Cycloalkylpyranones and Cycloalkyldihydropyrones as HIV Protease Inhibitors: Exploring the Impact of Ring Size on Structure−Activity Relationships

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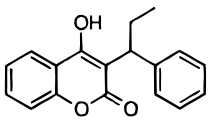
Received April 19, 1996[⊗]

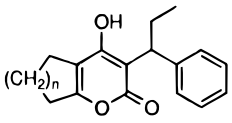
Previously, 3-substituted cycloalkylpyranones, such as **2d**, have proven to be effective inhibitors of HIV protease. In an initial series of 3-(1-phenylpropyl) derivatives with various cycloalkyl ring sizes, the cyclooctyl analog was the most potent. We became interested in exploring the influence of other structural changes, such as substitution on the phenyl ring and saturation of the 5,6-double bond, on the cycloalkyl ring size structure−activity relationship (SAR). Saturation of the 5,6-double bond in the pyrone ring significantly impacts the SAR, altering the optimal ring size from eight to six. Substitution of a sulfonamide at the meta position of the phenyl ring dramatically increases the potency of these inhibitors, but it does not change the optimal ring size in either the cycloalkylpyranone or the cycloalkyldihydropyrone series. This work has led to the identification of compounds with superb binding affinity for the HIV protease (K_i values in the 10−50 pM range). In addition, the cycloalkyldihydropyrones showed excellent antiviral activity in cell culture, with ED_{50} values as low as 1 μM.

One strategy for controlling human immunodeficiency virus (HIV) infection is inhibition of the HIV protease, which plays a critical role in viral maturation.[1] The search for an inexpensive inhibitor with good oral bioavailability at Pharmacia and Upjohn, Inc. began with a screening program, and this resulted in the discovery of a class of low molecular weight, non-peptidic lead structures, the 4-hydroxycoumarins (e.g. **1**).[2,3] One of the templates which we have since developed from this lead structure is the cyclooctylpyranone **2d**. We chose to replace the rigid benzene ring of the coumarin with conformationally-flexible cycloalkyl rings of various sizes, and when we prepared the first series of cycloalkylpyranone derivatives, this strategy resulted in a dramatic increase in enzyme binding affinity. In both this first series of analogs and a similar subsequent series, it was clear that the optimal size of the cycloalkyl ring was eight. X-ray crystal structures of **2c** and **2d** complexed with HIV protease were obtained, and these showed that the cycloalkyl ring folded into the S1 pocket of the protease. These crystal structures also illustrated that the cyclooctyl ring was better able to fill the S1 region than the cycloheptyl ring.[4] As we developed this new template, one of the issues of interest to us was the impact of structural changes in the cycloalkylpyranones, such as substitution on the phenyl ring and saturation of the 5,6-double bond in the pyrone ring, on the optimal size of the cycloalkyl ring.

Recently, substitution at the meta position on the phenyl ring of **2d** has been shown to allow substituents to reach the S3′ pocket, and this is associated with enhanced enzyme binding affinity.[5,6] In particular, use of an aryl sulfonamide substituent at this position has, in many cases, resulted in significant antiviral activity in addition to potent enzyme inhibitory activity.[6] We were interested in exploring the impact of this substitution on the optimal cycloalkyl ring size. A sulfonamide substituent which had been associated with good enzyme binding affinity in this and related templates[6b,7] was selected, and the compounds of interest were prepared in three steps from the known cycloalkylpyranones **3a**−**e**[8] (Scheme 1).[5,6] Substitution of the sulfonamide at the meta position dramatically improved the enzyme binding affinity of these analogs relative to the unsubstituted series.[9,10] The optimal cycloalkyl ring sizes, however, were unchanged, and the seven- and eight-membered derivatives (**5c,d**) were the most potent. Addition of a P3′ substituent does not appear to influence the structure−activity relationship (SAR) of the P1 substituent in this template.



1 K_i = 800 nM



2a	n = 1	K_i = 700 nM
2b	n = 2	K_i = 1100 nM
2c	n = 3	K_i = 480 nM
2d	n = 4	K_i = 75 nM
2e	n = 6	K_i > 2000 nM

We were also interested in the impact of a modification much closer to the P1 cycloalkyl ring: saturation of the 5,6-double bond in the pyrone ring. The cycloalkyldihydropyrones were formed by catalytic hydrogenation of the 5,6-double bond in the cycloalkylpyranones **3a**−**d** (Scheme 2). We found that hydrogenation of this tetrasubstituted double bond required fairly rigorous

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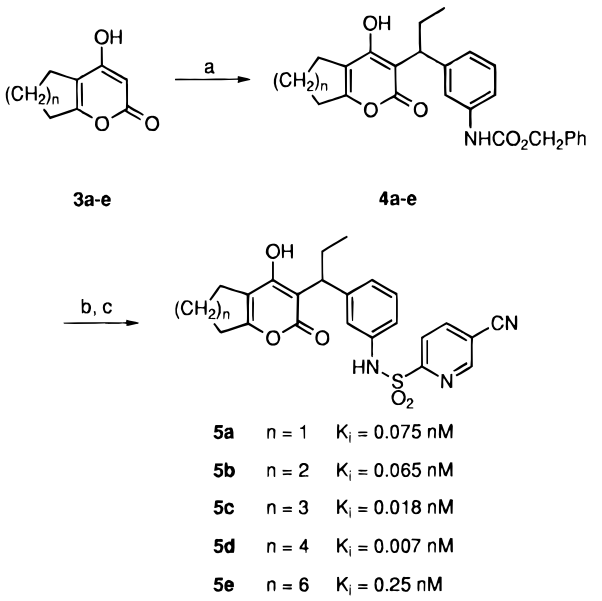
⊗ Abstract published in *Advance ACS Abstracts,* September 1, 1996.

