Structure–Activity Relationships of 2-(Benzothiazolylthio)acetamide Class of CCR3 Selective Antagonist

Akira NAYA,* Kensuke KOBAYASHI, Makoto ISHIKAWA, Kenji OHWAKI, Toshihiko SAEKI, Kazuhito NOGUCHI, and Norikazu OHTAKE

Banyu Tsukuba Research Institute; Okubo-3, Tsukuba, Ibaraki 300–2611, Japan.
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The structure activity relationships of novel selective CCR3 receptor antagonists, 2-(benzothiazolylthio)acetamimde derivatives were described. A lead structure (1a) was discovered from the screening of the focused library that was based on the structure of our dual antagonists for the human CCR1 and CCR3 receptors. Derivatization of 1a including incorporation of substituent(s) into each benzene ring of the benzothiazole and piperidine side chain resulted in the identification of potent and selective compounds (1b, r, s) exhibiting nano-molar binding affinity (IC_{50}s: 1.5—3.0 nM) and greater than 800-fold selectivity for the CCR3 receptor over the CCR1 receptor.

Key words chemokine; CCR3; 2-(benzothiazolylthio)acetamide

Chemokines, a large family of 8—14 kDa chemotactic cytokines, are generally classified into four sub-families, CXC chemokines, CC chemokines, C chemokine and CX_3C chemokine, based on the arrangement of the conserved cysteines in the N-terminal region.[1] These chemokines elicit their biological effects by activating a subset of the seven transmembrane G-protein-coupled receptors (GPCR). There have been 18 human chemokine receptors cloned and characterized to date; they are referred to as CCR1—11, CXCR1—5, XCR1 and CX_3CR1 receptors.

Selective accumulation of eosinophils into inflammatory sites is a characteristic in allergic diseases such as asthma.[2] Selective accumulation of eosinophils into allergic tissue suggests that there are chemoattractants and associated receptors that are specifically for eosinophils. There is a growing body of evidence that CC chemokines such as Eotaxin, RANTES, MCP-3 and MCP-4, which are ligands for the CCR3 receptor, are responsible for accumulation of eosinophils from the circulation to allergic sites.[3] Eotaxin is highly upregulated in animal models of allergic inflammation,[4,5] and both Eotaxin and CCR3 receptor are expressed in allergic disease in humans.[6—9] Furthermore, the antibody against Eotaxin reduced *in vivo* accumulation of eosinophils into the lung in response to ovalbumin,[10] and deletion of the Eotaxin gene led to the partial reduction of antigen-induced eosinophilia.[11] Thus, a CCR3 receptor-selective antagonist that suppresses the infiltration of eosinophils to inflammatory sites may have clinical potential in allergic diseases.

To identify an orally-active non-peptide antagonist that is selective for the CCR3 receptor, we initiated exploration of a lead structure. As a result of the screening of a focused library including 770 compounds which were designed based on our CCR1 and CCR3 mixed antagonist,[12] 2-(benzothiazolylthio)acetamide derivative (**1a**) was discovered. Derivatization of **1a** led to the identification of potent and selective antagonists such as compounds (**1b**, **r**, **s**). A preliminary account of this work has been presented previously.[13] Herein, we describe the detailed structure-activity relationships of 2-(benzothiazolylthio)acetamides that were derivatized from the lead structure (**1a**).

Chemistry

Preparation of compounds (**1b**—**s**) was summarized in Chart 1.

Chart 1 shows the synthesis of compounds (**1c**—**e**) which were prepared from 2-(benzothiazolylthio)acetamide (**2**) by reductive alkylation with appropriate benzaldehydes in 41—99% yields. Other derivatives (**1b**, **k**, **r**, **s**) were synthesized from 4-aminopiperidine (**3a**), which was reacted with bromoacetyl bromide and subsequently treated with 5-substituted benzothiazole to produce the compounds (**1b**, **k**) in 30—62% yields. Acylation of **1b** produced **1r** and **1s** in good yields. Compounds (**1f**—**j**) were prepared by condensation of carboxylic acids (**4a**—**e**) and the amines (**3a**, **b**) under a usual condition (WSC·HCl and 1-hydroxybenzotriazole (HOBt)).

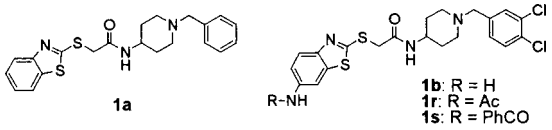
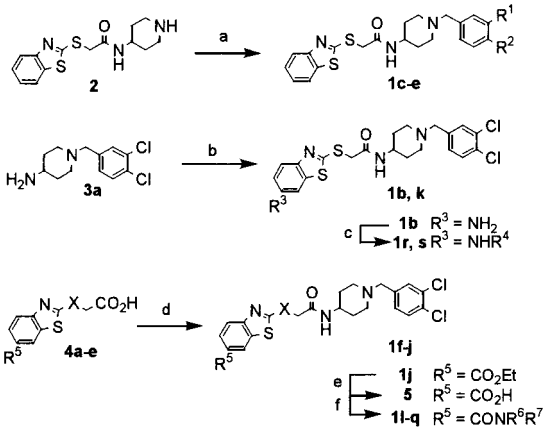


Fig. 1. The Structure of Compounds **1a**, **1b**, **1r**, and **1s**



Reagents: (a) RCHO, $NaBH(OAc)_3$; (b) (1) $BrCH_2COBr$, (2) Benzothiazole, $^iPr_2NEt$; (c) Ac_2O or PhCOCl; (d) **3a** or **3b**, WSC•HCl, HOBt, Et_3N; (e) NaOH; (f) HNR^6R^7, WSC•HCl, HOBt, Et_3N.

Chart 1

* To whom correspondence should be addressed. e-mail: nayaak@banyu.co.jp

Compounds (**1l**—**q**) were synthesized by condensation of the acid **5** and appropriate amines.

Chart 2 shows the synthesis of new amines (**2**, **3a**, **b**) and acids (**4b**—**e**).

