Targeting CXCR4 in HIV Cell-Entry Inhibition.

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Abstract: CXCR4 and CCR5 constitute the two major coreceptors for HIV-1 entry into host cells. In the course of an HIV-infection, a coreceptor switch takes place in approximately half of the patients – from R5 HIV-1 (CCR5 utilizing) strains to X4 HIV-1 (CXCR4 utilizing) strains. Treatment of HIV-infected individuals with CXCR4 antagonists delays the onset of AIDS by preventing the CCR5 to CXCR4 coreceptor switch. In addition to the endogenous CXCR4 and CCR5 ligands, other chemokines, for example the human herpesvirus 8 encoded CC-vCCL2, and modifications hereof, have proven efficient HIV-1 cell-entry inhibition through chemokine receptor interaction. However, pharmacokinetic and immunogenic drawbacks of chemokines and peptidic/peptoid compounds have brought the attention towards small-molecule antagonists, such as AMD3100, that displays high specificity and affinity towards CXCR4, but unfortunately no oral bioavailability. The hunt for orally active small-molecule CXCR4 antagonists led to the development of monocyclam-based compounds, and recently to the non-cyclam antagonist AMD070, which is orally active and currently in Phase II clinical trial as anti-HIV treatment. Current review provides an overview of the drug discovery within the field of anti-HIV treatment targeting CXCR4 spanning from natural occurring and modified chemokines, to HIV-mimicking peptides and peptoids ending at the non-peptide antagonists.

Keywords: CXCR4 antagonist, chemokine system, HIV-entry inhibition, 7TM receptors.

INTRODUCTION

Since its discovery as the etiological agent of acquired immunodeficiency syndrome (AIDS) in 1983, human immunodeficiency virus (HIV) has infected more than 64.9 million people worldwide and has killed more than 32 million people[1]. Tremendous effort has been put into the development of effective antiviral compounds for the treatment of HIV infected individuals and for a control of the pandemic spread of the virus. Many attempts have been made at targeting vulnerable steps in the replication cycle of HIV, resulting in enzyme-inhibitors in addition to fusion inhibitors. Combinations of three or more reverse transcriptase and protease inhibitors in the so-called highly active antiretroviral therapy (HAART) decrease viral load to below detectable levels, however unfortunately viral particles emerge from reservoirs where they have been stored upon termination of the therapy [1-3]. Moreover, current anti-HIV therapy is unaffordable in less developed countries, where the need for anti-HIV agents is highest. Furthermore, having a rapid viral turnover (10^7-10^{10} viral particles per day) and a high rate of incorrect nucleotide substitutions during reverse transcription (3×10^{-5} per nucleotide per cycle), HIV exhibits extensive heterogeneity which in turn leads to subsequent natural selection for resistant strains [4,5]. For these apparent reasons, there is a constant need for new and improved anti-HIV drugs. Targeting human components involved in the HIV infection, e.g. the 7TM receptors used for virus cell-entry CCR5 and CXCR4 (which obviously do not have the same error prone transcription rate as viral proteins), would be a safer approach for these reasons. In this review a special emphasis will be put on the description of the development of CXCR4 targeting ligands spanning from the chemokine-based ligands, to the peptide/peptoids ending with the non-peptide ligands (Table (**1**) and (**2**)).

HIV-1 CELL-ENTRY, REPLICATION AND COURSE OF INFECTION

HIV is a retrovirus from the lentivirus subfamily. The infection starts with virion-adsorption to the target cell membrane via CD4, and a 7TM coreceptor [6]. The chemokine receptors CXCR4 and CCR5 are the two major coreceptors, however also other chemokine receptors function as HIV cell-entry coreceptors [7-14]. Receptor binding initiates fusion of the cell membrane with the virion membrane and the virus gain entry into the target cell (Fig. (**1**)). The virus is now uncoated and the single-stranded viral RNA is released into the cytoplasm and immediately transcribed into double-stranded DNA by the viral reverse transcriptase enzyme [15]. The DNA enters the cell nucleus and is integrated into the host genome by the viral integrase enzyme [16,17]. In connection with the cellular DNA the integrated viral DNA is transcribed into mRNA and is even able to persist latently in a resting cell for many years until reactivation [1-3,15]. The viral mRNA is subsequently translated into proteins by

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[1]http://www.usaid.gov/our_work/global_health/aids/News/aidsfaq.html#deaths

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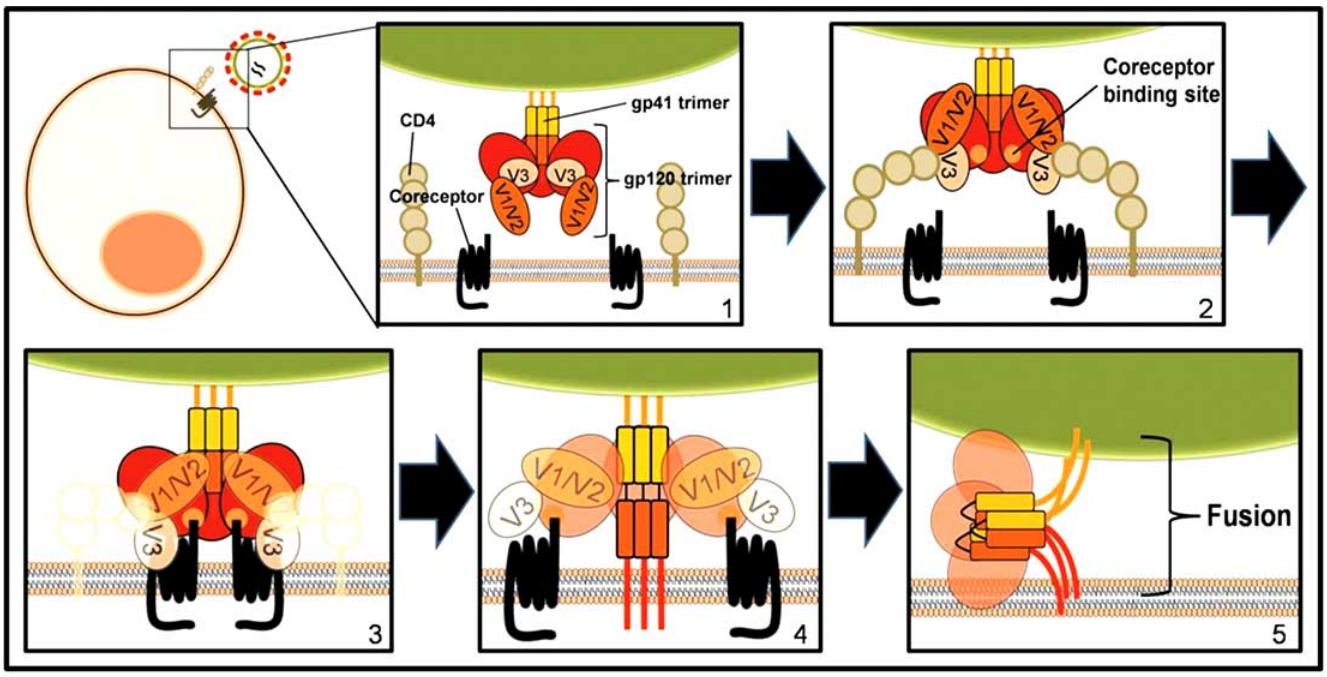

Fig. (1) The fusion/entry mechanism. The Envelope (Env) glycoproteins are the catalysts of the fusion between the cell membrane and the viral membrane [6]. Panel 1: A cellular protease cleaves gp160 into gp120 and gp41 that in turn are organized into heterotrimeric spikes (three subunits of gp120 and three of gp41) displayed on the virion surface [147,148]. Panel 2: gp120 binding to CD4 leads to pronounced rearrangement of the third variable loop (V3) [6,149]. This results in the exposure of the coreceptor-binding site and the affinity for the coreceptors increases [150,151]. Panel 3: Secondly, gp120 binds to the extracellular amino terminus of the coreceptor, which elicits conformational changes in the Env trimer required for further fusion [152]. Panel 4: Subsequently, gp41 is activated and the fusion peptide in gp41 is exposed in order to be inserted into the plasma membrane of the target cell [153]. Panel 5: gp41 folds into a hairpin-like structure and brings the amino terminal (adjacent to the cell membrane) and the carboxyl terminus (attached to the viral membrane) in close proximity [153]. Structural analysis of the two heptad repeat motifs in the N- and the C-terminus indicate that they pack in an antiparallel fashion to generate a highly stable six-helix bundle which brings the membranes into apposition and thus allows membrane fusion to occur via a fusion pore [153,154]. This allows the viral contents to be expelled into the cytoplasm.

proteins by the host cell machinery and the virus elements assemble into new virions at the cell membrane. The virion buds of the host cell membrane and the Gag and GagPol polyprotein precursors are cleaved by viral proteases, either during or after the virus release form the plasma membrane, to generate the mature Gag and Pol proteins [15]. The virus particle is released from the cell using the host membrane bilayer as an outer shell which is embedded with host cell proteins and spikes of the two viral envelope proteins, gp120 and gp41 [18] (Fig. (**1**)).

