Journal of Medicinal Chemistry

Article

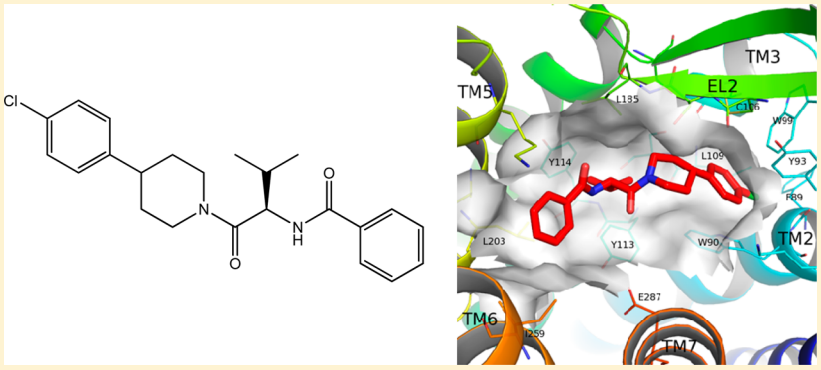
pubs.acs.org/jmc

Discovery and Lead Optimization of a Novel Series of CC Chemokine Receptor 1 (CCR1)-Selective Piperidine Antagonists via Parallel Synthesis

Cullen L. Cavallaro,* Stephanie Briceno, Jing Chen, Mary Ellen Cvijic, Paul Davies, John Hynes, Jr., Rui-Qin Liu, Sandhya Mandlekar, Anne V. Rose, Andrew J. Tebben, Katy Van Kirk, Andrew Watson, Hong Wu, Guchen Yang, and Percy H. Carter

Research and Development, Bristol-Myers Squibb Company, Route 206 and Provinceline Road, Princeton, New Jersey 08540, United States

Supporting Information



ABSTRACT: A series of novel, potent CCR1 inhibitors was developed from a moderately active hit using an iterative parallel synthesis approach. The initial hit (composed of three subunits: an amine, a central amino acid, and an N-terminal cap) became the basis for a series of parallel chemical libraries designed to generate SAR data. Libraries were synthesized that explored each of the three subunits; the CCR1 binding data obtained revealed the following: (1) changes to the amine are not well tolerated; (2) small alkylamino acids are preferred in the center of the molecule; (3) substitutions at the N-terminus are generally well tolerated. These data were used to drive the optimization of the series, ultimately providing a lead with a CCR1 binding IC_{50} of 28 nM (**48**). This lead demonstrates high selectivity for CCR1 over other CCR-family members, high microsomal stability, and good pharmacokinetics in mice.

INTRODUCTION

CC Chemokine receptor-1 (CCR1), a family A G protein-coupled receptor,[1] is expressed on the surface of monocytes/macrophages, osteoclasts, T-cells, and neutrophils. It serves as a receptor for 11 different chemokines, including MIP-1α, RANTES, and leukotactin-1. Through its interactions with these ligands, CCR1 helps mediate leukocyte activation and migration, processes that are critical for the progression of inflammatory diseases.

As with many other chemokine receptors,[2−4] extensive preclinical studies in mice have suggested that CCR1 should represent a viable target for the development of therapies for inflammatory disorders.[5] Accordingly, the drug discovery community has expended a considerable amount of effort in identifying small molecule antagonists of CCR1.[6,7] Indeed, a number of candidates have been advanced to clinical trials (Figure 1).[8] The first four candidates with publically disclosed clinical efficacy data were **1** (BX-471),[9] **2** (CP-481715),[10] **3** (AZD-4818),[11] and **4** (MLN-3897).[12] Although proof-of-mechanism was obtained with **2** in regards to blocking cellular migration,[13,14] the demonstration of clinical efficacy was not achieved in a phase 2 trial in patients with rheumatoid arthritis.[8] Likewise, **4** failed in its phase 2 rheumatoid arthritis trial.[15] Failed phase 2 trials have also been reported for **1** in multiple sclerosis[16] and **3** in chronic obstructive pulmonary disease.[17]

Although these phase 2 clinical experiences have been disappointing, it remains clear that CCR1 plays a role in the migration of cells involved in the pathogenesis of human rheumatoid arthritis.[13,18] Indeed, a number of authors have suggested that the problems with previous trials may stem from the fact that earlier compounds did not achieve complete blockade of CCR1 for the course of the trial.[4,18] On the basis of this hypothesis, Chemocentryx and GSK have continued to advance a compound[19] in phase 2 trials[20,21] and recently

Received: June 25, 2012
Published: October 17, 2012

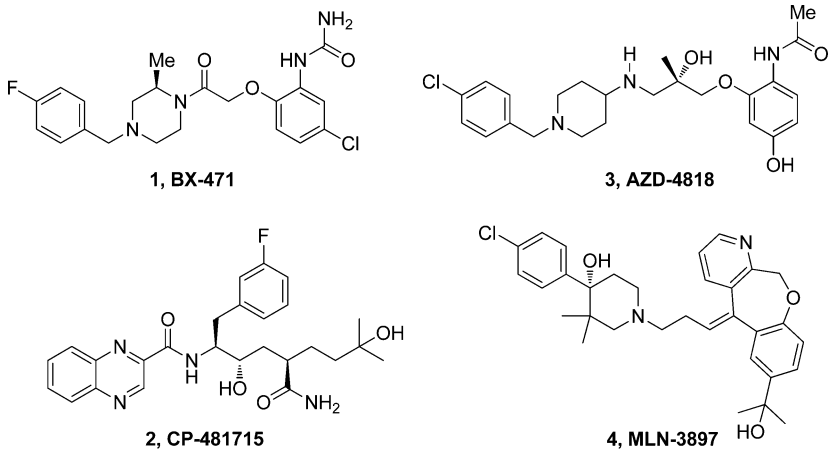


Figure 1. Structures of CCR1 antagonists with reported data from phase 2 clinical trials.

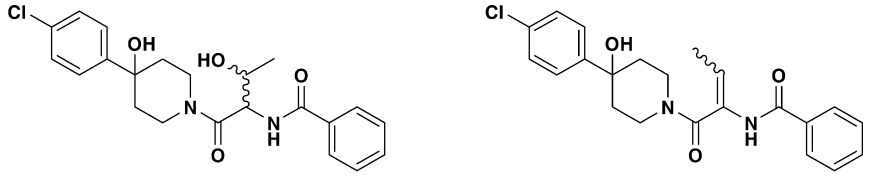
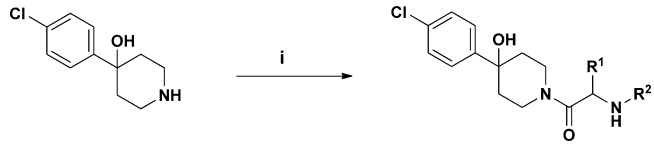


Figure 2. Screening hit **5** and its elimination product **6**.

disclosed favorable results from a 12-week phase 2 study in patients with rheumatoid arthritis.[22]

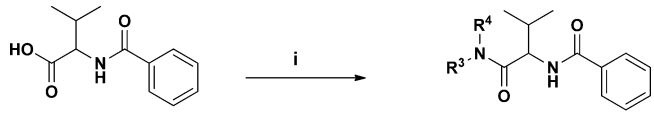
We had likewise been encouraged by the initial validation data for CCR1, especially for the treatment of rheumatoid arthritis, and set out to identify a potent, selective, and orally bioavailable antagonist of this target. A screen of our proprietary compound collection identified **5** as a moderately potent CCR1 antagonist (Figure 2). Further evaluation of this hit revealed it to be a mixture of compounds containing **5** and its elimination product **6**. The mixture had an IC_{50} of 332 nM in the CCR1 binding assay. Testing of the two isolated compounds showed that both were active against CCR1 but that **6** (CCR1 binding IC_{50} = 370 nM) was more potent than **5** (IC_{50} = 2832 nM) and therefore likely responsible for most of the activity of the original hit mixture. We were encouraged by the potency of these compounds and their structural differentiation from other CCR1 series (Figure 1).[6] Accordingly, they became the starting point for an iterative series of parallel synthetic libraries designed to improve CCR1 potency, explore structural tolerance, generate on-target SAR, and identify compound-related profiling liabilities.

Scheme 1. Synthesis of 4-(4-Chlorophenyl)-4-hydroxypiperidine Derivatives[a]



[a]Reagents and conditions: (i) $R^2NHCH(R^1)COOH$, EDC, HOBt, DIEA, DMF.

