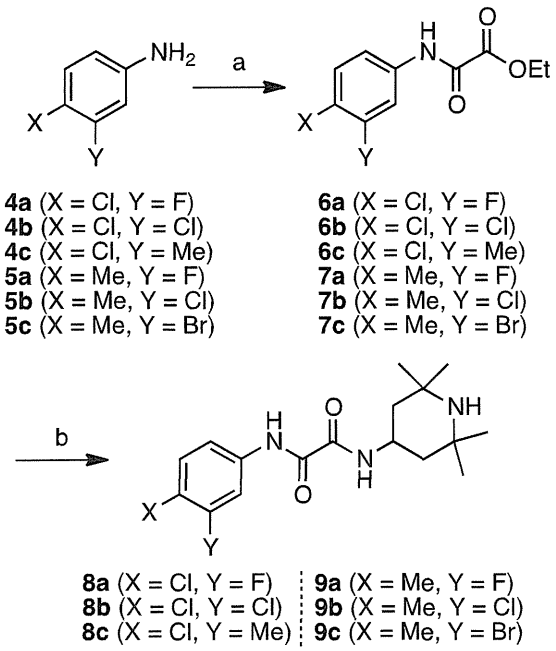
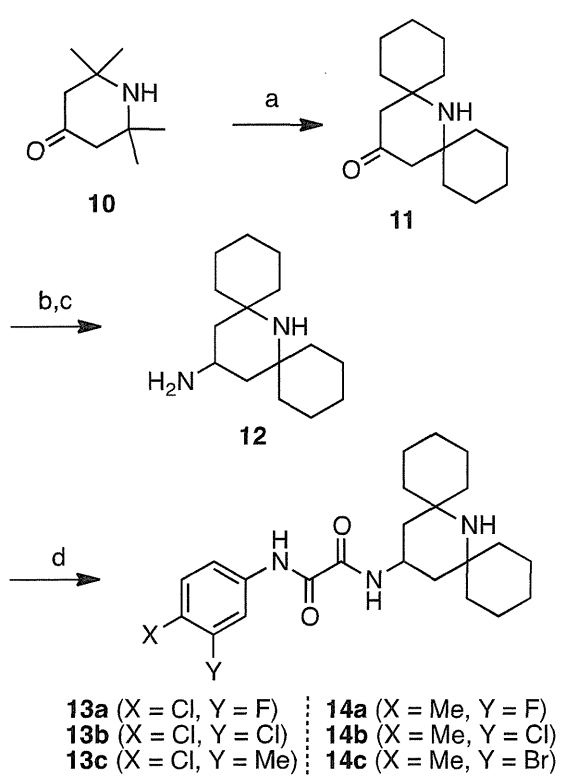


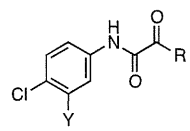
Scheme 1. Reagents and conditions: (a) ethyl chloroglyoxylate, Et_3N, THF; (b) 4-amino-2,2,6,6-tetramethylpiperidine, Et_3N, EtOH, 150 °C, microwave.

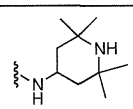
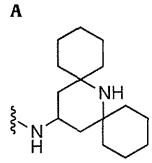


Scheme 2. Reagents and conditions: (a) cyclohexanone, NH_4Cl, DMSO, 60 °C; (b) *p*-methoxybenzylamine, $NaBH_3CN$, MeOH, then 1 M TMSBr in TFA; (c) CAN, CH_3CN/H_2O (*v*:*v* = 2:1); (d) **6** or **7**, Et_3N, EtOH, 150 °C, microwave.

of **1** = 0.61 μM and IC_{50} of **8a** = 0.32 μM). Compound **8b**[6a] having a *m*,*p*-dichlorophenyl group and compound **8c**[6a] (JRC-II-193) having a *p*-chloro-*m*-tolyl group showed moderate anti-HIV activity (IC_{50} of **8b** = 4.1 μM and IC_{50} of **8c** = 3.3 μM) but their potency was

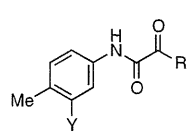
Table 1
Anti-HIV activity and cytotoxicity of compounds **8a–c** and **13a–c** containing a *p*-chlorophenyl group[a]

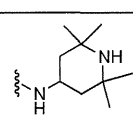
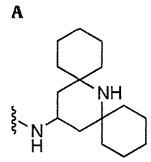


Compd	R	Y	IC_{50}[b] (μM) YTA48P	CC_{50}[c] (μM)
1		H	0.61	110
8a	**A**	F	0.32	94
8b	**A**	Cl	4.1	36
8c	**A**	Me	3.3	38
3		H	0.43	120
13a	**B**	F	0.23	11
13b	**B**	Cl	0.62	11
13c	**B**	Me	2.6	15

[a] All data are the mean values from three of more independent experiments.
[b] IC_{50} values of the multi-round assay are based on the inhibition of HIV-1-induced cytopathogenicity in PM1/CCR5 cells.
[c] CC_{50} values are based on the reduction of the viability of mock-infected PM1/CCR5 cells.

Table 2
Anti-HIV activity and cytotoxicity of compounds **9a–c** and **14a–c** containing a *p*-tolyl group[a]



Compd	R	Y	IC_{50}[b] (μM) YTA48P	CC_{50}[c] (μM)
2		H	9.0	260
9a	**A**	F	2.8	110
9b	**A**	Cl	3.2	62
9c	**A**	Br	>10	32
14a		F	0.54	91
14b	**B**	Cl	6.2	11
14c	**B**	Br	3.2	11

[a] All data are the mean values from three of more independent experiments.
[b] IC_{50} values of the multi-round assay are based on the inhibition of HIV-1-induced cytopathogenicity in PM1/CCR5 cells.
[c] CC_{50} values are based on the reduction of the viability of mock-infected PM1/CCR5 cells.

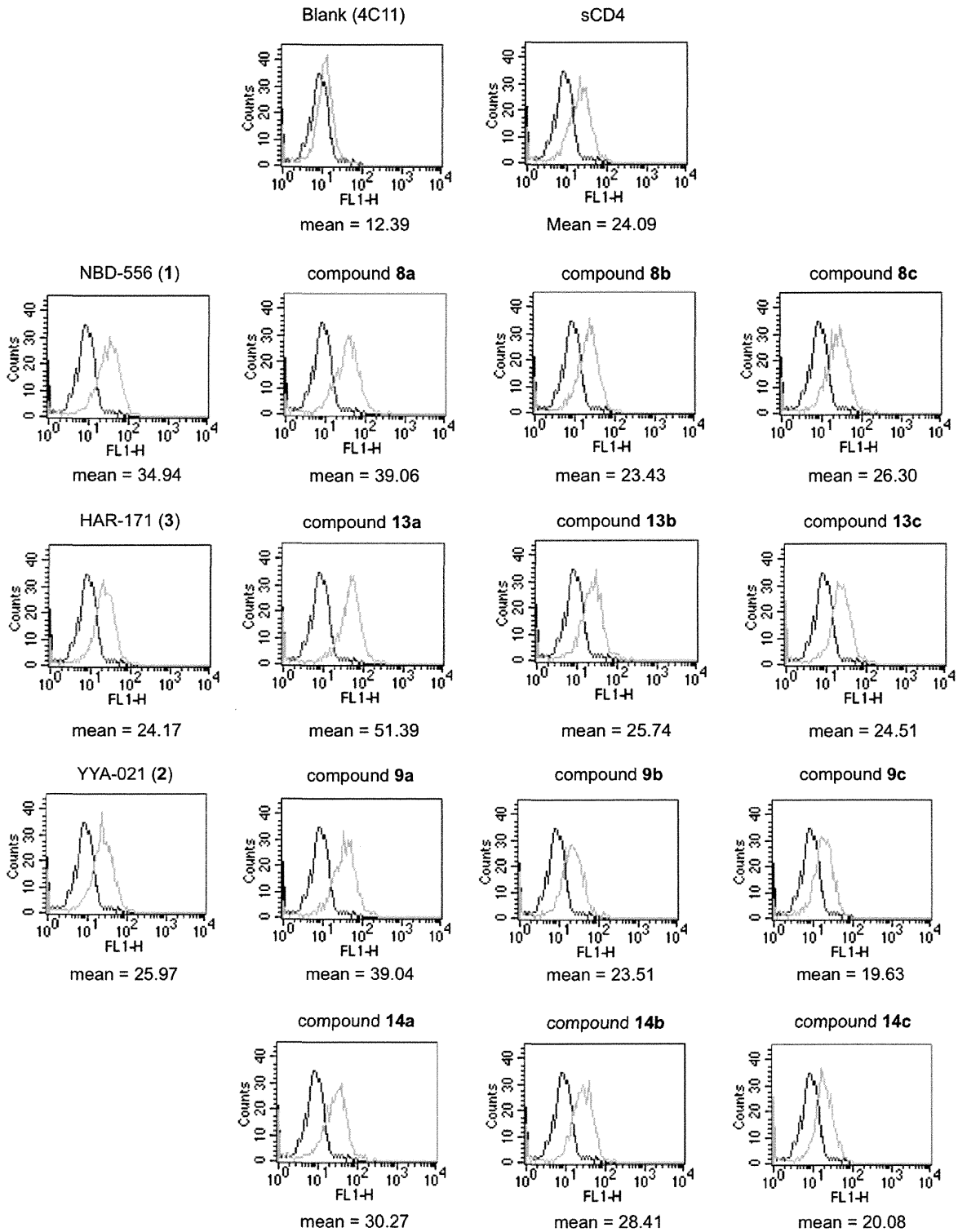


Figure 4. FACS analysis of synthetic compounds **8**, **9**, **13** and **14**.

