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Piperazine-Based CCR5 Antagonists as HIV-1 Inhibitors. I: 2(*S*)-Methyl Piperazine as a Key Pharmacophore Element

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Abstract—Optimization of the piperidino-piperazines **1** and **2** provided early leads **3** and **4**, which showed good activity in the CCR5–RANTES binding assay and in antiviral assays. A systematic study around these structures showed that the 2(*S*)-methyl piperazine is essential for CCR5 affinity, which is further enhanced by forming the 2,6-dimethyl benzamide of the piperidine.

The worldwide AIDS pandemic caused by the type 1 human immunodeficiency virus (HIV-1), with over 30 million infected individuals, continues to be a fatal medical condition.[1] Intensive efforts to combat this retrovirus have led to the adoption of combination therapy, using inhibitors of the viral protease and reverse transcriptase enzymes. Although effective in the short term, this approach suffers from patient compliance issues and incomplete suppression of the virus in the long term. This has led to the emergence of HIV strains resistant to the current regimen of drugs.[2] Hence, there is a need for new agents which act at different points in the viral life cycle.

It has been shown recently that binding to specific, cell-surface co-receptors is an essential process, when HIV-1 gains entry to the CD4+ cells of the immune system.[3] HIV-1 utilizes the chemokine receptor CCR5 on macrophages and T-cells, which are its primary targets. CCR5 is a G-protein coupled, 7-*trans*-membrane receptor whose endogenous ligands are the chemokines RANTES, MIP-1α and MIP-1β, which have been reported to suppress HIV-1 cell entry.[4]

Although CCR5 activation is involved in normal cell trafficking, the lack of functional CCR5 does not compromise the immune system in individuals who are homozygous for a defective genetic sequence for receptor coding. Further, such individuals are significantly resistant to HIV-1 infection.[5] These observations suggest that appropriate, small-molecule antagonists of the virus–CCR5 interaction should be novel anti-HIV-1 agents.[6] During the course of our studies, such a molecule, TAK-779, was described and shown to be an effective inhibitor of HIV-1 infection of T-cells.[7] Groups from Merck have also disclosed their work in this area.[8] In this paper, we describe the identification and elaboration of piperidino-piperazine lead compounds to obtain effective inhibitors of HIV-1 cell entry.

Three assays were used to evaluate compounds. The primary assay, also used in initial high-throughput library screening, measured the ability of compounds to inhibit ^{125}I-labeled RANTES binding to the CCR5 receptor on membranes.[9] Selected compounds were then evaluated in a viral entry assay,[10] in which a pseudotype virus bearing a reporter gene for luciferase was used to infect cells expressing CD4 and CCR5. Finally, antiviral activity was measured as the ability of compounds to inhibit the growth of primary HIV-1 isolates in human peripheral blood mononuclear cells.[11]

From our compound library, several structural types inhibited the RANTES–CCR5 interaction. Further evaluation for inhibition of viral entry at sub-cytotoxic

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levels revealed a series of compounds as exemplified by **1** and **2** (Fig. 1), originally prepared for our muscarinic receptor antagonist program.[12]

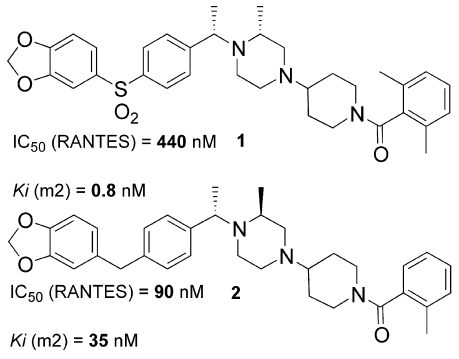
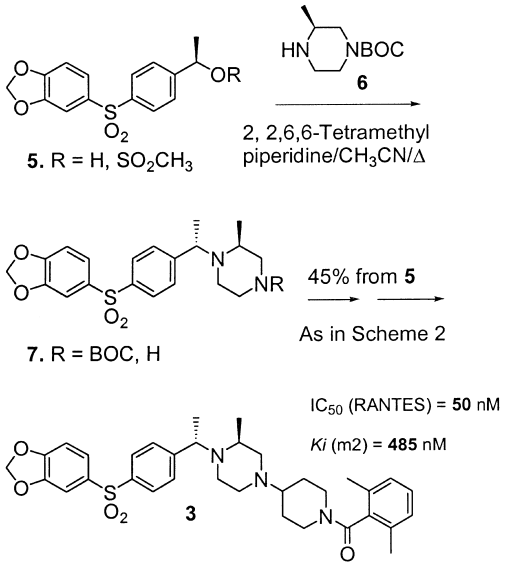


