Communications to the Editor

Discovery of a Novel Class of Potent HIV-1 Protease Inhibitors Containing the (*R*)-(Hydroxyethyl)urea Isostere

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The human immunodeficiency virus type-1 (HIV-1), the causative agent of AIDS (acquired immunodeficiency syndrome), encodes for a unique aspartyl protease.[1] Inactivation of this protease by site-directed mutagenesis of the catalytic aspartyl residues results in the production of noninfectious virions.[2] As a result, this protease represents an attractive target for the development of a therapeutic agent for the treatment of AIDS. Numerous examples of potent inhibitors of this protease, involving the incorporation of a variety of isosteres at the cleavage site, have been reported and recently reviewed.[3-7] We now wish to report our results on the development of a potent class of HIV-1 protease inhibitors which incorporate the (hydroxyethyl)urea isostere. To our knowledge no one has reported the utility of this isostere for inhibitors of the HIV-1 protease.[8] The inhibitors reported herein show a strong preference for the (*R*)-hydroxyl isomer[9-11] and exhibit a unique mode of binding to the enzyme.

The urea isostere can be envisioned as a modification of the hydroxyethylene isostere, wherein the P_1' chiral α-carbon center is replaced with a trigonal nitrogen (Figure 1). The initial targets chosen to determine the utility of this isostere were those shown in Table I (entries 1-4). The strategy undertaken was to maintain the left side of the inhibitor constant, systematically vary the R_1 and R_2 groups, and investigate the stereochemistry of the key hydroxyl group. The general synthetic route used is illustrated in Scheme I. The pure epoxides, **1a** or **1b**, were treated with an excess of amine (R_1NH_2) in refluxing 2-propanol to provide the crystalline amino alcohols **2**. These were then reacted with the appropriate isocyanate (R_2NCO) to complete the synthesis of the urea isostere. Removal of the protecting group of **3** and coupling of the free amine to *N*-Cbz-L-asparagine provided the initial targets **4**. Further elaboration of certain inhibitors involved removal of the Cbz group and addition of the quinoline-2-carboxamide (Qua) functionality.

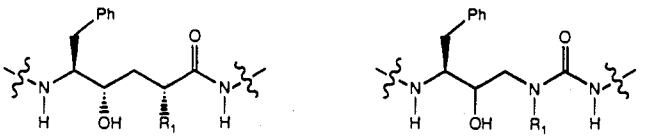
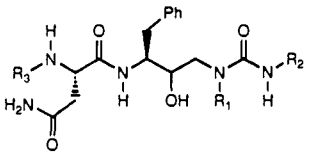


Figure 1. Isostere comparison.

Table I. HIV-1 Protease Inhibitors and Their IC_{50} Values



entry	R_1	R_2	R_3	IC_{50} (nM)[a] *R* alcohol	IC_{50} (nM)[a] *S* alcohol
1	CH_3	CH_3	Cbz[b]	*c*	*c*
2	CH_3	$CH_2CH_2CH_2CH_3$	Cbz	*c*	*c*
3	$CH_2CH(CH_3)_2$	CH_3	Cbz	1 500	*c*
4A	$CH_2CH(CH_3)_2$	$CH_2CH_2CH_2CH_3$	Cbz	940	*c*
4B	$CH_2CH(CH_3)_2$	$CH_2CH_2CH_2CH_3$	Qua[d]	126	
5	$CH_2CH(CH_3)_2$	$CH_2CH_2CH_3$	Cbz	518	
6	$CH_2CH(CH_3)_2$	CH_2CH_3	Cbz	330	
7	$CH_2CH(CH_3)_2$	$CH(CH_3)_2$	Cbz	260	
8A	$CH_2CH(CH_3)_2$	$C(CH_3)_3$	Cbz	35	54 000
8B	$CH_2CH(CH_3)_2$	$C(CH_3)_3$	Qua	6[e]	10 000
9A	$CH_2CH_2CH(CH_3)_2$	$C(CH_3)_3$	Cbz	13	
9B	$CH_2CH_2CH(CH_3)_2$	$C(CH_3)_3$	Qua	3	
10A	$CH_2C_6H_{11}$	$C(CH_3)_3$	Cbz	29	
10B	$CH_2C_6H_{11}$	$C(CH_3)_3$	Qua	5	
11A	CH_2Ph	$C(CH_3)_3$	Cbz	19	
11B	CH_2Ph	$C(CH_3)_3$	Qua	3	
12	(*R*)-$CH(CH_3)Ph$	$C(CH_3)_3$	Cbz	6 500	
13	(*S*)-$CH(CH_3)Ph$	$C(CH_3)_3$	Cbz	5 100	
14A	CH_2(4-pyridyl)	$C(CH_3)_3$	Cbz	105	
14B	CH_2(4-pyridyl)	$C(CH_3)_3$	Qua	19	

