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Discovery of a novel series of cyclic urea as potent CCR5 antagonists

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ABSTRACT

A novel series of cyclic urea-based CCR5 antagonists was designed aiming to resolve instability issue in the fasted simulated intestinal fluid (FSIF) associated with the acyclic urea moiety in **1**. This class of CCR5 compounds demonstrated high antiviral activities against HIV-1 infection in both HOS and PBL assays. Further evaluation of these compounds indicated that **16**-***R*** not only substantially enhanced its stability, but also exhibited excellent pharmacokinetics properties.

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Combating against HIV infection continues to be a challenge to public healthcare. Highly active anti-retrovirals therapy (HAART) has significantly increased the life expectancy of HIV patients and reduced morbidity, however, mutant resistance and patient compliance due to side effects have become serious issues. Discovery and development of new anti-HIV medicines with novel mode of action (MOA) promise to overcome these challenges. Since human chemokine receptor 5 (CCR5) was identified as one of viral entry co-receptors and plays an essential role in HIV viral replication,[1] efforts to discover human CCR5 antagonists as anti-HIV agents have been extensively pursued in the pharmaceutical industry,[2] which ultimately led to several small molecules in clinical development and one FDA approved drug (Maraviroc).[3]

We previously reported a series of acyclic urea as CCR5 antagonists, exemplified by **1**.[4] This series of compounds not only demonstrated potent anti-HIV activity in both human osteosarcoma (HOS) cell and peripheral blood lymphocytes (PBL) cell assays, but also exhibited promising DMPK profiles. However, compound **1** was found to have limited stability in fasted simulated intestinal fluid (FSIF) at pH 6.0 at 40 °C ($t_{1/2}$ = 334 h). Further degradation fragment analysis revealed that the acyclic urea portion of the molecule was responsible for its instability, and fragments **A**, **B** and **C** were detected by LC–MS, Figure 1. Since the urea deprotonation mechanistically precedes such a degradation, to rectify the issue, we designed the molecules that 'lack' the acidic proton in the NH moiety.

To that end, we first designed and synthesized carbamate **2**, which had IC_{50} = 29 nM in HOS cell assay against the Ba-L strain of HIV-1. Despite some loss of potency compared to **1**, carbamate **2** appeared to support our general strategy and that the acidic proton in the NH urea moiety was not a key pharmacophore. Meanwhile, we also prepared the benzyl-substituted urea **3** to probe different chemical space. Surprisingly, **3** was highly potent against HIV-1 infection with IC_{50}s = 1.7 nM and 0.9 nM in both HOS and PBL cell assays against the Ba-L strain of HIV-1,[5] respectively.

Following these results, we rationalized that five/six-member rings tying up both urea nitrogen atoms could be tolerated as pharmacophores and would also substantially improve the urea stability, Figure 2. To test this hypothesis, the imidazolidine-2, 4-dione racemic analogue **4** was designed and synthesized, Scheme 1. Thus, 5-amino-2,4-difluorobenamide **5** was treated with 4-nitro-phenyl chloroformate in the presence of *N*,*N*-dimethyl aniline to provide carbamate **6**, while the reductive alkylation of methyl amino(phenyl)acetate and Boc protected piperidione yielded amine **7**. Subsequent reaction of **7** with carbamate **6** produced imidazolidine-2, 4-dione **8** via spontaneous cyclization of transiently-forming acyclic urea intermediate. After the Boc protecting group was removed, the resulting piperidine **9** was reductively alkylated with aldehyde **10** to furnish **4** as a racemic mixture. Rewardingly, compound **4** had IC_{50} = 14 nM in HOS antiviral assay.

We next pursued the synthesis of the cyclic urea **16**, a partially reduced analogue of the imidazolidine-2, 4-dione analogue. Since the terminal sulfonamide moiety in **4** was found to be metaboli-