CHEMOKINE RECEPTORS AS CORECEPTORS FOR HIV CELL-ENTRY

CD4 was identified as an essential component of HIV-1 entry into host cells in 1984 [19,20]. It appeared however, that CD4 alone was insufficient to permit efficient HIV-1 infection [21-23]. It took more than 10 years to identify CCR5 and CXCR4 as the long sought cofactors for HIV cell-entry. Thus, in 1996 an orphan 7TM receptor was identified as a cofactor for HIV-1 strains [7]. It was designated "fusin" due to its role in HIV-1 env-mediated fusion. Analyses indicated that "fusin" acted as a cofactor for T cell line-tropic (T-tropic) HIV strains, but not for Macrophage-tropic (M-tropic) [7]. Among the known members of 7TM receptors, "fusin" had the strongest sequence homology to chemokine receptors [7,24]. This provided a direction for the identification of CCR5 as cofactor for M-tropic isolates. Moreover, a study from 1995 described that $CD8^+$ T cells released some HIV-1 suppressor factors which were identified as RANTES, MIP-1α and MIP-1β, respectively [25]. Not long after, a chemokine receptor (designated CCR5) was identified with specificity for these three chemokines and recognized soon after as the coreceptor for M-tropic HIV-1 [8,26-28]. Definitive evidence of CCR5 as a major prerequisite for HIV-1 infection came from the identification of a mutant allele of CCR5 (Δ32), in which a 32 basepair deletion prevents proper cell-surface expression [29,30]. $CD4^+$ T cells from homozygotes for this allele were highly resistant to infection with M-tropic HIV-1 but not T-tropic strains [29,30]. In 1996, SDF-1 was recognized as the ligand for "fusin", which hereafter was coined CXCR4 [9,31], and it was concomitant shown that SDF-1 inhibited infection by T-tropic HIV-1, but not CCR5-mediated infection by M-tropic HIV-1 [9,31]. The viral tropism for CCR5 and CXCR4 may shift during the infection. Thus viruses with a preference for CCR5 (R5 HIV) are found early in the infection and throughout all stages of the disease, whereas X4 HIV viruses (CXCR4-utilizing) or dual tropic R5X4, which can use both corecep-

tors, are detected in some individuals, and is associated with the late stages when the $CD4^+$ T-cell amount decreases substantially and the progression to AIDS begins [32].

BIOLOGICAL BACKGROUND OF CXCR4

Chemokines (chemotactic cytokines) consist of 60 to 100 amino acids of which many are basic [33]. They play prominent roles in chemotaxis as they are involved in leukocyte activation and trafficking during homeostasis as well as during inflammation [34,35]. The chemokine nomenclature is based on the arrangement of the first two of four highly conserved cysteines: CC indicates that they are adjacent and CXC that they are separated by a single residue. In addition, two minor classes (XC and CX3C) have been described [34,36,37]. Chemokines are in addition divided at a functional basis into *inducible* chemokines, that recruit leukocytes in response to pathophysiological events (also called *inflammatory* chemokines) and *constitutively expressed* chemokines, responsible for the basal leukocyte trafficking and the architecture of secondary lymphoid tissue (also called *homeostatic* chemokines) [34,35].

Chemokine receptors belong to the rhodopsin-like class A 7TM receptors [38]. The extracellular parts have an overall negative charge at physiological pH and are believed to be essential for chemokine binding. In contrast, the intracellular parts are important for the association with the G-protein (mainly $G_{\alpha i}$), and contain Ser and Thr residues that act as phosphorylation-sites during receptor activation and desensitisation [33,37,39]. CXCR4 is more broadly expressed than most other chemokine receptors [40-43] and was for a long time thought to be strictly monogamous in its relationship with the only known natural ligand, SDF-1 (also called CXCL12) [9,31]. However, recently CXCR7 (previously known as RDC) was deorphanized by the identification of SDF-1 as a ligand for this receptor [44]. Interesting, several groups have reported an interaction between CXCR4 and CXCR7, either in the form of heterodimerization [45,46], or in the control of signal-transduction pathways [47]. Furthermore, studies in zebrafish development have shown that CXCR4 and CXCR7 coordinate the migration of facial motor-neurons in the zebrafish hindbrain [48] and that these two receptors interplay in the control of the directionality of primordium migration [49].

The CXCR4/SDF-1 axis is involved in immune cell trafficking as it regulates chemotaxis of B cells, plasma cells, $CD4^+$-T cells, and dendritic cells *in vivo* and activates the tight adhesion of rolling T cells on activated epithelial cells and their subsequent transendothelial migration [50-56]. Furthermore, migration and positioning of B lymphocytes within a secondary lymphoid organ correlates with the CXCR4 response to SDF-1 [57]. Importantly, SDF-1 plays a pivotal role in the homing and anchorage of hematopoietic $CD34^+$ stem cells in the bone marrow [58]. These progenitor cells are mobilized from the bone marrow when SDF-1 drops below the normal threshold level for CXCR4 binding. Cytokines, such as the hematopoietic cytokine G-CSF cause a reduction in SDF-1 levels in the bone marrow, and thereby recruit stem-cells [59]. The mobilized $CD34^+$ cells are thereafter attracted to developmental sites or sites of tissue damage where the level of SDF-1 is upregulated [60]. Accordingly, the CXCR4/ SDF-1 axis is required for normal myelo- and lymphopoiesis and is in addition critical for proper embryonic development of numerous organ systems, and consequently, mice lacking CXCR4 have impaired hematopoiesis, derailed cerebellar neurone migration, defective formation of large vessels and cardiac ventricular septal defects [61-67]. In fact, gene knockout of SDF-1 is also lethal, which is rarely observed for deletion of genes for other 7TM receptors or ligands [62,66,67].

CXCR4 TARGETING BY CHEMOKINE-BASED LIGANDS

The initially discovered anti-HIV properties of the natural chemokines for CXCR4 and CCR5 were shown to be mediated by rapid and extensive receptor internalization [68,69]. Nevertheless, the idea of using natural chemokines as anti-HIV compounds is disturbed by the fact they have a very short half life (<10 min.) and by their potential inflammatory side-effects. To overcome these obstacles several modified chemokines have been designed [70,71]. For example, addition of a N-terminal Met residue to SDF-1 (Met-SDF-1) showed enhanced X4 HIV-1 inhibition which was correlated with prolonged down-regulation of CXCR4 [71]. However, *in vitro* studies with Met-SDF-1 demonstrated a more pronounced intracellular calcium signaling, indicating that *agonistic* chemokines will have inflammatory side-effects when used therapeutically [71]. For this reason, the focus was directed towards CXCR4 *antagonists* as described in details in the next sections.

vMIP2 Encoded by Human Herpesvirus 8 (HHV8)

Molecular mimicry within the chemokine system is widely used among herpes- and poxviruses, where several members contain genes for chemokine receptors and/or chemokine ligands - obtained through an ancient act of molecular piracy [72,73]. HHV8 encodes three CC-chemokine ligands, denoted vCCL1-3 [74-77], and one constitutively active CXC-chemokine receptor ORF74-HHV8 [78-80]. vMIP2 (alternatively known as vCCL2) is a broad acting antagonist that blocks CCR5 and CXCR4 among many other receptors with nano-molar potencies [75,81]. The interaction with CXCR4 (but not CCR5) has been shown to depend upon the N-terminus of vMIP2, as a peptide corresponding to residues 1-21 of vMIP2 retained CXCR4 binding with an IC_{50} of 190 nM in competition with ^{125}I- SDF-1, whereas it had no specific binding to CCR5 in competition with ^{125}I-MIP-1β (Fig. (**2**)) [82,83]. In fact, a more stable analogue of 1-21 vMIP2 consisting solely of residues with D-configuration (DV1) displayed an even higher affinity for CXCR4 (Table (**1**)) [84]. Further truncation of the N-terminus has indicated that the interaction with CXCR4 depends upon the five first residues [82,83]. Other variants of vMIP2 have been designed, for example a dimer of segment vMIP2 (1-11) (dimerized through Cys11) with a 180-fold lower inhibitory potency compared to native vMIP2 [85].

