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Discovery of 3,5-bis(trifluoromethyl)benzyl L-arylglycinamide based potent CCR2 antagonists

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Abstract—Systematic modification of a screening lead yielded a class of potent glycinamide based CCR2 antagonists. The best compound (**55**, (2*S*)-*N*-[3,5-bis(trifluoromethyl)benzyl]-2-{[2-(1-piperidinyl)ethyl]amino}-2-(3-thienyl)acetamide) displayed good binding affinity (IC_{50} = 30 and 39 nM) toward human monocytes and CHO cell expressing human CCR2b, respectively. Functionally, it blocked MCP-1 (CCL2)-induced calcium mobilization (IC_{50} = 50 nM) and chemotaxis mediated through the CCR2 receptor (9.6 nM). It is selective against other chemokine receptors tested.

Chemokines or chemotactic cytokines are a large family of small (~8–15 kDa) structurally related proteins that play an important role in leukocyte migration and activation.[1–5] Most members of this family belong to one of two major subfamilies which are distinguished based on the arrangement of the first two conserved cysteines in the sequence: the CC family which contains adjacent cysteines and the CXC family in which the cysteines are separated by a single intervening amino acid. Chemokines mediate their effects through activation of specific cell-surface seven-transmembrane spanning G-protein coupled receptors. Monocyte chemoattractant protein (MCP-1/CCL2) belongs to the CC chemokine subfamily and binds to CC chemokine receptor 2 (CCR2), which is expressed on the majority of blood born monocytes.[6] Interruption of the MCP-1/CCR2 axis in rodent models of inflammatory and autoimmune diseases by genetic deletion of either MCP-1[7] or CCR2[8–10] and use of peptidyl CCR2 antagonists,[11] or anti-MCP-1 antibodies[12] suggests that inhibition of CCR2 may provide potential therapies for a variety of diseases including rheumatoid arthritis,[11,12] multiple sclerosis[13–15], and atherosclerosis.[10,16–18] These prospects have prompted the search for small molecule MCP-1/CCR2 antagonists in many research laboratories.[19] Several examples have been reported in the scientific and patent literature as shown in Figure 1. Compound **1** was among the first reported CCR2 antagonists to exhibit micromolar binding affinity to the receptor and functional antagonism in the chemotaxis (CTX) assay.[20] Compound **2** showed higher affinity to the receptor but had much reduced functional antagonism.[21] Compound **3**, which bears structural resemblance to earlier reported CCR1 antagonists, was reported to have potent binding and functional activities.[22] A series of indolyl piperidine based CCR2b antagonists which also bind to 5-HT was reported (compound **4**).[23,24] In addition, a class of potent CCR5 antagonists have been reported to have low nanomolar binding to CCR2 and act as functional antagonists of the CCR2 receptor.[25,26] All of these are piperidine based with at least two aromatic rings as their pharmacophore. Two novel classes of CCR2 antagonists as represented by compounds **5** and **6** have also been disclosed. Compound **5** is a carboxylic acid which was reported to be a CCR2 antagonist with good binding affinity to the receptor.[27] The acyl urea **6**, however, inhibits MCP-1-induced chemotaxis of human monocytes in vitro and in vivo even though it does not displace the binding of MCP-1 to CCR2.[28,29] All these

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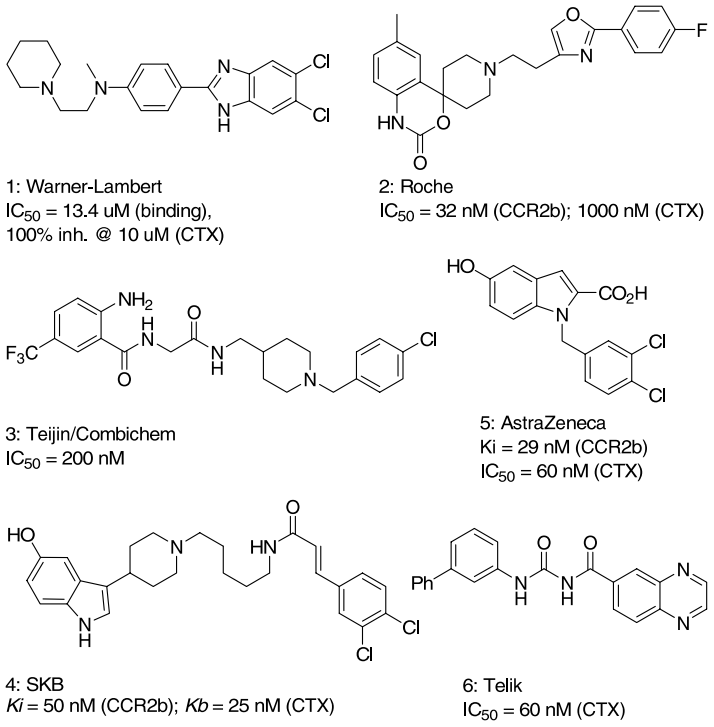


Figure 1. CCR2 antagonists.