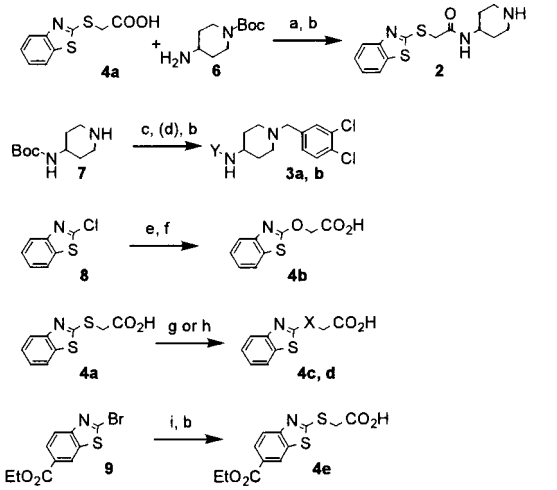
Result and Discussion

The synthesized compounds were tested for their inhibitory activity (IC_{50}) to ^{125}I-Eotaxin binding to CCR3 and to ^{125}I-MIP-1α binding to CCR1, which are expressed in CHO cells.

The optimization process of the lead (**1a**) was as follows. The lead compound was divided into three sub-structures (A-, B-, and C-parts), and optimization of each sub-structure was performed based on the previous results.[13] (Fig. 2)

First, effects of substituent(s) (A-part) on the benzene ring on the binding affinities to CCR3 and CCR1 receptors were examined (Table 1). Introduction of a chlorine atom into the 3-position of the benzene ring (**1c**) enhanced the binding affinity for the CCR3 receptor (IC_{50}: 280 nM) and almost lost all binding affinity for the CCR1 receptor. By contrast, incorporation of a chlorine atom (**1d**) at the 4-position resulted in approximately a 10-fold and 25-fold improvement in the binding affinity for the CCR1 and CCR3 receptors, respectively. Incorporation of chlorine atoms at both 3- and 4-positions (**1e**) further improved the CCR3 binding affinity.

With regard to the optimization of the B-part, replacement of the sulfur atom in **1e** with an oxygen atom (**1f**) or with oxidized sulfur atom (sulfoxide (**1g**) or sulfone (**1h**)) resulted in a great reduction in the binding affinity for the CCR3 receptor. *N*-Methylation (**1i**) of the amide moiety in **1e** greatly reduced the binding affinity for the CCR3 receptor, suggesting



Reagents: (a) WSC·HCl, HOBt, Et_3N; (b) TFA; (c) (3,4-Cl_2)PhCHO, $NaBH(OAc)_3$; (d) (1) NaH, (2) MeI; (e) (1) NaH, (2) $HOCH_2CO_2Et$; (f) NaOH; (g) Oxone; (h) Oxone, $NaHCO_3$, EDTA soln., NaOH; (i) (1) NaH, (2) $HSCH_2CO_2{}^tBu$;

Chart 2

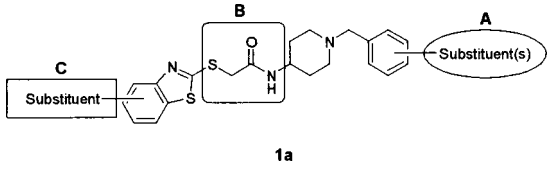
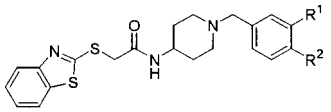


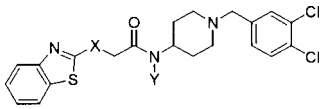
Fig. 2. Three Sub-Structures of the Lead Compound **1a**

Table 1. Binding Affinity of Compounds **1a**, **1c**—**e** to CCR3 and CCR1 Receptors



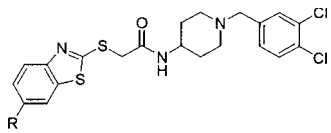
Compound	R^1	R^2	IC_{50} (nM)	
			CCR3	CCR1
1a	H	H	750	7200
1c	Cl	H	280	>10000
1d	H	Cl	79	260
1e	Cl	Cl	32	450

Table 2. Binding Affinity of Compounds **1e**—**i** to CCR3 and CCR1 Receptors



Compound	X	Y	IC_{50} (nM)	
			CCR3	CCR1
1e	S	H	32	450
1f	O	H	360	1600
1g	SO	H	930	590
1h	SO_2	H	6800	3600
1i	S	Me	4300	2500

Table 3. Binding Affinity of Compounds **1b**, **e**, **j**—**s** to CCR3 and CCR1 Receptors



Compound	R	IC_{50} (nM)		Selectivity
		CCR3	CCR1	
1e	H	32	450	14
1j	CO_2Et	48	>10000	>200
1k	OEt	20	3800	190
1l	$CONH_2$	3.4	3000	880
1m	CONHMe	4.0	9500	2400
1n	$CONMe_2$	3.4	>10000	>2900
1o	CONHBzl	1300	>10000	>7.7
1p	$CONHCH_2$(2-Py)	3.0	3800	1300
1q	$CONHCH_2$(3-Py)	4.8	>10000	>2100
1b	NH_2	2.3	1900	830
1r	NHCOMe	1.5	5400	3600
1s	NHCOPh	3.0	>10000	>3300

that the amide hydrogen would play an important role in the binding to the CCR3 and CCR1 receptors (Table 2).

