Synthesis and Cytotoxic Activity of Benzo[*a*]pyrano[3,2-*h*] and [2,3-*i*]xanthone Analogues of Psorospermine, Acronycine, and Benzo[*a*]acronycine

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Received February 20, 2006; accepted April 14, 2006

Condensation of 2-hydroxy-1-naphthalenecarboxylic acid with phloroglucinol afforded 9,11-dihydroxy-12*H*-benzo[*a*]xanthen-12-one (6). 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 6-hydroxy-3,3-dimethyl-3*H*,7*H*-benzo[*a*]pyrano[3,2-*h*]xanthen-7-one (12) and 5-hydroxy-2,2-dimethyl-2*H*,6*H*-benzo[*a*]pyrano[2,3-*i*]xanthen-6-one (13), which were methylated into 6-methoxy-3,3-dimethyl-3*H*,7*H*-benzo[*a*]pyrano[3,2-*h*]xanthen-7-one (14) and 5-methoxy-2,2-dimethyl-2*H*,6*H*-benzo[*a*]pyrano[2,3-*i*]xanthen-6-one (15), respectively. Osmium tetroxide oxidation of 14 and 15 gave the corresponding (±)-*cis*-diols 16 and 17, which afforded the corresponding esters 18—21 upon acylation. Similarly, condensation of 2-hydroxy-1-naphthalenecarboxylic acid with 3,5-dimethoxyaniline gave 11-amino-9-methoxy-12*H*-benzo[*a*]xanthen-12-one (23) which was converted into 11-amino-9-hydroxy-12*H*-benzo[*a*]xanthen-12-one (24) upon treatment with hydrogen bromide in acetic acid. Alkylation with 3-chloro-3-methyl-1-butyne followed by Claisen rearrangement afforded 6-amino-3,3-dimethyl-3*H*,7*H*-benzo[*a*]pyrano[3,2-*h*]xanthen-7-one (25) and 5-amino-2,2-dimethyl-2*H*,6*H*-benzo[*a*]pyrano[2,3-*i*]xanthen-6-one (26). The new benzopyranoxanthone derivatives only displayed marginal antiproliferative activity when tested against L1210 and KB-3-1 cell lines. The only compounds found significantly active against L1210 cell line, 16 and 20, belong to the benzo[*a*]pyrano[3,2-*h*]xanthen-7-one series, which possess a pyran ring fused angularly onto the xanthone basic core.

Key words benzopyranoxanthone; acronycine; synthesis; cytotoxicity

Natural products play a major role in anticancer drug discovery as a unique source of original structures which can provide models for future drug design. Relevant examples currently used in the clinic include teniposide and etoposide derived from naturally occurring podophyllotoxin, taxotere based on the structure of the yew diterpene paclitaxel, topotecan and irinotecan developed from camptothecin, and semisynthetic vinca alkaloids such as vinorelbine and vinflunine originating from the lead structure vinblastine.[1)] In this context, our group is interested in potential anticancer natural product-like compounds including an acridone or xanthone derived basic core and an additional fused dimethylpyran ring. Indeed, the fused dimethylpyran system, which arises in Nature from the condensation of a phenol with an active isoprene unit, appears as a privileged substructure present in numerous bioactive compounds exemplified by the insecticide chromenes precocenes,[2)] the HIV-1 reverse transcriptase inhibitors calanolides,[3)] and the antitumor acridone alkaloid acronycine (**1**).[4)] Based on this latter model, we recently prepared the pentacyclic analogues benzo[*a*]acronycine (6-methoxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*a*]-pyrano[3,2-*h*]acridin-7-one) (**2**) and and benzo[*b*]acronycine (6-methoxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]-pyrano[3,2-*h*]acridin-7-one) (**3**), together with several series of corresponding 1,2-dihydrodiol diesters, which displayed significantly enhanced *in vivo* antitumor activity when compared to the parent alkaloid.[5—8)] Such compounds are exemplified by *cis*-diacetate **4**, developed under the code S23906-1, which is currently undergoing phase I clinical trials.[9)] The mechanism of its action implies alkylation of the 2-amino group of DNA guanine residues by the carbocation resulting from the elimination of the ester leaving group at position 1 of the drug.[10—12)] This mechanism is related to that implied in the cytotoxic and antitumor properties of the natural furanoxanthone psorospermine (**5**), isolated from *Psorospermum febrifugum*.[13—15)] Actually, psorospermine has been shown to interact with DNA and to alkylate the *N*-7 of the guanine residues.[16,17)] In a continuation of our studies on the structure activity relationships in the acronycine series,[18—24)] we describe here the synthesis and biological activites of

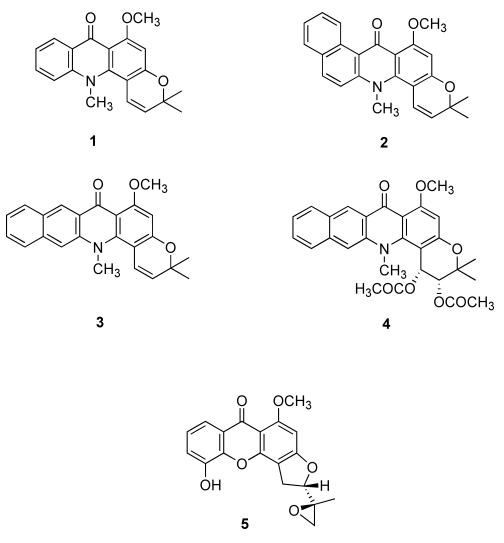

Chart 1

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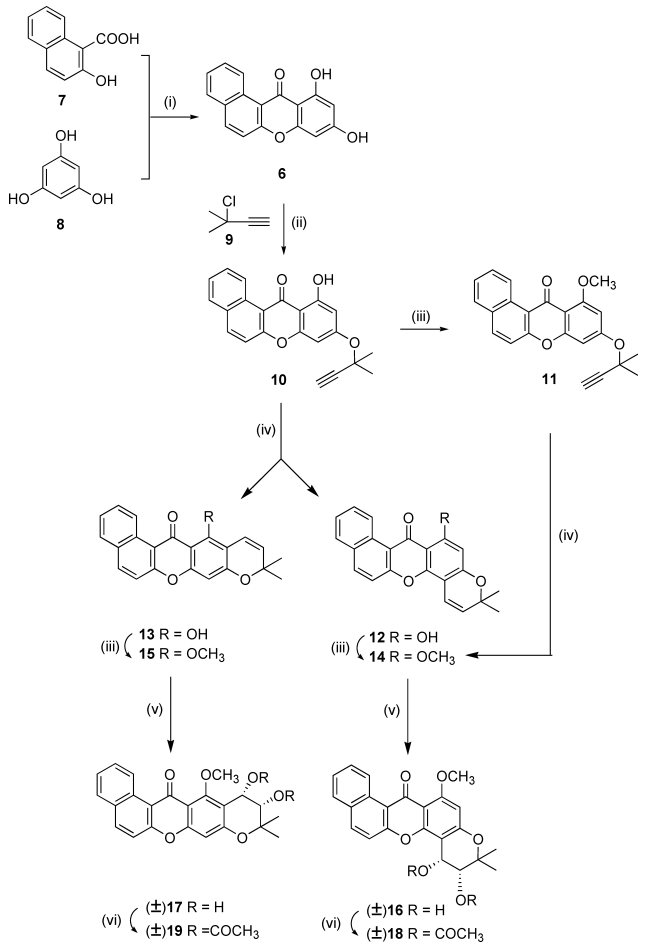
pentacyclic xanthone isosters and analogues of benzo[*a*]-acronycine, in the benzo[*a*]pyrano[3,2-*h*] and [2,3-*i*]xanthone series.

