Synthesis and Cytotoxic Activity of Pyranocoumarins of the Seselin and Xanthyletin Series

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The synthesis of known (**3**−**6**) and new (**7**−**10** and **14**−**22**) coumarins in the seselin and xanthyletin series is described. The cytotoxic activity of compounds **3**−**22** was carried out *in vitro* on L-1210 cells. The most active compounds were **9**, **16**, **18**, and **20** in the seselin series and **10**, **17**, and **19** in the xanthyletin series. Structure−activity relationships are discussed.

Coumarins constitute a major class of widely distributed O-heterocyclic natural products exhibiting a broad pharmacological profile,[1] including anticancer activity.[2] Naturally occurring pyranocoumarins show interesting cytotoxic[3−5] and antitumor-promoting activity.[5] Moreover, seselin (**1**) and xanthyletin (**2**) have been reported to act as DNA-damaging agents,[6] and 3′,4′-dihydroxy-3′,4′-dihydroseselin derivatives were found to display significant cytotoxic activity against P-388 lymphocytic leukemia.[3,4] As part of our research program on antitumor drugs from natural products, we synthesized known and new derivatives in the seselin and xanthyletin series.

Results and Discussion

The previously described[7−9] diols **3** and **4** have been obtained by catalytic osmium oxidation of **1** and **2**, respectively, using *N*-methylmorpholine *N*-oxide to regenerate the oxidizing agent (Scheme 1). Treatment of *cis* diols **3** and **4** with excess Ac_2O in pyridine afforded the corresponding diesters[8−10] **5** and **6**.

(±)-*trans*-4′-Hydroxy-3′-bromo-3′,4′-dihydroseselin (**7**) and (±)-*trans*-4′-hydroxy-3′-bromo-3′,4′-dihydroxanthyletin (**8**) were obtained by treatment of **1** and **2**, respectively, with NBS in aqueous THF solution.[11] The bromohydrins **7** and **8** were smoothly debrominated with tributyltin hydride,[11] to **9** and **10**, respectively. It is interesting to point out that 4′-hydroxy-3′,4′-dihydroseselin (**9**) has recently been described as praeroside V aglycon,[12] the enzymatic hydrolysis product of praeroside V (**11**), a new natural coumarin glycoside isolated from the roots of *Peucedanum praeruptorum*. However, careful examination of ^{1}H (Table 1) and ^{13}C NMR (Table 2) spectra of compound **9**, praeroside V (**11**), and praeroside V aglycon showed significant differences, particularly with respect to the signals of dihydropyran ring.

The ^{1}H NMR spectrum of **9** showed characteristic signals at $\delta = 5.02$ (1H, ddd, $J = 4.8$, 4.4, 3.0 Hz, dd, $J = 4.8$, 3.0 Hz after addition of D_2O), 5.30 (1H, d, $J = 4$ Hz, exch with D_2O), 2.05 (1H, dd, $J = 14.6$, 3.0 Hz) and $\delta = 1.93$ (1H, dd, $J = 14.6$, 4.8 Hz), which are in agreement with those described for other natural and hemisynthetic products containing the dimethylpyrano ring system[11,13] of **12** and **13**. The ^{13}C NMR spectrum of **9** also showed a signal pattern of the dimethylpyrano ring similar to that of **12**. On the basis of these results, the structure of praeroside V should be revised.

Treatment of bromohydrins **7** and **8** with excess Ac_2O or benzoic anhydride in pyridine afforded the corresponding esters **14**−**17**. In a similar way, alcohols **9** and **10** afforded the corresponding esters **18**−**21**. Finally, carbamate **22** was obtained by treatment of **7** with excess chloroethyl isocyanate in pyridine.

The cytotoxic activity of compounds **3**−**22** was measured *in vitro* using L-1210 leukemia.[14,15] The results (IC_{50}) are reported in Table 3. The most active compounds were **9**, **16**, **18**, and **20** in the seselin series and **10**, **17**, and **19** in the xanthyletin series. The perturbation of the cell cycle induced by the most active compounds was studied using the same cell line. Compounds **16** and **18** induced a partial accumulation of cells in the G_1 phase of the cell cycle. In contrast, the cell cycle was not modified by the most potent compounds **9**, **10**, and **19**. Considering the structure−cytotoxic activity relationships, it appears (with one exception) that compounds of the seselin series exhibit significantly more potent activity than compounds of the xanthyletin series. It appears also that compounds bearing a hydroxy or acetoxy group at the 4′-position and compounds bearing a bromo group at the 3′-position and a benzoxy group at the 4′-position are the most potent in inhibiting cell proliferation.

