Use of Medium-Sized Cycloalkyl Rings To Enhance Secondary Binding: Discovery of a New Class of Human Immunodeficiency Virus (HIV) Protease Inhibitors

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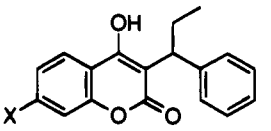
A unique strategy for the enhancement of secondary binding of an inhibitor to an enzyme has been demonstrated in the design of new human immunodeficiency virus (HIV) protease inhibitors. When the planar benzene ring of a 4-hydroxycoumarin lead compound (**1a**, K_i = 0.800 μM) was replaced with medium-sized (i.e., 7–9), conformationally-flexible, alkyl rings, the enzyme inhibitory activity of the resulting compounds was dramatically improved, and inhibitors with more than 50-fold better binding (e.g., **5d**, K_i = 0.015 μM) were obtained. X-ray crystal structures of these inhibitors complexed with HIV protease indicated the cycloalkyl rings were able to fold into the S1′ pocket of the enzyme and fill it much more effectively than the rigid benzene ring of the 4-hydroxycoumarin compound. This work has resulted in the identification of a promising lead structure for the design of potent, deliverable HIV protease inhibitors. Compound **5d**, a small (MW = 324), nonpeptidic structure, has already shown several advantages over peptidic inhibitors, including high oral bioavailability (91–99%), a relatively long half-life (4.9 h), and ease of synthesis (three steps).

At the end of 1993, the World Health Organization estimated 15 million people worldwide were already infected with human immunodeficiency virus (HIV), the causative agent of acquired immune deficiency syndrome (AIDS), and projected that number would grow to over 30 million by the turn of the century.[1] One potential therapeutic target is the HIV protease enzyme, which plays a key role in viral maturation.[2] Although promising in vitro enzyme inhibition and antiviral activity have been observed with several HIV protease inhibitors, most of the more potent candidates disclosed to date are peptide-derived compounds.[3] Clinical development of such compounds is often complicated by unfavorable pharmacokinetic parameters,[4] such as low oral bioavailability and rapid excretion, and by lengthy syntheses.[5]

One potential solution for the problems often encountered with peptide-derived inhibitors is use of a low molecular weight, nonpeptidic inhibitor. With this goal in mind, a screening program was developed at the Upjohn Co. to search for nonpeptidic inhibitors of HIV protease. Within a short period of time, the 4-hydroxycoumarin **1a** (K_i = 0.8 μM)[6] was identified as a lead structure.[7,8] Although concurrent research efforts have focused on development of coumarin and pyrone derivatives of the 4-hydroxycoumarin template,[7,9] we were interested using this template to identify other novel structures to serve as leads for design of new drug candidates. Ideally, the new lead structure would be significantly more active against HIV protease than **1a**, be easily accessible synthetically to facilitate subsequent structure–activity relationship (SAR) work, and retain the desirable pharmacokinetic characteristics of the 4-hydroxycoumarin class of compounds.[10]



1a X = H
1b X = OCH_3

Fortunately, X-ray crystal structures of **1a**[7] and related 4-hydroxycoumarins complexed with HIV proteases were available for use in drug design. Study of these inhibitor–protease complexes revealed that the pyrone ring was functioning as the pharmacophore by forming key hydrogen-bonding interactions at the active site. In addition, the ethyl group was filling the S1 region of the enzyme, and the phenyl group was occupying the S2 region of the enzyme.[11] The benzene ring of the coumarin, however, was only able to poorly interact with the protease in the S1′ region due to its rigidity. It was clear that enhancing the flexibility of the inhibitor was the most promising strategy for enhancing the secondary binding. However, instead of replacing the benzene ring with branched substituents at the C-6 position of the pyrone pharmacophore, as described in the pyrone inhibitor work cited above, we were interested in exploring an alternate strategy for increasing

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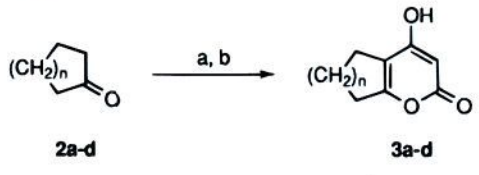
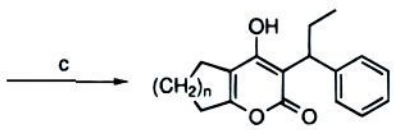
Scheme 1[a]



2a-d **3a-d**



no.	n	K_i (nM)
4a	1	700 nM
4b	2	1100 nM
4c	3	480 ± 70 nM
4d	4	75 ± 15 nM

[a] (a) Me_3SiCl, Et_3N, DMF, reflux; (b) $ClCOCH_2COCl$, Et_2O, −20 °C; (c) HOCH(Et)Ph, *p*-TSA, 3 Å sieves, toluene, reflux, 33−44%.

flexibility: replacing the benzene ring with saturated cycloalkyl rings. Consequently, the cycloalkylpyranones **4a−d** were selected as our initial set of targets. These new structures would allow us to retain the advantages of the binding pattern of **1a** while testing a new approach to avoiding the disadvantage of benzene ring rigidity.

Not only did the cycloalkylpyranones have the potential to demonstrate enhanced binding to the HIV protease, they also fulfilled one of our stated criteria for a new template—accessibility. This feature allowed the rapid synthesis and evaluation of **4a−d** (Scheme 1). First, the unsubstituted intermediates **3a−d** were prepared in two steps from the corresponding commercially-available cycloalkanones **2a−d**.[12] These intermediates were then readily alkylated at the C-3 position to form the target compounds **4a−d**. Although the cyclohexyl analog **4b** had comparable enzyme inhibitory activity to the coumarin lead compound, increase of the cycloalkyl ring size was accompanied by a dramatic improvement in the ability of the compounds to inhibit HIV-1 protease. In fact, the largest analog in the series, the cyclooctyl derivative **4d** (K_i = 75 nM), demonstrated a more than 10-fold improvement in activity over the lead structure.[13]

An X-ray crystal structure of inhibitor **4d** complexed with HIV-2 protease was obtained and compared to similar data from the complex of the 4-hydroxycoumarin inhibitor **1b** with HIV-2 protease.[14] As expected, the central pyrone ring of the cycloalkylpyranone **4d** functioned as the pharmacophore in a manner analogous to that of the pyrone ring in the 4-hydroxycoumarin inhibitor **1b**. In both cases, the pyrone ring was located in the active site of the protease (Figure 1). The 4-hydroxyl groups were within hydrogen-bonding distance of the two catalytic aspartic acid residues Asp 25 and Asp 25′, and the lactone carbonyls were within hydrogen-bonding distance of the N−H amide bonds of the two isoleucine residues on the flap of the enzyme, Ile 50 and Ile 50′. The binding in the active site, however, proved to be fairly flexible. The central pyrone ring of the cyclooctylpyranone was able to form these key hydrogen-bonding interactions with the active site of the protease even though it had rotated from the