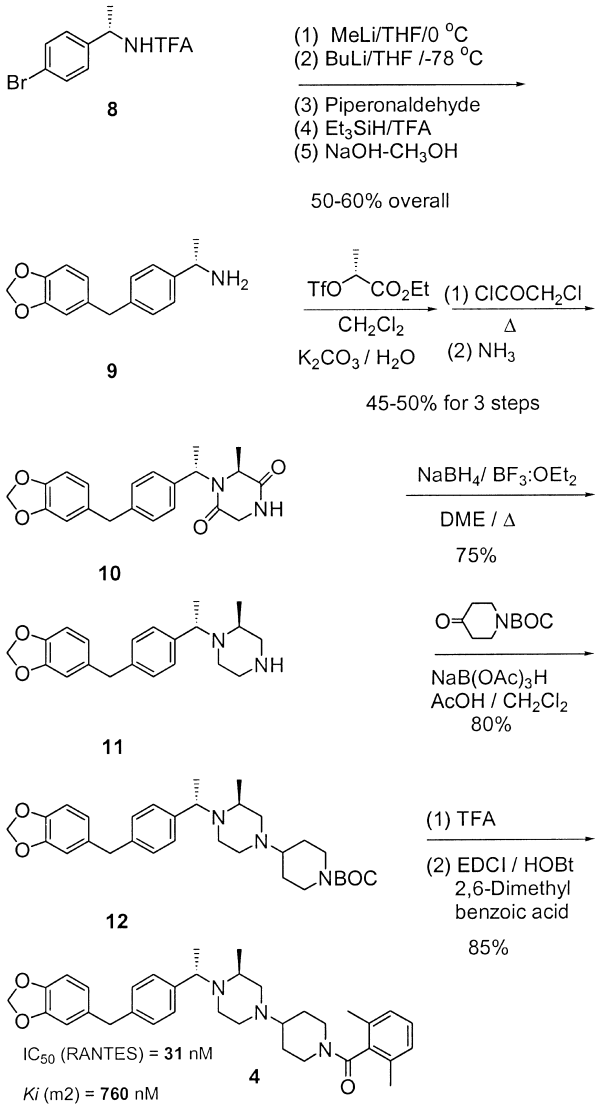
Figure 1.

Accordingly, we synthesized a series of compounds in which the methylation pattern and stereochemistry, the diaryl linking unit, and the amide substitution pattern were varied. The diaryl sulfones were prepared as outlined in Scheme 1. In this route, the benzylic chirality derives from the enantioselective reduction of a prochiral ketone.[13] The success of the subsequent S_N2 displacement reaction depends upon the presence of an electron-withdrawing *para* substituent, to suppress S_N1 chemistry at the benzylic center.



Scheme 1. S_N2 displacement route to CCR5 antagonists.

Alternatively, *p*-bromo α-methylbenzylamine was lithiated and treated with piperonal to obtain the diaryl methane **9** after deoxygenation of the initial carbinol. Reaction of **9** with (*R*)-methyl lactate led to the diketopiperazine **10** (Scheme 2). Reduction of the carbonyl groups in **10** and reductive amination with *N*-*tert*-butoxycarbonyl-4-piperidinone gave **12**. Coupling of the free piperidine from **12** with 2,6-dimethyl benzoic acid gave the target **4**. The mono- and bis-desmethyl compounds were similarly prepared.[14]

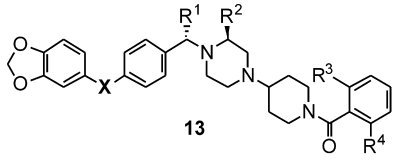


Scheme 2. The diketopiperazine route to CCR5 antagonists.

Results for the inhibition of RANTES binding (K_i) and viral entry (IC_{50}), as well as the M_2 receptor affinity (K_i),[12] for selected compounds are shown in Table 1.

Comparison of compounds **1** and **3** clearly shows that the chirality of the piperazine 2-substituent determines the selectivity: for effective CCR5 binding, 2(*S*) stereochemistry is needed.[9] In contrast, the 2(*R*) compounds such as **1** are M_2 antagonists. Further, any reduction in the level of methylation reduces the CCR5 affinity (compare compound **3** with **13c**–**e** and compound **4** with **13a**–**b**). The amide substitution pattern also affects potency: a 2,6-disubstituted aryl ring is very important (compare compound **2** with **4**). The choice of the groups R^3 and R^4 is critical: although methyl may be replaced by isosteric polar groups such as NH_2 or Cl (compounds **13f**–**h**), a smaller group (F) diminishes binding (compound **13i**). Finally, compound **14** shows that the 3,4-methylenedioxyphenyl ring can be replaced by 3-chlorophenyl ring, with only a modest potency loss in CCR5 binding, but a more significant reduction in the M_2 affinity.

