Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 14 (2004) 5413–5416
Bioorganic & Medicinal Chemistry Letters

Small molecule antagonists of the CCR2b receptor. Part 2: Discovery process and initial structure–activity relationships of diamine derivatives

Wilna J. Moree,[a,*] Ken-ichiro Kataoka,[b] Michele M. Ramirez-Weinhouse,[a,†] Tatsuki Shiota,[b] Minoru Imai,[b] Masaki Sudo,[b,‡] Takaharu Tsutsumi,[b] Noriaki Endo,[b] Yumiko Muroga,[b] Takahiko Hada,[b,§] Hiroko Tanaka,[b] Takuya Morita,[b] Jonathan Greene,[a] Doug Barnum,[a] John Saunders,[a,¶] Yoshinori Kato,[b] Peter L. Myers[a,‖] and Christine M. Tarby[a,**]

[a]*Deltagen Research Laboratories,[††] 4570 Executive Drive, Suite 400, San Diego, CA 92121, USA*
[b]*Teijin Institute for Bio-medical Research, 4-3-2 Asahigaoka, Hino, Tokyo 191-8512, Japan*

Received 6 October 2003; accepted 5 August 2004
Available online 16 September 2004

Abstract—Structure–activity relationships (SAR) of a weakly active class of CCR2b inhibitors were utilized to initiate a lead evolution program employing the Drug Discovery Engine™. Several alternative structural series have been discovered that display nanomolar activity in the CCR2b binding and CCR2b-mediated chemotaxis assays.

Chemokines (chemotactic cytokines) are a family of small molecular weight proteins involved in a variety of inflammatory responses via the chemoattraction and activation of leukocytes.[1] Monocyte chemoattractant protein-1 (MCP-1) is a 8–10 kDa protein belonging to the β- or CC chemokine subfamily and is postulated to be primarily responsible for the selective recruitment of leukocytes from the circulation to the site of inflammation[2] by binding to its 7-transmembrane G protein-coupled receptor (CCR2b) on the surface of monocytes and macrophages. Both CCR2b[3] and MCP-1[4] knockout mice as well as animal model studies with *anti*-MCP-1 have indicated that therapeutic intervention at the CCR2b receptor may have beneficial effects in a variety of inflammatory diseases including rheumatoid arthritis,[5] atherosclerosis,[6] glomerulonephritis,[7] and multiple sclerosis.[8] For this reason, we and others[9] have initiated programs to develop small molecule CCR2b antagonists. Screening of the Teijin sample collection, followed by hit to lead efforts, led to the identification of lead series **1** and **2** (Fig. 1).[10]

Despite optimization efforts, using both traditional medicinal chemistry and library-based approaches to identify potent CCR2b inhibitors in this series, binding

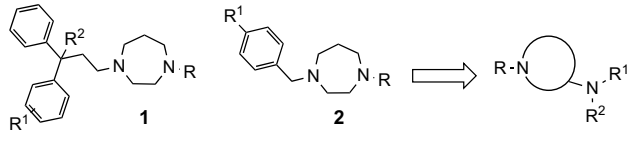


Figure 1. Homopiperazine lead series and alternative structural series.

* Corresponding author at present address: Neurocrine Biosciences, Inc., 12790 El Camino Real, San Diego, CA 92130, USA. Tel.: +1 858 617 7506; fax: +1 858 617 7998; e-mail: wmoree@neurocrine.com
† Present address: Pfizer Global Research and Development, La Jolla Laboratories, 3550 General Atomics Court, San Diego, CA 92121, USA.
‡ Present address: Pfizer Global Research and Development, Nagoya Laboratories, 5-2 Taketoyo, Aichi 470-2393, Japan.
§ Present address: Bizen Chemical Co., Ltd, Research and Development Department, 363-Tokutomi, Kumayama-Cho, Akaiwa-Gun, Okayama 709-0716, Japan.
¶ Present address: see *.
‖ Present e-mail address: myerspl@mindspring.com
** Present address: Bristol-Myers Squibb Company, PO Box 4000, Princeton, NJ 08543, USA.
†† Formerly CombiChem, Inc.

