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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 15 (2005) 5160–5164

Novel CCR1 antagonists with oral activity in the mouse collagen induced arthritis

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Received 15 July 2005; revised 16 August 2005; accepted 22 August 2005
Available online 28 September 2005

Abstract—Cinnamides as novel CCR1 antagonist chemotypes are described with high affinity to human and rodent receptors. **A1B1** and **A4B7** showed oral activity in the mouse collagen induced arthritis.

The chemokine receptor CCR1 and its major ligands CCL3 (MIP-1α) and CCL5 (RANTES) are believed to play a role in the pathogenesis of several inflammatory diseases including rheumatoid arthritis,[1,2] multiple sclerosis[3,4] and transplant rejection.[5,6] Small molecules as CCR1 antagonists are expected to be of great therapeutic value in these indications. The first clinical proof-of-concept with rheumatoid arthritis patients confirmed that a small molecule CCR1 antagonist can have relevant biological effects: reduction in the number of macrophages and CCR1+ cells in the synovium and general improvement after 14 days of treatment.[7] Several chemotypes have been described as CCR1 antagonists: hydroxyethylene peptide isosteres,[8–11] 4-hydroxypiperidines,[12,13] benzylpiperazines (e.g., BX-471 in clinical trial for multiple sclerosis),[14–17] arylpiperazines[18] and xanthene-9-carboxamides.[19–21] Many CCR1 antagonists reported above lack species cross-reactivity, in particular for rodent CCR1 which may limit their use, since many disease models are run in mice and rats.

Here, we wish to report our work directed towards the transformation of a modestly cross-reactive lead structure into novel CCR1 antagonists with equally high potency for human (h), rat (r) and mouse (m) CCR1 and their oral activity in animal models of inflammation and multiple sclerosis. BX-471[14–16] was chosen as the lead structure with its p-fluorobenzyl substituent kept unchanged, while the aryloxy acetic acid moiety **A** and the piperazine ring of **B** were the targets for bioisosteric substitution by the fragments **A1**–**A11** and **B1**–**B8** (Scheme 1).

Compounds **A1B1** through **A1B8** in Table 1 were prepared by coupling **A1-OH**[17] with **H-B1** through **H-B8** using EDCI, HOBt as standard coupling conditions. **A3B1** and **A3B7** were prepared similarly from **A3-OH**.[17] **A2B1** and **A4B7** were prepared from precursor **1**[17] (Scheme 2).

A5B1 was prepared from **A1B1** via $NaBH_4$ reduction in EtOH. Intramolecular Michael addition during ester hydrolysis of the urea derivative of aniline **2**[17] (Scheme 3) generated a cyclic urea intermediate, which was coupled with **H-B1** to render **A7B1**.

A6B1 was prepared from chloromethyl ketone **4**,[18] which was converted via phthalimide into the primary amine **5** (Scheme 4). *O*-Bromo nitrobenzene **3** and **5** were heated to 130 °C and delivered the piperazine analogue **6** in modest yield. $SnCl_2$-reduction of the nitro group and acylation generated **A6B1**.

A8B1 and **A9B1** were obtained by coupling **A8-OH**[22] and **A9-OH**[23] with **H-B1** under standard conditions. **A10B1** and **A11B1** were prepared as described previously.[17] **A11B7** was synthesized in analogy to the former two compounds using **H-B7**. From the eight piperazine

