Piperidine Carboxylic Acid Derivatives of 10*H*-Pyrazino[2,3-*b*][1,4]benzothiazine as Orally-Active Adhesion Molecule Inhibitors

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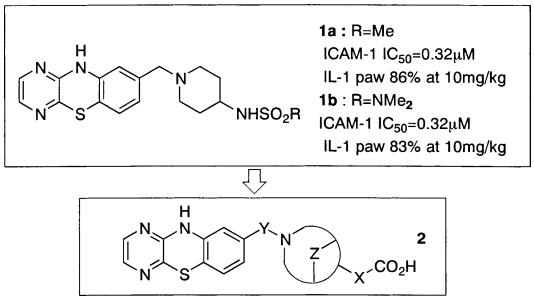
Novel piperidine carboxylic acid derivatives of 10*H*-pyrazino[2,3-*b*][1,4]benzothiazine were prepared and evaluated for their inhibitory activity on the upregulation of adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1). Replacement of the methanesulfonyl group on the piperidine ring of previously prepared derivatives with a carboxylic acid-containing moiety resulted in a number of potent adhesion molecule inhibitors. Of these, (*anti*) [3-(10*H*-pyrazino[2,3-*b*][1,4]benzothiazin-8-yl)methyl-3-azabicyclo[3.3.1]non-9-yl]acetic acid 2q (ER-49890), showed the most potent oral inhibitory activities against neutrophil migration in an interleukin-1 (IL-1) induced paw inflammation model using mice, and leukocyte accumulation in a carrageenan pleurisy model in the rat, and therapeutic effect on collagen-induced arthritis in rats.

Key words ER-49890; adhesion molecule inhibitor; 10*H*-pyrazino[2,3-*b*][1,4]benzothiazine

Adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1), E-selectin and vascular cell adhesion molecule-1 (VCAM-1), play a pivotal role in the inflammatory and immunological responses. They are initially upregulated on the endothelium by tumor necrosis factor-α (TNF-α) or interleukin-1 (IL-1) and go on to mediate the steps of leukocyte migration from the vasculature into the inflamed tissue, these leukocytes then promote the inflammatory response.[1] Also, the interaction of ICAM-1 and VCAM-1 expressed on antigen presenting cells with their ligands, such as lymphocyte function associated antigen-1 (LFA-1) and very late activation antigen-4 (VLA-4), has been involved in activation of T cells.[2—5] Recently, the antigen recognition step has been visualized and ICAM-1 identified as one important component of the immunological synapse.[6] Hence, it is hoped that interfering with these interactions will not only have an effect on leukocyte infiltration, but also modulate T cell response. Furthermore, it has been reported that anti-adhesion molecule antibodies can ameliorate the inflammatory reaction and immunological parameters in various animal models.[7] For these reasons the development of cell adhesion molecule inhibitors as novel therapeutics for inflammatory and autoimmune diseases has been widely anticipated, and a considerable literature on such compounds has appeared.[8—11]

We have reported that the piperidine sulfonamide and sulfamide derivatives of 10*H*-pyrazino[2,3-*b*][1,4]benzothiazine **1a** and **1b** have potent ICAM-1 upregulation inhibitory activity and suppresses neutrophil infiltration in an IL-1-induced paw inflammation model.[12] We report here the effect of replacing the piperidine sulfonamide moiety of **1a** in order to investigate further the structure–activity relationships of this family. Of particular interest to us was the introduction of a carboxylic acid group, a bioisostere of the sulfonamide function.



Chemistry We first prepared the right moieties of the compounds **2**. The piperidine carboxylates **4a**—**c** are either commercially available or known in the literature.[13,14] The other piperidine carboxylates **4d**—**i** and **4l**—**v** were prepared as shown in Charts 1—11. The syntheses of **4d** and **4e** are shown in Chart 1. Coupling the aldehyde **6**[15] with the appropriate Horner–Emmons reagent gave the unsaturated ester **7**. Hydrogenation of the resulting ester **7** using 10% palladium on carbon (Pd/C) followed by deprotection of the piperidine amine group with 4 N HCl/AcOEt provided saturated ester **4d**. The unsaturated ester **4e** was also obtained by removal of the Boc group of compound **7** using the same procedure.

The acetylenic ester **4f** was prepared as shown in Chart 2. Reaction of 4-piperidine aldehyde **9** with the Corey–Fuchs

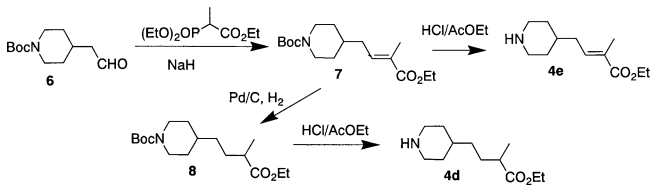


Chart 1

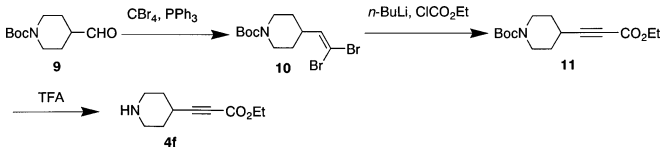


Chart 2

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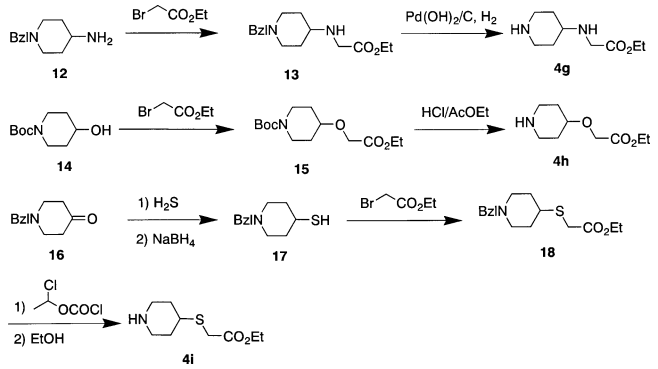


Chart 3

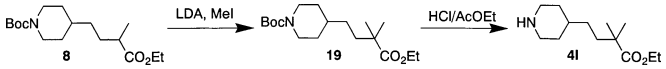


Chart 4

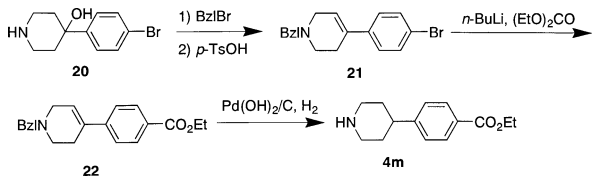


Chart 5

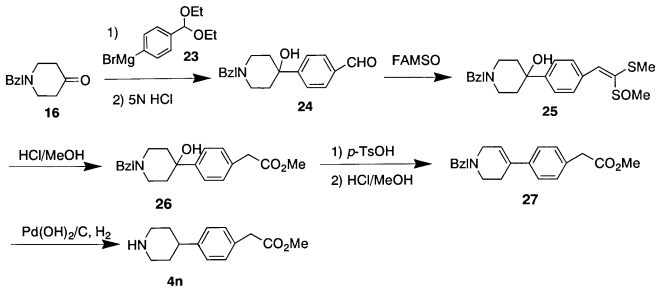


Chart 6

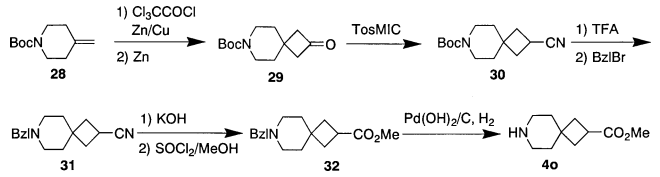


Chart 7

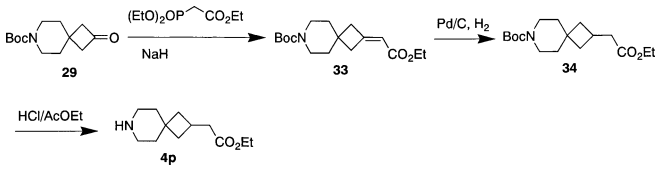


Chart 8

reagent provided dibromo compound **10**. This compound was treated with *n*-butyllithium (*n*-BuLi) to afford the acetylide, which was reacted with ethyl chloroformate.[16] Deprotection of **11** provided acetylene intermediate **4f**.

The amino-, oxy- and thio-acetic acid piperidine derivatives **4g**—**i** were prepared as shown in Chart 3. The appropriate amino- or hydroxy-piperidine **12** or **14** was alkylated with ethyl bromoacetate followed by deprotection to give compounds **4g** or **4h** respectively. Mercaptopiperidine **17** was synthesized using the method of Barrera as shown in Chart 3.[17] Benzyl piperidone **16** was converted to its dithiol, which on reduction with sodium borohydride gave **17**. Condensation of **17** with ethyl bromoacetate followed by removal of the benzyl group using 1-chloroethyl chloroformate (ACE-Cl) afforded compound **4i**.

The α,α-dimethyl ester **4l** was synthesized as shown in Chart 4. Methylation of carboxylate **8** with methyl iodide followed by deprotection using HCl in AcOEt gave **4l**.

Compound **4m** was prepared as shown in Chart 5. *N*-Alkylation of hydroxypiperidine derivative **20** with benzyl bromide followed by dehydration using *p*-toluenesulfonic acid (*p*-TsOH) in toluene gave 1,2,5,6-tetrahydropyridine derivative **21**. This bromide **21** was treated with *n*-BuLi followed by condensation with diethyl carbonate to furnish ester **22**. Hydrogenation of **22** using Pearlman's catalyst yielded the benzoate derivative of piperidine **4m**.

The synthesis of the phenylacetic acid derivative of piperidine **4n** was achieved as illustrated in Chart 6. Treatment of benzyl piperidone **16** with phenyl Grignard reagent **23** followed by deprotection furnished the benzaldehyde **24**. The phenylacetate **26** was obtained by condensation of aldehyde **24** with methyl methylsulfinylmethyl sulfide (FAMSO) and hydrolysis using 10% HCl/MeOH. Treatment of **26** with *p*-TsOH, followed by hydrogenation using $Pd(OH)_2/C$ afforded the piperidine phenylacetate **4n**.

The (7-azaspiro[3.5]non-2-yl)carboxylate **4o** was prepared as shown in Chart 7. The cycloaddition reaction between olefin **28**[18] and dichloroketene, generated *in situ* from trichloroacetyl chloride and zinc/copper, followed by reduction with zinc afforded the 7-azaspiro[3.5]nonane core of **29**. Treatment of this ketone **29** with tosylmethyl isocyanide (TosMIC) in the presence of potassium *tert*-butoxide afforded the cyanide **30**. Deprotection of the piperidine amino group with trifluoroacetic acid (TFA) followed by reprotection furnished the benzyl amine **31**. Hydrolysis of the cyano moiety provided the carboxylic acid. Treatment with thionyl chloride in MeOH, then debenzylation using $Pd(OH)_2/C$ gave amine **4o**.

Compound **4p** was synthesized as shown in Chart 8. Compound **33**, prepared by treatment of ketone **29** with the appropriate Horner–Emmons reagent, was hydrogenated over Pd/C to give saturated ester **34**. Deprotection of the piperidine amino group using HCl/AcOEt gave amine **4p**.

The syntheses of bicyclic amines **4q**—**v** are shown in Charts 9—11. Compounds **4q** and **4r** were prepared as shown in Chart 9. Hydrogenation of unsaturated ester **35**[19] using palladium on carbon provided a *anti–syn* mixture of **36** in the ratio of 3 : 1. This ratio was determined by integration of the equatorial protons of C-2 and C-4 in the ^{1}H-NMR spectra of the 3-azabicyclo[3.3.1]nonanes at 2.90 ppm (*anti*) and 2.62 ppm (*syn*) respectively. To separate the isomers, the protective group on the nitrogen was exchanged using vinyl chloroformate. The *anti*- and *syn*-isomers were isolated from the mixture using column chromatography (seven times)

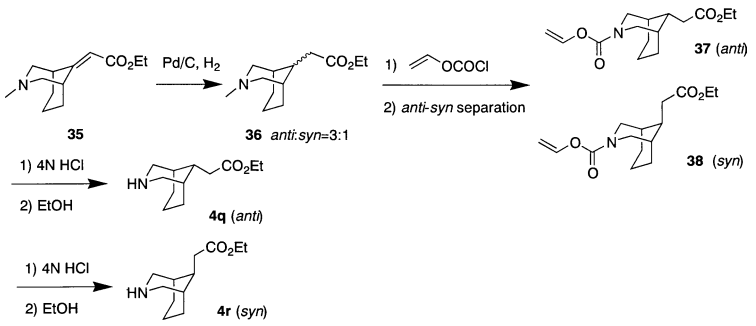


Chart 9

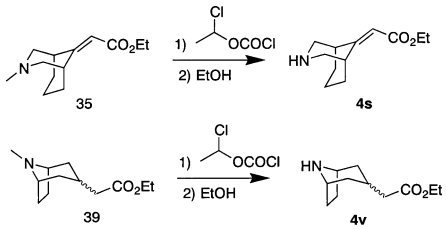


Chart 10

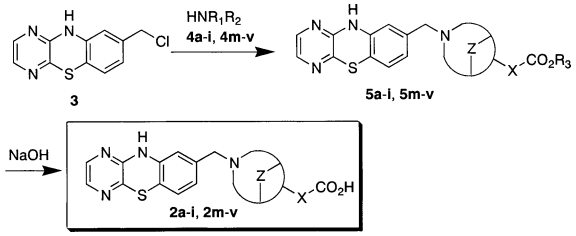


Chart 12

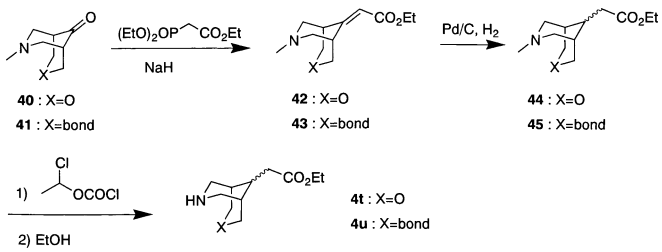


Chart 11

with a yield of 29% (**37**) and 13% (**38**) respectively. Chlorination of **37** or **38** with 4 N hydrochloric acid in dioxane, followed by hydrolysis afforded the important intermediates **4q** or **4r** respectively.

The syntheses of bicyclic intermediates **4s** and **4v** are shown in Chart 10. *N*-Demethylation of **35** or the known compound **39**[20] using ACE-Cl provided the corresponding secondary amine.

Compounds **4t** and **4u** were synthesized as shown in Chart 11. Unsaturated esters **42** and **43**, prepared by treatment of the ketones **40**[21] and **41**[22] with the appropriate Horner–Emmons reagent, were hydrogenated over Pd/C to give saturated esters **44** and **45**. Deprotection of the amino group of the bicyclic ring using ACE-Cl gave the corresponding amines **4t** and **4u**.

With the exceptions of **2j**, **2k** and **2l**, piperidine carboxylic acid derivatives of 10*H*-pyrazino[2,3-*b*][1,4]benzothiazine **2a**—**v** were synthesized as shown in Chart 12. Condensation of readily available chloride **3**[12] with the appropriate piperidine carboxylates **4a**—**i** and **4m**—**v** in DMF using K_2CO_3 provided the esters **5a**—**i** and **5m**—**v**. Hydrolysis of these esters under alkaline conditions provided the desired compounds **2a**—**i** and **2m**—**v**.

The synthesis of **2j** is shown in Chart 13. Protection of readily available ester **46**[12] with methoxymethyl chloride (MOMCl) gave the compound **47**, which was reduced with lithium aluminum hydride ($LiAlH_4$) and chlorinated to afford the intermediate **49**. Cyanation of **49** followed by hydrolysis and esterification gave **51**. Reduction of this ester and methanesulfonylation followed by condensation with **4d** gave **53**. Deprotection then hydrolysis of **53** using 5 N HCl followed by NaOH afforded target compound **2j**.

The synthesis of amide junction derivative **2k** is shown in Chart 14. Hydrolysis of ester **47** and subsequent condensation with piperidine ester **4d** *via* the acid anhydride gave piperidine amide **56**. Hydrolysis of ester **56** under alkaline conditions followed by deprotection with 5 N HCl gave carboxylic acid **2k**.

The synthesis of α,α-dimethyl carboxylic acid derivative **2l** is shown in Chart 15. Condensation between **49** and piperidine ester **4l** gave compound **58**. Hydrolysis of this ester **58** under alkaline conditions followed by deprotection of the *N*-methoxymethyl group of **59** with 5 N HCl gave carboxylic acid **2l**.

