Antitumor Agents. 166. Synthesis and Biological Evaluation of 5,6,7,8-Substituted-2-phenylthiochromen-4-ones†

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As a continuation of our structure−activity relationship study of substituted 2-phenyl-4-quinolones and flavonoids as antitumor and antiviral agents, a series of 5,6,7,8-substituted-2-phenylthiochromen-4-ones has been synthesized by condensation of substituted thiophenols and ethyl benzoylacetates. Target compounds were evaluated for biological activity. Among them, compounds **7**, **10**, **12**, and **13** displayed significant growth inhibitory action against a panel of tumor cell lines including human ileocecal carcinoma (HCT-8), murine leukemia (P-388), human melanoma (RPMI), and human central nervous system tumor (TE671) cells. Compounds **10**, **12**, and **19** displayed DNA topoisomerase I inhibitory activity *in vitro* and compound **11** was an *in vitro*, inhibitor of DNA topoisomerase II. Compound **11** was most active (ED_{50} value, 0.65 μM) against HIV in acutely infected H9 lymphocytes and had a therapeutic index of about 5.

As part of our continuing search for potential anticancer drug candidates in the 2-phenyl-4-quinolone series, we have synthesized a series of substituted 2-phenyl-4-quinolones and related compounds and evaluated them as cytotoxic compounds and as antimitotic agents interacting with tubulin. Among them, some compounds (**Q1** and **Q2**, Figure 1) were potent inhibitors of tubulin polymerization with activities comparable to those of the antimitotic natural products colchicine, podophyllotoxin, and combretastatin A-4.[2−4] For example, compound **Q2** totally inhibited the growth of about half of the NCI tumor cell lines at subnanomolar concentrations (log TGI < −9.00) and was also a potent inhibitor of tubulin polymerization with an IC_{50} value of 0.44 μM.

In our earlier studies, certain flavonoids (Figure 1) displayed antitumor activity or anti-HIV activity. For example, two flavonols isolated from *Polanisia dodecandra* [5,3′-dihydroxy-3,6,7,8,4′-pentamethoxyflavone (**F1**) and 5,4′-dihydroxy-3,6,7,8,3′-pentamethoxyflavone (**F2**)] displayed interesting antitumor activities.[7] [Compound **F1** was also previous isolated as a cytotoxic principle, centaureidin, from *Polymnia fruticosa* by Beutler et al.[5] and as an antimitotic agent from *Zieridium pseudobtusifolium* by Lichius et al.[6]] Compound **F1** was cytotoxic *in vitro* against a panel of cell lines derived from central nervous system cancers, nonsmall cell lung cancers, small cell lung cancers, ovarian cancers, colon cancers, renal cancers, a melanoma, and leukemia cells, with GI_{50} values in the low micromolar to nanomolar concentration range. Compound **F1** also inhibited tubulin polymerization ($IC_{50} = 0.83 \pm 0.2$ μM) and the binding of radiolabeled colchicine to tubulin (59% inhibition when present at equimolar concentration), and appears to be the first example of a flavonol displaying such bioactivity. Compound **F2** was also cytotoxic for medulloblastoma tumor cells with an ED_{50} value of 0.99 μg/mL. On the other hand, apigenin-7-*O*-β-D-glucopyranoside (**F3**) isolated from *Kummerowia striata*[8] and chrysin (**F4**) isolated from *Chrysanthemum morifolium*[9] as anti-HIV active principles displayed EC_{50} values of 1.8 μg/mL and 5 μM against H9 cell replication with therapeutic index value of >55.6 and 9, respectively.

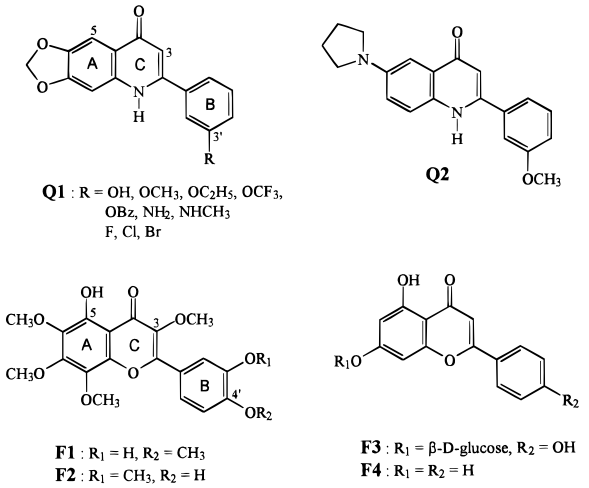

Figure 1. Bioactive 2-phenyl-4-quinolones and flavonoids.

Comparison of the structures between the bioactive 2-phenyl-4-quinolones and flavones discussed above revealed that these two types of compound share similar skeletons except they possess a different heteroatom at position 1 of the C ring. Therefore, similar or possible novel biological activities might be anticipated following the substitution of the heteroatom by a bioisostere such as sulfur.