approximately 10-fold lower than that of compound **8a**. The cytotoxicity of **8b** and **8c** is relatively stronger than that of **8a** (CC_{50} of **8b** = 36 μM and CC_{50} of **8c** = 38 μM). Compounds **13a–c** with hydrophobic cyclohexyl groups in the piperidine moiety showed more potent anti-HIV activity than the corresponding compounds **8a–c**, confirming the contribution of the bulky hydrophobic group(s) to an increase of antiviral activity. Our lead compound **3** showed significant anti-HIV activity comparable to that of compound **8a** (IC_{50} = 0.43 μM) but, consistent with previous results, exhibited lower cytotoxicity. In particular, compound **13a** with a *m*-fluoro-*p*-chlorophenyl group exhibited the highest anti-HIV activity. The IC_{50} value of **13a** was 0.23 μM, whose potency was

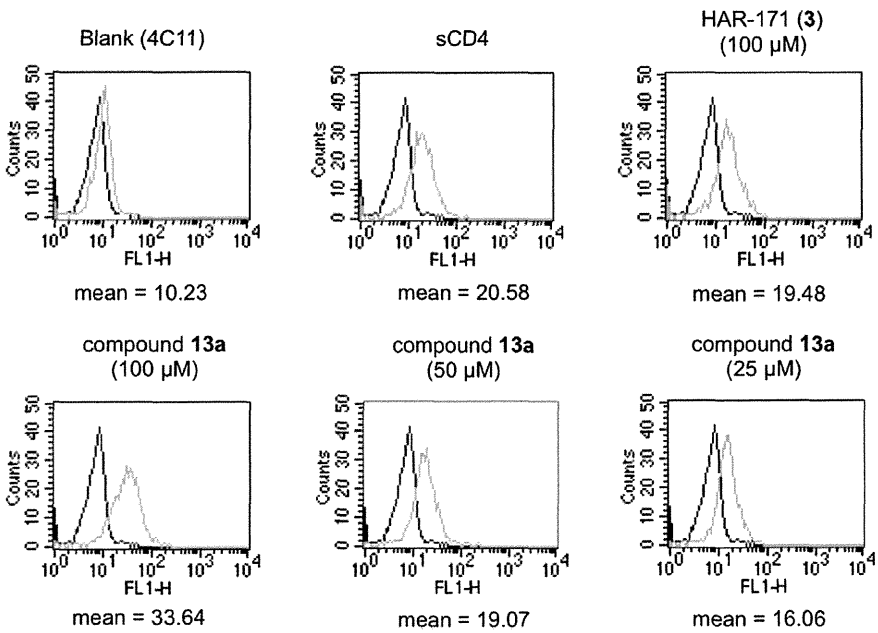


Figure 5. FACS analysis of **3** and **13a** in different concentrations.

approximately twice as high as that of compound **3**. Notably, compound **13b** with a *m,p*-dichlorophenyl group showed 7-fold more potent anti-HIV activity than the corresponding compound **8b**. Compound **13c**, which has a *p*-chloro-*m*-tolyl group, showed potent anti-HIV activity comparable to that of the corresponding compound **8c** and an increase of cytotoxicity (CC_{50} = 15 μM). We observed a tendency for compounds **13a–c** with both hydrophobic cyclohexyl groups and a *m,p*-disubstituted phenyl group to exhibit higher cytotoxicity than the corresponding tetramethyl-type compounds **8a–c**. No clear reason for an increase of cytotoxicity in the *m,p*-disubstituted phenyl group-containing compounds is apparent.

Assay results for the compounds **9a–c** and **14a–c** with a *p*-tolyl group are shown in Table 2. As expected, replacement of the *p*-chloro substituent with a *p*-methyl group resulted in somewhat reduction of anti-HIV activity. Compound **2**, YYA-021 has significant anti-HIV activity (IC_{50} = 9.0 μM) and exhibits the lowest cytotoxicity among all of the compounds tested (CC_{50} = 260 μM). These results are consistent with our previous SAR studies involving the aromatic ring. Introduction of a fluorine at the *meta*-position of the *p*-tolyl group, e.g. in compound **9a** and **14a**, improved the antiviral activity, as observed with **8a** and **13a** and a similar tendency was observed for compound **9b** with a *m*-chloro-*p*-tolyl group. In particular, compound **14a** with cyclohexyl groups and a *m*-fluoro-*p*-tolyl group showed slightly higher anti-HIV activity than the parent compound **1**. Among the compounds with *m*-bromo-*p*-tolyl groups, it was found that compound **9c**, with a 2,2,6,6-tetramethylpiperidine group, showed no anti-HIV activity at a concentration below 10 μM, whereas compound **14c** with hydrophobic cyclohexyl groups attached to the piperidine moiety, showed moderate activity (IC_{50} = 3.2 μM), indicating that the hydrophobic modification of piperidine ring can contribute to an increase in anti-HIV activity.

All the synthetic compounds were evaluated for their CD4 mimicry on the conformational changes in gp120 by fluorescence activated cell sorting (FACS) analysis, and the results are shown in Figure 4. The profile of binding of a CD4-induced (CD4i) monoclonal antibody (4C11) to the Env-expressing cell surface pretreated with the synthetic compounds was assessed in terms of the mean fluorescence intensity (MFI). The increase in binding affinity for 4C11 (by the pretreatment with synthetic compounds) suggests that those compounds can reflect the CD4 mimicry as a consequence of the conformational changes in gp120. Our previous studies disclosed that the profiles of the binding to the cell surface pretreated with **1**, **2**, or **3** were similar to those observed in pretreatment with soluble CD4, indicating that these compounds offer a significant enhancement of binding affinity for 4C11.[8] As shown in Figure 4, similar results were obtained with those compounds in this FACS analysis (MFI of **1**, **2**, and **3** = 34.94, 25.97, and 24.17, respectively). A notable increase in binding affinity for 4C11 was observed in essentially all the synthetic compounds. The compounds **8a**, **9a**, **13a** and **14a** with a *meta*-fluorine in the aromatic ring, showed significant anti-HIV activity, and produced a substantial increase in binding affinity for 4C11. These results suggested that the introduction of a fluorine group at the meta position of the aromatic ring is significant not only for the increase of anti-HIV activity, but also for the enhancement of a CD4 mimicry. In particular, a remarkable improvement in binding affinity for 4C11 was observed with **13a** (MFI = 51.39) which has twofold more potent anti-HIV activity than the lead compound **3** (HAR-171), and is the most active compound in terms of both anti-HIV activity and the CD4 mimicry resulting from the conformational change in gp120. The profiles of pretreatment of the cell surface with compounds **8b** and **13b** having a *m,p*-dichlorophenyl group, compounds **8c** and **13c** having a *p*-chloro-*m*-tolyl group, and compounds **9b** and **14b** with a *m*-chloro-*p*-tolyl group were similar to results obtained for **3**, suggesting that these compounds produced slightly lower enhancement compared to those of compounds **8a**, **9a**, **13a** and **14a** but significant levels of binding affinity for 4C11. On the other hand, pretreatment with compounds **9c**, which failed to show significant anti-HIV activity and **14c**, which had moderate anti-HIV activity resulted in a slight decrease of binding affinity for 4C11, suggesting that the introduction of a Br group at the *meta*-position of *p*-tolyl group is not advantageous to a CD4 mimicry, possibly due to the steric hindrance caused by the two bulky substituents. These results are consistent with previous observations that a limited size and electron-withdrawing ability of the aromatic substituents are required for potent anti-HIV activity and CD4 mimicry.[8a]

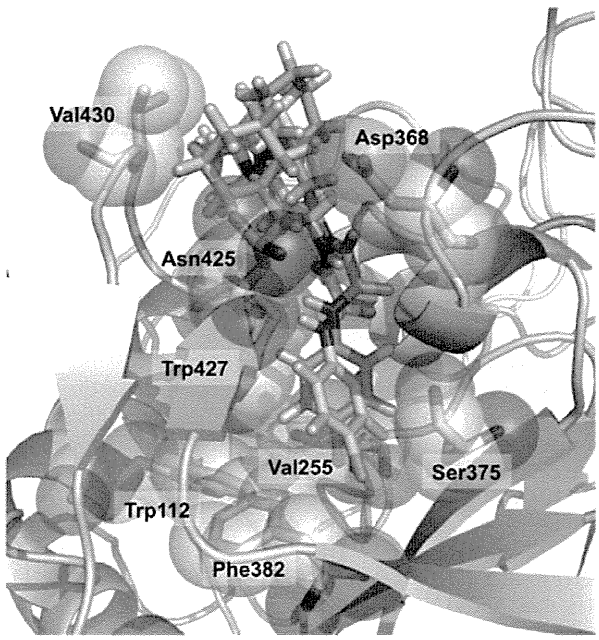


Figure 6. The modeled structure of **13a** (yellow carbon atoms) in the complex with the Phe43 cavity in gp120 (3TGS) overlaid with the modeled structure of **3** (green carbon atoms).

Since **13a** showed higher CD4 mimicry than the other compounds tested, the effect of the solution concentration of **13a** on the binding affinity for 4C11 was investigated. As shown in Figure 5, pretreatment of the cell surface with a 100 μM solution of **13a** produced a higher increase in the binding affinity for 4C11 than pretreatment with the same concentration of compound **3**. Interestingly, the profile pretreated with a 50 μM solution of **13a** was similar to that with a 100 μM of compound **3**, and even with a 25 μM solution of **13a** a potent enhancement of the binding affinity for 4C11 was observed: MFI of **13a** at concentrations of 50 μM and 25 μM = 19.07 and 16.06, respectively. This observation suggests that **13a** could serve as a novel lead compound for the development of envelope protein openers for the use combined with neutralizing antibodies because of its effectiveness at low concentrations.

The substantial increase in the CD4 mimicry of **13a** even at a low concentration is not easily explained because HAR-171 (**3**) and **13a** would be expected to form the similar binding modes with gp120. A probable contribution of **13a** is suggested by modeling studies docked into the Phe43 cavity in gp120 (3TGS) in which the depth and direction of the aromatic ring of **13a** is slightly different from those in compound **3** (Fig. 6), leading to the possible formation of appropriate interactions with the hydrophobic amino acid residues such as Val255 and Phe382, and therefore explaining the increased potency observed in the anti-HIV activity and CD4 mimicry of **13a**.

