 °C. The mobile phase consisted of 10 mM ammonium acetate (pH 4.0, solvent A) and acetonitrile (solvent B). Elution gradient conditions were as follows: [min, B%] = [0, 20] – [6.5, 80] – 8.5, 80] – [8.6, 20] – [12, 20], and flow rate was 0.2 mL/min. Mass spectrometric detection was performed using positive ionization electrospray. The selective reaction monitoring mode (*m*/*z*: precursor ion→product ion) was used to monitor ions.

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