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Letters

Design, Synthesis, and Progress toward Optimization of Potent Small Molecule Antagonists of CC Chemokine Receptor 8 (CCR8)

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Abstract: Activation of CCR8 by its ligand CCL1 may play an important role in diseases such as asthma, multiple sclerosis, and cancer. The study of small molecule CCR8 antagonists will help establish the validation of these hypotheses. We report the design, synthesis, and progress toward optimization of potent small molecule CCR8 antagonists identified from a high-throughput screen. These analogues exhibit good potency in binding and chemotaxis assays, show good selectivity versus the hERG channel, and have good eADME (early absorption, distribution, metabolism, and excretion) profiles.

Chemokines are chemotactic cytokines that regulate development, activation, and recruitment of leukocytes through binding and activation of seven transmembrane G-protein-coupled receptors (GPCRs). On the basis of the spacing of conserved cysteines at the N-terminus, chemokines are classified into CXC, CC, C, and CX3C subgroups.[1] Most chemokine receptors are activated by multiple chemokines. CCR8 is an exception because it has only one known natural ligand, CCL1 (also known as I-309). In addition, pox-viral chemokine MC148 and human herpes virus 8 (HHV-8) derived chemokine vMIP I have been identified as specific human CCR8 antagonists and agonists, respectively.[2]

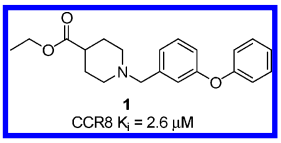

Figure 1. CCR8 screening lead.

CCR8 is expressed on a variety of cell types including monocytes and endothelial cells and is selectively up-regulated upon activation of T-helper-2 (Th2) cells.[3] Th2 cells are the main source of the cytokines IL-4, IL-5, and IL-13, which are major mediators of inflammation, airway hyperreactivity, and mucus hypersecretion in bronchial asthma.[4]

CCR8 is also expressed on phagocytic macrophages and activated microglial cells in the human central nervous system and is found in active demyelinating multiple sclerosis (MS) lesions, in progressive multifocalleukoencephalopathy (PML), and in cerebral ischemia.[5] Furthermore, endothelial-derived spindle cells of human Kaposi sarcoma biopsies express CCR8.[6] The CCR8 ligand, CCL1, has antiapoptotic activity in adult T-cell leukemia (ATL), caused by human T-cell leukemia virus type 1 (HTLV-1).[7]

Because of the expression pattern of CCR8, activation of the receptor by CCL1 may play an important role in various diseases including allergic asthma, multiple sclerosis, and cancer. Our research was directed toward identifying small molecule antagonists to understand the role of CCR8−CCL1 interactions in these diseases and to provide novel therapeutics for their treatment. Reports in the literature describing small molecule CCR8 antagonists are limited to a few published patent applications.[8] In this Letter, we describe the design, synthesis, and progress toward optimization of CCR8 antagonists.

Screening of our compound collection in a human CCR8 binding assay led to the discovery of biphenyl ether amine **1** (Figure 1) with a K_i of 2.6 μM as the initial lead. For SAR exploration, the lead structure was deconstructed into three basic fragments (Figure 2). We investigated all three fragments including (1) substituted piperidines and other amines, (2) the linker between the biphenyl ether and the amine, and (3) substituents on rings A and B along with biphenyl ether replacements. To achieve efficient optimization, our screening

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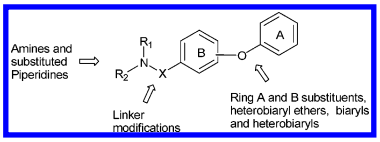
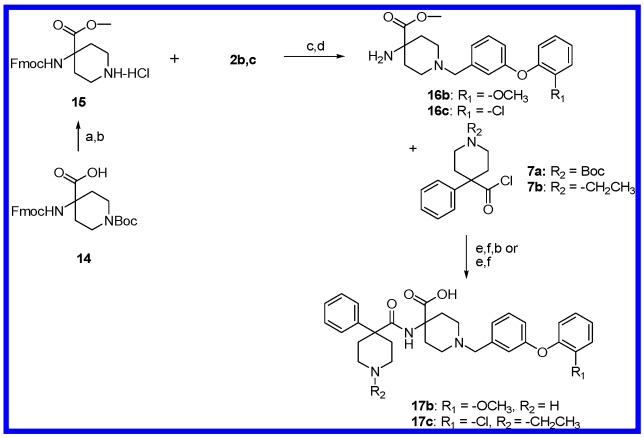


Figure 2. Chemistry strategy to address SAR.

strategy involved parallel evaluation of various properties in addition to potency, including in vitro selectivity and safety parameters. This allowed for early identification of issues such as hERG binding and metabolic stability.

