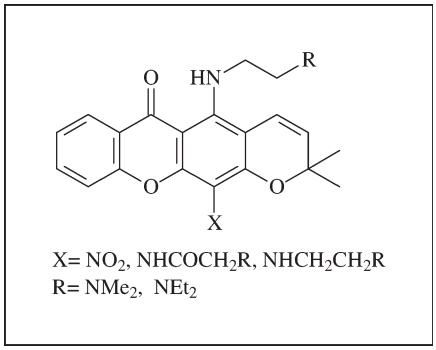
Design, Synthesis, and Cytotoxic Activity Evaluation of New Linear Pyranoxanthone Aminoderivatives

George Kolokythas,[a] Konstantinos Daniilides,[a] Nicole Pouli,[a] Panagiotis Marakos,[a]* Harris Pratsinis,[b] and Dimitris Kletsas[b]

[a]Division of Pharmaceutical Chemistry, Department of Pharmacy, University of Athens, Panepistimiopolis-Zografou, Athens 15771, Greece
[b]Laboratory of Cell Proliferation and Ageing, Institute of Biology, NCSR "Demokritos," 15310 Athens, Greece
*E-mail: marakos@pharm.uoa.gr

Received June 17, 2010
DOI 10.1002/jhet.670
Published online 21 April 2011 in Wiley Online Library (wileyonlinelibrary.com).



With the aim of enlightening some structure-activity correlation within the pyranoxanthenone series, we have designed and synthesized a number of new 5-aminosubstituted pyrano[3,2-*b*]xanthen-6-ones bearing various 12-substituents. *In vitro* cytotoxic potencies of the new derivatives toward the murine leukemia L1210 cell line, human colorectal adenocarcinoma (HT-29), and human uterine sarcoma (MES-SA and its 100-fold resistant to doxorubicin variant MES-SA/Dx5) cell lines, are described and compared with that of reference drugs. Among the studied compounds, those possessing a second aminosubstituted side-chain exhibit interesting cytotoxic activity against the solid tumor cell lines, and they retain activity against the multidrug resistant MES-SA/Dx5 subline. Their selective effect on a phase of the cell cycle was evaluated using HT-29 cells providing evidence that the compounds induce a G0/G1 arrest.

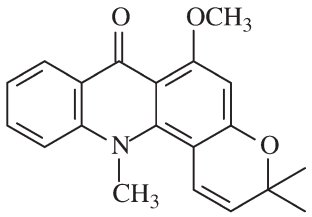
J. Heterocyclic Chem., **48**, 927 (2011).

INTRODUCTION

Compounds based on tricyclic planar chromophore framework represent a large and well-known family of multitargeted anticancer agents. A great number of natural and synthetic molecules, mainly anthraquinone, anthrapyrazole, and acridine derivatives have been tested for cytotoxicity and a number of them underwent clinical trials or have been approved for chemotherapy. Well-known examples include the clinically useful anticancer drug mitoxantrone, [1] 9-azaanthrapyrazoles, [2] imidazoacridones, [3] acridine-4-carboxamide derivatives [4], and pyrazoloacridine [5]. The mechanism of action of this diverse class of compounds is not fully understood, however, it is generally acknowledged to involve DNA intercalation and poisoning of critical enzymes, such as topoisomerases and telomerases. In addition, some of these molecules often display secondary effects on other biochemical pathways, including protein metabolism [6] and cell cycle regulation [7]. Recent evidence concerning the interaction with biological targets allows for the design of novel polycyclic compounds, endowed with better antiproliferative activity and/or the ability to overcome multidrug resistance. Within this concept, the position and the nature of the substituents on the heterocyclic nucleus are determinants of the biological activity observed [8,9]. Of particular importance is the presence of one or two flexible dialkylaminoalkylamino-substituents, which usually increase DNA binding affinity and solubility under physiological conditions, yielding in an improvement of the pharmacological response of several acridone derivatives [10].

We have been involved in the design, synthesis, and cytotoxic activity evaluation of a number of linear and angular pyranoxanthenone derivatives [11,12] possessing structural similarity with the pyranoacridone alkaloid acronycine (Fig. 1), which has shown promising antitumor properties on several murine solid tumor models [13].

In the course of the exploration of the structure-activity relationship studies in the pyranoxanthenone series,



acronycine

Figure 1. Structure of acronycine.

we have found that the replacement of the methoxy group of the lead compound by a flexible dialkylaminoethylamino side chain substitution results in a clear improvement of the antiproliferative activity towards both leukemia and solid tumor cell lines in the angular as well as in the linear pyranoxanthenones [14–17]. A similar approach, that is, the replacement of the methoxy group at C-6 by a basic dialkylaminoalkylamino side chain has also been performed in the acronycine series, and resulted in a significant increase of the cytotoxic activity against L1210 cells when compared with the parent compounds [18]. Furthermore, we have previously introduced a nitro group or a second basic side chain on the pyran ring of angular pyranoxanthenones and the biological evaluation of these derivatives provided evidence that extended conjugation of the xanthenone chromophore is in favor of the cell growth inhibitory activity [19]. As a continuation of this study, we decided to synthesize a number of structurally related linear analogues, possessing a dialkylaminoethylamino-side chain substitution and in position para- to this group a nitro- or an additional basic side chain, to study the impact of this structural modification on the biological activity of this class of compounds.

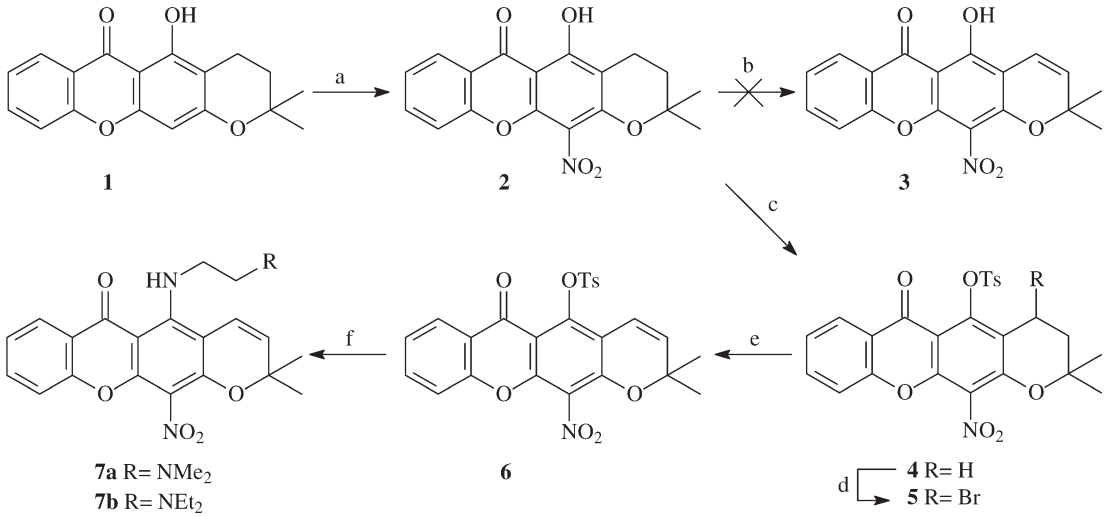
RESULTS AND DISCUSSION

Chemistry. For the preparation of the target derivatives, we used as starting material the dihydropyranoxanthenone **1** (Scheme 1), which was prepared according to known procedures, from 1,3-dihydroxyxanthenone upon reaction with 2-methyl-1,3-butadiene (isoprene), in the presence of orthophosphoric acid [20]. Compound **1** was then nitrated in high yield by treatment with fuming nitric acid in acetic acid, to provide the nitroderivative **2**.

