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Pharmacophore-based small molecule CXCR4 ligands

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ABSTRACT

Low molecular weight CXCR4 ligands were developed based on the peptide T140, which has previously been identified as a potent CXCR4 antagonist. Some compounds with naphthyl, fluorobenzyl and pyridyl moieties as pharmacophore groups in the molecule showed significant CXCR4-binding activity and anti-HIV activity. Structure–activity relationships were studied and characteristics of each of these three moieties necessary for CXCR4 binding were defined. In this way, CXCR4 ligands with two types of recognition modes for CXCR4 have been found.

The chemokine receptor CXCR4 is classified into a family of G protein-coupled receptors (GPCRs), and transduces signals of its endogenous ligand, CXCL12/stromal cell-derived factor-1 (SDF-1).[1] The CXCR4-CXCL12 axis plays a physiological role in chemotaxis,[2] angiogenesis[3] and neurogenesis[4] in embryonic stages. The CXCR4 receptor is linked to many disorders including HIV infection/AIDS,[5] metastasis of cancer cells,[6] leukemia cell progression,[7] rheumatoid arthritis.[8] Since CXCR4 is an important drug target in these diseases, it is thought that effective agents directed to this receptor may be useful leads for therapeutic agents. To date, we and others have developed several potent CXCR4 antagonists. A highly potent antagonist, T140, a 14-mer peptide with a disulfide bridge, and its downsized analogue, FC131, with a cyclic pentapeptide scaffold, and several other related compounds have been reported.[9] Based on T140 and FC131, small-sized linear anti-HIV agents such as ST34 (**1**) have been developed (Fig. 1).[10] AMD3100,[11] KRH-1636,[12] Dpa–Zn complex (**2**)[13] and other azamacrocyclic compounds such as **3**,[14] which like **1**, contain benzylamine and electron-deficient aromatic groups, have also been reported as nonpeptidic antagonists. Compound **1** possesses significant anti-HIV activity but does not have high CXCR4 binding affinity. In the present study, more effective linear CXCR4 antagonists derived from compound **1** have been examined, and structure–activity relationship studies of these compounds have been performed.

Initially, three segments of compound **1** were selected for structural modification to support the design of new synthetic compounds: replacement of the 4-trifluoromethylbenzoyl group (Fig. 2, R^1), modification of the stereochemistry of the 1-naphthylethylamine moiety (R^2) and introduction of pyridine moieties on the nitrogen atom (R^3). In a previous study of T140 analogues, 4-fluorobenzoyl was found to be superior to 4-trifluoromethylbenzoyl as an N-terminal moiety. Thus, 4-fluorobenzyl, 4-fluorobenzoyl and 4-fluorophenylethyl groups were used as substitutes for the 4-trifluoromethylbenzoyl group (R^1) in **1**. The (*R*)-1-naphthylethylamine moiety in **1** is also present in KRH-1636 where it has the (*S*)-stereochemistry and thus both the (*R*) and (*S*)-stereoisomers were investigated in the present study. Several CXCR4 antagonists such as KRH-1636,[12] Dpa-Zn complex (**2**)[13] and Dpa-cyclam compound (**3**),[14] contain pyridyl rings. Thus, 2, 3, or 4-pyridylmethyl and 2, 3, or 4-pyridylethyl groups were introduced on the nitrogen atom of the 4-aminomethylbenzoyl group (R^3). With these modifications, a total of $3 \times 2 \times 6 = 36$ compounds (**12**–**47**) were designed (Fig. 2).

The synthesis of the structural fragment, Unit 1 is shown in Scheme 1. N-nosylation of 4-amino-methylbenzoic acid (**4**) with 2-nitrobenzenesulfonyl chloride and subsequent esterification gave the *t*-butyl ester **5**. Introduction of an R^3 moiety by means of a Mitsunobu reaction followed by removal of the Ns group yielded amines **6A–F**. Introduction of either 4-fluorobenzyl or 4-fluorophenylethyl groups by reductive amination of **6A–F** produced amines **7Ai–Fi** or **7Aiii–Fiii**, respectively. Conversion of **6A–F** to the appropriate amide (**7Aii-Fii**), and subsequent deprotection of the *tert*-butyl group yielded Unit 1, **8Ai–Fiii**.

