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Identification of a novel series of potent and selective CCR6 inhibitors as biological probes

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ABSTRACT

CCR6 has been implicated in both autoimmune diseases and non-autoimmune diseases. Thus, inhibition of CCR6-dependent cell migration is an attractive strategy for their treatment. An orally available small molecule inhibitor of CCR6 could therefore be a useful biological probe for the pathophysiological studies. Initial SAR study of a hit compound provided potent *N*-benzenesulfonylpiperidine derivatives that suppressed CCL20-induced Gi signals. By subsequent scaffold morphing of the central ring and further optimization, we identified a novel series of 1,4-*trans*-1-benzenesulfonyl-4-aminocyclohexanes as potent and selective CCR6 inhibitors with good pharmacokinetic properties. Our compounds showed good correlation between Gi signal inhibitory activity and cell migration inhibitory activity in human CCR6-transfected CHO cells. In addition, representative compound **35** potently inhibited CCR6-dependent cell migration and the increase in ERK phosphorylation in human primary cells. Therefore, the compound could be used effectively as a biological probe against human CCR6.

Chemokines have essential roles in the homeostasis of immune systems. Among their receptors, CC chemokine receptor 6 (CCR6) is preferentially expressed on B cells, Th17 cells, and dendritic cell subsets.[1,2] CC chemokine ligand 20 (CCL20), also known as macrophage inflammatory protein-3α (MIP-3α), binds only to CCR6. Binding of CCL20 to CCR6 induces cell migration, an event reported to depend on the activation of both the extracellular signal-regulated kinase (ERK) and Gi signal pathways.[3] CCR6 regulates the migration of inflammatory cells,[4,5] and CCR6 antibody has been shown to inhibit CCL20-stimulated cell migration.[5] CCL20 is produced in tissues such as the skin, lung, and intestinal mucosa, and its expression is increased by various inflammatory stimuli. The association of CCR6 with autoimmune diseases such as rheumatoid arthritis[5,6] and psoriasis[7] has been demonstrated by genome-wide association study or in vivo study in disease models. CCR6 is also involved in non-autoimmune diseases such as cancer[8,9] and atherosclerosis.[10] Thus, inhibition of CCR6-dependent cell migration would be an attractive strategy for the treatment of various diseases, and an orally available small molecule inhibitor of CCR6 could be a useful biological probe for both in vitro and in vivo pathophysiological studies.

We began by searching for CCR6 inhibitors and verifying in vitro proof of concept (POC) that these inhibitors block CCR6-dependent migration of immune cells. First, high-throughput screening based on CCL20-induced Gi signal inhibitory activity assessed by measuring produced cyclic adenosine monophosphate in human CCR6-transfected CHO cells was performed. Consequently, *N*-benzenesulfonylpiperidine derivative **1** was identified as an inhibitor of human CCR6 (Fig. 1). Interestingly, this compound had high selectivity over human CCR1 and CCR7. Both CCR1 and CCR7 are also associated with cell migration,[11] and among CCRs, CCR7 has the highest homology to CCR6 (44% homology).[12] Therefore, in the functional analysis of CCR6, it is essential to use a biological probe that selects CCR6 over CCR1 and CCR7.

Based on these results, we first performed a structure-activity relationship (SAR) study of hit compound **1** to discover more potent inhibitors. Then, we modified the central piperidine ring, which led to the identification of a novel series of 1,4-*trans*-1-benzenesulfonyl-4-aminocyclohexane derivatives and their in vitro POC study. Our compounds demonstrated good correlation between Gi signal inhibitory activity and cell migration inhibitory activity in human CCR6-transfected CHO cells. Furthermore, representative compound **35** potently inhibited CCR6-dependent cell migration and the increase in ERK phosphorylation in human primary cells. Herein, we report the SAR studies of both the hit compound and the novel series of CCR6 inhibitors derived from it, and the biological activities of representative

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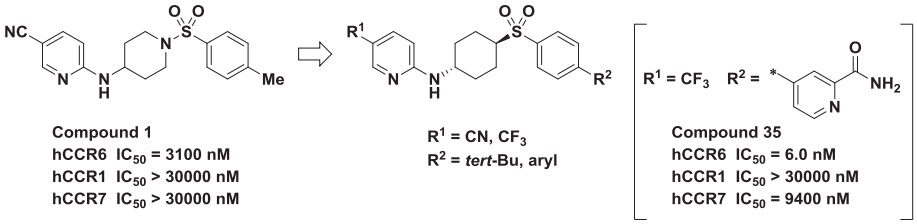
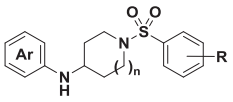
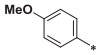


Fig. 1. Hit compound **1**, *N*-benzenesulfonylpiperidine derivative effective as a human CCR6 inhibitor, and design of a novel 1,4-*trans*-1-benzenesulfonyl-4-aminocyclohexane series.

Table 1
Human CCR6 inhibitory activity of *N*-benzenesulfonyl cyclic amine derivatives.



