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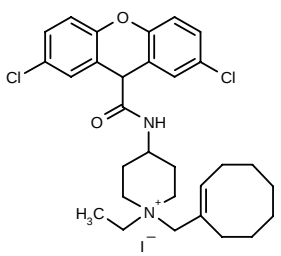
CCC: 0377-8282/2001

J-113863

Chemokine CCR1 Receptor Antagonist

1-(1-Cycloocten-1-ylmethyl)-4-(2,7-dichloroxanthen-9-ylcarboxamido)-1-ethylpiperidinium iodide



$C_{30}H_{37}Cl_2N_2O_2$ Mol wt: 655.4403

CAS: 202796-41-6 (as *cis*-isomer)
CAS: 202796-42-7 (as *trans*-isomer)

EN: 282962

Synthesis

The synthesis of J-113863 is shown in Scheme 1. Cyclooctanone tosylhydrazone was treated with *n*-BuLi and subsequently reacted with DMF to afford (II). Compound (IV) was prepared from 4-*tert*-butoxycarbonylpiperidine (III) by reductive alkylation with (II). Deprotection of (IV) and condensation with 2,7-dichloroxanthen-9-carboxylic acid (V) using 1,1'-carbonyldiimidazole (CDI) afforded (VI). Compound (VI) was quaternarized with iodoethane to provide a quaternary ammonium derivative as a mixture of two isomers (*cis* and *trans*) attributed to the 4-substituted piperidinium structure in a ratio of 2:1. Finally, the mixture was separated by silica gel column chromatography to give a major isomer, J-113863.

Introduction

Chemokines are a large family of 8-10 kDa chemotactic cytokines that play important roles in the selective recruitment of leukocytes to inflammatory lesions (1, 2) and may be pivotal proinflammatory mediators in various inflammatory disorders, such as allergic diseases, autoimmune diseases and ischemia (3, 4). Chemokines are divided into four subgroups (CC, CXC, C and CX3C) based on the position of the conserved cysteine residues. In the past decade, over 40 chemokines have been discovered. The specific effects of chemokines are mediated by their receptors, which belong to a family of seven transmembrane-spanning G-protein-coupled receptors with homology to the family of chemotactic receptors.

To date, 17 chemokine receptors (CCR1-10, CXCR1-5, XCR1 and CX3CR1) have been cloned and characterized (5), and have been viewed as attractive therapeutic targets by a number of pharmaceutical companies. At present, macrophage inflammatory protein-1α (MIP-1α), regulated upon activation normal T cell expressed (RANTES) and macrophage chemotactic protein-3 (MCP-3) are well known to be CC chemokines for CCR1 receptors.

Chronic inflammatory diseases such as multiple sclerosis and rheumatoid arthritis are characterized by the selective infiltration of T lymphocytes and macrophages into inflammatory sites, leading to extensive and chronic inflammation and tissue damage. Several reports have demonstrated the involvement of CCR1 receptors and their ligands, like MIP-1α and RANTES, in autoimmune diseases because of CCR1 receptor expression in T lymphocytes and macrophages. Treatment with anti-MIP-1α antibody was reported to ameliorate the severity of ongoing disease in a mouse experimental allergic encephalomyelitis (EAE) model of multiple sclerosis (6). CCR1 receptor-deficient mice had a significantly reduced incidence of disease compared with wild-type mice in a mouse EAE model (7). Furthermore, a CCR1 antagonist effectively reduced disease in a rat EAE model (8). These studies strongly suggest that CCR1 receptors play a role in the pathogenesis of EAE and may be involved in the pathogenesis of human multiple sclerosis.

In addition to multiple sclerosis, CCR1 receptors have been implicated in rheumatoid arthritis, which is characterized by the infiltration of memory T lymphocytes and monocytes into synovial tissues, causing chronic inflammation and loss of joint function. Synovial fibroblasts from patients with rheumatoid arthritis showed the upregulation of RANTES mRNA and protein (9). In addition, anti-RANTES antibody reduced the clinical score in a rat adjuvant-induced arthritis model (10). Furthermore, administration of Met-RANTES, which acts as a CCR1 antagonist, resulted in the delay of onset and amelioration of disease in a mouse collagen-induced arthritis

