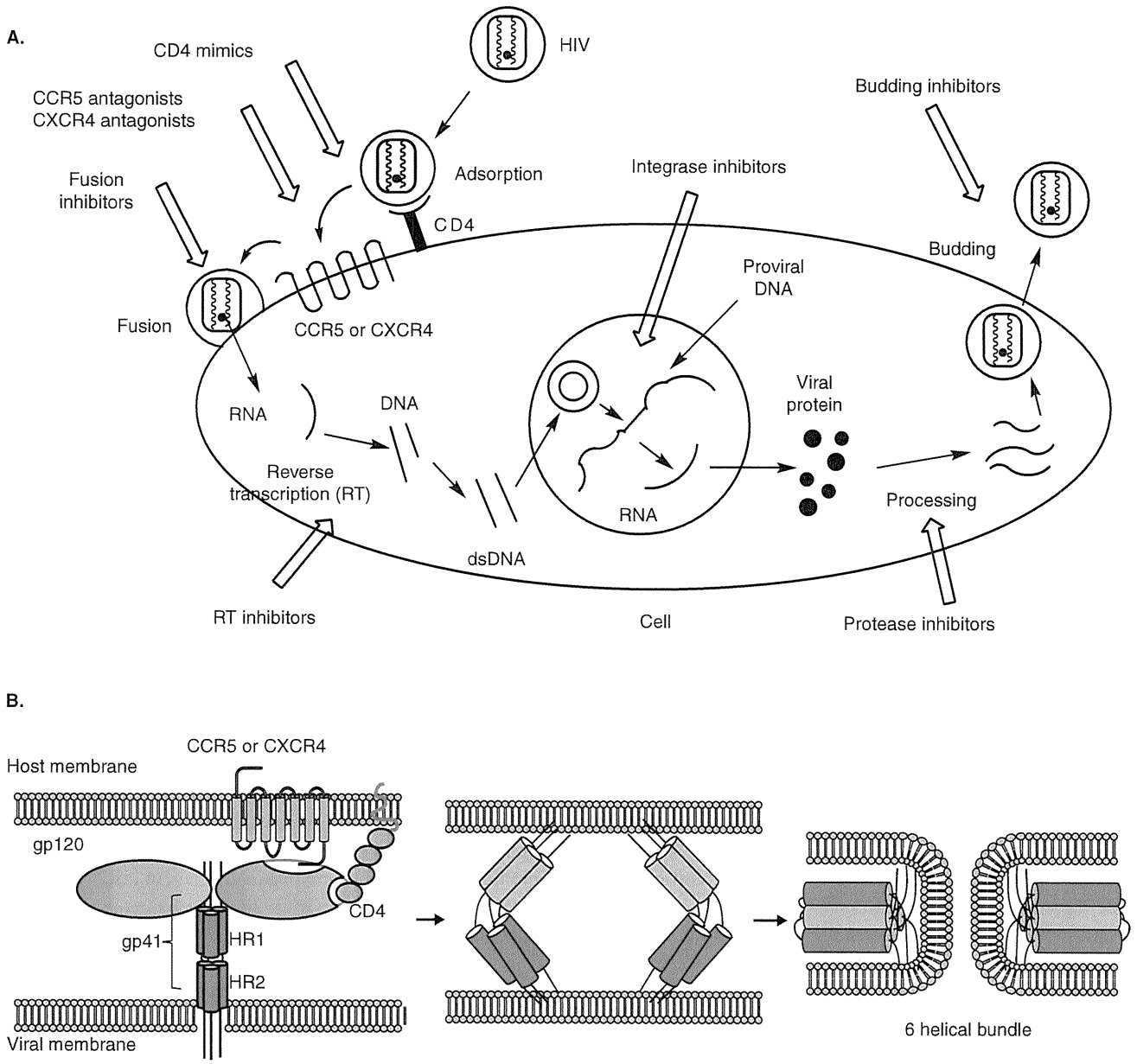
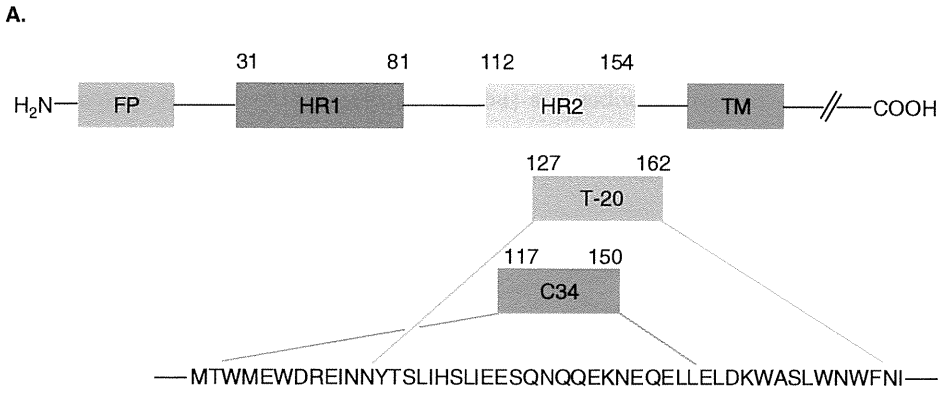



**Figure 1. A.** HIV-1 replication cycle and anti-HIV drugs that are effective at its various steps. **B.** Mechanisms of HIV-1 entry and fusion.

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



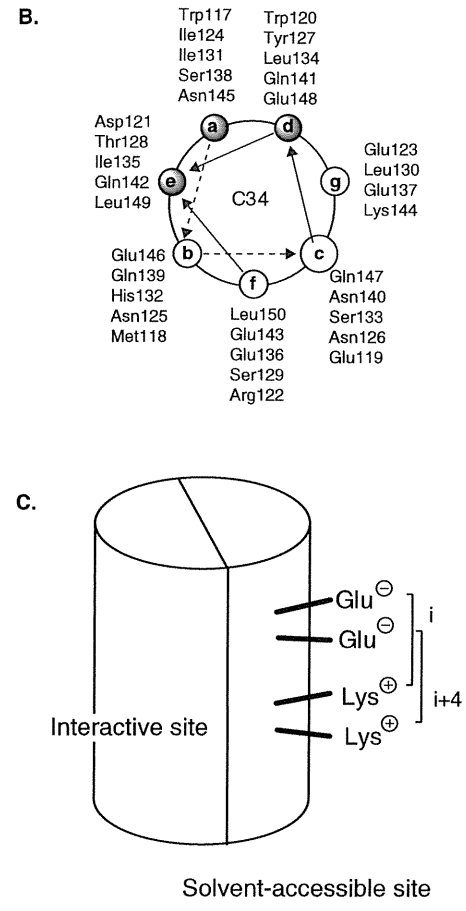




**Figure 2. A.** Schematic representation of gp41 and sequences of HR2 region peptides. **B.** Helical wheel representation of the C34 peptide. Amino acid residues are numbered according to gp41 of NL4-3 strain. **C.** The design concept of introduction of the Glu-Lys motif to the solvent-accessible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

