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ABT-538 is a potent inhibitor of human immunodeficiency virus protease and has high oral bioavailability in humans

(acquired immunodeficiency syndrome/antiviral/peptidomimetic)

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ABSTRACT **Examination of the structural basis for antiviral activity, oral pharmacokinetics, and hepatic metabolism among a series of symmetry-based inhibitors of the human immunodeficiency virus (HIV) protease led to the discovery of ABT-538, a promising experimental drug for the therapeutic intervention in acquired immunodeficiency syndrome (AIDS). ABT-538 exhibited potent *in vitro* activity against laboratory and clinical strains of HIV-1 [50% effective concentration (EC_{50}) = 0.022–0.13 μM] and HIV-2 (EC_{50} = 0.16 μM). Following a single 10-mg/kg oral dose, plasma concentrations in rat, dog, and monkey exceeded the *in vitro* antiviral EC_{50} for >12 h. In human trials, a single 400-mg dose of ABT-538 displayed a prolonged absorption profile and achieved a peak plasma concentration in excess of 5 μg/ml. These findings demonstrate that high oral bioavailability can be achieved in humans with peptidomimetic inhibitors of HIV protease.**

The pandemic spread and fatal prognosis of human immunodeficiency virus (HIV) infection demand new and effective antiretroviral therapies. Current agents, which target the HIV reverse transcriptase, suffer both from dose-limiting toxicities and from the selection of highly resistant mutant strains (1). Among other virus-encoded drug targets, the HIV protease commands attention by virtue of its clear and indispensable role in the proteolytic processing of the viral gag and gag-pol polyproteins (2). Viral progeny which lack a functional protease are not infectious (3), and chemical inhibitors of the protease efficiently block replication of HIV *in vitro* (4, 5). However, the discovery of protease inhibitors with utility in the treatment of human disease has been hampered by a number of significant obstacles. Foremost among these is the identification of inhibitors which simultaneously embody potent anti-HIV activity and high oral bioavailability. A virtual necessity for the long-term treatment of a chronic disease, high oral bioavailability must be accompanied by a long elimination half-life to yield sustained virus-suppressive drug levels in the blood and infected tissues. This requirement is prescribed by the profound ability of HIV to develop resistance under selective antiviral pressure. The identification of HIV protease inhibitors with optimal oral pharmacokinetic properties therefore represents a critical milestone on the path to therapeutic efficacy with this class of agents. Unfortunately, as a consequence of their peptidomimetic nature, most protease inhibitors have pharmacokinetic profiles that are characterized by low oral absorption and rapid elimination. The major liabilities in this regard are thought to include high molecular weight, low aqueous solubility, and susceptibility to proteolytic degradation, hepatic metabolism, and biliary extraction (6). Here we report the design and preclinical development of a potent inhibitor of HIV protease which overcomes these liabilities and exhibits an exceptional pharmacokinetic profile both in animal models and in humans.

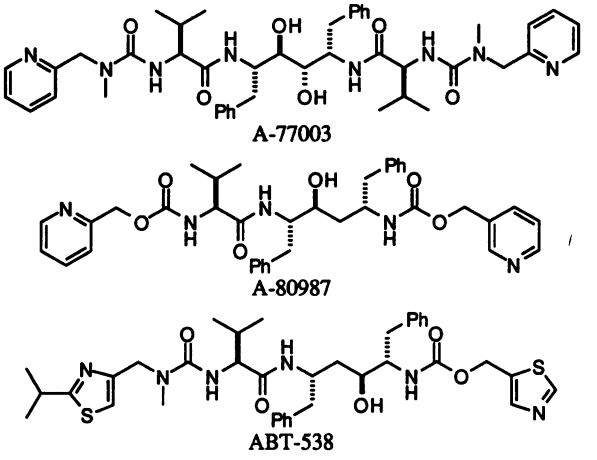

FIG. 1. Structures of A-77003, A-80987, and ABT-538; Ph, phenyl.

