Discovery and Structure−Activity Relationship of N-(Ureidoalkyl)-Benzyl-Piperidines As Potent Small Molecule CC Chemokine Receptor-3 (CCR3) Antagonists

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Structure−activity relationship (SAR) studies of initial screening hits from our corporate library of compounds and a structurally related series of CCR1 receptor antagonists were used to determine that an N-(alkyl)benzylpiperidine is an essential pharmacophore for selective CCR3 antagonists. Further SAR studies that introduced N-(ureidoalkyl) substituents improved the binding potency of these compounds from the micromolar to the low nanomolar range. This new series of compounds also displays highly potent, in vitro functional CCR3-mediated antagonism of eotaxin-induced Ca^{2+} mobilization and chemotaxis of human eosinophils.

Introduction

The normal immunological response to infection, tissue damage, or exposure to antigens is the recruitment of inflammatory cells to fight infection, remove damaged cells, and stimulate healing. While this is a normal process, the excessive recruitment of inflammatory cells is the hallmark of diseases such as asthma. A characteristic feature of asthma is leukocyte infiltration into the bronchial wall in which eosinophils predominate. Within the bronchial mucosa, eosinophils degranulate, releasing toxic proteins such as major basic protein, eosinophil cationic protein, and eosinophil peroxidase that cause tissue damage (e.g., epithelial injury and shedding). Eosinophil activation is thought to play a significant role in the underlying bronchial hyperreactivity that is characteristic of human asthma.[1]

Chemokines are chemotactic cytokines that play an important role in the directed migration of selected populations of leukocytes from the circulation to sites of inflammation, and thus, an integral part of the immune response. These small secreted proteins are classified into two main groups, CXC and CC, based on the position of the first two of their conserved N-terminal cysteine residues.[2] In general, the hallmark of the CC chemokines is their ability to chemoattract and activate inflammatory leukocytes, particularly subsets of lymphocytes, monocytes, eosinophils, and basophils, as well as some stromal cells, such as endothelial and smooth muscle cells. These activities of the CC chemokines are mediated through a family of receptors belonging to the seven transmembrane-spanning G protein-coupled receptors (7TM GPCR) on the surface of immune and inflammatory cells.[1]

Eosinophils are proinflammatory granulocytes that play a major role in allergic diseases, such as bronchial asthma[1f,g] and allergic rhinitis.[1h] Eotaxin, a CC chemokine[2b] with high selectivity for eosinophils, has recently been identified. Since the discovery of eotaxin, two other molecules, currently termed eotaxin-2[2c] and eotaxin-3,[2d] have been described. While they share the same name, the eotaxins are not closely related structurally (in fact, eotaxin and eotaxin-2 share only 39% amino acid identity) but appear to have a spectrum of activity identical to those of eotaxin. Although all three eotaxin molecules have a very similar range of biological activities, eotaxin-2 and eotaxin-3 are somewhat less potent than eotaxin. While many CC chemokines bind to several CC receptors, the eotaxins are exceptional in this regard and bind only to the CCR3 receptor. Furthermore, only a limited range of cells have been found to express CCR3, predominantly eosinophils and basophils. Thus, it appears that only the eotaxins can chemoattract and activate these target cells specifically.

Eosinophils express at least three chemokine receptors including CCR3, CCR1, and CXCR2. Of these, CCR3 achieves by far the highest expression levels and is thought to be the major eosinophil chemokine receptor.[1i] Eosinophils express lower levels of the closely related receptor CCR1, which binds and signals in response to MIP-1α, MCP-3, and RANTES. CCR1 and CCR3 share significant homology at the protein level (54% identity), with the majority of conservation occurring in the transmembrane helices.

The central role of CCR3 in allergic inflammation has made this receptor a major target for drug development[1d] with several reports of small molecule CCR3 antagonists appearing in the literature.[3,4] In this paper, we provide details of our efforts in this area.[5]

Results and Discussion

In our initial efforts to discover potential CCR3 antagonists, we screened our corporate library of compounds and identified several hits in the low micromolar range. Several of the compounds identified seemed to be structurally related, including compounds **1**−**3** as shown in Figure 1.

In addition to the screening hits, we synthesized several examples (Table 1) from a Takeda patent application,[6] which described a series of CCR1 receptor

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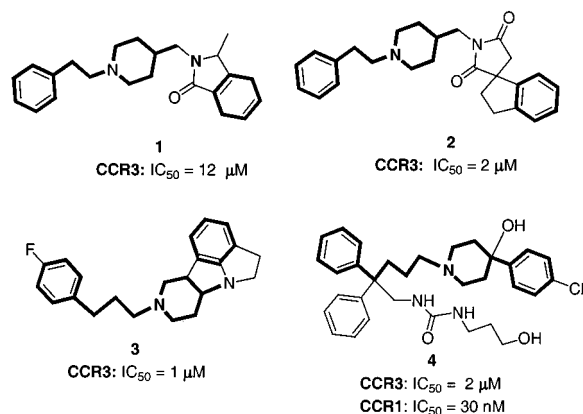
Figure 1. Initial hits identified from screening compound library. Common structural features are highlighted with bold bonds.

Table 1

compd	R	CCR1 IC$_{50}$ (μM)^a	CCR3 IC$_{50}$ (μM)^a
4	3-hydroxy-propyl	0.031	2.35
5	4-hydroxy-butyl	0.240	5.95
6	2-piperidin-1-yl-ethyl	0.360	2.20
7	1-benzyl-piperidin-4-yl	0.310	1.62
8	1-methoxycarbonyl-piperidin-4-yl	0.500	5.30
9	2-dimethylamino-ethyl	0.067	2.23

^{a} The value represents an average of usually three determinations with a standard deviation of ±20−50% of the mean value shown.

Table 2

compd	R	CCR1 IC$_{50}$ (μM)^a	CCR3 IC$_{50}$ (μM)^a
4	3-hydroxy-propyl	0.031	2.35
10	methyl	<0.78	8.3
11	isopropyl	<0.78	6.1
12	*tert*-butyl	0.071	3.0
13	2-(4-fluorophenyl)ethyl	0.295	2.2
14	benzyl	<0.78	8.3
15	cyclohexylmethyl	0.125	2.4
16	phenyl	<0.78	9.8
17	4-methoxy-phenyl	0.78	7.6
18	3-trifluoromethyl-phenyl	1.41	19.3
19	4-trifluoromethyl-phenyl	0.89	5.2
20	2,5-difluoro*o*-phenyl	1.34	5.7
21	4-pyridyl	0.167	3.5

^{a} The value represents an average of usually three determinations with a standard deviation of ±20−50% of the mean value shown.
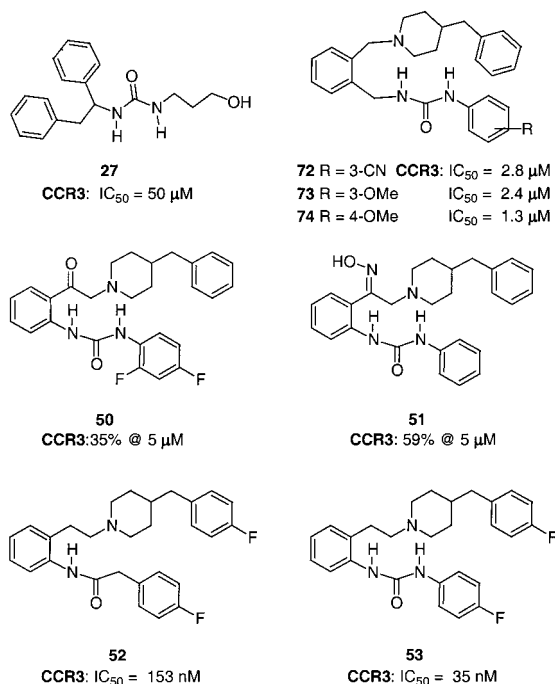
Table 3

compd	R^1	R^2	R^3	n	CCR3 IC$_{50}$ (μM)^a	CCR1 IC$_{50}$ (μM)^a
22	Ph	Ph	CH$_2$NH$_2$	3	12.0	0.76
23	Ph	Ph	CN	3	9.0	0.63
24	Ph	H	H	3	14.5	nt^b
25	Ph	H	H	1	19% at 10 μM	nt
26	Ph	H	H	0	18.7	nt

^{a} The value represents an average of usually three determinations with a standard deviation of ±20−50% of the mean value shown. ^{b} nt denotes not tested.

antagonists, as standards for our CCR1 counterscreen and the excellent potency of these compounds was confirmed in our assay (compound **4**, CCR1, IC$_{50}$ = 30 nM). Because the Takeda compounds had structural features similar to the screening hits **1**−**3**, they were tested in our CCR3 binding assay and found to have CCR3 binding affinity similar to the screening hits (**4**, CCR3, IC$_{50}$ = 2 μM). All of these compounds shared structural features that are highlighted with the bold bonds in Figure 1. Each contains a core 1,4-disubstituted piperidine with an N-phenethyl to N-phenbutyl group as the 1-substituent and a more variably linked aromatic group as the 4-substituent. Our initial analoging efforts concentrated on defining the essential pharmacophore features that were responsible for the CCR3 binding affinity of all of these compounds by trying to incorporate the features of one compound into the structure of the others. Because the Takeda compounds had the additional urea functionality that the screening hits lacked, we were especially interested in exploiting this functionality in our structure−activity relationship (SAR) studies.

To streamline the discussion for this paper, we will concentrate our comments in terms of Takeda compounds such as **4**. However, as results from concurrent work[7] in our labs starting with screening hit **3** indicate, because all of these compounds (**1**−**4**) have common structural features, all SAR studies beginning with any one compound would eventually lead to a common consensus structure as the key pharmacophore.