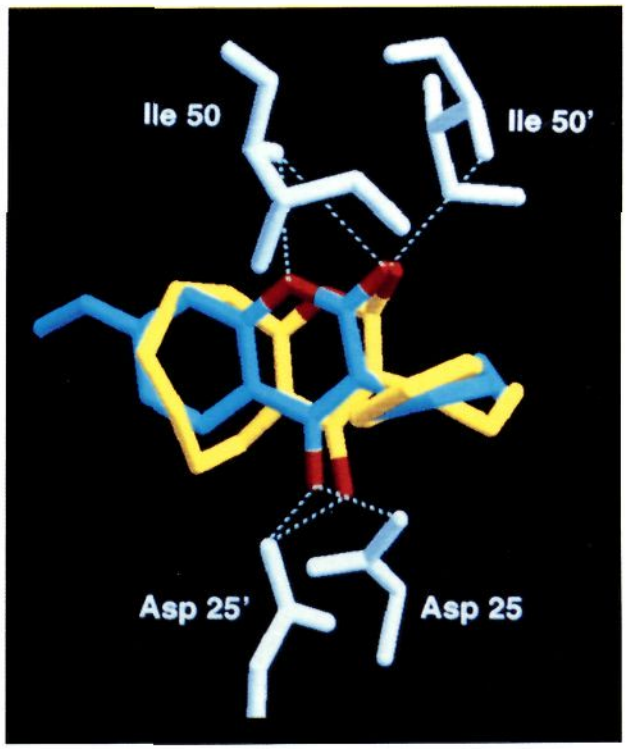


Figure 1. X-ray crystal structure of the cyclooctylpyranone inhibitor **4d** (yellow) bound to HIV-2 protease compared to an X-ray crystal structure of the 4-hydroxycoumarin **1b** (blue). The hydroxyl and lactone oxygens of the pharmacophores of both inhibitors (highlighted in red) form hydrogen-bonding interactions with the Ile 50, Ile 50′, Asp 25, and Asp 25′ residues in the active site of the HIV protease (shown in white).

position of the coumarin's pyrone ring. This rotation proved to play an important role in the binding of the new inhibitor by accommodating folding of the cyclooctyl ring into the S1′ pocket of the enzyme (Figure 2). In contrast, the rigidity of the planar coumarin inhibitor allowed only a poor fit into the S1′ pocket. A subsequent X-ray crystal structure of the cycloheptyl derivative **4c** complexed with HIV-2 protease showed a very similar pattern to the binding of **4d** (Figure 3). The central pyrone ring of **4c** formed the hydrogen-bonding interactions with Asp 25/25′ and Ile 50/50′, but this inhibitor had also rotated within the active site to allow its seven-membered ring to fold into the S1′ pocket. The dramatic enhancement of secondary binding observed for the cycloalkylpyranones is particularly intriguing in light of the conformational flexibility of the medium-sized cycloalkyl rings occupying the S1′ region of the protease. Monte Carlo calculations[15] of unbound **4d** indicated there were eight distinct conformations within 2.6 kcal of the minimum energy conformation, and similar calculations of the inhibitor bound to HIV-2 protease indicated there were six possible low-energy conformations of that ring within 3.2 kcal of the predicted lowest energy binding conformation.[16]

The use of a relatively-large, conformationally-flexible ring to improve the enzyme inhibitory activity of a lead structure is a unique strategy for achieving a proven method of enhancing the secondary binding of an inhibitor, increasing flexibility. The closest analogy to this strategy is use of a significantly less flexible saturated bridged ring system, a decahydroisoquinoline, at the P1′ position of peptide-derived protease inhibi-

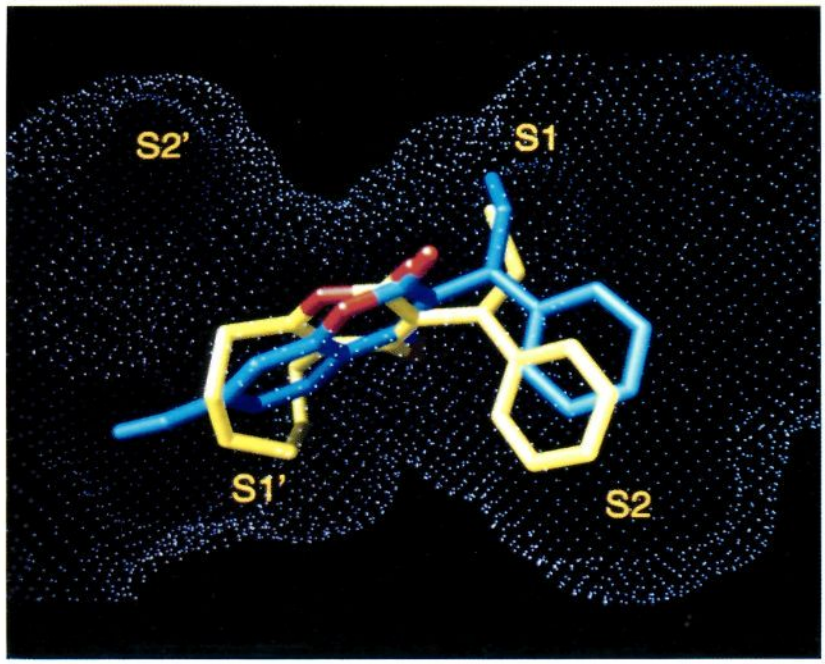


Figure 2. X-ray crystal structure of **4d** (yellow) complexed with HIV-2 protease (represented by the white dot surface) showing the ability of the inhibitor to fold its cyclooctyl ring into the enzyme's S1′ pocket. The planar 4-hydroxycoumarin inhibitor **1b** (blue) is only able to achieve a poor fit in the S1′ region.

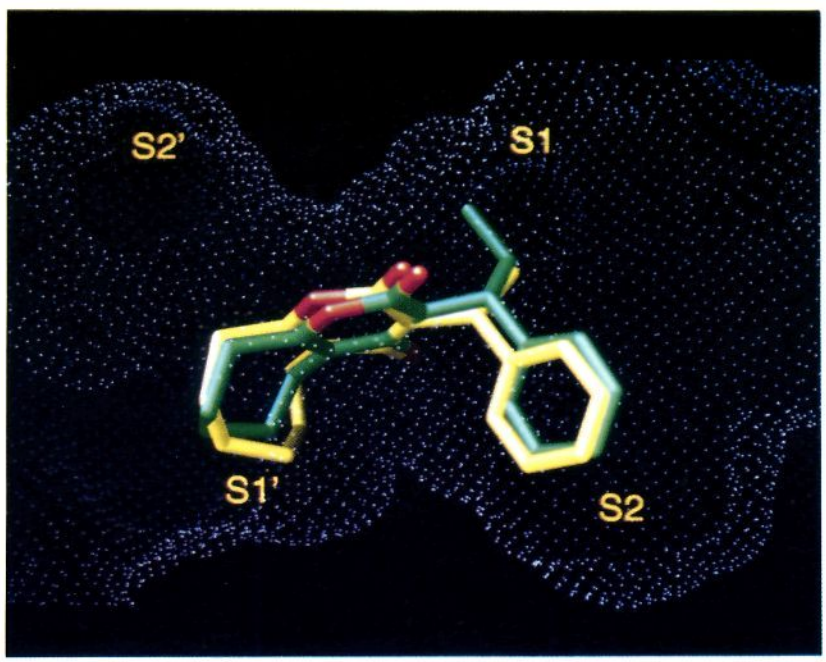
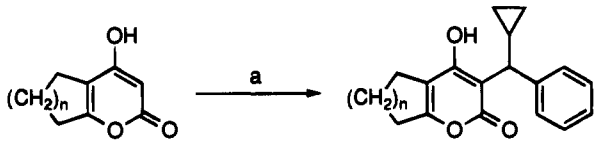


