Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 16 (2008) 4715–4732
Bioorganic & Medicinal Chemistry

Discovery, synthesis and biological evaluation of isoquinolones as novel and highly selective JNK inhibitors (1)

Yasutomi Asano,[a,*] Shuji Kitamura,[a] Taiichi Ohra,[a] Kazuyoshi Aso,[a] Hideki Igata,[c] Tomoko Tamura,[c] Tomohiro Kawamoto,[b] Toshimasa Tanaka,[b] Satoshi Sogabe,[b] Shin-ichi Matsumoto,[b] Masashi Yamaguchi,[b] Hiroyuki Kimura[b] and Fumio Itoh[a]

[a]*Medicinal Chemistry Research Laboratories, Pharmaceutical Research Division, Takeda Pharmaceutical Company, Ltd, 17-85, Jusohonmachi 2-chome, Yodogawa-ku, Osaka 532-8686, Japan*
[b]*Discovery Research Center, Pharmaceutical Research Division, Takeda Pharmaceutical Company, Ltd, 17-85, Jusohonmachi 2-chome, Yodogawa-ku, Osaka 532-8686, Japan*
[c]*Pharmacology Research Laboratories I, Pharmaceutical Research Division, Takeda Pharmaceutical Company, Ltd, 17-85, Jusohonmachi 2-chome, Yodogawa-ku, Osaka 532-8686, Japan*

Received 27 November 2007; revised 7 February 2008; accepted 8 February 2008
Available online 13 February 2008

Abstract—A novel series of 4-phenylisoquinolones were synthesized and evaluated as c-Jun N-terminal kinase (JNK) inhibitors. Initial modification at the 2- and 3-positions of the isoquinolone ring of hit compound **4**, identified from high-throughput screening, led to the lead compound **6b**. The optimization was carried out using a JNK1-binding model of **6b** and several compounds exhibited potent JNK inhibition. Among them, **11g** significantly inhibited cardiac hypertrophy in rat pressure-overload models without affecting blood pressure and the concept of JNK inhibitors as novel therapeutic agents for heart failure was confirmed.

1. Introduction

The c-Jun N-terminal kinases (JNKs)[1] form one subfamily of the mitogen-activated protein kinase (MAPK) group of serine–threonine protein kinases. JNK isoforms are created by alternative splicing of messenger RNA transcripts derived from three genes (*JNK1*, *JNK2*, *JNK3*). JNK1 and JNK2 exhibit a broad tissue distribution, whereas JNK3 is predominantly localized in the brain and testes. JNK activation leads to the phosphorylation of a number of transcription factors—most notably the c-Jun component of AP-1—and cellular proteins, particularly those associated with apoptosis (for example, Bcl2, p53 and so on). In this way, JNKs can serve as critical mediators of a variety of extra-cellular stimuli. Furthermore, activation of JNK in many diseases such as autoimmune and inflammatory diseases, neurological diseases, metabolic diseases and cancer has also been reported.[2]

Numerous companies have been pursuing discovery programs aimed at identifying small-molecule inhibitors of this enzyme. Recent progress in the X-ray crystal structure analysis of the members of the MAPK families such as ERK,[3] p38[4] and JNK[5] has also contributed to design of potent and selective JNK inhibitors. Several JNK inhibitors have been reported in the literature (Fig. 1). Celgene (Signal Pharmaceutical) reported the discovery of a series of pyrazoloanthrone derivatives **1** (SP600125)[6] as JNK inhibitors. Serono reported benzazole-acetonitrile derivatives **2** (AS601245)[7] which demonstrated efficacy by oral administration in an experimental model of rheumatoid arthritis. AstraZeneca also disclosed a series of aminoindazoles, represented by **3**,[8] as potent JNK inhibitors with good pharmacokinetic profiles. Additionally, some short JNK-inhibitory peptides derived from JNK-interacting protein 1 (JIP1), the protein 'scaffold' in the JNK pathway, have been identified to investigate the therapeutic potential of the JNK inhibitors.[9]

Keywords: JNK; JNK inhibitor; Isoquinolone; SAR; Modeling; Heart failure; Cardiac hypertrophy; X-ray crystallography.
* Corresponding author. Tel.: +81 6 6300 6836; fax: +81 6 6300 6306; e-mail: Asano_Yasutomi@takeda.co.jp

0968-0896/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2008.02.027

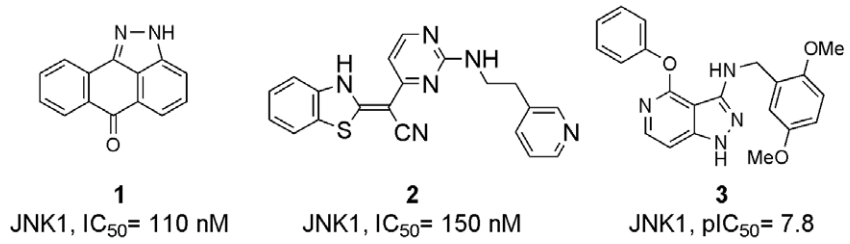
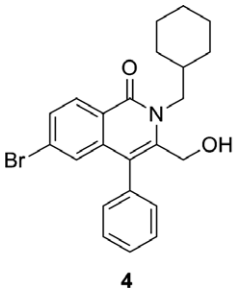


Figure 1. The structures of JNK inhibitors.

Recently, several reports have indicated that JNK plays important roles in the progression of heart failure. The development of cardiac hypertrophy in a murine pressure-overload model was suppressed without affecting blood pressure by JNK inhibition using dominant negative SEK-1, a kinase upstream of JNK.[10] Additionally, the MKK-7 over-expressing mice caused heart failure.[11] Moreover, JNK was activated in the heart of the patients with heart failure following myocardial infarction.[12] This accumulated evidence suggested that JNK inhibitors could be useful in the treatment of heart failure. On the other hand, some angiotensin II type 1 receptor blockers (ARB) also attenuate pressure-overload-induced cardiac hypertrophy in rats. However, this suppressive effect of ARB is accompanied by lowering blood pressure.[13] Therefore, we launched an investigation of JNK inhibitors as novel therapeutic agents for heart failure that should not affect blood pressure.

Isoquinolone derivative **4** was identified from high-throughput screening (HTS) for JNK1 inhibitors. The hit compound **4** showed relatively potent enzymatic inhibitory activity with an IC_{50} value of 1400 nM for JNK1, and it was also selective over two other closely related MAP kinases, p38 MAP kinase and ERK1 (Fig. 2).

During an initial SAR study of the hit compound **4**, the hydroxymethyl group at the 3-position and the cyclohexylmethyl group at the 2-position were modified due to their metabolic instability and the possibility of enhancing JNK-inhibitory activity (Table 1). Substitutions of the hydroxymethyl group for carboxylic acid (**5a**) and carboxylic acid methyl ester (**6a**) at the 3-position were performed. As a result, compounds **5a** and **6a** showed increased activity compared to the hit compound **4**. In addition, the cyclohexylmethyl group at 2-position of **5a** was converted to a benzyl (**6b**), phenethyl (**6c**) or phenylpropyl (**6d**) group. The inhibitory activity of **6b** was most potent (IC_{50} = 86 nM) after the initial modification. Compound **6b** was selected as a lead compound and further optimization was carried out.



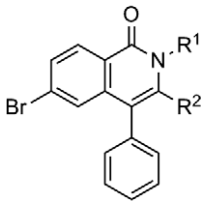
4

Kinase assay IC_{50} (nM)		
JNK1	p38α	ERK1
1400	>10000	>10000

Figure 2. Structure and enzymatic inhibitory activity of hit compound **4**.

The binding mode of lead compound **6b** to JNK1 was predicted by a docking study using a JNK1 model based on crystallographic data for JNK3. The amino acid residues near the ATP-binding site of JNK1 and JNK3 are well conserved and only two amino acid residues are different. Thus, Ser125 and Leu144 of JNK3 are substituted to Gly87 and Ile106 in JNK1, respectively. As for Ser125, the side chain is oriented toward the outside of the ATP pocket (solvent). Leu144 is situated at the back of the pocket and is out of range of hinge binders. It was assumed that compound **6b** was an ATP competitive inhibitor. As shown in Figure 3, the docking study suggested that compound **6b** filled the adenine-binding site by forming a hydrogen bond between the carbonyl oxygen of the isoquinolone group and the backbone NH group of Met111 in the hinge region of JNK1. Asp169 is close to the methoxycarbonyl group at the 3-position and the phenyl group at the 4-position. In addition to Asp169, other amino acid residues such as Lys55, Gln37 and Arg174 are near the phenyl group at the 4-position. The bromine atom at the 6-position occupies the hydrophobic pocket. Asn114 and Gln117 were found in front of the benzyl group, and it was thought that functionality at the 4-position of the benzyl

Table 1. Effect of substitutions on the isoquinolone ring



Compound	R^1	R^2	IC_{50}[a] (nM)		
			JNK1	p38α	ERK1
4	CH_2-*c*-Hex	CH_2OH	1400	>1100	>1100
5a	CH_2-*c*-Hex	CO_2H	860	>1100	>1100
6a	CH_2-*c*-Hex	CO_2Me	450	>1100	>1100
6b	CH_2Ph	CO_2Me	86	>1100	>1100
6c	$(CH_2)_2Ph$	CO_2Me	1100	>1100	>1100
6d	$(CH_2)_3Ph$	CO_2Me	190	>1100	>1100

[a] Enzymatic inhibitory activity.

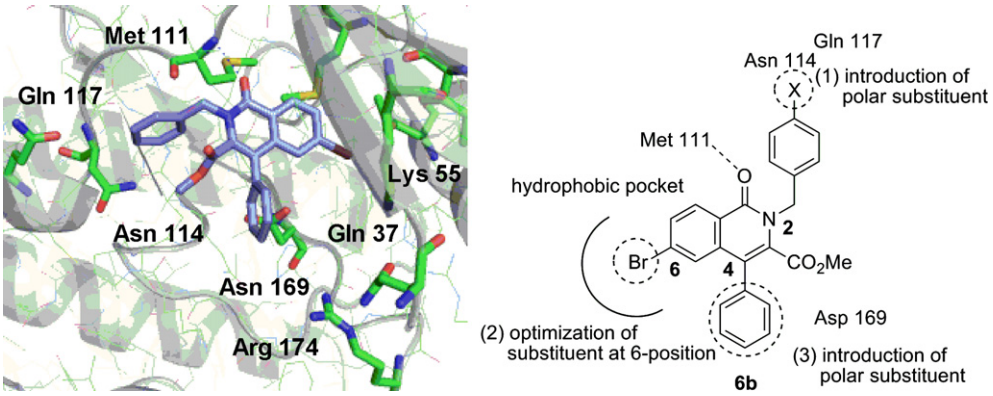


Figure 3. Predicated binding mode of lead compound **6b** to JNK1.

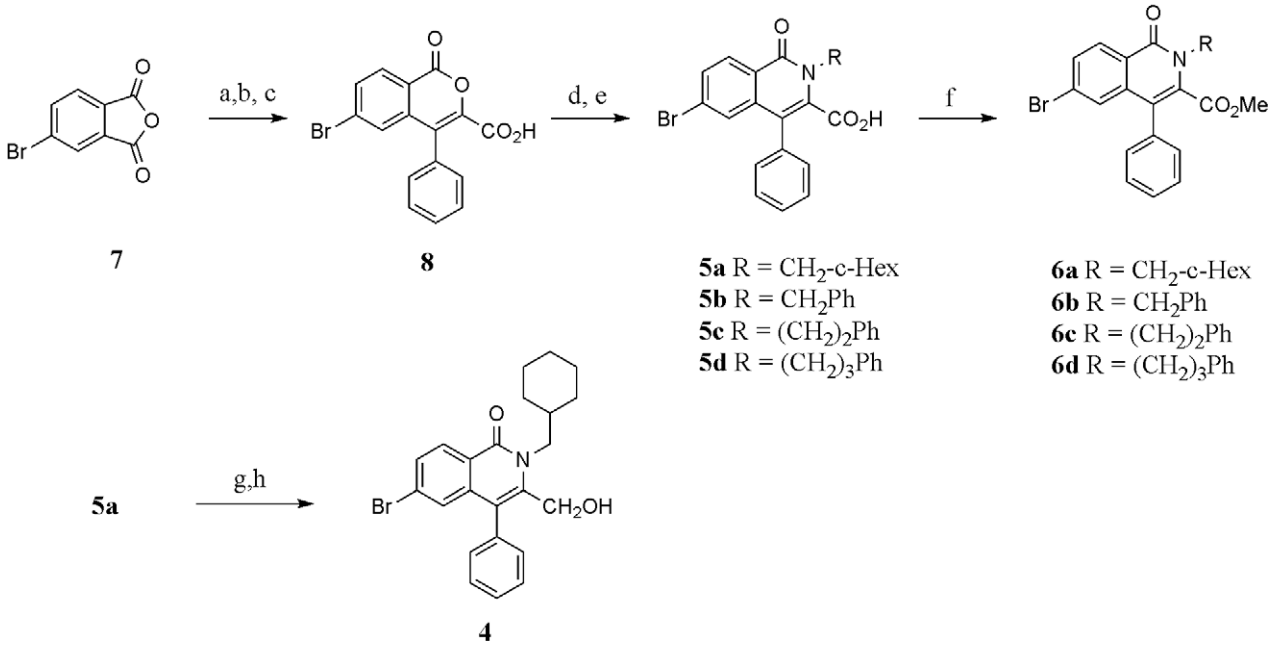
group could suitably form hydrogen bonds with Asn114 or Gln117.

