A Novel Cannabinoid Peripheral Cannabinoid Receptor-Selective Inverse Agonist Blocks Leukocyte Recruitment in Vivo


New Lead Discovery (C.A.L.) and Departments of Inflammation and Infectious Diseases (J.S.F., A.R.-T., J.V.J., X.F., W.G., M.A.S., S.K.N., D.J.L., R.W.H., L.A.B.), Allergy (T.T.K.), and Chemistry (B.L., J.A.K.), Schering-Plough Research Institute, Kenilworth, New Jersey

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ABSTRACT

The expression of the cannabinoid peripheral cannabinoid receptor (CB2) receptor on peripheral immune cells suggests that compounds specific for CB2 might be effective anti-inflammatory agents. In this report, we present the initial biological characterization of a CB2-selective dihydropyrazole, SR144528 (N-[1(S)-endo-1,3,3-trimethylbicyclo [2.2.1]heptan-2-yl]-5-(4-chloro-3-methylphenyl)-1-[4-methylphenyl(phenyl)-1H-pyrazole-3-carboxamide). In vitro, Sch.336 impairs the migration of CB2-expressing recombinant cell lines to the cannabinoid agonist 2-arachidonylglycerol. In vivo, the compound impairs migration of cells to cannabinoid agonist HU210 [(6αR)-trans-3-(1,1-dimethylheptyl)-6a,7,10,10a-tetrahydro-1-hydoxy-6,6-dimethyl-6H-dibenzo [b,d] pyran-9-methanol]. Oral administration of the Sch.336 significantly inhibited leukocyte trafficking in several rodent in vivo models, induced either by specific chemokines or by antigen challenge. Finally, oral administration of Sch.336 blocked ovalbumin-induced lung eosinophilia in mice, a disease model for allergic asthma. We conclude that selective cannabinoid CB2 inverse agonists may serve as novel immunomodulatory agents in the treatment of a broad range of acute and chronic inflammatory disorders in which leukocyte recruitment is a hallmark of disease pathology.

The identification of a second cannabinoid receptor present primarily in peripheral immune tissues and cells (Munro et al., 1993) suggested an immunomodulatory role for endocannabinoids independent of the effects mediated by their interaction with the “brain” cannabinoid CB1 receptor. Libraries of selective compounds now exist, based either on the structure of known ligands for the cannabinoid receptors or on results of random compound library screening (Huffman, 2000). With the help of CB2-specific compounds and by characterization of a CB2−/− mouse strain (Buckley et al., 2000),...
several functions have been reported for CB2. These functions include modulation of B-cell differentiation (Carayon et al., 1998), altered macrophage migration (Sacerdote et al., 2000), altered antigen processing (McCoy et al., 1999), and altered cannabinoid-mediated antitumor activity (Zhu et al., 2000). The proposed native ligand for CB2, 2-arachidonoyl-glycerol, may stimulate both chemotaxis (directional migration) and chemokinesis (random migration) in vitro (Jorda et al., 2002), and was recently shown to modulate 12-0-tetradecanoylphorbol-13-acetate-induced acute inflammation in the mouse ear (Oka et al., 2005). The CB2 receptor has also been implicated in pain sensitivity (Malan et al., 2003).

We recently reported the discovery of a new class of CB2-specific ligands, the triaryl bis-sulfones, an example of which is designated Sch.336. This compound exhibits nanomolar potency for the human cannabinoid CB2 receptor and marked selectivity versus the human CB1 receptor. We also showed that the compound behaves as an inverse agonist in a recombinant membrane-based GTPγS binding assay (Lavey et al., 2005). Here, we demonstrate that Sch.336 effectively blocks the migration of cells in response both to the cannabinoid agonist 2-arachidonoyl-glycerol in vitro and to more complex chemotactic signals in vivo. We show that oral administration of Sch.336 significantly impairs leukocyte trafficking in three diverse models of inflammation. These data suggest that the cannabinoid CB2 receptor plays a critical role in leukocyte recruitment to sites of inflammation and indicates that CB2-selective inverse agonists may provide a novel immunotherapeutic treatment of inflammatory diseases.

### Materials and Methods

**Mice.** Female B6D2F1 and CF-1 mice were obtained from Charles River Laboratories, Inc. (Wilmington, MA) and were used at 6 to 10 weeks of age. Male B6D2F1/J mice weighing 20 to 25 g were purchased from The Jackson Laboratory (Bar Harbor, ME). All experiments were carried out according to protocols and guidelines established by the Schering-Plough Research Institute Department of Animal Care and Use Committee, Schering-Plough Research Institute and are in accordance with appropriate Association for Assessment and Accreditation of Laboratory Animal Care guidelines.

