JOURNAL OF
MEDICINAL
CHEMISTRY

Volume 39, Number 22 October 25, 1996

Communications to the Editor

Structure-Based Design of HIV Protease Inhibitors: Sulfonamide-Containing 5,6-Dihydro-4-hydroxy-2-pyrones as Non-Peptidic Inhibitors¶

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Received July 25, 1996

The rapid spread of the acquired immunodeficiency syndrome (AIDS) epidemic has stimulated discovery of therapeutic agents to arrest the replication of the causative virus, human immunodeficiency virus (HIV). One promising possibility to interrupt the viral life cycle is the use of inhibitors of the virally encoded protease, which is indispensable for viral maturation.[1,2] Among the most potent inhibitors are peptidomimetic compounds containing transition-state inserts in place of the dipeptidic cleavage sites of the substrates.[3−6] The low oral bioavailability and rapid excretion of peptide-derived compounds[7] have limited their utility as potential therapeutic agents. Recent advances have resulted in HIV protease inhibitors with reduced peptidic character that are more orally bioavailable. An increasing number of HIV protease inhibitors[8−21] are currently undergoing clinical evaluations, some of which have already been approved as therapeutic agents for the treatment of HIV infection.

We previously reported[20] the identification of phenprocoumon (compound **1** in Figure 1, $K_i = 1\ \mu$M) from a broad screening program as an active HIV protease inhibitory template. It is noted that other independent studies[22−26] have also described 4-hydroxybenzopyran-2-ones and 4-hydroxypyran-2-ones as inhibitors of HIV protease. The increasing number of reported crystal structures of inhibitor/HIV protease complexes have provided numerous successful examples of structure-based designs of potent HIV protease inhibitors.[27,28] Our iterative cycles of structure-based design identified U-96988 (compound **2**, $K_i = 38$ nM, antiviral $IC_{50} = 3\ \mu$M) as the first clinical candidate.[20] Further structure-based drug design studies led to the discovery of the sulfonamide-containing U-103017 (compound **3**, $K_i < 1$ nM, antiviral $IC_{50} = 1-2\ \mu$M) as the second generation clinical candidate.[21] More recently, compounds in the 5,6-dihydro-4-hydroxy-2-pyrone template have demonstrated promising HIV protease inhibitory activity,[29,30] and we have previously reported HIV protease inhibitory activity ($K_i = 35$ nM) of compound **4**.[30] We now describe the structure−activity relationship study of a new series of sulfonamide-containing 5,6-dihydro-4-hydroxy-2-pyrones leading to the identification of the third-generation development candidate.

The preparation of a representative sulfonamide-containing 5,6-dihydro-4-hydroxy-2-pyrone **V** is shown in Scheme 1. The 5,6-dihydro-4-hydroxy-2-pyrone **5**[30] was condensed with *m*-nitrobenzaldehyde with alumi-

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S0022-2623(96)00541-9 CCC: $12.00

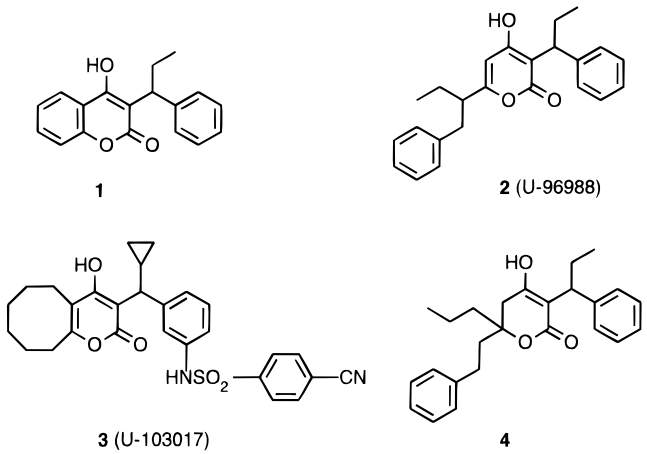
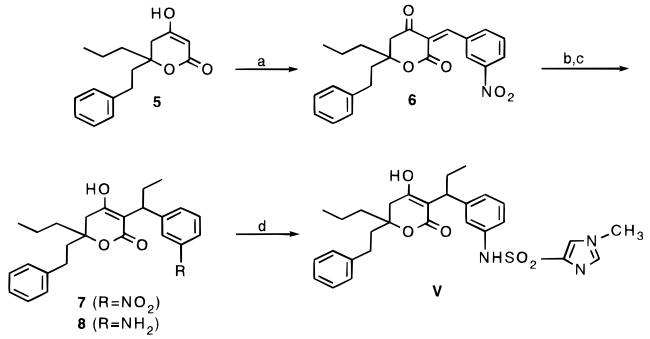


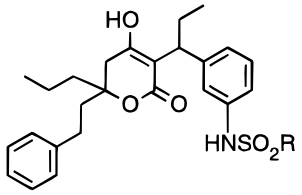
Figure 1. Structures of HIV protease inhibitors.

