Bioorganic & Medicinal Chemistry 19 (2011) 4953–4970

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Identification of 3-aminomethyl-1,2-dihydro-4-phenyl-1-isoquinolones: A new class of potent, selective, and orally active non-peptide dipeptidyl peptidase IV inhibitors that form a unique interaction with Lys554

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ARTICLE INFO

Article history:
Received 13 May 2011
Revised 20 June 2011
Accepted 20 June 2011
Available online 28 June 2011

Keywords:
Dipeptidyl peptidase IV (DPP-4)
Isoquinolone
Anti-diabetic effects

ABSTRACT

The design, synthesis, and structure–activity relationships of a new class of potent and orally active non-peptide dipeptidyl peptidase IV (DPP-4) inhibitors, 3-aminomethyl-1,2-dihydro-4-phenyl-1-isoquinolones, are described. We hypothesized that the 4-phenyl group of the isoquinolone occupies the S1 pocket of the enzyme, the 3-aminomethyl group forms an electrostatic interaction with the S2 pocket, and the introduction of a hydrogen bond donor onto the 6- or 7-substituent provides interaction with the hydrophilic region of the enzyme. Based on this hypothesis, intensive research focused on developing new non-peptide DPP-4 inhibitors has been carried out. Among the compounds designed in this study, we identified 2-[(3-aminomethyl-2-(2-methylpropyl)-1-oxo-4-phenyl-1,2-dihydro-6-isoquinolinyl)oxy]acetamide (**35a**) as a potent, selective, and orally bioavailable DPP-4 inhibitor, which exhibited in vivo efficacy in diabetic model rats. Finally, X-ray crystallography of **35a** in a complex with the enzyme validated our hypothesized binding mode and identified Lys554 as a new target-binding site available for DPP-4 inhibitors.

1. Introduction

Dipeptidyl peptidase IV (DPP-4, EC 3.4.14.5) is an extracellular membrane-bound serine protease expressed on the cell surface of a variety of organs, for example, intestine, kidney, and liver.[1] It catalyses the cleavage of N-terminal dipeptides from polypeptides with L-proline or L-alanine residues situated at the penultimate position of the substrates.[2,3] The unique aminopeptidase function of DPP-4 has been shown to modulate the biological activities of circulating regulatory peptides involved in the endocrine, neuroendocrine, and immune systems in vitro.[4] It has been revealed that DPP-4 plays a principal role in the inactivation of glucagon-like peptide-1 (GLP-1 or GLP-1[7–36]amide), which contributes to the control of blood glucose levels in vivo.[4–13]

GLP-1 is one of the gastrointestinal hormones (incretins) secreted by intestinal L-cells in response to the ingestion of nutrients.[14–16] It is known as the most potent insulinotropic hormone that increases glucose-stimulated insulin secretion. Furthermore, numerous studies have indicated the importance of GLP-1 not only as a potent insulin secretagogue, but also as a multifunctional hypoglycemic hormone responsible for the stimulation of β-cell growth, survival, and differentiation, the inhibition of glucagon release, the retardation of gastric emptying, etc. Actually, many clinical studies have shown that GLP-1 normalizes postprandial and fasting glycemia in subjects with type 2 diabetes.[17–21] On the basis of the above evidence, GLP-1 itself is one of the logical candidates for therapeutic agents in the treatment of diabetes.[22–25] However, active GLP-1 is rapidly metabolized by DPP-4 resulting in GLP-1[9–36]amide.[1] Therefore, alternative strategies to elicit the beneficial anti-diabetic effects of GLP-1 are required. One of the promising approaches is the inhibition of DPP-4 activity, which can extend the duration of GLP-1 action through the blockade of its degradation, resulting in improvement of the elevated glucose levels in diabetes. Accordingly, DPP-4 inhibitors have been expected to serve as a new type of glucose-lowering agent with little or no adverse effects observed with sulfonylureas. Many DPP-4 inhibitors, such as MK-403 (sitagliptin phosphate),[26] BMS-477118 (saxagliptin),[27,28] LAF-237 (vildagliptin),[28] and SYR-322

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doi:10.1016/j.bmc.2011.06.059

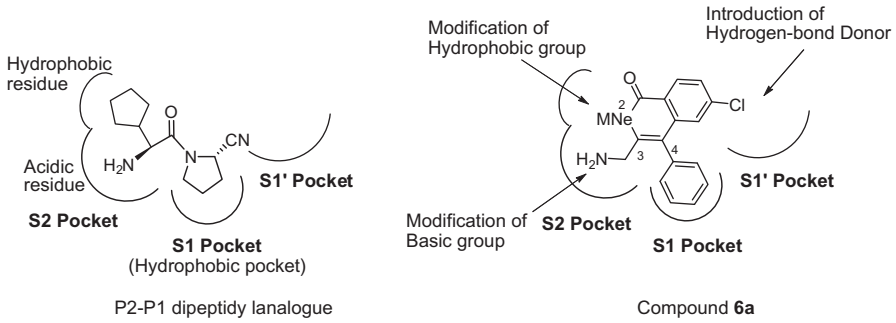


Chart 1. Binding mode of P2–P1 dipeptidyl analog of the substrate[30] and the lead compound **6a**.

(alogliptin benzoate),[29] have demonstrated improved blood sugar control in patients with type 2 diabetes. In our research for a new non-peptide DPP-4 inhibitor, high-throughput screening of the in-house chemical library led to the identification of an isoquinolone-based lead compound **6a** that showed potent inhibitory activity against human DPP-4 with a half-maximal inhibitory concentration (IC_{50}) value of 16 μM.

In this article, we describe the synthesis and structure–activity relationships (SARs) of the 1,2-dihydro-4-phenyl-1-isoquinolone derivatives, starting from the lead 3-(aminomethyl)-6-chloro-2-methyl-4-phenylisoquinolin-1(2*H*)-one (**6a**). The predicted binding mode of compound **6a** to DPP-4 is illustrated with the mode of a P2–P1 dipeptidyl analog of the substrate in Chart 1.[30] A docking study of compound **6a** with the enzyme indicated that the 3-aminomethyl and 4-phenyl moieties of the isoquinolone ring interacted with S2 and S1 pockets of the enzyme, respectively. On the basis of the results, we hypothesized that the 2-position of the isoquinolone would correspond to the hydrophobic moiety of the P2 fragment, and the introduction of a hydrogen bond donor onto the 6- or 7-position of the isoquinolone ring would increase the inhibitory activity by forming an interaction with the hydrophilic region of the enzyme. In order to verify our hypothesis, we first investigated the importance of the basic group at the 3-position and next investigated the effect of introducing an appropriate lipophilic moiety at the 2-position of the isoquinolone core and a hydrogen bond donor at the 6- or 7-position. Finally, we confirmed the binding mode of the isoquinolone derivative by using X-ray co-crystallography.

These studies revealed that the isoquinolone nucleus is an excellent template mimicking a P2–P1 dipeptidyl substrate and discovered the potent, selective, non-peptide DPP-4 inhibitor 2-{[3-(aminomethyl)-2-(2-methylpropyl)-1-oxo-4-phenyl-1,2-dihydroisoquinolin-6-yl]oxy}acetamide (**35a**). The results of co-crystallographic studies of **35a** and the pharmacological profiles are also reported.

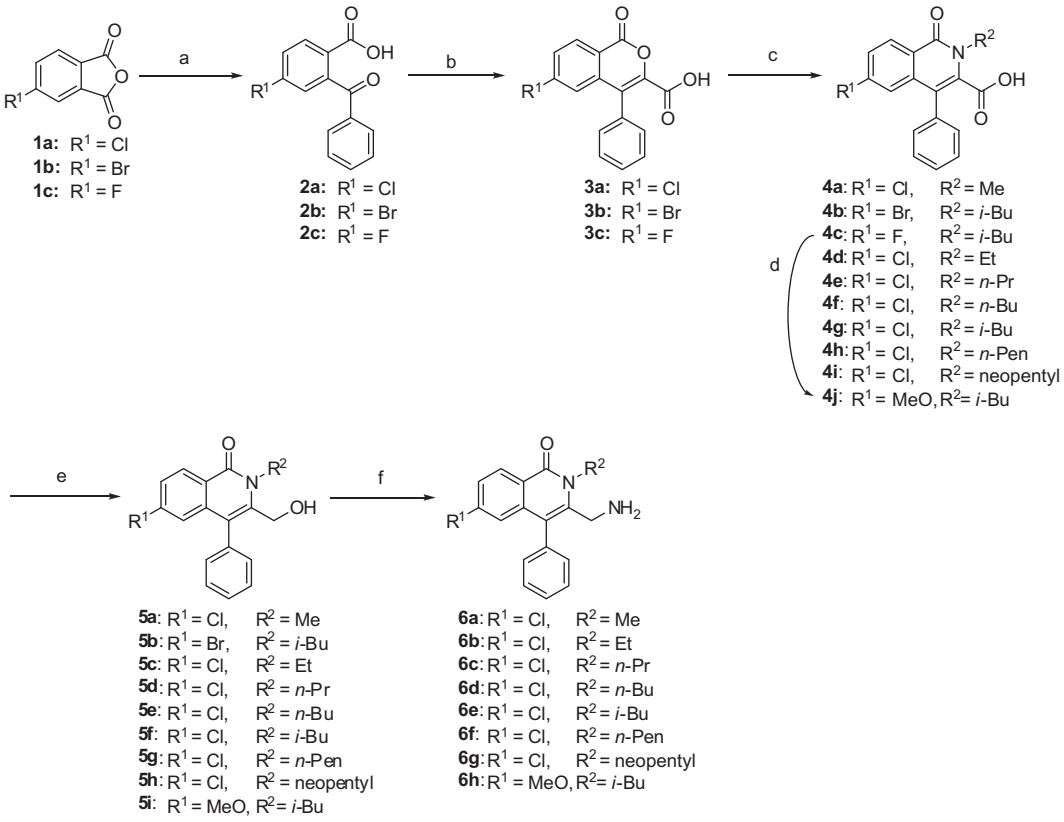
2. Chemistry

Construction of the 4-phenyl-1-isoquinolone nucleus was performed according to the previously reported procedure[31] shown in Scheme 1. Initially, commercially available 4-substituted phthalic anhydrides **1a–c** were reacted with benzene under Friedel–Crafts acylation conditions in order to afford 4-substituted-2-benzoylbenzoic acids **2a–c** together with the undesired regioisomers in an approximate 1:1 mixture. The undesired regioisomer, however, was easily removed by recrystallization in order to give pure compounds **2a–c**. After alkylation of **2a–c** with diethyl 2-bromomalonate, the resulting diesters were converted to isocoumarin-3-carboxylic acids **3a–c** by hydrolysis under reflux in concentrated hydrochloric acid–acetic acid and consequent decarboxylation and dehydration. A ring-opening reaction of **3a–c** with various primary amines and subsequent recyclization under acidic conditions furnished the 4-phenyl-1-isoquinolone-3-carboxylic acids **4a–i**. The fluorine atom of **4c** was replaced with a methoxy group to yield **4j**. The reduction of **4a**,**b** and **4d–j** with sodium borohydride with the corresponding acid chlorides provided hydroxymethyl derivatives **5a–i**. Methanesulfonylation or chlorination of **5a** and **5c**–**i** followed by a reaction with ethanolic ammonia in a sealed tube afforded the desired products **6a–h**.

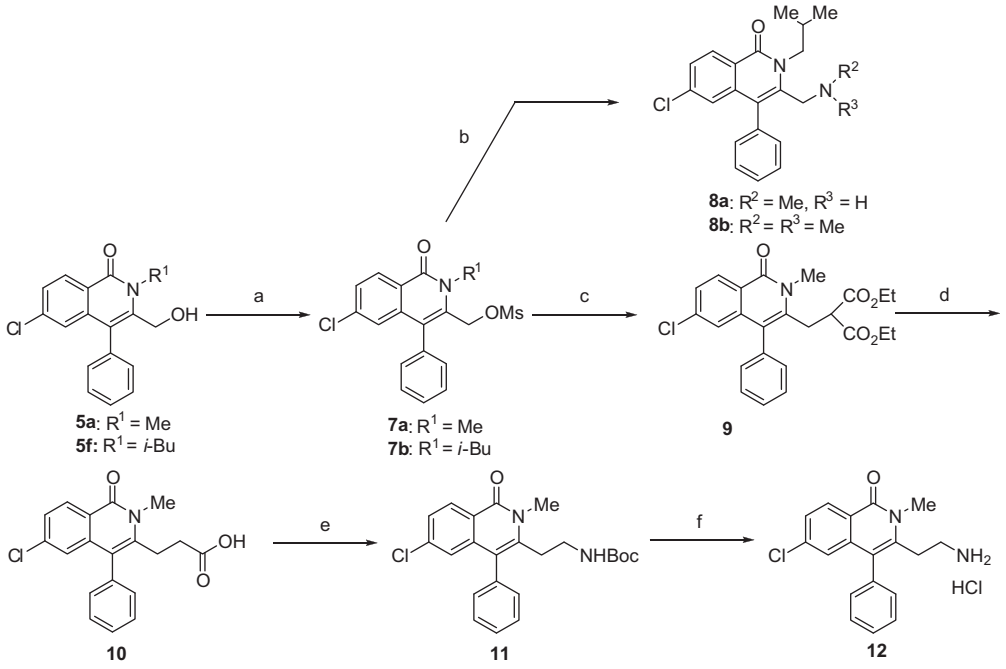
Modification of the basic group at the 3-position of the 4-phenyl-1-isoquinolones was conducted by the routes illustrated in Schemes 2 and 3. The hydroxymethyl derivatives **5a** and **5f** were converted to mesylates **7a** and **7b,** and then, the reaction of the compound **7b** with a large excess of methylamine or dimethylamine afforded the corresponding secondary amine **8a** or tertiary amine **8b**, respectively. Reaction of **7a** with diethyl malonate in the presence of sodium hydride gave the diester **9**, which was converted to propionic acid **10** by acidic hydrolysis with heating in concentrated hydrochloric acid and consequent decarboxylation. Curtius rearrangement reaction of the 3-carboxylic acid **10** with diphenylphosphoryl azide (DPPA) gave isocyanate, which was reacted with *tert*-butyl alcohol in one-pot to produce a primary amine protected by a *tert*-butoxycarbonyl (Boc) group. Next, deprotection of the Boc group of **11** provided the desired 3-(2-amino)ethyl analog **12**. The compound **4a** was derivatized to amine **13** by a similar procedure as that described above for the preparation of **12**.

The synthetic routes for 6-carboxylic acid **19a**, 6-carboxamides **19b–d**, 6-cyano **19e**, 6-acrylamide **23a,** and 6-propylamide **23b** are shown in Schemes 4 and 5. The compound **5b** was converted to the aminomethyl derivative, which was then protected by a Boc group to yield compound **14**. Palladium-catalyzed methoxycarbonylation of the bromide **14** using carbon monoxide and methanol afforded the methyl ester **15**.[32] Hydrolysis of the ester **15** gave carboxylic acid **16**, which was converted to the amides **17a–c** by condensation with the corresponding amines. The cyano derivative **18** was prepared by dehydration of **17a** using cyanuric chloride. Removal of the Boc group of **16**, **17a–c**, and **18** produced the aminomethyl derivatives **19a–e.** In addition, compound **14** was subjected to the Heck reaction with ethyl acrylate in the presence of the palladium catalyst to provide exclusively ethyl (*E*)-propenate **20a**.[33] Compound **20a** was then hydrogenated over palladium-charcoal to yield propionate **20b**. Hydrolysis of **20** gave the corresponding carboxylic acids **21**, which were converted to the desired carboxamide **23**, respectively, by amidation and subsequent deprotection.

The synthesis of 6- or 7-carbamoylmethoxy-1-isoquinolone **35** was carried out by the route depicted in Scheme 6. Commercially available 4-hydroxyphthalic acid **24** was converted to 4-benzyloxyphthalic anhydride **26** by benzylation, subsequent saponification, and dehydration using acetic anhydride. Nucleophilic attack



Scheme 1. Synthesis of compounds **6a–h**. Reagents: (a) $AlCl_3$, benzene; (b) (i) $BrCH(CO_2Et)_2$, K_2CO_3, DMF, acetone; (ii) concd HCl, AcOH; (c) (i) R^2NH_2, MeOH; (ii) 4 M HCl–AcOEt; (d) MeONa, MeOH; (e) (i) $(COCl)_2$, DMF, THF; (ii) $NaBH_4$, DME, THF; (f) (i) MsCl, Et_3N, CH_2Cl_2; (ii) 10% NH_3–MeOH, THF or 10% NH_3–EtOH, THF.

