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# Quinolone derivatives containing strained spirocycle as orally active glycogen synthase kinase 3β (GSK-3β) inhibitors for type 2 diabetics

Shigeki Seto [a,*], Kazuhiko Yumoto [a], Kyoko Okada [a], Yoshikazu Asahina [a], Aya Iwane [a], Maki Iwago [a], Reiko Terasawa [a], Kevin R. Shreder [b], Koji Murakami [a], Yasushi Kohno [a]

[a] Discovery Research Laboratories, Kyorin Pharmaceutical Co., Ltd, 2399-1, Nogi, Nogi-machi, Shimotsuga-gun, Tochigi 329-0114, Japan
[b] ActivX Biosciences, Inc., 11025 N. Torrey Pines Road, La Jolla, CA 92037, USA

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ABSTRACT

The design, synthesis, and evaluation of 6-6-7 tricyclic quinolones containing the strained spirocycle moiety aiming at the GSK-3β inhibitor were described. Among the synthesized compounds, **44**, having a cyclobutane ring on a spirocycle, showed excellent GSK-3β inhibitory activity in both cell-free and cell-based assays ($IC_{50}$ = 36 nM, $EC_{50}$ = 3.2 μM, respectively). Additionally, **44** decreased the plasma glucose concentration dose-dependently after an oral glucose tolerance test in mice.



## 1. Introduction

Glycogen synthase kinase 3 (GSK-3) is a serine/threonine protein kinase having two isoforms, GSK-3α and GSK-3β.[1] GSK-3 phosphorylates and inactivates glycogen synthase (GS), the late-limiting enzyme of glycogen formation.[2] Insulin has been demonstrated to cause inactivation of GSK-3, resulting in GS activation and the subsequent formation of glycogen.[3] In type 2 diabetics, liver and muscle glycogen synthesis is decreased, while GSK-3 expression and activity are increased.[4] Hence, the identification of small molecules aimed at GSK-3 inhibitors has become a focus of research for pharmaceutical companies during the past decade. For example, CHIR-99021 (**1**) displayed potent GSK-3 inhibitory activity and in vivo effects in Zucker diabetic fatty rats.[5]

In a previous communication, we described the discovery of quinolone derivative **2**, which shows not only potent GSK-3β inhibitory activity in a cell-free assay ($IC_{50}$ = 12 nM), but also has selective inhibitory activity for GSK-3β against 90 kinases (Fig. 1).[6] To further biologically evaluate the series derivatives, we evaluated the cellular effect of **2** by measuring glycogen synthesis activity in Hep G2 cells and found that **2** is less effective than **1** ($EC_{50}$ = 20 and 1.5 μM, respectively) in cell-based assays, perhaps because of insufficient cell permeability. We therefore focused next on an exploration of a potent compound in both cell-free and cell-based assays by structural modification of **2**. As an initial step, we tried to fit together compounds **1** and **2** as shown in Figure 2[7], and we

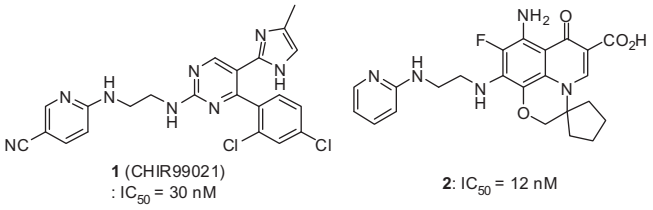


**Figure 1.** Structures of known GSK-3β inhibitors.

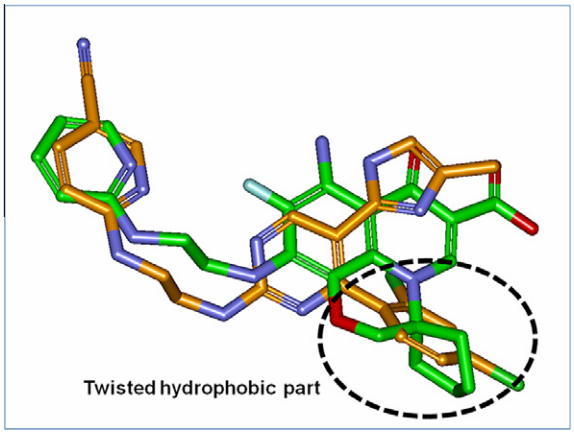


**Figure 2.** Structural comparison between **1** and **2**. Yellow shows **1** and green shows **2**.

* Corresponding author.
*E-mail address:* shigeki.seto@mb.kyorin-pharm.co.jp (S. Seto).

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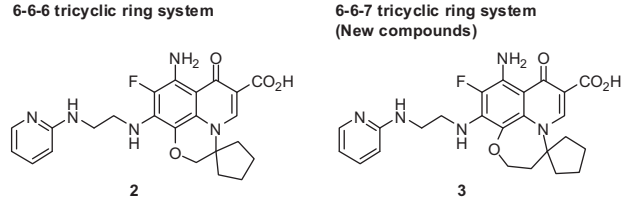



| | | |
|---|---|---|
| Cell-free | $IC_{50}$ = 12 nM | $IC_{50}$ = 250 nM |
| Cell-based | $EC_{50}$ = 20 μM | $EC_{50}$ = 13 μM |

**Figure 3.** Comparison between 6-6-6 and 6-6-7 tricyclic ring system.

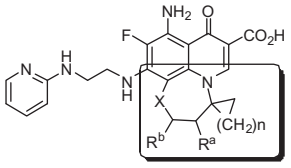


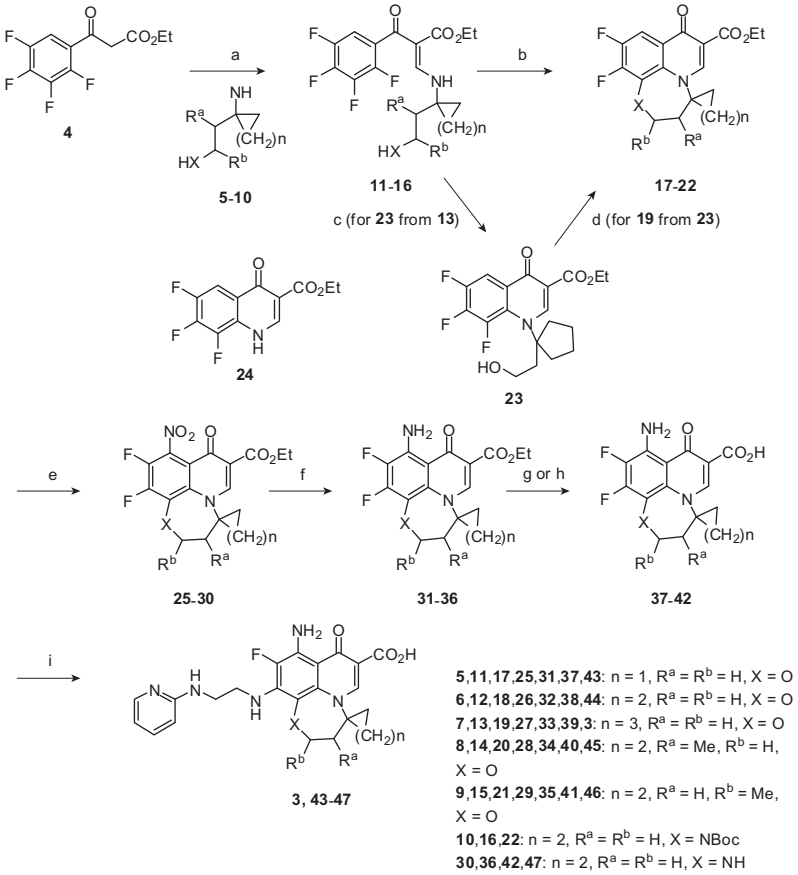
**Figure 4.** Design of 6-6-7 tricyclic quinolones having the strained spirocycle.

hypothesized that the bulkiness of the spirocycle moiety of **2**, which can overlap with the 2,4-dichlorophenyl moiety of **1**, could affect the GSK-3β inhibitory activity and cell permeability. As a preliminary study, we synthesized **3** having the 6-6-7 tricyclic ring system to compare with **2** as shown in Figure 3. Compound **3** having the 6-6-7 ring system is less active than compound **2** having the 6-6-6 ring system in the cell-free assay ($IC_{50}$ = 250 and 12 nM, respectively), whereas both compounds show almost the same activity in the cell-based assay ($EC_{50}$ = 13 and 20 μM, respectively). This result led us to perform a further optimization study using derivatives having the 6-6-7 ring system with spirocycles to obtain more cellular efficacious compounds, as shown in Figure 4. In addition, the synthesis of the spirocycle containing 6-6-7 tricyclic quinolones has not been reported as far as we know.

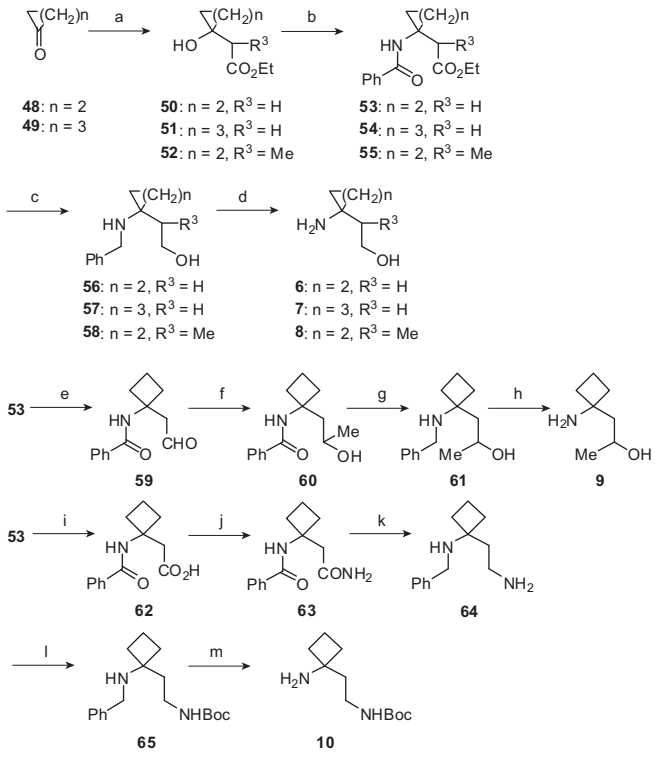
Herein, we report the synthesis and biological evaluation of novel 6-6-7 tricyclic quinolones with the strained spirocycles. Additionally, we show the in vivo result of a selected compound for an antidiabetic agent.

## 2. Chemistry

The synthesis of spirocycles **3, 43–47** is shown in Scheme 1. Grohe-heiter reaction[8,9] was used for the synthesis of the tetracyclic intermediates **17–22**. Condensation of ketoester **4** with triethylorthoformate, followed by treatment with aminoalcohols **5–9** or diamine **10**, afforded cyclization precursors **11–16**. The one-pot tandem cyclization reactions of **11–16** proceeded well except for that of **13**. Treatment of the precursors **11, 12, 14–16** with sodium hydride in DMF at 90 °C afforded spirocycles **17, 18, 20–22** in good to moderate yield. However, in the case of **13**, bicyclic compound **24** was the only product that was produced by the loss of cyclopentylethanol moiety when one-pot tandem cyclization reaction was




**Scheme 1.** Reagent and conditions: (a) (**1**) $HC(OEt)_3$, AcO, 120 °C, 3 h. (2) **5–10**, rt, 2 h, 68% (for **11**), 61% (for **12**), 75% (for **13**), 67% (for **14**), 83% (for **15**), 93% (for **16**); (b) NaH, DMF, rt, 1 h, then 90 °C, 1 or 0.5 h, 61% (for **17**), 26% (for **18**), 80% (for **20**), 67% (for **21**), 68% (for **22**); (c) NaH, DMF, rt, 1 h, 65% (d) NaH, DMF, 80 °C, 0.5 h, 31%; (e) $KNO_3$, $H_2SO_4$, 0 °C, 1 h, 41% (for **25**), 79% (for **26**), 46% (for **27**), 82% (for **28**), 93% (for **29**), 68% (for **30**); (f) 10% Pd-C, $H_2$, DMF, 50 °C, 1.5 h, 77% (for **31**), 79% (for **32**), 28% (for **33**), 91% (for **34**), 79% (for **35**), 88% (for **36**); (g) $H_2SO_4$, AcOH, $H_2O$, 100 °C, 1 h, 93% (for **37**), 90% (for **38**), 96% (for **39**), 98% (for **40**), 99% (for **41**); (h) NaOH, EtOH, 50 °C, 3 h, 83%; (i) *N*-2-pyridinyl-1,2-ethanediamine, $Et_3N$, DMSO, 100 °C, 2 h, 47% (for **43**), 66% (for **44**), 69% (for **3**), 44% (for **45**), 59% (for **46**), 64% (for **47**).



**Scheme 2.** Reagent and conditions: (a) $BrCH_2CO_2Et$ or $BrCH(Me)CO_2Et$, Zn, $Et_2O$, rt, 1 h, 54% (for **50**), 99% (for **51**), 100% (for **52**); (b) PhCN, *c*-$H_2SO_4$, 80 °C, 1 h, 51% (for **53**), 42% (for **54**), 46% (for **55**); (c) $LiAlH_4$, THF, reflux, 5 h, 99% (for **56**), 91% (for **57**), 96% (for **58**); (d) 10% Pd–C, $H_2$, EtOH, 5 kgf/cm$^2$, rt, 5 h, 73% (for **6**), 77% (for **7**), 74% (for **8**); (e) DIBAL-H, THF, −78 °C, 0.5 h, 32%; (f) MeMgCl, THF, rt, 5 h, 87%; (g) $LiAlH_4$, THF, reflux, 0.5 h, 91%; (h) 10% Pd-C, $H_2$, EtOH, 5 kgf/cm$^2$, rt, 5 h, 94%; (i) 3 N KOH, EtOH, rt, 1 h, 91%; (j) (**1**) $SOCl_2$, rt, 1 h. (2) 30% aq $NH_3$, THF, rt, 1 h, 93%; (k) $LiAlH_4$, THF, reflux, 5 h, 99%; (l) $Boc_2O$, 0 °C, 0.5 h, 54%; (m) 10% Pd-C, $H_2$, EtOH, 5 kgf/cm$^2$, rt, 5 h, 92%.

attempted. To diminish the yield of **24**, we tried a stepwise procedure. The intermediate **23** was isolated after the first cyclization reaction of **13** and then treated with sodium hydride in DMF at 80 °C in a short time to afford **19** with a minimum amount of undesired **24**.