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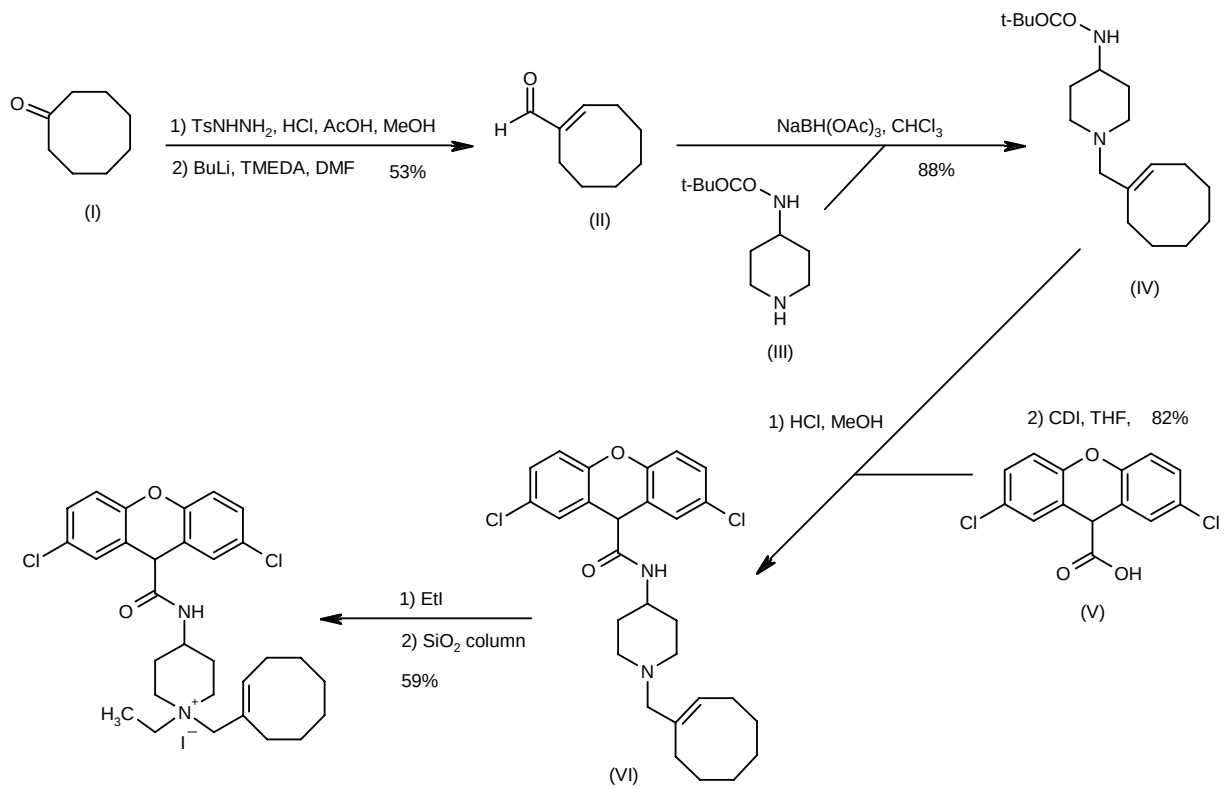
Scheme 1: Synthesis of J-113863



model (11). These studies suggest that the blockade of CCR1 receptors may be a therapeutic target for rheumatoid arthritis.

To further determine the roles of CCR1 receptors in chronic inflammatory diseases, we investigated nonpeptidyl CCR1 receptor antagonists and identified J-113863 as a highly potent antagonist for human and mouse CCR1 receptors. The compound was selected for further development as a potential agent for the treatment of chronic inflammatory diseases such as multiple sclerosis and rheumatoid arthritis.

CCR1 receptor antagonists

The first chemokine antagonists resulted from studies of the structure-activity relationship of chemokine amino acid sequences. The N-terminal truncation of MCP-3 and RANTES were reported to be antagonists, showing potent binding affinity for CCR1 receptors but having no effects on chemotaxis, enzyme release or intracellular Ca^{2+} concentrations (12). Another CCR1 receptor antagonist derived from a native chemokine was Met-RANTES, which was produced recombinantly in *E. coli* and added one methionyl residue in the N-terminal portion of RANTES (13). This analog was found to have an essentially equipotent binding affinity with native RANTES, but was not found to produce chemotactic activity or to increase intracellular Ca^{2+} concentrations. However, antagonists derived from native chemokines such as Met-RANTES showed a weak partial agonistic activity, depending on the assay systems (14).