The synthetic routes used to prepare analogues for SAR exploration are described in Schemes 1 and 2. Reductive amination of 3-phenoxybenzaldehyde **2a** and 4-carboxyethylpiperidine ($Na(OAc)_3BH$, 1% AcOH, 1,2-dichloroethane) readily provided our initial lead **1**. Hydrolysis of **1** produced the corresponding acid **3** (LiOH, EtOH/THF), which upon treatment with ethylamine in the presence of EDCI and HOBT yielded the bioisostere **4** (Table 1). To access substituted biaryl ethers, **2b**,**c**, 3-formylphenylboronic acid was treated with appropriately substituted phenols in the presence of copper acetate (Scheme 1).[9] Most reproducible yields for the biaryl ether synthesis were obtained when reactions were run open to air. Reductive amination of **2a**−**c** with 4*N*-Boc-aminopiperidine followed by removal of the Boc group with 4 M HCl/dioxane provided biaryl ether amines as dihydrochloride salts **5a**−**c**. Acylation of the dihydrochloride salts with various acid chlorides in the presence of diisopropylethylamine or with acids in the presence of EDCI and HOBT produced analogues **6a**−**d** (Table 1). Alternatively, acylation with commercially available *N*-Boc-4-phenylpiperidine-4-carboxylic acid (**7**) or *N*-Boc-3-phenylpyrrolidine-3-carboxylic acid (**8**)[10] in the presence of EDCI and HOBT and subsequent removal of the Boc protecting group provided biaryl ether amines **9b**, **9c**, and **10c** (Scheme 1). Alkylation of **9b** and **9c** with ethyl bromide in the presence of triethylamine yielded **11b** and **11c**, respectively. Alkylation of **9c** and **10c** with ethyl 2-bromoisobutyrate using potassium carbonate as base followed by hydrolysis of the corresponding esters with 6 N HCl gave piperidine **12c** and racemic pyrrolidine **13c**, respectively. Compounds **17b** and **17c,** derivatives of **9b** and **11c**, were prepared from commercially available piperidine **14** (Scheme 2). Esterification of **14** with TMS-diazomethane followed by removal of the Boc protecting group provided piperidine **15**. Reductive amination of **2b** with **15** followed by removal of the

Scheme 2. Synthesis of 4-Amino-4-carboxy Substituted Piperidine Biaryl Ethers **17b** and **17c**[a]



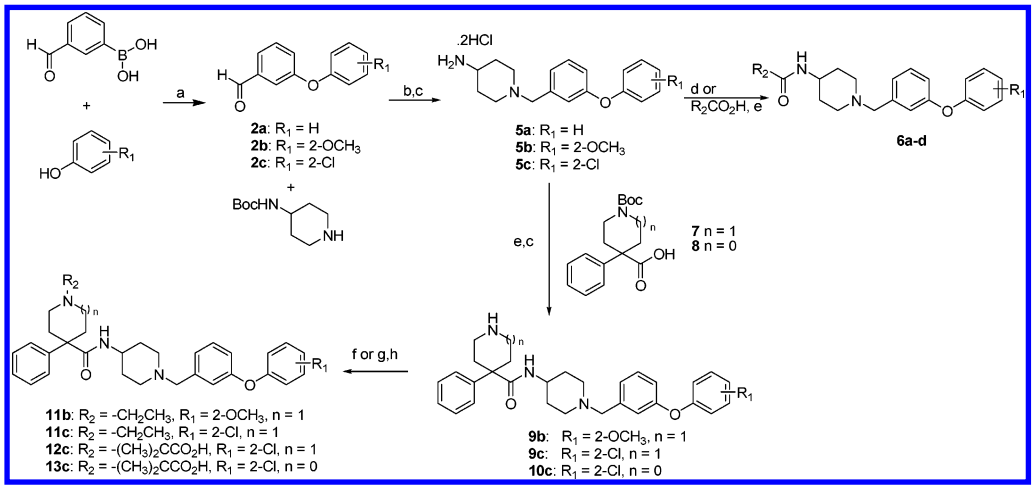
[a] Reagents and conditions: (a) $TMSCH_2N_2$, toluene/MeOH, room temp, 2 h; (b) 4 M HCl/dioxane, room temp, 2 h; (c) $Na(OAc)_3BH$, 1% AcOH, $ClCH_2CH_2Cl$, room temp, 18 h; (d) Et_2NH, DMF, room temp, 4 h; (e) Et_3N, CH_2Cl_2, room temp, 18 h; (f) 1.0 M NaOH(aq)/MeOH, 80 °C, 2 h.