From consideration of binding model described above, we planned the following strategy to increase the potency of compound **6b**: (1) installation of hydrogen bond-forming functionality at the 3- or 4-position of the benzyl group in anticipation of interaction with Asn114 and/or Gln117, (2) optimization of the 6-position substituent aimed at forming hydrophobic interaction with the hydrophobic pocket, (3) introduction of substituents onto the pendant phenyl ring at the 4-position in order to form interactions with polar amino acid residues such as Asp169, Lys55, Gln37 and Arg174. In this report we describe the design, synthesis, SAR, and pharmacological properties of the isoquinolone derivatives, and also a co-crystal structure of an isoquinolone derivative with JNK3.

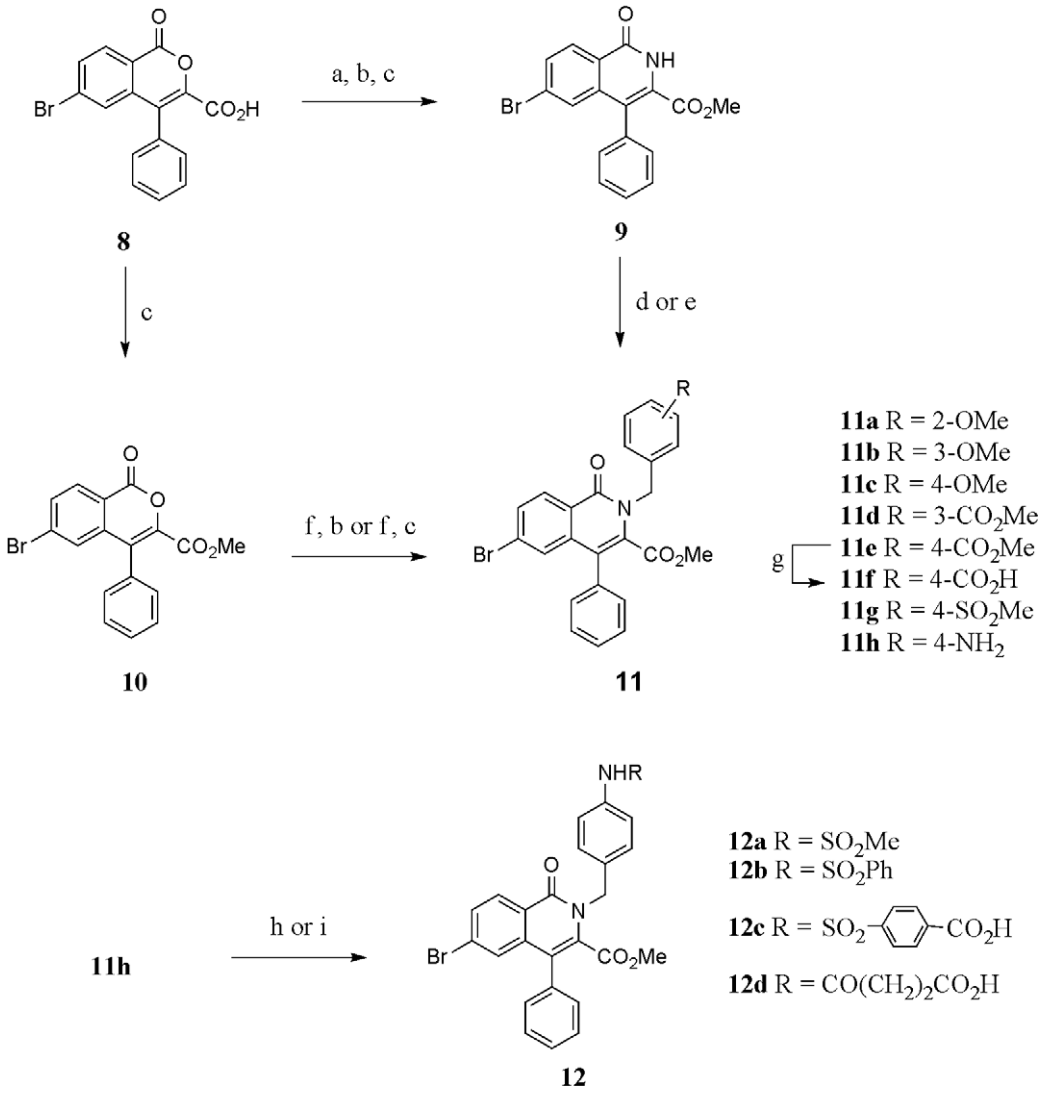
2. Chemistry

Initial modification at the 2- and 3-positions of isoquinolone ring was carried out as shown in Scheme 1. Friedel–Crafts reaction of commercially available 4-bromophthalic anhydride **7** with benzene and subsequent alkylation with diethyl bromomalonate followed by treatment with acetic acid and concentrated hydrochloric acid gave 1-oxoisochromene **8**. Reaction of **8** with the appropriate amines followed by treatment with 4 N hydrogen chloride in ethyl acetate afforded isoquinolones **5**. Methyl esters **6** were obtained by methylation of carboxylic acids **5** under basic condition. Conversion of carboxylic acid **5a** to the acid chloride followed by reduction with sodium borohydride yielded alcohol **4**.

Installation of various functional groups onto the 2-benzyl group was performed as shown in Scheme 2. Reaction of 1-oxoisochromene **8** with ammonia followed by



Scheme 1. Modification of substituent at 2 and 3-position. Reagents and conditions: (a) $AlCl_3$, benzene, rt; (b) K_2CO_3, $BrCH(CO_2Et)_2$, rt; (c) concd HCl, AcOH, 120 °C; (d) RNH_2, MeOH, rt; (e) 4 N-HCl in AcOEt, rt; (f) MeI, K_2CO_3, DMF, rt; (g) $(COCl)_2$, DMF, THF, rt; (h) $NaBH_4$, THF, 1,2-dimethoxyethane, 0 °C.

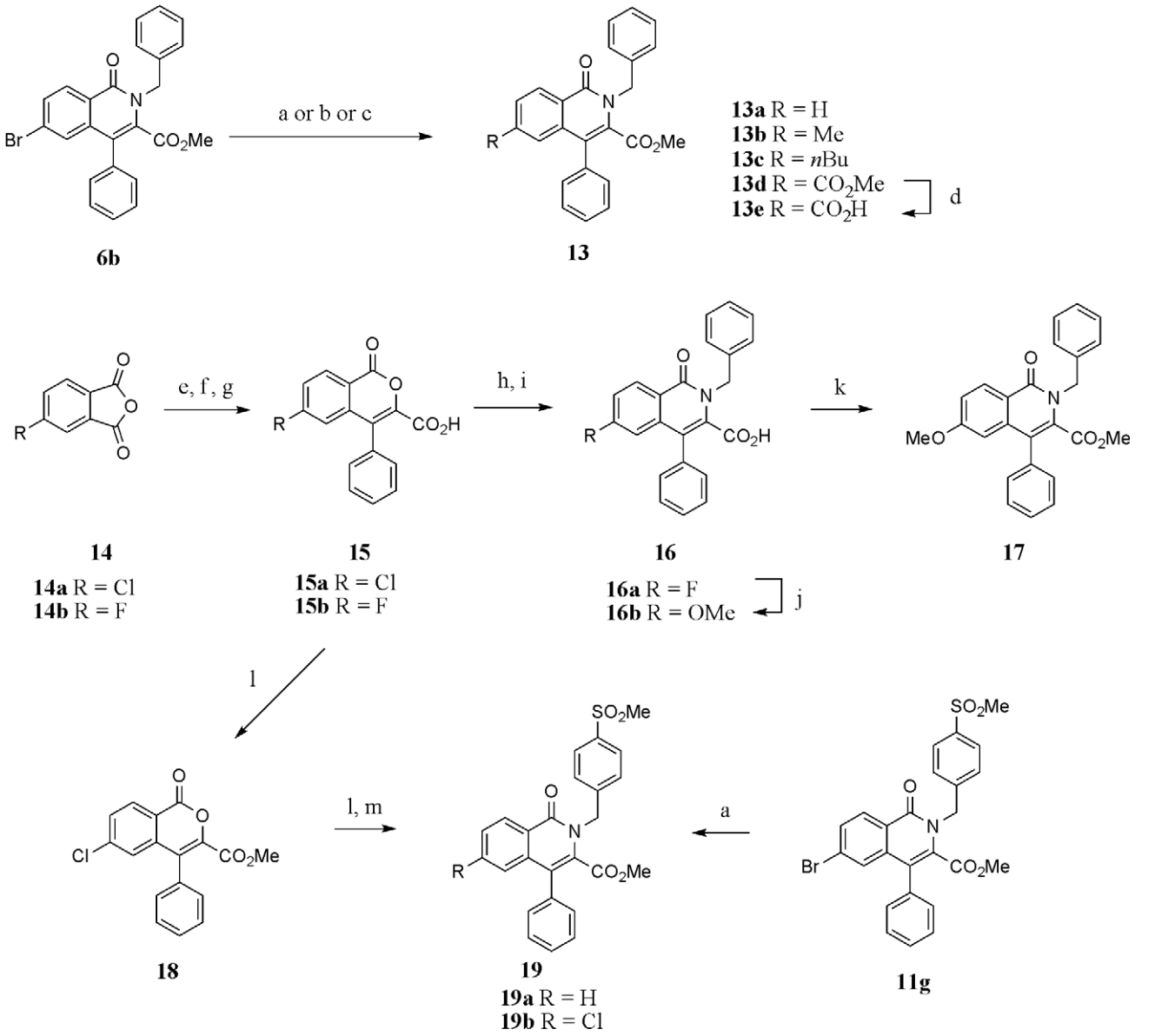


Scheme 2. Introduction of substituent on the benzene ring of 2-benzyl group. Reagents and conditions: (a) NH_3, MeOH, rt; (b) 4 N-HCl in AcOEt, rt; (c) H_2SO_4, MeOH, reflux; (d) $ArCH_2X$, basic resine, DMF, rt; (e) NaH, $ArCH_2Br$, DMF, rt; (f) $ArCH_2NH_2$, MeOH, rt; (g) NaOH, H_2O, MeOH, THF, rt; (h) RCl, Et_3N, THF, rt; (i) succinic anhydride, Et_3N, THF, rt.

dehydration under acidic conditions and esterification afforded isoquinolone **9**. Isoquinolones **11b–d** with substitutions at the 2-position were obtained by the reaction of isoquinolone **9** with the appropriate benzyl halide. Carboxylic acid **8** was esterified to give methyl ester **10**, which was reacted with benzylamine derivatives followed by dehydration under acidic conditions to afford isoquinolones **11a**, **e**, **g**, **h**. Alkaline hydrolysis of esters **11e** yielded carboxylic acids **11f**. Sulfonylation or acylation of amino derivative **11h** yielded sulfonyl or acyl derivatives **12a–d**.

Conversion of the substituent at the 6-position of isoquinolone derivatives is shown in Scheme 3. Catalytic hydrogenation of bromides **6b** and **11g** gave **13a** and **19a**, respectively. Suzuki coupling of **6b** with the appropriate alkylboronic acids yielded 6-alkylated compounds **13b**, **c**, respectively. Diester **13d** was also synthesized from **6b** by methoxycarbonylation using palladium catalyst. Alkaline hydrolysis of **13d** afforded carboxylic acid **13e**. Chlorine compound **19b** and fluorine compound **16a** were prepared by a similar reaction shown in Schemes 1 and 2 using commercially available phthalic anhydrides **14a** (Cl) and **14b** (F) as starting materials. Reaction of fluorine compound **16a** with sodium methoxide followed by methylation of the carboxylic acid afforded 6-methoxy compound **17**.

Introduction of substituents into the pendant phenyl ring at the 4-position was carried out as shown in Scheme 4. Successive treatment of 4-chlorophthalic anhydride **14a** with sodium methoxide, methyl *N*-(4-(methylsulfonyl)benzyl)glycinate and then sodium methoxide yielded isoquinolone **20**, which was reacted with sodium hydride and then *N*-phenyltrifluoromethanesulfonimide to give trifluoromethanesulfonate **21**. Suzuki coupling reaction of **21** with the appropriate phenyl boronic acids provided isoquinolones **22a** and **22c**, having a substituted pendant phenyl group at the 4-position. Reduction of aldehydes **22a** and **22c** using



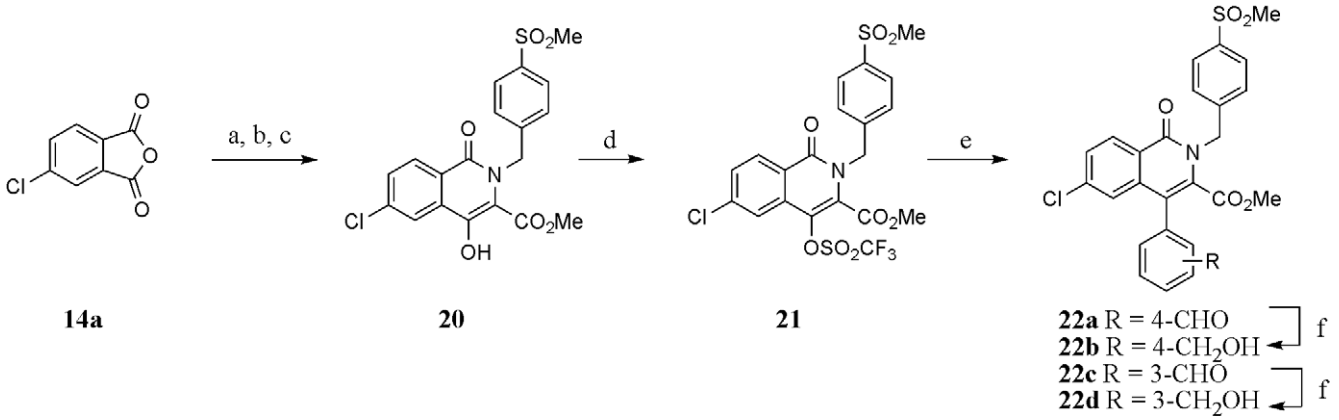
Scheme 3. Conversion of substituent at 6-position. Reagents and conditions: (a) 10% Pd–C, 1 atm H_2 gas, methanol, THF, rt, to **13a**; (b) $Rb(OH)_2$, $Pd(PPh_3)_4$, K_2CO_3, toluene, THF, 100 °C, to **13b**,**c**; (c) $Pd(OAc)_2$, DPPF, Et_3N, 1 atm CO_2 gas, MeOH, DMF, 50 °C, to **13d**; (d) 8 N NaOH, THF, rt; (e) $AlCl_3$, benzene, rt; (f) K_2CO_3, $BrCH(CO_2Et)_2$, rt; (g) concd HCl, AcOH, 120 °C; (h) $PhCH_2NH_2$, MeOH, rt; (i) 4 N HCl in AcOEt, rt; (j) 28% NaOMe in MeOH, reflux; (k) MeI, K_2CO_3, DMF, rt; (l) concd H_2SO_4 MeOH, reflux; (m) $ArCH_2NH_2$, MeOH, reflux.

sodium hydroborate gave hydroxymethyl derivatives **22b** and **22d**, respectively.

