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Discovery of a Potent and Orally Bioavailable CCR2 and CCR5 Dual Antagonist

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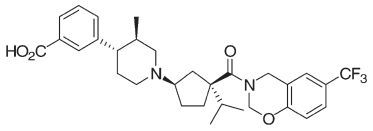
ABSTRACT This report describes the discovery of a potent, orally bioavailable CC chemokine receptor 2 (CCR2) antagonist which, while optimized for CCR2 potency, also had potent CC chemokine receptor 5 (CCR5) activity.

KEYWORDS CCR2, CCR5, chemokine, dual antagonist



Monocyte chemoattractant protein-1 (MCP-1, CCL2), included within the CC class of chemokines,[1] mediates chemotaxis of monocytes to inflammatory sites via interactions with its receptor, CCR2.[2] CCR2, a member of the G-protein-coupled seven-transmembrane receptor superfamily, is most abundantly expressed on monocytes. The CCL2/CCR2 axis has been implicated in various autoimmune or inflammation associated diseases[3−12] including rheumatoid arthritis (RA),[5] multiple sclerosis,[6] atherosclerosis,[7] chronic obstructive pulmonary disease (COPD),[8] asthma,[9] diabetes/obesity,[10,11] and neuropathic pain.[12] A number of small molecule CCR2 antagonists have already been described by our group[13−17] and by others.[18,19] Several small molecule CCR2 antagonists and a humanized monoclonal antibody targeting CCL2 have been advanced to clinical trials for treatment of RA, multiple sclerosis, atherosclerosis, COPD, pain, and diabetes.[18−22] While the outcomes from the earliest trials for CCR2 blockade in RA have so far been disappointing,[21,22] a trial in patients at high risk for cardiovascular disease having elevated high-sensitivity C-reactive protein (CRP) levels with Millenium's CCL2 monoclonal antibody (MLN-1202[23]) demonstrated lowering of CRP, consistent with a beneficial anti-inflammatory effect. This communication describes the discovery and detailed characteristics of 3-[(3*R*,4*S*)-1-((1*R*,3*S*)-3-isopropyl-3-{[6-(trifluoromethyl)-2*H*-1,3-benzoxazin-3(4*H*)-yl]carbonyl}cyclopentyl)-3-methylpiperidin-4-yl]-benzoic acid, a potent and orally bioavailable CCR2 antagonist that was selected as a clinical candidate.

We have previously described CCR2 antagonists based upon an aminocyclopentane carboxamide scaffold typified by **1**.[15,16] One drawback of compound **1** and its analogues was their high binding affinity at the outward delayed rectifier potassium channel (I_{Kr}). Blockade of I_{Kr} is associated with QTc prolongation *in vivo*, carrying the potential to induce cardiac arrhythmias.[24,25] Therefore, we set out to modify **1** to minimize the I_{Kr} inhibition, while retaining CCR2 potency. We previously reported that incorporation of a carboxyphenyl group onto the piperidine 4-position in a related series of CCR2 antagonists (**2**) dramatically decreased I_{Kr} binding affinity when compared to our standard 4-fluorophenylpiperidine-based analogues.[17] We applied this observation to compound **1**, giving target analogue **3** (Figure 1).

