Bioorganic & Medicinal Chemistry Letters 19 (2009) 597–601

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Discovery of trisubstituted cyclohexanes as potent CC chemokine receptor 2 (CCR2) antagonists

Robert J. Cherney [a,*], John B. Brogan [b], Ruowei Mo [a], Yvonne C. Lo [a], Gengjie Yang [a], Persymphonie B. Miller [a], Peggy A. Scherle [a], Bruce F. Molino [b], Percy H. Carter [a], Carl P. Decicco [a]

[a] Research and Development, Bristol-Myers Squibb Company, Princeton, NJ 08543-4000, USA
[b] AMRI, 26 Corporate Circle, PO Box 15098, Albany, NY 12212, USA

ARTICLE INFO

Article history:
Received 12 November 2008
Revised 12 December 2008
Accepted 16 December 2008
Available online 24 December 2008

Keywords:
CCR2 antagonist
Chemokines
MCP-1

ABSTRACT

A series of trisubstituted cyclohexanes was designed, synthesized and evaluated as CC chemokine receptor 2 (CCR2) antagonists. This led to the identification of two distinct substitution patterns about the cyclohexane ring as potent and selective CCR2 antagonists. Compound **36** exhibited excellent binding (CCR2 IC_{50} = 2.4 nM) and functional antagonism (calcium flux IC_{50} = 2.0 nM and chemotaxis IC_{50} = 5.1 nM).

Chemokines are endogenous proteins typically produced in small amounts to control the activation and migration of leukocytes.[1] However, in many autoimmune and inflammatory conditions chemokines are overexpressed, and hence continue to recruit inflammatory cells into the tissues and joints.[1] We have been interested in monocyte chemoattractant protein-1 (MCP-1 or CCL2), a CC chemokine,[2] which elicits a functional response by binding to its G-protein coupled receptor (GPCR), CC chemokine receptor 2 (CCR2).[3] With high levels of MCP-1 and CCR2 in many diseases, the pair is thought to play a role in rheumatoid arthritis,[4] atherosclerosis,[5] multiple sclerosis[6] and insulin resistance.[7] As a result, there has been significant interest in the design and synthesis of CCR2 antagonists as potential therapeutic agents.[8] In this communication, we describe our discovery and initial structure–activity relationship (SAR) study of trisubstituted cyclohexanes as novel CCR2 antagonists.

Recently, we described a series of disubstituted cyclohexanes and piperidines (Fig. 1, representative compounds **1** and **2**, respectively) as selective CCR2 antagonists.[9] Some piperidines were as much as 180-fold more active than the corresponding disubstituted cyclohexanes, and the piperidine nitrogen was suspected of contacting the glutamic acid residue at position 291 within the CCR2 receptor.[9b] In an attempt to optimize and explore this, we began an investigation of trisubstituted cyclohexanes **3** with an amino group in the 4- or 5-position, as shown in Figure 1.

The trisubstituted cyclohexanes **12–16** were synthesized in racemic form as shown in Scheme 1. Starting from the known racemic *cis*-epoxide **4**,[10] the epoxide was opened with sodium azide to give **5**. The azide was treated with triphenylphosphine and water to afford an amine, which was converted to carbamate **6**. The benzyl and trifluoroacetamide groups were removed and were replaced with a Cbz carbamate to furnish **7**. Inversion of the secondary alcohol to azide **8** was accomplished via a Mitsunobu reaction. The azide was converted to amine **9**, which was capped as benzamide **10**. The glycinamide was installed in one operation to give **12** or **13**, depending on the R^1 group of **11**. Compound **13** was deprotected with acid to yield **14**, which was converted to urea **15** via treatment with methyl isocyanate. The *iso*-propyl amine **16** was produced from **14** by a reductive amination.

As shown in Scheme 2, the synthesis of the racemic *cis*-targets **24–28** utilized an analogous sequence as above, however it started with racemic *trans*-epoxide **17**.[10]

Targets with substitution at the 4-position were realized by direct glycinamide coupling to racemic intermediate **9** (see Scheme 3). The resulting *bis*-amide **29** was converted to target **30** via carbamate removal and benzamide coupling. In addition, compound **30** was deprotected to **31**, which in turn was converted to **32** via the previously described reductive amination.

