Identification and Characterization of INCB9471, an Allosteric Noncompetitive Small-Molecule Antagonist of C-C Chemokine Receptor 5 with Potent Inhibitory Activity against Monocyte Migration and HIV-1 Infection

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Received January 20, 2011; accepted March 24, 2011

ABSTRACT

C-C chemokine receptor 5 (CCR5) is a clinically proven target for inhibition of HIV-1 infection and a potential target for various inflammatory diseases. In this article, we describe 5-[(4-{(3S)-4-[(1R,2R)-2-ethoxy-5-(trifluoromethyl)-2,3-dihydro-1H-inden-1-yl]-3-methylpiperazin-1-yl}-4-methylpiperidin-1-yl)carbonyl]-4,6-dimethylpyrimidine dihydrochloride (INCB9471), a potent and specific inhibitor of human CCR5 that has been proven to be safe and efficacious in viral load reduction in phase I and II human clinical trials. INCB9471 was identified using a primary human monocyte-based radioligand competition binding assay. It potently inhibited macrophage inflammatory protein-1/H9252-induced monocyte migration and infection of peripheral blood mononuclear cells by a panel of R5-HIV-1 strains. The results from binding and signaling studies using incremental amounts of INCB9471 demonstrated INCB9471 as a noncompetitive CCR5 inhibitor. The CCR5 residues that are essential for interaction with INCB9471 were identified by site-specific mutagenesis studies. INCB9471 rapidly associates with but slowly dissociates from CCR5. When INCB9471 was compared with three CCR5 antagonists that had been tested in clinical trials, the potency of INCB9471 in blocking CCR5 ligand binding was similar to those of 4,6-dimethyl-5-[[4-methyl-4-[(3S)-3-methyl-4-{(1R)-2-(methyloxy)-1-[4-(trifluoromethyl) phenyl]ethyl}-1-piperazinyl]-1-piperidinyl]carbonyl]pyridine (SCH-D; vicriviroc), 4-[[4-[[3R]-1-butyl-3-{(3R)-cyclohexyl-(hydroxyl)methyl}-2,5-dioxo-1,4,9-triazaspiro[5.5]undec-9-yl]methyl]phenyl]oxy]benzoic acid hydrochloride (873140; aplaviroc), and 4,4-difluoro-N-[[1S]-3-[(3-endo)-3-[3-methyl-5-(1-methyllethyl)-4H-1,2,4-triazol-4-yl]-8-azabicyclo[3.2.1]oct-8-yl]-1-phenylpropyl]cyclohexanecarboxamide (UK427857; maraviroc). Its inhibitory activity against CCR5-mediated Ca2+ mobilization was also similar to those of SCH-D and 873140. Further analysis suggested that INCB9471 and UK427857 may have different binding sites on CCR5. The significance of two CCR5 antagonists with different binding sites is discussed in the context of potentially overcoming drug-resistant HIV-1 strains.

Introduction

CCR5 was initially identified as a seven-transmembrane receptor for the chemokines RANTES, MIP-1α, and MIP-1β (Combadiere et al., 1996). The receptor is a 352-amino acid protein that belongs to the class A G protein-coupled receptor family with a high homology to rhodopsin and is most likely coupled to G proteins of the Gi/o subfamily because its activation in cells leads to inhibition of cAMP production, stimulation of Ca2+ ion release, and activation of mitogen-activated protein kinase family members (Onuffer and Horuk, 2002). CCR5 activation can also lead to phosphorylation of the Jak-Stat pathway, suggesting that the receptor could signal through other pathways in addition to the classic G protein-coupled mechanisms (Wong and Fish, 2003).

In the immune system CCR5 is expressed mainly on effector/memory T cells, monocytes, and dendritic cells, and its expression is up-regulated by activation (Lee et al., 1999). The receptor seems to play important roles in rejection in cardiac and islet allograft transplantation models (Gao et al., 2001; Abdi et al., 2002), and its increased expression seems to be positively associated with human renal and cardiac transplant rejection (Segerer et al., 2001; Fahmy et al., 2003). In both preclinical and clinical studies, CCR5 has been linked with rheumatoid arthritis (Zapico et al., 2000; Yang et al.,...
CCR5 also has been implicated in diseases of the central nervous system (Bajetto et al., 2002), and its deficiency may decrease susceptibility to experimental cerebral malaria (Beloue et al., 2003).

It is noteworthy that CCR5 is a coreceptor for HIV-1. The virus entry into human hematopoietic cells in vivo requires the cooperation of the viral subunit envelope glycoproteins gp120 and gp41, and two host-cell proteins, the CD4 receptor and either the CCR5 and CXCR4 coreceptor (Zhang and Moore, 1999). Binding of the viral envelope protein (Env) to CD4 induces conformational changes in the gp120 subunit that enable it to interact efficiently with the CCR5 or CXCR4 coreceptor (Wu et al., 1996). Although the structural consequences of coreceptor binding are not well understood, it is clear that CCR5 is essential for viral transmission and replication during the early, clinically latent phase of disease (Gonzalez et al., 2001). Even during the late stage of disease, CCR5-using virus was found exclusively in more than half of HIV-1-infected individuals (Doms, 2001). Genetic analysis has indicated that carriers of a 32-base pair deletion in CCR5 (CCR5Δ32 allele), which abolishes receptor expression in homoyzgotes, are resistant to HIV-1 transmission and disease progression (Dean et al., 1996). In addition, the experimental knockout of CCR5 in mice resulted in only minor defects in T cell and macrophage functions (Huffnagel et al., 1999). Furthermore, individuals carrying the CCR5Δ32 allele do not exhibit any overt immune dysfunctions (Dean et al., 1996). These results suggest that a CCR5-specific antagonist is not likely to cause any mechanism-based side effects. Finally, a number of CCR5 antagonists have been proven to be potent inhibitors of CCR5-mediated HIV-1 entry and the first CCR5 antagonist, maraviroc, was approved by the Food and Drug Administration and launched by Pfizer as an anti-HIV therapeutic agent in 2007. Taken together, CCR5 is an attractive therapeutic target for organ transplantation, autoimmune diseases, and HIV-1 infection.

