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Bioorganic & Medicinal Chemistry Letters 18 (2008) 2215–2221
Bioorganic & Medicinal Chemistry Letters

Identification of novel series of human CCR1 antagonists

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Received 14 June 2007; revised 19 September 2007; accepted 19 September 2007
Available online 25 September 2007

Abstract—A hit-to-lead optimization process was carried out on the high throughput screening hit compound **1** resulting in the identification of several potent and selective CCR1 receptor antagonists. Compound **37** shows the best overall profile with IC_{50} values of <100 nM in binding and functional assays.

Chemokines are a growing family of proteins that play an important role in leukocyte activation and migration.[1–3] They are divided into four subfamilies: CC, CXC, XC, and CX3C defined by the position of the conserved cysteine residues near the N terminus. The chemokines CCL3 (MIP-1α) and CCL5 (RANTES) bind to their shared receptor CCR1, which is expressed on a number of cell types including monocytes, macrophages, dendritic cells, and T cells. A substantial body of evidence has linked the chemokine receptor CCR1 and its major ligands to the pathogenesis of chronic inflammatory diseases including rheumatoid arthritis (RA),[4,5] multiple sclerosis (MS),[6,7] and transplant rejection.[8,9] The first clinical proof-of-concept in RA was obtained with a small molecule CCR1 antagonist that showed marked decrease in synovial inflammation and trend toward a decrease in clinical disease activity after 2 weeks of treatment.[10] Severe side effects were not reported from these studies. These results illustrate the therapeutic potential of small molecule CCR1 antagonists in these diseases, and thus make CCR1 an attractive target for drug discovery research. Here we report our work directed toward the identification of novel small molecule CCR1 antagonists.

Several chemotypes have been described as CCR1 antagonists: benzylpiperazines I (BX-471)[9,11] and II,[12] xanthene-9-carboxamides (III),[13] 4-hydroxypiperidines (IV and VI),[14,15] and hydroxyethylene peptide isosteres (VII)[16] (Chart 1). BX-471 and compound VI were advanced to the clinic for multiple sclerosis and rheumatoid arthritis, respectively. To identify novel chemotypes we screened a library of 4277 compounds using a fluorescent calcium flux as measured by fluoresecent imaging plate reader (FLIPR).[17] The initial 12 screening hits were confirmed by a competitive receptor binding assay.[18] The hydroxypiperidine compound **1** emerged as the best hit with moderate activity (IC_{50} < 6 μM; Fig. 1). Hit-to-lead modification strategies were developed around compound **1** to increase potency.

Strategically, compound **1** was divided into four discrete areas: hydrophobe 1 (right aromatic), amide functionality, aliphatic linker, and hydrophobe 2 (4-hydroxy-4-phenylpiperidine (Fig. 2)). Systematic structure–activity relationship (SAR) studies were conducted in each portion to improve the potency. Initial efforts were focused on the hydrophobe 1 and the amide functionality. To that end, 1-(3-aminopropyl)-4-(4-chlorophenyl)piperi-

Keywords: Chemokine receptor 1; CCR1 antagonist.
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0960-894X/$ - see front matter © 2008 Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2007.09.068