As the spirocycles **17–22** were in hand, the nitration and subsequent hydrogenation of the nitrogroup and the hydrolysis of the ester group afforded carboxylic acid **37–42**. Finally, the nucleophilic displacement reaction of **37–42** with *N*-2-pyridinyl-1,2-ethanediamine afforded the target compounds **3, 43–47**.

Scheme 2 shows the preparation of the intermediate aminoalcohols **6–9** and *N*-Boc-protected diamine **10**. Reformatsky reaction[10] of ketones **48** and **49** with α-bromoacetates provided β-hydroxy esters **50–52**. Subsequent Ritter reaction[11] of **50–52** with cyanobenzene gave *N*-benzoyl-3-amino acid esters **53–55**. Reduction of **53–55** with $LiAlH_4$, followed by hydrogenation with Pd–C, afforded corresponding aminoalcohols **6–8**.

The aminoalcohol **8** was prepared in four steps from the intermediate **53**. The methyl group was elongated via the reduction of **53** using DIBAL-H and subsequent methylation of aldehyde **59** with methyl magnesium chloride. Treatment of **60** in the same procedure with the synthesis of **6–8** afforded the desired aminoalcohol **9**.

The intermediate **53** was also used to prepare *N*-Boc-protected diamine **10**. *N*-benzyl-protected diamine **64** was formed in three steps via conversion to amide **63** though carboxylic acid **62**, followed by the reduction of two amide groups of **63**. Finally, *N*-Boc protection of **64** and subsequent debenzylation afforded *N*-Boc-protected diamine **10**.

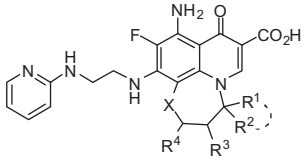
## 3. Results and discussion

### 3.1. GSK-3β inhibitory activity in cell-free and cell-based assays

Table 1 shows the results of the effects of newly synthesized compounds **3, 43–47** on cell-free GSK-3β kinase assay using recombinant full-length human GSK-3β and a cell-based assay using Hep G2 cells, together with the control compound **1**. A cell-free GSK-3β kinase assay detects inhibitors that interact directly with the polypeptide GSK-3β, while a cell-based assay can detect the ability on glycogen synthesis. Table 1 also shows anti-microbial activity to select a compound with minimal anti-bacterial activity that may not be desirable for GSK-3β inhibitors.[6]

To examine the optimal size of the ring connected with a tricyclic core, we examined the potency in terms of cell-free and cell-based assays of compounds **3**, **43**, **44**. Compared to compound

**Table 1**
Spirocycles SAR



| Compd | $R^1$ | $R^2$ | $R^3$ | $R^4$ | X | GSK-3β | | | Anti-bacterial activity: MIC (μM) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Cell-free assay | Cell-based assay | | | |
| | | | | | | $IC_{50}$ (nM) | $EC_{50}$[a] (μM) | Efficacy[b] (%) | *E. coli* | *S. aureus* |
| 3 | $-CH_2CH_2CH_2CH_2-$ | | H | H | O | 250 | 13 | 85 | >34 | >34 |
| **44** | $-CH_2CH_2CH_2-$ | | H | H | O | 36 | 3.2 | 65 | >34 | 18 |
| **43** | $-CH_2CH_2-$ | | H | H | O | 37 | —[c] | 42 | 9 | 5 |
| **45** | $-CH_2CH_2CH_2-$ | | Me | H | O | 10 | 1.4 | 48 | >34 | >34 |
| **46** | $-CH_2CH_2CH_2-$ | | H | Me | O | 250 | 2.1 | 94 | >34 | >34 |
| **47** | $-CH_2CH_2CH_2-$ | | H | H | NH | 550 | | 12 | >140 | >140 |
| CHIR99021 (**1**) | | | | | | 30 | 1.5 | 100 | —[d] | —[d] |

[a] $EC_{50}$ values were calculated when the efficacy showed more than 50. $EC_{50}$ values were the molar concentration of the test compounds that cause 50% of the efficacy. $n = 3$.
[b] Efficacy values were calculated as percentage of **1** (CHIR99021) at the dosage of 30 μM.
[c] Not certain.
[d] Not tested.

**3** with a cyclopentane ring, compounds **44** and **43**, having a cyclobutane ring and a cyclopropane ring, respectively, showed potent activity in the cell-free assay (**3**, $IC_{50}$ = 250 nM; **44**, $IC_{50}$ = 36 nM; **43**, $IC_{50}$ = 37 nM). As expected, in the cell-based assay, **44** showed more potent activity than **3**.

Next, we investigated the influence of the substitution of a methyl group on the seven-membered ring using compounds **45** and **46**, and the replacement of oxygen with nitrogen using **47**. Compared to the parent compound **44**, one regioisomer **45** showed 2- to 3-fold improved potency in both the cell-free and cell-based assays, while another regioisomer, **46**, exhibited weak potency in the cell-free assay. The replacement of oxygen with nitrogen was also disfavored, as demonstrated by comparing **44** and **47**.

With regard to antibacterial activity, the ring size of the spirocycle seems to be important. Compound **43**, having a cyclopropane ring, showed antibacterial activity with MIC values of 9 μM and 5 μM (*Escherichia* coli and *Staphylococcus aureus*, respectively). In contrast, other derivatives that had larger ring sizes than the cyclopropane ring showed no antibacterial activity.

Thus, we selected the cyclobutane derivatives **44** (cell-free, $IC_{50}$ = 36 nM; cell-based, $EC_{50}$ = 3.2 μM) and **45** (cell-free, $IC_{50}$ = 10 nM; cell-based, $EC_{50}$ = 1.4 μM) for further evaluation of their pharmacokinetic and pharmacological properties.

### 3.2. Pharmacokinetic and pharmacological study

The pharmacokinetic (PK) characteristics of **44**, **45**, and **1** at a dose of 300 mg/kg in ICR mice are shown in Table 2. The area under the curve (AUC) of **44** was 32-fold greater in liver than in plasma (760 and 24 μM h, respectively). A similar ratio was observed in the liver and plasma AUC of **45** (217 and 6.8 μM h, respectively), although these values were 3.5-fold smaller in **45** than in **44**. In contrast, **1** showed very large AUC in plasma, but the hepatic uptake of **1** was extremely low, 3.8 of the liver/plasma concentration ratio. Consequently, the AUC in liver of both **44** and **1** were almost the same (760 and 820 μM h, respectively).

Table 2 also shows the reducing effects on plasma glucose concentration after the oral glucose tolerance test (OGTT) at a dose of 300 mg/kg in ICR mice. Interestingly, the inhibition of the plasma glucose concentration was accompanied by the increase in liver AUC. Both **44** and **1** displayed very high liver AUC, and they also showed significantly lower plasma glucose concentrations than in control animals after the OGTT (31% and 38%, respectively).

Figure 5 shows the dose effects of **44** on OGTT. Compound **44** reduced the plasma glucose level dose-dependently by 20%, 32%, 39%, and 38% when **44** was treated at doses of 50, 100, 150, and 300 mg, respectively.

## 4. Conclusions

We successfully designed and synthesized a series of novel 6-6-7 tricyclic quinolones containing the strained spirocycle moiety aiming at the GSK-3β inhibitor. Among the synthesized compounds, **44**, having a cyclobutane ring on the spirocycle, showed excellent GSK-3β inhibitory activity in both cell-free and cell-based assays. Additionally, **44** showed a great reducing effect of plasma glucose concentration dose-dependently after the oral glucose tolerance test (OGTT). Further biological data on **44** will be reported elsewhere.

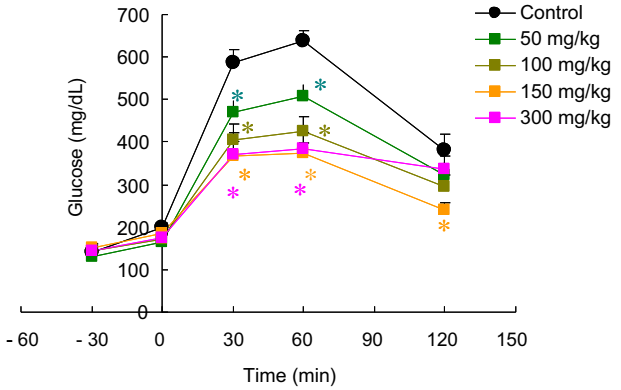



**Figure 5.** OGTT results. ICR mice were fasted overnight and treated with vehicle control or the indicated dosage of compound **44**, 30 min prior to the oral glucose tolerance test, using 5 g/kg body wt glucose feeding. Data are shown as means ± S.E. and $\Sigma Glucose_{30-60}$ inhibition %. $n$ = 5-6. $^{*}p$ <0.05 versus control.

## 5. Experimental section

### 5.1. Chemistry

Melting points were determined with a Yamato MP-500 melting point apparatus and are uncorrected. $^{1}H$ NMR spectra were measured in $CDCl_3$ or DMSO-$d_6$ with TMS and the solvent peak as internal standards, on a JEOL ECA-400 (400 MHz) spectrometer. Mass spectra (MS) were obtained on a Hitachi M-2000 mass spectrometer. Column chromatography was carried out on Merck silica gel 60. Analytical thin-layer chromatography (TLC) was performed on Merck precoated silica gel 60F254 plates, and the compounds were visualized by UV illumination (254 nm) or by heating after spraying with phosphomolybdic acid in ethanol. The data for elemental analysis were within ±0.4% of the theoretical values and were determined by a Yanaco CHN corder MT-5.

#### 5.1.1. Ethyl 3-{[1-(2-hydroxyethyl)cyclopropyl]amino}-2-(2,3,4,5,-tetrafluorobenzoyl)acrylate (11)

A solution of **4** (3.09 g, 11.7 mmol), $Ac_2O$ (6.7 mL, 70.9 mmol), and triethyl orthoformate (3.90 mL, 23.5 mmol) was heated at 120 °C for 3 h. The mixture was concentrated in vacuo and dried under high vacuum. The crude product was dissolved in anhydrous toluene (40 mL), and **5**[12] (1.82 g, 11.7 mmol) was added very slowly at 0 °C. The reaction mixture was stirred at room temperature for 5 h and diluted with toluene. The organic layer was

**Table 2**
PK profile and in vivo screening

| Compd | PK profile po[a] | | | OGTT[a] |
|---|---|---|---|---|
| | $AUC_{0-25\ h}$ (μM h) | Liver/plasma concentration ratio[b] | Liver AUC[c] (μM h) | Glucose inhibition (%) |
| **44** | 24 | 32 | 760 | 31 |
| **45** | 6.8 | 32 | 217 | 13 |
| 1 (CHIR99021) | 210 | 3.8 | 820 | 38 |

[a] ICR mice were treated with 300 mg/kg of the tested compound. Data are shown as means. $n$ = 5–6.
[b] Time point: 4 h.
[c] Liver AUC was calculated based on AUC in plasma and the concentration ratio of liver to plasma at 4 h after po administration.

washed with water and brine, and then was dried. The solvent was removed by evaporation, and the crude material was purified by flash column chromatography on silica gel (hexane:EtOAc = 2:1 to 1:1) to give **11** as a yellow solid (2.99 g, 68%).

Mp: 75–78 °C. $^1$H NMR (400 MHz, $CDCl_3$)$\delta$ 0.85–0.92 (2H, m), 0.94–1.11 (5H, m), 1.85–1.91 (2H, m), 3.85–3.89 (2H, m), 3.99–4.09 (2H, m), 6.95–7.13 (1H, m), 8.19 (1H, d, $J$ = 14.1 Hz), 9.84 and 11.2 (total 1H, each d, $J$ = 13.4 Hz). HRMS (EI) for $C_{17}H_{17}F_4NO_4$ ($M^+$): calcd, 375.1094; found, 375.1094.

### 5.1.2. Ethyl 3-{[1-(2-hydroxyethyl)cyclobutyl]amino}-2-(2,3,4,5-tetrafluorobenzoyl)acrylate (12)

A stirred solution of **4** (3.67 g, 13.9 mmol), $Ac_2O$ (7.89 mL, 83.5 mmol), and triethyl orthoformate (4.63 mL, 27.8 mmol) was heated at 120 °C for 3 h. The mixture was concentrated in vacuo and dried under high vacuum. To the mixture of the residue in anhydrous toluene (50 mL), was added **6** (1.60 g, 13.9 mmol) in anhydrous toluene (20 mL) very slowly at 0 °C and stirred at room temperature for 2 h. The solvent was removed by evaporation. The crude material was purified by flash column chromatography on silica gel (AcOEt:hexane = 1:1) to give **12** as a pale yellow solid (3.30 g, 61%).

Mp: 90–93 °C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 0.96 (0.6H, t, $J$ = 7.3 Hz), 1.09 (2.4H, t, $J$ = 7.3 Hz), 1.85–2.03 (12H, m), 2.06–2.13 (2H, m), 2.20–2.30 (2H, m), 2.32–2.43 (2H, m), 3.82–3.88 (2H, m), 4.02 (0.4H, q, $J$ = 7.3 Hz), 4.07 (1.6H, q, $J$ = 7.3 Hz), 6.94–7.03 (0.2H, m), 7.05–7.13 (0.8H, m), 8.23–8.30 (1H, m), 10.05–10.17 (0.2H, m), 11.36–11.50 (0.8H, m). HRMS (EI) for $C_{18}H_{19}F_4NO_4$ ($M^+$): calcd, 389.1250; found, 389.1291.

### 5.1.3. Ethyl 3-{[1-(2-hydroxyethyl)cyclopentyl]amino}-2-(2,3,4,5-tetrafluorobenzoyl)acrylate (13)

A stirred solution of **4** (20.3 g, 76.8 mmol), $Ac_2O$ (44.0 mL, 0.465 mol), and triethyl orthoformate (25.6 mL, 0.154 mol) was heated at 120 °C for 3 h. The mixture was concentrated in vacuo and dried under high vacuum. To the mixture of the residue in anhydrous toluene (200 mL), was added **7** (9.94 g, 76.9 mmol) in anhydrous toluene (50 mL) very slowly at 0 °C and stirred at room temperature for 2 h. The solvent was removed by evaporation. The crude material was purified by flash column chromatography on silica gel (AcOEt:hexane = 2:1) to give **13** as a colorless solid (23.2 g, 75%).

