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Original article

Design and synthesis of new pyranoxanthenones bearing a nitro group or an aminosubstituted side chain on the pyran ring. Evaluation of their growth inhibitory activity in breast cancer cells

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Abstract

Some new 2,6-disubstituted pyrano- and 1,2-dihydropyrano[2,3-*c*]xanthen-7-ones have been synthesized and their antiproliferative activity has been evaluated against MDA-MB-231 breast cancer cells. The antiproliferative activity evaluation of the compounds provided evidence that a dimethylamino substituted side chain and the presence of 1,2 double bond play a key role in cell growth inhibition. Among the tested derivatives the 6-dimethylaminoethylamino-2-nitropyranoxanthenone analogue possessed a significant inhibitory effect in a wide range of concentrations.

Keywords: 1,2-Dihydropyrano[2,3-*c*]xanthenones; Breast cancer cells; Growth inhibitory effect

1. Introduction

It has been previously shown that dialkylaminoalkyl groups present in the side chains of the anthracenedione antitumor drugs mitoxantrone and ametantrone (Fig. 1) play a key role in their biological activity, regulating DNA binding after metabolic activation [1]. The cytotoxic effects of the anthracenediones, similar to anthracyclines, are probably multimodal, although interaction with DNA and consequent damage are thought to be essential for their therapeutic effect [2]. Numerous anthracenedione derivatives have been synthesized in the past ten years, in an attempt to obtain new active drugs, showing better therapeutic efficacy, together with fewer and less pronounced side effects, e.g. cardiotoxicity and the development of resistance [3–5]. The chemical modification of the anthracenedione chromophore unit, the repositioning of the hydroxyl substituents and the variation of the alkylamino side chains have been extensively investigated and resulted in the preparation of very active derivatives, exemplified by the heterocyclic bis(2-aminoethyl)amino-substituted compound pixantrone dimaleate (Fig. 1) endowed with antileukemic activity comparable to mitoxantrone. Pixantrone showed no measurable cardiotoxicity and is currently undergoing phase III clinical trials for the treatment of non-Hodgkin's lymphoma [6–8]. All the above mentioned anthracenedione derivatives while being effective DNA intercalators, also interfere with the function of the DNA binding enzymes, such as DNA topoisomerases and DNA polymerases [9]. A common

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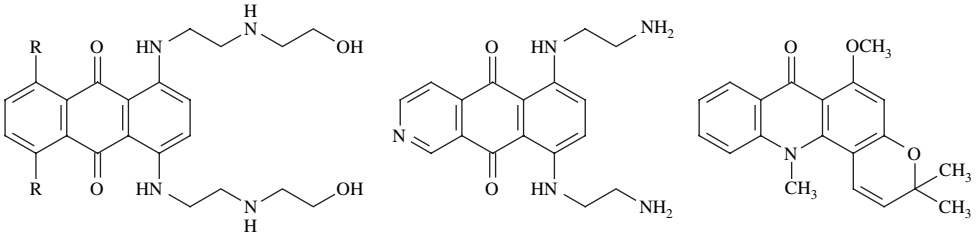

Fig. 1. Structures of mitoxantrone, ametantrone, piroxantrone and acronycine.

structural feature for these agents is the presence of one or two basic side chains, in a strictly defined orientation to their polycyclic chromophore moiety. This particular substitution pattern is thought to play an important role in modulating the affinity of each molecule for the nucleic acid and the sequence specific contacts along the double helical DNA. Similar substitution of the planar tricyclic acridone molecule in positions 1 and/or 4 with one or two analogous diaminoalkylo-residues, containing two or three methylene groups between amino groups, was found to play a crucial role in modulating both DNA binding properties and the pharmacological response of several acridone derivatives [10].

We have been involved in the synthesis and antiproliferative activity evaluation of a number of pyranoxanthenones [11] and azapyranoxanthenones [12,13] bearing structural similarity with the acridone alkaloid acronycine (Fig. 1), which was found to possess anticancer activity against a wide spectrum of experimental neoplasms in laboratory animals [14]. While the mechanism of action of acronycine at either cellular or molecular level has not been yet clearly established, it was shown that inhibition of DNA and RNA syntheses by this alkaloid is possibly the result of its potential capacity to intercalate into DNA [15]. Among numerous active acronycine derivatives, it has been reported that 2-nitroacronycine demonstrated high potency when tested against a panel of human tumor cells in vitro [15,16]. In previous studies we have found that the introduction of a flexible dialkylaminoethylamino side chain substitution in a number of (aza) pyranoxanthenones related to acronycine generally led to an improvement of the cytotoxicity against the murine leukemia L1210 and the colon adenocarcinoma HT-29 cell lines [13,17]. In accordance with this finding, the introduction of 6-dialkylaminoalkylamino side chain substitution on analogous pyranoacridones and benzo[*b*]pyranoacridones led to a significant increase of the cytotoxic activity against L1210 cells [18]. Prompted by these observations we decided to further investigate the effect on cytotoxicity caused by the insertion at the position 2 of the pyran ring of a nitro, a dialkylaminoethylamino side chain, or the less flexible dialkylaminomethylcarbonylamino group. We have also prepared analogues bearing a second basic side chain replacing the 6-methoxy group of the lead pyranoxanthenone chromophore, in an effort to study the structure–activity relationships within this class of compounds.

2. Chemistry

For the synthesis of the target derivatives 3,3-dimethyl-6-hydroxy-3*H*,7*H*-pyrano[2,3-*c*]xanthen-7-one **5** was used as key intermediate (Fig. 2). The preparation of this compound has already been reported [19], although in considerably low yield (11%), since it results from the Claisen rearrangement of the ether **2**, together with the corresponding linear isomer i.e. 2,2-dimethyl-5-hydroxy-2*H*,6*H*-pyrano[3,2-*b*]xanthen-6-one. We had previously observed that the methylation of the 1-OH group of compound **2**, prior to ring-closure improves the percentage of the angular isomer, presumably due to steric hindrance [20]. A slight modification of the reported procedure by the insertion of the bulky *p*-tosyl group into the free 1-OH of compound **2**, provided the ester **3**, which was subjected to thermal cyclization to furnish selectively the angular isomer **4** that was easily hydrolyzed to the required compound **5**. According to this methodology the hydroxyderivative **5** was prepared at 50% overall yield, starting from 1,3-dihydroxy-9*H*-xanthen-9-one **1**.

Methylation of the 6-OH group of compound **5** was then carried out, upon treatment with dimethyl sulfate in the presence of sodium hydride, to yield the 6-methoxyderivative **6** [20]. This analogue, was first converted into the *cis*-diol **7** (Fig. 3) by catalytic *syn*-dihydroxylation with osmium tetroxide and 4-morpholine *N*-oxide as oxidizing agent and then gave the 2-keto derivative **8**, upon treatment of **7** with a catalytic amount of *p*-toluenesulfonic acid in boiling toluene. An analogous methodology for the preparation of **8**, using cupric sulfate instead of *p*-toluenesulfonic acid, has been previously reported [20]. Compound **8** was then treated with the suitable 2-dialkylaminoethylamine and converted into the corresponding enamines **9a**,**b**. These enamines were highly unstable and upon the usual work-up were hydrolyzed back to the starting compound **8**. Consequently, they were not isolated, but were first treated with a saturated ethanolic HCl solution and the resulting hydrochlorides were then reduced by the reaction with sodiumcyanoborohydride to provide the target compounds **10a**,**b**.

For the preparation of the 6-aminosubstituted analogues of compounds **10**, we have attempted to apply the above mentioned methodology, using a suitable intermediate for the introduction of the amino substituted side chain. We have thus replaced the 6-methoxy group of compounds **10** by a tosyloxy

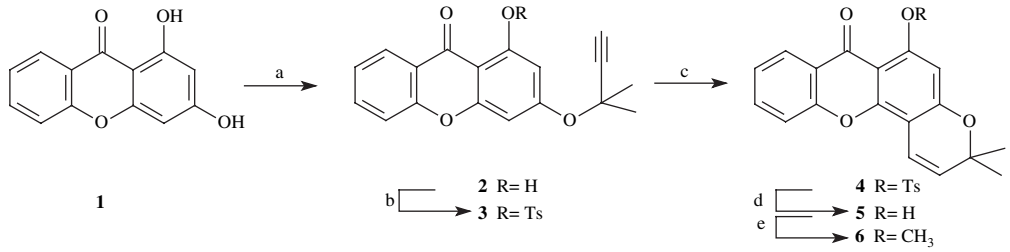

Fig. 2. a: 3-Chloro-3-methyl-1-butyne, K_2CO_3, KI, CuI, dry DMF, 70 °C, 26 h; b: TsCl, K_2CO_3, acetone dry, reflux, 1.5 h; c: DMF, reflux, 2 h; d: aq. NaOH, EtOH, r.t., 30 min; e: (1) NaH, dry THF, 1 h; (2) $(CH_3)_2SO_4$, dry THF, reflux, 3.5 h.

group, however, the subsequent displacement of the later by 2-dialkylaminoethylamines was unsuccessful.

Consequently, an alternative methodology was developed for the preparation of the target molecules. This involves initial nitration of compound **5** to the 2-nitroderivative **11** (Fig. 4), which was then converted to the tosylate **12**. The tosyloxy group of compound **12** turned out to be a very effective leaving group and was easily substituted by the reaction with appropriate 2-dialkylaminoethylamines to result in the aminoderivatives **13a**,**b**. Reduction of the double bond of the amines **13a**,**b** upon treatment with sodiumborohydride provided the nitro derivatives **14a**,**b** that were subjected to catalytic hydrogenation over Raney Ni in the presence of a 40% aqueous hydrazine solution, resulting in the 2-aminoderivatives **15a**,**b**. Reaction of these compounds with chloroacetic acid in the presence of *N*,*N*′-dicyclohexylcarbodiimide gave the chloroacetamides **16a**,**b**, which upon treatment with the suitable secondary amines furnished the target derivatives **17a**,**b**. On the other hand, the 2-aminoderivatives **15a**,**b** were treated with Boc-anhydride and converted to the carbamates **18a**,**b**, which reacted with the suitable dialkylaminoethylchlorides in the presence of sodium hydride. The resulting *N*-Boc analogues **19a**,**b** were deprotected by treatment with trifluoroacetic acid, to provide the target aminoderivatives **20a**,**b**.

By analogy to the above mentioned procedure, the nitroderivative **11** was methylated (Fig. 5) and the resulting compound **21** was successively reduced to give compounds **22** and **23**. The aminoderivative **23** was chloroacetylated and the resulting chloroacetamide **24** provided the target aminoderivatives **25a**,**b** upon reaction with the suitable secondary amines.

3. Results and discussion

The tested compounds were studied for their antiproliferative activity on the MDA-MB-231 breast cancer cell line, cultured in serum-containing medium. We choose to grow the cells in the presence of serum in order for our experimental model to be more similar to physiological conditions. Due to solubility problems in DMSO and ethanol, compounds **21** and **22** were not evaluated. The obtained IC_{50} values are presented in Table 1.

The 6-methoxy analogues **10a**,**b**, **23**, and **25a**,**b** showed an inhibitory effect (12–20% inhibition of cell growth,

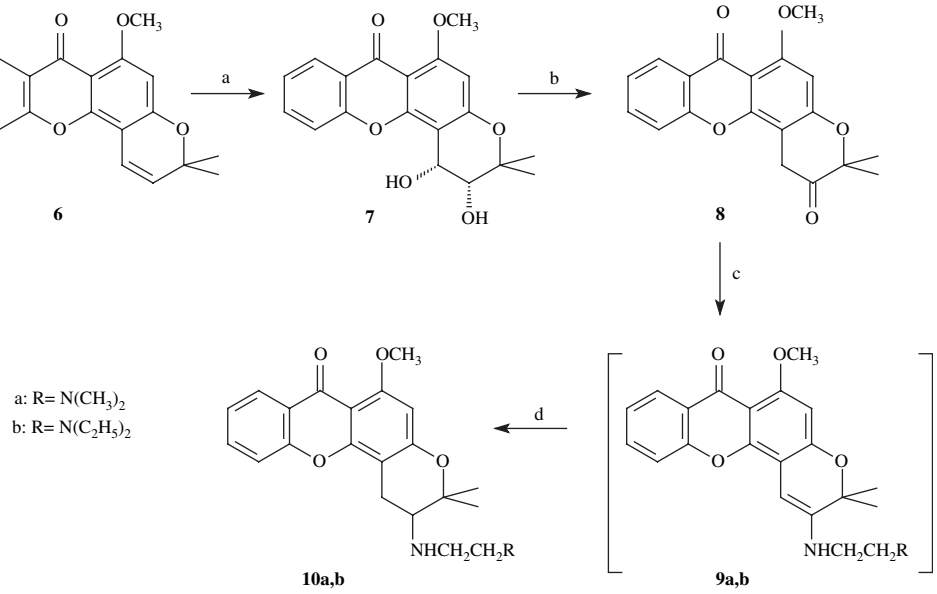

Fig. 3. a: (1) 2.5% OsO_4 in *tert*-butanol, *N*-methylmorpholine-*N*-oxide, *tert*-butanol/THF/H_2O, r.t., 48 h; (2) sat. aq. $NaHSO_3$, r.t., 2 h; b: *p*-TsOH × H_2O, toluene, reflux, 15 min; c: 2-dialkylaminoethylamine, abs. EtOH, reflux, 7 h; d: (1) EtOH sat. in HCl, $NaCNBH_3$, r.t., 1.5 h; (2) aq. 20% Na_2CO_3, r.t.

