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Potent CXCR4 Antagonists Containing Amidine Type Peptide Bond Isosteres

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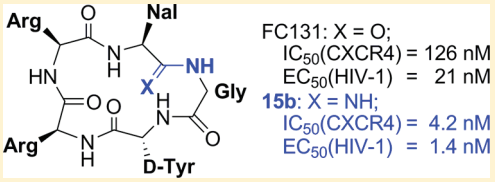
Supporting Information

ABSTRACT: A series of FC131 [*cyclo*(-D-Tyr-Arg-Arg-Nal-Gly-)] analogues containing amidine type peptide bond isosteres were synthesized as selective CXC chemokine receptor type 4 (CXCR4) antagonists. An isosteric amidine substructure was constructed by a macrocyclization process using nitrile oxide-mediated C−N bond formation. All of the amidine-containing FC131 analogues exhibited potent SDF-1 binding inhibition to CXCR4. The Nal-Gly-substituted analogue was characterized as one of the most potent cyclic pentapeptide-based CXCR4 antagonists reported to date. The improved activity against human immunodeficiency virus (HIV) type-1 X4 strains suggested that addition of another basic amidine group to the peptide backbone effectively increases the selective binding of the peptides to CXCR4 receptor.



KEYWORDS: Amidine, chemokine, CXCR4 antagonist, FC131, nitrile oxide, peptidomimetics

CXC chemokine receptor type 4 (CXCR4) is a G protein-coupled receptor[1] for stromal cell-derived factor 1 (SDF-1)[2] that plays a critical role in the metastasis of mammary carcinoma[3] and in human immunodeficiency virus (HIV) type-1 infection.[4] CXCR4 is an important therapeutic target for these diseases.[5] To date, several types of CXCR4 antagonists with a variety of scaffolds have been reported (Figure 1).[6−11] Although the scaffolds of these antagonists have little in common, the antagonists all contain a number of basic groups. For example, the polyphemusin II-derived anti-HIV peptide, T140 **1**,[6] has seven basic Arg and Lys residues. Another example is the small molecule antagonist AMD3100, which contains eight secondary or tertiary amino nuclei.[7] Crystal structure analysis and mutation experiments of the receptor indicated that the ion-pairing interaction between the basic functional groups of the antagonists and the acidic residues in CXCR4 contributes to the potent bioactivity.[12−14]

FC131 [*cyclo*(-D-Tyr-Arg-Arg-Nal-Gly-), Nal = 3-(2-naphthyl)-alanine] **2** is a highly potent CXCR4 antagonist (Figure 1).[15] Using the peptide library approach, the potent anti-HIV activity of T140 **1** was reproduced with the appropriate arrangement of basic and aromatic residues on the cyclic pentapeptide framework of FC131. Further systematic structure−activity studies, such as alanine-scanning or amino acid optimizations, have been conducted to identify the structural and electrostatic requirements for the bioactivity of FC131.[16] Substitution of an Arg residue in **2** with the epimeric *N*-methyl-D-arginine led to identification of cyclic pentapeptide-based CXCR4 antagonist, FC122 **3**, which is the most potent CXCR4 antagonist among the FC131 derivatives reported to date.[16] However, backbone modification of **2** using peptide bond isosteres did not improve the bioactivity.[17−19] For example, replacement of several peptide bonds with reduced amide bonds **5** or alkene dipeptide isosteres **6** resulted in greatly reduced bioactivity (Figure 2), which suggests that these isosteric substructures are not appropriate for modifications of FC131. On the basis of these previous studies of FC131 derivatives and the common structural features of highly potent CXCR4 antagonists, we envisioned that addition of basic functional group(s) onto FC131 could improve its potency.

Recently, we established a novel synthetic approach for amidine type peptide bond isosteres **7** using nitrile oxide-mediated C−N bond formation.[20] Amidine type peptide bond isosteres were designed based on substitution of the peptide bond carbonyl (C═O) group with an imino (C═NH) group.[21,22] Under physiological conditions, the positive charge of the protonated amidines **7′** is delocalized over two nitrogens. Substructure **7′** contributes both the double bond character of peptide bond **4** and the basic character of reduced amide bond isostere **5′**. Therefore, the addition of this acyclic amidine group to the framework was expected to enhance the bioactivity without inducing large conformational change in the backbone structure. Accordingly, amidine-containing FC131 analogues **15a**,**b** and **15d**−**f** were designed, in which each peptide bond was replaced with the amidine substructure (Table 1). Compounds **15c** and **15g** were also designed as epimers of **15b** (at the Nal position) and **15f** (at the Tyr position), respectively. In this study, we investigated the contribution of amidine units to the bioactivity of amidine-containing FC131 analogues **15a**−**g**.