[a] The positive control was MVT-101[14] (IC_{50} = 1.9 ± 0.4 μM). [b] Cbz = carbobenzyloxy. [c] Less than 50% inhibition at 10 μM. [d] Qua = quinolinyl-2-carboxamide. [e] IC_{50} = 6.3 ± 0.1 nM (n = 68).

In order to determine the stereochemical preference of the hydroxyl group, the diastereomeric epoxides **1a** and **1b** were required. Their synthesis is shown in Scheme II. The syn isomer **1a** (*R* alcohol precursor) was prepared by the reduction of *N*-Cbz-L-phenylalanyl chloromethyl ketone (**6**) with sodium borohydride to provide a 3:1 mixture of the isomeric chlorohydrins **7a** and **7b**, respectively. The desired isomer **7a** was obtained in pure form in 43% yield by trituration with hexane to remove **7b**, followed by recrystallization. Treatment of **7a** with potassium hydroxide in ethanol afforded crystalline **1a** in 90% yield.[12] The anti epoxide **1b** (*S* alcohol precursor) was prepared by the stereoselective epoxidation of olefin **9** with *m*-chloroperbenzoic acid (MCPBA) as previously described.[13]

IC_{50} values for inhibition of recombinant HIV protease were determined using the spectrofluorometric assay developed by Toth and Marshall.[14] The results obtained with the initial series of nine inhibitors are shown in Table I, entries 1-4. These results led to several conclusions. There is a marked preference for the *R* alcohol stereo-

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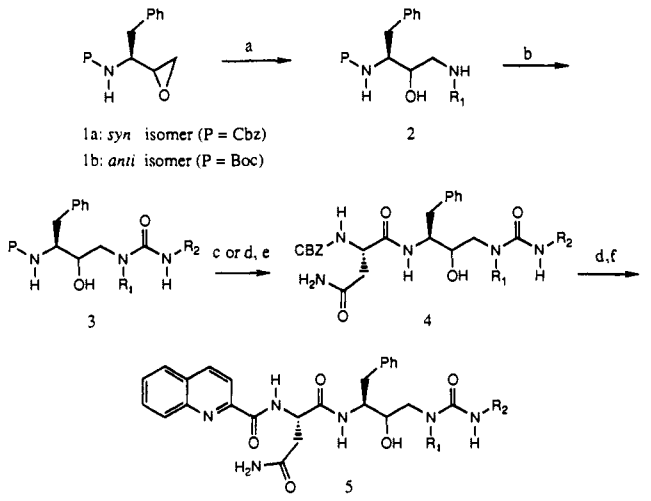
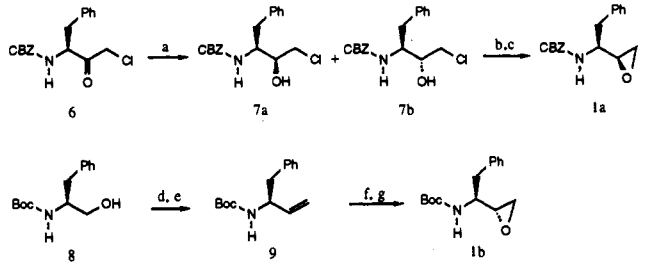
Scheme I[a]



[a] Reaction conditions: (a) 20 equiv of R_1NH_2, IPA, reflux; (b) R_2NCO, CH_2Cl_2, RT; (c) 4 N HCl/dioxane; (d) H_2, Pd(C), EtOH; (e) Cbz-L-Asn, EDC, HOBT, DMF; (f) quinoline-2-carboxylic acid hydroxysuccinimide ester, DMF.

