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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 17 (2007) 1722–1725

Oxazolidinones as novel human CCR8 antagonists

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Received 10 November 2006; revised 15 December 2006; accepted 18 December 2006
Available online 24 December 2006

Abstract—High-throughput screening of the corporate compound collection led to the discovery of a novel series of N-substituted-5-aryl-oxazolidinones as potent human CCR8 antagonists. The synthesis, structure-activity relationships, and optimization of the series that led to the identification of SB-649701 (**1a**), are described.

Human CCR8, a 7TM receptor formerly known as TER1, ChemR1, or CKR-L1, was first identified and cloned in 1996.[1] The CC chemokine I-309[2] functionally activates the receptor with high potency and binding affinity.[3] Three other CC chemokines, TARC, MIP-1β, and vMIP-I, were subsequently identified as the natural ligands of CCR8.[4] Murine CCR8 and its functional ligand TCA-3,[5] the mouse ortholog of I-309, were also identified and cloned.[6] Several lines of evidence suggest that CCR8 may represent a potential target for treatment of asthma and other allergic diseases although CCR8 knock-out mouse data were inconclusive.[7] CCR8 is preferentially expressed in Th2 lymphocytes, not Th1 lymphocytes.[6] I-309, a specific ligand for CCR8, is secreted by activated T lymphocytes.[2] Analysis of bronchial biopsies and bronchoalveolar lavage fluid before and following antigen challenge of atopic asthmatic patients revealed that TARC was highly upregulated in the airway epithelia after challenge.[8] In addition, CCR8 and its ligands are potentially implicated in viral infection and immune regulation.[4b,c,9] It was demonstrated that CCR8 served as a co-receptor for diverse T-cell tropic, dual-tropic, and macrophage-tropic HIV-1 strains and that I-309 was a potent inhibitor of HIV-1 envelope-mediated cell-cell fusion and virus infection.[9]

Several small molecule CCR8 antagonists have recently been reported in the literature.[10] Herein we describe the identification, synthesis, initial structure-activity relationships (SAR), functional activity, and initial development properties of a novel series of N-substituted-5-aryl-oxazolidinones as potent human CCR8 antagonists.

Keywords: CCR8; Antagonists; N-substituted-5-aryl-oxazolidinones; Asthma; HIV; Chemokine receptors.
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doi:10.1016/j.bmcl.2006.12.076

High-throughput screening (HTS) of our compound collection using a radioligand binding assay (measuring inhibition of [^{125}I] labeled human recombinant I-309 binding to human recombinant CCR8 stably expressed on Chinese hamster ovary (CHO) cell membranes)[11] led to the identification of SB-633852 (**2**), a racemic mixture, as a hit with a pIC_{50} of 5.8 (Fig. 1).[12,13] The compound was subsequently tested in a fluorometric imaging plate reader (FLIPR) assay (measuring inhibition of I-309-mediated $[Ca^{2+}]_i$-mobilization in rat basophilic leukemia (RBL) cells expressing human recombinant CCR8)[14] and found to be an antagonist with a pIC_{50} of 5.8. The FLIPR assay has since been used as the primary assay to support SAR work.

In order to explore the SAR around the three key regions of this chemical series, a general synthetic route outlined in Scheme 1 was developed.[15] Various aromatic aldehydes **3** were converted to the corresponding epoxides **4** using standard yield chemistry.[16] Ring opening of epoxides **4** with various mono-protected cyclic and acyclic diamines such as 4-amino-piperidine-1-carboxylic acid *tert*-butyl ester produced amino alcohols **5**, which were cyclized to afford oxazolidinones **6**. Removal of the Boc group followed by reductive amination of the corresponding amines with various aldehydes produced the desired compounds **7**. Using this five-step synthetic route, multiple regions of this chemical series were explored to improve potency and demonstrate sustainable SAR.

We first explored the central diamine (DA) moiety via preparing analogs containing a cyclic or acyclic linker. As shown in Table 1, compound **8**, a N–Me analog of SB-633852 (**2**), showed antagonist activity with a slight drop of potency, indicating that the NH of the propyl diamine moiety is not crucial. We then explored analogs containing a constrained (cyclic) diamine moiety and were extremely pleased to find that compound **9**, a 4-amino piperidine containing analog, had about 100-fold potency improvement comparing to SB-633852 (**2**). Other constrained analogs such as **10** containing a 4-aminomethyl piperidine moiety, **11** containing a 3-aminomethyl piperidine moiety, **12** containing a 3-amino pyrrolidine moiety, and **13** containing a 3-amino azepine moiety showed no potency in the CCR8 FLIPR assay—indicating that the configuration and the length of the central diamine linker is extremely important.

