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CCR5 receptor antagonists: Discovery and SAR of novel 4-hydroxypiperidine derivatives

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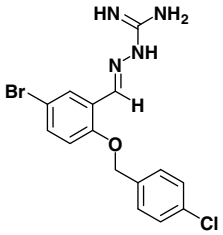
Abstract—The guanylhydrazone of 2-(4-chlorobenzyloxy)-5-bromobenzaldehyde, **1**, with an IC_{50} of 840 nM against the CCR5 receptor was identified using high-throughput screening. Optimization efforts led to the discovery of a novel piperidine series of CCR5 antagonists. In particular, the 4-hydroxypiperidine derivative, **6k**, had improved potency against CCR5, and was a starting point for further optimization. SAR elaboration using parallel synthesis led to the identification of **10h**, a potent CCR5 antagonist with an IC_{50} of 11 nM.

Inflammatory cells migrate into tissues in response to the production of a variety of factors including the family of 'chemotactic cytokines' also known as chemokines.[1] These proteins recruit and activate immune and inflammatory cells through interactions with specific chemokine receptors, a superfamily of seven transmembrane domain proteins that signal through coupled heterotrimeric G proteins.[1c,d,2] Chemokine receptors are key regulators of the immune response to infectious agents but are also critical for the initiation and maintenance of inflammatory reactions in a variety of chronic disease states, including asthma, rheumatoid arthritis, and allergic disease.

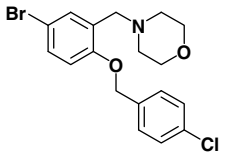
The chemokine receptor CCR5 has been shown to modulate the migration, proliferation, and immune functions in T cells and cells from the monocyte/macrophage lineage. CCR5 also functions as a key cell entry co-receptor for HIV-1 in concert with CD4.[1c,d,3] Endogenous high-affinity chemokine ligands/agonists for CCR5 include RANTES, MIP-1α, MIP-1β, and MCP-2.[4] A variety of evidence has implicated CCR5 and its chemokine ligands in the pathophysiology of T-helper (Th1) driven diseases such as multiple sclerosis (MS), organ transplant rejection, stroke, and psoriasis.[5] There has been much effort in the pharmaceutical industry to identify CCR5 antagonists.[6,7] A potent, selective, and orally available CCR5 antagonist would have therapeutic potential for the treatment of chronic inflammatory diseases as well as HIV.[1d,8]

In our previous manuscript,[9] we reported the discovery by high throughput screening and initial optimization of guanylhydrazone derivatives (e.g., **1**) as CCR5 antagonists. Further investigation led to the replacement of the guanylhydrazone with a tertiary amine such as morpholine (**2**). Herein, we describe the continuation of this work and the discovery and SAR of 4-hydroxypiperidine derivatives as CCR5 antagonists.



