Antiviral and Pharmacokinetic Properties of C_2 Symmetric Inhibitors of the Human Immunodeficiency Virus Type 1 Protease

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Specific processing of the human immunodeficiency virus (HIV) *gag* and *gag-pol* polyprotein gene products by the HIV protease is essential for the production of mature, infectious progeny virions. Inhibitors of HIV protease block this maturation and thus prohibit the spread of HIV in vitro. Previously, we reported a series of novel, symmetric inhibitors of HIV protease designed to match the C_2 symmetric structure of the active site of the enzyme. In response to the poor aqueous solubility of those lead compounds, we designed a series of analogs with substantially improved ($>10^4$ fold) solubility. These inhibitors showed anti-HIV activity in H9 and MT4 cells at 0.05 to 10 μM, and in most cases, they were noncytotoxic at concentrations in excess of 100 μM. Further examination of one inhibitor (A-77003) revealed broad-spectrum activity against both HIV types 1 and 2, including azidothymidine-resistant HIV, in a variety of transformed and primary human cell lines. After administration of the inhibitors to rats, short half-lives and, with two notable exceptions, moderate oral bioavailability were observed. Additional pharmacokinetic studies in dogs and monkeys revealed the potential utility of A-77003 as an intravenous anti-HIV agent.

The three major genes (*gag*, *pol*, and *env*) of human immunodeficiency virus type 1 (HIV-1) encode polyprotein products which undergo posttranslational proteolytic processing. In contrast to the *env* gene product gp160, which is cleaved by a cellular proteinase (6, 13), the *gag* and *gag-pol* polyproteins are specifically processed by a virally encoded aspartic proteinase (HIV protease) (2, 3, 14, 24). Site-directed mutagenesis of either the HIV protease active site or the *gag* and *pol* cleavage sites produces viral particles which are morphologically immature and noninfectious (5, 8, 11, 19). The inhibition of HIV protease has thus attracted widespread interest for potential use in the therapeutic intervention of AIDS (14, 24). We recently described (4, 7) novel, C_2 symmetric inhibitors designed to correspond to the C_2 symmetric active site of the HIV protease homodimer (18, 23). These compounds (Fig. 1), typified by A-74704 (4) and A-75925 (7), inhibited HIV protease activity at nanomolar and subnanomolar concentrations, respectively, and blocked the spread of infection of HIV-1 in vitro at submicromolar levels. However, the high lipophilicity and poor aqueous solubility (<10 ng/ml) of A-74704 and A-75925 presented difficulties for evaluation in vivo. We therefore designed a series of analogs (compounds 1 to 10) of symmetric inhibitors A-74704 and A-75925 with substantially improved aqueous solubility. This report describes the pharmacokinetic behavior and broad-spectrum anti-HIV activity of inhibitors 1 to 10.

MATERIALS AND METHODS

Chemical synthesis. Synthesis of the C_2 symmetric diaminoalcohol and diaminodiol intermediates for the preparation of inhibitors 1 to 10 have been described previously (7). α-Isocyanato-L-valine methyl ester, which was prepared by treatment of L-valine methyl ester hydrochloride with excess phosgene in toluene at 100°C, was condensed with 2-pyridylcarbinol, 3-pyridylcarbinol, 4-pyridylcarbinol, or 2-(*N*-methylamino)methylpyridine. The resulting esters were hydrolyzed and coupled to the diaminoalcohol or diaminodiol intermediates, either directly, using a water-soluble carbodiimide, or indirectly, via the corresponding *p*-nitrophenyl ester. Inhibitors were purified by chromatography on silica gel and were characterized by their proton nuclear magnetic resonance spectra, mass spectra, melting points, combustion analysis, and high-pressure liquid chromatographic purity. Experimental details of the syntheses and characterizations will be published elsewhere.

HIV protease inhibition. Inhibitor potencies were determined by inhibition of cleavage of the fluorogenic substrate DABCYL-Ser-Gln-Asn-Asp-Tyr-Pro-Ile-Val-Gln-EDANS (12) by using recombinant HIV-1 protease (20). The final concentrations of the components in the reaction buffer were 125 mM sodium acetate (pH 4.5), 1 M sodium chloride, 5 mM dithiothreitol, 0.5 mg bovine serum albumin per ml, 1.3 μM fluorogenic substrate, and 2% (vol/vol) dimethyl sulfoxide in a final volume of 300 μl. The reactions were initiated by the addition of a small aliquot of HIV protease (<1 nM). The fluorescence intensity (excitation, 340 nm; emission, 490 nm) was recorded as a function of time. The 50% inhibitory concentrations (IC_{50}s) were calculated directly from percent inhibition data.

