Bioorganic & Medicinal Chemistry Letters 18 (2008) 5063–5065

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and evaluation of *cis*-3,4-disubstituted piperidines as potent CC chemokine receptor 2 (CCR2) antagonists

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ARTICLE INFO

Article history:
Received 14 July 2008
Revised 29 July 2008
Accepted 31 July 2008
Available online 5 August 2008

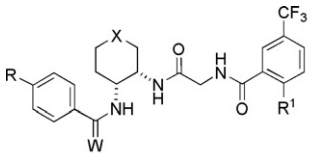
Keywords:
CCR2
CCR2 antagonist
Chemokine
GPCR

ABSTRACT

A series of *cis*-3,4-disubstituted piperidines was synthesized and evaluated as CC chemokine receptor 2 (CCR2) antagonists. Compound **24** emerged with an attractive profile, possessing excellent binding (CCR2 IC_{50} = 3.4 nM) and functional antagonism (calcium flux IC_{50} = 2.0 nM and chemotaxis IC_{50} = 5.4 nM). Studies to explore the binding of these piperidine analogs utilized a key CCR2 receptor mutant (E291A) with compound **14** and revealed a significant reliance on Glu291 for binding.

Chemokines are a large family of proteins involved in the activation and migration of leukocytes.[1] Our interest in chemokines has focused on a CC family member, monocyte chemoattractant protein-1 (MCP-1 or CCL2),[2] which is upregulated in many autoimmune and inflammatory conditions.[3] MCP-1 binds to its exclusive receptor, CC chemokine receptor 2 (CCR2),[4] which is a member of the G-protein coupled receptor (GPCR) family. The CCR2/MCP-1 pair has been implicated in a variety of diseases, including rheumatoid arthritis,[5] atherosclerosis,[6] multiple sclerosis,[7] and insulin resistance.[8] This has generated a great deal of interest in small molecule antagonists of CCR2 as potential therapeutic agents.[9] In this letter, we describe a novel series of disubstituted piperidines as potent CCR2 antagonists.

Recently, we described a series of disubstituted cyclohexanes **1** (Fig. 1) as selective CCR2 antagonists (see also derivative **2** in Table 1).[10] Looking to improve these antagonists, we became interested in the addition of a nitrogen within the cyclohexane to give a piperidine **3**. These piperidines were synthesized in racemic form, starting from dihydropyridine **4**, as shown in Scheme 1. Racemic epoxide **5** was produced via *m*-CPBA and was opened with sodium azide to give the regiomeric pair **6** and **7**.[11] This mixture was subjected to a hydrogenolysis (H_2, Pd/C) followed by carbamate formation and chromatography to afford the major isomer **8**. The secondary alcohol was then converted to an azide via a Mitsunobu reaction to yield **9**. This, in turn, was reduced to the amine **10** before it was coupled to the glycinamide **11** to give **12**. Hydrogenolysis gave the primary amine **13**, which was taken to either a target amine **14** or a target amide **15**. As shown in Scheme 2, for piperidine nitrogen substitution, **12** was treated with TFA to provide amine **16**. Selective alkylation with crotyl bromide (or other electrophiles) and subsequent hydrogenolysis gave **17**. As before, target amines **18** were produced via a reductive amination, and amides **19** were produced by a coupling reaction.



1 X = CH_2; W = O or H,H; R and R^1 = varied
3 X = NR^2; R, W, R^1 see Table 1 and 2

Figure 1. Exploration of piperidines as CCR2 antagonists.

The newly synthesized piperidines were evaluated using a radiolabeled MCP-1 displacement assay in peripheral blood mononuclear cells (PBMCs).[12] We were interested in selective CCR2 antagonists, and hence used a CCR3 binding assay[13] for an initial assessment of selectivity. Highly active compounds were also evaluated in two functional assays, the calcium flux assay[12] based on PBMCs and a chemotaxis assay[12] based on monocytes. With these highly active compounds, we also verified that the compounds did not induce calcium flux when incubated alone in PBMCs (without MCP-1 present), thus validating the compounds as antagonists and

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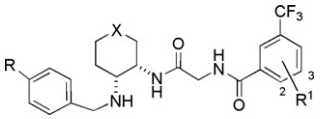
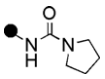
doi:10.1016/j.bmcl.2008.07.123

Table 1
Evaluation of benzylamine piperidines derivatives[a]



