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4-Amino-2-alkyl-butyramides as small molecule CCR2 antagonists with favorable pharmacokinetic properties

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Abstract—A systematic examination of the central aromatic portion of the lead (2*S*)-*N*-[3,5-bis(trifluoromethyl)benzyl]-2-(4-fluorophenyl)-4-(1′*H*-spiro[indene-1,4′-piperidin]-1′-yl)butanamide (**9**) led to the discovery of a novel class of CCR2 receptor antagonists, which carry small alicyclic groups such as *cyclo*propyl, *cylo*butyl, or *cyclo*propylmethyl attached at C_2 of the carbon backbone. The most potent compound discovered, namely (2*S*)-*N*-[3,5-bis(trifluoromethyl)benzyl]-2-*cyclo*propyl-4-[(1*R*,3′*R*)-3′-methyl-1′*H*-spiro[indene-1,4′-piperidin]-1′-yl]butanamide (**29**), showed very high binding affinity (IC_{50} = 4 nM, human monocyte) and excellent selectivity toward other related chemokine receptors. The excellent pharmacokinetic profile of this new lead compound allows for extensive in vivo evaluation.
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Chemokines (*Chemo*tactic cyto*kines*) are small molecular weight (8–10 kDa) water-soluble proteins composed of 340–380 amino acids that play a key role in immunomodulation and host-defense.[1,2] Their multiple effects are mediated through binding to a variety of related 7-transmembrane domain G-protein coupled receptors.[3] The observation that some chemokines and their respective receptors are upregulated during both chronic and acute inflammatory diseases, as well as in response to challenge of the immune system, suggested that they may present a viable target for drug intervention.[4,5]

Chemokines are classified as CC, CXC, CX3C, and XC based on the sequential relationship of the first two of four conserved cysteine residues.[6] Most of them appear to have redundant specificity since they bind to numerous receptors within the same family.[7] Monocyte Chemoattractant Protein 1 (CCL2/MCP-1), a member of the CC-subfamily, binds and activates the CC-chemokine receptor 2 (CCR2).[8] A large body of evidence accumulated in the recent past strongly suggests the involvement of the CCL2/CCR2 axis in arthritis,[9] multiple sclerosis, [10] and vascular disease[11] offering a logical platform for therapeutic intervention.[12] As a result of intensive research a number of small molecule CCR2 antagonists have been described in the scientific literature[13] and some representative examples from Takeda,[14,15] SmithKline,[16,17] Roche,[18] Teijin,[19] Johnson and Johnson,[20] and Astra-Zeneca[21] are shown in Figure 1.

High throughput screening of the Merck Sample Collection produced the initial lead **7** with a binding affinity[22] of 720 nM, Figure 2. Its optimization led to the discovery of the piperidine analog **8** with threefold improved potency.[23] Subsequently it was established that the basic nitrogen present in the backbone of the molecule can be omitted without significant loss of activity.[24] Intensive synthetic effort focusing on the amine portion of the lead structure produced further refinement of the piperidine ring. The pertinent SAR study revealed that introduction of a (substituted) phenyl ring at position 4 of the piperidine greatly enhances the observed binding affinities. Particularly interesting was the discovery of the spiroindene structural motif such as in **9**, which, albeit only slightly more potent than **8**, showed acceptable oral bioavailability.[25,26]

Herein, we wish to describe the results of an SAR study focusing on the central, aromatic portion of the lead structure **9**. This study was initiated by the observation

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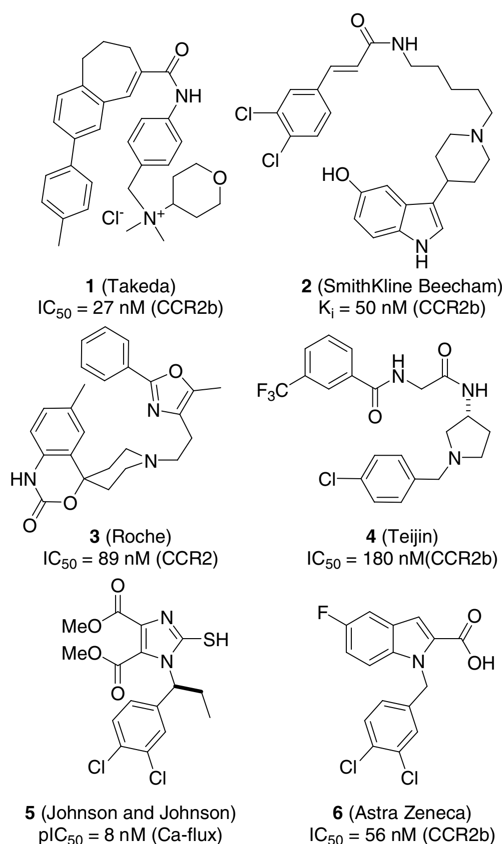


Figure 1. CCR2 antagonists.