3. Conclusion

CD4 mimics are attractive agents not only for the development of a novel class of HIV entry inhibitors but also as possible cooperating agents for the neutralizing antibodies—that is, envelope protein openers. In the present study, a structure–activity relationship study of a series of CD4 mimic compounds was performed with a view to improving the biological activity of HAR-171 (**3**), which was identified in our previous studies as a promising lead compound with anti-HIV activity, cytotoxicity and CD4 mimicry resulting from the conformational change in gp120. Systematic modification of the *meta*- and *para*-substituents of the aromatic ring of **3** led to some potent compounds. In particular, **13a**, which has a bulky hydrophobic group on its piperidine ring and a *m*-fluoro-*p*-chlorophenyl group, demonstrated twofold more potent anti-HIV activity and much higher CD4 mimicry than **2** following the conformational changes in gp120, although the cytotoxicity of **13a** is relatively high. Further structural modification studies of the aromatic ring and the oxalamide linker to improve pharmaceutical profiles will be the subject of future reports.

4. Experimentals

^{1}H NMR and ^{13}C NMR spectra were recorded using a Bruker Avance III spectrometer. Chemical shifts are reported in δ (ppm) relative to Me_4Si (in $CDCl_3$) as internal standard. Low- and high-resolution mass spectra were recorded on a Bruker Daltonics microTOF focus in the positive and negative detection mode. For flash chromatography, silica gel 60 N (Kanto Chemical Co., Inc.) was employed. Microwave reactions were performed in Biotage Microwave Reaction Kit (sealed vials) in an Initiator™ (Biotage). The wattage was automatically adjusted to maintain the desired temperature for the desired period of time.

4.1. Chemistry

4.1.1. Ethyl 2-((4-chloro-3-fluorophenyl)amino)-2-oxoacetate (6a)

To a stirred solution of 3-fluoroaniline (1.11 g, 10.0 mmol) in $CHCl_3$ (30.0 mL) was added dropwise *N*-chlorosuccinimide (NCS) in $CHCl_3$ (20.0 mL) at 0 °C. The mixture was stirred at 0 °C for 42 h. After the reaction mixture was concentrated under reduced pressure, the residue was dissolved in Et_2O. The mixture was washed with water, and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography over silica gel with EtOAc/*n*-hexane gave 4-chloro-3-fluoroaniline (259.4 g, 18% yield) as crystalline solids. To a stirred solution of the above aniline (259.4 mg, 1.78 mmol) in THF (8.9 mL) were added at 0 °C ethyl chloroglyoxylate (237.3 μL, 2.14 mmol) and Et_3N (296.6 μL, 2.14 mmol). The mixture was stirred at room temperature for 12 h. After the precipitate was filtrated off, the filtrate solution was concentrated under reduced pressure. The residue was dissolved in EtOAc, and washed with 1.0 M HCl, saturated $NaHCO_3$ and brine, then dried over $MgSO_4$. Concentration under reduced pressure to provide the title compound **6a** (435.2 mg, 99% yield) as brown crystals, which was used without further purification.

^{1}H NMR (500 MHz, $CDCl_3$) δ 1.44 (t, J = 7.50 Hz, 3H), 4.43 (q, J = 7.50 Hz, 2H), 7.24–7.25 (m, 1H), 7.35–7.40 (m, 1H), 7.70–7.75 (m, 1H), 8.93 (br, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 13.0, 64.1, 108.5 (d, J = 26.3 Hz), 115.9 (d, J = 3.75 Hz), 117.3 (d, J = 18.8 Hz), 130.9 (d, J = 10.0 Hz), 135.9, 153.9, 158.1 (d, J = 246.3 Hz), 160.5; HRMS (ESI), *m/z* calcd for $C_{10}H_{10}ClFNO_3$ (MH^-) 244.0182, found 244.0183.

4.1.2. Ethyl 2-((3,4-dichlorophenyl)amino)-2-oxoacetate (6b)

To a stirred solution of 3,4-dichloroaniline **4b** (1.94 g, 12.0 mmol) in THF (20.0 mL) were added ethyl chloroglyoxylate (1.11 mL, 10.0 mmol) and Et_3N (15.2 mL, 11.0 mmol) at 0 °C. The mixture was stirred at room temperature for 6 h. After the precipitate was filtrated off, the filtrate solution was concentrated under reduced pressure. The residue was dissolved in EtOAc, and washed with 1.0 M HCl, saturated $NaHCO_3$ and brine, then dried over $MgSO_4$. Concentration under reduced pressure to provide the title compound **6b** (1.58 g, 95% yield) as white powder, which was used without further purification.

^{1}H NMR (500 MHz, $CDCl_3$) δ 1.44 (t, J = 7.00 Hz, 3H), 4.43 (q, J = 7.00 Hz, 2H), 7.44 (d, J = 8.50 Hz, 1H), 7.49–7.51 (m, 1H), 7.87, 2.35 (d, J = 2.50 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.0, 64.0, 119.0, 121.5, 129.0, 130.8, 133.2, 135.7, 153.9, 160.5; HRMS (ESI), m/z calcd for $C_{10}H_{10}Cl_2NO_3$ (MH^+) 262.0038, found 262.0031.

4.1.3. Ethyl 2-((4-chloro-3-methylphenyl)amino)-2-oxoacetate (6c)

By use of a procedure similar to that described for the preparation of compound **6b**, the aniline **4c** (3.34 g, 24.0 mmol) was converted into the title compound **6c** (4.63 g, 96% yield) as white powder.

^{1}H NMR (500 MHz, $CDCl_3$) δ 1.43 (t, J = 7.00 Hz, 3H), 2.38 (s, 3H), 4.42 (q, J = 7.00 Hz, 2H), 7.33 (d, J = 8.50 Hz, 1H), 7.43–7.46 (m, 1H), 7.51–7.54 (m, 1H), 8.82 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.0, 20.2, 63.8, 118.5, 122.0, 129.7, 130.9, 134.8, 137.1, 153.8, 160.9; HRMS (ESI), m/z calcd for $C_{11}H_{13}ClNO_3$ (MH^+) 242.0578, found 242.0568.

4.1.4. Ethyl 2-((3-fluoro-4-methylphenyl)amino)-2-oxoacetate (7a)

By use of a procedure similar to that described for the preparation of compound **6b**, the aniline **5a** (3.00 g, 24.0 mmol) was converted into the title compound **7a** (4.24 g, 94% yield) as white powder.

^{1}H NMR (500 MHz, $CDCl_3$) δ 1.43 (t, J = 7.20 Hz, 3H), 2.25 (s, 3H), 4.42 (q, J = 6.80 Hz, 2H), 7.12–7.21 (m, 2H), 7.48–7.56 (m, 1H), 8.83 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.2 (2C), 63.8, 107.1 (d, J = 27.5 Hz), 115.0 (d, J = 10.0 Hz), 122.3 (d, J = 17.5 Hz), 131.6 (d, J = 6.25 Hz), 135.3 (d, J = 13.8 Hz), 153.8, 160.8, 161.1 (d, J = 243.8 Hz); HRMS (ESI), m/z calcd for $C_{11}H_{13}FNO_3$ (MH^+) 226.0879, found 226.0878.

4.1.5. Ethyl 2-((3-chloro-4-methylphenyl)amino)-2-oxoacetate (7b)

By use of a procedure similar to that described for the preparation of compound **6b**, the aniline **5b** (3.40 g, 24.0 mmol) was converted into the title compound **7b** (5.19 g, 94% yield) as white powder.

^{1}H NMR (500 MHz, $CDCl_3$) δ 1.43 (t, J = 7.00 Hz, 3H), 2.35 (s, 3H), 4.42 (q, J = 7.00 Hz, 2H), 7.22 (d, J = 8.50 Hz, 1H), 7.41–7.43 (m, 1H), 7.71 (d, J = 2.00 Hz, 1H), 8.83 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.0, 20.0, 63.8, 118.0, 120.3, 131.2, 133.3, 134.7, 135.0, 153.8, 160.8; HRMS (ESI), m/z calcd for $C_{11}H_{13}ClNO_3$ (MH^+) 242.0584, found 242.0573.

4.1.6. Ethyl 2-((3-bromo-4-methylphenyl)amino)-2-oxoacetate (7c)

By use of a procedure similar to that described for the preparation of compound **6b**, the aniline **5c** (4.47 g, 27.0 mmol) was converted into the title compound **7c** (6.24 g, 96% yield) as white powder.

^{1}H NMR (500 MHz, $CDCl_3$) δ 1.43 (t, J = 7.00 Hz, 3H), 2.38 (s, 3H), 4.42 (q, J = 7.00 Hz, 2H), 7.23 (t, J = 8.50 Hz, 1H), 7.48–7.53 (m, 1H), 7.83–7.90 (m, 1H), 8.80 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.0, 22.4, 63.9, 118.7, 123.4, 125.0, 131.0, 135.0, 135.2, 153.7, 160.8; HRMS (ESI), m/z calcd for $C_{11}H_{13}BrNO_3$ (MH^+) 286.0079, found 286.0068.

4.1.7. N^1-(4-Chloro-3-fluorophenyl)-N^2-(2,2,6,6-tetramethylpiperidin-4-yl)oxalamide (8a)

To a solution of compound **6a** (70.0 mg, 0.286) in EtOH (2.9 mL) were added Et_3N (0.200 mL, 1.45 mmol) and 2,2,6,6-tetramethylpiperidin-4-amine (0.150 mL, 0.870 mmol). The reaction mixture was stirred for 3 h at 150 °C under microwave irradiation. After being concentrated in vacuo, the residue was extracted with $CHCl_3$, and washed with saturated $NaHCO_3$ and brine, then dried over $MgSO_4$. Concentration under reduced pressure to provide the title compound **8a** (34.6 mg, 34% yield) as white powder.

