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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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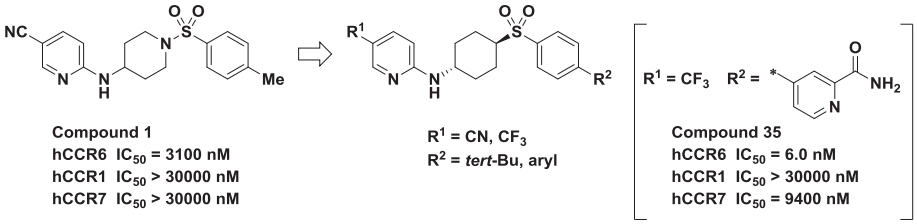
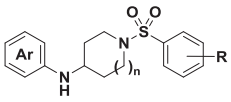
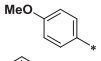


Fig. 1. Hit compound **1**, *N*-benzenesulfonylpiperidine derivative effective as a human CCR6 inhibitor, and design of a novel 1,4-*trans*-1-benzenesulfonyl-4-aminocyclohexane series.

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

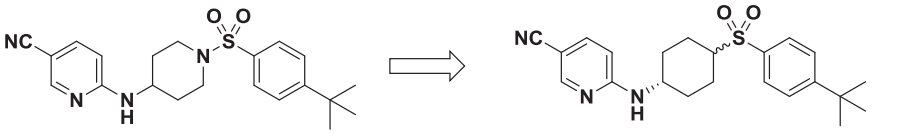
[a] Values shown in parentheses are 95% confidence intervals.
[b] Data of racemate.

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**)



14 IC_{50} = 220 nM (130-360 nM)

21 (1,4-*trans*) IC_{50} = 140 nM (96-220 nM)
22 (1,4-*cis*) IC_{50} >30000 nM

Fig. 2. Modification of the central ring from piperidine to cyclohexane. Values shown in parentheses are 95% confidence intervals.

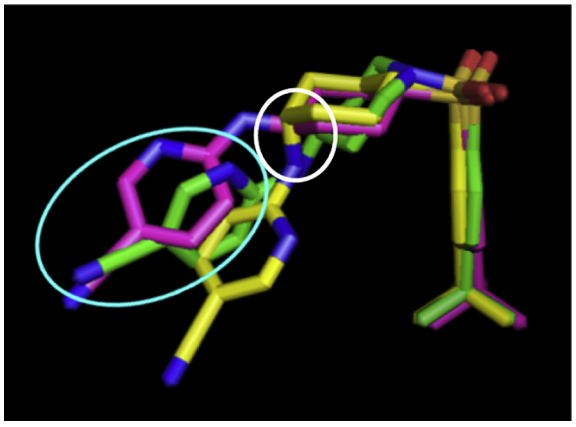


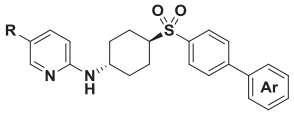
Fig. 3. Superposition of **14** (green), **21** (magenta), and **22** (yellow).

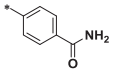
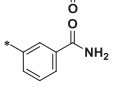
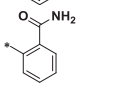
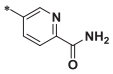
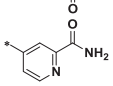
and 3-pyridyl group (**26**) exhibited potent activity, whereas that with 2-pyridyl group (**27**) had reduced activity, indicating the possible interaction of the moiety with the protein surface through hydrogen bonding. Encouraged by the observation, introduction of a carboxamide or a cyano group allowing the compound to come closer to the protein surface was next pursued. As for the carboxamide derivatives, both *para*- (**28**) and *meta*-substitution (**29**) demonstrated improved activity relative to the unsubstituted parent compound **24**. The result was contrary to that for *ortho*-substitution (**30**), but also consistent with the SAR for the pyridine derivatives mentioned above. On the other hand, there was little difference in the potency of the cyano derivatives; however, *para*-cyano derivative **31** showed relatively high activity. By putting the SAR information together, picolinamide derivatives (**34** and **35**) and picolinonitrile derivative (**36**) were prepared. They maintained excellent inhibitory activity in accordance with expectation; especially compound **35** had the most potent activity (IC_{50} = 6.0 nM) among this series of derivatives. Thus, we successfully discovered several tool candidates for in vitro evaluation with IC_{50} values of around 10 nM as represented by compounds **34** and **35**.

