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Spirodiketopiperazine-based CCR5 antagonist: Discovery of an antiretroviral drug candidate

Rena Nishizawa [a,*], Toshihiko Nishiyama [a], Katsuya Hisaichi [a], Chiaki Minamoto [a], Naoki Matsunaga [a], Yoshikazu Takaoka [a], Hisao Nakai [a], Stephen Jenkinson [c], Wieslaw M. Kazmierski [c], Hideaki Tada [b], Kenji Sagawa [b], Shiro Shibayama [b], Daikichi Fukushima [b], Kenji Maeda [d,e], Hiroaki Mitsuya [d,e]

[a] Medicinal Chemistry Research Laboratory, Ono Pharmaceutical Co., Ltd, Shimamoto, Mishima, Osaka 618-8585, Japan
[b] Exploratory Research Laboratory, Ono Pharmaceutical Co., Ltd, Ibaraki 300-424, Japan
[c] GlaxoSmithKline Research and Development, Five Moore Drive, Research Triangle Park, NC 27709-3398, USA
[d] Department of Internal Medicine II, Kumamoto University School of Medicine, Kumamoto 860-0811, Japan
[e] Experimental Retrovirology Section, HIV & AIDS Malignancy Branch, NCI, National Institutes of Health, Bethesda, MD 20892, USA

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ABSTRACT

Following the discovery that hydroxylated derivative **3** (Fig. 1) was one of the oxidative metabolites of the original lead **1**, it was found that hydroxylated compound **4** possesses higher in vitro anti-HIV potency than the corresponding non-hydroxylated compound **2**. Structural hybridation of **4** with the orally available analog **5** resulted in another orally-available spirodiketopiperazine CCR5 antagonist **6a** that possesses more favorable pharmaceutical profile for use as a drug candidate.

Since the United States Food and Drug Administration (US FDA) approval of the first antiretroviral drug in 1987, new agents have been added to the list of treatment options for human immunodeficiency virus type-1 (HIV-1) infection. Due to the increased incidence of drug-resistant viruses, there has been a growing need for novel treatments of HIV-1 infections. Therefore, the identification of new antiretroviral drugs that have unique mechanisms of action remains an important therapeutic objective.[1] In 1996, it was revealed that one of the *C–C* chemokine receptors, CCR5, is utilized by HIV-1 as one of the essential co-receptors and that the endogenous ligand showed anti-HIV-1 activity in vitro.[2] For such reasons, CCR5 antagonists that inhibit viral entry into the host cell are thought to be one of the most promising class of new antiviral drugs.

Since these discoveries, many pharmaceutical companies and academic institutions have been enthusiastically investigating novel CCR5 antagonists.[3] Thus far Maraviroc is currently the only approved CCR5 antagonist on the market for the treatment of HIV-1 infection. Due to its new mechanism of action, CCR5 antagonists may be utilized in salvage therapy in patients who have become resistant to one or multiple classes of antiretrovirals, in combination with other classes of active antiretroviral agents.

We have previously reported the discovery of spirodiketopiperazines **1** and **2** as structurally novel CCR5 antagonists (Fig. 1).[4] Both of these compounds showed potent anti-HIV activity, in vitro. After the incubation of **1** with human liver microsomes, the hydroxylated analog of 1′ position was identified as the biologically active metabolite.[5] Since the proposed structure of metabolite had two chiral centers, we synthesized all four possible stereo-isomers as their optically pure form from the corresponding β-hydroxylated leucine to evaluate their biological activities. Compound **3**, having a (3*R*,1′*R*)-configuration, exhibited approximately 4.5-12-fold higher potency than the other isomers in Ca assay. Based on the observation of this unexpectedly improved antagonist activity of compound **3**, we applied this information to compound **2** which showed more potent in vitro activity than compound **1**. Thus, it was found that the (3*R*, 1′*R*)-hydroxylated compound **4** also exhibited strong antagonist activity.[5,6] Although the hydroxylated analogs **3** and **4** could not improve the bioavailability in rodents[5], they showed some favorable pharmaceutical properties. For example, **4** did not show any significant inhibition of CYP 3A4 up to 30 μM (**2**: 9.3 μM of IC_{50}). Furthermore, aqueous solubility (pH 6.8) of **4** was significantly improved relative to the corresponding non-hydroxylated analog (**2**: <0.2 μg/mL; **4**: 2.7 μg/mL). Further assessment and optimization led us to the discovery of the

* Corresponding author. Tel.: +81 75 961 1151; fax: +81 75 962 9314.
E-mail address: r.nishizawa@ono.co.jp (R. Nishizawa).

