Novel Pyrrolidine Ureas as C−C Chemokine Receptor 1 (CCR1) Antagonists

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Monocyte infiltration is implicated in a variety of diseases including multiple myeloma, rheumatoid arthritis, and multiple sclerosis. C−C chemokine receptor 1 (CCR1) is a chemokine receptor that upon stimulation, particularly by macrophage inflammatory protein 1α (MIP-1α) and regulated on normal T-cell expressed and secreted (RANTES), mediates monocyte trafficking to sites of inflammation. High throughput screening of our combinatorial collection identified a novel, moderately potent CCR1 antagonist **3**. The library hit **3** was optimized to the advanced lead compound **4**. Compound **4** inhibited CCR1 mediated chemotaxis of monocytes with an IC_{50} of 20 nM. In addition, the compound was highly selective over other chemokine receptors. It had good microsomal stability when incubated with rat and human liver microsomes and showed no significant cytochrome P450 (CYP) inhibition. Pharmacokinetic evaluation of the compound in the rat showed good oral bioavailability.

Introduction

Chemokines, interacting through their respective receptors, are involved in mediating recruitment and activation of immune cells in response to physiological and pathological stimuli. Dysregulation of this process has been implicated in the initiation and progression of a number of important diseases including rheumatoid arthritis (RA[a]), multiple sclerosis (MS), allograft rejection, atherosclerosis, tissue fibrosis, airway hyper-responsiveness, remodeling, and mucus production. CCR1, which is expressed on human monocytes, T lymphocytes, dendritic cells, eosinophils and basophils, plays a key role in the development and progression of different diseases by interacting with its major ligands including C−C chemokine ligand (CCL) 3 (MIP1-α), CCL5 (RANTES), and CCL15 (leukotactin-1). Studies with CCR1 deficient mice, anti-MIP1-α, anti-RANTES, or small molecule inhibitors of CCR1 have demonstrated significant reduction of clinical scores in various animal disease models, such as experimental autoimmune encephalomyelitis,[1] adjuvant induced arthritis,[2,3] graft versus host disease,[4−6] renal fibrosis,[7] interleukin-13-induced lung fibrosis and mucus production,[8] and airway hyper-responsiveness and remodeling in response to infectious agents.[9] Taken together, accumulating evidence suggests that CCR1 is a key player in mediating leukocyte infiltration and activation and contributes to the pathogenesis of different diseases. As a result, small molecule antagonists of CCR1 may have therapeutic potential for the treatment of different immunological disorders without eliciting a general immunosuppressive effect. Therapeutic applications of CCR1 antagonists were further evaluated in clinical trials conducted by Pfizer with **1** (CP-481715)[10] and Berlex/Schering AG with **2** (BX471)[11] for RA and MS, respectively (Figure 1). Although efficacy was not observed in these trials, CCR1 still remains as an attractive therapeutic target for the treatment of a number of human diseases. Improvement of compound potency and physical/chemical properties represents a viable approach to identify new CCR1 antagonists suitable for clinical evaluation. In this study, we identified novel, selective, small molecule CCR1 antagonists with high oral bioavailability and potent activities in inhibiting receptor binding and monocyte chemotaxis. These compounds with clinically desired attributes represent a new chemical series for CCR1 antagonism and therapeutic intervention.

Upon screening of our combinatorial compound collection, we identified the pyrrolidine amide **3** as an initial lead. This compound was a moderate inhibitor of the CCR1 receptor with an IC_{50} of 660 nM. In addition, the compound possessed good stability in vitro with rat liver microsomes as well as good permeability as assessed in a Caco-2 assay. These latter results suggested that this class of compound might exhibit acceptable bioavailability and warrant further optimization.

Chemistry

Synthesis of these pyrrolidine derivatives was straightforward following the general procedures outlined in Schemes 1, 2, and 3. The amine derivatives **5a**−**o** were either commercially available or readily prepared as described. Amides were prepared by coupling of respective amines to the appropriate pyrrolidine carboxylic acid using *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDC)/1-hydroxybenzotriazole (HOBt) or benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBOP). Urea formation proceeded either through coupling of the commercially available isocyanate or via treatment of the corresponding aniline with triphosgene to form the isocyanate in situ.

Results and Discussion

In Vitro Structure−Activity Relationships (SAR). Initial SAR exploration focused on the alkylamide portion of the molecule. Truncation of the hydroxyethyl moiety to give the NH amide **8** resulted in complete loss of activity (Table 1). Replacement of hydroxyethyl with *n*-propyl **9** resulted in

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[a] Abbreviations: CCL, C−C chemokine ligand; CCR1, C−C chemokine receptor 1; CYP, cytochrome P450; DIEA, *N*,*N*-diisopropylethylamine; EDC, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide; HOBt, 1-hydroxybenzotriazole; MIP-1α, macrophage inflammatory protein 1α; MS, multiple sclerosis; PyBOP, benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate; RA, rheumatoid arthritis; RANTES, regulated on normal T-cell expressed and secreted; SAR, structure−activity relationships; TFA, trifluoroacetic acid.

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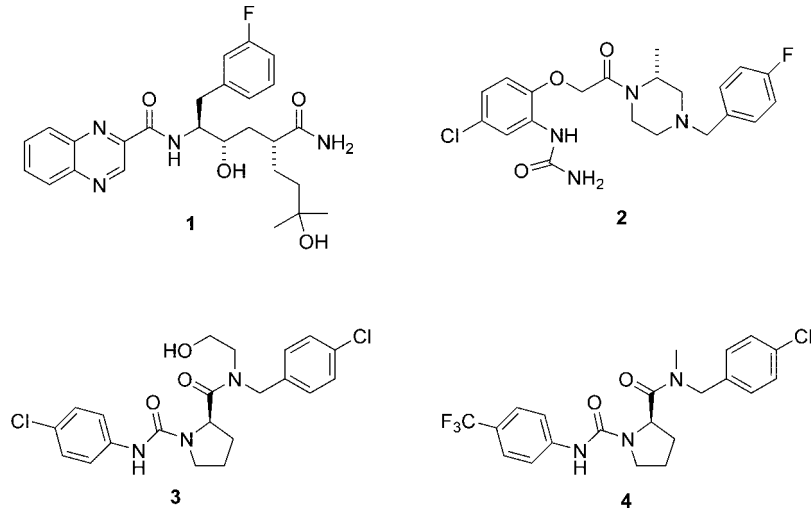
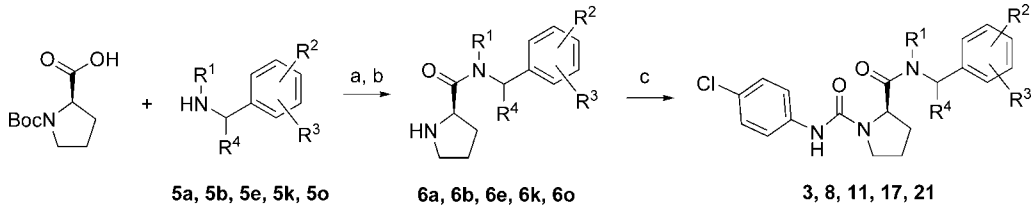