Figure 3. X-ray crystal structure of **4c** (green) complexed with HIV-2 protease compared to the X-ray crystal structure of inhibitor **4d** (yellow). The enzyme surface depicted here by the white dots is that of its complex with inhibitor **4d**. In both cases, the medium-sized cycloalkyl ring folds into the enzyme's S1′ pocket.

tors.[17] Although use of the decahydroisoquinoline is a popular and effective way to improve enzyme inhibition,[3] the group is not readily available; its synthesis requires several steps and involves the control of multiple chiral centers.[5a] The simple, medium-sized rings used to enhance the binding of the pyrone ring pharmacophore, however, are readily accessible, as illustrated above. Use of the medium-sized rings has the added appeal of enhancing binding without introducing asymmetric centers. This is an advantage over the pyrone inhibitors developed from the 4-hydroxycoumarin **1a**, which achieved best enzyme inhibition when the pyrone's C-6 substituent was branched in an asymmetric fashion.[7]

In light of the encouraging protease inhibitory activity in the initial set of compounds and the intriguing binding observations available from the X-ray data, a second, more extensive series of cycloalkylpyranone derivatives was synthesized and evaluated (**5a**–**g**; Scheme 2). In this case, a slight variation in substitution at the C-3 position was made. Use of α-cyclopropylbenzyl alcohol rather than 1-phenyl-1-propanol in the C-3 alkylation step generally resulted in better yields, presumably due to enhanced stabilization of the car-

Scheme 2[a]



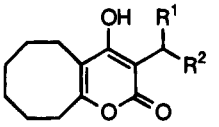
no.	n	no.	n	inhibition @ 1 μM	K_i (nM)[b]
3a	1	**5a**	1	40%	nd
3b	2	**5b**	2	62%	180
3c	3	**5c**	3	70%	96
3d	4	**5d**	4	95%	15 ± 5
3e	5	**5e**	5	94%	37
3f	6	**5f**	6	23%	560
3g	8	**5g**	8	<10%	nd

[a] (a) HOCH(cPr)Ph, *p*-TSA, 3 Å sieves, toluene, reflux, 32–78%. [b] nd indicates K_i value not determined due to relatively low enzyme inhibition.

bocationic intermediate by the cyclopropyl group relative to the ethyl group. In this second set of compounds, use of larger cycloalkyl rings was explored, in particular the 9-, 10-, and 12-membered rings. The results from the second group were very similar to the initial group described above: the optimal ring size of the cycloalkylpyranone inhibitors was clearly identified as eight (**5d**, K_i = 15 nM). The cyclooctylpyranone **5d** was, however, closely followed in activity by the nine-membered ring analog **5e** (K_i = 37 nM) and the seven-membered ring analog **5c** (K_i = 96 nM), implying that they too were able to effectively fill the S1′ pocket of the HIV protease. Derivatives with smaller (**5a**,**b**) or larger (**5f**,**g**) rings were much less active, most likely due to less favorable interactions with the S1′ region of the HIV protease. In summary, the second series of cycloalkylpyranones confirms the conclusion drawn from the first series. A unique strategy for improvement of secondary binding of an enzyme inhibitor, i.e., use of medium-sized, conformationally-flexible rings, resulted in the discovery of a potent new class of HIV protease inhibitors: the cyclooctylpyranones.

Changing the ethyl group in the C-3 substituent of **4d** to a cyclopropyl group (**5d**) improved the enzyme inhibitory activity of the cyclooctylpyranone inhibitor substantially, presumably by more efficient filling of the S1 pocket of the protease (see Figure 2). This observation prompted us to further investigate substitution at the C-3 position of the pyrone ring. Several analogs with various small alkyl groups designed to probe the nature of the S1 pocket of the protease were made and evaluated for HIV protease activity (Table 1).[18] All of the compounds with three-carbon substituents at the C-3-α position (**7**, **8**, and **5d**) were quite potent inhibitors. Substitution of slightly smaller (**4d**) or slightly larger (**9** and **10**) groups produced somewhat less active derivatives, but use of a methyl substituent (**6**) resulted in a dramatic loss of enzyme inhibitory activity. In addition to the derivatives made with varied alkyl substitution, a pair of symmetrical compounds was prepared and tested. The dicyclopropyl analog **12** (K_i = 57 nM) was still a fairly good inhibitor, but the diphenyl analog **11** (K_i = 180 nM) was significantly less active, possibly due to unfavorable steric factors in the S1 region.[19] Removal of the alkyl substituent at the C-3-α position of the pyrone ring was also explored (**13**–**15**). Deletion of the alkyl substituent (e.g., **13**) had a quite deleterious effect on the activity of the cycloalkylpyranone inhibitor, and extension of the chain resulted in only minor improvement in activity for **15**. These results demonstrate the importance of the interaction between the C-3-α substituent and the S1 region of the protease.

Table 1



no.	R^1	R^2	K_i (nM)[a]
6	Me	Ph	nd[b]
4d	Et	Ph	75 ± 15
7	*n*-Pr	Ph	29
8	*i*-Pr	Ph	22
5d	cPr	Ph	15 ± 5
9	*n*-Bu	Ph	62
10	*i*-Bu	Ph	90
11	Ph	Ph	180
12	cPr	cPr	57
13	H	Ph	nd[c]
14	H	(CH_2)Ph	nd[c]
15	H	$(CH_2)_2$Ph	nd[d]

[a] nd indicates K_i value not determined due to relatively low enzyme inhibition. [b] 10% inhibition at 1 μM. [c] <10% inhibition at 30 μM. [d] 38% inhibition at 30 μM.

The initial SAR work described above confirmed that the cyclooctylpyranone derivative **5d** was the most promising new lead structure for the design of new HIV protease inhibitors. This particular compound fulfills several of the criteria desirable in a new lead. It is a small (MW = 324), nonpeptidic compound with dramatically better (>50-fold) protease inhibitory activity than the 4-hydroxycoumarin inhibitor **1a**, and since it can be obtained in three steps from commercially-available materials, it is readily available. These characteristics prompted further investigation of the potential of **5d** as a lead structure.

Once inhibitor **5d** was identified as the compound of interest, its activity against HIV and other proteases was evaluated in more detail. First, the compound was separated into its two component enantiomers using chiral HPLC techniques. Both the (+)-enantiomer **16a** and the (−)-enantiomer **16b** were good inhibitors of HIV-1 protease with K_i values of 38 ± 3 and 13 ± 2 nM, respectively. In addition, **5d** was found to be quite effective against HIV-2 protease (K_i = 16 nM), and it demonstrated very good selectivity for the HIV proteases over other aspartyl proteases. In fact, the cyclooctyl compound showed no inhibition of the human enzymes renin, pepsin, cathepsin D, gastricsin, and cathepsin E at 25 μM.[20] Inhibitor **5d** was also evaluated for antiviral activity in HIV-1-infected H9 cells, where it demonstrated encouraging activity (ED_{50} = 57 μM).[21,22]

Inhibitor **5d** showed excellent pharmacokinetic behavior in rats. Time-course blood levels of the inhibitor after intravenous and oral administration are shown in Figure 4. The clearance values were relatively low (0.039 L/h/kg), and a half-life of 4.9 h was observed (Table 2). Excellent oral bioavailability was observed with both solution (F = 91%) and suspension (F = 99%) formulations of the inhibitor (Table 3). Thus, inhibitor **5d** appears to be avoiding the peptide-associated pitfalls of rapid clearance and low oral bioavailability.