Structurally, HIV protease functions as a C_2-symmetric homodimer with a single active site (7). Previously we reported the use of active-site symmetry as the basis for the design of potent selective inhibitors of HIV-1 protease (8, 9). While the goal of discovering inhibitors with good oral pharmacokinetic properties was not immediately realized, the pseudosymmetric inhibitor A-77003 (Fig. 1) possessed sufficient aqueous solubility for i.v. administration (10) and was examined in clinical trials (J.M.L., unpublished results). In the search for related inhibitors with improved oral bioavailability, we examined the subtle effects of molecular size, aqueous solubility, and hydrogen bonding capability on pharmacokinetic behavior (11). This study produced A-80987 (Fig. 1), which, although appreciably smaller than its predecessors, retained submicromolar antiviral activity. More importantly, this potent inhibition was accompanied by significant oral bioavailability in three animal species and in humans (see below). Unfortunately, the relatively short plasma half-life of A-80987 limited our ability to

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Abbreviations: HIV, human immunodeficiency virus; EC_{50}, 50% effective concentration; AZT, 3′-azido-3′-deoxythymidine; C_{max}, maximal plasma concentration; t_{max}, time to maximal plasma concentration.

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Table 1. Pharmacokinetic evaluation of A-80987 and ABT-538 in rat, monkey, and dog

Drug	Species	Dose* mg/kg	Dose* Route	$t_{1/2}$,† h	V_β,‡ liter/kg	CLp,§ liter/h·kg	C_{max},¶ μg/ml	t_{max},‖ h	F,** %	*n*
A-80987	Rat	5	i.v.	1.91	4.45	1.5 ± 0.5				4
		10	Oral				2.69 ± 0.69	0.25	26.4 ± 10.1	3
	Monkey	5	i.v.	2.00	4.36	1.4 ± 0.3				3
		10	Oral				0.65 ± 0.35	1.8	13.5 ± 8.5	9
	Dog	5	i.v.	1.54	4.97	1.0 ± 0.2				3
		10	Oral				2.75 ± 1.78	0.17	23.2 ± 14.9	6
ABT-538	Rat	5	i.v.	0.66	0.95	1.0 ± 0.2				4
		5	Oral	0.87			1.36 ± 0.32	1.0 ± 0.4	70.7 ± 19.9	4
		10	Oral	1.22			1.89 ± 0.67	2.0 ± 0.8	77.6 ± 14.2	6
	Monkey	5	i.v.	2.26	2.16	0.67 ± 0.24				6
		5	Oral	1.59			1.08 ± 0.74	2.0 ± 1.0	29.9 ± 17.4	6
		10	Oral	1.37			3.82 ± 2.47	2.1 ± 1.2	70.4 ± 37.6	6
	Dog	5	i.v.	1.07	0.34	0.22 ± 0.05				6
		5	Oral	0.86			3.58 ± 1.62	1.0 ± 0.5	37.4 ± 21.2	6
		10	Oral	1.50			16.60 ± 5.21	1.3 ± 0.8	100††	3
		5	Oral‡‡				7.20 ± 1.89	1.3 ± 0.3	94.1 ± 26.4	6

Data are expressed as mean ± SD.
*Unless noted otherwise, the vehicle for i.v. and oral dosing was ethanol/propylene glycol/dextrose 10% in water (20:30:50, by volume) containing two molar equivalents of methanesulfonic acid per mole of drug.
†Terminal phase plasma half-life after i.v. dose.
‡Volume of distribution.
§Clearance.
¶Maximal plasma concentration after oral dose.
‖Time of maximal plasma concentration after oral dose.
**Oral bioavailability based on the dose-normalized areas (under the concentration vs. time plot) for i.v. and oral doses.
††Because of nonlinearity, calculated oral bioavailability exceeded 100%.
‡‡Clinical capsule formulation.