Figure 1

Scheme 1[a]



[a] Reagents and conditions: (a) EDC, HOBt, CH_2Cl_2; or PyBOP, CH_2Cl_2; (b) trifluoroacetic acid (TFA), CH_2Cl_2; or 4 M HCl in dioxane; (c) 4-chlorophenyl isocyanate, *N*,*N*-diisopropylethylamine (DIEA), CH_2Cl_2.

significant loss of potency. Smaller alkyl groups however were well tolerated. The ethyl and methyl amides **10** and **11** were slightly more potent than the original hydroxyethyl lead.

Next our focus turned to the benzylic region. Shifting the chloro group to the 2- and 3-positions, **12** and **13**, was not tolerated. Also not tolerated was replacement of 4-chloro with 4-cyano **14**, 4-methoxy **15**, or 4-trifluoromethyl **16**. Replacement of 4-chloro with 4-fluoro **17** or 4-methyl **18** resulted in significant loss of potency. Further substitution of the 4-chlorobenzylamide with 2-methyl **19** and 3-methyl **20** resulted in loss or reduction of activity. Methyl substitution at the α-benzylic position, **21**, also reduced potency.

SAR around the 4-chlorobenzylamide appeared to be tightly constrained; thus, we shifted our investigations to the phenylurea portion of the molecule. Replacement of 4-chloro with 4-methyl **22**, 4-fluoro **23**, 4-cyano **24**, and 4-methoxy **25** resulted in modest loss of potency (Table 2). Substitution with 4-trifluoromethyl **4**, however, resulted in a compound with improved potency. This compound was also a potent inhibitor of MIP-1α-induced chemotaxis. Compound **4** was not as potent as the standard compound **2** which, when tested in the same assays, bound to CCR1 with $IC_{50} = 5.5 \pm 0.3$ nM and inhibited chemotaxis with $IC_{50} = 1.5 \pm 0.2$ nM.

Since substitution at the 3-position was not well-tolerated, as exemplified by compound **26**, we retained the 4-trifluoromethylphenylurea and investigated further substitution in the 2- and 6- positions. 2-Chloro substitution **27** had no impact on potency. Substitution of the 2-position with fluoro **28** and methyl **29** resulted in improved binding potency but showed no significant improvement for inhibition of chemotaxis. Only the 2,6-dimethyl-4-trifluorophenylurea **30** gave improved inhibition of chemotaxis with an IC_{50} of 8 nM.

With more optimal substituents now in place, we investigated the effect of changes in core ring size and stereochemistry. Expansion to the six-membered ring racemate **31** dramatically reduced potency (Figure 2). Contraction to the four-membered ring gave compound **32**, which was inactive at CCR1. Inversion of stereochemistry gave the *S*-pyrrolidine amide **33**, which also had poor activity.

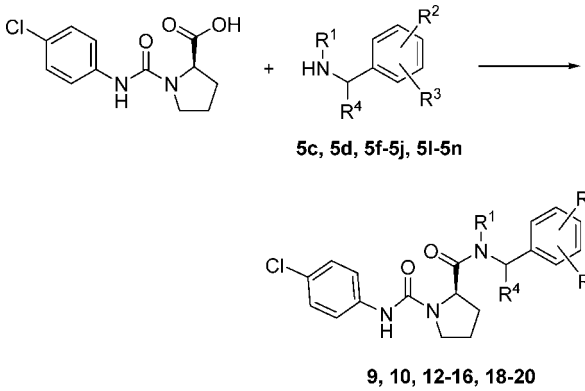
Further Testing. Having identified five reasonably potent compounds, we next assessed in vitro metabolic stability, permeability, and in vivo pharmacokinetics in rat. All of the compounds exhibited good stability in the presence of human and rat microsomes and good permeability in Caco-2 assays. When dosed orally in rats, compound **4** had the best exposure (Table 3). Further substitution at the 2-position with halogen or methyl resulted in decreased exposure. The most potent compound **30** also had the lowest exposure of the five.

Since it had good potency and acceptable oral exposure in the rat, compound **4** was selected for further study. The compound was found to have good pharmacokinetics in rat with 45% absolute oral bioavailability, $t_{1/2}$ of 4.4 h, and low plasma clearance of 4.2 (mL/min)/kg. No significant CYP inhibition was observed for 3A4, 2D6, or 1A2 up to 20 μM. The compound showed no significant activity against a broad panel of glycoprotein coupled receptors and ion channel receptors. In addition compound **4** was inactive versus other chemokine receptors including CCR2b, CCR4, CCR5, CXCR1, CXCR2, and CX3CR1. Pyrrolidine ureas such as **4** comprise a novel chemotype for antagonism of CCR1 and appear to be suitable for further pharmaceutical development. Further optimization of this series has been achieved and will be reported in due course.