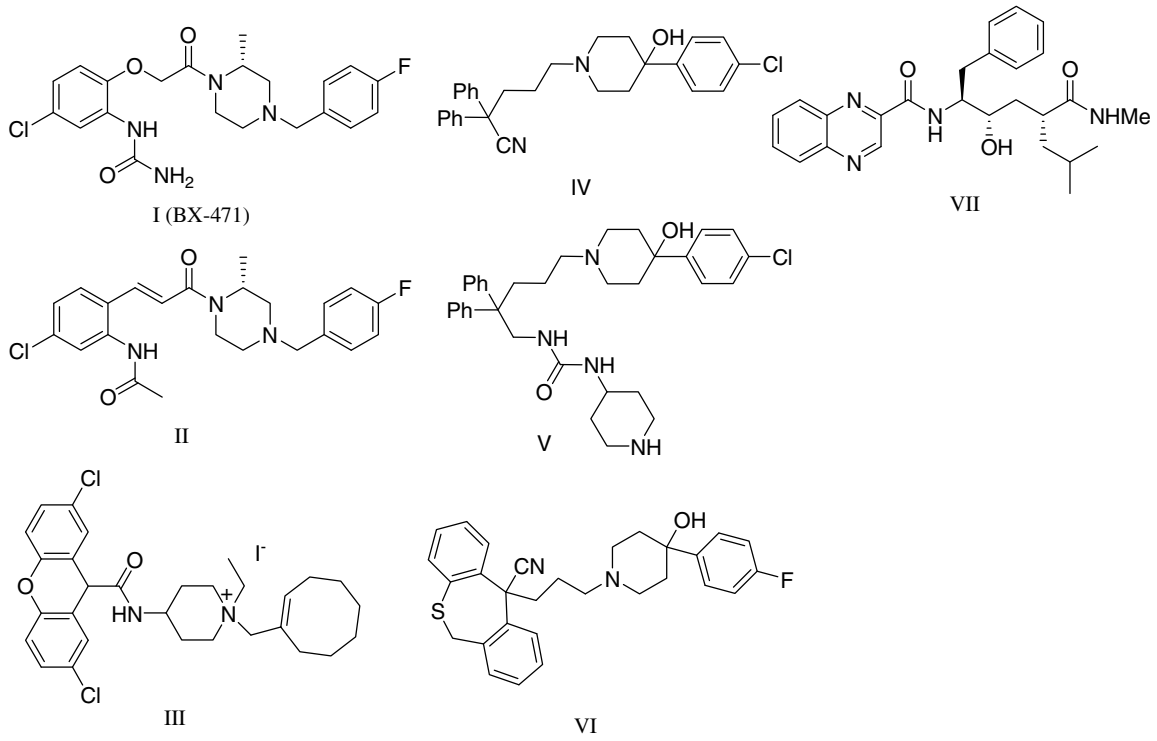


Chart 1. Structure of prototype CCR1 antagonists.

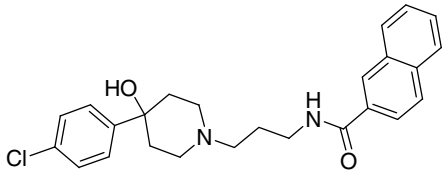


Figure 1. Structure of HTS hit.

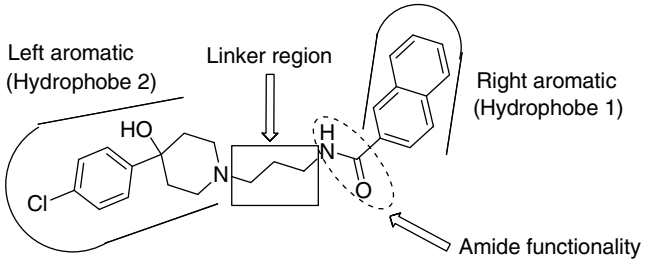
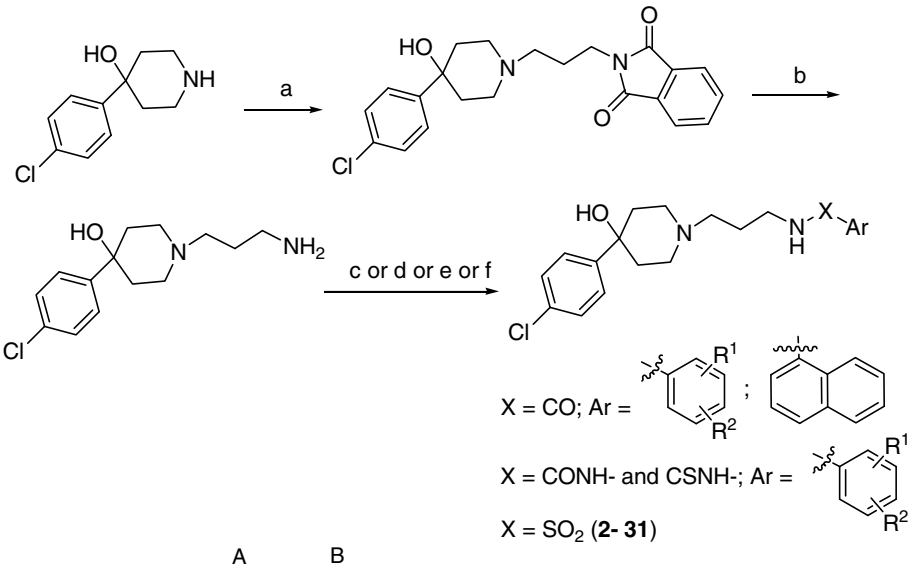