Versatile synthetic approaches to prepare *N*-benzenesulfonyl cyclic amine derivatives **A** (**2**–**20**) were developed as shown in Scheme 1. In the first route (Route 1), 1-Boc-protected cyclic amines (**37a**–**c**) were coupled with substituted 2-chloropyridines by either direct S_N2 substitution or palladium-catalyzed Buchwald reaction to afford arylamines (**38a**–**f**). After removal of the Boc group from the compound **38** series under acidic conditions, resultant amines (**39a**–**f**) were converted to target compounds **A** by sulfonylation with various substituted benzenesulfonyl chlorides. As the second route (Route 2), 4-(*N*-Boc-amino)piperidine (**40**) was firstly sulfonylated to provide compound **41**, which was next deprotected and neutralized to yield free amine **42**. Buchwald reaction of amine **42** using BrettPhos/BrettPhos – Palladacycle under microwave irradiation gave compounds **A**. Due to low reactivity of the corresponding aryl halide, compound **10** was synthesized via the alternative route (Route 3). That is, initial sulfonylation of 4-piperidinone hydrochloride (**43**) and subsequent reductive amination with arylamine and 2-picoline borane complex successfully provided the desired product **10**.

General synthetic route for the cyclohexane derivatives **B** (**21**, **22**, **50a** and **50b**) is illustrated in Scheme 2. Starting from tosylation of *cis*-

Table 2
Human CCR6 inhibitory activity of 1,4-*trans*-cyclohexane derivatives.

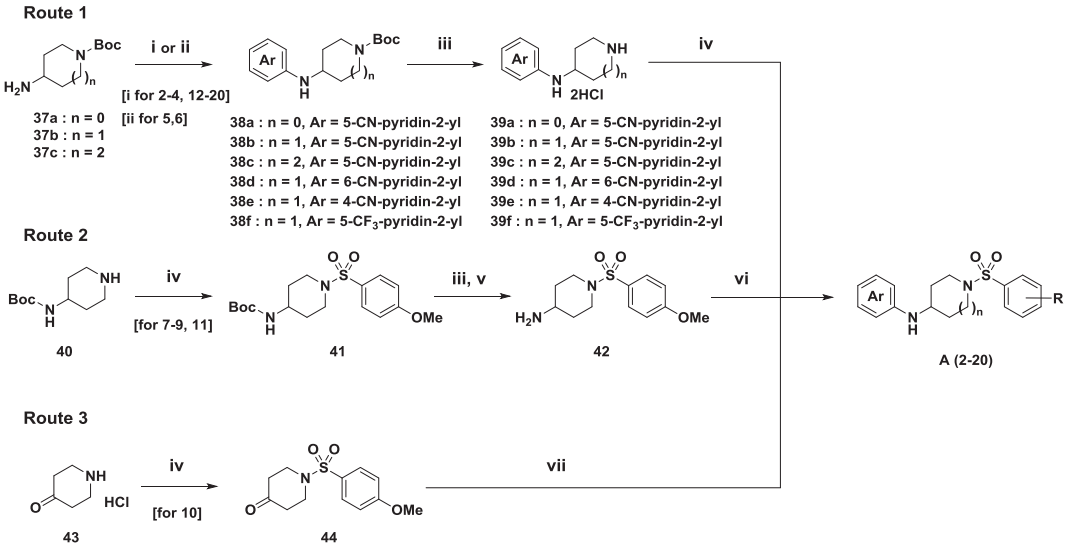


Cmpd	R	Ar	IC_{50}[a] (nM)
23	CN		190 (140–260)
24	CF_3		76 (56–100)
25	CF_3		24 (17–34)
26	CF_3		48 (34–67)
27	CF_3		460 (400–530)
28	CF_3		13 (10–17)
29	CF_3		12 (10–13)
30	CF_3		900 (750–1100)
31	CF_3		17 (13–22)
32	CF_3		31 (26–37)
33	CF_3		48 (42–55)
34	CF_3		17 (15–19)
35	CF_3		6.0 (5.0–7.1)
36	CF_3		27 (21–36)

