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Design and synthesis of novel amino-substituted xanthenones and benzo[*b*]xanthenones: Evaluation of their antiproliferative activity and their ability to overcome multidrug resistance toward MES-SA/D × 5 cells

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Abstract—A number of new xanthenone and benzo[*b*]xanthenone amino derivatives and their pyrazole-fused counterparts have been designed and synthesized possessing structural analogy to the potent anticancer agent 9-methoxypyrazoloacridine. The synthesis of the compounds proceeds through nucleophilic substitution of 1-chloro-4-nitroxanthenone or the corresponding benzo[*b*]xanthenone by the appropriately substituted amine or hydrazine, reduction of the nitro group, and conversion into the suitable dialkylaminoacetamides. This method cannot be applied for synthesis of the pyrazole-fused benzo[*b*]xanthenones, consequently a different, simple, and high-yielding synthetic procedure was developed for the preparation of the target molecules. 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/D × 5) cell lines are described and compared to those of reference drugs. The compounds exhibited significant cytotoxic activity against the tested cell lines and in addition they retain activity against the multidrug resistant MES-SA/D × 5 subline, showing resistant factors close to 1. A number of derivatives were found to posses DNA binding capacity, according to a standard ethidium bromide displacement assay. The majority of the studied compounds induce a G2/M arrest, although among them some G1 or S blockers have also been identified.

1. Introduction

Among different classes of chemotherapeutic agents used against malignant diseases, compounds that interact reversibly with DNA double helix have attracted particular attention, due to their high therapeutic potential. A number of them, such as the antitumor antibiotic actinomycin D, the 9-anilinoacridine amsacrine, and the anthracycline derivatives doxorubicin and daunomycin, have been extensively studied and were also used as lead compounds for the development of structurally related analogues. These drugs act by initially binding to cellular DNA sequences, which is usually followed by the formation of a ternary complex with DNA topoisomerase II.[1] This process produces a distortion of DNA structure, double strand break, perturbations of DNA synthesis, and ultimately they induce apoptosis in susceptible cells. Intercalation into DNA is considered to be an essential, but not the sole, determinant for their cell growth inhibitory activity.[2]

In recent years, a number of similarly acting aminoalkyl-substituted anthracenediones were developed exemplified by the drug mitoxantrone (**1**, Fig. 1) currently used in the treatment of acute leukemias and of breast cancers.[3,4] Unfortunately, the prolonged clinical application of quinone chemotypes has encountered problems, such

Keywords: Aminoxanthenones; Aminobenzo[*b*]xanthenones; Aminobenzopyrano[4,3,2-*cd*]indazoles; Aminobenzo[*g*]benzopyrano[4,3,2-*cd*]indazoles; Cytotoxic activity; DNA binding affinity; Cell-cycle selectivity; Multidrug resistance.
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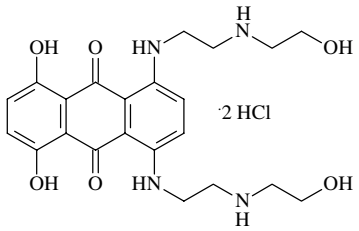
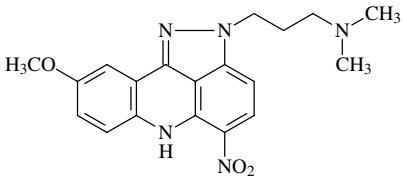


1 Mitoxantrone dihydrochloride



2 PZA

Figure 1. Structures of mitoxantrone and PZA.

as the development of cardiotoxicity[5] and multidrug resistance (MDR).[6] This led to the development of anthrapyrazoles[7] and benzothiopyranoindazoles,[8] which are characterized by the modification of the central quinone to a quasi-iminoquinone group, with an apparent suppression of redox cycling and free radical generation, thought to be responsible for cardiac damage.[9] The anthrapyrazoles demonstrate high activity,[12] but, in contrast to the anthracyclines, they are weak iron chelators[10] and appear considerably less cardiotoxic, in comparison to doxorubicin.[11] All these molecules share common structural features, such as a planar, or semi-planar, polycyclic chromophore and one or two polymethylenediamine fragments, as side chains bearing cationic charges, which improve solubility under physiological conditions and enable electrostatic binding to the phosphate moieties of DNA. Much effort has been devoted to the modification of the chromophores, in order to optimize their characteristics and a number of acridine derivatives, structurally related to the anthrapyrazoles, have also been developed. The most representative examples in this area include imidazoacridones,[13] pyrazoloacridines,[14] triazoloacridones[15], and pyrimidoacridines.[16]