Experimental Section

General Experimental Procedures. Spectra were recorded on the following apparatus: MS, Nermag R10-10C in desorption−chemical ionization, using NH_3 as reagent gas; NMR, Bruker AC200, ^{1}H NMR (200 MHz),^{13}C NMR (50 MHz) and a Bruker DRX400, ^{1}H NMR (400 MHz). Chemical shifts are given in δ with TMS as an internal standard. Coupling constants (J) are given in Hz. The signals of ^{1}H and ^{13}C spectra were unambiguously assigned by using 2D NMR techniques: ^{1}H−^{1}H-COSY, ^{13}C−^{1}H HETCOR, and HMBC. These 2D experiments were performed using standard

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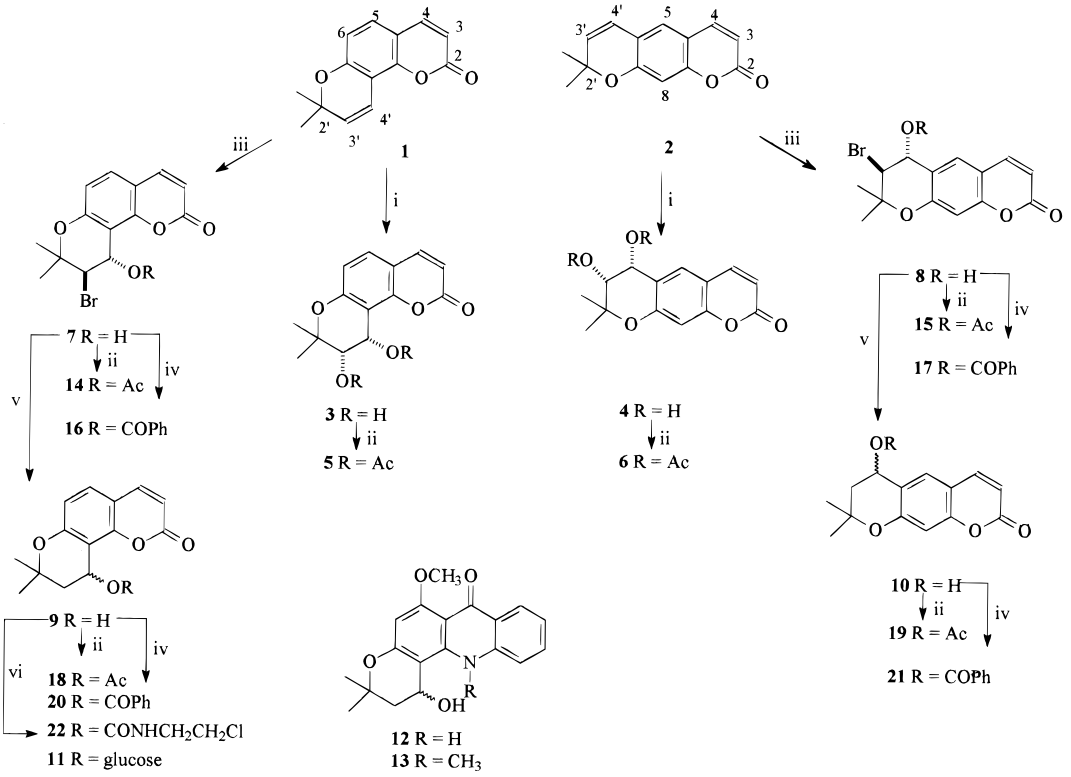
Scheme 1[a]



[a] Key: (i) OsO_4, *N*-methylmorpholine *N*-oxide, *t*-BuOH, THF, H_2O, rt; (ii) Ac_2O, Py, rt; (iii) NBS, THF, H_2O, 0 °C; (iv) $(PhCO)_2O$, Py, rt; (v) Bu_3SnH, AIBN, toluene, 110 °C; (vi) $ClCH_2CH_2CNO$, Py, Me_2CO, rt.