^{1}H NMR (500 MHz, $CDCl_3$) δ 0.99–1.50 (m, 15H), 1.92 (dd, J = 3.50, 9.00 Hz, 2H), 4.20–4.32 (m, 1H), 7.21–7.25 (m, 1H), 7.34–7.41 (m, 1H), 7.69–7.73 (m 1H), 9.31 (br, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 28.4, 34.8, 43.8, 44.5, 51.0, 108.3 (d, J = 26.3 Hz), 115.8 (d, J = 3.75 Hz), 117.1 (d, J = 17.5 Hz), 130.8, 136.2 (d, J = 8.75 Hz), 157.6, 158.1 (d, J = 247.5 Hz), 158.4; HRMS (ESI), m/z calcd for $C_{17}H_{24}ClFN_3O_2$ (MH^+) 356.1536, found 356.1548.

4.1.8. N^1-(3,4-Dichlorophenyl)-N^2-(2,2,6,6-tetramethylpiperidin-4-yl)oxalamide (8b)

By use of a procedure similar to that described for the preparation of compound **8a**, the compound **6b** (261.0 mg, 1.00 mmol) was converted into the title compound **8b** (520.0 mg, 70% yield) as white powder.

^{1}H NMR (500 MHz, $CDCl_3$) δ 1.07 (t, J = 12.0 Hz, 2H), 1.16 (s, 6H), 1.28 (s, 6H), 1.90–1.93 (m, 2H), 4.20–4.32 (m, 1H), 7.26 (m, 1H), 7.40–7.48 (m, 2H), 7.88 (s, 1H), 9.33 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 28.5 (2C), 34.9 (2C), 43.8, 44.6 (2C), 50.9 (2C), 119.0, 121.4, 128.7, 130.8, 133.1, 135.8, 157.7, 158.5; HRMS (ESI), m/z calcd for $C_{17}H_{22}Cl_2N_3O_2$ (MH^-) 370.1095, found 370.1105.

4.1.9. N^1-(4-Chloro-3-methylphenyl)-N^2-(2,2,6,6-tetramethylpiperidin-4-yl)oxalamide (8c)

By use of a procedure similar to that described for the preparation of compound **8a**, the compound **6c** (482.0 mg, 2.00 mmol) was converted into the title compound **8c** (364.0 mg, 49% yield) as white powder.

^{1}H NMR (500 MHz, $CDCl_3$) δ 1.07 (t, J = 12.0 Hz, 2H), 1.15 (s, 6H), 1.28 (s, 6H), 1.86–1.94 (m, 2H), 4.15–4.31 (m, 1H), 7.21–7.24 (m, 1H), 7.32–7.38 (m, 2H), 7.74 (s, 1H), 9.24 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 19.6, 28.5 (2C), 34.9 (2C), 43.7, 44.7 (2C), 50.9 (2C), 117.9, 120.2, 131.2, 133.1, 134.7, 135.1, 157.5, 158.8; HRMS (ESI), m/z calcd for $C_{18}H_{25}ClN_3O_2$ (MH^-) 350.1641, found 350.1656.

4.1.10. N^1-(3-Fluoro-4-methylphenyl)-N^2-(2,2,6,6-tetramethylpiperidin-4-yl)oxalamide (9a)

By use of a procedure similar to that described for the preparation of compound **8a**, the compound **7a** (225.0 mg, 1.00 mmol) was converted into the title compound **9a** (161.0 mg, 48% yield) as white powder.

^{1}H NMR (500 MHz, $CDCl_3$) δ 1.07 (t, J = 12.5 Hz, 2H), 1.15 (s, 6H), 1.28 (s, 6H), 1.92 (dd, J = 12.5, 3.50 Hz, 2H), 2.26 (s, 3H), 4.12–4.32 (m, 1H), 7.12–7.20 (m, 2H), 7.30–7.37 (m, 1H), 7.48–7.54 (m, 1H), 9.27 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.2, 28.5 (2C), 34.9 (2C), 43.7, 44.7 (2C), 50.9 (2C), 107.1 (d, J = 26.3 Hz), 115.0, 121.8 (d, J = 17.5 Hz) 131.6, 135.4, (d, J = 15.0 Hz), 157.5, 158.8, 161.1 (d, J = 242.5 Hz); HRMS (ESI), m/z calcd for $C_{18}H_{25}FN_3O_2$ (MH^-) 334.1936, found 334.1942.

4.1.11. N^1-(3-Chloro-4-methylphenyl)-N^2-(2,2,6,6-tetramethylpiperidin-4-yl)oxalamide (9b)

By use of a procedure similar to that described for the preparation of compound **8a**, the compound **7b** (482.0 mg, 1.00 mmol) was converted into the title compound **9b** (448.0 mg, 48% yield) as white powder.

^{1}H NMR (500 MHz, $CDCl_3$) δ 1.09 (t, J = 12.5 Hz, 3H), 1.18 (s, 6H), 1.30 (s, 6H), 1.93–1.95 (m, 2H), 2.41 (s, 3H), 4.20–4.34 (m, 1H), 7.30–7.37 (m, 2H), 7.44–7.46 (m, 1H), 7.53 (d, J = 2.50 Hz, 1H), 9.25 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.3, 28.5 (2C), 34.9 (2C), 43.7, 44.7 (2C), 50.9 (2C), 118.5, 122.0, 130.0, 130.7, 134.8, 137.1, 157.5, 158.8; HRMS (ESI), m/z calcd for $C_{18}H_{25}ClN_3O_2$ (MH^-) 350.1641, found 350.1636.

4.1.12. N^1-(3-Bromo-4-methylphenyl)-N^2-(2,2,6,6-tetramethylpiperidin-4-yl)oxalamide (9c)

By use of a procedure similar to that described for the preparation of compound **8a**, the compound **7c** (285.0 mg, 1.00 mmol) was converted into the title compound **9c** (157.0 mg, 40% yield) as white powder.

^{1}H NMR (500 MHz, $CDCl_3$) δ 1.07 (t, J = 12.5 Hz, 3H), 1.15 (s, 6H), 1.28 (s, 6H), 1.91 (dd, J = 8.00, 4.00 Hz, 2H), 2.38 (s, 3H), 3.70–3.75 (m, 1H), 7.22 (d, J = 8.50 Hz, 1H), 7.30–7.37 (m, 1H), 7.43–7.45 (m, 1H), 7.90 (d, J = 2.50 Hz, 1H), 9.25 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 22.4, 28.5 (2C), 34.9 (2C), 43.7, 44.7 (2C), 50.9 (2C), 118.6, 123.4, 125.0, 131.0, 134.9 (2C), 157.5, 158.8; HRMS (ESI), m/z calcd for $C_{18}H_{25}BrN_3O_2$ (MH^-) 394.1136, found 394.1158.

4.1.13. Amine (12)

The compound **11** was prepared according to the reported procedure.[14] To a stirred solution of piridone **11** (247.8 mg, 1.05 mmol) in MeOH (2.10 mL) was added *p*-methoxybenzylamine (0.41 mL, 3.15 mmol). After being stirred at room temperature for 23 h, sodium cyanoborohydride was added and stirred at room temperature for 48 h. The reaction mixture was poured into saturated $NaHCO_3$ and extracted with EtOAc, then dried over $MgSO_4$. After concentration under reduced pressure, the residue was treated with 1 M TMS in THF (4.8 mL). The mixture was stirred at 0 °C for 14 h. Concentration under reduced pressure followed by short chromatography with $CHCl_3$/MeOH gave the PMB-protected amine. To a solution of the above amine (584.0 mg, 1.64 mmol) in CH_3CN/H_2O (13.1 mL, v:v = 2:1) was added CAN (2.74 g, 8.2 mmol). The mixture was stirred at room temperature for 14 h. The reaction mixture was diluted with 0.5 M HCl and washed with CH_2Cl_2. The water layer was alkalized and extracted with EtOAc, then dried over Na_2SO_4. Concentration under reduced pressure followed by flash chromatography over silica gel with EtOAc–EtOH (4:1) to gave the title compound **12** (175.5 mg, 71% yield) as a yellow oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 1.15–1.85 (m, 24H), 2.95–3.05 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 22.2 (2C), 22.8 (2C), 26.2 (2C), 37.3 (2C), 42.3 (2C), 43.6 (2C), 47.0, 53.2 (2C); HRMS (ESI), m/z calcd for $C_{15}H_{29}N_2$ (MH^+) 237.2325, found 237.2321.

4.1.14. N^1-((4-Chloro-3-fluorophenyl)-N^2-(2,6-dicyclohexylpiperidin-4-yl)oxalamide (13a)

By use of a procedure similar to that described for the preparation of compound **8a**, the compound **6a** (36.8 mg, 0.150 mmol) was converted into the title compound **13a** (7.6 mg, 12% yield) as yellow powder.

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.71–2.28 (m, 24H), 2.03–2.20 (m, 2H), 4.02–4.16 (m, 1H), 7.13–7.18 (m, 1H), 7.27–7.33 (m, 1H), 7.62–7.66 (m, 1H), 9.25 (br, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.1, 22.0 (2C), 22.6 (2C), 25.8 (2C), 29.3, 29.7 (2C), 31.9, 70.5, 108.3 (d, J = 26.3 Hz), 115.8, 117.1 (d, J = 18.8 Hz), 130.8, 136.2 (d, J = 10.0 Hz), 157.6, 158.1 (d, J = 247.5 Hz), 158.6; HRMS (ESI), m/z calcd for $C_{23}H_{32}ClFN_3O_2$ (MH^+) 436.2162, found 436.2156.