Fmoc protecting group with diethylamine in DMF yielded the biaryl ether amine **16b**. Acylation of **16b** with *N*-Boc-4-phenylpiperidine-4-carbonyl chloride (**7a**) followed by hydrolysis of the methyl ester and removal of the Boc group provided **17b**. **17c** was prepared in a similar manner from **2c** using **7b**.

Our screening strategy was designed to identify compounds with good overall properties for evaluation in in vivo models as proof of concept studies for targeting CCR8. We tested all analogues in the primary assay (hCCR8 binding assay). On the basis of primary assay potency, selected compounds were tested in functional assays (hCCR8 chemotaxis), in a selectivity panel (other chemokine receptors and GPCR's), in early absorption, distribution, metabolism, and excretion (eADME) assays[11] such as P450 inhibition, permeability, and metabolic stability, and in safety-related assays such as hERG channel binding. Analogues with good overall in vitro properties were then profiled for their pharmacokinetics (PK) properties in two animal species (rodent and non-rodent).

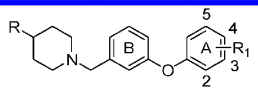
On the basis of our SAR plan (Figure 2), a survey of the pharmacophoric elements of the lead **1** revealed that the 3-biphenyl ether benzylamine moiety was required for binding activity. Acid **3** readily prepared from **1** showed loss in activity

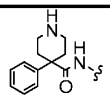
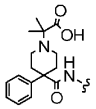
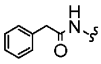
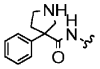
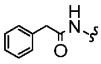
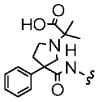
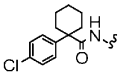
Scheme 1. General Synthesis of 4-Amino Substituted Biaryl Ether Piperidines and Pyrrolidines[a]



[a] Reagents and conditions: (a) $Cu(OAc)_2$, Et_3N, 4 Å molecular sieves, 1,2-dichoroethane, room temp, 18 h; (b) $Na(OAc)_3BH$, 1% AcOH, 1,2-dichloroethane, room temp, 18 h; (c) 4 M HCl/dioxane, room temp, 2 h; (d) R_2COCl, DIEA, CH_2Cl_2, room temp, 18 h; (e) EDCI, HOBT, NMM, DMF, room temp, 18 h; (f) CH_3CH_2Br, Et_3N, CH_2Cl_2, room temp, 18 h; (g) $Br(CH_3)_2CCO_2Et$, K_2CO_3, DMF, room temp, 18 h; (h) 6 N HCl, reflux, 20 h.

Table 1. Binding Activity (K_i) and Chemotaxis Inhibition of Biaryl Ether Piperidines



Entry	R	R_1	K_i [a,b] (μM)	Chemotaxis IC_{50} (μM) [b]	Entry	R	R_1	K_i [a,b] (μM)	Chemotaxis IC_{50} (μM) [b]
1		H	2.6 ± 0.9	ND	9b		2-OCH_3	0.062 ± 0.02	0.011 ± 0.002
3		H	>30	ND	11b		2-OCH_3	0.011 ± 0.001	0.056 ± 0.02
4		H	9.1 ± 5	ND	11c		2-Cl	0.015 ± 0.003	0.043 ± 0.02
6a		H	9.2 ± 1.8	ND	12c		2-Cl	0.009 ± 0.003	0.004 ± 0.002
6b		H	1.4 ± 0.2	ND	10c		2-Cl	0.027 ± 0.004	0.022 ± 0.005
6c		2-OCH_3	0.25 ± 0.03	0.055 ± 0.018	13c		2-Cl	0.006 ± 0.002	0.001 ± 0.0001
6d		2-OCH_3	0.25 ± 0.03	ND					

[a] Displacement of ^{125}I-I309 from human CCR8 expressed on L1.2 cells. [b] Values represent the mean of $n = 2$.

($K_i > 30$ μM). The ethylamide **4** and reversed amide **6a** showed decreased potency. The phenylacetylamide **6b** was slightly more potent than the initial lead **1**. An increase in binding potency was observed with substitution on the A ring of the biphenyl ether. Thus, **6c**, with a 2-methoxy substituent in the A ring inhibited hCCR8 binding with $K_i = 0.25$ μM. **6c** also blocked chemotaxis of CCR8 L1.2 transfected cells induced by I-309 with $IC_{50} = 0.055$ μM. In general, for this series of compounds, binding potency was within 3-fold of chemotaxis inhibition potency (see Table 1). Investigation of A and B ring substitution showed that the 2-position of the A ring, particularly methoxy and chloro groups, and the benzylic piperidine in the 3-position of the B ring provided optimal binding activity. Further piperidine modifications led to the spiro cyclohexyl analogue **6d**, which was equipotent to the unsubstituted benzylamide analogue **6c**. Incorporation of a spiro piperidine moiety, e.g., **9b**, improved potency in the whole-cell binding assay and the chemotaxis assay. The ethyl substituted piperidine **11b**, which was easily accessed from **9b**, gained a 4- to 6-fold improvement in potency. Further investigations revealed that the piperidine nitrogen could be substituted without loss of potency (additional data not shown). Thus, substitution on the piperidine nitrogen provided a handle to optimize other properties of the compounds.

