# Inhibitors of Adhesion Molecules Expression; The Synthesis and Pharmacological Properties of 10*H*-Pyrazino[2,3-*b*][1,4]benzothiazine Derivatives

Toshihiko KANEKO,*[,a] Richard S. J. CLARK,[a] Norihito OHI,[a] Tetsuya KAWAHARA,[a] Hiroshi AKAMATSU,[a] Fumihiro OZAKI,[a] Atsushi KAMADA,[a] Kazuo OKANO,[a] Hiromitsu YOKOHAMA,[a] Kenzo MURAMOTO,[a] Masayoshi OHKURO,[a] Osamu TAKENAKA,[a] and Seiichi KOBAYASHI[b]

[a] *Tsukuba Research Laboratories, Eisai Co., Ltd.; 5–1–3 Tokodai, Tsukuba, Ibaraki 300–2635, Japan: and* [b] *Eisai Research Institute of Boston, Inc.; 4 Corporate Drive, Andover, Massachusetts, 01810–2441, U.S.A.*
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**During a search for novel, orally-active inhibitors of upregulation of adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), we found a new series of 10*H*-pyrazino[2,3-*b*][1,4]benzothiazine derivatives to be potent ICAM-1 inhibitors. Of these compounds, *N*-[1-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)-piperidin-4-yl]-*N′*,*N′*-dimethylsulfamide 7p showed the potent oral inhibitory activities against neutrophil migration in a murine interleukin-1 (IL-1) induced paw inflammation model. The synthesis and structure–activity relationships of these amide derivatives are described.**

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

In various inflammatory and immune diseases leukocytes migrate from the vasculature into surrounding tissue, where they participate in the inflammatory response, resulting in tissue damage, swelling, pain and loss of function. This process is a cascade of events wherein the leukocytes adhere first transiently, then firmly to the endothelial cells lining blood vessels, then infiltrate through the vessel wall. Adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1),[1] E-selectin[2] and vascular cell adhesion molecule-1 (VCAM-1)[3,4] are upregulated on the endothelium by interleukin-1 (IL-1) or tumor necrosis factor-$\alpha$ (TNF-$\alpha$) and mediate steps in this cascade of events.[5,6]

It has also been established that activated lymphocytes play a pivotal role in the development and progression of inflammatory diseases such as rheumatoid arthritis (RA), colitis, psoriasis, multiple sclerosis *etc.*, in which immunological mechanisms are involved. For T cell activation and clonal expansion to occur, in addition to the stimulation of T cell receptor (TCR) with antigen-MHC complex expressed on antigen presenting cells, a costimulatory signal mediated by accessory molecules is also required, and TCR-mediated activation in the absence of such a signal can lead to T cell unresponsiveness. The interactions of adhesion molecules such as lymphocyte function associated antigen-1 (LFA-1)/ICAM-1[7—10] and very late antigen-4 (VLA-4)/VCAM-1[11,12] have been demonstrated to transmit this costimulatory signal, and hence it is expected that interfering with these interactions will lead not only to the inhibition of leukocyte infiltration outlined above, but also to unresponsiveness to antigen. Hence inhibitors of adhesion molecules appear to be an attractive means for both ameliorating the inflammatory response and achieving the long-term modulation of the immune response in autoimmune diseases.

It has been reported that anti-adhesion molecule antibodies are able to ameliorate the inflammatory reaction and immunological parameters in various animal models.[13,14] In clinical trials a monoclonal antibody to ICAM-1 and an ICAM-1 antisense oligonucleotide showed beneficial effects.[15—18] A number of inhibitors of cell adhesion molecules, especially ICAM-1, have been disclosed in the literature, however to date no such low-molecular weight compounds have been clinically evaluated.[19—23] Recently, antagonists of the ICAM-1/LFA-1 interaction and of VLA-4 have also been reported.[24—40]

During the course of our search for new orally-active adhesion molecules inhibitors, we discovered the 10*H*-pyrazino[2,3-*b*][1,4]benzothiazine derivative **7b** that inhibits ICAM-1 expression on human umbilical vein endothelial cells (HUVEC) with an $IC_{50}$ value of 0.32 $\mu$M. In this report we describe our initial structure activity studies which focus on modifications to the piperidine ring of **7b**.

**Chemistry** The 10*H*-pyrazino[2,3-*b*][1,4]benzothiazine derivatives shown in Tables 1—4 were prepared as shown in Charts 1—3. Compound **2** was synthesized from 4-chloro-3-nitrobenzoic acid **1** by displacement of chloride with sodium disulfide ($Na_2S_2$), reduction of the nitro group and esterification.[41,42] These intermediates, including aminothiophenol **2**, were unstable to purification. The 10*H*-pyrazino[2,3-*b*][1,4]-benzothiazine ring was constructed by condensation of **2** with 2,3-dichloropyrazine. The reduction of ester **3** with lithium aluminum hydride led to alcohol **4**, which on chlorination with methanesufonyl chloride afforded the key 10*H*-pyrazino[2,3-*b*][1,4]benzothiazine intermediate **5**. The ben-

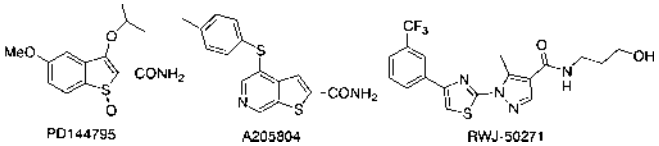
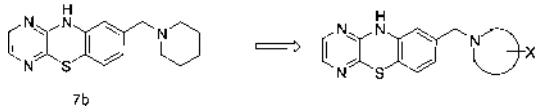



Fig. 1. Structures of the Known Small Molecule Cell Adhesion Molecule Expression Inhibitor (PD144795 and A205804) and ICAM-1/LFA-1 Binding Inhibitor (RWJ-50271)