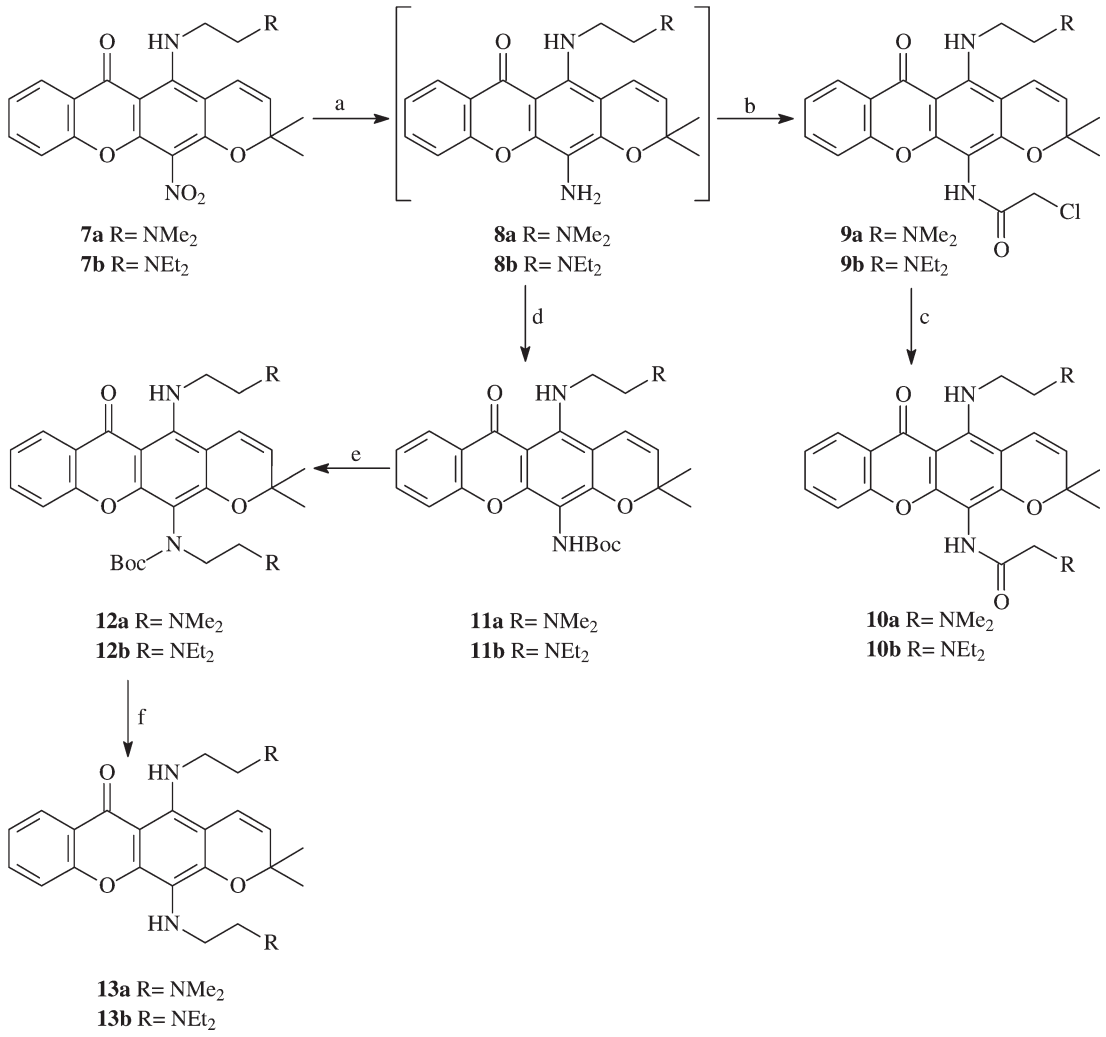
Our attempts to prepare the pyranoxanthenone **3** through treatment of compound **2** with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone in boiling toluene [21,22] or with 10% Pd/C in boiling diphenylether [23] were not successful. Consequently, **2** was converted to the tosylate **4**, which was treated with *N*-bromosuccinimide in the presence of 2,2′-diazodiisobutylnitrile (AIBN) to provide the bromide **5**, that upon dehydrohalogenation was converted into the tosylate **6**. The tosyloxy group of compound **6** was easily displaced by the suitable amines to result into the diamines **7a,b**.

Reduction of the nitroderivatives **7a,b** provided the amines **8a,b** (Scheme 2), which were not isolated due to instability but were converted to the chloracetamides **9a,b** upon treatment with chloracetylchloride. Subsequent reaction of the chloracetamides **9a,b** with an excess of the suitable secondary amine provided the target amides **10a,b**.

Scheme 1. Reagents and conditions: (a) Fuming nitric acid, glacial acetic acid, 60°C, 35 min; (b) 2,3-dichloro-5,6-dicyano-*p*-benzoquinone, toluene, reflux, 30 h, or 10% Pd/C, diphenylether, reflux, 24 h; (c) *p*-toluenesulfonyl chloride, potassium carbonate, acetone, reflux, 90 min; (d) *N*-bromosuccinimide, 2,2′-diazodiisobutylnitrile, carbon tetrachloride, reflux, 18 h; (e) triethylamine, 1,2-dichloroethane, 50°C, 17 h; (f) *N*,*N*-dialkylethylenediamine, abs. ethanol, reflux, 1 h.



Scheme 2. Reagents and conditions: (a) stannous chloride dihydrate, acetone, reflux, 5 h; (b) chloroacetylchloride, triethylamine, tetrahydrofuran, room temperature, 3h; (c) dialkylamine, abs. ethanol, reflux, 18 h; (d) di-*tert*-butyl dicarbonate, 4-dimethylaminopyridine, tetrahydrofuran, room temperature, 3 h; (e) sodium hydride, 2-dialkylaminoethylchloride, dimethylformamide, room temperature, 18 h; (f) trifluoroacetic acid, dichloromethane, room temperature, 1 h.



On the other hand, the free amino group of compounds **8a,b** was protected through the formation of the corresponding *t*-butyl carbamates **11a,b**. The suitable dialkylaminoethyl substitution was then introduced to the sodium hydride mediated anions of the above mentioned carbamates and the resulting derivatives **12a,b** were treated with trifluoroacetic acid to result into the target compounds **13a,b**.

For biological evaluation purposes, the free base forms of the amines **7a,b** were converted into their water-soluble hydrochloride addition salts by treatment with hydrochloric acid in methanol. Compounds **10a,b** and **13a** were tested at the free base form, since their hydrochloride, fumarate, or malonate addition salts were highly hygroscopic, whereas compound **13b** was converted into the corresponding stable difumarate addition salt.

Biological evaluation. The *in vitro* cytotoxic activity of the new compounds was evaluated in the established model of the murine leukemia cell line L1210, and in three human solid tumor cell lines: colorectal adenocarcinoma HT-29, uterine sarcoma MES-SA as well as its variant MES-SA/Dx5, reported to be 100-fold resistant to doxorubicin [24]. The results, including reference compounds mitoxantrone and doxorubicin, are presented in Table 1.

As a general remark, we assume that the 12-nitrosubstituted compounds **7a,b** proved to be less active than the corresponding derivatives **10a,b** and **13a,b**, which contain a second basic side chain at position 12. The nature of this side chain, that is, dialkylaminoacetamido-or dialkylaminoethylamino-, is not critical for the activity.

Concerning the activity against L1210 cell line compounds **10a,b** and **13a,b** possess IC_{50}'s in the range of

Table 1

Inhibition of proliferation of the amino substituted xanthenone derivatives (IC_{50} values in μM[a]).

Compound	L1210	HT-29	MES-SA	MES-SA/Dx5	RF[e]
7a[b]	37.2 (±5.7)	43.1 (±7.5)	30.2 (±2.6)	6.47 (±2.60)	0.2
7b[b]	>100	8.04 (±1.86)	94.8 (±1.7)	40.7 (±10.5)	0.4
10a[c]	17.1 (±3.2)	9.20 (±1.13)	17.0 (±5.33)	9.13 (±1.64)	0.5
10b[c]	8.83 (±2.21)	5.72 (±0.81)	12.2 (±4.71)	4.71 (±0.58)	0.4
13a[c]	10.4 (±1.8)	8.2 (±1.39)	11.0 (±0.9)	2.75 (±0.69)	0.4
13b[d]	11.0 (±1.2)	4.09 (±0.75)	11.3 (±1.6)	3.32 (±0.16)	0.3
Mitoxantrone	0.038 (±0.017)	0.025 (±0.008)	0.003 (±0.001)	0.028 (±0.002)	9.3
Doxorubicin	0.118 (±0.039)	0.153 (±0.076)	0.0097 (±0.0012)	0.704 (±0.337)	72.6

[a] The results represent the mean (± standard deviation) of three independent experiments and are expressed as IC_{50}. the concentration that reduced by 50% the optical density of treated cells with respect to untreated controls.
[b] Hydrochloride.
[c] Free base form.
[d] Difumarate.
[e] IC_{50} resistant cells/IC_{50} sensitive cells.

8.83–17.1 μM. It should be noticed that previously reported analogues, which lack a 12-substitution showed more interesting antiproliferative activity, with IC_{50} values close to 5 μM [14]. However, compounds **10a,b** and **13a,b** possess interesting antiproliferative activity against the colorectal adenocarcinoma HT-29 cell line. In this case, the insertion of a second basic side chain results in IC_{50}'s within the range of 4.09–9.2 μM and this provides an improvement over the antiproliferative activity reported for the corresponding analogues, which possess only a 5-(2-dialkylaminoethylamino)-side chain and show IC_{50}'s within the range of 8–23 μM [15]. Within the HT-29 cell line it is evident that the presence of diethylamino-substitution results in an increased growth inhibitory activity (IC_{50}'s 5.72 μM and 4.09 μM, for compounds **10b** and **13b**, respectively) when compared to their dimethylamino-counterparts (IC_{50}'s 9.2 μM and 8.2 μM, for compounds **10a** and **13a**, respectively) and this is in accordance with previous observations in the xanthenone series [15,16].

From a direct comparison of the activity toward the sensitive and resistant cell lines, it is evident that the compounds appear to be active against MES-SA cell line and simultaneously possess higher cytotoxicity against the doxorubicin resistant MES-SA/Dox-5 cell line. This finding is more pronounced for compounds **13**, especially **13b**. Worth mentioning, the RF to doxorubicin was found to be 72.6, that is, on the order of the expected value [24], whereas the RF to mitoxantrone was 9.3.

The incorporation of the pyran ring into the xanthenone skeleton, does not improve the antiproliferative activity of the compounds. Indeed, the corresponding 1-dialkylaminoethylamino-substituted 4-nitro- or 4-dialkylaminoacetamido-xanthenones, which have been previously reported [25] possess lower IC_{50} values against all three tested cell lines. However, the new compounds appear more active against the doxorubicine resistant phenotype (RF values 0.2–0.5) when compared with the above mentioned xanthenones.