Scheme 2. Synthesis of Bz-D,L-valine Derivatives[a]



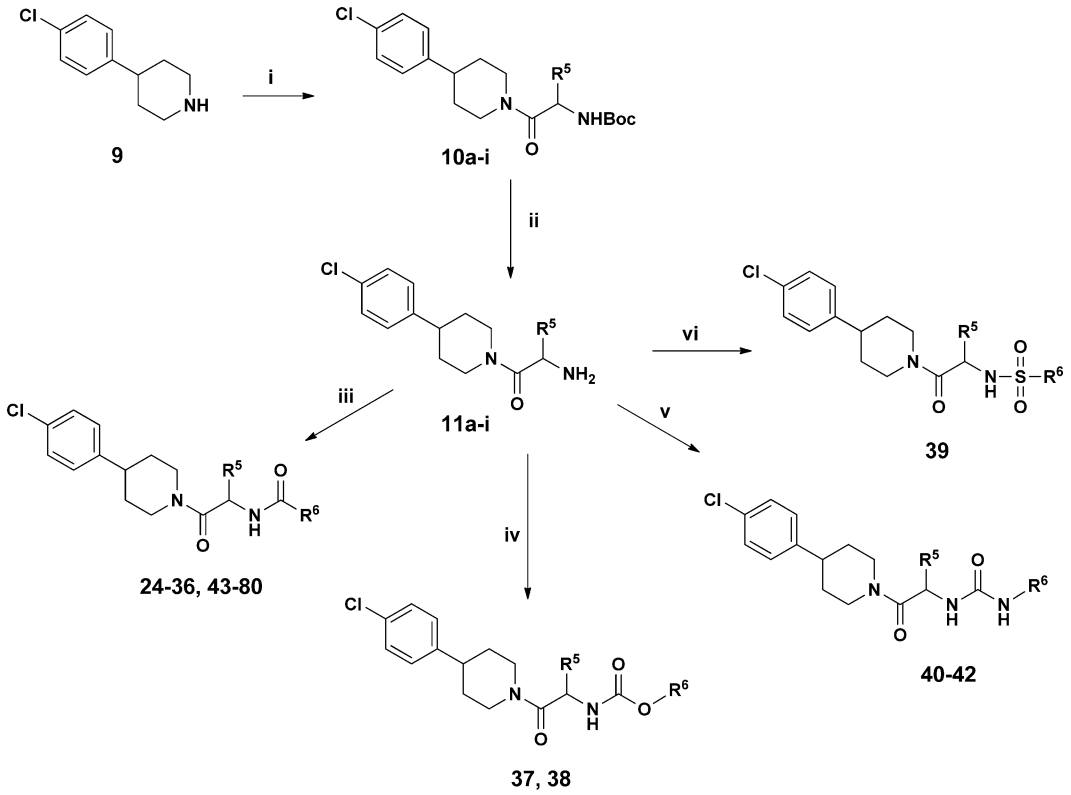
[a]Reagents and conditions: (i) R^3R^4NH, EDC, HOBt, DIEA, DMF.

CHEMISTRY

Libraries of potential inhibitors were synthesized by the general methods provided in Schemes 1, 2, and 3. Libraries containing compounds **12**–**16** were made by coupling 4-(4-chlorophenyl)-4-hydroxypiperidine (**7**) with various benzoyl- or Cbz-protected amino acid derivatives as shown in Scheme 1; screening hits **5** and **6** were also resynthesized by the same method. Libraries containing compounds **17**–**23** were synthesized from benzoyl-D,L-valine and a variety of amines (see Table 2 for examples of the final products) as shown in Scheme 2. For libraries containing compounds **24**–**80**, the Boc-protected intermediates (**10a**–**i**) were first made by EDC coupling of the appropriate Boc-protected amino acid (see Scheme 3 for a list of side chains) with 4-(4-chlorophenyl)-piperidine (**9**). These intermediates were deprotected with HCl in dioxane to provide **11a**–**i** which could then be coupled with the appropriate reagents to provide the final products as shown in Scheme 3. Amide derivatives **24**–**36** and **43**–**80** were synthesized from the corresponding acids using EDC, carbamates **37** and **38** were made from the carbamoyl chlorides, ureas **40**–**42** were made by reaction with isocyanates in dioxane, and sulfonamides (such as **39**) were made by reaction with sulfonyl chlorides (Scheme 3). Compounds **43** and **48** were made from L- and D-Boc-valine, respectively, by EDC coupling with **9**, followed by deprotection with HCl in dioxane and EDC coupling with benzoic acid. This method was found

dx.doi.org/10.1021/jm300896d | J. Med. Chem. 2012, 55, 9643–9653

Scheme 3. Synthesis of 4-(4-Chlorophenyl)piperidine Derivatives[a]



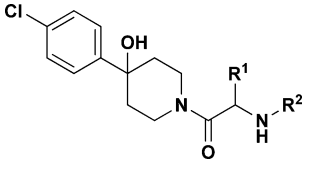
[a]Reagents and conditions: (i) BocNHCH(R^5)COOH, EDC, HOBt, DIEA, DMF; (ii) 4 M HCl in dioxane; (iii) R^6COOH, EDC, HOBt, DIEA, DMF; (iv) R^6OC(O)Cl, DIEA, THF; (v) R^6NCO, dioxane; (vi) R^6SO_2Cl, DIEA, THF, DCM. Side chains (R^5) for intermediates **10** and **11** are defined as follows: (a) *n*-Pr; (b) *i*-Pr; (c) *i*-Bu; (d) *t*-Bu; (e) (*R*)-Et; (f) (*R*)-*i*-Pr; (g) (*S*)-*i*-Pr; (h) (*R*)-CH_2(Me)Et; (i) (*R*)-cyclohexyl.

to minimize racemization, which was observed in direct couplings between enantiopure *N*-benzoyl-D-valine and **9**.

RESULTS AND DISCUSSION

The initial libraries built using 4-(4-chlorophenyl)-4-hydroxypiperidine (**7**) as a core revealed that a number of amino acid substitutions could be made in the center of the molecule that preserved CCR1 binding (Table 1). Although benzoyl-D,L-valine was favored (**12**, 180 nM), other amino acids with alkyl side chains also showed reasonable activity, for example in compounds **6** and **14**. Although less potent, *n*-propyl (**13**) and (methylthio)ethyl (**15**) side chains were tolerated. Significant drops in activity were observed when the N-terminal benzoyl group was replaced with Cbz (cf., **12** versus **16**) or acetyl (data not shown).

Table 1. CCR1 Binding Data for 4-(4-Chlorophenyl)-4-hydroxypiperidine Derivatives



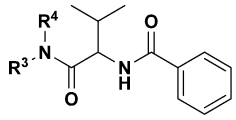
compound	R^1	R^2	CCR1 IC_{50} (nM)
5	CH(OH)Me	Bz	2832
6	=CHMe	Bz	370
12	*i*-Pr	Bz	180
13	Pr	Bz	521
14	*i*-Bu	Bz	324
15	CH_2CH_2SMe	Bz	539
16	*i*-Pr	Cbz	854

Efforts to replace the piperidine unit were more challenging, and only minor changes were tolerated in that portion of the molecule. Several libraries were synthesized from benzoyl-D,L-valine to explore this area, and although more than 70 amine replacements were investigated, the active compounds (Table 2) all closely resembled the original piperidine. Replacing the chlorine in **12** with fluorine (**17**) resulted in a significant drop in potency, but replacement with bromine resulted in an equipotent compound (**18**). Removing the 4-hydroxyl group from the piperidine led to a 3-fold improvement in potency (**21**); the fluoro analogue **20** was also active but not as potent as the chloro compound. The unsaturated piperidine **19** was nearly equipotent to the hydroxypiperidine derivative **12**. The other tolerated change was the replacement of the piperidine with a piperazine, and although this resulted in almost a 3-fold loss in potency (**22**), some of the potency could be regained with the addition of a second chlorine on the phenyl ring (**23**). All attempts to replace the aryl halogens with hydrogen, regardless of the nature of the heterocycle used, resulted in a complete loss in CCR1 activity (data not shown). These results are consistent with those published for other CCR1 chemotypes in which a haloaromatic group appears to be necessary for tight binding to the receptor.[23]