On the other hand, several nonpeptide CCR1 receptor antagonists with or without biological activities have been reported by pharmaceutical companies. A selected number of these antagonists with binding affinities are shown in Figure 1 and listed in Table I with binding inhibition data for [^{125}I]RANTES and[^{125}I]MIP-1α with human CCR1 receptors. As shown in compounds **1-4** of Figure 1, Takeda Chemical Industries claimed several compounds as CCR1 receptor antagonists (15-18). Among them, the diphenylmethane derivative (compound **1**) appeared to be the most potent CCR1 receptor antagonist. Compound **5** from LeukoSite Inc. (19) and compound **6** from Rhône-Poulenc Rorer (20) were also reported as CCR1 antagonists, but these antagonists had lower affinities for human CCR1 receptors than compound **1**. Moreover, compound **6** also showed a noncompetitive antagonist binding to an allosteric site of CCR1 receptors. Another prototypic CCR1 receptor antagonist that shared many structural features with compound **1** was discovered by Berlex Biosciences (compound **7**) (21). The researchers also

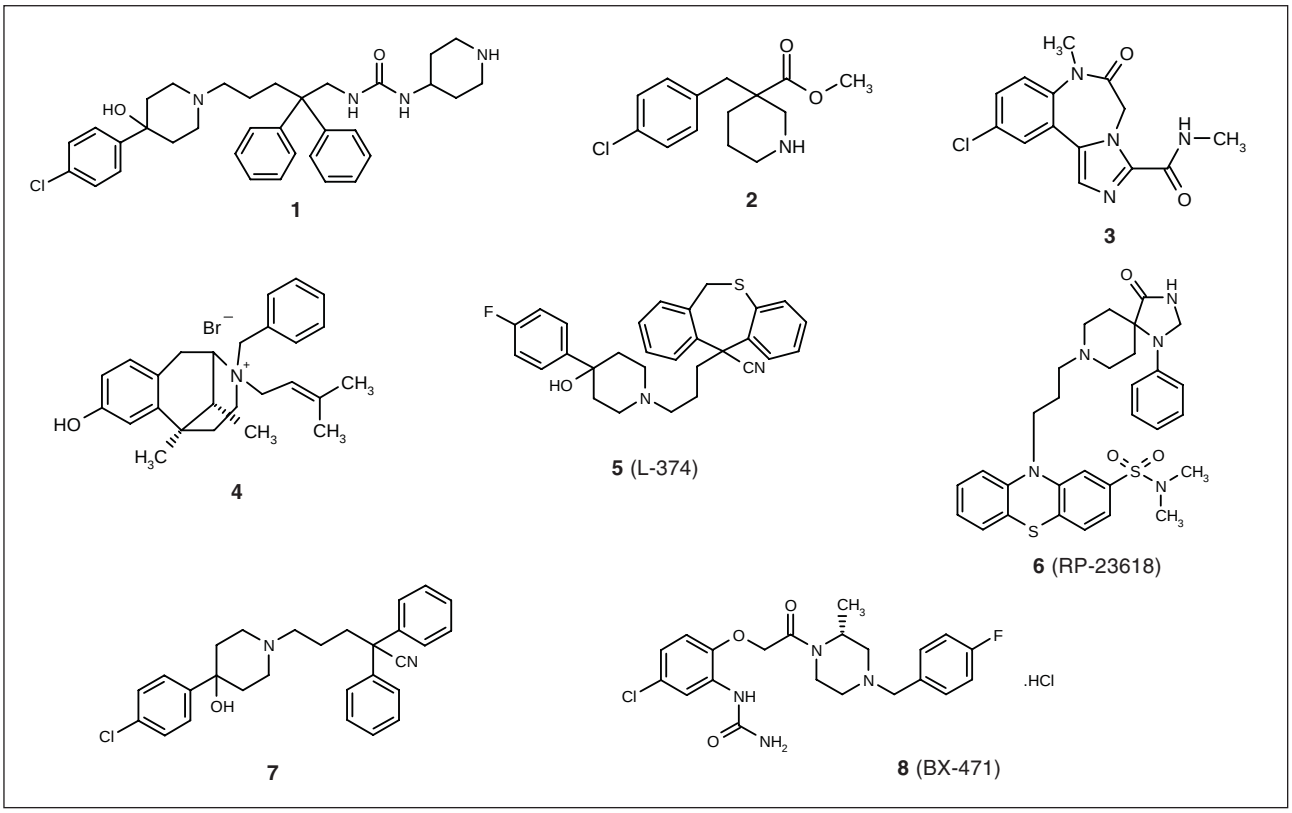


Fig. 1. Structures of reported CCR1 antagonists.

