Unraveling the Chemistry of Chemokine Receptor Ligands

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1. Introduction

Chemokines (for *chemo*attractant cyto*kines*) are a family of small (70−120 amino acids, 8−14 kDa), mostly basic, structurally related proteins. Chemokines share a high degree of homology in sequence, tertiary structure, and functions. They mainly act on neutrophils, monocytes, lymphocytes, and eosinophils and play a pivotal role in host defense mechanisms. Chemokines exhibit their biological effects by interacting with specific receptors on the cell surface of their target cells. These specific receptors, called chemokine receptors, belong to the G-protein-coupled receptors (GPCRs) superfamily.

1.1. New Classification and Nomenclature for Chemokine Ligands

At present, more than 45 chemokines and almost 20 chemokine receptors have been identified in humans. The discovery of new chemokines is still occurring at a rapid pace. Therefore, to avoid confusion, a new systematic classification and nomenclature system was devised for naming the new chemokines by Zlotnik and Yoshie[1] and has been implemented in recent publications. Table 1[1−6] illustrates this new classification and nomenclature, along with

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Table 1. Classification and Nomenclature of Chemokines and Chemokine Receptors[a]

receptor	cellular distribution	ligands by systematic name	common name of human ligands	common name of mouse ligands	presumed function	therapeutic potential
CC						
CCR1	N, M, MΦ, T, B, Eo, Ba, P, immature DC, mesangial cell	CCL3	MIP-1α, LD78α	MIP-1α	Th1 response	multiple sclerosis, rheumatoid arthritis, allograft rejection, viral myocarditis
		CCL5	RANTES	RANTES		
		CCL7	MCP-3	MARC?		
		CCL14	HCC-1	unknown		
		CCL15	HCC-2, Lkn-1, MIP-1δ	unknown		
		CCL16	HCC-4, LEC	LCC-1		
		CCL23	MPIF-1	unknown		
CCR2	M, MΦ, T, Ba, NK, immature DC, endothelium, fibroblast	CCL2	MCP-1, MCAF	JE	inflammation	multiple sclerosis, arthritis, asthma, glomerulonephritis, atherosclerosis
		CCL7	MCP-3	MARC?		
		CCL12	unknown	MCP-5		
		CCL13	MCP-4	unknown		
		CCL16	HCC-4, LEC	LCC-1		
CCR3	T, (Th2), Eo, Ba, MC, P, DC	CCL5	RANTES	RANTES	Th2 response	asthma, contact dermatitis
		CCL7	MCP-3	MARC?		
		CCL8	MCP-2	MCP-2?		
		CCL11	eotaxin	eotaxin		
		CCL13	MCP-4	unknown		
		CCL15	HCC-2, Lkn-1, MIP-1δ	unknown		
		CCL24	MPIF-2, eotaxin-2	unknown		
		CCL26	eotaxin-3	unknown		
		CCL28	MEC, CCK1	unknown		
CCR4	T(Th2), Ba, P, immature DC	CCL17	TARC	TARC	Th2 response	sepsis, asthma, contact dermatitis
		CCL22	MDC, STCP-1	ABCD-1		
CCR5	M, MΦ, T(Th1), NK, thymocyte, DC, aortic smooth muscle cell	CCL3	MIP-1α, LD78α	MIP-1α	Th1 response	multiple sclerosis, rheumatoid arthritis, intestinal inflammation, allograft rejection, HIV infection
		CCL4	MIP-1β	MIP-1β		
		CCL5	RANTES	RANTES		
		CCL8	MCP-2	MCP-2		
		CCL14	HCC-1	unknown		
CCR6	M, MΦ, T, B, immature DC	CCL20	MIP-3α, LARC, exodus-1	MIP-3α, LARC, exodus-1	dendritic cell function	psoriasis
CCR7	T, B, DC	CCL19	MIP-3β, ELC, exodus-3	MIP-3β, ELC, exodus-3	lymphocyte and dendritic cell migration to lymph nodes	cancer
		CCL21	6Ckine, SLC, exodus-2	6Ckine, SLC, exodus-2, TCA-4		
CCR8	N, M, T, B, thymocyte	CCL1	I-309	TAC-3, P500	Th2 response	atopic dermatitis, asthma
CCR9	T, thymocyte	CCL25	TECK	TECK	lymphocyte trafficking in thymus and small intestine	intestinal inflammation
CCR10	T, melanocyte, dermal endothelium, dermal fibroblast, Langerhans cell	CCL27	CTACK, ILC	ALP, CTACK, ILC, Eskine	lymphocyte trafficking in skin and colon	skin inflammation, ulcerative colitis
		CCL28	MEC, CCK1	unknown		
CXC						
CXCR1	N, T, M, MΦ, DC, astrocyte, endothelium	CXCL6	GCP-2	CKα-3	neutrophil recruitment	lung reperfusion injury, gout, psoriasis, cancer
		CXCL8	IL-8	unknown		
CXCR2	N, T, M, MΦ, DC, Eo, endothelium	CXCL1	GROα, MGSA-α	GRO, KC	neutrophil recruitment, angiogenesis	lung reperfusion injury, gout, psoriasis, cancer, atherosclerosis
		CXCL2	GROβ, MGSA-β	GRO, KC		
		CXCL3	GROγ, MGSA-γ	GRO, KC		
		CXCL5	ENA-78	LIX?		
		CXCL6	GCP-2	Ckα-3		
		CXCL7	NAP-2	unknown		
		CXCL8	IL-8	unknown		
CXCR3	N, T (Th1), B, DC, Eo, P, mesangial cell, smooth muscle cell	CXCL9	MIG	MIG	Th1 response, angiostasis	multiple sclerosis, rheumatoid arthritis, sarcoidosis, allograft rejection, cancer
		CXCL10	IP-10	IP-10		
		CXCL11	I-TAC	unknown		
CXCR4	N, M, MΦ, T, B, DC, P, astrocyte	CXCL12	SDF-1α, SDF-1β	SDF-1	organogenesis	HIV infection, cancer
CXCR5	M, MΦ, T, B, astrocyte, neuron	CXCL13	BLC, BCA-1	BLC, BCA-1	B-cell migration	not identified
CXCR6	T, DC	CXCL16			not known	not identified

Table 1 (Continued)

receptor	cellular distribution	ligands by systematic name	common name of human ligands	common name of mouse ligands	presumed function	therapeutic potential
CX3C						
CX3CR1	N, M, MΦ, neuron	CX3CL1	Fractalkine	Neurotactin	cell adhesion to endothelia and neurons	allograft rejection, glomerulonephritis, CNS inflammation
C						
XCR1	T	XCL1	Lymphotactin/ SCM1α/ATAC	Lymphotactin	not known	not identified
		XCL2	SCM-1β	unknown	not known	not identified

[a] We omitted the ligands (CCL6, CCL9/10, CCL18, CXCL4, CXCL14, CXCL15) of which the corresponding receptor(s) have not been identified. *Abbreviations:* B, B-cell; Ba, basophil; DC, dendritic cell; Eo, eosinophil; M, monocyte; MC, mast cell; MΦ, macrophage; N, neutrophil; NK, natural killer cell; P, platelet; T, T-cell; Th, T-helper cell. *Abbreviations for the ligands:* 6Ckine, 6-cysteine chemokine; BCA-1, B-cell-activating chemokine 1; BLC, B-lymphocyte chemoattractant; CTACK, cutaneous T-cell-attracting chemokine; DC-CK1, dendritic-cell chemokine 1; ELC, EBII (Epstein−Barr virus-induced gene 1)-ligand chemokine; ENA-78, epithelial-cell-derived neutrophil-activating peptide 78; GCP, granulocyte chemotactic protein; GRO, growth-regulated oncogene; HCC, hemofiltrate CC chemokine; IL-8, interleukin-8; ILC, interleukin-11 receptor α-locus chemokine; IP-10, interferon-inducible protein 10 (CXCL10); I-TAC, interferon-inducible T-cell α chemoattractant; LARC, liver and activation-regulated chemokine; LCC-1, liver CC chemokine-1; LEC, liver-expressed chemokine; LIX, lipopolysaccharide-induced CXC chemokine; Lkn-1, leukotactin-1; MARC, mast cell activation-related chemokine; MCAF, monocyte chemotactic and activating factor; MCP, monocyte chemoattractant protein; MDC, macrophage-derived chemokine; MEC, mucosal-associated chemokine; MGSA, melanocyte growth stimulatory activity peptide; MIG, monokine induced by γ-interferon (CXCL9); MIP, macrophage inflammatory protein; MIP-1α, macrophage inflammation protein 1α; MPIF-1, myeloid progenitor inhibitory factor 1; NAP, neutrophil-activating protein; PARC, pulmonary and activation-regulated chemokine; RANTES, regulated on activation, normal T-cell expressed and secreted; SDF-1, stromal-cell-derived factor 1; SLC, secondary lymphoid-tissue chemokine; STCP-1, stimulated T-cell chemotactic protein; TAC, T-cell-α chemoattractant; TARC, thymus and activation-regulated chemokine (CCL17); TCA-4, T-cell activation-4; TECK, thymus-expressed chemokine.

the corresponding protein names still used extensively in the literature. A list of abbreviations for the protein names in alphabetical order is part of the table footnote.

Chemokines have been divided into two major subfamilies on the basis of the arrangement of the two N-terminal cysteine residues, CXC and CC, depending on whether the first two cysteine residues have an amino acid between them (CXC) or are adjacent (CC). The genes for these families are currently designated *SCY* (small secreted cytokine), with *SCYa* corresponding to the CC subfamily and *SCYb* to the CXC subfamily. Two other classes of chemokines have been described as lymphotactin (C or *SCYc*) and fractalkine (CX3C or *SCYd*).

The chemokine nomenclature is based on the chemokine receptor nomenclature currently in use, which uses CC, CXC, XC, or CX3C followed by R (for *r*eceptor) and then a number. Thus, we have CCR1−10, CXCR1−6, XCR1 (the lymphotactin receptor), and CX3CR1 (the fractalkine receptor). Basically, the new nomenclature replaces R with L (ligand instead of receptor) to designate the ligands and uses CC for the *SCYa* subfamily, CXC for *SCYb*, XC for *SCYc*, and CX3C for *SCYd*. The numbering system is the one already in use to designate the genes encoding each chemokine. Thus, a given gene will have the *same number* as its protein ligand (for example, *ScyA 27* is the gene encoding CCL27), a correlation that should further simplify matters. It is obvious from Table 1 that many ligands bind to multiple receptors, while many receptors have multiple ligands.

For drug discovery, animal models are always needed. To correlate the in vitro and in vivo results, it is helpful to know the mouse chemokines that are homologous to human. Therefore, Table 1 lists the human chemokines with the related mouse chemokines, and the human chemokines are taken as "standard". The chemokines described in other species can be characterized in one of four ways: (a) Chemokines that unambiguously (based on a high degree of sequence homology, and chromosomal location to syntenic regions between species) correspond to a given human ligand; examples include RANTES (CCL5), TARC (CCL17), TECK (CCL25), etc. (b) Chemokines related to a human homologue but the exact homologue of which is not certain (indicated by a question mark in Table 1). (c) Those chemokines for which no human counterpart has been described; for example, MCP-5 (CCL12). (d) Human chemokines for which no mouse chemokine has been described; for example, IL-8 (CXCL8). These factors should be taken into consideration when developing an animal model for a disease targeting chemokine receptors.

So far, the classification and nomenclature are all based on the agonists of chemokine receptors. This is due to the fact that, historically, the identification and characterization of chemokine−receptor−ligand specificities was based on agonist activity. However, more and more examples have been reported showing that some chemokines act as agonists to chemokine receptors. On the other hand, the same chemokine can also act as antagonist or partial agonist to other chemokine receptors (for a review, see ref 8).

This review will focus on drugable small organic molecules as chemokine ligands, and therefore the discussion is organized on the basis of the receptors (see Table 1). Since the endogenous protein ligands mainly serve as the ligands for the assays in the screening of small molecules, the proteins' common names will be used for the nomenclature when discussing the protein ligands in this review (e.g., I-TAC instead of CXCL11, IL-8 instead of CXCL8). Table 1 provides the systematic names of the protein

ligands and their corresponding common names. The reader should consult Table 1 if the systematic names are desired.

1.2. Chemokine Receptors

Chemokines exert their biological functions through interaction with specific high- and low-affinity chemokine receptors which belong to the superfamily of seven transmembrane domain GPCRs. Chemokine receptors have been grouped into the rhodopsin-like family of GPCRs. (For details, see ref 9.)