While optimizing for binding potency, we investigated selectivity against other receptors and eADME[11] properties, including P450 CYP inhibition, metabolic stability, and permeability. In general, compounds of this class showed at least 300-fold selectivity vs GPCRs including chemokine receptors.[12] They did not significantly inhibit P450 isozymes including CYP 3A4 ($IC_{50} > 10$ μM). Caco-2 transport measurements revealed that our preferred compounds are highly permeable (e.g., **9b** Papp, $\times 10^6$ cm/s): A to B is 9, B to A is 20, ratio is 2.2). Metabolic stability of selected molecules was assessed by calculating in vitro hepatic clearance from microsomal incubation studies. Compounds of this class exhibited low in vitro hepatic clearance rates in human microsomal studies but higher rates in rat and dog (e.g., **9b** $Cl_h = 0.6$ L/h/kg (human), 3.1 L/h/kg (rat), and 1.4 L/h/kg (dog)). Some of the more potent CCR8 antagonists also potently inhibited [^{3}H]dofetilide binding to the hERG channel expressed in HEK293 cells (e.g., **9b** $K_i = 0.8$ μM). Our approach to dial out hERG activity was to introduce polar substituents in the molecule without disrupting target potency. Thus, substitution of the spiro piperidine **9b** with a carboxylic acid bearing group resulted in a potent compound, **12c**, with a 10-fold improvement in hERG window over binding K_i. Furthermore, introducing a carboxylic acid group in the 4 position of the aminopiperidine resulted in a substantial decrease in hERG binding, e.g., **17b** (hCCR8 $K_i = 0.06 \pm 0.003$ μM ($n = 2$), hERG $K_i > 10$ μM) and **17c** (hCCR8 $K_i = 0.023 \pm 0.005$ μM ($n = 2$), hERG $K_i > 10$ μM). Introduction of polar functionality into the molecule did not appreciably affect the permeability of the compound as measured by the Caco2 transport assay (e.g., **17c** Papp, $\times 10^6$ cm/s): A to B is 25, B to A is 83, ratio is 3.3). The spiro pyrrolidine version of **12c** was the most potent hCCR8 binder of the series ((±)-**13c** $K_i = 0.006$ μM).[13] Additionally, **13c** showed weaker hERG binding potency ($K_i > 10$ μM) than **12c**.

Applying our screening paradigm, we evaluated PK properties for selected compounds. The oral bioavailabilities of these compounds are low to moderate with high clearance rates, e.g., **9b** [rat (10 mpk po, 1 mpk iv, $F = 17\%$, $t_{1/2} = 0.7$ h, and Cl = 34 L/h/kg) and dog (10 mpk po, 1 mpk iv, $F = 20\%$, $t_{1/2} = 11$ h, and Cl = 4 L/h/kg)]. The high in vivo clearance rates in rats and dogs for **9b** can be attributed at least partly to P450 metabolism in the liver (based on the in vitro microsomal incubation studies). We are currently investigating the metabolic

fate of our preferred compounds and other clearance mechanisms to understand the high clearance rates and help resolve low oral exposure.

In summary, we have identified potent small molecule CCR8 antagonists. Our screening paradigm allowed for parallel evaluation of various properties in addition to potency including in vitro selectivity and safety. This approach helped for rapid and efficient optimization of the series. Compounds in this series potently block chemotaxis and possess good overall profiles including good hERG selectivity. We are currently focused on improving PK properties of this series while maintaining good potency, selectivity, and safety profiles.

Acknowledgment. The authors thank Nanda Gulavita, Marjorie Solomon, and May Zhu for analytical support and Tim Ocain for helpful suggestions with the manuscript.

Supporting Information Available: Description of binding, chemotaxis assays, and synthesis procedures including spectral data for **1**−**17**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) eADME assays: in vitro assays utilized in the hit to lead or lead optimization phase to study pharmacokinetic properties of compounds prior to testing in vivo.

(12) For example, for **12c**: % inhibition @ 1 μM 5HT2A is 14%, α_1 = 4%, β_1 = 10%, β_2 = 15%, % inhibition @ 10 μM CCR3 is 30%. See Supporting Information for additional data for **12c** and **17c**.

(13) All spiro pyrrolidines were tested as racemic mixtures. Separation of the enantiomers of **13c** and profiling of the eutomer are in progress.

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