Table 1. Binding (CCR5/M_2) and inhibition of HIV entry data for selected compounds



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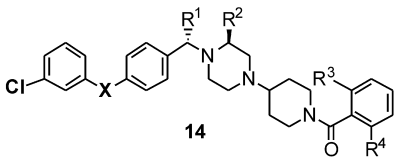

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Compd	X	R^1	R^2	R^3	R^4	K_i (nM)		IC_{50} (nM)
						CCR5	M_2	
3	**SO_2**	CH_3	**CH_3**	CH_3	CH_3	**28**	485	**2.5**
4	**CH_2**	CH_3	**CH_3**	CH_3	CH_3	**18**	760	**1.7**
13a	CH_2	H	CH_3	CH_3	CH_3	44	>40	ND
13b	CH_2	H	H	CH_3	CH_3	352	497	ND
13c	SO_2	H	CH_3	CH_3	CH_3	74	>40	ND
13d	SO_2	CH_3	H	CH_3	CH_3	442	**1**	ND
13e	SO_2	H	H	CH_3	CH_3	>1300	31	ND
13f	SO_2	CH_3	CH_3	CH_3	**NH_2**	31	100	**2**
13g	SO_2	CH_3	CH_3	Cl	NH_2	32	100	11
13h	SO_2	CH_3	CH_3	Cl	Cl	49	270	10
13i	SO_2	CH_3	CH_3	F	F	230	352	129
14	**CH_2**	CH_3	**CH_3**	CH_3	CH_3	45	**>1400**	12

Entry data is for JrFL/ADA strains of HIV-1.

For the more potent compounds in this series (**3**, **4**, **13f**), inhibition of the binding at CCR5 correlated well with inhibition of viral entry. It is important to note that IC_{50} values obtained from the entry assay were generally less than the K_i values determined from the RANTES binding assay. This probably reflects the differences in both the ligand (RANTES vs virus) and the target (cell membrane vs live cells) used in these assays.[15] Further, compound **4** inhibited the replication of a primary HIV-1 isolate (US-1) in PBMCs with a mean IC_{50} of 8 nM.[11] Compound **4** was also shown to be an antagonist at the CCR5 receptor, from effects on RANTES induced calcium flux.[5a] No appreciable binding was detected at other chemokine receptors (CCR1, CCR2 and CCR3).

In summary, these results clearly demonstrate that compounds containing the piperidino-2(*S*)-methyl piperazine pharmacophore prevent the entry of HIV-1 into target cells via inhibition of the binding of the virus to the co-receptor CCR5. The further development of this series to give potent, orally bioavailable CCR5 antagonists that inhibit HIV replication will be reported in the near future.

Acknowledgements

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References and Notes

1. UNAIDS. *Report on the Global HIV/AIDS Epidemic* **2000**. XIII International AIDS Conference, Durban, South Africa, 2000.
2. Boden, D.; Hruley, A.; Zhang, L.; Cao, Y.; Guo, Y.; Jones, E.; Tsay, J.; Ip, J.; Farthing, C.; Limoli, K.; Parkin, N.; Markowitz, M. *JAMA* **1999**, *282*, 1135.
3. (a) Littman, D. R. *Cell* **1998**, *93*, 677. (b) Murphy, P. M. *Nature Immunol.* **2001**, *2*, 116.
4. Cocchi, F.; DeVico, A. L.; Garzino Demo, A.; Arya, S.; Gallo, R.; Lusso, P. *Science* **1995**, *270*, 1811.
5. (a) 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. (b) Samson, M.; Libert, F.; Doranz, B. J.; Rucker, J.; Liesnard, C.; Farber, C.-M.; Saragosti, S.; Lapoumeroulle, C.; Cognaux, J.; Forceille, C.; Muyldermans, G.; Vehofstede, C.; Butonboy, G.; Georges, M.; Imai, T.; Rana, S.; Yi, Y.; Smyth, R. J.; Collman, R. G.; Doms, R. W.; Vassart, G.; Parmentier, M. *Nature* **1996**, *382*, 722.
6. (a) Hunt, S. W., III; LaRosa, G. J. *Annu. Rep. Med. Chem.* **1998**, *33*, 263. (b) Saunders, J.; Tarby, C. M. *Drug Discov. Today* **1999**, *4*, 80. (c) Blair, W. S.; Lin, P.-F.; Meanwell, N. A.; Wallace, O. B. *Drug Discov. Today* **2000**, *5*, 183.
7. (a) Shiraishi, M.; Aramaki, Y.; Imoto, H.; Nishikawa, Y.; Kanzaki, N.; Okamoto, M.; Sawada, H.; Nishimura, O.; Baba, M.; Fujino, M. *J. Med. Chem.* **2000**, *43*, 2049. (b) Baba, M.; Nishimura, O.; Kanzaki, N.; Okamoto, M.; Sawada, H.; Shiraishi, M.; Aramaki, Y.; Okanogi, K.; Meguro, K.; Fujino, M. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 5698.
8. (a) Dorn, C. P.; Finke, P. E.; Oates, B.; Budhu, R. J.; 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*, 259. (b) 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.
9. Binding assay: Membrane preparations from CHO cells expressing CCR5 were incubated with ^{125}I-RANTES in the presence or absence of compound for 1 h. Compounds were serially diluted over a range of 0.3–×3000 nM and tested in replicates of four. Reaction mixtures were harvested through glass fiber filters, and washed thoroughly. Total replicate