Utilizing the above strategy, racemic intermediate **22** was converted to benzamide **34** (see Scheme 4). Deprotection of **34** and glycinamide installation gave either **35** or **37**, depending on the R^1 of **11**. Compounds **36** and **38** were then produced via the deprotection of **35** and **37**, respectively.

* Corresponding author. Tel.: +1 609 252 3066; fax: +1 609 252 6601.
E-mail address: robert.cherney@bms.com (R.J. Cherney).

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.12.062

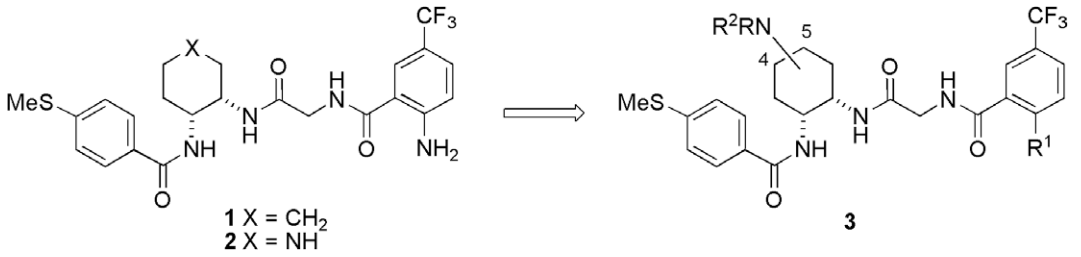
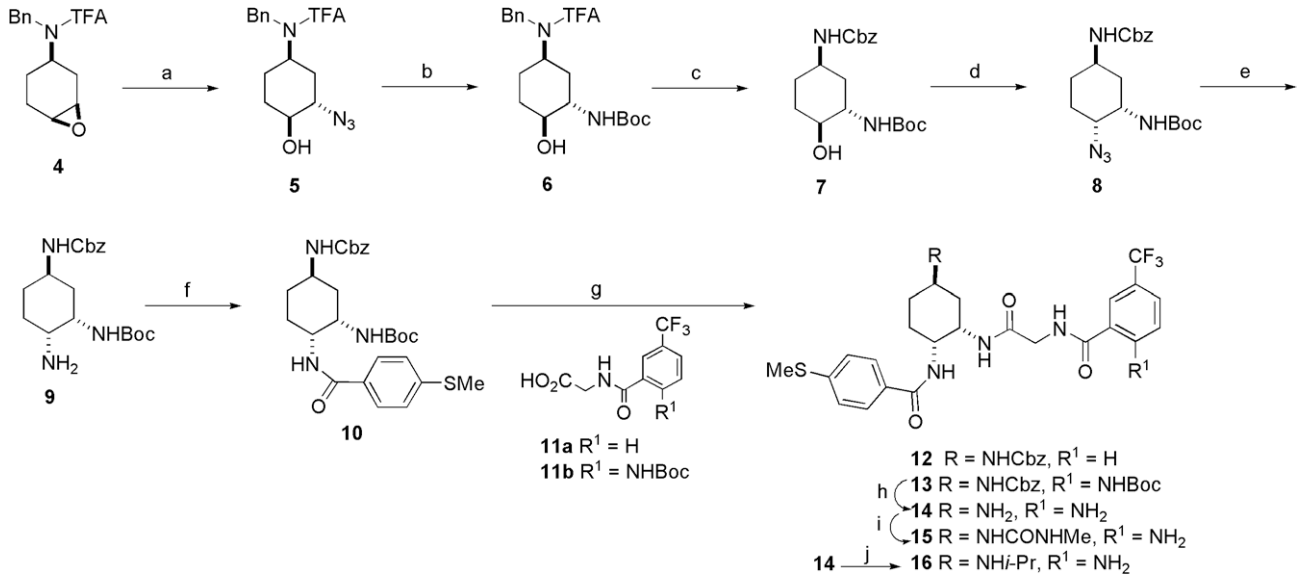
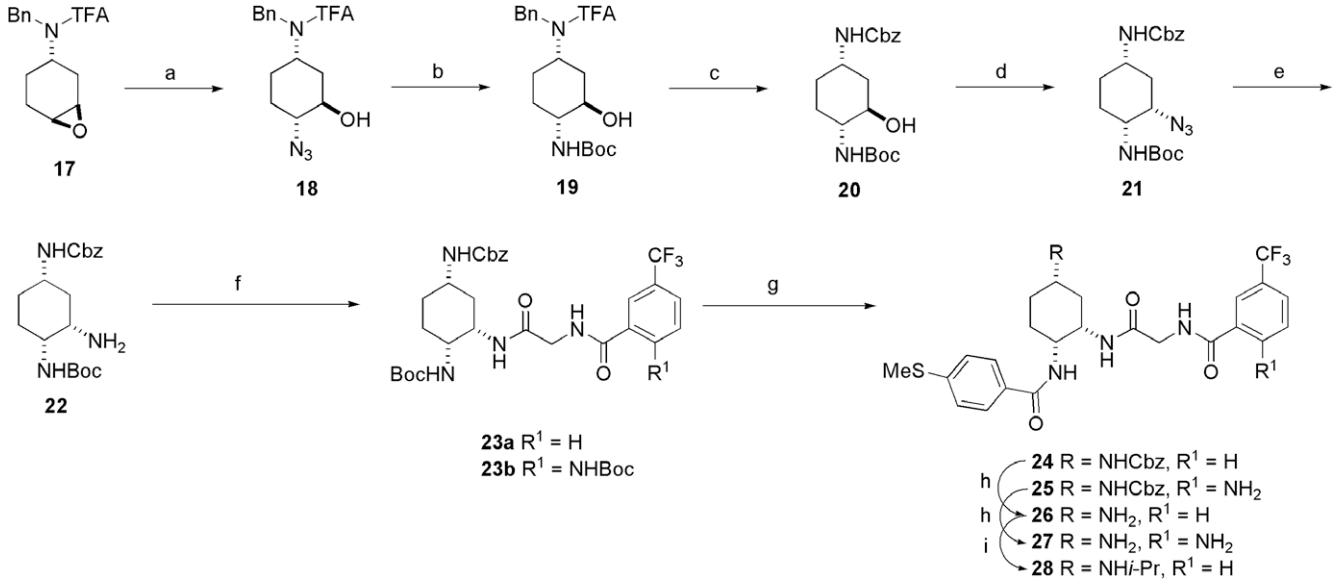