4-(4-Chlorophenyl)piperidine (**9**) became the basis for a library designed to generate additional SAR for the amino acid portion of the molecule (Table 3). A set of amino acids with small alkyl side chains was examined (racemic norvaline, valine,

dx.doi.org/10.1021/jm300896d | J. Med. Chem. 2012, 55, 9643−9653

Table 2. CCR1 Binding Data for Benzoyl-D,L-valine Derivatives



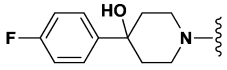
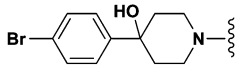
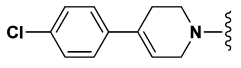
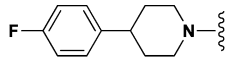
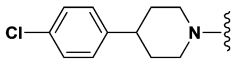
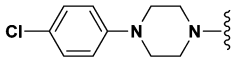
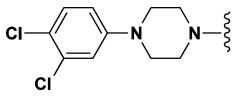
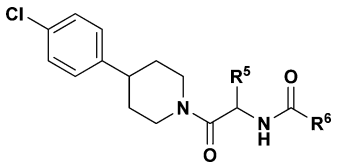
Compound	R^3R^4N-	CCR1 IC_{50} (nM)
17		933
18		172
19		185
20		423
21		55
22		525
23		375

Table 3. CCR1 Binding Data for 4-(4-Chlorophenyl)piperidine Derivatives

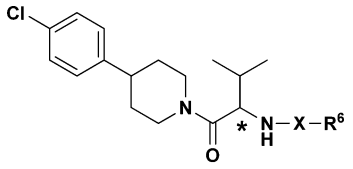


compound	R^5	R^6	CCR1 IC_{50} (nM)
24	*i*-Pr	*i*-Pr	388
25	*n*-Pr	Ph	120
21	*i*-Pr	Ph	55
26	*i*-Bu	Ph	130
27	*t*-Bu	Ph	259
28	*n*-Pr	2-MePh	152
29	*i*-Pr	2-MePh	36
30	*i*-Bu	2-MePh	88
31	*t*-Bu	2-MePh	262
32	*n*-Pr	2-naphth	191
33	*i*-Pr	2-naphth	80

tert-butyl glycine, and leucine) in combination with a set of acids to cap the N-terminus of the amino acid. The data from the library revealed that the valine analogues had superior potency in all cases (see **21**, **29**, and **33**).

Additional libraries were synthesized to generate SAR data for the N-terminus of the 4-(4-chlorophenyl)piperidine-valine core (Table 4). It is of interest to note that capping the N-terminus of this particular scaffold with the sulfonamide (**39**), carbamates (**37** and **38**), or ureas (**40**, **41**, and **42**) that are shown reduced CCR1 binding relative to the corresponding

Table 4. Comparison of CCR1 Binding Data for a Set of 4-(4-Chlorophenyl)piperidine Derivatives with Varying N-Terminal Capping Groups



X = C(O), C(O)O, SO_2 or C(O)NH

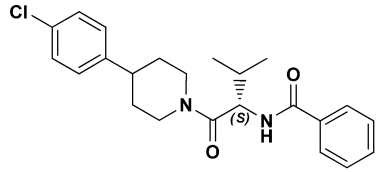
compound	X-R^6	*	CCR1 IC_{50} (nM)
34	$C(O)CH_2Ph$	D,L	40
35	$C(O)CH_2CH_2Ph$	D,L	35
36	$C(O)CH_2CH_2CH_2Ph$	D	95
37	C(O)OPh	D,L	265
38	$C(O)OCH_2Ph$	D,L	1077
39	SO_2CH_2Ph	D,L	303
40	C(O)NHPh	D	616
41	$C(O)NHCH_2Ph$	D	180
42	$C(O)NHCH_2CH_2Ph$	D	543

amide analogues (**34**, **35**, and **36**). Alkyl and aryl amides provided compounds with good potency against CCR1.

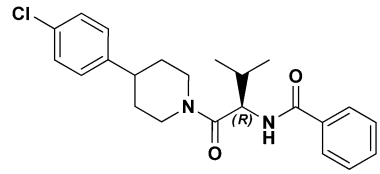
The synthesis of both benzoyl valine stereoisomers **43** and **48** revealed the preferred D-stereochemistry at the amino acid position (Figure 3) and the synthesis of a two-dimensional D-amino acid library (Table 5) reconfirmed earlier work in which the valine analogues were found to be the most potent. Isoleucine and aminobutyric acid analogues also showed very good activity.

The data from the analogues listed in Table 5 led to the synthesis of a series of libraries designed around a 4-(4-chlorophenyl)piperidine-D-valine core, which produced a large amount of SAR data for the N-terminal amides (Table 6). Alkyl, aryl, and heteroaryl substituents all provided compounds with acceptable CCR1 binding, with the alkyl series providing the most active compound (**61**). Small changes to this molecule affected binding negatively, but the resulting compounds still showed CCR1 binding of less than 200 nM. For example, changing the cyclohexyl ring in **61** to cyclopentyl (**60**) resulted in a 2.5-fold loss in binding, and a switch to cyclopropyl (**59**) resulted in about a 6-fold loss in binding; however, these compounds were still 20 and 46 nM, respectively. In the benzoic acid series, the binding seemed to stay in a tight range between 14 nM and 32 nM for most of the derivatives shown (**48** and Table 6, **62**–**68**), although there was a significant loss in potency for the 4-fluoro, 2,3-dimethyl, and 2,6-dimethyl analogues. The substituted phenylacetic acid analogues, in general, were less potent than the unsubstituted parent **52** except for the 2-fluoro compound **69** which showed a 2.5-fold improvement. In this series the addition of a 3-fluoro or 3-chloro substituent led to a slight loss in binding from the unsubstituted analogue **52** and more than a 9-fold loss when a 4-chloro or 4-methyl group was added. Activity was maintained upon further chain extension (**74**–**78**). As was observed with the phenylacetic acid analogues, 2-substitution also seemed to be preferred in the phenethyl series (**74** and **76**) and led to binding better than that of the unsubstituted analogue **55**, in addition to being superior to other substituted derivatives. Two heterocyclic compounds were also made, and the binding for benzothiazole derivative **79** was 18 nM; however, when the

dx.doi.org/10.1021/jm300896d | J. Med. Chem. 2012, 55, 9643–9653



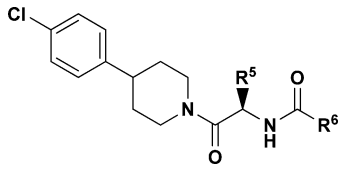
43
CCR1 IC_{50} 14,930 nM



48
CCR1 IC_{50} 28 nM

Figure 3. CCR1 binding data for the stereoisomers of **21**.

Table 5. Comparison of CCR1 Binding Data in a Series of 4-(4-Chlorophenyl)piperidine Derivatives with Varying Central Amino Acids



compound	R^5	R^6	CCR1 IC_{50} (nM)
44	Et	*i*-Bu	152
45	*i*-Pr	*i*-Bu	32
46	(*R*)-C(Me)Et	*i*-Bu	34
47	Et	Ph	121
48	*i*-Pr	Ph	28
49	(*R*)-C(Me)Et	Ph	45
50	cyclohexyl	Ph	121
51	Et	CH_2Ph	50
52	*i*-Pr	CH_2Ph	30
53	(*R*)-C(Me)Et	CH_2Ph	38
54	Et	CH_2CH_2Ph	85
55	*i*-Pr	CH_2CH_2Ph	40
56	(*R*)-C(Me)Et	CH_2CH_2Ph	66

benzothiazole was replaced with a benzothiophene (**80**), the binding IC_{50} value rose to 58 nM.

