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# Discovery, synthesis and biological evaluation of isoquinolones as novel and highly selective JNK inhibitors (2)

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**Abstract**—3-Metoxycarbonyl isoquinolone derivative **1** has been identified as a potent JNK inhibitor and significantly inhibited cardiac hypertrophy in a rat pressure-overload model. Herein, a series of isoquinolones with an imidazolylmethyl or a pyrazolylmethyl group at the 2-position were designed based on X-ray crystallographic analysis of the complex between the isoquinolone compound and JNK3, as wells as the relationship between compound lipophilicity (log *D*) and activity in a cell-based assay. The compounds prepared showed potent JNK1 inhibitory activities in a cell-based assay. Among them the isoquinolone derivative possessing 5-[(cyclopropylamino)carbonyl]-1-methyl-1*H*-pyrazole (**16e**) exhibited significant anti-hypertrophic activity at doses of more than 1 mg/kg (po) in a pressure-overload model.


## 1. Introduction

The c-Jun N-terminal protein kinases (JNKs) are a series of serine/threonine protein kinases of the mitogen-activated protein kinase (MAPK) family. JNK isoforms are created by alternative splicing of messenger RNA transcripts derived from three genes: *JNK1*, *JNK2* and *JNK3*.[1] It is reported that JNKs are activated in many diseases, such as diabetes, cancer, inflammation, stroke, etc. Hence, JNK inhibitors are expected to be effective therapeutic agents against a variety of these diseases. It is therefore not surprising that many pharmaceutical companies have been investigating potent and selective JNK inhibitors.[2] Recently, several reports indicated that JNK played important roles in the progression of heart failure.[3] Therefore, JNK inhibitors appear promising for the treatment of heart hypertrophy and heart failure.

Previous results from our laboratory demonstrated that isoquinolone derivative **1** selectively inhibited all three JNK isoforms with an $IC_{50}$ values of about 30 nM (>500-fold selectivity versus other kinases) and significantly decreased the left ventricular weight/body weight (LVW/BW) ratio at a dose of more than 10 mg/kg (qd, po) in a rat pressure-overload model. Detailed structure–activity relationship (SAR) studies of a series of compounds related to **1** are being reported elsewhere. And further efforts to increase JNK inhibitory activity led to 3-acyl-isoquinolone derivatives **2a** and **2d**. Compound **2d** showed more potent JNK inhibitory activity ($IC_{50}$ = 9.6 nM) than **1**, and good pharmacological properties. However, the in vivo activities of **1** and **2d** were less promising than their potent JNK inhibitory activities and PK profiles. We hypothesized that this discrepancy resulted from their weak inhibitory activities in

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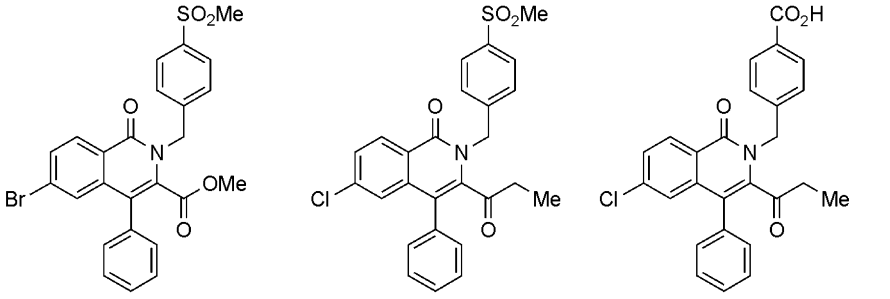
cell-based assay (Fig. 1). In order to increase their potency in our cell-based assay, we explored modifications of the 4-methylsulfonyl benzyl moiety at the isoquinolone 2-position, which has been shown to be tolerant of diverse modifications in SAR studies. In this report we describe the structure based drug design (SBDD), synthesis, JNK in vitro and in vivo evaluation of these isoquinolone derivatives.

## 2. Compound design

Co-crystallization of compound **2d** with JNK3 and subsequent X-ray crystallographic analysis resulted in the complex structure shown in Figure 2. Compound **2d** is bound in the ATP binding pocket of JNK3, with the carbonyl group of the isoquinolone ring forming a hydrogen bond with the main chain NH group of Met149 in the hinge region. The most important interaction observed is between the ligand carboxylic acid and the side chain amino group of Lys68, which is consistent with the observed increase in potency of compounds having a hydrogen bond donor or acceptor on the benzyl group. The two benzene rings, the isoquinolone ring and the pendant 4-phenyl group occupy hydrophobic pockets. The carbonyl of the 3-propionyl group also interacts with the side chain carbamoyl group of Asn152.

Our previous SAR studies on this series of 2-benzylisoquinolines also provided guidance for the design of improved inhibitors. We analyzed the $IC_{50}$ values from the cell-based assay in relation to several physical properties (solubility, log *D*, etc.) of the 2-benzyl isoquinolone derivatives. Among them the relationship between log *D* values measured by HPLC (calculated from retention time[4]) and the relative $IC_{50}$ values in cell-based and enzymatic assays suggested that a log *D* value of around 4.00 should yield potent JNK inhibitory activity in cells. And plotting the data indicated that compounds with low (<3.50) or high (>4.50) log *D* values showed a large difference in $IC_{50}$ values between cell-based and enzymatic assays (Fig. 3).

On the basis X-ray crystallographic and lipophilicity data, we designed compounds of type I, which have




**1**, JNK1 : $IC_{50}$ = 30 nM
H9c2 : $IC_{50}$ = 2600 nM

**2a**, JNK1 : $IC_{50}$ = 28 nM
H9c2 :not tested

**2d**, JNK1 : $IC_{50}$ = 9.6 nM
H9c2 : $IC_{50}$ = 2300 nM

**Figure 1.** Isoquinolone JNK inhibitors **1**, **2a**, and **2d**.

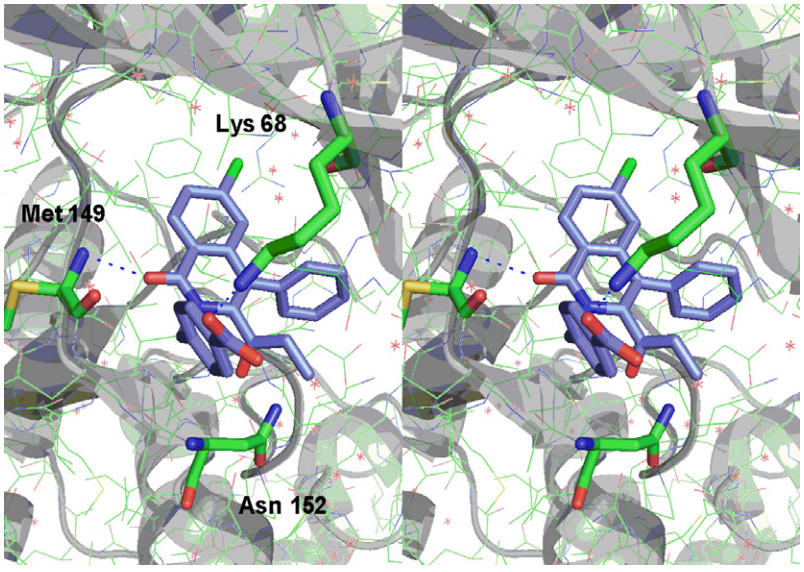



**Figure 2.** X-ray crystal structure of compound **2d** (blue) bound to JNK3 (green). Major interactions between the compound and protein are observed for Lys68, Met149, and Asn 152.

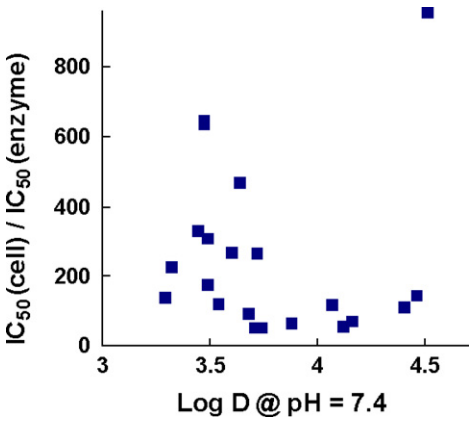



**Figure 3.** Relationship between the ratio of $IC_{50}$ values for cell assay enzyme assay and log *D* value at pH 7.4.

two substituents on the heteroaromatic ring attached to the isoquinolone 2-position, with the goal of increasing inhibitory activity in cells. For this designed compound, enhancement of inhibitory activity by interaction of a hydrogen bond acceptor (HBA) with the amino group of Lys68 and regulation of lipophilicity through the other substituent R can be expected. Additionally, a docking study based on the structural data for **2d** predicted that for the 2-isoquinoline aromatic ring a 5-membered ring would be preferred to a 6-membered ring (Fig. 4).

## 3. Chemistry

Synthesis of 3-methoxycarbonyl isoquinolone compound **1** is shown in Scheme 1. The coupling of 4-methylsulfonyl benzylamine **4** with isocoumarin **3**,[5] and subsequent dehydration with sulfuric acid gave **1** in good yield.

*N*-Arylmethyl aminoalcohols, the starting materials for 3-acyl-isoquinolone derivatives **2a**–**h**, were prepared as shown in Schemes 2–4. Coupling of 4-methylsulfonyl benzylamine **4** with 1,2-epoxybutane **5** gave aminoalcohol derivative **6a** (Scheme 2). Other aminoalcohol derivatives **6b**–**h** were prepared by reductive amination of aryl aldehydes **7b**–**h** with aminoalcohol **8** (Scheme 3). The aldehydes **7b**, **c** were prepared as shown in Scheme 4, and other aldehydes **7d**–**h** were prepared by a known procedure[6] or are commercially available.

The synthesis of 3-acyl-isoquinolone derivatives is shown in Scheme 5. Carboxylic acid **12** was condensed with aminoalcohols **6a**–**h**, and subsequently the hydroxy group oxidized to ketone by $SO_3$–pyridine complex and basic cyclodehydration conducted to yield **2a**–**h**. The reaction conditions for the preparation of each compound are summarized in Table 1.