Cmpd	Ar	n	R	IC_{50}[a] (nM)
1		1	4-Me	3100 (2800–3400)
2		1	4-MeO	2500 (2100–3000)
3		0	4-MeO	> 30000[b]
4		2	4-MeO	17,000 (15000–19000)[b]
5		1	4-MeO	7400 (6500–8300)
6		1	4-MeO	> 30000
7		1	4-MeO	940 (730–1200)
8		1	4-MeO	21,000 (18000–25000)
9		1	4-MeO	1900 (1300–2800)
10		1	4-MeO	> 30000
11		1	4-MeO	12,000 (8700–17000)
12		1	4-Cl	1500 (1300–1800)
13		1	4-CF_3	800 (390–1700)
14		1	4-*tert*-Bu	220 (130–360)
15		1	4-Ph	290 (220–370)
16		1	4-Ph	170 (110–250)
17		1	4-CN	12,000 (5900–24000)
18		1	4-AcNH	> 30000
19		1	3-Me	> 30000
20		1	3-Ph	> 30000

[a] Values shown in parentheses are 95% confidence intervals.
[b] Data of racemate.

compounds to show their potential as biological probes.

Our SAR exploration of the hit series focused on three moieties (the central cyclic amine, arylamine, and benzenesulfonyl moiety) to enhance inhibitory activity (Table 1). At first, we showed that 4-methoxyphenyl derivative **2** and compound **1** had similar inhibitory activity. Replacement of piperidine ring of compound **2** with pyrrolidine (**3**) or homopiperidine (**4**) led to a > 5-fold drop in potency. Then, as for the arylamino moiety at the 4-position of the piperidine ring, transposition of a cyano group at the 5-position on the pyridine ring to the 6-position (**5**) and 4-position (**6**) resulted in a loss of activity. Potency relative to that of compound **2** was maintained by introduction of a trifluoromethyl group (**7**) into the 5-position and was decreased by introduction of a methyl group (**8**), showing that electron-withdrawing groups at the 5-position enhanced potency. It could be considered that cyano and trifluoromethyl groups might affect the electron density of the aromatic ring or directly interact with the protein surface via hydrogen bonding to boost potency. A comparison of phenyl derivative **9** with compound **2** showed that the nitrogen atom of the pyridine ring was non-essential for activity but had critical effects to increase metabolic stability and reduce cytotoxicity [human metabolic stability (μL/min/mg): 19 (compound **2**), 69 (compound **9**); cytotoxicity (% of ATP content at 30 μM): 72.7 (compound **2**), 44.9 (compound **9**)]. 4-Methoxyphenyl (**10**) and unsubstituted phenyl derivative (**11**) were less potent than compound **9**. Next, effect of the substituents on the benzenesulfonyl moiety was investigated. Introduction of a lipophilic group such as a chloro, trifluoromethyl, *tert*-butyl, or phenyl group (**12–16**) at the 4-position afforded equipotent or enhanced activity relative to compound **1**. Especially, bulky groups such as the *tert*-butyl (**14**) and phenyl group (**15** and **16**) showed relatively high potency among them. On the other hand, the cyano (**17**) and acetamide group (**18**) had a detrimental effect on activity. *Meta*-substitution of a methyl (**19**) or phenyl group (**20**) also led to a decrease in activity. These results suggest that the tolerable polarity and position of substituents in the moiety are limited. As a result of initial SAR study, we identified several compounds (**14**, **15**, and **16**) that were 10-fold more potent than hit compound **1**.

We next conducted scaffold morphing of the hit series' central piperidine ring, which we replaced with a cyclohexane ring (Fig. 2). We envisioned that cyclohexane derivatives afford two isomers, 1,4-*cis* and *trans*, and if either isomer gets the better of the other in potency, favorable conformation is clarified. It was also expected that introduction of a cyclohexane ring might rigidify the conformation and thereby enhance potency. Consequently, the 1,4-*trans* isomer **21** exhibited as potent activity as the parent compound **14**, whereas the 1,4-*cis* isomer **22** was less potent. In order to verify the result, a docking study using Maestro was performed (Fig. 3).[13] The key pyridine ring with a cyano group at the 5-position overlaps considerably between potent compounds **14** and **21** (shown in a cyan circle). On the other hand, the 5-cyanopyridyl moiety of compound **22** cannot be placed in the proper direction due to its axial conformation (shown in a white circle).

Compound **21** showed not only equipotent activity but also higher solubility compared with the piperidine series [solubility in water and buffer solutions adjusted to pH 6.8 described in the Japanese Pharmacopoeia (μg/mL): < 0.10 (compound **14**), 3.8 (compound **21**)], and therefore we focused on the modification of this novel cyclohexane series to identify in vitro biological probes (Table 2). 4-Biphenyl sulfones **23** and **24** were equipotent to compound **21**. Especially, trifluoromethyl derivative **24** showed activity with an IC_{50} value of < 100 nM. Moreover, to exploit the surrounding environment of the biaryl moiety, the incorporation of a nitrogen atom into the terminal aryl group was examined. As a result, derivatives with 4-pyridyl (**25**)