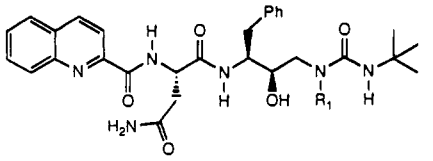
Scheme II[a]



[a] Reaction conditions: (a) $NaBH_4$, CH_3OH/THF (1:1); (b) separate; (c) KOH, EtOH; (d) SO_3·pyr; (e) CH_3PPh_3Br, $KN[Si(CH_3)_3]_2$; (f) MCPBA; (g) chromatography.

chemistry (entries 3 and 4A). An isobutyl group is preferred at the R_1 position, whereas at the R_2 position there is little difference between a methyl or an *n*-butyl group. Replacement of the Cbz by the Qua group (entry 4B, IC_{50} = 126 nM) led to a 7-fold increase in potency, consistent with the report by the Roche group for their hydroxyethylamine inhibitors.[9a] This limited study demonstrated that moderately potent inhibitors of the HIV-1 protease incorporating the (hydroxyethyl)urea isostere could be identified. The optimization of this initial lead then proceeded rapidly. Systematic variations of R_1 gave only minor potency changes, whereas variations of R_2 resulted in significant activity enhancements. As shown in Table I (entries 4–8), shortening of the *n*-butyl group of entry 4A led to a minor increase, with the ethyl group being most potent (entry 6). The introduction of branching by the addition of a single methyl (entry 7) did not effect potency, whereas the addition of a second methyl group (entry 8A) resulted in a substantial increase in activity and identified the *tert*-butyl group as optimal for this series of inhibitors. Addition of the Qua group (entry 8B) led to an increase in potency and provided a 6 nM enzyme inhibitor, designated SC-52151. In order to confirm that the stereochemical preference for the (*R*)-hydroxyl had not been altered, the (*S*)-hydroxyl isomer of SC-52151 was prepared and shown to inhibit the enzyme with an IC_{50} value of 10 000 nM. Thus for SC-52151, the *R* isomer is 1 700 times more potent than the *S* isomer. Moreover, SC-52151 is a selective inhibitor of the HIV protease, showing no significant inhibition against other aspartyl proteases (human renin, porcine pepsin, and bovine cathepsin D) at a concentration of 10 μM.[15]

Table II. Antiviral Activity of Selected HIV-1 Protease Inhibitors



entry	R_1	IC_{50} (nM)	EC_{50} (nM)[a]	TD_{50} (nM)[b]
8B	$CH_2CH(CH_3)_2$	6	21 ± 11[c]	50 000
9B	$CH_2CH_2CH(CH_3)_2$	3	10 ± 4[c]	50 000
11B	CH_2Ph	3	21 ± 4[c]	700 000
14B	CH_2(4-pyridyl)	19	30 ± 11[c]	300 000

[a] Effective concentration necessary to inhibit 50% HIV-induced cell death. [b] Toxic dose 50% in uninfected cells. [c] Average of two separate assays.

With the R_2 position optimized as a *tert*-butyl group, we then investigated the R_1 position. As seen in Table I this position readily accommodates a wide variety of substituents (entries 8–14), but prefers those without α-branching. For example, introduction of a benzyl group at this position led to a potent inhibitor (entry 11A, IC_{50} = 19 nM), but addition of an α-methyl group, regardless of the stereochemistry, provided moderate inhibitors (entries 12 and 13), which were 340 and 270 times less active, respectively. In all the cases examined in Table I, substitution of the Qua for the Cbz group resulted in improved potency.