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.08.009

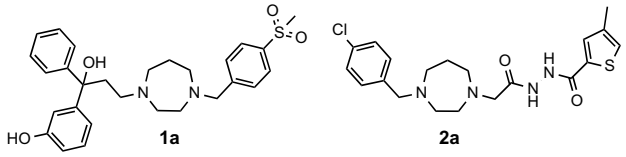
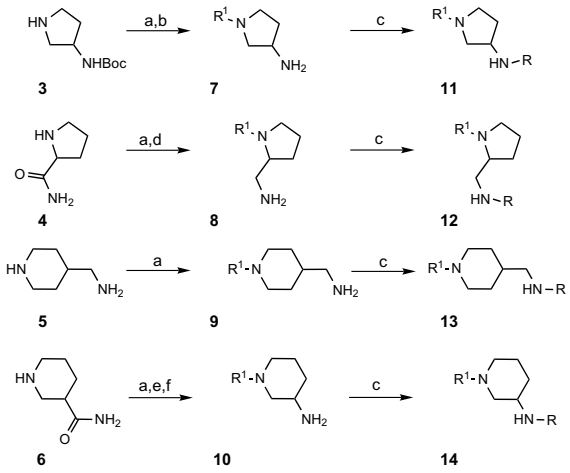


Figure 2. Most potent compounds in homopiperazine series **1** and **2**.

affinity remained in the micromolar range. These results required us to evolve away from this homopiperazine core and to develop alternative structural series. Our Drug Discovery Engine™ was utilized to assist in this lead evolution process,[11] leading to the discovery of several alternative diamine derived series with nanomolar activity in the CCR2b binding and CCR2b chemotaxis assays.

As described in our previous paper,[10] optimization of the homopiperazine series, using either traditional medicinal chemistry techniques or through library-based approaches, led to the identification of CCR2b antagonists with μM binding affinities. The most potent compounds identified in series **1** and **2** were **1a** with an IC_{50} value of 0.71 μM and **2a** with an IC_{50} value of 7.4 μM, respectively (Fig. 2). Although approximately 800 compounds were prepared in the homopiperazine series, further improvement of activity could not be established and alternative structural series were investigated using a process called the Drug Discovery Engine™.

For this purpose, screening data generated from the homopiperazine series together with screening data from ~200 known GPCR ligands were used to generate a computational model, composed of an ensemble of pharmacophore 'hypotheses'.[11b] Each of these pharmacophores contained two–four important features and was selected since they distinguished active from inactive compounds. The hypotheses were employed to filter a virtual library, which was computationally constructed by subjecting 20 different cores to a variety of in-silico reactions that would be applicable to high-throughput parallel synthesis. The cores and reactions were chosen such that the resulting compounds would contain a basic nitrogen, which was found to be a prerequisite for activity in the homopiperazine series as well as for most series described in the literature.[9a–c] The side chains were derived from commercially available reagents. In addition, side chains determined in the optimization of the homopiperazine series, such as the *p*-chlorobenzyl, benzhydryl, and 4-methyl-thiophene-2-carboxylic acid hydrazide, were included to allow for a direct comparison between templates. Examples of some of the reactions utilized were alkylation, acylation, sulfonylation, epoxide opening, Grignard addition, and (thio)urea formation. Generic examples of virtual library members **11**–**14** are shown in Scheme 1. Templates and chemistries were prioritized based on a relative score against the pharmacophore hypotheses[11b] as well as chemical feasibility. To facilitate high-throughput chemistry, high scoring compounds around each core were grouped and divided in subgroups based on a common substituent. The second substituent was varied using hypothesis matching entities as well as entities, which would probe a developing SAR. From the virtual library containing 83,000 members, a set of 820 compounds was synthesized around 11 different cores. Only the diamine derivatives **11**–**14** will be described herein, since the majority of active compounds were identified in those series (vide infra).



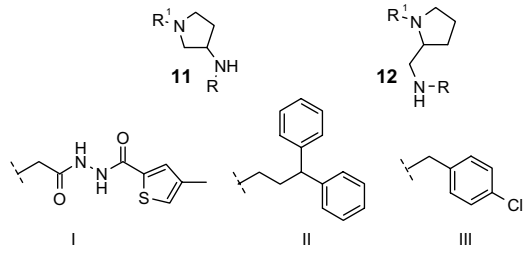
Scheme 1. Reagents and conditions: (a) R^1Cl or R^1Br, K_2CO_3, MeCN, 16 h, Δ; (b) TFA; (c) R^2CO_2H, EDC, HOBt, DIPEA, or R^2SO_2Cl, DIPEA, or R^2NCO, DIPEA, or R^2NCS, DIPEA, or RCl, or RBr, DIPEA; (d) BH_3; (e) Br_2, NaOH; (f) 20% HCl, Δ.