Compounds with an *N*-carbamoyl imidazolylmethyl or pyrazolylmethyl group were synthesized as shown in

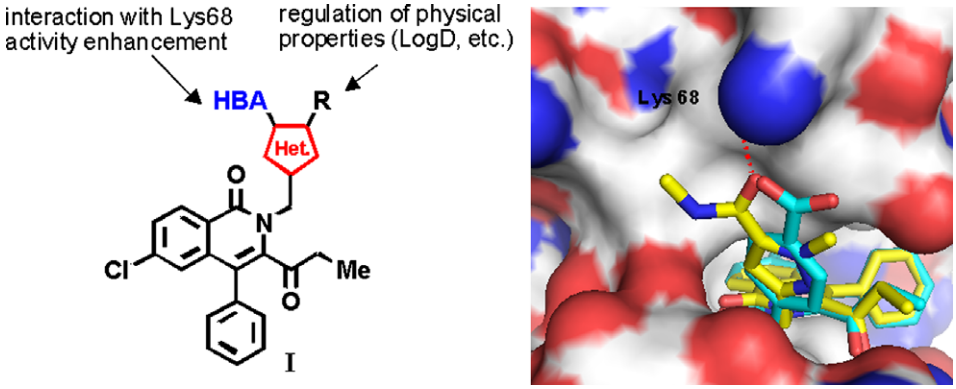
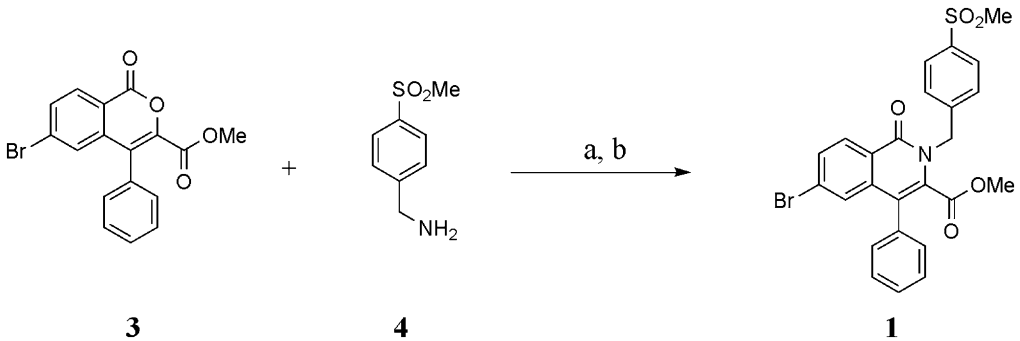
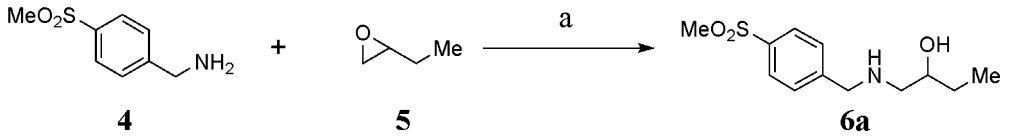



**Figure 4.** Designed compound **I** (left) and overlay view of **2d** (blue)—JNK3 crystal structure with **I** (yellow) (right). Compound **16c** was used as an example of **I**.

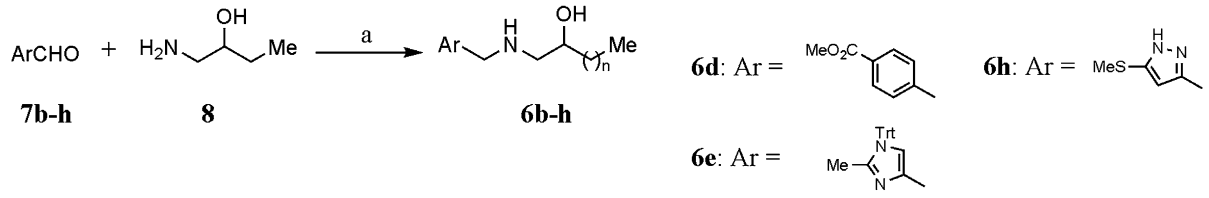



**Scheme 1.** Synthesis of 3-methoxycarbonyl isoquinolone **1**. Reagents: (a) $Et_3N$, MeOH; (b) $H_2SO_4$·MeOH.



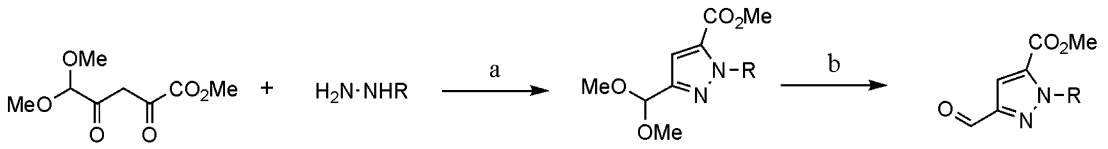

4 5 6a

**Scheme 2.** Synthesis of aminoalcohol **6a**. Reagents: (a) NaOMe, MeOH, THF.



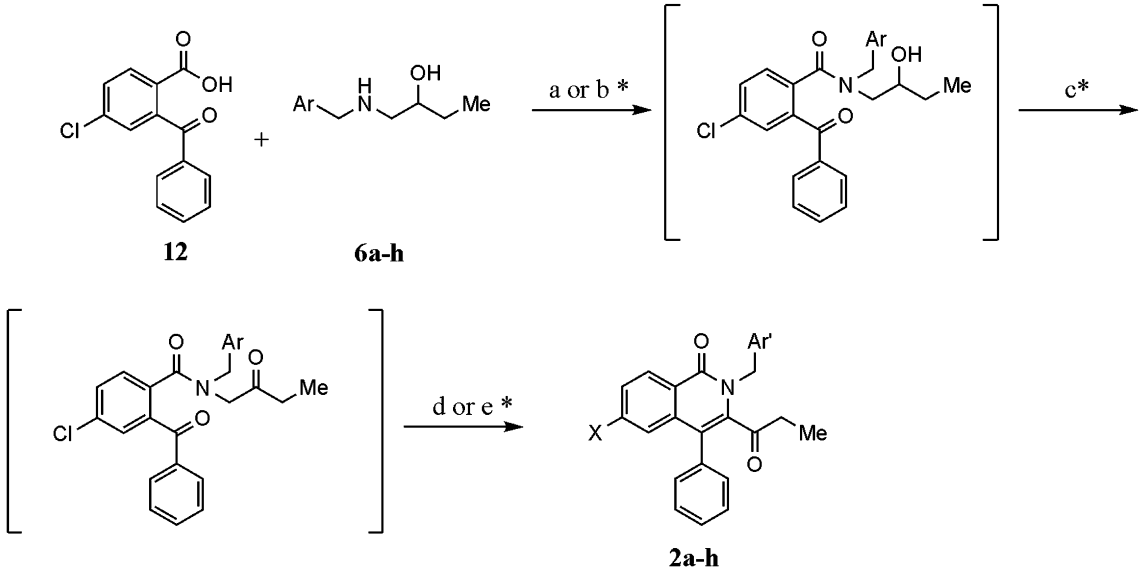

7b-h 8 6b-h

**Scheme 3.** Synthesis of aminoalcohols. Reagents: (a) $NaBH_4$, MeOH.




9 **10b:** R = H, **10c:** R = Me **11b:** R = H, **11c:** R = Me **7b:** R = H, **7c:** R = Me

**Scheme 4.** Synthesis of aldehydes used in preparation of aminoalcohols. Reagents: (a) MeOH; (b) 50% AcOH.



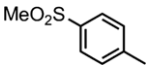
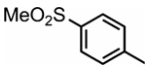
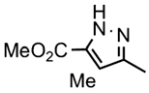
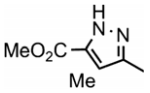
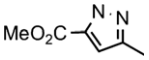
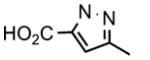
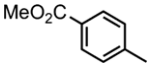
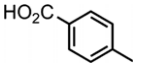
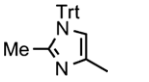
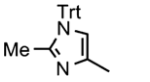
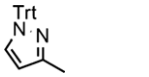
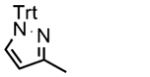
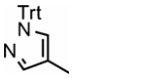
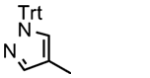
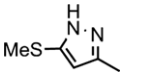
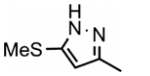

12 6a-h 2a-h

**Scheme 5.** Synthesis of isoquinolones. Reagents: (a) WSC, HOBt, $Et_3N$, DMF; (b) $SOCl_2$, toluene; **6**, toluene; (c) $SO_3$–Py, $ET_3N$, DMSO; (d) DBU, MeOH, THF; (e) KOH, EtOH. *See Table 1.

Scheme 6. The trityl group on azole nitrogen was readily removed by formic acid, and subsequent addition of alkyl isocyanate gave **14e–g**.

Hydrolysis of ester **2b** and oxidation of sulfide **2h** afforded carboxylic acid **15b** or sulfone **15h**, respectively, in good yields (Scheme 7).

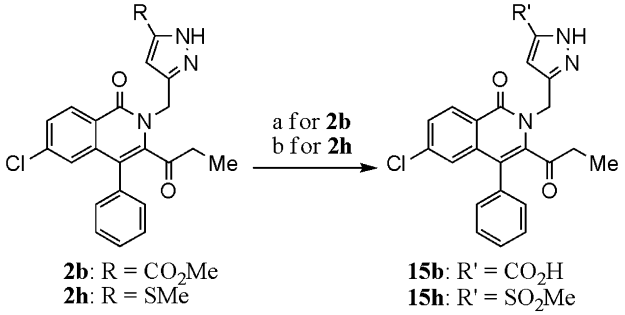
**Table 1.** Synthetic conditions for isoquinolones **2a–h**

| Compound | Ar for **6** | Ar′ for **2** | Reaction conditions |
|---|---|---|---|
| **2a** |  |  | a, c, d |
| **2b** |  |  | b, c, d |
| **2c** |  |  | b, c, e |
| **2d** |  |  | b, c, e |
| **2e** |  |  | b, c, e |
| **2f** |  |  | b, c, e |
| **2g** |  |  | b, c, e |
| **2h** |  |  | b, c, e |

Pyrazolecarboxamide derivatives **16a–e** were synthesized as shown in Scheme 8. Condensation of carboxylic acids **15b** or **2c** with appropriate amines via acid chloride intermediates gave amides **16a–e**.

Compounds possessing an ethyl group, difluoroethyl group and 3,3-dimethyl-3-hydroxypropyl group at the pyrazole ring 1-position were synthesized as shown in Scheme 9. Mitsunobu reaction between **15h** or **2b** and the appropriate primary alcohols gave **17a–c** regioselectively. Alkylation of **2b** with ethyl iodide also gave **17d**. The amide derivatives **18a**, **b** were obtained from **17c**, **d**, respectively, by hydrolysis and condensation with the appropriate amines.

Compounds possessing an acetic acid moiety at the pyrazole ring 1-position were prepared as shown in Scheme 10. Alkylation of **2h** with chloroacetone was carried out to give **19a**, which was oxidized to **20a** by treatment with *m*-chloroperbenzoic acid (*m*-CPBA). In a similar manner, **2h** was converted to **19b** by alkylation with *tert*-butyl bromoacetate. Deprotection of **19b** with hydrochloric acid followed by amidation with ammonia gave **20b**.



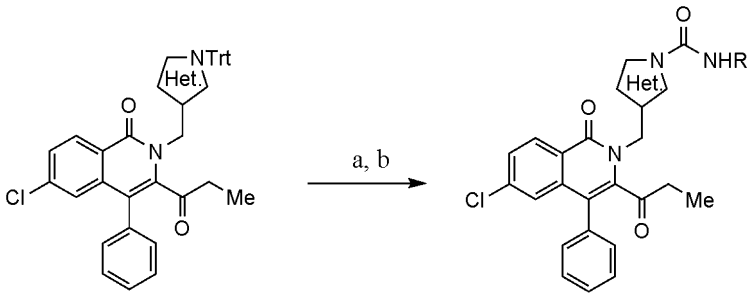

**Scheme 7.** Modification of pyrazolylmethyl moiety. Reagents: (a) 5 N NaOH, MeOH, THF; (b) *m*-CPBA, $CH_2Cl_2$.

## 4. Evaluation of biological activity

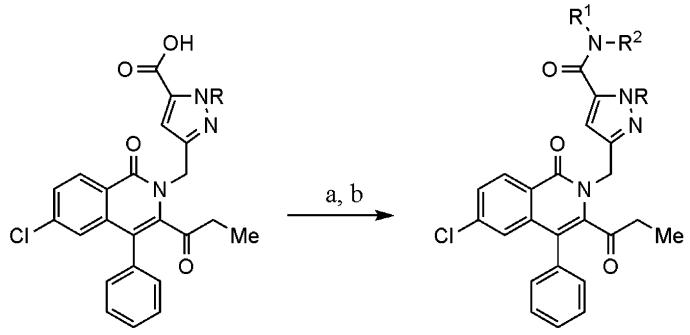
The compounds synthesized in this study were evaluated for their ability to inhibit JNK1 using radiometric assay and anisomycin-induced c-Jun phosphorylation in cardiomyocytes (H9c2 cells). These results are shown in Tables 2 and 3.

