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3-Amino-1-alkyl-cyclopentane carboxamides as small molecule antagonists of the human and murine CC chemokine receptor 2

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Abstract—A series of low molecular weight antagonists of both the human and murine CC chemokine receptor 2, containing a 1-alkyl-3-(3-methyl-4-spiroindenylpiperidine)-substituted cyclopentanecarboxamide, is described. A SAR study of the C_1 substituent revealed that short, branched alkyl groups such as isopropyl, isobutyl, or cyclopropyl are optimal for both human and murine CCR2 binding activity.

Chemokines (*chemo*tactic cyto*kines*) are important mediators of inflammation and host defense.[1–3] These low molecular weight proteins are secreted by pro-inflammatory cells, leukocytes, and endothelial cells in response to certain stimuli or insult to the immune system.[4–6] Chemokines can be divided into two major (CC and CXC) and two minor (CX3C and C) groups based on the number and spacing of the two conserved cysteine residues near the N-terminus of the molecule and the type of leukocytes they attract.[7]

The Monocyte Chemoattractant Protein 1 (MCP-1), a member of the CC family of chemokines, binds to and activates a seven transmembrane domain receptor known as CC chemokine receptor 2 (CCR2).[8] It has been strongly implicated in several inflammatory diseases, including rheumatoid arthritis[9] and atherosclerosis.[10,11] Studies with CCR2$^{-/-}$[12] and MCP-1$^{-/-}$[13,14] mice as well as with peptide MCP-1 antagonists[15] suggest that blocking the interaction of CCR2 and MCP-1 may be a viable approach for treatment of rheumatoid arthritis and atherosclerosis.[16]

Recently, a number of small molecule non-peptide CCR2 receptor antagonists from Roche,[17] Takeda,[18,19] and SmithKline[20,21] have been described. As part of our own effort, high throughput screening of the Merck Sample Collection produced the initial lead compound **1** (IC_{50} = 720 nM), which was elaborated into the piperidine analog **2** with approximately three fold improved binding affinity, Figure 1.[22] Introduction of a 3-methyl-4-spiroindenyl piperidine moiety afforded the second generation lead **3** (IC_{50} = 67 nM).[23] Additional refinement of the central region led to the discovery of cyclopropyl derivative **4** with outstanding binding affinity (IC_{50} = 4 nM), as well as oral bioavailability.[24]

Attempts to improve the pharmacological properties through cyclization within the central region of the scaffold afforded cyclopentane carboxamides such as **5a**, with a surprisingly high binding affinity (hIC_{50} = 20 nM), Figure 1.[25] Unfortunately, none of these exhibited sufficient affinity toward the murine CCR2 receptor,[26] (e.g., **5a**, *m*CCR2, 27% inhibition at 1 μM). A small molecule antagonist of both human and murine CCR2 activity would be highly desirable as it would greatly facilitate target validation and other studies in various rodent models.

Herein, we wish to describe the structure–activity studies which led to discovery of novel CCR2 receptor antagonists with excellent binding and functional activity at both the human as well as the murine derived CCR2 receptor.

Target compounds **5** and **6** (except for the cyclopropyl substituted cases **5k** and **6k**) were synthesized via one