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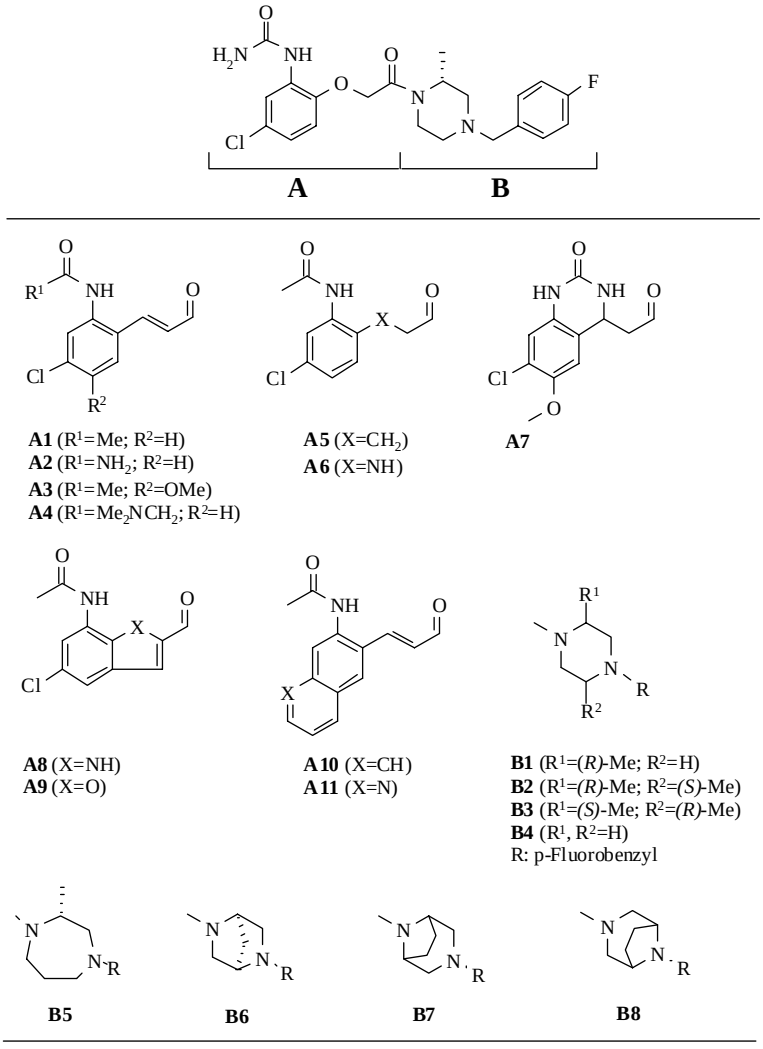
doi:10.1016/j.bmcl.2005.08.057



Scheme 1.

building blocks **H-B1** to **H-B8** (Scheme 1), **H-B4** was commercially available, while **H-B1**,[24] **H-B2**,[25] **H-B3**,[25] **H-B7**[26] and **H-B8**[26] were prepared according to literature procedures. **H-B6** was synthesized as described[27] using 4-fluorobenzylchloride. **H-B5** was obtained (Scheme 5) by coupling **13** with BOC-D-Ala-OH. The resulting product was deprotected under acidic conditions followed by cyclisation to **14** at pH 9. Reduction with BH_3 yielded the desired homopiperazine **H-B5**.

The ether functionality in BX-471 could favourably be substituted by the bioisosteric double bond in **A2B1**, which showed a 3- to 10-fold higher affinity for human, mouse and rat CCR1 (Table 1). The urea functionality of **A2B1** was replaced without loss of affinity by the acetyl group in **A1B1** and was kept through the whole series, assuming that the acetyl group was superior to the urea functionality considering pharmacokinetic properties. Among the piperazines **B1**–**B8** combined with **A1**, **A1B2** proved to be a potent hCCR1 antagonist with IC_{50} = 9 nM, but only modest affinities for the rodent receptors; its enantiomer **A1B3** was 10- to 100-fold weaker. Homopiperazine **A1B5** and methylene-bridged piperazine **A1B6** were considerably weaker with IC_{50}s of 1.6–1.7 μM. The unsubstituted piperazine derivative **A1B4** showed a 6-fold weaker potency than its methyl substituted analogue **A1B1**. Both ethylene-bridged piperazine fragments in **A1B7** and **A1B8** showed moderate IC_{50}s of 0.2–0.3 μM. However, in contrast to **B1**, **B7** had the remarkable property to increase the affinity for rodent receptors at least 10-fold, exemplified by the compounds **A3B7**, **A4B7** and **A11B7**. **A3B7** proved to be the most potent antagonist of rodent CCR1 within this series with IC_{50}s of 1 and 4 nM against mCCR1 and rCCR1. **A3B1**, on the other hand, had a slightly higher affinity for hCCR1, but was 10- to 20-fold weaker against rodent CCR1 compared to **A3B7**. It is noteworthy that the addition of a methoxy group in **A3** in comparison to **A1** resulted in a 10-fold higher affinity for human and rodent CCR1, exemplified by the pairs of compounds **A1B1**, **A3B1** and **A1B7**, **A3B7**. The dihydro analogue **A5B1** showed a 10-fold drop in affinity. The cyclic urea **A7B1** was weak with an IC_{50} = 0.8 μM, demonstrating either the wrong conformation of the urea group or the need for a specific conformation of a rigid side chain. Replacing the aryl ether in BX-471 by the