The aim of the present work was to further investigate the structure−activity relationships (SAR) of 2-phenyl-4-quinolones and flavonones by replacing the heteroatom in the C ring with a sulfur atom. Consequently, a series of 5,6,7,8-substituted-2-phenylthiochromen-4-ones and related 2-phenyl-4-quinolones have been synthesized and evaluated for cytotoxic, topoisomerase I and II inhibitory, and anti-HIV activities.

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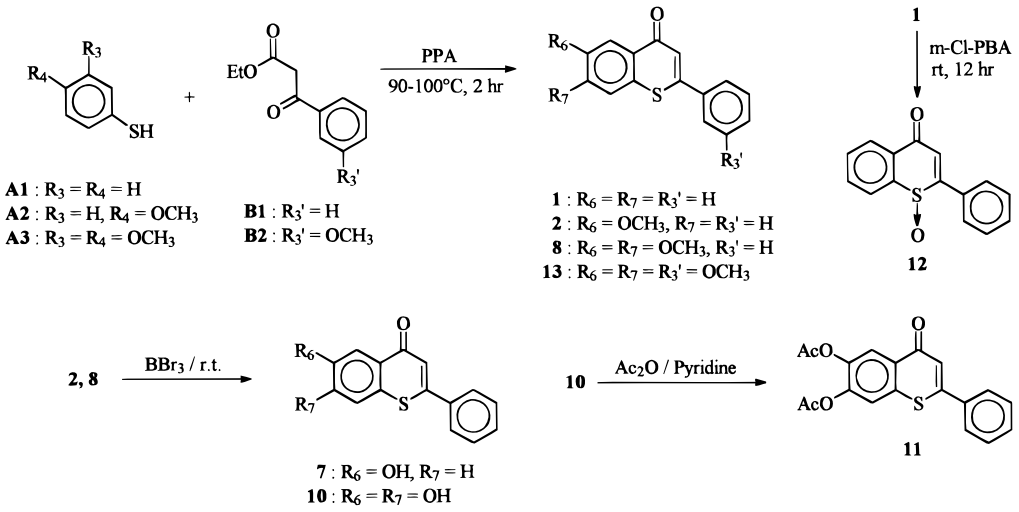
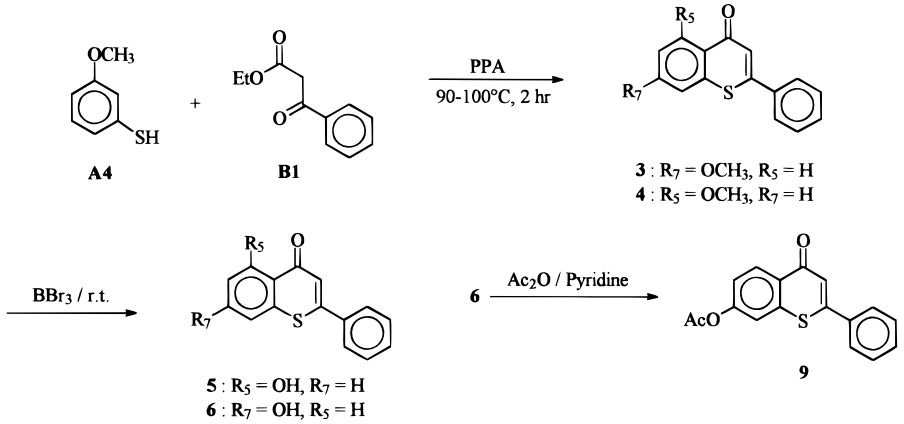
Scheme 1. Synthesis of 2-Phenylthiochromen-4-one Derivatives **1**, **2**, **7**, **8**, **10**, **11**, and **13**

