Nonpeptidic Potent HIV-1 Protease Inhibitors: (4-Hydroxy-6-phenyl-2-oxo-2*H*-pyran-3-yl)thiomethanes That Span P_1–P_2' Subsites in a Unique Mode of Active Site Binding

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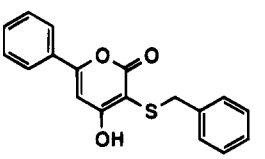
Using molecular modeling and the information derived from the X-ray crystal structure of HIV-1 protease (HIV PR) complexed with the pyran-2-one **1**, a series of (4-hydroxy-6-phenyl-2-oxo-2*H*-pyran-3-yl)thiomethanes was designed and analyzed as novel, nonpeptidic inhibitors of HIV PR. Structure–activity studies led to the discovery of inhibitor **19** having (*RS*)-1-(cyclopentylthio)-3-methylbutyl functionalization at the C-3 position, which exhibited a K_c of 33 nM. A X-ray crystallographic structure of **19** bound to HIV PR showed that structural water-301 (inhibitor–flap-bridging water) was displaced by the inhibitor. Interestingly, the enol moiety of the pyran-2-one formed a hydrogen bond directly with Asp125 and with Asp25 *via* a bridging water molecule, thus illustrating a unique mode of active site binding by an HIV PR inhibitor. The pendant cyclopentyl and isobutyl groups of **19** occupied the S_1' and S_2' binding sites, respectively, whereas the 6-phenyl group occupied a region in between the S_1 and S_3 pockets of HIV PR. Selected compounds were tested for antiviral activity on H9 cells infected with HIV-1_{IIIb}. A correlation between enzymatic activity and antiviral activity was not found in this series. The best antiviral compound in this series, **18**, contained (*RS*)-3-[cyclopentyl(cyclopentylthio)methyl] functionalization at the C-3 position of the pyran-2-one ring and exhibited a CIC_{50} of 14 μM and TC_{50} of 70 μM. These studies demonstrate that potent enzyme inhibition can be achieved by inhibitors that span only three subsites.

Introduction

Human immunodeficiency virus (HIV), a retrovirus, is the putative etiological agent of acquired immunodeficiency syndrome (AIDS).[1] A key enzyme, HIV protease (PR), which is essential for the replication and maturation of HIV, is an aspartic PR.[2,3] Over the last several years many groups have reported[4] potent pseudopeptide and peptidomimetic inhibitors of HIV PR. Although many of these peptide-based inhibitors have achieved very high binding affinity to HIV PR, they generally exhibit metabolic instability and/or poor oral bioavailability.[5,6] Furthermore, such compounds typically possess multiple chiral centers and consequently require stereocontrolled, multistep syntheses.[7] Until most recently, all nonpeptidic inhibitors of HIV PR which have been reported[8] exhibit low potency and/or possess complex structural features. Moreover, in many instances the mode of binding of these nonpeptidic inhibitors to HIV PR has not been intuitive.

HIV mutates at the level of PR to render resistance to antiviral drugs targeting the enzyme.[9] Consequently, there is a marked interest in developing structurally-diverse and/or small molecule leads which interact with a limited number of binding sites critical for HIV PR inhibition. Recently, we have reported[10] a series of 3-



1 IC_{50} : 1.7 μM

Figure 1.

and 6-substituted 4-hydroxypyran-2-ones as potent, achiral, and low molecular weight nonpeptidic HIV PR inhibitors. A X-ray crystallographic structure[10] of 4-hydroxy-3-(benzylsulfanyl)-6-phenylpyran-2-one, **1**, bound to HIV PR revealed that the 6-phenyl group of the inhibitor occupied the S_1 pocket[11] and the benzyl group occupied the S_1' pocket. The 4-hydroxyl group of the pyran-2-one moiety interacted with the catalytic Asp25 and Asp125 residues, and the lactone moiety interacted with the Ile50 and Ile150 residues in the homodimeric enzyme. Herein, we report the structure-guided design, synthesis, and activity of a series of (4-hydroxy-6-phenyl-2-oxo-2*H*-pyran-3-yl)thiomethanes[12] as potent second-generation analogs of **1**.

Design

Molecular modeling studies[13] and analysis of the X-ray structure of **1** bound to HIV PR suggested that branching at the C-3 position of the pyran-2-one ring might achieve simultaneous binding to both the S_1' and S_2' pockets of the enzyme. Transposition of the sulfur atom from the α-position of **1** to the β-position resulted in a ~50-fold decrease in binding affinity (compound **2**,

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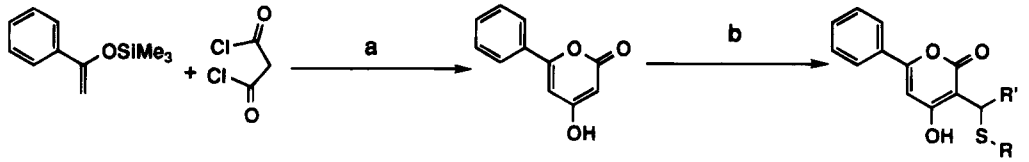
Scheme 1[a]



[a] Reaction conditions: (a) −78 °C for 1 h, followed by room temperature for 16 h; (b) RSH, R'CHO, piperidine, EtOH, reflux for 24 h.

Table 1. Pyran-2-one Inhibitors Having *S*-Aryl Functionalization at C-3 and Their Physical Properties and IC_{50} Values Tested Against HIV PR *in Vitro*

compd no.[a]	R	R'	mp (°C)	molecular formula[b]	elements analyzed	IC_{50} (μM)[c]
2	C_6H_5	H	211 dec	$C_{18}H_{14}O_3S_1 \cdot 0.2H_2O$	C,H	84.3
3	C_6H_5	C_6H_5	>220 dec	$C_{24}H_{18}O_3S_1 \cdot 0.2H_2O$	C,H	0.78
4	C_6H_5	2-naphthyl	98−101	$C_{28}H_{20}O_3S_1 \cdot 0.2H_2O$	C,H	7.82
5	C_6H_5	cyclohexyl	87−90	$C_{24}H_{24}O_3S_1$	C,H	2.44
6	C_6H_5	isobutyl	154−156	$C_{22}H_{22}O_3S_1 \cdot 0.15H_2O$	C,H	0.41
7	C_6H_5	isopentyl	144−145	$C_{23}H_{24}O_3S_1 \cdot 0.2H_2O$	C,H	0.39
8	2-naphthyl	C_6H_5	200 dec	$C_{28}H_{20}O_3S_1$	C,H	2.45
9	$CH_2C_6H_5$	C_6H_5	189−191	$C_{25}H_{20}O_3S_1$	*d*	0.48
10	$CH_2C_6H_5$	isobutyl	153−155	$C_{23}H_{24}O_3S_1$	C,H	0.26
11	$CH_2C_6H_5$	CH_2 cyclopropyl	59−61	$C_{23}H_{22}O_3S_1$	C,H	0.084

[a] All the compounds reported are racemic mixtures (1:1). [b] Water content was not experimentally determined. [c] Values are the average of at least two determinations. [d] Purity was determined by HPLC.

