# Structure-Based Design of Novel HIV Protease Inhibitors: Sulfonamide-Containing 4-Hydroxycoumarins and 4-Hydroxy-2-pyrones as Potent Non-Peptidic Inhibitors

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The low oral bioavailability and rapid biliary excretion of peptide-derived HIV protease inhibitors have limited their utility as potential therapeutic agents. Our broad screening program to discover non-peptidic HIV protease inhibitors previously identified compound **I** (phenprocoumon, $K_i = 1\ \mu M$) as a lead template. Structure-based design of potent non-peptidic inhibitors, utilizing crystal structures of HIV protease/inhibitor complexes, provided a rational basis for the previously reported carboxamide-containing 4-hydroxycoumarins and 4-hydroxy-2-pyrones. The amino acid containing compound **V** ($K_i = 4$ nM) provided an example of a promising new series of HIV protease inhibitors with significantly improved enzymatic binding affinity. In this report, further structure−activity relationship studies, in which the carboxamide is replaced by a sulfonamide functionality, led to the identification of another series of nonamino acid containing promising inhibitors with significantly enhanced enzyme binding affinity and *in vitro* antiviral activity. The most active diastereomer of the sulfonamide-containing pyrone **XVIII** ($K_i = 0.5$ nM) shows improved antiviral activity ($IC_{50} = 0.6\ \mu M$) and represents an example of a new design direction for the discovery of more potent non-peptidic HIV protease inhibitors as potential therapeutic agents for the treatment of HIV infection.

## Introduction

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 reported thus far 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 biliary 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 and also non-peptidic inhibitors that are more orally bioavailable, and an increasing number of HIV protease inhibitors[8−21] are currently undergoing clinical evaluations.

In a recent communication,[20] we reported the identification of phenprocoumon (3-(α-ethylbenzyl)-4-hydroxycoumarin, compound **I** 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] For our research program, the crystal structure of inhibitor **I**/HIV-1 protease complex formed the basis of iterative cycles of structure-based design of more active analogues. From that investigation, U-96988 (3-(α-ethylbenzyl)-6-(α-ethylphenethyl)-4-hydroxy-2*H*-pyran-2-one, compound **IV** in Figure 1, $K_i =$ 38 nM) was identified as the first clinical candidate in this promising series of non-peptidic HIV protease inhibitors as a potential therapeutic agent for the treatment of HIV infection.[20] The present report describes our continuing structure-based design effort which relied on the information from the crystal structures of HIV protease complexes with selected inhibitors.

Recently, we reported the identification of an appropriately attached carboxamide functionality to the pyrone-based non-peptidic inhibitors which resulted in more active inhibitors.[29] In the 4-hydroxycoumarin series, compound **II** ($K_i = 28$ nM) was reported to be the most active compound, while compound **V** ($K_i = 4$ nM) was the most active compound in the 4-hydroxy-2-pyrone series. Although these two potent inhibitors contain amino acid residues, it was noted that compound **III** ($K_i = 110$ nM) and compound **VI** ($K_i = 44$ nM) in the 4-hydroxycoumarin and 4-hydroxy-2-pyrone series, respectively, did not contain any amino acid residue, while showing reasonable enzyme inhibitory activity, and provided a basis for further analogue exploration. The carboxamide functional group was evaluated, and other functionalities were also explored in the cyclooctylpyranone template.[21,30] Of particular interest was the sulfonamide functionality which resulted in compounds with improved biological activities. In this report, we propose to evaluate a variety of sulfonamide-containing compounds in the 4-hydroxy-

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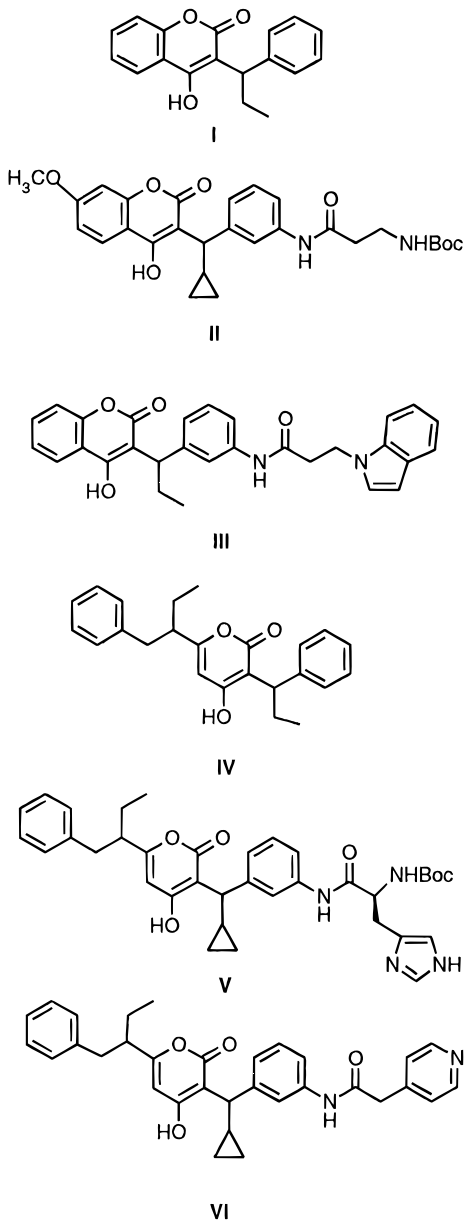


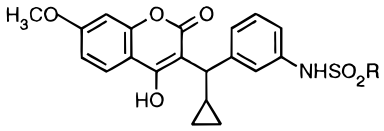

**Figure 1.** Structures of HIV protease inhibitors.

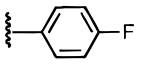
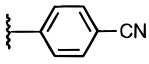
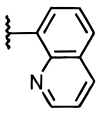
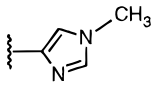
coumarin and the 4-hydroxy-2-pyrone series. We present these findings that result in the important identification of inhibitors with significantly improved enzyme binding affinity and *in vitro* antiviral activity and point to an additional new direction of research in the design of potent non-peptidic HIV protease inhibitors.

## Chemistry

The sulfonamide-containing 4-hydroxycoumarins **VII**−**X** (shown in Table 1) were prepared by the reaction of appropriate sulfonyl chlorides with the same penultimate amine precursor which was used in the preparation of the previously described compound **II**.[29] The preparation of the representative sulfonamide-containing 4-hydroxy-2-pyrone **XVIII** is shown in Scheme 1. The commercially available 4-hydroxy-6-methyl-2-pyrone (**1**) was condensed with the previously described benzylic alcohol **2**[29] under acidic catalysis to give the adduct **3**. Treatment with 3 equiv of lithium diisopropylamide and an electrophile resulted in an alkylation reaction which added a side chain at the C-6α position. Repeating the alkylation reaction with a second electrophile led to the addition of another side chain at the C-6α position. In this particular example, the use of ethyl bromide followed by benzyl bromide resulted in the generation of compound **4**. The carbobenzyloxy protecting group was removed by catalytic hydrogenolysis, and the resulting penultimate amine precursor **5** could be treated with a sulfonyl chloride to give the desired inhibitor. In this particular example, the use of 1-methylimidazole-4-sulfonyl chloride resulted in the isolation of compound **XVIII**. All the sulfonamide-containing 4-hydroxy-2-pyrones were prepared in a manner similar to the preparation of compound **XVIII**.

**Table 1.** Sulfonamide-Containing 4-Hydroxycoumarins



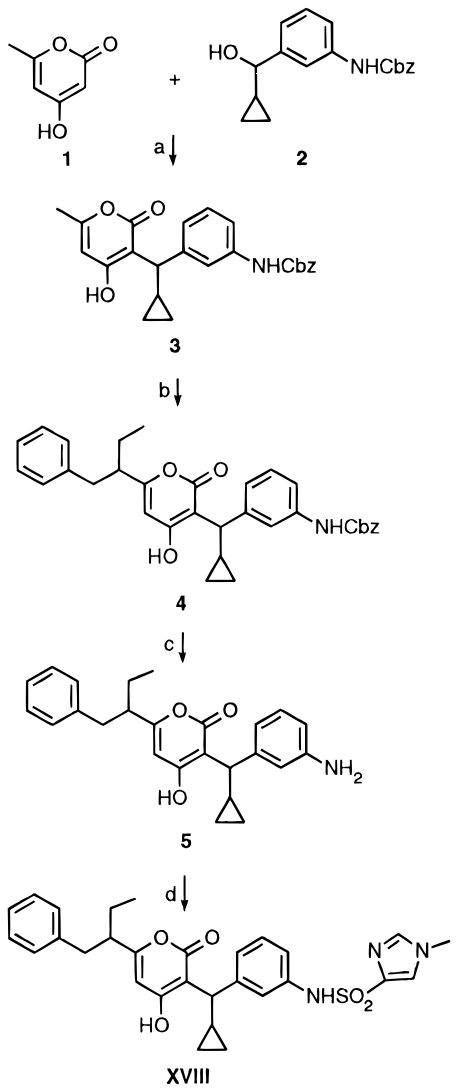
| Compound | R | $K_i$(nM) | $IC_{50}$(μM) |
|---|---|---|---|
| VII |  | 7.7 | >>10 |
| VIII |  | 5.8 | >>10 |
| IX |  | 4.9 | >>10 |
| X |  | 0.7 | >>10 |

## Results and Discussion

Tables 1−7 display the HIV-1 protease inhibitory activity ($K_i$ values) and the cell-culture (MT4/HIV-$1_{IIIB}$) antiviral activity ($IC_{50}$ or $IC_{90}$ values). These compounds were also tested for cytotoxicity in MT4 cells, and their $CCTD_{50}$ values were greater than 30 μM, and any observed antiviral activity of these compounds was not compromised by any significant residual cytotoxicity. In the 4-hydroxycoumarin template (see Table 1), compounds **VII**−**X** contain a group of representative aryl sulfonamides. On the basis of the previous research[21] on sulfonamide-containing compounds, these aryl groups are expected to bind into the $S_3'$ pocket[31] of the enzyme active site. Compound **VIII** incorporated the previously reported *p*-cyanophenyl group[21] and exhibited good enzyme inhibitory activity ($K_i$ = 5.8 nM) when compared to the carboxamide-containing compound **III** ($K_i$ = 110 nM) or even to the amino acid-containing compound **II** ($K_i$ = 28 nM). The *N*-methylimidazole-containing[32] compound **X** showed even higher enzyme inhibitory activity with a $K_i$ value of 0.7 nM. Unfortunately, these compounds with potent enzyme inhibitory activity showed very poor *in vitro* antiviral activity, and a concentration of 10 μM failed to show noticeable inhibition of viral replication in the cell culture assay.