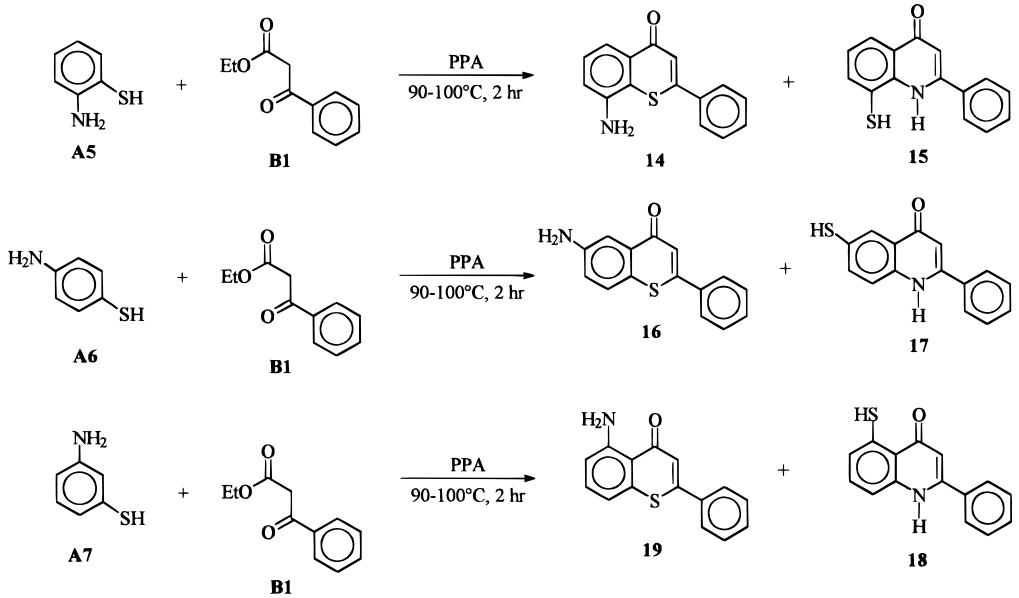


PPA: polyphosphoric acid; m-Cl-PBA: m-Chloro benzoic acid peroxide.

Scheme 2. Synthesis of 2-Phenylthiochromen-4-one Derivatives **3**−**6** and **9**



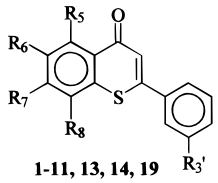
Scheme 3. Synthesis of 2-Phenylthiochromen-4-one Derivatives **14**−**19**



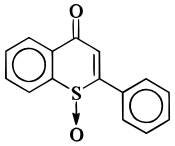
Chemistry

Compounds **1**−**19** were synthesized by condensation of commercially available substituted thiophenols (compounds **A1**-**A7**) and ethyl benzoylacetates (**B1**−**B2**) followed by demethylation, acetylation, or oxidation as shown in Schemes 1−3. Compounds **1**−**4**, **8**, **13**, and **14**−**19** were synthesized by the condensation of corresponding substituted thiophenols (compounds **A1**−**A7**) and ethylbenzoyl acetates (**B1**−**B2**) in heated (90−100 °C) polyphosphoric acid (PPA) for 2 h.[10] Compounds

Table 1. *In Vitro* Cytotoxic Activities of Substituted 2-Phenylthiochromen-4-ones in Various Tumor Cell



1-11, 13, 14, 19



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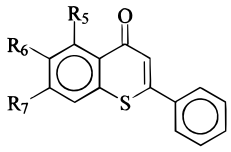
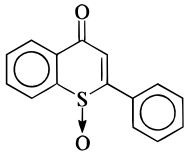
						ED_{50} (μg /mL)[a]					
compd no.	R_5	R_6	R_7	R_8	$R_{3'}$	KB[b]	A-549[b]	HCT-8[b]	P-388[b]	RPMI[b]	TE671[b]
1						–	–	–	–	–	–
2		OCH_3				–	–	–	–	–	–
3			OCH_3			5.5	–	9.16	–	–	–
4	OCH_3					–	–	–	–	5.5	–
5	OH					–	–	–	–	–	–
6			OH			–	–	–	–	–	–
7		OH				–	–	–	–	0.55	2.87
8		OCH_3	OCH_3			4.23	–	9.33	–	8.89	–
9			OAc			–	–	–	–	–	–
10		OH	OH			5.5	–	1.45	4.51	3.92	5.50
11		OAc	OAc			5.5	–	–	4.83	5.5	4.58
12						5.5	–	5.79	1.18	5.5	3.66
13		OCH_3	OCH_3		OCH_3	5.5	–	6.35	0.61	5.5	–
14				NH_2		–	–	–	7.46	5.5	–
19	NH_2					–	–	–	9.31	–	–

[a] EC_{50} was the concentration of drug which affords 50% reduction in cell number after a 3-day incubation. For significant activity of the pure compound, an $EC_{50} \leq 4.0$ μg/mL is required. [b] Human epidermoid carcinoma of the nasopharynx (KB), human lung carcinoma (A-549), human ileocecal carcinoma (HCT-8), murine leukimia (P-388), human melanoma (RPMI), and human CNS tumor (TE671). [c] "–" means inactive.

