Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 259–264

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Antagonists of the Human CCR5 Receptor as Anti-HIV-1 Agents. Part 1: Discovery and Initial Structure–Activity Relationships for 1-Amino-2-phenyl-4-(piperidin-1-yl)butanes

Conrad P. Dorn,[a] Paul E. Finke,[a,*] Bryan Oates,[a] Richard J. Budhu,[a] Sander G. Mills,[a] Malcolm MacCoss,[a] Lorraine Malkowitz,[b] Martin S. Springer,[b] Bruce L. Daugherty,[b] Sandra L. Gould,[b] Julie A. DeMartino,[b] Salvatore J. Siciliano,[b] Anthony Carella,[c] Gwen Carver,[c] Karen Holmes,[c] Renee Danzeisen,[c] Daria Hazuda,[c] Joseph Kessler,[c] Janet Lineberger,[c] Michael Miller,[c] William A. Schleif[c] and Emilio A. Emini[c]

[a]*Department of Medicinal Chemistry, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065, USA*
[b]*Department of Immunology Research, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065, USA*
[c]*Department of Antiviral Research, Merck Research Laboratories, PO Box 4, West Point, PA 19486, USA*

Received 18 September 2000; accepted 9 November 2000

Abstract—Screening of the Merck sample collection for compounds with CCR5 receptor binding afforded (2*S*)-2-(3,4-dichlorophenyl)-1-[*N*-(methyl)-*N*-(phenylsulfonyl)amino]-4-[spiro(2,3-dihydrobenzthiophene-3,4′-piperidin-1′-yl)]butane *S*-oxide (**4**) as a potent lead structure having an IC_{50} binding affinity of 35 nM. Herein, we describe the discovery of this lead structure and our initial structure–activity relationship studies directed toward the requirement for and optimization of the 1-amino fragment. © 2001 Published by Elsevier Science Ltd.

Human immunodeficiency virus type-1 (HIV-1) is an enveloped virus that must fuse its envelope with the plasma membrane of its host cell to gain cell entry.[1] Early studies had identified the T-lymphoid cell surface protein CD4 as a primary receptor involved in the necessary interaction with the gp120 viral envelope glycoprotein. However, while CD4 is required for HIV-1 entry into cells, CD4 alone was found not to be sufficient.[2] Thus, a search for a co-receptor was initiated.[3] It had been established that HIV-1 isolates can be divided into two general classes depending on their cellular tropism. M-tropic isolates can infect macrophages and primary T-cells, but not T-lymphoid cell lines, and are characterized as non-syncytium-inducing (NSI). T-tropic isolates infect T-lymphoid cell lines, as well as primary T-cells, but not macrophages, and are characterized as syncytium-inducing (SI). In 1995, the chemotactic cytokines (i.e., chemokines) MIP-1α, MIP-1β, and RANTES were reported to be suppressors of HIV-1 cell entry for the M-tropic variants, but not the T-tropic.[4] Subsequently, the chemokine receptor CCR5, which binds MIP-1α, MIP-1β, and RANTES, was identified as a co-receptor specific for the M-tropic HIV-1 isolates.[5–9] Previously, CXCR4, also a chemokine receptor, had been identified as the primary co-receptor for the T-tropic variants.[10] Thus, these co-receptors, along with CD4, interact with the viral gp120 to mediate the initial steps of the virus' host cell infection.

HIV-1 variants that exclusively use the CCR5 co-receptor, now known as R5 variants, largely predominate during the establishment and early stages of the infection in patients, although they appear to remain present throughout the course of the disease. Viral variants that utilize both the CCR5 and CXCR4 co-receptors, R5X4 variants, seem to arise during the course of infection and may be associated with enhanced disease progression. Isolates that exclusively use CXCR4, X4 variants, are rare in patients and arise through adaptation of viral replication to T-lymphoid laboratory cell lines.