Results and Discussion

The synthetic compounds were first evaluated for their *in vitro* inhibitory activity on the expression of the adhesion molecule ICAM-1 by TNF-α-stimulated human umbilical vein endothelial cells (HUVEC). The effect of the compounds on neutrophil accumulation after oral administration was evaluated in a mouse IL-1-induced paw inflammation model. In this model, myeloperoxidase activity was measured as the index of neutrophil infiltration.[12]

Table 1 summarizes the results of our initial studies with carboxylic acid derivatives. We first evaluated piperidine 4-carboxylic acid **2a**. The ICAM-1 inhibitory activity of this compound was only 3-fold less than that of sulfonamide **1a**. However **2a** showed only 61.5% inhibition of neutrophil accumulation in the IL-1-induced paw model even at 100 mg/kg *p.o.* Simple replacement of the methansulfon-

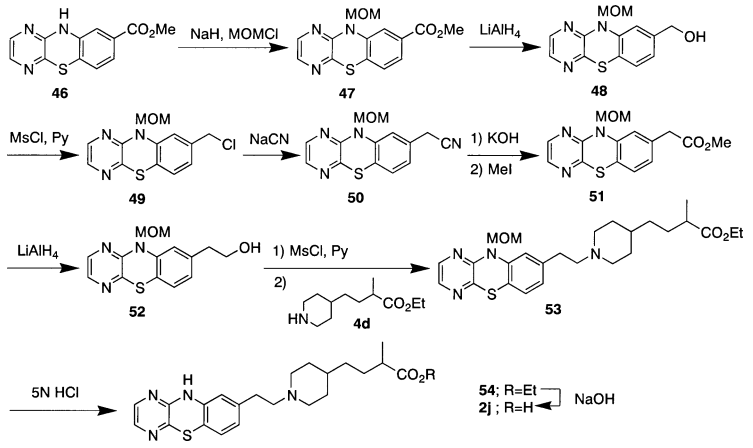


Chart 13

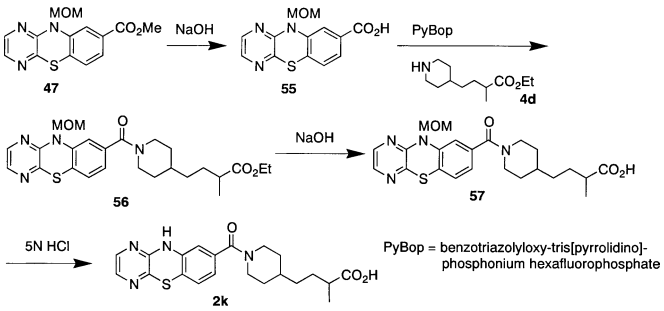


Chart 14

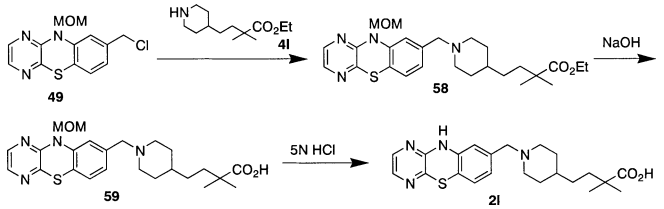


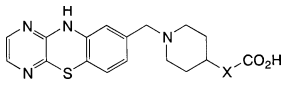
Chart 15

amide moiety of **1a** by a carboxylic acid was shown not to be suitable. We went on to look for suitable linkers between the piperidine ring and carboxylic acid. Methylene-elongation enhanced inhibitory activity both *in vitro* and *in vivo* by about 2—3-fold (**2b**—**d**). Double bond insertion was also acceptable, but resulted in a slight loss of inhibitory activity in the IL-1 paw model (**2e**). Triple bond insertion led to a loss in potency of ICAM-1 inhibitory activity (**2f**). Introduction of a heteroatom such as oxygen or nitrogen into this linker resulted in loss of potency (**2g**, **h**). Thio derivative **2i** also had only weak ICAM-1 inhibitory activity and low potency in the IL-1 paw model. Insertion of a heteroatom into this linker is clearly unfavorable.

Next, we modified the benzyl position Y between the 10*H*-pyrazino[2,3-*b*][1,4]benzothiazine core and the piperidine ring. The ethylene compound **2j** showed 5.6-fold lower *in vitro* activity than **2d** and had weak inhibitory activity in the IL-1 paw model at an oral dose of 30 mg/kg. The amide type **2k** exhibited complete loss of *in vitro* potency. Thus a single methylene is the most suitable linker. All of the above piperidine derivatives have only moderate potency even at a dose of 30 mg/kg in IL-1-induced paw model.

We further examined the linker between piperidine and

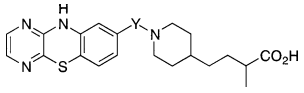
Table 1. Biological Data for Piperidine Carboxylic Acids (**2a**—**i**)



Compound No.	–X–CO_2H	ICAM-1[a] IC_{50} (μM)	IL-1 paw[b] (30 mg/kg, *p.o.*)	ClogP
2a	—CO_2H	1.0	61.5±4.4[c]	0.40
2b	CO_2H	0.53	71.9±11.7	1.02
2c	CO_2H	0.47	69.2±3.5	1.55
2d	Me, CO_2H	0.85	66.8±10.7	2.60
2e	Me, CO_2H	0.42	43.4±5.5	2.40
2f	—≡—CO_2H	>10	N.T.	1.14
2g	N, CO_2H	>10	N.T.	−0.26
2h	O, CO_2H	>10	N.T.	0.20
2i	S, CO_2H	4.8	14.5±15.9	0.80

a) Concentration of compound inhibiting ICAM-1 up-regulation by 50% of control value. *b*) Percentage inhibition of neutrophil infiltration in the mouse IL-1-induced paw inflammation model at a dose of 30 mg/kg *p.o.* Values are the mean of three animals. *c*) Percentage inhibition of neutrophil infiltration in the mouse IL-1-induced paw inflammation model at a dose of 100 mg/kg *p.o.* N.T.: not tested.

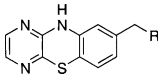
Table 2. Biological Data for Linker Y Modified Derivatives (**2j**, **k**)

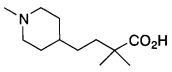
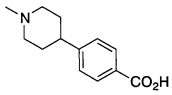
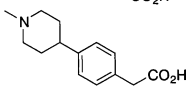
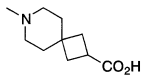
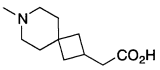
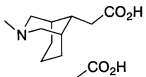
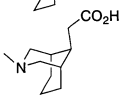


Compound No.	–Y–	ICAM-1[a] IC_{50} (μM)	IL-1 paw[b] (30 mg/kg, *p.o.*)	ClogP
2j		4.8	28.7±3.4	2.74
2k	O	>10	N.T.	3.58

a) Concentration of compound inhibiting ICAM-1 up-regulation by 50% of control value. *b*) Percentage inhibition of neutrophil infiltration in the mouse IL-1-induced paw inflammation model at a dose of 30 mg/kg *p.o.* Values are the mean of three animals. N.T.: not tested.

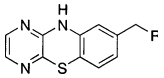
Table 3. Biological Data for Ring-Inserted Derivatives (**2l**—**r**)

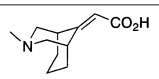
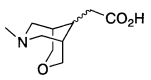
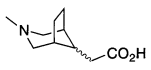


Compound No.	R	ICAM-1[a] IC_{50} (μM)	IL-1 paw[b] (10 mg/kg, *p.o.*)	ClogP
2l		0.88	21.7±16.1	3.00
2m		1.0	0	3.07
2n		0.71	88.0±0.9	2.59
2o		0.93	82.8±6.8	1.27
2p		0.90	88.8±2.0	1.89
2q		3.0	91.5±4.5	1.89
2r		2.3	80.6±8.6	1.89

a) Concentration of compound inhibiting ICAM-1 up-regulation by 50% of control value. *b*) Percentage inhibition of neutrophil infiltration in the mouse IL-1-induced paw inflammation model at a dose of 10 mg/kg *p.o.* Values are the mean of three animals.

Table 4. Biological Data for Bicyclic Ring-Inserted Derivatives (**2s**—**v**)



Compound No.	R	ICAM-1[a] IC_{50} (μM)	IL-1 paw[b] (10 mg/kg, *p.o.*)	ClogP
2s		9.4	N.T.	1.91
2t		9.6	0	0.24
2u		0.81	83.2±1.2	1.33
2v		>10	N.T.	1.33

a) Concentration of compound inhibiting ICAM-1 up-regulation by 50% of control value. *b*) Percentage inhibition of neutrophil infiltration in the mouse IL-1-induced paw inflammation model at a dose of 10 mg/kg *p.o.* Values are the mean of three animals.

carboxylic acid. We wished to prepare symmetric compounds that have significant activity in the IL-1 paw model as do **1a** and **1b**, but the α,α-dimethylcarboxylic acid derivative **2l** showed only 21.7% inhibition in this model at a dose of 10 mg/kg *p.o.* Hence we investigated ring insertion. Introduction of a phenyl ring, such as in benzoic acid **2m** and phenylacetic acid **2n**, gave compounds with the same *in vitro* potency as **2d**, though only **2n** showed significant activity in the IL-1 paw model at a dose of 10 mg/kg *p.o.* This *in vivo* activity of **2n** is 3-fold stronger than that of **2d** and is comparable to that of **1**. We speculated that aliphatic carboxylic acids may be more effective in providing *in vivo* active compounds than aromatic acids, and so 7-azaspiro[3.5]nonane derivatives such as **2o** and **2p** were prepared. These compounds retained ICAM-1 inhibitory activity and inhibited significantly neutrophil migration in the IL-1 paw model at a dose of 10 mg/kg *p.o.* The insertion of a rigid ring between the piperidine and aliphatic carboxylic acid is preferable for *in vivo* activity, and provides a good carrier moiety. We next focused on rigid ring systems such as bridged bicyclic derivatives in place of the piperidine ring. 3-Azabicyclo-[3.3.1]nonane ring derivatives **2q** and **2r** showed moderate *in vitro* inhibitory activities. Surprisingly, both compounds showed significant potency in the IL-1 induced paw model equivalent to that of **1b** and **2n**.

We further evaluated the potency of some carboxylic acid derivatives of 3-azabicyclo[3.3.1]nonane-like skeletons as shown in Table 4. α,β-Unsaturated carboxylic acid **2s** had 3-fold weaker ICAM-1 inhibitory activity compared to **2q**. The 7-oxo derivative of 3-azabicyclo[3.3.1]nonane **2t** also had 3-fold weaker potency than **2q**, and no inhibitory activity in IL-1 paw model at a dose of 10 mg/kg. The potency of other bicyclic derivatives was examined. 3-Azabicyclo[3.2.1]octane derivative **2u** showed significant activity both with respect to ICAM-1 inhibition and in the IL-1-induced paw inflammation model. But the tropane ring compound **2v** lost potency towards ICAM-1 inhibition.

In our previous report,[12] the preferable ClogP value of sulfonamide derivatives, **1a** and **1b** that were highly potent in the IL-1 paw inflammation model, were stated to be around 0.6 as calculated using a previous version of ClogP software. We have also calculated and compared the values for carboxylic acid derivatives. The values of ClogP for **2n**—**r** and **2u** were within the range 1.27—2.59, and especially those of **2p**—**r** were 1.9.[23] To compare the values for the two compound classes, the ClogP of **1a** and **1b** were re-calculated using the current version, and the results were 1.99 and 1.85, *i.e.* almost the same as the values of **2p**—**r** in the carboxylic acid series. These trends indicate that a value for the lipophilic parameter ClogP≒1.9 is a common important factor for activity for both types of compound in our *in vivo* models.

Of these compounds, we selected **2q** for further evaluation. The inhibitory activity of **2q** on other adhesion molecules was measured and the IC_{50} for inhibition of VCAM-1 found to be 2.5 μM, and for E-selectin found to be 4.5 μM.

Compared with **1b**, **2q** showed moderate *in vitro* ICAM-1 inhibitory activity but strong potency in the IL-1-induced paw model at a dose of 10 mg/kg. We examined the plasma concentration of each compound at the time when *in vivo* potency was evaluated. As shown in Table 5, **2q** showed about 28-fold higher plasma concentration than **1b**. Thus the strong inhibitory activity of **2q** likely results mainly from its much higher plasma concentrations.

Furthermore, the effects of **2q** and **1b** were evaluated in the carrageenan-induced pleurisy model in the rat. Intrapleural injection of carrageenan induces leukocyte infiltration into the pleural cavity. An antibody to ICAM-1 has been reported effective in reducing leukocyte infiltration in the pleural cavity in this model.[24] Compound **2q** significantly in-

Table 5. Biological Activities and One Point Plasma Concentrations of **1b** and **2q**

	ICAM-1 IC_{50} (μM)	IL-1 paw[a] 10 mg/kg *p.o.*	Concentration in plasma (μM)
1b	0.32	83.4±7.4	1.02±0.33
2q	3.0	91.5±4.5	28.8±2.5

a) Percentage inhibition of neutrophil infiltration in the mouse IL-1-induced paw inflammation model at a dose of 10 mg/kg *p.o.*

Table 6. Effects of **1b** and **2q** on Carrageenan-Induced Pleurisy Model

	Dose (mg/kg)	Leukocyte infiltration[a]
2q	1	36*±4
	3	49*±3
	10	67*±3
	30	91*±3
1b	3	35*±10
	10	51*±11

a) Percentage inhibition of leukocyte infiltration. Results were expressed as the mean±standard error of the mean (S.E.M.). Significant difference was obtained by one-way ANOVA, $*p<0.05$. Values are the mean of six animals.

Table 7. Pharmacokinetic Property of **2q** and **1b**[12] in Rats

	T_{max} (h)	C_{max} (μM)	$AUC_{0-24\,h}$ (μM·h)	$MRT_{0-24\,h}$ (h)	B.A.$_{0-24\,h}$ (%)
2q, 3 mg/kg, *p.o.*	1.0	13.6	90.1	5.14	98.5
1b, 10 mg/kg, *p.o.*	9.0	0.86	11.5	11.4	69.0

	$T_{1/2}(\alpha)$ (h)	$T_{1/2}(\beta)$ (h)	CL_t (ml/h/kg)	V_{dss} (ml/kg)
2q, 3 mg/kg, i.v.	0.69	4.17	84.5	287.4
1b, 3 mg/kg, i.v.	0.33	2.30	1335	3566

MRT is mean residence time.

hibited leukocyte infiltration from a dose of 1 mg/kg *p.o.* The potency of compound **1b** was 3-fold less than that of **2q**.

The effect of compound **2q** in the type II collagen-induced arthritis model in the rat was examined (Fig. 1). In an experiment examining therapeutic treatment, compound **2q** at doses of 2.5 to 20 mg/kg suppressed dose-dependently further increase in paw swelling.

Pharmacokinetic studies of **2q** were carried out in the fed male rat (3 mg/kg *p.o.*, 3 mg/kg i.v., $n=2$). This compound has a C_{max} of 13.6 μM, a mean residence time (*MRT*) of 5.14 h, and an oral bioavailability of 98.5%. Due to its better pharmacokinetics in the rat, **2q** also showed significant potency in the carrageenan pleurisy model in the rat, greater than might be predicted from its moderate *in vitro* ICAM-1 inhibition compared to that of **1b**.

Conclusion

We have studied derivatives of compound **1** in which a carboxylic acid-containing group has been used as a bioisostere of the sulfonamide on the piperidine ring. Simple replacement of the sulfonamide gave a derivative with only moderate potency in the IL-1-induced paw inflammation model

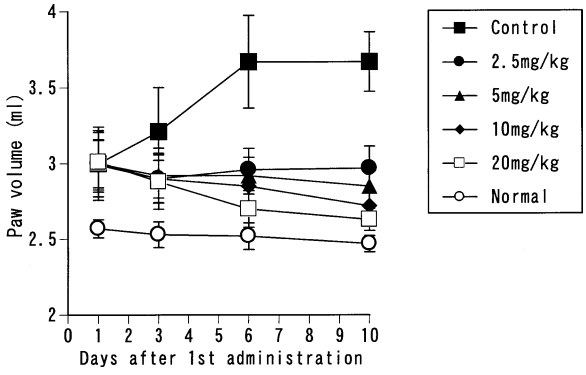


Fig. 1. Therapeutic Effect of **2q** on Type II Collagen-Induced Arthritis in Rats

Lewis female rats were immunized with an emulsion containing type II collagen and muramyl dipeptide in Freund's incomplete adjuvant. After 14 d, the rat were grouped with the same mean volume of paws at 3.0 ml. Compound **2q** was orally administered daily for 10 d from day 14 to day 23. Each points show mean±S.E.M. ($n=8$).

even at a dose of 100 mg/kg. By modifying the piperidine moiety, we found that rigid ring insertion, an aliphatic carboxylic acid and ClogP≒1.9 are important for high *in vivo* activities. In particular, **2q** significantly inhibited neutrophil migration in the mouse model at a dose of 10 mg/kg *p.o.* due to its good pharmacokinetic properties. In carrageenan-induced pleurisy model, **2q** also exhibited preferable potency in leukocyte infiltration. And furthermore, **2q** showed significant therapeutic effect in type II collagen-induced arthritis in rats. **2q** (ER-49890) was selected as a candidate for further evaluation of its pharmacological properties. This compound is expected to be a promising drug candidate for the treatment of autoimmune inflammatory diseases such as rheumatoid arthritis.