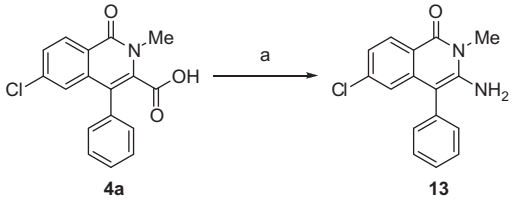


Scheme 2. Synthesis of compounds **8a,b** and **12**. Reagents: (a) MsCl, Et_3N, CH_2Cl_2; (b) R^2R^3NH, K_2CO_3, THF; (c) NaH, $CH_2(CO_2Et)_2$, THF; (d) concd HCl, AcOH; (e) (i) DPPA, Et_3N, DMF; (ii) toluene; (iii) *t*-BuOH; (f) 4 M HCl–AcOEt.

of *N*-isobutylglycine ethyl ester provided the corresponding carboxylic acids as a mixture of the regioisomers **27**. Hence, the mixture of the carboxylic acids **27** was then converted to the 6- or 7-benzyloxyisoquinolones **28a** and **28b** by alkylation and subsequent cyclization under basic conditions. The regioisomers **28a** and **28b** were successfully separated by column

chromatography. The 4-hydroxy-1-isoquinolone derivatives **28** were converted to triflates **29**. Suzuki coupling reaction of the triflates with phenylboronic acid was followed by saponification to give 4-phenyl-1-isoquinolone-3-carboxylic acids **31**. Compounds **31** were converted to Boc-protected derivatives **32** by using Gabriel synthesis. Catalytic hydrogenation of **32**, followed by the reaction with iodoacetamide gave carboxamides **34**. The target amines **35a**, **35b,** and **36** were readily obtained by the removal of the Boc group of **34a**, **34b,** and **33a**, respectively.

The synthesis of 7-carboxamide derivative **41** is shown in Scheme 7. Alkoxycarbonylation of triflate **37**, obtained from the 7-hydroxy derivative **33b**, was employed in a similar method to that used for the preparation of compound **15** in Scheme 4 to give methyl ester **38**. Compound **38** was then converted to the final product **41** by the same procedure described in Scheme 4.



Scheme 3. Synthesis of compound **13**. Reagents: (a) (i) DPPA, Et_3N, DMF; (ii) toluene; (iii) *t*-BuOH; (iv) 4 M HCl–AcOEt.

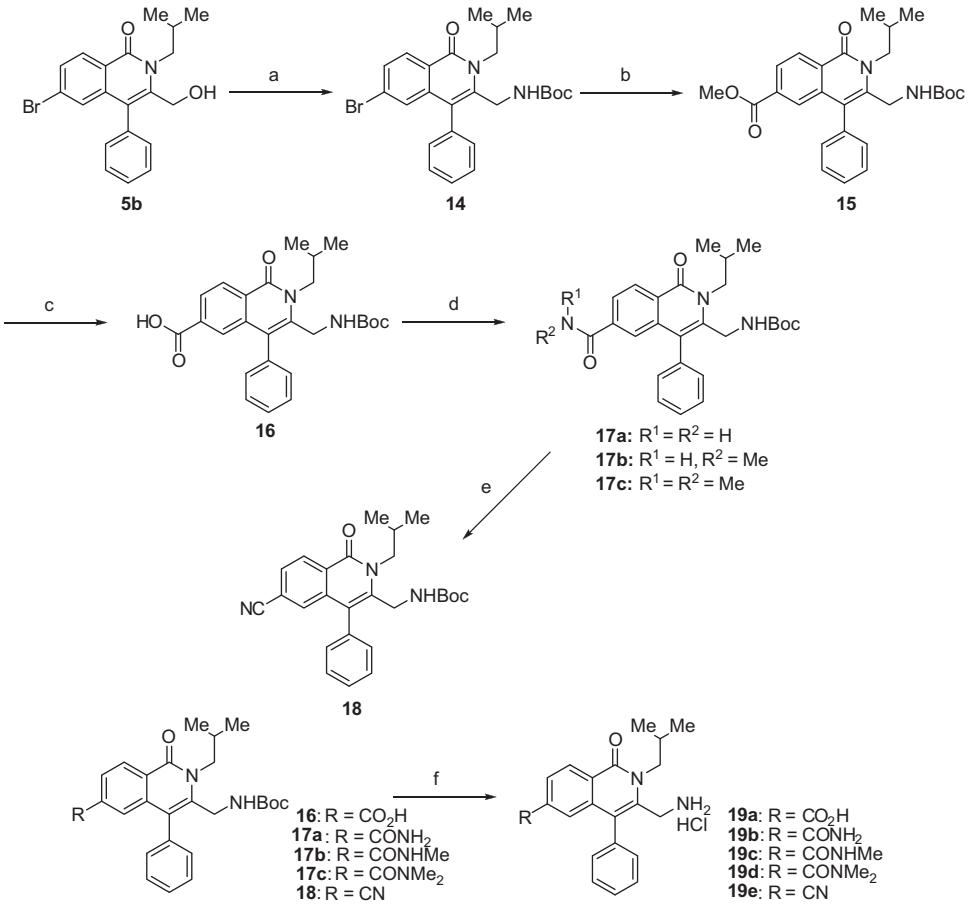
3. Results and discussion

3.1. In vitro enzyme inhibition studies

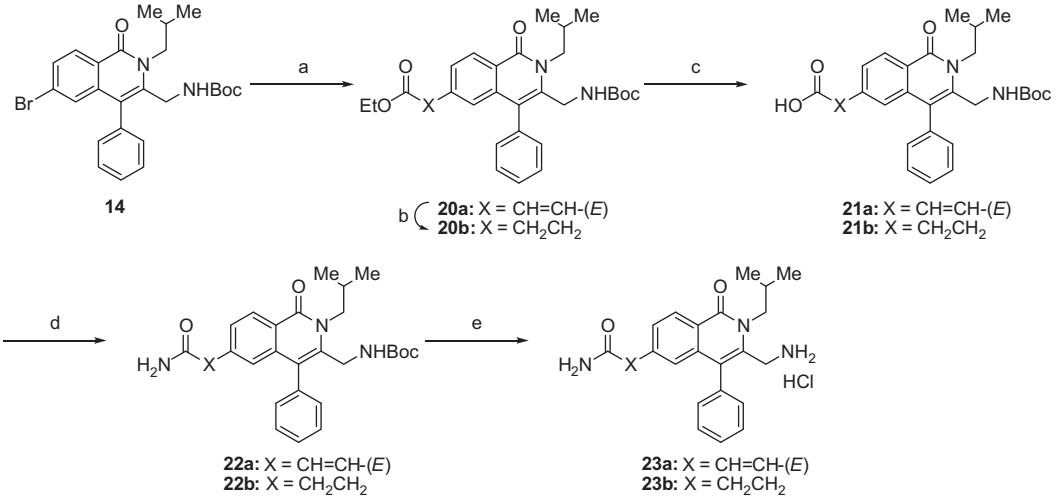
The 4-phenyl-1-isoquinolones described above were evaluated for their in vitro inhibition of human DPP-4, and the results are expressed as IC_{50} values.

First, a 3-substituent of the 4-phenyl-1-isoquinolone analog was investigated in order to confirm the role of the basic function of the lead compound **6a,** as presented in Table 1. Replacement of the aminomethyl moiety of **6a** with a hydroxymethyl group was unfavorable to its activity (**5a** vs **6a**). Shortening of the linker length between the C3 of the isoquinolone core and the nitrogen atom led to a decrease in activity (**13** vs **6a**). In addition, elongation of the linker length reduced the inhibitory activity (**12** vs **6a**). N-Methylation or N,N-dimethylation of **6e** resulted in a marked decrease in activity. These results indicated the importance of the unsubstituted aminomethyl group as the optimal basic moiety in this system. Hence, the aminomethyl group was selected for optimization in view of its inhibitory activity.

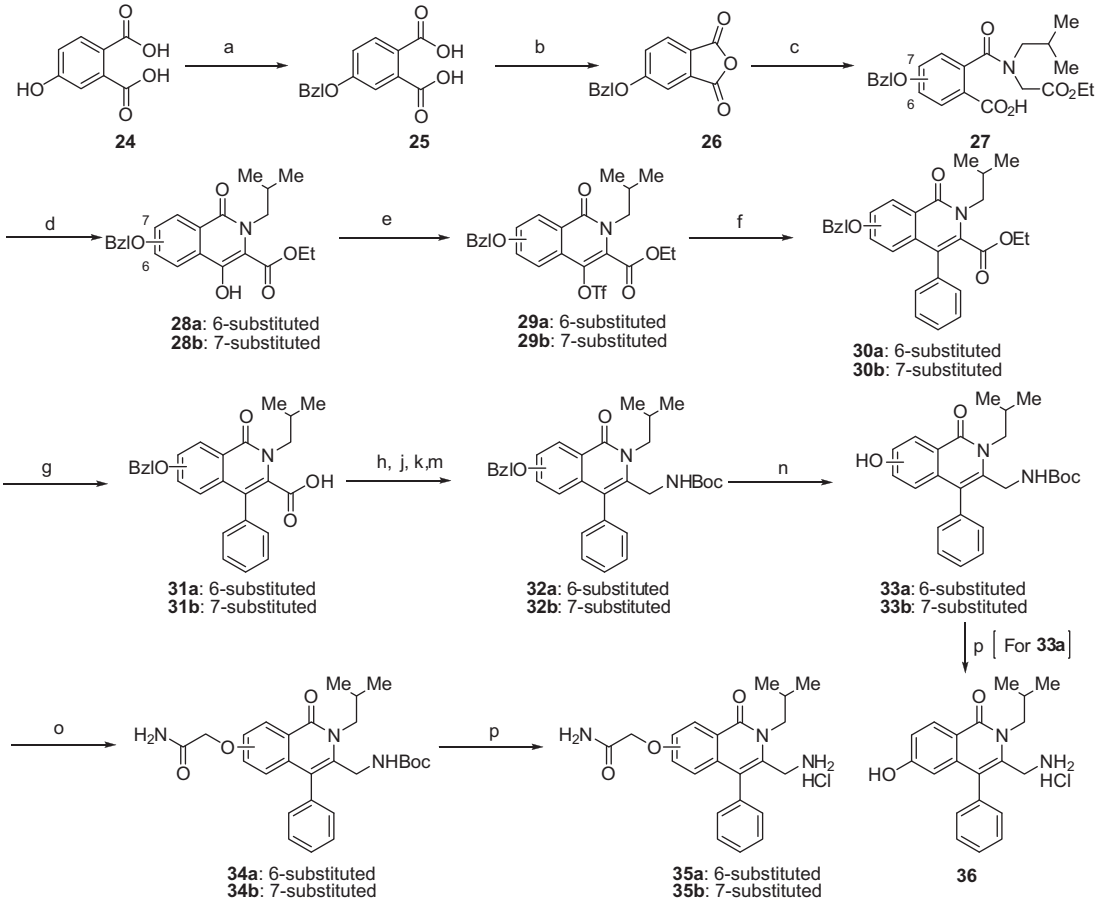
Next, the effect of the 2-substituent on the 4-phenyl-1-isoquinolone core was evaluated, as shown in Table 2. Introduction of a sterically bulky group resulted in further enhancement of the activity (**6e** and **6g** vs **6a**). These results suggest that the 2-alkyl substituent interacts with the hydrophobic residue in the target enzyme, which supports our hypothesis described above (Chart 1). Accordingly, the isobutyl group was chosen as the 2-substituent for the subsequent SAR studies.



Scheme 4. Synthesis of compounds **19a–e**. Reagents: (a) (i) $SOCl_2$, pyridine, THF, toluene; (ii) 2 M NH_3–EtOH; (iii) Boc_2O, DMAP, THF; (b) CO, $Pd(OAc)_2$, dppp, Et_3N, DMSO, MeOH; (c) NaOH, MeOH, THF; (d) HOBt·NH_3 or R^1R^2NH and HOBt, WSC, DMF; (e) cyanuric chloride, DMF; (f) 4 M HCl–AcOEt, THF.

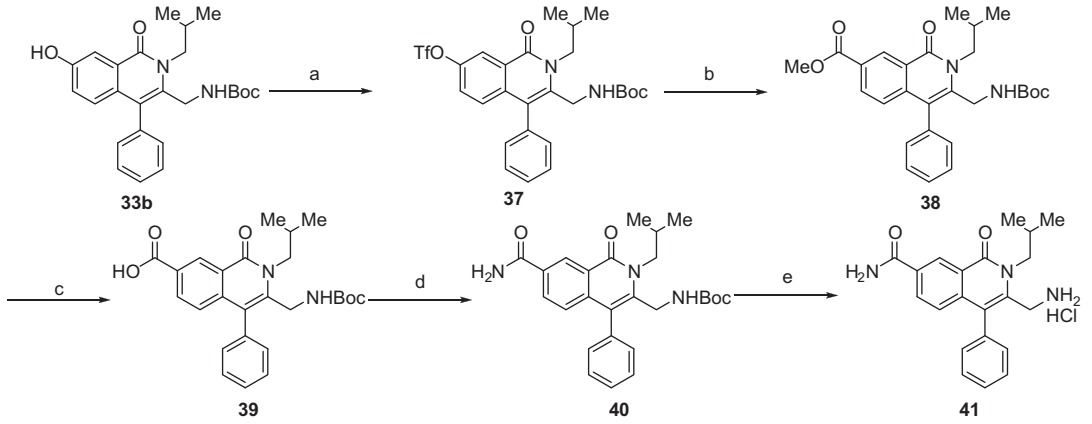


Scheme 5. Synthesis of compounds **23a** and **23b**. Reagents: (a) ethyl acrylate, $Pd(OAc)_2$, Et_3N, DMF; (b) 5% Pd–C, H_2, EtOH, THF; (c) NaOH, MeOH, THF; (d) WSC, HOBt·NH_3, DMF; (e) 4 M HCl–AcOEt.



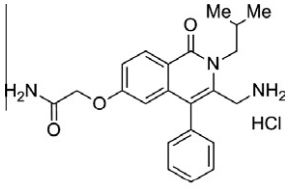
Scheme 6. Synthesis of compounds **35a,b** and **36**. Reagents: (a) (i) $PhCH_2Br$, K_2CO_3, DMF; (ii) NaOH, MeOH, THF; (b) Ac_2O; (c) $(CH_3)_2CHCH_2NHCH_2CO_2Et$,THF; (d) (i) EtI, K_2CO_3, DMF; (ii) 20% EtONa in EtOH; (e) NaH, $PhNTf_2$, DMF; (f) $PhB(OH)_2$, Na_2CO_3, $Pd(PPh_3)_4$, toluene, EtOH, H_2O; (g) NaOH, EtOH, THF; (h) (i) $(COCl)_2$, DMF, THF; (ii) $NaBH_4$, DME; (j) $SOCl_2$, toluene; (k) potassium phthalimide, DMF; (m) (i) $H_2NNH_2·H_2O$, EtOH; (ii) Boc_2O, THF; (n) 5% Pd–C, H_2, EtOH, THF; (o) ICH_2CONH_2, DBU, DMF; (p) 4 M HCl–AcOEt.

As mentioned above, we hypothesized that the introduction of an appropriate hydrogen bond donor into the 6- or 7-position of the 4-phenyl-1-isoquinolone would provide an interaction with the S1′ pocket. Effects of the 6- or 7-substituents were thus investigated. The results are summarized in Table 3. Replacement of the 6-chloro group of **6e** with a hydrophilic group, such as a carboxy (**19a**), a carbamoyl (**19b**), a cyano (**19e**), a hydroxy (**36**), and a methoxy group (**6h**), tended to reinforce the inhibitory potency.



