Discovery of 3-Piperidinyl-1-cyclopentanecarboxamide as a Novel Scaffold for Highly Potent CC Chemokine Receptor 2 Antagonists

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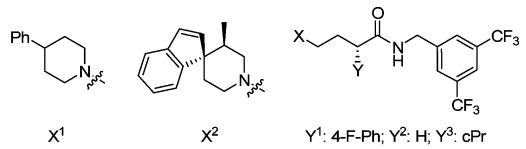
Abstract: Introduction of ring restrictions to a linear aminobutyramide CC chemokine receptor 2 (CCR2) antagonist lead (**2**) led to the discovery of a 1,3-disubstituted cyclopentane scaffold with enhanced hCCR2 receptor binding and antagonist activity. (1*S*,3*R*)-*N*-[3,5-Bis-(trifluoromethyl)benzyl]-1-methyl-3-[(1*R*,3′*R*)-methyl-1′*H*-spiro[indene-1,4′-piperidin]-1′-yl]cyclopentanecarboxamide (**16**) had IC_{50} of 1.3 nM (binding) and 0.45 nM (functional chemotaxis) against hCCR2. It also showed activity against the mouse CCR2 receptor with an IC_{50} of 130 nM. Compound **16** is selective against other chemokine receptors, including CCR5 (∼500-fold).

Chemokines, or chemotactic cytokines, are a large family of small structurally related proteins that play an important role in leukocyte migration and activation. Chemokines mediate their effect through binding to the specific cell-surface chemokine receptors, which belong to the superfamily of seven-transmembrane spanning G-protein-coupled receptors.[1−3] Monocyte chemoattractant protein (MCP-1,[a] CCL2) belongs to the CC chemokine family and binds to CC chemokine receptor 2 (CCR2), which is most abundantly expressed on monocytes. Studies have suggested that small-molecule CCR2 antagonists may provide potential therapies for a variety of diseases including rheumatoid arthritis, multiple sclerosis, and atherosclerosis.[4,5]

We have previously reported the identification of a class of submicromolar glycinamide-based CCR2 antagonists.[6] Optimization of the backbone led to the identification of a novel class of γ-aminobutyramide-based CCR2 receptor antagonists (**1**, Figure 1, IC_{50} shown as ability to displace MCP-1 binding to human CCR2b in Chinese hamster ovary cell line).[7] Extensive SAR studies on the phenylpiperidine identified that spiroindene-piperidine and particularly methyl substitutions improve potency (**2**).[7] Further modifications revealed that the 2-aryl substitution on the butyramide core can be eliminated with some loss of affinity (**3**) or replaced by small alkyl groups with improved potency (**4**).[8] While the butyramide leads were quite potent and orally bioavailable, the flexible backbone was an obvious target for medicinal chemistry manipulation to further improve potency and specificity. Furthermore, these acyclic leads lacked activity against the murine CCR2 receptor, which is critical for proof of concept studies in murine models. There has been one report on the pharmacological characterization of a rodent active CCR2 antagonist (INCB3344), but no structural information was given.[9] Herein, we report our systematic and rational approach that resulted in the identification of 3-amino-1-cyclopentanecarboxamide as the optimal scaffold for CCR2 antagonism. The rigid cyclopentane scaffold provided structural novelty and increased potency against human and murine CCR2 over the linear series.



1: X^1, Y^1: IC_{50} = 80 nM

2: X^2, Y^1: IC_{50} = 59 nM

3: X^2, Y^2: IC_{50} = 173 nM

4: X^2, Y^3: IC_{50} = 14 nM

Figure 1. 4-Aminobutyramide based hCCR2 antagonists.

Our approach to introduce structural rigidity was also partly inspired by the success of the earlier CCR5 antagonist program carried out at Merck, where a linear CCR5 antagonist lead similar to **1** (both were prepared as part of the NK1 antagonist effort) was transformed to a pyrrolidine scaffold with improved potency and selectivity.[10] Screening of the Merck CCR5 collection of more than 5000 compounds did not yield any CCR2 hits, suggesting that the structural requirements for the two receptors are quite different.

