Synthesis and Cytotoxic Activity of Benzopyranoxanthone Analogues of Benzo[*b*]acronycine and Psorospermine

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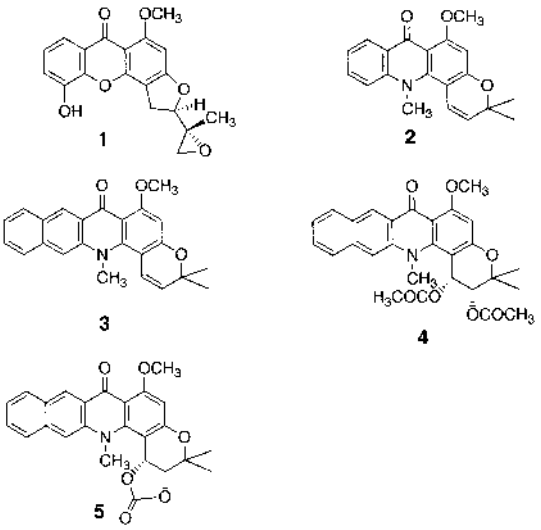
Condensation of 3-hydroxy-2-naphthalenecarboxylic acid with phloroglucinol afforded 1,3-dihydroxy-12*H*-benzo[*b*]xanthen-12-one. Construction of an additional dimethylpyran ring onto this skeleton, by alkylation with 3-chloro-3-methyl-1-butyne followed by Claisen rearrangement, gave access to a series of benzo[*b*]pyrano[2,3-*i*]xanthen-6-ones and benzo[*b*]pyrano[3,2-*h*]xanthen-7-ones related to psorospermine and benzo[*b*]acronycine. In contrast with what is observed in the pyridoacridone and benzopyridoacridone series, the linear benzo[*b*]-pyrano[2,3-*i*]xanthen-6-one derivatives were more potent than their angular benzo[*b*]pyrano[3,2-*h*]xanthen-7-one isomers. *cis*-3,4-Diacetoxy-5-methoxy-2,2-dimethyl-3,4-dihydro-2*H*,6*H*-benzo[*b*]pyrano[2,3-*i*]xanthen-6-one, the most active among the new compounds, was more potent than acronycine in inhibiting the proliferation of L1210 murine leukemia cells.

Key words benzopyranoxanthone; acronycine; synthesis; cytotoxicity

Several simple and fused xanthone derivatives have been reported as promising compounds in the search for new anticancer candidates. Indeed, xanthone-4-acetic acid derivatives induced tumor-growth delays and regressions when evaluated against the sc colon 38 model in mice.[1,2] Some natural xanthones have shown an intensive inhibitory effect against topoisomerases I and II in the course of *in vitro* experiments.[3] The furanoxanthone psorospermine (**1**), isolated from *Psorospermum febrifugum*,[4—6] exhibited significant cytotoxic and antineoplastic properties due to its interaction with DNA.[7,8] The tetracyclic basic core of psorospermine is closely related to that of the pyranoacridone alkaloid acronycine (**2**). This latter is an efficient anticancer agent, with a broad spectrum of activity including numerous solid tumors.[9—14] The structural relationships between the psorospermine and acronycine series stimulated the synthesis of pyrano[2,3-*c*]xanthen-7-one and pyrano[3,2-*b*]xanthen-6-one derivatives.[15,16] Some of those compounds exhibited cytotoxic activities within the same range of magnitude as acronycine, when tested against L1210 murine leukemia cells.[16]

Based on a hypothesis of bioactivation of acronycine into the corresponding epoxide[17,18] and of intercalation within DNA,[19] we recently prepared the pentacyclic 6-methoxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]pyrano[3,2-*h*]-acridin-7-one ("benzo[*b*]acronycine") (**3**) and a series of corresponding *cis*-1,2-dihydrodiol diesters, exemplified by diacetate **4** and carbonate **5**.[20] These latter compounds were 20 to 1000 fold more potent than acronycine in inhibiting L1210 cell proliferation. *In vivo*, against P388 leukemia, acronycine was only marginally active, while compounds **4** and **5** were significantly active at doses 16 fold lower. Against the sc colon 38 adenocarcinoma model in mice, compounds **4** and **5** were also highly efficient, inhibiting by more than 80% the tumor growth. These promising results prompted us to prepare pentacyclic xanthone isosters and analogues, in which the acridone nitrogen is replaced by an oxygen atom. We report here the synthesis and biological properties of benzo[*b*]-pyrano[3,2-*h*]xanthen-7-ones and benzo[*b*]pyrano[2,3-*i*]-xanthen-6-ones related to psorospermine and benzo[*b*]-acronycine.



Chemistry Construction of the tetracyclic 1,3-dihydroxy-12*H*-benzo[*b*]xanthen-12-one (**6**) basic core was obtained by condensation of 3-hydroxy-2-naphthalenecarboxylic acid (**7**) with phloroglucinol (**8**) in the presence of zinc chloride and phosphoryl chloride.[21,22] Chelation of the 1-hydroxy group of **6** by the carbonyl at the 12-position permitted the selective *O*-alkylation of the 3-hydroxy group with 3-chloro-3-methylbut-1-yne (**9**),[23,24] to give the corresponding propargylic ether **10**. Methylation of the 1-hydroxy group of **10**, carried out with dimethylsulfate in the presence of sodium hydride gave **11** in almost quantitative yield. When thermal cyclization was performed on the hydroxy ether **10**, for 2 h at 130 °C in dimethylformamide (DMF), the linear 5-hydroxy-2,2-dimethyl-2*H*,6*H*-benzo[*b*]pyrano[2,3-*i*]xanthen-6-one (**12**) was the major reaction product, which could be subsequently methylated to afford the desired 5-methoxy-