3. Results and discussion

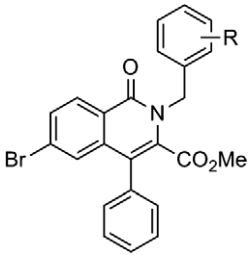
The synthesized isoquinolone derivatives were evaluated for their ability to inhibit JNK1 using a radiometric assay and results are shown in Tables 2–4.

Initially, various hydrogen bond-forming functional groups were installed into the benzyl group at the isoquinoline 2-position with the aim of increasing interactions with Asn114 and/or Gln117 (Table 2). A methoxy group, acting as a hydrogen bond acceptor at the benzyl 4-position (**11c**, IC_{50} = 42 nM) increased JNK-inhibitory activity twofold compared to lead compound **6b**. In contrast, a methoxy group at the 2- or 3-position of the benzyl group (**11a** and **11b**) did not increase the activity compared to **6b**. In a similar manner, methoxy carbonyl or carboxyl groups at the 4-position increased activity (IC_{50} values for **11e** and **11f** were 14 and 20 nM, respectively), while the methoxy carbonyl group at the 3-position showed no increase (**11d**, IC_{50} = 89 nM). Introduction of substituents at the 4-position that can interact as hydrogen bond acceptors, such as the methylsulfonyl group (**11g**, IC_{50} = 30 nM) also increased potency compared to **6b**. In the same way, substitution with an amino group at the 4-position increased JNK1-inhibitory activity (**11h**, IC_{50} = 43 nM) compared to **6b**, and furthermore methylsulfonylation of the amino group of **11h** further increased potency (**12a**, IC_{50} = 12 nM). Replacement of the methyl group of **12a** by a phenyl group maintained potent activity



Scheme 4. Introduction of substituent on the benzene ring at 4-position of isoquinolone ring. Reagents and conditions: (a) NaOMe, MeOH, rt; (b) $ArNHCH_2CO_2Me$, WSC, HOBt–H_2O, Et_3N, THF, CH_3CN, rt; (c) 28% NaOMe in MeOH, MeOH, rt; (d) NaH, $PhN(SO_2CF_3)_2$, DMF, rt; (e) $ArB(OH)_2$, $Pd(PPh_4)_3$, Na_2CO_3, H_2O, toluene, EtOH, 80 °C; (f) $NaBH_4$, MeOH, THF, 0 °C.

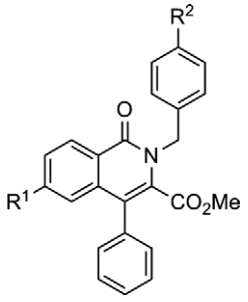
Table 2. Substituent effect on the benzene ring of 2-benzyl group



Compound	R	JNKI[a] IC_{50} (nM)
6b	H	86
11a	2-OMe	100
11b	3-OMe	79
11e	4-OMe	42
11d	3-CO_2Me	89
11e	4-CO_2Me	14
11f	4-CO_2H	20
11g	4-SO_2Me	30
11h	4-NH_2	43
12a	4-$NHSO_2$Me	12
12b	4-$NHSO_2$Ph	48
12c	4-$NHSO_2(C_6H_4$-4-$CO_2H)$	3.3
12d	4-$NHCO(CH_2)_2CO_2H$	9.2

[a] Inhibitory activity against JNK1.

Table 3. Substituent effect on 6-position of isoquinolone ring



Compound	R^1	R^2	JNKI[a] IC_{50} (nM)
13a	H	H	95
13b	Me	H	61
13c	*n*-Bu	H	1500
6b	Br	H	86
17	OMe	H	140
13e	CO_2H	H	8800
19a	H	SO_2Me	59
19b	Cl	SO_2Me	28
11g	Br	SO_2Me	30

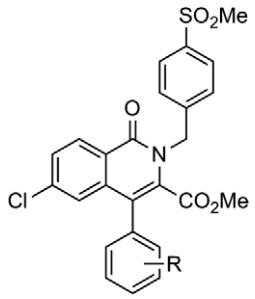
[a] Inhibitory activity against JNK1.

(**12b**, IC_{50} = 48 nM). Interestingly, introduction of a carboxyl group into the terminal benzene ring of **12b** increased the potency further and compound **12c** exhibited the most potent JNK1 inhibition (IC_{50} = 3.3 nM). Furthermore, compound **12d** possessing a methylene linker instead of a phenylene linker also showed similarly potent activity (IC_{50} = 9.2 nM).

Next, the substituent at the 6-position of the isoquinolone ring was modified for a series of compounds with benzyl or 4-methylsulfonyl benzyl at the 2-position (Table 3). Compounds with small lipophilic substituents such as hydrogen, methyl, or bromine showed potent activity (IC_{50} values of **13a**, **13b**, and **6b** were 95, 61 and 86 nM, respectively). Substitution with relatively hindered lipophilic groups such as *n*-butyl reduced potency (**13c**, IC_{50} = 1500 nM). Installation of more polar substituents like methoxy or carboxyl (**17** and **13e**) resulted in significant loss of potency. Furthermore, substituents that enhanced JNK-inhibitory activity in the benzyl series such as hydrogen and halogens were also introduced into the 4-methylsulfonylbenzyl series. The compounds prepared showed potent activity, in particular 6-chloro and 6-bromo derivatives **19b** and **11g** exhibited highly potent inhibition (IC_{50} values of 28 and 30 nM, respectively).

Lastly, various substituents on the pendant phenyl ring at the 4-position of isoquinolone ring were investigated, expecting interaction with polar amino acid residues of JNK1 such as Asp169, Lys55, Gln37 and Arg174. We prepared several compounds with substituted phenyl groups, and among them the 3- and 4-hydroxymethyl groups showed the same level of potency as non-substi-

Table 4. Substituent effect on pendant phenyl ring



Compound	R	JNK1[a] IC_{50} (nM)
19b	H	28
23b	4-CH_2OH	63
23a	3-CH_2OH	21

[a] Inhibitory activity against JNK1.

tuted compound **19b**. However, no remarkable improvement in potency was observed and it is unclear whether these substituents interact with the protein.

From the results of optimization studies of isoquinolone **6b**, we found that installation of hydrogen bond accepting and/or donating groups such as methanesulfonyl and carboxyl groups at 4-position of the 2-benzyl group increased JNK1-inhibitory activity, and that small lipophilic substituents such as chlorine and bromine atoms were favorable for substituents at the 6-position. However, no interaction with JNK1 was indicated by the modification of substituents on the phenyl group at the isoquinolone 4-position.

Due to its promising in vitro activities (including inhibition of anisomycin-induced c-Jun phosphorylation in H9c2 cells derived from rat myocardia), ADME profile and phamacokinetic parameters, compound **11g** was selected for further investigation of biological activity (Table 5).

Compound **11g** was evaluated for selectivity with respect to the JNK isoforms (JNK1-3) and other serine–threonine kinases. It showed similarly potent inhibition against all JNK subtypes (IC_{50} values: 30, 34 and 30 nM for JNK1, JNK2 and JNK3, respectively) and showed extremely high selectivity for JNK over other serine–threonine kinases tested (Table 6).

Compound **11g** was evaluated for suppressive effects on the development of cardiac hypertrophy in a rat pressure-overload model. It was orally administered to aortic-banded rats, once a day for 7 days in total. One day after final administration, the suppression of cardiac hypertrophy was evaluated by left ventricular weight/body weight ratio (LVW/BW). Compound **11g** exhibited significant suppression of cardiac hypertrophy at a dose of 10 mg/kg (Fig. 4). Additionally, the extent of c-Jun phosphorylation in the heart and the extent of cardiac hypertrophy in **11g**-treated and untreated pressure-overload rats were examined (Fig. 4). Compound **11g** also inhibited c-Jun phosphorylation at doses of 3 and

Table 5. JNK-inhibitory activity, ADME and PK profiles of **11g**

Compound	Enzyme JNK1[a] (IC_{50}: nM)	Cell-based H9c2[b] (IC_{50}: nM)	CYP3A4 (%inh at 10 μM)	Metabolic stability[c] (μL/min/mg)	Pharmacokinetic profile[d]					
					V_{dss} (ml/kg)	Cl_{total} (ml/h/kg)	AUC_{iv} (μg h/ml)	C_{max} (μg /ml)	AUC_{po} (μg h/ml)	t_{max} (h)	BA (%)
11g	30	2.6	0	0	2859	419	2.395	0.345	3.364	6.67	14

[a] Enzymatic inhibitory activity for JNK1.
[b] Inhibitory activity against AP-1 phosphorylation in H9c2 cells.
[c] Measured at 1 μM.
[d] Rat, iv; 1 mg/kg, po; 10 mg/kg.

Table 6. Inhibitory activities of **11g** against several serine–threonine kinases

Kinase	IC_{50} (nM)	% Inhibition 10 μM	Kinase	IC_{50} (nM)	% Inhibition 10 μM
JNK1	30		IKKβ	>11,000	20
JNK2	34		TAK1	>11,000	12
JNK3	30		PKCθ	>11,000	8
p38α	>11,000	18	MEKK	>11,000	8
ERK1	>11,000	2			

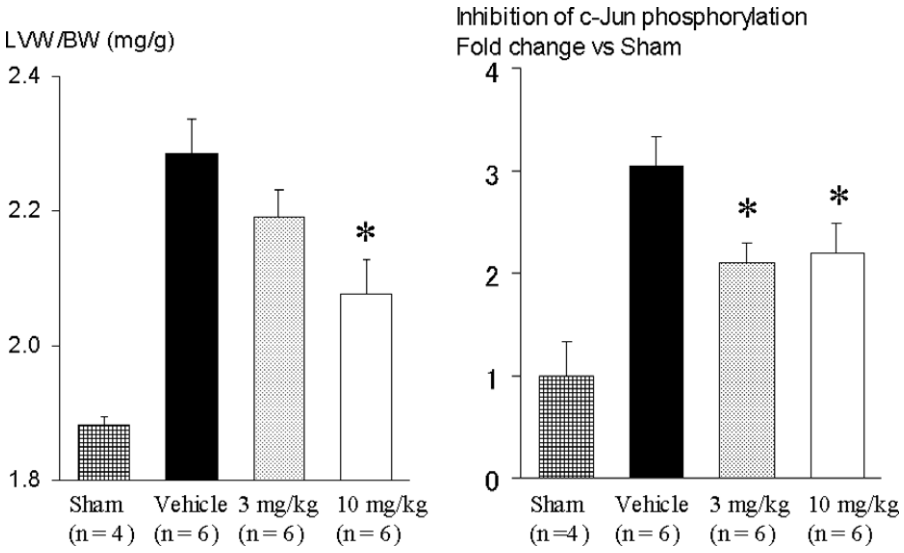


Figure 4. Inhibitory effect of **11g** on the c-jun phosphorylation in hearts and cardiac hypertorphy in aortic-banded rats. Data are represented as means ± SEM. $^{*}p < 0.025$ versus Vehicle (Williams test).

10 mg/kg even after 24 h of final administration. From these results, we concluded that inhibition of JNK activity in the heart led to the suppression of cardiac hypertrophy in our rat model.

The effects of **11g** on systolic blood pressure and cardiac hypertrophy in aortic-banded rats (30 mg/kg, po) were also checked. As shown in Figure 5, compound **11g** significantly inhibited cardiac hypertrophy, however, it did not affect systolic blood pressure even at a dose of 30 mg/kg. These results indicated the possibility of JNK inhibitor as novel therapeutic agents for heart failure without hypotensive activity.

Co-crystallization of isoquinolone-based JNK inhibitor and JNK3 was investigated and the crystal structure of the complex between **12d** and JNK3 was obtained. It showed that compound **12d** interacts with JNK3 in the ATP-binding site (Fig. 6). The binding mode of **12d** was slightly different from that of binding model shown in Figure 3. Met149 on the main chain of the hinge region of JNK3 (corresponding to Met111 for JNK1) forms a hydrogen bond with 1-position carbonyl oxygen of **12d** as predicted by the original binding model. An unexpected electrostatic interaction is observed between the 4-carboxyl group on the benzyl group of **12d** and Lys68 (corresponding to Lys30 of JNK1, that is positioned out of Fig. 3), but no interaction is observed with Asn152 (corresponding to Asn114 of JNK1). Introduction of a hydrogen bond acceptor at the 4-position of the 2-benzyl group led to potent JNK inhibition, suggesting that these functional groups might interact with Lys68. The crystal structure also showed a small hydrophobic space around the 6-bromine atom of **12d** and it could explain the decrease in inhibitory activity found for isoquinolones containing a hindered substituent or hydrophilic functional group at this position.

