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LACK OF STEREOSPECIFICITY IN THE BINDING OF THE P2 AMINO ACID OF RITONAVIR TO HIV PROTEASE

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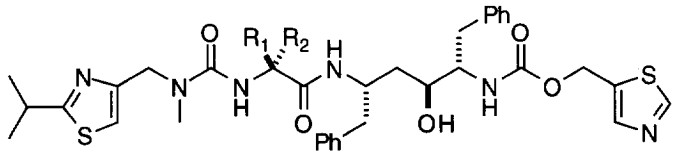
Abstract. The biological and pharmacokinetic properties of the HIV protease inhibitor ritonavir and its D-valinyl diastereomer, A-117673, were found to be indistinguishable. The X-ray crystal structure of the A-117673/HIV protease complex demonstrated similar binding modes for the two inhibitors, with a *ca* 1 Å difference in the backbone that allows the valine side chain of both compounds to project into the S2 subsite of the enzyme.

The binding of most potent inhibitors of the aspartic proteinase encoded by the human immunodeficiency virus (HIV protease) is specific with regard to the configuration at various stereocenters. Notable exceptions to this trend include the lack of stereospecificity in the binding of C_2-symmetry-based diaminodiols[1] and C-terminally truncated hydroxyethylamines[2] to HIV protease. In both cases, distinct conformations of diastereomeric inhibitors allow interaction of the hydroxyl group(s) with the catalytic aspartate residues.[2,3] Nonspecificity with regard to the configuration of hydrophobic P2 or P3 side chains in peptidomimetic HIV protease inhibitors is, to our knowledge, unprecedented. Indeed, modified D-amino acids have been utilized specifically to project the 'P2' side chain and 'P3' end group into the S3 and S2 subsites, respectively, thereby inverting the peptidomimetic orientations.[4] A lack of specificity has only been observed in nonpeptide, coumarin-based inhibitors wherein the nature of the P2 and P3 groups are similar in length and hydrophobicity.[5]

Ritonavir (ABT-538) is an orally bioavailable HIV protease inhibitor[6] that produces a profound decline in plasma HIV RNA in HIV-infected individuals accompanied by a significant enhancement in CD4 cell levels.[7,8] Ritonavir is highly potent (K_i = 15 pM) and specific for HIV protease.[6] Thus, we were surprised to discover in the course of evaluating diastereomeric structures for activity that A-117673, the D-valinyl diastereomer of ritonavir, also displayed potent inhibition. Here we report that the antiviral and pharmacokinetic properties of A-117673 are nearly indistinguishable from those of ritonavir. Further, we report the X-ray crystal structure of A-117673 bound to HIV protease, which displays a binding mode similar to that of ritonavir.



ritonavir (ABT-538): R_1 = *i*-Pr, R_2 = H
A-117673: R_1 = H, R_2 = *i*-Pr

Table 1. Anti-HIV activity of A-117673 (n = 4, mean ± S.D.).

	EC_{50} (μM)		$CCIC_{50}$ (μM)
Inhibitor	(0% human plasma)	(50% human plasma)	(0% human plasma)
A-117673	0.12 ± 0.03	0.88 ± 0.48	27 ± 4
Ritonavir	0.09 ± 0.03	1.07 ± 0.31	35 ± 10

The inhibitory potency of A-117673 against purified, recombinant HIV-1 protease was evaluated using a fluorogenic substrate.[9] The inhibitor served as an active site titrant, displaying 76.7% inhibition at 0.5 nM. In the same experiment, ritonavir showed 74.4% inhibition at the same concentration. The antiviral activity of A-117673 was evaluated against HIV-1_{3B} in MT4 cells using cytopathic effect as an endpoint. A direct comparison with ritonavir (n = 4) is shown in Table 1. Within experimental error, the activity of A-117673 was indistinguishable to that of ritonavir, both in the absence and presence of human serum, which is known to compromise the in vitro activity of some protease inhibitors.[10]

We next examined the pharmacokinetic properties of A-117673 in rats and dogs. The plasma concentrations of A-117673 and ritonavir following a 10 mg/kg (rats) or 5 mg/kg (dogs) oral dose are shown in Fig. 1. Pharmacokinetic parameters following a 5 mg/kg dose in three species are provided in Table 2. In both rat and dog, the pharmacokinetic properties of A-117673 were comparable or superior to that of ritonavir. In the monkey, the oral bioavailability and C_{max} of ritonavir were higher. In all three species, the pharmacokinetic profile of A-117673 was characterized by efficient absorption and prolonged maintenance of plasma concentrations, the latter of which is associated with a reduced rate of resistance emergence in vivo.[11]