Table 1. ^1H NMR (DMSO/TMS, 200 MHz, δ (ppm), *J* (Hz)

atom	9	**11** (praeroside V)[a]	praeroside V aglycon[a]
3	6.26, d, $J = 9.5$	6.23, d, $J = 9.5$	6.14, d, $J = 9.5$
4	7.97, d, $J = 9.5$	7.94, d, $J = 9.5$	7.89, d, $J = 9.5$
5	7.51, d, $J = 8.5$	7.43, d, $J = 8.5$	7.35, d, $J = 8.5$
6	6.77, d, $J = 8.5$	6.88, d, $J = 8.5$	6.79, d, $J = 8.5$
3′a	2.05, dd, $J = 3.0, 14.6$	3.01, d, $J = 6.1$ (2H: 3′a, 3′b)	2.93, dd, $J = 2.4, 13.6$ (2H: 3′a, 3′b)
3′b	1.93, dd, $J = 4.8, 14.6$		
4′	5.02, ddd, $J = 4.8, 4.4, 3.0$	3.92, t, $J = 6.1$	3.52, dd, $J = 2.4, 10.1$
Me	1.44 s	1.22 s	1.13, s (6H: Me1, Me2)
Me	1.37 s	1.17 s	
4′-OH	5.30, d, $J = 4.4$	G-1: 4.09, d, $J = 7.6$	

[a] From ref 12.

Table 2. ^{13}C NMR (DMSO/TMS, 50 MHz, δ ppm)

atom	**9**	**11**[a,b] (praeroside V)	praeroside V aglycon[a]
2	160.95	160.55	160.68
3	111.89	112.36	112.72
4	144.23	144.90	144.91
5	128.18	127.01	126.64
6	114.91	113.07	114.25
7	157.01	159.19	159.22
8	112.06	111.10	110.13
9	154.19	153.92	153.79
10	114.91	111.58	110.89
2′	76.24	71.58	71.77
3′	40.18	24.84	24.96
4′	59.02	85.04	76.94
Me	26.45	25.44	25.16
Me	27.95	25.94	25.65

[a] From ref 12. [b] Carbons of the glucose moiety are not mentioned.

Table 3. Cytotoxic Activity[a]

compd	IC_{50}, μM
3	>100
4	>100
5	>100
6	>100
7	>100
8	>100
9	0.9
10	6.5
14	60.3
15	>100
16	10.3
17	36.5
18	24.7
19	5.0
20	30.9
21	90.1
22	67.2

[a] Inhibition of L-1210 cell proliferation measured by the MMT assay (mean of 2 values obtained in independent experiments).

Bruker microprograms. Column chromatography was conducted using flash Si gel 60 Merck (40−63 μm), with an overpressure of 300 mbar. Medium-pressure liquid chromatography (MPLC) was performed with a Büchi model 688 apparatus on columns containing Si gel 60

Merck (20−40 μm). All new compounds gave satisfactory combustion analyses (C, H, N, within ±0.4% of calculated values). The murine leukemia L-1210 cell line was from the American Type Culture Collection (Rockville Pike, MD).

(±)-*cis*-3′,4′-Dihydroxy-3′,4′-dihydroseselin (3). Compound **3** was synthesized according to the published procedure.[10] ^{1}H and ^{13}C NMR.[10,16]

(±)-*cis*-3′,4′-Dihydroxy-3′,4′-dihydroxanthyletin (4). Treatment of **2** under conditions essentially the same as those described for the preparation of **3** afforded **4**: ^{1}H NMR (DMSO, 200 MHz) δ 1.25 (3H, s, Me), 1.40 (3H, s, Me), 3.63 (1H, t, J = 4.2 Hz, H-3′), 4.75 (1H, dd, J = 7.9, 4.2 Hz, H-4′), 5.09 (1H, d, J = 4.2 Hz, OH-3′), 5.34 (1H, d, J = 7.9 Hz, OH-4′), 6.22 (1H, d, J = 9.5 Hz, H-3), 6.60 (1H, s, H-8), 7.75 (1H, s, H-5), 8.00 (1H, d, J = 9.5 Hz, H-4); ^{13}C NMR (DMSO, 50 MHz) δ 24.45 (Me), 24.88 (Me), 63.62 (C-4′), 69.86 (C-3′), 79.81 (C-2′), 102.41 (C-8), 112.08 (C-3,7), 122.06 (C-6), 128.51 (C-5), 144.60 (C-4), 154.17 (C-9), 156.37 (C-10), 160.49 (C-2); MS-DCI m/z 280 $(M + NH_4)^+$.