* To whom correspondence should be addressed. e-mail: t3-kaneko@hhc.eisai.co.jp

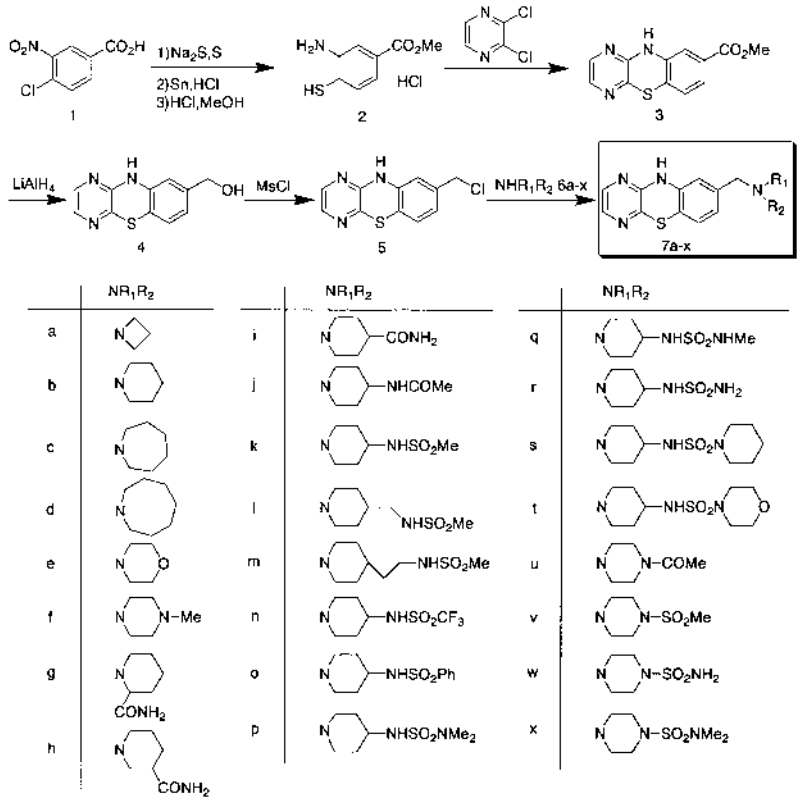


Chart 1

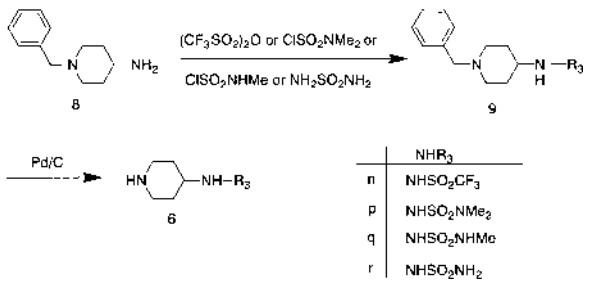


Chart 2

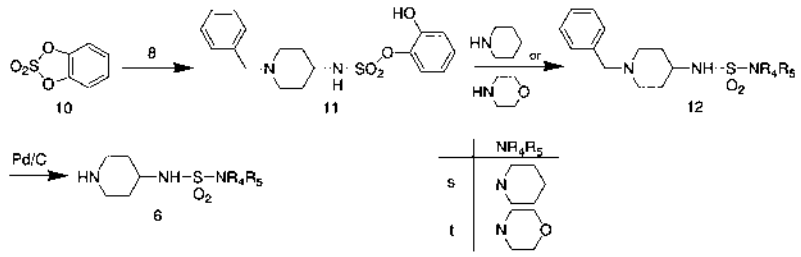


Chart 3

zyl chloride **5** was condensed with the amines **6a**—**x**, to obtain the target molecules **7a**—**x**.

With the exceptions of **6n** and **6p**—**t**, the secondary amines **6a**—**x** are either commercially available or known in the literature.[43—50] Sulfonamide **6n** and sulfamides **6p**—**r** were prepared as shown in Chart 2 by condensation of 4-amino-1-benzylpiperidine with trifluoromethanesulfonic anhydride, *N*,*N*-dimethylsulfamoyl chloride or *N*-methylsulfamoyl chloride[51] or sulfamide, followed by deprotection using 10% palladium on carbon (Pd/C). Sulfamides **6s** and **6t** were synthesized using the method of G. E. DuBois as shown in Chart 3.[52,53] Catechol sulfate **10** was condensed with *N*-benzyl piperidine to obtain sulfamate ester **11**. The key secondary amines **6s** and **6t** were obtained by coupling of this sulfamate ester **11** with piperidine or morpholine respectively, followed by debenzylation using Pd/C.

**Biological Assay** The 10*H*-pyrazino[2,3-*b*][1,4]benzothiazine derivatives were first evaluated for their inhibitory activity against the expression of adhesion molecules such as ICAM-1 on TNF-$\alpha$-stimulated HUVEC *in vitro*. A solution of the test compound was added to HUVEC, which was then stimulated with TNF-$\alpha$ (1 ng/ml) for 4 h. After fixing the HUVEC with glutaraldehyde, ICAM-1 expression on the cell surface was evaluated using an ELISA.[54] For *in vitro* studies the relative potencies are expressed as $IC_{50}$ values. Tests were run in duplicate, and the $IC_{50}$ value determinations were performed by the least-squares method using four concentrations of compound.

The effect of the compounds on neutrophil accumulation was next evaluated in a mouse IL-1-induced paw inflammation model. Briefly, the compounds to be tested were orally administered to BALB/c mice 30 min prior to a rat rIL-1 injection. Two hours after this injection, the injected paws were removed and myeloperoxidase (MPO) was measured spectrophotometrically as a marker enzyme for neutrophil content according to a literature method.[55]

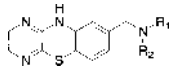
## Results and Discussion

Initially, the effect of ring size of the cyclic amine in compound **7** was investigated as shown in Table 1. The azetidine derivative **7a**, and 7- or 8-membered-ring (**7c**, **d**) compounds were 2—4-fold less active than piperidine **7b**. The morpholine derivative **7e** and piperazine derivative **7f** were also prepared to examine the possibility of introducing a heteroatom at the 4-position of piperidine, but were less active than the parent structure. These results suggest that the 6-membered-ring is favorable for activity.

As shown in Table 2 we next turned our attention to substituted piperidine derivatives, examining the optimal position for substitution using the carboxamide group as a probe. The 4-carboxamide **7i** showed good activity, with an $IC_{50}$ value of 0.69 $\mu$M, while the 3-derivative **7h** showed a slight loss of activity, with an $IC_{50}$ value of 1.02 $\mu$M. On the other hand, the 2-carboxamide **7g** showed a 17-fold loss in inhibitory activity compared to **7i**. These results suggest that steric hindrance around the benzylamine nitrogen is unfavorable. We decided to optimize further at the 4-position of the piperidine.

Next, modification of the carboxamide moiety of **7i** was investigated with results shown in Table 3. Retro-amide **7j** was twice as active *in vitro* as amide **7i**, while methanesulfonamide **7k**, used as a bioisostere of carboxamide, also showed 2-fold greater inhibitory activity than **7i**. We evaluated the inhibitory activity of **7k** against neutrophil infiltration in a mouse IL-1-induced paw inflammatory model at an oral dose of 10 mg/kg. In this model, antibody to ICAM-1 (KAT-1) has been found effective in reducing neutrophil infiltration in the IL-1 injected paw (data not shown). It showed significant inhibition of neutrophil migration in this model with an inhibition value of 85.9±4.0%. Hence, we next focused on sulfonamide derivatives in order to obtain stronger orally-active compounds. Compounds with an alkylene spacer between the piperidine and methanesulfonamide (**7l**, **m**) retained potency both *in vitro* and *in vivo*. Replacement of the methyl group of compound **7k** with a trifluoromethyl group **7n**, or phenyl group **7o** also led to retention of *in vitro* potency, but to loss in potency in the IL-1-induced paw model in mice at a dose of 10 mg/kg. These results suggest that the degree of lipophilicity of this terminal portion of compound **7k** may be critical for *in vivo* activity. To evaluate the lipophilicity of our compounds, we calculated their

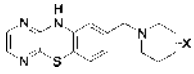
Table 1. ICAM-1 Inhibitory Activity of Simple Ring Derivatives (**7a**—**f**)



| Compound No. | $NR_1R_2$ | ICAM-1[a] $IC_{50}$ ($\mu$M) |
|---|---|---|
| **7a** |  | 0.49 |
| **7b** |  | 0.27 |
| **7c** |  | 0.83 |
| **7d** |  | 1.1 |
| **7e** |  | 2.0 |
| **7f** |  | 5.0 |

*a*) Concentration of compound inhibiting ICAM-1 up-regulation by 50% of control value.

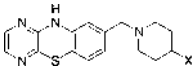
Table 2. ICAM-1 Inhibitory Activity of Piperidine Amide Derivatives (**7g**—**i**)



| Compound No. | X | Position | ICAM-1[a] $IC_{50}$ ($\mu$M) |
|---|---|---|---|
| **7g** | $CONH_2$ | 2 | 11.7 |
| **7h** | $CONH_2$ | 3 | 1.02 |
| **7i** | $CONH_2$ | 4 | 0.69 |

*a*) Concentration of compound inhibiting ICAM-1 up-regulation by 50% of control value.

