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Conformational studies of 3-amino-1-alkyl-cyclopentane carboxamide CCR2 antagonists leading to new spirocyclic antagonists

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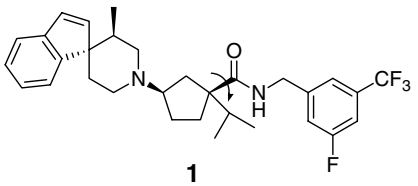
Abstract—In an effort to shed light on the active binding conformation of our 3-amino-1-alkyl-cyclopentane carboxamide CCR2 antagonists, we prepared several conformationally constrained analogs resulting from backbone cyclization. Evaluation of CCR2 binding affinities for these analogs gave insight into the optimal relative positions of the piperidine and benzylamide moieties while simultaneously leading to the discovery of a new, potent lead type based upon a spirocyclic acetal scaffold.

Chemokines are secreted by proinflammatory cells, leukocytes, and endothelial cells in response to a stimulus to the immune system. Monocyte chemoattractant protein-1 (MCP-1, CCL2), included within the CC class of chemokines,[1] mediates chemotaxis of monocytes to inflammatory sites primarily through interactions with its receptor, CCR2,[2] a member of the G-protein-coupled seven-transmembrane receptor superfamily. Numerous recent studies have linked CCL2 and CCR2 to various inflammatory diseases[3] including rheumatoid arthritis (RA)[4] and atherosclerosis.[5] Consequently, the therapeutic potential of CCR2 antagonists in treating inflammatory diseases has stimulated considerable interest.[6] Our group has recently disclosed the discovery and properties of a new class of CCR2 antagonists based upon a 3-amino-1-alkyl-cyclopentane carboxamide core typified by **1**.[7] A study of molecular models revealed that the bond connecting the cyclopentane to the amide in compound **1** can assume two types of lowest energy conformations which have similar energies: one in which the carbonyl projects over the plane of the cyclopentane ring and where the benzyl moiety is extended away, and one in which the carbonyl is roughly parallel to the cyclopentane, leaving the benzyl moiety above the plane of the cyclopentane. This letter describes the design, synthesis, and biological activity of analogs of **1** where our 3-fluoro-5-trifluoromethylbenzyl pharmacophore is locked into each of these two possible orientations.



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After studying models, we reasoned that backbone cyclization from the α-position to the carbonyl to the amide nitrogen (generating a spirocyclic core, **2**) would lock the 3-fluoro-5-trifluoromethylbenzyl group into an extended trajectory away from the cyclopentane subunit (Fig. 1).

Compound **2** as a mixture of two *syn*-diastereomers was prepared according to Scheme 1. Commercially available methyl (3-methylenecyclopentane)carboxylate **3** was deprotonated with LDA (−78 °C) and alkylated with allyl bromide (−78 °C to rt, 76%) to give **4**. Ozonolysis of both alkenes, followed by treatment with PPh_3, afforded ketoaldehyde **5** in 84% yield. Reductive

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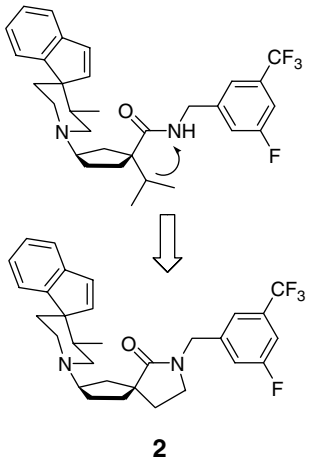


Figure 1. Cyclization locks benzyl in the extended conformation.