Table 1). However, introduction of a phenyl group at the α-position restored the binding affinity, by 100-fold (**2** vs **3**). This result was consistent with the hypothesis that branching at the C-3 position of the pyran-2-one ring could provide simultaneous binding at both the S_1' and S_2' sites.[14] Selected functionalizations having different steric and hydrophobic properties were introduced at the C-3 position to optimize the molecular recognition and the binding affinity. Since peptides containing hydrophobic amino acids at the peptide cleavage site (P_1-P_1') are good substrates for HIV PR,[15] we synthesized two series (Tables 1 and 2) of inhibitors containing aromatic and aliphatic functionalization on the sulfur atom.

Synthesis

The preparation of (4-hydroxy-6-phenyl-2-oxo-2*H*-pyran-3-yl)thiomethanes (**2**−**20**) was carried out as shown in Scheme 1. As a key intermediate, 4-hydroxy-6-phenylpyran-2-one was prepared by the reaction of 1-phenyl-1-[(trimethylsilyl)oxy]-1-ethylene with malonyl dichloride in diethyl ether at −78 °C for 1 h followed by stirring at room temperature overnight.[16] Analog synthesis then proceeded by the treatment of 4-hydroxy-6-phenylpyran-2-one with an alkyl (or aryl) aldehyde and an alkyl (or aryl) thiol in the presence of piperidine under ethanol reflux conditions, to give the final product as shown in Tables 2 and 3.[12]

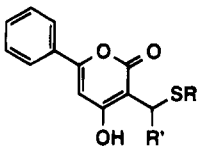
Biochemistry

All compounds were tested for their inhibition of purified recombinant HIV-1 PR using the peptide substrate His-Lys-Ala-Arg-Val-Leu-Phe(*p*-NO_2)-Glu-Ala-Nleu-Ser (Bachem Bioscience) as previously described.[17] The Leu-Phe(*p*-NO_2 bond of the substrate is cleaved by the enzyme, and substrate and products were separated by reversed-phase HPLC, absorbance being measured at 220 nm. Inhibitory activities (IC_{50}) of selected pyran-2-ones against both human cathepsin D and cathepsin E were measured by the hydrolysis of specific chromogenic substrates as described previously.[18] Renin inhibitory activity was determined by measuring generated angiotensin I with an RIA at pH 6.0 using human recombinant renin and a synthetic tetradecapeptide substrate as modified from Patt *et al.*[19] Selected inhibitors were also tested for their *in vitro* anti-HIV activity (Table 3). Specifically, H9 cells were infected with HIV-1_{IIIb} at an MOI of 0.01. Drugs were added to the cultures, and after 7 days postinfection, viral production was measured by an RT assay[20] and cytotoxicity by an XTT (tetrazolium salt XTT, sodium 3'-[1-[(phenylamino)carbonyl]-3,4-tetrazolium]bis(4-methoxy-6-nitrobenzenesulfonic acid) hydrate) assay.[21]

Results and Discussion

Pyran-2-ones Having *S*-Aryl Functionalization at C-3. Initial studies were focused on probing the S_1' and S_2' pockets in terms of aryl group-modified R' and SR groups of the C-3-substituted pyran-2-one template. Specifically, substitution of the phenyl moiety by 2-naphthyl (R' group, see Table 1), as in **4**, reduced activity by 10-fold compared with **3**. Saturation of the α-phenyl ring to cyclohexyl, to give **5**, affected a 3-fold decrease in binding affinity. To the extent that the S_2' pocket is known to accommodate branched aliphatic groups,[22] two inhibitors possessing isobutyl (**6**) or isopentyl (**7**) substituents to replace the α-phenyl group of **3** were synthesized. Each inhibitor was more potent than the parent compound **3**. Increasing the size of the *S*-phenyl ring (SR group) to the *S*-(2-naphthyl) ring, as in **8**, decreased the binding affinity by 3-fold (**8** vs **3**). Other inhibitors containing aromatic groups (*e.g.*, benzyl) to extend further into the enzyme pocket were also synthesized. This homologation afforded the pyrones **9**−**11**.

Table 2. Pyran-2-one Inhibitors Having *S*-Aliphatic Functionalization at C-3 and Their Physical Properties and IC_{50} Values Tested Against HIV PR *in Vitro*



compd no.[a]	R	R'	mp (°C)	molecular formula	elements analyzed	$IC_{50}(\mu M)^b$
12	cyclohexyl	C_6H_5	189–191	$C_{24}H_{24}O_3S_1$	CH	0.48
13	cyclohexyl	isobutyl	210–212	$C_{22}H_{28}O_3S_1$	CH	0.32
14	cyclohexyl	CH_2 cyclopropyl	>225	$C_{22}H_{26}O_3S_1$	CH	0.147
15	cyclohexyl	CH_2 cyclopentyl	157–160	$C_{24}H_{30}O_3S_1$	CH	0.541
16	cyclohexyl	neopentyl	>225	$C_{23}H_{30}O_3S_1$	CH	0.30
17	cyclohexylmethyl	isobutyl	75–80	$C_{23}H_{30}O_3S_1$	CH	0.848
18	cyclopentyl	cyclopentyl	139–142	$C_{22}H_{26}O_3S_1$	CH	0.225
19	cyclopentyl	isobutyl	146–149	$C_{21}H_{26}O_3S_1$	CH	0.058 (K_i = 33 nM)
20	cyclopentyl	CH_2 cyclopropyl	75–80	$C_{21}H_{24}O_3S_1$	CH	0.069

[a] All the compounds reported are racemic mixtures (1:1). [b] Values are the average of at least two determinations.

Inhibitor **9** showed a slight improvement of potency relative to **3**. Replacing the phenyl ring of **9** with an isobutyl group improved binding about 3-fold. Tying the ends of the isobutyl group to create a cyclopropylmethyl substituent (**11**), which provides π-character, gave a potent inhibitor (IC_{50} of 0.084 μM). In summary, relative to the *S*-aryl-substituted pyran-2-one lead **3**, these structure–activity studies showed that a cyclopropylmethyl group was the most effective substitution at the R' position, whereas both phenyl and benzyl groups were well-accommodated at the SR position.