Experimental Section

Mass spectrometry was conducted using a Thermo Finnigan LCQ classic ion trap or a PE SCIEX API 150EX single quadrupole. Liquid chromatography−mass spectrometry was conducted using

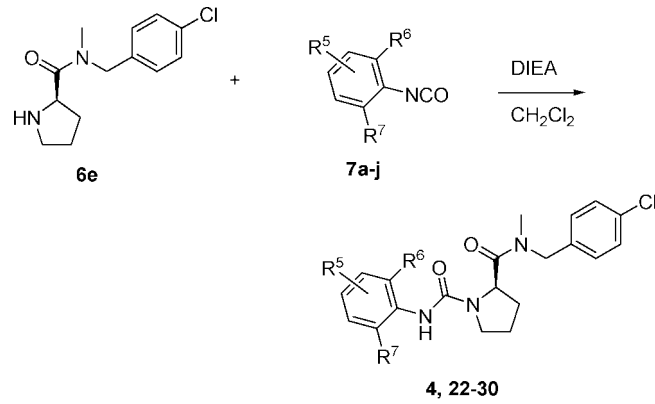
Scheme 2[a]



	R^1	R^2	R^3	R^4
a	CH_2CH_2OH	4-Cl	H	H
b	H	4-Cl	H	H
c	n-Pr	4-Cl	H	H
d	Et	4-Cl	H	H
e	Me	4-Cl	H	H
f	CH_2CH_2OH	2-Cl	H	H
g	CH_2CH_2OH	3-Cl	H	H
h	Me	4-CN	H	H
i	Me	4-OMe	H	H
j	Me	4-CF_3	H	H
k	Me	4-F	H	H
l	Me	4-Me	H	H
m	Me	4-Cl	2-Me	H
n	Me	4-Cl	3-Me	H
o	Me	4-Cl	H	Me

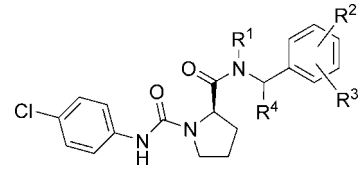
[a] Reagents and conditions: EDC, HOBt, CH_2Cl_2; or PyBOP, CH_2Cl_2.

Scheme 3



	R^5	R^6	R^7
7a	4-Me	H	H
7b	4-F	H	H
7c	4-CN	H	H
7d	4-OMe	H	H
7e	4-CF_3	H	H
7f	3-CF_3	H	H
7g	4-CF_3	Cl	H
7h	4-CF_3	F	H
7i	4-CF_3	Me	H
7j	4-CF_3	Me	Me

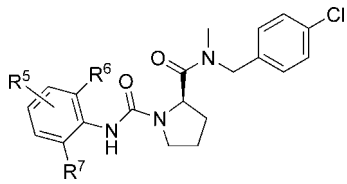
Table 1



compd	R^1	R^2	R^3	R^4	IC_{50} (nM)
3	CH_2CH_2OH	4-Cl	H	H	540 ± 210
8	H	4-Cl	H	H	>75000
9	*n*-Pr	4-Cl	H	H	3600 ± 710
10	Et	4-Cl	H	H	270 ± 160
11	Me	4-Cl	H	H	270 ± 20
12	CH_2CH_2OH	2-Cl	H	H	43000 ± 2800
13	CH_2CH_2OH	3-Cl	H	H	>100000
14	Me	4-CN	H	H	>13,000
15	Me	4-OMe	H	H	>77,000
16	Me	4-CF_3	H	H	>100000
17	Me	4-F	H	H	1300 ± 700
18	Me	4-Me	H	H	5700 ± 3300
19	Me	4-Cl	2-Me	H	>100000
20	Me	4-Cl	3-Me	H	4000 ± 280
21	Me	4-Cl	H	Me	4700 ± 700

a Waters 2690 with a 996 photodiode array detector linked to a Thermo Finnigan LCQ classic ion trap. High resolution mass spectrometry was conducted using a Micromass LCT oa-TOF with a Waters 2795 HPLC system and Waters 996 PDA. ^{1}H NMR spectroscopy was conducted using a Varian 300 MHz Gemini 2000 FT NMR. Elemental analyses were carried out by Robertson Microlit Laboratories. TLC was performed on silica gel plates purchased from Analtech Co. Flash column chromatography was performed using silica gel-60 (230−400 mesh). Preparative HPLC was carried out on a Rainin Dynamax system with Sunfire Prep C18 OBD, 5 μm, 19 mm × 100 mm column. All tested target compounds possessed a purity of at least 95% as determined by elemental analysis, when listed, or by HPLC−MS. HPLC for purity determinations were conducted using a Waters 2690 HPLC with a 996 photodiode array detector at wavelengths of 215 and 254 nm and a Phenomenex, Columbus, C18, 5 μm, 100 mm × 2.00 mm column. Solvent and gradient conditions for HPLC purity determinations were as follows: solvent A, 0.05% TFA in water; solvent B, 0.05%TFA in acetonitrile; flow rate = 0.4 mL/min; gradient, 0−0.50 min at 10% B, 0.50−5.50 min at 10% B → 90% B (linear), 5.50−7.50 min at 90% B, 7.50−8.00 min at 90% B → 10% B (linear), 8.00−10.00 min at 10% B; total run time = 10 min.

Table 2



compd	R^5	R^6	R^7	binding IC_{50} (nM)	chemotaxis IC_{50} (nM)
22	4-Me	H	H	680 ± 90	ND
23	4-F	H	H	700 ± 70	ND
24	4-CN	H	H	720 ± 330	ND
25	4-OMe	H	H	290 ± 20	440 ± 00
4	4-CF_3	H	H	140 ± 10	22 ± 9
26	3-CF_3	H	H	2950 ± 210	ND
27	4-CF_3	Cl	H	120 ± 7	35 ± 15
28	4-CF_3	F	H	23 ± 4	22 ± 7
29	4-CF_3	Me	H	50 ± 10	16 ± 4
30	4-CF_3	Me	Me	11 ± 00	6 ± 3

(*R*)-N^2-(4-Chlorobenzyl)-N^1-(4-chlorophenyl)-N^2-(2-hydroxyethyl)pyrrolidine-1,2-dicarboxamide (3). To a solution of **6a** in CH_3CN (2 mL) was added Et_3N (132 mg) and 4-chlorophenyl isocyanate (132 mg). The reaction mixture was stirred at room temperature for 16 h. The mixture was concentrated and purified by preparative HPLC to give pure compound (140 mg, 61%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.88−2.04 (m, 3H), 2.22 (m, 2H), 3.13 (m, 1H), 3.51 (m, 1H), 3.70 (m, 2H), 3.87 (m, 2H), 4.05 (m, 1H), 4.63−4.99 (m, 1H), 5.03−5.07 (m, 1H), 5.19 (m, 1H), 7.13−7.39 (m, 8H). Anal. ($C_{21}H_{23}Cl_2N_3O_3$) C, H. N: calcd, 9.63. Found, 7.22. HRMS (ESI) *m*/*z* calcd for $C_{21}H_{24}Cl_2N_3O_3$ $[M + H]^+$: 436.1195. Found: 436.1191.

