Synthesis and Biological Evaluation of Flavanones and Flavones Related to Podophyllotoxin

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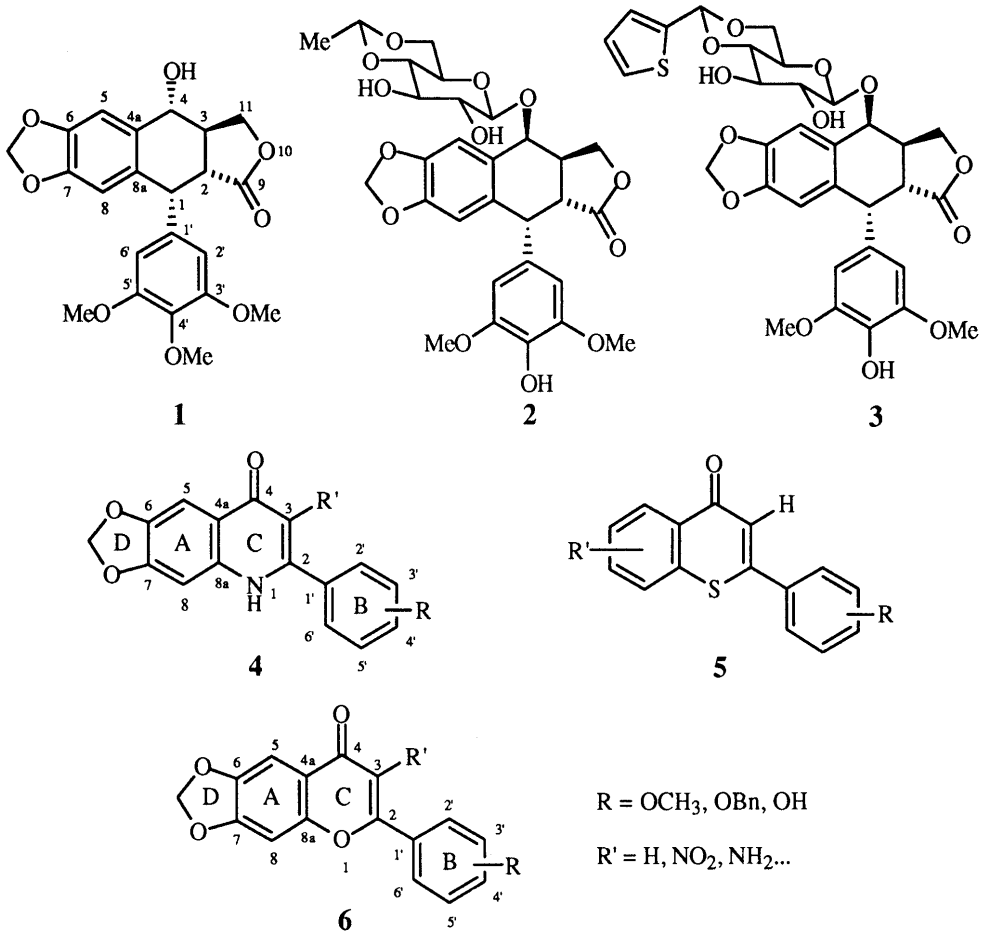
A series of novel flavonoids comprising structural elements present in the antineoplastic agents podophyllotoxin and etoposide was synthesized. These oxygen-containing analogues of antiproliferative quinolones exhibited moderate cytotoxicity towards L1210 and HT-29 cell lines.

Key words flavone; flavanone; podophyllotoxin analogue; quinolone analogue; cytotoxicity

Since the pioneering work of King and Sullivan in 1946[1] on the antitumor activity of podophyllotoxin (**1**), many derivatives of this lignan have been synthesized and examined as antiproliferative agents.[2] However, clinical trials of these compounds were disappointing because of severe side effects.[3] The related 4′-demethylepipodophyllotoxin derivatives were then developed, leading to potent drugs such as etoposide (VP16) (**2**) or teniposide (VM26) (**3**) which are still widely employed in cancer chemotherapy.[4] In contrast with the products derived from the podophyllotoxin framework (which act as inhibitors of mitosis by hindering microtubule polymerization[2c,5]), these epimeric analogues of **1** at the 4-position do not affect the cytoskeleton but induce dose-dependent DNA strand breakage associated with their ability to inhibit topoisomerase II.[6]

In the course of their search for new potential antitumor agents, Lee and co-workers have recently observed that 2-phenyl-4-quinolones (**4**) bearing a methylenedioxy group in the 6,7-positions and appropriate substituents on the aromatic B-ring exhibit a marked affinity for tubulin (involving the colchicine site, which is the site of action of podophyllotoxin).[7] The same group has also reported that several 2-phenylthiochromen-4-ones (**5**) are efficient *in vitro* inhibitors of DNA topoisomerase I or II.[8]

Here we describe the synthesis and evaluation of novel flavone derivatives as potential cytotoxic agents, based on the well-known fact that numerous flavonoids display antitumor effects.[9] The planned molecules (**6**) were designed to retain structural features considered to be



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important for activity, such as a methylenedioxy ring (D) in the 6,7-positions and an aromatic nucleus (B) bearing either three methoxy groups in the 3′-, 4′- and 5′-positions [analogues of podophyllotoxin (**1**)] or two methoxy substituents on the 3′- and 5′-carbons and a hydroxyl function in the 4′-position [analogues of etoposide (**2**)].