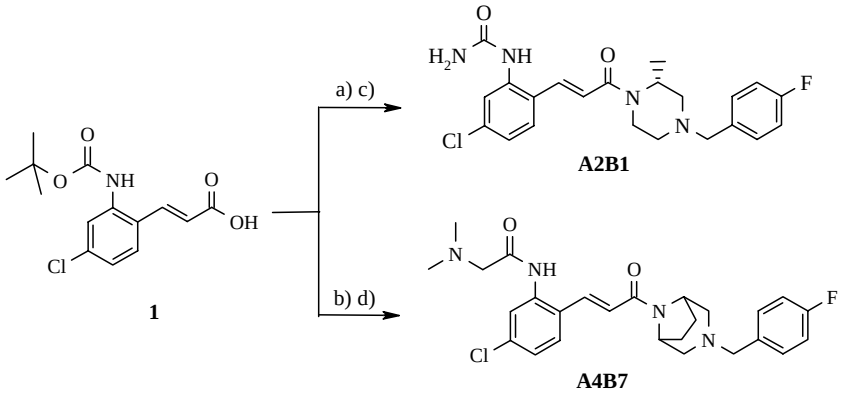
Table 1. CCR1 antagonists AB

Compound	hCCR1[a]	mCCR1[a]	rCCR1[a]	Ca^{2+b}
BX-471	0.040	1.5	0.55	0.0004
A1B1	0.030	0.58	0.32	0.001
A1B2	0.009	0.6	1.35	0.0001
A1B3	0.100	23.0	10.5	0.001
A1B4	0.190	0.448	0.15	0.058
A1B5	1.60	n.t.	n.t.	n.t.
A1B6	1.70	n.t.	n.t.	n.t.
A1B7	0.200	n.t.	n.t.	n.t.
A1B8	0.300	n.t.	n.t.	n.t.
A2B1	0.016	0.3	0.04	0.0009
A3B1	0.004	0.05	0.02	0.0015
A3B7	0.007	0.004	0.001	0.0000
A4B7	0.030	0.04	0.01	0.009
A5B1	0.360	n.t.	n.t.	n.t.
A6B1	0.050	3.0	1.7	0.0024
A7B1	0.800	n.t.	n.t.	n.t.
A8B1	0.560	3.0	1.0	0.0096
A9B1	0.120	4.8	1.0	0.028
A10B1	0.080	0.5	1.5	0.013
A11B1	0.03	1.00	0.3	0.020
A11B7	0.15	0.05	0.006	n.t.