Additional data were obtained for compound **48** and showed that it was highly selective for CCR1, relative to CCR2, CCR3, and CCR5 (Table 8). No inhibition was detected in the cytochrome P450 (CYP) panel (3A4, 2C9, 2C19, and 2D6). Compound **48** also showed reasonable stability to human, mouse, and rat liver microsomes and exhibited a high species selectivity, as it was potent against the human receptor, less active against the rabbit, and inactive in the murine and rodent assays. Species selectivity has been observed for other CCR1 chemotypes[24] and is believed to result from a number of small changes in the amino acid sequences of the receptors that cause subtle changes to the binding site.[25]

To understand the origins of selectivity for this series, a binding model of **48** in the orthosteric site of a CCR1 homology model built from a CXCR4 template (3OE9) was constructed. The model shows three sites of interaction corresponding to the three pharmacophoric elements of the small molecules. The first is an aromatic box composed of Phe89, Trp90, and Tyr93 from transmembrane region 2 (TM2), and Trp99 from extracellular loop 1 (EL1), and capped by a conserved cysteine, Cys106, on EL2. As would be expected from the SAR, in which the halophenyl moiety was found to be essential for CCR1 affinity, it fits tightly into this box, forming multiple contacts with these residues (Figure 4). The second region is a set of hydrophobic interactions between the isopropyl group, Tyr113 and Tyr114 from TM3, and Leu185 from EL2. Mutagenesis studies have shown Tyr113 and Tyr114 to be critical contacts for the CCR1 antagonist **1**.[26] In our own studies, when tested against the CCR1 mutant receptors Y113A and Y114A, the binding affinity of **48** was reduced 113-fold and 64-fold, respectively (data not shown). The terminal benzoyl moiety falls into the third region, a less defined hydrophobic surface between TM5 and TM6 open to solvent with key contacts to Leu203 and Ile259 from TM5 and TM6, respectively. Although the benzoyl group is packed against TM5, the pocket is large enough to tolerate a variety of groups, as was observed in the SAR described (Table 6). In contrast to other CCR1 antagonists, no interaction is observed with the conserved glutamic acid on TM7, Glu287.

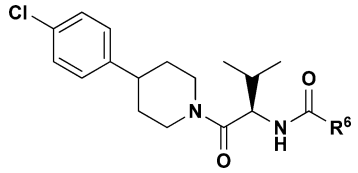
The amino acids predicted to be in contact with **48** and their counterparts in CCR2, CCR3, and CCR5, as well as the mouse, rat, and rabbit CCR1 orthologues, are tabulated in Table 7. Comparison of these residues provides some insight into the selectivity of this compound against CCR2, CCR3, and CCR5. The most substantial variation is found within the predicted ligand binding site of CCR2 where the key changes are as follows: Leu109 to phenylalanine (TM3), occluding the chlorophenyl pocket; Leu203 to arginine (TM5), disrupting the hydrophobic contact with the benzoyl moiety; contact with the isopropyl moiety by replacement of Tyr114 with the more polar histidine, and Leu185 to proline (EL2), altering the conformation of this loop and resulting in the loss of a hydrophobic interaction. The other CC receptors as well as the rat and mouse CCR1 have more subtle changes but, with the exception of rabbit CCR1, do show variability in position 185. The retention of activity in human and rabbit CCR1 suggests that Leu185 may be a critical interaction for the binding of **48** and a key determinant for specificity among the CC receptors. Further mutagenesis studies will be required to assess the contribution of this and additional residues to the binding of this series of compounds.

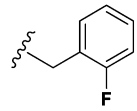
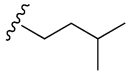
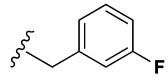
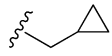
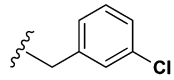
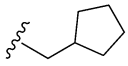
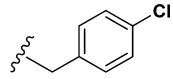
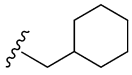
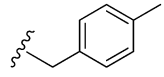
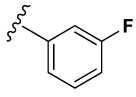
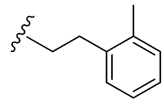
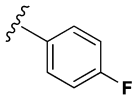
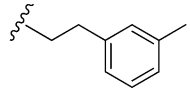
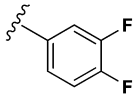
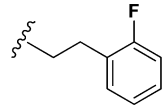
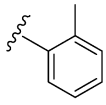
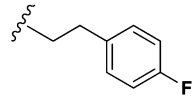
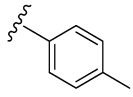
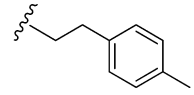
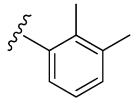
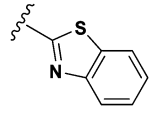
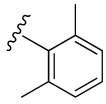
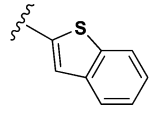
Overall, the chemical libraries that were synthesized provided significant SAR for all three areas of the initial lead molecule (amine, amino acid, and N-terminal cap) and numerous submicromolar CCR1 binders were discovered. Of these compounds, **48** proved to be the most viable lead. This compound was shown to be an antagonist of the CCR1 ligands it was tested against and demonstrated good functional activity in a cell migration assay (IC_{50} 78 nM, Table 8).

Mouse pharmacokinetic values for **48** are listed in Table 9. A clearance of 12.5 mL/min/kg, distribution volume of 4.8 L/kg, AUC of 5933 nM·h, and a half-life of 6.9 h were observed for an intravenous dose of 2.5 mg/kg. An orally administered dose of 5.2 mg/kg provided a C_{max} of 3129 nM, AUC of 6640 nM·h, half-life of 5.2 h, and a bioavailability of 52%.

dx.doi.org/10.1021/jm300896d | J. Med. Chem. 2012, 55, 9643−9653

Table 6. CCR1 Binding Data for 4-(4-Chlorophenyl)piperidine D-Valine Amide Derivatives



Compound	R^6	CCR1 IC_{50} (nM)	Compound	R^6	CCR1 IC_{50} (nM)
57		70	**69**		13
58		26	**70**		44
59		46	**71**		45
60		20	**72**		115
61		8	**73**		140
62		24	**74**		19
63		118	**75**		22
64		14	**76**		18
65		32	**77**		70
66		25	**78**		105
67		100	**79**		18
68		177	**80**		58

CONCLUSIONS

A series of novel, potent CCR1 inhibitors was developed from the moderately potent lead **6** using an iterative parallel synthesis strategy. SAR data generated for the three sections of the lead series (amine, amino acid, and N-terminal cap) revealed the following: (1) changes to the amine are not well tolerated; (2) small alkylamino acids are preferred in the center of the molecule, with valine providing the broadest and most consistently active compounds tested; (3) alterations at the N-terminus are often well tolerated. Optimization led to

dx.doi.org/10.1021/jm300896d | J. Med. Chem. 2012, 55, 9643−9653

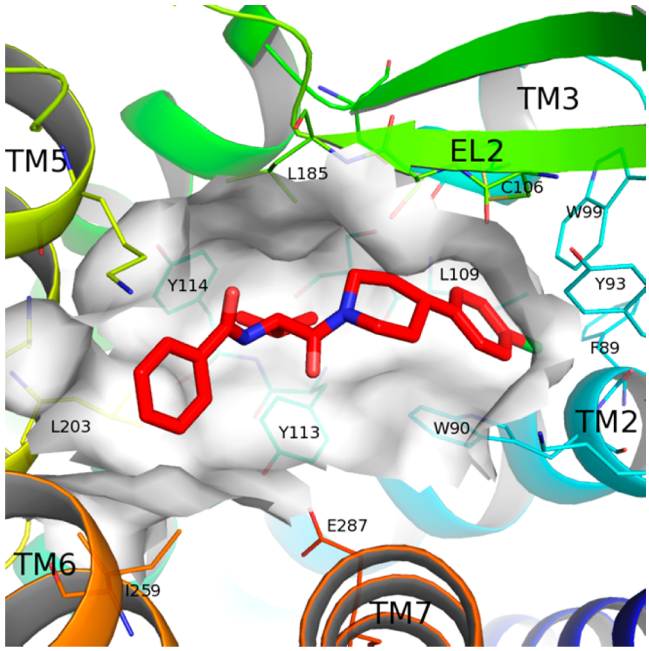


Figure 4. Extracellular view of the binding model of **48** (red tube) in the orthosteric site of a homology model of CCR1. Contact residues are shown as small sticks and labeled with a single letter amino acid code and sequence position. Helices are denoted by TMx and extracellular loop 2 labeled as EL2.

compound **48**, which inhibited CCR1 binding with an IC_{50} of 28 nM, was a functional antagonist of CCR1-mediated cellular migration, was selective against a variety of related CCR-family members, and showed good oral bioavailability.