However, the anthrapyrazoles cannot circumvent the problem of the development of acquired or intrinsic resistance (MDR).[17,18] Changes in intracellular drug metabolism or in the activity of target enzymes have been implicated in MDR, while physical obstacles to drug delivery might also play a role, due to deficient blood flow in growing solid tumors.[19] Nevertheless, the most important mechanism involves some ATP-binding membrane proteins, such as the product of human MDR-1 gene P-glycoprotein (Pgp), which are responsible for the active efflux of cytotoxic drugs out of resistant cells.[20] It has been reported that the anthrapyrazole-induced resistance of tumor cells can be overcome in vitro with the use of verapamil or other calcium blockers,[17] able to modulate the function of the above-mentioned exporting pumps.[21,22] The effectiveness of this drug combination has not yet been evaluated in vivo and is obviously far from therapeutic applications. On the other hand, intense research efforts have been made to design novel chemotherapeutic agents able to counteract the mechanisms of the undesirable MDR phenomenon.[23]

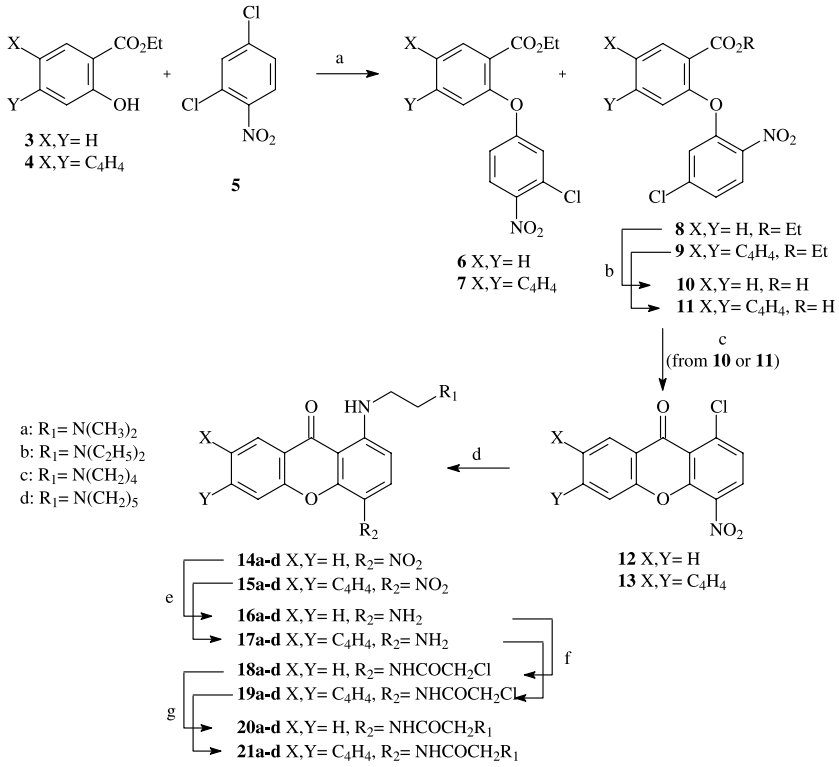
The incorporation of a fused five- or six-membered heterocyclic ring into the anthracenedione or acridine chromophore provided compounds able to overcome MDR.[24] Among them 9-methoxypyrazoloacridine (PZA, **2**, Fig. 1) was selected for clinical evaluation from a series of compounds that combine the DNA complexing activity of the acridine chromophore with the potential hypoxic cell selectivity derived from a reducible nitro group substitution on the ring.[25–27] Studies with PZA showed that bioreduction occurs, since the corresponding 5-amino derivative is the major metabolite of this drug in mice.[28] In addition to the experimentally confirmed hypoxia selectivity, PZA demonstrates unusual solid tumor selectivity and cytotoxicity to non-dividing cells,[29–31] while retaining full activity against resistant cell lines.[32–34] It has been reported that PZA targets topoisomerase I and II simultaneously, without stabilizing the topoisomerase–DNA cleavable complexes,[32,35] and has been thus suggested that it should act by a novel mechanism. The therapeutic potential of PZA is currently being evaluated in clinical combination studies, with the aim of circumventing drug resistance.[12,14,36–38]

