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Studies on the structure–activity relationship of 1,3,3,4-tetra-substituted pyrrolidine embodied CCR5 receptor antagonists. Part 2: Discovery of highly potent anti-HIV agents

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ABSTRACT

Modification of 1,3,3,4-tetra-substituted pyrrolidine embodied CCR5 receptor antagonists revealed that introducing a fluoro group at the 3-position of the 3-phenyl group to reduce metabolism did not adversely affect the high potency against HIV infection, and that replacing the piperidine ring with a tropane ring could deliver the most potent anti-HIV agents. Stereochemistry of the substituted tropane ring is essential for maintaining the potent anti-HIV activity because only *exo*-isomers displayed subnanomolar whole cell activity.

Since the β-chemokine receptor CCR5 was identified as an essential co-receptor for HIV-1 to dock and gain entry into $CD4^+$ macrophages and T-cells, great efforts have been directed to the discovery of selective CCR5 antagonists in order to develop promising agents for the treatment and/or prevention of HIV-1 infection.[1,2] These efforts have cumulated a variety of CCR5 antagonists that display significant activity against anti-HIV infection. Among the most potent compounds (Fig. 1), Takeda reported that TAK-652 (**1**) strongly inhibited the replication of six R5 HIV-1 clinical isolates (KK, CTV, HKW, HNK, HTN, and HHA) in human peripheral blood mononuclear cells (PBMCs), with EC_{50} values ranging from 0.024 to 0.089 nM.[3] Schering–Plough researchers described that vicriviroc (**2**) potently inhibit all the viral isolates tested, with geometric mean EC_{50} values ranging between 0.04 and 2.3 nM.[4] Roche's vicriviroc analog **3** also showed very potent antiviral activity with an EC_{50} of 0.5 nM.[5] Pfizer's maraviroc (**4**) showed potent antiviral activity against the 43 primary CCR5-tropic HIV-1 isolates tested, as reflected by a geometric mean EC_{90} of 2.0 nM (95% CI, 1.8–2.4 nM).[6,7]

During the studies on structure–activity relationship of 1,3,3,4-tetrasubstituted pyrrolidine embodied CCR5 receptor antagonists, some of our compounds have been improved to have single digit picomolar antiviral activity in PBMCs. These molecules may represent the most potent anti-HIV agents among the known CCR5 receptor antagonists. Herein we wish to disclose our results.

In a previous report, we found that modification of the N-substituents of nifeviroc (**5**, Fig. 2) led to the discovery of two urea analogs (**6** and **7**) and one amide analog (**8**) that retained potent anti-HIV activity. Based on these results, we planned to modify their benzene ring by introducing different substituents, and their piperidine ring by freezing its conformation by an ethylene bridge. Accordingly, the molecules **9** were designed as our new targets.

As depicted in Schemes 1 and 2, our desired compounds were assembled in a convergent manner. Baylis–Hillman reaction of methyl acrylate with α-keto esters **10** catalyzed by DABCO provided diesters **11** in with 32–47% yields.[8] Michael addition of (*R*)-α-methyl-benzylamine to these diesters followed by intramolecular condensation in refluxing dioxane afford enantiopure pyrrolidones **12** in 22–30% yields, upon crystallization from EtOAc solution. Hydrolysis of **12** accompanied by isomerization provided acids **13**. Reduction of **13** with LAH and subsequent hydrogenolysis produced free amines, which were reacted with cyclopentanecar-

