HIV Protease Inhibitors Possessing a Novel, High-Affinity, and Achiral P_1'/P_2' Ligand with a Unique Pattern of *in Vitro* Resistance. Importance of a Conformationally-Restricted Template in the Design of Enzyme Inhibitors

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Abstract: Achiral pyran-2-one analogs possessing a 3-*S*-(2-alkylphenyl) group were determined to be high-affinity inhibitors for human immunodeficiency virus (HIV) protease (PR). Crystallographic, modeling, and structure−activity studies led to the 3-*S*-(2-*tert*-butylphenyl) moiety as an apparent optimal group to access the S_2'/S_1' pockets of the enzyme. Further optimization led to an inhibitor, 3-[(2-*tert*-butylphenyl)sulfanyl]-4-hydroxy-6-(3-methylphenyl)-pyran-2-one (**14**), possessing a K_i of 3 nM. An X-ray crystallographic structure of an inhibitor, 4-hydroxy-3-[(2-isopropylphenyl)sulfanyl]-6-phenylpyran-2-one (**8**), bound to HIV PR showed that the 3-*S*-(2-isopropylphenyl) group occupied the P_2' and P_1' pockets, while other crucial interactions were common to those found with other pyran-2-one analogs. The high potency observed for this series may be due, in part, to the restrictions on the intramolecular collapsibility of these molecules in aqueous solution, leading to a highly favorable hydrophobic effect on binding. Herein we report a novel P_2'/P_1' achiral ligand which results in a tight-binding inhibitor that occupies only three pockets in the enzyme and exhibits a unique pattern of *in vitro* resistance.

Human immunodeficiency virus (HIV) protease[1] (PR) is one of the three crucial viral enzymes, *viz.*, reverse transcriptase (RT),[2] PR, and integrase (IN),[3] essential for the replication of HIV. It has been shown that the inhibition of HIV PR results in the production of virus particles that are immature and noninfectious, thereby preventing the spread of virus to other cells.[4] The design of potent and structurally-diverse HIV PR inhibitors has emerged over the past few years and has exemplified varying chemical templates, stereochemical complexity, and molecular recognition properties in binding to the target enzyme.[5,6] Although HIV PR inhibitors have been recently shown to be clinically effective,[7] a need remains for the development of structurally-diverse, easily synthesizable, small molecular weight, second-generation inhibitors. This is particularly important due to the occurrence of mutations in HIV PR and the subsequent inhibitor resistance.[8]

Recently, we reported[9] the discovery of a novel class of nonpeptidic HIV PR inhibitors, *viz.*, 4-hydroxypyran-2-one analogs, as achiral, low molecular weight, and conformationally-restricted P_1/P_1' peptidomimetics[10] (Figure 1, **1**). Subsequently, both monochiral[11] and bischiral[12] analogs that exploit branching at the C-3 (**2a** and **2b**) and/or C-6 (**3**) position of the pyran-2-one template were disclosed. Nevertheless, the discovery of a highly potent, achiral pyran-2-one analog has not yet been

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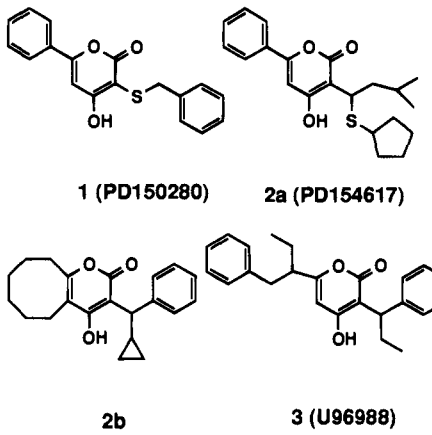


Figure 1.