Introduction of a substituent into the benzothiazole ring (C-part) in **1e** was performed to further improve the potency toward the CCR3 receptor and the selectivity for the CCR3 over the CCR1 receptor (Table 3). When an ethoxycarbonyl group was introduced into the 6-position, the resulting derivative (**1j**) retained potent binding affinity for the CCR3 re-

ceptor comparable to that of **1e**. However, its binding affinity for the CCR1 receptor was significantly reduced. As a result, its selectivity for the CCR3 over the CCR1 receptor was improved (CCR1/CCR3: >200 folds). An ethoxy group (**1k**) improved the binding affinity for the CCR3 receptor, while the selectivity was retained. Replacement of the ethyl ester moiety in **1j** with amide (**1l**), *N*-methylamide (**1m**), or *N*,*N*-dimethylamide (**1n**) brought about approximately a 10-fold improvement in the binding affinity (IC_{50}s: 3—4 nM) for the CCR3 receptor as compared to that of **1e**, while the binding affinity for the CCR1 receptor reduced with an increase in the number of *N*-substituents. The *N*,*N*-dimethylamide (**1n**) showed greater than 2900-fold selectivity over that of the CCR1 receptor. Interestingly, *N*-benzylamide (**1o**) greatly reduced the CCR3 binding affinity; however, the *N*-pyridylmethylamide (**1p**, **q**) recovered their CCR3 binding affinity, which was comparable to **1l**. These results suggest that an additional hydrogen binding site would exist in the antagonist binding site of the CCR3 receptor or the pyridine ring would electrically interact to the CCR3 receptor. Amine (**1b**) also retained low nano-molar binding affinity for the CCR3 receptor, and acetamide (**1r**) and benzamide (**1s**) maintained potent binding affinities. With respect to CCR3 potency and selectivity over the CCR1 receptor, the 6-acetamidobenzothiazole derivative (**1r**) with an IC_{50} of 1.5 nM and a 3600-fold selectivity over that of the CCR1 receptor would be the best compound of this series.

To determine the CCR3 antagonistic activity of the representative compound **1b**, we measured its ability to inhibit Eotaxin- and RANTES-induced Ca^{2+} responses with IC_{50} values of 27 and 13 nM, respectively.

Conclusion

We have discovered novel CCR3 selective antagonists (**1b**, **r**, **s**) by derivatization of a lead compound (**1a**), which was identified by screening the focused library (770 compounds of carboxamide derivatives). These compounds may be useful tools to elucidate the roles of CCR3 receptors in allergic diseases, especially in the selective accumulation and infiltration of eosinophils to inflammatory sites in these diseases.

Experimental

Materials and Methods All reagents and solvents were of commercial quality and were used without further purification unless otherwise noted. ^{1}H-NMR spectra were recorded on a Varian VXR 300 spectrometer with tetramethylsilane (δ=0) as an internal standard. The abbreviations for the signal patterns are as follows: s: singlet, d: doublet, t: triplet, q: quartet, dd: doublet of doublets, m: multiplet. Mass spectrometry was performed with a JEOL JMS-SX102A spectrometer. TLC was done with Merck Kieselgel F_{254} pre-coated plates. Silica gel column chromatography was carried out on Merck silica gel 60 (mesh 63—200 nm).

***N*-[1-(3-Chlorobenzyl)piperidin-4-yl]-(2-benzothiazolylthio)acetamide (1c)** To a solution of *N*-(Piperidin-4-yl)-(2-benzothiazolylthio)acetamide (**2**) (50 mg, 0.163 mmol) in CH_2Cl_2 (2.0 ml) was added 3-chlorobenzaldehyde (35 mg, 0.249 mmol) and $NaBH(OAc)_3$ (52 mg, 0.245 mmol) at room temperature, and the mixture was stirred for 20 h. After the addition of saturated $NaHCO_3$ solution, the mixture was extracted with $CHCl_3$. The organic layer was washed with brine, dried ($MgSO_4$), and concentrated *in vacuo*. The residue was purified by preparative TLC (5% MeOH in $CHCl_3$) to give **1c** (70 mg, 99%) as a colorless solid; ^{1}H-NMR ($CDCl_3$) δ: 1.34—1.53 (2H, m), 1.78—1.93 (2H, m), 2.05—2.22 (2H, m), 2.43—2.68 (2H, m), 3.30 (2H, s), 3.72—3.88 (1H, m), 3.93 (2H, s), 7.04—7.91 (9H, m); high resolution (HR)-MS *m*/*z*: 432.0983 (Calcd for $C_{21}H_{23}N_3OClS_2$, $(M+H)^+$: 432.0971).

The following compounds (**1d**, **e**) were prepared according to a procedure that was similar to that described for **1c** by using **2** and an appropriate aldehyde.

***N*-[1-(4-Chlorobenzyl)piperidin-4-yl]-(2-benzothiazolylthio)acetamide (1d)** This was prepared from 4-chlorobenzaldehyde (99%, colorless solid): ^{1}H-NMR ($CDCl_3$) δ: 1.32—1.50 (2H, m), 1.78—1.90 (2H, m), 2.03—2.19 (2H, m), 2.45—2.65 (2H, m), 3.30 (2H, s), 3.70—3.88 (1H, m), 3.92 (2H, s), 7.15 (2H, d, *J*=8.5 Hz), 7.24 (2H, d, *J*=8.5 Hz), 7.29—7.88 (5H, m); HR-MS *m*/*z*: 432.0983 (Calcd for $C_{21}H_{23}N_3OClS_2$, $(M+H)^+$: 432.0971).

***N*-[1-(3,4-Dichlorobenzyl)piperidin-4-yl]-(2-benzothiazolylthio)acetamide (1e)** This was prepared from 3,4-dichlorobenzaldehyde (41%, colorless solid): ^{1}H-NMR ($CDCl_3$) δ: 1.33—1.55 (2H, m), 1.80—1.93 (2H, m), 2.05—2.20 (2H, m), 2.42—2.65 (2H, m), 3.27 (2H, s), 3.70—3.88 (1H, m), 3.93 (2H, s), 7.07 (1H, dd, *J*=1.9, 8.5 Hz), 7.29—7.42 (3H, m), 7.48 (1H, t, *J*=7.8 Hz), 7.52 (1H, d, *J*=7.5 Hz), 7.79 (1H, d, *J*=7.8 Hz), 7.85 (1H, t, *J*=7.8 Hz); HR-MS *m*/*z*: 466.0575 (Calcd for $C_{21}H_{22}N_3OCl_2S_2$, $(M+H)^+$: 466.0581).

