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Azamacrocyclic Metal Complexes as CXCR4 Antagonists

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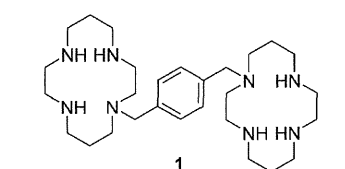
The chemokine receptor CXCR4 is a member of the seven transmembrane GPCR family, which is implicated in multiple diseases, including HIV infection, cancers, and rheumatoid arthritis. Low-molecular-weight nonpeptidic compounds, including AMD3100 and various pyridyl macrocyclic zinc(II) complexes, have been identified as selective antagonists of CXCR4. In the present study, structure–activity relationship studies were performed by combining the common structural features of alkylamino and pyridiyl macrocyclic antagonists. Several new zinc(II) or copper(II) complexes demonstrated potent anti-HIV activity, strong CXCR4-binding activity, and significant inhibitory activity against Ca^{2+} mobilization induced by CXCL12 stimulation. These results may prove useful in the design of novel CXCR4 antagonists, and the compounds described could potentially be developed as therapeutics against CXCR4-relevant diseases or chemical probes to study the biological activity of CXCR4.

Introduction

The chemokine receptor CXCR4, which transduces signals of its endogenous ligand, CXCL12/stromal cell-derived factor-1 (SDF-1),[1–4] is classified as a member of the seven transmembrane GPCR family, and plays a physiological role via its interaction with CXCL12 in chemotaxis,[5] angiogenesis,[6,7] and neurogenesis[8,9] in embryonic stages. CXCR4 is, however, relevant to multiple diseases including HIV infection/AIDS,[10,11] metastasis of several types of cancer,[12–14] leukemia cell progression,[15,16] and rheumatoid arthritis (RA),[17,18] and is considered an attractive drug target to combat these diseases. Thus, inhibitors targeting CXCR4 are expected to be useful for drug discovery.

Several CXCR4 antagonists have been reported,[19–35] including our discovery of the highly potent CXCR4 antagonist T140, a 14-mer peptide with a disulfide bridge, its smaller derivative, the 5-mer cyclic peptide FC131, and several other potent analogues.[19,24–26,28–30] Clinical development of these peptidic antagonists could be pursued using specific administration strategies involving biodegradable microcapsules.[14,36] However, herein we focus on novel nonpeptidic low-molecular-weight CXCR4 antagonists. To date, AMD3100 (**1**),[20,22] Dpa-Zn complex (**2**),[37] KRH-1636,[27] and other compounds[31–35] have been developed in this and other laboratories as low-molecular-weight nonpeptidic CXCR4 antagonists. The present study reports structure–activity relationship studies based on the combination of common structural motifs, such as xylene scaffolds and cationic moieties that are present in the aforementioned compounds.



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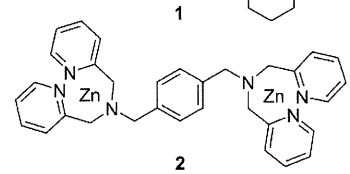

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Results and Discussion

In order to determine spatially suitable positioning of cationic moieties, *p*- and *m*-xylenes were utilized as spacers. Cationic moieties such as bis(pyridin-2-ylmethyl)amine (dipicolylamine), 1,4,7,10-tetraazacyclododecane (cyclen), and 1,4,8,11-tetraazacyclotetradecane (cyclam) were introduced as R^1 and R^2 (Figure 1). This combination of R^1, R^2, and spacer groups led to the design and synthesis of compounds **12–31**.

The CXCR4 binding activity of synthetic compounds was assessed based on the inhibition of [^{125}I]CXCL12 binding to Jurkat cells, which express CXCR4.[38] The percent inhibition of all compounds at 1 μM is shown in Table 1. Seven compounds (**16**, **17**, **20–22**, **28**, and **29**, Table 1) resulted in greater than 87% inhibition. The high activity of **16** is consistent with re-

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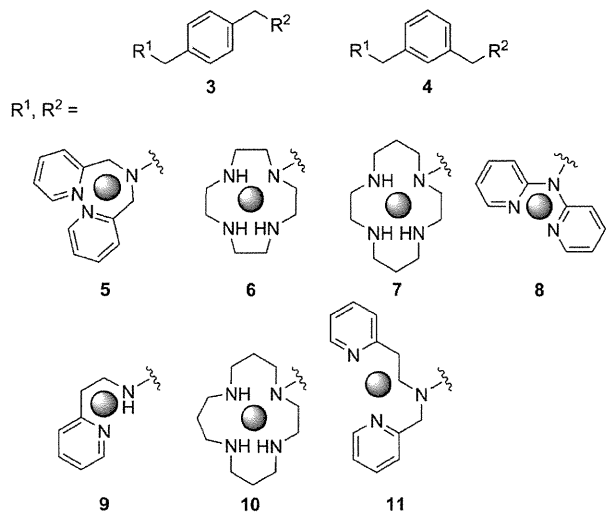

Figure 1. The structures of aromatic spacers (upper) and cationic moieties (R^1 and R^2). The shaded circle represents the position of the metal cation (Zn^{II} or Cu^{II}) in the chelate.

sults reported previously.[20,22] The anti-HIV activities of **17** and **29**, which contain only cyclam or cyclal rings, were reported by De Clercq et al.[39,40] Compounds with only pyridine and/or cyclen rings did not show any high binding activity. The presence of azamacrocyclic rings is presumably indispensable to the interaction of these compounds with CXCR4, and the size of rings appears to be important because not only compounds **16** and **17**, with two cyclam rings in the molecule, but also compounds **28** and **29**, with two cyclal rings, have remarkably more potent CXCR4 binding activity than compounds **14** and **15**, which have two cyclen rings. Compound **22**, with a *p*-xylene moiety, exhibited higher activity than compound **23**, which has an *m*-xylene moiety, indicating that *p*-xylene is more suitable than *m*-xylene as a spacer for approximate positioning of cationic moieties. At 0.1 μM, compound **22** resulted in 86% inhibition of [^{125}I]CXCL12 binding, while the other six compounds exhibited 37–66% inhibition. The IC_{50} value of compound **22** was estimated to be 37 nM.

$ZnCl_2$ was added to phosphate-buffered saline (PBS) solutions of these 20 compounds, **12–31**, to form zinc(II) complexes. The percent inhibition for each compound at 1 μM against [^{125}I]CXCL12 binding was determined and is given in Table 1. Zinc complexation of **12–15**, **18**, **19**, and **23** resulted in a remarkable increase in CXCR4 binding activity compared to the corresponding zinc-free compounds. These molecules contain dipicolylamine and/or cyclen moieties, suggesting that chelation of the nitrogen atoms with the zinc(II) ion significantly affects their interactions with CXCR4. The high activity of the zinc chelates of **12** and **13** is consistent with results provided in our previous paper.[37] Additionally, the anti-HIV activity of zinc complexes of **14** and **15** was reported by Kimura et al.[41] For compounds with only dipicolylamine and/or cyclen macrocycles as cationic moieties (**12–15**, **18**, and **19**), zinc complexation is critical to achieve high binding activity; the corresponding zinc-free compounds exhibit no significant activity. Compounds **16**, **17**, **20–22**, **28**, and **29** demonstrated high binding affinity in metal-free states as well as in zinc complexation states, indicating that zinc complexation of either of the macrocyclic rings in these compounds is not essential for high activity. The CXCR4 binding activity and anti-HIV activity of the zinc complex of **16** were reported previously.[42,43] Measured inhibition percentages for 0.1 μM of the zinc complexes of **12**, **14–23**, **28**, and **29** are given in Table 1. The zinc complexes of **20–22**, **28**, and **29** at 0.1 μM exhibited greater than 79% inhibition of [^{125}I]CXCL12 binding, and the other eight zinc complexes (of **12**, **14–19**, and **23**,) showed less than 55% inhibition. The IC_{50} values of zinc complexes of **20–22**, **28**, and **29** were estimated to be 11, 8.3, 22, 40, and 52 nM, respectively. Zinc complexes of compounds containing a combination of cyclen and cyclam moieties, **20** and **21**, had remarkably potent IC_{50} values.

To form chelates with a copper(II) cation, $CuCl_2$ was added to solutions in PBS of **12-31**. The inhibition percentages of all the compounds at 1 μM against [^{125}I]CXCL12 binding are shown in Table 1. Copper complexes of **14** and **15** exhibited a significant increase in CXCR4 binding activity as compared to the corresponding copper-free compounds, a phenomenon which is also seen in the zinc chelates. These compounds have two cyclen moieties in the molecules, suggesting that zinc or copper complexation is critical for high binding activity. Compounds **16**, **17**, and **20–22** showed high binding affinities in metal-free states and zinc- and copper-complexed states, indicating that metallic complexation of the cyclam rings in these compounds is not necessary for high activity. The CXCR4 binding activity of the copper complex of **16** was previously reported.[42] For compounds **17**, **22**, **23**, **28**, and **29**, copper complexation caused a significant decrease in binding activity compared to the corresponding copper-free compounds, whereas for compounds **14**, **15**, **18**, and **19**, copper complexation caused an increase in binding activity. This phenomenon may be due to the difference in ring sizes and structures of macrocycles, and was not observed upon zinc-complex formation. Inhibition at 0.1 μM of the copper complexes of **16** and **20–22**, which exhibited greater than 85% inhibition of [^{125}I]CXCL12 binding at 1 μM, are given in Table 1. The copper complexes of **16**, **20**, **21**, and **22** at 0.1 μM showed 39, 69, 88, and 39% inhibition, respectively, with the IC_{50} value of the copper complex of **21** estimated to be 16 nM.

Molecular modeling analysis of compound **21** and its zinc(II) and copper(II) complexes predicted that these complexes would form a stable coordinate conformation as shown in Figure 2. In general, zinc(II) complexes are predicted to adopt a tetrahedral conformation, while copper(II) complexes form a planar four coordinate/square conformation. The zinc(II) complex of **21** is predicted to have a tetrahedral conformation and the copper(II) complex a square planar conformation in both the cyclen and cyclam rings. The carboxyl group of either Asp 171 or Asp 262 in CXCR4 is thought to coordinate strongly with zinc ions but not copper ions in the complexes,[41–43] and as a consequence, the zinc complex of **21** would bind more strongly than **21** or its copper complex. This order of binding

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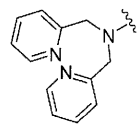
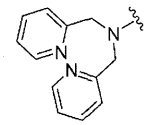
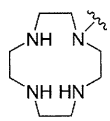
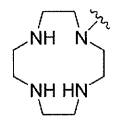
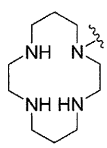
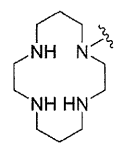
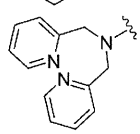
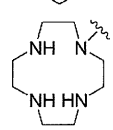
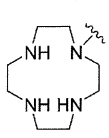
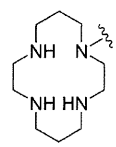
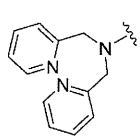
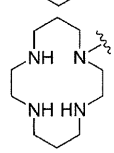
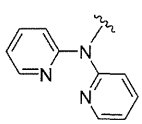
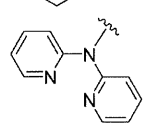
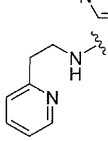
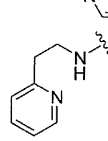
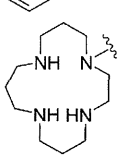
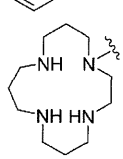
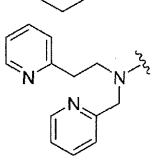
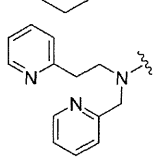
Table 1. CXCR4 binding activity of compounds **12–31** in the metal ion-free form, the zinc complex, and the copper complex.