Chemistry

The new flavonoids described herein were prepared by adapting a methodology developed in our laboratory starting from 2-hydroxy-4,5-methylenedioxybenzaldehyde (**7**) and appropriately substituted *Z*-2-chloro-2-nitroethenylbenzenes (**8a** or **8b**),[10] as depicted in Chart 1.

The intermediate 7-chloro-6,7-dihydro-7-nitro-6-(3,4,5-trimethoxyphenyl)-8*H*-1,3-dioxolo[4,5-*g*][1]benzopyran-8-ol (**9a**) and 7-chloro-6-[3,5-dimethoxy-4-(phenylmethoxy)phenyl]-6,7-dihydro-7-nitro-8*H*-1,3-dioxolo[4,5-*g*][1]benzopyran-8-ol (**9b**) were further oxidized with pyridinium chlorochromate (PCC) into the corresponding 7-chloro-6,7-dihydro-7-nitro-6-phenyl-8*H*-1,3-dioxolo-[4,5-*g*][1]benzopyran-8-ones **10a** and **10b**, then basic treatment with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) afforded the 3-nitroflavones **11a** and **11b**.[11] Because of the sparing solubility of these nitro derivatives in solvents commonly employed to carry out catalytic hydrogenations, satisfactory yields of the desired amino derivative **12a** or of its 4′-deprotected congener **12c** have been obtained in an unusual but efficient way by performing the reaction in chloroform, starting from **11a** or **11b**, respectively.

The flavanone **13a** or **13b**, as well as flavone **14a** or **14b**, was prepared by reducing the *gem*-chloronitrobenzopyranone **10a** or **10b** with tri-*n*-butyltin hydride in a radical chain process promoted by 2,2′-azobisisobutyronitrile (AIBN) in refluxing benzene.[12] A large excess of tin reagent was employed in order to replace both the chloro and nitro substituents in the 3-position of **10** with hydrogen atoms to provide the flavanones **13**, whereas the synthesis of the flavones **14** was achieved by using only one equivalent of reducing agent to give mainly the intermediate 3-chloroflavanones, which were immediately converted without further purification into the desired products by treatment with DBU in tetrahydrofuran (THF) at room temperature.

The 4′-hydroxyflavanone **13c** was next easily obtained in good yield by deprotecting the corresponding benzyloxy derivative **13b** by catalytic hydrogenolysis at atmospheric pressure. Such a procedure could not be employed to obtain the 4′-hydroxyflavone **14c** starting from **14b** because of the reduction of the 2,3-double bond of the flavone. In this case, the desired compound **14c** was prepared in 75% yield by heating the benzyloxy precursor

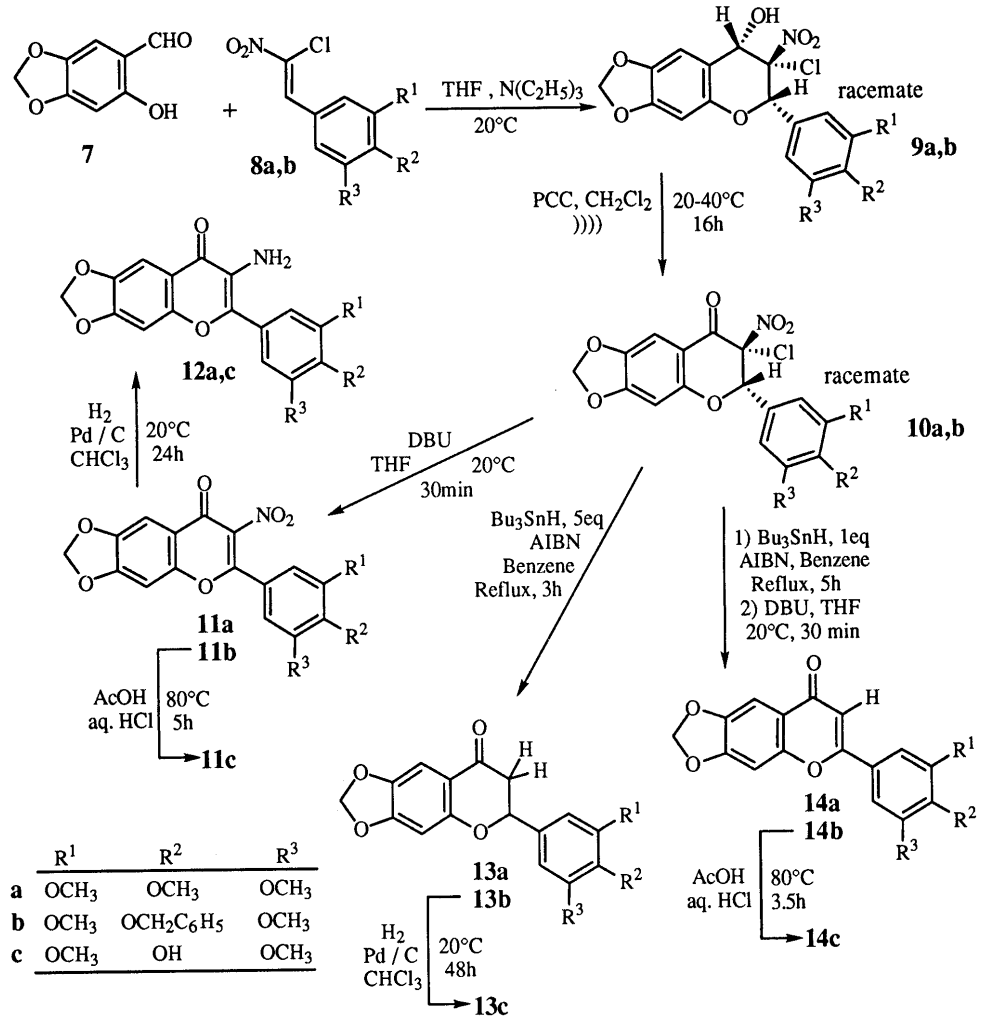

Chart 1

Table 1. Cytotoxicity of Compounds **10**—**14** on L1210 and HT-29 Cell Lines Compared to Adriamycin