***N*-[1-(3,4-Dichlorobenzyl)piperidin-4-yl]-(6-amino-2-benzothiazolylthio)acetamide (1b)** To a stirred solution of 4-amino-1-(3,4-dichlorobenzyl)piperidine (**3a**) (4.2 g, 16.2 mmol) in $CHCl_3$ (75 ml) was added bromoacetyl bromide (2.1 ml, 24.1 mmol) at 0 °C, and the mixture was stirred at the same temperature for 10 min. After the addition of saturated $NaHCO_3$ solution, the mixture was extracted with EtOAc. The organic layer was washed with brine, dried ($MgSO_4$), and concentrated *in vacuo*. The residue was diluted with $CHCl_3$ (100 ml), and to this solution were added 6-amino-2-mercaptobenzothiazole (3.0 g, 16.5 mmol) and *i*-Pr_2NEt (6.0 ml, 36.8 mmol) at room temperature. The mixture was stirred at a constant temperature for 18 h. After the addition of saturated $NaHCO_3$ solution, the mixture was extracted with $CHCl_3$. The organic layer was washed with brine, dried ($MgSO_4$), and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (0—3% MeOH in $CHCl_3$) to give **1b** (2.3 g, 30%) as a colorless solid: ^{1}H-NMR ($CDCl_3$) δ: 1.35—1.55 (2H, m), 1.74—1.92 (2H, m), 2.00—2.20 (2H, m), 2.36—2.65 (2H, m), 3.27 (2H, m), 3.65—3.90 (1H, m), 3.85 (2H, s), 6.80 (1H, dd, *J*=2.3, 8.7 Hz), 7.02 (1H, d, *J*=2.3 Hz), 7.00—7.12 (1H, m), 7.20—7.40 (2H, m), 7.50—7.70 (2H, m); HR-MS *m*/*z*: 481.0688 (Calcd for $C_{21}H_{23}N_4OCl_2S_2$, $(M+H)^+$: 481.0690).

***N*-[1-(3,4-Dichlorobenzyl)piperidin-4-yl]-(6-ethoxy-2-benzothiazolylthio)acetamide (1k)** This was prepared in a manner simillar to the procedure described for **1b** by using the amine **3a** and 6-ethoxy-2-mercaptobenzothiazole (62%, colorless solid): ^{1}H-NMR ($CDCl_3$) δ: 1.35—1.55 (2H, m), 1.46 (3H, t, *J*=7.0 Hz), 1.80—1.95 (2H, m), 2.05—2.25 (2H, m), 2.40—2.70 (2H, m), 3.28 (2H, s), 3.72—3.95 (1H, m), 3.89 (2H, s), 4.09 (2H, q, *J*=7.0 Hz), 6.96—7.12 (2H, m), 7.20—7.45 (3H, m), 7.55 (1H, d, *J*=8.9 Hz), 7.73 (1H, d, *J*=8.9 Hz); HR-MS *m*/*z*: 510.0837 (Calcd for $C_{23}H_{26}N_3O_2Cl_2S_2$, $(M+H)^+$: 510.0844).

***N*-[1-(3,4-Dichlorobenzyl)piperidin-4-yl]-(6-acetamido-2-benzothiazolylthio)acetamide (1r)** To a stirred solution of **1b** (9.6 mg, 0.020 mmol) in $CHCl_3$ (1.0 ml) were added Ac_2O (4.0 μl, 0.042 mmol) and Et_3N (10 μl, 0.072 mmol) at room temperature, and the mixture was stirred for 20 h. After the addition of saturated $NaHCO_3$ solution, the mixture was extracted with EtOAc. The organic layer was washed with water, dried ($MgSO_4$), and concentrated *in vacuo*. The residue was purified by preparative TLC (5% MeOH in $CHCl_3$) to give **1r** (7.0 mg, 67%) as a colorless solid; ^{1}H-NMR ($CDCl_3$) δ: 1.32—1.50 (2H, m), 1.76—1.91 (2H, m), 2.01—2.18 (2H, m), 2.22 (3H, s), 2.42—2.65 (2H, m), 3.28 (2H, s), 3.68—3.88 (1H, m), 3.90 (2H, s), 7.07 (1H, d, *J*=8.0 Hz), 7.19—7.50 (5H, m), 7.75 (1H, d, *J*=8.6 Hz), 8.36 (1H, s); HR-MS *m*/*z*: 523.0808 (Calcd for $C_{23}H_{25}N_4O_2Cl_2S_2$, $(M+H)^+$: 523.0796).

***N*-[1-(3,4-Dichlorobenzyl)piperidin-4-yl]-(6-benzamido-2-benzothiazolylthio)acetamide (1s)** This was prepared in a manner similar to the procedure described for **1r** by using **1b** and benzoyl chloride (61%, pale yellow solid); ^{1}H-NMR ($CDCl_3$) δ: 1.33—1.51 (2H, m), 1.76—1.91 (2H, m), 2.03—2.20 (2H, m), 2.41—2.65 (2H, m), 3.29 (2H, s), 3.70—3.88 (1H, m), 3.91 (2H, s), 7.08 (1H, d, *J*=8.2 Hz), 7.28—7.36 (2H, m), 7.38—7.62 (5H, m), 7.81 (1H, d, *J*=8.7 Hz), 7.85—7.95 (2H, m), 8.04 (1H, s), 8.52 (1H, d, *J*=2.0 Hz); HR-MS *m*/*z*: 585.0936 (Calcd for $C_{28}H_{27}N_4O_2Cl_2S_2$, $(M+H)^+$: 585.0953).

***N*-[1-(3,4-Dichlorobenzyl)piperidin-4-yl]-(6-ethoxycarbonyl-2-benzothiazolylthio)acetamide (1j)** To a stirred solution of (6-ethoxycarbonyl-2-benzothiazolylthio)acetic acid (**4e**) (123 mg, 0.41 mmol) and **3a** (130 mg, 0.50 mmol) in $CHCl_3$ (8.0 ml) were added HOBt (190 mg, 1.41 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSC·HCl) (120 mg, 0.63 mmol) at room temperature. After stirring for 20 h, the reaction mixture was diluted with EtOAc, washed with saturated $NaHCO_3$ solution and brine, dried ($MgSO_4$), and concentrated *in vacuo*. The residue was

purified by silica gel column chromatography (2% MeOH in $CHCl_3$) to give **1j** (217 mg, 98%) as a colorless solid: ^{1}H-NMR ($CDCl_3$) δ: 1.32—1.52 (2H, m), 1.43 (3H, t, J=7.1 Hz,), 1.80—1.92 (2H, m), 2.00—2.20 (2H, m), 2.45—2.68 (2H, m), 3.29 (2H, s), 3.70—3.90 (1H, m), 3.96 (2H, s), 4.43 (2H, d, J=7.1 Hz), 7.00—7.10 (1H, m), 7.20—7.40 (3H, m), 7.87 (1H, d, J=8.6 Hz), 8.16 (1H, dd, J=1.6, 8.9 Hz), 8.52 (1H, d, J=1.6 Hz); HR-MS m/z: 538.0792 (Calcd for $C_{24}H_{26}N_3O_3Cl_2S_2$, $(M+H)^+$: 538.0793).