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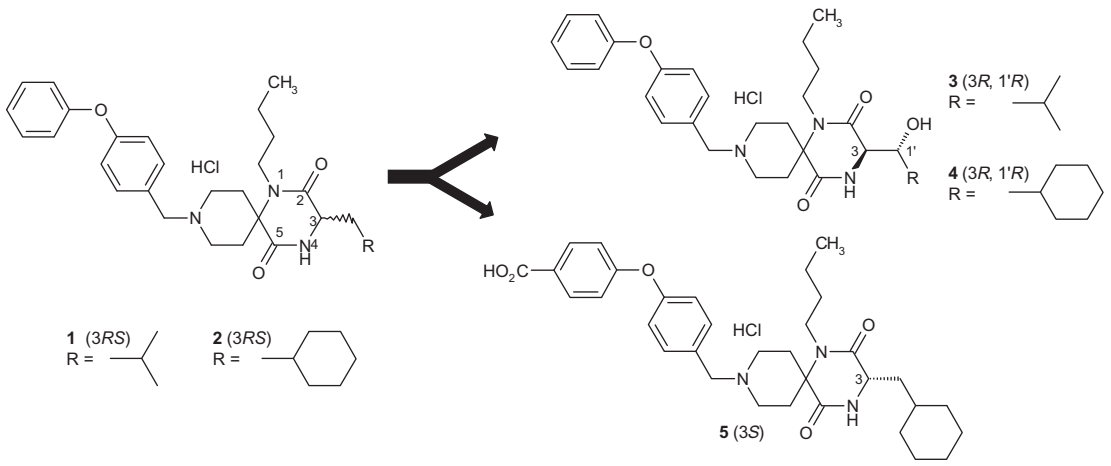


Figure 1. Lead compounds of CCR5 antagonists.

spirodiketopiperazine-based structure **5** as an orally-available CCR5 antagonist.[7] Based on the observation described above, we synthesized and evaluated **6a**, which was expected to have improved potency and oral availability with favorable pharmaceutical properties due of its hybrid structure possessing both of the features derived from **4** and **5**. The other possible stereoisomers **6b-d** were also synthesized and evaluated.

Test compounds **6a-d** listed in Table 1 were synthesized as outlined in Scheme 1. As shown in Scheme 1, the Ugi four component coupling reaction of *N*-benzylpiperizine-4-one, *n*-butyl amine, an appropriate optically-active amino acid among **7a–d** and 2-(4-morpholinyl)ethyl isonitrile followed by deprotection of *N*-Boc group and cyclization under acetic acid condition afforded the 9-*N*-benzyl spirodiketopiperazines is represented by the general formula **8** in 60–90% yield from **7a–d**. The stereochemical purity of the Ugi products was confirmed by NMR. Catalytic hydrogenation of **8** followed by reductive 9-*N*-alkylation with 4-(4-formylphenoxy)benzoic acid resulted in **6a–d** in 50–90% yield. Optically-active amino acids **7a–d** were prepared using Sharpless asymmetric epoxidation as previously reported.[8] The synthetic procedures of amino acid **7a** (2*R*, 3*R*) and **7c** (2*S*, 3*R*) are described as a representative example in Scheme 2. According to the same procedure as described for preparation of **7a** from **13a**, the amino acid **7d** (2*S*, 3*S*) was synthesized from the enantiomer of **13a**, which was prepared from **12** by Sharpless asymmetric epoxidation using another enantiomeric diethyl tartrate. According to the same procedure as described for the preparation of **7c** from **13a**, the amino acid **7b** (2*R*, 3*S*) was prepared from the enantiomer of **13a.**

Compounds **6a–d** listed in Table 1 were evaluated for their inhibitory activities against calcium mobilization of human CCR5 over-expressed CHO cell (hCCR5/CHO) stimulated by MIP-1α (Ca assay)[6a] and for their inhibitory activity of cell-cell fusion reaction between target cells expressing CD4/CCR5 and effector cells expressing the envelope protein of HIV-1.[9]