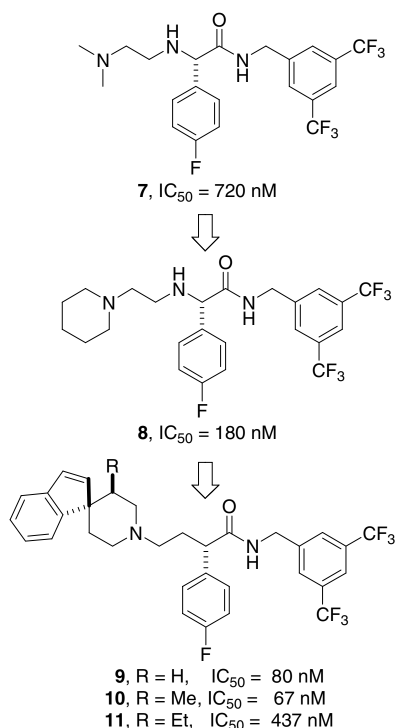


Figure 2. Lead evolution, CHO binding assay.

that omission of the 4-fluorophenyl pharmacophore in **9** had caused a less than expected decrease in binding affinity, approximately sevenfold. Furthermore, a properly placed methyl group, such as in compound **13**, led to almost full restoration of the activity, Figure 3.

We were interested to find out if the binding and functional activity of **13** could be further improved by the introduction of a pharmacophore with size and electronic characteristics between those of the 4-fluorophenyl group and a hydrogen atom.

The synthetic sequence utilized to access the desired compounds in a racemic form is illustrated for the example of the 2-*cyclo*propyl derivative **18**, Scheme 1.

The dianion of the commercially available *cyclo*propyl acetic acid (**14**) was alkylated with 2,2-dimethoxyethyl bromide. The amide was introduced using a 1-[(3-dimethylamino)propyl]-3-ethyl-carbodiimide (EDC) mediated coupling of acid **15** and the commercially available 3,5-bis(trifluoromethyl)-benzyl amine. The acetal was cleaved under acidic conditions at ambient temperature, and the liberated aldehyde was reductively aminated with spiro[indene-1,4′-piperidine].[27] Unfortunately, this sequence was only poorly reproducible as the intermediate aldehyde **17** readily underwent an intramolecular cyclization (**19**) followed by elimination of water (**20**) and decomposition.

In an attempt to block the lactam formation as well as facilitate access to single enatiomers, the acid was reacted via its pivaloyl anhydride with (4*R*)-4-benzyl-1,3-oxazolidin-2-one.[28] The two diastereoisomers were readily separated by flash column chromatography (silica gel, ethyl acetate–hexanes/3:7). Unfortunately, an attempt at hydrolytical cleavage of the oxazolidone failed to produce the acid **22**, as it readily underwent a γ-lactonization during the workup (**23**, **24**). To remedy this problem, the amine was introduced before the removal of the auxiliary, Scheme 2. Accordingly, the acetal was