Cell-cycle perturbations induced after incubation of exponentially growing colon adenocarcinoma HT-29 cells with the new compounds for 24 h are presented in Table 2. All studied compounds provoke an increase in the percentage of cells arrested at the G0/G1 phase of the cell cycle, in agreement with our previous observations for the mode of action of other pyranoxanthenone or azapyranoxanthenone aminoderivatives on HT-29 cells [15,16].

In summary, this study deals with the synthesis of a number of new 5,12-disubstituted pyrano[3,2-*b*]xanthen-6-ones. The evaluation of their antiproliferative activity against a panel of cell lines, showed that the 5,12-diaminosubstituted derivatives possess enhanced activity against the solid tumor cells and interestingly, they retain full cytotoxicity against the doxorubicin resistant cell line.

EXPERIMENTAL

All chemicals were purchased from Aldrich Chemical Co. (Munich, Germany). Melting points were determined on a Büchi apparatus and are uncorrected. ^{1}H-NMR spectra and 2D spectra were recorded on a Bruker Avance 400 instrument, whereas ^{13}C-NMR specra were recorded on a Bruker AC 200 spectrometer in deuterated solvents and were referenced to TMS (δ scale). The signals of ^{1}H and ^{13}C spectra were unambiguously assigned by using 2D NMR techniques: ^{1}H-^{1}H COSY, NOESY HMQC, and HMBC. Flash chromatography was performed on Merck (Darmstadt, Germany) 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.

Table 2

FACS analysis.

Compound	G0/G1	S	G2/M
7a	62.98	28.25	8.77
7b	61.85	28.18	9.96
10a	54.72	30.24	15.04
10b	68.87	21.21	9.92
13a	59.18	30.12	10.70
13b	59.35	40.65	0.00
ctrl	40.49	38.99	20.51

HT-29 cells were treated for 24 h with 20 μ*M* (except from **7a**. where 40 μ*M* were used) and then subjected to DNA content analysis. One representative determination out of three similar ones is demonstrated.

3,4-Dihydro-5-hydroxy-2,2-dimethyl-12-nitro-2*H*,6*H*-pyrano-[3,2-*b*]xanthen-6-one (2). Fuming nitric acid (64 μL, 1.55 mmol) was added dropwise to a solution of the pyranoxanthenone **1** (460 mg, 1.55 mmol) in glacial acetic acid (10 mL) and the resulting solution was stirred at 60°C for 35 min. The mixture was then poured into crushed ice (50 mL) and the precipitate was filtered, washed with water (3 × 15 mL) and dried over calcium chloride to give pure compound **2** (470 mg, 89%). mp: 162–164°C, (ethyl acetate-*n*-pentane). ^{1}H-NMR (400 MHz, deuteriochloroform) δ: 1.40 (s, 6H, 2 × gem CH_3), 1.90 (t, 2H, CH_2-3, J = 7 Hz), 2.72 (t, 2H, CH_2-4, J = 7 Hz), 7.35 (dt, 1H, H-8, J = 8 Hz, 2 Hz), 7.37 (dd, 1H, H-10, J = 8 Hz, 2 Hz), 7.70 (dt, 1H, H-9, J = 8 Hz, 2 Hz), 8.16 (dd,1H, H-7, J = 8 Hz, 2 Hz), 13.36 (s, 1H, D_2O exch., OH). ^{13}C-NMR (50 MHz, deuteriochloroform) δ: 15.81 (C-4), 26.54 (2 × gem CH_3), 31.09 (C-3), 78.98 (C-2), 101.15 (C-5a), 104.45 (C-4a), 117.91 (C-10), 120.11 (C-6a), 124.89 (C-8), 125.00 (C-12), 125.77 (C-7), 135.66 (C-9), 147.46 (C-11a), 153.89 (C-12a), 155.25 (C-10a), 161.39 (C-5), 180.35 (C-6). Anal. Calcd. for $C_{18}H_{15}NO_6$: C, 63.34; H, 4.43; N, 4.10. Found: C, 63.11; H, 4.38; N, 4.15.

3,4-Dihydro-2,2-dimethyl-5-(4-methylbenzenesulfonyloxy)-12-nitro-2*H*,6*H*-pyrano[3,2-*b*]xanthen-6-one (4). To a solution of compound **2** (740 mg, 2.17 mmol) in acetone (30 mL) was added potassium carbonate (1.50 g, 10.85 mmol) and *p*-toluenesulfonylchloride (1.24 g, 6.51 mmol) and the mixture was heated at reflux for 90 min. Upon cooling at room temperature the precipitate was filtered off, washed with acetone (2 × 20 mL), the filtrate was vacuum-evaporated and the residue was purified by column chromatography (silica gel) using a mixture of cyclohexane/ethyl acetate (85/15 v/v) as the eluent, to give compound **4** (910 mg, 85%) as a white solid. mp: 242–244°C, (ethyl acetate-*n*-pentane). ^{1}H-NMR (400 MHz, deuteriochloroform) δ: 1.38 (s, 6H, 2 × gem CH_3), 1.82 (t, 2H, CH_2-3, J = 7 Hz), 2.43 (s, 3H, $SO_2C_6H_4CH_3$), 2.82 (t, 2H, CH_2-4, J = 7 Hz), 7.34 (m, 4H, H-8, H-10, H-3′, H-5′), 7.65 (dt, 1H, H-9, J = 8 Hz, 2 Hz), 7.93 (d, 2H, H-2′, H-6′, J = 8 Hz), 8.06 (dd, 1H, H-7, J = 8 Hz, 2 Hz). ^{13}C-NMR (50 MHz, deuteriochloroform) δ: 18.01 (C-4), 21.76 ($SO_2C_6H_4CH_3$), 26.65 (2 × gem CH_3), 31.32 (C-3), 79.50 (C-2), 109.82 (C-5a), 116.14 (C-4a), 117.43 (C-10), 122.13 (C-6a), 124.78 (C-8), 126.69 (C-7), 128.93 (C-2′, C-6′), 129.41 (C-12), 129.74 (C-3′, C-5′), 133.16 (C-1′), 134.81 (C-9), 145.73 (C-4′), 146.39 (C-12a), 147.57 (C-11a), 151.46 (C-5), 154.22 (C-10a), 172.78 (C-6). Anal. Calcd. for $C_{25}H_{21}NO_8S$: C, 60.60; H, 4.27; N, 2.83. Found: C, 60.47; H, 4.21; N, 2.92.

4-Bromo-3,4-dihydro-2,2-dimethyl-5-(4-methylbenzenesulfonyloxy)-12-nitro-2*H*,6*H*-pyrano[3,2-*b*]xanthen-6-one (5). To a solution of the tosylate **4** (850 mg, 1.72 mmol) in dry carbon tetrachloride (30 mL) was added *N*-bromosuccinimide (321 mg, 1.80 mmol) and a catalytic amount of AIBN, and the mixture was stirred at room temperature for 15 min, and then was heated at reflux for 18 h. The organic solvent was vacuum-evaporated and the residue was purified by column chromatography (silica gel) using a mixture of cyclohexane/ethyl acetate (95/5, v/v) as the eluent, to result in compound **5** (730 mg, 74%). mp: 180–182°C, (ethyl acetate-*n*-pentane). ^{1}H-NMR (400 MHz, deuteriochloroform) δ: 1.46 (s, 3H, 1 × gem CH_3), 1.65 (s, 3H, 1 × gem CH_3), 2.49 (s, 3H, $SO_2C_6H_4CH_3$), 2.56 (d, 2H, CH_2-3, J = 5 Hz), 5.77 (t, 1H, H-4, J = 5 Hz), 7.38 (m, 2H, H-8, H-10), 7.44 (d, 2H, H-3′, H-5′, J = 8 Hz), 7.70 (dt, 1H, H-9, J = 8 Hz, 2 Hz), 8.11 (d, 2H, H-2′, H-6′, J = 8 Hz), 8.18 (dd, 1H, H-7, J = 8 Hz, 2 Hz). ^{13}C-NMR (50 MHz, deuteriochloroform) δ: 21.64 ($SO_2C_6H_4CH_3$), 27.30 (gem CH_3), 28.19 (gem CH_3), 34.65 (C-4), 41.42 (C-3), 79.68 (C-2), 111.00 (C-5a), 116.29 (C-4a), 117.29 (C-10), 121.81 (C-6a), 125.00 (C-8), 126.70 (C-7), 128.33 (C-2′, C-6′), 129.31 (C-12), 129.71 (C-3′, C-5′), 133.21 (C-1′), 135.04 (C-9), 146.07 (C-4′), 146.99 (C-12a), 148.71 (C-11a), 150.55 (C-5), 153.90 (C-10a), 172.20 (C-6). Anal. Calcd. for $C_{25}H_{20}BrNO_8S$: C, 52.27; H, 3.51; N, 2.44. Found: C, 52.51; H, 3.58; N, 2.41.