Compounds	IC_{50} (μM)	
	L1210	HT-29
10a	6.0	9.8
10b	10.0	10.4
11a	22.1	23.6
11b	5.9	126.3
11c	19.2	99.6
12a	93.0	56.6
12c	24.3	81.4
13a	25.5	41.3
13b	9.5	39.1
13c	24.0	43.1
14a	90.9	169.5
14b	89.5	95.2
14c	54.8	80.4
Adriamycin	0.025	0.07

14b in a mixture of acetic acid and concentrated hydrochloric acid. For a similar reason, the 3-nitro-2-(3′,5′-dimethoxy-4′-hydroxyphenyl)flavone **11c** was obtained in 90% yield by employing the same method starting from the 4′-benzyloxyflavone **11b**.

Biological Results

The antiproliferative activities of the prepared compounds, assessed using murine L1210 leukemia and human HT-29 colon adenocarcinoma culture cells,[13] are summarized in Table 1. The results were rather disappointing since the most cytotoxic compounds on both cultures were the intermediate chloronitro derivatives **10a** and **10b**; the planned flavanones **13a**—**c** and flavones **14a**—**c** only induced weak growth inhibition. Furthermore, the best response obtained for the murine cell line was obtained with the 3-nitroflavone **11b**, which was one of the less active compounds against human HT-29 cell culture. It is also worth pointing out that **10a** showed no specificity on the L1210 cell cycle and proved to be toxic at a 50 μM concentration.

Experimental

Melting points were measured on a Köfler hot stage apparatus and are uncorrected. Mass spectra were obtained with a Nermag-Ribermag R10-10C spectrometer applying a desorption chemical ionization (CI) technique using ammonia as the reagent gas. Infrared spectra were obtained with a Perkin-Elmer 1710 spectrophotometer for chloroform solutions or KBr discs. The ^{1}H-NMR (300 MHz) spectra were recorded on a Bruker AC 300 spectrometer. Chemical shifts are expressed as parts per million downfield from tetramethylsilane. Splitting patterns have been designated as follows: s (singlet), d (doublet), dd (doublet of doublet), m (multiplet) and br (broad signal). Coupling constants (*J* values) are listed in hertz (Hz). Reactions were monitored by analytical thin layer chromatography and products were visualized by exposure to UV light. Merck Silica gel (230—400 Mesh ASTM) was used for column chromatography. Methanol and CH_2Cl_2 employed as eluents were distilled on a rotary evaporator prior to use. Anhydrous benzene was obtained by distillation from calcium hydride. Dry THF was prepared by distillation from benzophenone/sodium.

6-Hydroxy-1,3-benzodioxole-5-carboxaldehyde (7)[14] This starting aldehyde was prepared on a 0.5 mol scale in an improved 98% yield by formylating sesamol using 1,1-dichlorodimethyl ether[15] in the presence of $TiCl_4$ in CH_2Cl_2, as described in comparable procedures.[16] mp 125—126 °C (from heptane).

***Z*-5-(2-Chloro-2-nitroethenyl)-1,2,3-trimethoxybenzene (8a)**[10] This compound was synthesized according to the previously described method,[10] starting from the corresponding aldehyde.

***Z*-5-(2-Chloro-2-nitroethenyl)-1,3-dimethoxy-4-(phenylmethoxy)-benzene (8b)** This β-chloro-β-nitrostyrene was obtained following the same procedure as used for **8a**,[10] starting from the appropriate benzaldehyde. mp 112—113 °C (recrystallized from a benzene/heptane mixture); ^{1}H-NMR ($CDCl_3$) δ: 3.89 (s, 6H), 5.13 (s, 2H), 7.12 (s, 2H), 7.19—7.49 (m, 5H), 8.33 (s, 1H); MS *m/z*: 350, 352 $(M+H)^+$, 367, 369 $(M+NH_4)^+$. *Anal.* Calcd for $C_{17}H_{16}ClNO_5$: C, 58.38; H, 4.61; N, 4.00. Found: C, 58.12; H, 4.56; N, 4.01. The starting 3,5-dimethoxy-4-(phenylmethoxy)benzaldehyde. mp 64—65 °C (recrystallized from hexane) was easily prepared in 83% yield by refluxing a mixture of syringaldehyde and benzyl bromide in dry acetone for 30 h in the presence of K_2CO_3.