**Reagents.** The CB2-selective bis-sulfone Sch.336 and the related CB2-selective bis-sulfones Sch.A and Sch.B were prepared by the Schering-Plough Research Institute Department of Chemical Research. Recombinant cytokines and fluorochrome-conjugated anti-chemokine receptor antibodies were purchased from R&D Systems (Minneapolis, MN). Anti-mouse leukocyte antibodies were purchased from Invitrogen. CB2 or CB1 transfectants at 1.5 × 10^6/ml were preincubated with designated test compounds in assay buffer (phenol red free-RPMI supplemented with 10% FBS) for 30 min at 37°C. Chemotactants (30 μl) diluted in assay buffer were dispensed into the bottom wells of disposable microchemotaxis plates (ChemoTx 101–5 sp; Neuroprobe Inc., Gaithersburg, MD). Twenty-five-microliter cell aliquots (50,000 cells) were then applied to filters (5-μm pore size) in the top plate. After incubation for 90 min, nonmigrated cells were removed using a cell scraper. Filters were washed with 25 μl of assay buffer. Migrated cells were collected in the bottom well by centrifuging the microplate assembly for 10 min at room temperature in a Sorvall RT Legend centrifuge equipped with an H-100B microplate rotor at 241.6g. Per manufacturer’s instructions, cells in the bottom well were transferred to wells of a flat-bottom Microlite (1+) luminometer plate (Thermo Electron Corporation, Waltham, MA) by centrifugation at 241.6g for 5 min. Eighty microliters of assay buffer and 100 μl of CellTiter Glo Reagent (Promega) were added per well. After incubation at room temperature for 10 min, luminescence intensity was measured using a luminometer (Thermo Electron Corporation) at an excitation time of 100 ms/well. In preliminary experiments, we have shown a linear relationship exists between luminescence intensity and cell number. Relative migration is reported as a percentage of total input cell number. Data are presented as the mean of triplicate determinations.

**Sponge Implantation and Cell Preparation.** In vivo chemotaxis to chemokine-soaked sponges was carried out as described previously (Fine et al., 2000). In brief, sterile 1-cm³ gel foam sponges (Henry Schein, Port Washington, NY) were soaked (1 h) with desired agent, then implanted into the peritoneal cavity of anesthetized female B6D2F1 mice. Sch.336 or vehicle (0.4% methylcellulose) were administered by oral gavage 1 h before and 3 to 4 h after sponge...
implantation. After 18 h, the sponges were removed and incubated for 1 h at 37°C in Cell Dissociation Buffer (Invitrogen) followed by mincing. Resulting single cell suspensions were recovered by centrifugation, washed with Ca/Mg-free Dulbecco's PBS, and fixed with 2.5% glutaraldehyde containing crystal violet dye (Sigma-Aldrich). Cell numbers were quantified microscopically using a hemocytometer and expressed as mean ± S.E.M., with at least five mice used per treatment group. Multiparameter flow cytometric evaluation was performed as described previously (Fine et al., 2000).

Delayed-Type Hypersensitivity Model. The model was carried out as described previously (Fine et al., 2003). Female CF-1 mice were immunized subcutaneously near the axilla with 0.2 ml of a 1:1:1 mixture of methylated BSA (mBSA; 50 mg/ml in saline), complete Freund's adjuvant (Sigma-Aldrich), and 0.5 mg of Mycobacterium tuberculosis dried cell walls (strains C, DT, and PN; Veterinary Laboratory Agency, Surrey, UK). Fourteen days later, anesthetized mice were challenged intrapleurally with 200 μg of mBSA in 0.2 ml of endotoxin-free saline (Abbott Laboratories, Abbott Park, IL). At specified times, mice were euthanized, the pleural cavity was exposed surgically, and exudates were harvested using 1 ml of chilled cell dissociation buffer (Invitrogen). Cell recovery, processing, and enumeration, as well as flow cytometric analyses, were performed as described previously (Fine et al., 2003).

Statistical Analysis. Differences between treatment groups were compared by Student's t test using InStat, version 3.00 for Windows 95 (GraphPad Software Inc.), with p < 0.05 being considered as a statistically significant difference.