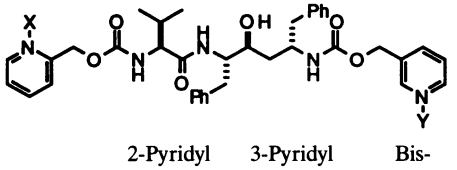
maintain plasma levels substantially in excess of the 95% effective concentration (EC_{95}) for viral replication *in vitro*. Intensive study of a series of A-80987 analogues has now yielded valuable insight into the relationship of chemical structure to antiviral activity, aqueous solubility, and hepatic metabolism. Application of these insights to compound design culminated in the discovery of ABT-538 (Fig. 1), an HIV protease inhibitor with enhanced antiviral activity and exceptional oral pharmacokinetics.

MATERIALS AND METHODS

Details of the synthesis of ABT-538 will be published elsewhere; prior to publication they may be obtained from D.J.K. The following reagents were obtained through the AIDS Research and Reference Reagent Program, AIDS Program, National Institute of Allergy and Infectious Diseases: MT4 cells, MT2 cells, HIV-1 MN/H9, H9/HIV-I RF NIH1983, HIV-2 MS/U937, AO18 H112-2 pre-3′-azido-3′-deoxythymidine (AZT) HIV isolate, and AO18 G910-6 post-AZT HIV isolate. Inhibition constants (K_i) for recombinant HIV-1 proteases were determined with a fluorometric assay as described previously (11). Inhibition of viral replication and compound cytotoxicity were determined in parallel, using a standard colorimetric assay (10, 12). Apparent aqueous solubility was measured as previously described (10).

Table 2. *In vitro* and *in vivo* metabolic distribution of A-80987

Compound, % of total



Species	X / Y	A-80987 —* / —*	2-Pyridyl *N*-oxide O / —*	3-Pyridyl *N*-oxide —* / O	Bis-*N*-oxide O / O
Rat†		69.3	5.2	20.5	5.0
Dog†		67.7	5.2	20.6	6.5
Human†		60.3	9.7	19.0	11.0
Rat (i.v.)‡		4.8	8.8	61.3	35.0
Dog (i.v.)‡		1.5	5.7	34.2	51.9

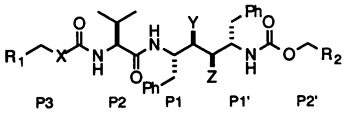
*Lone pair of electrons.
†Percentages after a 45-min incubation with hepatic microsomes.
‡Percentage of biliary carbon-14 recovered in a 0- to 2-h bile sample (n = 1).

Pharmacokinetic Analysis. Sprague–Dawley-derived rats (male, 0.25–0.35 kg), beagle dogs (male/female, 8–12 kg), and cynomolgus monkeys (*Macaca fascicularis*; female, 2.6–4 kg) received a 5-mg/kg i.v. bolus (over ≈60 s), or a 5-mg/kg or 10-mg/kg oral gavage. Human volunteers (male, nonfasted) received a single tablet of formulated A-80987·2HCl containing the equivalent of 500 mg of A-80987 or a capsule of formulated ABT-538 equivalent to 400 mg. Plasma samples, obtained as a function of time after dosing, were analyzed by reverse-phase HPLC as described previously (10).

Tissue Distribution. Male Sprague–Dawley-derived rats (0.25–0.35 kg) received a single 10-mg/kg (1-ml/kg) dose of ABT-538 administered orally by gavage. At selected times after dosing, three rats were killed, and the thymus, mesenteric lymph nodes, cervical lymph nodes, and brain were removed from each rat and placed on ice. The tissue samples were combined with 2 vol of 0.05 M sodium phosphate buffer (pH 4.4) and homogenized. Plasma was separated from a blood sample by centrifugation (4°C, 1820 × *g* for 10 min). Plasma and tissue homogenates were frozen (−30°C) until analysis. ABT-538 was quantified by HPLC.