7-Chloro-6,7-dihydro-7-nitro-6-(3,4,5-trimethoxyphenyl)-8*H*-1,3-dioxolo[4,5-*g*][1]benzopyran-8-ol (9a) and 7-Chloro-6-[3,5-dimethoxy-4-(phenylmethoxy)phenyl]-6,7-dihydro-7-nitro-8*H*-1,3-dioxolo[4,5-*g*][1]-benzopyran-8-ol (9b) These benzopyrans were obtained, starting from 0.1 mol of the suitable (2-chloro-2-nitroethenyl)benzene **8a** or **8b** and aldehyde **7**, according to a reported procedure.[10] They were employed without purification[17] in the subsequent oxidation step.

7-Chloro-6,7-dihydro-7-nitro-6-(3,4,5-trimethoxyphenyl)-8*H*-1,3-dioxolo[4,5-*g*][1]benzopyran-8-one (10a) The crude **9a**, directly collected from the above reaction after evaporation of the volatile materials, was taken up in dry CH_2Cl_2 (200 ml). This compound was then oxidized using pyridinium chlorochromate in an ultrasonically-assisted process according to the experimental protocol previously reported by one of us.[11] Purification over a silica gel column (350 g, eluent CH_2Cl_2), followed by recrystallization from a benzene/heptane mixture afforded pure **10a** in 30% overall yield based on **8a**.[18] mp 133—135 °C; ^{1}H-NMR ($CDCl_3$) δ: 3.87 (s, 9H), 6.11 (s, 2H), 6.17 (s, 1H), 6.64 (s, 1H), 6.67 (s, 2H), 7.35 (s, 1H); IR *v*: 1696 cm^{-1}; MS *m/z*: 438, 440 $(M+H)^+$, 455, 457 $(M+NH_4)^+$. *Anal.* Calcd for $C_{19}H_{16}ClNO_9$: C, 52.13; H, 3.68; N, 3.20. Found: C, 52.32; H, 3.56; N, 3.12.

7-Chloro-6-[3,5-dimethoxy-4-(phenylmethoxy)phenyl]-6,7-dihydro-7-nitro-8*H*-1,3-dioxolo[4,5-*g*][1]benzopyran-8-one (10b) Analytically pure **10b** was obtained, in 35% overall yield based on **8b**,[18] from crude **9b** by a procedure similar to that described for **10a**. mp 131—132 °C (recrystallized from a benzene/heptane mixture); ^{1}H-NMR ($CDCl_3$) δ: 3.83 (s, 6H), 5.04 (s, 2H), 6.11 (s, 2H), 6.16 (s, 1H), 6.64 (s, 1H), 6.66 (s, 2H), 7.32—7.48 (m, 5H), 7.35 (s, 1H); IR *v*: 1696 cm^{-1}; MS *m/z*: 531, 533 $(M+NH_4)^+$. *Anal.* Calcd for $C_{25}H_{20}ClNO_9$: C, 58.43; H, 3.92; N, 2.73. Found: C, 58.70; H, 4.01; N, 2.55.

7-Nitro-6-(3,4,5-trimethoxyphenyl)-8*H*-1,3-dioxolo[4,5-*g*][1]benzopyran-8-one (11a) This nitroflavone was prepared from **10a** (876 mg, 2 mmol) according to the procedure detailed in reference 11. Yield 98% (787 mg); mp 233—234 °C (recrystallized from a benzene/heptane mixture); ^{1}H-NMR ($CDCl_3$) δ: 3.90 (s, 9H), 6.19 (s, 2H), 6.92 (s, 2H), 7.00 (s, 1H), 7.59 (s, 1H); IR *v*: 1646 cm^{-1}; MS *m/z*: 402 $(M+H)^+$. *Anal.* Calcd for $C_{19}H_{15}NO_9$: C, 56.86; H, 3.77; N, 3.49. Found: C, 56.97; H, 3.77; N, 3.31.

6-[3,5-Dimethoxy-4-(phenylmethoxy)phenyl]-7-nitro-8*H*-1,3-dioxolo-[4,5-*g*][1]benzopyran-8-one (11b) Compound **11b** was obtained from **10b** (1.028 g, 2 mmol) in a similar manner to that employed for **11a**. Yield 99% (945 mg); mp 204—205 °C (recrystallized from a benzene/heptane mixture); ^{1}H-NMR ($CDCl_3$) δ: 3.86 (s, 6H), 5.11 (s, 2H), 6.18 (s, 2H), 6.90 (s, 2H), 6.99 (s, 1H), 7.31—7.49 (m, 5H); 7.58 (s, 1H); IR *v*: 1651 cm^{-1}; MS *m/z*: 478 $(M+H)^+$. *Anal.* Calcd for $C_{25}H_{19}NO_9$: C, 62.89; H, 4.01; N, 2.93. Found: C, 63.05; H, 4.09; N, 3.01.

6-(4-Hydroxy-3,5-dimethoxyphenyl)-7-nitro-8*H*-1,3-dioxolo[4,5-*g*][1]-benzopyran-8-one (11c) A mixture of **11b** (477 mg, 1 mmol), acetic acid (12.5 ml) and 12 N hydrochloric acid (25 ml) was heated at 80 °C for 5 h using an oil bath. The mixture was evaporated to dryness under reduced pressure to afford a residue, which was successively taken up with water (3 × 30 ml), acetone (3 × 30 ml), then CH_2Cl_2 (30 ml). The obtained solid after removal of CH_2Cl_2 was washed in refluxing ethanol, filtered and dried *in vacuo* to give **11c** (367 mg, yield 95%), mp >260 °C; ^{1}H-NMR (DMSO-d_6) δ: 3.80 (s, 6H), 6.31 (s, 2H), 7.01 (s, 2H), 7.44 (s, 1H), 7.52 (s, 1H), OH indiscernible; IR (KBr) *v*: 1637 cm^{-1}; MS *m/z*: 388 $(M+H)^+$. *Anal.* Calcd for $C_{18}H_{13}NO_9$: C, 55.82; H, 3.38; N, 3.62. Found: C, 55.93; H, 3.35; N, 3.65.