Table 2. Human DNA Topoisomerase I and II Inhibitory Activities of Substituted 2-Phenylthiochromen-4-ones



10, 11, 19



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				% inhibition (100 μ**M**)		cellular protein-linked
compd no.	R_5	R_6	R_7	topoisomerase I[a]	topoisomerase II[b]	DNA breaks (-fold)[d]
Q2[e]				ND	0	ND
10		OH	OH	100	0	1
11		OAc	OAc	0	100	1
12				100	0	1
19	NH_2			100	0	1

[a] Measured as ATP-independent relaxation of supercoiled plasmid DNA compared to enzyme and DNA control reactions. Camptothecin at 100 μm served as the positive inhibitor control. [b] Measured as ATP-dependent unknotting of P4 DNA compared to enzyme and DNA control reactions. VP-16 (100 μm) completely inhibited the unknotting activity. [c] Compounds (**6**, **9**, **13**–**17**) were not active at 100 μm. [d] Measured as potassium–SDS precipitable cpm in KB oral carcinoma cell cultures treated in triplicate at 50 μm for 1 h. VP-16 and camptothecin stimulated levels of protein–DNA complexes by (36 ± 5)- and (21 ± 2)-fold respectively. [e] See Figure 1; ND = not tested.

5–**7** and **10** were obtained by boron tribromide demethylation of **2**–**4** and **8**. Compounds **9** and **11** were generated by acetic anhydride–pyridine acetylation of **5** and **10** at room temperature. Peroxidation of compound **1** afforded compound **12** (Scheme 1). Using 3-methoxythiophenol (**A4**), both compounds **3** and **4** were generated due to condensation at either the 2- or the 6-position of the original thiophenol (Scheme 2). With the aminothiophenols **A5**-**A7**, the thiochromen-4-ones **14**, **16**, and **18** were accompanied by the corresponding 2-phenyl-4-quinolones **15**, **17**, and **19**, respectively (Scheme 3).

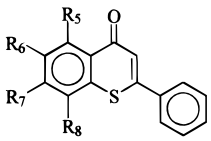
Results and Discussion

Assays for the inhibition of mammalian DNA topoisomerase I and II, for production of cellular protein-linked DNA breaks, for cytotoxicity in cancer cells, and for HIV-inhibitory effects were carried out according to the procedures described previously.[11–17]

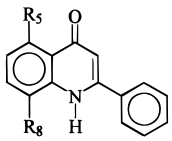
Table 1 shows the *in vitro* cytotoxic activities of 15 target compounds. Among these compounds, **1**, **2**, **5**, **6**, and **9** were inactive and **3**, **4**, **8**, **11**, **14**, and **19** were only weak cytotoxic agents. However, compounds **7**, **10**, **12**, and **13** displayed significant activity ($ED_{50} < 4.0$ μg/mL) against several tumor human cell lines including ileocecal carcinoma (HCT-8), murine leukemia (P-388), human melanoma (RPMI), and a central nervous system (CNS) tumor (TE671), respectively.

Table 2 shows the activity of target compounds against DNA topoisomerase I and II *in vitro*. Due to the insolubilities at the testing concentration, results for compounds **1**–**5**, **7**, **8**, and **18** were not obtained.

Table 3. HIV-Inhibitory Effects of Substituted 2-Phenylthiochromen-4-ones on Infected H9 Lymphocytes



4, 6, 7, 9-11, 14, 16, 19



15, 18

compd no.	R_5	R_6	R_7	R_8	IC_{50} (μg/mL)[a]	ED_{50} (μg /mL)[b]	therapeutic index
4[c]	OCH_3				7	7	1.0
6			OH		40	7	5.7
7		OH			40	9.5	4.2
9			OAc		45	80	0.6
10		OH	OH		1.8	0.8	2.3
11		OAc	OAc		3	0.65	4.6
14				NH_2	15	4	3.8
15				SH	35	12	2.9
16		NH_2			7.5	7	1.1
18	SH				35	6.5	5.4
19	NH_2				50	20	2.5

[a] Concentration which inhibits uninfected growth by 50%; AZT had an IC_{50} value of 2000 μM. [b] Concentration which inhibits virus replication growth by 50%; AZT had an ED_{50} value of 0.04 μM. [c] Crystals of the test agents were observed at both the 100 and 20 μg/mL concentration with compounds **1**, **3**, **8**, **10**, and **14**. The cells were dead at these concentrations. For compounds **5**, **6**, **7**, and **9**, crystals were observed at 100, 20, and 4 μg/mL, but cells were only dead at the 100 μg/mL concentration. For compounds **2**, **11**, **15**, **16**, **18**, and **19**, crystals were found at the 100 μg/mL concentration, and the cells were dead at that concentration. Compounds **13** and **17** did not dissolve in DMSO and could not be tested.

Compounds **10**, **12**, and **19** displayed topoisomerase I inhibitory activities, and compound **11** inhibited topoisomerase II activity. It is interesting that compounds **10** and **11** act selectively on different topoisomerases *in vitro*. Because the inhibition of neither topoisomerase I nor II was reported for either 2-phenyl-4-quinolones or flavonoids, the mechanism of inhibition for 2-phenylthiochromen-4-ones deserves further investigation. A preliminary study indicated that topoisomerase−DNA cleavable complexes were not induced in treated cells (Table 2).