Experimental

Melting points were measured using a Yanako melting-point apparatus and are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a Varian Unity 400 (400 MHz) spectrometer, and chemical shifts are expressed in ppm downfield from tetramethylsilane (TMS) as an internal reference. The following abbreviations are used: s=singlet, d=doublet, t=triplet, q=quartet, quint=quintet, sext=sextet, sept=septet, m=multiplet, br=broad. Mass spectra (MS) were obtained on a SSQ 7000 mass spectrometer. High resolution mass spectra (HR-MS) were obtained on a Q-Tof ultima global mass spectrometer. Elemental analysis was performed with a Elemental Analyzer vario EL III. Materials were used as bought without any special purification. Silica gel (Kieselgel 60, Merck) was used for column chromatography, and silica gel (Kieselgel 60 F_{254}, layer thickness 0.25 mm, Merck) for analytical thin layer chromatography (TLC). All organic extracts were dried over anhydrous $MgSO_4$, and solvents were removed with a rotary evaporator under reduced pressure.

Ethyl (*E*)-4-[1-(*tert*-Butoxycarbonyl)piperidin-4-yl]-2-methyl-2-butenoate (7) To a suspension of sodium hydride (NaH, 60% dispersion in mineral oil, 5.2 g, 130 mmol) in *N*,*N*-dimethylformamide (DMF, 200 ml) was added triethyl 2-phosphonopropionate (31 g, 130 mmol) at room temperature (rt), and the mixture was stirred at the same temperature for 30 min. To this solution was added a solution of **6**[15] (21 g, 92 mmol) in DMF (100 ml) at 0 °C, and the reaction mixture was stirred at rt for 12 h. The resulting mixture was poured into ice-water, and extracted with ethyl acetate (AcOEt). The extract was washed with brine, dried, and evaporated. The residue was chromatographed on silica gel (10% AcOEt–hexane) to give 26 g (90%) of **7** as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.15 (2H, dq, J=3.6, 12.0 Hz), 1.30 (3H, t, J=7.2 Hz), 1.46 (9H, s), 1.50—1.70 (1H, m), 1.67 (2H, br d, J=12.4 Hz), 1.83 (3H, s), 2.13 (2H, t, J=7.6 Hz), 2.68 (2H, br t, J=12.0 Hz), 4.00—4.20 (2H, m), 4.19 (2H, q, J=7.2 Hz), 6.76 (1H, t, J=7.6 Hz). ESI-MS *m/z*: 334 $(M+Na)^+$.

Ethyl 4-[1-(*tert*-Butoxycarbonyl)piperidin-4-yl]-2-methylbutanoate (8)

A suspension of **7** (13.8 g, 44.3 mmol) and 10% palladium on carbon (Pd/C, 0.95 g) in ethanol (EtOH, 100 ml) was stirred under a hydrogen atmosphere at rt for 2 d. The catalyst was removed by filtration and the filtrate was concentrated to give 12.1 g (87%) of **8** as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.00—1.15 (2H, m), 1.14 (3H, d, J=6.8 Hz), 1.16—1.30 (2H, m), 1.25 (3H, t, J=7.2 Hz), 1.30—1.50 (1H, m), 1.45 (9H, s), 1.50—1.74 (4H, m), 2.38 (1H, sext, J=6.8 Hz), 2.66 (2H, br t, J=12.0 Hz), 3.95—4.20 (2H, m), 4.13 (2H, q, J=7.2 Hz). ESI-MS m/z: 336 $(M+Na)^+$.

Ethyl 4-[1-(*tert*-Butoxycarbonyl)piperidin-4-yl]propynate (11) To a solution of carbon tetrabromide (13 g, 38 mmol) and triphenylphosphine (20 g, 76 mmol) in dichloromethane (CH_2Cl_2, 200 ml) was added dropwise a solution of **9** (4.0 g, 19 mmol) in CH_2Cl_2 (20 ml) at 0 °C, and the mixture was stirred at 0 °C for 1 h. This solution was diluted with diethyl ether (Et_2O, 200 ml), and the precipitate was filtered off. The filtrate was concentrated *in vacuo*. The residue was chromatographed on silica gel (6% AcOEt–hexane) to give 5.7 g (81%) of **10** as a colorless oil. To a solution of **10** (2.7 g, 7.3 mmol) in tetrahydrofuran (THF, 50 ml) was added *n*-butyllithium (*n*-BuLi, 1.0 M solution in hexane, 15 ml, 15 mmol) at −78 °C under a nitrogen atmosphere, and the mixture was stirred at −78 °C for 1 h and at 0 °C for 1 h. Ethyl chloroformate (2.5 ml, 26 mmol) was added at −78 °C, and the reaction mixture was allowed to warm to rt. The resulting mixture was poured into water, and extracted with AcOEt. The extract was washed with brine, dried and evaporated. The residue was chromatographed on silica gel (10% AcOEt–hexane) to give 2.1 g (quant.) of **11** as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.31 (3H, t, J=7.2 Hz), 1.45 (9H, s), 1.59—1.70 (2H, m), 1.78—1.87 (2H, m), 2.71 (1H, sept, J=4.0 Hz), 3.12—3.22 (2H, m), 3.64—3.78 (2H, m), 4.22 (2H, q, J=7.2 Hz). ESI-MS m/z: 304 $(M+Na)^+$.

Ethyl [1-(Benzyl)piperidin-4-yl]aminoacetate (13) To a suspension of 1-benzyl-4-aminopiperidine **12** (10 g, 53 mmol) and potassium carbonate (K_2CO_3, 11 g, 80 mmol) in DMF (50 ml) was added ethyl bromoacetate (5.8 ml, 52 mmol) at 0 °C over 20 min, and the mixture was stirred at rt for 12 h. The resulting mixture was poured into ice-water, and extracted with AcOEt. The extract was washed with brine, dried, and evaporated. The residue was chromatographed on NH silica gel (Fuji Silysia, DW2035, 10% AcOEt–hexane) to give 12 g (82%) of **13** as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.27 (3H, t, J=6.8 Hz), 1.36—1.48 (2H, m), 1.76—1.84 (2H, m), 2.01 (2H, dt, J=2.0, 12.0 Hz), 2.45 (1H, tt, J=4.0, 10.0 Hz), 2.80—2.88 (2H, m), 3.41 (2H, s), 3.49 (2H, s), 4.18 (2H, q, J=6.8 Hz), 7.20—7.34 (5H, m). ESI-MS m/z: 277 $(M+H)^+$.

Ethyl [1-(*tert*-Butoxycarbonyl)piperidin-4-yl]oxyacetate (15) To a solution of 1-*tert*-butoxycarbonyl-4-piperidinol **14** (8.0 g, 40 mmol) in DMF (100 ml) was added NaH (60% dispersion in mineral oil, 1.8 g, 45 mmol) at rt, and the mixture was stirred at rt for 2 h. Ethyl bromoacetate (5.3 ml, 48 mmol) was added dropwise at 0 °C, and the reaction mixture was stirred at rt for 2 h. The resulting mixture was poured into ice-water, and extracted with AcOEt. The extract was washed with brine, dried, and evaporated. The residue was chromatographed on silica gel (10% AcOEt–hexane) to give 4.1 g (36%) of **15** as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.29 (3H, t, J=7.2 Hz), 1.45 (9H, s), 1.52—1.62 (2H, m), 1.80—1.92 (2H, m), 3.02—3.12 (2H, m), 3.52—3.60 (1H, m), 3.70—3.86 (2H, m), 4.11 (2H, s), 4.22 (2H, q, J=7.2 Hz). ESI-MS m/z: 310 $(M+Na)^+$.

Ethyl [1-(Benzyl)piperidin-4-yl]thioacetate (18) Hydrogen sulfide was bubbled into a solution of 1-benzyl-4-piperidone **16** (18.9 g, 100 mmol) in isopropyl alcohol (*i*PrOH, 100 ml) at rt for 4 h. After stirring of the mixture at rt for 67 h, the solution was concentrated. To a solution of this residue in *i*PrOH (100 ml) was added sodium borohydride (5.7 g, 150 mmol) in portions at rt. After stirring of the mixture at 55 °C for 2 h, 1 N HCl was added and the solution was stirred at 55 °C for 1 h. The resulting mixture was poured into ice-water, and extracted with AcOEt. The extract was washed with brine, dried, and evaporated to give 21.0 g (quant.) of **17** as a pale yellow oil. To a suspension of **17** (21.0 g, 100 mmol) and K_2CO_3 (13.8 g, 100 mmol) in acetonitrile (200 ml) was added a solution of ethyl bromoacetate (10 ml, 90 mmol) in acetonitrile (50 ml) at rt over 1 h. After stirring of the mixture at rt for 16 h, the resulting mixture was poured into ice-water, and extracted with AcOEt. The extract was washed with 1 N NaOH, brine, dried, and evaporated. The residue was chromatographed on silica gel (20% AcOEt–hexane) to give 20.3 g (69%) of **18** as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.28 (3H, t, J=7.2 Hz), 1.55—1.70 (2H, m), 1.92—2.00 (2H, m), 2.07 (2H, br t, J=11.2 Hz), 2.75—2.90 (3H, m), 3.24 (2H, s), 3.48 (2H, s), 4.17 (2H, q, J=7.2 Hz), 7.20—7.34 (5H, m). ESI-MS m/z: 294 $(M+H)^+$.

Ethyl 4-[1-(*tert*-Butoxycarbonyl)piperidin-4-yl]-2,2-dimethylbutanoate (19) To a solution of diisopropylamine (5.4 ml, 39 mmol) in THF (50 ml) was added *n*-BuLi (2.5 M solution in hexane, 15 ml, 38 mmol) at −10 °C under a nitrogen atmosphere, and the mixture was stirred at 0 °C for 30 min. After cooling of the mixture to −78 °C, a solution of **8** (3.0 g, 9.6 mmol) in THF (10 ml) was added at −78 °C, and the mixture was stirred at 0 °C for 30 min. After recooling of the mixture to −78 °C, methyl iodide (3.0 ml, 48 mmol) was added, and the mixture was allowed to warm to rt. The resulting mixture was poured into ice-water, and extracted with AcOEt. The extract was washed with brine, dried, and evaporated. The residue was chromatographed on silica gel (10% AcOEt–hexane) to give 1.5 g (48%) of **19** as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.00—1.20 (1H, m), 1.06 (2H, dq, J=4.0, 12.4 Hz), 1.15 (6H, s), 1.20—1.38 (2H, m), 1.24 (3H, t, J=7.2 Hz), 1.45 (9H, s), 1.48—1.60 (2H, m), 1.64 (2H, br d, J=13.2 Hz), 2.66 (2H, br t, J=12.0 Hz), 4.00—4.16 (2H, m), 4.11 (2H, q, J=7.2 Hz). ESI-MS m/z: 350 $(M+Na)^+$.

4-(4-Bromophenyl)-1-benzyl-1,2,5,6-tetrahydropyridine (21) To a suspension of **20** (20 g, 78 mmol) and K_2CO_3 (16.2 g, 117 mmol) in DMF (100 ml) was added benzyl bromide (11.1 ml, 93.3 mmol) dropwise at 0 °C over 20 min, and the mixture was stirred at rt for 12 h. The reaction mixture was poured into ice-water and extracted with AcOEt. The extract was washed with brine, dried, and evaporated. The residue was chromatographed on silica gel (50% AcOEt–hexane) to give 27 g (quant.) of the benzyl amine as a colorless solid. A mixture of this amine (27 g, 78 mmol) and *p*-toluenesulfonic acid monohydrate (*p*-TsOH·H_2O, 30.0 g, 158 mmol) in toluene (100 ml) was refluxed under Dean–Stark conditions for 1 h. The resulting mixture was cooled to 0 °C, then was poured into a mixture of K_2CO_3–water–AcOEt, and the precipitated solid was filtered off. The filtered solution was extracted with AcOEt. The extract was washed with brine, dried, and evaporated to give 8.7 g (34%) of **21** as a colorless solid. mp 81—82 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.48—2.55 (2H, m), 2.71 (2H, t, J=5.6 Hz), 3.16 (2H, q, J=2.8 Hz), 3.63 (2H, s), 6.04—6.08 (1H, m), 7.11—7.45 (5H, m), 7.24 (2H, d, J=8.4 Hz), 7.42 (2H, d, J=8.4 Hz). ESI-MS m/z: 328 $(M)^+$.

Ethyl 4-(1-Benzyl-1,2,5,6-tetrahydropyridin-4-yl)benzoate (22) To a solution of **21** (8.7 g, 27 mmol) in THF (200 ml) was added *n*-BuLi (2.6 M solution in hexane, 13.5 ml, 35.1 mmol) at −78 °C under a nitrogen atmosphere, and the mixture was stirred at −78 °C for 15 min. To this solution was added diethyl carbonate (34.0 ml, 281 mmol) at −78 °C, and the mixture was stirred from −78 °C to rt over 3 h. The resulting mixture was poured into ice-water, and extracted with AcOEt. The extract was washed with brine, dried, and evaporated. The residue was chromatographed on silica gel (10% AcOEt–hexane) to give 2.4 g (28%) of **22** as a yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 1.39 (3H, t, J=7.2 Hz), 2.59 (2H, br s), 2.74 (2H, t, J=5.2 Hz), 3.14—3.28 (2H, m), 3.66 (2H, s), 4.37 (2H, q, J=7.2 Hz), 6.16—6.23 (1H, m), 7.22—7.50 (5H, m), 7.44 (2H, d, J=8.8 Hz), 7.98 (2H, d, J=8.8 Hz). ESI-MS m/z: 322 $(M+H)^+$.

4-(1-Benzyl-4-hydroxypiperidin-4-yl)benzaldehyde (24) To a suspension of magnesium (0.74 g, 32 mmol) in THF (20 ml) was added a solution of 4-bromobenzaldehyde diethyl acetal (5.0 ml, 25 mmol) in THF (10 ml) at 40 °C under a nitrogen atmosphere, and the mixture was stirred at rt for 30 min. After cooling of the mixture to 0 °C, a solution of 1-benzyl-4-piperidone **16** (5.6 g, 31 mmol) in THF (10 ml) was added at below 10 °C, and the mixture was stirred at rt for 1 h. To this reaction mixture was added first an aqueous solution of ammonium chloride (NH_4Cl, 50 ml) and then 5 N HCl until the pH 1 reached. The mixture was stirred at rt for 1 h, an aqueous solution of sodium hydrogen carbonate ($NaHCO_3$) was added, and then the mixture was extracted with AcOEt. The extract was washed with brine, dried, and evaporated. The residue was chromatographed on silica gel (70% AcOEt–hexane) to give 4.0 g (54%) of **24** as a yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 1.66—1.84 (3H, m), 2.18 (2H, dt, J=4.4, 13.2 Hz), 2.47 (2H, dt, J=2.4, 12.0 Hz), 2.78—2.90 (2H, m), 3.59 (2H, s), 7.20—7.44 (5H, m), 7.69 (2H, d, J=8.4 Hz), 7.85 (2H, d, J=8.4 Hz), 9.98 (1H, s). ESI-MS m/z: 296 $(M+H)^+$.

Methyl 4-(1-Benzyl-4-hydroxypiperidin-4-yl)phenylacetate (26) To a solution of **24** (11.2 g, 38 mmol) in THF (100 ml) was added methyl methylsulfinylmethyl sulfide (FAMSO, 39.6 g, 319 mmol) and 40% benzyltrimethylammonium hydroxide in methanol (40 ml) at rt, and the reaction mixture was refluxed for 4 h. After cooling of the mixture to rt, the resulting mixture was concentrated *in vacuo*. The residue was poured into water and extracted with AcOEt. The extract was washed with brine, dried, and evaporated. The residue was chromatographed on silica gel (20% MeOH–CH_2Cl_2) to give 26 g of crude **25** as a yellow oil. A solution of **25** (26 g, 38 mmol) in 10% HCl–MeOH (400 ml) was refluxed for 6 h. After cooling and evaporation of the solvent, the residue was poured into an aqueous solution of K_2CO_3 and extracted with AcOEt. The extract was washed with brine, dried, and evaporated. The residue was chromatographed on silica gel (70% AcOEt–hexane) to give 5.6 g (43%) of **26** as a yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 1.63 (1H, br s), 1.66—1.76 (2H, m), 2.15 (2H, dt, J=4.0, 12.8 Hz), 2.47 (2H, dt,

J=2.0, 12.0 Hz), 2.74—2.84 (2H, m), 3.58 (2H, s), 3.61 (2H, s), 3.69 (3H, s), 7.23—7.37 (7H, m), 7.44—7.49 (2H, m). ESI-MS m/z: 340 $(M+H)^+$.

Methyl 4-(1-Benzyl-1,2,5,6-tetrahydropyridin-4-yl)phenylacetate (27) A mixture of **26** (5.8 g, 17 mmol) and *p*-TsOH·H_2O (6.5 g, 34 mmol) in toluene (100 ml) was refluxed under Dean–Stark conditions for 30 min. After cooling to 0 °C, the mixture was poured into an aqueous solution of $NaHCO_3$ at 0 °C and the precipitate was filtered. The filtrate was extracted with AcOEt. The extract was washed with brine, dried, and evaporated. A solution of this residue and the filtrated precipitate in 10% HCl–MeOH (100 ml) was refluxed for 2 h. The reaction mixture was concentrated *in vacuo*, and poured into an aqueous solution of $NaHCO_3$ at 0 °C, then the mixture was extracted with AcOEt. The extract was washed with brine, dried, and evaporated. The residue was chromatographed on silica gel (30% AcOEt–hexane) to give 3.4 g (62%) of **27** as a pale yellow solid. mp 58—59 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.51—2.58 (2H, m), 2.71 (2H, t, J=5.6 Hz), 3.17 (2H, q, J=2.8 Hz), 3.60 (2H, s), 3.64 (2H, s), 3.68 (3H, s), 6.02—6.07 (1H, m), 7.21 (2H, d, J=8.0 Hz), 7.23—7.40 (7H, m). ESI-MS m/z: 322 $(M+H)^+$.