There are many ways of introducing ring restriction to **1**, especially if one considers potential attachment points on the two benzene rings and the piperidine. Furthermore, additional ring sizes are possible with 3−7 being the most used in medicinal chemistry. If all of these factors are considered, there are many structural possibilities with a large number being synthetically unattractive. We took a rational, stepwise approach to this problem by addressing substitution at the 2-position. Initial compounds showed 2-substitution to be potency enhancing but not required for CCR2 antagonism activity (Figure 1). This allowed us to work on the simple butyramide as the starting point. After scaffold modification, the 2-substitution can be reintroduced to further enhance potency. This allowed us to limit our attachment points to the five backbone atoms (from the benzylic position 2′ to the γ carbon). Second, we limited our ring size to 5 or 6, which would cover diverse conformations and made the problem much more manageable.

To introduce a five-membered ring between any two of the five backbone atoms, there are eight possibilities as shown in Figure 2 (2′−4 connection needs a minimum six-membered ring and 1′−4 connection gives rise to an acylated aminal that is generally considered unstable under mildly acidic conditions). Although it is feasible to make these 8 five-membered ring and 10 six-membered ring compounds, we felt that we could further triage them and set priorities for earlier success in finding an active scaffold.

We have previously reported that incorporation of methyl groups in **1** at positions 2 and 4 of the butyramide backbone led to decreases in potency while 3-substitution rendered the compound inactive.[7] Similarly, methylation of the amide nitrogen afforded more than a 40-fold decrease in potency and the introduction of methyl at the benzylic position of the benzylamide produced an inactive compound (unpublished data). On the basis of these results, we assumed that ring tethering at

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[a] Abbreviations: CCL2, chemokine (C−C motif) ligand 2; CCR2, chemokine (C−C motif) receptor 2; hCCR2, human CCR2; mCCR2, mouse CCR2; MCP-1, monocyte chemoattractant protein 1; NOE, nuclear Overhauser enhancement.

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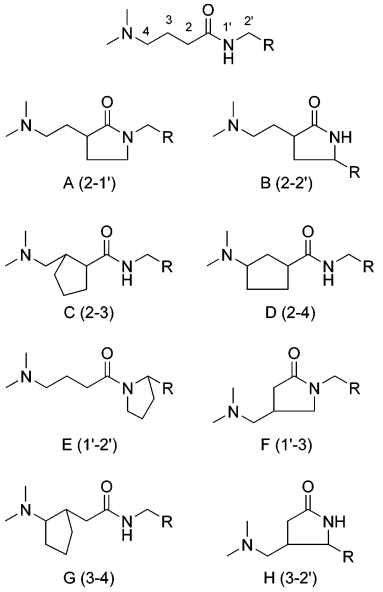
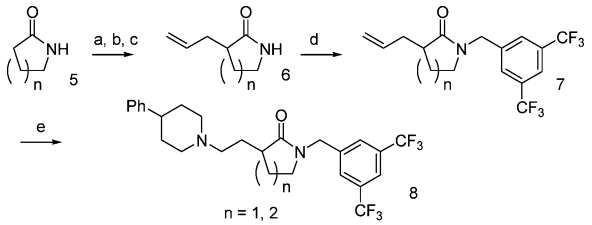


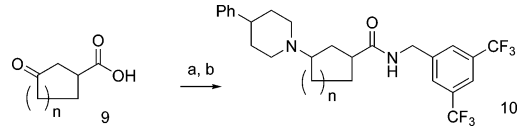
Figure 2. Chemically feasible five-membered ring constraint.

Scheme 1. Preparation of 2-Substituted Lactams[a]



[a] Reagents and conditions: Boc_2O, DMAP, CH_2Cl_2; (b) LHMDS, allyl bromide, THF, −78 °C to room temp; (c) HCl/dioxane−ether; (d) NaH, 3,5-bis-CF_3BnBr, THF/DMF; (e) O_3, CH_2Cl_2, −78 °C; $NaB(OAc)_3H$, 4-phenylpiperidine, CH_2Cl_2.

Scheme 2. Preparation of 1,3-Disubstituted Cyclocarboxamide[a]



[a] Reagents: (a) 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), 3,5-bis-CF3BnNH$_2$, CH_2Cl_2; (b) $NaB(OAc)_3H$, 4-phenylpiperidine, CH_2Cl_2.

the 3 and 2′ positions would probably not be tolerated. Therefore, these assumptions leave structures **A** and **D** (and their six-membered ring homologues) with the highest probability of yielding active compounds. We also prepared **C** and **E**, which turned out to be inactive as initially hypothesized. Structure **F** had been previously prepared and found to be inactive.

