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# 2,2-Dimethyl-2*H*-anthra[2,3-*b*]pyran-6,11-diones: A New Class of Cytotoxic Compounds

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**Abstract**—The synthesis and cytotoxic activity of some new 2,2-dimethyl-2*H*-anthra[2,3-*b*]pyran-6,11-diones is described. Certain compounds possess interesting activity against murine leukemia L-1210 cells. Relationships between the biological activity and the pyrano-ring conformations are discussed. 

## Introduction

Natural and synthetic quinones represent important antitumor drugs. The most active quinones are relatively large molecules possessing a large quinoid system, in particular that of anthracene-9,10-diones.[1] This group includes anthracycline antibiotics and aminoalkyl-functionalized anthraquinones such as mitoxanthrone (**1a**) and ametantrone (**1b**). On the other hand it is well documented that compounds possessing a furano or pyrano ring fused to a polycyclic, aromatic system such as acridone,[2–4] xanthone,[5,6] coumarin,[7] exhibit interesting cytotoxic and antitumor properties. A characteristic example is the natural alkaloid acronycine (**2**), which was found to be a very potent antitumor agent (possessing an $IC_{50}$ in leukemia cells in the order of 25 μM). Moreover recent studies have shown that hydroxylation of the pyrano-ring double bond of pyrano-xanthone, -coumarin and-acridone, yielded compounds, such as 1,2-dihydro-1-hydroxy-6-methoxy-3,5-dimethyl-3*H*,7*H*-pyran[2,3-*c*]xanthen-7-one (**3**), 1-hydroxy-1,2-dihydroseseline (**4**), and 1,2-diacetoxy-1,2-dihydroacronycine (**5**) (Chart 1), which exhibited promising cytotoxic and antitumor properties (possessing an $IC_{50}$ in leukemia cells in of 9, 0.9, and 4.2 μM, respectively). The above considerations guided our interest to develop simple and functionalized derivatives at positions 3, 4, 5, and 12 of 2,2-dimethyl-2*H*-anthra[2,3-*b*]pyran-6,11-dione as a new class of antiproliferative agents (Scheme 1). It is worthy to point out that pyranoanthraquinones are extremely rare in nature and the most characteristic examples are Ploiariquinones A and B (**6** and **7**) from the cicada tree, *Ploiarum* alternifolium,[8] although no biological evaluation has been reported for these compounds.

## Results and Discussion

The typical way of obtaining the key compound 5,12-dihydroxy-2,2-dimethyl-2*H*-anthra[2,3-*b*]pyran-6,11-dione (**8**) would have been treatment of purpurin (**9**) with 3-chloro-3-methyl-1-butyne in the presence of cuprous salt as catalyst and subsequent thermal cyclization. In our case this reaction proved unsuccessful, probably due to the inactivation of the C-3 hydroxyl group of purpurin by the presence of the neighbouring hydroxyl group. Therefore, the lead compound **8** was obtained by application of another methodology which was previously applied in the case of some natural pyranocoumarins[9] and pyranoanthaquinones[8] which involved reaction of the starting phenol with the corresponding α,β-unsaturated aldehyde. Therefore treatment of purpurin (**9**) with 3-methyl-2-butenal in dry pyridine yielded **8** (40%). The presence of the two phenolic hydroxyls caused a very important inactivation of the double bond of the pyran ring, making pyran ring substitution very difficult. In contrast, the functionalization of the 3,4-double bond could be achieved in the dimethoxy compound **10** obtained by methylation of **8**. Quinone **11** was obtained by treatment of **10** with NBS in aqueous THF solution.[10] The bromohydrin **11** was smoothly debrominated with tributyltin hydride,[10] to yield **12**. Treatment

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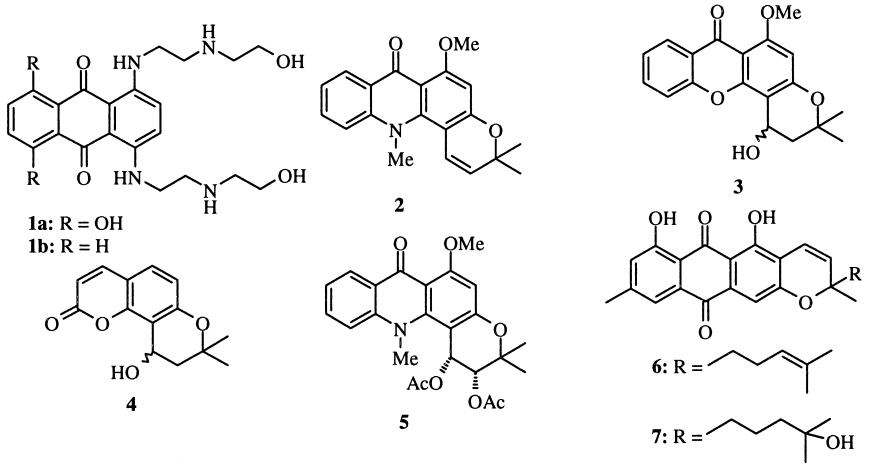
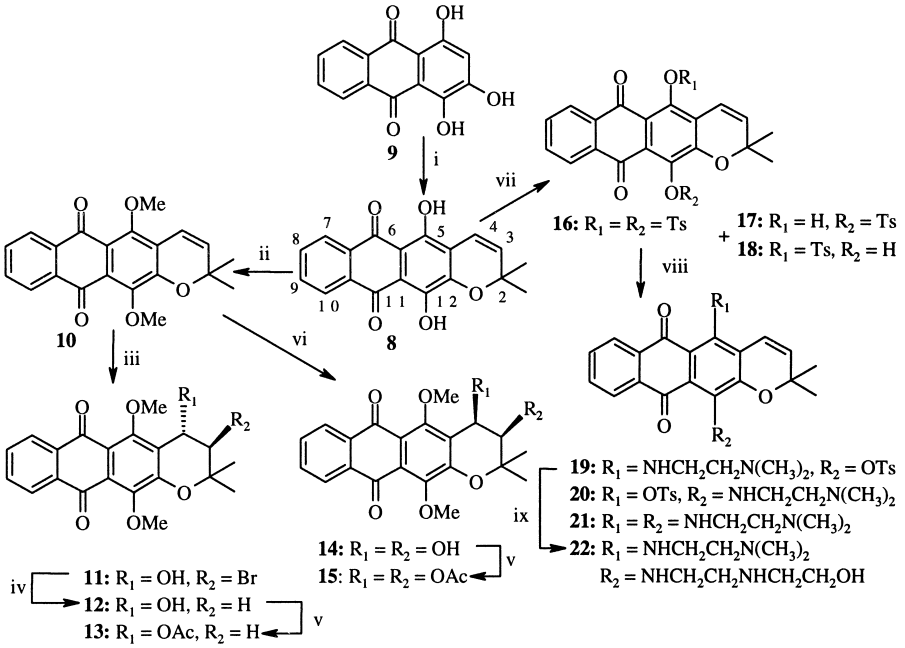



