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1,3,4-Trisubstituted Pyrrolidine CCR5 Receptor Antagonists. Part 1: Discovery of the Pyrrolidine Scaffold and Determination of Its Stereochemical Requirements

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Abstract—A series of 1,3,4-trisubstituted pyrrolidines was discovered to have the ability to displace [^{125}I]-MIP-1α from the CCR5 receptor expressed on Chinese hamster ovary (CHO) cell membranes. CCR5 activity was found to be dependent on the regiochemistry and the absolute stereochemistry of the pyrrolidine.

The outlook for patients infected with the human immunodeficiency virus (HIV) has improved in recent years due to the development of drugs that inhibit enzymes (reverse transcriptase and HIV protease) that are vital components of the viral life cycle.[1,2] While the use of combination therapy has resulted in decreased rates of mortality and morbidity in industrialized countries, individuals undergoing these treatments are still faced with significant issues regarding drug resistance (both to individual entities and classes of drugs): side-effect profiles, dose-limiting toxicities, difficult dosing regimens, and long-term costs.[3] Despite the fact that the number of newly reported cases of acquired immune deficiency syndrome (AIDS) in countries such as the United States has recently decreased,[4] this disease remains a serious worldwide public health problem and there continues to be an urgent need for new therapies.

The observation that the β-chemokines MIP-1α (macrophage inflammatory protein-1α), MIP-1β, and RANTES (regulation upon-activation, normal T-cell expressed and secreted) inhibit the infection of CD4$^+$ T-cells by primary nonsyncytium-inducing (NSI) strains of HIV-1[5] led in part to the discovery that the principal co-factor for the entry of macrophage-tropic strains of HIV-1 into monocytes, macrophages and primary T-cells is the β-chemokine receptor CCR5.[6] A small subset of the human population possesses a mutant allele for CCR5 which has a 32 base pair deletion in its coding region that results in a non-functional receptor. CD4$^+$ T-cells isolated from this population do not support the fusion of NSI strains of HIV and it appears that the presence of the mutant CCR5 allele is a primary genetic factor in the resistance to HIV infection observed in multiply exposed, uninfected individuals.[7,8] Initial reports describing the inhibition of viral replication with agents that interfere with viral cell entry, such as T-20[9] (a peptide designed to inhibit HIV transmembrane glycoprotein-mediated fusion) and small molecule human CCR5 receptor antagonists[10,11] indicate that this strategy shows potential as an AIDS therapy. In this communication, we wish to describe the discovery that appropriately functionalized 1,3,4-trisubstituted pyrrolidines are a new lead class in the search for antagonists of the CCR5 receptor.

Screening of the Merck Research Laboratories sample collection led to the identification of several 2-aryl-4-(piperidin-1-yl)butanamine analogues with affinity for the CCR5 receptor. One result from the development of the structure–activity relationships around these lead compounds[12,13] was the determination that compound **1** had an $IC_{50} = 68$ nM in an assay designed to measure the ability of test compounds to displace [^{125}I]-MIP-1α

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from the CCR5 receptor (see following section for assay details). As part of a larger effort to identify compounds that present the pharmacophoric elements of **1** on a rigid scaffold, pyrrolidines **2** were targeted as constrained analogues of **1** in which the *N*-methyl group was attached to C3 of the carbon backbone. The pyrrolidine scaffold was also chosen in part due to the literature precedent describing the preparation of 3,4-disubstituted pyrrolidines in a stereocontrolled manner.[14] While targeting the pyrrolidine did not result from any kind of molecular modeling studies, the ease of its synthesis was viewed to make it particularly attractive as it would allow for an expedient evaluation of a few key analogues and the viability of the scaffold as a lead structure for our CCR5 receptor antagonist program.