S0022-2623(96)00296-8 CCC: $12.00

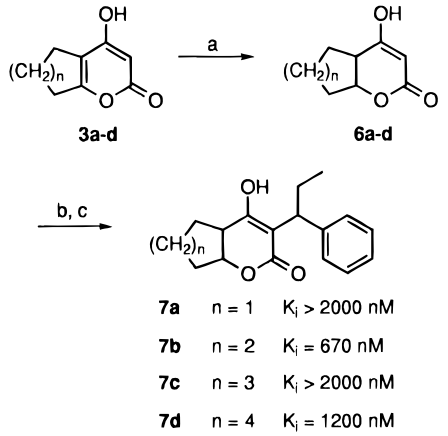
Scheme 1[a]



5a n = 1 K_i = 0.075 nM
5b n = 2 K_i = 0.065 nM
5c n = 3 K_i = 0.018 nM
5d n = 4 K_i = 0.007 nM
5e n = 6 K_i = 0.25 nM

[a] (a) 3-EtCH(OH)$C_6H_4NHCO_2CH_2Ph$, *p*-TsOH; (b) Pd/C, cyclohexene; (c) 4-cyano-2-pyridinesulfonyl chloride, pyridine, CH_2Cl_2.

Scheme 2[a]



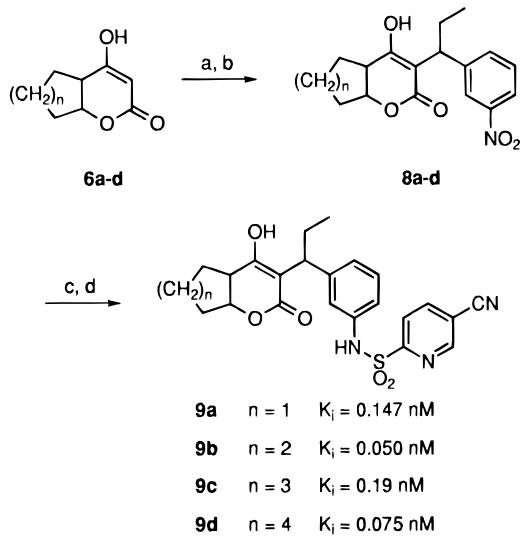
7a n = 1 K_i > 2000 nM
7b n = 2 K_i = 670 nM
7c n = 3 K_i > 2000 nM
7d n = 4 K_i = 1200 nM

[a] (a) PtO_2, H_2, HOAc; (b) PhCHO, $AlCl_3$; (c) Et_3Al, CuBr−Me_2S.

conditions, but we were able to selectively form the desired intermediates **6a**−**d.** ^{1}H NMR experiments indicate the cis isomer was formed exclusively. These intermediates were then substituted at the C-3 position via condensation with benzaldehyde, followed by conjugate addition of the C-3α ethyl substituent.[11] The enzyme binding affinity of analogs with ring sizes of five to eight is shown in Scheme 2.[12] In this case, the best inhibitor was the cyclohexyl derivative **7b**, not the cyclooctyl derivative **7d**. Clearly, saturation of the 5,6-double bond of the central pyrone ring has a significant impact on the ring size SAR.

To further explore the difference in ring size SAR for the saturated and unsaturated series, we turned to molecular modeling techniques. A Monte Carlo search for low-energy conformations on each ring was carried out with the ligand bound and unbound. The energy difference between the two forms was used as an approximate binding energy.[13] For the cycloalkylpyranones, binding energy calculations predicted the 8-membered ring analog **2d** would have the best binding affinity, and this was the case experimentally. For the cycloalkyldihydropyrones, binding energy calculations

Scheme 3[a]



9a n = 1 K_i = 0.147 nM
9b n = 2 K_i = 0.050 nM
9c n = 3 K_i = 0.19 nM
9d n = 4 K_i = 0.075 nM

[a] (a) 3-$NO_2C_6H_4CHO$, $AlCl_3$; (b) Et_3Al, CuBr−Me_2S; (c) Pd/C, H_2, EtOH; (d) 4-cyano-2-pyridinesulfonyl chloride; pyridine, CH_2Cl_2.

predicted the 6-membered ring compound **7d** would have the best binding affinity, and this also was in accord with the experimental results. In both series of compounds, visual inspection of the modeled lowest energy bound conformations indicated that the increased activity of the 8-membered unsaturated and the 6-membered saturated analogs was explained by the ability of the cycloalkyl ring to bend into the S1 pocket and achieve maximal hydrophobic contact without also inducing strain in the ring, as illustrated in Figures 1 and 2. The most potent compound in the unsaturated series (**2d**) had a better binding affinity than the most potent compound of the saturated series (**7b**), and again, this is most likely due to the greater degree of hydrophobic interaction between the cyclooctyl ring of **2d** and the S1 pocket of the enzyme relative to the cyclohexyl ring of **7b**, as illustrated in Figure 3.