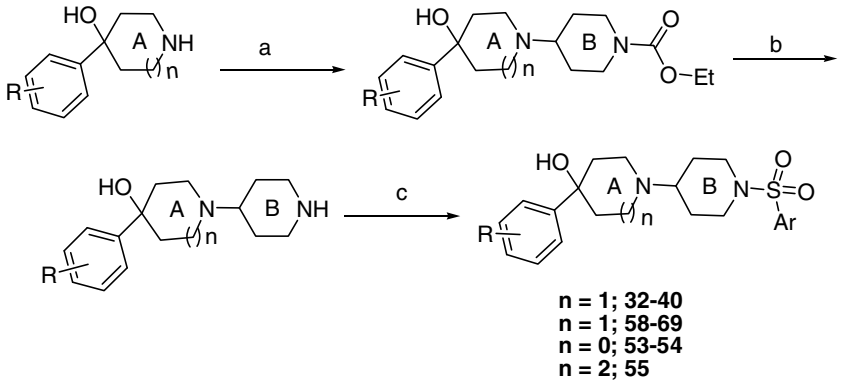
Figure 2. Hit-to-lead optimization strategy.

din-4-ol prepared in two steps[19] was derivatized via standard acylation reactions to provide amides and sulfonamides, **2**–**31** (Scheme 1). A small set of urea/thiourea compounds was prepared via the reaction of the above amine with desired isocyanates and isothiocyanates, respectively (Scheme 1). Compounds **32**–**40** were prepared from 4-(4-chlorophenyl)-1,4′-bipiperidin-4-ol by the treatment with requisite sulfonyl chlorides in a similar fashion (Scheme 2). The 4′-methyl substituted bipiperidine analogs **41**–**44** were synthesized from the corresponding amine, (4-chlorophenyl)-4′-methyl-1,4′-bipiperidin-4-ol[20] (Scheme 3). The synthesis of the corresponding 2′-methyl analogs, **45** and **46**, was accomplished in two steps starting from 2-methylpiperidin-4-one as shown in Scheme 4. The enantiomerically pure 1,3′-bipiperidin-4-ol analogs (**47**–**52**) were prepared by starting with chiral 4-(4-chlorophenyl)-1,3′-bipiperidin-4-ol intermediate (Scheme 5). Compounds (**53**–**55**) wherein the piperidine ring (A) was changed to pyrrolidine[21] and azepine[22] ring systems were prepared starting from the requisite amines in a manner similar to compounds in Scheme 2. Compounds **56** and **57** in which the hydroxyl group in the piperidine ring (A) was replaced with CN group were prepared from the requisite amine[23] according to Schemes 1 and 2, respectively. Compounds **58**–**69** were prepared by methods similar to Scheme 2.

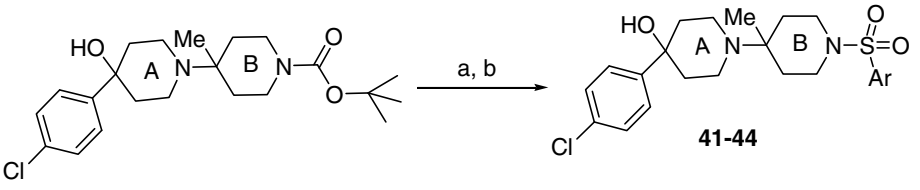
The analogs prepared in this study were evaluated for their inhibitory activity against ^{125}I-MIP-1α binding to human CCR1[18] and functional antagonist activity (Ca^{2+} flux) in MIP-1α stimulated THP-1 cells.[17] Initially, a small focused library was generated varying the right hand aromatic of the hit compound **1**, which offered no improvement in activity. Replacement of the amide group with other functionalities (e.g., urea, thiourea, and reverse amides; data not shown) resulted in compounds with significant loss in activity. Compound **2**, the sulfonamide analog of **1**, showed about 3-fold improvement in activity which was the starting point for detailed SAR studies. Table 1 lists all sulfonamide derivatives in the aminopropyl series. Compound **3**, the 1-naphthyl analog of **2**, retained similar potency.



