Bioorganic & Medicinal Chemistry Letters 20 (2010) 5477–5479

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Novel pyrrolidine heterocycles as CCR1 antagonists

J. Robert Merritt *, Ray James, Vidyadhar M. Paradkar, Chongwu Zhang, Ruiyan Liu, Jinqi Liu, Biji Jacob, Camelia Chiriac, Michael J. Ohlmeyer, Elizabeth Quadros, Pamela Wines, Jennifer Postelnek, Catherine M. Hicks, Weiqing Chen, Earl F. Kimble, Linda O'Brien, Nicole White, Hema Desai, Kenneth C. Appell, Maria L. Webb

Ligand Pharmaceuticals, Inc., 3000 Eastpark Blvd., Cranbury, NJ 08512, USA

ARTICLE INFO

Article history:
Received 1 June 2010
Revised 16 July 2010
Accepted 20 July 2010
Available online 24 July 2010

Keywords:
CCR1
Chemokine
CCL3

ABSTRACT

A novel series of pyrrolidine heterocycles was prepared and found to show potent inhibitory activity of CCR1 binding and CCL3 mediated chemotaxis of a CCR1-expressing cell line. A potent, optimized triazole lead from this series was found to have acceptable pharmacokinetics and microsomal stability in rat and is suitable for further optimization and development.

CCR1 is expressed on many human cell types including: monocytes, T lymphocytes, dendritic cells, eosinophils, and basophils.[1] When activated by chemokines such as CCL3 and CCL5, CCR1 mediates the directed trafficking of these cells in response to pathological and physiological stimuli. Interruption of this process with small-molecule antagonists could offer therapies for many disorders including multiple myeloma,[2,3] rheumatoid arthritis[4,5] (RA), and multiple sclerosis.[6,7]

Many small-molecule CCR1 antagonists have been reported previously and a number of these have entered human clinical trials. BX-471[8] and CP-481715,[9] Figure 1, were two of the first compounds to proceed through phase II trials for multiple sclerosis and RA, respectively. Both compounds failed to achieve a sufficient clinical score.[10,11] Other CCR1 compounds to enter the clinic have been: AZD-4818[12] for COPD, and MLN-3897[13] and CCX354[14] both for RA. Of these, only CCX354 remains in phase II trials at this time. While the reasons these compounds fail to achieve sufficient efficacy remain unclear, potent, bioavailable CCR1 antagonists may still be of therapeutic value particularly for other CCR1-mediated indications such as multiple myeloma.[15]

We previously disclosed our early lead CCR1 antagonist **1**.[16] This compound was obtained through optimization of a hit from our combinatorial compound collection. While enticing due to its good oral bioavailability in rats, this compound was not a sufficiently potent inhibitor of CCR1-mediated chemotaxis. We reasoned that replacement of the amide moiety with a bioisosteric

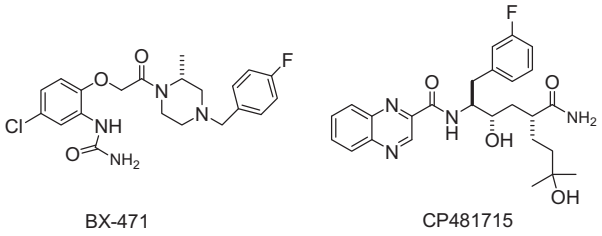


Figure 1. Disclosed structures for CCR1 antagonists to enter clinical trials.

heterocycle would offer rigidity which could result in improved potency

We prepared a series of heterocycle replacements for the amide moiety of compound **1** in which other sections of the molecule were conserved. In our previous exploration of the amide series, we observed that the 4-chlorobenzylamine moiety was critical for CCR1 potency, so this group was incorporated in the heterocycles. Likewise, the pyrrolidine core was retained, since changes in ring size and stereochemistry had resulted in drastic loss of potency. Also in the amide series, we found considerable tolerance for urea replacements and substitution. However, the 4-trifluoromethylphenylurea had provided the best combination of CCR1 potency and bioavailability.