4.1.15. N^1-(4-Chlorophenyl)-N^2-(2,6-dicyclohexylpiperidin-4-yl)oxalamide (13b)

By use of a procedure similar to that described for the preparation of compound **8a**, the compound **6b** (31.3 mg, 0.120 mmol) was converted into the title compound **13b** (28.0 mg, 52% yield) as white powder.

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.96 (t, J = 12.5 Hz, 2H), 1.10–1.84 (br, 20H), 2.05–2.19 (m, 2H), 4.08–4.21 (m, 1H), 7.23–7.33 (br, 1H), 7.39–7.46 (m, 2H), 7.88 (t, J = 1.00 Hz, 1H), 9.34 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.1, 22.1 (2C), 22.7 (2C), 26.1 (2C), 31.6, 37.2 (2C), 42.6, 43.0, 43.6, 52.6 (2C), 119.0, 121.4, 128.7, 130.8, 133.1, 135.8, 157.7, 158.5; HRMS (ESI), m/z calcd for $C_{23}H_{32}Cl_2N_3O_2$ (MH^+) 452.1872, found 452.1865.

4.1.16. N^1-((4-Chloro-3-methylphenyl)-N^2-(2,6-dicyclohexylpiperidin-4-yl)oxalamide (13c)

By use of a procedure similar to that described for the preparation of compound **8a**, the compound **6c** (121.0 mg, 0.500 mmol) was converted into the title compound **13c** (15.1 mg, 7% yield) as white powder.

^{1}H NMR (500 MHz, $CDCl_3$) δ 0.87–1.88 (br, 22H), 2.09–2.20 (m, 2H), 2.38 (s, 3H), 4.09–4.22 (m, 1H), 7.32–7.33 (m, 1H), 7.41–7.43 (m, 1H), 7.51 (d, J = 2.00 Hz, 1H), 7.73 (m, 1H), 9.24 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.2, 22.1 (2C), 22.7 (2C), 26.0 (2C), 29.7, 37.0, 42.3 (2C), 42.8 (2C), 43.4, 52.9 (2C), 118.4, 122.0, 130.0, 130.6, 134.8, 137.1, 157.5, 158.9; HRMS (ESI), m/z calcd for $C_{24}H_{35}ClN_3O_2$ (MH^+) 430.2267, found 430.2264.

4.1.17. N^1-(3-Fluoro-4-methylphenyl)-N^2-(2,6-dicyclohexylpiperidin-4-yl)oxalamide (14a)

By use of a procedure similar to that described for the preparation of compound **8a**, the compound **7a** (225.0 mg, 1.00 mmol) was converted into the title compound **14a** (27.5 mg, 7% yield) as white powder.

^{1}H NMR (500 MHz, $CDCl_3$) δ 0.971 (t, J = 12.5 Hz, 2H), 1.18–1.86 (m, 20H), 2.13–2.16 (m, 2H), 2.26 (s, 3H), 4.09–4.21 (m, 1H), 7.13–7.18 (m, 2H), 7.33 (d, J = 8.00 Hz, 1H), 7.50–7.53 (m, 1H), 9.27 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.2, 22.2 (2C), 22.8 (2C), 26.1 (2C), 37.2 (2C), 42.2 (2C), 43.3 (2C), 43.5, 52.6 (m, 2C), 107.0 (d, J = 27.5 Hz), 115.0 (d, J = 3.75 Hz), 121.8 (d, J = 17.5 Hz), 131.6 (d, J = 6.25 Hz), 135.4 (d, J = 10.0 Hz), 157.5, 158.9, 161.3 (d, J = 242.5 Hz); HRMS (ESI), m/z calcd for $C_{24}H_{33}FN_3O_2$ (MH^-) 414.2554, found 414.2562.

4.1.18. N^1-(3-Chloro-4-methylphenyl)-N^2-(2,6-dicyclohexylpiperidin-4-yl)oxalamide (14b)

By use of a procedure similar to that described for the preparation of compound **8a**, the compound **7b** (120.5 mg, 0.500 mmol) was converted into the title compound **14b** (12.9 mg, 6% yield) as white powder.

^{1}H NMR (500 MHz, $CDCl_3$) δ 0.973 (t, J = 12.5 Hz, 2H), 1.18–1.86 (br, 20H), 2.11–2.19 (m, 2H), 2.35 (s, 3H), 4.09–4.21 (m, 1H), 7.20–7.22 (m, 1H), 7.30–7.32 (m, 1H), 7.35–7.37 (d, J = 2.50 Hz, 1H), 7.73 (m, 1H), 9.22 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 19.6, 22.1 (2C), 22.7 (2C), 26.0 (2C), 29.7, 37.0, 42.1 (2C), 42.7 (2C), 43.2, 53.3 (2C), 118.0, 120.3, 131.2, 133.0, 134.7, 135.1, 157.5, 158.8; HRMS (ESI), m/z calcd for $C_{24}H_{33}ClN_3O_2$ (MH^+) 430.2267, found 430.2257.

4.1.19. N^1-(3-Bromo-4-methylphenyl)-N^2-(2,6-dicyclohexylpiperidin-4-yl)oxalamide (14c)

By use of a procedure similar to that described for the preparation of compound **8a**, the compound **7c** (142.0 mg, 0.500 mmol) was converted into the title compound **14c** (11.5 mg, 5% yield) as white powder.

^{1}H NMR (500 MHz, $CDCl_3$) δ 0.67–2.07 (br, 22H), 2.28 (br, 2H), 2.38 (s, 3H), 4.09–4.21 (m, 1H), 7.22 (d, J = 8.00 Hz, 1H), 7.28–7.38 (br, 1H), 7.43 (dd, J = 4.50, 2.50 Hz, 1H), 7.90 (d, J = 2.50 Hz, 1H), 9.21 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.1, 22.1 (2C), 22.4 (2C), 22.7 (2C), 25.9, 30.0, 31.6, 36.9 (2C), 42.7 (3C), 52.7, 52.9, 118.6, 123.4, 125.0, 131.0, 134.9, 135.1, 157.4, 158.8; HRMS (ESI), m/z calcd for $C_{24}H_{33}BrN_3O_2$ (MH^+) 474.1762, found 474.1746.

4.2. Antiviral assay and cytotoxicity assay

Anti-HIV activity and cytotoxicity measurements in PM1/CCR5 cells (Yoshimura et al., 2010) were based on viability of cells that

had been infected or not infected with 100 TCID50 of an R5 primary isolate YTA48P exposed to various concentrations of the test compound. After the PM1/CCR5 cells were incubated at 37 °C for 7 days. The 50% inhibitory concentration (IC_{50}) values and the 50% cytotoxic concentration (CC_{50}) were then determined using the Cell Counting Kit-8 assay (Dojindo Laboratories). All assays were performed in duplicate or triplicate.

4.3. FACS analysis

JR-FL (R5, Sub B) chronically infected PM1 cells were pre-incubated with 0.5 μg/mL of sCD4 or 100 μM of a CD4 mimic for 15 min, and then incubated with an anti-HIV-1 mAb, 4C11, at 4 °C for 15 min. The cells were washed with PBS, and fluorescein isothiocyanate (FITC)-conjugated mouse anti-human IgG antibody was used for antibody-staining. Flow cytometry data for the binding of 4C11 (green lines) to the Env-expressing cell surface in the presence of a CD4 mimic are shown among gated PM1 cells along with a control antibody (anti-human CD19: black lines). Data are representative of the results from a minimum of two independent experiments. The number at the bottom of each graph shows the mean fluorescence intensity (MFI) of the antibody 4C11.

4.4. Molecular modeling

Dockings of compounds **3** and **13a** were performed using Molecular Operating Environment modeling package (MOE 2008. 10, Canada), into the crystal structure of gp120 (PDB, entry 3TGS).

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Supplementary data

Supplementary data (NMR charts of compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2013.02.041.

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Low-Molecular-Weight CXCR4 Ligands with Variable Spacers

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Low-molecular-weight CXCR4 ligands based on known lead compounds including the 14-mer peptide T140, the cyclic pentapeptide FC131, peptide mimetics, and dipicolylamine-containing compounds were designed and synthesized. Three types of aromatic spacers, 1,4-phenylenedimethanamine, naphthalene-2,6-diyldimethanamine, and [1,1′-biphenyl]-4,4′-diyldimethanamine, were used to build four pharmacophore groups. As pharmacophore groups, 2-pyridylmethyl and 1-naphthylmethyl are present in all of the compounds, and several aromatic groups and a cationic group from 1-propylguanidine and 1,1,3,3-tetramethyl-2-propylguanidine were also used. Several compounds showed significant CXCR4 binding affinity, and zinc(II) complexation of bis(pyridin-2-ylmethyl)amine moieties resulted in a remarkable increase in CXCR4 binding affinity.