Since the binding affinity of the first series of cycloalkyldihydropyrones was relatively weak, we were interested in preparing the *m*-sulfonamide-substituted derivatives of these compounds. Synthesis of these analogs was similar to the unsubstituted analogs above (Scheme 3).[11] In this case, the saturated intermediates **6a**−**d** were condensed with 3-nitrobenzaldehyde. After the C-3α ethyl substituent was in place, the nitro group was reduced and the resulting aniline was coupled with 4-cyano-2-pyridinesulfonyl chloride to give **9a**−**d**. As expected, substitution of the sulfonamide at the meta position greatly enhanced the HIV protease inhibitory activity[9] of these compounds relative to the unsubstituted analogs, presumably via interaction of the arylsulfonamide with the S3′ region of the HIV protease. In addition, the optimal ring size of these compounds was six, just as it was in the unsubstituted series.

It is interesting to note that there appears to be some flexibility in the identity of the P1′ substituent of the cycloalkyldihydropyrones. We prepared the *tert*-butyl derivatives of both the unsubstituted (**10**) and the sulfonamide-substituted (**11**) cyclooctyldihydropyrone in a manner analogous to that described above.[15] In both cases, the enzyme binding affinity of these compounds was only somewhat less than that of the corresponding

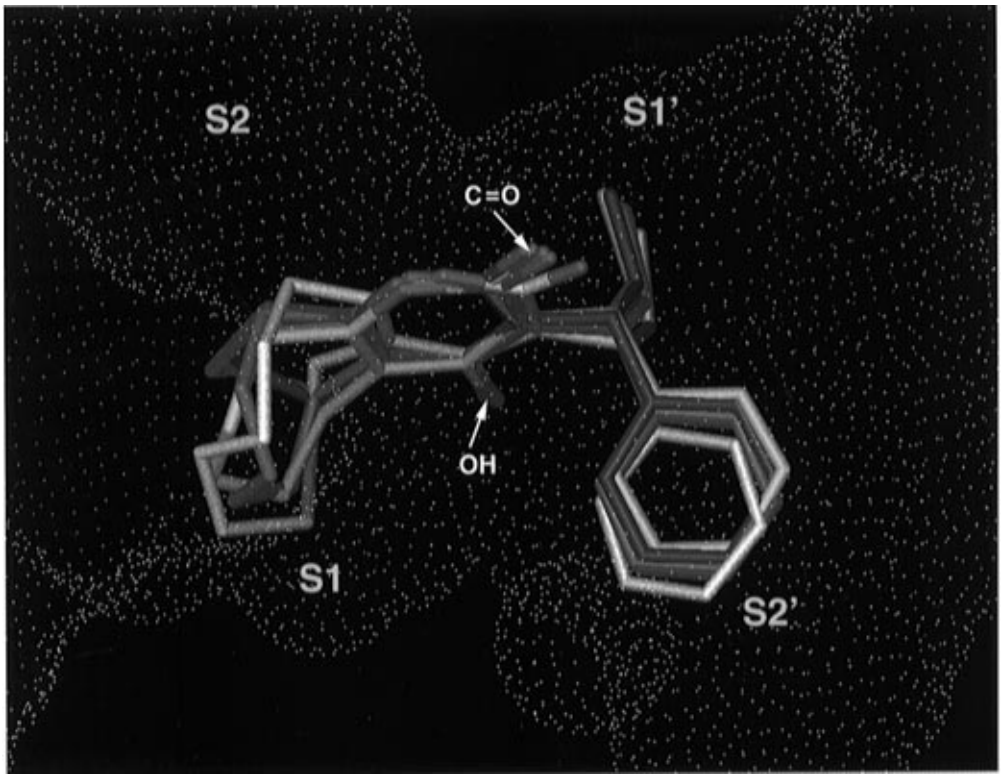
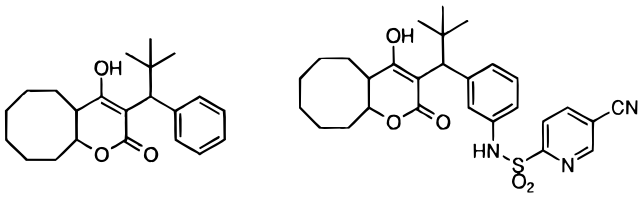
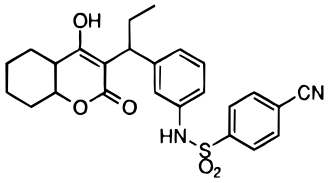