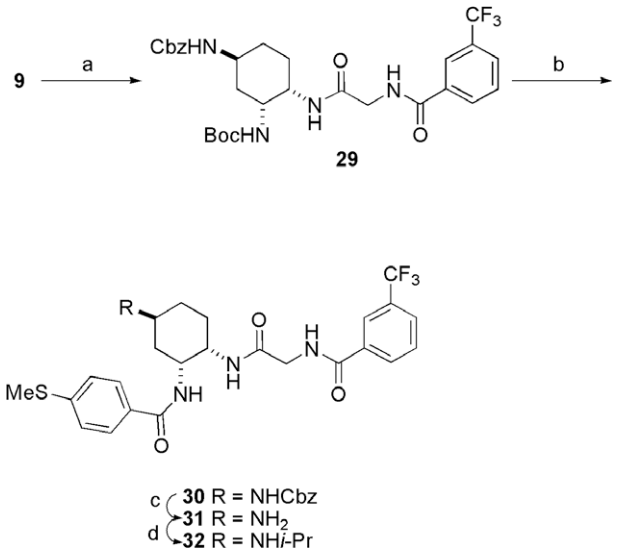
Figure 1. Proposed exploration of trisubstitued cyclohexanes **3** as CCR2 antagonists.



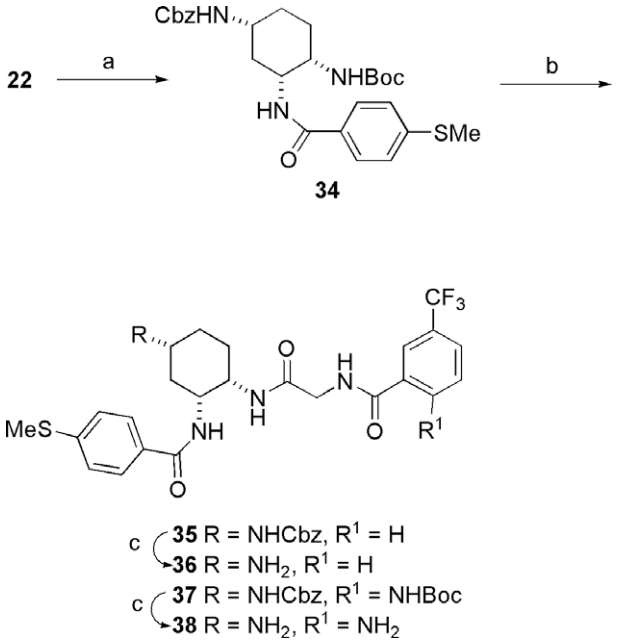
Scheme 1. Reagents and conditions: (a) NaN_3, MeOH/H_2O, Δ, 74%; (b) i—PPh_3, H_2O; ii—$(Boc)_2O$, $NaHCO_3$, THF/H_2O, 82% for two steps; (c) i—KOH, H_2O, MeOH; ii—H_2(50 psi), 5% Pd/C, MeOH; iii—CbzCl, $NaHCO_3$, THF/H_2O, quant; (d) HN_3, DEAD, PPh_3, THF/PhH, 5 °C, 93%; (e) PPh_3, H_2O, THF, 79%; (f) 4-(thiomethyl)benzoic acid, $(i\text{-Pr})_2$NEt, HATU, DMF, 94%; (g) i—TFA, CH_2Cl_2; ii—**11a** or **11b**, $(i\text{-Pr})_2$NEt, HATU, DMF, 87% for **13**; (h) 38% HBr/HOAc, 69%; (i) MeNCO, $(i\text{-Pr})_2$NEt, CH_2Cl_2, 27%; (j) acetone, $HC(OMe)_3$, $NaBH(OAc)_3$, 37%.