(±)-*cis*-3′,4′-Diacetoxy-3′,4′-dihydroseselin (5). To a solution of **3** (20 mg, 0.08 mmol) in dry pyridine (1.5 mL) was added Ac_2O (1.5 mL, 15 mmol). The reaction mixture was stirred for 24 h at room temperature, and then the reagents were removed under reduced pressure (using a high-vacuum pump). The residue was compound **5** (24 mg, 92%): ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.33 (3H, s, Me), 1.37 (3H, s, Me), 2.05 (3H, s, CH_3CO), 2.08 (3H, s, CH_3CO), 5.22 (1H, d, J = 5 Hz, H-3′), 6.16 (1H, d, J = 9.5 Hz, H-3), 6.45 (1H, d, J = 5 Hz, H-4′), 6.73 (1H, d, J = 8.5 Hz, H-6), 7.32 (1H, d, J = 8.5 Hz, H-5), 7.57 (1H, d, J = 9.5 Hz, H-4); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 20.46 (1-*C*H_3CO, 2-*C*H_3CO), 22.42 (Me), 24.57 (Me), 60.68 (C-4′), 69.95 (C-3′), 77.27 (C-2′), 106.51 (C-8), 112.39 (C-10), 112.86 (C-3), 114.25 (C-6), 129.17 (C-5), 143.31 (C-4), 153.67 (C-9), 156.37 (C-7), 159.79 (C-2), 169.83 (1-CH_3*C*O, 2-CH_3*C*O); MS-DCI m/z 364 $(M + NH_4)^+$.

(±)-*cis*-3′,4′-Diacetoxy-3′,4′-dihydroxanthyletin (6). Treatment of **4** (20 mg, 0.08 mmol) under conditions essentially similar to those described for the preparation of **5** afforded compound **6** (24 mg, 92%): ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.41 (6H, s, Me), 2.04 (3H, s, CH_3CO), 2.13 (3H, s, CH_3CO), 5.32 (1H, d, J = 4 Hz, H-3′), 6.16 (1H, d, J = 4 Hz, H-4′), 6.24 (1H, d, J = 9.5 Hz, H-3), 6.78 (1H,s, H-8), 7.28 (1H, s, H-5), 7.59 (1H, d, J = 9.5 Hz, H-4);^{13}C NMR ($CDCl_3$, 50 MHz) δ 20.60 (*C*H_3CO), 20.86 (*C*H_3CO), 24.37 (Me), 24.61 (Me), 64.83 (C-4′), 68.99 (C-3′), 77.99 (C-2′), 104.67 (C-8), 113.05 (C-7), 113.73 (C-3), 115.31 (C-6), 127.26 (C-5), 143.06 (C-4), 155.21 (C-9), 156.24 (C-10), 160.87 (C-2), 170.41 (CH_3*C*O), 170.61 (CH_3*C*O); MS-DCI m/z 364 $(M + NH_4)^+$.

(±)-*trans*-3′-Bromo-4′-hydroxy-3′,4′-dihydroseselin (7). To a solution of **1** (145 mg, 0.64 mmol) in THF (5 mL) and H_2O (5 mL) was added *N*-bromosuccinimide (117 mg, 0.66 mmol). The reaction mixture was stirred for 4 h at 0 °C, the reaction mixture was extracted with NaCl (saturated)−Et_2O, and the organic layer was collected. The solvent was removed under reduced pressure, and compound **7** was purified by crystallization with Et_2O (170 mg, 87%): ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.52 (3H, s, Me), 1.64 (3H, s, Me), 4.02 (1H, d, J = 5 Hz, OH-4′), 4.30 (1H, d, J = 5 Hz, H-3′), 5.41 (1H, t, J = 5 Hz, H-4′), 6.29 (1H, d, J = 9.5 Hz, H-3), 6.82 (1H, d, J = 8.5 Hz, H-6), 7.36 (1H, d, J = 8.5 Hz, H-5), 7.67 (1H, d, J = 9.5 Hz, H-4);^{13}C NMR ($CDCl_3$, 50 MHz) δ 24.27 (Me), 26.25 (Me), 57.53 (C-3′), 67.12 (C-4′), 78.85 (C-2′), 110.45 (C-8), 112.66 (C-3), 113.43 (C-10), 114.72 (C-6), 128.76 (C-5), 144.01 (C-4), 153.93 (C-9), 155.66 (C-7), 160.59 (C-2); MS-DCI m/z 344 $(M + NH_4)^+$, 342 $(M + NH_4)^+$.