Metabolism *in Vitro*. Rat, dog, or human microsomal protein (0.14–0.46 mg), prepared by differential centrifugation as described (13), was preincubated for 5 min at 37°C in an NADPH-generating system consisting of 1 mM $MgCl_2$, 5 mM glucose 6-phosphate, 1 mM $NADP^+$, and 2 units of glucose-6-phosphate dehydrogenase in 0.1 M potassium phosphate

Table 3. Anti-HIV activity of HIV protease inhibitors in MT4 cells and pharmacokinetic parameters following i.v. and oral dosing in rats



No.	R_1*	X	Y	Z	R_2*	EC_{50},† μM	$CCIC_{50}$,‡ μM	Solubility,§ μg/ml pH 7.4	Solubility,§ μg/ml pH 4	$t_{1/2}$,¶ h	C_{max},‖ μM	t_{max},** h	F,†† %	C_{max}/EC_{50}
A-80987	2-Pyr	O	OH	H	3-Pyr	0.22‡‡	>100	8.6	122	1.9	4.11	0.25	26	19
A-81525	2-Pyr	O	H	OH	5-Thz	0.54	>100	4.2	7.3	0.7	2.04	0.4	47	3.8
A-82200	4-Thz	NMe	OH	H	3-Pyr	0.73	83	53	240	1.4	3.13	0.4	35	4.3
A-83962	(2-iPr)-4-Thz	NMe	H	OH	3-Pyr	0.028	47	7.9	66	1.0	0.98	0.3	12	35
ABT-538	(2-iPr)-4-Thz	NMe	H	OH	5-Thz	0.025‡‡	57	5.3	6.9	1.2	2.62	2.0	78	105

*Pyr, pyridyl; Thz, thiazolyl; iPr, isopropyl.
†Values are geometric mean of two triplicate determinations at 10 and 32 tissue culture infectious doses ($TCID_{50}$) per well, respectively.
‡50% cytotoxic concentration.
§Estimated aqueous solubility.
¶Plasma half-life after a 5-mg/kg i.v. dose ($n \geq 3$).
‖Maximal plasma concentration after a 10-mg/kg oral dose ($n \geq 3$).
**Time of maximal plasma concentration after oral dose.
††Oral bioavailability based on the dose-normalized areas for 5-mg/kg i.v. and 10-mg/kg oral doses.
‡‡Mean of 36 triplicate determinations.

buffer (pH 7.4) at a final volume of 1 ml. Reaction was initiated by addition of the HIV protease inhibitor in 13–15 μl of 20% (vol/vol) ethanol containing 2 molar equivalents of methanesulfonic acid per mole of inhibitor. Incubations were interrupted after 0, 10, 20, 30, or 45 min by addition of 1 M Na_2CO_3. A 45-min no-NADPH control incubation was also performed for each protease inhibitor. The resulting mixture was extracted with ethyl acetate, acidified with 1 M HCl, and extracted with a second portion of ethyl acetate. The combined organic layers were concentrated to dryness under a N_2 stream and reconstituted for quantitative HPLC analysis.

Metabolism *in Vivo*. The bile ducts of male Sprague–Dawley rats and a male beagle dog were cannulated by using standard procedures. A 5-mg/kg i.v. dose of [*valine*-U-^{14}C]A-80987 or [*valine*-U-^{14}C]ABT-538 was administered, and bile was collected in 2-h intervals for 6 h. Parent drug and metabolites were quantitated by using reverse-phase HPLC with radioactivity flow detection.