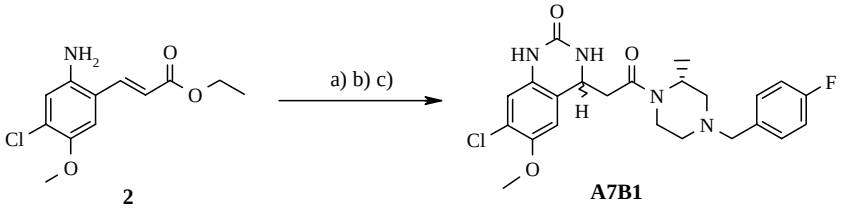
n.t., not tested.
[a] IC_{50} (μM).[29]
[b] IC_{50} (μM) of Ca^{2+} mobilization in MIP-1α stimulated THP-1 cells.[28]

arylamine in **A6B1** gave rise to a nearly equipotent compound, with slightly weaker activity on rodent receptors. Incorporation of the aniline nitrogen of **A7** into the indole ring in **A8B1** did not improve potency. Benzofuran **A9B1**—representing a rigidified **A** fragment of BX-471—led to a 3-fold loss in activity against h/m/rCCR1. The naphthyl- and quinolinyl building blocks in **A10B1** and **A11B1** showed properties similar to those of their benzene analogue **A1B1**. However, the quinoline analogue **A11B7** revealed—in agreement with all **B7**-derivatives—a more pronounced rCCR1 inhibitory profile, with an IC_{50} = 6 nM being 25-fold more potent against rCCR1 than against hCCR1. All compounds discussed were functional antagonists of hCCR1, demonstrated by their ability to inhibit the MIP-1α induced Ca^{2+} mobilization[28] in THP-1 cells (Table 1).

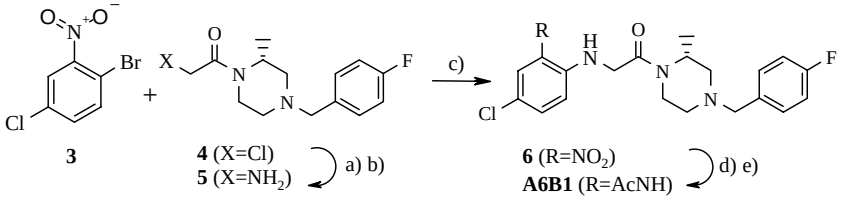
Pharmacokinetic data (Table 2) for BX-471 and **A2B1** revealed that the double bond in **A2** was superior to the aryl ether functionality in BX-471, leading to remarkably higher AUC-, C_{max}- and half life values. The acetyl group in **A1**—compared with the urea in **A2**—led to a further increase in AUC and half life, two important requirements for in vivo activity in rodent models. The methoxy group in **A3B1** demonstrated



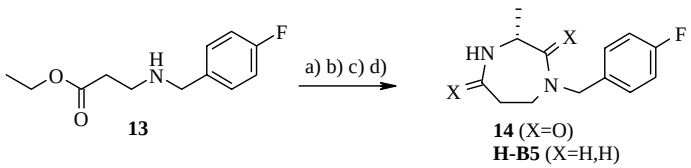
Scheme 2. Reagents and conditions: (a) **H-B1**, HOBt, EDCI.HCl, CH_2Cl_2, rt, 12 h, 63%; (b) **H-B7**, HOBt, EDCI.HCl, CH_2Cl_2, rt, 12 h, 66%; (c) NaOCN, HOAc, water, rt, 30 min, 65%; (d) i—Chloroacetyl chloride, THF, rt, 1 h, 85%, ii—$HNMe_2$, THF, 10 min rt, 95%.



Scheme 3. Reagents and conditions: (a) NaOCN, HOAc, water, rt, 30 min, 68%; (b) 2 N NaOH, EtOH, reflux, 10 min, 75%; (c) **H-B1**, HOBt, EDCI.HCl, CH_2Cl_2, rt, 12 h, 65%.



Scheme 4. Reagents and conditions: (a) Phthalimide-K, DMF, 120 °C, 30 min, 90%; (b) H_2NNH_2, MeOH, reflux 1.5 h, 96%; (c) neat, 130 °C, 45 min, 30%; (d) excess $SnCl_2$, EtOH/HClconcd/water 3/1/1 reflux 15 min, 93%; (e) Ac_2O, NEt_3, THF, rt 12 h, 27%.