Mp: 67–70 °C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 0.96 (0.6H, t, $J$ = 7.3 Hz), 1.09 (2.4H, t, $J$ = 7.3 Hz), 1.48–1.54 (1H, m), 1.74–1.90 (6H, m), 1.97–2.06 (4H, m), 3.78–3.85 (2H, m), 3.98–4.10 (2H, m), 6.95–7.12 (1H, m), 8.17–8.24 (1H, m), 9.81–9.94 (0.3H, m), 11.20–11.35 (0.7H, m). HRMS (ESI$^+$) for $C_{19}H_{22}F_4NO_4$ [M+H]$^+$: calcd, 404.14850; found, 404.14870. Anal. calcd for $C_{19}H_{21}F_4NO_4$ $0.2H_2O$: C, 56.07; H, 5.20; N, 3.44. Found: C, 55.92; H, 5.05; N, 3.37.

### 5.1.4. Ethyl 3-{[1-(1- hydroxypropane-2-yl)cyclobutyl]amino}-2-(2,3,4,5-tetrafluorobenzoyl)acrylate (14)

A stirred solution of **4** (6.13 g, 23.2 mmol), $Ac_2O$ (13.2 mL, 0.140 mol), and triethyl orthoformate (7.80 mL, 46.8 mmol) was heated at 120 °C for 3 h. The mixture was concentrated in vacuo and dried under high vacuum. To the mixture of the residue in anhydrous toluene (100 mL), was added **8** (3.00 g, 23.2 mmol) in anhydrous toluene (40 mL) very slowly at 0 °C and stirred at room temperature for 2 h. The solvent was removed by evaporation. The crude material was purified by flash column chromatography on silica gel (AcOEt:hexane = 1:1) to give **14** as a colorless solid (6.23 g, 67%).

Mp: 105–106 °C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 0.95 (0.6H, t, $J$ = 7.3 Hz), 0.97 (3H, d, $J$ = 7.3 Hz), 1.09 (2.4H, t, $J$ = 7.3 Hz), 1.54 (1H, t, $J$ = 4.9 Hz), 1.85–2.15 (3H, m), 2.19–2.50 (4H, m), 3.62–3.75 (2H, m), 4.02 (0.2H, q, $J$ = 7.3 Hz), 4.07 (0.8H, q, $J$ = 7.3 Hz), 6.97–7.13 (1H, m), 8.24 (0.8H, d, $J$ = 15.3 Hz), 8.25 (0.2H, d, $J$ = 15.3 Hz), 10.03 (0.2H, d, $J$ = 14.1 Hz), 11.40 (0.8H, d, $J$ = 14.1 Hz). HRMS (ESI$^+$) for $C_{19}H_{22}F_4NO_4$ [M+H]$^+$: calcd, 404.14850; found, 404.14862. Anal. calcd for $C_{19}H_{21}F_4NO_4$: C, 56.57; H, 5.25; N, 3.47. Found: C, 56.29; H, 5.12; N, 3.41.

### 5.1.5. Ethyl 3-{[1-(2- hydroxypropane-1-yl)cyclobutyl]amino}-2-(2,3,4,5-tetrafluorobenzoyl)acrylate (15)

A stirred solution of **4** (2.45 g, 9.27 mmol), $Ac_2O$ (5.30 mL, 56.1 mmol), and triethyl orthoformate (3.10 mL, 18.6 mmol) was heated at 120 °C for 3 h. The mixture was concentrated in vacuo and dried under high vacuum. To the mixture of the residue in anhydrous toluene (30 mL), was added **9** (1.20 g, 9.29 mmol) in anhydrous toluene (10 mL) very slowly at 0 °C and stirred at room temperature for 2 h. The solvent was removed by evaporation. The crude material was purified by flash column chromatography on silica gel (AcOEt:hexane = 1:1) to give **15** as a pale yellow solid (3.10 g, 83%).

Mp: 145–146 °C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 0.95 (0.6H, t, $J$ = 7.3 Hz), 1.09 (2.4H, t, $J$ = 7.3 Hz), 1.29 (3H, d, $J$ = 6.1 Hz), 1.51 (1H, br s), 1.83–2.05 (4H, m), 2.18–2.35 (3H, m), 2.41–2.53 (1H, m), 3.95–4.15 (3H, m), 6.96–7.13 (1H, m), 8.27 (0.8H, d, $J$ = 14.7 Hz), 8.31 (0.2H, d, $J$ = 14.7 Hz), 10.30 (0.2H, d, $J$ = 13.4 Hz), 11.56 (0.8H, d, $J$ = 13.4 Hz). HRMS (ESI$^+$) for $C_{19}H_{22}F_4NO_4$ [M+H]$^+$: calcd, 404.14850; found, 404.14759. Anal. calcd for $C_{19}H_{21}F_4NO_4$: C, 56.57; H, 5.25; N, 3.47. Found: C, 56.36; H, 5.04; N, 3.38.

### 5.1.6. Ethyl 3-{[1-(2-(tert-butoxycarbonylamino)ethyl)cyclobutyl]amino}-2-(2,3,4,5-tetrafluorobenzoyl)acrylate (16)

A stirred solution of **4** (5.28 g, 20.0 mmol), $Ac_2O$ (11.4 mL, 0.121 mol), and triethyl orthoformate (6.66 mL, 40.0 mmol) was heated at 120 °C for 3 h. The mixture was concentrated in vacuo and dried under high vacuum. To the mixture of the residue in anhydrous toluene (70 mL), was added **10** (4.29 g, 20.0 mmol) in anhydrous toluene (30 mL) very slowly at 0 °C and stirred at room temperature for 1 h. The solvent was removed by evaporation. The crude material was purified by flash column chromatography on silica gel (AcOEt:hexane = 2:1) to give **16** as a pale yellow oil (9.10 g, 93%).

$^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 0.98 (0.7H, t, $J$ = 7.3 Hz), 1.10 (2.3H, t, $J$ = 7.3 Hz), 1.43 (9H, s), 1.90–2.07 (4H, m), 2.17–2.38 (4H, m), 3.12–3.23 (2H, m), 4.00–4.15 (2H, m), 4.53–4.65 (1H, br), 6.95–7.03 (0.7H, m), 7.05–7.13 (0.3H, m), 8.13 (1H, d, $J$ = 14.1 Hz), 9.88 (0.3H, d, $J$ = 13.5 Hz), 11.29 (0.7H, d, $J$ = 13.5 Hz). HRMS (ESI$^+$) for $C_{18}H_{19}F_4NO_4$ [M+H]$^+$: calcd, 489.20126; found, 489.20200.

### 5.1.7. Ethyl 10′,11′-difluoro-2′,3′-dihydro-8′-oxo-spiro[cyclopropane-1,4′-[4*H*,8*H*]pyrido[1,2,3-*ef*][1,4]benzoxazepine]-7′-carboxylate (17)

A solution of NaH (617 mg, 15.4 mmol, 60% in oil) in DMF (30 mL) was cooled to 0 °C and treated dropwise with **11** (2.63 g, 7.01 mmol) in DMF (4 mL). The reaction mixture was stirred at room temperature for 1 h and for an additional hour at 80 °C. The reaction mixture was poured into ice water, and the resulting precipitate was removed by filtration and washed with water. The resulting solid was dissolved in 100 mL of EtOH and filtered. The filtrate was concentrated to 50 mL, and the resulting precipitate was removed by filtration, washed with EtOH, and then dried to give **17** as a pale brown solid (1.43 g, 61%).

Mp: 236–238 °C. $^1$H NMR (400 MHz, DMSO-$d_6$)$\delta$ 1.03 (2H, br), 1.18 (2H, br), 1.26 (3H, t, $J$ = 7.3 Hz), 2.03–2.73 (2H, br), 4.21 (2H, q, $J$ = 7.3 Hz), 7.66 (1H, dd, $J$ = 10.4, 7.9 Hz), 8.51 (1H, s). HRMS (EI) for $C_{17}H_{15}F_2NO_4$ ($M^+$): calcd, 335.0969; found, 335.0989. Anal calcd for $C_{17}H_{15}F_2NO_4$: C, 60.89; H, 4.51; N, 4.18; found: C, 60.83; H, 4.53; N, 4.16.

#### 5.1.8. Ethyl 10′,11′-difluoro-2′,3′-dihydro-8′-oxo-spiro[cyclobutane-1,4′-[4*H*,8*H*]pyrido[1,2,3-*ef*][1,4]benzoxazepine]-7′-carboxylate (18)

To an ice-cold solution of **12** (2.80 g, 7.19 mmol) in DMF (30 mL) was added NaH (350 mg, 8.75 mmol) and the mixture was stirred at room temperature for 0.5 h and the mixture was heated at 80 °C for 0.5 h. The mixture was treated portionwise at 0 °C with water and the resulting precipitate was combined by filtration, washed successively with water and then dried to give **18** as a colorless solid (655 mg, 26%).

Mp: 224–226 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 1.27 (3H, t, $J$ = 7.3 Hz), 1.67–1.79 (1H, m), 1.91–2.04 (1H, m), 2.42–2.53 (4H, m), 2.67 (2H, t, $J$ = 6.7 Hz), 4.24 (2H, q, $J$ = 7.3 Hz), 4.55 (2H, t, $J$ = 6.7 Hz), 7.72 (1H, dd, $J$ = 10.4 and 7.9 Hz), 8.41 (1H, s). HRMS (ESI$^+$) for $C_{18}H_{18}F_2NO_4$ [M+H]$^+$: calcd, 350.12039; found, 350.12110. Anal. calcd for $C_{18}H_{17}F_2NO_4$: C, 61.89; H, 4.91; N, 4.01. Found: C, 61.77; H, 4.94; N, 3.90.

#### 5.1.9. Ethyl 10′,11′-difluoro-2′,3′-dihydro-3′-methyl-8′-oxo-spiro[cyclobutane-1,4′-[4*H*,8*H*]pyrido[1,2,3-*ef*][1,4]benzoxazepine]-7′-carboxylate (20)

To an ice-cold solution of **14** (404 mg, 1.00 mmol) in DMF (4 mL) was added NaH (40 mg). After stirring at 0 °C for 1 h, the mixture was added NaH (40 mg). The mixture was stirred at 0 °C for 1 h, at room temperature for 1 h, at 70 °C for 1 h. The mixture was treated portionwise at 0 °C with water and the resulting precipitate was combined by filtration, washed successively with water and ethyl acetate, and then dried to give **20** as a pale yellow solid (289 mg, 80%).

Mp: 225–228 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 0.93 (3H, d, $J$ = 6.1 Hz), 1.27 (3H, t, $J$ = 7.3 Hz), 1.66–1.77 (1H, m), 1.85–2.00 (1H, m), 2.35 (1H, q, $J$ = 10.4 Hz), 2.53–2.69 (3H, m), 2.96–3.05 (1H, m), 3.82 (1H, t, $J$ = 11.6 Hz), 4.24 (2H, qd, $J$ = 7.3 and 2.4 Hz), 4.77 (1H, dd, $J$ = 12.9 and 8.9 Hz), 7.73 (1H, dd, $J$ = 10.4 and 8.6 Hz), 8.25 (1H, s). HRMS (ESI$^+$) for $C_{19}H_{20}F_2NO_4$ [M+H]$^+$: calcd, 364.13604; found, 364.13681. Anal. calcd for $C_{19}H_{19}F_2NO_4$ 0.8 $H_2O$: C, 60.41; H, 5.07; N, 3.71. Found: C, 60.16; H, 5.11; N, 3.69.

#### 5.1.10. Ethyl 10′,11′-difluoro-2′,3′-dihydro-2′-methyl-8′-oxo-spiro[cyclobutane-1,4′-[4*H*,8*H*]pyrido[1,2,3-*ef*][1,4]benzoxazepine]-7′-carboxylate (21)

To an ice-cold solution of **15** (404 mg, 1.00 mmol) in DMF (4 mL) was added NaH (40 mg). After stirring at 0 °C for 1 h, the mixture was added NaH (40 mg). The mixture was stirred at 0 °C for 1 h, at room temperature for 1 h, at 80 °C for 1 h. The mixture was treated portionwise at 0 °C with water and the resulting precipitate was combined by filtration, washed successively with water and $iPr_2O$, and then dried to give **21** as a colorless solid (244 mg, 67%).

Mp: 211–213 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 1.27 (3H, t, $J$ = 7.3 Hz), 1.43 (3H, d, $J$ = 6.1 Hz), 1.72 (1H, q, $J$ = 10.4 Hz), 1.90–2.04 (1H, m), 2.24 (1H, q, $J$ = 10.4 Hz), 2.34–2.43 (1H, m), 2.53–2.74 (4H, m), 4.24 (2H, q, $J$ = 7.3 Hz), 4.48–4.58 (1H, m), 7.72 (1H, qd, $J$ = 10.4 and 7.9 Hz), 8.40 (1H, s). HRMS (ESI$^+$) for $C_{19}H_{20}F_2NO_4$ [M+H]$^+$: calcd, 364.13604; found, 364.13588. Anal. calcd for $C_{19}H_{19}F_2NO_4$ 0.3$H_2O$: C, 61.88; H, 5.19; N, 3.80. Found: C, 61.87; H, 5.11; N, 3.85.

#### 5.1.11. Ethyl 1′-(tert-butoxycarbonyl)-10′,11′-difluoro-2′,3′-dihydro-8′-oxo-spiro[cyclobutane-1,4′-[4*H*,8*H*]pyrido[1,2,3-*ef*][1,4]benzodiazepine]-7′-carboxylate (22)

To an ice-cold solution of **16** (500 mg, 1.02 mmol) in DMF (5 mL) was added NaH (82.0 mg, 2.05 mmol) under iced-water cooling and the mixture was stirred on iced-water bath for 30 min and then at room temperature for 2 h. The mixture was treated portionwise at 0 °C with water and the resulting precipitate was combined by filtration, washed successively with water and diisopropyl ether and then dried to give **22** as a pale yellow solid (312 mg, 68%).