Table I: Binding affinities of reported human CCR1 receptor antagonists.

Compound	IC_{50} (nM) [^{125}I]RANTES	[^{125}I]MIP-1α
1	6	5000[b]
2	600	1000
3	100	
4	100	600
5	200	360
6	200	40
7	60	
8	2.8[a]	1.0[a]

[a]K_i values. [b]The binding affinity (18 nM) in our assay system is different from the value (5000 nM) reported by Takeda for MIP-1α binding.

determined the species differences in binding affinities of compound **7** for CCR1 receptors (22) and found that compound **7** showed binding affinities for human, rabbit and marmoset CCR1 receptors, with K_i values of 40, 245 and 451 nM, respectively. However, this compound had a negligible binding affinity for mouse CCR1 receptors. A promising CCR1 antagonist with oral availability was also reported by Berlex Biosciences (compound **8**), which had a high affinity for human CCR1 receptors (8) but a low affinity for mouse CCR1 receptors (23). Unfortunately, compound **8** was approximately 100 times less effective for rat CCR1 receptors than for human CCR1 receptors.

In general, it is well known that the previously reported chemokine receptor antagonists have crucial species differences in antagonism due to a lack of binding affinity for nonprimate CCR1 receptors. Since rodents are useful species for clarifying the involvement of CCR1 receptors in inflammatory and allergic disorders, species differences in antagonism for CCR1 receptors may limit the use of these CCR1 receptor antagonists. Therefore, we decided to seek a potent antagonist for both human and mouse CCR1 receptors in order to determine the pathophysiological role(s) of CCR1 receptors in mouse models of disease.

Discovery of J-113863

The discovery of our CCR1 receptor antagonist, J-113863 (24), is shown in Figure 2. Screening of our chemical collection for inhibition of [^{125}I]MIP-1α binding to Chinese hamster ovary (CHO) cells transfected with human CCR1 receptors led to the identification of a xanthen-9-carboxamide (compound **9a**) as the lead compound. Among the tertiary-amine analogs, compound **9b** showed the most potent binding affinity for human CCR1 receptors; however, binding affinity for mouse CCR1 receptors was not detected, even at a concentration of