RESULTS AND DISCUSSION

We have shown previously that the pharmacokinetic profiles of symmetry-based HIV protease inhibitors are related to a variety of physicochemical features. Within its structural class, A-80987 represented the optimal compromise between oral bioavailability and potency (11). The pharmacokinetic parameters for A-80987 in three species are provided in Table 1. Although a single 10 mg/kg oral dose of A-80987 in rat and dog provided peak plasma concentrations (C_{max}) at least 20-fold higher than the 50% effective concentration (EC_{50}) for anti-HIV activity *in vitro*, plasma levels following this early peak [time of maximal absorption (t_{max}) = 0.17–0.25 h] declined rapidly. In the monkey the absorption was more gradual (t_{max} = 1.8 h), but with substantially lower C_{max}. With the goal of identifying an agent that would provide more sustained virus-suppressive drug levels *in vivo*, we endeavored to design inhibitors with both enhanced anti-HIV activities and improved pharmacokinetic profiles. To this end, we embarked on a systematic study of the relationship of inhibitor structure both to antiviral activity and to pharmacokinetic behavior in rats. Initially, we examined the metabolism of A-80987 to understand the structural features responsible for rapid clearance.

Metabolism of A-80987. We studied the metabolic fate of A-80987 both *in vitro* and *in vivo*. The distribution of metabolites after incubation of A-80987 with rat, dog, and human hepatic microsomes is shown in Table 2. In each case, only three major metabolites were produced: the mono pyridine *N*-oxides resulting from oxidation at either the 2-pyridyl or 3-pyridyl group and the corresponding bis(pyridine *N*-oxide). We also examined the distribution of ^{14}C-labeled A-80987 after i.v. administration to bile-exteriorized dogs and rats. Within 2 h of a 5-mg/kg dose, 62% and 84% of the total radioactivity administered was recovered in the dog and rat bile, respectively. While only a small percentage (1.5–4.8%) of A-80987 was excreted unchanged, the three *N*-oxide metabolites constituted 92–95% of the total bile radioactivity in these two species (Table 2). Taken together, these results suggest that the plasma concentrations of A-80987 are limited in all species, including humans, by efficient metabolism of both pyridyl groups.

Design of Inhibitors with Improved Properties. With the above information in hand, we sought structural modifications of A-80987 which would improve its pharmacokinetic profile without sacrificing antiviral potency. We speculated that the rate of metabolism could be diminished by reducing the oxidation potential of the electron-rich pyridinyl groups. However, we observed that substantial changes in the basicity of the pyridyl groups reduced aqueous solubility and lowered oral absorption. Replacement of pyridine with thiazole, a less electron-rich bioisostere, was therefore pursued to balance aqueous solubility and metabolic stability. The activity, estimated aqueous solubility, and pharmacokinetic properties of key structural analogues leading to the identification of ABT-538 are provided in Table 3. All of the analogues inhibited purified HIV protease with $K_i < 1$ nM and showed similar *in vitro* anti-HIV dose–response curves, with EC_{90} values ≈2- to 3-fold higher than EC_{50} values. Antiviral activity decreased only slightly on placement of a 5-thiazolyl group into the P2′

Table 4. Anti-HIV activity of ABT-538 *in vitro*

Strain	EC_{50}, μM ABT-538	EC_{50}, μM AZT
HIV-1_{IIIB}	0.022	0.02
HIV-1_{MN}	0.009	0.01
HIV-1_{RF}	0.014	0.04
HIV-1_{TR17}	0.046	0.049
HIV-2_{MS}	0.16	0.02

Values represent a single triplicate assay.