Table 3 shows the HIV-inhibitory effects of the synthetic compounds. Due to the crystallization or insolubility of certain target compounds at the testing concentration, activities of **1**−**3**, **5**, **8**, **12**−**13**, and **17** could not be evaluated. Compounds **4**, **6**, **7**, **10**, **11**, **14**, **16**, and **18** displayed selective antiviral activity (ED_{50} 0.65−9.5 μg/mL) with therapeutic indexes in the range of 0.6−5.7 against HIV in acutely infected H9 lymphocytes. Among the active compounds, **11**, bearing acetoxy groups on both the 6- and 7-positions, was most active (ED_{50} value, 0.65 μg/mL) with a therapeutic index of about 5.

Compared with the 2-phenyl-4-quinolones, the 2-phenylthiochromen-4-ones exhibited improved solubility in organic solvents which made them easy to purify by crystallization, but it was more difficult to evaluate them in biological assays. Therefore, improved solubility of these synthetic compounds and the introduction of a wider variety of substituents will be necessary before clear SAR conclusions can be drawn. Despite the limitations of the current study, it is clear that heteroatom replacement by sulfur is tolerable because antitumor or anti-HIV activities, as observed with 2-phenyl-4-quinolones or flavonoids, were retained. The topoisomerase inhibitory effects observed *in vitro* were unexpected and the involvement of these enzymes as biochemical targets for **10**, **11**, **12**, and **19** are currently being evaluated.

Experimental Section

Melting points were determined on a Fisher-John melting point apparatus and are uncorrected. Elemental analyses were performed by Atlantic Microlabs, Atlantic, GA; ^{1}H NMR spectra were measured at 300 MHz on a Bruker 300 spectrometer and were recorded in $CDCl_3$, a mixture of $CDCl_3$ and CD_3OD, or DMSO-d_6. Chemical shifts are reported in δ (ppm) units relative to the internal reference Me_4Si. Infrared (IR) spectra were recorded on a Perkin-Elmer IR 400 spectrometer as KBr pellets.

General Procedure for the Synthesis of Compounds 1−4, 8, and 13−19. Thirteen grams of phosphorus pentoxide were added to 7 mL of concentrated phosphoric acid; the polyphosphoric acid (PPA) generated was heated to 90−100 °C, and the appropriate substituted thiophenol (compounds **A1**−**A7**, 0.01 mol) was added. The ethyl benzoylacetate (**B1**−**B2**, 0.01 mol) was added dropwise over 1.5 h at 90−100 °C, and the resulting mixture was further stirred for 30 min. After cooling, water was added and the precipitate was collected. The crude products were further purified by recrystallization or by silica gel column chromatographic separation ($CHCl_3$:CH_3OH = 100:1).

General Procedure for the Synthesis of Compounds 5−7 and10. Compound **8** (298 mg) was dissolved in 30 mL of CH_2Cl_2, and 2.0 mL of BBr_3 (1.0 M in hexane) was added with ice-cooling. The mixture was further stirred at room temperature for 30 min, and then 5 mL of CH_3OH was added slowly with ice-cooling to quench the reaction. After evaporation, the residue was washed with water and the precipitate was collected (95% yield). The product was recrystallized from $CHCl_3$ and CH_3OH to afford pure compound **10**. The same procedure was used for the synthesis of compounds **5**−**7**.

2-Phenylthiochromen-4-one (1): yield 95%; colorless needles; mp 121−122 °C; IR (KBr) ν 1610 (shoulder, conjugated C=O); ^{1}H NMR ($CDCl_3$) δ 7.27 (1H, s, H-3), 7.50−7.55 (3H, m, H-2′,4′,6′), 7.58 (1H, dd, J = 2.2, 8.1 Hz, H-8), 7.65 (1H, dt, J = 1.4, 8.1 Hz, H-6), 7.68−7.75 (3H, m, H-7,3′,5′), 8.57 (1H, dd, J = 1.4, 8.1 Hz, H-5). Anal. ($C_{16}H_{12}O_2S$) C, H.

6-Methoxy-2-phenylthiochromen-4-one (2): yield 96%; colorless needles; mp 147−148 °C; IR (KBr) ν 1610 (shoulder, conjugated C=O); ^{1}H NMR ($CDCl_3$) δ 3.97 (3H, s, OCH_3), 7.30 (1H, dd, J = 2.8, 8.8 Hz, H-7), 7.42 (1H, s, H-3), 7.50−7.54 (3H, m, H-2′,4′,6′), 7.63 (1H, d, J = 8.8 Hz, H-8), 7.71−7.75 (2H, m, H-3′,5′), 8.02 (1H, d, J = 2.8 Hz, H-5). Anal. ($C_{16}H_{12}O_2S$) C, H.