The compounds prepared exhibited potent JNK inhibitory activities in enzymatic assay. In particular, compounds in which the position adjacent to the carbamoyl group is carbon or alkylated nitrogen, such as **14e**, **14f**, **16b–d**, and **18b**, showed more potent activities; in contrast, compounds like **14g** and **16a**, with an unsubstituted nitrogen atom at this position showed less potent activity. Among these compounds, **16c** and **18b** exhibited particularly potent activity in both enzymatic and cell-based assays. The results for compounds **16b**, **16c**, and **18b** seem to support our hypothesis that a log *D* value near 4 is favorable for potent activity in the cell-based assay.

Compounds **14e**, **14f**, and **14g** are chemically unstable, and are easily hydrolyzed to give N-unsubstituted



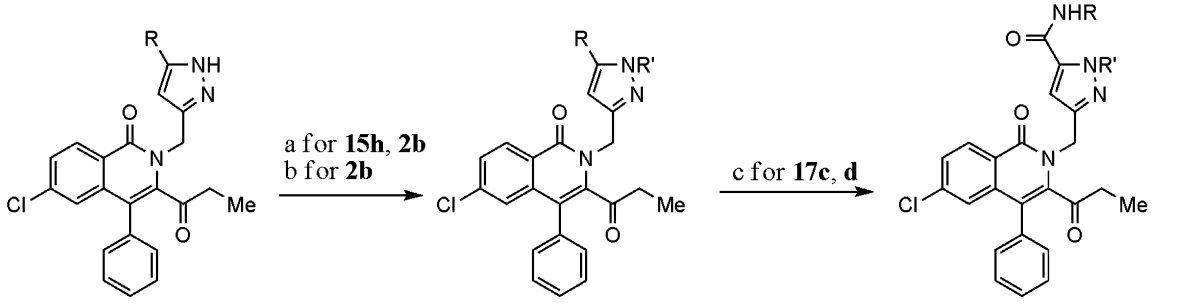

**Scheme 6.** Synthesis of *N*-acyl derivatives. Reagents: (a) HCOOH, THF; (b) RNCO, $Et_3N$, THF (R = Me for **13f**, R = Et for **13g** and **h**).




**15b**: R = H
**2c**: R = Me

**16a**: R = H, $R^1$ = H, $R^2$ = Me
**16b**: R = Me, $R^1$ = H, $R^2$ = H
**16c**: R = Me, $R^1$ = H, $R^2$ = Me
**16d**: R = Me, $R^1$ = Me, $R^2$ = Me
**16e**: R = Me, $R^1$ = H, $R^2$ = *c*Pr

**Scheme 8.** Modification of pyrazolylmethyl moiety. Reagents: (a) $(COCl)_2$, DMF, THF; (b) $NHR^1R^2$, THF.

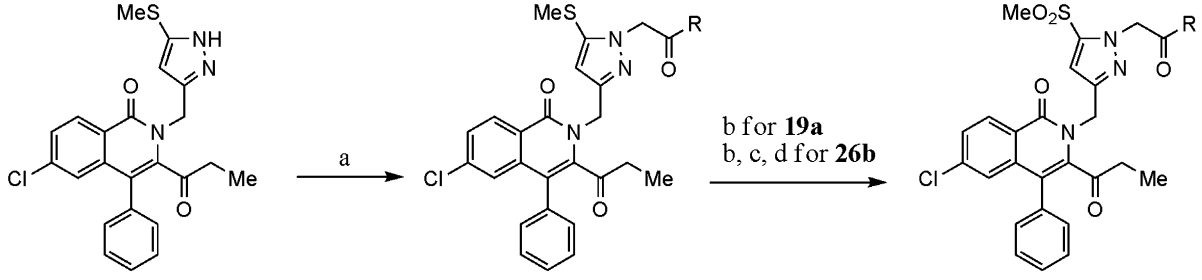



**15h**: R = $SO_2Me$
**2b**: R = $CO_2Me$

**17a**: R = $SO_2Me$, R' = $CH_2CHF_2$
**17b**: R = $SO_2Me$, R' = $(CH_2)_2CMe_2OH$
**17c**: R = $CO_2Me$, R' = $CH_2CHF_2$
**17d**; R = $CO_2Me$, R' = Et

**18a**: R = *c*Pr, R' = $CH_2CHF_2$
**18b**: R = H, R' = Et

**Scheme 9.** Modification of pyrazolylmethyl moiety. Reagents: (a) ADDP, $Bu_3P$, R′OH, toluene; (b) NaH, EtI, DMF; (c) i—5 N NaOH, MeOH, THF; ii—$(COCl)_2$, DMF, THF; iii—$RNH_2$, $CH_2Cl_2$.




**2h**

**19a**: R = Me
**19b**: R = $OBu^t$

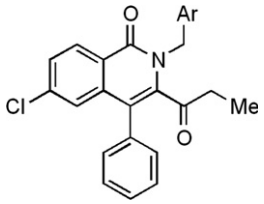
**20a**: R = Me
**20b**: R = $NH_2$

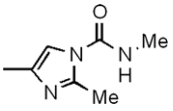
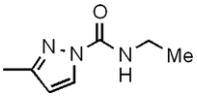
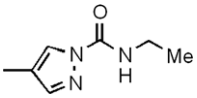
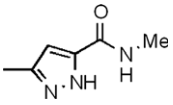
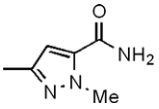
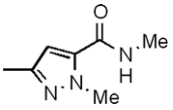
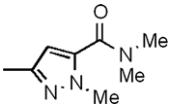
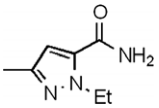
**Scheme 10.** Modification of pyrazolylmethyl moiety. Reagents: (a) NaH, $BrCH_2COR$, DMF; (b) *m*-CPBA, $CH_2Cl_2$; (c) HCl/AcOEt; (d) i—$(COCl)_2$, DMF, THF; (ii) $NH_4OH$, $CH_2Cl_2$.

imidazole or pyrazole compounds. The 5-carbamoyl pyrazole derivatives **16a–d**, **18b** showed good chemical stability. As described above, **16c** showed good potency in both enzymatic and cell-based assays, however it showed poor metabolic stability in both human and rat in vitro microsomes. Thus we sought to improve the metabolic stability of these pyrazole derivatives while retaining JNK1 inhibitory activity.

Next, optimization of the R1 and R2 substituents on the pyrazole ring was carried out and the results are shown in Table 3. Conversion of the methyl amide moiety of **16c** to the cyclopropyl amide of **16e** retained JNK1 inhibitory activity. Compound **16e** showed moderate metabolic stability with high potency in the H9c2 cell-based assay. Exchange of the methyl group of **16e** with the more metabolically stable difluoroethyl group im-

**Table 2.** In vitro JNKI inhibitory activities and properties of imidazolylmethyl or pyrazolylmethyl derivatives



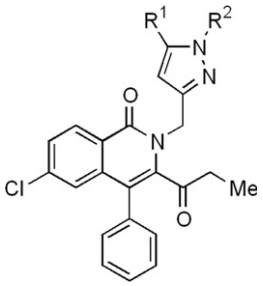

| Compound | Ar | JNKI[a] $IC_{50}$ (nM) | H9c2[b] $IC_{50}$ (μM) | Metabolic stability (μL/min/mg)[c] | | log *D* at pH 7.4 |
|---|---|---|---|---|---|---|
| | | | | Human | Rat | |
| **14e** |  | 6.8 | NT[d] | NT[d] | NT[d] | NT[d] |
| **14f** |  | 8.6 | NT[d] | NT[d] | NT[d] | 4.51 |
| **14g** |  | 43 | NT[d] | 43 | 60 | 4.45 |
| **16a** |  | 33 | NT[d] | 37 | 74 | 3.50 |
| **16b** |  | 7.3 | 2180 | 15 | 81 | 3.77 |
| **16c** |  | 9.4 | 260 | 79 | 177 | 3.97 |
| **16d** |  | 11 | NT[d] | 250 | 230 | 3.91 |
| **18b** |  | 11 | 670 | 35 | 110 | 4.08 |

[a] Inhibitory activity against JNK1.
[b] Inhibitory activity against AP-1 phosphorylation in Hyc2 cell.
[c] Measured at 1 μM.
[d] Not tested.

proved metabolic stability significantly (metabolic stability for **18a**: 14 μL/min/mg for human, 0 μL/min/mg for rat). The methylsulfonyl derivative having a difluoroethyl substituent on the nitrogen of pyrazole ring (**17a**) also showed high potency and good metabolic stability (JNK1 $IC_{50}$ = 10 nM, H9c2 $IC_{50}$ = 480 nM). Introduction a terminal hydroxy moiety resulted in increased H9c2 potency, but with reduced metabolic stability (**17 b**). Compound **20b**, having an acetoamide substituent, exhibited good metabolic stability but weaker cell-based activity than **17a**. In the pyrazole series, the relationship between the JNK1 cell activity and log *D* showed that a log *D* value of around 4.00 is ideal.

Among the compounds described above, we selected **16e** for further examination, since it showed high potency in vitro in cell-based assay ($IC_{50}$ value of 720 nM) and good metabolic stability (44 μL/min/mg for human, 89 μL/min/mg for rat). The pharmacokinetic parameters for **16e** with intravenous (iv) and oral (po) dosing in Wistar rats are shown in Table 4. Following iv administration of 0.3 mg/kg, **16e** demonstrated a total body clearance (CL) of 1281 mL/h/kg and a distribution volume ($V_d$) of

**Table 3.** In vitro JNK1 inhibitory activities and properties of pyrazolylmethyl derivatives




| Compound | $R^1$ | $R^2$ | JNK1[a] $IC_{50}$ (nM) | H9c2[b] $IC_{50}$ (nM) | Metabolic stability (μL/min/mg)[c] | | log *D* at pH 7.4 |
|---|---|---|---|---|---|---|---|
| | | | | | Human | Rat | |
| **16c** | CONHMe | Me | 9.4 | 260 | 79 | 177 | 3.97 |
| **16e** | CONH*c*-Pr | Me | 6.4 | 720 | 44 | 89 | 4.35 |
| **18a** | CONH*c*-Pr | $CH_2CF_2H$ | 10 | 1140 | 14 | 0 | 4.66 |
| **17a** | $SO_2Me$ | $CH_2CF_2H$ | 10 | 480 | 36 | 52 | 4.19 |
| **17b** | $SO_2Me$ | $(CH_2)_2CMe_2OH$ | 10 | 330 | 102 | 74 | 4.23 |
| **20a** | $SO_2Me$ | $CH_2COMe$ | 7.6 | 730 | 70 | 80 | 3.79 |
| **20b** | $SO_2Me$ | $CH_2CONH_2$ | 8.2 | 1050 | 14 | 0 | 3.42 |

[a] Inhibitory activity against JNK1.
[b] Inhibitory activity against AP-1 phosphorylation in H9c2 cell.
[c] Measured at 1 mM.