Compound	R	X	R^1	IC_{50}[b] (nM)			CCR3 binding % inh. at 10 μM[d]
				CCR2 binding	Ca flux	CTX mono[c]	
2	4-SMe	CH_2	2-NH_2	1180 ± 130 (3)	NT	NT	NT
14	4-SMe	NH	2-NH_2	6.3 ± 5.2 (2)	7.0 ± 1.4 (2)	285 ± 3.5 (2)	47.3
20	4-Cl	NH	2-NH_2	21.5 ± 7.7 (2)	19 (1)	NT	90
21	4-SEt	NH	2-NH_2	123 ± 21.2 (2)	NT	NT	NT
22	4-SMe	NH	H	97 ± 48.1 (2)	510 ± 70.7 (2)	NT	46
23	4 SMe	NMe	2-NH_2	0.95 ± 0.2 (2)	5.0 (1)	27.5 ± 12 (2)	56
24	4-SMe	NPr	2-NH_2	3.4 ± 2.3 (2)	2.0 (1)	5.4 ± 0.5 (2)	47
18	4 SMe	NBu	2-NH_2	7.9 ± 2.7 (2)	13.0 (1)	19.0 (1)	75
25	4-SMe	NPr	2-NHCONHMe	3.8 ± 1.1 (2)	3.0 (1)	8.0 ± 2.9 (2)	5.5 ± 3.9 (2)
26	4 SMe	NPr	2-NHCOpyrrolidine	8.7 ± 0.3 (2)	21 (1)	4.8 ± 0.6 (2)	38.2 ± 0.8 (2)
27	4-SMe	NPr	3-NH_2	6.1 ± 1.1 (2)	3.0 (1)	26.0 ± 21.6 (3)	49.3
28	4 SMe	NAc	H	463 ± 117.4 (2)	NT	NT	NT
29	4-SMe	NBoc	H	0% at 1 μM	NT	NT	NT

[a] Compounds are racemic, one enantiomer is displayed for illustrative purposes.
[b] IC_{50} values (n) are displayed as mean ± SD ($n = 2$) and mean ± SEM ($n > 2$).
[c] CTX mono, chemotaxis in monocytes.
[d] CCR3 % inhibition are $n = 1$, unless otherwise noted. NT, not tested. 2-NHCOpyrrolidine =



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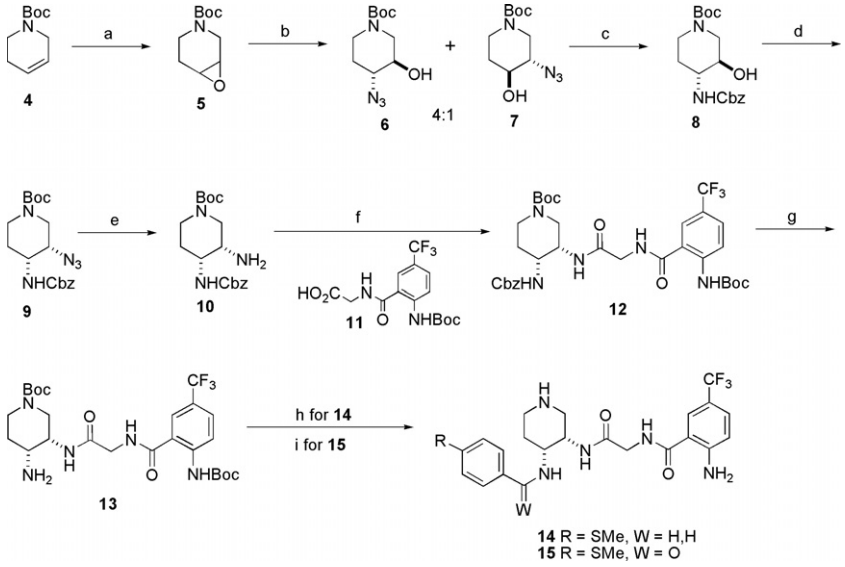
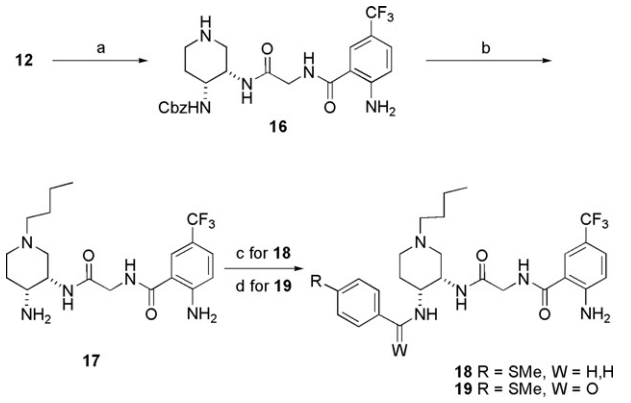