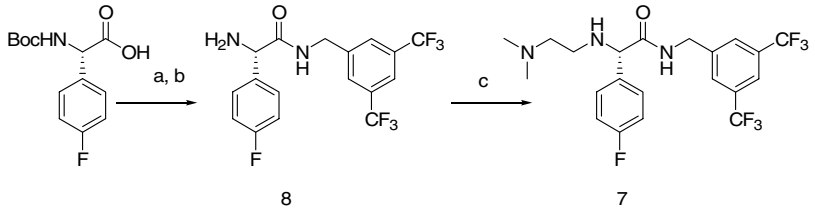
compounds suffer from various problems ranging from low binding affinity and/or low functional activity to poor selectivity. Herein we report a new class of glycinamide based potent CCR2 antagonists with good selectivity over other chemokine receptors.

Screening of the Merck sample collection resulted in a single hit **7** (IC_{50} = 770 nM, CCR2), which was originally prepared for the neurokinin antagonist program (IC_{50} = 650 nM, NK1). A modular approach was taken to develop this lead, namely identification of an optimal central amino acid, modification or replacement of the diamines, and investigation of the importance of the amide.

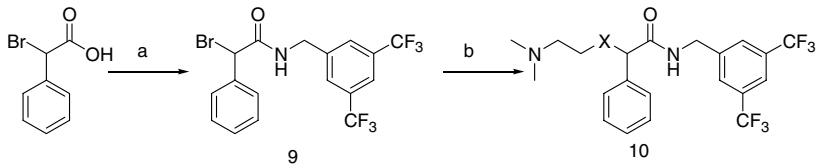
The synthetic approaches to these phenyl glycine derivatives were carried out either through the alkylation of the glycine or displacement of α-bromo-phenylacetamide. Scheme 1 shows a typical route starting from an N-protected phenyl glycine. EDC coupling with a benzyl amine provided the corresponding amide. After acidic deprotection, treatment of the resulting amine with an amino ethyl chloride in the presence of sodium bicarbonate in refluxing ethanol provided the desired compound. The stereochemistry of the amino acid was retained during the synthesis.

A second approach from α-bromo-phenylacetamide is depicted in Scheme 2. The desired α-bromo-phenylacetamide was prepared through the coupling of α-bromo-acetic acid with a benzylamine as described earlier. Treatment of the bromo phenylacetamide with neat diamines, amino thiols, and amino alcohols gave the desired compounds. This two-step synthesis allowed the facile preparation of compounds incorporating sulfur and oxygen as well as nitrogen at the backbone of the lead.

Compounds were initially evaluated for their ability to inhibit human ^{125}I-MCP-1 binding to the CCR2b receptor, stably expressed on CHO cells.[30] A binding assay based on human monocytes was later established and the two binding assays generally agree well in this lead class. Functional antagonism and efficacy of key compounds were studied in a calcium flux or chemotaxis assays, both using human primary monocytes.[31,32]



Scheme 1. Reagents and conditions: (a) EDC, HOBT, 3,5-bis(trifluoromethyl)benzyl amine, CH_2Cl_2, 0 °C; (b) HCl, EtOAc; (c) dimethylaminoethyl chloride HCl, $NaHCO_3$, EtOH, 80 °C.



Scheme 2. Reagents and conditions: (a) EDC, HOBT, 3,5-bis(trifluoromethyl)benzyl amine, CH_2Cl_2, 0 °C; (b) $Me_2N(CH_2)_2XH$ (X = NH, O, S; neat).

