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In Vitro Anti-Human Immunodeficiency Virus (HIV) Activity of XM323, a Novel HIV Protease Inhibitor

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XM323 represents a novel class of potent inhibitors of human immunodeficiency virus (HIV) protease. In vitro studies have shown that inhibition of this enzyme translates into potent inhibition of replication of HIV type 1 (HIV-1) and HIV-2. The inhibition of virus replication was assessed with three assays designed to measure the production of infectious virus, viral RNA, or p24 antigen. The production of mature infectious virions was measured with a yield reduction assay. By this assay, several strains and isolates of HIV-1 and HIV-2 were shown to be susceptible to XM323 in two lymphoid cell lines (MT-2 and H9) and in normal peripheral blood mononuclear cells, with a concentration required for 90% inhibition (IC_{90}) of 0.12 ± 0.04 μM (mean ± standard deviation). The production of HIV-1(RF) RNA was measured with an RNA hybridization-capture assay. With this assay, XM323 was shown to be a potent inhibitor of HIV-1(RF) replication, with an IC_{90} of 0.063 ± 0.032 μM. A third measure of virus replication, the production of p24 viral antigen, an essential protein component of the virion, was determined with the AIDS Clinical Trial Group-Department of Defense peripheral blood mononuclear cell consensus assay. This assay was used for expanded testing of XM323 against 28 clinical isolates and laboratory strains of HIV-1. XM323 was shown to be equally effective against zidovudine-susceptible and zidovudine-resistant isolates of HIV-1, with an overall IC_{90} of 0.16 ± 0.06 μM.

The aspartic acid protease encoded by human immunodeficiency virus (HIV) is critical for replication of the virus (4, 11). This enzyme is responsible for specific cleavages of the viral *gag* and *gag-pol* gene products, which are precursors of essential viral structural proteins and essential enzymes, including reverse transcriptase, integrase, and the protease itself (3, 17, 19). Inhibition of this enzyme by synthetic inhibitors during infection in vitro leads to a reduction in the number of infectious virus particles produced (13). This is presumably due primarily to incomplete processing of the $p55^{gag}$ polyprotein to the essential structural proteins p24, p17, p7, and p6.

To date, most inhibitors of the HIV aspartic acid protease have been transition state mimetics. These have included reduced amides (5, 14), hydroxyethylene isosteres (5, 18, 23, 24), statine analogs (5), phosphinic acid derivatives (6), and difluoroketone derivatives (5, 21).

XM323 represents a new class of protease inhibitors that are referred to as cyclic ureas. The description of the design, synthesis, and characteristics of this class of protease inhibitors will be published elsewhere (12). These compounds lack the amide bonds found in previous inhibitors but retain the symmetry of the potent C-2 symmetrical diols (10). XM323 is a potent inhibitor of purified HIV type 1 (HIV-1) protease, with a K_i of 0.27 nM (12). In this report, we present the anti-HIV activity of this compound as determined by three different measurements. Efficacy was determined by effects on the production of viral RNA, p24 antigen, and infectious virus. The susceptibilities of laboratory strains of HIV-1, HIV-2, and clinical isolates of HIV-1 were assessed.

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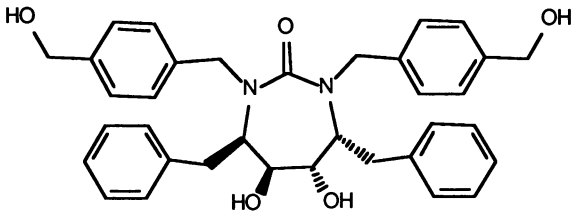
MATERIALS AND METHODS

Compounds. XM323 {[4*R*-(4α,5α,6β,7β)]-hexahydro-5,6-bis(hydroxy)-1,3-bis([4-(hydroxymethyl)phenyl]methyl)-4,7-bis(phenylmethyl)-2*H*-1,3-diazepin-2-one} was synthesized by chemists at DuPont Merck Pharmaceutical Co. (Fig. 1). Q8111 is a compound synthesized by DuPont Merck chemists to be equivalent to Ro31-8959 (18) from Roche. YY752 is a compound synthesized by DuPont Merck chemists to be equivalent to A80987 (9) from Abbott Laboratories. Zidovudine (AZT) was obtained from Burroughs Wellcome, and ddC was purchased from Sigma.