Chemistry

Construction of the tetracyclic 9,11-dihydroxy-12*H*-benzo[*a*]xanthen-12-one (**6**) basic skeleton was achieved by condensation of 2-hydroxy-1-naphthalenecarboxylic acid (**7**) with phloroglucinol (**8**) in the presence of zinc chloride and phosphoryl chloride.[25,26] Chelation of the 11-hydroxy group of **6** by the carbonyl at the 12-position permitted the selective *O*-alkylation of the 9-hydroxy group with 3-chloro-3-methyl-but-1-yne (**9**),[27] to give the corresponding propargylic ether **10**. Methylation of the 11-hydroxy group of **10**, carried out with dimethylsulfate in the presence of sodium hydride, gave **11**. When thermal cyclization was performed on the hydroxy ether **10** for 2 h at 130 °C in dimethylformamide, the two isomeric 6-hydroxy-3,3-dimethyl-3*H*,7*H*-benzo[*a*]pyrano[3,2-*h*]xanthen-7-one (**12**) and 5-hydroxy-2,2-dimethyl-2*H*,6*H*-benzo[*a*]pyrano[2,3-*i*]xanthen-6-one (**13**) were isolated in 57% and 30% yield, respectively, after separation by column chromatography. Benzopyranoxanthones **12** and **13** could be subsequently successfully converted into 6-methoxy-3,3-dimethyl-3*H*,7*H*-benzo[*a*]pyrano[3,2-*h*]xanthen-7-one (**14**) and 5-methoxy-2,2-dimethyl-2*H*,6*H*-benzo[*a*]pyrano[2,3-*i*]xanthen-6-one (**15**), upon treatment with dimethylsulfate in the presence of sodium hydride. In contrast, Claisen rearrangement of the methoxy ether **11** under similar conditions exclusively gave 6-methoxy-3,3-dimethyl-3*H*,7*H*-benzo[*a*]-pyrano[3,2-*h*]xanthen-7-one (**14**), with the pyran ring angularly fused onto the xanthone tricyclic system, 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 mode of fusion of the newly formed dimethylpyran ring onto the xanthone basic core, angular for **12** and **14**, and linear for **13** and **15**, was unambiguously ascribed for each compound from phase-sensitive NOESY experiments.[21,28]

In order to obtain dihydrodiols diesters similar to the highly active compounds in the benzo[*a*] and [*b*]acronycine series,[5—8] the (±)-*cis*-diols **16** and **17** were prepared by catalytic osmium tetroxide oxidation of **14** and **15**, respectively, using *N*-methylmorpholine *N*-oxide to regenerate the oxidizing agent.[29] The diols **16** and **17** were subsequently converted into the corresponding diacetates **18** and **19** upon treatment with acetic anhydride, and the cyclic carbonates **20** and **21**, when *N*,*N*′-carbonyldiimidazole was used as acylating agent.

In the acronycine and benzoacronycine series, interesting results in terms of cytotoxic activitiy were observed when the electron donating methoxy group at C-6 was replaced by an amino substituent.[30] Therefore, it appeared to us interesting



Reagents and conditions: (i) $POCl_3$, $ZnCl_2$, 65 °C; (ii) **9**, K_2CO_3, KI, DMF, 65 °C; (iii) $(CH_3)_2SO_4$, NaH, DMF, rt; (iv) DMF, 130 °C; (v) OsO_4, NMMO, *t*BuOH–THF–H_2O, rt; (vi) Ac_2O, C_5H_5N, rt.

Chart 2

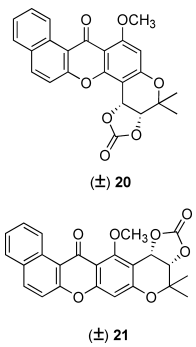
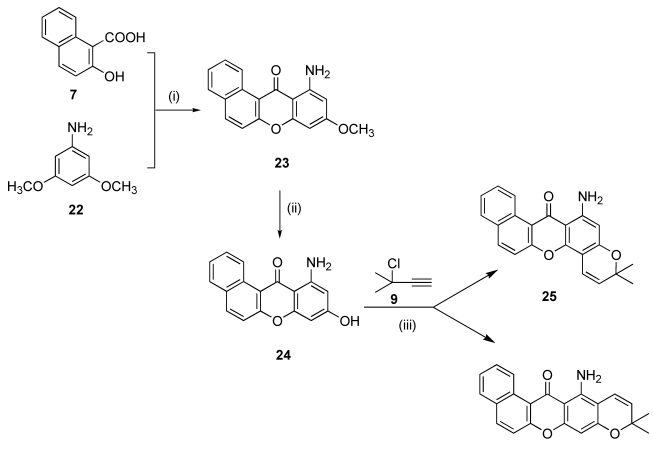


Chart 3



Reagents and conditions: (i) $POCl_3$, $ZnCl_2$, 65 °C; (ii) HBr, AcOH, Reflux; (iii) **9**, K_2CO_3, KI, DMF, 65 °C, then 130 °C.

Chart 4

Table 1. Inhibition of L1210 and KB-3-1 Cell Proliferation by Benzo[*a*]pyrano[3,2-*h*]xanthone Derivatives **14**, **16**, **18**, **20**, **25** and Benzo[*a*]pyrano[2,3-*i*]xanthone Derivatives **15**, **17**, **19**, **21**, **26** in Comparison with Acronycine (**1**), Benzo[*a*]acronycine (**2**), and Benzo[*b*]acronycine (**3**)

Compound	**1**	**2**	**3**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**25**	**26**
L1210 IC_{50} (μM)	23	2.5	2.0	22	21	1.0	16	42	19	3.0	46	40	27.5
KB-3-1 IC_{50} (μM)	37	8.5	3.4	34.5	30	28	19.5	7.5	50	37	30	19	31.5

to prepare compounds bearing a NH_2 group at the corresponding position in both benzo[*a*]pyrano[3,2-*h*] and [2,3-*i*]xanthone series. The synthetic strategy used was similar to that previously developed for the preparation of **12** and **13**. Accordingly, condensation of 2-hydroxy-1-naphthalenecarboxylic acid (**7**) with 3,5-dimethoxyaniline (**22**) gave 11-amino-9-methoxy-12*H*-benzo[*a*]xanthen-12-one (**23**), which was converted into 11-amino-9-hydroxy-12*H*-benzo[*a*]xanthen-12-one (**24**) upon treatment with hydrogen bromide in acetic acid. Alkylation with 3-chloro-3-methyl-1-butyne followed by Claisen rearrangement, afforded the desired 6-amino-3,3-dimethyl-3*H*,7*H*-benzo[*a*]pyrano[3,2-*h*]xanthen-7-one (**25**) and 5-amino-2,2-dimethyl-2*H*,6*H*-benzo[*a*]pyrano[2,3-*i*]xanthen-6-one (**26**).

Pharmacology

The new benzopyranoxanthone derivatives were evaluated *in vitro* for their cytotoxicity against two tumor cell lines, a murine leukemia cell line (L1210) and a human epidermoid carcinoma cell line (KB-3-1). The results (IC_{50}) are reported in Table 1. Most compounds only displayed marginal antiproliferative activity on both cell lines, within the same order of magnitude as acronycine itself. Nevertheless, diol **16** and carbonate **20** showed significant cytotoxicity when tested against L1210 cells.

Results and Discussion

All benzo[*a*]pyrano[2,3-*i*]xanthen-6-one derivatives, with a pyran ring linearly fused on the tricyclic xanthone system, are almost devoid of significant antiproliferative activity. In contrast, it is interesting to note that compounds **16** and **20**, found significantly active against L1210 cell line, as well as diacetate **18**, which exhibits cytotoxic properties against KB-3-1 cells, possess a pyran ring fused angularly onto the benzoxanthone basic core. This is a common structural feature with highly active acronycine and benzo[*a*] and [*b*]acronycine derivatives, which present a pyran ring angularly fused onto the acridone tricyclic system. Nevertheless, all the new benzo[*a*]pyrano[3,2-*h*]xanthen-7-one derivatives remain less potent than their benzo[*a*]pyrano[3,2-*h*]acridin-7-one and benzo[*b*]pyrano[3,2-*h*]acridin-7-one counterparts in inhibiting the proliferation of L1210 and KB-3-1 cells. Considering the structure activity relationships, the acridone moiety present in the natural alkaloid acronycine appears as an important structural requirement to maximize the cytotoxic effects in this series of drugs.

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 400 and 75 MHz, using Bruker Avance 400 and AC-300 spectrometers, respectively. When necessary, the signals were unambiguously assigned by 2D NMR techniques: ^{1}H–^{1}H COSY, ^{1}H–^{1}H NOESY, ^{13}C–^{1}H HMQC, and ^{13}C–^{1}H HMBC. 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.