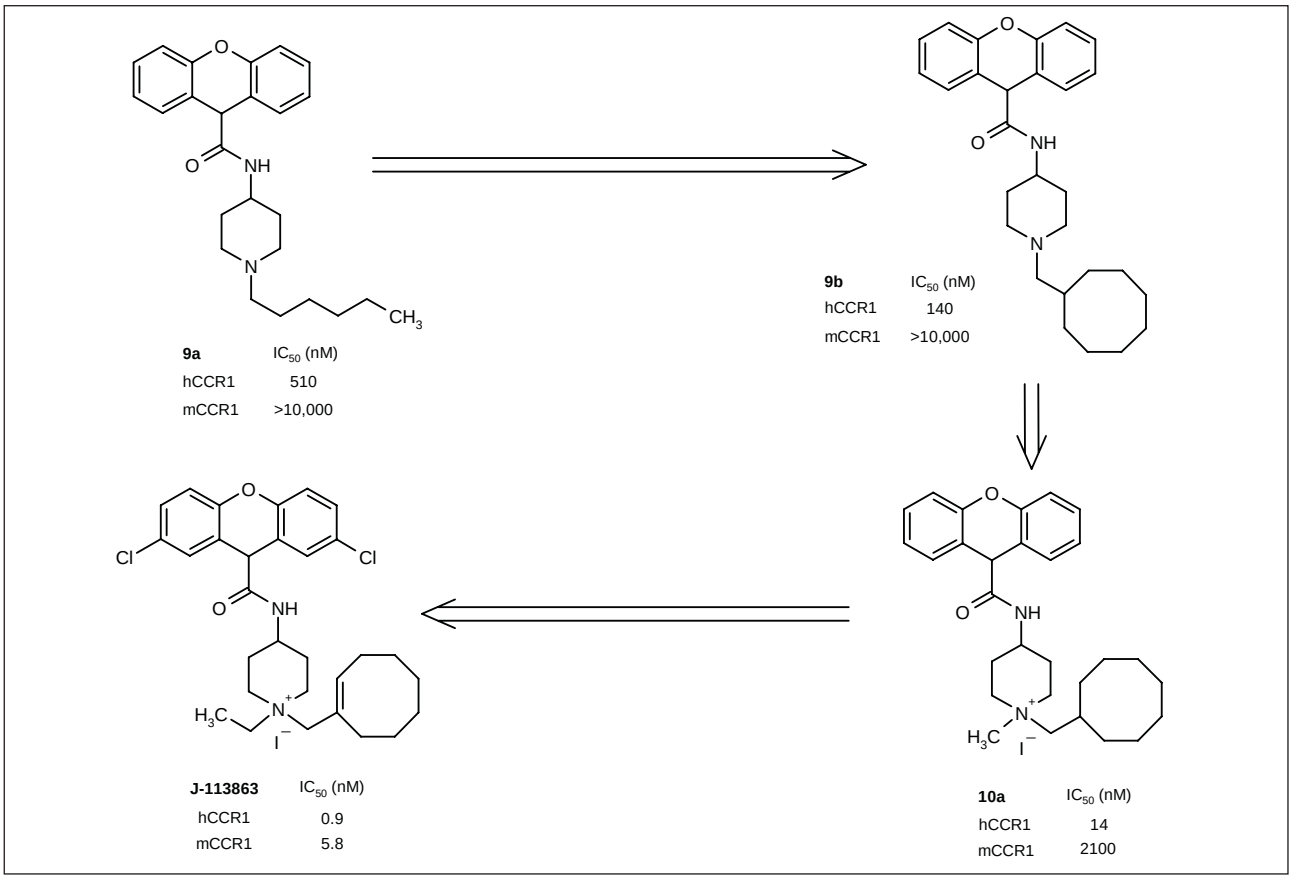


Fig. 2. Discovery of J-113863.

10,000 nM. We hypothesized that the piperidine nitrogen in this series of compounds was recognized as a cation binding site that was close to the hydrophobic site recognizing the cycloalkyl moiety in the antagonist binding pocket of CCR1 receptors. Therefore, a quaternary ammonium group could be used to replace the tertiary piperidine nitrogen. Following up this hypothesis, a quaternary ammonium (compound **10a**) was prepared and evaluated in the binding assay. Interestingly, compound **10a** showed greatly enhanced binding affinity not only for human CCR1 receptors but also for mouse CCR1 receptors. Derivatization of compound **10a** focusing on substitution on the piperidine nitrogen and installment of substituents into the xanthene group resulted in the discovery of J-113863, which showed potent antagonist activity not only for human CCR1 receptors but also for mouse CCR1 receptors.

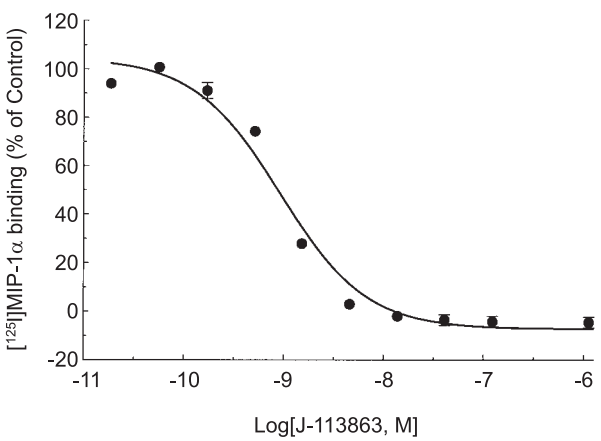


Fig. 3. Displacement of [^{125}I]MIP-1α binding to human CCR1 receptors by J-113863. The CHO cells (1 x 10^5 cells/tube) transfected with human CCR1 receptors were incubated with 50 pM [^{125}I]MIP-1α in the presence of increasing concentrations of J-113863 for 1 h at 37 °C. The data are expressed as a percent of the control value and are the mean ± SEM of three separate experiments.