**Chart 1.**




**Scheme 1.** Reagents and conditions: (i) 3-methyl-butenal, Py, 115 °C; (ii) $(Me)_2SO_4$, $K_2CO_3$, 56 °C; (iii) NBS, THF, $H_2O$, 0 °C; (iv) $Bu_3SnH$, AIBN, toluene, 110 °C; (v) $Ac_2O$, Py, rt; (vi) $OsO_4$, *N*-methyl-morpholine-*N*-oxide, *t*-BuOH, THF, $H_2O$, rt; (vii) TsCl, $K_2CO_3$, $Me_2CO$, 56 °C; (viii) $(CH_3)_2N(CH_2)_2NH_2$, Py, 60 °C; (ix) $HO(CH_2)_2NH(CH_2)_2NH_2$, Py, 60 °C.

of alcohol **12** with excess $Ac_2O$ in pyridine afforded the corresponding ester **13**. The diol **14** was obtained by catalytic osmium oxidation of **10** using *N*-methyl-morpholine-*N*-oxide to regenerate the oxidizing agent. Treatment of *cis*- diol **14** in a similar way to **12** afforded the corresponding diester **15**.

Introduction of the amino chains to position 5 and/or 12 was achieved via the intermediate 5,12-ditosyloxy derivative **16**.[11] This compound was obtained by treatment of **8** with tosyl chloride in the presence of $K_2CO_3$. The monotosylated compounds **17** and **18** were also obtained during the tosylation of **8.** Reaction of **16** with *N,N*-dimethylethylenediamine afforded the mono- **19** and **20** and the bi- **21** substituted derivatives. Further substitution of the most abundant mono-subsituted derivative **19** with 2-[(2-aminoethyl)amino]ethanol gave the unsymmetrical bi-substituted derivative **22**. The structures of all the synthetic compounds were established by the use of CI-MS and $^1H$, $^{13}C$, 1-D and 2-D NMR data. For the monotosylated compounds **17** and **18** and the corresponding aminated derivatives, **19** and **20**, structures were established unequivocally by application of long range heteronuclear correlation (HMBC). In the case of **18**, a $J_3$ correlation was observed between H-4 (6.36 ppm) of the pyran ring and the relatively shielded C-5 (137.1 ppm) and a $J_2$ correlation between C-12 (151.3 ppm) and OH (13.35 ppm). From these observations it was clear that the tosyloxy group was attached to C-5. In the case of **19**, a $J_3$ correlation was observed between H-1′ (3.46 ppm) of the side chain and C-5 (151.2 ppm), as well as between H-4 (6.53 ppm) of

the pyran ring and C-5. These observations confirmed that in isomer **19**, the side chain was attached to C-5 and not to C-12. Consequently, in the other isomer **20**, the side chain was attached to C-12.

The cytotoxic activity of compounds **8**–**22** was carried out in vitro using L-1210 leukemia cells.[12] The results ($IC_{50}$) are reported in Table 1. The most active compounds were **8**, **12**, **13**, **19**, **20** and **22** which are 15- to 3-fold more potent than acronycine.

The cytotoxic activity of these newly synthesized pyranoquinone derivatives is in the same order of magnitude with those of various pyrano-acridine -xanthone -coumarin derivatives. This could be explained by the presence of the dimethylpyrano ring, which is the main structural difference between our compounds and the previously described, highly cytotoxic anthraquinone derivatives.[13] This additional dimethylpyrano ring is probably, as in the case of acronycine, the active pharmacophhore site of the synthesized quinones described herein.

In order to study the cell cycle specificity of this new class of cytotoxic compounds, the lead compound **8** was evaluated for its ability to perturb the cell cycle in a specific way. Using the same cell line (L1210), compound **8** induced a partial accumulation of cells in the $G_2 + M$ phase of the cell cycle.