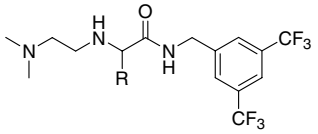
Structural optimization was carried out on each subunit of the screening lead **7**. The central 4-fluorophenyl glycine has the L or (*S*)-stereochemistry, while its D or (*R*)-enantiomer **11** is inactive (Table 1). Deletion of the aromatic ring also resulted in an inactive compound **12**. A survey of aromatic amino acids demonstrated that the aryl group is important for hCCR2 activity. The parent phenyl glycine derivative **13** is equipotent to the lead, but its homolog phenylalanine **14** was inactive. As a result, much of the early SAR was carried out with phenyl at this position for easier access of starting materials. A fully saturated cyclohexyl glycine **15** did not bind to the receptor suggesting very tight SAR in this region. Most substitutions on the phenyl group with the exception of small groups at the 4 position reduced potency as demonstrated by compounds **16**–**20**. The 4-fluorophenyl group can be replaced by a variety of small heteroaryl groups that retain or improve hCCR2 binding affinity. For example, the thiophene derivative **22** and furan analog **23** are more potent than the original lead.

The bis-trifluoromethylbenzyl group is extremely sensitive to modification (Table 2). Both of the CF_3 groups are critical for activity. Attempts to replace the bis-trifluoromethylbenzyl group with other substituted benzyl groups resulted in inactive compounds (**24**–**27**) as shown in Table 2. The introduction of a methyl at the benzylic position is a way of restricting the number of low-energy conformations at this region, potentially favoring a more active conformation. Unfortunately, in this instance it greatly reduced the binding of compound **28** as compared with the parent **13**.

Likewise, the secondary amide is also critical for receptor binding (Table 3). Replacement of the amide linker with *N*-methyl amide, reversed amide, sulfonamide, or ether linkers results in total loss in activity as shown in Table 3.

The effects of substitution and replacement of the glycine amine (NH) were investigated (Table 4). Simple methylation to convert it to a tertiary amine (**36**) abolished the binding affinity, as did acetylation (**37**). The imine (**38**), ether (**39**), thioether (**40**), and methylene (**41**) replacements all gave compounds with reduced binding affinity.

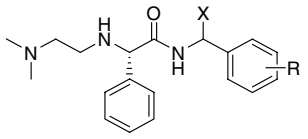
Table 1. Binding affinity to human CCR2 (CHO)



Compound	Stereo	R	Binding IC_{50} (nM)
7	L	4-F–Ph	770
11	D	4-F–Ph	8%
12	—	H	0%
13	L	Ph	1000
14	L	4-F–$PhCH_2$	1%
15	L	*c*-Hex	9%
16	DL	3,4-DiF–Ph	33%
17	DL	4-Cl–Ph	1100
18	DL	4-Br–Ph	25%
19	DL	3-Br–Ph	17%
20	L	4-MeO–Ph	47%
21	L	2-Thiophene	865
22	L	3-Thiophene	424
23	DL	2-Furan	592

% inhibition at 1 μM when no IC_{50}'s were measured.

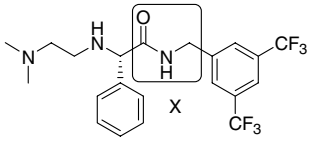
Table 2. Binding affinity to human CCR2 (CHO).



Compound	X	R	Binding IC_{50} (nM)
24	H	2-CF_3	1%
25	H	3-CF_3	5%
26	H	4-CF_3	7%
27	H	3,5-DiMe	0%
28	Me	3,5-DiCF_3	28%
13	H	3,5-DiCF_3	1000

% inhibition at 1 μM when no IC_{50}'s were measured.

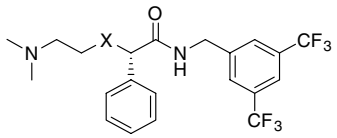
Table 3. Binding affinity to human CCR2 (CHO)



Compound	X	Binding IC_{50} (nM)
13	$CONHCH_2$	1000
29	$CONMeCH_2$	20%
30	CH_2NHCO	0%
31	CH_2NHSO_2	0%
32	CH_2NMeSO_2	0%
33	CH_2NAcCH_2	4%
34	CH_2NMsCH_2	0%
35	CH_2OCH_2	6%

% inhibition at 1 μM when no IC_{50}'s were measured.

Table 4. Binding affinity to human CCR2 (CHO)



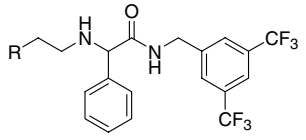
Compound	X	Binding IC_{50} (nM) (%)
36	MeN	8
37	AcN	0
38	–N=	10
39	O	0
40	S	25
41	CH_2	13

% inhibition at 1 μM when no IC_{50}'s were measured.

The dimethylamine unit on the left side of the molecule is essential for binding as replacement with non-basic groups generates inactive compounds (**44**, **45**, Table 5). Replacement of the dimethylamine group with other amino groups is tolerated and can lead to improved potency, with the piperidine group being optimal (**47**–**50**). Substitution on the piperidine group did not improve activity (**51**–**53**). Prolongation of the linker between the two amines reduced potency (**54**).