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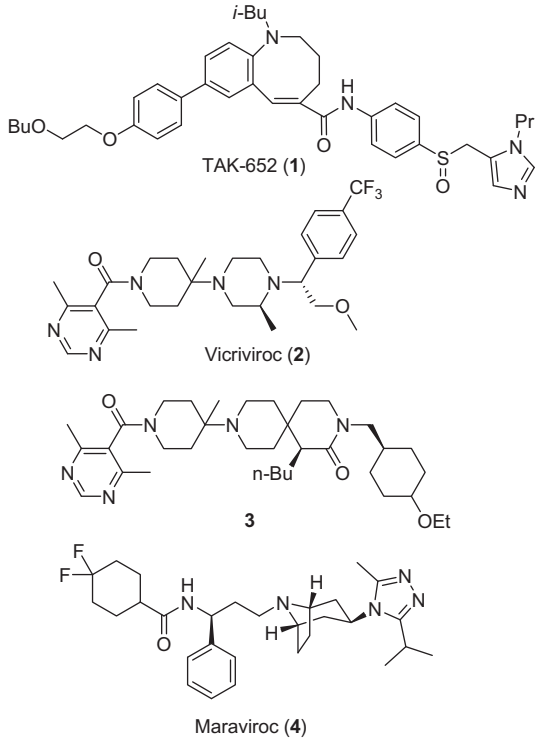


Figure 1. Structures of some CCR5 antagonists with potent anti-HIV activity.

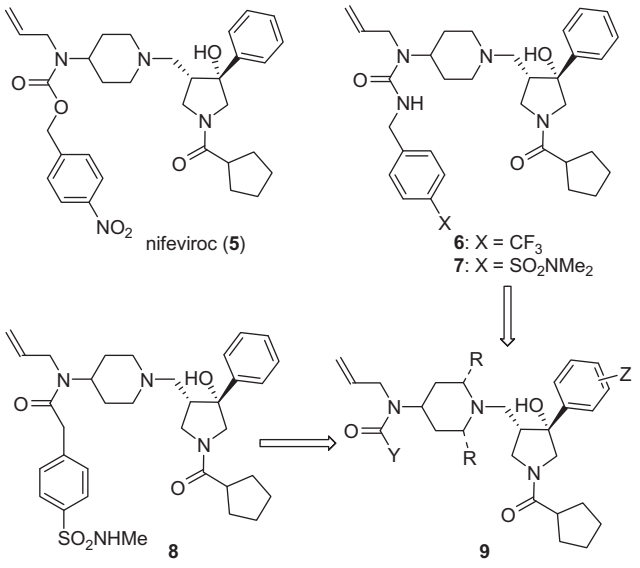
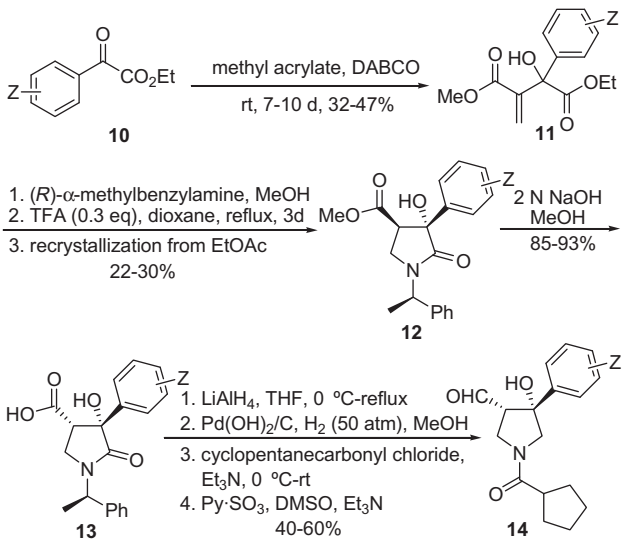


Figure 2. Structures of some pyrrolidine embodied anti-HIV agents and newly designed molecules.