1.2.1. Structural Characteristics of Chemokine Receptors

Chemokine receptors are typically 340−370 amino acids in length, with 25−80% homology among all known human chemokine receptors.[10] Chemokine receptors also share all of the highly conserved sequence motifs characteristic of the rhodopsin-like family of GPCRs. The overwhelming majority of these sequence motifs are located in the transmembrane region, suggesting the conservation of a common fold for this region throughout the entire rhodopsin-like family. The strong similarity of the transmembrane regions of chemokine receptors to those of other rhodopsin-like GPCRs suggests that these proteins undergo ligand-induced activation processes, involving analogous conformational changes with similar mechanisms. However, there is broad structural diversity among the extracellular region, despite a strong sequence conservation of the transmembrane helices. The structural adaptation of a receptor to its cognate ligand is expected to involve, in most cases, sequence specificity in the extracellular domains, and also in the transmembrane region, which holds the binding pocket of small ligands (for reviews, see refs 11 and 12). Therefore, each receptor must have evolved specific structural characteristics to exhibit the specific recognition of its cognate ligand to what is believed to constitute a common activation process (for examples, see refs 13 and 14). Understanding the structure of chemokine receptors and the activation mechanism would greatly facilitate drug discovery in terms of designing small molecules to target certain chemokine receptors and for co-screening against other chemokine receptors to evaluate the selectivity of development candidates. As is apparent in the main body of this review, many highly selective chemokine receptors antagonists have been discovered and reported.

1.2.2. A Brief Overview of the Expression of Chemokines and Chemokine Receptors

The expression patterns of chemokines and chemokine receptors provide clues for disease relevancy in targeting certain chemokine receptors with small drugable molecules. In general, chemokine receptors fall into two categories of expression: those expressed exclusively on a small number of leukocytes (e.g., CXCR1 is predominantly restricted to neutrophils) and those that are broadly expressed (e.g., CCR2 is expressed on monocytes, T-cells, natural killer cells, dendritic cells, and basophils).[6] Chemokine receptor expression is regulated by a variety of inflammatory stimuli or inhibited by the activation of other factors or receptors.

Expression of chemokines can be loosely grouped into two patterns. Chemokines involved in homeostatic trafficking, such as SDF-1, BAC-1, SLC, ELC, CLACK, and TECK, are expressed constitutively by many cell types in tissue-specific sites and contribute to homeostatic homing in these areas. In contrast, the expression of inflammatory chemokines is induced only under specific conditions, such as by inflammatory cytokines.[6] Additional information on the expression of chemokines and chemokine receptors will be presented in each section on specific chemokine receptors, which will serve as a basis for a rationale on targeting the corresponding receptors.

1.3. Potential Therapeutic Targets of Chemokine Receptors

Chemokines were originally identified by their ability to regulate the trafficking of immune cells.[15−17] However, the biological role of chemokines goes well beyond their ability to function as chemoattractants, and they have since been shown to be involved in many other biological processes,[7] including growth regulation,[18] hematopoiesis,[19−21] and angiogenesis,[22,23] and also play an important role in the central nervous system (CNS).[24,25] Many disease conditions have been related to the dis-regulation of chemokine networks, including rheumatoid arthritis (RA),[26] osteoarthritis,[27−30] multiple sclerosis (MS),[28] atherosclerosis,[31] Alzheimer's disease,[32] asthma,[33−34] chronic obstructive pulmonary disorder,[35] allergic disease,[36−40] and human immunodeficiency virus (HIV) infection.[41−49] Collectively, this information has provided a very strong rationale for the implementation of chemokine receptor antagonist research programs.

1.4. Scope of the Review

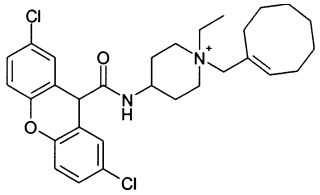
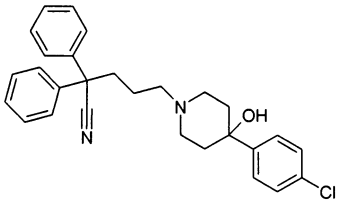
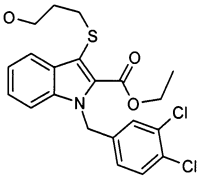
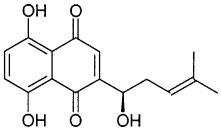
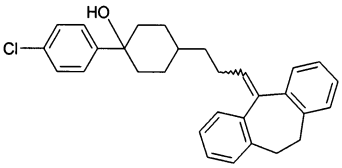
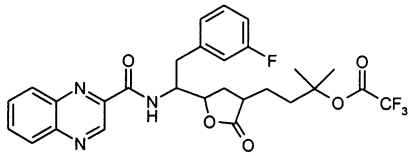
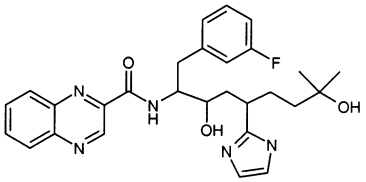
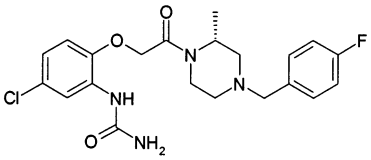
The study of chemokines and chemokine receptors is a very active, complex, and rapidly expanding field. The real excitement from a pharmaceutical perspective is that small-molecule chemokine receptor antagonists may be useful to treat certain diseases. This review will expand on a previous review by Trivedi et al.[50] and will focus on recent developments and progress made in medicinal chemistry toward the discovery of small-molecule chemokine receptor antagonists reported in the literature and patents since 2000.

2. CCR1 Receptor Antagonists

CCR1 is predominantly expressed on neutrophils, monocyte/macrophages, T-lymphocytes, natural killer cells, B-lymphocytes, mast cells, astrocytes, and neurons. Its high-affinity ligands include RANTES, MIP-1α, HCC-1, MCP-2, MCP-3, MIP-5, and Ckβ8. Several diseases have been associated with CCR1. These include, but are not limited to, MS,[51,52] allograft rejection,[53,54] and RA.[55,56]

Table 2 summarizes some examples of CCR1 ligands reported in the recent literature. Compound **1**[57] exhibits IC_{50} values of 0.9 and 5.8 nM respectively for human and murine CCR1 receptors. This com-

Table 2. CCR1 Antagonists

compound no.	structure	affinity	ref
1		h-CCR1, IC_{50} = 0.9 nM m-CCR1, IC_{50} = 5.8 nM	57
2		K_i = 52 ± 5 nM	58
3		K_i < 50 μM	59
4		IC_{50} = 2.63 and 2.57 μM (RANTES and MIP-1α, respectively)	60
5		IC_{50} = 1−1000 μM	61
6		IC_{50} < 25 μM	62
7		IC_{50} < 25 μM	62
8		IC_{50} = 1 nM vs MIP-1α on human CCR1; IC_{50} = 100 nM on rat; K_i = 50 nM on rabbit	63

pound is also a functional antagonist for CCR1, as demonstrated by its ability to inhibit the MIP-1α-induced Ca^{2+} response in U937 cells expressing human and mouse CCR1 receptors with IC_{50} values of 0.73 and 21 nM, respectively. Compound **1** displayed high selectivity over other CCR receptors (>1 μM for CCR2B, CCR4, CCR5, CXCR1,2, and CX3CR1).

Researchers at Berlex[58] reported compound **2** with a K_i value of 52 ± 5 nM. This binding constant was determined from competitive binding on CCR1 with [^{125}I]MIP-1α, as characterized in an MIP-1α-induced intracellular calcium mobilization assay using a fluorometric imaging plate reader (FLIPR) with human embryonic kidney (HEK)/293 cells that overexpress the human CCR1 receptor. The compound showed no significant agonist activity at the highest concentration tested (5 μM). The structure−activity relationship (SAR) of this series of compounds exhibited several interesting features. First, it appears that the molecule's chain length is related to its activity. An analogue of compound **2** with a chain length of two carbons had a reported K_i value of 4060 nM, while compound **2** (with three carbons) had a K_i value of 52 nM. Introduction of a four-carbon chain and five-carbon chain gave K_i's of 78 and 99 nM, respectively. Second, the quaternary ammonium salt of **2** had a 5−20-fold increase in binding affinity

compared to that of the corresponding parent piperidine derivatives.

Indolecarboxylate **3** was disclosed [59] as a CCR1 receptor antagonist with an $IC_{50} \leq 50$ μM for anti-inflammatory activity. Shikonin (**4**)[60] is a chemically characterized component of a traditional Chinese herbal medicine and has been shown to possess anti-inflammatory activity. Shikonin was shown to block radio-labeled RANTES and MIP-1α binding to human monocytes with IC_{50} values of 3.58 and 2.57 μM, respectively. In contrast, shikonin failed to inhibit SDF-1α binding to the cells at concentrations up to 17 μM. In addition, shikonin blocked RANTES and MIP-1α binding to stable CCR1-transfected HEK/293 cells with IC_{50} values of 2.63 and 2.57 μM, respectively. However, shikonin inhibited neither RANTES nor MIP-1α binding to CCR5-transfected HEK/293 cells. Shikonin inhibited RANTES-induced migration of HEK/293 cells transfected with CCR1 but did not inhibit the same type of cells induced by epidermal growth factor (EGF). Therefore, it could be envisioned that shikonin may be a good lead for the future design of more potent, highly selective therapeutics that could be useful as anti-inflammatory agents for selectively blocking the binding of CCR1 ligands.

Compound **5**[61] was disclosed as a compound for the treatment of diseases associated with aberrant leukocyte recruitment and/or activation. Chemokine binding activities of test compounds are reported with IC_{50} values ranging from <1 to <1000 μM. Compounds **6** and **7**[62] were reported as CCR1 antagonists for the treatment of inflammation and other immune diseases. These compounds were tested for inhibition of chemotaxis of various chemokines with inhibition of $IC_{50} < 25$ μM. Compound **8**[63] had an affinity of 1 nM vs MIP-1α on human CCR1 and was selective against other GPCRs.

3. CCR2 Receptor Antagonists

The expression of CCR2 in human monocytes can be stimulated by homocysteine, possibly involving an oxygen free radical.[31] Homocysteinemia is an independent risk factor for atherosclerosis. The development of atherosclerosis involves MCP-1-mediated monocyte recruitment to the lesion site. The action of MCP-1 is mostly via its interaction with MCP-1 receptor (CCR2), which is the major receptor for MCP-1 on the surface of monocytes. In addition, the C−C chemokines and their receptors CCR-2 and CCR-5 were found to be expressed in normal and osteoarthritis chondrocytes.[27] However, regulation of chemokine expression by IL-1β and TNF-α differed between normal and osteoarthritis chondrocytes. Intracellular staining revealed that approximately 20% of the chondrocytes contained CCR-2 and CCR-5 in the cytoplasm, whereas cell surface expression was detected less frequently. Interestingly, RANTES induced expression of its own receptor, CCR-5, suggests an autocrine/paracrine pathway of the chemokine within the cartilage milieu.

Finally, MCP-1 or RANTES not only induced MMP-3 expression, but also inhibited proteoglycan synthesis and enhanced proteoglycan release from the chondrocytes. The differential expression of chemokines and their receptors under the regulation of IL-1β and TNF-α suggests that the cytokine-triggered chemokine system may play a key role in the cartilage degradation of osteoarthritis, possibly by acting in an autocrine/paracrine manner.

Recently, several new compounds have been disclosed as CCR2 receptor ligands. For example, compound **9**[64] showed in vitro inhibition binding of MCP-1 with an IC_{50} value of 31 nM (Table 3). Compound **10**[65] had an IC_{50} value of ≤50 μM in the hMCP-1 receptor-binding assay. High-throughput screening (HTS) of the SmithKline Beecham (SKB) compound collection against the cloned human CCR2B receptor identified the indole derivative **11**[66] as a weak ligand ($K_i = 5.3$ μM). Extensive array SAR studies delivered compound **12**,[66] with a K_i value of 50 nM, that displayed selectivity against the closely related CCR5 receptor. Unfortunately, this compound was not developed due to its unwanted affinity for 5HT and dopamine, with the affinity comparable to that for the desired CCR2B receptor. Structure modification by conformational restriction of the indolopiperidine nucleus and the flexible C-5 linker chain led to compound **13**. This compound displayed comparable CCR2B affinity ($K_i = 40$ nM) relative to the more flexible analogue **12**, while having dramatically improved selectivity (1000-fold) against a number of 5-HT and dopaminergic receptors.[67]

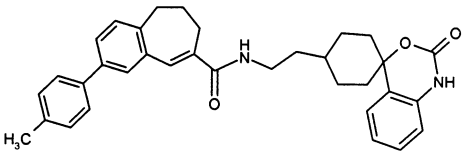
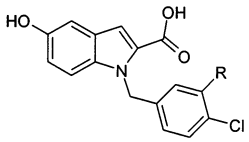
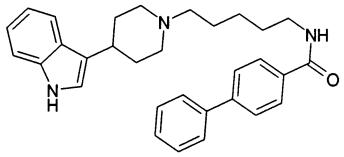
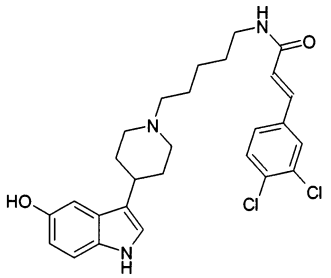
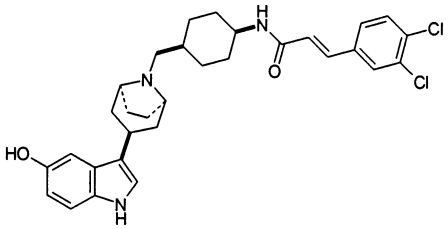
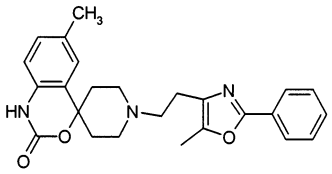
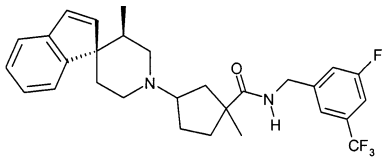
Compound **14**[68] is a potent antagonist to MCP-1 receptor with an IC_{50} value of 89 nM. An extensive SAR study disclosed that the central feature of this class of the compounds includes a pharmacophore defined by a basic nitrogen in the piperidine ring and an orthogonal relationship between the phenyl urethane system and a piperidine imposed by a spiro carbon atom. The hydrogen-bonding potential provided by the urethane functionality appears to be of key importance. Another dominant feature is the restriction in use of only small functional groups as substituents on the phenyl urethane heterocycle.

