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Activity of Different Bicyclam Derivatives against Human Immunodeficiency Virus Depends on Their Interaction with the CXCR4 Chemokine Receptor

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ABSTRACT

Bicyclams represent a novel class of selective anti-HIV inhibitors with potent activity against T-cell tropic strains of HIV. The prototype compound, the bicyclam AMD3100, has an EC_{50} of 1 to 10 ng/ml against different strains of HIV-1, including clinical isolates. AMD3100 was shown to interact with the CXC-chemokine receptor CXCR4, the main coreceptor used by T-cell tropic strains of HIV. Here we describe the interaction of different bicyclam derivatives with CXCR4. A close correlation (r^2 = 0.7) was found between the anti-HIV potency of the bicyclams and their ability to inhibit the binding of an anti-CXCR4 monoclonal antibody or the intracellular Ca^{++} signal induced by the stromal cell-derived factor-1α, the natural ligand of CXCR4. These results indicate that the mechanism of action of bicyclams is primarily mediated by their interaction with CXCR4. The most potent interaction with CXCR4 and thus anti-HIV activity was shown by bicyclam analogs with cyclam rings composed of fourteen members that are linked by an aromatic (phenyl) bridge. Elucidating the structural requirements for receptor interaction and the site(s) of interaction of bicyclams with CXCR4 will aid in the understanding of HIV-cell fusion.

The discovery of cellular cofactors involved in the entry of HIV into the host cell has renewed the interest in the early steps of virus replication as a target for therapeutic intervention (Cohen, 1997). These cofactors are selectively used by different HIV strains and belong to the family of G protein-coupled, 7-transmembrane proteins that function as receptors for chemokines (Deng et al., 1996; Feng et al., 1996).

Bicyclams are a class of antiviral compounds that act as potent and selective inhibitors of the replication of HIV-1 and HIV-2. Bicyclams are known to inhibit an early event of HIV replication that follows adsorption to the CD4 receptor but precedes reverse transcription (De Clercq, 1992). Thus, bicyclams were identified as HIV fusion/uncoating inhibitors (De Clercq et al., 1992). Recently AMD3100 [1,1′-[1,4-phenylenebis(methylene)]-bis(1,4,8,11-tetrazacyclotetradecane) octahydrochloride dihydrate], the prototype of the bicyclams (De Clercq et al., 1994), has been shown to selectively interact with CXCR4 (Schols et al., 1997a, b) the receptor for the CXC chemokine stromal cell-derived factor (SDF)-1 and also the main coreceptor used by T-tropic strains of HIV (referred as X4 strains, Berger et al., 1998) to enter their host cells (Feng et al., 1996; Oberlin et al., 1996). Small molecules such as AMD3100 that can be readily synthesized and easily administered may have a clear advantage for clinical development. Moreover, the understanding of the mode of action of AMD3100 and bicyclams in general may help to develop newer anti-HIV agents directed to CXCR4 or other chemokine coreceptors used by HIV to enter the cells.

Bridger et al. (1995) and Joao et al. (1995) have shown that the antiviral activity of bicyclam analogs is restricted to the presence of two macrocyclic structures of 12 to 14 members per cyclam ring although identical rings are not required. Furthermore, the distance between the two macrocyclic rings as reflected by the length of the linker and specific substitutions on the phenylenebis(methylene) linker are important requirements for the anti-HIV potency of bicyclams. The structural requirements for the anti-HIV activity of the bicyclam analogs are summarized in Table 1.

In this study we investigated the previous structure-func-

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ABBREVIATIONS: DS, dextran sulfate; mAb, monoclonal antibody; MTT, 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide; PBS, phosphate-buffered saline; SDF, stromal cell-derived factor

tion relationship of the bicyclam analogs for their interaction with CXCR4. From a comparative analysis of the structure-function relationship of the bicyclams from their interaction with CXCR4 and their anti-HIV activity, we conclude that the anti-HIV activity of the bicyclam derivatives primarily depends on their affinity for CXCR4.

Materials and Methods

Compounds. The bicyclam analogs described in Figs. 1 and 2 were synthesized at Johnson Matthey (West Chester, PA) as described previously (Bridger et al., 1995, 1996). The chemokine SDF-1α was purchased from R&D Systems (Abingdon, UK).

