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Communications to the Editor

Inhibitors of HIV-1 Protease Containing the Novel and Potent (*R*)-(Hydroxyethyl)sulfonamide Isostere

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Replication of the HIV virus requires processing of the proteins encoded by the *gag* and *gag-pol* genes by a virally encoded aspartyl protease (HIV protease).[1] As such, inhibition of the HIV protease offers an attractive target for the treatment of acquired immunodeficiency syndrome (AIDS).[2-4] Numerous examples of potent inhibitors of this enzyme have been reported.[5-7] We have previously reported the discovery of a series of potent HIV-1 protease inhibitors containing the (*R*)-(hydroxyethyl)urea isostere.[8] We now report a novel series of highly potent HIV-1 protease inhibitors incorporating the (*R*)-(hydroxyethyl)sulfonamide isostere. While sulfur-[9] and phosphorus-based[10] isosteres have been reported previously, the sulfur or phosphorus groups were incorporated as scissile bond replacements. The (*R*)-(hydroxyethyl)sulfonamides described here contain the sulfamido moiety in place of the P1'P2' amide linkage.

A variety of (hydroxyethyl)sulfonamides were synthesized to determine the optimal binding of this group within the S1' and S2' subsites and the preferred stereochemistry of the hydroxyl group.

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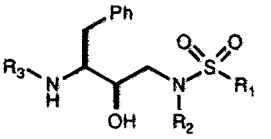
Table 1. HIV-1 Protease Inhibitors IC_{50} and K_i Values



compd	R_1	R_2	R_3	−OH[a]	IC_{50} (nM)[b]	K_i (nM)
4	CH_3	$CH_2CH_2CH(CH_3)_2$	Cbz[c]	*R*	3200	1872
5	*n*-C_3H_7	$CH_2CH_2CH(CH_3)_2$	Cbz	*R*	210	128
6a	C_6H_5	$CH_2CH_2CH(CH_3)_2$	Cbz	*R*	16	10
6b	C_6H_5	$CH_2CH_2CH(CH_3)_2$	Cbz	*S*	450	295
8	$CH_2C_6H_5$	$CH_2CH_2CH(CH_3)_2$	Cbz	*R*	10000	
9	*o*-ClC_6H_4	$CH_2CH_2CH(CH_3)_2$	Cbz	*R*	380	227
10	*m*-ClC_6H_4	$CH_2CH_2CH(CH_3)_2$	Cbz	*R*	92	61
11	*p*-ClC_6H_4	$CH_2CH_2CH(CH_3)_2$	Cbz	*R*	10	4.1
12	C_6H_5	$CH_2CH(CH_3)_2$	Cbz	*R*	6	3.2
13	C_6H_5	$CH_2C_6H_5$	Cbz	*R*	190	125
14	C_6H_5	$CH_2C_6H_{11}$	Cbz	*R*	14	6.7
15	CH_3	$CH_2CH_2CH(CH_3)_2$	CbzAsn[d]	*R*	100	57
16	CH_3	$CH_2CH_2CH(CH_3)_2$	QuiAsn[e]	*R*	27	13
17	C_6H_5	$CH_2CH_2CH(CH_3)_2$	CbzAsn	*R*	2.2	0.5
19	C_6H_5	$CH_2CH_2CH(CH_3)_2$	QuiAsn	*R*	1.5	1.0

[a] Configuration of the hydroxy group. [b] The positive control was MVT-101 ($IC_{50} = 1.9 \pm 0.4$ μM). [c] Cbz, carbobenzyloxy. [d] CbzAsn, *N*-Cbz-L-asparagine. [e] QuiAsn, *N*-quinolinyl-2-carboxy-L-asparagine.

A general synthetic route for the preparation of compound **19** is shown in Scheme 1. The pure epoxide **2** was prepared from Cbz-L-phenylalanyl chloromethylketone as previously described.[8,11] The epoxide was treated with an excess of isoamylamine in refluxing 2-propanol to provide the crystalline amino alcohol **3**. Alternatively, the isoamylamine could be replaced with other primary amines. The amino alcohol **3** was then allowed to react with benzenesulfonyl chloride to generate **6a**. Compounds **4-14** were prepared in a similar manner. Hydrogeneration of the Cbz protecting group was readily accomplished, and the resulting free amine **7** could be smoothly coupled to *N*-Cbz-L-asparagine using HOBt and EDC to generate **17**. Deprotection of **17** and coupling to quinaldic acid as the hydroxysuccinimde ester generated **19**. Compound **16** was prepared in a similar manner.

