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Low-Molecular-Weight CXCR4 Ligands with Variable Spacers

Tetsuo Narumi,[a] Haruo Aikawa,[a] Tomohiro Tanaka,[a] Chie Hashimoto,[a] Nami Ohashi,[a] Wataru Nomura,[a] Takuya Kobayakawa,[a] Hikaru Takano,[a] Yuki Hirota,[a] Tsutomu Murakami,[b] Naoki Yamamoto,[c] and Hirokazu Tamamura*[a]

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-

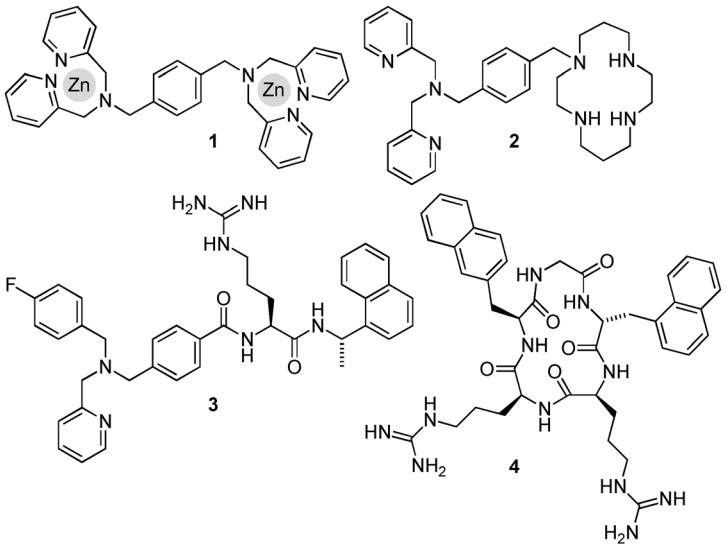

Figure 1. Reported low-molecular-weight CXCR4 antagonists.

[a] *Dr. T. Narumi, Dr. H. Aikawa, Dr. T. Tanaka, C. Hashimoto, Dr. N. Ohashi, Dr. W. Nomura, T. Kobayakawa, H. Takano, Y. Hirota, Prof. H. Tamamura*
Institute of Biomaterials and Bioengineering
Tokyo Medical and Dental University
2-3-10 Kandasurugadai, Chiyoda-ku, Tokyo 101-0062 (Japan)
E-mail: tamamura.mr@tmd.ac.jp

[b] *Dr. T. Murakami*
AIDS Research Center, National Institute of Infectious Diseases
1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8640 (Japan)

[c] *Prof. N. Yamamoto*
Department of Microbiology, Yong Loo Lin School of Medicine
National University of Singapore
Block MD4, 5 Science Drive 2, Singapore 117597 (Singapore)

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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

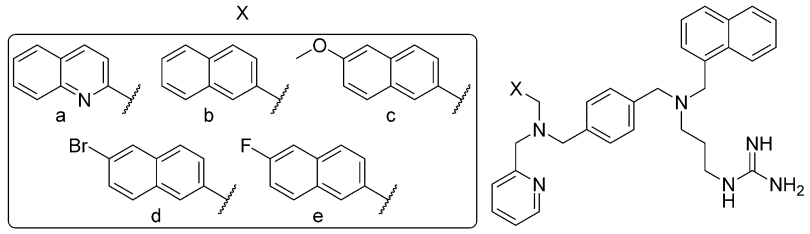


Figure 2. New compounds containing the 1,4-phenylenedimethanamine structure.

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**.

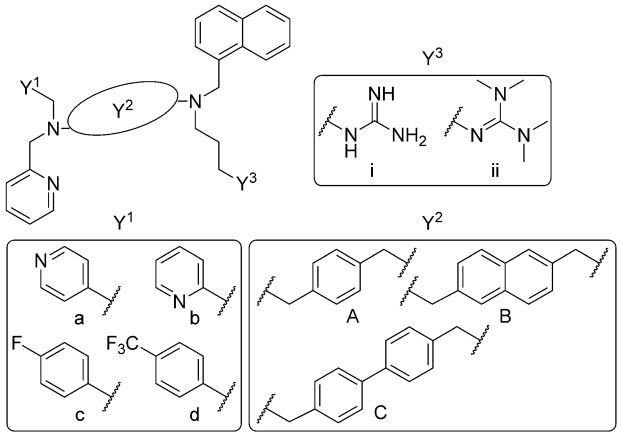


Figure 3. New compounds containing the 1,4-phenylenedimethanamine, naphthalene-2,6-diyldimethanamine, and [1,1′-biphenyl]-4,4′-diyldimethanamine structures.