HIV yield reduction assay. MT-2, a human T-cell leukemia virus type 1-transformed human T-cell line, was cultured in RPMI 1640 medium supplemented with 5% (vol/vol) heat-inactivated fetal bovine serum (FBS), 2 mM L-glutamine, and 5 μg of gentamicin per ml. HIV strains HIV-1(IIIB), HIV-1(RF), and HIV-2(ROD) were propagated in H9 cells, a T-cell lymphoma cell line, in RPMI 1640 with 5% FBS. Routine testing for mycoplasma (2) ensured that all experiments were performed with mycoplasma-free MT-2 and H9 cells. Poly-L-lysine (Sigma)-coated cell culture plates were prepared according to the method of Harada et al. (7). MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was obtained from Sigma.

Test compounds were dissolved in dimethyl sulfoxide (DMSO) to 5 mg/ml and serially diluted into RPMI 1640 medium to 10× the desired final concentration for a final concentration of DMSO of ≤0.5%. This concentration of DMSO was found to have no effect on the yield of infectious virus in this assay. MT-2 cells (5×10^5/ml) in 2.3 ml were mixed with 0.3 ml of the appropriate test compound solution and incubated for 30 min at room temperature. Virus (approximately 5×10^5 PFU/ml) in 0.375 ml was added to the cells and compound mixtures for a multiplicity of infection (MOI) of 0.2 PFU per cell and was incubated for 1 h at 36°C. The cultures were centrifuged at ~300 × *g* for 10 min, and



XM323

FIG. 1. Structure of {[4*R*-(4α,5α,6β,7β)]-hexahydro-5,6-bis(hydroxy)-1,3-bis([4-(hydroxymethyl)phenyl]methyl)-4,7-bis(phenylmethyl)-2*H*-1,3-diazepin-2-one}.

the supernatant fluids containing unattached virus were discarded. The cell pellets were suspended in fresh RPMI 1640 containing the appropriate concentrations of test compound and placed in an incubator at 36°C (3% CO_2). Virus was allowed to replicate for 3 days. Cultures were centrifuged for 10 min at 1,000 rpm, and the supernatant fluids containing cell-free progeny virus were removed for plaque assay.

The titers of progeny virus produced in the presence or absence of test compounds were determined by a plaque assay (22) similar to that described by Nakashima et al. (15). Suspensions of progeny virus were serially diluted in RPMI 1640, and 1 ml of each dilution was added to 9 ml of MT-2 cells in RPMI 1640. Cells and virus were incubated for 3 h at 36°C to allow efficient attachment of the virus to cells. Each virus-cell mixture was divided equally between two wells of a six-well poly-L-lysine-coated culture plate and incubated overnight at 36°C in 3% CO_2. Liquid and unattached cells were removed prior to the addition of 1.5 ml of RPMI 1640 with 0.75% (wt/vol) SeaPlaque agarose (FMC Corp.) and 5% FBS. Plates were incubated for 3 days, and a second RPMI 1640-agarose overlay was added. After an additional 3 days at 36°C in 3% CO_2, a final overlay of phosphate-buffered saline (PBS [Sigma]) with 0.75% SeaPlaque agarose and 1 mg of MTT per ml was added. The plates were incubated overnight at 36°C. Clear plaques on a purple background were counted, and the number of PFU of virus was calculated for each sample. The antiviral activity of test compounds was determined by the percent reduction in the virus titer compared with the amount of virus grown in the absence of any inhibitors.

Isolation of HIV-1 from clinical samples. HIV-1 was isolated from patient peripheral blood mononuclear cells (PBMCs) by cocultivation with phytohemagglutinin- and interleukin 2 (IL-2) (Roche)-stimulated PBMCs from normal donors. Target PBMCs from normal HIV-seronegative donors were obtained by Ficoll-Hypaque gradient centrifugation of heparinized blood. After the cells were washed once in PBS and twice in RPMI 1640, the cells were resuspended at a density of approximately 2×10^6/ml in RPMI 1640 with 10% FBS in an appropriately sized tissue culture flask. Phytohemagglutinin was added to a concentration of 5 μg/ml and IL-2 was added to a concentration of 1,000 Roche-Cetus U/ml. The cells were incubated at 36°C in 5% CO_2 for 72 h to induce blast transformation.

