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Bioorganic & Medicinal Chemistry Letters 17 (2007) 231–234
Bioorganic & Medicinal Chemistry Letters

CCR5 receptor antagonists: Discovery and SAR study of guanylhydrazone derivatives

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Received 31 August 2006; revised 16 September 2006; accepted 19 September 2006
Available online 1 November 2006

Abstract—High throughput screening (HTS) led to the identification of the guanylhydrazone of 2-(4-chlorobenzyloxy)-5-bromobenzaldehyde as a CCR5 receptor antagonist. Initial modifications of the guanylhydrazone series indicated that substitution of the benzyl group at the para-position was well tolerated. Substitution at the 5-position of the central phenyl ring was critical for potency. Replacement of the guanylhydrazone group led to the discovery of a novel series of CCR5 antagonists.
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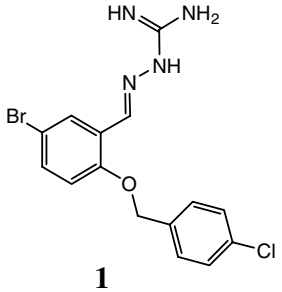
Chemokines are a large family of chemotactic proteins that play an important role in the immune and inflammatory response of various diseases and disorders including asthma and allergic disease as well as autoimmune diseases such as rheumatoid arthritis (RA). Chemokines regulate leukocyte activation and recruitment to sites of inflammation via interaction with a family of GPCRs, the chemokine receptors. The chemokine receptor CCR5 functions physiologically as a receptor for the leukocyte chemoattractants RANTES, MIP-1α, and MIP-1β, and it has also recently been shown to function pathologically as one of the key cell entry co-receptors for HIV-1.[1] In lesions of multiple sclerosis (MS), CCR5 has been detected on activated myeloid microglial cells and infiltrating T cells. CCR5 antagonists might, therefore, be useful in suppressing the chronic inflammatory symptoms of this disease.

Since the discovery of CCR5 as a co-receptor for HIV-1 cell entry, there has been an increased effort in the pharmaceutical industry to develop CCR5 antagonists.[2] During the course of our work in this area, we became aware that another group screening for CCR5 antagonists had identified the same initial lead **1** as our group (Fig. 1).[3] This report indicated that the aminoguanidine group of this compound was required for potency. In contradiction to this conclusion,[3] we have demonstrated that the aminoguanidine functional group is not required and suitable replacements can be identified. Herein, we report our discovery and initial SAR of guanylhydrazones of 2-benzyloxybenzaldehyde as CCR5 receptor antagonists. Potent, orally available, small molecule CCR5 antagonists are potential therapeutic agents for the treatment of chronic inflammatory diseases and HIV.

From high throughput screening of our internal compound library using a ^{125}I-MIP-1α binding assay on a human CCR5/CD4 transfected HEK293 cell line, 14 compounds with IC_{50} values of less than 5 μM were identified. Among these, three compounds were shown



1

CCR5 binding: IC_{50} 0.84 μM
Ca^{2+} flux: IC_{50} 2.2 μM

Figure 1. CCR5 antagonist HTS lead **1**.

Keywords: CCR5; Chemokine receptor antagonists.
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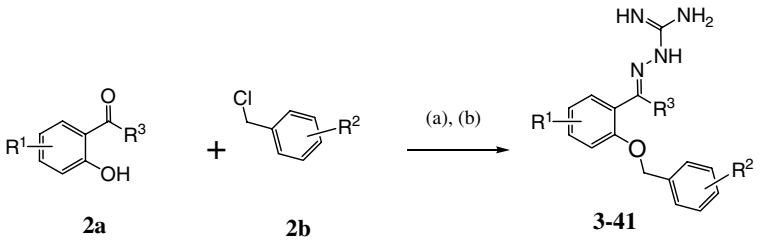
doi:10.1016/j.bmcl.2006.09.052



Scheme 1. Reagents and conditions: (a) Cs_2CO_3, DMF, 60°C, 2 h; (b) aminoguanidine hydrogencarbonate, EtOH, reflux.

to be functional antagonists of CCR5. Compound **1**, the guanylhydrazone of 2-benzyloxybenzaldehyde, was validated to be a moderately potent CCR5 antagonist, exhibiting an IC_{50} value of 0.84 and 2.2 μM in the binding and Ca^{2+} flux assays, respectively. These preliminary results prompted us to explore lead optimization and to develop a structure–activity relationship (SAR) for the series.