7-Amino-6-(3,4,5-trimethoxyphenyl)-8*H*-1,3-dioxolo[4,5-*g*][1]benzopyran-8-one (12a) A solution of **11a** (401 mg, 1 mmol) in freshly distilled chloroform (25 ml) was stirred for 24 h, under hydrogen, in the presence

of 10% Pd–C (250 mg), at atmospheric pressure and at room temperature. After filtration on a pad of Celite and rinsing of the solid several times with methanol, the filtrate was evaporated to provide the desired compound **12a** (364 mg, yield 98%); mp 208—210 °C (recrystallized from a benzene/heptane mixture); ^{1}H-NMR ($CDCl_3$) δ: 3.94 (s, 9H), 3.99 (s, 2H, exch. D_2O), 6.10 (s, 2H), 6.89 (s, 1H), 7.12 (s, 2H), 7.56 (s, 1H); IR ν: 1619 cm^{-1}; MS m/z: 372 $(M+H)^+$. *Anal.* Calcd for $C_{19}H_{17}NO_7$: C, 61.45; H, 4.61; N, 3.77. Found: C, 61.19; H, 4.56; N, 3.67.

7-Amino-6-(4-hydroxy-3,5-dimethoxyphenyl)-8*H*-1,3-dioxolo[4,5-*g*]-[1]benzopyran-8-one (12c) Compound **12c** was synthesized from **11b** (477 mg, 1 mmol) in the same manner as described for the synthesis of **12a**. However, in the present case, better results in the purification step were obtained using a Soxhlet apparatus for 24 h in methanol as a solvent. In this way, after evaporation to dryness, **12c** was obtained with satisfactory purity in 89% yield (318 mg). mp >260 °C; ^{1}H-NMR (DMSO-d_6) δ: 3.86 (s, 6H), 4.60 (br s, 2H, exch. D_2O), 6.21 (s, 2H), 7.22 (s, 2H), 7.30 (s, 1H), 7.35 (s, 1H), 9.01 (s, 1H, exch. D_2O); IR (KBr) ν: 1636 cm^{-1}; MS m/z: 358 $(M+H)^+$. *Anal.* Calcd for $C_{18}H_{15}NO_7$: C, 60.51; H, 4.23; N, 3.92. Found: C, 60.47; H, 4.21; N, 3.87.

6,7-Dihydro-6-(3,4,5-trimethoxyphenyl)-8*H*-1,3-dioxolo[4,5-*g*][1]-benzopyran-8-one (13a) This flavanone was synthesized by adding tri-*n*-butyltin hydride (1.46 g, 1.35 ml, 5 mmol) to a boiling solution of the precursor **10a** (438 mg, 1 mmol) and AIBN (206 mg, 1.25 mmol) in dry benzene (15 ml), under an argon atmosphere. The reflux was continued for 3 h and the solvent was distilled off. The residue was taken up with acetonitrile (30 ml) and hexane (8 ml). After decantation, the lower acetonitrile phase was extracted with hexane (5 × 8 ml), and the acetonitrile solution was evaporated to dryness. The obtained crude material was chromatographed over a silica gel column (70 g, eluent CH_2Cl_2, then CH_2Cl_2/methanol 99/1 and, lastly, CH_2Cl_2/methanol 98/2) to provide pure flavanone **13a** (269 mg, yield 75%). mp 148—149 °C (recrystallized from a benzene/heptane mixture); ^{1}H-NMR ($CDCl_3$) δ: 2.77—3.10 (AB part of ABX system, 2H, $\delta_A=2.82$, $J_{AX}=2.9$, $J_{AB}=16.9$, $\delta_B=3.02$, $J_{BX}=13.5$), 3.87 (s, 3H), 3.90 (s, 6H), 5.32—5.39 (X part of ABX system, 1H), 6.01 (d, 2H, $J=3.4$), 6.53 (s, 1H), 6.69 (s, 2H), 7.31 (s, 1H); IR ν: 1674 cm^{-1}; MS m/z: 359 $(M+H)^+$, 376 $(M+NH_4)^+$. *Anal.* Calcd for $C_{19}H_{18}O_7$: C, 63.68; H, 5.06. Found: C, 63.49; H, 5.04.

6-[3,5-Dimethoxy-4-(phenylmethoxy)phenyl]-6,7-dihydro-8*H*-1,3-dioxolo[4,5-*g*][1]benzopyran-8-one (13b) The flavanone **13b** was prepared from **10b** (514 mg, 1 mmol) in a similar manner to that employed for **13a**. Yield 91% (395 mg); mp 136—137 °C (recrystallized from a benzene/heptane mixture); ^{1}H-NMR ($CDCl_3$) δ: 2.77—3.08 (AB part of ABX system, 2H, $\delta_A=2.82$, $J_{AX}=2.7$, $J_{AB}=16.9$, $\delta_B=3.02$, $J_{BX}=13.7$), 3.87 (s, 6H), 5.03 (s, 2H), 5.32—5.38 (X part of ABX system, 1H), 6.01 (d, 2H, $J=2.9$); 6.53 (s, 1H), 6.68 (s, 2H); 7.30—7.40 (m, 3H), 7.31 (s, 1H), 7.51 (dd, 2H, $J=1.5, 7.7$); IR ν: 1674 cm^{-1}; MS m/z: 435 $(M+H)^+$. *Anal.* Calcd for $C_{25}H_{22}O_7$: C, 69.12; H, 5.10. Found: C, 69.26; H, 5.11.