4. Conclusion

With the goal of discovering novel JNK inhibitors, we carried out the initial optimization of isoquinolone derivatives based on the hit compound **4** found by HTS. As a result, we discovered that a hydrogen bond-accepting functional group at the 4-position of the benzyl group led to an increase of JNK inhibition.

Among the isoquinolone derivatives synthesized, methylsulfonyl derivative **11g** showed potent inhibitory activity against JNK1 and was found to be a JNK selective and ATP competitive inhibitor. From an X-ray crystal structure analysis of the complex of **12d** and JNK3, interactions between the isoquinolone derivatives and the surrounding amino acid residue of JNK3 were clarified. Methylsulfonyl derivative **11g** showed significant suppression of cardiac hypertrophy in a rat model after oral administration, as well as no hypotensive effect at a

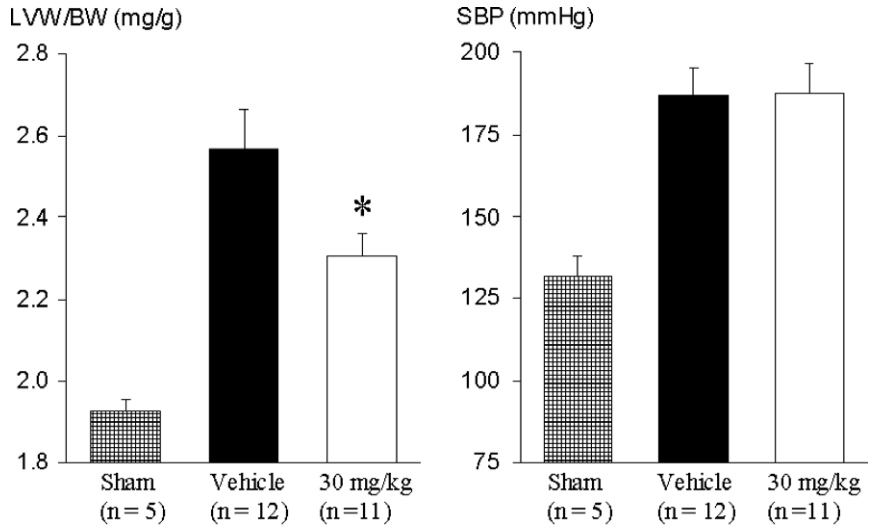


Figure 5. Inhibitory effect of **11g** on the c-jun phosphorylation in hearts and cardiac hypertroph in aortic-banded rats. Data are represented as means ± SEM. $^{*}p < 0.025$ versus Vehicle (Student's t test).

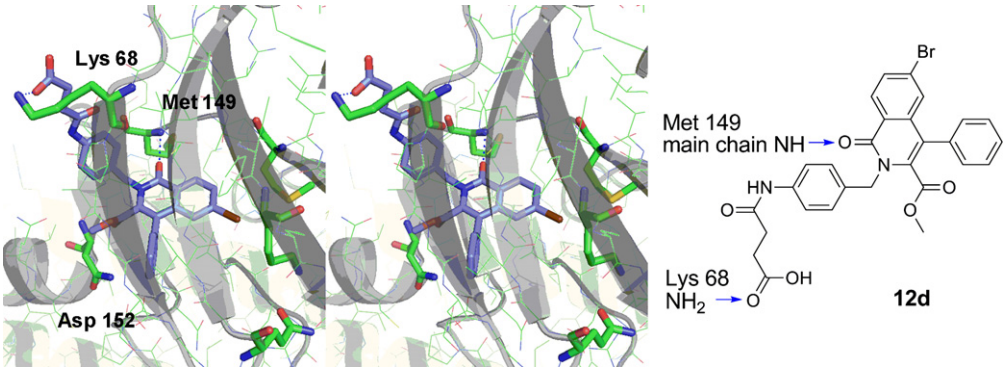


Figure 6. The co-crystal structure of **12d** and JNK3.

dose of 30 mg/kg. These results suggested that JNK inhibitor can be a novel therapeutic agent for heart failure without affecting blood pressure.

5. Experimental

5.1. Chemistry

Melting points were determined on a Yanagimoto micro melting point apparatus and were not corrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on Varian Gemini-200 (200 MHz), Varian Mercury-300 (300 MHz), or Bruker DPX-300 (300 MHz) instruments. Chemical shifts are reported as δ values (ppm) down field from internal TMS of the indicated organic solutions. Peak multiplicities are expressed as follows: Abbreviations are used as follows: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublet; dt, doublet of triplet; br s, broad singlet; m, multiplet. Coupling constants (J values) are given in hertz (Hz). Elemental analyses were carried out by Takeda Analytical Laboratories. LC/MS (ESI$^+$) was performed on a Micromass ZMD instrument, using a CAPCELL PAK UG-120 ODS (Shiseido Co., Ltd) column (2.0 mm i.d. × 50 mm) with acetonitrile/water mobile phase, and a HP-1100 (Agilent Technologies) apparatus for monitoring at 220 nm. Preparative reversed-phase high-performance liquid chromatography (Preparative RPHPLC) was performed on a Gilson, Inc. high-throughput purification system with acetonitrile/water mobile phase. Reaction progress was determined by thin-layer chromatography (TLC) analysis on silica gel 60 F_{254} plates (Merck). Chromatographic purification was carried out on silica gel columns (Kieselgel 60, 0.063–0.22 mm, Merck) or on Purif-Pack (SI 60 μm or NH 60 μm, Fuji Silysia, Ltd). Reagents and solvents were obtained from commercial sources and used without further purification. Abbreviations are used as follows: $CDCl_3$, deuterated chloroform; DMSO-d_6, dimethyl sulfoxide-d_6; AcOEt, ethyl acetate; DMF, *N*,*N*-dimethylformamide; MeOH, methanol; THF, tetrahydrofuran; EtOH, ethanol; DMSO, dimethyl sulfoxide.

5.1.1. 6-Bromo-1-oxo-4-phenyl-1*H*-isochromene-3-carboxylic acid (8). To a solution of 4-bromophthalic acid anhydride **7** (100 g, 0.441 mol) in benzene (500 ml) was added aluminum chloride (118 g, 0.885 mol), and the mixture was stirred at room temperature for 1 h and

subsequently refluxed for 30 min. After cooling the reaction mixture, it was diluted with AcOEt (200 ml) and poured into ice water. Concentrated hydrochloric acid (50 ml) was added and the mixture was stirred at room temperature for 30 min. The organic layer was separated and washed successively with 4 N hydrochloric acid, water and brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure until crystals precipitated. The crude crystals were collected by filtration, and recrystallized from toluene–AcOEt to give 2-benzoyl-4-bromobenzoic acid (59.0 g, 44%) as colorless crystals. To a solution of 2-benzoyl-4-bromobenzoic acid (57.6 g, 0.189 mol) in acetone (500 ml) was added potassium carbonate (26.1 g, 0.189 mol), and the mixture was stirred at room temperature for 10 min to allow precipitation of potassium salt. Then diethyl bromomalonate (54.2 g, 0.226 mol) and DMF (25 ml) were added, and the mixture was stirred at room temperature for 20 h. The reaction mixture was concentrated under reduced pressure, and AcOEt (200 ml) and water (200 ml) were added to the residue. The mixture was stirred at room temperature for 30 min. The organic layer was separated and washed with water and brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure. A mixed solution of acetic acid (200 ml) and concentrated hydrochloric acid (200 ml) was added to the obtained residue, and the mixture was stirred at 120 °C for 15 h. After cooling, the reaction mixture was concentrated under reduced pressure, and the residue was dissolved in AcOEt, washed with water and brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The obtained residue was crystallized from diisopropyl ether and collected by filtration to give **8** (53.0 g, 81%) as colorless crystals. ^{1}H NMR ($CDCl_3$) δ: 7.18–7.30 (3H, m), 7.46–7.56 (3H, m), 7.77 (1H, dd, J = 1.8, 8.4 Hz), 8.26 (1H, d, J = 8.4 Hz).

5.1.2. 2-Benzyl-6-bromo-1-oxo-4-phenyl-1,2-dihydroisoquinoline-3-carboxylic acid (5), typical procedure. To a solution of **8** (1.00 g, 29.0 mmol) in MeOH (20 ml) was added benzylamine (3.16 ml, 28.9 mmol), and the mixture was stirred at room temperature for 2 h. The solvent was evaporated under reduced pressure and 1 N hydrochloric acid was added. The mixture was extracted with AcOEt. The extract was washed successively with 1 N hydrochloric acid and saturated brine and anhydrous magnesium sulfate was added to dry the mixture. The solvent was evaporated under reduced pressure, and 4 N hydrogen chloride–AcOEt solution (17 ml) was added to the obtained residue, and the mixture stirred at room temperature for 18 h. The solvent was evaporated under reduced pressure, and addition of water gave crystal precipitation. The obtained crystals were collected by filtration, washed with water and ether and dried under reduced pressure at 50 °C to give **5b** (921 mg, 73%). ^{1}H NMR (DMSO-d_6) δ: 5.28 (2H, s), 7.15–7.40 (8H, m), 7.45–7.55 (3H, m), 7.77 (1H, dd, J = 1.8, 8.4 Hz), 8.26 (1H, d, J = 8.4 Hz). LC/MS (ESI^+): 434, 436.

The following compounds (**5a**, **5c**, **5d**) were prepared from **8** in a manner similar to that used for **5b**.

Compound **5a**: mp 221–223 °C. ^{1}H NMR (DMSO-d_6) δ: 0.80–1.30 (6H, m), 1.50–1.80 (4H, m), 1.80–2.00 (1H, m), 3.93 (2H, d, J = 7.2 Hz), 7.14 (1H, d, J = 1.8 Hz), 7.30–7.40 (2H, m), 7.45–7.60 (3H, m), 7.74 (1H, dd, J = 1.8, 8.4 Hz), 8.25 (1H, d, J = 8.4 Hz). LC/MS (ESI^+): 440, 442. Anal. Calcd for $C_{23}H_{22}BrNO_3$: C, 62.74; H, 5.04; N, 3.18. Found: C, 62.40; H, 5.15; N, 3.14.

Compound **5c**: ^{1}H NMR (DMSO-d_6) δ: 2.95–3.10 (2H, m), 4.05–4.20 (2H, m), 7.17 (1H, d, J = 1.8 Hz), 7.20–7.40 (7H, m), 7.45–7.55 (3H, m), 7.78 (1H, dd, J = 1.8, 8.8 Hz), 8.28 (1H, d, J = 8.8 Hz). LC/MS (ESI^+): 448, 450.

Compound **5d**: ^{1}H NMR (DMSO-d_6) δ: 2.06 (2H, m), 2.66 (2H, t, J = 7.2 Hz), 4.01 (2H, t, J = 7.8 Hz), 7.10–7.40 (7H, m), 7.45–7.55 (4H, m), 7.74 (1H, dd, J = 1.8, 8.7 Hz), 8.24 (1H, d, J = 8.7 Hz). LC/MS (ESI^+): 462, 464.

5.1.3. 6-Bromo-2-cyclohexylmethyl-3-hydroxymethyl-4-phenylisoquinolin-1(2*H*)-one (4). To a solution of **5a** (200 mg, 0.454 mmol) in THF (5 ml) were added oxalyl chloride (0.119 ml, 1.41 mmol) and DMF (0.2 ml), and the mixture was stirred at room temperature for 1 h. The solvent was evaporated under reduced pressure. A solution of the residue in THF (3 ml) was added dropwise to a suspension of sodium borohydride (52 mg, 1.37 mmol) in 1,2-dimethoxyethane. The mixture was stirred under ice-cooling for 1 h. and poured into 1 N hydrochloric acid and the mixture was extracted with AcOEt. The extract was washed successively with saturated aqueous sodium hydrogen carbonate and saturated brine, and dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography (hexane/AcOEt) to give **4** (10.0 mg, 5.2%). Mp 181–182 °C. ^{1}H NMR ($CDCl_3$) δ: 1.00–1.30(4H, m), 1.60–2.00 (7H, m), 4.22 (2H, d, J = 6.8 Hz), 4.45 (2H, s), 7.12 (1H, d, J = 1.8 Hz), 7.25–7.35 (2H, m), 7.40–7.55 (4H, m), 8.27 (1H, d, J = 8.8 Hz). LC/MS (ESI^+): 426, 428. Anal. Calcd for $C_{23}H_{24}BrNO_2$: C, 64.79; H, 5.67; N, 3.29. Found: C, 64.97; H, 5.74; N, 3.40.

5.1.4. Methyl 2-benzyl-6-bromo-1-oxo-4-phenyl-1,2-dihydroisoquinoline-3-carboxylate (6b), typical procedure. A mixture of **5b** (150 mg, 0.345 mmol), potassium carbonate (95.0 mg, 0.687 mmol), iodomethane (0.043 ml, 0.69 mmol) and DMF (5 ml) was stirred at room temperature for 3 h. After diluting with water, the mixture was extracted with ether and the extract was washed with saturated brine, and dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure. AcOEt and hexane were added to the residue, and the mixture was crystallized to give **6b** (96.0 mg, 62%). Mp 142.5–143.5 °C. ^{1}H NMR ($CDCl_3$) δ: 3.19 (3H, s), 5.42 (2H, s), 7.20–7.30 (7H, m), 7.35–7.50 (4H, m), 7.66 (1H, dd, J = 2.0, 8.8 Hz), 8.41 (1H, d, J = 8.4 Hz). LC/MS (ESI^+): 448, 450. Anal. Calcd for $C_{24}H_{18}BrNO_3$: C, 64.30; H, 4.05; N, 3.12. Found: C, 64.54; H, 4.02; N, 3.13.

The following compounds (**6a**, **6c**, **6d**) were prepared in a manner similar to that used for **6b**.