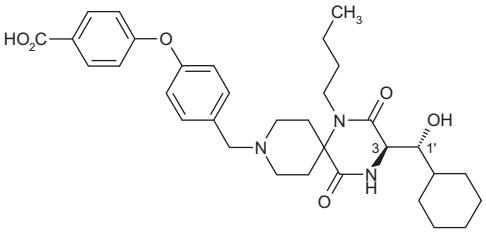
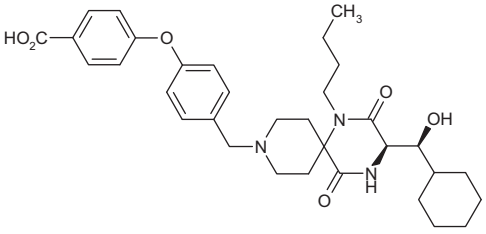
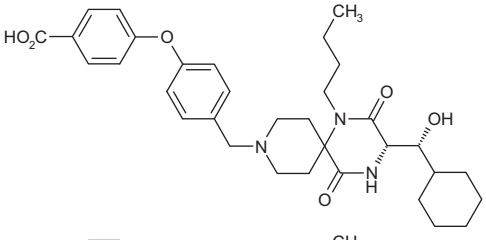
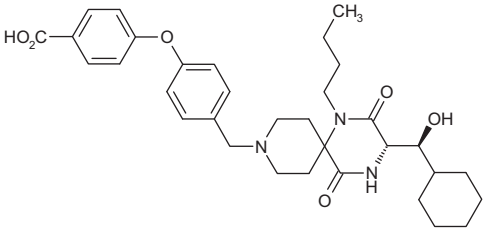
As shown in Table 1, we evaluated **6a-d** to investigate the structure–activity relationship (SAR) of the four stereoisomers. In our previous paper,[5] we reported that the (3*R*, 1′*R*)-isomer **4** was identified to show the most potent in vitro activities among the four possible stereoisomers. Based on the SAR of **4** and its possible three stereoisomers, **6a** was predicted to show the most potent antagonistic activity. Unexpectedly, however, isomers **6a**, **6b** and **6d** showed nearly equipotent antagonistic activity, while **6c** (3*S*, 1′*R*) showed nearly 10-fold less antagonistic activity. Regarding the 1′*R*-isomers, the 3*R*-isomer **6a** showed more potency than the corresponding 3*S*-isomer **6c**. Regarding the 1′*S*-isomers, both the 3*R*-isomer **6b** and 3*S*-isomer **6d** exhibited nearly equipotent activity. This specific SAR was consistent with the SAR of **4** and its three stereoisomers.[5]

According to the results of the fusion assay, **6a** showed nearly two orders of magnitude more potency in the pIC_{50} values relative to those of other three stereoisomers **6b–d**. This lack of correlation between anti-chemokine activity and anti-HIV activity has been previously reported, and is attributed to the allosteric nature of CCR5 inhibition.[10] Such a discrepancy observed between the calcium assay and the fusion assay was considered to be because of the following reasons. The calcium assay (receptor antagonist activity) was conducted using the natural ligand (MIP-1α), while the fusion assay (inhibitory effect on HIV envelope glycoprotein-mediated cell–cell fusion) was conducted using envelope glycoprotein. To more extensively estimate the activity of these compounds, the inhibition of virus entry mediated by membrane fusion and/or the HIV replication inhibition should be evaluated in addition to the receptor antagonist activity. The HIV replication inhibition results are described further in the article.