For the high-throughput parallel library synthesis of diamine derivatives **11**–**14**, intermediates **7**–**10** were prepared by alkylation of starting materials **3**–**6**. For 4-aminomethylpiperidine **5**, selective alkylation on the secondary nitrogen was achieved by the use of a large excess of diamine to generate intermediate **9**. Alkylated prolinamide required an additional reduction step with borane to give intermediate **8**, whereas alkylated 3-(Boc-amino)pyrrolidine was treated with TFA to remove the Boc group to give **7**. Finally, alkylated nipecotamide was subjected to a Hofmann rearrangement, followed by hydrolysis of the formed isocyanate to give intermediate **10**.

All intermediates **7**–**10** were either alkylated using alkyl halides, acylated with acids using EDC/HOBt coupling conditions, reacted with sulfonylchlorides, or treated with (thio)isocyanates to give the library compounds **11**–**14**, where R is variably alkyl, $-COR^2$, $-SO_2R^2$, $-CONHR^2$, or $-CSNHR^2$. A variety of purification techniques was employed, which included acid–base extractions, ion exchange chromatography, resin scavenging techniques and silica gel column chromatography. Compounds that were less than 85% pure were subsequently subjected to reverse phase HPLC/MS purification using mass triggered fraction collection.[12]

The biological activity of the compounds was initially assessed in a radioligand binding assay using human ^{125}I-MCP-1 and THP-1 cells.[13] The results for selected compounds are summarized in Tables 1–3.

Table 1. Binding affinities of dialkylated diamines to CCR2b



Compound	R^1	R	IC_{50} (μM)[a]
11a	**II**	**I**	33
11b	**III**	**I**	41
11c	**I**	**II**	17
11d	**I**	**III**	7.8 ($n = 3$)[b]
12a	**I**	**II**	26

[a] Assayed with ^{125}I-MCP-1 ($n = 1$, unless indicated otherwise).
[b] SEM = 2.2 μM.

The new diamine cores substituted with the same combination of alkyl side chains as used on the homopiperazine core in various orientations led in some cases to equipotent compounds (i.e., **11c–d**, **12a**) indicating that the homopiperazine core could be replaced (Table 1).

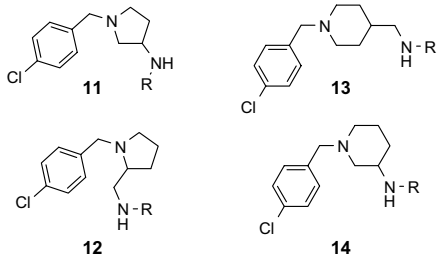
Table 3. Chemotaxis to MCP-1 compared to binding affinity

Compound	Binding to CCR2b IC_{50} (μM)	Chemotaxis to MCP-1 EC_{50} (μM)[a]
11i(*S*)	1.20	0.360 ± 0.09
11i(*R*)	0.18	0.024 ($n = 2$)
12i(*S*)	3.10	0.630 ± 0.17
12i(*R*)	0.57	0.810 ± 0.268
13i	0.70	0.043 ($n = 2$)
14i	0.66	0.610 ± 0.153

[a] Ave ($n = 3$) ± SEM unless indicated otherwise.

Substitution of one of the alkyl side chains by a sulfonamide or a (thio)urea resulted in very weak to inactive compounds for all the templates (data not shown). Although replacement of one of the alkyl side chains by an amide eliminated activity in the homopiperazine series (data not shown), this was not true for some of the other diamine templates. Both 3-aminopyrrolidine and 2-aminomethylpyrrolidine derivatives **11e** and **12e** maintained micromolar activity containing a 5-oxo-5-phenyl pentanoyl amide sidechain (Table 2). Modification of this side chain to a *N*-(carbamoylethyl) benzamide did not affect activity for most templates (**11f**, **12f**, **13f**, **14f**). It was rationalized that a contracted side chain such as *N*-(carbamoylmethyl) benzamide could