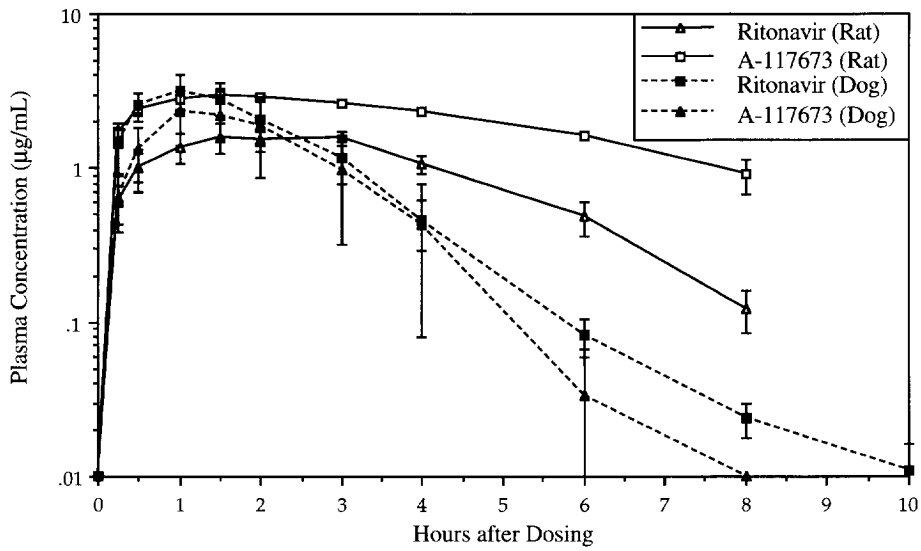


Fig. 1. Mean (± S.E.M.) plasma concentrations of A-117673 (n = 2 - 3) and ritonavir (n = 5 - 7) in rats and dogs following a 10 mg/kg or 5 mg/kg oral dose, respectively.

Table 2. Pharmacokinetic properties of A-117673.

Species	Dose (5 mg/kg)	Property	A-117673		Ritonavir	
Rat	IV	$T_{1/2}$	1.8		0.66	
		Cl_p	0.47		1.04	
	Oral	C_{max}	2.99	(0.22)	1.36	(0.16)
		AUC	19.03	(0.25)	3.53	(0.50)
		F	86.2	(1.13)	70.7	(10.0)
Dog	IV	$T_{1/2}$	1.41		1.07	
		Cl_p	0.36		0.22	
	Oral	C_{max}	2.36	(0.94)	3.58	(0.72)
		AUC	6.06	(3.21)	8.06	(1.93)
		F	42.5	(22.5)	37.4	(9.5)
Monkey	IV	$T_{1/2}$	0.63		2.26	
		Cl_p	1.10		0.67	
	Oral	C_{max}	0.56	(0.22)	1.08	(0.30)
		AUC	1.07	(0.46)	2.57	(0.77)
		F	21.8	(9.3)	29.9	(7.1)

Values are expressed as mean (standard error). $T_{1/2}$: terminal phase half-life (hours); Cl_p: clearance (μg·h/mL); C_{max}: maximum plasma concentration following oral dosing (μg/mL); AUC: area under the plasma concentration curve (μg·h/mL); F: oral bioavailability (%).

The favorable pharmacokinetic profile of ritonavir is associated with potent inhibition of cytochrome P450 (CYP), the major oxidative enzymes in the liver and intestine.[12,13] We compared the spectroscopic interaction of A-117673 and ritonavir with cytochrome P450 in human liver microsomal preparations.[14] Both inhibitors elicited identical 'Type II' difference spectra (wavelength maxima and minima at 424 nm and 395 nm, respectively), indicating that each is able to interact directly with the CYP heme via the unhindered 5-thiazolyl nitrogen atom.[12] Both compounds also inhibited the terfenadine hydroxylase activity representing the most abundant form of cytochrome P450 (CYP3A4) present in human liver with an IC_{50} of 0.25 μM. Taken together, these results suggest that coadministration of other HIV protease inhibitors with A-117673 might significantly enhance the plasma levels of those inhibitors in a manner similar to that observed after coadministration with ritonavir.[12]