7-Methoxy-2-phenylthiochromen-4-one (3): yield 94%; colorless needles; mp 135 °C; IR (KBr) ν 1610 (shoulder, conjugated C=O); ^{1}H NMR ($CDCl_3$) δ 3.93 (3H, s, OCH_3), 7.07 (1H, d, J = 2.3 Hz, H-8), 7.11 (1H, dd, J = 2.3, 8.9 Hz, H-6), 7.18 (1H, s, H-3), 7.50−7.52 (3H, m, H-2′,4′,6′), 7.67−7.70 (2H, m, H-3′,5′), 8.48 (1H, d, J = 8.9 Hz, H-5). Anal. ($C_{16}H_{12}O_2S$) C, H.

5-Methoxy-2-phenylthiochromen-4-one (4): yield 92%; colorless needles; mp 200−201 °C; IR (KBr) ν 1610 (shoulder, conjugated C=O); ^{1}H NMR ($CDCl_3$) δ 4.01 (3H, s, OCH_3), 6.98 (1H, d, J = 8.2 Hz, H-8), 7.14 (1H, s, H-3), 7.24 (1H, t, J = 8.2 Hz, H-7), 7.50−7.52 (3H, m, H-2′,4′,6′), 7.53 (1H, d, J = 8.2 Hz, H-6), 7.68−7.71 (2H, m, H-3′,5′). Anal. ($C_{16}H_{12}O_2S$) C, H.

5-Hydroxy-2-phenylthiochromen-4-one (5): yield 97%; yellow needles; mp 155−156 °C; IR (KBr) ν 3400 (OH), 1610 (shoulder, conjugated C=O); ^{1}H NMR ($CDCl_3$) δ 6.97 (1H, br d, J = 8.0 Hz, H-8), 7.13 (1H, br d, J = 8.0 Hz, H-6), 7.14 (1H, s, H-3), 7.51 (1H, t, J = 8.0 Hz, H-7), 7.52−7.60 (3H, m, H-2′,4′,6′), 7.68−7.73 (2H, m, H-3′,5′), 14.01 (1H, s, O*H*). Anal. ($C_{15}H_{10}O_2S$) C, H.

7-Hydroxy-2-phenylthiochromen-4-one (6): yield 95%; colorless needles; mp 268−269 °C dec; IR (KBr) ν 3400−3200 (OH), 1610 (shoulder, conjugated C=O); ^{1}H NMR ($CDCl_3$:CD_3OD = 5:1) δ 6.97 (1H, dd, J = 2.2, 8.8 Hz, H-6), 7.00 (1H, d, J

= 2.2 Hz, H-8), 7.12 (1H, s, H-3), 7.38−7.44 (3H, m, H-2′,4′,6′), 7.57−7.61 (2H, m, H-3′,5′), 8.28 (1H, d, J = 8.8 Hz, H-5). Anal. ($C_{15}H_{10}O_2S$) C, H.

6-Hydroxy-2-phenylthiochromen-4-one (7): yield 96%; yellowish needles; mp 288−289 °C; IR (KBr) ν 3400−3200 (OH), 1610 (shoulder, conjugated C═O); ^{1}H NMR ($CDCl_3$:CD_3OD = 5:1) δ 7.05 (1H, s, H-3), 7.08 (1H, dd, J = 2.8, 8.8 Hz, H-7), 7.31−7.36 (3H, m, H-2′,4′,6′), 7.45 (1H, d, J = 8.8 Hz, H-8), 7.51−7.54 (2H, m, H-3′,5′), 7.68 (1H, d, J = 2.8 Hz, H-5). Anal. ($C_{15}H_{10}O_2S$) C, H.

6,7-Dimethoxy-2-phenylthiochromen-4-one (8): yield 92%; colorless needles; mp 171−172 °C; IR (KBr) ν 3400−3200 (OH), 1610 (shoulder, conjugated C═O); ^{1}H NMR ($CDCl_3$) δ 4.03, 4.04 (3H each, both s, $OCH_3 \times 2$), 7.04 (1H, s, H-8), 7.24 (1H, s, H-3), 7.51−7.53 (3H, m, H-2′,4′,6′), 7.68−7.71 (2H, m, H-3′,5′), 7.99 (1H, s, H-5). Anal. ($C_{17}H_{14}O_3S$) C, H.

7-Acetyl-2-phenylthiochromen-4-one (9): yield 99%; colorless needles; mp 228−229 °C; IR (KBr) ν 1750 (acetyl C═O), 1610 (shoulder, conjugated C═O); ^{1}H NMR ($CDCl_3$) δ 2.37 (3H, s, OAc), 7.37 (1H, s, H-3), 7.46 (1H, dd, J = 2.5, 8.8 Hz, H-6), 7.51−7.56 (3H, m, H-2′,4′,6′), 7.71−7.73 (2H, m, H-3′,5′), 7.73 (1H, d, J = 8.8 Hz, H-5), 8.28 (1H, d, J = 2.5 Hz, H-8). Anal. ($C_{17}H_{12}O_3S$) C, H.