![Image](https://example.com/image.png)

**Materials and Methods**

**Test Compounds**

4,6-Dimethyl-5-[[4-(methylyl-4-((3S)-3-methyl-4-((1R)-2-(methyloxy)-1-(4-trifluoromethyl) phenyl)(ethyl)-1-piperazinyl)-1-piperidinyl)carboxy]l]pyrimidine (SCH-D, 4,4-difluoro-N-((1S)-3-(3-endo)-3-[3-methyl-5-(1-methylthyl)4H-1,2,4-triazol-4-yl]-8-azabicyclo[3.2.1]oct-8-yl]-1-phenylpropyl)cyclohexanecarboxamide (UK427857), 4-(4-(3R)-1-buty1-3-(R)-cyclohexyl(hydroxyl)methyl)-2,5-dioxio, 1,4,9-triazaspiro[5,5]undec-9-yl]methylphenyloxy]benzoic acid hydrochloride (873140), and INCB9471 were synthesized using procedures disclosed in the literature (Baroum et al., 2000; Perros et al., 2001; Mitsuya et al., 2002; Xue et al., 2005).

**Cloning of G16, CCR5, and CXCR3 cDNAs**

The cDNA encoding G16 protein was purchased from Molecular Devices (Sunnyvale, CA) and subcloned into pCDNA3.1 (Invitrogen, Carlsbad, CA). Full-length cDNAs for human CCR5 and CXCR3 were amplified from a human spleen cDNA library (Clontech, Mountain View, CA) by polymerase chain reaction using specific primers (forward, 5’-ccacatggattatcaagttgaatg-3’ and backward, 5’-tttggtcatacagccagatattctg-3’ for CCR5; forward, 5’-aagttgcaacctgctgggtgtgaccacga-3’ and backward, 5’-gctgatacetaacgctgagaggtcagctg-3’ for CXCR3). The amplified cDNAs were subcloned into the mammalian cell expression vector, pFLAG-CMV3 (Sigma-Aldrich, St. Louis, MO) and verified by sequencing both strands. All point mutations in CCR5 were introduced using a QuikChange Site-Directed Mutagenesis kit (Stratagene, La Jolla, CA).

**Cell Culture, DNA Transfection, and Receptor Expression**

**Human and Cynomolgus Monkey Monocytes**

To prepare human monocytes, leukopheresis (Biological Specialty, Colmar, PA) was performed on healthy, drug-free donors, and PBMCs were isolated via density gradient centrifugation. Human monocytes were further isolated via centrifugal elutriation. After being washed with phosphate-buffered saline, the monocytes were resuspended at 10⁶ cells/ml with RPMI medium 1640 (Invitrogen) supplemented with 10% fetal bovine serum (HyClone Laboratories, Logan, UT) and either incubated at 4°C overnight (for CCR2 assay) or treated with 10 to 20 ng/ml of rhIL-10 (R&D Systems, Minneapolis, MN) at 37°C with 5% CO2 for 24 to 48 h (for CCR5 assay). CCR5 expression on the rhIL-10-treated human monocytes was verified by staining the cells with a PE-conjugated anti-human CCR5 antibody (2D7) (BD Pharmingen, San Diego, CA), followed by FACS analysis using FACSCalibur (BD Biosciences Discovery Labware, Bedford, MA). To prepare cynomolgus monkey monocytes, whole blood from disease-free cynomolgus monkeys was collected (Covance, Denver, PA). After being isolated with ficoll hypaque density gradient centrifugation, the cynomolgus monkey monocytes were incubated with nonpimate anti-CD14 beads (Miltenyi Biotec Inc., Auburn, CA) to allow monocytes to be purified using an AutoMACs sorter (Miltenyi Biotec). For CCR5 expression, the cynomolgus monkey monocytes were treated with 10 to 20 ng/ml of rhIL-10 (R&D Systems) at 37°C with 5% CO2 for 24 h or 48 h.

**ABBREVIATIONS:** CCR5, C-C chemokine receptor 5; CXCR4, C-X-C chemokine receptor 4; CXCR3, C-X-C chemokine receptor 3; DMEM, Dulbecco’s modified Eagle’s medium; FACS, fluorescence-activated cell sorting; HEK, human embryonic kidney; MCP-1, monocyte chemoattractant protein-1; MIP-1α, macrophage inflammatory protein-1α; PBMC, peripheral blood mononuclear cell; PE, phycoerythrin; RANTES, regulated on activation, normal T cell expressed and secreted; rhIL-10, recombinant human interleukin-10; TM, transmembrane domain; SCH-D, 4,6-dimethyl-5-[(4-trifluoromethyl) phenyl(ethyl)-1-piperazinyl]-1-piperidinyl[carboxyl]l]pyrimidine; UK287587, 4-(4-(3R)-1-buty1-3-(R)-cyclohexyl(hydroxy)methyl]-2,5-dioxio, 1,4,9-triazaspiro[5,5]undec-9-yl]methylphenyloxy]benzoic acid hydrochloride; INCB9471, 5-[(4-(3S)-4-((1R,2R)-2-ethoxy-5-(trifluoromethyl)phenyl)-1H-inden-1-yl]-3-methylpiperazin-1-yl]-4-methylpiperidin-1-yl]carbonyl-4,6-dimethylpyrimidine dihydrochloride; INCB9471, 5-[(4-(3S)-4-((1R,2R)-2-ethoxy-5-(trifluoromethyl))phenyl)-1H-inden-1-yl]-3-methylpiperazin-1-yl]-4-methylpiperidin-1-yl]carbonyl-4,6-dimethylpyrimidine dihydrochloride; TAK779, N,N-dimethyl-N-[4-[[2-(4-methoxyphenyl)-(ethoxymino)methyl]-1’-[(2,4-dimethyl-3-pyridyl)]carbonyl]-4’-methyl-1,4’-bipiperidine N-oxide; AD101, [2,4-dimethylpyridin-3-yl]-[4-methyl-4-((3S)-3-methyl-4-((1S)-1-[4-(trifluoromethyl)phenyl]ethyl)piperazin-1-yl)]-1-piperidyl)methanone.
Stable Cell Lines. To generate stable cell lines that heterologously express wild-type CCR5 or CXCR3 proteins, pFLAG-CMV3-CCR5 or pFLAG-CMV3-CXCR3, respectively, was transfected into HEK293SFM cells (Invitrogen) with Lipofectamine 2000 (Invitrogen). Twenty-four hours after transfection, Geneticin (G418) (Invitrogen) was added to the culture medium (DMEM with 10% fetal bovine serum) at 1 mg/mL. After selection for 3 weeks, mixed stable cell lines expressing either CCR5 or CXCR3 were established. Expression of CCR5 and CXCR3 on the cells was confirmed by staining the cells with PE-conjugated anti-human CCR5 antibody (BD Pharmingen) and M2 anti-FLAG monoclonal antibody, respectively, followed by FACS analysis using a flow cytometer (BD Biosciences Discovery Labware). The HEK293SFM cell lines expressing various CCR5 mutants were established in the same way as the wild-type receptor.