2,2-Dimethyl-5-(4-methylbenzenesulfonyloxy)-12-nitro-2*H*, 6*H*-pyrano[3,2-*b*]xanthen-6-one (6). To a solution of compound **5** (420 mg, 0.73 mmol) in 1,2-dichloroethane (25 mL) was added triethylamine (3 mL) and the mixture was heated at 50°C for 17 h. The organic solvent was vacuum-evaporated and dichloromethane (50 mL) was added to the residue. The organic phase was washed with a 9% hydrochloric acid solution (2 × 30 mL), dried (sodium sulfate) and concentrated to dryness. The residue was purified by column chromatography using a mixture of cyclohexane/dichloromethane (1/1, v/v) as the eluent, to provide compound **6** (284 mg, 79%) as a white solid. mp: 193–195°C, (ethyl acetate-*n*-pentane). ^{1}H-NMR (400 MHz, deuteriochloroform) δ: 1.47 (s, 6H, 2 × gem CH_3), 2.42 (s, 3H, $SO_2C_6H_4CH_3$), 5.64 (d, 1H, H-3, J = 10 Hz), 6.36 (d, 1H, H-4, J = 10 Hz,), 7.34 (m, 4H, H-8, H-10, H-3′, H-5′), 7.66 (dt, 1H, H-9, J = 8 Hz, 2 Hz), 7.90 (d, 2H, H-2′, H-6′, J = 8 Hz,), 8.14 (dd, 1H, H-7, J = 8 Hz, 2 Hz). ^{13}C-NMR (50 MHz, deuteriochloroform) δ: 21.58 ($SO_2C_6H_4CH_3$), 27.93 (2 × gem CH_3), 80.56 (C-2), 110.56 (C-5a), 114.12 (C-4a), 115.04 (C-4), 117.24 (C-10), 121.88 (C-6a), 124.93 (C-8), 126.54 (C-7), 128.71 (C-2′, C-6′), 129.49 (C-12), 129.74 (C-3′, C-5′), 131.76 (C-3), 132.35 (C-1′), 134.78 (C-9), 142.90 (C-12a), 145.76 (C-4′), 148.45 (C-11a), 150.03 (C-5), 153.89 (C-10a), 172.48 (C-6). Anal. Calcd. for $C_{25}H_{19}NO_8S$: C, 52.37; H, 3.34; N, 2.44. Found: C, 52.23; H, 3.29; N, 2.47.

***N*,*N*-Dimethyl-*N*′-[2,2-dimethyl-12-nitro-6-oxo-2*H*,6*H*-pyrano[3,2-*b*]xanthen-5-yl]ethanediamine (7a).** 2-Dimethylaminoethylamine (560 μL, 5.10 mmol) was added to a solution of compound **6** (250 mg, 0.51 mmol) in absolute ethanol (30 mL) and the mixture was heated at reflux for 1 h. Ethanol was vacuum-evaporated and dichloromethane (50 mL) was added to the residue. The organic phase was washed with water (3 × 20 mL), dried (sodium sulfate) and concentrated to dryness. The residue was purified by column chromatography using a

mixture of dichloromethane/methanol (98/2, v/v) as the eluent, to give compound **7a** (150 mg, 72%) as a light yellow oil. mp (hydrochloride): 252–253°C, (ethanol). ^{1}H-NMR (400 MHz, deuteriochloroform) δ: 1.50 (s, 6H, 2 × gem CH_3), 2.33 [s, 6H, $N(CH_3)_2$], 2.59 (t, 2H, $NHCH_2CH_2NMe_2$, J = 7 Hz), 3.60 (q, 2H, $NHCH_2CH_2NMe_2$, J = 7 Hz, 5 Hz), 5.53 (d, 1H, H-3, J = 10 Hz), 6.52 (d, 1H, H-4, J=10 Hz), 7.35 (m, 2H, H-8, H-10), 7.63 (dt, 1H, H-9, J = 8 Hz, 2 Hz), 8.20 (dd, 1H, H-7, J = 8 Hz, 2 Hz), 10.30 (t, 1H, NH, J = 5 Hz, D_2O exch.). ^{13}C-NMR (50 MHz, deuteriochloroform) δ: 26.83 (2 × gem CH_3), 45.50 [$N(CH_3)_2$], 46.79 ($NHCH_2CH_2NMe_2$), 59.65 ($NHCH_2CH_2NMe_2$), 78.36 (C-2), 101.73 (C-4a), 102.21 (C-5a), 117.28 (C-10), 119.93 (C-4), 120.79 (C-12), 121.54 (C-6a), 124.59 (C-8), 124.71 (C-3), 126.06 (C-7), 134.41 (C-9), 150.80 (C-11a), 152.01 (C-5), 152.64 (C-12a), 154.03 (C-10a), 177.89 (C-6). Anal. Calcd. for $C_{22}H_{23}N_3O_5$ HCl: C, 59.26; H, 5.43; N, 9.42. Found: C, 59.01; H, 5.39; N, 9.68.

***N*,*N*-Diethyl-*N*′-[2,2-dimethyl-12-nitro-6-oxo-2*H*,6*H*-pyrano[3,2-*b*]xanthen-5-yl]ethanediamine (7b).** The synthetic route was analogous to the one used for the synthesis of compound **7a**. Yield: 74%. mp: (hydrochloride): 216–217°C, (ethanol). ^{1}H-NMR (400 MHz, deuteriochloroform) δ: 1.02 [t, 6H, $N(CH_2CH_3)_2$, J = 7 Hz], 1.49 (s, 6H, 2 × gem CH_3), 2.58 [q, 4H, $N(CH_2CH_3)_2$, J = 7 Hz], 2.66 (t, 2H, $NHCH_2CH_2NEt_2$, J = 7 Hz), 3.55 (q, 2H, $NHCH_2CH_2NEt_2$, J = 7 Hz, 5 Hz), 5.50 (d, 1H, H-3, J = 10 Hz), 6.60 (d, 1H, H-4, J = 10 Hz), 7.34 (m, 2H, H-10, H-8), 7.62 (dt, 1H, H-9, J = 8 Hz, 2 Hz), 8.18 (dd, 1H, H-7, J = 8 Hz, 2 Hz,), 10.25 (t, 1H, NH, J = 5 Hz, D_2O exch.). ^{13}C-NMR (50 MHz, deuteriochloroform) δ: 11.54 [$N(CH_2CH_3)_2$], 26.83 (2 × gem CH_3), 47.16 ($NHCH_2CH_2NEt_2$) and [$N(CH_2CH_3)_2$], 53.33 ($NHCH_2CH_2NEt_2$), 78.32 (C-2), 101.77 (C-4a), 102.29 (C-5a), 117.24 (C-10), 120.04 (C-4), 120.77 (C-12), 121.58 (C-6a), 124.56 (C-3, C-8), 125.99 (C-7), 134.33 (C-9), 150.80 (C-11a), 152.05 (C-5), 152.60 (C-12a), 154.03 (C-10a), 177.74 (C-6). Anal. Calcd. for $C_{24}H_{27}N_3O_5$ HCl H_2O: C, 58.59; H, 6.15; N, 8.54. Found: C, 58.42; H, 6.06; N, 8.38.

2-Chloro-*N*-[5-(2-dimethylaminoethylamino)-2,2-dimethyl-6-oxo-2*H*,6*H*-pyrano[3,2-*b*]xanthen-12-yl]acetamide (9a). To a solution of compound **7a** (130 mg, 0.32 mmol) in acetone was added stannous chloride dihydrate (289 mg, 1.28 mmol) and the mixture was heated at reflux for 5 h. The solvent was then vacuum-evaporated and a 20% sodium carbonate solution (50 mL) was added to the residue. The aqueous phase was washed with ethyl acetate (3 × 50 mL) and the organic extracts were dried (sodium sulfate) and concentrated to dryness, to provide the amine **8a** as oil, which was not particularly stable, but could be spectroscopically characterized. ^{1}H-NMR (400 MHz, deuteriochloroform) δ: 1.45 (s, 6H, 2 × gem CH_3), 2.23 [s, 6H, $N(CH_3)_2$], 2.49 (t, 2H, $NHCH_2CH_2NMe_2$, J = 7 Hz), 3.33 (q, 2H, $NHCH_2CH_2NMe_2$, J = 7 Hz, 5 Hz), 5.51 (d, 1H, H-3, J = 10 Hz), 6.57 (d, 1H, H-4, J = 10 Hz), 7.23 (dt, 1H, H-8, J = 8 Hz, 2 Hz), 7.31 (dd, 1H, H-10, J = 8 Hz, 2 Hz), 7.54 (dt, 1H, H-9, J = 8 Hz, 2 Hz), 8.18 (dd, 1H, H-7, J = 8 Hz, 2 Hz), 8.62 (t, 1H, $NHCH_2CH_2NMe_2$, J = 5 Hz, D_2O exch.). The amine without any further purification was dissolved in dry tetrahydrofuran (15 mL), triethylamine (108 μL, 0.78 mmol), and chloroacetylchloride (41 μL, 0.52 mmol) were added to the solution at 0°C, and the mixture was stirred at room temperature for 3 h. The organic solvent was vacuum-evaporated, a 10% sodium carbonate solution (50 mL) was added to the residue and was extracted with dichloromethane (3 × 50 mL). The combined organic extracts were dried (sodium sulfate) and concentrated to dryness and the residue was purified by column chromatography using a mixture of dichloromethane/methanol (95/5, v/v) as the eluent, to provide the chloride **9a** (93 mg, 64%) as a light yellow oil. ^{1}H-NMR (400 MHz, deuteriochloroform) δ: 1.44 (s, 6H, 2 × gem CH_3), 2.30 [s, 6H, $N(CH_3)_2$], 2.55 (t, 2H, $NHCH_2CH_2NMe_2$, J = 7 Hz), 3.50 (q, 2H, $NHCH_2CH_2NMe_2$, J = 7 Hz, 5 Hz), 4.27 (s, 2H, $NHCOCH_2Cl$), 5.45 (d, 1H, H-3, J = 10 Hz), 6.52 (d, 1H, H-4, J = 10 Hz), 7.27 (m, 2H, H-8, H-10), 7.55 (dt, 1H, H-9, J = 8 Hz, 2 Hz), 7.70 (s, 1H, $NHCOCH_2Cl$, D_2O exch.,), 8.15 (dd, 1H, H-7, J = 8 Hz, 2 Hz), 9.68 (t, 1H, $NHCH_2CH_2NMe_2$, J = 5 Hz, D_2O exch.,). ^{13}C-NMR (50 MHz, deuteriochloroform) δ: 27.22 (2 × gem CH_3), 44.78 [$N(CH_3)_2$], 45.95 ($NHCH_2CH_2NMe_2$), 48.35 ($NHCOCH_2Cl$), 58.22 ($NHCH_2CH_2NMe_2$), 77.24 (C-2), 103.23 (C-12), 105.03 (C-4a), 105.10 (C-5a), 116.32 (C-10), 119.03 (C-4), 121.65 (C-6a), 123.25 (C-8), 123.93 (C-3), 125.32 (C-7), 133.24 (C-9), 149.12 (C-5), 153.90 (C-11a), 154.92 (C-10a), 156.52 (C-12a), 163.42 ($NHCOCH_2Cl$), 179.11 (C-6). Anal. Calcd. for $C_{24}H_{26}ClN_3O_4$: C, 63.22; H, 5.75; N, 9.22. Found: C, 63.40; H, 5.71; N, 9.05.