Compound **3** was prepared in an analogous fashion to that described previously for its close analogue **2**.[17] CCR2 binding affinities were determined by measuring inhibition of ^{125}I-MCP-1 binding to the endogenous CCR-2 receptor on human monocyte whole cells.[13] I_{Kr} binding data was obtained as described previously.[26] While the I_{Kr} binding affinity for analogue **3** was successfully reduced by 600-fold (I_{Kr} IC_{50} = 21 μM), the CCR2 binding affinity was also reduced, albeit by only 7-fold to 29 nM. In an effort retrieve the lost CCR2 potency, we decided to incorporate beneficial modifications found in other series explored within our program. In particular, we have observed that substitution of an oxygen atom in place of carbon at the 4-position of the 7-trifluoromethyltetrahydroisoquinoline subunit of **1** can improve potency. We decided to incorporate this feature, and the synthesis of the corresponding target compound **17** is described in Scheme 1. Commercially available fluoride **4** was treated with potassium *tert*-butoxide to give the ether **5**.[27] Reduction with hydrogen and catalytic Raney Ni provided the corresponding benzyl amine **6**. Commercially available ketoacid **7** was protected as its *tert*-butyl ester[28]/dimethylacetal in two steps. Deprotonation of **8**, followed by addition of 2-iodopropane, gave **9**. Treatment with a 5:1 mixture of 4 M HCl in dioxane and water removed both protecting groups to afford ketoacid **10**. Conversion to the corresponding acid chloride, followed by coupling with benzyl alcohol, provided the benzyl ester **11**. The ester was resolved using preparative chiral HPLC (Chiralcel OD column, Chiral Technologies, Inc., 15% IPA/hexanes). The first peak to elute was identified as having the desired *S* absolute

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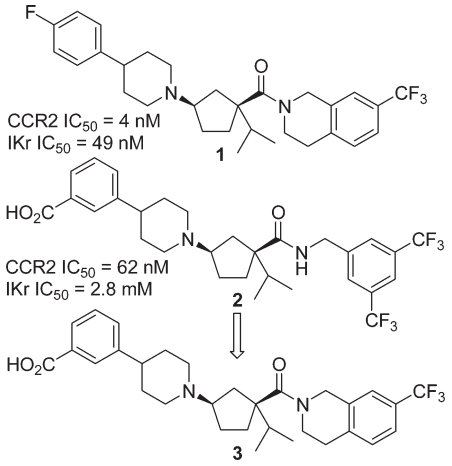


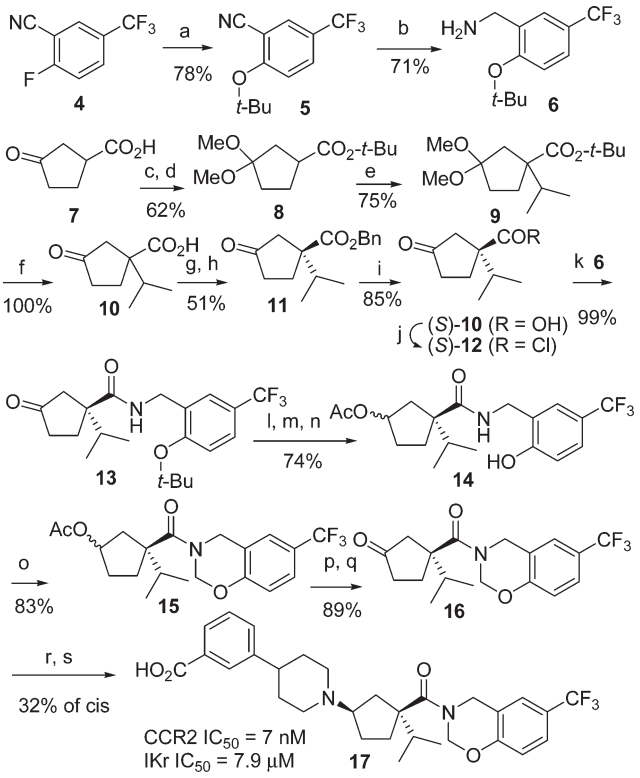
Figure 1. Leads **1** and **2**, and derived target analogue **3**.