Figure 1. Overlay of the lowest energy bound conformations found using Monte Carlo searching of models of cycloalkylpyranones in the binding site of HIV protease. The 4-hydroxypyrone ring, which forms hydrogen-bonding interactions with the catalytic aspartic acids (C=O) and the isoluecine residues (OH) in the active site of the protease, is in the center of each molecule, with oxygen atoms highlighted in red. The cycloalkyl rings are on the left side. Molecule coloring is according to the size of the cycloalkyl ring: 5 (**2a**) is purple, 6 (**2b**) is green, 7 (**2c**) is yellow, 8 (**2d**) is blue, and 10 (**2e**) is white. The dot surface represents the van der Waals surface of the HIV protease binding site as determined in an X-ray crystal structure of the protease with **2d**. The labels S2 through S2′ denote the location of binding pockets typically occupied by side chains of peptidic inhibitors. According to these models, the cyclooctyl ring (blue) achieves the best hydrophobic interaction with the S1 region.

ethyl analogs **7d** and **9d**, respectively. The identity of the sulfonamide substituent, however, appears to be more important to the enzyme binding affinity of the cycloalkyldihydropyrones. Compound **12**, which has a 4-cyanophenyl substituent, is far less potent than the corresponding 4-cyano-2-pyridyl analog **9b**.[16]



10 K_i = 1500 nM **11** K_i = 0.20 nM



12 K_i = 15 nM

All of the compounds prepared above were screened for antiviral activity in HIV-1 infected H-9 cells.[17] As expected, the unsubstituted analogs **2a**−**e**, **7a**−**d**, and **10** showed little or no antiviral activity at concentrations up to 30 μM. The results for the sulfonamide-substituted compounds, listed in Table 1, were somewhat surprising. Despite excellent enzyme binding affinity, the cycloalkylpyranones **5a**−**e** on the whole showed little antiviral activity. The exception was the 8-membered ring analog **5d** (ED_{50} = 2.6 μM). In contrast, the cycloalkyldihydropyrones generally demonstrated very good antiviral activity.[18] The most potent compound was the 6-membered ring analog **9b** (ED_{50} = 0.95 μM; ED_{90} = 2.5 μM). The observed antiviral activity is not due to cytotoxicity, as the $CCTD_{50}$ values for all of the compounds in Table 1 were above 30 μM.

In summary, saturation of the 5,6-double bond in the pyrone ring of the cycloalkylpyranone template significantly impacts the cycloalkyl ring SAR, altering the optimal ring size from eight to six. Substitution of a sulfonamide at the meta position of the phenyl ring, however, does not change the optimal ring size in either the cycloalkylpyranone or the cycloalkyldihydropyrone series. Both of the sulfonamide-substituted series demonstrated superb binding affinity for the HIV protease, with K_i values for the best compounds in the 10−50 pM range. In addition, the cycloalkyldihydropyrones showed excellent antiviral activity in cell culture.

Experimental Section

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 Analytical Chemistry group of Pharmacia and Upjohn, Inc. Flash chromatography was performed on 230−400 mesh silica gel 60.

4-Cyano-2-pyridinesulfonyl Chloride.[19] 5-Cyano-2-mercaptopyridine[20] (2.5 g, 18.4 mmol) was suspended in 1 N HCl (35 mL) and cooled in an ice bath. Chlorine was bubbled through the suspension for 1 h. The reaction mixture was then