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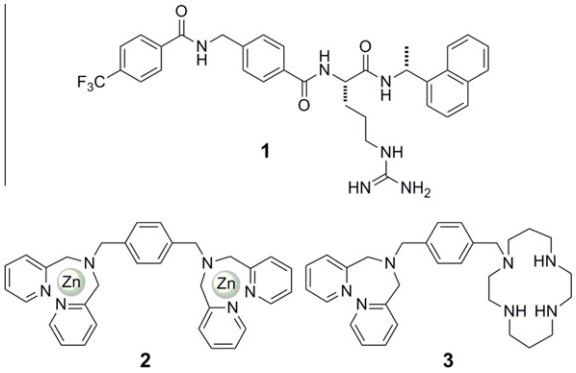


Figure 1. The structures of **1** (ST34), Dpa-Zn complex (**2**) and Dpa-cyclam compound (**3**).

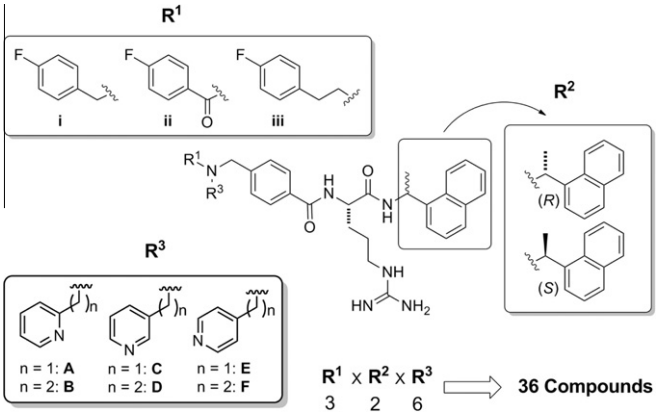
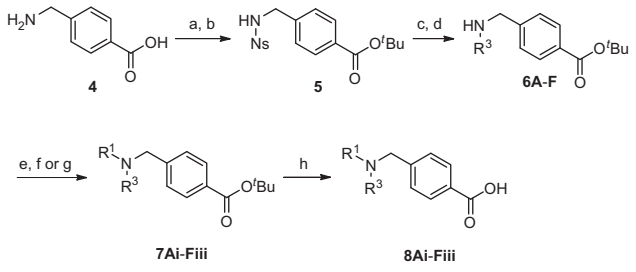


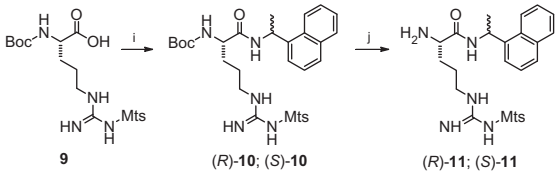
Figure 2. The structures of substituents for three parts of compound **1** in the design of new compounds.



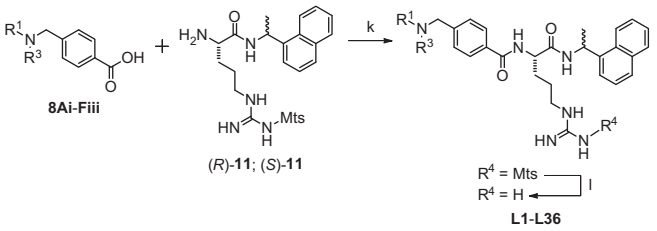
Scheme 1. The synthetic scheme of Unit 1, compounds **8Ai–Fiii**. Reagents and conditions and yields: (a) NsCl, Et_3N, THF/H_2O (1/1); (b) isobutene, THF/H_2SO_4 (10/1), 39% (2 steps); (c) PPh_3, DEAD, R^3OH, THF; (d) PhSH, K_2CO_3, DMF, 42–92% (2 steps); (e) $NaBH(OAc)_3$, 4-fluorobenzaldehyde, CH_2Cl_2; (f) $NaBH(OAc)_3$, (4-fluorophenyl)acetaldehyde, CH_2Cl_2; g) 4-fluorobenzoyl chloride, Et_3N, CH_2Cl_2, 51-94%; (h) TFA then 4 M HCl/EtOAc, quantitative; The structures of R^1 and R^3 are shown in Fig. 2 as i–iii and **A**–**F**, respectively. Ns = 2-nitrobenzenesulfonyl, ^{t}Bu = *tert*-butyl, DEAD = diethyl azodicarboxylate.