Scheme 2. Reagents and conditions: (a) NaN_3, MeOH/H_2O, Δ, 57%; (b) i—PPh_3, H_2O; ii—$(Boc)_2O$, $NaHCO_3$, THF/H_2O, 80% for two steps; (c) i—KOH, H_2O, MeOH; ii—H_2(50 psi), 5% Pd/C, MeOH; iii—CbzCl, $NaHCO_3$, THF/H_2O, 88%; (d) HN_3, DEAD, PPh_3 THF/PhH, 5 °C, 82%; (e) PPh_3, H_2O, THF, 90%; (f) **11a** or **11b**, $(i\text{-Pr})_2$NEt, HATU, DMF, 62% for **23b**; (g) i-TFA, CH_2Cl_2, ii—4-(thiomethyl)benzoic acid, $(i\text{-Pr}_2)$NEt HATU, DMF, 87%; (h) 38% HBr/HOAc; (i) actone, $HC(OMe)_3$, $NaBH(OAc)_3$, 68%.



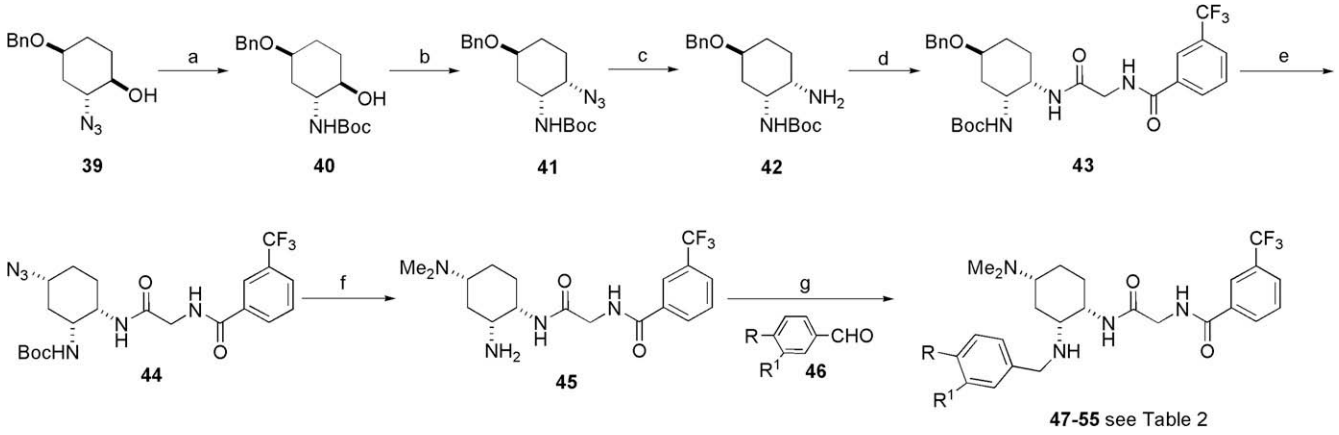
Scheme 3. Reagents and conditions: (a) **11a**, $(i\text{-Pr})_2$NEt, HATU, DMF, 86%; (b) i—TFA, CH_2Cl_2, ii—4-(thiomethyl)benzoic acid, $(i\text{-Pr})_2$NEt, HATU, DMF, 38%; (c) HBr/HOAc, 90%; (d) acetone, $HC(OMe)_3$, $NaBH(OAc)_3$, $NaBH(OAc)_3$, 81%.