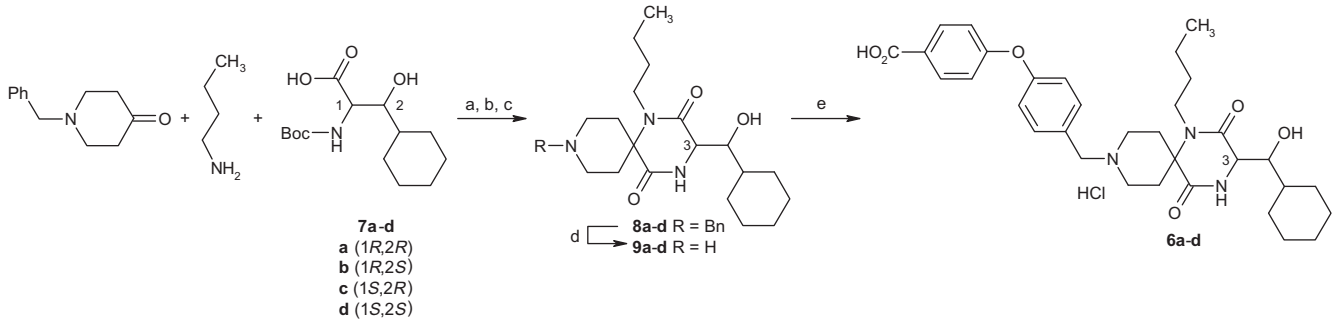
Isomers **6a-d** were investigated for their inhibitory activity on cytochrome P450 (2C9, 2D6 and 3A4 isomers) as shown in Table 2. For comparative purposes, the profiles of **1**-**5** were included to demonstrate the improvement shown with **6a-d**. The initial leads **1** and **2** showed P450 inhibition on all the enzymes tested, while the hydroxylated analogs **3** and **4** showed no inhibition on 3A4 up to 30 μM. Analog **5** which also possessed the *p*-carboxylic acid functional group still showed inhibitory activity on 2C9 and 3A4, while it did not show inhibitory activity on 2D6 up to 30 μM. The analog **6a** that possesses the best potential as a drug candidate did not show inhibitory activity on all the tested enzymes up to 30 μM. As described above, introduction of the hydrophilic functions such as hydroxyl and/or carboxylic acid functions was effective to remove or reduce these inhibitory activities. Among the tested compounds, only (3*R*, 1′*S*)-isomer **6b** showed inhibitory activity against 2C9. While this result was not able to be explained rationally, (3*R*, 1′*S*)-configuration was estimated to have a tendency to show relatively more affinity to the enzyme 2C9 than the other three configurations.

The (3*R*, 1′*R*)-configuration of **6a** was identified as possessing the most optimized stereochemistry regarding the biological potency, while the *p*-carboxylic acid function was found to be necessary for oral bioavailability, as illustrated by **5**. For the simultaneous optimization of the biological potency and oral bioavailability, the (3*R*, 1′*R*)-configuration and the *p*-carboxylic acid function are both needed, as indicated by the area under the curve (AUC) values (**6a** > **6b–d**) after oral dosing described in Table 3.

Table 1
Activity profiles of stereoisomers **6a–d**

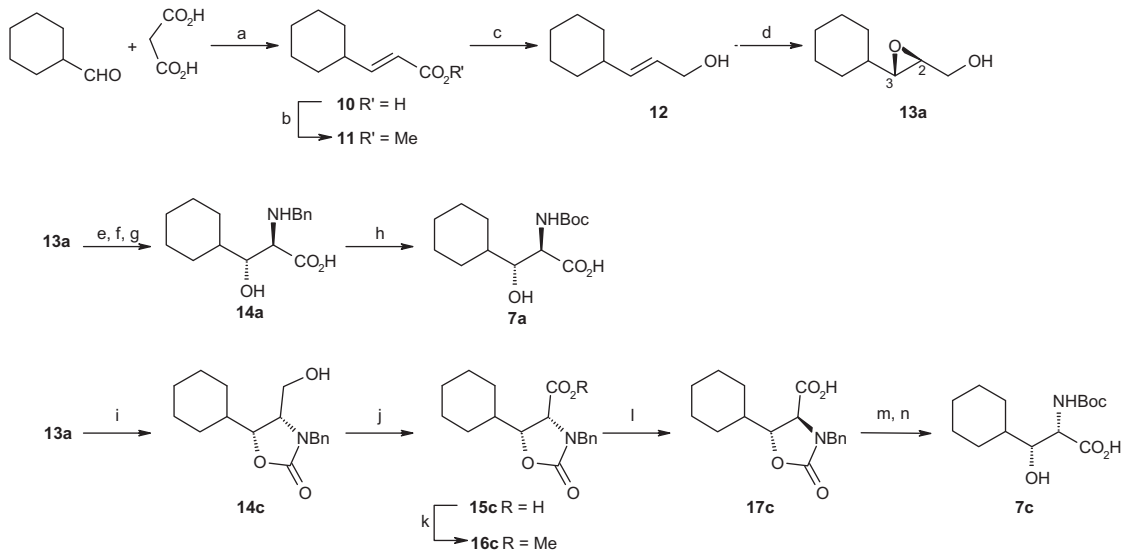
Compound	Ca assay IC_{50} (nM)	Fusion assay pIC_{50}
1	94	6.07
2	28	ND[a]
3	84	6.64
4	53	8.11
5	13	7.06
6a(3*R*, 1′*R*) 	34	8.25
6b(3*R*, 1′*S*) 	44	6.74
6c(3*S*, 1′*R*) 	470	6.42
6d(3*S*, 1′*S*) 	51	6.35