Scheme 1. Preparation of Sulfonamide-Containing 5,6-Dihydro-4-hydroxy-2-pyrones[a]



[a] (a) *m*-$NO_2C_6H_4CHO$, $AlCl_3$, THF; (b) Et_3Al, $CuBr(CH_3)_2S$, THF; (c) 10% Pd/C, NH_4HCO_2, CH_3OH; (d) RSO_2Cl, pyridine.

Table 1. Sulfonamide-Containing 5,6-Dihydro-4-hydroxy-2-pyrones



R	compd	K_1 (nM)	IC_{50} (μM)	IC_{90} (μM)
phenyl	**I**	17	1.6	7.3
4-cyanophenyl	**II**	8	1.1	4.2
4-fluorophenyl	**III**	6	1.2	4.3
1-quinolin-8-yl	**IV**	3	1.0	3.0
1-methylimidazol-4-yl	**V**	1	0.5	0.9

num trichloride catalysis to give the benzylidene intermediate **6**. Conjugate addition to that benzylidene intermediate **6** with triethylaluminum in the presence of copper(I) bromide dimethyl sulfide complex gave adduct **7**. The nitro functionality was then reduced with transfer hydrogenolysis using ammonium formate and 10% palladium on carbon as a catalyst. The resulting amine **8** was reacted with a 1-methylimidazole-4-sulfonyl chloride in the presence of pyridine to give the desired product **V**.

The compounds in Table 1 are mixtures of four diastereomers. The R groups of the sulfonamides affected both the HIV protease inhibitory activity and also the resulting *in vitro* antiviral activity. The imidazole sulfonamide (compound **V**) showed enhanced activity

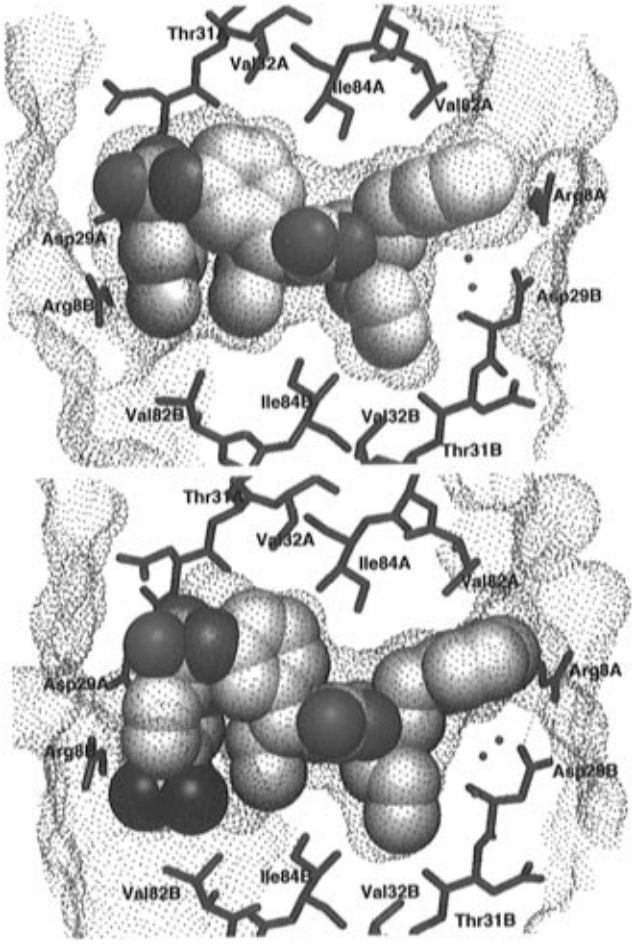
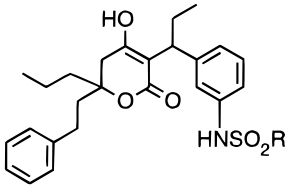