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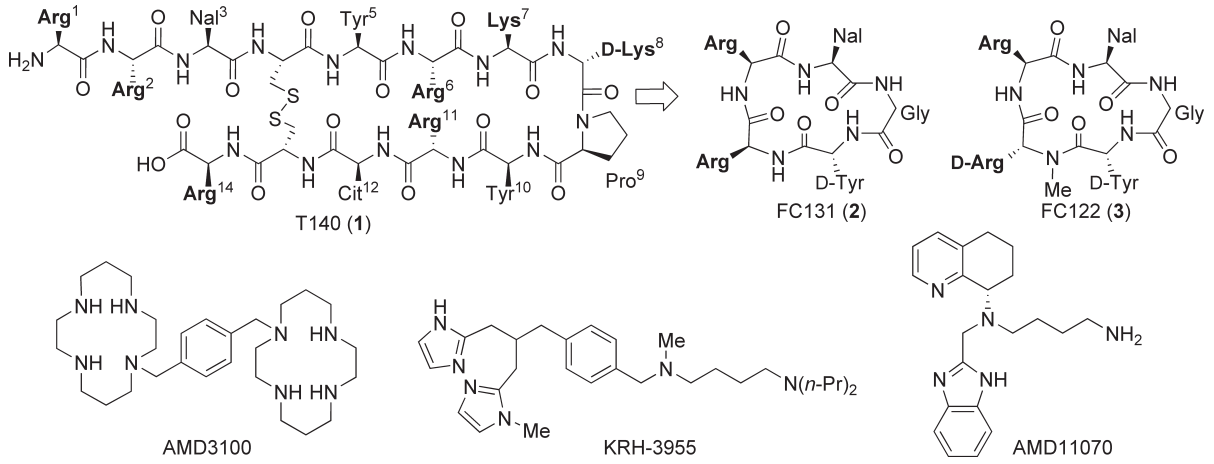

Figure 1. Structures of reported CXCR4 antagonists. Bold residues are basic residues. Nal = 3-(2-naphthyl)alanine.

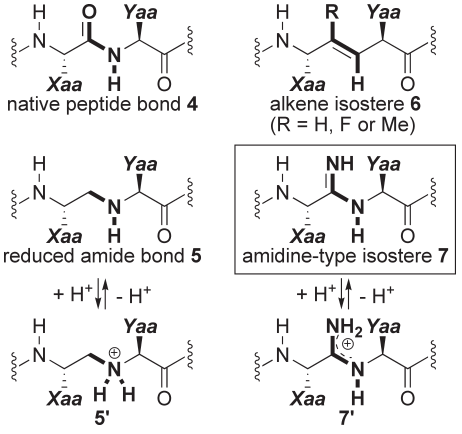

Figure 2. Structures of the peptide bond and the mimetics.

Table 1. Inhibitory Activity of FC131 and the Derivatives 15a−g against [^{125}I]-SDF-1 Binding to CXCR4

peptide	sequence[a]	IC_{50} (nM)[b]
FC131 (**2**)	*cyclo*(-D-Tyr-Arg-Arg-Nal-Gly-)	126 ± 68
FC122 (**3**)	*cyclo*(-D-Tyr-D-MeArg-Arg-Nal-Gly-)	37 ± 20
15a	*cyclo*(-D-Tyr-Arg-Arg-Nal-Gly-Ψ-)	9.4 ± 3.0
15b	*cyclo*(-D-Tyr-Arg-Arg-Nal-Ψ-Gly-)	4.2 ± 0.31
15c	*cyclo*(-D-Tyr-Arg-Arg-D-Nal-Ψ-Gly-)	4.9 ± 1.1
15d	*cyclo*(-D-Tyr-Arg-Arg-Ψ-Nal-Gly-)	11 ± 2.9
15e	*cyclo*(-D-Tyr-Arg-Ψ-Arg-Nal-Gly-)	16 ± 7.2
15f	*cyclo*(-D-Tyr-Ψ-Arg-Arg-Nal-Gly-)	679 ± 132
15g	*cyclo*(-Tyr-Ψ-Arg-Arg-Nal-Gly-)	334 ± 6.2

[a] Ψ indicates the ψ[−C(=NH)−NH−] substructure. Nal, 3-(2-naphthyl)alanine. [b] IC_{50} values are the concentrations for 50% inhibition of the [^{125}I]-SDF-1α binding to CXCR4 transfectant of HEK293 cells.