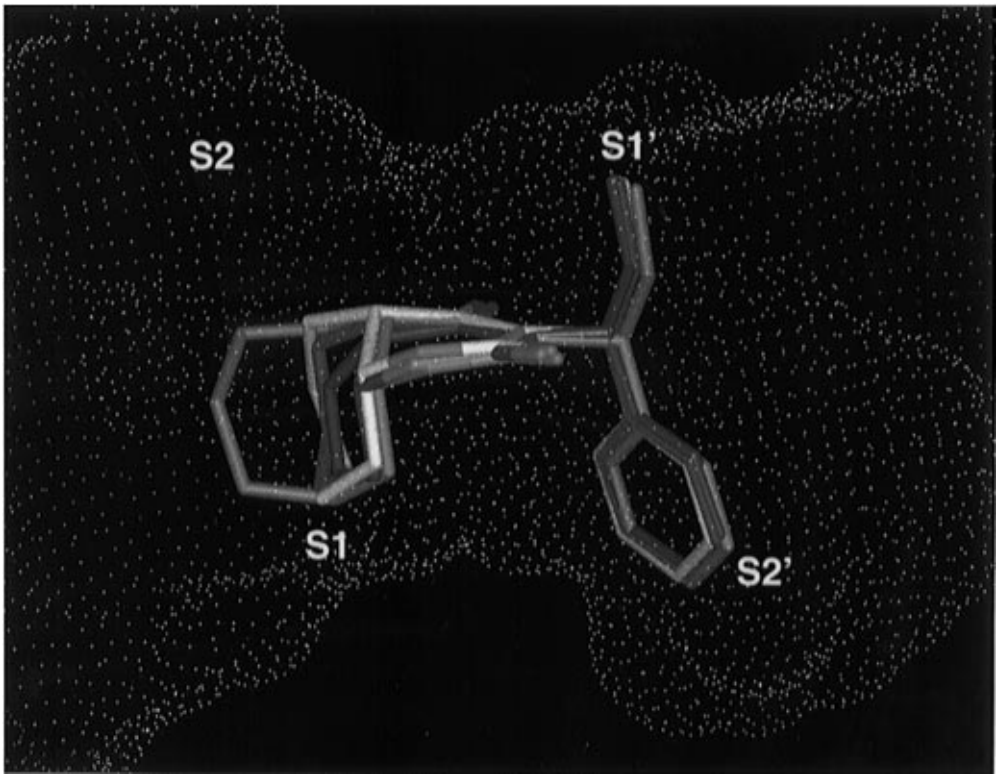


Figure 2. Overlay of the lowest energy bound conformations found using Monte Carlo searching of models of cycloalkyldihydropyrones in the binding site of HIV protease. Labeling is analogous to that used in Figure 1, with the molecules colored according to ring size: 5 (**7a**) is purple, 6 (**7b**) is green, 7 (**7c**) is yellow, and 8 (**7d**) is blue. Relative to Figure 1, the view is rotated slightly around the horizontal axis to allow a better view of the cycloalkyl rings. According to these models, the cyclohexyl ring (green) achieves the best hydrophobic interaction with the S1 region.

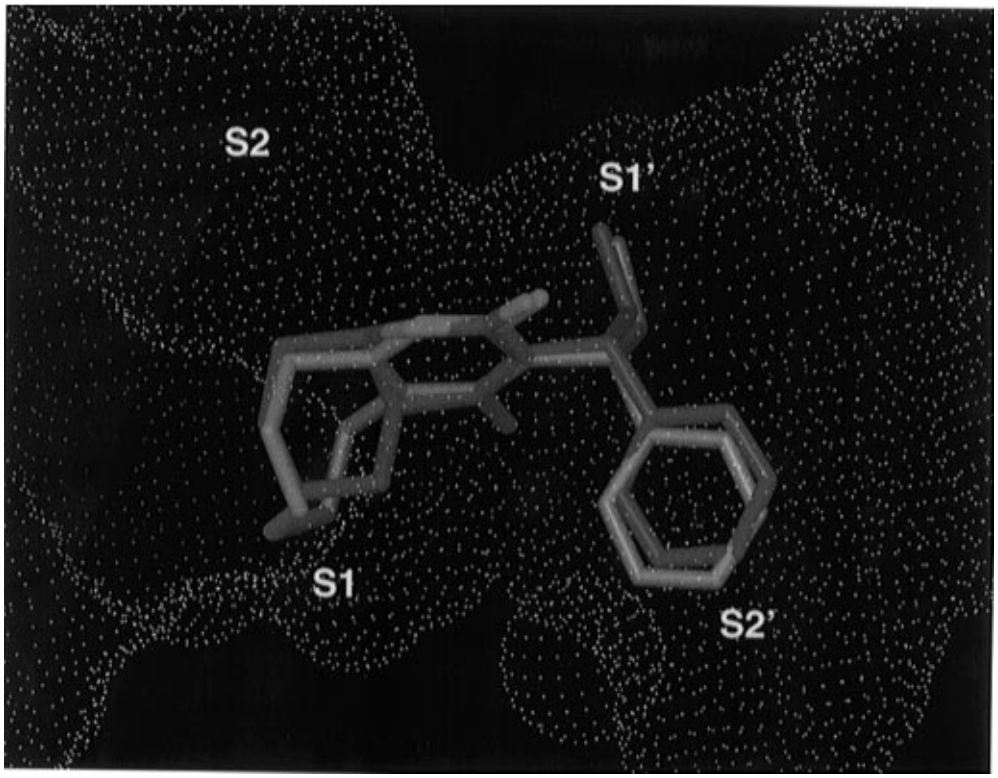


Figure 3. Modeled binding orientation of the 8-membered ring analog **2d** (blue), predicted to have the tightest binding in the cycloalkylpyrone series, and the 6-membered analog **7b** (green), predicted to have the tightest binding in the cycloalkyldihydropyrone series. The apparent greater degree of projection of the blue ring into the S1 pocket relative to the green ring may account for the higher potency of **2b** measured in the enzyme inhibitory activity assay.

filtered, and the filter cake was washed with ice water (100 mL). After drying *in vacuo*, a pale yellow solid (2.27 g) was obtained: mp 51−54 °C. This intermediate displayed limited stability and was therefore used immediately in the next reaction. Alternatively, it could be stored at −78 °C under nitrogen for up to several months with only marginal loss in

Table 1. Biological Activity of *m*-Sulfonamide-Substituted Analogs

compd	K_i (nM)[a]	ED_{50} (μM)[b]
5a	0.075	8.6
5b	0.065	>3[c]
5c	0.018	>3[c]
5d	0.007	2.6
5e	0.25	>3[c]
9a	0.147	2.9
9b	0.050	0.95
9c	0.19	2.0
9d	0.175	2.9
11	0.20	2.5
12	15	>3[c]

[a] HIV protease inhibitory activity. [b] Antiviral activity in HIV-1-infected H-9 cells. [c] <10% inhibition at 3 μM.

chemical integrity. However, decomposition with evolution of gas was noted occasionally upon warming to room temperature.