Antiviral activity. The following reagents were obtained through the AIDS Research and Reference Reagent Program, AIDS Program, National Institute of Allergy and Infectious Diseases: H9/HTLV-III$_{MN}$ NIH 1984 and H9/HTLV-III$_{RF}$ NIH 1983 from Robert Gallo; HUT 78/HIV-1$_{SF2}$ from Jay Levy; U937/HIV-2$_{MS}$ from Phyllis Kanki; A3.01/LAV.04 from Malcolm Martin; CEM-T4 cells from J. P. Jacobs; and MT2 cells, A018 H112-2 pre-azidothymi-

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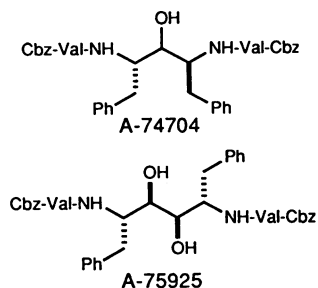
FIG. 1. Structures of C_2 symmetric HIV protease inhibitors (Cbz, benzyloxycarbonyl; Val, L-valinyl; Ph, phenyl).

dine (pre-AZT) HIV isolate, and A018 G910-6 post-AZT HIV isolate from Douglas Richman.

HIV antigen assay. H9, CEM, or MT4 cells (4×10^5 cells) were incubated with 0.1 ml (100 infectious units) of HIV stock on a shaker for 2 h at 37°C. The resulting culture was washed three times and resuspended into 2 ml of medium containing 10 μl of inhibitor at twofold dilutions in dimethyl sulfoxide. The control culture was treated in an identical manner, except that no inhibitor was added to the medium. Aliquots of culture supernatants were removed at three time points, usually 4, 7, and 10 days, and were monitored for HIV antigen (HIVAG-1; Abbott Laboratories) (16). Cytotoxicity was determined by cell viability on day 7 by trypan blue dye exclusion, and cells were refed with medium containing inhibitor (except for control wells, which were refed with medium only) on day 4. The percent inhibition of HIV by the compound was determined by comparing HIV antigen levels in the supernatants of infected cells incubated with inhibitor with those in the supernatants from the control culture without compound. The 50% effective concentration (EC_{50}) was the concentration of inhibitor that gave 50% inhibition of HIV activity. Typically, data from day 7 are reported; data from day 4 or 10 were used to maintain consistency when variations in virus replication and/or cell growth occurred. The $CCIC_{50}$ was the concentration of inhibitor at which 50% of the cells remained viable.

CPE assay. HIV-1_{3B} stock ($10^{4.7}$ 50% tissue culture infective doses per ml) was diluted 100-fold and was incubated with MT4 cells at 4×10^5 cells per ml for 1 h at 37°C (multiplicity of infection, 0.001 50% tissue culture infective dose per cell). The resulting culture was washed twice, resuspended to 10^5 cells per ml of medium, seeded in a 96-well plate at 100 μl per well, and treated with an equal volume of a 1% dimethyl sulfoxide solution of inhibitor in a series of half-log-unit dilutions in medium in triplicate. The virus control culture was treated in an identical manner, except that no inhibitor was added to the medium. The cell control was incubated in the absence of inhibitor or virus. The optical density (OD) was measured at day 5 by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) in a colorimetric assay (17). Virus and cell control OD values were the averages of six determinations. The cell control OD averaged 0.93 ± 0.05, and the ratio of cell control OD/virus control OD measured 4.71 ± 0.69. Percent inhibition of HIV cytopathic effect (CPE) was calculated by the following formula: [(average OD − virus control OD)/(cell control OD − virus control OD)] × 100. Cytotoxicity was determined by incubation in duplicate with serial dilutions of inhibitor in the absence of virus. Percent cytotoxicity was determined according to the following formula: (average

OD/cell control OD) × 100. The EC_{50} was the concentration of inhibitor that gave 50% inhibition of the cytopathic effect. The $CCIC_{50}$ was the concentration of inhibitor which gave a 50% cytotoxic effect.