Synthesis of the L-Nal-Gly-substituted analogue **15b** is shown in Scheme 1 as a representative preparation of peptides **15a−g**. The first Nal residue was loaded onto aminooxy-2-chlorotrityl resin **8**[20] by treatment with Fmoc-3-(2-naphthyl)alaninal **9b** under acid-free conditions to give aldoxime resin **10b**. To prevent possible intramolecular cyclization between side chain guanidino and aldehyde groups in the preparation of aldoxime resins **10d** and **10e**, di-Boc-protected arginine [Arg(Boc)$_2$]-derived aldehyde was utilized for the preparation of Arg-Arg- and Arg-Nal-substituted analogues **15d** and **15e**. Peptide elongation was performed by the standard Fmoc-based solid-phase synthesis using *N*,*N*′-diisopropylcarbodiimide (DIC)/*N*-hydroxybenzotriazole (HOBt) in DMF. The cleavage of peptide aldoxime resin **11b** provided the linear peptide aldoxime **12b**, which was treated with *N*-chlorosuccinimide and triethylamine to afford cyclic amidoxime (*N*-hydroxyamidine) **13b**.[20] After Raney Ni-mediated reduction to the amidine **14b**, deprotection with a cocktail of 1M TMSBr, thioanisole/TFA, *m*-cresol, and 1,2-ethanedithiol gave the desired amidine-containing FC131 analogue **15b**. The analogues **15a** and **15c−g** were synthesized by the same procedure. During this nitrile oxide-mediated cyclization, significant epimerizations of the activated C termini of the peptides were not observed.[23]

The potency of the resulting FC131 analogues **15a−g** to inhibit [^{125}I]-SDF-1 binding to CXCR4 was evaluated (Table 1). Peptides **15a−e** were more potent than the control peptides **2** and **3**. This indicates that the basic amidine units had the expected effect of increasing the affinity with the receptor. By contrast, substitution of the Tyr-Arg dipeptide decreased the CXCR4 antagonistic activity (**15f** and **15g**). These observations were consistent with our previous study, in that the D-Tyr-Arg peptide bond is an indispensable functional group that is required to maintain the peptide conformation and the interaction with the receptor. Potent bioactivity of D-MeArg-substituted peptide (**3**) indicated that the amide hydrogen of Arg is not critical to the bioactivity,[16] while the local backbone conformation, particularly with respect to the orientation of D-Tyr carbonyl oxygen, may contribute to the receptor binding. Less potent bioactivity of **15f** and **15g** supports the significant contribution of D-Tyr carbonyl group in peptides **2** and **3**.

Nal-Gly-modified analogues **15b** and **15c** were the most potent inhibitors of the compounds synthesized in this study. At this Nal-Gly dipeptide position, the amidine substructure was more appropriate than the reduced amide motif (−CH$_2$−NH−), which exhibited slightly lower bioactivity than FC131 in our previous study.[17] It is interesting that modification at the Arg-Nal dipeptide (**15d**) gave potent bioactivity, whereas replacement of this dipeptide with the reduced amide bond in our earlier study reduced receptor binding.[17] This indicates that the high bioactivity of **15d** could be caused by conformational advantage rather than

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Scheme 1. Synthesis of Amidine-Containing FC131 Analogue 15b