Compound **6a**: mp 181–183 °C. ^{1}H NMR ($CDCl_3$) δ: 0.95–1.30 (6H, m), 1.60–1.90 (5H, m), 3.46 (3H, s), 3.96 (2H, d, J = 7.4 Hz), 7.25–7.40 (3H, m), 7.40–7.50 (3H, m), 7.63 (1H, dd, J = 1.8, 8.4 Hz), 8.36 (1H, d, J = 8.4 Hz). LC/MS (ESI^+): 454, 456. Anal. Calcd for $C_{24}H_{24}BrNO_3$: C, 63.44; H, 5.32; N, 3.08. Found C, 63.40; H, 5.46; N, 3.00.

Compound **6c**: mp 179–180 °C. ^{1}H NMR ($CDCl_3$) δ: 3.12 (2H, t, J = 8.1 Hz), 3.50 (3H, s), 4.16 (2H, t, J = 8.1 Hz), 7.20–7.35 (7H, m), 7.38 (1H, d, J = 1.8 Hz), 7.45–7.50 (3H, m), 7.66 (1H, dd, J = 1.8, 8.4 Hz), 8.39 (1H, d, J = 8.4 Hz). LC/MS (ESI^+): 462, 464. Anal. Calcd for $C_{25}H_{20}BrNO_3$: C, 64.95; H, 4.36; N, 3.03. Found: C, 64.73; H, 4.46; N, 3.08.

Compound **6d**: mp 176.5–177 °C. ^{1}H NMR ($CDCl_3$) δ: 2.05–2.25 (2H, m), 2.74 (2H, t, J = 7.2 Hz), 3.74 (3H, s), 3.95–4.10 (2H, m), 7.15–7.40 (8H, m), 7.45–7.50 (3H, m), 7.65 (1H, dd, J = 1.8, 8.4 Hz), 8.37 (1H, d, J = 8.4 Hz). LC/MS (ESI^+): 476, 478. Anal. Calcd for $C_{26}H_{22}BrNO_3$: C, 65.55; H, 4.66; N, 2.94. Found: C, 64.35; H, 4.77; N, 3.01.

5.1.5. Methyl 6-bromo-1-oxo-4-phenyl-1,2-dihydroisoquinoline-3-carboxylate (9). To a solution of **8** (15.0 g, 43.5 mmol) in MeOH (100 ml) was added 12% ammonia/MeOH solution (60 ml), and the mixture was stirred at room temperature for 24 h. The reaction mixture was concentrated under reduced pressure, and 1 N hydrochloric acid was added to the obtained residue. The mixture was extracted with AcOEt. The organic layer was dried and concentrated under reduced pressure and 4 N hydrochloric acid AcOEt solution (30 ml) was added to the obtained oily substance. The mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated under reduced pressure. The obtained crystal was washed with water, and dried under reduced pressure to give 6-bromo-1-oxo-4-phenyl-1,2-dihydroisoquinoline-3-carboxylic acid as colorless crystals. To the obtained crystals was added a solution of concentrated sulfuric acid (40 ml) in MeOH (400 ml), and the mixture was refluxed for 20 h. The reaction mixture was concentrated under reduced pressure, and the residue was neutralized with aqueous potassium carbonate solution and extracted with $CHCl_3$. The organic layer was washed with water and brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to give **9** (6.51 g, 42%) as colorless crystals. ^{1}H NMR ($CDCl_3$) δ: 3.64 (3H, s), 7.20–7.27 (2H, m), 7.32 (1H, d, J = 1.8 Hz), 7.45–7.55 (3H, m), 7.71 (1H, dd, J = 1.8, 8.4 Hz), 8.37 (1H, d, J = 8.4 Hz), 9.35 (1H, br s).

5.1.6. Methyl 6-bromo-2-(4-methoxybenzyl)-1-oxo-4-phenyl-1,2-dihydroisoquinoline-3-carboxylate (11c), typical procedure. Compound **9** (54 mg, 0.15 mmol), 4-methoxybenzylbromide (34 mg, 0.17 mmol) and 2-*tert*-butylimino-2-diethylamino-1,3-dimethyl-perhydro-1,3,2-diazaphosphorine resin (2.2 mmol/g, 0.11 g, 0.24 mmol) were suspended in DMF (2 ml), and the suspension was shaken at room temperature for 15 h. using a shaking apparatus. After filtration, the solvent was evaporated under reduced pressure, and the obtained residue was purified by preparative RPHPLC to give **11c** (10 mg, 14%). Mp 198–199 °C. ^{1}H NMR ($CDCl_3$) δ: 3.24 (3H, s), 3.76 (3H, s), 5.35 (2H, s), 6.81 (2H, d, J = 8.7 Hz), 7.13–7.32 (4H, m), 7.33–7.49 (4H, m), 7.64 (1H, dd, J = 1.8, 8.5 Hz), 8.40 (1H, d, J = 8.5 Hz). LC/MS (ESI^+): 478, 480. Anal. Calcd for $C_{25}H_{20}BrNO_4$: C, 62.77; H, 4.21; N, 2.93. Found: C, 62.90; H, 4.22; N, 2.88.

11b was prepared from **9** in a manner similar to that used for **8c**.

Compound **11b**: mp 127–129 °C. ^{1}H NMR ($CDCl_3$) δ: 3.22 (3H, s), 3.75 (3H, s), 5.38 (2H, s), 6.72–6.88 (3H, m), 7.20 (1H, t, J = 7.8 Hz), 7.24–7.33 (2H, m), 7.34–7.50 (4H, m), 7.65 (1H, dd, J = 1.9, 8.3 Hz), 8.40 (1H, d, J = 8.3 Hz). LC/MS (ESI^+): 478, 480. Anal. Calcd for $C_{25}H_{20}BrNO_4$: C, 62.77; H, 4.21; N, 2.93. Found: C, 62.82; H, 4.35; N, 3.03.

5.1.7. Methyl 6-bromo-2-[3-(methoxycarbonyl)benzyl]-1-oxo-4-phenyl-1,2-dihydroisoquinoline-3-carboxylate (11d). To a solution of **9** (1.30 g, 3.63 mmol) in DMF (20 ml) was added sodium hydride (60% in mineral oil, 216 mg, 5.40 mmol), and the mixture was stirred at room temperature for 20 min. Methyl 3-(bromomethyl)benzoate (990 mg, 4.32 mmol) was added, and the mixture was stirred at room temperature for 5 h. The solvent was evaporated under reduced pressure, and 1 N hydrochloric acid was added. The mixture was extracted with AcOEt. The extract was washed with saturated brine and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure, and the obtained residue was purified by silica gel column chromatography (hexane/AcOEt) to give **11d** (719 mg, 39%). Mp 151–152 °C. ^{1}H NMR ($CDCl_3$) δ: 3.23 (3H, s), 3.89 (3H, s), 5.44 (2H, s), 7.26–7.53 (8H, m), 7.99 (1H, dd, J = 1.8, 8.4 Hz), 7.91–7.95 (2H, m), 8.40 (1H, d, J = 8.7 Hz). LC/MS (ESI^+): 506, 508. Anal. Calcd for $C_{26}H_{20}BrNO_5$: C, 61.67; H, 3.98; N, 2.77. Found: C, 61.66; H, 4.00; N, 2.67.

5.1.8. Methyl 6-bromo-1-oxo-4-phenyl-1*H*-isochromene-3-carboxylate (10). To a solution of **8** (10 g, 29.0 mmol) in methanol (200 ml) was added sulfuric acid (20 ml) and the mixture was refluxed for 2 h. The reaction mixture was concentrated, and the residue was neutralized with aqueous sodium hydrogen carbonate. The resulting mixture was extracted with AcOEt. The organic layer was washed with water and brine, dried over sodium sulfate and concentrated. The residue was crystallized from isopropyl ether to give **10** (9.76 g, 94%) as colorless crystals. ^{1}H NMR ($CDCl_3$) δ: 3.72 (3H, s), 7.22–7.30 (3H, m), 7.50–7.57 (3H, m), 7.75 (1H, dd, J = 1.8, 8.4 Hz), 8.26 (1H, d, J = 8.4 Hz).

5.1.9. Methyl 6-bromo-2-(4-methylsulfonylbenzyl)-1-oxo-4-phenyl-1,2-dihydroisoquinoline-3-carboxylate (11g), typical procedure. A mixture of **10** (575 mg, 1.60 mmol),

1-(4-(methylsulfonyl)phenyl)methanamine (356 mg, 1.92 mmol) and MeOH (4 ml) was heated under reflux for 12 h. Concentrated sulfuric acid (0.4 ml) was added to the reaction mixture under ice-cooling, and the mixture was heated under reflux for 2 h. The reaction mixture was concentrated under reduced pressure, and water was added. The mixture was extracted with AcOEt, and the organic layer was washed with water, saturated aqueous sodium hydrogen carbonate solution, water and brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was purified by recrystallization from hexane–AcOEt to give **11g** (329 mg, 39%) as colorless crystals: mp 201–203 °C; ^{1}H NMR ($CDCl_3$) δ: 3.02 (3H, s), 3.26 (3H, s), 5.42 (2H, s), 7.26–7.51 (8H, m), 7.69 (1H, dd, J = 1.8, 8.7 Hz), 7.87–7.91 (2H, m), 8.38 (1H, d, J = 8.7 Hz); LCMS (ESI$^+$) 526, 528; Anal. Calcd for $C_{25}H_{20}BrNO_5S$: C, 57.04; H, 3.83; N, 2.66. Found: C, 57.00; H, 3.75; N, 2.54.

The following compounds (**11a**, **11h**) were prepared from **10** in a manner similar to that used for **11g**.

Comound **11a**: mp 237–239 °C. ^{1}H NMR ($CDCl_3$) δ: 3.22 (3H, s), 3.80 (3H, s), 5.39 (2H, s), 6.82–6.89 (2H, m), 7.04 (1H, m), 7.19–7.48 (7H, m), 7.66 (1H, dd, J = 1.8, 8.7 Hz), 8.37 (1H, d, J = 8.7 Hz). LC/MS (ESI$^+$): 478, 480. Anal. Calcd for $C_{25}H_{20}NO_4Br$–H_2O: C, 60.50; H, 4.47; N, 2.82. Found: C, 60.35; H, 4.08; N, 2.83.

Compound **11h**: mp 203.5–204 °C. ^{1}H NMR ($CDCl_3$) δ: 3.24 (3H, s), 3.62 (2H, s), 5.31 (2H, s), 6.58 (2H, d, J = 8.7 Hz), 7.07 (2H, d, J = 8.7 Hz), 7.26–7.48 (6H, m), 7.64 (1H, dd, J = 1.8, 8.4 Hz), 8.40 (1H, d, J = 8.4 Hz). LC/MS (ESI$^+$): 463, 465. Anal. Calcd for $C_{24}H_{19}BrN_2O_3$: C, 62.22; H, 4.13; N, 6.05. Found: C, 62.07; H, 4.07; N, 6.10.

5.1.10. Methyl 6-bromo-2-(4-methoxycarbonylbenzyl)-1-oxo-4-phenyl-1,2-dihydroisoquinoline-3-carboxylate (11e). To a solution of **10** (3.60 g, 10.0 mmol) in MeOH (50 ml) was added methyl 4-(aminomethyl)benzoate (3.16 g, 19.1 mmol), and the mixture was stirred at room temperature for 18 h. The solvent was evaporated under reduced pressure, and 1 N hydrochloric acid was added. The resulting mixture was extracted with AcOEt. The extract was washed with saturated brine, and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure, and the obtained residue was dissolved in MeOH (100 ml). Concentrated sulfuric acid (10 ml) was added, and the mixture was heated under reflux for 3 h. The solvent was evaporated under reduced pressure, water was added under ice-cooling, and the mixture was neutralized with potassium carbonate. The resulting mixture was extracted with AcOEt, and the extract was washed with saturated brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The obtained residue was recrystallized from MeOH to give **11e** (3.39 g, 67%). Mp 172–174 °C. ^{1}H NMR ($CDCl_3$) δ: 3.18 (3H, s), 3.89 (3H, s), 5.45 (2H, s), 7.24-7.47 (8H, m), 7.67 (1H, dd, J = 1.8, 8.4 Hz), 7.97 (2H, d, J = 8.4 Hz), 8.40 (1H, d, J = 8.4 Hz). LC/MS (ESI$^+$): 506, 508. Anal. Calcd for $C_{26}H_{20}BrNO_5$: C, 61.67; H, 3.98; N, 2.77. Found: C, 61.58; H, 3.99; N, 2.75.

5.1.11. 4-((6-Bromo-3-methoxycarbonyl-1-oxo-4-phenylisoquinolin-2(1*H*)-yl)methyl)benzoic acid (11f). To a mixture of **11e** (2.00 g, 3.95 mmol) in MeOH (10 ml) and THF (20 ml) was added 8 N aqueous sodium hydroxide solution (1.00 ml, 8.00 mmol), and the mixture was stirred at room temperature for 12 h. The solvent was evaporated under reduced pressure, and water was added. The mixture was acidified with 1 N hydrochloric acid, and extracted with AcOEt. The extract was washed with saturated brine, and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure, and then the obtained residue was recrystallized from MeOH to give **11f** (1.29 g, 66%). Mp 240–241 °C. ^{1}H NMR ($CDCl_3$) δ: 3.21 (3H, s), 5.46 (2H, s), 7.25–7.49 (8H, m), 7.68 (1H, dd, J = 1.8, 8.7 Hz), 8.02 (2H, d, J = 7.0 Hz), 8.40 (1H, d, J = 8.4 Hz). LC/MS (ESI$^+$): 492, 494. Anal. Calcd for $C_{25}H_{18}BrNO_5$: C, 60.99; H, 3.69; N, 2.85. Found: C, 60.76; H, 3.82; N, 2.69.