Encouraged by this key finding, we then explored the preferred stereochemistry at the 5-position of the oxazolidinone. Compound **9**, a racemic mixture, was separated via chiral HPLC to afford SB-649701 (**1a**) with 96.5% ee and **1b** with 95% ee.[17] As shown in Figure 2, the R enantiomer, **1a** (96.5% ee), was greater than 100-fold more potent than the S enantiomer, **1b** (95% ee). The observed FLIPR potency of **lb** could be due to the presence of 2.5% of the R enantiomer in **1b** as the enantiomeric excess of **1b** is 95%.

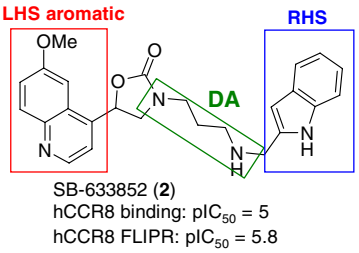
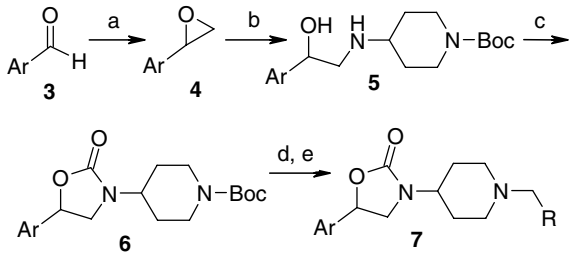
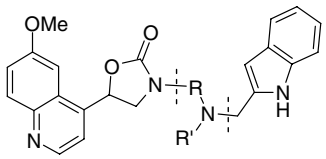


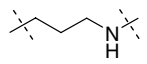
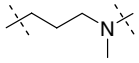
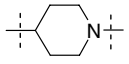
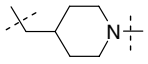
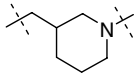
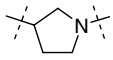
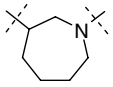
Figure 1. Structure of HTS hit SB-633852 (**2**).



Scheme 1. Reagents and conditions: (a) Me_2S, Me_2SO_4, NaOMe, CH_3CN, rt; (b) 4-amino-piperidine-1-carboxylic acid *tert*-butyl ester, $LiClO_4$, CH_3CN, reflux; (c) 1,1-carbonyldiimidazole, 4-dimethylamino pyridine, CH_2Cl_2, rt; (d) 30% trifluoroacetic acid in CH_2Cl_2, rt; (e) RCHO, $Na(OAc)_3BH$, CH_2Cl_2, rt.

Table 1. SAR of the central diamine moiety



Compound		hCCR8 FLIPR[a] (pIC_{50})
2		5.8
8		5.2
9		7.6
10		<4.3
11		<4.3
12		<4.3
13		<4.3

[a] Mean of at least 2 determinations with standard deviation of <±0.3.

We next turned our attention to exploring the left-hand side (LHS) aromatic moiety. Changing the 6-methoxy quinoline-4-yl group (**9**) to 2-methoxy-1,5-naphthyridin-8-yl (**14a**) resulted in no or little potency loss (Table 2). The des-methoxy compound, **14b**, was about 30-fold less potent. Further structural changes such as switching the

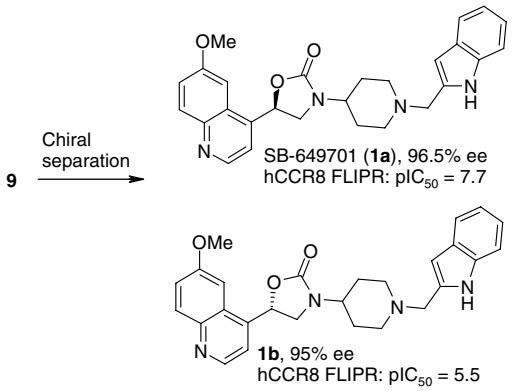
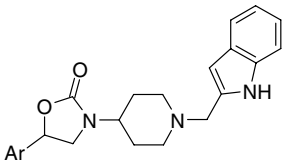


Figure 2. Preferred stereochemistry.

Table 2. SAR of the left-hand side (LHS) aromatic moiety



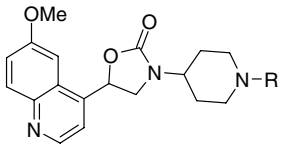
Compound	Ar	hCCR8 FLIPR[a] (pIC_{50})
9	6-Methoxyquinolin-4-yl	7.6
14a	2-Methoxy-1,5-naphthyridin-8-yl	7.5
14b	Quinolin-4-yl	6.0
14c	1-Naphthyl	<4.3
14d	Quinolin-3-yl	<4.3
14e	Quinolin-2-yl	<4.3
14f	Pyridin-4-yl	<4.3

[a] Mean of at least 2 determinations with standard deviation of <±0.3.