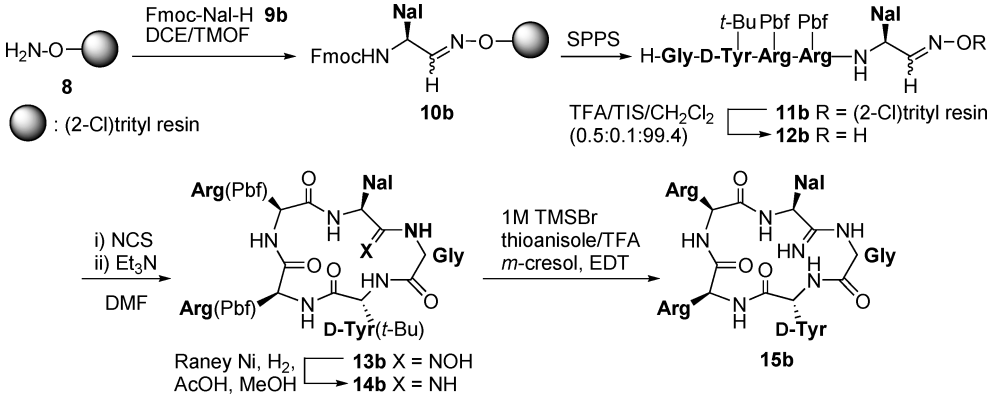

Table 2. Anti-HIV Activity of FC131 and the Derivatives 15a−g

	EC_{50} (nM)[a]		
peptide	NL4-3	IIIB	Ba-L
FC131 (**2**)	21 ± 4.3	21 ± 5.9	–[b]
FC122 (**3**)	7.6 ± 0.34	7.6 ± 1.1	–[b]
15a	1.3 ± 0.43	0.61 ± 0.10	–[b]
15b	1.4 ± 0.44	1.0 ± 0.23	–[b]
15c	2.2 ± 0.04	2.0 ± 0.59	–[b]
15d	4.4 ± 1.0	6.3 ± 0.47	–[b]
15e	1.9 ± 0.47	1.2 ± 0.29	–[b]
15f	300 ± 57	258 ± 47	–[b]
15g	248 ± 55	238 ± 37	–[b]

[a] EC_{50} is the concentration that blocks HIV-1 infection by 50%. [b] No inhibitory activity was observed at 10 μM.

the basicity. The partial double bond character of the amidine motif in **15d** might favorably constrain the cyclic configuration and place the side chains in the appropriate spatial orientations of the pharmacophore.

Recent reports of the docking model of FC131-CXCR4 interactions indicated that the amino group of the Gly-D-Tyr peptide bond forms a hydrogen bond to the carbonyl group of Ala95.[24,25] It was also suggested that the Arg-Arg dipeptide is surrounded by acidic residues in CXCR4 (Glu288 and Asp262).[24,25] The potent bioactivities of Gly-D-Tyr- and Arg-Arg-substituted analogues **15a** and **15e** may support the presence of these favorable interactions, which were enhanced by the introduction of positively charged amidine motifs. In particular, the amidine in the Arg-Arg dipeptide could form salt bridge(s) with the negatively charged residues.

Interestingly, both stereoisomers of Nal-Gly- and Tyr-Arg-modified analogues (**15b,c** and **15f,g**) showed similar antagonistic activities [**15b** (IC_{50} = 4.2 nM) and **15c** (IC_{50} = 4.9 nM); **15f** (IC_{50} = 679 nM) and **15g** (IC_{50} = 334 nM)]. This is in contrast to the suggestion that the bioactivity of FC131 derivatives is sensitive to the configurations of the component residues.[16,17] These results may suggest that the local conformation around the amidine motif is more flexible in cyclic pentapeptides than in the original peptide bond. Of note, none of the peptides **15a−g** showed inhibition against SDF-1-CXCR7 interaction (data not shown), which is reported to be an alternative receptor of SDF-1.

Anti-HIV activity based on inhibition of human immunodeficiency virus type 1 (HIV-1) entry into target cells was examined by the MAGI assay using NL4-3, IIIB, and Ba-L strains (Table 2). NL4-3 and IIIB strains use CXCR4 for entry into cells, and the FC131 analogues **15a−e** showed very potent anti-HIV activity against these strains. The two Tyr-Arg-substituted peptides **15f** and **15g** only moderately inhibited infection with these two strains, which was similar to their inhibitory effects against SDF-1-CXCR4 binding. The Ba-L strain uses CCR5 for entry to cells, and none of the peptides showed inhibitory activity against this strain even with the peptides at 10 μM. This result indicates that peptides **15a−g** show similar target specificity to FC131 as selective CXCR4 antagonists.[26] The cytotoxicity of analogues **15a−g** was not observed even at 10 μM in the MAGI assay.

In conclusion, we developed novel potent cyclic pentapeptide-based CXCR4 antagonists containing amidine type peptide bond isosteres. Substitutions of four peptide bonds in FC131, except for the D-Tyr-Arg position, with an amidine motif, improved the inhibitory activity against SDF-1 binding and HIV-1 infection by X4 strains. It was also demonstrated that the analogues were selective antagonists for CXCR4 and not for CXCR7 and CCR5, which are the targets shared by SDF-1 (CXCR7) and HIV-1 (CCR5). Further studies to understand the binding mode of these peptidomimetics and to develop derivatives with multiple amidine motifs in a single molecule are in progress.

■ ASSOCIATED CONTENT

Supporting Information. Experimental procedures and characterization data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS

CXCR4, CXC chemokine receptor type 4; HIV-1, human immunodeficiency virus type 1; Nal, 3-(2-naphthyl)alanine; DIC, *N*,*N*′-diisopropylcarbodiimide; HOBt, *N*-hydroxybenzotriazole; SDF-1, stromal cell-derived factor 1

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