Scheme 7. Synthesis of compound **41**. Reagents: (a) NaH, $PhNTf_2$, DMF; (b) CO, $Pd(OAc)_2$, dppp, Et_3N, DMSO, MeOH; (c) NaOH, MeOH, THF; (d) WSC, HOBt·NH_3, DMF; (e) 4 M HCl–AcOEt.

Table 1
SAR summary for the 3-substituents on the isoquinolone derivatives

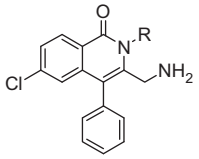


35a

Compound	R	DPP-IV inhibition (IC_{50}, μM)[a]
5a	CH_2OH	>100
6a	CH_2NH_2	16
12	$CH_2CH_2NH_2$	87
13	NH_2	>100
6e	CH_2NH_2	1.6
8a	CH_2NHMe	>100
8b	CH_2NMe_2	>100

[a] Inhibitory activity against human DPP-4.

Table 2
SAR summary for the 2-substituents on the isoquinolone derivatives

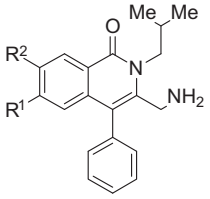


Compound	R	DPP-IV inhibition (IC_{50}, μM)[a]
6b	Et	8.3
6c	*n*-Pr	2.4
6d	*n*-Bu	2.4
6e	*i*-Bu	1.6
6f	*n*-Pen	3.8
6g	Neopentyl	1.9

[a] Inhibitory activity against human DPP-4.

Remarkably, compound **19b** with a hydrogen-bond donating carbamoyl group exhibited submicromolar inhibitory activity. A significant potency-enhancing effect was achieved by the insertion of an oxymethylene linker between the carbamoyl moiety and the C6 of the isoquinolone core (**35a**), which led to a fivefold improvement in potency when compared with **6e**. A similar improvement was found with an unsaturated ethylene derivative (**23a**). In contrast, compounds bearing a bulkier substituent, such

Table 3
SAR summary for the 6- or 7-substituents on the isoquinolone derivatives



Compound	R^1	R^2	DPP-IV inhibition (IC_{50}, μM)[a]
6e	Cl	H	1.6
19a	CO_2H	H	0.98
19b	$CONH_2$	H	0.36
19e	CN	H	1.1
41	H	$CONH_2$	2.4
19c	CONHMe	H	1.9
19d	$CONMe_2$	H	4.0
36	OH	H	0.79
6h	OMe	H	0.87
35a	OCH_2CONH_2	H	0.24
35b	H	OCH_2CONH_2	1.1
23a	$CH{=}CHCONH_2$ (*E*)	H	0.28
23b	$CH_2CH_2CONH_2$	H	1.5

[a] Inhibitory activity against human DPP-4.

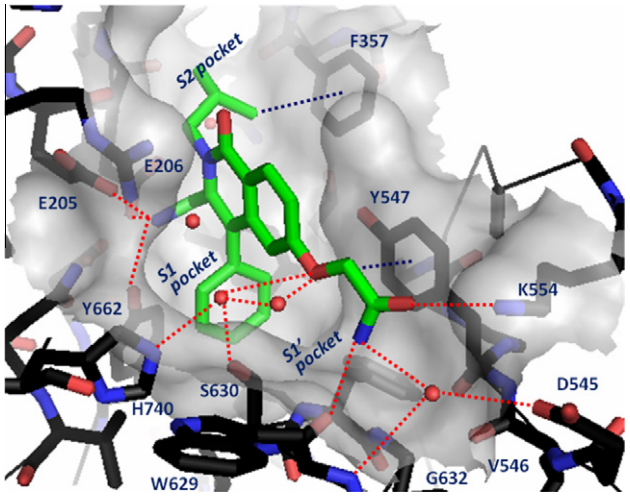


Figure 1. X-ray structure of compound **35a** in complex with human DPP-4. The amino acids (black carbons, blue texts) in the binding sites of **35a** (green carbons) are indicated. Major interactions are depicted as dotted lines (hydrophobic, blue; hydrophilic, red).

Table 4
Selectivity for other peptidases

Enzyme	DPP-4 (human) IC_{50}[a] (μM)	DPP-4 (rat plasma) IC_{50}[a] (μM)	DPP-2 (rat spleen) IC_{50}[a] (μM)	Cathepsin C (bovine spleen) IC_{50}[a] (μM)	Prolyl endopeptidase (*Flavobacterium* sp.) IC_{50}[a] (μM)	Proline iminopeptidase (*Bacillus* sp.) IC_{50}[a] (μM)	Leucine aminopeptidase (porcine kidney) IC_{50}[a] (μM)
19b	0.36	0.55	38	>30	>300	>300	>300
23a	0.28	0.27	74	170	>300	>300	>300
35a	0.24	0.15	61	>100	>300	>300	>300

[a] IC_{50} values shown are means of duplicate measurements.

as a mono- or dialkylcarbamoyl (**19c** and **19d**), and the insertion of a saturated linker (**23b**) led to a slight decrease in activity compared to **19b**. Moreover, transposition of the 6-carbamoyl and the 6-carbamoylmethoxy groups onto the 7-position (**35b** and **41**, respectively) caused a deleterious effect on the activity. These results suggest the importance of a hydrogen-bond donating moiety linked through an appropriate linker to the C6 of the isoquinolone core and show that the carbamoylmethoxy group of **35a** and the (*E*)-2-carbamoylethenyl group of **23a** are the optimal substituents at the 6-position.

The X-ray structure of compound **35a** in complex with human DPP-4 was obtained (Fig. 1). The 3-aminomethyl group interacts with the Glu-motif (Glu205 and Glu206) that are located on the α-helix that protrudes from the β-propeller domain into the active site. The bulky end of the 2-isobutyl group makes a hydrophobic interaction with Phe357, and the 4-phenyl ring occupies the well-defined hydrophobic S1 pocket. In addition, the 6-carbamoylmethoxy moiety exerts van der Waals interactions with Tyr547, and its amide NH_2 groups are assumed to interact weakly with Trp629. The most interesting interaction is the 3.1–3.4 Å hydrogen bond formed between the carbonyl oxygen on the 6-carbamoylmethoxy moiety and the side chain of Lys554 residue. This crystallography identified a potential and useful binding site that has not been reported or targeted before. Thus, this study indicates that the binding mode of the isoquinolone derivative to DPP-4 hypothesized by our docking study is consistent with that observed in the co-crystals of **35a**.

Consequently, the carbamide (**19b**), oxyacetamide (**35a**), and acrylamide (**23a**) analogs were further evaluated for in vitro enzyme selectivity. As presented in Table 4, compounds **19b**, **23a,** and **35a** displayed high selectivity for DPP-4 over closely related peptidases, such as cathepsin C, prolyl endopeptidase, proline iminopeptidase, and DPP-2. We selected compounds **23a** and **35a** on the basis of safety pharmacology. The compounds **23a** and **35a** analogs showed excellent oral bioavailability in rats (data not shown). Thus, **23a** and **35a** are promising compounds for exerting potent in vivo effects when administered orally.

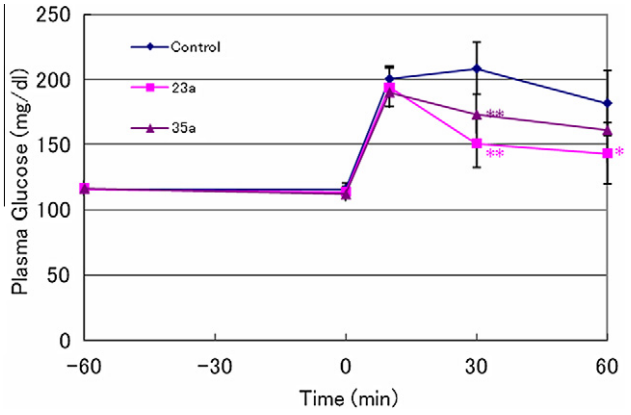


Figure 2. Oral glucose tolerance test in female Wistar fatty rats. Plasma glucose levels of female Wistar fatty rats ($n = 6$) administered 1 mg/kg of compounds or 0.5% MC 60 min before oral glucose load (1 g/kg). Data points indicate means, and the error bars indicate SD ($n = 6$). $^{*}p < 0.05$, $^{**}p < 0.01$ versus control by Dunnet test.

3.2. In vivo studies

The in vivo hypoglycemic effects of compounds **23a** and **35a** were evaluated in an oral glucose tolerance test (OGTT) using female Wistar fatty rats, which are one of the type 2 diabetic animal models with obesity, and the results are shown in Figure 2. Oral administration of **23a** and **35a** at a dose of 1 mg/kg significantly reduced plasma glucose levels in female Wistar fatty rats.

The dose-dependent effects of compound **35a** in the OGTT are shown in Figure 3. The results indicate that compound **35a** produced a dose-dependent improvement of glucose tolerance in Wistar fatty rats after oral administration and its minimum effective dose was 0.3 mg/kg (Fig. 3 A). Simultaneously, plasma insulin levels increased in a dose-dependent manner, which was consistent with the glucose-lowering effect of **35a** (Fig. 3 B). Therefore, the potent, selective, and orally active DPP-4 inhibitor **35a** is expected to provide a potential therapeutic efficacy for the treatment of type 2 diabetes.

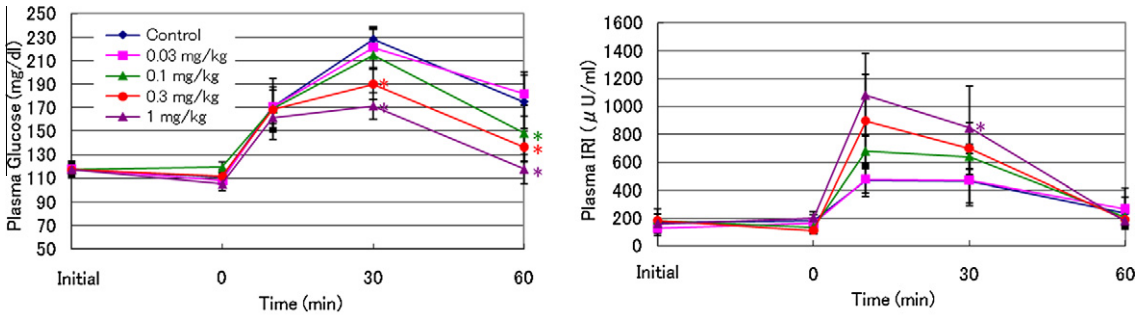


Figure 3. Oral glucose tolerance test of compound **35a** in female Wistar fatty rats. Plasma glucose (A) and plasma insulin levels (B) of female Wistar fatty rats administered 0.03–1 mg/kg of compound **35a** or 0.5% MC 60 min before oral glucose load (1 g/kg). Data points indicate means, and the error bars indicate SD ($n = 6$). ∗: $p < 0.025$ versus control by Williams test.

4. Conclusion

Starting from the lead compound **6a**, a series of 1,2-dihydro-4-phenyl-1-isoquinolones was designed, synthesized, and evaluated as DPP-4 inhibitors. Significant improvement in in vitro activity for this class has been accomplished by the introduction of an appropriate lipophilic side chain and a hydrogen bond donor into the 2- and 6-position of the isoquinolone nucleus, respectively. These studies revealed that the isoquinolone core is an excellent template mimicking the dipeptidyl backbone and side-chains. Finally, we discovered a potent, selective, and orally bioavailable non-peptide DPP-4 inhibitor, 2-{[3-(aminomethyl)-2-(2-methylpropyl)-1-oxo-4-phenyl-1,2-dihydroisoquinolin-6-yl]oxy}acetamide (**35a**). The co-crystallographic analysis of **35a** in complex with DPP-4 validated our hypothesized binding mode, and moreover identified Lys554 as a new target-binding site available for DPP-4 inhibitors.

5. Experimental section

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

5.1. 4-Chloro-2-(phenylcarbonyl)benzoic acid (2a)

To a solution of 4-chlorophthalic anhydride (5.0 g, 27.4 mmol) in benzene (25 mL) was added aluminum chloride (7.3 g, 54.7 mmol) in small portions at 0 °C, and the resulting mixture was stirred at room temperature for 40 h. The mixture was quenched with water at 0 °C and extracted with AcOEt. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The resulting solid was filtered, washed with hexane–AcOEt and dried to give **2a** (6.7 g, 47%) as white crystals. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.31–7.73 (7H, m), 8.02 (1H, d, J = 8.4 Hz).

5.2. 4-Bromo-2-(phenylcarbonyl)benzoic acid (2b)

To a solution of 4-bromophthalic anhydride (50 g, 220 mmol) in benzene (500 mL) was added aluminum chloride (60 g, 450 mmol) in small portions at 0 °C, and the resulting mixture was stirred at room temperature for 24 h. The mixture was poured into ice-water, and extracted with AcOEt and THF. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 70/30), and the residue was recrystallized from AcOEt–IPE to afford **2b** (33 g, 49%) as white crystals. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.36–7.73 (7H, m), 7.94 (1H, d, J = 8.4 Hz). Mp 185–187 °C.

5.3. 4-Fluoro-2-(phenylcarbonyl)benzoic acid (2c)

Compound **2c** was prepared from 4-fluorophthalic anhydride in a manner similar to that described for the synthesis of **2a** in 33% yield as a white solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.03–7.72 (7H, m), 8.10 (1H, dd, J = 5.2, 8.8 Hz).

5.4. 6-Chloro-1-oxo-4-phenyl-1*H*-isochromene-3-carboxylic acid (3a)

A mixture of **2a** (1.3 g, 5 mmol), potassium carbonate (0.71 g, 5.1 mmol), diethyl bromomalonate (1.3 g, 5.4 mmol), acetone (35 mL) and *N*,*N*-dimethylformamide (1 mL) was stirred at room temperature for 1.5 h. The solvent was evaporated under reduced pressure. The residue was poured into water and extracted with AcOEt. The AcOEt extract was washed with water, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was added a mixture of acetic acid (25 mL) and concentrated hydrochloric acid (25 mL), and the mixture was stirred at 110 °C for 3 h. After evaporation of the solvent, the residue was poured into water and extracted with AcOEt. The extract was washed with brine, dried with anhydrous $MgSO_4$ and concentrated in vacuo to give **3a** (0.89 g, 59%) as crystals. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.07 (1H, d, J = 1.8 Hz), 7.21–7.25 (2H, m), 7.47–7.53 (3H, m), 7.61 (1H, dd, J = 1.8, 8.4 Hz), 8.35 (1H, d, J = 8.4 Hz).

5.5. 6-Bromo-1-oxo-4-phenyl-1*H*-isochromene-3-carboxylic acid (3b)

A mixture of **2b** (25 g, 82 mmol), potassium carbonate (12 g, 87 mmol), diethyl bromomalonate (22 g, 92 mmol), acetone (450 mL) and *N*,*N*-dimethylformamide (8 mL) was stirred at room temperature for 15 h. The solvent was evaporated under reduced pressure. The residue was poured into water and extracted with AcOEt. The AcOEt extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was crystallized from hexanes, and collected by filtration. The obtained crystals were added to a mixture of acetic acid (235 mL) and concentrated hydrochloric acid (360 mL), and the mixture was stirred at 120 °C for 8 h. The reaction mixture was cooled and concentrated. The residue was poured into water and extracted with AcOEt. The extract was washed with brine, dried with anhydrous $MgSO_4$, and concentrated under reduced pressure. The precipitated crystals were collected by filtration, washed with IPE and dried to give **3b** (17 g, 60%) as crystals. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.20–7.28 (3H, m), 7.47–7.55 (3H, m), 7.77 (1H, dd, J = 1.8, 8.6 Hz), 8.26 (1H, d, J = 8.6 Hz). Mp 205–206 °C.

5.6. 6-Fluoro-1-oxo-4-phenyl-1*H*-isochromene-3-carboxylic acid (3c)

Compound **3c** was prepared in a manner similar to that described for **3a** in 69% yield as a white solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.73 (1H, dd, J = 1.8, 9.8 Hz), 7.26–7.57 (6H, m), 8.41 (1H, dd, J = 5.4, 8.8 Hz).