It is interesting to point out that the pyranoanthraquinones **12** and **13** which bear a hydroxy or acetoxy group at position 4, such as **12** and **13**, had significant cytotoxicity (3- and 8-fold more potent than acronycine) as in the case of pyranocoumarins and pyranoxanthones. In contrast, the 3,4-diacetoxy derivative **15** was completely devoided of cytotoxic activity. Taking into account the crucial role of D-ring conformation to the biological activity of acronycine derivatives,[14] it seemed interesting to investigate the D-ring conformation of 3,4-dihydro-3,4-dihydroxy-5,12-dimethoxy-2,2-dimethyl-2*H*-anthra[2,3-*b*]pyran-6,11-dione derivatives. This was carried out by the performance of NOESY experiments and computational conformation analysis. The conformation analysis was performed using molecular mechanics calculations and the two lowest energy conformations (structures I and II for compound **14**, Fig. 1) were predicted. Both conformations were half-chair and their calculated energy difference was small, suggesting that they could possibly both be present in solution. If compound **14** existed under conformation I, a correlation between H-4 and the β-pyran methyl group should be observed in the NOESY experiment. The absence of such a correlation suggested that 3,4-dihydro-3,4-dihydroxy derivatives exist predominantly under conformation II in solution. In this conformation, the substituents on C-3 and C-4 are relatively hindered by the pyran methyl groups, since the substituent on C-3 is *gauche* with both methyl groups and the substituent on C-4 is homoaxial with the α-$CH_3$. It is noteworthy that the pyran ring of the highly active 1,2-dihydro-1,2-dihydroxyacronycine derivatives, predominantly adopts the conformation I,[14] where the substituent on C-3 has a pseudoaxial orientation and both C-3 and C-4 substituents are less hindered by the pyran methyl groups. This could possibly explain the fact that the synthesized 3,4-dihydro-3,4-dihydroxy derivatives (**14** and **15**) are less potent than the corresponding acronycine derivatives.

In regard to the amino functionalized derivatives the most active was the monosubstituted **20** (15-fold more potent than acronycine and 2-fold more potent than ametantrone)[15] and the unsymmetrically substituted **22.** It is noteworthy that the symmetrically bisubstituted compound **21** was inactive.

The development of new analogues in the mitoxantrone series is currently in progress in our laboratory.

**Table 1.** Cytotoxic activity[a]

| Compound | $IC_{50}$, μM |
|---|---|
| **8** | 7.8 |
| **10** | 47.6 |
| **11** | 33.3 |
| **12** | 7.7 |
| **13** | 3.1 |
| **14** | > 50 |
| **15** | > 50 |
| **16** | > 50 |
| **17** | > 50 |
| **19** | 6.6 |
| **20** | 1.7 |
| **21** | > 50 |
| **22** | 3.4 |
| **2** | 25 |
| **1b** | 3.6[b] |

[a]Inhibition of L-1210 cell proliferation measured by the MMT assay (mean of 2 values obtained in independent experiments).
[b]From ref 15.

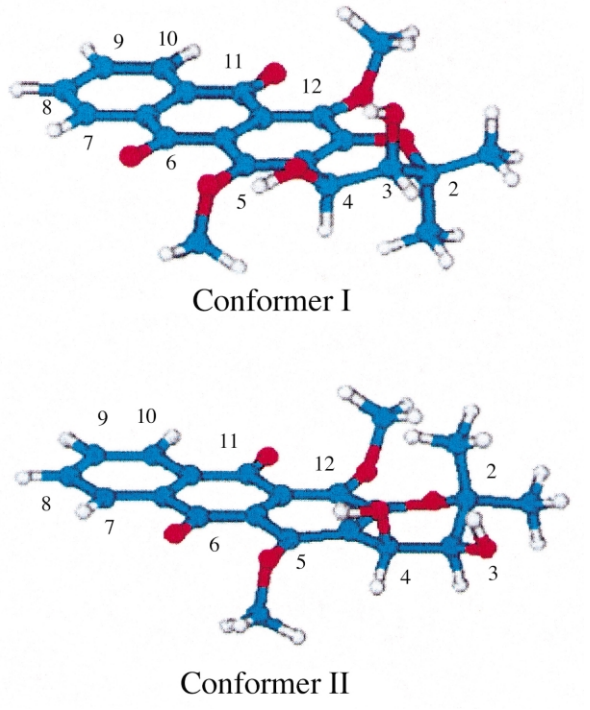



**Figure 1.** Conformers I and II for compound **14**.

## Experimental

### General

Spectra were recorded on the following apparatus: MS, Nermag R10-10C in disorption-chemical ionization, using $NH_3$ as reagent gas, IR, Paragon 500, NMR, Bruker AC 200, $^1H$ NMR (200 MHz), $^{13}C$ NMR (50 MHz) and a Bruker DRX400. Chemical shifts are given in δ values with TMS as an internal standard. Coupling constants (*J*) are given in Hz. The signals of $^1H$ and $^{13}C$ spectra were unambiguously assigned by use of 2-D NMR techniques: $^1H$-$^1H$-COSY, $^{13}C$-$^1H$ HETCOR and HMBC. These 2-D experiments were performed using standard Bruker microprograms. Column chromatography was conducted using flash Silica gel 60 Merck (40–63 μm), with an overpressure of 300 mbar. All new compounds gave satisfactory combustion analyses (C, H, N, within ± 0.4% of calcd values).