5.1.12. Methyl 6-bromo-2-(4-(methylsulfonylamino)benzyl)-1-oxo-4-phenyl-1,2-dihydroisoquinoline-3-carboxylate (12a), typical procedure. To a solution of **11h** (139 mg, 0.300 mmol) in THF (5 ml) was added triethylamine (0.083 ml, 0.56 mmol), and then methanesulfonyl chloride (0.028 ml, 0.36 mmol) was added. The mixture was stirred at room temperature for 3 h. The solvent was evaporated under reduced pressure, 1 N hydrochloric acid was added, and the mixture was extracted with CH_2Cl_2. The extract was washed with saturated brine, and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure, and the obtained residue was purified by silica gel column chromatography (hexane/AcOEt) to give **12a** (90 mg, 55%). Mp 246–247 °C. ^{1}H NMR ($CDCl_3$) δ: 2.97 (3H, s), 3.27 (3H, s), 5.34 (2H, s), 6.60 (1H, s), 7.14 (2H, d, J = 8.7 Hz), 7.26–7.49 (8H, m), 7.66 (1H, dd, J = 1.8, 8.4 Hz), 8.38 (1H, d, J = 8.4 Hz). LC/MS (ESI$^+$): 541, 543. Anal. Calcd for $C_{25}H_{21}N_2O_5SBr$: C, 55.46; H, 3.91; N, 5.17. Found: C, 55.67; H, 4.03; N, 5.04.

The following compounds (**12b**, **12c**) were prepared from **11h** by a manner similar to that used for **12a**.

Compound **12b**: mp 197–198 °C. ^{1}H NMR ($CDCl_3$) δ: 3.15 (3H, s), 5.31 (2H, s), 6.89 (1H, s), 6.98 (2H, d, J = 8.7 Hz), 7.12 (2H, d, J = 8.7 Hz), 7.24–7.27 (2H, m), 7.37–7.54 (7H, m), 7.65 (1H, dd, J = 1.8, 8.7 Hz), 7.74 (2H, d, J = 8.7 Hz), 8.37 (1H, d, J = 8.7 Hz). LC/MS (ESI$^+$): 603, 604. Anal. Calcd for $C_{30}H_{23}N_2O_5SBr$: C, 59.71; H, 3.84; N, 4.64. Found: C, 59.69; H, 3.84; N, 4.60.

Compound **12c**: mp >285 °C. ^{1}H NMR (DMSO-d_6) δ: 3.31 (3H, s), 5.26 (2H, s), 7.10–7.37 (5H, m), 7.40–7.55 (3H, m), 7.65–7.93 (7H, m), 8.31 (1H, d, J = 8.4 Hz), 10.24 (1H, s). LCMS (ESI$^+$): 647 (M+H), 649. Anal. Calcd for $C_{31}H_{23}BrN_2O_7S$-1.7H_2O: C, 54.91; H, 3.92; N, 4.13. Found: C, 54.63; H, 3.55; N, 4.11.

5.1.13. 4-[(4-{[6-Bromo-3-(methoxycarbonyl)-1-oxo-4-phenylisoquinolin-2(1*H*)-yl]methyl}phenyl)amino]-4-oxobutanoic acid (12d). To a solution of **11h** (139 mg, 0.300 mmol) in THF (5 ml) were added triethylamine (0.080 ml, 0.57 mmol) and succinic anhydride (45 mg, 0.45 mmol), and the mixture was stirred at room temperature for 24 h. The solvent was evaporated under reduced pressure, and water was added. The mixture was extracted with AcOEt. The extract was washed with saturated brine, and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure, and the obtained residue was crystallized from diisopropyl ether to give **12d** (85 mg, 50%). Mp 204–205 °C. ^{1}H NMR ($CDCl_3$) δ: 2.59–2.80 (4H, m), 3.24 (3H, s), 5.35 (2H, s), 6.37 (1H, s), 7.18–7.58 (9H, m), 7.60–7.75 (2H, m), 8.38 (1H, d, J = 8.4 Hz). LC/MS (ESI$^+$): 563, 564. Anal. Calcd for $C_{28}H_{23}BrN_2O_6$: C, 59.69; H, 4.11; N, 4.97. Found: C, 59.74; H, 4.27; N, 4.94.

5.1.14. Methyl 2-benzyl-1-oxo-4-phenyl-1,2-dihydroisoquinoline-3-carboxylate (13a). A mixture of **6b** (400 mg, 0.892 mmol), 10% palladium/carbon (100 mg), MeOH (5 ml) and THF (5 ml) was stirred at room temperature under 1 atm hydrogen pressure for 12 h. Palladium carbon was filtered off, and the filtrate was concentrated under reduced pressure. The obtained residue was purified by silica gel column chromatography (AcOEt), and recrystallized (AcOEt–hexane) to give **13a** (230 mg, 57%). Mp 136–138 °C. ^{1}H NMR ($CDCl_3$) δ: 3.19 (3H, s), 5.46 (2H, s), 7.14–7.68 (13H, m), 8.50–8.66 (1H, m). LC/MS (ESI$^+$): 370. Anal. Calcd for $C_{24}H_{19}NO_3$: C, 78.03; H, 5.18; N, 3.79. Found: C, 77.87; H, 5.12; N, 3.49.

5.1.15. Methyl 2-benzyl-6-methyl-1-oxo-4-phenyl-1,2-dihydroisoquinoline-3-carboxylate (13b). A mixture of **6b** (300 mg, 0.669 mmol), methylboronic acid (200 mg, 3.34 mmol), tetrakis(triphenylphosphine)palladium(0) (80.0 mg, 0.0692 mmol), potassium carbonate (280 mg, 2.03 mmol), toluene (6 ml) and THF (3 ml) was stirred at 100 °C under a nitrogen atmosphere for 12 h. Water was added to the reaction mixture, and the mixture was extracted with AcOEt. The organic layer was washed with water and saturated brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The obtained residue was purified by silica gel column chromatography (hexane → hexane/AcOEt) and recrystallized (AcOEt/hexane) to give **13b** (210 mg, 82%). Mp 112–115 °C. ^{1}H NMR ($CDCl_3$) δ: 2.37 (3H, s), 3.18 (3H, s), 5.44 (2H, s), 7.01 (1H, s), 7.14–7.54 (11H, m), 8.45 (1H, d, J = 8.2 Hz). LCMS (ESI$^+$): 384. Anal. Calcd for $C_{25}H_{21}NO_3$: C, 78.31; H, 5.52; N, 3.65. Found: C, 78.30; H, 5.50; N, 3.57.

5.1.16. Methyl 2-benzyl-6-butyl-1-oxo-4-phenyl-1,2-dihydroisoquinoline-3-carboxylate (13c). A mixture of **6b** (300 mg, 0.669 mmol), *n*-butylboronic acid (340 mg, 3.34 mmol), tetrakis(triphenylphosphine)palladium(0) (80.0 mg, 0.0692 mmol), potassium carbonate (280 mg, 2.03 mmol), toluene (6 ml) and THF (3 ml) was stirred at 100 °C under a nitrogen atmosphere for 12 h. Water was added to the reaction mixture, and the mixture was extracted with AcOEt. The organic layer was washed with water and saturated brine. The solvent was dried over anhydrous sodium sulfate, and evaporated under reduced pressure. The obtained residue was purified by silica gel column chromatography (hexane/AcOEt), and crystallized from hexane to give **13c** (210 mg, 74%). Mp 95–97 °C. ^{1}H NMR ($CDCl_3$) δ: 0.88 (3H, t, J = 7.3 Hz), 1.14–1.66 (4H, m), 2.61 (2H, t, J = 7.7 Hz), 3.18 (3H, s), 5.44 (2H, s), 6.94–7.06 (1H, m), 7.14–7.50 (11H, m), 8.47 (1H, d, J = 8.4 Hz). LC/MS (ESI$^+$): 426. Anal. Calcd for $C_{28}H_{27}NO_3$: C, 79.03; H, 6.40; N, 3.29. Found: C, 79.02; H, 6.37; N, 3.27.

5.1.17. Dimethyl 2-benzyl-1-oxo-4-phenyl-1,2-dihydroisoquinoline-3,6-dicarboxylate (13d). A mixture of **6b** (300 mg, 0.669 mmol), palladium(II) acetate (8.0 mg, 0.036 mmol), 1,1′-bis(diphenylphosphino)ferrocene (20.0 mg, 0.0361 mmol), triethylamine (0.230 ml, 1.65 mmol), DMF (3 ml) and MeOH (3 ml) was stirred at 50 °C under 1 atm carbon monoxide for 24 h. Water was added to the reaction mixture, and the mixture was extracted with AcOEt. The organic layer was washed with water and saturated brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The obtained residue was purified by silica gel column chromatography (hexane → 1:1 hexane/AcOEt), and recrystallized (AcOEt/hexane) to give **13d** (180 mg, 63%). Mp 164–166 °C. ^{1}H NMR ($CDCl_3$) δ: 3.20 (3H, s), 3.88 (3H, s), 5.45 (2H, s), 7.14–7.54 (10H, m), 7.94–8.00 (1H, m), 8.16 (1H, dd, J = 1.4, 8.4 Hz), 8.58–8.68 (1H, m). LC/MS (ESI$^+$): 428. Anal. Calcd for $C_{26}H_{21}NO_5$: C, 73.06; H, 4.95; N, 3.28. Found: C, 72.92; H, 4.86; N, 3.09.

5.1.18. 2-Benzyl-3-methoxycarbonyl-1-oxo-4-phenyl-1,2-dihydroisoquinoline-6-carboxylic acid (13e). To a solution of **13c** (1.75 g, 4.23 mmol) in THF (10 ml) were added MeOH (10 ml) and 8 N aqueous sodium hydroxide solution (1.00 ml, 8.00 mmol) at room temperature, and the mixture was stirred for 12 h. The reaction mixture was concentrated under reduced pressure, and water was added, after which conc. hydrochloric acid (1 ml) was added. The mixture was extracted with AcOEt. The organic layer was washed with water and saturated brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The obtained residue was recrystallized (AcOEt/hexane) to give **13e** (1.67 g, 99%). Mp 245–248 °C. ^{1}H NMR ($CDCl_3$) δ: 3.21 (3H, s), 5.46 (2H, s), 7.16–7.56 (10H, m), 7.94–8.06 (1H, m), 8.19 (1H, dd, J = 1.8, 8.4 Hz), 8.65 (1H, d, J = 8.4 Hz). LCMS (ESI$^+$): 414. Anal. Calcd for $C_{25}H_{19}NO_5$·0.2AcOEt: C, 71.89; H, 4.82; N, 3.25. Found: C, 71.63; H, 4.77; N, 2.99.

5.1.19. 6-Chloro-1-oxo-4-phenyl-1*H*-isochromene-3-carboxylic acid (15a). To a solution of 4-chlorophthalic anhydride (25.0 g, 0.137 mol) in benzene (200 ml) was added aluminum chloride **14a** (36.5 g, 0.274 mol), and the mixture was stirred at room temperature for 1 h. and subsequently refluxed for 30 min. After cooling the reaction mixture, it was diluted with AcOEt (200 ml), and poured into ice water. Concentrated hydrochloric acid (20 ml) was added, and the mixture

was stirred at room temperature for 30 min. After separating the organic layer, the layer was washed successively with 4 N hydrochloric acid, water and brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure until the crystals precipitated. The crystals were collected by filtration, and washed with diisopropyl ether to give 2-benzoyl-4-chlorobenzoic acid (16.0 g, 45%) as colorless crystals. To a solution of 2-benzoyl-4-chlorobenzoic acid (13.0 g, 49.9 mmol) in acetone (200 ml) was added potassium carbonate (6.90 g, 49.9 mmol), and the mixture was stirred at room temperature for 10 min to allow precipitation of potassium salt. Then diethyl bromomalonate (13.2 g, 55.2 mmol) and DMF (10 ml) were added, and the mixture was stirred at room temperature for 20 h. The reaction mixture was concentrated under reduced pressure, and AcOEt (100 ml) and water (100 ml) were added to the residue. The mixture was stirred at room temperature for 30 min. The organic layer was separated and washed with water and brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. A mixed solution of acetic acid (100 ml) and conc. hydrochloric acid (100 ml) was added to the obtained residue, and the mixture was stirred for 20 h at 120 °C. After cooling, the reaction mixture was concentrated under reduced pressure, and the residue was dissolved in AcOEt, washed with water and brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The obtained residue was crystallized from diisopropyl ether and collected by filtration to give **15a** (9.78 g, 65%) as colorless crystals. ^{1}H NMR ($CDCl_3$) δ: 7.07 (1H, d, J = 1.8 Hz), 7.20–7.28 (2H, m), 7.45–7.54 (3H, m), 7.60 (1H, dd, J = 1.8, 8.4 Hz), 8.34 (1H, d, J = 8.4 Hz).

Compound **15b** was prepared from 4-fluorophthalic anhydride **14b** in a manner similar to that used for **15a**.

Compound **15b**: ^{1}H NMR ($CDCl_3$) δ: 6.77 (1H, dd, J = 2.2, 9.4 Hz), 7.08–7.62 (6H, m), 8.45 (1H, dd, J = 5.6, 8.8 Hz).

5.1.20. 2-Benzyl-6-fluoro-1-oxo-4-phenyl-1,2-dihydroisoquinoline-3-carboxylic acid (16a). A mixture of **15b** (300 mg, 1.06 mmol), benzylamine (230 mg, 2.14 mmol), triethylamine (0.300 ml, 2.15 mmol) and MeOH (3 ml) was stirred at 50 °C for 36 h. The reaction mixture was concentrated under reduced pressure, 1 N hydrochloric acid was added, and the mixture was extracted with AcOEt. The organic layer was washed with water and saturated brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. A 4 N solution of hydrochloric acid in AcOEt (3 ml) was added to the residue at room temperature, and the mixture was stirred for 12 h. The reaction mixture was concentrated under reduced pressure, and the obtained crystals were washed with (diisopropyl ether) to give **16a** (300 mg, 76%). Mp 232–236 °C. ^{1}H NMR (DMSO-d_6) δ: 5.28 (2H, m), 6.72 (1H, dd, J = 2.6, 10.2 Hz), 7.12-7.72 (11H, m), 8.41 (1H, dd, J = 6.0, 9.0 Hz). Anal. Calcd for $C_{23}H_{16}FNO_3$: C, 73.99; H, 4.32; N, 3.75. Found: C, 73.77; H, 4.50; N, 3.62.