For the 4-hydroxy-2-pyrone series, the template of the carboxamide-containing compound **VI** was used as a starting point in which the carboxamide functionality was replaced with the *N*-methylimidazole-containing

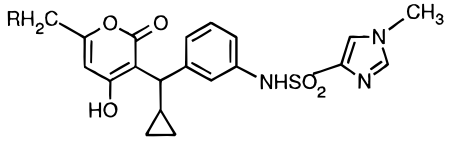
**Scheme 1.** Preparation of Compound **XVIII**[a]




XVIII

[a] (a) $TsOH,CH_2Cl_2$; (b) 3 equiv of $LiNPr^i_2$, $CH_3CH_2Br$; 3 equiv of $LiNPr^i_2$, $C_6H_5CH_2Br$; (c) $(NH_4)(HCO_2)$, Pd/C, $CH_3OH$; (d) 2 equiv of pyridine, 1 equiv of 1-methylimidazole-4-sulfonyl chloride.

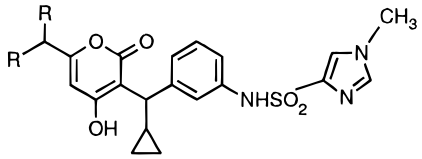
**Table 2.** Sulfonamide-Containing 4-Hydroxy-2-pyrones




| compd | R | $K_i$ (nM) | $IC_{50}$ ($\mu$M) |
|---|---|---|---|
| **XI** | H | 14 | ≫3 |
| **XII** | $CH_3CH_2$ | 1.4 | ≫3 |
| **XIII** | $C_6H_5CH_2$ | 0.97 | ≫3 |
| **XIV** | $H_3C(OCH_2CH_2)_2$ | 1.6 | ≫3 |

sulfonamide functionality. Table 2 shows a series of compounds in which there is no α-branching at the C-6 position. The simplest inhibitor **XI**, with a methyl group at C-6, showed good inhibitory activity ($K_i$ = 14 nM). Extending the substitution at C-6 with an alkyl or aryl group led to compounds with significantly improved inhibitory activity as represented by compounds **XII** and **XIII** ($K_i \approx 1$ nM). The oligoether-containing compound **XIV** was prepared to enhance water solubility and maintained enzyme inhibitory potency. Also unfortunately, these compounds with high enzyme inhibitory activity showed poor *in vitro* antiviral activity. Even though they showed some antiviral activity at 3 $\mu$M, the inhibition of viral replication was less than 50%.

**Table 3.** Sulfonamide-Containing 4-Hydroxy-2-pyrones



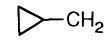
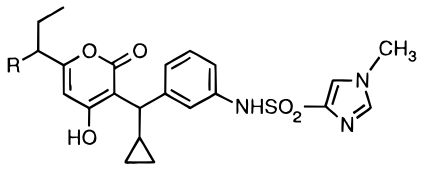

| Compound | R | $K_i$(nM) | $IC_{50}$(μM) |
|---|---|---|---|
| **XV** | $CH_3CH_2$ | 0.54 | >>3 |
| **XVI** |  | 1.0 | 1.2 |
| **XVII** | $H_3C(OCH_2CH_2)_2$ | 4.2 | >>3 |

**Table 4.** Sulfonamide-Containing 4-Hydroxy-2-pyrones



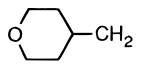
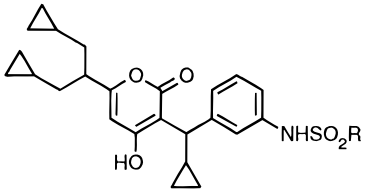

| Compound | R | $K_i$ (nM) | $IC_{50}$ (μM) |
|---|---|---|---|
| **XV** | $CH_3CH_2$ | 0.54 | >>3 |
| **XVIII** | $C_6H_5CH_2$ | 1.3 | 1.3 |
| **XIX** |  | 0.48 | 4.2 |
| **XX** | $H_3C(OCH_2CH_2)_2$ | 0.88 | 5.8 |

Table 3 shows a few representative compounds in the *N*-methylimidazolesulfonamide containing 4-hydroxy-2-pyrones in which the C-6α position contains two identical substituents. Compounds **XV** and **XVI** showed potent inhibitory activity ($K_i$ = 0.5−1 nM). Although this enzyme inhibitory activity did not improve over that for compounds in Table 2, compound **XVI** showed significant *in vitro* antiviral activity ($IC_{50}$ = 1.2 $\mu$M). The dioligoether-containing compound **XVII** showed reduced enzyme inhibitory activity and no *in vitro* antiviral activity.

Table 4 shows representative compounds in the *N*-methylimidazolesulfonamide containing 4-hydroxy-2-pyrones in which there are two substituents at the C-6α position and one of which is an ethyl group. Even though compound **XVIII** showed enzyme inhibitory activity comparable to that of compound **XV**, the benzyl group in compound **XVIII** resulted in a compound with significant *in vitro* antiviral activity ($IC_{50}$ = 1.3 $\mu$M). Attempt to increase hydrophilicity in compounds **XIX** and **XX**, while maintaining the enzyme inhibitory activity, led to some loss of antiviral activity.

Since compound **XVI** showed antiviral activity among the analogues in Table 3, further evaluation of other arenesulfonamides was explored. Some representative compounds are shown in Table 5. However, compounds **XXI**−**XXIV** all showed weaker enzyme inhibitory activ-

**Table 5.** Sulfonamide-Containing 4-Hydroxy-2-pyrones



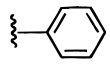
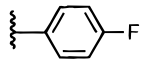
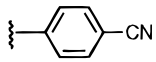
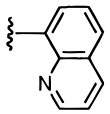
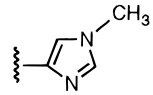
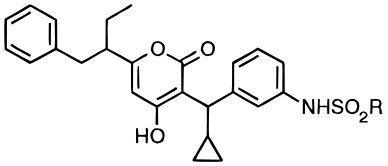
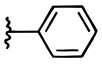
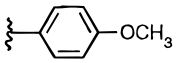
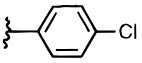
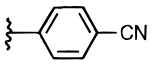
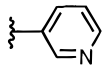
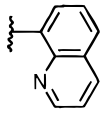
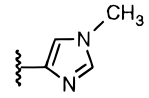

| Compound | R | $K_i$ (nM) | $IC_{50}$ (μM) | $IC_{90}$ (μM) |
|---|---|---|---|---|
| **XXI** |  | 23 | 4.1 | 12.1 |
| **XXII** |  | 16 | 2.2 | 8.0 |
| **XXIII** |  | 5.4 | 2.2 | 8.9 |
| **XXIV** |  | 5.3 | 2.3 | 6.5 |
| **XVI** |  | 1.0 | 1.2 | 4.6 |

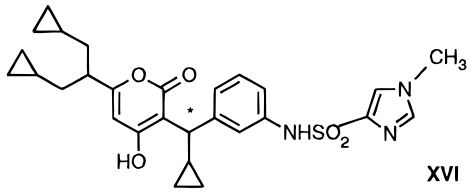
**Table 6.** Sulfonamide-Containing 4-Hydroxy-2-pyrones




| Compound | R | $K_i$ (nM) | $IC_{50}$ (μM) | $IC_{90}$ (μM) |
|---|---|---|---|---|
| **XXV** | $CH_2CH_2CH_3$ | 40 | 3.5 | 11.1 |
| **XXVI** |  | 17 | 1.4 | 7.6 |
| **XXVII** |  | 22 | 1.8 | 7.6 |
| **XXVIII** |  | 15 | 2.0 | 6.2 |
| **XXIX** |  | 5.7 | 1.6 | 9.9 |
| **XXX** |  | 12 | 2.6 | 28.7 |
| **XXXI** |  | 3.5 | 2.3 | 6.0 |
| **XVIII** |  | 1.3 | 1.3 | 4.1 |

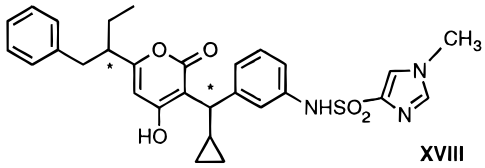
ity and poorer antiviral activity as compared to compound **XVI**. Similar study for the active compound **XVIII** is summarized in Table 6, where a variety of arenesulfonamides were assessed. Compounds **XXV**–**XXXI** showed weaker activity than compound **XVIII**.

**Table 7.** Individual Isomers of Sulfonamide-Containing 4-Hydroxy-2-pyrones




**XVI**

| Compound | $K_i$ (nM) | $IC_{50}$ (μM) | $IC_{90}$ (μM) |
|---|---|---|---|
| racemate | 1.0 | 1.2 | 4.6 |
| enantiomer 1 | 0.74 | 2.4 | 10.4 |
| enantiomer 2 | 0.84 | 1.3 | 6.9 |




**XVIII**

| Compound | $K_i$ (nM) | $IC_{50}$ (μM) | $IC_{90}$ (μM) |
|---|---|---|---|
| mixture of 4 diastereomers | 1.3 | 1.3 | 4.1 |
| diastereomer 1 | 0.52 | 0.6 | 4.5 |
| diastereomer 2 | 0.73 | 1.6 | 5.7 |
| diastereomer 3 | 1.9 | 2.2 | 6.8 |
| diastereomer 4 | 1.4 | 0.9 | 6.0 |

Since compounds **XVI** and **XVIII** emerged as compounds of interest, and, as with all other compounds discussed so far, they are mixture of stereoisomers, we had interest in studying the activity of individual isomers of these two compounds. A chiral HPLC technique was utilized in the separation and preparation of the individuals isomers of compounds **XVI** and **XVIII**. The components were studied for their enzyme inhibitory and *in vitro* antiviral activities, and the results are summarized in Table 7. For compound **XVI**, the two individual enantiomers were shown to be very close in activity and differed by less than a factor of 2. The racemic **XVI** was, therefore, not experimentally distinguishable in activity from the "more active" enantiomer. For compound **XVIII**, two diastereomers (1 and 2) were found to be slightly more active than the remaining two diastereomers (3 and 4). Diastereomers 1 and 2 possess the same absolute stereochemistry at C-6α. Compound **XVIII**, which is a mixture of four diastereomers, showed experimentally similar activity to the "most active" diastereomer (1). From these two sets of compounds, it is suggested that HIV protease does not have high preference for the absolute stereochemistry at the two chiral centers in these series of inhibitors.