Table 3. Biological Activities of Piperidine Sulfonamides and Sulfamides (**7j**—**t**)



| Compound No. | X | ICAM-1[a] $IC_{50}$ ($\mu$M) | IL-1 paw[b] (10 mg/kg, *p.o.*) | ClogP |
|---|---|---|---|---|
| **7j** | NHCOMe | 0.34 | N.T. | — |
| **7k** | $NHSO_2Me$ | 0.32 | 85.9±4.0 | 0.67 |
| **7l** | $CH_2NHSO_2Me$ | 0.36 | 77.6±13.0 | 1.29 |
| **7m** | $CH_2CH_2NHSO_2Me$ | 0.36 | 89.7±4.0 | 1.82 |
| **7n** | $NHSO_2CF_3$ | 0.30 | 14.2±3.2 | 2.78 |
| **7o** | $NHSO_2Ph$ | 0.37 | 11.6±3.2 | 2.46 |
| **7p** | $NHSO_2NMe_2$ | 0.32 | 83.4±7.4 | 0.53 |
| **7q** | $NHSO_2NHMe$ | 0.32 | 47.1±15.3 | 1.40 |
| **7r** | $NHSO_2NH_2$ | 0.26 | 10.1±8.0 | 0.25 |
| **7s** |  | 0.32 | 0 (30)[c] | 1.72 |
| **7t** |  | 0.30 | 66.1±11.9 (30)[c] | 0.87 |

*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. *c*) Percentage inhibition at a dose of 30 mg/kg *p.o.* N.T.: not tested.

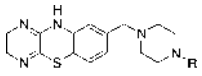
ClogP, the values for **7k**, **7n** and **7o** being 0.67, 2.78 and 2.46 respectively.[56] We speculated that the preferred ClogP value is around 0.6 for these 4-aminopiperidine derivatives and went on to prepare and examine the low ClogP sulfamide derivatives **7p**—**t** shown in Table 3. *In vitro*, all these derivatives showed equivalent potency. Of these compounds, the *N*,*N*-dimethyl derivative **7p** was as active in the IL-1 paw model as the methanesulfonamide **7k**. The ClogP value of **7p** is also around 0.6, the value suggested above. Furthermore, the morpholinesulfamide **7t** showed 66.1±11.9% inhibition in the IL-1 paw model at 30 mg/kg, while the activity of the equivalent piperidine sulfamide **7s** was low. Taken together these results suggest that introduction of a "carrier" sulfonamide or sulfamide moiety into these types of compounds to bring their ClogP to around 0.6 can be an important factor in achieving *in vivo* activity in the IL-1 paw model.

We next reexamined the piperazine scaffold, preparing amides **7u**—**x**. Carboxamide **7u** showed weak activity *in vitro* compared with the piperidine carboxamide **7j**, the same trend as observed in the simple ring compounds **7b** and **7f**. However, both sulfonamide **7v** and sulfamide **7w** showed good *in vitro* activities. These compounds also suppressed neutrophil migration moderately in the IL-1-induced paw inflammation model. **7x**, the analog of **7p**, showed no inhibitory activity in this model even at a dose of 30 mg/kg. The activities of all the piperazine series were less potent than those of the corresponding piperidine analogues.

The inhibitory activity on other adhesion molecules was studied. The compound **7p** suppressed the up-regulation of E-selectin with an $IC_{50}$ value of 0.55 $\mu$M and that of VCAM-1 with an $IC_{50}$ value of 0.36 $\mu$M.

The pharmacokinetic characteristics of dimethylsulfamide **7p** were also evaluated in fed male rats as shown in Table 5 (10 mg/kg *p.o.*, 3 mg/kg i.v., $n=3$). Compound **7p** has a $C_{max}$ of 0.86±0.17 $\mu$M, a mean residence time (*MRT*) of 11.4±2.1 h, and oral bioavailability of 69.0±1.9%. In this study, the demethyl metabolite, shown as **7q**, was also observed with a $C_{max}$ of 0.52±0.05 $\mu$M, and a *MRT* of 11.4±1.7 h.

Table 4. Biological Activities of Piperazine Amide Derivatives (**7u**—**x**)



| Compound No. | R | ICAM-1[a] $IC_{50}$ ($\mu$M) | IL-1 paw[b] (10 mg/kg) | ClogP |
|---|---|---|---|---|
| **7u** | –COMe | 1.4 | 18.6±5.9 | 1.16 |
| **7v** | $-SO_2Me$ | 0.39 | 53.4±11.8 | 1.58 |
| **7w** | $-SO_2NH_2$ | 0.58 | 41.8±22.4 | 1.46 |
| **7x** | $-SO_2NMe_2$ | 1.0 | 0 (30)[c] | 1.61 |

*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. *c*) Percentage inhibition at a dose of 30 mg/kg *p.o.*

Table 5. Pharmacokinetic Characteristics of **7p**

| | $T_{max}$ (h) | $C_{max}$ ($\mu$M) | $AUC_{0-24\,h}$ ($\mu$M·h) | $MRT_{0-24\,h}$ (h) | B.A.$_{0-24\,h}$ (%) |
|---|---|---|---|---|---|
| 10 mg/kg, *p.o.* | 9.00±7.51 | 0.86±0.17 | 11.5±0.3 | 11.4±2.1 | 69.0±1.9 |

| | $T_{1/2}(\alpha)$ (h) | $T_{1/2}(\beta)$ (h) | $CL_t$ (ml/h/kg) | $V_{dss}$ (ml/kg) |
|---|---|---|---|---|
| 3 mg/kg, i.v. | 0.33±0.26 | 2.30±0.37 | 1335±26 | 3566±198 |

*MRT* is mean residence time.

## Conclusion

In order to develop an orally-active inhibitor of adhesion molecules expression, 10*H*-pyrazino[2,3-*b*][1,4]benzothiazine derivatives were synthesized and their pharmacological properties were evaluated. We found 8-methylpiperidine to be a prefered substituent, and that 4-sulfonamide- and 4-sulfamide-substituted piperidine derivatives showed especially potent *in vivo* activities. In particular, *N*-[1-(10*H*-pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)piperidin-4-yl]-*N*′,*N*′-dimethylsulfamide **7p** significantly inhibited neutrophil migration in the mouse IL-1-induced paw inflammation model after oral administration at a dose of 10 mg/kg. This compound has also good oral bioavailability. This may prove to be valuable therapeutic agent in the treatment of chronic disorders such as rheumatoid arthritis. Efforts to discover novel adhesion molecule inhibitors possessing more potent oral activity are ongoing.

### Experimental

Melting points were measured using a Yanako melting-point apparatus and are uncorrected. The 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, m=multiplet, br=broad. Mass spectra (MS) were obtained on a JEOL JMS-HX100 mass spectrometer. High resolution mass spectra (HR-MS) were obtained on a JEOL JMS-SX102AQQ mass spectrometer. Elemental analysis was performed with a Heraeus Elemental Analyzer CHN-O-RAPID. 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.

**Methyl 10*H*-Pyrazino[2,3-*b*][1,4]benzothiazine-8-carboxylate (3)** To a suspension of 4-chloro-3-nitrobenzoic acid **1** (500 g, 2.46 mol) in ethanol (2250 ml) was added dropwise a solution of sodium hydroxide (67 g, 1.7 mol) in water (125 ml), followed by portion-wise addition of a solution of $Na_2S_2$ (prepared from sodium sulfide/9$H_2O$ (600 g, 2.50 mol) and sulfur (80 g, 2.5 mol)). The resulting mixture was refluxed for 30 min, cooled to room temperature (rt), then filtered to obtain 450 g of a dark-green solid precipitate.

To a suspension of this crude compound (450 g) and tin (1098 g, 9.25 mol) in ethanol (2500 ml) was added dropwise conc.HCl until reaction was complete by TLC. The tin residue was removed by filtration, and the filtrate was evaporated. Conc.HCl and ethanol were added to the residue and filtration of the precipitate gave 270 g of a pale yellow powder.

This powder (270 g) was added to a hydrogen chloride saturated methanol solution (900 ml). The resulting mixture was refluxed for 7 h, then was concentrated *in vacuo* to give the crude pale yellow solid (320 g).