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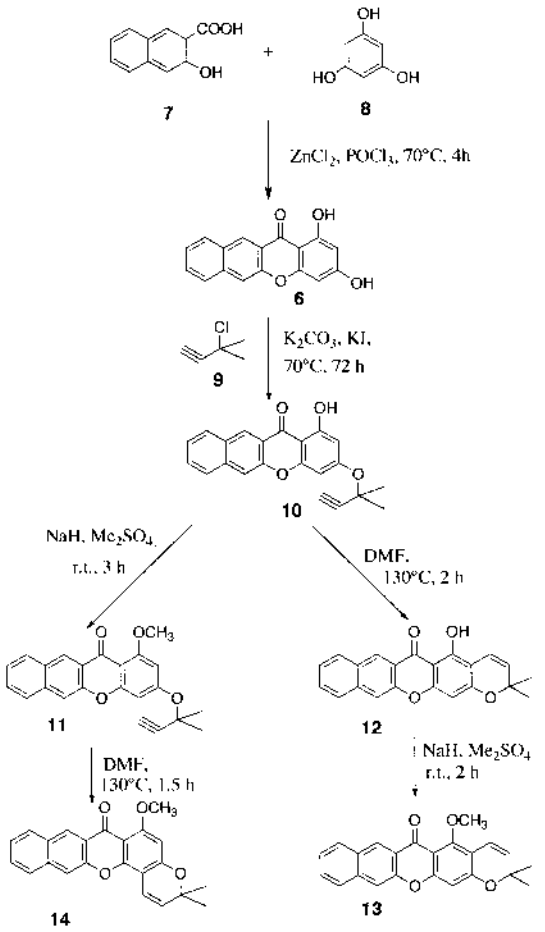

Chart 1. Synthesis of Benzo[*b*]pyrano[3,2-*h*]xanthen-7-ones and Benzo-[*b*]pyrano[2,3-*i*]xanthen-6-ones

2,2-dimethyl-2*H*,6*H*-benzo[*b*]pyrano[2,3-*i*]xanthen-6-one (**13**). In contrast, Claisen rearrangement of the methoxy ether **11**, under similar conditions, exclusively gave the angular 6-methoxy-3,3-dimethyl-3*H*,7*H*-benzo[*b*]pyrano[3,2-*h*]xanthen-7-one (**14**), most probably due to the increased steric hindrance of the methoxy group at 5-position of **11**, when compared with the chelated hydroxy group of **10**. The linear structures of benzopyranoxanthones **12** and **13**, and the angular structure of benzopyranoxanthone **14** were unambiguously ascribed from phase-sensitive nuclear Overhauser and exchange spectroscopy (NOESY) experiments performed on these three compounds.[20,25] The (±)-*cis*-diol **15** was conveniently obtained by catalytic osmium tetroxide oxidation of **14**, using *N*-methylmorpholine *N*-oxide to regenerate the oxidizing agent.[26] Isosters of the highly active compounds **4** and **5** were obtained by treatment of diol **15** with either acetic anhydride or *N*,*N*′-carbonyldiimidazole, affording the diacetate **16** and the cyclic carbonate **17**, respectively. Under similar conditions, oxidation of the linear 5-methoxy-2,2-dimethyl-2*H*,6*H*-benzo[*b*]pyrano[2,3-*i*]xanthen-6-one (**13**) gave the corresponding *cis*-diol **18**, which was subsequently converted into the diacetate **19** and the cyclic carbonate **20**.

Pharmacology The study of the biological properties of the new benzopyranoxanthone derivatives was carried out *in vitro* on L1210 murine leukemia cell line. The results (IC_{50}) are reported in Table 1. Most angular compounds were devoid of significant antiproliferative activity. In contrast, linear derivatives significantly inhibited the proliferation of L1210 cells. The most cytotoxic compound, *cis*-3,4-diacetoxy-5-methoxy-2,2-dimethyl-3,4-dihydro-2*H*,6*H*-benzo[*b*]pyrano-[2,3-*i*]xanthen-6-one (**19**), was more potent than acronycine in inhibiting the proliferation of L1210 murine leukemia cells.