We have previously reported on the synthesis and antiproliferative activity of a number of xanthenone and thioxanthenone amino derivatives, as well as their pyrazole-fused counterparts. These derivatives showed interesting cytotoxicity against the murine leukemia L1210 cell line, as well as against some human solid tumor cell lines.[39–42] In a preliminary report, we have described the synthesis of some amino-substituted xanthenone and benzopyrano[4,3,2-*cd*]indazole nitro derivatives, with direct structural similarity to PZA.[43] These compounds exhibited interesting cytotoxic properties against a panel of cell lines and retain activity against the multidrug resistant MES-SA/D × 5 subline. Prompted by these findings we decided to further explore the above-mentioned scaffolds and present here the preparation and biological evaluation of some xanthenone and benzo[*b*]xanthenone amino derivatives bearing either a nitro substitution or a second basic side chain that could possibly assist in more effective DNA-binding.

2. Results and discussion

2.1. Chemistry

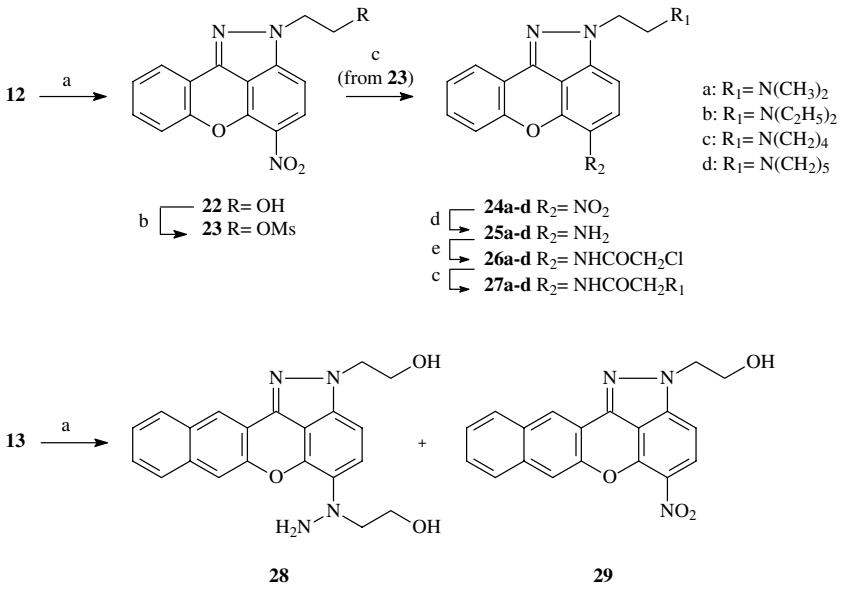
Commercially available ethyl salicylate (**3**) or ethyl 3-hydroxy-2-naphthoate (**4**) was used as starting material (Scheme 1). Treatment of each ester with 2,4-dichloronitrobenzene (**5**) resulted in a mixture of isomeric diaryle-