Analogues in the **A** series were prepared according to Scheme 1. The available lactam **5** was N-Boc protected, then alkylated and deprotected to give the allyl analogue **6**. Subsequent N-benzylation gave *N*-benzyl lactam **7**. The double bond was cleaved with ozone, and the crude ozonide was decomposed in the presence of 4-phenylpiperidine and sodium triacetoxyborohydride to effect direct reductive amination.

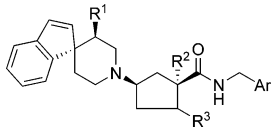
Analogues in the **D** series were prepared in two steps from the available keto acids as described in Scheme 2. Coupling of the keto acid with 3,5-bis(trifluoromethyl)benzylamine followed by reductive amination with a substituted piperidine provided the desired compounds as a mixture of four isomers.

The synthesized compounds were screened for their ability to displace ^{125}I labeled MCP-1 in isolated human primary monocytes or mouse CCR2 expressed in Chinese hamster ovary cell line. Functional antagonism and efficacy of key compounds were studied in a calcium flux or in chemotaxis assays, both using human primary monocytes.[11]

Table 1. Binding Affinity to Human Monocyte

compd	*n*	stereochemistry	IC_{50}
8a	1	racemic	inactive
8b	2	racemic	inactive
10a	1	mixture of four	65 nM
10b	2	mixture of four	23% @ 1 μM
10a-1	1	cis-(1*R*,3*S*)	30% @ 1 μM
10a-2	1	cis-(1*S*,3*R*)	36 nM
10a-3	1	trans-(1*S*,3*S*)	44% @ 1 μM
10a-4	1	trans-(1*R*,3*R*)	25% @ 1 μM

Table 2. Effect of a Methyl on the Binding Affinity to Human and Mouse Monocyte



compd	R^1	R^2	R^3	hCCR2 IC_{50} (nM)	mCCR2 IC_{50} (nM)
11	H	H	H	7.5	inactive
12	Me	H	H	1.4	27% @ 1 μM
16	Me	Me	H	1.3	130
17	Me	H	Me	>1000	inactive
18	H	Me	H	47	27% @ 1μM

Neither five- nor six-membered-ring lactams (**8a**, **8b**) showed any activity (Table 1). On the other hand, the 1,3-disubstituted cyclopentane **10a** had an IC_{50} of 65 nM on the hCCR2 receptor as a mixture of four isomers. Interestingly, its homologue **10b** was totally inactive, indicating very stringent stereo requirement for the backbone. Compound **10a** was a mixture of four diastereomers in unequal ratios that proved to be inseparable on chiral HPLC columns in a single run. Fortunately, the two sets of enatiomers (3:1) can first be separated by a Chiralcel OD column (hexane/ethanol 96:4). NOE measurements indicated that the major isomer had a 1,3-cis arrangement. Single enatiomers were then obtained using a Chiralcel AD column (hexane/ethanol 96:4). The absolute stereochemistry of active cis isomers (**10a-2**) was later established to be 1*S*,3*R* by resynthesis using known (*S*)-3-oxocyclopentane carboxylic acid as the starting material. Binding results for the four isomers are listed in Table 1. **10a-2** was the only active isomer, showing 36 nM potency in the human monocyte binding assay.

In the linear butyramide series, we had previously demonstrated that replacing the phenylpiperidine with spiroindene-piperidine and methylspiroindene-piperidine enhanced the binding affinity.[7,8] Incorporation of this feature in the cyclopentane series, according to Scheme 2, resulted in **11** and **12**, respectively, after chiral separation. Compound **11** (single active isomer) appeared to be 4-fold more active than **10a-2**, while single isomer **12** was more than 20-fold more active (Table 2). Compound **12** binds to the hCCR2 receptor with high affinity (IC_{50} = 1.4 nM), and functionally, it inhibited MCP-1 induced calcium flux (IC_{50} = 2.3 nM) and chemotaxis (IC_{50} = 57 nM) in human monocytes.

Having established 1,3-disubstitutedcyclopentane as the optimal template, we turned our attention to further increasing potency through substitution. To take advantage of the observed potency enhancing effects of the 2-substitutents in the linear system, there are two possible positions for substitution in the cyclopentane template, as shown in Figure 3. The 1-substituted system is similar to the unsubsituted in terms of stereochemistry,

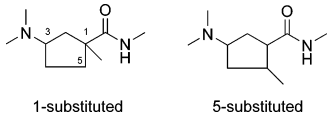
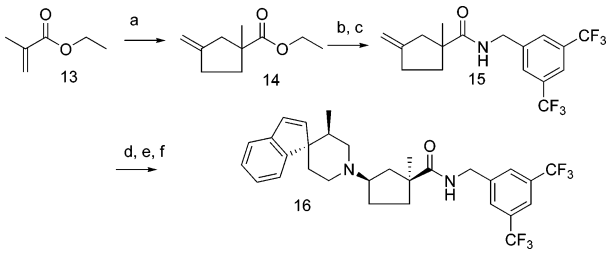


Figure 3. Possible substitution patterns.