Compd	Spacer	R^1	R^2	Metal free			Zinc complex			Copper complex		
				Inhibition[a] [%]		IC_{50}[b]	Inhibition[a] [%]		IC_{50}[b]	Inhibition[a] [%]		IC_{50}[b]
				1 μM	0.1 μM	[nM]	1 μM	0.1 μM	[nM]	1 μM	0.1 μM	[nM]
12	*p*-xylene			0	n.d.	n.d.	83±2	24±5	n.d.	10±4	n.d.	n.d.
13	*m*-xylene			0	n.d.	n.d.	31±3	n.d.	n.d.	0	n.d.	n.d.
14	*p*-xylene			30±4	n.d.	n.d.	87±4	0	n.d.	60±2	n.d.	n.d.
15	*m*-xylene			33±2	n.d.	n.d.	94±1	13±6	n.d.	80±3	n.d.	n.d.
16	*p*-xylene			94±4	59±6	n.d.	97±5	28±3	n.d.	98±1	39±3	n.d.
17	*m*-xylene			95±3	49±9	n.d.	98±4	55±7	n.d.	75±1	n.d.	n.d.
18	*p*-xylene			32±0.7	n.d.	n.d.	97±6	0	n.d.	52±3	n.d.	n.d.
19	*m*-xylene			17±5	n.d.	n.d.	91±4	0	n.d.	22±6	n.d.	n.d.
20	*p*-xylene			89±3	62±3	n.d.	>100	79±1	11	>100	69±3	n.d.
21	*m*-xylene			89±3	66±3	n.d.	92±3	>100	8.3	>100	88±1	16
22	*p*-xylene			94±3	86±3	37	99±8	79±0.6	22	85±3	39±3	n.d.
23	*m*-xylene			58±8	n.d.	n.d.	90±17	37±0.3	n.d.	48±4	n.d.	n.d.
24	*p*-xylene			3±0.9	n.d.	n.d.	0	n.d.	n.d.	0	n.d.	n.d.
25	*m*-xylene			4±3	n.d.	n.d.	0	n.d.	n.d.	0	n.d.	n.d.
26	*p*-xylene			14±2	n.d.	n.d.	10±3	n.d.	n.d.	0	n.d.	n.d.
27	*m*-xylene			10±3	n.d.	n.d.	10±4	n.d.	n.d.	0	n.d.	n.d.
28	*p*-xylene			91±0.4	37±0.9	n.d.	97±4	>100	40	57±4	n.d.	n.d.
29	*m*-xylene			87±2	50±1	n.d.	>100	91±4	52	55±1	n.d.	n.d.
30	*p*-xylene			0	n.d.	n.d.	14±3	n.d.	n.d.	14±3	n.d.	n.d.
31	*m*-xylene			24±2	n.d.	n.d.	20±3	n.d.	n.d.	0	n.d.	n.d.
FC-131	*cyclo*-[D-Tyr-Arg-Arg-Nal-Gly-]			100	100	1.8	–	–	–	–	–	–

[a] CXCR4 binding activity was assessed based on inhibition of [^{125}I]CXCL12 binding to Jurkat cells. Percent inhibition for all compounds at 1 and 0.1 μM were calculated relative to the percent inhibition by FC131 (100%). [b] IC_{50} values are the concentrations which correspond to 50% inhibition of [^{125}I]CXCL12 binding to Jurkat cells. All data are mean values ±SEM of at least three independent experiments. n.d. = not determined.

affinities is commonly seen for these compounds and their zinc(II) or copper(II) complexes.

We investigated the CXCR4 antagonistic activity of compound **22** and the zinc complexes of **20**, **21**, **22**, and **28**, all of which possess strong CXCR4 binding activity. The CXCR4 antagonistic activity was assessed based on the inhibitory activity of the compounds against Ca^{2+} mobilization induced by CXCL12 stimulation through CXCR4 (figure S1 in the Support-

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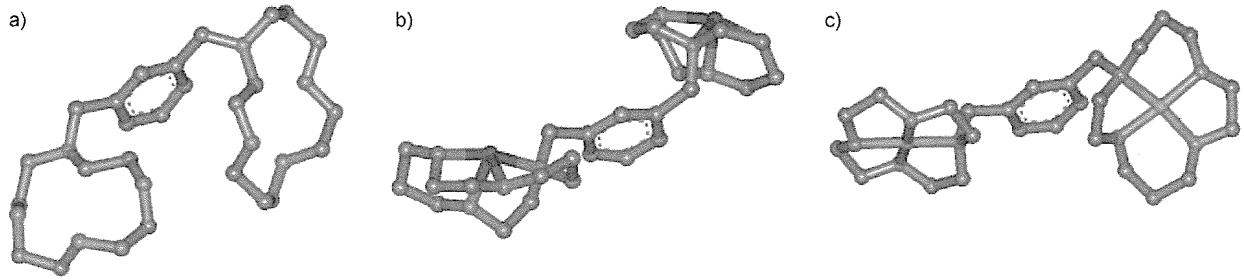

Figure 2. Structures calculated by molecular modeling of a) compound **21**, and its b) zinc and c) copper complexes. Atom color code: nitrogen = blue, carbon = gray, zinc = red, copper = light red.

ing Information). All of the tested compounds showed significant antagonistic activity at 1 μM.

The representative compounds **14**, **16**, **20–23**, **28**, and **29**, as well as their zinc chelates, were evaluated for anti-HIV activity. CXCR4 is the major co-receptor for the entry of T-cell-line-tropic (X4) HIV-1.[10,11] Inhibitory activity against X4-HIV-1 (NL4-3 strain)-induced cytopathogenicity in MT-4 cells was assessed and is shown in Table 2.[38] A correlation between CXCR4 binding activity and anti-HIV activity was observed. For compound **16** and its zinc complex, anti-HIV activity was significantly stronger than CXCR4 binding activity, and for the zinc complexes of compounds **20–22**, the CXCR4 binding activity is two to four-times stronger than the anti-HIV activity. The anti-HIV activity of the zinc complex of **16** was the most potent (EC_{50} = 8.2 nM). This is comparable to the anti-HIV activities of **16** and its zinc complex that were reported previously.[20,22,42,43] The zinc complex of **21**, which was the most active compound in terms of CXCR4 binding activity, also exhibited potent anti-HIV activity (EC_{50} = 36 nM).

Taken together, these results show that all of the compounds exhibiting CXCR4 binding activity also showed significant anti-HIV activity (EC_{50} values < 300 nM), and none of the tested compounds exhibited significant cytotoxicity (CC_{50} values > 10 μM; Table 2). Conversely, zinc complexes of **20**, **21**, **22**, and **28** did not exhibit significant anti-HIV activity against macrophage-tropic (R5) HIV-1 (NL(AD8) strain)-induced cytopathogenicity in PM-1 cells at concentrations below 10 μM. Since R5-HIV-1 strains use CCR5 instead of CXCR4 as the major co-receptor for entry, this suggests that these compounds do not bind CCR5 but rather are highly selective for CXCR4.

Table 2. Anti-HIV activity and cytotoxicity of representative compounds in the metal ion-free and zinc chelates.

Compd	Metal ion-free		Zinc chelate	
	EC_{50}[a] [nM]	CC_{50}[b] [μM]	EC_{50}[a] [nM]	CC_{50}[b] [μM]
14	200	> 10	200	> 10
16	21	> 10	8.2	> 10
20	38	> 10	39	> 10
21	50	> 10	36	> 10
22	93	> 10	48	> 10
23	290	> 10	220	> 10
28	36	> 10	56	> 10
29	130	> 10	42	> 10
FC131	93	> 10		
AZT	69	> 100		

[a] EC_{50} values are the concentrations corresponding to 50% protection from X4-HIV-1 (NL4–3 strain)-induced cytopathogenicity in MT-4 cells. [b] CC_{50} values are the concentrations at which the viability of MT-4 cells is reduced by 50%. All data are mean values from at least three independent experiments.

Conclusions

The present study introduces a new class of low-molecular-weight CXCR4 antagonists and their zinc(II) or copper(II) complexes, which contain pyridyl or azamacrocycle moieties with *p*-xylene or *m*-xylene spacers. These compounds demonstrated strong CXCR4 binding activity. Zinc complexes of **20** and **21**, which were the two most active compounds, contain cyclen and cyclam rings with *p*- and *m*-xylene spacers and exhibited remarkably potent IC_{50} values (11 and 8.3 nM, respectively). These compounds showed significant CXCR4 antagonistic activity, based on inhibitory activity against Ca^{2+} mobilization induced by CXCL12 stimulation through CXCR4, as well as potent anti-HIV activity, as assessed by protection from X4-HIV-1-induced cytopathogenicity in MT-4 cells. These results provide useful insights into the future design of novel CXCR4 antagonists, complementing information from other CXCR4 antagonists such as T140, FC131, and KRH-1636. Furthermore, these new compounds are useful for the development of therapeutic strategies for CXCR4-relevant diseases and chemical probes to study the biological activity of CXCR4.

Experimental Section

Chemistry

Compounds **12–17**, **20**, **21**, **24**, **25**, **27–29**, and **31** were synthesized as previously reported.[20,22,37,40,41,44–47] Compounds **18**, **19**, **22**, **23**, **26**, and **30** were synthesized in the present study; details are provided in the Supporting Information. A representative compound, **18**, was synthesized by coupling *p*-dibromoxylene (1,4-bis-(bromomethyl)benzene) with tri-Boc-protected 1,4,7,10-tetraazacyclododecane, followed by treatment with trifluoroacetic acid and subsequent coupling with bis(pyridin-2-ylmethyl)amine. All crude compounds were purified by RP-HPLC and identified by FAB/ESI-

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HRMS. Zinc(II) or copper(II) complex formation was accomplished by treatment of the above compounds with 10 equiv of $ZnCl_2$ or $CuCl_2$ in PBS. All zinc(II) or copper(II) complexes were characterized by chemical shifts of their methylene protons in ^1H NMR analysis. The pyridyl zinc(II) complex was characterized previously,[37] and zinc(II) or copper(II) complex formation with these macrocyclic compounds has been reported elsewhere.[41,42,48,49] Detailed procedures and data are provided in the Supporting Information.

Biological assays

A CXCR4 binding assay for compounds, based on the inhibition of [^{125}I]CXCL12 binding to Jurkat cells, was performed as reported by Tanaka et al.[38] CXCR4 antagonistic activity was evaluated as described by Ichiyama et al[27], measuring inhibitory activity against Ca^{2+} mobilization induced by CXCL12 stimulation in HOS cells expressing CXCR4. Anti-HIV activity was determined by inhibitory activity against X4-HIV-1(NL4–3)-induced cytopathogenicity in MT-4 cells as reported by Tanaka et al.[38] An X4 HIV-1 infectious molecular clone (pNL4–3) was obtained from the AIDS Research and Reference Reagent Program. The virus NL4–3 was obtained from the culture supernatant of 293T cells transfected with pNL4–3.

Molecular modeling

Molecular modeling calculations were performed using Sybyl (version 7.0, Tripos). Energy minimization was performed using the Tripos force field and Gasteiger–Hückel charge parameters. The lowest energy conformation was obtained by random search methods.