## Crystallographic Study

It is important to obtain crystal structures of the enzyme complexes of some representative compounds to evaluate whether there has been any significant change in the binding conformation of a new class of compounds. Crystallization experiments with a few compounds against both HIV-1 and HIV-2 proteases

were attempted, and the crystal of the HIV-2 protease complexed with the diastereomer 1 of compound **XVIII** was suitable for a crystallographic study. Even though HIV-1 and HIV-2 proteases show 50% sequence homology, with homology being much higher in the inhibitor-binding regions, we realize that these can be subtle differences in the binding conformation of an inhibitor in HIV-1 or HIV-2 proteases. The diastereomer 1 of compound **XVIII** inhibited HIV-2 protease with a $K_i$ value of 1.0 nM, which is comparable to its inhibition of HIV-1 protease ($K_i = 0.52$ nM). The crystal structure revealed that this ligand bound to HIV-2 protease in two orientations related by the pseudo 2-fold symmetry axis of the protease dimer; equal occupancies to the atoms of the inhibitor in the two orientations were observed. Figure 2 displays diastereomer 1 of compound **XVIII** in only one of the two possible orientations. Figure 2a shows the ligand occupying the $S_2'$ to the $S_3$ subsites of the enzyme active site. The C-3α cyclopropyl group was positioned in the $S_1$ pocket, while the C-3α phenyl group was directed toward the $S_2$ pocket. The *meta* substituent on the C-3α phenyl group, viz., the *N*-methylimidazolesulfonamide group, was located in the $S_3$ pocket, as was seen previously with the *p*-cyanobenzenesulfonamide group.[21] Free rotation about the C-6/C-6α inhibitor bond allowed for two possible orientations of the groups attached to the C-6α atom. However, the difference electron density map favored the conformation in which the ethyl group was placed in the $S_1'$ pocket while the benzyl group spanned the $S_2'$ pocket. Figure 2b highlights the notable hydrogen-bonding arrangements between the ligand and the enzyme active site. The C-4 hydroxyl group of compound **XVIII** was positioned within hydrogen-bonding distance to the two catalytic aspartate groups (2.8 and 3.0 Å, D25a and D25b, respectively). The lactone carbonyl oxygen atom of the ligand replaced the ubiquitous water molecule found in most complexes of peptide-derived inhibitors;[27,28] this oxygen atom was positioned within hydrogen-bonding distance (3.0 Å) to the amide nitrogen atom of the flap residue Ile 50b. These results compared well with those previously observed for the HIV-1 protease/inhibitor **I** complex.[20] One of the sulfonamide oxygen atoms exhibited bifurcated hydrogen-bonding interactions with Asp30b (3.1 Å for O2[SO2]−HN[D30b] and 2.9 Å for O2[SO2]−OD-[D30b]). The unsubstituted nitrogen of the imidazole ring had a strong hydrogen-bonding interaction (2.7 Å) with the amide nitrogen of aspartic acid D29b, which was not possible for the phenyl moiety of the *p*-cyanobenzenesulfonamide.[21] This additional interaction may partially explain the increase of enzymatic potency of compound **XVIII** ($K_i = 1.3$ nM) compared to inhibitor **XXIX** ($K_i = 5.7$ nM). Even though the observation of this latter interaction in the crystal structure was with an inhibitor in HIV-2 protease, we also observed the same hydrogen-bonding interaction in the crystal structures of HIV-1 protease complexed with other imidazole-containing pyrone-based inhibitors.

## Summary

From a broad screening program to discover non-peptidic HIV protease inhibitors, phenprocoumon (compound **I**, $K_i = 1$ μM) was previously identified as a lead template, and a structure-based design effort led to the identification of the first-generation drug candidate (compound **IV**, $K_i = 38$ nM). Molecular modeling studies based on crytal structures of HIV protease/inhibitor complexes suggested the incorporation of a carboxamide functionality at the *meta* position of the benzyl side chain at C-3. Carboxamide-containing 4-hydroxycoumarin (compound **II**, $K_i = 28$ nM) and 4-hydroxy-2-pyrone (compound **V**, $K_i = 4$ nM) were shown to have improved inhibitory activity. In this study, the replacement of the carboxamide group with a sulfonamide functionality resulted in non amino acid containing compounds with improved activity. The most active diastereomer of compound **XVIII** showed potent HIV protease inhibitory activity with a $K_i$ value of 0.5 nM. A crystal structure of this compound in the enzyme complex was determined and confirmed the expected conformation of the ligand in the enzyme active site. This inhibitor also showed improved *in vitro* antiviral activity ($IC_{50} = 0.6$ μM) over previously reported carboxamide-containing 4-hydroxy-2-pyrones which exhibited $IC_{50}$ values above 3 μM. This finding of novel inhibitors with improved enzyme binding affinity and antiviral activity provides a new direction for the preparation of a new promising series of potent and non-peptidic HIV protease inhibitors for the treatment of HIV infection.

## Experimental Section

**Chemistry.** Mass spectra, infrared spectra, and combustion analyses were obtained by the Structural, Analytical and Medicinal Chemistry Department of Pharmacia & Upjohn. $^1$H NMR spectra were recorded at 300 MHz with a Bruker Model AM-300 spectrometer. Chemical shifts were reported as δ units relative to tetramethylsilane as internal standard. Thin-layer chromatography was conducted with Analtech 0.25-mm glass plates precoated with silica gel GF. Chromatography used E. Merck silica gel 60 (70−230 mesh for column chromatography and 230−400 mesh for flash chromatography). All solvents for chromatography were reagent grade.

Reagents were from commercial sources and used without further purification unless otherwise noted. Diethyl ether was Mallinkrodt anhydrous grade. Dichloromethane was dried over 4A molecular sieves. Diisopropylethylamine and benzene were distilled from calcium hydride. Diethyl cyanophosphonate was distilled before use. Tetrahydrofuran was distilled under argon from sodium metal in the presence of benzophenone.

***N*-[3-[Cyclopropyl(4-hydroxy-7-methoxy-2-oxo-2*H*-1-benzopyran-3-yl)methyl]phenyl]-4-fluorobenzenesulfonamide (VII).** To a cold (0 °C), stirred solution of 51 mg (0.15 mmol) of 3-[(3-aminophenyl)cyclopropylmethyl]-4-hydroxy-7-methoxybenzopyran-2-one[29] and 24 μL (0.30 mmol) of pyridine in 0.5 mL of dichloromethane was added 30 mg (0.15 mmol) of 4-fluorobenzenesulfonyl chloride. The solution was stirred for 18 h, diluted with ethyl acetate, washed with dilute aqueous HCl and brine, and then dried ($MgSO_4$). Following removal of solvent *in vacuo*, the residue was flash chromatographed with elution using 1−4% methanol in dichloromethane to afford 64.6 mg (0.13 mmol, 86%) of *N*-[3-[cyclopropyl(4-hydroxy-7-methoxy-2-oxo-2*H*-1-benzopyran-3-yl)methyl]phenyl]-4-fluorobenzenesulfonamide (**VII**): $^1$H NMR δ 0.18 (m, 1H), 0.43 (m, 1H), 0.56 (m, 1H), 0.70 (m, 1H), 1.46 (m, 1H), 3.85 (s, 3H), 3.85 (d, 1H), 6.72 (d, 1H), 6.83 (dd, $J = 2.4$ Hz, $J = 8.9$ Hz, 1H), 6.9−7.0 (m, 3H), 7.2−7.3 (m, 4H), 7.34 (s, 1H), 7.6−7.7 (m, 3H); MS (EI) *m*/*z* 495 (calcd 495 for $C_{26}H_{22}NO_6FS$); TLC $R_f$ 0.25 (3% methanol in dichloromethane).

Compounds **VIII**−**X** were prepared in a manner similar to that of the preparation of compound **VII**; their physical data are summarized as follows.

***N*-[3-[Cyclopropyl(4-hydroxy-7-methoxy-2-oxo-2*H*-1-benzopyran-3-yl)methyl]phenyl]-4-cyanobenzenesulfonamide (VIII)**: $^1$H NMR δ 0.18 (m, 1H), 0.38 (m, 1H), 0.55 (m,