**5,12-Dihydroxy-2,2-dimethyl-2*H*-anthra[2,3-*b*]pyran-6,11-dione (8).** To a solution of **9** (1.000 g, 3.90 mmol) in dry pyridine (5 mL), 3-methyl-2-butenal (3.75 mL, 39 mmol) was added. The reaction mixture was stirred for 5 h at 115 °C under argon and then the reagents were removed under reduced pressure (using a high vacuum pump). The residue was purified by flash chromatography on Silica gel with $CH_2Cl_2$ to give compound **8** (500 mg, 40%). $^1H$ NMR (200 MHz, $CDCl_3$) δ 1.57 (6H, s, 2×Me), 5.80 (1H, d, $J$ = 10 Hz, H-3), 6.75 (1H, d, $J$ = 10 Hz, H-4), 7.80 (2H, m, H-8, 9), 8.29 (2H, m, H-7, 10), 13.85 (1H, s, 5-OH), 13.30 (1H, s, 12-OH); $^{13}C$ NMR (50 MHz, $CDCl_3$) δ 28.34 (2×Me), 79.20 (C-2), 115.30 (C-4), 106.33 (C-11a), 112.20 (C-4a), 116.84 (C-5a), 126.84 (C-7, 10), 131.69 (C-3), 133.31 (C-6a, 10a), 133.82 (C-9), 134.33 (C-8), 149.26 (C-12), 150.14 (C-12a), 156.16 (C-5), 184.70 (C-6), 186.60 (C-11). MS-DCI $m/z$ 323 $(M+H)^+$.

**5,12-Dimethoxy-2,2-dimethyl-2*H*-anthra[2,3-*b*]pyran-6,11-dione (10).** To a solution of **8** (100 mg, 0.31 mmol) in dry acetone (12 mL) was added dimethylsulfate (2 mL) and dry $K_2CO_3$ (1 g). The reaction mixture was stirred for 1 h at 56 °C. Then the reagents were removed by filtration and by evaporation under reduced pressure (using a high vacuum pump). The residue was purified by flash chromatography on Silica gel with cyclohexane:$CH_2Cl_2$ (50:50) to $CH_2Cl_2$ to give compound **7** (90 mg, 81%). $^1H$ NMR (200 MHz, $CDCl_3$) δ 1.51 (6H, s, 2×Me), 3.89 (3H, s, 5-OMe), 3.94 (3H, s, 12-OMe), 5.82 (1H, d, $J$ = 10 Hz, H-3), 6.71 (1H, d, $J$ = 10 Hz, H-4), 7.67 (2H, m, H-8, 9), 8.12 (2H, m, H-7, 10); $^{13}C$ NMR (50 MHz, $CDCl_3$) δ 28.19 (2×Me), 61.12 (12-OMe), 62.52 (5-OMe), 78.03 (C-2), 116.18 (C-4), 119.41 (C-11a), 121.76 (C-4a), 126.40 (C-7, 10), 127.72 (C-5a), 133.15 (C-8, 9), 133.38 (C-3), 134.19 (C-6a, 10a), 145.73 (C-12), 153.08 (C-12a), 153.37 (C-5), 181.82 (C-6), 183.07 (C-11). IR ($CHCl_3$) $\nu_{max}$ 2933, 1670, 1596, 1344, 1130 $cm^{-1}$.MS-DCI $m/z$ 351 $(M+H)^+$.

**(±)-*trans*-3-Bromo-3,4-dihydro-5,12-dimethoxy-2,2-dimethyl-4-hydroxy-2*H*-anthra[2,3-*b*]pyran-6,11-dione (11).** To a solution of **10** (70 mg, 0.20 mmol) in THF (5 mL) and $H_2O$ (5 mL) was added *N*-bromo-succinimide (38 mg, 0.21 mmol). The reaction mixture was stirred for 4 h at 0 °C and then the reaction mixture was extracted with NaCl (satd)-$Et_2O$ and the organic layer was collected. The solvent was removed under reduced pressure and compound **11** was purified by flash chromatography on Silica gel with $CH_2Cl_2$:MeOH (99.5:0.5) (73 mg, 82%). $^1H$ NMR (200 MHz, $CDCl_3$) δ 1.57 (3H, s, Me), 1.66 (3H, s, Me), 3.94 (3H, s, 5-OMe), 3.96 (1H, d, $J$ = 2.5 Hz, OH-4), 4.02 (3H, s, 12-OMe), 4.26 (1H, d, $J$ = 6 Hz, H-3), 5.23 (1H, dd, $J$ = 6, 2.5 Hz, H-4), 7.69 (2H, m, H-8, 9), 8.12 (2H, m, H-7, 10); $^{13}C$ NMR (50 MHz, $CDCl_3$) δ 23.77 (Me), 26.35 (Me), 56.49 (C-3), 61.19 (12-OMe), 62.74 (5-OMe), 68.18 (C-4), 79.50 (C-2), 118.61 (C-11a), 123.46 (C-4a), 126.55 (C-7, 10), 128.24 (C-5a), 133.46 (C-9), 133.61 (C-8), 133.97 (C-6a, 10a), 146.40 (C-12), 152.65 (C-12a), 156.76 (C-5), 181.24 (C-6), 182.86 (C-11). MS-DCI $m/z$ 449, 447 $(M+H)^+$.