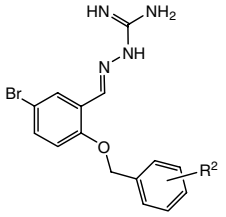
Guanylhydrazone analogs of our initial lead compound **1** were easily prepared in two steps (Scheme 1). Alkylation of the phenol (**2a**) with benzyl chloride or bromide (**2b**) using cesium carbonate provided the benzyloxy aryl ether. Treatment with aminoguanidine hydrogencarbonate led to the formation of guanylhydrazone **3**.

In an attempt to improve the potency of compound **1**, we first explored variations of the benzyl group (Table 1) with a series of substituted benzyloxy aryl ether derivatives. The unsubstituted compound **3** was slightly less potent than compound **1**. Different halogen substitutions had little effect on potency (**1**, **4**, and **5**). Compounds **8** and **9**, with nitro groups at the 3- or 4-position exhibited activity similar to **1**, whereas **10** with a nitro group at the 2-position showed sevenfold lower activity. Compound **6** with a 4-cyano group exhibited fourfold higher potency than **7** with a 3-cyano group. Compounds **11** and **12** with methyl ester groups at the 3- or 4-position were as potent as **1**, but **13** with a 3-carboxylic acid group was significantly less active. Electron-donating groups at the 4-position (**14**, **16**, and **17**) were well tolerated. Compound **14** with a 4-methyl group was threefold more potent than **15** with a 3-methyl group. Compound **18** with a 4-phenyl substituent was only twofold less potent than **1**. Disubstituted analogs were prepared and tested. The 2,4-difluoro analog, **20**, exhibited fourfold less inhibitory activity than the 4-fluoro analog, **4**. The 3-nitro-6-methoxy analog, **21**, exhibited fivefold less inhibitory activity than the 3-nitro analog, **9**. It was interesting that **19** with a fused benzene ring demonstrated potency comparable to the initial compound **1**. From these results, it was clear that substitution at the 4-position of the benzyl group was generally well tolerated and was more favorable than substitution at the 3-position.

We next examined substitution of the central aryl ring of the guanylhydrazone template (Table 2). Removal of the 5-bromo substituent from **1** afforded **22**, which was found to be inactive. Compound **23** with a 5-chloro substituent showed activity similar to **1**, but **25** with a 5-fluoro substituent was fourfold less active. Electron-withdrawing groups (Cl, F, NO_2, and CN) at the 5-position (**23**–**25**, and **29**) were tolerated. Larger substituents at the 5-position, methoxycarbonyl group (**32**) or electron-donating groups such as amino, diethylamino, and *t*-butyl (**34**, **33**, and **28**) yielded inactive compounds. Halogen substitution at the 3- or 4-position (**27**, **30**, and **31**) without substitution at the 5-position also resulted in a loss of inhibitory activity. The 3,5-dichloro substituted analog, **26**, was fourfold less potent than compound **1**, but the 5-bromo-3-methoxy substituted analog, **35**, was inactive. From the SAR study of the aryl ring, it could be determined that substitution at the 5-position with bromo, chloro, nitro or cyano group was preferred.

The preparation of ketone guanylhydrazones was also explored (Table 3). Guanylhydrazones of ketones,

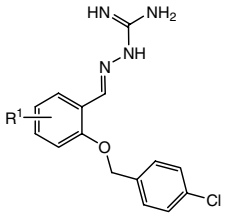
Table 1. Optimization of the benzyl group of guanylhydrazones



Compound	R^2	IC_{50}[a] (μM)
1	4-Cl	0.84
3	H	1.9
4	4-F	0.8
5	4-Br	0.6
6	4-CN	0.48
7	3-CN	2
8	4-NO_2	0.9
9	3-NO_2	1.7
10	2-NO_2	5.7
11	4-CO_2Me	1.3
12	3-CO_2Me	1.6
13	3-CO_2H	39
14	4-Me	0.5
15	3-Me	1.7
16	4-OMe	0.7
17	4-OBn	2.9
18	4-Ph	1.7
19	2,3- 	1.1
20	2,4-diF	3.7
21	3-NO_2-6-OMe	8.7

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

Table 2. Optimization of aryl group of guanylhydrazones

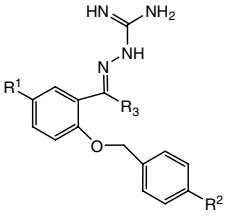


Compound	R^1	IC_{50}[a] (μM)
1	5-Br	0.84
22	H	>30
23	5-Cl	0.6
24	5-NO_2	1.2
25	5-F	3.4
26	3,5-diCl	3.1
27	3-F	>30
28	5-CMe_3	>30
29	5-CN	0.6
30	4-Cl	>30
31	3-Cl	>30
32	5-CO_2Me	>30
33	5-NEt_2	>30
34	5-NH_2	>30
35	3-OMe-5-Br	>30