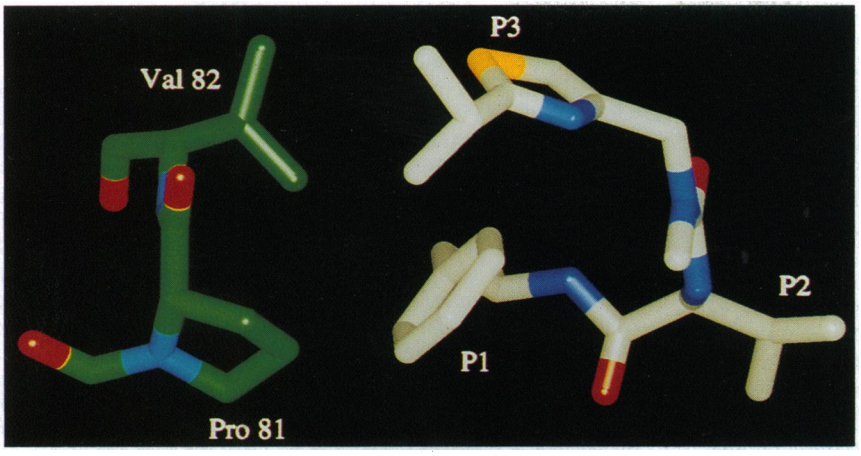


FIG. 2. Preliminary x-ray crystal structure, refined to 1.8 Å, of ABT-538/HIV-1 protease, showing a hydrophobic cluster between Pro-81, Val-82, and the P3 isopropylthiazolyl and P1 phenyl groups of ABT-538. Space group is $P2_12_12$.

position (A-81525) and a 4-thiazolyl group into the P3 position (A-82200). The high specificity for a 5-thiazolyl group vs. 4-thiazolyl or 2-thiazolyl (data not shown) at P2′ suggested specific hydrogen bonding of the thiazolyl nitrogen to the backbone NH of Asp-30 similar to that observed in the x-ray structure of A-80987 bound to HIV protease (C.H.P., unpublished results) and provided support for the bioisosteric relationship of the two heterocycles in this series of inhibitors. Aqueous solubility, which declined upon replacement of pyridine with the less basic thiazolyl group, was adjusted by use of an *N*-methylurea linkage between the P3 and P2 groups, a feature previously shown to confer increased solubility (10). Importantly, the oral bioavailability of both A-81525 and A-82200 exceeded that of A-80987. Encouraged by this initial success, we examined methods to improve the antiviral potency of this series. Structural modifications at the P2′ position led to reduced activity; however, substitution of hydrophobic groups at the 2 position of the P3 thiazolyl ring enhanced potency substantially (A-83962). The combination of these changes led to the identification of ABT-538 (Fig. 1), which showed an ≈9-fold enhancement of activity against HIV-1_{3B} in MT4 cells over A-80987. More importantly, administration of a 10-mg/kg oral dose of ABT-538 to rats resulted in prolonged absorption (t_{max} = 2.0 h), peak plasma levels > 100-fold in excess of the EC_{50}, and a calculated oral bioavailability of 78%.

Properties of ABT-538. The excellent pharmacokinetic properties of ABT-538 in rat were confirmed in monkey and dog (Table 1). In all species, the time to maximal plasma concentration was delayed ($t_{max} \geq$ 1.0 h) compared with A-80987. Following a 5-mg/kg oral (solution formulation) dose in rat, dog, and monkey, plasma concentrations of ABT-538 exceeded the *in vitro* EC_{50} for anti-HIV activity by >50-fold at the C_{max}, and they remained above the EC_{50} for ≥6–8 h. The change in the area of the plasma concentration vs. time curve was nonlinear between 5 and 10 mg/kg in both dog and monkey but not in the rat. In each species, ≥30% oral bioavailability was observed at all doses. Administration of a single 5-mg/kg dose of the proprietary clinical formulation (L. Al-Razzak, personal communication) in dog provided a mean C_{max} of >7 μg/ml with plasma levels of ABT-538 in excess of the EC_{50} for 10 h.

Several factors appear to contribute to the superior properties of ABT-538. Enhancement of antiviral potency over A-80987 is predominantly a result of a lower K_i for HIV protease (ABT-538, 15 pM; A-80987, 164 pM). Modeling studies of ABT-538 based on the crystal structure of A-77003 (14) suggested an additional hydrophobic interaction between the isopropyl substituent on the P3 thiazolyl group of ABT-538 and the side chains of Pro-81 and Val-82 of HIV protease. The energetic importance of this interaction was experimentally substantiated by the preliminary x-ray structure of ABT-538 bound to HIV protease (Fig. 2) and by the selection of Val-82 mutants upon *in vitro* passage of HIV in the presence of increasing concentrations of ABT-538 (15). The precise physicochemical features responsible for the pharmacokinetic profile of ABT-538 are less easily defined. Because of the lower basicity of the thiazolyl groups, the aqueous solubilities of ABT-538 and A-80987 were similar in pH 7.4 buffer but nearly 20-fold different at pH 4. This difference may be responsible in part for the prolonged absorption of ABT-538. The delicate balance between solubility and oral absorption was emphasized by the carbamate analogue of ABT-538, which was insoluble in the dosing vehicle and, given orally as a suspension, provided only trace plasma levels. Furthermore, ABT-538, administered to dogs as a solid capsule, generated very low plasma concentrations, suggesting that dissolution of the inhibitor was critical for good bioavailability.