**Table 4.** Pharmacokinetic profile of JNK inhibitor **16e**[a]

| Compound | $V_d$(SS) (mL/kg) | $CL_{total}$ (mL/h/kg) | $AUC_{iv}$ (ng h/mL) | $C_{max}$ (ng/mL) | $AUC_{po}$ (ng h/mL) | $t_{max}$ (h) | BA (%) |
|---|---|---|---|---|---|---|---|
| **16e** | 3538 | 1281 | 234.4 | 47.1 | 340.5 | 2.00 | 15 |

[a] Dose: iv, 0.3 mg/kg; po, 3 mg/kg.

3538 mL/kg. Compound **16e** was relatively well-absorbed ($t_{max}$ = 2.0 h, $C_{max}$ = 47.1 ng/mL) following oral administration of 3 mg/kg and was found to be orally bioavailable (BA = 15%). Compound **16e** was evaluated for suppressive effects in the development of cardiac hypertrophy in the rat pressure-overload induced cardiac hypertrophy model. **16e** was administered orally in aortic-banded rats, once a day for 7 days in total. One day after final administration, the suppression of cardiac hypertrophy was evaluated by calculation of the left ventricular weight/body weight ratio (LVW/BW). These ratios are shown as the % suppression from the control values. Compound **16e** significantly decreased the LVW/BW ratio at a dose of 1 mg/kg (Fig. 5). This compound showed significantly more potent efficacy than **1**.

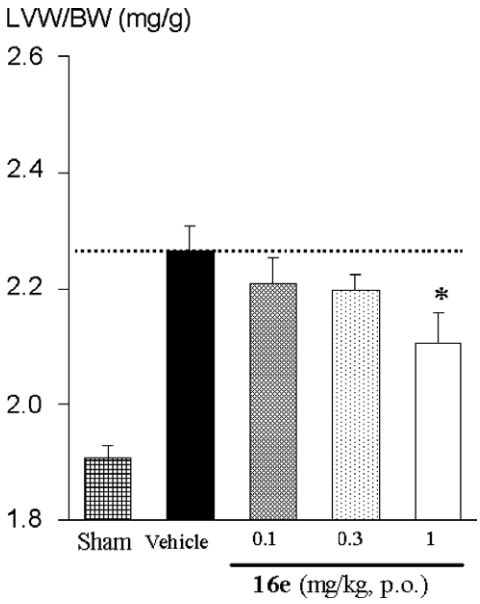



**Figure 5.** Effect of compound **16e** on LVW/BW ratio in rat pressure-overload model. Data are represented as means ± SEM ($n$ = 4–9). $^*p < 0.025$ versus vehicle (Student's $t$ test).

## 5. Conclusions

Based on the information derived from co-crystal structure analysis of JNK3 and **2d**, and the optimal relationship between the lipophilicity (log *D* value) and the relative cell-based and enzymatic potencies, 2-pyrazolylmethylisoquinolone compounds were designed and prepared. The 5-carbamoyl pyrazole **16e** showed highly potent inhibition of JNK1 activity in a cell-based assay. Oral doses of compound **16e** demonstrated significant efficacy in suppressing hypertrophy of the left ventricle in a rat pressure-overload induced cardiac hypertrophy model. These compounds are expected to exhibit efficacy in progression to heart failure.

## 6. Experimental

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 $\delta$ 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; brs, 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-through-put 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.

### 6.1. Methyl-6-bromo-2-[4-(methylsulfonyl)benzyl]-1-oxo-4-phenyl-1,2-dihydroisoquinoline-3-carboxylate (1)

A mixture of **3** (575 mg, 1.6 mmol), **4** (356 mg, 1.9 mmol) and methanol (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 (hexane/AcOEt) to give **1** (329 mg, 63%) as colorless crystals: mp 201–203 °C; $^1$H NMR ($CDCl_3$) $\delta$: 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); LC/MS (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.

### 6.2. 1-{[4-(Methylsulfonyl)benzyl]amino}butan-2-ol (6a)

A solution of **4** (3.0 g, 13.5 mmol) and 1,2-epoxybutane **5** (1.1 g, 15.3 mmol) in 2-propanol (50 ml) was heated under reflux for 12 h. The reaction mixture was concentrated under reduced pressure, and the obtained residue was purified by silica gel column chromatography (EtOAc/MeOH) to give **6a** (1.7 g, 49%) as a colorless oil: $^1$H NMR ($CDCl_3$) $\delta$: 0.96 (3H, t, $J$ = 7.2 Hz), 1.34–1.60 (2H, m), 2.49 (1H, dd, $J$ = 9.2, 12.0 Hz), 2.77 (1H, dd, $J$ = 2.9, 12.0 Hz), 3.06 (3H, s), 3.48–3.70 (1H, m), 3.87 (1H, d, $J$ = 14.3 Hz), 3.96 (1H, d, $J$ = 14.3 Hz), 7.54 (2H, d, $J$ = 8.5 Hz), 7.91 (2H, d, $J$ = 8.5 Hz).

### 6.3. Methyl-3-(dimethoxymethyl)-1*H*-pyrazole-5-carboxylate (11b)

To a solution of **9** (71.5 g, 0.35 mol) in methanol (350 ml) was added hydrazine monohydrate **10b** (79.3 g, 0.385 mol) at 0 °C. After being stirred at room temperature for 2 h, the reaction mixture was concentrated in vacuo. The residue was dissolved in ethyl acetate, and the solution was dried over anhydrous magnesium sulfate. The resulting solution was filtered through silica gel pad, and the solvent was concentrated in vacuo to give **11b** (51.8 g, 96%) as a pale yellow oil: $^1$H NMR ($CDCl_3$) $\delta$: 3.37 (6H, s), 3.93 (3H, s), 5.60 (1H, s), 6.87 (1H, s), 11.80 (1H, brs).

### 6.4. Methyl-3-(dimethoxymethyl)-1-methyl-1*H*-pyrazole-5-carboxylate (11c)

To a solution of **9** (110.3 g, 0.54 mol) in methanol (550 ml) was added methylhydrazine **10c** (24.9 g, 0.54 mol) at 0 °C over 10 min. After being stirred at room temperature overnight, the reaction mixture was concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt) to give **11c** (68.6 g, 59%) as a pale yellow oil: $^1$H NMR ($CDCl_3$) $\delta$: 3.38 (6H, s), 3.88 (3H, s), 4.18 (3H, s), 5.48 (1H, s), 6.88 (1H, s).

### 6.5. Methyl-3-formyl-1*H*-pyrazole-5-carboxylate (7b)

A solution of **11b** (40 g, 0.2 mol) in 50% aqueous acetic acid (400 ml) was at 60 °C for 1 h. The resulting mixture was concentrated in vacuo to give **7b** (27.0 g, 88%) as a colorless amorphous powder: $^1$H NMR ($CDCl_3$) $\delta$: 3.92 (3H, s), 7.34 (1H, s), 10.0 (1H, s), 11.1 (1H, s).

### 6.6. Methyl-3-formyl-1-methyl-1*H*-pyrazole-5-carboxylate (7c)

The compound **7c** was prepared from **11c** by the same method as that described for **7b**. A pale yellow amorphous powder (96%): $^1$H NMR ($CDCl_3$) $\delta$: 3.93 (3H, s), 4.28 (3H, s), 7.32 (1H, s), 9.96 (1H, s).

### 6.7. Methyl 3-{[(2-hydroxybutyl)amino]methyl}-1*H*-pyrazole-5-carboxylate (6b)

To a solution of **7b** (20 g, 0.13 mol) in methanol (400 ml) was added 1-amino-2-butanol **8** (13.9 g, 0.156 mol) and the resulting mixture was stirred for 1 h. Sodium borohydride (5.46 g, 0.13 mol) was added and the mixture was stirred for 1 h. The reaction mixture was concen-

trated in vacuo. To the residue was added saturated aqueous sodium hydrogen carbonate, and the mixture was extracted with AcOEt. The organic layer was dried over anhydrous sodium sulfate, and the solvent was evaporated under reduced pressure to give **6b** (21.1 g, 71%) as colorless amorphous powder: $^1$H NMR (DMSO-$d_6$) $\delta$: 0.82 (3H, t, $J$ = 7.4 Hz), 1.24–1.39 (3H, m), 2.34–2.50 (2H, m), 3.33–3.49 (2H, m), 3.66–3.74 (2H, m), 3.77 (3H, s), 6.61 (1H, s).

The aminoalcohols **6c**–**h** were prepared from **8** and the corresponding aldehydes **7c**–**h** prepared by the same method as that described for **6b**.

### 6.8. Methyl 3-{[(2-hydroxybutyl)amino]methyl}-1-methyl-1*H*-pyrazole-5-carboxylate (6c)

A colorless amorphous powder (79%): $^1$H NMR (DMSO-$d_6$) $\delta$: 0.84 (3H, t, $J$ = 7.3 Hz), 1.15–1.52 (2H, m), 2.36–2.52 (2H, m), 3.35–3.50 (1H, m), 3.58–3.73 (2H, m), 3.82 (3H, s), 4.03 (3H, s), 4.45 (1H, brs), 6.79 (1H, s).

### 6.9. Methyl-4-{[(2-hydroxybutyl)amino]methyl}benzoate (6d)

A colorless oil (84%): $^1$H NMR (DMSO-$d_6$) $\delta$: 0.86 (3H, t, $J$ = 7.6 Hz), 1.25–1.48 (2H, m), 2.73 (1H, m), 2.94 (1H, m), 3.76 (1H, m), 3.87 (3H, s), 4.18–4.32 (2H, m), 7.73 (2H, d, $J$ = 8.4 Hz), 8.00 (2H, d, $J$ = 8.4 Hz), 9.17 (2H, brs).

### 6.10. 1-{[(2-Methyl-1-trityl-1*H*-imidazol-4-yl)methyl]amino}butan-2-ol (6e)

A colorless amorphous powder (98%): $^1$H NMR ($CDCl_3$) $\delta$: 0.95 (3H, t, $J$ = 7.4 Hz), 1.39–1.51 (2H, m), 1.62 (3H, s), 2.47 (1H, dd, $J$ = 9.6, 12.2 Hz), 2.77 (1H, dd, $J$ = 3.0, 12.2 Hz), 3.54 (1H, m), 3.65 (2H, s), 6.55 (1H, s), 7.07–7.17 (6H, m), 7.23–7.38 (10H, m).

### 6.11. 1-{[(1-Trityl-1*H*-pyrazol-3-yl)methyl]amino}butan-2-ol (6f)

A colorless amorphous powder (80%): $^1$H NMR ($CDCl_3$) $\delta$: 0.92 (3H, t, $J$ = 7.5 Hz), 1.35–1.45 (2H, m), 2.38 (1H, dd, $J$ = 9.6, 12.0 Hz), 2.70 (1H, dd, $J$ = 3.0, 12.0 Hz), 3.47 (1H, m), 3.81 (2H, s), 6.14 (1H, d, $J$ = 2.4 Hz), 7.09–7.16 (6H, m), 7.20–7.32 (10H, m).