Solubility evaluation. A weighed amount (~1 mg) of inhibitor was combined with 1 ml of 0.05 M phosphate buffer (pH 7.4) containing 0.9% (wt/vol) NaCl. Samples were vortexed vigorously for 1 min and were then sonicated for 10 min. The sample was filtered through a 0.45-μm-pore-size syringe filter (Millipore). The filtrate was diluted with mobile phase in triplicate; this was followed by high-pressure liquid chromatographic analysis. The concentration of the solubility sample was calculated by linear least-squares regression analysis of the spiked aqueous standards versus concentration; standards were assayed simultaneously with the samples.

Animal studies. The in vivo behaviors of the protease inhibitors were evaluated in male Sprague-Dawley-derived rats (weight, 250 to 400 g; Sasco Animal Laboratories, Oregon, Wis.). The animals were fasted overnight prior to dosing but were permitted water ad libitum. For intravenous dosing, the rats were anesthetized with ether, and a small incision was made on the ventral surface of the neck. The dose was administered into the jugular vein as a slow bolus over a 40- to 45-s time interval. The animals were permitted to recover from the anesthetic. Oral dosing was by gavage. Groups of four rats received either a 5-mg/kg (of body weight) (1-ml/kg) intravenous dose or a 10-mg/kg (2-ml/kg) oral dose. The protease inhibitors were prepared as 5-mg/ml solutions in a 20% ethanol–5% glucose in water vehicle containing two molar equivalents of methanesulfonic acid. In selected instances, the vehicle contained 30% (by volume) propylene glycol to aid in solubilization. Heparinized blood samples (~0.4 ml) were obtained from a tail vein of each rat at 0.1, 0.25, 0.5, 1, 1.5, 2, 3, 4, and 6 h after dosing. The blood samples were immediately placed in an ice bath; plasma was separated from the erythrocytes by centrifugation (Eppendorf model 5402; 3 min, 13,500 rpm, 4°C) within 1 h of collection and stored frozen (−30°C) until analysis.

The pharmacokinetic behavior of A-77003 in beagle dogs was evaluated following a single oral or intravenous dose. Six beagle dogs of either sex (weight, 7.8 to 10.2 kg; Marshall Research Animals) were randomly assigned to one of two groups, with each group containing three animals. All animals were fasted overnight prior to dosing and throughout the study period, but they were permitted free access to water. Animals were housed individually in stainless steel cages in accordance with the specifications of the Institutional Animal Care and Use Committee (IACUC). A-77003 was administered intravenously as a slow bolus (~60 s) via the cephalic vein to three dogs at a dose of 5 mg/kg (1 ml/kg) and orally by gavage to three dogs at a dose of 10 mg/kg (2 ml/kg). A-77003 was prepared as a 5-mg/ml solution as described above for both oral and intravenous dosing. Heparinized blood samples (~3 ml) were obtained from the jugular vein of each animal prior to dosing and at 0.08 (intravenous administration only), 0.17, 0.33, 0.5, 0.75, 1.0, 1.5, 2, 3, 4, 6, and 8 h after drug administration. The samples were promptly chilled in an ice bath. Plasma was separated from the erythrocytes by centrifugation (2,500 rpm, 20 min, 4°C) within 2 h of collection. The plasma was transferred to clean glass culture tubes and stored frozen (−30°C) until analysis.

The pharmacokinetic behavior of A-77003 was also evaluated in monkeys following a single oral or intravenous dose. Six female cynomolgus monkeys (weight, 2.5 to 3.4 kg)

TABLE 1. Potency and solubility of HIV protease inhibitors[a]
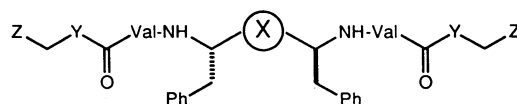
Compound no.	A no.	X	Y	Z	IC_{50} (nM)	Solubility at pH 7.4 (μg/ml)
1	A-75912	CH(OH)	O	2-pyridyl	4.8	51
2	A-75918	CH(OH)	O	3-pyridyl	6.8	18
3	A-75917	CH(OH)	O	4-pyridyl	26	7.8
4	A-77272	CH(OH)	NCH$_3$	2-pyridyl	43	167
5	A-76792	(R,R)-CH(OH)-CH(OH)	O	2-pyridyl	<1[b]	5.0
6	A-77212	(R,S)-CH(OH)-CH(OH)	O	2-pyridyl	<1[b]	2.4
7	A-76890	(S,S)-CH(OH)-CH(OH)	O	2-pyridyl	<1[b]	0.21
8	A-76889	(R,R)-CH(OH)-CH(OH)	NCH$_3$	2-pyridyl	1.7	225
9	A-77003	(R,S)-CH(OH)-CH(OH)	NCH$_3$	2-pyridyl	<1[b]	197
10	A-76928	(S,S)-CH(OH)-CH(OH)	NCH$_3$	2-pyridyl	<1[b]	3.6

[a] Val, L-valinyl; Ph, phenyl.