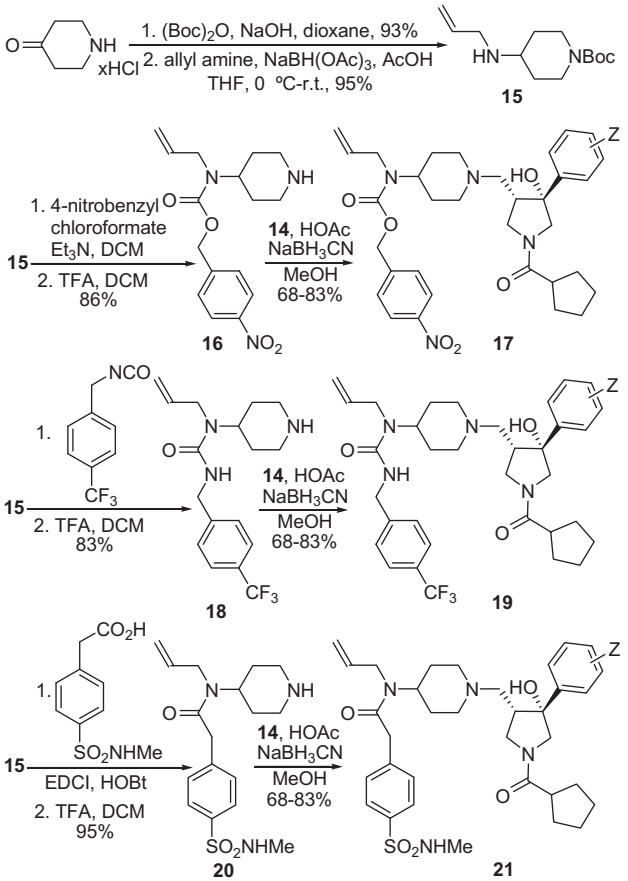
bonyl chloride and then oxidized with Py·SO_3 to deliver aldehydes **14**.

In a parallel procedure, protection of 4-piperidinone followed by reductive amination afforded allyl amine **15**. Condensation of **15** with 4-nitrobenzyl chloroformate, or an isocyanate or an acid and subsequent Boc deprotection with TFA produced amines **16**, **18**, and **20**. These amines were subjected to reductive amination with the aldehydes **14** to give the target molecules **17**, **19**, and **21** (Scheme 2).

All the compounds synthesized were tested for both their inhibition of RANTES-stimulated [^{35}S]GTPγS binding to CCR5-expressing CHO cell membranes and their antiviral activity in human peripheral blood mononuclear cells (R5-tropic HIV-1Ba-L strain). As summarized in Table 1, it is obvious that introduction of a methoxy or fluoro group at the 4-position of the phenyl ring resulted in a dramatic loss of antiviral activity and binding inhibition (**17a**, **17b**, **19a**, and **21a**). However, adding a fluoro group at the 3-position still delivered highly potent molecules (**17c**, **19b**, and **21b**).



Scheme 1.



Scheme 2.

Table 1
Biological data for compounds **5**, **17**, **19**, and **21**

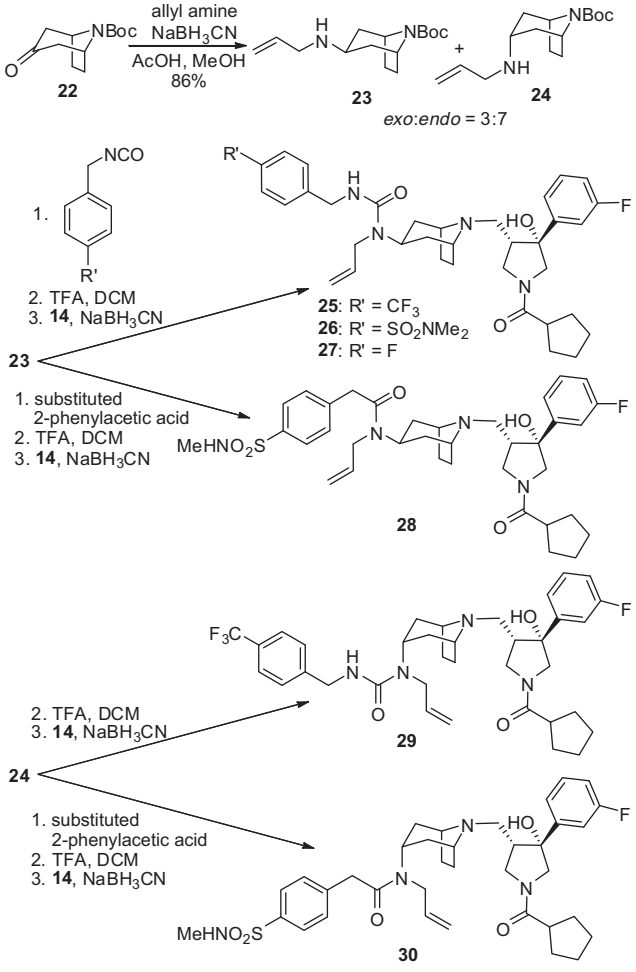
Compound	IC_{50}[a] (nM)	EC_{50}[b] (nM)
5	2.9	<0.4
17a: Z = 4-OMe	318	>100
17b: Z = 4-F	30	>100
17c: Z = 3-F	8.85	<0.4
19a: Z = 4-F	38.3	20
19b: Z = 3-F	1.38	6
21a: Z = 4-F	11.17	>100
21b: Z = 3-F	2.98	4.5