stereochemistry by elaboration to a known pure CCR2 analogue. Hydrogenolysis of the benzyl ester with hydrogen (1 atm) and Pd/C in methanol afforded (*S*)-ketoacid **10**. Conversion of (*S*)-**10** to acid chloride (*S*)-**12**, followed by addition of amine **6** and triethylamine, provided amide **13**. Reduction of the ketone with sodium borohydride was followed by formation of the corresponding acetate ester. Cleavage of the *tert*-butyl ether to the phenol **14** was achieved by treatment with 4 M HCl in dioxane. Then, addition of an excess of paraformaldehyde and catalytic TsOH in toluene, followed by azeotropic removal of the generated water under reflux, provided the cyclized benzoxazine product **15**. A more direct approach where cyclization was attempted in the presence of the cyclopentanone failed to give any product. Hydrolysis of the ester group in **15**, followed by Swern oxidation, gave ketoamide **16**. Reductive amination with ethyl 3-piperidin-4-yl benzoate[17] gave a mixture (~1:1) of cis- and *trans*-isomers which were separable by preparative TLC. The higher eluting compound was presumed to be the *cis*-isomer on the basis of the CCR2 binding affinity of both esters (higher eluting IC_{50} 7.2 nM, lower eluting 41% inhibition at 1 μM). We have previously reported that, of the 4 possible 3-amino-1-carboxamide-cyclopentane stereoisomers, the *cis*-(3*R*,1*S*)-isomer alone has potent activity on CCR2.[15] We have never observed an exception to this stereochemistry preference. Hydrolysis of the benzoate ester using excess lithium hydroxide gave the target analogue **17**.

As anticipated, benzoxazine analogue **17** was more potent compared to **3** in our binding assay (CCR2 IC_{50} = 7 nM). In addition, **17** demonstrated weak binding on the I_{Kr} channel (IC_{50} = 7.9 μM), corresponding to a >1000-fold selectivity window. In a functional assay measuring inhibition of MCP-1 mediated chemotaxis of monocytes[13] **17** was a potent functional antagonist (IC_{50} of 0.5 nM). In addition, compound **17** was orally bioavailable in rats (*F* = 21%), beagles (82%), and rhesus monkeys (*F* = 60%).

Encouraged, we decided to explore the possibility of further potency enhancements. In particular, we knew from earlier work that incorporation of a *trans*-(3*R*)-methyl group into the 4-arylpiperidine subunit can improve potency.[14,15]

Scheme 1. Synthesis of Benzoxazine Analogue **17**[a]



[a] (a) KO-*t*-Bu (1.5 equiv), THF, 0 °C−rt; (b) H_2, Raney Ni, EtOH, 37% aq NH_4OH; (c) H_2SO_4, $MgSO_4$, *t*-BuOH, DCM; (d) TMOF, TsOH, MeOH, DCM; (e) LDA, THF; 2-iodopropane, −78 °C−rt; (f) 4 M HCl/dioxane, H_2O (5:1); (g) oxalyl chloride, DMF (cat.), DCM; BnOH, Et_3N, DCM, DMAP; (h) chiral HPLC, Chiralcel OD column, 15% IPA/hexanes, faster eluting peak; (i) H_2, Pd/C, MeOH, 1 atm; (j) oxalyl chloride, DMF (cat.), DCM; (k) Et_3N (2 equiv), **6**, DCM; (l) $NaBH_4$, MeOH, 0 °C−rt; (m) Ac_2O, Et_3N, DMAP (cat.); (n) 4 M HCl/dioxane, rt; (o) paraformaldehyde (~1 g/3 mmol substrate), TsOH, toluene, Dean−Stark trap, reflux 3.5 h; (p) LiOH, EtOH, H_2O; (q) oxalyl chloride, DMSO, substrate, Et_3N, −78 °C−rt; (r) ethyl 3-piperidin-4-ylbenzoate,[17] $NaB(OAc)_3H$, 4 Å molecular sieves, 3−5 days; (s) LiOH (10 equiv), EtOH, water, 50 °C, 1.5 h.