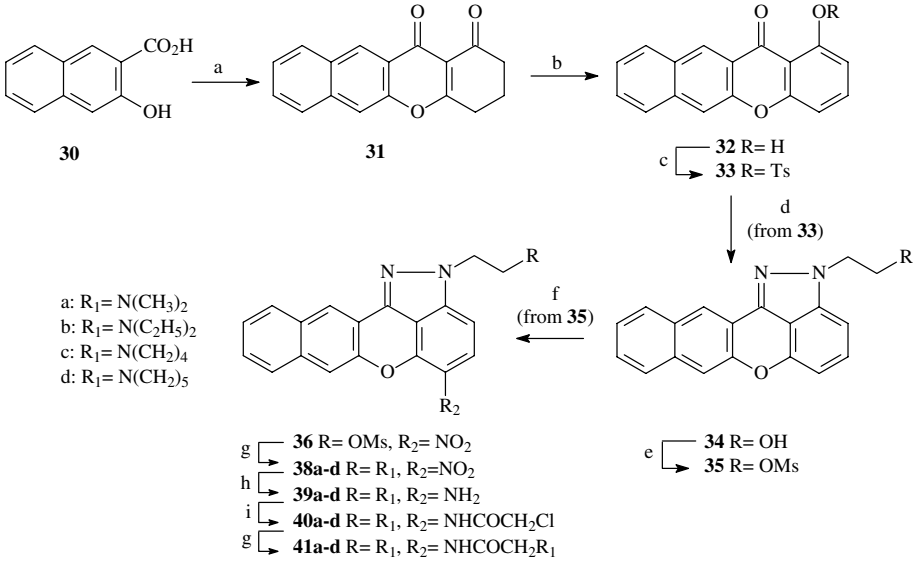
Scheme 1. Reagents and conditions: (a) K_2CO_3, Cu_2O, DMF, 110 °C, 8 h; (b) EtOH, NaOH 40%, rt, 30 min; (c) PPA 110 °C, 1 h; (d) $H_2NCH_2CH_2R_1$, pyridine, reflux, 1–3 h; (e) H_2, Pd/C 10%, EtOH, 50 psi, rt, 3–4h; (f) $ClCOCH_2Cl$, Et_3N, THF, rt, 90 min; (g) secondary amine, EtOH abs, reflux, 10–12 h.

thers (**6**, **8** and **7**, **9**, respectively). The mixture of the esters **6** and **8** was separated by column chromatography and each ester was isolated in pure form and identified by means of ^1H and ^{13}C NMR spectral data, using both direct and long-range heteronuclear correlation experiments (HMBC and HMQC sequencies).[43] The formation of both naphthoates **7** and **9** was evident from an ^1H NMR spectrum recorded in their mixture, however we could isolate pure only the desired isomer **9** by trituration, since all our attempts to separate this mixture by column chromatography were unsuccessful. Each one of the isomers **8** and **9** was then saponified under mild conditions and the resulting carboxylic acids, **10** and **11**, respectively, without further purification, were ring closed upon treatment with PPA providing the 1-chloro-4-nitroxanthenones **12** and **13**. The nitro derivatives **14a**–**14d** and **15a**–**15d** were prepared by the nucleophilic substitution of the chloro group of **12** or **13**, respectively, by appropriately substituted dialkylaminoethylamines. The 4-nitro group was then easily reduced by hydrogenation over palladium on activated carbon to provide the amino derivatives **16a**–**16d** and **17a**–**17d**. Since these derivatives were highly unstable in solution, probably due to fast air oxidation, they were not isolated, but converted to the corresponding chloracetamides **18a**–**18d** and **19a**–**19d**, by treatment with chloracetyl chloride. Reaction of these amides with the suitable secondary amines resulted in the target compounds **20a**–**20d** and **21a**–**21d**.

In order to prepare the corresponding pyrazole-fused amino derivatives, **12** was reacted with 2-hydroxyethylhydrazine to provide the carbinol **22** (Scheme 2).[43] Compound **22** was converted to the mesylate **23**, which was treated with the appropriately substituted secondary amines to result in the amino derivatives **24a**–**24d**. Reduction of the nitro group of compounds **24a**–**24d** provided the intermediate amines **25a**–**25d**, which were again not isolated or identified due to instability, but converted into the chloracetamides **26a**–**26d**. Reaction of the compounds **26a**–**26d** with the suitable secondary amines gave the benzopyranoindazoles **27a**–**27d**. Our attempts to apply an analogous methodology for the preparation of the corresponding benzoxanthenone analogues were not successful because treatment of the chloride **13** with 2-hydroxyethylhydrazine provided only minor amounts of the desired carbinol **29** (Scheme 2), the major product being **28**. This compound should have resulted from the displacement of the nitro group by the reagent, presumably upon initial formation of **29**, which renders the nitro group of this heterocyclic system highly susceptible to nucleophilic substitution. Consequently, we have developed a new methodology for the preparation of the desired derivatives. We have thus used 3-hydroxy-2-naphthoic acid (**30**, Scheme 3), which was treated with 1,3-cyclohexanedione to result in the diketone **31**. Oxidation of **31** in the presence of DDQ in boiling toluene provided the phenol **32** in 78% overall yield. The preparation of the phenol **32** has been previously reported through two different methodologies. The first involves the reaction of 3-hydroxy-2-naphthoic acid with resorcinol in the presence of zinc chloride and provided the product in 27% yield.[44] The second proceeds through the oxidation of