2-Chloro-*N*-[5-(2-diethylaminoethylamino)-2,2-dimethyl-6-oxo-2*H*,6*H*-pyrano[3,2-*b*]xanthen-12-yl]acetamide (9b). The amine **8b** was first prepared according to the method reported for the synthesis of the amine **8a**. Analogously, this compound was used to the next step without further purification and was roughly spectroscopically characterized. ^{1}H-NMR (400 MHz, deuteriochloroform) δ: 0.99 [t, 6H, $N(CH_2CH_3)_2$, J = 7 Hz], 1.48 (s, 6H, 2 × gem CH_3), 2.53 [q, 4H, $N(CH_2CH_3)_2$, J = 7 Hz,], 2.63 (t, 2H, $NHCH_2CH_2NEt_2$, J = 7 Hz), 3.35 (q, 2H, $NHCH_2CH_2NEt_2$, J = 7 Hz, 5 Hz), 5.53 (d, 1H, H-3, J = 10 Hz), 6.63 (d, 1H, H-4, J = 10 Hz), 7.27 (dt, 1H, H-8, J = 8 Hz, 2 Hz), 7.36 (dd, 1H, H-10, J = 8 Hz, 2 Hz), 7.58 (dt, 1H, H-9, J = 8 Hz, 2 Hz), 8.21 (dd, 1H, H-7, J = 8 Hz, 2 Hz), 8.56 (t, 1H, $NHCH_2CH_2NEt_2$, J = 5 Hz, D_2O exch.). Compound **9b** was then prepared as a light yellow oil, with a method analogous to the one used for the preparation of **9a**. Yield: 67%. ^{1}H-NMR (400 MHz, deuteriochloroform) δ: 1.07 [t, 6H, $N(CH_2CH_3)_2$, J = 7 Hz], 1.45 (s, 6H, 2 × gem CH_3), 2.65 [q, 4H, $N(CH_2CH_3)_2$, J = 7 Hz], 2.73 (t, 2H, $NHCH_2CH_2NEt_2$, J = 7 Hz), 3.54 (q, 2H, $NHCH_2CH_2NEt_2$, J = 7 Hz, 5 Hz), 4.28 [s, 2H, $NHCOCH_2Cl$), 5.48 (d, 1H, H-3, J = 10 Hz), 6.54 (d, 1H, H-4, J = 10 Hz), 7.28 (m, 2H, H-10, H-8), 7.57 (dt, 1H, H-9, J = 8 Hz, 2 Hz), 7.71 (s, 1H, $NHCOCH_2Cl$, D_2O exch.), 8.16 (dd, 1H, H-7, J = 8 Hz, 2 Hz,), 9.62 (t, 1H, $NHCH_2CH_2NEt_2$, J = 5 Hz, D_2O exch.). ^{13}C-NMR (50 MHz, deuteriochloroform) δ: 10.34 [$N(CH_2CH_3)_2$], 27.10 (2 × gem CH_3), 41.93 ($NHCOCH_2Cl$), 46.39 [$N(CH_2CH_3)_2$], 46.49 ($NHCH_2CH_2NEt_2$), 52.52 ($NHCH_2CH_2NEt_2$), 77.89 (C-2), 102.36 (C-12), 104.21 (C-4a), 104.49 (C-5a), 116.17 (C-10), 119.74 (C-4), 121.82 (C-6a), 122.99 (C-8), 123.73 (C-3), 125.36 (C-7), 132.92 (C-9), 151.08 (C-5), 153.98 (C-11a), 154.95 (C-10a), 156.54 (C-12a), 165.57 ($NHCOCH_2Cl$), 178.75 (C-6). Anal. Calcd. for $C_{26}H_{30}ClN_3O_4$: C, 64.52; H, 6.25; N, 8.68. Found: C, 64.23; H, 6.14; N, 8.72.

2-Dimethylamino-*N*-[5-(2-dimethylaminoethylamino)-2,2-dimethyl-6-oxo-2*H*,6*H*-pyrano[3,2-*b*]xanthen-12-yl]acetamide (10a). Dimethylamine (33% solution in ethanol, 232 μL, 1.7 mmol) was added to a solution of the chloride **9a** (80

mg, 0.17 mmol) in ethanol (30 mL) and the resulting solution was heated at reflux for 18 h. The mixture was vacuum-evaporated and the residue was purified by column chromatography using a mixture of dichloromethane/methanol (92/8, v/v) as the eluent, to give compound **10a** (70 mg, 86%) as a light yellow oil ^{1}H-NMR (400 MHz, deuteriochloroform) δ: 1.46 (s, 6H, 2 × gem CH_3), 2.34 [s, 6H, $NHCH_2CH_2N(CH_3)_2$], 2.48 [s, 6H, $NHCOCH_2N(CH_3)_2$], 2.61 (t, 2H, $NHCH_2CH_2NMe_2$, J = 7 Hz), 3.18 (s, 2H, $NHCOCH_2NMe_2$), 3.54 (q, 2H, $NHCH_2CH_2NMe_2$, J = 7 Hz, 5 Hz), 5.50 (d, 1H, H-3, J = 10 Hz), 6.58 (d, 1H, H-4, J = 10 Hz), 7.28 (m, 2H, H-8, H-10), 7.57 (dt, 1H, H-9, J = 8 Hz, 2 Hz), 8.19 (dd, 1H, H-7, J = 8 Hz, 2 Hz,), 8.33 (s, 1H, $NHCOCH_2NMe_2$, D_2O exch.), 9.62 (t, 1H, $NHCH_2CH_2NMe_2$, J = 5 Hz, D_2O exch.). ^{13}C-NMR (50 MHz, deuteriochloroform) δ: 27.38 (2 × gem CH_3), 44.80 [$NHCH_2CH_2N(CH_3)_2$], 45.23 [$NHCOCH_2N(CH_3)_2$], 46.70 ($NHCH_2CH_2NMe_2$), 58.99 ($NHCH_2CH_2NMe_2$), 63.03 ($NHCOCH_2NMe_2$), 76.37 (C-2), 103.35 (C-12), 104.97 (C-4a), 105.05 (C-5a), 117.24 (C-10), 119.71 (C-4), 121.58 (C-6a), 123.93 (C-8), 124.71 (C-3), 126.03 (C-7), 134.15 (C-9), 149.26 (C-5), 153.67 (C-11a), 154.66 (C-10a), 156.24 (C-12a), 169.51 ($NHCOCH_2NMe_2$), 179.03 (C-6). Anal. Calcd. for $C_{26}H_{32}N_4O_4$: C, 67.22; H, 6.94; N, 12.06. Found: C, 67.39; H, 6.85; N, 11.92.