In order to obtain the initial targets (the racemic 3,4-*cis*- and 3,4-*trans*-pyrrolidine compounds, **2**) free of isomeric impurities, the synthesis of these analogues was designed to minimize manipulations of carbonyl-containing intermediates that might be prone to epimerization (Scheme 1). The dipolar cycloaddition reaction between methyl *trans*-cinnamate and *N*-benzyl azomethine ylide proceeded smoothly to afford pyrrolidine **3** in quantitative yield. Uneventful ester reduction and debenzylation of **3** was followed by a concomitant *N*- and *O*-phenylsulfonylation, which afforded **4** in low yield. A significant amount of elimination occurred on the attempted alkylation of spiro[2,3-dihydrobenzothiophene]-3,4′-piperidine[15] with sulfonate ester **4**. This was readily circumvented by first converting **4** to **5**; when the iodide was employed to alkylate the piperidine, the reaction afforded **6** without complication. Selective *S*-oxidation[16] of **6** was carried out to give both sulfoxide **7** and sulfone **8**. Racemic 3,4-*cis*-pyrrolidine analogues **9**–**11** were prepared in an analogous manner starting from methyl *cis*-cinnamate.

The individual enantiomers of sulfone **8** were synthesized after it became apparent that the 3,4-*trans*-pyrrolidine scaffold afforded an active series of compounds. While only modest success has been reported for the asymmetric dipolar cycloaddition of azomethine ylides to cinnamates,[17,18] employing *trans*-cinnamoyl oxazolidinone **12**[19] as a substrate did provide a convenient method for the resolution of a synthetic intermediate as the 3-(*S*),4-(*R*) diastereomer **13** and the 3-(*R*),4-(*S*) diastereomer **14** were readily separable using silica gel chromatography (Scheme 2). A series of straightforward functional group transformations that could be adapted to prepare analogues containing various piperidine pharmacophores from a late stage intermediate was used to convert alcohol **15** to **18** and **19**. An orthogonal sequence that would allow for the variation of *N*-substituents while holding a given piperidine pharmacophore constant in subsequent analogues was used to prepare **23** from alcohol **20** (Scheme 3).

An assay to measure the ability of test compounds to displace ligand from the CCR5 receptor expressed in Chinese hamster ovary (CHO) cell membranes was developed for the purposes of analogue screening.[20] While the most appropriate ligand for such an assay would presumably be the HIV surface glycoprotein (gp120) complexed with solubilized primary HIV

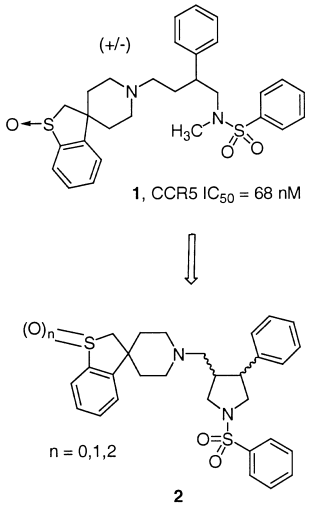
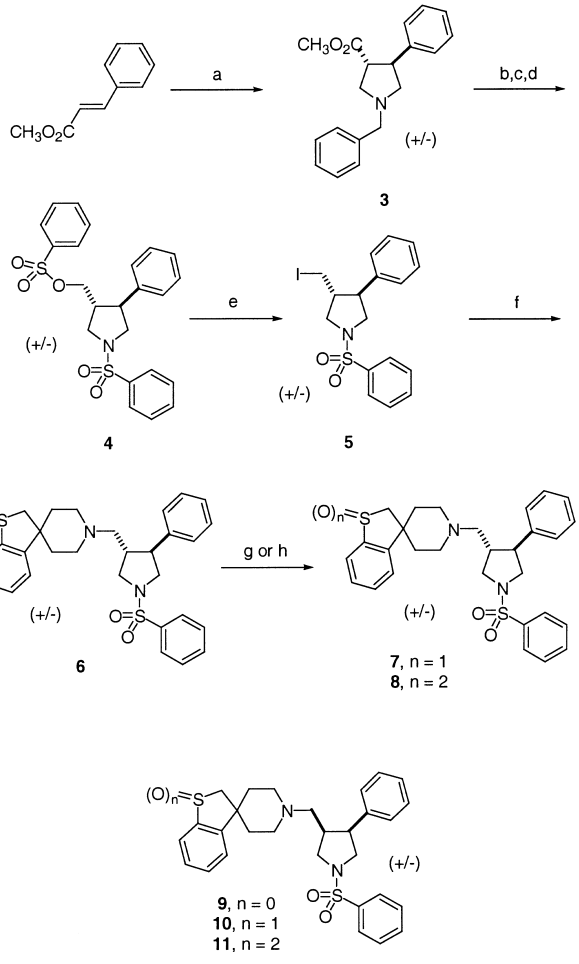
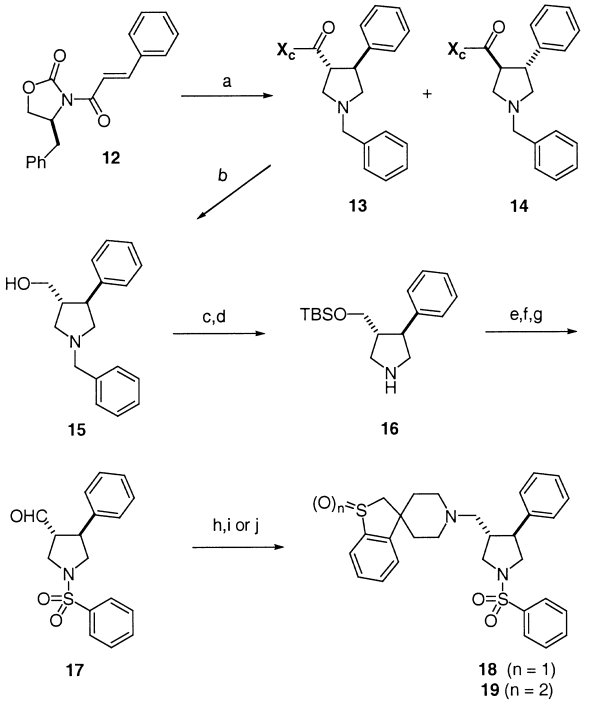