Scheme 2. Reagents and conditions: (a) $H_2NNHCH_2CH_2OH$, pyridine, rt, 12 h; (b) CH_3SO_2Cl, CH_2Cl_2, Et_3N, rt, 4 h; (c) secondary amine, EtOH abs, reflux, 10–12 h; (d) H_2, Pd/C 10%, EtOH, 50 psi, rt, 6 h; (e) $ClCOCH_2Cl$, THF, Et_3N, rt, 1–2 h.



Scheme 3. Reagents and conditions: (a) PPA, 1,3-cyclohexanedione, 150 °C, 2 h; (b) DDQ, toluene, reflux, 40 min; (c) 4-toluenesulfonylchloride, acetone, Na_2CO_3, reflux, 3.5 h; (d) $H_2NNHCH_2CH_2OH$, pyridine, reflux, 9 h; (e) CH_3SO_2Cl, CH_2Cl_2, Et_3N, rt, 4 h; (f) HNO_3 fuming, CH_3CO_2H, rt, 30 min; (g) secondary amine, EtOH abs, reflux, 8–10 h; (h) H_2, Pd/C 10%, EtOH, 50 psi, rt, 5 h; (i) $ClCOCH_2Cl$, Et_3N, THF, rt, 1–2 h.

1-hydroxy-5a,6,11,11a-tetrahydrobenzo[*b*]xanthene-12-one, derived from the cycloaddition reaction of the suitable chromene carboxaldehyde.[45] Nevertheless, our procedure is quite simple, high yielding, and can be used as a general scope reaction for the preparation of 1-hydroxyxanthenones. The phenol **32** was then easily converted to the tosylate **33**, which was reacted with 2-hydroxyethylhydrazine. The resulting carbinol **34** was treated with mesylchloride to provide the mesylate **35**, which was then nitrated to give the 5-nitro derivative **36** in good yield, together with a small amount of the 3-nitro isomer **37** (Fig. 2). Compound **36** was used for the preparation of the target nitroamines **38a**–**38d**, as well as for the synthesis of the diamines **41a**–**41d** via a procedure analogous to the one presented for the corresponding pyrazoloxanthenones **27a**–**27d**. The air-sensitive amines **39a**–**39d** were not isolated, but converted in one step into the chloracetamides **40a**–**40d**.

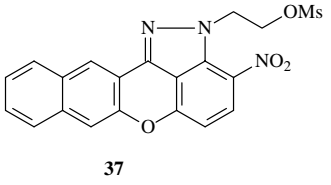
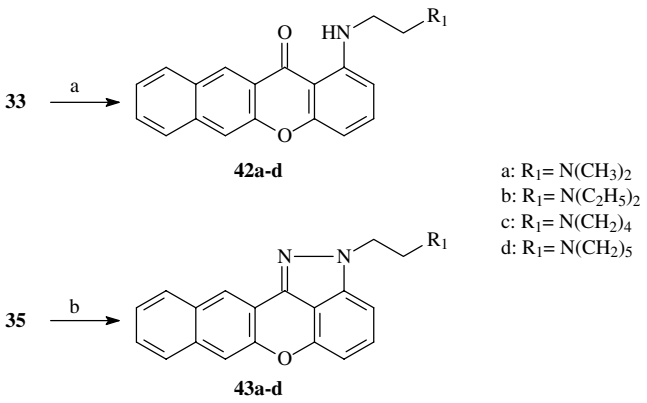


Figure 2. Structure of the nitro-isomer **37**.

Taking advantage of the intermediates **33** and **35**, we were also able to prepare the non-substituted amino



Scheme 4. Reagents and conditions: (a) $H_2NCH_2CH_2R_1$, pyridine, reflux, 16 h; (b) secondary amine, EtOH abs, reflux, 10 h.