The importance of CCR5 for the establishment of the initial infection in humans was demonstrated by human genetic studies of high-risk individuals.[11] Individuals

*Corresponding author. E-mail: paul_finke@merck.com

0960-894X/01/$ - see front matter © 2001 Published by Elsevier Science Ltd.
PII: S0960-894X(00)00637-5

homozygous for a 32-base pair deletion in the gene for CCR5 do not express functional receptor on their cell surfaces and have been identified as being highly resistant to HIV-1 infection,[12] while infected individuals heterozygous for the defective gene appear to exhibit delayed disease progression.[13] Given the importance of CCR5 for the establishment, and possible maintenance, of HIV-1 infection in vivo, and the lack of an overt detrimental phenotype in humans that do not express functional CCR5, we initiated an effort to identify suitable CCR5 antagonists for use as potential anti-HIV-1 therapeutic agents.[14–16]

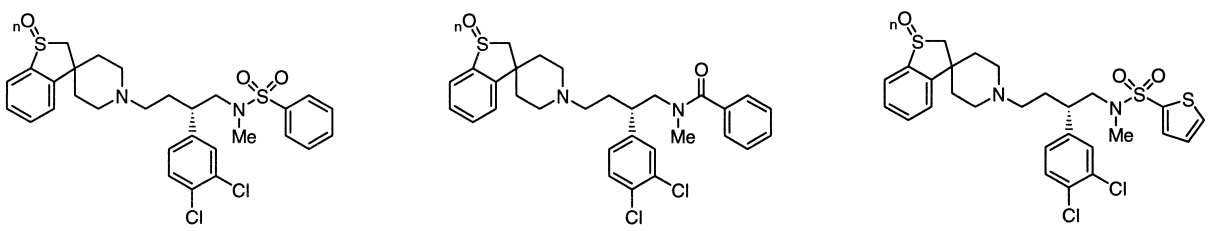
CCR5 is a member of the seven-transmembrane G-protein coupled receptor superfamily and is one of 18 currently known chemokine receptors. There are over 50 reported chemokine ligands that are classified according to the pattern of their first two conserved cysteine residues. The two main groups of chemokines are the CC (or β) chemokines, having adjacent cysteines, and the CXC (or α) chemokines, having one intervening non-cysteine residue.[17]

The identification of CCR5 as a primary HIV-1 co-receptor has initiated a flurry of activity to discover potent antagonists in an effort to validate this mechanism of viral entry as a viable therapeutic target. In addition, there has been an explosion of research in the chemokine area in general with several studies indicating that a potent CCR5 antagonist might also be effective in the clinical treatment of other disorders, such as rheumatoid arthritis,[18] asthma,[19] and multiple sclerosis.[20,21] To date, there have been several reports of CCR5 antagonists in the patent literature.[1,22] The most potent structurally characterized compound is Takeda's TAK-779 (**1**),[23] which is actually a dual CCR5 and CCR2b antagonist having binding affinities of 1.4 and 27 nM, respectively. Herein, the discovery of a series of CCR5 selective 1-(*N*-(alkyl)-*N*-(arylsulfonyl)amino)-2-(3,4-dichlorophenyl)-4-(4-(substituted)piperidin-1-yl)butane structures (**2**) and our initial SAR pertaining to the C-1 *N*-(alkyl)-*N*-(arylsulfonyl)amino moiety of **2** is described.[24]

Once CCR5 was identified as a primary HIV-1 co-receptor, an extensive screening of the Merck sample collection was initiated. To accomplish this, a high-throughput binding assay of [^{125}I]-MIP-1α to stably expressed human CCR5 receptors in Chinese hamster ovary (CHO) cells was developed.[25] From this screening effort, a number of 1-(*N*-alkyl-*N*-phenylsulfonylamino)-2-(3,4-dichlorophenyl)-4-(4-(substituted)piperidin-1-yl)butane derivatives (**2**)[26] were discovered as possible lead structures (see Fig. 1). The best compound identified was (2*S*)-2-(3,4-dichlorophenyl)-1-[*N*-(methyl)-*N*-(phenylsulfonyl)-amino]-4-[spiro(2,3-dihydrobenzthiophene-3,4′-piperidin-1′-yl)]butane *S*-oxide (**4**, 1:1 mixture of sulfoxide diastereomers) with an IC_{50} = 35 nM. The related sulfone **5** was also quite potent with an IC_{50} = 100 nM, while the parent sulfide **3** was much less active (IC_{50} = 1000 nM). The interaction of **4** with the CCR5 receptor was found to be enantioselective at C-2, as evidenced by the much lower affinity of its (2*R*) diastereomers (IC_{50} = 870 nM). The CCR5 binding enantioselectivity of the sulfoxide isomers was not determined. Compounds **4** and **5** were also found to be selective CCR5 antagonists in that their IC_{50} values for CCR1, CCR2, and CCR3 were all greater than 1000 nM (data not shown).