5.7. 6-Chloro-2-methyl-1-oxo-4-phenyl-1,2-dihydroisoquinoline-3-carboxylic acid (4a)

A mixture of **3a** (6.0 g, 20 mmol), 40% methylamine in MeOH (40 mL, 392 mmol) and MeOH (80 mL) was stirred at room temperature for 4 h. After evaporation of the solvent, 4 M hydrogen chloride in AcOEt (98 mL) was added to the residue. The mixture was stirred at room temperature for 24 h. After evaporation of

the solvent, the resulting crystals were filtered, washed sequentially with water and Et_2O, and dried in vacuo to give **4a** (6.0 g, 96%) as crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.61 (3H, s), 7.17 (1H, d, J = 1.9 Hz), 7.30–7.34 (2H, m), 7.40–7.55 (4H, m), 8.36 (1H, d, J = 4.8 Hz).

5.8. 6-Bromo-2-(2-methylpropyl)-1-oxo-4-phenyl-1,2-dihydroisoquinoline-3-carboxylic acid (4b)

A solution of **3b** (8.0 g, 23 mmol) and isobutylamine (23 mL, 230 mmol) in MeOH (120 mL) was stirred at room temperature for 15 h. The solvent was evaporated under reduced pressure, and the residue was acidified with concentrated hydrochloric acid and extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. To the residue was added 4 M hydrogen chloride in AcOEt (150 mL), and the resulting mixture was stirred at room temperature for 3 h. The solvent was evaporated under reduced pressure, and the precipitated crystals were collected by filtration and washed with water. The crystals were washed with water and dried to give **4b** (8.1 g, 87%) as crystals. ^{1}H NMR(200 MHz, $CDCl_3$) δ 0.92 (6H, d, J = 6.6 Hz), 2.21 (1H, m), 3.99 (2H, d, J = 7.6 Hz), 7.32–7.38 (3H, m), 7.42–7.47 (3H, m), 7.60 (1H, dd, J = 2.0, 8.4 Hz), 8.26 (1H, d, J = 8.4 Hz). Mp 233–235 °C.

Compounds **4c**–**i** were prepared in a manner similar to that described for **4b**.

5.9. 6-Fluoro-2-(2-methylpropyl)-1-oxo-4-phenyl-1,2-dihydroisoquinoline-3-carboxylic acid (4c)

A white solid (35%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.95 (1H, d, J = 6.6 Hz), 2.22–2.36 (1H, m), 4.03–4.07 (2H, m), 6.76 (1H, dd, J = 2.6, 10.2 Hz), 7.14–7.47 (5H, m) 7.95 (1H, dd, J = 4.8, 8.6 Hz), 8.48 (1H, dd, J = 6.0, 8.8 Hz).

5.10. 6-Chloro-2-ethyl-1-oxo-4-phenyl-1,2-dihydroisoquinoline-3-carboxylic acid (4d)

Colorless crystals (61%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.43 (3H, d, J = 7.0 Hz), 4.15 (2H, q, J = 7.0 Hz), 7.16 (1H, d, J = 1.8 Hz), 7.30–7.49 (6H, m), 8.42 (1H, dd, J = 2.4, 8.6 Hz). Mp 242–243 °C.

5.11. 6-Chloro-1-oxo-4-phenyl-2-propyl-1,2-dihydroisoquinoline-3-carboxylic acid (4e)

Colorless crystals (70%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.96 (3H, t, J = 7.3 Hz), 1.75–1.93 (2H, m), 3.96–4.07 (2H, m), 7.15 (1H, d, J = 1.9 Hz), 7.33–7.36 (2H, m), 7.43–7.50 (4H, m), 8.40 (1H, d, J = 8.7 Hz).

5.12. 2-Butyl-6-chloro-1-oxo-4-phenyl-1,2-dihydroisoquinoline-3-carboxylic acid (4f)

A white solid (79%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.94 (3H, t, J = 7.3 Hz), 1.35–1.46 (2H, m), 1.70–1.87 (2H, m), 3.97–4.09 (2H, m), 7.15 (1H, d, J = 1.9 Hz), 7.31–7.39 (2H, m), 7.42–7.49 (4H, m), 8.37 (1H, d, J = 8.7 Hz).

5.13. 6-Chloro-2-(2-methylpropyl)-1-oxo-4-phenyl-1,2-dihydroisoquinoline-3-carboxylic acid (4g)

A white solid (82%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.92 (6H, d, J = 6.8 Hz), 2.16–2.26 (1H, m), 3.99 (2H, d, J = 7.5 Hz), 7.14 (1H, d, J = 1.9 Hz), 7.31–7.38 (2H, m), 7.42–7.50 (4H, m), 8.37 (1H, d, J = 8.7 Hz).

5.14. 6-Chloro-1-oxo-2-pentyl-4-phenyl-1,2-dihydroisoquinoline-3-carboxylic acid (4h)

A white solid (91%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.84–0.95 (3H, m), 1.24–1.42 (4H, m), 1.76–1.86 (2H, m), 3.94–4.10 (2H, m), 7.15 (1H, d, J = 2.3 Hz), 7.31–7.38 (2H, m), 7.42–7.49 (4H, m), 8.37 (1H, d, J = 8.7 Hz).

5.15. 6-Chloro-2-(2,2-dimethylpropyl)-1-oxo-4-phenyl-1,2-dihydroisoquinoline-3-carboxylic acid (4i)

A white solid (82%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.02 (9H, s), 1.74 (1H, t, J = 6.2 Hz), 4.51 (2H, d, J = 6.0 Hz), 6.94 (1H, d, J = 1.9 Hz), 7.27–7.30 (2H, m), 7.35 (1H, dd, J = 1.9, 8.7 Hz), 7.48–7.54 (3H, m), 8.33 (1H, d, J = 8.7 Hz).

5.16. 6-Methoxy-2-(2-methylpropyl)-1-oxo-4-phenyl-1,2-dihydroisoquinoline-3-carboxylic acid (4j)

A mixture of **4c** (5.0 g, 14.7 mmol) and 28% sodium methoxide in MeOH (40.0 g, 207 mmol) was refluxed for 15 h. After evaporation of the solvent, the residue was poured into water, and extracted with AcOEt. The extract was washed with brine, dried with anhydrous $MgSO_4$, and concentrated in vacuo to give **4j** (4.0 g, 77%) as crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.94 (1H, d, J = 6.6 Hz), 2.22–2.36 (1H, m), 3.71 (3H, s), 4.02 (2H, d, J = 7.8 Hz), 6.50 (1H, d, J = 2.2 Hz), 7.06 (1H, dd, J = 2.6, 8.8 Hz), 7.39–7.48 (5H, m), 8.39 (1H, d, J = 8.8 Hz).

5.17. 6-Chloro-3-(hydroxymethyl)-2-methyl-4-phenylisoquinolin-1(2*H*)-one (5a)

To a solution of compound **4a** (17.8 g, 56.7 mmol) in THF (270 mL) was added oxalyl chloride (10 mL, 117 mmol) and *N,N*-dimethylformamide (5 drops). The mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated under reduced pressure, and the residue was dissolved in THF (130 mL). The resulting mixture was added dropwise to a suspension of sodium tetrahydroborate (6.5 g, 172 mmol) in 1,2-dimethoxyethane (170 mL) at 0 °C. The obtained mixture was stirred at 0 °C for 2 h. The reaction mixture was poured into 1 M hydrochloric acid and extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The resulting crystals were washed with IPE to give **5a** (14.0 g, 82%) as crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.97 (1H, br s), 3.82 (3H, s), 4.45 (1H, d, J = 4.8 Hz), 6.98 (1H, d, J = 2.0 Hz), 7.28–7.55 (7H, m), 8.37 (1H, d, J = 8.6 Hz). ESI-HRMS calcd for $C_{17}H_{14}ClN_2O$ *m/z* calcd 300.0786 (M+H), found 300.0771 (M+H).

Compounds **5b**–**5i** were prepared in a manner similar to that described for **5a**.

5.18. 6-Bromo-3-(hydroxymethyl)-2-(2-methylpropyl)-4-phenylisoquinolin-1(2*H*)-one (5b)

Colorless crystals (74%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (6H, d, J = 6.6 Hz), 2.22 (1H, m), 2.35 (1H, t, J = 5.8 Hz), 4.21 (2H, d, J = 7.6 Hz), 4.44 (2H, d, J = 5.8 Hz), 7.10 (1H, d, J = 1.8 Hz), 7.30–7.35 (2H, m), 7.47 (1H, dd, J = 1.8, 8.4 Hz), 7.50–7.56 (3H, m), 8.20 (1H, d, J = 8.4 Hz). Mp 176–177 °C.

5.19. 6-Chloro-2-ethyl-3-(hydroxymethyl)-4-phenylisoquinolin-1(2*H*)-one (5c)

A white solid (71%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.42 (3H, t, J = 7.0 Hz), 1.71 (1H, t, J = 5.7 Hz), 4.33–4.50 (4H, m), 6.98 (1H, d,

J = 1.9 Hz), 7.28–7.31 (2H, m), 7.40 (1H, dd, *J* = 2.1, 8.5 Hz), 7.46–7.57 (3H, m), 8.41 (1H, d, *J* = 8.7 Hz). Mp 166–167 °C.

5.20. 6-Chloro-3-(hydroxymethyl)-4-phenyl-2-propylisoquinolin-1(2*H*)-one (5d)

A white solid (74%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.04 (3H, t, *J* = 7.5 Hz), 1.68 (1H, t, *J* = 5.8 Hz), 1.75–1.91 (2H, m), 4.21–4.32 (2H, m), 4.38–4.47 (2H, m), 6.97 (1H, d, *J* = 1.9 Hz), 7.27–7.31 (2H, m), 7.39 (1H, dd, *J* = 2.3, 8.7 Hz), 7.47–7.58 (3H, m), 8.40 (1H, d, *J* = 8.7 Hz).

5.21. 2-Butyl-6-chloro-3-(hydroxymethyl)-4-phenylisoquinolin-1(2*H*)-one (5e)

A white solid (62%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.99 (3H, t, *J* = 7.3 Hz), 1.40–1.54 (2H, m), 1.68 (1H, t, *J* = 6.0 Hz), 1.72–1.86 (2H, m), 4.24–4.37 (2H, m), 4.43 (2H, d, *J* = 6.0 Hz), 6.97 (1H, d, *J* = 1.9 Hz), 7.28–7.31 (2H, m), 7.39 (1H, dd, *J* = 2.1, 8.5 Hz), 7.47–7.57 (3H, m), 8.40 (1H, d, *J* = 8.7 Hz).

5.22. 6-Chloro-3-(hydroxymethyl)-2-(2-methylpropyl)-4-phenylisoquinolin-1(2*H*)-one (5f)

A white solid (77%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.99 (6H, d, *J* = 6.4 Hz), 1.70 (1H, t, *J* = 5.8 Hz), 2.17–2.37 (1H, m), 4.21 (2H, d, *J* = 7.5 Hz), 4.45 (2H, d, *J* = 5.3 Hz), 6.96 (1H, d, *J* = 1.9 Hz), 7.28–7.32 (2H, m), 7.38 (1H, dd, *J* = 1.9, 8.7 Hz), 7.47–7.56 (3H, m), 8.38 (1H, d, *J* = 8.7 Hz).

5.23. 6-Chloro-3-(hydroxymethyl)-2-pentyl-4-phenylisoquinolin-1(2*H*)-one (5g)

A white solid (71%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87–0.99 (3H, m), 1.30–1.50 (4H, m), 1.66 (1H, t, *J* = 5.8 Hz), 1.75–1.85 (2H, m), 4.22–4.37 (2H, m), 4.43 (2H, d, *J* = 6.0 Hz), 6.97 (1H, d, *J* = 1.9 Hz), 7.27–7.31 (2H, m), 7.39 (1H, dd, *J* = 2.1, 8.5 Hz), 7.47–7.56 (3H, m), 8.40 (1H, d, *J* = 8.7 Hz).

5.24. 6-Chloro-2-(2,2-dimethylpropyl)-3-(hydroxymethyl)-4-phenylisoquinolin-1(2*H*)-one (5h)

A white solid (71%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.96 (9H, s), 4.14 (2H, br s), 7.11 (1H, d, *J* = 1.9 Hz), 7.30–7.33 (2H, m), 7.42–7.50 (4H, m), 8.35 (1H, d, *J* = 8.7 Hz).

5.25. 3-(Hydroxymethyl)-6-methoxy-2-(2-methylpropyl)-4-phenylisoquinolin-1(2*H*)-one (5i)

A white solid (63%). ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.99 (6H, d, *J* = 6.8 Hz), 1.81 (1H, t, *J* = 6.0 Hz), 2.20–2.34 (1H, m), 3.67 (3H, s), 4.20 (2H, d, *J* = 7.6 Hz), 4.45 (2H, d, *J* = 5.7 Hz), 6.34 (1H, d, *J* = 2.6 Hz), 7.00 (1H, dd, *J* = 2.5, 8.9 Hz), 7.28–7.36 (2H, m), 7.44–7.57 (3H, m), 8.38 (1H, d, *J* = 8.7 Hz).

5.26. 3-(Aminomethyl)-6-chloro-2-methyl-4-phenylisoquinolin-1(2*H*)-one (6a)

To a solution of **5a** (6.0 g, 20 mmol) in CH_2Cl_2 (200 mL) was added triethylamine (5.6 mL, 40 mmol) and methanesulfonyl chloride (1.9 mL, 24.5 mmol). The mixture was stirred at room temperature for 30 min. The reaction mixture was poured into 1 M hydrochloric acid and extracted with CH_2Cl_2. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure to give (6-chloro-2-methyl-1-oxo-4-phenyl-1,2-dihydroisoquinolin-3-yl)methyl methanesulfonate. A mixture of the (6-chloro-2-methyl-1-oxo-4-phenyl-1,2-dihydroisoquinolin-3-yl)methylmethanesulfonate, 10% NH_3 in MeOH (40 mL), MeOH (10 mL) and THF (50 mL) was heated in a sealed tube at 150 °C for 6 h. After evaporation of the solvent, the reaction mixture was poured into water and extracted with Et_2O. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was collected by filtration, washed with water and Et_2O to give **6a** (3.20 g, 54%) as crystals. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.41 (2H, br s), 3.66 (2H, s), 3.85 (3H, s), 6.92 (1H, d, *J* = 2.3 Hz), 7.22–7.29 (2H, m), 7.35–7.41 (1H, m), 7.43–7.57 (3H, m), 8.36–8.46 (1H, m). ESI-HRMS calcd for $C_{17}H_{15}ClN_2O$ *m/z* 299.0946 (M+H), found 299.0946 (M+H).

5.27. 3-(Aminomethyl)-2-ethyl-6-chloro-4-phenylisoquinolin-1(2*H*)-one (6b)

To a solution of 6-chloro-2-ethyl-3-(hydroxymethyl)-4-phenylisoquinolin-1(2*H*)-one (0.25 g, 0.8 mmol) in CH_2Cl_2 (5 mL) was added triethylamine (0.2 mL, 1.4 mmol) and methanesulfonyl chloride (0.1 mL, 1.3 mmol) at 0 °C, and the resulting mixture was stirred at room temperature for 30 min. The reaction mixture was poured into 1 M hydrochloric acid and extracted with CH_2Cl_2. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. A mixture of the residue, 10% NH_3 in EtOH (10 mL), and THF (10 mL) was heated in a sealed tube at 130 °C for 3 h. The reaction mixture was poured into water and extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 50/50) to give **6b** (0.08 g, 38%) as crystals. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.39 (2H, br s), 1.42 (3H, t, *J* = 7.0 Hz), 3.64 (2H, s), 4.43 (2H, q, *J* = 7.0 Hz), 6.91 (1H, d, *J* = 2.2 Hz), 7.23–7.31 (2H, m), 7.38 (1H, dd, *J* = 2.2, 8.4 Hz), 7.45–7.57 (3H, m), 8.41 (1H, d, *J* = 8.4 Hz). Mp 126–127 °C. Anal. Calcd for $C_{18}H_{17}ClN_2O{\cdot}1/4H_2O$: C, 68.62; H, 5.51; N, 8.89. Found: C, 68.61; H, 5.40; N, 8.84. ESI-HRMS calcd for $C_{18}H_{17}ClN_2O$ *m/z* 313.1102 (M+H), found 313.1085 (M+H).