In addition to improving the CCR3 binding potency of the initial hits, the dissection of the structure and the synthetic studies also focused on determining the structural features responsible for receptor selectivity in order to reverse the selectivity found in **4** from CCR1 to CCR3.[8] In an effort to improve potency and selectivity for CCR3, we first examined changing the type of urea substituent from the polar alkyl preferred in the Takeda patent[6] (examples in Table 1) to more lipophilic alkyls or aryl substituents. As the data summarized in Table 2 show, changing the urea substituents of **4** to small alkyl (**10**), branched alkyl (**12**), cycloalkyl (**15**), or phenyl (**16**) and substituted phenyls (**17**−**20**) had only a small effect on CCR1 selectivity or CCR3 potency.

To identify the essential pharmacophore responsible for the CCR3 activity, the Takeda compounds were systematically simplified by incorporating some of the structural features found in the screening hits **1**−**3** (simple N-alkylphenyl substituents). Removal of the urea functionality in Takeda compound **4** to give **22** (and **23**, Table 3) had only a modest effect on CCR3 potency or CCR1 selectivity. That the urea functionality did contribute to the potency for CCR3 was evidenced by the synthesis of several urea compounds, such as **27** (Figure 2), which although weak, still had some CCR3 binding affinity. As the data in Table 3 summarizes, the benzhydrylpropyl motif of the Takeda compounds (**22**) could be further simplified to the N-benzyl analogue **26**

Figure 2. Structure of analogues discussed in the text.

Table 4

compd	R^1	R^2	R^3	X	n	CCR3 IC$_{50}$ (μM)^a	CCR1 IC$_{50}$ (μM)^a
28	Ph	Ph	CN	C	3	4.6	>50
29	Ph	Ph	CN	N	3	14.1	>50
30	Ph	H	H	C	3	3.8	>50
31	Ph	H	H	N	3	8.4	nt^b
32	Ph	H	H	C	1	7.1	>50
33	Ph	H	H	C	0	8.3	>50

^{a} The value represents an average of usually three determinations with a standard deviation of ±20–50% of the mean value shown. ^{b} nt denotes not tested.

and still maintain significant binding affinity for the CCR3 receptor.

Next, an examination of a variety of other substituted piperidines was undertaken to study the importance of the 4-hydroxy-4-phenylpiperidine motif found in the Takeda analogue **22** (Table 3). Several replacements were synthesized, including the substituted piperidine found in screening hit **3**, and all were found to be less potent (data not shown). However, the 4-benzylpiperidine substituent, as in **28** (Table 4), provided compounds that are more potent and selective for CCR3 over CCR1. The corresponding piperazine analogues (**29**), while less potent, also provided CCR3 selectivity. Similar to the Takeda-like analogues (in Table 3), the benzhydryl-propyl motif of **28** could be simplified to the phenethyl (**32**) or benzyl analogue (**33**), while maintaining binding affinity and selectivity for the CCR3 receptor as summarized in Table 4.

Having established that an N-benzyl- or N-phenethyl-substituted 4-benzylpiperdine was required for optimal CCR3 binding affinity and selectivity, we then reincorporated the urea functionality to improve the potency. The initial incorporation of the urea functionality into the simplest N-benzyl analogue (**33**, Table 4) at the ortho position of the N-benzyl ring was mainly driven

Table 5

compd	R	m	n	CCR3 IC$_{50}$ (μM)^a	CCR1 IC$_{50}$ (μM)^a
34	1-benzyl-piperidin-4-yl	0	1	3.9	>50
35	2-piperidin-1-yl-ethyl	0	1	7.6	>50
36	3-hydroxy-propyl	0	1	2.6	>10
37	benzyl	0	1	3.4	>10
38	phenyl	0	1	0.90	>10
39	2,5-difluoro-phenyl	0	1	1.75	> 50
40	3-methoxy-phenyl	0	1	0.60	>10
41	4-methoxy-phenyl	0	1	0.75	>10
42	3-cyano-phenyl	0	1	0.75	>10
43	5-indazolyl	0	1	0.45	>10
44	6-indazolyl	0	1	0.45	>10
45	3-cyano-phenyl	0	2	0.14	>10
46	3-methoxy-phenyl	0	2	0.18	>10
47	2,5-difluoro-phenyl	1	1	0.41	>10
48	3-methoxy-phenyl	1	1	0.081	>10
49	3-cyano-phenyl	1	1	0.049	>10

^{a} The value represents an average of usually three determinations with a standard deviation of ±20–50% of the mean value shown.

by synthetic expediency to give the analogues summarized in Table 5. The use of the polar alkyl urea substituents (**34–36**, Table 5) originally found in the Takeda compounds (of Table 1) did not improve the CCR3 binding affinity. However, these compounds did maintain selectivity for the CCR3 receptor as shown in Table 5. On the other hand, phenyl and substituted phenyl urea analogues provided compounds that were more potent, with the IC$_{50}$ improving to below the micromolar range (**38**, Table 5). The substitution pattern on the phenyl urea was important since analogues with a 2-substituent (**39** and **47**) were less potent than phenyl ureas with 3- or 4-substituents (**40** and **41**). The heterocyclic ureas (**43** and **44**) showed a further improvement in potency.

An increase in potency was also observed in changing the length of the linker between the piperidine ring and the urea functionality from the benzyl **42** to the phenethyl **45** or the hydroxyethylphenyl **49** as shown in Table 5. However, the acetylphenyl **50** or the oxime **51** (Figure 2) containing linkers were significantly less potent than the other phenethyl linkers. These changes affect not only the spacing but also the basicity of the piperidine nitrogen and suggest a preference for a more basic amine.[9]

Alternatives to the urea functionality were examined briefly and were found less potent. A typical result is exemplified by the amide **52**, which is about 5-fold less potent than the corresponding urea analogue **53**, as shown in Figure 2.

Further structure–activity studies were carried out to investigate the regiochemical requirements of the linker phenyl group to optimize the relative spatial orientation between the 4-benzylpiperidine and the urea substituents. The data in Table 6 show that analogues with the para substitution pattern are clearly less potent (compare **41** vs **55** or **42** vs **57**). Ortho- or meta-substituted analogues are in general of similar potency (compare **34** vs **54**). However, with the more potent (phenethyl linked) analogues, there seems to be a

Table 6
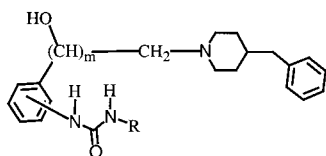
compd	R	m	position	CCR3 IC$_{50}$ (μM)[a]
34	1-benzyl-piperidin-4-yl	0	ortho	3.9
54	1-benzyl-piperidin-4-yl	0	meta	3.7
41	4-methoxy-phenyl	0	ortho	0.75
55	4-methoxy-phenyl	0	para	48% at 5 μM
42	3-cyano-phenyl	0	ortho	0.75
56	3-cyano-phenyl	0	meta	0.45
57	3-cyano-phenyl	0	para	47% at 5 μM
43	5-indazolyl	0	ortho	0.45
58	5-indazolyl	0	meta	0.62
59	phenyl	1	ortho	0.249
60	phenyl	1	meta	71% at 5 μM

[a] The value represents an average of usually three determinations with a standard deviation of ±20−50% of the mean value shown.

Table 7
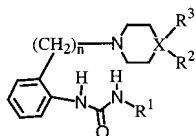
| compd	R^1	R^2	R^3	X	n	CCR3 IC$_{50}$ (μM)[a]
39	2,5-difluoro-phenyl	CH$_2$Ph	H	C	1	1.75
61	2,5-difluoro-phenyl	C(O)Ph	H	C	1	4.65
62	2,5-difluoro-phenyl	Ph	H	C	1	11.0
63	2,5-difluoro-phenyl	4-Cl-Ph	OH	C	1	21% at 5 μM
64	2,5-difluoro-phenyl	CH$_2$Ph		N	1	28% at 5 μM
65	2,5-difluoro-phenyl	CH$_2$Ph	H	C	0	9% at 5 μM
42	3-cyano-phenyl	CH$_2$Ph	H	C	1	0.75
45	3-cyano-phenyl	CH$_2$Ph	H	C	2	0.14
66	2-(4-fluorophenyl)ethyl	CH$_2$Ph		N	1	9.20
67	2-(4-fluorophenyl)ethyl	CH$_2$Ph	H	C	1	3.80
68	benzo[1,3]dioxol-5-ylmethyl	CH$_2$Ph	H	C	1	1.75
69	benzo[1,3]dioxol-5-ylmethyl	C(O)Ph	H	C	1	7.35
70	2,2-diphenyl-ethyl	CH$_2$Ph	H	C	1	2.65
71	2,2-diphenyl-ethyl	C(O)Ph	H	C	1	3.90

[a] The value represents an average of usually three determinations with a standard deviation of ±20−50% of the mean value shown.

preference for the ortho substitution pattern (compare **59** vs **60**).

With the SAR of the urea and linker portions established, alternatives to the 4-benzylpiperidine were again explored to confirm that it was still optimal. As shown in Table 7, all replacements that were examined resulted in reduced binding potency. This included 4-benzoyl-, 4-phenyl-, and 4-hydroxy-4-phenyl-substituted piperidines, as well as 4-benzylpiperazine, all of which showed lower binding affinity (compare **39** to **61**−**64**). Having the 4-benzyl-piperidine directly attached to the phenyl ring also caused a significant decrease in binding potency (**65**, Table 7). Insertion of a methylene group between the linker phenyl ring and the urea functionality (as in **72**−**74**, Figure 2) also decreased the binding affinity. As noted earlier, an ethyl spacer between the piperidine ring and the phenyl group (**45**, Table 7) gave the most potent compounds.