Pyran-2-ones Having *S*-Aliphatic Functionalization at C-3. Pseudopeptide and peptidomimetic inhibitors substituted with cyclohexyl side chain-modified amino acids (e.g., cyclohexylalanine) binding at the P_1 site have been reported to be potent inhibitors of HIV PR.[4,23] To explore such functionalization in our pyran-2-one template, a series of branched pyran-2-ones containing different *S*-aliphatic groups were synthesized, and their *in vitro* IC_{50} values are shown in Table 2. Replacement of the *S*-phenyl moiety with an *S*-cyclohexyl to give **12** again increased the binding affinity relative to **3**. With the *S*-cyclohexyl group conserved, we then more fully examined the R' position with varying functionalization. Briefly, it was found that cyclopropylmethyl (**14**) was superior to isobutyl (**13**), whereas cyclopentyl (**15**) or neopentyl (**16**) functionalization was essentially equipotent to **12**. Further homologation of the *S*-cyclohexyl moiety to *S*-cyclohexylmethyl (analogous to the *S*-phenyl to *S*-benzyl modification *vide supra*) resulted in **17**, which exhibited decreased binding affinity of about 2–3-fold relative to **13**. However, replacement of the *S*-cyclohexyl by *S*-cyclopentyl (**19**) enhanced the affinity by 5–6-fold relative to **13**. Similarly, R' site substitution of the α-isobutyl by cyclopropylmethyl (**20**) provided high binding affinity. In summary, *S*-alicyclic compounds, particularly those possessing a cyclopentyl substituent, resulted in a second series of potent inhibitors. Unlike the *S*-aryl inhibitors, both branched and cyclic aliphatic substituents impart potency at the R' position.

Kinetics and Selectivity. Kinetic analysis[24] of **19** revealed it to be a competitive inhibitor (Figure 2), with a K_i value of 33 nM. Inhibitors **11** and **18**, each representing the most potent analogs of the above series, were tested against other human aspartic proteases. It was found that these inhibitors were more than 100-fold selective for HIV PR relative to renin, cathepsin E, and cathepsin D (Table 3).

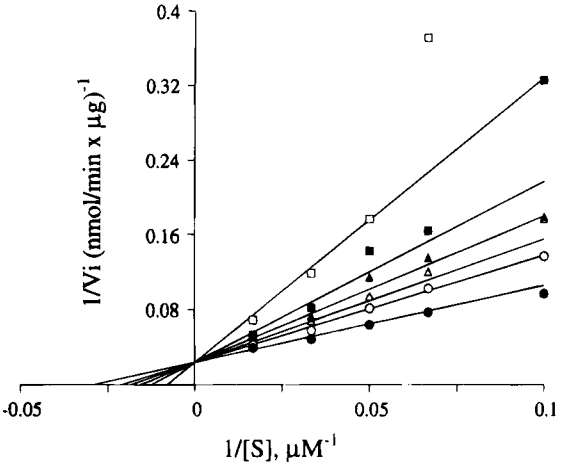


Figure 2. Double-reciprocal plot for inhibition of HIV-1 protease by compound **19**. Inhibitor concentration, nM: (□) 90, (■) 45, (△) 30, (▲) 20, (○) 13, (●) 0.0 The data are shown to fit to a competitive inhibition model by a nonlinear least-squares algorithm, with $K_m = 34 \pm 4.3$ μM (standard error), $k_{cat} = 15 \pm 1.0$ s^{-1}, and $K_i = 33 \pm 3.9$ nM.

Table 3. Selected Pyran-2-ones and Their IC_{50} Values Tested Against Human Aspartic Proteases

	IC_{50} (μM)		
compd no.	recombinant renin	native cathepsin E	native cathepsin D
11	>10	>40	>40
19	>10	>40	>40

X-Ray Crystal Structure. The X-ray crystallographic structure of **19** bound to HIV PR was determined at 2.0 Å resolution.[25] A racemic mixture of **19** was used for crystallization studies, but only the *S* enantiomer of the inhibitor was observed in the complex.[26] Analysis of the X-ray crystallographic structure showed that the *S*-cyclopentyl and isobutyl groups of **19** occupied the S_1' and S_2' binding sites, respectively, as predicted from modeling studies (Figures 3 and 4). The 6-phenyl group of the pyran-2-one straddles the S_1 and S_3 pockets of the enzyme. Structural water-301 (inhibitor–flap-bridging water), which is present in all known HIV PR–peptidomimetic inhibitor complexes,[27,28] was displaced by the lactone carbonyl of the pyran-2-one ring, which formed a direct hydrogen bond with the

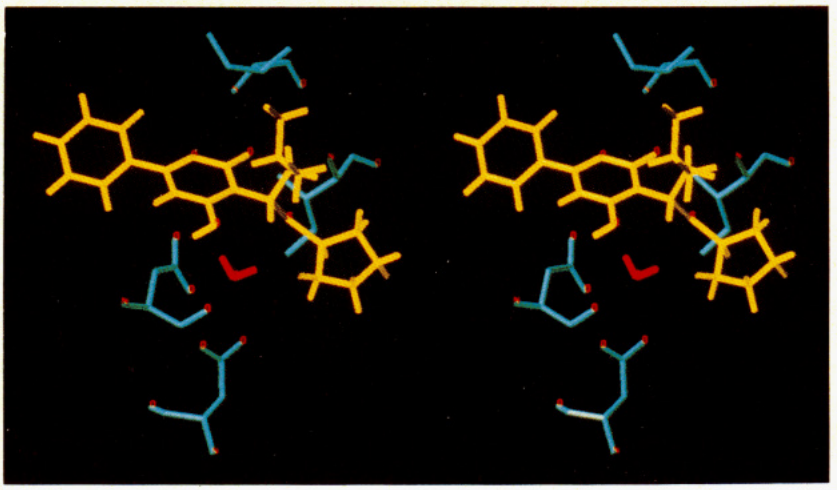


Figure 3. Relaxed stereorepresentation of the X-ray crystal structure (yellow) of compound **19** bound to HIV PR. Asp25/125 and Ile50/150 from the crystal complex are shown in cyan, and bridged water is shown in red.