9,11-Dihydroxy-12*H*-benzo[*a*]xanthen-12-one (6) To a mixture of 2-hydroxy-1-naphthalenecarboxylic acid (**7**) (18.8 g, 100 mmol), anhydrous phloroglucinol (**8**) (12.6 g, 100 mmol), and freshly fused zinc chloride (41 g), phosphoryl chloride (100 ml) was added. The reaction mixture was stirred at 65 °C for 4 h, poured onto ice (1500 g) and left overnight. After filtration, the dark red 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** (6.31 g, 23%) as yellow needles, mp 194—195 °C (acetone/ethyl acetate 4 : 1). IR (KBr) cm^{-1}: 3380, 3270, 3100, 1639, 1573, 1511, 1437, 1336, 1235, 1153, 819. UV λ_{max} (MeOH) nm ($\log \varepsilon$): 238 (sh.), 273 (4.63), 329 (4.30), 367 (sh.). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 6.23 (1H, d, J=2 Hz, C10-H), 6.41 (1H, d, J=2 Hz, C8-H), 7.61 (1H, td, J=8, 1 Hz, C3-H), 7.63 (1H, d, J=9 Hz, C6-H), 7.74 (1H, td, J=8, 1 Hz, C2-H), 8.03 (1H, dd, J=8, 2 Hz, C4-H), 8.31 (1H, d, J=9 Hz, C5-H), 9.91 (1H, dd, J=8, 1 Hz, C1-H), 10.99 (1H, s, D_2O exch., C9-OH), 13.31 (1H, s, D_2O exch., C11-OH). ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 182.7 (C-12), 165.6 (C-11), 163.3 (C-9), 157.7 (C-6a), 156.7 (C-7a), 138.0 (C-5), 130.5 (2C, C-12b, C-4a), 130.0 (C-2), 129.4 (C-4), 126.7 (C-6), 126.4 (C-1), 118.3 (C-3), 112.6 (C-12a), 104.1 (C-11a), 99.1 (C-10), 93.9 (C-8). DCI-MS m/z: 279 $[MH]^+$. *Anal.* Calcd for $C_{17}H_{10}O_4$: C, 73.37; H, 3.62. Found: C, 73.25; H, 3.74.

11-Hydroxy-9-(1,1-dimethylpropargyloxy)-12*H*-benzo[*a*]xanthen-12-one (10) A solution of 9,11-dihydroxy-12*H*-benzo[*a*]xanthen-12-one (**6**) (3.48 g, 12.5 mmol) in dry *N*,*N*-dimethylformamide (140 ml) was stirred and heated at 65 °C for 24 h under nitrogen, in the presence of potassium carbonate (3.45 g), potassium iodide (4.15 g) and 3-chloro-3-methylbut-1-yne (**9**) (10.25 g, 100 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** (3.19 g, 74%) as a pale yellow amorphous solid. IR (KBr) cm^{-1}: 3245, 2920, 1654, 1581, 1503, 1448, 1355, 1247, 1142, 1025, 831, 749. UV λ_{max} (MeOH) nm ($\log \varepsilon$): 274 (4.45), 324 (4.23). DCI-MS m/z: 345 $[MH]^+$. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.26 (6H, s, $C(CH_3)_2$), 2.74 (1H, s, C3′-H), 6.74 (1H, d, J=2 Hz, C10-H), 6.89 (1H, d, J=2 Hz, C8-H), 7.54 (1H, d, J=9 Hz, C6-H), 7.62 (1H, td, J=8, 1 Hz, C3-H), 7.79 (1H, td, J=8, 1 Hz, C2-H), 7.93 (1H, dd, J=8, 1 Hz, C4-H), 8.15 (1H, d, J=9 Hz, C5-H), 9.98 (1H, dd, J=8, 1 Hz, C1-H), 13.40 (1H, s, D_2O exch., C11-OH). *Anal.* Calcd for $C_{22}H_{16}O_4$: C, 76.73; H, 4.68. Found: C, 76.85; H, 4.69.

11-Methoxy-9-(1,1-dimethylpropargyloxy)-12*H*-benzo[*a*]xanthen-12-one (11) Sodium hydride (0.07 g of 50% oil dispersion, 1.5 mmol) was added to an ice-cooled solution of 11-hydroxy-9-(1,1-dimethylpropargyloxy)-12*H*-benzo[*a*]xanthen-12-one (**10**) (0.172 g, 0.5 mmol) in *N*,*N*-dimethylformamide (5 ml). Dimethylsulfate (0.19 ml, 2 mmol) was added and the reaction mixture was stirred under nitrogen at room temperature for 3.5 h. After addition of water (50 ml) and neutralization with 10% sulfuric acid, the mixture was extracted with dichloromethane (4×20 ml). The organic layers were evaporated under reduced pressure. Flash chromatography (solvent: cyclohexane, then cyclohexane/acetone 99 : 1 to 90 : 10) gave **11** (0.14 g, 78%) as pale yellow prisms, mp 173—174 °C (acetone/ethyl acetate 4 : 1). IR (KBr) cm^{-1}: 3200, 3114, 2932, 1635, 1611, 1577, 1511, 1433, 1351, 1246, 1134, 819, 753. UV λ_{max} (MeOH) nm ($\log \varepsilon$): 263 (sh.), 270 (4.86), 306 (4.47), 319 (4.50), 341 (sh.), 375 (sh.). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.73 (6H, s, $C(CH_3)_2$), 2.46 (1H, s, C3′-H), 4.05 (3H, s, OCH_3), 6.64 (1H, d, J=1 Hz, C10-H), 7.06 (1H, d, J=1 Hz, C8-H), 7.61 (1H, td, J=8, 1 Hz, C3-H), 7.67 (1H, d, J=9 Hz, C6-H), 7.73 (1H, td, J=8, 1 Hz, C2-H), 8.04 (1H, dd, J=8, 1 Hz, C4-H), 8.28 (1H, d, J=9 Hz, C5-H), 9.94 (1H, dd, J=8, 1 Hz, C1-H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 177.8 (C-12), 161.4

(C-11), 160.5 (C-9), 157.5 (C-6a), 156.1 (C-7a), 135.6 (C-5), 131.1 (C-4a), 130.3 (C-12b), 129.0 (C-2), 128.2 (C-4), 127.2 (C-1), 125.8 (C-3), 117.3 (C-6), 115.6 (C-12a), 109.5 (C-11a), 99.0 (C-10), 98.2 (C-8), 84.9 (C-2′), 75.2 (C-3′), 72.6 (C-1′), 56.4 (OCH_3), 29.6 (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, 76.99; H, 5.08.

6-Hydroxy-3,3-dimethyl-3*H*,7*H*-benzo[*a*]pyrano[3,2-*h*]xanthen-7-one (12) and 5-Hydroxy-2,2-dimethyl-2*H*,6*H*-benzo[*a*]pyrano[2,3-*i*]xanthen-6-one (13) A solution of 11-hydroxy-9-(1,1-dimethylpropargyloxy)-12*H*-benzo[*a*]xanthen-12-one (**10**) (1 g, 2.9 mmol) in *N*,*N*-dimethylformamide (60 ml) was heated under nitrogen at 130 °C for 2 h. Water (50 ml) was added and the mixture was extracted with dichloromethane (3×50 ml). The organic layers were evaporated under reduced pressure. Flash chromatography (solvent: cyclohexane, then cyclohexane/acetone 99 : 1 to 95 : 5) gave successively 5-hydroxy-2,2-dimethyl-2*H*,6*H*-benzo[*a*]pyrano[2,3-*i*]xanthen-6-one (**13**) (0.301 g, 30%) and 6-hydroxy-3,3-dimethyl-3*H*,7*H*-benzo[*a*]-pyrano[3,2-*h*]xanthen-7-one (**12**) (0.572 g, 57%) as pale yellow amorphous solids.