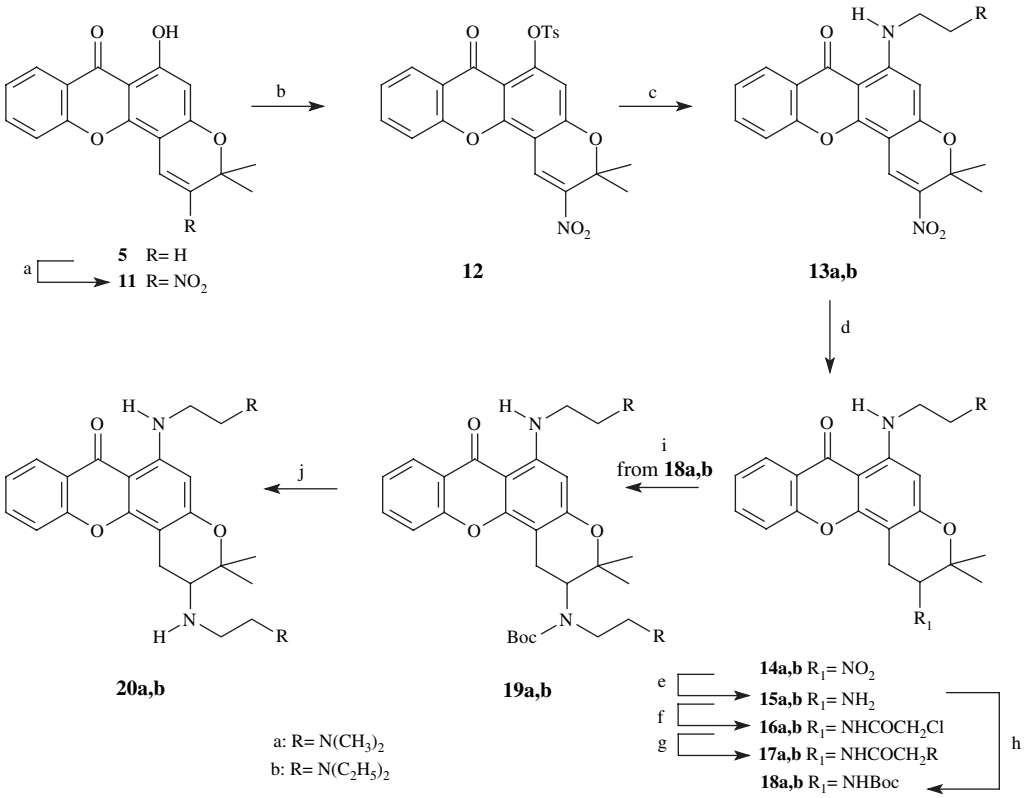

Fig. 4. a: 65% HNO_3, glacial AcOH, r.t., 30 min; b: TsCl, K_2CO_3, acetone, reflux, 1 h; c: 2-dialkylaminoethylamine, abs. EtOH, reflux, 2 h; d: $NaBH_4$, MeOH/CH_2Cl_2, r.t., 30 min; e: Raney Ni wet, aq. hydrazine 40%, EtOH, 45 °C, 15 min; f: $ClCH_2COOH$, DCC, CH_2Cl_2, r.t., 30 min; g: dialkylamine, EtOH, reflux, 10 h; h: Boc_2O, Et_3N, dry THF, r.t., 3 h; i: (1) NaH, dry DMF, r.t., 30 min; (2) 2-dialkylaminoethylchloride, dry DMF, r.t., 18 h; j: CF_3COOH, CH_2Cl_2, r.t., 1 h.

depending on the compound) shown even from the lowest concentration tested (1 μM). This inhibitory pattern remained for the concentration of 3 μM. Using 10 and 30 μM only moderately increased (15–30%) the inhibition of cell growth. Significant inhibitory effect (74–80%) was detected at the highest concentration (100 μM) tested. However, this effect is probably due to cytotoxicity, since a large number of cells lost contact with the culture flask and the remaining adherent cells underwent morphological changes.

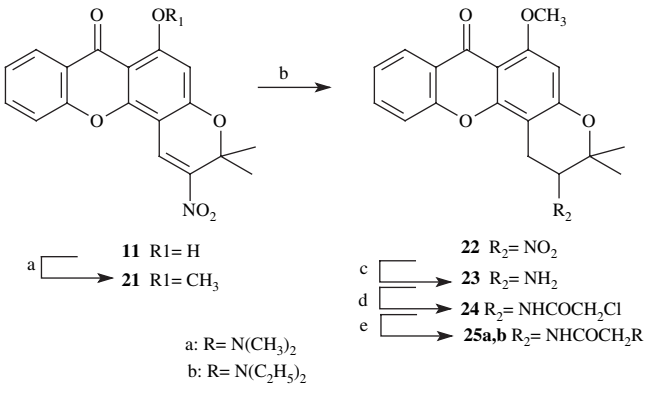

Fig. 5. a: (1) NaH, dry THF, 30 min; (2) $(CH_3)_2SO_4$, dry THF, reflux, 3 h; b: $NaBH_4$, MeOH/CH_2Cl_2, r.t., 30 min; c: Raney Ni wet, aq. hydrazine 40%, EtOH, 45 °C, 15 min; d: $ClCH_2COOH$, DCC, CH_2Cl_2, 30 min; e: dialkylamine, EtOH, reflux, 6 h.

The antiproliferative activities of the 2-nitropyranoxanthenones **13a**,**b** bearing a 6-amino-substituted side chain are shown in Fig. 6. The dimethylamino analogue **13a** exhibited a significant inhibitory effect in a wide range of concentrations, showing an IC_{50} value of 21 μM. On the contrary, the

Table 1
Antiproliferative activity of the synthesized compounds (IC_{50} values in μM)[a] against MDA-MB-231 breast cancer cells

Compound	IC_{50} (μM)
10a	57 (6.1)
10b	44 (4.8)
13a	21 (1.4)
13b	>100
14a	>100
14b	>100
15a	38 (2.3)
15b	62 (6.8)
17a	50 (6.0)
17b	68 (6.3)
20a	46 (5.5)
20b	73 (8.1)
23	53 (4.2)
25a	51 (3.1)
25b	52 (4.3)

[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.

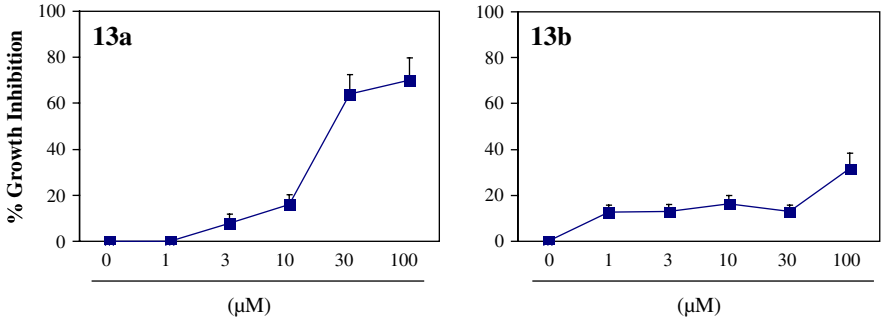

Fig. 6. Inhibitory effects of **13a** and **13b** on human breast cancer MDA-MB-231 cell line. The cells were incubated in serum-containing medium for 72 h in the presence of increasing concentrations (1, 3, 10 and 100 μM). Cell proliferation was assessed by the WST-1 assay. The results are presented as percentage of growth inhibition in respect to controls. Control values did not exhibit significant changes as compared to DMSO vehicle. Each point represents the average ± standard deviation of three individual experiments, performed in three replicates.

diethylamino nitroderivative **13b** did not cause significant inhibition even at high concentrations.

The antiproliferative effects of the 1,2-dihydropyranoxanthenones **14a**, **15a**, **17a** and **20a** which are substituted with a 6-dimethylaminoethylamino side chain are presented in Fig. 7. An inhibitory effect up to 20% was obtained for all compounds for the concentrations 1, 3 and 10 μM. At higher concentrations a significant antiproliferative activity was observed for the 2-amino analogue **15a**, the diamine **20a** and the acetamide **17a**. The respective IC_{50} values were 38, 46 and 50 μM.

For the corresponding 1,2-dihydropyranoxanthenones **14b**, **15b**, **17b** and **20b** bearing a 6-diethylaminoethylamino side chain, two patterns of antiproliferative activity were observed. The 2-nitroderivative **14b**, similarly as its structural analogue **14a**, caused a low inhibition (approximately 20–30%) at the concentrations of 30 and 100 μM. Compounds **15b**, **17b** and **20b** possessed a similar inhibitory effect as **14a** at the concentration of 30 μM, but they reached 75–80% inhibition at a concentration of 100 μM.

The above results indicate that the presence of the dimethylamino substitution increases the growth inhibitory activity as compared to the diethylamino one. It is worth noticing that this slight structural modification resulted in an improved antiproliferative activity in the whole respective analogues series. Another potentially important finding is the antiproliferative effect of the 1,2 unsaturation. Both factors, i.e. the dimethylamino substitution and the presence of 1,2 double bond in combination seem to be the key players for the growth inhibitory effects observed for this group of compounds.

4. Experimental protocols

4.1. Chemistry

All chemicals were purchased from Aldrich Chemical Co. Melting points were determined on a Büchi apparatus and are uncorrected. ^{1}H NMR spectra and 2D spectra were

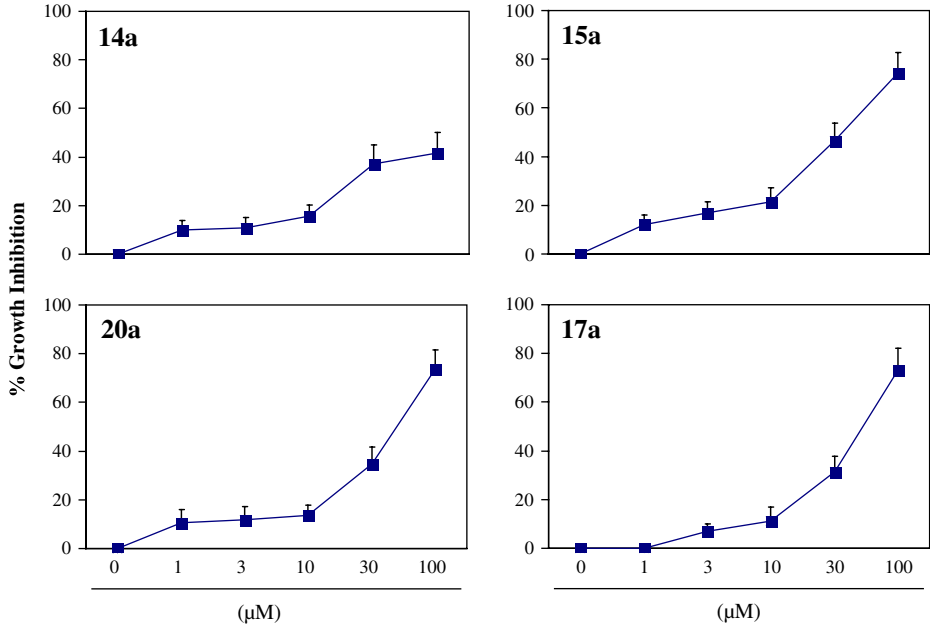

Fig. 7. Study of the antiproliferative effects of **14a**, **15a**, **17a** and **20a** on breast cancer cells. For details see legend of Fig. 6.

recorded on a Bruker Avanche 400 instrument, whereas ^{13}C NMR spectra 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 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 performed on a Perkin–Elmer PE 240C Elemental Analyzer (Norwalk, CT) and are within ±0.4% of the theoretical values.