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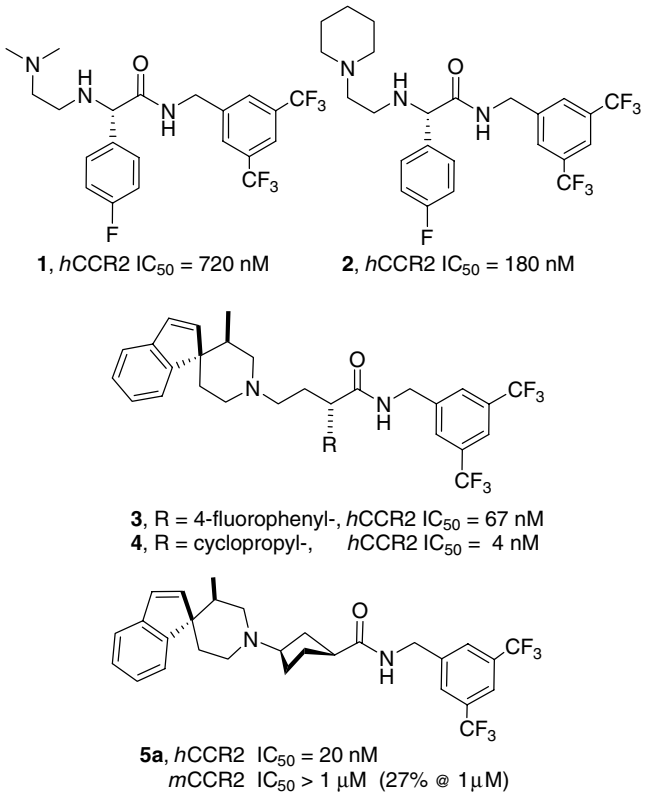
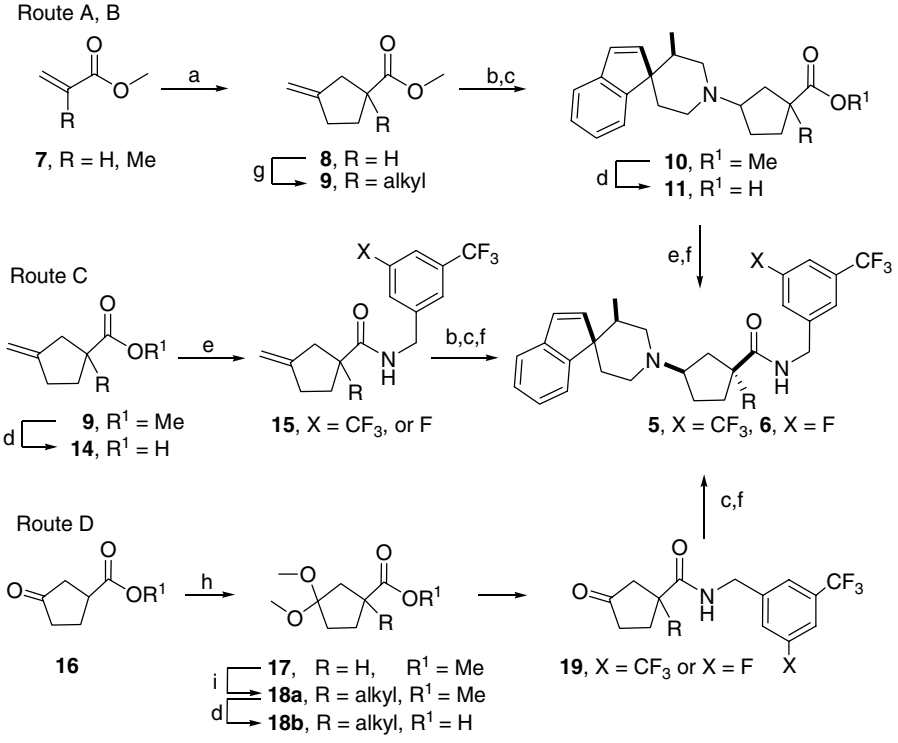


Figure 1. CCR2 antagonists.

of the four principal routes depicted in Scheme 1. According to Route A (compounds **5a**, **6b**, Scheme 1), methyl acrylate (**7**, R = H) or methyl methacrylate (**7**, R = Me) and 3-(trimethylsilyl)methyl-2-propen-1-yl acetate were subjected to a palladium-catalyzed 3 + 2 cycloaddition.[27,28] The double bond in esters **8** and **9** was cleaved with ozone and the crude ozonides were subjected to a reductive amination with enantiomerically pure 3-methyl-4-spiroindenyl-piperidine[23] without isolation of the respective ketones. A base-catalyzed hydrolysis of the esters **10** yielded the respective acids **11**, which were coupled with 3,5-bistrifluoromethylbenzylamine (**12**) or 3-fluoro-5-trifluoromethylbenzylamine (**13**) to yield the final amides as a mixture of four diastereoisomers. The enantiomerically pure[29] compounds **5a** and **6b** were obtained by an HPLC separation of the diastereoisomers using either a Chiralcel OD or Chiralpak AD column.[30]