1

IC_{50}: 0.84μM



2

IC_{50}: 1.0μM

Keywords: CCR5 receptor antagonists; Hydroxypiperidine; Chemokine.
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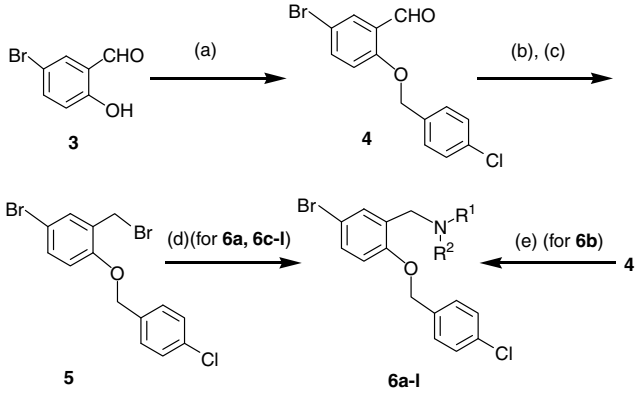
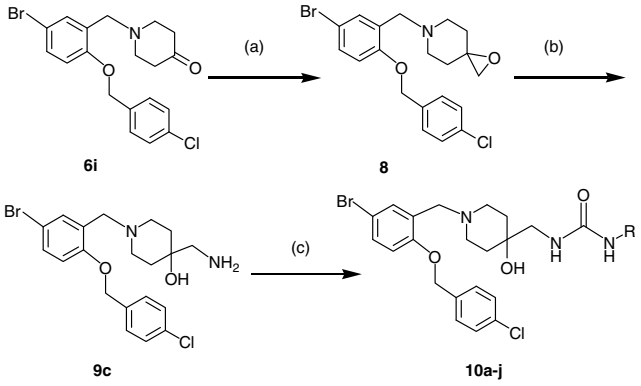
Analogs were conveniently synthesized from the common intermediate **5** (Scheme 1). Alkylation of 5-bromosalicylaldehyde **3** with 4-chlorobenzyl chloride afforded aldehyde **4**. Reduction of **4** to the alcohol with $NaBH_4$, followed by bromination with CBr_4 and PPh_3 in CH_2Cl_2, yielded intermediate **5** (77% for three steps from **3**). Displacement of the bromide with various secondary amines afforded compounds **6c–l**. Reaction of **5** with NH_4OH gave rise to **6a** as well as overalkylated side-products. Compound **6b** was prepared from aldehyde **4** by reductive amination with $NaBH(OAc)_3$ and ethylamine.

The ureas, **10a–j**, were prepared according to Scheme 2. The starting material, **6i**, was converted to **8** in high yield (97%) with trimethylsulfonium iodide and potassium *tert*-butoxide in DMSO. The epoxide was treated with excess ammonium hydroxide in MeOH in a sealed tube and heated at 60 °C for 6 h to give the amino-alcohol **9c** (81%). The ureas (**10a–j**) were obtained by reacting **9c** with the corresponding isocyanate and triethylamine in dichloromethane at room temperature for 2 h.



Scheme 1. Preparation of benzyl amine. Reagents and conditions: (a) 4-chlorobenzyl chloride, K_2CO_3, DMF, 120 °C, 4 h, 98%; (b) $NaBH_4$, MeOH, rt; (c) CBr_4, PPh_3, CH_2Cl_2, 79% (two steps from **4**); (d) R_1R_2NH, K_2CO_3, DMF, rt; (e) $EtNH_2$, $NaBH(OAc)_3$, CH_2Cl_2, rt, overnight.

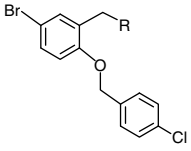


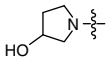
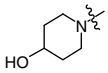
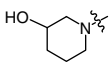
Scheme 2. Preparation of urea analogs. Reagents and conditions: (a) $Me_3S^+I^-$, KO^{t}Bu, DMSO, rt, 6 h, 94%; (b) in sealed tube, NH_4OH, MeOH, 60 °C, 5 h, 81%; (c) RNCO, CH_2Cl_2, Et_3N, rt, 2 h.

The compounds used to explore the SAR of different simple amines are outlined in Table 1. The compounds with small substituents (**6c–d**) had similar activity to **2**, but **6e**, with a larger substituent had decreased activity. The compounds with a primary or secondary amine also had similar activity (**6a** and **6b**, respectively). Pyrrolidine derivative **6f** also had similar activity to the morpholine analog **2**, however, both piperazine-derived compounds were inactive (**6g–h**). Improved activity was seen for hydroxy and keto substituted pyrrolidine and piperidine analogs (**6i–l**). The 4-hydroxypiperidine analog, **6k**, gave the best result with a 20-fold improvement in potency over **2**.

The exciting discovery of **6k** led us to further explore this position to seek the optimal substituent on the piperidine (Table 2). Replacement of the hydroxy with an alkoxy (**7a**) and fluorine (**7b**) decreased potency 10- and 5-fold, respectively. Replacement with a nitrogen containing substituent had variable effects. Compounds containing a basic nitrogen (**7c**, **7d**, and **7g**) lost greater than 25-fold potency, but the piperazine, **7h**, only lost 7-fold. The amide and carbamate (**7e** and **7f**) were more potent than the respective amine, but still less potent than **6k**. The ester and acid (**7i** and **7j**) also lost significant activity.