The following compounds (**1f**—**i**) were prepared in a manner similar to the procedure described for **1j** by using the amine **3a** or **3b** and an appropriate acid.

***N*-[1-(3,4-Dichlorobenzyl)piperidin-4-yl]-(2-benzothiazolyloxy)acetamide (1f)** This was prepared from (2-benzothiazolyloxy)acetic acid (**4b**) and **3a** (64%, colorless oil): ^{1}H-NMR ($CDCl_3$) δ: 1.40—1.64 (2H, m), 1.86—2.05 (2H, m), 2.05—2.22 (2H, m), 2.62—2.85 (2H, m), 3.41 (2H, s), 3.82—4.00 (1H, m), 5.02 (2H, s), 6.25 (1H, d, J=8.2 Hz), 7.12 (1H, dd, J=2.0, 8.2 Hz), 7.22—7.50 (4H, m), 7.64—7.80 (2H, m); HR-MS m/z: 450.0804 (Calcd for $C_{21}H_{22}N_3O_2Cl_2S$, $(M+H)^+$: 450.0810).

***N*-[1-(3,4-Dichlorobenzyl)piperidin-4-yl]-(2-benzothiazolylsulfinyl)acetamide (1g)** This was prepared from (2-benzothiazolylsulfinyl)acetic acid (**4c**) and **3a** (59%, colorless solid): ^{1}H-NMR ($CDCl_3$) δ: 1.36—1.54 (2H, m), 1.70—2.20 (4H, m), 2.60—2.80 (2H, m), 3.41 (2H, s), 3.70—3.90 (1H, m), 3.88 (1H, d, J=14.7 Hz), 4.12 (1H, d, J=14.7 Hz), 6.86 (1H, d, J=7.7 Hz,), 7.13 (1H, dd, J=1.9, 8.2 Hz), 7.37 (1H, d, J=8.2 Hz), 7.41 (1H, d, J=1.9 Hz), 7.51 (1H, t, J=7.4 Hz), 7.58 (1H, t, J=7.4 Hz), 7.99 (1H, d, J=7.4 Hz), 8.09 (1H, d, J=7.4 Hz); HR-MS m/z: 482.0537 (Calcd for $C_{21}H_{22}N_3O_2Cl_2S_2$, $(M+H)^+$: 482.0531).

***N*-[1-(3,4-Dichlorobenzyl)piperidin-4-yl]-(2-benzothiazolylsulfonyl)acetamide (1h)** This was prepared from (2-benzothiazolylsulfonyl)acetic acid (**4d**) and **3a** (48%, colorless oil): ^{1}H-NMR ($CDCl_3$) δ: 1.42—1.64 (2H, m), 1.70—2.00 (2H, m), 2.05—2.22 (2H, m), 2.60—2.80 (2H, m), 3.41 (2H, s), 3.70—3.89 (1H, m), 4.39 (2H, s), 6.68 (1H, d, J=7.8 Hz), 7.13 (1H, dd, J=2.0, 8.2 Hz), 7.37 (1H, d, J=8.2 Hz), 7.41 (1H, d, J=2.0 Hz), 7.58—7.72 (2H, m), 8.02 (1H, dd, J=2.0, 7.2 Hz), 8.21 (1H, dd, J=2.0, 7.2 Hz); HR-MS m/z: 498.0508 (Calcd for $C_{21}H_{22}N_3O_3Cl_2S_2$, $(M+H)^+$: 498.0480).

***N*-[1-(3,4-Dichlorobenzyl)piperidin-4-yl]-*N*-methyl-(2-benzothiazolylthio)acetamide (1i)** This was prepared from **4a** and **3b** (81%, colorless solid): ^{1}H-NMR ($CDCl_3$) δ: 1.50—2.18 (6H, m), 2.78—2.95 (2H, m), 2.89 & 3.06 (3H, s), 3.36 & 3.43 (2H, s), 3.72—3.85 & 4.39—4.57 (1H, m), 4.39 & 4.44 (2H, s), 7.08—7.18 (1H, m), 7.23—7.48 (4H, m), 7.72—7.99 (2H, m); HR-MS m/z: 480.072 (Calcd for $C_{22}H_{24}N_3OCl_2S_2$, $(M+H)^+$: 480.0738).

***N*-[1-(3,4-Dichlorobenzyl)piperidin-4-yl]-(6-carboxyl-2-benzothiazolylthio)acetamide (5)** To a stirred solution of **1j** (200 mg, 0.37 mmol) in THF (2.0 ml) and MeOH (2.0 ml) was added 1 M NaOH solution (0.50 ml, 0.50 mmol) at room temperature. After stirring for 3 h, the residue was adjusted to pH 5—6 with 1 M HCl, extracted with EtOAc, washed with brine, and dried ($MgSO_4$). The solvent was removed *in vacuo* to give **5** (140 mg, 74%) as pale yellow solid, which was used for the next step without further purification.

***N*-[1-(3,4-Dichlorobenzyl)piperidin-4-yl]-(6-carbamoyl-2-benzothiazolylthio)acetamide (1l)** This was prepared in a similar manner to the procedure described for **1j** by using **5** and ammonium chloride. (5%, colorless solid): ^{1}H-NMR ($CDCl_3$) δ: 1.32—1.50 (2H, m), 1.79—1.91 (2H, m), 2.02—2.18 (2H, m), 2.48—2.67 (2H, m), 3.30 (2H, s), 3.70—3.88 (1H, m), 3.94 (2H, s), 5.50—6.34 (2H, m), 7.06 (1H, d, J=8.0 Hz), 7.19—7.38 (3H, m), 7.81—7.90 (2H, m), 8.33 (1H, s); HR-MS m/z: 509.0634 (Calcd for $C_{22}H_{23}N_4O_2Cl_2S_2$, $(M+H)^+$: 509.0640).