[b] IC_{50}s obtained for compounds 5 to 7 and compounds 9 and 10 indicated that all were roughly equipotent and suggested inhibition constants substantially less than 1 nM. Because of uncertainties in determining these values when inhibitor concentrations that approximated the concentration of enzyme in the assay were used, these values are reported as <1 nM. The results of a kinetic analysis by using methods for tightly binding inhibitors to determine true kinetic constants are described elsewhere (9).

obtained from an established colony were randomly assigned to one of two groups, with each group containing three animals. All animals were fasted overnight prior to dosing and through the first 3 h after dosing; animals were permitted free access to water. Food (Purina monkey chow) was provided to all animals after the 3-h sampling time point. Animals were housed individually in stainless steel cages in accordance with the specifications of IACUC. A-77003 was administered intravenously as a slow bolus (~60 s) via the saphenous vein to three monkeys at a dose of 5 mg/kg (1 ml/kg). A-77003 was administered orally via a nasoesophageal tube to three monkeys at a dose of 10 mg/kg (2 ml/kg); an aliquot of water (~5 to 8 ml) was given before and after the oral dose. A-77003 was prepared as a 5-mg/ml solution, as described above, for both oral and intravenous dosing. Heparinized blood samples (~2.0 to 2.5 ml) were obtained from the femoral artery or vein of each animal at the time points identical to those selected for the dog study.

Sample analysis. The inhibitors were selectively removed from rat plasma by using liquid-liquid extraction under alkaline conditions. A plasma aliquot (0.2 ml) was combined with an equal volume of an appropriate internal standard, 0.5 ml of 0.5 M Na$_2$CO$_3$ and 5 ml of ethyl acetate–hexane (7:3 [vol/vol]). Samples were vortexed vigorously for 15 s followed by centrifugation (Eppendorf model 5402; 10 min, 2,500 rpm, 4°C). The organic layer was transferred to a conical centrifuge tube and was evaporated to dryness with a gentle stream of dry air over low heat (~35°C). Samples were reconstituted in 0.2 ml of the mobile phase (17:5:78, by volume; see below) with vortexing. The analytical method for the quantitation of A-77003 in monkey and dog plasma samples was similar to that described above, but a larger plasma volume (0.5 ml) was used for extraction.

The parent inhibitors and respective internal standards were separated from plasma contaminants on a 3-μm Spherisorb ODS-2 column (5 cm by 4.6 mm; Regis Chemical Co.) with an acetonitrile–methanol–0.1% trifluoroacetic acid in 0.01 M tetramethylammonium perchlorate mobile phase run in an isocratic manner at 1.0 ml/min with UV detection at 205 nm. The drug concentration in each plasma sample was calculated by least-squares linear regression (unweighted)

analysis of the peak area ratio (inhibitor/internal standard) of the spiked plasma standards versus concentration. The spiked plasma standards were assayed simultaneously with the samples. The assays for each inhibitor were linear (correlation coefficients, >0.998) over the concentration range of 0 to 10 μg/ml, with a mean percent standard deviation of <3% for the analysis of triplicate standards at six separate concentrations and an estimated limit of quantitation of ~0.01 (dog, monkey) to 0.02 (rat) μg/ml. Initial estimates of the pharmacokinetic parameters for NONLIN (22) following intravenous dosing of the inhibitors were obtained with the program CSTRIP (21). Model selection was based on comparisons of data fitted to biexponential and triexponential equations by using the Akaike information criterion (1, 25). The initial weighting scheme was concentration^{-1}; in selected instances concentration^{-2} was used as the weighting format. Area under the curve (AUC) values were calculated by the trapezoidal rule over the time course of the study. The terminal-phase rate constant (β) was used to extrapolate from the last time point (6 to 8 h after dosing) in the calculation of the AUC from 0 h to infinity (AUC$_{0-\infty}$). The peak plasma concentrations (C_{max}) and time to peak plasma concentration (T_{max}) were observed experimental values. The total plasma clearance (CL$_P$) was calculated by dividing the dose by the AUC. The volume of the central compartment (V$_1$) was calculated by dividing the dose by the extrapolated concentration at time zero (C_0). Assuming dose proportionality and correcting for the differences in dosing, a comparison of the AUC following oral dosing with that obtained following intravenous dosing provided an estimate of the bioavailability (F).