We decided to install the corresponding *trans*-4-(3-carboxyphenyl)-3-methylpiperidine **21** (Scheme 2) to determine if the same potency enhancement would apply in this series. The synthesis began with Boc protected ethyl 3-piperidin-4-ylbenzoate **18**.[17,29] Oxidation with ruthenium(IV) oxide and sodium periodate proceeded over 11 days to afford the lactam **19**.[30] Deprotonation, followed by treatment with iodomethane, gave the *trans*-3-methylpiperidinone **20**. Removal of the Boc group, followed by reduction with borane−dimethylsulfide complex, gave the corresponding piperidine **21**. Difficulties with the purification of **21** following the reduction step led us to protect the amine (Boc), whereupon purification was readily accomplished. The Boc group was then removed to provide clean **21** as a mixture of two *trans*-isomers. Reductive alkylation with ketone **16** afforded a mixture of all four possible diastereomeric products (~5:5:6:6 ratio, 80% total yield). The four isomers were separated by preparative chiral HPLC using a Chiralcel OD column (Chiral Technologies, Inc.). The third (18%) and

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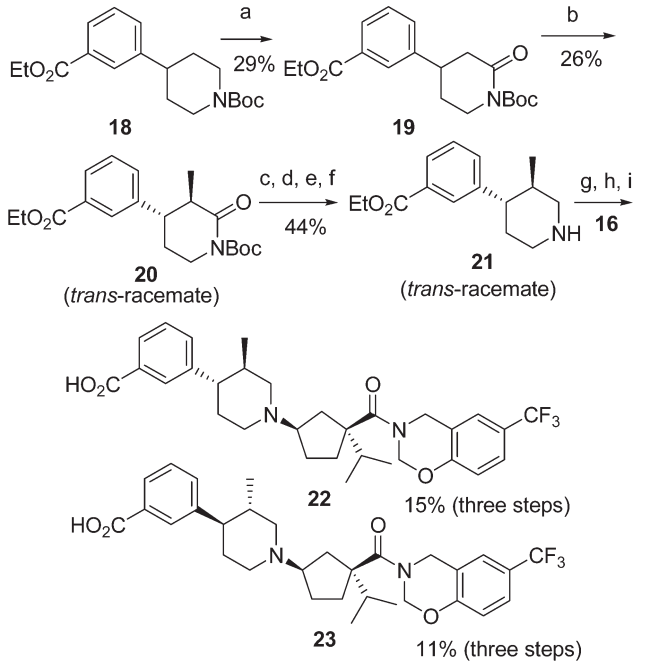
Scheme 2. Synthesis of Analogues **22** and **23**[a]



[a] (a) Ruthenium(IV) oxide hydrate (0.2 equiv), $NaIO_4$ (3.2 equiv), $CHCl_3$/H_2O, 11 days at rt;[31] (b) KHMDS, THF; MeI, −78 °C; (c) 4 M HCl in dioxane; (d) BH_3 DMS, THF, rt; 0.5 M HCl/EtOH, 50 °C, 4 h; (e) Boc_2O, DIEA, DCM, DMAP (cat.); (f) 4 M HCl in dioxane; (g) **17**, $NaB(OAc)_3H$, 4 Å mol sieves powder, DIEA, 4 days, rt; (h) preparative chiral HPLC (Chiralcel OD column, 8% EtOH/hexanes, *cis*-peaks were third and fourth to elute); (i) LiOH, EtOH, H_2O, rt, 22 h.

fourth (17%) peaks to elute were determined to be the desired *cis*-cyclopentane isomers. Hydrolysis of the esters collected from the third and fourth peaks afforded the target acids **22** and **23**. We initially assigned the piperidine stereochemistries for **22** and **23** (as shown in Scheme 2) on the basis of their potencies; by analogy to an earlier series we knew that while both *trans*-isomers maintain potency, the (3*R*,4*S*)-4-aryl-3-methylpiperidine stereochemistry is optimal.[14] We later definitively confirmed the tentatively assigned stereochemistry of **22** by application of an alternative synthetic route to the (4*R*)-carboxyphenyl-(3*S*)-methyl isomer of piperidine intermediate **21**, where the stereochemistry was established though use of a chiral starting material of known absolute stereochemistry.[31] Similarly, the *cis*-cyclopentane stereochemistry was confirmed by a later process synthesis using chiral starting materials of known absolute stereochemistry.

