Novel Series of Achiral, Low Molecular Weight, and Potent HIV-1 Protease Inhibitors

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Human immunodeficiency virus protease (HIV PR) is an enzyme in the HIV essential for viral replication.[1] Although many peptide-based HIV PR inhibitors possessing subnanomolar potency and initial clinical success are reported,[2] as a chemical class they have liabilities with respect to metabolism and/or possess low bioavailability.[3,4] Furthermore, their preparation typically has required multistep syntheses.[5,6] On the other hand, many of the nonpeptidic inhibitors[7-9] reported to date have low potency and/or complex structural (stereochemical) features. Herein, we report the discovery and structure-based design of a series of achiral (hitherto unknown), nonpeptide inhibitors of HIV PR that incorporate a novel structural scaffold relative to other known peptidomimetics.[10]

Mass (high volume) screening of compounds from the Parke-Davis collection resulted in the identification of 4-hydroxy-6-phenyl-3-(phenylthio)pyran-2-one (**1**) as a HIV PR inhibitor with low micromolar affinity.[11] By chemical analogy with known transition-state analogues,[12,13] **1** was viewed as a conformationally-restricted P_1–P_1' peptidomimetic (Figure 1). Specifically, it was

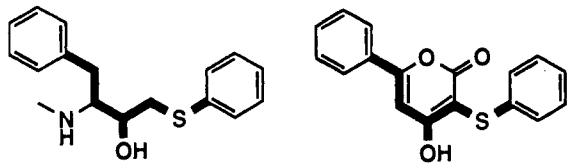

Figure 1. Comparison of hydroxyethyl sulfide isostere with compound **1**.

postulated that the enol moiety of **1** might hydrogen bond with two Asp residues present in the enzyme active site and that its two phenyl groups might occupy the P_1 and P_1' pockets. Kinetic studies of **1** and **2** showed competitive inhibition, which confirmed that these compounds were binding at the active site of the enzyme. To investigate the possible binding modes of **1** and **2** in HIV-1 protease, a number of docking experiments were conducted. Early trials involved the use of molecular dynamics as implemented in SYBYL,[14] and, for comparison, a Monte Carlo-based docking technique, Autodock, described by Goodsell and Olson.[15] The protein structure from the HIV-1/MVT101 crystal complex[16] was used in the docking studies, and thus, in the Autodock simulations,[17] the flap regions remained in the closed orientation. Several low-energy structures of **1** and **2** bound in the HIV-1 protease emerged from these studies, some of which proved to be closely related to the binding mode of **2** in the protease X-ray crystal complex. (Figure 2; *vide infra*).

One of the compounds in this series, **2**, was determined in its HIV PR bound state *via* X-ray crystallography (Figures 2 and 3). This complex confirmed our original hypothesis regarding the mode of binding and the active site interactions of **2**. In the X-ray structure, the critical water molecule-301, which is generally present in HIV PR inhibitor complexes[18] and forms hydrogen bonds to flaps as well as inhibitor atoms, was replaced by the lactone moiety in the pyrone structure. Specifically, the lactone carbonyl and oxygen groups formed hydrogen bonds to Ile50 and Ile150 of the enzyme.[19] Comparison of our findings with recent reports by DuPont-Merck[20] of a cyclic urea-based HIV PR inhibitor show similar molecular recognition interactions vis-à-vis water-301 replacement. Furthermore, the enol hydroxyl group formed the predicted interactions with the two catalytic Asp25 and Asp125 residues.[21] Multiple binding modes for HIV PR inhibitor complexes have been observed in hydroxyethylamine-incorporated peptide-based inhibitors and C_2-symmetry-based diol inhibitors, where the configuration of the hydroxyl group is quite different.[22] Herein, we report a case where the hydroxyl group is enolic in nature.

Since peptides containing hydrophobic amino acids near the peptide cleavage site (P_1–P_1') are known to be good substrates

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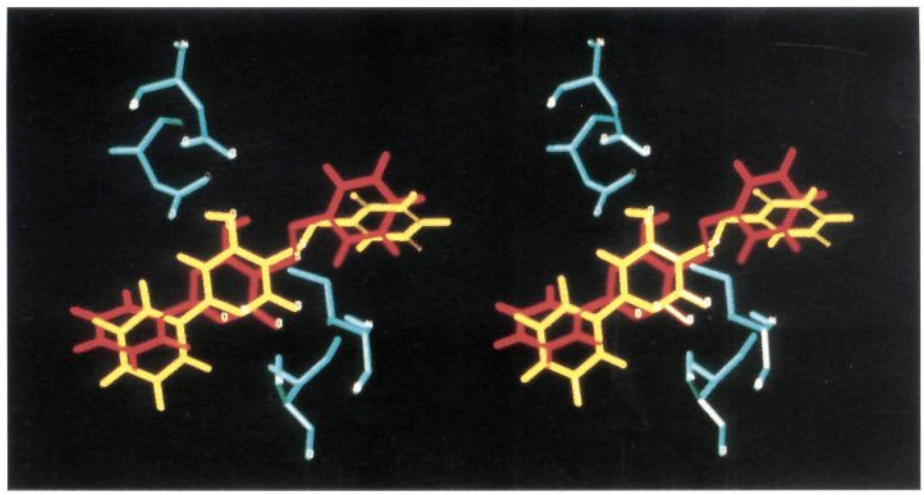