EXPERIMENTAL SECTION

Materials, Methods, and Equipment. Analytical LC-MS: data for purity and compound identification were obtained with a Shimadzu Discovery VP system equipped with a Waters ZQ mass spectrometer using the following column and conditions: Waters SunFire C18 column (4.6 × 50 mm, 5 um particle size); 10 μL injection volume; 4 mL/min flow rate; 4 min gradient from 0 to 100% B, where A is 1:9 MeOH/H_2O with 0.1% TFA, and B is 9:1 MeOH/H_2O with 0.1% TFA; UV detection at 220 nM; electrospray ionization (positive). Unless otherwise indicated, all compounds tested were of at least 95% purity when tested by this method. Preparative LC-MS: unless otherwise indicated, all compounds were purified with a Shimadzu Discovery VP preparative system using the following column and conditions: Waters SunFire C18 OBD column (19 × 100 mm, 5 μm particle size); 2 mL injection volume; 20 mL/min flow rate; 10 min gradient from 20% to 100% B, where A is 1:9 MeOH/H_2O with 0.1% TFA, and B is 9:1 MeOH/H_2O with 0.1% TFA; UV detection at 220 nM; mass triggered collection by electrospray ionization (positive). ^{1}H NMR: 400 MHz spectra were taken using a Bruker DRX-400 spectrometer; 500 MHz spectra were taken using a JEOL Eclipse spectrometer or a Bruker Avance. Miniblocks were obtained from Mettler-Toledo AutoChem (Columbia, MD). Reagents and solvents were obtained from commercial suppliers and used without further purification. Unless otherwise indicated, all biological assays were run in duplicate.

General Procedure A for the Synthesis of Amide Libraries from an Acid Core. A Miniblock XT was equipped with 16 × 100 mm glass reaction tubes. To each tube were added 120 μL of a 0.5 M stock solution of **8** in a 1:3 mixture of DIEA/DMF, 75 μL of a 1 M solution of HOBt in DMF, and 300 μL of a 0.25 M solution of EDC in DMF. A different amine was then added to each well (300 μL of a 0.25 M solution of amine in DMF). The reactions were agitated overnight on a platform shaker at room temperature. Heterogeneous reaction mixtures were filtered, and the crude mixtures were purified by preparative LC-MS without further workup.

General Procedure B for the Synthesis of Amide Libraries from an Amine Core. A Miniblock XT was equipped with 16 × 100 mm glass reaction tubes. To each tube were added a different acid (300 μL of a 0.25 M solution of acid in DMF), 75 μL of a 1 M solution of HOBt in DMF, and 300 μL of a 0.25 M solution of EDC in DMF. A

Table 8. Additional Data for Compound 48

	48
CCR1 binding IC_{50}, nM (MIP-1α)	28 ± 5
CCR1 Ca flux IC_{50}, nM (MIP-1α)	15[a]
CCR1 Ca flux IC_{50}, nM (CK-8β)	85[a]
CCR1 chemotaxis IC_{50} (MIP-1α), nM	78 ± 10[b]
CCR2 binding IC_{50}, nM	>20 000
CCR3 binding IC_{50}, nM	>30 000
CCR5 binding IC_{50}, nM	>20 000
human LM (% remaining)	100
mouse LM (% remaining)	73
rat LM (% remaining)	83
mouse CCR1 binding IC_{50}, nM (MIP-1α)	>25 000
rabbit CCR1 binding IC_{50}, nM (MIP-1α)	550 ± 125[c]
rat CCR1 binding IC_{50}, nM (MIP-1α)	>30 000
CYP IC_{50}, nM (3A4, 2C9, 2C19, 2D6)	>40 000
hERG patch clamp IC_{50}, nM	7000
solubility, pH 6.5 buffer (mg/mL)	0.001

[a]Based on a single determination. [b]n = 11 determinations. [c]n = 5 determinations.

Table 9. Pharmacokinetic Data for Compound 48[a] in Mice

	iv, 2.5 mg/kg	po, 5.2 mg/kg
CL (mL/min/kg)	12.5	NA
V_{ss} (L/kg)	4.8	NA
C_{max} (nM)	NA	3100
AUC (nM h)	5900	6600
$T_{1/2}$ (h)	6.9	5.2
F%	NA	52

[a]Vehicle: 10% DMA, 30% PEG400, 60% HCl. **48** was insoluble in the dosing vehicle at 1 mg/mL; n = 3 mice.

Table 7. Contact Residues from the CCR1 Model

receptor	89	90	93	99	109	113	114	183	185	203	259	287
CCR1	F	W	Y	W	L	Y	Y	C	L	L	I	E
CCR1 rat	F	W	Y	W	L	Y	Y	C	P	L	V	E
CCR1mouse	F	W	Y	W	L	Y	Y	C	P	L	V	E
CCR1 rabbit	F	W	Y	W	L	Y	Y	C	L	L	L	E
CCR2	F	W	S	W	F	Y	H	C	P	R	I	E
CCR3	F	W	Y	W	L	Y	H	C	A	M	I	E
CCR5	F	W	Y	W	L	Y	F	C	S	I	L	E

dx.doi.org/10.1021/jm300896d | J. Med. Chem. 2012, 55, 9643−9653

solution of **11b** was then added to each well (300 μL of a 0.25 M solution in 1:5 mixture of DIEA/DMF). The reactions were agitated overnight on a platform shaker at room temperature. Heterogeneous reaction mixtures were filtered, and the crude mixtures were purified by preparative LC-MS without further workup.

General Procedure C for the Synthesis of Sulfonamide Libraries. A Miniblock XT was equipped with 16 × 100 mm glass reaction tubes. To each tube were added 75 μmol of a different sulfonyl chloride, 50 μL of DIEA, 300 μL of DCM, and 300 μL of a 0.2 M solution of **11b** in THF. The reactions were agitated overnight on a platform shaker at room temperature. The reaction mixtures were diluted with 1 mL of MeOH, and the crude mixtures were purified by preparative LC-MS without further workup.

General Procedure D for the Synthesis of Carbamate Libraries. A Miniblock XT was equipped with 16 × 100 mm glass reaction tubes. To each tube was added 75 μmol of a different carbamoyl chloride, 250 μL of a 0.2 M solution of **11f** in THF, 250 μL of THF, and 50 μL of DIEA. The reactions were agitated overnight on a platform shaker at room temperature. The reaction mixtures were diluted with 1 mL of MeOH, and the crude mixtures were purified by preparative LC-MS without further workup.

General Procedure E for the Synthesis of Urea Libraries. A Miniblock XT was equipped with 16 × 100 mm glass reaction tubes. To each tube were added 500 μL of a 0.2 M solution of an isocyanate in dioxane and 250 μL of a 0.2 M solution of **11f** in dioxane. The reactions were agitated overnight on a platform shaker at room temperature. The reaction mixtures were diluted with 1 mL of MeOH, and the crude mixtures were purified by preparative LC-MS without further workup.

***N*-(1-(4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl)-3-hydroxy-1-oxobutan-2-yl)benzamide (5).** This compound was resynthesized by the method that follows: HOBt (1.25 equiv), EDC (1.25 equiv), DIEA (3 equiv), and **7** (1.25 equiv) were added to a solution of *N*-benzoyl-L-threonine (56 mg, 0.25 mmol) in 2 mL of 1:1 DCE/DMF. The mixture was stirred for 16 h at rt and then was purified by preparative LC-MS. The fraction containing **5** was concentrated, treated with saturated aqueous $NaHCO_3$, and extracted with $CHCl_3$. The organic portion was then concentrated and dried in vacuo. The crude material was dissolved in MeOH and purified by preparative HPLC (UV collection). ^{1}H NMR (400 MHz, CD_3OD) δ 7.81−8.00 (m, 2H), 7.43−7.67 (m, 5H), 7.26−7.41 (m, 2H), 5.06−5.20 (m, 1H), 4.53 (br d, J = 12.72 Hz, 1H), 4.06−4.32 (m, 2H), 3.58−3.77 (m, 1H), 3.13−3.28 (m, 1H), 1.91−2.20 (m, 2H), 1.71−1.90 (m, 2H), 1.22−1.39 (m, 3H); purity 93%; LC-MS (ESI) 417.23 m/z $(M + H)^+$.

***N*-(1-(4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl)-1-oxobut-2-en-2-yl)benzamide (6).** Isolated as a byproduct from the synthesis **5**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.84 (s, 1H), 7.90−8.03 (m, 2H), 7.45−7.61 (m, 6H), 7.36−7.44 (m, 2H), 5.51−5.63 (m, 1H), 5.20−5.30 (m, 1H), 4.15−4.27 (br m, 1H), 4.02−4.12 (br m, 1H), 3.40−3.55 (m, 1H), 3.14−2.98 (br m, 1H), 2.01−2.15 (br m, 1H), 1.72−1.79 (m, 3H), 1.47−1.64 (m, 2H); LC-MS (ESI) 399.26 m/z $(M + H)^+$.