4.1.1. 3-(1,1-Dimethylpropyn-2-oxy)-1-[(4-methylphenyl) sulfonyloxy)]-9H-xanthen-9-one (**3**)

To a solution of the xanthenone **2** [19] (4 g, 13.60 mmol) in dry acetone (100 mL), were added *p*-toluenesulfonyl chloride (7.77 g, 40.80 mmol) and potassium carbonate (9.38 g, 68.00 mmol) and the resulting mixture was heated at reflux under argon for 90 min. The inorganic precipitate was then filtered off and the filtrate was evaporated to dryness. Flash chromatography on silica gel using a mixture of cyclohexane–EtOAc 80:20 as the eluent, provided the title compound **3** (5.30 g, 87%). Mp 146–148 °C (EtOAc–*n*-hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.72 (s, 6H, 2 × gemCH_3), 2.38 (s, 3H, $SO_2C_6H_4CH_3$), 2.73 (s, 1H, H-3′), 7.02 (d, $J = 2$ Hz, 1H, H-2), 7.26 (d, $J = 2$ Hz, 1H, H-4), 7.30 (m, 3H, H-3″, H-5″ and H-7), 7.35 (dd, $J = 8$ Hz, 2 Hz, 1H, H-5), 7.62 (dt, $J = 8$ Hz, 2 Hz, 1H, H-6), 7.96 (d, $J = 8$ Hz, 2H, H-2″ and H-6″), 8.20 (dd, $J = 8$ Hz, 2 Hz, 1H, H-8); ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm): 21.69 ($SO_2C_6H_4CH_3$), 29.43 (2 × gemCH_3), 73.28 (C-2′), 75.87 (C-3′), 84.06 (C-1′), 105.39 (C-4), 111.44 (C-2), 117.09 (C-9a), 117.24 (C-5), 122.48 (C-8a), 124.10 (C-7), 126.84 (C-8), 128.93 (C-2″ and C-6″), 129.58 (C-3″ and C-5″), 132.98 (C-1″), 134.34 (C-6), 145.30 (C-4″), 148.29 (C-1), 155.14 (C-10a), 158.04 (C-4a), 160.14 (C-3), 174.19 (C-9). Anal. Calcd. for $C_{25}H_{20}O_6S$. Calcd. (%): C: 66.95, H: 4.49. Found (%): C: 67.23, H: 4.16.

4.1.2. 3,3-Dimethyl-6-[(4-methylphenyl)sulfonyloxy]-3H,7H-pyrano[2,3-c]xanthen-7-one (**4**)

A solution of compound **3** (5.30 g, 11.83 mmol) in dry DMF (20 mL) was heated at reflux for 2 h. The solvent was then vacuum-evaporated and the residue was purified by column chromatography (silica gel, cyclohexane–EtOAc 8:2) to provide compound **4** (4.72 g, 89%). Mp 188–190 °C (Et_2O–*n*-pentane) (Lit. [17] 188–190 °C).

4.1.3. 3,3-Dimethyl-6-hydroxy-3H,7H-pyrano[2,3-c] xanthen-7-one (**5**)

A solution of sodium hydroxide (4.21 g, 105.30 mmol) in water (5 mL) was added dropwise at 0 °C to a solution of compound **4** (4.72 g, 10.53 mmol) in ethanol (80 mL) and the resulting mixture was stirred at room temperature for 30 min. Ethanol was then vacuum-evaporated and the residue was extracted with EtOAc (3 × 60 mL). The organic extracts were washed with brine, dried (Na_2SO_4) and concentrated to dryness. The residue was purified by column chromatography (silica gel, cyclohexane–EtOAc 95:5), to provide compound **5** (2.69 g, 87%). Mp 176–177 °C (Et_2O–*n*-hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.47 (s, 6H, 2 × gemCH_3), 5.60 (d, $J = 10.5$ Hz, 1H, H-2), 6.26 (s, 1H, H-5), 6.83 (d, $J = 10.5$ Hz, 1H, H-1), 7.34 (dt, $J = 8$ Hz, 0.5 Hz, 1H, H-9), 7.44 (dd, $J = 8$ Hz, 0.5 Hz, 1H, H-11), 7.70 (dt, $J = 8$ Hz, ~1 Hz, 1H, H-10), 8.22 (dd, $J = 8$ Hz, ~1 Hz, 1H, H-8), 13.15 (s, 1H, D_2O exch., OH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm): 28.19 (2 × gemCH_3), 78.13 (C-3), 99.13 (C-5), 100.55 (C-12b), 103.44 (C-6a), 114.78 (C-1), 117.31 (C-11), 120.19 (C-7a), 123.91 (C-9), 125.57 (C-8), 126.95 (C-2), 134.75 (C-10), 151.52 (C-11a), 155.44 (C-12a), 160.70 (C-4a), 162.93 (C-6), 180.53 (C-7).

4.1.4. N,N-Dimethyl-N′-[1,2-dihydro-3,3-dimethyl-6-methoxy-7-oxo-3H,7H-pyrano[2,3-c]xanthen-2-yl]ethane-1,2-diamine (**10a**)

2-Dimethylaminoethylamine (340 μL, 3.1 mmol) was added to a solution of compound **8** [20] (100 mg, 0.31 mmol) in absolute ethanol (40 mL) and the resulting mixture was heated at reflux under argon for 7 h. After completion of the reaction, as indicated upon TLC monitoring, the mixture was cooled at 0 °C and a saturated ethanolic HCl solution was added (1.5 mL). The mixture was stirred at this temperature for few minutes, sodiumcyanoborohydride (97 mg, 1.55 mmol) was then added and the resulting mixture was stirred at room temperature for 90 min. A 20% solution of sodium carbonate (20 mL) was added, the reaction mixture was vacuum-evaporated, extracted with EtOAc–water, the organic layer was dried (Na_2SO_4) and the solvent was evaporated to dryness. The residue was purified by column chromatography (silica gel, CH_2Cl_2:CH_3OH 9:1) to furnish compound **10a** (80 mg, 64%). Mp (dihydrochloride) > 250 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.31 (s, 3H, 1 × gemCH_3), 1.46 (s, 3H, 1 × gemCH_3), 2.37 [s, 6H, $N(CH_3)_2$], 2.61 (m, 3H, H-1a and $NHCH_2CH_2NMe_2$), 2.78 [m, 2H, $NHCH_2CH_2NMe_2$ and H-2), 3.05 (m, 1H, $NHCH_2CH_2NMe_2$), 3.15 (dd, $J = 14$ Hz, 5 Hz, 1H, H-1b), 3.91 (s, 3H, CH_3O), 6.25 (s, 1H, H-5), 7.29 (dt, $J = 8$ Hz, 2 Hz, 1H, H-9), 7.37 (dd, $J = 8$ Hz, 2 Hz, 1H, H-11), 7.59 (dt, $J = 8$ Hz, 2 Hz, 1H, H-10), 8.25 (dd, $J = 8$ Hz, 2 Hz, 1H, H-8); ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm): 20.73 (1 × gemCH_3), 23.19 (C-1), 26.39 (1 × gemCH_3), 44.88 [$N(CH_3)_2$], 45.50 ($NHCH_2CH_2NMe_2$), 56.09 (CH_3O), 57.81 (C-2), 58.73 ($NHCH_2CH_2NMe_2$), 79.39 (C-3), 95.67 (C-5), 100.41 (C-12b), 106.77 (C-6a), 116.84 (C-11), 122.83 (C-7a), 123.68 (C-9), 126.54 (C-8), 133.38 (C-10), 154.66 (C-11a), 156.61 (C-12a), 158.66 (C-4a), 160.10 (C-6), 175.42 (C-7). Anal. Calcd. for $C_{23}H_{28}N_2O_4 \cdot 2HCl$. Calcd. (%): C: 58.85, H: 6.44, N: 6.46. Found (%): C: 58.76, H: 6.38, N: 6.25.

4.1.5. N,N-Diethyl-N′-[1,2-dihydro-3,3-dimethyl-6-methoxy-7-oxo-3H,7H-pyrano[2,3-c]xanthen-2-yl]ethane-1,2-diamine (**10b**)

Compound **10b** was prepared by a procedure analogous to that of **10a**. Yield: 68%; mp (dihydrochloride) > 250 °C

(EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.00 [t, $J = 7$ Hz, 6H, $N(CH_2CH_3)_2$], 1.29 (s, 3H, 1 × gemCH_3), 1.44 (s, 3H, 1 × gemCH_3), 2.56 [m, 7H, $N(CH_2CH_3)_2$, $NHCH_2CH_2NEt_2$ and H-1a], 2.69 (m, 1H, $NHCH_2CH_2NEt_2$), 2.76 (dd, $J = 14$ Hz, 5 Hz, 1H, H-2), 2.93 (m, 1H, $NHCH_2CH_2NEt_2$), 3.14 (dd, $J = 14$ Hz, 5 Hz, 1H, H-1b), 3.91 (s, 3H, CH_3O), 6.26 (s, 1H, H-5), 7.29 (dt, $J = 8$ Hz, 2 Hz, 1H, H-9), 7.38 (dd, $J = 8$ Hz, 2 Hz, 1H, H-11), 7.60 (dt, $J = 8$ Hz, 2 Hz, 1H, H-10), 8.26 (dd, $J = 8$ Hz, 2 Hz, 1H, H-8); ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm): 11.57 [$N(CH_2CH_3)_2$], 20.65 (1 × gemCH_3), 23.22 (C-1), 26.42 (1 × gemCH_3), 46.05 ($NHCH_2CH_2NEt_2$), 46.75 [$N(CH_2CH_3)_2$], 52.67 ($NHCH_2CH_2NEt_2$), 56.16 (CH_3O), 57.92 (C-2), 79.46 (C-3), 95.71 (C-5), 100.60 (C-12b), 106.88 (C-6a), 116.85 (C-11), 122.95 (C-7a), 123.72 (C-9), 126.70 (C-8), 133.42 (C-10), 154.78 (C-11a), 156.69 (C-12a), 158.75 (C-4a), 160.18 (C-6), 175.58 (C-7). Anal. Calcd. for $C_{25}H_{32}N_2O_4 \cdot 2HCl$. Calcd. (%): C: 60.36, H: 6.89, N: 6.06. Found (%): C: 60.27, H: 6.82, N: 5.91.

4.1.6. 3,3-Dimethyl-6-hydroxy-2-nitro-3H,7H-pyrano [2,3-c]xanthen-7-one (**11**)

To a suspension of **5** (500 mg, 1.70 mmol) in glacial acetic acid (30 mL) at 0 °C, was added dropwise a solution of nitric acid 65% (165 μL, 1.70 mmol) in glacial acetic acid (1 mL). After 30 min, the reaction mixture was poured into ice-water and the resulting mixture was neutralized with 10% sodium carbonate, extracted with EtOAc (3 × 50 mL), dried (Na_2SO_4) and the solvent was evaporated to dryness. Flash chromatography on silica gel, using a mixture of cyclohexane–EtOAc 95:5 as the eluent, provided compound **11** (323 mg, 56%). Mp 168–170 °C (EtOAc–*n*-pentane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.79 (s, 6H, 2 × gemCH_3), 6.28 (s, 1H, H-5), 7.43 (dt, $J = 8$ Hz, 2 Hz, 1H, H-9), 7.52 (dd, $J = 8$ Hz, 2 Hz, 1H, H-11), 7.77 (dt, $J = 8$ Hz, 2 Hz, 1H, H-10), 8.21 (s, 1H, H-1), 8.23 (dd, $J = 8$ Hz, 2 Hz, 1H, H-8), 13.34 (s, 1H, D_2O exch., OH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm): 26.43 (2 × gemCH_3), 80.62 (C-3), 98.33 (C-12b), 99.66 (C-5), 104.49 (C-6a), 117.78 (C-11), 120.58 (C-7a), 122.66 (C-1), 125.12 (C-9), 126.03 (C-8), 135.71 (C-10), 143.34 (C-2), 154.59 (C-12a), 155.54 (C-11a), 161.01 (C-4a), 167.35 (C-6), 180.59 (C-7). Anal. Calcd. for $C_{18}H_{13}NO_6$. Calcd. (%): C: 63.72, H: 3.86, N: 4.13. Found (%): C: 63.64, H: 3.70, N: 4.01.