5.28. 3-(Aminomethyl)-6-chloro-2-propyl-4-phenylisoquinolin-1(2*H*)-one (6c)

Compound **6c** was prepared in a manner similar to that described for **6a** in 80% yield as a white solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.04 (3H, t, *J* = 7.4 Hz), 1.17 (2H, br s), 1.71–1.93 (2H, m), 3.63 (2H, s), 4.28 (2H, t, *J* = 7.6 Hz), 6.90 (1H, d, *J* = 2.2 Hz), 7.21–7.32 (2H, m), 7.37 (1H, dd, *J* = 2.2, 8.4 Hz), 7.41–7.58 (3H, m), 8.40 (1H, d, *J* = 8.4 Hz). Mp 146–147 °C. Anal. Calcd for $C_{19}H_{19}ClN_2O$: C, 69.83; H, 5.86; N, 8.57. Found: C, 69.97; H, 5.90; N, 8.49. ESI-HRMS calcd for $C_{19}H_{19}ClN_2O$ *m/z* 327.1259 (M+H), found 327.1247 (M+H).

5.29. 3-(Aminomethyl)-2-butyl-6-chloro-4-phenylisoquinolin-1(2*H*)-one (6d)

Compound **6d** was prepared in a manner similar to that described for **6a** in 32% yield as a white solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.00 (3H, t, *J* = 7.4 Hz), 1.23 (2H, br s), 1.38–1.60 (2H, m), 1.68–1.84 (2H, m), 3.63 (2H, s), 4.33 (2H, t, *J* = 7.6 Hz), 6.90 (1H, d, *J* = 2.0 Hz), 7.22–7.31 (2H, m), 7.38 (1H, dd, *J* = 2.0, 8.8 Hz), 7.45–7.58 (3H, m), 8.41 (1H, d, *J* = 8.8 Hz). Anal. Calcd for $C_{20}H_{21}ClN_2O$: C, 70.48; H, 6.21; N, 8.22. Found: C, 70.27; H, 6.18; N, 8.09. ESI-HRMS calcd for $C_{20}H_{21}ClN_2O$ *m/z* 341.1415 (M+H), found 341.1391 (M+H).

5.30. 3-(Aminomethyl)-6-chloro-2-(2-methylpropyl)-4-phenylisoquinolin-1(2*H*)-one (6e)

Compound **6e** was prepared in a manner similar to that described for **6a** in 42% yield as a white solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.00 (6H, d, J = 6.6 Hz), 1.18 (2H, br s), 2.12–2.38 (1H, m), 3.66 (2H, s), 4.20 (2H, d, J = 7.4 Hz), 6.90 (1H, d, J = 1.6 Hz), 7.21–7.31 (2H, m), 7.37 (1H, dd, J = 1.6, 8.4 Hz), 7.45–7.58 (3H, m), 8.40 (1H, d, J = 8.4 Hz). Mp 123–124 °C. Anal. Calcd for $C_{20}H_{21}ClN_2O$: C, 70.48; H, 6.21; N, 8.22. Found: C, 70.35; H, 6.07; N, 8.10. ESI-HRMS calcd for $C_{20}H_{21}ClN_2O$ *m/z* 341.1415 (M+H), found 341.1395 (M+H).

5.31. 3-(Aminomethyl)-6-chloro-2-pentyl-4-phenylisoquinolin-1(2*H*)-one (6f)

Compound **6f** was prepared in a manner similar to that described for **6a** in 56% yield as a white solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.93 (3H, t, J = 7.4 Hz), 1.20–1.53 (6H, m), 1.70–1.90 (2H, m), 3.63 (2H, s), 4.31 (2H, t, J = 8.0 Hz), 6.90 (1H, d, J = 2.2 Hz), 7.22–7.31 (2H, m), 7.37 (1H, dd, J = 2.2, 8.4 Hz), 7.45–7.58 (3H, m), 8.40 (1H, d, J = 8.4 Hz). Anal. Calcd for $C_{21}H_{23}ClN_2O$: C, 71.07; H, 6.53; N, 7.89. Found: C, 70.82; H, 6.34; N, 7.72. ESI-HRMS calcd for $C_{21}H_{23}ClN_2O$ *m/z* 355.1572 (M+H), found 355.1550 (M+H).

5.32. 3-(Aminomethyl)-6-chloro-2-(2,2-dimethylpropyl)-4-phenylisoquinolin-1(2*H*)-one (6g)

Compound **6g** was prepared in a manner similar to that described for **6a** in 81% yield as a white solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.02 (9H, s), 1.23 (2H, br s), 3.71 (2H, s), 4.30 (2H, br s), 6.90 (1H, d, J = 2.2 Hz), 7.20–7.30 (2H, m), 7.37 (1H, dd, J = 2.2, 8.4 Hz), 7.42–7.68 (3H, m), 8.39 (1H, d, J = 8.4 Hz). Mp 173–174 °C. Anal. Calcd for $C_{21}H_{23}ClN_2O$: C, 71.07; H, 6.53; N, 7.89. Found: C, 70.89; H, 6.54; N, 7.61. ESI-HRMS calcd for $C_{21}H_{23}ClN_2O$ *m/z* 355.1572 (M+H), found 355.1539 (M+H).

5.33. 3-(Aminomethyl)-6-methoxy-2-(2-methylpropyl)-4-phenylisoquinolin-1(2*H*)-one (6h)

Compound **6h** was prepared in a manner similar to that described for **6a** in 84% yield as white needles (84%). ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.00 (6H, d, J = 6.6 Hz), 1.26 (2H, br s), 2.15–2.38 (1H, m), 3.65 (2H, s), 3.67 (3H, s), 4.18 (2H, d, J = 7.2 Hz), 6.30 (1H, d, J = 2.4 Hz), 7.02 (1H, dd, J = 2.4, 8.8 Hz), 7.23–7.34 (2H, m), 7.39–7.57 (3H, m), 8.41 (1H, d, J = 8.8 Hz). Anal. Calcd for $C_{21}H_{24}N_2O_2$: C, 74.97; H, 7.19; N, 8.33. Found: C, 74.73; H, 7.40; N, 8.32. ESI-HRMS calcd for $C_{21}H_{24}N_2O_2$ *m/z* 337.1911 (M+H), found 337.7879 (M+H).

5.34. 6-Chloro-3-[(methylamino)methyl]-2-(2-methylpropyl)-4-phenylisoquinolin-1(2*H*)-one (8a)

Compound **8a** was prepared in a manner similar to that described for **6b** in 38% yield as a white solid.

^{1}H NMR (200 MHz, $CDCl_3$) δ 1.00 (6H, d, J = 6.6 Hz), 2.16–2.29 (4H, m), 3.45 (2H, s), 4.22 (2H, d, J = 7.2 Hz), 6.91 (1H, d, J = 1.8 Hz), 7.21–7.26 (2H, m), 7.38 (1H, dd, J = 1.8, 8.4 Hz), 7.48–7.52 (3H, m), 8.41 (1H, d, J = 8.4 Hz). ESI-HRMS calcd for $C_{21}H_{23}ClN_2O$ *m/z* 355.1572 (M+H), found 355.1542 (M+H).

5.35. 6-Chloro-3-[(dimethylamino)methyl]-2-(2-methylpropyl)-4-phenylisoquinolin-1(2*H*)-one (8b)

Compound **8b** was prepared in a manner similar to that described for **6b** in 38% yield as a white solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.97 (6H, d, J = 7.0 Hz), 2.06 (6H, s), 2.13–2.33 (1H, m), 3.28 (2H, s), 4.35 (2H, d, J = 7.4 Hz), 6.91 (1H, d, J = 1.6 Hz), 7.16–7.22 (2H, m), 7.38 (1H, dd, J = 1.6, 8.4 Hz), 7.44–7.52 (3H, m), 8.42 (1H, d, J = 8.4 Hz). ESI-HRMS calcd for $C_{22}H_{25}ClN_2O$ *m/z* 369.1728 (M+H), found 369.1728 (M+H).

5.36. Diethyl [(6-chloro-2-methyl-1-oxo-4-phenyl-1,2-dihydroisoquinolin-3-yl)methyl]-propanedioate (9)

To a solution of **7a** (2.69 g, 8.4 mmol) and ethyl malonate (1.35 g, 8.4 mmol) in THF (35 mL) was added sodium hydride (0.34 g, 8.5 mmol) at 0 °C, and the resulting mixture was stirred at room temperature for 48 h. The mixture was quenched with water at 0 °C and extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was crystallized from AcOEt–IPE to give **9** (3.25 g, 87%) as crystals. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.13 (6H, t, J = 7.2 Hz), 3.30–3.47 (3H, m), 3.69 (3H, s), 4.06 (2H, q, J = 7.2 Hz), 6.88 (1H, d, J = 2.2 Hz), 7.19–7.23 (2H, m), 7.37 (1H, dd, J = 2.2, 8.4 Hz), 7.45–7.54 (3H, m), 8.39 (1H, d, J = 8.4 Hz). Mp 120–121 °C. Anal. Calcd for $C_{24}H_{24}ClNO_5$: C, 65.23; H, 5.47; N, 3.17. Found: C, 65.12; H, 5.37; N, 3.38.

5.37. 3-(6-Chloro-2-methyl-1-oxo-4-phenyl-1,2-dihydroisoquinolin-3-yl)propanoic acid (10)

A mixture of **9** (3.25 g, 7.4 mmol), concentrated hydrochloric acid (35 mL, 401 mmol) and acetic acid (35 mL, 611 mmol) was heated at 120 °C for 2 h. After evaporation of the solvent, the residue was extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was crystallized from AcOEt–IPE to give **10** (2.32 g, 92%) as crystals. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.39–2.47 (2H, m), 2.81–2.89 (2H, m), 3.71 (3H, s), 6.84 (1H, d, J = 2.2 Hz), 7.21–7.26 (2H, m), 7.35 (1H, dd, J = 2.2, 8.4 Hz), 7.46–7.55 (3H, m), 8.35 (1H, d, J = 8.8 Hz).

5.38. *tert*-Butyl [2-(6-chloro-2-methyl-1-oxo-4-phenyl-1,2-dihydroisoquinolin-3-yl)ethyl]carbamate (11)

To a solution of **10** (0.15 g, 0.4 mmol) in *N,N*-dimethylformamide (3 mL) was added DPPA (0.1 mL, 0.5 mmol) and triethylamine (0.07 mL, 0.5 mmol), and the resulting mixture was stirred at room temperature for 5 h. The mixture was poured into water and extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was dissolved in toluene (8 mL), and the solution was refluxed for 1.5 h. The solution was added *tert*-BuOH (5 mL), and the resulting mixture was stirred at 85 °C for 15 h. After evaporation of the solvent, the residue was extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 80/20) to give **11** (0.10 g, 57%) as needles. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.39 (9H, s), 2.69–2.83 (2H, m), 3.14–3.28 (2H, m), 3.81 (3H, s), 4.51 (1H, br s), 6.85 (1H, d, J = 1.9 Hz), 7.21–7.25 (2H, m), 7.33–7.39 (1H, m), 7.45–7.57 (3H, m), 8.40 (1H, d, J = 8.7 Hz). Anal. Calcd for $C_{23}H_{25}N_2O_3 \cdot 1/2H_2O$: C, 65.47; H, 6.21; N, 6.64. Found: C, 65.74; H, 6.17; N, 7.13.

5.39. 3-(2-Aminoethyl)-6-chloro-2-methyl-4-phenylisoquinolin-1(2*H*)-one hydrochloride (12)

To a solution of **11** (0.07 g, 0.17 mmol) in AcOEt (2 mL) was added 4 M hydrogen chloride in AcOEt (2 mL), and the resulting mixture was stirred at room temperature for 8 h. After evaporation

of the solvent, the resulting crystals was filtered and washed with IPE to give **12** (0.04 g, 69%) as needles. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.87 (4H, br s), 3.67 (3H, s), 6.69 (1H, d, J = 2.3 Hz), 7.29–7.39 (2H, m), 7.47–7.65 (4H, m), 7.89 (3H, br s), 8.29 (1H, d, J = 8.7 Hz). Anal. Calcd for $C_{18}H_{17}ClN_2O{\cdot}HCl{\cdot}1/2H_2O$: C, 60.34; H, 5.35; N, 7.82. Found: C, 60.39; H, 5.24; N, 7.79. ESI-HRMS calcd for $C_{18}H_{17}ClN_2O$ m/z 313.1102 (M+H), found 313.1073 (M+H).

5.40. 3-Amino-6-chloro-2-methyl-4-phenylisoquinolin-1(2*H*)-one (13)

Compound **13** was prepared in a manner similar to that described for **12** in 38% yield as a yellow solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.66 (3H, s), 3.94 (2H, br s), 6.89 (1H, d, J = 1.9 Hz), 7.12 (1H, dd, J = 2.3, 8.7 Hz), 7.29–7.36 (2H, m), 7.40–7.50 (1H, m), 7.50–7.60 (2H, m), 8.27 (1H, d, J = 8.7 Hz). ESI-HRMS calcd for $C_{16}H_{13}ClN_2O$ m/z 283.0644 (M−H), found 283.0632 (M−H).

5.41. *tert*-Butyl {[6-bromo-2-(2-methylpropyl)-1-oxo-4-phenyl-1,2-dihydroisoquinolin-3- yl]methyl}carbamate (14)

To a solution of **5b** (8.7 g, 22.5 mmol) and pyridine (5 drops) in THF (30 mL) and toluene (30 mL) was added thionyl chloride (3.4 mL, 47.3 mmol). The obtained mixture was stirred at room temperature for 3 h. The reaction mixture was concentrated, poured into water and extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure to give 6-bromo-3-(chloromethyl)-2-(2-methylpropyl)-4-phenylisoquinolin-1(2*H*)-one (8.2 g, 90%) as crystals. 6-bromo-3-(chloromethyl)-2-(2-methylpropyl)-4-phenylisoquinolin-1(2*H*)-one (10 g, 24.7 mmol) in THF (20 mL) and a solution of 2 M NH_3 in ethanol (200 mL) were sealed in a stainless tube and stirred at 140 °C for 5 h. The reaction mixture was cooled and concentrated. The residue was poured into water and extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was crystallized from Et_2O and the precipitated crystals were collected by filtration to afford 3-(aminomethyl)-6-bromo-2-(2-methyl)propyl-4-phenylisoquinolin-1(*2H*)-one (5.6 g, 53%) as crystals. To a solution of 3-(aminomethyl)-6-bromo-2-(2-methyl)propyl-4-phenylisoquinolin-1(*2H*)-one (5.6 g, 14.5 mmol) and 4-dimethylaminopyridine (20 mg) in THF (50 mL) was added di-*t*-butyl dicarbonate (6.3 g, 2.9 mmol). The obtained mixture was stirred at room temperature for 1 h. The reaction mixture was poured into water and extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was crystallized from Et_2O to give **14** (6.6 g, 94%) as crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.00 (6H, d, J = 7.0 Hz), 1.43 (9H, s), 2.23 (1H, m), 4.06 (2H, d, J = 7.8 Hz), 4.19 (2H, d, J = 5.4 Hz), 4.50 (1H, br s), 7.08 (1H, d, J = 2.0 Hz), 7.21–7.25 (2H, m), 7.48–7.56 (4H, m), 8.30 (1H, d, J = 8.8 Hz).

5.42. Methyl 3-{[(*tert*-butoxycarbonyl)amino]methyl}-2-(2-methylpropyl)-1-oxo-4-phenyl- 1,2-dihydroisoquinoline-6-carboxylate (15)

A mixture of **14** (3.0 g, 6.2 mmol), 1,3-bis(diphenylphosphino)propane (0.45 g, 1.1 mmol) and triethylamine (0.69 g, 6.8 mmol) in DMSO (30 mL) and MeOH (15 mL) was stirred under a carbon monoxide atmosphere at room temperature for 30 min. Then the resulting mixture was added palladium acetate (0.25 g, 1.1 mmol), and the mixture was stirred under carbon monoxide atmosphere at 80 °C for 15 h. The reaction mixture was poured into water and extracted with AcOEt. The extract was washed with water, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/AcOEt = 70/30) to give **15** (1.92 g, 83%) as crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.01 (6H, d, J = 6.6 Hz), 1.43 (9H, s), 2.26 (1H, m), 3.85 (3H, s), 4.09 (2H, d, J = 7.6 Hz), 4.22 (2H, d, J = 5.6 Hz), 4.47 (1H, br s), 7.23–7.28 (2H, m), 7.49–7.56 (3H, m), 7.66 (1H, d, J = 1.0 Hz), 8.05 (1H, dd, J = 1.7, 8.0 Hz), 8.52 (1H, d, J = 8.0 Hz). Mp 205–206 °C. Anal. Calcd for $C_{27}H_{32}N_2O_5$: C, 69.81; H, 6.94; N, 6.03. Found: C, 69.71; H, 6.80; N, 6.13.