Certain inhibitors were evaluated for their antiviral properties against the $HTLV_{IIIB}$ strain of HIV-1 in a CEM cell line.[16] As shown in Table II, these compounds are very effective antiviral agents and show a good correlation between their IC_{50} and EC_{50} values, suggestive of effective cell penetration and stability to the assay conditions. SC-52151 (entry 8B) has also been shown to be equally effective against fresh clinical isolates of HIV-1, including AZT-resistant strains, in human peripheral blood mononuclear cells (PBMC).[17]

The conformation of one of these inhibitors (entry 4B) bound to the active site of recombinant HIV-1 protease has been determined by X-ray diffraction at 2.3-Å resolution.[18] As shown in Figure 2, the inhibitor binds in an extended conformation with the (*R*)-hydroxyl group positioned symmetrically between the two catalytic aspartates of the enzyme. Both the P_2 asparagine carbonyl and the urea carbonyl hydrogen bond to the critical water molecule observed in all reported structures to date. The trisubstituted urea portion of these inhibitors provides a rigid framework from which the isobutyl and *n*-butyl groups project. Interestingly, the isobutyl group is *not* bound in the S_1' subsite but instead resides in the S_2' subsite. Similarly, the *n*-butyl group is not in the S_2' subsite, but rather in the S_1' subsite. This unprecendented juxtapositioning (Figure 3) of substituents is in contrast to a previously reported structure of a urea-containing renin inhibitor bound to the aspartyl protease endothiapepsin.[8c] Additional structures of inhibitors related to entry 4B, but with the optimized *tert*-butyl group present, demonstrate that this unique binding mode is a general feature of these urea isosteres when bound to the HIV-1 protease.[19,20]

In summary, a novel series of HIV-1 protease inhibitors has been developed which utilizes the (hydroxyethyl)urea isostere. The preferred stereochemistry for the key

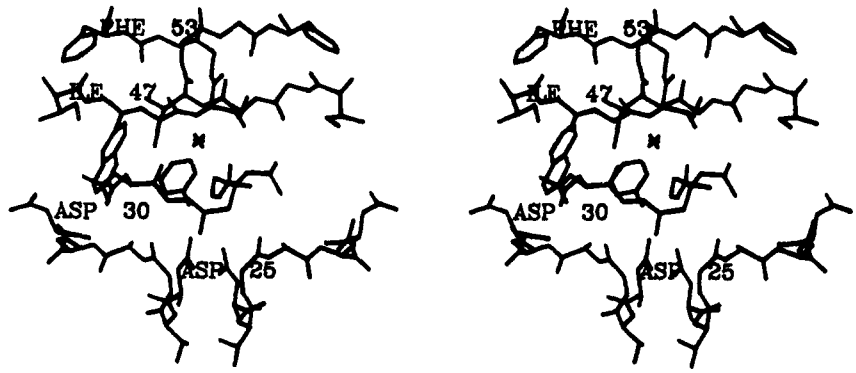


Figure 2. Bound conformation of entry 4B. A stereoview approximately along the local diad axis of the protease dimer. Residues 25–30 and 47–53 from each of the two subunits of the protein are shown, as well as the inhibitor and the key structural water molecule. As anticipated, the inhibitor molecule binds in an extended conformation, but the P2′ *n*-butyl substituent of the urea moiety is in the S1′ binding pocket, trans to the P1 benzyl substituent.