The synthesis of Unit 2 is shown in Scheme 2. Condensation of Boc-Arg(Mts)-OH (**9**) and (*R*)-1-naphthylethylamine or its (*S*) isomer produced amides (*R*)-**10** or (*S*)-**10**. Removal of the Boc group gave Unit 2, (*R*)-**11** and (*S*)-**11**.

Compounds **12**–**47** were synthesized by amide condensation of Unit 1, **8Ai–Fiii**, with Unit 2, (*R*)-**11** and (*S*)-**11**, and subsequent deprotection of the Mts group, as shown in Scheme 3.[15] All the synthetic compounds were purified by preparative reverse phase HPLC. In cases where peaks derived from side products appeared around the target peaks on the HPLC profile, the precise analysis was accomplished, giving rise to lower yields (Scheme 3, l).



Scheme 2. Synthetic schemes of Unit 2, compounds (***R*/*S*)-11**. Reagents and conditions: (i) EDCI·HCl, $HOBt·H_2O$, Et_3N, (*R*/*S*)-(+/−)-1-(1-naphthyl)ethylamine, CH_2Cl_2, 83–97%; (j) TFA then 4 M HCl/EtOAc, quantitative; EDCI·HCl = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, $HOBt·H_2O$ = 1-hydroxybenzotriazol monohydrate, Mts = 2,4,6-trimethylphenylsulfonyl, Boc = *tert*-butoxycarbonyl.



Scheme 3. Synthetic schemes of compounds **12**–**47**. Reagents and conditions: (k) EDCI·HCl, $HOBt·H_2O$, Et_3N, DMF, 36-95%; (l) TMSBr, *m*-cresol, 1,2-ethanedithiol, thioanisole, TFA, 4–54%. The structures of R^1 and R^3 are shown in Figure 2 as i–iii and **A**–**F**, respectively.

The CXCR4-binding activity of synthetic compounds was assessed in terms of the inhibition of [^{125}I]-CXCL12 binding to Jurkat cells, which express CXCR4.[16] The percent inhibition of all the compounds at 10 μM is shown in Table 1. Several of the compounds showed significant binding affinity. In general, compounds in which the 1-naphthylethylamine moiety (R^2) has the (*S*)-stereochemistry, as in KRH-1636, are more potent than the (*R*)-stereoisomers. Ten compounds (**26–28**, **30**, **33**, **36**, **39**, **44**, **45** and **47**, Table 1) were found to induce at least 30% inhibition and compounds **26**, **27** and **33**, which have a pyridyl group with a nitrogen atom at the β-position, showed more than 60% inhibition. It is noteworthy that compounds **26** and **27** in which R^2 is a (*R*)-1-naphthylethylamine moiety, are both more potent than the corresponding (*S*)-stereoisomers **44** and **45.** Compounds **26**, **27** and **33**, have a 4-fluorobenzyl or 4-fluorophenylethyl group, which rather than an amide, is a reductive alkyl type (R^1). As can be seen from Table 1, there is a tendency for compounds with a pyridyl group with a nitrogen atom at the β-position (R^3: C or D), to be more potent in terms of CXCR4-binding activity than the corresponding compounds, which have a pyridyl group with a nitrogen atom at the α- or γ- position (R^3: A, B, E or F), and those with a reductive alkyl 4-fluorobenzyl or 4-fluorophenylethyl group (R^1: i or iii), to be more potent in CXCR4-binding activity than the corresponding compounds, with a 4-fluorobenzoyl group (R^1: ii).

Compounds were next evaluated for anti-HIV activity and cytotoxicity. CXCR4 is the major co-receptor for the entry of T-cell line-tropic (X4-) HIV-1.[5] Accordingly, inhibitory activity against X4-HIV-1 (NL4-3 strain)-induced cytopathogenicity in MT-4 cells (anti-HIV activity), and reduction of the viability in MT-4 cells (cytotoxicity) were assessed[16] and are shown in Table 1. Compounds **26** and **33**–**35** showed significant anti-HIV activity with EC_{50} values in the micromolar range. Compounds **26** and **33** showed both potent CXCR4-binding activity (79% and 60% inhibition at 10 μM, respectively) and anti-HIV activity (EC_{50} = 11 and 13 μM, respectively), the two activities being highly correlated. Compounds **34** and **35** have significant anti-HIV activity with EC_{50} values of 8 and 10 μM, respectively, which is higher than CXCR4-binding activities, which are 16% and 20% inhibition at 10 μM, respectively. Compound **27**, which showed relatively high CXCR4-binding activity (69% inhibition at 10 μM), failed to show

