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Spirodiketopiperazine-based CCR5 antagonists: Improvement of their pharmacokinetic profiles

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ABSTRACT

Spirodiketopiperazine-based CCR5 antagonists, showing improved pharmacokinetic profiles without reduction in antagonist activity, were designed and synthesized. We also demonstrate the anti-HIV activity of a representative compound **12**, as measured in a p24 assay.

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Chemokines, which were initially identified as chemoattractants in the context of leukocyte trafficking to sites of inflammation, exert a variety of biological activities by binding to their receptors on the surface of specific cells. Chemokines are a large family of small cytokines that selectively control the adhesion, chemotaxis, and activation of various leukocyte populations and are known to be involved in the initiation and progress of inflammation and allergic disease.[1] They are classified into two main groups—CC chemokines and CXC chemokines—based on their conserved N-terminal cysteine residues. Additionally, CC chemokine receptor 5 (CCR5) and CXC chemokine receptor 4 (CXCR4) have attracted substantial interest because they form portals of cellular entry for human immunodeficiency viruses (HIV-1 and HIV-2) and related simian or feline retroviruses.[2] Thus, identifying the CCR5 receptor antagonist, which inhibits HIV from binding to the specific receptor, is one of the most promising approaches for the treatment of AIDS, especially at the early stage of infection.[3] Due to its novel mode of action, the CCR5 antagonist could be one of the final agents utilized in salvage therapy in combination with other active antiviral agents. Maraviroc is the only approved CCR5 receptor antagonist on the market for treating HIV-1 infection.[4] In this study, we report the optimization process to find 4-(4-{[(3*S*)-1-butyl-3-(cyclohexylmethyl)-2,5-dioxo-1,4,9-triazaspiro[5.5]undec-9-yl]methyl}phenoxy)benzoic acid hydrochloride (**12**) with the aim of improving the antiviral activity and pharmacokinetics of the newly found chemical leads **1a** and **1b** (Fig. 1).

Compounds **1a**, **1b**, **2a**, **2b**, **3b** and **4a** were synthesized by the previously reported solid-phase method[5a] and compounds **6**–**12** were synthesized by the solution-phase method described in Scheme 1. A mixture of 1*N*-benzylpiperidin-4-one, *n*-butylamine, *N*-Boc-amino acid and 2-(morpholin-1-yl)ethylisocyanide in methanol was stirred at 55 °C.[6] The Boc protecting group of an amino acid was removed by treatment with concentrated HCl without isolation of the Ugi product,[6] cyclization of which, by heating in toluene in the presence of acetic acid at 80 °C, afforded **13**. Removal of the benzyl group of **13** by catalytic hydrogenation produced the cyclized spirodiketopiperazine **14**, which was isolated as HCl salt in 60–70% yield in four steps. Reductive alkylation of compound **14** resulted in a good yield (50–90%) of the desired products. Synthesis of **5a** is described in Scheme 2. N-Alkylation of **14** with a tosylate, which was prepared by the tosylation of 4-phenoxyphenylethyl alcohol with polymer-supported tosyl chloride, afforded **5a**. These basic compounds were isolated as their HCl salts.

Compounds listed in Tables 1–4 were evaluated for their inhibitory activities against calcium mobilization of human CCR5

