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SYNTHESIS AND ANTIVIRAL ACTIVITY OF A NOVEL CLASS OF HIV-1 PROTEASE INHIBITORS CONTAINING A HETEROCYCLIC P_1'-P_2' AMIDE BOND ISOSTERE

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Abstract: A novel series of hydroxyethylene-based peptidomimetics that contain 2-substituted nitrogen heterocycles as P_1'-P_2' amide bond isosteres has been prepared and evaluated as inhibitors of HIV-1 protease and *in vitro* HIV-1 replication. Many of these compounds exhibit inhibition constants in the low to sub-nanomolar range. Structure-activity relationships are discussed.

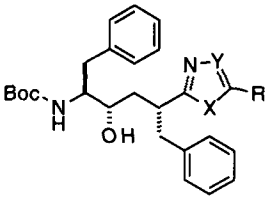
Human immunodeficiency virus type-1 (HIV-1), a member of the *Retroviridae* family, is the causative agent of acquired immunodeficiency syndrome (AIDS).[1] A crucial step in the replicative cycle of the virus involves the processing by a virus-encoded protease of the viral *gag* and *gag-pol* polyproteins into structural proteins and enzymes essential for the proper assembly and maturation of fully infectious virions.[2] The HIV-1 protease is therefore an attractive target for the design of antiviral agents for the treatment of AIDS.

To date, a number of different potent classes of HIV-1 protease inhibitors have been reported.[3] With the exception of a recently reported series of non-peptide cyclic ureas,[4] these inhibitors are peptide analogs in which the scissile amide bond has been replaced by a non-hydrolyzable isostere. Additionally, pseudosymmetric[5] and C_2 symmetric[4,6] inhibitors which take advantage of the C_2 symmetric nature of the enzyme have been reported. Each of these classes has provided extremely potent compounds that inhibit HIV-1 protease in the nanomolar and sub-nanomolar range and are also quite effective as inhibitors of HIV-1 replication in cell cultures.[5a,7] Although the scissile amide bond in these inhibitors has been replaced with an isostere, most of them still contain a number of labile amide bonds as a consequence of their peptidic nature. We sought to design an improved antiviral drug by replacement of one or more additional amide bonds with isosteres that are hydrolytically stable. Our approach involved the substitution of such an amide replacement for the P_1'-P_2' (nomenclature of Schechter and Berger[8]) amide bond.

The development of an isostere for the P_1'-P_2' amide bond presents a formidable challenge within the context of inhibition of HIV-1 protease. The correct *trans* orientation about the amide bond portion of the isostere, as well as its capability to function as a hydrogen bond acceptor for the bound water molecule are required. Bearing in mind that the isostere must also be hydrolytically stable, a nitrogen heterocycle such as that shown in Figure 1 appeared to be a promising candidate.[9] The present report describes the evaluation of

three different nitrogen heterocycles, triazole, thiazole and imidazole, as P_1'-P_2' amide bond replacements in hydroxyethylene-based HIV-1 protease inhibitors.