**(±)-3,4-Dihydro-5,12-dimethoxy-2,2-dimethyl-4-hydroxy-2*H*-anthra[2,3-*b*]pyran-6,11-dione (12).** Compound **11** (57 mg, 0.13 mmol) was dissolved in anhydrous toluene (7.5 mL) and the solution was refluxed for 15 min under argon. Then was added AIBN (azo-bis-2, 2′(methyl-2-propionitrile)) (9 mg) and after 5 min a solution of tributyltin hydride (0.3 mL in 4 mL of toluene) 0.5 mL/5 min for 40 min. The reaction mixture was refluxed for 2 h. Then the solvent was evaporated and the residue was purified by flash chromatography on Silica gel with cyclohexane:EtOAc (80:20) to give compound **12** (34 mg, 71%). $^1H$ NMR (200 MHz, $CDCl_3$) δ 1.48 (3H, s, Me), 1.49 (3H, s, Me), 2.12 (2H, d, $J$ = 5.5 Hz, H-3), 3.52 (1H, d, $J$ = 2 Hz, OH-4), 3.93 (3H, s, 5-OMe), 4.02 (3H, s, 12-OMe), 5.06 (1H, td, $J$ = 5.5, 2 Hz, H-4), 7.70 (2H, m, H-8, 9), 8.16 (2H, m, H-7, 10); $^{13}C$ NMR (50 MHz, $CDCl_3$) δ 26.43 (Me), 27.75 (Me), 39.66 (C-3), 60.31 (C-4), 61.12 (12-OMe), 62.52 (5-OMe), 77.60 (C-2), 117.65 (C-11a), 125.07 (C-4a), 126.47 (C-7, 10), 127.79 (C-5a), 133.38 (C-9), 133.53 (C-8), 134.11 (C-6a, 10a), 146.68 (C-12), 154.18 (C-12a), 157.34 (C-5), 181.53 (C-6), 183.14 (C-11). MS-DCI $m/z$ 369 $(M+H)^+$.

**(+)-4-Acetoxy-3,4-dihydro-5,12-dimethoxy-2,2-dimethyl-2*H*-anthra[2,3-*b*]pyran-6,11-dione (13).** To a solution of **12** (12 mg, 0.03 mmol) in dry pyridine (1.5 mL) was added $Ac_2O$ (1.5 mL, 15 mmol). The reaction mixture was stirred for 24 h at room temperature and then the reagents were removed under reduced pressure (using a high vacuum pump). The residue was compound **13** (13 mg, 96%). $^1H$ NMR (200 MHz, $CDCl_3$) δ 1.45 (3H, s, Me), 1.53 (3H, s, Me), 2.09 (3H, s, $CH_3CO$), 2.15 (2H, m, H-3), 3.90 (3H, s, 5-OMe), 3.94 (3H, s, 12-OMe), 6.22 (1H, dd, $J$ = 5, 3 Hz, H-4), 7.70 (2H, m, H-8, 9), 8.16 (2H, m, H-7,10); $^{13}C$ NMR (50 MHz, $CDCl_3$) δ 21.23 ($CH_3CO$), 25.42 (Me), 29.40 (Me), 38.33 (C-3), 61.23 (C-4), 61.23 (12-OMe), 62.57 (5-OMe), 76.15 (C-2), 117.74 (C-11a), 120.30 (C-4a), 126.41 (C-7), 126.62 (C-10), 128.56 (C-5a), 133.27 (C-9), 133.60 (C-8), 134.15 (C-6a), 134.24 (C-10a), 146.28 (C-12), 155.27 (C-12a), 158.13 (C-5), 169.95 ($CH_3CO$), 181.23 (C-6), 183.35 (C-11). MS-DCI $m/z$ 410 $(M+H)^+$.

**(±)-3,4-Dihydro-3,4-dihydroxy-5,12-dimethoxy-2,2-dimethyl-2*H*-anthra[2,3-*b*]pyran-6,11-dione (14).** To a solution of **10** (40 mg, 0.11 mmol) in 7 mL of *t*-BuOH:THF:

$H_2O$ (10:3:1) was added *N*-methyl-morpholine-*N*-oxide (28 mg) and $OsO_4$ (2.5% in *t*-BuOH) (0.2 mL). The reaction mixture was stirred for 48 h at room temperature. Then $NaHSO_3$ (satd) was added and the mixture was stirred for 1 h. Then the reaction mixture was extracted with $CH_2Cl_2/H_2O$ and the organic layer was collected. The solvent was removed under reduced pressure and compound **14** was purified by flash chromatography on Silica gel with cyclohexane:EtOAc (60:40) (29 mg, 69%).$^1$H NMR (200 MHz, $CDCl_3$) δ 1.42 (3H, s, Me), 1.53 (3H, s, Me), 3.20 (1H, d, $J=5$ Hz, OH-3), 3.86 (1H, t, $J=5$ Hz, H-3), 3.96 (3H, s, 5-OMe), 4.02 (3H, s, 12-OMe), 4.21 (1H, d, $J=2$ Hz, OH-4), 5.08 (1H, dd, $J=5$, 2 Hz, H-4), 7.70 (2H, m, H-8, 9), 8.15 (2H, m, H-7,10); $^{13}$C NMR (50 MHz, $CDCl_3$) δ 22.45 (Me), 24.44 (Me), 61.12 (12-OMe), 62.59 (5-OMe, C-4), 70.31 (C-3), 79.43 (C-2), 118.09 (C-11a), 123.09 (C-4a), 126.55 (C-7, 10), 128.09 (C-5a), 133.46 (C-9), 133.61 (C-8), 134.12 (C-6a, 10a), 146.47 (C-12), 153.60 (C-12a), 157.50 (C-5), 181.39 (C-6), 183.01 (C-11). MS-DCI *m*/*z* 385 $(M+H)^+$.