6-Hydroxy-3,3-dimethyl-3*H*,7*H*-benzo[*a*]pyrano[3,2-*h*]xanthen-7-one (**12**): IR (KBr) cm^{-1}: 3250, 3064, 2963, 2920, 1647, 1577, 1518, 1448, 1344, 1274, 1145, 1114, 827, 746. UV λ_{max} (MeOH) nm (log ε): 276 (3.81), 380 (2.94). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.51 (6H, s, C(CH_3)$_2$), 5.62 (1H, d, *J*=10 Hz, C2-H), 6.30 (1H, s, C5-H), 6.82 (1H, d, *J*=10 Hz, C1-H), 7.45 (1H, d, *J*=9 Hz, C13-H), 7.56 (1H, td, *J*=8, 1 Hz, C10-H), 7.73 (1H, td, *J*=8, 1 Hz, C9-H), 7.85 (1H, dd, *J*=8, 1 Hz, C11-H), 8.07 (1H, d, *J*=9 Hz, C12-H), 9.89 (1H, dd, *J*=8, 1 Hz, C8-H), 13.54 (1H, s, D_2O exch., C6-OH). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 183.1 (C-7), 163.2 (C-6), 160.1 (C-4a), 157.4 (C-13a), 150.7 (C-14a), 137.0 (C-12), 130.8 (C-7b), 130.3 (C-11a), 129.6 (C-9), 128.6 (C-11), 127.2 (C-2), 126.9 (C-8), 126.3 (C-10), 117.5 (C-13), 115.3 (C-1), 113.2 (C-7a), 105.1 (C-6a), 100.6 (C-14b), 99.8 (C-5), 78.2 (C-3), 28.4 (2C, C3-($\underline{C}H_3$)$_2$). DCI-MS *m/z*: 345 $[MH]^+$. *Anal.* Calcd for $C_{22}H_{16}O_4$: C, 76.73; H, 4.68. Found: C, 76.85; H, 4.63.

5-Hydroxy-2,2-dimethyl-2*H*,6*H*-benzo[*a*]pyrano[2,3-*i*]xanthen-6-one (**13**): IR (KBr) cm^{-1}: 3247, 3083, 2966, 2932, 1647, 1592, 1514, 1448, 1297, 1239, 1138, 823, 757. UV λ_{max} (MeOH) nm (log ε): 251 (4.49), 261 (4.52), 298 (4.66), 349 (4.28). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.51 (6H, s, C(CH_3)$_2$), 5.63 (1H, d, *J*=10 Hz, C3-H), 6.42 (1H, s, C14-H), 6.80 (1H, d, *J*=10 Hz, C4-H), 7.51 (1H, d, *J*=9 Hz, C12-H), 7.61 (1H, td, *J*=8, 1 Hz, C9-H), 7.78 (1H, td, *J*=8, 1 Hz, C8-H), 7.91 (1H, dd, *J*=8, 1 Hz, C10-H), 8.13 (1H, d, *J*=9 Hz, C11-H), 9.98 (1H, dd, *J*=8, 1 Hz, C7-H), 13.82 (1H, s, D_2O exch., C5-OH). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 183.0 (C-6), 163.1 (C-14a), 160.0 (2C, C-5, C-12a), 157.3 (C-13a), 136.8 (C-11), 130.8 (C-6b), 130.2 (C-10a), 129.5 (C-8), 128.5 (C-10), 127.1 (C-3), 126.8 (C-7), 126.2 (C-9), 117.4 (C-12), 114.9 (C-4), 113.3 (C-6a), 105.2 (C-5a), 100.5 (C-4a), 99.7 (C-14), 78.1 (C-2), 28.3 (2C, C3-($\underline{C}H_3$)$_2$). DCI-MS *m/z*: 345 $[MH]^+$. *Anal.* Calcd for $C_{22}H_{16}O_4$: C, 76.73; H, 4.68. Found: C, 76.62; H, 4.73.

6-Methoxy-3,3-dimethyl-3*H*,7*H*-benzo[*a*]pyrano[3,2-*h*]xanthen-7-one (14) Method A: A solution of 11-methoxy-9-(1,1-dimethylpropargyloxy)-12*H*-benzo[*a*]xanthen-12-one (**11**) (0.716 g, 2 mmol) in *N*,*N*-dimethylformamide (50 ml) was heated under nitrogen at 130 °C for 1.5 h. The cooled mixture was diluted with water (200 ml) and extracted with ethyl acetate (3×50 ml). The organic layers were evaporated under reduced pressure. Flash chromatography (solvent: cyclohexane/acetone 4 : 1) gave **14** (0.634 g, 88%) as pale yellow prisms, mp 202—203 °C (cyclohexane/acetone 4 : 1). IR (KBr) cm^{-1}: 3060, 2963, 2932, 1645, 1635, 1569, 1507, 1429, 1340, 1243, 1114, 815, 773. UV λ_{max} (MeOH) nm (log ε): 246 (4.44), 277 (4.66), 299 (sh.), 326 (4.11), 362 (3.88). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.52 (6H, s, C3-(CH_3)$_2$), 4.03 (3H, s, OCH_3), 5.63 (1H, d, *J*=10 Hz, C2-H), 6.38 (1H, s, C5-H), 6.92 (1H, d, *J*=10 Hz, C1-H), 7.48 (1H, d, *J*=9 Hz, C13-H), 7.56 (1H, td, *J*=8, 1 Hz, C10-H), 7.73 (1H, td, *J*=8, 1 Hz, C9-H), 7.87 (1H, dd, *J*=8, 1 Hz, C11-H), 8.10 (1H, d, *J*=9 Hz, C12-H), 10.18 (1H, dd, *J*=8, 1 Hz, C8-H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 178.0 (C-7), 161.6 (C-6), 158.2 (C-4a), 155.9 (C-13a), 152.6 (C-14a), 135.7 (C-12), 131.3 (C-7b), 130.5 (C-11a), 129.2 (C-9), 128.3 (C-11), 127.4 (C-2), 127.3 (C-8), 125.9 (C-10), 117.3 (C-13), 115.6 (C-7a), 115.5 (C-1), 108.7 (C-6a), 102.0 (C-14b), 96.2 (C-5), 78.2 (C-3), 56.6 ($O\underline{C}H_3$), 28.4 (2C, C3-($\underline{C}H_3$)$_2$). DCI-MS *m/z*: 359 $[MH]^+$. *Anal.* Calcd for $C_{23}H_{18}O_4$: C, 77.08; H, 5.06. Found: C, 77.01; H, 5.12.

Method B: Sodium hydride (0.52 g of 50% oil dispersion, 10.8 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**) (1.25 g, 3.6 mmol) in *N*,*N*-dimethylformamide (60 ml). Dimethylsulfate (1.0 ml, 10.8 mmol) was added and the reaction mixture was stirred under nitrogen at room temperature for 2 h. After addition of ice (100 g), the mixture was extracted with ethyl acetate (3×50 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 90 : 10) gave **14** (1.03 g, 80%) as pale yellow prisms, mp 202—203 °C.

5-Methoxy-2,2-dimethyl-2*H*,6*H*-benzo[*a*]pyrano[2,3-*i*]xanthen-6-one (15) Methylation of 5-hydroxy-2,2-dimethyl-2*H*,6*H*-benzo[*a*]pyrano[2,3-*i*]xanthen-6-one (**13**) (1.25 g, 3.6 mmol), under conditions similar to those described for the preparation of **14** from **12**, afforded **15** (1.01 g, 78%) as a yellow amorphous solid. IR (KBr) cm^{-1}: 3052, 2971, 2924, 1643, 1604, 1511, 1433, 1243, 1146, 823, 749. UV λ_{max} (MeOH) nm (log ε): 261 (sh.), 282 (4.75), 340 (4.37). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.51 (6H, s, C2-(CH_3)$_2$), 4.02 (3H, s, OCH_3), 5.73 (1H, d, *J*=10 Hz, C3-H), 6.70 (1H, s, C14-H), 6.80 (1H, d, *J*=10 Hz, C4-H), 7.47 (1H, d, *J*=9 Hz, C12-H), 7.56 (1H, td, *J*=8, 1 Hz, C9-H), 7.74 (1H, td, *J*=8, 1 Hz, C8-H), 7.87 (1H, dd, *J*=8, 1 Hz, C10-H), 8.05 (1H, d, *J*=9 Hz, C11-H), 10.06 (1H, dd, *J*=8, 1 Hz, C7-H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 177.4 (C-6), 158.5 (C-5), 157.2 (C-13a), 156.3 (2C, C-14a, C-12a), 135.8 (C-11), 131.2 (C-6b), 130.4 (2C, C-3, C-10a), 129.3 (C-8), 128.5 (C-10), 127.0 (C-7), 125.9 (C-9), 117.6 (C-12), 115.4 (2C, C-4, C-6a), 112.8 (C-5a), 112.4 (C-4a), 100.3 (C-14), 77.9 (C-2), 62.9 ($O\underline{C}H_3$), 28.5 (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, 76.92; H, 5.15.

