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POTENT PIPERAZINE HYDROXYETHYLAMINE HIV PROTEASE INHIBITORS CONTAINING NOVEL P_3 LIGANDS

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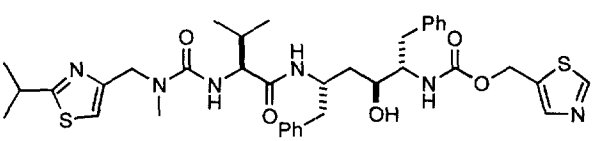
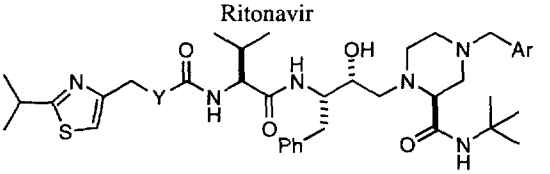
Abstract. The 2-isopropyl thiazolyl group is a highly optimized P_3 ligand for C_2 symmetry-based HIV protease inhibitors, as exemplified in the drug ritonavir. Here we report that incorporation of this P_3 ligand into a piperazine hydroxyethylamine series also yielded novel, highly potent inhibitors. In tissue culture assays, the presence of human serum was less deleterious to the activity of these inhibitors than to that of ritonavir. Furthermore, potent activity against ritonavir resistant HIV was observed.

Human immunodeficiency virus (HIV) protease is responsible for the cleavage of the *gag* and *gag-pol* polyproteins for the maturation of infectious virus. Recent clinic studies have demonstrated the benefit in blocking this critical viral enzyme for preventing death and disease progression for advanced AIDS patients.[1-3] A wide variety of classes of peptidomimetic inhibitors have been reported based upon HIV protease substrate sequences and on the three-dimensional structure of the C_2-symmetric, homodimeric enzyme active site.[4] As part of our program to explore inhibitors structurally related to ritonavir,[5-7] we were interested in incorporating the 2-isopropyl-4-thiazolyl P_3 group, which is highly optimized for the C_2 symmetry-based HIV protease inhibitors,[8] into a hydroxyethylamine series of inhibitors.[9,10] We anticipated that the presence of the basic nitrogen atoms of the hydroxyethylamine would produce compounds with greater aqueous solubility than ritonavir. Additionally, we anticipated that use of the piperazine hydroxyethylamine transition state isostere, typified by structure **I**, might improve antiviral potency over ritonavir by projecting N_4-substituents into the hydrophobic S_3' subsite. Here we report that inhibitors of structure **I** display up to 20-fold greater anti-HIV activity than ritonavir in the presence of human serum.



Ritonavir



I, Y = O or NCH_3

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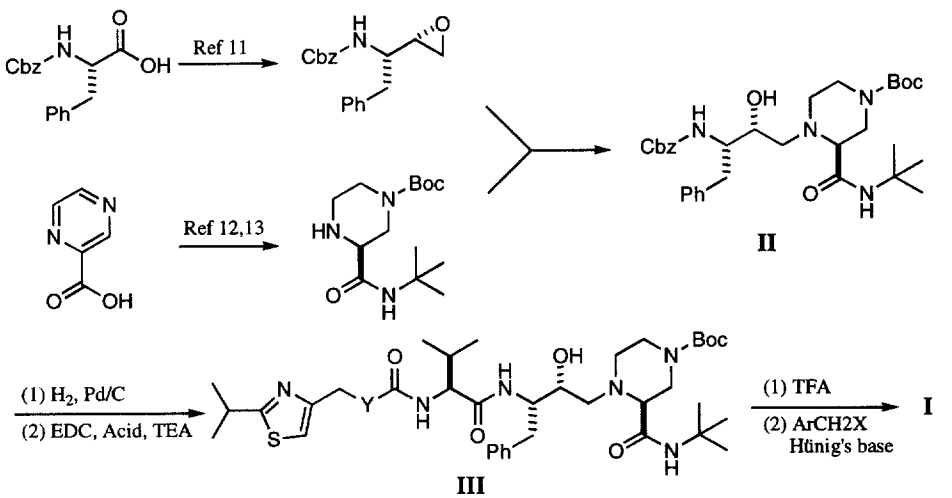
The fully protected piperazine core **II** was synthesized as previously reported.[11-13] After removal of the Cbz protecting group of **II** by catalytic hydrogenation, carbodiimide coupling to different P_2-P_3 acids yielded intermediates **III**. The desired inhibitors **I** were then generated by cleaving the Boc protecting group of **III** under acidic conditions and subsequently alkylating with activated alkyl halides (Scheme I). All inhibitors showed satisfactory purity by ^1H NMR and mass spectral analysis.