Figure 2. Relaxed stereo representation of Autodock suggested bound structure (red) of compound **2** overlaid on the X-ray crystal structure (yellow) of compound **2** bound to HIV-PR. Asp25/125 and Ile50/150 from the crystal complex are shown in cyan.

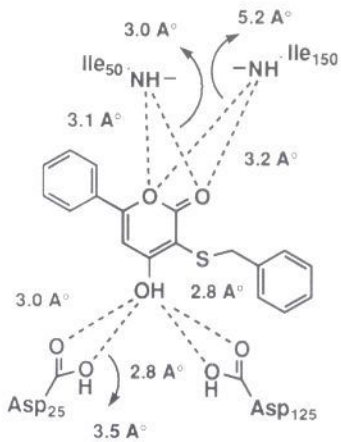
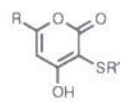


Figure 3. Interatomic distances of compound **2** binding to HIV PR (from X-ray crystal structure).

for HIV PR,[23] the SPh compound **1** was homologated to SCH_2Ph (**2**), SCH_2CH_2Ph (**3**), and $SCH_2CH_2CH_2Ph$ (**4**) to better fit the P_1' pocket. A 2–3-fold enhancement in binding affinity[11] was observed (Table 1). The hydroxyl substitution at the para position of the 6-aryl ring as seen in **5**, in an effort to mimic the Tyr-Phe cleavage site, enhanced the binding affinity by another 2-fold relative to **3**. Subsequent molecular modeling studies[14] of **1** further suggested that the S_3 pocket might be accessable *via* a tethering approach[24] and that an inhibitor could be designed to interact with Arg8, which is present in this pocket. This concept was tested by preparing an analogue containing a OCH_2CO_2H substituent at the para position of the 6-phenyl ring to form a charged complex with Arg8. The resulting competitive inhibitor, **7**, showed a significant enhancement in HIV PR affinity. The corresponding ester **6**, which cannot form a charge complex, was ~3 times less active and exhibited a potency comparable to the 4-hydroxyl analogue **5**. Most importantly, compound **7** possesses a low molecular weight, has no chiral centers, and is accessible in three synthetic steps. Synthetic efforts are now directed toward increased potency and improved antiviral activity.

Table 1. Pyrone Inhibitors and Their IC_{50} Values Tested against HIV PR *in vitro*



compd no.	R	R′	IC_{50} μM[a]
1	C_6H_5	C_6H_5	3.0 (K_i = 1.1 μM)
2	C_6H_5	$CH_2C_6H_5$	1.67 (K_i = 0.7 μM)
3	C_6H_5	$CH_2CH_2C_6H_5$	1.26
4	C_6H_5	$CH_2CH_2CH_2C_6H_5$	1.41
5	C_6H_4(4-OH)	$CH_2CH_2C_6H_5$	0.52
6	C_4H_4(4-$OCH_2CO_2C_2H_5$)	$CH_2CH_2C_6H_5$	0.57
7	C_6H_4(4-OCH_2CO_2H)	$CH_2CH_2C_6H_5$	0.16 (K_i = 0.051 μM)

[a] For the details of the assay see ref 11. Values are the average of at least two determinations.

In conclusion, starting from a low-affinity mass screening lead and a predicted mode of binding, followed by peptide-derived inhibitor structure–activity relationship analysis and molecular modeling studies, we have advanced a novel, low molecular weight, and readily synthesizable series of nonpeptide HIV PR inhibitors lacking chiral centers. Rationalization of the potencies of these compounds might be due to replacement of two water molecules in the active site, and the likely adoption of a stable conformation in aqueous media may explain the observed binding affinities of these pyran-2-ones, as related to concepts of collective substrate[25] and hydrophobic collapse.[26] Since these inhibitors replace the water molecule (301) which is unique to most HIV PR inhibitor complexes, it is reasonable to predict that these inhibitors would be selective to HIV PR inhibition.[27] The interactions of the lactone moiety with HIV PR are similar to the interactions observed for the lactone portion of the coumarin analogue[19] and indicated that the lactone group may represent a useful and novel pharmacophoric core for further design of HIV PR inhibitors.

Supplementary Material Available: Experimental details and characterization data for **1**–**7** (4 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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