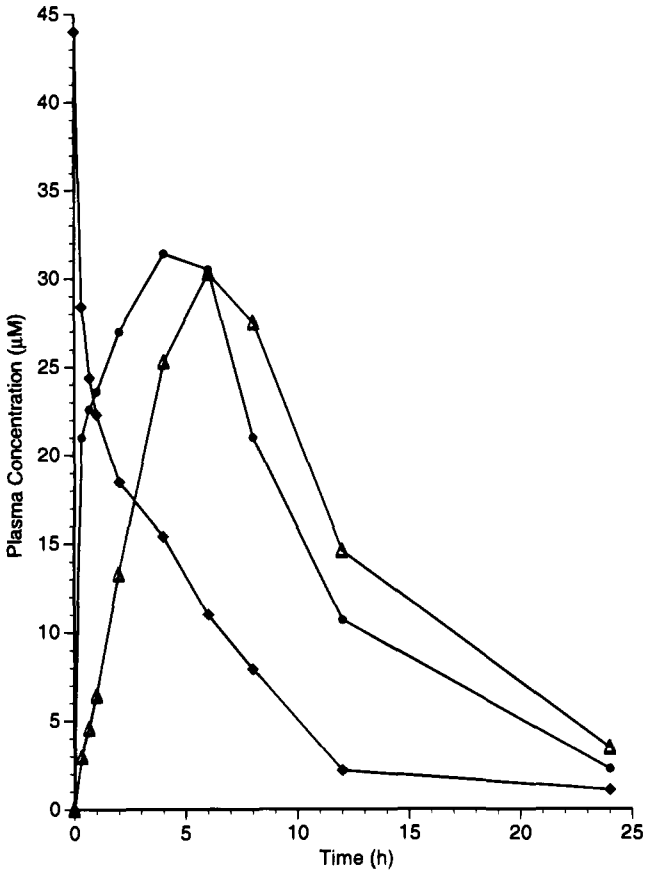


Figure 4. Plasma–time profile of inhibitor **5d** in rats. The mean plasma concentrations are plotted as a function of time. Three groups of rats received either a 2.5 mg/kg intravenous dose of **5d** (diamonds), a 5 mg/kg oral dose of a solution formulation of **5d** (circles), or a 5 mg/kg oral dose of a suspension formulation of **5d** (triangles).

Table 2. Selected Pharmacokinetic Parameters for **5d** in Rats Calculated from Time-Course Plasma Concentrations (Mean ± SD)[a]

n	dose (mg/kg)	$t_{1/2\beta}$ (h)	V_{ss} (L/kg)	Cl_p (L/h/kg)
3	2.5 (iv)	4.9 ± 1.3	0.25 ± 0.02	0.039 ± 0.011

[a] The plasma concentration–time data were fitted to mathematical equations using nonlinear regression analysis, and the distribution rate constant (β) and half-life ($t_{1/2\beta}$) were estimated. The clearance (Cl_p) and steady state volume of distribution (V_{ss}) were also calculated.

Table 3. Selected Pharmacokinetic Parameters for **5d** in Rats Calculated from Time-Course Plasma Concentrations (Mean ± SD, all rats received a dose of 5 mg/kg po)

formulation	n	AUC	C_{max} (μM)	T_{max} (h)	F^a (%)
solution[b]	3	374 ± 232	40.8 ± 18.6	3.7 ± 2.1	91 ± 5
suspension[c]	3	388 ± 132	30.3 ± 7.2	6	99 ± 4

[a] Oral bioavailability calculated using an average of the iv AUC (dose adjusted). [b] **5d** dissolved in 80% propylene glycol/20% 0.1 N NaOH. [c] **5d** suspended in 0.05 M phosphate buffer with 0.25% methyl cellulose.

In summary, a unique strategy for the improvement of an inhibitor's secondary binding to the HIV protease enzyme has been used to identify a small (MW = 324), nonpeptidic lead structure (**5d**). The dramatic binding enhancement observed with use of the conformationally-flexible seven-, eight-, and nine-membered rings reveals a simple, effective method for improving enzyme inhibitory activity without adding to the structure's stereochemical complexity. The cyclooctylpyranone lead structure is a promising starting point in our development of an effective, deliverable HIV protease inhibitor. Inhibitor **5d** is not only quite potent against the HIV proteases (K_i = 15–16 nM), but it is also easily accessible and has demonstrated excellent pharmacokinetic parameters. Investigation of the potential of this new class of compounds is continuing.

Table 4. Summary of Selected Diffraction Data and Refinement Statistics for Three HIV-2 Protease Inhibitor Complexes[a]

	complex		
	1	2	3
inhibitor	**1b**	**4c**	**4d**
space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
unit cell			
a (Å)	34.02	33.89	33.38
b (Å)	46.00	45.67	45.28
c (Å)	134.18	134.58	133.36
resolution (Å)	2.5	2.35	2.3
no. of observations	21 608	27 701	33 887
unique reflections	6651	7557	8579
% complete	85	76	90
R_{merge}	0.068	0.071	0.064
R-factor (refinement)	0.208	0.185	0.189

[a] Additional details may be found in the Brookhaven Protein Data Bank entries (see ref 29).

Experimental Section

X-ray Crystallography. Crystallization. The preparation and purification of the recombinant HIV-2 protease used for this work has been described elsewhere.[23] The protein preparation contains a Lys[57]/Leu mutation but has been found to be indistinguishable in activity and specificity from the wild type enzyme. To prepare crystalline complexes, about 0.2 mg of the selected inhibitor dissolved in 2 μL of DMSO was added to 200 μL aliquots of HIV-2 protease at ca. 5 mg/mL and equilibrated on ice for 30 min. Undissolved inhibitor was removed by centrifugation, and the protein solution was concentrated to 8–10 mg/mL. Crystals were grown at room temperature in 4–7 μL hanging drops by vapor diffusion against a precipitant of 30–35% (w/v) PEG 4000 in 0.1 Hepes, pH 6.8–7.6. Larger single crystals could occasionally be grown by addition of 1–3% n-butanol to the well solution.

Data Collection. A single crystal of each complex was used for data collection. Diffraction data to 2.0 Å were collected using a Siemens area detector, with X-rays generated by a Siemens rotating anode source operating at 45 kV, 96 mA. Measurements were made as a series of 0.25° frames, with an exposure time of 240 s/frame. Data sets were processed using XENGEN data reduction software.[24] Table 4 summarizes the data collection statistics of the three data sets along with statistics from the refinement of the models. The effective resolution of each crystal was taken as the maximum resolution for which the mean I/σ was greater than 2.0. Data beyond this maximum were discarded and not used.