(±)-*cis*-1,2-Dihydroxy-6-methoxy-3,3-dimethyl-1,2-dihydro-3*H*,7*H*-benzo[*a*]pyrano[3,2-*h*]xanthen-7-one (16) Compound **14** (0.537 g, 1.5 mmol) was added to a solution of osmium tetroxide (2.5% in 2-methyl-2-propanol, 1.08 ml) and 4-methylmorpholine *N*-oxide monohydrate (0.204 g, 1.5 mmol) in *t*-BuOH–THF–H_2O (10 : 3 : 1, 12 ml). The reaction mixture was stirred at room temperature for 48 h. After addition of saturated aqueous solution of sodium bisulfite (30 ml), the mixture was stirred for 1 h and then extracted with dichloromethane (4×25 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/acetone 99 : 1 to 90 : 10) to give **15** (0.537 g, 90%) as a yellowish amorphous solid. IR (KBr) cm^{-1}: 3425, 2971, 2932, 1645, 1635, 1580, 1511, 1433, 1278, 1204, 1107, 819, 753. UV λ_{max} (MeOH) nm (log ε): 260 (sh.), 271 (4.63), 320 (4.28), 358 (sh.). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 1.41 (6H, s, C3-(CH_3)$_2$), 3.67 (1H, dd, *J*=8, 5 Hz, C2-H), 3.87 (3H, s, OCH_3), 5.02 (1H, t, *J*=5 Hz, C1-H), 5.03 (1H, d, *J*=8 Hz, D_2O exch., C2-OH), 5.43 (1H, d, *J*=5 Hz, D_2O exch., C1-OH), 6.39 (1H, s, C5-H), 7.61 (1H, td, *J*=8, 1 Hz, C10-H), 7.73 (1H, td, *J*=8, 1 Hz, C9-H), 7.75 (1H, d, *J*=9 Hz, C13-H), 8.04 (1H, dd, *J*=8, 1 Hz, C11-H), 8.29 (1H, d, *J*=9 Hz, C12-H), 9.84 (1H, dd, *J*=8, 1 Hz, C8-H). ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 177.0 (C-7), 161.6 (C-6), 158.4 (C-4a), 157.1 (C-13a), 156.5 (C-14a), 136.6 (C-12), 131.3 (C-7b), 131.1 (C-11a), 129.9 (C-9), 129.7 (C-11), 127.0 (C-8), 126.8 (C-10), 119.1 (C-13), 115.6 (C-7a), 108.9 (C-6a), 105.3 (C-14b), 97.1 (C-5), 80.4 (C-3), 75.2 (C-2), 65.1 (C-1), 57.1 ($O\underline{C}H_3$), 27.9 (C3-$\underline{C}H_3$), 22.0 (C3-$\underline{C}H_3$). DCI-MS *m/z*: 393 $[MH]^+$. *Anal.* Calcd for $C_{23}H_{20}O_6$: C, 70.40; H, 5.14. Found: C, 70.32; H, 5.11.

(±)-*cis*-3,4-Dihydroxy-5-methoxy-2,2-dimethyl-3,4-dihydro-2*H*,6*H*-benzo[*a*]pyrano[2,3-*i*]xanthen-6-one (17) Oxidation of **15** (0.309 g, 0.86 mmol) under conditions similar to those described for the preparation of **16** from **14**, afforded **17** (0.286 g, 85%) as a yellowish amorphous solid. IR (KBr) cm^{-1}: 3440, 3052, 2974, 2940, 1639, 1604, 1511, 1433, 1235, 1130, 827, 749. UV λ_{max} (MeOH) nm (log ε): 261 (sh.), 269 (4.70), 298 (4.21), 324 (4.45), 360 (sh.). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 1.42 (3H, s, C2-CH_3), 1.51 (3H, s, C2-CH_3), 3.26 (1H, d, D_2O exch., *J*=4.5 Hz, C3-OH), 3.89 (1H, t, *J*=4.5 Hz, C3-H), 4.16 (3H, s, OCH_3), 4.26 (1H, d, D_2O exch., *J*=1 Hz, C4-OH), 5.17 (1H, dd, *J*=4.5, 1 Hz, C4-H), 6.78 (1H, s, H-14), 7.49 (1H, d, *J*=9 Hz, C12-H), 7.59 (1H, td, *J*=8, 1 Hz, C9-H), 7.77 (1H, td, *J*=8, 1 Hz, C8-H), 7.90 (1H, dd, *J*=8, 1 Hz, C10-H), 8.09 (1H, d, *J*=9 Hz, C11-H), 10.02 (1H, dd, *J*=8, 1 Hz, C7-H). ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 177.3 (C-6), 160.8 (C-5), 158.0 (C-13a), 157.3 (C-14a), 156.6 (C-12a), 136.3 (C-11), 131.1 (C-6b), 130.4 (C-10a), 129.5 (C-8), 128.6 (C-10), 126.8 (C-7), 126.1 (C-9), 117.6 (C-12), 115.2 (C-6a), 114.2 (C-5a), 111.7 (C-4a), 101.0 (C-14), 79.0 (C-2), 70.8 (C-3), 62.8 (C-4), 62.6 ($O\underline{C}H_3$), 24.6 (C2-$\underline{C}H_3$), 23.0 (C2-$\underline{C}H_3$). DCI-MS *m/z*: 393 $[MH]^+$. *Anal.* Calcd for $C_{23}H_{20}O_6$: C, 70.40; H, 5.14. Found: C, 70.27; H, 5.16.

(±)-*cis*-1,2-Diacetoxy-6-methoxy-3,3-dimethyl-1,2-dihydro-3*H*,7*H*-benzo[*a*]pyrano[3,2-*h*]xanthen-7-one (18) An ice-cooled mixture of acetic anhydride (0.8 ml, 8 mmol) and pyridine (4 ml) was added to (±)-*cis*-1,2-dihydroxy-6-methoxy-3,3-dimethyl-1,2-dihydro-3*H*,7*H*-benzo[*a*]-pyrano[3,2-*h*]xanthen-7-one (**16**) (0.078 g, 0.2 mmol). After stirring at room temperature for 3 d, the mixture was poured into 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** (0.076 g, 80%) as an amorphous white solid. IR (KBr) cm^{-1}:

3052, 2978, 2936, 1747, 1647, 1584, 1475, 1375, 1247, 1134, 1041, 1021, 823. UV λ_{max} (MeOH) nm (log ε): 270 (4.56), 319 (4.22). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.48 (3H, s, C3-CH_3), 1.53 (3H, s, C3-CH_3), 2.16 (6H, s, C1-OCOCH_3, C2-OCOCH_3), 4.05 (3H, s, OCH_3), 5.32 (1H, d, J=5 Hz, C2-H), 6.38 (1H, s, C5-H), 6.70 (1H, d, J=5 Hz, C1-H), 7.36 (1H, d, J=9 Hz, C13-H), 7.57 (1H, td, J=8, 1 Hz, C10-H), 7.73 (1H, td, J=8,1 Hz, C9-H), 7.88 (1H, dd, J=8, 1 Hz, C11-H), 8.06 (1H, d, J=9 Hz, C12-H), 10.07 (1H, dd, J=8, 1 Hz, C8-H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 177.5 (C-7), 170.4 (O<u>C</u>OCH_3), 170.0 (O<u>C</u>OCH_3), 162.5 (C-6), 158.3 (C-4a), 156.2 (C-13a), 155.8 (C-14a), 136.1 (C-12), 131.1 (C-7b), 130.6 (C-11a), 129.4 (C-9), 128.4 (C-11), 127.3 (C-8), 126.1 (C-10), 117.2 (C-13), 115.7 (C-7a), 109.2 (C-6a), 98.9 (C-14b), 96.2 (C-5), 78.2 (C-3), 71.3 (C-2), 60.9 (C-1), 56.6 (OCH_3), 26.1 (C3-<u>C</u>H_3), 21.9 (OCO<u>C</u>H_3), 21.0 (OCO<u>C</u>H_3), 20.8 (C3-<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*-3,4-Diacetoxy-5-methoxy-2,2-dimethyl-3,4-dihydro-2*H*,6*H*-benzo[*a*]pyrano[2,3-*i*]xanthen-6-one (19) Acetylation of **17** (0.080 g, 0.204 mmol) under conditions similar to those described for the preparation of **18** from **16**, gave **19** (0.077 g, 79%) as a white amorphous solid. IR (KBr) cm^{-1}: 3056, 2990, 2936, 1744, 1650, 1608, 1514, 1437, 1375, 1235, 1091, 1045, 827, 757. UV λ_{max} (MeOH) nm (log ε): 251 (3.89), 274 (4.22), 299 (4.00), 322 (4.21). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.45 (3H, s, C2-CH_3), 1.49 (3H, s, C2-CH_3), 2.12 (6H, s, C3-OCOCH_3, C4-OCOCH_3), 4.03 (3H, s, OCH_3), 5.26 (1H, d, J=5 Hz, C3-H), 6.48 (1H, d, J=5 Hz, C4-H), 6.75 (1H, s, C14-H), 7.47 (1H, d, J=9 Hz, C12-H), 7.57 (1H, td, J=8, 1 Hz, C9-H), 7.74 (1H, td, J=8, 1 Hz, C8-H), 7.87 (1H, dd, J=8, 1 Hz, C10-H), 8.06 (1H, d, J=9 Hz, C11-H), 10.00 (1H, dd, J=8, 1 Hz, C7-H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 177.2 (C-6), 170.2 (O<u>C</u>OCH_3), 170.0 (O<u>C</u>OCH_3), 161.6 (C-5), 158.1 (C-13a), 157.9 (C-14a), 156.4 (C-12a), 136.2 (C-11), 131.0 (C-6b), 130.4 (C-10a), 129.5 (C-8), 126.9 (C-7), 126.1 (C-9), 117.5 (C-12), 115.2 (C-6a), 112.2 (C-5a), 110.9 (C-4a), 100.6 (C-14), 77.0 (C-2), 71.5 (C-3), 62.8 (OCH_3), 61.0 (C-4), 26.3 (C2-<u>C</u>H_3), 21.9 (C2-<u>C</u>H_3), 21.0 (OCO<u>C</u>H_3), 20.8 (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, 68.05; H, 5.14.

(±)-*cis*-1,2-Di-*O*-carbonyloxy-6-methoxy-1,2-dihydro-3,3-dimethyl-3*H*,7*H*-benzo[*a*]pyrano[3,2-*h*]xanthen-7-one (20) To a solution of (±)-*cis*-1,2-dihydroxy-6-methoxy-3,3-dimethyl-1,2-dihydro-3*H*,7*H*-benzo[*a*]-pyrano[3,2-*h*]xanthen-7-one (**16**) (0.137 g, 0.35 mmol) in 2-butanone (10 ml), *N*,*N*′-carbonyldiimidazole (0.36 g, 2.1 mmol) was added. The reaction mixture was refluxed for 24 h and after cooling, 5% aqueous Na_2CO_3 (7 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/acetone 99 : 1 to 90 : 10) to give **20** (0.069 g, 47%) as a white amorphous solid. IR (KBr) cm^{-1}: 3052, 2975, 2940, 1794, 1658, 1580, 1476, 1243, 1173, 1106, 823. UV λ_{max} (MeOH) nm (log ε): 270 (4.56), 318 (4.21). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 1.40 (3H, s, C3-CH_3), 1.66 (3H, s, C3-CH_3), 4.05 (3H, s, OCH_3), 4.85 (1H, d, J=8 Hz, C2-H), 6.18 (1H, d, J=8 Hz, C1-H), 6.41 (1H, s, C5-H), 7.54 (1H, d, J=9 Hz, C13-H), 7.60 (1H, td, J=8, 1 Hz, C10-H), 7.75 (1H, td, J=8, 1 Hz, C9-H), 7.91 (1H, dd, J=8, 1 Hz, C11-H), 8.11 (1H, d, J=9 Hz, C12-H), 10.06 (1H, dd, J=8, 1 Hz, C8-H). ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 177.2 (C-7), 163.3 (C-6), 158.0 (C-4a), 156.7 (C-13a), 155.8 (C-14a), 154.0 (O–<u>C</u>O–O), 136.2 (C-12), 131.0 (C-7b), 130.7 (C-11a), 129.5 (C-9), 128.5 (C-11), 127.2 (C8), 126.3 (C-10), 117.3 (C-13), 115.8 (C-7a), 110.0 (C-6a), 97.1 (C-14b), 96.7 (C-5), 78.2 (2C, C-2, C-3), 68.3 (C-1), 56.8 (OCH_3), 24.1 (C3-<u>C</u>H_3), 22.6 (C3-<u>C</u>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, 4.39.

(±)-*cis*-3,4-Di-*O*-carbonyloxy-5-methoxy-2,2-dimethyl-3,4-dihydro-2*H*,6*H*-benzo[*a*]pyrano[2,3-*i*]xanthen-6-one (21) Treatment of **17** (0.105 g, 0.27 mmol) with *N*,*N*′-carbonyldiimidazole (0.220 g, 1.35 mmol) under conditions similar to those described for the preparation of **20**, gave **21** (0.083 g, 73%) as a white amorphous solid. IR (KBr) cm^{-1}: 3087, 2986, 2944, 1817, 1647, 1608, 1511, 1433, 1375, 1243, 1161, 1087, 831, 765. UV λ_{max} (MeOH) nm (log ε): 251 (3.72), 274 (4.09), 299 (3.80), 321 (3.98). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.31 (3H, s, C2-CH_3), 1.65 (3H, s, C2-CH_3), 4.18 (3H, s, OCH_3), 4.80 (1H, d, J=8 Hz, C3-H), 6.11 (1H, d, J=8 Hz, C4-H), 6.82 (1H, s, C14-H), 7.50 (1H, d, J=9 Hz, C12-H), 7.61 (1H, td, J=8, 1 Hz, C9-H), 7.78 (1H, td, J=8, 1 Hz, C8-H), 7.91 (1H, dd, J=8, 1 Hz, C10-H), 8.12 (1H, d, J=9 Hz, C11-H), 9.99 (1H, dd, J=8, 1 Hz, C7-H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 177.0 (C-6), 162.5 (C-5), 158.6 (C-13a), 157.6 (C-14a), 156.5 (C-12a), 154.0 (O–<u>C</u>O–O), 136.6 (C-11), 131.0 (C-6b), 130.5 (C-10a), 129.7 (C-8), 128.6 (C-10), 126.8 (C-7), 126.3 (C-9), 117.4 (C-12), 115.4 (C-6a), 113.0 (C-5a), 109.2 (C-4a), 101.8 (C-14), 78.2 (C-3), 76.3 (C-2), 68.6 (C-4), 64.2 (OCH_3), 24.3 (C2-<u>C</u>H_3), 22.5 (C2-<u>C</u>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, 69.01; H, 4.25.