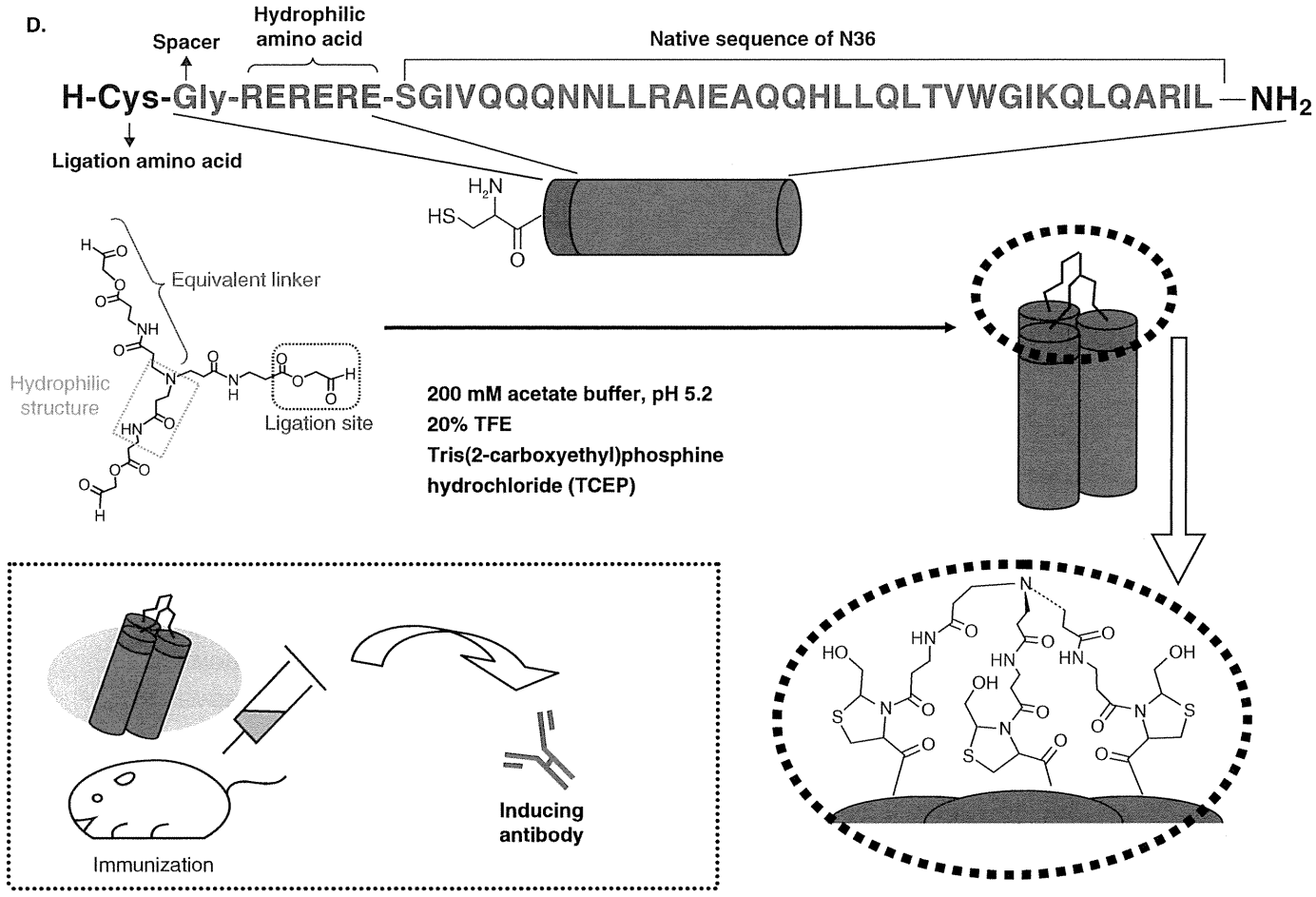



**Figure 2. (continued). A.** Schematic representation of gp41 and sequences of HR2 region peptides. **B.** Helical wheel representation of the C34 peptide. Amino acid residues are numbered according to gp41 of NL4-3 strain. **C.** The design concept of introduction of the Glu-Lys motif to the solvent-accessible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

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.,

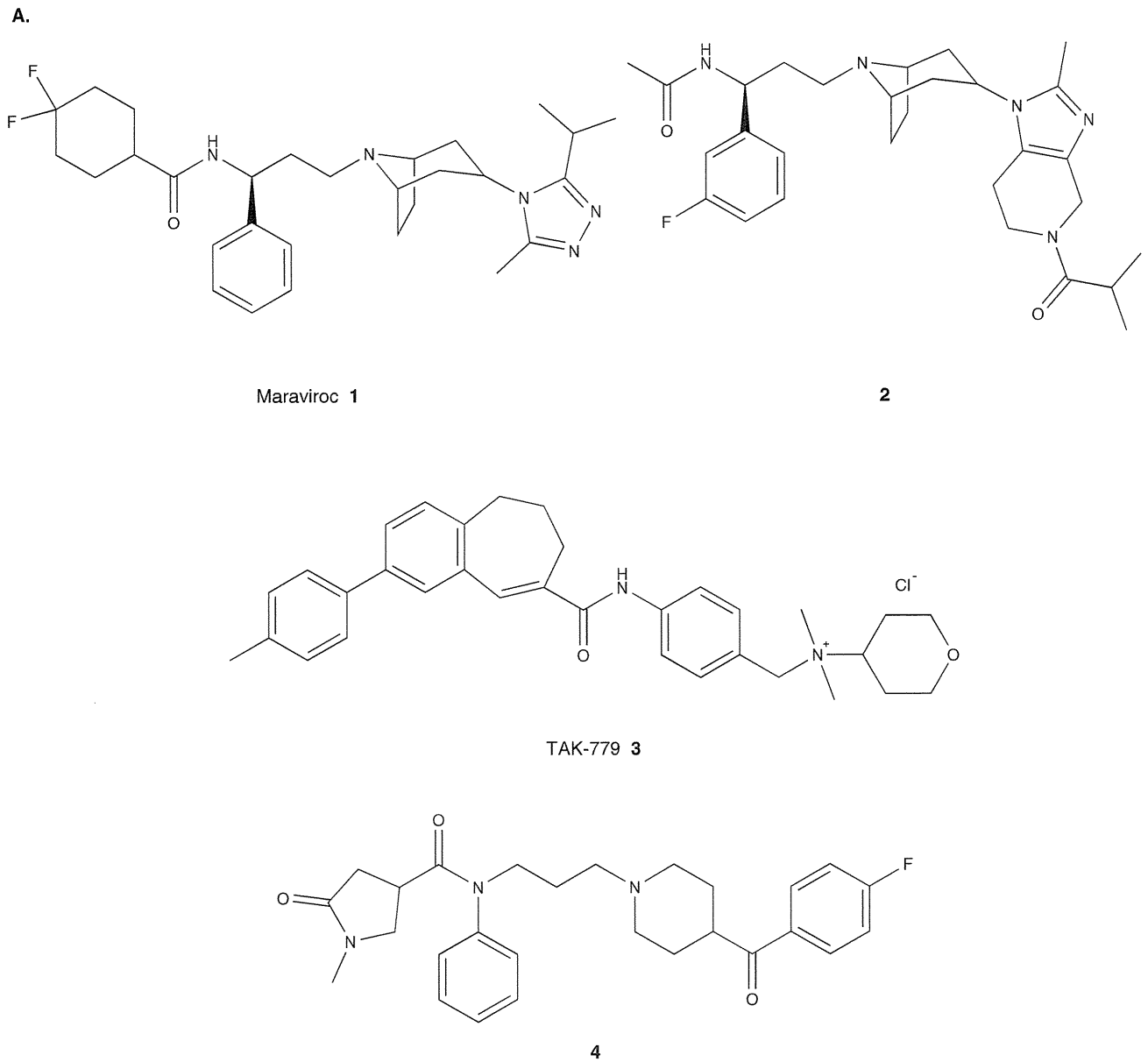
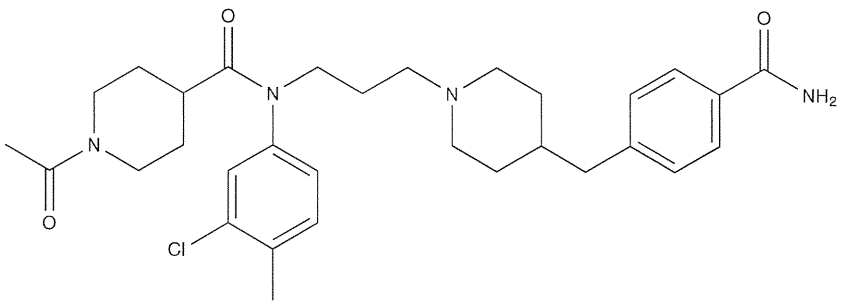