Structure Refinement. Since the space group of these protease–inhibitor complexes was the same as ones previously refined,[23] refinement of these protease models could be initiated without resolving the position of the molecule in the cell. Structure refinements on complex 1 and complex 2 were performed using the PROLSQ[25] least-squares method with periodic manual rebuilding using FRODO interactive graphics,[26] based on $2|F_o - F_c|$ and $|F_o - F_c|$ electron density maps. Electron density maps were calculated using the XTAL package of crystallographic programs.[27] Complex 3 refinement was carried out using CEDAR.[28] Diffraction data having intensities greater than 2σ were used in the calculations for complex 1, but all data were used for complexes 2 and 3. The inhibitors and solvent molecules were added during later stages of the refinements. The atomic coordinates of these structures have been deposited with the Protein Data Bank.[29] Complex 1 was

found in two overlapping orientations related by the pseudo-2-fold axis relating the two protein monomers. Complexes 2 and 3 are found in only one orientation so that much better structures of the inhibitor conformation could be determined.

Pharmacokinetic Evaluation. Three fasted male rats received a 2.5 mg/kg bolus intravenous injection of inhibitor **5d** formulated in 10% propylene glycol and 22.5% hydroxypropyl-β-cyclodextrin in Tris (0.74 mg/mL). A second group of three rats received a 5 mg/kg oral dose of inhibitor **5d** formulated as a solution in 80:20 propylene glycol/0.1 N aqueous NaOH (1.49 mg/mL). A third group of three rats received a 5 mg/kg oral dose of inhibitor **5d** formulated as a suspension in 0.05 M phosphate buffer with 0.25% methyl cellulose. Blood samples were collected from the superior vena cava through an indwelling cannula. Plasma was harvested by centrifugation at 12000*g* for 5 min and stored frozen until analysis. For HPLC analysis of plasma drug concentrations, a 50 μL sample aliquot was mixed with 100 μL of acetonitrile containing an internal standard. The plasma proteins precipitated, and the sample was centrifuged. The supernatant was then mixed with 100 μL of aqueous 0.1% (v/v) trifluoroacetic acid. The prepared samples were chromatographed on a reverse-phase column (Zorbax 15B-C8, 150 × 4.6 mm, 5 μm particle size) with a mobile phase of 55:45 (vol ratio) acetonitrile:0.1% (v/v) trifluoroacetic acid delivered at 1.0 mL/min. The UV absorbance of the column effluent was monitored at 295 nm.

Chemical Synthesis. Melting points are uncorrected. ^{1}H NMR spectra were measured on a Bruker AM 300 (300 MHz) instrument using tetramethylsilane as an internal standard. All other physical data were measured by the Physical and Analytical Chemistry unit of Upjohn Laboratories. Flash chromatography was performed on 230–400 mesh silica gel 60.

Representative Procedure for Preparation of 4a–d, 5a–g, and 7–12. 3-(Cyclopropylphenylmethyl)-5,6,7,8,9,-10-hexahydro-4-hydroxy-2*H*-cycloocta[*b*]pyran-2-one (5d). A 50 mL, three-necked, round-bottomed flask with a 10 mL, pressure-equalizing addition funnel filled with activated 3 Å molecular sieves and fitted with a reflux condenser and nitrogen inlet was charged with **3d**[12] (0.753 g, 3.86 mmol), *p*-toluenesulfonic acid (0.184 g, 0.97 mmol), and 10 mL of toluene. α-Cyclopropylbenzyl alcohol (0.55 mL, 5.79 mmol) was added, and the reaction mixture was warmed to ca. 140 °C. Additional α-cyclopropylbenzyl alcohol (0.37 mL, 3.86 mmol) was added after 2.5 and 5 h. After a total of 7 h, the reaction mixture was diluted with 1 N NaOH and washed with ether. The pH of the aqueous layer was adjusted from 14 to 1 with concentrated HCl. A white precipitate formed, which was extracted with CH_2Cl_2. The CH_2Cl_2 layers were then combined, dried over $MgSO_4$, filtered, and concentrated to give 1.128 g of a white solid. Column chromatography on 50 g of silica gel (elution with 20% ethyl acetate–hexane) yielded 0.859 g (69%) of **5d** as a white solid: mp 174–176 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51 (d, *J* = 7.7 Hz, 2 H), 7.38 (dd, *J* = 7.3, 7.7 Hz, 2 H), 7.28 (d, *J* = 7.3 Hz, 1 H), 6.26 (s, 1 H), 3.99 (d, *J* = 8.8 Hz, 1 H), 2.62 (t, *J* = 6.2 Hz, 2 H), 2.44 (t, *J* = 6.2 Hz, 2 H), 1.80–1.72 (m, 2 H), 1.60–1.32 (m, 7 H), 0.78–0.70 (m, 1 H), 0.64–0.56 (m, 2 H), 0.32–0.26 (m, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.7, 164.0, 161.2, 140.9, 129.1, 127.8, 127.3, 110.7, 106.2, 43.6, 30.6, 29.1, 28.8, 26.2, 25.8, 22.0, 13.1, 4.8, 3.7; IR (mineral oil) 3155, 3080, 1665, 1559 cm^{-1}; MS (EI) *m/z* 324 (M^+). Anal. ($C_{21}H_{24}O_3$) C, H.

6,7-Dihydro-4-hydroxy-3-(1-phenylpropyl)cyclopenta-[*b*]pyran-2(5*H*)-one (4a): 0.285 g (33%) of a pale yellow solid; mp 183–186 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43 (d, *J* = 7.7 Hz, 2 H), 7.34 (dd, *J* = 7.7, 7.3 Hz, 2 H), 7.24 (d, *J* = 7.3 Hz, 1 H), 6.29 (br s, 1 H), 4.30 (t, *J* = 7.9 Hz, 1 H), 2.76 (t, *J* = 7.9 Hz, 2 H), 2.62–2.57 (m, 2 H), 2.22–2.00 (m, 4 H), 1.00 (t, *J* = 7.3 Hz, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.7, 163.6, 163.2, 142.2, 129.0, 127.6, 126.9, 111.3, 104.9, 41.7, 31.1, 25.4, 23.9, 19.8, 12.4; IR (mineral oil) 3130, 3079, 3056, 3022, 1672, 1662, 1627, 1561 cm^{-1}; MS (EI) *m/z* 270 (M^+). Anal. ($C_{17}H_{18}O_3$) C, H.

5,6,7,8-Tetrahydro-4-hydroxy-3-(1-phenylpropyl)-2*H*-1-benzopyran-2-one (4b): 0.549 g (44%) of a white solid; mp 178–180 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45–7.35 (m, 4 H), 7.28 (d, *J* = 6.8 Hz, 1 H), 5.76 (br s, 1 H), 4.35 (t, *J* = 7.7 Hz, 1 H), 2.48 (t, *J* = 6.3 Hz, 2 H), 2.24–2.20 (m, 2 H), 2.18–1.99 (m, 2 H), 1.81–1.70 (m, 2 H), 1.69–1.63 (m, 2 H), 1.03 (t, *J* = 7.3 Hz, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.03, 163.9, 158.4, 141.8, 129.2, 127.5, 127.1, 108.4, 106.0, 41.2, 27.1, 23.9, 21.5, 21.4, 20.2, 12.3; IR (mineral oil) 3156, 3085, 3058, 3025, 1668, 1634, 1559 cm^{-1}; MS (EI) *m/z* 284 (M^+). Anal. ($C_{18}H_{20}O_3$) C, H.