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Keywords: azamacrocycles · Ca^{2+} mobilization · CXCR4 · HIV · structure–activity relationships

[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] C. C. Bleul, R. C. Fuhlbrigge, J. M. Casanovas, A. Aiuti, T. A. Springer, *J. Exp. Med.* **1996**, *184*, 1101–1109.
[6] 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.
[7] T. Nagasawa, S. Hirota, K. Tachibana, N. Takakura, S. Nishikawa, Y. Kitamura, N. Yoshida, H. Kikutani, T. Kishimoto, *Nature* **1996**, *382*, 635–638.
[8] Y. Zhu, Y. Yu, X. C. Zhang, T. Nagasawa, J. Y. Wu, Y. Rao, *Nat. Neurosci.* **2002**, *5*, 719–720.
[9] R. K. Stumm, C. Zhou, T. Ara, F. Lazarini, M. Dubois-Dalcq, T. Nagasawa, V. Hollt, S. Schulz, *J. Neurosci.* **2003**, *23*, 5123–5130.
[10] 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.
[11] Y. Feng, C. C. Broder, P. E. Kennedy, E. A. Berger, *Science* **1996**, *272*, 872–877.
[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] 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.
[18] H. Tamamura, M. Fujisawa, K. Hiramatsu, M. Mizumoto, H. Nakashima, N. Yamamoto, A. Otaka, N. Fujii, *FEBS Lett.* **2004**, *569*, 99–104.
[19] 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.
[20] D. Schols, S. Struyf, J. Van Damme, J. A. Este, G. Henson, E. DeClarcq, *J. Exp. Med.* **1997**, *186*, 1383–1388.
[21] B. J. Doranz, K. Grovit-Ferbas, M. P. Sharron, S.-H. Mao, M. Bidwell Goetz, E. S. Daar, R. W. Doms, W. A. O'Brien, *J. Exp. Med.* **1997**, *186*, 1395–1400.
[22] G. A. Donzella, D. Schols, S. W. Lin, J. A. Este, K. A. Nagashima, *Nat. Med.* **1998**, *4*, 72–76.
[23] O. M. Z. Howard, J. J. Oppenheim, M. G. Hollingshead, J. M. Covey, J. Bigelow, J. J. McCormack, R. W. Buckheit, Jr., D. J. Clanton, J. A. Turpin, W. G. Rice, *J. Med. Chem.* **1998**, *41*, 2184–2193.
[24] 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.
[25] 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.
[26] 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.
[27] 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.
[28] H. Tamamura, N. Fujii, *Curr. Drug Targets-Infectious Disorders* **2004**, *4*, 103–110.
[29] 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.
[30] 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.
[31] G. C. Valks, G. McRobbie, E. A. Lewis, T. J. Hubin, T. M. Hunter, P. J. Sadler, C. Pannecouque, E. De Clercq, S. J. Archibald, *J. Med. Chem.* **2006**, *49*, 6162–6165.
[32] W. Zhan, Z. Liang, A. Zhu, S. Kurtkaya, H. Shim, J. P. Snyder, D. C. Liotta, *J. Med. Chem.* **2007**, *50*, 5655–5664.
[33] A. Khan, G. Nicholson, J. Greenman, L. Madden, G. McRobbie, C. Pannecouque, E. De Clercq, R. Ullom, D. L. Maples, R. D. Maples, J. D. Silversides, T. J. Hubin, S. J. Archibald, *J. Am. Chem. Soc.* **2009**, *131*, 3416–3417.
[34] G. J. Bridger, R. T. Skerlj, P. E. Hernandez-Abad, D. E. Bogucki, Z. Wang, Y. Zhou, S. Nan, E. M. Boehringer, T. Wilson, J. Crawford, M. Metz, S. Hatse, K. Princen, E. De Clercq, D. Schols, *J. Med. Chem.* **2010**, *53*, 1250–1260.

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[35] R. T. Skerlj, G. J. Bridger, A. Kaller, E. J. McEachern, J. B. Crawford, Y. Zhou, B. Atsma, J. Langille, S. Nan, D. Veale, T. Wilson, C. Harwig, S. Hatse, K. Princen, E. De Clercq, D. Schols, *J. Med. Chem.* **2010**, *53*, 3376–3388.
[36] M. Takenaga, H. Tamamura, K. Hiramatsu, N. Nakamura, Y. Yamaguchi, A. Kitagawa, S. Kawai, H. Nakashima, N. Fujii, R. Igarashi, *Biochem. Biophys. Res. Commun.* **2004**, *320*, 226–232.
[37] 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.
[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] G. J. Bridger, R. T. Skerlj, D. Thornton, S. Padmanabhan, S. A. Martellucci, G. W. Henson, M. J. Abrams, N. Yamamoto, K. De Vreese, R. Pauwels, E. De Clercq, *J. Med. Chem.* **1995**, *38*, 366–378.
[40] G. J. Bridger, R. T. Skerlj, S. Padmanabhan, S. A. Martellucci, G. W. Henson, M. J. Abrams, H. C. Joao, M. Witvrouw, K. De Vreese, R. Pauwels, E. De Clercq, *J. Med. Chem.* **1996**, *39*, 109–119.
[41] Y. Inouye, T. Kanamori, T. Yoshida, T. Koike, M. Shionoya, H. Fujioka, E. Kimura, *Biol. Pharm. Bull.* **1996**, *19*, 456–458.
[42] L. O. 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.
[43] H. F. Egberink, E. De Clercq, A. L. Van Vliet, J. Balzarini, G. J. Bridger, G. Henson, M. C. Horzinek, D. Schols, *J. Virol.* **1999**, *73*, 6346–6352.
[44] M. Le Baccon, F. Chuburu, L. Toupet, H. Handel, M. Soibinet, I. Dechamps-Olivier, J.-P. Barbier, M. Aplincourt, *New J. Chem.* **2001**, *25*, 1168–1174.
[45] B. Antonioli, D. J. Bray, J. K. Clegg, K. Gloe, K. Gloe, O. Kataeva, L. F. Lindoy, J. C. McMurtrie, P. J. Steel, C. J. Sumby, M. Wenzel, *Dalton Trans.* **2006**, 4783–4794.
[46] S. P. Foxon, D. Utz, J. Astner, S. Schindler, F. Thaler, F. W. Heinemann, G. Liehr, J. Mukherjee, V. Balamurugan, D. Ghosh, R. Mukherjee, *Dalton Trans.* **2004**, 2321–2328.
[47] S. Mandal, F. Lloret, R. Mukherjee, *Inorg. Chim. Acta* **2009**, *362*, 27–37.
[48] M. Soibinet, I. De'champs-Olivier, E. Guillon, J.-P. Barbier, M. Aplincourt, F. Chuburu, M. Le Baccon, H. Handel, *Eur. J. Inorg. Chem.* **2003**, 1984–1994.
[49] R. W. Hay, M. T. Tarafder, *Transition Met. Chem.* **1990**, *15*, 490–492.

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Expert Opinion

The successes and failures of HIV drug discovery

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Introduction: To date, several anti-human immunodeficiency virus (HIV) drugs, including reverse transcriptase inhibitors and protease inhibitors, have been developed and used clinically for the treatment of patients infected with HIV. Recently, novel drugs have been discovered which have different mechanisms of action from those of the above inhibitors, including entry inhibitors and integrase (IN) inhibitors; the clinical use of three of these inhibitors has been approved. Other inhibitors are still in development.

Areas covered: This review article summarizes the history of the development of anti-HIV drugs and also focuses on successes in the development of these entry and IN inhibitors, along with looking at exploratory approaches for the development of other inhibitors.

Expert opinion: Currently used highly active antiretroviral therapy can be subject to a loss of efficacy, due to the emergence of multi-drug resistant (MDR) strains; a change of regimens of the drug combination is required to combat this, along with careful monitoring of the virus and CD4 in the blood, by methods such as cellular tropism testing. In such a situation, entry inhibitors such as CCR5/CXCR4 antagonists, CD4 mimics, fusion inhibitors and IN inhibitors might be optional agents for an expansion of the drug repertoire available to patients at all stages of HIV infection.

Keywords: AIDS, CCR5, CD4 mimic, chemokine receptor, CXCR4, fusion, HIV, integrase

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1. Introduction

The human immunodeficiency virus (HIV) is the cause of acquired immunodeficiency syndrome (AIDS), which was discovered by Montagnier and colleagues in 1983 [1]. HIV infects human host cells and destroys immune systems, subsequently causing immunodeficiency. To date, the number of people worldwide infected with HIV is certainly in excess of 30 million.

Several anti-HIV drugs have been reported in the last 25 years (**Figure 1A**). HIV is a retrovirus, which is an RNA virus that is replicated in a host cell via the enzyme reverse transcriptase to produce DNA from its RNA genome. This DNA is then incorporated into the host genome by an integrase (IN) enzyme. These inhibitors block the action of reverse transcriptase, and include nucleoside reverse transcriptase inhibitors (NRTIs) such as azidothymidine (AZT) and non-nucleoside reverse-transcriptase inhibitors (NNRTIs). The class of anti-HIV drugs that were initially approved for clinical use is reverse transcriptase inhibitors such as AZT [2]. The class of drugs that were subsequently approved is protease inhibitors, which prevent the cleavage of HIV precursor proteins into active proteins, a process that normally occurs in viral replication. This family of drugs includes Saquinavir (Invirase/Fortovase, Roche, Basel, Switzerland) and Ritonavir (Norvir, Abbott Laboratories, IL, USA), which have been used clinically in HIV/AIDS treatment. These drugs are usually administered as part of a two- or three-drug cocktail, accompanied by one or more

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Article highlights.

- The highly active anti-retroviral therapy (HAART) involving use of reverse transcriptase inhibitors and protease inhibitors has led to great success in clinical treatment of human immunodeficiency virus (HIV)-infected patients.
- Brand-new drugs with different action mechanisms have been discovered to date.
- Enfuvirtide, a fusion inhibitor, Maraviroc, a co-receptor CCR5 antagonist and Raltegravir, an integrase (IN) inhibitor have successively been approved for clinical use.
- The potential of new inhibitors from novel drug categories such as entry inhibitors including CCR5/CXCR4 antagonists and CD4 mimics, fusion inhibitors and IN inhibitors might be critical.
- Optional agents are valuable for an expansion of the drug repertoire available to patients because in case of loss of efficacy of HAART, change of regimens of the drug combination in HAART is required.

This box summarizes key points contained in the article.

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NRTIs. Such cocktail therapies are known as highly active anti-retroviral therapy (HAART), which has brought great success and hope in the clinical treatment of HIV-infected patients [2]. HAART is capable of lowering the HIV level in the blood until it cannot be detected with current methods. Side effects associated with protease inhibitors include a lipodystrophy syndrome in which abnormal distribution of fat occurs and the face, arms and legs become thin but the therapy involves more serious clinical problems such as the emergence of multi-drug resistant (MDR) HIV-1 strains, and considerable expense. These drawbacks encouraged us to develop brand-new drugs with novel mechanisms of action.