4.1.7. 3,3-Dimethyl-6-[(4-methylphenyl)sulfonyloxy)]-2-nitro-3H,7H-pyrano[2,3-c]xanthen-7-one (**12**)

Compound **12** was prepared according to the procedure described for the preparation of **3**, starting from **11**. Yield: 92%; mp > 250 °C (EtOAc–*n*-hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.81 (s, 6H, 2 × gemCH_3), 2.42 (s, 3H, $SO_2C_6H_4CH_3$), 6.76 (s, 1H, H-5), 7.34 (d, $J = 8$ Hz, 2H, H-3′ and H-5′), 7.37 (dt, $J = 8$ Hz, 2 Hz, 1H, H-9), 7.46 (dd, $J = 8$ Hz, 2 Hz, 1H, H-11), 7.69 (dt, $J = 8$ Hz, 2 Hz, 1H, H-10), 7.98 (d, $J = 8$ Hz, 2H, H-2′ and H-6′), 8.20 (s, 1H, H-1), 8.21 (dd, $J = 8$ Hz, 2 Hz, 1H, H-8); ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm): 21.74 ($SO_2C_6H_4CH_3$), 26.47 (2 × gemCH_3), 80.87 (C-3), 105.96 (C-12b), 108.68 (C-5), 110.42 (C-6a), 117.22 (C-11), 121.45 (C-1), 122.39 (C-7a), 124.94 (C-9), 126.94 (C-8), 128.93 (C-2′ and C-6′), 129.69 (C-3′ and C-5′), 132.66 (C-1′), 134.80 (C-10), 145.74 (C-4′), 146.57 (C-2), 151.68 (C-6), 154.46 (C-11a), 156.37 (C-12a), 157.30 (C-4a), 173.24 (C-7). Anal. Calcd. for $C_{25}H_{19}NO_8S$. Calcd. (%): C: 60.85, H: 3.88, N: 2.84. Found (%): C: 61.11, H: 4.17, N: 2.56.

4.1.8. N,N-Dimethyl-N′-[3,3-dimethyl-2-nitro-7-oxo-3H,7H-pyrano[2,3-c]xanthen-6-yl]ethane-1,2-diamine (**13a**)

A solution of **12** (153 mg, 0.31 mmol) and 2-dimethylaminoethylamine (340 μL, 3.1 mmol) in absolute ethanol (7 mL) was refluxed for 2 h. The reaction mixture was then vacuum-evaporated, extracted with CH_2Cl_2–water, the organic layer was dried (Na_2SO_4) and the solvent was evaporated to dryness. The residue was purified by column chromatography (silica gel, CH_2Cl_2:CH_3OH 98:2) to furnish compound **13a** (110 mg, 87%). Mp (hydrochloride) > 250 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.69 (s, 6H, 2 × gemCH_3), 2.27 [s, 6H, $N(CH_3)_2$], 2.57 (t, $J = 7$ Hz, 2H, $NHCH_2CH_2NMe_2$), 3.20 (q, $J = 7$ Hz, 5 Hz, 2H, $NHCH_2CH_2NMe_2$), 5.72 (s, 1H, H-5), 7.21 (dt, $J = 8$ Hz, 2 Hz, 1H, H-9), 7.25 (dd, $J = 8$ Hz, 2 Hz, 1H, H-11), 7.53 (dt, $J = 8$ Hz, 2 Hz, 1H, H-10), 8.03 (dd, $J = 8$ Hz, 2 Hz, 1H, H-8), 8.07 (s, 1H, H-1), 10.16 (t, $J = 5$ Hz, 1H, D_2O exch., NH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm): 26.38 (2 × gemCH_3), 41.17 ($NHCH_2CH_2NMe_2$), 45.48 [$N(CH_3)_2$], 57.38 ($NHCH_2CH_2NMe_2$), 80.03 (C-3), 91.42 (C-5), 95.67 (C-12b), 102.50 (C-6a), 117.03 (C-11), 121.89 (C-7a), 124.36 (C-9), 124.61 (C-1), 126.01 (C-8), 134.21 (C-10), 140.93 (C-2), 154.47 (C-11a), 155.63 (C-6), 156.36 (C-12a), 159.72 (C-4a), 177.79 (C-7). Anal. Calcd. for $C_{22}H_{23}N_3O_5 \cdot HCl$. Calcd. (%): C: 59.26, H: 5.43, N: 9.42. Found (%): C: 59.12, H: 5.39, N: 9.37.

4.1.9. N,N-Diethyl-N′-[3,3-dimethyl-2-nitro-7-oxo-3H,7H-pyrano[2,3-c]xanthen-6-yl]ethane-1,2-diamine (**13b**)

Compound **13b** was prepared by a procedure analogous to that of **13a**. Yield: 89%; mp (hydrochloride) > 250 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.07 [t, $J = 7$ Hz, 6H, $N(CH_2CH_3)_2$], 1.74 (s, 6H, 2 × gemCH_3), 2.61 [q, $J = 7$ Hz, 4H, $N(CH_2CH_3)_2$], 2.75 (t, $J = 7$ Hz, 2H, $NHCH_2CH_2NEt_2$), 3.24 (q, $J = 7$ Hz, 5 Hz, 2H, $NHCH_2CH_2NEt_2$), 5.79 (s, 1H, H-5), 7.27 (dt, $J = 8$ Hz, 2 Hz, 1H, H-9), 7.32 (dd, $J = 8$ Hz, 2 Hz, 1H, H-11), 7.59 (dt, $J = 8$ Hz, 2 Hz, 1H, H-10), 8.09 (dd, $J = 8$ Hz, 2 Hz, 1H, H-8), 8.15 (s, 1H, H-1), 10.21 (t, $J = 5$ Hz, 1H, D_2O exch., NH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm): 11.87 [$N(CH_2CH_3)_2$], 26.36 (2 × gemCH_3), 41.37 ($NHCH_2CH_2NEt_2$), 46.95 [$N(CH_2CH_3)_2$], 50.97 ($NHCH_2CH_2NEt_2$), 80.12 (C-3), 91.64 (C-5), 95.65 (C-12b), 102.64 (C-6a), 117.12 (C-11), 121.84 (C-7a), 124.28 (C-9), 124.93 (C-1), 126.02 (C-8), 134.05 (C-10), 140.86 (C-2), 154.48 (C-11a), 155.70 (C-6), 156.75 (C-12a), 159.71 (C-4a), 177.69 (C-7). Anal. Calcd. for $C_{24}H_{27}N_3O_5 \cdot HCl$. Calcd. (%): C: 60.82, H: 5.95, N: 8.87. Found (%): C: 60.69, H: 5.78, N: 8.99.

4.1.10. N,N-Dimethyl-N′-[1,2-dihydro-3,3-dimethyl-2-nitro-7-oxo-3H,7H-pyrano[2,3-c]xanthen-6-yl]ethane-1,2-diamine (**14a**)

To a solution of **13a** (70 mg, 0.17 mmol) in a 3/1 mixture of $CH_3OH-CH_2Cl_2$ (10 mL) at 0 °C was added sodiumborohydride (20 mg, 0.51 mmol), and the resulting mixture was stirred at room temperature for 30 min. The mixture was then poured into ice-water, extracted with CH_2Cl_2 (3×20 mL), dried (Na_2SO_4) and the solvent was evaporated to dryness. Flash chromatography on silica gel, using a mixture of CH_2Cl_2:CH_3OH 95:5 as the eluent, provided compound **14a** (57 mg, 82%). Mp (hydrochloride) 243–245 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.41 (s, 3H, $1 \times$ gemCH_3), 1.46 (s, 3H, $1 \times$ gemCH_3), 2.31 [s, 6H, $N(CH_3)_2$], 2.65 (t, $J = 7$ Hz, 2H, $NHCH_2$-CH_2NMe_2), 3.26 (m, 3H, $NHCH_2CH_2NMe_2$ and H-1a), 3.35 (dd, $J = 14$ Hz, 5 Hz, 1H, H-1b), 4.86 (t, $J = 5$ Hz, 1H, H-2), 5.84 (s, 1H, H-5), 7.25 (m, 2H, H-9 and H-11), 7.53 (dt, $J = 8$ Hz, 2 Hz, 1H, H-10), 8.14 (dd, $J = 8$ Hz, 2 Hz, 1H, H-8), 9.41 (t, $J = 5$ Hz, 1H, D_2O exch., NH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm): 21.46 (C-1), 22.18 ($1 \times$ gemCH_3), 25.75 ($1 \times$ gemCH_3), 40.77 ($NHCH_2CH_2NMe_2$), 45.42 [$N(CH_3)_2$], 57.94 ($NHCH_2CH_2NMe_2$), 74.75 (C-3), 85.31 (C-2), 91.39 (C-5), 91.47 (C-12b), 102.83 (C-6a), 116.60 (C-11), 121.97 (C-7a), 123.58 (C-9), 125.90 (C-8), 133.59 (C-10), 151.30 (C-6), 154.69 (C-11a), 155.95 (C-12a), 158.09 (C-4a), 177.94 (C-7). Anal. Calcd. for $C_{22}H_{25}N_3O_5 \cdot HCl$. Calcd. (%): C: 59.12, H: 5.64, N: 9.40. Found (%): C: 58.96, H: 5.59, N: 9.21.

4.1.11. N,N-Diethyl-N′-[1,2-dihydro-3,3-dimethyl-2-nitro-7-oxo-3H,7H-pyrano[2,3-c]xanthen-6-yl]ethane-1,2-diamine (**14b**)

Compound **14b** was prepared by a procedure analogous to that of **14a**. Yield: 90%; mp (hydrochloride) > 250 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.04 [t, $J = 7$ Hz, 6H, $N(CH_2CH_3)_2$], 1.39 (s, 3H, $1 \times$ gemCH_3), 1.45 (s, 3H, $1 \times$ gemCH_3), 2.59 [q, $J = 7$ Hz, 4H, $N(CH_2CH_3)_2$], 2.74 (t, $J = 7$ Hz, 2H, $NHCH_2CH_2NEt_2$), 3.20 (m, 3H, $NHCH_2CH_2NEt_2$ and H-1a), 3.32 (dd, $J = 14$ Hz, 7 Hz, 1H, H-1b), 4.84 (t, $J = 7$ Hz, 1H, H-2), 5.81 (s, 1H, H-5), 7.21 (m, 2H, H-9 and H-11), 7.50 (dt, $J = 8$ Hz, 2 Hz, 1H, H-10), 8.11 (dd, $J = 8$ Hz, 2 Hz, 1H, H-8), 9.35 (t, $J = 5$ Hz, 1H, D_2O exch., NH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm): 11.56 [$N(CH_2CH_3)_2$], 21.43 (C-1), 21.88 ($1 \times$ gemCH_3), 25.81 ($1 \times$ gemCH_3), 41.08 ($NHCH_2CH_2NEt_2$), 47.07 [$N(CH_2CH_3)_2$], 51.20 ($NHCH_2CH_2NEt_2$), 75.15 (C-3), 85.47 (C-2), 91.46 (C-5), 91.93 (C-12b), 103.02 (C-6a), 116.64 (C-11), 122.01 (C-7a), 123.66 (C-9), 125.94 (C-8), 133.57 (C-10), 151.33 (C-6), 154.84 (C-11a), 156.08 (C-12a), 158.14 (C-4a), 178.17 (C-7). Anal. Calcd. for $C_{24}H_{29}N_3O_5 \cdot HCl$. Calcd. (%): C: 60.56, H: 6.35, N: 8.83. Found (%): C: 60.41, H: 6.32, N: 8.68.

4.1.12. N,N-Dimethyl-N′-[2-amino-1,2-dihydro-3,3-dimethyl-7-oxo-3H,7H-pyrano[2,3-c]xanthen-6-yl]ethane-1,2-diamine (**15a**)

To a solution of **14a** (150 mg, 0.36 mmol) in ethanol (10 mL) at 45 °C were added Raney nickel (36 mg) and hydrazine 40% (197 μL), and the resulting mixture was stirred at this temperature for 15 min. The reaction mixture was then filtered hot, the precipitate was washed with CH_2Cl_2 (2×40 mL) and CH_3OH (40 mL) and the filtrate was concentrated to dryness. Flash chromatography on silica gel using a mixture of CH_2Cl_2:CH_3OH 94:6 as the eluent, provided compound **15a** (130 mg, 95%). Mp (dihydrochloride) > 250 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.30 (s, 3H, $1 \times$ gemCH_3), 1.40 (s, 3H, $1 \times$ gemCH_3), 1.81 (s, D_2O exch., 2H, NH_2), 2.31 [s, 6H, $N(CH_3)_2$], 2.56 (dd, $J = 17$ Hz, 7 Hz, 1H, H-1a), 2.64 (t, $J = 7$ Hz, 2H, $NHCH_2CH_2NMe_2$), 3.03 (m, 2H, H-1b and H-2), 3.25 (q, $J = 7$ Hz, 5 Hz, 2H, $NHCH_2CH_2NMe_2$), 5.85 (s, 1H, H-5), 7.26 (dt, $J = 8$ Hz, 2 Hz, 1H, H-9), 7.31 (dd, $J = 8$ Hz, 2 Hz, 1H, H-11), 7.55 (dt, $J = 8$ Hz, 2 Hz, 1H, H-10), 8.19 (dd, $J = 8$ Hz, 2 Hz, 1H, H-8), 9.39 (t, $J = 5$ Hz, 1H, D_2O exch., NH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm): 21.04 ($1 \times$ gemCH_3), 26.03 (C-1), 26.53 ($1 \times$ gemCH_3), 41.19 ($NHCH_2CH_2NMe_2$), 45.47 [$N(CH_3)_2$], 51.35 (C-2), 58.13 ($NHCH_2CH_2NMe_2$), 78.81 (C-3), 91.65 (C-5), 94.68 (C-12b), 102.71 (C-6a), 116.79 (C-11), 122.32 (C-7a), 123.39 (C-9), 125.25 (C-8), 133.55 (C-10), 151.21 (C-6), 155.13 (C-11a), 156.74 (C-12a), 159.95 (C-4a), 178.31 (C-7). Anal. Calcd. for $C_{22}H_{27}N_3O_3 \cdot 2HCl$. Calcd. (%): C: 58.15, H: 6.43, N: 9.25. Found (%): C: 58.22, H: 6.39, N: 9.05.