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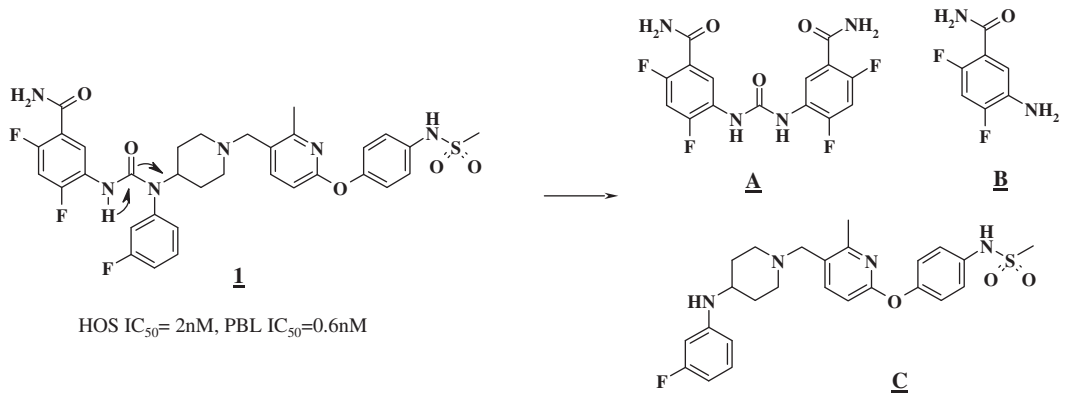


Figure 1. Proposed degradation mechanism of **1** under the simulated physiological conditions.

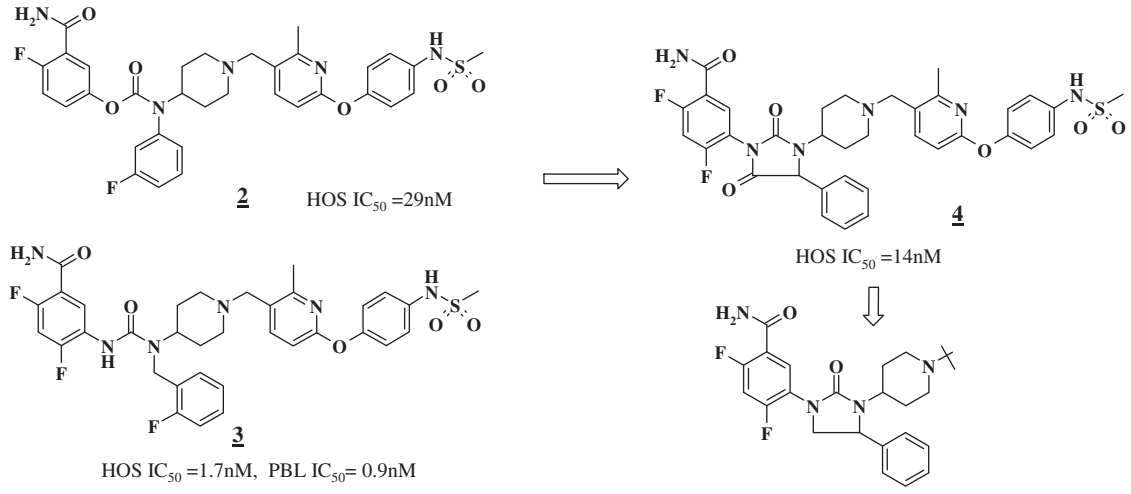
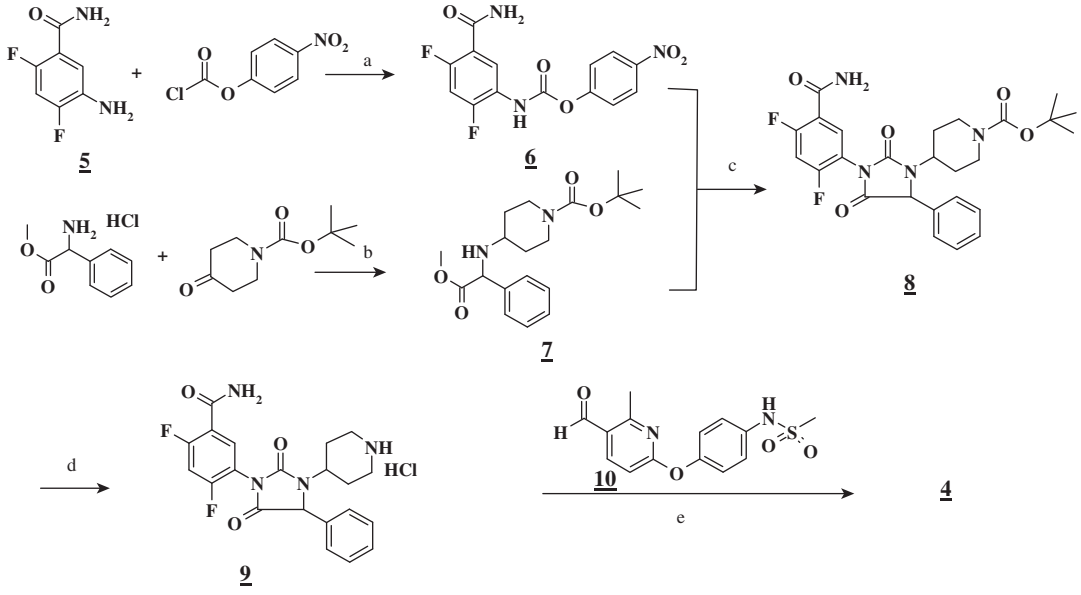
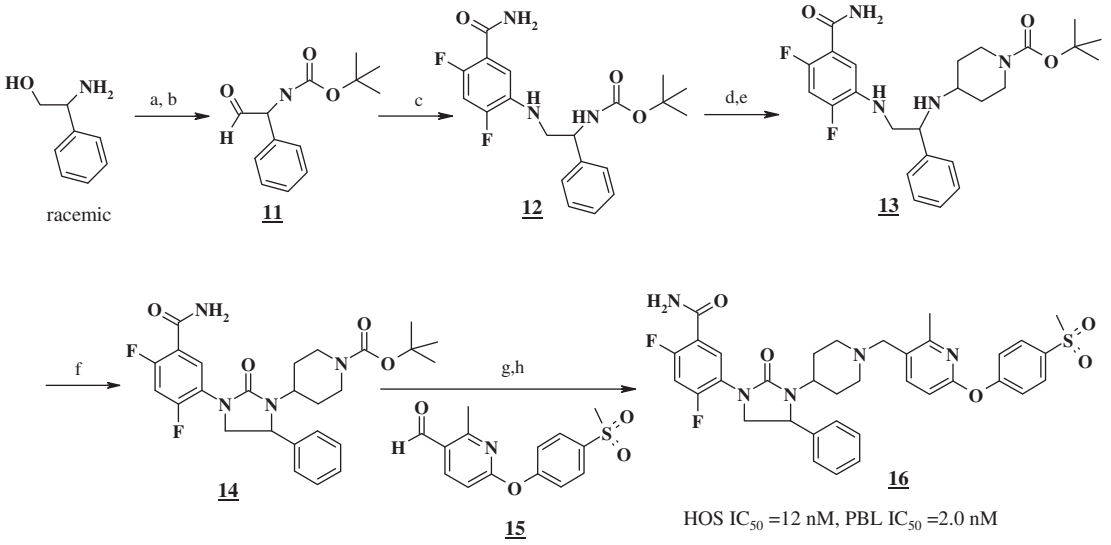