5.43. 3-{[(*tert*-Butoxycarbonyl)amino]methyl}-2-(2-methylpropyl)-1-oxo-4-phenyl-1,2- dihydroisoquinoline-6-carboxylic acid (16)

To a solution of **15** (0.28 g, 0.6 mmol) in THF (10 mL) and MeOH (10 mL) was added 1 M sodium hydroxide (5 mL). The obtained mixture was stirred at room temperature for 2 h. The reaction mixture was poured into water, acidified with 1 M hydrochloric acid, and extracted with AcOEt. The AcOEt extract was washed with water, dried over $MgSO_4$, and concentrated in vacuo. The obtained crystals were recrystallized from AcOEt–Et_2O to give **16** (1.54 g, 98%) as crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.96 (6H, d, J = 6.6 Hz), 1.07 (3H, t, J = 7.4 Hz), 1.49 (9H, s), 1.50–1.72 (2H, m), 1.84–1.98 (2H, m), 2.14–2.21 (1H, m), 3.90 (2H, t, J = 6.4 Hz), 4.00 (2H, d, J = 6.8 Hz), 4.55 (2H, d, J = 5.0 Hz), 5.37 (1H, br s), 8.08–8.13 (1H, m), 8.35–8.46 (2H, m). Anal. Calcd for $C_{26}H_{30}N_2O_5$: C, 69.31; H, 6.71; N, 6.22. Found: C, 69.17; H, 6.59; N, 6.27.

5.44. *tert*-Butyl {[6-carbamoyl-2-(2-methylpropyl)-1-oxo-4-phenyl-1,2-dihydroisoquinolin-3- yl]methyl}carbamate (17a)

A solution of **16** (0.6 g, 1.3 mmol), WSC (0.5 g, 2.6 mmol) and HOBt·NH_3 (0.4 g, 2.6 mmol) in *N,N*-dimethylformamide (3 mL) was stirred at room temperature for 3 h. The reaction mixture was poured into water and extracted with AcOEt. The AcOEt extract was washed with aqueous sodium hydrogen carbonate solution and brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was crystallized from Et_2O–hexane to give **17a** (0.5 g, 86%) as crystals. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.00 (6H, d, J = 6.6 Hz), 1.43 (9H, s), 2.24 (1H, m), 4.08 (2H, d, J = 7.2 Hz), 4.20 (2H, d, J = 5.4 Hz), 4.68 (1H, br s), 5.73 (1H, br s), 6.08 (1H, br s), 7.24–7.29 (2H, m), 7.38 (1H, d, J = 2.0 Hz), 7.47–7.56 (3H, m), 7.74 (1H, dd, J = 2.0, 8.8 Hz), 8.45 (1H, d, J = 8.8 Hz).

5.45. *tert*-Butyl {[6-(methylcarbamoyl)-2-(2-methylpropyl)-1-oxo-4-phenyl-1,2- dihydroisoquinolin-3-yl]methyl}carbamate (17b)

Compound **17b** was prepared in a manner similar to that described for **17a** in 45% yield as crystals. ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.99 (6H, d, J = 6.6 Hz), 1.43 (9H, s), 2.20–2.24 (1H, m), 2.94 (3H, d, J = 2.4 Hz), 4.08 (2H, d, J = 7.0 Hz), 4.20 (2H, d, J = 5.0 Hz), 4.72 (1H, br s), 6.19 (1H, br s), 7.24–7.34 (3H, m), 7.49–7.52 (3H, m), 7.64–7.68 (1H, m), 8.42 (1H, d, J = 8.0 Hz).

5.46. *tert*-Butyl {[6-cyano-2-(2-methylpropyl)-1-oxo-4-phenyl-1,2-dihydroisoquinolin-3- yl]methyl}carbamate (18)

A mixture of **17a** (0.3 g, 0.67 mmol), cyanuric chloride (0.37 g, 2 mmol) and *N,N*-dimethylformamide (3 mL) was stirred at 0 °C for 30 min. The mixture was poured into water and extracted with AcOEt. The AcOEt extract was washed with aqueous sodium hydrogen carbonate solution and brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 70/30) to give **18** (0.28 g, 97%) as crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.01

(6H, d, J = 7.0 Hz), 1.43 (9H, s), 2.24 (1H, m), 4.09 (2H, d, J = 7.0 Hz), 4.23 (2H, d, J = 5.4 Hz), 4.43 (1H, br s), 7.21–7.29 (3H, m), 7.51–7.59 (3H, m), 7.65 (1H, dd, J = 1.4, 8.0 Hz), 8.55 (1H, d, J = 8.0 Hz).

5.47. 3-(Aminomethyl)-2-(2-methylpropyl)-1-oxo-4-phenyl-1,2-dihydroisoquinoline-6-carboxylic acid hydrochloride (19a)

To a solution of **16** (0.15 g, 0.33 mmol) in THF (6 mL) was added a solution of 4 M hydrogen chloride in dioxane (10 mL). The obtained solution was stirred at room temperature for 15 h. The reaction mixture was concentrated under reduced pressure, and the residue was crystallized from Et_2O to give **19a** (0.09 g, 69%) as crystals. ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.89 (6H, d, J = 6.6 Hz), 1.01 (3H, t, J = 7.4 Hz), 1.50–1.69 (2H, m), 1.80–1.93 (2H, m), 1.99–2.12 (1H, m), 3.97 (2H, t, J = 6.4 Hz), 3.99 (2H, d, J = 7.6 Hz), 4.21 (2H, s), 8.09 (1H, dd, J = 1.4, 8.4 Hz), 8.34 (1H, d, J = 1.4 Hz), 8.38 (1H, d, J = 8.4 Hz), 8.69 (3H, br s). ESI-HRMS calcd for $C_{21}H_{22}N_2O_3$ m/z 349.1558 (M−H), found 349.1571 (M−H).

5.48. 3-(Aminomethyl)-2-(2-methylpropyl)-1-oxo-4-phenyl-1,2-dihydroisoquinoline-6-carboxamide hydrochloride (19b)

Compound **19b** was prepared in a manner similar to that described for **19a** in 85% yield as colorless crystals. ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.92 (6H, d, J = 6.6 Hz), 2.11 (1H, m), 3.87 (2H, d, J = 5.6 Hz), 4.10 (2H, d, J = 7.2 Hz), 7.39 (1H, d, J = 1.4 Hz), 7.40–7.43 (2H, m), 7.56–7.62 (4H, m), 8.00 (1H, dd, J = 1.4, 8.4 Hz), 8.16 (1H, s), 8.37 (1H, d, J = 8.4 Hz), 8.61 (3H, br s). Mp 240–242 °C. ESI-HRMS calcd for $C_{21}H_{23}N_3O_2$ m/z 350.1863 (M+H), found 350.1842 (M+H).

5.49. 3-(Aminomethyl)-*N*-methyl-2-(2-methylpropyl)-1-oxo-4-phenyl-1,2-dihydro- isoquinoline-6-carboxamide hydrochloride (19c)

Compound **17c** was prepared in a manner similar to that described for **17a** then compound **17c** was converted to **19c** in a manner similar to that described for **19a** in 70% yield as an amorphous powder. ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.93 (6H, d, J = 6.6 Hz), 2.01–2.17 (1H, m), 2.72 (3H, d, J = 4.8 Hz), 3.87 (2H, s), 4.09 (2H, d, J = 6.8 Hz), 7.39–7.43 (3H, m), 7.54–7.60 (3H, m), 7.95 (1H, dd, J = 1.6, 8.6 Hz), 8.39 (1H, d, J = 8.6 Hz), 8.60 (3H, br s), 8.64 (1H, t, J = 4.8 Hz). ESI-HRMS calcd for $C_{22}H_{25}N_3O_2$ m/z 364.2020 (M+H), found 364.1988 (M+H).

5.50. 3-(Aminomethyl)-*N*,*N*-dimethyl-2-(2-methylpropyl)-1-oxo-4-phenyl-1,2- dihydroisoquinoline-6-carboxamide hydrochloride (19d)

Compound **19d** was prepared in a manner similar to that described for **19a** in 86% yield as a white solid. ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.93 (6H, d, J = 6.6 Hz), 2.01–2.17 (1H, m), 2.78 (3H, s), 2.91 (3H, s), 3.88 (2H, s), 4.08 (2H, d, J = 7.4 Hz), 6.84 (1H, d, J = 1.0 Hz), 7.39–7.43 (2H, m), 7.54–7.61 (4H, m), 8.37 (1H, d, J = 8.6 Hz), 8.50 (3H, br s). ESI-HRMS calcd for $C_{23}H_{27}N_3O_2$ m/z 378.2176 (M+H), found 378.2150 (M+H).

5.51. 3-(Aminomethyl)-2-(2-methylpropyl)-1-oxo-4-phenyl-1,2-dihydroisoquinoline-6-carbonitrile hydrochloride (19e)

Compound **19e** was prepared in a manner similar to that described for **19a** in 88% yield as colorless crystals. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.93 (6H, d, J = 6.6 Hz), 1.98–2.19 (1H, m), 3.89 (2H, s), 4.11 (2H, d, J = 7.4 Hz), 7.22 (1H, d, J = 1.6 Hz), 7.42–7.46 (2H, m), 7.58–7.61 (3H, m), 7.97 (1H, dd, J = 1.6, 8.4 Hz), 8.48 (1H, d, J = 8.4 Hz), 8.67 (3H, br s). ESI-HRMS calcd for $C_{21}H_{21}N_3O$ m/z 332.1757 (M+H), found 332.1727 (M+H).

5.52. Ethyl (2*E*)-3-[3-{[(*tert*-butoxycarbonyl)amino]methyl}-2-(2-methylpropyl)-1-oxo-4- phenyl-1,2-dihydroisoquinolin-6-yl]prop-2-enoate (20a)

To a solution of **14** (9.71 g, 20 mmol), ethyl acrylate (10.2 g, 100 mmol), and triethylamine (27.9 mL, 200 mmol) in *N*,*N*-dimethylformamide (100 mL) was added $Pd(OAc)_2$ under argon atmosphere, and the resulting mixture was stirred at 80 °C under argon atmosphere for 8 h. The mixture was poured into water and extracted with AcOEt. The AcOEt extract was washed with aqueous sodium hydrogen carbonate solution and brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 70/30) to give **20a** (9.11 g, 90%) as an amorphous powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.01 (6H, d, J = 7.0 Hz), 1.31 (3H, t, J = 7.3 Hz), 1.43 (9H, s), 2.18–2.31 (1H, m), 4.08 (2H, d, J = 7.4 Hz), 4.13–4.28 (4H, m), 4.53 (1H, br s), 6.37 (1H, d, J = 16.2 Hz), 7.00 (1H, d, J = 1.4 Hz), 7.18–7.28 (2H, m), 7.44–7.67 (5H, m), 8.44 (1H, d, J = 8.0 Hz). Anal. Calcd for $C_{30}H_{36}N_2O_5$: C, 71.40; H, 7.19; N, 5.55. Found: C, 71.37; H, 7.15; N, 5.43.

5.53. Ethyl 3-[3-{[(*tert*-butoxycarbonyl)amino]methyl}-2-(2-methylpropyl)-1-oxo-4-phenyl- 1,2-dihydroisoquinolin-6-yl]propanoate (20b)

A mixture of **20a** (0.90 g, 1.8 mmol), 5% palladium on carbon (0.5 g), EtOH (10 mL) and THF (10 mL) was stirred under atmospheric pressure of hydrogen. After palladium on carbon was removed by filtration, the filtrate was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/AcOEt = 70/30) to give **20b** (0.82 g, 90%) as an amorphous powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.99 (6H, d, J = 6.6 Hz), 1.19 (3H, t, J = 7.1 Hz), 1.42 (9H, s), 2.18–2.29 (1H, m), 2.51 (2H, t, J = 7.8 Hz), 2.90 (2H, t, J = 7.8 Hz), 4.01–4.12 (4H, m), 4.19 (2H, d, J = 5.6 Hz), 4.40 (1H, br s), 6.74 (1H, d, J = 1.6 Hz), 7.21–7.26 (2H, m), 7.31 (1H, dd, J = 1.6, 8.4 Hz), 7.46–7.55 (3H, m), 8.39 (1H, d, J = 8.4 Hz).

5.54. (2*E*)-3-[3-{[(*tert*-Butoxycarbonyl)amino]methyl}-2-(2-methylpropyl)-1-oxo-4-phenyl- 1,2-dihydroisoquinolin-6-yl]prop-2-enoic acid (21a)

To a solution of **20a** in THF (10 mL) and EtOH (10 mL) was added 1 M sodium hydroxide, and the resulting mixture was stirred at room temperature for 1 h. The mixture was poured into water, acidified with 1 M hydrochloric acid, and extracted with AcOEt. The AcOEt extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was crystallized from AcOEt–hexane to **21a** (0.56 g, 85%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.99 (6H, d, J = 6.6 Hz), 1.48 (9H, s), 2.16–2.24 (1H, m), 4.06 (2H, d, J = 7.2 Hz), 4.15 (2H, d, J = 4.0 Hz), 5.62 (1H, br s), 6.27 (1H, d, J = 16.0 Hz), 6.82 (1H, s), 7.33–7.40 (3H, m), 7.48–7.58 (3H, m), 8.28 (1H, d, J = 8.8 Hz). Mp 172–173 °C. Anal. Calcd for $C_{28}H_{32}N_2O_5$: C, 70.27; H, 7.16; N, 5.85. Found: C, 70.08; H, 6.80; N, 5.65.

5.55. 3-[3-{[(*tert*-Butoxycarbonyl)amino]methyl}-2-(2-methylpropyl)-1-oxo-4-phenyl-1,2-dihydroisoquinolin-6-yl]propanoic acid (21b)

Compound **21b** was prepared in a manner similar to that described for **21a** in 91% yield as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.98 (6H, d, J = 6.9 Hz), 1.42 (9H, s), 2.15–2.27 (1H, m),

2.58 (2H, t, J = 7.8 Hz), 2.91 (2H, t, J = 7.8 Hz), 4.06 (2H, d, J = 6.0 Hz), 4.19 (2H, d, J = 5.1 Hz), 4.53 (1H, br s), 6.76 (1H, s), 7.22–7.25 (2H, m), 7.31 (1H, d, J = 8.8 Hz), 7.46–7.52 (3H, m), 8.37 (1H, d, J = 8.8 Hz). Mp 182–183 °C. Anal. Calcd for $C_{28}H_{34}N_2O_5$: C, 70.12; H, 7.36; N, 5.84. Found: C, 70.21; H, 7.55; N, 5.68.

5.56. *tert*-Butyl ({6-[(1*E*)-3-amino-3-oxoprop-1-en-1-yl]-2-(2-methylpropyl)-1-oxo-4-phenyl- 1,2-dihydroisoquinolin-3-yl}methyl)carbamate (22a)

A mixture of **21a** (0.33 g, 0.7 mmol), WSC (0.27 g, 1.4 mmol), HOBt·NH_3 (0.21 g, 1.4 mmol) and *N,N*-dimethylformamide (10 mL) was stirred at room temperature for 2 h. The mixture was poured into water and extracted with AcOEt. The AcOEt extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was crystallized from AcOEt–IPE to give **22a** (0.31 g, 94%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.00 (6H, d, J = 6.6 Hz), 1.44 (9H, s), 2.16–2.24 (1H, m), 4.07 (2H, d, J = 7.4 Hz), 4.19 (2H, d, J = 5.6 Hz), 4.96 (1H, br s), 5.75 (1H, br s), 6.38 (1H, d, J = 15.6 Hz), 6.94 (1H, s), 7.26–7.30 (2H, m), 7.40–7.56 (5H, m), 8.29 (1H, d, J = 8.4 Hz). Mp 146–147 °C. Anal. Calcd for $C_{28}H_{33}N_3O_4{\cdot}1/4H_2O$: C, 70.05; H, 7.03; N, 8.75. Found: C, 70.08; H, 7.09; N, 8.64.