After obtaining written informed consent from selected Medical Center of Delaware Infectious Disease Clinic patients, 14 to 21 ml of heparinized blood was drawn for virus isolation. Patient PBMCs were obtained by Ficoll-Hypaque separation as described above. The previously stimulated normal donor PBMC target cells were washed in fresh media and resuspended at a density of 1×10^6 to 2×10^6/ml. Approximately 10^7 patient PBMCs were added to an equal number of the target cells in RPMI 1640 with 10% FBS and 1,000 U of IL-2 per ml. The cultures were incubated at 36°C in 5% CO_2 in a T-25 tissue culture flask. Approximately 10^7 fresh target cells were added every 7 days, and half of the medium was changed twice weekly. Cultures were routinely expanded to a T-75 flask during the second week. Infections were monitored for formation of syncytia visually and through p24 antigen determinations at least twice weekly as described below. Cultures were maintained for at least 4 weeks before being discarded as negative. Aliquots of positive cultures were transferred to 1.8-ml cryovials (Nalge Co., Rochester, N.Y.) and stored in liquid nitrogen.

Titration of virus stock for PBMC assay. Virus stocks were titrated after serial fourfold dilutions in a 96-well microtiter plate. Normal donor phytohemagglutinin- and IL-2-stimulated PBMCs in RPMI 1640 medium (10% FBS, 1,000 U of IL-2 per ml) were added to each well at 2×10^5 cells per well. Plates were incubated at 36°C in 5% CO_2. On day 4, cells were resuspended and approximately half of the culture was replaced with fresh medium. On day 7, Triton X-100 was added to a final concentration of 0.5% to lyse cells and inactivate virus. Plates with cell lysates were frozen at −70°C until p24 antigen could be determined. After plates were thawed, 100 μl of supernatant fluid was transferred to a fresh 96-well microtiter plate containing 100 μl of RPMI 1640 per well. The p24 antigen concentration was determined with the DuPont, NEN HIV-1 p24 antigen capture assay. Wells were scored positive if the p24 concentration was >50 pg/ml, and 50% tissue culture infective doses for the virus stocks were determined.

AIDS Clinical Trials Group-Department of Defense PBMC consensus assay. The consensus assay used in this study was developed cooperatively by members of the AIDS Clinical Trials Group and the U.S. Department of Defense (8). Test compounds were prepared as described above. Phytohemagglutinin- and IL-2-stimulated donor PBMCs (1.6×10^7) in RPMI 1640 were infected with 1.6×10^4 50% tissue culture infective doses of virus (MOI, ~0.001) and incubated at 36°C for 1 h. Unadsorbed virus in the supernatant fluid was removed after centrifugation. The infected cells were resuspended in fresh medium, and aliquots were distributed in microtiter plate wells (0.2×10^6 cells per 100 μl per well), which had been prepared by the addition of 100 μl of graded concentrations of test compounds per well in triplicate. Plates were incubated at 36°C in 5% CO_2 for 4 days, at which time 125 μl of cells plus medium was removed and 150 μl of RPMI 1640 containing the appropriate concentration of compound was added. After incubation for an additional 3 days, 20 μl of the supernatant fluid was serially diluted to a final dilution of 1:156 in medium with a final concentration of Triton X-100 of 0.5%. Plates were stored frozen at −70°C until p24 antigen assays were performed. The p24 antigen values of the untreated controls and sample wells were measured by the DuPont, NEN enzyme-linked immunosorbent assay. The optical density readings of the no-drug controls were averaged to determine the value for the untreated control. The concentrations of compound producing a 50 or 90% reduction in the amount of p24 antigen (IC_{50} or IC_{90}, respectively) were determined.