Results

Pharmacology of Sch.336. The initial characterization of Sch.336 as an inverse agonist was based on the ability of this compound to decrease GTP<sub>S</sub> binding to membranes containing recombinant human CB<sub>2</sub> receptors in vitro (Lavey et al., 2005). We have expanded upon this observation using three independent assay systems. Figure 1A shows that Sch.336 competes with [3H]CP55,940 for binding to human CB<sub>2</sub> on Sf9 cell membranes with K<sub>i</sub> = 1.8 nM. This value is near equivalent to that of the cannabinoid agonist HU210 (K<sub>i</sub> = 3.2 nM) and superior to the CB<sub>2</sub>-selective inverse agonist SR144528 (K<sub>i</sub> = 14.9 nM) and the CB<sub>1</sub>-selective inverse agonist SR141716A (K<sub>i</sub> = 2596 nM). These same compounds were then analyzed for ligand-induced GTP<sub>S</sub> binding to these same membranes. Figure 1B shows that Sch.336 decreases GTP<sub>S</sub> binding on human CB<sub>2</sub>-containing membranes with an EC<sub>50</sub> = 2 nM. SR144528 decreases GTP<sub>S</sub> binding with a decreased potency (EC<sub>50</sub> = 8.9 nM) and roughly 50% decreased efficacy. In contrast, the cannabinoid agonist HU210 increases GTP<sub>S</sub> binding with an EC<sub>50</sub> = 0.9 nM. As expected, Sch.336 shows decreased potency on CB<sub>2</sub>-containing membranes (Fig. 1C), with EC<sub>50</sub> = 200 nM versus that of the CB<sub>1</sub>-specific inverse agonist SR141716A (EC<sub>50</sub> = 5.6 nM).

Competitive ligand binding in the presence of GTP<sub>S</sub> is expected to increase the affinity of inverse agonists for its receptor (de Ligt et al., 2000). Figure 2 shows that the addition of 100 μM GTP<sub>S</sub> increased the potency of Sch.336...
binding to the human CB2, from an EC\textsubscript{50} of 3.2 to 0.23 nM (Fig. 2A). A similar experiment carried out using recombinant Sf9 membranes in the absence (○) or presence (●) of 100 μM GTP-γS as described. Nonlinear regression analysis of saturation data and of concentration-response data were performed with Prism 2.0 software (GraphPad Software Inc.) to calculate K\textsubscript{d} and B\textsubscript{max}. Data show [\textsuperscript{3}H]CP55,940 binds to CB2 with a K\textsubscript{d} = 1.0 ± 0.3 nM (n = 2) in the absence of GTP-γS, and with K\textsubscript{d} = 5.8 ± 0.3 nM (n = 2) in the presence of GTP-γS.

Fig. 2. Effect of GTP-γS on saturation binding to the human CB2 receptor. Saturation binding of [\textsuperscript{3}H]CP55,940 to human CB2 receptor was carried out using recombinant Sf9 membranes in the absence (○) or presence (●) of 100 μM GTP-γS as described. Nonlinear regression analysis of saturation data and of concentration-response data were performed with Prism 2.0b software (GraphPad Software Inc.) to calculate K\textsubscript{d} and B\textsubscript{max}. Data show [\textsuperscript{3}H]CP55,940 binds to CB2 with a K\textsubscript{d} = 1.0 ± 0.3 nM (n = 2) in the absence of GTP-γS, and with K\textsubscript{d} = 5.8 ± 0.3 nM (n = 2) in the presence of GTP-γS.

Sch.336 Inhibits Recombinant Cell Chemotaxis in Vitro. Having documented the potency, selectivity, and pharmacology of Sch.336 for the human CB2 receptor, we examined the effect of Sch.336 on human peripheral blood mononuclear cell and murine T cell proliferation and T cell and monocyte cytokine production and surface marker up-regulation. These activities are associated with immune modulation ascribed to cannabinoid CB2 agonists (Kaplan et al., 2003). In each case, Sch.336 and related congeners were either inactive or demonstrated limited activity at concentrations >5 μM, which is inconsistent with a CB2-mediated response. The compound showed no apparent toxicities in any in vitro cellular system tested (data not shown).

