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CCR5 Antagonists: Bicyclic Isoxazolidines as Conformationally Constrained *N*-1-Substituted Pyrrolidines

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Abstract—A series of CCR5 antagonists containing bicyclic isoxazolidines was generated through a nitrone mediated cycloaddition with olefins bearing the preferred pharmacophores previously described. Potent antagonists (**3** and **16**) were generated with enhanced affinity for the CCR5 receptor while maintaining antiviral activity against HIV. © 2002 Elsevier Science Ltd. All rights reserved.

The human immunodeficiency virus (HIV) makes specific contacts between the gp120 viral envelope glycoprotein and both the CD4 surface protein and a chemokine receptor of its host prior to fusion and initiation of infection.[1,2] The utilization of a chemokine coreceptor by HIV has led to the classification of viral isolates based on the coreceptor used to initiate infection. Thus, R5 isolates use the chemokine receptor CCR5 to establish an infection in host cells and infect macrophages and primary T-cells, and X4 isolates use the chemokine receptor CXCR4 and target T-lymphoid cell lines.[3] A 32-base pair deletion within the CCR5 gene has been reported to provide a delayed onset of AIDS in heterozygotes and high resistance to initial infection for homozygotes.[4] These findings have encouraged the pursuit of CCR5 antagonists (e.g., TAK-779[5] and SCH-C[6]) as novel agents for the treatment and/or prevention of HIV infection.

Recent reports from these laboratories described a series of 1,3,4-trisubstituted pyrrolidine CCR5 antagonists as novel antivirals against HIV (e.g., **1**).[7–9] A survey of *N*-1 substituents on the pyrrolidine revealed a flexible SAR in this portion of the molecule, which tolerated both *N*-alkyl and *N*-acyl groups. In order to explore the conformational preferences about the C–N bond via rigidified analogues, we envisioned replacing the pyrrolidine in **1** with a bicyclic isoxazolidine, generating target structures such as **2** and **3**. This constraint would hopefully mimic a bioactive amide rotamer of the pyrrolidine and provide insight for the requirements for potency.

An expedient route into this scaffold was employed through a nitrone mediated cycloaddition reaction with olefins bearing the preferred *N*-1 pharmacophores previously described (e.g., cyclohexyl and phenyl).[10] The assembly of the bicyclic targets began with the chiral pyrrolidine **4** (Scheme 1).[7] A mixture of nitrones was generated using the oxidizing conditions of Petrini.[11] Cycloaddition of the nitrones with either styrene or methylenecyclohexane gave a separable mixture of two major products **5A** and **5B**. The silyl ethers were cleaved

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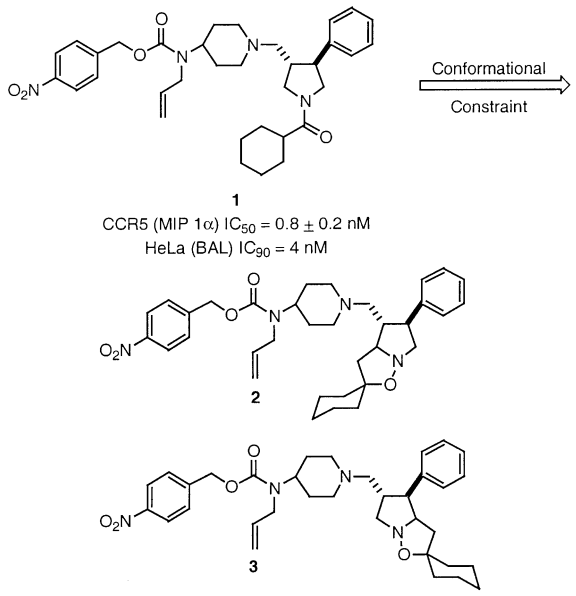


