Design and Synthesis of Some New Pyranoxanthenones with Cytotoxic Activity

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As part of a research program directed towards the design and synthesis of pyranoxanthones structurally related to acronycine, we present here the synthesis and cytotoxic activity of the pyranoxanthones **3** and **4** (X= H, Br; R= H, OMe; R'= H, Ac). Some of these compounds inhibit L1210 cell proliferation.

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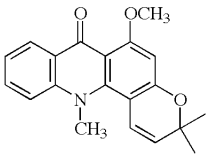
The alkaloid acronycine (Figure 1) isolated from several species of Rutaceae [1], has been reported to exhibit a broad spectrum antitumor activity in experimental animals [2]. However, in clinical trials success was limited, partially due to its extremely low water solubility [3]. In a search for compounds with better selectivity and higher potency, several structural modifications of acronycine have been reported, involving substitutions on the acridone chromophore, like 11-methoxyacronycine and the pyran moiety of the molecule as well [4-6].

The above mentioned considerations have prompted us to initiate a research program for the preparation of analogs, replacing the acridine chromophore by the xanthone nucleus, in order to investigate the influence of this isosteric alteration on biological activity. It should be noted that a number of xanthones have already been reported to possess cytotoxic and antitumor properties. Derivatives of 9-oxo-9*H*-xanthene-4-acetic acid are among the most potent compounds yet reported against mice bearing murine colon 38 tumors [7], while the furanoxanthone psorospermin (Figure 1) isolated from the African plant *Psorospermum febrifugum* [8], shows significant promise as an antileukemic agent [9].

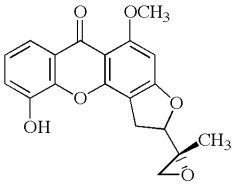
We have previously prepared 1,2-dihydro-2-hydroxy-6-methoxy-3,3-dimethyl-3*H*,7*H*-pyrano[2,3-*c*]xanthen-7-one (Figure 1) and found that, it was almost as potent as acronycine against leukemia L1210 cell line [10]. On the basis of these findings and in connection with our research on the pyranoxanthenone ring manipulation, we have prepared the pyrano[2,3-*c*]xanthen-7-ones **3Aa-d** and **3Ba-d** and the pyrano[3,2-*b*]xanthen-6-ones **4Aa-d** and **4Ba-d**, to better understand the structure activity relationships and the biological properties of this class of compounds. A number of analogs, bearing a methoxyl group on the xanthone chromophore were also prepared, in order to mimic the structure of psorospermin and the cytotoxic 11-methoxyacronycine and determine if the presence of a small substituent on the ring system effects biological activity.

The derivatives structures are outlined in Scheme 1. The preparation of starting materials **1** (angular isomers) and **2** (linear isomers), have been reported elsewhere. We have made a slight modification to the reported procedure in order to ameliorate the yield of the desired compounds [10-13].

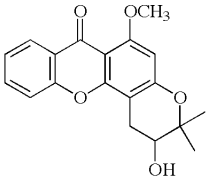
The reaction of the derivatives **1** and **2** with *N*-bromosuccinimide (NBS) in water produced the *trans*-bromohydrins **3Aa**, **3Ac** and **4Aa**, **4Ac** respectively. Reductive debromination of the above mentioned bromohydrins, *via* free radical reaction with tri-*n*-butyltinhydride in the presence of 2,2'-diazodiisobutylnitrile (AIBN), furnished the 1- and 4-monohydroxy- analogs **3Ba**, **3Bc** and **4Ba**,



Acronycine



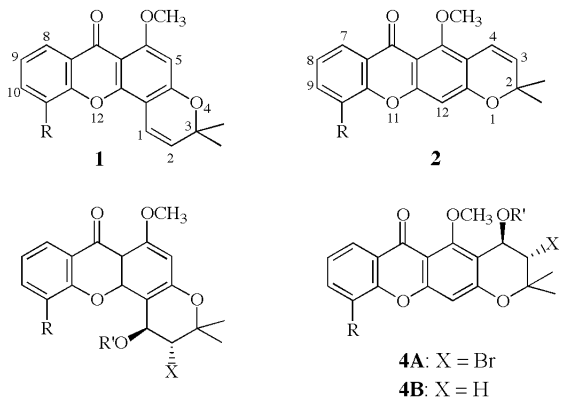
Psorospermin



1,2-dihydro-2-hydroxy-6-methoxy-3,3-dimethyl-3*H*,7*H*-pyrano[2,3-*c*]xanthen-7-one

Figure 1

Scheme 1



1 **2**

3A: X = Br
3B: X = H

4A: X = Br
4B: X = H

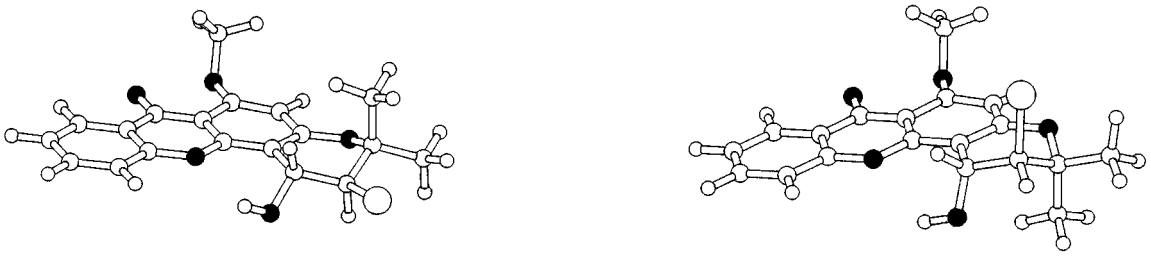
3,4	R	R'
a	H	H
b	H	Ac
c	OCH_3	H
d	OCH_3	Ac

4Bc respectively. The corresponding acetates were subsequently prepared upon treatment with excess acetic anhydride in the presence of pyridine.

