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Discovery of a Novel CCR3 Selective Antagonist

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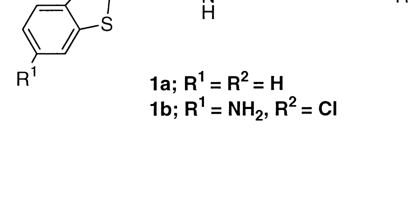
Abstract—In searching for a novel CCR3 receptor antagonist, we designed a library that included a variety of carboxamide derivatives based on the structure of our potent antagonists for human CCR1 and CCR3 receptors, and screened the new compounds for inhibitory activity against ^{125}I-Eotaxin binding to human CCR3 receptors expressed in CHO cells. Among them, two 2-(benzothiazolethio)acetamide derivatives (**1a** and **2a**) showed binding affinities with IC_{50} values of 750 and 1000 nM, respectively, for human CCR3 receptors. These compounds (**1a** and **2a**) also possessed weak binding affinities for human CCR1 receptors. We selected **1a** as a lead compound for derivatization to improve in vitro potency and selectivity for CCR3 over CCR1 receptors. Derivatization of **1a** by incorporating substituents into each benzene ring of the benzothiazole and piperidine side chain resulted in the discovery of a compound (**1b**) exhibiting 820-fold selectivity for CCR3 receptors (IC_{50} = 2.3 nM) over CCR1 receptors (IC_{50} = 1900 nM). This compound (**1b**) also showed potent functional antagonist activity for inhibiting Eotaxin (IC_{50} = 27 nM)- or RANTES (IC_{50} = 13 nM)-induced Ca^{2+} increases in eosinophils.

Introduction

Chemokines, a large sub-family of chemoattractant cytokines, are generally classified into four sub-families, CXC chemokines, CC chemokines, C chemokine and CX_3C chemokine, based on the arrangement of the cysteines in the N-terminal region.[1] These chemokines elicit their biological effects by activation of a subset of the seven transmembrane G-protein-coupled receptors (GPCRs). There have been 16 human chemokine receptors cloned and characterized to date, which are referred to as CCR1–11, CXCR1–5, XCR1 and CX_3CR1 receptors.

Selective accumulation of eosinophils to inflammatory sites is characteristic in allergic diseases such as asthma.[2] Therefore, eosinophils are thought to play an important role in the initiation and progression of these diseases. 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 the recruitment of eosinophils from the circulation to allergic sites.[3] It should also be noted that enhanced expression of Eotaxin and CCR3 receptor mRNA was observed in bronchial biopsies from patients with atopic asthma.[4] 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 selective for CCR3 receptors, we initiated exploration of a lead structure. Screening of a focused library including 770 compounds to search for compounds exhibiting greater than 50% inhibition at a concentration of 1 μM to ^{125}I-Eotaxin binding led to the selection of a 2-(benzothiazolethio)acetamide derivative (**1a**). In this paper, we describe the identification of a lead CCR3 receptor antagonist from a focused library and the structure–activity relationships of the 2-(benzothiazolethio)acetamides that were derived from the lead structure (**1a**).



1a; $R^1 = R^2 = H$
1b; $R^1 = NH_2$, $R^2 = Cl$

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Results and Discussion

Chemistry

Our strategy to identify a lead structure for a potent CCR3 selective antagonist involved the design of a library constituted from carboxamide derivatives and the derivatization of selected compounds.