***N*-(1-(4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl)-3-methyl-1-oxobutan-2-yl)benzamide (12).** Prepared from commercially available **7** and **8** according to general procedure A. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.45−8.58 (m, 1H), 7.88−7.94 (m, 2H), 7.33−7.57 (m, 7H), 5.29 (d, J = 11.39 Hz, 1H), 4.74−4.82 (m, 1H), 4.38 (br d, J = 12.39 Hz, 1H), 4.01−4.19 (m, 1H), 3.40−3.53 (m, 1H), 2.92−3.09 (m, 1H), 2.15−2.28 (m, 1H), 1.80−1.94 (m, 1H), 1.63−1.80 (m, 2H), 1.49−1.63 (m, 1H), 0.89−1.01 (m, 6H); LC-MS (ESI) 437.24 m/z $(M + Na)^+$.

***N*-(1-(4-(4-Chlorophenyl)piperidin-1-yl)-3-methyl-1-oxobutan-2-yl)benzamide (21).** Prepared from commercially available **8** and **9** according to general procedure A. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.45−8.55 (m, 1H), 7.84−7.96 (m, 2H), 7.50−7.60 (m, 1H), 7.42−7.50 (m, 2H), 7.30−7.40 (m, 2H), 7.28 (d, J = 8.92 Hz, 1H), 7.21 (d, J = 8.42 Hz, 1H), 4.72−4.85 (m, 1H), 4.59 (br d, J = 12.88 Hz, 1H), 4.24−4.40 (m, 1H), 3.08−3.21 (m, 1H), 2.78−2.88 (m, 1H), 2.60−2.76 (m, 1H), 2.13−2.27 (m, 1H), 1.72−1.87 (m, 2H), 1.35−1.59 (m, 2H), 0.87−1.01 (m, 6H); LC-MS (ESI) 398.20 m/z $(M + H)^+$.

***(R)*-2-Amino-1-(4-(4-chlorophenyl)piperidin-1-yl)-3-methylbutan-1-one (11f, R^5 = iPr).** HOBt (1.1 equiv), EDC (1.1 equiv), DIEA (3 equiv), and **9** (1.1 equiv) were added to a solution of Boc-D-valine (1300 mg, 6.0 mmol) in chloroform (8 mL). The mixture was stirred for 16 h at rt and then was washed with 2% aqueous HCl and saturated aqueous $NaHCO_3$. The organic portion was dried over $MgSO_4$, concentrated, and dried in vacuo to provide a pale yellow gum, which was treated with 20 mL of 4 M HCl in dioxane. The solution was stirred for 2 h at rt, concentrated, and dried in vacuo to provide a waxy yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.34−7.41 (m, 2H), 7.24−7.31 (m, 2H), 4.57 (br d, J = 7.93 Hz, 1H), 3.81−3.94 (m, 1H), 3.08−3.20 (m, 1H), 2.78−2.91 (m, 1H), 2.62−2.75 (m, 1H), 1.76−1.94 (m, 3H), 1.36−1.60 (m, 2H), 0.96 (dd, J = 6.69, 11.64 Hz, 3H), 0.87 (dd, J = 6.94, 17.83 Hz, 3H); LC-MS (ESI) 295.15 $(M + H)^+$.

***(R)*-*N*-(1-(4-(4-Chlorophenyl)piperidin-1-yl)-3-methyl-1-oxobutan-2-yl)benzamide (48).** HOBt (1.1 equiv), DIEA (3 equiv), and benzoic acid (1.1 equiv) were added to a solution of **11f** (41 mg, 0.13 mmol) in 1 mL of DMF. After 5 min of stirring, EDC (1.1 equiv) was added and the solution was stirred for 16 h. The solution was purified by preparative LC-MS without any workup. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82−7.92 (m, 2H), 7.44−7.59 (m, 3H), 7.26−7.37 (m, 2H), 7.15 (dd, J = 12.7, 8.6 Hz, 2H), 5.15 (ddd, J = 10.4, 8.6, 6.4 Hz, 1H), 4.75−4.88 (m, 1H), 4.25−4.37 (m, 1H), 3.27 (qd, J = 13.0, 2.3 Hz, 1H), 2.68−2.87 (m, 2H), 1.87−2.24 (m, 3H), 1.54−1.82 (m, 2H), 0.98−1.13 (m, 6H); LC-MS (ESI) 399.17 m/z $(M + H)^+$.

CCR1 Binding Assay. For radioligand competition studies, a final concentration of 1 × 10^5 THP-1 monocytic leukemia cells were combined with 100 μg of LS WGA PS beads (Amersham cat. no. RPNQ 0260) in 40 μL of assay buffer (RPMI 1640 without phenol red, 50 mM HEPES, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 0.1% BSA). The THP-1 cell/bead mixture was added to each well of a 384-well assay plate (PerkinElmer cat. no. 6007899) containing test compound in 3-fold serial dilution, with final concentrations ranging from 8 μM to 140 pM. A final concentration of 0.1 nM [^{125}I]-MIP-1α (PerkinElmer cat. no. NEX298) in 20 μL of assay buffer was added to the reaction. Sealed assay plates were incubated at room temperature for 12 h and then analyzed by LEADseeker. Each experiment was run in duplicate. The competition data of the test compound over a range of concentrations was plotted as percentage inhibition of radioligand specific bound in the absence of test compound (percent of total signal). After correction for nonspecific binding, IC_{50} values were determined. The IC_{50} value is defined as the concentration of test compound needed to reduce radioligand specific binding by 50% and is calculated using the four-parameter logistic equation to fit the normalized data. Assay variability was ±15% and was measured using a standard control.

CCR1 Calcium Flux Assay. THP-1 cells were labeled with calcium fluorophore (fluo-3 a.m., Molecular Probes cat. no. F-1241), resuspended in assay buffer containing probenecid, and transferred to plates. Calcium flux was monitored in a FLIPR (Molecular Devices, CA) following addition of MIP-1α (R&D 270-LD, 20 nM). Effects of compound were determined as changes in the base-to-peak excursion relative to control.

CCR1 Chemotaxis Assay. THP-1 cells were fluorescently labeled with calcein-AM (10 μg/mL; Molecular Probes, Eugene, OR). The labeled cells were then washed and resuspended in chemotaxis buffer and added to the top chamber of a chemotaxis plate (Neuroprobe cat. no. 101-8; 8 μm pore size) with human MIP-1α (R&D 270-LD, 1 nM) in the bottom well. Compounds were added to upper and lower wells. The plate was incubated for 60 min at 37 °C after which fluorescence in the lower wells was read at 485 nm/530 nm excitation/emission.

CCR2 Binding Assay. Radioligand competition binding assays were used for assessment of binding affinity of test compounds to the CCR2 receptor. For radioligand competition studies, 100 μL containing 2.5 × 10^5 THP-1 cells/well (in assay buffer containing 50 mM HEPES, pH 7.4, 5 mM $MgCl_2$, 1 mM $CaCl_2$, and 0.5% BSA) were added to 96-well assay plates containing the test compounds in 3-

fold serial dilution, with final concentrations ranging from 5 μM to 100 pM. Subsequently, 50 μL ^{125}I-MCP-1 radioligand at a final concentration of 0.2 nM in assay buffer was added to the reaction. After a 90 min incubation period at room temperature, the binding reaction was terminated by harvesting on GF/B filter plates (PerkinElmer cat. no. 6005177) followed by washing with ice-cold wash buffer (50 mM HEPES, pH 7.4, 0.1% BSA, 0.5 M NaCl) to remove unbound ligand. After washing, the plate was dried for 45 min at 60 °C followed by addition of 40 μL MicroScint 20 scintillation fluid, sealed, and analyzed by a Packard TopCount reader.

CCR3 Binding Assay. Ligand binding to the human CCR3 receptor was determined by a scintillation proximity assay (SPA) established using a CHO CCR3/2 chimeric cell line. Cells were grown in F-10 cell culture medium (Invitrogen cat. no. 11550-043), 10% fetal bovine serum (Invitrogen cat. no. 26140-122), 1% penicillin/streptomycin (Invitrogen cat. no. 15140-122), and 600 μg/mL Geneticin (Invitrogen cat. no. 10131-027). For competition studies, a 96-well Corning plate (Costar cat. no. 3912) containing compounds of interest at concentration−response curves with final concentrations ranging from 40 μM to 0.7 nM (3-fold, 11-point serial dilution) was prepared. A 100 μL volume of assay buffer (25 mM HEPES, pH 7.6, 1 mM $CaCl_2$, 5 mM $MgCl_2$, 120 mM NaCl, 0.5% BSA) containing 2.0×10^5 CHO CCR3/2 cells and 200 μg of PVT-WGA-PS beads (Amersham cat. no. RPNQ0001) were then added to each well of the assay plate. Subsequently, 50 μL of ^{125}I-Eotaxin (PerkinElmer cat. no. NEC314) at a 0.2 nM final concentration was added to the reaction. The assay plate was sealed and incubated for 3 h at room temperature followed by analysis on a Packard TopCount. The control for nonspecific binding was a 100 nM final concentration of Eotaxin (R&D Systems cat. no. 320-EO-020). Each experiment was run in duplicate.