11-Amino-9-methoxy-12*H*-benzo[*a*]xanthen-12-one (23) To a mixture of 2-hydroxy-1-naphthalenecarboxylic acid (**7**) (5.64 g, 30 mmol), 3,5-dimethoxyaniline (**22**) (12.6 g, 100 mmol), and freshly fused zinc chloride (12 g), phosphoryl chloride (50 ml) was added. The reaction mixture was stirred at 65 °C for 1.5 h, poured onto ice (500 g) and left overnight. After filtration, the red precipitate was washed with saturated aqueous sodium bicarbonate and water, and dried in vacuum over P_2O_5. Purification by flash column chromatography (solvent: cyclohexane, then cyclohexane/acetone 99 : 1 to 80 : 20) gave **23** (2.04 g, 23%) as yellow needles, mp 186—187 °C (cyclohexane/acetone 9 : 1). IR (KBr) cm^{-1}: 3379, 3270, 3103, 2998, 1639, 1573, 1514, 1441, 1239, 1157, 819. UV λ_{max} (MeOH) nm (log ε): 242 (4.52), 285 (4.45), 299 (sh.), 381 (3.86). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 3.84 (3H, s, OCH_3), 6.02 (1H, d, J=2 Hz, C10-H), 6.26 (1H, d, J=2 Hz, C8-H), 6.82 (2H, br s, D_2O exch., C11-NH_2), 7.44 (1H, d, J=9 Hz, C6-H), 7.56 (1H, td, J=8, 1 Hz, C3-H), 7.74 (1H, td, J=8, 1 Hz, C2-H), 7.88 (1H, dd, J=8, 2 Hz, C4-H), 8.05 (1H, d, J=9 Hz, C5-H), 10.06 (1H, dd, J=8, 1 Hz, C1-H). ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 181.1 (C-12), 164.6 (C-9), 158.1 (C-6a), 156.6 (C-7a), 152.3 (C-11), 135.7 (C-5), 131.2 (C-12b), 130.2 (C-4a), 129.0 (C-2), 128.4 (C-4), 126.8 (C-1), 125.6 (C-3), 117.6 (C-6), 114.4 (C-12a), 104.7 (C-11a), 95.1 (C-10), 89.5 (C-8), 55.4 (OCH_3). DCI-MS m/z: 292 $[MH]^+$. *Anal.* Calcd for $C_{18}H_{13}NO_3$: C, 74.22; H, 4.50; N, 4.81. Found: C, 74.17; H, 4.49; N, 4.72.

11-Amino-9-hydroxy-12*H*-benzo[*a*]xanthen-12-one (24) To a solution of **23** (2.04 g, 7 mmol) in acetic acid (70 ml) was added 48% hydrogen bromide aqueous solution (45 ml). The reaction mixture was refluxed for 4 d. The cooled mixture was poured onto ice water (500 ml). The brown precipitate was filtered, washed with water (4×100 ml), and dried in vacuum over P_2O_5. Column chromatography (solvent: dichloromethane, then dichloromethane/methanol, 99 : 1 to 95 : 5) gave **24** (1.50 g, 77%) as bright yellow needles, mp 235—236 °C (dichloromethane/methanol 9 : 1). IR (KBr) cm^{-1}: 3476, 3359, 3309, 3091, 1643, 1549, 1514, 1464, 1347, 1169, 819, 742. UV λ_{max} (MeOH) nm (log ε): 241 (4.43), 286 (4.36), 382 (3.77). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 6.05 (2H, s, C8-H, C10-H), 7.56 (1H, d, J=9 Hz, C6-H), 7.58 (1H, td, J=8, 1 Hz, C3-H), 7.70 (2H, br s, D_2O exch., C11-NH_2), 7.71 (1H, td, J=8, 1 Hz, C2-H), 8.01 (1H, dd, J=8, 2 Hz, C4-H), 8.23 (1H, d, J=9 Hz, C5-H), 9.97 (1H, dd, J=8, 1 Hz, C1-H), 10.31 (1H, s, D_2O exch., C9-OH). ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 181.2 (C-12), 164.6 (C-9), 158.9 (C-6a), 157.3 (C-7a), 154.7 (C-11), 137.1 (C-5), 132.0 (C-12b), 131.3 (C-4a), 130.1 (C-2), 130.0 (C-4), 127.4 (C-1), 126.9 (C-3), 118.9 (C-6), 114.8 (C-12a), 103.9 (C-11a), 97.0 (C-10), 91.2 (C-8). DCI-MS m/z: 278 $[MH]^+$. *Anal.* Calcd for $C_{17}H_{11}NO_3$: C, 73.64; H, 4.00; N, 5.05. Found: C, 74.71; H, 4.06; N, 4.97.

6-Amino-3,3-dimethyl-3*H*,7*H*-benzo[*a*]pyrano[3,2-*h*]xanthen-7-one (25) and 5-amino-2,2-dimethyl-2*H*,6*H*-benzo[*a*]pyrano[2,3-*i*]xanthen-6-one (26) A solution of **24** (0.277 g, 1 mmol) in dry *N*,*N*-dimethylformamide (15 ml) was stirred and heated at 65 °C for 15 min, under argon, in the presence of anhydrous potassium carbonate (0.276 g, 2 mmol). Dry potassium iodide (0.332 g, 2 mmol) and 3-chloro-3-methylbut-1-yne (0.308 g, 3 mmol) were added and the mixture was stirred for 24 h at 65 °C, then was heated at 130 °C for 3 h. The cooled reaction mixture was diluted with water (40 ml) and extracted with ethyl acetate (3×30 ml). The combined organic layers were washed with water and evaporated under reduced pressure. Purification by flash column chromatography (solvent: cyclohexane, then cyclohexane/acetone 99 : 1 to 95 : 5) gave successively 5-amino-2,2-dimethyl-2*H*,6*H*-benzo[*a*]pyrano[2,3-*i*]xanthen-6-one (**26**) (0.064 g, 19%) and 6-amino-3,3-dimethyl-3*H*,7*H*-benzo[*a*]pyrano[3,2-*h*] xanthen-7-one (**25**) (0.045 g, 13%) as bright yellow crystals.

6-Amino-3,3-dimethyl-3*H*,7*H*-benzo[*a*]pyrano[3,2-*h*]xanthen-7-one (**25**): Yellow sheets, mp 175—176 °C (dichloromethane/methanol 4 : 1). IR (KBr) cm^{-1}: 3301, 3056, 2971, 1635, 1569, 1514, 1433, 1340, 1243, 1142, 823, 753. UV λ_{max} (MeOH) nm (log ε): 243 (4.45), 288 (4.60), 305 (sh.), 401 (3.72). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.50 (6H, s, C(CH_3)$_2$), 5.54 (1H, d, J=10 Hz, C2-H), 5.97 (1H, s, C5-H), 6.87 (1H, d, J=10 Hz, C1-H), 6.88 (2H, br s, D_2O exch., C6-NH_2), 7.46 (1H, d, J=9 Hz, C13-H), 7.57 (1H, td, J=8, 1 Hz, C10-H), 7.73 (1H, td, J=8, 1 Hz, C9-H), 7.87 (1H, dd, J=8, 1 Hz, C11-H), 8.04 (1H, d, J=9 Hz, C12-H), 10.05 (1H, dd, J=8, 1 Hz, C8-H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 181.3 (C-7), 158.7 (C-4a), 156.4 (C-13a), 152.2 (C-14a), 152.0 (C-6), 135.8 (C-12), 131.3 (C-7b), 130.4 (C-11a), 129.2 (C-9), 128.5 (C-11), 126.9 (C-8), 125.7 (C-10), 125.7 (C-2), 117.6 (C-13), 115.8 (C-1), 114.7 (C-7a), 104.4 (C-6a), 95.8 (C-14b), 97.1 (C-5), 77.6 (C-3), 28.4 (2C, C3-(<u>C</u>H_3)$_2$). DCI-MS m/z: 344 $[MH]^+$. *Anal.* Calcd for

$C_{22}H_{17}NO_3$: C, 76.95; H, 4.99; N, 4.08. Found: C, 77.07; H, 4.92; N, 3.99.