Preparation of [3H]INCB9471

[3H]INCB9471 was custom-prepared by GE Healthcare (Chalfont St. Giles, Buckinghamshire, UK). In brief, INCB9471 (5 mg) and palladium black (15 mg) were ground together to give an intimate mixture and this was heated in a sealed glass tube at 150°C with tritium gas (5 Ci) for 30 min. The crude [3H]INCB9471 was purified by high-performance liquid chromatography on a Luna C18 (250 × 4.6 mm) column eluting in a water/acetonitrile/trifluoroacetic acid gradient system. The material was evaporated to dryness and redissolved in ethanol. [3H]INCB9471 was analyzed by high-performance liquid chromatography; the radiochemical purity was 99.4%. Mass spectrometry of [3H]INCB9471 gave a spectrum that corresponded to the inactive INCB9471 and labeling achieved a specific activity of 109 Ci/mmol.

Whole-Cell Binding Assay

All of the whole-cell binding assays were done by using 96-well MultiScreen filter plates (Millipore Corporation, Billerica, MA). For 125I-MIP-1β saturation binding to CCR5 assay, 3 × 105 rhIL-10-treated monocytes per well in 150 μL of RPMI medium 1640 (Invitrogen) with 20 μM HEPES (Invitrogen) and 0.3% bovine serum albumin (Sigma-Aldrich) were incubated at room temperature for 1 h with a range of concentrations of 125I-MIP-1β (PerkinElmer Life and Analytical Sciences, Waltham, MA) in the presence or absence of an excessive amount of unlabeled MIP-1β (R&D Systems). To screen for CCR5 inhibitors, 3 × 105 rhIL-10-treated monocytes per well were incubated with 0.25 nM 125I-MIP-1β (PerkinElmer Life and Analytical Sciences) and a series of concentrations of compound inhibitors. Nonspecific binding was determined by incubating the cells with 0.3 μM unlabeled MIP-1β (R&D Systems). For 125I-MIP-1β, 125I-MIP-1α, and 125I-RANTES (PerkinElmer Life and Analytical Sciences) binding displacement studies using the recombinant HEK293SFM/CCR5 cells, 2 × 105 cells per well were mixed with each of the radioligands at 0.25 nM and a series of concentrations of inhibitors. For counter screening against CCR2, CXCR4, and CXCR3, 2 × 105 monocytes, SUP-T1 cells (American Type Culture Collection, Manassas, VA) or the recombinant 293SFM/CXCR3 cells were incubated with 0.25 nM 125I-MCP-1, 125I-stromal cell-derived factor-1α, and 125I-IP-10 (10-kDa interferon-inducible protein) (PerkinElmer Life and Analytical Sciences), respectively, and a range of concentrations of compound inhibitors. The binding reaction was terminated by harvesting the cells onto filter plates on a vacuum manifold (Millipore Corporation). The filter was then washed five times with RPMI medium 1640 (Invitrogen) supplemented with 20 mM HEPES (Invitrogen), 0.3% bovine serum albumin (Sigma-Aldrich), and 0.4 M NaCl on the vacuum manifold, air dried, and peeled from the plate. The filters corresponding to the sample wells were punched out using the Millipore Punch System (Millipore Corporation). The amount of bound radioactivity on each filter was determined by counting on a gamma counter. Specific binding was defined as the total binding minus the nonspecific binding. All experiments were performed in duplicate. The binding data were evaluated with Prism (GraphPad Software Inc., San Diego, CA).

Membrane Binding Assay

The recombinant HEK293SFM cells expressing either wild-type CCR5 or various mutants were harvested in 50 mM ice-cold Tris-HCl, pH 7.5, and processed with a homogenizer (setting 3, 30 s; Polytrom system PT 1200C, Kinematics, Littau-Lucerne, Switzerland). The homogenate was centrifuged for 5 min at 10,000g to remove nuclei and unbroken cells. The supernatant was centrifuged at 50,000g for 10 min, and the resulting pellet was resuspended in 50 mM ice-cold Tris-HCl, pH 7.5. The protein concentration of the membrane preparation was measured by using Protein Assay Reagent (Bio-Rad Laboratories, Hercules, CA).

For [3H]INCB9471 saturation binding assay, membrane proteins (10 μg) were incubated in a total volume of 150 μL of 50 mM Tris-HCl, pH 7.5, with a range of [3H]INCB9471 concentrations for 1.5 h at room temperature in the presence or absence of excess unlabeled compound. For binding displacement assays, membrane protein (10 μg) was incubated in a total volume of 150 μL of 50 mM Tris-HCl, pH 7.5, with 10 nM [3H]INCB9471 in the presence or absence of different amounts of inhibitors for 1.5 h at room temperature. Nonspecific binding was determined by inclusion of 5 μM INCB9471. The bound radioactivity was separated by filtration through polyethyleneimine-treated GF/B filters (PerkinElmer Life and Analytical Sciences) with a Filtermate 196 harvester (PerkinElmer Life and Analytical Sciences). The filters were washed eight times with 50 mM ice-cold Tris-HCl, pH 7.5, and radioactivity retained on the filters was measured by liquid scintillation counting with a TopCount (PerkinElmer Life and Analytical Sciences) at 35% efficiency. All experiments were performed in duplicate. The binding data were evaluated with Prism (GraphPad Software Inc.).

Ca2+ Flux Assay

HEK293SFM cells were transiently cotransfected in DMEM and 10% fetal calf serum with CCR5 cDNA in pFLAG-CMV3 (0.5 μg/cm2) and Gα16 protein cDNA in pCDNA3 (0.05 μg/cm2) using Lipofectamine 2000 reagent (1.5 μg/cm2) (Invitrogen). Twenty-four hours after transfection, the cells were harvested and resuspended at 4 × 104 cells/25 μL/well in DMEM and 10% fetal calf serum in poly(D-lysine)-treated, 384, clear-bottomed black plates (BD Biosciences Discovery Labware). Forty-eight hours after transfection, the cells were loaded with 1 h with Calcium 3 dye (Molecular Devices). CCR5 antagonists were then evaluated in the cells for inhibitory activity against MIP-1α-, MIP-1β-, or RANTES-induced intracellular Ca2+ mobilization using a fluorescence imaging plate reader (Molecular Devices). All experiments were performed in duplicate. The data (relative fluorescent unit change) were analyzed with Prism (GraphPad Software Inc.).