Antiviral Assay and Cytotoxicity Assay. Anti-HIV activity and cytotoxicity measurements in MT-4 cells (Harada et al., 1985) were based on viability of cells that had been infected or not infected with HIV-1 exposed to various concentrations of the test compound. After the MT-4 cells were allowed to proliferate for 5 days, the number of viable cells was quantified by a tetrazolium-based colorimetric method as described by Pauwels et al. (1988). Anti-HIV activity in SUP-T1 cells (Smith et al., 1984) was based on inhibition of HIV-1 induced cytopathic effect observed microscopically. Anti-HIV activity in MAGI-CCR5 cells (Chackerian et al., 1997) was determined as follows: cells (1×10^5/ml) were infected with 3000 ng/ml p24 antigen of HIV-1 BaL in the presence of varying concentrations of the test compound. Five days after infection, the cells were washed with phosphate-buffered saline (PBS) and evaluated for beta-galactosidase activity as described earlier (Esté et al., 1995).

The HIV-1 NL4-3 virus (Adachi et al., 1986) is a molecular clone obtained from the National Institutes of Health (Bethesda, MD). The R5X4 HIV-1 RF strain (Alkhatib et al., 1996; Doms et al., 1997) was obtained from the Medical Research Council (London, UK) through the AIDS Reagent Project. The AMD3100-resistant strain (De Vreese et al., 1996a, b) was derived after sequential passage of the NL4-3 virus in the presence of increasing concentrations of AMD3100 in MT-4 cells. The X4 HIV-1 strain AOM is a low-passage clinical isolate from our cohort of HIV positive patients. HIV-1 AOM was able to induce syncytium formation in MT-2 cells and uses CXCR4 as its main entry coreceptor. HIV-1 168.10 (De Jong et al., 1992) is a molecular clone virus that uses CXCR4 and CCR5 as entry coreceptors. HIV-1 BaL (Gartner et al., 1986) is a macrophage tropic strain of R5 phenotype (Doms et al., 1997).

Flow Cytometric Analyses. SUP-T1 cells were incubated with the anti-CXCR4 monoclonal antibody (12G5 mAb) (R&D Systems) for 45 min at 4°C in the presence or absence of 0.5 μg/ml test compound. Then the cells were washed with PBS and incubated with fluorescein isothiocyanate-conjugated goat-anti-mouse antibody (Becton Dickinson, San Jose, CA) for 30 min. The cells were washed with PBS and analyzed by flow cytometry in a FACScalibur system (Becton Dickinson, San Jose, CA). Data were acquired and analyzed with CellQuest software (Becton Dickinson) on an Apple Macintosh computer.

Correlation between the EC_{50} of each drug in the 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide (MTT) assay and the IC_{50} of each drug the 12G5-labeled cells was evaluated using a simple linear regression model with $IC_{50-12G5}$ as the dependent variable. The slope (β), the 95% confidence interval of the slope (95% CI), the Pearson correlation coefficient (r^2), and their statistical significance (p) were calculated.

Measurement of Intracellular Calcium Concentrations. The intracellular calcium concentrations $[Ca^{++}]_i$ were determined as described previously (Wuyts et al., 1997). Briefly, SUP-T1 cells were loaded with Fura-2 (Molecular Probes, Leiden, the Netherlands) or Fluo-3 (Sigma, St. Louis, MO). Fluorescence was measured in a luminescence spectrophotometer fitted with a water-thermostable, stirred 4-position cuvette holder (Perkin-Elmer, Norwalk, CT) or a Fluoroskan Ascent fluorometer (Labsystems, Helsinki, Finland). Cells were first stimulated with dilution buffer (control) or test compound at different concentrations. SDF-1α was used as a second stimulus to induce $[Ca^{++}]_i$ increase; it was added 100 sec after the first stimulus. The compound concentration required to inhibit the $[Ca^{++}]_i$ increase by 50% ($IC_{50\ [Ca++]i}$) was calculated.

TABLE 1
Structural requirements of antivirally active Bis-macrocycles

Requirements
Molecules require two chelating macrocyclic rings for high activity
Distance between metal-binding centers must be 9.5 to 11.5 Å
Plane torsion of −60 to 30° and 120 to 140° are allowed
Plane angles of 40 to 70° and 110 to 140° are allowed
Maximize metal affinity for each macrocyclic ring
Optimum ring size for cyclam rings: 14 atoms
Features to avoid
Plane torsion of 70 to 110°
Plane angles 0 to 35° and 160 to 180°

Adapted from Joao et al. (1995).