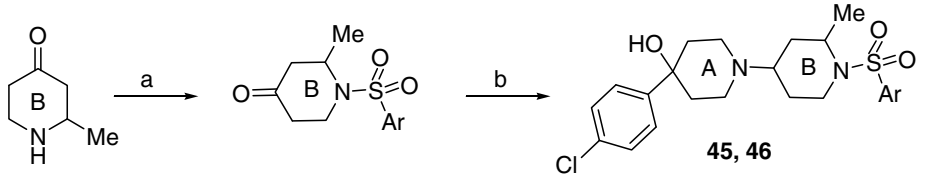
Scheme 1. Reagents and conditions: (a) *N*-(3-bromopropyl)phthalimide, Cs_2CO_3, KI, DMF, rt, 18 h; (b) H_2NNH_2, MeOH, rt, 18 h; (c) ArCOCl, DIEA, CH_2Cl_2, rt, 1 h; (d) ArNCO, DIEA, CH_2Cl_2, rt, 18 h; (e) ArNCS, DIEA, CH_2Cl_2, rt, 18 h; (f) $ArSO_2Cl$, DIEA, CH_2Cl_2, rt, 1 h.



Scheme 2. Reagents and conditions: (a) ethyl 4-oxopiperidine-1-carboxylate, $Na(OAc)_3BH$, CH_2Cl_2, rt, overnight; (b) H_2NNH_2, 50% aq KOH, EtOH, reflux, 18 h; (c) $ArSO_2Cl$, DIEA, CH_2Cl_2, rt, 1.5 h.

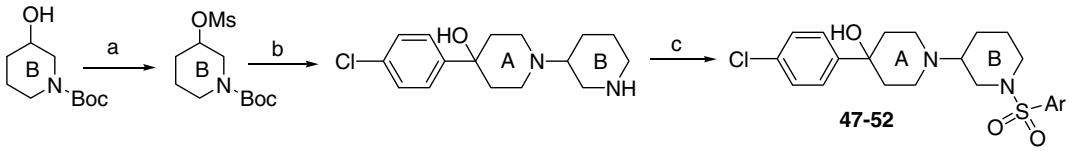


Scheme 3. Reagents and conditions: (a) 50% TFA in CH_2Cl_2, rt, 30 min; (b) $ArSO_2Cl$, DIEA, CH_2Cl_2, rt, 1.5 h.



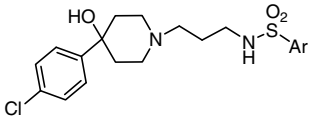
Scheme 4. Reagents and conditions: (a) $ArSO_2Cl$, DIEA, CH_2Cl_2, rt, 1.5 h; (b) 4-(4-chlorophenyl)piperidin-4-ol, $Na(OAc)_3BH$, CH_2Cl_2, rt, 18 h.

The naphthyl moiety in **3** could favorably be replaced with the phenyl (**4**) with a 2-fold improvement in potency. The substitution on the phenyl ring was evaluated next. Addition of halogens (**5**, **6**, and **9**) in the 2-position of **4** maintained potency; halogens at the 4-position were less favored (compare **8** and **10** vs **6** and **9**). Similar ef-



Scheme 5. Reagents and conditions: (a) MsCl, TEA, THF, 0 °C, 3 h; (b) 4-(4-chlorophenyl)piperidin-4-ol, DMSO, 100 °C, 18 h; (c) $ArSO_2Cl$, DIEA, CH_2Cl_2, rt, 2 h.

Table 1. SAR of substitution on the aryl moiety in the aminopropyl series



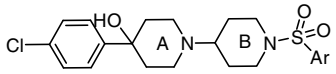
Compound	Ar	CCR1 binding[a] IC_{50} (μM)	Ca^{2+} flux[b] IC_{50} (μM)
2	2-Naphthyl	1.15	0.52
3	1-Naphthyl	0.68	0.18
4	Ph	0.30	0.09
5	2-Br–Ph	0.14	0.17
6	2-Cl–Ph	0.11	0.04
7	3-Cl–Ph	0.38	0.10
8	4-Cl–Ph	0.80	0.37
9	2-F–Ph	0.58	0.08
10	4-F–Ph	0.91	0.21
11	2-Me–Ph	0.15	0.03
12	4-Me–Ph	0.43	0.12
13	2-CF_3–Ph	0.25	0.05
14	4-CF_3–Ph	9.4	2.2
15	2-OCF_3–Ph	0.35	0.19
16	4-OCF_3–Ph	9.0	3.65
17	4-OMe–Ph	3.88	0.83
18	2-Ph–Ph	0.6	0.084
19	4-Ph–Ph	>30	>30
20	2-Th	0.16	0.07
21	3-Th	0.57	0.385
22	2-(2-Th)–Ph	0.24	0.23
23	2-(3-Th)–Ph	0.35	0.11
24	2,3-DiCl–Ph	0.51	0.35
25	2,4-DiCl–Ph	0.41	0.36
26	2,5-DiCl–Ph	0.54	0.28
27	3,4-DiCl–Ph	1.85	2.46
28	3,5-DiCl–Ph	0.78	1.34
29	2,6-DiCl–Ph	0.08	0.03
30	2-Cl, 4-F–Ph	0.36	0.10
31	2-Cl, 6-Me–Ph	0.09	0.04