The IC_{50} values for inhibition of recombinant HIV-1 protease were determined using the spectrofluorometric

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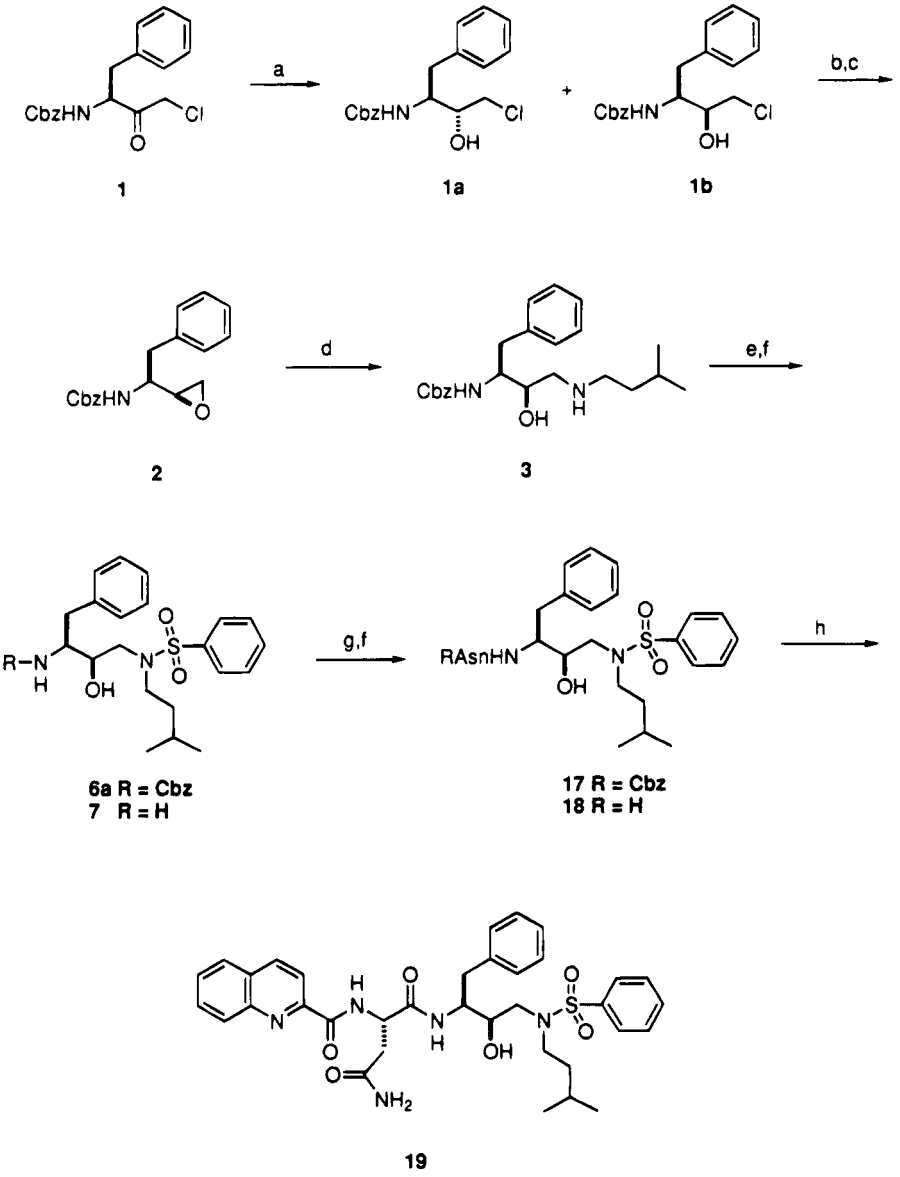
Scheme 1[a]