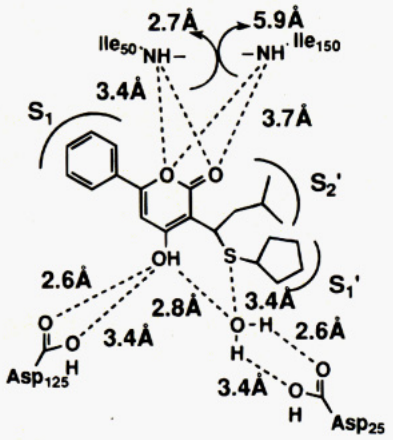


Figure 4. Interatomic distances of compound **19** binding to HIV PR as derived from the X-ray crystal structure. Distances are measured between heavy atoms.

NH of Ile50 only. This is in contrast to the X-ray crystal structure of the structurally-related inhibitor **1**[10] in which the lactone was positioned more symmetrically such that it formed hydrogen bonds with both Ile50 and Ile150. The enol moiety of **19** was observed to form a hydrogen bond with Asp125 and to interact indirectly with Asp25 *via* a bridging water molecule. *This water has not been observed in any of the HIV PR crystal structures reported*[28] *to date*. Moreover, this water molecule is 3.4 Å away from the sulfur atom present in the inhibitor. The resulting intermolecular hydrogen bond network of the **19**–HIV PR complex exemplifies a unique mode of binding (Figure 4).

Molecular Modeling. The unique binding mode observed for **19** in the HIV PR X-ray crystal structure raises questions regarding the modes of binding for the other branched inhibitors (**2**–**18** and **20**). From preliminary modeling, it is not obvious that each branched analog would interact with the enzyme in the manner observed with **19**.[13] Supporting this assessment is a recent paper reporting the X-ray crystal structure of HIV PR bound with a similar branched pyran-2-one inhibitor.[14] In this analog, the α-carbon bound to C-3 of the pyran-2-one is substituted with a phenyl ring and an ethyl group, while a phenethyl moiety is attached at the 6-position. The binding mode for the analog is related to that observed for **1** cocrystallized with the protease, in which no bridging water is found.[10] These results suggest that the binding mode of the inhibitors described above would be dependent on the groups which make up the branched system. Various side chains that differ in size, shape, flexibility, and electronic properties are present in this series of inhibitors. These variables affect the ability of the inhibitor to maneuver within the protease and interact favorably with a particular enzyme site. Therefore, each compound would have to be evaluated individually to study the effects of these variables on binding. Furthermore, the role which sulfur plays in the binding of the bridging water molecule, which is positioned 3.4 Å away, is not evident. Although sulfur is considered a weak hydrogen acceptor,[29] is its interaction with water strong enough to favor the binding mode observed with **19**? Would this dictate the binding of the other inhibitors in the series? Multiple X-ray structures might answer that question. At present, predicting the bound conformations for this series is quite challenging.

Table 4. Selected Pyran-2-ones and Their Antiviral Activities[a]

compd no.	CIC_{50} (μM)	TC_{50} (μM)
9	16	61
10	28	67
11	33	68
14	25	67
18	14	70
19	21	28

[a] Antiviral assay using HIV-1_{IIIb}-infected H9 cells.

Antiviral Activity. Selected pyran-2-ones tested for *in vitro* antiviral activity using H9-infected cells showed moderate activity (Table 4). AZT showed an IC_{50} of 34 ± 29 nM in a side-by-side antiviral assay. The observed binding affinity toward HIV PR does not correlate well with antiviral activity. The toxicity was consistently about 60–70 μM for all compounds except **19** (Table 4). While we remain somewhat puzzled by their poor antiviral activity, the fact that this series of potent (HIV PR bindingwise) pyran-2-ones occupies only three binding sites of the enzyme is nevertheless encouraging. Further structural modification of these lead compounds is predicted to enhance their cellular antiviral efficacy.

Conclusions

The information derived from the molecular modeling and X-ray crystal structure of a single pyran-2-one-based analog, **1**, complexed with HIV PR,[10] led to the

design and analysis of a series of second-generation analogs predicted to interact with three binding sites of the target enzyme. Our results further demonstrate that the pyran-2-one template is a versatile nonpeptide scaffold that can be substituted to mimic related peptide-based inhibitors of HIV PR. These pyran-2-ones described here possess only one chiral center, exhibit low molecular weight, and are accessible in two synthetic steps. Such work extends previous studies[30] in the design and discovery of nonpeptide ligands at receptor sites as derived from β-D-glucose, steroid, and pyrrolidinone templates. Also, a recent disclosure[31] of the C_2 symmetric cyclic ureido diol inhibitors of HIV PR illustrates a significant achievement in the *de novo* design of a series of potent compounds. Compound **19** is selective for HIV PR and shows competitive inhibition. The design of more potent inhibitors of HIV PR using iterative structure-based design strategies[32] is in progress.

Experimental Section

Biological Assays. For determination of IC_{50} values, affinity-purified HIV-1 protease (Bachem Bioscience), 1.1 nM final concentration, was added to a solution (100 mL final volume) containing inhibitor, 40 μM peptide substrate (His-Lys-Ala-Arg-Val-Leu-(*p*-NO_2-Phe)-Glu-Ala-Nleu-Ser (Bachem Bioscience)), and 1.0% dimethyl sulfoxide in assay buffer: 1.0 mM dithiothreitol, 0.1% poly(ethylene glycol) (MW 8000), 80 mM sodium acetate, 160 mM sodium chloride, 1.0 mM EDTA, all at pH 4.7. Poly(ethylene glycol) was used in the assay for glycerol since it has been reported to be a more effective stabilizing agent of the protease.[33] The solution was mixed and incubated for 25 min at 37 °C and the reaction quenched by the addition of trifluoroacetic acid, 2% final concentration. The Leu-Phe(*p*-NO_2) bond of the substrate was cleaved by the enzyme. The cleavaged products and substrate were separated by reversed-phase HPLC. Absorbance was measured at 220 nm, peak areas were determined, and percent conversion to product was calculated using relative peak areas. The data were plotted as percent control (the ratio of percent conversion in the presence and absence of inhibitor) vs inhibitor concentration and fit with the equation $Y = 100/1 + (X/IC_{50})^A$, where IC_{50} is the inhibitor concentration at 50% inhibition and A is the slope of the inhibition curve. A peptidic HIV-1 protease inhibitor reported by Roche, compound **XIV** (prepared according to standard methods), was used as a standard,[34] yielding an average $IC_{50} = 10$ nM in this assay. The peptidic inhibitor is a close analog of Ro 31-8959, with the decahydro-3-isoquinolinecarbonyl replaced by piperidine-2-carbonyl.