Results

Antiviral Activity of Bicyclams against HIV-1 Strains. The antiviral activity as measured by the MTT method (Pauwels et al, 1988) is shown for a series of bicyclam analogs (Table 2). The prototype compound AMD3100 proved to be the most potent inhibitor of HIV-1 NL4 to 3 replication. If the bridge between the two cyclam rings was eliminated as in compound AMD3120, or if the cyclam rings were linked by an aliphatic bridge as in compound AMD2763, instead of an aromatic [phenylenebis(methylene)] bridge, the anti-HIV activity was markedly reduced (1436- and 70-fold respectively). The distance, as measured by the number of atoms in the bridge between the two cyclam rings, also had an influence on the anti-HIV activity even if the aromatic linker was maintained; compound AMD3390 was > 6000-fold less active than AMD3100.

Modifications of the phenyl linker of AMD3100 had various effects on the anti-HIV activity against the NL4-3 strain; inclusion of substituents attached to the aromatic ring in the linker (compounds AMD3068, AMD3196, AMD3128, AMD3203, AMD3207, AMD3208, and AMD3209) resulted in reduced or no antiviral activity. This effect was less if at least two (compounds AMD3207 and AMD3166 were 4-fold and 7-fold less active than AMD3100, respectively) or four substituents (compound AMD3070, was 18-fold less active than AMD3100) were included in the phenyl linker. The reason for reduced activity of these bicyclam analogs appears to result purely from steric hindrance effects and restricted rotation of the macrocyclic rings upon the size of the substituent as demonstrated previously (Bridger et al., 1995).

Alteration of the disposition of nitrogen atoms in the macrocycles also had a detrimental effect on the anti-HIV potency of the compound. AMD6037 and AMD6038 were less active than AMD3100 (40-fold and 8-fold, respectively).

There was a strong correlation between the antiviral activity of the bicyclam analogs tested against HIV-1 NL4-3 and their activity against the low-passage clinical isolate HIV-1 AOM ($r^2 = 0.8$) and the strains HIV-1 168.10 ($r^2 = 0.7$) or HIV-1 RF ($r^2 = 0.7$); therefore, subsequent experiments were correlated to the anti-HIV activity of compounds against HIV-1 NL4-3. Compounds that were highly potent against NL4-3 were evaluated against the R5 strain BaL. Only AMD3479 (the zinc complex of AMD3100) was active against HIV-1 BaL albeit 1000-fold higher EC_{50} of that required to inhibit NL4-3 replication (Table 2).