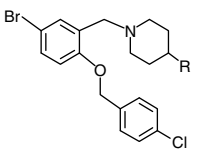
Table 1. SAR study of amine



Compound	R	IC_{50}[a] (μM)
6a	H_2N–	1.7
6b	EtNH–	1.1
6c	Me_2N–	0.6
6d	Et_2N–	1.4
6e	MeBnN–	10.1
6f		1.3
2		1.0
6g		>30
6h		>30
6i		0.15
6j		0.35
6k		0.049
6l		0.15

[a] Inhibition of ^{125}I-labeled MIP-1α binding to human CCR5/CD4 transfected HEK-293 cells.

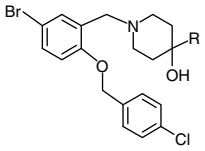
Table 2. Piperidine derivatives

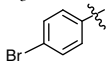
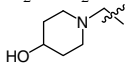
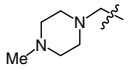


Compound	R	IC_{50}[a] (μM)
6k	HO–	0.049
7a	EtO–	0.45
7b	F–	0.28
7c	H_2N–	4.7
7d	EtHN–	3.1
7e	AcHN–	0.39
7f	MeO(CO)NH–	0.4
7g	H_2NCH_2–	1.3
7h		0.35
7i	EtO_2C–	1.3
7j	HO_2C–	8.5

[a] Inhibition of ^{125}I-labeled MIP-1α binding to human CCR5/CD4 transfected HEK-293 cells.

Table 3. 4-Piperidine derivatives



Compound	R	IC_{50}[a] (μM)
6k	H–	0.049
9a	F_3C–	0.58
9b		4.8
9c	H_2NCH_2–	0.58
9d		1.1
9e		0.21

[a] Inhibition of ^{125}I-labeled MIP-1α binding to human CCR5/CD4 transfected HEK-293 cells.

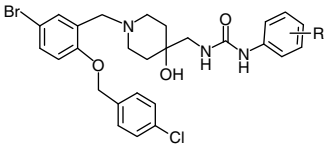
These results suggest that the hydroxyl group plays an important role in the binding of this series of compounds to the CCR5 receptor. In subsequent compounds we retained the hydroxyl group at the 4-position of the piperidine and introduced additional substituents (Table 3). Unfortunately, all of the tertiary alcohols initially examined displayed greatly reduced activity. Addition of a trifluoromethyl or aromatic group (**9a** and **9b**) caused a loss of 10-fold in activity. Addition of an aminomethyl function (**9c**–**e**) also decreased activity, but the range of results obtained with various substitutions suggested the possibility for further optimization.

The free amino group in **9c** provided a handle for further functionalization and potential optimization by library production. Therefore a library of ureas was prepared from **9c** (181 compounds). Several compounds from this library gave >90% inhibition at a concentration of 0.25 μM. Resynthesis of these hits gave the results in Table 4. Compared with the free amine **9c**, the 4-F-phenyl urea (**10a**) was 3-fold less potent, but the 2,6-dihalo-substituted phenyl ureas had up to 10-fold higher potency (**10b** and **10c**). 2,4,6-Trisubstituted phenyl ureas had lower potency than the corresponding 2,6-disubstituted phenyl ureas (**10d**–**f**). The most potent compounds were 2,6-dialkyl or dialkoxy substituted phenyl ureas (**10g**–**j**) with the diethyl compound, **10h**, having an IC_{50} of 11 nM.