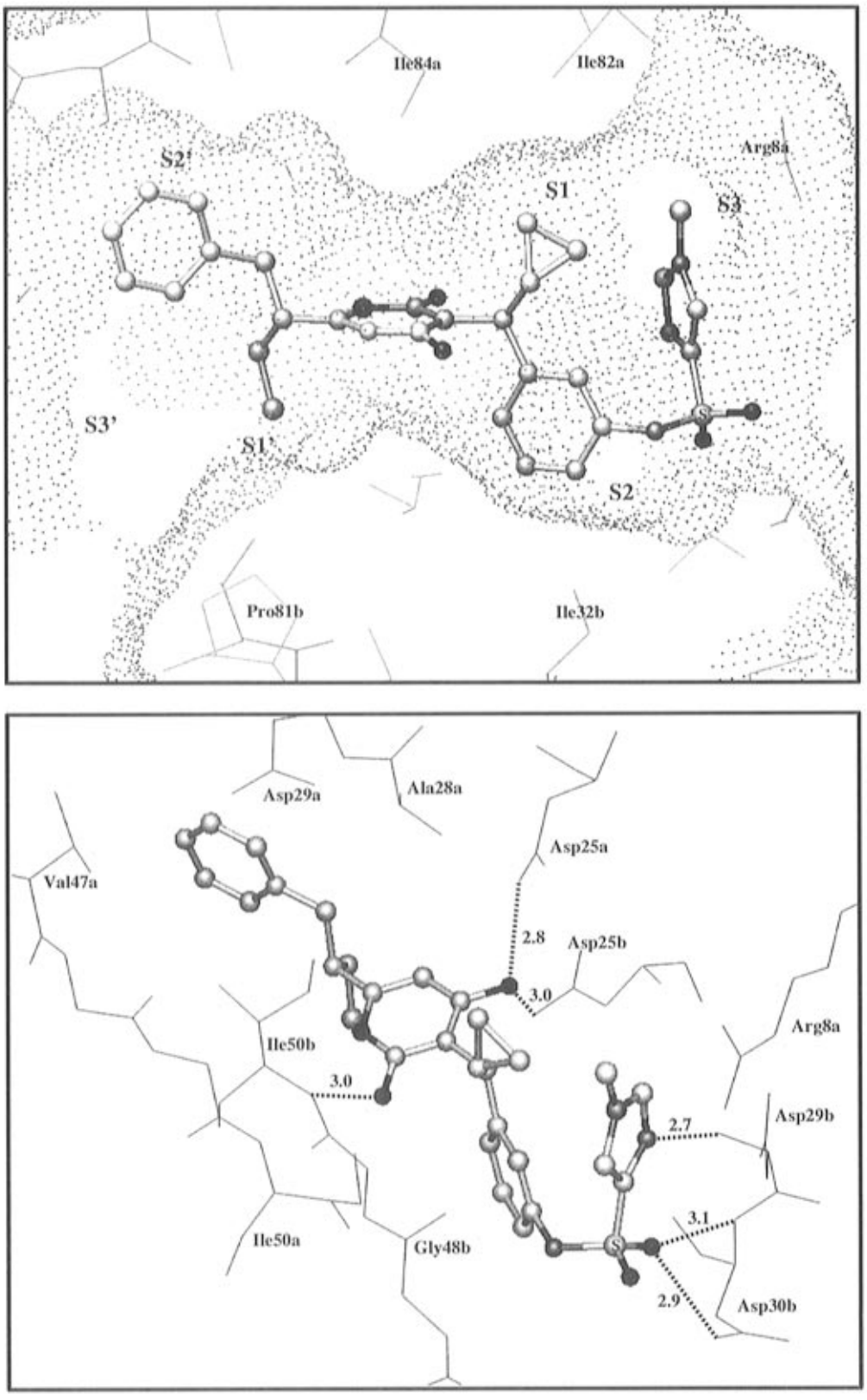



**Figure 2.** (a) Crystal structure of the diastereomer 1 of compound **XVIII**/HIV-2 protease complex. The dot surface is a smoothed representation of the van der Waals surface of the HIV-2 protease binding site, as it appears in complex with compound **XVIII**. The labels S3′ through S3 approximately locate the six subsites commonly filled by peptidic inhibitors. (b) Interatomic distances of potential hydrogen bonding atoms in the HIV-2 protease/**XVIII** complex. See description in the text.

1H), 0.68 (m, 1H), 1.55 (m, 1H), 3.79 (d, $J$ = 8.8 Hz, 1H), 3.85 (s, 3H), 6.71 (d, $J$ = 2.4 Hz, 1H), 6.86 (dd, $J$ = 2.4 Hz, $J$ = 8.9 Hz, 1H), 7.01 (m, 1H), 7.20 (t, $J$ = 7.8 Hz, 1H), 7.3 (m, 1H), 7.53 (d, 2H), 7.69 (m, 2H), 7.78 (d, 2H); HRMS (EI) $m/z$ 502.1192 found (calcd 502.1198 for $C_{27}H_{22}N_2O_6S$); TLC $R_f$ 0.24 (3% methanol in dichloromethane).

***N*-[3-[Cyclopropyl(4-hydroxy-7-methoxy-2-oxo-2*H*-1-benzopyran-3-yl)methyl]phenyl]-8-quinolinesulfonamide (IX)**: $^1$H NMR −0.03 (m, 1H), 0.33 (m, 1H), 0.5 (m, 2H), 1.24 (m, 1H), 3.70 (d, $J$ = 8.8 Hz, 1H), 3.88 (s, 3H), 6.76 (d, $J$ = 2.4 Hz, 1H), 6.85 (dd, $J$ = 2.4 Hz, $J$ = 8.8 Hz, 1H), 6.92 (br, 1H), 7.1−7.3 (m, 4H), 7.44 (m, 1H), 7.54 (d, 8.8 Hz, 1H), 7.82

(dd, $J$ = 1.5 Hz, $J$ = 8.3 Hz, 1H), 8.05 (dd, $J$ = 1.8 Hz, $J$ = 8.4 Hz, 1H), 8.22 (dd, $J$ = 1.5 Hz, $J$ = 7.3 Hz, 1H), 9.04 (dd, $J$ = 1.8 Hz, $J$ = 4.3 Hz, 1H); HRMS (EI) $m/z$ 528.1345 found (calcd 528.1355 for $C_{29}H_{24}N_2O_6S$); TLC $R_f$ 0.36 (3% methanol in dichloromethane).

***N*-[3-[Cyclopropyl(4-hydroxy-7-methoxy-2-oxo-2*H*-benzopyran-3-yl)methyl]phenyl]-1-methyl-1*H*-imidazole-4-sulfonamide (X)**: $^1$H NMR $\delta$ 0.18 (m, 1H), 0.35 (m, 1H), 0.50 (m, 1H), 0.63 (m, 1H), 1.6 (m, 1H), 3.51 (s, 3H), 3.67 (d, $J$ = 9.3 Hz, 1H), 3.84 (s, 3H), 6.74 (d, $J$ = 2.4 Hz, 1H), 6.80 (dd, $J$ = 2.5 Hz, $J$ = 8.9 Hz, 1H), 7.1−7.4 (m, 7H), 7.71 (d, $J$ = 8.8 Hz, 1H); HRMS (EI) $m/z$ 481.1301 found (calcd 481.1307 for $C_{24}H_{23}N_3O_6S$); TLC $R_f$ 0.29 (8% methanol in dichloromethane).

**3-[α-Cyclopropyl-*m*-[(benzyloxycarbonyl)amino]benzyl]-4-hydroxy-6-methylpyran-2-one (3).** A mixture of 493 mg (3.91 mmol) of 4-hydroxy-6-methyl-2-pyrone (**1**), 56 mg (0.29 mmol) of *p*-toluenesulfonic acid monohydrate, and 592 mg (1.99 mmol) of *m*-[(benzyloxycarbonyl)amino]phenylcyclopropylcarbinol[29] (**2**) in 20 mL of dichloromethane was refluxed through an addition funnel containing 3 Å molecular sieves under an argon atmosphere for 6 h. The mixture was cooled to room temperature and concentrated under reduced pressure. The residue was adsorbed onto silica gel and purified by flash column chromatography eluting with 30−50% ethyl acetate in dichloromethane to afford 470 mg (1.16 mmol, 58%) of 3-[α-cyclopropyl-*m*-[(benzyloxycarbonyl)amino]-benzyl]-4-hydroxy-6-methylpyran-2-one (**3**) as a tan foam: TLC $R_f$ 0.52 (ethyl acetate); $^1$H NMR ($CDCl_3$) $\delta$ 0.23 (m, 2H), 0.43 (m, 1H), 0.66 (m, 1H), 1.78 (m, 1H), 2.04 (s, 3H), 3.41 (d, $J$ = 10.2 Hz, 1H), 5.09 (m, 2H), 5.89 (s, 1H), 7.00 (s, 1H), 7.14 (m, 2H), 7.3 (m, 5H), 7.37 (s, 1H), 10.1 (br s, 1H); MS (EI) $m/z$ = 405 ($M^+$). Anal. ($C_{24}H_{23}NO_5$) C, H, N.

**3-[α-Cyclopropyl-*m*-[(benzyloxycarbonyl)amino]benzyl]-4-hydroxy-6-propylpyran-2-one (3a).** To a stirred solution of 0.45 mL (3.21 mmol) of diisopropylamine in 3 mL of anhydrous tetrahydrofuran at −78 °C under an argon atmosphere was added 2.0 mL (3.2 mmol) of a 1.6 M solution of *n*-butyllithium in hexane. The mixture was allowed to warm to 0 °C for 15 min and then recooled to −78 °C. The resulting solution was treated with 405 mg (1.00 mmol) of 3-[α-cyclopropyl-*m*-[(benzyloxycarbonyl)amino]benzyl]-4-hydroxy-6-methylpyran-2-one (**3**) as a solution in 4 mL of anhydrous tetrahydrofuran. After 1 h at −78 °C, the mixture was treated with 85 μL (1.14 mmol) of ethyl bromide. The reaction mixture was allowed to stir for 3 h at −78 °C, and then the reaction was quenched with excess 1 N aqueous hydrochloric acid. The mixture was partioned between ethyl acetate and aqueous pH4 phosphate buffer. The layers were separated, and the aqueous phase was extracted twice with additional portions of ethyl acetate. The combined organic extract was dried over magnesium sulfate and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography eluting with 10% to 20% ethyl acetate in dichloromethane to afford 277 mg (0.64 mmol, 64%) of 3-[α-cyclopropyl-*m*-[(benzyloxycarbonyl)amino]benzyl]-4-hydroxy-6-propylpyran-2-one (**3a**) as a pale yellow foam: $R_f$ 0.33 (10% ethyl acetate in dichloromethane); $^1$H NMR ($CDCl_3$) $\delta$ 0.24 (m, 2H), 0.45 (m, 1H), 0.65 (m, 1H), 0.88 (t, $J$ = 7.4 Hz, 3H), 1.54 (hextet, $J$ = 7.5 Hz, 2H), 1.8 (m, 1H), 2.28 (t, $J$ = 7.6 Hz, 2H), 3.42 (d, $J$ = 10.2 Hz, 1H), 5.1 (m, 2H), 5.95 (s, 1H), 6.89 (s, 1H), 7.15 (m, 2H), 7.3 (m, 5H), 7.35 (s, 1H), 10.0 (br s, 1H); MS (EI) $m/z$ = 433 ($M^+$). Anal. ($C_{26}H_{27}NO_5$) C, H, N.

**3-[α-Cyclopropyl-*m*-[(benzyloxycarbonyl)amino]benzyl]-6-(α-ethylphenethyl)-4-hydroxy-2*H*-pyran-2-one (4).** To a stirred solution of 210 mg (0.48 mmol) of the 3-[α-cyclopropyl-*m*-[(benzyloxycarbonyl)amino]benzyl]-4-hydroxy-6-propylpyran-2-one (**3a**) in 3 mL of anhydrous tetrahydrofuran at −78 °C under an argon atmosphere was added 1.0 mL (1.5 mmol) of a 1.5 M solution of lithium diisopropylamide mono(tetrahydrofuran) in cyclohexane. The mixture was warmed to −20 °C over 45 min and then recooled to −78 °C. The resulting orange suspension was treated with 60 μL (0.50 mmol) of benzyl bromide and was warmed to −20 °C over 1 h to afford a yellow solution. The reaction was then quenched with excess 1 N aqueous hydrochloric acid and partioned between diethyl ether and aqueous pH = 4 phosphate buffer. The layers were separated, and the aqueous phase was extracted twice with additional portions of diethyl ether. The combined organic extract was dried over sodium sulfate and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography eluting with 5−10% ethyl acetate in dichloromethane to afford 153 mg (0.29 mmol, 60%) of 3-[α-cyclopropyl-*m*-[(benzyloxycarbonyl)amino]-benzyl]-6-(α-ethylphenethyl)-4-hydroxy-2*H*-pyran-2-one (**4**) as a white solid: $^1$H NMR ($CDCl_3$) $\delta$ 0.26 (m, 2H), 0.46 (m, 1H), 0.66 (m, 1H), 0.78 (2t, $J$ = 7.4 Hz, 3H), 1.6 (m, 2H), 1.8 (m, 1H), 2.4 (m, 1H), 2.7 (m, 1H), 2.9 (m, 1H), 3.45 (d, $J$ = 9.9 Hz, 1H), 5.12 (s, 2H), 5.85 (s, 1H), 6.79 (br, 1H), 6.9−7.4 (m, 14H), 9.56 (br, 1H); EI HRMS $m/z$ = 523.2350 (calcd for $C_{33}H_{33}NO_5$ 523.2359); TLC $R_f$ 0.27 (5% ethyl acetate in dichloromethane).