The CCR2 binding, functional, and I_{Kr} binding data for compounds **22**, **23**, as well as analogues **1**, **3**, and **17**, are presented in Table 1. While analogues **22** and **23** are both potent, analogue **22** appears slightly more potent than both **23** and **17**. Compound **22** exhibited similar binding potency for mouse CCR2 (IC_{50} = 4 nM). Direct equilibrium binding experiments using ^{3}H-labeled **22** and monocytes demonstrate that **22** has a K_D for the receptor of 0.7 nM. ^{3}H-22 had a very slow association and disassociation at the receptor giving a receptor disassociation time ($T_{1/2}$) which was

Table 1. CCR2 Binding and Functional Data and I_{Kr} Binding Data for Analogues **1**, **3**, **17**, **22**, and **23**[a]

	IC_{50} (nM)		
analogue	CCR2	chemotaxis	I_{Kr}
1	4 (3)	2 (2)	49 (2)
3	29 (1)		21,000 (2)
17	7 (16)	0.5 (2)	7,900 (2)
22	4 (5)	0.3 (3)	33,000 (2)
23	6 (3)	2 (2)	7,300 (2)

[a] Numbers in parentheses represent numbers of determinations. Standard deviations, when calculable, are always less than 25% of the measured value.

difficult to measure at room temperature, but was greater than 9 h. Analogue **22** is a potent inhibitor of MCP-1 mediated chemotaxis of monocytes (IC_{50} = 0.3 nM). An *in vitro* human whole blood shape change assay[32] measuring MCP-1-induced changes in monocyte cell shape was used to measure the potency of compound **22** to functionally antagonize CCR2 on monocytes in whole blood. In this assay compound **22** has an IC_{50} of 15 nM with a preincubation of 30 min. Interestingly, when **22** was preincubated in whole blood for 24 h, the IC_{50} improved to 0.1 nM, a shift in over 2 orders of magnitude. This is likely due to an underestimation of potency under standard 30 min preincubation conditions as a result of a combination of the slow receptor kinetics and the high level of plasma protein binding. Reversible binding to plasma proteins *in vitro* was 98, 98, 97, and 97% in rat, dog, monkey, and human, respectively (at 0.1 μM). When the compound was allowed ample time to come to equilibrium with the receptor on monocytes during the overnight incubation before whole blood stimulation with MCP-1, the compound's true potency could be determined.

The 3-methyl group in **22** appears to confer improved selectivity against the I_{Kr} channel (I_{Kr}/CCR2 > 8,000). Compound **22** displayed potent activity against the closely related CCR5 receptor. However, the potency of **22** at CCR5 was at least 2.5-fold less than that observed for CCR2 and the compound exhibited much faster disassociation kinetics at CCR5.[33] No immune system deficits have been described in a group of individuals deficient for CCR5 (CCR5 delta32 homozygous individuals). Therefore, CCR5 blockade by **22** is unlikely to be a treatment liability. Interestingly, the IC_{50} of **22** on murine CCR5 is > 10 μM. Counterscreening against a panel of 136 receptors, enzymes, and ion channels (MDS Pharma Services) established **22** to be remarkably selective for CCR2. In addition to the known CCR5 activity, **22** displayed weak activity against the M2 and M4 subtypes of the muscarinic receptors ($IC_{50} \geq 10$ μM for both). Compound **22** did not inhibit any of five human CYP marker enzymes assayed (> 100 μM on CYP3A4, CYP2C9, CYP2D6, CYP1A2, and CYP2C19). As shown in Table 2, **22** had excellent oral bioavailability in rats (F = 48%), dogs (F = 63%), and monkeys (F = 66%).