CXCR4 TARGETING BY PEPTIDIC DERIVED / PEPTOID ANTAGONISTS

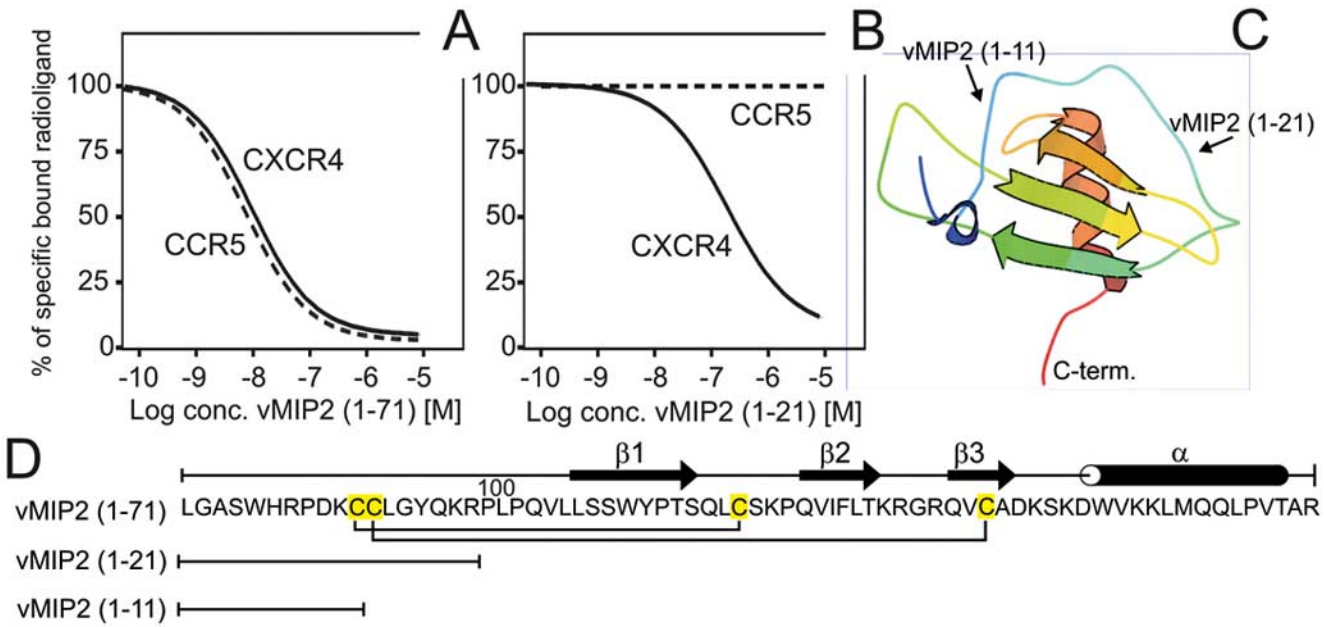

Fig. (2) vMIP2 interaction with CXCR4. (A) and **(B)** Competition binding with vMIP2 (1-71) **(A)** and the N-terminal fragment vMIP2 (1-21) **(B)** in CXCR4 and CCR5 expressing cells, using ^{125}SDF-1 and MIP-1β, respectively as radioligands (adapted and redrawn from [75,82,83]). **(C)** NMR structure ***1hfg*** of vMIP2 (1-71), imported from the Protein Data Base (www.rcsb.org), and drawn by KiNG [85]. Arrows indicate the position for truncation of the two vMIP2 frements (vMIP2 (1-11) and vMIP2 (1-21). **(D)** Primary structure of vMIP2 (1-71) with indications of the two cystein-bridges, the three β-strands, the α-helix, and the two N-terminal fragments vMIP2 (1-11) and vMIP2 (1-21).

T22 is an 18-mer derivative of polyphemusin II - isolated from the American horseshoe crab (*Limulus polyphemus*) - with a potent inhibition of CXCR4 through an interaction with the N-terminus and extracellular loop 1 and 2 (ECL1 and ECL2) [86-88]. However, T22 is highly basic with five Arg and three Lys residues and thereby also troublesome with regard to oral bioavailability. Smaller, more potent, derivatives of T22, referred to as T134 and T140 (both 14-mer), have been constructed in which some of the basic residues were replaced [89-92] (summarized in Table (**1**)). In addition, T140 has been conjugated with the clinically used NRTI (nucleoside reverse transcriptase inhibitor) AZT (azidothymidine), also known as zidovudine or retrovir by a succinate linker [93] in order to combine two different approach for HIV inhibition. Thus a series of AZT-conjugated T140 products have been described, of which the most potent compounds inhibit HIV cytopathicity with nanomolar potencies [93] (Table (**1**)). The binding of T140 nvolves residues in TM-IV (Asp^{171}), TM-VI (Asp^{262}) and in ECL2, Arg^{188} and Tyr^{190} in particular [94].

Other classes of peptidic agents have been described, some of which were initially thought to inhibit the Tat/TAR binding and thus inhibit HIV RNA transcription, as exemplified by CGP64222 [95] and ALX40-4C [96]. CGP64222 is a an nine-residue Arg and Lys-enriched peptoid, that exerts its anti-HIV activities through an inhibition of the interaction between Tat and the *cis*-acting RNA sequence, the transactivation response element (TAR), located downstream of the transcriptional initiation site. In more details, CGP64222 competes with Tat for binding to TAR and furthermore induces a conformational change in TAR through a direct contact between an *N*-Arg side chain in CGP64222 and G26 and U23 from TAR [95]. In addition, CGP64222 interacts directly with CXCR4 and hence also block the CXCR4-medited HIV cell-entry [97]. ALX40-4C is a polypeptide of nine Arg residues stabilized by terminal protection and inclusion of D-amino acids [98]. It interacts specifically with CXCR4 presumably by binding to the negatively charged domains of CXCR4 in competition with the V3 loop of the viral glycoprotein gp120 [98,99] and has been in phase I/II clinical trials [100].

FC131 is a cyclic pentapeptide [cyclo(-Arg^1-Arg^{2}Nal^3-Gly^4-D-Tyr^5-)] derived from the critical residues of T140 for CXCR4 inhibition (Arg^2, L-3-(2-naphthyl)alanine (Nal)^3, Tyr^5 and Arg^{14}) [101]. FC131 displays similar affinity and anti-HIV potency as compared to T140 [91,102-104], and several analogs of FC131 have been described (Table (**1**)) [101,104,105].

A common disadvantage of the antagonists mentioned above is their complex and expensive synthesis. The lack of oral bioavailability is another problem for the majority of these compounds. KRH-1636 and analogs represent orally active peptoid compounds. KRH-1636 contains N-pyridinylmethylene, Arg and naphthalene moieties that interacts selectively with CXCR4 and thereby inhibits X4 HIV strains [106] (Table (**1**)). KRH-2731 is an alkyl-amine analogue of KRH-1636 with higher affinity towards CXCR4, and improved anti-HIV activity in addition to improved oral bioavailability [107].