(±)-*trans*-3′-Bromo-4′-hydroxy-3′,4′-dihydroxanthyletin (8). Treatment of **2** (145 mg, 0.64 mmol) under conditions essentially the same as those described for the preparation of **7** afforded compound **8** (160 mg, 83%): ^{1}H NMR (DMSO, 200 MHz) δ 1.45 (3H, s, Me), 1.60 (3H, s, Me), 4.30 (1H, d, J = 8 Hz, H-3′), 4.80 (1H, t, J = 8 Hz, H-4′), 6.30 (1H, d, J = 9.5 Hz, H-3), 6.78 (1H, s, H-8), 7.75 (1H, s, H-5), 8.04 (1H, d, J = 9.5 Hz, H-4);^{13}C NMR (DMSO, 50 MHz) δ 21.87 (Me), 27.59 (Me), 60.99 (C-3′), 68.05 (C-4′), 79.71 (C-2′), 103.22 (C-8), 113.04 (C-3,7), 121.91 (C-6), 129.04 (C-5), 144.25 (C-4), 154.31 (C-9), 154.90 (C-10), 160.11 (C-2); MS-DCI m/z 344 $(M + NH_4)^+$, 342 $(M + NH_4)^+$.

(±)-*trans*-3′-Bromo-4′-acetoxy-3′,4′-dihydroseselin (14). Treatment of **7** (31 mg, 0.09 mmol) under conditions essentially the same as those described for the preparation of **5** afforded **14** (33 mg, 95%): ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.57 (6H, s, Me), 2.13 (3H, s, CH_3CO), 4.31 (1H, d, J = 4.5 Hz, H-3′), 6.23 (1H, d, J = 9.5 Hz, H-3), 6.49 (1H, d, J = 4.5 Hz, H-4′), 6.80 (1H, d, J = 8.5 Hz, H-6), 7.37 (1H, d, J = 8.5 Hz, H-5), 7.60 (1H, d, J = 9.5 Hz, H-4);^{13}C NMR ($CDCl_3$, 50 MHz) δ 20.79 (*C*H_3CO), 25.36 (Me), 26.13 (Me), 53.96 (C-3′), 66.82 (C-4′), 77.93 (C-2′), 105.94 (C-8), 112.63 (C-10), 113.27 (C-3), 114.48 (C-6), 129.35 (C-5), 143.34 (C-4), 153.87 (C-9), 156.22 (C-7), 159.88 (C-2), 169.82 (CH_3*C*O); MS-DCI m/z 386 $(M + NH_4)^+$, 384 $(M + NH_4)^+$.

(±)-*trans*-3′-Bromo-4′-acetoxy-3′,4′-dihydroxanthyletin (15). Treatment of **8** (20 mg, 0.06 mmol) under conditions essentially the same as those described for the preparation of **5** afforded **15** (21 mg, 95%): ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.50 (3H, s, Me), 1.62 (3H, s, Me), 2.21 (3H, s, CH_3CO), 4.22 (1H, d, J = 7.8 Hz, H-3′), 6.23 (1H, d, J = 9.5 Hz, H-3), 6.26 (1H, d, J = 7.8 Hz, H-4′), 6.76 (1H, s, H-8), 7.23 (1H, s, H-5), 7.57 (1H, d, J = 9.5 Hz, H-4); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 21.06 (*C*H_3-CO), 22.26 (Me), 27.46 (Me), 54.87 (C-3′), 70.38 (C-4′), 79.65 (C-2′), 105.00 (C-8), 113.45 (C-7), 114.12 (C-3), 116.91 (C-6), 128.11 (C-5), 142.93 (C-4), 155.36 (C-9), 155.80 (C-10), 160.58 (C-2), 170.87 (CH_3*C*O); MS-DCI m/z 386 $(M + NH_4)^+$, 384 $(M + NH_4)^+$.