3-[(Benzyloxycarbonyl)amino]-α-ethylbenzyl Alcohol. A mixture of 3-nitropropiophenone (7.17 g, 40.0 mmol) and 10% Pd/C (0.5 g) in MeOH (150 mL) was placed on a Parr hydrogenation apparatus (initial pressure 50 psi) for 3 h. The reaction mixture was then filtered through Celite and concentrated *in vacuo* to give 6.09 g of crude 1-(3-aminophenyl)-1-propanol as a brown oil, which was used without further purification. This intermediate was immediately dissolved in 150 mL of CH_2Cl_2, and $(i\text{-Pr})_2NEt$ (8.4 mL, 48.0 mmol) was added. Benzyl chloroformate (6.3 mL, 44.0 mmol) was then added slowly, and the resulting solution was stirred at room temperature for 18 h. Cold HCl (0.5 N) was added, and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (100 mL), and the combined organic layers were concentrated to give 13.8 g of a brown oil. Column chromatography on 200 g of silica gel (elution with 40−50% Et_2O/hexane) gave an oil which was crystallized from EtOAc/hexane to yield 10.0 g (88%) of the title compound as a white crystalline solid: mp 88−91 °C; ^{1}H NMR ($CDCl_3$) δ 7.42−7.33 (m, 6 H), 7.29−7.24 (m, 2 H), 7.06−7.03 (m, 1 H), 6.71 (br s, 1 H), 5.20 (s, 2 H), 4.57 (t, J = 6.5 Hz, 1 H), 1.91 (br s, 1 H), 1.84−1.68 (m, 2 H), 0.91 (t, J = 7.4 Hz, 3 H) ppm.

Representative Procedure for the Preparation of 2e and 4a−e. [3-[1-(6,7-Dihydro-4-hydroxy-2-oxo-2(5*H*)-cyclopenta[*b*]pyran-3-yl)propyl]phenyl]carbamic Acid, Phenylmethyl Ester (4a). A mixture of **3a** (0.806 g, 5.3 mmol), 3-[(benzyloxycarbonyl)amino]-α-ethylbenzyl alcohol (1.51 g, 5.3 mmol), and *p*-toluenesulfonic acid (0.32 g, 1.6 mmol) in toluene (50 mL) was heated to reflux for 5 h in a flask equipped with a Dean−Stark trap. The reaction mixture was then diluted with EtOAc, washed with water and saturated $NaHCO_3$, and concentrated to 2.17 g of an oil. Column chromatography on 75 g of silica gel (elution with 20−50% EtOAc/hexane and 5% MeOH/$CHCl_3$) gave 0.317 g (14%) of **4a** as a beige solid: mp 84−88 °C; ^{1}H NMR ($CDCl_3$) δ 7.37−7.23 (m, 8 H), 7.13 (d, J = 7.4 Hz, 1 H), 6.70 (s, 1 H), 5.18 (s, 2 H), 4.24 (t, J = 7.8 Hz, 1 H), 2.77−2.72 (m, 2 H), 2.60−2.55 (m, 2 H), 2.16−1.98 (m, 4 H), 0.98 (t, J = 7.3 Hz, 3 H) ppm; ^{13}C NMR ($CDCl_3$) δ 166.6, 163.6, 163.4, 153.3, 143.7, 138.4, 135.9, 129.8, 128.6, 128.3, 128.2, 122.8, 118.0, 117.3, 111.3, 104.7, 67.0, 41.8, 31.2, 25.5, 24.0, 19.8, 12.5 ppm; MS (EI) m/z 419 (M^+). Anal. ($C_{25}H_{25}NO_5$) C, H, N.

Representative Procedure for the Preparation of 5a−e. 4-Cyano-*N*-[3-[1-(6,7-dihydro-4-hydroxy-2-oxo-2(5*H*)-cyclopenta[*b*]pyran-3-yl)propyl]phenyl]-2-pyridinesulfonamide (5a). A mixture of **4a** (0.23 g, 0.55 mmol) and 10% Pd/C (0.15 g) in cyclohexene (4 mL) and EtOH (4 mL) was warmed to reflux for 1.5 h. The mixture was then filtered through Celite, washed with EtOH, and concentrated to give 0.17 g (100%) of an aniline intermediate as a yellow oil: ^{1}H NMR (CD_3OD) δ 7.23−7.16 (m, 1 H), 6.96 (t, J = 7.7 Hz, 1 H), 6.84−6.78 (m, 1 H), 6.56−6.52 (m, 1 H), 4.10−4.05 (m, 1 H), 2.76−2.62 (m, 4 H), 2.36−2.21 (m, 2 H), 2.12−2.02 (m, 2 H), 0.87 (t, J = 7.3 Hz, 3 H) ppm; MS (EI) m/z 285 (M^+). A mixture of the above aniline intermediate (0.17 g, 0.55 mmol), 4-cyano-2-pyridinesulfonyl chloride (0.11 g, 0.55 mmol), and pyridine (0.089 mL, 1.10 mmol) in CH_2Cl_2 (3 mL) was stirred at room temperature for 1.5 h. Column chromatography of the crude reaction mixture on 60 g of silica gel (elution with 1−2.5% MeOH/$CHCl_3$) gave 0.100 g of a white foam. Crystallization from CH_2Cl_2/hexane gave 0.051 g (21%) of **5a** as white crystals: mp 128−131 °C; ^{1}H NMR ($CDCl_3$) δ 8.98−8.92 (m, 1 H), 8.17−7.99 (m, 2 H), 7.22−7.17 (m, 3 H), 7.08−7.02 (m, 1 H), 6.89 (br s, 1 H), 5.75 (s, 1 H), 4.11 (t, J = 7.9 Hz, 1 H), 2.85−2.75 (m, 2 H), 2.67−2.59 (m, 2 H), 2.16−2.01 (m, 4 H), 0.89 (t, J = 7.3 Hz, 3 H) ppm; MS (EI) m/z 451 (M^+). Anal. ($C_{23}H_{21}N_3O_5S$) C, H, N.