Table 1. Chemokine-Based and Peptides/Peptidomimetic Compounds Targeting CXCR4. From Left: Classification of Compound Based on Structure, Compound Name, Compound Affinity Measured by Competition Binding Using Either Iodinated SDF-1 or 12G5 (Indicated in Brackets) as Radioligand, Inhibitory Potency Measured by Compound Inhibition of SDF-1 Induced CXCR4 Activation Anti-HIV Potency

Classification	Name	Affinity IC_{50} or K_d*	Potency		Ref.
			Inhibition of SDF-1-Induced Signaling.	Anti-HIV Activity (IC_{50})	
Peptides					
CXC-chemokine	**SDF-1**	3.6 nM*, (SDF-1)	Agonist: 1.1 nM (50% induction of $[Ca^{2+}]_i$ release)	79 nM (LTR-driven transcription of viral genes in $CD4^+$ HeLa cells, SI HIV)	[156]
CXC-chemokine	**SDF-1β**	2.2 nM* (SDF-1β)	Agonist: 1.0 nM (50% induction of $[Ca^{2+}]_i$ release)	40 nM (LTR-driven transcription of viral genes in $CD4^+$ HeLa cells, SI HIV)	[156]
Modified Chemokine	**Met-SDF-1β**	12.4 nM*, (SDF-1) [71]	N/A	1.04 μg/ml (p24 antigen production in PBMCs, III_B HIV)[157]	[71] [157]
SDF-1-derived peptide	**1-9[P2G] dimer**	2580 nM*, (SDF-1)	50 μM (50% inhibition of cell migration)	N/A	[158]
SDF-1-derived peptide	**5-14[C9W, F13-14f] dimer**	290 nM, (SDF-1)	N/A	130 nM (reporter gene in $CD4^+$ HeLa cells, CEM infected cells)	[159]
HIV-derived peptide	**SPC3**	38 nM*, (SDF-1) [160]	N/A	70 nM ($p24^{gag}$ concentration in CEM cells, LAI, NDK, ROD HIV)[161]	[160] [161]
HHV8-encoded CC-chemokine	**vMIP2**	5.8 nM, (SDF-1) [75] 14.8 nM, (SDF-1) [82]	1 μM (100% block of $[Ca^{2+}]_i$ release)[75]	≈ 50 nM (p24 production in US87.CD4 cells, 89.6 HIV)[75]	[75] [82]
vMIP2-derived peptide	**vMIP-II (1-11) dimer**	≈3 μM (12G5)	N/A	N/A	[85]
vMIP2-derived peptide	**V1 (1-21 vMIP2)**	190 nM (SDF-1) [82]	200 μM (100% block of $[Ca^{2+}]_i$ release)[82]	< 50 μM (p24 production in MT-4 cells, III_B HIV)[83]	[82] [83]
vMIP2-derived peptide	**D-V1 (1-21 vMIP2)**	13 nM (SDF-1)	N/A	< 2.5 μM (replication in PBMC, HXBc2 HIV)	[84]
Polyphemusin II-derivative	**T22**	4.8 nM (12G5) [89]	3 μM (100% block of $[Ca^{2+}]$i release)[162]	2.9 nM (HIV-entry in U87.CD4.CXCR4 cells, HXB-2 HIV)[89]	[89] [162]
T22-analog	**T140**	2.5 nM, (12G5) [89] 16 nM, (SDF-1) [90] 4.5 nM, (SDF-1) [91]	N/A	0.18 nM (HIV-entry in U87.CD4.CXCR4 cells, HXB-2 HIV)[89]	[89-91]
T22-analog	**T134**	4.1 nM (12G5)[89]	N/A	9 nM (cytopathicity in MT-4 cells III_B HIV)[92] 2.7 nM (HIV-entry in U87.CD4.CXCR4 cells, 89.6 HIV)[89]	[89] [92]
AZT-conjugated T140	**TZ14003**	N/A	N/A	4.6 nM (cythopathicity in MT-4 cells, III_B HIV)	[93]

Table 1. Contd….

Classification	Name	Affinity IC_{50} or K_d*	Potency		Ref.
			Inhibition of SDF-1-Induced Signaling.	Anti-HIV Activity (IC_{50})	
AZT-conjugated T140	**TZ14007**	N/A	N/A	6.1 nM (cythopathicity in MT-4 cells, III_B HIV)	[93]
	ALX40-4C	3.2 μM, (12G5) [89] 717 nM, (SDF-1) [90]	20 nM (50% inhibition of $[Ca^{2+}]_i$ release)[100]	3.0 nM (replication in HUT78 T cell line, PBMC, NL4.3, HIV)[99] 400 nM (HIV-entry in U87.CD4.CXCR4 cells, HXB-2 HIV)[89]	[89] [90] [100] [99]
T140-derived peptoid (CPP)	**FC131**	3.2 nM, (SDF-1) [91] 4.5 nM, (SDF-1) [163] 4 nM, (SDF-1) [105]	N/A	73 - 88 nM (cytopathicity in MT-4 cells) [91,104]	[91] [163] [105] [104]
FC131 derivative (CPP)	**Ala^3FC131**	63 nM (SDF-1)	N/A	N/A	[101]
FC131 derivative (CPP)	**D-NMe-Ala^3FC131**	42 nM (SDF-1)	N/A	N/A	[101]
FC131 derivative (CPP)	***trans*- 4-guanidino-Pro^3FC131**	10 nM (SDF-1)	N/A	N/A	[101]
FC131 derivative (CPP)	***cis*- 4-guanidino-Pro^3FC131**	10 nM (SDF-1)	N/A	N/A	[101]
FC131 derivative (CPP)	**D-Arg^3FC131**	8 nM (SDF-1) [105]	N/A	0.3 μM (cytopathicity in MT-4 cells) [104]	[104] [105]
FC131 derivative (CPP)	**D-Arg^3-D-Nal^5FC131**	16 nM (SDF-1) [105]	N/A	1.0 μM (cytopathicity in MT-4 cells) [104]	[104] [105]
Basic Peptidomimetic oligomer	**CGP64222**	36 μM (12G5)	50 μg/ml (100% block of $[Ca^{2+}]_i$ release)	8.4 μg/ml (replication in MT-4 cells, III_B HIV)	[97]
Arg-based peptidomimitic	**KRH-1636**	13 nM (SDF-1)	10 μM (strong inhibition of $[Ca^{2+}]_i$ release)	19 nM (replication in MT-4 cells, III_B HIV) 25 – 152 nM (p24 production in PBMCs, NL4.3, YU-6, YU-10, YU-11, HTLV III_B HIV)	[106]
Arg-based peptidomimitic	**KRH-2731**	<1 nM (SDF-1) [107]	10 nM (almost 100% block of $[Ca^{2+}]_i$ release)[107]	1 – 4.2 nM (p24 production in PBMCs, NL4.3, 92HT599, 92HT593, 89.6 HIV) [107]	[107]

CXCR4 TARGETING BY NON-PEPTIDE ANTAGONISTS

The non-peptide antagonists represent an important class of CXCR4 antagonists. Among these, the bicyclams and derivatives of these have displayed the highest potency and specificity. However, the lack of oral bioavailability for the original bicyclams due to the positive charge of +2 for each of the cyclam moieties at physiological pH has set of a chemical treasure hunt for the identification of compounds with improved oral bioavailability.