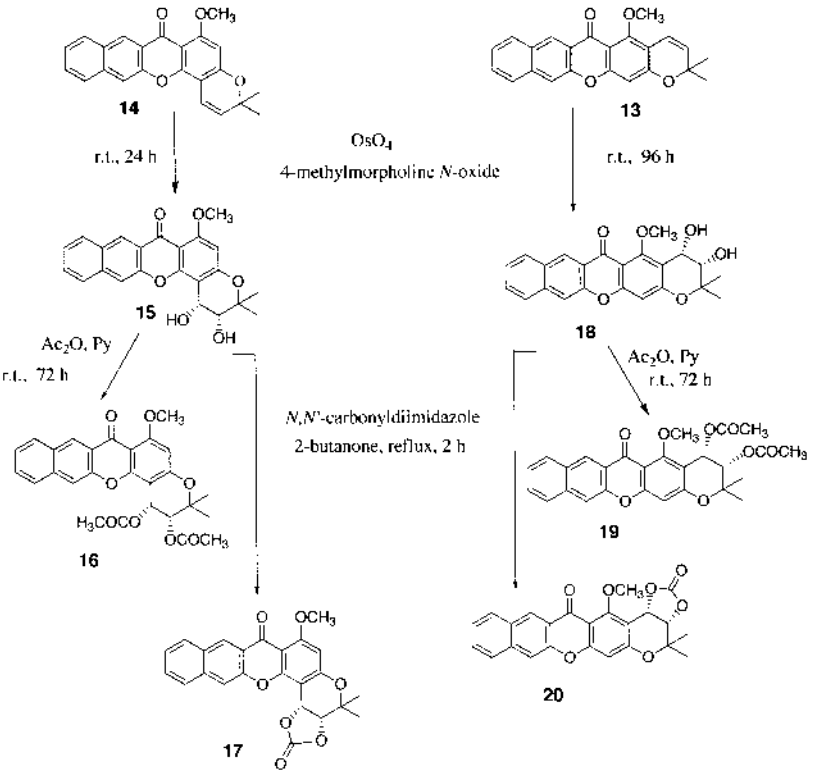

Chart 2. Synthesis of 6-Methoxy-3,3-dimethyl-3,7-dihydrobenzo[*b*]pyrano[3,2-*h*]xanthen-6-one and of 5-Methoxy-2,2-dimethyl-2,6-dihydrobenzo[*b*]-pyrano[2,3-*i*]xanthen-6-one Analogues

Table 1. Inhibition of L1210 Cell Proliferation by Benzoxanthone **6** and Benzopyranoxanthones **11**—**20** in Comparison with Acronycine (**2**)

Compound	IC_{50} (μM)
2	27
6	100
11	109
12	Insoluble
13	42
14	96
15	55
16	13
17	52
18	28
19	7
20	16

Results and Discussion

Considering the structure–activity relationships in the acronycine series, it appears that angular pyranoxanthone derivatives are less potent than their acridone counterparts in inhibiting the proliferation of L1210 cells. Indeed, the only significantly active compound, *cis*-1,2-diacetoxy-6-methoxy-3,3-dimethyl-1,2-dihydro-3*H*,7*H*-benzo[*b*]pyrano[3,2-*h*]xanthen-7-one (**16**), was less potent than the corresponding 1,2-diacetoxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydrobenzo[*b*]pyrano[3,2-*h*]acridin-7-one (**4**), belonging to the benzo[*b*]acronycine series.[20]

In strong contrast with what is observed in the pyridoacridone (acronycine, benzo[*b*]acronycine) series, all the linear pyranoxanthone derivatives were more potent than their angular isomers. For instance, both diacetate **19** and cyclic carbonate **20** were more potent than their angular counterparts **16** and **17**, and also than acronycine (**2**) in inhibiting the proliferation of L1210 murine leukemia cells. This suggests that the mechanism of action of pyranoxanthone derivatives should be different from that of the corresponding pyranoacridones.

Experimental

Chemistry The melting points were determined on a Leica VM apparatus and are not corrected. IR spectra (ν_{max} in cm^{-1}) were obtained on a Perkin-Elmer 257 instrument. UV spectra (λ_{max} in nm) were determined in spectroscopic grade MeOH on a Beckman Model 34 spectrophotometer. ^{1}H-NMR [δ (ppm), J (Hz)] and ^{13}C-NMR spectra were recorded at 300 and 75 MHz respectively, using a Bruker AC-300 spectrometer. When necessary, the signals were unambiguously assigned by two-dimensional (2D) NMR techniques: ^{1}H–^{1}H correlation spectroscopy (COSY), ^{1}H–^{1}H NOESY, ^{13}C–^{1}H heteronuclear chemical shift correlation (HETCOR), and ^{13}C–^{1}H correlation spectroscopy *via* long range couplings (COLOC). These experiments were performed using standard Bruker microprograms. Mass spectra were recorded with a Nermag R-10-10C spectrometer using electron impact (MS) and/or desorption chemical ionization (DCI-MS; reagent gas: NH_3) techniques. Flash column chromatographies were conducted using Silica gel 60 Merck (35—70 μm) with an overpressure of 300 mbars.

1,3-Dihydroxy-12*H*-benzo[*b*]xanthen-12-one (6) To a mixture of 3-hydroxy-2-naphthalenecarboxylic acid (**7**) (5 g, 26 mmol), phloroglucinol (**8**) (3.55 g, 26 mmol) and freshly fused zinc chloride (11 g), phosphoryl chloride (50 ml) was added. The reaction mixture was stirred at 70 °C for 4 h, poured onto ice (150 g) and left overnight. After filtration, the precipitate was washed with saturated aqueous sodium bicarbonate and water, and dried. Purification by flash column chromatography (solvent: cyclohexane, then cyclohexane/acetone 99 : 1 to 80 : 20) gave **6** (6.52 g, 90%) as reddish crystals, mp 278—279 °C. IR (KBr) cm^{-1}: 3355, 3055, 1664, 1603, 1480, 1344, 1252, 1158, 1079, 899, 813, 738. UV λ_{max} (MeOH) nm (log ε): 259 (4.89), 330 (sh.), 344 (4.28). ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 6.17 (1H, d, J=2 Hz, C2-H), 6.36 (1H, d, J=2 Hz, C4-H), 7.52 (1H, t, J=8 Hz, C9-H), 7.66 (1H, t, J=8 Hz, C8-H), 7.97 (1H, s, C6-H), 7.99 (1H, d, J=8 Hz, C7-H), 8.17 (1H, d, J=8 Hz, C10-H), 8.76 (1H, s, C11-H), 11.16 (1H, s, D_2O exch., C3-OH), 12.87 (1H, s, D_2O exch., C1-OH). ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 181.4 (s, C-12), 167.6 (s, C-3), 164.5 (s, C-1), 158.9 (s, C-4a), 152.3 (s, C-5a), 137.4 (s, C-6a), 130.7 (d, C-10), 130.5 (d, C-8), 130.2 (s, C-10a), 128.2 (2d, C-7, C-11), 127.0 (d, C-9), 120.7 (s, C-11a), 114.2 (d, C-6), 102.8 (s, C-12a), 98.9 (d, C-2), 95.4 (d, C-4). DCI-MS m/z: 279 $[MH]^+$. *Anal.* Calcd for $C_{17}H_{10}O_4$: C, 73.37; H, 3.62. Found: C, 73.15; H, 3.54.