**(±)-3,4-Diacetoxy-3,4-dihydro-5,12-dimethoxy-2,2-dimethyl-2*H*-anthra[2,3-*b*]pyran-6,11-dione (15).** To a solution of **14** (20 mg, 0.05 mmol) in dry pyridine (1.5 mL) was added $Ac_2O$ (1.5 mL, 15 mmol). The reaction mixture was stirred for 24 h at room temperature and then the reagents were removed under reduced pressure (using a high vacuum pump). The residue was compound **15** (24 mg, 96%). $^1$H NMR (200 MHz, $CDCl_3$) δ 1.49 (6H, s, 2×Me), 1.53 (3H, s, Me), 2.09 (6H, s, 2×$COCH_3$), 3.88 (3H, s, 5-OMe), 3.95 (3H, s, 12-OMe), 5.22 (1H, d, $J=5$ Hz, H-3), 6.39 (1H, d, $J=5$ Hz, H-4), 7.70 (2H, m, H-8, 9), 8.15 (2H, m, H-7, 10); $^{13}$C NMR (50 MHz, $CDCl_3$) δ 20.62 ($CH_3CO$), 20.71 ($CH_3CO$), 21.54 (Me), 25.98 (Me), 60.64 (C-4), 61.33 (12-OMe), 62.64 (5-OMe), 70.82 (C-3), 77.63 (C-2), 118.55 (C-11a), 120.13 (C-4a), 126.44 (C-7), 126.64 (C-10), 128.95 (C-5a), 133.36 (C-9), 133.68 (C-8), 134.12 (C-6a, 10a), 146.10 (C-12), 154.02 (C-12a), 157.91 (C-5), 169.76 (2×$CH_3CO$), 181.14 (C-6), 183.14 (C-11). MS-DCI *m*/*z* 469 $(M+H)^+$.

**2,2-Dimethyl-5,12-ditosyloxy-2*H*-anthra[2,3-*b*]pyran-6,11-dione (16), 2,2-dimethyl-5-hydroxy-12-tosyloxy-2*H*-anthra[2,3-*b*]pyran-6,11-dione (17), 2,2-dimethyl-12-hydroxy-5-tosyloxy-2*H*-anthra[2,3-*b*]pyran-6,11-dione (18).** To a solution of **8** (100 mg, 0.31 mmol) in dry acetone (12 mL) was added tosyl chloride (215 mg, 1.12 mmol) and dry $K_2CO_3$ (1 g). The reaction mixture was stirred for 6 h at 56 °C. Then the reagents were removed by filtration and by evaporation under reduced pressure. The residue was purified by flash chromatography on Silica gel with cyclohexane:EtOAc (95:5) to (80:20) to give compound **16** (90 mg, 47%), **17** (15 mg, 10%) and **18** (6 mg, 4%).

**16.** $^1$H NMR (200 MHz, $CDCl_3$) δ 1.25 (6H, s, 2×Me), 2.40 (3H, s, 4′-Me), 2.43 (3H, s, 4″-Me), 5.65 (1H, d, $J=10$ Hz, H-3), 6.43 (1H, d, $J=10$ Hz, H-4), 7.30 (2H, d, $J=8.5$ Hz, H- 3′, 5′), 7.32 (2H, d, $J=8.5$ Hz, H- 3″, 5″), 7.69 (2H, m, H-8, 9), 7.82 (2H, d, $J=8.5$ Hz, H- 2′, 6′), 7.84 (2H, d, $J=8.5$ Hz, H- 2″, 6″), 7.98 (2H, m, H-7, 10); $^{13}$C NMR (50 MHz, $CDCl_3$) δ 21.51 (4″-Me, 4′-Me), 27.75 (2×Me), 79.14 (C-2), 115.93 (C-4, 11a), 121.55 (C-5a), 123.06 (C-4a), 126.51 (C-7), 126.58 (C-10), 128.38 (C-2″, 6″), 128.86 (C-2′, 6′), 129.52 (C-3″, 5″), 129.52 (C-3′, 5′), 132.43 (C-1′, 1″), 133.35 (C-8), 133.53 (C-9), 134.19 (C-3), 134.60 (C-6a, 10a), 141.58 (C-5, 12), 144.85 (C-4′), 145.69 (C-4″), 152.05 (C-12a), 180.24 (C-6), 180.72 (C-11). MS-DCI *m*/*z* 631 $(M+H)^+$.

**17.** $^1$H NMR (200 MHz, $CDCl_3$) δ 1.23 (6H, s, 2×Me), 2.41 (3H, s, 4′-Me), 5.66 (1H, d, $J=10$ Hz, H-3), 6.71 (1H, d, $J=10$ Hz, H-4), 7.29 (2H, d, $J=8.5$ Hz, H-3′, 5′), 7.73 (2H, m, H-8, 9), 7.83 (2H, d, $J=8.5$ Hz, H-2′, 6′), 8.09 (1H, m, H-7), 8.21 (1H, m, H-10), 13.67 (1H, s, 5-OH). MS-DCI *m*/*z* 477 $(M+H)^+$.