[a] ND : Not determined.



Scheme 1. Synthesis of spirodiketopiperazines **6a–d**. Reagents and conditions: (a) 2-(4-morpholinyl)-ethyl isonitrile, MeOH, 55 °C; (b) concd HCl, 55 °C; (c) AcOH/toluene, 80 °C; (d) H_2, $Pd(OH)_2/C$, EtOH, 50 °C, then 4 N HCl/AcOEt, 60–90% yield in four-steps; (e) 4-(4-formylphenoxy)benzoic acid, $NaBH(OAc)_3$, AcOH, DMF and then 4 N HCl/AcOEt, 50–90% yield.

Analogs **6a-d** were evaluated for their pharmacokinetics (PK) values after their single dose oral administration and intravenous administration in rats. These data are summarized in Table 3. Among the four tested compounds, **6a** showed the best oral exposure (AUC = 3422 ng h/mL) and maximum plasma concentration (C_{max} = 2360 ng/mL). A marked improvement of the AUC value of **6a** relative to the chemical lead **1** (Table 5) after oral dosing was estimated to be due to the marked reduction of the clearance (CL) and tissue distribution (V_{ss}) after intravenous dosing. Thus, stereochemistry of position-3 and position-1′ of **6a** (3*R*, 1′*R*) was thought to be involved as one of the factors for the improvement of PK values as illustrated by the lower C_{max} and AUC values after



Scheme 2. Synthetic method of optically-active β-hydroxy-*N*-Boc-α-amino acids **7a** and **7c**. Reagents and conditions: (a) piperidine, pyridine, 97%; (b) concd H_2SO_4, MeOH, 87%; (c) DIBAL, THF, 90%; (d) (−)-diethyl-D-tartrate, $Ti(Oi\text{-}Pr)_4$, cumene hydroperoxide, MS4A, CH_2Cl_2, 85%; (e) SO_3.pyridine, Et_3N, DMSO; (f) 2-methyl-2-butene, $NaClO_2$, NaH_2PO_4, MeCN, H_2O; (g) $BnNH_2$, 5.0 M NaOH, H_2O, 35% from **13**; h) H_2, $Pd(OH)_2$/C, MeOH, then Boc_2O, 1.0 M NaOH, 97%; (i) benzyl isocyanate, NaH, THF, 28%; (j) Jones reagent, acetone; (k) TMS-diazomethane, diethyl ether, 62% from **14c**; (l) KOH, EtOH, 98%; (m) 2.0 M KOH; (n) H_2, $Pd(OH)_2$/C then Boc_2O, 84% from **17c**.

Table 2
P450 inhibition of **1**–**5** and **6a**–**d**

Compound	P450 inhibition IC_{50} (μM)		
	2C9	2D6	3A4
1	5.9	4.6	19.7
2	5.5	7.8	9.3
3	>30	>30	>30
4	3.7	9.8	>30
5	8.8	>30	24.9
6a	>30	>30	>30
6b	18	>30	>30
6c	>30	>30	>30
6d	>30	>30	>30

oral dosing of **6b** (3*R*, 1′*S*), **6c** (3*S*, 1′*R*), and **6d** (3*S*, 1′*S*). Stability of these test compounds in rat liver microsomes was investigated but all isomers **6a**-**d** did not show significant differences between each other in in vitro metabolic stability, Caco2 permeability, and solubility, as shown in Table 4. In vitro metabolic stability and in vivo clearance of these test compounds did not show a strong relationship. Therefore, one cannot rule out other clearance processes in addition to metabolic ones to explain the mismatch in C_{max}/AUC across the 4 isomers. The differences in their susceptibility to the systemic metabolism, transporter recognition and/or the changes in V_{ss} may suggest additional processes.