5.1.21. 2-Benzyl-6-methoxy-1-oxo-4-phenyl-1,2-dihydroisoquinoline-3-carboxylic acid (16b). To a solution of sodium methoxide in MeOH (28%, 4 ml) was added **16a** (350 mg, 0.937 mmol), and the mixture was heated under reflux for 6 h. To the reaction mixture were added water and 10% hydrochloric acid to acidify the aqueous layer of the mixture and the mixture was extracted with AcOEt. The organic layer was washed with water and saturated brine and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure. The obtained residue was crystallized (AcOEt) to give **16b** (260 mg, 72%). Mp 254–258 °C. ^{1}H NMR (DMSO-d_6) δ: 3.69 (3H, s), 5.26 (2H, m), 6.46 (1H, d, J = 2.6 Hz), 7.12–7.58 (11H, m), 8.27 (1H, d, J = 8.8 Hz). Anal. Calcd for $C_{24}H_{19}NO_4$: C, 74.79; H, 4.97; N, 3.63. Found: C, 74.44; H, 5.02; N, 3.54.

5.1.22. Methyl 2-benzyl-6-methoxy-1-oxo-4-phenyl-1,2-dihydroisoquinoline-3-carboxylate (17). Compound **16b** (160 mg, 0.415 mmol) was dissolved in DMF (3 ml), and iodomethane (0.060 ml, 0.96 mmol) and potassium carbonate (120 mg, 0.868 mmol) were added at room temperature. The mixture was stirred for 12 h. Water was added to the reaction mixture, and the mixture was extracted with AcOEt. The organic layer was washed with water and saturated brine. The solvent was dried over anhydrous sodium sulfate, and evaporated under reduced pressure, and the obtained residue was crystallized (AcOEt/hexane) to give **17** (120 mg, 72%). Mp 133–135 °C. ^{1}H NMR ($CDCl_3$) δ: 3.18 (3H, s), 3.73 (3H, s), 5.42 (2H, s), 6.60 (1H, d, J = 2.6 Hz), 7.12 (1H, dd, J = 2.4, 9.0 Hz), 7.14–7.50 (10H, m), 8.49 (1H, d, J = 8.8 Hz). LC/MS (ESI$^+$): 400 (M+H). Anal. Calcd for $C_{25}H_{21}NO_4$: C, 75.17; H, 5.30; N, 3.51. Found: C, 75.19; H, 5.28; N, 3.42.

5.1.23. Methyl 6-chloro-1-oxo-4-phenyl-1*H*-isochromene-3-carboxylate (18). To a solution of **15a** (9.0 g, 30 mmol) in MeOH (200 ml) was added conc. sulfuric acid (20 ml) and the mixture was refluxed for 2 h. The reaction mixture was concentrated under reduced pressure, and the residue was neutralized with aqueous sodium hydrogen carbonate, and extracted with AcOEt. The organic layer was washed with water and brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was crystallized (diisopropyl ether) to give **18** (8.7 g, 92%) as colorless crystals. ^{1}H NMR ($CDCl_3$) δ: 3.72 (3H, s), 7.08 (1H, d, J = 2.2 Hz), 7.22–7.30 (2H, m), 7.48–7.55 (3H, m), 7.59 (1H, dd, J = 2.2, 8.4 Hz), 8.35 (1H, d, J = 8.4 Hz).

5.1.24. Methyl 2-(4-methylsulfonylbenzyl)-1-oxo-4-phenyl-1,2-dihydroisoquinoline-3-carboxylate (19a). A mixture of **11g** (200 mg, 0.380 mmol), 10% palladium/carbon (20 mg) and MeOH (4 ml) was stirred under a hydrogen atmosphere for 1 h. The catalyst was removed by filtration, and the filtrate was concentrated under reduced pressure to give **19a** (127 mg, 75%) as a colorless powder. Mp 184.5–186 °C. ^{1}H NMR ($CDCl_3$) δ: 3.02 (3H, s), 3.26 (3H, s), 5.45 (2H, s), 7.26–7.34 (3H, m), 7.41–7.52 (5H, m), 7.56–7.67 (2H, m), 7.89 (2H, d, J = 8.4 Hz), 8.54 (1H, m). LC/MS (ESI$^+$): 448. Anal.

Calcd for $C_{25}H_{21}NO_5S$: C, 67.10; H, 4.73; N, 3.13. Found: C, 66.88; H, 4.75; N, 3.04.

5.1.25. Methyl 6-chloro-2-(4-methylsulfonylbenzyl)-1-oxo-4-phenyl-1,2-dihydroisoquinoline-3-carboxylate (19b). A mixture of **18** (200 mg, 0.635 mmol), 1-(4-(methylsulfonyl)phenyl)methanamine (240 mg, 1.30 mmol) and MeOH (4 ml) was heated under reflux for 12 h. Concentrated sulfuric acid (0.4 ml) was added to the reaction mixture under ice-cooling, and the mixture was heated under reflux for 2 h. The reaction mixture was concentrated under reduced pressure, and water was added. The mixture was extracted with AcOEt, and the organic layer was washed with water, saturated aqueous sodium hydrogen carbonate solution, water and saturated brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was purified by recrystallization (AcOEt/hexane) to give **19b** (200 mg, 65%). Mp 201–203 °C. ^{1}H NMR ($CDCl_3$) δ: 3.02 (3H, s), 3.26 (3H, s), 5.42 (2H, s), 7.18–7.62 (9H, m), 7.89 (2H, d, J = 8.4 Hz), 8.46 (1H, d, J = 8.4 Hz). LC/MS (ESI$^+$): 482, 484. Anal. Calcd for $C_{25}H_{20}ClNO_5S$: C, 62.30; H, 4.18; N, 2.91. Found: C, 62.10; H, 4.12; N, 2.88.

5.1.26. Methyl 6-chloro-4-hydroxy-2-[4-(methylsulfonyl)benzyl]-1-oxo-1,2-dihydroisoquinoline-3-carboxylate (20). To a solution (50 ml) of 4-chloro phthalic anhydride **14a** (12.1 g, 66.3 mmol) in MeOH was added sodium methoxide (3.60 g, 66.6 mmol) and the mixture stirred at room temperature for 12 h. The solvent was evaporated under reduced pressure, and the residue was partitioned between AcOEt and 1 N hydrochloric acid. The organic layer was dried over anhydrous magnesium sulfate and concentrated under reduced pressure to give the mixture of 4-chloro-2-(methoxycarbonyl)benzoic acid and 5-chloro-2-(methoxycarbonyl)benzoic acid. To a solution of this mixture in CH_3CN (70 ml) was added methyl *N*-[4-(methanesulfonyl)benzyl]glycinate (17.0 g, 66.0 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (16.5 g, 86.1 mmol) and 1-hydroxybenzotriazole monohydrate (11.2 g, 73.1 mmol), and the mixture was stirred at room temperature for 12 h. The solvent was evaporated under reduced pressure, and the residue was partitioned between AcOEt and 1 N hydrochloric acid. The organic layer was dried over anhydrous magnesium sulfate and concentrated under reduced pressure. This product was dissolved in MeOH (100 ml), and 28% sodium methoxide/MeOH solution (26.0 g, 135 mmol) was added at room temperature with stirring. The mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated under reduced pressure, and the residue was partitioned between AcOEt and 1 N hydrochloric acid. The organic layer was dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/AcOEt) and recrystallized (hexane/AcOEt) to give **20** (5.57 g, 20%) as yellow crystals. ^{1}H NMR ($CDCl_3$) δ: 3.03 (3H, s), 3.76 (3H, s), 5.55 (2H, s), 7.32 (2H, d, J = 8.7 Hz), 7.69 (1H, dd, J = 2.1, 8.7 Hz), 7.88 (2H, d, J = 8.7 Hz), 8.19 (1H, d, J = 2.1 Hz), 8.40 (1H, d, J = 8.7 Hz), 11.29 (1H, s).

5.1.27. Methyl 6-chloro-2-[4-(methylsulfonyl)benzyl]-1-oxo-4-{[(trifluoromethyl)sulfonyl]oxy}-1,2-dihydroisoquinoline-3-carboxylate (21). To a solution of **20** (5.48 g, 13 mmol) in DMF (45 ml) was added sodium hydride (60% in mineral oil, 630 mg, 16 mmol) at 0 °C and the mixture was stirred for 30 min. Then, *N*-phenyltrifluoromethanesulfonimide (5.6 g, 16 mmol) was added and the mixture was stirred at room temperature for 3 h. The solvent was evaporated under reduced pressure. The residue was partitioned between AcOEt and water, and the organic layer was dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/AcOEt) to give **21** (3.46 g, 48%) as colorless crystals. ^{1}H NMR ($CDCl_3$) δ: 3.02 (3H, s), 3.79 (3H, s), 5.49 (2H, s), 7.40 (2H, d, J = 8.4 Hz), 7.65 (1H, dd, J = 1.8, 8.4 Hz), 7.79 (1H, d, J = 1.8 Hz), 7.89 (2H, d, J = 8.4 Hz), 8.42 (1H, d, J = 8.4 Hz).

5.1.28. Methyl 6-chloro-4-(4-formylphenyl)-2-[4-(methylsulfonyl)benzyl]-1-oxo-1,2-dihydroisoquinoline-3-carboxylate (22a). A mixture of **20** (116 mg, 0.21 mmol), sodium carbonate (45 mg, 0.43 mmol), 4-formylphenylboronic acid (37 mg, 0.25 mmol), tetrakis(triphenylphosphine)palladium(0) (12 mg, 0.010 mmol), toluene (2.0 ml), water (0.4 ml) and EtOH (0.4 ml) was stirred at 80 °C for 12 h under an argon atmosphere. After cooling, the reaction mixture was partitioned between water and AcOEt. The organic layer was dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was purified by preparative RPHPLC to give **22a** (63 mg, 59%) as a colorless powder. ^{1}H NMR ($CDCl_3$) δ: 3.02 (3H, s), 3.27 (3H, s), 5.42 (2H, s), 7.13 (1H, d, J = 2.1 Hz), 7.44–7.52 (4H, m), 7.55 (1H, dd, J = 2.1, 8.7 Hz), 7.89 (2H, d, J = 8.7 Hz), 7.99 (2H, d, J = 8.4 Hz), 8.47 (1H, d, J = 8.7 Hz), 10.10 (1H, s). LC/MS (ESI$^+$): 510, 512.

Compound **22c** was prepared from **20** and 3-formylphenylboronic acid in a manner similar to that used for **22a**.

Compound **21c**: ^{1}H NMR ($CDCl_3$) δ: 3.02 (3H, s), 3.27 (3H, s), 5.41 (2H, d, J = 2.4 Hz), 7.10 (1H, d, J = 1.8 Hz), 7.47 (2H, d, J = 8.7 Hz), 7.51–7.61 (2H, m), 7.66 (1H, t, J = 7.5 Hz), 7.82 (1H, m), 7.89 (2H, d, J = 8.7 Hz), 7.98 (1H, dt, J = 1.8, 7.5 Hz), 8.47 (1H, d, J = 9.0 Hz), 10.06 (1H, s). LC/MS (ESI$^+$): 510, 512.

5.1.29. Methyl 6-chloro-4-[4-(hydroxymethyl)phenyl]-2-[4-(methylsulfonyl)benzyl]-1-oxo-1,2-dihydroisoquinoline-3-carboxylate (22b). To a mixture of **22a** (76 mg, 0.15 mmol), MeOH (1.0 ml) and THF (1.0 ml) was added sodium borohydride (6.0 mg, 0.16 mmol) at 0 °C with stirring, and the mixture was stirred for 1 h. The reaction mixture was concentrated under reduced pressure, and the residue was partitioned between 1 N hydrochloric acid and AcOEt. The organic layer was dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was purified by preparative RPHPLC to give **22b** (35 mg, 45%) as a colorless powder. Mp 252–253 °C. ^{1}H NMR ($CDCl_3$) δ: 1.84 (1H, t, J = 5.7 Hz), 3.03 (3H, s), 3.30 (3H, s),

4.80 (2H, d, J = 5.7 Hz), 5.41 (2H, s), 7.21–7.36 (3H, m), 7.43–7.57 (5H, m), 7.89 (2H, d, J = 8.7 Hz), 8.45 (1H, d, J = 8.7 Hz). MS (ESI^+): 512, 514. Anal. Calcd for $C_{26}H_{22}ClNO_6S$: C, 60.99; H, 4.33; N, 2.74. Found: C, 61.23; H, 4.47; N, 2.67.

Compound **22d** was prepared from **22c** and 3-formylphenylboronic acid in a manner similar to that used for **22b**.

Compound **21d**: mp 214–215 °C. ^1H NMR ($CDCl_3$) δ: 1.82 (1H, t, J = 5.4 Hz), 3.03 (3H, s), 3.29 (3H, s), 4.77 (2H, d, J = 5.1 Hz), 5.38 (1H, d, J = 16.5 Hz), 5.45 (1H, d, J = 16.5 Hz), 7.18–7.36 (3H, m), 7.41–7.66 (5H, m), 7.89 (2H, d, J = 8.4 Hz), 8.46 (1H, d, J = 8.4 Hz). LC/MS (ESI^+): 512, 514. Anal. Calcd for $C_{26}H_{22}ClNO_6S$: C, 60.99; H, 4.33; N, 2.74. Found: C, 61.08; H, 4.46; N, 2.60.