Several laboratories (Sacerdote et al., 2000; Kishimoto et al., 2003) have shown that cannabinoid agonists can induce cellular migration. Of particular note, Jorda et al. (2002) demonstrated that splenic B lymphocytes migrate to 2-arachidonylglycerol, an effect blocked by CB2 antagonists but not by CB1 antagonists. Therefore, we tested the affect of Sch.336 on directional cell migration of cells expressing cannabinoid receptors. Figure 4A shows that BaF/3 cells expressing human CB2, as described under Materials and Methods. Figure 3 shows that the Sch.336 potentiates or increases the forskolin-stimulated cAMP accumulation in recombinant CHO cells with an EC\textsubscript{50} = 1.9 nM. This result is similar to the IC\textsubscript{50} = 0.6 nM value obtained using GTP-γS binding to recombinant Sf9 membranes (Lavey et al., 2005) and is more potent than the Sanofi CB2 inverse agonist SR144528, which exhibits an EC\textsubscript{50} = 23 nM. In contrast, the two cannabinoid agonists WIN55,212-2 and HU210 inhibited the accumulation of cAMP in the forskolin-stimulated recombinant CHO cells, exhibiting IC\textsubscript{50} values of 4.6 and 2.9 nM, respectively. Thus, Sch.336 seems to be a more effective CB2-selective inverse agonist than SR144528 (Rinaldi-Carmona et al., 1998).

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Sch.336 inhibits BaF3/CB1 migration to 100 nM 2-AG with an IC_{50} = 324 nM, whereas SR141716A inhibits the migration with an IC_{50} = 525 nM (Fig. 4D), consistent with the CB2-selective nature of Sch.336. We found potency of this inhibitory effect to be dependent on the concentration of the chemoattractant-increasing 2-AG concentration to 1 mM results in a corresponding increase in IC_{50} for both selective inverse agonists (Fig. 4, C and D).

Sch.336 Inhibits Leukocyte Migration in Vivo. Having demonstrated that Sch.336 blocks chemotaxis to cannabinoid agonists in vitro, we sought to extend this activity to an in vivo setting. For these experiments, we used an implantable sponge model for cell recruitment, which allowed us to control the distribution of the chemoattractant agent and allowed easy quantification of infiltrating cells (Fine et al., 2000). Sch.336 and SR144528 were administered to female B6D2/F1 mice 3 h after i.p. implantation of a 1-cm-diameter gel foam sponge soaked with HU210 (30 μg/ml). After 18 h, the mice were sacrificed, and the sponges were retrieved. Cells entering the sponges were quantified as described under Materials and Methods. In Fig. 5, we show that both Sch.336 and the CB2-selective dihydropyrazole, SR144528, attenuate total cell recruitment to HU210 in a dose-dependent manner, with a maximal effect at 0.2 mg/kg. We next examined whether oral administration of Sch.336 could attenuate migration mediated by a chemokine, CCL2. This monocyte and macrophage chemoattractant has been implicated in the pathogenesis of various chronic inflammatory immune diseases including atherosclerosis (Charo and Taubman, 2004), multiple sclerosis (Mahad and Ransohoff, 2003), arthritis (Quinones et al., 2005), and inflammatory airway disease (Owen, 2001). Figure 6A shows that oral administratio-
tion of Sch.336 significantly inhibited the migration of leukocytes into the CCL2-soaked gel foam sponge, with an IC\textsubscript{50} at 2 to 5 mg/kg, equivalent to the nonsteroidal anti-inflammatory drug indomethacin. Flow cytometric analysis of cells recovered from CCL2-soaked sponges (Fine et al., 2000) revealed that recruitment of monocytes/macrophages (Gr-1\textsuperscript{+}CD11b\textsuperscript{+}), granulocytes (Gr-1\textsuperscript{+}CD11b\textsuperscript{+}), and lymphocytes (CD3\textsuperscript{+}CD11b\textsuperscript{−}) was inhibited in a dose-dependent manner (Fig. 6B). This effect does not seem to be mediated by a direct effect on the CCR2 receptor because we have found that 2 \mu g/ml Sch.336 did not alter \textsuperscript{[125I]}CCL2 binding to membranes expressing recombinant CCR2 (data not shown). The CB1-selective inverse agonist SR141716A (Shire et al., 1999) at 10 to 20 mg/kg neither had any effect on cell influx to the CCL2-soaked sponge nor was it able to interfere with the ability of 2 mg/kg Sch.336 to inhibit recruitment. These results reinforce the notion that inverse agonism at CB2, but not CB1, is responsible for the blockade of leukocyte migration (Fig. 6C). Moreover, the ability of Sch.336 to block cell migration was directly dependent on its relative potency for CB2 because structurally related congeners of Sch.336 did not ameliorate cell recruitment (Fig. 7). We emphasize that compounds in this experiment were given i.p. to ensure adequate drug availability.