In support of our design hypothesis, the improved pharmacokinetic profile of ABT-538 also correlated with the rate of metabolism in dog hepatic microsomes. We examined the metabolic lability of three inhibitors which differed in the number of pyridinyl and thiazolyl end groups. The relative rates of conversion of parent compound to metabolites we observed (A-80987, 1.0; A-83962, 0.2; and ABT-538, 0.05) are entirely consistent with the decreased susceptibility of the thiazolyl groups toward oxidation relative to the pyridinyl groups. Furthermore, the rate of biliary excretion of ABT-538 in bile-exteriorized dogs after intravenous dosing was roughly half that of A-80987 (39.8% vs. 76.8%, respectively, of the dose excreted within 6 h). Taken together, these results suggest that the improved pharmacokinetic profile of ABT-538 is directly related to increased hepatic stability.

In anticipation of clinical studies, the biochemical, antiviral, and pharmacokinetic properties of ABT-538 were examined in detail. High (>10^5) specificity for HIV protease over the human aspartic proteinases pepsin, renin, and gastricin was observed. In contrast, ABT-538 inhibited human cathepsins D and E with K_i values of 20 nM and 8 nM, respectively (J. Kay, personal communication). Nonetheless, these values still represent a specificity of ≥500-fold for HIV protease. The spectrum of antiviral activity of ABT-538 against four laboratory strains of HIV-1 (IIB, MN, RF, and TR17) and one strain of HIV-2 (MS) was examined in MT4 cells. As shown in Table 4, activity was comparable or superior to that of AZT against HIV-1 but ≈1/8 of that against HIV-2. We also examined the effect of ABT-538 toward a panel of clinical isolates by cocultivation of uninfected and infected peripheral blood lymphocytes (PBLs). The average EC_{50} for reduction of p24 antigen production on day 7 for seven isolates was 0.045 μM (high, 0.13; low, <0.01). Cytotoxicity in uninfected PBLs, as measured by trypan blue exclusion, was observed only at much higher concentrations [50% cytotoxic concentration ($CCIC_{50}$) = 49 μM]. As anticipated for a protease inhibitor,

ABT-538 demonstrated equal potency (EC_{50} = 0.013 and 0.019 μM, respectively) against pre- and post-AZT-resistant HIV in MT2 cells (16).

Recent reports have suggested that the lymph system is a primary site of HIV replication *in vivo* (17, 18). We therefore examined the distribution of ABT-538 into lymph organs. Following a single 10-mg/kg oral dose in rats, the concentration of parent compound in the thymus, cervical lymph node, and mesenteric lymph node exceeded the *in vitro* 90% effective concentration (EC_{90}) for >12 h (t_{max} = 3 h, C_{max} = 1.64, 2.26, and 2.29 μg/ml, respectively) and declined more slowly than plasma levels. Tissue distribution studies following a 50-mg/kg oral dose of ^{14}C-labeled ABT-538 in rats (J.F.D., unpublished data) also provided tissue-to-plasma ratios ≥1 for total radioactivity in the brachial and submaxillary lymph nodes after 9 h (6.17 and 7.07 μg equivalents, respectively). Taken together, these results suggest that trough levels in human lymph organs, where the replication of HIV is high, will equal or exceed those observed in plasma on repeated dosing. Concentrations of ABT-538 in rat brain following a single 50-mg/kg dose were low, affording tissue-to-plasma ratios ≤1 over 24 h and suggesting little propensity for accumulation in the central nervous system upon chronic treatment.