Table 4
In vitro metabolic stability, Caco2 permeability, and solubility of **6a**–**d**

Compound	% Remaining[a]		Caco2 × 10^{-6} (cm/s)	Solubility[d] (μM)
	HLM[b]	RLM[c]		
6a (3*R*, 1′*R*)	36	39	3.8	70
6b (3*R*, 1′*S*)	90.1	76.5	1.6	81
6c (3*S*, 1′*R*)	88.1	74.8	1.6	85
6d (3*S*, 1′*S*)	69.8	52.7	2.1	80

[a] The data show the remaining% 15 min after incubating with the 0.5 mg/mL liver microsomes.
[b] Human liver microsomes
[c] Rat liver microsomes
[d] Kinetic solubility

Table 5
Anti-HIV activity of the representative compounds

Compound	Anti-HIV-1 activity IC_{50} (nM)[a]	
	HIV-$1_{Ba\text{-}L}$ (R5)	HIV-1_{MM} ($R5_{MDR}$)
6a	0.4 ± 0.3	0.6 ± 0.2
TAK-779	28 ± 32	14 ± 8
SCH-351125	4 ± 2	3 ± 0.5
Zidovudine[b]	7 ± 4	250 ± 98
Nelfinavir[c]	12 ± 8	>1000

[a] IC_{50} values are based on the inhibition of HIV p24 antigen expression in PBMC.
[b] Zidovudine is a reverse transcriptase inhibitor.
[c] Nerfinavir is a HIV-1 protease inhibitor

Table 3
Pharmacokinetic profiles of **6a**–**d**

Compound	10 mg/kg, po				3 mg/kg, iv			
	C_{max} (ng/mL)	$T_{1/2}$ (min)	AUC (ng h/mL)	BA (%)	AUC (ng h/mL)	$T_{1/2}$ (min)	CL (mL/min/kg)	V_{ss} (mL/kg)
1	33.3[a]	75.7	96.7[a]	1.3	372	13	137	2349
2	5.6[a]	103	24.8[a]	1.9	400	19.9	113	2542
5	2400	48.4	10532	34	3091	11.1	16	145
6a (3*R*, 1′*R*)	2360	120	3422	23	4402	21.8	11.3	132
6b (3*R*, 1′*S*)	70	35.2	67.5	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]
6c (3*S*, 1′*R*)	280	14.4	126	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]
6d (3*S*, 1′*S*)	260	19.2	213	6.5	988	16.2	50.9	836

[a] C_{max} and AUC are normalized to a dose of 10 mg/kg.
[b] ND: Not determined.

Non-hydroxylated analog **2**, hydroxylated analog **4**, and hydroxylated benzoic acid analog **6a** were evaluated for their anti-HIV activity using CCR5-tropic (R5) HIV-1 and multidrug-resistant HIV-1 virus, as shown in Tables 1 and 5. Analogs **4** and **6a** showed potent anti-HIV activity as indicated by a significant decrease in virus p24 production relative to **2**. These compounds potently inhibited not only the replication of laboratory and primary HIV-1 R5 strains, but also that of various multidrug-resistant monocyte/macrophage tropic (R5) HIV-1.[6b] These compounds were inactive against T-cell-tropic (X4) HIV-1. These data support the hypothesis that spirodiketopiperazines, such as **2**, **4**, and **6a,** show potent anti-HIV activity through their antagonistic effects on CCR5.

In summary, the design and synthesis of the structurally novel spirodiketopiperazines for use as orally-available CCR5 antagonists was performed based on the information previously reported by our group. Structural hybridization of the hydroxylated derivative **4** possessing more favorable pharmaceutical profiles and the orally-available non-hydroxylated derivative **5** resulted in the discovery of **6a**, which shows potential as an orally-available anti-HIV drug candidate. Compound **6a** possessing (3*R*, 1′*R*)-configuration exhibited potent CCR5 antagonist activity and the most potent activity by fusion assay among the tested four stereoisomers. For its biological potency and oral availability, both the optimized (3*R*, 1′*R*)-configuration and the *p*-carboxylic acid function on the terminal phenoxy moiety were found to be required. More detailed SAR including PK data will be reported in due course.

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