[a] Reaction conditions: (a) $NaBH_4$, MeOH/THF (1:1); (b) separate; (c) KOH, EtOH; (d) 20 equiv of isoamylamine; (e) $C_6H_5SO_2Cl$, Et_3N, CH_2Cl_2; (f) 10% Pd/C, H_2, MeOH; (g) Cbz-L-Asn, HOBt, EDC, DMF; (h) quinoline-2-carboxylic acid hydroxysuccinimide ester.

assay developed by Toth and Marshall and are shown in Table 1.[12,13]

It was observed that the in vitro potency increases significantly as sulfonyl group R_1 is changed from methyl to *n*-propyl to phenyl (compounds **4**, **5**, and **6a**). Replacing the phenyl group with benzyl (compound **8**), however, causes a significant reduction in affinity. Introduction of a chlorine atom into the para position of the sulfonamide phenyl ring (**11**) resulted in a compound of similar potency to the unsubstituted compound (**6a**). In contrast, the introduction of a chlorine atom into the ortho or meta positions (**9** or **10**) resulted in a decrease in potency.[14] Several variations at R_2 were investigated (**6a**, **12**, **13**, and **14**). The compounds with the isoamyl (**6a**) and cyclohexylmethyl (**14**) side chains had similar potency, whereas the isobutyl analog (**12**) exhibited a modest increase in potency. In contrast, the benzyl analog (**13**) was about 10-fold less active than the corresponding cyclohexylmethyl compound.

With respect to the stereochemistry of the hydroxyl group, compounds **6a** and **6b** demonstrate a clear preference for the *R* alcohol in this series.[15]

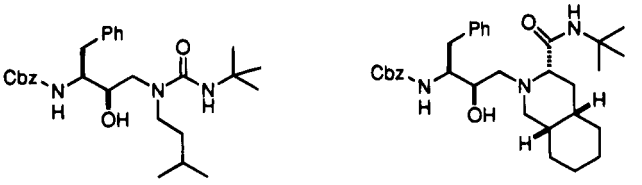


Figure 1. Structures for a (*R*)-(hydroxyethyl)urea isostere and (*R*)-(hydroxyethyl)amino isostere containing a Cbz group at P2, comparable to **12**, and their IC_{50} values.

Replacement of the carbobenzyloxy group with Cbz-L-asparagine led to increased potency (compare compounds **4** and **15** and **6a** and **17**) and, in the case of **15**, the subsequent replacement of the Cbz group with quinaldic acid resulted in an additional increase in binding affinity (**16**).

These results demonstrate that utilization of the (*R*)-(hydroxyethyl)sulfonamide isostere can provide potent

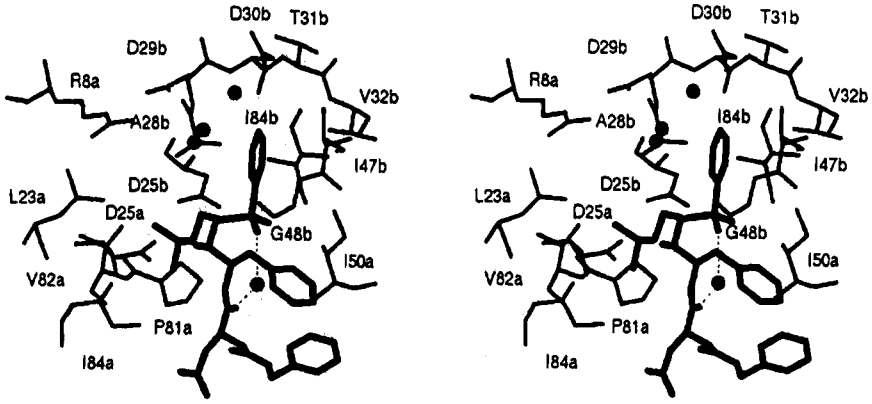
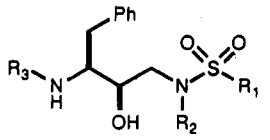


Figure 2. Stereoview of the binding of **17** to HIV-1 protease. The single-letter amino acid code is used and residues from opposing chains of the dimeric enzyme are labeled a and b; the (*R*)-(hydroxyethyl)sulfonamide inhibitor is drawn with thicker lines. A conserved solvent ligand forms a hydrogen bond (2.76 Å) with the P2 carbonyl oxygen and with the *pro-R* oxygen of the sulfonamide (2.76 Å); the *pro-S* oxygen and amide nitrogen atoms of the sulfonamide moiety do not participate in hydrogen bonding. Some of the atoms of the P1′ isoamyl group and P2′ phenyl ring are solvent accessible, and three ordered water molecules that lead to the surface of the complex are illustrated.