Recently, the molecular mechanism of HIV-1 replication has been elucidated in detail. A dynamic supramolecular mechanism is associated with HIV entry/fusion steps. First, an HIV envelope protein gp120 interacts with a host-cell surface protein CD4, which causes gp120 to undergo a conformational change subsequently binding to the second cellular receptors, a chemokine receptor such as CCR5 [3-7] and CXCR4 [8]. The binding triggers the exposure of another envelope protein gp41 whose N-terminus penetrates the cell membrane. This is followed by the formation of the gp41 trimer-of-hairpins structure, which leads to fusion of HIV/cell membranes, completing the infection process [9]. The description of this dynamic molecular machinery has encouraged researchers to develop inhibitors which block the HIV entry/fusion steps targeting the receptors, CD4, CCR5 and CXCR4, and the viral protein gp41. In 2003, the Food and Drug Administration (FDA) approved Enfuvirtide (Fuzeon/T-20, Roche, Basel, Switzerland/Trimeris, Durham, NC, USA) as the first 'fusion inhibitor' for use in combination with other anti-HIV drugs to treat advanced HIV-1 infection [10]. In 2007, the FDA approved a CCR5 co-receptor antagonist, Maraviroc (Pfizer, New York, NY, USA), for use in combination with other anti-HIV drugs for the treatment of patients infected with CCR5-tropic HIV-1 [11]. In the same year, the FDA approved Raltegravir (Merck Sharp & Dohme Corp., NJ, USA) for use in combination with other antiretroviral agents in treatment-experienced adult patients who present with evidence of viral replication and HIV-1 strains resistant to multiple HAART agents [12,13]. Subsequently in 2009, the FDA granted expanded approval of Raltegravir for use in all patients. Numerous reviews exist concerning reverse transcriptase and protease inhibitors, and this review will focus on the success of a fusion inhibitor, Enfuvirtide, a CCR5 antagonist, Maraviroc and an IN inhibitor, Raltegravir, as well as the development of other anti-HIV agents including CXCR4 antagonists and CD4 mimics.

2. HIV fusion inhibitors such as Enfuvirtide

The binding of gp120 to CCR5 or CXCR4 triggers the formation of the trimer-of-hairpins structure of gp41 and subsequent fusion of the HIV/cell membranes, as described above. The trimer-of-hairpins structure is a six-helical bundle consisting of a central parallel trimer of the N-terminal helical region (HR1 region) surrounded by the C-terminal helical region (HR2 region) oriented in an antiparallel, hairpin fashion (**Figure 1B**) [9]. A subdomain is composed of two peptides, a 51-mer from the HR1 region and a 43-mer from the HR2 region, designated as N51 and C43, respectively [14]. There have been numerous reports that several HR2 region peptides inhibit bundle formation of six alpha-helices by the binding to the inner three-stranded coiled coils of the HR1 region thereby inhibiting membrane fusion (**Figure 2A**) [15]. An HR2 region peptide, C34, with 34 residues from the native sequence of gp41, has potent inhibitory activity against HIV-1 fusion [16]. In addition, a 36-residue peptide, T-20, which has the native sequence of gp41 and 24 residues in common with C34, shows potent anti-HIV activity, and its clinical use as the first entry/fusion inhibitor [10] in HIV/AIDS treatment was approved by the FDA in 2003 designated as Enfuvirtide. Enfuvirtide, in combination with other antiretroviral agents was approved for the treatment of advanced HIV-1 infection in adults and children aged 6 years or older with evidence of HIV-1 replication notwithstanding current therapy, and of resistance to current anti-HIV drugs. In view of the clinical use of Enfuvirtide, the dynamic supramolecular mechanism involving membrane fusion is a valid and rational target for inhibitors of HIV-1 replication. The success of Enfuvirtide has encouraged the development of entry/fusion inhibitors as a new class of anti-HIV drugs distinct from the first- and second-generation drugs. While reverse transcriptase inhibitors and protease inhibitors work internally in T cells to inhibit functions of viral enzymes, entry and fusion inhibitors work extracellularly, preventing HIV from invading cells. C34 has an exact interface that is capable of interacting with the inner three-stranded coiled coils of the gp41 HR1 region, compared with Enfuvirtide, which has a poorly delineated interface. However, a disadvantage

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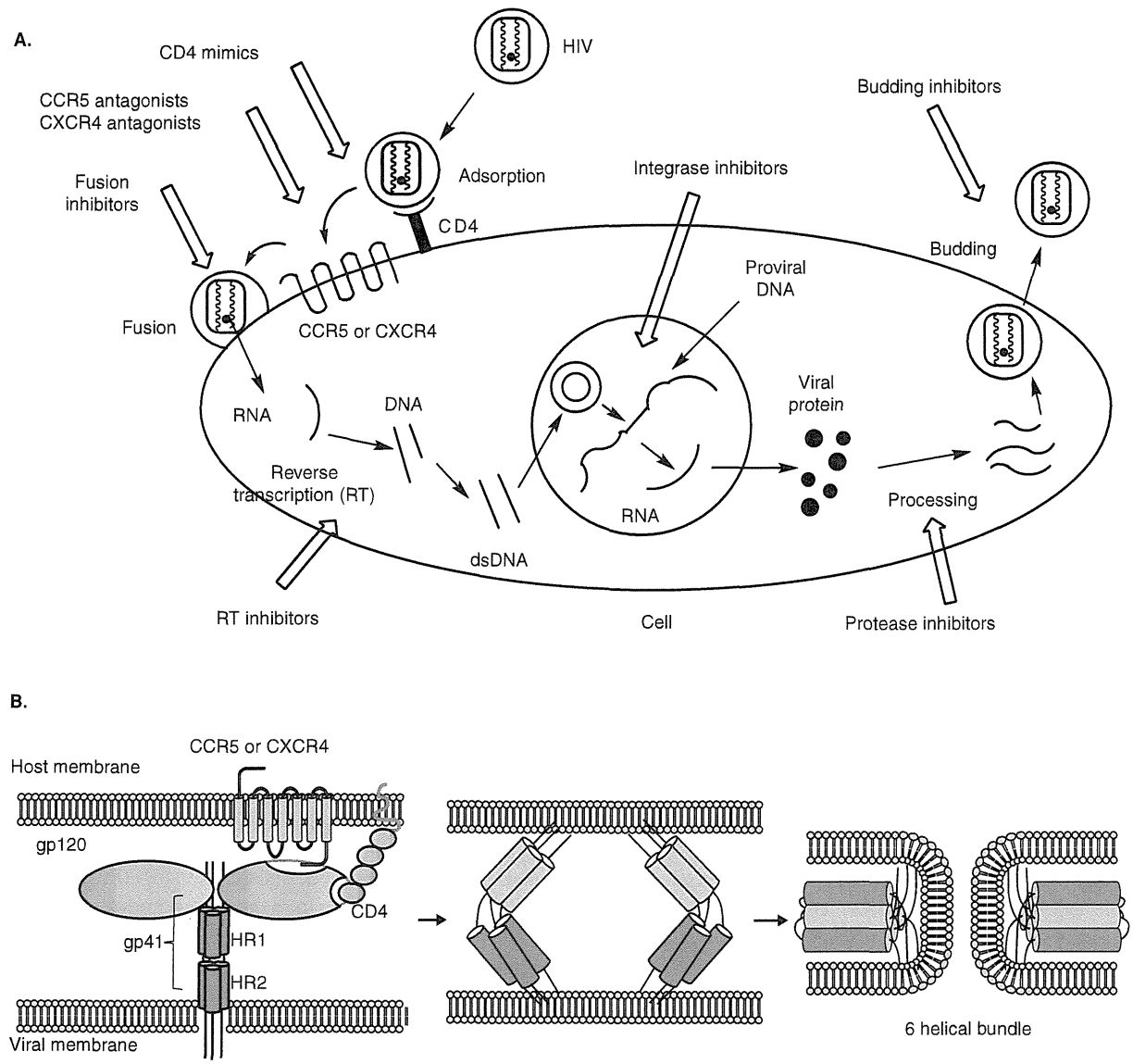

Figure 1. A. HIV-1 replication cycle and anti-HIV drugs that are effective at its various steps. **B.** Mechanisms of HIV-1 entry and fusion.

of C34 is its poor aqueous solubility and highly soluble C34 analogs, known as subcutaneous glucogon-like peptides (SC) peptides, were developed by artificial remodeling of C34 by Otaka *et al.* (**Figure 2**) [17]. In the design of SC peptides, the amino acid residues at the *a*, *d* and *e* positions of the helical wheel diagram of C34, which are essential for interaction with the inner coiled coils of HR1, were maintained with no substitution, while non-conserved residues at the *b*, *c*, *f* and *g* positions, which are located in the exterior region, were replaced by Glu or Lys. Several Glu-Lys side-chain ion charge pairs formed between i and i + 4 positions enhance solubility and alpha-helicity of the C34 analogs. The aqueous solubility of the SC peptides, SC34 and SC34EK, is more than three orders of magnitude greater than that of the original C34 peptide. Anti-HIV activities of these SC peptides were superior or comparable with that of the original C34 peptide, and an order of magnitude greater than that of Enfuvirtide [18]. Furthermore, SC peptides were even effective against an Enfuvirtide-resistant strain. The C34 and SC peptide sequences lack the C-terminal lipid binding domain of Enfuvirtide and it has been suggested that SC peptides have a mechanism of action distinct from that of Enfuvirtide [19]. Thus, these SC peptides are leads for further refinement and clinical development. C34, T-649 [20], Enfuvirtide and SC peptides are all 34- to 36-mer peptides derived from the HR2 region of

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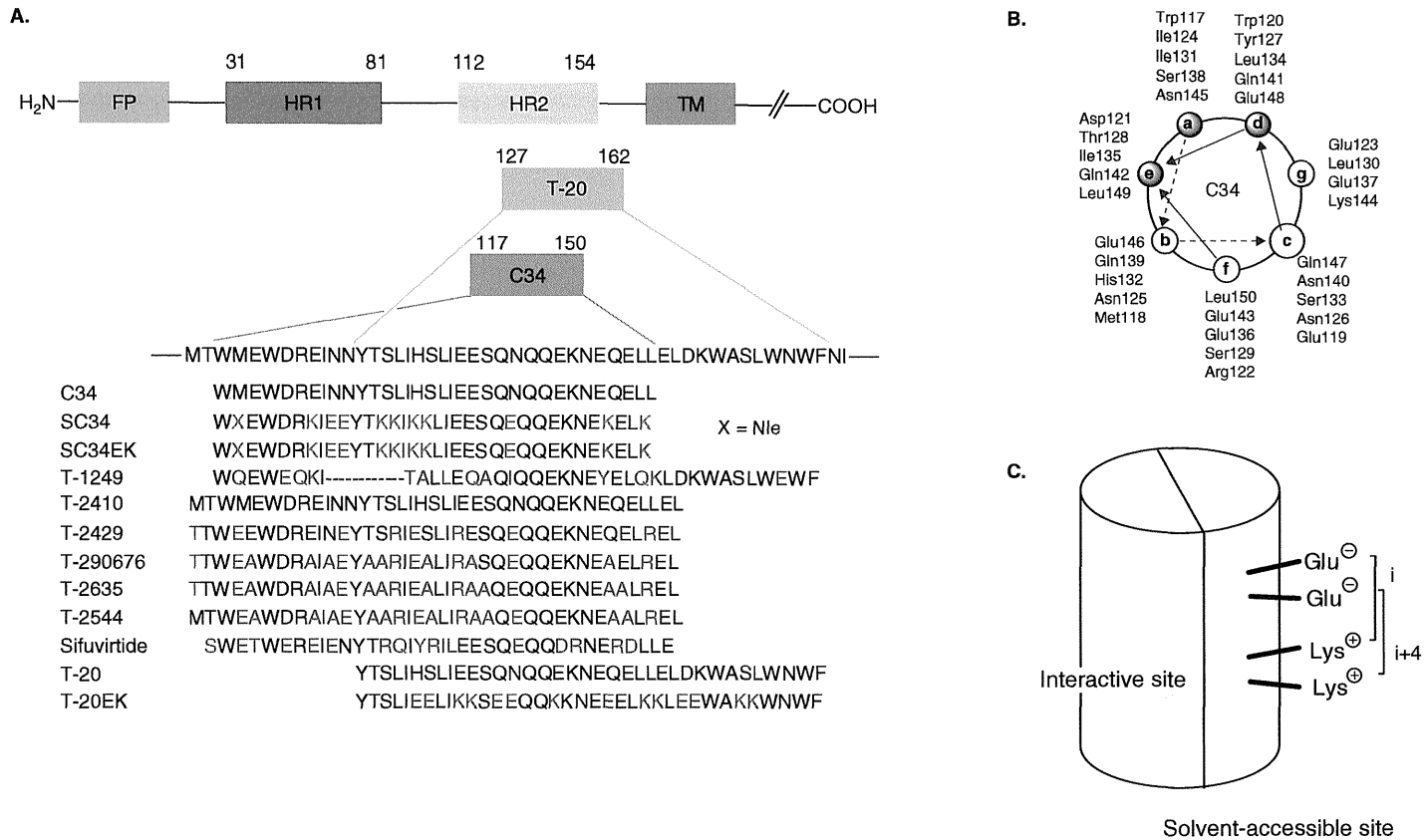

Figure 2. A. Schematic representation of gp41 and sequences of HR2 region peptides. **B.** Helical wheel representation of the C34 peptide. Amino acid residues are numbered according to gp41 of NL4-3 strain. **C.** The design concept of introduction of the Glu-Lys motif to the solvent-accessible site. **D.** Remodeling of dynamic structures of HR1 regions leads to synthetic antigen molecules inducing neutralizing antibodies.