Table 1. NOE data observed for regioisomers **7** and **8**

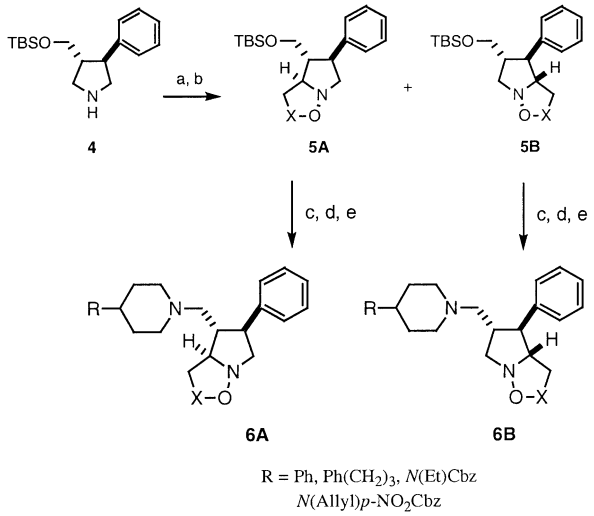
NOE's	NOE's
H2 to H3β	H2 to H4
H2 to H4	H3β to H8
H2 to H6β	H3β to *ortho* H's on phenyl
H3α to H8	H5 to H8
H5 to H8	H8 to *ortho* H's on phenyl

The proton signals were assigned by COSY and HSQC NMR experiments (600 MHz, $CDCl_3$). Energy minimized models were generated (Sybyl) and were found to be consistent with the NOE data and confirmed the stereochemical assignments.

with TBAF to provide the intermediate alcohols. Following a Swern oxidation, the intermediate aldehydes were subjected to reductive aminations with the previously disclosed 4-substituted piperidines (see Tables 2 and 3).[8,9]

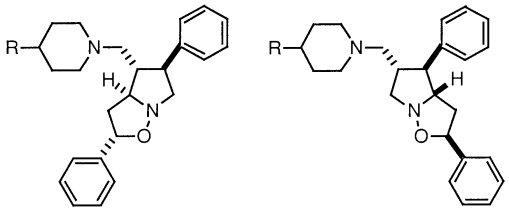
The stereochemistry of the nitrone cyclizations was initially inferred from literature precedents invoking an *exo*-transition state.[12] This proposal assumed the dipolarophile would approach the nitrone from the least hindered face. In the case of regioisomer **5A**, the cycloaddition should occur opposite to the TBS-group. Whereas in regioisomer **5B**, the dipolarophile should approach the face opposite the phenyl substituent. More conclusive evidence was obtained through COSY, NOESY and HSQC NMR experiments with alcohols **7** and **8** (derived from styrene), which supported the depicted stereochemistry. The resulting NOE's listed in Table 1 confirmed the original assumptions.

The CCR5 receptor affinity and antiviral data for the compounds are presented in Tables 2 and 3. Initially analogues were screened for their ability to displace [^{125}I]-labeled MIP-1α from the CCR5 receptor expressed on CHO cell membranes.[13] The most potent compounds were further evaluated as antivirals in a HeLa cell anti-infectivity assay versus the BAL strain of HIV.[14] Upon initial evaluation of the data, a preference for regioisomer **B** was clearly defined (**16** vs **12** and **3** vs **2**). Although this trend was present in both series, the spiro-cyclohexyl analogues showed a more distinct difference (Table 3). In addition, the data supported the



Scheme 1. Reagents and conditions: (a) Na_2WO_4, H_2O_2urea, MeOH (79%); (b) olefin, toluene, reflux; (X = PhCH 55%; X = C_6H_{10} 23%); (c) TBAF, THF (X = PhCH A 69%, B 82%; X = C_6H_{10} A 80%, B 82%); (d) $(COCl)_2$, DMSO, DIEA, CH_2Cl_2 (X = PhCH A 73%, B 90%; X = C_6H_{10} A 86%, B 77%); (e) piperidine analogue, $NaB(OAc)_3H$, CH_2Cl_2.