Scheme 1. Reagents and conditions: (a) *m*-CPBA, CH_2Cl_2, 0 °C; (b) NaN_3, NH_4Cl, MeOH/H_2O, Δ; (c) i—H_2, Pd/C, MeOH; ii—Cbz_2O, TEA, THF/H_2O; iii—separation of diastereomers; (d) HN_3, DEAD, PPh_3, THF/PhH, 5 °C; (e) PPh_3, H_2O, THF, 65 °C; (f) **11**, NMM, HATU, DMF; (g) H_2, Pd/C, MeOH; (h) i—4-methylthiobenzaldehyde, $NaBH_3CN$, $ZnCl_2$, MeOH; ii—TFA; (i) i—4-methylthiobenzoic acid, NMM, HATU, DMF; ii—TFA.

not partial agonists. As shown in Table 1, the addition of a nitrogen to form the piperidine was very beneficial. When compared to the cyclohexyl derivative **2**,[10] piperidine **14** displayed a 180-fold improvement in binding affinity for CCR2. Piperidine **14** also had excellent activity in the calcium flux assay ($IC_{50} = 7$ nM) and the chemotaxis assay (28.5 nM), along with good selectivity over CCR3. In order to optimize this CCR2 activity, we examined other areas of piperidine **14**. The methyl sulfide substituent was optimized in the cyclohexyl series,[10] and again proved to be sensitive toward substitution as shown with the chloro-derivative **20** (CCR2 $IC_{50} = 21.5$ nM) and the ethyl sulfide **21** (CCR2 $IC_{50} = 123$ nM). The 2-amino substituent[14] of the benzamide was known to be important for binding affinity and functional activity,[10,15] and its removal in compound **22** (CCR2 $IC_{50} = 97$ nM) proved to be detrimental when compared to **14**. As shown by derivatives **18**, **23**, and **24**, the CCR2 affinity was retained by substituting the piperidine nitrogen with small alkyl groups. This could be effectively matched with urea substitutions on the benzamide (derivatives **25** and **26**) or the incorporation of a 3-amino group (derivative **27**). However, conversion of the piperidine nitrogen to a non-basic moiety was unfavorable as observed for acetamide **28** (CCR2 $IC_{50} = 463$ nM) and carbamate **29** (CCR2 $IC_{50} > 1000$ nM).

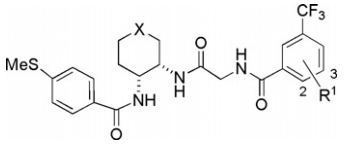


Scheme 2. Reagents and conditions: (a) TFA, CH_2Cl_2; (b) i—crotyl bromide, K_2CO_3, CH_3CN; ii—H_2, Pd/C, MeOH; (c) 4-methylthiobenzaldehyde, $NaBH_3CN$, $ZnCl_2$, MeOH; (d) 4-methylthiobenzoic acid, NMM, HATU, DMF.

Unlike the piperidine nitrogen, the benzyl amine could be converted to an amide and retain significant CCR2 affinity. As shown in Table 2, the unsubstituted piperidine derivative **15** lost only 4-fold in CCR2 affinity when compared to its benzyl amine analog **14**. The amide derivatives with small *N*-alkyl piperidines were essentially equipotent to their benzyl amine counterparts as shown by *N*-butyl **19**, *N*-methyl **30**, and *N*-propyl **31**. As before, these derivatives lost all CCR2 activity when a carbamate was installed on the piperidine nitrogen (see derivative **32**).