6-methoxy quinoline-4-yl group to 1-naphthyl (**14c**), quinolin-3-yl (**14d**), quinolin-2-yl (**14e**), and pyridin-4-yl (**14f**) resulted in complete potency loss. The SAR in this region indicated a limited degree of tolerability for structural variation.

We then investigated the right-hand side (RHS) N-capping group. A number of analogs containing a variety of capping groups were prepared and evaluated in the CCR8 FLIPR assay (Table 3). Adding methyl to the indole nitrogen resulted in about 100-fold potency loss (**9** vs **15a**). Changing the 1-*H*-indol-2-ylmethyl group to the 1-*H*-indol-3-ylmethyl (**15b**) abolished CCR8 antagonist activity completely. Other fused bicyclic aromatic groups such as benzofuran-2-ylmethyl (**15c**), benzothiophen-2-ylmethyl (**15d**), quinolin-2-ylmethyl (**15e**), and naphthalen-2-ylmethyl (**15f**) were moderately potent in the FLIPR assay. In addition to fused bicyclic aromatic groups, a number of alkyl and benzyl groups were also explored. While methyl (**15g**) was inactive, hexyl (**15h**) was moderately potent and cyclohexylmethyl (**15i**) was weakly active. Although benzyl (**15j**) showed only weak potency, extending the chain length from 1 (**15j**) to 3 (**15k**) resulted in 50-fold potency increase. The preferred substitution pattern for the benzyl sub-series is: *para* > *meta* > *ortho* (**15l** vs **15m** vs **15n**). Several 4-substituted benzyl groups (**15o**–**15r**) were subsequently investigated

Table 3. SAR of the right-hand side (RHS) N-capping group



Compound	R	hCCR8 FLIPR[a] (pIC_{50})
9	1-*H*-Indol-2-ylmethyl	7.6
15a	1-Methyl-indol-2-ylmethyl	5.5
15b	1-*H*-indol-3-ylmethyl	<4.3
15c	Benzofuran-2-ylmethyl	5.9
15d	Benzothiophen-2-ylmethyl	6.1
15e	Quinolin-2-ylmethyl	5.5
15f	Naphthalen-2-ylmethyl	5.9
15g	Methyl	<4.3
15h	1-Hexyl	6.3
15i	Cyclohexan-1-ylmethyl	5.1
15j	Benzyl	4.9
15k	Phen-1-ylpropyl	6.6
15l	4-Chlorobenzyl	6.2
15m	3-Chlorobenzyl	5.2
15n	2-Chlorobenzyl	<4.3
15o	4-Methoxybenzyl	5.9
15p	4-Cyanobenzyl	4.9
15q	4-Bromobenzyl	7.3
15r	4-Phenylbenzyl	<4.3
15s	3,4-Dimethylbenzyl	7.4
15t	3,4-Dichlorobenzyl	6.9
15u	3,4-Dichlorophen-1-ylcarbonyl	5.2
15v	3,4-Dichlorophen-1-ylsulfonyl	<4.3

[a] Mean of at least 2 determinations with standard deviation of <±0.3.

and 4-bromobenzyl (**15q**) was found to be optimal with a pIC_{50} of 7.3. The 3,4-disubstituted benzyl analogs such as **15s** and **15t** were also highly potent antagonists with a pIC_{50} of 7.4 and 6.9, respectively. We also explored amide and sulfonamide capping groups and found that amide **15u** and sulfonamide **15v** were dramatically less potent compared to amine **15t**.[18] In general, modifications in this region were well tolerated and several potential sub-series with a different type of N-capping group (e.g., fused bicyclic aromatic, mono- and di-substituted benzyl, phenylpropyl) have been identified.

In addition to human CCR8 FLIPR assay, SB-649701 (**1a**) was also evaluated in two distinct chemotaxis assays. SB-649701 (**1a**) was a functional antagonist in a HUT78 (a human T cell line) chemotaxis assay (measuring the I-309 mediated migration of HUT78 through 3 μm ChemoTX filter systems)[19] with a pIC_{50} of 6.3 ($n = 3$, SD = 0.28). In an activated Th2 chemotaxis assay (measuring the I-309 mediated migration of activated Th2 cells),[20] SB-649701 (**1a**) was also a potent functional antagonist with a pIC_{50} of 7.0 ($n = 4$, SD = 0.14). SB-649701 (**1a**) was also tested in mouse, rat, and guinea pig CCR8 FLIPR assays and found to have no activity. The lack of rodent ortholog activity might be due to the significant sequence differences between human and rodent receptors (e.g., mouse CCR8 shows only 71% homology to human CCR8[6]).