Chemistry

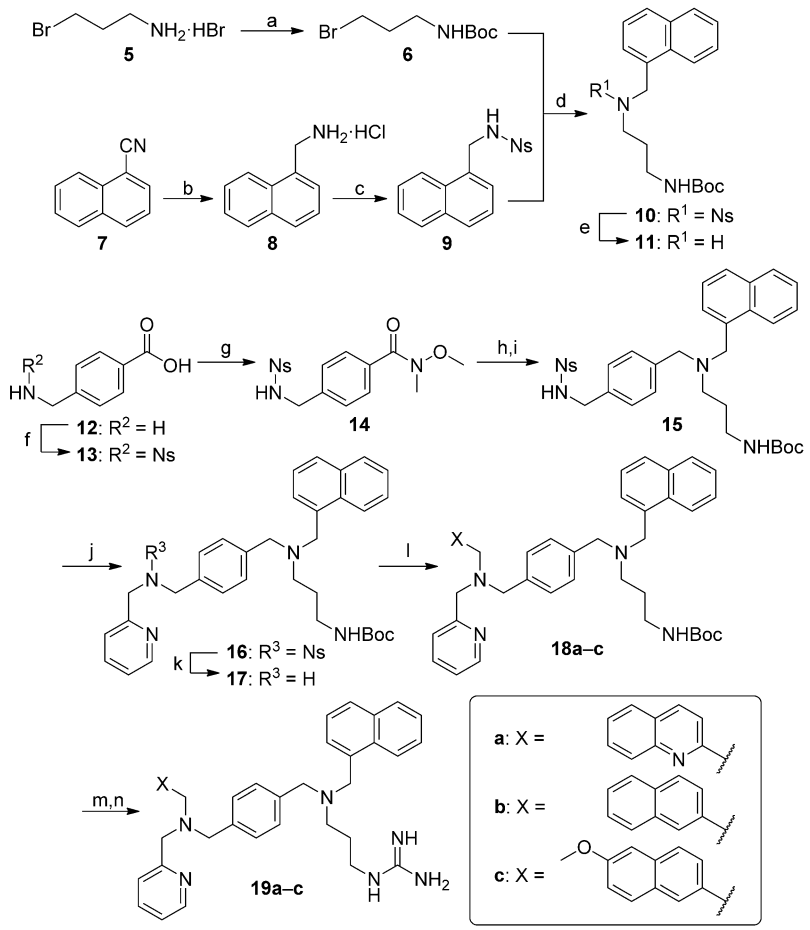
The synthesis of compounds **19a–c** is shown in Scheme 1. Condensation of *N*-Boc-3-aminopropylbromide (**6**) and *N*-Ns-aminonaphthalen-1-yl-methane (**9**; Ns = 2-nitrobenzenesulfonyl) followed by removal of the Ns group produced the amine **11**. The *N*-Ns-4-aminomethylbenzoic acid derived Weinreb 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) 4 M 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.

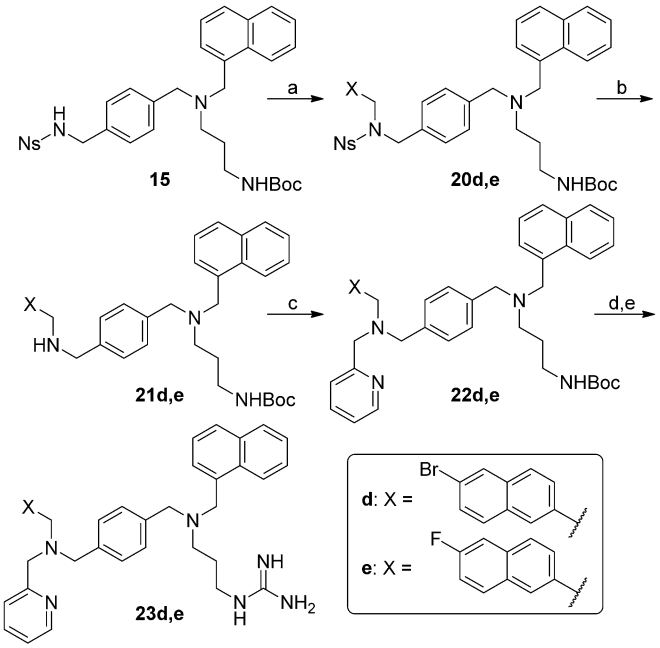
Table 1. CXCR4 binding affinities of compounds **19a–c** and **23d,e**.