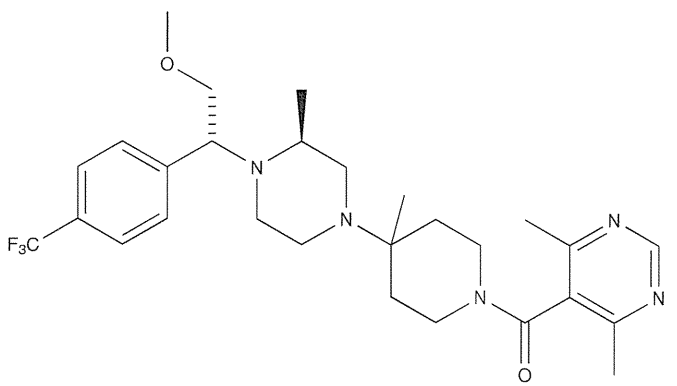

**Figure 3. A.** Structures of CCR5 antagonists. **B.** Structures of CXCR4 antagonists. **C.** Development of non-peptidic CXCR4 antagonists. **D.** Structures of bivalent CXCR4 ligands. A maximum increase in binding affinity for CXCR4 was observed in (**25**), n = 20 and (**26**), m = 12.

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].

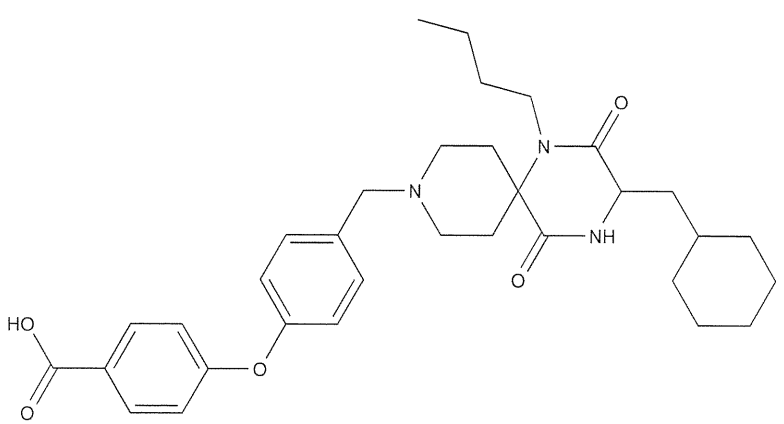
AMD3100 (**10**) (Genzyme Corp., Cambridge, MA, USA), a non-peptidic bicyclam-containing small molecular CXCR4 antagonist, was the first CXCR4 antagonist to enter clinical trials for the treatment of HIV-1-infected patients (**Figure 3B**) [51,52]. It was found, however, to have adverse cardiovascular 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*



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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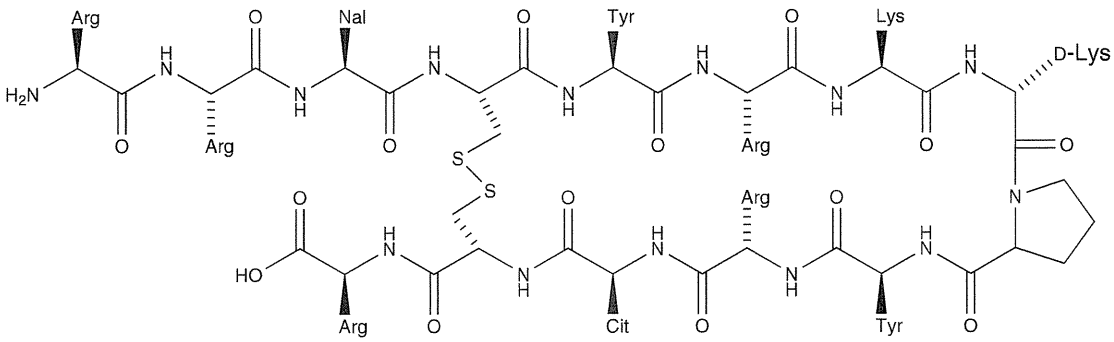
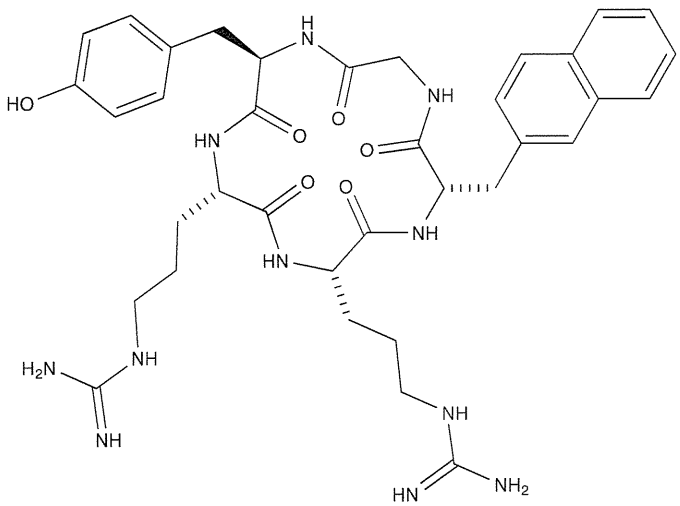
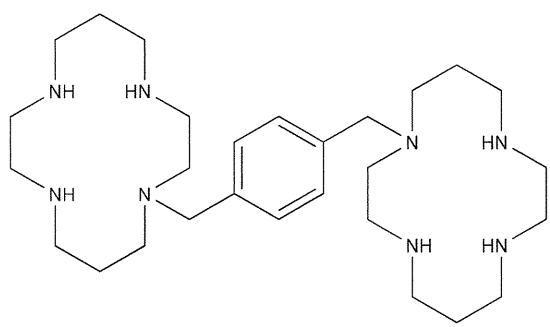


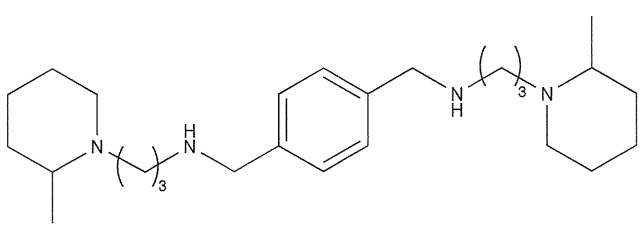

T140 **8**



FC131 **9**



AMD3100 **10**



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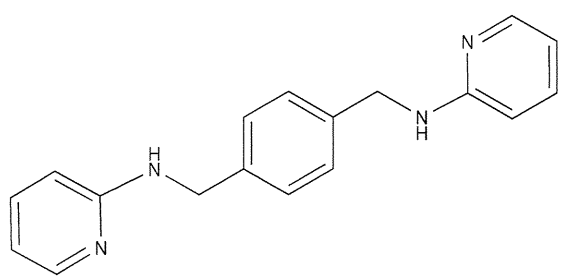
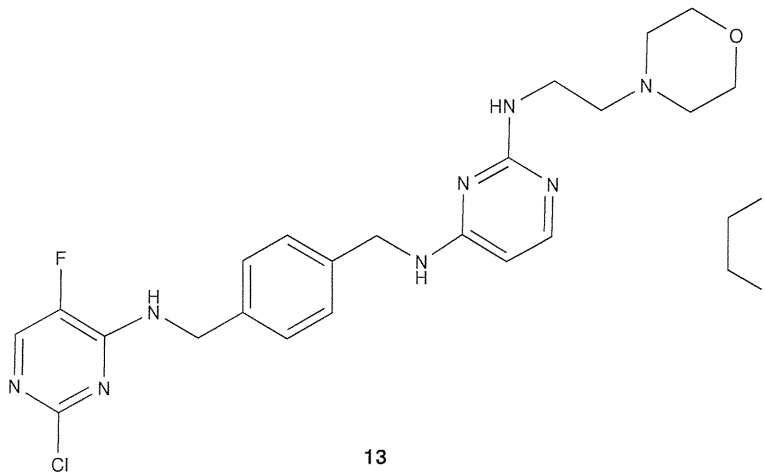