6,7,8,9-Tetrahydro-4-hydroxy-3-(1-phenylpropyl)cyclohepta[*b*]pyran-2(5*H*)-one (4c): 0.172 g (41%) of a white solid; mp 72–75 °C dec; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44–7.36 (m, 4 H), 7.31–7.26 (m, 1 H), 5.84 (br s, 1 H), 4.38 (t, *J* = 7.8 Hz, 1 H), 2.71–2.67 (m, 2 H), 2.42–2.38 (m, 2 H), 2.23–1.96 (m, 2 H), 1.77–1.73 (m, 2 H), 1.70–1.62 (m, 2 H), 1.56–1.49 (m, 2 H), 1.03 (t, *J* = 7.3 Hz, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.0, 163.8, 141.5, 129.4, 127.5, 127.3, 113.1, 105.6, 41.2, 33.8, 31.3, 26.1, 24.4, 23.8, 22.1, 12.2; IR (mineral oil) 3126, 3085, 3058, 3025, 1657, 1627, 1548 cm^{-1}; MS (EI) *m/z* 298 (M^+). Anal. ($C_{19}H_{22}O_3$) C, H.

5,6,7,8,9,10-Hexahydro-4-hydroxy-3-(1-phenylpropyl)-2*H*-cycloocta[*b*]pyran-2-one (4d): 0.404 g (34%) of a white solid; mp 189–191 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44–7.35 (m, 4 H), 7.26 (d, *J* = 7.0 Hz, 1 H), 5.76 (s, 1 H), 4.37 (t, *J* = 7.7 Hz, 1 H), 2.61 (t, *J* = 6.2 Hz, 2 H), 2.42–2.37 (m, 2 H), 2.21–1.98 (m, 2 H), 1.77–1.71 (m, 2 H), 1.53–1.38 (m, 6 H), 1.02 (t, *J* = 7.3 Hz, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.5, 163.8, 161.2, 141.5, 129.3, 127.5, 127.2, 110.5, 106.2, 41.2, 30.6, 29.1, 28.8, 26.1, 25.7, 23.9, 22.0, 12.2; IR (mineral oil) 3174, 3086, 3063, 3027, 1664, 1640, 1566 cm^{-1}; MS (EI) *m/z* 312 (M^+). Anal. ($C_{20}H_{24}O_3$) C, H.

3-(Cyclopropylphenylmethyl)-6,7-dihydro-4-hydroxycyclopenta[*b*]pyran-2(5*H*)-one (5a): 0.695 g (62%) of a white solid; mp 183–185 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50 (d, *J* = 7.6 Hz, 2 H), 7.35 (dd, *J* = 7.2, 7.6 Hz, 2 H), 7.26 (d, *J* = 7.2 Hz, 1 H), 6.51 (s, 1 H), 3.84 (d, *J* = 9.1 Hz, 1 H), 2.77 (t, *J* = 7.7 Hz, 2 H), 2.67–2.56 (m, 2H), 2.11–2.00 (m, 2 H), 1.51–1.40 (m, 1 H), 0.78–0.70 (m, 1 H), 0.69–0.49 (m, 2 H), 0.31–0.23 (m, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.9, 163.6, 163.3, 141.6, 128.9, 127.8, 127.0, 111.5, 105.1, 44.3, 31.1, 25.5, 19.8, 13.0, 5.1, 3.8; IR (mineral oil) 3142, 3076, 3065, 3024, 3001, 1661, 1612, 1566, 1544 cm^{-1}; MS (E) *m/z* 282 (M^+). Anal. ($C_{18}H_{18}O_3$) C, H.

3-(Cyclopropylphenylmethyl)-5,6,7,8-tetrahydro-4-hydroxy-2*H*-1-benzopyran-2-one (5b): 0.513 g (49%) of a white solid; mp 180–181 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 (d, *J* = 7.7 Hz, 2 H), 7.35 (dd, *J* = 7.7, 7.1 Hz, 2 H), 7.26 (d, *J* = 7.1 Hz, 1 H), 6.14 (s, 1 H), 3.92 (d, *J* = 9.2 Hz, 1 H), 2.49 (t, *J* = 6.3 Hz, 2 H), 2.30–2.26 (m, 2 H), 1.82–1.74 (m, 2 H), 1.72–1.65 (m, 2 H), 1.40–1.31 (m, 1 H), 0.79–0.70 (m, 1 H), 0.63–0.56 (m, 2 H), 0.32–0.24 (m, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.3, 163.9, 158.4, 141.2, 129.1, 127.8, 127.3, 108.6, 106.1, 43.7, 27.1, 21.6, 21.4, 20.2, 13.0, 5.0, 3.8; IR (mineral oil) 3151, 3073, 3060, 3025, 1668, 1634, 1564 cm^{-1}; MS (EI) *m/z* 296 (M^+). Anal. ($C_{19}H_{20}O_3$) C, H.

3-(Cyclopropylphenylmethyl)-6,7,8,9-tetrahydro-4-hydroxycyclohepta[*b*]pyran-2(5*H*)-one (5c): 0.896 g (69%) of a white solid; mp 85–88 °C dec; ^{1}H NMR (300 Mhz, $CDCl_3$) δ 7.52 (d, *J* = 7.7 Hz, 2 H), 7.37 (dd, *J* = 7.7, 7.3 Hz, 2 H), 7.28 (d, *J* = 7.3 Hz, 1 H), 6.24 (s, 1 H), 3.97 (d, *J* = 8.9 Hz, 1 H), 2.72–2.68 (m, 2 H), 2.47–2.43 (m, 2 H), 1.82–1.75 (m, 2 H), 1.71–1.64 (m, 2 H), 1.59–1.52 (m, 2 H), 1.37–1.29 (m, 1 H), 0.78–0.71 (m, 1 H), 0.65–0.57 (m, 2 H), 0.32–0.26 (m, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.3, 163.9, 163.8, 140.9, 129.1, 127.9, 127.4, 113.2, 105.7, 43.7, 33.8, 31.3, 26.1, 24.4, 22.1, 13.0, 4.9, 3.8; IR (mineral oil) 3138, 3076, 3060, 3025, 1658, 1628, 1549 cm^{-1}; MS (EI) *m/z* 310 (M^+). Anal. ($C_{20}H_{22}O_3$) C, H.

3-(Cycopropylphenylmethyl)-6,7,8,9,10,11-hexahydro-4-hydroxycyclonona[*b*]pyran-2(5*H*)-one (5e): 0.194 g (63%) of a white solid; mp 82–84 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51 (d, *J* = 7.3 Hz, 2 H), 7.37 (dd, *J* = 7.3, 7.2 Hz, 2 H), 7.29 (d, *J* = 7.2 Hz, 1 H), 6.22 (s, 1 H), 4.01 (d, *J* = 8.6 Hz, 1 H), 2.65–2.61 (m, 2 H), 2.46–2.43 (m, 2 H), 1.79–1.78 (m, 2 H), 1.58–1.31 (m, 9 H), 0.79–0.70 (m, 1 H), 0.66–0.57 (m, 2 H), 0.33–0.25 (m, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.6, 164.2, 161.1, 140.8, 129.1, 127.9, 127.4, 111.6, 106.3, 43.5, 30.2, 25.9,

25.7, 25.0, 24.9, 24.0, 22.9, 13.1, 4.8, 3.7; IR (mineral oil) 3133, 3075, 3060, 3024, 1659, 1632, 1550 cm^{-1}; MS (EI) *m/z* 338 (M^+); HRMS calcd for $C_{22}H_{26}O_3$ 338.1882, found 338.1872; HPLC (3.9 × 300 mm μ-Bondapak C18 column, 10–85% CH_3CN in pH 3 aqueous buffer, 2.0 mL/min) t_R = 12.8 min.