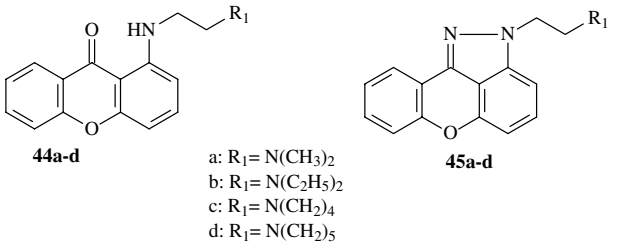


Figure 3. Structure of the previously reported[43] amino-substituted xanthenones **44a–44d** and benzopyranoindazoles **45a–45d**.

derivatives **42a–42d** and **43a–43d**, respectively (Scheme 4), which would be useful for comparative reasons, concerning the structure–activity relationship studies. We also cite herein complete experimental data of the aminoxanthenones **14a–14d** and **44a–44d** as well as of their pyrazole-fused analogues **24a–24d** and **45a–45d** (Fig. 3), which have been reported previously, in a preliminary communication.[43]

For biological evaluation purposes, the free base forms of the majority of the target amines were converted into their water-soluble hydrochloride addition salts by treatment with hydrochloric acid in methanol. However, compounds **21d** and **41a–41d** were tested at the free base form, since their hydrochloride, fumarate or malonate addition salts were highly hygroscopic.

2.2. Biological activity

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/D × 5, reported to be 100-fold resistant to doxorubicin.[46] The results, including reference compounds mitoxantrone and doxorubicin, are presented in Tables 1 and 2. The previously reported cytotoxicity data of the structurally related analogues **14a–14d**, **24a–24d**, **44a–44d**, and **45a–45d**[43] are also included in the Tables, in order to manifest clearly the structure–activity relationships within this class of compounds.

The vast majority of the new compounds showed interesting cytotoxic activity against all the tested cell lines, with IC_{50} values varying typically within the range of 0.3–25 μM.

In general, the xanthenone and benzo[*b*]xanthenone nitro-substituted amino derivatives (**14a–14d** and **15a–15d**), as well as the corresponding dialkylaminoacetamides (**20a–20d** and **21a–21d**), appear considerably more active when compared with the corresponding non-substituted analogues (**42a–42d** and **44a–44d**). In the case of the corresponding pyrazole-fused analogues, the substituted (**24a–24d**, **27a–27d**, **38a–38d**, and **41a–41d**) and non-substituted (**43a–43d** and **45a–45d**) derivatives possess comparable cytotoxicity. The data indicate that the replacement of the nitro group with a second basic side chain affords a similar or a slightly higher cytotoxicity in the tested cell lines. Furthermore, the insertion of a fourth benzene ring into the xanthenone chromophore gives compounds that retain cytotoxicity, although a clear improvement in the activity of the parent molecules is not observed. Noticeably, the non-substituted compounds **43a–43d** are considerably weaker than **45a–45d** against the L1210 cell line.

The most promising derivatives against the L1210 cell line appear to be the dialkylaminoacetamido-substituted benzo[*b*]xanthenones **21a–21d** and **41a–41d**. The pyrrolidine analogue **21c** emerges as the most active, showing strong cytotoxicity with an IC_{50} value of 0.36 μM, directly comparable with the corresponding value of PZA, which has been reported to be 0.424 μM.[26]

The compounds possess an interesting antiproliferative activity against the colorectal adenocarcinoma HT-29 cell line. In this case, the pyrazole-fused xanthenones and benzo[*b*]xanthenones bearing the dialkylaminoacetamido side chain (**27a–27d** and **41a–41d**, respectively) are the most potent compounds within the series.

Concerning the activity against the uterine sarcoma MES-SA and MES-SA/D × 5 cell lines, on average the pyrazole-fused derivatives are more active than the corresponding amino-substituted analogues. Furthermore, the majority of the nitro-substituted derivatives and the dialkylaminoacetamides exhibit an interesting profile of IC_{50} values, below 3 μM. Among them the pyrazole-fused analogues **38b**, **38c**, and **27c** proved to be the most cytotoxic compounds, possessing antiproliferative activity in the submicromolar range (IC_{50} values of 0.55, 0.57, and 0.59 μM, respectively).