HIV RNA hybridization assay. The HIV RNA hybridization assay was performed as follows (1). MT-2 cells were maintained in RPMI 1640 supplemented with 5% FBS, 2 mM glutamine, and 5 μg of gentamicin per ml. HIV-1(RF) was propagated in H9 cells in the same medium. For evaluation

of antiviral efficacy, cells to be infected were subcultured 1 day prior to infection. On the day of infection, cells were resuspended at 2×10^6 cells per ml in a low-biotin medium (either Dulbecco's modified Eagle's medium or RPMI 1640 medium minus biotin [GIBCO, custom formulation]) with 5% FBS for infection in microtiter plates.

Test compounds were dissolved in DMSO to 5 mg/ml and diluted in culture medium to twice the highest concentration to be tested and a maximum DMSO concentration of 2%. Further serial dilutions of the compound in culture medium were performed directly in microtiter plates. After compound dilution, MT-2 cells (50 μl) were added to a final concentration of 5×10^5/ml (10^5 per well). Cells were incubated with compounds for 30 min at 37°C in a CO_2 incubator. HIV-1(RF) in 50 μl (approximately 3×10^5 PFU as determined by plaque assay on MT-2 cells) was added to cells and test compound in microtiter wells. The final volume in each well was 200 μl.

After 3 days of culture at 37°C, cells were lysed and the amount of HIV RNA that had accumulated in the cells was determined by a sandwich hybridization assay utilizing two modified oligonucleotide probes complementary to adjacent regions in the HIV-1 *gag* gene. A 5 M combination of 5 M guanidinium isothiocyanate (Sigma), 0.1 M EDTA, and 10% dextran sulfate containing biotinylated *gag* capture probe was added to the cells in order to liberate RNA and allow hybridization of HIV RNA with the *gag* capture probe. Hybridization was carried out in the same microplate well for 16 to 20 h in a 37°C incubator. The hybridization solution was diluted threefold with distilled water and transferred to a streptavidin-coated microtiter plate (DuPont Biotechnology Systems, Boston, Mass.). Binding of RNA-capture probe hybrids to the streptavidin was allowed to proceed for 2 h at room temperature, after which the plates were washed six times with plate wash buffer (PBS with 0.5% Tween 20). A second hybridization of an alkaline phosphatase-conjugated *gag* reporter probe to the immobilized complex of capture probe and hybridized target RNA was carried out in the washed streptavidin-coated well. After hybridization for 1 h at 37°C, the plate was again washed. Immobilized alkaline phosphatase activity was detected after addition of a fluorogenic substrate (4-methylumbelliferyl phosphate [JBL Scientific]) and incubation at 37°C. Fluorescence at 450 nm was measured with a microplate fluorometer (Dynatech) exciting at 365 nm. The quantity of HIV RNA present in a sample was proportional to the net signal (signal from cell lysates containing HIV RNA minus noise from wells containing uninfected cell lysate) in the RNA hybridization assay. The concentration of compound which reduced the net signal in the RNA assay by 90% was designated the IC_{90}.

Cytotoxicity assay. MT-2 cells and PBMCs were cultured as described above. Cell viability was determined by the metabolism of the tetrazolium dye XTT {3,3′-[1-[(phenylamino)carbonyl]-3,4-tetrazolium]bis-(4-methoxy-6-nitrobenzene-sulfonic acid)} (Sigma) (20). In this assay, metabolically active cells transform XTT to a soluble, colored formazan product. Microtiter plates (96 wells) were seeded with 10^5 cells per well, and graded concentrations of test compound in RPMI 1640 plus 5% FBS were added. Cells were allowed to grow at 36°C in the presence of compound for 3 or 5 days depending on the cell type. Each well received 0.05 ml of 1 mg of XTT per ml in PBS containing 0.025 mM phenazine methosulfate. Plates were incubated for an additional 4 h at 36°C, and A_{450} was measured. The absorption was proportional to the number of viable cells in the well. The concentration of compound which produced a 50% reduction in the number of viable cells was designated the TC_{50}.