A very important finding was that the analogous amides were essentially inactive against CCR5 as illustrated with the set of compounds **6**–**8**.[26] Thus, these initial binding results seemed to indicate that the sulfonamide has a distinct CCR5 receptor interaction and/or can uniquely provide access to an important orientation for the phenyl moiety which is capable of imparting the desired selective receptor binding interactions. Based on the activity of the 2-thienyl derivatives **9** and **10** (IC_{50} = 65 and 120 nM, respectively), replacement of the phenyl with different heterocyles also appeared reasonable.

Further characterization of these initial leads was also obtained from HIV-1 viral replication inhibition studies in isolated peripheral blood mononuclear cells (PBMCs).[27] In this assay, compounds **4** and **9** showed consistent inhibition with IC_{95} values of 6–12 μM while weaker



3 n = 0 CCR5 IC_{50} = 1,000 nM
4 n = 1 CCR5 IC_{50} = 35 nM
5 n = 2 CCR5 IC_{50} = 100 nM

6 n = 0 CCR5 % I = 0 @ 4,000 nM
7 n = 1 CCR5 % I = 22% @ 4,000 nM
8 n = 2 CCR5 % I = 2% @ 4,000 nM

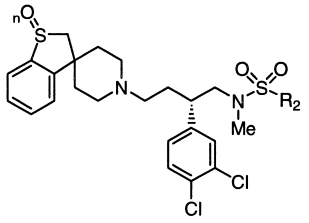
9 n = 1 CCR5 IC_{50} = 60 nM
10 n = 2 CCR5 IC_{50} = 120 nM

Figure 1. Structures and CCR5 activities of lead compounds.

CCR5 antagonists gave lower or no inhibition. Consistent with selective CCR5 inhibition, **4** was ineffective when an X4-tropic HIV-1 strain was used (data not shown). These inhibition studies provided encouragement to pursue these newly identified lead structures.

The synthesis of these and related compounds[26,28] (see Table 1) started from the chiral acid **11** and utilized a synthetic sequence previously reported from these laboratories (Scheme 1).[29] Conversion to an appropriate amide **12** (R^1 = H, Me, Et) allowed for introduction of the desired final C-1 *N*-alkyl group. Reduction of these amides afforded the amines **13**, which were protected as the Boc derivatives **14** while the left hand piperidine was incorporated. Thus, the two-step oxidation of the allyl group with catalytic osmium tetroxide and *N*-methylmorpholine *N*-oxide (NMO) followed by periodate cleavage of the intermediate diols was carried out to afford the aldehyde **15**. Reductive alkylation of the spiro-piperidine **16** with aldehydes **15** using either sodium cyanoborohydride in methanol or sodium triacetoxyborohydride in 1,2-dichloroethane[30] afforded **17**. Removal of the Boc protecting group with HCl in methanol and sulfonylation afforded the sulfide derivatives **19**. Selective monooxidation of the sulfides with 1 equiv of Oxone®[31] at −20 °C for 2–5 min in methanol afforded the final sulfoxide compounds **20** as shown in Figure 2 and Table 1. The sulfoxides were all isolated as 1:1 inseparable mixtures of the *R* and *S* stereoisomers at sulfur. Alternatively, use of excess Oxone® for extended time at room temperature gave the corresponding spiro-sulfones **21**.

Table 1. Structure and CCR5 binding activities for the sulfonyl derivatives **4**, **5**, **20**, and **21**



Compound	Structure		CCR5[a]
	n	R^2	IC_{50} (nM)[b]
3	0	Phenyl	1000
4	1	Phenyl	35
5	2	Phenyl	100
9	1	2-Thienyl	60
10	2	2-Thienyl	120
20c	1	NMe_2	340
20d	1	Benzyl	590
20e	1	Methyl	850
20f	1	*n*-Octyl	1400
20g	1	Cyclopentyl	100
20h	1	Cyclohexyl	100
20i	1	2-Cl-Phenyl	80
20j	1	3-Cl-Phenyl	70
20k	1	4-Cl-Phenyl	40
21l	2	3-NO_2-Phenyl	150
21m	2	4-NO_2-Phenyl	60
20n	1	4-MeO-Phenyl	40
20o	1	4-Ph-Phenyl	40
20p	1	Naphth-1-yl	360
20q	1	Naphth-2-yl	60
20r	1	Indan-5-yl	70
20s	1	3-Thienyl	>4000[c]
20t	1	Pyridin-3-yl	100
20u	1	Quinolin-8-yl	90
20v	1	Quinolin-3-yl	120
20w	1	1-Me-Imidazol-4-yl	340