[a] Results shown are mean values of triplicate samples in a single experiment.
[b] Results shown are the mean values of at least two independent experiments.

fects were observed with Me, CF_3, OCF_3, and Ph substitutions at 2-position (**11**, **13**, **15**, and **18**) versus 4-position (**12**, **14**, **16**, and **19**) of the phenyl ring (**4**), respectively. The complete loss in potency of **19** implicates limited space around that site. Replacement of the phenyl in **4** by thienyl (Th) was tolerated; the 2-thienyl analog **20** was slightly more potent than the 3-thienyl analog **21**. Compounds **22** and **23**, the 2- and 3-thienyl substituted phenyl analogs, retained potency similar to the 2-biphenyl analog **18**. Among the disubstituted compounds, 2,6-diCl (**29**) and 2-Cl, 6-Me (**31**) showed the best potency (IC_{50} values of <100 nM in binding and Ca^{2+} flux assays) (Table 1).

Compounds in the 1,4-bipiperidine linker series (Table 2) were, in general, slightly less potent than the aminopropyl linker series. Best activity was seen with the 2-Cl (**33**) and 2-Br (**37**) substituted analogs, respectively. From these results it was apparent that the SAR trend in the aminopropyl series could be applied to the bipiperidine series and other variations to the bipiperidine

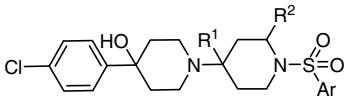
Table 2. SAR of substitution on the aryl moiety in the 1,4′-bipiperidin-4-ol linker series



Compound	Ar	CCR1 binding[a] IC_{50} (μM)	Ca^{2+} flux[b] IC_{50} (μM)
32	Ph	1.27	1.24
33	2-Cl–Ph	0.20	0.13
34	2,6-DiCl–Ph	0.42	0.42
35	2-Me–Ph	0.5	0.18
36	2-Cl, 6-Me–Ph	0.37	0.43
37	2-Br–Ph	0.09	0.09
38	2-Br, 4,6-DiF–Ph	1.12	0.33
39	2-Th	0.97	1.02
40	2,3,4-TriCl–Ph	3.6	0.67

[a] Results shown are mean values of triplicate samples in a single experiment.
[b] Results shown are the mean values of at least two independent experiments.

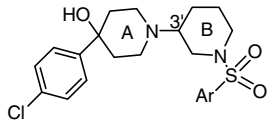
Table 3. SAR of substitution on the B-ring of the 1,4′-bipiperidin-4-ol linker



Compound	Ar	R^1	R^2	CCR1 binding[a] IC_{50} (μM)	Ca^{2+} flux[b] IC_{50} (μM)
41	2-Cl–Ph	Me	H	5.15	2.26
42	2,6-DiCl–Ph	Me	H	4.2	4.65
43	2-Cl, 6-Me–Ph	Me	H	3.57	2.26
44	2-Th	Me	H	>10	7.33
45	2-Cl–Ph	H	Me	0.32	0.28
46	2-Br–Ph	H	Me	0.22	0.12

[a] Results shown are mean values of triplicate samples in a single experiment.
[b] Results shown are the mean values of at least two independent experiment.