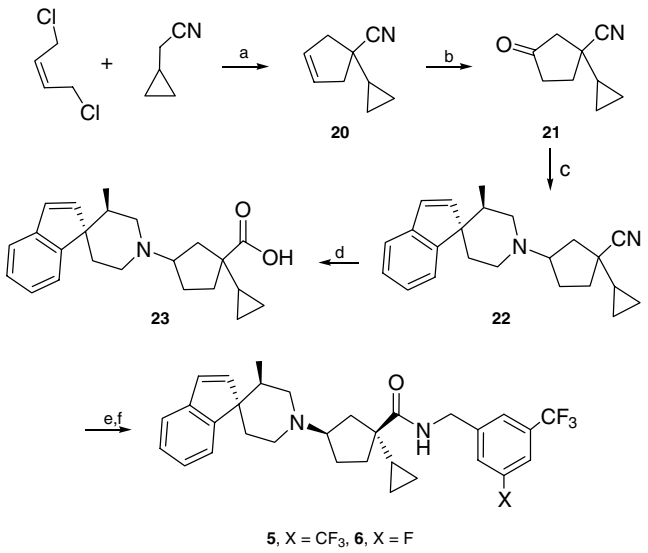
The majority of the target compounds (**5c**, **d**, **f**, **h**–**j**, and **6b**–**i**) were synthesized following Route B. Thus, the enolate formed from the ester **8** using lithium diisopropylamide (LDA) was alkylated with the respective haloalkane to yield esters **9**, which were subjected to ozonolysis and a reductive amination as described above. Once again, EDC mediated amide formation followed by an HPLC separation of the isomers completed the synthesis.

Route C (compounds **5b**, **e**, and **g**) involved an early installation of the amide: the methyl 1-alkyl-3-methyl-



Scheme 1. Reagents and conditions: (a) methyl acrylate, or methyl methacrylate, 3-(trimethylsilyl)methyl-2-propen-1-yl acetate, $Pd(AcO)_2$, $(i\text{-}PrO)_3P$, toluene, reflux; (b) O_3, CH_2Cl_2, −78 °C; (c) 3-methyl-4-spiroindenylpiperidine hydrochloride, $NaHB(OAc)_3$, DIEA, 4 Å molecular sieves, CH_2Cl_2; (d) LiOH or NaOH, H_2O, rt or 80 °C; (e) **12** or **13** 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC), 1-hydroxy-7-azabenzotriazole (HOAT), CH_2Cl_2; (f) Chiralcel OD or Chiralpak AD preparative column, EtOH/Hexanes at 9.0 mL/min; (g) alkyl halide, LDA, THF, −78 to 0 °C; (h) TMOF, *p*TSA, CH_2Cl_2; (i) chloromethyl methyl sulfide or dimethyl disulfide, LDA, THF, −78 to 0 °C; (j) TFA, CH_2Cl_2.

enecyclopentane carboxylates **9** were subjected to a base-catalyzed hydrolysis and the resulting acids **14** were coupled with amine **12** or **13** to yield amides **15**. As described in Route A, the double bond was cleaved with ozone, and the crude ozonides were subjected to a reductive amination.



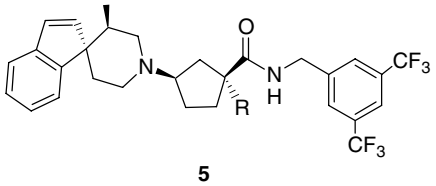
Scheme 2. Reagents and conditions: (a) LHMDS, DME, 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone, (10:1); (b) B_2H_6, −78 °C, THF; PCC, $MgSO_4$, CH_2Cl_2; (c) 3-methyl-4-spiroindenylpiperidine hydrochloride, $NaB(OAc)_3H$, DIEA, 4 Å molecular sieves, dichloroethane; (d) NaOH, H_2O, dioxane, 90 °C; (e) **12** or **13**, EDC, HOAT, CH_2Cl_2; (f) Chiralcel OD or Chiralpak AD preparative column, EtOH/Hexanes at 9.0 mL/min.

The target compounds containing sulfur in the side chain (**5l**, **m**, **6l**, **m**) were synthesized via Route D. Thus, 3-oxocyclopentanecarboxylic acid[31] (**16**) was converted to the ester acetal **17** with trimethyl orthoformate (TMOF). The respective lithium enolate was alkylated with chloromethyl methyl sulfide (**5l**, **6l**) or dimethyl disulfide (**5m**, **6m**). The esters **18a** were hydrolyzed, and the resulting acids were coupled to amines **12** or **13**. The acetal protecting group was removed under acidic conditions and the keto amides **19** were reductively aminated as described above to yield the final products. The respective isomers were separated by HPLC.

For the synthesis of the cyclopropyl substituted target compounds **5k** and **6k** an alternative strategy (Route E) had to be adopted as shown in Scheme 2. The commercially available cyclopropylacetonitrile was alkylated with 1,4-dichloro-*cis*-2-butene in the presence of lithium bis(trimethylsilyl)amide (LHMDS) to form the cyclopentenecarbonitrile **20**. Hydroboration, followed by a pyridinium chlorochromate (PCC) oxidation yielded the ketone **21**, which was subjected to reductive amination with 3-methyl-4-spiroindenyl piperidine. The aminonitrile **22** was hydrolyzed, and a standard amide-forming step installed the 3,5-bistrifluoromethylbenzylamide- and 3-fluoro-5-trifluoromethylbenzylamide groups, respectively. The single enantiomers were obtained via a HPLC separation, as described above.