6,7-Dihydro-6-(4-hydroxy-3,5-dimethoxyphenyl)-8*H*-1,3-dioxolo[4,5-*g*][1]benzopyran-8-one (13c) The flavanone **13c** was prepared by reducing **13b** (232 mg, 0.534 mmol) in a similar manner to that employed to synthesize **12a**. Yield 81% (149 mg); mp 192—193 °C with allotropic change at 170—175 °C (recrystallized from a benzene/heptane mixture); ^{1}H-NMR ($CDCl_3$) δ: 2.76—3.09 (AB part of ABX system, 2H, $\delta_A=2.81$, $J_{AX}=2.8$, $J_{AB}=16.9$, $\delta_B=3.03$, $J_{BX}=13.4$), 3.93 (s, 6H), 5.31—5.37 (X part of ABX system, 1H), 5.59 (s, 1H), 6.01 (d, 2H, $J=2.5$); 6.52 (s, 1H), 6.70 (s, 2H), 7.30 (s, 1H); IR ν: 1673 cm^{-1}: MS m/z: 345 $(M+H)^+$, 362 $(M+NH_4)^+$. *Anal.* Calcd for $C_{18}H_{16}O_7$: C, 62.79; H, 4.68. Found: C, 62.75; H, 4.66.

6-(3,4,5-Trimethoxyphenyl)-8*H*-1,3-dioxolo[4,5-*g*][1]benzopyran-8-one (14a) This flavone was obtained by adding, under an argon atmosphere, tri-*n*-butyltin hydride (320 mg, 0.3 ml, 1.1 mmol) to a boiling solution of the relevant precursor **10a** (438 mg, 1 mmol) and AIBN (41 mg, 0.25 mmol) in dry benzene (15 ml). When the starting material had completely reacted (about 4 h as judged by TLC), the same work-up as for **13a** or **13b** (see above) provided a residue mainly constituted of the corresponding 3-chloroflavanone (7-chloro-6,7-dihydro-6-(3,4,5-trimethoxyphenyl)-8*H*-1,3-dioxolo[4,5-*g*][1]benzopyran-8-one). This crude material was dissolved in anhydrous THF (15 ml) then treated, at 20 °C in an inert atmosphere, with DBU (183 mg, 0.18 ml, 1.2 mmol) for 30 min. The reaction medium was acidified with aqueous 0.1 N hydrochloric acid then extracted with CH_2Cl_2 (60 ml, then 3 × 20 ml). The combined organic extracts were dried ($MgSO_4$) and evaporated. The residue was taken up in CH_2Cl_2 (10 ml). The flavone **14a** is scarcely soluble and, in this particular case, a first crop of the desired product was obtained by direct filtration. Evaporation of the filtrate, followed by purification over a silica gel column (80 g, eluent CH_2Cl_2, then CH_2Cl_2/methanol 99/1), left a solid, which was recrystallized together with the previously filtered compound. Yield 58% (207 mg); mp >260 °C (recrystallized from a benzene/heptane mixture); ^{1}H-NMR ($CDCl_3$) δ: 3.94 (s, 9H), 6.13 (s, 2H), 6.72 (s, 1H), 7.00 (s, 1H), 7.09 (s, 2H), 7.55 (s, 1H); IR ν: 1639 cm^{-1}; MS m/z: 357 $(M+H)^+$. *Anal.* Calcd for $C_{19}H_{16}O_7$: C, 64.04; H, 4.53. Found: C, 63.80; H, 4.50.

6-[3,5-Dimethoxy-4-(phenylmethoxy)phenyl]-8*H*-1,3-dioxolo[4,5-*g*]-[1]benzopyran-8-one (14b) The flavone **14b** was prepared starting from **10b** (514 mg, 1 mmol) in a similar manner to that employed for **14a**. However, **14b** is more soluble than **14a**, and the whole crude material was chromatographed under the same conditions as for **14a**. Yield 52% (225 mg); mp 207—208 °C (recrystallized from a benzene/heptane mixture); ^{1}H-NMR ($CDCl_3$) δ: 3.92 (s, 6H), 5.11 (s, 2H), 6.13 (s, 2H), 6.72 (s, 1H), 6.99 (s, 1H), 7.07 (s, 2H), 7.33—7.51 (m, 5H), 7.55 (s, 1H); IR ν: 1639 cm^{-1}; MS m/z: 433 $(M+H)^+$. *Anal.* Calcd for $C_{25}H_{20}O_7$: C, 69.44; H, 4.66. Found: C, 69.73; H, 4.80.