5.57. *tert*-Butyl {[6-(3-amino-3-oxopropyl)-2-(2-methylpropyl)-1-oxo-4-phenyl-1,2-dihydroisoquinolin-3-yl]methyl}carbamate (22b)

Compound **22b** was prepared in a manner similar to that described for **22a** in 94% yield as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.99 (6H, d, J = 6.6 Hz), 1.42 (9H, s), 2.13–2.29 (1H, m), 2.43 (2H, t, J = 8.0 Hz), 2.92 (2H, t, J = 8.0 Hz), 4.05 (2H, d, J = 7.2 Hz), 4.19 (2H, d, J = 5.2 Hz), 4.54 (1H, br s), 5.42 (2H, br s), 6.75 (1H, d, J = 1.4 Hz), 7.21–7.30 (3H, m), 7.46–7.57 (3H, m), 8.33–8.37 (1H, m). Mp 239–240 °C. Anal. Calcd for $C_{28}H_{35}N_3O_4{\cdot}1/4H_2O$: C, 69.76; H, 7.42; N, 8.72. Found: C, 69.54; H, 7.41; N, 8.58.

5.58. (2*E*)-3-[3-(Aminomethyl)-2-(2-methylpropyl)-1-oxo-4-phenyl-1,2-dihydroisoquinolin-6-yl]prop-2-enamide hydrochloride (23a)

Compound **23a** was prepared in a manner similar to that described for **19a** in 95% yield as a pale yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.92 (6H, d, J = 6.9 Hz), 2.06–2.16 (1H, m), 3.87 (2H, d, J = 4.8 Hz), 4.08 (2H, s), 6.56 (1H, d, J = 16.0 Hz), 6.97 (1H, d, J = 1.2 Hz), 7.19 (1H, br s), 7.31 (1H, d, J = 16.0 Hz), 7.42–7.44 (2H, m), 7.54–7.63 (3H, m), 7.69 (1H, br s), 7.78 (1H, dd, J = 1.2, 8.8 Hz), 8.35 (1H, d, J = 8.8 Hz), 8.62 (3H, br s). Mp 223–225 °C. Anal. Calcd for $C_{23}H_{26}Cl\ N_3O_2{\cdot}1.5H_2O$: C, 62.93; H, 6.66; N, 9.57. Found: C, 63.15; H, 6.66; N, 9.34. ESI-HRMS calcd for $C_{23}H_{25}N_3O_2$ *m/z* 376.2020 (M+H), found 376.1989 (M+H).

5.59. 3-[3-(Aminomethyl)-2-(2-methylpropyl)-1-oxo-4-phenyl-1,2-dihydroisoquinolin-6- yl]propanamide hydrochloride (23b)

Compound **23b** was prepared in a manner similar to that described for **19a** in 95% yield as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.91 (6H, d, J = 6.3 Hz), 2.02–2.16 (1H, m), 2.26 (2H, t, J = 7.2 Hz), 2.76 (2H, t, J = 7.2 Hz), 3.86 (2H, s), 4.08 (2H, s), 6.72 (1H, s), 6.75 (1H, br s), 7.29 (1H, br s), 7.38–7.40 (2H, m), 7.45 (1H, d, J = 8.4 Hz), 7.55–7.59 (3H, m), 8.25 (1H, d, J = 8.4 Hz), 8.57 (3H, br s). Mp 186–187 °C. ESI-HRMS calcd for $C_{23}H_{27}N_3O_2$ *m/z* 378.2176 (M+H), found 378.2150 (M+H).

5.60. 5-(Benzyloxy)-2-benzofuran-1,3-dione (26)

To a suspension of **24** (45.5 g, 250 mmol), K_2CO_3 (103.7 g, 750 mmol) and *N,N*-dimethylformamide (500 mL) was added benzylbromide (107 mL, 900 mmol), and the resulting mixture was stirred at 70 °C for 6 h. The mixture was poured into water and extracted with AcOEt. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was dissolved in THF (200 mL) and MeOH (200 mL), then added to a solution of sodium hydroxide (25.0 g, 625 mmol) in water (200 mL). The mixture was refluxed for 6 h. After being cooled, the mixture was poured into water and extracted with Et_2O. The water layer was acidified with 1 M hydrochloric acid, and extracted with AcOEt. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The resulting crystals were collected by filtration to give 4-(benzyloxy)benzene-1,2-dicarboxylic acid **25** (61.0 g, 90%) as white crystals. A mixture of **25** (61.0 g, 224 mmol) and acetic anhydride (200 mL) was stirred at 150 °C for 2 h. After evaporation of the solvent, the resulting crystals were collected by filtration to give **26** (51.4 g, 90%) as white crystals. ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.23 (2H, s), 7.37–7.49 (7H, m), 7.91 (1H, d, J = 8.8 Hz). Mp 124–125 °C. Anal. Calcd for $C_{15}H_{10}O_4$: C, 70.86; H, 3.96. Found: C, 70.79; H, 3.91.

5.61. Ethyl 6-(benzyloxy)-4-hydroxy-2-(2-methylpropyl)-1-oxo-1,2-dihydroisoquinoline-3- carboxylate (28a) and ethyl 7-(benzyloxy)-4-hydroxy-2-(2-methylpropyl)-1-oxo-1,2-dihydroisoquinoline-3-carboxylate (28b)

A mixture of **26** (4.07 g, 16 mmol) and ethyl 2-(isobutylamino)acetate (2.86 g, 18 mmol) in THF (30 mL) was stirred at room temperature for 1 h. The reaction mixture was poured into water and extracted with AcOEt. The AcOEt extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo to give compound **27**. The residue was dissolved in *N,N*-dimethylformamide (30 mL), then iodoethane (1.5 mL, 19.2 mmol) and K_2CO_3 (2.30 g, 12 mmol) were added to the mixture, and the resulting mixture was stirred at room temperature for 3 h. The reaction mixture was poured into water and extracted with AcOEt. The AcOEt extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was dissolved in ethanol (50 mL), then 20% sodium ethoxide in EtOH (10.9 g, 32 mmol) was added, and the mixture was stirred at room temperature for 1 h. The reaction mixture was poured into 1 M hydrochloric acid (70 mL) and extracted with AcOEt. The AcOEt extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 70/30) and the component eluted earlier was concentrated to give **28b** (4.21 g, 67%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.82 (6H, d, J = 6.6 Hz), 1.45 (3H, t, J = 7.1 Hz), 1.77–1.91 (1H, m), 4.42–4.52 (4H, m), 7.31–7.58 (6H, m), 7.97 (1H, d, J = 2.6 Hz), 8.10 (1H, d, J = 8.8 Hz), 11.45 (1H, s).

The component eluted later was concentrated to give **28a** (0.80 g, 13%) as crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.81 (6H, d, J = 6.6 Hz), 1.46 (3H, t, J = 7.2 Hz), 1.73–1.87 (1H, m), 4.39 (2H, d, J = 7.2 Hz), 4.48 (2H, q, J = 7.2 Hz), 5.21 (2H, s), 7.28–7.49 (6H, m), 7.60 (1H, d, J = 2.6 Hz), 8.38 (1H, d, J = 8.8 Hz), 11.23 (1H, s). Mp 92–93 °C. Anal. Calcd for $C_{23}H_{25}NO_5$: C, 70.90; H, 6.90; N, 3.31. Found: C, 70.84; H, 6.85; N, 3.11.

5.62. Ethyl 6-(benzyloxy)-2-(2-methylpropyl)-1-oxo-4-{[(trifluoromethyl)sulfonyl]oxy}- 1,2-dihydroisoquinoline-3-carboxylate (29a)

To a solution of **28a** (3.95 g, 10 mmol) in *N,N*-dimethylformamide (40 mL) was added portionwise sodium hydride (0.48 g,

12 mmol) at 0 °C, and the resulting mixture was stirred at 0 °C for 30 min. To the obtained mixture was added *N*-phenyltrifluoromethanesulfonimide (4.50 g, 12 mmol), and the resulting mixture was stirred at room temperature for 6 h. The mixture was poured into water and extracted with AcOEt. The AcOEt extract was washed with water, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 70/30) and concentrated to give **29a** (5.24 g, quant.) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.89 (6H, d, J = 6.6 Hz), 1.43 (3H, t, J = 7.1 Hz), 1.93–2.09 (1H, m), 4.03 (2H, d, J = 7.6 Hz), 4.46 (2H, q, J = 7.1 Hz), 5.19 (2H, s), 7.22–7.49 (7H, m), 8.36 (1H, d, J = 8.8 Hz).

5.63. Ethyl 7-(benzyloxy)-2-(2-methylpropyl)-1-oxo-4-{[(trifluoromethyl)sulfonyl]oxy}-1,2-dihydroisoquinoline-3-carboxylate (29b)

Compound **29b** was prepared in a manner similar to that described for **29a** in 91% yield as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (6H, d, J = 6.6 Hz), 1.44 (3H, t, J = 7.1 Hz), 1.96–2.07 (1H, m), 4.12 (2H, d, J = 7.2 Hz), 4.45 (2H, q, J = 7.1 Hz), 5.21 (2H, s), 7.30–7.48 (6H, m), 7.74 (1H, d, J = 9.0 Hz), 7.96 (1H, d, J = 2.4 Hz).

5.64. 6-(Benzyloxy)-2-(2-methylpropyl)-1-oxo-4-phenyl-1,2-dihydroisoquinoline-3-carboxylic acid (31a)

A mixture of **29a** (5.24 g, 10 mmol), phenylboronic acid (1.44 g, 12 mmol), Na_2CO_3 (3.18 g, 30 mmol), tetrakis(triphenylphosphine)palladium (0.69 g, 2.25 mmol), toluene (50 mL), MeOH (10 mL) and water (10 mL) was refluxed for 10 h under argon atmosphere. After being cooled, the mixture was poured into water and extracted with AcOEt. The AcOEt extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 70/30) and concentrated to give **30a** as white crystals. The obtained **30a** was dissolved in EtOH (50 mL) and THF (50 mL), then a solution of sodium hydroxide (1.20 g, 30 mmol) in water (20 mL) was added. The resulting mixture was refluxed for 3 h. The mixture was poured into water, acidified with 1 M hydrochloric acid, and extracted with AcOEt. The AcOEt extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo to give **31a** (2.95 g, 69.1%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.85 (6H, d, J = 6.6 Hz), 2.09–2.14 (1H, m), 3.83 (2H, d, J = 7.5 Hz), 4.96 (2H, s), 6.58 (1H, d, J = 2.4 Hz), 7.09–7.44 (11H, m), 8.29 (1H, d, J = 8.7 Hz).

5.65. 7-(Benzyloxy)-2-(2-methylpropyl)-1-oxo-4-phenyl-1,2-dihydroisoquinoline-3-carboxylic acid (31b)

Compound **31b** was prepared in a manner similar to that described for **31a** in 75% yield as an amorphous powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (6H, d, J = 6.8 Hz), 2.14–2.28 (1H, m), 4.01 (2H, d, J = 7.8 Hz), 5.15 (2H, s), 7.11–7.48 (12H, m), 7.66 (1H, d, J = 1.8 Hz).

5.66. *tert*-Butyl {[6-(benzyloxy)-2-(2-methylpropyl)-1-oxo-4-phenyl-1,2-dihydroisoquinolin- 3-yl]methyl}carbamate (32a)

To a solution of **31a** (3.42 g, 8 mmol) in THF (30 mL) were added oxalyl chloride (0.84 mL, 9.6 mmol) and *N*,*N*-dimethylformamide (3 drops), and the mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated under reduced pressure and the residue was dissolved in THF (20 mL). The obtained solution was added dropwise to a suspension of sodium tetrahydroborate (1.06 g, 28 mmol) in 1,2-dimethoxyethane (20 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h. The reaction mixture was poured into 1 M hydrochloric acid and extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The obtained crystals were recrystallized from THF-IPE to give 6-(benzyloxy)-3-(hydroxymethyl)-2-(2-methylpropyl)-4-phenylisoquinolin-1(2*H*)-one (2.92 g, 88.5%) as crystals. To a suspension of 6-(benzyloxy)-3-(hydroxymethyl)-2-(2-methylpropyl)-4-phenylisoquinolin-1(2*H*)-one (2.89 g, 7 mmol) in toluene (30 mL) was added thionyl chloride (1.0 mL, 14 mmol). The obtained mixture was refluxed under heating for 2 h. The reaction mixture was poured into saturated aqueous sodium hydrogen carbonate solution and extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo to give 6-(benzyloxy)-3-(chloromethyl)-2-(2-methylpropyl)-4-phenylisoquinolin-1(2*H*)-one (2.61 g, 86%) as crystals. A mixture of 6-(benzyloxy)-3-(chloromethyl)-2-(2-methylpropyl)-4-phenylisoquinolin-1(2*H*)-one (2.59 g, 6 mmol) and potassium phthalimide (1.67 g, 9 mmol) and *N*,*N*-dimethylformamide (30 mL) was stirred at room temperature for 6 h. The reaction mixture was poured into water and extracted with AcOEt. After washing with water, the extract was dried over anhydrous $MgSO_4$, and concentrated in vacuo. The obtained crystals were recrystallized from AcOEt–IPE to give 2-{[6-(benzyloxy)-2-(2-methylpropyl)-1-oxo-4-phenyl-1,2-dihydroisoquinolin-3-yl]methyl}-1*H*-isoindole-1,3(2*H*)-dione (3.04 g, 94%) as crystals. To a solution of 2-{[6-(benzyloxy)-2-(2-methylpropyl)-1-oxo-4-phenyl-1,2-dihydroisoquinolin-3-yl]methyl}-1*H*-isoindole-1,3(2*H*)-dione (2.98 g, 5.5 mmol) in EtOH (30 mL) was added hydrazine monohydrate (0.8 mL, 16.5 mmol). The obtained mixture was refluxed under heating for 1 h. The reaction mixture was poured into saturated aqueous sodium hydrogen carbonate solution and extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was dissolved in THF (20 mL) and di-*t*-butyl dicarbonate (1.9 mL, 8.3 mmol) was added thereto. The obtained mixture was stirred at room temperature for 1 h. The reaction mixture was poured into water and extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The obtained crystals were recrystallized from AcOEt–IPE to give **32a** (2.29 g, 81%) as crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.99 (6H, d, J = 7.0 Hz), 1.42 (9H, s), 2.16–2.30 (1H, m), 4.03 (2H, d, J = 7.4 Hz), 4.17 (2H, d, J = 5.6 Hz), 4.45 (1H, br s), 4.92 (2H, s), 6.35 (1H, d, J = 2.4 Hz), 7.09 (1H, dd, J = 2.4, 9.0 Hz), 7.17–7.37 (7H, m), 7.47–7.52 (3H, m), 8.38 (1H, d, J = 9.0 Hz). Mp 141–142 °C. Anal. Calcd for $C_{32}H_{35}N_2O_4$: C, 74.97; H, 7.08; N, 5.46. Found: C, 74.60; H, 7.13; N, 5.45.

5.67. *tert*-Butyl {[7-(benzyloxy)-2-(2-methylpropyl)-1-oxo-4-phenyl-1,2-dihydroisoquinolin- 3-yl]methyl}carbamate (32b)

Compound **32b** was prepared in a manner similar to that described for **32a** in 75% yield as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.01 (6H, d, J = 6.8 Hz), 1.43 (9H, s), 2.20–2.31 (1H, m), 4.08 (2H, d, J = 7.4 Hz), 4.19 (2H, d, J = 5.2 Hz), 4.55 (1H, br s), 5.16 (2H, s), 6.89 (1H, d, J = 8.8 Hz), 7.15 (1H, dd, J = 2.8, 8.8 Hz), 7.24–7.55 (10H, m), 7.96 (1H, d, J = 2.8 Hz). Mp 181–182 °C. Anal. Calcd for $C_{32}H_{36}N_2O_4$: C, 74.97; H, 7.08; N, 5.46. Found: C, 74.94; H, 7.14; N, 5.31.