Results from concurrent work[7] in our labs with the propyl linker CCR3 antagonist showed that a 4-fluorobenzyl piperidine substituent had a dramatic effect on

Table 8
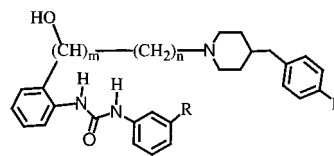
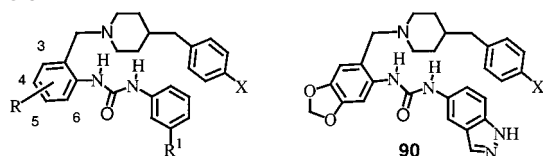| compd	R	m	n	CCR3 IC$_{50}$ (μM)[a]
75	H	0	1	0.361
76	cyano	0	1	0.310
77	acetyl	0	1	0.125
78	H	1	1	0.024
79	methoxy	1	1	0.021
80	cyano	1	1	0.011
81	cyano	0	2	0.014
82	methoxy	0	2	0.011
83	acetyl	0	2	0.0026

[a] The value represents an average of usually three determinations with a standard deviation of ±20−50% of the mean value shown.

the binding potency of CCR3 antagonists. To examine whether the effect was also observed in the phenyl linker series, several compounds incorporating this group were synthesized. As shown in Table 8, the 4-fluorobenzyl substituent has a significant effect on binding potency. There is about a 3-fold improvement in CCR3 binding potency with the benzyl linker (compare **38**, Table 5, vs **75**, Table 8) and about a 10-fold improvement with the phenethyl linker analogues (compare **45**, Table 7, vs **81**, Table 8).

With the SAR on the piperidine and urea portions established, the focus shifted to the phenyl ring in the linker portion. The synthetic effort concentrated on the more accessible benzyl linker analogues, and the results are summarized in Table 9. Systematic substitution of the four possible positions on the phenyl ring was examined. Substitutions at the 5- and 6-positions were either equivalent or less potent than the unsubstituted analogues (compare **42**, Table 5, vs **87**, Table 9). Substituents at the 3- and 4-positions showed a dramatic improvement in CCR3 binding potency. In particular, the carboxylic acid derivatives (methyl esters and amides) at the 3-position consistently showed single digit nanomolar affinities (compare **77**, Table 8, vs **107** and **115**, Table 9).

Selected analogues were characterized in various functional assays of human CCR3 receptor activity. Two main assays were used; the first is an eotaxin-induced intracellular calcium mobilization assay using FLIPR (fluorometric imaging plate reader) technology and the second, an eotaxin-induced eosinophil chemotaxis assay. Both of these assays use freshly isolated human eosinophils. The results for a few of the more potent analogues are summarized in Table 10. These analogues inhibited eotaxin-induced Ca^{2+} mobilization and eosinophil chemotaxis. The compounds themselves did not cause Ca^{2+} mobilization or chemotaxis at concentrations up to 10 μM (results not shown) indicating that these molecules are functional antagonists of the human CCR3 receptor in human eosinophils with an IC$_{50}$ in the low nanomolar range in the calcium assay and between 30 and 80 nM in the chemotaxis assay. These compounds are not only potent receptor antagonists of CCR3 but also selective for CCR3. All of the compounds listed in Table 10, as well as all compounds in this study

Table 9

compd	R	position	R^1	X	CCR3 IC$_{50}$ (μM)^a
84	phenyl	6-5 fused	methoxy	F	0.423
85	phenyl	6-5 fused	cyano	F	0.529
86	CO$_2$Me	5	methoxy	H	0.777
87	CO$_2$Me	5	cyano	H	72% at 5 μM
88	CH$_2$OH	5	methoxy	H	0.360
89	CH$_2$OH	5	cyano	H	0.114
90	dioxymethylene	5-4 fused	5-indazolyl	H	0.260
91	dioxymethylene	5-4 fused	acetyl	H	0.228
92	dioxymethylene	5-4 fused	cyano	H	0.237
93	dioxymethylene	5-4 fused	cyano	F	0.085
94	dioxymethylene	5-4 fused	acetyl	F	0.040
95	dioxymethylene	5-4 fused	methoxy	F	0.141
96	CO$_2$Me	4	cyano	F	0.067
97	CO$_2$Me	4	methoxy	F	0.064
98	CO$_2$Me	4	acetyl	F	0.042
99	CH$_2$OH	4	cyano	F	0.055
100	CH$_2$OH	4	methoxy	F	0.047
101	CONH$_2$	4	cyano	F	0.008
102	CONH$_2$	4	acetyl	F	0.003
103	CONHMe	4	acetyl	F	0.008
104	CO$_2$Me	3	H	F	0.009
105	CO$_2$Me	3	cyano	F	0.007
106	CO$_2$Me	3	methoxy	F	0.011
107	CO$_2$Me	3	acetyl	F	0.008
108	CH$_2$OH	3	H	F	0.053
109	COOH	3	H	F	0.017
110	CONH$_2$	3	cyano	F	0.003
111	CONH$_2$	3	acetyl	F	0.002
112	CONHMe	3	H	F	0.002
113	CONHMe	3	cyano	F	0.001
114	CONHMe	3	methoxy	F	0.002
115	CONHMe	3	acetyl	F	0.001

^{a} The value represents an average of usually three determinations with a standard deviation of $\pm$20$-$50% of the mean value shown.

Table 10

compd	CCR3 (IC$_{50}$, nM)^a	intracellular calcium mobilization (IC$_{50}$, nM)	eotaxin-induced chemotaxis (IC$_{50}$, nM)
83	2.6	nt^b	71^c
102	3.0	9.5^c	nt
103	8.0	11.8^c	nt
107	8.0	8.2^c	76^c
111	2.0	5.9^c	46^c
112	2.0	4.3 $\pm$ 2.2	nt
113	1.0	5.3 $\pm$ 2.2	nt
114	2.0	4.2 $\pm$ 1.1	nt
115	1.0	1.4 $\pm$ 0.8	30^c

^{a} The value represents an average of usually three determinations with a standard deviation of $\pm$20$-$50% of the mean value shown. ^{b} nt denotes not tested. ^{c} Value represents a single determination.

with CCR3 binding affinities less than 20 nM, were examined against a panel of other chemokine receptors and found to be selective for CCR3 by displaying low binding affinity for CCR1, CCR2, or CCR5 (IC$_{50}$ > 10 μM).

Finally, reports of the improved binding affinities of quaternary ammonium salts in CCR1[12] and CCR3[3c] receptor antagonists prompted us, early in our program, to explore the use of salts in our series. Several quaternary piperidinium salts were synthesized, and

Table 11
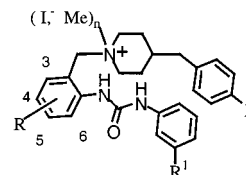
compd	R	position	R^1	n	X	CCR3 IC$_{50}$ (μM)^a
92	dioxymethylene	5-4 fused	cyano	0	H	0.237
117	dioxymethylene	5-4 fused	cyano	1	H	0.011
118	HO	4	cyano	0	H	0.277
119	HO	4	cyano	1	H	0.021
93	dioxymethylene	5-4 fused	cyano	0	F	0.085
120	dioxymethylene	5-4 fused	cyano	1	F	0.002
94	dioxymethylene	5-4 fused	acetyl	0	F	0.040
121	dioxymethylene	5-4 fused	acetyl	1	F	0.001

^{a} The value represents an average of usually three determinations with a standard deviation of $\pm$20$-$50% of the mean value shown.

they indeed demonstrated a 10$-$40-fold increase in binding affinity as compared to the parent piperidine derivatives as shown in Table 11.

Interestingly, the quaternary piperdinium salts listed in Table 11 were found to be potent functional agonists in the calcium and chemotaxis assays, while the corresponding parent piperidine derivatives (in Table 11) were themselves antagonists. For example, compound **121** (Table 11) caused Ca^{2+} mobilization at a concentration of 7 nM. The quaternary piperdinium salt pharmacophore does not appear to be a prerequisite for agonist activity, since, while several salts were synthesized, only some were agonists. In addition, some parent piperidine derivatives (nonpiperdinium salts) were found to be weak agonists (data not shown). All of the agonists displayed a rather flat SAR profile for agonist activity. The structural requirements are limited mainly to the oxygenated phenyl linker analogues listed in Table 11 and an ester urea derivative with much weaker activity. Because the potential of quaternary salts is limited due to pharmacokinetic liabilities (low oral bioavailability) and the therapeutic value of a CCR3 agonist is questionable, these compounds were not pursued much further. However, the data are presented in this paper as an interesting observation that other workers might find useful as a research tool.

Chemistry

The syntheses of the compounds are summarized in Schemes 1$-$4. Examples from the Takeda patent were synthesized from the key intermediate **23** (Scheme 1), which was obtained following the procedure described in the patent.[6] The cyano group was reduced to the amine **22** by hydrogenaton over Raney Nickel. The desired ureas were then obtained by reaction of the amine **22** with commericially available isocyanates. When the isocyanates were not available, **22** was first converted to the phenylcarbamate **122** with phenyl chloroformate (Scheme 1) and then treated with the appropriate amine to give the ureas shown in Tables 1 and 2. Similar chemistry was used to synthesize the compounds shown in Tables 3 and 4.

The benzyl and phenethyl linker analogues were synthesized as shown in Schemes 2 and 3. Commercially available 4-benzylpiperidine was alkylated with 2-ni-

Scheme 1[a,b]
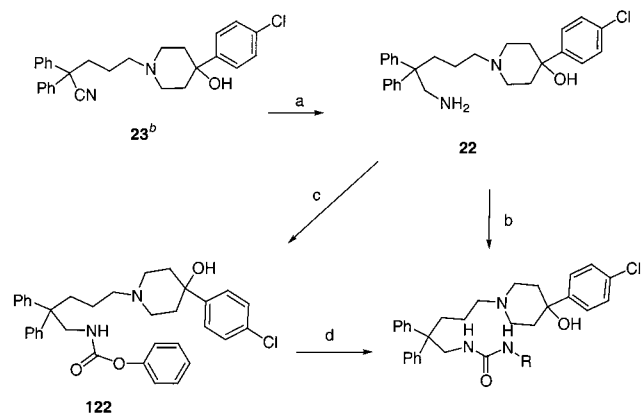
[a] Conditions: (a) Raney Ni, H$_2$ 60 psi, NH$_3$/EtOH, 80%. (b) OCNR, THF, room temperature, 70−80%. (c) ClC(O)OPh, THF, 70%. (d) NH$_2$R, THF, 70−80%. [b]Synthesized according to ref 6.