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%).

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-



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

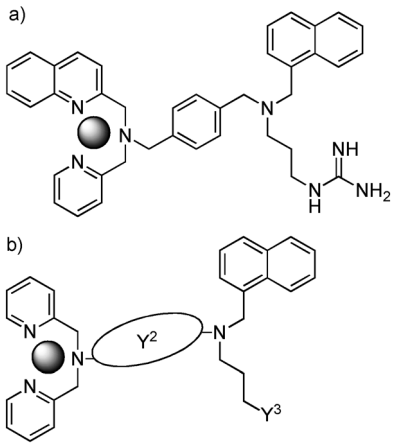


Figure 4. Zinc complexes of a) **19 a** and b) **37 b**, **38 b**, **39 b**, **40 b**, **41 b**, and **42 b**. The shaded circle represents the position of the zinc cation in the chelate. The structures of Y^2 and Y^3 are shown in Figure 3 as A–C and i–ii, respectively.

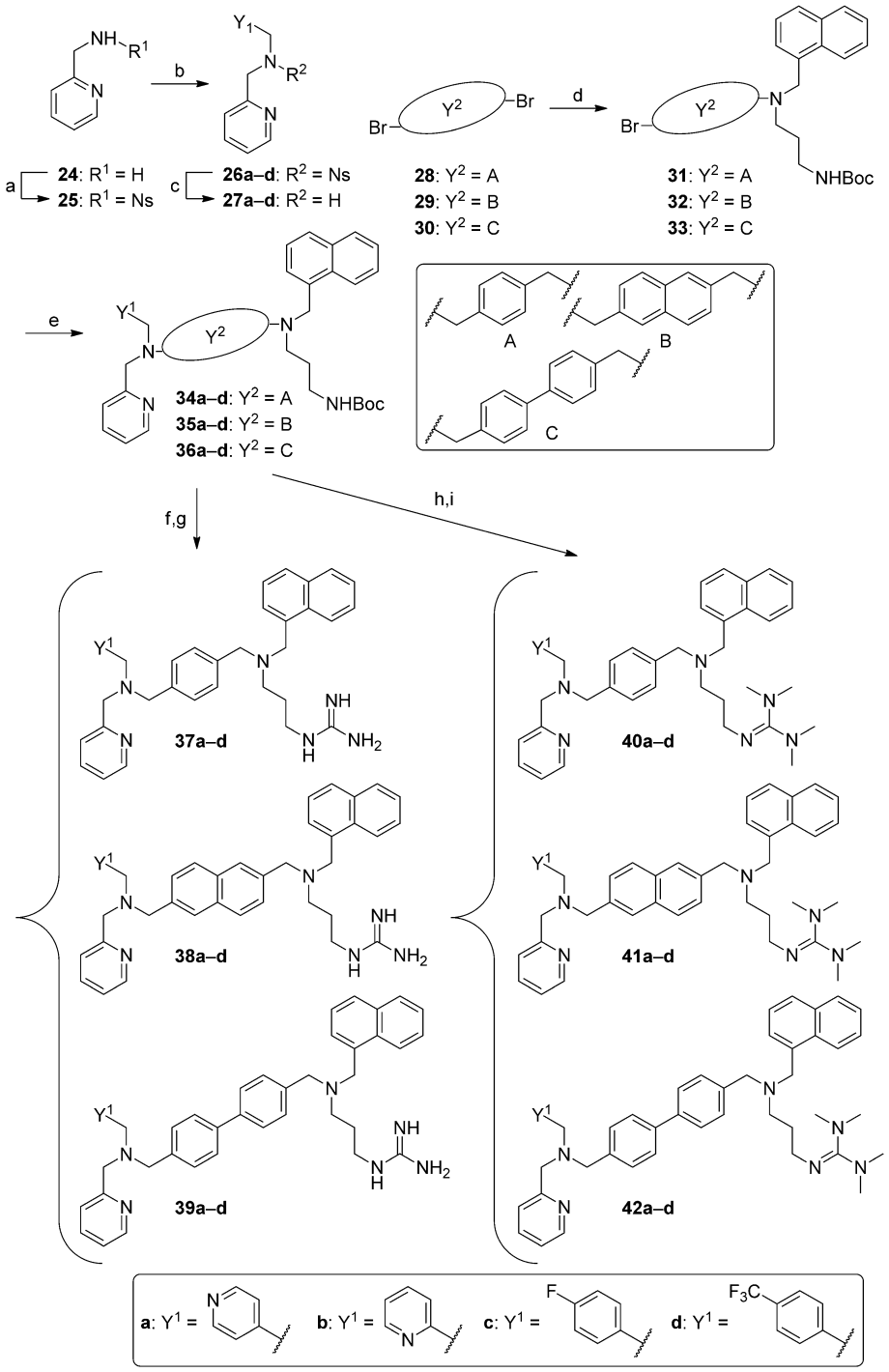
Table 2. CXCR4 binding affinities of compounds **37 a**–**42 d**.