Scheme 4. Reagents and conditions: (a) 4-(thiomethyl)benzonic acid, $(i\text{-Pr}_2)$NEt, HATU, DMF, 98%; (b) i—TFA, CH_2Cl_2 ; ii—**11a** or **11b**, $(i\text{-Pr})_2$NEt, HATU, DMF, 85%; (c) 38% HBr/HOAc, 48%.

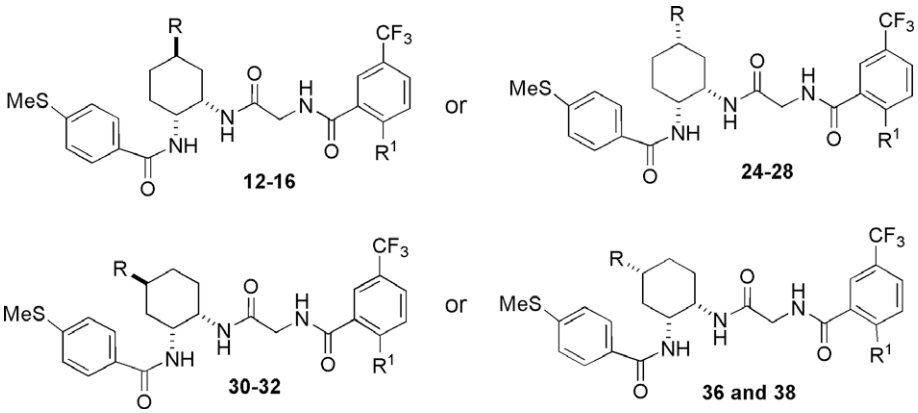
Benzyl amine targets were synthesized according to the sequence illustrated in Scheme 5. Starting with the known azido alcohol **39**,[11] reduction and carbamate formation were accomplished in one step to give **40**. The alcohol was converted to a mesylate prior to an azide displacement to afford **41**. Reduction to amine **42** was followed by glycinamide formation to provide **43**. After hydrogenolysis of the benzyl group, another alcohol to azide sequence gave **44**. The azide was reduced and was dimethylated before the final carbamate removal revealed **45**. The final targets **47–55** were realized via reductive amination of **45** with the appropriate aldehyde.

The trisubstituted cyclohexanes were evaluated in vitro, using peripheral blood mononuclear cells (PBMCs) in a radiolabeled MCP-1 displacement assay.[12] We were interested in selective CCR2 antagonists and initially used a CCR3 binding assay[13] for a quick assessment of selectivity. Compounds with CCR2 binding IC_{50}s <20 nM were also evaluated in two functional assays: a calcium flux assay[12,14] and a chemotaxis assay.[12] As shown in Table 1, the first compounds tested in the CCR2 binding assay, trisubstituted analogs **12** and **13**, did manage some affinity for CCR2, even though the carbamate groups were still in place. Removal of the carbamate group from **13** resulted in a 4-fold increase in CCR2 affinity for amine **14**. Two beneficial substitutions were the urea **15** and the *iso*-propyl **16**. Compound **16** was significant as it displayed a 41-fold increase and a 7-fold increase in affinity for CCR2 as compared to the previously described disubstituted cyclohexane **1**[9a] and piperidine **2**,[9b] respectively. Compound **16** also demonstrated excellent activity in the calcium flux assay while remaining selective against CCR3. In general, inversion of the 5-position (compounds **24–28**) did not improve affinity for CCR2, although the *iso*-propyl amine **28** displayed a CCR2 binding IC_{50} = 135.7 nM. The *trans*-compounds in the 4-position (**30**–**32**) were also lacking in CCR2 activity with the primary amine **31** (CCR2 IC_{50} = 227.5 nM) representing the best in this class. However, inversion at the 4-position gave the *cis*-compound **36**, which had surprising activity in binding (CCR2 IC_{50} = 2.4 nM), calcium (CCR2 IC_{50} = 2.0 nM), and chemotaxis (CCR2 CTX IC_{50} = 5.1 nM). The 2-amino compound **38** did serve as a direct comparison to