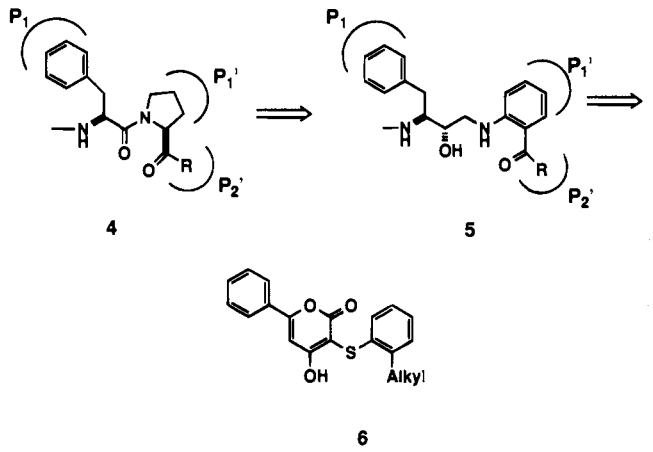
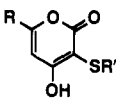


Figure 2.

achieved. Our efforts were focused on eliminating chirality, thus providing facile synthetic accessibility relative to chiral HIV PR inhibitors,[5,13] and simultaneously improving the binding affinity.

α-Alkylbenzamides have been incorporated into HIV substrates and peptidomimetic inhibitors as P_1' proline mimics[14,15] and have led to compounds of type **5** (Figure 2). The α-carbonyl amide in **5** forms hydrogen bonds with the critical water molecule (water-301) found in HIV PR inhibitor complexes.[6,16] Since the lactone moiety of the pyran-2-one ring displaces water-301 in our inhibitors, a simple alkyl group (preferably branched alkyl group[17]) as shown in **6** was thought to be sufficient to occupy the S_2' pocket. Molecular modeling[18] of **8**, using the X-ray crystal structure of **1** and manual docking, indicated that the *S*-phenyl ring would occupy the S_2' site and the isopropyl group would be directed toward the S_1' pocket. This prediction was based on the steric compatibility of the ligand with the enzyme binding region.

Table 1. Pyran-2-one Inhibitors and Their IC_{50} Values Tested against HIV PR *in Vitro*



entry	R	R′	IC_{50}[a] (μM)	K_i[a] (μM)
1	C_6H_5	$CH_2C_6H_5$	1.67	0.7[b]
7	C_6H_5	C_6H_5	3.	1.1[b]
8	C_6H_5	C_6H_4(2-iPr)	0.037	0.016
9	C_6H_5	C_6H_4(2-Me)	0.424	0.016
10	C_6H_5	C_6H_4(2-Et)	0.169	0.072
11	C_6H_5	C_6H_4(2-tBu)	0.017	0.0073
12	benzodioxin-6-yl	C_6H_4(2-tBu)	0.013	0.0056
13	C_6H_4(3-Cl)	C_6H_4(2-tBu)	0.022	0.0094
14	C_6H_4(3-Me)	C_6H_4(2-tBu)	0.007	0.003

[a] Values are the average of at least two determinations. For the details of the assay see ref 11. [b] Taken from ref 9.

In order to examine this hypothesis, several pyran-2-one analogs, **8**–**14** (Table 1, *vide infra*), were synthesized by the condensation of the 2-S-substituted malonic esters with the corresponding trimethylsilyl enol ether at 150 °C.[19] Inhibitor **8** (IC_{50} of 37 nM) showed a 100-fold increase in binding affinity[20] to HIV PR when compared to the parent compound **7** (Table 1). The *o*-methyl and *o*-ethyl analogs **9** and **10** were not as effective, showing only a 7-fold and 18-fold increase in potency, respectively, compared to **7**. Interestingly, the *o*-*tert*-butyl group containing analog **11** enhanced the potency by another 2-fold relative to **8**. Thus, the structure–activity relationship of the above compounds clearly demonstrates that *ortho* substitution on the *S*-phenyl ring has a dramatic effect on the binding affinity to HIV PR. Substitution of the 6-phenyl moiety in **11** with benzodioxin-6-yl, 3-chlorophenyl, and 3-methylphenyl groups resulted in **12**, **13**, and **14**, respectively, with a similar or 2-fold increase in binding affinity.[21] Of particular significance is the fact that these inhibitors are nonchiral and of low molecular weight and can be synthesized in three steps.