Given the 180-fold enhancement in CCR2 binding affinity of piperidine **14** as compared to the cyclohexyl derivative **2**, it was suspected that the piperidine nitrogen of **14** was making a new contact within the CCR2 receptor. In order to gain additional insight into this interaction, we examined the binding of piperidine **14** in a CCR2 receptor mutant. Of the GPCRs, chemokine receptors are unique in that they contain a conserved glutamic acid (Glu) in transmembrane domain-7 (TM-7).[16] In CCR2, this is Glu291, which has been shown to be critical for small molecule antagonist binding via site-directed receptor mutagenesis with the Glu291 to Ala291 mutant (E291A).[17] Piperidine **14** was examined in wild-type CCR2 and the E291A mutant in order to assess the involvement of Glu291 in its binding (Table 3). As evident from the 123-fold change in binding between wild-type CCR2 and the E291A mutant, piperidine **14** had a significant reliance on Glu291 for its binding affinity. A second derivative, **15**, was examined in the E291A mutant, and it also displayed a large reliance on Glu291 (32-fold shift, Table 3). This represents a departure from the cyclohexyl derivatives, which did not have a large reliance on Glu291 when examined in the E291A mutant, as reported previously.[10]

Table 2
Evaluation of benzamide piperidine derivatives[a]



Compound	X	R^1	IC_{50}[b] (nM)			CCR3 binding % inh. at 10 μM[d]
			CCR2 binding	Ca flux	CTX mono[c]	
15	NH	2-NH_2	28 ± 7.1 (2)	75 ± 0.7 (2)	109 ± 58.0 (2)	19.7
19	NBu	2-NH_2	4.3 ± 0.73 (3)	13 (1)	17.5 ± 2.1 (2)	77
30	NMe	2-NH_2	1.8 ± 0.3 (2)	2 (1)	38.5 ± 9.2 (2)	43.1
31	NPr	3-NH_2	6.2 ± 0.9 (2)	6.0 (1)	25.0 ± 19.8 (2)	NT
32	NBoc	2-NHBoc	0% at 1 μM	NT	NT	NT

[a] Compounds are racemic, one enantiomer is displayed for illustrative purposes.
[b] IC_{50} values (n) are displayed as mean ± SD (n = 2) and mean ± SEM (n > 2).
[c] CTX mono, chemotaxis in monocytes.
[d] CCR3 % inhibition are n = 1. NT, not tested.

Table 3
Evaluation of **14** and **15** versus the E291A CCR2 mutant

Compound	WT CCR2 IC_{50} [a] (nM)	E291A IC_{50} [a] (nM)	E291A fold change
14	6.1	749.3	123
15	12	380.8	32

[a] IC_{50} values are n = 1.

In conclusion, we have described the synthesis and evaluation of novel piperidine derivatives as potent CCR2 antagonists. As observed for the comparison of cyclohexane **2** to piperidine **14**, addition of the piperidine nitrogen alone can afford a significant enhancement in CCR2 affinity. From a binding study with the E291A CCR2 mutant, this enhanced affinity appears to be attributed to an interaction with Glu291 within the CCR2 receptor.

Acknowledgments

We thank Mr. Dayton T. Meyer and Ms. Ruowei Mo for several of the benzoic acids used in this work. We also thank Dr. Joel C. Barrish for a critical review of the manuscript.