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

36–**41**, were obtained as mixtures of *E*- and *Z*-isomers.[4] When R^3 was substituted by a methyl group, the *E*/*Z* ratio was around 19:1 by HPLC analysis. Methyl substituted compound **36** was fourfold more potent than **1**. The *E*-isomer, **37E**[5], was 60-fold more potent than the *Z*-isomer, **37Z**.[5] Compound **38** (R^2=CN) exhibited the highest activity in this series with an IC_{50} value of 0.093 μM. The ethyl substituted compound, **39**, showed twofold reduced potency relative to **36**. The *n*-Bu analog, **40**, was 20-fold less active than **36**. However, the *i*-Bu substituted analog, **41** was inactive. Both compounds **40** and **41**, contained additional substitutions at R^1 and R^2. These results indicated that the methyl group substitution was optimal at the R^3 position and the E-configuration was preferred.

Table 3. Optimization of guanylhydrazones of ketones

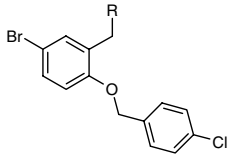


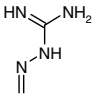
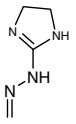
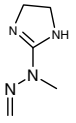
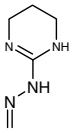
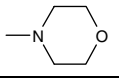
Compound	R^1	R^2	R^3	IC_{50}[a] (μM)
1	Br	Cl	H	0.84
36	Br	Cl	Me	0.2
37Z[5]	Cl	Cl	Me	8.5
37E[5]	Cl	Cl	Me	0.14
38	Br	CN	Me	0.093
39	Br	Cl	Et	0.4
40	Cl	F	*n*-Bu	4.2
41	Cl	F	*i*-Bu	>30

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

It was previously suggested[3] that the guanylhydrazone group was necessary for the activity of this series, as the corresponding benzaldehyde was inactive. The guanylhydrazone moiety is a basic group and may function as the positively charged moiety that is present in many CCR5 antagonists.[6] As such, we initially sought to replace this group with other similar functional groups. We then further explored replacement of the guanylhydrazone group with tertiary amines (Table 4). We found that the 4,5-dihydro-1*H*-imidazolyl guanylhydrazone **42** exhibited the same activity as analog **1**, while the corresponding *N*-methyl substituted guanylhydrazone, **43**, resulted in diminished activity by 20-fold. 1,4,5,6-Tetrahydropyrimidinyl guanylhydrazone, **44**, exhibited twofold less potent inhibitory activity than analog **1**. Reductive amination of the benzaldehyde with dimethylamine afforded compound **45**, which demonstrated similar activity to compound **1**. The *N*,*N*-diethyl analog **46**, and morpholine analog **47** also exhibited activity

Table 4. Guanylhydrazone replacement



Compound	R	IC_{50}[a] (μM)
1		0.84
42		0.75
43		14
44		2.0
45	NMe_2	0.6
46	NEt_2	1.4
47		1.0

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

similar to compound **1**. Based upon these results, it was clear that the presence of the guanylhydrazone group is not necessary for inhibitory activity and that this position of the molecule is amenable to further optimization efforts.

In conclusion, modification and optimization of the various groups of the initial HTS lead compound **1** led to the discovery of a new series of CCR5 antagonists. It was found that the guanylhydrazone moiety itself was not required for CCR5 antagonistic potency as suitable substitutions could be identified. Further investigations to improve the potency and explore the PK profile of novel CCR5 antagonists based upon compound **45** from this series will be reported in due course.

Acknowledgments

The authors thank Dr. Jerry Dallas for structural elucidation of compound **37E** and **37Z** using ^{13}C NMR and Dr. John Bauman for helpful suggestions and discussion.

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5. **37E** and **37Z** was separated by HPLC with solvent A, 0.1% TFA in H_2O; solvent B, 0.1% TFA in MeCN as eluents. The configuration of **37E** and **37Z** isomer could be clearly deduced by the comparison of their ^{13}C chemical shifts, specifically the methyl carbon atom. The ^{13}C chemical shift (δ = 18.0 ppm) of the methyl carbon atom in **37E** is upfield (δ = 24.4 ppm) of the methyl carbon atom in **37Z**. These data were consistent with those from reference 4.
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