Determination of K_i was performed similarly, with the following modifications. The substrate concentrations used were 10, 15, 20, 30, and 60 μM with an HIV-1 PR concentration of 1.1 nM. To measure accurate initial velocities, the incubation time was varied with substrate concentration as follows: 10 μM, 10.5 min; 15 μM, 12 min; 20 μM, 14.5 min; 30 μM, 18 min; 60 μM, 27 min. This resulted in consistent conversion values (products/substrate + produts) of 25−30% without inhibitor. The data were initially fit to a mixed noncompetitive inhibition model, described by the equation $V_i = V_{max}[S]/([S](1 + [I]/K_{i2}) + K_m(1 + [i]/K_{i1}))$, using a nonlinear least-squares algorithm. The value determined for K_{i2} was insignificant, suggesting no inhibition of V_{max} by this inhibitor. Inhibitor **19** is therefore a competitive inhibitor, and the data in Figure 2 is shown to fit the equation for competitive inhibition, $V_i = V_{max}[S]/([S] + K_m(1 + [I]/K_i))$.

The substrates used to assay human cathepsin D and cathepsin E were Lys-Pro-Ile-Glu-Phe(*p*-NO_2)-Arg-Leu and Pro-Pro-Thr-Ile-Phe(*p*-NO_2)-Arg-Leu, respectively, and inhibitions were determined as previously reported earlier.[18] *In vitro* human renin inhibition was measured according to methods modified from Patt *et al.*[19]

Antiviral Assay. Selected pyran-2-ones from Tables 1 and 2 were tested for antiviral activity. A batch of H9 cells were infected with HIV-1_{IIIb} at an MOI of 0.01 and distributed into a 96-well plate. A duplicate plate of uninfected H9 cells was prepared for the cytotoxicity assay. Drugs were serially diluted 1/3.16 in DMSO, transferred to media at an 8× concentration, and then added to the cultures in triplicate. The final DMSO concentration was 0.002 (0.2%). At 7 days of postinfection, viral production was measured by RT assay and cytotoxicity by XTT assay. The RT assay was performed as a modification of Borroto-Esoda and Boone[20] and quantified using a Molecular Dynamics phosphoimager. The XTT assay was performed as a modification of Roehm *et al.*[21]

Chemical Synthesis. Melting points were determined in open capillary tubes on a Hoover melting point apparatus and are uncorrected. Infrared (IR) spectra were determined in KBr pellet on a Nicolet FT IR SX-20 spectrophotometer. Proton magnetic resonance (NMR) spectra were recorded on Bruker AM 250 and 400 spectrometers, and chemical shifts are reported in δ units relative to internal tetramethylsilane. All mass spectra (MS) were obtained on a Finnigan 4500 GCMS or VG analytical 7070E/F spectrometer. Elemental analyses were performed on a Perkin-Elmer Model 240 elemental analyzer, and all compounds gave analytical results of ±0.4% of theoretical values. Flash column or medium pressure chromatography was performed using silica gel, 230−400 mesh, and concentrations were performed *in vacuo* at 10−30 mmHg.

Preparation of 6-Phenyl-4-hydroxy-2*H*-pyran-2-one. The trimethylsilyl enol ether of acetophenone (Aldrich; 50 g, 260 mmol) was dissolved in anhydrous ethyl ether (800 mL) and cooled to −78 to −40 °C. To it was added dropwise malonyl dichloride (18.32 g, 130 mmol). The reaction mixture was warmed up gradually to room temperature and stirred overnight. The solid obtained was filtered and washed with anhydrous ether. Isolated yield: 87%. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 5.42 (s, 1H), 6.78 (s, 1H), 7.53 (m, 3H), 7.83 (m, 2H). CI-MS (*m/z*): 189 (100), 160 (14), 147 (17), 105 (20).

General Procedure for the Preparation of (6-Phenyl-4-hydroxy-2-oxo-2*H*-pyran-3-yl)(arylthio)methanes. To the 6-phenyl-4-hydroxy-2*H*-pyran-2-one in ethanol were added the appropriate aldehyde (1.0−1.1 equiv), thiol (2.0−3 equiv), piperidine, and acetic acid. The reaction mixture was kept at 80 °C for 24 h. The ethyl alcohol was evaporated and acidified with 1 N hydrochloric acid, and the residue was extracted into ethyl acetate. After evaporation of the solvent, the residue was purified by chromatography (silica gel, 230−400 mesh) to yield 35−60% of the desired product.

Examples. 4-Hydroxy-6-phenyl-3-[(phenylthio)methyl]-2*H*-pyran-2-one (2). The title compound was prepared according to the general procedure using 4-hydroxy-6-phenyl-2*H*-pyran-2-one (1.0 g, 5.31 mmol), ethanol (10 mL), paraformaldehyde (0.175 g, 5.8 mmol), thiophenol (1.4 mL, 13.8 mmol), piperidine (0.5 mL), and acetic acid (0.5 mL). IR (KBr): 3059, 2930, 2642, 1647, 1622, 1568, 1496, 1408, 1267, 1170, 1026, 911, 914, 835, 767, 690, 567, 472 cm^{-1}. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 3.98 (s, 2H), 6.73 (s, 1H), 7.17 (m, 1H), 7.30 (m, 2H), 7.37 (m, 2H), 7.54 (m, 3H), 7.77 (m, 2H), 12.05 (bs, 1H). CI-MS (*m/z*): 311 (2, M + H), 229 (4), 210 (93), 1.72 (5), 159 (2), 1.47 (17), 123 (4), 111 (100), 105 (37), 91 (13), 84 (12).

(±)-4-Hydroxy-6-phenyl-3-[phenyl(phenylthio)methyl]-2*H*-pyran-2-one (3). The compound was prepared according to the general procedure using 4-hydroxy-6-phenyl-2*H*-pyran-2-one (1.0 g, 5.31 mmol), ethanol (10 mL), benzaldehyde (0.593 mL, 5.84 mmol), thiophenol (1.40 mL, 13.8 mmol), piperidine (0.5 mL), and acetic acid (0.5 mL). IR (KBr): 3429, 3061, 2924, 1649, 1568, 1494, 1452, 1408, 1336, 1273, 767, 742, 692 cm^{-1}. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 5.80 (s, 1H), 6.70 (s, 1H), 7.23 (m, 8H), 7.54 (m, 5H), 7.74 (m, 2H). FABMS (*m/z*): 387 (9, M + H), 322 (5), 277 (100), 248 (5).