Bicyclam Compounds Targeting CXCR4

Shortly after the discovery of HIV as the causative agent of AIDS in 1983, one group discovered potent *in vivo* anti-HIV activity of the polyoxometalate HPA-23 [108]. Subse-

quently, multiple polyoxometalates were tested for their antiviral potency which generally was characterized by an EC_{50} of 1 μg per ml [109]. However, in spite of their potent activities, further research on these compounds were halted by the fact that they had to be administered by a parenteral route for a prolonged period of time and thus it was feared that they might be deposited somewhere in the body, e.g. in the liver or kidneys [110,111]. The attention was turned towards the cyclam chelator (1,4,8,11-tetraazacyclotetradecane) based on the rationale that incorporation of the metal ions into an organic molecule instead of a polyoxo shell would be more suitable [112]. Interestingly, the initial testing of different cyclam samples in the absence of metal-ions uncovered a surprisingly high anti-HIV activity with an EC_{50} of 10 μg per ml for one compound [112,113]. Because none of the other samples showed potency values near this range, it was suspected that the anti-HIV activity was caused by an impurity. Indeed, that turned out to be the case, and a huge increase (50-100-fold) in anti-HIV potency was observed upon a purification of this impurity to homogeneity (EC_{50} value of 0.1-0.2 μg per ml) [114]. The impurity turned out to be the bicyclam JM1657, consisting of two cyclam moieties linked by a carbon-carbon bridge (Fig. (**3**)). JM1657 could not be resynthesized; however, instead another potent bicyclam was constructed (JM2763) in which the cyclam rings were tethered by an aliphatic linker (Fig. (**3**)) [115]. Shortly hereafter, the bicyclam JM3100, with an aromatic linker, was presented [116]. This structural constrainment as compared to JM2763 resulted in a huge improvement of the anti-HIV activity through CXCR4 inhibition [89,90,116-118].

Metal-ion Chelating Properties of the Cyclam Compounds

Going back to the starting point, it was tested whether metal-ion chelation of the cyclam rings would enhance the activity of JM3100 (which in the meantime was renamed AMD3100). In short terms, is was found that the ability to inhibit HIV infection was essentially unchanged when AMD3100 was complexed to zinc or nickel and further decreased upon chelation of cobber or cobalt, whereas is was virtually inactive in complex with palladium (Table (**2**)) [119,120]. The affinity to CXCR4 of the AMD3100-Zn

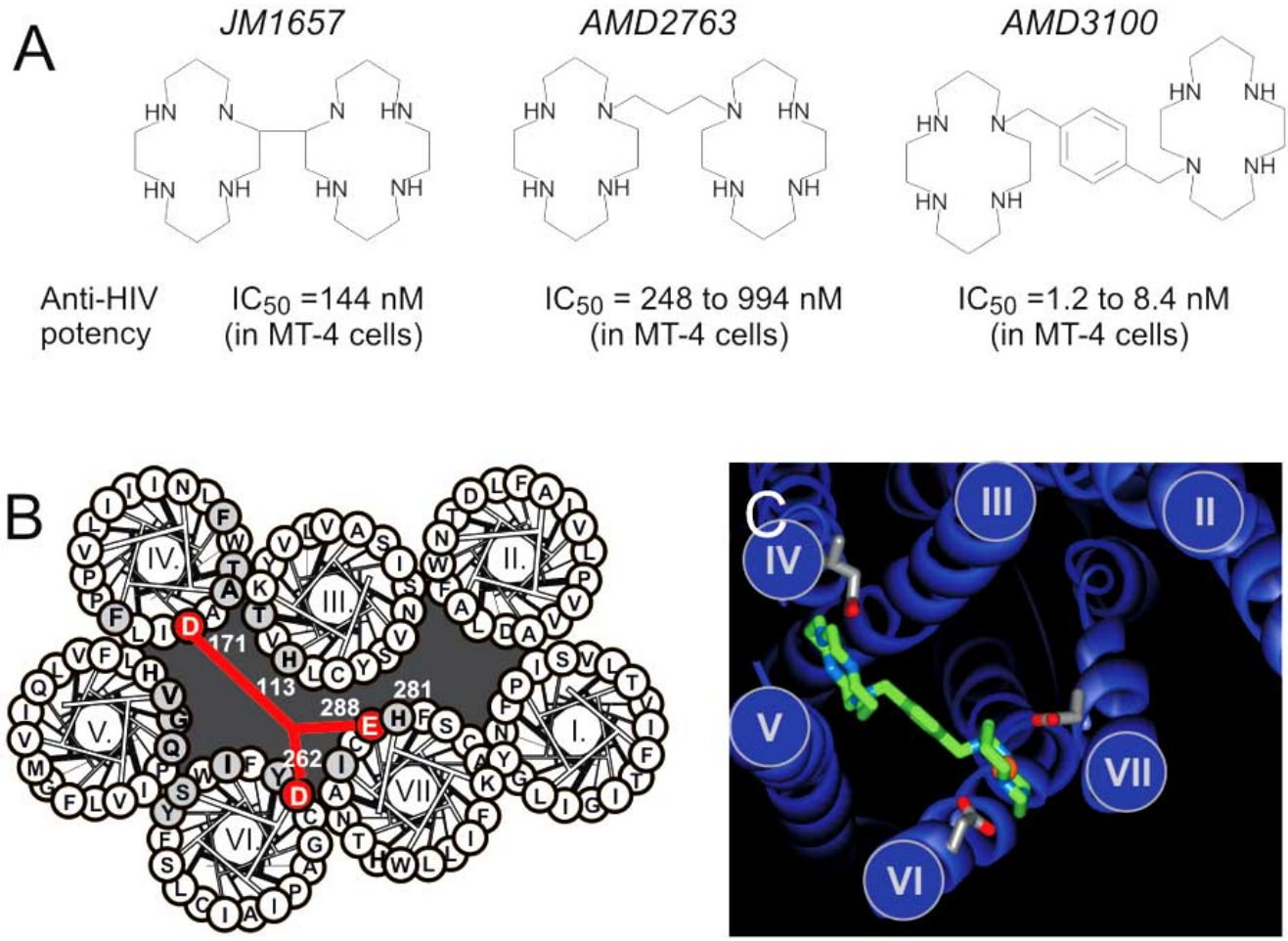

Fig. (3) Bicyclam "evolution" and interaction with CXCR4. (**A**) The initial identified impurity (JM1657), shown together AMD2763 and AMD3100, with indications of the anti-viral properties of these three molecules through CXCR4 inhibition (adapted from Table (**2**)). **(B)** Helical wheel model of CXCR4 (seen from outside the cell) with red-coloring of the three key-residues for AMD3100 binding (Asp^{171}, Asp^{262} and Glu^{288}). All grey residues have been mutated in the mapping of bi- and mono-cyclams in CXCR4, and the positions of His^{281} in TM-VII and His^{113} in TM-III (both with selective importance for AMD3465, but not AMD3100) are indicated. **(C)** Model of the tri-acidic anchorpoint of AMD3100 in CXCR4, based on the crystal structure of rhodopsin [155].

Table 2. Non-Peptide Compounds Targeting CXCR4 From Left: Classification of Compound Based on Structure, Compound Name, Compound Affinity Measured by Competition Binding Using Either Iodinated SDF-1 or 12G5 (Indicated in Brackets) as RADIOLIGAND, Inhibitory Potency Measured by Compound Inhibition of SDF Induced CXCR4 Activation Anti-HIV Potency