Scheme 5. Reagents and conditions: (a) EDCI, HOBt, BOC-D-Ala-OH, THF, rt 5 h, 90%; (b) 4 N HCl dioxane, rt, 16 h, 39%; (c) pH 9, water, rt; (d) BH_3 THF, 60 °C, 97%.

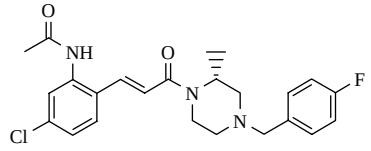
Table 2. Pharmacokinetics in rats and chemotaxis

Compound	F (%)	C_{max} [a]	$T_{1/2}$ (h)	AUC [b]	CCL3 chemotaxis [c]
BX-471	100	0.400	1.9	139	2.36
A1B1	95	0.85	16.5	784	9.97
A2B1	90	0.934	11.0	487	45.00
A3B1	100	0.396	4.1	750	69.7
A3B7	83	0.080	5.1	158	7.50
A4B7	77	0.407	2.92	1071	41.00

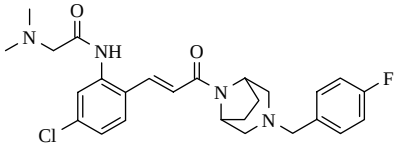
F, bioavailability; $T_{1/2}$, half life; AUC, area under the curve.
[a] μM/L; dose normalized.
[b] ng h/mL.
[c] IC_{50} (nM) of MIP-1α (CCL3) induced transwell chemotaxis.[30]

a 2- to 4-fold drop of C_{max} and half lives, while replacement of **B1** by **B7** reduced all parameters, except the half life in **A3B7**. However, combining **B7** and **A4** led to the highest AUC value of the series in **A4B7**.

Based on their promising pharmacokinetic properties in rats and potency to inhibit CCL3- induced chemotaxis,[30] **A1B1**, **A4B7** and **A3B7** were tested in the LPS-accelerated collagen-induced arthritis model in mice.[31] Due to their shorter half lives, **A3B7** and **A4B7** were dosed 2×30 mg/kg p.o. per day, while **A1B1** with a longer half life was dosed once per day 60 mg/kg p.o. **A4B7** reduced the clinical severity score by 58%, **A1B1** by 48%, while **A3B7** was inactive. The lack of activity of **A3B7** can be explained by its lower exposure (AUC = 158; Table 1) in comparison to **A1B1** (AUC = 784) and **A4B7** (AUC = 1071). At a dose of 100 mg/kg p.o. **A1B1** (plasma levels after 4 h: 8.15 μM; after 18 h: 1.4 μM) significantly reduced histological scores of inflammation, pannus formation, cartilage degradation and bone resorption. As a representative of the novel cinnamide chemotype CCR1 antagonists, **A1B1** underwent a GPCR selectivity screen including



A1B1



A4B7

related chemokine receptors, muscarinic, adrenergic and opioid receptors and proved to be highly selective.[32] Since most GPCRs tested for selectivity were of human origin, animal in vivo data have to be interpreted with caution. More work is needed—e.g., chemotaxis experiments with rat and mouse cells—to relate in vivo activity with inhibition of the CCR1 receptor.

In summary, our pilot set aiming at the discovery of novel CCR1 antagonists with cross-reactivity for human and rodent receptors yielded a number of potent antagonists. Two compounds—**A1B1** and **A4B7**—demonstrated oral efficacy in a model of rheumatoid arthritis in the mouse.