Sch.336 also inhibited leukocyte recruitment in a murine model of delayed-type hypersensitivity (Fine et al., 2003). In this model, a local inflammatory cellular response is elicited by intrapleural challenge with mBSA in sensitized animals. Figure 8A shows that oral administration of 10 mg/kg Sch.336 significantly inhibited leukocyte accumulation in the pleural space as early as 6 h after antigen challenge and that this response persisted through 72 h. This effect was equivalent to that obtained with the dihydropyrazole CB\textsubscript{2} inverse agonist SR144528 and with methotrexate (Fig. 8B), as well as with the nonsteroidal anti-inflammatory compound piroxicam (data not shown). These data indicate that CB\textsubscript{2}-selective inverse agonists can impair leukocyte recruitment in vivo in an antigen-mediated response.

**Antigen-Induced Lung Eosinophilia Is Suppressed by Sch.336.** Finally, we tested the efficacy of Sch.336 in an antigen-induced allergic mouse model in which sensitized
animals are challenged with aerosolized ovalbumin (Kung et al., 1994). The ovalbumin-induced lung model is an accepted model for the inflammatory component of human asthma featuring hallmarks of the disease, such as lung eosinophilia and pulmonary influx of CD4+ Th2 T lymphocytes (Gonzalo et al., 1996; Cohn et al., 2004; Kasserra et al., 2004; Munitz and Levi-Schaffer, 2004). Oral administration of Sch.336 at 2 mg/kg b.i.d. 1 h before ovalbumin challenge resulted in a significant decrease in the total number of cells (Fig. 9A) and eosinophils (Fig. 9B) present in the bronchoalveolar lavage (BAL) of allergic mice. The efficacy of Sch.336 was similar to that observed after treatment with the PDE4 inhibitor rolipram. No effect of cell recruitment was observed in animals dosed with 0.5 mg/kg Sch.336.

Discussion

It is a general contention that pharmacological manipulation of leukocyte recruitment in target tissues will have broad application for modulating chronic inflammatory diseases such as asthma, multiple sclerosis, and rheumatoid arthritis. For example, antibodies targeting \( \alpha 4 \beta 1/ \alpha 4 \beta 7 \) integrin-dependent pathways have been shown to block leukocyte recruitment and to dampen cell influx in a variety of preclinical models of chronic inflammation (Sircar et al., 2002). In addition, administration of a humanized anti-\( \alpha 4 \) monoclonal antibody has demonstrated efficacy in the treatment of multiple sclerosis and Crohn’s disease (Ghosh et al., 2003; Miller et al., 2003). Significant efforts have been devoted to the discovery and development of antagonists for specific chemokine receptors (Chen et al., 2004), such as CCR1 for multiple sclerosis, rheumatoid arthritis, and transplantation (Horuk, 2003); CCR3 for eosinophil migration accompanying asthma (Varnes et al., 2004); CXCR2 antagonists for chronic obstructive pulmonary disease, arthritis, and reperfusion injury (Widdowson et al., 2004); and CCR5 (Horuk, 2003) and CXCR4 (De Clercq, 2003).

Several laboratories have reported the ability of cannabinoid agonists to affect the chemotactic activity of immune cells. For example, the cannabinoid agonist CP55,940 enhanced rat peritoneal macrophage chemotaxis by a mechanism that was shown to be CB2-dependent (Sacerdote et al., 2000). Two groups (Jorda et al., 2002; Kishimoto et al., 2003) have reported enhanced chemotactic activities in mouse splenocytes and human peripheral blood monocytes to 2-arachidonoylglycerol. Both studies concluded that these responses were mediated through the cannabinoid CB2 receptor. More recently, it was shown by Oka et al. (2004) that 2-arachidonoylglycerol could stimulate human eosinophil migration, again through the CB2 receptor. These studies point to a role for CB2 agonism in mediating enhanced cell migration. Our studies support the contention that CB2 inverse agonists can directly inhibit cellular chemotaxis, both in vitro and in vivo. In this report, we demonstrate that a novel class of CB2-specific compounds, the triaryl bis-sulfones, modulates endocannabinoid-induced chemotaxis of recombinant cells in vitro (Fig. 4), modulates endocannabinoid- and chemokine-induced cell recruitment to an implanted gel foam sponge in vivo (Figs. 5 and 6), and modulates antigen-induced cell influx into the peritoneal cavity and into the lung (Figs. 8 and 9). The compounds, identified through by compound library screening and exemplified by Sch.336, behave as inverse agonists in vitro, as shown by an ability to decrease ligand-induced GTP\(_S\) binding to the hCB2 receptor (Fig. 1), sensitivity to binding in the presence of GTP\(_S\) (Fig. 2), and their capacity to increase forskolin-stimulated cAMP production (Fig. 3). Moreover, the compound can also attenuate the cell chemotaxis in vitro to a potent cannabinoid agonist 2-arachidonoylglycerol in a CB2-dependent fashion (Fig. 4).