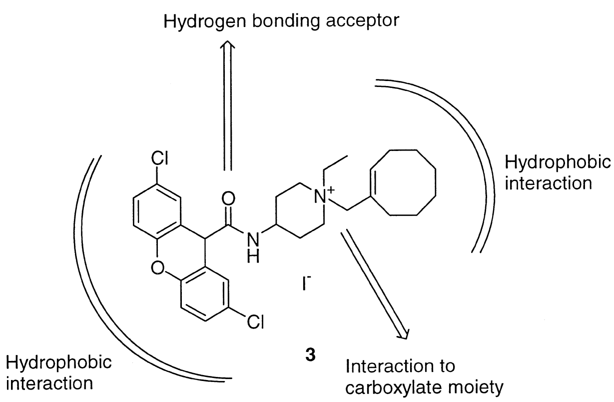
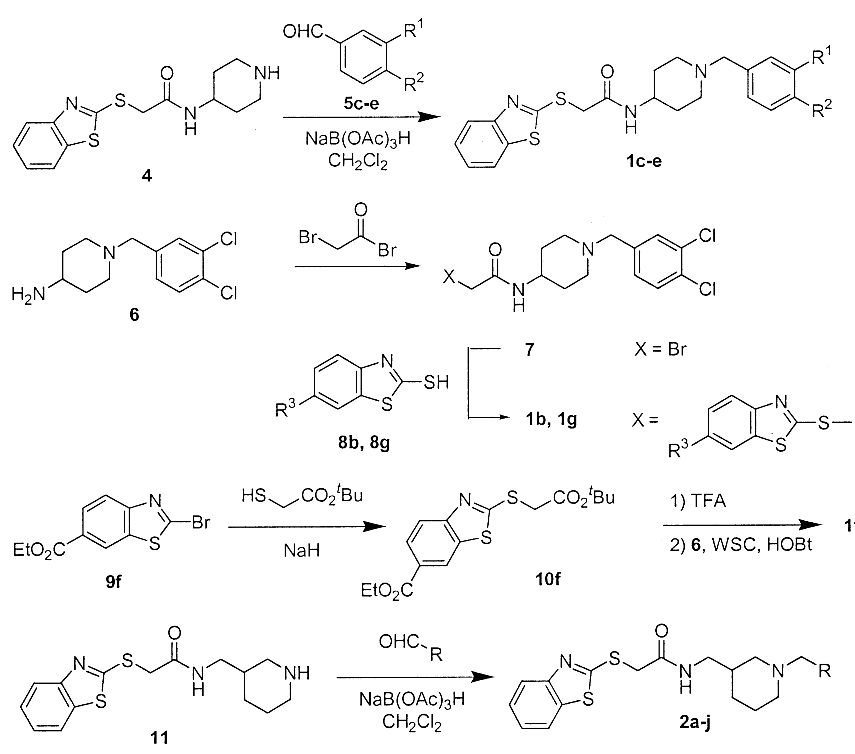


Figure 1.

Previously we reported that a xanthenecarboxamide (**3**) showed potent inhibitory activity against both human CCR1 ($IC_{50} = 0.9$ nM) and CCR3 receptors ($IC_{50} = 0.58$ nM).[5] Based on the structure of **3**, we hypothesized that the following functional groups are also required for CCR3 inhibitory activity: (1) a carbonyl group as a hydrogen bonding acceptor; (2) an amine moiety for electrostatic interaction to the target receptor; and (3) an aromatic group for a hydrophobic interaction site, and that the quaternary ammonium structure which was inappropriate for oral absorption would be replaceable with a basic amine group. Following up on this hypothesis, we designed carboxamides with aromatic group(s) in the acid part and with a benzylamine in the amine part (vide infra). To construct a library, we selected 70 kinds of commercially available carboxylic acids[6] from our in-house database after clustering, based on the binary Tanimoto coefficient[7a] calculated by the MDL keys[7b] as a structural descriptor, and used 11 structurally diverse diamines. These carboxylic acids and diamines were coupled by a parallel synthesis method using EDCI-HOBt as a coupling reagent to produce a total of 770 carboxamides. The structure of each compound was elucidated by its molecular weight measured by LC–MS.

Preparation of derivatives (**1b–g**, **2a–j**) is summarized in Scheme 1.[8] Derivatives (**1c–e**) were prepared from secondary amine (**4**) by reductive alkylation with substituted benzaldehydes (**5c–e**) in >90% yields. Other derivatives (**1b** and **1g**) were synthesized from 4-aminopiperidine (**6**), which was reacted with bromoacetyl bromide to produce bromoacetamide (**7**). Treatment of **7** with substituted benzothiazole (**8b** and **8g**) yielded the desired compounds in 55–65% yields (two steps). Compound **1f** was synthesized from 2-bromobenzothiazole (**9f**), which was reacted with mercaptoacetate to produce 2-(benzothiazolethio)acetate (**10f**) in 42% yields. Deprotection of **10f** and coupling with **6** led to **1f** in 98% yields (two steps). Also, derivatives (**2a–j**) were prepared from an amine (**11**) by reductive alkylation with appropriate aldehydes.



Scheme 1.