3-(Cyclopropylphenylmethyl)-5,6,7,8,9,10,11,12-octahydro-4-hydroxy-2*H*-cyclodeca[*b*]pyran-2-one (5f): 0.315 g (53%) of a white solid; mp 88–90 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 7.51 (d, *J* = 7.6 Hz, 2 H), 7.38 (dd, *J* = 7.6, 7.2 Hz, 2 H), 7.28 (d, *J* = 7.2 Hz, 1 H), 6.20 (s, 1 H), 4.02 (d, *J* = 8.5 Hz, 1 H), 2.74–2.68 (m, 2 H), 2.50 (t, *J* = 6.6 Hz, 2 H), 1.88–1.84 (m, 2 H), 1.67–1.57 (m, 2 H), 1.45–1.40 (m, 4 H), 1.38–1.31 (m, 3 H), 1.29–1.21 (m, 2 H), 0.77–0.71 (m, 1 H), 0.67–0.56 (m, 2 H), 0.33–0.27 (m, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.5, 164.6, 160.3, 140.8, 129.1, 127.8, 127.4, 111.1, 106.2, 43.4, 28.8, 26.9, 25.7, 25.4, 25.3, 22.7, 21.1, 20.7, 13.0, 4.8, 3.6; IR (mineral oil) 3158, 3084, 3076, 3024, 1660, 1632, 1547 cm^{-1}; MS (EI) *m/z* 352 (M^+). Anal. ($C_{23}H_{28}O_3$) C, H.

3-(Cyclopropylphenylmethyl)-5,6,7,8,9,10,11,12,13,14-decahydro-4-hydroxy-2*H*-cyclododeca[*b*]pyran-2-one (5g): 0.245 g (32%) of a white solid; mp 98–99 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 7.51 (d, *J* = 7.4 Hz, 2 H), 7.37 (dd, *J* = 7.4, 7.3 Hz, 2 H), 7.29 (d, *J* = 7.3 Hz, 1 H), 6.20 (s, 1 H), 3.97 (d, *J* = 8.8 Hz, 1 H), 2.55–2.49 (m, 2 H), 2.32 (t, *J* = 6.9 Hz, 2 H), 1.88–1.83 (m, 2 H), 1.57–1.50 (m, 2 H), 1.40–1.33 (m, 10 H), 1.31–1.23 (m, 3 H), 0.77–0.70 (m, 1 H), 0.65–0.58 (m, 2 H), 0.32–0.26 (m, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.3, 164.7, 160.7, 140.9, 129.1, 127.9, 127.4, 111.7, 106.4, 43.6, 27.5, 26.8, 25.0, 24.9, 24.8, 23.7, 22.5, 22.1, 21.9, 14.0, 13.0, 4.9, 3.9; IR (mineral oil) 3131, 3076, 3060, 3024, 1658, 1632, 1546 cm^{-1}; MS (EI) *m/z* 380 (M^+). Anal. ($C_{25}H_{32}O_3$) C, H.

5,6,7,8,9,10-Hexahydro-4-hydroxy-3-(1-phenylbutyl)-2*H*-cycloocta[*b*]pyran-2-one (7): 0.52 g (16%) of a white solid; mp 173–175 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 7.44–7.25 (m, 5 H), 5.84 (s, 1 H), 4.45 (t, *J* = 7.7 Hz, 1 H), 2.60 (m, 2 H), 2.40 (m, 2 H), 2.12–1.96 (m, 2 H), 1.73 (m, 2 H), 1.60–1.33 (m, 8 H), 0.98 (t, *J* = 7.3 Hz, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.4, 163.7, 161.1, 141.7, 129.3, 127.4, 127.2, 110.5, 106.5, 39.4, 33.2, 30.6, 29.1, 28.8, 26.1, 25.7, 22.0, 20.8, 14.1; IR (mineral oil) 3158, 3086, 3063, 3027, 1663, 1601, 1564 cm^{-1}; MS (EI) *m/z* 326 (M^+). Anal. ($C_{21}H_{26}O_3$) C, H.

5,6,7,8,9,10-Hexahydro-4-hydroxy-3-(2-methyl-1-phenylpropyl)-2*H*-cycloocta[*b*]pyran-2-one (8): 0.493 g (38%) of a white solid; mp 214–216 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 7.46 (d, *J* = 7.5 Hz, 2 H), 7.32 (dd, *J* = 7.2, 7.5 Hz, 2 H), 7.23 (d, *J* = 7.2 Hz, 1 H), 6.02 (br s, 1 H), 3.94 (d, *J* = 10.6 Hz, 1 H), 2.86–2.79 (m, 1 H), 2.58 (t, *J* = 6.2 Hz, 2 H), 2.47–2.40 (m, 2 H), 1.73–1.71 (m, 2 H), 1.59–1.54 (m, 2 H), 1.48–1.46 (m, 2 H), 1.41–1.39 (m, 2 H), 0.98 (d, *J* = 6.4 Hz, 3 H), 0.97 (d, *J* = 6.4 Hz, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.3, 163.5, 161.3, 142.2, 129.0, 128.2, 126.8, 110.0, 106.4, 48.5, 30.8, 29.2, 28.9, 28.3, 26.3, 25.8, 22.2, 22.1, 21.0; IR (mineral oil) 3159, 3085, 3063, 3028, 1658, 1632, 1621, 1541 cm^{-1}; MS (EI) *m/z* 326 (M^+). Anal. ($C_{21}H_{26}O_3$) C, H.

5,6,7,8,9,10-Hexahydro-4-hydroxy-3-(1-phenylpentyl)-2*H*-cycloocta[*b*]pyran-2-one (9): 0.68 g (22%) of a white solid; mp 161–164 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 7.44–7.24 (m, 5 H), 5.85 (br s, 1 H), 4.42 (m, 1 H), 2.61 (m, 2 H), 2.40 (m, 2 H), 2.17–2.01 (m, 1 H), 2.04–1.94 (m, 1 H), 1.73 (m, 2 H), 1.64–1.31 (m, 10 H), 0.90 (t, *J* = 6.9 Hz, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.4, 163.7, 161.1, 141.7, 129.3, 127.5, 127.2, 110.5, 106.5, 39.6, 30.6, 29.7, 29.1, 28.9, 28.8, 26.1, 25.7, 22.7, 22.0, 13.9; IR (mineral oil) 3132, 3086, 3058, 3028, 1656, 1634, 1618, 1545, 1534 cm^{-1}; MS (EI) *m/z* 340 (M^+). Anal. ($C_{22}H_{28}O_3$) C, H.