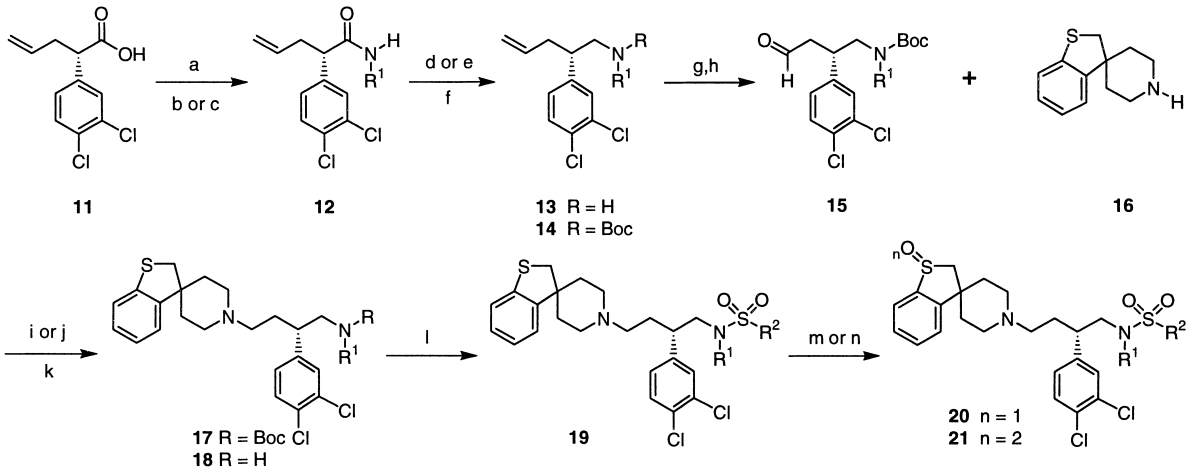
[a]See ref 25 for the procedure.
[b]The IC_{50} results are an average of three independent titrations with calculated standard errors of less than 15%. The assay-to-assay variation was generally ±2-fold based on the results of a standard compound.
[c]Compound **20s** gave 20% inhibition at 4000 nM, the highest concentration tested.

Other nitrogen-based derivatives were also prepared (Scheme 2). Oxidation of the Boc intermediate **17** (R^1 = Me) gave the sulfoxide **22**, which on deprotection with TFA afforded the *N*-methylamine derivative **23**. Reductive alkylation of **18** (R^1 = Me) with benzaldehyde led to the benzylic amine **24**, which on oxidation as above afforded the sulfoxide **25** and sulfone **26**. Alternatively, acylation of **18** with a variety of acid chlorides, chloroformates, isocyanates and carbamoyl chlorides yielded several carbonyl derivatives which on oxidation as above afforded the sulfoxides **27**–**30**.

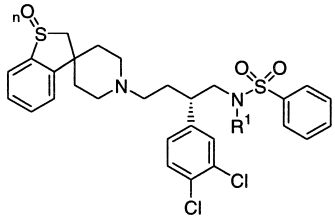
The preparation of some oxygen-based derivatives also started with acid **11** (Scheme 3). Reduction with lithium aluminum hydride afforded the alcohol **31**, which could be alkylated to give the ether **32** or acylated to the ester **33**. These were then carried through the same sequence of reactions as above to give the sulfoxides **34** and **35**, respectively.

The above compounds were evaluated for CCR5 binding activity in the described [^{125}I]-MIP-1α assay.[25] Initial modification of the *N*-alkyl indicated that methyl was optimal since the *N*-H derivatives **19a** and **20a** were considerably less potent and extension to the *N*-ethyl analogues **19b**, **20b**, and **21b** was also detrimental as shown in Figure 2. While all three sulfur oxidation states were usually screened, in almost all cases the sulfoxide derivative was preferred and these derivatives will be emphasized in the following discussion. Since the sulfoxide isomers were not separated, any possible sulfoxide stereochemical preference for CCR5 interaction was not determined. The corresponding sulfones were usually equipotent or slightly less active while the sulfides were much poorer inhibitors, or inactive.