(*R*)-N^2-(4-Chlorobenzyl)-N^2-methyl-N^1-[4-(trifluoromethyl)phenyl]pyrrolidine-1,2-dicarboxamide (4). To a solution of **6e** (50 mg, 0.2 mmol) in dichloromethane (2 mL) were added 4-(trifluoromethyl)phenyl isocyanate (0.030 mL, 0.2mmol) and DIEA (0.050 mL). The reaction mixture was stirred for 12 h and then evaporated to dryness. Purification by silica gel column chromatography (ethyl acetate/hexane) afforded the desired product as a white amorphous solid (40.9 mg, 47%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.66 (m, 1H), 1.84 (m, 2H), 2.12 (m, 1H), 2.91 (s, 3H), 3.50 (m, 2H), 4.30 (m, 1H), 4.47 (m, 1H), 4.71 (m, 1H), 7.24 (m, 4H), 7.49 (d, 2H), 7.63 (m, 2H), 8.58 (s, 1H). Anal. ($C_{21}H_{21}ClF_3N_3O_2$) C, H, N. MS calcd: 440.1 $(M + H)^+$. Found: 439.8.

(*R*)-N^2-(4-Chlorobenzyl)-N^1-(4-chlorophenyl)pyrrolidine-1,2-dicarboxamide (8). 8 was prepared from **6b** and 4-chlorophenyl isocyanate by the same procedure described for **4**. Yield: 48%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.94−2.03 (m, 3H), 2.21−2.26 (m, 1H), 3.57 (m, 1H), 3.75 (m, 1H), 4.38 (m, 2H), 4.47 (m, 1H), 7.38−7.48 (m, 6H), 7.67−7.72 (m, 2H), 8.48 (s, 1H), 8.57 (t, 1H). Anal. ($C_{19}H_{19}Cl_2N_3O_2$) C, H, N. MS calcd for $C_{19}H_{20}Cl_2N_3O_2$: 392.1 $(M + H)^+$. Found: 391.9.

General Procedure B for Amide Formation. To a solution of (*R*)-1-[(4-chlorophenyl)carbamoyl]pyrrolidine-2-carboxylic acid (1 equiv) and the appropriate amine **5** (1 equiv) in dichloromethane was added EDC hydrochloride (2.5 equiv) and catalytic HOBt. The mixture was stirred at room temperature overnight, then filtered and concentrated to give the crude product which was purified by flash chromatography.

General Procedure C for Amide Formation. To a solution of (*R*)-1-[(4-chlorophenyl)carbamoyl]pyrrolidine-2-carboxylic acid (1 equiv) and the appropriate amine **5** (1 equiv) in dichloromethane was added PyBOP (1.5 equiv). The mixture was stirred at room temperature overnight, then concentrated to give the crude product which was purified by flash chromatography.

(*R*)-N^2-(4-Chlorobenzyl)-N^1-(4-chlorophenyl)-N^2-propylpyrrolidine-1,2-dicarboxamide (9). 9 was prepared from **5c** according to general procedure B. Yield: 6%. ^{1}H NMR (300 MHz, CD_3OD) δ 0.82−0.94 (m, 3H), 1.46−2.40 (m, 6H), 3.03−3.46 (m, 2H), 3.52−3.72 (m, 2H), 4.52−4.64 (m, 2H), 4.76−4.82 (m, 1H), 7.22−7.28 (m, 4H), 7.34−7.43 (m, 4H). HRMS (ESI) *m*/*z* calcd for $C_{22}H_{26}Cl_2N_3O_2$ $[M + H]^+$: 434.1402. Found: 434.1394.

(*R*)-N^2-(4-Chlorobenzyl)-N^1-(4-chlorophenyl)-N^2-ethylpyrrolidine-1,2-dicarboxamide (10). 10 was prepared from **5d** according to general procedure B. Yield: 3%. ^{1}H NMR (300 MHz, CD_3OD) δ 1.07 (t, 1H), 1.25 (t, 2H), 1.80−2.40 (m, 4H), 3.42−3.72 (m, 4H), 4.50−4.64 (m, 2H), 7.26 (m, 5H), 7.38 (m, 3H). HRMS (ESI) *m*/*z* calcd for $C_{21}H_{24}Cl_2N_3O_2$ $[M + H]^+$: 420.1246. Found: 420.1235.

(*R*)-N^2-(4-Chlorobenzyl)-N^1-(4-chlorophenyl)-N^2-methylpyrrolidine-1,2-dicarboxamide (11). 11 was prepared from **6e** and 4-chlorophenyl isocyanate by the same procedure described for **4**. Yield: 65%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.66 (m, 1H), 1.79 (m, 2H), 1.96−2.20 (m, 1H), 2.79−2.89 (m, 3H), 3.45 (m, 2H), 4.29−4.77 (m, 3H), 7.12−7.20 (m, 4H), 7.27 (m, 1H), 7.33 (m, 1H), 7.46 (m, 2H), 8.28 (m, 1H). Anal. ($C_{20}H_{21}Cl_2N_3O_2$) C, H, N. MS calcd for $C_{20}H_{22}Cl_2N_3O_2$: 406.1 $(M + H)^+$. Found: 405.8.

(*R*)-N^2-(2-Chlorobenzyl)-N^1-(4-chlorophenyl)-N^2-(2-hydroxyethyl)pyrrolidine-1,2-dicarboxamide (12). 12 was prepared from **5f** according to general procedure C. Yield: 5%. ^{1}H NMR (300 MHz, CD_3OD) δ 1.93−2.36 (m, 4H), 3.53−3.76 (m, 6H), 4.61−4.88 (m, 2H), 5.05 (m, 1H), 7.25 (m, 4H), 7.30−7.45 (m, 4H). HRMS (ESI) *m*/*z* calcd for $C_{21}H_{24}Cl_2N_3O_3$ $[M + H]^+$: 436.1195. Found: 436.1208.

(*R*)-N^2-(3-Chlorobenzyl)-N^1-(4-chlorophenyl)-N^2-(2-hydroxyethyl)pyrrolidine-1,2-dicarboxamide (13). 13 was prepared from **5g** according to general procedure B. Yield: 8%. ^{1}H NMR (300 MHz, CD_3OD) δ 1.82−2.40 (m, 4H), 3.37−3.76 (m, 6H), 4.56−4.82 (m, 2H), 5.05 (m, 1H), 7.26 (m, 4H), 7.30−745 (m, 4H). HRMS (ESI) *m*/*z* calcd for $C_{21}H_{24}Cl_2N_3O_3$ $[M + H]^+$: 436.1795. Found: 436.1181.