References and notes

1. (a) D'Elios, M. M.; Del Prete, G.; Amedei, A. *Expert Opin. Ther. Patents* **2008**, *18*, 309; (b) Horuk, R. *ScientificWorldJournal* **2007**, *7*, 224; (c) Charo, I. F.; Ransohoff, R. M. *N. Eng. J. Med.* **2006**, *354*, 610.
2. (a) Allen, S. J.; Crown, S. E.; Handel, T. M. *Annu. Rev. Immunol.* **2007**, *25*, 787; (b) Murphy, P. M.; Baggiolini, M.; Charo, I. F.; Hebert, C. A.; Horuk, R.; Matsushima, K.; Miller, L. H.; Oppenheim, J. J.; Power, C. A. *Pharmacol. Rev.* **2000**, *52*, 145.
3. Daly, C.; Rollins, B. J. *Microcirculation* **2003**, *10*, 247.
4. Feria, M.; Diaz-Gonzalez, F. *Expert Opin. Ther. Patents* **2006**, *16*, 49.
5. Tak, P. P. *Best Pract. Res. Clin. Rheumatol.* **2006**, *20*, 929.
6. (a) Coll, B.; Alonso-Villaverde, C.; Joven, J. *Clin. Chim. Acta* **2007**, *383*, 21; (b) Peters, W.; Charo, I. F. *Curr. Opin. Lipidol.* **2001**, *12*, 175.
7. Mahad, D. J.; Ransohoff, R. M. *Semin. Immunol.* **2003**, *15*, 23.
8. Kamei, N.; Tobe, K.; Suzuki, R.; Ohsugi, M.; Watanabe, T.; Kubota, N.; Ohtsuka-Kowatari, N.; Kumagai, K.; Sakamoto, K.; Kobayashi, M.; Yamauchi, T.; Ueki, K.; Oishi, Y.; Nishimura, S.; Manabe, I.; Hashimoto, H.; Ohnishi, Y.; Ogata, H.; Tokuyama, K.; Tsunoda, M.; Ide, T.; Murakami, K.; Nagai, R.; Kadowaki, T. *J. Biol. Chem.* **2006**, *281*, 26602.
9. (a) Carter, P. H.; Cherney, R. J.; Mangion, I. K. *Annu. Rep. Med. Chem.* **2007**, *42*, 211; (b) Dawson, J.; Miltz, W.; Mir, A. K.; Wiessner, C. *Expert Opin. Ther. Targets* **2003**, *7*, 35.
10. Cherney, R. J.; Mo, R.; Meyer, D. T.; Nelson, D. J.; Lo, Y. C.; Yang, G.; Scherle, P. A.; Mandlekar, S.; Wasserman, Z. R.; Jezak, H.; Solomon, K. A.; Tebben, A. J.; Carter, P. H.; Decicco, C. P. *J. Med. Chem.* **2008**, *51*, 721.
11. (a) Marquis, R. W.; Ru, Y.; Zeng, J.; Trout, R. E. L.; LoCastro, S. M.; Gribble, A. D.; Witherington, J.; Fenwick, A. E.; Garnier, B.; Tomaszek, T.; Tew, D.; Hemling, M. E.; Quinn, C. J.; Smith, W. W.; Zhao, B.; McQueney, M. S.; Janson, C. A.; D'Alessio, K.; Veber, D. F. *J. Med. Chem.* **2001**, *44*, 725; (b) D'Andrea, S. V.; Michalson, E. T.; Freeman, J. P.; Chidester, C. G.; Szmuszkovicz, J. *J. Org. Chem.* **1991**, *56*, 3133.
12. Carter, P. H.; Cherney, R. J. Diamines as modulators of chemokine receptor activity. WO 2002050019, **2002**.
13. Wacker, D. A.; Santella, J. B., III; Gardner, D. S.; Varnes, J. G.; Estrella, M.; DeLucca, G. V.; Ko, S. S.; Tanabe, K.; Watson, P. S.; Welch, P. K.; Covington, M.; Stowell, N. C.; Wadman, E. A.; Davies, P.; Solomon, K. A.; Newton, R. C.; Trainor, G. L.; Friedman, S. M.; Decicco, C. P.; Duncia, J. V. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1785.
14. Moree, W. J.; Kataoka, K.; Ramirez-Weinhouse, M. M.; Shiota, T.; Imai, M.; Tsutsumi, T.; Sudo, M.; Endo, N.; Muroga, Y.; Hada, T.; Fanning, D.; Saunders, J.; Kato, Y.; Myers, P. L.; Tarby, C. M. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1869.
15. Carter, P. H.; Brown, G. D.; Friedrich, S. R.; Cherney, R. J.; Tebben, A. J.; Lo, Y. C.; Yang, G.; Jezak, H.; Solomon, K. A.; Scherle, P. A.; Decicco, C. P. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5455.
16. Rosenkilde, M. M.; Schwartz, T. W. *Curr. Top. Med. Chem.* **2006**, *6*, 1319.
17. (a) Berkhout, T. A.; Blaney, F. E.; Bridges, A. M.; Cooper, D. G.; Forbes, I. T.; Gribble, A. D.; Groot, P. H. E.; Hardy, A.; Ife, R. J.; Kaur, R.; Moores, K. E.; Shillito, H.; Willetts, J.; Witherington, J. *J. Med. Chem.* **2003**, *46*, 4070; (b) Mirzadegan, T.; Diehl, F.; Ebi, B.; Bhakta, S.; Polsky, I.; McCarley, D.; Mulkins, M.; Weatherhead, G. S.; Lapierre, J.-M.; Dankwardt, J.; Morgans, D., Jr.; Wilhelm, R.; Jarnagin, K. *J. Biol. Chem.* **2000**, *275*, 25562.