**18.** $^1$H NMR (200 MHz, $CDCl_3$) δ 1.54 (6H, s, 2×Me), 2.40 (3H, s, 4′-Me), 5.67 (1H, d, $J=10$ Hz, H-3), 6.36 (1H, d, $J=10$ Hz, H-4), 7.28 (2H, d, $J=8.5$ Hz, H-3′, 5′), 7.73 (2H, m, H-8, 9), 7.81 (2H, d, $J=8.5$ Hz, H-2′, 6′), 8.08 (1H, m, H-7), 8.22 (1H, m, H-10), 13.35 (1H, s, 5-OH); $^{13}$C NMR (50 MHz, $CDCl_3$) δ 21.69 (4′-Me), 28.07 (2×Me), 78.46 (C-2), 115.45 (C-11a), 116.39 (C-4), 118.78 (C-5a), 122.95 (C-4a), 126.42 (C-7), 127.34 (C-10), 128.89 (C-2′, 6′), 129.74 (C-3′, 5′), 132.14 (C-10a), 132.82 (C-1′), 133.48 (C-8), 133.67 (C-9), 134.60 (C-10a), 134.74 (C-3), 137.11 (C-5), 145.48 (C-4′), 147.40 (C-12a), 151.31 (C-12), 179.86 (C-11), 188.55 (C-6). MS-DCI *m*/*z* 477 $(M+H)^+$.

**2,2-dimethyl-5-[*N*-[2-(dimethylamino)ethyl]amino]-12-tosyloxy-2*H*-anthra[2,3-*b*]pyran-6,11-dione (19), 2,2-dimethyl-12-[*N*-[2-(dimethylamino)ethyl]amino]-5-tosyloxy-2*H*-anthra[2,3-*b*]pyran-6,11-dione (20), 2,2-dimethyl-5,12-bis[*N*-[2-(dimethylamino)ethyl] amino]-2*H*-anthra[2,3-*b*]pyran-6,11-dione (21).** To a solution of **16** (100 mg, 0.16 mmol) in dry pyridine (4 mL) was added *N,N*-dimethylethylenediamine (1 mL). The reaction mixture was stirred for 2 h at 60 °C. Then the reagents were removed under reduced pressure (using a high vacuum pump). The residue was purified by flash chromatography on Silica gel with $CH_2Cl_2$:MeOH (99:1) to (80:20) to give compound **19** (43 mg, 49%), **20** (13 mg, 15%) and **21** (26 mg, 36%).

**19.** $^1$H NMR (200 MHz, $CDCl_3$) δ 1.37 (6H, s, 2×Me), 2.29 (6H, s, 2′-N$(Me)_2$), 2.37 (3H, s, 4″-Me), 2.50 (2H, t, $J=6.5$ Hz, H-2′), 3.46 (2H, q, $J=6.5$ Hz, H-1′), 5.55 (1H, d, $J=10$ Hz, H-3), 6.53 (1H, d, $J=10$ Hz, H-4), 7.23 (2H, d, $J=8.5$ Hz, H- 3″, 5″), 7.62 (2H, m, H-8, 9), 7.79 (2H, d, $J=8.5$ Hz, H- 2″, 6″), 7.88 (1H, dd, $J=7.5$, 1.5 Hz, H-7), 8.17 (1H, dd, $J=7.5$, 1.5 Hz, H-10), 10.58 (1H, t, $J=6.5$ Hz, N-H); $^{13}$C NMR (50 MHz, $CDCl_3$) δ 21.46 (4″-Me), 26.36 (2×Me), 45.73 (2′-N$(Me)_2$), 47.60 (C-1′), 60.20 (C-2′), 77.24 (C-2), 110.42 (C-11a), 113.70 (C-5a), 114.35 (C-4a), 120.42 (C-4), 126.01 (C-7, 10), 127.66 (C-3), 128.28 (C-2″, 6″), 129.29 (C-3″, 5″), 132.76 (C-8), 133.67 (C-9), 134.18 (C-6a, 10a), 134.80 (C-1″), 135.40 (C-12), 144.46 (C-4″), 151.23 (C-5), 153.56 (C-12a), 182.50 (C-6), 183.44 (C-11). MS-DCI *m*/*z* 547 $(M+H)^+$.

**20.** $^1$H NMR (200 MHz, $CDCl_3$) δ 1.50 (6H, s, 2×Me), 2.33 (9H, s, 2′-N$(Me)_2$, 4″-Me), 2.62 (2H, t, $J=6.5$ Hz, H-2′), 3.88 (2H, q, $J=6.5$ Hz, H-1′), 5.65 (1H, d, $J=10$ Hz, H-3), 6.50 (1H, d, $J=10$ Hz, H-4), 7.22 (2H,

d, $J=8.5$ Hz, H- 3″, 5″), 7.61 (1H, td, $J=8$, 1.5 Hz, H-8), 7.66 (1H, td, $J=8$, 1.5 Hz, H-9), 7.78 (2H, d, $J=8.5$ Hz, H- 2″, 6″), 7.87 (1H, dd, $J=8$, 1.5 Hz, H-7), 8.19 (1H, dd, $J=8$, 1.5 Hz, H-10), 10.27 (1H, t, $J=6.5$ Hz, N-H); $^{13}$C NMR (50 MHz, $CDCl_3$) δ 21.66 (4″-Me), 27.65 (2×Me), 44.63 (C-1′), 45.69 (2′-N(Me)$_2$), 60.21 (C-2′), 77.05 (C-2), 110.52 (C-11a), 113.50 (C-5a), 117.39 (C-4),121.21 (C-4a), 126.25 (C-7), 126.39 (C-10), 128.97 (C-2″, 6″), 129.59 (C-3″, 5″), 131.94 (C-3), 132.57 (C-8), 132.79 (C-1″), 133.08 (C-9), 133.85 (C-6a), 134.26 (C-10a), 135.21 (C-5), 143.37 (C-12), 145.14 (C-4″), 146.79 (C-12a), 181.89 (C-6), 184.16 (C-11). MS-DCI $m/z$ 547 $(M+H)^+$.