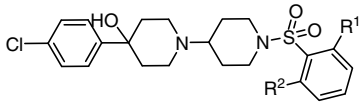
Table 4. SAR of substitution on the aryl moiety in the 1,3′-bipiperidin-4-ol linker series



Compound	3′-Enantiomer	Ar	CCR1 binding[a] IC_{50} (μM)	Ca^{2+} flux[b] IC_{50} (μM)
47	R	2-Cl, 6-Me–Ph	0.39	0.27
48	S	2-Cl, 6-Me–Ph	0.43	0.48
49	R	2-Cl–Ph	1.98	1.29
50	S	2-Cl–Ph	1.9	2.04
51	R	2-Br–Ph	1.4	1.4
52	S	2-Br–Ph	0.46	1.3

[a] Results shown are mean values of triplicate samples in a single experiment.
[b] Results shown are the mean values of at least two independent experiments.

Table 5. Effects of hydroxypiperidine ring size modifications



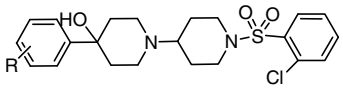
Compound	N	R^1	R^2	CCR1 binding[a] IC_{50} (μM)	Ca^{2+} flux[b] IC_{50} (μM)
33	1	Cl	H	0.201	0.13
53	0	Cl	H	>30	>10
54	0	Cl	Me	>30	>10
55	2	Cl	H	0.40	0.13

[a] Results shown are mean values of triplicate samples in a single experiment.
[b] Results shown are the mean values of at least two independent experiments.

linker were undertaken. Addition of a small group, for example, a methyl in the 4′-position of the piperidine ring B (Table 3), was detrimental (compare **41**–**44** with **33**, **34**, **36**, and **39**) whereas similar substitution at the 2′-position retained potency (**45** and **46** vs **33** and **37**) suggesting the importance of the conformation of the molecule for binding to the receptor.

A small number of enantiomerically pure compounds were prepared in the 1,3′-bipiperidine linker series incorporating the best aryl sulfonamide moieties (Table 4). However, no discrimination between *R*- and *S*-enantiomers was observed with these analogs. The potency was maintained with the 2-Cl, 6-Me di-substituted analogs

Table 6. SAR of substitution on the left aromatic in the 1,4′-bipiperidin-4-ol linker series



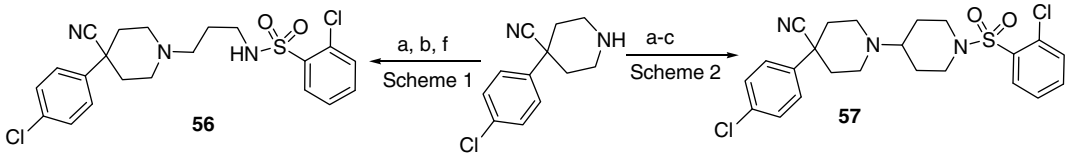
Compound	R	CCR1 binding[a] IC_{50} (μM)	Ca^{2+} flux[b] IC_{50} (μM)
58	4-F	1.17	1.25
59	4-Br	0.44	0.18
60	3-Cl	7.0	6.21
61	3-Cl, 4-F	0.51	0.59
62	3, 4-DiCl	0.89	0.18
63	3-F, 4-Cl	0.43	0.61
64	3,4,5-TriF	1.33	2.55
65	4-*t*-Bu	>10	>10
66	4-Ph	>10	>10
67	4-OMe	>10	>10
68	4-SMe	>10	7.98
69	4-OPh	>10	>10

[a] Results shown are mean values of triplicate samples in a single experiment.
[b] Results shown are the mean values of at least two independent experiments.

(**47** and **48**) and significant decrease was noted with the 2-Cl (**49** and **50**) and 2-Br (**51** and **52**) analogs. One possible explanation could be that the rotation of the phenyl group was more hindered with 2,6-di-substitution, and the molecule was locked in one conformation that resulted in tight binding with the receptor.

Interesting results were observed with the compounds wherein the piperidine (A) ring size was changed. The potency of **53** and **54** (pyrrolidine analogs of **33** and **36**; Table 5) was severely compromised whereas only marginal loss in potency was observed with **55** (azipine analog of **33**).

It is conceivable that the H-bond required for activity could not be realized in **53** and **54** due to the shorter distance between the ring nitrogen and the polar group (OH). By contrast, the slightly longer distance in **55** may still fall within the range of H-bond contact with the receptor. Similar observations were reported in the haloperidol SAR studies.[22] The importance of the –OH group was also recognized from the 5-fold reduction in potency observed with the –CN analogs **56** and **57** (IC_{50} binding: 0.6 and 1 μM) (Scheme 6) in comparison to **6** and **33** (IC_{50} binding: 0.11 and 0.2 μM), respectively. The substitution on the left aromatic ring was investigated next (Table 6). Compound **58**, the 4-F analog of **33** showed 6-fold reduced potency whereas marginal potency loss was observed with the corre-



Scheme 6. Reagents and conditions: see Schemes 1 and 2.