As alluded to above, the sulfonamide appeared to be a critical factor for activity since the related amides **6**–**8** (and all substituted benzamide analogues as well,[26] data not shown), were essentially inactive. However, there was a hint of activity with the phenylacetamide **27** and the carbamate **28** (CCR5 IC_{50} = 640 and 780 nM, respectively). The other nitrogen based derivatives that were prepared in Scheme 2 were also found to be poor inhibitors, such as the *t*-butyl carbamate **22** (35% I @ 1000 nM) and ureas **29** and **30** (69 and 73% I @ 10,000 nM). Deletion of the entire phenylsulfonamide as in the *N*-methyl amine **23** resulted in complete loss of activity. Removal of just the sulfonyl moiety as in the benzylamines **25** (40% I @ 1000 nM) and **26** (IC_{50} = 1000 nM) also led to greatly diminished activity, indicating that a basic moiety was not tolerated or that the sulfonamide was a key



Scheme 1. Reagents: (a) $(COCl)_2$, DMF (cat), DCM, rt; (b) R^1NH_2 (aq, 5 equiv), THF, rt; (c) NH_4OH, THF, rt; (d) LAH, THF, rt; (e) DIBAL-H, THF, rt; (f) $(Boc)_2O$, DIPEA, DCM, rt; (g) OsO_4 (cat), NMO, 2:1:1 v/v/v acetone/*t*-butanol/water, rt; (h) $NaIO_4$, 4:1 v/v THF/water, rt; (i) **16**-HCl, $NaBH(OAc)_3$, DIPEA, DCE, rt; (j) **16**-HCl, $NaCNBH_3$, DIPEA, MeOH, rt; (k) HCl, MeOH, rt; (l) R^2SO_2Cl, DIPEA, DCM, rt; (m) Oxone® (1.2 equiv), MeOH, −20 °C, 2–5 min; (n) Oxone® (3 equiv), MeOH, rt.



CCR5 IC_{50} (nM)

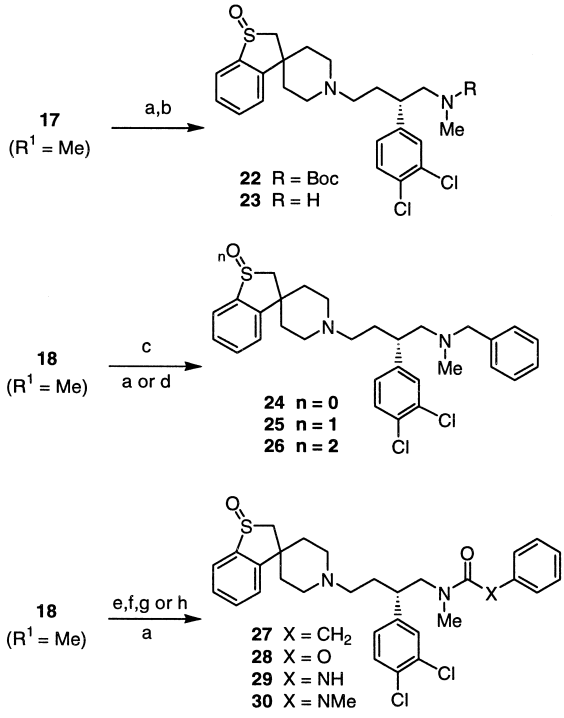
	R^1 = H	R^1 = Me	R^1 = Et
n = 0	**19a** 1,600	**3** 1,000	**19b** 10,000
n = 1	**20a** 230	**4** 35	**20b** 130
n = 2		**5** 100	**21b** 160

Figure 2. CCR5 activities of *N*-alkyl derivatives.

pharmacophore for this scaffold. In agreement with the latter, the corresponding oxygen derivatives **34** and **35** were also found to have poor or no affinity (41 and 4% I @ 1000 nM, respectively).

The phenyl was also determined to be important for potent CCR5 binding affinity in that the *N*,*N*-dimethylsulfonylurea **20c**, the benzylsulfonamide **20d** and the alkyl sulfonamides **20e–h** were all less active (Table 1), although the cycloalkyl derivatives **20g** and **20h** displayed moderate CCR5 affinity. Substitution on the phenyl ring of the lead compound **4** was then investigated. From this work it was clear that *ortho* and *meta* substitution was not desirable (i.e., compounds **20i**, **20j**, and **21l**), but *para* substituents with a broad range of structural properties were tolerated (i.e., compounds **20k**, **21m**, **20n**, and **20o**). In accord with this SAR, the β-naphthyl derivative **20q** was better than the α-naphthyl **20p**. Also, 3,4-alkyl disubstitution as with indane **20r** afforded slightly diminished binding, presumably due to the *meta* effect.