Scheme 2[a,b]
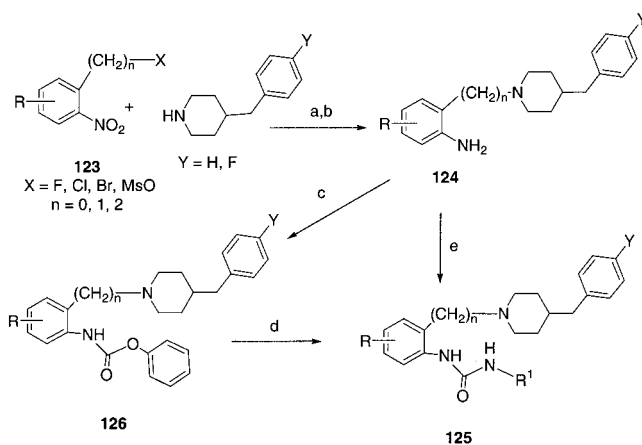
[a] Conditions: (a) For $n = 1$: K$_2$CO$_3$, DMF, room temperature, 70−90%; or for $n = 2$: THF, 3 days, room temperature, 75%. (b) 10% Pd−C, H$_2$ 50 psi, MeOH, 80−90%. (c) ClC(O)OPh, Et$_3$N, THF, 70−80%. (d) NH$_2$R^1, DMF, 50 °C, 70−80%. (e) OCNR^1, THF, room temperature, 70−80%. [b]Same synthetic scheme was used starting with the 3- and 4-NO$_2$ isomers of compound **123**.

trobenzyl bromide **123** in dimethyl formamide (DMF) to give the N-alkylated product. Catalytic hydrogenation of the nitro group gave the aniline **124**, which was converted to the desired ureas **125** by reaction with isocyanates or via the carbamate **126**. The alkylation of 4-benzylpiperidine with 2-nitrophenethyl bromide required tetrahydrofuran (THF) as the solvent to minimize styrene formation, which was the main product observed in DMF.

To preclude styrene formation, as well as to access substituted phenethyl derivatives, 4-benzylpiperidine was alkylated with the phenacyl bromide **127** (Scheme 3) to give the alkylated product. The nitro group was then hydrogenated to give the aniline **128**. The ketone of **128** was reduced with NaBH$_4$ to the aniline alcohol **130**, and subsequent treatment with isocyanates yielded the ureas **131**. Alternately, the aniline alcohol **130** was obtained from the reaction of nitro-epoxide **132** with 4-benzylpiperidine followed by catalytic hydrogenation. The epoxide **132** was obtained from the phenacyl bromide **127** by NaBH$_4$ reduction and cyclizing the resulting bromohydrin with NaOH.

The synthesis of the carboxylic acid derivative-substituted phenyl linker analogues is summarized in

Scheme 3[a,b]
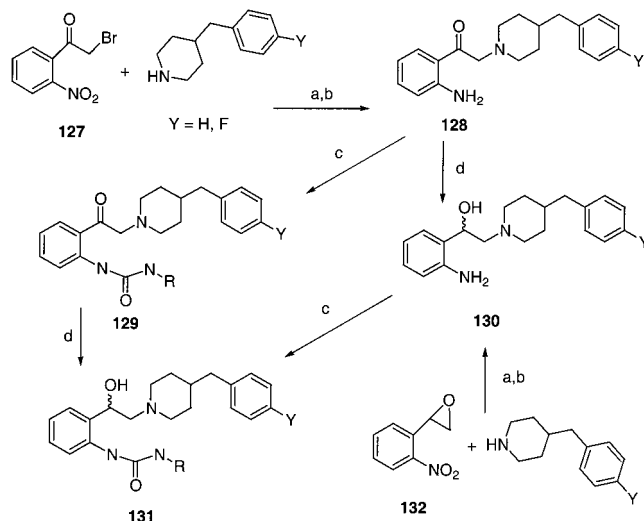
[a] Conditions: (a) K$_2$CO$_3$, DMF, room temperature, 80−90% or (a) K$_2$CO$_3$, DMF, room temperature, 50 °C, 70−80%. (b) 10% Pd−C, H$_2$ 50 psi, MeOH, 90%. (c) OCNR, THF, room temperature, 70−80%. (d) NaBH$_4$, MeOH, room temperature, 90%. [b]Same synthetic scheme was used starting with the 3-NO$_2$ isomers of compound **127**.

Scheme 4[a]
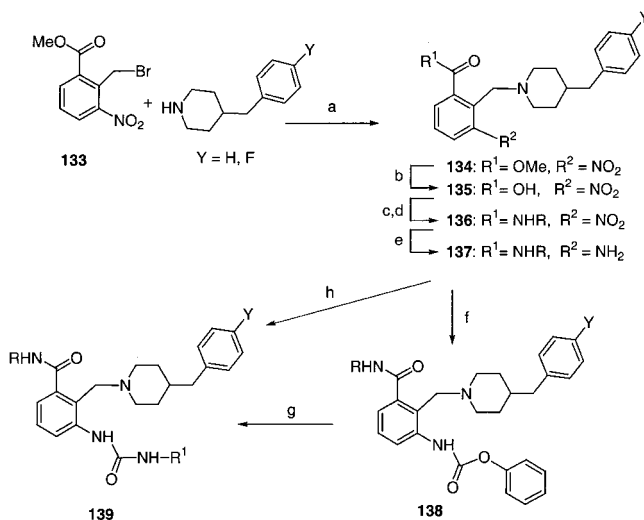
[a] Conditions: (a) K$_2$CO$_3$, DMF, room temperature, 80−90%. (b) NaOH, MeOH, reflux − 2 h, 90%. (c) Oxalyl chloride, CH$_2$Cl$_2$, room temperature. (d) RNH$_2$, CH$_2$Cl$_2$, 80−90%, 2 steps. (e) 10% Pd−C, H$_2$ 50 psi, MeOH, 90%. (f) ClC(O)OPh, Et$_3$N, THF, 65−75%. (g) NH$_2$R^1, DMF, 50 °C, 70−80%. (h) OCNR^1, THF, room temperature, 70−80%.

Scheme 4. Alkylation of the benzyl bromide **133** (obtained from NBS bromination of the commercially available toluene) with 4-benzylpiperidine gave the ester **134**. The ester was hydrolyzed, and the resulting acid **135** was converted to the acid chloride (oxalyl chloride) and treated with the appropriate amine to give the desired amide **136**. The nitro group of **136** was hydrogenated to give the amine **137**. The amine was converted to the desired urea by either direct treatment with the appropriate isocyanate or via the carbamate intermediate **138**.

Conclusion

In search of novel CCR3 antagonists, we identified several hits with micromolar potency from our corporate

compound library. Using these hits and a structurally related CCR1 antagonist reported in a Takeda patent[6] as a starting point, we initiated a series of SAR studies that determined that an essential pharmacophore for CCR3 binding was an N-(alkyl)benzylpiperidine core. Introduction of N-(ureidoalkyl) substituents improved the binding potency of these compounds to sub-micromolar. Further SAR studies were carried out and led to the identification of a potent series of CCR3 antagonists with single-digit nanomolar potency. These potent N-(ureidoalkyl)benzylpiperidines displayed in vitro functional antagonism of CCR3-mediated eotaxin-induced calcium mobilization and chemotaxis of human eosinophils. Studies are continuing on this class of compounds to improve their functional potency and will be reported in due course.

Experimental Section

General. All reactions were carried out under an atmosphere of dry nitrogen. Commercial reagents were used without further purification. ^{1}H NMR (300 MHz) spectra were recorded using tetramethylsilane (TMS) as an internal standard. Thin-layer chromatography (TLC) was performed on E. Merck 15710 silica gel plates. Medium-pressure liquid chromatography (MPLC) was carried out using EM Science silica gel 60 (230–400 mesh). High-performance liquid chromatography (HPLC) chromatography was carried out using Jasco PV-987 pumps, Jasco UV-975 detectors, and DuPont Zorbax Sil or Zorbax NH$_2$ one inch preparative columns. All final targets were obtained as noncrystalline amorphous solids unless specified otherwise. Mass spectra were measured with HP5988A Mass Spectrometer with particle beam interface using NH$_3$ for chemical ionization or Finnigan MAT 8230 Mass Spectrometer with NH$_3$-DCI or VG TRIO 2000 for ESI. High-resolution mass spectra were measured on a VG 70-VSE instrument with NH$_3$ chemical ionization. Elemental analysis was performed by Quantitative Technologies, Inc., Bound Brook, NJ. For compounds where analysis was not obtained, HPLC analysis was used, and purity was determined to be >95%.

Chemistry. The synthesis of illustrative examples are given below. A complete listing of all of the compounds synthesized for this work can be found in the Supporting Information.

1-{5-[4-(4-Chloro-phenyl)-4-hydroxy-piperidin-1-yl]-2,2-diphenyl-pentyl}-3-[2-(4-fluoro-phenyl)ethyl]urea (13). A solution of carbamate **122**[6] (100 mg, 0.176 mmol) in DMF was treated with 4-fluorophenylethylamine (48 mg, 0.35 mmol) and K$_2$CO$_3$ (130 mg, 0.90 mmol) and stirred overnight at room temperature. The mixture was diluted with water, and the precipitated solid was filtered on a buchner funnel. The solid was washed on the funnel with water and 1 N NaOH and allowed to air-dry to give 90 mg (85%) of urea **13**. ^{1}H NMR (300 MHz, CDCl$_3$) δ (TMS): 7.44–7.42(m, 2 H), 7.39–7.16 (m, 12 H), 7.08–6.81 (m, 4 H), 4.16 (bt, 1 H), 3.91 (m, 3 H), 3.25 (m, 2 H), 2.76–2.60 (m, 3 H), 2.41–1.80 (m, 10 H), 1.64 (m, 2 H), 1.33–1.20 (m, 2 H). MS ESI (M + H)$^+$ = 614.2. Anal. (C$_{37}$H$_{41}$N$_3$O$_2$ClF−(CDCl$_3$)$_{0.1}$) C, H, N.