Scheme 3. Synthesis of 1-Substituted Analogues[a]



[a] Reagents and conditions: (a) 2-[(trimethylsilyl)methyl]-2-propen-1-yl acetate, $Pd(OAc)_2$, $(^iPrO)_3P$, THF, reflux; (b) aqueous LiOH, dioxane, water, 80 °C; (c) EDC, 3,5-bis-CF_3BnNH_2; (d) O_3, CH_2Cl_2/MeOH, −78 °C; (e) $NaB(OAc)_3H$, 4-phenylpiperidine, CH_2Cl_2; (f) chiral column HPLC separation.

while the 5-substituted system introduced an extra chiral center with a possibility of eight isomers.

Both series were prepared using similar 2 + 3 cycloaddition chemistry with the appropriate acrylate as the starting olefin.[12] Only the synthesis of the 1-substituted analogue is shown in Scheme 3. The introduction of 1-methyl began with a palladium-catalyzed cycloaddition of 2-[(trimethylsilyl)methyl]-2-propen-1-yl acetate to ethyl 1-methylacrylate **13** as shown. The volatile ester **14** was used without any further purification and was hydrolyzed and converted to the amide **15** as described in Scheme 2. Ozonolysis and reductive amination in one pot, as described in Scheme 1, afforded a mixture of four diastereoisomers, which were separated using chiral column HPLC as described for **10a** to yield the single isomer **16**. Its relative stereochemistry was established through NMR analysis and comparisons with compound **10a-2**, and the absolute stereochemistry was confirmed by an alternative synthetic route starting from (*S*)-3-oxocyclopentanecarboxylic acid. Similarly, the 5-methyl analogue **17** was prepared starting from crotonic acid derivatives.

As in the series described earlier, only one (**16**) of the four isolated isomers was active. Although the introduction of 1-methyl did not significantly increase the hCCR2 binding affinity (**16**, IC_{50} = 1.3 nM) over **12**, it showed substantial improvement in the functional chemotaxis assay (0.45 nM). Such discrepancies between the binding and functional assay may be due to the binding assay having bottomed out in the low nanomolar range, thus making the functional chemotaxis assay a better predictor with this class of hCCR2 antagonists.

It had been a project goal to discover compounds with murine CCR2 receptor antagonism to validate the target in murine models; however, our previous reported compounds were devoid of mCCR2 activity. Therefore, the discovery that **16** binds to the mCCR2 with an IC_{50} of 130 nM was very encouraging. Table 2 illustrates the important role that a methyl group played in CCR2 activity. The introduction of a methyl to the 5-position totally eliminated CCR2 activity (all eight isomers were prepared). Removal of the methyl group on the piperidine (**18**) resulted in loss of human and mouse CCR2 activity. Later publications from our group will detail the SAR that led to more potent mCCR2 compounds for proof of concept studies in mouse.

In counterscreens, **16** also showed good selectivity versus neurokinin receptors NK1 and NK2, and it has ~500-fold selectivity against CCR5 receptor (IC_{50} = 630 nM, MIP-1α binding), a marked improvement over the linear analogues. In addition, **16** has no affinity for other chemokine receptors tested (no significant binding at 1 μM on CCR1, CCR3, CCR4, CXCR3, CXCR4, and CCR8). It has a modest oral bioavailability in rats (F = 15%), moderately high clearance (Cl_p = 50 mL min^{-1} kg^{-1}), a large volume of distribution (V_{dss} = 6 L kg^{-1}), and a long half-life ($t_{1/2}$ = 8.1 h).

In summary, we have described SAR studies that have resulted in the identification of 1,3-disubstituted cyclopentane as a novel scaffold for the preparation of potent and selective CCR2 antagonist. This lead showed modest mCCR2 activity and served as a starting point for all of our subsequent efforts at finding potent and selective CCR2 antagonists.

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Supporting Information Available: Experimental details for the synthesis of **16**. This material is available free of charge via the Internet at http://pubs.acs.org.

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