Chemotaxis Assay

The rhIL-10-treated human monocytes were prepared as described above. A total of 5 × 105 cells in RPMI medium 1640 (Invitrogen) with or without various concentrations of INCB9471 in RPMI medium 1640 were loaded in the wells on top of an 8-μm polycarbonate filter in a 96-well modified Boyden chamber (NeuroProbe, Gaithersburg, MD). Beneath the filter, 30 nM human MIP-1β (R&D Systems) with or without INCB9471 or media was placed in a corresponding 96-well plate. The sealed chambers were incubated for 45 min at 37°C, 5% CO2. Filters were washed and stained with Wright Giemsa (Sigma-Aldrich), and the number of monocytes that migrated toward MIP-1β in the bottom chamber was counted by microscopy. Specific migration is defined as the total migration minus the background migration. All experiments were performed in triplicate, and the data were analyzed with Prism (GraphPad Software Inc.).

Cerep Selectivity Panel

A panel of 50 different biogenic amine receptors, neuropeptide receptor, ion channel binding sites, and neurotransmitter trans-
porter binding assays were done by Cerep, Inc (Redmond, WA). All assays were run using recombinant human receptors except where noted. The full methods and references can be found on the Cerep website (www.cerep.com). The assays were run at 1 μM INCB9471, and the percentage of inhibition is given as the average of three determinations.

**Anti-HIV-1 Infection Assay**

ImQuest BioSciences, Inc (Frederick, MD), WPS Pharmaceuticals and Laboratory Services (Vienna, VA), and Lab 21 Ltd (Cambridge, UK) were used to assess the potency of INCB9471 against a number of different R5 tropic HIV strains. Infectivity assays were performed using phytohemagglutinin-activated human PBMCs. Infectivity was determined by measurement of viral p24 core protein in culture supernatants. Both 50 and 90% inhibitory concentrations were determined (IC50 and IC90). Drug-induced cytotoxicity was simultaneously determined by measurement of the reduction of the formazan dye 3(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (Promega, Madison, WI), which is catalyzed by mitochondrial succinate dehydrogenase.

**Results**

**Development of a Human Monocyte-Based CCR5 Radioligand Binding Assay.** Freshly isolated human monocytes express CCR5 at low level and do not bind 125I-MIP-1β well. It is noteworthy that a previous study indicated that rhIL-10 treatment could further up-regulate CCR5 expression in human monocytes, possibly through a transcriptional mechanism, and increase chemotactic response to MIP-1β (Houle et al., 1999). We verified the study by treating freshly isolated human monocytes with 10 to 20 ng/ml of rhIL-10 overnight and examining CCR5 expression on these cells by flow cytometry, using the PE-conjugated 2D7 monoclonal anti-CCR5 antibody. The receptor surface expression was indeed slightly increased by the rhIL-10 treatment, compared with the untreated cells (data not shown). More importantly, the rhIL-10 treatment dramatically enhanced 125I-MIP-1β binding to monocytes.

To characterize 125I-MIP-1β binding to CCR5 on the rhIL-10-treated human monocytes, saturation binding assays were performed by incubating the cells with serial concentrations of 125I-MIP-1β. The receptor demonstrated a single class of specific binding sites for the radioligand with an apparent Kd of 0.4 nM (Fig. 1A). In a homologous competition experiment, in which the monocytes were mixed with a single concentration of 125I-MIP-1β (0.25 nM) in the presence of different concentrations of the unlabeled MIP-1β, the apparent Ki value of the unlabeled MIP-1β was also approximately 0.4 nM (Fig. 1B) [Ki = IC50/(1 + L/Kd), where L is the 125I-MIP-1β concentration and Kd is the apparent dissociation constant obtained from the saturation experiments]. These results indicate that IL-10-treated human monocytes can be a reliable source of CCR5 for compound screening using a binding assay.

**Identification of INCB9471.** Having established the high affinity binding of 125I-MIP-1β to CCR5 on rhIL-10-treated human monocytes, we used the binding assay to screen for CCR5 inhibitors by measuring binding of 125I-MIP-1β in the presence or absence of test compounds. INCB9471 was identified as a result of a series of internal structure-activity relationship studies (Xue et al., 2010). It is a piperazino-piperidine constrained compound with a molecular weight of 559.677 (Fig. 2A) and has demonstrated potent

![Fig. 1](https://example.com/f1.png)

**Fig. 1.** A, saturation binding of 125I-MIP-1β to rhIL-10-treated human monocytes. Apparent Kd is the radioligand concentration responsible for half of the theoretical maximal binding. B, inhibition of 125I-MIP-1β (0.25 nM) binding to rhIL-10-treated monocytes by different concentrations of unlabeled MIP-1β. Data shown are representative of three independent experiments. Each data point is the mean of duplicate determinations. The data were analyzed by nonlinear regression analysis using a single site-binding (hyperbola) model (A) and one-site competition model (B).

![Fig. 2](https://example.com/f2.png)

**Fig. 2.** INCB9471 chemical structure and inhibitory activity against 125I-MIP-1β binding to rhIL-110-treated human and cynomolgus monkey monocytes. A, chemical structure of INCB9471. B, INCB9471 inhibition of 125I-MIP-1β binding to rhIL-10-treated human and cynomolgus monkey monocytes. Binding of 125I-MIP-1β (0.25 nM) to the human and cynomolgus monkey cells in the presence of different concentrations of INCB9471 was measured. Data shown are representative of more than 60 independent assays, and the value of each data point is an average of duplicates. The data were analyzed by nonlinear regression analysis using a one-site competition model.
inhibitory activities against 125I-MIP-1β binding to both human and cynomolgus monkey monocytes treated with IL-10, with IC50 values of 7 and 4.7 nM, respectively (Fig. 2B). INCB9471 did not inhibit 125I-MIP-1β binding to mouse CCR5 (data not shown).

**Selectivity of INCB9471.** The selectivity of INCB9471 for CCR5 was first tested in radioligand binding assays for 50 other targets by Cerep. These targets represent major classes of biogenic amine receptors, neuropeptide receptors, ion channel binding sites, and neurotransmitter transporters, including two chemokine receptors (CCR1 and CXCR2). INCB09471 at 1 μM did not inhibit radioligand binding to any one of these targets by more than 25% (data not shown).