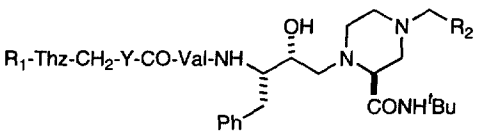
Scheme I. Synthesis of piperazine hydroxyethylamine HIV protease inhibitors



The biological activities of the inhibitors are summarized in Table 1. The IC_{50} values for the inhibitors against HIV protease and the anti-HIV activity (EC_{50}) and cytotoxicity ($CCIC_{50}$) values in MT4 cells using a cytopathic effect assay were measured according to reported methods.[14] In general, the substituted 4-thiazoles which are highly optimized P_3 ligands for C_2 symmetry-based HIV protease inhibitors, combined with the piperazine hydroxyethylamine core unit to give potent inhibitors. Compounds attached at the 4-position of the thiazole ring were uniformly more potent in the presence of human serum than analogs attached at the 5-position, regardless of the alkyl substitution at the 2-position of the thiazole ring (Example **22**, **24**, **26** vs. **23**, **25**, **27**). The 2-isopropyl group remained the optimal substituent for the 4-thiazole. Analogs (**6** and **7**) with unsubstituted 5-thiazole as a P_3 ligand maintained comparable antiviral activities to compounds with isopropyl substituted 4-thiazoles (**2** and **9**). In the ritonavir SAR studies,[15] inhibitors with carbamate linkages between the P_2 and P_3 ligand produced slightly better antiviral activities than inhibitors with N-methyl urea linkages. However, ritonavir has better aqueous solubility profiles than the corresponding inhibitor with a carbamate linkage. A similar SAR trend was observed in the current series. Inhibitors **2**, **6**, and **9** showed favorable protease activities as well as antiviral activities in the MT4 cell culture assay compared to inhibitors **1**, **4**, and **10**, respectively. Although the aqueous solubility of the inhibitors in the current series was not measured, it is

anticipated that the basic nitrogen atoms of the piperazine will improve the aqueous solubility and also compensate the solubility disadvantage of the carbamate linkages.

Table 1. Inhibition of HIV protease by piperazine hydroxyethylamine analogs of **I**.