The X-ray crystal structure of **8** bound to HIV PR was determined to evaluate the binding mode of this compound. As was observed in the X-ray crystal structure of **1** bound to HIV PR, water-301, which forms bridging hydrogen bonds between the flaps and the peptidomimetic inhibitor backbone, was displaced by the lactone moiety of the pyran-2-one template (Figure 3). In contrast to our initial hypothesis, but in line with the molecular modeling prediction, the isopropyl group present on the 3-*S*-phenyl group occupied the S_1' pocket, whereas the 3-*S*-phenyl group partially filled the S_2' pocket. A small molecule X-ray crystal structure of **8** was also obtained. An overlay of the crystal structures of the bound *vs* unbound molecule is shown in Figure 4. In comparing the bound structure to the unbound structure, the *S*-phenyl ring is oriented on opposite sides of the pyran-2-one ring system. Although there exists a pseudosymmetry in these conformations, this

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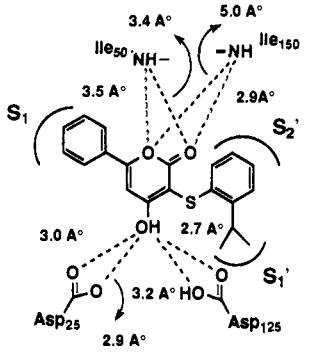

Figure 3. Interatomic distances of compound **8** bound to HIV PR (from the X-ray crystal structure).

relative orientation is considered real since the hydrogens are clearly defined in the small molecule structure, and when the analog is bound in the PR, the hydroxyl group would be expected to bind at the catalytic site. *Ab initio* calculations indicate that the two conformers of **8** were energetically equivalent to within 3.3 kJ/mol.[22] Monte Carlo conformational analysis of **8** using Macromodel[23] indicates pairs of essentially energetically equivalent conformers with the *S*-phenyl ring above or below the plane of the pyrone ring.

Although the potent inhibitors **8**–**14** occupy only three binding pockets, their small size and lack of conformational flexibility result in a favorable entropic effect for binding. Conformational analysis of 1000 randomly-generated conformers of **8**, carried out in H_2O, resulted in only 27 unique structures within a 50 kJ/mol range (after a constrained minimization in H_2O, the energy of bound conformation was within this range). The limited flexibility of **8** also restricts the hydrophobic aggregation of the nonpolar groups prevalent in the inhibitor that is normally considered detrimental to binding.[24] These results emphasize the importance of entropic considerations for HIV PR inhibitor design.[25]

Antiviral activity of these inhibitors in cellular assays correlated poorly with enzymatic inhibition.[11a] These inhibitors probably exist in anionic form at physiological pH; the pK_a of inhibitor **8**, for example, was found to be 4.1. The X-ray crystal structure of **8** bound to HIV PR showed that the enolic hydroxyl group is within the hydrogen bonding distance to both catalytic aspartic acids (*vide supra*, Figure 3). Kinetic as well as molecular simulation studies of HIV PR showed that one of the aspartic acids present in the active site is in the protonated form, whereas the other catalytic aspartic acid is in the unprotonated form.[26] Preliminary energy calculations indicate that the inhibitor binds in the enol form, and thus binding at neutral pH would impose a thermodynamic protonation, a penalty for compounds with acidic pK_a values. In support of this hypothesis the *in vitro* inhibitory activities of the pyran-2-ones against HIV PR were found to be pH sensitive. For example, inhibitors **8** and **14** tested at pH 6.2 have IC_{50} values of 0.700 and 0.092 μM, respectively, which are 19- and 13-fold higher than those measured at 4.7. Experiments were also performed to answer the question as to whether these inhibitors would penetrate infected cell membranes? The permeability of inhibitor **8** was relatively high, i.e., 145×10^{-4} cm/min, across monolayers composed of human colon adenocarcinoma cell lines (CACO-2), a value higher than previously observed in studies with a wide spectrum of compounds.[27] Clearly, penetration of cell membranes was not a major issue. We next tested the serum protein binding properties of these compounds. The pyran-2-ones with the enolic hydroxylic group sandwiched between hydrophobic groups structurally resemble the anticoagulant warfarin, which is known to bind to serum proteins.[28] Hence, it was hypothesized that these pyran-2-ones bind strongly to serum proteins, which are present in the antiviral assay.[29] Appropriate experiments thus performed showed that greater than 98% of inhibitor **8** was bound to plasma proteins at pH 7.4. Thus, the protein binding nature of these inhibitors results in a decreased concentration of the free drug available for antiviral activity. The combination of high protein binding and ionization of the hydroxyl group at near neutral pH may be responsible for the poor antiviral activities of these compounds as measured in a cell culture.