7-(*tert*-Butoxycarbonyl)-7-azaspiro[3.5]nonan-2-one (29) To a suspension of zinc–copper couple (51.0 g, 781 mmol) and **28**[18] (11.4 g, 57.9 mmol) in Et_2O (180 ml) was added a solution of trichloroacetyl chloride (35.0 ml, 314 mmol) in 1,2-dimethoxyethane (DME, 100 ml) dropwise at rt under a nitrogen atmosphere. After stirring of the mixture at rt for 12 h, the reaction mixture was poured into an aqueous solution of $NaHCO_3$ at 0 °C, and filtrated. The filtrate was extracted with AcOEt. The extract was washed with brine, dried, and evaporated. The residue was passed though silica gel (20% AcOEt–hexane) to afford 29 g of the crude dichloroketone as a pale brown oil. To a solution of this oil in saturated NH_4Cl–MeOH (400 ml) was added zinc (40.0 g, 612 mmol) portion wise at rt. The reaction mixture was stirred at rt for 8 h, and then filtered. The filtrate was concentrated *in vacuo*. The residue was chromatographed on silica gel (20% AcOEt–hexane) to give 14 g (quant.) of **29** as a colorless solid. mp 55—56 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.47 (9H, s), 1.70 (4H, t, J=5.2 Hz), 2.82 (4H, s), 3.41 (4H, t, J=5.2 Hz). ESI-MS m/z: 262 $(M+Na)^+$.

[7-(*tert*-Butoxycarbonyl)-7-azaspiro[3.5]non-2-yl]carbonitrile (30) To a suspension of **29** (6.0 g, 25 mmol) and *p*-tosylmethyl isocyanide (TosMIC, 5.4 g, 28 mmol) in DME (50 ml) was added a solution of potassium *tert*-butoxide (5.6 g, 50 mmol) in *tert*-butanol (25 ml)-DME (25 ml) at 10 °C-rt over 1 h under a nitrogen atmosphere. After stirring of the mixture at rt for 12 h, the reaction mixture was poured into ice-water, then extracted with AcOEt. The extract was washed with brine, dried, and evaporated. The residue was chromatographed on silica gel (20% AcOEt–hexane) to give 2.1 g (33%) of **30** as a colorless solid. mp 65—66.5 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.45 (9H, s), 1.50—1.60 (2H, m), 1.60—1.66 (2H, m), 2.13—2.30 (4H, m), 3.07 (1H, quint, J=8.0 Hz), 3.25—3.36 (4H, m). ESI-MS m/z: 273 $(M+Na)^+$.

(7-Benzyl-7-azaspiro[3.5]non-2-yl)carbonitrile (31) To a solution of **30** (6.4 g, 26 mmol) in CH_2Cl_2 (40 ml) was added trifluoroacetic acid (40 ml, 520 mmol) at 0 °C, and the reaction mixture was stirred at rt for 2.5 h. The resulting mixture was poured into an aqueous solution of $NaHCO_3$, and extracted with AcOEt. The extract was washed with brine, dried, and evaporated to give 3.7 g (quant.) of crude secondary amine as a pale brown oil. To a solution of this amine and triethylamine (8.2 ml, 59 mmol) in CH_2Cl_2 (50 ml) was added benzyl bromide (3.1 ml, 26 mmol) at 0 °C, and the mixture was stirred at rt for 3 h. The resulting mixture was poured into water, and extracted with AcOEt. The extract was washed with brine, dried, and evaporated. The residue was chromatographed on NH silica gel (10% AcOEt–hexane) to give 1.9 g (31%) of **31** as a colorless solid. mp 38—39 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.60 (2H, t, J=5.6 Hz), 1.69 (2H, t, J=5.6 Hz), 2.08—2.26 (4H, m), 2.20—2.40 (4H, m), 3.02 (1H, quint, J=8.8 Hz), 3.45 (2H, s), 7.12—7.34 (5H, m). ESI-MS m/z: 241 $(M+H)^+$.

Methyl (7-Benzyl-7-azaspiro[3.5]non-2-yl)carboxylate (32) A mixture of **31** (3.8 g, 16 mmol) and KOH (7.0 g, 130 mmol) in ethylene glycol (30 ml) was stirred at 180 °C for 10 h. After cooling of the mixture to rt, sodium dihydrogenphosphate (NaH_2PO_4) was added to a suspension of the residue in MeOH (500 ml), and the salt was filtrated off. The filtrate was concentrated *in vacuo*. To a solution of this carboxylic acid in MeOH (500 ml) was added thionyl chloride (10 ml, 140 mmol) at 0 °C. The resulting mixture was stirred at rt for 3 d, then concentrated *in vacuo*. The residue was poured into an aqueous solution of $NaHCO_3$, and extracted with AcOEt. The extract was washed with brine, dried, and evaporated. The residue was chromatographed on NH silica gel (10% AcOEt–hexane) to give 3.7 g (84%) of **32** as a pale brown solid. mp 41.5—42.5 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.59 (2H, t, J=5.6 Hz), 1.63 (2H, t, J=5.6 Hz), 2.02 (4H, d, J=8.8 Hz), 2.20—2.40 (4H, m), 3.05 (1H, quint, J=8.8 Hz), 3.45 (2H, s), 3.67 (3H, s), 7.19—7.36 (5H, m). ESI-MS m/z: 274 $(M+H)^+$.

Ethyl [7-(*tert*-Butoxycarbonyl)-7-azaspiro[3.5]non-2-ylidene]acetate (33) To a suspension of NaH (60% dispersion in mineral oil, 0.59 g, 15 mmol) in DMF (50 ml) was added triethyl phosphonoacetate (3.3 g, 15 mmol) at rt, and the mixture was stirred at rt for 30 min. After cooling of the mixture to 0 °C, a solution of **29** (2.5 g, 10 mmol) in DMF (10 ml) was added, and the mixture was stirred at rt for 12 h. The resulting mixture was poured into water, and extracted with AcOEt. The extract was washed with brine, dried, and evaporated. The residue was chromatographed on silica gel (10% AcOEt–hexane) to give 3.2 g (quant.) of **33** as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.28 (3H, t, J=7.2 Hz), 1.46 (9H, s), 1.56—1.62 (4H, m), 2.57 (2H, br s), 2.85—2.90 (2H, m), 3.27—3.43 (4H, m), 4.15 (2H, q, J=7.2 Hz), 5.70 (1H, quint, J=2.4 Hz). ESI-MS m/z: 332 $(M+Na)^+$.

Ethyl [7-(*tert*-Butoxycarbonyl)-7-azaspiro[3.5]non-2-yl]acetate (34) A suspension of **33** (3.2 g, 10 mmol) and 10% Pd/C (0.9 g) in EtOH (50 ml) was stirred under a hydrogen atmosphere at rt for 48 h. The catalyst was removed by filtration and the filtrate was concentrated to give 3.2 g (quant.) of **34** as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.25 (3H, t, J=7.2 Hz), 1.45 (9H, s), 1.42—1.51 (3H, m), 1.52—1.65 (3H, m), 1.98—2.08 (2H, m), 2.41 (2H, d, J=8.0 Hz), 2.63 (1H, sept, J=8.0 Hz), 3.25 (2H, t, J=5.6 Hz), 3.34 (2H, t, J=5.6 Hz), 4.11 (2H, q, J=7.2 Hz). ESI-MS m/z: 334 $(M+Na)^+$.

Ethyl (3-Methyl-3-azabicyclo[3.3.1]non-9-yl)acetate (36) A suspension of **35**[19] (0.30 g, 1.3 mmol) and Pd/C (0.10 g) in EtOH (30 ml) was stirred under a hydrogen atmosphere at rt for 12 h. The catalyst was removed by filtration and the filtrate was concentrated to give 0.30 g (quant.) of a mixture of the *anti* and *syn* isomers of **36** (*anti* : *syn*=3 : 1) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.25 (3H, t, J=7.2 Hz), 1.37—1.88 (7H, m), 1.92—2.08 (1H, m), 2.13 (3H, s), 2.23 (3/2H, br d, J=11.2 Hz), 2.36 (1/2H, br d, J=11.2 Hz), 2.32—2.58 (1H, m), 2.41 (1/2H, d, J=8.0 Hz), 2.48 (3/2H, d, J=8.0 Hz), 2.62 (1/2H, br d, J=11.2 Hz), 2.90 (3/2H, br d, J=11.2 Hz), 4.13 (2H, q, J=7.2 Hz). ESI-MS m/z: 226 $(M+H)^+$.

Ethyl (*anti*)-(3-Vinyloxycarbonyl-3-azabicyclo[3.3.1]non-9-yl)acetate (37) and Ethyl (*syn*)-(3-Vinyloxycarbonyl-3-azabicyclo[3.3.1]non-9-yl)acetate (38) To a solution of **36** (2.2 g, 9.8 mmol) in 1,2-dichloroethane (20 ml) was added vinyl chloroformate (4.2 ml, 49 mmol) at 0 °C. After stirring of the mixture at 0 °C for 30 min, the mixture was refluxed for 3 h. After cooling of the reaction mixture to rt, the volatiles were removed under reduced pressure. The residue was poured into an aqueous solution of $NaHCO_3$ and extracted with AcOEt. The extract was washed with brine, dried and evaporated. The residue was chromatographed on silica gel (10% AcOEt–toluene) seven times to give 0.80 g (29%) of **37** and 0.35 g (13%) of **38** as a colorless oil. **37**; ^{1}H-NMR ($CDCl_3$) δ: 1.26 (3H, t, J=7.2 Hz), 1.42—1.52 (1H, m), 1.54—1.86 (7H, m), 2.20 (1H, t, J=7.2 Hz), 2.52 (2H, d, J=7.2 Hz), 3.14 (1H, br d, J=13.2 Hz), 3.22 (1H, br d, J=13.2 Hz), 4.08—4.26 (2H, m), 4.14 (2H, q, J=7.2 Hz), 4.44 (1H, dd, J=1.6, 6.4 Hz), 4.78 (1H, dd, J=1.6, 14.0 Hz), 7.23 (1H, dd, J=6.4, 14.0 Hz). ESI-MS m/z: 282 $(M+H)^+$. **38**; ^{1}H-NMR ($CDCl_3$) δ: 1.26 (3H, t, J=7.2 Hz), 1.48—1.56 (1H, m), 1.66—1.82 (5H, m), 1.84—1.93 (2H, m), 2.16 (1H, t, J=8.0 Hz), 2.46 (2H, d, J=8.0 Hz), 3.20—3.27 (1H, m), 3.28—3.36 (1H, m), 3.92 (2H, dd, J=6.8, 13.2 Hz), 4.15 (2H, q, J=7.2 Hz), 4.45 (1H, dd, J=1.6, 6.4 Hz), 4.79 (1H, dd, J=1.6, 14.4 Hz), 7.25 (1H, dd, J=6.4, 14.4 Hz). ESI-MS m/z: 282 $(M+H)^+$.

Ethyl (3-Methyl-7-oxo-3-azabicyclo[3.3.1]non-9-ylidene)acetate (42) In the same manner as described for the preparation of **33**, **42** was obtained from **40**[21] and triethyl phosphonoacetate as a colorless oil (59%). ^{1}H-NMR ($CDCl_3$) δ: 1.29 (3H, t, J=7.2 Hz), 2.26 (4H, s), 2.41—2.50 (2H, m), 3.14—3.22 (2H, m), 3.72—3.82 (2H, m), 3.97 (1H, s), 4.17 (2H, q, J=7.2 Hz), 4.06—4.22 (2H, m), 5.74 (1H, s). ESI-MS m/z: 226 $(M+H)^+$.

Ethyl (3-Methyl-3-azabicyclo[3.2.1]oct-8-ylidene)acetate (43) In the same manner as described for the preparation of **33**, **43** was obtained from **41**[22] and triethyl phosphonoacetate as a colorless oil (42%). ^{1}H-NMR ($CDCl_3$) δ: 1.24—1.32 (3H, m), 1.60—1.77 (2H, m), 1.78—1.98 (2H, m), 2.14—2.32 (2H, m), 2.24 (3H, s), 2.47 (1H, br s), 2.80—2.92 (2H, m), 3.74 (1H, br s), 4.10—4.22 (2H, m), 5.63 (1H, s). ESI-MS m/z: 210 $(M+H)^+$.

Ethyl (3-Methyl-7-oxo-3-azabicyclo[3.3.1]non-9-yl)acetate (44) In the same manner as described for the preparation of **34**, **44** (*anti* : *syn*=5 : 1) was obtained from **42** as a colorless oil (77%). ^{1}H-NMR ($CDCl_3$) δ: 1.27 (3H, t, J=7.2 Hz), 1.52—1.68 (2H, m), 2.06—2.15 (1H, m), 2.27 (3H, s), 2.33 (2H, br d, J=11.2 Hz), 2.49 (1/3H, d, J=7.6 Hz), 2.63 (5/3H, d, J=7.6 Hz), 2.87 (1/3H, br d, J=11.6 Hz), 3.11 (5/3H, br d, J=11.6 Hz), 3.80 (2H, br d, J=12.0 Hz), 3.95 (5/3H, br d, J=11.6 Hz), 4.07 (1/3H, br d, J=11.6 Hz), 4.15 (2H, q, J=7.2 Hz). ESI-MS m/z: 228 $(M+H)^+$.

Ethyl (3-Methyl-3-azabicyclo[3.2.1]oct-8-yl)acetate (45) In the same

manner as described for the preparation of **34**, **45** (*anti* : *syn*=1 : 1) was obtained from **43** as a colorless oil (90%). ^{1}H-NMR ($CDCl_3$) δ: 1.26 (3H, t, J=7.2 Hz), 1.66—2.10 (7H, m), 2.09 (1H, br d, J=11.2 Hz), 2.16 (1H, d, J=7.2 Hz), 2.23 (3/2H, s), 2.25 (3/2H, s), 2.34 (1H, br d, J=11.2 Hz), 2.49 (1H, dd, J=3.6, 10.8 Hz), 2.57 (1H, d, J=7.2 Hz), 2.72 (1H, dd, J=3.6, 10.8 Hz), 4.13 (1H, q, J=7.2 Hz), 4.14 (1H, q, J=7.2 Hz). ESI-MS m/z: 212 $(M+H)^+$.

Ethyl [1-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)piperidin-4-yl]carboxylate (5a) A suspension of **3** (1.0 g, 4.0 mmol), ethyl isonipecotate (1.3 g, 8.3 mmol), and K_2CO_3 (1.7 g, 12 mmol) in DMF (30 ml) was stirred at 60 °C for 3 h. After cooling of the mixture to rt, the resulting mixture was poured into ice-water, and extracted with AcOEt. The extract was washed with brine, dried, and evaporated. The residue was chromatographed on silica gel (3% MeOH–CH_2Cl_2) to give 1.0 g (67%) of **5a** as a yellow solid. mp 126—127 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.25 (3H, t, J=7.2 Hz), 1.68—1.82 (2H, m), 1.87 (2H, dd, J=3.2, 13.2 Hz), 2.00 (2H, dt, J=2.4, 11.2 Hz), 2.28 (1H, tt, J=4.0, 11.2 Hz), 2.81 (2H, br d, J=11.2 Hz), 3.31 (2H, s), 4.13 (2H, q, J=7.2 Hz), 6.52 (1H, s), 6.53 (1H, d, J=1.2 Hz), 6.74 (1H, dd, J=1.2, 8.0 Hz), 6.81 (1H, d, J=8.0 Hz), 7.57 (1H, d, J=2.8 Hz), 7.68 (1H, d, J=2.8 Hz). ESI-MS m/z: 371 $(M+H)^+$.

[1-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)piperidin-4-yl]carboxylic Acid (2a) To a solution of **5a** (0.50 g, 1.3 mmol) in EtOH (10 ml)–THF (10 ml) was added an aqueous solution (5 ml) of NaOH (0.17 g, 4.3 mmol) under a nitrogen atmosphere, and the mixture was stirred at 60 °C for 1 h. After cooling of the mixture to 0 °C, the reaction mixture was poured slowly into an aqueous solution of NaH_2PO_4 and AcOEt at 0 °C. The organic layer was separated, and the aqueous phase was concentrated *in vacuo* to 1/2 volume. The residue was chromatographed on MCI GEL (MITSUBISHI CHEMICAL, CHP20P, water to MeOH) to give 0.35 g (78%) of **2a** as a yellow solid. mp 257—258 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.44—1.58 (2H, m), 1.74 (2H, br d, J=11.2 Hz), 1.91 (2H, br t, J=11.2 Hz), 2.06—2.20 (1H, m), 2.69 (2H, br d, J=12.0 Hz), 3.23 (2H, s), 6.67 (1H, d, J=8.0 Hz), 6.75 (1H, s), 6.82 (1H, d, J=8.0 Hz), 7.61 (1H, d, J=2.8 Hz), 7.63 (1H, d, J=2.8 Hz), 9.42 (1H, s). FAB-MS m/z: 343 $(M+H)^+$. *Anal.* Calcd for $C_{17}H_{18}N_4O_2S\cdot 1.3H_2O$: C, 55.81; H, 5.68; N, 15.31. Found: C, 55.89; H, 5.49; N, 15.34.