The structure determination of both isomers of **1** and **2**, as well as of all the new derivatives, was based on the complete characterization of the carbon chemical shifts using 2D NMR experiments (HMBC, HMQC). The pronounced three bond correlation between the C-5 and the 4-hydrogen atom was observed in the HMBC of the linear isomer, whereas it is of course absent in the case of the angular isomer. Another useful observation for the identification of each pair of isomers is the difference between the chemical shifts of the C-5 of the angular isomer (95 ppm), versus the C-12 of the linear one (101 ppm). Furthermore, the spectroscopic study of the compounds revealed that the chemical shifts of carbons 8, 9, 10 and 11a of the 10-methoxy angular derivatives **3Ab**, **3Ad**, **3Bb**, **3Bd**, appeared upfield when compared to the corresponding shifts of the 10-unsubstituted compounds. An analogous observation was made in the case of the linear isomers and is probably due to the electronic influence of the methoxyl group on the D-ring. It would be of interest to notice that the *trans*-derivatives **3Aa-d** exhibited ^3J (H_1, H_2) coupling constants in the range of 3-7 Hz when the spectra were recorded in $CDCl_3$, but these values decreased dramatically (1-3 Hz) when we used DMSO-d_6 as the solvent. In contrast, higher values, ranging from 8-9.5 Hz, were observed for the corresponding acronycine analogs and these values were not influenced by the solvent [14]. The above mentioned observations could suggest a difference in the D-ring conformation of the two classes of compounds. Indeed, in the case of the xanthone derivatives, the conformation analysis, performed using molecular mechanics calculations, predicted two low energy conformations (Figure 2, structures I and II for compound **3Aa**), which are both half-chair and their calculated energy difference is small enough (0.5 kcal.mol^{-1}) to suggest that they should be both present in solution. This hypothesis is supported by the observed ^3J (H_1, H_2) coupling constants. On the other hand, in the case of the acronycine derivatives the energy difference between the corresponding conformers I and II was significantly higher at 1.4 kcal.mol^{-1}, suggesting that I is the predominant conformation [14]. In this conformer the H-1 and H-2 adopt a *trans* diaxial orientation, resulting in the somewhat high ^3J (H_1, H_2) experimental coupling constants.

The study of cytotoxic activity and cell cycle selectivity, were carried out *in vitro* using the L1210 leukemic cell line, with acronycine as the reference compound. The results of these experiments are presented in Table 1. As far as their cytotoxicity is concerned, a number of the new compounds possessed interesting biological properties. From the pyrano[3,2-*b*]xanthen-6-one series, compounds, **4Aa** and **4Ba**, were more potent than acronycine and **4Ab** was almost as potent as the reference compound. On the contrary, the derivatives bearing the 10-methoxyl group were devoid of antiproliferative activity.

From the pyrano[2,3-*c*]xanthen-7-one series, the 11-methoxy derivatives again proved to be inactive, suggesting that this kind of substitution of the D ring does



Conformer I Conformer II

Figure 2

Table 1

Compound	IC_{50}, μ*M* L1210	Cell Cycle Effect (L1210) % of cells in the indicated phase.
3Aa	> 60	N.T. [a]
3Ab	18.8	G2+M (54% at 100 μ*M*)
3Ac	> 60	N.T.
3Ad	> 60	N.T.
3Ba	0.9	G2+M (50% at 2.5 μ*M*) [b]
3Bb	39.3	N.T.
3Bc	> 60	N.T.
3Bd	> 60	N.T.
4Aa	12.4	G1 (60% at 50 μ*M*) [c]
4Ab	25.1	N.T.
4Ac	> 60	N.T.
4Ad	> 60	N.T.
4Ba	18.7	G1 (54% at 100 μ*M*) [c]
4Bb	53.9	N.T.
4Bc	> 60	N.T.
4Bd	> 60	N.T.
ACRONYCINE	25	G2+M (46% at 50 μ*M*)

[a] N.T.: Not tested
[b] 27% of untreated cells were in the G2+M phase of the cell cycle.
[c] 38% of untreated cells were in the G1 phase of the cell cycle.

not favour the cytotoxicity. On the other hand, compounds **3Ab** and **3Ba** were more potent than acronycine and compound **3Bb** was slightly less potent than acronycine.

The perturbation of the cell cycle induced by the more potent compounds was studied on the same cell line. Compounds **3Ab** and **3Ba**, which possess a structural similarity with acronycine, induced a partial accumulation of cells in the G2+M phase of the cell cycle. Since it has been reported that the same type of perturbation was observed with acronycine, this result could suggest a similar mechanism of action at the molecular level [15]. It is noticeable that the linear analogs **4Aa** and **4Ba** induced a partial accumulation of cells in the G1 phase of the cell cycle.

The hydroxy derivative **3Ba**, proved to be by far the most interesting compound, with an IC_{50} equal to 0.9 μ*M* (that is 28 times more potent than acronycine against L1210 cells) and inducing a partial blockade in the G_2 + M phase (50%) of the cell cycle at 2.5 μ*M*.

Further biological evaluation of the new compounds is currently in progress.

EXPERIMENTAL

Melting points were determined on a Büchi apparatus and are uncorrected. ^{1}H-NMR spectra and 2-D spectra were recorded on a Bruker Avance 400 instrument, whereas ^{13}C-NMR spectra were recorded on a Bruker AC 200 spectrometer in deuterated solvents and were referenced to tetramethylsilane (δ scale). Flash chromatography was performed on Merck silica gel 60 (0.040-0.063 mm). Analytical thin layer chromatography (TLC) was carried out on precoated (0.25 mm) Merck silica gel F-254 plates. Elemental analyses were within ± 0.4% of the theoretical values.