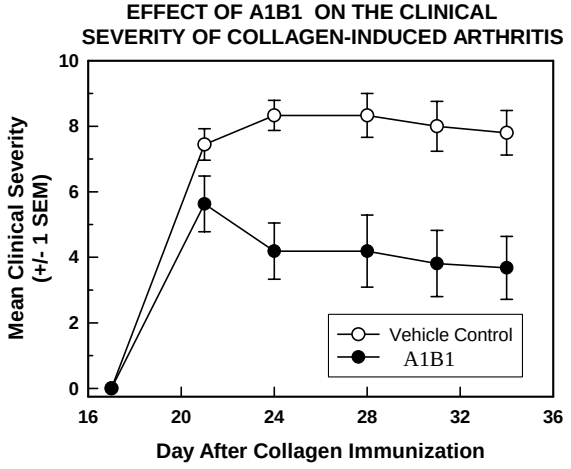
Acknowledgment

The authors wish to thank the Analytical Department of Novartis Pharma AG for NMR, MS, and IR spectra including their interpretation.

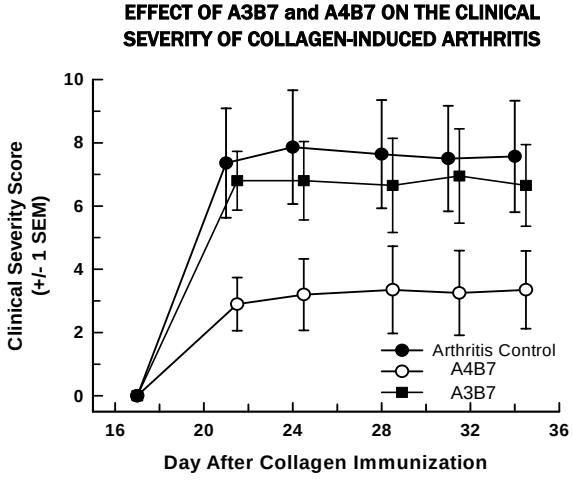
References and notes

1. Godessart, N.; Kunkel, S. L. *Curr. Opin. Immunol.* **2001**, *13*, 670.
2. Loetscher, P.; Moser, B. *Arthritis Res.* **2002**, *4*, 233.
3. Karpus, W. J.; Kennedy, K. J. *J. Leukocyte Biol.* **1997**, *62*, 691.
4. Godiska, R.; Chantry, D.; Dietsch, G. N.; Gray, P. N. *J. Neuroimmunol.* **1995**, *58*, 167.
5. Horuk, R.; Shurey, S.; Ng, H. P.; May, K.; Baumann, J. G.; Islam, I.; Ghannam, A.; Buckmann, B.; Wei, G. P.; Xu, W.; Liang, M.; Rosser, M.; Dunning, L.; Hesselgesser, J.; Snider, R. M.; Morrissey, M. M.; Perez, H. D.; Green, C. *Immunol. Lett.* **2001**, *76*, 193.
6. Horuk, R.; Clayberger, C.; Krensky, A. M.; Wang, Z.; Grone, H. J.; Weber, C.; Weber, K. S.; Nelson, P. J.; May, K.; Rosser, M.; Dunning, L.; Liang, M.; Buckmann, B.; Ghannam, A.; Ng, H. P.; Islam, I.; Baumann, J. G.; Wei, G. P.; Monahan, S.; Xu, W.; Snider, R. M.; Morrissey, M. M.; Hesselgesser, J.; Perez, H. D. *J. Biol. Chem.* **2001**, *276*, 4199.
7. Haringman, J. J.; Kraan, M. C.; Smeets, T. J. M.; Zwinderman, K. H.; Tak, P. P. *Ann. Rheum. Dis.* **2003**, *62*, 715.
8. Brown, M. F.; Avery, M.; Brissette, W. H.; Chang, J. H.; Colizza, K.; Conklyn, M.; DiRico, A. P.; Gladue, R. P.; Kath, J. C.; Krueger, S. S.; Lira, P. D.; Lillie, B. M.; Lundquist, G. D.; Mairs, E. N.; McElroy, E. B.; McGlynn, M. A.; Paradis, T. J.; Poss, C. S.; Rossulek, M. I.; Shepard, R. M.; Sims, J.; Strelevitz, T. J.; Truesdell, S.; Tylaska, L. A.; Yoon, K.; Zheng, D. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2175.
9. Kath, J. C.; Brissette, W. H.; Brown, M. F.; Conklyn, M.; DiRico, A. P.; Dorff, P.; Gladue, R. P.; Lillie, B. M.; Lira,