Representative Procedure for the Preparation of 6a−d. Tetrahydrocyclopenta[*b*]pyran-2,4(3*H*,4a*H*)-dione (6a). A solution of PtO_2 (2.3 g) and **3a** (9.13 g, 60.0 mmol) in HOAc (150 mL) was hydrogenated at 50 psi for 1.5 h. The reaction mixture was then filtered through Celite and concentrated to a brown solid. Column chromatography on 150 g of silica gel (elution with 1−10% MeOH/$CHCl_3$) gave 2.91 g of a yellow solid. Crystallization from CH_2Cl_2/hexane gave 2.32 g (25%) of **6a** as white crystals: mp 127−132 °C; ^{1}H NMR ($CDCl_3$) δ 5.10−5.06 (m, 1 H), 3.57 (d, J = 17.3 Hz, 1 H), 3.38 (d, J = 17.3 Hz, 1 H), 2.91−2.84 (m, 1 H), 2.21−2.08 (m, 2 H), 1.99−1.78 (m, 4 H) ppm; ^{13}C NMR (CD_3OD) δ 178.0, 171.9, 83.7, 42.8, 34.2, 31.1, 24.2 ppm; MS (EI) m/z 154 (M^+). Anal. ($C_8H_{10}O_3$) C, H.

Representative Procedure for the Preparation of 7a−d, 8a−d, and 10. 5,6,7,7a-Tetrahydro-4-hydroxy-3-(1-phenylpropyl)-4a*H*-cyclopenta[*b*]pyran-2-one (7a). A solution of $AlCl_3$ (0.346 g, 2.59 mmol) in THF (4 mL) was added to a solution of **6a** (0.200 g, 1.30 mmol) and benzaldehyde (0.13 mL, 1.31 mmol) in THF (8 mL). The resulting mixture was stirred at room temperature for 2.5 h, $Na_2CO_3 \cdot 10H_2O$ (0.50 g) was added, and the mixture was stirred an additional 20 min. The mixture was then dried over $MgSO_4$, filtered through Celite, and concentrated to yield 0.263 g of yellow oil. This material was immediately dissolved in a solution of CuBr−Me_2S (0.80 g, 0.389 mmol) in THF (4 mL), and Et_3Al (1.4 mL of 1.0 M in hexane) was added. The reaction mixture was then stirred at room temperature for 18 h, quenched with water, and diluted with Et_2O. The organic layer was separated, filtered through Celite, and concentrated to give 0.145 g of clear oil. Column chromatography (elution with 10−30% EtOAc/hexane) yielded 0.043 g of white solid. Crystallization from CH_2Cl_2/hexane gave 0.026 g (7%) of **7a** as white crystals: mp 94−98 °C; ^{1}H NMR ($CDCl_3$) δ 7.38−7.19 (m, 5 H), 4.12−4.09 (m, 0.4 H), 3.67−3.65 (m, 0.6 H), 3.28−3.20 (m, 2 H), 2.61−2.50 (m, 2 H), 2.14−1.87 (m, 6 H), 0.72−0.67 (m, 3 H) ppm; MS (EI) m/z 272 (M^+); HRMS (EI) calcd for $C_{17}H_{20}O_3$ 272.1412, found 272.1410.