**12**

**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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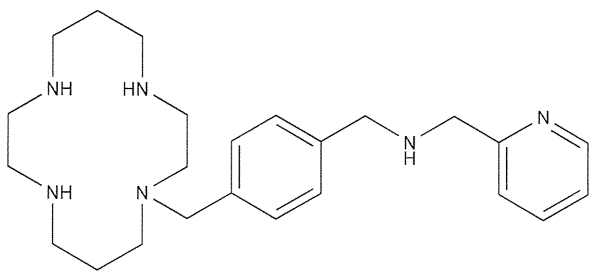
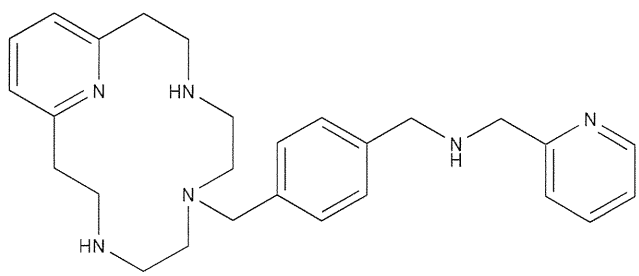


AMD3465 **14**



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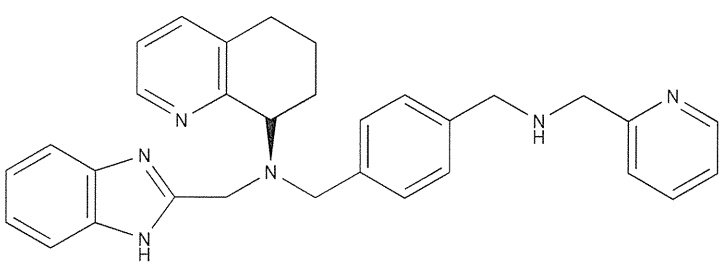

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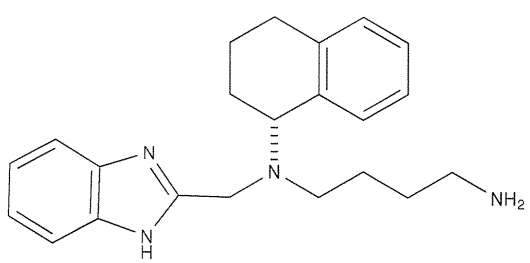
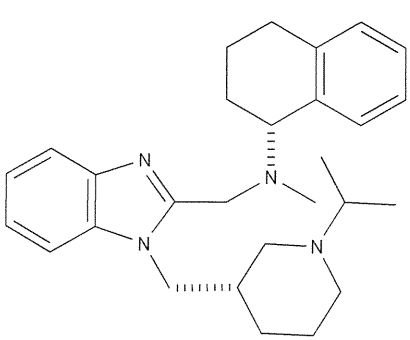


AMD070 **17**



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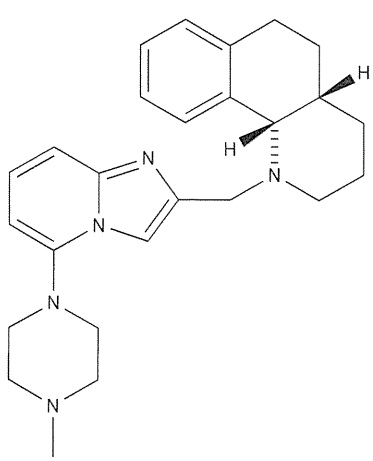

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**Figure 3. (continued). A.** Structures of CCR5 antagonists. **B.** Structures of CXCR4 antagonists. **C.** Development of non-peptidic CXCR4 antagonists. **D.** Structures of bivalent CXCR4 ligands. A maximum increase in binding affinity for CXCR4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

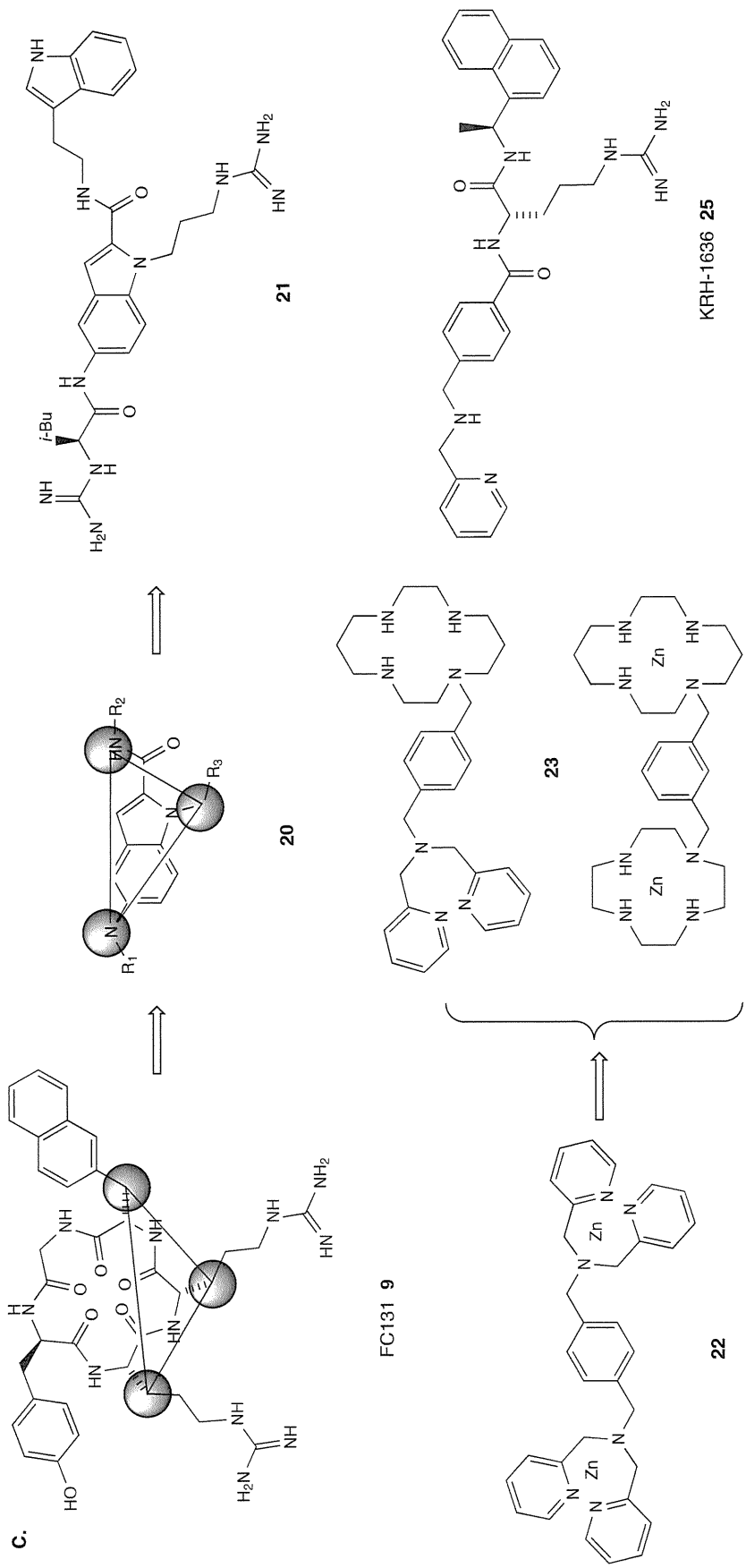



**Figure 3. (continued). A.** Structures of CCR5 antagonists. **B.** Structures of CXCR4 antagonists. **C.** Development of non-peptidic CXCR4 antagonists. **D.** Structures of bivalent CXCR4 ligands. A maximum increase in binding affinity for CXCR4 was observed in (25), n = 20 and (26), m = 12.