In vitro activity

To determine the binding affinity of J-113863, we set up a [^{125}I]MIP-1α binding assay system with CHO cells transfected with human CCR1 receptors. As shown in Figure 3 and Table II, J-113863 dose-dependently and

Table II: Binding affinities in human and mouse CCR1 receptors.

	IC_{50} (nM)	
	Human	Mouse
J-113863	0.9	5.8
Compound **1**	18	>10,000
Compound **7**	48	5000
MIP-1α	1.5	0.8

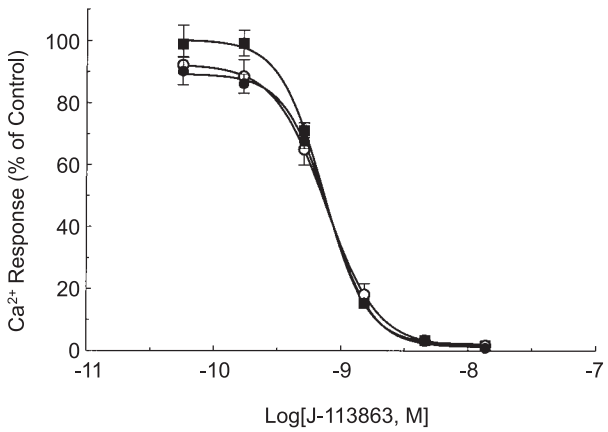


Fig. 4. Effects of J-113863 on CCR1 chemokine-induced increases in intracellular Ca^{2+} concentrations in U937 cells transfected with human CCR1 receptors. Fura2-AM-loaded U937 cells transfected with human CCR1 receptors were pretreated with J-113863 for 5 min and then stimulated with MIP-1α (●), RANTES (○) and MCP-3 (■). In each experiment, the Ca^{2+} increase in the presence of vehicle (DMSO) was determined as 100% control.

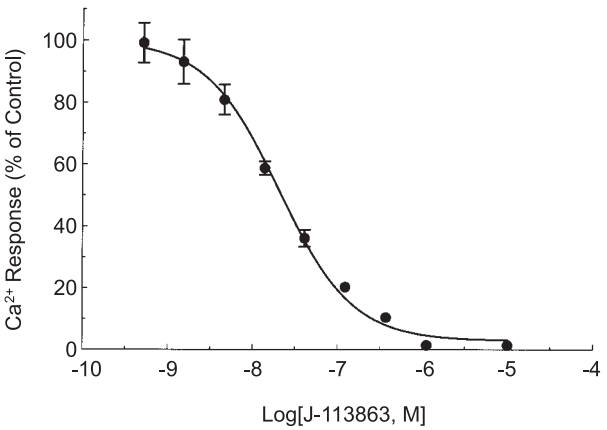


Fig. 5. Effect of J-113863 on MIP-1α-induced increases in intracellular Ca^{2+} concentrations in U937 cells transfected with mouse CCR1 receptors. Fura2-AM-loaded U937 cells transfected with mouse CCR1 receptors were pretreated with J-113863 for 5 min and then stimulated with MIP-1α.

completely inhibited [^{125}I]MIP-1α binding. The IC_{50} value of J-113863 was 0.9 nM, demonstrating a binding affinity that was almost comparable to that of cold MIP-1α (IC_{50} = 1.5 nM). In addition, we determined the binding affinity of other CCR1 receptor antagonists with the same binding assay system. The IC_{50} values of compounds **1** and **7** were 18 and 48 nM, respectively, indicating that J-113863 has a potent binding affinity for human CCR1 receptors. In addition, in the binding assay system with CHO cells transfected with mouse CCR1 receptors, the IC_{50} values of J-113863 and compounds **1** and **7** were 5.8, >10,000 and 5000 nM, respectively (Table II). These data indicate that J-113863, but not compounds **1** or **7**, has a promising binding affinity for mouse CCR1 receptors in addition to human CCR1 receptors.