The ability of all the tested compounds to overcome the multidrug resistance of the MES-SA/D × 5 cell line is clearly indicated by the resistant factor (RF) values, which are all practically equal to 1. From a comparison of any group of analogously substituted derivatives, it is evident that they retain full antiproliferative activity against P-glycoprotein-overexpressing cells. Worth mentioning, the RF to doxorubicin was found to be 98.25 as expected,[46] while the RF to mitoxantrone was 11.55.

Table 1. Inhibition of proliferation of the amino-substituted xanthenone and benzo[*b*]xanthenone derivatives (IC_{50} values in μM[a]) and DNA binding (EC_{50} values in μM)

Compound	R_1	R_2	L1210	HT-29	MES-SA	MES-SA/D × 5	RF[e]	EC_{50} (μM[f])
14a[b,i]	$N(CH_3)_2$	NO_2	3.13 (±0.31)	4.66 (±0.84)	1.56 (±0.62)	0.95 (±0.20)	0.61	[g]
14b[b]	$N(C_2H_5)_2$	NO_2	4.96 (±1.16)	3.20 (±0.45)	1.25 (±0.09)	2.02 (±0.29)	1.62	[g]
14c[d,i]	$N(CH_2)_4$	NO_2	1.48 (±0.67)	2.53 (±0.39)	0.78 (±0.11)	0.55 (±0.07)	0.70	7.9 (±0.31)
14d[b,i]	$N(CH_2)_5$	NO_2	5.88 (±1.47)	6.49 (±2.29)	1.59 (±0.18)	1.56 (±0.12)	0.98	[g]
15a[b]	$N(CH_3)_2$	NO_2	1.93 (±0.88)	1.81 (±0.71)	2.38 (±0.66)	1.1 (±0.39)	0.46	[h]
15b[b]	$N(C_2H_5)_2$	NO_2	3.90 (±0.74)	9.43 (±2.05)	6.31 (±1.56)	11.6 (±3.19)	1.84	[g]
15c[b]	$N(CH_2)_4$	NO_2	3.24 (±0.75)	3.43 (±1.08)	3.01 (±0.84)	1.23 (±0.51)	0.41	[h]
15d[b]	$N(CH_2)_5$	NO_2	3.00 (±0.60)	2.26 (±0.64)	7.81 (±2.27)	4.55 (±1.75)	0.58	[g]
20a[c]	$N(CH_3)_2$	$NHCOCH_2R_1$	1.09 (±0.11)	7.23 (±4.50)	1.59 (±0.35)	1.56 (±0.41)	0.98	9.0 (±0.83)
20b[c]	$N(C_2H_5)_2$	$NHCOCH_2R_1$	3.90 (±0.86)	5.99 (±2.34)	2.72 (±0.47)	2.69 (±0.39)	0.99	[g]
20c[c]	$N(CH_2)_4$	$NHCOCH_2R_1$	3.58 (±0.23)	5.05 (±3.14)	1.70 (±0.07)	1.84 (±0.29)	1.08	10.9 (±0.95)
20d[c]	$N(CH_2)_5$	$NHCOCH_2R_1$	4.34 (±1.12)	7.45 (±1.97)	1.84 (±0.61)	1.43 (±0.37)	0.78	[g]
21a[c]	$N(CH_3)_2$	$NHCOCH_2R_1$	2.13 (±0.69)	4.16 (±1.15)	2.34 (±0.75)	3.06 (±0.44)	1.31	1.2 (±0.05)
21b[c]	$N(C_2H_5)_2$	$NHCOCH_2R_1$	1.24 (±0.23)	1.22 (±0.41)	1.16 (±0.15)	1.07 (±0.11)	0.92	[g]
21c[c]	$N(CH_2)_4$	$NHCOCH_2R_1$	0.36 (±0.08)	1.44 (±0.40)	2.40 (±0.11)	1.25 (±0.09)	0.52	3.3 (±0.45)
21d[d]	$N(CH_2)_5$	$NHCOCH_2R_1$	5.92 (±2.91)	9.68 (±4.12)	6.71 (±2.00)	5.41 (±1.76)	0.81	[g]
42a[b]	$N(CH_3)_2$	H	26.1 (±4.69)	23.4 (±4.91)	23.4 (±6.21)	8.51 (±1.38)	0.36	[h]
42b[b]	$N(C_2H_5)_2$	H	9.31 (±0.18)	10.1 (±0.45)	6.40 (±0.98)	7.10 (±1.32)	1.11	[g]
42c[b]	$N(CH_2)_4$	H	9.19 (±2.10)	11.1 (±0.88)	7.60 (±0.90)	5.10 (±0.54)	0.67	[h]
42d[b]	$N(CH_2)_5$	H	9.66 (±1.38)	13.8 (±3.17)	6.60 (±0.75)	5.70 (±0.97)	0.86	[g]
44a[b,i]	$N(CH_3)_2$	H	6.58 (±1.65)	21.6 (±7.51)	9.03 (±2.61)	6.62 (±1.96)	0.73	[g]
44b[b]	$N(C_2H_5)_2$	H	5.00 (±1.55)	10.9 (±3.88)	8.38 (±1.71)	6.68 (±1.27)	0.80	[g]
44c[b,i]	$N(CH_2)_4$	H	9.00 (±1.88)	26.6 (±2.55)	14.61 (±4.16)	7.76 (±1.80)	0.53	43.2 (±5.91)
44d[b,i]	$N(CH_2)_5$	H	4.59 (±0.97)	26.5 (±5.11)	8.31 (±2.19)	6.04 (±1.75)	0.73	[g]
Mx			0.077 (±0.010)	0.020 (±0.004)	0.003 (±0.000)	0.030 (±0.020)	11.55	0.7 (±0.75)
Dx			0.080 (±0.005)	0.320 (±0.180)	0.016 (±0.008)	1.56 (±0.10)	98.25	