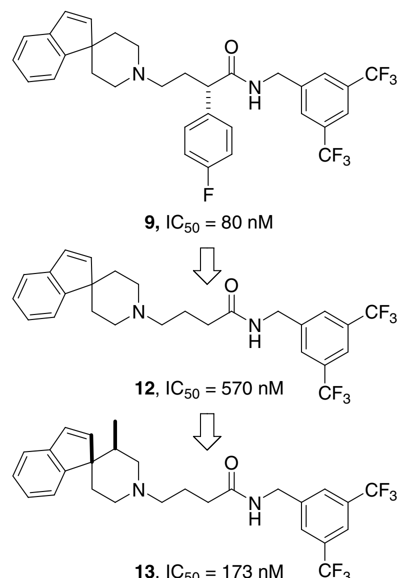
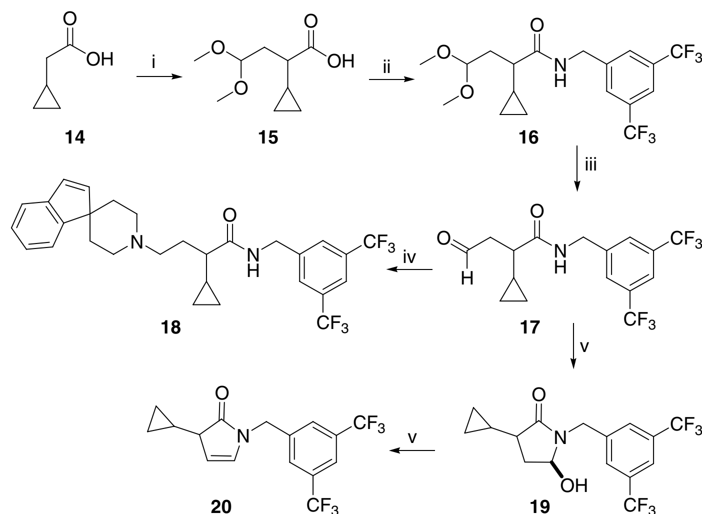
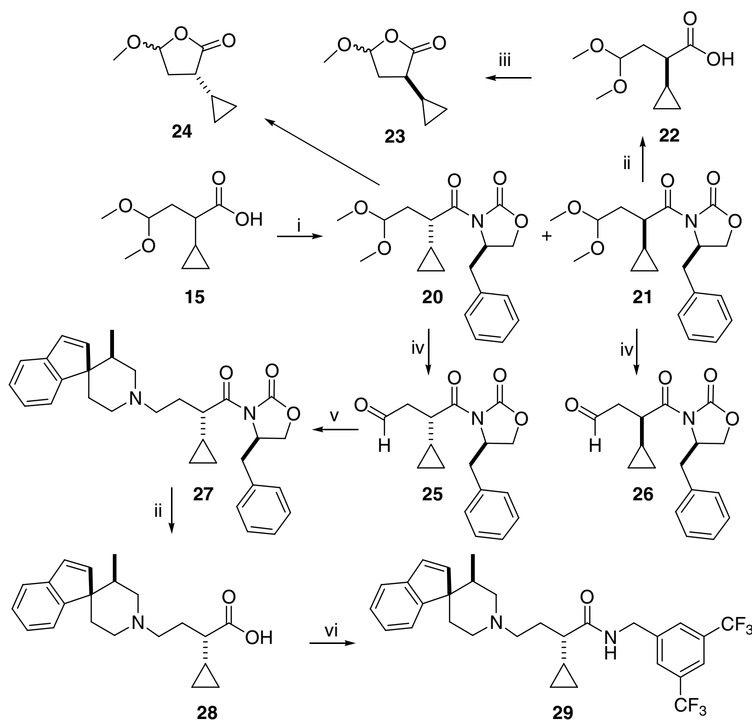


Figure 3. Central region SAR: initial observations, CHO binding assay.



Scheme 1. Reagents and conditions: (i) 2,2-dimethoxyethyl bromide, LDA, THF, −78 to +40 °C; (ii) 3,5-(bistrifluoromethyl)benzylamine, EDC, HOAT, DMF; (iii) aq TFA, rt 10 min; (iv) spiroindene, $NaHB(OAc)_3$, dichloroethane; (v) spontaneous.