Introduction

CXCR4 is a chemokine receptor that transduces signals of its endogenous ligand, CXCL12/stromal cell-derived factor-1 (SDF-1).[1–4] This receptor is a member of the seven-transmembrane GPCR family, and has been reported to exist and function as an oligomer,[5] which was elucidated by our molecular ruler approach.[6] The CXCR4–CXCL12 axis plays a physiological role in embryonic stages in chemotaxis,[7] angiogenesis,[8,9] and neurogenesis.[10,11] CXCR4 is associated with many disorders including cancer cell metastasis,[12–14] leukemia cell progression,[15,16] HIV infection/AIDS,[17,18] and rheumatoid arthritis;[19,20] it is therefore a major target in the discovery of chemotherapeutic treatments for these diseases. To date, many researchers, including ourselves, have developed potent CXCR4 antagonists. A 14-mer peptide, T140, and a cyclic pentapeptide, FC131, have been found to be potent CXCR4 antagonists.[21–27] In addition, downsizing of these peptides has led to the development of active small-molecular peptide mimetics.[28] Another peptide mimetic, KRH-1636,[29] and a bicyclam, AMD3100,[30,31] have also been reported. Furthermore, several compounds based on monocyclams[32] and noncyclams[33,34] have been reported. Other aza-macrocyclic compounds such as the Dpa–Zn complex **1**[35] and the Dpa–cyclam compound **2**[36] have been developed as non-peptide leads (Figure 1). These lead compounds have 1,4-phenylenedimethanamine structures with amino groups presenting basic/aromatic moieties. We recently developed small-molecular peptide mimetics containing benzyl and 2-pyridylmethyl amino groups, such as compound **3**[37] and cyclic pentapeptide FC131 derivatives containing two naphthalene moieties (e.g., **4**).[38] In the study presented herein, we tried to develop more effective small mole-

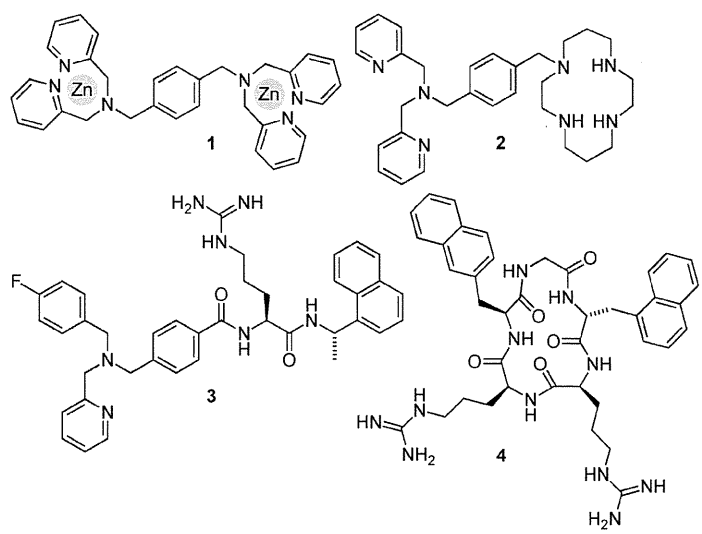

Figure 1. Reported low-molecular-weight CXCR4 antagonists.

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cules based on these lead compounds and to perform appropriate structure–activity relationship studies.

Results and Discussion

Design

We initially designed compounds that contain 1,4-phenylenedimethanamine, one amino group of which is linked to guanidine and naphthalene moieties, and the other to 2-pyridylmethyl and naphthalene analogues, as shown in Figure 2. The

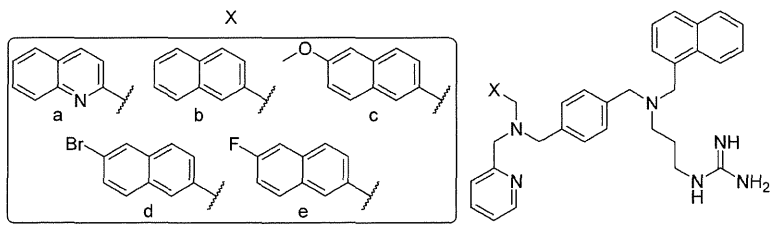


Figure 2. New compounds containing the 1,4-phenylenedimethanamine structure.

adoption of these functional moieties is based on structures of compound **3**, which contains 4-fluorobenzyl and 2-pyridylmethyl amino groups, and compound **4**, which contains two naphthalene moieties. Thus, 2-methylquinoline, 2-methylnaphthalene, 2-methoxy-6-methylnaphthalene, 2-bromo-6-methylnaphthalene, and 2-fluoro-6-methylnaphthalene (X-CH_2) moieties were introduced on a nitrogen atom of the 1,4-phenylenedimethanamino group in compounds **19a–c** and **23d,e**. Furthermore, compounds with 1,4-phenylenedimethanamine, naphthalene-2,6-diyldimethanamine, and [1,1′-biphenyl]-4,4′-diyldimethanamine structures as spacer templates (H_2N-Y^2-NH_2) were designed as shown in Figure 3 to refine the spacers. Monocyclic aromatic groups, 4- or 2-pyridylmethyl, 4-fluorobenzyl, and 4-trifluoromethylbenzyl groups (Y^1-CH_2) were introduced on a nitrogen atom of the above spacer templates, and guanidino and tetramethylguanidino groups were used as substituents for Y^3 in compounds **37a–42d**.

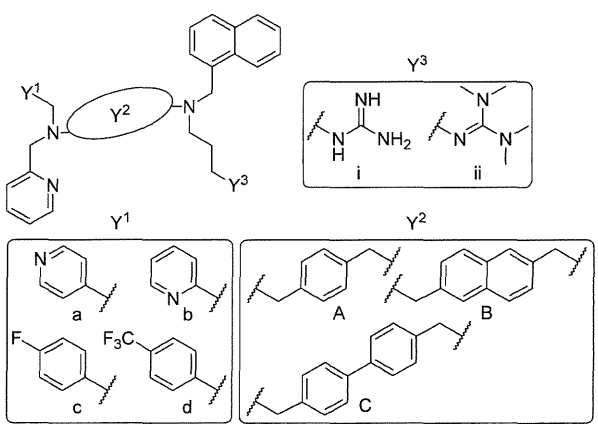


Figure 3. New compounds containing the 1,4-phenylenedimethanamine, naphthalene-2,6-diyldimethanamine, and [1,1′-biphenyl]-4,4′-diyldimethanamine structures.

Chemistry

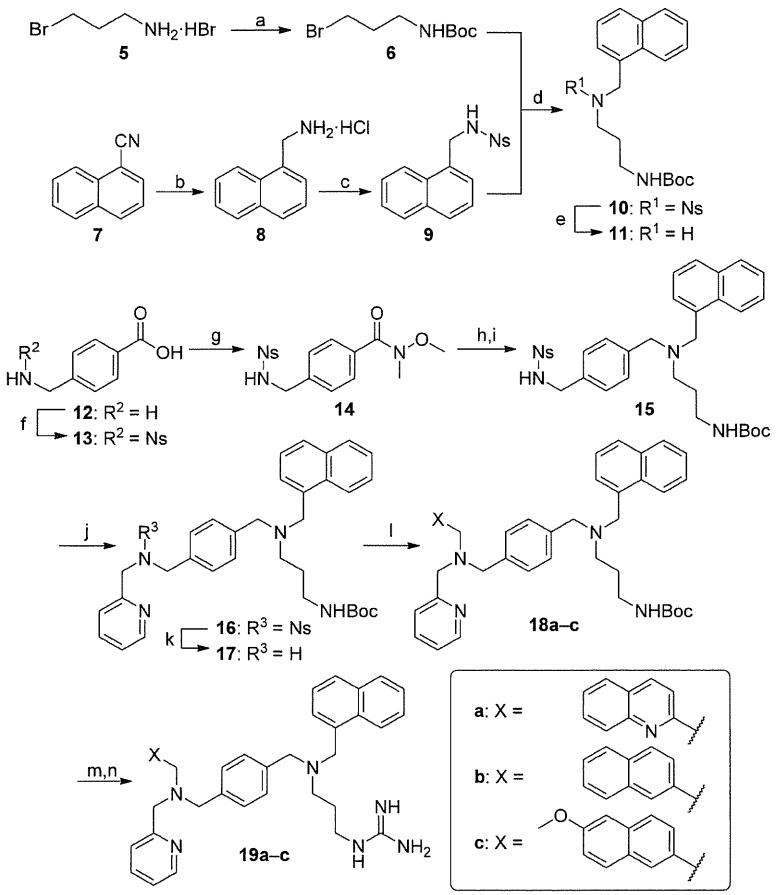
The synthesis of compounds **19a–c** is shown in Scheme 1. Condensation of *N*-Boc-3-aminopropylbromide (**6**) and *N*-Ns-aminonaphthalen-1-yl-methane (**9**; Ns = 2-nitrobenzenesulfonyl) followed by removal of the Ns group produced the amine **11**. The *N*-Ns-4-aminomethylbenzoic acid derived Weinreb amide **14** was treated with DIBAL to afford the corresponding aldehyde, the reductive amination of which was performed by treatment with amine **11** to afford the tertiary amine **15**. Introduction of a 2-pyridinylmethyl group into **15** by means of Mitsunobu reaction followed by removal of the Ns group yielded amine **17**. Introduction of 2-methylquinoline, 2-methylnaphthalene, and 2-methoxy-6-methylnaphthalene groups by reductive amination of **17** produced amines **18a–c**, respectively, and subsequent removal of the Boc group followed by N-guanylation yielded the desired compounds **19a–c**.

As shown in Scheme 2, introduction of 2-bromo-6-methylnaphthalene and 2-fluoro-6-methylnaphthalene moieties into **15** by Mitsunobu reaction followed by removal of the Ns group yielded amines **21d** and **21e**, respectively. Introduction of a 2-pyridinylmethyl group by reductive amination of **21d** and **21e** produced amines **22d** and **22e**, respectively, and subsequent removal of the Boc group followed by N-guanylation yielded the desired compounds **23d** and **23e**.

Scheme 3 shows the synthesis of **37a–39d** and **40a–42d**. Introduction of 4-pyridylmethyl, 2-pyridylmethyl, or 4-fluorobenzyl and 4-trifluoromethylbenzyl groups into *N*-Ns-(pyridin-2-ylmethyl)amide **25** by Mitsunobu reaction followed by removal of the Ns group yielded amines **27a–d**, respectively. Treatment of 1,4-phenylenedimethane, naphthalene-2,6-diyldimethane, and [1,1′-biphenyl]-4,4′-diyldimethane-derived dibromides **28–30** with amine **11** afforded the tertiary amines **31–33**, respectively. Subsequent treatment of **31–33** with amines **27a–d** yielded amines **34a–36d**. Subsequent removal of the Boc group followed by N-guanylation and N-tetramethylguanylation yielded the desired compounds **37a–39d** and **40a–42d**, respectively.