6,7-Dihydroxy-2-phenylthiochromen-4-one (10): yield 91%; yellow needles; mp 259−261 °C dec; IR (KBr) ν 3450−3200 (broad, OH), 1610 (shoulder, conjugated C═O); ^{1}H NMR ($CDCl_3$ + CD_3OD = 5 + 1) δ 7.21 (1H, s, H-3), 7.41−7.46 (3H, m, H-2′,4′,6′), 7.44 (1H, s, H-8), 7.59−7.62 (2H, m, H-3′,5′), 7.79 (1H, s, H-5). Anal. ($C_{15}H_{10}O_3S$) C, H.

6,7-Diacetyl-2-phenylthiochromen-4-one (11): yield 98%; colorless needles; mp 171−172 °C; IR (KBr) ν 1755 (acetyl C═O), 1610 (shoulder, conjugated C═O); ^{1}H NMR ($CDCl_3$) δ 2.36 (6H, s, OAc × 2), 7.26 (1H, s, H-3), 7.50−7.55 (3H, m, H-2′,4′,6′), 7.61 (1H, s, H-8), 7.68−7.70 (2H, m, H-3′,5′), 8.36 (1H, s, H-5). Anal. ($C_{19}H_{14}O_5S$) C, H.

2-Phenylthiochromen-4-one 1-oxide (12): yield 90%. Compound **1** (100 mg) was dissolved in 5 mL of CH_2Cl_2, and *m*-chloroperbenzoic acid (300 mg) was added. The mixture was further stirred at room temperature for 12 h. The reaction solution was introduced directly to a silica gel column (eluent of $CHCl_3$:MeOH = 100:1) to afford compound **12** (99 mg, yield 93%): colorless needles; mp 126−127 °C; IR (KBr) ν 1645 (conjugated C═O), 1145 (S═O); ^{1}H NMR ($CDCl_3$) δ 6.85 (1H, s, H-3), 7.51−7.62 (3H, m, H-2′,4′,6′), 7.79 (1H, t, J = 8.0 Hz, H-6), 7.87−7.93 (2H, m, H-3′,5′), 7.90 (1H, d, J = 8.0 Hz, H-7), 8.13 (1H, d, J = 8.0 Hz, H-8), 8.24 (1H, d, J = 8.0 Hz, H-5). Anal. ($C_{15}H_{10}O_2S$) C, H.

6,7,3′-Trimethoxy-2-phenylthiochromen-4-one (13): yield 89%; colorless needles; mp 189−190 °C; IR (KBr) ν 1610 (shoulder, conjugated C═O); ^{1}H NMR ($CDCl_3$) δ 3.89, 4.02, 4.04 (3H each, all s, $OCH_3 \times 3$), 7.04 (1H, s, H-3), 7.05 (1H, dd, J = 2.0, 8.0 Hz, H-4′), 7.21 (1H, t, J = 2.0 Hz, H-2′), 7.25 (1H, s, H-8), 7.28 (1H, dd, J = 2.0, 8.0 Hz, H-6′), 7.42 (1H, t, J = 8.0 Hz, H-5′), 7.98 (1H, s, H-5). Anal. ($C_{18}H_{16}O_4S$) C, H.

8-Amino-2-phenylthiochromen-4-one (14): yield 55%; colorless needles; mp 254 °C; IR (KBr) ν 3350 and 3250 (NH_2), 1610 (shoulder, conjugated C═O); ^{1}H NMR ($CDCl_3$) δ 6.71 (1H, s, H-3), 7.18 (1H, dt, J = 1.5, 7.0 Hz, H-6), 7.48 (1H, dd, J = 1.5, 7.0 Hz, H-7), 7.50−7.62 (6H, m, H-5,2′−6″), 12.15 (1H, br s, N*H*). Anal. ($C_{15}H_{11}NOS$) C, H, N.

8-Mercapto-2-phenyl-4-quinolone (15): yield 26%; colorless needles; mp 105−106 °C; IR (KBr) ν 1610 (shoulder, conjugated C═O); ^{1}H NMR ($CDCl_3$) δ 7.41 (1H, t, J = 8.0 Hz, H-6), 7.52 (1H, s, H-3), 7.50−7.55 (3H, m, H-2′,4′,6′), 7.93 (1H, d, J = 8.0 Hz, H-7), 8.13 (1H, d, J = 8.0 Hz, H-5), 8.11−8.16 (2H, m, H-3′,5′). Anal. ($C_{15}H_{11}NOS$) C, H, N.