Figure 2. Design rationale of the cyclic urea.



Scheme 1. Reagents and conditions: (a) *N*,*N*-dimethylaniline, CH_2Cl_2, 40 °C, overnight, 97%; (b) $NaHB(OAc)_3$, CH_2Cl_2, rt, quant.; (c) iPr_2NEt, CH_3CN, 75 °C, overnight, 65%; (d) HCl (4 M in dioxane), CH_2Cl_2, rt quant.; (e) 10, $NaBH(OAc)_3$, CH_2Cl_2, rt, 33%.

cally labile in vivo, it was replaced by a sulfonyl group, without affecting the potency of analogues. After protection of the amino group in racemic 2-amino-2-phenyl ethanol with Boc, the remaining free hydroxyl was oxidized by Dess–Martin periodinane. The



Scheme 2. Reagents and conditions: (a) Boc_2O, CH_2Cl_2, rt, 3 h; (b) Dess–Martin periodinanes, 2 h, 65% over two steps; (c) **5**, $NaBH(OAc)_3$, CH_2Cl_2, rt, 4 h, 33%; (d), HCl (4 M in dioxane), CH_2Cl_2; (e) $NaBH(OAc)_3$, Et_3N, CH_2Cl_2, rt, 4 h, 92%; (f) triphosgene, Et_3N, THF, −78 °C to rt, 2 h, 83%; (g) HCl (4 M in dioxane), CH_2Cl_2, rt quant.; (h) **15**, $NaBH(OAc)_3$, CH_2Cl_2, rt, 65%.