1-Hydroxy-3-(1,1-dimethylpropargyloxy)-12*H*-benzo[*b*]xanthen-12-one (10) A solution of 1,3-dihydroxy-12*H*-benzo[*b*]xanthen-12-one (**6**) (2 g, 7.19 mmol) in dry DMF (160 ml) was stirred and heated at 70 °C for 3 d under nitrogen, in the presence of potassium carbonate (2 g), potassium iodide (2.4 g) and 3-chloro-3-methylbut-1-yne (**9**) (3.7 g, 21 mmol). Water (100 ml) was added and the precipitate was filtered and dried. Flash column chromatography (solvent: cyclohexane/dichloromethane 50 : 50 to 5 : 95, then dichloromethane) gave **10** (1.31 g, 53%) as a yellow amorphous solid. IR (NaCl film) cm^{-1}: 3055, 2979, 2924, 1684, 1639, 1600, 1472, 1446, 1193, 1176, 813. UV λ_{max} (MeOH) nm (log ε): 263 (3.43), 284 (3.25), 343 (3.00). DCI-MS m/z: 345 $[MH]^+$. ^{1}H-NMR (300 MHz, C_6D_6) δ: 1.60 (6H, s, $C(CH_3)_2$), 3.10 (1H, s, C3′-H), 7.02 (1H, d, J=2 Hz, C2-H), 7.10 (1H, d, J=2 Hz, C4-H), 7.14 (1H, td, J=8,1 Hz, C9-H), 7.27 (1H, td, J=8, 1 Hz, C8-H), 7.50 (1H, dd, J=8, 1 Hz, C7-H), 7.52 (1H, s, C6-H), 7.64 (1H, dd, J=8, 1 Hz, C10-H), 8.96 (1H, s, C11-H), 13.64 (1H, s, D_2O exch., C1-OH). *Anal.* Calcd for $C_{22}H_{16}O_4$: C, 76.73; H, 4.68. Found: C, 76.75; H, 4.59.

1-Methoxy-3-(1,1-dimethylpropargyloxy)-12*H*-benzo[*b*]xanthen-12-one (11) Sodium hydride (0.3 g of 50% oil dispersion, 6 mmol) was slowly added to an ice-cooled suspension of 1-hydroxy-3-(1,1-dimethylpropargyloxy)-12*H*-benzo[*b*]xanthen-12-one (**10**) (1 g, 2.9 mmol) in DMF (50 ml). Dimethylsulfate (1.1 ml, 12 mmol) was added and the reaction mixture was stirred under nitrogen at room temperature for 3 h. After addition of water (100 ml) and neutralization with 10% sulfuric acid, the mixture was extracted with dichloromethane (4×50 ml). The organic layers were evaporated under reduced pressure. Flash chromatography (solvent: cyclohexane, then cyclohexane/acetone 99 : 1 to 90 : 10) gave **11** (0.85 g, 82%) as a pale yellow amorphous solid. IR (KBr) cm^{-1}: 3200, 3003, 2944, 2106, 1739, 1658, 1628, 1600, 1576, 1466, 1350, 1261, 1141, 1113, 873, 820, 744. UV λ_{max} (MeOH) nm (log ε): 259 (4.91), 288 (sh.), 328 (4.33). ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 1.80 (6H, s, $C(CH_3)_2$), 2.79 (1H, s, C3′-H), 4.02 (3H, s, OCH_3), 6.55 (1H, d, J=2 Hz, C2-H), 7.03 (1H, d, J=2 Hz, C4-H), 7.45 (1H, td, J=8, 1 Hz, C9-H), 7.56 (1H, td, J=8, 1 Hz, C8-H), 7.76 (1H, s, C6-H), 7.86 (1H, dd, J=8, 1 Hz, C7-H), 8.04 (1H, dd, J=8, 1 Hz, C10-H), 8.85 (1H, s, C11-H). ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 175.8 (s, C-12), 161.8 (s, C-1), 161.7 (s, C-3), 159.4 (s, C-4a), 151.5 (s, C-5a), 136.0 (s, C-6a), 129.6 (d, C-10), 128.5 (2d, C-8, C-11), 128.0 (d, C-7), 126.8 (s, C-10a), 125.1 (d, C-9), 122.5 (s, C-11a), 112.4(d, C-6), 106.9 (s, C-12a), 98.4 (d, C-4), 97.8 (d, C-2), 84.7 (s, C-2′), 75.3 (s, d, C-1′, C-3′), 56.3 (q, OCH_3), 29.5 (q , 2C, C-2′ ($\underline{C}H_3)_2$). DCI-MS m/z: 359 $[MH]^+$. *Anal.* Calcd for $C_{23}H_{18}O_4$: C, 77.08; H, 5.06. Found: C, 77.02; H, 4.98.