4.1.13. N,N-Diethyl-N′-[2-amino-1,2-dihydro-3,3-dimethyl-7-oxo-3H,7H-pyrano[2,3-c]xanthen-6-yl]ethane-1,2-diamine (**15b**)

Compound **15b** was prepared by a procedure analogous to that of **15a**. Yield: 94%; mp (dihydrochloride) > 250 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.09 [t, $J = 7$ Hz, 6H, $N(CH_2CH_3)_2$], 1.30 (s, 3H, $1 \times$ gemCH_3), 1.39 (s, 3H, $1 \times$ gemCH_3), 1.81 (s, D_2O exch., 2H, NH_2), 2.57 (dd, $J = 17$ Hz, 7 Hz, 1H, H-1a), 2.67 [q, $J = 7$ Hz, 4H, $N(CH_2CH_3)_2$], 2.83 (t, $J = 7$ Hz, 2H, $NHCH_2CH_2NEt_2$), 3.04 (m, 2H, H-1b and H-2), 3.31 (q, $J = 7$ Hz, 5 Hz, 2H, $NHCH_2CH_2NEt_2$), 5.87 (s, 1H, H-5), 7.27 (dt, $J = 8$ Hz, 2 Hz, 1H, H-9), 7.34 (dd, $J = 8$ Hz, 2 Hz, 1H, H-11), 7.57 (dt, $J = 8$ Hz, 2 Hz, 1H, H-10), 8.18 (dd, $J = 8$ Hz, 2 Hz, 1H, H-8), 9.35 (t, $J = 5$ Hz, 1H, D_2O exch., NH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm): 11.66 [$N(CH_2CH_3)_2$], 20.66 ($1 \times$ gemCH_3), 26.18 (C-1), 26.34 ($1 \times$ gemCH_3), 41.12 ($NHCH_2CH_2NEt_2$), 47.25 [$N(CH_2CH_3)_2$], 51.55 (C-2), 51.62 ($NHCH_2CH_2NEt_2$), 78.96 (C-3), 91.85 (C-5), 94.72 (C-12b), 102.49 (C-6a), 117.01 (C-11), 122.33 (C-7a), 123.56 (C-9), 126.22 (C-8), 133.58 (C-10), 151.38 (C-6), 155.06 (C-11a), 156.50 (C-12a), 159.97 (C-4a), 178.38 (C-7). Anal. Calcd. for $C_{24}H_{31}N_3O_3 \cdot 2HCl$. Calcd. (%): C: 59.75, H: 6.89, N: 8.71. Found (%): C: 59.61, H: 6.83, N: 8.50.

4.1.14. 2-Chloro-N-[1,2-dihydro-6-(2-dimethylaminoethylamino)-3,3-dimethyl-7-oxo-3H,7H-pyrano[2,3-c]xanthen-2-yl]acetamide (**16a**)

To a solution of **15a** (40 mg, 0.10 mmol) in dry CH_2Cl_2 (4 mL) were added chloroacetic acid (10 mg, 0.11 mmol) and *N*,*N*′-dicyclohexylcarbodiimide (22 mg, 0.11 mmol), and the

resulting mixture was stirred at room temperature for 30 min. The reaction mixture was then filtered, the precipitate was washed with CH_2Cl_2 (2 × 10 mL) and the filtrate was concentrated to dryness. Flash chromatography on silica gel using a mixture of CH_2Cl_2:CH_3OH 95:5 as the eluent, provided compound **16a** (43 mg, 94%). Mp 228–230 °C (CH_2Cl_2–*n*-pentane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.36 (s, 3H, 1 × gemCH_3), 1.41 (s, 3H, 1 × gemCH_3), 2.34 [s, 6H, N$(CH_3)_2$], 2.67 (t, $J = 7$ Hz, 2H, NHCH$_2$CH_2NMe$_2$), 2.90 (dd, $J = 16$ Hz, 3 Hz, 1H, H-1a), 3.06 (dd, $J = 16$ Hz, 5 Hz, 1H, H-1b), 3.23 (q, $J = 7$ Hz, 5 Hz, 2H, NHCH_2CH$_2$NMe$_2$), 4.07 (s, 2H, NHCOCH_2Cl), 4.35 (m, 1H, H-2), 5.81 (s, 1H, H-5), 6.88 (d, $J = 9$ Hz, 1H, N*H*COCH$_2$Cl), 7.29 (dt, $J = 8$ Hz, 2 Hz, 1H, H-9), 7.32 (dd, $J = 8$ Hz, 2 Hz, 1H, H-11), 7.59 (dt, $J = 8$ Hz, 2 Hz, 1H, H-10), 8.19 (dd, $J = 8$ Hz, 2 Hz, 1H, H-8), 9.40 (t, $J = 5$ Hz, 1H, D_2O exch., N*H*CH$_2$CH$_2$NMe$_2$); ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm): 23.63 (C-1), 24.56 (2 × gemCH_3), 40.61 (NH*C*H$_2$CH$_2$NMe$_2$), 42.68 (NHCO-*C*H$_2$Cl), 45.29 [N$(CH_3)_2$], 48.46 (C-2), 57.68 (NHCH$_2$-*C*H$_2$NMe$_2$), 77.18 (C-3), 91.90 (C-5), 92.65 (C-12b), 103.10 (C-6a), 116.90 (C-11), 122.10 (C-7a), 123.69 (C-9), 126.07 (C-8), 133.66 (C-10), 151.03 (C-6), 154.95 (C-11a), 156.94 (C-12a), 159.28 (C-4a), 165.89 (NH*C*OCH$_2$Cl), 178.33 (C-7). Anal. Calcd. for $C_{24}H_{28}ClN_3O_4$. Calcd. (%): C: 62.94, H: 6.16, N: 9.18. Found (%): C: 62.85, H: 6.02, N: 8.91.

*4.1.15. 2-Chloro-N-[6-(2-diethylaminoethylamino)-1,2-dihydro-3,3-dimethyl-7-oxo-3H,7H-pyrano[2,3-c]xanthen-2-yl]acetamide (**16b**)*

Compound **16b** was prepared by a procedure analogous to that of **16a**. Yield: 95%; mp 184–186 °C (CH_2Cl_2–*n*-pentane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.28 [t, $J = 7$ Hz, 6H, N$(CH_2CH_3)_2$], 1.35 (s, 3H, 1 × gemCH_3), 1.39 (s, 3H, 1 × gemCH_3), 2.88 (dd, $J = 17$ Hz, 3 Hz, 1H, H-1a), 3.03 (dd, $J = 17$ Hz, 5 Hz, 1H, H-1b), 3.12 [q, $J = 7$ Hz, 4H, N$(CH_2CH_3)_2$], 3.20 (t, $J = 7$ Hz, 2H, NHCH$_2$CH_2NEt$_2$), 3.64 (q, $J = 7$ Hz, 5 Hz, 2H, NHCH_2CH$_2$NEt$_2$), 4.04 (s, 2H, NHCOCH_2Cl), 4.32 (m, 1H, H-2), 5.92 (s, 1H, H-5), 6.87 (d, $J = 9$ Hz, 1H, N*H*COCH$_2$Cl), 7.28 (m, 2H, H-9 and H-11), 7.58 (dt, $J = 8$ Hz, 2 Hz, 1H, H-10), 8.12 (dd, $J = 8$ Hz, 2 Hz, 1H, H-8), 9.46 (t, $J = 5$ Hz, 1H, D_2O exch., N*H*CH$_2$CH$_2$NEt$_2$); ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm): 8.81 [N$(CH_2CH_3)_2$], 23.58 (C-1), 24.40 (1 × gemCH_3), 24.63 (1 × gemCH_3), 38.03 (NH*C*H$_2$CH$_2$NEt$_2$), 42.66 (NHCO*C*H$_2$Cl), 46.34 [N$(CH_2CH_3)_2$], 48.43 (C-2), 49.66 (NHCH$_2$*C*H$_2$NEt$_2$), 77.42 (C-3), 92.19 (C-5), 93.96 (C-12b), 103.09 (C-6a), 117.09 (C-11), 121.77 (C-7a), 123.90 (C-9), 125.95 (C-8), 133.94 (C-10), 150.28 (C-6), 154.93 (C-11a), 156.98 (C-12a), 159.52 (C-4a), 165.97 (NH*C*OCH$_2$Cl), 178.61 (C-7). Anal. Calcd. for $C_{26}H_{32}ClN_3O_4$. Calcd. (%): C: 64.25, H: 6.64, N: 8.65. Found (%): C: 64.03, H: 6.52, N: 8.57.

*4.1.16. 2-Dimethylamino-N-[1,2-dihydro-6-(2-dimethylaminoethylamino)-3,3-dimethyl-7-oxo-3H,7H-pyrano[2,3-c]xanthen-2-yl]acetamide (**17a**)*

To a solution of the chloride **16a** (442 mg, 1 mmol) in absolute ethanol (10 mL), was added a 33% solution of dimethylamine in ethanol (1 mL) and the resulting solution was heated at reflux for 10 h. Upon cooling, the solvent was vacuum-evaporated and the residue was purified by column chromatography (silica gel) using a mixture of CH_2Cl_2:CH_3OH 8:1 as the eluent, to provide compound **17a** as an oil (396 mg, 88%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.37 (s, 3H, 1 × gemCH_3), 1.38 (s, 3H, 1 × gemCH_3), 2.21 [s, 6H, NHCOCH$_2$N(C$H_3)_2$], 2.56 [s, 6H, NHCH$_2$CH$_2$-N(C$H_3)_2$], 2.86 (dd, $J = 17$ Hz, 4 Hz, 1H, H-1a), 2.95 (m, 4H, NHCOCH_2NMe$_2$ and NHCH$_2$CH_2NMe$_2$), 3.04 (dd, $J = 17$ Hz, 5 Hz, 1H, H-1b), 3.50 (q, $J = 7$ Hz, 5 Hz, 2H, NHCH_2CH$_2$NMe$_2$), 4.32 (m, 1H, H-2), 5.93 (s, 1H, H-5), 7.30 (dt, $J = 8$ Hz, 2 Hz, 1H, H-9), 7.34 (d, $J = 9$ Hz, 1H, N*H*COCH$_2$NMe$_2$), 7.35 (dd, $J = 8$ Hz, 2 Hz, 1H, H-11), 7.61 (dt, $J = 8$ Hz, 2 Hz, 1H, H-10), 8.18 (dd, $J = 8$ Hz, 2 Hz, 1H, H-8), 9.49 (t, $J = 5$ Hz, 1H, D_2O exch., N*H*CH$_2$CH$_2$NMe$_2$); ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm): 73.76 (C-1), 24.10 (1 × gemCH_3), 25.01 (1 × gemCH_3), 39.30 (NH*C*H$_2$CH$_2$-NMe$_2$), 44.34 [NHCH$_2$CH$_2$N(*C*H$_3)_2$], 45.76 [NHCOCH$_2$N-(*C*H$_3)_2$], 47.62 (C-2), 56.82 (NHCH$_2$*C*H$_2$NMe$_2$), 62.85 (NHCO*C*H$_2$NMe$_2$), 78.34 (C-3), 92.20 (C-5), 94.31 (C-12b), 103.01 (C-6a), 117.08 (C-11), 121.95 (C-7a), 123.80 (C-9), 125.95 (C-8), 133.87 (C-10), 150.36 (C-6), 155.00 (C-11a), 156.94 (C-12a), 159.74 (C-4a), 170.11 (NH*C*OCH$_2$NMe$_2$), 178.68 (C-7). Anal. Calcd. for $C_{26}H_{34}N_4O_4$. Calcd. (%): C: 66.93, H: 7.35, N: 12.01. Found (%): C: 66.81, H: 7.20, N: 11.87.