Table 1
CXCR4-binding activity, anti-HIV activity and cytotoxicity of compounds **12**–**47**

Compd no.	R^1 [a]	R^2 [b]	R^3 [c]	Inhibition[d] (%)	EC_{50}[e] (μM)	CC_{50}[f] (μM)	Compd no.	R^1 [a]	R^2 [b]	R^3 [c]	Inhibition[d] (%)	EC_{50}[e] (μM)	CC_{50}[f] (μM)
12	i	(*R*)	A	0	>20	35	**30**	i	(*S*)	A	30 ± 1.1	>4	11
13	i	(*R*)	B	4 ± 1.7	>4	23	**31**	i	(*S*)	B	25 ± 3.3	>20	24
14	i	(*R*)	C	6 ± 0.7	>20	37	**32**	i	(*S*)	C	27 ± 1.7	>20	41
15	i	(*R*)	D	24 ± 1.7	n.d.	n.d.	**33**	i	(*S*)	D	60 ± 1.5	13	65
16	i	(*R*)	E	12 ± 3.0	>20	39	**34**	i	(*S*)	E	16 ± 1.2	8	44
17	i	(*R*)	F	16 ± 2.2	n.d.	n.d.	**35**	i	(*S*)	F	20 ± 1.3	10	44
18	ii	(*R*)	A	3 ± 0.9	>20	38	**36**	ii	(*S*)	A	36 ± 1.8	>20	37
19	ii	(*R*)	B	6 ± 3.9	>20	41	**37**	ii	(*S*)	B	0	>20	43
20	ii	(*R*)	C	11 ± 0.8	>20	45	**38**	ii	(*S*)	C	14 ± 1.4	>20	57
21	ii	(*R*)	D	22 ± 4.1	n.d.	n.d.	**39**	ii	(*S*)	D	32 ± 8.4	n.d.	n.d.
22	ii	(*R*)	E	6 ± 2.7	>20	45	**40**	ii	(*S*)	E	13 ± 15	>20	51
23	ii	(*R*)	F	12 ± 1.9	n.d.	n.d.	**41**	ii	(*S*)	F	25 ± 13	>20	47
24	iii	(*R*)	A	15 ± 2.1	n.d.	n.d.	**42**	iii	(*S*)	A	16 ± 5.1	>4	9.9
25	iii	(*R*)	B	13 ± 0.6	>20	27	**43**	iii	(*S*)	B	23 ± 14	>4	13
26	iii	(*R*)	C	79 ± 14	11	47	**44**	iii	(*S*)	C	36 ± 13	n.d.	n.d.
27	iii	(*R*)	D	69 ± 5.0	>11	11	**45**	iii	(*S*)	D	35 ± 5.2	n.d.	n.d.
28	iii	(*R*)	E	44 ± 5.4	n.d.	n.d.	**46**	iii	(*S*)	E	26 ± 23	n.d.	n.d.
29	iii	(*R*)	F	0	n.d.	n.d.	**47**	iii	(*S*)	F	51 ± 6.6	n.d.	n.d.
KRH-1636				100	0.33	80	FC131				100	0.16	>10
AMD3100				n.d.	0.062	55	1 (ST34)				n.d.	7.4	66
AZT				n.d.	0.058	100							

[a,c] The structures of R^1 and R^3 are shown in Fig. 2 as i–iii and **A–F**, respectively.
[b] The absolute configuration in stereochemistry of R^2 shown in Fig. 2 is described.
[d] CXCR4-binding activity was assessed based on the inhibition of the [^{125}I]-CXCL12 binding to Jurkat cells. Inhibition percentages of all the compounds at 10 μM were calculated relative to the inhibition percentage by T140 (100%).
[e] EC_{50} values are the concentrations for 50% protection from X4-HIV-1 (NL4-3 strain)-induced cytopathogenicity in MT-4 cells.
[f] CC_{50} values are the concentrations for 50% reduction of the viability of MT-4 cells. All data are the mean values from at least three independent experiments.