Scheme 5. Reagents and conditions: (a) H_2, $Pd(OH)_2$, $(Boc)_2O$, EtOAc, 89%; (b) i—MsCl, Et_3N, CH_2Cl_2, ii—NaN_3, CH_2Cl_2, DMSO, Δ, 65%; (c) H_2, 10% Pd/C, MeOH; (d) **11a**, BOP, NMM, DMF, 95%; (e) i—H_2 (50 psi), $Pd(OH)_2$, MeOH; ii—MsCl, Et_3N, CH_2Cl_2; iii—NaN_3, DMSO, Δ, 50%; (f) i—H_2, 10% Pd/C, MeOH; ii—37% HCHO, $NaBH_3CN$, MeOH, iii—TFA; (g) $NaBH(OAc)_3$, THF.

Table 1
Evaluation of trisubstituted cyclohexane derivatives[a]

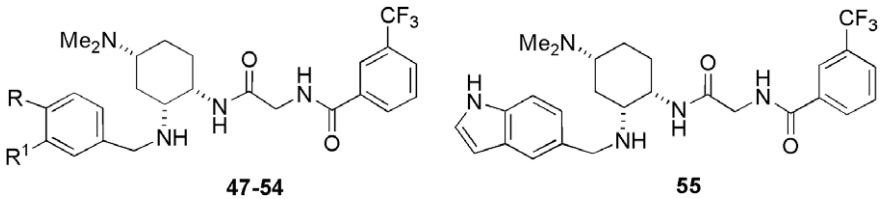


Compound	R	R^1	IC_{50}[b] (nM)			CCR3 binding %Inh at 10 μM[c]
			CCR2 binding	Ca^{2+} flux	Chemotaxis	
1	See Figure 1		155 ± 55.2 (2)	NT	NT	NT
2	See Figure 1		28 ± 7.1 (2)	75 ± 0.7 (2)	109 ± 58.0 (2)	19.7
12	NHCbz	H	1605.0 ± 572.0 (2)	NT	NT	NT
13	NHCbz	NHBoc	600.0 ± 247.5 (2)	NT	NT	NT
14	NH_2	NH_2	167.5 ± 74.2 (2)	NT	NT	NT
15	NHCONHMe	NH_2	112.0 ± 14.1 (2)	NT	NT	NT
16	NH*i*-Pr	NH_2	3.8 ± 1.1 (2)	2.0 (1)	NT	27.2 ± 4.9 (2)
24	NHCbz	H	0% at 1 μM	NT	NT	NT
25	NHCbz	NH_2	3.1% at 1 μM	NT	NT	NT
26	NH_2	H	13% at 1 μM	NT	NT	NT
27	NH_2	NH_2	28.1% at 1 μM	NT	NT	NT
28	NH*i*-Pr	H	135.7 ± 8.5 (4)	NT	NT	NT
30	NHCbz	H	14% at 1 μM	NT	NT	NT
31	NH_2	H	227.5 ± 34.6 (2)	NT	NT	NT
32	NH*i*-Pr	H	31.4% at 1 μM	NT	NT	NT
36	NH_2	H	2.4 ± 0.9 (3)	2.0 (1)	5.1 ± 3.8 (2)	7.0 ± 6.2 (2)
38	NH_2	NH_2	4.8 ± 2.5 (2)	6.0 (1)	13.5 ± 8.2 (3)	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] CCR3% inhibition are n = 1, unless otherwise noted; NT, not tested.

cyclohexane **1** and piperidine **2**, where compound **38** displayed 32-fold and 6-fold more CCR2 affinity than the disubstituted compounds, respectively. Significantly, the 2-amino-benzamide compound **38** did not improve CCR2 functional antagonism as compared to compound **36**. In this regard, compound **36** is unusual, as previous studies[9a,15] have shown a reliance on the 2-amino benzamide group (of the glycinamide) for potent functional activity.