(±)-*trans*-3′-Bromo-4′-benzoyloxy-3′,4′-dihydroseselin (16). To a solution of **7** (31 mg, 0.09 mmol) in dry pyridine (1.5 mL) was added benzoic anhydride (62 mg, 0.29 mmol). The reaction mixture was stirred for 48 h at room temperature, and then the reagents were removed under reduced pressure (using a high-vacuum pump). The residue was extracted with EtOAc−$NaHCO_3$ (saturated), and the organic layer was collected. The solvent was removed under reduced pressure, and the remaining residue was purified by flash chromatography on Si gel with cyclohexane−EtOAc (90:10) to give compound **16** (31 mg, 76%): ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.67 (6H, s, Me), 4.52 (1H, d, J = 3 Hz, H-3′), 6.23 (1H, d, J = 9.5 Hz, H-3), 6.78 (1H, d, J = 3,

H-4′), 6.90 (1H, d, J = 8.5 Hz, H-6), 7.41 (2H, tt, J = 7, 1.5 Hz, H-3″, 5″) 7.44 (1H, d, J = 8.5 Hz, H-5), 7.55 (1H, tt, J = 7, 1.5 Hz, H-4″), 7.63 (1H, d, J = 9.5 Hz, H-4), 8.02 (2H, dt, J = 7, 1.5 Hz, H-2″, 6″); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 24.76 (Me), 28.03 (Me), 53.48 (C-3′), 67.17 (C-4′), 77.00 (C-2′), 106.00 (C-8), 112.65 (C-10), 113.48 (C-3), 114.58 (C-6), 128.48 (C-3″, 5″), 129.50 (C-1″), 129.55 (C-2″, 6″), 129.85 (C-5), 133.36 (C-4″), 143.18 (C-4), 154.37 (C-9), 156.38 (C-7), 159.77 (C-2), 165.05 (*O*C*O*); MS-DCI m/z 448 $(M + NH_4)^+$, 446 $(M + NH_4)^+$.

(±)-*trans*-3′-Bromo-4′-(benzoyloxy)-3′,4′-dihydroxanthyletin (17). Treatment of compound **8** (31 mg, 0.09 mmol) under conditions essentially the same as those described for the preparation of **16** afforded compound **17** (29 mg, 71%): ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.58 (3H, s, Me), 1.70 (3H, s, Me), 4.41 (1H, d, J = 7.5 Hz, H-3′), 6.23 (1H, d, J = 9.5, H-3), 6.52 (1H, d, J = 7.5 Hz, H-4′), 6.82 (1H, s, H-8), 7.35 (1H, s, H-5), 7.46 (2H, tt, J = 7, 1.5 Hz, H-3″, 5″), 7.54 (1H, d, J = 9.5 Hz, H-4), 7.61 (1H, tt, J = 7, 1.5 Hz, H-4″), 8.07 (2H, dt, J = 7, 1.5 Hz, H-2″, 6″); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 22.99 (Me), 27.27 (Me), 54.73 (C-3′), 70.83 (C-4′), 79.43 (C-2′), 105.07 (C-8), 113.43 (C-7), 114.10 (C-3), 116.66 (C-6), 128.64 (C-5, 5″, 3″), 129.00 (C-1″), 130.00 (C-2″, 6″), 133.82 (C-4″), 143.00 (C-4), 155.43 (C-9), 155.95 (C-10), 160.64 (C-2), 166.24 (*O*C*O*); MS-DCI m/z 448 $(M + NH_4)^+$, 446 $(M + NH_4)^+$.

(±)-4′-Hydroxy-3′,4′-dihydroseselin (9). Compound **7** (90 mg,0.28 mmol) was dissolved in anhydrous toluene (10 mL), and the solution was refluxed for 15 min under argon. Then AIBN (azo-bis-2,2′(methyl-2-propionitrile)) (9 mg) was added, and after 5 min a solution of tributyltin hydride (0.5 mL in 4 mL of toluene) was added for 40 min. The reaction mixture was refluxed for 1 h. Then the solvent was evaporated and the residue was purified by flash chromatography on Si gel with cyclohexane−EtOAc (70:30) to give compound **6** (55 mg, 78%): ^{1}H NMR (DMSO, 200 MHz) Table 1; ^{13}C NMR (DMSO, 50 MHz) Table 2; MS-DCI m/z 264 $(M + NH_4)^+$.

(±)-4′-Hydroxy-3′,4′-dihydroxanthyletin (10). Treatment of compound **8** (90 mg, 0.28 mmol) under conditions essentially the same as those described for the preparation of **9** afforded **10** (50 mg, 71%): ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.30 (3H, s, Me), 1.47 (3H, s, Me), 1.87 (1H, dd, J = 14, 8 Hz, H-3′a), 2.20 (1H, dd, J = 14, 6 Hz, H-3′b), 2.74 (1H, d, J = 8 Hz,OH-4′), 4.85 (1H, dd, J = 14, 8 Hz, H-4′), 6.08 (1H, d, J = 9.5 Hz, H-3), 6.60 (1H, s, H-8), 7.53 (1H, d, J = 9.5 Hz, H-4), 7.57 (1H, s, H-5); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 25.67 (Me), 29.21 (Me), 42.24 (C-3′), 62.81 (C-4′), 77.28 (C-2′), 104.24 (C-8), 112.40 (C-7), 112.64 (C-3), 122.59 (C-6), 127.35 (C-5), 143.67 (C-4), 154.69 (C-9), 156.97 (C-10), 161.68 (C-2); MS-DCI m/z 264 $(M + NH_4)^+$.