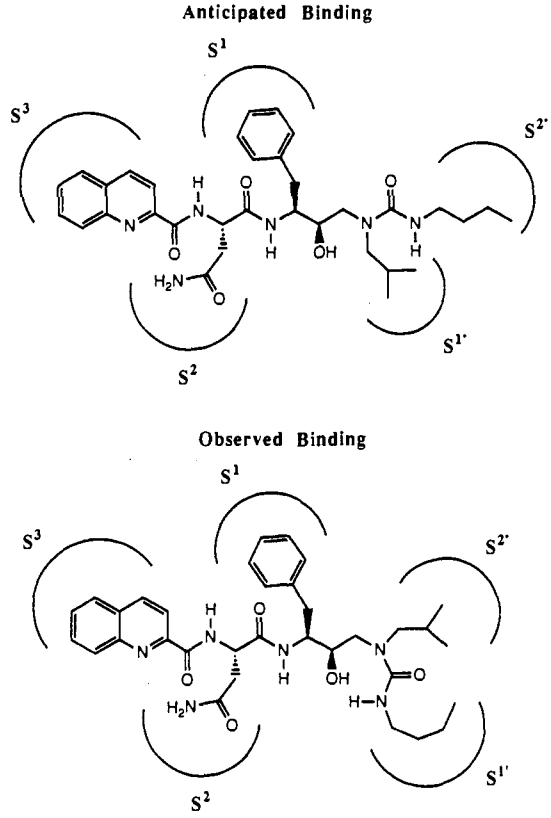


Figure 3. Representation of entry 4B bound to HIV-1 protease.

hydroxy group is *R*. This isostere can be readily synthesized in four steps from commercially available materials. The inhibitors reported herein are potent and selective inhibitors of the HIV-1 protease and show excellent antiviral properties in tissue culture. As determined by X-ray diffraction studies, these inhibitors bind to the enzyme in a previously unreported manner. One of these inhibitors, SC-52151, is currently being developed for clinical trials as a therapeutic agent for the treatment of AIDS.

Acknowledgment. The authors wish to express their gratitude to Professors Daniel H. Rich (University of Wisconsin Madison) and Garland Marshall (Washington University) for their overall contributions to our protease inhibitor project. We are also indebted to the Searle Chemical Development Group for large quantities of epoxide **1a**.

Supplementary Material Available: Experimental procedures for the preparation of SC-52151 and its analogs, as well as further details of the biological assays and X-ray crystal structure (10 pages). Ordering information is given on any current masthead page.

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(15) For the different enzymes, the % inhibition observed was as follows: porcine pepsin (7% at 10 μM), bovine cathepsin D (4% at 10 μM), and human renin (0% at 10 μM).

(16) Drug candidates were evaluated against the $HTLV_{IIIB}$ strain of HIV-1 in CEM cells at a multiplicity of infection of 0.1 for their ability to prevent HIV-induced cell death. Compounds were evaluated in triplicate at varying doses and compared to (1) untreated uninfected cell control samples, (2) drug treated, uninfected cell toxicity control samples, and (3) untreated, infected virus control samples. Drug was added on days 1, 2, and 5; the assay terminated on day 7; and cell death assessed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). This assay detects drug-induced suppression of viral CPE, as well as drug cytotoxicity, by measuring the generation of MTT-formazan by surviving cells. 3′-Azido-3′-deoxythymidine (AZT) and 2′,3′-dideoxyinosine (DDI) were included as positive control compounds and typically provided EC_{50} values of 0.001–0.005 and 0.25–1.0 μg/mL, respectively.

(17) The details of these results and the effect of SC-52151 on protein processing will be the subject of a future paper.

(18) The inhibitor complex crystallized in the $P2_12_12_1$ space group at pH 6.2 from a 44% saturated ammonium sulfate solution. The lattice parameters were a = 52.0, b = 59.1, c = 62.3 Å. The final data set included 99 135 observations of 8 644 independent diffraction intensities; the agreement index, R_{sym}, is 0.072 and is representative of pairwise differences between the measured intensities of equivalent reflections. The model now includes the two subunits of the enzyme, the inhibitor, and 75 solvent ligands. The current crystallographic residual is 0.21 (rms deviations from ideal bond and angle distances of 0.02 and 0.045 Å, respectively) for the 7919 reflections greater than $2.0\sigma(F)$.

(19) The details of this and related X-ray structures will be reported in a separate paper.

(20) It is interesting to note that although both SC-52151 and Ro 31-8959 contain a *tert*-butyl group, they bind very differently. The *tert*-butyl urea of SC-52151 occupies the S_1' subsite, whereas the *tert*-butylamide of Ro 31-8959 occupies the S_2' subsite of the enzyme.[9b]