TABLE 1. Inhibition of HIV-1 and HIV-2 determined by yield reduction assay

Virus	Cell type	Mean IC_{90} (SD [μM]) of[a]:	
		XM323	AZT
HIV-1(RF)	MT-2	0.15 (0.02)	0.19 (0.07)
	H-9	0.04 (0.02)	0.24 (0.06)
	PBMCs	0.14 (0.02)	0.13 (0.03)
HIV-1(IIIb)	MT-2	0.11 (0.03)	0.04 (0.01)
	H-9	0.14 (0.02)	7.5 (2.4)
	PBMCs	0.14 (0)	0.3 (0.03)
HIV-1 clinical isolates[b]			
AZTr E	MT-2	0.14 (0)	>4
AZTr H	MT-2	0.09 (0.04)	>11
A018A pre	MT-2	0.14 (0.02)	0.3 (0)
A018C post	MT-2	0.32 (0.16)	>11
HIV-2(ROD)	MT-2	0.050 (0.005)	0.32 (0.11)

[a] Each value is the mean of two to four experiments run in duplicate. SD, estimated standard deviation based on the range according to the method of Pearson and Hartley (16) for small sample size.

[b] AZTr, AZT resistant; pre and post are paired isolates with AZT-sensitive (pre) and AZT-resistant (post) phenotypes.

RESULTS

Efficacy in yield reduction assay against laboratory strains and clinical isolates of HIV-1 and HIV-2. The ability of XM323 to inhibit the replication of two laboratory strains of HIV-1, HIV-1(RF) and HIV-1(IIIb), four clinical isolates of HIV-1, and one strain of HIV-2, HIV-2(ROD), was determined by yield reduction assay. In this assay, the yield of infectious virus is measured over a range of compound concentrations and the concentration of compound sufficient to reduce the yield of virus by 90% (IC_{90}) is determined. Since the host cells may have an effect on the potency of an inhibitor, the IC_{90} of XM323 was determined with two transformed T-lymphocyte cell lines (MT-2 and H9) and normal PBMCs. Table 1 shows the IC_{90}s from these assays.

XM323 is equally potent against the strains of HIV-1 and HIV-2 tested. IC_{90}s for HIV-1 ranged from 0.04 to 0.32 μM. More important, XM323 shows a dose-dependent inhibition of virus replication over a wide range of concentrations (Fig. 2). XM323 reduced the yield of HIV-1(RF) by 3 to 4 log units at concentrations as low as 1.8 μM (1 μg/ml).

Efficacy against laboratory strains and clinical isolates of HIV-1 by PBMC assay. The ability of XM323 and AZT to inhibit the replication of laboratory strains and clinical isolates of HIV-1 in the AIDS Clinical Trial Group-Department of Defense PBMC consensus assay is shown in Table 2. In this assay, p24 antigen production from virus-infected PBMCs is measured over a range of compound concentrations and the IC_{50} and IC_{90} are determined.

XM323 was a potent inhibitor of common laboratory strains and diverse clinical isolates of HIV-1. The IC_{50} for each of the three laboratory strains was 0.018 μM, and the IC_{90}s ranged from 0.07 to 0.16 μM. XM323 was effective against both nucleoside-susceptible and nucleoside-resistant isolates of HIV. IC_{50}s ranged from <0.018 to 0.13 μM, and IC_{90}s ranged from 0.05 to 0.29 μM. Dose-dependent inhibition of p24 production occurred over a wide range of XM323