### 6.12. 1-[(1-Trityl-1*H*-pyrazol-4-yl)amino]butan-2-ol (6g)

A colorless amorphous powder (99%): $^1$H NMR ($CDCl_3$) $\delta$: 0.95 (3H, t, $J$ = 7.4 Hz), 1.45 (2H, m), 2.43 (1H, dd, $J$ = 9.4, 12.1 Hz), 2.74 (1H, dd, $J$ = 3.0, 12.1 Hz), 2.96 (1H, s), 3.52 (1H, m), 3.58–3.78 (2H, m), 7.08–7.21 (6H, m), 7.23–7.37 (9H, m), 7.60 (1H, s).

### 6.13. 1-({[5-(Methylthio)-1*H*-pyrazol-3-yl]methyl}amino)butan-2-ol (6h)

A colorless amorphous powder (99%): $^1$H NMR (DMSO-$d_6$) $\delta$: 0.93 (3H, t, $J$ = 7.4 Hz), 1.38–1.51 (2H, m), 2.49 (3H, s), 2.51–2.58 (1H, m), 2.65–2.75 (1H, m), 3.62 (1H, m), 3.85 (2H, s), 6.11 (1H, s).

### 6.14. 6-Chloro-2-[4-(methylsulfonyl)benzyl]-4-phenyl-3-propionylisoquinolin-1(2*H*)-one (2a)

To a solution of **6a** (739 mg, 2.86 mmol), **12** (782 mg, 3 mmol) and 1-hydroxy-1*H*-benzotriazole (463 mg, 3.43 mmol) in DMF (20 ml) was added triethylamine (0.6 ml, 4.29 mmol), and then 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (822 mg, 4.29 mmol) was added at room temperature, and the mixture was stirred at room temperature for 15 h. The solvent was evaporated under reduced pressure, and the residue was partitioned between AcOEt and water, washed with 1 N hydrochloric acid, aqueous sodium hydrogen carbonate and brine, and then dried by adding sodium sulfate. The solvent was evaporated under reduced pressure and the obtained residue was purified by silica gel column chromatography (hexane/AcOEt). The obtained colorless amorphous form was dissolved in dimethylsulfoxide (30 ml) and triethylamine (10 ml) was added. Under ice-cooling, pyridine–sulfur trioxide complex (1.91 g, 12 mmol) was added, and the mixture was stirred at room temperature for 8 h. Water was added, and the mixture was extracted with AcOEt, washed with 1 N hydrochloric acid, aqueous sodium hydrogen carbonate and brine, and dried over sodium sulfate. The solvent was evaporated under reduced pressure, and the obtained residue was suspended in methanol, crystallized and filtered. The obtained colorless amorphous form was dissolved in methanol (20 ml) and THF (10 ml), and then 1,8-diazabicyclo[5.4.0]-7-undecene (0.90 ml, 6 mmol) was added. The mixture was heated under reflux for 30 h. The solvent was evaporated under reduced pressure, and the residue was partitioned between AcOEt and 1 N hydrochloric acid, washed with aqueous sodium hydrogen carbonate and brine, and then sodium sulfate was added to dry the mixture. The solvent was evaporated under reduced pressure and the obtained residue was purified by silica gel column chromatography (hexane/AcOEt) to give **2a** (317 mg, 22%) as colorless crystals: mp 238–240 °C; $^1$H NMR ($CDCl_3$) $\delta$: 0.51 (3H, t, $J$ = 7.0 Hz), 1.73 (2H, q, $J$ = 7.0 Hz), 3.02 (3H, s), 5.38 (2H, s), 7.16–7.64 (9H, m), 7.89 (2H, d, $J$ = 8.4 Hz), 8.48 (1H, d, $J$ = 8.8 Hz); LC/MS (ESI$^+$) 480, 482; Anal. calcd for $C_{26}H_{22}ClNO_4S$: C, 65.06; H, 4.62; Cl, 7.39; N, 2.92; O, 13.33; S, 6.68. Found: C, 64.90; H, 4.59; N, 3.02.

### 6.15. Methyl-3-((6-chloro-1-oxo-4-phenyl-3-propionyl-2(1*H*)-isoquinolinyl)methyl)-1*H*-pyrazole-5-carboxylate (2b)

To a suspension of **12** (2.22 g, 8.5 mmol) in toluene (20 ml) was added DMF (0.1 ml) and then thionyl chloride (0.74 ml, 10 mmol) was added, and the mixture was stirred at 60 °C for 3 h. The solvent was evaporated under reduced pressure, toluene (50 ml) was added and the mixture was re-concentrated. The obtained residue was suspended in toluene (20 ml) and *N*-ethyldiisopropylamine (4.4 ml, 25.5 mmol) was added, and then **6b** (1.59 g, 7 mmol) was added. After being stirred at 90 °C for 2 h, water was added and extracted with

AcOEt. The organic layer was washed with 1 N hydrochloric acid, aqueous sodium hydrogen carbonate and brine, and dried over sodium sulfate. The solvent was evaporated under reduced pressure, and the obtained residue was dissolved in dimethylsulfoxide (20 ml) and triethylamine (15.6 ml) was added. Under ice-cooling, pyridine–sulfur trioxide complex (8.9 g, 55.9 mmol) was added, and the mixture was stirred at room temperature for 2 h. Water was added, and the mixture was extracted with AcOEt, washed with 1 N hydrochloric acid, aqueous sodium hydrogen carbonate and brine, and dried by adding sodium sulfate. The solvent was evaporated under reduced pressure, and the obtained residue was purified by silica gel column chromatography (hexane/AcOEt). The obtained colorless amorphous form was dissolved in methanol (20 ml) and THF (10 ml), and then 1,8-diazabicyclo[5.4.0]-7-undecene (2.55 ml, 17.1 mmol) was added. The mixture was heated under reflux for 12 h. The solvent was evaporated under reduced pressure, and the residue was partitioned between AcOEt and 1 N hydrochloric acid, washed with aqueous sodium hydrogen carbonate, and brine and sodium sulfate was added to dry the mixture. The solvent was evaporated under reduced pressure, and the obtained residue was purified by silica gel column chromatography (hexane/AcOEt) to give **2b** (802 mg, 25%) as colorless crystals: $^1$H NMR ($CDCl_3$) $\delta$: 0.73 (3H, t, $J$ = 7.1 Hz), 2.10 (2H, q, $J$ = 7.1 Hz), 3.89 (3H, s), 5.19 (2H, s), 6.84 (1H, s), 7.20–7.33 (3H, m), 7.43–7.58 (4H, m), 8.46 (1H, d, $J$ = 8.7 Hz).

### 6.16. 3-((6-Chloro-1-oxo-4-phenyl-3-propionyl-2(1*H*)-isoquinolinyl)methyl)-1-methyl-1*H*-pyrazole-5-carboxylic acid (2c)

To a suspension of **12** (2.22 g, 8.5 mmol) in toluene (20 ml) was added DMF (0.1 ml) and then thionyl chloride (0.74 ml, 10 mmol) was added, and the mixture was stirred at 60 °C for 3 h. The solvent was evaporated under reduced pressure, toluene (50 ml) was added, and the mixture was re-concentrated. The obtained residue was suspended in toluene (20 ml) and *N*-ethyldiisopropylamine (4.4 ml, 25.5 mmol) was added, and then **6c** (1.68 g, 7 mmol) was added. After being stirred at 90 °C for 2 h, water was added, and extracted with AcOEt. The organic layer was washed with 1 N hydrochloric acid, aqueous sodium hydrogen carbonate and brine, and dried over sodium sulfate. The solvent was evaporated under reduced pressure, and the obtained residue was dissolved in dimethylsulfoxide (20 ml), and then triethylamine (15.6 ml) was added. Under ice-cooling, pyridine–sulfur trioxide complex (8.9 g, 55.9 mmol) was added, and the mixture was stirred at room temperature for 2 h. Water was added, and the mixture was extracted with AcOEt, washed with 1 N hydrochloric acid, aqueous saturated sodium hydrogen carbonate and brine, and dried over sodium sulfate. The solvent was evaporated under reduced pressure, and the obtained residue was purified by silica gel column chromatography (hexane/AcOEt). To the obtained colorless amorphous material was added 0.1 N KOH solution in methanol (30 ml), and the mixture was heated under reflux for 12 h. The solvent was evaporated under reduced pressure, and the residue was partitioned between AcOEt and 1 N hydrochloric acid, washed with aqueous sodium hydrogen carbonate and brine, and sodium sulfate was added to dry the mixture. The solvent was evaporated under reduced pressure, and the obtained residue was recrystallized from methanol to give **2c** (881 mg, 28%) as colorless crystals: $^1$H NMR ($CDCl_3$) $\delta$: 0.69 (3H, t, $J$ = 7.0 Hz), 2.10 (2H, q, $J$ = 7.0 Hz), 4.07 (2H, s), 5.28 (2H, s), 7.02 (1H, s), 7.26 (5H, m), 7.48 (4H, m), 8.51 (1H, d, $J$ = 8.7 Hz).

The isoquinolones **2d**–**h** were prepared from **12** and the corresponding aminoalcohols (**6d**–**h**) prepared by the same method as that described for **2c**.

### 6.17. 4-[(6-Chloro-1-oxo-4-phenyl-3-propionylisoquinolin-2(1*H*)-yl)methyl]benzoic acid (2d)

Colorless crystals (69%): mp 230–231 °C; $^1$H NMR ($CDCl_3$) $\delta$: $^1$H NMR ($CDCl_3$) $\delta$: 0.44 (3H, t, $J$ = 7.2 Hz), 1.58 (2H, q, $J$ = 7.2 Hz), 5.43 (2H, s), 7.24–7.30 (5H, m), 7.43–7.55 (4H, m), 8.01 (2H, d, $J$ = 7.8 Hz), 8.50 (1H, d, $J$ = 8.4 Hz); LC/MS ($ESI^+$) 446, 448; Anal. calcd for $C_{26}H_{20}ClNO_4$: C, 70.03; H, 4.52; Cl, 7.95; N, 3.14; O, 14.35. Found: C, 69.79; H, 4.54; N, 3.11.

### 6.18. 6-Bromo-2-((2-methyl-1-trityl-1*H*-imidazol-4-yl)methyl)-4-phenyl-3-propionyl-1(2*H*)-isoquinolinone (2e)

Colorless crystals (9%): $^1$H NMR ($CDCl_3$) $\delta$: 0.57 (3H, t, $J$ = 7.1 Hz), 1.54 (3H, s), 2.06 (2H, q, $J$ = 7.1 Hz), 5.23 (1H, s), 6.60 (1H, s) 7.00–7.14 (6H, m), 7.20–7.35 (12H, m), 7.39 (1H, d, $J$ = 1.7 Hz), 7.42–7.51 (3H, m), 7.59 (1H, dd, $J$ = 1.9, 8.7 Hz), 8.37 (1H, d, $J$ = 8.7 Hz).

### 6.19. 6-Chloro-4-phenyl-3-propionyl-2-((1-trityl-1*H*-pyrazol-3-yl)methyl)-1(2*H*)-isoquinolinone (2f)

Colorless crystals (18%): $^1$H NMR ($CDCl_3$) $\delta$: 0.29 (3H, t, $J$ = 7.1 Hz), 1.74 (2H, q, $J$ = 7.1 Hz), 5.24 (2H, s), 6.32 (1H, d, $J$ = 2.5 Hz), 6.98–7.08 (6H, m), 7.15–7.30 (13H, m), 7.37–7.50 (4H, m), 8.45 (1H, d, $J$ = 8.7 Hz).