The following compounds (**1m**—**q**) were prepared in a manner similar to the procedure described for **1l** by using **5** and an appropriate amine.

***N*-[1-(3,4-Dichlorobenzyl)piperidin-4-yl]-(6-methylcarbamoyl-2-benzothiazolylthio)acetamide (1m)** This was prepared from methylamine hydrochloride (7%, colorless solid): ^{1}H-NMR ($CDCl_3$) δ: 1.32—1.56 (2H, m), 1.79—1.92 (2H, m), 2.03—2.20 (2H, m), 2.50—2.68 (2H, m), 3.06 (3H, d, J=4.9 Hz), 3.31 (2H, s), 3.70—3.88 (1H, m), 3.95 (2H, s), 6.10—6.25 (1H, m), 7.07 (1H, d, J=8.2 Hz), 7.19—7.39 (3H, m), 7.79 (1H, dd, J=1.7, 8.5 Hz), 7.87 (1H, d, J=8.5 Hz), 8.29 (1H, d, J=1.7 Hz); HR-MS m/z: 523.0820 (Calcd for $C_{23}H_{25}N_4O_2Cl_2S_2$, $(M+H)^+$: 523.0796).

***N*-[1-(3,4-Dichlorobenzyl)piperidin-4-yl]-(6-dimethylcarbamoyl-2-benzothiazolylthio)acetamide (1n)** This was prepared from dimethylamine hydrochloride (2%, colorless solid): ^{1}H-NMR ($CDCl_3$) δ: 1.31—1.50 (2H, m), 1.80—1.92 (2H, m), 2.03—2.19 (2H, m), 2.50—2.69 (2H, m), 2.95—3.20 (6H, m), 3.33 (2H, s), 3.71—3.85 (1H, m), 3.94 (2H, s), 7.03—7.12 (1H, m), 7.29—7.40 (3H, m), 7.49—7.54 (1H, m), 7.80—7.90 (2H, m); HR-MS m/z: 537.0981 (Calcd for $C_{24}H_{27}N_4O_2Cl_2S_2$, $(M+H)^+$: 537.0953).

***N*-[1-(3,4-Dichlorobenzyl)piperidin-4-yl]-(6-benzylcarbamoyl-2-benzothiazolylthio)acetamide (1o)** This was prepared from benzylamine (36%, colorless solid): ^{1}H-NMR ($CDCl_3$) δ: 1.31—1.49 (2H, m), 1.78—1.91 (2H, m), 2.02—2.18 (2H, m), 2.48—2.65 (2H, m), 3.30 (2H, s), 3.69—3.86 (1H, m), 3.94 (2H, s), 4.68 (2H, d, J=5.6 Hz), 6.48 (1H, t, J=5.6 Hz), 7.06 (1H, d, J=8.2 Hz), 7.20—7.42 (8H, m), 7.78—7.88 (2H, m), 8.31 (1H, s); HR-MS m/z: 599.1097 (Calcd for $C_{29}H_{29}N_4O_2Cl_2S_2$, $(M+H)^+$: 599.1109).

***N*-[1-(3,4-Dichlorobenzyl)piperidin-4-yl]-[6-(2-pyridylmethyl)carbamoyl-2-benzothiazolylthio]acetamide (1p)** This was prepared from 2-(aminomethyl)pyridine (40%, colorless solid): ^{1}H-NMR ($CDCl_3$) δ: 1.34—1.50 (2H, m), 1.75—1.91 (2H, m), 2.02—2.19 (2H, m), 2.45—2.68 (2H, m), 3.29 (2H, s), 3.68—3.87 (1H, m), 3.95 (2H, s), 4.79 (2H, d, J=4.3 Hz), 7.06 (1H, d, J=8.2 Hz), 7.19—7.38 (5H, m), 7.65—7.80 (2H, m), 7.85—8.00 (2H, m), 8.38 (1H, s), 8.58 (1H, d, J=4.0 Hz); HR-MS m/z: 600.1044 (Calcd for $C_{28}H_{28}N_5O_2Cl_2S_2$, $(M+H)^+$: 600.1061).

***N*-[1-(3,4-Dichlorobenzyl)piperidin-4-yl]-[6-(3-pyridylmethyl)carbamoyl-2-benzothiazolylthio]acetamide (1q)** This was prepared from 3-(aminomethyl)pyridine (8%, colorless solid): ^{1}H-NMR ($CDCl_3$) δ: 1.32—1.50 (2H, m), 1.79—1.91 (2H, m), 2.03—2.20 (2H, m), 2.51—2.67 (2H, m), 3.31 (2H, s), 3.70—3.90 (1H, m), 3.95 (2H, s), 4.71 (2H, d, J=5.5 Hz), 6.56 (1H, t, J=5.5 Hz), 7.07 (1H, d, J=1.9, 8.2 Hz), 7.20—7.41 (4H, m), 7.74 (1H, d, J=7.9 Hz), 7.83 (1H, dd, J=1.6, 8.5 Hz), 7.88 (1H, d, J=8.5 Hz), 8.32 (1H, d, J=1.6 Hz), 8.57 (1H, dd, J=1.4, 4.7 Hz), 8.64 (1H, s); HR-MS m/z: 600.1073 (Calcd for $C_{28}H_{28}N_5O_2Cl_2S_2$, $(M+H)^+$: 600.1061).