(±)-*trans*-2-Bromo-1,2-dihydro-1-hydroxy-6-methoxy-3,3-dimethyl-3*H*,7*H*-pyran[2,3-*c*]xanthen-7-one (**3Aa**).

N-Bromosuccinimide (80 mg, 0.448 mmole) was added at 0 °C to a 1:1, tetrahydrofuran:water solution (6 ml) of 6-methoxy-3,3-dimethyl-3*H*,7*H*-pyran[2,3-*c*]xanthen-7-one (**1a**, [11] 138 mg, 0.448 mmole) and the mixture was stirred for 4 hours at room temperature. At the end of this time, a saturated sodium chloride solution was added (2 ml) and stirring was continued for 10 minutes. The organic solvent was then vacuum-evaporated and the precipitate was filtered, air-dried and purified by column chromatography (silica gel, 30x1 cm) using a mixture of cyclohexane-ethyl acetate (7:1, v/v) as the eluent, to give (±)-*trans*-2-bromo-1,2-dihydro-1-hydroxy-6-methoxy-3,3-dimethyl-3*H*,7*H*-pyran[2,3-*c*]xanthen-7-one as a solid, which was recrystallized from dichloromethane - *n*-pentane (143 mg, 79 %), mp 208 °C; ^{1}H nmr (400 MHz, deuteriochloroform): δ 1.59 (3H, s, 1 x gemCH_3) 1.73 (3H, s, 1 x gemCH_3), 3.45 (1H, d, J = 4 Hz, deuterium oxide-exchangeable, 1-OH), 3.96 (3H, s, 6-OCH_3), 4.38 (1H, d, J = 5 Hz, H-2), 5.28 (1H, dd, J = 5 Hz, 4 Hz, H-1), 6.27 (1H, s, H-5), 7.38 (1H, td, J = 8 Hz, 0.5 Hz, H-9), 7.42 (1H, dd, J = 8 Hz, 0.5 Hz, H-11), 7.68 (1H, td, J = 8 Hz, 1.5 Hz, H-10), 8.30 (1H, dd, J = 8 Hz, 1.5 Hz, H-8); ^{13}C nmr (50 MHz, deuteriochloroform): δ 26.45 (CH_3), 29.55 (CH_3), 56.32 (6-OCH_3), 57.93 (C-2), 67.58 (C-1), 79.02 (C-3), 95.76 (C-5), 102.37 (C-12b), 107.46 (C-6a), 116.83 (C-11), 122.82 (C-7a), 122.81 (C-9), 126.82 (C-8), 133.94 (C-10), 154.34 (C-11a), 157.58 (C-12a), 157.98 (C-4a), 161.72 (C-6), 175.20 (C-7).

Anal. Calcd. for $C_{19}H_{17}BrO_5$: C, 56.31; H, 4.23. Found: C, 56.59; H, 3.98.

(±)-*trans*-2-Bromo-1,2-dihydro-1-hydroxy-6,11-dimethoxy-3,3-dimethyl-3*H*,7*H*-pyran[2,3-*c*]xanthen-7-one (**3Ac**).

This compound was prepared according to the procedure described for **3Aa**, as white solid (dichloromethane - *n*-pentane), in 76 % yield, mp: 214-215 °C; ^{1}H nmr (400 MHz, deuteriochloroform): δ 1.50 (3H, s, 1 x gemCH_3), 1.64 (3H, s, 1 x gemCH_3), 3.93 (3H, s, OCH_3), 3.96 (3H, s, OCH_3), 4.08 (1H, d, J = 1 Hz, deuterium oxide-exchangeable, 2-OH), 4.27 (1H, d, J = 7 Hz, H-2), 5.38 (1H, dd, J = 7 Hz, 1 Hz, H-1), 6.27 (1H, s, H-5), 7.11 (1H, dd, J = 8 Hz, 1.5 Hz, H-10), 7.23 (1H, t, J = 8 Hz, H-9), 7.79 (1H, dd, J = 8 Hz, 1.5 Hz, H -8); ^{13}C nmr (50 MHz, deuteriochloroform): δ 22.66 (CH_3), 27.14 (CH_3), 56.33 (11-OCH_3), 56.40 (6-OCH_3), 57.88 (C-2), 68.06 (C-1), 79.90 (C-3), 95.89 (C-5), 103.31 (C-12b), 107.43 (C-6a), 114.23 (C-9), 117.55 (C-8), 123.79 (C-7a), 123.83 (C-10), 144.42 (C-11), 147.87 (C-11a), 157.01 (C-4a), 157.67 (C-12a), 161.75 (C-6), 174.99 (C-7).

Anal. Calcd. for $C_{20}H_{19}BrO_6$: C, 55.18; H, 4.40. Found: C, 55.00; H, 4.24.

(±)-*trans*-3-Bromo-3,4-dihydro-4-hydroxy-5-methoxy-2,2-dimethyl-2*H*,6*H*-pyran[3,2-*b*]xanthen-6-one (**4Aa**).