Compd	$Y^{1[a]}$	$Y^{2[b]}$	$Y^{3[c]}$	Inhibition [%][d]	Compd	$Y^{1[a]}$	$Y^{2[b]}$	$Y^{3[c]}$	Inhibition [%][d]
37 a	a	A	i	9.6 ± 1.9	**40 a**	a	A	ii	0
37 b	b	A	i	21.4 ± 2.8	**40 b**	b	A	ii	41.5 ± 4.8
37 c	c	A	i	8.5 ± 1.8	**40 c**	c	A	ii	12.7 ± 4.0
37 d	d	A	i	22.3 ± 1.4	**40 d**	d	A	ii	23.8 ± 6.0
38 a	a	B	i	0	**41 a**	a	B	ii	3.2 ± 2.2
38 b	b	B	i	4.7 ± 1.3	**41 b**	b	B	ii	21.6 ± 2.6
38 c	c	B	i	4.2 ± 6.0	**41 c**	c	B	ii	13.2 ± 1.5
38 d	d	B	i	4.1 ± 4.1	**41 d**	d	B	ii	18.4 ± 1.2
39 a	a	C	i	8.1 ± 1.1	**42 a**	a	C	ii	8.8 ± 1.0
39 b	b	C	i	18.0 ± 1.1	**42 b**	b	C	ii	0
39 c	c	C	i	26.0 ± 3.0	**42 c**	c	C	ii	26.6 ± 4.4
39 d	d	C	i	27.9 ± 5.2	**42 d**	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 **39 b** 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 **37 b** and **40 b** containing 1,4-phenylenedimethanamine were evaluated to be 2.1 μM. In comparing the CXCR4 binding affinity of the zinc complexes of **37 b**, **38 b**, **39 b**, **40 b**, **41 b**, and **42 b**, 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 **37 b**, **38 b**, **39 b**, **40 b**, **41 b**, and **42 b**, 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 **37 b** and **40 b** 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