Having established the optimal moieties at each position, we incorporated them into one molecule (Table 6). The

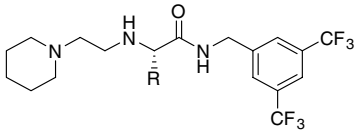
Table 5. Binding affinity to human CCR2 (CHO)



Compound	Stereo	R	Binding IC_{50} (nM)
42	DL	H_2N	14%
43	DL	Me_2N	1477
44	DL	*i*-Pr	0%
45	DL	MeO	0%
46	DL	Et_2N	678
47	DL	1-pyrrolidinyl	521
48	DL	1-piperidinyl	342
49	L	1-homopiperidinyl	400
50	DL	1-morpholinyl	575
51	L	2-Me-1-piperidinyl	838
52	L	4-Me-1-piperidinyl	756
53	L	4-Ph-1-piperidinyl	822
54	L	1-piperidinylmethyl	26%

% inhibition at 1 μM when no IC_{50}'s were measured.

Table 6. Binding affinity to human CCR2 (CHO)



Compound	R	Binding IC_{50} (nM)
55	3-Thiophene	39
56	Ph	219
57	4-F–Ph	175

Table 7. Receptor selectivity profile of compound **55**

Receptor	Binding IC_{50} (nM)	Receptor	Binding IC_{50} (nM)
CCR1	>1000 (−19%)	CCR8	>1000 (15%)
CCR3	>1000 (2%)	NK1	>100 (31%)
CCR4	>2000 (6%)	NK2	>1000 (20%)
CCR5	>1000 (49%)	NK3	>1000 (10%)

3-thiopheneglycine derivative **55** showed potent binding affinity to the human CCR2 receptor. For comparison, the phenylglycine and 4-*F*-phenylglycine analogs were 4-fold less active. Furthermore, compound **55** showed similar binding affinities to human monocytes with an IC_{50} of 30 nM. It inhibited MCP-1-induced calcium flux in human monocytes with an IC_{50} of 50 nM. More importantly, it inhibited MCP-1-induced chemotaxis of human monocytes with an IC_{50} of 9.6 nM. In addition, compound **55** shows good selectivity against other chemokine receptors as well as neurokinin receptors (Table 7). These data indicate that **55** is a CCR2-specific antagonist.

The PK profile of compound **55** was evaluated in rats. Oral bioavailability was low ($F = 4.5\%$), clearance was high (Clp = 80 mL/min/kg), and half-life was modest ($t_{1/2} = 1.8$ h). A study was carried out to see if the low oral bioavailability was due to high first pass metabolism. It was orally administered to rats (3 mg/kg), and portal and systemic levels are measured at several time points. It exhibited good oral absorption as demonstrated by high portal levels (over 300 ng/mL at 1 h), but limited systemic exposure (<20 ng/mL) suggests that first pass metabolism is responsible for the low oral bioavailability.

In conclusion, we have described SAR studies that have resulted in the identification of compound **55** as a novel and potent non-peptidyl CCR2 antagonist with comparable binding (cloned receptor and human monocyte) and functional chemotaxis and calcium flux activities. Compound **55** shows remarkable selectivity toward other chemokine receptors as well as the neurokinin receptors. This lead has served as a starting point for the design and synthesis of additional CCR2 antagonists. Progress in this area will be reported in due course.

References and notes

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30. Initially, the binding affinities were evaluated in the CHO assay; later the human monocyte based assay was used. Radioligand competition binding assays: Human monocytes (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 at 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.
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.
32. For a description of the chemotaxis assay, see: Ayala, J.M.; Goyal, S.; Liverton, N.J.; Claremon, D.A.; O'Keefe, S.J.; Hanlon, W.A. J Leukoc Biol 2000, 67, 869–75. Briefly: assays were performed in 96-well disposable chemotaxis plates (ChemoTx, NeuroProbe, Inc.) with a 5 m pore size (5.7 mm diameter). Monocytes (1×10^7 cells/ml) were incubated with 2 M Calcein-AM (Molecular Probes) in Hanks' balanced salt solution containing 0.01% BSA at 37 °C for 30 min. The dye-loaded cells were washed and resuspended at 6×10^6 cells/ml in RPMI 1640 (lacking phenol red) containing 0.01% BSA. Assay was performed with 1.5×10^5 cells/well.