Representative Procedure for the Preparation of 9a−d, 11, and 12. 4-Cyano-*N*-[3-[1-(5,6,7,7a-tetrahydro-4-hydroxy-2-oxo-4a*H*-cyclopenta[*b*]pyran-3-yl)propyl]phenyl]-2-pyridinesulfonamide (9a). A solution of **8a** (0.300 g, 0.945 mmol) and 10% Pd/C (0.15 g) in EtOH (50 mL) was hydrogenated at an initial pressure of 50 psi for 2 h. The reaction mixture was then filtered through Celite and concentrated *in vacuo* to give 0.269 g (99%) of an aniline intermediate as a gray foam: mp 75−79 °C; ^{1}H NMR (CD_3OD) δ 6.94 (t, J = 7.7 Hz, 1 H), 6.83−6.72 (m, 2 H), 6.52−6.50 (m, 1 H), 4.89−4.71 (m, 1 H), 3.95−3.89 (m, 1 H), 2.67−2.56 (m, 1 H), 2.29−2.07 (m, 2 H), 2.07−1.86 (m, 4 H), 1.82−1.59 (m, 2 H), 0.87 (dt, J = 1.5, 7.3 Hz, 3 H) ppm; MS (EI) m/z 287 (M^+). The aniline (0.254 g, 0.884 mmol) was promptly dissolved in CH_2Cl_2 (5 mL), and 4-cyano-2-pyridinesulfonyl chloride (0.179 g, 0.884 mmol) and pyridine (0.143 mL, 1.77 mmol) were added to the solution. The resulting mixture was stirred at room temperature for 18 h and then purified by column chromatography on 60 g of silica gel (elution with 0−5% MeOH/$CHCl_3$) to give 0.233 g of a white foam. Crystallization from CH_2Cl_2 gave 0.167 g (42%) of **9a** as white crystals: mp 124−127 °C; ^{1}H NMR ($CDCl_3$) δ 9.00−8.94 (m, 1 H), 8.12−7.95 (m, 2 H), 7.51 (s, 0.5 H), 7.39 (s, 0.5 H), 7.24−7.01 (m, 4 H), 5.11−5.08 (m, 1 H), 3.84−3.81 (m, 1 H), 3.24−3.21 (m, 1 H), 2.92−2.87 (m, 1 H), 2.15−2.10 (m, 1 H), 2.00−

1.61 (m, 6 H), 0.69−0.63 (m, 2 H) ppm; MS (EI) *m/z* 453 (M^+). Anal. ($C_{23}H_{23}N_3O_5S$) C, H, N.

Supporting Information Available: Yields and spectral data for compounds **2e**, **4b**−**e**, **5b**−**e**, **6b**−**d**, **7b**−**d**, **8a**−**d**, **9b**−**d**, and **10**−**12** (9 pages). Ordering information is given on any current masthead page.

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(9) In vitro enzyme kinetics were performed with a fused recombinant enzyme instead of the dimeric native protease to obviate enzyme dissociation. To obtain these lower K_i values, assays were incubated for 72 h at a lower enzyme concentration (0.2−0.5 nM). Otherwise the assay conditions were as described in ref 2. We note that this is not an initial rate assay, but an end-point assay. In the absence of inhibitor, more than 99.5% of the substrate is converted to product; the percent conversion in the presence of inhibitor depends on inhibitor concentration. In our assay, the end points are measured for up to 96 different inhibitor concentrations, and the K_i value is then calculated using an equation in which the enzyme rate equation is solved as a function of substrate concentration. When many enzyme concentrations are used, this technique allows accurate calculations of K_i values much less than half the enzyme concentration, the usual lower limit for IC_{50} determination. Although we cannot calculate the IC_{50} values using this method, since these inhibitors are competitive (as shown by early kinetics work[2] and by crystal structures of inhibitors complexed with the active site of HIV protease[4−6]), by definition the IC_{50} values are identical to the K_i values.

(10) The dramatic increase in binding affinity of the meta sulfonamide substituted derivatives is presumably due to the ability of the sulfonamide aryl substituent, in this case the 4-cyano-2-pyridyl group, to efficiently interact with the S3′ pocket of the protease. In addition, X-ray crystal structures of similar compounds have shown that the aryl group occupying the S3′ region is able to form π-stacking interactions with the Arg 8 residue of the enzyme. See ref 6.

(11) These synthetic procedures were adapted from routes developed for related templates. Romero, D. L.; Tommasi, R. A.; Janakiraman, M. N.; Strohbach, J. W.; Turner, S. R.; Biles, C.; Morge, R. A.; Johnson, P. D.; Aristoff, P. A.; Tomich, P. K.; Lynn, J. C.; Horng, M.-M.; Chong, K.-T.; Hinshaw, R. R.; Howe, W. J.; Finzel, B. C.; Watenpaugh, K. D.; Thaisrivongs, S. *J. Med. Chem.*, submitted.

(12) In vitro enzyme kinetics for these compounds were performed as described in ref 2.

(13) Energy-based conformational searching was carried out with the Monte Carlo multiple minimum (MCMM) facility of Batchmin v. 4.5,[14] using the Amber* united atom forcefield, the PRCG minimizer, and the surface area solvation approximation, as implemented in BatchMin. A model of each compound was constructed in the HIV-2 protease binding site, starting from the X-ray crystal structure of the complex of **2c**. Each model was then subjected to 1000 steps of torsional variation of the cycloalkyl ring bonds, followed by energy minimization of the ligand within the field of an 8 Å nonmoving shell of protein atoms, to arrive at an energy-ordered list of "bound" conformations for each ligand. The ligand model was then moved away from the protein and the procedure was repeated. The nonmoving shell of protein atoms contributed a solvation component to the overall "unbound" energies, even though it did not contribute an interaction energy with the ligand. The difference between the lowest bound and unbound energies became the approximate binding energy for that compound. The entire procedure was repeated, using the X-ray structure of the complex of **2d** as the source of protein atom positions. The binding energies calculated in the two runs were then averaged.

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JM960296C