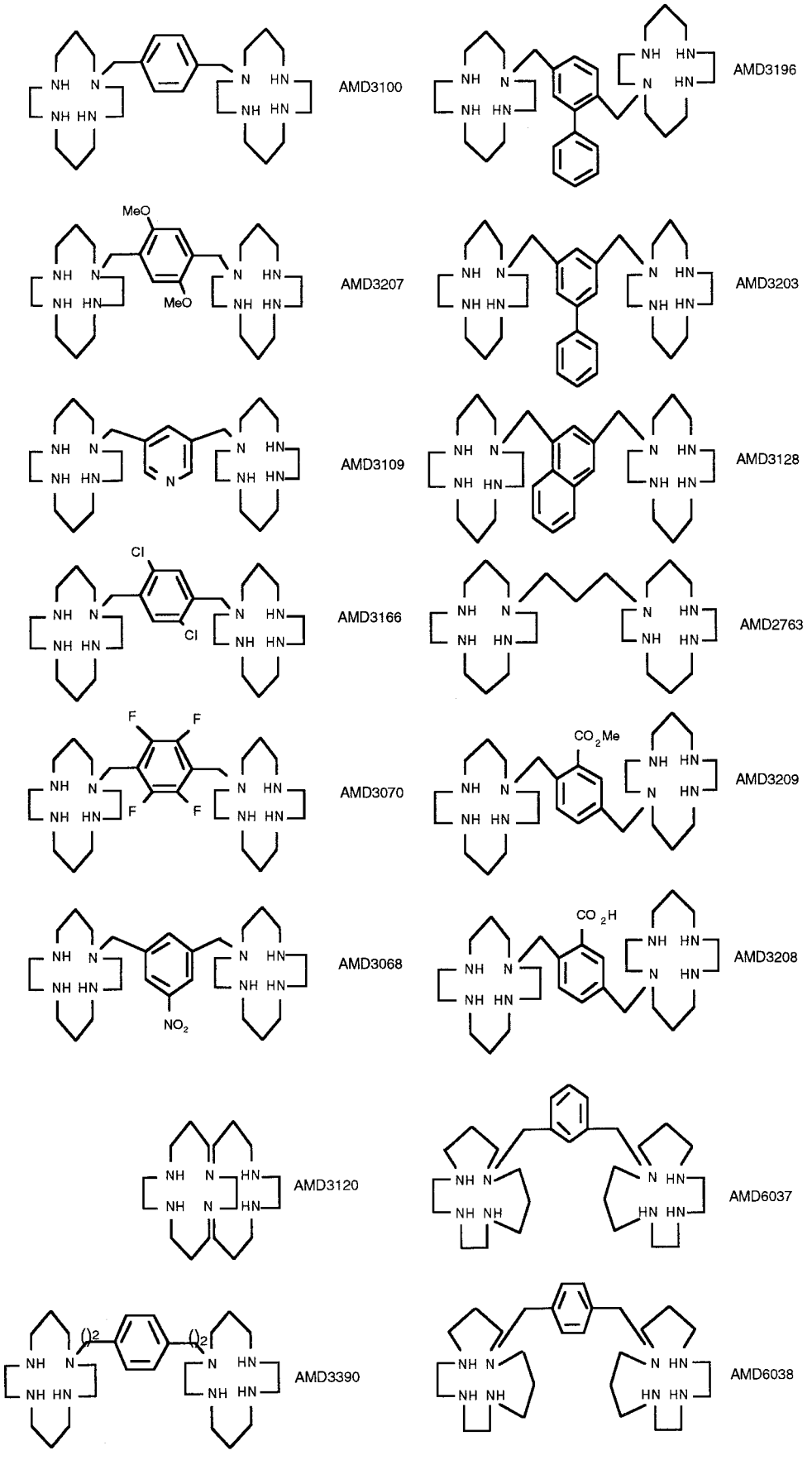

Fig. 1. A, structures of Bis-macrocyclic (bicyclam) analogs.

Antiviral Activity of Bicyclams against NL4-3 AMD3100-Resistant Strain. The AMD3100-resistant strain showed reduced sensitivity to AMD3100 (82-fold resistance as compared with the wild type strain) and was insensitive to SDF-1α at its highest concentration tested (2 μg/ml). The resistant virus was also cross-resistant to all the bicyclam analogs tested, although with different magnitudes. The AMD3100-resistant strain could replicate in the presence of the most potent compounds, as it showed reduced sensitivity to the drugs (43-fold, 33-fold, 12-fold, 19-fold, and 66-fold, against AMD3479, AMD3462, AMD3207, AMD3166, and AMD6038, respectively) (Table 2). Compounds with lesser activity against the wild type strain were less active (AMD3109, AMD3070, AMD3068, and AMD3196 were 50-, 5-, 21-, and 89-fold less active, respectively) or became completely inactive (AMD3128, AMD2763, AMD3209, AMD3120, AMD3208, AMD3158, AMD3469, AMD3390, AMD3461, and AMD6037) against the AMD3100-resistant strain. Notably, compound AMD3203 was only 2-fold less active against the AMD3100-resistant strain.

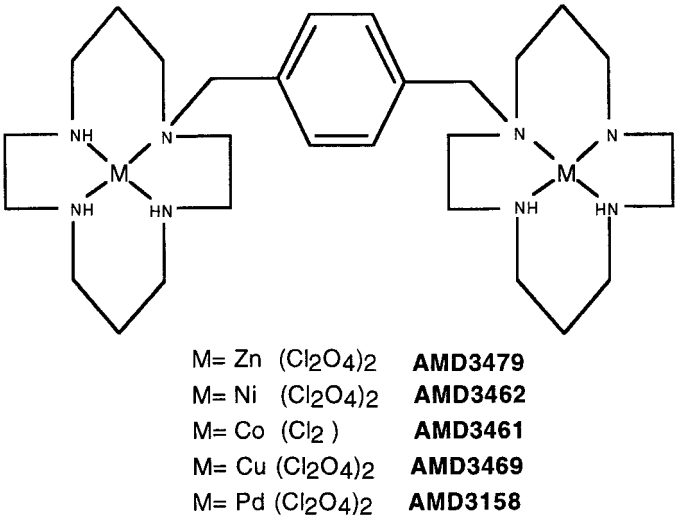

Fig. 2. Structure of transition metal complex analogs of AMD3100.