To evaluate the CCR1 antagonistic activity of J-113863, we tested its inhibitory effect on the Ca^{2+} response in U937 cells transfected with human CCR1. MIP-1α produced a remarkable increase in intracellular Ca^{2+} concentrations in U937 cells transfected with CCR1, but not in untransfected host U937 cells. As shown in Figure 4, J-113863 completely blocked the MIP-1α (10 nM)-induced Ca^{2+} response of U937 cells transfected with CCR1 receptors, with an IC_{50} value of 0.73 nM. In addition, J-113863 completely inhibited the Ca^{2+} response induced by the CCR1 receptor agonists RANTES (100 nM) and MCP-3 (10 nM), with IC_{50} values of 0.72 and 0.77 nM, respectively. In contrast, J-113863 up to 10 μM had no inhibitory effect on interleukin-8- and fMLP-induced Ca^{2+} flux in human neutrophils (unpublished data), indicating that J-113863 has no antagonistic activity for human CXCR1/2 or fMLP receptors. We also found an antagonistic activity of J-113863 for mouse CCR1 receptors in U937 cells transfected with mouse CCR1 receptors, indicating that J-113863 is a potent antagonist with an IC_{50} value of 21 nM (Fig. 5). Thus, J-113863 shows potent antagonistic activity for human and mouse CCR1 receptors.

To further clarify the specificity of J-113863, we tested various enzyme and receptor assay systems involved in inflammation and allergic disorders. As shown in Table III, J-113863 showed a high affinity for human CCR3 receptors, with an IC_{50} value of 5.8 nM, but did not interact with other molecules, including CCR2b receptors, CCR5 receptors, cyclooxygenases (COXs), phosphodiesterases (PDEs), platelet activating factor (PAF) receptors and leukotriene D_4 (LTD_4) receptors. To further determine the CCR3 antagonistic activity of J-113863, we tested the inhibitory effects of J-113863 on eotaxin-induced intracellular Ca^{2+} concentrations and chemotaxis in human eosinophils. As shown in Figure 6, J-113863 showed potent antagonistic activity, with IC_{50} values of 6.1 nM and 27 nM, respectively. Thus, J-113863 is a nonpeptidyl and potent CCR1 antagonist with high affinity for human CCR3 receptors.

Recently, Sabroe *et al.* reported on the biological characteristics of J-113863 (referred to by them as UCB-35625) (25). However, the structure they described as J-113863 was quite different in some points from

Table III: Specificity of J-113863.

Receptor/Enzyme	Ligand/Substrate	IC_{50} (nM)
CCR2b	[^{125}I]MCP-1	>1000
CCR3	[^{125}I]Eotaxin	0.58
CCR3 (mouse)	[^{125}I]Eotaxin	460
CCR5	[^{125}I]MIP-1β	>1000
IL-8 receptor	[^{125}I]IL-8	>10,000
PAF receptor	[^{3}H]PAF	>1000
LTB_4 receptor	[^{3}H]LTB_4	>10,000
LTD_4 receptor	[^{3}H]LTD_4	>10,000
TNFα receptor	[^{125}I]TNFα	>10,000
COX-1	Arachidonic acid	>300,000
COX-2	Arachidonic acid	>300,000
LTA_4 hydrolase	LTA_4	>100,000
LTC_4 synthetase	LTA_4	>1,000,000
15-lipoxygenase	Linoleic acid	>30,000
PDE-I, II, III, IV, V	[^{3}H]cAMP	>100,000
Elastase	MeOSuc-AAPV-pNa	>30,000
Thromboxane synthetase	PGG_2	>100,000