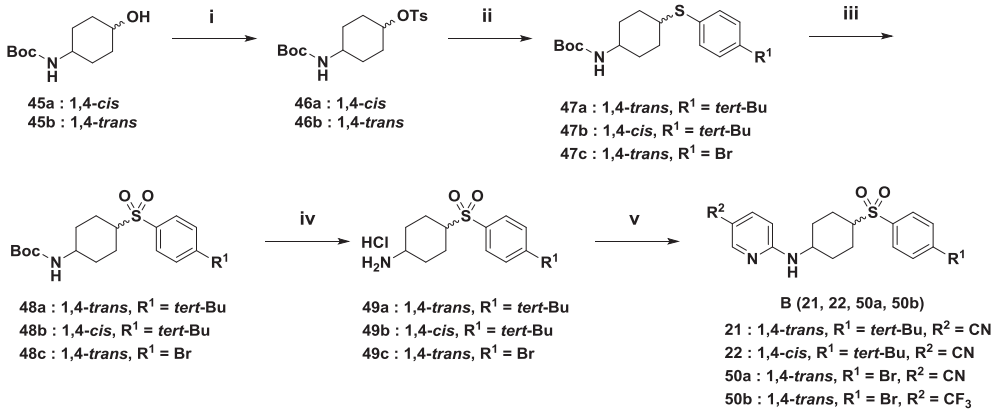
[a] Values shown in parentheses are 95% confidence intervals.

and *trans*-4-*N*-Boc-1-cyclohexanols (**45a** and **45b**), subsequent S_N2 substitution reaction with thiophenols successfully proceeded with stereo-chemical inversion to afford corresponding *trans*- and *cis*-4-*N*-Boc-1-phenylthiocyclohexanes (**47a**–**c**), respectively. Then, oxidation of the sulfide group of compound **47** series using *m*-CPBA followed by deprotection of Boc group yielded cyclohexylamines (**49a**–**c**). Finally, coupling reaction of amines **49** with 2-halopyridines provided the desired compounds **B**.

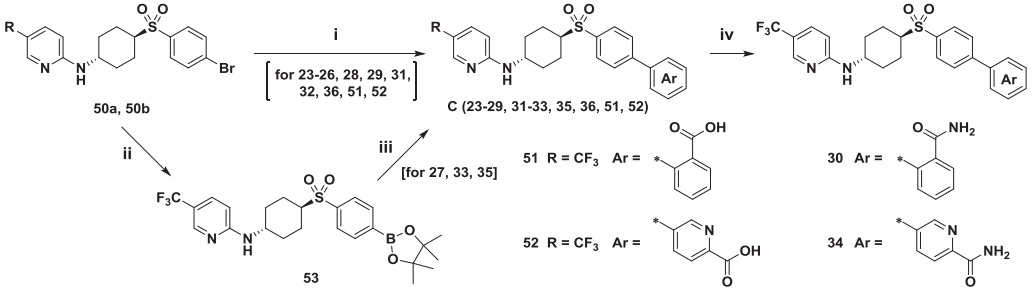
Among cyclohexane derivatives, biaryl sulfone compounds were prepared as depicted in Scheme 3. 4-Bromophenyl sulfones (**50a** and **50b**) were subjected to Suzuki-Miyaura coupling reaction with various boronic acids or boronic acid pinacol esters to afford target compounds **C**. As an alternative route, compound **50b** was firstly converted to phenylboronic acid pinacol ester **53** using bis(pinacolato)diboron and a palladium catalyst. Then, subsequent coupling reaction with aryl



Scheme 1. Reagents and Conditions: (i) Ar-Cl (Ar = 2-pyridyl), DIEA, DMF, 60–120 °C; (ii) Ar-Cl (Ar = 2-pyridyl), $Pd_2(dba)_3$, BINAP, Cs_2CO_3, toluene or DME, 80 °C; (iii) 4 M HCl (EtOAc or CPME solution), EtOAc or EtOH, rt or 50 °C; (iv) R-$C_6H_4SO_2Cl$, Et_3N or DIEA, THF, 0 °C or rt; (v) $NaHCO_3$, H_2O, EtOAc, THF, rt; (vi) Ar-Br (Ar = 2-pyridyl, phenyl), BrettPhos, BrettPhos–Palladacycle, Cs_2CO_3 or sodium *tert*-butoxide, DMF, 130 °C (under microwave irradiation); (vii) Ar-NH_2, 2-picoline borane complex, AcOH, MeOH, rt.