CCR2 is a chemokine receptor that is closely related to CCR5. CXCR3, like CCR5, is a marker of Th1 cells. CXCR4 is a coreceptor for HIV with X4 tropism. To counter-screen CCR5, CXCR3, like CCR5, is a coreceptor for HIV with X4 tropism. To counter-screen CCR5. CXCR3, like CCR5, is a coreceptor for HIV with X4 tropism. To counter-screen CCR5, CXCR3, like CCR5, is a coreceptor for HIV with X4 tropism. To counter-screen CCR5, CXCR3, like CCR5, is a coreceptor for HIV with X4 tropism. INCB09471 against these additional chemokine receptors, assays were established to assess binding to human CCR2, CXCR3, and CXCR4 (see Materials and Methods). Although binding of the radioligands to these three receptors could by easily displaced by the unlabeled natural ligands or internal small-molecule inhibitors specific for CCR2 or CXCR3, no inhibitory activity of INCB9471 at 1 μM was detected (data not shown). Taken together, these results indicate that INCB09471 is a CCR5-selective inhibitor.

**INCB9471 Inhibition of MIP-1β-Induced Monocyte Migration.** One of the cellular consequences of CCR5 signaling is migration of the receptor expressing cells toward the chemokine ligands for CCR5. The inhibitory effect of INCB9471 on the migration of rhIL-10-treated human monocytes toward MIP-1β was studied using Boyden chambers. The monocytes moved toward a gradient of MIP-1β. INCB9471 inhibited the migration process potently, with IC50 values of approximately 5 nM (Fig. 3).

**In Vitro Anti-HIV Activity of INCB9471.** Imquest Biosciences, Inc., WPS Pharmaceuticals, and Laboratory Services and Lab 21 Ltd were used to assess the potency of INCB9471 against a panel of nine different R5 tropic HIV-1 strains in human PBMCs. The concentration of INCB9471 showing 50 and 90% inhibition, along with the tropism and clade designation of the tested strains, is shown in Table 1. In these assays, the IC50 values of INCB9471 ranged from 3 to 25 nM. As expected, INCB9471 did not exhibit antiviral activity against two HIV-1 strains that use CXCR4 as a coreceptor for viral entry, thereby further confirming target specificity of INCB9471 for CCR5. Under similar conditions, INCB9471 had no effect on human PBMC viability at concentrations up to 1 μM (data not shown).

**Inhibition Mode of INCB9471 against 125I-MIP-1β Binding to Native CCR5.** To understand the mode of INCB9471 inhibition of 125I-MIP-1β binding to CCR5, we examined 125I-MIP-1β saturation binding to CCR5 on the rhIL-10-treated-monocytes in the presence of different concentrations of the compound. As shown in Fig. 4A, the total 125I-MIP-1β binding to the receptor (Bmax) was reduced 5-fold by INCB9471 ranging from 0 to 20 nM. However, the affinity (Kd) of the radioligand for the receptor was not significantly affected (less than 1-fold; from 0.63 to 0.91 nM) by the compound in the same concentration range. We also compared the inhibitory activities of INCB9471 and unlabeled MIP-1β against the binding of four different concentrations of 125I-MIP-1β to CCR5. The IC50 values of unlabeled MIP-1β increased more than 5-fold with a 10-fold increase in the

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**TABLE 1**

Inhibitory potency of INCB9471 against HIV-1 infection of human PBMCs

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<td>R5</td>
<td>G</td>
<td>2.0±1.1</td>
<td>20±8.7</td>
<td>11±3.5</td>
<td>3</td>
<td>&gt;900*</td>
<td>&gt;150</td>
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<td>IIIIB</td>
<td>X4</td>
<td>B</td>
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Average for all R5 HIV-1 strains

Average for R5 HIV-1 strains-clade B

Average for R5 HIV-1 strains-clade C

Average for R5 HIV-1 strains-clade E

Average for R5 HIV-1 strains-clade G

a IC50, cytotoxic concentration that reduces the number of viable cells by 50%.

b TI, therapeutic index, defined as CC50/IC50.

c Highest concentration tested.
amount of $^{125}$I-MIP-1β (Fig. 4B). By contrast, less than a 2-fold change in the IC$_{50}$ values of INCB9471 was found using the same concentration range of $^{125}$I-MIP-1β (Fig. 4C). Together, these data demonstrate that INCB9471, relative to protein ligands, is a noncompetitive inhibitor of CCR5.

Inhibition of $^{125}$I-MIP-1β, $^{125}$I-MIP-1α, and $^{125}$I-RANTES Binding to Recombinant CCR5 by INCB9471, SCH-D, UK427857, and UK427857, are three of the CCR5 antagonists that have been tested in clinical trials for HIV entry inhibition. We compared INCB9471 with these three compounds for CCR5 binding using the recombinant HEK293SFMC/CCR5 cells. The rank order potency of these four compounds varied depending on the radioligand used, with the difference in the IC$_{50}$ values generally being within 3-fold (Fig. 6). Like INCB9471, SCH-D, UK427857, and 873140 could not completely displace the bound $^{125}$I-RANTES (30–40% of the total bound cpm undisplaceable) (Fig. 6C). Compound 873140 was previously reported as being able to inhibit $^{125}$I-RANTES binding to CCR5 by only 20% (Watson et al., 2005). In our assay, approximately 60% inhibition of $^{125}$I-RANTES binding was observed with the same compound (Fig. 6C).

Saturation Binding of $[^3H]$INCB9471 to CCR5. To directly measure the binding of INCB9471 to CCR5, the compound was labeled with tritium by gas exchange with a final specific activity of approximately 110 Ci/mmol. $[^3H]$INCB9471 demonstrated high affinity for the recombinant CCR5 in a saturation binding assay using membranes from HEK293SFMC/CCR5 cells with an apparent K$_d$ of 3 nM (Fig. 7). We explored how INCB9471 and the other three antagonists bind CCR5 relative to each other by examining the saturation binding of $[^3H]$INCB9471 to the recombinant CCR5 in the presence of increasing concentrations of INCB9471, SCH-D, UK427857, or 873140. Total $[^3H]$INCB9471 binding ($B_{max}$) was not appreciably affected by either unlabeled INCB9471, SCH-D, or 873140 (Fig. 7, A, C, and D). Each of these three unlabeled compounds dose-dependently decreased the affinity of $[^3H]$INCB9471 for the receptor, showing approximately a 10-fold increase in K$_d$ value when the concentration of the INCB9471,
SCH-D, or 873140 was increased from 0 to 30 nM. In contrast, UK427857 dose-dependently reduced the total binding of \(^{3}H\)INCB9471 to CCR5 and was less effective in reducing the affinity of \(^{3}H\)INCB9471 for CCR5 than the other three compounds (Fig. 7B). These data suggest that INCB9471 and UK427587 may use distinct binding modes on CCR5.