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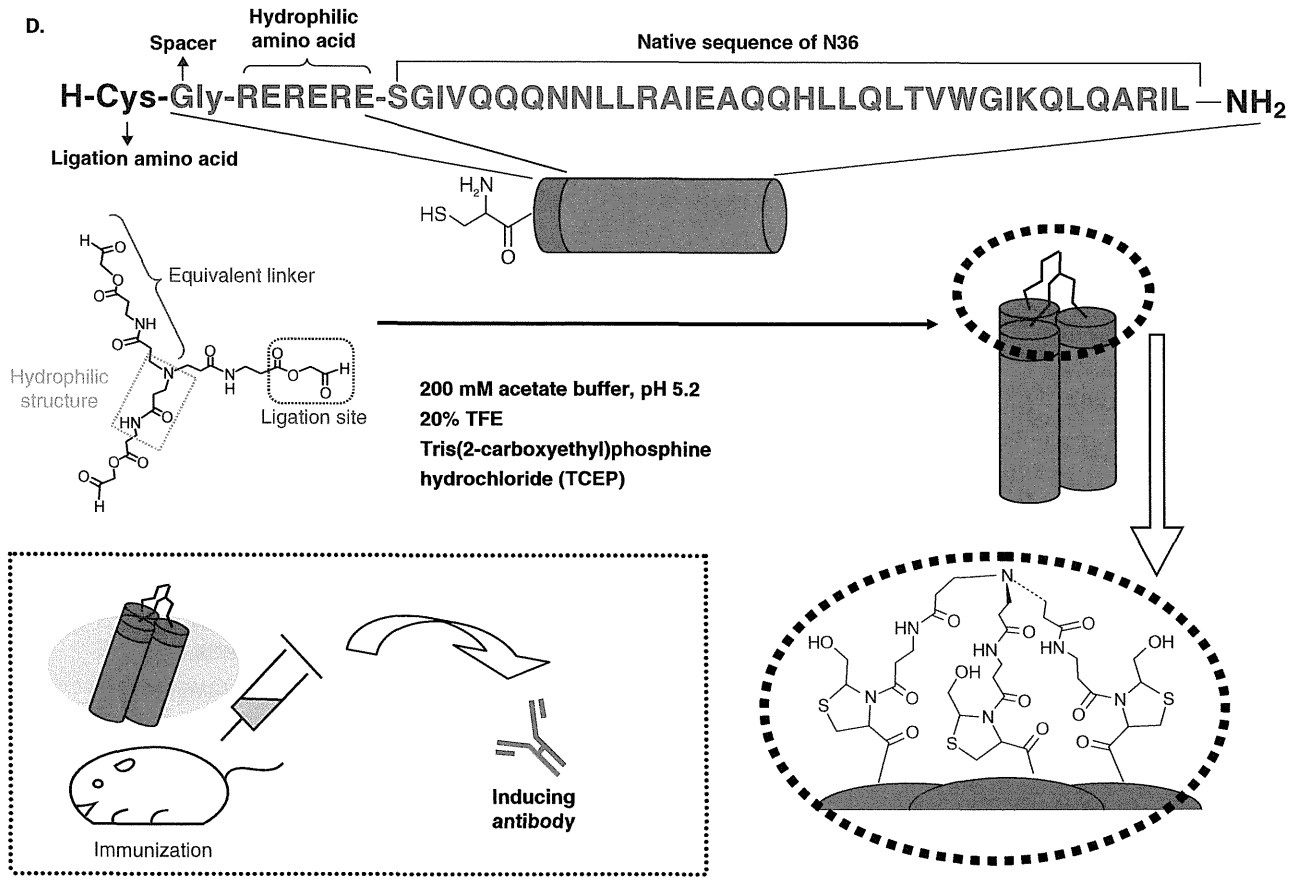

Figure 2. (continued). A. Schematic representation of gp41 and sequences of HR2 region peptides. **B.** Helical wheel representation of the C34 peptide. Amino acid residues are numbered according to gp41 of NL4-3 strain. **C.** The design concept of introduction of the Glu-Lys motif to the solvent-accessible site. **D.** Remodeling of dynamic structures of HR1 regions leads to synthetic antigen molecules inducing neutralizing antibodies.

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gp41. T-1249 (Roche, Basel, Switzerland/Trimeris, Durham, NC, USA), a successor to Enfuvirtide [21], was much anticipated as its hydrophobic C-terminal sequence inhibits HIV-1 fusion by interacting with lipid bilayers in a manner similar to the interaction enjoyed by Enfuvirtide, but its clinical trial was discontinued in January 2005 because of formulation problems. In addition, attempts have been made to develop small non-peptide inhibitors that block gp41 activation [22-24]. To date, development of highly potent and useful low molecular weight inhibitors has been difficult, although non-natural binding elements that contribute to the formation of a stable complex with the inner coiled coils have been identified using a biased combinatorial chemistry library [25]. The development of small organic compounds as useful fusion inhibitors is continuing.

The HR1 region is critical for the development of AIDS vaccine antigens because the six-helical bundle structure is formed by interactive approach to the central trimer of the HR1 regions by the HR2 regions of three strands coiled coils. In the design of artificial antigens that induce broadly neutralizing antibodies, a useful strategy is to synthesize molecules that mimic the natural trimer on the virion surface. Using an original template with C3-symmetric linkers, we designed and synthesized a novel three-helical bundle mimetic corresponding to the trimeric form of N36 [26] (**Figure 2D**). The antiserum produced by immunization of the N36 trimeric form antigen in mice showed structural preference for binding to the N36 trimer and more potent neutralizing activity against HIV-1 infection than the N36 monomer. The exposed timing of epitopes was limited during HIV-1 entry, and carbohydrates, which could disturb accession of antibodies to epitopes, were not included in the amino acid sequences of the native protein [27]. These two advantages in the design based on the HR1 region of gp41 could further enhance the potential of a vaccine design based on the HR1 region.

3. HIV co-receptor inhibitors such as Maraviroc

Interaction of CD4 with the HIV envelope protein gp120 causes a conformational change in the latter and its subsequent binding to the second cellular receptors, CCR5 [3-7] and CXCR4 [8] as described above, in Section 1. Macrophage-tropic (R5) HIV-1 strains, which constitute a majority in the early stage of HIV infection, use CCR5 as a co-receptor, whereas T cell line-tropic (X4) HIV-1 strains, which are the major species in the late stage of HIV infection and AIDS, use CXCR4 as a co-receptor. CCR5 and CXCR4 play physiological roles as the receptors for endogenous ligands, or chemokines. Several chemokine antagonists of CCR5 and CXCR4 have been developed as entry inhibitors. The validity of development of CCR5 antagonists is supported by the finding that individuals with the CCR5-Δ32 deletion mutation are healthy and strongly resistant to HIV-1 infection [28]. To date, several pharmaceutical companies have investigated novel CCR5 antagonists with suitable pharmaceutical properties. One CCR5-selective antagonist, Maraviroc (**1**) is the first CCR5 antagonist to be approved by the FDA as described above (**Figure 3A**) [11] and is used for the treatment of patients infected with CCR5-tropic HIV-1. Several other CCR5 antagonists are currently in clinical trials. Maraviroc has relatively high oral bioavailability (23%) with food effects in humans [29,30]. During the process of further improvement of Maraviroc's pharmacological profile, compound **2** was found to have potent fusion inhibitory activity (IC_{50} < 0.1 nM) and a pharmacological profile identical to that of Maraviroc [31]. Compound **2** showed high anti-HIV activity even against a Maraviroc-resistant mutant. Thus, compound **2** might be a desirable second-generation CCR5 antagonist. Takeda Pharmaceutical Co. Ltd., Osaka, Japan developed TAK-779 (**3**), a CCR5 antagonist with minimal bioavailability for i.v. use [32,33] but clinical development was discontinued because of local reactions at s.c. injection sites. High throughput screening has been used to develop an orally bioavailable CCR5 antagonist, and has led to the discovery of a novel lead compound **4** with a scaffold structure different from that of TAK-779 [34]. Subsequent optimization resulted in the development of TAK-220, a new CCR5 antagonist with a piperidine-4-carboxamide structure (**5**) (Takeda Pharmaceutical Co. Ltd., Osaka, Japan/Tobira Therapeutics, Inc.), which has high CCR5 binding activity and resistance to metabolic modification [35]. Merck/Schering-Plough Corp., which became Merck & Co., NJ, USA in a merger, reported a piperidinopiperazine series such as Vicriviroc (SCH-D/SCH417690) (**6**) [36]. In its Phase III studies, the safety and efficacy of Vicriviroc in the addition to optimized or HAART regimens was assessed based on established criteria. Vicriviroc, however, failed to show that its addition to the current regimens for the further treatment of HIV-1 infectious patients with evidence of HIV-1 replication is more effective than background regimens. As a result, Merck & Co. suspended its support of the FDA approval for Vicriviroc in treatment-experienced subjects [37,38]. A CCR5 antagonist with a spirodiketopiperazine scaffold, ONO-4128/873140 (**7**) (GSK, Middlesex, UK/Ono Pharmaceutical Co. Ltd., Osaka, Japan), has been developed by combinatorial chemistry utilizing solid-phase techniques [39]. ONO-4128/873140 has CCR5 binding activity in the nanomolar range and potent Ca^{2+} mobilization inhibitory activity. The spirodiketopiperazine is an attractive scaffold because it is likely to lead to more diverse derivatives. ONO-4128/873140 was advanced into the Phase II studies, but not into Phase III studies on account of its hepatotoxicity.

The other co-receptor for HIV-1 entry is CXCR4 [8], which is also relevant to mediation of the metastasis of a variety of cancer cells [40-42], leukemia [43,44] and rheumatoid arthritis [45,46]. Thus, CXCR4 is an important target for drug discovery, because CXCR4 antagonists might overcome these diseases. To date, several CXCR4 antagonists, peptidic and non-peptidic have been developed.