5-Hydroxy-2,2-dimethyl-2*H*,6*H*-benzo[*b*]pyrano[2,3-*i*]xanthen-6-one (12) A solution of 1-hydroxy-3-(1,1-dimethylpropargyloxy)-12*H*-benzo[*b*]xanthen-12-one (**10**) (0.5 g, 1.45 mmol) in DMF (40 ml) was heated under nitrogen at 130 °C for 2 h. Water (50 ml) was added and the mixture was extracted with dichloromethane (3×40 ml). The organic layers were evaporated under reduced pressure. Flash chromatography (solvent: cyclohexane, then cyclohexane/acetone 99 : 1 to 95 : 5) gave **12** (0.42 g, 85%) as a yellow amorphous solid. IR (KBr) cm^{-1}: 3455, 3066, 2974, 2925, 2857, 1724, 1639, 1612, 1472, 1343, 1243, 1176, 1087, 834, 777. UV λ_{max} (MeOH) nm (log ε): 235 (sh.), 265 (4.58), 285 (4.48), 343 (4.15). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.45 (3H, s, C2-CH_3), 1.52 (3H, s, C2-CH_3), 5.61 (1H, d, J=10 Hz, C3-H), 6.34 (1H, s, C14-H), 6.74 (1H, d, J=10 Hz, C4-H), 7.47 (1H, td, J=8, 1 Hz, C9-H), 7.59 (1H, td, J=8, 1 Hz, C10-H), 7.77 (1H, s, C12-H), 7.86 (1H, dd, J=8, 1 Hz, C11-H), 8.01 (1H, dd, J=8, 1 Hz, C8-H), 8.78 (1H, s, C7-H), 13.22 (1H, s, D_2O exch., C5-OH). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 181.2 (s, C-6), 161.4 (s, C-5), 158.1 (s, C-14a), 157.5 (s, C-13a), 151.8 (s, C-12a), 136.6 (s, C-11a), 129.6 (d, C-8), 129.5 (s, C-7a), 129.1 (d, C-10), 127.4 (d, C-7), 127.2 (d, C-3), 127.0 (d, C-11), 125.6 (d, C-9), 120.1 (s, C-6a), 115.4 (d, C-4), 113.2 (d, C-12), 104.2 (2s, C-4a, C-5a), 95.2 (d, C-14), 78.4 (s, C-2), 28.4 (q ,C2-$\underline{C}H_3$), 26.9 (q, C2-$\underline{C}H_3$). DCI-MS m/z: 345 $[MH]^+$. *Anal.* Calcd for $C_{22}H_{16}O_4$: C, 76.73; H, 4.68. Found: C, 76.65; H, 4.68.

5-Methoxy-2,2-dimethyl-2*H*,6*H*-benzo[*b*]pyrano[2,3-*i*]xanthen-6-one (13) Sodium hydride (0.19 g of 50% oil dispersion, 4 mmol) was slowly added to an ice-cooled solution of 5-hydroxy-2,2-dimethyl-2*H*,6*H*-benzo[*b*]-pyrano[2,3-*i*]xanthen-6-one (**12**) (0.69 g, 2 mmol) in DMF (50 ml). Di-

methylsulfate (0.56 ml, 6 mmol) was added and the reaction mixture was stirred under nitrogen at room temperature for 2 h. After addition of water (200 ml), the mixture was extracted with ethyl acetate (3×30 ml). The organic layers were washed with 10% aqueous NaOH and evaporated under reduced pressure. Flash chromatography (solvent: cyclohexane, then cyclohexane/acetone 99 : 1 to 96 : 4) gave **13** (0.63 g, 88%) as a yellow amorphous solid. IR (KBr) cm^{-1}: 3062, 2980, 2930, 2857, 1735, 1662, 1627, 1601, 1470, 1364, 1257, 1078, 888, 832, 777. UV λ_{max} (MeOH) nm (log ε): 238 (sh.), 279 (4.69), 323 (4.14). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.50 (6H, s, C2-$(CH_3)_2$), 4.00 (3H, s, OCH_3), 5.71 (1H, d, J=10 Hz, C3-H), 6.64 (1H, s, C14-H), 6.76 (1H, d, J=10 Hz, C4-H), 7.46 (1H, td, J=8, 1 Hz, C9-H), 7.57 (1H, td, J=8, 1 Hz, C10-H), 7.77 (1H, s, C12-H), 7.86 (1H, dd, J=8, 1 Hz, C11-H), 8.20 (1H, dd, J=8, 1 Hz, C8-H), 8.85 (1H, s, C7-H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 175.5 (s, C-6), 159.6 (s, C-5), 159.0 (s, C-14a), 156.6 (s, C-13a), 151.4 (s, C-12a), 136.1 (s, C-11a), 129.8 (d, C-3), 129.6 (d, s, C-8, C-7a), 128.5 (d, C-10), 127.9 (d, C-7), 126.8 (d, C-11), 125.2 (d, C-9), 122.1 (s, C-6a), 115.9 (d, C-4), 112.6 (d, C-12), 111.7 (s, C-4a), 109.5 (s, C-5a), 100.6 (d, C-14), 77.9 (s, C-2), 62.6 (q, O<u>C</u>H$_3$), 28.4 (q, 2C, C-2(<u>C</u>H$_3$)$_2$). DCI-MS *m*/*z*: 359 $[MH]^+$. *Anal.* Calcd for $C_{23}H_{18}O_4$: C, 77.08; H, 5.06. Found: C, 76.92; H, 5.15.