*4.1.17. 2-Diethylamino-N-[6-(2-diethylaminoethylamino)-1,2-dihydro-3,3-dimethyl-7-oxo-3H,7H-pyrano[2,3-c]xanthen-2-yl]acetamide (**17b**)*

Compound **17b** was prepared by a procedure analogous to that of **17a**. Yield: 87%; mp (dihydrochloride) > 250 °C (EtOH–Et_2O); ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.84 [t, $J = 7$ Hz, 6H, NHCOCH$_2$N(CH$_2$C$H_3)_2$], 1.11 [t, $J = 7$ Hz, 6H, NHCH$_2$CH$_2$N(CH$_2$C$H_3)_2$], 1.36 (s, 6H, 2 × gemCH_3), 2.42 [q, $J = 7$ Hz, 4H, NHCOCH$_2$N(CH_2CH$_3)_2$], 2.70 [q, $J = 7$ Hz, 4H, NHCH$_2$CH$_2$N(CH_2CH$_3)_2$], 2.85 (m, 3H, NHCH$_2$CH_2NEt$_2$ and H-1a), 2.92 (d, $J = 17$ Hz, 1H, NHCOCH_2NEt$_2$), 3.01 (dd, $J = 17$ Hz, 5 Hz, 1H, H-1b), 3.05 (d, $J = 17$ Hz, 1H, NHCOCH_2NEt$_2$), 3.34 (q, $J = 7$ Hz, 5 Hz, 2H, NHCH_2CH$_2$NEt$_2$), 4.27 (m, 1H, H-2), 5.93 (s, 1H, H-5), 7.29 (dt, $J = 8$ Hz, 2 Hz, 1H, H-9), 7.34 (dd, $J = 8$ Hz, 2 Hz, 1H, H-11), 7.59 (dt, $J = 8$ Hz, 2 Hz, 1H, H-10), 7.74 (d, $J = 9$ Hz, 1H, N*H*COCH$_2$NEt$_2$), 8.20 (dd, $J = 8$ Hz, 2 Hz, 1H, H-8), 9.39 (t, $J = 5$ Hz, 1H, D_2O exch., N*H*CH$_2$CH$_2$NEt$_2$); ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm): 9.57 [NHCH$_2$-CH$_2$N(CH$_2$*C*H$_3)_2$], 11.16 [NHCOCH$_2$N(CH$_2$*C*H$_3)_2$], 23.59 (C-1), 23.86 (1 × gemCH_3), 24.95 (1 × gemCH_3), 38.74 (NH*C*H$_2$CH$_2$NEt$_2$), 47.23 [NHCH$_2$CH$_2$N(*C*H$_2$CH$_3)_2$], 47.73 (C-2), 48.73 [NHCOCH$_2$N(*C*H$_2$CH$_3)_2$], 50.20 (NHCH$_2$*C*H$_2$-NEt$_2$), 57.07 (NHCO*C*H$_2$NEt$_2$), 77.53 (C-3), 92.01 (C-5), 94.28 (C-12b), 102.85 (C-6a), 117.05 (C-11), 121.77 (C-7a), 123.77 (C-9), 125.85 (C-8), 133.88 (C-10), 150.22 (C-6), 154.93 (C-11a), 156.88 (C-12a), 159.70 (C-4a), 171.63 (NH*C*OCH$_2$NEt$_2$), 178.57 (C-7). Anal. Calcd. for $C_{30}H_{42}$

$N_4O_4 \cdot 2HCl$. Calcd. (%): C: 60.50, H: 7.45, N: 9.41. Found (%): C: 60.34, H: 7.39, N: 9.27.

4.1.18. *tert*-Butyl-N-[1,2-dihydro-6-(2-dimethylaminoethylamino)-3,3-dimethyl-7-oxo-3H,7H-pyrano[2,3-c]xanthen-2-yl]carbamate (**18a**)

To a solution of **15a** (128 mg, 0.32 mmol) in dry THF (10 mL) at 0 °C were added triethylamine (223 μL, 1.6 mmol) and di-*tert*-butyl dicarbonate (73 μL, 0.32 mmol) and the resulting solution was stirred at room temperature for 3 h. The reaction mixture was then vacuum-evaporated, extracted with EtOAc–water, the organic layer was dried (Na_2SO_4) and the solvent was evaporated to dryness. The residue was purified by column chromatography (silica gel, CH_2Cl_2:CH_3OH 95:5) to furnish compound **18a** (145 mg, 94%) as an oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.35 (s, 3H, 1 × gemCH_3), 1.39 (s, 3H, 1 × gemCH_3), 1.41 [s, 9H, C(CH_3)$_3$], 2.32 [s, 6H, N(CH_3)$_2$], 2.65 (t, $J = 7$ Hz, 2H, NHCH_2CH_2NMe_2), 2.86 (dd, $J = 17$ Hz, 7 Hz, 1H, H-1a), 3.03 (dd, $J = 17$ Hz, 5 Hz, 1H, H-1b), 3.26 (q, $J = 7$ Hz, 5 Hz, 2H, NHCH_2CH_2NMe_2), 4.00 (m, 1H, H-2), 4.72 (d, $J = 10$ Hz, 1H, D_2O exch., NHCO), 5.87 (s, 1H, H-5), 7.28 (dt, $J = 8$ Hz, 2 Hz, 1H, H-9), 7.34 (dd, $J = 8$ Hz, 2 Hz, 1H, H-11), 7.59 (dt, $J = 8$ Hz, 2 Hz, 1H, H-10), 8.21 (dd, $J = 8$ Hz, 2 Hz, 1H, H-8), 9.43 (t, $J = 5$ Hz, 1H, D_2O exch., NHCH_2CH_2NMe_2); ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm): 23.92 (C-1), 24.01 (1 × gemCH_3), 24.90 (1 × gemCH_3), 28.63 [C(CH_3)$_3$], 40.79 (NHCH_2CH_2NMe_2), 45.49 [N(CH_3)$_2$], 49.22 (C-2), 57.85 (NHCH_2CH_2NMe_2), 77.88 [C(CH_3)$_3$], 79.62 (C-3), 91.77 (C-5), 93.15 (C-12b), 102.95 (C-6a), 116.87 (C-11), 121.97 (C-7a), 123.74 (C-9), 126.09 (C-8), 133.54 (C-10), 150.99 (C-6), 154.92 (C-11a), 155.90 (C-12a), 156.88 (NHCO), 159.62 (C-4a), 178.45 (C-7). Anal. Calcd. for $C_{27}H_{35}N_3O_5$. Calcd. (%): C: 67.34, H: 7.33, N: 8.73. Found (%): C: 67.72, H: 7.64, N: 8.43.

4.1.19. *tert*-Butyl-N-[6-(2-diethylaminoethylamino)-1,2-dihydro-3,3-dimethyl-7-oxo-3H,7H-pyrano[2,3-c]xanthen-2-yl]carbamate (**18b**)

Compound **18b** was prepared by a procedure analogous to that of **18a**. Yield: 86%; isolated as an oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.05 [t, $J = 7$ Hz, 6H, N(CH_2CH_3)$_2$], 1.32 (s, 3H, 1 × gemCH_3), 1.36 (s, 3H, 1 × gemCH_3), 1.38 [s, 9H, C(CH_3)$_3$], 2.61 [q, $J = 7$ Hz, 4H, N(CH_2CH$_3$)$_2$], 2.77 (t, $J = 7$ Hz, 2H, NHCH$_2$CH_2NEt$_2$), 2.82 (dd, $J = 17$ Hz, 7 Hz, 1H, H-1a), 3.00 (dd, $J = 17$ Hz, 5 Hz, 1H, H-1b), 3.25 (q, $J = 7$ Hz, 5 Hz, 2H, NHCH_2CH_2NMe_2), 3.98 (m, 1H, H-2), 4.79 (d, $J = 10$ Hz, 1H, D_2O exch., NHCO), 5.85 (s, 1H, H-5), 7.24 (dt, $J = 8$ Hz, 2 Hz, 1H, H-9), 7.29 (dd, $J = 8$ Hz, 2 Hz, 1H, H-11), 7.55 (dt, $J = 8$ Hz, 2 Hz, 1H, H-10), 8.16 (dd, $J = 8$ Hz, 2 Hz, 1H, H-8), 9.34 (t, $J = 5$ Hz, 1H, D_2O exch., NHCH$_2$CH$_2$NEt$_2$); ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm): 11.30 [N(CH$_2$CH_3)$_2$], 23.40 (C-1), 23.48 (1 × gemCH_3), 24.81 (1 × gemCH_3), 28.45 [C(CH_3)$_3$], 40.95 (NHCH_2-CH$_2$NEt$_2$), 47.21 [N(CH_2CH$_3$)$_2$], 49.23 (C-2), 51.04 (NHCH$_2$CH_2NEt$_2$), 77.87 [C(CH$_3$)$_3$], 79.49 (C-3), 91.79 (C-5), 93.21 (C-12b), 102.89 (C-6a), 117.01 (C-11), 122.06 (C-7a), 123.67 (C-9), 126.09 (C-8), 133.56 (C-10), 151.11 (C-6), 154.94 (C-11a), 155.42 (C-12a), 156.96 (NHCO), 159.58 (C-4a), 178.34 (C-7). Anal. Calcd. for $C_{29}H_{39}N_3O_5$. Calcd. (%): C: 68.35, H: 7.71, N: 8.24. Found (%): C: 68.07, H: 8.04, N: 7.92.

4.1.20. *tert*-Butyl-N-(2-dimethylaminoethyl)-N-[1,2-dihydro-6-(2-dimethylaminoethylamino)-3,3-dimethyl-7-oxo-3H,7H-pyrano[2,3-c]xanthen-2-yl]carbamate (**19a**)

To a solution of **18a** (73 mg, 0.15 mmol) in dry DMF (5 mL) was added NaH under argon (80% in hexanes, 30 mg, 0.75 mmol) and the mixture was stirred at room temperature for 30 min. A solution of 2-dimethylaminoethylchloride (160 mg, 1.5 mmol) in dry DMF (3 mL) was then added dropwise and the mixture was stirred for 18 h. The solvent was then vacuum-evaporated, the residue was extracted with EtOAc (3 × 60 mL), and the combined organic extracts were washed with brine, dried (Na_2SO_4), and concentrated to dryness. The residue was purified by column chromatography (silica gel) eluting with CH_2Cl_2:CH_3OH 95:5, to provide compound **19a** (65 mg, 78%) as an oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.33 (s, 3H, 1 × gemCH_3), 1.36 (s, 3H, 1 × gemCH_3), 1.46 [s, 9H, C(CH$_3$)$_3$], 1.98 [s, 6H, N(Boc)CH$_2$CH$_2$N(CH_3)$_2$], 2.31 [s, 6H, NHCH$_2$CH$_2$N(CH_3)$_2$], 2.64 [m, 4H, NHCH$_2$CH_2NMe_2 and N(Boc)CH$_2$CH_2NMe_2], 2.90 (dd, $J = 17$ Hz, 7 Hz, 1H, H-1a), 3.20 (m, 5H, N(Boc)CH_2CH$_2$NMe_2 and H-1b and NHCH_2CH$_2$NMe_2), 4.64 (dd, $J = 7$ Hz, 5 Hz, 1H, H-2), 5.86 (s, 1H, H-5), 7.29 (dt, $J = 8$ Hz, 2 Hz, 1H, H-9), 7.33 (dd, $J = 8$ Hz, 2 Hz, 1H, H-11), 7.59 (dt, $J = 8$ Hz, 2 Hz, 1H, H-10), 8.20 (dd, $J = 8$ Hz, 2 Hz, 1H, H-8), 9.40 (t, $J = 5$ Hz, 1H, D_2O exch., NHCH$_2$CH$_2$NMe_2); ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm): 21.99 (C-1), 24.50 (1 × gemCH_3), 25.88 (1 × gemCH_3), 28.63 [C(CH_3)$_3$], 40.99 (NHCH_2CH$_2$NMe_2), 41.09 [N(Boc)CH_2CH$_2$NMe_2], 45.12 [N(Boc)CH$_2$CH$_2$N(CH_3)$_2$], 45.57 [NHCH$_2$CH$_2$N(CH_3)$_2$], 51.53 (C-2), 57.94 (NHCH$_2$-CH_2NMe_2), 58.32 [N(Boc)CH$_2$CH_2NMe_2], 79.01 [C(CH$_3$)$_3$], 80.19 (C-3), 92.06 (C-5), 94.36 (C-12b), 103.06 (C-6a), 117.03 (C-11), 122.30 (C-7a), 123.67 (C-9), 126.19 (C-8), 133.52 (C-10), 151.15 (C-6), 155.05 (C-11a), 156.07 (C-12a), 156.19 [COOC(CH$_3$)$_3$], 160.08 (C-4a), 178.41 (C-7). Anal. Calcd. for $C_{31}H_{44}N_4O_5$. Calcd. (%): C: 67.37, H: 8.02, N: 10.14. Found (%): C: 67.69, H: 7.75, N: 10.43.