counts were averaged and IC_{50} values calculated as the amount of compound required to inhibit 50% of total ^{125}I-RANTES binding. The binding affinity constant, K_i, was determined using prism software analysis. The standard error was 10% and variability was 2–3 fold from assay-to-assay. For the compounds **1** and **2** from high-throughput screening, K_i values are not available.

10. Entry assay: Connor, R. I.; Chen, B. K.; Choe, S.; Landau, N. R. *Virology* **1995**, *206*, 935.

11. Replication assay: Trkola, A.; Ketas, T. J.; Nagashima, K. A.; Zhao, L.; Cilliers, T.; Morris, L.; Moore, J. P.; Maddon, P. J.; Olson, W. C. *J. Virol.* **2001**, *75*, 579.

12. Kozlowski, J. A.; Lowe, D. B.; Guzik, H. S.; Zhou, G.; Ruperto, V. B.; Duffy, R. A.; McQuade, R.; Crosby, G., Jr.; Taylor, L. A.; Billard, W.; Binch, H., III; Lachowicz, J. E. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2255.

13. (a) Corey, E. J.; Bakshi, R. K.; Shibata, S. *J. Am. Chem. Soc.* **1987**, *109*, 5551. (b) Mathre, D. J.; Jones, T. K.; Xavier, L. C.; Blacklock, T. J.; Reamer, R. A.; Mohan, J. J.; Turner-Jones, E. T.; Hoogsteen, K.; Baum, M. W.; Grabowski, E. J. J. *J. Org. Chem.* **1991**, *56*, 751.

14. All compounds reported in here were fully characterized by ^{1}H NMR (300 MHz, $CDCl_3$), MS and HRMS. Selected data is shown below.

Compound **3**: ^{1}H NMR δ 0.86 (m, 3H), 1.12 (br s, 3H), 1.20 (br m, 2H), 1.25 (br m, 3H), 1.78 (br s, 1H), 2.10 (m, 4H), 2.19 (s, 3H), 2.27 (s, 3H), 2.78 (m, 2H), 2.93 (m, 2H), 3.44 (br d, 1H), 4.10 (m, 1H), 4.86 (br d, 1H), 6.05 (s, 2H), 6.87 (d, $J=8.6$ Hz, 1H), 7.02 (m, 2H), 7.14 (dd, $J=7.3$ Hz, 7.3 Hz, 1H), 7.32 (d, $J=1.8$ Hz, 1H), 7.55 (d, $J=8.6$ Hz, 3H) and 7.83 (d, $J=7.9$ Hz, 2H). HRMS calcd for $C_{34}H_{42}N_3O_5S$ ($M+H^+$) 604.2845. Found 604.2861.

Compound **4**: ^{1}H NMR δ 1.38 (d, $J=3$ Hz, 3H), 1.50 (br s, 3H), 1.6–1.8 (br m, 2H), 1.9–2.2 (br m, 6H), 2.27 (s, 3H), 2.28 (s, 3H), 2.4–3.1 (br m, 6H), 3.2–3.5 (m, 2H), 3.8 (s, 2H), 4.9 (br d, 1H), 5.8 (s, 2H), 6.6 (s, 1H), 6.65 (d, $J=6$ Hz, 1H), 6.75 (d, $J=6$ Hz, 1H), 6.9–7.2 (m, 5H) and 7.45 (br s, 2H). HRMS calcd for $C_{35}H_{44}N_3O_3$ ($M+H^+$) 554.3383. Found 554.3383.

15. Compounds from this series have similar effects on the activity of other CCR5 ligands such as MIP-1β.