With the other sections of the structure thus conserved, we prepared the collection of heterocycles shown in Figure 2 utilizing synthetic approaches similar to that exemplified in Scheme 1 for compound **7**.[17] Introduction of imidazole as a racemate, compound

* Corresponding author. Tel.: +1 908 737 7226.
E-mail address: jmerritt@kean.edu (J.R. Merritt).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.07.082

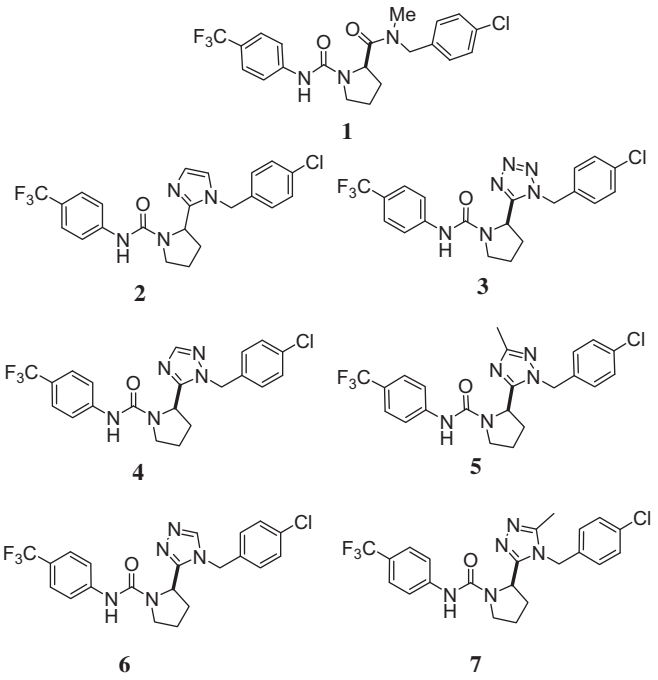
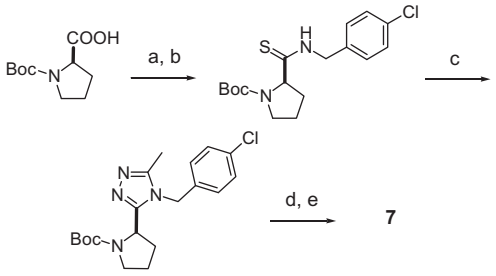


Figure 2. Amide lead and bioisosteric heterocycles for CCR1.



Scheme 1. Reagents and conditions: (a) 4-chlorobenzylamine, HBTU, DIEA, DMF; (b) Lawesson's reagent, toluene, refluxed 2 h; (c) acetohydrazide, $Hg(OAc)_2$, MeCN; (d) 4 M HCl in dioxane; (e) 1-isocyanato-4-(trifluoromethyl)benzene, DCM.

2, afforded similar potency as for the original lead **1**, Table 1. However, as is typical for imidazoles,[18] this compound had poor microsomal stability which translated to poor pharmacokinetics. The tetrazole **3** likewise had poor microsomal stability but significantly improved potency. The 1,2,4-triazole, **4**, showed marked improvement in microsomal stability which translated to improved pharmacokinetics. However, this compound was no more potent for inhibition of chemotaxis than the original amide lead **1**. Further substitution of this triazole with a methyl group at position 3, compound **5**, dramatically reduced CCR1 binding potency.

The most significant overall improvement was observed with 1,3,4-triazole. Compound **6** was significantly more potent for inhibition of binding and chemotaxis than the original amide lead. It also exhibited moderate microsomal stability and pharmacokinetics. Interestingly, further substitution of this triazole, did not reduce potency as it had for the 1,2,4-triazole **5**. Indeed compound **7** had an IC_{50} of 2.9 nM for inhibition of chemotaxis. Furthermore, introduction of the methyl group at position 2 improved microsomal stability and pharmacokinetics.

Compound **7** was selected for further evaluation. Additional pharmacokinetic studies in rat showed that this compound has a plasma half-life of 3.8 h, 26% bioavailability and low clearance of 2.8 mL/min/kg. T_{max} was at 1.1 h with a C_{max} of 1124 ng/mL. CYP profiling showed no inhibition at less than 10 μM for 3A4, 2D6, 2C9, 1A2, and 2C19 isoforms.[23]

In summary, further optimization of our original amide lead for CCR1 was achieved. A 1,3,4-triazole replacement for the amide moiety resulted in improved potency while retaining good bioavailability and metabolic profile.