### 6.20. 6-Chloro-4-phenyl-3-propionyl-2-((1-trityl-1*H*-pyrazol-4-yl)methyl)-1(2*H*)-isoquinolinone (2g)

Colorless crystals (44%): $^1$H NMR ($CDCl_3$) $\delta$: 0.50 (3H, $J$ = 7.2 Hz), 1.81 (2H, q, $J$ = 7.2 Hz), 5.15 (2H, s), 7.02–7.16 (6H, m), 7.20–7.31 (12H, m), 7.34 (1H, s), 7.41–7.53 (4H, m), 7.63 (1H, s), 8.45 (1H, d, $J$ = 8.7 Hz).

### 6.21. 6-Chloro-2-{[5-(methylthio)-1*H*-pyrazol-3-yl]methyl}-4-phenyl- 3-propionylisoquinolin-1(2*H*)-one (2h)

Colorless crystals (78%): $^1$H NMR ($CDCl_3$) $\delta$: 0.72 (3H, t, $J$ = 7.1 Hz), 2.09 (2H, q, $J$ = 7.1 Hz), 2.46 (3H, s), 5.14 (2H, s), 6.22 (1H, s), 7.22–7.31 (3H, m), 7.44–7.53 (4H, m), 8.45 (1H, d, $J$ = 8.7 Hz), 10.93 (1H, s).

### 6.22. 4-[(6-Chloro-1-oxo-4-phenyl-3-propionylisoquinolin-2(1*H*)- yl)methyl]-N,2-dimethyl-1*H*-imidazole-1-carboxamide (14e)

To a solution of **13e** (346 mg, 0.5 mmol) in THF (5 ml) was added formic acid (5 ml), and the mixture was stirred under reflux for 12 h. The solvent was evaporated under reduced pressure, and the residue partitioned between AcOEt and 1 N sodium hydroxide aqueous solution. The organic layer was washed with brine and dried over sodium sulfate. The solvent was evaporated under reduced pressure, and the obtained residue was dissolved in THF (10 ml) and methyl isocyanate (0.04 ml, 0.75 mmol) was added at 0 °C. After being stirred at that temperature for 3 h, the resulting mixture was partitioned between AcOEt and water. The organic layer was washed with brine, and dried over sodium sulfate. The solvent was evaporated under reduced pressure, and the obtained residue was purified by silica gel column chromatography (hexane/AcOEt) to give **14e** (99 mg, 43%) as colorless crystals: mp 210–212 °C; $^1$H NMR ($CDCl_3$) $\delta$: 0.72 (3H, t, $J$ = 7.1 Hz), 2.36 (2H, q, $J$ = 7.0 Hz), 2.53 (3H, s), 2.91 (3H, d, $J$ = 4.7 Hz), 5.06 (2H, s), 6.09 (1H, m), 7.19–7.34 (4H, m), 7.39–7.55 (4H, m), 8.33 (1H, d, $J$ = 8.7 Hz); LC/MS (ESI$^+$) 463, 465; Anal. calcd for $C_{25}H_{23}ClN_4O_3$: C, 64.86; H, 5.01; N, 12.10. Found: C, 64.82; H, 4.96; N, 12.05.

The isoquinolones **14f** and **g** were prepared from ethyl isocyanate and the corresponding isoquinolones (**13f**,**g**) by the same method as that described for **14e**.

### 6.23. 3-[(6-Chloro-1-oxo-4-phenyl-3-propionylisoquinolin-2(1*H*)-yl)methyl]-*N*-ethyl-1*H*-pyrazole-1-carboxamide (14f)

Colorless crystals (88%): mp 199–201 °C; $^1$H NMR ($CDCl_3$) $\delta$: 0.61 (3H, t, $J$ = 7.2 Hz), 1.22 (3H, t, $J$ = 7.2 Hz), 1.85 (2H, q, $J$ = 7.0 Hz), 3.34–3.49 (2H, m), 5.34 (2H, s), 6.40 (1H, d, $J$ = 2.6 Hz), 6.96 (1H, m), 7.22–7.33 (3H, m), 7.41–7.59 (4H, m), 8.12 (1H, d, $J$ = 2.6 Hz), 8.48 (1H, d, $J$ = 8.7 Hz); LC/MS (ESI$^+$) 463, 465; Anal. calcd for $C_{25}H_{23}ClN_4O_3$: C, 64.86; H, 5.01; N, 12.10. Found: C, 64.79; H, 4.95; N, 12.15.

### 6.24. 4-[(6-Chloro-1-oxo-4-phenyl-3-propionylisoquinolin-2(1*H*)- yl)methyl]-*N*-ethyl-1*H*-pyrazole-1-carboxamide (14g)

Colorless crystals (63%): mp 197–198 °C; $^1$H NMR ($CDCl_3$) $\delta$: 0.68 (3H, t, $J$ = 7.2 Hz), 1.24 (3H, t, $J$ = 7.2 Hz), 1.98 (2H, q, $J$ = 7.2 Hz), 3.36–3.51 (2H, m) 5.09 (2H, s), 7.06 (1H, t, $J$ = 5.6 Hz), 7.20–7.33 (3H, m), 7.42–7.53 (4H, m), 7.66 (1H, s), 8.23 (1H, s), 8.46 (1H, d, $J$ = 8.5 Hz); LC/MS (ESI$^+$) 463, 465; Anal. calcd for $C_{25}H_{23}ClN_4O_3$: C, 64.86; H, 5.01; N, 12.10. Found: C, 64.81; H, 5.06; N, 12.02.

### 6.25. 3-((6-Chloro-1-oxo-4-phenyl-3-propionyl-2(1*H*)-isoquinolinyl)methyl)-1*H*-pyrazole-5-carboxylic acid (15b)

To a solution of **2b** (359 mg, 0.82 mmol) in THF (5 ml) and methanol (5 ml) was added 5 N sodium hydroxide aqueous solution (2 ml, 10 mmol), and the mixture was stirred at room temperature for 22 h. The solvent was evaporated under reduced pressure, and the residue was partitioned between 1 N hydrochloric acid and AcOEt. The organic layer was washed with brine, and dried over sodium sulfate. The solvent was evaporated under reduced pressure, and the obtained residue was recrystallized (EtOH) to give **15b** (172 mg, 49%) as colorless crystals: $^1$H NMR (DMSO-$d_6$) $\delta$: 0.49 (3H, t, $J$ = 7.1 Hz), 2.02 (2H, q, $J$ = 7.1 Hz), 3.16 (1H, s), 5.14 (2H, s), 6.62 (1H, s), 7.08 (1H, d, $J$ = 1.9 Hz), 7.26–7.37 (2H, m), 7.44–7.59 (3H, m), 7.67 (1H, dd, $J$ = 2.1, 8.7 Hz), 8.36 (1H, d, $J$ = 8.7 Hz).

### 6.26. 6-Chloro-2-{[5-(methylsulfonyl)-1*H*-pyrazol-3-yl]methyl}-4-phenyl-3-propionylisoquinolin-1(2*H*)-one (15h)

To a solution of **2h** (876 mg, 2 mmol) in dichloromethane (20 ml) was added *m*-chloroperbenzoic acid (65%) (1.38 g, 12.3 mmol) at 0 °C and the mixture was stirred at room temperature for 2 h. To the resulting mixture was added 10% aqueous solution of sodium thiosulfate (20 ml), and stirred at room temperature for 30 min. The resulting mixture was extracted with dichloromethane, and the organic layer was washed with brine, and dried over sodium sulfate. The solvent was evaporated under reduced pressure, and the obtained residue was purified by silica gel column chromatography (hexane/AcOEt) to give **15h** (650 mg, 69%) as colorless crystals: $^1$H NMR ($CDCl_3$) $\delta$: 0.80 (3H, t, $J$ = 7.1 Hz), 2.18 (2H, q, $J$ = 7.1 Hz), 3.18 (3H, s), 5.09 (2H, s), 6.85 (1H, s), 7.24–7.31 (3H, m), 7.48–7.57 (4H, m), 8.46 (1H, d, $J$ = 8.5 Hz), 11.82 (1H, s).

### 6.27. 3-[(6-Chloro-1-oxo-4-phenyl-3-propionylisoquinolin-2(1*H*)-yl)methyl]-*N*-methyl-1*H*-pyrazole-5-carboxamide (16a)

To a solution of **15b** (109 mg, 0.25 mmol) in THF (5 ml) were added oxalyl chloride (0.06 ml, 0.75 mmol) and DMF (1 drop), and the mixture was stirred at room temperature for 1 h. The solvent was evaporated under reduced pressure, toluene (10 ml) was added, and the mixture was re-concentrated. The residue was dissolved in dichloromethane (5 ml), and a 2 M solution of methylamine in THF (1.25 ml, 2.5 mmol) was added. After being stirred at room temperature for 1 h, water was added, and the residue was extracted with dichloromethane. The organic layer was washed with brine, and dried over sodium sulfate. The solvent was evaporated under reduced pressure, and the obtained residue was purified by silica gel column chromatography (hexane/AcOEt) to give **16a** (38 mg, 34%) as colorless crystals: mp 259–261 °C; $^1$H NMR ($CDCl_3$) $\delta$: 0.76 (3H, t, $J$ = 7.1 Hz), 2.14 (2H, q, $J$ = 7.1 Hz), 2.91 (3H, d, $J$ = 5.3 Hz), 5.17 (2H, s), 6.75 (1H, s), 7.12 (1H, s) 7.20–7.29 (4H, m), 7.42–7.55 (4H, m), 8.44 (1H, d, $J$ = 8.5 Hz); LC/MS (ESI$^+$) 449, 451; Anal. calcd for $C_{24}H_{21}ClN_4O_3$–$H_2O$–0.2AcOEt: C, 61.47; H, 5.12; N, 11.56. Found: C, 61.69; H, 4.81; N, 11.27.

The isoquinolones **16b**–**e** were prepared from **2c** and the corresponding amines by the same method as that described for **16a**.

### 6.28. 3-[(6-Chloro-1-oxo-4-phenyl-3-propionylisoquinolin-2(1*H*)-yl)methyl]-1-methyl-1*H*-pyrazole-5-carboxamide (16b)

Colorless crystals (63%): mp 235–237 °C; $^1$H NMR ($CDCl_3$) $\delta$: 0.70 (3H, t, $J$ = 7.1 Hz), 2.11 (2H, q, $J$ = 7.1 Hz), 4.06 (3H, s), 5.24 (2H, s), 5.73 (1H, s), 6.21 (1H, s), 6.75 (1H, s), 7.14–7.34 (3H, m), 7.41–7.55 (4H, m), 8.41 (1H, d, $J$ = 8.5 Hz); LC/MS ($ESI^+$) 449, 451; Anal. calcd for $C_{24}H_{21}ClN_4O_3$–0.2$H_2O$: C, 63.70; H, 4.77; N, 12.38. Found: C, 63.97; H, 4.94; N, 12.02.