2-Diethylamino-*N*-[5-(2-diethylaminoethylamino)-2,2-dimethyl-6-oxo-2*H*,6*H*-pyrano[3,2-*b*]xanthen-12-yl]acetamide (10b). The synthetic route is analogous to the one followed for the preparation of compound **10a**. Oil. Yield: 88%. ^{1}H-NMR (400 MHz, deuteriochloroform) δ: 1.10 [t, 6H, $NHCH_2CH_2N(CH_2CH_3)_2$, J = 7 Hz], 1.19 [t, 6H, $NHCOCH_2N(CH_2CH_3)_2$, J = 7 Hz], 1.45 (s, 6H, 2 × gem CH_3), 2.72 [m, 10H, $NHCH_2CH_2N(CH_2CH_3)_2$, $NHCOCH_2N(CH_2CH_3)_2$, $NHCH_2CH_2NEt_2$], 3.25 (s, 2H, $NHCOCH_2NEt_2$), 3.57 (q, 2H, $NHCH_2CH_2NEt_2$, J = 7 Hz, 5 Hz), 5.51 (d, 1H, H-3, J = 10 Hz), 1H, 6.58 (d, 1H, H-4, J = 10 Hz), 7.25 (m, 2H, H-10 and H-8), 7.57 (dt, 1H, H-9, J = 8 Hz, 2 Hz), 8.17 (dd, 1H, H-7, J = 8 Hz, 2 Hz), 8.66 (s, 1H, $NHCOCH_2NEt_2$, D_2O exch.), 9.54 (t, 1H, $NHCH_2CH_2NEt_2$, J = 5 Hz, D_2O exch.). ^{13}C-NMR (50 MHz, deuteriochloroform) δ: 11.06 [$NHCH_2CH_2N(CH_2CH_3)_2$], 12.64 [$NHCOCH_2N(CH_2CH_3)_2$], 27.27 (2 × gem CH_3), 47.30 [$NHCH_2CH_2N(CH_2CH_3)_2$, $NHCH_2CH_2NEt_2$], 49.18 [$NHCOCH_2N(CH_2CH_3)_2$], 53.29 ($NHCH_2CH_2NEt_2$), 57.56 ($NHCOCH_2NEt_2$), 76.37 (C-2), 102.43 (C-12), 104.01 (C-4a), 104.56 (C-5a), 117.02 (C-10), 120.48 (C-4), 121.76 (C-6a), 123.68 (C-8), 124.56 (C-3), 125.99 (C-7), 133.78 (C-9), 150.21 (C-5), 153.78 (C-11a), 154.62 (C-10a), 156.20 (C-12a), 171.46 ($NHCOCH_2NEt_2$), 178.70 (C-6). Anal. Calcd. for $C_{30}H_{40}N_4O_4$: C, 64.79; H, 7.25; N, 10.08. Found: C, 64.65; H, 7.37; N, 10.24.

***N*,*N*-Dimethyl-*N*′-[12-(*t*-butoxycarbonylamino)-2,2-dimethyl-6-oxo-2*H*,6*H*-pyrano[3,2-*b*]xanthen-5-yl]ethanediamine (11a).** To a solution of the amine **8a** (100 mg, 0.26 mmol) in dry tetrahydrofuran (20 mL) at 0°C, were added 4-dimethylaminopyridine (47 mg, 0.38 mmol) and di-*tert*-butyl dicarbonate (73 μL, 0.32 mmol) and the mixture was stirred at room temperature for 3 h. The solvent was vacuum-evaporated, the residue was dissolved in ethyl acetate (50 mL) and washed with water (2 × 50 mL). The organic extracts were dried (sodium sulfate) and concentrated to dryness and the residue was purified by column chromatography using a mixture of dichloromethane/methanol (98/4, v/v) as the eluent, to provide compound **11a** (86 mg, 68%) as a light yellow oil. ^{1}H-NMR (400 MHz, deuteriochloroform) δ: 1.35 [s, 9H, $NHCOOC(CH_3)_3$], 1.43 (s, 6H, 2 × gem CH_3), 2.29 [s, 6H, $NHCH_2CH_2N(CH_3)_2$], 2.56 (t, 2H, $NHCH_2CH_2NMe_2$, J = 7 Hz), 3.55 (q, 2H, $NHCH_2CH_2NMe_2$, J = 7 Hz, 5 Hz), 5.50 (d, 1H, H-3, J = 10 Hz), 6.63 (d, 1H, H-4, J = 10 Hz), 7.32 (m, 2H, H-8, H-10), 7.59 (dt,1H, H-9, J = 8 Hz, 2 Hz), 8.22 (dd, 1H, H-7, J = 8 Hz, 2 Hz), 9.72 (t, 1H, $NHCH_2CH_2NMe_2$, J = 5 Hz, D_2O exch.). ^{13}C-NMR (50 MHz, deuteriochloroform) δ: 26.98 (2 × gem CH_3), 27.79 [$NHCOOC(CH_3)_3$], 45.58 [$NHCH_2CH_2N(CH_3)_2$], 47.41 ($NHCH_2CH_2NMe_2$), 59.95 ($NHCH_2CH_2NMe_2$), 76.89 (C-2), 81.89 [$NHCOOC(CH_3)_3$], 103.39 (C-4a), 103.87 (C-5a), 106.00 (C-12), 116.95 (C-10), 120.55 (C-4), 121.76 (C-6a), 123.68 (C-8), 124.34 (C-3), 126.10 (C-7), 133.82 (C-9), 150.47 (C-5), 151.35 [$NHCOOC(CH_3)_3$], 153.30 (C-11a), 154.51 (C-10a), 155.95 (C-12a), 178.44 (C-6). Anal. Calcd. for $C_{27}H_{33}N_3O_5$: C, 67.62; H, 6.94; N, 8.76. Found: C, 67.47; H, 7.11; N, 8.93.

***N*,*N*-Diethyl-*N*′-[12-(t-butoxycarbonylamino)-2,2-dimethyl-6-oxo-2*H*,6*H*-pyrano[3,2-*b*]xanthen-5-yl]ethanediamine (11b).** The synthetic route is analogous to the one followed for the preparation of compound **11a**. Oil. Yield: 69%. ^{1}H-NMR (400 MHz, deuteriochloroform) δ: 1.00 [t, 6H, $NHCH_2CH_2N(CH_2CH_3)_2$, J = 7 Hz], 1.34 [s, 9H, $NHCOOC(CH_3)_3$], 1.42 (s, 6H, 2 × gem CH_3), 2.56 [q, 4H, $NHCH_2CH_2N(CH_2CH_3)_2$, J = 7 Hz], 2.67 (t, 2H, $NHCH_2CH_2NEt_2$, J = 7 Hz), 3.52 (q, 2H, $NHCH_2CH_2NEt_2$, J = 7 Hz, 5 Hz), 5.47 (d, 1H, H-3, J = 10 Hz), 6.63 (d, 1H, H-4, J = 10 Hz), 7.30 (m, 2H, H-8, H-10), 7.59 (dt, 1H, H-9, J = 8 Hz, 2 Hz), 8.20 (dd, 1H, H-7, J = 8 Hz, 2 Hz), 9.66 (t, 1H, $NHCH_2CH_2NEt_2$, J = 5 Hz, D_2O exch.). ^{13}C-NMR (50 MHz, deuteriochloroform) δ: 11.61 [$NHCH_2CH_2N(CH_2CH_3)_2$], 26.94 (2 × gem CH_3), 27.74 [$NHCOOC(CH_3)_3$], 47.19 [$NHCH_2CH_2N(CH_2CH_3)_2$], 47.78 ($NHCH_2CH_2NEt_2$), 53.55 ($NHCH_2CH_2NEt_2$), 76.85 (C-2), 81.81 [$NHCOOC(CH_3)_3$], 103.35 (C-4a), 103.87 (C-5a), 105.85 (C-12), 116.92 (C-10), 120.59 (C-4), 121.77 (C-6a), 123.64 (C-8), 124.23 (C-3), 126.00 (C-7), 133.75 (C-9), 150.51 (C-5), 151.36 [$NHCOOC(CH_3)_3$], 153.27 (C-11a), 154.48 (C-10a), 155.92 (C-12a), 178.34 (C-6). Anal. Calcd. for $C_{29}H_{37}N_3O_5$: C, 68.62; H, 7.35; N, 8.28. Found: C, 68.85 H, 7.47; N, 8.12.

***N*,*N*-Dimethyl-*N*′-[12-[*N*-(*t*-butoxycarbonyl)-*N*-(2-dimethylaminoethyl)amino]-2,2-dimethyl-6-oxo-2*H*,6*H*-pyrano[3,2-*b*]xanthen-5-yl]ethanediamine (12a).** Sodium hydride (60% in hexanes, 30 mg, 0.75 mmol) was added under argon at 0°C to a solution of compound **11a** (70 mg, 0.15 mmol) in dry dimethylformamide (5 mL) and the mixture was stirred at room temperature for 30 min. It was then cooled at 0°C, a solution of 2-dimethylaminoethylchloride (160 mg, 1.5 mmol) in dimethylformamide (3 mL) was added dropwise and the resulting mixture was stirred for 18 h. Water (100 mL) was then added and the aqueous phase was extracted with ethyl acetate (3 × 50 mL). The organic extracts were dried (sodium sulfate) and concentrated to dryness and the residue was purified by column chromatography using a mixture of dichloromethane/methanol (90/10, v/v) as the eluent to give compound **12a** (50 mg, 62%) as a light yellow oil. ^{1}H-NMR (400 MHz, deuteriochloroform) δ: 1.25 [s, 9H, $NHCOOC(CH_3)_3$], 1.46 (s, 6H, 2 × gem CH_3), 2.28 [s, 6H, $N(Boc)CH_2CH_2N(CH_3)_2$], 2.30 [s, 6H, $NHCH_2CH_2N(CH_3)_2$], 2.58 [m, 4H, $NHCH_2CH_2NMe_2$, $N(Boc)CH_2CH_2N(CH_3)_2$], 3.54 (q, 2H, $NHCH_2CH_2NMe_2$, J =