Finally, a vast range of hydrophobicities and sizes are acceptable for substituents on the phenethyl portion of the antagonists exemplified by **14**. Compound **15**[69] was claimed as a CCR2 antagonist for anti-inflammatory and anti-rheumatic agents in a recent patent.

4. CCR3 Receptor Antagonists

Selective accumulation of eosinophils to inflammatory sites is characteristic in allergic diseases such as asthma.[70] Eosinophils are therefore thought to play an important role in the initiation and progression of these diseases. On the other hand, CC chemokines such as eotaxin, RANTES, MCP-3, and MCP-4, which are ligands for the CCR3 receptor, are responsible for the recruitment of eosinophils from the circulation to allergic sites.[71] Eotaxin also plays an important role in eosinophil-dependent inflammation in nasal mucosa. Blocking eotaxin or CCR-3 might be useful as a new therapeutic tool for treatment of allergic rhinitis. It has been demonstrated[72] that in subjects with allergic rhinitis, allergen challenge led to parallel increases in eosinophil counts, eosinophil protein X (EPX) level, and eotaxin con-

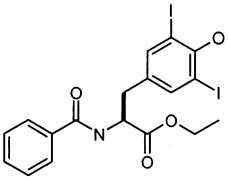
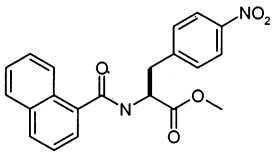
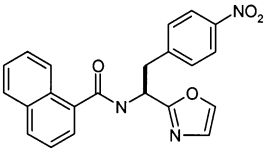
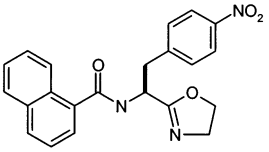
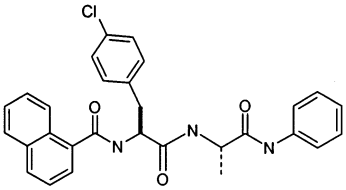
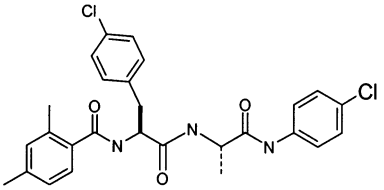
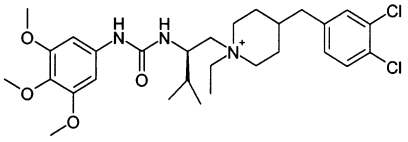
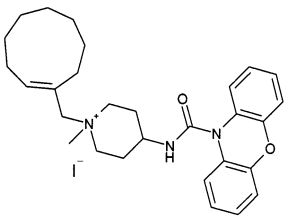
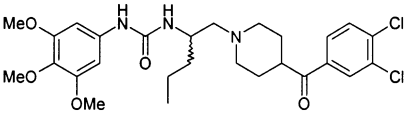
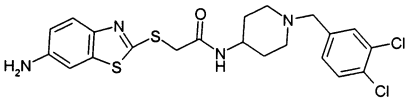
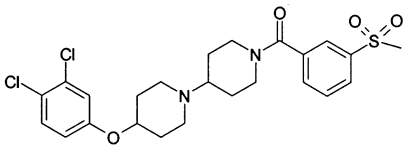
Table 3. CCR2 Antagonists

compound no.	structure	affinity	ref
9		$IC_{50} = 0.031\ \mu M$ vs MCP-1	64
10		hMCP-1, $IC_{50} < 50\ \mu M$ (R = OMe, CF_3)	65
11		$K_i = 5.3\ \mu M$	66
12		CCR2B, $K_i = 50$ nM	66
13		CCR2, $K_i = 40$ nM	67
14		CCR2B, $IC_{50} = 89$ nM	68
15		CCR2 antagonist (no data)	69

centrations in nasal lavage fluid. The levels of eotaxin in lavage samples showed a strong correlation with lavage levels of eosinophil counts and EPX. Normal subjects had few, if any, eosinophils and EPX as well as the measured parameters in their nasal lavage fluids before and after antigen challenge. In the experiments of eosinophil endothelial transmigration (TEM) assay using the nasal microvascular endothelial cells, eotaxin showed the most potent effect among various eosinophil chemoattractants. In addition, treatment of eosinophils with anti-CCR-3 mAb significantly blocked eosinophil TEM induced by homogenate of nasal mucosa.

The discovery of a series of phenylalanine-derived CCR3 antagonists was reported recently.[73] HTS using a fluorescence imaging plate reader (FLIPR) to track the intracellular calcium changes led to the identification of **16** as a modestly effective antagonist and an initial lead. In the binding assay using [^{125}I]human eotaxin as the radioligand and purified human eosinophils as the CCR3 source, **16** was found to have reasonable CCR3 affinity ($IC_{50} = 535$ nM) (Table 4). Parallel, solution-phase library synthesis was utilized to delineate the SAR, leading to the synthesis of a highly potent, freely reversible, CCR3-selective antagonist **17**,[73] with an IC_{50} value of 5 nM. Compound **17** effectively blocks calcium mobilization ($IC_{50} = 38$ nM) and chemotaxis mediated by eotaxin and MCP-4 with potency identical to that of its inhibition of eotaxin, indicating that it will block all CCR3 ligand interactions. However, the presence of a metabolically labile ester functionality precluded the evaluation of **17** in models of inflammatory disease in vivo. A more stable ester biostere was

Table 4. CCR3 Antagonists

compound no.	structure	affinity	ref
16		$IC_{50} = 0.535\ \mu M$	73
17		$IC_{50} = 5$ nM	73
18		$IC_{50} > 33\ \mu M$	74
19		$IC_{50} = 563$ nM	74
20		$IC_{50} = 5$ nM	74
21		$IC_{50} = 45$ nM	74
22		$IC_{50} = 0.21\ \mu M$	75
23		$IC_{50} = 13$ nM	76
24		$IC_{50} = 0.6-10\ \mu M$	77
25		$IC_{50} = 2.3$ nM	78
26		no data	79

investigated. Simple primary and secondary amides as ester replacements were unsuccessful and generally resulted in a significant loss of receptor binding affinity. The greatest loss of CCR3 affinity was observed upon replacing the ester group with simple heterocyclic alternatives (e.g., **18**),[74] except for dihydrooxazole **19**, which did retain some receptor affinity. Extension of the chain with the esters or amides was found to restore the affinity of some of the inactive templates. Further modification of the structure led to the discovery of compounds **20** and **21** as potent antagonists. In the functional assay, compound **20** was found to inhibit eosinophil chemotaxis with an IC_{50} value of 15 nM. It showed no effect on the C5a-induced eosinophil chemotaxis, indicating that compound **20** appears to be acting via CCR3 antagonism. Compounds **23**[76] and **24**[77] were also disclosed as CCR3 antagonists with various degrees of affinity.

Screening of a focused library of 770 carboxamides led to the discovery of compound **25**[78] as a potent CCR3 antagonist. This library was derived from 70 different commercially available carboxylic acids selected on the basis of the binary Tanimoto coeffcient calculated by the MDL keys as a structural descriptor, and 11 structurally diverse diamines. Compound **25** had an IC_{50} value of 2.3 nM for CCR3 and 1900 nM for CCR1. Compound **25** also showed potent functional antagonism activity for inhibiting eotaxin (IC_{50} = 27 nM) or RANTES (IC_{50} = 13 nM)-induced Ca^{2+} increases in eosinophils. Compound **26**[79] was tested in an osteoarthritis assay for Ca^{2+} flux, human eosinophil chemotaxis, and H1 antagonism.

5. CCR5 Receptor Antagonists

The identification of CCR5 and CXCR4 as coreceptors with CD4 for HIV-1 and other lentiviruses has spurred a flurry of activity to discover potent antagonists in an effort to validate this mechanism of viral entry as a viable therapeutic target. In addition, there has been an explosion of research in the chemokine area in general, with several studies indicating that a potent CCR5 antagonist might also be effective in the clinical treatment of other disorders, such as rheumatoid arthritis,[80] asthma,[81] and multiple sclerosis.[82−83] The observation that the chemokines MIP-1α, MIP-1β, and RANTES inhibit the infection of $CD4^+$ T-cells by primary non-syncytium-inducing (NSI) strains of HIV-1 led in part to the discovery that the principal cofactor for the entry of macrophage-tropic strains of HIV-1 into monocytes, macrophages, and primary T-cells is the chemokine receptor CCR5. The fact that a small subset of the human population possesses a mutant allele for CCR5 which has a 32-base-pair deletion in its coding region that results in a nonfunctional receptor implies that CCR5 is an excellent target for therapeutic intervention in HIV. $CD4^+$ T-cells isolated from this population do not support the fusion of NSI strains of HIV, and it appears that the presence of the mutant CCR5 allele is a primary genetic factor in the resistance to HIV infection

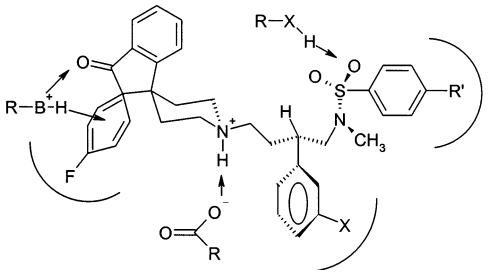


Figure 1. Pharmacophore model for CCR5.

observed in multiply exposed, uninfected individuals.[84]

To date, there have been many reports of CCR5 antagonists in the literature. Merck researchers have carried out an extensive screening program using a high-throughput assay of [^{125}I]-MIP-1α to obtain stable expression of human CCR5 receptors in Chinese hamster ovary cells, which led to the discovery of compound **27**[85] as a possible lead structure. This structural type has some interesting characteristics. Both the corresponding sulfone (IC_{50} = 100 nM) and subsequent sulfide analogues (IC_{50} = 1000 nM) were significantly less active (Table 5). The analogous amides were essentially inactive. Extensive chemical modification around the SAR led to the optimized lead structure **28**,[86] which has an IC_{50} value of 10 nM. Compound **28** also was demonstrated to have good selectivity. The IC_{50}'s for CCR1, CCR2, CCR3, and CXCR4 were all >10 μM. It was hypothesized that the spiropiperidine was not required for activity and that a simple 4-phenylpiperidine might have similar binding while being a much simpler structure. This modification led to the discovery of compound **29**.[86] In an isolated peripheral mononuclear cell (PBMC) viral replication assay using the R5-tropic HIV-1 YU-2 isolate, compound **28** gave an IC_{95} value as low as 1500 nM, while the initial lead compound **27** had an IC_{95} value of 6−12 μM in the same assay. Compound **29** showed an IC_{95} value of 6000 nM, and compound **28** displayed modest pharmacokinetics in the rat at 1 mg/kg iv and 10 mg/kg oral ($t_{1/2}$ = 0.7 and 0.8 h, F = 3%).

The spiropiperidine and 4-phenylpiperidine series were further explored and optimized to provide compound **30**,[87] with an IC_{50} value of 5 nM. As a result of these investigations, a CCR5 pharmacophore model[87] was proposed as shown in Figure 1.