Mp: 237–238 °C. $^1$H NMR1H NMR (400 MHz, DMSO-$d_6$) $\delta$ 1.28 (3H, t, $J$ = 7.3 Hz), 1.42 (5H, s), 1.54 (4H, s), 1.67–1.80 (1H, m), 1.80–1.95 (1H, m), 2.10–2.22 (1H, m), 2.22–2.37 (2H, m), 2.40–2.56 (1H, m), 2.65–2.80 (2H, m), 4.17–4.37 (3H, m), 7.93–8.01 (1H, m), 8.47 (0.44H, s), 8.51 (0.56H, s). HRMS (ESI$^+$) for $C_{23}H_{27}F_2N_2O_5$ [M+H]$^+$: calcd, 449.18880; found, 449.18905. Anal. calcd for $C_{23}H_{26}F_2N_2O_5$: C, 61.60; H, 5.84; N, 6.25. Found: C, 61.61; H, 5.93; N, 6.21.

#### 5.1.12. Ethyl 1,4-dihydro-1-[1-(2-hydroxyethyl)cyclopropyl]-4-oxo-6,7,8-trifluoro-3-quinolinecarboxylate (23)

To an ice-cold solution of **13** (22.7 g, 56.3 mmol) in THF (200 mL) was added NaH (3.60 g, 90.0 mmol). The mixture was stirred at room temperature for 1 h. The mixture was treated portionwise at 0 °C with water. The resulting mixture was extracted with ethyl acetate. The combined extracts were concentrated in vacuo. The crude material was purified by flash column chromatography on silica gel (AcOEt:hexane = 2:1) to give **23** as a colorless solid (14.1 g, 65%).

Mp: 230–233 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 1.27 (3H, t, $J$ = 7.3 Hz), 1.53–1.75 (4H, m), 2.10–2.26 (4H, m), 2.31–2.44 (2H, m), 4.23 (2H, q, $J$ = 7.3 Hz), 4.55–4.60 (1H, m), 7.99–8.07 (1H, m), 8.80 (1H, s). HRMS (ESI$^+$) for $C_{19}H_{21}F_3NO_4$ [M+H]$^+$: calcd, 384.14227; found, 384.14170. Anal. calcd for $C_{19}H_{20}F_3NO_4$ 0.1$H_2O$: C, 59.25; H, 5.23; N, 3.64. Found: C, 59.17; H, 5.20; N, 3.61.

#### 5.1.13. Ethyl 10′,11′-difluoro-2′,3′-dihydro-8′-oxo-spiro[cyclopentane-1,4′-[4*H*,8*H*]pyrido[1,2,3-*ef*][1,4]benzoxazepine]-7′-carboxylate (19)

To a solution of **23** (3.84 g, 10.0 mmol) in DMF (40 mL) was added NaH (480 mg, 12.0 mmol), and the mixture was heated at 80 °C for 0.5 h. The mixture was treated portionwise at 0 °C with water. The resulting mixture was extracted with ethyl acetate. The combined extracts were concentrated in vacuo. The crude material was purified by flash column chromatography on silica gel (AcOEt:hexane = 2:1) to give **19** as a colorless solid (1.12 g, 31%).

Mp: 185–187 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 1.42 (3H, t, $J$ = 7.3 Hz), 1.52–1.64 (2H, m), 1.75–1.88 (2H, m), 2.11–2.21 (2H, m), 2.27–2.37 (2H, m), 2.47 (2H, t, $J$ = 6.7 Hz), 4.39 (2H, q, $J$ = 7.3 Hz), 4.47 (2H, t, $J$ = 6.7 Hz), 7.98 (1H, dd, $J$ = 9.8 and 7.9 Hz), 8.67 (1H, s). HRMS (ESI$^+$) for $C_{19}H_{20}F_2NO_4$ [M+H]$^+$: calcd, 364.13604; found, 364.13274. Anal. calcd for $C_{19}H_{19}F_2NO_4$: C, 62.80; H, 5.27; N, 3.85. Found: C, 62.52; H, 5.23; N, 3.83.

#### 5.1.14. Ethyl 10′,11′-difluoro-2′,3′-dihydro-9′-nitro-8′-oxo-spiro[cyclopropane-1,4′-[4*H*,8*H*]pyrido[1,2,3-*ef*][1,4]benzoxazepine]-7′-carboxylate (25)

A solution of **17** (1.2 g, 3.58 mmol) in concentrated $H_2SO_4$ (15 mL) was treated portionwise at 0 °C with solid $KNO_3$ (510 mg, 5.04 mmol). After stirring at 0 °C for 2 h, the reaction mixture was poured into ice-water and the resulting precipitate was removed by filtration. The resulting solid was washed with EtOH and dried to give **25** as a brown solid (554 mg, 41%).

Mp: 310–313 °C. $^1$H NMR (400 MHz, DMSO-$d_6$)$\delta$ 1.02 (2H, br), 1.10–1.27 (5H, m), 1.91–2.86 (2H, br), 4.21 (2H, q, $J$ = 7.3 Hz), 4.61 (2H, br), 4.52 (2H, br), 8.58 (1H, s). HRMS (EI) for $C_{17}H_{14}F_2N_2O_6$ (M$^+$): calcd, 380.0820; found, 380.0856. Anal calcd for $C_{17}H_{14}F_2N_2O_6$: C, 53.69; H, 3.71; N, 7.37; found: C, 53.58; H, 3.57; N, 7.36.

#### 5.1.15. Ethyl 10′,11′-difluoro-2′,3′-dihydro-9′-nitro-8′-oxo-spiro[cyclobutane-1,4′-[4*H*,8*H*]pyrido[1,2,3-*ef*][1,4]benzoxazepine]-7′-carboxylate (26)

A solution of **18** (925 mg, 2.65 mmol) in concentrated $H_2SO_4$ (11 mL) was treated portionwise at 0 °C with solid $KNO_3$

(363 mg, 3.59 mmol). After stirring at 0 °C for 2 h, the reaction mixture was poured into ice-water and the resulting precipitate was combined by filtration, washed with water. The crude material was purified by flash column chromatography on silica gel ($CH_2Cl_2$: MeOH = 10: 1) to give **26** as a yellow solid (822 mg, 79%).

Mp: 340–345 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 1.27 (3H, t, $J$ = 7.3 Hz), 1.68–1.78 (1H, m), 1.90–2.03 (1H, m), 2.40–2.58 (4H, m), 2.69 (2H, t, $J$ = 6.7 Hz), 4.24 (2H, q, $J$ = 7.3 Hz), 4.63 (2H, t, $J$ = 6.7 Hz), 8.44 (1H, s HRMS (ESI$^+$) for $C_{18}H_{17}F_2N_2O_6$ [M+H]$^+$: calcd, 395.10547; found, 395.10611. Anal. calcd for $C_{18}H_{16}F_2N_2O_6$ 0.1$H_2O$: C, 54.81; H, 4.07; N, 7.07. Found: C, 54.31; H, 3.95; N, 6.96.

### 5.1.16. Ethyl 10′,11′-difluoro-2′,3′-dihydro-9′-nitro-8′-oxo-spiro[cyclopentane-1,4′-[4*H*,8*H*]pyrido[1,2,3-*ef*][1,4]benzoxazepine]-7′-carboxylate (27)

A solution of **19** (50.0 g, 0.138 mmol) in concentrated $H_2SO_4$ (1 mL) was treated portionwise at 0 °C with solid $KNO_3$ (20.0 mg, 0.199 mmol). After stirring at 0 °C for 1 h, the reaction mixture was poured into ice-water and the resulting precipitate was combined by filtration, washed with water and dissolved in $CH_2Cl_2$-MeOH (5:1 v/v). The combined extracts were concentrated in vacuo. The crude material was purified by flash column chromatography on silica gel ($CH_2Cl_2$: MeOH = 10: 1) to give **27** as a yellow solid (26.0 mg, 46%).

Mp: 243–246 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 1.37 (3H, t, $J$ = 7.3 Hz), 1.52–1.66 (2H, m), 1.78–1.90 (2H, m), 2.15–2.30 (4H, m), 2.50 (2H, t, $J$ = 6.7 Hz), 4.36 (2H, q, $J$ = 7.3 Hz), 4.55 (2H, t, $J$ = 6.7 Hz), 8.69 (1H, s). HRMS (ESI$^+$) for $C_{19}H_{19}F_2N_2O_6$ [M+H]$^+$: calcd, 409.12112; found, 409.12385. Anal. calcd for $C_{19}H_{18}F_2N_2O_6$ 0.2$H_2O$: C, 55.40; H, 4.40; N, 6.80. Found: C, 55.34; H, 4.56; N, 6.51.

### 5.1.17. Ethyl 10′,11′-difluoro-2′,3′-dihydro-3′-methyl-9′-nitro-8′-oxo-spiro[cyclobutane-1,4′-[4*H*,8*H*]pyrido[1,2,3-*ef*][1,4]benzoxazepine]-7′-carboxylate (28)

A solution of **20** (1.00 g, 2.75 mmol) in concentrated $H_2SO_4$ (12 mL) was treated portionwise at 0 °C with solid $KNO_3$ (377 mg, 3.73 mmol). After stirring at 0 °C for 2 h, the reaction mixture was poured into ice-water and the resulting precipitate was combined by filtration, washed with water. The crude material was purified by recrystalization of the cake from DMF (20 mL) to give **28** as a yellow solid (923 mg, 82%).

Mp: 330–333 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 0.94 (3H, d, $J$ = 6.1 Hz), 1.26 (3H, t, $J$ = 7.3 Hz), 1.67–1.78 (1H, m), 1.83–1.98 (1H, m), 2.40–2.64 (4H, m), 2.92–3.05 (1H, m), 3.98 (1H, t, $J$ = 11.6 Hz), 4.24 (2H, t, $J$ = 7.3 Hz), 4.80 (1H, dd, $J$ = 11.6 and 7.9 Hz), 8.25 (1H, s). HRMS (ESI$^+$) for $C_{19}H_{19}F_2N_2O_6$ [M+H]$^+$: calcd, 409.12112; found, 409.12181. Anal. calcd for $C_{19}H_{18}F_2N_2O_6$: C, 55.88; H, 4.44; N, 6.86. Found: C, 55.75; H, 4.37; N, 6.91.

### 5.1.18. Ethyl 10′,11′-difluoro-2′,3′-dihydro-2′-methyl-9′-nitro-8′-oxo-spiro[cyclobutane-1,4′-[4*H*,8*H*]pyrido[1,2,3-*ef*][1,4]benzoxazepine]-7′-carboxylate (29)

A solution of **21** (182 mg, 0.501 mmol) in concentrated $H_2SO_4$ (2 mL) was treated portionwise at 0 °C with solid $KNO_3$ (72.0 mg, 0.712 mmol). After stirring at 0 °C for 2 h, the reaction mixture was poured into ice-water and the resulting precipitate was combined by filtration, washed with water and then dried to give **29** as a pale yellow solid (190 mg, 93%).

Mp: 300–303 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 1.27 (3H, t, $J$ = 7.3 Hz), 1.45 (3H, d, $J$ = 6.1 Hz), 1.61–1.77 (1H, m), 1.90–2.03 (1H, m), 2.29–2.48 (3H, m), 2.57–2.72 (3H, m), 4.24 (2H, q, $J$ = 7.3 Hz), 4.62–4.73 (1H, m), 8.43 (1H, s). HRMS (ESI$^+$) for $C_{19}H_{19}F_2N_2O_6$ [M+H]$^+$: calcd, 409.12112; found, 409.12140. Anal. calcd for $C_{19}H_{18}F_2N_2O_6$: C, 55.88; H, 4.44; N, 6.86. Found: C, 55.68; H, 4.29; N, 6.78.

### 5.1.19. Ethyl 10′,11′-difluoro-2′,3′-dihydro-9′-nitro-8′-oxo-spiro[cyclobutane-1,4′-[4*H*,8*H*]pyrido[1,2,3-*ef*][1,4]benzodiazepine]-7′-carboxylate (30)

A solution of **22** (5.50 g, 12.3 mmol) in concentrated $H_2SO_4$ (60 mL) was treated portionwise at 0 °C with solid $KNO_3$ (1.37 g, 13.6 mmol). After stirring at 0 °C for 1 h, the reaction mixture was poured into ice-water and the resulting precipitate was combined by filtration, washed with water. The crude material was purified by flash column chromatography on silica gel ($CH_2Cl_2$: AcOEt = 1: 1) to give **30** as a yellow solid (3.28 g, 68%).

Mp: 245–248 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 1.26 (3H, t, $J$ = 7.3 Hz), 1.63–1.75 (1H, m), 1.85–1.98 (1H, m), 2.35–2.57 (6H, m), 3.47–3.55 (2H, m), 4.22 (2H, q, $J$ = 7.3 Hz), 6.96–7.02 (1H, m), 8.32 (1H, s). HRMS (ESI$^+$) for $C_{18}H_{18}F_2N_3O_5$ [M+H]$^+$: calcd, 394.12145; found, 394.12213. Anal. calcd for $C_{18}H_{17}F_2N_3O_5$: C, 54.96; H, 4.36; N, 10.68. Found: C, 55.07; H, 4.39; N, 10.48.

### 5.1.20. Ethyl 9′-amino-10′,11′-difluoro-2′,3′-dihydro-8′-oxo-spiro[cyclopropane-1,4′-[4*H*,8*H*]pyrido[1,2,3-*ef*][1,4]benzoxazepine]-7′-carboxylate (31)

A solution of **25** (500 mg, 1,31 mmol) and 10% Pd/C (100 mg) in DMF (30 mL) was stirred under a hydrogen atmosphere at 50 °C for 1.5 h. The catalyst was removed by filtration over Celite, and the filtrate was concentrated in vacuo. The resulting solid was dissolved in $CH_2Cl_2$ and EtOH (3:1, 65 mL), and filtered. After the $CH_2Cl_2$ was removed, the precipitate was isolated by filtration, washed with EtOH, and dried to give **31** as a brown solid (355 mg, 77%).

Mp: 274–277 °C. $^1$H NMR (400 MHz, DMSO-$d_6$)$\delta$ 0.99 (2H, br), 1.13 (2H, br), 1.25 (3H, t, $J$ = 7.3 Hz), 1.64–2.91 (2H, br), 4.18 (2H, q, $J$ = 7.3 Hz), 4.35 (2H, br), 7.46 (2H, br), 8.35 (1H, s). HRMS (EI) for $C_{17}H_{16}F_2N_2O_4$ (M$^+$): calcd, 350.1078; found, 350.1033. Anal calcd for $C_{17}H_{16}F_2N_2O_4$: C, 58.28; H, 4.60; N, 8.00; found: C, 58.21; H, 4.47; N, 7.87.