Though initial clinical results using HIV PR inhibitors are encouraging, in general, they show viral resistance resulting in a considerable challenge for the use of HIV PR therapy for AIDS treatment.[30] Consequently, combination therapy, using inhibitors either targeting different stages of the HIV life cycle or aiming at the same target, is an increasingly popular strategy for the treatment of AIDS.[31] The various classes of PR inhibitors possessing different binding interactions could potentially lead to different resistance patterns.[32,33] Since the present pyran-2-one inhibitors possess a novel and simple structure, we have begun to evaluate their inhibitory activity against mutant enzymes. Inhibitor **8** was tested against a series of HIV PR mutants reported to be associated with the development of resistance to HIV PR inhibitors[8,34] (Table 2). The *in vitro* inhibitory activity of **8** is only moderately affected (3–5 fold) by the R8Q, I84V, and V82F subsite mutants of HIV PR, and is actually more potent (~2-fold) against the V32I and V82A mutants. The initial activity results with mutant PR and the simple, novel structure of these inhibitors are encouraging. Optimization of these pyran-2-ones for antiviral activity is in progress.

In conclusion, by a simple modification of a mass screening lead, **7**, i.e., the addition of a methyl group on the 6-phenyl ring and a *tert*-butyl group on the 3-*S*-phenyl ring to yield **14**, activity was enhanced 500-fold. Inhibitor **14** occupies only three

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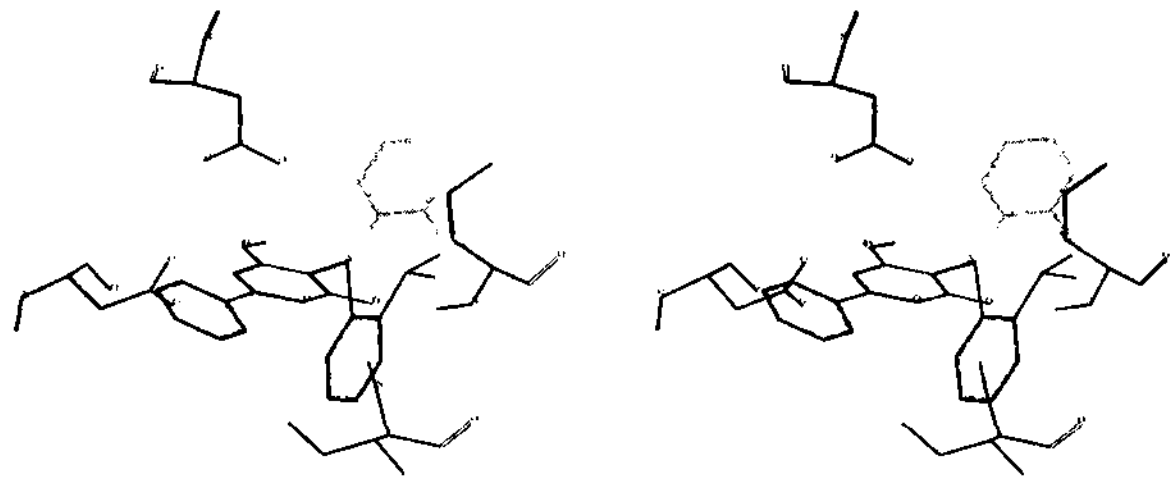

Figure 4. Stereoview of an overlay of the X-ray crystal structure of **8** bound in the HIV PR (black lines) and the small molecule X-ray crystal structure of **8** (gray lines). The residues Asp25/Asp125 and Ile50/150 are also shown.