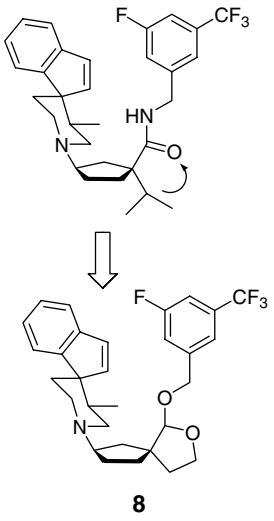
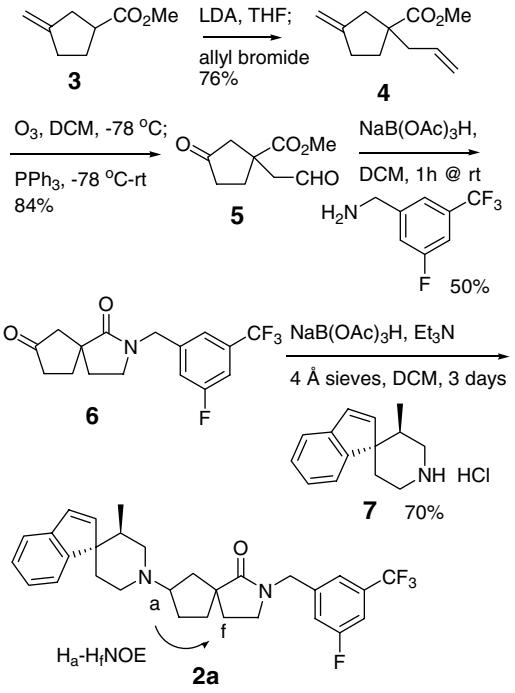


Figure 2. Spiroacetal locks benzyl above the cyclopentane.



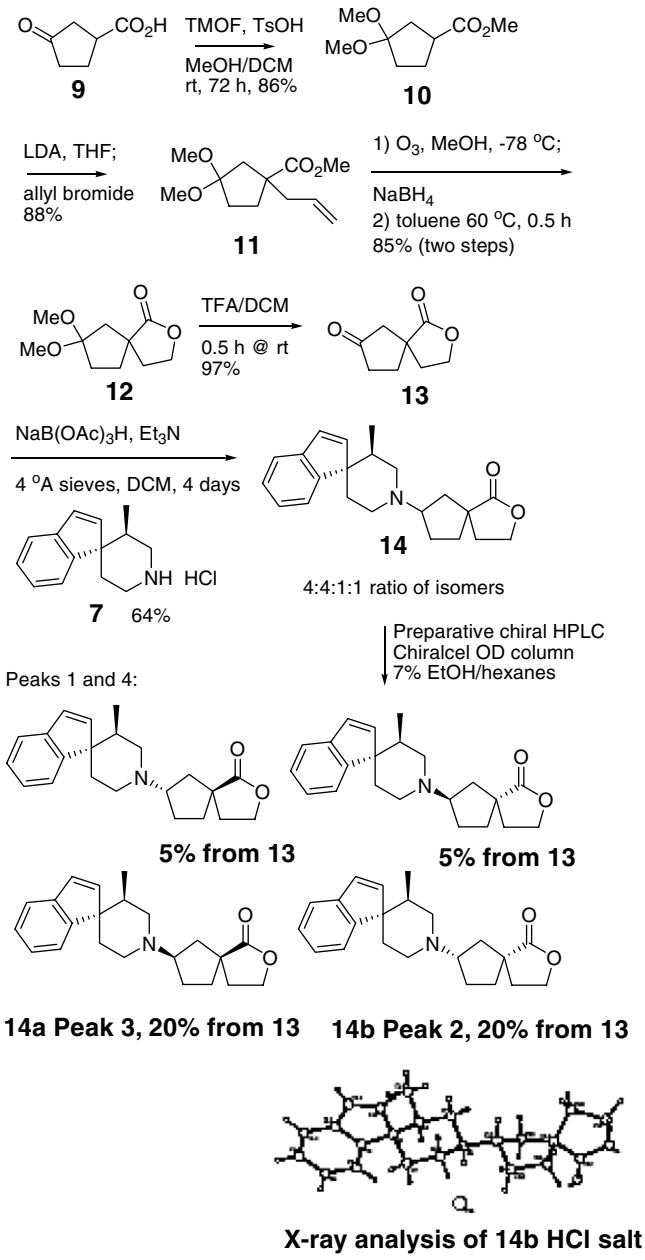
Scheme 1. Synthesis of *syn*-spirolactams **2a** (mix two *syn*-diastereomers).