### 5.1.21. Ethyl 9′-amino-10′,11′-difluoro-2′,3′-dihydro-8′-oxo-spiro[cyclobutane-1,4′-[4*H*,8*H*]pyrido[1,2,3-*ef*][1,4]benzoxazepine]-7′-carboxylate (32)

A solution of **26** (770 mg, 1.95 mmol) in DMF (70 mL) was hydrogenated under atmospheric pressure over 10% Pd/C (200 mg) at 50 °C for 1 h. The catalyst was removed by filtration over Celite and the filtrate was concentrated in vacuo. The crude material was purified by flash column chromatography on silica gel ($CH_2Cl_2$: MeOH = 10: 1) to give **32** as a colorless solid (559 mg, 79%).

Mp: 257–260 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 1.26 (3H, t, $J$ = 7.3 Hz), 1.65–1.77 (1H, m), 1.87–2.01 (1H, m), 2.35–2.62 (6H, m), 4.21 (2H, q, $J$ = 7.3 Hz), 4.31–4.42 (2H, brt), 7.40–7.60 (2H, br s), 8.23 (1H, s). HRMS (ESI$^+$) for $C_{18}H_{19}F_2N_2O_4$ [M+H]$^+$: calcd, 365.13129; found, 365.13127. Anal. calcd for $C_{18}H_{18}F_2N_2O_4$ 0.2$H_2O$: C, 58.76; H, 4.93; N, 7.61. Found: C, 58.70; H, 4.84; N, 7.40.

### 5.1.22. Ethyl 9′-amino-10′,11′-difluoro-2′,3′-dihydro-8′-oxo-spiro[cyclopentane-1,4′-[4*H*,8*H*]pyrido[1,2,3-*ef*][1,4]benzoxazepine]-7′-carboxylate (33)

A solution of **27** (2.00 g, 4.90 mmol) in DMF (120 mL) was hydrogenated under atmospheric pressure over 10% Pd/C (400 mg) at 50 °C for 1.5 h. The catalyst was removed by filtration over Celite and the filtrate was concentrated in vacuo. The crude material was purified by flash column chromatography on silica gel ($CH_2Cl_2$: MeOH = 10: 1) to give **33** as a yellow solid (527 mg, 28%).

Mp: 144–146 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 1.26 (3H, t, $J$ = 7.3 Hz), 1.37–1.50 (2H, m), 1.65–1.77 (2H, m), 2.06–2.25 (4H, m), 2.36 (2H, t, $J$ = 6.7 Hz), 4.20 (2H, q, $J$ = 7.3 Hz), 4.30 (2H, t,

$J$ = 6.7 Hz), 7.53 (2H, br s), 8.47 (1H, s). HRMS (ESI$^+$) for $C_{19}H_{21}F_2N_2O_4$ [M+H]$^+$: calcd, 379.14694; found, 379.15131. Anal. calcd for $C_{19}H_{20}F_2N_2O_4$: C, 60.31; H, 5.33; N, 7.40. Found: C, 60.06; H, 5.23; N, 7.40.

#### 5.1.23. Ethyl 9′-amino-10′,11′-difluoro-2′,3′-dihydro-3′-methyl-8′-oxo-spiro[cyclobutane-1,4′-[4*H*,8*H*]pyrido[1,2,3-*ef*][1,4] benzoxazepine]-7′-carboxylate (34)

A solution of **28** (900 mg, 2.20 mmol) in DMF (55 mL) was hydrogenated under atmospheric pressure over 10% Pd/C (180 mg) at 50 °C for 3 h. The catalyst was removed by filtration over Celite and the filtrate was concentrated in vacuo. The residue was washed with EtOH and collected by filtration and then dried to give **34** as a pale yellow solid (756 mg, 91%).

Mp: 248–250 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 0.89 (3H, br s), 1.26 (3H, t, $J$ = 7.3 Hz), 1.65–1.77 (1H, m), 1.80–1.95 (1H, m), 2.30–2.67 (4H, m), 2.82–2.94 (1H, m), 3.52–3.66 (1H, m), 4.15–4.28 (2H, m), 4.58–4.70 (1H, m), 7.40–7.65 (2H, br), 8.05 (1H, s). HRMS (ESI$^+$) for $C_{19}H_{21}F_2N_2O_4$ [M+H]$^+$: calcd, 379.14694; found, 379.14659.

#### 5.1.24. Ethyl 9′-amino-10′,11′-difluoro-2′,3′-dihydro-2′-methyl-8′-oxo-spiro[cyclobutane-1,4′-[4*H*,8*H*]pyrido[1,2,3-*ef*][1,4] benzoxazepine]-7′-carboxylate (35)

A solution of **29** (1.13 g, 2.77 mmol) in DMF (70 mL) was hydrogenated under atmospheric pressure over 10% Pd/C (230 mg) at 50 °C for 2 h. The catalyst was removed by filtration over Celite and the filtrate was concentrated in vacuo. The residue was washed with EtOH and collected by filtration and then dried to give **35** as a pale yellow solid (824 mg, 79%).

Mp: 211–213 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 1.26 (3H, t, $J$ = 7.3 Hz), 1.36 (3H, d, $J$ = 6.1 Hz), 1.70 (1H, q, $J$ = 10.4 Hz), 1.86–2.00 (1H, m), 2.25 (1H, q, $J$ = 10.4 Hz), 2.30–2.39 (1H, m), 2.42–2.63 (4H, m), 4.14–4.27 (2H, m), 4.27–4.37 (1H, m),7.49 (2H, br s), 8.22 (1H, s). HRMS (ESI$^+$) for $C_{19}H_{21}F_2N_2O_4$ [M+H]$^+$: calcd, 379.14694; found, 379.14645. Anal. calcd for $C_{19}H_{20}F_2N_2O_4$: C, 60.31; H, 5.33; N, 7.40. Found: C, 60.34; H, 5.23; N, 7.22.

#### 5.1.25. Ethyl 9′-amino-10′,11′-difluoro-2′,3′-dihydro-8′-oxo-spiro[cyclobutane-1,4′-[4*H*,8*H*]pyrido[1,2,3-*ef*][1,4]benzodiaze pine]-7′-carboxylate (36)

A solution of **22** (1.23 g, 3.13 mmol) in DMF (77 mL) was hydrogenated under atmospheric pressure over 10% Pd/C (250 mg) at 50 °C for 1 h. The catalyst was removed by filtration over Celite and the filtrate was concentrated in vacuo. The crude material was purified by flash column chromatography on silica gel ($CH_2Cl_2$: MeOH = 2: 1) to give **36** as a yellow solid (1.00 g, 88%).

Mp: 176–178 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 1.25 (3H, t, $J$ = 7.3 Hz), 1.62–1.73 (1H, m), 1.82–1.96 (1H, m), 2.24–2.41 (4H, m), 2.45–2.60 (2H, m), 3.27–3.38 (2H, m), 4.19 (2H, q, $J$ = 7.3 Hz), 5.28–5.34 (1H, m), 7.10 (2H, br s), 8.12 (1H, s). HRMS (ESI$^+$) for $C_{18}H_{20}F_2N_3O_3$ [M+H]$^+$: calcd, 364.14727; found, 364.14797. Anal. calcd for $C_{18}H_{19}F_2N_3O_3$ 0.2$H_2O$: C, 58.91; H, 5.22; N, 11.45. Found: C, 58.91; H, 5.20; N, 11.06.

#### 5.1.26. 9′-Amino-10′,11′-difluoro-2′,3′-dihydro-8′-oxo-spiro[cyclopropane-1,4′-[4*H*,8*H*]pyrido[1,2,3-*ef*][1,4]benzoxazepine]-7′-carboxylic acid (37)

A solution of **31** (320 mg, 0.913 mmol) in mixture of AcOH-$H_2O$-$H_2SO_4$ (6:4:1 v/v, 5.3 mL) was heated at reflux for 1 h. The reaction mixture was poured into ice water and a precipirtate was collected by filtration, washed with water and then dried to give the title compound **37** as a yellow solid (274 mg, 93%).

Mp: 356–365 °C. $^1$H NMR (400 MHz, DMSO-$d_6$)$\delta$ 0.63–1.55 (4H, br), 1.90–3.11 (2H, br), 3,78–4.96 (2H, br), 7.40 (2H, s), 8.64 (1H, s), 14.5 (1H, s). HRMS (EI) for $C_{15}H_{12}F_2N_2O_4$ (M$^+$): calcd, 322.0765; found, 322.0773. Anal calcd for $C_{15}H_{12}F_2N_2O_4$: C, 55.90; H, 3.75; N, 8.69; found: C, 55.52; H, 3.71; N, 8.36.

#### 5.1.27. 9′-Amino-10′,11′-difluoro-2′,3′-dihydro-8′-oxo-spiro [cyclobutane-1,4′-[4*H*,8*H*]pyrido[1,2,3-*ef*][1,4]benzoxazepine]-7′-carboxylic acid (38)

To a mixture of **32** (513 mg, 1.41 mmol) in EtOH (14 mL) was added 2 N NaOH (7.0 mL, 14.0 mmol) at room temperature and the mixture was heated at 50 °C for 3 h. The reaction mixture was added 2 N HCl (7.0 mL) and water. A precipitate formed and was collected by filtration, washed successively with water and then dried to give **38** as a colorless solid (427 mg, 90%).

Mp: 330–333 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 1.64–1.77 (1H, m), 1.89–2.02 (1H, m), 2.56–2.70 (2H, br), 4.30–4.50 (2H, br), 7.35–7.52 (2H, br s), 8.47 (1H, s), 14.60 (1H, s). HRMS (ESI$^+$) for $C_{16}H_{15}F_2N_2O_4$ [M+H]$^+$: calcd, 337.09999; found, 337.10033. Anal. calcd for $C_{16}H_{14}F_2N_2O_4$ 0.1 $H_2O$: C, 56.84; H, 4.17; N, 8.29. Found: C, 56.91; H, 4.25; N, 7.99.

#### 5.1.28. 9′-Amino-10′,11′-difluoro-2′,3′-dihydro-8′-oxo-spiro [cyclopentane-1,4′-[4*H*,8*H*]pyrido[1,2,3-*ef*][1,4]benzoxazepine]-7′-carboxylic acid (39)

To a mixture of **33** (482 mg, 1.27 mmol) in EtOH (13 mL) was added 2 N NaOH (6.5 mL, 13.0 mmol) at room temperature and the mixture was heated at 50 °C for 3 h. The reaction mixture was added 2 N HCl (6.5 mL) and water. A precipitate formed and was collected by filtration, washed successively with water and then dried to give **39** as a colorless solid (425 mg, 96%).

Mp: 242–245 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 1.35–1.52 (2H, m), 1.65–1.75 (2H, m), 2.10–2.30 (4H, m), 2.43 (2H, t, $J$ = 6.7 Hz), 4.34 (2H, t, $J$ = 6.7 Hz), 7.50 (2H, br s), 8.67 (1H, s), 14.5 (1H, br s). HRMS (ESI$^+$) for $C_{17}H_{17}F_2N_2O_4$ [M+H]$^+$: calcd, 351.11564; found, 351.11965. Anal. calcd for $C_{17}H_{16}F_2N_2O_4$: C, 58.28; H, 4.60; N, 8.00. Found: C, 58.21; H, 4.60; N, 7.77.

#### 5.1.29. 9′-Amino-10′,11′-difluoro-2′,3′-dihydro-3′-methyl-8′-oxo-spiro[cyclobutane-1,4′-[4*H*,8*H*]pyrido[1,2,3-*ef*][1,4]benzoxaze pine]-7′-carboxylic acid (40)

To a mixture of **34** (720 mg, 1.90 mmol) in EtOH (20 mL) was added 2 N NaOH (10.0 mL) at room temperature and the mixture was heated at 50 °C for 3 h. The reaction mixture was added 2 N HCl (10.0 mL) and water. A precipitate formed and was collected by filtration, washed successively with water and then dried to give **40** as a yellow solid (654 mg, 98%).

Mp: 305–308 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 0.90 (3H, d, $J$ = 5.5 Hz), 1.64–1.76 (1H, m), 1.81–1.96 (1H, m), 2.35–2.72 (4H, m), 2.88–3.02 (1H, m), 3.64 (1H, t, $J$ = 11.6 Hz), 4.60–4.75 (1H, m), 7.46 (2H, br s), 8.26 (1H, s), 14.60 (1H, br s). HRMS (ESI$^+$) for $C_{17}H_{17}F_2N_2O_4$ [M+H]$^+$: calcd, 351.11564; found, 351.11536.

#### 5.1.30. 9′-Amino-10′,11′-difluoro-2′,3′-dihydro-2′-methyl-8′-oxo-spiro[cyclobutane-1,4′-[4*H*,8*H*]pyrido[1,2,3-*ef*][1,4]ben zoxazepine]-7′-carboxylic acid (41)

To a mixture of **35** (768 mg, 2.03 mmol) in EtOH (20 mL) was added 2 N NaOH (10.0 mL) at room temperature and the mixture was heated at 50 °C for 3 h. The reaction mixture was added 2 N HCl (10.0 mL) and water. A precipitate formed and was collected by filtration, washed successively with water and then dried to give **41** as a yellow solid (703 mg, 99%).

Mp: 297–300 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 1.37 (3H, d, $J$ = 6.1 Hz), 1.70 (1H, q, $J$ = 11.0 Hz), 1.88–2.02 (1H, m), 2.31 (1H, q, $J$ = 11.0 Hz), 2.37–2.69 (5H, m), 4.30–4.42 (1H, m), 7.43 (1H, br s), 8.47 (1H, s), 14.61 (1H, br s). HRMS (ESI$^+$) for $C_{17}H_{17}F_2N_2O_4$ [M+H]$^+$: calcd, 351.11564; found, 351.11474. Anal. calcd for

$C_{17}H_{16}F_2N_2O_4$ 0.15$H_2O$: C, 57.84; H, 4.57; N, 7.94. Found: C, 58.10; H, 4.52; N, 7.65.

#### 5.1.31. 9′-Amino-10′,11′-difluoro-2′,3′-dihydro-8′-oxo-spiro [cyclobutane-1,4′-[4*H*,8*H*]pyrido[1,2,3-*ef*][1,4]benzodiazepine]-7′-carboxylic acid (42)

To a mixture of **36** (515 mg, 1.41 mmol) in EtOH (15 mL) was added 2 N NaOH (7.0 mL, 14.0 mmol) at room temperature and the mixture was heated at 50 °C for 3 h. The reaction mixture was added 2 N HCl (7.0 mL) and water. A precipitate formed and was collected by filtration, washed successively with water and then dried to give **42** as a yellow solid (397 mg, 83%).