To a suspension of this solid (320 g, 1.72 mol) in *N*,*N*-dimethylformamide (DMF, 320 ml) was added dropwise 2,3-dichloropyrazine (256 g, 1.72 mol). The resulting mixture was stirred at 100 °C for 30 min, cooled to rt, and added to 640 ml of water, then the precipitate was filtered off and washed with water and ether to give 128 g of **3** as a yellow powder (20%, from **1**). mp 265—268 °C. $^1$H-NMR (DMSO-$d_6$) $\delta$: 3.78 (3H, s), 7.02 (1H, d, $J$=8.2 Hz), 7.29 (1H, dd, $J$=1.9, 8.2 Hz), 7.31 (1H, d, $J$=1.9 Hz), 7.64 (1H, d, $J$=2.9 Hz), 7.65 (1H, d, $J$=2.9 Hz), 9.63 (1H, s). FAB-MS *m*/*z*: 259 $(M)^+$.

**10*H*-Pyrazino[2,3-*b*][1,4]benzothiazine-8-methanol (4)** To a stirred suspension of lithium aluminum hydride (40 g, 1.1 mol) in tetrahydrofuran (THF, 1 l) was added dropwise a solution of **3** (200 g, 771 mmol) in THF

(2.5 l) over 1 h at below 15 °C under a nitrogen atmosphere. After completion of addition, the resulting mixture was stirred for 1 h at this temperature, then was cooled with ice-water, and water was added dropwise (40 ml), followed by 15% aqueous sodium hydroxide solution (40 ml), and finally water (120 ml), all at under 20 °C. Stirring was continued for a further 30 min, then the reaction mixture were filtered. The aluminum residue was washed well with 4×1 l of THF, then the organic phases were evaporated to give 125 g (70%) of **4** as a yellow crystalline solid. mp 187—189 °C. $^1$H-NMR (DMSO-$d_6$) $\delta$: 4.30 (2H, d, $J$=6.0 Hz), 5.17 (1H, t, $J$=6.0 Hz), 6.70 (1H, d, $J$=7.9 Hz), 6.75 (1H, s), 6.83 (1H, d, $J$=7.9 Hz), 7.61 (1H, d, $J$=2.6 Hz), 7.63 (1H, d, $J$=2.6 Hz), 9.50 (1H, s). FAB-MS $m/z$: 231 $(M)^+$.

**8-Chloromethyl-10*H*-pyrazino[2,3-*b*][1,4]benzothiazine (5)** To a solution of **4** (7.0 g, 30 mmol) and pyridine (6.1 ml, 76 mmol) in DMF (50 ml) was added dropwise methanesulfonyl chloride (5.9 ml, 76 mmol) under nitrogen at 0 °C. The resulting mixture was stirred for 1 h at rt, then was poured into a mixture of $NaHCO_3$–water–$CH_2Cl_2$, and extracted with ethyl acetate (AcOEt). The extract was washed with brine, dried, and evaporated. The precipitate was washed with ether to give 4.7 g (62%) of **5** as a yellow powder. mp 161—162 °C. $^1$H-NMR (DMSO-$d_6$) $\delta$: 4.58 (2H, s), 6.78—6.80 (1H, m), 6.80—6.84 (1H, m), 6.90 (1H, dd, $J$=1.7, 7.9 Hz), 7.63—7.66 (2H, m), 9.58 (s, 1H). FAB-MS $m/z$: 249 $(M)^+$.

***N*-(1-Benzylpiperidin-4-yl)trifluoromethanesulfonamide (9n)** To a solution of 4-amino-1-benzylpiperidine (6.8 g, 36 mmol) in $CH_2Cl_2$ (100 ml) was added triethylamine ($NEt_3$, 7.4 ml, 53 mmol) and trifluoromethanesulfonic anhydride (10 g, 36 mmol) at −78 °C. The reaction mixture was stirred for 2 h at −78 °C—rt, water 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 (40% AcOEt–hexane) to give 7.2 g (63%) of **9n** as a colorless solid. mp 131—132 °C. $^1$H-NMR (DMSO-$d_6$) $\delta$: 1.46—1.60 (2H, m), 1.75 (2H, br d, $J$=10 Hz), 1.90—2.10 (2H, m), 2.76 (2H, br d, $J$=11.6 Hz), 3.18—3.38 (1H, m), 3.44 (2H, s), 7.20—7.34 (5H, m), 9.41 (1H, s).

***N*-(1-Benzylpiperidin-4-yl)-*N*′,*N*′-dimethylsulfamide (9p)** To a solution of 4-amino-1-benzylpiperidine (3.0 g, 16 mmol) in $CH_2Cl_2$ (100 ml) was added pyridine (4.0 ml) and *N*,*N*-dimethylsulfamoyl chloride (2.1 ml, 19 mmol) at 0 °C. The reaction mixture was stirred for 12 h at rt, water was added, and then the mixture was extracted with AcOEt. The extract was washed with brine, dried, and evaporated. The residue was chromatographed on NH silica gel (4% MeOH–$CH_2Cl_2$) to afford **9p** (4.5 g, 94%) as a pale yellow solid. mp 105—106 °C. $^1$H-NMR ($CDCl_3$) $\delta$: 1.65—1.85 (2H, m), 2.07 (2H, br d, $J$=11.2 Hz), 2.33 (2H, br s), 2.78 (6H, s), 3.00 (2H, br d, $J$=11.2 Hz), 3.30 (1H, br s), 3.70 (2H, s), 4.50—4.80 (1H, m), 7.26—7.44 (5H, m).

***N*-(1-Benzylpiperidin-4-yl)-*N*′-methylsulfamide (9q)** To a solution of *N*-methylsulfamoyl chloride[51] (5.8 g, 45 mmol) and $NEt_3$ (9.5 ml, 68 mmol) in toluene (50 ml) was added 4-amino-1-benzylpiperidine (7.7 g, 41 mmol) at 0 °C. The reaction mixture was stirred for 12 h at rt, an aqueous solution of $K_2CO_3$ was added, and then the mixture was extracted with AcOEt. The extract was washed with brine, dried, and evaporated. The residue was chromatographed on NH silica gel (70% AcOEt–hexane) to afford **9q** (3.5 g, 30%) as a colorless solid. mp 135.5—136.5 °C. $^1$H-NMR (DMSO-$d_6$) $\delta$: 1.34—1.50 (2H, m), 1.77 (2H, br d, $J$=11.2 Hz), 1.91 (2H, br t, $J$=11.2 Hz), 2.40 (3H, d, $J$=4.8 Hz), 2.71 (2H, br d, $J$=11.2 Hz), 2.84—2.96 (1H, m), 3.40 (2H, s), 6.57 (1H, q, $J$=5.2 Hz), 6.85 (1H, d, $J$=7.6 Hz), 7.18—7.33 (5H, m).

***N*-(1-Benzylpiperidin-4-yl)sulfamide (9r)** To a solution of 4-amino-1-benzylpiperidine (9.9 g, 52 mmol) in 1,2-dimethoxyethane (50 ml) was added sulfamide (5.0 g, 52 mmol) at rt. The reaction mixture was stirred at 100 °C for 10 min and at 130 °C for 2 h, then concentrated *in vacuo*. The residue was chromatographed on NH silica gel (60% AcOEt–hexane) to afford **9r** (3.8 g, 27%) as a colorless solid. mp 90—91 °C. $^1$H-NMR (DMSO-$d_6$) $\delta$: 1.34—1.46 (2H, m), 1.81 (2H, br d, $J$=10.8 Hz), 1.92 (2H, br t, $J$=11.6 Hz), 2.72 (2H, br d, $J$=11.6 Hz), 2.96—3.08 (1H, m), 3.40 (2H, s), 6.45 (2H, s), 6.51 (1H, d, $J$=7.6 Hz), 7.18—7.32 (5H, m).