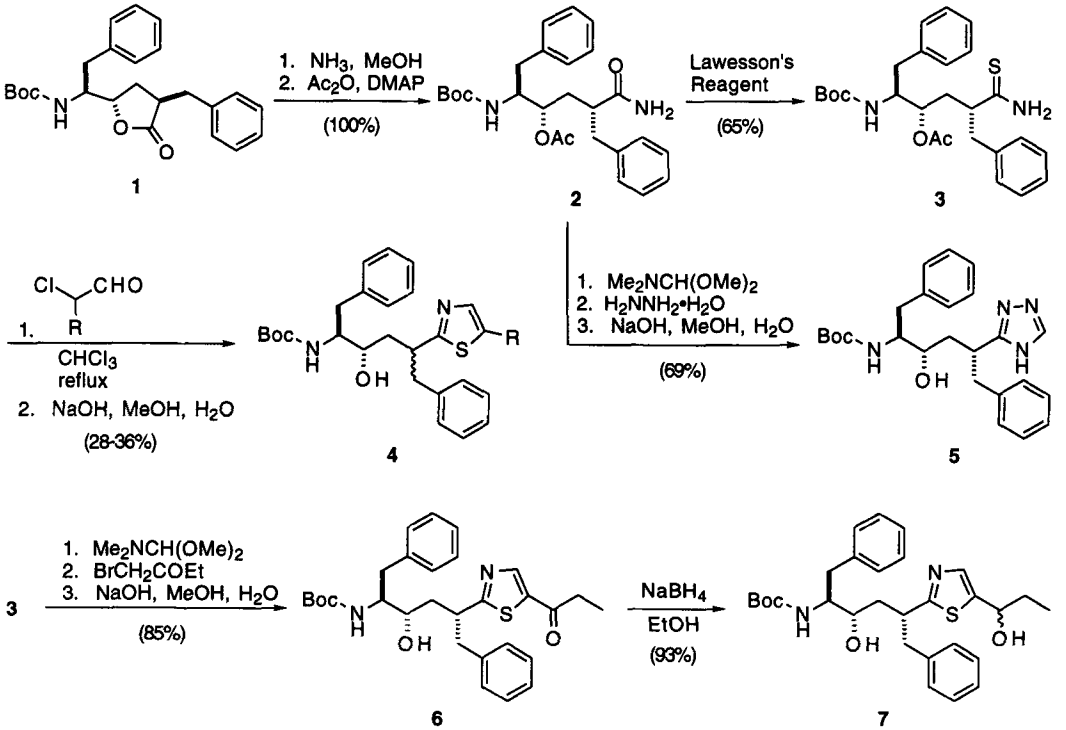
Figure 1



X = NH, S; Y = CH, N

The thiazole and triazole-containing inhibitors were prepared from the known lactone **1**[10] as shown in Scheme I. Ammoniation of **1** followed by acetylation of the resulting hydroxy amide provided amide **2**. Condensation of **2** with N,N-dimethylformamide dimethyl acetal, followed by treatment with hydrazine hydrate[11] and subsequent basic hydrolysis of the acetate ester afforded the desired triazole **5**. The preparation of the thiazole-containing inhibitors involved first the conversion of amide **2** into thioamide **3** by treatment with Lawesson's Reagent.[12] Treatment of **3** with α-chloroaldehydes in refluxing chloroform[13] and subsequent