RESULTS

In order to enhance the aqueous solubility of lead compounds A-74704 and A-75925, the terminal phenyl residues were modified to pyridyl groups. The IC_{50}s for inhibition of recombinant HIV protease by a fluorogenic assay (12) and estimated solubilities in phosphate buffer (pH 7.4) for the resulting analogs 1 to 10 are given in Table 1. Comparison of the three regioisomeric pyridyl analogs of A-74704, com-

TABLE 2. Anti-HIV$_{3B}$ activity and cellular toxicity of HIV protease inhibitors

Compound no.	H9 cells[a]		EC$_{50}$ (μM) for CEM cells[a,b]	MT4 cells[c]	
	EC$_{50}$ (μM)[d]	CCIC$_{50}$ (μM)		EC$_{50}$ (μM)[e]	CCIC$_{50}$ (μM)
1	1.5	65	1.3	4.4 ± 1.0	57
2	0.8	120	ND[f]	3.0 ± 1.7	62
3	2.7	125	ND	5.6	>100
4	10	>100	ND	17	>100
5	0.12	>100	0.12	0.67 ± 0.59	>100
6	<0.06	>100	0.02	0.14 ± 0.08	>100
7	0.045	100	0.07	0.18 ± 0.07	>100
8	0.37	>100	0.28	1.54 ± 0.29	>100
9	0.14	>100	0.07	0.20 ± 0.14	150
10	0.06	>100	0.09	0.17 ± 0.13	>100

[a] HIV antigen assay.

[b] With the exception of compounds 9 and 10 (averages of 14 assays), values represent the result of a single triplicate assay. A typical standard deviation is represented by compound 9: 0.073 ± 0.043 (n = 14).

[c] CPE assay.

[d] With the exception of compounds 3, 4, and 6 (single assays), values represent the averages of two to four single replicate assays. A typical standard deviation is represented by compound 9: 0.14 ± 0.05 (n = 3).

[e] With the exceptions of compound 3 (average of two assays) and 4 (single assay), values represent the average ± standard deviation of three or more triplicate assays.

[f] ND, not done.

pounds 1 to 3, revealed that 2-pyridyl substitution (compound 1) provided the most potent inhibition as well as the highest aqueous solubility. The 2-pyridyl group was therefore used in the vicinal diol-based inhibitors 5 to 10. Like A-75925 (7), diol inhibitors 5 to 7 were substantially more active than the corresponding "mono-ol"-based inhibitor 1. However, aqueous solubility was at least an order of magnitude lower. The solubilities of the diol inhibitors could be improved significantly by modifying the carbamate linkages to N-methyl ureas (compounds 8 to 10). While N-methyl urea substitution did not significantly affect the potency of (R,S) and (S,S) stereoisomers 9 and 10 in the protease assay, this structural change was clearly deleterious to the (R,R) diol 8 (versus compound 5), which no longer inhibited HIV protease at subnanomolar concentrations. Similarly, mono-ol-based urea 4 was a substantially weaker inhibitor than the corresponding carbamate 1. Interestingly, the solubilities of the three diastereomeric diols (compounds 5 to 7 and 8 to 10) followed the trend (R,R) > (R,S) ≫ (S,S).