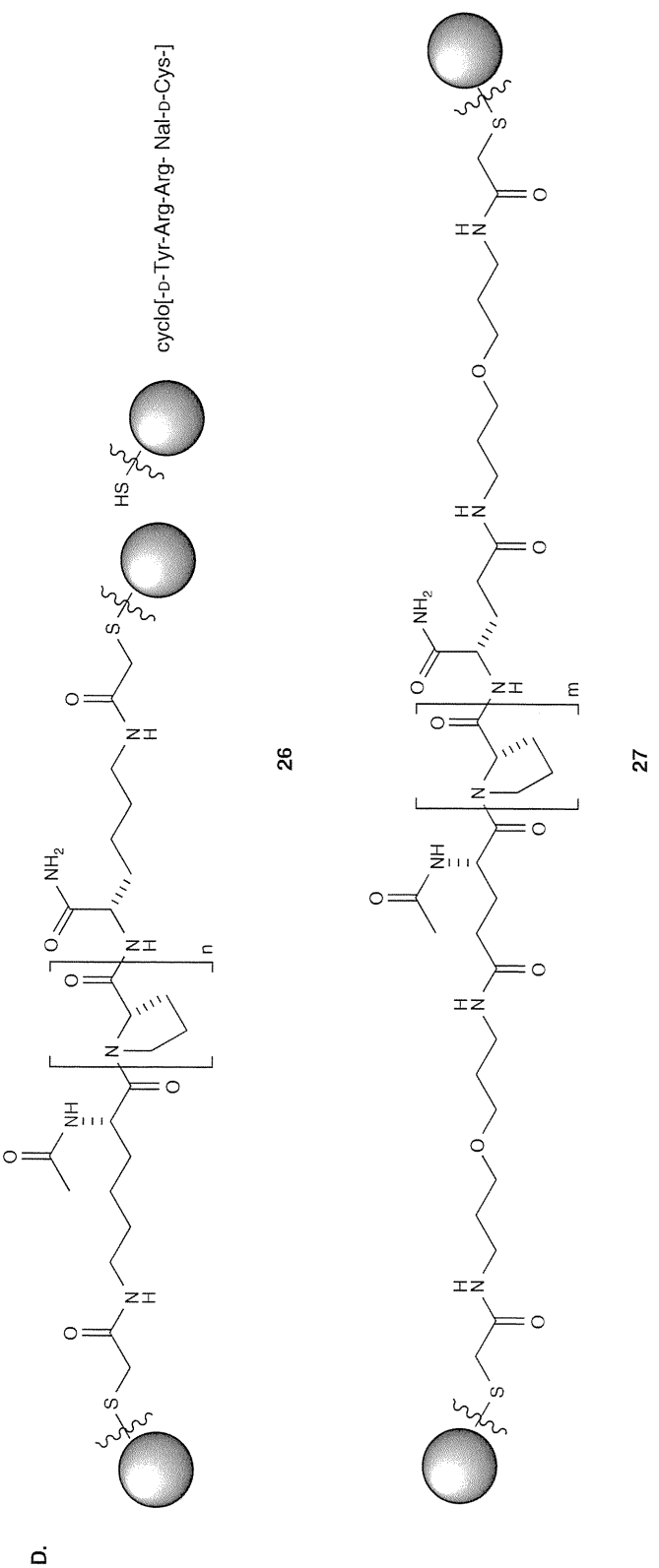



**Figure 3. (continued).** **A.** Structures of CCR5 antagonists. **B.** Structures of CXCR4 antagonists. **C.** Development of non-peptidic CXCR4 antagonists. **D.** Structures of bivalent CXCR4 ligands. A maximum increase in binding affinity for CXCR4 was observed in (25), n = 20 and (26), m = 12.

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

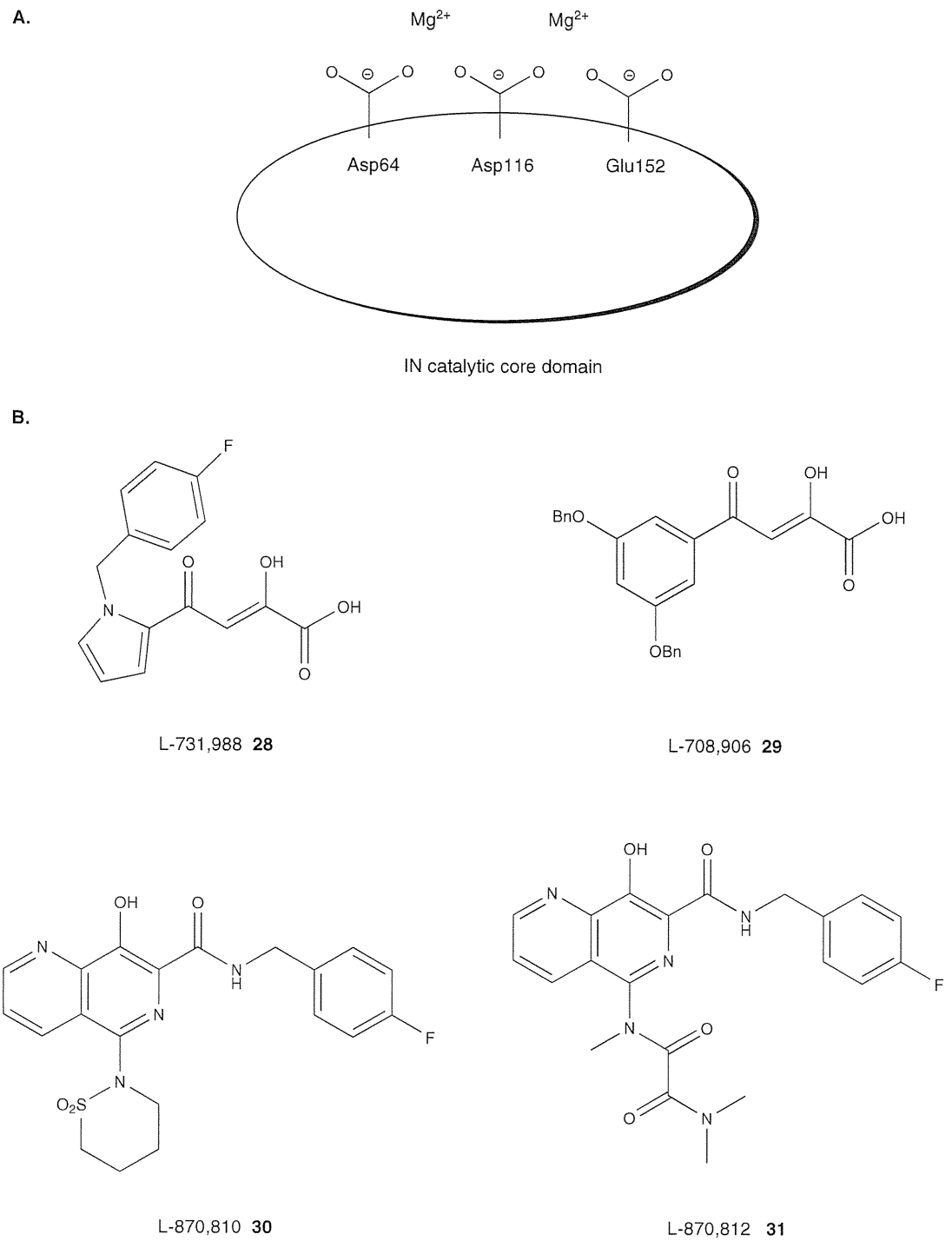
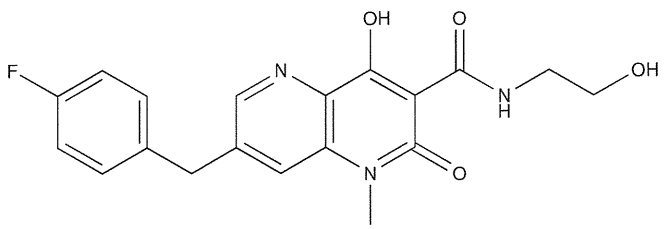