(*R*)-N^2-(4-Cyanobenzyl)-N^1-(4-chlorophenyl)-N^2-methylpyrrolidine-1,2-dicarboxamide (14). 14 was prepared from **5h** according to general procedure B. Yield: 6%. ^{1}H NMR (300 MHz, CD_3OD) δ 1.85−2.40 (m, 4H), 2.90−3.12 (m, 3H), 3.56−3.72 (m, 2H), 4.52−4.67 (m, 1H), 4.80−4.96 (m, 2H), 7.24−7.26 (d, 2H), 7.40−7.76 (m, 6H). HRMS (ESI) *m*/*z* calcd for $C_{21}H_{22}ClN_4O_2$ $[M + H]^+$: 397.1431. Found: 397.1441.

(*R*)-N^2-(4-Methoxybenzyl)-N^1-(4-chlorophenyl)-N^2-methylpyrrolidine-1,2-dicarboxamide (15). 15 was prepared from **5i** according to general procedure C. ^{1}H NMR (300 MHz, CD_3OD) δ 1.82−2.34 (m, 4H), 2.86−3.01 (m, 3H), 3.55−3.70 (m, 2H), 3.74 (m, 3H), 4.53 (m, 1H), 4.90 (m, 2H), 6.83−6.95 (m, 2H), 7.16−7.32 (m, 4H), 7.39−7.42 (m, 2H). HRMS (ESI) *m*/*z* calcd for $C_{21}H_{25}ClN_3O_3$ $[M + H]^+$: 402.1584. Found: 402.1584.

(*R*)-N^2-[4-(Trifluoromethyl)benzyl]-N^1-(4-chlorophenyl)-N^2-methylpyrrolidine-1,2-dicarboxamide (16). 16 was prepared from **5j** according to general procedure C. Yield: 5%. ^{1}H NMR (300 MHz, CD_3OD) δ 1.86−2.42 (m, 4H), 2.92−3.06 (m, 3H), 3.56−3.74 (m, 2H), 4.56−4.82 (m, 2H), 4.92−4.98 (m, 1H), 7.24−7.27 (m, 2H), 7.45 (m, 4H), 7.60−7.63 (m, 2H). HRMS (ESI) *m*/*z* calcd for $C_{21}H_{22}ClF_3N_3O_2$ $[M + H]^+$: 440.1353. Found: 440.1345.

(*R*)-N^2-(4-Fluorobenzyl)-N^1-(4-chlorophenyl)-N^2-methylpyrrolidine-1,2-dicarboxamide (17). 17 was prepared from **6k** and 4-chlorophenyl isocyanate by the same procedure described for **4**. Yield: 52%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.65 (m, 1H), 1.85 (m, 2H), 1.95−2.20 (m, 1H), 2.63−2.89 (m, 3H), 3.41−3.50 (m, 2H), 4.29−4.47 (m, 2H), 4.74 (m, 1H), 7.00−7.20 (m, 4H), 7.35 (m, 1H), 7.46 (m, 2H), 8.29 (s, 1H). Anal. ($C_{20}H_{21}ClFN_3O_2$) C, H, N. MS calcd for $C_{20}H_{22}ClFN_3O_2$: 390.1 $(M + H)^+$. Found: 389.9.

(*R*)-N^2-(4-Methylbenzyl)-N^1-(4-chlorophenyl)-N^2-methylpyrrolidine-1,2-dicarboxamide (18). 18 was prepared from **5l** according to general procedure B. Yield: 9%. ^{1}H NMR (300 MHz, CD_3OD) δ 1.83−2.14 (m, 4H), 2.30−2.32 (m, 3H), 2.88−3.04 (m, 3H), 3.56−3.73 (m, 2H), 4.48−4.88 (m, 2H), 4.92 (m, 1H), 7.13−7.25 (m, 6H), 7.40−7.42 (m, 2H). HRMS (ESI) *m*/*z* calcd for $C_{21}H_{25}ClN_3O_2$ $[M + H]^+$: 386.1680. Found: 386.1688.

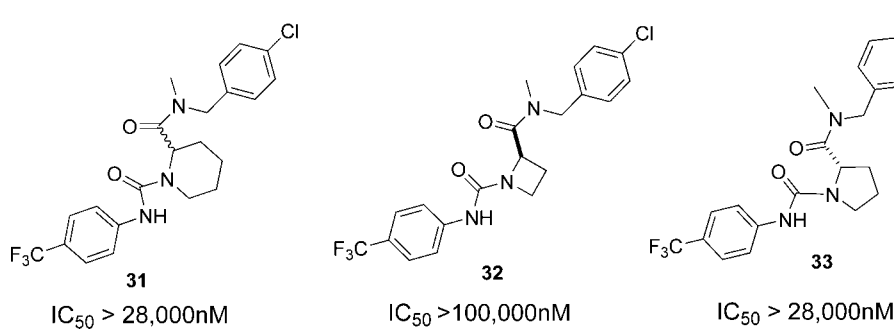


Figure 2

Table 3

compd	HLM (% remaining)	RLM (% remaining)	$AUC_{(0-\infty)}$ po (ng/(mL·h))	C_{max} (ng/mL)
4	78	74	9390 ± 1590	834 ± 299
27	73	68	2060 ± 391[a]	437 ± 53
28	93	76	1240 ± 452[a]	291 ± 89
29	58	81	2590 ± 428[a]	699 ± 153
30	74	84	611 ± 221	195 ± 33

[a] $AUC_{(0-6)}$.

(*R*)-*N*²-(4-Chloro-2-methylbenzyl)-*N*¹-(4-chlorophenyl)-*N*²-methylpyrrolidine-1,2-dicarboxamide (19). 19was prepared from **5m** according to general procedure C. Yield: 9%. ^{1}H NMR (300 MHz, CD_3OD) δ 1.83−2.40 (m, 7H), 1.80−2.46 (m, 4H), 2.92−3.05 (m, 3H), 3.55−3.70 (m, 2H), 4.59 (m, 2H), 4.92−4.96 (m, 1H), 7.14−7.26 (m, 5H), 7.38−7.42 (m, 2H). HRMS (ESI) *m*/*z* calcd for $C_{21}H_{24}Cl_2N_3O_2$ $[M + H]^+$: 420.1246. Found: 420.1249.