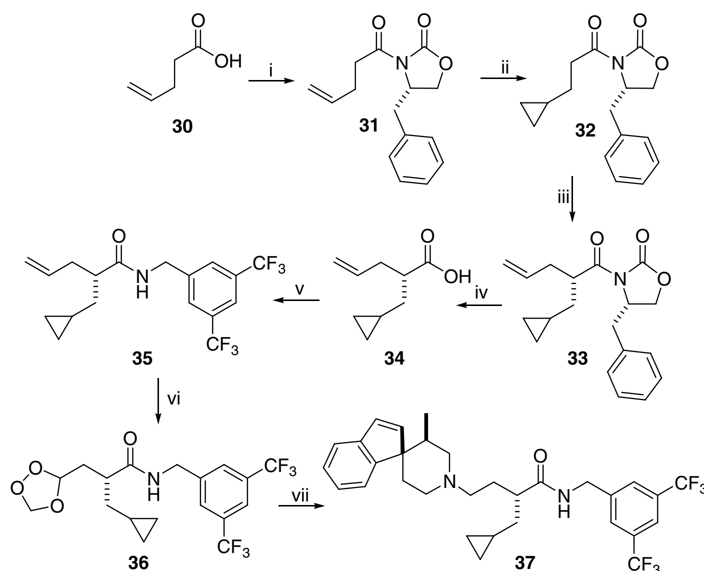


Scheme 2. Reagents and conditions: (i) *t*BuCOCl, DIEA, THF, lithium (4*R*)-4-benzyl-1,3-oxazolidin-2-one; (ii) LiOOH, 0 °C, THF, water; (iii) spontaneous; (iv) aq TFA; (v) (1*R*,3′*R*)-3′-methyl-spiro[indene-1,4′-piperidine], $NaHB(OAc)_3$, dichloroethane; (vi) 3,5-bis(trifluoromethyl)benzylamine, EDC, HOAT, DCM.

cleaved (93% yield) and aldehydes **25** and **26** were reductively aminated with (1*R*,3′*R*)-3′-methyl-spiro[indene-1,4′-piperidine].[27] The auxiliary was removed under standard conditions and the target compound **29** was obtained in an EDC-mediated amide forming step, Scheme 2.

The chiral oxazolidinone is uniquely suited to control absolute stereochemistry at the newly formed C_2-center.[29] We envisioned that replacing the 2,2-dimethoxyethyl bromide with the more reactive allyl chloride would further streamline the synthesis. This strategy is illustrated in Scheme 3, which describes the preparation of the methylene homolog of **29**, compound **37**.

To this end, the commercially available 4-pentenoic acid (**30**) was coupled with (4*S*)-4-benzyl-1,3-oxazolidin-2-one (vide ante) and the *cyclo*propyl group introduced via a palladium-catalyzed cyclo-propanation of the olefin with diazomethane.[30] The LDA mediated allylation of **32** produced stereoselectively the (4*S*)-4-benzyl-3-[(2*S*)-2-(*cyclo*propylmethyl)pent-4-enoyl]-1,3-oxazolidin-2-

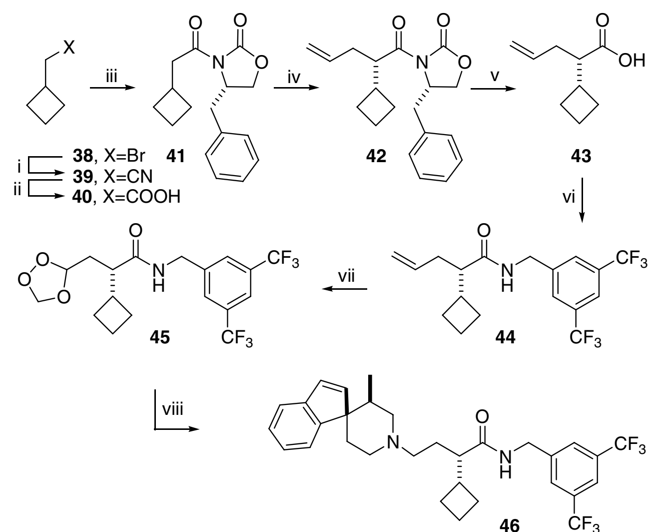


Scheme 3. Reagents and conditions: (i) *t*BuCOCl, DIEA, THF, lithium (4*R*)-4-benzyl-1,3-oxazolidin-2-one; (ii) CH_2N_2, $Pd(OAc)_2$, Et_2O; (iii) allyl bromide, LDA, −78 to −15 °C, THF; (iv) LiOOH, THF, water, 0 °C; (v) 3,5-(bistrifluoromethyl)benzyl-amine, EDC, HOAT, DCM; (vi) O_3, −78 °C, DCM; (vii) crude ozonide **36**, (1*R*,3′*R*)-3′-methylspiro[indene-1,4′-piperidine], $NaHB(OAc)_3$, dichloroethane.

one (**33**). Hydrolytic cleavage of the oxazolidone with lithium hydroperoxide afforded, albeit in low yield (27%), the desired acid **34**. The facile introduction of the amide (**35**) was followed by an oxidative cleavage of the olefin (O_3, −78 °C). The intermediate ozonide could be reduced to the respective aldehyde under standard conditions (e.g., with dimethyl sulfide), however the reaction was slow allowing sufficient time for the unwanted intramolecular cyclization (such as **19**, Scheme 1) to prevail. On the other hand, when the ozonide reduction was performed in the presence of the amine component (sodium triacetoxyborohydride, ambient temperature) the formed aldehyde was trapped as an enamine, and the subsequent in situ reductive amination completed the synthesis.