A 14-mer peptide T140 (**8**) derived from polyphemusin II, has been reported to be a potent CXCR4 antagonist (**Figure 3B**) [47] by us (Kyoto University, Kyoto, Japan). Biostable T140 analogs [48,49] (Biokine Therapeutics Ltd.,

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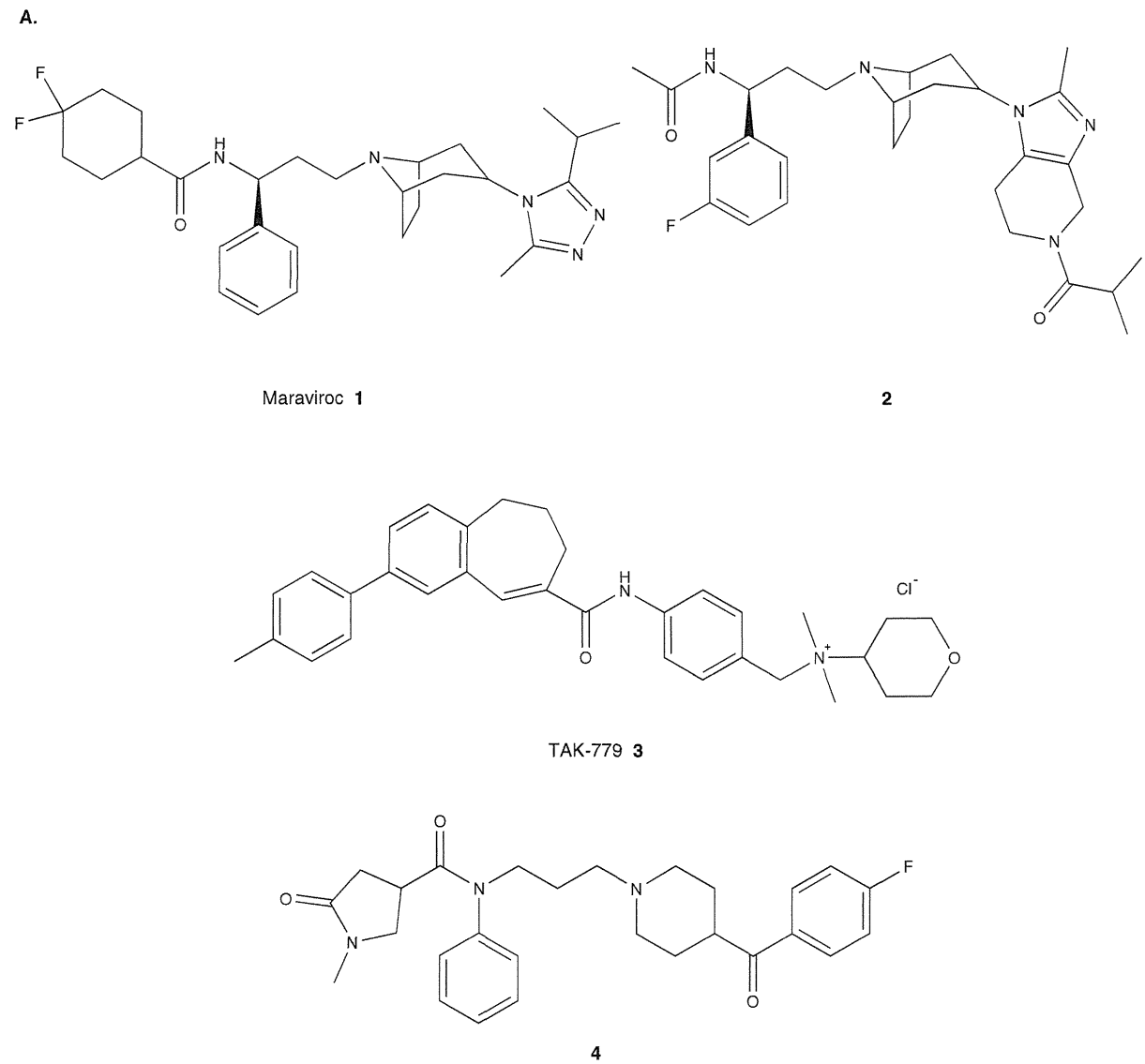

Figure 3. **A.** Structures of CCR5 antagonists. **B.** Structures of CXCR4 antagonists. **C.** Development of non-peptidic CXCR4 antagonists. **D.** Structures of bivalent CXCR4 ligands. A maximum increase in binding affinity for CXCR4 was observed in (**25**), n = 20 and (**26**), m = 12.

Rehovot, Israel) have significant inhibitory activity not only against HIV infection *in vitro* but also against tumor metastasis *in vivo* [41,42]. FC131 (**9**) was developed by downsizing of **8** [50].

AMD3100 (**10**) (Genzyme Corp., Cambridge, MA, USA), a non-peptidic bicyclam-containing small molecular CXCR4 antagonist, was the first CXCR4 antagonist to enter clinical trials for the treatment of HIV-1-infected patients (**Figure 3B**) [51,52]. It was found, however, to have adverse cardiovascular effects and its use as an anti-AIDS drug was discontinued. AMD3100 has two cyclam moieties tethered by *p*-xylene templates. Teixidó and colleagues have constructed combinatorial libraries based on the structure of AMD3100 and containing: 1) at least two nitrogen atoms on each side of the *p*-xylene template; and 2) separation between these nitrogen atoms similar to that in cyclam [53]. As a result, the non-cyclam compound **11** with potent anti-HIV activity was found. Liotta and colleagues have screened various compounds in which two basic centers (e.g., guanidine, hydrazone or pyridine groups) were connected by a phenyl-containing bridge. A bisymmetric pyridine-containing compound **12** was found to have 10-nM potency [54] but further preclinical studies showed that compound **12** failed to exhibit any *in vivo*

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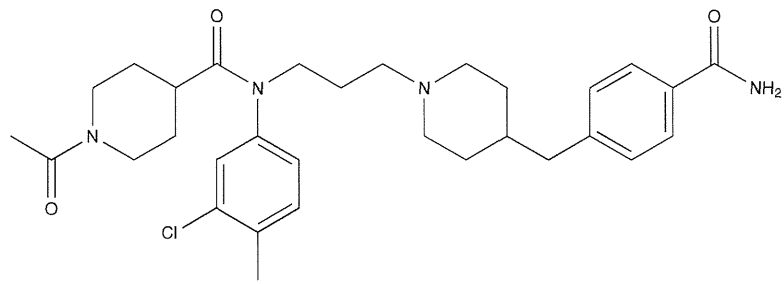
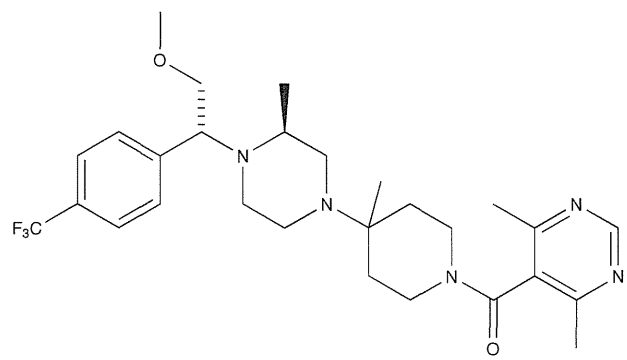
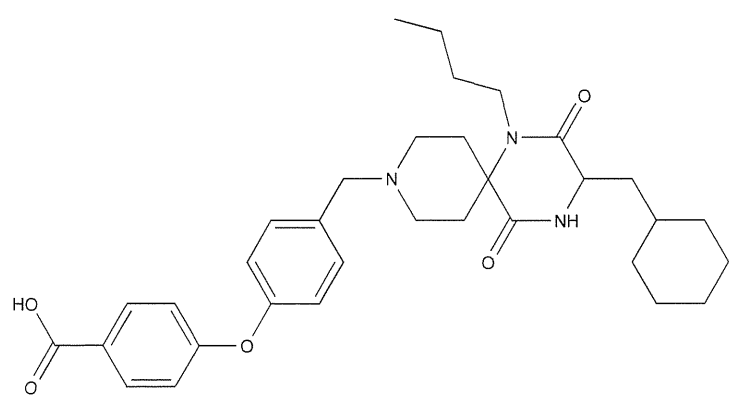


TAK-220 **5**



Vicriviroc/SCH-D (SCH417690) **6**



ONO-4128/873140 **7**

Figure 3. (continued). **A.** Structures of CCR5 antagonists. **B.** Structures of CXCR4 antagonists. **C.** Development of non-peptidic CXCR4 antagonists. **D.** Structures of bivalent CXCR4 ligands. A maximum increase in binding affinity for CXCR4 was observed in (25), n = 20 and (26), m = 12.

efficacy due to poor biostability. On the hypothesis that a poor pharmacokinetic profile of compound 12 might be a result of rapid oxidative metabolism, further structure–activity relationship studies of compound 12 were conducted and led to the development of compound 13 with a nanomolar level of CXCR4 antagonistic activity [55]. To develop non-cyclam CXCR4 antagonists, De Clercq and colleagues have carried out structure–activity relationship studies on AMD3100 (10) [56,57]. First, according to the hypothesis that both rings of 10 are not essential structural requirements, they synthesized and evaluated single cyclam analogs containing an aromatic ring instead of another cyclam ring. The

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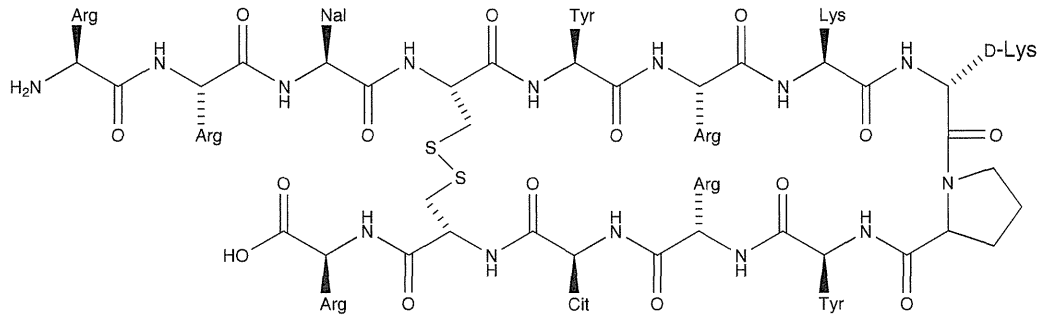
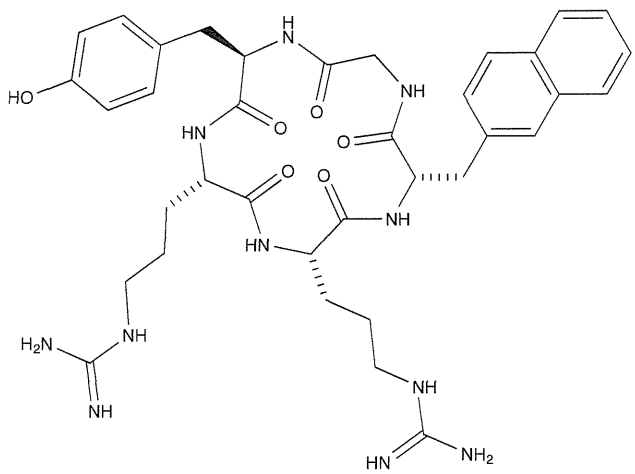
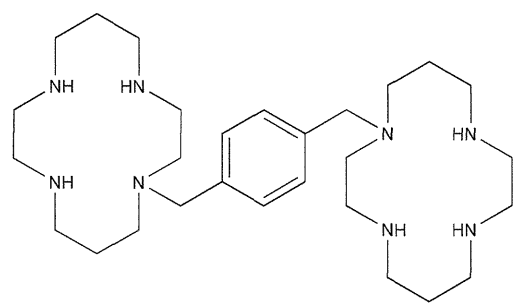


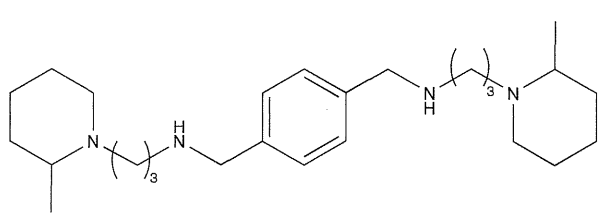
T140 **8**



FC131 **9**



AMD3100 **10**



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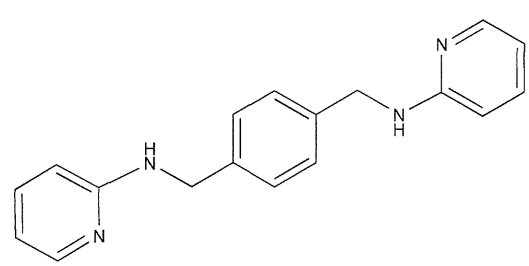

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Figure 3. (continued). A. Structures of CCR5 antagonists. **B.** Structures of CXCR4 antagonists. **C.** Development of non-peptidic CXCR4 antagonists. **D.** Structures of bivalent CXCR4 ligands. A maximum increase in binding affinity for CXCR4 was observed in (25), n = 20 and (26), m = 12.