**3-(α-Cyclopropyl-*m*-aminobenzyl)-6-(α-ethylphenethyl)-4-hydroxy-2*H*-pyran-2-one (5).** A mixture of 2.43 g (4.64 mmol) of 3-[α-cyclopropyl-*m*-[(benzyloxycarbonyl)amino]benzyl]-6-(α-ethylphenethyl)-4-hydroxy-2*H*-pyran-2-one (**4**), 2.93 g (46 mmol) of ammonium formate, and 250 mg of 5% palladium on carbon in 20 mL of methanol was stirred under argon for 1.5 h. An additional 250 mg of catalyst was added, and stirring was continued for another hour, at which time TLC indicated completion of reaction. The mixture was filtered through Celite and the filtrate concentrated under reduced pressure. Following trituration with dichloromethane to separate product from ammonium salts, the crude amine was flash chromatographed on silica gel 60 (230−400 mesh) using 35−50% ethyl acetate in dichloromethane to give 1.65 g (91%) of 3-(α-cyclopropyl-*m*-aminobenzyl)-6-(α-ethylphenethyl)-4-hydroxy-2*H*-pyran-2-one (**5**): $^1$H NMR ($CDCl_3$) $\delta$ 0.3 (m, 2H), 0.5 (m, 1H), 0.65 (m, 1H), 0.81 (2t, $J$ = 7.3 Hz, 3H), 1.6 (m, 3H), 2.5 (m, 1H), 2.7 (m, 1H), 2.9 (m, 1H), 3.46 (2d, $J$ = 9.8 Hz, 1H), 5.81 (2s, 1H), 6.49 (d, $J$ = 7.9 Hz, 1H), 6.80 (s, 1H), 6.9−7.3 (m, 7H); TLC $R_f$ 0.38 (30% ethyl acetate in dichloromethane); EI MS $m/z$ 389, 361 ($C_{24}H_{27}NO_2$), 360 ($C_{23}H_{22}NO_3$).

***N*-[3-[Cyclopropyl[4-hydroxy-2-oxo-6-[1-(phenylmethyl)propyl]-2*H*-pyran-3-yl]methyl]phenyl]-1-methyl-1*H*-imidazole-4-sulfonamide (XVIII).** To a cold (0 °C), stirred solution of 78 mg (0.20 mmol) of 3-(α-cyclopropyl-*m*-aminobenzyl)-6-(α-ethyl-phenethyl)-4-hydroxy-2*H*-pyran-2-one (**5**) and 32 μL (0.40 mmol) of pyridine in 1 mL dichloromethane was added 38 mg (0.21 mmol) of 1-methylimidazole-4-sulfonyl chloride. The mixture was stirred for several hours and then flash column chromatographed using 5% methanol in dichloromethane to provide 82.8 mg (0.16 mmol, 77%) of *N*-[3-[cyclopropyl[4-hydroxy-2-oxo-6-[1-(phenylmethyl)propyl]-2*H*-pyran-3-yl]methyl]phenyl]-1-methyl-1*H*-imidazole-4-sulfonamide (**XVIII**): $^1$H NMR $\delta$ 0.19 (m, 2H), 0.46 (m, 1H), 0.65 (m, 1H), 0.86 (t, $J$ = 7.4 Hz, 3H), 1.5−1.9 (m, 3H), 2.51 (m, 1H), 2.8 (2dd, 1H), 2.9 (2dd, 1H), 3.28 (d, $J$ = 10.3 Hz, 1H), 3.61 (s, 3H), 5.74 (s, 1H), 6.9−7.4 (m, 12H); HRMS (EI) 533.1988 found (calcd 533.1984 for $C_{29}H_{31}N_3O_5S$); TLC $R_f$ 0.29 (5% methanol in dichloromethane).

Compounds **XI**−**XVII** and **XIX**−**XXXI** were prepared in a manner similar to that of the preparation of compound **XVIII**; their physical data are summarized as follows.

***N*-[3-[Cyclopropyl(4-hydroxy-6-methyl-2oxo-2*H*-pyran-3-yl)methyl]phenyl]-1-methyl-1*H*-imidazole-4-sulfonamide (XI)**: $^1$H NMR ($CDCl_3$−$CD_3OD$) $\delta$ 0.19 (m, 2H), 0.43 (m, 1H), 0.67 (m, 1H), 1.82 (m, 1H), 2.18 (s, 3H), 3.29 (d, $J$ = 10.4 Hz, 1H), 3.67 (s, 3H), 5.92 (s, 1H), 6.93 (m, 1H), 7.10 (t, $J$ = 8.0 Hz, 1H), 7.2 (m, 2H), 7.37 (s, 1H), 7.47 (s, 1H); HRMS (FAB) 416.1296 found (calcd 416.1280 for $C_{20}H_{22}N_3O_5S$); TLC $R_f$ 0.13 (5% methanol in dichloromethane). Anal. ($C_{20}H_{22}N_3O_5S$) C, H, N.

***N*-[3-[Cyclopropyl(4-hydroxy-2-oxo-6-propyl-2*H*-pyran-3-yl)methyl]phenyl]-1-methyl-1*H*-imidazole-4-sulfonamide (XII)**: $^1$H NMR ($CDCl_3$−$CD_3OD$) $\delta$ 0.2 (m, 2H), 0.45 (m, 1H), 0.65 (m, 1H), 0.97 (t, $J$ = 7.4 Hz, 3H), 1.65 (m, 2H), 1.8 (m, 1H), 2.39 (t, $J$ = 7.6 Hz, 2H), 3.28 (d, $J$ = 10.4 Hz, 1H), 3.66 (s, 3H), 5.89 (s, 1H), 6.93 (m, 1H), 7.10 (t, $J$ = 7.8 Hz, 1H), 7.2 (m, 2H), 7.34 (s, 1H), 7.44 (s, 1H); HRMS (FAB) 444.1589 found (calcd 444.1593 for $C_{22}H_{26}N_3O_5S$); TLC $R_f$ 0.17 (5% methanol in dichloromethane).

***N*-[3-[Cyclopropyl[4-hydroxy-2-oxo-6-(2-phenylethyl)-2*H*-pyran-3-yl]methyl]phenyl]-1-methyl-1*H*-imidazole-4-sulfonamide (XIII)**: $^1$H NMR $\delta$ 0.21 (m, 2H), 0.45 (m, 1H), 0.63 (m, 1H), 1.80 (m, 1H), 2.69 (m, 2H), 2.92 (m, 2H), 3.30 (d, $J$ = 10.3 Hz, 1H), 3.59 (s, 3H), 5.89 (s, 1H), 6.9 (m, 1H), 7.08 (t, 1H), 7.1−7.4 (m, 9H); HRMS (EI) *m/z* 505.1672 found (calcd 505.1671 for $C_{27}H_{27}N_3O_5S$); TLC $R_f$ 0.27 (5% methanol in dichloromethane).

***N*-[3-[Cyclopropyl[6-[3-(2-methoxyethoxy)propyl]-4-hydroxy-2-oxo-2*H*-pyran-3-yl]methyl]phenyl]-1-methyl-1*H*-imidazole-4-sulfonamide (XIV)**: $^1$H NMR ($CDCl_3$) $\delta$ 0.10 (m, 1H), 0.26 (m, 1H), 0.44 (m, 1H), 0.60 (m, 1H), 1.75 (m, 1H), 1.86 (quintet, $J$ = 7.5 Hz, 2H), 2.47 (t, $J$ = 7.5 Hz, 2H), 3.32 (d, $J$ = 10.2 Hz, 1H), 3.35 (s, 3H), 3.4−3.6 (m, 4H), 3.59 (s, 3H), 5.96 (s, 1H), 6.98 (m, 1H), 7.11 (t, $J$ = 7.8 Hz, 1H), 7.2 (m, 2H), 7.28 (s, 1H), 7.44 (s, 1H); HRMS (EI) *m/z* 517.1874 found (calcd 517.1883 for $C_{25}H_{31}N_3O_7S$); TLC $R_f$ 0.20 (5% methanol in dichloromethane).

***N*-[3-[Cyclopropyl[6-(1-ethylpropyl)-4-hydroxy-2-oxo-2*H*-pyran-3-yl]methyl]phenyl]-1-methyl-1*H*-imidazole-4-sulfonamide (XV)**: $^1$H NMR ($CDCl_3$) $\delta$ 0.14 (m, 2H), 0.39 (m, 1H), 0.58 (m, 1H), 0.79 (t, $J$ = 7.3 Hz, 6H), 1.55 (m, 4H), 1.75 (m, 1H), 2.1 (m, 1H), 3.22 (d, $J$ = 10.3 Hz, 1H), 3.58 (s, 3H), 5.85 (s, 1H), 6.85 (m, 1H), 7.02 (t, $J$ = 7.8 Hz, 1H), 7.15 (m, 2H), 7.29 (s, 1H), 7.38 (s, 1H); HRMS (FAB) 472.1924 found (calcd 472.1906 for $C_{24}H_{30}N_3O_5S$); TLC $R_f$ 0.76 (20% methanol in dichloromethane).