Interaction with CXCR4 Receptor. To elucidate whether the anti-HIV activity of bicyclam analogs is due to their interaction with CXCR4, we tested the capacity to inhibit the binding of a mAb to CXCR4 of different bicyclam analogs with anti-HIV-1 activity ranging from highly active (EC_{50} values in the ng/ml range as for AMD3100) to analogs selected because of their marginal or no anti-HIV activity (EC_{50} values greater than 10 μg/ml or not active even at 250 μg/ml). SDF-1α, the natural ligand of CXCR4, and active as an HIV-1 inhibitor, was included for comparison to the activity of the bicyclam analogs. Figure 3 shows the correlation for twenty-one bicyclam derivatives between the antiviral activity for HIV-1 NL4-3 (EC_{50}) and the interaction with CXCR4 as measured by the $IC_{50-12G5}$. Compounds showing high affinity for CXCR4 (as measured by the inhibition of 12G5 binding to SUP-T1 cells) exhibited potent anti-HIV activity. A clear correlation was seen between the anti-HIV potency expressed as $\log_{10}$ EC_{50} and the $IC_{50-12G5}$. The correlation coefficient was 0.8 and the calculated r^2 value was 0.7 ($p < .01$). Compared with the bicyclam analogs, SDF-1α at 0.5 μg/ml (roughly the same concentration as its EC_{50} for anti-HIV activity) inhibited by 50% the binding of 12G5 mAb to SUP-T1 cells. Similarly, a close correlation was found between the anti-HIV potency of the compounds tested and their capacity to inhibit the intracellular Ca^{++} signal induced by SDF-1α in SUP-T1 cells ($r^2 = 0.7$) (Fig. 4).

Effect of Metal Complexes to AMD3100. For transition metal complexes of the prototype AMD3100, the anti-HIV-1

TABLE 2
Anti-HIV-1 activity of the different bicyclam analogues and the CXC-chemokine SDF-1α

Compound	EC_{50}[a] (μg/ml)						CC_{50}[b] (μg/ml)
	NL4-3	AOM	RF	168.10[c]	BaL[d]	AMD3100-resistant NL4-3	
AMD2763	0.35	0.19	1.26	0.2	>25	>25	>250
AMD3068	0.134	0.65	0.07	0.02	NT[e]	3.00	>250
AMD3070	0.090	0.067	0.08	0.02	15	0.48	>250
AMD3100	0.005	0.05	0.13	0.002	>25	0.41	>250
AMD3109	0.100	0.05	0.08	0.24	>25	5.00	>250
AMD3120	7.18	86.7	>250	6.0	>25	>50	>250
AMD3128	0.20	0.22	0.36	0.24	>25	>25	>250
AMD3158	25.8	23.4	125	2.0	NT	>100	>250
AMD3166	0.036	0.086	0.12	0.048	NT	0.68	>250
AMD3196	0.134	0.34	0.39	0.24	>25	12.10	>250
AMD3203	0.20	0.08	0.1	0.12	NT	0.42	>250
AMD3207	0.020	0.03	0.07	0.01	NT	0.24	185
AMD3208	>250	>10.0	>10	NT	NT	>250	>250
AMD3209	0.81	0.87	1.4	1.2	NT	>25	>250
AMD3390	30.4	5.6	>250	1.2	NT	>5	>250
AMD3461	11.1	7.98	>250	6.0	NT	>50	>250
AMD3462	0.008	0.028	0.08	0.048	NT	0.26	>250
AMD3469	0.023	0.074	0.27	NT	NT	>1	>250
AMD3479	0.007	0.015	0.023	<0.08	7	0.30	>250
AMD6037	0.20	0.56	1.94	0.24	>25	>25	>250
AMD6038	0.04	0.14	0.22	0.04	>25	2.65	>250
SDF-1α	0.30	1.06	1.36	0.2	NT	>2.00	>5

[a] EC_{50} as measured by the MTT assay.
[b] CC_{50}: 50% cytotoxic concentration, or concentration of the compound required to reduce the viability of MT-4 cells by 50%, as measured by the MTT assay.
[c] EC_{50} in SUP-T1 cells.
[d] EC_{50} based on the inhibition of HIV-1-induced β-galactosidase activity in MAGI-CD4-CCR5 cells.
[e] NT, not tested.

activity depended on the bound metal (Table 3). The Zn^{++} complex (AMD3479) was slightly more active (10-fold) than AMD3100; the Ni^{++} complex (AMD3462) was as active as AMD3100 in their capacity to inhibit 12G5 binding. The Cu^{++} (AMD3469) and Co^{+++} (AMD3461) complexes were 5-fold and 2220-fold less active, respectively, than AMD3100. The Pd^{++} complex (AMD3158) was virtually inactive. Similar differential inhibitory effects were noted for the metal complexes on the binding of the mAb with CXCR4; the IC_{50} for 12G5 binding to SUP-T1 cells closely paralleled the EC_{50} for anti-HIV activity ($r^2 = 0.8$). Similarly, the EC_{50} for antiviral activity of metal complexes correlated with the IC_{50} for inhibition of the Ca^{++} flux induced by SDF-1α indicating the dependence on the interaction of metal-complexed bicyclams with CXCR4 for their respective anti-HIV activity.