The low-yielding lithium hydroperoxide mediated auxiliary cleavage was addressed during the synthesis of the *cyclo*butyl derivative **46**, Scheme 4. According to this, the commercially available *cyclo*butyl bromide was reacted with potassium cyanide in DMF at elevated temperature, and the nitrile **39** was hydrolyzed. The chiral auxiliary was attached under the usual conditions and the LDA derived anion was allylated at −16 °C overnight. Saponification of the oxazolidone **42** with less nucleophilic potassium hydroxide required somewhat forceful conditions, and we were pleased to find that no scrambling at the C_2-chiral center had occurred. The synthesis of the *cyclo*butyl analog **46** was then completed as described above.

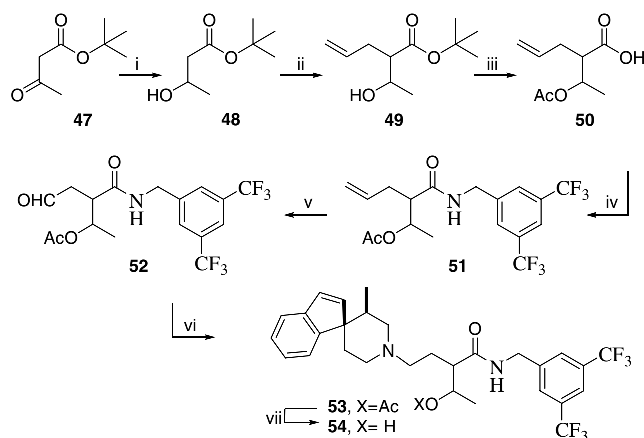
The synthesis of the hydroxyethyl derivative **54** utilized a Lemieux–Johnson oxidation of the double bond in **51** to unmask the aldehyde group, Scheme 5. Due to the presence of an intramolecular hydrogen bond between the ester oxygen and the amide nitrogen, no γ-lactam formation was observed and the aldehyde **52** was isolated in nearly quantitative yield. An uneventful



Scheme 4. Reagents and conditions: (i) KCN, DMF, 80 °C; (ii) aq KOH, 80 °C; (iii) *t*BuCOCl, DIEA, THF, lithium (4*R*)-4-benzyl-1,3-oxazolidin-2-one; (iv) allyl bromide, LDA, −78 to −15 °C, THF; (v) aq KOH, EtOH 60 °C; (vi) 3,5-(bistrifluoromethyl)benzyl-amine, EDC, HOAT, DCM; (vii) O_3, −78 °C, DCM; (viii) crude ozonide **45**, (1*R*,3′*R*)-3′-methylspiro[indene-1,4′-piperidine], NaHB $(OAc)_3$, dichloroethane.

reductive amination followed by a base-catalyzed ester hydrolysis completed the synthesis of **54**.

The target compounds in which the C_2-substituent is linked to the main backbone of the lead scaffold through a nitrogen linker are depicted in Scheme 6. The commercially available 1-amino-4-pentenoic acid (**55**) was reacted with phthalanhydride and esterified with diazomethane (**57**). The olefin was oxidatively cleaved, and the ozonide was reduced at ambient temperature



Scheme 5. Reagents and conditions: (i) $NaBH_4$, EtOH; (ii) allyl bromide, LDA, −78 to −15 °C, THF; (iii) Ac_2O, Py; (iv) TFA, DCM, anisole; (v) 3,5-(bistrifluoromethyl)benzyl-amine, PyBroP, DCM; (vi) OsO_4, $NaIO_4$, THF, MeOH; (vii) (1*R*,3′*R*)-3′-methylspiro[indene-1,4′-piperidine], $NaHB(OAc)_3$, dichloroethane; (viii) aq K_2CO_3, MeOH.

with dimethyl sulfide. A routine reductive amination (**59**) was followed by ester hydrolysis and the amide formed as described above. The phthalide protecting group in **60** was cleaved with hydrazine at elevated temperature, and 2-amino intermediate **61** was used then as a synthetic relay to access the respective amides and/or sulfonamides.