Table 7. Chemotaxis activity of selected compounds

Compound	Chemotaxis[a] IC_{50} (μM)
18	0.151
22	0.296
33	0.183
37	0.042

[a] Results shown are the mean values of at least two independent experiments.

sponding 4-Br analog (**59**). Moving the chloro group to the 3-position of the phenyl ring (**60**) was detrimental; however addition of either a fluorine (**61**) or a chlorine (**62**) at the 4-position in **60** recovered 8- to 14-fold activity. Compound **63**, where the positions of the halogens were switched, retained comparable potency (**63** vs **61**) suggesting the importance of 4-halogen on the phenyl ring. By contrast, replacement of the 4-chloro with the bulky *t*-Bu (**65**) and phenyl (**66**) groups resulted in total loss of affinity, suggesting a space restriction around this site. All other substituents (e.g., OMe, SMe, and OPh) led to inactive compounds. Some of the potency improvement could be lipophilicity driven.

Receptor selectivity against CCR2, CCR3, CCR4, and CCR5 (>100-fold) was confirmed with selected compounds in Table 7. Several analogs retained potency to inhibit CCL3-induced chemotaxis (Table 7).[24] Compound **37** showed the best overall profile.

In summary, hit-to-lead optimization on an in-house hit generated several novel, potent, and selective human CCR1 receptor antagonists. However, these compounds showed weak affinity for the mouse receptor. Further SAR development was discontinued because of other series of actives with improved profile were identified.[25]

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17. THP-1 cells in RPMI-1640 were adjusted to 2×10^6/ml, mixed 1:1 with Calcium Assay Kit buffer (Molecular Devices, Sunnyvale, CA), and incubated for 30 min at 37 °C in the presence of 2.5 mM probenicid (Sigma). Dye-loaded cells were then collected by centrifugation at 1000 rpm for 5 min, resuspended in assay buffer (RPMI-1640 containing 0.1% BSA), and seeded at 1.5×10^5/well on poly-D-lysine-coated 96-well plates (Molecular Devices) with or without test compounds. The plates were centrifuged at 1000 rpm for 5 min. After stimulation with 5 nM human MIP-1α, changes in intracellular free Ca^{2+} concentration were measured using FLIPR (Molecular Devices).
18. THP-1 cells expressing CCR1 were harvested and washed once with PBS. Cells (5×10^6/ml) were mixed with test compound or vehicle and 50 pM human [^{125}I]MIP-1α in a 96-well tissue culture plate in a total volume of 120 μl of binding buffer which contained 50 mM Hepes, pH 7.4, 5 mM $MgCl_2$, 1 mM $CaCl_2$, and 0.5% BSA (Hepes–BSA). The mixture was incubated at room temperature for 90 min and transferred to 96-well glass fiber filter plate pre-coated with 0.5% polyethyleneimine (PEI). Cells were separated by vacuum aspiration and washed twice with Hepes–BSA containing 0.5 M NaCl. Radioactivity was measured on TopCount plate reader with 40 μl Microscint 40 scintillation fluid. Non-specific binding was determined in the presence of 150 nM unlabeled human MIP-1α.
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24. THP-1 cells at 5×10^6/ml were incubated with 1 μM calcein (Molecular Devices) for 30 min at 37 °C. Cells were collected by centrifugation, washed once with RPMI-1640, and resuspended at 5×10^6/ml. The calcein-labeled cells were then mixed with compound or DMSO control for

30 min at 37 °C and loaded in the wells on the top of a 5 μm polycarbonate filter in a 96-well Boyden chamber (NeuroProbe), in which 0.5 nM human MIP-1α with or without compounds had been added to the corresponding wells beneath the filter. The sealed chamber was incubated for 2 h at 37 °C. Non-migrated cells on top of the filter were removed by wiping. The filter was reversed and read at 485/530 nm emission/excitation wavelengths with FlexStation II (Molecular Devices).

25. Yun Feng Xie et al. Unpublished results.