Scheme 2. Reagents and Conditions: (i) *p*-TsCl, DMAP, pyridine, rt; (ii) 4-R^1-C_6H_4SH, K_2CO_3, DMF, 100 °C or Cs_2CO_3, acetone, 60 °C; (iii) *m*-CPBA, EtOAc, rt; (iv) 4 M HCl EtOAc solution, EtOAc, rt; (v) 2-chloro-5-cyanopyridine or 2-fluoro-5-(trifluoromethyl)pyridine, DIEA, NMP or DMF, 100–120 °C.



Scheme 3. Reagents and Conditions: (i) Ar-$B(OR)_2$ (R = H or CMe_2), $Pd(PPh_3)_4$, K_2CO_3, 1,4-dioxane, EtOH, H_2O, 90–100 °C (in some cases, under microwave irradiation); (ii) bis(pinacolato)diboron, $Pd(dppf)Cl_2{\cdot}CH_2Cl_2$ or $Pd(PPh_3)_4$, AcOK, DME or 1,4-dioxane, 90 °C; (iii) Ar-Br, $Pd(PPh_3)_4$, K_2CO_3, 1,4-dioxane, EtOH, H_2O, 80–90 °C, or Na_2CO_3, DME, H_2O, 100 °C, (iv) NH_4Cl, HATU, Et_3N, DMF, rt.

bromides gave compounds **C** as well. Carboxamides **30** and **34** were prepared by amidation of carboxylic acids **51** and **52** with NH_4Cl and HATU.

Representative compounds were evaluated in the migration assay using human CCR6-transfected CHO cells, and also their activity against monkey CCR6 and subclass selectivity were assessed (Table 3). In regard to human CCR6, good correlation between Gi signal inhibitory activity and migration inhibitory activity was observed. Among them, compound **35** exhibited the most potent activity in the migration assay as well as the Gi signal assay. All the compounds maintained high human CCR6 selectivity over human CCR1 and CCR7. In terms of disease model animals, we chose monkeys because the amino acid sequence of human CCR6 has higher homology with monkey CCR6 (> 94%) than with rodent CCR6 [75% (mouse) and 76% (rat), respectively].[14] Thus, we established a Gi signal assay using monkey CCR6-transfected CHO cells. As expected, the inhibitory activity against

Table 3
Inhibitory activity against human CCR6-dependent migration and monkey CCR6, and subclass selectivity of representative compounds.

Cmpd	IC_{50}[a] (nM)				
	Migration assay	Gi signal assay			
	human CCR6	human CCR6	monkey CCR6	human CCR1	human CCR7
1	1400 (630–3200)	3100 (2800–3400)	1300 (1000–1600)	> 30000	> 30000
15	240 (170–340)	290 (220–370)	280 (250–320)	> 30000	> 30000
28	41 (29–59)	13 (10–17)	16 (11–22)	> 30000	> 30000
31	36 (27–49)	17 (13–22)	21 (14–31)	> 30000	> 30000
34	42 (25–69)	17 (15–19)	9.5 (6.5–14)	> 30000	18,000 (13000–26000)
35	< 30	6.0 (5.0–7.1)	0.45 (0.30–0.69)	> 30000	9400 (6300–14000)

[a] Values shown in parentheses are 95% confidence intervals.