Further SAR studies led to the discovery of a new class of non-spiropiperidine CCR5 antagonists containing a 4-(*N*-alkyl-*N*-(alkoxycarbonyl)amino)piperidine moiety, as represented by compound **32**,[89] which had an IC_{50} of 0.75 nM. In the PBMC cell-based assay, compound **32** had an IC_{95} of 250 nM. The corresponding amide analogue had an IC_{95} of 50 nM, the best efficacy in the PBMC assay in the series. Another modification was attempted in which constrained analogues of compound **30** were made, leading to pyrrolidine **31**.[88] Incorporation of the favorable pharmacophore elements from the acyclic compounds into the pyrrolidines resulted in analogue **33**,[90] with an IC_{50} value of 0.8 ± 0.5 nM. Compound **33** had an IC_{95} value of 31 nM, *a* V_{dss} of 1.7 L/kg, a $t_{1/2}$ of 1.2 h, and bioavailability of 39% in the rat. The

Table 5. CCR5 Antagonists

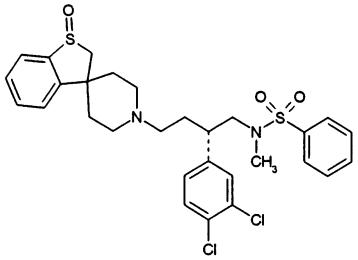
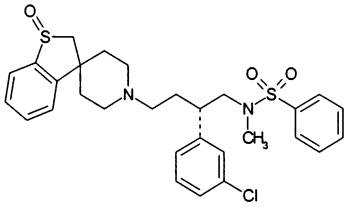
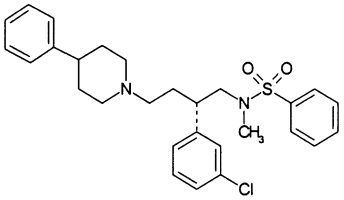
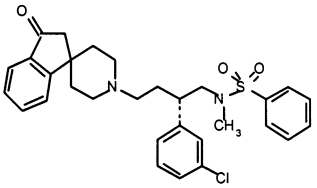
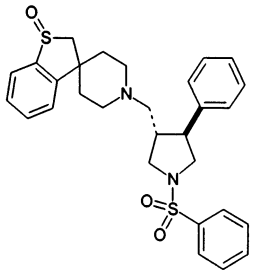
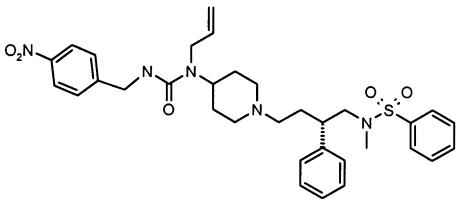
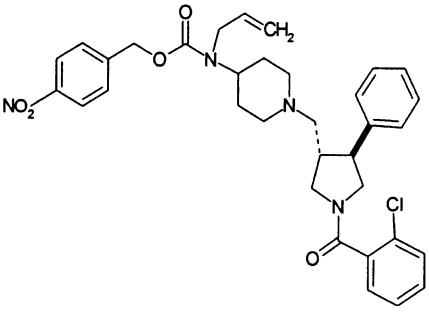
compound no.	structure	affinity	ref
27		CCR5, IC_{50} = 35 nM	85
28		CCR5, IC_{50} = 10 nM CCR1, 2, 3, 4, CXCR4, IC_{50} > 10 μM	86
29		IC_{50} = 30 nM	86
30		IC_{50} = 5 nM	87
31		IC_{50} = 26 nM	88
32		IC_{50} = 0.75 nM	89
33		IC_{50} = 0.8 nM	90

Table 5 (Continued)

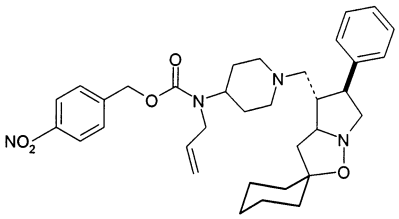
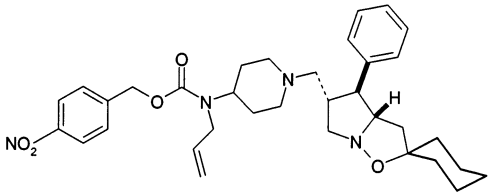
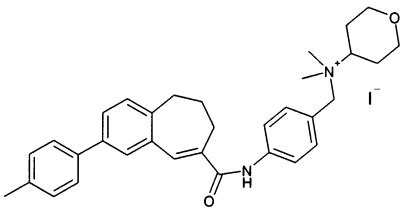
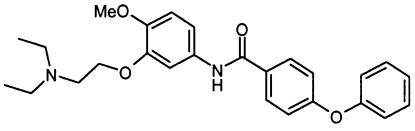
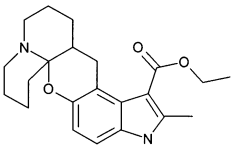
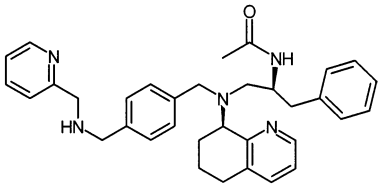
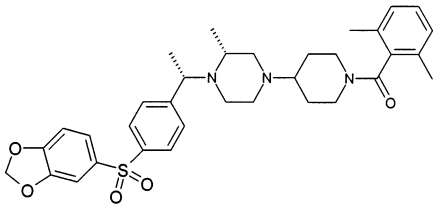
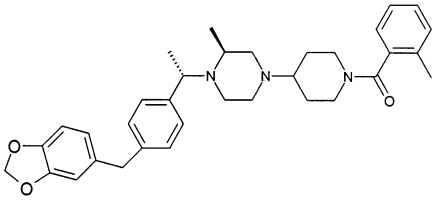
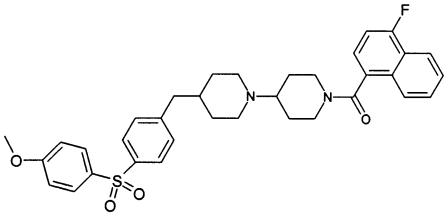
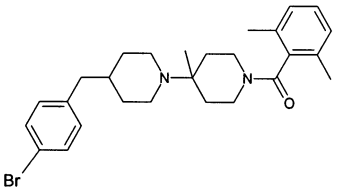
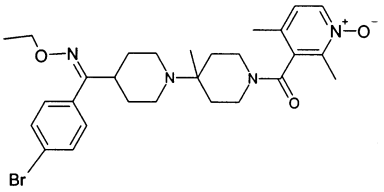
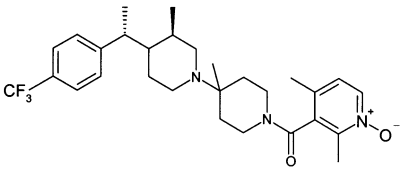
compound no.	structure	affinity	ref
34		$IC_{50} = 14 \pm 2$ nM	91
35		$IC_{50} = 0.13 \pm 0.08$ nM	91
36	 TAK-779	$IC_{50} = 1.4$ nM	92
37		$IC_{50} = 0.1$ nM	94
38		no data	95
39		$EC_{50} < 20\mu$g/mL	96
40		$IC_{50} = 440$ nM (vs RANTES) $K_i = 0.8$ nM (vs M2)	97
41		$IC_{50} = 90$ nM (vs RANTES) $K_i = 0.35$ nM (vs M2)	97
42		$K_i = 1.0\ \mu$M $K_i = 1.3$ nM (vs M2)	97

Table 5 (Continued)

compound no.	structure	affinity	ref
43		$K_i = 66$ nM $IC_{50} = 10$ nM rat PK (10 mg/kg, po), $AUC_{0-6h} = 0.04$ h·mg/mL	98
44		$K_i = 2.1$ nM $IC_{50} = 0.6$ nM rat PK (10 mg/kg, po), $AUC_{0-6h} = 6.5$ h·mg/mL	98
45		$K_i = 7$ nM $IC_{50} = 1.0$ nM rat PK (10 mg/kg, po), $AUC_{0-6h} = 9.243$ h·mg/mL	99

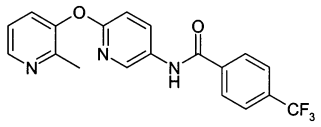
conformational preference about the C−N bond was explored by preparing more rigid analogues. The pyrrolidine in compound **33** was replaced with a bicyclic isoxazolidine, generating the desired compounds **34** and **35**.[91] This constraint mimicked the bioactive rotamer of the pyrrolidine and provided insight for a required feature for potency. The fact that compound **35** is much more potent than compound **34** clearly indicates a rotamer preference. Compound **35** was further characterized in a cellular microphysiometer assay using CHO cells expressing human CCR5 receptors. Neither compound **35** nor its close analogue elicited agonist activity, as demonstrated by the lack of change in the extracellular pH in comparison to the agonist control (MIP-1α). These results, coupled with the binding data, indicate that these compounds are true antagonists of the CCR5 receptors. It is interesting to note that although compound **35** has better receptor affinity than compound **33**, the antiviral activity of **35** is an order of magnitude less potent when compared to **33**. This suggests that blocking MIP-1α binding might not completely impart antiviral activity, which may require different interactions for blocking HIV in comparison with the chemokines.

TAK-779 (**36**)[92] is an antagonist of the CCR5 chemokine receptor with an IC_{50} of 1.4 nM. TAK-779 is under investigation by Takeda and Kagoshima University for the potential treatment of HIV. Reports on the pharmacokinetics of TAK-779 showed that the human plasma protein binding of TAK-779 was 97−98%, and its anti-HIV activity in the presence of 50% human serum was one-seventh the activity in the presence of 10% fetal calf serum. When TAK-779 was administered to rats at a dose of 1 mg/kg iv, its plasma half-life and AUC were 8.7 h and 3.5 mg·h/mL, respectively. The plasma half-life and AUC in lymph nodes were 22.9 h and 18.3 mg·h/mL, respectively. Preclinical data on TAK-779 were also reported. In cultures of HIV-1-infected macrophages, TAK-779 inhibited syncytium formation at concentrations of 1 μg/mL and inhibited replication by as much as 99% at 2 μg/mL. The compound also inhibited MIP-1β-stimulated calcium signaling in both monocytes and CCR5-transfected cells.[93]

Some other structure types, exemplified by compounds **37**[94] and **38**,[95] were also disclosed as CCR5 antagonists. Compound **39**[96] was claimed to have protective effects against infection by HIV through binding to chemokine receptors, including CXCR4 and CCR5, thus inhibiting the subsequent binding of their natural ligands. The compound demonstrated inhibition of HIV-1 NL4.3 or IIIB replication in MT-4 cells with an EC_{50} value of less than 20 μg/mL.

Three assays were used to evaluate compounds in Schering Plough's anti-HIV program. The primary assay used for the initial HTS measured the ability of compounds to inhibit [^{125}I]-labeled RANTES binding to the CCR5 receptor on membranes. Selected compounds were then evaluated in a viral entry assay, in which a pseudo-type virus bearing a receptor gene for luciferase was used to infect cells expressing CD4 and CCR5. Finally, antiviral activity was measured as the ability of compounds to inhibit the growth of primary HIV-1 isolates in human peripheral blood mononuclear cells.[97] Using these assays, several structural types found in their compound library inhibited the RANTES−CCR5 interaction. Further evaluation for inhibition of viral entry at sub-cytotoxic levels revealed a series of compounds exemplified by compounds **40** and **41**,[96] which were originally prepared for a muscarinic receptor antagonist program. Subsequent SAR investigations using the knowledge gained from concurrent efforts in a related piperazino−piperidine amide series yielded the low-molecular-weight, early lead compound **43** (CCR5, $K_i = 66$ nM; M2, $K_i = 1323$ nM).[98] Investigation of this series improved not only the CCR5 binding but also the selectivity with respect to the M2 muscarinic receptor. SAR development of oximino−piperidino−piperidine amides derived from the lead compound **43** led to the discovery of the

Table 6. Examples of CCR10 Antagonists

compound no.	structure	activity	ref
46		$IC_{50} < 5\ \mu M$	103

clinical candidate SCH 351125 (**44**),[97] a potent and orally bio-available CCR5 antagonist. This compound entered Phase I trials in 2001. Researchers at Schering Plough identified several second-generation compounds, such as compound **45** (SCH 350634)[99] to replace SCH-351125, as Phase I trials were suspended in April 2001. Detailed pharmacokinetic evaluation in beagle dogs and cynomolgus monkeys clearly demonstrated that compound **45** (amorphous HCl salt) is capable of achieving and sustaining high blood levels following oral administration. The major route of excretion is through the urine in rats and through the bile in dogs and in monkeys. The major metabolite is a result of oxidative cleavage of the CH_3-$CHCH_2$ region of the chiral piperazine. Reduction of the *N*-oxide (**45**) back to the nicotinamide was not observed. Compound **45** shows 30−50-fold selectivity for CCR5 over the M_1 (K_i = 350 nM) and the M_2 (K_i = 250 nM) muscarinic receptors. It has no appreciable affinity for other related receptors of current interest. There is neither inhibition nor induction of the liver enzymes with this compound. In the PBMC-based assay, compound **45** inhibited the replication of HIV-1 isolates with IC_{50} values in the 2−20 nM range. The cytotoxicity of **45** is >40 μM in PBMC cultures using MTS cell titer 96 protocol. In rats, mean blood pressure, heart rate, and ECG were clean after oral administration of this compound up to a dose of 30 mg/kg, and there were no negative effects on CNS-related behavior at the lowest tested dose of 3 mg/kg. At higher doses, some effects were noted in the gastrointestinal emptying and transit experiments, indicating its effect on muscarinic receptors.[100]

6. CCR10 Receptor Antagonists

Chemokine receptor CCR10 is a known receptor for ligands CTACK, which is primarily expressed in skin by keratinocytes, and CCL28, which is expressed in epithelial cells and a wide variety of other tissues.[101] Together they have been found to be important homing molecules for T-cell migration to the skin. Inhibition of CTACK suppresses some skin-inflammatory responses, which suggests that drugs targeting CCR10 could be used to treat human skin discorders.[102] It is suspected that at least one of the CCR10 or CCR4 pathways must be functional for T-cell recruitment, and therefore an antagonist that blocks both CCR10 and CCR4 might be the most effective means to investigate delayed-type hypersensitivity reactions in skin. Most skin-infiltrating lymphocytes in patients suffering from psoriasis and atopic or allergic-contact dermatitis express CCR10. This could mean that several inflammatory skin diseases such as psoriasis and contact dermatitis would be suitable for CTACK/CCR10 targeted therapy. There have been very few reports on small molecules that target CCR10. Also, there is no evidence that validation of a chemokine receptor model in the mouse will hold true for humans.