Ethyl [1-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)piperidin-4-yl]acetate (5b) In the same manner as described for the preparation of **5a**, **5b** was obtained from **3** and ethyl (piperidin-4-yl)acetate[13] as a yellow solid (73%). mp 116—117 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.25 (3H, t, J=7.2 Hz), 1.22—1.36 (2H, m), 1.60—1.74 (2H, m), 1.70—1.86 (1H, m), 1.96 (2H, dt, J=2.4, 11.6 Hz), 2.22 (2H, d, J=6.8 Hz), 2.82 (2H, br d, J=11.6 Hz), 3.31 (2H, s), 4.12 (2H, q, J=7.2 Hz), 6.47—6.57 (1H, m), 6.53 (1H, d, J=1.6 Hz), 6.75 (1H, d, J=8.0 Hz), 6.82 (1H, d, J=8.0 Hz), 7.57 (1H, d, J=2.8 Hz), 7.69 (1H, d, J=2.8 Hz). ESI-MS m/z: 385 $(M+H)^+$.

[1-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)piperidin-4-yl]acetic Acid (2b) In the same manner as described for the preparation of **2a**, **2b** was obtained from **5b** as a pale brown solid (86%). mp 127—128 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.05—1.25 (2H, m), 1.59 (3H, br d, J=11.2 Hz), 1.85 (2H, br t, J=11.2 Hz), 2.10 (2H, d, J=6.8 Hz), 2.71 (2H, br d, J=11.2 Hz), 3.22 (2H, s), 6.67 (1H, dd, J=1.6, 8.0 Hz), 6.74 (1H, d, J=1.6 Hz), 6.81 (1H, d, J=8.0 Hz), 7.61 (1H, d, J=2.8 Hz), 7.62 (1H, d, J=2.8 Hz), 9.42 (1H, s). FAB-MS m/z: 357 $(M+H)^+$. *Anal.* Calcd for $C_{18}H_{20}N_4O_2S\cdot 1.8H_2O$: C, 55.59; H, 6.12; N, 14.41. Found: C, 55.37; H, 6.11; N, 14.37.

Ethyl 3-[1-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)piperidin-4-yl]propionate (5c) In the same manner as described for the preparation of **5a**, **5c** was obtained from **3** and ethyl 3-(piperidin-4-yl)propionate[14] as a yellow solid (63%). mp 111—112 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.25 (3H, t, J=6.8 Hz), 1.20—1.35 (3H, m), 1.53—1.65 (2H, m), 1.60—1.73 (2H, m), 1.85—2.00 (2H, m), 2.31 (2H, t, J=8.0 Hz), 2.85 (2H, br d, J=10.0 Hz), 3.33 (2H, s), 4.12 (2H, q, J=6.8 Hz), 6.54 (1H, br s), 6.56 (1H, s), 6.75 (1H, dd, J=1.0, 8.0 Hz), 6.82 (1H, d, J=8.0 Hz), 7.58 (1H, d, J=2.8 Hz), 7.69 (1H, d, J=2.8 Hz). ESI-MS m/z: 399 $(M+H)^+$.

3-[1-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)piperidin-4-yl]propionic Acid (2c) To a solution of **5c** (0.50 g, 1.3 mmol) in EtOH (10 ml)–THF (10 ml) was added 1 N NaOH (3.9 ml, 3.9 mmol) under a nitrogen atmosphere, and the mixture was stirred at 60 °C for 1 h. After cooling of the mixture to rt, the reaction mixture was concentrated *in vacuo*. The residue was diluted with water, and washed with AcOEt. The water phase was concentrated to 1/2 volume. The residue was poured slowly into an aqueous solution of NaH_2PO_4. After stirring of the mixture at rt for 20 min, the precipitate was collected and dried to obtain 0.39 g (81%) of **2c** as a yellow solid. mp 135—136 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.00—1.24 (3H, m), 1.41 (2H, q, J=7.2 Hz), 1.58 (2H, br d, J=11.2 Hz), 1.81 (2H, br t, J=11.2 Hz), 2.18 (2H, t, J=7.2 Hz), 2.72 (2H, br d, J=11.2 Hz), 3.22 (2H, s), 6.67 (1H, d, J=8.0 Hz), 6.74 (1H, s), 6.81 (1H, d, J=8.0 Hz), 7.61 (1H, d, J=2.8 Hz), 7.62 (1H, d, J=2.8 Hz), 9.41 (1H, s), 12.0 (1H, s). ESI-MS m/z: 371 $(M+H)^+$. *Anal.* Calcd for $C_{19}H_{22}N_4O_2S\cdot 1.5H_2O$: C, 57.41; H, 6.34; N, 14.10. Found: C, 57.25; H, 6.55; N, 14.08.

Ethyl 4-[1-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)piperidin-4-yl]-2-methylbutanoate (5d) (1) To a solution of **8** (1.0 g, 3.2 mmol) in AcOEt (10 ml) was added 4 N HCl–AcOEt (2.4 ml, 9.6 mmol) at 0 °C. The resulting mixture was stirred at rt for 12 h, and concentrated *in vacuo* to give 0.80 g (quant.) of **4d** as a colorless oil. ^{1}H-NMR (DMSO-d_6) δ: 1.07 (3H, d, J=6.8 Hz), 1.18 (3H, t, J=7.2 Hz), 1.10—1.60 (7H, m), 1.75 (2H, br d, J=13.2 Hz), 2.38 (1H, sext, J=6.8 Hz), 2.70—2.85 (2H, m), 3.19 (2H, br d, J=12.4 Hz), 4.06 (2H, q, J=7.2 Hz).

(2) A suspension of this oil **4d** (0.80 g, 3.2 mmol), **3** (0.40 g, 1.6 mmol) and K_2CO_3 (1.1 g, 8.0 mmol) in DMF (20 ml) was stirred at 60 °C for 3 h. After cooling of the mixture to 0 °C, the resulting mixture was poured into ice-water, and extracted with AcOEt. The extract was washed with brine, dried, and evaporated. The residue was chromatographed on silica gel (2% MeOH–CH_2Cl_2) to give 0.36 g (53%) of **5d** as a yellow solid. mp 78—79 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.14 (3H, d, J=6.8 Hz), 1.25 (3H, t, J=6.8 Hz), 1.10—1.33 (5H, m), 1.35—1.50 (1H, m), 1.50—1.73 (3H, m), 1.83—2.00 (2H, m), 2.38 (1H, sext, J=6.8 Hz), 2.84 (2H, br d, J=10.0 Hz), 3.33 (2H, s), 4.13 (2H, q, J=6.8 Hz), 6.39 (1H, s), 6.56 (1H, s), 6.76 (1H, d, J=8.0 Hz), 6.83 (1H, d, J=8.0 Hz), 7.57 (1H, d, J=2.8 Hz), 7.69 (1H, d, J=2.8 Hz). ESI-MS m/z: 427 $(M+H)^+$.

4-[1-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)piperidin-4-yl]-2-methylbutanoic Acid (2d) In the same manner as described for the preparation of **2c**, **2d** was obtained from **5d** as a yellow solid (79%). mp 187—188 °C. ^{1}H-NMR (DMSO-d_6) δ: 0.96—1.24 (5H, m), 1.03 (3H, d, J=6.8 Hz), 1.26—1.40 (1H, m), 1.46—1.64 (1H, m), 1.59 (2H, br d, J=10.4 Hz), 1.84 (2H, br t, J=10.4 Hz), 2.26 (1H, sext, J=6.8 Hz), 2.74 (2H, br d, J=11.2 Hz), 3.23 (2H, s), 6.69 (1H, d, J=8.0 Hz), 6.76 (1H, s), 6.83 (1H, d, J=8.0 Hz), 7.64 (2H, s), 9.43 (1H, s), 12.0 (1H, s). FAB-MS m/z: 399 $(M+H)^+$. *Anal.* Calcd for $C_{21}H_{26}N_4O_2S\cdot 0.6H_2O$: C, 61.62; H, 6.70; N, 13.69. Found: C, 61.46; H, 6.74; N, 13.68.

Ethyl (*E*)-4-[1-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)-piperidin-4-yl]-2-methylbutenoate (5e) In the same manner as described for the preparation of **5d**, **5e** was obtained from **7** and **3** as a yellow solid (44%). mp 120—121 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.20—1.40 (2H, m), 1.29 (3H, t, J=7.2 Hz), 1.34—1.52 (1H, m), 1.56—1.72 (2H, m), 1.82 (3H, s), 1.92 (2H, br t, J=10.0 Hz), 2.12 (2H, t, J=7.2 Hz), 2.84 (2H, br d, J=11.6 Hz), 3.32 (2H, s), 4.19 (2H, q, J=7.2 Hz), 6.47 (1H, s), 6.54 (1H, s), 6.72—6.80 (2H, m), 6.83 (1H, d, J=7.6 Hz), 7.57 (1H, d, J=2.8 Hz), 7.69 (1H, d, J=2.8 Hz). ESI-MS m/z: 425 $(M+H)^+$.

(*E*)-4-[1-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)piperidin-4-yl]-2-methylbutenoic Acid (2e) In the same manner as described for the preparation of **2a**, **2e** was obtained from **5e** as a yellow solid (50%). mp 136—137 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.19 (2H, dq, J=2.8, 12.0 Hz), 1.30—1.46 (1H, m), 1.59 (2H, br d, J=11.2 Hz), 1.72 (3H, d, J=1.6 Hz), 1.91 (2H, br t, J=10.8 Hz), 2.09 (2H, t, J=7.2 Hz), 2.77 (2H, br d, J=11.2 Hz), 3.28 (2H, s), 6.65 (1H, dt, J=1.6, 7.2 Hz), 6.69 (1H, dd, J=1.6, 8.0 Hz), 6.76 (1H, d, J=1.6 Hz), 6.83 (1H, d, J=8.0 Hz), 7.62 (1H, d, J=2.8 Hz), 7.63 (1H, d, J=2.8 Hz), 9.44 (1H, s). FAB-MS m/z: 397 $(M+H)^+$. *Anal.* Calcd for $C_{21}H_{24}N_4O_2S\cdot 0.8H_2O$: C, 61.38; H, 6.28; N, 13.63. Found: C, 61.60; H, 6.23; N, 13.64.

Ethyl 3-[1-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)piperidin-4-yl]propynate (5f) To a solution of **11** (0.80 g, 2.8 mmol) and anisole (5 ml) in CH_2Cl_2 (5 ml) was added trifluoroacetic acid (5.0 ml, 65 mmol) at rt, and the mixture was stirred at rt for 30 min. The resulting mixture was poured into $NaHCO_3$–ice-water, and extracted with AcOEt. The extract was dried, and evaporated to give crude **4f** as a yellow oil. A mixture of this oil, **3** (0.54 g, 2.2 mmol) and K_2CO_3 (0.91 g, 6.6 mmol) in DMF (20 ml) was stirred at 60 °C for 3 h. After cooling to 0 °C, the mixture was poured into ice-water, and extracted with AcOEt. The extract was washed with brine, dried, and evaporated. The residue was chromatographed on silica gel (60% AcOEt–CH_2Cl_2) to give 0.53 g (61%) of **5f** as a yellow solid. mp 129—130 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.31 (3H, t, J=7.2 Hz), 1.68—1.80 (2H, m), 1.84—1.94 (2H, m), 2.12—2.25 (2H, m), 2.50—2.60 (1H, m), 2.62—2.74 (2H, m), 3.33 (2H, s), 4.22 (2H, q, J=7.2 Hz), 6.46 (1H, s), 6.53 (1H, s), 6.75 (1H, dd, J=1.2, 8.0 Hz), 6.83 (1H, d, J=8.0 Hz), 7.58 (1H, d, J=2.8 Hz), 7.69 (1H, d, J=2.8 Hz). ESI-MS m/z: 395 $(M+H)^+$.

3-[1-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)piperidin-4-yl]propynoic Acid (2f) In the same manner as described for the prepara-

tion of **2a**, **2f** was obtained from **5f** as a yellow solid (94%). mp 166—167 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.48—1.64 (2H, m), 1.77—1.88 (2H, m), 2.22 (2H, br s), 2.50—2.75 (3H, m), 3.38 (2H, s), 6.73 (1H, dd, J=1.6, 8.0 Hz), 6.77 (1H, s), 6.87 (1H, d, J=8.0 Hz), 7.64 (1H, d, J=2.8 Hz), 7.65 (1H, d, J=2.8 Hz), 9.49 (1H, s). ESI-MS m/z: 367 (M+H)$^+$. *Anal.* Calcd for $C_{19}H_{18}N_4O_2S \cdot 3.6H_2O$: C, 52.91; H, 5.89; N, 12.99. Found: C, 52.77; H, 5.79; N, 13.02.

Ethyl [1-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)piperidin-4-yl]aminoacetate (5g) (1) A suspension of **13** (2.5 g, 9.0 mmol) and 20% $Pd(OH)_2$/C (0.9 g) in EtOH (30 ml) was stirred at rt for 6 h under a hydrogen atmosphere (4 kg/cm^2). The catalyst was removed by filtration and the filtrate was concentrated to give 1.7 g (quant.) of **4g** as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.28 (3H, t, J=7.2 Hz), 1.20—1.32 (2H, m), 1.67 (2H, br s), 1.84 (2H, br d, J=11.6 Hz), 2.50—2.66 (3H, m), 3.09 (2H, br d, J=12.8 Hz), 3.44 (2H, s), 4.20 (2H, q, J=7.2 Hz).

(2) In the same manner as described for the preparation of **5a**, **5g** was obtained from **4g** and **3** as yellow oil (74%). ^{1}H-NMR ($CDCl_3$) δ: 1.28 (3H, t, J=7.2 Hz), 1.37—1.50 (2H, m), 1.83 (2H, br d, J=11.2 Hz), 2.02 (2H, br t, J=10.8 Hz), 2.44—2.54 (1H, m), 2.81 (2H, br d, J=11.6 Hz), 3.34 (2H, s), 3.42 (2H, s), 4.19 (2H, q, J=7.2 Hz), 6.55 (1H, s), 6.63 (1H, s), 6.75 (1H, d, J=8.0 Hz), 6.82 (1H, d, J=8.0 Hz), 7.57 (1H, d, J=2.4 Hz), 7.69 (1H, d, J=2.4 Hz). ESI-MS m/z: 400 (M+H)$^+$.

[1-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)piperidin-4-yl]aminoacetic Acid (2g) In the same manner as described for the preparation of **2a**, **2g** was obtained from **5g** as a yellow solid (85%). mp 234—235 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.40—1.54 (2H, m), 1.78—1.92 (4H, m), 2.70—2.85 (3H, m), 3.10 (2H, s), 3.23 (2H, s), 6.67 (1H, dd, J=1.6, 8.0 Hz), 6.72 (1H, d, J=1.6 Hz), 6.83 (1H, d, J=8.0 Hz), 7.62 (1H, d, J=2.8 Hz), 7.63 (1H, d, J=2.8 Hz), 9.45 (1H, s). ESI-MS m/z: 372 (M+H)$^+$. *Anal.* Calcd for $C_{18}H_{21}N_5O_2S \cdot 1.2H_2O$: C, 55.00; H, 6.00; N, 17.82. Found: C, 55.09; H, 6.06; N, 17.82.

Ethyl [1-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)piperidin-4-yl]oxyacetate (5h) In the same manner as described for the preparation of **5d**, **5h** was obtained from **15** and **3** as a yellow solid (90%). mp 85—86 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.28 (3H, t, J=7.2 Hz), 1.55—1.75 (2H, m), 1.85—1.98 (2H, m), 2.05—2.20 (2H, m), 2.68—2.78 (2H, m), 3.33 (2H, s), 3.37—3.48 (1H, m), 4.10 (2H, s), 4.21 (2H, q, J=7.2 Hz), 6.48 (1H, s), 6.55 (1H, s), 6.75 (1H, dd, J=1.2, 8.0 Hz), 6.83 (1H, d, J=8.0 Hz), 7.58 (1H, d, J=2.8 Hz), 7.69 (1H, d, J=2.8 Hz). ESI-MS m/z: 401 (M+H)$^+$.

[1-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)piperidin-4-yl]oxyacetic Acid (2h) In the same manner as described for the preparation of **2a**, **2h** was obtained from **5h** as a yellow solid (62%). mp 137—138 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.43 (2H, br q, J=9.6 Hz), 1.74—1.87 (2H, m), 2.02 (2H, br t, J=9.6 Hz), 2.54—2.68 (2H, m), 3.25 (2H, s), 3.30—3.42 (1H, m), 3.97 (2H, s), 6.70 (1H, d, J=8.0 Hz), 6.77 (1H, s), 6.84 (1H, d, J=8.0 Hz), 7.63 (1H, d, J=2.8 Hz), 7.65 (1H, d, J=2.8 Hz), 9.45 (1H, s). ESI-MS m/z: 373 (M+H)$^+$. *Anal.* Calcd for $C_{18}H_{20}N_4O_3S \cdot 0.7H_2O$: C, 56.15; H, 5.60; N, 14.55. Found: C, 55.89; H, 5.70; N, 14.52.