No.	R_1	Thz	Y	Val	R_2	% Inhib. @ (nM)	EC_{50} (μM) (0%HS)	EC_{50} (μM) (50%HS)	$CCIC_{50}$ (μM)
Ritonavir						78 (0.5)	0.07	0.81	56
1	*i*-Pr	4	NMe	L	5-Thz	60 (0.5)	0.013	0.084	>100
2	*i*-Pr	4	O	L	5-Thz	67 (0.5)	0.012	0.067	57
3	*i*-Pr	4	O	D	5-Thz	50 (27)	6	26	18
4	H	5	NMe	L	5-Thz	50 (0.5)	0.259	0.211	>100
5	H	5	NMe	D	5-Thz	50 (97)	NA	NA	NA
6	H	5	O	L	5-Thz	45 (0.5)	0.057	0.075	>100
7	H	5	O	L	Ph	40 (0.5)	0.017	0.104	53
8	H	5	NMe	D	Ph	50 (120)	3	7	56
9	*i*-Pr	4	O	L	Ph	61 (0.5)	0.022	0.08	45
10	*i*-Pr	4	NMe	L	Ph	66 (0.5)	0.048	0.306	56
11	*i*-Pr	4	O	L	4-F-Ph	64 (0.5)	0.021	0.174	15
12	Me	4	O	L	4-F-Ph	50 (0.5)	0.011	0.071	39
13	*i*-Pr	4	O	L	3-OH-Ph	53 (0.5)	0.04	0.142	17
14	*i*-Pr	4	O	L	3-Pyr	69 (0.5)	0.012	0.067	59
15	*i*-Pr	4	O	L	3,4-OCH_2O-Ph	60 (0.5)	0.007	0.040	13
16	*i*-Pr	4	O	L	2-Quinolinyl	70 (0.5)	0.014	0.125	19
17	*i*-Pr	4	NMe	L	3-MeO, 4-OH-Ph	63 (0.5)	0.041	0.242	20
18	*i*-Pr	4	O	L	4-Pyr	73 (0.5)	0.017	0.076	56
19	*i*-Pr	4	O	L	4-OH-Ph	54 (0.5)	0.047	0.226	19
20	*i*-Pr	4	O	L	2-Benzimidazolyl	65 (0.5)	0.046	1.055	18
21	Me	4	O	L	3,4-OCH_2O-Ph	45 (0.5)	0.011	0.074	56
22	Me	4	O	L	3,4-Di-(MeO)-Ph	49 (0.5)	0.01	0.042	57
23	Me	5	O	L	3,4-Di-(MeO)-Ph	44 (0.5)	0.011	0.059	53
24	Et	4	O	L	3,4-Di-(MeO)-Ph	82 (0.5)	0.019	0.042	45
25	Et	5	O	L	3,4-Di-(MeO)-Ph	37 (0.5)	0.016	0.132	50
26	*i*-Pr	4	O	L	3,4-Di-(MeO)-Ph	62 (0.5)	0.017	0.046	18
27	*i*-Pr	5	O	L	3,4-Di-(MeO)-Ph	51 (0.5)	0.017	0.086	21
28	*i*-Pr	4	O	L	4-t-HBF	69 (0.5)	0.005	0.042	18

HS = human serum; NA = not active; Thz = thiazolyl; Pyr = pyridyl; 4-t-HBF = 4-tetrahydroisobenzofuranyl.

Unlike ritonavir, which lacks of stereospecificity in the binding of the P_2 amino acid to HIV protease,[16] the D-valine analogs in the present series cause drastic loss of antiviral activities (compounds **3** vs. **2** and **5** vs. **4**). SAR studies also identified several novel P_3' groups in this series. The inhibitors (**15** and **28**) possessing 3,4-(methylenedioxy)phenyl and tetrahydroisobenzofuran as P_3' groups were most active (EC_{50} = 7 and 5 nM respectively) in the antiviral assay. In the presence of human serum, the antiviral activity of ritonavir declines by >10-fold in vitro due to binding to α_1-acid glycoprotein and human serum albumin.[17] With the exception of **20**, the potency of compounds **1-28** in MT4 cells was attenuated only 2- to 9-fold in the presence of 50% human serum. This observation is consistent with the low protein binding reported for indinavir, which also contains a piperazinyl hydroxyethylamine.[9]

Table 2. Inhibition of human liver microsomal CYP3A4.

Protease Inhibitor	Ritonavir	2	14	15	22	25	28
IC_{50} (μM)	0.25	15	2.3	9.6	120	35	12

The favorable pharmacokinetic profile of ritonavir has been associated with potent inhibition of the 3A4 isozyme of cytochrome P450 (CYP) in the liver and/or intestine.[18] Ritonavir interacts directly with the CYP heme via the unhindered P_2'-5-thiazolyl nitrogen atom. We therefore examined the inhibitory effect of selective members of this series of compounds (Table 2). Compound **14** was the most active inhibitor against CYP3A4 with an IC_{50} of 2.3 μM (10-fold less active than ritonavir). Interestingly, the CYP active site can apparently accommodate the ring structure presented in the backbone, however, more potent inhibition was observed with the 3-pyridyl group of **14** than with the 5-thiazolyl group of **2**.

Table 3. Susceptibility of patient isolates to ritonavir and compound **15**.