[1] T. Nagasawa, H. Kikutani, T. Kishimoto, *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 2305 – 2309.
[2] C. C. Bleul, M. Farzan, H. Choe, C. Parolin, I. Clark-Lewis, J. Sodroski, T. A. Springer, *Nature* **1996**, *382*, 829 – 833.
[3] E. Oberlin, A. Amara, F. Bachelerie, C. Bessia, J. L. Virelizier, F. Arenzana-Seisdedos, O. Schwartz, J. M. Heard, I. Clark-Lewis, D. L. Legler, M. Loetscher, M. Baggiolini, B. Moser, *Nature* **1996**, *382*, 833 – 835.
[4] K. Tashiro, H. Tada, R. Heilker, M. Shirozu, T. Nakano, T. Honjo, *Science* **1993**, *261*, 600 – 603.
[5] J. Wang, L. He, C. A. Combs, G. Roderiquez, M. A. Norcross, *Mol. Cancer Ther.* **2006**, *5*, 2474 – 2483.
[6] T. Tanaka, W. Nomura, T. Narumi, A. Masuda, H. Tamamura, *J. Am. Chem. Soc.* **2010**, *132*, 15899 – 15901.
[7] C. C. Bleul, R. C. Fuhlbrigge, J. M. Casanovas, A. Aiuti, T. A. Springer, *J. Exp. Med.* **1996**, *184*, 1101 – 1109.
[8] K. Tachibana, S. Hirota, H. Iizasa, H. Yoshida, K. Kawabata, Y. Kataoka, Y. Kitamura, K. Matsushima, N. Yoshida, S. Nishikawa, T. Kishimoto, T. Nagasawa, *Nature* **1998**, *393*, 591 – 594.
[9] T. Nagasawa, S. Hirota, K. Tachibana, N. Takakura, S. Nishikawa, Y. Kitamura, N. Yoshida, H. Kikutani, T. Kishimoto, *Nature* **1996**, *382*, 635 – 638.
[10] Y. Zhu, T. Yu, X.-C. Zhang, T. Nagasawa, J. Y. Wu, Y. Rao, *Nat. Neurosci.* **2002**, *5*, 719 – 720.
[11] R. K. Stumm, C. Zhou, T. Ara, F. Lazarini, M. Dubois-Dalcq, T. Nagasawa, V. Hollt, S. Schulz, *J. Neurosci.* **2003**, *23*, 5123 – 5130.
[12] T. Koshiba, R. Hosotani, Y. Miyamoto, J. Ida, S. Tsuji, S. Nakajima, M. Kawaguchi, H. Kobayashi, R. Doi, T. Hori, N. Fujii, M. Imamura, *Clin. Cancer Res.* **2000**, *6*, 3530 – 3535.
[13] A. Müller, B. Homey, H. Soto, N. Ge, D. Catron, M. E. Buchanan, T. McClanahan, E. Murphy, W. Yuan, S. N. Wagner, J. L. Barrera, A. Mohar, E. Verastegui, A. Zlotnik, *Nature* **2001**, *410*, 50 – 56.
[14] H. Tamamura, A. Hori, N. Kanzaki, K. Hiramatsu, M. Mizumoto, H. Nakashima, N. Yamamoto, A. Otaka, N. Fujii, *FEBS Lett.* **2003**, *550*, 79 – 83.
[15] N. Tsukada, J. A. Burger, N. J. Zvaifler, T. J. Kipps, *Blood* **2002**, *99*, 1030 – 1037.
[16] J. Juarez, K. F. Bradstock, D. J. Gottlieb, L. J. Bendall, *Leukemia* **2003**, *17*, 1294 – 1300.
[17] H. K. Deng, R. Liu, W. Ellmeier, S. Choe, D. Unutmaz, M. Burkhart, P. D. Marzio, S. Marmon, R. E. Sutton, C. M. Hill, C. B. Davis, S. C. Peiper, T. J. Schall, D. R. Littman, N. R. Landau, *Nature* **1996**, *381*, 661 – 666.
[18] Y. Feng, C. C. Broder, P. E. Kennedy, E. A. Berger, *Science* **1996**, *272*, 872 – 877.
[19] T. Nanki, K. Hayashida, H. S. El-Gabalawy, S. Suson, K. Shi, H. J. Girschick, S. Yavuz, P. E. Lipsky, *J. Immunol.* **2000**, *165*, 6590 – 6598.
[20] H. Tamamura, M. Fujisawa, K. Hiramatsu, M. Mizumoto, H. Nakashima, N. Yamamoto, A. Otaka, N. Fujii, *FEBS Lett.* **2004**, *569*, 99 – 104.
[21] T. Murakami, T. Nakajima, Y. Koyanagi, K. Tachibana, N. Fujii, H. Tamamura, N. Toshida, M. Waki, A. Matsumoto, O. Yoshie, T. Kishimoto, N. Yamamoto, T. Nagasawa, *J. Exp. Med.* **1997**, *186*, 1389 – 1393.
[22] H. Tamamura, Y. Xu, T. Hattori, X. Zhang, R. Arakaki, K. Kanbara, A. Omagari, A. Otaka, T. Ibuka, N. Yamamoto, H. Nakashima, N. Fujii, *Biochem. Biophys. Res. Commun.* **1998**, *253*, 877 – 882.