amination with 1.0 equivalent of 3-fluoro-5-trifluoromethylbenzylamine took place selectively at the aldehyde and occurred with concomitant cyclization to the dihydropyrolidinone **6** in 50% yield. A second reductive amination reaction with (1*R*,3′*R*)-3′-methylspiro[indene-1,4′-piperidine][8] over 3 days at rt afforded the target analog **2a** as a mixture of two *syn*-isomers. The stereochemistry was determined by a ROESY 2D NMR experiment which revealed an NOE between the methine hydrogen H_a and the methylene hydrogens H_f (see Scheme 1).

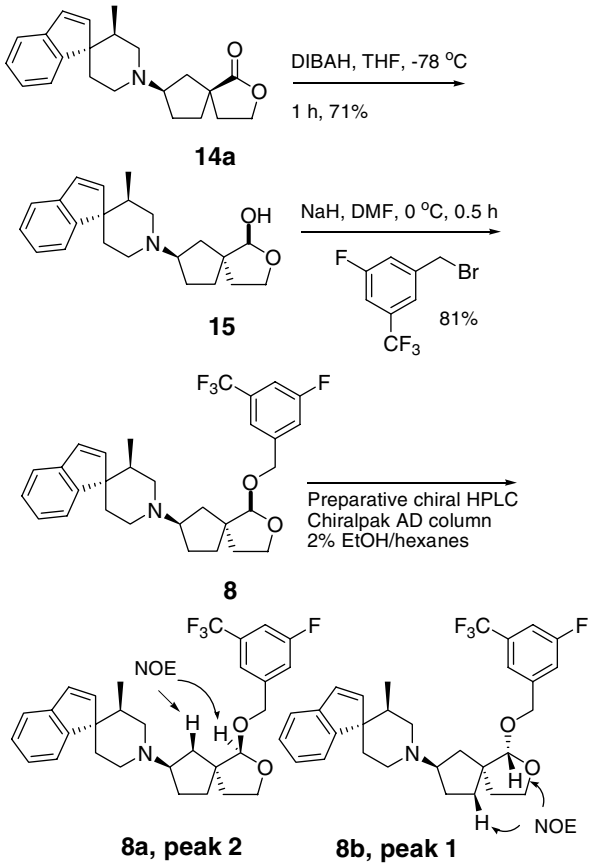
Toward preparing the alternative locked analog we envisioned a spirocyclic acetal scaffold **8**, which according to models, positions the benzyl subunit above the cyclopentyl and in closer proximity to the piperidine fragment. In addition, the acetal ring oxygen can serve as an isoelectronic substitute for the original carbonyl oxygen (Fig. 2).

The synthesis of the two epimeric acetal isomers of **8** was accomplished as described in Schemes 2 and 3. According to Scheme 2, the commercially available ketoacid **9** is fully protected by treatment with trimethylorthoformate and catalytic TsOH in MeOH/DCM to give **10** (86%). Alkylation as described previously afforded **11** in 88% yield. Ozonolysis followed by treatment with sodium borohydride gave the corresponding alcohol, which upon warming in toluene cyclized to provide the lactone **12** (85%, two steps). Treatment with TFA to give ketone **13** was followed by reductive amination with piperidine **7**, as described previously, to give aminolactone **14** as a 4:4:1:1 mixture of 4 diastereomers in 64% yield (ratio determined by chiral analytical HPLC). All four isomers were separated by chiral preparative HPLC using a Chiralcel OD column (2 cm × 25 cm, purchased from Chiral Technologies, Inc), using 7% ethanol/hexanes as the eluent. One of the two major isomers (2nd peak to elute) was converted to its HCl salt and crystals grown from DCM/heptane were subjected to X-ray crystallographic analysis (see Scheme 2 for 3D structure, $R = 3.3\%$). The resulting coordinates (deposited with the Cambridge crystallographic Data Centre for small molecules: 670426) indicated that the cyclopentane stereocenters had the opposite configuration to what we required. From this we deduced that the other major isomer (3rd peak to elute) should have the correct *syn*-(1*R*,3*S*)-stereochemistry corresponding to our original lead **1** since the reductive amination is selective for the *syn*-isomers.