***N*-[3-[Cyclopropyl[6-[2-cyclopropyl-1-(cyclopropylmethyl)ethyl]-4-hydroxy-2-oxo-2*H*-pyran-3-yl]methyl]phenyl]-1-methyl-1*H*-imidazole-4-sulfonamide (XVI)**: $^1$H NMR ($CDCl_3$−$CD_3OD$) $\delta$ −0.02 (m, 4H), 0.21 (m, 2H), 0.4 (m, 5H), 0.65 (m, 3H), 1.4 (m, 2H), 1.6 (m, 2H), 1.8 (m, 1H), 2.6 (m, 1H), 3.31 (d, $J$ = 10.8 Hz, 1H), 3.67 (s, 3H), 6.02 (s, 1H), 6.89 (m, 1H), 7.07 (t, $J$ = 7.7 Hz, 1H), 7.12 (m, 1H), 7.25 (s, 1H), 7.44 (s, 1H), 7.57 (s, 1H); HRMS (EI) 523.2142 found (calcd 523.2141 for $C_{28}H_{33}N_3O_5S$); TLC $R_f$ 0.32 (5% methanol in dichloromethane).

The two individual enantiomers of compound **XVI** were obtained via chiral HPLC preparative work; their physical data are summarized as follows.

***N*-[3-[[*R* or *S*]-Cyclopropyl[6-[2-cyclopropyl-1-(cyclopropylmethyl)ethyl]-4-hydroxy-2-oxo-2*H*-pyran-3-yl]methyl]phenyl]-1-methyl-1*H*-imidazole-4-sulfonamide** (enantiomer 1 of **XVI**): $^1$H NMR ($CDCl_3$−$CD_3OD$) $\delta$ −0.07 (m, 4H), 0.15 (m, 2H), 0.35 (m, 5H), 0.57 (m, 3H), 1.35 (m, 2H), 1.55 (m, 2H), 1.75 (m, 1H), 2.52 (m, 1H), 3.24 (d, $J$ = 10.2 Hz, 1H), 3.60 (s, 3H), 5.88 (s, 1H), 6.85 (m, 1H), 7.04 (t, $J$ = 7.9 Hz, 1H), 7.15 (m, 2H), 7.27 (s, 1H), 7.37 (s, 1H); HRMS (EI) 523.2149 found (calcd 523.2141 for $C_{28}H_{33}N_3O_5S$); TLC $R_f$ 0.32 (5% methanol in dichloromethane).

***N*-[3-[[*S* or *R*]-Cyclopropyl[6-[2-cyclopropyl-1-(cyclopropylmethyl)ethyl]-4-hydroxy-2-oxo-2*H*-pyran-3-yl]methyl]phenyl]-1-methyl-1*H*-imidazole-4-sulfonamide** (enantiomer 2 of **XVI**): $^1$H NMR ($CDCl_3$−$CD_3OD$) $\delta$ −0.07 (m, 4H), 0.15 (m, 2H), 0.35 (m, 5H), 0.57 (m, 3H), 1.35 (m, 2H), 1.55 (m, 2H), 1.75 (m, 1H), 2.52 (m, 1H), 3.24 (d, $J$ = 10.3 Hz, 1H), 3.60 (s, 3H), 5.88 (s, 1H), 6.85 (m, 1H), 7.04 (t, $J$ = 8.0 Hz, 1H), 7.15 (m, 2H), 7.27 (s, 1H), 7.37 (s, 1H); HRMS (EI) 523.2137 found (calcd 523.2141 for $C_{28}H_{33}N_3O_5S$); TLC $R_f$ 0.32 (5% methanol in dichloromethane).

***N*-[3-[Cyclopropyl[6-[1-[2-(methoxyethoxy)ethyl]-3-(2-methoxyethoxy)propyl]-4-hydroxy-2-oxo-2*H*-pyran-3-yl]methyl]phenyl]-1-methyl-1*H*-imidazole-4-sulfonamide (XVII)**: $^1$H NMR ($CDCl_3$) $\delta$ 0.07 (m, 1H), 0.29 (m, 1H), 0.46 (m, 1H), 0.61 (m, 1H), 1.7−2.0 (m, 5H), 2.72 (m, 1H), 3.32 (s, 3H), 3.33 (s, 3H), 3.3−3.6 (m, 13H), 3.64 (s, 3H), 6.03 (s, 1H), 6.98 (m, 1H), 7.1−7.3 (m, 4H), 7.51 (s, 1H); HRMS (EI) *m/z* 619.2578 found (calcd 619.2563 for $C_{30}H_{41}N_3O_9S$); TLC $R_f$ 0.21 (5% methanol in dichloromethane). Anal. ($C_{30}H_{41}N_3O_9S$) C, H, N.

The four individual diastereomers of compound **XVIII** were obtained via chiral HPLC preparative work; their physical data are summarized as follows.

***N*-[3-[[*R* or *S*]-Cyclopropyl[4hhydroxy-2-oxo-6-[1(*R*)-(phenylmethyl)propyl]-2*H*-pyran-3-yl]methyl]phenyl]-1-methyl-1*H*-imidazole-4-sulfonamide** (diastereomer 1 of **XVIII**): $^1$H NMR $\delta$ 0.17 (m, 2H), 0.43 (m, 1H), 0.62 (m, 1H), 0.83 (t, $J$ = 7.4 Hz, 3H), 1.6 (m, 2H), 1.75 (m, 1H), 2.49 (m, 1H), 2.78 (dd, $J$ = 7.0 Hz, $J$ = 13.5 Hz, 1H), 2.90 (dd, $J$ = 8.0 Hz, $J$ = 13.5 Hz, 1H), 3.28 (d, $J$ = 10.2 Hz, 1H), 3.55 (s, 3H), 5.75 (s, 1H), 6.9−7.4 (m, 11H); HRMS (EI) *m/z* 533.1983 found (calcd 533.1984 for $C_{29}H_{31}N_3O_5S$); TLc $_f$ 0.32 (5% methanol in dichloromethane).

***N*-[3-[[*S* or *R*]-Cyclopropyl[4-hydroxy-2-oxo-6-[1(*R*)-(phenylmethyl)propyl]-2*H*-pyran-3-yl]methyl]phenyl]-1-methyl-1*H*-imidazole-4-sulfonamide** (diastereomer 2 of **XVIII**): $^1$H NMR $\delta$ 0.19 (m, 2H), 0.42 (m, 1H), 0.62 (m, 1H), 0.84 (t, $J$ = 7.4 Hz, 3H), 1.6 (m, 2H), 1.75 (m, 1H), 2.50 (m, 1H), 2.78 (dd, $J$ = 7.0 Hz, $J$ = 13.5 Hz, 1H), 2.91 (dd, $J$ = 7.9 Hz, $J$ = 13.5 Hz, 1H), 3.28 (d, $J$ = 10.2 Hz, 1H), 3.55 (s, 3H), 5.74 (s, 1H), 6.9−7.4 (m, 11H); HRMS (EI) *m/z* 533.1993 found (calcd 533.1984 for $C_{29}H_{31}N_3O_5S$); TLC $R_f$ 0.30 (5% methanol in dichloromethane).

***N*-[3-[[*R* or *S*]-Cyclopropyl[4-hydroxy-2-oxo-6-[1(*S*)-(phenylmethyl)propyl]-2*H*-pyran-3-yl]methyl]phenyl]-1-methyl-1*H*-imidazole-4-sulfonamide** (diastereomer 3 of **XVIII**): $^1$H NMR $\delta$ 0.19 (m, 2H), 0.43 (m, 1H), 0.62 (m, 1H), 0.84 (t, $J$ = 7.4 Hz, 3H), 1.6 (m, 2H), 1.75 (m, 1H), 2.50 (m, 1H), 2.78 (dd, $J$ = 7.0 Hz, $J$ = 13.5 Hz, 1H), 2.91 (dd, $J$ = 7.9 Hz, $J$ = 13.5 Hz, 1H), 3.28 (d, $J$ = 10.2 Hz, 1H), 3.58 (s, 3H), 5.74 (s, 1H), 6.9−7.4 (m, 11H); HRMS (EI) *m/z* 533.1993 found (calcd 533.1984 for $C_{29}H_{31}N_3O_5S$); TLC $R_f$ 0.30 (5% methanol in dichloromethane).

***N*-[3-[[*S* or *R*]-Cyclopropyl[4-hydroxy-2-oxo-6-[1(*S*)-(phenylmethyl)propyl]-2*H*-pyran-3-yl]methyl]phenyl]-1-methyl-1*H*-imidazole-4-sulfonamide** (diastereomer 4 of **XVIII**): $^1$H NMR $\delta$ 0.19 (m, 2H), 0.44 (m, 1H), 0.62 (m, 1H), 0.83 (t, $J$ = 7.4 Hz, 3H), 1.6 (m, 2H), 1.75 (m, 1H), 2.50 (m, 1H), 2.79 (dd, $J$ = 7.0 Hz, $J$ = 13.5 Hz, 1H), 2.91 (dd, $J$ = 7.9 Hz, $J$ = 13.5 Hz, 1H), 3.30 (d, $J$ = 10.2 Hz, 1H), 3.53 (s, 3H), 5.80 (s, 1H), 6.9−7.4 (m, 11H); HRMS (EI) *m/z* 533.1990 found (calcd 533.1984 for $C_{29}H_{31}N_3O_5S$).

***N*-[3-[Cyclopropyl[4-hydroxy-2-oxo-6-[1-[(tetrahydro-2*H*-pyran-4-yl)methyl]propyl]-2*H*-pyran-3-yl]methyl]phenyl]-1-methyl-1*H*-imidazole-4-sulfonamide (XIX)**: $^1$H NMR $\delta$ 0.12 (m, 1H), 0.26 (m, 1H), 0.45 (m, 1H), 0.60 (m, 1H), 0.82 (t, $J$ = 7.4 Hz, 3H), 1.1−1.7 (m, 9H), 1.77 (m, 1H), 2.35 (m, 1H), 3.3 (m, 3H), 3.58 (s, 3H), 3.86 (m, 2H), 6.00 (s, 1H), 6.9−7.5 (m, 5H), 7.59 (m, 1H); HRMS (EI) *m/z* 541.2238 found (calcd 541.2246 for $C_{28}H_{35}N_3O_6S$); TLC $R_f$ 0.24 (5% methanol in dichloromethane).