### 6.29. 3-[(6-Chloro-1-oxo-4-phenyl-3-propionylisoquinolin-2(1*H*)-yl)methyl]-*N*,1-dimethyl-1*H*-pyrazole-5-carboxamide (16c)

Colorless crystals (61%): mp 236–237 °C; $^1$H NMR ($CDCl_3$) $\delta$: 0.69 (3H, t, $J$ = 7.1 Hz), 2.08 (2H, m), 2.89 (3H, d, $J$ = 4.9 Hz), 4.06 (3H, s), 5.22 (2H, s), 6.35 (1 H, s), 6.66 (1H, s), 7.15–7.33 (3H, m), 7.36–7.54 (4H, m), 8.38 (1H, d, $J$ = 8.7 Hz); LC/MS ($ESI^+$) 463, 465; Anal. calcd for $C_{25}H_{23}ClNO_3$: C, 64.86; H, 5.01; N, 12.10. Found: C, 64.80; H, 5.02; N, 12.04.

### 6.30. 3-[(6-Chloro-1-oxo-4-phenyl-3-propionylisoquinolin-2(1*H*)-yl)methyl]-*N*,*N*,1-trimethyl-1*H*-pyrazole-5-carboxamide (16d)

Colorless crystals (45%): mp 176–178 °C; $^1$H NMR ($CDCl_3$) $\delta$: 0.66 (3H, t, $J$ = 7.1 Hz), 2.04 (2H, q, $J$ = 7.1 Hz), 3.03–3.13 (6H, m), 3.86 (3H, s), 5.29 (2H, s), 6.44 (1H, s), 7.20–7.30 (3H, m), 7.41–7.51 (4H, m), 8.44 (1H, d, $J$ = 8.7 Hz); LC/MS ($ESI^+$) 477, 479; Anal. calcd for $C_{26}H_{25}ClN_4O_3$: C, 65.47; H, 5.28; N, 11.75. Found: C, 65.48; H, 5.27; N, 11.78.

### 6.31. 3-[(6-Chloro-1-oxo-4-phenyl-3-propionylisoquinolin-2(1*H*)-yl)methyl]-*N*-cyclopropyl-1-methyl-1*H*-pyrazole-5-carboxamide (16e)

Colorless crystals (61%): mp 194–196 °C; $^1$H NMR ($CDCl_3$) $\delta$: 0.53–0.64 (2H, m), 0.69 (3H, t, $J$ = 7.1 Hz), 0.79–0.88 (2H, m), 2.09 (2H, q, $J$ = 7.1 Hz), 2.74–2.82 (1H, m), 4.06 (3H, s), 5.22 (2H, s), 6.16 (1H, s), 6.57 (1H, s), 7.22–7.30 (3H, m), 7.42–7.52 (4H, m), 8.43 (1H, d, $J$ = 8.5 Hz); LC/MS ($ESI^+$) 489, 491; Anal. calcd for $C_{27}H_{25}ClN_4O_3$: C, 66.32; H, 5.15; N, 11.46. Found: C, 66.27; H, 5.15; N, 11.47.

### 6.32. 6-Chloro-2-{[1-(2,2-difluoroethyl)-5-(methylsulfonyl)-1*H*-pyrazol-3-yl]methyl}-4-phenyl-3-propionylisoquinolin-1(2*H*)-one (17a)

To a solution of **15h** (118 mg, 0.25 mmol) in toluene (10 ml) were added 2,2-difluoroethanol (0.024 ml, 0.38 mmol), tributylphophine (0.12 ml, 0.5 mmol) and 1,1′-(azodicarbonyl)dipiperidine (126 mg, 0.5 mmol), and the mixture was stirred at room temperature for 24 h. The insoluble materials were filtered off, and the filtrate was partitioned between AcOEt and water. The organic layer was washed with 1 N hydrochloric acid aqueous saturated sodium hydrogen carbonate and brine, and dried over sodium sulfate. The solvent was evaporated under reduced pressure, and the obtained residue was purified by silica gel column chromatography (hexane/AcOEt) to give **17a** (82 mg, 61%) as colorless crystals: mp 203–204 °C; $^1$H NMR ($CDCl_3$) $\delta$: 0.67 (3H, t, $J$ = 7.2 Hz), 2.05 (2H, q, $J$ = 7.2 Hz), 3.18 (3H, s), 4.80 (2H, td, $J$ = 13.3, 4.1 Hz), 5.26 (2H, s), 6.13 (1H, dt, $J$ = 4.1, 55.6 Hz), 7.03 (1H, s), 7.24–7.31 (3H, m), 7.43–7.56 (4H, m), 8.45 (1H, d, $J$ = 8.7 Hz); LC/MS ($ESI^+$) 534, 536; Anal. calcd for $C_{26}H_{24}ClF_2N_3O_5S$: C, 56.23; H, 4.15; N, 7.87. Found: C, 56.31; H, 4.12; N, 7.79.

The isoquinolones **17b**,**c** were prepared from **15h** or **2b** and the corresponding alcohol by the same method as that described for **17a**.

### 6.33. 6-Chloro-2-{[1-(3-hydroxy-3-methylbutyl)-5-(methylsulfonyl)-1*H*-pyrazol-3-yl]methyl}-4-phenyl-3-propionylisoquinolin-1(2*H*)-one (17b)

Colorless crystals (56%): mp 184–186 °C; $^1$H NMR ($CDCl_3$) $\delta$: 0.68 (3H, t, $J$ = 7.1 Hz), 1.24 (6H, s), 1.63 (1H, s), 1.97–2.09 (4H, m), 3.18 (3H, s), 4.48–4.57 (2H, m), 5.25 (2H, s), 6.89 (1H, s), 7.20–7.36 (3H, m), 7.43–7.54 (4H, m), 8.45 (1H, d, $J$ = 8.9 Hz); LC–MS ($ESI^+$) 556, 558; Anal. calcd for $C_{28}H_{30}ClN_3O_5S$: C, 60.48; H, 5.44; N, 7.56. Found: C, 60.31; H, 5.42; N, 7.35.

### 6.34. Methyl-3-[(6-chloro-1-oxo-4-phenyl-3-propionylisoquinolin-2(1*H*)-yl)methyl]-1-(2,2-difluoroethyl)-1*H*-pyrazole-5-carboxylate (17c)

Colorless crystals (56%): $^1$H NMR ($CDCl_3$) $\delta$: 0.66 (3H, t, $J$ = 7.1 Hz), 2.06 (2H, q, $J$ = 7.1 Hz), 3.86 (3H, s), 4.85 (2H, td, $J$ = 13.1, 4.4 Hz), 5.27 (2H, s), 6.05 (1H, tt, $J$ = 55.7, 4.4 Hz), 6.99 (1H, s), 7.23–7.31 (3H, m), 7.42–7.52 (4H, m), 8.46 (1H, d, $J$ = 8.7 Hz).

### 6.35. Methyl-3-[(6-chloro-1-oxo-4-phenyl-3-propionylisoquinolin-2(1*H*)-yl)methyl]-1-ethyl-1*H*-pyrazole-5-carboxylate (17d)

To a suspension of NaH (60% dispersion) (48 mg, 1.2 mmol) in DMF (10 ml) was added **2b** (540 mg, 1.2 mmol) at 0 °C and the mixture was stirred at that temperature for 30 min. To the resulting mixture was added iodoethane (0.19 ml, 2.4 mmol) at 0 °C. After being stirred at room temperature overnight, the solvent was evaporated under reduced pressure, and the residue was partitioned between 1 N hydrochloric acid and AcOEt. The organic layer was washed with saturated sodium hydrogen carbonate and brine, and dried over sodium sulfate. The solvent was evaporated under reduced pressure and the obtained residue was purified by silica gel column chromatography (hexane/AcOEt) to give **17d** (413 mg, 72%) as colorless crystals: $^1$H NMR ($CDCl_3$) $\delta$: 0.66 (3H, t, $J$ = 7.1 Hz), 0.93 (3H, t, $J$ = 7.4 Hz), 2.03 (2H, q, $J$ = 7.1 Hz), 3.86 (3H, s), 4.48

(2H, q, $J$ = 7.2 Hz), 5.22–5.36 (2H, m), 6.87 (1H, s), 7.19–7.34 (3H, m), 7.41–7.51 (4H, m), 8.48 (1H, d, $J$ = 8.7 Hz).

### 6.36. 3-[(6-Chloro-1-oxo-4-phenyl-3-propionylisoquinolin-2(1*H*)-yl)methyl]-*N*-cyclopropyl-1-(2,2-difluoroethyl)-1*H*-pyrazole-5-carboxamide (18a)

To a solution of **17c** (260 mg, 0.5 mmol) in THF (5 ml) and methanol (5 ml) was added 5 N sodium hydroxide aqueous solution (2 ml, 10 mmol), and the mixture was stirred at room temperature for 22 h. The solvent was evaporated under reduced pressure and the residue was partitioned between 1 N hydrochloric acid and AcOEt. The organic layer was washed with brine, and dried by adding sodium sulfate. The solvent was evaporated under reduced pressure. To a solution of obtained residue in THF (5 ml) were added oxalyl chloride (0.13 ml, 1.5 mmol) and DMF (1 drop), and the mixture was stirred at room temperature for 1 h. The solvent was evaporated under reduced pressure, toluene (10 ml) was added, and the mixture was re-concentrated. The residue was dissolved in dichloromethane (5 ml) and cyclopropylamine (0.07 ml, 1 mmol) was added. After being stirred at room temperature for 1 h, water was added, and the residue was extracted with dichloromethane. The organic layer was washed with brine, and dried over sodium sulfate. The solvent was evaporated under reduced pressure, and the obtained residue was purified by silica gel column chromatography (hexane/AcOEt) to give **18a** (96 mg, 36%) as colorless crystals: mp 184–186 °C; $^1$H NMR ($CDCl_3$) $\delta$: 0.54–0.63 (2H, m), 0.69 (3H, t, $J$ = 7.1 Hz), 0.79–0.90 (2H, m), 2.13 (2H, q, $J$ = 7.1 Hz), 2.74–2.82 (1H, m), 4.83 (2H, td, $J$ = 4.3, 13.2 Hz), 5.23 (2H, s), 5.89–6.41 (2H, m), 6.71 (1H, s), 7.22–7.30 (3H, m), 7.42–7.52 (4H, m), 8.41 (1H, d, $J$ = 8.7 Hz); LC/MS (ESI$^+$) 539, 540; Anal. calcd for $C_{28}H_{25}ClF_2N_4O_3$: C, 62.40; H, 4.68; N, 10.40. Found: C, 62.42; H, 4.68; N,10.35.

### 6.37. 3-{4-[(6-Chloro-1-oxo-4-phenyl-3-propionylisoquinolin-2(1*H*)-yl)methyl]phenyl}propanoic acid (18b)

The compound **18b** was prepared from **17d** and 28% $NH_3$ by the same method as that described for **18a**. colorless crystals (46%): mp 144–146 °C; $^1$H NMR ($CDCl_3$) $\delta$: 0.69 (3H, t, $J$ = 7.1 Hz), 1.35 (3H, t, $J$ = 7.2 Hz), 2.10 (2H, q, $J$ = 7.1 Hz), 4.49 (2H, q, $J$ = 7.2 Hz), 5.26 (2H, s), 5.41 (1H, s), 5.86 (1H, s), 6.67 (1H, s), 7.23–7.30 (3H, m), 7.39–7.56 (4H, m), 8.45 (1H, d, $J$ = 8.7 Hz).; LC/MS (ESI$^+$) 462, 465; Anal. calcd for $C_{25}H_{23}ClN_4O_3$–0.5AcOEt–0.1hexane: C, 64.29; H, 5.55; N, 10.87. Found: C, 64.46; H, 5.74; N, 10.68.