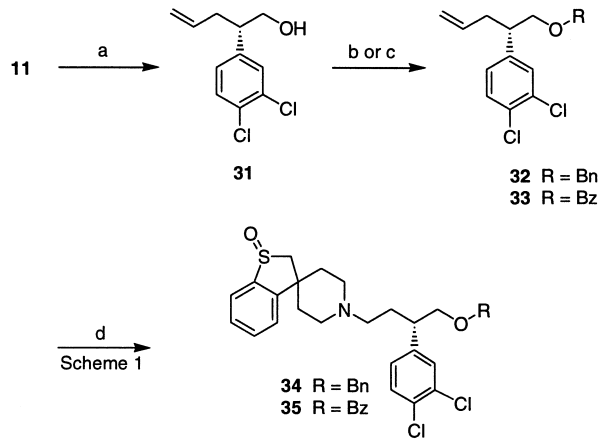
Based on the initial screening result for the 2-thienyl derivative **9** (IC_{50} = 60 nM), it was hoped that the phenyl could be replaced with a variety of other aromatic heterocycles. However, as also shown in Table 1, even the



Scheme 2. Reagents: (a) Oxone® (1.2 equiv), MeOH, −20 °C, 2–5 min; (b) TFA, rt; (c) benzaldehyde, $NaHB(OAc)_3$, DIPEA, DCE, rt; (d) Oxone® (3 equiv), MeOH, rt; (e) $PhCH_2COCl$, DIPEA, DCM, rt; (f) PhOCOCl, DIPEA, DCM, rt; (g) PhNCO, DIPEA, DCM, rt; (h) PhN(Me)COCl, DIPEA, DCM, rt.

3-thienyl isomer **20s** indicated a dramatic loss in binding (20% I @ 4000 nM). A variety of heterocycles, for example pyridine **20t**, quinolines **20u** and **20v** and imidazole **20w**, were also prepared and were shown to have only moderate activity. Thus, the simple *N*-(methyl)-*N*-(phenylsulfonyl)amino moiety of the original lead **4** remained the optimal functionality at C-1 of this butane scaffold to achieve potent CCR5 binding.

The initial lead structures were assayed in a modified PBMC-based viral replication assay as previously



Scheme 3. Reagents: (a) LAH, THF, rt; (b) BnBr, NaH, DMF, rt; (c) BzCl, DIPEA, DCM, rt; (d) see Scheme 1, steps g–m.

described.[27] Using the R5-tropic HIV-1 YU-2 strain, compounds **4** and **9** yielded IC_{95} values in this assay of 6–12 μM, which initially indicated that the desired inhibition of viral entry is possible with a small molecule antagonist. As indicative of the weaker CCR5 binding data for most of the subsequent derivatives, poorer results were generally seen in this screen. However, the 4-substituted derivatives **20k**, **21m**, **20n**, and **20o** appeared equipotent to **4** and **9**.

In summary, screening of the Merck sample collection identified several potent lead structures for the inhibition of [^{125}I]-MIP-1α binding to CCR5. The pivotal nature of the C-1 *N*-(methyl)-*N*-(phenylsulfonyl)amino moiety of the original lead structure **4** was confirmed in an extensive study of other nitrogen- as well as oxygen-based derivatives. Alkyl substituents for the phenyl were also found to be less active. Further elaboration of the phenyl indicated that *para* substitution was possible with a variety of functionality and without loss in binding activity, which might be important for future modifications to achieve proper pharmacokinetic or biological properties. While several heterocyclic replacements for the phenyl were prepared, their binding activity was not improved over **4**. Viral growth inhibition was also demonstrated with **4** and **9**, as well as several analogous, equipotent compounds. Thus, the *N*-(methyl)-*N*-(phenylsulfonyl)amino moiety was identified as an important subunit for selective CCR5 binding with this scaffold and its further utilization will be discussed in several subsequent publications related to the modification of the 2-phenyl and 4-piperidine moieties.[32]