In vitro anti-HIV activity. Inhibitors 1 to 10 were evaluated for their ability to block HIV antigen production in acutely infected H9 and CEM cultures and to inhibit the CPE of HIV-1$_{3B}$ in MT4 cells. In vitro efficacy, expressed as EC$_{50}$s, and cytotoxicity, expressed as CCIC$_{50}$s, are provided in Table 2. Steep dose-response curves in the range of the EC$_{50}$ were observed for all of the inhibitors by both assays. However, the amount of compound needed to suppress antigen production in H9 and CEM cells was generally severalfold lower than that required to block CPE in MT4 cells. To a first approximation, anti-HIV activity in vitro reflected the IC$_{50}$ against purified HIV protease in that the diol inhibitors as a class (compounds 5 to 10) were generally ≥10-fold more potent than mono-ol inhibitors 1 to 4. Thus, with the exception of compound 8 in MT4 cells, all of the diol inhibitors blocked the spread of HIV at submicromolar concentrations. Within the sets of diastereomeric diols, activity followed the trend (S,S) ≈ (R,S) > (R,R). The cytotoxic levels of inhibitors 5 to 10 in H9 and MT4 cells

TABLE 3. Pharmacokinetic parameters of HIV protease inhibitors in rat following a single dose of 5 (intravenous) or 10 (oral) mg/kg[a]

Compound no.	Intravenous[b]		Oral		
	$t_{1/2\beta}$ (h)	V_1 (liters/kg)	C_{max} (μM)	T_{max} (h)	F (%)
1	1.9	0.71	1.6 ± 0.36	0.25	18.9 ± 3.5
2	0.31	1.04	0.22 ± 0.07	0.1	2.1 ± 0.40
3	0.57	0.38	0.20 ± 0.05	0.1	0.9 ± 0.29
4	0.45	0.41	1.5 ± 0.30	0.66	11.1 ± 3.1
5	1.8[b]	0.79	0.14 ± 0.01	0.1	2.6 ± 1.1
6	NS[c]		0.06 ± 0.03	0.14	[d]
7	NS		<0.02		[d]
8	0.66	0.53	<0.05 ± 0.02	0.67	<1
9	0.47	0.37	0.15 ± 0.08	0.18	0.7 ± 0.25
10	0.47	0.70	0.30 ± 0.06	0.14	1.5 ± 0.09

[a] The values represent the mean ± standard error of the mean for four rats.

[b] The intravenous dose was administered as a fine suspension.

[c] NS, insufficient solubility to permit intravenous dosing; the oral dose was administered as a suspension.

[d] There was very low bioavailability and no intravenous reference.

were greater than 100 μM, providing in vitro selectivity indices of >100 to >2,000.

Pharmacokinetics. Pharmacokinetic parameters for inhibitors 1 to 10 in rats are provided in Table 3. Compounds 6 and 7 were too insoluble to permit intravenous dosing; trace concentrations (<0.06 μM) were noted following oral dosing of a suspension. The plasma concentration profiles of the remaining inhibitors after intravenous dosing were described by a biexponential elimination model, characterized by a rapid distribution half-life ($t_{1/2}$) (<5 min) and apparent V_1 values (<1 liters/kg). The terminal elimination $t_{1/2}$s ($t_{1/2\beta}$s) ranged from a low of 0.31 h (compound 2) to a high of 1.9 h (compound 1). The protease inhibitors were rapidly absorbed after oral dosing, with peak concentrations in plasma generally observed in the first 15 min. Low concentrations (<0.3 μM) of parent drug were noted in 8 of the 10 compounds examined in this series. However, significantly higher concentrations (C_{max}, >1.5 μM) were noted after oral dosing of the 2-pyridyl mono-ol inhibitors 1 and 4, providing apparent F values of 18.9 and 11.1%, respectively.

Evaluation of A-77003. On the basis of a combination of favorable solubility and antiviral activity, we selected A-77003 (compound 9; Fig. 2) for further in vitro and pharmacokinetic evaluation. Table 4 gives the activity of A-77003 against various laboratory and primary strains of HIV. Depending on the cell line, virus strain, virus load, and assay method, EC$_{50}$s ranged from 30 to 300 nM. Excellent antiviral activity was uniformly maintained against a variety of laboratory strains of HIV-1 and HIV-2 in various T-cell lines (entries 1 to 12). Similar activity was observed against both HIV-1$_{3B}$ (entry 13) and a primary strain of HIV-1 (entry 14) in primary blood lymphocytes. Notably, A-77003 was active at similar levels against HIV-1 isolates from a single patient prior to (entry 15) and after (entry 16) the development of clinical resistance to AZT (10).
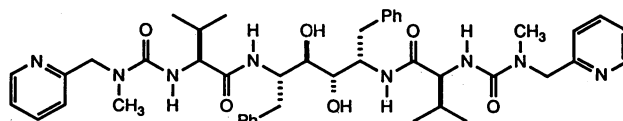

FIG. 2. Structure of A-77003 (Ph, phenyl).