Table 2
Evaluation of trisubstituted cyclohexane derivatives[a]



Compound	R	R^1	IC_{50}[b] (nM)		
			CCR2 binding	Ca^{2+} flux	Chemotaxis
47	SMe	H	2.4 ± 0.6 (2)	6.0 (1)	22.5 ± 7.8 (2)
48	Me	H	9.9 ± 8.5 (2)	5.5 ± 0.7 (2)	12.6 ± 4.8 (3)
49	*i*-Pr	H	16.5 ± 0.7 (2)	19 (1)	NT
50	OMe	H	12.5 + 0.7 (2)	8.0 (1)	41.4 ± 26.6 (4)
51	Cl	H	16.0 ± 0.0 (2)	7.0 (1)	NT
52	Cl	Cl	61.5 ± 4.9 (2)	NT	NT
53	H	CF_3	32.5 ± 17.7 (2)	27 (1)	NT
54	NHAc	H	7.7 ± 3.0 (2)	NT	17.4 ± 4.5 (6)
55	See structure		3.1 ± 1.1 (2)	8.0 (1)	23.0 ± 17.0 (2)

[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). NT, not tested.

As a result of this activity, we investigated benzyl amines, as shown in Table 2. The 4-thiomethyl of **47** remained one of the better substituents, although 4-methyl **48** and 4-*N*-acetamide **54** only lost 4-fold in affinity for CCR2 as compared to **47**. Other substituents at the 4-position (**49**, **50**, **51**) lost additional affinity as compared to **47**. Substitution as 3,4-dichloro (**52**) or 3-trifluoromethyl (**53**) was not favorable; however the indole **55** was approximately equipotent to **47** in both binding and functional activity.

In summary, we have described the use of selective epoxide openings as a unified approach toward the synthesis of four new trisubstituted scaffolds as novel CCR2 antagonists. All four substitution patterns afforded CCR2 antagonists with binding $IC_{50} < 250$ nM. A substantial enhancement in CCR2 binding affinity was observed for two of the trisubstituted scaffolds (see compounds **16** and **36**) as compared to the disubstituted cyclohexane antagonists reported earlier. Significantly, compound **36** displayed potent functional antagonism of CCR2 without resorting to the large lipophilic groups needed in previously described studies.

Acknowledgment

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. *Sci. World J.* **2007**, *7*, 224; (c) Charo, I. F.; Ransohoff, R. M. *N Eng. J. Med.* **2006**, *354*, 610.
2. For chemokine nomenclature, see: 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. (a) Daly, C.; Rollins, B. J. *Microcirculation* **2003**, *10*, 247; (b) Feria, M.; Diaz-Gonzalez, F. *Expert Opin. Ther. Patents* **2006**, *16*, 49.
4. Tak, P. P. *Best Pract. Res. Clin. Rheumatol.* **2006**, *20*, 929.
5. (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.
6. Mahad, D. J.; Ransohoff, R. M. *Semin. Immunol.* **2003**, *15*, 23.
7. 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.
8. a Carter, P. H.; Cherney, R. J.; Mangion, I. K. Annual Reports in Medicinal Chemistry. **2007,** 42, 211.; (b) Dawson, J.; Miltz, W.; Mir, A. K.; Wiessner, C. *Expert Opin. Ther. Targets* **2003**, *7*, 35.
9. (a) 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; (b) Cherney, R. J.; Nelson, D. J.; Lo, Y. C.; Yang, G.; Scherle, P. A.; Jezak, H.; Solomon, K. A.; Carter, P. H.; Decicco, C. P. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5063.
10. Fletcher, S. R.; Baker, R.; Chambers, M. S.; Herbert, R. H.; Hobbs, S. C.; Thomas, S. R.; Verrier, H. M.; Watt, A. P.; Ball, R. G. *J. Org. Chem.* **1994**, *59*, 1771.
11. Chini, M.; Crotti, P.; Flippin, L. A.; Macchia, F. *J. Org. Chem.* **1991**, *56*, 7043.
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. For all compounds tested in the calcium flux assay, calcium flux inhibition was unchanged when the compound was incubated alone (without MCP-1 present), thus validating the compound as an antagonist and not a partial agonist.
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.