The above results indicate that the biological and pharmacokinetic properties of A-117673 and ritonavir are equivalent. If, however, the binding of each inhibitor to the active site of HIV protease were distinct, the activity of each against resistant HIV mutants might be expected to differ, offering the prospect of combination therapy using both diastereomers. We therefore examined the activity of A-117673 against mutant HIV strains from patients undergoing monotherapy with ritonavir[11] (Table 3). Although minor differences between the two inhibitors were noted (e.g. in the activity against the V82F and V82S mutant strains), the significant loss of activity against the multiply mutated (M36I,I54V,A71V,V82T) strain from patient 129 suggested that viral strains with high level resistance to ritonavir[11] would be substantially cross-resistant to A-117673.

Table 3. Activity of A-117673 against HIV selected during ritonavir therapy.

Patient No.	Viral Genotype[a]	EC_{50} (nM) A-117673	Ritonavir
302	baseline	46	23
302	V82A	43	32
313	baseline	35	35
313	V82F	43	123
337	baseline	19	35
337	V82S	22	143
304	baseline	35	19
304	M36M/I, V82V/A	59	54
129	baseline	5	5
129	M36I, I54V, A71V, V82T	25	65

[a]Variation in HIV protease from the consensus B sequence that did not pre-exist in the baseline sequence.

We further examined the activity of A-117673 against HIV molecular clones containing the initial mutations in HIV protease selected by ritonavir monotherapy[11] (Table 4). The 2- to 3-fold loss of susceptibility of each mutant to A-117673 was similar to that observed with ritonavir, again suggesting a significant level of cross resistance between the two agents.

Table 4. Activity of A-117673 against mutant HIV molecular clones.

VIRUS	EC_{50} (μM) A-117673	Ritonavir
wt (pNL4-3)	0.137	0.055
V82A	0.121	0.230
V82F	0.276	0.308
V82T	0.352	0.316

The high degree of similarity in the activity of A-117673 and ritonavir toward both wild-type and mutant HIV suggested that the binding modes for the two compounds to the HIV protease active site were not dissimilar in spite of the different configuration at the P2 valinyl group. The preliminary X-ray crystal structure of A-117673 bound to HIV protease was solved at 1.8 Å resolution (P_{61}). An overlay of the two inhibitors and a close-up of the P2 valinyl units of A-117673 and ritonavir (2.0 Å, P_{61}) are shown in Fig. 2a and 2b. In spite of the opposite configuration at the valine α-carbon, the ϕ and φ angles of the valine residue of the two inhibitors are similar (-95 and +82 for ritonavir and -108 and +58 for A-117673, respectively). A *ca* 1-1.3 Å shift of the backbone of A-117673 relative to ritonavir permits both inhibitors to project the valine side chain into the hydrophobic P2 subsite. The isopropyl side chain of each compound adopts a low-energy conformation with the two methyl groups anti to either the C_α-N or C_α-CO backbone bonds. As a result, corresponding methyl groups