[a] SPA GTPγS assay.
[b] Antiviral activity in PBMC.

Table 2
Biological data for compounds **25**–**30**

Compound	IC_{50}[a] (nM)	EC_{50}[b] (nM)	hERG binding[c]	CC_{50}[d] (μM)
25	6.77	<0.005	89.5	12.5
26	11.4	0.0055	31.3	55
27	30	1.75	—	42
28	3.98	16	—	65
29	14.3	>100	88.6	102.7
30	27.1	>100	—	50

[a] SPA GTPγS assay.
[b] Antiviral activity in PBMC.
[c] % inhibition at 1 μM.
[d] Cellular toxicity (PBMC).



Scheme 3.

For modification of the piperidine part, we planned to replace this unit with the tropane moiety, and hoped its rigid conformation would make the resulting molecules more potent. The synthesis of our target molecules was outlined in Scheme 3, *N*-Boc protected 8-aza-bicyclo[3.2.1]octan-3-one **22** was reacted with allylamine under reductive amination conditions to produced *exo*-isomer **23** and *endo*-isomer **24** which were separated in a ratio of 3:7. Following the same procedures as indicated in Scheme 2, these two amines were converted into the desired urea analogs **25**–**27** and **29**, and amide analogs **28** and **30**.

All the tropane-bridged derivatives showed good CCR5 binding inhibition, whether they are *exo*- or *endo*-configuration (Table 2). However, their antiviral activity was very different: all the *exo*-compounds kept good antiviral activity in PBMCs while the *endo*-configuration was detrimental to the anti-HIV-1 activity (cf. **25** and **29**, **28** and **30**). Gratifyingly, compound **25** showed excellent potency and the mean EC_{50} value was less than 5 pM. Inspired by the surprising results, some substituents on the left benzyl ring were examined in the *para*-position. The $SO_2N(CH_3)_2$ substituted analog **26** had an EC_{50} of which was 5.5 pM which is similar potency to compound **25**.

The compounds were also nontoxic with CC_{50}'s >10 μM, giving a selectivity index of greater than 2×10^6 However, substituting of *p*-CF_3 benzyl by fluoro in **27** decreased the antiviral activity, but the compound still kept good potency (EC_{50} = 1.75 nM). The key finding from this study was that the piperidine ring of our compounds could be substituted by an *exo*-tropane ring.

In summary, through further SAR studies on 1,3,3,4-tetrasubstituted pyrrolidine embodied CCR5 receptor antagonists, we have demonstrated for the original piperidine based series that introducing a substituent at the 3-position of the phenyl ring was possible without loss of activity, while substituents in the 4-position were not tolerated. Significantly, by replacing the piperidine ring with a tropane ring, two *exo*-analogs with single digital picomolar potency for antiviral activity and nanomolar binding affinity, were discovered. Their excellent potency was found to be highly dependent on the stereochemistry of the substituted tropane ring, because *endo*-isomers showed considerably low potency for anti-HIV activity, although binding inhibition ability to CCR5 receptor could not distinguish between them.

Acknowledgments

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