6-Methoxy-3,3-dimethyl-3*H*,7*H*-benzo[*b*]pyrano[3,2-*h*]xanthen-7-one (14) A solution of 1-methoxy-3-(1,1-dimethylpropargyloxy)-12*H*-benzo[*b*]xanthen-12-one (**11**) (0.6 g, 1.67 mmol) in DMF (40 ml) was heated under nitrogen at 130 °C for 1.5 h. The cooled mixture was diluted with water (50 ml) and extracted with ethyl acetate (3×50 ml). The organic layers were evaporated under reduced pressure. Flash chromatography (solvent: cyclohexane, then cyclohexane/acetone 99 : 1 to 70 : 30) gave **14** (0.56 g, 93%) as a yellowish amorphous solid. IR (KBr) cm^{-1}: 3064, 2984, 2941, 2857, 1738, 1665, 1632, 1576, 1459, 1393, 1258, 1141, 873, 828, 754. UV λ_{max} (MeOH) nm (log ε): 261 (4.60), 285 (sh.), 328 (3.96). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.51 (6H, s, C3-$(CH_3)_2$), 4.02 (3H, s, OCH_3), 5.63 (1H, d, J=10 Hz, C2-H), 6.29 (1H, s, C5-H), 6.93 (1H, d, J=10 Hz, C1-H), 7.45 (1H, td, J=8, 1 Hz, C10-H), 7.56 (1H, td, J=8, 1 Hz, C11-H), 7.77 (1H, s, C13-H), 7.85 (1H, dd, J=8, 1 Hz,, C12-H), 8.03 (1H, dd, J=8, 1 Hz, C9-H), 8.84 (1H, s, C8-H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 175.9 (s, C-7), 162.1 (s, C-6), 159.4 (s, C-4a), 154.1 (s, C-14a), 151.3 (s, C-13a), 136.0 (s, C-12a), 129.7 (d, C-9), 128.5 (d, s, C-11, C-8a), 128.1 (d, C-8), 127.0 (d, C-2), 126.8 (d, C-12), 125.2 (d, C-10), 122.5 (s, C-7a), 115.5 (d, C-1), 112.4 (d, C-13), 106.2 (s, C-6a), 102.3 (s, C-14b), 95.3 (d, C-5), 78.3 (s, C-3), 56.4 (q, O<u>C</u>H$_3$), 28.3 (q, 2C, C3-(<u>C</u>H$_3$)$_2$). DCI-MS *m*/*z*: 359 $[MH]^+$. *Anal.* Calcd for $C_{23}H_{18}O_4$: C, 77.08; H, 5.06. Found: C, 77.02; H, 5.14.

(±)-*cis*-1,2-Dihydroxy-6-methoxy-3,3-dimethyl-1,2-dihydro-3*H*,7*H*-benzo[*b*]pyrano[3,2-*h*]xanthen-7-one (15) Compound **14** (0.2 g, 0.556 mmol) was added to a solution of osmium tetroxide (2.5% in 2-methyl-2-propanol, 0.4 ml) and 4-methylmorpholine *N*-oxide monohydrate (0.076 g, 2.7 mmol) in *tert*-BuOH–tetrahydrofuran (THF)–H_2O (10 : 3 : 1, 15 ml). The reaction mixture was stirred at room temperature for 24 h. After addition of saturated aqueous solution of sodium bisulfite (20 ml), the mixture was stirred for 1 h and then extracted with dichloromethane (6×15 ml). The combined organic layers were dried with sodium sulfate, filtered and evaporated under reduced pressure. The crude product was purified by flash chromatography (solvent: dichloromethane, then dichloromethane/methanol 99 6: 1 to 90 : 10) to give **15** (0.13 g, 59%) as a pale yellow amorphous solid. IR (NaCl film) cm^{-1}: 3329, 2924, 1658, 1599, 1581, 1477, 1455, 1400, 1300, 1268, 1111, 870, 814. UV λ_{max} (MeOH) nm (log ε): 260 (3.56), 330 (3.00). ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 1.40 (3H, s, C3-CH_3), 1.43 (3H, s, C3-CH_3), 3.67 (1H, dd, J=8, 4 Hz, C2-H), 3.85 (3H, s, OCH_3), 5.02 (1H, t, J=4 Hz, C1-H), 5.08 (1H, d, J=8 Hz, D_2O exch., C2-OH), 5.43 (1H, d, J=4 Hz, D_2O exch., C1-OH), 6.33 (1H, s, C5-H), 7.53 (1H, ddd, J=9, 8, 1 Hz, C10-H), 7.64 (1H, ddd, J=9, 8, 1 Hz, C11-H), 8.02 (1H, dd, J=9, 1 Hz, C12-H), 8.07, (1H, s, C13-H), 8.20 (1H, dd, J=9, 1 Hz, C9-H), 8.71 (1H, s, C8-H). DCI-MS *m*/*z*: 393 $[MH]^+$. *Anal.* Calcd for $C_{23}H_{20}O_6$: C, 70.40; H, 5.14. Found: C, 70.22; H, 5.21.