P. D.; Mairs, E. N.; Martin, W. H.; McElroy, E. B.; McGlynn, M. A.; Paradis, T. J.; Poss, C. S.; Stock, I. A.; Tylaska, L. A.; Zheng, D. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2169.
10. Kath, J. C.; DiRico, A. P.; Gladue, R. P.; Martin, W. H.; McElroy, E. B.; Stock, I. A.; Tylaska, L. A.; Zheng, D. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2163.
11. Gladue, R. P.; Tylaska, L. A.; Brissette, W. H.; Lira, P. D.; Kath, C. S.; Poss, M. F.; Brown, M. F.; Paradis, T. J.; Conklyn, M. J.; Ogborne, M. A.; McGlynn, M. A.; Lillie, B. M.; DiRico, A. P.; Mairs, E. N.; McElroy, E. B.; Martin, W. H.; Stock, I. A.; Shepard, R. M.; Showell, H. J.; Neote, K. S. *J. Biol. Chem.* **2003**, *278*, 40473.
12. Hesselgesser, J.; Ng, H. P.; Liang, M.; Zheng, W.; May, K.; Baumann, J. G.; Monahan, S.; Islam, I.; Wei, G. P.; Ghannam, A.; Taub, D. D.; Rosser, M.; Snider, R. M.; Morrissey, M. M.; Perez, H. D.; Horuk, R. *J. Biol. Chem.* **1998**, *273*, 15687.
13. Ng, H. P.; May, K.; Bauman, J. G.; Ghannam, A.; Islam, I.; Liang, M.; Horuk, R.; Hesselgesser, J.; Snider, R. M.; Perez, H. D.; Morrissey, M. M. *J. Med. Chem.* **1999**, *42*, 4680.
14. Liang, M.; Mallari, C.; Rosser, M.; Ng, H. P.; May, K.; Monahan, S.; Bauman, J. G.; Islam, I.; Ghannam, A.; Buckman, B.; Shaw, K.; Wei, G. P.; Xu, W.; Zhao, Z.; Ho, E.; Shen, J.; Oanh, H.; Subramanyam, B.; Vergona, R.; Taub, D.; Dunning, L.; Harvey, S.; Snider, R. M.; Hesselgesser, J.; Morrissey, M. M.; Perez, H. D.; Horuk, R. *J. Biol. Chem.* **2000**, *275*, 19000.
15. Horuk, R.; Clayberger, C.; Krensky, A. M.; Wang, Z.; Gröne, H. J.; Weber, C.; Weber, K. S. C.; Nelson, P. J.; May, K.; Rosser, M.; Dunning, L.; Liang, M.; Buckman, B.; Ghannam, A.; Ng, H. P.; Islam, I.; Bauman, J. G.; Wei, G. P.; Monahan, S.; Xu, W.; Snider, R. M.; Morrissey, M. M.; Hesselgesser, J.; Perez, H. D. *J. Biol. Chem.* **2001**, *276*, 4199.
16. Horuk, R.; Shurey, S.; Ng, H. P.; May, K.; Bauman, J. G.; Islam, I.; Ghannam, A.; Buckman, B.; Wei, G. P.; Xu, W.; Liang, M.; Rosser, M.; Dunning, L.; Hesselgesser, J.; Snider, R. M.; Morrissey, M. M.; Daniel Perez, H. D.; Green, C. *Immunol. Lett.* **2001**, *76*, 193.
17. Bollbuck, B.; Eder, J.; Heng, R.; Revesz, L.; Schlapbach, A.; Waelchli, R. WO 2004037796. *Chem. Abstr*. **2004**, *140*, 391295.
18. Hilger, C. S.; Johannsen, B.; Steinbach, J.; Maeding, P.; Halks-Miller, M.; Horuk, R.; Dinter, H.; Mohan, R.; Hesselgesser, J. E. WO 2002036581 *Chem. Abstr*. **2002**, *136*, 379069.
19. Pennell, A. M. K.; Aggen, J. B.; Wright, J. J. K.; Sen, S.; McMaster, B. E.; Dairaghi, D. J. WO 2003105853. *Chem. Abstr*. **2004**, *140*, 42209.