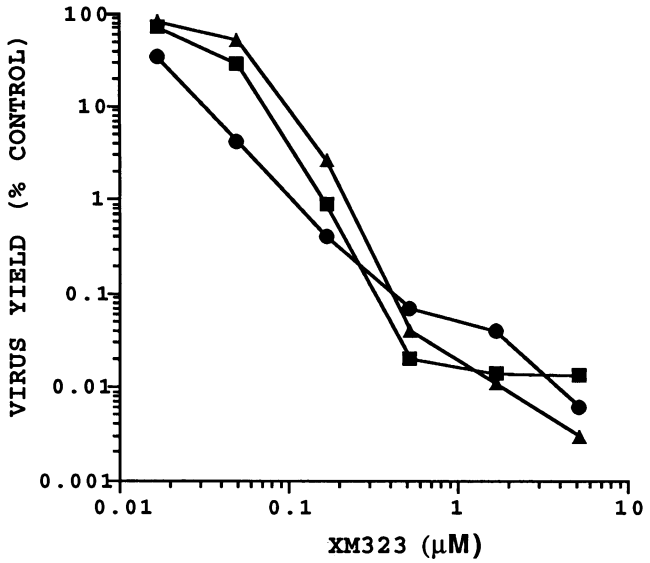


FIG. 2. Effect of XM323 on HIV-1(RF) in different cells. Three different cells were infected with HIV-1(RF) in the presence of various concentrations of XM323 as described in Materials and Methods. At each concentration, the yield of virus after 3 days of replication was determined by plaque assay with MT-2 cells. The yield of virus is plotted as the percentage of yield of the respective untreated infected control cultures. ■, MT-2; ●, H9; ▲, PBMCs.

concentrations, with ≥95% inhibition of p24 antigen production at 1.8 μM.

Cytotoxicity assay. The ability of cells to proliferate over 3 days (MT-2 cells) or 5 days (PBMCs) in the presence of XM323 was determined by XTT dye transformation. XM323 was tolerated by MT-2 and PBMCs with a TC_{50} of 77 μM. This concentration was over 600 times the IC_{90} for HIV-1 in these cells.

Efficacy against HIV-1(RF) by the RNA hybridization assay. The ability of XM323 to inhibit the replication of HIV-1(RF) in MT-2 cells was determined with the HIV RNA hybridization assay. In this assay, the accumulation of HIV *gag* RNA sequences in infected cells was measured over a range of compound concentrations, and the IC_{90} was determined. The antiviral efficacy of XM323 in this assay was compared with the antiviral efficacies of two inhibitors of the viral reverse transcriptase (AZT and ddC) and two additional inhibitors of the virally encoded protease, Q8111 (a compound synthesized by DuPont Merck to be equivalent to Ro31-8959) (18) and YY752 (a compound synthesized by DuPont Merck to be equivalent to A80987) (9). Table 3 shows the IC_{90}s from these assays. The IC_{90} for XM323 against HIV-1(RF) was 0.063 μM. XM323 was more potent than the nucleoside analogs AZT and ddC, slightly more potent than the protease inhibitor YY752, but less potent than the protease inhibitor Q8111.

Effect of MOI on the anti-HIV activity of XM323. With many antiviral agents, the ability to inhibit virus replication is inversely proportional to the inoculum size. If cells are challenged at a high MOI, more compound is often required to achieve a given percentage of inhibition than if the cells are challenged with less virus. In order to determine the effect of MOI on the potency of XM323, the standard yield reduction assay was performed but with different levels of viral inoculum. For comparison, the assays were conducted with AZT as well. Table 4 shows the results of these tests.

TABLE 2. Inhibition of HIV-1 in PBMCs as determined by p24 antigen production

Isolate	Inhibition (μM) of HIV-1 by[a]:			
	XM323		AZT	
	IC_{50}	IC_{90}	IC_{50}	IC_{90}
HIV-1 laboratory strains				
HIV-1(RF)	0.018	0.16	0.002	0.03
HIV-1(IIIb)	0.018	0.07	0.005	0.07
HIV-1(HXB2)	0.018	0.14	0.008	0.5
HIV-1 clinical isolates[b]				
A012B pre	0.018	0.07	0.004	0.08
A012D post	0.07	0.25	2.3	>5.0
A018A pre	0.07	0.24	0.06	0.1
A018C post	0.13	0.25	3.5	>5.0
B	0.04	0.14	0.05	0.8
C	0.11	0.18	0.4	0.9
D1	0.07	0.25	>5.0	>5.0
D2	0.018	0.13	1.0	>5.0
D3	0.05	0.16	4.4	>5.0
E	0.018	0.05	0.04	1.3
G	0.11	0.16	0.05	0.6
H	0.11	0.18	2.1	>5.0
K	0.05	0.16	0.07	0.7
L	0.11	0.18	0.06	0.09
N	<0.018	0.05	0.4	>5.0
P	0.11	0.18	>5.0	>5.0
R	0.04	0.09	0.18	4.3
V	0.07	0.18	3.0	>5.0
W	0.04	0.11	0.36	2.5
X	0.09	0.29	>5.0	>5.0
Y	0.04	0.14	0.009	0.08
PB	0.04	0.13	0.2	3.8
1	0.07	0.18	>5.0	>5.0
2	0.04	0.16	4.8	>5.0
9156	0.09	0.16	0.005	0.05