Table 2. IC_{50} values (μM) of diamine derivatives for CCR2b receptor



R	IC_{50} (μM)[a] series **11**	IC_{50} (μM)[a] series **12**	IC_{50} (μM)[a] series **13**	IC_{50} (μM)[a] series **14**
e	11[c] ($n = 5$)	19 ($n = 2$)	50	87
f	22	40 ($n = 2$)	64	74
g	42	66 ($n = 2$)	11 ($n = 2$)	NA[b]
h	6.9 ($n = 2$)	14 ($n = 2$)	5.5 ($n = 2$)	17
i	0.37, (*S*) 1.2, (*R*) 0.18 ($n = 2$)	2.3, (*S*) 3.1, (*R*) 0.57	0.7[d] ($n = 12$)	0.66

[a] Assayed with ^{125}I-MCP-1 ($n = 1$, unless indicated otherwise).
[b] Not active.
[c] SEM = 2.7 μM.
[d] SEM = 0.08 μM.

improve activity for the more extended template **13** compared to **11e**. Indeed binding affinity of compound **13g** was significantly better than of **13e** or **13f**. Substitution of the aromatic ring in *N*-(carbamoylmethyl) benzamide side chain with a *m*-methyl group (**13h**) improved activity further by 2-fold and with a *m*-trifluoromethyl (**13i**) by 12-fold. This trend was observed for all the diamine templates **11**–**14**. The substitution effect was most dramatic for the 3-aminopyrrolidine derivatives (**11**) and 3-aminopiperidine derivatives (**14**). In both series binding affinity improved approximately 100-fold by substitution of the *N*-(carbamoylmethyl) benzamide in **11g**, **14g** with a *m*-CF_3 group (**11i**, **14i**). In series **12** this effect was less pronounced and only a 29-fold difference in binding affinity was observed between **12g** containing *N*-(carbamoylmethyl) benzamide and **12i** with an additional *m*-CF_3 substituent. The observation that *N*-(carbamoylmethyl)-3-trifluoromethyl benzamide has a similar effect on binding affinity of all series is somewhat surprising since series **11**, **12**, and **14** exhibit a constrained ethylene diamine core, but this structural motif is conspicuously absent in series **13**.

Chiral resolution indicated a preference for the *R* enantiomer over the *S* enantiomer in both series **11** and **12**. Compounds **11i**(*R*) and **12i**(*R*) were 5- to 7-fold more active than **11i**(*S*), and **12i**(*S*). Compounds containing the *N*-(carbamoylmethyl)-3-trifluoromethyl benzamide side chain were not only potent CCR2b binders but also showed potent inhibition of cell chemotaxis caused by MCP-1 using THP-1 cells as the chemotactic cell line.[14] The results are summarized in Table 3. In addition, potent compounds in series **11** inhibited chemokine induced Ca^{2+} immobilization in a calcium flux $[Ca^{2+}]_i$ assay (compounds and data not shown). The effect of all potent compounds in series **11**–**14** on CCR1 was measured using [^{125}I]-MIP-1α and THP cells indicating a moderate selectivity, for example, 49% inhibition at 10 μM was observed for **11i**(*R*) ($IC_{50} > 5\,\mu M$), corresponding to a ~30-fold selectivity on the related chemokine receptor.

In conclusion, we described a lead evolution process that led to the generation of alternative structural series to the homopiperazines as CCR2b antagonists. A synergistic approach between computational library design and traditional medicinal chemistry in high-throughput format led to the identification of four novel lead series that displayed submicromolar activity in CCR2b binding and chemotaxis assays. Interestingly, the *N*-(carbamoylmethyl)-3-trifluoromethyl benzamide side chain led to a dramatic increase in binding affinity in all series shown in Table 3. Optimization of the lead series and detailed SAR studies will be the subject of our next communication.

Acknowledgements

The authors wish to thank Dr. Dan Kassel, Dr. Lu Zeng, and Ms. Xiaoli Wang for their technical expertise in setting up systems for HPLC purification of compounds described in this paper and Dr. Takeshi Hara and Dr. Seizi Kurozumi for general support.