**2-Hydroxyphenyl *N*-(1-Benzylpiperidin-4-yl)sulfamate (11)** This sulfamate was prepared according to the procedure of DuBois.[53] To a solution of 1-benzyl-4-aminopiperidine (9.1 g, 48 mmol) and $NEt_3$ (5.6 ml, 40 mmol) in DMF (120 ml) was added a solution of catecol sulfate[52] (9.0 g, 52 mmol) in $CH_2Cl_2$ (20 ml) at 0 °C. The reaction mixture was stirred for 2.5 h at 0 °C, poured into a 1% NaCl solution (500 ml), then extracted with $Et_2O$. The extract was washed with brine, dried, and evaporated. The residue was chromatographed on silica gel (5% MeOH–$CH_2Cl_2$) to afford **11** (17 g, 99%) as a colorless oil. $^1$H-NMR ($CDCl_3$) $\delta$: 1.58 (2H, dq, $J$=4.0, 12 Hz), 1.96 (2H, d, $J$=12 Hz), 2.08 (2H, t, $J$=12 Hz), 2.81 (2H, m), 3.44 (1H, m), 3.48 (2H, s), 4.95 (2H, br s), 6.83 (1H, dt, $J$=2.0, 8.0 Hz), 6.91 (1H, dd, $J$=2.0, 8.0 Hz), 7.10 (1H, dt, $J$=2.0, 8.0 Hz), 7.22 (1H, dd, $J$=2.0, 8.0 Hz), 7.24—7.32 (5H, m).

***N*-(1-Benzylpiperidin-4-yl)-*N*′,*N*′-pentamethylenesulfamide (12s)** This sulfamide was prepared according to the procedure of DuBois.[53] A solution of **11** (8.7 g, 24 mmol) and piperidine (2.4 g, 29 mmol) in 1,4-dioxane (50 ml) was refluxed for 4 h under a nitrogen atmosphere. The reaction mixture was allowed to cool and then poured into water, and extracted with AcOEt. The extract was washed with brine, dried, and evaporated. The residue was chromatographed on silica gel (4% MeOH–$CH_2Cl_2$) to afford **12s** (4.9 g, 60%) as a pale red solid. mp 82—83 °C. $^1$H-NMR ($CDCl_3$) $\delta$: 1.47—1.60 (4H, m), 1.55—1.70 (4H, m), 1.90—2.05 (2H, m), 2.10 (2H, br t, $J$=10.8 Hz), 2.79 (2H, d, $J$=11.6 Hz), 3.15 (4H, t, $J$=5.6 Hz), 3.10—3.30 (1H, m), 3.48 (2H, s), 4.00—4.15 (1H, m), 7.22—7.40 (5H, m).

***N*-(1-Benzylpiperidin-4-yl)-*N*′,*N*′-(3-oxapentamethylene)sulfamide (12t)** In the same manner as described for the preparation of **12s**, **12t** was obtained as a pale red solid (62%). mp 99—100 °C. $^1$H-NMR ($CDCl_3$) $\delta$: 1.48—1.60 (2H, m), 1.98 (2H, br d, $J$=12 Hz), 2.10 (2H, br t, $J$=11.6 Hz), 2.80 (2H, br d, $J$=12 Hz), 3.17 (4H, t, $J$=4.8 Hz), 3.15—3.30 (1H, m), 3.49 (2H, s), 3.74 (4H, t, $J$=4.8 Hz), 4.06—4.16 (1H, m), 7.20—7.35 (5H, m).

**8-(Piperidin-1-ylmethyl)-10*H*-pyrazino[2,3-*b*][1,4]benzothiazine (7b)** A mixture of **5** (0.50 g, 2.0 mmol), piperidine (0.20 g, 2.3 mmol), $K_2CO_3$ (0.83 g, 6.0 mmol) and DMF (20 ml) was stirred at 60 °C for 3 h. The resulting mixture was poured into water, and was then extracted with AcOEt. The extract was washed with brine, dried, and evaporated. The residue was chromatographed on silica gel (5% MeOH–$CH_2Cl_2$) to give the product (0.45 g, 75%) as a yellow solid. mp 178.5—179.5 °C. $^1$H-NMR (DMSO-$d_6$) $\delta$: 1.30—1.43 (2H, m), 1.35—1.53 (4H, m), 2.17—2.35 (4H, m), 3.20 (2H, s), 6.67 (1H, d, $J$=8.0 Hz), 6.74 (1H, s), 6.81 (1H, d, $J$=8.0 Hz), 7.58—7.66 (2H, m), 9.42 (1H, s). FAB-MS $m/z$: 299 $(M+H)^+$. *Anal.* Calcd for $C_{16}H_{18}N_4S$: C, 64.40; H, 6.08; N, 18.78. Found: C, 64.21; H, 5.90; N, 18.56.

**8-(Azetidin-1-ylmethyl)-10*H*-pyrazino[2,3-*b*][1,4]benzothiazine (7a)** In the same manner as described for the preparation of **7b**, **7a** was obtained as yellow solid (68%). mp 158—161 °C. $^1$H-NMR (DMSO-$d_6$) $\delta$: 1.96 (2H, quint., $J$=6.8 Hz), 3.08 (4H, t, $J$=6.8 Hz), 3.32 (2H, s), 6.67 (1H, dd, $J$=1.6, 7.6 Hz), 6.72 (1H, d, $J$=2.0 Hz), 6.82 (1H, d, $J$=7.6 Hz), 7.62—7.66 (2H, m), 9.45 (1H, s). FAB-MS $m/z$: 271 $(M+H)^+$. *Anal.* Calcd for $C_{14}H_{14}N_4S$: C, 62.20; H, 5.22; N, 20.72. Found: C, 62.04; H, 5.14; N, 20.85.

**8-(Hexamethyleniminomethyl)-10*H*-pyrazino[2,3-*b*][1,4]benzothiazine (7c)** In the same manner as described for the preparation of **7b**, **7c** was obtained as yellow solid (69%). mp 161—163 °C. $^1$H-NMR (DMSO-$d_6$) $\delta$: 1.54 (8H, s), 2.43—2.55 (4H, m), 3.38 (2H, s), 6.70 (1H, dd, $J$=1.6, 8.0 Hz), 6.80 (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), 9.45 (1H, s). HR-MS (FAB) $m/z$: Calcd for $C_{17}H_{21}N_4S$ $(M+H)^+$: 313.1487. Found: 313.1494.

**8-(Heptamethylenimnomethyl)-10*H*-pyrazino[2,3-*b*][1,4]benzothiazine (7d)** In the same manner as described for the preparation of **7b**, **7d** was obtained as yellow solid (66%). mp 133—135 °C. $^1$H-NMR (DMSO-$d_6$) $\delta$: 1.45—1.66 (10H, m), 2.46 (4H, t, $J$=5.6 Hz), 3.38 (2H, s), 6.74 (1H, d, $J$=8.0 Hz), 6.83 (1H, d, $J$=1.6 Hz), 6.84 (1H, d, $J$=7.6 Hz), 7.60—7.68 (2H, m), 9.54 (1H, s). HR-MS (FAB) $m/z$: Calcd for $C_{18}H_{23}N_4S$ $(M+H)^+$: 327.1643. Found: 327.1609.

**8-[(Morpholin-4-yl)methyl]-10*H*-pyrazino[2,3-*b*][1,4]benzothiazine (7e)** In the same manner as described for the preparation of **7b**, **7e** was obtained as yellow solid (67%). mp 165—166 °C. $^1$H-NMR (DMSO-$d_6$) $\delta$: 2.30 (4H, br s), 3.25 (2H, s), 3.54 (4H, br t, $J$=4.4 Hz), 6.70 (1H, d, $J$=7.5 Hz), 6.75 (1H, s), 6.84 (1H, d, $J$=7.5 Hz), 7.56—7.66 (2H, m), 9.45 (1H, s). ESI-MS $m/z$: 301 $(M+H)^+$. *Anal.* Calcd for $C_{15}H_{16}N_4OS \cdot 0.1H_2O$: C, 59.62; H, 5.40; N, 18.54. Found: C, 59.58; H, 5.26; N, 18.48.