(±)-4-Hydroxy-3-[2-naphthalenyl(phenylthio)methyl]-6-phenyl-2*H*-pyran-2-one (4). The compound was prepared according to the general procedure using 4-hydroxy-6-phenyl-2*H*-pyran-2-one (1.0 g, 5.31 mmol), ethanol (10 mL), 2-naphthaldehyde (0.912 g, 5.84 mmol), thiophenol (1.40 mL, 13.8 mmol), piperidine (0.5 mL), and acetic acid (0.5 mL). IR (KBr): 3435, 3026, 2924, 1639, 1602, 1568, 1494, 1452, 1408, 756, 698 cm^{-1}. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 5.96 (s, 1H),

6.73 (s, 1H), 7.18 (t, 1H), 7.36 (m, 5H), 7.52 (m, 3H), 7.75 (m, 3H), 7.88 (m, 3H), 8.07 (s, 1H). FABMS (*m/z*): 437 (2, M + H), 327 (100), 325 (11), 299 (9), 249 (11).

(±)-3-[Cyclohexyl(phenylthio)methyl]-4-hydroxy-6-phenyl-2*H*-pyran-2-one (5). The compound was prepared as described in the general procedure using 4-hydroxy-6-phenyl-2*H*-pyran-2-one (1.0 g, 5.31 mmol), ethanol (10 mL), cyclohexancarboxaldehyde (0.707 mL, 5.84 mmol), thiophenol (1.40 mL, 13.8 mmol), piperidine (0.5 mL), and acetic acid (0.5 mL). IR (KBr): 3427, 3061, 2926, 2850, 1649, 1566, 1496, 1450, 1406, 1319, 1280, 1230, 767, 690 cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 1.46 (m, 5H), 1.61 (m, 4H), 2.15 (m, 1H), 2.31 (d, 1H), 4.26 (d, 1H), 6.65 (s, 1H), 7.16 (t, 1H), 7.27 (t, 2H), 7.37 (d, 2H), 7.52 (m, 3H), 7.74 (m, 2H), 11.80 (bs, 1H). FABMS (*m/z*): 393 (2, M + H), 267 (3), 253 (2), 241 (3), 231 (1).

(±)-4-Hydroxy-3-[3-methyl-1-(phenylthio)butyl]-6-phenyl-2*H*-pyran-2-one (6). The compound was prepared according to the general procedure using 4-hydroxy-6-phenyl-2*H*-pyran-2-one (1.0 g, 5.31 mmol), ethanol (10 mL), isovaleraldehyde (0.626 mL, 5.84 mmol), thiophenol (1.40 mL, 13.8 mmol), piperidine (0.5 mL), and acetic acid (0.5 mL). IR (KBr): 3070, 2955, 2931, 2868, 1653, 1568, 1496, 1452, 1406, 1367, 1309, 1287, 1230, 1126, 767, 744, 690 cm^{-1}. ^{1}H-NMR (400 MHz, acetone-d_6): δ 0.89 (d, 3H), 0.93 (d, 3H), 1.63 (m, 1H), 1.80 (m, 1H), 2.32 (m, 1H), 4.82 (dd, 1H), 6.70 (s, 1H), 7.24 (m, 3H), 7.48 (m, 5H), 7.82 (m, 2H), 10.49 (bs, 1H). FABMS (*m/z*): 367 (5, M + H), 258 (21), 257 (100), 255 (7), 241 (3).

(±)-4-Hydroxy-3-[4-methyl-1-(phenylthio)pentyl]-6-phenyl-2*H*-pyran-2-one (7). The compound was prepared according to the general procedure using 4-hydroxy-6-phenyl-2*H*-pyran-2-one (1.0 g, 5.31 mmol), ethanol (10 mL), 4-methylpentanal (0.584 mL, 5.84 mmol), thiophenol (1.40 mL, 13.8 mmol), piperidine (0.5 mL), and acetic acid (0.5 mL). IR (KBr): 3070, 2955, 2928, 2868, 1651, 1566, 1496, 1452, 1406, 1367, 1290, 1267, 1230, 1124, 767, 742, 690 cm^{-1}. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 0.80 (d, 3H), 0.81 (d, 3H), 1.07 (m, 1H), 1.18 (m, 1H), 1.49 (m, 1H), 1.89 (m, 1H), 2.19 (m, 1H), 4.51 (dd, 1H), 6.68 (s, 1H), 7.19 (t, 1H), 7.29 (t, 2H), 7.35 (d, 2H), 7.53 (m, 3H), 7.76 (m, 2H). FABMS (*m/z*): 381 (10, M + H), 271 (100).

(±)-4-Hydroxy-3-[(2-naphthalenylthio)phenylmethyl]-6-phenyl-2*H*-pyran-2-one (8). The compound was prepared according to the general procedure using 4-hydroxy-6-phenyl-2*H*-pyran-2-one (1.0 g, 5.31 mmol), ethanol (10 mL), benzaldehyde (0.593 mL, 5.84 mmol), 2-naphthalenethiol (2.21 g, 13.8 mmol), piperidine (0.5 mL), and acetic acid (0.5 mL). IR (KBr): 3435, 3026, 2922, 1647, 1566, 1494, 1452, 1408, 765, 696 cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 5.9 (s, 1H), 6.71 (s, 1H), 7.20 (m, 5H), 7.44 (m, 7H), 7.75 (m, 3H), 7.82 (m, 2H). FABMS (*m/z*): 437 (1, M + H), 429 (2), 387 (2), 323 (14), 291 (5), 277 (100), 249 (8).

(±)-4-Hydroxy-6-phenyl-3-[phenyl[(phenylmethyl)-thio]methyl-2*H*-pyran-2-one (9). The title compound was prepared by the general procedure described above using 4-hydroxy-6-phenyl-2*H*-pyran-2-one (1.0 g, 5.31 mmol), ethanol (10 mL), benzaldehyde (0.593 mL, 5.84 mmol), benzyl mercaptan (1.62 mL, 13.8 mmol), piperidine (0.5 mL), and acetic acid (0.5 mL). IR (KBr): 3086, 3026, 2924, 1651, 1568, 1494, 1452, 1406, 1338, 1271, 1109, 846, 769, 696, 572 cm^{-1}. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 3.70 (dd, 2H), 5.29 (s, 1H), 6.65 (s, 1H), 7.23 (m, 8H), 7.50 (m, 5H), 7.73 (m, 2H), 11.96 (bs, 1H). FABMS (*m/z*): 401 (15, M + H), 323 (3), 293 (3), 277 (100).