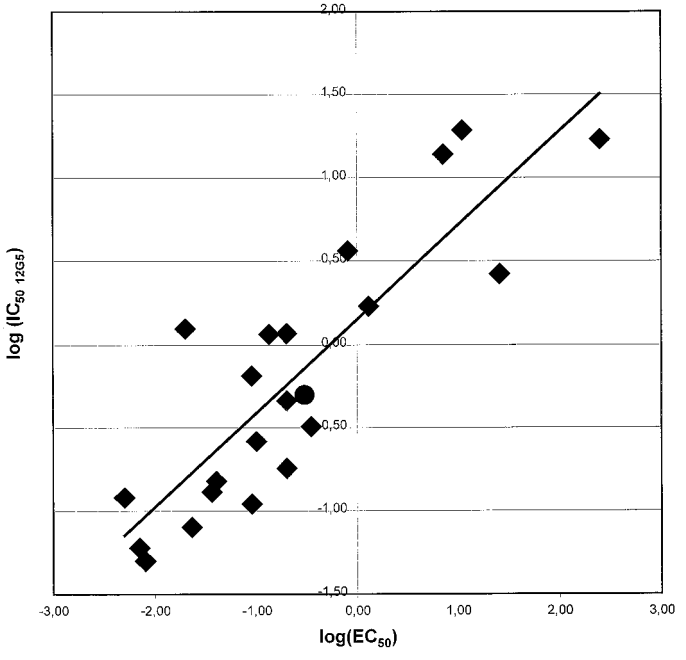

Fig. 3. Correlation of the anti-HIV-1 (NL4–3) activity and interaction with the CXCR4 receptor of the different bicyclam analogs as assessed by linear regression analysis. Anti-HIV-1 activity [log(EC_{50})] is plotted against the mean fluorescence intensity of SUP-T1 cells labeled with the CXCR4 mAb (12G5) and FITC-conjugated anti-mouse antibody in the presence of 0.5 μg/ml SDF-1α (●) or each of the bicyclam analogs presented in Table 2 (♦).

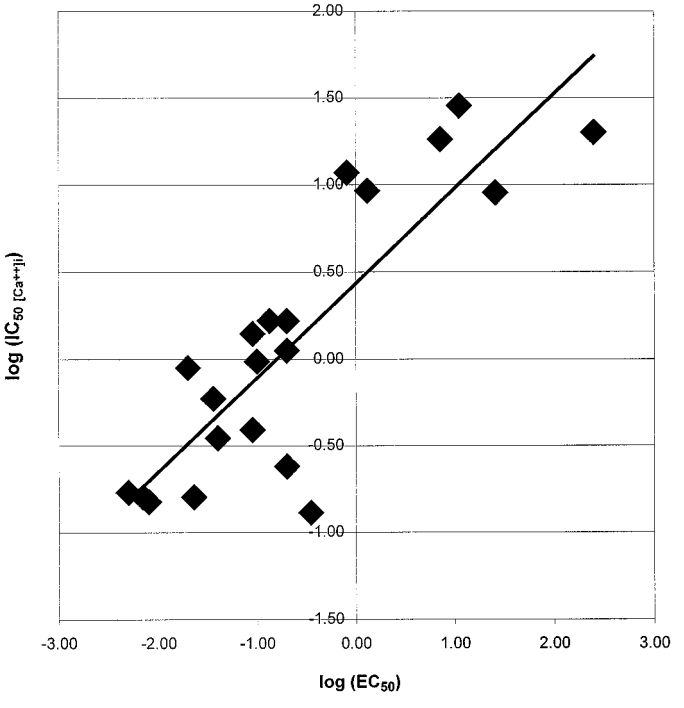

Fig. 4. Correlation of the anti-HIV-1 (NL4–3) activity and interaction with the CXCR4 receptor of different bicyclam analogs as assessed by linear regression analysis. Anti-HIV-1 activity [log(EC_{50})] is plotted against the inhibition of SDF-1α-dependent intracellular Ca^{++} mobilization [log ($[Ca^{++}]_i$)] in SUP-T1 cells.