The homochiral lactone **14a** was treated with DIBAH (1 M in hexanes) at −78 °C to afford a 3:2 mixture of diastereomeric lactols **15** in 71% yield (Scheme 3). Alkylation with 3-fluoro-5-trifluoromethylbenzyl bromide (NaH, DMF, 0.5 h, 0 °C) gave acetal **8** as a 3:2 mixture of diastereomers (81%). Preparative chiral HPLC sepa-



Scheme 2. Synthesis of aminolactone **14a**.



Scheme 3. Completion of **8a** and **8b**.

ration using a Chiralpak AD column (purchased from Chiral Technologies, Inc., 2 cm × 25 cm), and eluting with 2% ethanol/hexanes afforded the two homochiral acetals **8a** and **8b**. The absolute configuration of the acetal stereocenter in both **8a** and **8b** (shown in Scheme 3) was determined by NOESY 2D NMR experiments after all spectral assignments were obtained by a combination of COSY, TOCSY, and HSQC 2D experiments to assign all protons. The key NOE signals indicated in Scheme 3 correlate with models to support the assigned stereochemistry in each case.

The conformationally constrained analogs **2a**, **8a**, and **8b**, along with our initial lead compound **1**, were evaluated in our CCR2 binding assay (inhibition of ^{125}I-MCP-1 binding to endogenous receptor on human monocyte whole cells).[9] The results are listed in Table 1 below. The extended analog **2a** was inactive in our binding assay. In contrast, both analogs **8a** and **8b** were potent, with **8b** in particular having a very high binding affinity comparable to the original lead **1**. Moreover, when **8b** was evaluated in a functional assay measuring inhibition of MCP1 mediated chemotaxis of monocytes,[9] it proved to be a potent functional antagonist with an IC_{50} of 2.5 nM. These data strongly support the hypothesis that the active binding conformation of CCR2 antagonists typified by **1** is one in which the benzyl group is positioned above (and roughly perpendicular) to the cyclopentyl ring, forming a cup shaped structure with the piperidine subunit in close proximity to the benzyl subunit.

In summary we have prepared two conformationally constricted analogs of CCR2 antagonist **1** in an effort to shed light on its active conformation. The first analog prepared, **2b**, in which the 3-fluoro-5-trifluoromethylbenzyl pharmacophore was projected away from the

Table 1. Binding affinities for original lead and new analogs

Compound	Human monocytes IC_{50}[a]
1	1.4 nM
2a	18% at 1 μM[b]
8a	115 nM
8b	4.6 nM

[a] For details on this binding assay see Ref. 9.
[b] % Inhibition at 1 μM.

cyclopentyl core, was inactive in our binding assay. The second analog, based on a spiroacetal framework **8b**, and in which the 3-fluoro-5-trifluoromethylbenzyl pharmacophore is positioned over and roughly perpendicular to the cyclopentyl core, proved to be a potent CCR2 antagonist. This led us to infer that the active conformation of **1** is analogous to **8b** where the benzyl group is over the cyclopentane core. In addition, use of the spirocyclic acetal as a tool for studying the binding conformation of our CCR2 antagonists has led to the discovery of a new structural class of CCR2 antagonist which shows potent binding and functional activity.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.01.016.

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