5.68. *tert*-Butyl {[6-hydroxy-2-(2-methylpropyl)-1-oxo-4-phenyl-1,2-dihydroisoquinolin-3- yl]methyl}carbamate (33a)

A suspension of **32a** (2.05 g, 4 mmol) and 5% palladium on carbon (0.6 g) in THF (10 mL) and EtOH (10 mL) was stirred under a hydrogen atmosphere at room temperature for 2 h. The catalyst

was filtered off and the filtrate was concentrated under reduced pressure. The obtained crystals were recrystallized from AcOEt–IPE to give **33a** (1.56 g, 92.3%) as crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (6H, d, J = 6.8 Hz), 1.42 (9H, s), 2.14–2.24 (1H, m), 4.02 (2H, d, J = 7.2 Hz), 4.18 (2H, d, J = 5.4 Hz), 4.47 (1H, br s), 6.33 (1H, d, J = 2.4 Hz), 7.03 (1H, dd, J = 2.4, 8.8 Hz), 7.20–7.27 (2H, m), 7.43–7.46 (3H, m), 7.97 (1H, br s), 8.30 (1H, d, J = 8.8 Hz). Mp 218–219 °C. Anal. Calcd for $C_{25}H_{30}N_2O_4$: C, 71.07; H, 7.16; N, 6.63. Found: C, 70.85; H, 7.10; N, 6.62.

5.69. *tert*-Butyl {[7-hydroxy-2-(2-methylpropyl)-1-oxo-4-phenyl-1,2-dihydroisoquinolin-3- yl]methyl}carbamate (33b)

Compound **33b** was prepared in a manner similar to that described for **33a** in 97% yield as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.02 (6H, d, J = 7.0 Hz), 1.42 (9H, s), 2.21–2.35 (1H, m), 4.11 (2H, d, J = 7.2 Hz), 4.22 (2H, d, J = 5.2 Hz), 4.52 (1H, br s), 6.91 (1H, d, J = 8.8 Hz), 7.16 (1H, dd, J = 2.6, 8.8 Hz), 7.23–7.28 (2H, m), 7.44–7.55 (3H, m), 8.52 (1H, d, J = 2.8 Hz), 8.90 (1H, s). Mp 232–233 °C. Anal. Calcd for $C_{25}H_{30}N_2O_4$: C, 71.07; H, 7.16; N, 6.63. Found: C, 70.81; H, 7.22; N, 6.35.

5.70. *tert*-Butyl {[6-(2-amino-2-oxoethoxy)-2-(2-methylpropyl)-1-oxo-4-phenyl-1,2- dihydroisoquinolin-3-yl]methyl}-carbamate (34a)

A solution of **33a** (0.63 g, 1.5 mmol), 2-iodoacetamide (0.43 g, 2.3 mmol) and 1,8-diazabicyclo[5.4.0]-7-undecene (0.34 mL, 2.3 mmol) in *N*,*N*-dimethylformamide (10 mL) was stirred at 80 °C for 10 h. The reaction mixture was poured into water and extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 50/50) to give **34a** (0.32 g, 44%) as crystals. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.89 (6H, d, J = 6.6 Hz), 1.38 (9H, s), 2.07–2.21 (1H, m), 3.88 (2H, d, J = 6.6 Hz), 3.95 (2H, d, J = 4.0 Hz), 4.34 (2H, s), 6.30 (1H, d, J = 2.4 Hz), 7.13 (1H, dd, J = 2.4, 8.8 Hz), 7.34–7.52 (8H, m), 8.24 (1H, d, J = 8.8 Hz). Mp 226–227 °C. Anal. Calcd for $C_{27}H_{33}N_3O_5$: C, 67.62; H, 6.94; N, 8.76. Found: C, 67.36; H, 6.73; N, 8.60.

5.71. *tert*-Butyl {[7-(2-amino-2-oxoethoxy)-2-(2-methylpropyl)-1-oxo-4-phenyl-1,2- dihydroisoquinolin-3-yl]methyl}-carbamate (34b)

Compound **34b** was prepared in a manner similar to that described for **34a** in 40% yield as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.00 (6H, d, J = 6.6 Hz), 1.43 (9H, s), 2.18–2.34 (1H, m), 4.08 (2H, d, J = 7.4 Hz), 4.20 (2H, d, J = 5.4 Hz), 4.51 (1H, br s), 4.58 (2H, s), 5.86 (1H, br s), 6.57 (1H, br s), 6.94 (1H, d, J = 8.8 Hz), 7.13 (1H, d, J = 8.8 Hz), 7.23–7.27 (2H, m), 7.49–7.52 (3H, m), 7.90 (1H, s). Mp 211–212 °C. Anal. Calcd for $C_{27}H_{33}N_3O_5$: C, 67.62; H, 6.94; N, 8.76. Found: C, 67.38; H, 6.69; N, 8.87.

5.72. 2-{[3-(Aminomethyl)-2-(2-methylpropyl)-1-oxo-4-phenyl-1,2-dihydroisoquinolin-6- yl]oxy}acetamide hydrochloride (35a)

Compound **35a** was prepared in a manner similar to that described for **19a** in 95% yield as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.91 (6H, d, J = 6.6 Hz), 2.01–2.18 (1H, m), 3.37 (2H, br s), 3.85 (2H, br s), 4.05 (2H, d, J = 6.8 Hz), 4.36 (2H, s), 6.30 (1H, d, J = 2.0 Hz), 7.19 (1H, dd, J = 2.0, 8.8 Hz), 7.36–7.40 (2H, m), 7.52–7.58 (3H, m), 8.28 (1H, d, J = 8.8 Hz), 8.55 (3H, s). Mp 185–186 °C. Anal. Calcd for $C_{22}H_{26}ClN_3O_3 \cdot 1/2H_2O$: C, 62.23; H, 6.44; N, 9.75. Found: C, 62.18; H, 6.40; N, 9.89. ESI-HRMS calcd for $C_{22}H_{25}N_3O_3$ *m/z* 380.1969 (M+H), found 380.1946 (M+H).

5.73. 2-{[3-(Aminomethyl)-2-(2-methylpropyl)-1-oxo-4-phenyl-1,2-dihydroisoquinolin-7-yl]oxy}acetamide hydrochloride (35b)

Compound **35b** was prepared in a manner similar to that described for **19a** in 91% yield as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.92 (6H, d, J = 6.6 Hz), 1.99–2.19 (1H, m), 3.85 (2H, d, J = 4.2 Hz), 4.09 (2H, d, J = 7.0 Hz), 4.58 (2H, s), 6.85 (1H, d, J = 8.8 Hz), 7.33 (1H, dd, J = 2.6, 8.8 Hz), 7.36–7.41 (2H, m), 7.56–7.60 (3H, m), 7.69 (1H, br s), 7.72 (1H, d, J = 2.6 Hz), 8.64 (3H, s). Mp 244–245 °C. ESI-HRMS calcd for $C_{22}H_{25}N_3O_3$ *m/z* 380.1969 (M+H), found 380.1943 (M+H).

5.74. 3-(Aminomethyl)-6-hydroxy-2-(2-methylpropyl)-4-phenylisoquinolin-1(2*H*)-one hydrochloride (36)

Compound **36** was prepared in a manner similar to that described for **19a** in 89% yield as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.90 (6H, d, J = 6.6 Hz), 1.99–2.13 (1H, m), 3.82 (2H, d, J = 5.6 Hz), 4.03 (2H, d, J = 7.0 Hz), 6.20 (1H, d, J = 2.4 Hz), 7.02 (1H, dd, J = 2.4, 8.6 Hz), 7.36–7.40 (2H, m), 7.47–7.60 (3H, m), 8.04 (1H, br s), 8.16 (1H, d, J = 8.6 Hz), 8.61 (3H, br s). Mp 249–251 °C. ESI-HRMS calcd for $C_{20}H_{22}N_2O_2$ *m/z* 321.1609 (M−H), found 321.1623 (M−H).

5.75. 3-{[(*tert*-Butoxycarbonyl)amino]methyl}-2-(2-methylpropyl)-1-oxo-4-phenyl-1,2- dihydroisoquinolin-7-yl trifluoromethanesulfonate (37)

Compound **37** was prepared in a manner similar to that described for **29a** in 90% yield as an amorphous powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.01 (6H, d, J = 6.6 Hz), 1.43 (9H, s), 2.20–2.29 (1H, m), 4.09 (2H, d, J = 7.5 Hz), 4.23 (2H, d, J = 5.4 Hz), 4.46 (1H, br s), 7.06 (1H, d, J = 9.0 Hz), 7.22–7.27 (2H, m), 7.36 (1H, dd, J = 2.7, 9.0 Hz), 7.42–7.56 (3H, m), 8.34 (1H, d, J = 2.7 Hz).

5.76. Methyl 3-{[(*tert*-butoxycarbonyl)amino]methyl}-2-(2-methylpropyl)-1-oxo-4-phenyl- 1,2-dihydroisoquinoline-6-carboxylate (38)

Compound **38** was prepared in a manner similar to that described for **15** in 74% yield as crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.01 (6H, d, J = 6.6 Hz), 1.43 (9H, S), 2.05–2.28 (1H, m), 3.93 (3H, s), 4.10 (2H, d, J = 7.5 Hz), 4.22 (2H, d, J = 5.4 Hz), 4.61 (1H, br s), 6.98 (1H, d, J = 8.7 Hz), 7.24–7.28 (2H, m), 7.46–7.57 (3H, m), 8.02 (1H, d, J = 8.7, 1.8 Hz), 9.10 (1H, d, J = 1.8 Hz). Mp 134–135 °C. Anal. Calcd for $C_{27}H_{32}N_2O_5$: C, 69.81; H, 6.94; N, 6.03. Found: C, 69.46; H, 7.04; N, 5.81.

5.77. 3-{[(*tert*-Butoxycarbonyl)amino]methyl}-2-(2-methylpropyl)-1-oxo-4-phenyl-1,2- dihydroisoquinoline-7-carboxylic acid (39)

Compound **39** was prepared in a manner similar to that described for **16** in 92% yield as crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.91 (6H, d, J = 6.6 Hz), 1.38 (9H, s), 2.11–2.26 (1H, m), 3.91 (2H, d, J = 6.6 Hz), 3.99 (2H, d, J = 4.2 Hz), 6.99 (1H, d, J = 8.8 Hz), 7.34 (1H, br s), 7.39–7.42 (2H, m), 7.46–7.56 (3H, m), 8.09 (1H, dd, J = 2.0, 8.8 Hz), 8.87 (1H, d, J = 2.0 Hz). Mp 246 °C. Anal. Calcd for $C_{26}H_{30}N_2O_5 \cdot 1/4H_2O$: C, 68.62; H, 6.76; N, 6.16. Found: C, 68.81; H, 6.83; N, 5.87.

5.78. 3-{[(*tert*-Butoxycarbonyl)amino]methyl}-2-(2-methylpropyl)-1-oxo-4-phenyl-1,2- dihydroisoquinoline-7-carboxamide (40)

Compound **40** was prepared in a manner similar to that described for **17a** in 96% yield as crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.99 (6H, d, J = 6.6 Hz), 1.43 (9H, s), 2.10–2.24 (1H, m), 4.08 (2H, d, J = 7.0 Hz), 4.22 (2H, d, J = 5.2 Hz), 4.76 (1H, br s), 5.96 (1H, br s), 6.74 (1H, br s), 7.02 (1H, d, J = 8.6 Hz), 7.25–7.30 (2H, m), 7.45–7.56 (3H, m), 8.05 (1H, dd, J = 1.4, 8.6 Hz), 8.78 (1H, d, J = 1.4 Hz). Mp 232–233 °C. Anal. Calcd for $C_{26}H_{31}N_3O_4{\cdot}1/2H_2O$: C, 68.10; H, 7.03; N, 9.16. Found: C, 68.31; H, 7.07; N, 8.75.

5.79. 3-(Aminomethyl)-2-(2-methylpropyl)-1-oxo-4-phenyl-1,2-dihydroisoquinoline-7-carboxamide hydrochloride (41)

Compound **41** was prepared in a manner similar to that described for **19a** in 95% yield as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.94 (6H, d, J = 6.6 Hz), 2.04–2.19 (1H, m), 3.89 (2H, d, J = 4.4 Hz), 4.13 (2H, d, J = 7.0 Hz), 6.94 (1H, d, J = 8.6 Hz), 7.39–7.44 (2H, m), 7.54–7.64 (4H, m), 8.16 (1H, dd, J = 1.8, 8.6 Hz), 8.30 (1H, br s), 8.67 (3H, br s). Mp 254–256 °C. Anal. Calcd for $C_{21}H_{23}N_3O_2{\cdot}HCl{\cdot}H_2O$: C, 63.87; H, 6.38; N, 10.64. Found: C, 63.76; H, 6.29; N, 10.30. ESI-HRMS calcd for $C_{21}H_{23}N_3O_2$ m/z 350.1863 (M+H), found 350.1828 (M+H).

5.80. DPP4 + compound 35a structure determination

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

Table 5
Summary of crystallographic analysis

	Compound **35a**
PDB code	3OPM
Data collection	
Space group	*P*21
Unit cell lengths (Å)	121.8; 122.6; 144.7
Unit cell angles (°)	90.0; 115.0; 90.0
Resolution (Å)	2.72
Observations	413,990
Unique	100,745
Completeness (%)	97.5 (82.7)
I/σ_I	10.9 (1.5)
R_{sym} (%)	0.114 (0.590)
Model refinement	
Reflections (work/free)	95,647/5038
R_{factor} (work/free %)	19.17/25.57
Protein molecules per ASU	4
Solvent molecules	506
Mean B value (Å^2)	49.2
RMSD ideal bond lengths (Å)	0.009
RMSD ideal bond angles (°)	1.24

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

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

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

Rat DPP-2 was partially purified from rat kidney according to the method previously reported. One microliter of compounds dissolved in DMSO was mixed with 29 μL of distilled water, 10 μL of 1 mol/L 3,3-dimethylglutamic acid buffer (pH 5.5), and 10 μL of the DPP-2 fraction. After the mixture was incubated at room temperature for 20 min, the reaction was initiated by adding 50 μL of 1 mmol/L of H-Lys-Ala-pNA·2HCl and run at 37 °C for 60 min. Human DPP-8 and DPP-9 were purified, respectively by affinity chromatography from the 293-F cells expressing each FLAG-tagged protein. One microliter of compounds dissolved in DMSO was mixed with 29 μL of distilled water, 10 μL of 1 mol/L Tris–HCl buffer (pH 7.5), and 10 μL of the enzyme fraction. After the mixture was incubated at room temperature for 20 min, the reaction was initiated by adding 50 μL of 2 mmol/L of Gly-Pro-pNA·Tos for DPP-8 or 4 mmol/L of Gly-Pro-pNA·Tos for DPP-9 and run at 37 °C for 90 min. Absorbance at 405 nm of each reaction mixture was measured using a microplate reader at the initial time and the end of the reaction. The well containing substrate alone was used as a basal control. The well containing the substrate and the enzyme without the compound was used as a total reaction.

5.82. Oral glucose tolerance test in female Wistar fatty rats

Prior to the start of the study, rats were fasted overnight and divided into six groups based on plasma glucose and body weight. Each group was administered vehicle (0.5% methylcellulose) or compound orally. One hour after drug or vehicle administration,

all animals received an oral glucose load (1 g/kg). Blood samples were collected before the glucose load (time 0), and 10, 30, 60 and 120 min after the glucose load and plasma glucose and plasma immuno-reactive insulin (IRI) were analyzed.

Acknowledgments

The authors thank Dr. Hidefumi Yukimasa, Dr. Kaneyoshi Kato, and Dr. Akio Miyake for helpful discussions. We also thank ALS, which is supported by the Director, Office of Science, Office of Basic Energy Sciences, Materials Sciences Division of the US Department of Energy under contract DE-AC03-76SF00098 at Lawrence Berkeley National Laboratory.

A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.06.059. These data include MOL files and InChiKeys of the most important compounds described in this article.

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