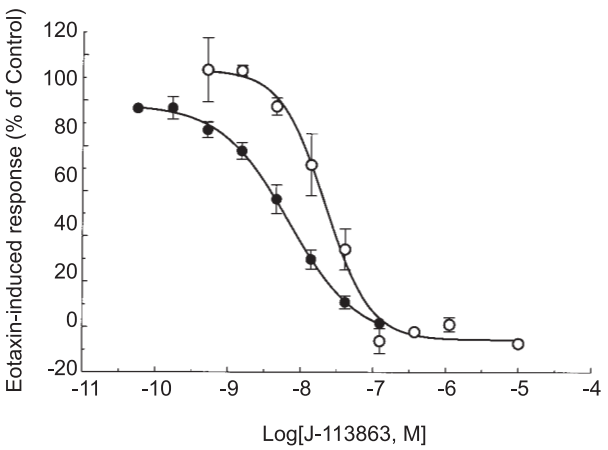


Fig. 6. Inhibitory effects of J-113863 on eotaxin-induced increases in intracellular Ca^{2+} concentrations and chemotaxis in human peripheral blood eosinophils. Fura2-AM-loaded human eosinophils (●) were pretreated with J-113863 for 5 min and then challenged with eotaxin (10 nM). Eosinophil chemotaxis (○) was assessed in a 96-well chemotaxis chamber (Neuro Probe, Cabin John, MD) using polycarbonate membranes with 5-μm pores. The antagonist was added to both upper and lower wells. Migration was allowed to proceed for 3 h at 37 °C in 5% CO_2. The number of eosinophils that migrated into the lower well was determined using CyQUANT cell proliferation assay kit (Molecular Probes, Inc, Eugene, OR, USA).

the compound that we presented. J-113863 has a (1-cyclooctenyl)methyl group on the nitrogen atom, while UCB-35625 has a cycloheptylmethyl group. In addition, they reported that UCB-35625 is a trans isomer, whereas we claimed for convenience that J-113863 has a *cis*-configuration. We have not yet determined the absolute configuration of J-113863. Thus, the biological properties of J-113863 reported in this review appear to be correct.

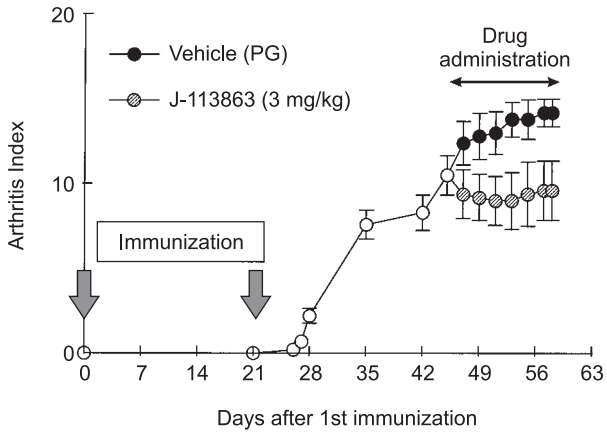


Fig. 7. Effect of J-113863 on clinical score of arthritis in type II collagen-induced arthritis models. Male DBA/1J mice (8 weeks old) were immunized by 100 μg bovine type II collagen with Freund's incomplete adjuvant on days 0 and 21. J-113863 was dissolved in propylene glycol (PG) and subcutaneously administered at a dose of 3 mg/kg/day from days 45-58. The clinical arthritis score was graded on a scale of 0-5 : 0 = no arthritis, 1 = redness, 2 = light swelling (1-2 digits), 3 = medium swelling (3-5 digits), 4 = severe swelling (paw), 5 = rigor.

In vivo activity

Antibodies against MIP-1α and RANTES and the RANTES-derived antagonist Met-RANTES were reported to reduce the severity of disease in animal models of rheumatoid arthritis. To evaluate the *in vivo* CCR1 antagonistic activity of J-113863, we determined the effect of the compound in a type II collagen-induced arthritis model in DBA/1J mice. As shown in Figure 7, the clinical arthritis score, assessed from the symptoms of arthritis, was increased after second immunization of type II collagen as an antigen. Administration of J-113863 (3 mg/kg/day) after the onset of disease significantly reduced the development of arthritis in this disease model, as seen in clinical arthritis scores. This result suggests that J-113863 may be a promising CCR1 antagonist even *in vivo*, although further extensive studies are necessary to determine the usefulness of J-113863.

Conclusions

Recently, several nonpeptidyl CCR1 antagonists have been discovered by various pharmaceutical companies. Unfortunately, most of these antagonists, excluding BX-471 (compound **8**), might be unacceptable for concept studies with animal models of disease due to a lack of binding affinity for nonprimate CCR1 receptors. We describe herein a highly potent and nonpeptidyl CCR1 antagonist, J-113863, for human and mouse species that demonstrates an inhibitory effect in a mouse arthritis model. To further clarify the roles of CCR1 in chronic inflammatory diseases, it is necessary to test various

CCR1 antagonists with structural diversity in animal models of diseases. Thus, J-113863, which has a high affinity for murine CCR1 receptors, may be a useful tool for elucidating the roles of CCR1 in chronic inflammatory diseases.

Acknowledgements

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Manufacturer

Banyu Pharmaceutical Co., Ltd. (JP).

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