CCR5 Binding Assay. The human CCR5 ligand binding scintillation proximity assay (SPA) was established using the Athersys HT1080 human fibrosarcoma cell line, which stably expresses a RAGE-activated human CCR5 receptor, and ^{125}I-MIP-1β was used as tracer ligand. HT1080-CCR5 cells were grown in alpha MEM (Mediatech cat. no. 15-012-CV) supplemented with 10% dialyzed fetal bovine serum, 2% penicillin/streptomycin/glutamine (Invitrogen cat. no. 0378-016), and 500 μg/mL hygromycin B (Invitrogen cat. no. 25300-062). For CCR5 cell membrane preparation, 1×10^8 cells/pellet were suspended in buffer (50 mM HEPES, 5 mM $MgCl_2$, 1 mM $CaCl_2$), homogenized on ice with a Polytron homogenizer, and centrifuged at 48 000g for 10 min at 4 °C. The cell pellet was resuspended in buffer and protein concentration determined by the Bradford assay. For competition studies, a 96-well Corning plate (Costar cat. no. 3912) containing compounds of interest at concentration−response curves with final concentrations ranging from 40 μM to 0.7 nM (3-fold, 11-point serial dilution) was prepared. Ten micrograms of membrane preparation protein/well and 200 μg/well PVT-WGA-PS beads (Amersham cat. no. RPNQ0001) were mixed in 100 μL total volume and then added to each well of the assay plate. Subsequently, 50 μL of ^{125}I-MIP-1β at 0.1 nM final concentration was added to the reaction. The assay plate was sealed and incubated for 4 h at room temperature followed by analysis on a Packard TopCount.

Liver Microsome Assays. Compound metabolic stability was determined in human, rat, and mouse liver microsomes by a previously reported method.[27]

Cross Species Assays. Binding was assessed using human ^{125}I-MIP-1α (NEN/Perkin-Elmer cat. no. NEX298) diluted to a final concentration of 0.08 nM in assay buffer. Nonspecific binding was determined in the presence of excess unlabeled ligand. Plates were incubated for 60 min at room temperature and washed. After air-drying, filters were removed and gamma-counted. Cell sources for the assays were as follows: mouse, WEHI 274.1 cell line (ATCC cat. no. CRL-1679); rat, thioglycollate-elicited peritoneal cells; rabbit, peripheral blood mononuclear cells.

CYP Assays. Cyp inhibition potential was assayed against a panel of recombinant CYP microsomes (Supersomes) derived from baculovirus-infected insect cells obtained from BD Biosciences (Woburn, MA). A 2.5 mL aliquot of a prewarmed 2-fold-concentrated mixture of appropriate fluorogenic substrate and P450 enzyme in potassium phosphate assay buffer was added to each well of the assay-ready plates (containing 10 nL of test substance). Plates were then prewarmed at 37 °C for 30 min. Reactions were initiated by the addition of 2.5 mL of prewarmed 2-fold-concentrated NADPH-regenerating system in the same assay buffer. Assay plates were incubated at 37 °C. Following incubation, reactions were quenched/stopped by the addition of 3 mL of a quench buffer (80% acetonitrile, 20% 0.5 M TRIS-base).

hERG Patch Clamp Assay. A whole-cell patch-clamp was used to directly measure hERG tail currents in HEK-293 cells stably expressing the cloned hERG potassium channel α subunit. Effects of compounds were calculated by measuring inhibition of peak tail current. Experiments were carried out using an aqueous buffer with pH 7.4 at room temperature. There was no protein in the assay buffer.

Solubility. Aqueous solubility was determined using a thermodynamic equilibrium solubility assay. A solution of 0.5 to 0.7 mg of the compound of interest was dissolved in 5 mL of methanol (or DMSO/methanol if required), diluted in a serial fashion, and used to create a standard calibration curve from the UV data obtained by HPLC analysis at 210 or 254 nM. A test sample was created by adding 1 mL of aqueous potassium phosphate (50 mM, pH 6.5) to 1 mL of the standard solution. This solution was vortexed, sonicated for 30 s, and then agitated on an orbital shaker for 15−24 h at room temperature. The sample was then centrifuged for 2 min at 10 000 rpm and analyzed by HPLC with the same method as that used for the standard. Solubility was determined by comparison of the result with the standard calibration curve.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Details of intermediate compound synthesis and analytical data (^{1}H NMR and LC-MS) for the remaining compounds from the tables. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: (609) 252-4170; e-mail: cullen.cavallaro@bms.com.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors thank Gerry Everlof for providing solubility data, Bob Grafstrom for his contributions to the mutagenesis work, and Mian Gao for the plasmid production performed in support of the mutagenesis effort.

■ ABBREVIATIONS USED

CCR1, CC chemokine receptor 1; CHO, Chinese hamster ovary; DIEA, *N,N*-diisopropylethylamine; EDC, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide; FLIPR, fluorometric imaging plate reader; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HOBt, 1-hydroxybenzotriazole; LM, liver microsomes; MCP, monocyte chemotactic protein; MIP, macrophage inflammatory protein; RANTES, regulated upon activation normal T-cell expressed and secreted, also known as CC chemokine ligand 5 (CCL5)

■ REFERENCES

(1) Allen, S. J.; Crown, S. E.; Handel, T. M. Chemokine:receptor structure, interactions, and antagonism. *Annu. Rev. Immunol.* **2007**, *25*, 787−820.

