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Spirodiketopiperazine-based CCR5 antagonists: Lead optimization from biologically active metabolite

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Abstract—Hydroxylated derivatives were designed and synthesized based on the information of oxidative metabolites. Compounds derived from β-substituted (2*R*,3*R*)-2-amino-3-hydroxypropionic acid showed improved inhibitory activities against the binding of MIP-1α to human CCR5, compared with the non-hydroxylated derivatives and the other isomers.

Millions of people in the world are still suffering from acquired immune deficiency syndrome (AIDS).[1] Although the highly active antiretroviral therapy (HAART), a cocktail of protease and reverse transcriptase inhibitors, has been useful for many patients, several issues still remain for anti-HIV therapy: a gradual spread of drug-resistant strains, severe adverse effects, expensive therapeutic cost, etc.[2] These issues require new anti-HIV drugs to have a different mode of action from conventional drugs.

Agents inhibiting HIV entry into target cells are one of the most promising approaches to treat AIDS.[3] A number of potential sites for therapeutic intervention become accessible during the narrow window between virus attachment and the subsequent fusion of viral envelope with the cell membrane. In 1996, it was revealed that one of the C–C chemokine receptor 5 (CCR5) is utilized by HIV-1 as an essential co-receptor and that the endogenous ligand showed anti-HIV-1 activity in vitro.[4] CCR5 belongs to the superfamily of G protein-coupled receptors (GPCRs), which greater than 30% of all known marketed medicines modulate the function of.[5] After these reports, many pharmaceutical companies and academic institutions have been enthusiastically investigating novel antagonists against CCR5 with suitable pharmaceutical properties.[6]

We previously reported the identification of several spirodiketopiperazine derivatives, for example, **1** (Fig. 1), from a combinatorial library targeting chemokine receptors.[7] Compound **1** not only selectively inhibited the binding of macrophage inflammatory protein (MIP)-1α to human CCR5 receptor, but also potently blocked the infectivity and replication of laboratory and clinical strains of HIV as well as those of highly drug-resistant HIV variants with minimal cytotoxicity.[8] Although

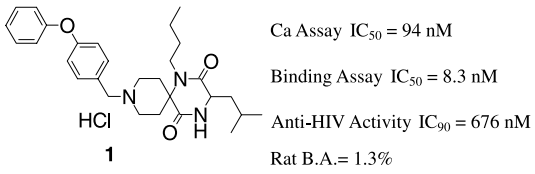

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Figure 1. The structure of lead compound **1**.

Keywords: CCR5; HIV-1; Active metabolite.
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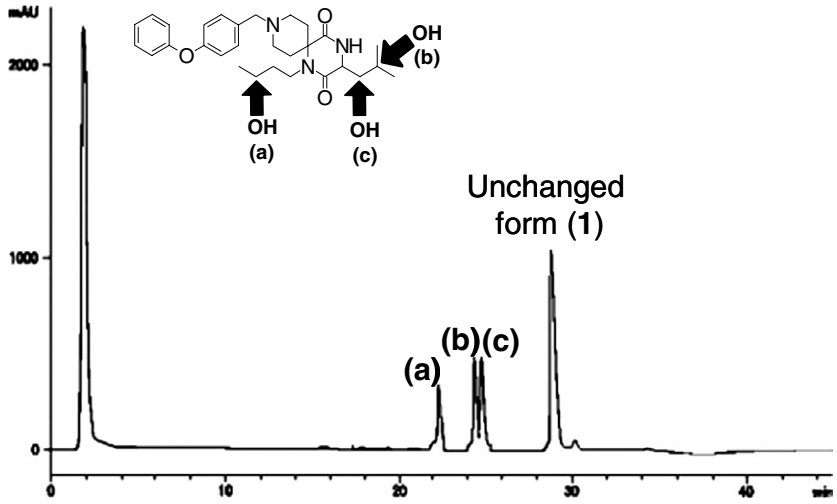