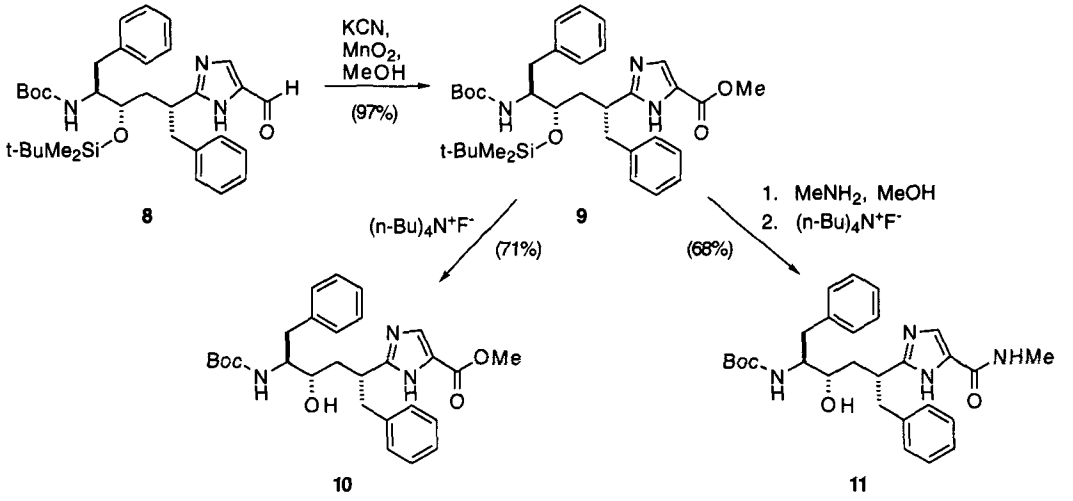
Scheme I



basic hydrolysis of the acetate esters provided 5-alkylthiazoles **4** with loss of stereochemistry at the carbon bearing the benzyl substituent. Alternatively, condensation of **3** with N,N-dimethylformamide dimethyl acetal, followed by reaction with 1-bromo-2-butanone[14] and subsequent basic hydrolysis of the acetate ester provided 5-propionyl thiazole **6**. Finally, reduction of **6** with sodium borohydride provided 5-(1-hydroxypropyl)thiazole **7**.

The 4(5)-acylimidazoles were synthesized as previously described[15] while the 4(5)-carbomethoxyimidazole (**10**) and 4(5)-carboxamidoimidazole (**11**) were prepared as shown in Scheme II. Treatment of 4(5)-formylimidazole **8** with potassium cyanide and MnO_2 in methanol[16] provided compound **9**, which was deprotected to afford 4(5)-carbomethoxyimidazole **10**. Alternatively, amination with methylamine in methanol followed by deprotection of the *t*-butyldimethylsilyl ether provided 4(5)-carboxamidoimidazole **11**. The purity and identity of all final compounds were ascertained by proton NNR and mass spectral analysis.

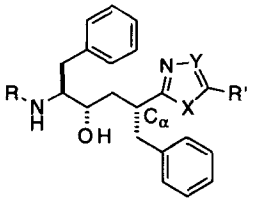
Scheme II



All three of the heterocycles studied were effective isosteres for the P_1'-P_2' amide bond in a hydroxyethylene-based HIV-1 protease inhibitor (Table 1). The inhibitors incorporating imidazoles were the most potent, with those incorporating a thiazole or triazole being significantly less potent. This difference in potency is most likely a result of the greater capability of the imidazole ring relative to the thiazole or triazole to act as a hydrogen bond acceptor because of its greater basicity. The replacement of an acyl substituent on a heterocycle with an alkyl group is also expected to significantly increase the basicity of the ring and therefore make it a stronger hydrogen bond acceptor. Evidence in support of this theory is given by the greater potency of a 5-(1-hydroxyalkyl)thiazole (entry 9) relative to that of a 5-acylthiazole (entry 8).

The imidazole-containing series, because it possessed the most potent inhibitors, was selected for further SAR studies. The effect of the acyl substituent on the imidazole was investigated first. The HIV-1 inhibitory potency in this series increased with increasing size of the acyl group, up to a chain length of three carbons.[15]

Table 1. Inhibition of HIV-1 Protease[a] and *In Vitro* Viral Replication[b] by Heterocycle-Containing Hydroxyethylene Isosteres.



Entry	X	Y	R	R'	$K_{i,app}$ (nM)	IC_{50} (nM)
1	NH	N	Boc	H	5800	NT (e)
2	S	CH	Boc	H	3000 (c)	NT
3	S	CH	Boc	Et	2000 (c)	NT
4	S	CH	Boc	*n*-Pr	700 (c)	NT
5	S	CH	Boc	*n*-Pr	4600 (d)	NT
6	S	CH	Boc	*n*-Pr	410 (d)	NT
7	S	CH	Boc	*n*-Bu	26000 (c)	NT
8	S	CH	Boc	COEt	3700	NT
9	S	CH	Boc	CH(OH)Et	720	NT
10	NH	CH	Boc	COEt	92	NT
11	NH	CH	Boc	CO-*s*-Bu	53	950 (f)
12	NH	CH	Boc	CO-3-pentyl	46	850
13	NH	CH	Boc	CO-*c*-pentyl	150	NT
14	NH	CH	Boc	COPh	7800	NT
15	NH	CH	Boc	CO_2Me	1800	NT
16	NH	CH	Boc	CONHMe	3700	NT
17	NH	CH	Cbz-Val	*i*-Pr	0.6	100
18	NH	CH	MeOCO-Val	*i*-Pr	2.1	50
19	NH	CH	EtOCO-Val	*i*-Pr	2.8	77
20	NH	CH	*i*-PrOCO-Val	*i*-Pr	7.9	134
21	NH	CH	*t*-BuOCO-Val	*i*-Pr	300	NT
22	NH	CH	Ac-L-Val	*i*-Pr	1.0	40
23	NH	CH	Ac-D-Val	*i*-Pr	150	NT
24	NH	CH	CHO-Val	*i*-Pr	4.6	NT
25	NH	CH	Cbz-Thr	*i*-Pr	1.4	11