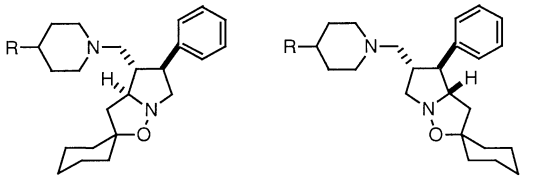
Table 2. CCR5 receptor affinity and antiviral activity of the styrene derived isoxazolidines



R	Compd	MIP 1α[a] (HeLa)[b]	Compd	MIP 1α (HeLa)[b]
Ph	**9**	30 ± 7 (NT)[c]	**13**	27 ± 5 (NT)[c]
$Ph(CH_2)_3$	**10**	11 ± 1 (NT)[c]	**14**	5 ± 0.5 (> 300)
N(Et)Cbz	**11**	46 ± 12 (NT)[c]	**15**	~27[d] (NT)[c]
N(Allyl)*p*-NO_2Cbz	**12**	6 ± 2 (> 300)	**16**	2.5 ± 0.3 (300)

[a]Displacement of [^{125}I]-labeled MIP-1α from the CCR5 receptor expressed on CHO cell membranes (IC_{50}, nM). Data are reported as mean ± SD for n = three determinations. See ref 13 for assay protocol.
[b]IC_{90} values obtained in the HeLa cell anti-infectivity assay versus BAL. See ref 14 for assay protocol.
[c]NT, not tested.
[d]Approximate IC_{50}; 100% inhibition was not obtained at 1 μM.

Table 3. CCR5 receptor affinity and antiviral activity of the methylenecyclohexane derived isoxazolidines



R	Compd	MIP 1α[a] (HeLa)[b]	Compd	MIP 1α (HeLa)[b]
Ph	**17**	33±3 (NT)[c]	**20**	43±6 (NT)[c]
$Ph(CH_2)_3$	**18**	69% @ 1 μM (NT)[c]	**21**	8±1 (>300)
N(Et)Cbz	**19**	~86[d] (NT)[c]	**22**	5±1 (>300)
N(Allyl)*p*-NO_2Cbz	**2**	14±2 (NT)[c]	**3**	0.13±0.08 (33)

[a]Displacement of [^{125}I]-labeled MIP-1α from the CCR5 receptor expressed on CHO cell membranes (IC_{50}, nM). Data are reported as mean ±SD for n = three determinations. See ref 13 for assay protocol.
[b]IC_{90} values obtained in the HeLa cell anti-infectivity assay versus BAL. See ref 14 for assay protocol.
[c]NT, not tested.
[d]Approximate IC_{50}; 100% inhibition was not obtained at 1 μM.

previous observations[8,9] that preferred piperidine substituents can increase antiviral potency, as may be seen comparing the potencies of the 4-(3-phenpropyl)-piperidines with those of the 4-[*N*-alkyl(benzyloxycarbonyl)amino]piperidines (e.g., **20**, **21**, **22**, and **3**). In general, detectable antiviral activity was observed with CCR5 affinities less than 5 nM (**16** and **3**).

Since compounds **3** and **16** were the most potent antivirals produced in this series of analogues, the functional activity was characterized in a cellular microphysiometer assay using CHO cells expressing human CCR5 receptors. Neither compound elicited agonist activity as demonstrated by the lack of change in the extracellular pH in comparison to an agonist control (MIP-1α). These results coupled with the binding data given above indicated that both **3** and **16** were antagonists of the CCR5 receptor.[15]

Although compound **3** has better receptor affinity then **1**, the antiviral activity of **3** is an order of magnitude less potent when compared to **1**. This suggests that blocking MIP-1α binding might not completely impart antiviral activity, which may require different interactions for blocking HIV in comparison with the chemokines.

In conclusion, we have presented a case for using bicyclic isoxazolidines as a conformational constraint for *N*-substituted pyrrolidines. This approach should find further use in exploring the conformational preferences and controlling the rotation about the C–N bond within other heterocycles. With this strategy, we were able to generate potent antagonists (**3** and **16**) with enhanced affinity for the CCR5 receptor while maintaining antiviral activity against HIV.

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