4.1.21. *tert*-Butyl-N-(2-diethylaminoethyl)-N-[6-(2-diethylaminoethylamino)-1,2-dihydro-3,3-dimethyl-7-oxo-3H,7H-pyrano[2,3-c]xanthen-2-yl]carbamate (**19b**)

Compound **19b** was prepared by a procedure analogous to that of **19a** and was isolated as an oil. Yield: 81%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.74 [t, $J = 7$ Hz, 6H, N(Boc)CH$_2$-CH$_2$N(CH$_2$CH_3)$_2$], 1.03 [t, $J = 7$ Hz, 6H, NHCH$_2$CH$_2$N-(CH$_2$CH_3)$_2$], 1.31 (s, 3H, 1 × gemCH_3), 1.34 (s, 3H, 1 × gemCH_3), 1.44 [s, 9H, C(CH$_3$)$_3$], 2.18 [m, 4H, N(Boc)CH$_2$-CH$_2$N(CH_2CH$_3$)$_2$], 2.32 [m, 2H, N(Boc)CH$_2$CH_2NEt$_2$], 2.58 [q, $J = 7$ Hz, 4H, NHCH$_2$CH$_2$N(CH_2CH$_3$)$_2$], 2.75 [t, $J = 7$ Hz, 2H, NHCH$_2$CH_2NEt$_2$], 2.87 (dd, $J = 17$ Hz, 5 Hz, 1H, H-1a), 3.05 [m, 2H, N(Boc)CH_2CH$_2$NEt$_2$], 3.18 [m, 3H, NHCH_2CH$_2$NEt$_2$ and H-1b], 4.63 (dd, $J = 7$ Hz, 5 Hz, 1H, H-2), 5.84 (s, 1H,

H-5), 7.25 (dt, $J = 8$ Hz, 2 Hz, 1H, H-9), 7.29 (dd, $J = 8$ Hz, 2 Hz, 1H, H-11), 7.55 (dt, $J = 8$ Hz, 2 Hz, 1H, H-10), 8.17 (dd, $J = 8$ Hz, 2 Hz, 1H, H-8), 9.34 (t, $J = 5$ Hz, 1H, D_2O exch., N*H*$CH_2CH_2NEt_2$); ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm): 11.91 [$NHCH_2CH_2N(CH_2CH_3)_2$], 11.99 [N(Boc)CH_2-$CH_2N(CH_2CH_3)_2$], 21.98 (C-1), 24.58 (1 × gemCH_3), 25.98 (1 × gemCH_3), 28.58 [$C(CH_3)_3$], 41.37 ($NHCH_2CH_2NEt_2$), 41.84 [N(Boc)$CH_2CH_2NEt_2$], 47.17 [$NHCH_2CH_2N(CH_2$-$CH_3)_2$], 47.25 [$N(Boc)CH_2CH_2N(CH_2$-$CH_3)_2$], 51.33 ($NHCH_2$-CH_2NEt_2), 51.56 [N(Boc)$CH_2CH_2NEt_2$], 51.98 (C-2), 79.15 [$C(CH_3)_3$], 80.14 (C-3), 92.34 (C-5), 94.33 (C-12b), 102.93 (C-6a), 116.92 (C-11), 122.32 (C-7a), 123.71 (C-9), 126.31 (C-8), 133.71 (C-10), 151.30 (C-6), 155.09 (C-11a), 156.29 (C-12a), 156.49 [$COOC(CH_3)_3$], 160.09 (C-4a), 178.28 (C-7). Anal. Calcd. for $C_{35}H_{52}N_4O_5$. Calcd. (%): C: 69.05, H: 8.61, N: 9.20. Found (%): C: 68.82, H: 8.94, N: 8.92.

*4.1.22. N,N′-Bis(2-dimethylaminoethyl)-1,2-dihydro-3,3-dimethyl-7-oxo-3H,7H-pyrano[2,3-c]xantheno-2,6-diamine (**20a**)*

To a solution of **19a** (50 mg, 0.09 mmol) in dry CH_2Cl_2 (5 mL) at 0 °C was added trifluoroacetic acid (0.5 mL) and the resulting mixture was stirred at room temperature for 1 h. The reaction mixture was then made alkaline with a 10% $NaHCO_3$ solution and extracted with CH_2Cl_2 (3 × 60 mL). The combined organic extracts were washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure and the residue was purified by column chromatography (silica gel, CH_2Cl_2:MeOH 9:1) to afford compound **20a** (35 mg, 86%). Mp (trihydrochloride) > 250 °C (CH_2Cl_2–Et_2O); ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.28 (s, 3H, 1 × gemCH_3), 1.44 (s, 3H, 1 × gemCH_3), 2.22 [s, 6H, 2-$NHCH_2CH_2N(CH_3)_2$], 2.30 [s, 6H, 6-$NHCH_2CH_2N(CH_3)_2$], 2.41 (t, $J = 7$ Hz, 2H, 2-$NHCH_2CH_2NMe_2$), 2.53 (dd, $J = 17$ Hz, 5 Hz, 1H, H-1a), 2.62 [t, $J = 7$ Hz, 2H, 6-$NHCH_2CH_2NMe_2$], 2.72 [m, 2H, H-2 and 2-$NHCH_2CH_2NMe_2$], 2.93 (m, 1H, 2-$NHCH_2CH_2NMe_2$), 3.10 (dd, $J = 17$ Hz, 7 Hz, 1H, H-1b), 3.25 (q, $J = 7$ Hz, 5 Hz, 2H, 6-$NHCH_2CH_2NMe_2$), 5.85 (s, 1H, H-5), 7.28 (dt, $J = 8$ Hz, 2 Hz, 1H, H-9), 7.36 (dd, $J = 8$ Hz, 2 Hz, 1H, H-11), 7.58 (dt, $J = 8$ Hz, 2 Hz, 1H, H-10), 8.21 (dd, $J = 8$ Hz, 2 Hz, 1H, H-8), 9.38 (t, $J = 5$ Hz, 1H, D_2O exch., 6-N*H*$CH_2CH_2NMe_2$); ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm): 20.85 (1 × gemCH_3), 23.10 (C-1), 26.55 (1 × gemCH_3), 40.90 (6-$NHCH_2CH_2NMe_2$), 44.97 [2-$NHCH_2CH_2N(CH_3)_2$], 45.50 [6-$NHCH_2CH_2N(CH_3)_2$], 45.54 [2-$NHCH_2CH_2NMe_2$], 57.91 (6-$NHCH_2CH_2NMe_2$), 58.22 (C-2), 58.84 (2-$NHCH_2$-CH_2NMe_2), 79.01 (C-3), 91.61 (C-5), 94.84 (C-12b), 102.61 (C-6a), 116.92 (C-11), 122.18 (C-7a), 123.50 (C-9), 126.11 (C-8), 133.35 (C-10), 151.11 (C-6), 155.04 (C-11a), 156.54 (C-12a), 159.99 (C-4a), 178.32 (C-7). Anal. Calcd. for $C_{26}H_{36}N_4O_3 \cdot 3HCl$. Calcd. (%): C: 55.57, H: 7.00, N: 9.97. Found (%): C: 55.28, H: 6.82, N: 10.25.

*4.1.23. N,N′-bis(2-Diethylaminoethyl)-1,2-dihydro-3,3-dimethyl-7-oxo-3H,7H-pyrano[2,3-c]xantheno-2,6-diamine (**20b**)*

Compound **20b** was prepared by a procedure analogous to that of **20a**. Yield: 87%; mp (trihydrochloride) > 250 °C (EtOH); ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.03 [t, $J = 7$ Hz, 6H, 2-$NHCH_2CH_2N(CH_2CH_3)_2$], 1.06 [t, $J = 7$ Hz, 6H, 6-$NHCH_2CH_2N(CH_2CH_3)_2$], 1.28 (s, 3H, 1 × gemCH_3), 1.43 (s, 3H, 1 × gemCH_3), 2.53 (dd, $J = 17$ Hz, 7 Hz, 1H, H-1a), 2.60 [m, 10H, 6-$NHCH_2CH_2N(CH_2CH_3)_2$, 2-$NHCH_2CH_2N(CH_2CH_3)_2$ and 2-$NHCH_2CH_2NEt_2$], 2.73 [m, 2H, H-2 and 2-$NHCH_2CH_2NEt_2$], 2.78 (t, $J = 7$ Hz, 2H, 6-$NHCH_2CH_2NEt_2$), 2.95 (m, 1H, 2-$NHCH_2CH_2NEt_2$), 3.08 (dd, $J = 17$ Hz, 5 Hz, 1H, H-1b), 3.25 (q, $J = 7$ Hz, 5 Hz, 2H, 6-$NHCH_2CH_2NEt_2$), 5.86 (s, 1H, H-5), 7.27 (dt, $J = 8$ Hz, 2 Hz, 1H, H-9), 7.36 (dd, $J = 8$ Hz, 2 Hz, 1H, H-11), 7.58 (dt, $J = 8$ Hz, 2 Hz, 1H, H-10), 8.20 (dd, $J = 8$ Hz, 2 Hz, 1H, H-8), 9.34 (t, $J = 5$ Hz, 1H, D_2O exch., 6-N*H*$CH_2CH_2NEt_2$); ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm): 11.61 [2-$NHCH_2$-$CH_2N(CH_2CH_3)_2$], 11.81 [6-$NHCH_2CH_2N(CH_2CH_3)_2$], 20.76 (1 × gemCH_3), 23.18 (C-1), 26.57 (1 × gemCH_3), 41.24 (6-$NHCH_2CH_2NEt_2$), 46.04 (2-$NHCH_2CH_2NEt_2$), 46.97 [2-NH-$CH_2CH_2N(CH_2CH_3)_2$], 47.21 [6-$NHCH_2CH_2N(CH_2CH_3)_2$], 51.44 (6-$NHCH_2CH_2NEt_2$), 52.90 (2-$NHCH_2CH_2NEt_2$), 58.30 (C-2), 79.02 (C-3), 91.60 (C-5), 94.95 (C-12b), 102.61 (C-6a), 116.88 (C-11), 122.24 (C-7a), 123.41 (C-9), 126.07 (C-8), 133.29 (C-10), 151.15 (C-6), 155.10 (C-11a), 156.51 (C-12a), 160.06 (C-4a), 178.24 (C-7). Anal. Calcd. for $C_{30}H_{44}N_4O_3 \cdot 3HCl$. Calcd. (%): C: 58.30, H: 7.66, N: 9.07. Found (%): C: 58.12, H: 7.59, N: 8.86.

*4.1.24. 3,3-Dimethyl-6-methoxy-2-nitro-3H,7H-pyrano[2,3-c]xanthen-7-one (**21**)*

To a solution of **11** (143 mg, 0.42 mmol) in dry THF (5 mL) was added NaH (60% in hexanes, 25 mg, 0.813 mmol) and the mixture was stirred at room temperature, under argon for 30 min. A solution of dimethyl sulfate (55 mg, 0.43 mmol) in dry THF (3 mL) was then added dropwise and the mixture was refluxed for 3 h. After cooling, the excess of NaH was destroyed by the addition of EtOH (1 mL) and the precipitate was filtered off. The filtrate was concentrated under reduced pressure and the residue was purified by column chromatography (silica gel), elution with cyclohexane–EtOAc 4:1, to provide compound **21**. Yield: 76%; mp 224–226 °C (CH_2Cl_2–Et_2O); ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.79 (s, 6H, 2 × gemCH_3), 4.00 (s, 3H, CH_3O), 6.31 (s, 1H, H-5), 7.35 (dt, $J = 8$ Hz, 2 Hz, 1H, H-9), 7.44 (dd, $J = 8$ Hz, 2 Hz, 1H, H-11), 7.66 (dt, $J = 8$ Hz, 2 Hz, 1H, H-10), 8.24 (dd, $J = 8$ Hz, 2 Hz, 1H, H-8), 8.25 (s, 1H, H-1); ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm): 26.45 (2 × gemCH_3), 56.85 (OCH_3), 80.54 (C-3), 95.44 (C-5), 100.11 (C-12b), 107.83 (C-6a), 117.03 (C-11), 122.91 (C-7a), 123.21 (C-1), 124.70 (C-9), 126.81 (C-8), 134.22 (C-10), 143.85 (C-2), 154.47 (C-11a), 155.96 (C-12a), 159.69 (C-4a), 165.84 (C-6), 174.59 (C-7). Anal. Calcd. for $C_{19}H_{15}NO_6$. Calcd. (%): C: 64.59, H: 4.28, N: 3.96. Found (%): C: 64.49, H: 4.22, N: 4.08.