(±)-4′-Acetoxy-3′,4′-dihydroseselin (18). Treatment of compound **9** (31 mg, 0.13 mmol) under conditions essentially the same as those described for the preparation of **5** afforded **18** (34 mg, 95%): ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.40 (6H, s, Me), 2.05 (3H, s, CH_3CO), 2.15 (2H, d, J = 5 Hz, H-3′a, 3′b), 6.20 (1H, d, J = 9.5 Hz, H-3), 6.25 (1H, d, J = 5 Hz, H-4′), 6.75 (1H, d, J = 8.5 Hz, H-6), 7.31 (1H, d, J = 8.5 Hz, H-5), 7.58 (1H, d, J = 9.5 Hz, H-4);^{13}C NMR ($CDCl_3$, 50 MHz) δ 21.09 (*C*H_3CO), 25.9 (Me), 28.48 (Me), 38.33 (C-3′), 61.21 (C-4′), 75.78 (C-2′), 107.56 (C-8), 112.07 (C-10), 112.77 (C-3), 114.75 (C-6), 128.98 (C-5), 143.54 (C-4), 153.98 (C-9), 157.71 (C-7), 160.42 (C-2), 170.36 (CH_3*C*O); MS-DCI m/z 306 $(M + NH_4)^+$.

(±)-4′-Acetoxy-3′,4′-dihydroxanthyletin (19). Treatment of **10** (14 mg, 0.06 mmol) under conditions essentially the same as those described for the preparation of **5** afforded compound **19** (16 mg, 95%). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.40 (3H, s, Me), 1.45 (3H, s, Me), 1.99 (1H, dd, J = 14, 6 Hz, H-3′a), 2.13 (3H, s, CH_3CO), 2.23 (1H, dd, J = 14, 6 Hz, H-3′b), 6.00 (1H, t, J = 6 Hz, H-4′), 6.21 (1H, d, J = 9.5 Hz, H-3), 6.73 (1H, s, H-8), 7.35 (1H, s, H-5), 7.58 (1H, d, J = 9.5 Hz, H-4); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 21.33 (*C*H_3CO), 27.15 (Me), 27.61 (Me), 38.34 (C-3′), 64.99 (C-4′), 77.20 (C-2′), 104.87 (C-8), 112.69 (C-7), 113.42 (C-3), 117.43 (C-6), 128.53 (C-5), 143.24 (C-4), 155.28 (C-9), 157.37 (C-10), 161.02 (C-2), 170.87 (CH_3*C*O); MS-DCI m/z 306 $(M + NH_4)^+$.

(±)-4′-Benzoyloxy-3′,4′-dihydroseselin (20). Treatment of **9** (31 mg, 0.13 mmol) under conditions essentially the same as those described for the preparation of **16** afforded compound **20** (31 mg, 68%): ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.67 (6H, s, Me), 4.52 (2H, d, J = 3 Hz, H-3′a, 3′b), 6.23 (1H, d, J = 9.5 Hz, H-3), 6.78 (1H, d, J = 3 Hz, H-4′), 6.90 (1H, d, J = 8.5 Hz, H-6), 7.41 (2H, tt, J = 7, 1.5 Hz, H-3″, 5″) 7.44 (1H, d, J = 8.5 Hz, H-5), 7.55 (1H, tt, J = 7, 1.5 Hz, H-4″), 7.63 (1H, d, J = 9.5 Hz, H-4), 8.02 (2H,dt, J = 7, 1.5 Hz, H-2″, 6″); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 25.60 (Me), 29.20 (Me), 38.35 (C-3′), 61.50 (C-4′), 75.57 (C-2′), 107.44 (C-8), 111.97 (C-10), 112.93 (C-3), 114.75 (C-6), 128.33 (C-3″, 5″), 129.10 (C-5), 129.70 (C-2″, 6″), 130.09 (C-1″), 132.95 (C-4″), 143.32 (C-4), 154.44 (C-9), 157.79 (C-7), 160.21 (C-2), 165.59 (*O*C*O*); MS-DCI m/z 368 $(M + NH_4)^+$.