### 6.38. 6-Chloro-2-{[5-(methylsulfonyl)-1-(2-oxopropyl)-1*H*-pyrazol-3-yl]methyl}-4-phenyl-3-propionylisoquinolin-1(2*H*)-one (20a)

To a suspension of NaH (60% dispersion) (2.64 g, 66 mmol) in DMF (100 ml) was added dropwise a solution of **2h** (26.3 g, 60 mmol) in DMF (350 ml) at 0 °C and the mixture was stirred at that temperature for 30 min. To the resulting mixture a solution of chloroacetone (7.18 ml, 90 mmol) in DMF (50 ml) was added dropwise at 0 °C. After being stirred at room temperature overnight, the solvent was evaporated under reduced pressure and the residue was partitioned between 1 N hydrochloric acid and AcOEt. The organic layer was washed with saturated sodium hydrogen carbonate and brine and dried over sodium sulfate. The solvent was evaporated under reduced pressure and the obtained residue was purified by silica gel column chromatography (hexane/AcOEt) to give crude mixture of **19a**, which was dissolved in dichloromethane (200 ml). To the solution was added dropwise a solution of *m*-chloroperbenzoic acid (65%) (33.3 g, 144.6 mmol) in dichloromethane (400 ml) at 0 °C, and the mixture was stirred at room temperature for 3 h. To the resulting mixture was added dropwise 10% aqueous solution of sodium thiosulfate (500 ml) at 0 °C, and stirred at room temperature for 1 h. The resulting mixture was extracted with dichloromethane, and the organic layer was washed with saturated sodium hydrogen carbonate and brine, and dried over sodium sulfate. The solvent was evaporated under reduced pressure and the obtained residue was purified by silica gel column chromatography (hexane/AcOEt) to give **20a** (13.8 g, 44%) as colorless crystals: mp 192–194 °C; $^1$H NMR ($CDCl_3$) $\delta$: 0.62 (3H, t, $J$ = 7.1 Hz), 1.94 (2H, q, $J$ = 7.1 Hz), 2.24 (3H, s), 3.11 (3H, s), 5.23–5.34 (4H, m), 7.00 (1H, s), 7.24–7.30 (3H, m), 7.43–7.53 (4H, m), 8.46 (1H, d, $J$ = 8.7 Hz); LC/MS (ESI$^+$) 526, 528; Anal. calcd for $C_{26}H_{24}ClN_3O_5S$: C, 59.37; H, 4.60; N, 7.99. Found: C, 59.26; H, 4.60; N,8.02.

### 6.39. *tert*-Butyl-[3-[(6-chloro-1-oxo-4-phenyl-3-propionylisoquinolin-2(1*H*)-yl)methyl]-5-(methylthio)-1*H*-pyrazol-1-yl]acetate (19b)

To a suspension of NaH (60% dispersion) (876 mg, 22 mmol) in DMF (20 ml) was added **2h** (1.11 g, 2 mmol) at 0 °C and the mixture was stirred at that temperature for 30 min. To the resulting mixture was added *tert*-butyl bromoacetate (0.44 ml, 3 mmol) at 0 °C. After being stirred at room temperature overnight, the solvent was evaporated under reduced pressure, and the residue was partitioned between 1 N hydrochloric acid and AcOEt. The organic layer was washed with saturated sodium hydrogen carbonate and brine and dried over sodium sulfate. The solvent was evaporated under reduced pressure, and the obtained residue was purified by silica gel column chromatography (hexane/AcOEt) to give **19b** (576 mg, 52%) as colorless crystals: $^1$H NMR ($CDCl_3$) $\delta$: 0.59 (3H, t, $J$ = 7.0 Hz), 1.42 (9H, s), 1.97 (2H, q, $J$ = 7.0 Hz), 2.34 (3H, s), 4.76 (2H, s), 5.31 (2H, s), 6.40 (1H, s), 7.19–7.33 (3H, m), 7.39–7.51 (4H, m), 8.48 (1H, d, $J$ = 8.7 Hz).

### 6.40. 2-[3-[(6-Chloro-1-oxo-4-phenyl-3-propionylisoquinolin-2(1*H*)-yl)methyl]-5-(methylsulfonyl)-1*H*-pyrazol-1-yl]acetamide (20b)

To a solution of **19b** (560 mg, 0.96 mmol) in dichloromethane (10 ml) was added *m*-chloroperbenzoic acid (65%) (1.38 mg, 2.88 mmol) at 0 °C, and the mixture was stirred at room temperature for 2 h. To the resulting

mixture was added 10% aqueous solution of sodium thiosulfate (20 ml), and stirred at room temperature for 30 min. The resulting mixture was extracted with dichloromethane, and the organic layer was washed with brine, and dried over sodium sulfate. The solvent was evaporated under reduced pressure, and the obtained residue was purified by silica gel column chromatography (3:2 to 7:3 hexane/AcOEt). To the obtained residue, a 4 N solution of hydrochloric acid in AcOEt (20 ml) was added at 0 °C and the mixture was stirred at room temperature for 3 h. The solvent was evaporated under reduced pressure, toluene (10 ml) was added, and the mixture was re-concentrated. To a solution of obtained residue in THF (5 ml) were added oxalyl chloride (0.11 ml, 1.3 mmol) and DMF (1 drop), and the mixture was stirred at room temperature for 1 h. The solvent was evaporated under reduced pressure, toluene (10 ml) was added, and the mixture was re-concentrated. The residue was dissolved in dichloromethane (10 ml) and saturated ammonia aqueous solution (5 ml) was added. After being stirred at room temperature for 1 h, water was added, and the residue was extracted with dichloromethane. The organic layer was washed with brine and dried by adding sodium sulfate. The solvent was evaporated under reduced pressure, and the obtained residue was purified by silica gel column chromatography (hexane/AcOEt) to give **20b** (816 mg, 16%) as colorless crystals: mp 247–248 °C; $^1$H NMR ($CDCl_3$) $\delta$: 0.66 (3H, t, $J$ = 7.1 Hz), 2.04 (2H, q, $J$ = 7.1 Hz), 3.21 (3H, s), 5.16 (2H, s), 5.26 (2H, s), 5.54 (1H, s), 6.03 (1H, s), 7.00 (1H, s), 7.24–7.30 (3H, m), 7.44–7.53 (4H, m), 8.45 (1H, d, $J$ = 8.7 Hz); LC/MS ($ESI^+$) 527, 529; Anal. calcd for $C_{25}H_{23}ClN_4O_5S$: C, 56.98; H, 4.40; N, 10.63. Found: C, 56.94; H, 4.35; N,10.67.

### 6.41. 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 [$\gamma$ – $^{32}$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. To clarify the inhibitory mode of **1**, the $IC_{50}$ value was determined at each of ATP concentrations of 0.05, 0.5, 5 and 50 μM.

### 6.42. 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-1 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.

### 6.43. 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 22 G 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. The injection needle was then 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. Test 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 atrium 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%.

### 6.44. Statistical analysis

Data are expressed and presented as means ± SE. 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.

### 6.45. 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, and multiple reaction monitoring mode.

### 6.46. Crystallization and structure determination

JNK3 was cloned and purified following the published protocol.[7] Compound **2d** was incubated with the enzyme with a 3-fold 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:AMP-PNP complex. Crystals were then 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: 2ZDT 2ZDU).

## References and notes


1. Gupta, S.; Barrett, T.; Whitmarsh, A. J.; Cavanagh, J.; Sluss, H. K.; Derijard, B.; Davis, R. J. *EMBO J.* **1996**, *15*, 2760–2770.
2. (a) 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; (b) 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; (c) Stocks, M. J.; Barber, S.; Ford, R.; Leroux, F.; St-Gallay, S.; Teague, S.; Xue, Y. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3459–3462; (d) Liu, G.; Xin, Z.; Liu, M.; Wang, S.; Clampit, J. E.; Gum, R. J.; Haasch, D. L.; Trevillyan, J. M.; Sham, H. L.; Abad-Zapatero, C.; Fry, E. H. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2590–2594; (e) Liu, G.; Zhao, H.; Liu, B.; Xin, Z.; Liu, M.; Kosogof, C.; Szczepankiewicz, B. G.; Wang, S.; Clampit, J.; Gum, R. J.; Haasch, D. L.; Trevillyan, J. M.; Sham, H. L. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5723–5730; (f) Zhao, H.; Serby, M. D.; Xin, Z.; Szczepankiewicz, B. G.; Liu, M.; Kosogof, C.; Liu, B.; Nelson, L. T. J.; Johnson, E. F.; Wang, S.; Pederson, T.; Gum, R. J.; Clampit, J. E.; Haasch, D. L.; Abad-Zapatero, C.; Fry, E. H.; Rondinone, C. M.; Trevillyan, J. M.; Sham, H. L.; Liu, G. *J. Med. Chem.* **2006**, *49*, 4455–4458; (g) Szczepankiewicz, B. G.; Kosogof, C.; Nelson, L. T. J.; Liu, G.; Liu, B.; Zhao, H.; Serby, M. D.; Xin, Z.; Liu, M.; Gum, R. J.; Haasch, D. L.; Wang, S.; Clampit, J. E.; Johnson, E. F.; Lubben, T. H.; Stashko, M. A.; Olejniczak, E. T.; Sun, C.; Dorwin, S. A.; Haskins, K.; Abad-Zapatero, C.; Fry, E. H.; Hutchins, C. W.; Sham, H. L.; Rondinone, C. M.; Trevillyan, J. M. *J. Med. Chem.* **2006**, *49*, 3563–3580.
3. (a) Choukron, G.; Hajjar, R.; Fry, S.; Monte, F. D.; Haq, S.; Guerrero, J. L.; Picard, M.; Rosenzweig, A.; Force, T. *J. Clin. Invest.* **1999**, *104*, 391–398; (b) Gong, X.; Petrich, B. G.; Liao, P.; Wang, Y. *Circulation* **2000**, *102*, 216–217; (c) Petrich, B. G.; Gong, X.; Lerner, D. L.; Wang, X.; Brown, J. H.; Saffitz, J. E.; Wang, Y. . *Circ. Res.* **2002**, *91*, 640–647; (d) 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; (e) Cook, S. A.; Sugden, P. H.; Clerk, A. *J. Mol. Cell. Cardiol.* **1999**, *31*, 1429–1434.
4. Yamagami, C.; Ogura, T.; Takao, N. *J. Chromatogr.* **1990**, *514*, 123–136.
5. Natsugari, H.; Tawada, H.; Ikeda, H. U.S. Patent 198462, 1993.
6. (a) Lee, H. T.; O'Brian, P. M.; Picard, J. A.; Purchase, C. F., Jr.; Roth, B. D.; Sliskovic, D. R.; White, A. D. EP 1203767, 2002; (b) Miyake, H.; Take, K.; Shigenaga, S.; Azami, H.; Sasaki, H.; Eikyu Y.; Nakai, K.; Ishida, J.; Manabe, T.; Konishi, N.; Terasaka, T. WO 1998/57954, 1998; (c) Mahata, Pranab K.; Syam Kumar, U. K.; Sriram, V.; Ila, H.; Junjappa, H. *Tetrahedron* **2003**, *59*, 2631–2640.
7. 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.