7 Hz, 5 Hz), 3.70 [t, 2H, N(Boc)$CH_2$$CH_2$$NMe_2$, J = 7 Hz], 5.49 (d, 1H, H-3, J = 10 Hz), 6.60 (d, 1H, H-4, J = 10 Hz), 7.31 (m, 2H, H-8, H-10), 7.61 (dt, 1H, H-9, J = 8 Hz, 2 Hz), 8.22 (dd, 1H, H-7, J = 8 Hz, 2 Hz), 9.69 (t, 1H, N$H$$CH_2$$CH_2$$NMe_2$, J = 5 Hz, D_2O exch.). ^{13}C-NMR (50 MHz, deuteriochloroform) δ: 27.38 (2 × gem CH_3), 28.19 [NHCOOC(CH_3)$_3$], 45.39 [N(Boc)$CH_2$$CH_2$N($CH_3$)$_2$], 45.65 [NH$CH_2$$CH_2$N($CH_3$)$_2$], 46.53 [N(Boc)$CH_2$$CH_2$$NMe_2$], 47.64 (NH$CH_2$$CH_2$$NMe_2$), 56.90 [N(Boc)$CH_2$$CH_2$$NMe_2$], 60.09 (NH$CH_2$$CH_2$$NMe_2$), 76.38 (C-2), 79.43 [NHCOOC(CH_3)$_3$], 103.57 (C-4a), 104.12 (C-5a), 108.17 (C-12), 116.95 (C-10), 120.74 (C-4), 121.88 (C-6a), 123.71 (C-8), 124.15 (C-3), 126.21 (C-7), 133.86 (C-9), 150.36 (C-5), 154.40 (C-10a), 154.62 [NHCOOC(CH_3)$_3$], 155.50 (C-11a), 156.75 (C-12a), 178.62 (C-6). Anal. Calcd. for $C_{31}H_{42}N_4O_5$: C, 67.61; H, 7.69; N, 10.17. Found: C, 67.42; H, 7.57; N, 9.93.

***N*,*N*-Diethyl-*N*′-[12-[*N*-(*t*-butoxycarbonyl)-*N*-(2-diethylaminoethyl)amino]-2,2-dimethyl-6-oxo-2*H*,6*H*-pyrano[3,2-*b*]xanthen-5-yl]ethanediamine (12b).** The synthetic route is analogous to the one followed for the preparation of compound **12a**. Oil. Yield: 65%. ^{1}H-NMR (400 MHz, deuteriochloroform) δ: 1.00 [m, 12H, NHCH$_2$CH$_2$N(CH$_2$$CH_3$)$_2$, N(Boc)CH$_2CH_2$N-(CH$_2$$CH_3$)$_2$], 1.24 [s, 9H, NHCOOC($CH_3$)$_3$], 1.44 (s, 6H, 2 × gem CH_3), 2.56 [m, 8H, NHCH$_2$CH$_2$N(CH_2CH$_3$)$_2$, N(Boc)CH$_2$-CH$_2$N(CH_2CH$_3$)$_2$], 2.68 [t, 2H, NHCH$_2$$CH_2NEt_2$, J = 7 Hz), 2.78 [t, 2H, N(Boc)CH$_2$$CH_2NEt_2$, J = 7 Hz), 3.51 (q, 2H, NHCH_2CH$_2$NEt$_2$, J = 7 Hz, 5 Hz), 3.66 (t, 2H, N(Boc)-CH_2CH$_2$NEt$_2$, J = 7 Hz), 5.47 (d, 1H, H-3, J = 10 Hz), 6.60 (d, 1H, H-4, J = 10 Hz), 7.30 (m, 2H, H-8, H-10), 7.59 (dt, 1H, H-9, J = 8 Hz, 2 Hz), 8.20 (dd, 1H, H-7, J = 8 Hz, 2 Hz), 9.62 (t, 1H, NHCH$_2$CH$_2$NEt$_2$, J = 5 Hz, D_2O exch.). ^{13}C-NMR (50 MHz, deuteriochloroform) δ: 11.65 [NHCH$_2$CH$_2$-N(CH$_2$$CH_3$)$_2$, N(Boc)CH$_2CH_2$N(CH$_2$$CH_3$)$_2$], 27.31 (2 × gem CH_3), 28.15 [NHCOOC(CH_3)$_3$], 46.16 [N(Boc)CH_2CH$_2$NEt$_2$], 47.30 [NHCH$_2$CH$_2$N(CH_2CH$_3$)$_2$, N(Boc)CH$_2$CH$_2$N(CH_2CH$_3$)$_2$], 47.89 (NHCH_2CH$_2$NEt$_2$), 50.35 [N(Boc)CH$_2$$CH_2NEt_2$], 53.66 (NHCH$_2$$CH_2NEt_2$), 76.42 (C-2), 79.43 [NHCOOC(CH_3)$_3$], 103.54 (C-4a), 104.16 (C-5a), 108.17 (C-12), 116.95 (C-10), 120.77 (C-4), 121.84 (C-6a), 123.71 (C-8), 124.04 (C-3), 126.10 (C-7), 133.86 (C-9), 150.36 (C-5), 154.25 (C-10a), 154.55 [NHCOOC(CH_3)$_3$], 155.47 (C-11a), 156.64 (C-12a), 178.55 (C-6). Anal. Calcd. for $C_{35}H_{50}N_4O_5$: C, 69.28; H, 8.31; N, 9.23. Found: C, 69.03; H, 8.27; N, 8.89.

***N*,*N*′-Bis(2-dimethylaminoethyl)-2,2-dimethyl-6-oxo-2*H*,6*H*-pyrano[3,2-*b*]xantheno-5,12-diamine (13a).** To a solution of compound **12a** (50 mg, 0.09 mmol) in dry dichloromethane (5 mL) at 0°C was added trifluoroacetic acid (0.5 mL) and the mixture was stirred, at room temperature for 1 h. The organic phase was then washed with a 20% sodium carbonate solution (2 × 30 mL), dried (sodium sulfate) and concentrated to dryness and the residue was purified by column chromatography using a mixture of dichloromethane/methanol (85/15, v/v) as the eluent to give compound **13a** (33 mg, 84%) as a light yellow oil. ^{1}H-NMR (400 MHz, deuteriochloroform) δ: 1.48 (s, 6H, 2 × gem CH_3), 2.28 [s, 6H, 5-NHCH$_2$CH$_2$N(CH_3)$_2$], 2.31 [s, 6H, 12-NHCH$_2$CH$_2$N(CH_3)$_2$], 2.55 [m, 4H, 5-NHCH$_2$$CH_2NMe_2$, 12-NHCH$_2$$CH_2$N(CH$_3$)$_2$], 3.24 (q, 2H, 12-NH$CH_2CH_2NMe_2$, J = 7 Hz, 5 Hz), 3.42 (t, 2H, 5-NHCH_2CH$_2$NMe$_2$, J = 7 Hz), 5.52 (d, 1H, H-3, J = 10 Hz), 6.59 (d, 1H, H-4, J = 10 Hz), 7.27 (dt, 1H, H-8, J = 8 Hz, 2 Hz), 7.39 (dd, 1H, H-10, J = 8 Hz, 2 Hz), 7.58 (dt, 1H, H-9, J = 8 Hz, 2 Hz), 8.20 (dd, 1H, H-7, J = 8 Hz, 2 Hz), 8.98 (t, 1H, 5-NHCH$_2$CH$_2$NMe$_2$, J = 5 Hz, D_2O exch.). ^{13}C-NMR (50 MHz, deuteriochloroform) δ: 27.24 (2 × gem CH_3), 45.24 [12-NHCH$_2$CH$_2$N(CH_3)$_2$], 45.72 [5-NHCH$_2$CH$_2$N(CH_3)$_2$], 45.87 (12-NHCH_2CH$_2$NMe$_2$), 48.41 (5-NHCH_2CH$_2$NMe$_2$), 59.06 (12-NHCH$_2$$CH_2NMe_2$), 60.09 (5-NHCH$_2$$CH_2NMe_2$), 76.63 (C-2), 105.34 (C-4a), 105.48 (C-5a), 116.84 (C-12), 117.02 (C-10), 120.74 (C-4), 121.80 (C-6a), 123.46 (C-8), 124.89 (C-3), 126.21 (C-7), 133.71 (C-9), 146.21 (C-5), 149.95 (C-11a), 152.01 (C-12a), 154.81 (C-10a), 179.03 (C-6). Anal. Calcd. for $C_{26}H_{34}N_4O_3$: C, 69.31; H, 7.61; N, 12.44. Found: C, 69.18; H, 7.50; N, 12.22.