**21.** $^1$H NMR (200 MHz, $CDCl_3$) δ 1.57 (6H, s, 2×Me), 2.35 (6H, s, 2″-N(Me)$_2$), 2.43 (6H, s, 2′-N(Me)$_2$), 2.60 (2H, t, $J=6.8$ Hz, H-2″), 2.76 (2H, t, $J=6.5$ Hz, H-2′), 2.49 (2H, t, $J=6.8$ Hz, H-1′), 3.93 (2H, t, $J=6.5$ Hz, H-1″), 5.68 (1H, d, $J=10$ Hz, H-3), 6.58 (1H, d, $J=10$ Hz, H-4), 7.67 (2H, m, H-8, 9), 8.32 (2H, m, H-7,10); $^{13}$C NMR (50 MHz, $CDCl_3$) δ 26.84 (2×Me), 42.45 (C-1″), 44.33 (2″-N(Me)$_2$), 44.70 (2′-N(Me)$_2$), 45.61 (C-1′), 59.03 (C-2′), 59.43 (C-2″), 77.10 (C-2), 109.32 (C-11a), 112.70 (C-5a), 117.02 (C-4a), 120.30 (C-4), 125.84 (C-7, 10), 126.32 (C-3), 128.78 (C-8, 9), 132.75 (C-6a), 132.82 (C-10a), 141.86 (C-12), 147.63 (C-5), 149.50 (C-12a), 182.91 (C-6), 186.68 (C-11). MS-DCI $m/z$ 447 $(M+H)^+$.

**2,2-dimethyl-5-[*N*-[2-(dimethylamino)ethyl]amino]-12-[*N*-[2-[(2-hydroxyethyl)amino] ethyl]amino]-2*H*-anthra[2,3-*b*]pyran-6,11-dione (22).** To a solution of **19** (18 mg, 0.03 mmol) in dry pyridine (3 mL) was added 2-[(2-aminoethyl)amino]ethanol (0.3 mL). The reaction mixture was stirred for 24 h at 60 °C. Then the reagents were removed under reduced pressure (using a high vacuum pump). The residue was purified by flash chromatography on Silica gel with $CH_2Cl_2$:MeOH (99:1) to (80:20) to give compound **22** (10 mg, 69%).$^1$H NMR (200 MHz, $CDCl_3$) δ 1.55 (6H, s, 2×Me), 2.28 (6H, s, 2′-N(Me)$_2$), 2.53 (2H, t, $J=5.5$ Hz, H-2′), 2.88 (2H, t, $J=4$ Hz, H-3″), 2.95 (2H, t, $J=5.5$ Hz, H-2″), 3.44 (2H, t, $J=5.5$ Hz, H-1′), 3.67 (2H, t, $J=4$ Hz, H-10a), 3.88 (2H, t, $J=5.5$ Hz, H-1″), 5.64 (1H, d, $J=10$ Hz, H-3), 6.57 (1H, d, $J=10$ Hz, H-4), 7.64 (2H, m, H-8, 9), 8.29 (2H, m, H-7,10); $^{13}$C NMR (50 MHz, $CDCl_3$) δ 26.78 (2×Me), 45.75 (2′-N(Me)$_2$), 46.66 (C-1″), 47.46 (C-1′), 49.30 (C-2″), 50.76 (C-3″), 60.30 (C-2′), 60.95 (C-4″), 77.10 (C-2), 109.32 (C-11a), 112.70 (C-5a), 116.27 (C-4a), 121.29 (C-4), 126.14 (C-7), 126.29 (C-10), 127.29 (C-3), 132.04 (C-8), 132.23 (C-9), 134.36 (C-6a), 134.70(C-10a), 141.13 (C-12), 148.92 (C-5), 150.43 (C-12a), 181.77 (C-6), 182.54 (C-11). MS-DCI $m/z$ 479 $(M+H)^+$.

## Cell culture and cytotoxicity

L-1210 cells were cultivated in RPMI 1640 medium (Gibco) supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 units/mL penicillin, 100 μg/mL streptomycin, and 10 mM HEPES buffer (pH 7.4). Cytotoxicity was measured by the microculture tetrazolium assay.[12] Cells were exposed to graded concentrations of tested compound (nine serial dilutions in triplicate) for 48 h. Results are expressed as $IC_{50}$, the concentration needed to reduce by 50% the optical density of treated cells with respect to the optical density of untreated controls.

For the cell-cycle analysis L-1210 cells ($5\times10^5$ cells/mL) were incubated for 21 h with various concentrations of tested compounds. Cells were then fixed by 70% EtOH (v/v), washed, and incubated with PBS containing 100 μg/mL RNAse and 25 μg/mL propidium iodide for 30 min at 20 °C. For each sample, 10,000 cells were analyzed on an ATC3000 flow cytometer (Bruker, Wissenbourg, France).

## 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 (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.

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