(*R*)-*N*²-(4-Chloro-3-methylbenzyl)-*N*¹-(4-chlorophenyl)-*N*²-methylpyrrolidine-1,2-dicarboxamide (20). 20 was prepared from **5n** according to general procedure C. Yield: 6%. ^{1}H NMR (300 MHz, CD_3OD) δ 1.83−2.39 (m, 7H), 2.88−3.07 (m, 3H), 3.53−3.74 (m, 2H), 4.40−4.74 (m, 2H), 4.80 (m, 1H), 7.04−7.36 (m, 5H), 7.40−7.44 (m, 2H). HRMS (ESI) *m*/*z* calcd for $C_{21}H_{24}Cl_2N_3O_2$ $[M + H]^+$: 420.1246. Found: 420.1244.

(2*R*)-*N*¹-(4-Chlorophenyl)-*N*²-[1-(4-chlorophenyl)ethyl]-*N*²-methylpyrrolidine-1,2-dicarboxamide (21). 21 was prepared from **6o** and 4-chlorophenyl isocyanate by the same procedure described for **4**. Yield: 34%. ^{1}H NMR (300 MHz, CD_3OD) δ 1.47−1.70 (m, 3H), 1.80−2.46 (m, 4H), 2.56−2.64 (m, 1H), 2.77−2.80 (m, 2H), 3.55−3.73 (m, 4H), 5.36−5.90 (m, 1H), 7.21−7.49 (m, 8H). Anal. ($C_{21}H_{23}Cl_2N_3O_2$) C, H, N. MS calcd: 420.1 $(M + H)^+$. Found: 419.9.

(*R*)-*N*²-(4-Chlorobenzyl)-*N*²-methyl-*N*¹-*p*-tolylpyrrolidine-1,2-dicarboxamide (22). 22 was prepared from **6e** and 4-methylphenyl isocyanate by the same procedure described for **4**. Yield: 69%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.67 (m, 1H), 1.78−2.13 (m, 6H), 2.64−2.89 (m, 3H), 3.47 (m, 2H), 4.29−4.75 (m, 3H), 6.94 (m, 2H), 7.13 (m, 2H), 7.23−7.33 (m, 4H), 8.02 (s, 1H). Anal. ($C_{21}H_{24}ClN_3O_2$) C, H, N. MS calcd for $C_{21}H_{25}ClN_3O_2$: 386.1 $(M + H)^+$. Found: 385.9.

(*R*)-*N*²-(4-Chlorobenzyl)-*N*¹-(4-fluorophenyl)-*N*²-methylpyrrolidine-1,2-dicarboxamide (23). 23 was prepared from **6e** and 4-fluorophenyl isocyanate by the same procedure described for **4**. Yield: 77%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.65 (m, 1H), 1.81−2.16 (m, 3H), 2.63−2.89 (m, 3H), 3.40−3.48 (m, 2H), 4.29−4.76 (m, 3H), 6.97 (t, 2H), 7.13 (d, 1H), 7.25 (d, 1H), 7.37−7.42 (m, 4H), 8.17 (s, 1H). Anal. ($C_{20}H_{21}ClFN_3O_2$) C, H, N. MS calcd for $C_{20}H_{22}ClFN_3O_2$: 390.1 $(M + H)^+$. Found: 389.9.

(*R*)-*N*²-(4-Chlorobenzyl)-*N*¹-(4-cyanophenyl)-*N*²-methylpyrrolidine-1,2-dicarboxamide (24). 24 was prepared from **6e** and 4-cyanophenyl isocyanate by the same procedure described for **4**. Yield: 71%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.68 (m, 1H), 1.82−1.88 (m, 2H), 1.98−2.19 (m, 1H), 2.65−2.91 (m, 3H), 3.45−3.56 (m, 2H), 4.28−4.78 (m, 3H), 7.13 (d, 1H), 7.24 (d, 1H), 7.34 (t, 2H), 7.43 (d, 2H), 7.62−7.65 (m, 2H), 8.56 (s, 1H). Anal. ($C_{21}H_{21}ClN_4O_2$) C, H, N. MS calcd for $C_{21}H_{22}ClN_4O_2$: 397.1 $(M + H)^+$. Found: 396.8.

(*R*)-*N*²-(4-Chlorobenzyl)-*N*¹-(4-methoxyphenyl)-*N*²-methylpyrrolidine-1,2-dicarboxamide (25). 25 was prepared from **6e** and 4-methoxyphenyl isocyanate by the same procedure described for **4**. Yield: 87%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.61−1.67 (m, 1H), 1.79−1.94 (m, 2H), 2.08−2.15 (m, 1H), 2.64−2.89 (m, 3H), 3.23 (s, 3H), 3.39−3.51 (m, 2H), 4.30−4.75 (m, 3H), 6.73 (d, 2H), 7.13−7.33 (m, 6H), 7.97 (s, 1H). Anal. ($C_{21}H_{24}ClN_3O_3$) C, H, N. MS calcd for $C_{21}H_{25}ClN_3O_3$: 402.1 $(M + H)^+$. Found: 401.8.

(*R*)-*N*²-(4-Chlorobenzyl)-*N*²-methyl-*N*¹-(3-(trifluoromethyl)phenyl)pyrrolidine-1,2-dicarboxamide (26). 26 was prepared from **6e** and 3-(trifluoromethyl)phenyl isocyanate by the same procedure described for **4**. Yield: 74%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.67 (m, 1H), 1.82−2.18 (m, 3H), 2.65−2.91 (m, 3H), 3.47−3.51 (m, 2H), 4.28−4.77 (m, 3H), 7.14−7.40 (m, 6H), 7.65−7.73 (m, 1H), 7.88 (d, 1H), 8.50 (d, 1H). Anal. ($C_{21}H_{21}ClF_3N_3O_2$) C, H, N. MS calcd for $C_{21}H_{22}ClF_3N_3O_2$: 440.1 $(M + H)^+$. Found: 439.8.

(*R*)-*N*²-(4-Chlorobenzyl)-*N*¹-[2-chloro-4-(trifluoromethyl)phenyl]-*N*²-methylpyrrolidine-1,2-dicarboxamide (27). 27 was prepared from **6e** and 2-chloro-4-(trifluoromethyl)phenyl isocyanate by the same procedure described for **4**. Yield: 99%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.70 (m, 1H), 1.88 (m, 2H), 1.98−2.22 (m, 1H), 2.65−2.90 (m, 3H), 3.51 (m, 2H), 4.30−4.88 (m, 3H), 7.13 (m, 1H), 7.27−7.35 (m, 3H), 7.57 (d, 1H), 7.76 (m, 1H), 7.99 (d, 1H). Anal. ($C_{21}H_{20}Cl_2F_3N_3O_2$) H, N. C: calcd, 53.17. Found: 54.27. HRMS (ESI) *m*/*z* calcd for $C_{21}H_{21}Cl_2F_3N_3O_2$ $[M + H]^+$: 474.0963. Found: 474.0965.