Figure 2. Crystal structures of the HIV-1 protease triple mutant (Q7K/L33I/L63I) complexed with inhibitors **V** (a, top) and **VIIb** (b, bottom). The inhibitors are depicted in space-filling representation, with carbon (gray), oxygen (red), nitrogen (blue), sulfur (yellow), and fluorine (purple) atoms as shown. The enzyme surface at the active site is depicted as dotted surface, and selected amino acid residues surrounding the active site are also shown and labeled. See text for discussion of binding of inhibitors in the active site.

over the substituted phenyl analogues. The crystal structure of the HIV-1 protease triple mutant (Q7K/L33I/L63I) complexed with inhibitor **V** was determined. This particular protease construct provided an enzyme with significantly improved stability for structural studies; its preparation has been described elsewhere.[31] Although inhibitor **V** is a mixture of four diastereomers, only the 3α*R*,6*R* diastereomer could be fitted into the difference electron density at the active site. The crystal structure showed nearly symmetrical hydrogen bonding between the 4-hydroxyl group and the catalytic aspartic acids. The carbonyl oxygen also made nearly symmetrical hydrogen-bonding interactions with the NH groups of the flap residues Ile 50A and Ile 50B and replaced the ubiquitous water molecule found in complexes of peptide-derived inhibitors. As shown in Figure 2a, the phenethyl and the propyl groups at C-6 could be found in the S_1' and the S_2' subsites, respectively. The C-3α ethyl and phenyl groups occupied the S_1 and the S_2 subsites, respectively, with the imidazole substituent curling into the S_3 subsite. The sulfonamide functionality showed strong hydrogen bonding to a few residues at the active site.

Since compound **V** showed promising activity ($K_i = 1$ nM, $IC_{50} = 0.5$ μM), the four individual stereoisomers

Table 2. Individual Diastereomers of Sulfonamide-Containing 5,6-Dihydro-4-hydroxy-2-pyrones



R	compd	stereochemistry	K_i (nM)	IC_{50} (μM)	IC_{90} (μM)
1-methylimidazol-4-yl	**Va**	3α*R*,6*S*	0.12	0.58	1
	Vb	3α*R*,6*R*	0.06	0.13	0.56
	Vc	3α*S*,6*S*	1.0	>>1	>>1
	Vd	3α*S*6*R*	0.3	>>1	>>1
5-cyano-2-pyridyl	**VIa**	3α*R*,6*S*	0.04	0.11	0.89
	VIb	3α*R*,6*R*	0.007	0.04	0.26
	VIc	3α*S*,6*S*	0.12	>>1	>>1
	VId	3α*S*,6*R*	0.10	0.49	1.0
5-(trifluoromethyl)-2-pyridyl	**VIIa**	3α*R*,6*S*	0.018	0.14	0.84
	VIIb	3α*R*,6*R*	0.008	0.03	0.10
	VIIc	3α*S*,6*S*	0.22	1.7	3.0
	VIId	3α*S*,6*R*	0.032	0.41	1.8

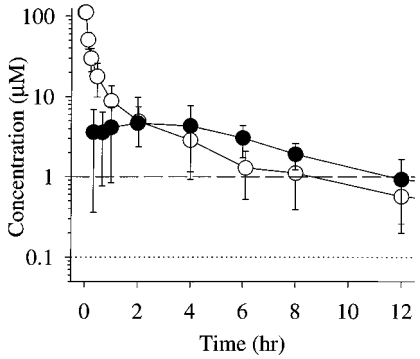


Figure 3. Time-course blood levels of inhibitor **VIIb** after intravenous (opened circles) and oral (closed circles) administrations to rats. The intravenous dose ($n = 3$ rats) was 5 mg/kg given in 10% propylene glycol and 22.5% (hydroxypropyl)-β-cyclodextrin at 2 mg/g concentration. The oral dose ($n = 9$ rats) was 10 mg/kg given in 0.01 M sodium hydroxide solution (pH 10) at 2 mg/g concentration. The dotted line denotes the cell-culture antiviral IC_{90} value of 0.1 μM in cell culture medium containing 10% fetal bovine serum, while the dashed line denotes the IC_{90} value of 1 μM in cell culture medium containing 10% fetal bovine serum and 75% human plasma.