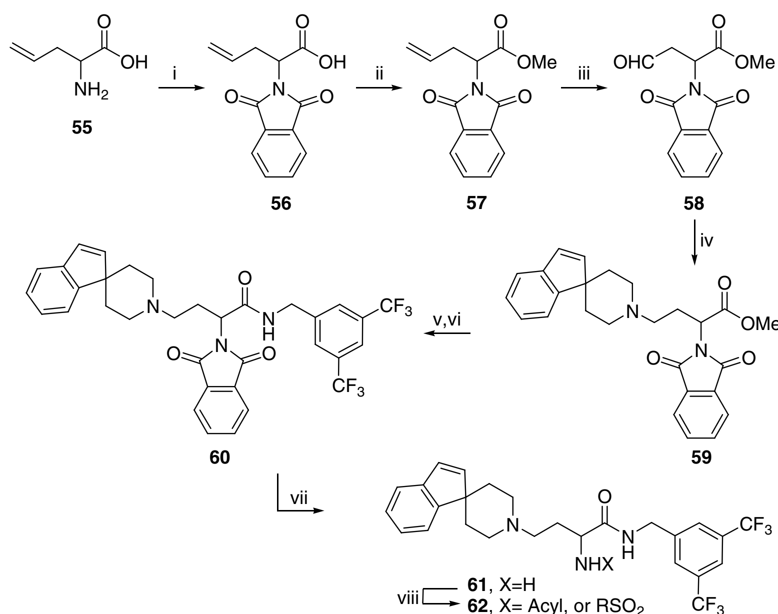
The synthesized target compounds were initially assessed by their capacity to displace radiolabeled MCP-1 from CCR2b receptors expressed in a CHO cell line. Later, a human monocyte-based assay was employed. The two respective data sets parallel each other quite well, with the monocyte assay indicating approximately a fourfold higher binding affinity.

The observation that compound **12** retained some activity (IC_{50} = 570 nM, Table 1), seemed to suggest that less prominent, perhaps metabolically more robust groups could take up the role of the aromatic ring in the 4-fluorophenyl derivative **9**.

We were pleased to find that attachment of a simple methyl group to the carbon-chain backbone at C_2 increased the binding activity by approximately twofold (**63**, IC_{50} = 231 nM). Comparison of the activities of the two stereoisomeric methyl derivatives **63** and **64** (Table 1) confirmed that the substituent has to be of the same absolute orientation (*R*) as in the lead scaffold. Further increasing the chain length to an allyl (**65**, IC_{50} = 193 nM), or early branching (isopropyl, **66**, IC_{50} = 204 nM), had only a marginal effect. Similarly, a lipophilic *cyclo*hexyl group (**69**, IC_{50} = 160 nM, racemate) did not seem to offer an advantage.

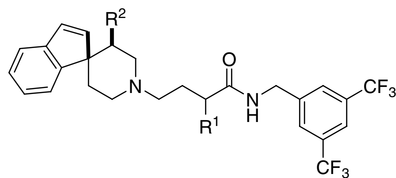
Introduction of polar groups seemed to decrease potency regardless of the size. The activity of the small amino analog **61** (Table 2) was quite low (IC_{50} > 1 μM) and similarly ineffective were the small heterocycles. Only the two smallest sulfonamides **74** (methanesulfonamide, IC_{50} = 244 nM, racemic) and **75** (trifluoromethanesulfonamide, IC_{50} = 125 nM, racemic) showed some activity.

On the other hand, small *cyclo*alkyl groups, particularly *cyclo*propyl (**68**, IC_{50} = 98 nM, Table 1), increased the binding potency dramatically. These small alicycles, particularly in conjunction with the strategically placed methyl group (3′*R* on spiro[indene-1,4′-piperidine]),



Scheme 6. Reagents and conditions: (i) phthalanhydride, toluene, reflux, 6 h; (ii) CH_2N_2, Et_2O; (iii) O_3, DCM, −78 °C, then DMS, rt; (iv) spiro[indene-1,4′-piperidine], $NaHB(OAc)_3$, dichloroethane; (v) aq LiOH, dioxane; (vi) 3,5-(bistrifluoromethyl)benzyl-amine, EDC, HOAT, DCM; (vii) NH_2NH_2, EtOH, reflux, 30 min; (viii) acyl chloride or anhydride or RSO_2Cl, DIEA, DCM.