Ethyl [1-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)piperidin-4-yl]thioacetate (5i) (1) To a solution of **18** (3.0 g, 10 mmol) in 1,2-dichloroethane (19 ml) was added 1-chloroethyl chloroformate (2.8 ml, 26 mmol) dropwise at 0 °C over 30 min under a nitrogen atmosphere. After stirring at 0 °C for 15 min, the mixture was heated to 100 °C for 1 h, then concentrated *in vacuo*. A solution of this residue in EtOH (300 ml) was refluxed for 2 h, then the solvent was removed under reduced pressure to give 2.4 g (quant.) of secondary amine **4i** as a pale brown solid. ^{1}H-NMR (DMSO-d_6) δ: 1.20 (3H, t, J=7.2 Hz), 1.56—1.72 (2H, m), 2.02—2.12 (2H, m), 2.84—2.98 (2H, m), 3.00—3.12 (1H, m), 3.18—3.29 (2H, m), 3.43 (2H, s), 4.10 (2H, q, J=7.2 Hz).

(2) A suspension of this amine **4i** (1.2 g, 5.0 mmol), **3** (1.0 g, 4.0 mmol) and K_2CO_3 (2.2 g, 16 mmol) in DMF (20 ml) was stirred at 70 °C for 3 h. After cooling to 0 °C, the mixture was poured into ice-water and extracted with AcOEt. The extract was washed with brine, dried and evaporated. The residue was chromatographed on silica gel (70% AcOEt–CH_2Cl_2) to give 0.86 g (51%) of **5i** as a yellow solid. mp 87—88 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.28 (3H, t, J=7.2 Hz), 1.55—1.68 (2H, m), 1.98 (2H, br d, J=12.8 Hz), 2.07 (2H, br t, J=10.8 Hz), 2.77—2.87 (3H, m), 3.25 (2H, s), 3.32 (2H, s), 4.19 (2H, q, J=7.2 Hz), 6.42—6.58 (1H, m), 6.53 (1H, s), 6.75 (1H, dd, J=1.2, 8.0 Hz), 6.83 (1H, d, J=8.0 Hz), 7.58 (1H, d, J=2.8 Hz), 7.69 (1H, d, J=2.8 Hz). ESI-MS m/z: 417 (M+H)$^+$.

[1-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)piperidin-4-yl]thioacetic Acid (2i) In the same manner as described for the preparation of **2c**, **2i** was obtained from **5i** as a yellow solid (83%). mp 137—138 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.36—1.50 (2H, m), 1.90 (2H, br d, J=12.0 Hz), 2.00 (2H, br t, J=11.2 Hz), 2.64—2.84 (1H, m), 2.72 (2H, br d, J=10.8 Hz), 3.26 (2H, s), 3.27 (2H, s), 6.70 (1H, d, J=8.0 Hz), 6.77 (1H, s), 6.85 (1H, d, J=8.0 Hz), 7.64 (1H, d, J=2.8 Hz), 7.65 (1H, d, J=2.8 Hz), 9.45 (1H, s). ESI-MS m/z: 389 (M+H)$^+$. *Anal.* Calcd for $C_{18}H_{20}N_4O_2S_2 \cdot 1.5H_2O$: C, 52.03; H, 5.58; N, 13.48. Found: C, 51.93; H, 5.69; N, 13.47.

Ethyl 4-[1-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)piperidin-4-yl]benzoate (5m) (1) A suspension of **22** (1.8 g, 5.6 mmol) and 20% $Pd(OH)_2$/C (0.9 g) in EtOH (50 ml) was stirred at rt for 3 d under a hydrogen atmosphere. The catalyst was removed by filtration and the filtrate was concentrated to give 1.3 g (quant.) of **4m** as a colorless solid. ^{1}H-NMR ($CDCl_3$) δ: 1.39 (3H, t, J=7.2 Hz), 1.90—2.00 (4H, m), 2.67—2.82 (1H, m), 2.82—2.96 (2H, m), 3.42 (2H, br d, J=12.8 Hz), 4.37 (2H, q, J=7.2 Hz), 7.30 (2H, d, J=8.4 Hz), 8.00 (2H, d, J=8.4 Hz).

(2) A suspension of this amine **4m** (1.3 g, 5.6 mmol), **3** (1.1 g, 4.4 mmol) and K_2CO_3 (1.8 g, 13 mmol) in DMF (20 ml) was stirred at 60 °C for 3 h under a nitrogen atmosphere. After cooling to rt, the mixture was poured into ice-water and extracted with AcOEt. The extract was washed with brine, dried and evaporated. The residue was chromatographed on silica gel (50% AcOEt–CH_2Cl_2) to give 1.3 g (65%) of **5m** as a yellow solid. mp 165—166 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.38 (3H, t, J=6.8 Hz), 1.70—1.90 (4H, m), 2.00—2.15 (2H, m), 2.50—2.64 (1H, m), 3.00 (2H, br d, J=11.2 Hz), 3.40 (2H, s), 4.36 (2H, q, J=6.8 Hz), 6.42 (1H, s), 6.59 (1H, s), 6.79 (1H, d, J=8.0 Hz), 6.85 (1H, d, J=8.0 Hz), 7.29 (2H, d, J=8.0 Hz), 7.58 (1H, d, J=2.8 Hz), 7.70 (1H, d, J=2.8 Hz), 7.98 (2H, d, J=8.0 Hz). ESI-MS m/z: 447 (M+H)$^+$.

4-[1-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)piperidin-4-yl]benzoic Acid (2m) In the same manner as described for the preparation of **2c**, **2m** was obtained from **5m** as a yellow solid (70%). mp 286—287 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.58—1.74 (2H, m), 1.76 (2H, br d, J=10.4 Hz), 2.04 (2H, br t, J=10.4 Hz), 2.50—2.64 (1H, m), 2.90 (2H, br d, J=11.2 Hz), 3.33 (2H, s), 6.74 (1H, d, J=8.0 Hz), 6.81 (1H, s), 6.86 (1H, d, J=8.0 Hz), 7.37 (2H, d, J=8.0 Hz), 7.64 (1H, d, J=2.8 Hz), 7.65 (1H, d, J=2.8 Hz), 7.87 (2H, d, J=8.0 Hz), 9.46 (1H, s). ESI-MS m/z: 419 (M+H)$^+$. *Anal.* Calcd for $C_{23}H_{22}N_4O_2S \cdot 1.7H_2O$: C, 61.51; H, 5.70; N, 12.47. Found: C, 61.41; H, 5.78; N, 12.42.

Methyl 4-[1-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)-piperidin-4-yl]phenylacetate (5n) (1) In the same manner as described for the preparation of **5m**, step 1, **4n** was obtained from **27** as a colorless oil (83%). ^{1}H-NMR ($CDCl_3$) δ: 1.50—1.76 (3H, m), 1.81 (2H, br d, J=12.8 Hz), 2.53—2.64 (1H, m), 2.66—2.78 (2H, m), 3.17 (2H, br d, J=12.0 Hz), 3.59 (2H, s), 3.68 (3H, s), 7.17 (2H, d, J=8.0 Hz), 7.20 (2H, d, J=8.0 Hz).

(2) In the same manner as described for the preparation of **5m**, step 2, **5n** was obtained from **4n** and **3** as a yellow solid (84%). mp 142—143 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.68—1.84 (4H, m), 2.06 (2H, dt, J=3.2, 11.2 Hz), 2.43—2.54 (1H, m), 2.97 (2H, br d, J=12.4 Hz), 3.38 (2H, s), 3.60 (2H, s), 3.69 (3H, s), 6.48—6.60 (1H, m), 6.58 (1H, s), 6.79 (1H, dd, J=1.6, 8.0 Hz), 6.85 (1H, d, J=8.0 Hz), 7.18 (2H, d, J=8.4 Hz), 7.22 (2H, d, J=8.4 Hz), 7.58 (1H, d, J=2.8 Hz), 7.69 (1H, d, J=2.8 Hz). ESI-MS m/z: 447 (M+H)$^+$.

4-[1-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)piperidin-4-yl]phenylacetic Acid (2n) In the same manner as described for the preparation of **2c**, **2n** was obtained from **5n** as a yellow solid (67%). mp 259—260 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.62 (2H, dq, J=3.2, 12.0 Hz), 1.72 (2H, br d, J=10.8 Hz), 1.96—2.06 (2H, m), 2.40—2.56 (1H, m), 2.88 (2H, br d, J=11.2 Hz), 3.30 (2H, s), 3.49 (2H, s), 6.72 (1H, dd, J=1.6, 8.0 Hz), 6.80 (1H, d, J=1.6 Hz), 6.84 (1H, d, J=8.0 Hz), 7.17 (4H, s), 7.62 (1H, d, J=2.8 Hz), 7.63 (1H, d, J=2.8 Hz), 9.44 (1H, s). ESI-MS m/z: 433 (M+H)$^+$. *Anal.* Calcd for $C_{24}H_{24}N_4O_2S \cdot 0.3H_2O$: C, 65.82; H, 5.66; N, 12.79. Found: C, 65.74; H, 5.65; N, 12.85.

Methyl [7-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)-7-azaspiro[3.5]non-2-yl]carboxylate (5o) (1) In the same manner as described for the preparation of **5m**, step 1, **4o** was obtained from **32** as a colorless oil (88%). ^{1}H-NMR ($CDCl_3$) δ: 1.53 (2H, t, J=5.2 Hz), 1.58 (2H, t, J=5.2 Hz), 1.87 (1H, br s), 2.04 (4H, d, J=8.8 Hz), 2.66—2.73 (2H, m), 2.74—2.80 (2H, m), 3.07 (1H, quint, J=8.8 Hz), 3.68 (3H, s).

(2) In the same manner as described for the preparation of **5m**, step 2, **5o** was obtained from **4o** and **3** as a yellow solid (80%). mp 183—184 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.59 (2H, t, J=5.2 Hz), 1.64 (2H, t, J=5.2 Hz), 2.02 (4H, d, J=8.8 Hz), 2.14—2.44 (4H, m), 3.05 (1H, quint, J=8.8 Hz), 3.29 (2H, s), 3.67 (3H, s), 6.43 (1H, br s), 6.54 (1H, s), 6.75 (1H, dd, J=1.6, 7.6 Hz), 6.82 (1H, d, J=7.6 Hz), 7.56—7.60 (1H, m), 7.67—7.72 (1H, m). ESI-MS m/z: 397 (M+H)$^+$.

[7-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)-7-aza-

spiro[3.5]non-2-yl]carboxylic Acid (2o) In the same manner as described for the preparation of **2a**, **2o** was obtained from **5o** as a yellow solid (64%). mp 150—151 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.46 (2H, t, J=5.2 Hz), 1.50—1.60 (2H, m), 1.80—1.90 (2H, m), 1.88—1.98 (2H, m), 2.06—2.34 (4H, m), 2.98 (1H, quint, J=8.4 Hz), 3.21 (2H, s), 6.69 (1H, d, J=8.0 Hz), 6.76 (1H, d, J=1.6 Hz), 6.83 (1H, d, J=1.6, 8.0 Hz), 7.62—7.66 (2H, m), 9.44 (1H, s). FAB-MS m/z: 383 $(M+H)^+$. *Anal.* Calcd for $C_{20}H_{22}N_4O_2S \cdot 0.7H_2O$: C, 60.80; H, 5.97; N, 14.18. Found: C, 60.61; H, 6.01; N, 14.18.

Ethyl [7-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)-7-azaspiro[3.5]non-2-yl]acetate (5p) (1) In the same manner as described for the preparation of **5d**, step 1, **4p** was obtained from **34** as a colorless solid (quant.). ^{1}H-NMR (DMSO-d_6) δ: 1.16 (3H, t, J=7.2 Hz), 1.44—1.53 (2H, m), 1.63 (2H, t, J=5.6 Hz), 1.73 (2H, t, J=5.6 Hz), 1.94—2.02 (2H, m), 2.41 (2H, d, J=8.0 Hz), 2.45—2.59 (1H, m), 2.86 (2H, t, J=5.6 Hz), 2.95 (2H, t, J=5.6 Hz), 4.02 (2H, q, J=7.2 Hz).

(2) In the same manner as described for the preparation of **5d**, step 2, **5p** was obtained from **4p** and **3** as a yellow solid (75%). mp 144—145 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.24 (3H, t, J=7.2 Hz), 1.44 (2H, dd, J=8.4, 12.0 Hz), 1.52 (2H, t, J=5.2 Hz), 1.64 (2H, t, J=5.2 Hz), 1.96—2.06 (2H, m), 2.24 (2H, br s), 2.33 (2H, br s), 2.39 (2H, d, J=8.0 Hz), 2.60 (1H, sept, J=8.0 Hz), 3.29 (2H, s), 4.10 (2H, q, J=7.2 Hz), 6.47 (1H, br s), 6.54 (1H, s), 6.75 (1H, dd, J=1.2, 7.6 Hz), 6.82 (1H, d, J=7.6 Hz), 7.57 (1H, d, J=2.8 Hz), 7.69 (1H, d, J=2.8 Hz). ESI-MS m/z: 425 $(M+H)^+$.

[7-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)-7-azaspiro[3.5]non-2-yl]acetic Acid (2p) In the same manner as described for the preparation of **2c**, **2p** was obtained from **5p** as a yellow solid (84%). mp 124—125 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.38 (2H, dd, J=8.8, 12.0 Hz), 1.44 (2H, t, J=5.2 Hz), 1.50—1.60 (2H, m), 1.89 (2H, br t, J=10.8 Hz), 2.16 (2H, br s), 2.24 (2H, br s), 2.30 (2H, d, J=7.6 Hz), 2.40—2.56 (1H, m), 3.20 (2H, s), 6.69 (1H, dd, J=1.6, 8.0 Hz), 6.76 (1H, d, J=1.6 Hz), 6.83 (1H, d, J=8.0 Hz), 7.63 (1H, d, J=2.8 Hz), 7.64 (1H, d, J=2.8 Hz), 9.43 (1H, s). FAB-MS m/z: 397 $(M+H)^+$. *Anal.* Calcd for $C_{21}H_{24}N_4O_2S \cdot 1.5H_2O$: C, 59.55; H, 6.43; N, 13.23. Found: C, 59.66; H, 6.48; N, 13.33.

Ethyl (*anti*)-[3-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)-3-azabicyclo[3.3.1]non-9-yl]acetate (5q) (1) A solution of **37** (0.50 g, 1.8 mmol) in 4 N HCl–dioxane (2.0 ml, 8.0 mmol) was stirred at rt for 1 h. To this solution was added EtOH (30 ml) and the mixture was then refluxed for 1 h. After evaporating the volatiles at reduced pressure, the residue was poured into ammonia–ice-water and extracted with CH_2Cl_2. The extract was washed with brine, dried and evaporated to give 0.36 g (95%) of **4q** as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.25 (3H, t, J=7.2 Hz), 1.48—1.65 (5H, m), 1.76—1.90 (3H, m), 2.06—2.23 (2H, m), 2.48 (2H, d, J=8.0 Hz), 3.00—3.16 (4H, m), 4.13 (2H, q, J=7.2 Hz).

(2) A suspension of this amine **4q** (0.36 g, 1.8 mmol), **3** (0.28 g, 1.1 mmol) and K_2CO_3 (0.70 g, 5.1 mmol) in DMF (10 ml) was stirred at 80 °C for 3 h under a nitrogen atmosphere. After cooling to 0 °C, the mixture was poured into ice-water and extracted with AcOEt. The extract was washed with brine, dried and evaporated. The residue was chromatographed on silica gel (15% AcOEt–toluene) to give 0.27 g (57%) of **5q** as a yellow solid. mp 142—143 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.14 (3H, t, J=7.2 Hz), 1.34—1.48 (3H, m), 1.56 (2H, br s), 1.60—1.75 (2H, m), 1.82—1.92 (1H, m), 2.15 (2H, br d, J=11.2 Hz), 2.43 (2H, d, J=8.0 Hz), 2.40—2.60 (1H, m), 2.85 (2H, br d, J=9.2 Hz), 3.15 (2H, s), 4.02 (2H, q, J=7.2 Hz), 6.67 (1H, dd, J=1.6, 8.0 Hz), 6.71 (1H, d, J=1.6 Hz), 6.82 (1H, d, J=8.0 Hz), 7.61 (1H, d, J=2.8 Hz), 7.62 (1H, d, J=2.8 Hz), 9.55 (1H, s). FAB-MS m/z: 425 $(M+H)^+$.

(*anti*)-[3-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)-3-azabicyclo[3.3.1]non-9-yl]acetic Acid (2q) In the same manner as described for the preparation of **2c**, **2q** was obtained from **5q** as a yellow solid (64%). mp 230—231 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.34—1.48 (3H, m), 1.58 (2H, br s), 1.60—1.76 (2H, m), 1.80—1.90 (1H, m), 2.14 (2H, br d, J=10.0 Hz), 2.33 (2H, d, J=8.0 Hz), 2.44—2.60 (1H, m), 2.85 (2H, br d, J=10.0 Hz), 3.15 (2H, s), 6.67 (1H, d, J=8.0 Hz), 6.72 (1H, s), 6.82 (1H, d, J=8.0 Hz), 7.60 (1H, d, J=2.8 Hz), 7.62 (1H, d, J=2.8 Hz), 9.54 (1H, s). FAB-MS m/z: 397 $(M+H)^+$. *Anal.* Calcd for $C_{21}H_{24}N_4O_2S \cdot 0.1H_2O$: C, 63.32; H, 6.12; N, 14.07. Found: C, 63.19; H, 6.07; N, 13.90.