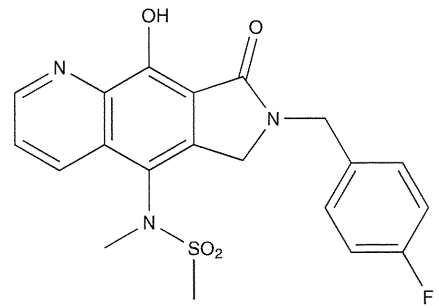

**Figure 4. A.** Brief presentation of the integrase (IN) catalytic core domain with triad carboxylate residues of Asp64, Asp116 and Glu152, critical for coordination of two magnesium ions. **B.** Structures of DKA type and DKA mimic IN inhibitors. **C.** Structures of naphthyridinone and pyrimidinone-related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

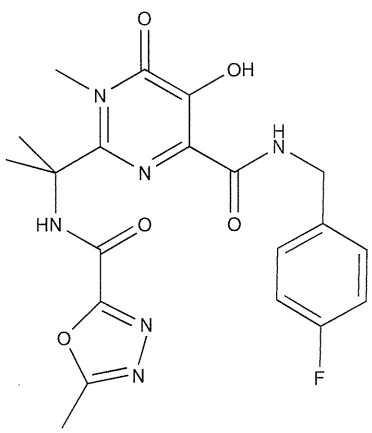
C.



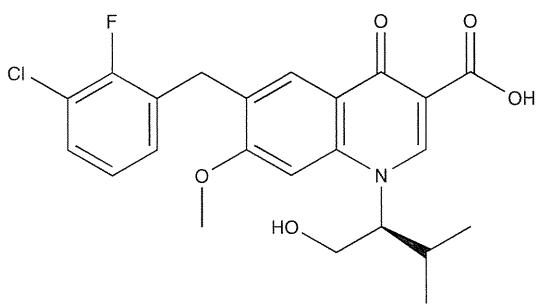
S/GSK364735 **32**



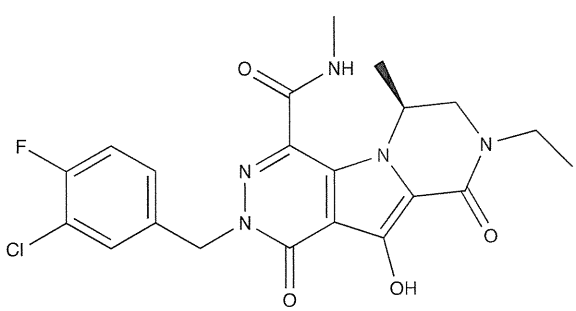
GS-9160 **33**



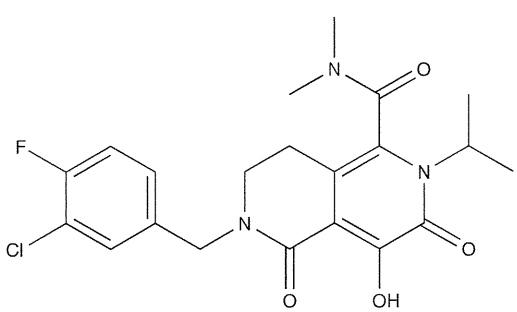
Raltegravir **34**



Elvitegravir **35**



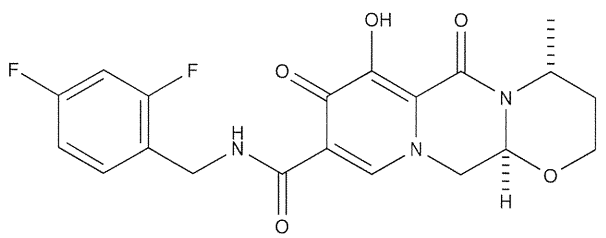
MK-2048 **36**



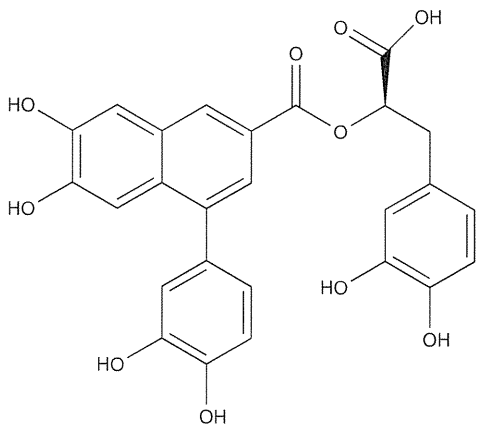
MK-0536 **37**

**Figure 4. (continued). 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.

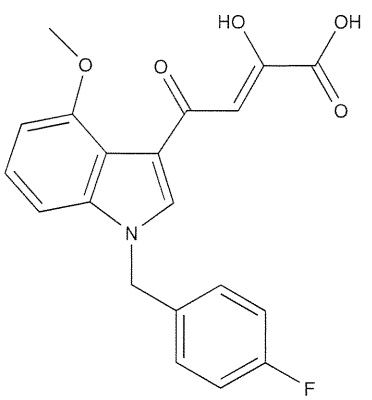
but its clinical trial was discontinued due to hepatotoxicity. A tricyclic analog with a quinoline template in combination with a lactam ring and a benzyl moiety, GS-9160 (33) (Gilead Sciences, Inc., Foster, CA, USA), was reported [80]. This compound has a very low $EC_{50}$ and a very high selectivity index of ~ 2000. In addition, it showed synergistic effects in combination with protease inhibitors, NNRTIs and NRTIs. Viral resistance selections with GS-9160 obtained mutations within the catalytic core domain of IN but its pharmacokinetic profile in individuals with once-daily dosing did not achieve antiviral



Dolutegravir **38**



Globoidnan A **39**



CHI-1043 **40**

**Figure 4. (continued). 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.

efficacy and the clinical trial of this compound was terminated after Phase I studies.

Raltegravir (34), a pyrimidinone derivative, was the first IN inhibitor to be approved by the FDA [12,13]. This compound has a *p*-fluorobenzyl branch as a common structure and a five-membered heterocyclic ring. In clinical trials, doses of 200, 400 and 600 mg were studied, and the recommended dose of Raltegravir for adults is 400 mg twice a day. In clinical trials patients treated with Raltegravir achieved viral loads of less than 50 copies/ml sooner than those with a dosage of protease inhibitors or NNRTIs. In 2007, Raltegravir was initially approved only for use in patients with resistance to other HAART drugs. However, in 2009, the FDA expanded approval of Raltegravir for use in all patients in combination with other anti-HIV agents. Monotherapy with Raltegravir is unlikely to show durability. Research of effects on latent viral reservoirs and eradication of HIV is in progress. Possible side effects are diarrhea, nausea, headache, fever, rash, Stevens–Johnson syndrome and depression. Concerning emergence of resistant mutants, Raltegravir is likely to lose efficacy due to a major viral mutation compared with protease inhibitors requiring more mutations. A new pyrimido-azepine derivative (PYRAZ) (Merck & Co., NJ, USA), which shows less cross-resistance with Raltegravir-resistant strains, was reported [81]. Elvitegravir (GS-9137/JTK-303) (35) (Gilead Sciences, Inc., Foster, CA, USA/JT, Tokyo, Japan) with a quinolone template also advanced into Phase III studies as the second candidate of IN inhibitors [82]. This compound has nanomolar levels of IN inhibitory and anti-HIV activities together with moderate bioavailability and low clearance. Boosting by a CYP450 inhibitor ritonavir is useful for efficacy of viral load reductions [83]. In addition, another booster agent GS-9350, itself with no antiviral activity, has been tried in a combinational regimen [84]. Elvitegravir showed cross-resistance with Raltegravir-resistant strains [85]. Another potential inhibitor MK-2048 (36) (Merck & Co., NJ, USA) showed