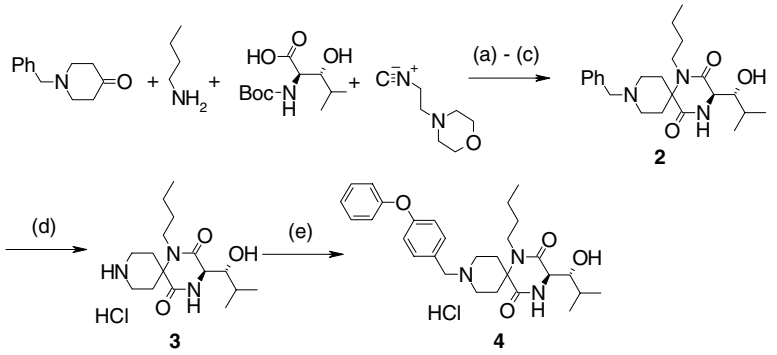
Figure 2. Metabolites of the compound **1**. Three main metabolites were identified on LC-MS after incubation with human liver microsome (5.0 mg protein/ml) for 1.5 h.

compound **1** showed potent activity in vitro, oxidative metabolism in liver microsomes resulted in low bioavailability in rodents.

After the incubation with human liver microsomes, metabolites of **1** were purified by HPLC. The three major isolated metabolites (a, b, c in Fig. 2) were analyzed by LC-MS and ^{1}H NMR, and found to be compounds hydroxylated on the *n*-butyl and/or the *i*-butyl group.[9] Additionally, we evaluated the in vitro antagonistic activity of each isolated metabolite. Fortunately, the samples of the peaks (b) and (c) showed significant antagonistic activities (data not shown). This information prompted us to try the introduction of hydroxyl group on side chains to improve the in vitro activity as well as pharmaceutical properties. Herein, we describe the preliminary structure–activity relationship (SAR) of the hydroxylated-spirodiketopiperazines and the unexpected improvement on the activities, especially in vitro anti-HIV activities.

The compounds **1**, **5**, **7**, **8**, and **12** were synthesized from the *N*-alloc-4-piperidone, the corresponding amine, the corresponding *N*-Boc-amino acid, and 4-phenoxybenzaldehyde by the reported solid-phase synthesis.[7] The compounds **9**–**11** and **13** were synthesized from the corresponding amino acid derivatives by the identical procedure to the synthesis of compound **4** shown in Scheme 1. The mixture of 1-benzylpiperidone, butylamine, *N*-Boc-β-hydroxy-D-leucine, and 2-(4-morpholinyl)ethylisocyanide[10] in methanol was stirred at 55 °C.[11] The enantiomerically pure β-hydroxy-α-amino acids were prepared according to the reported method through Sharpless asymmetric epoxidation from the corresponding allyl alcohol.[12] The Boc protecting group of amino acid was removed by the treatment of concentrated HCl without isolation of the Ugi product. Cyclization of the obtained crude product by heating in toluene in the presence of acetic acid at 80 °C followed by the removal of the benzyl group by catalytic hydrogenation afforded the cyclized spirodiketopiperazine, and compound **3** was isolated as a HCl salt in acceptable yield. Reductive alkylation of compound **3** resulted in desired product **4** in high yields.

The compounds listed in Tables 1–3 were evaluated for their inhibitory activities against calcium mobilization of human CCR5 overexpressed CHO cell (hCCR5/



Scheme 1. Typical synthetic route for spirodiketopiperazines. Reagents and condition: (a) MeOH, 55 °C; (b) concd HCl, 55 °C; (c) AcOH/toluene, 80 °C; (d) H_2, $Pd(OH)_2$/C, EtOH, 55 °C then 4 N HCl/AcOEt (60–70% in four steps); (e) 4-phenoxybenzaldehyde, $NaBH(OAc)_3$, AcOH, DMF then 4 N HCl/AcOEt (80%).