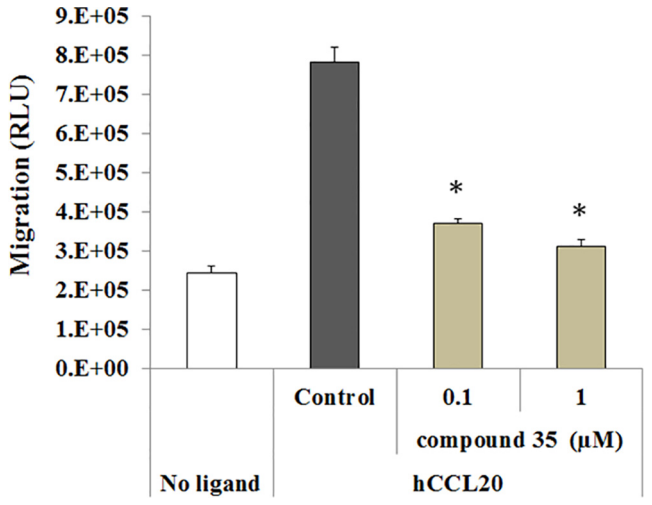


Fig. 4. Effect of compound **35** in CCL20-induced human B cell migration assay. The vertical axis is indicated by ATP content measured in relative luminescence units (RLUs). Statistical analysis was performed using the Williams test. *: $P < 0.025$ versus control. Error bars indicate SEM (N = 3).

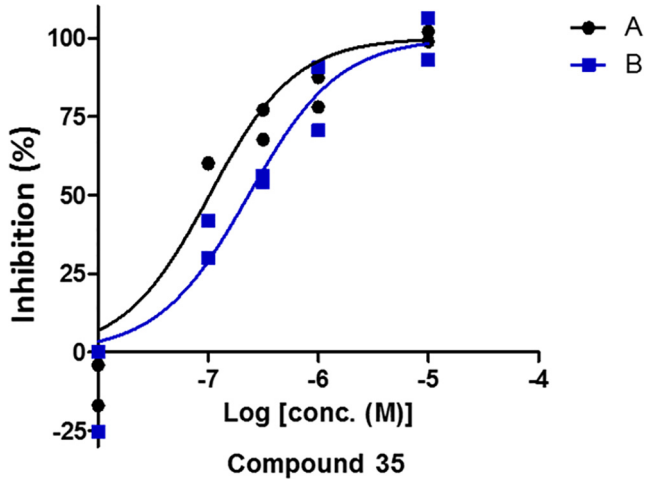


Fig. 5. Effect of compound **35** in a human whole blood assay. CCL20-induced increase in ERK phosphorylation was evaluated in B cells. A and B are different donors (N = 2).

monkey CCR6 was well correlated with that against human CCR6, and compound **35** showed the greatest activity against monkey CCR6 as well.

In order to validate its potency as a biological probe, representative compound **35** was subjected to in vitro evaluation in human primary cell assays. Firstly, compound **35** significantly inhibited CCL20-induced human B cell migration at 0.1 and 1 μM (Fig. 4). Efficacy of the compound was also confirmed in a human whole blood assay, in which CCL20-induced increase in ERK phosphorylation was detected in B cells (Fig. 5). Compound **35** potently inhibited ERK phosphorylation in a concentration-dependent manner. Thus, we confirmed that our compounds could work effectively in vitro as biological probes against human CCR6.

Next, we conducted pharmacokinetic study of compound **35** in cynomolgus monkeys (0.1 mg/kg, iv and 1 mg/kg, po, male, N = 3) for further application in vivo. As a result, compound **35** displayed area under the curve values [$AUC_{0\text{-}24h}$ (ng * h/mL): 1552 (iv), 10,050 (po)], volume of distribution (Vd_{ss}: 704 mL/kg), low clearance (CL_t: 66 mL/h/kg), plasma unbound fraction (f_{up}: 0.01), and good bioavailability (BA: 65%). We also assessed off target profile of compound **35**. Compound **35** did not exhibit remarkable inhibitory activities against CYPs (1A2, 2C8, 2D6, 3A4: < 50% at 10 μM; 2C9: 51% at 10 μM) and hERG (< 50% at 10 μM with/without BSA). Those results suggested that compound **35** would be usable as an in vivo probe as well.

In conclusion, we have identified a novel series of potent and selective human CCR6 inhibitors, 1,4-*trans*-1-benzenesulfonyl-4-aminocyclohexanes. Among them, compound **35** potently inhibited both CCL20-induced CCR6-dependent migration and ERK phosphorylation in human primary cell assays. They can be utilized as in vivo biological probes, when, for instance, monkeys are utilized as disease model animals. We think that CCR6 inhibitor has an opportunity as a novel therapeutic option for diseases including various autoimmune diseases and cancer where CCR6-dependent cell migration contributes to the pathophysiology, and our compounds help further understanding of the pathophysiology of these diseases.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.bmcl.2018.07.042.

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