(2) Viola, A.; Luster, A. D. Chemokines and their receptors: drug targets in immunity and inflammation. *Annu. Rev. Pharmacol. Toxicol.* **2008**, *48*, 171−197.
(3) Horuk, R. Chemokine receptor antagonists: overcoming developmental hurdles. *Nat. Rev. Drug Discovery* **2009**, *8*, 23−33.
(4) Schall, T. J.; Proudfoot, A. E. Overcoming hurdles in developing successful drugs targeting chemokine receptors. *Nat. Rev. Immunol.* **2011**, *11*, 355−363.
(5) Horuk, R. Targeting CCR1. *Methods Princ. Med. Chem.* **2011**, *46* (Chemokine Receptors as Drug Targets), 323−338.
(6) Review: Cheng, J.-F.; Jack, R. CCR1 antagonists. *Mol. Diversity* **2008**, *12*, 17−23.
(7) For leading references to patent and public literature since that described in ref 6, see: (a) Carter, P. H.; Hynes, J. N-aryl pyrazoles, indazoles and azaindazoles as antagonists of CC chemokine receptor 1: patent cooperation treaty applications WO2010/036632, WO2009/134666 and WO2009/137338. *Expert Opin. Ther. Pat.* **2010**, *20*, 1609−1618. (b) Merritt, J. R.; James, R.; Paradkar, V. M.; Zhang, C.; Liu, R.; Liu, J.; Jacob, B.; Chiriac, C.; Ohlmeyer, M. J.; Quadros, E.; Wines, P.; Postelnek, J.; Hicks, C. M.; Chen, W.; Kimble, E. F.; O'Brien, L.; White, N.; Desai, H.; Appell, K. C.; Webb, M. L. Novel pyrrolidine heterocycles as CCR1 antagonists. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5477−5479.
(8) Gladue, R. P.; Brown, M. F.; Zwillich, S. H. CCR1 antagonists: what have we learned from clinical trials. *Curr. Top. Med. Chem.* **2010**, *10*, 1268−1277.
(9) 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. Identification and characterization of a potent, selective, and orally active antagonist of the CC chemokine receptor-1. *J. Biol. Chem.* **2000**, *275*, 19000−19008.
(10) 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. CP-481,715, a potent and selective CCR1 antagonist with potential therapeutic implications for inflammatory diseases. *J. Biol. Chem.* **2003**, *278*, 40473−40480.
(11) Structure suggested from a patent analysis, as described in: Norman, P. AZD-4818, a chemokine CCR1 antagonist: WO2008103126 and WO2009011653. *Expert Opin. Ther. Pat.* **2009**, *19*, 1629−1633.
(12) Lu, C.; Balani, S. K.; Qian, M. G.; Prakash, S. R.; Ducray, P. S.; von Moltke, L. L. Quantitative prediction and clinical observation of a CYP3A inhibitor-based drug-drug interactions with MLN3897, a potent C-C chemokine receptor-1 antagonist. *J. Pharm. Exp. Ther.* **2010**, *332*, 562−568.
(13) Haringman, J. J.; Kraan, M. C.; Smeets, T. J. M.; Zwinderman, A. H.; Tak, P. P. Chemokine blockade and chronic inflammatory disease: proof of concept in patients with rheumatoid arthritis. *Ann. Rheum. Dis.* **2003**, *62*, 715−721.
(14) Borregaard, J.; Skov, L.; Wang, L.; Ting, N.; Wang, C.; Beck, L. A.; Sonne, J.; Clucas, A. Evaluation of the effect of the specific CCR1 antagonist CP-481715 on the clinical and cellular responses observed following epicutaneous nickel challenge in human subjects. *Contact Dermatitis* **2008**, *59*, 212−219.
(15) Vergunst, C. E.; Gerlag, D. M.; von Moltke, L.; Karol, M.; Wyant, T.; Chi, X.; Matzkin, E.; Leach, T.; Tak, P. P. MLN3897 plus methotrexate in patients with rheumatoid arthritis: safety, efficacy, pharmacokinetics, and pharmacodynamics of an oral CCR1 antagonist in a phase IIa, double-blind, placebo-controlled, randomized, proof-of-concept study. *Arthritis Rheum.* **2009**, *60*, 3572−3581.
(16) 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. CCR1 Antagonist Study Group. Blockade of chemokine signaling in patients with multiple sclerosis. *Neurology* **2006**, *67*, 1880−1883.
(17) Kerstjens, H. A.; Bjermer, L.; Eriksson, L.; Dahlstrom, K.; Vestbo, J. Tolerability and efficacy of inhaled AZD4818, a CCR1 antagonist, in moderate to severe COPD patients. *Respir. Med.* **2010**, *104*, 1297−1303.
(18) Lebre, M. C.; Vergunst, C. E.; Choi, I. Y. K.; Aarrass, S.; Oliveira, A. S. F.; Wyant, T.; Horuk, R.; Reedquist, K. A.; Tak, P. P. Why CCR2 and CCR5 blockade failed and why CCR1 blockade might still be effective in the treatment of rheumatoid arthritis. *PLoS One* **2011**, *6*, e21772.
(19) The structure has not been formally disclosed, but several patent applications from Chemocentryx claim a single compound. For example, see: Li, L.; Pennell, A. M. K.; Zhang, P. 3-(Imidazolyl)-pyrazolo[3,4b]-pyridines. US Patent application US 2010/0173911 A1.
(20) Dairaghi, D. J.; Zhang, P.; Wang, Y.; Seitz, L. C.; Johnson, D. A.; Miao, S.; Ertl, L. S.; Zeng, Y.; Powers, J. P.; Pennell, A. M.; Bekker, P.; Schall, T. J.; Jaen, J. C. Pharmacokinetic and pharmacodynamic evaluation of the novel CCR1 antagonist CCX354 in healthy human subjects: Implications for selection of clinical dose. *Clinical Pharm. Ther.* **2011**, *89*, 726−734.
(21) CCX354 is listed as in an active clinical trial on www.clinicaltrials.gov (accessed Aug 10, 2011). See trial identifier NCT01242917, "A Study to Evaluate the Safety and Efficacy of CCX354-C in Subjects With Rheumatoid Arthritis Partially Responsive to Methotrexate Therapy (CARAT-2)".
(22) Tak, P. P.; Balanescu, A.; Tseluyko, V.; Bojin, S.; Drescher, E.; Dairaghi, D.; Miao, S.; Marchesin, V.; Jaen, J.; Bekker, P.; Schall, T. J. Safety and efficacy of oral chemokine receptor 1 antagonist CCX354-C in a phase 2 rheumatoid arthritis study. Presented at the ACR/ARHP Scientific Meeting, Chicago, IL, November 5−9, 2011; Presentation L11.
(23) (a) Xie, Y. F.; Sircar, I.; Lake, K.; Komandla, M.; Ligsay, K.; Li, J.; Xu, K.; Parise, J.; Schneider, L.; Huang, D.; Liu, J.; Sakurai, N.; Barbosa, M.; Jack, R. Identification of novel series of human CCR1 antagonists. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2215−2221. (b) 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. Novel pyrrolidine ureas as C-C chemokine receptor 1 (CCR1) antagonists. *J. Med. Chem.* **2009**, *52*, 1295−1301.
(24) For chemotypes that show selectivity for the human receptor over the murine receptor, see: (a) Naya, A.; Sagara, Y.; Ohwaki, K.; Saeki, T.; Ichikawa, D.; Iwasawa, Y.; Noguchi, K.; Ohtake, N. Design, synthesis, and discovery of a novel CCR1 antagonist. *J. Med. Chem.* **2001**, *44*, 1429−1435. (b) Xie, Y. F.; Lake, K.; Ligsay, K.; Komandla, M.; Sircar, I.; Nagarajan, G.; Li, J.; Xu, K.; Parise, J.; Schneider, L.; Huang, D.; Liu, J.; Dines, K.; Sakurai, N.; Barbosa, M.; Jack, R. Structure-activity relationships of novel, highly potent, selective, and orally active CCR1 antagonists. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3367−3372. Chemotypes are also known that show little selectivity between the human and murine receptors; for example, see: Revesz, L.; Bollbuck, B.; Buhl, T.; Eder, J.; Esser, R.; Feifel, R.; Heng, R.; Hiestand, P.; Jachez-Demange, B.; Loetscher, P.; Sparrer, H.; Schlapbach, A.; Waelchli, R. Novel CCR1 antagonists with oral activity in the mouse collagen induced arthritis. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5160−5164.
(25) (a) Liang, M.; Rosser, M.; Ng, H. P.; May, K.; Bauman, J. G.; Islam, I.; Ghannam, A.; Kretschmer, P. J.; Pu, H.; Dunning, L.; Snider, R. M.; Morrissey, M. M.; Hesselgesser, J.; Perez, H. D.; Horuk, R. Species selectivity of a small molecule antagonist for the CCR1 chemokine receptor. *Eur. J. Pharmacol.* **2000**, *389*, 41−49. (b) Onuffer, J.; McCarrick, M. A.; Dunning, L.; Liang, M.; Rosser, M.; Wei, G.-P.; Ng, H.; Horuk, R. Structure function differences in nonpeptide CCR1 antagonists for human and mouse CCR1. *J. Immunol.* **2003**, *170*, 1910−1916.
(26) Vaidehi, N.; Schlyer, S.; Trabanino, R. J.; Floriano, W. B.; Abrol, R.; Sharma, S.; Kochanny, M.; Koovakat, S.; Dunning, L.; Liang, M.; Fox, J. M.; Lopes de Mendonca, F.; Pease, J. E.; Goddard, W. A., III;

Horuk, R. Predictions of CCR1 chemokine receptor structure and BX 471 antagonist binding followed by experimental validation. *J. Biol. Chem.* **2006**, *281*, 27613−27620.

(27) Wu, S. C.; Yoon, D.; Chin, J.; van Kirk, K.; Seethala, R.; Golla, R.; He, B.; Harrity, T.; Kunselman, L. K.; Morgan, N. N.; Ponticiello, R. P.; Taylor, J. R.; Zebo, R.; Harper, T. W.; Li, W.; Wang, M.; Zhang, L.; Sleczka, B. G.; Nayeem, A.; Sheriff, S.; Camac, D. M.; Morin, P. E.; Everlof, J. G.; Li, Y.-X.; Ferraro, C. A.; Kieltyka, K.; Shou, W.; Vath, M. B.; Zvyaga, T. A.; Gordon, D. A.; Robl, J. A. Discovery of 3-hydroxy-4-cyano-isoquinolines as novel, potent, and selective inhibitors of human 11β-hydroxydehydrogenase 1 (11β-HSD1). *Bioorg. Med. Chem. Lett.* **2011**, *21*, 6693−6698.