[a] Each value is the mean of two determinations.
[b] Pre and post, paired isolates with AZT-susceptible (pre) and AZT-resistant (post) phenotypes.

For XM323, increasing the inoculum of HIV-1(RF) from 0.02 PFU per cell to 20 PFU per cell resulted in a change in the IC_{90} from 0.05 μM to 0.48 μM. Over the same range, the IC_{90} for AZT changed similarly from 0.1 to 0.46 μM.

DISCUSSION

XM323 represents a novel class of potent inhibitors of HIV protease. By using a rational drug design approach, a class of inhibitors unlike any previously described that are cyclic in structure and contain no peptide bonds was designed. Crystallographic structural studies of HIV-1 protease-cyclic urea complexes have shown that these inhibitors bind with high affinity in a unique manner. In addition to

TABLE 3. Inhibition of HIV-1(RF) replication determined by RNA hybridization assay

Compound (no. of assays)	Mean IC_{90}[a] (μM) ± SD
XM323 (23)	0.06 ± 0.03
Q8111 (7)	0.009 ± 0.003
YY752 (3)	0.14 ± 0.03
AZT (5)	0.34 ± 0.26
ddC (70)	0.7 ± 0.3

[a] Each value is the mean ± standard deviation (SD) of multiple assays.

TABLE 4. Effect of viral inoculum size on antiviral activity of XM323 and AZT in yield reduction assay

MOI (PFU/cell)	IC_{90} (μM) of [a]:	
	XM323	AZT
0.02	0.05	0.1
0.2	0.1	0.1
2.0	0.2	0.2
20.0	0.48	0.46

[a] Each value is the mean of two assays.

forming specific interactions at the substrate binding subsites and hydrogen bonds with the active site aspartic acids, these cyclic ureas incorporate the binding features of an important enzyme-bound water molecule (12). Enzyme inhibition studies have shown that these unique binding features result in potent enzyme inhibition. The studies reported here demonstrate that this enzyme inhibition translates into potent inhibition of the replication of several strains of HIV-1 and HIV-2. In the yield reduction assay, which measures the production of mature infectious virions, XM323 is equally potent against AZT-susceptible and AZT-resistant strains of HIV-1. Against all strains of HIV-1 tested by yield reduction, XM323 reduced virus production, with an IC_{90} of 0.12 ± 0.04 μM (0.068 ± 0.02 μg/ml) (mean ± standard deviation). Two other measures of virus replication, HIV RNA levels and p24 antigen levels, also indicated that XM323 is a potent inhibitor of laboratory strains and clinical isolates of HIV.

The potency of XM323, like that of AZT, is sensitive to the viral inoculum size, with larger inocula requiring more compound to achieve IC_{90}. A 1,000-fold increase in the size of the viral inoculum resulted in a 10-fold increase in the IC_{90} of XM323 and a 5-fold increase in the IC_{90} of AZT.

Cytotoxicity studies with MT-2 cells and PBMCs indicated that these cells were equally tolerant of XM323, with a TC_{50} of 77 μM. This provides an in vitro therapeutic ratio (TC_{50}/IC_{90}) of over 600 for these cells, suggesting that XM323 has little cytotoxic potential and a great degree of anti-HIV selectivity.

The data presented here describe a compound with potent antiviral activity and little cytotoxicity. Other studies reported elsewhere (12) have shown that XM323 and related compounds have good oral bioavailability, which is an important improvement over previously described protease inhibitors. The combination of potent enzyme inhibition, antiviral activity, and oral bioavailability makes the cyclic urea compounds exciting new candidates for the treatment of HIV disease.

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