**8-[(4-Methylpiperazin-1-yl)methyl]-10*H*-pyrazino[2,3-*b*][1,4]benzothiazine (7f)** In the same manner as described for the preparation of **7b**, **7f** was obtained as yellow solid (63%). mp 191—192 °C. $^1$H-NMR (DMSO-$d_6$) $\delta$: 2.12 (3H, s), 2.10—2.50 (8H, br s), 3.23 (2H, s), 6.68 (1H, d, $J$=8.0 Hz), 6.74 (1H, s), 6.82 (1H, d, $J$=7.6 Hz), 7.62 (2H, s), 9.43 (1H, s). ESI-MS $m/z$: 314 $(M+H)^+$. *Anal.* Calcd for $C_{16}H_{19}N_5S \cdot 0.51H_2O$: C, 59.57; H, 6.25; N, 21.71. Found: C, 59.24; H, 5.87; N, 21.52.

**1-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)piperidine-2-carboxamide (7g)** In the same manner as described for the preparation of **7b**, **7g** was obtained as yellow solid (44%). mp 209—211 °C. $^1$H-NMR (DMSO-$d_6$) $\delta$: 1.14—1.28 (1H, m), 1.24—1.44 (1H, m), 1.44—1.60 (2H, m), 1.54—1.70 (1H, m), 1.64—1.76 (1H, m), 1.80—1.92 (1H, m), 2.60—2.70 (1H, m), 2.68—2.78 (1H, m), 2.91 (1H, d, $J$=13.6 Hz), 3.56 (1H, d, $J$=13.6 Hz), 6.77 (1H, s), 6.74—6.84 (1H, m), 6.83 (1H, d, $J$=8.4 Hz), 7.04 (1H, s), 7.09 (1H, s), 7.62 (1H, d, $J$=2.8 Hz), 7.63 (1H, d, $J$=2.4 Hz), 9.40 (1H, s). FAB-MS

*m/z*: 342 $(M+H)^+$. *Anal.* Calcd for $C_{17}H_{19}N_5OS \cdot 0.5H_2O$: C, 58.26; H, 5.75; N, 19.98. Found: C, 58.17; H, 5.77; N, 19.91.

**1-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)piperidine-3-carboxamide (7h)** In the same manner as described for the preparation of **7b**, **7h** was obtained as brown oil (59%). $^1$H-NMR (DMSO-$d_6$) $\delta$: 1.20—1.40 (1H, m), 1.30—1.50 (1H, m), 1.54—1.65 (1H, m), 1.65—1.78 (1H, m), 1.78—1.94 (1H, m), 1.93 (1H, t, $J$=10.8 Hz), 2.22—2.34 (1H, m), 2.62—2.80 (2H, m), 3.24 (1H, d, $J$=13.6 Hz), 3.28 (1H, d, $J$=13.2 Hz), 6.70 (1H, dd, $J$=1.6, 8.0 Hz), 6.74 (1H, d, $J$=1.6 Hz), 6.76 (1H, s), 6.85 (1H, d, $J$=8.0 Hz), 7.27 (1H, s), 7.64 (1H, d, $J$=3.2 Hz), 7.65 (1H, d, $J$=2.8 Hz), 9.46 (1H, s). HR-MS (FAB) *m/z*: Calcd for $C_{17}H_{20}N_5OS$ $(M+H)^+$: 342.1389. Found: 342.1346.

**1-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)piperidine-4-carboxamide (7i)** In the same manner as described for the preparation of **7b**, **7i** was obtained as yellow solid (56%). mp 225—228 °C. $^1$H-NMR (DMSO-$d_6$) $\delta$: 1.53 (2H, br t, $J$=10.8 Hz), 1.50—1.70 (2H, m), 1.84 (2H, br t, $J$=10.8 Hz), 1.94—2.10 (1H, m), 2.66—2.84 (2H, m), 3.23 (2H, s), 6.68 (1H, d, $J$=8.0 Hz), 6.60—6.75 (1H, br s), 6.76 (1H, s), 6.82 (1H, d, $J$=7.6 Hz), 7.19 (1H, br s), 7.62 (2H, s), 9.44 (1H, br s). FAB-MS *m/z*: 342 $(M+H)^+$. *Anal.* Calcd for $C_{17}H_{19}N_5OS \cdot 0.3H_2O$: C, 58.87; H, 5.70; N, 20.19. Found: C, 58.89; H, 5.49; N, 20.00.

***N*-[1-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)piperidin-4-yl]acetamide (7j)** In the same manner as described for the preparation of **7b**, **7j** was obtained as yellow solid (61%). mp 212—214 °C. $^1$H-NMR (DMSO-$d_6$) $\delta$: 1.25—1.40 (2H, m), 1.66 (2H, br d, $J$=10.4 Hz), 1.75 (3H, s), 1.86—2.00 (2H, m), 2.69 (2H, br d, $J$=10.4 Hz), 3.23 (2H, s), 3.40—3.54 (1H, m), 6.68 (1H, d, $J$=7.6 Hz), 6.73 (1H, s), 6.82 (1H, d, $J$=7.6 Hz), 7.62 (2H, s), 7.73 (1H, d, $J$=6.8 Hz), 9.44 (1H, s). FAB-MS *m/z*: 356 $(M+H)^+$. *Anal.* Calcd for $C_{18}H_{21}N_5OS \cdot 0.35H_2O$: C, 59.76; H, 6.05; N, 19.36. Found: C, 59.46; H, 5.68; N, 19.17.

***N*-[1-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)piperidin-4-yl]methanesulfonamide (7k)** In the same manner as described for the preparation of **7b**, **7k** was obtained as yellow solid (65%). mp 220—223 °C. $^1$H-NMR (DMSO-$d_6$) $\delta$: 1.34—1.52 (2H, m), 1.79 (2H, br d, $J$=11.6 Hz), 1.88—2.04 (2H, m), 2.71 (2H, br d, $J$=11.2 Hz), 2.90 (3H, s), 3.02—3.18 (1H, m), 3.25 (2H, s), 6.70 (1H, d, $J$=7.6 Hz), 6.76 (1H, s), 6.85 (1H, d, $J$=7.6 Hz), 7.07 (1H, d, $J$=7.2 Hz), 7.61—7.69 (2H, m), 9.46 (1H, s). ESI-MS *m/z*: 392 $(M+H)^+$. *Anal.* Calcd for $C_{17}H_{21}N_5O_2S_2 \cdot 0.1H_2O$: C, 51.91; H, 5.43; N, 17.81. Found: C, 51.89; H, 5.32; N, 17.59.

***N*-[[1-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)piperidin-4-yl]methyl]methanesulfonamide (7l)** In the same manner as described for the preparation of **7b**, **7l** was obtained as yellow solid (53%). mp 218—221 °C. $^1$H-NMR (DMSO-$d_6$) $\delta$: 1.04—1.18 (2H, m), 1.30—1.44 (1H, m), 1.65 (2H, br d, $J$=11.2 Hz), 1.85 (2H, br t, $J$=11.6 Hz), 2.76 (2H, br d, $J$=11.2 Hz), 2.80 (2H, t, $J$=6.0 Hz), 2.86 (3H, s), 3.25 (2H, s), 6.70 (1H, dd, $J$=1.6, 8.0 Hz), 6.76 (1H, d, $J$=2.0 Hz), 6.84 (1H, d, $J$=8.0 Hz), 6.98 (1H, t, $J$=6.4 Hz), 7.62—7.66 (2H, m), 9.45 (1H, s). ESI-MS *m/z*: 406 $(M+H)^+$. *Anal.* Calcd for $C_{18}H_{23}N_5O_2S_2 \cdot 0.2H_2O$: C, 52.84; H, 5.76; N, 17.12. Found: C, 52.86; H, 5.56; N, 17.44.