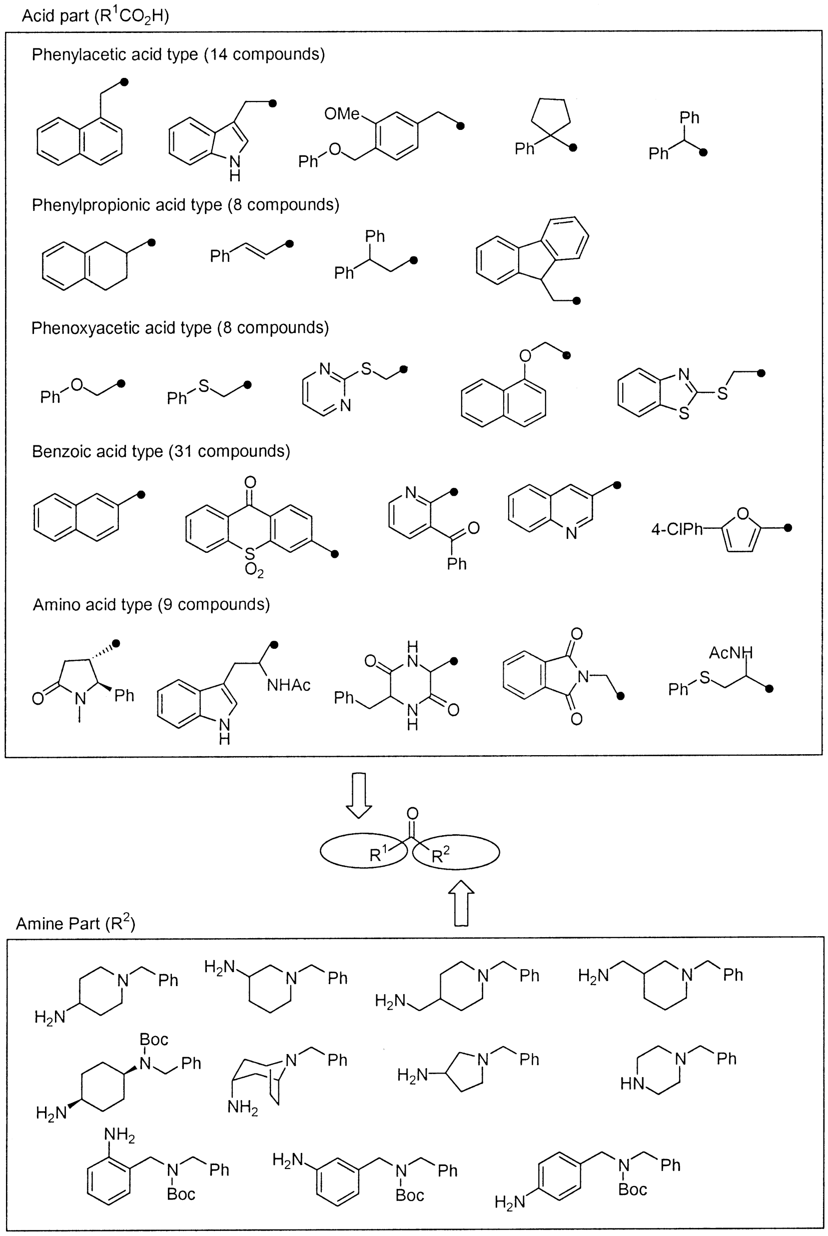


Figure 2.

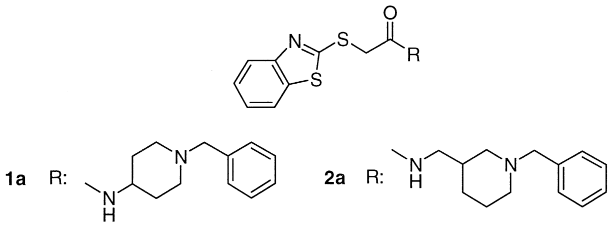
Biological properties

The primary screening of compounds was conducted by 50% inhibition at 1 μM to ^{125}I-Eotaxin binding to CCR3 receptors expressed in CHO cells. Selected compounds showing greater than 50% inhibition at 1 μM were subsequently purified or re-synthesized, and tested in the binding assay for human CCR3 receptors. Among the compounds tested, only two 2-(benzothiazolethio)acetamide derivatives (**1a** and **2a**) showed more than 50% inhibition, and their IC_{50} values were 0.75 and 1.0 nM, respectively (Table 1). Unfortunately, these compounds also showed potent inhibitory activity against human CCR1 receptors.