TABLE 4. In vitro anti-HIV activity of A-77003

Entry	Cell line	Assay	Virus strain	EC_{50} $(\mu M)^a$	$CCIC_{50}$ (μM)
1	H9	Antigen	$HIV-1_{3B}$	0.15	>100
2	CEM	Antigen	$HIV-1_{3B}$	0.07	>100
3	CEM	Antigen	$HIV-1_{MN}$	0.03	>100
4	CEM	Antigen	$HIV-1_{RF}$	0.055	>100
5	CEM	Antigen	$HIV-1_{CDC451}$	0.07	>100
6	H9	Antigen	$HIV-1_{SF}$	0.30	>100
7	MT4	Antigen	$HIV-2_{ROD}$	0.27	150
8	MT4	CPE	$HIV-1_{3B}$	0.20	150
9	MT4	CPE	$HIV-1_{MN}$	0.12	150
10	MT4	CPE	LAV^b	0.24	150
11	CEM	CPE	LAV	0.15	>100
12	MT4	CPE	$HIV-2_{MS}$	0.24	150
13	PBL	Antigen	$HIV-1_{3B}$	0.06	100
14	PBL	Antigen	HIV^c	0.03	100
15	MT2	CPE	Pre-AZT $HIV_1{}^d$	0.12	>100
16	MT2	CPE	Post-AZT $HIV_1{}^e$	0.21	>100

[a] Values represent the results of single triplicate or quadruplicate assays.
[b] LAV, lymphadenopathy-associated virus.
[c] Primary patient isolate from PBL.
[d] $HIV-1_{A018}$ H112-2 pre-AZT isolate; EC_{50} (AZT) = 0.11 μM.
[e] $HIV-1_{A018}$ G910-6 post-AZT isolate; AZT had no effect up to 10 μM.

The pharmacokinetic behavior of A-77003 in rats, dogs, and monkeys following a single oral or intravenous dose is compared in Table 5. Concentrations of the parent drug in plasma distributed rapidly following intravenous dosing in three species. The terminal $t_{1/2\beta}$ in the rat (0.47 hours) was faster than that noted for the dog ($t_{1/2\beta}$, 1.1 h); a significantly longer $t_{1/2\beta}$ (3.2 h) was observed in monkeys. A-77003 was rapidly absorbed in the rats and dogs, with peak concentrations in plasma noted in the first 15 min after dosing. A lag time was recorded following oral dosing in the monkeys with C_{max} values noted 1 to 2 h after dosing in two of the three monkeys; no trace of the parent drug was found in one of the orally dosed monkeys. Concentrations in plasma significantly in excess of the in vitro anti-HIV EC_{50} were recorded in the dogs (C_{max}, 0.59 μM; Fig. 3), in contrast to the lower peak concentrations in plasma noted in the rats and monkeys (C_{max}, <0.16 μM). In each of the three species, F averaged less than 3%.

DISCUSSION

We reasoned that introduction of polar, solubilizing functionality into the aromatic rings of the terminal benzyloxycarbonyl groups of A-74704 and A-75925 might provide analogs which retained activity against HIV protease since, in the X-ray crystal structure of the HIV protease–A-74704
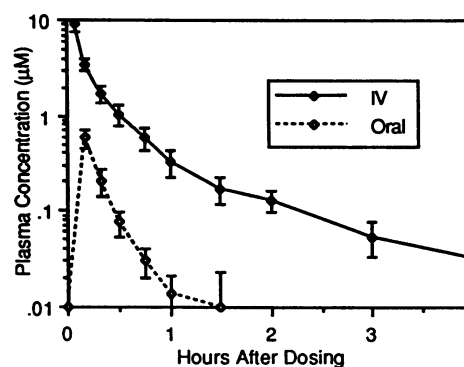

FIG. 3. Mean ± standard error of the mean (n = 3) concentrations of A-77003 in plasma after a dose of 5 (intravenous [IV]) or 10 (oral) mg/kg in dogs.