***N*-(Piperidin-4-yl)-(2-benzothiazolylthio)acetamide (2)** To a stirred solution of **4a** (450 mg, 2.00 mmol) and 4-amino-1-*tert*-butoxycarbonylpiperidine (**6**)[15] (400 mg, 2.00 mmol) in $CHCl_3$ (20 ml) were added HOBt (400 mg, 2.96 mmol) and WSC·HCl (575 mg, 3.00 mmol) at room temperature. After stirring for 20 h, the reaction mixture was diluted with EtOAc, washed with saturated $NaHCO_3$ solution, 10% citric acid solution, and water, dried ($MgSO_4$), and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (50—70% EtOAc in *n*-hexane) to give *N*-(1-*tert*-butoxycarbonylpiperidin-4-yl)-(2-benzothiazolylthio)acetamide (814 mg, 100%) as a colorless solid. A solution of the solid (814 mg, 2.00 mmol) in 10% HCl–MeOH (20 ml) was stirred at room temperature for 20 h. The mixture was concentrated *in vacuo*, and the residue was basified with 1 M NaOH, and extracted with $CHCl_3$. The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to give **2** (485 mg, 79%) as a colorless solid: ^{1}H-NMR ($CDCl_3$) δ: 1.18—1.37 (2H, m,), 1.80—1.94 (2H, m), 2.59—2.72 (2H, m), 2.92—3.05 (2H, m), 3.78—3.94 (1H, m), 3.92 (2H, s), 7.35 (1H, t, J=7.8 Hz), 7.45 (1H, t, J=7.8 Hz), 7.45—7.70 (1H, m), 7.78 (1H, d, J=7.8 Hz), 7.84 (1H, d, J=7.8 Hz).

4-Amino-1-(3,4-dichlorobenzyl)piperidine (3a) To a solution of **7** (6.0 g, 30 mmol) in $CHCl_3$ (80 ml) was added 3,4-dichlorobenzaldehyde (6.3 g, 36 mmol) and $NaBH(OAc)_3$ (7.6 g, 36 mmol) at room temperature, and the mixture was stirred for 20 h. After the addition of saturated $NaHCO_3$ solution, the mixture was extracted with $CHCl_3$. The organic layer was dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (25—50% EtOAc in *n*-hexane) to give 4-*tert*-butoxycarbonylamino-1-(3,4-dichlorobenzyl)piperidine (6.9 g, 64%) as a colorless solid. A solution of the solid (6.9 g, 19.2 mmol) in 10% HCl–MeOH (50 ml) was stirred at room temperature for 20 h. The mixture was concentrated *in vacuo*, and the residue was basified 1 M NaOH and extracted with $CHCl_3$. The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to give **3a** (4.23 g, 85%) as a colorless oil: ^{1}H-NMR ($CDCl_3$) δ: 1.31—1.48 (2H, m), 1.75—1.87 (2H, m), 1.96—2.10 (2H, m), 2.60—2.84 (3H, m), 3.42 (2H, s), 7.15 (1H, dd, J=2.0, 8.2 Hz), 7.37 (1H, d, J=8.2 Hz), 7.42 (1H, d, J=2.0 Hz).

1-(3,4-Dichlorobenzyl)-4-(methylamino)piperidine (3b) To a solution of **7** (2.0 g, 10 mmol) in $CHCl_3$ (40 ml) was added 3,4-dichlorobenzaldehyde (1.9 g, 10.8 mmol) and $NaBH(OAc)_3$ (3.2 g, 15 mmol) at room temperature, and the mixture was stirred for 20 h. After the addition of saturated $NaHCO_3$ solution, the mixture was extracted with $CHCl_3$. The organic layer was dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (25—50% EtOAc in *n*-hexane) to give 4-*tert*-butoxycarbonylamino-1-(3,4-dichlorobenzyl)piperidine (2.6 g, 72%) as a colorless solid. To a stirred solution of the solid (51 mg, 0.142 mmol) in THF (1.0 ml) was added 60% NaH in oil (10 mg, 0.25 mmol) at room temperature, and the resulting solution was stirred at the same temperature for 30 min. To this solution was added MeI (50 ml, 0.80 mmol), and the mixture was stirred for 3.5 h. The reaction was quenched by adding saturated $NaHCO_3$, and the mixture was extracted with EtOAc. The organic layer was washed with water, dried ($MgSO_4$), and concentrated *in vacuo*. The residue

was purified by preparative TLC (5% MeOH in $CHCl_3$) to give 4-(*tert*-butoxycarbonyl)(methyl)amino-1-(3,4-dichlorobenzyl)piperidine (9.0 mg, 17%) as a colorless solid. A solution of the solid (9.0 mg, 0.024 mmol) in 10% HCl–MeOH (1.0 ml) was stirred at room temperature for 1.0 h. The mixture was concentrated *in vacuo*, and the residue was basified with 1 M NaOH, and extracted with $CHCl_3$. The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to give **3b** (5.8 mg, 88%) as a colorless oil: ^{1}H-NMR ($CDCl_3$) δ: 1.30—1.48 (2H, m), 1.80—1.96 (2H, m), 1.96—2.14 (2H, m), 2.30—2.42 (1H, m), 2.44 (3H, s), 2.70—2.90 (2H, m), 3.42 (2H, s), 7.15 (1H, dd, J=2.0, 8.2 Hz), 7.37 (1H, d, J=8.2 Hz), 7.42 (1H, d, J=2.0 Hz).

(2-Benzothiazolyloxy)acetic Acid (4b) To a stirred solution of 2-chlorobenzothiazole (**8**) (187 mg, 1.10 mmol) and ethyl glycolate (138 mg, 1.33 mmol) in tetrahydrofuran (THF) (5.0 ml) was added 60% NaH in oil (66 mg, 1.65 mmol) at room temperature, and the resulting solution was refluxed for 1 h. The reaction was quenched by adding water, and the mixture was extracted with EtOAc. The organic layer was washed with brine, dried ($MgSO_4$), and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (0—3% MeOH in $CHCl_3$) to give ethyl (2-benzothiazolyloxy)acetate (233 mg, 89%) as a colorless solid. To a solution of the solid (18 mg, 0.076 mmol) in MeOH (2.0 ml) was added 3 M NaOH (1.0 ml, 3.0 mmol), and the resulting solution was stirred for 20 h. The mixture was acidified with 1 M HCl, and extracted with $CHCl_3$. The organic layer was washed with brine, dried ($MgSO_4$), and concentrated *in vacuo* to give **4b** (15 mg, 95%) as a colorless oil: ^{1}H-NMR ($CDCl_3$) δ: 5.17 (2H, s), 7.26 (1H, d, J=7.6 Hz), 7.38 (1H, d, J=7.6 Hz), 7.66 (1H, d, J=7.6 Hz), 7.69 (1H, d, J=7.6 Hz).