(*R*)-*N*²-(4-Chlorobenzyl)-*N*¹-[2-fluoro-4-(trifluoromethyl)phenyl]-*N*²-methylpyrrolidine-1,2-dicarboxamide (28). 28 was prepared from **6e** and 2-fluoro-4-(trifluoromethyl)phenyl isocyanate by the same procedure described for **4**. Yield: 22%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.96−2.28 (m, 4H), 2.95−3.03 (m, 3H), 3.56 (m, 1H), 3.76 (m, 1H), 4.48−4.67 (m, 2H), 4.82−4.94 (m, 1H), 6.69 (m, 1H), 7.16−7.36 (m, 5H), 8.35 (m, 1H). Anal. ($C_{21}H_{20}ClF_4N_3O_2$) H, N. C: calcd, 55.08. Found, 54.16. HRMS (ESI) *m*/*z* calcd for $C_{21}H_{21}ClF_4N_3O_2$ $[M + H]^+$: 458.1258. Found: 458.1260.

(*R*)-*N*²-(4-Chlorobenzyl)-*N*²-methyl-*N*¹-[2-methyl-4-(trifluoromethyl)phenyl]pyrrolidine-1,2-dicarboxamide (29). 2-Methyl-4-(trifluoromethyl)phenyl isocyanate **7i** was generated in situ by dropwise addition of triphosgene (44.5 mg, 0.15 mmol) in CH_2Cl_2 (1 mL) to a solution of 2-methyl-4-(trifluoromethyl)benzenamine (79 mg, 0.45 mmol) in dichloromethane (1 mL). The mixture was stirred at room temperature for 3 h. The resulting suspension was added dropwise to a solution of **6e** (116 mg, 0.40 mmol) and DIEA (0.175 mL, 1.0 mmol) in dichloromethane (2 mL), stirred for 10 min, and purified by preparative HPLC to give the title product as light-yellow oil (57.3 mg, 32%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.97−2.02 (m, 1H), 2.10−2.14 (m, 1H), 2.24−2.35 (m, 5H), 2.99 (s, 1H), 3.06 (s, 2H), 3.54 (m, 1H), 3.70 (m, 1H), 4.59−4.67 (m, 2H), 4.89−4.98 (m, 1H), 7.17 (d, 1H), 7.24−7.38 (m, 3H), 7.44 (d, 2H), 7.81 (t, 1H). Anal. ($C_{22}H_{23}ClF_3N_3O_2$) C, H, N. MS calcd: 454.1 $(M + H)^+$. Found: 453.9.

(*R*)-*N*²-(4-Chlorobenzyl)-*N*¹-[2,6-dimethyl-4-(trifluoromethyl)phenyl]-*N*²-methylpyrrolidine-1,2-dicarboxamide (30). To a solution of **7j** in dichloromethane was added **6e** (45 mg, 0.18 mmol)

and DIEA (0.063 mL, 0.36 mmol) in dichloromethane (0.5 mL), in one portion, and the mixture was stirred at room temperature overnight. The mixture was concentrated and purified by HPLC to afford 17.3 mg of the desired product. Yield: 24%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.01−2.46 (m, 10H), 3.02−3.09 (m, 3H), 3.61−3.68 (m, 1H), 3.78−3.85 (m, 1H), 4.54−4.79 (m, 2H), 4.89−5.03 (m, 1H), 7.21−7.41 (m, 6H). Anal. ($C_{23}H_{25}ClF_3N_3O_2$) H, N. C: calcd, 59.03. Found, 58.03. HRMS (ESI) *m*/*z* calcd for $C_{23}H_{26}ClF_3N_3O_2$ $[M + H]^+$: 468.1666. Found: 468.1662.

***N*^{2}-(4-Chlorobenzyl)-*N*^{2}-methyl-*N*^{1}-[4-(trifluoromethyl)phenyl]piperidine-1,2-dicarboxamide (31).** 1-(*tert*-Butoxycarbonyl)piperidine-2-carboxylic acid (2.29 g, 10 mmol) and *N*-(4-chlorobenzyl)-*N*-methylamine (1.56 g, 10 mmol) were subjected to the procedure described for **6e** to afford *N*-(4-chlorobenzyl)-*N*-methylpiperidine-2-carboxamide. Yield: 55%. The product (53 mg, 0.2 mmol) was combined with DIEA (50 μL), 4-(trifluoromethyl)phenyl isocyanate (30 μL, 0.21 mmol), and dichloromethane (2 mL) and treated as described for **4**. Yield: 29%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.80−2.00 (m, 6H), 3.12 (s, 3H), 3.66 (m, 1H), 4.04 (m, 1H), 4.52−4.68 (m, 2H), 5.24−5.36 (m, 1H), 7.36 (m, 1H), 7.44 (m, 2H), 7.70−7.82 (m, 4H), 8.00 (m, 1H), 9.03 (s, 1H). Anal. ($C_{22}H_{23}ClF_3N_3O_2$) C, H, N. MS calcd for $Na^+C_{22}H_{23}ClF_3N_3O_2$: 476.1 $(M + Na)^+$. Found: 475.9.

***(R)*-*N*^{2}-(4-Chlorobenzyl)-*N*^{2}-methyl-*N*^{1}-[4-(trifluoromethyl)phenyl]azetidine-1,2-dicarboxamide (32).** (*R*)-1-(*tert*-Butoxycarbonyl)azetidine-2-carboxylic acid (100 mg, 0.49 mmol) and *N*-(4-chlorobenzyl)-*N*-methylamine (76 mg, 0.49 mmol) were subjected to the procedure described for **6e** to afford (*R*)-*N*-(4-chlorobenzyl)-*N*-methylazetidine-2-carboxamide. Yield: 51%. The product (30 mg, 0.13 mmol) was combined with DIEA (44 μL), 4-(trifluoromethyl)phenyl isocyanate (19 μL, 0.13 mmol), and CH_2Cl_2 (2 mL) and treated as described for **4**. Yield: 62%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.41−2.55 (m, 2H), 2.86−2.98 (m, 3H), 3.72−3.79 (m, 1H), 4.02−4.14 (m, 1H), 4.35−4.46 (m, 1H), 4.74−4.79 (d, 1H), 5.04 (t, 1H), 7.09−7.37 (m, 4H), 7.44−7.51 (m, 4H). Anal. ($C_{20}H_{19}ClF_3N_3O_2$) H, N. C: calcd, 56.40. Found: 55.28. HRMS (ESI) *m*/*z* calcd for $C_{20}H_{20}ClF_3N_3O_2$ $[M + H]^+$: 426.1196. Found: 426.1193.