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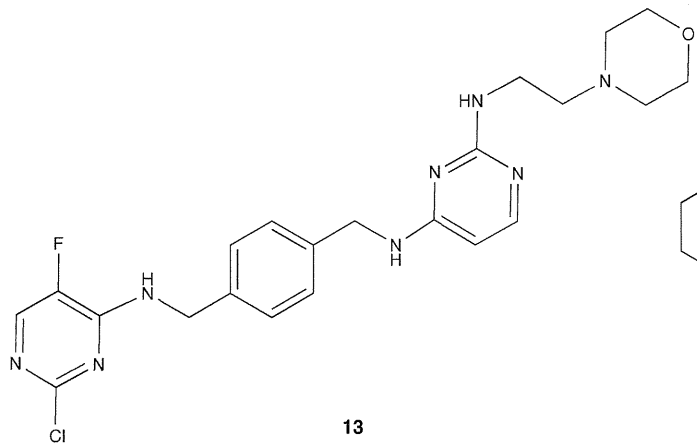

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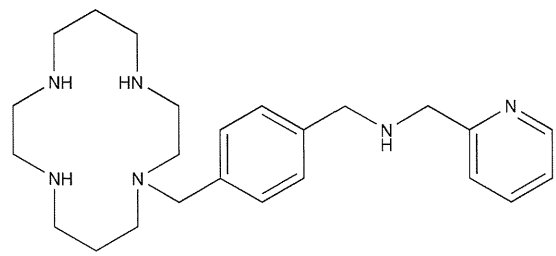
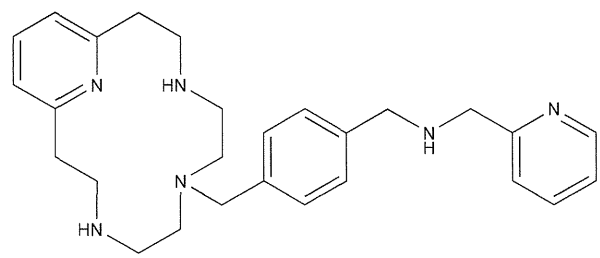


AMD3465 **14**



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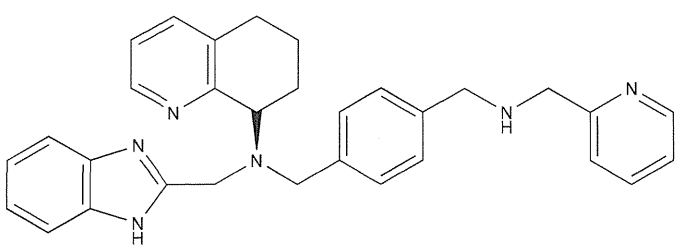

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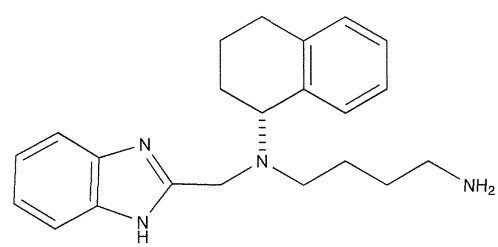
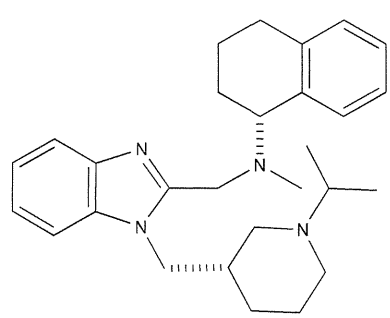


AMD070 **17**



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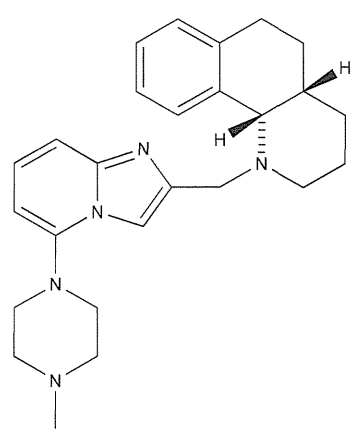

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Figure 3. (continued). **A.** Structures of CCR5 antagonists. **B.** Structures of CXCR4 antagonists. **C.** Development of non-peptidic CXCR4 antagonists. **D.** Structures of bivalent CXCR4 ligands. A maximum increase in binding affinity for CXCR4 was observed in (25), n = 20 and (26), m = 12.

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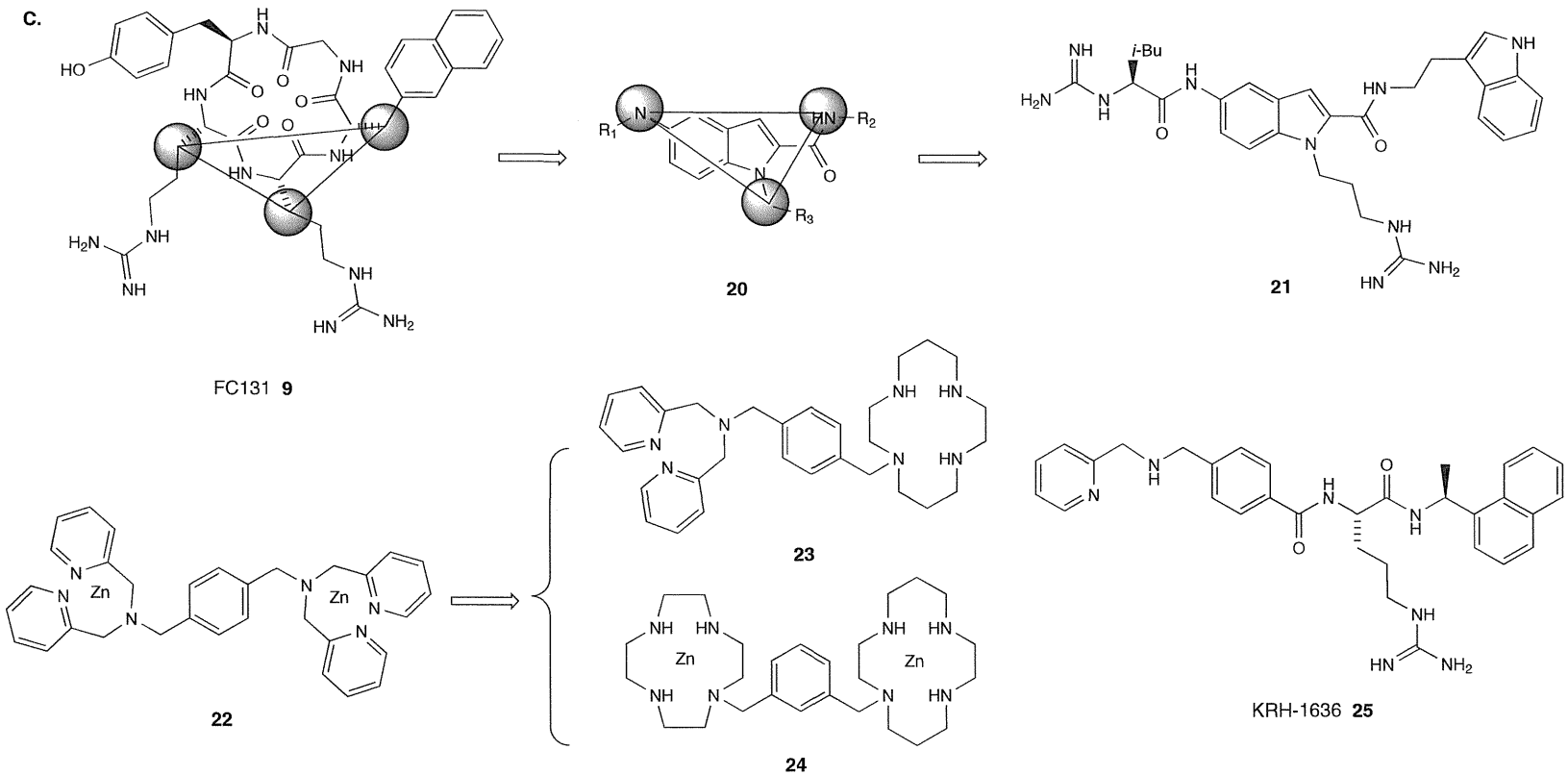

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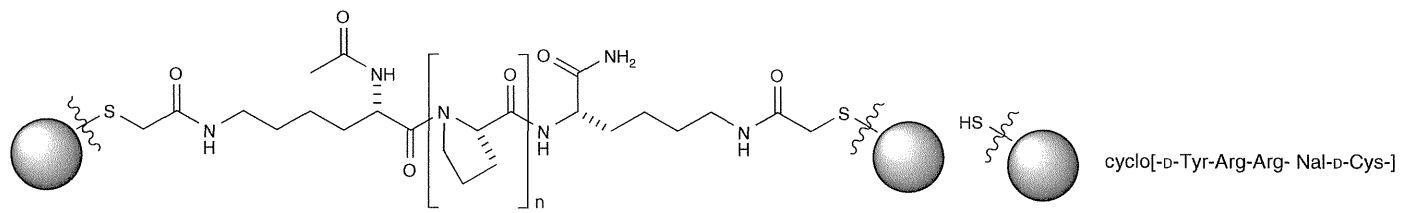

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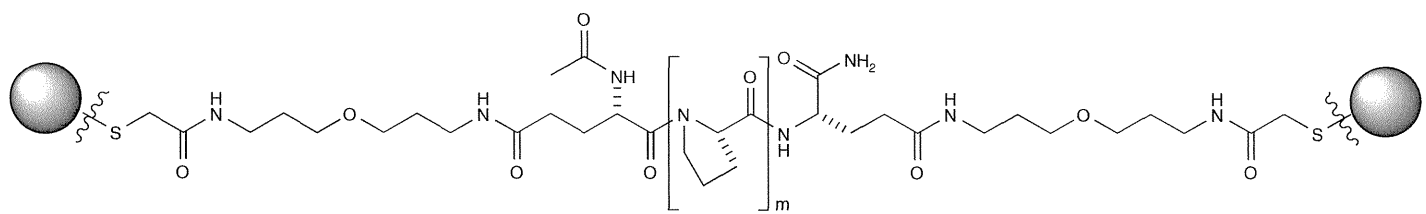

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Figure 3. (continued). **A.** Structures of CCR5 antagonists. **B.** Structures of CXCR4 antagonists. **C.** Development of non-peptidic CXCR4 antagonists. **D.** Structures of bivalent CXCR4 ligands. A maximum increase in binding affinity for CXCR4 was observed in (25), n = 20 and (26), m = 12.