significant anti-HIV activity at concentrations below 11 μM because of high cytotoxicity (CC_{50} = 11 μM). With the exception of **27**, **30**, **42** and **43**, the tested compounds showed no significant cytotoxicity (CC_{50} >20 μM, Table 1). On the other hand, compounds **26**, **27**, **33**, **34** and **35** at concentrations below 100 μM failed to show significant protective activity against macrophage-tropic (R5-) HIV-1 (NL(AD8) strain)-induced cytopathogenicity in PM-1/CCR5, whereas the EC_{50} of the CCR5 antagonist SCH-D[17] in this assay was 0.055 μM (data not shown). Since instead of CXCR4, R5-HIV-1 strains use the chemokine receptor CCR5, a member of the GPCR family, as the major co-receptor for their entry, this suggests that these compounds do not bind to CCR5. Thus, compounds **26**, **27**, **33**, **34** or **35** have highly selective affinity for CXCR4. Compounds **34** and **35**, which have significant anti-HIV activity, have a pyridyl group with a nitrogen atom at the γ-position, in contrast to compounds **26**, **27** and **33** which also show CXCR4-binding activity, but have a pyridyl group with a nitrogen atom at the β-position. Furthermore, compounds **34** and **35** have R^1 = 4-fluorobenzyl and R^2 = (*S*)-1-naphthylethylamine. A possible explanation of these observations is that compounds **34** and **35** compete with HIV-1 in binding to CXCR4 while compounds **26** and **33** compete with HIV-1 and CXCL12. Compound **27** does not compete with HIV-1 because of its high cytotoxicity. This suggests that the CXCR4 binding site used by compounds **34** and **35** differs slightly from that used by compounds **26**, **27** and **33**.

Low molecular weight CXCR4 ligands with two types of recognition modes for CXCR4 have been obtained in this study: one causes competition with HIV-1 on CXCR4 whereas the other causes competition with HIV-1 and CXCL12. These compounds have selective affinity for CXCR4 because they do not significantly bind to CCR5. Further structural modification studies of these CXCR4 ligands are the subject of an ongoing project.

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15. *For example, the synthesis of compound* ***30***: To a stirred solution of **8Ai** (176 mg, 0.415 mmol, HCl salt) in DMF (4 mL) were added EDCI·HCl (104 mg, 0.454 mmol), HOBt·H_2O (58.4 mg, 0.381 mmol), Et_3N (301 μL, 2.16 mmol) and ***(S)*-11** (320 mg, 0.657 mmol, HCl salt) at 0 °C. The mixture was stirred at room temperature for 43 h. The reaction mixture was diluted with $CHCl_3$ and washed with saturated citric acid, saturated $NaHCO_3$ and brine, and dried over $MgSO_4$. Concentration under reduced pressure followed by flash column chromatography over silica gel with $CHCl_3$/MeOH (20/1) gave the condensation product (175 mg, 0.208 mmol, 50% yield) as white powder. To this compound were added *m*-cresol (75.0 μL, 0.714 mmol), 1,2-ethanedithiol (225 μL, 2.68 mmol), thioanisole (225 μL, 1.91 mmol), TFA (3 mL) and bromotrimethylsilane (495 μL, 3.82 mmol) with stirring at 0 °C, and the stirring was continued at room temperature for 3.5 h under N_2. The reaction mixture was concentrated under reduced pressure, followed by addition of Et_2O to precipitate the product. After washing with Et_2O, the crude product was purified by preparative HPLC and lyophilized to give the compound **30** (15.6 mg, 0.0236 mmol, 13%) as white powder. ^{1}H NMR δ_H (400 MHz; DMSO-d_6) 1.49 (m, 2H), 1.51 (d, J = 7.2 Hz, 3H), 1.80–1.62 (m, 2H), 3.07 (dd, J = 6.4, 12.8 Hz, 2H), 3.85 (s, 2H), 3.91 (s, 4H), 4.54 (m, 1H), 5.72 (m, 1H), 7.13 (t, J = 8.8 Hz, 2H), 7.40 (m, 1H), 7.60–7.45 (m, 10H), 7.75–7.95 (m, 5H), 8.10 (m, 1H), 8.40 (d, J = 8.0 Hz, 1H), 8.58 (m, 1H), 8.65 (d, J = 7.6 Hz, 1H); LRMS (ESI), m/z calcd for $C_{39}H_{42}FN_7O_2$ $(MH)^+$ 660.34, found 660.31.
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