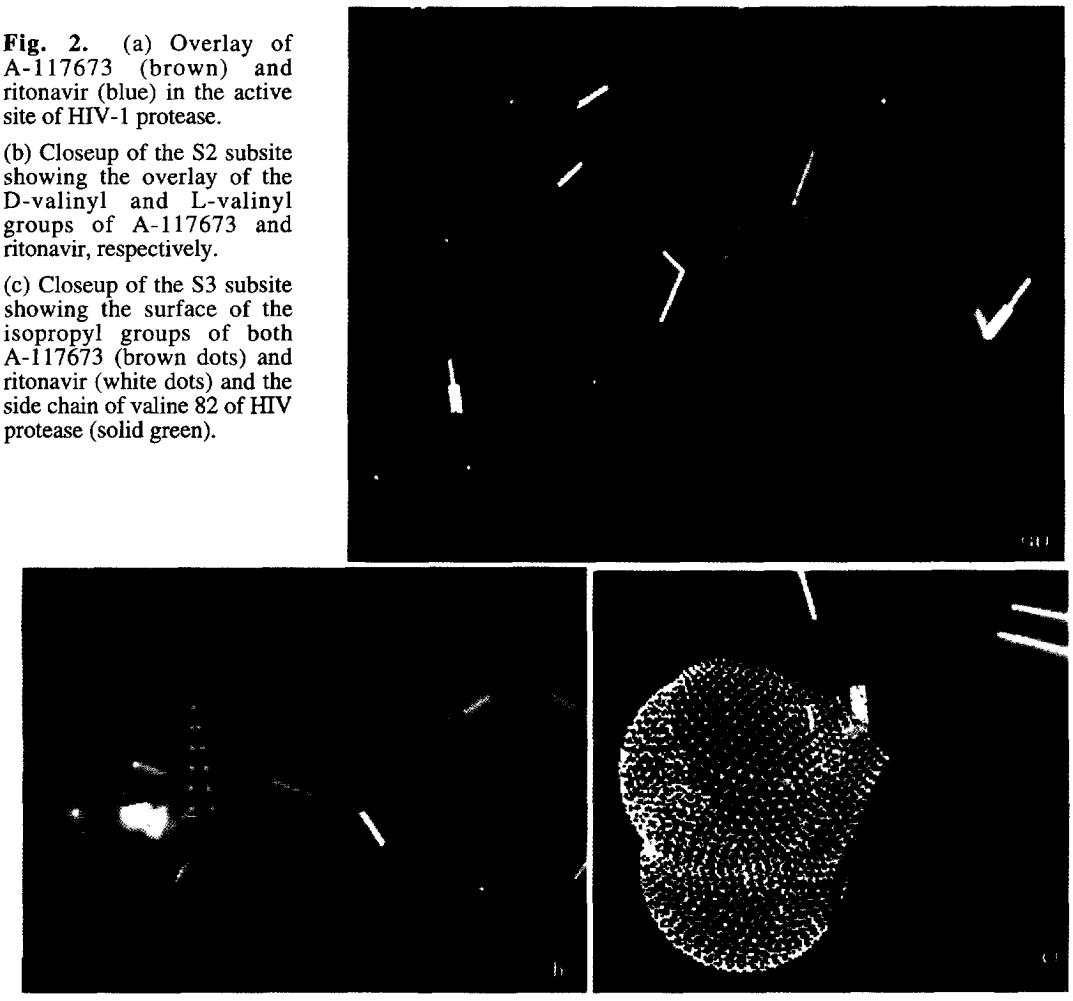


Fig. 2. (a) Overlay of A-117673 (brown) and ritonavir (blue) in the active site of HIV-1 protease.

(b) Closeup of the S2 subsite showing the overlay of the D-valinyl and L-valinyl groups of A-117673 and ritonavir, respectively.

(c) Closeup of the S3 subsite showing the surface of the isopropyl groups of both A-117673 (brown dots) and ritonavir (white dots) and the side chain of valine 82 of HIV protease (solid green).

between the two inhibitors are ≤0.6 Å apart when overlaid. Importantly, the three hydrogen bonding interactions observed between ritonavir and HIV protease in the S2 subsite region are preserved in the A-117673 complex: (1) the valine carbonyl oxygen accepts a hydrogen bond from H_2O 301, (2) the valine NH donates a hydrogen bond to the Gly 48 carbonyl oxygen, and (3) the urea carbonyl oxygen accepts a hydrogen bond from Asp 29 NH. No significant differences in the positions of the enzyme S2 subsite residues were noted between the two structures; thus, A-117673 does not induce a conformational change in the enzyme upon binding. In summary, A-117673 binds remarkably similarly to the canonical backbone conformation orientation observed for many peptide- or C_2 symmetry-based inhibitors, with the above described departure in the S2 subsite. The P3 isopropylthiazolyl group of A-117673 is only minimally displaced relative to that of ritonavir, preserving a significant interaction

with Val 82. Figure 2c shows the hydrophobic interaction of both isopropyl groups with the side chain of Val 82, which is associated with the high potency of ritonavir against wild-type HIV protease.[6] The similarity in the position of this isopropyl group between the two inhibitors is consistent with the similar profile of both toward V82 mutants.

In summary, A-117673, the D-valinyl isomer of ritonavir, displays biological and pharmacokinetic properties that are indistinguishable from those of ritonavir. Comparison of the X-ray structures of both inhibitors in the active site of HIV protease illustrates two similar binding modes that project the P2 side chain of each into the same location. The overall similarity of the two structures is unprecedented in peptidomimetic HIV protease inhibitors, and is consistent with the similar profiles of the two agents against mutant HIV.

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