This compound was prepared according to the procedure described for **3Aa**, as white solid (dichloromethane - *n*-pentane), in 80 % yield, mp: >250 °C; ^{1}H nmr (400 MHz, deuteriochloroform): δ 1.55 (3H, s, 1 x gemCH_3) 1.66 (3H, s, 1 x gemCH_3), 3.93 (1H, d, J=2 Hz, deuterium oxide-exchangeable, 4-OH), 4.11 (3H, s, 5-OCH_3), 4.27 (1H, d, J = 6.5 Hz, H-3), 5.28 (1H, dd, J = 6.5 Hz, 2 Hz, H-4), 6.77 (1H, s, H-12), 7.38 (1H, td, J = 8 Hz, 0.5 Hz, H-8), 7.43 (1H, dd, J = 8 Hz, 0.5 Hz, H-10), 7.70 (1H, td, J = 8 Hz, 1.5 Hz, H-9), 8.30 (1H, dd, J = 8 Hz, 1.5

Hz, H-7); ^{13}C nmr, (50 MHz, deuteriochloroform): δ 23.24 (CH_3), 26.96 (CH_3), 57.35 (C-3), 62.78 (5-OCH_3), 68.12 (C-4), 79.41 (C-2), 101.51 (C-12), 110.05 (C-5a), 114.29 (C-4a), 117.26 (C-10), 122.40 (C-6a), 123.96 (C-8), 126.63 (C-7), 134.42 (C-9), 155.15 (C-10a), 157.76 (C-11a), 158.55 (C-12a), 160.30 (C-5), 175.07 (C-6).

Anal. Calcd. for $C_{19}H_{17}BrO_5$: C, 56.31; H, 4.23. Found: C, 56.47; H, 4.12.

(±)-*trans*-3-Bromo-3,4-dihydro-4-hydroxy-5,10-dimethoxy-2,2-dimethyl-2*H*,6*H*-pyran[3,2-*b*]xanthen-6-one (**4Ac**).

This compound was prepared according to the procedure described for **3Aa**, as white solid (dichloromethane - *n*-pentane), in 83 % yield, mp: 211-212 °C; ^{1}H nmr, (400 MHz, deuteriochloroform): δ 1.48 (3H, s, 1 x gemCH_3) 1.61 (3H, s, 1 x gemCH_3), 3.95 (1H, d, J =2 Hz, deuterium oxide-exchangeable, 4-OH), 3.98 (3H, s, OCH_3), 4.06 (3H, s, OCH_3), 4.22 (1H, d, J = 6.5 Hz, H-3), 5.22 (1H, dd, J = 6.5 Hz, 2 Hz, H-4), 6.81 (1H, s, H-12), 7.16 (1H, dd, J = 8 Hz, 2 Hz, H-9), 7.24 (1H, t, J = 8 Hz, H-8), 7.81 (1H, dd, J = 8 Hz, 2 Hz, H-7); ^{13}C nmr, (50 MHz, deuteriochloroform): δ 23.06 (CH_3), 27.01 (CH_3), 56.42 (C-3), 57.48 (10-OCH_3), 62.74 (5-OCH_3), 68.14 (C-4), 79.39 (C-2), 101.77 (C-12), 110.41 (C-5a), 114.49 (C-4a), 115.19 (C-8), 117.46 (C-7), 123.24 (C-6a), 123.42 (C-9), 145.47 (C-10), 148.12 (C-10a), 157.75 (C-11a), 158.30 (C-12a), 160.17 (C-5), 174.94 (C-6).

Anal. Calcd. for $C_{20}H_{19}BrO_6$: C, 55.18; H, 4.40. Found: C, 55.48; H, 4.23.

(±)-1,2-Dihydro-1-hydroxy-6-methoxy-3,3-dimethyl-3*H*,7*H*-pyran[2,3-*c*]xanthen-7-one (**3Ba**).

A mixture of tributyltin hydride (0.21 ml) and 2,2'-diazodiisobutylnitrile (6 mg) in dry toluene (5 ml) was added under argon, at 40 °C, to a solution of the bromohydrin **3Aa** (69 mg, 0.17 mmole) in dry toluene (5 ml) and the resulting mixture was then heated at 90 °C for 3 hours. The solvent was vacuum-evaporated and the residue was purified by column chromatography (dry pack, silica gel 15x1.5 cm) using a mixture of cyclohexane - ethyl acetate (2:1, v/v) as the eluent, to afford (±)-1,2-dihydro-1-hydroxy-6-methoxy-3,3-dimethyl-3*H*,7*H*-pyran[2,3-*c*]xanthen-7-one as a solid, which was recrystallized from ethanol (43 mg, 77 %), mp: 243 °C; ^{1}H nmr, (400 MHz, deuteriochloroform): δ 1.49 (3H, s, 1 x gemCH_3), 1.56 (3H, s, 1 x gemCH_3), 2.13 (1H, dd, J = 14.5 Hz, 5.6 Hz, H-2a), 2.17 (1H, dd, J = 14.5 Hz, 3.6 Hz, H-2b), 2.85 (1H, d, J = 2 Hz, deuterium oxide-exchangeable, 1-OH), 3.98 (3H, s, 6-OCH_3), 5.29 (1H, ddd, J = 5.6 Hz, 3.6 Hz, 2 Hz, H-1), 6.30 (1H, s, H-5), 7.37 (1H, td, J = 8 Hz, 0.5 Hz, H-9), 7.42 (1H, dd, J = 8 Hz, 0.5 Hz, H-11), 7.66 (1H, td, J = 8 Hz, 2 Hz, H-10), 8.31 (1H, td, J = 8 Hz, 2 Hz, H-8); ^{13}C nmr, (50 MHz, deuteriochloroform): δ 26.18 (CH_3), 28.77 (CH_3), 56.27 (6-OCH_3), 40.07 (C-2), 59.47 (C-1), 77.64 (C-3), 96.05 (C-5), 104.05 (C-12b), 106.90 (C-6a), 116.72 (C-11), 122.98 (C-7a), 124.21 (C-9), 126.88 (C-8), 133.68 (C-10), 154.40 (C-11a), 157.75 (C-12a), 159.18 (C-4a), 161.51 (C-6), 175.16 (C-7).

Anal. Calcd. for $C_{19}H_{18}O_5$: C, 69.92; H, 5.57. Found: C, 69.74; H, 5.46.