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**Fig. 5.** Inhibitory activities of INCB9471 against binding of \(^{125}I\)-MIP-1\(\alpha\), \(^{125}I\)-MIP-1\(\beta\), and \(^{125}I\)-RANTES to heterologously expressed recombinant CCR5. A, validation of the recombinant CCR5 binding assay. Homologous competition binding of \(^{125}I\)-MIP-1\(\alpha\), \(^{125}I\)-MIP-1\(\beta\), and \(^{125}I\)-RANTES (0.25 nM each) to human CCR5 expressed on HEK293 cells were measured in the presence of different amounts of unlabeled chemokines. B, INCB9471 inhibition of \(^{125}I\)-MIP-1\(\alpha\), \(^{125}I\)-MIP-1\(\beta\), and \(^{125}I\)-RANTES (0.25 nM each) binding to the recombinant CCR5. The compound was titrated over the concentration range indicated, and binding of the radioligands to the HEK293/CCR5 cells was measured. The value of each data point is an average of duplicates. Data shown are representative of two independent assays. The data were analyzed by nonlinear regression analysis using a one-site competition model.

**Fig. 6.** Inhibitory potencies of different CCR5 antagonists against binding of \(^{125}I\)-MIP-1\(\alpha\) (A), \(^{125}I\)-MIP-1\(\beta\) (B), and \(^{125}I\)-RANTES (C) to recombinant CCR5. Binding of the radioligands (0.25 nM each) to HEK293/CCR5 cells was measured in the presence of CCR5 inhibitors titrated over the concentration range indicated. The value of each data point is an average of duplicates. Each curve is representative of two independent assays. The data were analyzed by nonlinear regression analysis using a one-site competition model.
Site-Specific Mutagenesis. Several residues of CCR5 were predicted to be located in a putative small-molecule inhibitor binding pocket believed to be essential for HIV-1 infection inhibitory activity of two small-molecule antagonists [4-[(Z)-(4-bromophenyl)-(ethoximino)methyl]-1-[(2,4-dimethyl-3-pyridinyl)carbonyl]-4'-methyl-1,4'-bipiperidine N-oxide (SCH-C) and (2,4-dimethylpyridin-3-yl)-[4-methyl-3-(35)-3-methyl-4-[[1S]-1-[4-(trifluoromethyl)phenyl]ethyl]piperazin-1-yl]-1-piperidyl)methanone (AD101)] (Tsamis et al., 2003). These residues include Leu33 and Tyr37 in TM1, Phe79 and Trp86 in TM2, Tyr108 and Phe113 in TM3, Ile198 in TM5, and Glu283 in TM7. As part of the study to understand the molecular mechanism by which INCB9471 interacts with CCR5, the ability of alanine mutants of each of these residues to bind [3H]INCB9471 was examined using the membrane binding assay. All mutants were expressed at a level similar to that of the wild type (data not shown). Alanine mutation of Glu283 in TM7 dramatically reduced the ability of the receptor to interact with the compound because very little binding of [3H]INCB9471 to the mutant receptor could be observed. Structural alteration caused by each of the Ile198 → Ala, Tyr108 → Ala, and Phe113 → Ala mutations also significantly affected INCB9471 binding to the receptor with the $B_{\text{max}}$ values being reduced by more than 3-fold, and $K_d$ values increased by more than three times. Leu33 → Ala, Tyr37 → Ala, Phe79 → Ala, and Trp86 → Ala did not appreciably change the ability of the receptor to bind the compound (Table 2).

Kinetics of [3H]INCB9471 Binding to CCR5. The rate at which [3H]INCB9471 associates with and dissociates from CCR5 was measured using membranes of HEK293SF/M CCR5 cells. To investigate the association rate, a single concentration of 10 nM [3H]INCB9471 was incubated with 10 μg of CCR5 membrane in the presence or absence of an excessive amount of unlabeled INCB9471 for different periods of time (up to 1.5 h). A rapid binding rate was observed within the first 5 min, which gradually leveled off, reaching a plateau (up to 1.5 h). A rapid binding rate was observed within the first 5 min, which gradually leveled off, reaching a plateau (up to 1.5 h). A rapid binding rate was observed within the first 5 min, which gradually leveled off, reaching a plateau (up to 1.5 h). A rapid binding rate was observed within the first 5 min, which gradually leveled off, reaching a plateau (up to 1.5 h). A rapid binding rate was observed within the first 5 min, which gradually leveled off, reaching a plateau (up to 1.5 h). A rapid binding rate was observed within the first 5 min, which gradually leveled off, reaching a plateau (up to 1.5 h). A rapid binding rate was observed within the first 5 min, which gradually leveled off, reaching a plateau (up to 1.5 h).

TABLE 2

<table>
<thead>
<tr>
<th>Mutant</th>
<th>$K_d$ (nM)</th>
<th>$B_{\text{max}}$ (cpm)</th>
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<td>Wild type</td>
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<td>8.8 ± 0.2</td>
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<td>Leu33 → Ala (TM1)</td>
<td>5.9 ± 0.4</td>
<td>4.6 ± 0.2</td>
</tr>
<tr>
<td>Tyr37 → Ala (TM1)</td>
<td>8.2 ± 0.5</td>
<td>5.1 ± 0.3</td>
</tr>
<tr>
<td>Phe79 → Ala (TM2)</td>
<td>4.9 ± 1.4</td>
<td>8.7 ± 0.9</td>
</tr>
<tr>
<td>Trp86 → Ala (TM2)</td>
<td>8.3 ± 1.7</td>
<td>7.1 ± 0.7</td>
</tr>
<tr>
<td>Tyr108 → Ala (TM3)</td>
<td>16.1 ± 3.3</td>
<td>2.7 ± 0.3</td>
</tr>
<tr>
<td>Phe113 → Ala (TM3)</td>
<td>12.9 ± 7</td>
<td>0.9 ± 0.4</td>
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<tr>
<td>Ile198 → Ala (TM5)</td>
<td>10.7 ± 0.7</td>
<td>2.4 ± 0.2</td>
</tr>
<tr>
<td>Glu283 → Ala (TM7) Not measurable</td>
<td>Not measurable</td>
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Fig. 7. Saturation binding of [3H]INCB9471 to CCR5. Saturation binding of [3H]INCB9471 to the membrane preparations of the recombinant HEK293/CCR5 cells was measured in the absence or presence of different concentrations of unlabeled INCB9471 (A), 874130 (B), SCH-D (C), and UK427857 (D). $B_{\text{max}}$ measures the theoretic maximal binding achievable. $K_d$ is the [3H]INCB9471 concentration responsible for half of the theoretical maximal binding. The value of each data point is an average of duplicates. Data shown are representative of two independent assays. The data were analyzed by nonlinear regression analysis using a one binding site (hyperbola) model.
of the total bound [3H]INCB9471 was still associated with the receptor 24 h after dissociation was allowed to occur.