Table 1. Activity of the compounds **5** and **6**

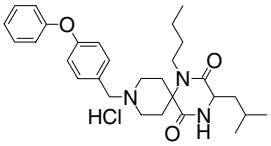
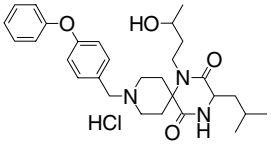
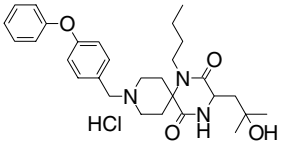
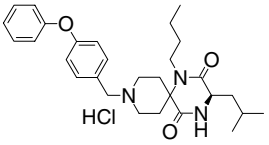
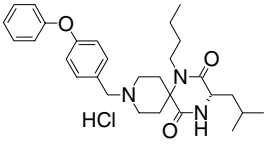
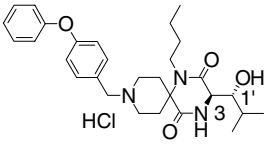
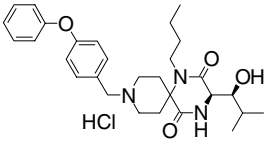
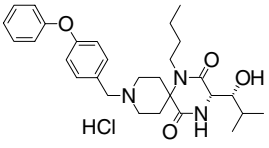
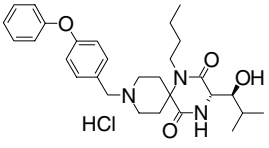
Compound	Structure	IC_{50}	
		Binding assay (nM)	Ca assay (nM)
1		8.3	94
5		Not tested	Ca. 10,000
6		28	79

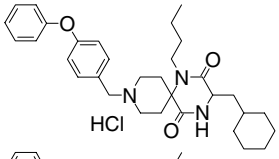
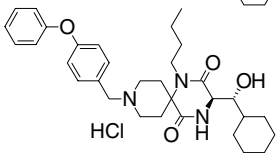
Table 2. Activity of the stereoisomers **4** and **7**–**11**

Compound	Structure	IC_{50}	
		Binding assay (nM)	Ca assay (nM)
7 *R* form		29	130
8 *S* form		11	84
4 (3*R*,1′*R*) form		3.5	33
9 (3*R*,1′*S*) form		24	210
10 (3*S*,1′*R*) form		68	400
11 (3*S*,1′*S*) form		53	150

CHO) stimulated by MIP-1α (Ca assay) and for their CCR5 binding affinity by the inhibition of ^{125}I-MIP-1α binding to hCCR5/CHO (binding assay).[8,13]

The compound **5**, the proposed structure of metabolite (a), was synthesized from the 3-hydroxy-1-butylamine and *N*-Boc-leucine as a mixture containing the same

Table 3. Antagonistic activity and anti-HIV activity in compounds **12** and **13**

Compound	Structure	Binding assay IC_{50} (nM)	Ca assay IC_{50} (nM)	Anti-HIV IC_{50} (nM)	MAGI assay IC_{50} (nM)
12		6.1	28	31	337
13		1.1	53	0.6	6.0

quantity of all possible four isomers. The compound **6**, the proposed structure of metabolite (b), was prepared from the *N*-Boc-aspartic acid derivative in racemic form.[14] Compounds **5** and **6** were evaluated for their activities and the results are summarized in Table 1. Whereas the compound **5** showed significant decrease of activity in calcium mobilization assay, the compound **6** showed a comparable activity to the parent compound **1**.[15]