Scientists at Millenium Pharmaceuticals have filed a patent on a series of substituted N-heterocyclyl benzamides as G-protein-coupled heptahelical receptor binding compounds.[103] The described compounds were prepared for the treatment of disorders ranging from neurological, immunological, inflammatory, cancer, and other chemokine-related disorders. In a time-resolved fluorescence (TRF) assay, compound **46** (Table 6) showed high binding affinity for the CCR10 receptor, with an IC_{50} of <5 μM.

7. CXCR1/CXCR2 Receptor Antagonists

CXCR1 and CXCR2 were the first chemokine receptors to be cloned in 1990[104,105] for interleukin-8 (IL-8), a potent pro-inflammatory CXC chemokine originally characterized as a neutrophil chemoattractant that has been identified in the molecular pathway of a wide range of diseases, including RA, osteoarthritis, acute respiratory distress syndrome (ARDS),[106,107] chronic obstructive pulmonary disease (COPD),[108,109] and promotion of tumor growth and metastasis.[110] IL-8 is found in numerous cell types, among them neutrophils, eosinophils, basophils, and peripheral blood T lymphocytes.[111−113] Neutrophils migrate to the lung in response to various chemoattractants, such as CXC chemokine IL-8. These chemotactic agents interact with cell surface chemokines receptors, such as CXCR1 and CXCR2 on neutrophils. In neutrophils, when the receptor is occupied, intracellular calcium flux and pro-inflammatory responses occur.[114,115] The role of the receptor in the mechanism of these diseases is still unknown. CXCR1 serves as a receptor for both IL-8 and granulcyte chemotactic protein-2 (GCP-2). CXCR2 not only binds IL-8 and GCP-2 but also is associated with a number of other Glu-Leu-Arg proteins. The ligands of CXCR2 include IL-8, KC, MIP2, and GRO-α. There is still direct evidence for a functional role for CXCR2 on astrocytes (non-neuronal cells of the brain and spinal cord). CXCR1 selectively binds IL-8 but does not bind the GRO-related proteins, and there is no report of CXCR1 expression in the CNS. Although small-molecule CXCR1 and CXCR2 antagonists could have potential therapeutic value, relatively few have been reported in the literature.

The small-molecule CXCR2 antagonist, SKF83589 (Figure 2), a 500 nM hit (IC_{50} for inhibition of IL-8 binding to CXCR2), was identified by researchers at GlaxoSmithKline using HTS. Chemical optimization of this hit using combinatorial techniques, to randomly place substituents on both of the aromatic

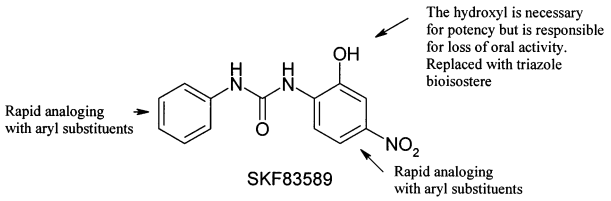


Figure 2.

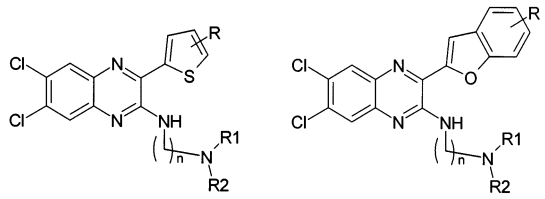


Figure 3.

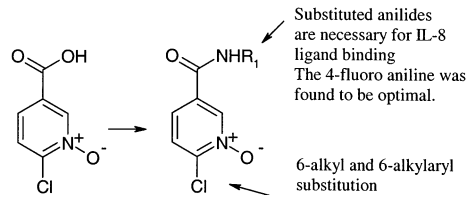


Figure 4.

rings simultaneously and independently to improve binding affinity, led to compound **47** (SB 225002) (Table 7). Compound **47** inhibited IL-8 binding to CHO−CXCR2 transfected membranes with an affinity of 22 nM (IC_{50} of IL-8-CXCR2) and was found to be selective by 150-fold against CXCR1 and several other GPCRs. This selectivity was attributed to its lack of activity (IC_{50} > 10 μM) in inhibiting IL-8-mediated Ca^{2+} flux in human neutrophils, since this cell type expresses both CXCR1 and CXCR2 receptors. Although selective binding and high affinity were observed, the compound lacked oral activity in animal models and had poor PK properties (it was rapidly cleared at 60−120 mL kg^{-1} min^{-1}).[116,117] The high clearance was attributed to the phenolic hydroxyl which was found to undergo glucuronidation during metabolism. Since the phenolic hydroxyl group in its unprotected state was found to be necessary for activity, a bioisostere replacement had to be designed into the molecule that would maintain the potency while improving the ADME and PK properties. This was accomplished by incorporating a triazole ring at the 2,3-position in place of the 2-hydroxyl group of the aromatic ring. Systematic SAR evaluation led to the synthesis of *N*-(3-alkyl-4-cyano-2-hydroxyphenyl)-*N*′-phenyl ureas using a Claisen rearrangement to install the 3-substitutent. Compound **48** (SB265610) is potent, with an IC_{50} of 39 nM, a good serum half-life (146 min/290 min), oral bio-availability, and in vivo activity in neutrophil animal models.[109] Compound **48** was found to be orally active in rabbit airway inflammation and a mouse atherosclerosis model.[118] The analogue with chloride replacing bromide was less active (IC_{50} = 90 nM). Compound **48** was found to have good in vivo activity as well. In the rabbit model of LPS-induced neutrophilia, inhibitor **48** had an ED_{50} of 10 mg/kg. At high dose (100 mg kg^{-1} day^{-1}), the compound was found to reduce atherosclerotic lesions by 40% in LDL receptor-deficient mice, with oral bioavailability of 71% in rat and 25% in rabbit. In vitro and in vivo pharmacology of SB 265610 indicates that it may be useful for defining the role of neutrophils and CXCR2 in lung inflammation.

The latest patent activity on theses compounds prepared by SKB show patent applications for 3-sulfonamido-substituted diphenyl ureas related to compound **47**.[116]

Table 7 summarizes some examples of the CXCR2 receptor (ligands) antagonists reported in the literature recently. Researchers at Pfizer reported a series of 2-((alkylaminoalkyl)amino)-3-aryl-6,7-dichloroquinoxalines compounds (Figure 3) that are claimed as selective antagonists of IL-8 binding and neutrophil chemotaxis, with IC_{50}'s of 100−400 nM.[120,121] Chemical optimization led to the synthesis and identification of compound **49** as a selective antagonist of IL-8 binding (IC_{50} = 110 nM) and potent IC_{50} inhibitor of neutrophil chemotaxis (IC_{50} = 170 nM).[124]

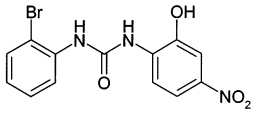
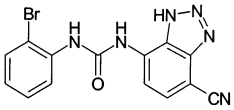
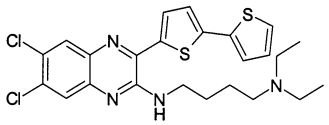
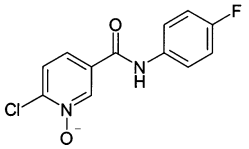
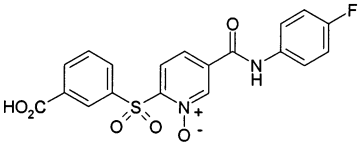
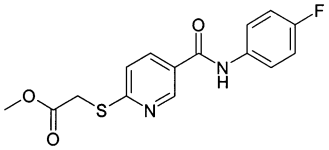
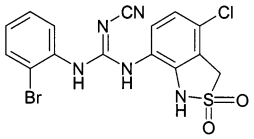
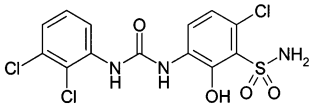
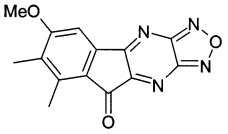
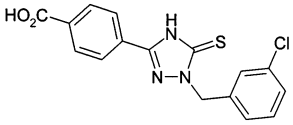
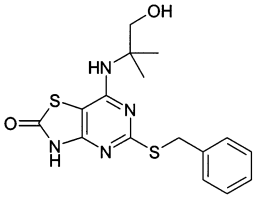
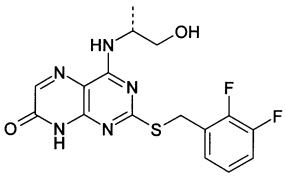
A series of nicotinamide *N*-oxides[122] and a related class of compounds, 6-substituted nicotinanilides,[123] were identified by researchers at Celltech as potent and selective inhibitors of GRO-α-driven human neutrophil chemotaxis. Compounds of this class could be useful for the treatment of acute inflammatory, auto-immune, and allergic disorders associated with neutrophil infiltration. Initially the series prepared were 6-chloronicotinamide *N*-oxides (Figure 4). This evolved to a series of 6-sulfonyl-1-oxy-nicotinamides as part of the lead optimization strategy as the compounds were screened for their ability to antagonize the binding of IL-8 to CXCR2 and also block GRO-α-driven human neutrophil chemotaxis.

Structure optimization for SAR focused on the amide modification in the 3-position of the pyridine. It was found that several substituted anilides blocked GRO-α-driven neutrophil chemotaxis in the low micromolar activity range. When the other amides where prepared and tested, the compounds had significantly decreased activity.

As a result of this optimization strategy, the 4-fluoroanilide **50** was found to possess the necessary recognition element for blocking the IL-8/CXCR2 interaction.[122] Continued SAR studies revealed a disparity between the ligand binding affinity (IL-8 binding activity) and the functional activity (chemotaxis assay) when substituting various anilines. Although a number of substituted anilines blocked GRO-α-driven neutrophil chemotaxis, only the 4-fluoroanilide was found to give acceptable activity in the IL-8 ligand binding assay. It is theorized that there is a possibility of multiple binding domains for small molecule−ligand contact with CXCR2 that could independently block high-affinity binding or receptor activation. It is proposed that the CXCR2 amino acid residues required for cell activation are not necessarily the same residues required for ligand binding.[125] Divergence in the regions of the ligand contact on CXCR2 for binding and cell activation allow distinct antagonist to independently block one or the other or both.[126]

To complement the SAR around the 3-position, modification of the 6-position of the *N*-oxide scaffold

Table 7. Examples of CXCR1/CXCR2 Antagonists

compound no.	structure	affinity	ref
47		SB225002 CXCR2, IC_{50} = 22 nM CL = 60/120 mL kg^{-1} min^{-1}	116, 117
48		SB265610 CXCR2, IC_{50} = 39 nM CL = 12/24 mL kg^{-1} min^{-1} $T_{1/2}$ = 146 min/290 min	119
49		PD 0220245 IC_{50} = 110 nM (vs IL-8) neutrophil chemotaxis IC_{50} = 170 nM	120, 121, 124
50		GRO-α chemotaxis IC_{50} = 1.1 μM IC_{50} = 1.0 μM (vs IL-8)	122
51		inhibition of chemotaxis IC_{50} > 20 μM IC_{50} = 32 nM (vs IL-8)	122
52		inhibition of chemotaxis IC_{50} = 42 nM	123
53		no data	127
54		IC_{50} < 30 nM	128
55		IC_{50} = 0.05−12 μM	129
56		IC_{50} < 10 μM (vs h[125I]IL-8)	130
57		CXCR2 antagonists in human neutrophils at μM levels	131
58		antagonists of CXCR2 receptor in human neutrophils at μM levels	132

was also explored. A diverse set of 6-alkyl- and alkylaryl-substituted nicotinamide *N*-oxide sulfones were prepared and found to provide potent analogues in the ligand binding assay. Compound **51** was the most potent in IL-8 ligand binding but was functionally inactive. It is speculated that this compound may

interact at sites on CXCR2 that specifically disrupt IL-8 binding or may bind at a subsite on CXCR2 not be required for GRO-α-stimulated receptor activation. Although these compounds are effective CXCR2 antagonists, they were also found to inhibit CXCR1 to a lesser extent. This suggests that these inhibitors are in fact interacting with CXCR2 rather than binding to IL-8. Additional evaluation of these compounds in in vitro functional and ligand binding assays continues along with pharmacological profiling.

In further investigation of the SAR around substitution on the 6-position of the nicotinanilide series, the Celltech group found they could optimize the GRO-α chemotaxis activity by incorporating a thioglycolate ester pharmacophore as represented by compound **52**. Extension of the methylene unit of the thioglycolate functionality by even one carbon resulted in a significant drop in potency. Compound **52** was found to be a selective inhibitor for CXCR2 against GRO-α-induced calcium flux in human neutrophils. The free acid and subsequent amide analogues were inactive. This may be due to the facts that they may be less able to pass through the cell membrane and there is increased enzymatic stability toward hydrolysis in the cell that could result in lower intracellular concentrations. In the case of the active analogues, the ester may act as a prodrug until hydrolyzed in the cell. Finally, it may be that they just have a reduced affinity for the CXCR2 receptor.