***N*,*N*′-Bis(2-diethylaminoethyl)-2,2-dimethyl-6-oxo-2*H*,6*H*-pyrano[3,2-*b*]xantheno-5,12-diamine (13b).** The synthetic route is analogous to the one followed for the preparation of compound **13a**. Yield: 86%. mp (difumarate): 125–126°C, (ethanol). ^{1}H-NMR (400 MHz, deuteriochloroform) δ: 1.00 [t, 6H, 5-NHCH$_2$CH$_2$N(CH$_2$$CH_3$)$_2$, J = 7 Hz], 1.05 [t, 6H, 12-NHCH$_2$CH$_2$N(CH$_2$$CH_3$)$_2$, J = 7 Hz], 1.47 (s, 6H, 2 × gem CH_3), 2.56 [t, 4H, 5-NHCH$_2$CH$_2$N(CH_2CH$_3$)$_2$, J = 7 Hz], 2.65 [m, 6H, 5-NHCH$_2$$CH_2NEt_2$, 12-NHCH$_2CH_2$N($CH_2CH_3$)$_2$], 2.74 (t, 2H, 12-NHCH$_2$$CH_2NEt_2$, J = 7 Hz), 3.28 (t, 2H, 12-NHCH_2CH$_2$NEt$_2$, J = 7 Hz), 3.40 (q, 2H, 5-NHCH_2CH$_2$NEt$_2$, J = 7 Hz, 5 Hz), 5.52 (d, 1H, H-3, J = 10 Hz), 6.59 (d, 1H, H-4, J = 10 Hz), 7.26 (dt, 1H, H-8, J = 8 Hz, 2 Hz), 7.37 (dd, 1H, H-10, J = 8 Hz, 2 Hz), 7.58 (dt, 1H, H-9, J = 8 Hz, 2 Hz), 8.19 (dd, 1H, H-7, J = 8 Hz, 2 Hz), 8.92 (t, 1H, 5-NHCH$_2$CH$_2$NEt$_2$, J = 5 Hz, D_2O exch.). ^{13}C-NMR (50 MHz, deuteriochloroform) δ: 11.51 [5-NHCH$_2$CH$_2$N(CH$_2$$CH_3$)$_2$, 12-NHCH$_2CH_2$N(CH$_2$$CH_3$)$_2$], 27.27 (2 × gem CH_3), 45.47 [12-NHCH_2CH$_2$NEt$_2$], 46.60 [12-NHCH$_2$CH$_2$N(CH_2CH$_3$)$_2$], 47.34 [5-NHCH$_2$CH$_2$N(CH_2CH$_3$)$_2$], 48.63 (5-NHCH_2CH$_2$NEt$_2$), 52.82 (12-NHCH$_2$$CH_2NEt_2$), 53.62 (5-NHCH$_2$$CH_2NEt_2$), 76.60 (C-2), 105.45 (C-4a), 105.63 (C-5a), 116.98 (C-12), 117.02 (C-10), 120.74 (C-4), 121.76 (C-6a), 123.42 (C-8), 124.93 (C-3), 126.14 (C-7), 133.71 (C-9), 145.80 (C-5), 149.44 (C-11a), 151.46 (C-12a), 154.81 (C-10a), 179.03 (C-6). Anal. Calcd. for $C_{30}H_{42}N_4O_3$. $2C_4H_4O_4$: C, 61.77; H, 6.82; N, 7.58. Found: C, 61.98; H, 6.69; N, 7.44.

Cell culture and assessment of cytotoxicity. The new compounds were tested for their cytotoxic activity on the murine leukemia cell line L1210 (American Type Culture Collection, Rockville, MD), as well as on the following human solid tumor cell lines: colorectal adenocarcinoma HT-29, uterine sarcoma MES-SA, and its 100-fold resistant to doxorubicin subline MES-SA/Dx5 (European Collection of Cell Cultures, Salisbury, U.K.) [24]. L1210 cells were cultured in RPMI 1640 medium (Gibco BRL, Paisley, U.K.) supplemented with penicillin (100 U/mL), streptomycin (100 μg/mL), and 10% fetal bovine serum (media and antibiotics from Biochrom KG, Berlin, Germany) in an environment of 5% CO_2, 85% humidity, and 37°C. HT-29 cells were cultured in Dulbecco's minimal essential medium supplemented with antibiotics and serum (as above), and routinely subcultured using a trypsin 0.25%-EDTA 0.02% solution. MES-SA and MES-SA/Dx5 cells were cultured in McCoy's 5A medium supplemented with antibiotics and serum (as above), and subcultured by means of 0.03% EDTA. The cytotoxicity assay was performed by a modification of the MTT method. Briefly, the cells were plated at a density of ~5,000 cells/well in 96-well flat-bottomed microplates, and after 24 h the test compounds were added,

appropriately diluted with DMSO. After a 72-h incubation, the medium was replaced with MTT (Sigma) dissolved at a final concentration of 1 mg/mL in serum-free, phenol-red-free RPMI (Biochrom KG) for a further 4-h incubation. Then, the MTT formazan was solubilized in 2-propanol and the optical density was measured with a microplate analyzer at a wavelength of 550 nm (reference wavelength 690 nm). Doxorubicin and mitoxantrone were included in the experiments as positive controls. The results represent the mean of three independent experiments and are expressed as IC_{50}, the concentration that reduced by 50% the optical density of treated cells with respect to untreated controls. Furthermore, the resistance factor was calculated as the ratio between the IC_{50} of MES-SA/Dx5 cells and the IC_{50} of MES-SA cells.

Cell cycle analysis. Cell-cycle analysis was performed following incubation of exponentially growing HT-29 cells with the indicated concentrations of the test substances for 24 h. Treated cultures were then washed in PBS, fixed in 50% ethanol, and stained with an RNAse-containing propidium iodide solution. DNA content was analyzed on a FACS Calibur (Becton Dickinson, San Jose, CA) flow cytometer using the ModFit software (Verity Software House, Topsham, ME).

Acknowledgment. This project is co-financed within Op. Education by the ESF (European Social Fund) and National Resources.

REFERENCES AND NOTES

[1] Faulds, D.; Balfour, J. A.; Chrisp, P.; Langtry, H. D. Drugs 1991, 41, 400.

[2] Krapcho, A. P.; Menta, E. Drugs Fut 1997, 22, 641.

[3] Cholody, W. M.; Horowska, B.; Paradziej-Lukowicz, J.; Martelli, S.; Konopa, J. J. Med Chem 1996, 39, 1028.

[4] Atwell, G. J.; Rewcastle, G. W.; Baguley, B. C.; Denny, W. A. J Med Chem 1987, 30, 664.

[5] Adjei, A. A. Invest New Drugs 1999, 17, 43.

[6] Vispé, S.; Vandenberghe, I.; Robin, M.; Annereau, J. P.; Créancier, L.; Pique, V.; Galy, J. P.; Kruczynski, A.; Barret, J. M.; Bailly, C. Biochem Pharmacol 2007, 73, 1863.

[7] Otsuka, Y.; Tanaka, T.; Uchida, D.; Noguchi, Y.; Saeki, N.; Saito, Y.; Tatsuno, I. Neurosci Lett 2004, 365, 180.

[8] Wakelin, L. P. G.; Adams, A.; Denny, W. A. J Med Chem 2002, 45, 894.

[9] Antonini, I.; Polucci, P.; Kelland, L. R.; Menta, E.; Pescalli, N.; Martelli, S. J Med Chem 1999, 42, 2535.

[10] Konopa, J. Pure Appl Chem 2001, 73, 1421.

[11] Ghirtis, K.; Pouli, N.; Marakos, P.; Skaltsounis, A.-L.; Leonce, S.; Gaignard, D. H.; Atassi, G. Heterocycles 2000, 53, 93.

[12] Ghirtis, K.; Pouli, N.; Marakos, P.; Skaltsounis, A.-L.; Leonce, S.; Atassi, G.; Gaignard, D. H. J Heterocycl Chem 2001, 38, 147.

[13] Svoboda, G. H.; Poore, G. A.; Simpson, P. J.; Boder, G. B. J. Pharm Sci 1966, 55, 758.

[14] Kostakis, I. K.; Ghirtis, K.; Pouli, N.; Marakos, P.; Skaltsounis, A. L.; Leonce, S.; Gaignard, D. H.; Atassi, G. Il Farmaco 2000, 55, 455.

[15] Kolokythas, G.; Kostakis, I. K.; Pouli, N.; Marakos, P.; Skaltsounis, A.-L.; Pratsinis, H. Bioorg Med Chem Lett 2002, 12, 1443.

[16] Kolokythas, G.; Kostakis, I. K.; Pouli, N.; Marakos, P.; Kletsas, D.; Pratsinis, H. Bioorg Med Chem 2003, 11, 4591.

[17] Kolokythas, G.; Pouli, N.; Marakos, P.; Pratsinis, H.; Kletsas, D. Eur J Med Chem 2006, 41, 71.

[18] Costes, N.; Elomri, A.; Dufat, H.; Michel, S.; Seguin, E.; Koch, M.; Tillequin, F.; Pfeiffer, B.; Renard, P.; Léonce, S.; Pierré, A. Oncology Res 2002, 13, 191.

[19] Kolokythas, G.; Kostakis, I. K.; Pouli, N.; Marakos, P.; Kousidou, O. Ch.; Tzanakakis, G. N.; Karamanos, N. K. Eur J Med Chem 2007, 42, 307.

[20] Ahluwalia, K. V.; Jolly, S. R.; Tehim, K. A. J Chem Soc Perkin Trans 1 1983, 1229.

[21] Cardilo, G.; Cricchio, R.; Merlini, L. Tetrahedron 1968, 24, 4825.

[22] Ahluwalia, K. V.; Arora, K. K. Tetrahedron 1981, 37, 1437.

[23] Hernandez-Galan, R.; Salva, J.; Massanet, G. M.; Collado, I. G. Tetrahedron 1993, 49, 1701.

[24] Harker, W. G.; Sikic, B. I. Cancer Res 1985, 45, 4091.

[25] Kostakis, I. K.; Pouli, N.; Marakos, P.; Skaltsounis, A.-L.; Pratsinis, H.; Kletsas, D. Bioorg Med Chem 2006, 14, 2910.