Since the proposed structure of metabolite (c) had two chiral centers, we synthesized all four possible enantiomers **4** and **9**–**11** in optically pure form from the corresponding β-hydroxylated-leucine to evaluate their biological activities (Table 2). Whereas there was no remarkable difference between the activities of the two enantiomers, **7** and **8**, in lead compound **1**, there was a significant difference among the hydroxylated stereoisomers (**4** versus **9**–**11**). Compound **4** having 3*R*,1′*R*-configuration exhibited approximately 10-fold more potency than the other isomers **9**–**11**. This result indicates that introducing hydroxyl group on the side chain at the 3-position of diketopiperazine ring could lead to a significant improvement in the interaction with the receptor. Based on the observation of this unexpectedly improved antagonistic activity of compound **4**, we applied this information to compound **12** which showed more potent anti-HIV activity than compound **1**. It was found that the (3*R*,1′*R*)-hydroxyl compound **13** exhibited strong inhibitory activities in both binding and Ca assays. Furthermore, investigating anti-HIV activity in the next step, compound **13** showed 6 nM of IC_{90} value in anti-HIV assay ($CCR5^+$ MAGI cell anti-infectivity single cycle assay versus the BAL strain of HIV,[8a]), which was 50-fold stronger than non-hydroxyl analogue **12**.[16]

We also evaluated the oral bioavailability of compounds **4** and **13** in rat. Unfortunately, the bioavailability of both compounds was less than 1% (data not shown). Further assessment and optimization are required to identify promising clinical candidates with acceptable pharmaceutical profile.

In conclusion, using metabolite data of lead compound **1**, we discovered the excellent enhancement on the activity in binding and anti-HIV assays by the introduction of a β-hydroxyl group. Although the role of the hydroxyl group is still unclear, two hypotheses have been made to explain the increase in activity. One is the formation of a new hydrogen bond between the hydroxyl group and CCR5. The other is restricting the conformation of the compound to favorably orient the side chains. Further investigations are in progress.

Introduction of the hydroxyl group could not improve the bioavailability in rodent. However, the introduction of a hydrophilic moiety on the molecule showed some favorable pharmaceutical properties.[17] Further optimization of these compounds to improve their oral absorption and metabolic stability which are necessary to provide CCR5 antagonists suitable for clinical use will be discussed in our future reports.

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9. ^{1}H NMR spectra of metabolites (a) and (c) indicated to be a single compound, respectively. However, it was difficult to determine the stereochemistry at each asymmetric center. As for the metabolite (c), the ^{1}H NMR data were identical to those of the compounds **4** and **11**, which indicates that this metabolite has *anti*-configuration.
10. We initially used several isocyanides, for example, benzylisocyanide, for this reaction to yield the desired product. However, most of isocyanides were difficult to be handled because of the offensive odor. It was found that a commercially available isocyanide, 2-(4-morpholinyl)ethylisocyanide, is not malodorous, and we decided to employ this reagent for the further derivatization.
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12. (a) Caldwell, C. G.; Bondy, S. S. *Synthesis* **1990**, 34; (b) Nagamitsu, T.; Sunazuka, T.; Tanaka, H.; Omura, S.; Sprengeler, P. A.; Smith, A. B., III *J. Am. Chem. Soc.* **1996**, *118*, 3584, Compound **13** was synthesized from the β-hydroxy-cyclohexylalanine, which was obtained from the 3-cyclohexyl-allyl alcohol by an identical procedure to that reported in Ref. 12a.
13. All IC_{50} values represent the average of two or more determinations, and the standard deviations were no greater than 30% from the mean.
14. Compound **6** was prepared by the reaction of methylmagnesium bromide with the benzyl ester derivative, which was obtained from the *N*-alloc-piperidone, butylamine, *N*-Boc-γ-benzyl-aspartic acid, and 4-phenoxybenzaldehyde according to the solid-phase synthesis reported in Ref. 7.
15. Compounds described herein showed selective CCR5 antagonistic activities over other chemokine receptors, for example, CCR1 and CXCR4.
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17. For example, whereas compound **12** inhibited CYP 3A4 at 9.3 μM of IC_{50}, compound **13** did not show any significant inhibition at 30 μM. Furthermore, compound **13** showed an improved aqueous solubility (pH 6.8) compared to compound **12** (**12**, <0.2 μg/ml; **13**, 2.7 μg/ml).