***N*-[3-[Cyclopropyl[6-[1-ethyl-3-(2-methoxyethoxy)propyl]-4-hydroxy-2-oxo-2*H*-pyran-3-yl]methyl]phenyl]-1-methyl-1*H*-imidazole-4-sulfonamide (XX)**: $^1$H NMR ($CDCl_3$) $\delta$ 0.05 (m, 1H), 0.3 (m, 1H), 0.5 (m, 1H), 0.6 (m, 1H), 0.83 (t, $J$ = 7.3 Hz, 3H), 1.5−1.9 (m, 5H), 2.45 (m, 1H), 3.31 and 3.33 (2 s, 3H total), 3.3−3.6 (m, 7H), 3.62 (s, 3H), 6.01 (s, 1H), 6.98 (m, 1H), 7.1−7.3 (m, 4H), 7.48 (s, 1H); HRMS (EI) *m/z* 545.2186 found (calcd 545.2196 for $C_{27}H_{35}N_3O_7S$); TLC $R_f$ 0.24 (5% methanol in dichloromethane); Anal. ($C_{27}H_{35}N_3O_7S$) C, H, N.

***N*-[3-[Cyclopropyl[6-[2-cyclopropyl-1-(cyclopropylmethyl)ethyl]-4-hydroxy-2-oxo-2*H*-pyran-3-yl]methyl]phenyl]benzenesulfonamide (XXI)**: $^1$H NMR ($CDCl_3$) $\delta$ −0.06 (m, 4H), 0.11 (m, 1H), 0.3−0.7 (m, 9H), 1.3 (m, 2H), 1.4 (m, 2H), 1.65 (m, 1H), 2.55 (m, 1H), 3.43 (d, $J$ = 9.7 Hz, 1H), 6.06 (s, 1H), 6.9 (m, 1H), 7.1−7.5 (m, 7H), 7.72 (m, 2H); HRMS (EI) 519.2078 found (calcd 519.2079 for $C_{30}H_{33}NO_5S$); TLC $R_f$ 0.36 (5% ethyl acetate in dichloromethane). Anal. ($C_{30}H_{33}NO_5S$) C, H, N.

***N*-[3-[Cyclopropyl[6-[2-cyclopropyl-1-(cyclopropylmethyl)ethyl]-4-hydroxy-2-oxo-2*H*-pyran-3-yl]methyl]phenyl]-4-fluorobenzenesulfonamide (XXII)**: $^1$H NMR ($CDCl_3$) $\delta$ −0.08 (m, 4H), 0.1−0.6 (m, 10H), 1.4−1.6 (m, 4H), 1.65 (m, 1H), 2.55 (m, 1H), 3.39 (d, $J$ = 9.9 Hz, 1H), 6.11 (s, 1H), 6.9−7.3 (m, 7H), 7.72 (m, 2H), 9.7 (br s, 1H); HRMS (EI) 537.1977 found (calcd 537.1985 for $C_{30}H_{32}NO_5SF$); TLC $R_f$ 0.39 (10% ethyl acetate in dichloromethane).

***N*-[3-[Cyclopropyl[6-[2-cyclopropyl-1-(cyclopropylmethyl)ethyl]-4-hydroxy-2-oxo-2*H*-pyran-3-yl]methyl]phenyl]-4-cyanobenzenesulfonamide (XXIII)**: $^1$H NMR ($CDCl_3$) $\delta$ −0.03 (m, 4H), 0.1−0.7 (m, 10H), 1.4 (m, 2H), 1.6

(m, 2H), 1.75 (m, 1H), 2.58 (m, 1H), 3.33 (d, $J = 10.2$ Hz, 1H), 5.98 (s, 1H), 6.9 (m, 1H), 7.11 (t, $J = 8.1$ Hz, 1H), 7.2 (m, 2H), 7.67 (m, 2H), 7.82 (m, 2H); HRMS (EI) 544.2035 found (calcd 544.2032 for $C_{31}H_{32}N_2O_5S$); TLC $R_f$ 0.30 (10% ethyl acetate in dichloromethane).

***N*-[3-[Cyclopropyl[6-[2-cyclopropyl-1-(cyclopropylmethyl)ethyl]-4-hydroxy-2-oxo-2*H*-pyran-3-yl]methyl]phenyl]-8-quinolinesulfonamide (XXIV)**: $^1$H NMR ($CDCl_3$) $\delta$ −0.07 (m, 4H), 0.18 (m, 1H), 0.3−0.6 (m, 9H), 1.3−1.6 (m, 5H), 2.53 (m, 1H), 3.32 (d, $J = 9.7$ Hz, 1H), 5.96 (s, 1H), 6.87 (m, 1H), 7.0 (m, 2H), 7.13 (s, 1H), 7.5 (m, 2H), 7.9 (m, 2H), 8.2 (m, 2H), 9.05 (m, 1H); HRMS (EI) 570.2188 found (calcd 570.2188 for $C_{33}H_{34}N_2O_5S$); TLC $R_f$ 0.40 (10% ethyl acetate in dichloromethane). Anal. ($C_{33}H_{34}N_2O_5S$) C, H, N.

***N*-[3-[Cyclopropyl[4-hydroxy-2-oxo-6-[1-(phenylmethyl)propyl]-2*H*-pyran-3-yl]methyl]phenyl]propanesulfonamide (XXV)**: $^1$H NMR $\delta$ 0.24 (m, 2H), 0.47 (m, 1H), 0.68 (m, 1H), 0.80 (t, $J = 7.4$ Hz, 3H), 0.92 (2t, $J = 7.4$ Hz, 3H), 1.55 (m, 2H), 1.75 (m, 3H), 2.5 (m, 1H), 2.75 (m, 1H), 2.85 (m, 1H), 3.0 (m, 2H), 3.44 (2d, 1H), 5.92 (s, 1H), 6.9−7.4 (m, 9H), 9.5 (br, 1H); IR (thin film) 3252, 2967, 1661, 1574, 1415, 1285, 1146 cm$^{-1}$; HRMS (EI) *m/z* 495.2074 found (calcd 495.2079 for $C_{28}H_{33}NO_5S$); TLC $R_f$ 0.18 (10% ethyl acetate in dichloromethane).

***N*-[3-[Cyclopropyl[4-hydroxy-2-oxo-6-[1-(phenylmethyl)propyl]-2*H*-pyran-3-yl]methyl]phenyl]benzenesulfonamide (XXVI)**: $^1$H NMR $\delta$ 0.08 (m, 1H), 0.24 (m, 1H), 0.45 (m, 1H), 0.57 (m, 1H), 0.78 (t, $J = 7.2$ Hz, 3H), 1.5−1.7 (m, 3H), 2.49 (m, 1H), 2.75 (dd, 1H), 2.85 (dd, 1H), 3.41 (2d, 1H), 5.84 (s, 1H), 6.8−7.5 (m, 12H), 7.7 (m, 1H), 8.9 (br, 1H); IR (thin film) 3253, 2964, 1661, 1572, 1414, 1284, 1158 cm$^{-1}$; HRMS (EI) *m/z* 529.1927 found (calcd 529.1923 for $C_{31}H_{31}NO_5S$); TLC $R_f$ 0.23 (10% ethyl acetate in dichloromethane).

***N*-[3-[Cyclopropyl[4-hydroxy-2-oxo-6-[1-(phenylmethyl)propyl]-2*H*-pyran-3-yl]methyl]phenyl]-4-methoxybenzenesulfonamide (XXVII)**: $^1$H NMR $\delta$ 0.11 (m, 1H), 0.24 (m, 1H), 0.44 (m, 1H), 0.58 (m, 1H), 0.78 (t, $J = 7.5$ Hz, 3H), 1.6 (m, 3H), 2.49 (m, 1H), 2.74 (dd, 1H), 2.86 (dd, 1H), 3.40 (2d, 1H), 3.73 (s, 3H), 5.85 (s, 1H), 6.7−7.3 (m, 12H), 7.65 (m, 2H), 9.0 (br, 1H); IR (thin film) 3253, 2965, 1661, 1579, 1415, 1262, 1155 cm$^{-1}$; HRMS (EI) *m/z* 559.2019 found (calcd 559.2028 for $C_{32}H_{33}NO_6S$); TLC $R_f$ 0.20 (10% ethyl acetate in dichloromethane).

***N*-[3-[Cyclopropyl[4-hydroxy-2-oxo-6-[1-(phenylmethyl)propyl]-2*H*-pyran-3-yl]methyl]phenyl]-4-chlorobenzenesulfonamide (XXVIII)**: $^1$H NMR $\delta$ 0.11 (m, 1H), 0.22 (m, 1H), 0.44 (m, 1H), 0.58 (m, 1H), 0.78 (t, 3H), 1.5−1.7 (m, 3H), 2.49 (m, 1H), 2.7 (m, 1H), 2.85 (m, 1H), 3.38 (d, $J = 9.8$ Hz, 1H), 5.91 (s, 1H), 6.8−7.3 (m, 12H), 7.62 (m, 2H), 9.5 (br, 1H); IR (thin film) 3255, 2965, 1660, 1574, 1414, 1281, 1163, 1094 cm$^{-1}$; HRMS (EI) *m/z* 563.1528 found (calcd 563.1533 for $C_{31}H_{30}NO_5ClS$); TLC $R_f$ 0.30 (10% ethyl acetate in dichloromethane).

***N*-[3-[Cyclopropyl[4-hydroxy-2-oxo-6-[1-(phenylmethyl)propyl]-2*H*-pyran-3-yl]methyl]phenyl]-4-cyanobenzenesulfonamide (XXIX)**: $^1$H NMR $\delta$ 0.12 (m, 1H), 0.23 (m, 1H), 0.45 (m, 1H), 0.59 (m, 1H), 0.80 (t, 3H), 1.6 (m, 3H), 2.5 (m, 1H), 2.7−2.9 (m, 2H), 3.38 (d, $J = 9.8$ Hz, 1H), 5.87 (s, 1H), 6.8−7.2 (m, 9H), 7.45 (br, 1H), 7.57 (m, 2H), 7.78 (m, 2H), 9.27 (br, 1H); IR (thin film) 3253, 2965, 1661, 1573, 1415, 1282, 1165 cm$^{-1}$; HRMS (EI) *m/z* 554.1878 found (calcd 554.1875 for $C_{32}H_{30}N_2O_5S$); TLC $R_f$ 0.23 (10% ethyl acetate in dichloromethane).

***N*-[3-[Cyclopropyl[4-hydroxy-2-oxo-6-[1-(phenylmethyl)propyl]-2*H*-pyran-3-yl]methyl]phenyl]-3-pyridinesulfonamide (XXX)**: $^1$H NMR $\delta$ 0.11 (m, 1H), 0.22 (m, 1H), 0.45 (m, 1H), 0.59 (m, 1H), 0.84 (t, $J = 7.4$ Hz, 3H), 1.5−1.8 (m, 3H), 2.51 (m, 1H), 2.81 (dd, $J = 7.0$ Hz, $J = 13.5$ Hz, 1H), 2.92 (2dd, $J = 7.9$ Hz, $J = 13.5$ Hz, 1H), 3.28 (2d, $J = 10.3$ Hz, 1H), 5.69 (s, 1H), 6.9−7.4 (m, 10H), 8.05 (m, 1H), 8.60 (m, 1H), 8.83 (s, 1H); HRMS (EI) *m/z* 530.1879 found (calcd 530.1875 for $C_{30}H_{30}N_2O_5S$); TLC $R_f$ 0.25 (4% methanol in dichloromethane).