(±)-1,2-Dihydro-1-hydroxy-6,11-dimethoxy-3,3-dimethyl-3*H*,7*H*-pyran[2,3-*c*]xanthen-7-one (**3Bc**).

This compound was prepared according to the procedure described for **3Ba**, as white solid (ethanol), in 80 % yield, mp: 196-197 °C; ^{1}H nmr, (400 MHz, deuteriochloroform): δ 1.40 (3H, s, 1 x gemCH_3), 1.49 (3H, s, 1 x gemCH_3), 2.10 (1H, dd, J = 14 Hz, 6 Hz, H-2b), 2.20 (1H, dd, J = 14 Hz, 6 Hz, H-2a), 3.73 (1H, d, J = 2 Hz deuterium oxide-exchangeable, 1-OH), 3.94 (3H, s, OCH_3), 3.98 (3H, s, OCH_3), 5.27 (1H, td, J = 6 Hz, 2 Hz, H-1), 6.26 (1H, s, H-5), 7.13 (1H, dd, J = 8 Hz, 1.5 Hz, H-10), 7,24 (1H, t, J = 8 Hz, H-9), 7.83 (1H, dd, J = 8 Hz, 1.5 Hz, H-8); ^{13}C nmr, (50 MHz, deuteriochloroform): δ 27.13 (2xCH_3), 40.32 (C-2), 56.28 (2xOCH_3), 60.26 (C-1), 76.37 (C-3), 96.07 (C-5), 104.65 (C-12b), 106.50 (C-6a), 114.02 (C-9), 114.70 (C-7a), 117.61 (C-8), 123.57 (C-10), 144.53 (C-11), 147.88 (C-11a), 157.53 (C-4a), 158.99 (C-12a), 161.32 (C-6), 170.97 (C-7).

Anal. Calcd. for $C_{20}H_{20}O_6$: C, 67.40; H, 5.66. Found: C, 67.22; H, 5.61.

(±)-3,4-Dihydro-4-hydroxy-5-methoxy-2,2-dimethyl-2*H*,6*H*-pyran[3,2-*b*]xanthen-6-one (**4Ba**).

This compound was prepared according to the procedure described for **3Ba**, as white solid (ethanol), in 73 % yield, mp: 190 °C; ^{1}H nmr, (400 MHz, deuteriochloroform): δ 1.44 (3H, s, 1 x gemCH_3), 1.50 (3H, s, 1 x gemCH_3), 2.11 (1H, dd, J = 14 Hz, 6 Hz, H-3a), 2.17 (1H, dd, J = 14 Hz, 6 Hz, H-3b), 3.52 (1H, d, J = 1.5 Hz, deuterium oxide-exchangeable, 4-OH), 4.11 (3H, s, 5-OCH_3), 5.13 (1H, td, J = 5.5 Hz, 1.5 Hz, H-4), 6.71 (1H, s, H-12), 7.36 (1H, td, J = 8 Hz, 1 Hz, H-8), 7.42 (1H, dd, J = 8 Hz, 1 Hz, H-10), 7.68 (1H, td, J = 8 Hz, 1.5 Hz, H-9), 8.30 (1H, dd, J = 8 Hz, 1.5 Hz, H-7); ^{13}C nmr, (50 MHz, deuteriochloroform): δ 26.85 (CH_3), 27.79 (CH_3), 39.98 (C-3), 59.98 (C-4), 62.53 (5-OCH_3), 77.62 (C-2), 101.31 (C-12), 109.77 (C-5a), 115.68 (C-4a), 117.21 (C-10), 122.41 (C-6a), 123.96 (C-8), 126.59 (C-7), 134.15 (C-9), 155.18 (C-10a), 158.36 (C-11a), 159.40 (C-12a), 160.50 (C-5), 175.18 (C-6).

Anal. Calcd. for $C_{19}H_{18}O_5$: C, 69.92; H, 5.57. Found: C, 69.73; H, 5.34.

(±)-3,4-Dihydro-4-hydroxy-5,10-dimethoxy-2,2-dimethyl-2*H*,6*H*-pyran[3,2-*b*]xanthen-6-one (**4Bc**).

This compound was prepared according to the procedure described for **3Ba**, as white solid (ethanol), in 87 % yield, mp: 166-167 °C; ^{1}H nmr, (400 MHz, deuteriochloroform): δ 1.39 (3H, s, 1 x gemCH_3), 1.43 (3H, s, 1 x gemCH_3), 2.08 (2H, d, J = 5.5 Hz, H-3), 3.47 (1H, d, J = 1.5 Hz, deuterium oxide-exchangeable, 4-OH), 3.98 (3H, s, OCH_3), 4.05 (3H, s, OCH_3), 5.08 (1H, td, J = 5.5 Hz, 1.5 Hz, H-1), 6.77 (1H, s, H-12), 7.15 (1H, dd, J = 8 Hz, 2 Hz, H-9), 7.22 (1H, t, J = 8 Hz, H-8), 7.82 (1H, dd, J = 8 Hz, 2 Hz, H-7); ^{13}C nmr, (50 MHz, deuteriochloroform): δ 26.83 (CH_3), 27.80 (CH_3), 40.00 (C-3), 56.40 (10-OCH_3), 59.99 (C-4), 62.50 (5-OCH_3), 77.49 (C-2), 101.62 (C-12), 109.63 (C-5a), 115.04 (C-8), 115.82 (C-4a), 117.51 (C-7), 123.21 (C-9), 123.29 (C-6a), 145.53 (C-10), 148.13 (C-10a), 158.15 (C-11a), 159.43 (C-12a), 160.38 (C-5), 175.11 (C-6).

Anal. Calcd. for $C_{20}H_{20}O_6$: C, 67.40; H, 5.66. Found: C, 67.34; H, 5.39.