Mp: 220–225 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 1.61–1.73 (1H, m), 1.82–1.97 (1H, m), 2.30–2.44 (4H, m), 2.55–2.69 (2H, m), 3.25–3.50 (2H, m), 5.53–5.59 (1H, br), 7.03 (2H, br s), 8.35 (1H, s), 14.75 (1H, s). HRMS (ESI$^+$) for $C_{16}H_{15}F_2N_3O_3$ [M+H]$^+$: calcd, 336.11597; found, 336.11648.

Anal. calcd for $C_{16}H_{15}F_2N_3O_3$ 0.1 $H_2O$: C, 57.01; H, 4.48; N, 12.46. Found: C, 57.22; H, 4.51; N, 12.08.

#### 5.1.32. 9′-Amino-10′-fluoro-2′,3′-dihydro-8′-oxo-11′-[2-(2-pyridylamino)ethylamino]spiro[cyclopropane-1,4′-[4*H*,8*H*] pyrido[1,2,3-*ef*][1,4] benzoxazepine]-7′-carboxylic acid (43)

A solution of **37** (150 mg, 0.465 mmol), triethylamine (0.100 mL, 0.717 mmol) and *N*-2-(pyridinyl)-1,2-ethanediamine (95.5 mg, 0.696 mmol) in DMSO (2 mL) was stirred at 100 °C for 3 h. The reaction mixture was poured into ice water and the resulting precipitate was removed by filtration and washed with ethanol. The resulting solid was dissolved in DMF and filtered. The filtrate was poured into water and the resulting precipitate was removed by filtration, washed with water and dried to give **43** as a dark yellow solid (95.6 mg, 47%).

Mp: 237–239 °C $^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$ 0.81–1.50 (4H, m), 3.40–3.51 (2H, m), 3.55–3.66 (2H, m), 3.80–4.60 (4H, m), 6.32 (1H, br s), 6.40–6.50 (2H, m), 6.70 (1H, t, *J* = 5.5 Hz), 9.97 (2H, br s), 7.30–7.40 (1H, m), 7.90–8.00 (1H, m), 15.14 (1H, s). HRMS (ESI) for $C_{22}H_{23}FN_5O_4$ [M+H]$^+$: calcd, 440.17340; found, 440.17315. Anal. Calcd for $C_{22}H_{22}FN_5O_4$, 0.2$H_2O$: C, 59.64; H, 5.10; N, 15.81. Found: C, 59.44; H, 5.14; N, 15.61.

#### 5.1.33. 9′-Amino-10′-fluoro-2′,3′-dihydro-8′-oxo-11′-[2-(2-pyridylamino)ethylamino]spiro[cyclobutane-1,4′-[8*H*]pyrido [1,2,3-*ef*][1,4]benzoxazepine]-7′-carboxyic acid (44)

A solution of **38** (186 mg, 0.553 mmol), Jiwen (Jim) Liu, Amgen Inc., 1120 Veterans Boulevard, South San Francisco, CA 94080, USA2-pyridinyl-1,2-ethanediamine (115 mg, 0.838 mmol) and triethylamine (117 μL) in DMSO (2.5 mL) was stirred at 120 °C for 4 h. The reaction mixture was added portionwise at 0 °C with ice-water and the mixture was added 2 N HCl (3 drops). The resulting precipitate was combined by filtration. The cake washed with EtOH and collected by filtration and then dried to give **44** as a yellow solid (165 mg, 66%).

Mp: 173–175 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 1.61–1.74 (1H, m), 1.84–2.00 (1H, m), 2.30–2.65 (6H, m), 3.40–3.50 (2H, m), 3.52–3.62 (2H, m), 4.10–4.33 (2H, br), 6.20–6.30 (1H, m), 6.42–6.51 (2H, m), 6.69 (1H, t, *J* = 5.5 Hz), 6.90–7.08 (2H, br), 7.32–7.40 (1H, m), 7.97 (1H, dd, *J* = 4.3 and 1.2 Hz), 8.26 (1H, s), 15.23 (1H, s). HRMS (ESI$^+$) for $C_{23}H_{25}FN_5O_4$ [M+H]$^+$: calcd, 454.18906; found, 454.18677. Anal. calcd for $C_{23}H_{24}FN_5O_4$ 0.2$H_2O$: C, 60.44; H, 5.29; N, 15.32. Found: C, 60.56; H, 5.35; N, 15.07.

#### 5.1.34. 9′-Amino-10′-fluoro-2′,3′-dihydro-8′-oxo-11′-[2-(2-pyridylamino)ethylamino]spiro[cyclopentane-1,4′-[8*H*]pyrido[1,2,3-*ef*][1,4]benzoxazepine]-7′-carboxyic acid (3)

A solution of **39** (200 mg, 0.571 mmol) and *N*-2-pyridinyl-1,2-ethanediamine (118 mg, 0.860 mmol) in DMSO (2.5 mL) was stirred at 100 °C for 4 h. The reaction mixture was added portionwise at 0 °C with ice-water and the resulting precipitate was combined by filtration, washed with water and i$Pr_2O$. The cake washed with EtOH and collected by filtration and then dried to give **3** as a pale yellow solid (185 mg, 69%).

Mp: 180–183 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 1.34–1.49 (2H, m), 1.60–1.75 (2H, m), 2.03–2.23 (4H, m), 2.34 (2H, t, *J* = 6.7 Hz), 3.40–3.55 (2H, m), 3.55–3.64 (2H, m), 4.17 (2H, t, *J* = 6.7 Hz), 6.25–6.33 (1H, br), 6.43–6.51 (2H, m), 6.69 (1H, t, *J* = 5.5 Hz), 7.06 (2H, br), 7.35 (1H, ddd, *J* = 6.7, 6.7 and 1.8 Hz), 8.86 (1H, dd, *J* = 4.9 and 1.2 Hz), 8.49 (1H, s), 15.1 (1H, br s). HRMS (ESI$^+$) for $C_{24}H_{27}FN_5O_4$ [M+H]$^+$: calcd, 468.20471; found, 4678.20118. Anal. calcd for $C_{24}H_{26}FN_5O_4$ 0.1$H_2O$: C, 61.42; H, 5.58; N, 14.92. Found: C, 61.32; H, 5.75; N, 14.63.

#### 5.1.35. 9′-Amino-10′-fluoro-2′,3′-dihydro-3′-methyl-8′-oxo-11′-[2-(2-pyridylamino)ethylamino]spiro[cyclobutane-1,4′-[8*H*]pyrido[1,2,3-*ef*][1,4]benzoxazepine]-7′-carboxyic acid (45)

A solution of **40** (177 mg, 1.29 mmol) and triethylamine (180 μL) in DMSO (4 mL) was stirred at 120 °C for 5 h. The reaction mixture was added portionwise at 0 °C with ice-water and the mixture was extracted with $CH_2Cl_2$. The combined extracts were dried over anhydrous $Na_2SO_4$, filtered, and then concentrated in vacuo. The crude material was purified by flash column chromatography on silica gel ($CH_2Cl_2$: MeOH = 10: 1) to give **45** as a yellow amorphous solid (177 mg, 44%).

Mp: 201–204 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 0.86 (3H, d, *J* = 5.5 Hz), 1.62–1.73 (1H, m), 1.79–1.93 (1H, m), 2.25–2.38 (1H, m), 2.38–2.64 (3H, m), 2.80–2.93 (1H, m), 3.27–3.50 (3H, m), 3.52–3.14 (2H, m), 4.50–4.60 (1H, m), 6.28 (1H, br s), 6.42–6.52 (2H, m), 6.69 (1H, t, *J* = 5.5 Hz), 7.01 (2H, br s), 7.32–7.40 (1H, m), 7.96 (1H, dd, *J* = 4.9 and 1.2 Hz), 8.07 (1H, s), 15.24 (1H, s). HRMS (ESI$^+$) for $C_{24}H_{27}FN_5O_4$ [M+H]$^+$: calcd, 468.20471; found, 468.20923. Anal. calcd for $C_{24}H_{26}FN_5O_4$: C, 61.61; H, 5.61; N, 14.98. Found: C, 61.39; H, 5.51; N, 14.70.

#### 5.1.36. 9′-Amino-10′-fluoro-2′,3′-dihydro-2′-methyl-8′-oxo-11′-[2-(2-pyridylamino)ethylamino]spiro[cyclobutane-1,4′-[8*H*] pyrido[1,2,3-*ef*][1,4]benzoxazepine]-7′-carboxyic acid (46)

A solution of **41** (351 mg, 1.00 mmol), *N*-2-pyridinyl-1,2-ethanediamine (210 mg, 1.53 mmol) and triethylamine (0.22 mL) in DMSO (5 mL) was stirred at 120 °C for 5 h. The reaction mixture was added portionwise at 0 °C with ice-water and the mixture was extracted with $CH_2Cl_2$. The combined extracts were dried over anhydrous $Na_2SO_4$, filtered, and then concentrated in vacuo. The crude material was purified by flash column chromatography on silica gel ($CH_2Cl_2$: MeOH = 10: 1) to gave **46** as a yellow amorphous solid (278 mg, 59%).

Mp: 106–108 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 1.29 (3H, d, *J* = 6.1 Hz), 1.67 (1H, q, *J* = 10.4 Hz), 1.84–1.99 (1H, m), 2.21 (1H, q, *J* = 10.4 Hz), 2.30–2.40 (1H, m), 2.40–2.62 (4H, m), 3.40–3.50 (2H, m), 3.53–3.67 (2H, m), 4.08–4.20 (1H, m), 5.91 (1H, br s), 6.42–6.50 (2H, m), 6.65 (1H, t, *J* = 5.5 Hz), 7.00 (2H, br s), 7.32–7.38 (1H, m), 7.94 (1H, dd, *J* = 4.9 and 1.2 Hz), 8.25 (1H, s), 15.24 (1H, s). HRMS (ESI$^+$) for $C_{24}H_{27}FN_5O_4$ [M+H]$^+$: calcd, 467.20471; found, 467.20453. Anal. calcd for $C_{24}H_{26}FN_5O_4$ 0.4$H_2O$: C, 60.72; H, 5.52; N, 14.75. Found: C, 60.61; H, 5.49; N, 14.67.

#### 5.1.37. 9′-Amino-10′-fluoro-2′,3′-dihydro-8′-oxo-11′-[2-(2-pyridylamino)ethylamino]spiro[cyclobutane-1,4′-[8*H*]pyrido[1,2,3-*ef*][1,4]benzodiazepine]-7′-carboxyic acid (47)

A solution of **42** (300 mg, 0.895 mmol), *N*-2-pyridinyl-1,2-ethanediamine (185 mg, 1.35 mmol) and triethylamine (0.23 mL) in DMSO (4 mL) was stirred at 120 °C for 5 h. The reaction mixture was added portionwise at 0 °C with iced-water and the mixture was extracted with $CH_2CH_2$. The organic layer was dried over

anhydrous $Na_2SO_4$, and concentrated in vacuo. The crude material was purified by flash column chromatography on silica gel ($CH_2Cl_2$: MeOH = 10: 1) to give **47** as a yellow solid (259 mg, 64%).

Mp: 110–115 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 1.58–1.70 (1H, m), 1.78–1.92 (1H, m), 2.21–2.38 (4H, m), 2.43–2.60 (2H, m), 3.19–3.37 (2H, m), 3.37–3.51 (4H, m), 4.50 (1H, t, $J$ = 5.5 Hz), 5.50–5.58 (1H, br), 6.42–6.51 (2H, m), 6.65 (1H, t, $J$ = 5.5 Hz), 6.82 (2H, br s), 7.36 (1H, td, $J$ = 6.7 and 1.8 Hz), 7.95 (1H, dd, $J$ = 4.9 and 1.2 Hz), 8.18 (1H, s), 15.28 (1H, s). HRMS (ESI$^+$) for $C_{23}H_{26}FN_6O_3$ [M+H]$^+$: calcd, 453.20504; found, 453.20466. Anal. calcd for $C_{23}H_{25}FN_6O_3$ 0.3$H_2O$: C, 60.33; H, 5.50; N, 18.35. Found: C, 60.47; H, 5.58; N, 18.06.

### 5.1.38. Ethyl 1-hydroxycyclobutylacetate (50)

Trimethylchlorosilane (1.14 mL, 8.92 mmol) was added from a syringe to a suspension of zinc powder (7.97 g, 0.122 mol) in $Et_2O$ (200 mL). The mixture was stirred for 15 min at room temperature and then heated to reflux. The heating was stopped, and ethyl bromoacetate (10.3 mL, 92.9 mmol) was added at such a rate that the ether boiled gently. After being heated to reflux for 1 h, the mixture was stirred for 1 h at room temperature. A solution of **48** (6.00 g, 75.9 mmol) in $Et_2O$ (30 mL) was added while the temperature of the mixture was maintained at 19-20 °C by intermittent cooling. After being stirred for 1 h at room temperature, the mixture was poured into iced 25% ammonia (400 mL). The aqueous phase was extracted with ether, the combined phases were dried on $K_2CO_3$ and evaporated, and **50** was obtained as a colorless oil (6.50 g, 54%).

$^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.29 (3H, t, $J$ = 7.3 Hz), 1.47–1.64 (1H, m), 1.76–1.87 (1H, m), 1.93–2.06 (2H, m), 2.12–2.22 (2H, m), 2.67 (2H, s), 3.70 (1H, s), 4.19 (2H, q, $J$ = 7.3 Hz). HRMS (CI$^+$) for $C_8H_{15}O_3$ [M+H]$^+$: calcd, 159.1021; found, 159.0994.

### 5.1.39. Ethyl 1-hydroxycyclopentylacetate (51)

Trimethylchlorosilane (3.60 mL, 28.2 mmol) was added from a syringe to a suspension of zinc powder (25.2 g, 0.385 mol) in $Et_2O$ (500 mL). The mixture was stirred for 15 min at room temperature and then heated to reflux. The heating was stopped, and ethyl bromoacetate (32.4 mL, 0.292 mol) was added at such a rate that the ether boiled gently. After being heated to reflux for 1 h, the mixture was stirred for 1 h at room temperature. A solution of **49** (20.2 g, 0.240 mol) in $Et_2O$ (30 mL) was added while the temperature of the mixture was maintained at 19-21 °C by intermittent cooling. After being stirred for 1 h at room temperature, the mixture was poured into iced 25% ammonia (500 mL). The aqueous phase was extracted with ether, the combined phases were dried on $K_2CO_3$ and evaporated, and **51** was obtained as a colorless oil (41.0 g, 99%).