Table 2. Antiviral Activity of Selected HIV-1 Protease Inhibitors



entry	R_1	R_2	R_3	EC_{50} (nM)[a]	TD_{50} (nM)[b]
6a	C_6H_5	$CH_2CH_2CH(CH_3)_2$	Cbz	55	26000
12	C_6H_5	$CH_2CH(CH_3)_2$	Cbz	113	8000
16	CH_3	$CH_2CH_2CH(CH_3)_2$	QuiAsn[c]	53	45000
19	C_6H_5	$CH_2CH_2CH(CH_3)_2$	QuiAsn	5	203000

[a] Concentration necessary to inhibit 50% HIV-induced cell death. [b] Toxic dose 50% in uninfected cells. [c] QuiAsn, *N*-quinolinyl-2-carboxy-L-asparagine.

inhibitors of the HIV-1 protease, even with a carbobenzyloxy group in the P2 position. This is in contrast to the (*R*)-(hydroxyethyl)urea isostere[8] and the (*R*)-(hydroxyethyl)amino isostere.[16] For example, **12** has an IC_{50} = 6 nM, while compounds **20** and **21**, Figure 1, exhibit IC_{50} values of 112 and 62 nM, respectively, in our enzyme assay.

X-ray crystallography of the enzyme-inhibitor complex with **17** (Figure 2) indicates that the inhibitor binds in an extended conformation. The Cbz group occupies S3, the asparagine is in S2, and the benzyl side chain of the Phe isostere is in S1 as expected. The *N*-isoamyl side chain of the sulfonamide occupies S1′ and the aryl ring of the sulfonamide occupies S2′ in a normal extended fashion. As expected, the hydroxyl group binds to the catalytic aspartates. The *pro-R* oxygen of the sulfonamide along with the P2/P1 amide carbonyl forms a strong hydrogen bonding array to the structural water molecule found in most enzyme–inhibitor complexes.[17] The *pro-S* oxygen and the amide nitrogen of the sulfonamide do not participate in hydrogen bonding to the enzyme.

Several of these compounds were screened for antiviral activity against the HIV_{IIIB} strain of HIV-1 in a CEM cell based assay.[18] As shown in Table 2, these compounds have excellent antiviral activity and minimal toxicity. There is good correlation between IC_{50}'s and EC_{50}'s in the longer compounds (**16** and **19**), suggesting good cell penetration and stability to the assay conditions.

In conclusion, we have discovered a series of novel and highly potent HIV-1 protease inhibitors, based on the (*R*)-(hydroxyethyl)sulfonamide isostere, which show excellent antiviral properties in vitro. Certain members of this class represent some of the simplest, most potent enzyme inhibitors reported to data.[19]

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Supplementary Material Available: Experimental procedures for the preparation of **19** and its analogues and, also, 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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(13) K_i's were determined using the program FastKi, written by Dr. Petr Kuzmic, Biokin, Ltd., P.O. Box 9240, Madison, WI 53715.

(14) Similar results were observed with other substituents, for example, methoxy and nitro (data not shown).

(15) For examples of *R* vs *S* alcohol comparisons in other inhibitor series, see ref 8 and references contained therein.

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(17) X-ray crystallography of other inhibitor complexes show this binding mode to be typical of the (*R*)-(hydroxyethyl)arylsulfonamide isostere.

(18) Drug candidates were evaluated against the HIV_{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 0, 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 μg/mL and 0.25–1.0 μg/mL, respectively. For comparison, Ro 31-8959, prepared in-house, exhibited an EC_{50} = 8 nM in this assay.[16]

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