***(S)*-*N*^{2}-(4-Chlorobenzyl)-*N*^{2}-methyl-*N*^{1}-[4-(trifluoromethyl)phenyl]pyrrolidine-1,2-dicarboxamide (33). 33** was prepared from (*S*)-1-(*tert*-butoxycarbonyl)pyrrolidine-2-carboxylic acid by the same procedure described for **4**. Yield: 71%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.65 (m, 1H), 1.82−2.16 (m, 3H), 2.64−2.90 (m, 3H), 3.50 (m, 2H), 4.27−4.78 (m, 3H), 7.11−7.37 (m, 4H), 7.48 (m, 2H), 7.63 (m, 2H), 8.56 (s, 1H). Anal. ($C_{21}H_{21}ClF_3N_3O_2$) C, H, N. MS calcd for $C_{21}H_{22}ClF_3N_3O_2$: 440.1 $(M + H)^+$. Found: 439.8.

Biological Assays: Materials and Methods. Recombinant membranes were prepared by cloning human CCR1 into an episomal expression vector (Horlick-2000)[12] and stably expressing in HEK293 cells. [^{125}I]MIP1α was obtained from Perkin-Elmer (Waltham, MA), and SPA beads were obtained from GE healthcare (Piscataway, NJ). The human monocytic cell line, THP-1, was purchased from ATCC (Manassas, VA). Recombinant human MIP1α was obtained from R&D Systems (Minneapolis, MN). Plates used for chemotaxis in 96-well format (5 μm) were obtained from Corning (Corning, NY). CellTiter-Glo was obtained from Promega, Madison WI.

Receptor and Ligand Binding Assay. The receptor binding assay was conducted in 384-well plates by preparing membrane and SPA bead mixture in assay buffer (130 mM NaCl, 5 mM KCl, 1 mM $MnCl_2$, 50 mM Tris HCl, 7.4, 0.1% BSA) at 50 μg/mL membrane and 10 mg/mL SPA beads. An amount of 10 μL of the membrane/SPA mixture was transferred to the assay plate along with 10 μL of different concentrations of testing compounds. Then 5 μL of [^{125}I]MIP1α was added at a final concentration of 0.1 nM. The plate was spun at 2500 rpm for 2 min and incubated for 4 h and read (1 min/well count time) on a MicroBeta TriLux (Perkin-Elmer).

Chemotaxis Assay. Chemotaxis was conducted in 96-well chemotaxis chambers from Corning. Briefly, agonists were diluted in assay buffer (RPMI plus 0.1% BSA) and then added to the bottom wells of the chamber. THP1 cells with a density of 2×10^6 cells/mL were added to the top chamber in the presence or absence of various concentrations of compounds. The apparatus was incubated for 3 h in a 5% CO_2-humidified incubator at 37 °C. After the incubation period, the migrating cells from the bottom chamber were quantified by transferring equal volumes of cells into CellTiter-Glo and read on PerkinElmer Victor multilable counter using luminescence protocol.

Rat and Human Liver Microsome Stability Assays. Assay mixtures typically contained rat or human microsomes (300 nM cytochrome P450, BD Gentest, Woburn, MA), phosphate buffer (pH 7.4), 1 μM test compound, 1 mM NADPH in a final assay volume of 0.1 mL. Incubations were for 30 min at 37 °C and were terminated by the addition of 0.2 mL of acetonitrile. Samples were centrifuged at 2000*g* and analyzed by LC/MS. The percentage of compound remaining at 30 min was calculated.

Caco-2 Permeability. Caco-2 cells were obtained from ATCC (Rockville, MD). The cells were seeded onto BIOCOAT insert plates (BD Biosciences, San Jose, CA) at a density of 250 000 cells/well in basal seeding medium containing MITO+ serum extender for 3 days followed by the treatment with Entero-STIM medium containing MITO+ serum extender for 2 days. Permeability studies were performed with monolayers cultured for 5 days. The cell monolayer integrity was monitored by transepithelial electrical resistance and by permeation of Lucifer yellow. Permeability studies were initiated by the addition of test compound (20 μM) in Hank's buffered salt solution to either the apical or basolateral side of the monolayer to measure apical to basolateral or basolateral to apical transport, respectively. Plates were incubated at 37 °C and samples taken from the donor solution at time zero and 90 min and from the basolateral solution at 90 min. The concentration of test compound was determined by HPLC/UV.

Cytochrome P450 Inhibition. Human recombinant cytochrome P450 isoenzymes (BD Gentest, Woburn, MA) were tested (CYP1A2, 2C9, 2C19, 2D6, and 3A4). Each isoenzyme converts a specific fluorogenic substrate to a fluorescent product. Test compound (up to 20 μM) was added, and the fluorescence was read, after a defined reaction time, using a Victor V2 Wallac 1420 multilabel counter. The percentage inhibition at each concentration was calculated, and the IC_{50} was determined by fitting the plot of percent inhibition vs logarithmic concentration values (μM).

Pharmacokinetics Studies in the Rat. In preliminary screening studies, test compounds were dosed orally by gavage (5 mg/kg in 0.5% methyl cellulose) to three to four male Sprague−Dawley rats, either as single compounds or as part of a cassette. Serial blood samples were collected from an indwelling arterial cannula up to 6 or 24 h. For bioavailability studies, test compound (1 mg/kg) was also administered intravenously via an indwelling catheter. Plasma was separated, and samples were prepared by protein precipitation and analyzed for parent compound by LC/MS/MS. Pharmacokinetic parameters were determined using noncompartmental analysis (WinNonlin, Pharsight Corp., Mountain View, CA).

Supporting Information Available: Details of intermediate compound synthesis and elemental analysis data. This material is available free of charge via the Internet at http://pubs.acs.org.

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