were prepared for biological activity evaluation. The amine **8** was converted to the corresponding carbobenzyloxy analogue, which could then be resolved on a preparative chiral HPLC column[32] into the four individual stereoisomers.[33] The carbobenzyloxy protecting group was then removed by catalytic hydrogenolysis, and the resulting four diastereomers of the amine **8** were individually treated with the sulfonyl chloride to afford the four separate stereoisomers. The results of the biological testings are shown in Table 2. Due to the high enzymatic inhibitory activity of these compounds, the K_i value determination required the use of a tandemly linked HIV protease[34] since the native dimeric enzyme dissociated at low enzyme concentration. Although all four diastereomers (compounds **Va**−**d**) showed HIV protease inhibitory activity, the two diastereomers with the *R* stereochemistry at the C-3α center are the more active components. Between compounds **Va** and **Vb**, the diastereomer with the *R* stereochemistry at the C-6 center (**Vb**) showed higher activity. Additional variation in sulfonamides was then evaluated, and the examples with substituted pyridyl group (compounds **VI** and **VII**) showed the most promising activity. Compounds **VIb** and **VIIb**, with the 3α*R* and 6*R* stereochemistry, were the most active. Both compounds showed highly potent HIV protease inhibitory activity, with K_i values below 10 pM, and IC_{50} values of 30 nM in the HIV-1_{IIIB}-infected H9 cells. Early safety studies suggested advantages of compound **VIIb** over compound **VIb**; therefore, compound **VIIb** (U-140690) was selected as a candidate.

The crystal structure of the HIV-1 protease triple mutant (Q7K/L33I/L63I) complexed with inhibitor **VIIb** was determined. Difference electron density confirmed the 3α*R*,6*R* stereochemistry, and the inhibitor was found to bind in one clear orientation (see Figure 2b), with features which were nearly identical to those already described for inhibitor **V**.

U-140690 also inhibited HIV-2 protease with high potency ($K_i < 1$ nM). It was shown to be highly selective for HIV proteases since it inhibited other selected aspartyl proteases only weakly, with K_i values of 2, 15, and 9 μM against human pepsin, human cathepsin D, and human cathepsin E, respectively.[35] U-140690 was also shown to be very effective against a panel of 10 AZT-resistant HIV-1 clinical isolates in primary PBMC, with a mean IC_{90} value of 0.15 ± 0.07 μM.[36] Since there are reports indicating that reduction of in vitro antiviral activity[11] and lack of in vivo antiviral activity[37] may be attributed to extensive binding of drug to plasma proteins, we also evaluated the effect of protein binding on the in vitro antiviral activity of U-140690. In the HIV-1_{IIIB}-infected H9 cell assay containing 10% fetal bovine serum and 75% human plasma, U-140690 still showed significant antiviral activity with an IC_{90} value of 1 μM. Since laboratory ritonavir[11] resistant isolates have been described,[38] studies with these HIV-1 isolates,[39] which were highly resistant to ritonavir, showed them to be highly resistant to saquinavir,[8] indivanir,[12] and viracept,[18] with 47- to >125-fold increases in IC_{90} values as compared to matched, parental isolates. These isolates, however, remained significantly sensitive to U-140690, with only a 6-fold increase in IC_{90} value as compared to the wild-type isolate.[40] Moreover, an evaluation of four ritonavir resistant clinical isolates (32−67-fold increase in IC_{90} value for ritonavir) indi-

cated only a 2−3-fold increase in IC_{90} value for U-140690.[40]

After 5 mg/kg intravenous dosing to rats, $CL_{tot} = 0.17 \pm 0.10$ (L/h)/kg, $V_{ss} = 0.51 \pm 0.14$ L/kg, and $t_{1/2} = 5.4 \pm 0.3$ h. After 10 mg/kg oral dosing to rats, $F = 30\%$ (relative to the 5 mg/kg intravenous dosing). Time-course blood levels of U-140690 after iv and po dosings in rats are shown in Figure 3. Importantly, the blood levels of U-140690 exceeded 1 μM (the in vitro IC_{90} value in the presence of 10% fetal bovine serum and 75% human plasma) for 8−12 h. Additional extensive preclinical and safety studies are underway in order to initiate clinical trials of U-140690 as the third-generation development candidate for the treatment of HIV infection.

Supporting Information Available: Physical data for compounds **7**, **8**, **VIIa**, **VIIb**, **VIIc**, and **VIId**; descriptions of the HIV protease inhibition and cell-culture antiviral assays; crystallization procedure for the recombinant triple mutant of HIV-1 protease (Q7K/L33I/L63I) complexed with compounds **V** and **VIIb**; data collection and structure refinement description and a table summary of selected diffraction data collection and refinement statistics for the two crystal structures (7 pages). Ordering information is given on any current masthead page.

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JM960541S