As illustrated in Table 1, a substituent attached at position C_1 of the cyclopentane ring has beneficial effect

Table 1. Human and murine binding affinities in the 3,5-bistrifluoromethylbenzylamide series



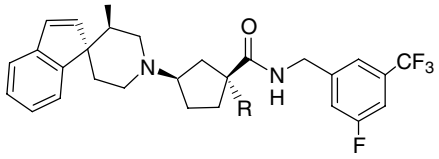
5

Entry	Substituent R	Synthetic method	Binding IC_{50} (nM) or % at 1 μM (SEM, *n*)[34]	
			*h*CCR2[a]	*m*CCR2[b]
5a	H–	A	20.0 (±1.41, 2)	27 % (n/a)
5b	Me–	C	9.0 (1)	126.0 (±33.08, 3)
5c[c]	Et-	B	10.0 (1)	17.5 (±2.12, 2)
5d	*i*-Pr–	B	7.6 (±2.19, 1)	15.0 (1)
5e	*n*-Pr–	C	16.0 (1)	44.3 (±23.5, 3)
5f	*i*-Bu–	B	8.0 (1)	26.0 (±1.41, 2)
5g[c]	*c*-PrCH_2–	C	20.0 (1)	63.5 (±19.1, 2)
5h	*c*-BuCH_2–	B	19.0 (±8.49, 2)	64.0 (1)
5i[c]	$CH_3(CH_2)_5$–	B	231.0 (±167.6, 2)	279.0 (1)
5j[c]	CH_3OCH_2–	B	15.5 (±0.71, 2)	93.0 (1)
5k[c]	*c*-Pr–	E	6.5 (±2.12, 2)	45.0 (1)
5l	CH_3SCH_2–	D	13.0 (1)	65.5 (±21.9, 2)
5m	CH_3S–	D	28.0 (1)	351.0 (1)

[a] Human CHO cell.
[b] Mouse CHO cell.
[c] Mixture of two 1,3-*cis*-cyclopentane isomers.

on the *h*CCR2 activity as evidenced by an approximate twofold increase of the human CCR2 binding affinity when a hydrogen was replaced by a methyl group (entries **5a** vs **5b**, Table 1). However, the same change induced a dramatic increase in murine CCR2 binding. In this case, the affinity improved from inhibition of 27% at 1 μM concentration to IC_{50} of 126 nM. The observed activities after an increase in chain-length from methyl (**5b**) to ethyl (**5c**), *n*-propyl (**5e**), and *n*-hexyl (**5i**) suggest that the optimal chain

Table 2. Human and murine binding affinities in the 3-fluoro-5-trifluoromethylbenzylamide series



6

Entry	Substituent R	Synthetic method	Binding IC_{50} (nM) or % at 1 μM (SEM, *n*)[34]	
			h-CCR2[a]	*m*-CCR2[b]
6b	Me–	B	4.0 (1)	57.0 (±29.7, 2)
6c[c]	Et–	B	3.3 (±2.48, 2)	3.5 (±0.71, 2)
6d	*i*-Pr–	B	3.1 (±1.56, 2)	5.0 (1)
6e[c]	*n*-Pr–	B	5.0 (3)	17.0 (1)
6f	*i*-Bu–	B	3.9 (±2.97, 2)	7.5 (±3.01, 10)
6g[c]	*c*-$PrCH_2$–	B	5.5 (±0.71, 2)	15.0 (1)
6h[d]	*c*-$BuCH_2$–	B	6.3 (±1.06, 2)	28.0 (±5.66, 2)
6i[e]	$CH_3(CH_2)_5$–	B	27.0 (1)	128.0 (1)
6j[e]	CH_3OCH_2–	B	8.5 (±0.71, 2)	74.0 (1)
6k	*c*-Pr–	E	1.9 (±1.41, 2)	8.0 (1)
6l	CH_3SCH_2–	D	3.5 (±2.12, 2)	17.0 (±1.71, 2)
6m	CH_3S–	D	67.0 (1)	193.0 (1)

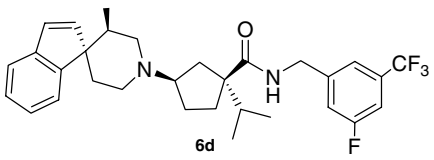
[a] Human CHO cell.
[b] Mouse CHO cell.
[c] Mixture of two 1,3-*cis*-cyclopentane isomers.
[d] The binding affinities of the remaining three isomers were determined to be 32, 135, and 229 nM for the human-, and 147, 559 nM, and 49% (at 1 μM) for the murine derived CCR2 receptor.
[e] Mixture of four 1,3-cis/trans-cyclopentane isomers.