To avoid the liability of sulfur oxidation during metabolism, the oxygen isosteres were also prepared. These analogues retained sub-micromolar potency and were also selective for GRO-α.

SmithKline Beecham reported a series of benzoisothiazoline *S*,*S*-dioxide derivatives[127] related to compound **53** and hydroxydiphenylurea sulfonamides related to compound **54** as IL-8 receptor antagonists.[128] These compounds are described as being active in chemokine-mediated diseases related to IL-8, GROα, ENA-78, and NAP involved in chemotaxis, neutrophil granule release, and respiratory burst. Compound **54** exhibited activity against IL-8 and GRO-α in receptor binding assays, with $IC_{50} < 30$ nM.

A group at Warner Lambert reported a series of diazafluorenones **55** as IL-8 receptor antagonists with activity of $IC_{50} = 0.05-12$ μM for IL-8.[129]

Astrazeneca has a great effort toward research for CXCR2 antagonists. They describe the preparation of 1*H*-[1,2,4]triazole-3-thiones **56** as chemokine receptor antagonists for treatment of psoriasis, angiogenesis, and COPD attributed to high CXCR2 levels.[130] A group in the UK have patented novel thiazolo[4,5-*d*]pyrimidines, represented by compound **57**, as modulators of chemokine receptors, particularly antagonists of the CXCR2 receptor in human neutrophils.[131] The same group also investigated pteridine compounds, as represented by compound **58**, for the treatment of psoriasis mediated by CXCR2. These compounds were also found to be antagonists of the CXCR2 receptor in human neutrophils.[132]

8. CXCR4 Receptor Antagonists

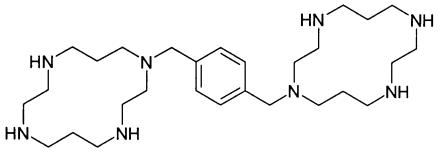
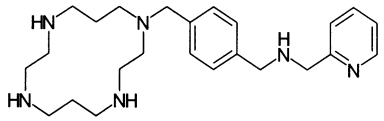
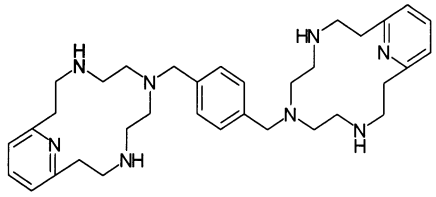
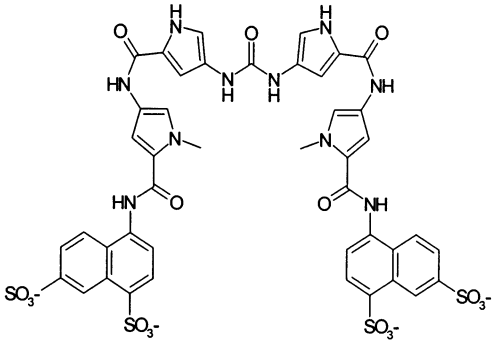
CXCR4, along with CCR5, which is a key co-receptor for HIV-1, represents a new target class for drug design for an anti-HIV agent.[133] Since strains of HIV have shown drug resistance to protease or reverse transcriptase for HIV, this new target class is of great interest.[134]

Researchers at Johnson Matthey (AnorMED Inc.) have developed JM3100 (AMD-3100), **59** (Table 8), which has been shown to block this receptor.[135−138] These phenylenebis(methylene)-linked bis-azamacrocycles have been shown to inhibit HIV-1 and HIV-2 replication by antagonism of the CXCR4 chemokine receptor.[137] A series of analogues were synthesized which contain a neutral heteroatom (oxygen, sulfur) or heteroatom of lower pK_a than a secondary amine (Figure 5) as replacements for the amino groups of AMD 3100. These analogues were less active against HIV-1 and HIV-2. The bis-sulfur analogue also showed cytotoxicity to MT-4 cells. Introduction of a single pyridine into the macrocyclic scaffold gave a compound with activity comparable to that of the saturated aliphatic counterpart but without the MT-4 cell toxoicity.[137] Replacing the pyridine with a pyrazine or a pyridine with electron-withdrawing or -donating groups substituted in the 4-position either reduced their antiviral potency or increased MT-4 cell toxicity. Inhibitor **59**, although potent, lacked oral bio-availability in humans.[139] Therefore, researchers at AnorMed chemically optimized this structure to afford a series of bis-tetraazamacrocycles, as represented by inhibitor **60**, which was demonstrated to be 50% orally bio-available in rats and rabbits.[140] Due to cardiovascular side effects (antagonism of the CXCR4-mediated receptor activity on atrial smooth muscle), clinical development of compound **60** was abandoned.

Finally, a series of macrocyclic analogues were synthesized incorporating a pyridine in both macrocyclic rings and varying ring size from 12 to 16 members per ring to give a *p*-phenylene-bis(methylene-linked) dimer. As a result, lead compound **61** (AMD 3329) was prepared which displayed the best antiviral activity, with EC_{50}'s against HIV-1 and HIV-2 replication of 0.8 and 1.6 nM, respectively, 3-fold better than those of AMD 3100. Compound **61** also inhibited binding of mAb 12G5 to CXCR4 and Ca^{2+} flux induced by SDF-1α, a CXC-chemokine and the natural ligand for CXCR4.[136] It showed no effect on binding of monoclonal antibody 2D7 to CCR5. In addition to a possible treatment for HIV, it is speculated that CXCR4 inhibitors could have a use in the treatment of arthritis and also prove to be an effective treatment for cancer. AnorMed has been conducting studies in both arthritis and cancer models over the last year.[141−142]

An analogue of Distamycin, **62**, was found to inhibit, in vitro and in vivo, HIV replication in a murine model by acting on a chemokine receptor which acts as a co-receptor for HIV-1 entry into human cells.[143] Inhibition was reported to be attributed to specific competition for the ligand receptor sites in CCR5, CCR3, CCR1, and CXCR4 but not CXCR2 or CCR2b and blocked chemokine-induced

Table 8. Examples of CXCR4 Antagonists

compound no.	structure	affinity	ref
59		AMD-3100 $IC_{50} = 0.031\ \mu M$ (vs MCP-1)	136
60		AMD-8664 $IC_{50} < 50\ \mu M$ (vs hMCP-1)	140
61		AMD-3329 HIV-1 and HIV-2 replication $EC_{50} = 0.8, 1.6$ nM	137
62		NSC 651016 inhibition of HIV-1 replication at 88% at 200 μM HIV-1, $EC_{50} = 5.5\ \mu M$ HIV-2, $EC_{50} = 13.9\ \mu M$	143

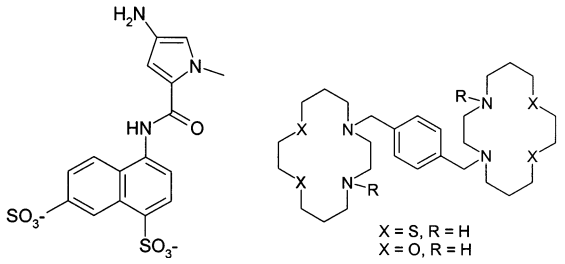


Figure 5.

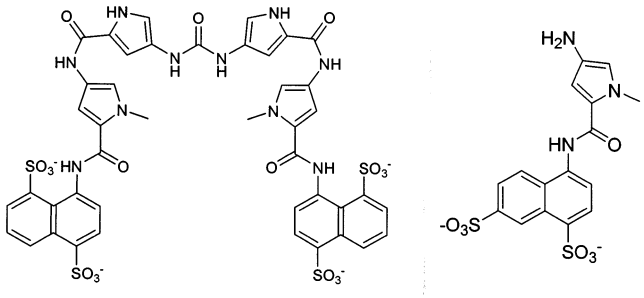


Figure 6.

calcium flux. The monomeric form of compound **62**, NSC 655720, was not active, as well as analogue NSC 645795, which has a similar structure and charge (Figure 6). Further chemical optimization of **62** is said to be continuing for evaluation as a treatment of HIV-1 infection and as a microbicide to prevent the sexual transmission of HIV-1.

9. Future Perspectives and Conclusions

The study of chemokine receptors and their ligands is a rapidly growing and intense area of research in drug discovery. There is now extensive evidence for the involvement of chemokines in many disease pathways, and therefore continued research to discover small-molecule chemokine receptor antagonists for these targets is warranted.

To date, there has been significant progress in the identification of small-molecule nanomolar chemokine antagonists that are bioavailable. It is extremely encouraging that this class of GPCRs can be antagonized with low-molecular-weight small organic compounds, although it appears that some chemokines such as CXCR2 are more amiable to the discovery of selective antagonists than others, such as CXCR1 and CXCR3. This could be due to inadequate screens that are usually based only on binding inhibition.

However, the jury is still out on whether there are any real potential therapeutic advantages of molecules which act on chemokine receptors. Clinical efficacy still remains to be demonstrated for these chemokine receptor antagonists. It will be in the clinic that our understanding of chemokine biology will be challenged. Important questions, such as predictions for pharmacology (based on cell response, tissue distribution, and knockout mice) and the relevance of redundancy in the system, still remain to be answered. It is almost certain that there will be some unexpected observations and results. The clinical data will definitely provide the critical information for better understanding of chemokine biol-

ogy, which will then better define the selective requirements for the different chemokines in the subfamily. The new generation of the chemokine receptor antagonists based on these modified models and selectivity profiles may provide the future drugs for many diseases.