(±)-*trans*-1-Acetoxy-2-bromo-1,2-dihydro-6-methoxy-3,3-dimethyl-3*H*,7*H*-pyran[2,3-*c*]xanthen-7-one (**3Ab**).

Acetic anhydride (1.5 ml), was added to a solution of the bromohydrin **3Aa** (24 mg, 0.059 mmole) in dry pyridine (3 ml) and the mixture was stirred at room temperature overnight. The solvent was vacuum-evaporated and the residue was purified by column chromatography (silica gel 15x1.5 cm) using a mixture of cyclohexane - ethyl acetate (5:1, v/v) as the eluent, to give

(±)-*trans*-1-acetoxy-2-bromo-1,2-dihydro-6-methoxy-3,3-dimethyl-3*H*,7*H*-pyran[2,3-*c*]xanthen-7-one as a solid, which was recrystallized from diethyl ether – *n*-hexane (23 mg, 87 %), mp: 204 °C; ^{1}H nmr, (400 MHz, deuteriochloroform): δ 1.64 (3H, s, 1 x gemCH_3), 1.66 (3H, s, 1 x gemCH_3), 2.17 (3H, s, CH_3CO), 4.01 (3H, s, 6-OCH_3), 4.34 (1H, d, J = 4 Hz, H-2), 6.37 (1H, s, H-5), 6.66 (1H, d, J = 4 Hz, H-1), 7.33 (1H, td, J = 8 Hz, 0.5 Hz, H-11), 7.36 (1H, dd, J = 8 Hz, 0.5 Hz, H-9), 7.66 (1H, td, J = 8 Hz, 1.5 Hz, H-10), 8.30 (1H, dd, J = 8 Hz, 1.5 Hz, H-8); ^{13}C nmr, (50 MHz, deuteriochloroform): δ 21.07 (*C*H_3CO), 25.32 (CH_3), 26.53 (CH_3), 54.32 (C-2), 56.44 (6-OCH_3), 67.14 (C-1), 78.18 (C-3), 95.65 (C-5), 98.05 (C-12b), 108.20 (C-6a), 116.87 (C-11), 122.85 (C-7a), 124.36 (C-9), 126.68 (C-8), 133.99 (C-10), 154.48 (C-11a), 157.62 (C-12a), 158.63 (C-4a), 162.40 (C-6), 169.83 (CH_3*C*O), 175.12 (C-7).

Anal. Calcd. for $C_{21}H_{19}BrO_6$: C, 56.39; H, 4.28. Found: C, 56.26; H, 4.20.

(±)-*trans*-1-Acetoxy-2-bromo-1,2-dihydro-6,11-dimethoxy-3,3-dimethyl-3*H*,7*H*-pyran[2,3-*c*]xanthen-7-one (**3Ad**).

This compound was prepared according to the procedure described for **3Ab**, as white solid (diethyl ether – *n*-hexane), in 93 % yield, mp: 179-180 °C; ^{1}H nmr, (400 MHz, deuteriochloroform): δ 1.58 (3H, s, 1 x gemCH_3), 1.61 (3H, s, 1 x gemCH_3), 2.11 (3H, s, CH_3CO), 3.93 (3H, s, OCH_3), 3.96 (3H, s, OCH_3), 4.32 (1H, d, J = 3 Hz, H-2), 6.34 (1H, s, H-5), 6.52 (1H, d, J = 3 Hz, H-1), 7.10 (1H, dd J = 8 Hz, 1.5 Hz, H-10), 7.23 (1H, t, J = 8 Hz, H-9), 7.80 (1H, dd, J = 8 Hz, 1.5 Hz, H-8); ^{13}C nmr, (50 MHz, deuteriochloroform): δ 20.63 (*C*H_3CO), 24.36 (CH_3), 28.00 (CH_3), 54.17 (C-2), 55.85 (10-OCH_3), 56.31 (6-OCH_3), 67.44 (C-1), 77.56 (C-3), 95.63 (C-5), 97.52 (C-12b), 107.61 (C-6a), 114.35 (C-9), 117.31 (C-8), 123.55 (C-10), 123.77 (C-7a), 145.20 (C-11), 148.14 (C-11a), 157.63 (C-12a), 158.47 (C-4a), 162.31 (C-6), 169.85 (CH_3*C*O), 175.02 (C-7).

Anal. Calcd. for $C_{22}H_{21}BrO_7$: C, 55.36; H, 4.44. Found: C, 55.41; H, 4.38.

(±)-*trans*-4-Acetoxy-3-bromo-3,4-dihydro-5-methoxy-2,2-dimethyl-2*H*,6*H*-pyran[3,2-*b*]xanthen-6-one (**4Ab**).

This compound was prepared according to the procedure described for **3Ab**, as white solid (diethyl ether – *n*-hexane), in 87 % yield, mp: 200 °C; ^{1}H nmr, (400 MHz, deuteriochloroform): δ 1.62 (3H, s, 1 x gemCH_3), 1.64 (3H, s, 1 x gemCH_3), 2.13 (3H, s, CH_3CO), 4.00 (3H, s, 5-OCH_3), 4.36 (1H, d, J = 3.5 Hz, H-3), 6.45 (1H, d, J = 3.5 Hz, H-4), 6.72 (1H, s, H-12), 7.37 (1H, td, J = 8 Hz, 0.5 Hz, H-8), 7.42 (1H, dd, J = 8 Hz, 0.5 Hz, H-10), 7.68 (1H, td, J = 8 Hz, 1.5 Hz, H-9), 8.29 (1H, dd, J = 8 Hz, 1.5 Hz, H-7); ^{13}C nmr, (50 MHz, deuteriochloroform): δ 21.04 (*C*H_3CO), 25.24 (CH_3), 27.71 (CH_3), 54.08 (C-3), 62.66 (5-OCH_3), 67.04 (C-4), 77.44 (C-2), 101.19 (C-12), 108.67 (C-4a), 110.83 (C-5a), 117.19 (C-10), 122.40 (C-6a), 123.92 (C-8), 126.67 (C-7), 134.34 (C-9), 155.10 (C-10a), 158.56 (C-11a), 159.08 (C-12a), 162.17 (C-5), 169.74 (CH_3*C*O), 174.98 (C-6).