Discussion

Earlier studies have shown that bicyclams, while being very potent inhibitors of HIV-1 replication, fail to inhibit virus-cell binding and are ineffective in blocking the viral reverse transcriptase or protease in cell-free systems (De Clercq et al., 1992, 1994). From this earlier work it was suggested that bicyclams must interfere with a postbinding event coinciding with the virus fusion/uncoating process. De Vreese et al. (1996a, b) selected, after prolonged passage of the HIV-1 NL4-3 strain in MT-4 cells in the presence of increasing concentrations of AMD3100, a mutant strain that was approximately 100-fold resistant to the compound. Resistance to AMD3100 was mapped to the envelope gp120 molecule. Several mutations leading to amino acid substitutions were found in the V3-V5 domain; they appeared to be particularly clustered at or near the V3 loop (De Vreese et al., 1996b). Thus, the HIV glycoprotein gp120 was suggested as the target of bicyclams but the specific site and mode of interaction with gp120 remained elusive.

With the discovery of the chemokine receptors as cofactors for the entry of HIV into $CD4^+$ cells, the mode of action of bicyclams has become clearer. We have shown that AMD3100 selectively interacts with CXCR4 (Schols et al., 1997a, b) which pointed to the direct interaction of CXCR4 with bicyclams as the mode of action of this class of compounds. The correlation shown here between the anti-HIV activity of the different bicyclam analogs and their interaction with the CXCR4 receptor (as monitored by inhibition of mAb 12G5 binding to cells and inhibition of SDF-1α-dependent intracellular Ca^{++} flux) strongly suggests that blockade of the interaction between HIV and CXCR4 is the primary site of intervention of the bicyclams.

All bicyclam analogs that showed activity against HIV-1 NL4-3 were also active against an X4 HIV-1 clinical isolate

TABLE 3
Anti-HIV-1 activity, inhibition of 12G5 mAb binding and inhibition of $[Ca++]_i$ flux of AMD3100 and its different transition metal complexes

Compound (bound metal)	EC_{50}[a] for HIV-1 (IIIB)	IC_{50}[b] 12G5 binding	$IC_{50\ [Ca++]i}$[c]
		$\mu g/ml$	
AMD3100 (free)	0.009	0.01	0.005
AMD3479 (Zn)	0.008	0.001	0.003
AMD3462 (Ni)	0.008	0.016	0.002
AMD3469 (Cu)	0.048	0.2	0.05
AMD3461 (Co)	0.74	0.5	0.6
AMD3158 (Pd)	68.62	12.5	70

[a] EC_{50} as measured by the MTT assay.
[b] IC_{50}: 50% inhibitory concentration, or concentration of the compound required to inhibit by 50% the binding of 12G5 mAb to CXCR4+ SUP-T1 cells.
[c] $IC_{50\ [Ca2+]i}$ by SDF-1α in SUP-T1 cells.

AOM and the HIV-1 RF and 168.10 strains that primarily uses CXCR4 as coreceptor but can enter cells by CCR5 as cofactor (Alkhatib et al., 1996, data not shown). There was a close correlation between the antiviral activity of the different compounds against these three HIV-1 strains. However, the most active compounds (AMD3100, AMD3462, AMD3479, AMD3207, and AMD3469) were slightly less active against HIV-1 AOM and RF. Clinical isolates of HIV are composed of a heterogeneous population, whereas the RF strain may use CCR5 (although inefficiently) to enter cells (Alkhatib et al., 1996; Doms et al., 1997). The anti-HIV activity of bicyclams would be attenuated by the ability of HIV-1 to use other coreceptors; nevertheless, the correlation found between the anti-HIV activity against NL4-3, AOM, RF, and 168.10 reiterates that bicyclams interfere with HIV replication through a similar mode of action. Furthermore, the lack of activity shown by different bicyclam analogs against the R5 strain BaL indicates that bicyclams are only active against those strains that can use CXCR4 as entry coreceptor.

We have clearly shown that the interaction of bicyclams with CXCR4 (monitored by inhibition of 12G5 mAb binding) follows a similar structure-activity relationship as found earlier for inhibition of HIV-1 replication (Bridger et al., 1995, 1996). The interaction with the CXCR4 receptor appears to depend on the size of the tetraazamacrocyclic rings (which should be restricted to no more than fourteen members) and the linker [preferably phenylenebis(methylene)]. Also, for the metal-AMD3100 complexes, a close correlation was found between the anti-HIV activity and CXCR4 interaction, the order of decreasing activity being Zn > Ni > Cu > Co > Pd. Furthermore, the anti-HIV activity of bicyclams also parallels their capacity to inhibit the intracellular Ca^{++} signal induced by SDF-1α, suggesting that bicyclams inhibit HIV-1 replication through a similar mode of action as SDF-1.