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11. References

(1) Zlotnik, A.; Yoshie, O. *Immunity* **2000**, *12*, 121.
(2) Onuffer, J. J., Horuk, R. *Trends Pharmcol. Sci.* **2002**, *23*, 459.
(3) International Union of Immunological Society/World Health Organization. Subcommittee on Chemokine Nomenclature. 2001 Chemokine/chemokine receptor nomenclature. *J. Leukocyte Biol.* **2001**, *70*, 465.
(4) http://cytokine.medic.kumamoto-u.ac.jp/CFC/CK/Chemokine.html
(5) http://www.cmbi.kun.nl/7tm/multali.html
(6) Olson, T. S.; Ley, K. *Am. J. Physiol.: Regul. Integr. Comp. Physiol.* **2001**, *283*, R7.
(7) Luster, A. D. *New Engl. J. Med.* **1998**, *338*, 436.
(8) Loetscher, P.; Clark-Lewis, I. *J. Leukocyte Biol.* **2001**, *69*, 881.
(9) Flower, D. R. *Biochim. Biophys. Acta* **1999**, *1422*, 207.
(10) Page, R. D. *Comput. Appl. Biosci.* **1996**, *12*, 357.
(11) Gether, U. *Endocr. Rev.* **2000**, *21*, 90.
(12) Ji, T. H.; Grossmann, M.; Ji, I. *J. Biol. Chem.* **1998**, *273*, 17299.
(13) Govaerts, C.; Blanpain, C.; Deupi, X.; Ballet, S.; Ballesteros, J. A.; Wodak, S. J.; Vassart, G.; Pardo, L.; Parmentier, M. *J. Biol. Chem.* **2001**, *276*, 13217.
(14) Govaerts, C.; Bondue, A.; Springael, J.-Y.; Olivella, M.; Deupi, X.; Poul, E. L.; Wodak, S. J.; Parmentier, M.; Pardo, L.; Blanpain, C. *J. Biol. Chem.* **2003**, *278*, 1892.
(15) Glass, W. G.; Chen, B. P.; Liu, M. T.; Lane, T. E. *Viral Immunol.* **2002**, *15*, 261
(16) Christopherson, K., II; Hromas, R.; et al. *Stem Cells* **2001**, *19*, 388.
(17) Cyster, J. G. *Science* **1999**, *286*, 2098.
(18) Horuk, R. *Cytokine Growth Factor Rev.* **2001**, *12*, 313.
(19) Broxmeyer, H. E. *Int. J. Hematol.* **2001**, *74*, 9.
(20) Mohle, R.; Bautz, F.; Denzlinger, C.; Kanz. L. *Ann. N.Y. Acad. Sci.* **2001**, *938*, 26.
(21) Youn, B. S.; Mantel, C.; Broxmeyer, H. E. *Immunol. Rev.* **2000**, *177*, 150.
(22) Marra, F. *Front. Biosci.* **2002**, *7*, d1899.
(23) Gillitzer, R.; Goebeler, M. *J. Leukocyte Biol.* **2001**, *69*, 513.
(24) De Groot, C. J.; Woodroofe, M. N. *Prog. Brain Res.* **2001**, *132*, 533.
(25) Bajetto, A.; Bonavia, R.; Barbero, S.; Florio, T.; Schettini, G. *Front. Neuroendocrinol.* **2001**, *22*, 147.
(26) Matsui, T.; Akahoshi, T.; Namai, R.; Hashimoto, A.; Kurihara, Y.; Rana, M.; Nishimura, A.; Endo, H.; Kitasato, H.; Kawai, S.; Takagishi, K.; Kondo, H. *Clin. Exp. Immunol.* **2001**, *125*, 155.
(27) Yuan, G. H.; Masuko-Hongo, K.; Sakata, M.; Tsuruha, J.; Onuma, H.; Nakamura, H.; Aoki, H.; Kato, T.; Nishioka, K. *Arthritis Rheumatism* **2001** *44*, 1056.
(28) Van Der Voorn, P.; Tekstra, J.; Beelen, R. H. J.; Tensen, C. P.; Van Der Valk, P.; De Groot, C. J. A. *Am. J. Pathol.* **1999**, *154*, 45.
(29) Sorensen, T. L.; Tani, M.; Jensen, J.; Pierce, V.; Lucchinetti, C.; Folcik, V. A.; Qin, S.; Rottman, J.; Sellebjerg, F.; Strieter, R. M.; Frederiksen, J. L.; Ransohoff, R. M. *J. Clin. Invest.* **1999**, *103*, 807.
(30) Balashov, K. E.; Rottman, J. B.; Weiner, H. L.; Hancock, W. W. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 6873.
(31) Wang G.; O, K. *Biochem. J.* **2001**. *357*, 233.
(32) Nakatani, T.; Kaburagi, Y.; Shimada, Y.; Inaoki, M.; Takehara, K.; Mukaida, N.; Sato, S. *J. Allergy Clin. Immunol.* **2001**, *107*, 353.
(33) Abi-Younes, S.; Si-Tahar, M.; Luster, A. D. *Thrombosis Res.* **2001**, *101*, 279.
(34) Stellato, C.; Brummet, M. E.; Plitt, J. R.; Shahabuddin, S.; Baroody, F. M.; Liu, M. C.; Ponath, P. D. *Beck J. Immunol.* **2001**, *166*, 1457.
(35) Owen, C. *Pulm. Pharmacol. Ther.* **2001**, *14*, 193.
(36) Corrigan C. *Curr. Opin. Invest. Drugs.* **2000**, *1*, 321.
(37) Klein, A.; Talvani, A.; Silva, P. M.; Martins, M. A.; Wells, T. N.; Proudfoot, A.; Luckacs, N. W.; Teixeira, M. M. *J. Immunol.* **2001**, *167*, 524.
(38) Bandeira-Melo, C.; Sugiyama, K.; Woods, L. J.; Weller, P. F. *J. Immunol.* **2001**, *166*, 4813.
(39) Sabroe, I.; Williams, T. J.; Pease, J. E. *Clin. Sci.* **2001**, *100*, 359.
(40) Kaplan, A. P. *Int. Arch. Allergy Immunol.* **2001**, *124*, 423.
(41) Heveker, N. *Curr. Drug Targets* **2001**, *2*, 21.
(42) Marone, G.; de Paulis, A.; Florio, G.; Petraroli, A.; Rossi, F. W.; Triggiani, M. *Int. Arch. Allergy Immunol.* **2001**, *125*, 89.
(43) Murakami, T.; Yamamoto, N. *Int. J. Hematol.* **2000**, *72*, 412.
(44) Baggiolini, M. *Nature* **1998**, *392*, 565.
(45) Gerard, C.; Rollins, B. J. *Nat. Immunol.* **2001**, *2*, 108.
(46) Proudfoot, A. E. I. *Nat. Rev. Immunol.* **2002**, *2*, 106.
(47) Hatse, S.; Princen, K.; Bridger, G.; De, C. E.; Schols, D.; et al. *FEBS Lett.* **2002**, *527*, 255.
(48) Isgro, A.; Aiuti, A.; Mezzaroma, I.; Addesso, M.; Riva, E.; Giovannetti, A.; Mazzetta, F.; Alario, C.; Mazzone, A.; Ruco, L.; Aiuti, F.; et al. *Br. J. Haematol.* **2002**, *118*, 864.
(49) Govaerts, C.; Bondue, A.; Springael, J.-Y.; Olivella, M.; Deupi, X.; Le-Poul, E.; Wodak, S.-J.; Parmentier, M.; Pardo, L.; Blanpain, C. *J. Biol. Chem.* **2003**, *278*, 1892.
(50) Trivedi, B. K.; Low, J. E.; Carson, K.; LaRosa, G. J. *Ann. Rep. Med. Chem.* **2000**, *35*, 191.
(51) Karpus, W. J.; Kennedy, K. J. *J. Leukocyte Biol.* **1997**, *62*, 681.
(52) Godiska, R.; Chantry, D.; Dietsch, G. N.; Gray, P. N. *J. Neuroimmunol.* **1995**, *58*, 167.
(53) Horuk, R.; Shurey, S.; Ng, H. P.; May, K.; Bauman, J. G.; Islam, I.; Ghannam, A.; Buckman, B.; Wei, G. P.; Xu W.; Liang, M.; Rosser, M.; Dunning, L.; Hesselgesser, J.; Snider, R. M.; Morrissey, M. M.; Perez, H. D.; Green, C. *Immunol. Lett.* **2001**, *76*, 193.
(54) Horuk, R.; Clayberger, C.; Krensky, A. M.; Wang, Z.; Grone, H. J.; Weber, C.; Weber, K. S.; Nelson, P. J.; May, K.; Rosser, M.; Dunning, L.; Liang, M.; Buckman, B.; Ghannam, A.; Ng, H. P.; Islam, I.; Bauman, J. G.; Wei, G. P.; Monahan, S.; Xu, W.; Snider R. M.; Morrissey, M. M.; Hesselgesser, J.; Perez, H. D. *J. Biol. Chem.* **2001,** *276*, 4199.
(55) Koch, A. E.; Kunkel, S. L.; Strieter, R. M. *J. Invest. Med.* **1995**, *43*, 28.
(56) Rathanaswami, P.; Hachicha, M.; Sadick, M.; Schall, T. J.; Mccoll, S. R. *J. Biol. Chem.* **1993**, *268*, 5834.
(57) Naya, A.; Sagara, Y.; Ohwaki, K.; Saeki, T.; Ichikawa, D.; Iwasawa, Y.; Noguchi, K.; Ohtake, N. *J. Med. Chem.* **2001**, *44*, 1429.
(58) Ng, H. P.; May, K.; Bauman, J. G.; Ghannam, A.; Islam, I.; Liang, M.; Horuk, R.; Hesselgesser, J.; Snider, R. M.; Perez, H. D.; Morrissey, M. M. *J. Med. Chem.* **1999**, *42*, 4680.
(59) Faull, A. W.; Kettle, J. WO Patent 0046198, 2001; *Chem. Abstr.* **2001**, *133*, 150462.
(60) Chen, X.; Oppenheim, J.; Howard, O. M. *Int. Immunopharmacol.* **2001**, *1*, 229.
(61) Luly, J. R.; Nakasato, Y.; Ohshima, E. U.S. Patent 6329385, 2001; *Chem. Abstr.* **2001**, *134*, 163016.
(62) Brown, M. F.; Poss, C. S. WO Patent 0157023, 2001; *Chem. Abstr.* **2001**, *135*, 152821.
(63) Bauman, J. G.; Buckman, B. O.; Ghannam, A. F.; Hesselgesser, J. E.; Horuk, R.; Islam, I.; Liang M.; May, K. B.; Monahan, S. D.; Morrissey, M. M.; Ng, H. P.; Wie, G. P.; Xu. W.; Zheng, W. WO Patent 09856771, 1999; *Chem. Abstr.* **1999**, *130*, 66509.
(64) Orino, H.; Ishiyama, T.; Tatematsu, T.; Makino, C.; Ochiai, Y.; Kito, K.; Kanetani, N.; Nishida, K. JP Patent 2000-200089267, 2000; *Chem. Abstr.* **2001**, *135*, 283187.
(65) Faull, A. W.; Kettle, J. G. WO Patent 0151467, 2001; *Chem. Abstr.* **2001**, *135*, 107249.
(66) Forbes, I. T.; Cooper, D. G.; Dodds, E. K.; Hickey, D. M. B.; Ife, R. J.; Meeson, M.; Stockley, M.; Berkhout, T. A.; Gohil, J.; Groot, P. H. E.; Moores, K. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1803.
(67) Witherington, J.; Bordas. V.; Cooper. D. G.; Forbes, I. T.; Gribble A. D.; Ife, R. J.; Berkhout, T.; Gohil. J.; Groot, P. H. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2177.
(68) Mirzadegan, T.; Diehl, F.; Ebi, B.; Bhakta, S.; Polsky, I.; McCarley, D.; Mulkins, M.; Weatherhead, G. S.; Lapierre, J.; Dankwardt, J.; Morgans, D. Jr.; Wilhelm, R.; Jarnagin, K. *J. Biol. Chem.* **2000**, *275*, 25562.
(69) Yang, L.; Butora, G.; Parsons, W. H.; Pasternak, A. WO Patent 0213824, 2002; *Chem. Abstr.* **2002**, *136*, 200102.
(70) Desreumaux, P.; Capron, M. *Curr. Opin. Immunol.* **1996**, *8*, 790.
(71) Heath, H.; Qin, S. X.; Rao, P.; Wu, L. J.; Larosa, G.; Kassam, N.; Ponath, P. D.; Mackay, C. R. *J. Clin. Invest.* **1997**, *99*, 178.
(72) Terada, N.; Hamano, N.; Kim, W. J.; Hirai, K.; Nakajima, T.; Yamada, H.; Kawasaki, H.; Yamashita, T.; Kishi, H.; Nomura, T.; Numata, T.; Yoshie, O.; Konno, A. *Am. J. Respir. Crit. Care Med.* **2001**, *164*, 575.
(73) Dhanak, D.; Christmann, L. T.; Darcy, M. G.; Jurewicz, A. J.; Keenan, R. M.; Lee, J.; Sarau, H. M.; Widdowson, K. L.; White, J. R. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1441.
(74) Dhanak, D.; Christmann, L. T.; Darcy, M. G.; Keenan, R. M.; Knight, S. D.; Lee, J.; Ridgers, L. H.; Sarau, H. M.; Shah, D. H.; White, J. R.; Zhang, L. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1445.
(75) Hirschfeld, D. R.; Smith, D. B.; Kertesz, D. J. DE Patent 19955793, 2000; *Chem. Abstr.* **2000**, *133*, 4602.