(±)-*cis*-1,2-Diacetoxy-6-methoxy-3,3-dimethyl-1,2-dihydro-3*H*,7*H*-benzo[*b*]pyrano[3,2-*h*]xanthen-7-one (16) An ice-cooled mixture of acetic anhydride (0.5 ml, 5 mmol) and pyridine (2 ml) was added to (±)-*cis*-1,2-dihydroxy-6-methoxy-3,3-dimethyl-1,2-dihydro-3*H*,7*H*-benzo[*b*]pyrano[3,2-*h*]xanthen-7-one (**15**) (0.035 g, 0.09 mmol). After stirring at room temperature for 3 d, the mixture was poured onto cold water (10 ml). The precipitate was filtered, washed with water (2×5 ml) and dried *in vacuo* over P_2O_5 to give **16** (0.016 g, 38%) as an amorphous pale yellow solid. IR (NaCl film) cm^{-1}: 3058, 2948, 2363, 2342, 1760, 1667, 1600, 1588, 1515, 1481, 1420, 1342, 1215, 1110, 906, 878, 821, 747. UV λ_{max} (MeOH) nm (log ε): 260 (3.54), 327 (3.00). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.48 (3H, s, C3-CH_3), 1.56 (3H, s, C3-CH_3), 2.15 (6H, s, C1-OCOCH_3, C2-OCOCH_3), 4.01 (3H, s, OCH_3), 5.31 (1H, d, J=5 Hz, C2-H), 6.32 (1H, s, C5-H), 6.71 (1H, d, J=5 Hz, C1-H), 7.48 (1H, ddd, J=9, 8, 1 Hz, C10-H), 7.59 (1H, ddd, J=9, 8, 1 Hz, C11-H), 7.68 (1H, s C13-H), 7.87 (1H, dd, J=8, 1 Hz, C12-H), 8.05 (1H, dd, J=8, 1 Hz, C9-H), 8.85 (1H, s, C8-H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 179.6 (s, C-7), 170.0 (s, C2-O<u>C</u>OCH$_3$), 169.6 (s, C1-O<u>C</u>OCH$_3$), 162.8 (s, C-6), 159.0 (2s, C-4a, C-14a), 151.0 (s, C-13a), 136.1 (s, C-12a), 129.7 (d, s, C-9, C-8a), 128.7 (d, C-11), 128.1 (d, C-8), 127.0 (d, C-12), 125.4 (d, C-10), 122.3 (s, C-7a), 112.6 (d, C-13), 107.3 (s, C-6a), 103.4 (s, C-14b), 95.3 (d, C-5), 76.6 (s, C-3), 71.2 (d, C-2), 60.9 (d, C-1), 54.6 (q, OCH_3), 26.0 (q, C3-<u>C</u>H$_3$), 21.7 (q, C3-<u>C</u>H$_3$), 20.9 (q, C2-OCO<u>C</u>H$_3$), 20.7 (q, C1-OCO<u>C</u>H$_3$). DCI-MS *m*/*z*: 477 $[MH]^+$. *Anal.* Calcd for $C_{27}H_{24}O_8$: C, 68.06; H, 5.08. Found: C, 68.12; H, 5.21.

(±)-*cis*-1,2-Di-*O*-carbonyloxy-6-methoxy-3,3-dimethyl-1,2-dihydro-3*H*,7*H*-benzo[*b*]pyrano[3,2-*h*]xanthen-7-one (17) To a solution of (±)-*cis*-1,2-dihydroxy-6-methoxy-3,3-dimethyl-1,2-dihydro-3*H*,7*H*-benzo[*b*]pyrano[3,2-*h*]xanthen-7-one (**15**) (0.1 g, 0.25 mmol) in 2-butanone (10 ml), *N*,*N*′-carbonyldiimidazole (0.33 g, 1.7 mmol) was added. The reaction mixture was refluxed for 24 h and after cooling, 5% aqueous Na_2CO_3 (10 ml) was added. The mixture was extracted with EtOAc (3×10 ml). The combined organic layers were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The crude product was purified by flash chromatography (solvent: dichloromethane, then dichloromethane/methanol 99 : 1 to 90 : 10) to give **17** (0.018 g, 17%) as a pale yellow amorphous solid. IR (NaCl film) cm^{-1}: 3010, 2982, 1787, 1668, 1630, 1582, 1484, 1416, 1382, 1260, 1194, 1110, 888, 838, 780. UV λ_{max} (MeOH) nm (log ε): 260 (2.87), 326 (2.46). ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 1.37 (3H, s, C3-CH_3), 1.60 (3H, s, C3-CH_3), 3.96 (3H, s, OCH_3), 5.22 (1H, d, J=8 Hz, C2-H), 6.40 (1H, d, J=8 Hz, C1-H), 6.52 (1H, s, C5-H), 7.56 (1H, ddd, J=9, 8, 1 Hz, C10-H), 7.67 (1H, ddd, J=9, 8, 1 Hz, C11-H), 8.04 (1H, s, C13-H), 8.07 (1H, dd, J=8, 1 Hz, C12-H), 8.18 (1H, dd, J=8, 1 Hz, C9-H), 8.76 (1H, s, C8-H). DCI-MS *m*/*z*: 419 $[MH]^+$. *Anal.* Calcd for $C_{24}H_{18}O_7$: C, 68.90; H, 4.34. Found: C, 68.82; H, 4.27.

(±)-*cis*-3,4-Dihydroxy-5-methoxy-2,2-dimethyl-3,4-dihydro-2*H*,6*H*-benzo[*b*]pyrano[2,3-*i*]xanthen-6-one (18) Oxidation of **13** (0.14 g, 0.39 mmol) under conditions similar to those described for the preparation of **15** from **14**, afforded **18** (0.05 g, 33%) as a pale yellow amorphous solid. IR (KBr) cm^{-1}: 3450, 2980, 2934, 1664, 1445, 1353, 1248, 1187, 1136, 1107, 834, 754, 689. UV λ_{max} (MeOH) nm (log ε): 259 (4.66), 328 (4.18). ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 1.45 (6H, s, C3-$(CH_3)_2$), 3.60 (1H, dd, J=7, 5 Hz, C3-H), 3.94 (3H, s, OCH_3), 4.83 (1H, t, J=5 Hz, C4-H), 5.14 (2H, m, D_2O exch., C3-OH, C4-OH), 6.68 (1H, s, H-14), 7.52 (1H, t, J=8 Hz, C9-H), 7.65 (1H, t, J=8 Hz, C10-H), 7.98 (1H, s, H-12), 8.00 (1H, d, J=8 Hz, C11-H), 8.18 (1H, d, J=8 Hz, C8-H), 8.78 (1H, s, H-7). DCI-MS *m*/*z*: 393 $[MH]^+$. *Anal.* Calcd for $C_{23}H_{20}O_6$: C, 70.40; H, 5.14. Found: C, 70.29; H, 5.06.