		EC_{50} (nM) (Fold Change over Pretreatment Baseline)	
Patient Sample No.	**Viral Genotype**	**Ritonavir**	**Compound 15**
129-week 8	M36I, I54V, A71V, V82T	149 (14)	10 (2)
129-week 16	K20K/R, 36M/I, I54V, A71A/V, V82T	368 (33)	33 (7)
131-week 28	K20K/R, M36M/I, I54V/I, L63L/P, V82/A	365 (20)	29 (7)
313-day 57	M36M/I, V82F	340 (9)	24 (3)
313-day 85	M36M/I, I54I/V, V82F/A/S/T	575 (14)	27 (3)
235-week 36	I54I/V, A71V, V82A, L90L/M	87 (4)	24 (2)

Because of its high potency against wild-type HIV, the activity of compound **15** (A-160621) was assessed against viral strains isolated from patients undergoing monotherapy with ritonavir[19] (Table 3). The antiviral effect of A-160621 was maintained against molecular clones containing a single point mutation at position 82 (data not shown). While the potency of ritonavir declined significantly (14-fold) against the

multiply mutated (M36I, I54V, A71V, V82T) strain from patient 129, A-160621 displayed an EC_{50} of 10 nM, which is only twofold higher than the EC_{50} against pretreatment virus. Because the antiviral activity of A-160621 is only attenuated by sixfold in the presence of human serum, the modest (two to sevenfold) change in EC_{50} for A160621 against ritonavir-resistant HIV suggests that plasma levels of 1 μM or greater may be suppressive of resistant HIV in vivo.

Previous studies have demonstrated that coadministration with ritonavir significantly enhances the plasma levels of other protease inhibitors.[19] Accordingly, we examined the levels of A-160621 in rats and dogs (Figure 1) after an equal (10 mg/kg and 5 mg/kg, respectively) co-dose of ritonavir. The AUC of A-160621 was elevated by sixfold and 24-fold, respectively. Notably, even after 8 h the plasma levels of A-160621 exceeded the EC_{50} in the presence of human serum (0.04 nM) by 50-fold and >eightfold, respectively, in these species. Co-therapy with A-160621 and ritonavir may therefore be effective for suppressing HIV in vivo.

In conclusion, we have identified several new P_3 ligands and also incorporated substituted 4-thiazoles as P_3' ligands into the piperazine hydroxyethylamine-based HIV protease inhibitors. Some members of this series displayed a 20-fold increase in antiviral activity against HIV in the presence of 50% human serum over ritonavir in vitro. A-160621 maintained excellent potency against ritonavir-resistant, multiply mutated viral strains. Additional modifications that further improve the pharmacokinetic profile of members of this structural class may produce compounds with suitable properties for clinical evaluation.

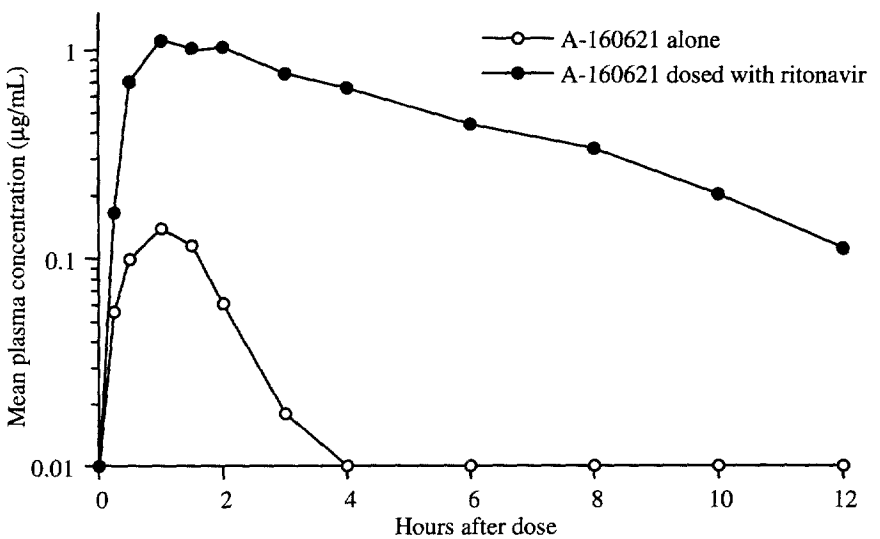


Figure 1. Mean plasma concentrations of A-160621 (compound **15**) after a 5 mg/kg oral dose in dogs (n = 3) alone or co-dosed with 5 mg/kg of ritonavir.

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