6-(4-Hydroxy-3,5-dimethoxyphenyl)-8*H*-1,3-dioxolo[4,5-*g*][1]benzopyran-8-one (14c) This hydroxyflavone was synthesized from **14b** (252 mg, 0.58 mmol) using the methodology employed to prepare **11c**, but with heating only for 3.5 h. Yield 75% (149 mg); mp >260 °C; ^{1}H-NMR (DMSO-d_6) δ: 3.88 (s, 6H), 6.22 (s, 2H), 6.98 (s, 1H), 7.07 (s, 3H), 7.46 (s, 1H), OH indiscernible; IR ν: 1635 cm^{-1}; MS m/z: 343 $(M+H)^+$. *Anal.* Calcd for $C_{18}H_{14}O_7$: C, 63.16; H, 4.12. Found: C, 63.05; H, 4.03.

Antiproliferative Assays Adriamycin, used as a reference cytotoxic compound, was purchased from Farmitalia and was solubilized at 10^{-2} M in water and diluted in complete culture medium.

Cell Culture and Cytotoxicity: L1210 cells (murine leukemia) were provided by the NCI, Frederick, U.S.A. and HT29 cells (human colon carcinoma) by ATCC, Rockville, MD, U.S.A. They were cultivated in RPMI 1640 medium (Gibco) supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 units/ml penicillin, 100 mg/ml streptomycin, and 10 mM HEPES buffer (pH = 7.4). Cytotoxicity was measured by the microculture tetrazolium assay as described.[19] Cells were exposed to graded concentrations of the compounds (nine serial dilutions in triplicate) for 48 h for L1210 and 96 h for HT29. Results were expressed as IC_{50}, the concentration which reduced by 50% the optical density of treated cells with respect to untreated controls.

Cell Cycle Analysis: For the cell cycle analysis, L1210 cells (2.5×10^5 cells/ml) were incubated for 21 h with various concentrations of the compound. Cells were then fixed in 70% ethanol (v/v), washed and incubated in Dulbecco's phosphate buffered saline (D-PBS) containing 100 mg/ml RNAse and 25 mg/ml propidium iodide for 30 min at 20 °C. For each sample, 10^4 cells were analyzed on an ATC3000 flow cytometer (Bruker, France) using an argon laser (Spectra-Physics) emitting 400 mW at 488 nm. The fluorescence of propidium iodide was collected through a 615 nm long-pass filter.

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References and Notes

1) King L., Sullivan M., *Science*, **104**, 244—245 (1946).
2) *a*) Stähelin H., Cerletti A., *Schweiz. Med. Wschr.*, **94**, 1490—1502 (1964); *b*) Gensler W. J., Murthy C. D., Trammell M. H., *J. Med. Chem.*, **20**, 635—644 (1977); *c*) Loike J. D., Brewer C. F., Sternlicht H., Gensler W. J., Horwitz S. B., *Cancer Res.*, **38**, 2688—2693 (1978); *d*) Stähelin H., von Wartburg A., *Prog. Drug. Res.*, **33**, 169—266 (1989); *e*) Jardine I., "Anticancer Agents Based on Natural Products Models," ed. by Cassady J. M., Douros J. D., Academic Press, New York, 1980, pp. 319—351; *f*) Stähelin H. F., von Wartburg A., *Cancer Res.*, **51**, 5—15 (1991).
3) *a*) Falkson G., Sandison A. G., Vanzyl, J., *S. Afr. J. Radiol.*, **2**, 1—7 (1964); *b*) Vaitkevicius V. K., Reed M. I., *Cancer Chemother. Rep.*, **50**, 565—571 (1966).
4) *a*) Stähelin H., *Eur. J. Cancer*, **6**, 303—311 (1970); *b*) Idem, *ibid.*, **9**, 215—221 (1973); *c*) Cohen M. H., Broder L. E., Fossieck B. E., Ihde D. C., Minna J. D., *Cancer Treat. Rep.*, **61**, 489—490 (1977); *d*) Aisner J., Wiernik P. H., *Cancer*, **46**, 2543—2549 (1980); *e*) Issell B. F., *Cancer Chemother. Pharmacol.*, **7**, 73—80 (1982); *f*) Taylor R. E., Mc Elwain T. J., Barrett A., Peckham M. J., *ibid.*, **7**, 175—177

(1982); *g*) Laubenstein L. J., Krigel R. L., Hymes K. B., Muggia F. M., *Proc. Am. Soc. Clin. Oncol.*, **2**, 228 (1983); *h*) Issell B. F., Muggia F. M., Carter S. K., "[VP16] Current Status and New Developments," Academic Press, Orlando, 1984, pp. 1—353. *i*) O'Dwyer P. J., Leyland-Jones B., Alonso M. T., Marsoni S., Wittes R. E., *N. England J. Med.*, **312**, 692—700 (1985); *j*) Williams S. D., Birch R., Einhorn L. H., Irwin L., Greco F. A., Loehrer P. J., *ibid.*, **316**, 1435—1440 (1987); *k*) Zhang Y., Lee K.-H., *Chin. Pharm. J.*, **46**, 319—369 (1994).

5) *a*) Cornman I., Cornman M. E., *Ann. N. Y. Acad. Sci.*, **51**, 1443—1487 (1951); *b*) Kelly M. G., Hartwell J. L., *J. Natl. Cancer Inst.*, **14**, 967—1010 (1954); *c*) Wilson L., Bryan *J. Adv. Cell Mol. Biol.*, **3**, 21—72 (1974); *d*) Cortese F., Bhattacharyya B., Wolff J., *J. Biol. Chem.*, **252**, 1134—1140 (1977); *e*) Andreu J. M., Timasheff S. N., *Biochemistry*, **21**, 6465—6476 (1982); *f*) Sackett D. L., *Pharm. Ther.*, **59**, 163—228 (1993).