We also evaluated this series in selectivity, CYP450 inhibition, hERG, and in vitro and in vivo PK studies.

SB-649701 (**1a**) showed at least 100-fold selectivity vs a number of 7TM receptors including chemokine receptors. SB-649701 (**1a**) and **15s** did not significantly inhibit all six major P450 isozymes (**1a**, $pIC_{50} < 5.1$; **15s**, $pIC_{50} < 5.0$). In hERG binding assay, SB-649701 (**1a**) and **15s** had a pIC_{50} of 5.8 and 5.2, respectively, which provided a respective 79- and 158-fold hERG window over CCR8 IC_{50}. In in vitro human and rat liver microsome stability studies, SB-649701 (**1a**) showed moderate intrinsic clearance (human: 7 mL/min/g liver; rat 7 mL/min/g liver). SB-649701 (**1a**) also demonstrated moderate clearance and low to moderate oral bioavailability (Clb = 38 mL/min/kg, $F = 18\%$, $T_{1/2} = 1$ h, $V_{dss} = 2.4$ L/kg) in in vivo mouse PK studies (1 mg/kg iv, 2 mg/kg po). Overall, SB-649701 (**1a**) possessed good selectivity and initial development properties.

In summary, SAR exploration of a novel oxazolidinone series identified via HTS led to the discovery of potent human CCR8 antagonists such as SB-649701 (**1a**) with good chemotaxis activity, selectivity, DMPK, and hERG properties. The further optimization of this series will be the subject of future publications.

Acknowledgments

We thank Bing Wang, Chad Quinn, Qian Jin, and Carl Bennett for NMR, analytical LC/MS, and preparative HPLC support, and Arun Kaura for chiral HPLC separation.

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11. Binding assay method: assays were performed in a 384-well microtiter plate format. Each reaction well contained: 0.35 nM [^{125}I]-I309, 3.0 μg/mL of human CCR8 CHO membranes prebound to 60 μg of LEADseeker WGA-PS SPA (Wheatgerm–agglutinin coupled polystyrene scintillation proximity imaging) beads in 25 mM bis-trispropane buffer, 2 mM $MnCl_2$, 1 mM EDTA, 100 mM NaCl, 7.5% glycerol, 0.05% CHAPS at pH 7.5, and appropriate concentration of a test compound.
12. The chiral centers in the structures shown in the paper are racemic unless the chirality is explicitly illustrated.
13. (a) The biological assay results in the paper are a mean of at least 2 determinations with standard deviation of $<\pm 0.3$ unless otherwise noted; (b) $pIC_{50} = -\log IC_{50}$ (M).
14. For FLIPR assay details, see Jin, J.; Kerns, J. K.; Wang, F.; Wang, Y. WO Patent 2004032856-A2, 2004; Jin, J.; Kerns, J. K.; Wang, F.; Wang, Y. *Chem. Abstr.* **2004**, *140*, 357326.
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17. (a) Chiral separation conditions: Chiralcel AD (20×250 mm, 10 μM) eluting at 15 ml/min 30% eluent "A": 70% eluent "B" gave two components at $R_T = 17.7$ and 33 min. "A" = EtOH:MeOH:isopropylamine = 50:50:0.1, "B" = hexanes:isopropylamine = 99.9:0.1; (b) Stereochemistry was assigned by converting the (R)-epoxide to the final product and chiral HPLC R_T comparison. For the (R)-epoxide synthesis, see: Davies, D. T.; Markwell, R. E.; Pearson, N. D.; Takle, A. K. WO Patent 2000078748-A1, 2004; Davies, D. T.; Markwell, R. E.; Pearson, N. D.; Takle, A. K. *Chem. Abstr.* **2000**, *134*, 71500.
18. Amide **15u** and sulfonamide **15v** were prepared via reaction of the piperidine intermediate with the corresponding acid and sulfonyl chloride, respectively.
19. (a) For HUT78 chemotaxis assay details, see Jin, J.; Kerns, J. K.; Wang, F.; Wang, Y. WO Patent 2004073619-A2, 2004; Jin, J.; Kerns, J. K.; Wang, F.; Wang, Y. *Chem. Abstr.* **2004**, *141*, 243573; (b) Jin, J.; Kerns, J. K.; Wang, F.; Wang, Y. WO Patent 2004074438-A2, 2004; Jin, J.; Kerns, J. K.; Wang, F.; Wang, Y. *Chem. Abstr.* **2004**, *141*, 243574.
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