To investigate the role of substituent on the piperidine nitrogen in the inhibitory activity against CCR3 receptors, the phenyl ring of **2a** was replaced with a variety of substituents (Table 2). Evidently, only a benzyl group was found to be effective in enhancing the activity. Considering the potency of **1a** and **2a** for CCR3 receptors and selectivity for CCR3 over CCR1 receptors, we prioritized to further derivatize **1a** (Table 3).

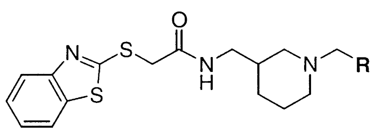
First, the effects of substituents on the benzene ring of the piperidine nitrogen in **1a** on inhibitory activity were examined. Although substituents such as a methyl, methoxy, or nitro group at the 4-position resulted in a decrease in the potency for CCR3 receptors, introduction

Table 1. Binding affinity of compounds **1a** and **2a** to CCR3 and CCR1 receptors



Compound	IC$_{50}$ (μM)	
	CCR3	CCR1
1a	0.75	7.1
2a	1.0	4.2

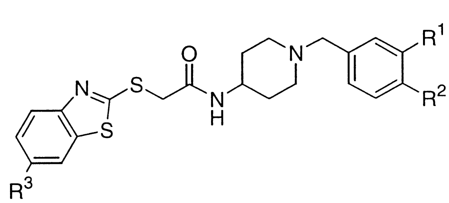
Table 2. Binding affinity of compounds **2a–j** to CCR3 receptors



Compound	R	IC$_{50}$ (nM)
		CCR3
2a	Ph	1000
2b	Cyclohexyl	30%@
2c	Et	0%@
2d	*n*-Butyl	0%@
2e	2-Thiazolyl	0%@
2f	3-Pyridyl	0%@
2g	4-Pyridyl	0%@
2h	2-Naphthyl	5%@
2i	1-Naphthyl	15%@
2j	CH_2Ph	0%@

@: inhibition% at 1 μM.

Table 3. Binding affinity of compounds **1a–g** to CCR3 and CCR1 receptors



Compound	R^1	R^2	R^3	IC$_{50}$ (nM)	
				CCR3	CCR1
1a	H	H	H	750	7200
1c	H	Cl	H	79	260
1d	Cl	H	H	280	> 10,000
1e	Cl	Cl	H	32	450
1f	Cl	Cl	COOEt	48	> 10,000
1g	Cl	Cl	OEt	20	3600
1b	Cl	Cl	NH_2	2.3	1900

of a chlorine atom at the 4-position of the benzene ring (**1c**) greatly enhanced the binding affinity for both CCR1 and CCR3 receptors. Therefore, the selectivity of **1c** for CCR3 receptors was not improved. By contrast, incorporation of a chlorine atom at the 3 position (**1d**) led to approximately 3-fold improvement in the binding affinity for CCR3 receptors, while reducing the affinity for CCR1 receptors. Although a 3,4-dichlorophenyl derivative (**1e**) seemed most potent, its selectivity for CCR3 over CCR1 receptors also remained to be improved.

Next, we tried to introduce a substituent into the benzothiazole ring of **1e**. When an ethoxycarbonyl group was incorporated into the 6 position, the resulting derivative (**1f**) showed potent binding affinity for CCR3 receptors comparable to that of **1e**. By contrast, the affinity of **1f** for CCR1 receptors was much reduced as compared to that of **1e**. It should be noted that **1f**, in which a substituent was incorporated at the benzothiazole ring, showed 200-fold selectivity for CCR3 over CCR1 receptors. An ethoxy moiety (**1g**) resulted in improvement in affinity for both CCR1 and CCR3 receptors, while retaining the selectivity. Further derivatization at this part revealed that the most effective functional group seemed to be an amino moiety (**1b**), which brought about 10-fold improvement in the binding affinity for CCR3 receptors as compared to that of **1e**. The 6-aminobenzothiazole derivative (**1b**) showed IC$_{50}$ values of 2.3 and 1900 nM for human CCR3 and CCR1 receptors, respectively, and 820-fold selectivity for CCR3 over CCR1 receptors.