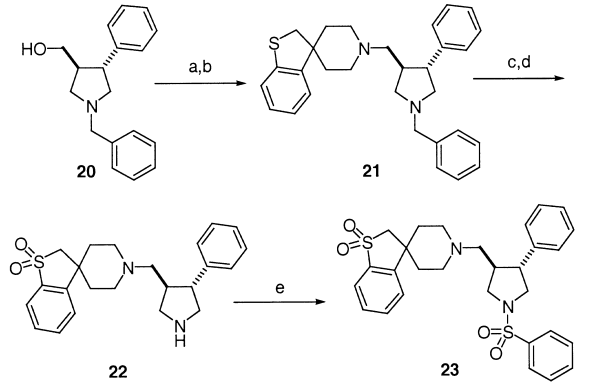
Figure 1. Pyrrolidine targets based on CCR5 receptor antagonist **1**.



Scheme 1. Reagents: (a) *N*-methoxymethyl-*N*-trimethylsilylmethyl benzyl amine, cat. TFA, CH_2Cl_2, 0 °C (99%); (b) DIBALH, THF, 0 °C (85%); (c) 50 psi H_2, $Pd(OH)_2/C$, MeOH (99%); (d) 2.5 equiv $PhSO_2Cl$, DMAP, pyridine, 0 °C to rt (23%); (e) NaI, acetone, reflux (86%); (f) spiro[2,3-dihydrobenzothiophene]-3,4′-piperidine, *i*PrCN, 100 °C (77%); (g) 1 equiv Oxone®, THF/H_2O, 0 °C (83%); (h) 2.5 equiv Oxone®, THF/H_2O, rt (45%).



Scheme 2. Reagents: (a) *N*-methoxymethyl-*N*-trimethylsilylmethyl benzyl amine, cat. TFA, CH_2Cl_2, 0 °C to rt (88% total yield, *ds* = 1:1); (b) $LiAlH_4$, THF, 0 °C to rt (69%); (c) TBS-Cl, DIEA, CH_2Cl_2, rt (100%); (d) $NH_4{}^+HCO_2{}^-$, $Pd(OH)_2/C$, MeOH, 55 °C (99%); (e) $PhSO_2Cl$, DIEA, CH_2Cl_2, 0 °C to rt (82%); (f) TBAF, THF, 0 °C to rt (95%); (g) $(COCl)_2$, DMSO, DIEA, CH_2Cl_2, −78 °C to 0 °C (95%); (h) spiro[2,3-dihydrobenzothiophene]-3,4′-piperidine× HCl, TEA, $NaB(OAc)_3H$, CH_2Cl_2 (88%); (i) 1.0 equiv Oxone®, THF/H_2O, 0 °C (73%); (j) 2.5 equiv Oxone®, THF/H_2O (97%).