Classification	Name	Affinity IC_{50} or K_d*	Potency		Ref.
			Inhibition of SDF-1-Induced Signaling.	Anti-HIV Activity (IC_{50})	
Polyoxometalate	**HPA-23**	N/A	N/A	0.04 – 0.6 μM (cytopathicity in MT-4 cells, HE, ROD, RF, III_B, EHO HIV)	[109]
Bicyclam	**JM1657**	N/A	N/A	144 nM (cytopath. in MT-4 cells, III_B HIV) [115]	[115]
Bicyclam	**JM2762**	N/A	N/A	19 μM (cytopath. in MT-4 cells, III_B HIV)	[115]
Bicyclam	**JM2763 (AMD2763)**	N/A	N/A	248 – 994 nM (cytopath. in MT-4 cells, III_B HIV) [115,141] 0.2- 1.3 μg/ml (cytopath. in MT-4 cells, NL4-3, AOM, RF HIV) [119] 0.2 μg/ml (cytopath. in SUP-T1 cells, 168.10 HIV) [119]	[115] [141] [119]
Bicyclam	**JM2849**	N/A	N/A	616 nM (cytopath. in MT-4 cells, III_B HIV)	[164]
Bicyclam	**JM2936**	N/A	N/A	2.0 μM (cytopath. in MT-4 cells, III_B HIV)	[115]
Bicyclam	**AMD3100 (JM3100)**	13 μM (12G5)[89] 0.1 μM (SDF-1)[90] 33 nM (SDF-1)[141] ≈0.1 μM (12G5)[142] ≈1 μM (12G5)[128]	100 ng/ml (100% block of$[Ca^{2+}]_i$ release)[117] 58 nM (50% inhibition of $[Ca^{2+}]_i$ release)[141] 0.2 μM (50% inhibition of PI turnover)[142] 0.1 μM (50% inhibition of PI turnover)[128]	1.2-8.4 nM (cytopath. in MT-4 cells, RF, HE, III_B, EHO, ROD HIV)[116] 1.1 nM (HIV-entry in U87.CD4.CXCR4 cells, HXB-2 HIV) 12.4 – 99.4 nM (cytopath. in MT-4 cells, III_B HIV) [115,141] 0.005 – 0.13 μg/ml (cytopath. in MT-4 cells, NL4-3, AOM, RF HIV) [119] 0.002 μg/ml (cytopath. in SUP-T1 cells, 168.10 HIV) [119] 3.0 – 14.0 nM (cytopath. in U87.CD4 cells, CI No. 10, NDK, NL4.3 HIV) [142] 0.9 nM (cell fusion between P4-R5 MAGI cells and CHO-K1 cells expressing NL4.3 gp120) [145]	[89] [90] [141] [119] [142] [128] [117] [116] [145]
Bicyclam + metal ions	**AMD3158 (AMD3100(Pd)$_2$)**	12.5 μg/ml (12G5)	70 μg/ml (50% inhibition of $[Ca^{2+}]_i$)	23.4 – 125 μg/ml (cytopath. in MT-4 cells, AOM, NL4.3, III_B, RF HIV) 2.0 μg/ml (cytopath. in SUP-T1 cells, 168.10 HIV)	[119]
Bicyclam + metal ions	**AMD3461 (AMD3100(Co)$_2$)**	0.5 μg/ml (12G5)	0.6 μg/ml (50% inhibition of $[Ca^{2+}]_i$)	0.74 – 11.1 μg/ml (cytopath. in MT-4 cells, III_B, AOM, NL4.3 HIV-1) 6.0 μg/ml (cytopath. in SUP-T1 cells, 168.10 HIV)	[119]
Bicyclam + metal ions	**AMD3462 (AMD3100(Ni)$_2$)**	0.016 μg/ml (12G5)	0.002 μg/ml (50% inhibition of $[Ca^{2+}]_i$)	0.008 – 0.08 μg/ml (cytopath. in MT-4 cells, NL4.3, III_B, AOM, RF HIV) 0.048 μg/ml (cytopath. in SUP-T1 cells, 168.10 HIV)	[119]
Bicyclam + metal ions	**AMD3469 (AMD3100 (Cu)$_2$)**	0.2 μg/ml (12G5)	0.05 μg/ml (50% inhibition of $[Ca^{2+}]_i$)	0.023 – 0.27 μg/ml (cytopath. in MT-4 cells, NL4.3, III_B, AOM, RF HIV)	[119]

Table 2. Contd….

Classification	Name	Affinity IC_{50} or K_d*	Potency		
			Inhibition of SDF-1-Induced Signaling.	Anti-HIV Activity (IC_{50})	
Bicyclam + metal ions	**AMD3479 ($AMD3100(Zn)_2$)**	0.001 μg/ml (12G5)[119] ≈0,01 μM (Met-SDF-1)[121] ≈0,01 μM (12G5)[121]	0.003 μg/ml (50% inhibition of $[Ca^{2+}]_i$)[119]	0.007 – 0.023 μg/ml (cytopath. in MT-4 cells, NL4.3, III_B, AOM, RF HIV)[119] <0.08 μg/ml (cytopath. in SUP-T1 cells, 168.10 HIV)[119]	[119] [121]
Monocyclam	**JM1498**	N/A	N/A	399 μM (cytopath. in MT-4 cells, III_B HIV)[115]	[115]
Monocyclam	**AMD3389**	170 nM (SDF-1) [159]	20 uM (50% inhibition of PI- turnover) [142]	1μM (replication in MT-4 cells, HIV III_B HIV)	[141] [142]
Monocyclam	**AMD3451**	420 nM (Met-SDF-1)	10-40 μM (50% inhibition of Ca^{2+} mobilization)	1.2 μM (replication in MT-4 cells, HIV III_B HIV)	[139]
Monocyclam	**AMD3465**	18 nM (SDF-1) [141] ≈0,1 μM (12G5) [142]	4 nM (50% inhibition of Ca^{2+} mobilization) [141] 9.8 nM (50% inhibition of PI-turnover) [142]	12.3 nM (cytopath. in MT-4 cells, III_B HIV) [141] 2.7 – 36 nM (cytopath. in U87.CD4 cells, NDK, CI No. 10, NL4.3 HIV) [142] 0.4 nM (cell fusion between P4-R5 MAGI cells and CHO-K1 cells expressing NL4.3 gp120) [145]	[141] [142] [145]
Monocyclam	**AMD3529**	N/A	1.7 μM (50% inhibition of PI-turnover) [142]	N/A	[142]
Monocyclam + metal ions	**AMD8721 (AMD3465(Cu))**	N/A	42 nM (50% inhibition of PI- turnover) [142]	N/A	[142]
Monocyclam+ metal ions	**AMD8899 (AMD3465(Ni))**	N/A	11 nM (50% inhibition of PI- turnover) [142]	N/A	[142]
Non-cyclam	**AMD070**	12.5 nM (SDF-1) [145]	9.0 nM (inhibition of Ca^{2+} mobilization) [145]	1.5 nM (cell fusion between P4-R5 MAGI cells and CHO-K1 cells expressing NL4.3 gp120) [145] 1-10 nM (replication in 5 different CD4+ T cell lines, CXCR4 cell lines and PBMCs) [144]	[145] [144]
Non-peptides	**SCR0009418 SCR0014730 SCR0014732 SCR0020108 SCR0024508**	N/A	1-10 μM (inhibition of CXCR4 redistribution) [167]	N/A	[165]

complex was however increased 6-36-fold as compared to AMD3100 alone based on an improved interaction of one of the cyclam rings with the carboxylate group of Asp^{262} in TM-VI (position VI:23) [119,121]. Moreover, in complex with different metal ions, a close correlation was found between the anti-HIV activity and the CXCR4 interaction, including inhibition of SDF-1 induced intracellular Ca^{2+} signaling, the order of decreasing activity being Zn > Ni > Cu > Co > Pd [119]. Zn^{2+} is located in the center of the cyclam ring and coordinates the four nitrogen atoms in a planar fashion [122]. The conditional dissociation constant (K_d) for zinc binding to cyclam is approximately 0.1 pM in blood plasma (pH = 7.4) and the affinity of the alkyl-substituted cyclams is likely to be even higher. Since the level of free Zn(II) in plasma is ≈ 1 nM it is therefore reasonable to assume that $AMD3100(Zn)_2$ can exist *in vivo* and AMD3100 might in fact act as a prodrug [123]. Detailed 1D and 2D NMR studies have shown that metal-ion chelation of the cyclam rings stabilizes the thermodynamically favored cis-V/trans-I and trans-III conformations [123,124].

Quantitative structure-activity relationship (QSAR) analysis of the many different bicyclam-compounds with variable linker-length and -constrainment revealed that the chemical requirements for maximal anti-HIV activity and minimal toxicity appeared to be 1) two metal-chelating macrocyclic rings, not necessarily identical, with an optimum ring size of 14 atoms, and 2) a distance of 9.5-11.5 Å between the metal binding centers [120,125]. Moreover, it was found that compounds with an aromatic linker, such as AMD3100, exhibit superior CXCR4 antagonism and anti-HIV activity as compared to compounds that contain an aliphatic bridge between the two cyclam rings [113,119,125]. X-ray and neutron diffraction structures have shown that the protonated cyclam ring has the propensity to form a direct, hydrogen-bonded stabilized complex with carboxylic acid groups [126].