$^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.13 (3H, t, $J$ = 7.3 Hz), 1.34–1.52 (4H, m), 1.61–1.75 (4H, m), 2.45 (2H, s), 3.22 (1H, s), 4.03 (2H, q, $J$ = 7.3 Hz). HRMS (CI$^+$) for $C_9H_{17}O_3$ [M+H]$^+$: calcd, 173.1178; found, 173.1181.

### 5.1.40. Ethyl 2-(1-hydroxycyclobutyl)propionate (52)

Trimethylchlorosilane (950 μL, 7.43 mmol) was added from a syringe to a suspension of zinc powder (6.65 g, 0.102 mol) in abs. $Et_2O$ (170 mL). The mixture was stirred for 15 min at room temperature and then heated to reflux. The heating was stopped, and ethyl 2-bromopropionate (10.1 mL, 77.8 mmol) was added at such a rate that the ether boiled gently. After being heated to reflux for 1 h, the mixture was stirred for 1 h at room temperature. A solution of **48** (5.00 g, 63.2 mmol) in $Et_2O$ (10 mL) was added while the temperature of the mixture was maintained at 19-20 °C by intermittent cooling. After being stirred for 1 h at room temperature, the mixture was poured into iced 25% ammonia (400 mL). The aqueous phase was extracted with ether, the combined phases were dried on $K_2CO_3$ and evaporated, and **52** was obtained as a colorless oil (12.9 g, 100%).

$^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.21 (3H, d, $J$ = 6.8 Hz), 1.29 (3H, t, $J$ = 7.3 Hz), 1.51–1.64 (1H, m), 1.80–1.90 (1H, m), 1.96–2.17 (4H, m), 2.70 (1H, q, $J$ = 7.3 Hz), 3.44 (1H, s), 4.18 (2H, qd, $J$ = 7.3 and 1.2 Hz). HRMS (EI) for $C_9H_{16}O_3$ (M$^+$): calcd, 172.1099; found, 172.1133.

### 5.1.41. Ethyl 1-(benzoylamino)cyclobutylacetate (53)

To a mixture of **50** (6.45 g, 40.8 mmol) and benzonitrile (40 mL, 0.392 mol), $H_2SO_4$ (2.20 mL, 41.3 mmol) was slowly added at room temperature. The mixture was stirred for 1 h at room temperature and then at 80 °C for 1 h. The mixture was cooled on iced water, to which was added 2 N NaOH solution until pH reached 7. The mixture was extracted with ethyl acetate. The combined extracts were dried over anhydrous $Na_2SO_4$, filtered, and then concentrated in vacuo. The crude material was purified by flash column chromatography on silica gel (AcOEt:hexane = 5:1) to give **53** as a colorless solid (5.40 g, 51%).

Mp: 44–46 °C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.22 (3H, t, $J$ = 7.3 Hz), 1.84–2.07 (2H, m), 2.22–2.32 (2H, m), 2.44–2.55 (2H, m), 3.05 (2H, s), 4.11 (2H, q, $J$ = 7.3 Hz), 6.73 (1H, s), 7.39–7.52 (3H, m), 7.72–7.79 (2H, m). HRMS (EI) for $C_{15}H_{19}NO_3$ (M$^+$): calcd, 261.1365; found, 261.1360. Anal. calcd for $C_{15}H_{19}NO_3$ 0.1$H_2O$: C, 68.47; H, 7.28; N, 5.32. Found: C, 68.75; H, 7.28; N, 5.03.

### 5.1.42. Ethyl 1-(benzoylamino)cyclopentylacetate (54)

To a mixture of **51** (17.3 g, 0.100 mol) and benzonitrile (200 mL, 1.96 mol), $H_2SO_4$ (5.40 mL, 0.101 mol) was slowly added at room temperature. The mixture was stirred for 1 h at room temperature, and then at 80 °C for 1 h. The mixture was cooled on iced water, to which was added 2 N NaOH solution until pH reached 7. The mixture was extracted with ethyl acetate. The combined extracts were dried over anhydrous $Na_2SO_4$, filtered, and then concentrated in vacuo. The crude material was purified by flash column chromatography on silica gel (AcOEt:hexane = 5:1) to give **54** as a colorless amorphous mass (11.65 g, 42%).

$^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.20 (3H, t, $J$ = 7.3 Hz), 1.65–1.87 (6H, m), 2.23–2.33 (2H, m), 2.97 (2H, s), 4.10 (2H, q, $J$ = 7.3 Hz), 6.45 (1H, s), 7.38–7.50 (3H, m), 7.72–7.76 (2H, m). HRMS (ESI$^+$) for $C_{16}H_{22}NO_3$ [M+H]$^+$: calcd, 276.15997; found, 276.15872. Anal. calcd for $C_{16}H_{21}NO_3$ 0.1$H_2O$: C, 69.34; H, 7.64; N, 5.05. Found: C, 69.36; H, 7.59; N, 4.88.

### 5.1.43. Ethyl 2-[1-(benzoylamino)cyclobutyl]propionate (55)

To a mixture of **52** (12.5 g, 72.6 mmol) and benzonitrile (75 mL), $H_2SO_4$ (3.90 mL, 73.2 mmol) was slowly added at room temperature. The mixture was stirred for 1 h at room temperature, and then at 80 °C for 1 h. The mixture was cooled on iced water, to which was added 2 N NaOH solution until pH reached 7. The mixture was extracted with ethyl acetate. The combined extracts were dried over anhydrous $Na_2SO_4$, filtered, and then concentrated in vacuo. The crude material was purified by flash column chromatography on silica gel (AcOEt:hexane = 5:1) to give **55** as a pale yellow solid (9.24 g, 46%).

Mp: 55–58 °C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.25 (3H, t, $J$ = 7.3 Hz), 1.30 (3H, d, $J$ = 7.3 Hz), 1.73–1.87 (1H, m), 1.98–2.09 (2H, m), 2.21–2.32 (1H, m), 2.53–2.63 (1H, m), 2.84–2.94 (1H, m), 3.11 (1H, q, $J$ = 7.3 Hz), 4.08–4.20 (2H, m), 6.88 (1H, br s), 7.40–7.52 (3H, m), 7.75–7.80 (2H, m). HRMS (APCI$^+$) for $C_{16}H_{22}NO_3$ [M+H]$^+$: calcd, 276.15997; found, 276.15976. Anal. calcd for $C_{16}H_{21}NO_3$ 0.3 $H_2O$: C, 68.45; H, 7.54; N, 4.49. Found: C, 68.45; H, 7.77; N, 4.80.

### 5.1.44. 1-(Benzylamino)-1-(2-hydroxyethyl)cyclobutane (56)

To a solution of **53** (5.30 g, 20.3 mmol) in THF (100 mL) was added $LiAlH_4$ (3.88 g, 0.102 mol) at room temperature. The mixture

was stirred for 1 h, then refluxed for 1 h. The mixture was cooled on ice-water bath, to which was dropped a little water. The mixture was diluted with ethyl acetate, then dried over anhydrous $Na_2SO_4$, filtered, and concentrated in vacuo. The crude material was purified by distillation to give **56** as a colorless oil (2.53 g, 61%).

bp 177-178 °C (12 Torr). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.69–1.86 (2H, m), 1.88 (2H, t, $J$ = 5.5 Hz), 1.92–2.09 (4H, m), 3.73 (2H, s), 3.87 (2H, t, $J$ = 5.5 Hz), 7.22–7.35 (5H, m). HRMS (CI$^+$) for $C_{13}H_{20}NO$ [M+H]$^+$: calcd, 206.1545; found, 206.1510.

### 5.1.45. 1-(Benzylamino)-1-(2-hydroxyethyl)cyclopentane (57)

To a solution of **54** (11.6 g, 42.1 mmol) in THF (200 mL) was added $LiAlH_4$ (8.00 g, 0.211 mol) at room temperature and the mixture was stirred for 0.5 h, then, refluxed 1 h. The mixture was cooled on ice-water bath, to which was dropped a little water. The mixture was diluted with ethyl acetate, then dried over anhydrous $Na_2SO_4$, filtered, and then concentrated in vacuo. The crude material was purified by distillation to give **57** as a colorless oil (8.41 g, 91%).

bp 184-185 °C (9.2-9.0 Torr). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.57–1.85 (10H, m), 3.76 (2H, s), 3.87–3.92 (2H, m), 7.22–7.35 (5H, m). HRMS (EI) for $C_{14}H_{21}NO$ (M$^+$): calcd, 219.1623; found, 219.1623.

### 5.1.46. 1-(Benzylamino)-1-(1-hydroxypropane-2-yl)cyclobutane (58)

To a solution of **55** (9.07 g, 32.9 mmol) in THF (160 mL) was added $LiAlH_4$ (6.30 g, 0.166 mol) at room temperature and the mixture was stirred for 1 h, then, refluxed 1 h. The mixture was cooled on ice-water bath, to which was dropped a little water. The mixture was diluted with ethyl acetate, then dried over anhydrous $Na_2SO_4$, filtered, and then concentrated in vacuo. The crude material was purified by distillation to give **58** as a pale yellow oil (6.92 g, 96%).

$^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.00 (3H, d, $J$ = 6.7 Hz), 1.66–1.77 (1H, m), 1.81–1.97 (2H, m), 2.02–2.10 (1H, m), 2.10–2.24 (3 H, m), 3.59 (1H, dd, $J$ = 11.0 and 7.9 Hz), 3.71 (1H, d, $J$ = 11.6 Hz), 3.74 (1H, dd, $J$ = 11.0 and 3.7 Hz), 3.89 (1H, d, $J$ = 11.6 Hz), 7.23–7.36 (5H, m). HRMS (CI$^+$) for $C_{14}H_{22}NO$ [M+H]$^+$: calcd, 220.1701; found, 220.1670.

### 5.1.47. 1-Amino-1-(2-hydroxyethyl)cyclobutane (6)

To a solution of **56** (4.00 g, 19.5 mmol) in EtOH (100 mL) was added 10% Pd-C (500 mg). The mixture was stirred under $H_2$ gas, 0.5 MPa, at room temperature for 6 h. The mixture was filtered, and the filtrate was concentrated in vacuo. The crude material was purified by distillation to give **6** as a colorless oil (1.65 g, 73%).

bp 71-72 °C (2.3 Torr). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.60–1.72 (2H, m), 1.75 (2H, t, $J$ = 5.5 Hz), 1.77–1.87 (2H, m), 2.03–2.13 (2H, m), 3.81 (2H, q, $J$ = 5.5 Hz). HRMS (CI$^+$) for $C_6H_{14}NO$ [M+H]$^+$: calcd, 116.1075; found, 116.1032.

### 5.1.48. 1-Amino-1-(2-hydroxyethyl)cyclopentane (7)

To a solution of **57** (8.40 g, 38.3 mmol) in EtOH (150 mL) was added 10% Pd-C (1.70 g). The mixture was stirred under $H_2$ gas 5 kgf/cm$^2$ at room temperature for 2 h. The mixture was filtered, and the filtrate was concentrated in vacuo. The crude material was purified by distillation to give **7** as a colorless oil (3.82 g, 77%).

bp 99-102 °C (6.2-5.8 Torr). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.48–1.82 (10H, m), 2.00–3.00 (2H, br), 3.82–3.89 (2H, m). HRMS (CI$^+$) for $C_7H_{16}NO$ [M+H]$^+$: calcd, 130.1232; found, 130.1203.

### 5.1.49. 1-Amino-1-(1-hydroxypropane-2-yl)cyclobutane (8)

To a solution of **58** (6.81 g, 31.1 mmol) in EtOH (150 mL) was added 10% Pd-C (700 mg). The mixture was stirred under $H_2$ gas 5 kgf/cm$^2$ at room temperature for 6 h. The mixture was filtered, and the filtrate was concentrated in vacuo. The crude material was purified by distillation to give **8** as a colorless oil (2.96 g, 74%).

$^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.03 (3H, d, $J$ = 7.3 Hz), 1.63–1.74 (3H, m), 1.78–1.94 (2H, m), 2.10–2.28 (2H, m), 3.50 (1H, dd, $J$ = 11.0 and 4.3 Hz), 3.91 (1H, dd, $J$ = 11.0 and 3.1 Hz). HRMS (CI$^+$) for $C_7H_{16}NO$ [M+H]$^+$: calcd, 130.1232; found, 130.1254.

### 5.1.50. 1-(Benzoylamino)cyclobutylacetaldehyde (59)

To a stirred solution of **53** (6.86 g, 26.3 mmol) in THF (130 mL), DIBAL (40.0 mL, 1 M solution in toluene) was added dropwise at −78 °C for 0.5 h. After stirring at −78 °C for 6 h, to the mixture was added dropwise MeOH (10 mL) at −78 °C for 0.5 h, to which was added saturated aqueous $NH_4Cl$ (20 mL). The mixture was stirred at room temperature for 1 h and then extracted with $Et_2O$. The combined extracts were dried over anhydrous $Na_2SO_4$, filtered, and then concentrated in vacuo. The crude material was purified by flash column chromatography on silica gel (hexane:AcOEt = 2:1) to give **59** as a colorless solid (1.82 g, 32%).

Mp: 87–89 °C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.87–2.07 (2H, m), 2.25–2.34 (2H, m), 2.36–2.45 (2H, m), 3.28 (2H, s), 6.57 (1H, br s), 7.39–7.45 (2H, m), 7.46–7.53 (1H, m), 7.70–7.76 (2H, m), 9.79 (1H, s). HRMS (APCI$^+$) for $C_{13}H_{16}N_2O_2$ [M+H]$^+$: calcd, 218.11810; found, 218.11823. Anal. calcd for $C_{13}H_{15}N_2O_2$: C, 71.87; H, 6.96; N, 6.45. Found: C, 71.77; H, 7.16; N, 6.40.

### 5.1.51. 1-(Benzoylamino)-1-(2-hydroxypropane-1-yl)cyclobutane (60)

To a stirred solution of **59** (2.82 g, 13.0 mmol) in THF (30 mL), methylmagnesium chloride (14.0 mL, 3 M solution in THF) was added at 0 °C, and then the mixture was stirred at room temperature for 5 h. To this mixture was added saturated aqueous $NH_4Cl$ (10 mL) at 0 °C, and then extracted with ethyl acetate. The combined extracts were dried over anhydrous $Na_2SO_4$, filtered, and then concentrated in vacuo. The crude material was purified by flash column chromatography on silica gel (hexane:AcOEt = 2: 1) to give **60** as a colorless oil (2.65 g, 87%).