Ethyl (*syn*)-[3-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)-3-azabicyclo[3.3.1]non-9-yl]acetate (5r) (1) In the same manner as described for the preparation of **5q**, step 1, **4r** was obtained from **38** as a colorless oil (quant.). ^{1}H-NMR ($CDCl_3$) δ: 1.26 (3H, t, J=7.2 Hz), 1.54 (2H, br s), 1.62—1.74 (1H, m), 1.76—1.94 (4H, m), 2.10—2.34 (3H, m), 2.55 (2H, d, J=8.0 Hz), 2.81—2.89 (2H, m), 3.14—3.24 (2H, m), 4.13 (2H, q, J=7.2 Hz).

(2) In the same manner as described for the preparation of **5q**, step 2, **5r** was obtained from **4r** and **3** as a yellow solid (65%). mp 115—116 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.25 (3H, t, J=7.2 Hz), 1.50—1.90 (7H, m), 2.00—2.10 (1H, m), 2.39 (2H, br d, J=12.0 Hz), 2.43 (2H, d, J=7.6 Hz), 2.50—2.70 (1H, m), 2.64 (2H, br d, J=10.4 Hz), 3.22 (2H, s), 4.12 (2H, q, J=7.2 Hz), 6.34—6.42 (1H, m), 6.48 (1H, d, J=1.6 Hz), 6.75 (1H, dd, J=1.6, 8.0 Hz), 6.81 (1H, d, J=8.0 Hz), 7.55 (1H, d, J=2.8 Hz), 7.67 (1H, d, J=2.8 Hz). ESI-MS m/z: 425 $(M+H)^+$.

(*syn*)-[3-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)-3-azabicyclo[3.3.1]non-9-yl]acetic Acid (2r) In the same manner as described for the preparation of **2c**, **2r** was obtained from **5r** as a yellow solid (93%). mp 236—237 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.38—1.48 (1H, m), 1.54—1.68 (4H, m), 1.68—1.80 (2H, m), 1.87 (1H, t, J=7.6 Hz), 2.30 (2H, d, J=7.6 Hz), 2.24—2.38 (2H, m), 2.55 (2H, br d, J=10.8 Hz), 2.50—2.70 (1H, m), 3.16 (2H, s), 6.67 (1H, d, J=7.6 Hz), 6.71 (1H, s), 6.82 (1H, d, J=7.6 Hz), 7.61 (1H, d, J=2.8 Hz), 7.62 (1H, d, J=2.8 Hz), 9.54 (1H, s). FAB-MS m/z: 397 $(M+H)^+$. *Anal.* Calcd for $C_{21}H_{24}N_4O_2S$: C, 63.61; H, 6.10; N, 14.13. Found: C, 63.24; H, 6.07; N, 14.09.

Ethyl [3-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)-3-azabicyclo[3.3.1]non-9-ylidene]acetate (5s) To a solution of **35** (2.0 g, 9.0 mmol) in 1,2-dichloroethane (8.4 ml) was added 1-chloroethyl chloroformate (2.9 ml, 27 mmol) dropwise at 0 °C for 30 min under a nitrogen atmosphere. After stirring at 0 °C for 15 min, the mixture was heated to 100 °C for 1 h, then concentrated *in vacuo*. A solution of this residue in EtOH (100 ml) was refluxed for 2 h, then the solvent was removed under reduced pressure to give 2.2 g (quant.) of secondary amine **4s** as a pale yellow oil. A suspension of this amine **4s** (2.2 g, 9.0 mmol), **3** (1.5 g, 6.0 mmol) and K_2CO_3 (3.3 g, 24 mmol) in DMF (20 ml) was stirred at 80 °C for 3 h under a nitrogen atmosphere. After cooling to 0 °C, the mixture was poured into ice-water and extracted with AcOEt. The extract was washed with brine, dried and evaporated. The residue was chromatographed on silica gel (15% AcOEt–toluene) to give 1.6 g (64%) of **5s** as a yellow solid. mp 45—46 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.28 (3H, t, J=7.2 Hz), 1.48—1.58 (1H, m), 1.75—1.90 (2H, m), 1.93—2.03 (2H, m), 2.25—2.32 (3H, m), 2.71—2.86 (1H, m), 2.96—3.04 (2H, m), 3.19 (1H, d, J=13.2 Hz), 3.25 (1H, d, J=13.2 Hz), 3.94 (1H, s), 4.15 (2H, q, J=7.2 Hz), 5.63 (1H, s), 6.39 (1H, s), 6.52 (1H, s), 6.79 (1H, dd, J=1.2, 8.0 Hz), 6.85 (1H, d, J=8.0 Hz), 7.56—7.59 (1H, m), 7.67—7.72 (1H, m). ESI-MS m/z: 423 $(M+H)^+$.

[3-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)-3-azabicyclo[3.3.1]non-9-ylidene]acetic Acid (2s) In the same manner as described for the preparation of **2c**, **2s** was obtained from **5s** as a yellow solid (1.7%). mp 126—127 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.40—1.50 (1H, m), 1.62—1.78 (2H, m), 1.88—2.00 (2H, m), 2.17 (2H, br t, J=12.8 Hz), 2.33 (1H, s), 2.70—2.88 (1H, m), 2.93—3.02 (2H, m), 3.16 (1H, br d, J=13.2 Hz), 3.21 (1H, br d, J=13.2 Hz), 3.86 (1H, s), 5.57 (1H, s), 6.72 (1H, d, J=8.0 Hz), 6.77 (1H, s), 6.87 (1H, d, J=8.0 Hz), 7.60—7.68 (2H, m), 9.58 (1H, s), 11.98 (1H, s). HR-MS (ESI) m/z: Calcd for $C_{21}H_{23}N_4O_2S$ $(M+H)^+$: 395.1542. Found: 395.1551.

Ethyl [3-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)-7-oxo-3-azabicyclo[3.3.1]non-9-yl]acetate (5t) In the same manner as described for the preparation of **5s**, **5t** (*anti* : *syn*=5 : 1) was obtained from **44** and **3** as a yellow oil (88%). ^{1}H-NMR ($CDCl_3$) δ: 1.26 (3H, t, J=7.2 Hz), 1.70 (2H, br s), 2.12 (1H, t, J=8.0 Hz), 2.39 (5/3H, br d, J=11.2 Hz), 2.50 (1/3H, d, J=8.0 Hz), 2.54 (1/3H, br d, J=11.2 Hz), 2.65 (5/3H, d, J=8.0 Hz), 2.78 (1/3H, br d, J=11.6 Hz), 3.02 (5/3H, d, J=11.6 Hz), 3.35 (2H, s), 3.75 (5/3H, br d, J=11.6 Hz), 3.81 (1/3H, d, J=11.6 Hz), 3.96 (5/3H, br d, J=11.6 Hz), 4.01 (1/3H, d, J=11.6 Hz), 4.14 (2H, q, J=7.2 Hz), 6.62 (1H, br s), 6.69 (1H, s), 6.76—6.84 (1H, m), 6.81 (1H, s), 7.57 (1H, d, J=3.2 Hz), 7.67 (1H, d, J=3.2 Hz). ESI-MS m/z: 427 $(M+H)^+$.

[3-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)-7-oxo-3-azabicyclo[3.3.1]non-9-yl]acetic Acid (2t) In the same manner as described for the preparation of **2a**, **2t** (*anti* : *syn*=5 : 1) was obtained from **5t** as a yellow solid (85%). mp 168—169 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.52 (2H, br s), 1.90 (1H, t, J=7.2 Hz), 2.20 (5/3H, br d, J=10.8 Hz), 2.40 (1/3H, br d, J=10.8 Hz), 2.30—2.54 (2H, m), 2.58—2.94 (2H, m), 3.22 (2H, s), 3.56 (5/3H, br d, J=11.2 Hz), 3.60 (1/3H, br d, J=11.2 Hz), 3.77 (5/3H, br d, J=10.0 Hz), 3.82 (1/3H, br d, J=10.0 Hz), 6.70—6.80 (2H, m), 6.82 (1H, d, J=7.6 Hz), 7.60 (1H, d, J=2.8 Hz), 7.61 (1H, d, J=2.8 Hz), 9.50 (1H, s). FAB-MS m/z: 399 $(M+H)^+$. *Anal.* Calcd for $C_{20}H_{22}N_4O_3S \cdot 1.8H_2O$: C, 55.75; H, 5.99; N, 13.00. Found: C, 55.64; H, 5.83; N, 12.76.

Ethyl [3-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)-3-azabicyclo[3.2.1]oct-8-yl]acetate (5u) In the same manner as described for the preparation of **5s**, **5u** (*anti* : *syn*=1 : 3) was obtained from **45** and **3** as a yellow solid (19%). mp 121—122 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.25 (3H, t, J=7.2 Hz), 1.62—1.82 (4H, m), 1.92—2.18 (3H, m), 2.11 (1/2H, br d,

J=10.4 Hz), 2.16 (1/2H, d, J=7.6 Hz), 2.36 (3/2H, br d, J=10.4 Hz), 2.42 (3/2H, dd, J=2.8, 10.8 Hz), 2.57 (3/2H, d, J=7.6 Hz), 2.65 (1/2H, dd, J=2.8, 10.8 Hz), 3.31 (1/2H, s), 3.33 (3/2H, s), 4.12 (1/2H, q, J=7.2 Hz), 4.13 (3/2H, q, J=7.2 Hz), 6.39 (1H, s), 6.51 (1H, s), 6.76 (1H, d, J=7.6 Hz), 6.81 (1H, d, J=7.6 Hz), 7.56 (1H, d, J=2.8 Hz), 7.67 (1H, d, J=2.8 Hz). ESI-MS m/z: 411 $(M+H)^+$.

[3-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)-3-azabicyclo[3.2.1]oct-8-yl]acetic Acid (2u) In the same manner as described for the preparation of **2a**, **2u** (*anti* : *syn*=1 : 3) was obtained from **5u** as a yellow solid (60%). mp 134—135 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.54—2.10 (5H, m), 2.24—2.56 (4H, m), 2.44 (2H, d, J=7.2 Hz), 2.40—2.64 (2H, m), 3.25 (1/2H, s), 3.27 (3/2H, s), 6.68 (1H, d, J=8.0 Hz), 6.78 (1H, s), 6.81 (1H, d, J=8.0 Hz), 7.61 (1H, d, J=2.8 Hz), 7.62 (1H, d, J=2.8 Hz), 9.51 (1H, s). FAB-MS m/z: 383 $(M+H)^+$. *Anal.* Calcd for $C_{20}H_{22}N_4O_2S \cdot 0.7H_2O$: C, 60.80; H, 5.97; N, 14.18. Found: C, 60.86; H, 5.81; N, 14.18.

Ethyl [8-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)-8-azabicyclo[3.2.1]oct-3-yl]acetate (5v) In the same manner as described for the preparation of **5s**, **5v** (*exo* : *endo*=1 : 5) was obtained from **39**[20] and **3** as a yellow oil (58%). ^{1}H-NMR ($CDCl_3$) δ: 1.26 (3H, t, J=7.2 Hz), 1.20—1.33 (2H, m), 1.50—1.75 (2H, m), 1.95—2.08 (2H, m), 2.10—2.23 (2H, m), 2.25—2.40 (1H, m), 2.45 (2H, d, J=8.0 Hz), 3.13 (2H, br s), 3.37 (5/3H, s), 3.40 (1/3H, s), 4.13 (2H, q, J=7.2 Hz), 6.48 (1H, s), 6.60 (1H, s), 6.79 (1H, d, J=8.0 Hz), 6.82 (1H, d, J=8.0 Hz), 7.54—7.60 (1H, m), 7.63—7.72 (1H, m). ESI-MS m/z: 411 $(M+H)^+$.

[8-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)-8-azabicyclo[3.2.1]oct-3-yl]acetic Acid (2v) In the same manner as described for the preparation of **2a**, **2v** (*exo* : *endo*=1 : 5) was obtained from **5v** as a yellow solid (63%). mp 148—149 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.21 (2H, br d, J=13.6 Hz), 1.16—1.62 (2H, m), 1.88—2.09 (4H, m), 2.10—2.21 (1H, m), 2.34 (2H, d, J=8.4 Hz), 3.04 (2H, s), 3.29 (5/3H, s), 3.30 (1/3H, s), 6.74 (1H, dd, J=1.6, 7.6 Hz), 6.82 (1H, d, J=7.6 Hz), 6.86 (1H, d, J=1.6 Hz), 7.63 (1H, d, J=2.8 Hz), 7.64 (1H, d, J=2.8 Hz), 9.47 (1H, s). FAB-MS m/z: 383 $(M+H)^+$. *Anal.* Calcd for $C_{20}H_{22}N_4O_2S \cdot 0.8H_2O$: C, 60.52; H, 5.99; N, 14.12. Found: C, 60.39; H, 6.10; N, 14.06.

Methyl 10-Methoxymethyl-10*H*-pyrazino[2,3-*b*][1,4]benzothiazine-8-carboxylate (47) To a suspension of **46**[12] (415 g, 1.60 mol) in DMF (2.5 l) was added NaH (60% dispersion in mineral oil, 66.0 g, 1.65 mol) portion wise at −10—0 °C, and the mixture was stirred at 0 °C for 1 h. To this solution was added chloromethyl methyl ether (110 g, 1.37 mol) at below 5 °C, and the reaction mixture was stirred at rt for 2 h. The resulting mixture was poured into ice-water, and extracted with CH_2Cl_2. The extract was washed with water, dried, and evaporated. The precipitate was washed with diisopropyl ether to give 335 g (69%) of **47** as a yellow solid. mp 151—152 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.55 (3H, s), 3.91 (3H, s), 5.31 (2H, s), 7.06 (1H, d, J=8.0 Hz), 7.62 (1H, dd, J=1.6, 8.0 Hz), 7.74 (1H, d, J=1.6 Hz), 7.85 (2H, s). FAB-MS m/z: 303 $(M)^+$.

10-Methoxymethyl-10*H*-pyrazino[2,3-*b*][1,4]benzothiazine-8-methanol (48) To a suspension of $LiAlH_4$ (0.20 g, 5.3 mmol) in THF (20 ml) was added a solution of **47** (2.0 g, 6.6 mmol) in THF (30 ml) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h, and water was added dropwise (0.2 ml), followed by 15% aqueous sodium hydroxide solution (0.2 ml), and finally water (0.6 ml). After stirring for 30 min, the reaction mixture was filtered and the filtrate was evaporated. The residue was chromatographed on silica gel (60% AcOEt–hexane) to give 1.6 g (86%) of **48** as a yellow solid. mp 92—93 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.93 (1H, br s), 3.53 (3H, s), 4.62 (2H, s), 5.29 (2H, s), 6.96 (1H, d, J=8.0 Hz), 6.99 (1H, d, J=8.0 Hz), 7.14 (1H, s), 7.83 (2H, s). ESI-MS m/z: 275 $(M)^+$.

8-Chloromethyl-10-methoxymethyl-10*H*-pyrazino[2,3-*b*][1,4]benzothiazine (49) To a solution of **48** (10 g, 36 mmol) and pyridine (7.4 ml, 92 mmol) in DMF (70 ml) was added dropwise methanesulfonyl chloride (7.0 ml, 90 mmol) at 0 °C under a nitrogen atmosphere. The resulting mixture was stirred at rt for 1 h, then was poured into an aqueous solution of $NaHCO_3$ at 0 °C, and extracted with AcOEt. The extract was washed with brine, dried, and evaporated. The precipitate was washed with ether to give 7.1 g (67%) of **49** as a yellow solid. mp 125—126 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.55 (3H, s), 4.52 (2H, s), 5.29 (2H, s), 6.98—7.02 (2H, m), 7.17 (1H, s), 7.84 (1H, d, J=2.8 Hz), 7.85 (1H, d, J=2.8 Hz). FAB-MS m/z: 293 $(M)^+$.

10-Methoxymethyl-10*H*-pyrazino[2,3-*b*][1,4]benzothiazine-8-acetonitrile (50) A suspension of **49** (7.1 g, 24 mmol) and sodium cyanide (2.4 g, 49 mmol) in DMSO (50 ml) was stirred at rt for 12 h. The resulting mixture was poured into water, and extracted with AcOEt. The extract was washed with brine, dried, and evaporated. The residue was chromatographed on silica gel (20% AcOEt–hexane) to give 5.2 g (76%) of **50** as a yellow solid. mp 121—122 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.55 (3H, s), 3.69 (2H, s), 5.28 (2H, s), 6.94 (1H, dd, J=2.0, 8.0 Hz), 7.02 (1H, d, J=8.0 Hz), 7.10 (1H, d, J=2.0 Hz), 7.85 (1H, d, J=2.8 Hz), 7.87 (1H, d, J=2.8 Hz). ESI-MS m/z: 284 $(M)^+$.