***N*-[2-[1-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)piperidin-4-yl]ethyl]methanesulfonamide (7m)** In the same manner as described for the preparation of **7b**, **7m** was obtained as yellow solid (46%). mp 168—170 °C. $^1$H-NMR (DMSO-$d_6$) $\delta$: 1.03—1.18 (2H, m), 1.20—1.40 (1H, m), 1.38 (2H, q, $J$=7.2 Hz), 1.61 (2H, br d, $J$=12.0 Hz), 1.85 (2H, br t, $J$=10.8 Hz), 2.75 (2H, br d, $J$=10.4 Hz), 2.87 (3H, s), 2.94 (2H, q, $J$=6.8 Hz), 3.24 (2H, s), 6.70 (1H, d, $J$=8.0 Hz), 6.76 (1H, d, $J$=1.6 Hz), 6.84 (1H, d, $J$=8.0 Hz), 6.91 (1H, t, $J$=6.0 Hz), 7.62—7.66 (2H, m), 9.45 (1H, s). ESI-MS *m/z*: 420 $(M+H)^+$. *Anal.* Calcd for $C_{19}H_{25}N_5O_2S_2$: C, 54.39; H, 6.01; N, 16.69. Found: C, 54.25; H, 5.79; N, 16.66.

***N*-[1-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)piperidin-4-yl]trifluoromethanesulfonamide (7n)** To a solution of **9n** (7.0 g, 22 mmol) in ethanol (100 ml), 10% Pd/C (1.0 g) was added. The flask was then placed under a hydrogen atmosphere and stirred at rt for 12 h. The catalyst was removed by filtration and the filtrate was concentrated to give **6n** as a colorless solid (0.9 g, 18%). A mixture of **5** (0.5 g, 2.0 mmol), **6n** (0.56 g, 2.4 mmol) and $K_2CO_3$ (0.83 g, 6.0 mmol) in DMF (30 ml) was stirred at 60 °C for 3 h. The resulting mixture was poured into water, and then 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 **7n** (0.35 g, 39%) as a yellow solid. mp 199—201 °C. $^1$H-NMR (DMSO-$d_6$) $\delta$: 1.43—1.60 (2H, m), 1.75 (2H, br d, $J$=10.4 Hz), 1.96 (2H, br t, $J$=10.4 Hz), 2.73 (2H, br d, $J$=11.2 Hz), 3.25 (2H, s), 3.15—3.40 (1H, m), 6.67 (1H, d, $J$=7.6 Hz), 6.73 (1H, s), 6.83 (1H, d, $J$=7.6 Hz), 7.62 (1H, d, $J$=3.2 Hz), 7.63 (1H, d, $J$=3.2 Hz), 9.45 (2H, s). ESI-MS *m/z*: 446 $(M+H)^+$. *Anal.* Calcd for $C_{17}H_{18}F_3N_5O_2S_2 \cdot 1.7H_2O$: C, 42.89; H, 4.53; N, 14.71. Found: C, 42.93; H, 4.28; N, 14.70.

***N*-[1-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)piperidin-4-yl]benzenesulfonamide (7o)** In the same manner as described for the preparation of **7b**, **7o** was obtained as yellow solid (55%). mp 157—158 °C. $^1$H-NMR (DMSO-$d_6$) $\delta$: 1.25—1.48 (2H, m), 1.40—1.60 (2H, m), 1.74—1.96 (2H, m), 2.50—2.70 (2H, m), 2.85—3.00 (1H, m), 3.20 (2H, s), 6.65 (1H, d, $J$=7.6 Hz), 6.71 (1H, s), 6.82 (1H, d, $J$=8.0 Hz), 7.53—7.70 (5H, m), 7.70—7.83 (1H, m), 7.75—7.90 (2H, m), 9.44 (1H, s). ESI-MS *m/z*: 454 $(M+H)^+$. *Anal.* Calcd for $C_{22}H_{23}N_5O_2S_2 \cdot 0.3H_2O$: C, 57.57; H, 5.18; N, 15.26. Found: C, 57.56; H, 4.94; N, 15.22.

***N*-[1-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)piperidin-4-yl]-*N′*,*N′*-dimethylsulfamide (7p)** In the same manner as described for the preparation of **7n**, **7p** was obtained as yellow solid (50%). mp 202—203 °C. $^1$H-NMR (DMSO-$d_6$) $\delta$: 1.35—1.50 (2H, m), 1.70—1.80 (2H, m), 1.84—1.96 (2H, m), 2.61 (6H, s), 2.64—2.74 (2H, m), 2.90—3.00 (1H, m), 3.22 (2H, s), 6.66 (1H, d, $J$=8.0 Hz), 6.73 (1H, s), 6.82 (1H, d, $J$=8.0 Hz), 7.19 (1H, d, $J$=8.0 Hz), 7.62 (2H, s), 9.44 (1H, s). ESI-MS *m/z*: 421 $(M+H)^+$. *Anal.* Calcd for $C_{18}H_{24}N_6O_2S_2$: C, 51.41; H, 5.75; N, 19.98. Found: C, 51.29; H, 5.64; N, 20.05.

***N*-[1-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)piperidin-4-yl]-*N′*-methylsulfamide (7q)** In the same manner as described for the preparation of **7n**, **7q** was obtained as yellow solid (47%). mp 209—210 °C. $^1$H-NMR (DMSO-$d_6$) $\delta$: 1.34—1.48 (2H, m), 1.77 (2H, br d, $J$=10 Hz), 1.82—1.94 (2H, m), 2.41 (3H, d, $J$=4.8 Hz), 2.70 (2H, br d, $J$=12 Hz), 2.84—2.96 (1H, m), 3.22 (2H, s), 6.58 (1H, q, $J$=4.8 Hz), 6.67 (1H, d, $J$=8.4 Hz), 6.73 (1H, s), 6.82 (1H, d, $J$=7.6 Hz), 6.87 (1H, d, $J$=8.0 Hz), 7.62 (1H, d, $J$=2.8 Hz), 7.63 (1H, d, $J$=2.4 Hz), 9.44 (1H, s). ESI-MS *m/z*: 407 $(M+H)^+$. *Anal.* Calcd for $C_{17}H_{22}N_6O_2S_2$: C, 50.23; H, 5.45; N, 20.67. Found: C, 50.04; H, 5.30; N, 20.68.

***N*-[1-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)piperidin-4-yl]sulfamide (7r)** In the same manner as described for the preparation of **7n**, **7r** was obtained as yellow solid (44%). mp 196—199 °C. $^1$H-NMR (DMSO-$d_6$) $\delta$: 1.32—1.48 (2H, m), 1.81 (2H, br d, $J$=11.6 Hz), 1.90 (2H, br t, $J$=11.2 Hz), 2.70 (2H, br d, $J$=11.2 Hz), 2.94—3.10 (1H, m), 3.22 (2H, s), 6.45 (2H, s), 6.53 (1H, d, $J$=7.2 Hz), 6.67 (1H, d, $J$=8.0 Hz), 6.73 (1H, s), 6.82 (1H, d, $J$=8.0 Hz), 7.62 (2H, s), 9.44 (1H, s). ESI-MS *m/z*: 393 $(M+H)^+$. *Anal.* Calcd for $C_{16}H_{20}N_6O_2S_2 \cdot 0.3H_2O$: C, 48.30; H, 5.22; N, 21.12. Found: C, 48.27; H, 5.09; N, 21.23.

***N*-[1-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)piperidin-4-yl]-*N′*,*N′*-pentamethylenesulfamide (7s)** In the same manner as described for the preparation of **7n**, **7s** was obtained as yellow solid (45%). mp 56—57 °C. $^1$H-NMR (DMSO-$d_6$) $\delta$: 1.35—1.54 (4H, m), 1.44—1.56 (4H, m), 1.77 (2H, br d, $J$=11.6 Hz), 1.92 (2H, br d, $J$=11.6 Hz), 2.71 (2H, br d, $J$=11.6 Hz), 2.98 (5H, t, $J$=5.6 Hz), 3.24 (2H, s), 6.69 (1H, d, $J$=7.6 Hz), 6.75 (1H, d, $J$=1.6 Hz), 6.84 (1H, d, $J$=8.0 Hz), 7.20 (1H, d, $J$=7.6 Hz), 7.64 (1H, d, $J$=2.8 Hz), 7.65 (1H, d, $J$=2.8 Hz), 9.46 (1H, s). FAB-MS *m/z*: 461 $(M+H)^+$. *Anal.* Calcd for $C_{21}H_{28}N_6O_2S_2$: C, 54.76; H, 6.13; N, 18.25. Found: C, 54.74; H, 5.82; N, 18.51.