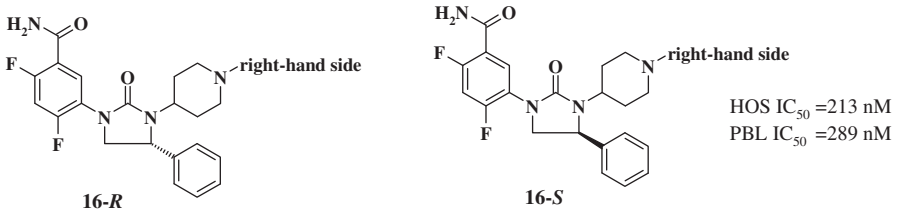
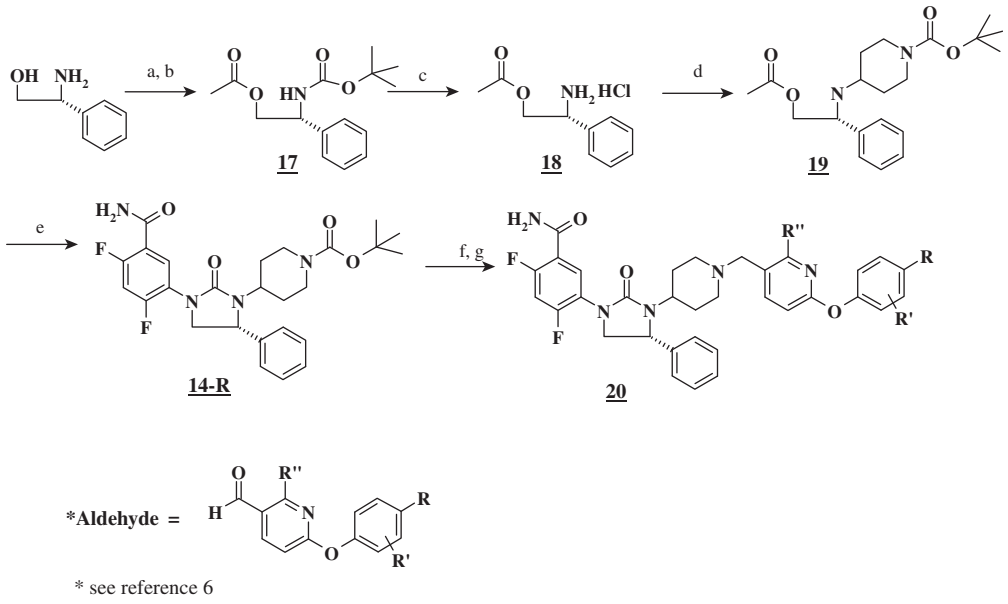


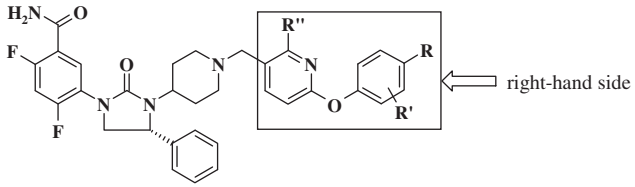
Figure 3. Antiviral activities of **16-*R*** and **16-*S***.

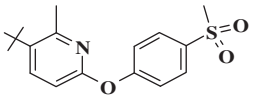
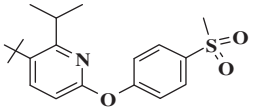
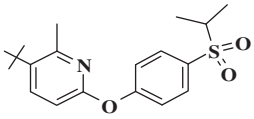
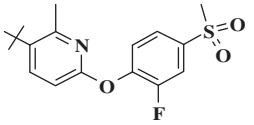
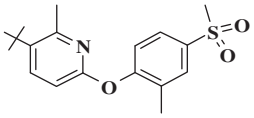
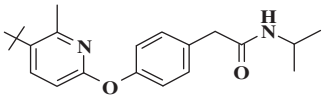


Scheme 3. Reagents and conditions: (a) Boc_2O, CH_2Cl_2, rt, overnight; (b) Ac_2O, Et_3N, rt, 2 h; (c) HCl (4 M in dioxane), CH_2Cl_2, rt, quant.; (d) 1,1-dimethylethyl-4-oxo-1-piperidnecarboxylate, $Na(OAc)_3$, Et_3N, CH_2Cl_2, rt, overnight, 83%; (e) **6**, DBU, toluene, 60 °C, overnight, 58%; (f) HCl (4 M in dioxane), CH_2Cl_2; (g) Aldehyde, $NaHB(OAc)_3$, Et_3N, CH_2Cl_2, rt, overnight, 40–65%. (See above-mentioned references for further information.)