1-{5-[4-(4-Chloro-phenyl)-4-hydroxy-piperidin-1-yl]-2,2-diphenyl-pentyl}-3-(2,4-difluoro-phenyl)urea (20). A solution of amine **22**[6] (200 mg, 0.44 mmol) in THF (5 mL) was treated with 2,4-difluorophenyl isocyanate (69 mg, 0.44 mmol) and stirred for 30 min at room temperature. The reaction mixture was concentrated under vacuum, and the residue was chromatographed on silica gel (EtOAc) to give 180 mg (70%) of **20** as a white solid. ^{1}H NMR (300 MHz, CDCl$_3$) δ (TMS): 7.83 (m, 1 H), 7.42–7.39 (m, 2 H), 7.37–7.20 (m, 12 H), 6.92 (m, 1 H), 6.60 (m, 1 H), 6.34 (bs, 1 H), 4.38 (bs, 1 H), 4.04 (d, J = 6 Hz, 2 H), 2.67 (m, 2 H), 2.36–2.25 (m, 4 H), 2.20–2.15 (m, 2 H), 2.13–1.95 (m, 2 H), 1.63–1.45 (m, 3 H), 1.40–1.20 (m, 2 H). MS ESI (M + H)$^+$ = 604.4. Anal. (C$_{35}$H$_{36}$N$_3$O$_2$ClF$_2$−(H$_2$O)$_{0.5}$) C, H, N.

1-{5-[4-(4-Chloro-phenyl)-4-hydroxy-piperidin-1-yl]-2,2-diphenyl-pentyl}-3-pyridin-4-yl-urea (21). A solution of amine **22**[6] (200 mg, 0.44 mmol) in DMF was treated with the carbamate, pyridin-4-yl-carbamic acid 4-nitro-phenyl ester (obtained from the reaction of *p*-nitrophenyl chloroformate with 4-amino pyridine) (110 mg, 0.44 mmol), and K$_2$CO$_3$ (300 mg, 2.2 mmol) and stirred overnight at room temperature. The mixture was diluted with water, and the precipitated solid was extracted into EtOAc and washed with water and brine. The solvent was removed on a rotary evaporator, and the residue was chromatographed on silica gel (30%MeOH/EtOAc) to give 150 mg (60%) of **21**. ^{1}H NMR (300 MHz, CDCl$_3$) δ (TMS): 7.40–7.36 (m, 2 H), 7.30–7.16 (m, 13 H), 6.92–6.80 (m, 4 H), 3.81 (d, J = 6 Hz, 2 H), 3.67 (bt, 1 H), 2.66 (m, 2 H), 2.36–2.29 (m, 4 H), 2.08–1.95 (m, 4 H), 1.80 (bs, 1 H), 1.64 (m, 2 H), 1.27 (m, 2 H). MS ESI (M + H)$^+$ = 569.4. Anal. (C$_{34}$H$_{37}$N$_4$O$_2$-Cl−(CDCl$_3$)$_{0.25}$) C, H, N.

5-(4-Benzyl-piperidin-1-yl)-2,2-diphenyl-pentanenitrile (28). A solution of 5-bromo-2,2-diphenyl-pentanenitrile[6] (200 mg, 0.64 mmol), 4-benzylpiperidine (113 mg, 0.64 mmol), and K$_2$CO$_3$ in DMF was stirred at room temperature overnight. The mixture was diluted with water, and the precipitated solid was extracted into EtOAc and washed with water and brine. The solvent was removed on a rotary evaporator, and the residue was chromatographed on silica gel (70% EtOAc/hexanes) to give 200 mg (77%) of **28** as a thick oil. ^{1}H NMR (300 MHz, CDCl$_3$) δ (TMS): 7.40–7.06 (m, 15 H), 2.77 (m, 2 H), 2.50 (d, J = 7 Hz, 2 H), 2.47–2.30 (m, 4 H), 1.78 (dt, J = 2 Hz, J = 12 Hz, 2 H), 1.69–1.57 (m, 4 H), 1.45 (m, 1 H), 1.31–1.18 (m, 4 H). MS ESI (M + H)$^+$ = 409.3.

1-[1-(Phenylmethyl)-4-piperidinyl]-3-[2-[[4-(phenyl-methyl)-1-piperidinyl]methyl]phenyl]urea (34). A solution of 4-benzylpiperidine (1.75 g, 10 mmol) in 25 mL of DMF was treated with 2-nitrobenzyl bromide (2.16 g, 10 mmol) and K$_2$CO$_3$ (1.38 g, 10 mmol), and the reaction mixture was stirred at room temperature for 2 h. The mixture was diluted with water and extracted into ethyl acetate. The organic extracts were washed successively with water and brine, and the organic solvent was removed under vacuum on a rotary evaporator to give 4-benzyl-1-(2-nitro-benzyl)piperidine (Scheme 2, R, Y = H) as a yellow oil. Crude product was redissolved in ethyl acetate (50 mL) and treated with 10% Pd/C and hydrogenated at 50 psi at room temperature for 40 min. The solution was filtered, the solvent was removed under vacuum, and the residue was purified by chromatography (MPLC, 40% ethyl acetate/hexane; silica gel) to give 2.0 g of 2-(4-benzyl-piperidin-1-ylmethyl)phenylamine **124** (Scheme 2, R, Y = H) as a white solid.

A solution of aniline **124** (1.2 g, 4.3 mmol) in THF was treated with Et$_3$N (1.0 g, 10 mmol) and cooled in an ice bath to °0 C. Phenyl chloroformate (0.71 g, 4.5 mmol) was added to the mixture and stirred for 1 h. The mixture was diluted with water and extracted into ethyl acetate. The extracts were washed with water and brine, and the solvent was removed under vacuum to give [2-(4-benzyl-piperidin-1-ylmethyl)phenyl]carbamic acid phenyl ester **126** as an off-white solid. The crude product was used without further purification.

A solution of phenylcarbamate **126** (0.2 g, 0.5 mmol) in DMF was treated with 4-amino-1-benzylpiperidine (95 mg, 0.5 mmol) and K$_2$CO$_3$ (138 mg, 1 mmol), and the mixture was heated at 50 °C for 2 h. The mixture was diluted with water and extracted into ethyl acetate. The extracts were washed with water and brine, and the solvent was removed under vacuum. The residue was purified by chromatography (MPLC, 0–25% MeOH/EtOAc; silica gel) to give 200 mg of **34** (83%) as a white solid. ^{1}H NMR (300 MHz, CDCl$_3$) δ (TMS): 9.60 (bs, 1 H), 7.93 (d, J = 8 Hz, 1 H), 7.33–7.21 (m, 9 H), 7.13 (d, J = 7 Hz, 2 H), 7.02 (d, J = 6 Hz, 1 H), 6.89 (m, 1 H), 4.26 (bs, 1 H), 3.68 (m, 1 H), 3.52 (s, 2 H), 3.51 (s, 2 H), 2.83 (m, 4 H), 2.54 (d, J = 7 Hz, 2 H), 2.19 (m, 2 H), 2.02–1.92 (m, 4 H), 1.88–1.45 (m, 5 H), 1.24–1.15 (m, 2 H). MS ESI (M + H)$^+$ = 497. Anal. (C$_{32}$H$_{40}$N$_4$O$_0$−(CDCl$_3$)$_{0.12}$) C, H, N.

1-[2-(4-Benzyl-piperidin-1-ylmethyl)phenyl]-3-(2,4-di-fluoro-phenyl)urea (39). A solution of aniline **124** (as

described above for the synthesis of **34**) (140 mg, 0.5 mmol) in THF was treated with 2,5-difluorophenyl isocyanate (80 mg, 0.5 mmol) at room temperature for 1 h. The solvent was removed under vacuum, and the residue was purified by chromatography (20% EtOAc/Hexane, silica gel) to give 150 mg (68%) of urea **39** as a white solid. ^{1}H NMR (300 MHz, CDCl$_3$) δ (TMS): 11.0 (bs, 1 H), 8.06 (d, $J = 8$ Hz, 1 H), 8.12−8.02 (m, 1 H), 7.30−6.92 (m, 9 H), 6.67 (m, 1 H), 6.58 (bs, 1 H), 3.59 (s, 2 H), 2.88 (m, 2 H), 2.54 (d, $J = 7$ Hz, 2 H), 1.95 (m, 2 H), 1.72 (m, 2 H), 1.60 (m, 1 H), 1.30−1.15 (m, 2 H). MS ESI (M + H)$^+$ = 436.3. Anal. (C$_{26}$H$_{27}$N$_3$OF$_2$) C, H, N.

3-[4-(4-Fluoro-benzyl)piperidin-1-ylmethyl]-4-[3-(3-methoxy-phenyl)ureido]benzoic Acid Methyl Ester (97). The same procedure as for **39** was followed (see Scheme 2) except starting with 3-bromomethyl-4-nitro-benzoic acid methyl ester and 4-(4-fluorobenzyl)piperidine. ^{1}H NMR (300 MHz, CDCl$_3$) δ (TMS): 10.63 (bs, 1 H), 8.24 (d, $J = 9$ Hz, 1 H), 7.95 (dd, $J = 2$ Hz, $J = 9$ Hz, 1 H), 7.74 (d, $J = 2$ Hz, 1 H), 7.26 (m, 1 H), 7.09−7.00 (m, 2 H), 6.98−6.93 (m, 2 H), 6.85 (dd, $J = 1.5$ Hz, $J = 8$ Hz, 1 H), 6.71 (dd, $J = 2$ Hz, $J = 8$ Hz, 1 H), 6.26 (s, 1 H), 3.88 (s, 3 H), 3.83 (s, 3 H), 3.55 (s, 2 H), 2.71 (m, 2 H), 2.44 (d, $J = 7$ Hz, 2 H), 1.87 (m, 2 H), 1.54 (m, 2 H), 1.46 (m, 1 H), 0.94 (m, 2 H). MS ESI (M + H)$^+$ = 506.3. Anal. (C$_{29}$H$_{32}$N$_3$O$_4$F−(H$_2$O)$_{0.25}$) C, H, N.