(±)-4-Hydroxy-3-[1-[(phenylmethyl)thio]-3-methylbutyl]-6-phenyl-2*H*-pyran-2-one (10). The compound was prepared as described in the general procedure using 4-hydroxy-6-phenyl-2*H*-pyran-2-one (1.5 g, 7.98 mmol), isovaleraldehyde (0.69 g, 7.98 mmol), benzyl mercaptan (1.98 g, 15.96 mmol), piperidine (1.0 mL), and acetic acid (1.0 mL). IR (KBr): 3106, 2928, 2851, 1659, 1568, 1404, 1302, 1125, 997, 847, 766, 689, 569 cm^{-1}. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 0.64 (d, 3H), 0.81 (d, 3H), 1.25 (m, 1H), 1.53 (m, 1H), 2.04 (m, 1H), 3.69 (ABXq, 2H), 4.22 (q, 1H), 6.64 (s, 1H), 7.17 (t, 1H), 7.25 (t, 2H), 7.33 (d, 2H), 7.53 (m, 3H), 7.75 (m, 2H). CI-MS (*m/z*): 325 (2, M + H), 285 (4), 259 (50), 257 (49), 201 (46), 189 (16), 147 (8), 116 (8), 105 (28), 83 (100).

(±)-3-[2-Cyclopropyl-1-[(phenylmethyl)thio]ethyl]-4-hydroxy-6-phenyl-2*H*-pyran-2-one (11). The title compound was prepared according to the general procedure using 4-hydroxy-6-phenyl-2*H*-pyran-2-one (1.5 g, 7.98 mmol), ethanol (10 mL), cyclopropylmethanecarboxaldehyde (0.67 g, 7.98 mmol), benzyl mercaptan (1.98 g, 15.96 mmol), piperidine (0.5 mL), and acetic acid (0.5 mL). IR (KBr): 3061, 2919, 2613, 1649, 1564, 1404, 1267, 766, 691 cm^{-1}. ^{1}H-NMR (400 MHz, DMSO-d_6): δ −0.97 (m, 2H), 0.28 (m, 2H), 0.58 (m, 1H), 1.61 (m, 1H), 2.01 (m, 1H), 3.72 (ABXq, 2H), 4.22 (q, 1H), 6.67 (s, 1H), 7.18 (t, 1H), 7.25 (d, 2H), 7.31 (t, 2H), 7.53 (m, 3H), 7.75 (m, 2H). CI-MS (*m/z*): 255 (19), 201 (5), 147 (2).

3-[(Cyclohexylthio)phenylmethyl]-4-hydroxy-6-phenyl-2*H*-pyran-2-one (12). The compound was prepared according to the general procedure using 4-hydroxy-6-phenyl-2*H*-pyran-2-one (1.0 g, 5.31 mmol), ethanol (10 mL), benzaldehyde (0.593 mL, 5.84 mmol), cyclohexyl mercaptan (1.68 mL, 13.8 mmol), piperidine (0.5 mL), and acetic acid (0.5 mL). IR (KBr): 3104, 3026, 2928, 2852, 1701, 1649, 1564, 1496, 1450, 1404, 1338, 1273, 1111, 765, 744, 699 cm^{-1}. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.21 (m, 5H), 1.52 (m, 1H), 1.66 (m, 2H), 1.91 (m, 2H), 2.58 (m, 1H), 5.37 (s, 1H), 6.70 (s, 1H), 7.17 (t, 1H), 7.27 (t, 2H), 7.53 (m, 5H), 7.74 (m, 2H), 11.96 (bs, 1H), FABMS (*m/z*): 393 (8, M + H), 309 (6), 277 (100), 273 (9).

(±)-3-[1-(Cyclohexylthio)-3-methylbutyl]-4-hydroxy-6-phenyl-2*H*-pyran-2-one (13). The title compound was prepared by the general procedure described above using 4-hydroxy-6-phenyl-2*H*-pyran-2-one (1.5 g, 7.98 mmol), isovaleraldehyde (0.76 g, 8.78 mmol), cyclohexyl mercaptan (2.04 g, 17.56 mmol), piperidine (1.0 mL), acetic acid (1.0 mL), and ethanol (20 mL). IR (KBr): 3086, 3030, 2955, 2868, 1651, 1566, 1497, 1404, 1312, 1275, 1127, 1028, 912, 844, 766, 700 cm^{-1}. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 0.89 (t, 6H), 1.36 (m, 6H), 1.44 (m, 1H), 1.56 (m, 2H), 1.69 (m, 2H), 1.81 (m, 1H), 2.08 (m, 2H), 2.61 (bm, 1H), 4.22 (m, 1H), 6.67 (s, 1H), 7.53 (m, 3H), 7.78 (m, 2H). CI-MS (*m/z*): 285 (3, M + H), 259 (24), 257 (39), 201 (19), 105 (8), 85 (100).

(±)-3-[1-(Cyclohexylthio)-2-cyclopropylethyl]-4-hydroxy-6-phenyl-2*H*-pyran-2-one (14). The compound was prepared according to the general procedure using 4-hydroxy-6-phenyl-2*H*-pyran-2-one (1.0 g, 5.31 mmol), ethanol (10 mL), cyclopropylmethanecarboxaldehyde (0.67 g, 5.84 mmol), cyclohexyl mercaptan (1.68 mL, 13.8 mmol), piperidine (0.5 mL), and acetic acid (0.5 mL). IR (KBr): 3074, 3001, 2928, 2850, 2658, 1707, 1649, 1566, 1496, 1450, 1406, 1315, 1267, 1228, 1130, 1113, 1016, 918, 827, 767, 690 cm^{-1}. ^{1}H-NMR (400 MHz, DMSO-d_6): δ −0.02 (m, 1H), 0.05 (m, 1H), 0.34 (m, 2H), 0.64 (m, 2H), 1.22 (m, 5H), 1.52 (m, 1H), 1.67 (m, 3H), 1.84 (m, 1H), 1.97 (m, 2H), 2.64 (m, 1H), 4.21 (t, 1H), 6.69 (s, 1H), 7.52 (m, 3H), 7.75 (m, 2H). MS-CI (*m/z*): 371 (1, M + H), 279 (1), 255 (14), 201 (2), 147 (1), 116 (6), 105 (2), 83 (100).

(±)-3-[1-(Cyclohexylthio)-2-cyclopentylethyl]-4-hydroxy-6-phenyl-2*H*-pyran-2-one (15). The compound was prepared as described in the general procedure using 4-hydroxy-6-pheny-2*H*-pyran-2-one (1.0 g, 5.31 mmol), ethanol (10 mL), cyclopentylmethanecarboxaldehyde (0.65 g, 5.84 mmol), cyclohexyl mercaptan (1.68 mL, 13.8 mmol), piperidine (0.5 mL), and acetic acid (0.5 mL). IR (KBr): 3103, 3038, 2930, 2852, 1656, 1568, 1496, 1446, 1404, 1300, 1265, 1228, 1122, 1028, 912, 850, 765, 688, 677, 570 cm^{-1}. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.44 (m, 19H), 2.01 (m, 1H), 2.19 (m, 1H), 2.60 (m, 1H), 4.16 (m, 1H), 6.68 (s, 1H), 7.53 (m, 3H), 7.75 (m, 2H), 11.66 (bs, 1H). FABMS (*m/z*): 399 (5, M + H), 392 (22), 283 (100).