Further characterization was performed on two key compounds: **6k** and **10h**. Plasma concentrations of both compounds after oral administration at 2 mg/kg (**6k**) and 5 mg/kg (**10h**) were below the low limit of quantification. The profiles of plasma concentration versus time of both compounds are shown in Figures 1 and 2, respectively. Compound **6k** was evaluated in rats by dosing 0.5 mg/kg iv and 2 mg/kg po. Unfortunately, this compound showed a short terminal phase half-life (0.72 h) and high clearance (146 mL/min/kg). The pharmacokinetics study of **10h** in rats by dosing 1 mg/kg iv and 5 mg/kg po revealed high clearance (60 mL/min/kg) and an elevated volume of distribution (22 L/kg). Compound **10h** was found to be highly metabolized by human liver microsomes with 11% of the parent compound remaining after a 1 h incubation.

In conclusion, initial variation of the amine moiety of this series resulted in the discovery of compound **6k**. Further optimization of **6k** led to the key intermediate **9c**. Using automated chemical synthesis, a urea library was prepared from intermediate **9c** containing compounds with high potency. The urea library led to the discovery of a potent CCR5 antagonist (**10h**), but further work on this series was terminated due to its poor pharmacokinetic properties.

Table 4. 4-Hydroxypiperidine urea analog



Compound	R	IC_{50}[a] (μM)
10a	4-F	1.5
10b	2,6-di F	0.32
10c	2,6-di Cl	0.057
10d	2,4,6-tri Cl	0.45
10e	4-Br-2,6-di Me	0.17
10f	2,4,6-tri Me	0.10
10g	2,6-di Me	0.047
10h	2,6-di Et	0.011
10i	2,6-di ^{i}Pr	0.035
10j	2,6-di MeO	0.027

[a] Inhibition of ^{125}I-labeled MIP-1α binding to human CCR5/CD4 transfected HEK-293 cells.

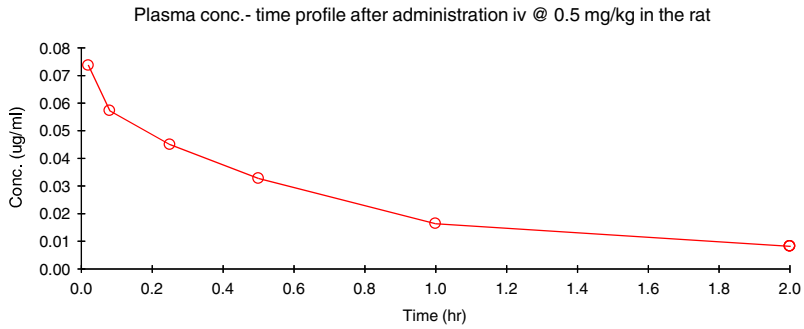


Figure 1. Plasma concentrations–time profile of **6k** after iv administration at 0.5 mg/kg in the rat. PK (rat): $t_{1/2}$ = 0.72 h; $AUC_{(all)}$ = 0.056 μg h/mL; V_z = 9.1 L/kg, Cl = 146 mL/min/kg.

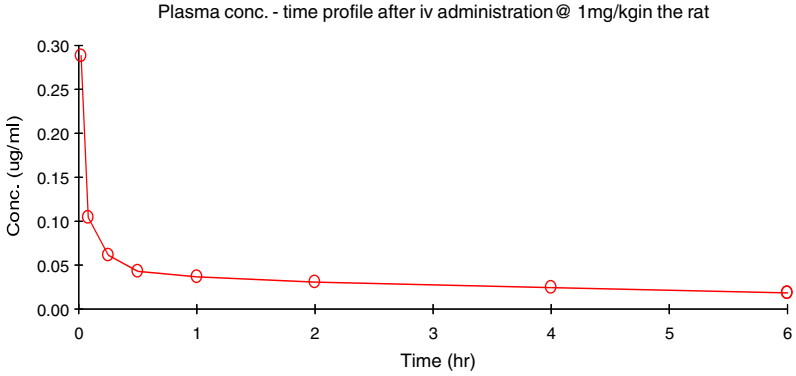


Figure 2. Plasma concentrations–time profile of **10h** after iv administration at 1 mg/kg in the rat. PK (rat): $t_{1/2}$ = 4.3 h; $AUC_{(all)}$ = 0.326 μg h/mL; V_z = 22 L/kg, Cl = 60 mL/min/kg.

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