improved potency against mutant strains [86]. Viral mutations in resistance to MK-2048 are different from those with Raltegravir or Elvitegravir. MK-2048 is superior to Raltegravir in terms of retention since it inhibits IN four times longer. Thus, MK-2048 has advanced into Phase III studies. MK-0536 (37) (Merck & Co., NJ, USA) also has a good retention of anti-HIV activity [87]. Dolutegravir (S/GSK1349572) (38) (Shionogi, Osaka, Japan-GSK, Middlesex, UK) is a new generation IN inhibitor with potent anti-HIV activity, a low clearance and good oral bioavailability and which advanced into Phase III trials in February 2011 [88,89]. In evaluation using mutation site-directed mutants, Dolutegravir showed a resistant profile that was different from those of Raltegravir and Elvitegravir, suggesting less cross-resistance with those drugs and a genetic barrier to resistance. Even once-daily monotherapy in humans with Dolutegravir without booster drugs showed efficient reduction in RNA levels, high retention of blood concentrations and good pharmacokinetic profiles. Currently, three-drug combinational use with two NRTIs, Abacavir (ABC) and 3TC, is awaiting approval. Globoidnan A (39), a lignan found in *Eucalyptus globoidea* in Australia, has been found to inhibit the action of HIV-IN [90] but it is not known whether it inhibits other retroviral INs. Since it has a novel structure, this possibility will be investigated further. Future IN inhibitors require a high genetic barrier to resistance, low dose and once-daily dose with good pharmacokinetic profiles in the absence of booster drugs. Structural analysis of the complex of IN and DNA should be useful for the design of new IN inhibitors but structural elucidation of HIV-1 IN has not been succeeded yet in spite of numerous efforts. The crystal structure of human foamy virus IN with viral DNA can be used as an alternative [91,92]. Practically, the binding modes of a new IN inhibitor with a benzylindole derivative, CHI-1043 (40), which has a nanomolar range of inhibitory activity against strand transfer reaction, was analyzed using the above crystal structure of the complex, suggesting that CHI-1043 has the same binding modes as Raltegravir and Elvitegravir [93]. Development of HIV-1 IN inhibitors such as Raltegravir has recently been advanced in AIDS chemotherapy but combinational dosing regimens are necessary because emergence of resistant mutants against Raltegravir has been reported. Investigational drugs such as Elvitegravir and Dolutegravir are anticipated for clinical use.

Recently, we have discovered different types of IN inhibitors [94,95]. By screening a random library of overlapping peptides derived from HIV-1 gene products we have found three Vpr-derived 15-mer peptides with significant IN inhibitory activity, indicating that IN inhibitors exist in the viral preintegration complex (PIC) (**Figure 5A**). These inhibitory peptides are consecutive overlapping peptides. Peptidic 12- and 18-mers from the above original Vpr-sequence with the addition of an octa-arginyl group into the C-terminus to enhance cell membrane permeability have IN inhibitory activity and anti-HIV activity. The detailed mechanism of action of these inhibitors has not been disclosed although it is thought that they may bind to the cleft between the amino-terminal domain and the core domain of HIV-1 IN. This region is distinct from the nucleic acid interacting surfaces, indicating that the Vpr-derived peptides inhibit IN function in an allosteric manner. These data are useful for the development of different types of potent HIV-1 IN inhibitors based on Vpr-derived peptides.

## 5. CD4 mimics as HIV entry inhibitors

The binding of gp120 to the host-cell surface protein CD4 causes gp120 to undergo a conformational change subsequently binding to the co-receptor CCR5 or CXCR4, as described in Section 1. Thus, CD4-related molecules including soluble CD4 (sCD4) could be inhibitors of HIV entry, although unsuccessful attempts have been made to develop sCD4. Recently, several small CD4 mimics have been found by us and others. These include NBD-556 (41) [96,97], YYA-021 (42) [98-100], JRC-II-191 (43) [101] and BMS806 (44) (**Figure 5B**) [102]. NBD-556, YYA-021 and JRC-II-191 cause a conformational change of gp120 and thereby block binding of HIV virion to CCR5 or CXCR4. On the other hand, BMS806 binds to gp120 and blocks the CD4 induction of the HR1 exposure without any significant effect on CD4 binding. YYA-021 also induces a highly synergistic interaction in the combinational use with the CXCR4 antagonist T140 or the neutralizing anti-V3 monoclonal antibody KD-247 and exerts a pronounced effect on the dynamic supramolecular mechanism of HIV-1 entry. CD4 mimics are essential probes directed to HIV entry, and might be important leads for the cocktail therapy of AIDS.

## 6. Conclusion

Since the discovery of AIDS in 1983, several inhibitory drugs against HIV replication have been developed and used clinically for treatment of patients with AIDS and HIV infection. Use of reverse transcriptase inhibitors and protease inhibitors in combination, designated HAART, has provided great success in clinical treatments. Recently, novel drugs including entry inhibitors and IN inhibitors, which belong to categories distinct from the above drugs, have been approved for clinical use. Fusion inhibitors such as Enfuvirtide, co-receptor CCR5 antagonists such as Maraviroc and IN inhibitors such as Raltegravir have successively been developed in company with the potential of other inhibitors including CXCR4 antagonists and CD4 mimics.

## 7. Expert opinion

In the three decades since the discovery of AIDS, the number of HIV people infected with HIV has surpassed 30 million. In the early era of the discovery of AIDS, it was thought that AIDS/HIV infectious syndrome was a lethal disease. However, with the appearance of second-generation drugs,