(76) Naya, A.; Owada, Y.; Saeki, T.; Ohwaki, K.; Iwasawa, Y. WO Patent 9804554, 1998; *Chem. Abstr.* **1998**, *128*, 16735.
(77) Gong, L.; Kertesz, D. J.; Smith, D. B.; Wilhelm, R. S. WO Patent 0029377, 2000; *Chem. Abstr.* **2000**, *132*, 347495.
(78) Naya, A.; Kobayashi, K.; Ishikawa, M.; Ohwaki, K.; Saeki, T.; Noguchi, K.; Ohtake, N. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1219.
(79) Lawrence, L.; Rigby, A.; Sanganee, H.; Springthorpe, B. WO Patent 0177101, 2001; *Chem. Abstr.* **2001**, *135*, 318419.
(80) Pipitone, N.; Pitzalis, C. *Curr. Opin. Anti-inflammat. Immunomodulat. Invest. Drugs* **2000**, *2*, 9.
(81) Hall, I. P.; Wheatley, A.; Christie, G.; McDougall, C.; Hubbard, R.; Helms, P. J. *Res. Lett.* **1999**, *354*, 9186.
(82) Barcellos, L. F.; Schito, A. M.; Rimmler, J. B.; Vittinghoff, E.; Shih, A.; Lincoln, R.; Callier, S.; Elkins, M. K.; Goodkin D. E, Haines, J. L.; Pericak-Vance, M. A.; Hauser, S. L.; Oksenberg, J. R. *Immunogenetics* **2000**, *51*, 281.
(83) Sellebjerg, F.; Madsen, H. O.; Jensen, C. V.; Jensen, J.; Garred, P. *J. Neuroimmunol.* **2000**, *102*, 98.
(84) Deng, H.; Liu, R.; Ellmeier, W.; Choe, S.; Unutmaz, D.; Burkhart, M.; Di Marzio, P.; Marmon, S.; Sutton, R. E.; Hill, C. M.; Davis, C. B.; Peiper, S. C.; Schall, T. J.; Littman, D. R.; Landau, N. R. *Nature* **1996**, *381*, 661; Samson, M.; Libert, F.; Doranz, B. J.; Rucker, J.; Liesnard, C.; Farber, C.-M.; Saragosti, S.; Lapoumeroulie, C.; Cognaux, J.; Forceille, C.; Muyldermans, G.; Verhofstede, C.; Burtonboy, G.; Georges, M.; Imai, T.; Rana, S.; Yi, Y.; Smyth, R. J.; Collman, R. G.; Doms, R. W.; Vassart, G.; Parmentier, M. *Nature* **1996**, *382*, 722. Liu, R.; Paxton, W. A.; Choe, S.; Ceradini, D.; Martin, S. R.; Horuk, R.; MacDonald, M. E.; Stuhlmann, H.; Koup, R. A.; Landau, N. R. *Cell* **1996**, *86*, 367.
(85) Dorn, C. P.; Finke, P. E.; Oates, B.; Budhu, R. J.; Mills, S. G.; MacCoss, M.; Malkowitz, L.; Springer, M. S.; Daugherty, B. L.; Gould, S. L.; DeMartino, J. A.; Siciliano, S. J.; Carella, A.; Carver, G.; Holmes, K.; Danzeisen, R.; Hazuda, D.; Kessler, J.; Linberger, J.; Miller, M.; Schleif, W. A.; Emini, E. A. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2469.
(86) Finke, P. E.; Meurer, L. C.; Oates, B.; Mills, S. G.; MacCoss, M.; Malkowitz, L.; Springer, M. S.; Daugherty, B. L.; Gould, S. L.; DeMartino, J. A.; Siciliano, S. J.; Carella, A.; Carver, G.; Holmes, K.; Danzeisen, R.; Hazuda, D.; Kessler, J.; Lineberger, J.; Miller, M.; Schleif, W. A.; Emini, E. A. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 265.
(87) Finke, P. E.; Meurer, L. C.; Oates, B.; Shah, S. K.; Loebach, J. L.; Mills, S. G.; MacCoss, M.; Castonguay, L.; Malkowitz, L.; Springer, M. S.; Gould, S. L.; DeMartino, J. A. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2469.
(88) Hale, J. J.; Budhu, R. J.; Mills, S. G.; MacCoss, M.; Malkowitz, L.; Siciliano, S.; Gould, S. L.; DeMartino, J. A.; Springer, M. S. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1437.
(89) Finke, P. E.; Oates, B.; Mills, S. G.; MacCoss, M.; Malkowitz, L.; Springer, M. S.; Gould, S. L.; DeMartino, J. A.; Carella, A.; Carver, G.; Holmes, K.; Danzeisen, R.; Hazuda, D.; Kessler, J.; Lineberger, J.; Miller, M.; Schleif, W. A.; Emini, E. A. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2475.
(90) Hale, J. J.; Budhu, R. J.; Holson, E. B.; Finke, P. E.; Oates, B.; Mills, S. G.; MacCoss, M.; Gould, S. L.; DeMartino, J. A.; Springer, M. S.; Siciliano, S.; Schleif, W. A.; Hazuda, D.; Miller, M.; Kessler, J.; Danzeisen, R.; Holmes, K.; Lineberger, J.; Carella, A.; Carver, G.; Emini, E. A. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2741.
(91) Lynch, C. L.; Gentry, A. L.; Hale, J. J.; Mills, S. G.; MacCoss, M.; Springer, M. S.; Gould, S. L.; DeMartino, J. A.; Siciliano, S.; Casciert, M. A.; Doss, G.; Carella, A.; Carver, G.; Holmes, K.; Schleif, W. A.; Danzeisen, R.; Hazuda, D.; Kessler, J.; Lineberger, J.; Miller, M.; Emini, E. A. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 677.
(92) Shiraishi, M.; Aramaki, Y.; Seto, M.; Imoto, H.; Nishikawa, Y.; Kanzaki, N.; Okamoto, M.; Sawada, H.; Nishimura, O.; Baba, M.; Fujino, M. *J. Med. Chem.* **2000**, *43*, 2049. Este, J. A. *Curr. Opin. Invest. Drugs* **2001**, *2*, 354.
(93) Aquaro, S.; Hatse, S.; Princen, K.; Baba, M.; Perno, C. F.; DeClercq, E.; Schols, D. *Antiviral Res.* **2002**, *53*, 3.
(94) Ku, T. W.; Bondinell, W. E.; Neeb, M. J. WO Patent 0006146, 2000; *Chem. Abstr.* **2000**, *132*, 151564.
(95) Harriman, G. C.; Kolz, C. N.; Luly, J. R.; Roth, B. D.; Song, Y.; Trivedi, B. K. WO Patent 0042045, 2000; *Chem. Abstr.* **2000**, *133*, 105029.
(96) Bridger, G.; Skerlj, R.; Kaller, A.; Harwig, C.; Bogucki, D.; Wilson, T. R.; Crawford, J.; McEachern, E. J.; Atsma, B.; Nan, S.; Zhou, Y.; Schols, D. WO Patent 0056729, 2000; *Chem. Abstr.* **2000**, *133*, 266589.
(97) Tagat, J. R.; McCombie, S. W.; Steensma, R. W.; Lin, S.-I.; Nazareno, D. V.; Baroudy, B.; Vantuno, N.; Xu, S.; Liu, J. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2143.
(98) Palani, A.; Shapiro, S.; Clader, J. W.; Greenlee, W. J.; Cox, K.; Strizki, J.; Endres, M.; Baroudy, B. M. *J. Med. Chem.* **2001**, *44*, 3339.
(99) Tagat, J. R.; Steensma, R. W.; McCombie, S. W.; Nazareno, D. V.; Lin, S.; Neustadt, B. R.; Cox, K.; Xu, S.; Wojcik, L.; Murray, M. G.; Vantuno, N.; Baroudy, B. M.; Strizki, J. *J. Med. Chem.* **2001**, *44*, 3343.
(100) Eyles, A. *IDDB Meeting Rep.* **2002**, April 7−11.
(101) Wang, W.; Soto, H.; Oldham, E. R.; Buchanan, M. E.; Homey, B.; et al. *J. Biol. Chem.* **2000**, *275*, 22313.
(102) Homey, B.; Alenius, H.; Mueller, A.; Soto, H.; Bowman, E. P.; Yuan, W.; McEvoy, L.; Lauerma, A. I.; Assmann, T.; et al. *Nature Med.* **2002**, *7*, 157.
(103) Shiosaki, K.; Fleming, P. WO Patent 0046203, 2000; *Chem. Abstr.* **2000**, *133*, 164005.
(104) Holmes, W. E.; Lee, J.; Kuang, W. J.; Rice, G. C.; Wood, W. I. *Science* **1991**, *253*, 1278.
(105) Murphy, P. M.; Tiffany, H. L. *Science* **1991**, *253*, 1280.
(106) Miller, E. J.; Cohen, A. B.; Nagao, S.; et al. *Am. Rev. Respir. Dis.* **1992**, *146*, 427.
(107) Broaddus, V. C.; Hebert, C. A.; Vitangcol, R. V.; et al. *Am. Rev. Respir. Dis.* **1992**, *146*, 825.
(108) Widdowsen, K. L.; Jin, Q. WO Patent 068570, 2001; *Chem. Abstr.* **2001**, *135*, 242017; WO Patent 068569, 2001; *Chem. Abstr.* **2001**, *135*, 257047; WO Patent 068568, 2001; *Chem. Abstr.* **2001**, *135*, 257156; WO Patent 068084, 2001; *Chem. Abstr.* **2001**, *135*, 257046; WO Patent 035442, 2000; *Chem. Abstr.* **2000**, *133*, 58620.
(109) Leckie, M. J.; Jenkins, G. R.; Khan. J.; Smith, S. J.; Walker, C.; Barnes, P. J.; Hansel, T. T. *Thorax* **2003**, *58*, 23.
(110) Moore, B. B.; Arenberg, D. A.; Strieter, R. M. *Trends Cardiovas. Med.* **1998**, *8*, 51.
(111) Larsen, C. G.; Anderson, A. O.; Appella, E.; Oppenheim, J. J.; Matsushima, K. *Science* **1989**, *243*, 1464.
(112) Warringa, R. A.; Koenderman, L.; Kok, P. T.; Kreukniet, J.; Bruijnzeel, P. L. *Blood* **1991**, *77*, 2694.
(113) White, M. V.; Yoshimura, T.; Hook, W.; Kaliner, M. A.; Leonard, E. J. *Immunol. Lett.* **1989**, *22*, 151.
(114) Walz, A.; Dewald, B.; von Tscharner, V.; Baggiolini, M. *J. Exp. Med.* **1989**, *170*, 1745.
(115) Thelen, M.; Peveri, P.; Kernen, P.; von Tschararer, V.; Walz, A.; Baggiolini, M. *FASEB J.* **1988**, *2*, 2702.
(116) White, J. R.; Lee, J. M.; Young, P. R.; Hertzberg, R. P.; Jurewicz, A. J.; Chaikin, M. A.; et al. *J. Biol. Chem.* **1998**, *273*, 10095.
(117) Widdowson, K. L.; Jin, Q. WO Patent 068084, 2001; *Chem. Abstr.* **2001**, *135*, 257046.
(118) Auten, R.; et al. *J. Pharmacol. Exp. Ther.* **2001**, *299*, 90.
(119) Jin, Q.; et al. *Abstracts of Papers*, 221st ACS National Meeting, April 1−5, 2001, San Diego, CA; American Chemical Society: Washington, DC, 2001; MEDI-258.
(120) Patent Evaluation, Warner-Lambert Co. WO9942463. *Exp. Opin. Ther. Patents* **2000**, *10*, 121.
(121) Carson, K. G.; Connor, D. T.; Li, J. J.; Low, J. E.; Luly, J. R.; Miller, S. R.; Roth, B. D.; Trivedi, B. K. WO Patent 9942461, 1999; *Chem. Abstr.* **1999**, *131*, 165309; WO 9942463, 1999; *Chem. Abstr.* **1999**, *131*, 170359.
(122) Cutshall, N. S.; Ursino, R.; Kucera, K. A.; Latham, J.; Ihle, N. C. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1951.
(123) Cutshall, N. S.; Kucera, K. A.; Ursino, R.; Latham, J.; Ihle, N. C. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1517.
(124) Li, J. J.; Yue, W. S.; Trivedi, B. K.; Miller, S. R.; Connor, D. T.; Roth, B. D.; Low, J. E.; Heilig, D. J.; Hunt, S.; Carson, K. G.; Glynn, R. A.; Ye, Q.; Luly, J. R.; Yang, W.; Qin, S. *Abstracts of Papers*, 220th ACS National Meeting, Aug 20−24, 2000, Washington, DC; American Chemical Society: Washington, DC, 2000; MEDI-144.
(125) Katancik, J. A.; Sharma, A.; De Nardin, E. *Cytokine* **2000**, *12*, 1480.
(126) Ahuja, S. K.; Lee, J. C.; Murphy, P. M. *J. Biol. Chem.* **1996**, *271*, 225.
(127) Bryan, D. L.; Widdowson, K. L. WO Patent 9936069, 1999; *Chem. Abstr.* **1999**, *131*, 116232; WO Patent 034141, 2001; *Chem. Abstr.* **2001**, *134*, 131392.
(128) Jin, Q.; McCleland, B. W.; Palovich, M. R.; Widdowson, K. L. WO Patent 0035442, 2000; *Chem. Abstr.* **2000**, *133*, 58620.
(129) Bratton, L. D.; Connor, D. T., Miller, S. R.; Trivedi, B. K.; Unangst, P. C. WO Patent 0179209, 2001; *Chem. Abstr.* **2001**, *132*, 318518.
(130) Cage, P.; Teobald, B. WO Patent 0177087, 2001; *Chem. Abstr.* **2001**, *135*, 303896.
(131) Willis, P. A.; Bonnert, R. V.; Hunt, S. F.; Walters, I. A. S. WO Patent 0125242, 2001; *Chem. Abstr.* **2001**, *134*, 280857.
(132) Bonnert, R.; Gardiner, S.; Hunt, F.; Walters, I. WO Patent 162758, 2001; *Chem. Abstr.* **2001**, *135*, 211056.
(133) Berger, E. A. *AIDS* **1997**, *11* (Suppl. A), 3.
(134) Michael, N. L.; Moore, J. P. *Nature Med.* **1999**, *5*, 740.
(135) Schols, D.; Struyf, S.; Van Damme, J.; Este, J. A.; Henson, G.; De Clercq, E.. *J. Exp. Med.* **1997**, *186*, 1383.
(136) Donzella, G. A.; et al. *Nature Med.* **1998**, *4*, 72.
(137) Bridger, G. J.; Skerlj, R. T.; Padmanabhan, S.; Martellucci, S. A.; Henson, G. W.; Struyf, S.; Witvrouw, M.; Schols, D.; De Clercq, E. *J. Med. Chem.* **1999**, *42*, 3971.
(138) Hendrix, C. W.; et al. *Antimicrob. Agents Chemother.* **2000**, *44*, 1667.

(139) Hendrix, C. W.; Flexner, C.; MacFarland, R. T.; Giandomenico, C.; Fuchs, E. J.; Redpath, E.; Bridger, G.; Henson, G. W. *Antimicrob. Agents Chemother*. **2000**, *44*, 1667.
(140) MacFarland, R. T.; et al. Presented at the 40th Interscience Conference on Antimicrobial Agents and Chemotherapy, Toronto, 2000; Poster 1845.
(141) Matthys, P.; et al. *J. Immunol*. **2001**, *167*, 4686.
(142) Muller, A.; et al. *Nature* **2001**, *410*, 50.
(143) Howard, O. M. Z.; Oppenheim, J. J.; Hollingshead, M. G.; Covey, J. M.; Bigelow, J.; McCormack, J. J.; Buckheit, R. W., Jr.; Clanton, D. J.; Turpin, J. A.; Rice, W. G. *J. Med. Chem*. **1998**, *41*, 2184.

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