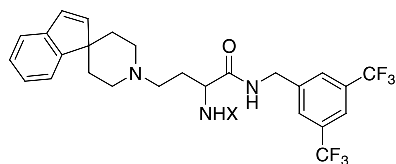
Table 1. Spiroindene class: binding affinities



Compound (stereo)	R^1	R^2	CCR2 IC_{50}, nM	
			CHO	*h*Monocyte
12	–H	H	570	80%[a]
63*R*	Methyl–	H	231	37
64*S*	Methyl–	H	605	86
65*R*	Allyl–	H	193	37
66*S*	*i*Propyl–	H	204	47
67*R*	*i*Propyl–	H	349	72
18*R*/*S*	*c*Propyl–	H	69	38
68*S*	*c*Propyl–	H	98	15
69*R*/*S*	*c*Hexyl–	H	160	73
70*R*/*S*	MeO_2SN	H	783	70%[a]
71*R*/*S*	AcN	H	660	80
72*R*/*S*	Benzyl–	H	298	232
13	H	Methyl	173	93
29*S*	*c*Propyl–	Methyl	14	4
73*R*	*c*Propyl–	Methyl	80	13
46*S*	*c*Butyl–	Methyl	28	12
37*S*	*c*Propylmethyl–	Methyl	25	9
54*R*/*S*	1-Hydroxyethyl–[b]	Methyl	n/a	48

[a] % Inhibition at 1 μM.
[b] Both *R* and *S* isomers present within the side chain.

Table 2. Spiroindene class: C_2-amides, binding affinities



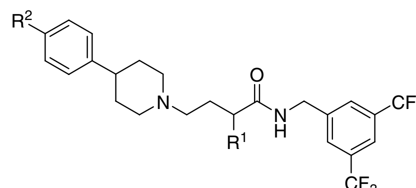
Compound[a]	X	CCR2 IC_{50} (nM) CHO assay
61	H	33%[b]
74	$MeSO_2$–	244
75	CF_3SO_2–	125
76	MeOCO–	805
77	–CH=O	25%[b]
78	S, SO_2-	20%[b]
79	N, S, AcHN, SO_2-	45%[b]
80	SO_2-	7%[b]
81	HN, N H, O, CO-	525

[a] All compounds in this series were racemic.
[b] % Inhibition at 1 μM.

produced some of the most active antagonists of the CCR2b receptor described to date, Table 4.

The C_2-*cyclo*propyl analog **29** exhibited remarkable activity of 14 nM (IC_{50}, CHO assay) and it appeared to be even more active (IC_{50} = 4 nM) in the *h*Monocyte assay. Slightly less active were the respective *cyclo*butyl (**46**, IC_{50} = 28 nM, CHO, IC_{50} = 11 nM *h*Monocyte)

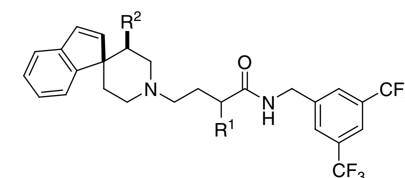
Table 3. 4-Phenylpiperidine class: binding affinities



Compound	R^1	R^2	CCR2 IC_{50}, nM	
			CHO	*h*Monocyte
82*S*	*c*Propyl	F	280	72
83*S*	*c*Propyl	H	285	129
84*R*	*n*Propyl	H	126	42
85*R*	Allyl	H	642	216
86*S*	$MeOCOCH_2$–	H	42[a]	n/a
87*R*	$MeOCOCH_2$–	H	38[a]	n/a
88*R*/*S*	1-Hydroxyethyl–[b]	F	n/a	462

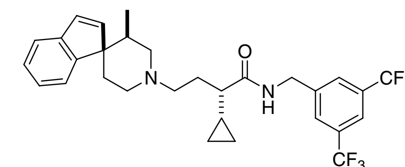
[a] % Inhibition at 1 μM.
[b] Both *R* and *S* isomers present within the side chain.

Table 4. Functional activities of selected compounds



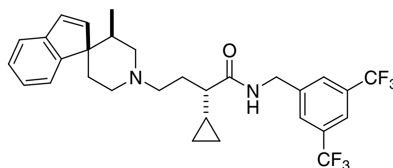
Compound	R^1	R^2	Ca-Flux IC_{50} (nM)
29*S*	*c*Propyl–	Methyl	4
46*S*	*c*Butyl–	Methyl	11
37*S*	*c*Propylmethyl–	Methyl	3

Table 5. Selectivity profile of the *cyclo*propyl analog **29**



Receptor	Inhibition (%)	Concentration (μM)
hCCR1	−5	1
hCCR3	3	4
hCCR4	−18	2
hCXCR4	8	1
CCR5 (MIP1alpha)	38	1
CCR8	9	1

Table 6. Pharmacokinetic properties of the *cyclo*propyl derivative **29** (Sprague–Dawley rats)



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	4.17	7.36	2.19	4.11			
p.o.	3.00	4.08				1.58	4.67	98

and *cyclo*propylmethyl (**37**, IC_{50} = 25 nM, CHO, IC_{50} = 9 nM *h*Monocyte) analogs.