***N*-[3-[Cyclopropyl[4-hydroxy-2-oxo-6-[1-(phenylmethyl)propyl]-2*H*-pyran-3-yl]methyl]phenyl]-8-quinolinesulfonamide (XXXI)**: $^1$H NMR $\delta$ −0.13 (m, 1H), 0.13 (m, 1H), 0.34 (m, 2H), 0.77 (t, $J = 7.3$ Hz, 3H), 1.5 (m, 3H), 2.47 (m, 1H), 2.7−2.9 (m, 2H), 3.23 (d, $J = 9.8$ Hz, 1H), 5.79 (s, 1H), 6.8−7.2 (m, 10H), 7.41 (t, 1H), 7.5 (m, 1H), 7.89 (d, $J = 8.2$ Hz, 1H), 8.2 (m, 2H), 9.01 (br d, 1H); IR (thin film) 3258, 2964, 1659, 1567, 1413, 1166, 1146 cm$^{-1}$; HRMS (EI) *m/z* 580.2028 found (calcd 580.2032 for $C_{34}H_{32}N_2O_5S$); TLC $R_f$ 0.48 (20% ethyl acetate in dichloromethane).

**Table 8.** Summary of Selected Diffraction Data Collection and Refinement Statistics of HIV-2 Protease−**XVIIIa** Complex

| | |
|---|---|
| space group | $P2_12_12_1$ |
| unit cell: *a*, Å | 33.671 |
| *b*, Å | 45.671 |
| *c*, Å | 131.300 |
| resolution, Å | 2.2 |
| no. of observations | 35912 |
| unique reflections | 10225 |
| % completeness | 93.6 |
| *R* merge | 0.088 |
| *R* factor [refinement] | 0.181 |
| rms deviations: distance, Å | 0.034 |
| angle, deg | 3.800 |
| fixed dihedrals, deg | 12.607 |
| flexible dihedrals, deg | 16.112 |

**Chiral HPLC conditions.** Compound **XVI** was separated into the two individual enantiomers on a 2.0 × 25 cm (R,R) Whelk-O 1 column, using 30% isopropyl alcohol, 0.1% acetic acid, and 0.2% water in hexane at 6.0 mL/min flow rate, monitoring at 290 nm. On the analytical column (0.46 × 25 cm Chiralpak AD) using 30% isopropyl alcohol, 0.1% acetic acid, and 0.2% water in hexane at 0.5 mL/min flow rate, monitoring at 290 nm, the two enantiomers exhibited retention times of 24.1 and 33.4 min, respectively.

The four diastereomers of compound **4** were obtained on a 1.0 × 25 cm (R,R) Whelk-O 1 column, using 25% isopropyl alcohol and 0.05% acetic acid in hexane at 9.0 mL/min flow rate, monitoring at 310 nm. On the analytical column (0.46 × 25 cm (R,R) Whelk-O 1 column) using 25% isopropyl alcohol and 0.05% acetic acid in hexane at 1.0 mL/min flow rate, monitoring at 290 nm, the four diastereomers exhibited retention times of 19.5, 26.5, 40.7, and 42.5 min, respectively. These four individual diastereomers were separately carried through the reaction sequence to produce the four individual diastereomers of compound **XVIII**.

**Crystallography: Crystallization.** Preparation and purification of the recombinant HIV-2 protease has been described previously.[33] Protein preparation of HIV-2 protease contains a Lys$^{57}$/Leu mutation but has been found to be indistinguishable in activity and specificity from the wild type enzyme. Crystals of HIV-2 protease complexed with diastereomer 1 of compound **XVIII** were obtained by co-crystallization experiments in which 2 $\mu$L of the inhibitor solution (0.1 mg/$\mu$L concentration) in DMSO was added to 130 $\mu$L of the freshly thawed, ice-cold protease solution (ca. 6 mg/mL concentration) and the mixture equilibrated on ice for 60 min. The undissolved inhibitor that precipitates upon mixing was removed by centrifugation. Crystals were grown at room temperature in 10-$\mu$L hanging drops (equal volumes of protein and precipitant) by vapor diffusion against a precipitant of 30−35% (w/v) PEG 4000 at pH 6.6−7.0 (0.1 M imidazole) and at pH 7.2−7.6 (0.1 M HEPES buffer).

**Data Collection.** A single crystal of the inhibitor/protease complex with approximate dimensions of 0.3 × 0.2 × 0.2 mm was used for data collection. Diffraction data 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 180 s per frame. Data sets were processed using XENGEN data reduction software.[34] Table 8 summarizes the data collection along with statistics from the refinement of the models. The effective resolution was taken as the maximum resolution for which the mean $I/\sigma$ was greater than 2.0. Data beyond this maximum were discarded.

**Structure Refinement.** Since the space group of the protease/inhibitor complex was the same as one previously refined in our laboratory,[33] refinement of the protease model

could be initiated without resolving the position of the molecule in the cell. Structural refinement was carried out using CEDAR,[39] with periodic manual rebuilding using the interactive graphics program CHAIN,[37] 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.[38] Inhibitor and solvent molecules were added during later stages of the refinements. Atomic coordinates of this structure are being deposited with the Protein Data Bank.[40]

**HIV-1 and HIV-2 Protease Inhibitory Assays and $K_i$ Value Determination.** HIV-1 protease[41] and HIV-2 protease[33] were purified and refolded from *E. coli* inclusion bodies. The substrate used spans the p17−p24 processing site (R-V-S-Q-N-Y-P-I-V-Q-N-K) and was derivatized with biotin and fluorescein isothiocyanate at the amino and carboxy termini, respectively. The reaction was performed in assay buffer at substrate concentrations below $K_m$ concentrations for 90 min at room temperature in the dark. The assay buffer consists of 0.1 M sodium acetate, 1.0 M NaCl, 0.05% NP40. Enzyme and substrate concentrations were 10 and 50 nM, respectively. Inhibitors were dissolved in dimethyl sulfoxide, and the amount in the assay was 2%. After incubation the reaction was stopped by addition of fluoricon avidin beads at 0.5% (w/v). The residual bound fluorescence was obtained by processing on an IDEXX Screen Machine from which percent inhibition values were calculated. A range of inhibitor concentrations was used. Determination of $K_i$ values with this assay required analysis under conditions in which substrate concentration resided substantially below $K_m$ and the inhibitor concentrations greatly exceeded the $K_i$ value and the enzyme concentration. One can solve the remaining substrate after prolonged incubation times as a quadratic, exponential function in terms of inhibitor concentrations and time to generate calculated values for the kinetic constants $K_m$, $k_{cat}$, and $K_i$ as well as the enzyme concentration.

**MTT Cytotoxicity Assay.**[42] Into appropriate wells of a 96-well microtiter plate was added 125 $\mu$L of 2× drug dissolved in RPMI Complete medium, followed by 125 $\mu$L of suspension of MT4 cells containing $2 \times 10^4$ cells, and mixed gently. Various drug concentrations were tested in triplicate. Positive controls contained cells and RPMI Complete without drug, while negative controls contained medium only. Each microplate was incubated for 4−7 days at 37 °C in 5% $CO_2$. After completion of the incubation period, 100 $\mu$L of supernatant was removed, with minimal disturbance of the cells at the bottom of each well. To each well was added 10 $\mu$L of a freshly prepared MTT solution (5 mg/mL in PBS, Sigma). The plate was incubated for 4 h at 37 °C in 5% $CO_2$. An additional 50 $\mu$L was removed from all wells, and the cells were mixed thoroughly by vigorously pipetting up and down to resuspend the formazan dye precipitate. To each well was added 100 $\mu$L of 0.04 N HCl/2-propanol solution with extensive mixing. The plate was read within 15 min on a dual-wavelength microplate reader using a test wavelength of 570 nm and a reference wavelength of 650 nm. The cytotoxic activity of a test compound was calculated by dividing the mean OD at each drug concentration by the mean OD of the positive controls, subtracting the result from 1, and multiplying by 100. The $CCTD_{50}$ (cell culture toxicity dose$_{50}$) value was determined to be the calculated compound concentration required to inhibit 50% of cell metabolism when OD's between compound-exposed cells and compound-free control cells were compared. The $CCTD_{50}$ value was calculated by linear regression or by straight-line extrapolation.

**Antiviral Activity (MT4/HIV-$1_{IIIB}$ Assay).**[43] MT4 cells were washed and resuspended in an inoculum of HIV-$1_{IIIB}$ at a MOI of approximately 0.005. Adsorption occurred for 2 h at 37 °C in 5% $CO_2$. Unabsorbed virus was removed by low-speed centrifugation (200*g*, 10 min) of the cells followed by removal of the supernatant. The cell pellet was resuspended to a concentration of $8 \times 10^5$ cells/mL in RPMI Complete medium. In appropriate wells of 24-well tissue culture plates (Corning no. 25820), 0.5 mL of infected MT4 cells and 0.5 mL of 2× test compound dissolved in RPMI Complete plus 0.2% DMSO were combined. Each drug concentration was tested in triplicate. Virus control wells received RPMI Complete plus 0.2% DMSO without compound. Final DMSO concentration in each culture was 0.1%. Plates were incubated for 7 days at 37 °C in 5% $CO_2$. At the end of the 7-day incubation period, 100 $\mu$L of supernatant was removed from each test well and live virus inactivated by the addition of lysis buffer (Coulter Diagnostics, Hialeah, FL) containing 5% Triton X-100. The amount of HIV p24 core antigen was quantified with an ELISA procedure by following the manufacturer's direction. The $IC_{50}$ (inhibitory concentration$_{50}$), the amount of drug necessary to reduce the concentration of p24 in drug-containing cultures by 50% when compared to drug-free controls, was calculated by comparing the quantity of p24 produced in drug-containing supernatants with that of DMSO-containing drug-free supernatants. This was accomplished by using the linear portion of the plot of $\log_{10}$ drug concentration versus percent p24 inhibition and utilizing linear regression or straight line extrapolation to calculate the drug concentration necessary to inhibit 50% of non-drug-treated p24 antigen.

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