Methyl 10-Methoxymethyl-10*H*-pyrazino[2,3-*b*][1,4]benzothiazine-8-acetate (51) A suspension of **50** (3.7 g, 13 mmol) and KOH (2.2 g, 39 mmol) in EtOH (70 ml) was refluxed for 16 h. After cooling to rt, the mixture was poured into a cold aqueous solution of NaH_2PO_4, and extracted with AcOEt. The extract was washed with brine, dried, and evaporated to afford 3.8 g (97%) of crude carboxylic acid as a black oil. A suspension of this oil (3.8 g, 13 mmol), methyl iodide (1.2 ml, 19 mmol) and K_2CO_3 (3.5 g, 25 mmol) in DMF (50 ml) was stirred at rt for 12 h. The resulting mixture was poured into water, and extracted with AcOEt. The extract was washed with brine, dried, and evaporated. The residue was chromatographed on silica gel (15% AcOEt–hexane) to give 1.8 g (44%) of **51** as a yellow solid. mp 85—86 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.54 (3H, s), 3.57 (2H, s), 3.71 (3H, s), 5.28 (2H, s), 6.89 (1H, dd, J=1.6, 7.6 Hz), 6.97 (1H, d, J=7.6 Hz), 7.07 (1H, d, J=1.6 Hz), 7.83 (1H, d, J=2.8 Hz), 7.84 (1H, d, J=2.8 Hz). ESI-MS m/z: 317 $(M)^+$.

10-Methoxymethyl-10*H*-pyrazino[2,3-*b*][1,4]benzothiazine-8-ethanol (52) To a suspension of $LiAlH_4$ (76 mg, 2.0 mmol) in THF (20 ml) was added a solution of **51** (0.90 g, 2.8 mmol) in THF (10 ml) at 0 °C. The resulting mixture was stirred at 0 °C for 30 min, and water was added dropwise (0.08 ml), followed by 15% aqueous sodium hydroxide solution (0.08 ml), and finally water (0.24 ml). After stirring for 30 min, the reaction mixture was filtered and the filtrate was evaporated. The residue was chromatographed on silica gel (60% AcOEt–hexane) to give 0.70 g (86%) of **52** as a yellow solid. mp 64—65 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.82 (2H, t, J=6.4 Hz), 3.54 (3H, s), 3.85 (2H, t, J=6.4 Hz), 5.28 (2H, s), 6.86 (1H, dd, J=1.2, 8.0 Hz), 6.96 (1H, d, J=8.0 Hz), 7.03 (1H, d, J=1.2 Hz), 7.83 (1H, d, J=2.8 Hz), 7.84 (1H, d, J=2.8 Hz). ESI-MS m/z: 289 $(M)^+$.

Ethyl 4-[1-[2-(10-Methoxymethyl-10*H*-pyrazino[2,3-*b*][1,4]benzothiazin-8-yl)ethyl]piperidin-4-yl]-2-methylbutanoate (53) To a solution of **52** (1.2 g, 4.1 mmol) and pyridine (0.73 ml, 9.0 mmol) in CH_2Cl_2 (30 ml) was added dropwise methanesulfonyl chloride (0.63 ml, 8.1 mmol) at 0 °C under a nitrogen atmosphere. The resulting mixture was stirred at rt for 12 h, and concentrated *in vacuo*. The residue was chromatographed on silica gel (50% AcOEt–hexane) to give 1.5 g (quant.) of the mesylate as a yellow solid. In the same manner as described for the preparation of **5d**, **53** was obtained from this mesylate and **4d** as a yellow oil (22%). ^{1}H-NMR ($CDCl_3$) δ: 1.14 (3H, d, J=7.2 Hz), 1.25 (3H, t, J=7.2 Hz), 1.20—1.34 (5H, m), 1.36—1.50 (1H, m), 1.56—1.76 (3H, m), 1.97 (2H, br t, J=10.4 Hz), 2.39 (1H, sext, J=7.2 Hz), 2.48—2.60 (2H, m), 2.70—2.80 (2H, m), 2.97 (2H, br d, J=10.8 Hz), 3.53 (3H, s), 4.13 (2H, q, J=7.2 Hz), 5.26 (2H, s), 6.82 (1H, dd, J=1.2, 7.6 Hz), 6.92 (1H, d, J=7.6 Hz), 7.01 (1H, d, J=1.2 Hz), 7.82 (1H, d, J=2.8 Hz), 7.83 (1H, d, J=2.8 Hz). ESI-MS m/z: 485 $(M+H)^+$.

Ethyl 4-[1-[2-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-yl)ethyl]-piperidin-4-yl]-2-methylbutanoate (54) To a solution of **53** (0.43 g, 0.89 mmol) in THF (10 ml) was added dropwise 5 N HCl (1.0 ml, 5.0 mmol) at 0 °C. The resulting mixture was stirred at rt for 3 h, then was poured into a mixture of $NaHCO_3$–water, and extracted with AcOEt. The extract was washed with brine, dried, and evaporated. The residue was chromatographed on silica gel (10% MeOH–AcOEt) to give 0.24 g (62%) of **54** as a pale brown oil. ^{1}H-NMR ($CDCl_3$) δ: 1.14 (3H, d, J=7.2 Hz), 1.10—1.35 (5H, m), 1.25 (3H, t, J=7.2 Hz), 1.35—1.50 (1H, m), 1.55—1.75 (3H, m), 1.95 (2H, br t, J=10.4 Hz), 2.38 (1H, sext, J=7.2 Hz), 2.45—2.55 (2H, m), 2.60—2.70 (2H, m), 2.94 (2H, br d, J=10.8 Hz), 4.12 (2H, q, J=7.2 Hz), 6.33 (1H, br s), 6.35 (1H, d, J=1.6 Hz), 6.66 (1H, dd, J=1.6, 8.0 Hz), 6.79 (1H, d, J=8.0 Hz), 7.55 (1H, d, J=2.8 Hz), 7.67 (1H, d, J=2.8 Hz). ESI-MS m/z: 441 $(M+H)^+$.

4-[1-[2-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-yl)ethyl]piperidin-4-yl]-2-methylbutanoic Acid (2j) In the same manner as described for the preparation of **2c**, **2j** was obtained from **54** as a yellow solid (64%). mp 239—240 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.01 (3H, d, J=6.8 Hz), 0.96—1.20 (5H, m), 1.24—1.38 (1H, m), 1.46—1.60 (1H, m), 1.57 (2H, br d, J=10.8 Hz), 1.85 (2H, br t, J=10.4 Hz), 2.24 (1H, sext, J=6.8 Hz), 2.32—2.42 (2H, m), 2.42—2.56 (2H, m), 2.83 (2H, br d, J=11.2 Hz), 6.60 (1H, d, J=1.6 Hz), 6.63 (1H, dd, J=1.6, 7.6 Hz), 6.77 (1H, d, J=7.6 Hz), 7.60 (1H, d, J=2.8 Hz), 7.62 (1H, d, J=2.8 Hz), 9.40 (1H, s). FAB-MS m/z: 413 $(M+H)^+$. *Anal.* Calcd for $C_{22}H_{28}N_4O_2S \cdot 0.8H_2O$: C, 61.89; H, 6.99; N, 13.12. Found: C, 61.78; H, 6.97; N, 13.13.

10-Methoxymethyl-10*H*-pyrazino[2,3-*b*][1,4]benzothiazine-8-carboxylic Acid (55) A suspension of **47** (3.0 g, 9.9 mmol) and NaOH (0.80 g, 20 mmol) in EtOH (20 ml)–water (15 ml) was stirred at rt for 12 h. After evaporation of the solvent, the residue was diluted with an aqueous solution

of NaH_2PO_4, and extracted with AcOEt. The extract was washed with brine, dried, and evaporated to give 2.8 g (97%) of **55** as a yellow solid. mp 204—205 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.40 (3H, s), 5.27 (2H, s), 7.22 (1H, d, J=7.6 Hz), 7.52 (1H, dd, J=1.2, 7.6 Hz), 7.64 (1H, d, J=1.2 Hz), 7.93 (1H, d, J=2.4 Hz), 7.97 (1H, d, J=2.4 Hz), 13.10 (1H, s). ESI-MS m/z: 289 $(M)^+$.

Ethyl 4-[1-[(10-Methoxymethyl-10*H*-pyrazino[2,3-*b*][1,4]benzothiazin-8-yl)carbonyl]piperidin-4-yl]-2-methylbutanate (56) To a solution of **55** (1.0 g, 3.5 mmol), **4d** (1.0 g, 4.0 mmol) and *N*,*N*-diisopropylethylamine (iPr$_2$EtN, 3.0 ml, 17 mmol) in CH_2Cl_2 (30 ml) was added benzotriazolyloxy-tris[pyrrolidino]-phosphonium hexafluorophosphate (PyBop, 2.2 g, 4.2 mmol), and the mixture was stirred at rt for 12 h. The reaction mixture was poured into water and extracted with AcOEt. The extract was washed with brine, dried, and evaporated. The residue was chromatographed on silica gel (50% AcOEt–hexane) to give 1.2 g (71%) of **56** as a yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 1.06—1.34 (4H, m), 1.15 (3H, d, J=7.2 Hz), 1.26 (3H, t, J=7.2 Hz), 1.36—1.60 (2H, m), 1.60—1.90 (3H, m), 2.39 (1H, sext, J=7.2 Hz), 2.75 (1H, br s), 2.97 (1H, br s), 3.51 (3H, s), 3.75 (1H, br s), 4.13 (2H, q, J=7.2 Hz), 4.65 (1H, br s), 5.26 (2H, s), 6.98 (1H, dd, J=1.2, 7.6 Hz), 7.03 (1H, d, J=7.6 Hz), 7.18 (1H, d, J=1.2 Hz), 7.85 (1H, d, J=2.8 Hz), 7.86 (1H, d, J=2.8 Hz). ESI-MS m/z: 485 $(M+H)^+$.

4-[1-[(10-Methoxymethyl-10*H*-pyrazino[2,3-*b*][1,4]benzothiazin-8-yl)carbonyl]piperidin-4-yl]-2-methylbutanoic Acid (57) To a solution of **56** (0.70 g, 1.4 mmol) in EtOH (10 ml)–THF (10 ml) was added 5 N NaOH (1.2 ml, 6.0 mmol) under a nitrogen atmosphere, and the mixture was stirred at 80 °C for 2 h. After cooling of the mixture to 0 °C, the reaction mixture was poured into an aqueous solution of NaH_2PO_4. The mixture was extracted with AcOEt. The extract was washed with brine, dried, and evaporated. The residue was chromatographed on silica gel (80% AcOEt–hexane) to give 0.48 g (75%) of **57** as a yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 1.08—1.40 (4H, m), 1.20 (3H, d, J=7.2 Hz), 1.40—1.60 (2H, m), 1.60—1.90 (3H, m), 2.40—2.52 (1H, m), 2.74 (1H, br s), 2.98 (1H, br s), 3.51 (3H, s), 3.78 (1H, br s), 4.65 (1H, br s), 5.26 (2H, s), 6.99 (1H, dd, J=1.6, 8.0 Hz), 7.03 (1H, d, J=8.0 Hz), 7.18 (1H, d, J=1.6 Hz), 7.85 (1H, d, J=2.8 Hz), 7.87 (1H, d, J=2.8 Hz). ESI-MS m/z: 456 $(M)^+$.

4-[1-[(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-yl)carbonyl]piperidin-4-yl]-2-methylbutanoic Acid (2k) To a solution of **57** (0.13 g, 0.28 mmol) in THF (10 ml) was added dropwise 5 N HCl (1.5 ml, 7.5 mmol) at 0 °C. The resulting mixture was stirred at rt for 2 h, then was concentrated *in vacuo*. The residue was diluted with water, and stirred at rt for 20 min. The precipitate was collected to give 0.083 g (72%) of **2k** as a yellow solid. mp 103—104 °C. ^{1}H-NMR (DMSO-d_6) δ: 0.96—1.10 (2H, m), 1.05 (3H, d, J=6.8 Hz), 1.14—1.27 (2H, m), 1.31—1.42 (1H, m), 1.42—1.80 (4H, m), 2.29 (1H, sext, J=6.8 Hz), 2.68 (1H, br s), 2.96 (1H, br s), 3.59 (1H, br s), 4.37 (1H, br s), 6.73—6.77 (2H, m), 6.94—6.98 (1H, m), 7.66—7.68 (2H, m), 9.59 (1H, s), 12.0 (1H, s). FAB-MS m/z: 413 $(M+H)^+$. *Anal.* Calcd for $C_{21}H_{24}N_4O_3S\cdot 0.5H_2O$: C, 59.84; H, 5.98; N, 13.29. Found: C, 60.10; H, 5.96; N, 13.31.

Ethyl 4-[1-(10-Methoxymethyl-10*H*-pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)piperidin-4-yl]-2,2-dimethylbutanoate (58) In the same manner as described for the preparation of **5d**, **58** was obtained from **19** and **49** as a yellow oil (67%). ^{1}H-NMR ($CDCl_3$) δ: 1.10—1.30 (5H, m), 1.14 (6H, s), 1.24 (3H, t, J=7.2 Hz), 1.46—1.56 (2H, m), 1.64 (2H, br d, J=12.4 Hz), 1.98 (2H, br t, J=11.2 Hz), 2.84 (2H, br d, J=11.2 Hz), 3.41 (2H, s), 3.53 (3H, s), 4.10 (2H, q, J=7.2 Hz), 5.29 (2H, s), 6.93 (1H, dd, J=1.2, 8.0 Hz), 6.96 (1H, d, J=8.0 Hz), 7.10 (1H, s), 7.83 (1H, d, J=3.2 Hz), 7.83 (1H, d, J=3.2 Hz). ESI-MS m/z: 485 $(M+H)^+$.

4-[1-(10-Methoxymethyl-10*H*-pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)piperidin-4-yl]-2,2-dimethylbutanoic Acid (59) In the same manner as described for the preparation of **57**, **59** was obtained from **58** as a yellow oil (72%). ^{1}H-NMR ($CDCl_3$) δ: 1.10—1.25 (3H, m), 1.17 (6H, s), 1.25—1.40 (2H, m), 1.42—1.50 (2H, m), 1.74 (2H, br d, J=13.2 Hz), 2.16 (2H, br t, J=11.2 Hz), 3.22 (2H, br d, J=11.2 Hz), 3.51 (3H, s), 3.71 (2H, s), 5.27 (2H, s), 6.93 (1H, d, J=8.0 Hz), 6.97 (1H, d, J=8.0 Hz), 7.03 (1H, s), 7.83 (1H, d, J=2.8 Hz), 7.85 (1H, d, J=2.8 Hz). ESI-MS m/z: 457 $(M+H)^+$.

4-[1-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)piperidin-4-yl]-2,2-dimethylbutanoic Acid (2l) To a solution of **59** (0.14 g, 0.31 mmol) in THF (20 ml) was added dropwise 5 N HCl (1.0 ml, 5.0 mmol) at 0 °C. The resulting mixture was stirred at rt for 2 h, then was concentrated *in vacuo*. The residue was diluted with 1 N NaOH, and this aqueous phase was chromatographed on MCI GEL (water to MeOH) to give 0.096 g (75%) of **2l** as a yellow solid. mp 247—248 °C. ^{1}H-NMR (DMSO-d_6) δ: 0.92 (6H, s), 0.98—1.14 (5H, m), 1.26—1.34 (2H, m), 1.55 (2H, br d, J=9.2 Hz), 1.80 (2H, br t, J=10.0 Hz), 2.71 (2H, br d, J=10.8 Hz), 3.20 (2H, s), 6.67 (1H, dd, J=1.6, 8.0 Hz), 6.75 (1H, d, J=1.6 Hz), 6.80 (1H, d, J=8.0 Hz), 7.61 (1H, d, J=2.8 Hz), 7.62 (1H, d, J=2.8 Hz), 9.45 (1H, s). FAB-MS m/z: 413 $(M+H)^+$. *Anal.* Calcd for $C_{22}H_{28}N_4O_2S\cdot H_2O$: C, 61.37; H, 7.02; N, 13.01. Found: C, 61.16; H, 6.93; N, 12.88.

Carrageenan-Induced Pleurisy in the Rat[24] Male F344 Rats (6—7 week old, Charles River Japan Inc., Atsugi, Japan) were anesthetized with Et_2O, and λ-carrageenan (0.2 ml/pleural cavity, 1% dissolved in saline, Zushikagaku Laboratory, Inc., Kanagawa, Japan) was injected into the pleural cavity of the rats. The test compound was orally administered 30 min before the injection of carrageenan. After 5 h, the rats were sacrificed and the exudate in the cavity was carefully collected by pipette and its weight was measured. The numbers/volume of viable leukocytes in the exudate fluid were counted with a hemocytometer. The numbers of infiltrated leukocytes in the exudate fluid were calculated by the formula; (exudates volume)×(numbers/volume of infiltrated leukocytes).

Type II Collagen-Induced Arthritis in the Rat Lewis female rats were supplied by Charles River Japan. Type II collagen solution (0.16%; Cosmo-Bio, Japan) containing muramyl dipeptide (0.4 mg/ml) was emulsified with an equal volume of Freund's incomplete adjuvant (FIA; Difco Laboratories). 1 ml of this emulsion was injected intradermally at various sites in the back of the animals. After 14 d, the rats were grouped with the same mean volume of paws at 3.0 ml. Compound **2q** was orally administered daily for 10 d from day 14 to day 23 as a suspension in a 0.5% methylcellulose solution. The severity of collagen-induced arthritis was assessed by quantification of hind paw swelling (n=8).

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