*4.1.25. 1,2-Dihydro-3,3-dimethyl-6-methoxy-2-nitro-3H,7H-pyrano[2,3-c]xanthen-7-one (**22**)*

Compound **22** was prepared by a procedure analogous to that of **14a**. Yield: 88%; mp > 250 °C (CH_2Cl_2–Et_2O); ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.49 (s, 3H, 1 × gemCH_3),

1.51 (s, 3H, 1 × gemCH_3), 3.36 (dd, $J = 17$ Hz, 6 Hz, 1H, H-1a), 3.51 (dd, $J = 17$ Hz, 6 Hz, 1H, H-1b), 3.95 (s, 3H, CH_3O), 4.91 (t, $J = 6$ Hz, 1H, H-2), 6.33 (s, 1H, H-5), 7.33 (dt, $J = 8$ Hz, 2 Hz, 1H, H-9), 7.38 (dd, $J = 8$ Hz, 2 Hz, 1H, H-11), 7.63 (dt, $J = 8$ Hz, 2 Hz, 1H, H-10), 8.28 (dd, $J = 8$ Hz, 2 Hz, 1H, H-8); ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm): 22.83 (1 × gemCH_3), 22.92 (C-1), 25.96 (1 × gemCH_3), 56.61 (OCH_3), 75.72 (C-3), 85.06 (C-2), 96.10 (C-5), 97.76 (C-12b), 108.53 (C-6a), 117.09 (C-11), 123.16 (C-7a), 124.27 (C-9), 127.03 (C-8), 133.94 (C-10), 154.65 (C-11a), 156.66 (C-12a), 157.41 (C-4a), 161.00 (C-6), 175.36 (C-7). Anal. Calcd. for $C_{19}H_{17}NO_6$. Calcd. (%): C: 64.22, H: 4.82, N: 3.94. Found (%): C: 63.95, H: 4.77, N: 3.75.

4.1.26. 2-Amino-1,2-dihydro-3,3-dimethyl-6-methoxy-3H,7H-pyrano[2,3-c]xanthen-7-one (**23**)

Compound **23** was prepared by a procedure analogous to that of **15a**. Yield: 92%; mp > 250 °C (EtOH–Et_2O); ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.33 (s, 3H, 1 × gemCH_3), 1.40 (s, 3H, 1 × gemCH_3), 1.91 (s, 2H, D_2O exch., NH_2), 2.66 (dd, $J = 17$ Hz, 6 Hz, 1H, H-1a), 3.11 (m, 2H, H-1b and H_2), 3.90 (s, 3H, CH_3O), 6.24 (s, 1H, H-5), 7.28 (dt, $J = 8$ Hz, 2 Hz, 1H, H-9), 7.33 (dd, $J = 8$ Hz, 2 Hz, 1H, H-11), 7.58 (dt, $J = 8$ Hz, 2 Hz, 1H, H-10), 8.25 (dd, $J = 8$ Hz, 2 Hz, 1H, H-8); ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm): 20.83 (1 × gemCH_3), 25.78 (1 × gemCH_3), 26.14 (C-1), 50.99 (C-2), 56.21 (OCH_3), 78.88 (C-3), 95.74 (C-5), 100.19 (C-12b), 107.02 (C-6a), 116.89 (C-11), 122.93 (C-7a), 123.83 (C-9), 126.74 (C-8), 133.57 (C-10), 154.77 (C-11a), 156.77 (C-12a), 158.59 (C-4a), 160.25 (C-6), 175.47 (C-7). Anal. Calcd. for $C_{19}H_{19}NO_4 \cdot HCl$. Calcd. (%): C: 63.07, H: 5.57, N: 3.87. Found (%): C: 62.90, H: 5.52, N: 4.04.

4.1.27. 2-Chloro-N-[1,2-dihydro-3,3-dimethyl-6-methoxy-7-oxo-3H,7H-pyrano[2,3-c]xanthen-2-yl]acetamide (**24**)

Compound **24** was prepared by a procedure analogous to that of **16a**. Yield: 94%; mp 214–216 °C (CH_2Cl_2–*n*-pentane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.34 (s, 3H, 1 × gemCH_3), 1.45 (s, 3H, 1 × gemCH_3), 2.93 (dd, $J = 17$ Hz, 3 Hz, 1H, H-1a), 3.05 (dd, $J = 17$ Hz, 5 Hz, 1H, H-1b), 3.82 (s, 3H, CH_3O), 4.18 (s, 2H, NHCOCH_2Cl), 4.40 (m, 1H, H-2), 6.11 (s, 1H, H-5), 7.11 (d, $J = 9$ Hz, 1H, NHCOCH_2Cl), 7.26 (dt, $J = 8$ Hz, 2 Hz, 1H, H-9), 7.43 (dd, $J = 8$ Hz, 2 Hz, 1H, H-11), 7.48 (dt, $J = 8$ Hz, 2 Hz, 1H, H-10), 8.17 (dd, $J = 8$ Hz, 2 Hz, 1H, H-8); ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm): 23.48 (C-1), 24.52 (1 × gemCH_3), 24.70 (1 × gemCH_3), 42.84 (NHCOCH$_2$Cl), 48.00 (C-2), 56.05 (CH_3O), 78.04 (C-3), 95.86 (C-5), 98.67 (C-12b), 107.04 (C-6a), 116.81 (C-11), 122.60 (C-7a), 123.95 (C-9), 126.53 (C-8), 133.82 (C-10), 154.45 (C-11a), 157.39 (C-12a), 158.34 (C-4a), 160.06 (C-6), 166.71 (NHCO), 175.30 (C-7). Anal. Calcd. for $C_{21}H_{20}ClNO_5$. Calcd. (%): C: 62.77, H: 5.02, N: 3.49. Found (%): C: 62.44, H: 5.27, N: 3.62.

4.1.28. 2-Dimethylamino-N-[1,2-dihydro-3,3-dimethyl-6-methoxy-7-oxo-3H,7H-pyrano[2,3-c]xanthen-2-yl]acetamide (**25a**)

Compound **25a** was prepared by a procedure analogous to that of **17a**. Yield: 87%; mp (hydrochloride) 204–206 °C, (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.35 (s, 3H, 1 × gemCH_3), 1.37 (s, 3H, 1 × gemCH_3), 2.17 [s, 6H, NHCOCH_2N(CH_3)$_2$], 2.90 (dd, $J = 17$ Hz, 3 Hz, 1H, H-1a), 2.93 (s, 2H, NHCOCH_2NMe$_2$), 3.07 (dd, $J = 17$ Hz, 5 Hz, 1H, H-1b), 3.90 (s, 3H, CH_3O), 4.33 (m, 1H, H-2), 6.28 (s, 1H, H-5), 7.27 (dt, $J = 8$ Hz, 2 Hz, 1H, H-9), 7.32 (m, 2H, H-11 and NHCOCH$_2$NMe$_2$), 7.56 (dt, $J = 8$ Hz, 2 Hz, 1H, H-10), 8.22 (dd, $J = 8$ Hz, 2 Hz, 1H, H-8); ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm): 23.83 (C-1), 24.07 (1 × gemCH_3), 24.93 (1 × gemCH_3), 45.83 [NHCOCH_2N(CH_3)$_2$], 47.38 (C-2), 56.18 (CH_3O), 62.98 (NHCOCH_2NMe$_2$), 77.89 (C-3), 95.89 (C-5), 99.07 (C-12b), 107.30 (C-6a), 116.95 (C-11), 122.90 (C-7a), 123.92 (C-9), 126.61 (C-8), 133.62 (C-10), 154.68 (C-11a), 156.96 (C-12a), 158.30 (C-4a), 160.30 (C-6), 170.44 (NHCO), 175.49 (C-7). Anal. Calcd. for $C_{23}H_{26}N_2O_5 \cdot HCl$. Calcd. (%): C: 61.81, H: 6.09, N: 6.27. Found (%): C: 61.69, H: 6.01, N: 6.13.

4.1.29. 2-Diethylamino-N-[1,2-dihydro-3,3-dimethyl-6-methoxy-7-oxo-3H,7H-pyrano[2,3-c]xanthen-2-yl]acetamide (**25b**)

Compound **25b** was prepared by a procedure analogous to that of **17a**. Yield: 89%; mp (hydrochloride) 211–213 °C, (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.76 [t, $J = 7$ Hz, 6H, NHCOCH_2N(CH_2CH_3)$_2$], 1.32 (s, 3H, 1 × gemCH_3), 1.33 (s, 3H, 1 × gemCH_3), 2.35 [q, $J = 7$ Hz, 4H, NHCOCH_2N(CH_2CH$_3$)$_2$], 2.88 (m, 2H, H-1a and NHCOCH_2NEt$_2$), 3.01 (m, 2H, H-1b and NHCOCH_2NEt$_2$), 3.88 (s, 3H, CH_3O), 4.25 (m, 1H, H-2), 6.26 (s, 1H, H-5), 7.24 (dt, $J = 8$ Hz, 2 Hz, 1H, H-9), 7.30 (dd, $J = 8$ Hz, 2 Hz, 1H, H-11), 7.55 (dt, $J = 8$ Hz, 2 Hz, 1H, H-10), 7.70 (d, $J = 9$ Hz, 1H, NHCOCH$_2$NEt$_2$), 8.20 (dd, $J = 8$ Hz, 2 Hz, 1H, H-8); ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm): 12.43 [NHCOCH$_2$N(CH$_2$CH_3)$_2$], 23.85 (C-1), 24.19 (1 × gemCH_3), 24.82 (1 × gemCH_3), 47.41 (C-2), 48.76 [NHCOCH_2-N(CH_2CH$_3$)$_2$], 56.23 (CH_3O), 57.45 (NHCOCH_2NEt$_2$), 77.86 (C-3), 95.79 (C-5), 99.03 (C-12b), 107.30 (C-6a), 117.00 (C-11), 122.88 (C-7a), 123.93 (C-9), 126.66 (C-8), 133.67 (C-10), 154.71 (C-11a), 157.07 (C-12a), 158.33 (C-4a), 160.38 (C-6), 171.85 (NHCO), 175.55 (C-7). Anal. Calcd. for $C_{25}H_{30}N_2O_5 \cdot HCl$. Calcd. (%): C: 63.22, H: 6.58, N: 5.90. Found (%): C: 6.48, H: 6.50, N: 5.77.

4.2. Biological evaluation

4.2.1. Materials

Eagle's minimal essential medium (EMEM), foetal bovine serum (FBS), sodium pyruvate, sodium bicarbonate, L-glutamine, nonessential amino acids, penicillin, streptomycin, amphoterecin B and gentamycin were all obtained from Biochrom KG (Berlin, Germany). Insulin was obtained from

Sigma Chemicals (Steinhelm, Germany). All other chemicals used were of the best commercially available grade.

4.2.2. Cell culture conditions

MDA-MB-231 (HTB 26: human breast adenocarcinoma, ER-negative, high invasive potential) was obtained from the American Type Culture Collection (ATCC) and cultured as monolayers at 37 °C in a humidified atmosphere of 5% (v/v) CO_2 and 95% air. Cells were seeded in 75-cm^2 plastic tissue culture flasks and cultured in EMEM supplemented with 10% FBS, 2 mM L-glutamine, 1.0 mM sodium pyruvate, 1.5 g/L sodium bicarbonate, 0.1 mM nonessential amino acids, 0.01 mg/mL of insulin and a cocktail of antimicrobial agents (100 IU/mL penicillin, 100 μg/mL streptomycin, 10 μg/mL gentamicin sulfate and 2.5 μg/mL amphoterecin B) [21]. According to pilot experiments with respect to growth rate and doubling time, the medium was changed every three days. The cells were harvested after treatment with 0.25% (w/v) trypsin in PBS, containing 0.1% (w/v) Na_2EDTA.

4.2.3. Cell proliferation

In order to evaluate the effects of the new animoderivatives on cell proliferation, cells were seeded in the presence of serum into 96-well plates at a density of 1×10^4 cells/well. Twenty-four hours after plating, new medium supplemented with the aminoderivatives (1.0, 3.0, 10.0, 30.0 and 100.0 μM) to be tested was added. The compounds were diluted in DMSO and/or ethanol as a stock reagent and remained stored at −20 °C. To achieve the desirable concentrations for the experiments, the stock reagents were diluted to appropriate final concentrations in the culture media. After 72 h incubation, the medium was replaced with WST-1 (water-soluble tetrazolium salt). Cells were incubated for 3 h and the quantification of the formazan dye in the microplate was measured with an ELISA plate reader at 450 nm (reference wavelength at 650 nm). IC_{50} represents the concentration that reduces by 50% the optical density of treated cells with respect to untreated controls.

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