The effectiveness of such small aliphatic substituents was also evaluated in simpler 4-phenyl piperidines, Table 3. In this series, the activities of the synthesized compounds did not reach the level of those obtained in the spiroindene class. The most potent compound in this series (propyl, **84**, IC_{50} = 126 nM, CHO, IC_{50} = 42 nM *h*Monocyte) was approximately ten-fold less active, than the best spiroindenes.

The functional activity of selected compounds was evaluated using the calcium flux based FLIPR assay.[31] The results are summarized in Table 4. Once again, in agreement with the binding data, the *cyclo*propyl derivative **29** and its methylene homolog **37** exhibited particularly high functional activities.

The *cyclo*propyl analog **29** also showed quite remarkable selectivity against other closely related chemokine receptors, Table 5. Except for the CCR5 receptor, which it showed an inhibition of 38% at a concentration of 1 mM, affinities to all other chemokine receptors were negligible.

The *cyclo*propyl derivative **29** was also evaluated in a rat pharmacokinetic model. Excellent drug levels were observed after both intravenous (1.0 mg/kg, AUCn = 4.17 μM) as well as oral (3.0 mg/kg, AUCn = 4.08 μM) administration. The compound showed a slow clearance rate of 7.36 mL/min/kg, low volume of distribution (2.19 L/kg), and an outstanding oral bioavailability of 98%, Table 6.

A systematic examination of the central part of the lead structure **10** demonstrated that the aromatic ring can be successfully replaced with small, alicyclic groups. The present study led to the discovery of (2*S*)-*N*-[3,5-bis(trifluoromethyl)benzyl]-2-*cyclo*propyl-4-[(1*R*,3′*R*)-3′-methyl-1′*H*-spiro[indene-1,4′-piperidin]-1′-yl]butanamide (**29**), a high affinity antagonist of the CCR2b receptor with an excellent selectivity profile and outstanding pharmacokinetic properties.

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22. Initially, the binding affinities were evaluated in the CHO assay, later the Human Monocyte-based assay was used. Radioligand Competition Binding Assays: Human mono-

cytes (2×10^5), or CHO cells expressing human CCR2b (5×10^4) were incubated with ^{125}I-hMCP-1 (20–50 pM) and various concentrations of unlabeled chemokines in binding buffer for 60 min at room temperature. The binding buffer contains 50 mM HEPES, 5 mM $MgCl_2$, and 1 mM $CaCl_2$, pH 7.4. 125I-hMCP-1 was purchased from Perkin Elmer Life Sciences, Inc., with a specific activity of 2200 Ci/mmol. The assay was terminated by filtration of the reaction mixture through GF/B filter plates (presoaked in 0.1% polyethyleneimine) using a Packard Cell Harvester. The filter plates were washed with 25 mM HEPES, pH 7.5, containing 500 mM NaCl and dried in an incubator for @ 37 °C for 30 min. The plates were loaded with Microscint 0 (Packard) and counted in a Topcount NXT (Packard). The software program Prism (GraphPad) was used for all calculations. All data represent mean values for at least two separate experiments.

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31. CHO cells expressing hCCR2B (4×10^4) were incubated in cell culture medium containing Fluo-3, AM fluorescent dye (5 μg/mL, Molecular Probes), and probenicid (710 μg/mL, Sigma) for 1 h at 37 °C. Cells were washed with Hanks buffer containing HEPES (20 mM), BSA (0.1%), and probenicid, and then were resuspended in 90 μL of the same buffer. A ligand addition plate was prepared by adding 135 μL of chemokines at various concentrations for the titration. To test inhibition of calcium release by TAK-779, 2 μL of this antagonist at various concentrations was added to the cells upon resuspension and 20 nM hMCP-1 was used to activate CCR2B receptors. Both plates were placed into the Fluorescence Imaging Plate Reader (FLIPR, Coherent, Inc.). Chemokines were dispensed from addition plate into the cell plate and calcium release was measured by the Argon Laser at an excitation wavelength of 488 nm and an emission wavelength of 530 nm.