resulting aldehyde **11** was subsequently coupled with 5-amino-2,4-difluorobenzamide **5** via reductive alkylation to yield **12**. Subsequent treatment of **12** with HCl followed by a second reductive alkylation with Boc protected piperidione produced the diamine **13**. Next, key intermediate **14** was obtained by reacting **13** with triphosgene in presence of triethyl amine. With the cyclic urea

Table 1
Antiviral potency of **16-*R***, **20a–e** in HOS and PBL cell assays



Compound	Right-hand side	HOS IC50 (nM)	PBL IC50 (nM)
16-*R*		4.0	1.0
20a		2.6	1.6
20b		3.6	1.1
20c		5.2	0.6
20d		5.4	NT[a]
20e		3.3	0.5

[a] NT: not tested.

intermediate in hand, final compound **16** was obtained by a two-step sequence similar to the synthesis of **4**. The racemic cyclic urea **16** turned out to be very potent against HIV-l in both HOS and PBL assays, Scheme 2. Chiral resolution of **16** was subsequently pursued to afford both ***R*** and ***S*** optical isomers, and the *R*-isomer (**16-*R***) turned out to be bioactive one (IC_{50} = 1.0 nM in PBL and 4.0 nM in HOS assays), Figure 3.

A chiral synthesis of cyclic urea portion **14-*R*** was also executed. In order to selectively acetylate the hydroxyl group of (2*R*)-2-amino-2-phenylethanol, its amino group was first protected with Boc and was then unmasked with HCl after acetylation. The resulting intermediate **18** was treated with 1,1-dimethylethyl-4-oxo-1-piperidnecarboxylate under routine reductive amination condition to give **19**. The subsequent cyclization was carried out very smoothly by reacting with the carbamate **6** to yield an acyclicurea intermediate that subsequently underwent the cyclization promoted by DBU under elevated temperature to afford **14-*R***. Final compounds **20a–e** were furnished in a two-step sequence similar to the synthesis of **4**, Scheme 3.

The compounds were tested for their antiviral activity in both HOS ($n = 2$) and PBL ($n \geqslant 4$) assays and their cellular toxicity was investigated with CellTiter-Glo reagent (Promega). None of the compounds induced cell death up to 1 μM (data not shown) indicating that the observed potency is the result of an antiviral effect and not cell toxicity, Table 1. Similarly to **16-*R***, other compounds in Table 1 exhibited potent antiviral activity against HIV-1 infection in both HOS and PBL assays. Slight increases of alkyl substituent size at either central pyridine ring (**20a**) or terminal sulfone group (**20b**, *i*-Pr vs Me) had little effect on potency. Additional substitution on the terminal phenyl ring were also well-tolerated (**20c** and **20d**). Replacement of the sulfonyl group with carboxamide (**20e)** also gave the similar result.

The stability of **16-*R*** in FSIF was next determined. As anticipated, the replacement of NH-moiety nearing acyclic urea with

Table 2
Pharmacokinetics of **16-*R*** in dog and monkey at 1 mg/kg dose[a]

Compound	Dog			Monkey		
	$t_{1/2}$ (hr) (i.v.)	Clearance (mL/min/kg)	F%	$t_{1/2}$ (hr) (i.v.)	Clearance (mL/min/kg)	F%
16-*R*	6.9	2.1	76.0	1.4	20.2	110.0

[a] Dose vehicle: 5% mannitol with 0.05% acetic acid in DMSO.

the cyclic urea motif in **16-*R*** very substantially increased compound stability in FSIF with no sign of substantial compound decomposition within 1000 h.

Selected compounds from Table 1 were also evaluated in vivo PK models. Most compounds had short half-life (<1 h) and high clearance in rat, however, **16-*R*** exhibited medium clearance (27.0 mL/min/kg) and was therefore advanced to dog and monkey PK studies. Compound **16-*R*** had an excellent PK profile in dog with a low clearance and a high bioavailablity, Table 2. Despite that the in vivo half-life in monkey was short, **16-*R*** still had a high oral exposure, which is consistent with its high absorbance observed in dog.

In summary, we designed and developed practical syntheses of a novel class of cyclic urea-based CCR5 antagonists (**16-*R***, **20a–e**).[7] This class of the compounds demonstrated high antiviral activity in both HOS and PBL assays. The evolution of the acyclic to the cyclic urea not only resolved the chemical stability issue, but also discovered **16-*R*** that had very good in vivo PK properties. Additional lead optimization and preclinical characterization of this series will be published in due time.

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