[a]Enzyme inhibition assays were conducted at 37 °C in a buffer (pH 6.0) composed of 50 mM 2-(N-morpholinoethanesulfonic acid, 1 mM EDTA, 200 mM NaCl, 1 mM dithiothreitol, 0.1% triton X-100, and 10% (V/V) DMSO (MENDT buffer) in reaction mixtures containing 1-10 mM concentrations of the substrate Ac-RASQNYPVV-NH_2, variable concentrations of the inhibitor and 20 nM of HIV-1 protease (Ref. 17). [b]*In vitro* antiviral assays were conducted using MOLT-4 cells, adding compound on days 0, 2 and 4, and assaying for reverse transcriptase activity on day 7. [c]1:1 mixture of diastereomers at C_α. [d]One of diastereomeric pair. [e]Not Tested. [f]Assay conditions as above except no compound was added on days 2 and 4 post-infection.

α,α-Disubstitution was well tolerated and actually increased potency in some cases (entries 11 and 12 vs. entry 10). An sp^2 α-carbon was not well tolerated, however (entry 14). Furthermore, carboxylate or carboxamido substitutions on the imidazole ring resulted in dramatic losses in potency (entries 15 and 16). These structure-activity data add further support to the concept that the alkyl portion of the acyl substituent binds in the hydrophobic $S_{2'}$ subsite of the enzyme, as is observed in the X-ray structure of HIV-1 protease complexed with SB 206343 (entry 17).

Finally, the effect of addition of an N-protected L-valine or L-threonine residue onto the N-terminus of the inhibitor was investigated. Incorporation of either of these residues into the inhibitor resulted in a

tremendous increase in potency to low nanomolar and sub-nanomolar levels. A D-valine substitution in this position, however, does not enhance potency (entry 22 vs. 23). Furthermore, the nature of the N-terminal acyl group does not affect the potency of the inhibitors. N-terminal amides and carbamates show similar potency and the level of inhibition is essentially independent of the size of the acyl group (entries 17-22, 24), although a carbamate derived from a tertiary alcohol is not well-tolerated (entry 21). These findings are consistent with the crystallographic data which indicate that the N-terminal acyl group does not contribute to any positive binding interactions beyond hydrogen bonding to the carbonyl oxygen, but that a quaternary carbon alpha to the carbamate oxygen results in severe negative van der Waals interactions between the inhibitor and the enzyme.[15] These inhibitors are also quite effective towards the *in vitro* inhibition of viral replication, with IC_{50} values in the 10-100 nM range.

In summary, we have developed a novel series of potent, hydroxyethylene-based inhibitors of HIV-1 protease and *in vitro* HIV-1 replication that contain a 2-substituted nitrogen heterocycle as a P_1'-P_2' amide bond isostere. SAR data have revealed that the 4(5)-acylimidazole-containing inhibitors are the most potent and that this particular heterocycle is an excellent amide bond mimetic when incorporated into a hydroxyethylene-based HIV-1 protease inhibitor.

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