References and Notes

1. For a review of HIV-1 entry mechanisms and current entry inhibitors, see Blair, W. S.; Lin, P.-F.; Meanwell, N. A.; Wallace, O. B. *Drug Discovery Today* **2000**, *5*, 183.
2. Maddon, P. J.; Dalgleish, A. G.; McDougal, J. S.; Clapham, P. R.; Weiss, R. A.; Axal, R. *Cell* **1986**, *47*, 333.
3. For a review of this co-receptor search, see Fauci, A. S. *Nature* **1996**, *384*, 529.
4. Cocchi, F.; DeVico, A. L.; Garzino Demo, A.; Arya, S. K.; Gallo, R. C.; Lusso, P. *Science* **1995**, *270*, 1811.
5. Choe, H.; Farzan, M.; Sun, Y.; Sullivan, N.; Rollins, B.; Ponath, P. D.; Wu, L.; Mackay, C. R.; LaRosa, G.; Newman, W.; Gerard, N.; Gerard, C.; Sodroski, J. *Cell* **1996**, *85*, 1135.
6. Doranz, B. J.; Rucker, J.; Yi, Y.; Symth, R. J.; Samson, M.; Peiper, S. C.; Parmentier, M.; Collman, R. G.; Doms, R. W. *Cell* **1996**, *85*, 1149.
7. Dragic, T.; Litwin, V.; Allaway, G. P.; Martin, S. R.; Huang, Y.; Nagashima, K. A.; Cayanan, C.; Maddon, P. J.; Koup, R. A.; Moore, J. P.; Paxton, W. A. *Nature* **1996**, *381*, 667.
8. Deng, H.; Liu, R.; Ellmeier, W.; Choe, S.; Unutmaz, D.; Burkhart, M.; Di Marzio, P.; Marmon, S.; Sutton, R. E.; Hill, C. M.; Davis, C. B.; Peiper, S. C.; Schall, T. J.; Littman, D. R.; Landau, N. R. *Nature* **1996**, *381*, 661.
9. Alkhatib, G.; Combadiere, C.; Broder, C. C.; Feng, Y.; Kennedy, P. E.; Murphy, P. M.; Berger, E. A. *Science* **1996**, *272*, 1955.
10. Feng, Y.; Broder, C. C.; Kennedy, P. E.; Berger, E. A. *Science* **1996**, *272*, 872.
11. Paxton, W. A.; Martin, S. R.; Tse, D.; O'Brien, T. R.; Skurnick, J.; VanDevanter, N. L.; Padian, N.; Braun, J. F.; Kotler, D. P.; Wolinsky, S. M.; Koup, R. A. *Nature Med.* **1996**, *2*, 412.
12. Liu, R.; Paxton, W. A.; Choe, S.; Ceradini, D.; Martin, S. R.; Horuk, R.; MacDonald, M. E.; Stuhlmann, H.; Koup, R. A.; Landau, N. R. *Cell* **1996**, *86*, 367.
13. Michael, N. L.; Chang, G.; Louie, L. G.; Mascola, J. R.; Dondero, D.; Birx, D. L.; Sheppard, H. W. *Nature Med.* **1997**, *3*, 338.
14. Bates, P. *Cell* **1996**, *86*, 1.
15. Moore, J. P. *Science* **1997**, *276*, 51.
16. Cohen, O. J.; Kinter, A.; Fauci, A. S. *Immunol. Rev.* **1997**, *159*, 31.
17. For a recent chemokine review, see Baggiolini, M.; Dewald, B.; Moser, B. *Annu. Rev. Immunol.* **1997**, *15*, 675.
18. Pipitone, N.; Pitzalis, C. *Curr. Opin. Anti-inflammat. Immunomodulat. Investigat. Drugs* **2000**, *2*, 9.
19. Hall, I. P.; Wheatley, A.; Christie, G.; McDougall, C.; Hubbard, R.; Helms, P. J. *Res. Lett.* **1999**, *354*, 9186.
20. Barcellos, L. F.; Schito, A. M.; Rimmler, J. B.; Vittinghoff, E.; Shih, A.; Lincoln, R.; Callier, S.; Elkins, M. K.; Goodkin, D. E.; Haines, J. L.; Pericak-Vance, M. A.; Hauser, S. L.; Oksenberg, J. R. *Immunogenetics* **2000**, *51*, 281.
21. Sellebjerg, F.; Madsen, H. O.; Jensen, C. V.; Jensen, J.; Garred, P. *J. Neuroimmunology* **2000**, *102*, 98.
22. Horuk, R.; Ng, H. P. *Med. Res. Rev.* **2000**, *2*, 155.
23. Shiraishi, M.; Aramaki, Y.; Seto, M.; Imoto, H.; Nishikawa, Y.; Kanzaki, N.; Okamoto, M.; Sawada, H.; Nishimura, O.; Baba, M.; Fujino, M. *J. Med. Chem.* **2000**, *43*, 2049.
24. Dorn, C. P.; Finke, P. E.; Oates, B.; Budhu, R. J.; Caldwell, C. G.; MacCoss, M.; Mills, S. G.; Malkowitz, L.; Springer, M. S.; Schleif, W. A.; Carella, A.; Carver, G.; Holmes, K.; Emini, E. A. *Abstracts of Papers, Part 2*, 219th National Meeting of the American Chemical Society, San Francisco, CA, March 26–30, 2000; American Chemical Society: Washington, DC, 2000; MEDI #117.
25. The CCR5 binding assay was a modification of that described in ref 33. In summary, the CCR5 binding assay utilized [^{125}I]-MIP-1α as the ligand and was carried out by mixing 200 μL of 50 mM Hepes buffer, pH 7.4, containing 5 mM $MgCl_2$, 1 mM $CaCl_2$, 0.5% BSA, 10 μg/mL each of the protease inhibitors aprotinin, chymostatin and leupeptin, 0.1 mM PMSF, and 0.01 mM phosphoramidon with 5 μL of compound at increasing concentrations or DMSO for the background, 20 μL of [^{125}I]-MIP-1α (2×10^4 cpm, 2200 Ci/mmol) and 10 μL of buffer or unlabeled MIP-1α (100 nM final concentration). Assays were initiated by adding intact CHO cells