6) *a*) Loike J. D., Horwitz S. B., *Biochemistry*, **15**, 5435—5443 (1976); *b*) Idem, *ibid.*, **15**, 5443—5448 (1976); *c*) Grieder A., Maurer R., Stähelin H., *Cancer Res.*, **37**, 2998—3005 (1977); *d*) Long B. H., Minocha A., *Proc. Am. Assoc. Cancer Res.*, **24**, 321 (1983); *e*) Glisson B. S., Smallwood S. E., Ross W. E., *Biochim. Biophys. Acta*, **783**, 74—79 (1984); *f*) Minocha A., Long B. H., *Biochem. Biophys. Res. Commun.*, **122**, 165—170 (1984); *g*) Liu L. F., *Annu. Rev. Biochem.*, **58**, 351—375 (1989); *h*) MacDonald T. L., Lehnert E. K., Loper J. T., Chow K.-C., Ross W. E., "DNA Topoisomerases in Cancer," ed. by Potmesil M., Kohn K. W., Oxford University Press, New York, 1991, pp. 199—213; *i*) Osheroff N., Robinson M. J., Zechiedrich L., *ibid.*, pp. 230—239; *j*) Wang, J. C., *Annu. Rev. Biochem.*, **65**, 635—692 (1996); *k*) Burden D. A., Kingma P. S., Froelich-Ammon S. J., Bjornsti M.-A., Patchan M. W., Thompson R. B., Osheroff N., *J. Biol. Chem.*, **271**, 29238—29244 (1996); *l*) Pommier Y., "Cancer Therapeutics: Experimental and Clinical Agents," ed. by Teicher B. A., Humana Press Inc., Totowa, New Jersey, 1997, pp. 153—174.

7) *a*) Li L., Wang H.-K., Kuo S.-C., Wu T.-S., Lednicer D., Lin C. M., Hamel E., Lee K.-H., *J. Med. Chem.*, **37**, 1126—1135 (1994); *b*) Li L., Wang H.-K., Kuo S.-C., Wu T.-S., Mauger A., Lin C. M., Hamel E., Lee K.-H., *ibid.*, **37**, 3400—3407 (1994).

8) Wang H.-K., Bastow K. F., Cosentino L. M., Lee K.-H., *J. Med. Chem.*, **39**, 1975—1980 (1996).

9) *a*) Pathak D., Pathak K., Singla A. K., *Fitoterapia*, **62**, 371—389 (1991); *b*) Cody V., Middleton E., Harborne J. B. (eds.), "Progress in Clinical and Biological Research, Vol 213, Plant Flavonoids in Biology and Medicine," Alan R. Liss Inc., New York, 1986; *c*) Cody V., Middleton E., Harborne J. B., Beretz A. (eds.), "Progress in Clinical and Biological Research, Vol 280, Plant Flavonoids in Biology and Medicine II," Alan R. Liss Inc., New York, 1988; *d*) Cushman M., Nagarathnam D., *J. Natl. Products*, **54**, 1656—1660 (1991); *e*) Post J. F. M., Varma R. S., *Cancer Lett.*, **67**, 207—213 (1992); *f*) Cunningham B. D. M., Threadgill M. D., Groundwater P. W., Dale I. L., Hickman J. A., *Anti Cancer Drug Des.*, **7**, 365—384 (1992); *g*) Middleton E., Jr., Kandaswami C., "The Flavonoids, Advances in Research Since 1986," ed. by Harborne J. B., Chapman & Hall, London, 1993, pp. 619—652; *h*) Sedlacek H. H., Czech J., Naik R., Kaur G., Worland P., Losiewicz M., Parker B., Carlson B., Smith A., Senderowicz A., Sausville E., *Int. J. Oncol.*, **9**, 1143—1168 (1996).

10) Dauzonne D., Demerseman P., *Synthesis*, **1990**, 66—70.

11) Dauzonne D., Grandjean C., *Synthesis*, **1992**, 677—680.

12) *a*) Kuivila H. G., *Synthesis*, **1970**, 499—509; *b*) Ono N., Kaji A., *ibid.*, **1986**, 693—704; *c*) Neumann W. P., *ibid.*, **1987**, 665—683; *d*) Rosini G., Ballini R., *ibid.*, **1988**, 833—847.

13) Fogh J., Trempe G., "Human Tumour Cells *in Vitro*," ed. by Fogh J., Plenum Press, New York, 1975, p. 119.

14) Fukui K., Nakayama M., *Bull. Chem. Soc. Jpn.*, **35**, 1321—1323 (1962).

15) Gross H., Rieche A., Matthey G., *Chem. Ber.*, **96**, 308—313 (1963).

16) Royer R., Buisson J.-P., *Eur. J. Med. Chem.-Chim. Ther.*, **15**, 275—278 (1980).

17) Particularly in the case of **9a**, chromatography must be avoided because an important retro-reaction occurs on the column.

18) These moderate yields do not constitute an important drawback, since it is possible to recover unreacted salicylaldehyde **7** almost quantitatively (according to reference 10) and to recycle it.

19) Pierre A., Kraus-Berthier L., Atassi G., Cros S., Poupon M.-F., Lavielle G., Berlion M., Bizarri J.-P., *Cancer Res.*, **51**, 2312—2318 (1991).