We have shown that HIV binding inhibitors such as dextran sulfate (DS) and the oligonucleotide AR177 (Zintevir) are no longer able to inhibit the binding of DS-resistant and AR177-resistant viruses (Esté et al., 1997, 1998) thus confirming the mode of action of these compounds (i.e., inhibition of virus adsorption to the cells). Mutations required to generate partial resistance to AMD3100 (De Vreese et al., 1996b) also lead to cross-resistance to polyanions such as DS (Esté et al., 1996) and to the chemokine SDF-1α (Schols et al., 1998). DS-resistant NL4-3 (Esté et al., 1997), AR177-resistant NL4-3 (Esté et al., 1998), and SDF-1α-resistant NL4-3 (Schols et al., 1998) show mutations in the gp120 that are also present in the AMD3100-resistant strain. At first glance these results suggest that polyanions may share similarities in their mode of action to bicyclams, that is, polyanions such as DS or AR177 could interact with postbinding events. However, the cross-resistance observed could be explained by an indirect effect on virus binding to $CD4^+$ cells as an consequence of the virus escaping the antagonism of AMD3100 on CXCR4 through mutations in the gp120 glycoprotein.

To escape the antiviral activity of bicyclams, the AMD3100-resistant strain could have switched coreceptor use or (as it has been demonstrated with different HIV-1 and HIV-2 strains) it could be using CXCR4 differently than the parental NL4-3 virus (Brelot et al., 1997). The results presented here do not address this issue; however, the AMD3100-resistant strain was cross-resistant to all the bicyclam analogs tested. This indicates that all the bicyclams share the same mode of action with AMD3100, that is, they "see" CXCR4 in a similar fashion. If different virus strains interact with CXCR4 in a different fashion (i.e., the AMD3100-resistant virus as opposed to the NL4–3 virus) that allows them to escape the anti-HIV activity of AMD3100, then all bicyclam analogs will show a reduced inhibitory capacity because they all appear to interact in a similar fashion. Nevertheless, their specific activity against 12G5 binding and SDF-1α-induced intracellular Ca^{++} flux points to their inhibition of the HIV-fusion process through interaction with the HIV entry cofactor CXCR4.

The bicyclam derivatives exhibit a mode of anti-HIV activity that is clearly different from that of the other anti-HIV agents presently used or considered for use in the treatment of HIV infection. However, after the report by Schols et al. (1997a) on the AMD3100-CXCR4 interaction, two other groups described newly identified CXCR4 antagonists: 1) ALX40-4C, a polycationic nonapeptide solely existing of arginine residues (Doranz et al., 1997) and 2) T22 (Murukami et al., 1997), an 18-residue peptide which has eight positive charges. As AMD3100 is also positively charged, it appears that the cationic nature of these compounds is necessary for their activity. Furthermore, the restriction on the number and position of amino groups in the bicyclam structure suggests that specific disposition of positive charges is required for strong interaction with CXCR4. Furthermore, T22, like AMD3100, may form a Zn^{++} complex that is 4-fold more active than T22 itself (Tamamura et al., 1996). Because the Zn^{++} complex of AMD3100 was 10 times more potent than AMD3100 in its interaction with CXCR4, it is possible that Zn^{++} complex formation of these antagonists of CXCR4 may be of importance.

The recent studies by Tachibana et al. (1998) and Zou et al. (1998) have revealed that CXCR4 and SDF-1 are important in embryonic development and could have nonredundant functions in adults. This poses serious concerns on the use of CXCR4 antagonists as therapeutic agents against HIV. A possible toxic effect was not reported after administration of AMD3100 (10 mg/kg/day b.i.d.) to SCID-hu Thy/Liv mice for 28 days, in spite of a significant decrease in HIV viral load in the infected mice (Datema et al., 1996). Furthermore, homozygosity for an SDF-1 gene variant that has been associated with a delayed progression to AIDS is found in about 3% of healthy individuals studied (Winkler et al., 1998). Although this finding appears to be controversial (Mummidi et al., 1998) alterations of the SDF/CXCR4 systems may not necessarily induce an adverse condition in healthy individuals. In turn, low but significant levels of a CXCR4 antagonist could block the development of X4 strains that are clearly associated with disease progression (Fauci, 1996; Glushakova et al., 1998).

Bicyclams not only demonstrate the feasibility of developing nonpeptidic, small-molecule antagonists to the chemokine receptors but may serve, through the understanding of the structural components that are required for coreceptor interaction, for the development of new compounds against a broader spectrum of HIV-1 strains.

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