Table 1
In vitro CCR1 binding, chemotaxis, liver microsome stability and in vivo rat AUC for selected compounds (data shown are ±standard deviation of two or more measurements)

Compd	CCR1 binding[19] IC_{50} (nM)	CCR1 chemotaxis[20] IC_{50} (nM)	HLM[21] % remaining	RLM % remaining	$AUC_{(0-\infty)}$[22] po (ng/ml h)
1	140 ± 10	22 ± 9	78	74	9390 ± 1590
2	65 ± 22	16 ± 4	39	55	100 ± 36
3	20 ± 6	6.5 ± 2.0	59	25	ND
4	32 ± 8	22 ± 9	61	81	4459 ± 2453
5	810 ± 127	ND	ND	ND	ND
6	17 ± 5	4 ± 2	52	41	657 ± 289
7	69 ± 0.2	2.9 ± 0.8	100	87	7649 ± 4033

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.07.082.

References

1. Furuichi, K.; Ji-Liang Gao, J.; Horuk, R.; Wada, T.; Kaneko, S.; Murphy, P. M. *J. Immunol.* **2008**, *81*, 8670.
2. Menu, E.; De Leenheer, E.; De Raeve, H.; Coulton, L.; Imanishi, T.; Miyashita, K.; Van Valckenborgh, E.; Van Riet, I.; Van Camp, B.; Horuk, R.; Croucher, P.; Vanderkerten, K. *Clin. Exp. Metastasis* **2006**, *23*, 291.
3. Oba, Y.; Lee, J.; Ehrlich, L.; Chung, H.; Jelinek, N.; Callender, N.; Horuk, R.; Choi, S.; Roodman, G. *Exp. Hematol.* **2005**, *33*, 272.
4. Barnes, D. A.; Tse, J.; Kaufhold, M.; Owen, M.; Hesselgesser, J.; Strieter, R.; Horuk, R. *J. Clin. Invest.* **1998**, *101*, 2910.
5. Snowden, N.; Hajeer, A.; Thomson, W.; Ollier, B. *Lancet* **1994**, *343*, 547.
6. Rottman, J. B.; Slavin, A. J.; Silva, R.; Weiner, H. L.; Gerard, C. G.; Hancock, W. W. *Eur. J. Immunol.* **2000**, *30*, 2372.
7. Baranzini, S. E.; Elfstrom, C.; Chang, S. Y.; Butunoi, C.; Murray, R.; Higuchi, R.; Oksenberg, J. R. *J. Immunol.* **2000**, *165*, 6576.
8. Horuk, R. *Rev. Med. Chem.* **2005**, *5*, 791.
9. Gladue, R. P.; Tylaska, L. A.; Brissette, W. H.; Lira, P. D.; Kath, J. C.; Poss, C. S.; Brown, M. F.; Paradis, T. J.; Conklyn, M. J.; Ogborne, K. T.; 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. *J. Biol. Chem.* **2003**, *278*, 40473.
10. Gladue, R. P.; Zwillich, S. H.; Clucas, A. T.; Brown, M. F. *Curr. Opin. Investig. Drugs* **2004**, *5*, 499.
11. Zipp, F.; Hartung, H. P.; Hillert, J.; Schimrigk, S.; Trebst, C.; Stangel, M.; Infante-Duarte, C.; Jakobs, P.; Wolf, C.; Sandbrink, R.; Pohl, C.; Filippi, M. *Neurology* **2006**, *67*, 1880.
12. Norman, P. *Expert Opin. Ther. Pat.* **2009**, *19*, 1629.
13. Vergunst, C. E.; Gerlag, D. M.; von Moltke, L.; Karol, M.; Wyant, T.; Chi, X.; Matzkin, E.; Leach, T.; Tak, P. P. *Arthritis Rheum.* **2009**, *60*, 3572.
14. http://clinicaltrials.pharmaceutical-business-review.com/news/chemocentryx_begins_phase_2_clinical_trial_for_ccx354_091216/ (last accessed 3rd May 2010).
15. Vallet, S.; Raje, N.; Ishitsuka, K.; Hideshima, T.; Podar, K.; Chhetri, S.; Pozzi, S.; Breitkreutz, I.; Kiziltepe, T.; Yasui, H.; Ocio, E. M.; Shiraishi, N.; Jin, J.; Okawa, Y.; Ikeda, H.; Mukherjee, S.; Vaghela, N.; Cirstea, D.; Ladetto, M.; Boccadoro, M.; Anderson, K. C. *Blood* **2007**, *110*, 3744.
16. Merritt, J. R.; Liu, J.; Quadros, E.; Morris, M. L.; Liu, R.; Zhang, R.; Jacob, B.; Postelnek, J.; Hicks, C. M.; Chen, W.; Kimble, E. F.; Rogers, W. L.; O'Brien, L.; White, N.; Desai, H.; Bansal, S.; King, G.; Ohlmeyer, M. J.; Appell, K. C.; Webb, M. L. *J. Med. Chem.* **2009**, *52*, 1295.
17. Synthetic details for compounds **2**–**7** are available in the Supplementary data.
18. Tang, C.; Chiba, M.; Nishime, J.; Hochman, J. H.; Chen, I. W.; Williams, T. M.; Lin, J. H. *Drug Metab. Dispos.* **2000**, *28*, 680.
19. Biological Assays: Materials and methods. Recombinant membranes were prepared by cloning human CCR1 into an episomal expression vector (Horlick-2000) and stably expressing in HEK293 cells. [^{125}I]-MIP1α was obtained from Perkin–Elmer (Waltham, MA) and SPA beads were obtained from GE healthcare (Piscataway, NJ). The human monocytic cell line, THP-1, was purchased from ATCC (Manassas, VA). Recombinant human MIP1α was obtained from R&D Systems (Minneapolis, MN). Plates used for chemotaxis