(±)-4′-Benzoyloxy-3′,4′-dihydroxanthyletin (21). Treatment of **10** (31 mg, 0.13 mmol) under conditions essentially the same as those described for the preparation of **16** afforded compound **21** (31 mg, 68%): ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.48 (3H, s, Me), 1.58 (3H, s, Me), 2.15 (1H, dd, J = 14, 6 Hz, H-3′a), 2.28 (1H, dd, J = 14, 6 Hz, H-3′), 6.21 (1H, d, J = 9.5 Hz, H-3), 6.29 (1H, t, J = 6 Hz, H-4′), 6.78 (1H, s, H-8), 7.44 (1H, s, H-5), 7.46 (2H, tt, J = 7, 1.5 Hz, H-3″, 5″), 7.56 (1H, tt, J = 7, 1.5 Hz, H-4″), 7.56 (1H, d, J = 9.5 Hz, H-4), 8.03 (2H, dt, J = 7, 1.5 Hz, H-2″, 6″); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 27.49 (Me, Me), 38.55 (C-3′), 65.39 (C-4′), 77.00 (C-2′), 105.00 (C-8), 112.81 (C-7), 113.46 (C-3), 117.44 (C-6), 128.50 (C-1″), 128.53 (C-5″, 3″), 128.96 (C-5), 129.70 (C-2″, 6″), 133.43 (C-4″), 143.30 (C-4), 155.36 (C-9), 157.48 (C-10), 161.02 (C-2), 166.31 (*O*C*O*); MS-DCI m/z 368 $(M + NH_4)^+$.

(±)-4′-(2-Chloroethylcarbamate)-3′,4′-dihydroseselin (22). To a solution of **9** (30 mg, 0.12 mmol) in dry pyridine (1 mL) and dry Me_2CO was added 2-chloroethyl isocyanate (0.5 mL, 5.8 mmol). The reaction mixture was stirred for 24 h at room temperature, and then the reagents were removed under reduced pressure (using a high-vacuum pump). The residue was submitted to flash chromatography on Si with cyclohexane−EtOAc (95:5−50:50) to give compound **22** (18 mg, 50%): ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.42 (6H, s, Me), 2.14 (1H, dd, J = 15, 5 Hz, H-3′b), 2.26 (1H, dd, J = 15, 4 Hz, H-3′a), 3.60 (4H, m, H-6′,7′), 5.10 (1H, br, N−H), 6.20 (1H, d, J = 9.5 Hz, H-3), 6.20 (1H, dd, J = 5, 4 Hz,

H-4′), 6.74 (1H, d, $J = 8.5$ Hz, H-6), 7.30 (1H, d, $J = 8.5$ Hz, H-5), 7.57 (1H, d, $J = 9.5$ Hz, H-4); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 25.79 (Me), 28.57 (Me), 38.68 (C-3′), 42.88 (C-7′), 44.41 (C-6′), 61.94 (C-4′), 75.79 (C-2′), 107.85 (C-8), 111.96 (C-10), 112.79 (C-3), 114.73 (C-6), 128.90 (C-5), 143.44 (C-4), 153.50 (C-9), 155.68 (O*C*O), 157.60 (C-7), 160.32 (C-2); MS-DCI *m*/*z* 369 $(M + NH_4)^+$.

Cell Culture and Cytotoxicity. L-1210 cells were cultivated in RPMI 1640 medium (Gibco) supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 units/mL penicillin, 100 μg/mL streptomycin, and 10 mM HEPES buffer (pH = 7.4). Cytotoxicity was measured by the microculture tetrazolium assay.[14] Cells were exposed to graded concentrations of drug (nine serial dilutions in triplicate) for 48 h. Results are expressed as IC_{50}, the concentration needed to reduce by 50% the optical density of treated cells with respect to the optical density of untreated controls.

For the cell-cycle analysis, L-1210 cells (5×10^5 cells/mL) were incubated for 21 h with various concentrations of drugs. Cells were then fixed by 70% EtOH (v/v), washed, and incubated with PBS containing 100 μg/mL RNAse and 25 μg/mL propidium iodide for 30 min at 20 °C. For each sample, 10 000 cells were analyzed on an ATC3000 flow cytometer (Bruker, Wissenbourg, France).

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