Table 2. Inhibitor **8** and Its K_i Values Tested against Substite Mutants of HIV PR *in Vitro*

enzyme	K_i[a] (μM)	K_i(mutant)/K_i(wild type)	enzyme	K_i[a] (μM)	K_i(mutant)/K_i(wild type)
WT	0.2	1.0	V82A	0.1	0.5
R8Q	1.1	4.7	V82F	1.3	5.7
V32I	0.1	0.4	I84V	0.7	3.1

[a] K_i values were measured at pH 6.2 using a fluorogenic substrate as described in ref 34.

subsites of the enzyme, possesses a novel P_1'/P_2' ligand, has no chiral centers, and binds with low nanomolar affinity (K_i = 0.003 μM) to HIV PR.[35] Inhibitor 8 exhibited a novel pattern of *in vitro* resistance to HIV PR subsite mutants. The high potency observed for this series may be partly due to the inherent stability of extended conformations of these compounds in aqueous solution. In addition, the limited number of freely rotatable bonds is advantageous from entropy considerations. The concept of hydrophobic collapse in the design of biologically active compounds has recently gained the attention of medicinal chemists.[36] Consideration of the design of hydrophobically-noncollapsible or conformationally-restricted molecules for enzyme inhibitors or receptor antagonists might lead to new generations of structurally unique and synthetically simpler molecules.

Experimental Section

Melting points were determined in open capillary tubes on a Hoover melting point apparatus and are uncorrected. Infrared (IR) spectra were determined using KBr pellets on a Nicolet FT IR SX-20 spectrophotometer. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AM 250 spectrometer, and chemical shifts are reported in δ units relative to internal tetramethylsilane. Alt mass spectra (MS) were obtained on a Finnigan 4500 GC–MS or a VG Analytical 7070E/F spectrometer. Elemental analyses were performed on a Perkin-Elmer Model 240 elemental analyzer, and all compounds had 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 determined *in vacuo* at 10–30 mmHg.

The *ab initio* geometry optimizations were performed using the Dgauss program (DFT method) with the DZVP basic set and nonlocal gradient corrected functionality for energy calculations.[22] The Monte Carlo simulation for **8** was carried out using the Macromodel software (Version 4.5).[21] The simulation was performed in H_2O, using the Amber* force field, starting with an unconstrained minimized structure of the bound conformation of **8**. A constrained minimization of the bound conformation of **8** was also carried out in H_2O using the Amber* force field.

General Procedure. Pyrones described are synthesized according to the procedure described previously.[19] To the ketone (1 equiv) taken in dichloromethane and cooled to 0 °C was added triethylamine (1.5–2.2 equiv), followed by trimethylsilyl triflate (1–1.2 equiv). The reaction was slowly warmed to room temperature and allowed to stir for 30 min. The reaction was quenched with saturated sodium bicarbonate and diluted with diethyl ether. The ethereal layer was washed with sodium bicarbonate and brine and then dried over sodium sulfate. The solvents were removed under vacuum. The crude silyl enol ether thus prepared as described above was treated with the corresponding diethyl 2-substituted propane-1,3-dioate (1–0.5 equiv). The reaction mixture was heated to 135–150 °C for 16 h. The reaction was cooled to room temperature and subjected to flash column silica gel chromatography.