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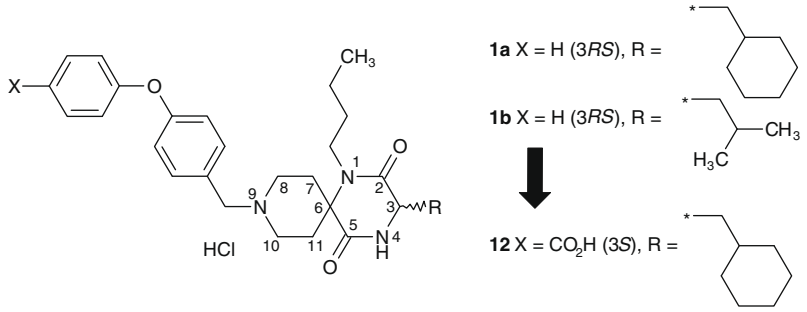
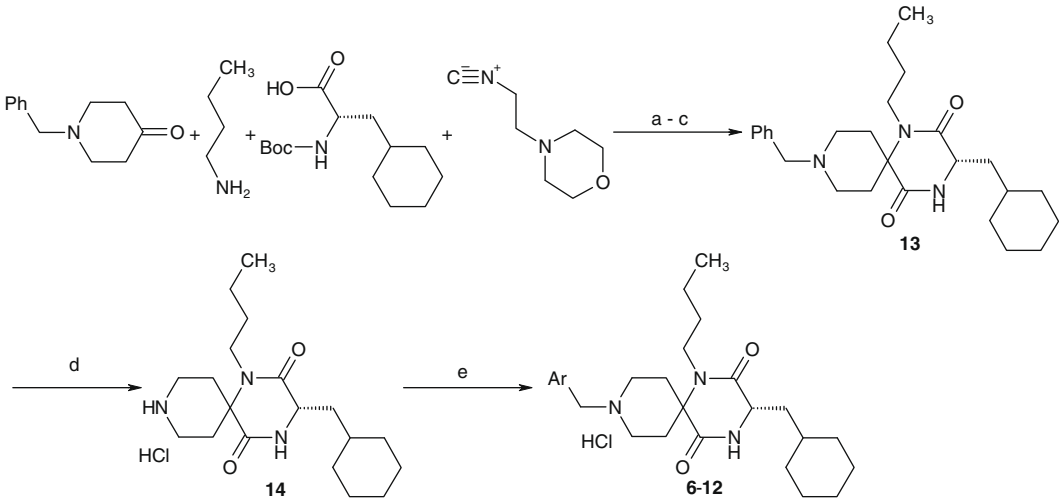
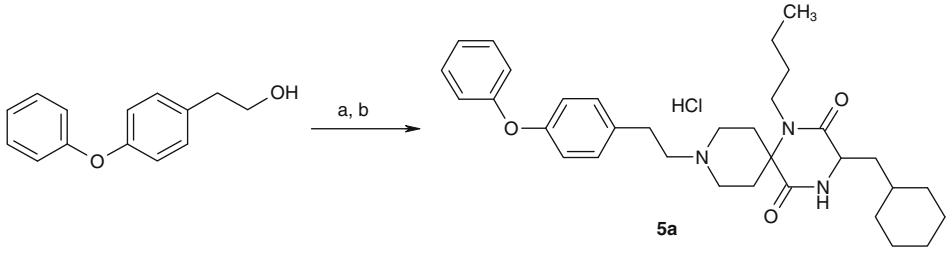


Figure 1. Discovery of an orally active chemical lead.



Scheme 1. Typical synthetic route of spirodiketopiperazines. Reagents and conditions: (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) Ar-CHO, $NaBH(OAc)_3$, AcOH, DMF, then 4 N HCl/AcOEt (50–90%).



Scheme 2. Synthesis of **5a**. Reagents and conditions: (a) polystyrene-supported tosylchloride, pyridine, CH_2Cl_2; (b) **14**, iPr_2NEt, MeCN, then 4 N HCl/AcOEt (48% in two steps).

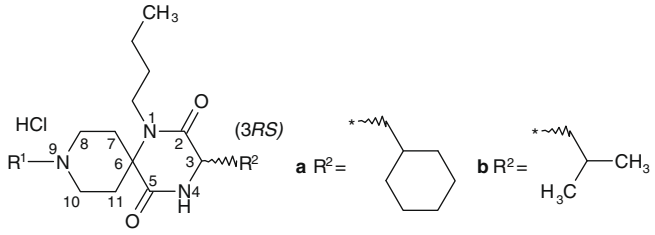
overexpressed CHO cells (hCCR5/CHO) stimulated by MIP-1α (Ca assay).[7]

We previously reported the discovery of spirodiketopiperazines **1a**, **1b**, **2a**, and **2b** (Table 1), as the novel chemical leads of CCR5 antagonist from our combinatorial library targeting G-protein coupled receptors (GPCRs).[5] Analogues **1a** and **2a** tended to show stronger activity compared with the corresponding 3-isobutyl analogues **1b** and **2b**, respectively. Although **1a**, **1b**, **2a**, and **2b** showed from potent to moderate antagonist activity in vitro, they were not expected to display potent activity in vivo because of their unfavorable PK data, including poor area under the concentration-time curve (AUC), high clearance (CL) and distribution values (V_{ss}), as shown in Table 5. Accordingly, structural optimization was focused not only on increased activity but also on the improvement of these PK values. Our optimization process was initiated with chemical modification of the N-substituents at the 9-position using readily available spirodiketopiperazine **14** as a key intermediate.

As shown in Table 2, reductive amination of **14** with commercially available benzaldehyde and *p*-methoxybenzaldehyde provided **3b** and **4a**, respectively. Although reduction of the molecular weight was expected to be a promising strategy for improving oral absorption, this approach resulted in reduced activity. N-Alkylation of **14** with 4-phenoxyphenylethyltosylate afforded **5a**, also with remarkable reduction in activity relative to **1a**. As a result, the 9-*N*-phenylmethyl group was found to be one of the structural requirements for antagonist activity. Introduction of a *p*-phenoxy substituent into the 9-*N*-phenylmethyl group of **3b** tended to show increased activity, as illustrated by the increased potency of **1b** relative to **3b**.