$^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.25 (3H, d, $J$ = 6.1 Hz), 1.77–2.12 (5H, m), 2.28–2.40 (1H, m), 2.52 (1H, q, $J$ = 10.4 Hz), 2.75–2.85 (1H, m), 3.26 (1H, d, $J$ = 3.7 Hz), 3.97–4.07 (1H, m), 6.91 (1H, br s), 7.39–7.46 (2H, m), 7.46–7.53 (1H, m), 7.75–7.82 (2H, m). HRMS (APCI$^-$) for $C_{14}H_{18}NO_2$ [M-H]$^+$: calcd, 232.11375; found, 232.13421.

### 5.1.52. 1-(Benzylamino)-1-(2-hydroxypropane-1-yl)cyclobutane (61)

To a solution of **60** (2.64 g, 11.3 mmol) in THF (40 mL) was added $LiAlH_4$ (1.20 g, 31.6 mmol) at room temperature and the mixture was stirred for 1 h, then, refluxed 0.5 h. The mixture was cooled on ice-water bath, to which was dropped a little water. The mixture was diluted with ethyl acetate, then dried over anhydrous $Na_2SO_4$, filtered, and then concentrated in vacuo. The crude material was purified by flash column chromatography on silica gel (AcOEt) to give **61** as a colorless oil (2.25 g, 91%).

$^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.21 (3H, d, $J$ = 6.1 Hz), 1.63 (1H, dd, $J$ = 13.7 and 2.4 Hz), 1.73–1.90 (5H, m), 2.04–2.13 (1H, m), 2.18–2.27 (1H, m), 3.59 (1H, d, $J$ = 11.6 Hz), 3.81 (1H, d, $J$ = 11.6 Hz), 4.07–4.16 (1H, m), 7.22–7.35 (5H, m). HRMS (CI$^+$) for $C_{14}H_{22}NO$ [M+H]$^+$: calcd, 220.1701; found, 220.1693.

### 5.1.53. 1-Amino-1-(2-hydroxypropane-1-yl)cyclobutane (9)

To a solution of **61** (2.24 g, 10.2 mmol) in EtOH (50 mL) was added 10% Pd-C (300 mg) and the mixture was stirred under $H_2$ gas 5 kgf/cm$^2$ at room temperature for 10 h. The mixture was filtered, and the filtrate was concentrated in vacuo. The crude material was purified by distillation to give **9** as a pale yellow oil (1.24 g, 94%).

$^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.17 (3H, d, $J$ = 6.1 Hz), 1.43 (1H, ddd, $J$ = 14.7, 10.4 and 1.2 Hz), 1.61–1.69 (1H, m), 1.72 (1H, dd, $J$ = 14.7 and 1.8 Hz), 1.75–1.90 (3H, m), 1.93–2.03 (1H, m), 2.12–2.20 (1H, m), 4.00–4.08 (1H, m). HRMS ($CI^+$) for $C_7H_{16}NO$ $[M+H]^+$: calcd, 130.1232; found, 130.1227.

#### 5.1.54. 1-(Benzoylamino)cyclobutylacetic acid (62)

To a solution of **53** (3.87 g, 14.8 mmol) in EtOH (30 mL) was added 3 N KOH (10 mL, 30 mmol) at room temperature. The mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated in vacuo and added to 6 N HCl (5.0 mL). The precipitate was collected by filtration, washed successively with water and diisopropyl ether, and then dried to give **62** as a colorless solid (3.15 g, 91%).

Mp: 183–185 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 1.76–1.89 (2H, m), 2.16–2.26 (2H, m), 2.28–2.40 (2H, m), 2.90 (2H, s), 7.43 (2H, t, $J$ = 6.7 Hz), 7.50 (1H, t, $J$ = 6.7 Hz), 7.83 (2H, d, $J$ = 6.7 Hz), 8.45 (1H, s), 12.00 (1H, br s). HRMS ($CI^+$) for $C_{13}H_{16}NO_3$ $[M+H]^+$: calcd, 234.1130; found, 234.1115. Anal. calcd for $C_{13}H_{15}NO_3$: C, 66.94; H, 6.48; N, 6.00. Found: C, 66.84; H, 6.52; N, 6.01.

#### 5.1.55. 1-(Benzoylamino)cyclobutylacetamide (63)

The mixture of **62** (1.00 g, 4.29 mmol) and thionyl chloride (3.20 mL, 43.9 mmol) was stirred at room temperature for 1 h. The mixture was concentrated in vacuo. The residue in THF (30 mL) was added to 25% aqueous ammonia (30 mL) cooled on ice water. The mixture was stirred at room temperature for 1 h. The mixture was diluted with AcOEt–MeOH (3:1 v/v) and then washed with water. The organic layer was dried over anhydrous $Na_2SO_4$, filtered, and then concentrated in vacuo to give **63** as a colorless solid (925 mg, 93%).

Mp: 215–217 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 1.75–1.87 (2H, m), 2.14–2.23 (2H, m), 2.37–2.47 (2H, m), 2.70 (2H, s), 6.80 (1H, s), 7.29 (1H, s), 7.43 (2H, t, $J$ = 6.7 Hz), 7.50 (1H, t, $J$ = 6.7 Hz), 7.82 (2H, d, $J$ = 6.7 Hz), 8.39 (1H, s). HRMS ($CI^+$) for $C_{13}H_{17}N_2O_2$ $[M+H]^+$: calcd, 233.1290; found, 233.1265. Anal. calcd for $C_{13}H_{16}N_2O_2$ 0.5H2O: C, 64.71; H, 6.68; N, 11.61. Found: C, 64.42; H, 6.69; N, 11.53.

#### 5.1.56. 1-(2-Aminoethyl)-1-(benzylamino)cyclobutane (64)

To a solution of **63** (687 mg, 3.00 mmol) in THF (30 mL) was added portionwise $LiAlH_4$ (570 mg, 15.0 mmol) at room temperature for 30 min and the mixture was heated to reflux for 5 h. The mixture was cooled on ice-water bath, to which was dropped aqueous THF and a little water. The mixture was filtered on celite, and the solution dried over anhydrous $Na_2SO_4$. The solution was concentrated in vacuo to give **64** as a pale yellow oil (609 mg, 99%).

$^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.65–1.80 (2H, m), 1.82 (2H, t, $J$ = 7.3 Hz), 1.86–2.02 (4H, m), 2.82 (2H, t, $J$ = 7.3 Hz), 3.65 (2H, s), 7.20–7.38 (5H, m). HRMS ($CI^+$) for $C_{13}H_{21}N_2$ $[M+H]^+$: calcd, 205.1705; found, 205.1743.

#### 5.1.57. 1-(Benzylamino)-1-[2-(*tert*-butoxycarbonylamino)ethyl]cyclobutane (65)

To a solution of **64** (205 mg, 1.00 mmol) in THF (1 mL) was added $Boc_2O$ (230 mg, 1.05 mmol) under ice-water cooling. The mixture was stirred in an ice-water bath for 30 min. The mixture was then concentrated in vacuo. The crude material was purified by flash column chromatography on silica gel (AcOEt:hexane = 2:1) to give **65** as a colorless solid (164 mg, 54%).

Mp: 67–70 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 1.36 (9H, s), 1.60–1.84 (6H, m), 1.84–1.94 (2H, m), 2.92–3.04 (2H, m), 3.53 (2H, s), 6.83 (1H, t, $J$ = 5.5 Hz), 7.21 (1H, t, $J$ = 7.3 Hz), 7.29 (2H, t, $J$ = 7.3 Hz), 7.35 (2H, d $J$ = 7.3 Hz). HRMS ($CI^+$) for $C_{18}H_{29}N_2O_2$ $[M+H]^+$: calcd, 305.2229; found, 305.2228. Anal. calcd for $C_{18}H_{28}N_2O_2$ 0.1$H_2O$: C, 70.60; H, 9.22; N, 9.15. Found: C, 70.38; H, 9.06; N, 8.92.

#### 5.1.58. 1-Amino-1-[2-(tert-butoxycarbonylamino)ethyl]cyclobutane (10)

To a solution of **65** (117 mg, 0.384 mmol) in EtOH (5 mL) was added 10% Pd-C (35 mg) and the mixture was stirred under $H_2$ gas 5 kgf/cm$^2$ at room temperature for 5 h. The mixture was filtered, and the filtrate was concentrated in vacuo. The crude material was purified by distillation to give **10** as a colorless solid (76.0 mg, 92%).

Mp: 53–55 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 1.37 (9H, s), 1.49–1.58 (2H, m), 1.62–1.77 (4H, m), 1.82–1.91 (2H, m), 2.92–3.01 (2H, m), 6.78 (1H, t, $J$ = 5.5 Hz). HRMS ($CI^+$) for $C_{11}H_{23}N_2O_2$ $[M+H]^+$: calcd, 215.1760; found, 215.1747. Anal. calcd for $C_{11}H_{22}N_2O_2$: C, 61.65; H, 10.35; N, 13.07. Found: C, 61.53; H, 10.29; N, 12.98.

### 5.2. Biology

#### 5.2.1. GSK-3β inhibitory assay[13]

First, 10–25 ng of recombinant full-length human GSK-3β is incubated in the presence or absence of the compound at varying concentrations for 1 h at 30 °C in 20 mM MOPS, pH 7.0, 10 mM magnesium acetate, 0.2 mM EDTA, 2 mM EGTA, 30 mM magnesium chloride, 62.5 μM phospho-glycogen synthase peptide-2, 5 μM ATP, 10 mM β-glycerol phosphate, 1 mM sodium orthovanadate, and 1 mM dithiothreitol. Proceed to KinaseGlo luciferase reaction (Promega), after which an equal volume of KinaseGlo luciferase reagent (Promega) is added. The luminescence is then read using a luminescence plate reader within 5–10 min. Compound activity is expressed as a percentage of inhibition relative to the maximal inhibition observed at the maximal dose. $IC_{50}$ values are calculated from the resulting curve using curve-fitting software (GraphPad Prizm).

#### 5.2.2. Glycogen synthesis assay in Hep G2 cells

Hep G2 cells were obtained from the Japanese Collection of Research Bioresources and were grown in standard culture medium: a low-glucose Dulbecco's modified Eagle's medium (DMEM), containing 10% fetal calf serum supplemented with 100 U/mL penicillin and 100 μg/mL streptomycin, in a humidified and 5% $CO_2$ atmosphere kept at 37 °C. The Hep G2 cells were harvested with 0.25% trypsin solution containing 1 mM EDTA and were seeded on 12-well plates at $1 \times 10^5$ cells per well. Following a culture for 3 days, the cells were washed once with phosphate-buffered saline (PBS), and were incubated with serum-free low-glucose DMEM supplemented with 100 U/mL penicillin and 100 μg/mL streptomycin. Following a culture for 3 h, the compounds provided herein at various concentrations and 2.5 μCi/mL D-[2-$^3$H]glucose (PerkinElmer, Boston, MA, USA) were added to the serum-free low-glucose DMEM. A vehicle control of DMSO (0.3%, final concentration) was also used. The total volume per well of the reaction medium was 1.0 mL of serum-free low-glucose DMEM. After incubation at 37 °C for 3 h, the medium was aspirated and the cells were washed twice with PBS, after which 0.25 mL of 1 N KOH containing 0.4 mg/mL carrier glycogen was added. After incubation at 37 °C for 30 min, 0.25 mL of 48.8% (w/v) KOH was added to each well for cell lysis. After incubation at 95 °C for 30 min, 1.5 mL of 95% (v/v) ethanol was added to the cell lysate. Total glycogen was precipitated overnight at −20 °C. Glycogen precipitates were recovered by centrifugation at 19,000 x g for 30 min at 4 °C. Precipitates were washed once with 1 mL of 70% (v/v) ethanol, and were re-suspended in 0.5 mL water. [$^3$H]Glucose incorporation into glycogen was assessed using a liquid scintillation counter (Packard Instrument, Meriden, CT, USA). The $EC_{50}$ values of the tested

compounds were derived from the curve fitting using the Prism program (GraphPad Software, San Diego, CA). The standard reference compound is **1** (CHIR99021) ($EC_{50}$ = 1.5 μM, 100% maximum activation at 30 μM).

#### 5.2.3. Anti-bacterial activity

Anti-bacterial activity was measured by determining MIC values using standard microdilution methods recommended by the Clinical and Laboratory Standards Institute with Mueller–Hinton Broth (Becton Dickinson, Cockeysville, MD). The MIC was defined as the lowest concentration of the compound that inhibited visible growth after incubation for 18 h at 35 °C. See: Wikler, M. A.; Cockerill, F. R., III; Bush, K.; Dudley, M. N.; Eliopoulos, G. M.; Hardy, D. J.; Hindler, J. F.; Patel, J. B.; Powell, M.; Turnidge, J. D.; Weinstein, M. P.; Zimmer, B. L.; Ferraro, M. J.; Swenson, J.M. in Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard-Eighth Edition. Clinical and Laboratory Standards Institute, 2009, M07-A8.

#### 5.2.4. Animal study: oral glucose tolerance test

Male Crlj:CD1 (ICR) mice were obtained from Charles River Laboratories Japan (Yokohama, Japan). All mice were given a standard diet (Clea Japan, Tokyo, Japan) and tap water *ad libitum*. All institutional guidelines for animal care and use were applied in this study. Test compounds were suspended in 0.3% carboxymethylcellulose sodium salt (CMC-Na; Sigma, St. Louis, MO). After fasting for 15–17 h, the test compound (3, 10, 30, 100, or 300 mg/kg) or vehicle (0.3% CMC-Na) was orally administered to 7-week-old ICR mice. A glucose solution (5 g/kg) was orally administered at 30 min after the test compound treatment. Blood samples were collected from the tail vein using capillary tubes containing EDTA•2 K before test compound treatment, as well as at 0, 0.5, 1, and 2 h after glucose load. The blood samples were centrifuged at 2,500×*g* for 5 min, and separated plasma was kept on ice and analyzed on the same day. Plasma glucose levels were determined using the glucose C II-test (Wako Pure Chemical Industries, Osaka, Japan). The sum of plasma glucose levels at 0.5 and 1 h after glucose load was compared to that of vehicle treatment, and the results were presented as a percentage decrease.

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