Biological studies

The CXCR4 binding affinity of the synthesized compounds was assessed through inhibition of [^{125}I]CXCL12 binding to Jurkat cells, which express CXCR4.[38] The activity was evaluated for compounds **19a–c** containing 2-methylquinoline, 2-methylnaphthalene, 2-methoxy-6-methylnaphthalene, and **23d,e**,



Scheme 1. *Reagents and conditions*: a) Boc_2O, Et_3N, MeOH/MeCN (1:1), 98%; b) $LiAlH_4$, THF, 0°C, 89%; c) NsCl, Et_3N, THF, 78%; d) K_2CO_3, DMF, 60°C, 96%; e) PhSH, K_2CO_3, DMF, 95%; f) NsCl, Et_3N, THF, 88%; g) EDCI·HCl, HOBt·H_2O, $NHCH_3(OCH_3)$·HCl, Et_3N, DMF, 88%; h) DIBAL/*n*-hexane, CH_2Cl_2, −78°C; i) $NaBH(OAc)_3$, AcOH, amine **11**, 1,2-dichloroethane, 43% (two steps); j) PPh_3, DEAD, 2-pyridinemethanol, THF, 76%; k) PhSH, K_2CO_3, DMF, 87%; l) $NaBH(OAc)_3$, AcOH, X-CHO, 1,2-dichloroethane, 70% (**18a**), 69% (**18b**), 49% (**18c**); m) 4M HCl/dioxane; n) *N*,*N*-diisopropylethylamine, 1-amidinopyrazole·HCl, DMF, 28% (**19a**), 58% (**19b**), 46% (**19c**) (two steps). Ns = 2-nitrobenzenesulfonyl.

with 2-bromo-6-methylnaphthalene and 2-fluoro-6-methylnaphthalene moieties, respectively (X-CH_2), introduced on a nitrogen atom of the 1,4-phenylenedimethanamino group. The percent inhibition data for all compounds at 10 μM are listed in Table 1. With the exception of **19c**, which contains a 2-methoxynaphthalene group, the compounds showed significant but very weak binding affinity. With an electron-donating methoxy group, the 2-methoxynaphthalene moiety is an electron-rich aromatic group. The quinoline, 2-bromonaphthalene, and 2-fluoronaphthalene moieties are electron-deficient aromatic groups because of the electron-deficient pyridine ring and electron-withdrawing fluorine and bromine atoms. It is suggested that when X represents bicyclic or electron-rich aromatic groups, the compounds are unlikely to be potent ligands.

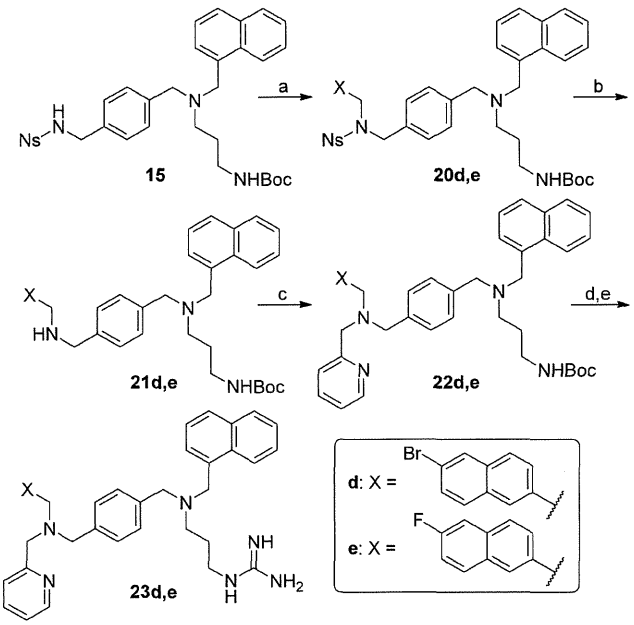
Because some compounds containing bicyclic or electron-rich aromatic groups at the group X position in Figure 2 do not have high binding affinity for CXCR4, compounds in Figure 3 in which Y^1 is a monocyclic and electron-deficient aromatic group were designed: 4-pyridylmethyl, 2-pyridylmethyl, 4-fluorobenzyl, and 4-trifluoromethylbenzyl groups (Y^1-CH_2) were introduced onto the nitrogen atom. In addition, as spacer templates (H_2N-Y^2-NH_2) 1,4-phenylenedimethanamine, naphthalene-2,6-diyldimethanamine, and [1,1′-biphenyl]-4,4′-diyldimethanamine structures were introduced to refine the spacer structures, and guanidino and tetramethylguanidino groups were used as Y^3 substituents. The CXCR4 binding affinities of compounds **37a–42d** were evaluated (Table 2). None of these compounds showed more than 50% inhibition at 10 μM. In general, 4-trifluoromethylbenzyl, [1,1′-biphenyl]-4,4′-diyldimethanamine, and tetramethylguanidino moieties seem to be more suitable as candidates for Y^1-CH_2, H_2N-Y^2-NH_2, and Y^3, respectively. Among these synthetic compounds, **40b**, containing 2-pyridylmethyl, 1,4-phenylenedimethanamine and tetramethylguanidino groups, and **42d** containing 4-trifluoromethylbenzyl, [1,1′-biphenyl]-4,4′-diyldimethanamine and tetramethylguanidino groups, have the highest binding affinity for CXCR4.

As described above in the Introduction, aza-macrocyclic compounds such as the Dpa–Zn complex **1**[35] and the Dpa–cyclam compound **2**[36] have high binding affinities toward CXCR4. The zinc complex of **2** also has a higher CXCR4 binding affinity. Thus, the CXCR4 binding affinities of the zinc complexes of **19a**, containing 2-pyridylmethyl and 2-methylquino-

Table 1. CXCR4 binding affinities of compounds **19a–c** and **23d,e**.

Compd	X[a]	Inhibition [%][b]
19a	a	14.4±1.0
19b	b	7.0±0.6
19c	c	0
23d	d	9.0±2.2
23e	e	9.5±1.3
FC131	–	100

[a] The structures of X (a–e) are shown in Figure 2. [b] CXCR4 binding affinity was assessed based on inhibition of [^{125}I]CXCL12 binding to Jurkat cells; percent inhibition values for all compounds at 10 μM were calculated relative to that of FC131 (100%).



Scheme 2. *Reagents and conditions*: a) PPh_3, DEAD, X-CH_2OH, THF, RT, 97% (**20d**), 59% (**20e**); b) PhSH, K_2CO_3, DMF, RT, 42% (**21d**), 64% (**21e**); c) $NaBH(OAc)_3$, AcOH, 2-pyridinecarbaldehyde, 1,2-dichloroethane, RT, 78% (**22d**), 85% (**22e**); d) 4 M HCl/dioxane, RT; e) DIPEA, 1-amidinopyrazole·HCl, DMF, RT, 24% (**23d**), 18% (**23e**) (two steps).

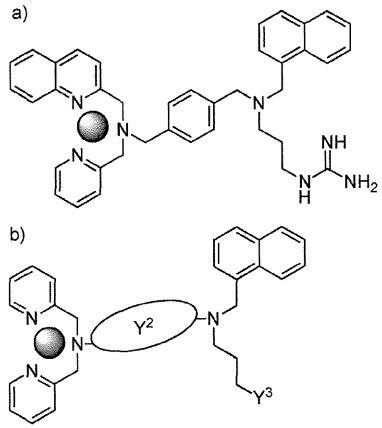


Figure 4. Zinc complexes of a) **19a** and b) **37b**, **38b**, **39b**, **40b**, **41b**, and **42b**. The shaded circle represents the position of the zinc cation in the chelate. The structures of Y^2 and Y^3 are shown in Figure 3 as A–C and i–ii, respectively.

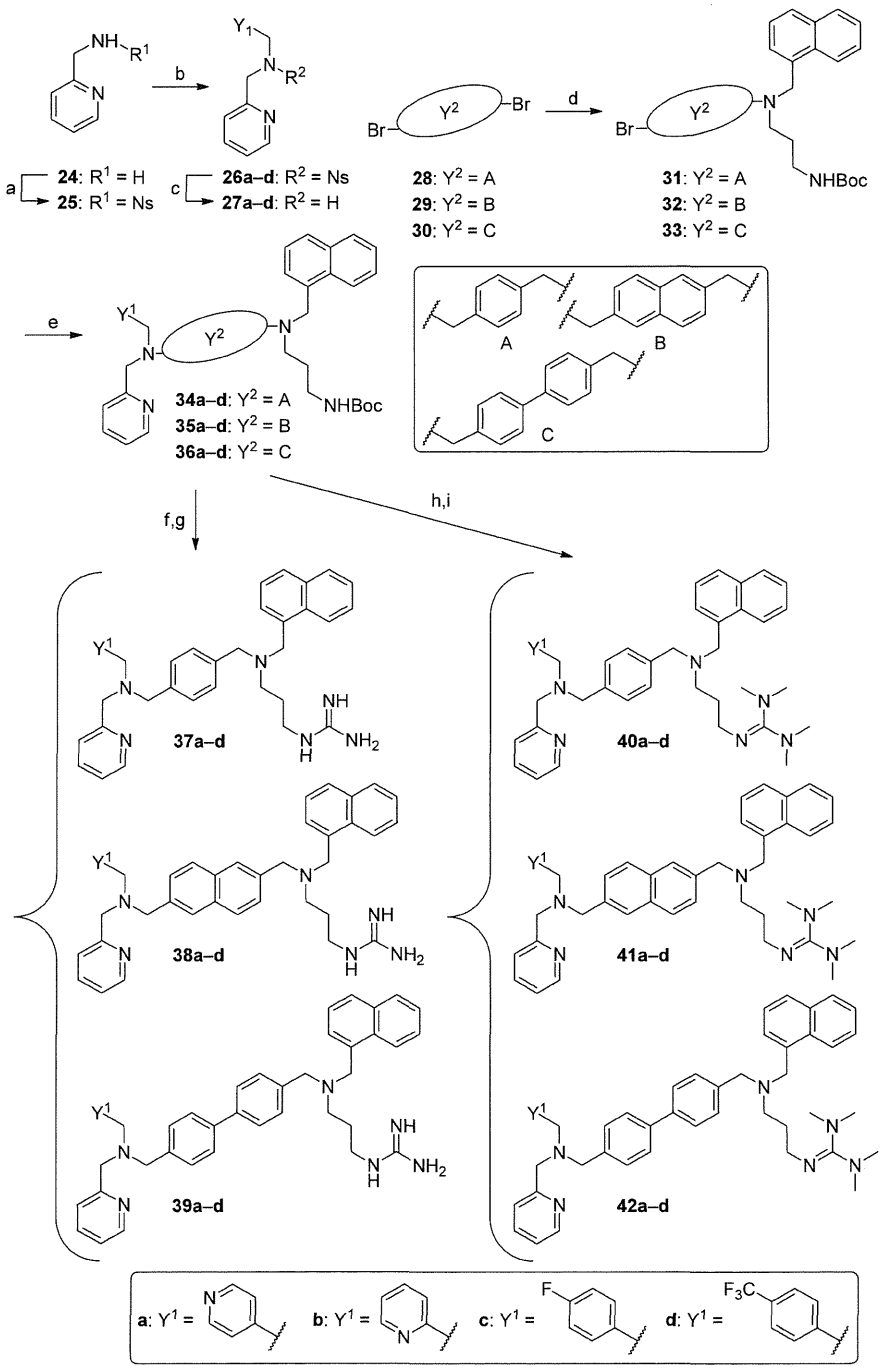
Table 2. CXCR4 binding affinities of compounds **37a–42d**.