As shown in Table 3, the effect of chemical modification of the linker X on activity profiles was investigated. Replacement of the 9-*N*-4-phenoxyphenylmethyl moiety of **1a** with 9-*N*-4-phenylthiophenylmethyl afforded a diphenyl sulfide analogue **6** with less potent activity. Replacement of the sulfide moiety of **6** with sulfone

Table 1
Hits from the newly designed G-protein coupled receptor (GPCR) directed library



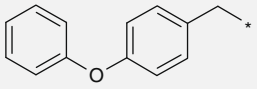
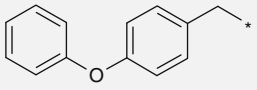
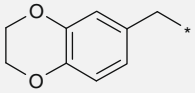
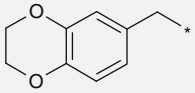
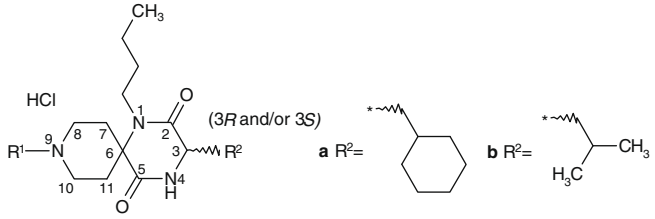
Compds	R^1	IC_{50} (nM) Ca assay
1a		28
1b		94
2a		50
2b		180

Table 2
Effect of the spirodiketopiperazines 9-*N*-substituents on activity profiles



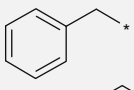
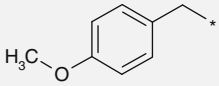
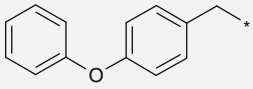
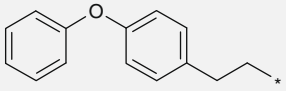
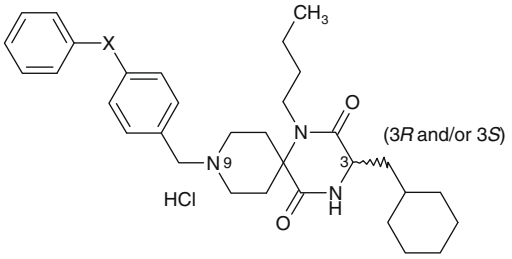
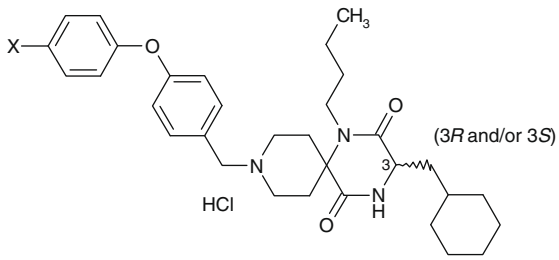
Compds	R^1	C3 configuration (3*R* and/or 3*S*)	IC_{50} (nM) Ca assay
3b		(3*RS*)	900
4a		(3*RS*)	120
1a		(3*RS*)	28
1b		(3*RS*)	94
5a		(3*S*)	ca. 10,000

Table 3
Effect of chemical modification of a linker X on activity profiles



Compds	R^1	C3 configuration (3*R* and/or 3*S*)	IC_{50} (nM) Ca assay
6		(3*S*)	170
7		(3*S*)	86
1a		(3*RS*)	28

Table 4
Effect of the *p*-substituent of the biphenyl ether residue on activity profiles



Compds	X	IC_{50} (nM) Ca assay	C3 configuration (3*R* and/or 3*S*)	Stability in rat liver microsomes% remaining at 15 min
8	Me–	79	(3*S*)	48
9	MeO–	130	(3*S*)	34
10	HO–	42	(3*S*)	22
11	F–	92	(3*S*)	53
12	HO_2C–	13	(3*S*)	22
1a	H–	28	(3*RS*)	33

afforded **7**, which showed slightly less potent activity relative to the corresponding ether analogue **1a**. For this reason, the following optimization was focused on the synthetic work of diphenyl ether derivatives.