(2-Benzothiazolylsulfinyl)acetic Acid (4c) To a stirred solution of **4a** (126 mg, 0.56 mmol) in THF (6.0 ml) was added a solution of OXONE® (520 mg) in water (6.0 ml) at 0 °C, and the resulting solution was stirred at room temperature for 4 h. The reaction was diluted with water, and the mixture was extracted with $CHCl_3$. The organic layer was washed with brine and dried ($MgSO_4$). The solvent was removed *in vacuo* to give **4c** (135 mg, 100%) as a colorless solid: ^{1}H-NMR ($CDCl_3$) δ: 3.88 (1H, d, J=14.6 Hz), 4.12 (1H, d, J=14.6 Hz), 7.51 (1H, t, J=7.4 Hz), 7.58 (1H, t, J=7.4 Hz), 7.99 (1H, d, J=7.4 Hz), 8.09 (1H, d, J=7.4 Hz).

(2-Benzothiazolylsulfonyl)acetic Acid (4d) To a stirred solution of **4a** (272 mg, 1.21 mmol) and NaOH (58 mg, 1.45 mmol) and $NaHCO_3$ (800 mg, 9.52 mmol) in water (6.0 ml) and acetone (1.0 ml) was added a solution of OXONE® (970 mg) in 0.4 mM EDTA solution (6.0 ml) at 0 °C, and the resulting solution was stirred at room temperature for 20 h. The reaction mixture was added to Na_2SO_3, and the mixture was acidified with 2 M HCl and extracted with $CHCl_3$. The organic layer was washed with brine, dried ($MgSO_4$), and evaporated *in vacuo*. The residue was recrystallized from $CHCl_3$ and *n*-hexane to give **4d** (108 mg, 35%) as a colorless solid: ^{1}H-NMR ($CDCl_3$) δ: 4.62 (2H, s), 7.62 (1H, t, J=7.0 Hz), 7.67 (1H, t, J=7.0 Hz), 8.03 (1H, d, J=7.0 Hz), 8.23 (1H, d, J=7.0 Hz).

(6-Ethoxycarbonyl-2-benzothiazolylthio)acetic Acid (4e) To a stirred solution of the ethyl 2-bromobenzothiazole-6-carboxylate (**9**)[16] (286 mg, 1.00 mmol) and 60% NaH in oil (50 mg, 1.25 mmol) in THF (3.0 ml) was added *tert*-butyl 2-mercaptoacetate[17] (155 mg, 1.05 mmol) at room temperature, and the resulting solution was refluxed for 2 h. The reaction mixture was poured into ice, and the mixture was extracted with EtOAc. The organic layer was washed with brine and dried ($MgSO_4$). The solvent was removed *in vacuo* to give ethyl 2-*tert*-butoxycarbonylmethylthiobenzothiazole-6-carboxylate (108 mg, 35%) as a colorless solid. A solution of the solid (150 mg, 0.42 mmol) in $CHCl_3$ (2.0 ml) was added to trifluoroacetic acid (1.0 ml), and the resulting solution was stirred at room temperature for 15 h. The solvent was removed *in vacuo* to give **4e** (123 mg, 99%) as a colorless solid: ^{1}H-NMR ($CDCl_3$) δ: 1.33 (3H, t, J=7.1 Hz), 4.27 (2H, s), 4.33 (2H, q, J=7.1 Hz), 7.90 (1H, d, J=8.5 Hz), 8.01 (1H, dd, J=1.7, 8.5 Hz), 8.68 (1H, d, J=1.7 Hz).

Construction of Receptor Expression Vectors and Stable Transfection into CHO Cells The cDNA fragments for human CCR3 and CCR1 were designed according to sequences from GenBank™ (submissions: U28694 and L09230, respectively). The cDNA fragments were sequenced and subcloned into the expression vector pRc/CMV or pcDNA3 (Invitrogen, San Diego, CA, U.S.A.). For stable expression into CHO cells, the cells were transfected with the constructed vectors using LipefectAMINE (Life Technologies, Inc.). After selection with 500 μg/ml of G418 for 2 weeks, G418-resistant cells were cloned and used for binding studies.

^{125}I-Chemokine Binding Study The cell-based binding assays were performed in 96-well microplates in a total volume of 400 μl. CHO cells transfected with CCR receptors were detached by phosphate buffered saline (PBS) (−) containing 2 mM EDTA and resuspended in binding buffer (Krebs–Linger Phosphate Buffer containing 0.1% bovine serum albunin (BSA) and 0.1% glucose). CHO cells (1×10^5 cells) were incubated with 50 pM ^{125}I-chemokine and an antagonist or an unlabeled chemokine for 1 h at 37 °C in the binding buffer to reach equilibrium. Nonspecific binding was determined in the presence of 100 nM of unlabeled chemokine. After incubation, the ice-cold binding buffer was added to the binding reaction. Then, the binding reaction was filtered by GF/C glass fiber filter (Whatman International Ltd., Maidstone, U.K.) presoaked with 1% polyethylenimine to reduce nonspecific binding to the glass filter. The radioactivity on the glass filter was determined with a gamma counter (COBRA 5002, Packard, Downers Grove, IL, U.S.A.).

Calcium Response Study Eosinophils were loaded with 1 μM Fura-2 acetoxymethyl ester (Molecular Probes Inc., Eugene, OR, U.S.A.) for 30 min at 37 °C. After two washes, the cells were resuspended at a concentration of 1×10^6 cells/ml in Krebs–Heseleit–Hopes buffer containing 0.1% BSA. The cell suspension (500 μl) was transferred into cuvettes with constant stirring. Changes in fluorescence were monitored at 37 °C using a spectrophotometer (CAF-110; JASCO Corp., Tokyo, Japan) at excitation wave-lengths of 340 and 380 nm and an emission wavelength of 510 nm. Calculation of Ca^{2+} concentration was performed using a K_d for Ca^{2+} binding of 224 nM. The antagonist was added to the cuvette 5 min prior to the addition of the chemokine.

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