Our contention that Sch.336 functions through mediation of cellular chemotaxis relies primarily in vivo models of cellular recruitment. We chose this approach to insure that the compound could impact the complex chemokine environments and diverse cytokine signals that influence efficacy in vivo (Paoletti et al., 2005). To this end, in vivo models selected were designed to evaluate potencies in systems of
increasing biological complexity. We were able to demonstrate that HU210, a cannabinoid agonist, would induce cell migration into the sponge, and that this effect was attenuated by the CB2 inverse agonist Sch.336 (Fig. 5). We next demonstrated that oral administration of Sch.336 attenuated monocyte/macrophage, granulocyte, and lymphocyte migration to a CCL2-soaked gel foam sponge (Fig. 6). This effect was dose-dependent, unaffected by coadministration with a CB1-specific antagonist SR141716A, and required that the triaryl bis-sulfone bind to the CB2 receptor (Fig. 7). These results, when coupled with the inability of Sch.336 to alter binding of CCL2 to CCR2 or to down-regulate CCR2 expression (data not shown), support our contention that Sch.336 exerts its bioactivity through the CB2 receptor. We next demonstrate that SCH 336 can dampen pleuritis induced in a murine model of delayed type hypersensitivity (Fig. 8). Finally, we show that oral administration of Sch.336 attenuates ovalbumin-induced lung eosinophilia, a system that mimics certain pathobiological hallmarks of human asth-

Fig. 8. Inhibition of pleural cavity allergic delayed-type hypersensitivity reaction. A, methylated BSA sensitized female CF-1 mice were challenged with 200 μg of mBSA i.p. after being dosed with 10 mg/kg piroxicam or Sch.336 at designated times (arrows). At specified times, cells from the pleural cavity were collected as described under Materials and Methods and quantified microscopically. Total cells are expressed as mean ± S.E.M., with at least five mice used per treatment group. ■, mBSA sensitized, mBSA challenge, dose with MC vehicle; □, mBSA sensitized, mBSA challenge, dose with piroxicam; ▲, mBSA sensitized, saline challenge, dose with MC vehicle; △, mBSA sensitized, mBSA challenge, dose with Sch.336. B, methylated BSA sensitized female CF-1 mice were challenged with 200 μg of mBSA i.p. after being dosed with designated concentrations of SR144528 (■), Sch.336 (△), or methotrexate (▲). As described, cells from the pleural cavity were collected and quantified microscopically. Total cells are expressed as mean ± S.E.M. *p < 0.01 from control; **p < 0.001 from challenge.

Unlike selective chemokine receptor antagonists, Sch.336 seems to interfere with the ability of diverse leukocyte populations to migrate to sites of inflammation (Fig. 6). However, treatment with the compound did not completely eliminate chemotactic activity, which may suggest that immunosuppression may not be an overriding issue with chronic treatment. To date, the biochemical mechanism by which Sch.336 exerts its activity is unclear. We have been unable to demonstrate an effect of Sch.336 on any secondary chemotactic effector; although not conclusive, we hypothesize that Sch.336 functions directly on leukocytes. Sch.336 (at 2 μg/ml) was unable to block chemokine interaction with its cognate receptor and does not seem to modulate expression of a panel of chemokine receptors on white blood cells (data not shown). Initial studies have shown effects of Sch.336 on chemokine-induced polarization of human peripheral blood mononuclear cells (L.A. Bober, data not shown). Mechanisms
involving modification of heterologous interactions between cannabinoid and chemokine receptors and/or modulation of downstream receptor signaling have been proposed. For example, the first CB₂ inverse agonist, the biarylpyrazole SR-144528, was shown to alter p42/p44 mitogen-activated protein kinase signaling mediated by receptors to insulin and LPA (Bouaboula et al., 1999). Heterologous desensitization of chemokine receptors via opioid receptor signaling has also been documented (for review, see Steele et al., 2002). In the case of opioids, desensitization of chemokine receptors seems to be mediated by phosphorylation via calcium-independent protein kinase C isotypes (Zhang et al., 2003). Preliminary data in our laboratory indicate that specific phosphorylation events may be modulated by CB₂-specific triaryl bis-sulfones (C. A. Lunn, data not shown).

References