The *in vivo* efficacy of compound **22** was evaluated in an *ex vivo* rhesus whole blood shape change assay in which **22** was dosed at 2 mg/kg orally. Comparison of the plasma levels with the inhibition of monocyte shape change produced a

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Table 2. Pharmacokinetic Properties of Compound **22**[a]

species	dose (iv/po; mg/kg)	F (%)	AUC (po; μM·h)	Vdss (L/kg)	Clp (mL/min/ kg)	$t_{1/2}$ (h)
rat	1/3	48	5.3	0.4	8.1	0.9
dog	1/2	63	33	0.5	1.1	5.5
rhesus	1/2	66	4.7	0.9	8.3	4.9

[a] Rat pk was analyzed in blood; dog and mouse were analyzed in plasma.

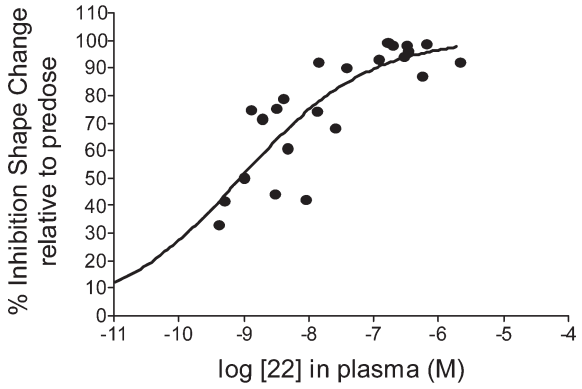

Figure 2. *In vivo* potency of **22** determined by *ex vivo* rhesus whole blood shape change assay.[34]

concentration responsive curve that gave an estimated IC_{50} of 0.9 nM (Figure 2).[34] This is consistent with the potency of **22** on rhesus CCR2 determined in *in vitro* whole blood spiking experiments in rhesus blood (IC_{50}'s of 30 nM and 0.2 nM with 30 min and 24 h preincubations, respectively). Our data suggest that subnanomolar potency can be achieved *in vivo*.

In summary, we have improved upon the I_{Kr} selectivity and CCR2 potency of antagonist lead **1** to give antagonists **17** and **22** which are orally bioavailable, potent functional antagonists of CCR2. Compound **22** was highly selective for CCR2, with the exception of related chemokine receptor CCR5, where it also had potent activity. The potent *in vivo* efficacy of compound **22** was demonstrated using an *ex vivo* rhesus whole blood shape change assay. On the basis of its potency, *in vivo* efficacy, safety, and oral bioavailability, compound **22** was selected as a clinical candidate for the CCR2 program at Merck.

SUPPORTING INFORMATION AVAILABLE Experimental details for the synthesis and characterization of CCR2 antagonists **3**, **17**, and **22**. This material is available free of charge via the Internet at http://pubs.acs.org.

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ABBREVIATIONS CCR2, CC chemokine receptor 2; CCL2, CC chemokine ligand 2; CCR5, CC chemokine receptor 5; TLC, thin layer chromatography.

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(31) The Merck process chemistry group devised a synthesis of the (4*R*)-carboxyphenyl-(3*S*)-methyl piperidine subunit starting from pure methyl (*S*)-(+)-3-hydroxy-2-methylpropionate. They also devised a synthesis of the *cis*-(3*R*,1*S*)-cyclopentane core also using starting materials of known absolute stereochemistry. This improved synthesis will be described elsewhere.

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(33) Compound **22** inhibits binding of ^{125}I-MIP-1α to CCR5 with IC_{50} = 25 nM. K_D as measured by direct binding of ^{3}H-22 to recombinant CCR5 expressing cells was 1.8 nM compared to 0.7 nM for CCR2. The receptor $T_{1/2}$ of ^{3}H-22 at CCR5 was 32 min at 37 °C, compared to a $T_{1/2}$ of 5 h at 37 °C for CCR2.

(34) Compound **22** was dosed orally in 5 rhesus at 2 mg/kg and plasma concentration of **22** and MCP-1 whole blood monocyte shape change was determined at various time points postdosing (6, 24, 36, 48, 72 h). % inhibition shape change was determined by comparing postdose values to predose values in the same animals. See also ref 32.

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