The excellent pharmacokinetic profile of ABT-538 was confirmed in phase I human trials (J.M.L., unpublished results). Fig. 3 compares the plasma levels obtained in healthy male volunteers after a single 400-mg (5.9-mg/kg) dose of formulated ABT-538 with those obtained with a 500-mg (6.6-mg/kg) dose of A-80987. ABT-538 exhibited peak plasma levels of 5.3 ± 1.8 μg/ml, a >300-fold excess over its *in vitro* EC_{50}, and a dose-normalized area under the curve which was 14-fold greater than that of A-80987. Furthermore, the absorption profile in humans (t_{max} = 3.4 ± 1.4 h) extended well beyond that observed in any of the animal models. Indeed, plasma concentrations in humans exceeded the *in vitro* EC_{90} for >24 h. These results establish that the favorable pharmacokinetic properties exhibited by ABT-538 in animals constituted a sound basis for its selection for human clinical trials.

In the absence of reliable animal models for AIDS, the simultaneous optimization of both *in vitro* anti-HIV activity and pharmacokinetic behavior of antiviral agents provides the most reliable measure of potential activity *in vivo*. A pharmacokinetic profile which maintains systemic levels sufficient to completely block viral replication *in vivo* may be particularly important to prevent or delay the onset of resistance. Through systematic studies of the relationship of structure to activity, pharmacokinetics, and metabolism, we have identified ABT-538, a peptidomimetic inhibitor of HIV protease with a unique combination of potency and oral bioavailability. In accord with recent reports of both renin (19) and HIV protease inhibitors (20) with promising pharmacokinetic properties, these results establish that peptidomimetic agents with high oral bioavailability in animals and humans can be discovered. As observed with another HIV protease inhibitor (21), the antiviral activity of ABT-538 in vivo is anticipated to be attenuated by ≥10-fold due to high (99%) binding to human plasma proteins. Nevertheless, the prolonged pharmacokinetic profile in humans should provide trough concentrations consistently in excess of the estimated *in vivo* EC_{95}. Indeed, in early trials in AIDS patients, orally administered ABT-538 has produced profound declines in plasma viral RNA with concomitant increases in CD4 cells (22, 23). The continued investigation of ABT-538 for treatment of HIV infection is therefore strongly warranted.

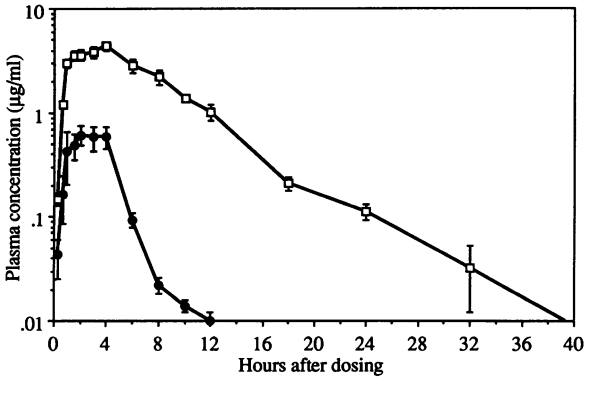


FIG. 3. Mean (±SEM) plasma levels of ABT-538 and A-80987 following oral dosing in humans. □, Single 400-mg dose of ABT-538 (n = 8); ●, single 500-mg dose of A-80987 (n = 10).

We are grateful to Prof. John Kay (University of Wales) for inhibition studies on human cathepsin D and E. The synthesis of ^{14}C-labeled A-80987 and ABT-538 by Dr. John Uchic is gratefully acknowledged. We are grateful to Ms. Jean Patterson for assistance in inhibitor synthesis. This work was supported in part by Grant AI 27720 from the National Institute of Allergy and Infectious Diseases.

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