INCB9471 Inhibition of CCR5-Mediated Ca$^{2+}$ Flux.

The effect of INCB9471 on CCR5-mediated signaling was examined by measuring ligand-induced Ca$^{2+}$ fluorescence response in HEK293SFM cells transiently cotransfected with the constructs expressing CCR5 and G$\alpha_{i,16}$ protein. MIP-1α, MIP-1β, and RANTES induced Ca$^{2+}$ mobilization in these cells in a dose-dependent manner (Fig. 9, B, D, and F). INCB9471 itself could not induce any Ca$^{2+}$ flux in the same cells (data not shown) but could block the Ca$^{2+}$ signaling of all three ligands, with an IC$_{50}$ value that was slightly lower for MIP-1β than for MIP-1α and RANTES (Fig. 9, A, C, and E). It is noteworthy that when INCB9471 was tested against a range of concentrations of MIP-1α, MIP-1β, or RANTES (61–1000 nM) its IC$_{50}$ values remained constant (Fig. 9, A, C, and E). Moreover, increasing the concentration of INCB9471 resulted in a remarkable reduction of total ligand-induced Ca$^{2+}$ fluorescence response, but the potency of each of the three ligands for receptor activation, measured as EC$_{50}$, was not affected by the change in INCB9471 concentration (Fig. 9, B, D, and F). We compared the inhibitory effect of INCB9471 with the other CCR5 antagonists on the chemokine ligand-induced Ca$^{2+}$ flux in the 293SFM/CCR5 cells. Whereas INCB9471, SCH-D, and 873140 demonstrated similar potencies against each of the three ligands, UK427857 was consistently approximately 10 times more potent than the other three compounds (Fig. 10), again suggesting that UK427857 may bind to CCR5 differently than the other molecules.

Discussion

Abundant evidence from studies using either genetic approaches or pharmaceutical intervention strongly support CCR5 as a therapeutic target for HIV-1 infection and, possibly, for inflammatory diseases. In this study, we have developed a $^{125}$I-MIP-1β competition binding assay using rhIL-10-treated primary human monocytes as the source of CCR5. The binding assay is robust (30- to 40-fold window) and enabled us to identify a number of different small-molecule CCR5 inhibitors, among which was INCB9471.

INCB9471 has all of the properties necessary for being classified as a selective CCR5 antagonist. It binds to the recombinant CCR5 with high affinity ($K_d \approx$3 nM) and potently inhibits binding of $^{125}$I-MIP-1α, $^{125}$I-MIP-1β, and $^{125}$I-RANTES to the receptor. It dose-dependently inhibits ligand-induced Ca$^{2+}$ flux in the recombinant CCR5 cells without showing any agonist activity by itself. It seems to be highly specific for CCR5. More than 50 membrane proteins, including several chemokine receptors, were counter-screened with radioligand binding assays for inhibition by INCB9471 and little or no activity was found. It is very potent in inhibiting MIP-β-induced human monocyte migration and R5 tropic HIV-1 infection of human PBMCs with IC$_{50}$ values in the low or sub nanomolar range. Moreover, it has favorable pharmacokinetic profiles in animals, including good oral bioavailability (Xue et al., 2010). These results corroborate well with the observed safety and pharmacokinetic profiles and anti-HIV-1 infection efficacy in clinical trials of the compound.

Efforts were made to understand the mode by which INCB9471 interacts with CCR5 and inhibits the function of the receptor. Previously, a noncompetitive allosteric model has been described whereby binding of the antagonist precludes binding of the tracer ligand in a noncompetitive manner (Ehlert 1988; Watson et al., 2005). This model predicts depression of the maxima of saturation binding curves of the tracer ligand by the inhibitor without concomitant change of the affinity of the tracer ligand. In the current study, an inspection of the relationship between the $^{125}$I-MIP-1β and INCB9471 in saturation binding assays using rhIL-10-treated human monocytes revealed a general reduction in the maximal $^{125}$I-MIP-1β binding with increasing amounts of INCB9471 while the affinity (measured as $K_d$) of $^{125}$I-MIP-1β for the receptor remained the same. Consistent with these results from the saturation binding assays, the maximal response in Ca$^{2+}$ flux induced by MIP-1α, MIP-1β, or RANTES in the recombinant CCR5 cells was found to be decreased in the presence of increasing amounts of INCB9471 but the potency (measured as EC$_{50}$ values) of each of the three ligands was not changed. These results fit the noncompetitive model as explained by Ehlert (1988) and Watson et al. (2005) and, therefore, suggest that INCB9471 is an allosteric noncompetitive CCR5 inhibitor.