Binding Mode of Bicyclams in CXCR4

Initial attempts to describe the molecular interaction between AMD3100 and CXCR4 focused on His and Asp residues in CXCR4 due to the propensity of these residues to binds metal ions (chelated with the cyclam-rings) [127]. The results suggested that each of the cyclam rings is positioned in close proximity to an Asp residue in each end of the major binding pocket, namely the Asp^{171} in TM-IV (in position IV:20) and Asp^{262} in TM-VI (in position VI:23), respectively. Two years later the same group performed an exhaustive receptor mutagenesis study of essentially all residues facing into the major binding pocket of CXCR4 and identified Glu^{288} (in position VII:06) as being highly important for the binding of AMD3100 [128] in addition to the previously identified AspIV:20 and AspVI:23. A survey of the major binding pocket of all human 7TM receptors revealed that the combination of these three acidic residues is unique to CXCR4, consistent with the CXCR4-specificity for AMD3100. It was, however, possible to transfer the binding-site of AMD3100 to the otherwise distantly related CXCR3 [128]. Based on the mutational analysis in CXCR4 and the transfer of the tri-dentate acidic binding site to CXCR3 is was suggested, that one cyclam rings interacts with AspIV:20, whereas the other is sandwiched between the carboxylic groups of AspVI:23 and GluVII:06) [127,128].

Clinical trials of AMD3100 revealed poor efficacy and side effects when high doses were given and in combination with poor oral bioavailability further testing of AMD3100 as an anti-HIV drug was discontinued [129]. The detailed knowledge of the binding mechanism of AMD3100 has served as the basis for the design of mono- and non-cyclam compounds mimicking the action of AMD3100 (see later).

An Unexpected Advantage of AMD3100 Treatment

The first human studies of AMD3100 uncovered a dose-dependent increase in white blood cell count due to stem cell mobilization from the bone marrow [130,131]. As mentioned above, $CXCR4^+$ progenitor cells are mobilized to other tissues in the absence of SDF-1 in the bone marrow [58-60]. Thus, prevention of SDF-1 binding to CXCR4 in the bone marrow is the presumed mechanism behind the stem cell mobilizing properties of AMD3100 and recent clinical studies have shown that AMD3100 efficiently mobilize the hematopoietic progenitor cells from the bone marrow into the peripheral blood ready to be collected and used for transplantation [132,133]. Furthermore, AMD3100 has been shown to synergize with G-CSF to achieve a greater mobilization than either compound alone [134,135]. Presently, AMD3100 has been given the generic name Plerixafor and has recently passed through phase III clinical trials and has been approved by the FDA for autologous transplantation in patients with non-Hodgkin's lymphoma and multiple myeloma[2]. The reader is referred to two recent reviews for further details regarding the use of AMD3100 (Plerixafor) in stem-cell recruitment [136,137].

Monocyclam Compounds Targeting CXCR4

AMD3100 is very efficient provided it is given intravenously. For the clinical use as a HIV-entry inhibitor targeting CXCR4, oral availability would be a great advantage due to the global use of such a compound. In order to improve the oral bioavailability and to reduce the putative cyto-toxic positive charge of the aza-macrocyclic compounds, the number of basic amine groups and the overall charge at physiological pH has been reduced. The initial characterizations of the cyclam ring JM1498 (Fig. (**4**)) uncovered a much lower CXCR4 binding affinity and poor anti-HIV activity (Table (**2**)) as compared to the bicyclams [115]. Almost 10 years later, a surprisingly high-affine monocyclam-based compound AMD3389 was presented with a K_i value almost similar to AMD3100 (K_i= 170 nM vs. 74 nM, respectively) [127]. However, at the same time, the potency with regard to the anti-HIV-1 activity ($IC_{50} \approx 1$ μM) was approximately 1000-fold lower [127,138]. Another interesting monocyclam AMD3451 was described in 2004 with antiviral activities against X4, R5 and dualtropic HIV [139]. Thus, AMD3451 acts as an antagonist of CXCR4 as well as CCR5, however unfortunately with low potencies in both cases [139]. Interestingly, both AMD3389 and AMD3451 increased the binding of iodinated 12G5 (a CXCR4-specific monoclonal antibody) [127,139], in contrast to the displacement observed by AMD3100, indicating that both monocyclams affect the position of extracellular loop 2 (which holds important epitopes for 12G5 [140]) in a way that favors antibody recognition [127,139]. As for the binding mode of these simple monocyclams: cyclam alone (JM1498), AMD3389 and AMD3451 mutational analysis have shown that the cyclam ring is affected by substitution of AspIV:20, but not of AspVI:23, indicating that AspIV:20 is the main interaction point of these molecules in CXCR4 [120,127,139].

AMD3465 is the prototype monocyclam with *improved* affinity and inhibitory potency for CXCR4 compared to AMD3100 [141]. It was presented initially in 2005 as an analog of AMD3100, in which one of the cyclam rings was replaced by a 2-pyridylmethylamine moiety (Fig. (**4**)). By different binding assays using fluorescent-labeled 12G5 and iodinated SDF-1, AMD3465 was shown to bind CXCR4 with 8-fold higher affinity compared to AMD3100 [141]. It was furthermore able to antagonize SDF-1-induced CXCR4 activation (Ca^{2+}-release, migration and inositol-phosphate