Anal. Calcd. for $C_{21}H_{19}BrO_6$: C, 56.39; H, 4.28. Found: C, 56.24; H, 4.19.

(±)-*trans*-4-Acetoxy-3-bromo-3,4-dihydro-5,10-dimethoxy-2,2-dimethyl-2*H*,6*H*-pyran[3,2-*b*]xanthen-6-one (**4Ad**).

This compound was prepared according to the procedure described for **3Ab**, as white solid (diethyl ether – *n*-hexane), in 91 % yield, mp: 181 °C; ^{1}H nmr, (400 MHz, deuteriochloroform): δ 1.57 (3H, s, 1 x gemCH_3), 1.59 (3H, s, 1 x gemCH_3), 2.10 (3H, s, CH_3CO), 3.94 (3H, s, OCH_3), 3.98 (3H, s, OCH_3), 4.28 (1H, d, J = 3.5 Hz, H-3), 6.40 (1H, d, J = 3.5 Hz, H-4), 6.84 (1H, s, H-12), 7.16 (1H, dd, J = 8 Hz, 2 Hz, H-9), 7.24 (1H, t, J = 8 Hz, H-8), 7.81 (1H, dd, J = 8 Hz, 2 Hz, H-7); ^{13}C nmr, (50 MHz, deuteriochloroform): δ 21.03 (*C*H_3CO), 25.32 (CH_3), 27.49 (CH_3), 54.19 (C-3), 56.46 (10-OCH_3), 62.66 (5-OCH_3), 67.10 (C-4), 77.47 (C-2), 101.30 (C-12), 108.92 (C-5a), 110.72 (C-4a), 115.22 (C-8), 117.59 (C-7), 123.38 (C-6a), 123.40 (C-9), 145.52 (C-10), 148.13 (C-10a), 158.59 (C-11a), 158.87 (C-12a), 162.02 (C-5), 169.72 (CH_3*C*O), 174.90 (C-6).

Anal. Calcd. for $C_{22}H_{21}BrO_7$: C, 55.36; H, 4.44. Found: C, 55.61; H, 4.44.

(±)-1-Acetoxy-1,2-dihydro-6-methoxy-3,3-dimethyl-3*H*,7*H*-pyran[2,3-*c*]xanthen-7-one (**3Bb**).

This compound was prepared according to the procedure described for **3Ab**, as white solid (diethyl ether – *n*-hexane), in 75 % yield, mp: 210 °C; ^{1}H nmr, (400 MHz, deuteriochloroform): δ 1.52 (6H, s, 2 x gemCH_3), 2.11 (1H, dd, J = 15 Hz, 4.4 Hz, H-2a), 2.13 (3H, s, CH_3CO), 2.17 (1H, dd, J = 15 Hz, 4.4 Hz, H-2b), 4.00 (3H, s, 6-OCH_3), 6.31 (1H, s, H-5), 6.48 (1H, t, J = 4 Hz, H-1), 7,36 (1H, dd, J = 8 Hz, 0.8 Hz, H-11), 7.36 (1H, td, J = 8 Hz, 0.8 Hz, H-9), 7.65 (1H, td, J = 8 Hz, 1.5 Hz, H-10), 8.31 (1H, dd, J = 8 Hz, 1.5 Hz, H-8); ^{13}C nmr, (50 MHz, deuteriochloroform): δ 21.29 (*C*H_3CO), 25.55 (CH_3), 29.22 (CH_3), 38.76 (C-2), 56.35 (6-OCH_3), 61.11 (C-1), 77.62 (C-3), 95.80 (C-5), 99.82 (C-12b), 108.20 (C-6a), 116.72 (C-11), 122.91 (C-7a), 124.07 (C-9), 126.68 (C-8), 133.78 (C-10), 154.42 (C-11a), 157.63 (C-12a), 160.00 (C-4a), 162.16 (C-6), 165.97 (CH_3*C*O), 170.36 (C-7).

Anal. Calcd. for $C_{21}H_{20}O_6$: C, 68.47; H, 5.47. Found: C, 68.36; H, 5.30.

(±)-1-Acetoxy-1,2-dihydro-6,11-dimethoxy-3,3-dimethyl-3*H*,7*H*-pyran[2,3-*c*]xanthen-7-one (**3Bd**).

This compound was prepared according to the procedure described for **3Ab**, as white solid (diethyl ether – *n*-hexane), in 65 % yield, mp: 247 °C (dec); ^{1}H nmr, (400 MHz, deuteriochloroform): δ 1.45 (6H, s, 2 x gemCH_3), 2.08 (3H, s, CH_3CO), 2.20 (2H, d, J = 4 Hz, H-2), 3.94 (3H, s, OCH_3), 3.95 (3H, s, OCH_3), 6.27 (1H, s, H-5), 6.32 (1H, t, J = 4 Hz, H-1), 7.10 (1H, dd, J = 8 Hz, 1.5 Hz, H-10), 7.22 (1H, t, J = 8 Hz, H-9), 7.82 (1H, dd, J = 8 Hz, 1.5 Hz, H-8); ^{13}C nmr, (50 MHz, deuteriochloroform): δ 21.01 (*C*H_3CO), 25.48 (CH_3), 28.90 (CH_3), 38.87 (C-2), 55.97 (11-OCH_3), 56.33 (6-OCH_3), 61.64 (C-1), 76.15 (C-3), 95.82 (C-5), 99.95 (C-12b), 107.05 (C-6a), 114.31 (C-9), 117.39 (C-8), 123.43 (C-10), 123.82 (C-7a), 145.06 (C-11), 148.27 (C-11a), 157.44 (C-12a), 159.93 (C-4a), 162.04 (C-6), 170.53 (CH_3*C*O), 175.31 (C-7).