[23] H. Tamamura, A. Omagari, S. Oishi, T. Kanamoto, N. Yamamoto, S. C. Peiper, H. Nakashima, A. Otaka, N. Fujii, *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2633 – 2637.
[24] N. Fujii, S. Oishi, K. Hiramatsu, T. Araki, S. Ueda, H. Tamamura, A. Otaka, S. Kusano, S. Terakubo, H. Nakashima, J. A. Broach, J. O. Trent, Z. Wang, S. C. Peiper, *Angew. Chem.* **2003**, *115*, 3373 – 3375; *Angew. Chem. Int. Ed.* **2003**, *42*, 3251 – 3253.
[25] H. Tamamura, K. Hiramatsu, S. Ueda, Z. Wang, S. Kusano, S. Terakubo, J. O. Trent, S. C. Peiper, N. Yamamoto, H. Nakashima, A. Otaka, N. Fujii, *J. Med. Chem.* **2005**, *48*, 380 – 391.
[26] H. Tamamura, T. Araki, S. Ueda, Z. Wang, S. Oishi, A. Esaka, J. O. Trent, H. Nakashima, N. Yamamoto, S. C. Peiper, A. Otaka, N. Fujii, *J. Med. Chem.* **2005**, *48*, 3280 – 3289.
[27] C. Hashimoto, T. Tanaka, T. Narumi, W. Nomura, H. Tamamura, *Expert Opin. Drug Discovery* **2011**, *6*, 1067 – 1090.
[28] H. Tamamura, H. Tsutsumi, H. Masuno, S. Mizokami, K. Hiramatsu, Z. Wang, J. O. Trent, H. Nakashima, N. Yamamoto, S. C. Peiper, N. Fujii, *Org. Biomol. Chem.* **2006**, *4*, 2354 – 2357.
[29] K. Ichiyama, S. Yokoyama-Kumakura, Y. Tanaka, R. Tanaka, K. Hirose, K. Bannai, T. Edamatsu, M. Yanaka, Y. Niitani, N. Miyano-Kurosaki, H. Takaku, Y. Koyanagi, N. Yamamoto, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 4185 – 4190.
[30] E. De Clercq, N. Yamamoto, R. Pauwels, J. Balzarini, M. Witvrouw, K. De Vreese, Z. Debyser, B. Rosenwirth, P. Peichl, R. Datema, *Antimicrob. Agents Chemother.* **1994**, *38*, 668 – 674.
[31] D. Schols, S. Struyf, J. Van Damme, J. A. Esté, G. Henson, E. De Clercq, *J. Exp. Med.* **1997**, ***186***, 1383 – 1388.
[32] S. Hatse, K. Princen, E. De Clercq, M. M. Rosenkilde, T. W. Schwartz, P. E. Hernandez-Abad, R. T. Skerlj, G. J. Bridger, D. Schols, *Biochem. Pharmacol.* **2005**, *70*, 752 – 761.
[33] Z. Liang, W. Zhan, A. Zhu, Y. Yoon, S. Lin, M. Sasaki, J. M. A. Klapproth, H. Yang, H. E. Grossniklaus, J. Xu, M. Rojas, R. J. Voll, M. M. Goodman, R. F. Arrendale, J. Liu, C. C. Yun, J. P. Snyder, D. C. Liotta, H. Shim, *PLoS One* **2012**, *7*, e34038.
[34] S. Pettersson, V. I. Pérez-Nueno, L. Ros-Blanco, R. Puig de La Bellacasa, M. O. Rabal, X. Batllori, B. Clotet, I. Clotet-Codina, M. Armand-Ugón, J. A. Esté, J. I. Borrell, J. Teixidó, *ChemMedChem* **2008**, *3*, 1549 – 1557.
[35] H. Tamamura, A. Ojida, T. Ogawa, H. Tsutsumi, H. Masuno, H. Nakashima, N. Yamamoto, I. Hamachi, N. Fujii, *J. Med. Chem.* **2006**, *49*, 3412 – 3415.
[36] T. Tanaka, T. Narumi, T. Ozaki, A. Sohma, N. Ohashi, C. Hashimoto, K. Itotani, W. Nomura, T. Murakami, N. Yamamoto, H. Tamamura, *ChemMedChem* **2011**, *6*, 834 – 839.

[37] T. Narumi, T. Tanaka, C. Hashimoto, W. Nomura, H. Aikawa, A. Sohma, K. Itotani, M. Kawamata, T. Murakami, N. Yamamoto, H. Tamamura, *Bioorg. Med. Chem. Lett.* **2012**, *22*, 4169 – 4172.
[38] T. Tanaka, H. Tsutsumi, W. Nomura, Y. Tanabe, N. Ohashi, A. Esaka, C. Ochiai, J. Sato, K. Itotani, T. Murakami, K. Ohba, N. Yamamoto, N. Fujii, H. Tamamura, *Org. Biomol. Chem.* **2008**, *6*, 4374 – 4377.
[39] L. Ole Gerlach, J. S. Jakobsen, K. P. Jensen, M. R. Rosenkilde, R. T. Skerlj, U. Ryde, G. J. Bridger, T. W. Schwartz, *Biochemistry* **2003**, *42*, 710 – 717.
[40] M. M. Rosenkilde, L.-O. Gerlach, J. S. Jakobsen, R. T. Skerlj, G. J. Bridger, T. W. Schwartz, *J. Biol. Chem.* **2004**, *279*, 3033 – 3041.

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