1-{2-[4-(4-Fluoro-benzyl)piperidin-1-ylmethyl]-4-hydroxymethyl-phenyl}-3-(3-methoxy-phenyl)urea (100). Compound **100** was obtained from the LiBH$_4$ reduction of **97**. ^{1}H NMR (300 MHz, CDCl$_3$) δ (TMS): 10.13 (bs, 1 H), 8.02 (d, $J = 8$ Hz, 1 H), 7.28−7.20 (m, 2 H), 7.08 (m, 4 H), 6.96 (m, 2 H), 6.85 (dd, $J = 1.5$ Hz, $J = 8$ Hz, 1 H), 6.71 (dd, $J = 2$ Hz, $J = 8$ Hz, 1 H), 6.26 (s, 1 H), 4.61 (s, 2 H), 3.83 (s, 3 H), 3.49 (s, 2 H), 2.72 (m, 2 H), 2.43 (d, $J = 7$ Hz, 2 H), 1.85 (m, 2 H), 1.62 (bs, 1 H), 1.54 (m, 2 H), 1.42 (m, 1 H), 0.94 (m, 2 H). MS ESI (M + H)$^+$ = 478.3.

1-{2-[2-(4-Benzyl-piperidin-1-yl)acetyl]phenyl}-3-(2,4-difluoro-phenyl)urea (50). To an ice-cold solution of 2-bromo-2′-nitro-acetophenone **127** (Scheme 3) (2.4 g, 10 mmol) in DMF was added 4-benzylpiperidine (1.75 g, 10 mmol), and it was stirred for 30 min. The solution was poured into a mixture of K$_2$CO$_3$ (1.38 g, 10 mmol) in water/ice and extracted into ethyl acetate. The ethyl acetate extract was washed several times with water. The resultant ethyl acetate solution of crude nitro-ketone was treated with 10% Pd/C and hydrogenated at 50 psi hydrogen at room temperature for 40 min. The solution was then filtered, the solvent was removed under vacuum, and the residue was purified by chromatography (MPLC, 30% ethyl acetate/hexane; silica gel) to give 1.9 g (63%) of 1-(2-amino-phenyl)-2-(4-benzyl-piperidin-1-yl)ethanone **128** (Scheme 3) as a tan/brown solid.

A solution of aniline **128** (310 mg, 1.0 mmol) in THF was treated with 2,5-difluoroisocyanate (160 mg, 1.0 mmol) at room temperature for 1 h. The solvent was removed under vacuum, and the residue was purified by chromatography (MPLC, 20% EtOAc/Hexane, silica gel) to give 420 mg (91%) of the desired urea-ketone **50** as a white solid. ^{1}H NMR (300 MHz, CDCl$_3$) δ (TMS): 11.5 (bs, 1 H), 8.55 (d, $J = 8$ Hz, 1 H), 8.09−8.02 (m, 1 H), 7.98 (dd, $J = 21$ Hz, $J = 8$ Hz, 1 H), 7.56 (m, 1 H), 7.30−6.98 (m, 8 H), 6.89 (bs, 1 H), 6.67 (m, 1 H), 3.82 (s, 2 H), 2.95 (m, 2 H), 2.55 (d, $J = 7$ Hz, 2 H), 2.09 (m, 2 H), 1.65 (m, 2 H), 1.55 (m, 1 H), 1.45−1.25 (m, 2 H). MS ESI (M + H)$^+$ = 464.3. Anal. (C$_{27}$H$_{27}$N$_3$O$_2$F$_2$−(H$_2$O)$_{0.25}$) C, H, N.

1-{2-[2-(4-Benzyl-piperidin-1-yl)-1-hydroxy-ethyl]phenyl}-3-(2,4-difluoro-phenyl)urea (47). A solution of the urea-ketone **50** (260 mg, 0.56 mmol) in MeOH was treated with NaBH$_4$ (400 mg, 11 mmol) at room temperature for 1 h. The solvent was removed under vacuum, and the residue was treated with 1 N NaOH and extracted into EtOAc. The extracts were washed with water and brine, and the solvent was removed under vacuum to give 0.25 g (96%) of the desired alcohol **47** as a white solid. ^{1}H NMR (300 MHz, CDCl$_3$) δ (TMS): 8.95 (bs, 1 H), 8.55 (d, $J = 8$ Hz, 1 H), 8.06−7.99 (m, 1 H), 7.86 (d, $J = 8$ Hz, 1 H), 7.33−6.98 (m, 9 H), 6.85 (bs, 1 H), 6.66 (m, 1 H), 4.81 (abx dd, $J = 10$ Hz, $J = 4$ Hz, 1 H), 3.02 (m, 1 H), 2.84 (m, 1 H), 2.78 (abx dd, $J = 10$ Hz, $J = 17$

Hz, 1 H), 2.55 (d, $J = 7$ Hz, 2 H), 2.51 (abx dd, $J = 4$ Hz, $J = 10$ Hz, 1 H), 2.30 (m, 1 H), 2.02 (m, 1 H), 1.68 (m, 2 H), 1.57 (m, 1 H), 1.43−1.25 (m, 2 H). MS ESI (M + H)$^+$ = 466.4. Anal. (C$_{27}$H$_{29}$N$_3$O$_2$F$_2$−(H$_2$O)$_{0.5}$) C, H, N.

1-{2-[2-(4-Benzyl-piperidin-1-yl)ethyl]phenyl}-3-(3-cyano-phenyl)urea (45). A solution of 4-benzylpiperidine (1.0 g, 5.7 mmol) in THF was treated with 2-nitrophenethyl bromide (1.0 g, 4.3 mmol), and the reaction mixture was stirred at room temperature for 3 days. The solvent was removed under vacuum, and the residue was partitioned between 0.5 N NaOH and chloroform. The organic extract was washed successively with water and brine. The solvent was removed under vacuum on a rotary evaporator, and the residue was chromatographed (MPLC, 50% ethyl acetate/hexane; silica gel) to give 0.5 g (36%) of 4-benzyl-1-[2-(2-nitro-phenyl)ethyl]-piperidine (Scheme 2, $n = 2$, R = H) as a yellow oil. ^{1}H NMR (300 MHz, CDCl$_3$) δ (TMS): 7.88 (d, $J = 8$ Hz, 1 H), 7.51 (m, 1 H), 7.37−7.13 (m, 7 H), 3.09 (m, 2 H), 2.94 (m, 2 H), 2.61 (m, 2 H), 2.54 (d, $J = 7$ Hz, 2 H), 2.01 (m, 2 H), 1.65 (m, 2 H), 1.53 (m, 1 H), 1.38−1.24 (m, 2 H).

The oil (0.5 g, 1.5 mmol) was redissolved in ethyl acetate (50 mL) and treated with 10% Pd/C and hydrogenated at 50 psi at room temperature for 45 min. The solution was filtered, and the solvent was removed under vacuum to give 0.4 g (89%) of 2-[2-(4-benzyl-piperidin-1-yl)ethyl]phenylamine **124** (Scheme 2, $n = 2$, R = H) as a white solid. The aniline was used without further purification. A solution of 2-[2-(4-benzyl-piperidin-1-yl)ethyl]phenylamine (240 mg, 0.81 mmol) in THF was treated with 3-cynanophenyl isocyanate (144 mg, 1.0 mmol) and stirred at room temperature for 2 h. The solvent was removed under vacuum, and the residue was purified by chromatography (MPLC, 50% EtOAc/Hexane to 100% EtOAc, silica gel) to give 250 mg (71%) of the desired urea **45** as a white solid. ^{1}H NMR (300 MHz, CDCl$_3$) δ (TMS): 9.85 (bs, 1 H), 7.88 (bs, 1 H), 7.77 (d, $J = 8$ Hz, 1 H), 7.65 (m, 1 H), 7.41−7.03 (m, 11 H), 3.11 (m, 2 H), 2.86 (m, 2 H), 2.65 (m, 2 H), 2.60 (d, $J = 7$ Hz, 2 H), 2.13 (m, 2 H), 1.79 (m, 2 H), 1.63−1.45 (m, 3 H). MS ESI (M + H)$^+$ = 439.3 Anal. (C$_{28}$H$_{30}$N$_4$O−(H$_2$O)$_{0.5}$) C, H, N.

1-[2-(4-Benzyl-piperidin-1-yl)phenyl]-3-(2,4-difluoro-phenyl)urea (65). A solution of 4-benzyl piperidine (3.5 g, 20 mmol) in DMF was cooled in an ice bath and treated with NaH (60% oil dispersion, 0.8 g, 20 mmol) and stirred for 30 min, removed from the ice bath, and stirred for an additional 30 min. The solution was again cooled in an ice bath and treated with 2-fluoro-nitrobenzene (2.82, 20 mmol) and stirred for 2 h. The mixture was removed from the bath and stirred at room temperature overnight. The solution was diluted with water and extracted into EtOAc. The organic extract was washed with water and brine and concentrated, and the residue was chromatographed (Hexane to 5% EtOAc/hexane, silica gel) to give 5.8 g (98%) of 4-benzyl-1-(2-nitro-phenyl)-piperidine as a yellow oil. The oil obtained was dissolved in EtOAc, treated with 1.1 g of 10% Pd/C, and hydrogenated at 50 psi for 1 h. The solution was filtered, and the solvent was removed under vacuum to give 5.2 g of 2-(4-benzyl-piperidin-1-yl)phenylamine, which was used without further purification. A solution of 2-(4-benzyl-piperidin-1-yl)phenylamine (133 mg, 0.50 mmol) in THF was treated with 2,4-difluorophenyl isocyanate (80 mg, 0.52 mmol) at room temperature for 30 min. The solvent was removed under vacuum, and the residue was chromatographed (5−10% EtOAc/hexane, silica gel) to give 160 mg (76%) of **65** as a white solid. ^{1}H NMR (300 MHz, CDCl$_3$) δ (TMS): 8.01−7.92 (m, 2 H), 7.79 (bs, 1 H), 7.32−7.00 (m, 10 H), 6.71 (m, 1 H), 3.02 (m, 2 H), 2.64 (m, 2 H), 2.57 (d, $J = 7$ Hz, 2 H), 1.76 (m, 2 H), 1.68 (m, 1 H), 1.59 (m, 2 H). MS ESI (M + H)$^+$ = 422.3 Anal. (C$_{25}$H$_{25}$N$_3$O$_2$F$_2$) C, H, N.