Compd	$Y^{1[a]}$	$Y^{2[b]}$	$Y^{3[c]}$	Inhibition [%][d]	Compd	$Y^{1[a]}$	$Y^{2[b]}$	$Y^{3[c]}$	Inhibition [%][d]
37a	a	A	i	9.6±1.9	**40a**	a	A	ii	0
37b	b	A	i	21.4±2.8	**40b**	b	A	ii	41.5±4.8
37c	c	A	i	8.5±1.8	**40c**	c	A	ii	12.7±4.0
37d	d	A	i	22.3±1.4	**40d**	d	A	ii	23.8±6.0
38a	a	B	i	0	**41a**	a	B	ii	3.2±2.2
38b	b	B	i	4.7±1.3	**41b**	b	B	ii	21.6±2.6
38c	c	B	i	4.2±6.0	**41c**	c	B	ii	13.2±1.5
38d	d	B	i	4.1±4.1	**41d**	d	B	ii	18.4±1.2
39a	a	C	i	8.1±1.1	**42a**	a	C	ii	8.8±1.0
39b	b	C	i	18.0±1.1	**42b**	b	C	ii	0
39c	c	C	i	26.0±3.0	**42c**	c	C	ii	26.6±4.4
39d	d	C	i	27.9±5.2	**42d**	d	C	ii	45.0±3.0

[a–c] The structures of Y^1, Y^2, and Y^3 are shown in Figure 3 as a–d, A–C, and i–ii, respectively. [d] CXCR4 binding affinity was assessed based on the inhibition of [^{125}I]CXCL12 binding to Jurkat cells; percent inhibition values for all compounds at 10 μM were calculated relative to that of FC131 (100%).

except **39b** is observed if the inhibitory activities of the zinc complexes at 5 μM (Table 3) are compared with those of the corresponding metal-free compounds at 10 μM (Tables 1 and 2). The high activity of the zinc complexes is consistent with results reported in our previous work,[35,36] and suggests that the formation of chelates of the nitrogen atoms in the compounds with the zinc(II) ion might enhance their interaction with CXCR4. Fixation of the functional moieties by zinc(II) chelation, progression of electron deficiency of the aromatic moieties, interaction of the zinc(II) ion with residues on CXCR4, etc., might be considered as reasons for the enhanced CXCR4 binding affinity of the zinc complexes. According to previous reports,[39,40] in the case of chelation of the zinc complexes of AMD3100, a divalent metal ion such as zinc(II) in one of the bicyclam rings increased this compound's affinity for CXCR4 through a specific interaction with the carboxylate of Asp262 of CXCR4. A similar phenomenon could be occurring in the zinc complexes of the present compounds. The IC_{50} values of the zinc complexes of **37b** and **40b** containing 1,4-phenylenedimethanamine were evaluated to be 2.1 μM. In comparing the CXCR4 binding affinity of the zinc complexes of **37b**, **38b**, **39b**, **40b**, **41b**, and **42b**, 1,4-phenylenedimethanamine is the most suitable spacer template (H_2N-Y^2-NH_2), and naphthalene-2,6-diyldimethanamine is the second most effective. As substituents for Y^3, the tetramethylguanidino group is more appropriate than guanidine. The reason for this property has not been clarified yet; however, the tetramethyl group might stabilize a positively charged nitrogen atom, or might enhance a hy-

line groups, and **37b**, **38b**, **39b**, **40b**, **41b**, and **42b**, containing the Dpa group, were evaluated (Figure 4). $ZnCl_2$ (10 equiv relative to each compound) was added to phosphate-buffered saline (PBS) solutions of these compounds to form zinc(II) complexes. Chelation of the nitrogen atoms of **37b** and **40b** with the zinc(II) ion has been demonstrated by changes in NMR chemical shifts upon $ZnCl_2$ titration as zinc chelates as described in our previous studies.[35,36] The percent inhibition of the zinc complexes at 5 μM is listed in Table 3. A remarkable increase in CXCR4 binding affinity of all the zinc complexes



Scheme 3. *Reagents and conditions*: a) NsCl, Et_3N, THF, 84%; b) Y^1-CH_2OH, DEAD, PPh_3, THF, 53% (**26a**), 92% (**26b**), 70% (**26c**), 97% (**26d**); c) PhSH, K_2CO_3, DMF, 97% (**27a**), 74% (**27b**), 91% (**27c**), 91% (**27d**); d) KI, K_2CO_3, **11**, MeCN, 78% (**31**), 53% (**32**), 71% (**33**); e) KI, K_2CO_3, amine **27a–d**, MeCN, 25% (**34a**), 78% (**34b**), 80% (**34c**), 90% (**34d**), 38% (**35a**), 75% (**35b**), 67% (**35c**), 55% (**35d**), 23% (**36a**), 59% (**36b**), 80% (**36c**), 80% (**36d**); f) 4 M HCl/dioxane; g) DIPEA, 1-amidinopyrazole·HCl, DMF, 19% (**37a**), 49% (**37b**), 52% (**37c**), 30% (**37d**), 42% (**38a**), 56% (**38b**), 62% (**38c**), 44% (**38d**), 39% (**39a**), 48% (**39b**), 87% (**39c**), 50% (**39d**) (two steps); h) 4 M HCl/dioxane; i) DIPEA, 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, DMF, 24% (**40a**), 36% (**40b**), 31% (**40c**), 32% (**40d**), 31% (**41a**), 14% (**41b**), 47% (**41c**), 27% (**41d**), 37% (**42a**), 25% (**42b**), 27% (**42c**), 44% (**42d**) (two steps).

drophobic interaction with residues on CXCR4. Comparison of the CXCR4 binding affinity of the zinc complexes of **19a** and **37b** shows that the 2-pyridylmethyl group is more suitable than the 2-methylquinoline group as X-CH_2 or Y^1-CH_2 introduced on the nitrogen atom.

Conclusions

New low-molecular-weight CXCR4 ligands were designed and synthesized. The most potent compounds are **37b** and **40b**, zinc complexes with a Dpa group on the 1,4-phenylenedimethanamine spacer template. The distances between all the functional moieties of the compounds linked by the 1,4-phenylenedimethanamine spacer might be appropriate for interaction with CXCR4. These compounds exhibited IC_{50} values at micromolar levels in CXCR4 binding affinity. Zinc complexation of Dpa-containing compounds resulted in a remarkable increase in CXCR4 binding affinity relative to the corresponding zinc-free compounds. The results reported herein might provide useful insight into the design of novel CXCR4 ligands, complementing information from other compounds such as T140, FC131, and KRH-1636. These compounds will be useful for the development of future therapeutic strategies for CXCR4-relevant diseases.

Experimental Section

Chemistry

Synthetic strategies of compounds reported in the present study are described in Results and Discussion above, and details are provided in the Supporting Information. Zn^{II} complex formation was performed by treatment of the compounds with 10 equiv $ZnCl_2$ in PBS. The Zn^{II} complexes were characterized by the chemical shifts of their methylene protons in ^1H NMR spectroscopic analysis. The Dpa–Zn^{II} complex was characterized previously.[35] Detailed data are provided in the Supporting Information.

Table 3. CXCR4 binding affinities of compounds **19a**, **37b**, **38b**, **39b**, **40b**, **41b**, and **42b** in zinc(II) complex.

Compd	Inhibition [%][a]	IC_{50} [nM][b]
19a	34.5±6.5	ND
37b	93.4±6.4	2100
38b	25.6±2.4	ND
39b	0	ND
40b	98.0±1.0	2100
41b	80.7±0.8	ND
42b	35.9±0.9	ND
FC131[c]	100	15.9

[a] CXCR4 binding affinity was assessed based on the inhibition of [^{125}I]CXCL12 binding to Jurkat cells; percent inhibition values for all zinc complexes at 5 μM were calculated relative to that of FC131 (100%). [b] IC_{50}: zinc complex concentration required for 50% inhibition of [^{125}I]CXCL12 binding to Jurkat cells; all data are the mean values from at least three independent experiments; ND: not determined. [c] Metal free.

Biological assays

CXCR4 binding assays of compounds based on the inhibition of [^{125}I]CXCL12 binding to Jurkat cells were performed as reported by Tanaka et al.[38]

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Keywords: aza-macrocycles · chemokine receptors · CXCR4 · low-molecular-weight ligands · zinc complexes

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