5,6,7,8,9,10-Hexahydro-4-hydroxy-3-(3-methyl-1-phenylbutyl)-2*H*-cycloocta[*b*]pyran-2-one (10): 0.38 g (13%) of a white solid; mp 191–195 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 7.47–7.24 (m, 5 H), 5.91 (br s, 1 H), 4.53 (t, *J* = 7.9 Hz, 1 H), 2.60 (m, 2 H), 2.41 (m, 2 H), 2.05–1.84 (m, 2 H), 1.73 (m, 2 H), 1.64–1.36 (m, 7 H), 0.98 (d, *J* = 6.5 Hz, 6 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.2, 163.7, 161.1, 142.1, 129.2, 127.5, 127.1, 110.4, 106.7, 40.2, 37.5, 30.6, 29.1, 28.8, 26.1, 25.9, 25.7, 22.9, 22.7, 22.0; IR (mineral oil) 3124, 3086, 3058, 3025, 1661, 1638, 1625, 1557, 1531 cm^{-1}; MS (EI) *m/z* 340 (M^+). Anal. ($C_{22}H_{28}O_3$) C, H.

3-(Diphenylmethyl)-5,6,7,8,9,10-hexahydro-4-hydroxy-2*H*-cycloocta[*b*]pyran-2-one (11): 0.30 g (81%) of a white solid; mp 209–212 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 7.39–7.26 (m, 6 H), 7.21 (d, *J* = 6.9 Hz, 4 H), 5.94 (s, 1 H), 5.87 (s, 1 H), 2.66–2.61 (m, 2 H), 2.47–2.42 (m, 2 H), 1.79–1.71 (m, 2 H), 1.60–1.43 (m, 6 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.1, 164.7, 161.7, 140.4, 129.3, 128.7, 127.6, 110.9, 105.4, 46.9, 30.8, 29.4, 28.9, 26.4, 25.9, 22.2; IR (mineral oil) 3087, 3061, 3054, 3029, 1671, 1649, 1623, 1612, 1602, 1549, 1515, 1496 cm^{-1}; MS (EI) *m/z* 360 (M^+). Anal. ($C_{22}H_{28}O_3$) C, H.

3-(Dicyclopropylmethyl)-5,6,7,8,9,10-hexahydro-4-hydroxy-2*H*-cycloocta[*b*]pyran-2-one (12): 0.34 g (48%) of a white solid; mp 197–198 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 7.44 (br s, 1 H), 2.68 (t, *J* = 6.4 Hz, 1 H), 2.64–2.60 (m, 2 H), 2.56–2.52 (m, 2 H), 1.79–1.71 (m, 2 H), 1.69–1.61 (m, 2 H), 1.55–1.41 (m, 6 H), 1.04–0.93 (m, 2 H), 0.60–0.51 (m, 2 H), 0.48–0.42 (m, 4 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.7, 164.3, 160.8, 110.4, 105.4, 39.7, 30.6, 29.2, 28.8, 26.2, 26.0, 22.1, 12.4, 3.6, 2.4; IR (mineral oil) 3078, 3008, 1658, 1610, 1547 cm^{-1}; MS (EI) *m/z* 288 (M^+). Anal. ($C_{22}H_{28}O_3$) C, H.

Separation of 5d into Component Enantiomers. Separation was carried out using a 2.0 × 25 cm Chiralcel OD column as the chiral stationary phase and 4% ethanol and 0.1% acetic acid in hexane as the mobile phase (16 mL/min). A UV detector at 295 nm was used to monitor the separation of 20 mg of racemate/run. Enantiomeric purity was determined by HPLC using a 0.46 × 25 cm Chiralcel OD column as the chiral stationary phase and 4% ethanol and 0.05% acetic acid in hexane as the mobile phase (0.75 mL/min).

(+)-3-(Cyclopropylphenylmethyl)-5,6,7,8,9,10-hexahydro-4-hydroxy-2*H*-cycloocta[*b*]pyran-2-one (16a): 98% ee; t_R = 18.7 min; [α] = +68°. Anal. ($C_{21}H_{24}O_3$) C, H.

(−)-3-(Cyclopropylphenylmethyl)-5,6,7,8,9,10-hexahydro-4-hydroxy-2*H*-cycloocta[*b*]pyran-2-one (16b): 92% ee; t_R = 22.6 min; [α] = −76°. Anal. ($C_{21}H_{24}O_3$) C, H.

Registry Numbers Supplied by Author: α-Cyclopropylbenzyl alcohol [1007-03-0], 1-phenyl-1-propanol [93-54-9], 2-methyl-1-phenyl-1-propanol [611-69-8], 1-phenyl-1-butanol [614-14-2], 3-methyl-1-phenyl-1-butanol [1565-86-2], 1-phenyl-1-pentanol [583-03-9].

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(13) Since these new compounds were derived from a 4-hydroxycoumarin lead, **4d** was screened for anticoagulant activity in rats. When dosed with 200 μg/kg/day of **4d** iv for 5 days or 1 mg/kg for 3 days, no anticoagulant activity, as measured by the prothrombin time ratio, was observed. In contrast, warfarin demonstrated significant anticoagulant activity in the 200 μg/kg/day study and was lethal in the 1 mg/kg/day study. We thank Dr. Ronald Shebuski and Ann Wiltse for this data.

(14) Due to relatively weak binding of **1a** to the HIV-2 protease, a clear X-ray crystal structure of the complex could not be obtained. An X-ray crystal structure of the 7-methoxy derivative **1b** (K_i = 0.5 μM in HIV-1, 0.22 μM in HIV-2) complexed with HIV-2 protease, however, was available and is used here as a representative structure to illustrate the binding of the 4-hydroxycoumarin inhibitors.

(15) The calculations were carried out with the Monte Carlo multiple minimum facility of BatchMin 3.5, using the AMBER force field; see: Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Sill, W. C. MacroModel – An Integrated Software System for Modeling Organic and Bioorganic Molecules Using Molecular Mechanics. *J. Comput. Chem.* **1990**, *11*, 440–467.

(16) The cyclooctyl ring has to distort very little, if at all, in order to achieve a conformation suitable for binding to the enzyme. The bound conformation of **4d** observed in the X-ray crystal structure is only 0.3 kcal above the lowest energy unbound conformation, as determined by Monte Carlo calculations.

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(18) Compounds **7–12** were prepared from **3c** using the alkylation methodology described in Scheme 1 and the appropriate secondary alcohol. Synthesis of **6** and **13–15** was conducted as described in Gammill, R. B.; Judge, T. M.; Phillips, G.; Zhang, Q.; Sowell, C. G.; Cheney, B. V.; Mizsak, S. A.; Dolak, L. A.; Seest, E. P. Asymmetric Synthesis of an HIV Protease Inhibitor via a Novel α-Oxoketene/ketene [4 + 2] Cycloaddition Reaction. *J. Am. Chem. Soc.* **1994**, *116*, 12113–12114. Full experimental details for **6** and **13–15** will be reported shortly in a full paper describing the cycloaddition chemistry. Compounds **6** and 14 were first prepared by Q. Zhang in the laboratory of Dr. Ronald B. Gammill.

(19) We thank Paul Johnson for the preparation of 11.

(20) We thank Dr. Alfredo G. Tomasselli for renin inhibition data and Professor Ben M. Dunn, University of Florida, Gainesville, FL, for the remaining human enzyme inhibition data.

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