(3×10^4) stably expressing 10^6 CCR5 receptors per cell and incubated for 1 h at 24 °C. Separation of free from bound [^{125}I]-MIP-1α was carried out on a Packard Filtermate 196 using GF/C filters pre-soaked in 0.33% PEI. IC_{50} values were calculated using standard methods from the binding results using final concentrations of test compound in the range from 0.13 to 10,000 nM. The titrations were usually done in triplicate with the average IC_{50} value being reported. The calculated standard errors were normally less than 15% in a given assay. Assay-to-assay variability was within ±2-fold based on a standard compound.[32]

26. MacCoss, M.; Mills, S. G.; Shah, S. K.; Chiang, Y.-C. P.; Dunn, P. T.; Koyama, H. US Patent 6,013,652, 2000; *Chem. Abstr*. **2000**, *132*, 78470s.

27. Condra, J. H.; Schleif, W. A.; Blahy, O. M.; Gabryelski, L. J.; Graham, D. J.; Quintero, J. C.; Rhodes, A.; Robbins, H. L.; Roth, E.; Shivaprakash, M.; Titus, D.; Yang, T.; Teppler, H.; Squires, K. E.; Deutsch, P. J.; Emini, E. A. *Nature* **1995**, *374*, 569.

28. Mills, S. G.; Springer, M. S.; MacCoss, M. WO Patent 98/25,605, 1998; *Chem. Abstr*. **1998**, *129*, 81760e.

29. Hale, J. J.; Finke, P. E.; MacCoss, M. *Bioorg. Med. Chem. Lett*. **1993**, *3*, 319.

30. Abdel-Magid, A. F.; Carson, K. G.; Harris, B. D.; Maryanoff, C. A.; Shah, R. D. *J. Org. Chem*. **1996**, *61*, 3849.

31. Trost, B. M.; Curran, D. P. *Tetrahedron Lett*. **1981**, *22*, 1287.

32. Finke, P. E.; Meurer, L. C.; Oates, B.; Mills, S. G.; MacCoss, M.; Malkowitz, L.; Springer, M. S.; Daugherty, B. L.; Gould, S. L.; DeMartino, J. A.; Siciliano, S. J.; Carella, A.; Carver, G.; Holmes, K.; Danzeisen, R.; Hazuda, D.; Kessler, J.; Lineberger, J.; Miller, M.; Schleif, W. A.; Emini, E. A. *Bioorg. Med. Chem. Lett*. **2001**, *11*, 265.

33. Siciliano, S. J.; Kuhmann, S. E.; Weng, Y.; Madani, N.; Springer, M. S.; Lineberger, J. E.; Danzeisen, R.; Miller, M. D.; Kavanaugh, M. P.; DeMartino, J. A.; Kabat, D. *J. Biol. Chem*. **1999**, *274*, 1905.