6-Amino-2-phenylthiochromen-4-one (16): yield 35%; colorless needles; mp 252−253 °C; IR (KBr) ν 3350 and 3250 (NH_2), 1610 (shoulder, conjugated C═O); ^{1}H NMR ($CDCl_3$) δ 6.80 (1H, s, H-3), 7.48 (1H, br d, J = 7.0 Hz, H-7), 7.49 (1H, d, J = 7.0 Hz, H-8), 7.54−7.58 (3H, m, H-2′,4′,6′), 7.61−7.63 (2H, m, H-3′,5′), 7.66 (1H, br s, H-5), 12.42 (1H, br s, N*H*). Anal. ($C_{15}H_{11}NOS$) C, H, N.

6-Mercapto-2-phenyl-4-quinolone (17): yield 24%; fine crystals; mp 263−264 °C; IR (KBr) ν 1610 (shoulder, conjugated C═O); ^{1}H NMR ($CDCl_3$:CD_3OD = 5:1) δ 6.61 (1H, s, H-3), 7.55−7.57 (3H, m, H-2′,4′,6′), 7.61 (1H, br d, J = 8.0 Hz, H-7), 7.66 (1H, d, J = 8.0 Hz, H-8), 7.75−7.77 (2H, m, H-3′,5′), 8.22 (1H, br s, H-5). Anal. ($C_{15}H_{11}NOS$) C, H.

5-Mercapto-2-phenyl-4-quinolone (18): yield 49%; orange crystals; mp 141−142 °C; IR (KBr) ν 1610 (shoulder, conjugated C═O); ^{1}H NMR ($CDCl_3$) δ 7.12 (1H, s, H-3), 7.15 (1H, d, J = 8.0 Hz, H-6), 7.18 (1H, d, J = 8.0 Hz, H-8), 7.45 (1H, t, J = 8.0 Hz, H-7), 7.52−7.55 (3H, m, H-2′,4′,6′), 7.67−7.70 (2H, m, H-3′,5′). Anal. ($C_{15}H_{11}NOS$) C, H, N.

5-Amino-2-phenylthiochromen-4-one (19): yield 21%; fine crystals; mp >300 °C dec; IR (KBr) ν 3350 and 3250 (NH_2), 1610 (shoulder, conjugated C═O); ^{1}H NMR (DMSO-d_6) δ 6.40 (1H, s, H-3), 7.41 (1H, d, J = 7.5 Hz, H-6), 7.51 (1H, t, J = 7.5 Hz, H-7), 7.56 (1H, d, J = 7.5 Hz, H-8), 7.60−7.61 (3H, m, H-2′,4′,6′), 7.83−7.86 (2H, m, H-3′,5′). Anal. ($C_{15}H_{11}NOS$) C, H, N.

Biological Assays. The *in vitro* cytotoxicity assay was carried out according to procedures described in Geran et al.[11] and Ferguson et al.[12] The assay against KB (nasal pharyngeal carcinoma), A-549 (human lung cancer), HCT-8 (human colon carcinoma), PRMI-7951 (human melanoma), TE-671 (human medulloblastoma), and P-388 (murine leukemia) tumor cells was based on a method reported in Lee et al.[13]

Assays for the *in vitro* inhibition of DNA topoisomerases I and II and for production of cellular protein-linked DNA breaks were carried out according to the procedures described previously.[14,15]

HIV inhibition was measured as described previously.[16,17] The H9 T cell line was maintained in continuous culture with complete medium (RPMI 1640 and 10% fetal calf serum) at 5% CO_2 and 37 °C and was used in experiments only when in log phase of growth. The cells were incubated with HIV-1 (IIIB isolate, $TCID_{50}$ 10^4 IU/mL, at a multiplicity of infection of 0.1−0.01 IU/cell) for 1 h at 37 °C and 5% CO_2. The cells then were washed thoroughly to remove unabsorbed virus and resuspended at 4×10^5 cells/mL in complete medium. Aliquots (1 mL) were placed in wells of 24-well culture plates containing an equal volume of test compound (diluted in the culture medium). After a 4-day incubation at 37 °C, cell density of uninfected cultures was determined by counting cells in a Coulter counter to assess toxicity of the test compound. A p24 antigen ELISA assay was used to determine the level of virus released in the medium of the HIV-infected cultures. The p24 antigen assay uses an HIV-1 anti-p24 specific monoclonal antibody as the capture antibody coated on 96-well plates. Following a sample incubation period, rabbit serum containing antibodies for HIV-1 p24 is used to tag any p24 "captured" onto the microtiter well surface. Peroxidase conjugated goat anti-rabbit serum is then used to tag HIV-1 p24 specific rabbit antibodies that have complexed with captured p24. The presence of p24 in test samples is then revealed by addition of substrate. The cutoff for the p24 ELISA assay is 12.5 pg/mL. P24 in the culture medium was quantitated against a standard curve containing known amounts of p24. The effective (EC_{50}) and inhibitory (IC_{50}) concentrations (for anti-HIV activity and cytotoxicity, respectively) were determined.

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