Examples. 4-Hydroxy-3-[(2-isopropylphenyl)sulfanyl]-6-phenylpyran-2-one (8). The compound was prepared using 1-phenyl-1-[(trimethylsilyl)oxy]ethylene (1.24 g, 6.45 mmol) and diethyl 2-[(2-isopropylphenyl)thio]propane-1,3-dioate (1.0 g, 3.23 mmol). Isolated yield: 45%. Mp: 197–199 °C dec. IR (KBr): 3117, 2963, 1661, 1551, 1406, 1101, 760 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.25 (d, 6H), 3.42 (m, 1H), 6.89 (s, 1H), 6.92 (dd, 1H), 7.06 (t, 1H), 7.13 (t, 1H), 7.28 (d, 1H), 7.56 (m, 3H), 7.85 (m, 2H). Cl-MS (*m/z*): 339 (100), 305 (4), 219 (25), 189 (11), 147 (9), 105 (9). Anal. Calcd for $C_{20}H_{18}O_3S_1$: C, 70.98; H, 5.36. Found: C, 70.82; H, 5.24.

4-Hydroxy-3-[(2-methylphenyl)sulfanyl]-6-phenylpyran-2-one (9). The compound was prepared using 1-phenyl-1-[(trimethylsilyl)oxy]-ethylene (1.95 g, 10.14 mmol) and diethyl 2-[2-methylphenyl)thio]-propane-1,3-dioate (1.43 g, 5.07 mmol). Isolated yield: 62%. Mp: 210–211 °C dec. IR(KBr): 3426, 3057, 1632, 1539, 1406, 1348, 1105, 763, 750, 526 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.47 (s, 3H), 6.82 (s, 1H), 6.86 (d, 1H), 7.04 (m, 2H), 7.17 (d, 1H), 7.56 (m, 3H), 7.86 (m, 2H). Cl-MS (*m/z*): 311 (100), 219 (29), 189 (37), 147 (27), 125 (40), 105 (24). Anal. Calcd for $C_{18}H_{14}O_3S_1$: C, 69.66; H, 4.55. Found: C, 69.54; H, 4.45.

4-Hydroxy-3-[(2-ethylphenyl)sulfanyl]-6-phenylpyran-2-one (10). The compound was prepared using 1-phenyl-1-[(trimethylsilyl)oxy]-ethylene (4.17 g, 21.27 mmol) and diethyl 2-[2-ethylphenyl)thio]-propane-1,3-dioate (1.5 g, 10.86 mmol). Isolated yield: 62%. Mp: 190–192 °C dec. IR (KBr): 3356, 1728, 1628, 1547, 1219, 671 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.25 (t, 3H), 2.78 (q, 2H), 6.89 (s, 1H), 6.92 (m, 1H), 7.08 (m, 2H), 7.2 (m, 1H), 7.58 (m, 3H), 7.86 (m, 2H). Cl-MS (*m/z*): 325 (100), 219 (26), 189 (11), 147 (11), 139 (15), 105 (19). Anal. Calcd for $C_{19}H_{16}O_3S_1$: C, 70.35; H, 4.97. Found: C, 70.10; H, 5.07.

3-[(2-*tert*-Butylphenyl)sulfanyl]-4-hydroxy-6-phenylpyran-2-one (11). The compound was prepared using 1-phenyl-1-[(trimethylsilyl)oxy)ethylene (1.19 g, 6.17 mmol) and diethyl 2-[2-*tert*-butylphenyl)-thio]propane-1,3-dioate (1.00 g, 3.09 mmol). Isolated yield: 48%.

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Mp: 139−141 °C dec. IR (KBr): 3063, 2955, 1686, 1626, 1543, 1408, 1098, 764 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 1.53 (s, 9H), 6.9 (s, 1H), 6.97 (m, 1H), 7.05 (m, 2H), 7.33 (m, 1H), 7.54 (m, 3H), 7.85 (m, 2H), 7.96 (dd, 1H). CI-MS (*m/z*): 353 (100), 297 (35), 219 (70), 189 (37), 147 (45), 105 (48), 91 (30). Anal. Calcd for $C_{21}H_{20}O_3S_1$: C, 71.56; H, 5.72. Found: C, 71.32; H, 6.02.