Scheme 3. Reagents: (a) $(COCl_2)_2$, DMSO, DIEA, CH_2Cl_2, −78 to 0 °C (95%); (b) spiro[2,3-dihydrobenzothiophene]-3,4′-piperidine× HCl, TEA, $NaB(OAc)_3H$, CH_2Cl_2 (92%); (c) 2.5 equiv Oxone®, THF/H_2O (85%); (d) $NH_4{}^+HCO_2{}^-$, $Pd(OH)_2/C$, MeOH, 55 °C (65%); (e) $PhSO_2Cl$, DIEA, CH_2Cl_2, 0 °C to rt (69%).

receptor CD4,[21] the readily available β-chemokine [^{125}I]-MIP-1α was chosen as a suitable surrogate due to the reported partial overlap of CCR5 binding sites of the β-chemokines and HIV envelope glycoprotein[22] and the fact that β-chemokines can inhibit the entry of HIV into T-cells.[23,24]

Determination of the binding constants of the racemic 3,4-*trans* compounds (**6**–**8**) and 3,4-*cis* compounds (**9**–**11**) in the [^{125}I]-MIP-1α CCR5 assay indicated that the 3,4-*trans* compounds were not only appreciably more active than the corresponding 3,4-*cis* analogues, but that compounds **7** and **8** also had binding affinities comparable to the lead acyclic compound, **1** (Table 1). In addition, the binding constants of the 3-(*S*),4-(*S*) enantiomers of sulfoxide **7** and sulfone **8** served to demonstrate that the CCR5 affinity of these pyrrolidine compounds was also enantiospecific as **18** and **19** were both approximately equipotent to **1** while the 3-(*R*),4-(*R*)-sulfone **23** was inactive.

Table 1. Inhibition of [^{125}I]-MIP-1α binding to CCR5[a]

Compound	CCR5 IC_{50} (nM)
1	68 ± 6
6	> 10,000
7[b]	120 ± 10
8	170 ± 15
9	> 10,000
10[b]	3500 ± 340
11	> 10,000
18[b]	26 ± 2
19	49 ± 7
23	> 10,000

[a]Displacement of [^{125}I]-labeled MIP-1α from the CCR5 receptor on CHO cell membranes. Data are reported as mean ± SD for $n = 3$ determinations. See ref 20 for assay details.
[b]1:1 mixture of sulfoxide diastereomers.

An increase in extracellular acidity due to ATP utilization or alteration in Na^+/H^+ exchange is indicative of receptor activation.[25] Measurement of changes in local pH using a microphysiometer[26] allows for the identification of activation via any operative signal transduction pathway(s). Compound **18** was determined to not significantly alter the extracellular pH of CHO cells expressing CCR5 as compared to an agonist control (MIP-1α) indicating that receptor activation had not occurred and that **18** is an antagonist of CCR5 in this context.

A series of 1,3,4-trisubstiuted pyrrolidine compounds based on the acyclic lead compound **1** was prepared. The ability of the pyrrolidine compounds to displace [^{125}I]-MIP-1α from the CCR5 receptor was demonstrated to be dependent on the stereochemistry of the pyrrolidine scaffold. The optimization of pyrrolidine compounds based on sulfone **19** will be the subject of future reports.

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26. Chinese hamster ovary cells stably transfected with human CCR5 were seeded onto Transwell cell capsule cups (Molecular Devices) at a density of 0.33×10^6 cells/mL/cup in MEM alpha medium. Following overnight culture, the capsules were transferred to microphysiometer sensor chambers (Cytosensor, Molecular Devices) and allowed to equilibrate for 30 min–2 h during which time they were perfused with running medium [1 mM phosphate-buffered RPMI-1640 medium, pH 7.4 (Molecular Devices) + 0.1 BSA]. Once stable acidification rates were established, the cells were exposed to either 1 nM MIP-1α or 1 μM **18** for 6 min at a flow rate of 0.1 mL/min of running medium. Perfusion was stopped at 2 min intervals, during which time acidification rates were measured for 30 s. The data collected was normalized using the Cytosensor microphysiometer software. Compound **18** at a concentration of 1 μM was found to elicit 11% of the initial response of MIP-1α at its EC_{50} (1–2 nM).