5-Amino-2,2-dimethyl-2*H*,6*H*-benzo[*a*]pyrano[2,3-*i*]xanthen-6-one (**26**): Yellow needles, mp 193—194 °C (ethyl acetate/acetone 4 : 1). IR (KBr) cm^{-1}: 3289, 2971, 1639, 1584, 1514, 1445, 1340, 1239, 1142, 823, 746. UV λ_{max} (MeOH) nm (log ε): 252 (4.51), 260 (sh.), 317 (4.68), 389 (3.76). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.48 (6H, s, $C(CH_3)_2$), 5.62 (1H, d, J=10 Hz, C3-H), 6.23 (1H, s, C14-H), 6.42 (1H, d, J=10 Hz, C4-H), 7.11 (2H, br s, D_2O exch., C6-NH_2), 7.44 (1H, d, J=9 Hz, C12-H), 7.56 (1H, td, J=8, 1 Hz, C9-H), 7.73 (1H, td, J=8, 1 Hz, C8-H), 7.87 (1H, dd, J=8, 1 Hz, C10-H), 8.04 (1H, d, J=9 Hz, C11-H), 10.06 (1H, dd, J=8, 1 Hz, C7-H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 181.1 (C-6), 158.8 (C-14a), 157.8 (C-12a), 156.5 (C-13a), 147.0 (C-5), 135.8 (C-11), 131.3 (C-6b), 130.4 (C-10a), 129.1 (C-8), 128.5 (C-10), 127.4 (C-3), 126.9 (C-7), 125.7 (C-9), 117.7 (C-12), 115.7 (C-4), 114.5 (C-6a), 104.7 (C-5a), 101.8 (C-4a), 92.7 (C-14), 76.9 (C-2), 27.9 (2C, C3-($\underline{C}H_3)_2$). DCI-MS *m*/*z*: 344 $[MH]^+$. *Anal.* Calcd for $C_{22}H_{17}NO_3$: C, 76.95; H, 4.99; N, 4.08. Found: C, 76.85; H, 4.96; N, 4.09.

Pharmacology Cytotoxicity: Murine leukemia L1210 cells from the American Type Culture Collection (Rockville Pike, MD, U.S.A.) were grown in RPMI medium 1640 supplemented with 10% fetal calf serum, 2 mM L-glutamine, penicillin 100 U/ml, streptomycin 100 μg/ml and 10 mM HEPES buffer (pH 7.4). The cytotoxicity was measured using the microculture tetrazolium assay essentially as described.[31] Cells were exposed for 48 h to nine graded concentrations in triplicate of the test drug. Results are expressed as IC_{50} values (mean, n=3), which are defined as the drug concentration inhibiting the absorbance by 50% with respect to that of untreated cells.

References

1) Lee K.-H., *J. Biomed. Sci.*, **6**, 236—250 (1999).
2) Bowers W. S., *Am. Zool.*, **21**, 737—742 (1981).
3) Zembower D. E., Liao S., Flavin M. T., Xu Z.-Q., Stup T. L., Buckheit R. W., Khilevich A., Mar Y. Y., Sheinkman A. K., *J. Med. Chem.*, **40**, 1005—1017 (1997).
4) Tillequin F., Michel S., Skaltsounis A.-L., "Alkaloids: Chemical and Biological Perspectives," Vol. 12, ed. by Pelletier S. W., Elsevier, New York, 1998, pp. 1—102.
5) Nguyen Tuan M., Sittisombut C., Boutefnouchet S., Lallemand M.-C., Michel S., Koch M., Tillequin F., Mazinghien R., Lansiaux A., David-Cordonnier M.-H., Pfeiffer B., Kraus-Berthier L., Léonce S., Pierré A., *J. Med. Chem.*, **49**, 3383—3394 (2006).
6) Costes N., Le Deit H., Michel S., Tillequin F., Koch M., Pfeiffer B., Renard P., Léonce S., Guilbaud N., Kraus-Berthier L., Pierré A., Atassi Gh., *J. Med. Chem.*, **43**, 2395—2402 (2000).
7) Doan Thi Mai H., Gaslonde T., Michel S., Tillequin F., Koch M., Bongui J.-B., Elomri A., Seguin E., Pfeiffer B., Renard P., David-Cordonnier M.-H., Laine W., Bailly C., Kraus-Berthier L., Léonce S., Hickman J. A., Pierré A., *J. Med. Chem.*, **46**, 3072—3082 (2003).
8) Doan Thi Mai H., Gaslonde T., Michel S., Koch M., Tillequin F., Pfeiffer B., Renard P., Kraus-Berthier L., Léonce S., Pierré A., *Chem. Pharm. Bull.*, **52**, 293—297 (2004).
9) Guilbaud N., Léonce S., Tillequin F., Koch M., Hickman J. A., Pierré A., *Anti-Cancer Drugs*, **13**, 445—449 (2002).
10) David-Cordonnier M.-H., Laine W., Lansiaux A., Kouach M., Briand G., Pierré A., Hickman J. A., Bailly C., *Biochemistry*, **41**, 9911—9920 (2002).
11) David-Cordonnier M.-H., Laine W., Joubert A., Tardy C., Goossens J.-F., Kouach M., Briand G., Doan Thi Mai H., Michel S., Tillequin F., Koch M., Léonce S., Pierré A., Bailly C., *Eur. J. Biochem.*, **270**, 2848—2859 (2003).
12) David-Cordonnier M.-H., Laine W., Kouach M., Briand G., Vezin H., Gaslonde T., Michel S., Doan Thi Mai H., Tillequin F., Koch M., Léonce S., Pierré A., Bailly C., *Bioorg. Med. Chem.*, **12**, 23—29 (2004).
13) Kupchan S. M., Streelman D. R., Sneden A. T., *J. Nat. Prod.*, **43**, 296—301 (1980).
14) Habib A. M., Ho D. K., Masuda S., McCloud T., Reddy K. S., Aboushoer M., McKenzie A., Byrn S. R., Chang C.-J., Cassady J. M., *J. Org. Chem.*, **52**, 412—418 (1987).
15) Abou-Shoer M., Boettner F. E., Chang C.-J., Cassady J. M., *Phytochemistry*, **27**, 2795—2800 (1988).
16) Permana P. A., Ho D. K., Cassady J. M., Snapka R. M., *Cancer Res.*, **54**, 3191—3195 (1994).
17) Hansen M., Lee S.-J., Cassady J. M., Hurley L. H., *J. Am. Chem. Soc.*, **118**, 5553— 5561 (1996).
18) Michel S., Seguin E., Tillequin F., *Curr. Med. Chem.*, **9**, 1689—1700 (2002).
19) David-Cordonnier M.-H., Laine W., Gaslonde T., Michel S., Tillequin F., Koch M., Léonce S., Pierré A., Bailly C., *Curr. Med. Chem.-Anti-Cancer Agents*, **4**, 82—92 (2004).
20) Michel S., Gaslonde T., Tillequin F., *Eur. J. Med. Chem.*, **39**, 649—655 (2004).
21) Sittisombut C., Costes N., Michel S., Koch M., Tillequin F., Pfeiffer B., Renard P., Pierré A., Atassi Gh., *Chem. Pharm. Bull.*, **49**, 675—679 (2001).
22) Tran Thi H. A., Nguyen Thi T., Michel S., Tillequin F., Koch M., Pfeiffer B., Pierré A., Trinh Van-Dufat H., *Chem. Pharm. Bull.*, **52**, 540—545 (2004).
23) Doan Thi Mai H., Gaslonde T., Michel S., Koch M., Tillequin F., Bailly C., David- Cordonnier M.-H., Pfeiffer B., Léonce S., Pierré A., *Chem. Pharm. Bull.*, **53**, 919—922 (2005).
24) Bongui J.-B., Elomri A., Cahard D., Tillequin F., Pfeiffer B., Pierré A., Seguin E., *Chem. Pharm. Bull.*, **53**, 1540—1546 (2005).
25) Grover P. K., Shah G. D., Shah R. C., *J. Chem. Soc.*, **1955**, 3982—3985 (1955).
26) Pillai R. K. M., Naiksatam P., Johnson F., Rajagopalan R., Watts P. C., Cricchio R., Borras S., *J. Org. Chem.*, **51**, 717—723 (1986).
27) Hennion G. F., Boisselle A. P., *J. Org. Chem.*, **26**, 725—727 (1961).
28) Mikros E., Mitaku S., Skaltsounis A.-L., Libot F., Tillequin F., Koch M., *Magn. Reson. Chem.*, **37**, 498—506 (1999).
29) Rheenen V. V., Kelly R. C., Cha D. Y., *Tetrahedron Lett.*, **1976**, 1973—1976 (1976).
30) Costes N., Elomri A., Trinh Van-Dufat H., Michel S., Seguin E., Koch M., Tillequin F., Pfeiffer B., Renard P., Léonce S., Pierré A., *Oncol. Res.*, **13**, 191—197 (2003).
31) Pierré A., Kraus-Berthier L., Atassi Gh., Cros S., Poupon M. F., Lavielle G., Berlion M., Bizarri J. P., *Cancer Res.*, **51**, 2312—2318 (1991).