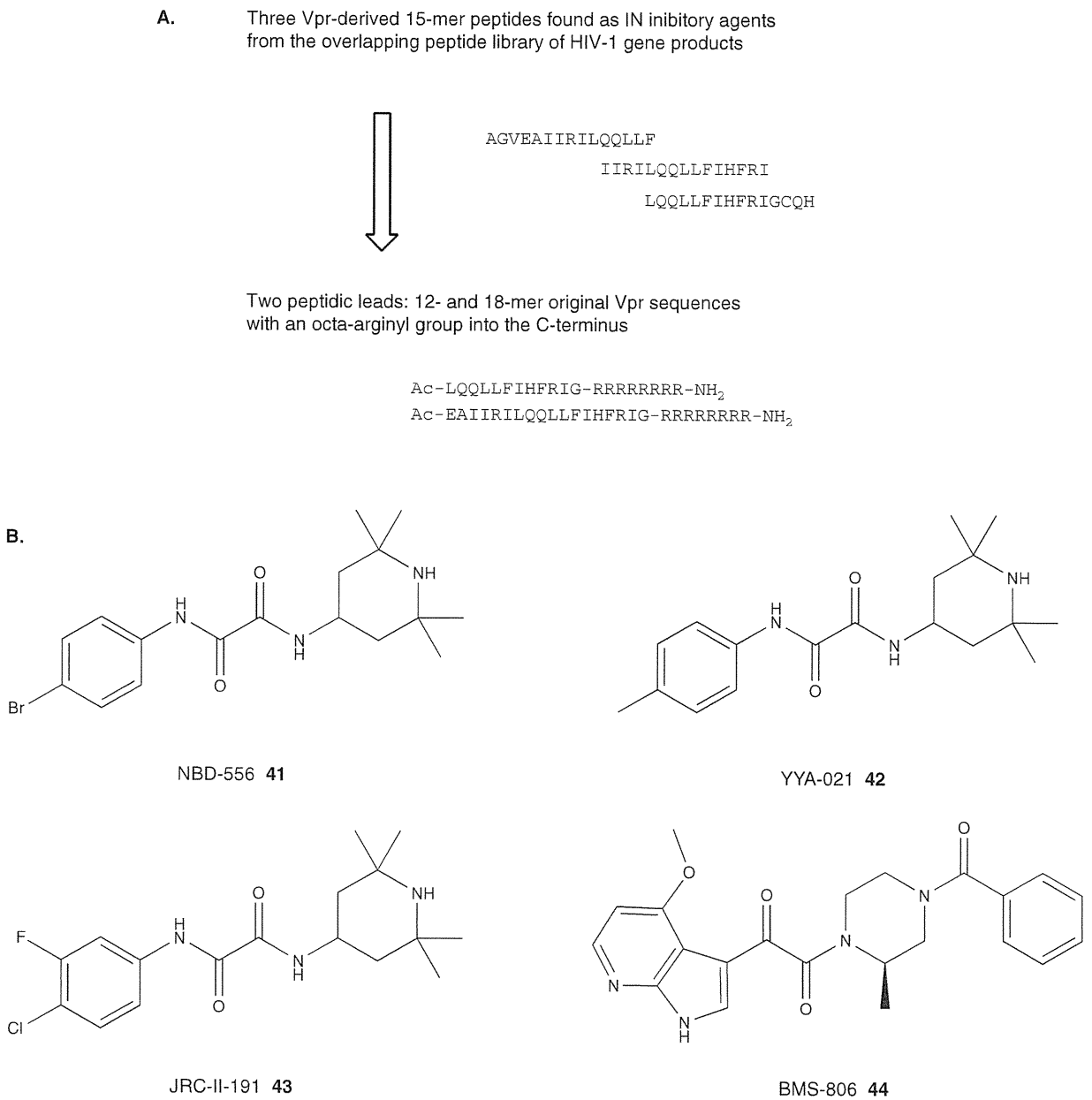



**Figure 5. A.** Vpr-derived IN inhibitors with an allosteric mechanism. **B.** Structures of small-sized CD4 mimics.

the protease inhibitors and introduction of a cocktail therapy (HAART), AIDS has become a curable disease. HAART can reduce the concentrations of HIV in blood to undetectable levels. There are, however, serious clinical problems including side effects, the emergence of MDR strains and high costs. Thus, brand-new drugs with novel mechanisms of action continue to be sought.

Since 1995, the molecular mechanisms underlying the HIV-1 replication have been elucidated in detail, in particular for the dynamic supramolecular mechanism associated with HIV entry/fusion steps. Elucidation of the mechanism led to the development of Enfuvirtide, which was the first entry/fusion inhibitor approved by the FDA. This drug is now used as an additional drug in the cocktail therapy for patients with evidence of HIV infection and resistance to other drugs. Enfuvirtide is not the first-choice drug, and it is not used as a monotherapy. Appearance of Enfuvirtide has an important impact in terms of its role in a repertoire of anti-HIV drugs, because it can be used even for treatment of advanced infection. Whereas reverse transcriptase inhibitors and protease inhibitors work inside of cells to inhibit functions of viral enzymes, the fusion inhibitor Enfuvirtide works extracellularly to prevent HIV from invading cells. Since fusion inhibitors are not required to penetrate cells, cell penetration is not required in drug design

and development. Entry and fusion inhibitors have this advantage. In addition, the HR1 and HR2 regions of gp41 have highly conserved sequences among various strains, without modifications of carbohydrates, suggesting that fusion inhibitors such as Enfuvirtide are likely to be able to access HIV virion of diverse strains. The HR1 region is, therefore, also critical for the development of AIDS vaccines and we have synthesized an artificial antigen molecule consisting of a novel three-helical bundle mimetic, corresponding to the trimeric form of N36. The exposed timing of its epitopes is limited during HIV-1 entry, and carbohydrates, which disturb access of antibodies to its epitopes, are not included. These two advantages could further enhance the potential of a vaccine design based on the HR1 region. Enfuvirtide and several reported C34 analogs are peptidic compounds, and development of non-peptide low molecular weight inhibitors is desirable although this is difficult and has not succeeded to date. The success of Enfuvirtide has encouraged development of entry/fusion inhibitors as a new class of anti-HIV drugs. Therefore, Maraviroc (1) was developed as the first CCR5 antagonist to be approved by the FDA. Since individuals with the CCR5-32 deletion mutation are healthy and strongly resistant to HIV-1 infection [28], it was thought that CCR5 antagonists have suitable pharmaceutical properties. Further, it might be difficult to generate resistant viruses in a use of drugs, which target host proteins such as CCR5. Accordingly, many CCR5 antagonists have been developed and some are now in clinical trials. Appearance of new CCR5 antagonists following the development of Maraviroc would be desirable. The discovery of CXCR4 has provoked vigorous research on drug development with its correlation to another co-receptor for HIV entry. However, blocking of the CXCL12–CXCR4 axis might be dangerous because CXCR4 is constitutively expressed in several organs and tissues, and CXCR4 plays a critical role in embryogenesis, homeostasis and inflammation in the fetus especially in the embryonic development of hemopoietic, cardiovascular and central nervous systems. It also plays a role in the homing of immune cells in inflammation. Knockout of CXCL12 or CXCR4 is known to be embryonically lethal [103] and thus one must carefully consider the risks associated with blockade of the CXCL12–CXCR4 axis. As anti-HIV agents, CXCR4 antagonists play a critical role in HIV-infected patients who have X4 HIV-1 strains that emerge late in the HIV infectious disease process. CXCR4 antagonists might suppress the appearance of X4 or dual-tropic strains in patients who have R5 strains that constitute a majority in the early stages of HIV infection. Combinational use of CXCR4 antagonists with CCR5 antagonists has shown potent synergism against a 1:1 mixture of X4 and R5 strains *in vitro* [104]. As anticancer agents, CXCR4 antagonists which block the CXCL12–CXCR4 interactions might represent a novel and useful chemotherapy of cancer metastasis and leukemia. CXCR4 antagonists might be useful for mobilization of hemopoietic stem cells from the bone marrow [105]. The interaction between CXCL12 and CXCR4 is correlated with the retention of stem cells in the bone marrow, and blocking this interaction results in mobilization of stem cells. AMD3100 induces not only rapid mobilization of hemopoietic stem cells [106], but also adverse cardiovascular effects. Its use as an anti-AIDS drug has been discontinued, but its development as an agent for stem cell mobilization continues [107]. In the year (2007) that Maraviroc received approval, the FDA also approved Raltegravir as the first IN inhibitor for use in combination with other antiretroviral agents in treatment-experienced patients with HIV-1 strains resistant to multiple HAART agents. Subsequently, in 2009 the FDA granted expanded approval of Raltegravir for use in combinational dosing regiments in all patients. The design of these IN inhibitors is thought to be rational since it is based on scaffold structures with a two magnesium-binding pharmacophore such as DKA, naphthyridinone and pyrimidinone-related templates. Appearance of IN inhibitors after Raltegravir for clinical use is desired. We have found Vpr-derived peptides which inhibit IN function in an allosteric manner. In future designs, these inhibitors are attractive and useful leads because the binding site is different from that used by Raltegravir. CD4 has long been a target for AIDS chemotherapy and anti-HIV drugs, and recently, several small-sized CD4 mimics have been found. Since these compounds target the dynamic supramolecular mechanism of HIV-1 entry and induce highly synergistic effects when combined with the CXCR4 antagonist T140 or the anti-V3 antibody KD-247, they might transpire to be important leads for the cocktail therapy of AIDS.

A methodology for finding of anti-HIV leads using random libraries such as overlapping peptide libraries derived from HIV-1 gene products is a useful strategy which has led to the discovery of new allosteric-type HIV IN inhibitors [94,95]. Recently, a combination therapy including an HIV protease dimerization inhibitor, Darunavir, Tibotec Pharmaceuticals, Co Cork, Ireland [108,109], and an IN inhibitor, Raltegravir, is a major first choice for drug combination regimens. In case of loss of efficacy of HAART due to the emergence of MDR strains, change of regimens of the drug combination in HAART is required with monitoring of the virus and CD4 in blood including cellular tropism testing. In such a situation, the number of available potent anti-HIV drugs is critical. Entry inhibitors such as CCR5/CXCR4 antagonists and 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. Today, 20-year-old HIV-positive persons in wealthy countries starting HAART drugs can expect to live up to 69 years of age [110].

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## Declaration of interest

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