5.2. Measurement of JNK1 activity

All test compounds were dissolved in DMSO. The kinase reaction mixture was preincubated with test compound for 5 min at 30 °C. Kinase reactions were performed at 30 °C for 60 min in reaction buffer (25 mM HEPES, pH 7.5, 10 mM magnesium acetate, 1 mM dithiothreitol) containing 50 ng activated human JNK1, 0.1 μCi [γ-^{32}P]ATP, 500 nM ATP and 1 μg GST-c-Jun (1–79) with test compounds. After incubation, the reactions were terminated by adding 10% (final concentration) trichloroacetic acid (Wako, Japan). Phosphorylated proteins were filtrated in GF/C filter plates (PerkinElmer, USA) with a Cell Harvester (PerkinElmer, USA), and washed with 250 mM phosphoric acid. The plates were then incubated for 60 min at 45 °C, followed by the addition of 40 μL of MicroScint-O (PerkinElmer, USA). The radioactivity was counted by a TopCount scintillation counter (PerkinElmer, USA). The IC_{50} values were calculated as the concentration of the test compound at which the c-Jun phosphorylation was reduced to 50% of the control value. The IC_{50} values for JNK1 inhibitors were calculated from a dose–response curve at compound concentrations of 0.001, 0.01, 0.1, 1 and 10 μM in the presence of 0.5 μM ATP.

5.3. H9c2 cell based assay

H9c2 cells (serum free/DMEM) derived from rat myocardium were seeded in 6 well plates and then incubated with test compound for 30 min. Subsequently, they were stimulated with anisomycin (50 ng/ml, Sigma, US) for 30 min. After being washed with iced PBS, the H9c2 cells were dissolved in 30 μL of Cell Lysis buffer (CST, US). After centrifugation, the solution of cells was measured by a human AP-I Transcription Factor Assay Kit (Active Motif). The IC_{50} values were calculated as the concentration of test compound at which c-Jun phosphorylation was reduced to 50% of the control value.

5.4. Preparation of the pressure-overloaded cardiac hypertrophy model induced by abdominal aortic stenosis in rats

Male 9-week-old Wistar rats were used. Under xylazine (10 mg/kg, ip) and ketamine (50 mg/kg, ip) anesthesia, the abdominal aorta was exposed by median laparotomy. The left and right renal arteries were detached from the back, a 22G injection needle was put along the artery and was ligated together with the aorta using a 2-0 silk thread just below the right renal artery. Then, the injection needle was removed to make a stenosis in the abdominal aorta. The abdomen was closed and the rats were housed together. For a sham group, the abdomen was closed without ligation (no stenosis) with a silk thread. A compound was suspended in a 0.5% methyl cellulose solution (2 ml/kg) and orally administered before aortic stenosis and once a day from day 1 (the next day of stricture) to day 7 after operation. Vehicle (0.5% methyl cellulose solution) was administered instead of the compound to the sham group and control group. At day 7 after the preparation of aortic stenosis, body weight was measured and the heart was removed and then extracted under pentobarbital (50 mg/kg, ip) anesthesia. The right and left artium and right ventricle were excised from the heart and the wet weight of the left ventricle was measured. The left ventricle weight to body weight ratio of each rat was calculated and an increase from the average value of the sham group was calculated. The rate of inhibition for each group to cardiac hypertrophy was calculated based on the control group as 100%. Systolic blood pressure (SBP) was monitered before the heart was extracted under pentobarbital (50 mg/kg, ip) anesthesia. The venous catheter was passed through the right carotid artery and connected to a pressure transducer coupled to a polygraph system (NEC San-ei Instruments, Nihon Kohden Corporation).

5.5. Measurement of phosphorylated c-Jun in the heart of cardiac hypertrophy model rats

The left ventricle was homogenized in 5.0 ml of ice-cooled lysis buffer (20 mM Tris–HCl, pH 7.4, 1% NP40, 0.1% SDS, 150 mM NaCl, 1 mM EDTA, 0.1 mg/ml PMSF, 0.3 TIU aprotinin, 1 mM sodium orthovanadate), and centrifuged at 12,000 rpm (15,800g), 4 °C for 30 min. After centrifugation, the supernatant was recovered and used as a tissue extract solution. The protein amount was measured by the Bradford method (Bio-Rad, Protein Assay). The tissue extract solution containing 15 mg of protein was added to 8 μg of anti-c-Jun rabbit polyclonal antibody (H79, Santa Cruz) and 40 μl of Protein A-Agarose (Santa Cruz), and the mixture was stirred overnight at 4 °C. The mixture was centrifuged at 2500 rpm, 4 °C for 5 min, and after removal of supernatant, washed three times with TBS buffer (50 mM Tris–HCl, pH 7.4, 150 mM NaCl, 0.1 mg/ml phenylmethanesulfonyl fluoride (PMSF), 0.3 Trypsin inhibitor unit (TIU) aprotinin, 1 mM sodium orthovanadate), and dissolved in 30 μl of Laemmli sample buffer (Bio-Rad). A 10 μl aliquot of the dissolved sample was loaded onto a 10% separation gel (Bio-Rad) and electrophoresed at 150 V for 60 min. The protein on the gel was transferred onto PVDF membrane (Bio-Rad) after methanol treatment by wet blotting at 100 V for 1 h. The membrane was washed with TBS-T (0.05% Tween 20), blocked with 5% skim milk/TBS-T (blocking buffer) at room temperature for 1 h.

and reacted with anti-phosphorylated c-Jun (ser63) mouse monoclonal antibody (KM-1 diluted 250-fold with blocking buffer, Santa Cruz) at room temperature for 1.5 h. The membrane was washed with TBS-T (5 min, two times), reacted with HRP labeled-anti mouse IgG secondary antibody (Amersham) diluted 2000-fold with blocking buffer at room temperature for 1 h. and washed with TBS-T (5 min, three times) and TBS. The membrane was reacted with 2 ml of ECL reactive solution (ECL Plus, Amersham) for 5 min, exposed to X-ray film for 1 min, and the film was immersed in a developing solution for 90 s, washed with water and immersed in a fixing solution to develop the film. The developed X-ray film was scanned by DeskscanII, automatically corrected, and further automatically corrected by Photoshop to reverse the color tone. The density of the band seen at the objective molecular weight was digitalized on the histogram of Photoshop.

5.6. Statistical analysis

Data are expressed and presented in the figure as means ± SD. A Williams' test or Student's *t* test was used to compare the means of normally distributed continuous variables. A value of $p < 0.05$ was chosen as the limit of statistical significance.

5.7. Pharmacokinetic analysis in rats

Plasma samples were deproteinized with acetonitrile, and the resulting protein precipitate was removed by centrifugation. The compound concentrations in the supernatant were measured by HPLC-UV or LC/MS/MS. A Shimadzu Vp series HPLC system (Shimadzu) and a MS/MS API 3000 triple quadruple mass spectrometer (MDS Sciex) were used. The HPLC conditions were as follows: L-column ODS (4.6 × 250 mm), 0.01 mol/L of 4:6 ammonium acetate/acetonitrile mobile phase, 1 ml/min flow rate, 40 °C column temperature, and 306 nm UV detection. The LC/MS/MS conditions were as follows: Capcell PAK C8 (2.1 × 50 mm) column, 0.01 mol/L of 48:52 ammonium formate/acetonitrile mobile phase (adjusted to pH 3 with formic acid), 0.2 ml/min flow rate, 40 °C column temperature, turbo ion spray source, positive ion mode, multiple reaction monitoring mode.

5.8. Crystallization and structure determination

JNK3 was cloned and purified following the published protocol.[14] Compound **12d** was incubated with the enzyme with a threefold excess of inhibitor for 3 h in ice. Crystals of the complex were grown by hanging-drop co-crystallization under the following conditions: 20% pentaerythritol ethoxylate (15/4 EO/OH) reservoir, 0.1 M ammonium sulfate in 0.1 M Bis–Tris, pH 6.5, and the use of the micro-seeding method using crystals of the JNK3:AMPPNP complex. Then, crystals were transferred to the reservoir solution containing 15% glycol as cryoprotectant and frozen with liquid nitrogen. They belong to the orthorhombic space group $P2_12_12_1$, with unit cell parameters $a = 57.0$ Å, $b = 70.3$ Å, $c = 108.0$ Å and one molecule per asymmetric unit. X-ray diffraction data were collected on beamline BL32B2 at the facilities of the Pharmaceutical Consortium at SPring-8 and processed to an R_{merge} value of 0.100 and completeness of 98.3 at 2.5 Å resolution. The structure was solved by molecular replacement with CNX (Accelrys, USA) using the coordinates of the JNK3:AMPPNP complex (PDB code: 1JNK) as a search model. Initial difference Fourier electron density maps were used to determine the binding conformation of the ligand. Refinement was carried out using alternating cycles of manual rebuilding using the graphic software QUANTA (Accelrys, USA) and computer-based refinement using CNX (Accelrys, USA). The crystallographic *R*-factor/free-*R* were refined to 0.266/0.331, with RMSD bond length/angle of 0.008 Å/1.5° at 2.5 Å resolution. Coordinates are deposited in the Protein Data Bank (PDB ID: 2ZDU).

References and notes

1. (a) Pulverer, B. J.; Kyriakis, J. M.; Avruch, J.; Nikolakaki, E.; Woodgety, J. R. *Nature* **1991**, *353*, 670–674; (b) Hibi, M.; Lin, A.; Smeal, T.; Minden, A.; Karine, M. *Genes Dev.* **1993**, *7*, 2135–2148; (c) Sugden, P. H.; Clerk, A. *Circ. Res.* **1998**, *83*, 345–352; (d) Bogoyevitch, M. A. *Rev. Cardiovasc. Res.* **2000**, *45*, 826–842; (e) Bogoyevitch, M. A.; Boehm, I.; Oaklay, A.; Ketterman, A. J.; Ball, R. K. *Biochim. Biophys. Acta* **2004**, *1697*, 89–101.
2. Manning, A. M.; Davis, R. J. *Nat. Rev. Drug Disc.* **2003**, *2*, 554–565.
3. Zhang, F.; Strand, A.; Robbins, D.; Cobb, M. H.; Goldsmith, E. J. *Nature* **1994**, *367*, 704–711.
4. Willson, K. P.; Fitzgibbon, M. J.; Caron, P. R.; Griffith, J. P.; Chen, W.; McCaffrey, P. G.; Chambers, S. P.; Su, M. S.-S. *J. Biol. Chem.* **1996**, *271*, 27696–27700.
5. Xie, X.; Gu, Y.; Fox, T.; Coll, J. T.; Fleming, M. A.; Markland, W.; Caron, P. R.; Wilson, K. P.; Su, M. S.-S. *Structure* **1998**, *6*, 983–991.
6. Bennett, B. L.; Sasaki, D. T.; Murray, B. W.; O'Leary, E. C.; Sakata, S. T.; Xu, W.; Leisten, J. C.; Motiwala, A.; Pierce, S.; Satoh, Y.; Bhagwat, S. S.; Manning, A. M.; Anderson, D. W. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 13681–13686.
7. Gaillard, P.; Jeanclaude-Etter, I.; Ardissone, V.; Arkinstall, S.; Cambert, Y.; Camps, M.; Chabert, C.; Church, D.; Cirillo, R.; Gretener, D.; Halazy, S.; Nichols, A.; Szyndralewiez, C.; Vitte, P.; Gotteland, J. *J. Med. Chem.* **2005**, *48*, 4596–4607.
8. Stocks, M. J.; Barber, S.; Ford, R.; Leroux, F.; St-Gallay, S.; Teague, S.; Xue, Y. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3459–3462.
9. Bogoyevitch, M. A. *Trends Mol. Med.* **2005**, *11*, 232–239.
10. Choukron, G.; Hajjar, R.; Fry, S.; Monte, F. D.; Haq, S.; Guerrero, J. L.; Picard, M.; Rosenzweig, A. *J. Clin. Invest.* **1999**, *104*, 391–398.
11. (a) Gong, X.; Petrich, B. G.; Liao, P.; Wu, B.; Chien, K. R.; Jolla, L.; Wang, Y. *Circulation* **2000**, *102*, 216–217; (b) Petrich, B. G.; Gong, X.; Lerner, D. L.; Wang, X.; Brown, J. H.; Saffitz, J. E.; Wang, Y. *Cir. Res.* **2002**, *91*, 640–647; (c) Petrich, B. G.; Eloff, B. C.; Lerner, D. L.; Kovacs, A.; Saffitz, J. E.; Rosenbaum, D. S.; Wang, Y. *J. Biol. Chem.* **2004**, *279*, 15330–15338.

12. Cook, S. A.; Sugden, P. H.; Clerk, A. *J. Mol. Cell Cardiol.* **1999**, *31*, 1429–1434.
13. (a) Mukawa, H.; Toki, Y.; Shimauchi, A.; Matsui, H.; Morishima, I.; Okumura, K.; Ito, T.; Hayakawa, T. *J. Hypertens.* **1997**, *15*, 1027–1032; (b) Obayashi, M.; Yano, M.; Kohno, M.; Kobayashi, S.; Yamamoto, T.; Ohkusa, T.; Konishi, M.; Matsuzaki, M. *Am. J. Hypertens.* **1999**, *12*, 381–387; (c) Mukawa, H.; Toki, Y.; Miyazaki, Y.; Matsui, H.; Okumura, K.; Ito, T. *Hypertens. Res.* **2003**, *26*, 89–95.
14. Xie, X.; Gu, Y.; Fox, T.; Coll, J. T.; Fleming, M. A.; Markland, W.; Caron, P. R.; Wilson, K. P.; Su, M. S. *Structure* **1998**, *6*, 983–991.