References and notes

1. (a) Rollins, B. J. *Blood* **1997**, *90*, 909; (b) Howard, O. M. Z.; Ben-Baruch, A.; Oppenheim, J. J. *Trends Biotechnol.* **1996**, *14*, 46; (c) Saunders, J.; Tarby, C. M. *Drug Discovery Today* **1999**, *4*, 80; (d) Murphy, P. M.; Baggiolini, M.; Charo, I. F.; Hebert, C. A.; Horuk, R.; Matsushima, K.; Miller, L. H.; Oppenheim, J. J.; Power, C. A. *Pharmacologic. Rev.* **2000**, *52*, 145; (e) Horuk, R. *Cytokine Growth Factor Rev.* **2001**, *12*, 313.
2. (a) Rollins, B. J.; Morrison, E. D.; Stiles, C. D. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 3738; (b) Charo, I. F.; Myers, S. J.; Herman, A.; Franci, C.; Connolly, A. J.; Coughlin, S. R. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 2752.
3. Boring, L.; Gosling, J.; Chensue, S. W.; Kunkel, S. L.; Farese, R. V., Jr.; Broxmeyer, H. E.; Charo, I. F. *J. Clin. Invest.* **1997**, *100*, 2552.
4. Lu, B.; Rutledge, B. J.; Gu, L.; Fiorillo, J.; Lukacs, N. W.; Kunkel, S. L.; North, R.; Gerard, C.; Rollins, B. J. *J. Exp. Med.* **1998**, *187*, 601.
5. (a) Ogata, H.; Takeya, M.; Yoshimura, T.; Takagi, K.; Takahashi, K. *J. Pathol.* **1997**, *182*, 106; (b) Gong, J.-H.; Ratkay, L. G.; Waterfield, J. D.; Clark-Lewis, I. *J. Exp. Med.* **1997**, *186*, 131.
6. (a) Boring, L.; Gosling, J.; Cleary, M.; Charo, I. F. *Nature* **1998**, *394*, 894; (b) Dawson, T. C.; Kuziel, W. A.; Osahar, T. A.; Maeda, N. *Atherosclerosis* **1999**, *143*, 205.
7. Lloyd, C. M.; Minto, A. W.; Dorf, M. E.; Proudfoot, A.; Wells, T. N. C.; Salant, D. J.; Gutierrez-Ramos, J.-C. *J. Exp. Med.* **1997**, *185*, 1371.
8. Kennedy, K. J.; Strieter, R. M.; Kunkel, S. L.; Lukacs, N. W.; Karpus, W. J. *J. Neuroimmunol.* **1998**, *92*, 98.
9. (a) 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; (b) Forbes, I. T.; Cooper, D. G.; Dodds, E. K.; Hickey, D. M. B.; Ife, R. J.; Meeson, M.; Stockley, M.; Berkhout, T. A.; Gohil, J.; Groot, P. H. E.; Moores, K. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1803; (c) Witherington, J.; Bordas, V.; Cooper, D. G.; Forbes, I. T.; Gribble, A. D.; Ife, R. J.; Berkhout, T.; Gohil, J.; Groot, P. H. E. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2177; For short reviews, see: (d) Trivedi, B. K.; Low, J. E.; Carson, K.; LaRosa, G. J. *Ann. Reports Med. Chem.* **2000**, *35*, 191; (e) Howard, O. M. Z.; Yoshimura, T. *Expert Opin. Ther. Patents* **2001**, *11*(7), 1147.
10. Imai, M.; Shiota, T.; Kataoka, K.; Tarby, C. M.; Moree, W. J.; Tsutsumi, T.; Sudo, M.; Ramirez-Weinhouse, M. M.; Comer, D.; Sun, C.-M.; Yamagami, S.; Tanaka, H.; Morita, T.; Hada, T.; Greene, J.; Barnum, D.; Saunders, J.; Myers, P. L.; Kato, Y.; Endo, N. *Bioorg. Med. Chem. Lett.* **2004**, *14*, preceding paper. doi:10.1016/j.bmcl.2004.08.008.
11. (a) Myers, P. L.; Greene, J. W.; Saunders, J.; Teig, S. L. *Today's Chemist Work* **1997**, *6*(7), 46; (b) Bradley, E. K.; Beroza, P.; Penzotti, J. E.; Grootenhuis, P. D. J.; Spellmeyer, D. C.; Miller, J. L. *J. Med. Chem.* **2000**, *43*, 2770.
12. Zeng, L.; Kassel, D. B. *Anal. Chem.* **1998**, *70*, 4380.
13. See Ref. 10 in preceding paper.
14. Falk, W.; Goodwin, R. H., Jr.; Leonard, E. J. *J. Immunol. Methods* **1980**, *3*, see Ref. 11 in preceding paper.