(±)-*cis*-3,4-Diacetoxy-5-methoxy-2,2-dimethyl-3,4-dihydro-2*H*,6*H*-benzo[*b*]pyrano[2,3-*i*]xanthen-6-one (19) Acetylation of **18** (0.03 g, 0.076 mmol) under conditions similar to those described for the preparation of **16**, gave **19** (0.026 g, 72%) as a pale yellow amorphous solid. IR (KBr) cm^{-1}: 3010, 2934, 1747, 1663, 1606, 1464, 1375, 1355, 1241, 1191, 1142, 1103, 1055, 838, 756, 671. UV λ_{max} (MeOH) nm (log ε): 260 (4.74), 324 (4.24). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.48 (3H, s, C2-CH_3), 1.50 (3H, s, C2-CH_3), 2.12 (6H, s, C3-OCOCH_3, C4-OCOCH_3), 4.02 (3H, s, OCH_3), 5.26 (1H, d, J=5 Hz, C3-H), 6.46 (1H, d, J=5 Hz, C4-H), 6.73 (1H, s, C14-H), 7.49 (1H, td, J=8, 1 Hz, C9-H), 7.61 (1H, td, J=8, 1 Hz, C10-H), 7.80 (1H, s C12-H), 7.89 (1H, dd, J=8, 1 Hz, C11-H), 8.05 (1H, dd, J=8, 1 Hz, C8-H), 8.85 (1H, s, C7-H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 181.2 (s, C-6), 169.9 (2s, C1-O<u>C</u>OCH$_3$, C2-O<u>C</u>OCH$_3$), 162.2 (s, C-5), 159.7 (s, C-14a), 159.3 (s, C-13a), 151.5 (s, C-12a), 136.5 (s, C-11a), 129.8 (d, C-8), 128.8 (s, C-7a), 128.3 (2d, C-10, C-7), 127.0 (d, C-11), 125.4 (d, C-9), 122.1 (s, C-6a), 112.7 (d, C-12), 110.0 (s, C-4a), 109.9 (s, C-5a), 101.0 (d, C-14), 77.0 (s, C-2), 71.3 (d, C-3), 62.6 (q, OCH_3), 60.9 (d, C-4), 26.2 (q, C2-<u>C</u>H$_3$), 21.9 (q, C2-<u>C</u>H$_3$), 20.9 (q, C3-OCO<u>C</u>H$_3$), 20.6 (q, C4-OCO<u>C</u>H$_3$). DCI-MS *m*/*z*: 477 $[MH]^+$. *Anal.* Calcd for $C_{27}H_{24}O_8$: C, 68.06; H, 5.08. Found: C, 68.15; H, 4.99.

(±)-*cis*-3,4-Di-*O*-carbonyloxy-5-methoxy-2,2-dimethyl-3,4-dihydro-2*H*,6*H*-benzo[*b*]pyrano[2,3-*i*]xanthen-6-one (20) Treatment of **18** (0.1 g, 0.255 mmol) with *N*,*N*′-carbonyldiimidazole (0.207 g, 1.275 mmol) under conditions similar to those described for the preparation of **17**, gave **20** (0.05 g, 47%) as a white yellowish amorphous solid. IR (KBr) cm^{-1}: 3057, 2944, 1812, 1663, 1613, 1468, 1375, 1357, 1252, 1190, 1175, 1078, 1031, 918, 878, 847, 758. UV λ_{max} (MeOH) nm (log ε): 262 (4.58), 324 (4.03). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.32 (3H, s, C2-CH_3), 1.65 (3H, s, C2-CH_3),

4.16 (3H, s, OCH_3), 4.79 (1H, d, J=8 Hz, C3-H), 6.08 (1H, d, J=8 Hz, C4-H), 6.77 (1H, s, C14-H), 7.50 (1H, td, J=8, 1 Hz, C9-H), 7.61 (1H, td, J=8, 1 Hz, C10-H), 7.81 (1H, s C12-H), 7.90 (1H, dd, J=8, 1 Hz, C11-H), 8.05 (1H, dd, J=8, 1 Hz, C8-H), 8.88 (1H, s, C7-H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 175.5 (s, C-6), 163.0 (s, C-5), 160.4 (s, C-14a), 158.7 (s, C-13a), 153.8 (s, O–CO–O), 151.4 (s, C-12a), 136.5 (s, C-11a), 129.7 (2d, C-7, C-8), 129.1 (d, C-10), 128.4 (d, C-7), 127.0 (d, C-11), 125.7 (d, C-9), 121.9 (s, C-6a), 113.0 (d, C-12), 110.8 (s, C-5a), 108.4 (s, C-4a), 102.2 (d, C-14), 78.0 (d, C-3), 76.4 (s, C-2), 68.4 (d, C-4), 64.2 (q, OCH_3), 24.2 (q, C2-$\underline{C}H_3$), 22.4 (q, C2-$\underline{C}H_3$). DCI-MS m/z: 419 $[MH]^+$. *Anal.* Calcd for $C_{24}H_{18}O_7$: C, 68.90; H, 4.34. Found: C, 68.92; H, 4.40.

Biological Pharmacology Cytotoxicity: Murine leukemia L1210 cells from the American Type Culture Collection (Rockville Pike, MD) were grown in RPMI medium 1640 supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin and 10 mM HEPES buffer (pH 7.4). The cytotoxicity was measured by microculture tetrazolium assay essentially as described.[21] Cells were exposed for 48 h to nine graded concentrations in triplicate of the test drug. Results are expressed as IC_{50} (mean, n=3), which is defined as the drug concentration inhibiting the absorbance by 50% with respect to that of untreated cells.

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