Table 3. Functional activities of selected compounds

Entry	Substituent R	Chemotaxis[a] IC_{50} (nM) (SEM, *n*)	Ca^{2+} Flux[b] IC_{50} (nM) (SEM, *n*)[34]
6d	*i*-Pr–	0.15 (±0.1096, 2)	0.71 (1)
6f	*i*-Bu–	0.32 (±0.2302, 39)	2.04 (±0.198, 2)
6k	*c*-Pr–	0.23 (±0.1768, 2)	0.63 (1)

[a] Human MCP-1 monocytes.
[b] Human monocytes.

Table 4. Pharmacokinetic properties of the isopropyl derivative **6d** (male Sprague–Dawley rats)



6d

Route	Dose (mg/kg)	AUCn (μM h)	Clearance (mL/min/kg)	Vol. distrib. (L/kg)	$t_{1/2}$ (h)	C_{max} (μM)	T_{max} (h)	*F* (%)
iv	1.00	1.637	19.4	2.99	2.5			
po	3.00	0.492			2.0	0.414	0.83	32

length is two to three carbon atoms. The presence of a heteroatom within the chain (**5j**, **5l**, and **5m**) did not seem to have a dramatic influence on the binding affinity. Conversely, branching in the side chain (**5d** or **5f**) improved both the murine as well as human CCR2 activities. With additional restriction of the aliphatic substituent (**5k**, cyclopropyl, and **5g**, cyclopropylmethyl) both the human and murine affinities remained approximately the same.

A similar overall trend was observed in the analogous 5-fluoro-3-trifluoromethylbenzyl series (compounds **6a**–**m**, Table 2), except that both the human and murine activities were approximately twofold higher. Within this series, isopropyl (**6d**), isobutyl (**6f**), and cyclopropyl (**6k**) substitution yielded compounds with activities in the nanomolar range.

Compounds which were found to be the most active in the respective binding assays were also the most potent in the chemotaxis[32] and calcium flux[33] functional assays as shown in Table 3. In fact, the shorter, branched alkyl substituted cyclopentanes **6d**, **f**, and **k** were generally subnanomolar in the monocyte chemotaxis and calcium flux assays.

The pharmacokinetic properties of selected compounds were evaluated in Sprague–Dawley Rats. For example, the isopropyl derivative **6d** exhibited excellent drug levels after both intravenous (1.0 mg/kg, AUCn = 1.637 μM) and oral (3.0 mg/kg, AUCn = 0.492 μM, normalized value) administration. The compound showed a moderate clearance rate of 19.4 mL/min/kg, low volume of distribution (2.99 L/kg), and good oral bioavailability of 32%, Table 4.

In conclusion, systematic variation of the aliphatic side chain attached at C_1 of the cyclopentane core in lead structure **5** and **6** yielded compounds with low nanomolar affinities for both the human and murine CCR2 receptor. Some of these compounds, especially those belonging to the 3-fluoro-5-trifluoro-methylbenzamide series (e.g., **6d**, **6f**, and **6k**) were ideally suited for target validation and other biological studies requiring a rodent model.

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26. CHO cells (5×10^4) expressing either human CCR2B I40L or murine CCR2B receptor were incubated with ^{125}I-hMCP-1 (20–25 pM, 2200 Ci/mmol; Dupont/New England Nuclear) or ^{125}I-JE (20–25 pM, 2200 Ci/mmol; Dupont/New England Nuclear) at room temperature for 45 min in buffer containing Hepes (50 mM), $MgCl_2$ (5 mM), $CaCl_2$ (1 mM), pH 7.4, 0.5% BSA, and protease inhibitor cocktail. The reactions were terminated by filtration over GF/B filters that had been presoaked in 0.10% polyethyleneimine using a Packard Cell Harvester to separate bound from free ligand. The filters were washed with 25 mM Hepes, pH 7.5, containing 500 mM NaCl and the plates dried. The plates were counted for ^{125}I radioactivity using Microscint 0 (Packard) and a Topcount NXT (Packard).

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34. Standard Error Margins were not calculated when the datapoints were obtained from the same experiment using different dilutions. In this instance the number of experiments (*n*) is considered 1 and the IC_{50} value is calculated as an average of two datapoints.