2-[4-(4-Fluoro-benzyl)piperidin-1-ylmethyl]-3-(3-phenyl-ureido)benzoic Acid Methyl Ester (104). A solution of 2-methyl-3-nitro-benzoic acid methyl ester (10.0 g, 51 mmol) in CCl$_4$ was heated to reflux and treated with NBS (9.1 g, 51 mmol) and a catalytic amount of benzoyl peroxide. The mixture was heated to reflux for 24 h, cooled to room temperature, and filtered. The filtrate was concentrated under vacuum, and the residue was chromatographed (5−10% EtOAc/hexane, silica

gel) to give 10.3 g (73%) of the benzyl bromide **133** (see Scheme 4), 2-bromomethyl-3-nitro-benzoic acid methyl ester. ^{1}H NMR (300 MHz, CDCl$_3$) δ (TMS): 8.11 (dd, $J = 1$ Hz, $J = 8$ Hz, 1 H), 7.97 (dd, $J = 1$ Hz, $J = 8$ Hz, 1 H), 7.53 (t, $J = 8$ Hz, 1 H), 5.16 (s, 2 H), 4.00 (s, 3 H). A solution of the above benzyl bromide **133** (4.9 g, 18 mmol) in DMF was treated with 4-(4-fluorobenzyl)piperidine HCl (4.2 g, 18 mmol) and K$_2$CO$_3$ (2.76 g, 20 mmol), and the reaction mixture was stirred at room temperature for 6 h. The mixture was diluted with water and extracted into ethyl acetate. The organic extracts were washed successively with water and brine, and the organic solvent was removed under vacuum on a rotary evaporator to give 6.9 g (100%) of **134** (Scheme 4), 2-[4-(4-fluoro-benzyl)piperidin-1-ylmethyl]-3-nitro-benzoic acid methyl ester, as a yellow oil. This was used without further purification. ^{1}H NMR (300 MHz, CDCl$_3$) δ (TMS): 7.34−7.69 (m, 2 H), 7.40 (t, $J = 8$ Hz, 1 H), 7.08−7.01 (m, 2 H), 6.97−6.90 (m, 2 H), 3.88 (s, 3 H), 3.77 (s, 2 H), 2.61 (m, 2 H), 2.45 (d, $J = 7$ Hz, 2 H), 1.93 (dt, $J = 11$ Hz, $J = 2$ Hz, 2 H), 1.47 (m, 2 H), 1.40 (m, 1 H), 1.12 (m, 2 H).

A solution of the above crude nitrobenzene **134** (1.1 g, 2.8 mmol) in EtOAc (50 mL) was treated with 10% Pd/C (0.4 g) and hydrogenated at 50 psi at room temperature for 45 min. The solution was then filtered, and the solvent was removed under vacuum to give the aniline as a thick oil. The aniline was used without further purification. ^{1}H NMR (300 MHz, CDCl$_3$) δ (TMS): 7.11−6.93 (m, 6 H), 6.74 (d, $J = 8$ Hz, 1 H), 5.11 (bs, 2 H), 3.84 (s, 3 H), 3.71 (s, 2 H), 2.82 (m, 2 H), 2.48 (d, $J = 7$ Hz, 2 H), 2.00 (dt, $J = 11$ Hz, $J = 2$ Hz, 2 H), 1.59 (m, 2 H), 1.51 (m, 1 H), 1.23 (m, 2 H). A solution of above aniline (210 mg, 0.59 mmol) in THF was treated with phenyl isocyanate (72 mg, 0.60 mmol) and stirred at room temperature for 2 h. The solvent was removed under vacuum, and the residue was purified by chromatography (MPLC, 50% EtOAc/Hexane to 100% EtOAc, silica gel) to give 200 mg (74%) of the desired urea **104** as a white solid. ^{1}H NMR (300 MHz, CDCl$_3$) δ (TMS): 10.5 8 (bs, 1 H), 8.15 (d, $J = 8$ Hz, 1 H), 7.40−7.23 (m, 6 H), 7.15 (m, 1 H), 7.04 (m, 2 H), 6.95 (m, 2 H), 6.30 (bs, 1 H), 3.87 (s, 3 H), 3.78 (s, 2 H), 2.72 (m, 2 H), 2.43 (d, $J = 7$ Hz, 2 H), 2.00 (m, 2 H), 1.52 (m, 2 H), 1.45 (m, 1 H), 0.92 (m, 2 H). MS ESI (M + H)$^+$ = 476.3 Anal. (C$_{28}$H$_{30}$N$_3$O$_3$F) C, H, N.

3-[3-(3-Cyano-phenyl)ureido]-2-[4-(4-fluoro-benzyl)piperidin-1-ylmethyl]benzamide (110). A solution of 2-[4-(4-fluoro-benzyl)piperidin-1-ylmethyl]-3-nitro-benzoic acid methyl ester **134** (described above for the synthesis of **104**) (2.6 g, 6.7 mmol) in 75 mL of MeOH was treated with 21 mL of 1 N NaOH, and the mixture was heated to reflux for 2 h. The solution was concentrated under vacuum, and the residue was diluted with water and neutralized with 20 mL of 1 N HCl (to a pH of about 6) to give a precipitate. The solid was extracted into EtOAc, washed with water and brine, and dried over MgSO$_4$. The solvent was removed under vacuum to give 2.5 g (100%) of the acid **135** (Scheme 4) as a yellow foam. A solution of the acid **135** (1.0 g, 2.68 mmol) in dichloromethane was treated dropwise with oxalyl chloride (0.55 g, 4.3 mmol) at room temperature [immediate bubbling and precipitate formations were observed] and stirred for 1.5 h. The solvent and excess oxalyl chloride were removed under vacuum to give the acid chloride as a yellow foam. The crude acid chloride was treated with a saturated solution of ammonia in dioxane at room temperature for 20 min. The solution was concentrated under vacuum, and the residue was partitioned between water and EtOAc. The organic extract was washed with water and brine and dried over MgSO$_4$. The solvent was removed under vacuum to give 0.9 g (90%) of the corresponding amide **136** (Scheme 4) as a yellow foam. This was used without further purification. A solution of the above crude amide **136** (0.9 g, 2.4 mmol) in THF (50 mL) was treated with 10% Pd/C (0.3 g) and hydrogenated at 50 psi at room temperature for 30 min. The solution was then filtered, and the solvent was removed under vacuum to give the aniline **137** (Scheme 4) as a white solid. The aniline was used without further purification. A solution of aniline **137** (Scheme 4) (100 mg, 0.29 mmol) in THF was treated with 3-cyanophenyl isocyanate (42 mg, 0.35 mmol) at room temperature for 1 h. The solvent was removed under

vacuum, and the residue was purified by chromatography (MPLC, EtOAc−10% MeOH/EtOAc, silica gel) to give 75 mg (54%) of the desired urea **110** as a white solid. ^{1}H NMR (300 MHz, CDCl$_3$) δ (TMS): 8.01 (d, $J = 8$ Hz, 1 H), 7.83 (s, 1 H), 7.62 (d, $J = 8$ Hz, 1 H), 7.42−7.17 (m, 4 H), 7.06 (m, 2 H), 6.95 (m, 2 H), 6.70 (bs, 1 H), 5.75 (bs, 1 H), 3.74 (s, 2 H), 2.86 (m, 2 H), 2.50 (d, $J = 7$ Hz, 2 H), 2.25 (m, 2 H), 1.68 (m, 2 H), 1.58 (m, 1 H), 1.16 (m, 2 H). MS ESI (M + H)$^+$ = 486.4 Anal. (C$_{28}$H$_{28}$N$_5$O$_2$F−(CDCl$_3$)$_{0.12}$) C, H, N.

4-Benzyl-1-{6-[3-(3-cyano-phenyl)ureido]benzo[1,3]-dioxol-5-ylmethyl}-1-methyl-piperidinium; Iodide (117). A solution of **92** (200 mg, 0.42 mmol) in AcCN (10 mL) was treated with MeI (0.052 mL, 0.84 mmol) and heated at 40 °C overnight. The solvent was removed under vacuum, and the residue was chromatographed (MPLC, EtOAc, silica gel) to give 200 mg of solid. This was recrystallized from 80% EtOAc/Hexane to give 120 mg (47%) of light yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ (TMS): 9.16 (bs, 1 H), 8.32 (bs, 1 H), 7.96 (m, 1 H), 7.63 (m, 1 H), 7.46 (m, 1 H), 7.43 (m, 1 H), 7.29 (m, 2 H), 7.22−7.05 (m, 5 H), 6.12 (s, 2 H), 4.45 (s, 2 H), 3.30 (m, 4 H), 2.92 (s, 3 H), 2.58 (d, $J = 7$ Hz, 2 H), 1.60−1.50 (m, 5 H). MS ESI (M − I)$^+$ = 483.4 Anal. (C$_{29}$H$_{31}$N$_4$O$_3$I) C, H, N.