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analogs bearing one cyclam ring and one 2-pyridyl ring such as AMD3465 (**14**) (Genzyme Corp., Cambridge, MA, USA) had CXCR4-binding activity at nanomolar levels [58]. Second, they have fixed the 2-pyridyl ring moiety and replaced the cyclam ring by various azamacrocyclic rings. Compound **15**, which contains a pyr-[14]aneN$_4$ ring instead of the cyclam ring, showed potent nanomolar anti-HIV activity [56]. Compound **16**, which contains a tetrahydroquinoline and benzoimidazole moiety, showed relatively high anti-HIV activity, although it does not have a cyclam ring [57]. A tetrahydroquinoline compound AMD070 (**17**) (Genzyme Corp., Cambridge, MA, USA) has been found to be a CXCR4 antagonist by recent antiviral evaluation and pharmacokinetic analysis [59,60]. This compound has a protein-adjusted EC_{50} value of 125 nM against HIV-1 in MT-4 cells and bioavailability of over 20% in rats and approximately 80% in dog and is now in Phase I/II clinical trials. Recently, compounds **18** and **19**, which were designed based on the structure of AMD070 (**17**), have been reported by GlaxoSmithKline Co. Ltd. (GSK) [61,62]. Compound **19** has anti-HIV activity comparable with that of AMD070, relatively low bioavailability in rat (16%) and dog (30%) but a suitable cytochrome P450 profile. Screening against a panel of enzymes and receptors, suggests that compound **19** has little risk of unexpected enzyme and receptor inhibition and it has progressed into toxicology studies. Development of the cyclic pentapeptide FC131 has led to non-peptidic CXCR4 antagonists. In one case, the peptide backbone of FC131 (**9**) was entirely replaced by an indole template, which enabled reproduction of the disposition of the pharmacophore moieties in the original peptide (**Figure 3C**). A structure–activity relationship study using modified indoles, for example, **20** identified novel small-molecule antagonists with three appropriately linked pharmacophore moieties such as compound **21** which binds to CXCR4 with micromolar activity [63]. Non-peptide compounds having the dipicolylamine (DPA)–zinc(II) complex structure, utilized as chemosensors that can sense phosphorylated peptide surfaces, were identified as potent and selective antagonists against CXCR4 [64]. A DPA–Zn complex with a xylene scaffold **22** binds to CXCR4 with 50 nM activity and has micromolar anti-HIV activity. Structure–activity relationship studies performed by combining the common structural features of alkylamino and pyridiyl macrocyclic antagonists including DPA–Zn complex (**22**) and AMD3100 (**10**) led to new lead compounds **23** and **24** with 30 and 10 nM activity for binding to CXCR4, respectively [65]. Compounds **23** and **24** also have anti-HIV activity of 90 and 30 nM levels, respectively. These are attractive and useful leads for the future development of non-peptidic CXCR4 antagonists. A low molecular weight compound, KRH-1636 (**25**) (Kureha Chemical, Tokyo, Japan & Daiichi Sankyo Co. Ltd., Tokyo, Japan), derived by intensive modification of the N-terminal tripeptide of T140, Arg-Arg-Nal, was reported to be an orally bioavailable and duodenally absorbable CXCR4 antagonist and X4 HIV-1 inhibitor [66]. Continuous efforts to find more effective CXCR4 inhibitors have recently led to identification of KRH-2731, an orally bioavailable CXCR4 antagonist [67]. Finally, although their structures have not been disclosed yet, the KRH-1636 derivatives KRH-2731 and KRH-3955, which are in the preclinical stage, may be promising as novel inhibitory drugs for treatment of cancer metastasis as well as for HIV-1 infection.

CXCR4 belongs to the G protein-coupled receptor (GPCR) family, and several GPCRs can function *in vitro* as monomers, many of them, including the chemokine receptors, presumably existing *in vivo* as dimers and/or higher order oligomers. Chemokine receptors such as CXCR4 can form homodimers and/or heterodimers with other chemokine receptors [68,69]. Accordingly, we designed and synthesized CXCR4 bivalent ligands consisting of two molecules of an FC131 analog, (*cyclo*(-D-Tyr-Arg-Arg-Nal-D-Cys-)), connected by various lengths of poly(L-proline) or PEGylated poly(L-proline) linkers (**26,27**) (**Figure 3D**) [70]. A maximum increase in binding affinity for CXCR4 was observed for bivalent ligands of the two linker types with suitable lengths (5.5 – 6.5 nm). As a result, we have presented experimental results concerning the elucidation of the native state of the CXCR4 dimer as a function of the distance between the ligand-binding sites (5.5 – 6.5 nm). Fluorescent-labeled bivalent ligands have, however, been shown to be powerful tools for cancer diagnosis as a result of their ability to distinguish the density of CXCR4 on the surface of cancer cells.

4. HIV integrase inhibitors such as Raltegravir

The enzyme HIV-1-IN is critical to the stable infection of host cells since, by means of 3′-end processing and strand transfer reactions, it catalyzes the insertion of reverse-transcribed viral double-stranded DNA into the chromosomal genome of host cells. It is a 32-kDa protein consisting of 288 amino acid residues, and is divided into N-terminal, C-terminal and catalytic core domains [71,72]. The catalytic core domain has a triad of carboxylate residues, of Asp64, Asp116 and Glu152, which are critical for coordination of two magnesium ions to catalyze breaking and formation of DNA phosphodiester bonds (**Figure 4A**), and which are designated as 3′-end processing and strand transfer reactions, respectively [73-75]. Thus, several IN strand transfer inhibitors possessing a two magnesium-binding pharmacophore, which target the carboxylate triad, have been developed. Initially, diketo acids (DKAs) and their analogs, such as L-731,988 (**28**) and L-708,906 (**29**) (Merck & Co., NJ, USA), which have a two magnesium-binding pharmacophore, have been found as first-generation IN inhibitors (**Figure 4B**) [76]. This design is based on an interactive model of the binding of these inhibitors to the carboxylate triad through coordination of two magnesium ions. However, some DKA compounds lacked sufficient potency for binding to IN and pharmacokinetic properties. New heterocyclic DKA analogs with the two magnesium-binding pharmacophore including the naphthyridine

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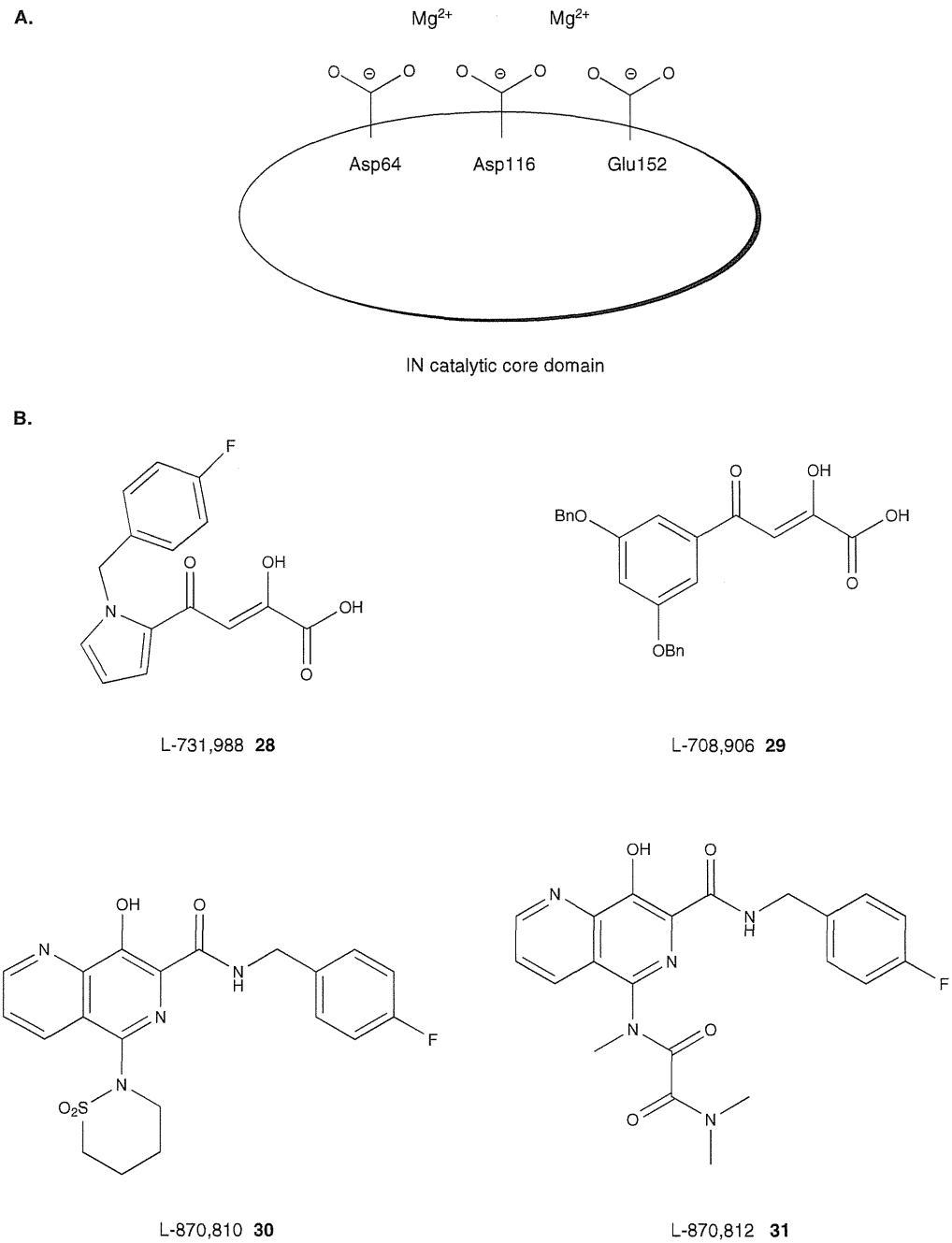

Figure 4. A. Brief presentation of the integrase (IN) catalytic core domain with triad carboxylate residues of Asp64, Asp116 and Glu152, critical for coordination of two magnesium ions. **B.** Structures of DKA type and DKA mimic IN inhibitors. **C.** Structures of naphthyridinone and pyrimidinone-related and other IN inhibitors.

carboxamides, L-870,810 (**30**) and L-870,812 (**31**) (Merck & Co., NJ, USA), have been developed and have shown efficacy in a human and a rhesus simian-human immunodeficiency virus (SHIV) model [77,78]. L-870,810 advanced into the Phase IIa studies and showed viral load reduction, but the trials were terminated due to hepatotoxicity. Subsequent candidate compounds include a naphthyridinone scaffold with a benzyl moiety, such as S/GSK364735 (**32**) (Shionogi-GSK), which has potent anti-HIV activity and HIV-1 RNA reduction activity (**Figure 4C**) [79]. This compound progressed to Phase IIa studies,

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