20. Akira, N.; Toshihiko, S. *Drugs Future* **2001**, *26*, 121.
21. Naya, A.; Sagara, Y.; Ohwaki, K.; Saeki, T.; Ichikawa, D.; Iwasawa, Y.; Noguchi, K.; Ohtake, N. *J. Med. Chem.* **2001**, *44*, 1429.
22. Katti, H. A.; Siddappa, S. *Ind. J. Chem. Section B* **1983**, *22*, 1205.
23. Kori, M.; Miki, T.; Nishimoto, T.; Tozawa, R. WO 2001098282 A1. *Chem. Abstr*. **2002**, *136*, 69829.
24. Bolos, J.; Gubert, S.; Anglada, L.; Planas, J. M.; Burgarolas, C.; Castello, J. M.; Sacristan, A.; Ortiz, J. A. *J. Med. Chem.* **1996**, *39*, 2962.
25. Mavunkel, B. J.; Chakravarty, S.; Perumattam, J. J.; Dugar, S.; Lu, Q.; Liang, X. WO 2000071535. *Chem. Abstr*. **2001**, *134*, 17503.
26. Blumberg, L. C.; Brown, M. F.; Glaude, R. P.; Poss, C. S. WO 2002032901. *Chem. Abstr*. **2002**, *136*, 340711.
27. Smith, David William; Yocca, Frank D.; Yevich, Joseph Paul; Mattson, Ronald John. EP 345808 *Chem. Abstr*. **1991**, *113*, 172049.
28. THP-1 cells naturally expressing CCR1 were used and preincubated with CCR1 antagonists for 30 min. at RT, followed by stimulation with 3 nM MIP-1α and subsequent measurement of the fluo-4 fluorescence.
29. CHO-K1 cells were stably transfected with hCCR1, mCCR1 and rCCR1. A scintillation proximity assay was performed using CHO-K1 membranes and radioactively labelled ligand MIP-1α.
30. Mouse pre-B cells were stably transfected with hCCR1 and used to assess the inhibitory effect of CCR1 antagonists on the transwell chemotaxis induced by 1 nM MIP-1α.
31. Collagen induced arthritis in mice was introduced with chicken type II collagen followed 17 days later by injection of LPS. CCR1 antagonists were administered by gavage one day prior to LPS injection. Thirty-five days after immunization mice were killed and all four paws were removed and processed for histological examination.
32. Receptor selectivity profile of **A1B1**. Receptor (human, except where stated): IC_{50} (μM). CCR2b: >10. CCR3: >10. CCR5 (mouse): >10. CCR6: >100. Serotonin 5HT1A: >1. 5HT1B: >1. 5HT2A: >1. 5HT2B: >1. 5HT2C : >1. 5HT3: >1. 5HT6: >1. 5HT7: >1. Muscarinic M1: >1. M2: >1. M3: >1. M4: >1. M5: >1. Dopamine D1: >1. D2: >1. D3: >1. D4.4: >1. Adrenergic A1: >1. A2A: >1. A2C: >1. A2B: >1. B1: >1. B2: >1. Opiate mu: >1. kappa: >1. delta: >1.



M A1B1 was administered at a dose of 60 mg/kg in 5% aerosil in water. Treatment was initiated once daily prior to LPS challenge and continued until study termination on day 34.



A4B7 and A3B7 were administered at a dose of 2 × 30 mg/kg in 5% aerosil in water. Treatment was initiated prior to LPS challenge and continued until study termination on day 34.