1-(1,2-Diphenyl-ethyl)-3-(3-hydroxy-propyl)urea (27). This was obtained from the reaction of (1,2-diphenyl-ethyl)-carbamic acid phenyl ester with 3-amino-propan-1-ol. ^{1}H NMR (300 MHz, DMSO-d_6) δ (TMS): 7.29−7.12 (m, 10 H), 6.40 (d, $J = 8$ Hz, 1 H), 5.8 (m, 1 H), 4.86 (m, 1 H), 4.42 (t, $J = 5$ Hz), 3.44 (m, 2 H), 2.96 (m, 2 H), 2.92 (m, 2 H), 1.43 (m, 2 H). MS ESI (M + H)$^+$ = 299.2. HRMS Calcd for C$_{18}$H$_{23}$N$_2$O$_2$ (M + H)$^+$, 299.1760; found, 299.1771. Anal. (C$_{18}$H$_{22}$N$_2$O$_2$) C, H, N.

1-[2-(4-Benzyl-piperidin-1-ylmethyl)benzyl]-3-(3-cyano-phenyl)urea (72). A similar procedure as described above for **39** was followed. Thus, 4-benzylpiperidine was alkylated with 2-bromomethyl-benzonitrile (in DMF) to give 2-(4-benzyl-piperidin-1-ylmethyl)benzonitrile. The nitrile was reduced using Raney Nickel in EtOH/NH$_3$ (50 psi H$_2$/room temperature/3 days) to give 2-(4-benzyl-piperidin-1-ylmethyl)benzyl-amine. The resulting benzylamine was treated with 3-cyanophenyl isocyanate (in THF) to give the urea **72**. ^{1}H NMR (300 MHz, CDCl$_3$) δ (TMS): 7.73 (m, 1 H), 7.49−7.10 (m, 13 H), 5.99 (bs, 1 H), 4.42 (s, 2 H), 3.50 (s, 2 H), 2.92 (m, 2 H), 2.55 (d, $J = 7$ Hz, 2 H), 2.03 (m, 2 H), 1.76 (m, 2 H), 1.66 (m, 1 H),1.20−1.04 (m, 2 H). MS ESI (M + H)$^+$ = 439.3. Anal. (C$_{28}$H$_{30}$N$_4$O) C, H, N.

1-{2-[2-(4-Benzyl-piperidin-1-yl)-1-hydroxyimino-eth-yl]phenyl}-3-phenyl-urea (51). Following the procedure detailed above for **50** except using phenyl isocyanate provided 1-{2-[2-(4-benzyl-piperidin-1-yl)acetyl]phenyl}-3-phenyl-urea **129** (Scheme 3). A solution of **129** (310 mg, 0.73 mmol) in EtOH was treated with hydroxylamine hydrochloride (300 mg, 4.3 mmol) and heated to reflux for 2 h. The solution was diluted with water and extracted into EtOAc. The extract was washed with water and brine and concentrated under vacuum, and the residue was chromatographed (MPLC, 50%−70% EtOAc/hexane, silica gel) to give 200 mg (63%) of the oxime **51** (Figure 2) as a solid. ^{1}H NMR (300 MHz, CDCl$_3$) δ (TMS): 7.89 (d, $J = 8$ Hz, 1 H), 7.75 (bs, 1 H), 7.59(s, 1 H), 7.46−6.99 (m, 12 H), 6.89 (m, 2 H), 3.43 (s, 2 H), 2.93 (m, 2 H), 2.26 (d, $J = 7$ Hz, 2 H), 1.93 (m, 2 H), 1.47 (m, 2 H), 1.36 (m, 1 H), 1.25−1.10 (m, 2 H). MS ESI (M + H)$^+$ = 443.4. Anal. (C$_{27}$H$_{30}$N$_4$O$_2$−(H$_2$O)$_{0.25}$) C, H, N.

1-(2-{2-[4-(4-Fluoro-benzyl)-piperidin-1-yl]ethyl}-phenyl)-3-(4-fluoro-phenyl)urea (53). This compound was obtained from the reaction of 2-{2-[4-(4-fluoro-benzyl)piperidin-1-yl]ethyl}phenylamine (detailed above for the synthesis of **81/45**) with 4-fluorophenyl isocyanate. ^{1}H NMR (300 MHz, CDCl$_3$) δ (TMS): 9.34 (bs, 1 H), 7.77 (d, $J = 8$ Hz, 1 H), 7.39−7.20 (m, 4 H), 7.15−6.92 (m, 8 H), 3.03 (m, 2 H), 2.81 (m, 2 H), 2.58 (m, 2 H), 2.50 (d, $J = 7$ Hz, 2 H), 2.02 (m, 2 H), 1.68 (m, 2 H), 1.56−1.25 (m, 3 H). MS ESI (M + H)$^+$ = 450.3 Anal. (C$_{27}$H$_{29}$N$_3$-OF$_2$−(H$_2$O)$_{0.5}$) C, H, N.

N-(2-{2-[4-(4-Fluoro-benzyl)piperidin-1-yl]ethyl}phenyl)-2-(4-fluoro-phenyl)acetamide (52). This compound was obtained from the reaction of 2-{2-[4-(4-fluoro-benzyl)piperidin-

1-yl]ethyl}phenylamine (detailed above for the synthesis of **81**/**45**) with (4-fluoro-phenyl)acetyl chloride. ^{1}H NMR (300 MHz, CDCl$_3$) δ (TMS): 10.15 (bs, 1 H), 7.91 (d, $J = 8$ Hz, 1 H), 7.39–6.92 (m, 11 H), 3.67 (s, 2 H), 2.88 (m, 2 H), 2.65 (m, 2 H), 2.54 (m, 2 H), 2.45 (d, $J = 7$ Hz, 2 H), 2.00 (m, 2 H), 1.60 (m, 2 H), 1.50 (m, 1 H), 1.25 (m, 2 H). MS ESI (M + H)$^+$ = 449.3. Anal. (C$_{28}$H$_{30}$N$_2$OF$_2$) C, H, N.

Biological Assays

CCR3 Receptor Binding. Millipore filter plates (no. MABVN1250) were treated with 5 μg/mL protamine in phosphate-buffered saline, pH 7.2, for 10 min at room temperature. Plates were washed three times with phosphate-buffered saline and incubated with phosphate-buffered saline for 30 min at room temperature. For binding, 50 μL of binding buffer (0.5% bovine serum albumin, 20 mM HEPES buffer, and 5 mM magnesium chloride in RPMI 1640 media) with or without a test concentration of a compound present at a known concentration was combined with 50 μL of 125-I-labeled human eotaxin (to give a final concentration of 150 pM radioligand) and 50 μL of cell suspension in binding buffer containing 5×10^5 total cells. Cells used for the binding assay were a Chinese hamster ovary cell line transfected with a gene expressing human CCR3 as described by Daugherty.[13] The mixture of compound, cells, and radioligand was incubated at room temperature for 30 min. Plates were placed onto a vacuum manifold, the vacuum was applied, and plates were washed three times with binding buffer with 0.5 M NaCl added. The plastic skirt was removed from the plate, the plate was allowed to air-dry, the wells were punched out, and the CPM was counted. The percent inhibition of binding was calculated using the total count obtained in the absence of any competing compound or chemokine ligand, and the background binding was determined by addition of 100 nM eotaxin in place of the test compound.

Human Eosinophil Chemotaxis Assay. Neuroprobe MBA96 96 well chemotaxis chambers with Neuroprobe poly(vinylpyrrolidone)-free polycarbonate PFD5 5 μm filters in place were warmed in a 37 °C incubator prior to assay. Freshly isolated human eosinophils[14] were suspended in RPMI 1640 with 0.1% bovine serum albumin at 1×10^6 cells/mL and warmed in a 37 °C incubator prior to assay. A 20 nM solution of human eotaxin in RPMI 1640 with 0.1% bovine serum albumin was warmed in a 37 °C incubator prior to assay. The eosinophil suspension and the 20 nM eotaxin solution were each mixed 1:1 with prewarmed RPMI 1640 with 0.1% bovine serum albumin with or without a dilution of a test compound that was at least 2-fold the desired final concentration. The filter was separated, and the eotaxin/compound mixture was placed into the bottom part of the chemotaxis chamber. The filter and upper chamber were assembled, and a 200 μL volume of the cell suspension/compound mixture was added to the appropriate wells of the upper chamber. The upper chamber was covered with a plate sealer, and the assembled unit was placed in a 37 °C incubator for 45 min. After incubation, the plate sealer was removed and all of the remaining cell suspension was aspirated off. The chamber was disassembled, unmigrated cells were washed away with phosphate-buffered saline, and then, the filter was wiped with a rubber-tipped squeegee. The

filter was allowed to completely dry and was stained with Wright Giemsa. Migrated cells were enumerated by microscopy.

Calcium Mobilization Assay. Intracellular calcium flux was measured as the increase in fluorescence emitted by the calcium-binding fluorophore, fluo-3, when preloaded cells were stimulated with CCR3 ligand. Freshly isolated eosinophils were loaded with fluorophore by resuspending them in a HEPES-buffered phosphate-buffered saline containing 5 μM fluo-3 and incubating for 60 min at 37 °C. After they were washed twice to remove excess fluorophore, cells were resuspended in binding buffer (without phenol red) and plated into 96 well plates at 2×10^5/well. Plates were placed individually in a FLIPR-1 (Molecular Devices, CA) that uses an argon-ion laser to excite the cells and robotically adds reagents while monitoring changes in fluorescence in all wells simultaneously. To determine the IC$_{50}$, compound or buffer alone was added and cells were incubated for 5 min; eotaxin was then added to a final concentration of 10 nM. The fluorescence shift was monitored, and the base-to-peak excursion was computed automatically. All conditions were tested in duplicate, and the mean shift per condition was determined. The inhibition achieved by graded concentrations of compound was calculated as a percentage of the compound-free eotaxin control.

Supporting Information Available: Complete experimentals including analytical data (NMR, MS) for all of the compounds in this study. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM0201767