complex (4), those groups do not interact specifically with hydrophobic pockets of the active site. Of the three possible regioisomeric pyridyl groups, the 2-pyridyl group provided the greatest benefit in increasing solubility and in maintaining both HIV protease inhibitory potency and in vitro anti-HIV activity. This benefit was also realized in vivo for compounds 1 and 4, both of which gave significant levels in plasma upon oral administration in rats. However, the maximum concentrations in plasma obtained after oral dosing failed to exceed the concentrations required for anti-HIV activity in vitro. We therefore investigated 2-pyridyl-substituted analogs of the more potent diol inhibitor A-75925. With the exception of compound 8, inhibitors 5 to 10 maintained most of the high potency of A-75925 (7) against both purified HIV protease and HIV in vitro, with compound 9 (A-77003) providing the highest activity among the inhibitors with reasonable (>100 $\mu g/ml$) aqueous solubility. Efforts to further increase solubility generally led to compounds with inferior antiviral activity, even in cases in which HIV protease inhibitory potency was maintained. Unfortunately, the substantial concentrations achieved in plasma after oral administration of compounds 1 and 4 were not realized with any of the potent diol inhibitors 5 to 10, which uniformly gave absolute F values of less than 3%. The oral F did not appear to be directly related to aqueous solubility, since both compounds 8 and 9 were more soluble than inhibitor 1. Efforts are under way to understand the subtle structural features which affect the solubilities and pharmacokinetic behaviors of these inhibitors, in hopes that both high activity and bioavailability can be achieved.

Although oral efficacy is the ultimate goal for inhibitors of

TABLE 5. Pharmacokinetic comparison of A-77003 in rats, dogs, and monkeys following a single dose of 5 mg/kg intravenously or 10 mg/kg orally^a

Species	Intravenous dose				Oral dose		
	$t_{1/2\beta}$ (h)	V_1 (liters/kg)	V_β (liters/kg)	CL_P (liters/h)	C_{max} (μM)	T_{max} (h)	F (%)
Rat^b	0.47	0.37	1.5	0.49 ± 0.02	0.15 ± 0.08	0.18	0.7 ± 0.09
Dog^c	1.13	0.17	2.7	14.9 ± 2.26	0.59 ± 0.13	0.17	2.0 ± 0.11
Monkey^c	3.19	0.40	5.0	2.93 ± 0.12	0.16 ± 0.08	1.5	2.5 ± 1.6

[a] V_β, volume of distribution of the terminal phase; CL_P, total plasma clearance. The abbreviations of the other pharmacokinetic parameters were defined in the text.
[b] Reported values represent the mean ± standard error of the mean for four animals.
[c] Reported values represent the mean ± standard error of the mean for three animals.

HIV protease, the favorable balance of solubility and antiviral activity of A-77003 (compound 9) presented the possibility of its usefulness as an intravenous agent. We therefore investigated the spectrum of anti-HIV activity of A-77003 in greater detail as well as its pharmacokinetic behavior in dogs and monkeys. The results shown in Table 4 demonstrate that A-77003 has broad antiviral activity against a wide variety of HIV strains in a number of different human cell lines. The maintenance of activity of A-77003 against AZT-resistant HIV suggests a complementarity of A-77003 with inhibitors of reverse transcriptase on the basis of different mechanisms of antiviral action. Moreover, the results of cocultivation experiments (15) demonstrate a substantial advantage of A-77003 over AZT in the ability to block the spread of HIV infection in vitro. Although it is not well absorbed orally, the increased intravenous $t_{1/2}$ of A-77003 observed in dogs and monkeys argues favorably for its clinical usefulness as an intravenous agent. Indeed, a single 5-mg/kg bolus injection in the monkey resulted in levels of A-77003 in plasma which exceeded the EC_{50} in MT4 cells for >2 h and the EC_{50} in primary blood lymphocytes for >8 h. It is therefore likely that continuous intravenous infusion could provide concentrations in plasma sufficient for anti-HIV efficacy in vivo.

In summary, the aqueous solubilities of two series of lipophilic C_2 symmetric HIV protease inhibitors were substantially improved without significantly sacrificing in vitro anti-HIV activity by incorporating a 2-pyridyl residue at each terminus of the inhibitors. Although these changes led in one series to compounds (compounds 1 to 4) with significant oral bioavailability in the rat, those inhibitors lacked the in vitro potency deemed necessary for clinical effectiveness. In the more active diol series (compounds 5 to 10), the inhibitors were uniformly poorly bioavailable; however, A-77003 emerged as a compound with sufficient aqueous solubility and in vitro anti-HIV efficacy for consideration as an intravenous agent.

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