in 96-well format (5 microns) were obtained from Corning (Corning, NY). Cell titer-glo was obtained from Promega, Madison WI. The receptor binding assay was conducted in 384-well plates by preparing membrane and SPA bead mixture in assay buffer (130 mM NaCl, 5 mM KCl, 1 mM $MnCl_2$, 50 mM Tris–HCl, 7.4, 0.1% BSA) at 50 μg/ml membrane and 10 mg/ml SPA beads. The membrane/SPA (10 μl) mixture was transferred to the assay plate along with 10 μl of different concentrations of testing compounds. [^{125}I]-MIP1α (5 μl) was then added at final concentration of 0.1 nM. The plate was spun at 2500 rpm for 2 min and incubated for 4 h and read (1 min/well count time) on a MicroBeta® TriLux (Perkin–Elmer, USA).

20. Chemotaxis was conducted in 96-well chemotaxis chambers from Corning. Briefly, agonists were diluted in assay buffer (RPMI plus 0.1% BSA) and then added to the bottom wells of the chamber. THP1 cells with a density of 2×10^6 cells/ml were added to the top chamber in the presence or absence of various concentrations of compounds. The apparatus was incubated for 3 h in a 5% CO_2-humidified incubator at 37 °C. After the incubation period, the migrating cells from the bottom chamber were quantified by transferring equal volumes of cells into cell titer-glo and read on Perkin–Elmer Victor™ multilable counter using luminescence protocol.
21. Rat and human liver microsome stability assays. Assay mixtures typically contained rat or human microsomes (300 nM cytochrome P450, BD Gentest, Woburn, MA), phosphate buffer (pH 7.4), 1 μM test compound, 1 mM NADPH in a final assay volume of 0.1 mL. Incubations were for 30 min at 37 °C and were terminated by the addition of 0.2 ml of acetonitrile. Samples were centrifuged at 2000*g* and analyzed by LC/MS. The percentage of compound remaining at 30 min was calculated.
22. Pharmacokinetics studies in the rat. In preliminary screening studies, test compounds were dosed orally by gavage (5 mg/kg in 0.5% methyl cellulose) to 3–4 male Sprague–Dawley rats, either as single compounds or as part of a cassette. Serial blood samples were collected from an indwelling arterial cannula up to 6 or 24 h. For bioavailability studies, test compound (1 mg/kg) was also administered intravenously via an indwelling catheter. Plasma was separated and samples were prepared by protein precipitation and analyzed for parent compound by LC/MS/MS. Pharmacokinetic parameters were determined using non-compartmental analysis (WinNonlin, Pharsight Corp., Mountain View, CA).
23. Cytochrome P450 inhibition. Human recombinant cytochrome P450 isoenzymes (BD Gentest, Woburn, MA) were tested (CYP1A2, 2C9, 2C19, 2D6, and 3A4). Each isoenzyme converts a specific fluorogenic substrate to a fluorescent product. Test compound (up to 20 μM) was added and the fluorescence was read, after a defined reaction time, using a Victor V2 Wallac 1420 Multilabel Counter. The percentage inhibition at each concentration was calculated and the IC_{50} was determined by fitting the plot of percent inhibition versus logarithmic concentration values (μM).