[2] http://www.nci.nih.gov/cancertopics/druginfo/fda-plerixafor

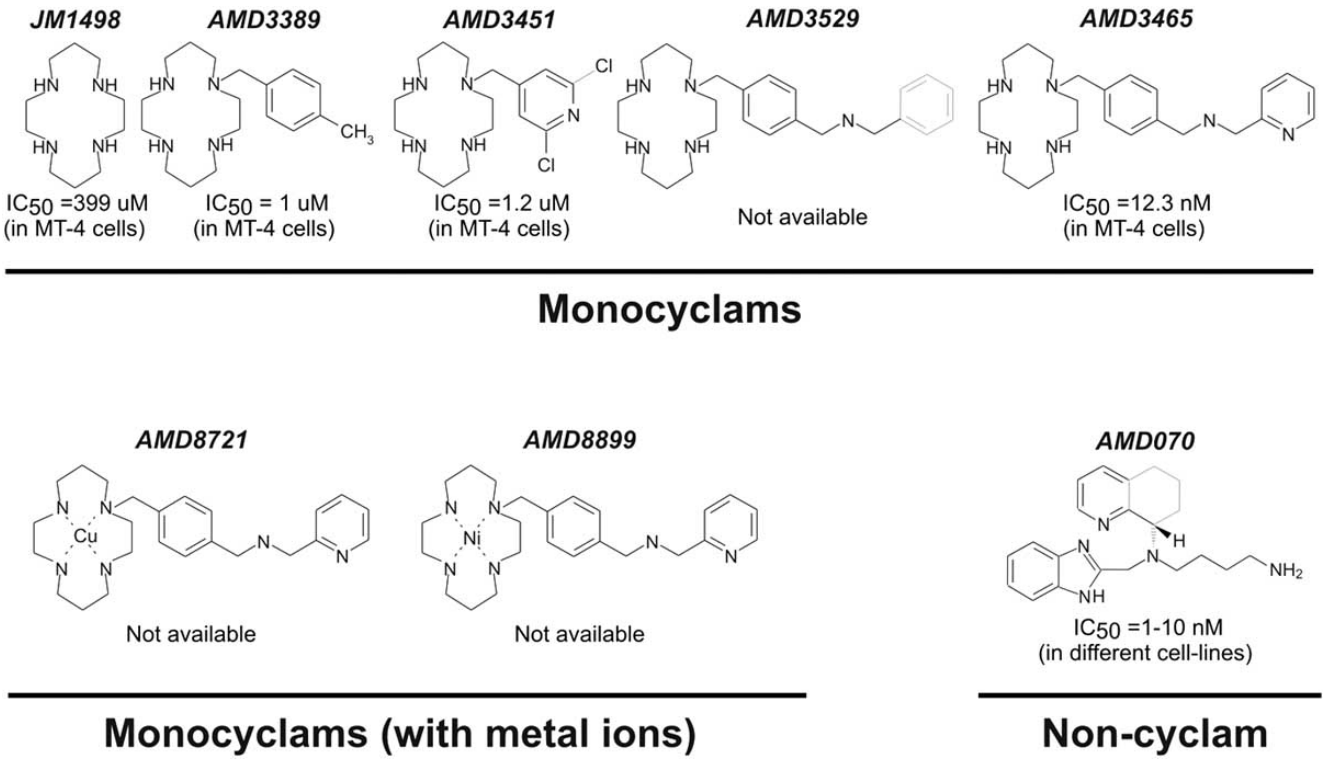

Fig. (4) Selected monocyclams and the non-cyclam AMD070. Upper panel (monocyclams) from left: JM1498, AMD3389, AMD3451 (dual-tropic, but with low affinities towards CCR5 as well as CXCR4), AMD3529 and AMD3465. Lower panel (monocyclams with metal ions): AMD8721 (AMD3465 with Cu) and AMD8899 (AMD3465 with Ni), together with the noncyclam AMD070. The anti-viral properties of the compounds (if available) are indicated below each compound (values are also presented in Table 2).

accumulation) with >20-fold higher potency compared to AMD3100 [142]. With respect to the anti-viral properties, AMD3465 inhibited three different X4 viral strains with potencies similar to or even higher than AMD3100 [141].

Binding Mode of AMD3465 in CXCR4

The improved CXCR4 binding of AMD3465, as compared to AMD3100, inspired a rather comprehensive mapping project. Thus, by employing a library of 23 mutations covering the major ligand-binding pocket of CXCR4 (Fig. (**3**)), the molecular interaction of AMD3465 was found to be dependent on the same three acidic residues as AMD3100 (Fig. (**3**)) [142]. Interestingly, 7 of the 23 mutations were found to impair AMD3465 selectively, with the largest impact of an Ala-substitution of His^{281} (located in ECL3 close to TM-VII (in position VII:-02)). This single substitution resulted in a >4500 fold decreased binding affinity of AMD3465 compared to wt CXCR4. A similar selectivity was observed by Ala-substitution of HisIII:05 (23-fold decrease in affinity) [142].

A series of different monocyclams was employed to determine the relative binding mode of the cyclam part versus the non-cyclam part of AMD3465 (Fig. (**4**)). The compounds varied in the "cyclam" part (e.g. by chelation of a copper-ion in AMD8721 or a nickel-ion in AMD8899) and in the "non-cyclam" part (AMD3389 with no substitution and AMD3529 with a phenyl-ring instead of the missing cyclam ring). The analysis of AMD3389 and AMD3529 in comparison with AMD3465 showed that the absence of an aromatic ring (AMD3389) results in a highly impaired inhibitory potency, and that a phenyl ring (AMD3529) is insufficient as compared to the 2-pyridylmethylamine moiety in AMD3465. At the same time, all analogs appeared to have similar dependency of residues surrounding AspIV:20, which is consistent with the previous observations for simple cyclams being dependent upon AspIV:20 [120,127,139]. Consequently, it was suggested, that the cyclam part of AMD3465 interacts with AspIV:20, and that the non-cyclam part interacts more broadly with residues in the top of TM-VI and VII, of which the His^{281} and Glu^{288} are suggested as interaction partners for the pyridine ring. Thus, both AMD3100 and AMD3465 interact with CXCR4 in a way that prevents proper movement of the outer half of TM-VI towards TM-III, in accordance with the recently proposed "Global Toggle Switch Mechanism" for the activation of rhodopsin-like 7TM receptors [143]. However, like the bicyclam AMD3100, AMD3465 contains a cyclam ring which prevents it from oral bioavailability. Therefore, exploiting the monocyclams is only a step on the way towards developing orally active non-cyclam CXCR4-targeting anti-HIV agents, exemplified by AMD070 [144,145].

Noncyclam Compound Targeting CXCR4

AMD070 is an oral active CXCR4-selective non-peptide antagonist, developed from the series of mono-cyclams (Fig. (**4**)) where the second cyclam ring has been substituted with a more drug-like chemical moiety (Fig. (**4**)). It strongly in-

hibits X4 HIV-1 infectivity with an EC_{50} of 1-10 nM in five different $CD4^+$ T cell lines, CXCR4-transfected cell lines and peripheral blood mononuclear cells (PBMCs) [144]. The non-cyclam was additive or synergistic in its effects when used in combination with other known antiretrovirals and equally active against (non nucleoside reverse transcriptase inhibitors) NNRTI-, (nucleoside reverse transcriptase inhibitors) NRTI- and (protease inhibitors) PI-resistant X4 viruses [60]. Recently, a comparative study suggested partly overlapping binding sites between AMD3100, AMD3465 and AMD070 (in the study referred to as AMD11070) by determining binding-affinities for a series of mutations flanking the major as well as minor binding pocket with ^{125}I-SDF-1 as radioligand [145]. AMD070 is currently in Phase II clinical trials as HIV cell-entry inhibitor.

CONCLUDING REMARKS

An important issue in developing CXCR4 antagonists is the essential role of the CXCR4/SDF-1 axis. What could the potential detrimental effects be of antagonizing such a crucial receptor/ligand axis? As stated, gene knockout experiments in mice of CXCR4 or SDF-1 are lethal *in utero* with harmful defects in the embryonic development, However, these concerns are related to developmental issues and the fact that Plerixafor is well tolerated in the developed organism eliminates the concerns in post-developed individuals. Naturally, it could be argue that blocking the CXCR4/SDF-1a axis is chronic in treatment of HIV and this may provoke side effects not apparent in short-term administration. Phase III and IV studies will elucidate the extent of such putative long-term side-effects. On the other hand, several experiments have shown that the CXCR4 antagonists have additive or even synergistic effects when used in combination with anti-retrovirals targeting other steps of the viral life cycle [144,146]. Hence, the chemokine-targeting HIV inhibitors represent an important complement to existing antiviral therapeutics, and due to the different roles of CCR5 and CXCR4 in the course of an HIV infection, the ideal solution would be to administer antagonists against CCR5 and CXCR4 simultaneously - maybe even as one compound with high affinity for both receptors.

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ABBREVIATIONS

AIDS	=	Acquired Immunodeficiency Syndrome
AZT	=	Azidothymidine
CCR5	=	CC chemokine receptor 5
CPP	=	Cyclopentapeptide
CXCR4	=	CXC chemokine receptor 4
ECL1-3	=	Extracellular loop 1-3
Env	=	Envelope
G-CSF	=	Granulocyte-Colony Stimulating Factor
HAART	=	Highly Active Antiretroviral Therapy
HIV	=	Human Immunodeficiency Virus
HHV8	=	Human Herpesvirus 8 (alternatively known as Kaposi's Sarcoma Associated Herpesvirus
KSHV), IC_{50}	=	Effective concentration for 50% inhibition of maximum activity
ICL1-3	=	Intracellular loop 1-3
M-tropic	=	Macrophage-tropic
MIP-1α	=	Macrophage Inflammatory Protein 1α (= CCL3)
MIP-1β	=	Macrophage Inflammatory Protein 1β (= CCL4)
NRTI	=	Nucleoside Reverse Transcriptase Inhibitor
PBMC	=	Peripheral Blood Mononuclear Cell
R5	=	Virus variants that use CCR5 as coreceptor
R5X4	=	Virus variants that use both CCR5 and CXCR4 as coeceptors (dualtropic)
RANTES	=	Regulated upon Activation, Normal T-cell Expressed, and Secreted (= CCL5)
SDF-1	=	Stromal Derived Factor 1 (= CXCL12)
SI	=	Syncytium Inducing
T-tropic	=	T-cell tropic
TAR	=	transactivation response element
V1-V5	=	Variable regions 1-5 of the HIV gp120-protein
X4	=	Virus variants that use CXCR4 as coreceptor

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