Anal. Calcd. for $C_{22}H_{22}O_7$: C, 66.32; H, 5.57. Found: C, 66.04; H, 5.49.

(±)-4-Acetoxy-3,4-dihydro-5-methoxy-2,2-dimethyl-2*H*,6*H*-pyran[3,2-*b*]xanthen-6-one (**4Bb**).

This compound was prepared according to the procedure described for **3Ab**, as white solid (diethyl ether – *n*-hexane), in 86 % yield, mp: 182 °C; ^{1}H nmr, (400 MHz, deuteriochloroform): δ 1.47 (3H, s, 1 x gemCH_3), 1.49 (3H, s, 1 x gemCH_3), 2.11 (3H, s, CH_3CO), 2.18 (1H, dd, J = 15.3 Hz, 5 Hz, H-3a), 2.22 (1H, dd, J = 15.3 Hz, 2.7 Hz, H-3b), 4.00 (3H, s, 5-OCH_3), 6.26 (1H, dd

J = 5 Hz, 2.7 Hz, H-4), 6.71 (1H, s, H-12), 7.36 (1H, td, J = 8 Hz, 0.7 Hz, H-8), 7.41 (1H, dd, J = 8 Hz, 0.7 Hz, H-10), 7.67 (1H, td, J = 8 Hz, 1.5 Hz, H-9), 8.29 (1H, td, J = 8 Hz, 1.5 Hz, H-7); ^{13}C nmr, (50 MHz, deuteriochloroform): δ 21.42 (*C*H_3CO), 25.79 (CH_3), 29.53 (CH_3), 38.60 (C-3), 61.44 (C-4), 62.64 (5-OCH_3), 77.66 (C-2), 101.06 (C-12), 110.16 (C-4a), 110.72 (C-5a), 117.17 (C-10), 122.46 (C-6a), 123.79 (C-8), 126.67 (C-7), 134.19 (C-9), 155.13 (C-10a), 158.97 (C-11a), 160.13 (C-12a), 161.76 (C-5), 170.21 (CH_3*C*O), 175.08 (C-6).

Anal. Calcd. for $C_{21}H_{20}O_6$: C, 68.47; H, 5.47. Found: C, 68.11; H, 5.19.

(±)-4-Acetoxy-3,4-dihydro-5,10-dimethoxy-2,2-dimethyl-2*H*,6*H*-pyran[3,2-*b*]xanthen-6-one (**4Bd**).

This compound was prepared according to the procedure described for **3Ab**, as white solid (diethyl ether – *n*-hexane), in 83 % yield, mp: 192 °C; ^{1}H nmr, (400 MHz, deuteriochloroform): δ 1.45 (6H, s, 2 x gemCH_3), 2.08 (3H, s, CH_3CO), 2.19 (2H, d, J = 3 Hz, H-3a, 3b), 3.94 (3H, s, OCH_3), 3.97 (3H, s, OCH_3), 6.20 (1H, t, J = 3 Hz, H-4), 6.76 (1H, s, H-12), 7.13 (1H, dd, J = 8 Hz, 1.5 Hz, H-9), 7.21 (1H, t, J = 8 Hz, H-8), 7.80 (1H, dd, J = 8 Hz, 1.5 Hz, H-7); ^{13}C nmr, (50 MHz, deuteriochloroform): δ 21.30 (*C*H_3CO), 25.66 (CH_3), 29.38 (CH_3), 38.52 (C-3), 56.35 (10-OCH_3), 61.40 (C-4), 62.52 (5-OCH_3), 75.62 (C-2), 101.28 (C-12), 109.94 (C-4a), 110.88 (C-5a), 114.97 (C-8), 117.51 (C-7), 123.17 (C-9), 123.24 (C-6a), 145.40 (C-10), 148.03 (C-10a), 158.66 (C-11a), 160.06 (C-12a), 161.57 (C-5), 170.08 (CH_3*C*O), 174.89 (C-6).

Anal. Calcd. for $C_{22}H_{22}O_7$: C, 66.32; H, 5.57. Found: C, 65.99; H, 5.34.

Molecular Calculations.

Molecular calculations were performed using the MM+ force field of the HyperChem program (HyperChem is developed and licensed from Hypercube; the MM+ force field used in this software for molecular mechanics calculations is an extension of MM2 using the MM2 (1991) parameters and atom types with the 1997 functional form). The Polak - Ribiere (conjugate gradient) minimization method with an energy convergence criterion of 0.01 kcal.mol^{-1} was used for geometry optimization.

Cytotoxicity.

Murine leukemia L1210 cells from the American Type Culture Collection (Rockville Pike, MD) were grown in RPMI 1640 medium supplemented with 10% fetal calf serum, 2 m*M L*-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin and 10 m*M* HEPES buffer (pH 7.4). The cytotoxicity was measured by the microculture tetrazolium assay essentially as described [16,17]. Cells were exposed for 48 hours to nine graded concentrations of the test compound. 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.

Cell Cycle Analysis.

L1210 cells ($2.5x10^5$/ml) were incubated for 21 hours (approximately two doubling times) with various concentrations of cytotoxic drugs. Cells were then fixed by 70% ethanol, washed twice with phosphate-buffered saline (PBS) and incubated in PBS containing 100 µg/ml RNase and 25 µg/ml propidium iodide (PI) for 30 minutes. For each sample, 10,000 cells were analyzed on an Epics XL Coulter flow cytometer.

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