Effect of a *p*-substitution of the predicted metabolic site of the terminal phenyl moiety of **1a** on stability in rat liver microsomes was investigated.[8] As shown in Table 4, introduction of *p*-methyl group and *p*-methoxy group as electron-donating substituents into the terminal phenyl moiety of **1a** afforded 4-(*p*-methylphenoxy)phenylmethyl and 4-(*p*-methoxyphenoxy)phenylmethyl analogues **8** and **9**, respectively with a tendency of showing slightly less potent activity relative to **1a**, while demethylation of **9** afforded an analogue **10** with a tendency of showing slightly more potent activity relative to the corresponding methoxy analogue **9**. Introduction of a *p*-fluoro group as an electron-withdrawing group instead of *p*-methyl and *p*-methoxy groups afforded **11** with a slightly less potent activity relative to **1a**. Introduction of a carboxylic acid group as an electron-withdrawing and hydrophilic substituent as a *p*-substituent afforded **12** with an increased activity. Stability of these test compounds in the rat liver microsomes was investigated but these in vitro data did not indicate a significant improvement in metabolic stability in rat liver microsomes.[9]

PK data obtained after single-dose oral administration of the initial chemical lead **1a** and the representative compound **12** to rats are presented in Table 5. As described previously, the initial lead **1a** showed very poor bioavailability (1.9%). Other PK values such as the maximum plasma concentration (C_{max}), plasma elimination half-life ($T_{1/2}$) and AUC were also very poor. The probable

Table 5
Pharmacokinetic data for compounds **1a** and **11** in rats

Compds	30 mg/kg, po				30 mg/kg, iv			
	C_{max} ng/mL	$T_{1/2}$ min	AUC ng h/m (0–∞)	BA%	AUC ng h/mL (0–∞)	$T_{1/2}$ min	CL mL/min/mL	V_{ss} mL/kg
1a	16.7[a]	103	74.4[a]	1.9	400	19.9	113	2542
12	7200	48.4	10,532	34.1	3091	11.1	16	145

[a] Dose normalized AUC and C_{max} to 30 mg/kg.

Table 6
Anti-HIV activity of the representative compounds

Compds	Anti-HIV-1 activity in p24 assay HIV-1Ba-L (R5) IC50 (nM)
1b	160
12	39
Zidovudine[a]	60
Nelfinavir[b]	12

[a] Zidovudine is reverse transcriptase inhibitor.
[b] Nelfinavir is HIV-1 protease inhibitor.

reasons for such poor PK values of **1a** were the large clearance (CL = 113 mL/min/kg) and distribution volumes (V_{ss} = 2542 mL/kg) which were unfavorable for drugs which show efficacy in the blood, such as anti-HIV drugs. However, benzoic acid analogue **12** showed significantly improved PK values such as C_{max} (7200 ng/mL), oral exposure (AUC = 10532 ng h/mL) and bioavailability (BA = 34.1%) after its oral dosing. Remarkable improvement of solubility (26 μM)[9] and Caco2 permeability (26.4×10^{-6} cm/s)[9] of **12** relative to **1a** (solubility: less than 5 μM and Caco2 permeability: not detected) was estimated to be one of the most plausible reasons. The marked reduction in clearance (CL = 16 mL/min/kg) and distribution volume (V_{ss} = 145 mL/kg) after intravenous dosing was considered to be another plausible reason for the improved AUC and BA. The marked reduction of CL of **12** strongly suggested in vivo metabolic stabilization, although in vitro studies did not indicate a significant improvement in metabolic stability.

Furthermore the representative compound **12**, PK profiles of which were significantly improved relative to the initial lead **1b** without reduction of the potent antagonist activity, was investigated for its anti-HIV activity using a launched reverse transcriptase inhibitor Zidovudine as a positive control. Results are summarized in Table 6. Compound **12** showed an IC_{50} value of 39 nM in an anti-HIV-1 p24 assay (using PBMC as the target cells[7]).

In conclusion, starting with the initial hit compounds **1a** and **1b** which showed unfavorable PK profiles, we identified compound **12** which showed significant improvement in bioavailability and oral exposure (AUC) without reduction in activity. Compound **12** was produced by introducing a carboxylic acid group into the *p*-position of the terminal phenyl moiety. Although the role of carboxylic acid is still unclear, compound **12** is thought to show improved C_{max}, AUC and BA after oral dosing because of its much improved solubility and Caco2 permeability. However, its oral absorption process has not yet been elucidated. The significant reduction of CL and V_{ss} of compound **12** was also considered to be another plausible reason for the increased C_{max}, AUC and BA. As such, introduction of carboxylic acid into the *p*-position of the terminal phenoxy moiety was found to be effective not only to block metabolic deactivation but also to improve PK profiles. The representative compound **12** showed more potent activity than **1b** in the p24 assay (with the BAL strain of HIV). Further optimization of compound **12** to improve its activity and PK profile, will be reported in near future. These findings will contribute further to the development of CCR5 antagonists for clinical use.

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9. Full details of the experimental will be reported very soon in the full paper which we are preparing.