To determine whether **1b** showed functional antagonism of CCR3 receptors, we measured its ability to inhibit Eotaxin- and RANTES-induced Ca^{2+} increases in eosinophils. **1b** inhibited the Ca^{2+} responses with IC$_{50}$ values of 27 and 13 nM, respectively. These results indicated that **1b** is a potent antagonist selective for CCR3 receptors. Details on the biological properties of this compound will be reported in the near future.

Conclusions

We discovered a novel CCR3 selective antagonist (**1b**) by modifying the lead compound (**1a**), which was identified from the screening of a focused library (770 carboxamide derivatives). Since **1b** possessed potent functional antagonism against Eotaxin- and RANTES-induced Ca^{2+} increases in eosinophils as well as high potency in the binding assay, it may be a useful tool to elucidate the role of CCR3 receptors in allergic diseases, especially in the selective accumulation of eosinophils to inflammatory sites.

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5. Naya, A.; Sagara, Y.; Ohwaki, K.; Saeki, T.; Ichikawa, D.; Iwasawa, Y.; Noguchi, K.; Ohtake, N. *J. Med. Chem.* in press.
6. The other carboxylic acids were as follows: 2-naphthylacetic acid, 1-phenyl-1-cyclopropanecarboxylic acid, 2-methoxy-2-trifluoromethylphenylacetic acid, ketoprofen, benzoylformic acid, 2,3-diphenylpropionic acid, 9-hydroxyfluorene-9-carboxylic acid, 2-(cyclopentyl)phenylacetic acid, 9-xanthenecarboxylic acid, coumarin-3-carboxylic acid, 3-methylindene-2-carboxylic acid, *trans*-2-phenyl-1-cyclopropanecarboxylic acid, 3-(4-methylbenzoyl)acrylic acid, 3-phenoxypropionic acid, 2-naphthoxyacetic acid, bis(4-chlorophenoxy)acetic acid, 1-naphthoic acid, 2,3-dihydrobenzofuran-7-carboxylic acid, 3-phenoxybenzoic acid, 3-*n*-propoxypicolinic acid, 4-(1*H*-pyrroll-1-yl)benzoic acid, 4-(3-methyl-5-oxo-2-pyrozolin-1-yl)benzoic acid, 2-bibenzylcarboxylic acid, 2-benzoylbenzoic acid, phthalic acid monobenzyl ester, 2-phenoxybenzoic acid, 4-phenylbenzoic acid, 4-(2-cyclohexenyloxy)benzoic acid, indole-2-carboxylic acid, 1-fluorenecarboxylic acid, 2-phenylbenzoic acid, 4-acetamidobenzoic acid, 2-(3-carboxyanilino)-4,6-dichloro-1,3,5-triazine, *N*-(1-naphthyl)phthalamic acid, 2-phenyl-4-quinolinecarboxylic acid, nalidixic acid, oxolinic acid, 3,5-dibenzyloxybenzoic acid, furosemide, phthalylsulfathiazole, probenecid, indole-3-carboxylic acid, *cis*-2-benzamidocyclohexanecarboxylic acid, (*s*)-2-phenyl-*N*-(trifluoroacetyl)glycine, *N*-benzoylphenylalanine, dansylglycine.
7. (a) McGregor, M. J.; Pallai, P. V. *J. Chem. Inf. Comput. Sci.* **1997**, *37*, 443. (b) MDL keys use a predefined dictionary of either 166 or 960 structural features and register their presence or absence in a compound by a 1 or 0 in the corresponding position in a bit string. Software and documentation available from Molecular Design Ltd., San Leandro, CA.
8. All compounds gave satisfactory spectral data. For example, see: **1b:** ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.35–1.55 (m, 2H), 1.74–1.92 (m, 2H), 2.00–2.20 (m, 2H), 2.36–2.65 (m, 2H), 3.27 (s, 2H), 3.65–3.90 (m, 1H), 3.85 (s, 2H), 6.80 (dd, $J = 2.3$, 8.7 Hz, 1H), 7.02 (d, $J = 2.3$ Hz, 1H), 7.00–7.12 (m, 1H), 7.20–7.40 (m, 2H), 7.50–7.70 (m, 2H); HRMS calcd for $C_{21}H_{23}N_4O^{35}Cl_2S_2$ $(M+H)^+$: 481.0690, found 481.0688.