***N*-[1-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)piperidin-4-yl]-*N′*,*N′*-(3-oxapentamethylene)sulfamide (7t)** In the same manner as described for the preparation of **7n**, **7t** was obtained as yellow solid (45%). mp 189—190 °C. $^1$H-NMR (DMSO-$d_6$) $\delta$: 1.36—1.48 (2H, m), 1.77 (2H, br d, $J$=10.8 Hz), 1.91 (2H, br t, $J$=10.8 Hz), 2.70 (2H, br d, $J$=11.6 Hz), 2.94 (4H, t, $J$=4.4 Hz), 2.90—3.06 (1H, m), 3.22 (2H, s), 3.60 (4H, t, $J$=4.4 Hz), 6.67 (1H, d, $J$=7.6 Hz), 6.73 (1H, s), 6.82 (1H, d, $J$=7.6 Hz), 7.36 (1H, d, $J$=8.0 Hz), 7.62 (2H, s), 9.44 (1H, s). FAB-MS *m/z*: 463 $(M+H)^+$. *Anal.* Calcd for $C_{20}H_{26}N_6O_3S_2$: C, 51.93; H, 5.67; N, 18.17. Found: C, 51.67; H, 5.47; N, 18.19.

***N*,*N*-[3-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)-3-azapentamethylene]acetamide (7u)** In the same manner as described for the preparation of **7b**, **7u** was obtained as yellow solid (62%). mp 195—196 °C. $^1$H-NMR (DMSO-$d_6$) $\delta$: 1.95 (3H, s), 2.25 (2H, t, $J$=4.8 Hz), 2.31 (2H, t, $J$=4.8 Hz), 3.29 (2H, s), 3.34—3.43 (4H, m), 6.70 (1H, d, $J$=8.4 Hz), 6.75 (1H, s), 6.84 (1H, d, $J$=8.0 Hz), 7.60—7.66 (2H, m), 9.45 (1H, s). FAB-MS *m/z*: 341 $(M)^+$. *Anal.* Calcd for $C_{17}H_{19}N_5OS \cdot 0.7H_2O$: C, 57.67; H, 5.81; N, 19.78. Found: C, 57.59; H, 5.72; N, 19.87.

***N*,*N*-[3-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)-3-azapentamethylene]methanesulfonamide (7v)** In the same manner as described for the preparation of **7b**, **7v** was obtained as yellow solid (54%). mp 221—222 °C. $^1$H-NMR (DMSO-$d_6$) $\delta$: 2.38—2.45 (4H, m), 2.85 (3H, s), 3.04—3.12 (4H, m), 3.32 (2H, s), 6.71 (1H, dd, $J$=1.6, 7.6 Hz), 6.74 (1H, d, $J$=1.6 Hz), 6.84 (1H, d, $J$=7.6 Hz), 7.62 (1H, d, $J$=2.8 Hz), 7.63 (1H, d, $J$=2.4 Hz), 9.45 (1H, s). FAB-MS *m/z*: 377 $(M)^+$. *Anal.* Calcd for

$C_{16}H_{19}N_5O_2S_2$: C, 50.91; H, 5.07; N, 18.55. Found: C, 50.78; H, 5.06; N, 18.41.

***N*,*N*-[3-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)-3-azapentamethylene]sulfamide (7w)** In the same manner as described for the preparation of **7b**, **7w** was obtained as yellow solid (50%). mp 215—217 °C. $^1$H-NMR (DMSO-$d_6$) $\delta$: 2.36—2.46 (4H, m), 2.90—3.00 (4H, m), 3.32 (2H, s), 6.72 (1H, dd, $J$=1.6, 8.0 Hz), 6.78 (3H, s), 6.86 (1H, d, $J$=8.0 Hz), 7.62—7.68 (2H, m), 9.48 (1H, s). HR-MS (FAB) *m/z*: Calcd for $C_{15}H_{19}N_6O_2S_2$ $(M+H)^+$: 379.1011. Found: 379.0991.

***N*,*N*-[3-(10*H*-Pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)-3-azapentamethylene]-*N*′,*N*′-dimethylsulfamide (7x)** In the same manner as described for the preparation of **7b**, **7x** was obtained as yellow solid (55%). mp 171—173 °C. $^1$H-NMR (DMSO-$d_6$) $\delta$: 2.33—2.40 (4H, m), 2.73 (6H, s), 3.08—3.16 (4H, m), 3.30 (2H, s), 6.70 (1H, d, $J$=8.0 Hz), 6.75 (1H, m), 6.84 (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*: 407 $(M+H)^+$. *Anal.* Calcd for $C_{17}H_{22}N_6O_2S_2$: C, 50.23; H, 5.45; N, 20.67. Found: C, 50.21; H, 5.36; N, 20.78.

## Pharmacology

**Quantification of Adhesion Molecule Expression on HUVEC by ELISA** The quantification of adhesion molecule expression on HUVEC by ELISA was performed according to the methods previously described.[54] The test compounds (or vehicle) were added to confluent HUVEC (Sanko Junyaku, Tokyo, Japan) monolayer cultures in 96 well-plates, and 0.25 h later, the cells were stimulated by the addition of TNF-$\alpha$ (final concentration: 1 ng/ml) or vehicle. After an incubation period of 4 h at 37 °C, HUVEC were washed twice with 100 $\mu$l of PBS (phosphate-buffered saline), and then fixed with 100 $\mu$l of PBS containing 0.025% glutaraldehyde for 5 min at room temperature. After the fixation, the cells were washed three times with 200 $\mu$l of PBS containing 0.1% BSA (PBSA). An aliquot of 100 $\mu$l of washing buffer containing 1 $\mu$g/ml of either mouse anti-human ICAM-1 (R&D, Minneapolis, MN, U.S.A.), mouse anti-human E-selectin (R&D, Minneapolis, MN, U.S.A.), or mouse anti-human VCAM-1 antibody (R&D, Minneapolis, MN, U.S.A.) was added to each well, and then the cells were stored at room temperature. Thirty minutes later, the cells were washed twice with 200 $\mu$l of PBSA. An aliquot of 100 $\mu$l of peroxidase-conjugated goat anti-mouse IgG (Fc) polyclonal antibody solution (Cosmo Bio Co., Ltd., Tokyo, Japan) which was diluted (1 : 1000) with washing PBSA, was added to each well. After an incubation period of 30 min at room temperature, the cells were washed three times with 200 $\mu$l of washing buffer. An aliquot of 100 $\mu$l of TMB substrate solution was applied to each well and, after adequate color development, the reaction was quenched by the addition of 100 $\mu$l of 1 M phosphoric acid. The absorbance at wavelength 450 nm was measured using an automated microplate reader. ($n$=2).

**IL-1-Induced Paw Inflammation Model in the Mouse** Pathogen-free Balb/c mice (6 week old, Charles River laboratories, Astugi, Japan) were orally dosed with vehicle (0.5% hydroxypropyl methyl cellulose) or the test compounds dissolved or suspended in vehicle. Thirty minutes later, the left hind paw of each mouse was injected subcutaneously with rat recombinant IL-1$\alpha$ (rrIL-1$\alpha$, obtained in our laboratories) (5 ng/50 $\mu$l/site). Two hours post rrIL-1$\alpha$ injection, the animals were sacrificed and the injected paws were removed with scissors. The paws were homogenized with 0.5% hexadecyl trimethylammonium bromide (HTAB) in potassium phosphate buffer (pH 6), and the MPO activities of the homogenates were evaluated using *o*-dianisidine–$H_2O_2$, as a marker enzyme for neutrophil content. MPO activity in the inflamed paw tissue was measured by the spectrophotometric method reported previously.[55] ($n$=3).

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