Benzodioxin-6-yl-3-[(2-*tert*-butylphenyl)sulfanyl]-4-hydroxypyran-2-one (12). The compound was prepared using benzodioxin-6-yl methyl ketone (2.0 g, 11.2 mmol), trimethylsilyl triflate (2.5 g, 11.2 mmol), triethylamine (1.7 g, 16.8 mmol), and diethyl 2-[2-*tert*-butylphenyl)thio]propane-1,3-dioate (1.81 g, 5.6 mmol). Isolated yield: 63%. Mp: 240−242 °C dec. IR (KBr): 3401, 2965, 1729, 1653, 1542, 1506, 1287, 1067, 757 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 1.53 (s, 9H), 4.33 (m, 4H), 6.75 (s, 1H), 6.97 (m, 1H), 7.08 (m, 3H), 7.33 (m, 3H). CI-MS (*m/z*): 410 (100), 355 (15), 277 (23), 219 (25), 205 (32), 163 (50). Anal. Calcd for $C_{23}H_{22}O_5S_1 \cdot 0.2H_2O$: C, 66.65; H, 5.31. Found: C, 66.34; H, 5.11.

3-[(2-*tert*-Butylphenyl)sulfanyl]-6-(3-chlorophenyl)-4-hydroxypyran-2-one (13). The compound was prepared using 3-chloroacetophenone (1.0 g, 6.5 mmol), trimethylsilyl triflate (1.58 g, 7.1 mmol), triethylamine (1.44 g, 14.2 mmol), and diethyl 2-[2-*tert*-butylphenyl)-thio]propane-1,3-dioate (1.05 g, 3.25 mmol). Isolated yield: 54%. Mp: 182−185 °C. IR (KBr): 3401, 2965, 1729, 1653, 1542, 1506, 1287, 1067, 757 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 1.53 (s, 9H), 4.33 (m, 4H), 6.75 (s, 1H), 6.97 (m, 1H), 7.08 (m, 3H), 7.33 (m, 3H). CI-MS (*m/z*): 386 (100), 387 (83), 351 (26), 331 (31), 255 (18), 253 (46), 225 (16), 223 (32), 183 (10), 181 (28), 151 (24), 149 (32), 139 (29), 91 (23). Anal. Calcd for $C_{21}H_{19}O_3S_1Cl_1 \cdot 0.5H_2O$: C, 63.65; H, 5.05. Found: C, 63.64; H, 4.94.

3-[(2-*tert*-Butylphenyl)sulfanyl]-4-hydroxy-6-(3-methylphenyl)pyran-2-one (14). The compound was prepared using 3-methylacetophenone (1.0 g, 2.63 mmol), trimethylsilyl triflate (1.17 g, 5.26 mmol), triethylamine (0.53 g, 5.26 mmol), and diethyl 2-[2-*tert*-butylphenyl)thio]propane-1,3-dioate (1.00 g, 2.63 mmol). Isolated yield: 48%. Mp: 172−173 °C dec. IR (KBr : 2967, 1617, 1537, 1406, 1335, 1250, 754 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 1.53 (s, 9H), 6.83 (s, 1H), 7.08 (m, 2H), 7.36 (m, 1H), 7.42 (m, 1H), 7.47 (m, 1H), 7.85 (m, 2H), 7.66 (m, 2H). CI-MS (*m/z*): 367(100), 311(33), 233 (50), 203 (51), 161 (43), 119 (59), 91 (43). Anal. Calcd for $C_{22}H_{22}O_3S_1 \cdot 0.2H_2O$: C, 71.34; H, 5.94. Found: C, 71.49; H, 6.37.

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Supporting Information Available: Text describing single-crystal X-ray analysis and tables giving crystallographic data, bond distances and angles, torsion angles, positional parameters, and least-squares planes (12 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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