(±)-3-[1-(Cyclohexylthio)-3,3-dimethylbutyl]-4-hydroxy-6-phenyl-2*H*-pyran-2-one (16). The compound was prepared as described in the general procedure using 4-hydroxy-6-phenyl-2*H*-pyran-2-one (1.0 g, 5.31 mmol), ethanol (10 mL), 3,3-dimethylbutanal (0.73 mL, 5.84 mmol), cyclohexyl mercaptan (1.86 mL, 13.8 mmol), piperidine (0.5 mL), and acetic acid (0.5 mL). IR (KBr): 3103, 3040, 2930, 2852, 2658, 1656, 1568, 1496, 1450, 1404, 1363, 1284, 1120, 997, 914, 848, 765, 688, 576 cm^{-1}. ^{1}H-NMR (400 MHz, DMSO-d_6): 0.85 (s, 9H),

1.25 (m, 6H), 1.65 (m, 7H), 4.30 (m, 1H), 6.69 (s, 1H), 7.54 (m, 3H), 7.75 (m, 2H). FABMS (*m/z*): 387 (11, M + H), 314 (3), 271 (100).

(±)-3-[1-[(Cyclohexylmethyl)thio]-3-methylbutyl]-4-hydroxy-6-phenyl-2*H*-pyran-2-one (17). The compound was prepared according to the general procedure using 4-hydroxy-6-phenyl-2*H*-pyran-2-one (1.0 g, 5.31 mmol), ethanol (10 mL), isovaleraldehyde (0.462 mL, 5.84 mmol), cyclohexylmethanethiol (1.79 g, 13.8 mmol), piperidine (0.5 mL), and acetic acid (0.5 mL). IR (KBr): 3088, 3040, 2926, 2852, 1660, 1566, 1496, 1450, 1404, 1311, 1286, 1267, 1124, 912, 846, 765, 689 cm^{-1}. ^1H-NMR (400 MHz, DMSO-d_6): δ 0.58 (d, 6H), 1.11 (m, 5H), 1.57 (m, 8H), 2.07 (m, 1H), 2.28 (dd, 1H), 2.38 (dd, 1H), 4.17 (dd, 1H), 6.69 (s, 1H), 7.54 (m, 3H), 7.75 (m, 2H), 11.71 (bs, 1H). FABMS (*m/z*): 387 (2, M + H), 256 (100).

(±)-3-[Cyclopentyl(cyclopentylthio)methyl]-4-hydroxy-6-phenyl-2*H*-pyran-2-one (18). The compound was prepared according to the general procedure using 4-hydroxy-6-phenyl-2*H*-pyran-2-one (1.0 g, 5.31 mmol), ethanol (10 mL), cyclopentanecarboxaldehyde (0.78 g, 7.96 mmol), cyclopentyl mercaptan (1.43 mL, 13.8 mmol), piperidine (0.5 mL), and acetic acid (0.5 mL). IR (KBr): 3063, 3038, 2956, 2866, 1706, 1647, 1565, 1496, 1451, 1404, 1312, 1277, 1227, 1148, 913, 767, 690 cm^{-1}. ^1H-NMR (400 MHz, DMSO-d_6): δ 1.03 (m, 1H), 1.64 (m, 15H), 2.64 (m, 1H), 3.0 (m, 1H), 3.84 (d, 1H), 6.69 (s, 1H), 7.52 (m, 3H), 7.76 (m, 2H), 11.55 (bs, 1H). FABMS (*m/z*): 371 (20, M + H), 337 (2), 325 (3), 301 (6), 269 (100).

(±)-3-[1-(Cyclopentylthio)-3-methylbutyl]-4-hydroxy-6-phenyl-2*H*-pyran-2-one (19). The title compound was prepared according to the general procedure using 4-hydroxy-6-phenyl-2*H*-pyran-2-one (1.0 g, 5.31 mmol), ethanol (10 mL), isovaleraldehyde (0.62 mL, 5.84 mmol), cyclopentyl mercaptan (1.43 mL, 13.8 mmol), piperidine (0.5 mL), and acetic acid (0.5 mL). IR (KBr): 3103, 3040, 2955, 2868, 1651, 1568, 1496, 1452, 1404, 1365, 1309, 1286, 1267, 1236, 1124, 1028, 912, 848, 765, 688, 569 cm^{-1}. ^1H-NMR (400 MHz, DMSO-d_6): δ 0.85 (d, 3H), 0.87 (d, 3H), 1.32 (m, 1H), 1.54 (m, 7H), 1.85 (m, 1H), 2.0 (m, 2H), 3.04 (m, 1H), 4.20 (dd, 1H), 6.69 (s, 1H), 7.53 (m, 3H), 7.76 (m, 2H), 11.69 (bs, 1H). FABMS (*m/z*): 359 (3, M + H), 301 (2), 257 (100).

(±)-3-[1-(Cyclopentylthio)-2-cyclopropylethyl]-4-hydroxy-6-phenyl-2*H*-pyran-2-one (20). The compound was prepared according to the general procedure using 4-hydroxy-6-phenyl-2*H*-pyran-2-one (1.0 g, 5.31 mmol), ethanol (10 mL), cyclopropylmethanecarboxaldehyde (0.892 g, 10.62 mmol), cyclopentyl mercaptan (1.43 mL, 13.8 mmol), piperidine (0.5 mL), and acetic acid (0.5 mL). IR (KBr): 3074, 3001, 2955, 2868, 2636, 1709, 1649, 1564, 1496, 1452, 1406, 1317, 1288, 1267, 1230, 1126, 1028, 1016, 916, 829, 767, 690 cm^{-1}. ^1H-NMR (400 MHz, DMSO-d_6): δ 0.04 (m, 2H), 0.07 (m, 2H), 0.66 (m, 1H), 1.53 (m, 7H), 1.94 (m, 3H), 3.19 (m, 1H), 4.21 (dd, 1H), 6.71 (s, 1H), 7.54 (m, 3H), 7.76 (m, 2H). FABMS (*m/z*): 357 (9, M + H), 301 (3), 289 (2), 269 (2), 255 (100), 241 (4).

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