The conclusion that INCB9471 is an allosteric noncompetitive CCR5 inhibitor is further supported by three other pieces of evidence obtained in this study. First, the INCB9471 IC$_{50}$ values observed in the $^{125}$I-MIP-1β binding displacement assay and MIP-1α, MIP-β, or RANTES-induced Ca$^{2+}$ flux assay...
infection, which predicts an allosteric mechanism as the only
function of the receptor in an allosteric fashion (Tsamis et al.,
SCH-C and 873140 have also been suggested to inhibit the
INCB9471 was able to displace only approximately 70% of
This finding is in line with our belief that INCB9471 binds
log [INCB9471] (nM) of MIP-1β and RANTES (F) in inducing Ca2+
formation time of approximately 15 to 20 min (data not shown).
that CCR5, the bound 3H-
inhibits binding of125I-MIP-1β to CCR5, the bound 3H-INCB9471 could not be displaced by MIP-1β (data not shown).
was not affected by a 10-fold change in the dose of either the
tracer or the chemokine ligands. Second, although INCB9471
inhibited binding of 125I-MIP-1β to CCR5, the bound 3H-INCB9471 could not be displaced by MIP-1β (data not shown).
Third, incomplete displacement of receptor radioligand is often considered as a hallmark of allosteric interaction. In the 125I-RANTES binding inhibition experiments, INCB9471 was able to displace only approximately 70% of the total bound radioligand. Small-molecule CCR5 inhibitors SCH-C and 873140 have also been suggested to inhibit the function of the receptor in an allosteric fashion (Tsamis et al., 2003; Watson et al., 2005). In fact, one would not expect orthosteric hindrance as a mode of interaction between CCR5 and any small-molecule inhibitors. The natural ligands of CCR5 are proteins. The size of a small molecule would be hardly sufficient to block the protein-protein interaction unless the inhibitor and ligand happen to share the same key residues for binding. For the interaction between CCR5 and HIV-1, mutational studies have indicated that many regions of both CCR5 and gp120 need to interact to promote HIV infection, which predicts an allosteric mechanism as the only possible explanation for a low molecular weight antagonist of CCR5 to be effective in blocking HIV-1 entry (Kazmierski et al., 2002). Our conclusion that INCB9471, a potent inhibitor of CCR5 signaling and HIV-1 infection, is an allosteric inhibitor of CCR5 is completely consistent with this concept. Consistent with this conclusion, a recent study indicates that N,N-dimethyl-N-[4-[[2-(4-methylphenyl)-6,7-dihydro-5H-benzo
carbonyl]amino]benzyl]tetrahydro-2H-pyran-4-amination chloride (TAK779) and UK427857 both inhibit HIV-1 infection by a noncompetitive and allosteric mechanism (Garcia-Perez et al., 2011).
Time-course studies on the binding of [3H]INCB9471 to CCR5 revealed an approximately two-phase curve, with a rapid phase within the first 5 min, which was then followed by a much slower one. The amount of the radiolabeled compound bound during the first rapid phase accounts for only approximately 20% of the maximal binding, which requires approximately a 90-min incubation time. A possible explanation for the two-phase INCB9471 binding curve is that CCR5, like many other G protein-coupled receptors, does not really exist in a singular state (see review by Kenakin, 2004).
Therefore, at any time, only a portion of the total CCR5 on the cell membrane is in the “right” conformation for INCB9471 to bind. After the correct sites are occupied by the compound, it may take some time for the remainder of the receptor population to adopt the pertinent conformation. In the Ca2+ flux assay, if INCB9471 was added simultaneously with ligand to the recombinant CCR5 cell it did not interfere with the signaling at all. The inhibitory effect of the compound did not appear unless the compound had been preincubated with the cells for 2 to 3 min before addition of the ligand. Stabilization of its EC50 values required a preincubation time of approximately 15 to 20 min (data not shown). This finding is in line with our belief that INCB9471 binds
CCR5 at a site different from that of the ligands and allosterically causes a conformation change in the receptor.

We followed the dissociation time course of INCB9471 from CCR5 for approximately 24 h. Based on the data generated by the study, it could not be accurately estimated what the t_{1/2} for reversal of INCB9471 binding really is, but the off-rate of INCB9471 seems to be very slow. Fifty percent of initially bound [3H]-INCB9471 still remained associated with the receptor 24 h after the dissociation process started. Because it would be predicted that the protection from HIV infection requires constant allosteric modulation of the receptor, the slow dissociation rate of INCB9471 from CCR5 is expected to be beneficial to the therapeutic value of the compound.

We also compared the potency of INCB9471 with that of SCH-D, UK427857, and 873140 in blocking binding of 125I-RANTES to CCR5. No meaningful differences in IC_{50} values were found among these four compounds. When tested in the MIP-1α, MIP-1β, or RANTES-induced Ca^{2+} flux assay, UK427857 is approximately 10 times more potent than INCB9471, 873140, and SCH-D. The IC_{50} values of the latter three compounds are comparable. To explain this discrepancy, we hypothesized that UK427857 has a binding site on CCR5 that is different from that of INCB9471, SCH-D, and INCB9471 and therefore engagement of this binding site by UK427857 can result in a different conformational change in CCR5, which renders the receptor less efficient in G protein coupling. To test this hypothesis we monitored the change in K_{d} and B_{max} values in [3H]INCB9471 saturation binding to CCR5 in the presence of unlabeled INCB9471, SCH-D, 843140, or UK427857. As a positive control, we showed that the affinity of [3H]INCB9471 for CCR5 was reduced with increasing amounts of unlabeled INCB9471 (reduction in K_{d} values) but the B_{max} values were not changed, a result expected if the radiolabeled and unlabeled compound share the same binding site. Similar data were obtained for SCH-D and 873140, suggesting these two compounds and INCB9471 share a similar binding pocket on CCR5.

It is noteworthy that adding UK427857 to the [3H]INCB9471 saturation binding assay reduced the B_{max} values but not as much the affinity of the radioligand for CCR5, supporting our hypothesis that UK427857 and INCB9471 share a similar binding site. This phenomenon of two different small-molecular CCR5 antagonists using two different binding sites is apparently not unique for INCB9471 and UK427857. The CCR5 binding site for three structurally related compounds SCH-C, AD101, and TAK779 was mapped to a similar pocket formed between TM1, TM2, TM3, and TM7 (Dragic et al., 2000; Tsamis et al., 2003; Seibert et al., 2006) but members of another set of chemically unrelated small-molecular inhibitors bind to a CCR5 pocket formed between TM2, TM3, TM6, and TM7 (Castonguay et al., 2003). Site-specific mutagenesis studies were performed by analyzing
the binding of [3H]INCB9471 to several alanine mutants of the CCR5 receptor. Our results indicated that Glu283 in TM7 is absolutely required for INCB9471 binding to CCR5. Ile198 in TM5 and Tyr108, Phe113 in TM3 are important but not essential. Further study is still needed to fully map out the INCB9471 binding pocket on CCR5.

There are therapeutic implications of INCB9471 and UK427857 having different binding sites on CCR5 that pertain to the use of these two antagonists in the treatment of HIV. HIV is known to mutate rapidly, resulting in sequence changes in its Env complex with no concomitant loss of function (Poignard et al., 2001), and resistance can occur through the production of an escape mutant in the presence of an antagonist over time (Trkola et al., 2002; Kuhmann et al., 2004). INCB9471 and UK427857 may induce different conformational changes in CCR5 because of their separate binders, and determine whether the two antagonists will generate different profiles of resistant mutant viruses.

Acknowledgments
We thankLisaZhu, LynnLeffet, PatFeldman, andDawnEllis for excellent technical assistance.

AuthorshipContributions
Participated in research design: Shin, Solomon, Baribaud, Friedman, Scherer, and Newton.
Contributed new reagents or analytic tools: Xue and Metcalf.
Performed data analysis: Shin, Solomon, Covington, and Baribaud.
Erickson-Vitanen, Contel, Liu, Burn, Hollis, Yelleswaran, Vaddi, Metcalf, Friedman, Scherler, and Newton.
Write or contributed to the writing of the manuscript: Shin, Reference:

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