[a] The results represent means (±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] Dihydrochloride.
[d] Free base form.
[e] IC_{50}-resistant cells/IC_{50}-sensitive cells.
[f] The results represent means of two individual experiments (±1–10%) and are expressed as EC_{50}, the concentration of the compound that causes a 50% reduction in the fluorescence of the calf thymus DNA/ethidium bromide complex.
[g] Not tested.
[h] >100 μM.
[i] Results previously reported in Ref. 43.

A competitive fluorescence displacement assay was performed to determine the binding capacity of selected compounds to calf thymus DNA. However, this assay does not provide evidence concerning the exact mode of interaction with the nucleic acids (e.g., intercalation and /or groove binding mechanism). The results are expressed in EC_{50} values and are presented in the last column of Tables 1 and 2. The data on binding indicate that the xanthenone and benzo[*b*]xanthenone amino derivatives that possess a dialkylaminoacetamido substitution (**20a**, **20c**, **21a**, and **21c**) are effective DNA ligands capable of displacing ethidium bromide (Table 1). On the other hand, Table 2 shows that the pyrazole-fused analogues are weak DNA ligands, with the exception of the dialkylaminoacetamido-xanthenones **27a** and **27c**. Even if there is no quantitative correspondence between binding with DNA and in vitro cytotoxicity, it seems that the most cytotoxic derivatives possess high affinity for DNA.

Cell-cycle perturbations induced after incubation of exponentially growing MES-SA uterine sarcoma cells with a number of new compounds for 24 h are presented in Table 3. Most of the studied compounds provoke, as expected, a G2/M arrest. It is known that pyrazoloacridine and mitoxantrone block the cell cycle in the G2 phase.[33,47] Two compounds (**42a** and **44c**) with IC_{50}s higher than the concentration used for FACS analysis had, indeed, no significant effect on the cell-cycle phase distribution. On the other hand, the two benzo[*b*]xanthenone amino derivatives bearing the nitro group (**15a** and **15c**) tend to cause accumulation of MES-SA cells in the G1 phase, while the dimethylaminoethylamino-substituted xanthenylacetamide **20a** and its rigid, pyrazole-fused analogue **27a**, proved to be even more potent G1 blockers. Furthermore, the pyrrolidinethylamino-substituted benzo[*b*]-xanthenylacetamide **21c** and the corresponding pyrazole-fused derivative **41c** induced an S-phase arrest of the cells. Especially in the series of the amino-substituted compounds (Table 1), a high percentage of cells accumulated in the G2 or S phases correlates with high DNA-binding capacity (e.g., **21a**, **21c** or **14c**), while the most potent G1 blockers **20a** and **27a** bind also with high affinity to DNA. Interestingly, **21c**, as well as, **21a**, **15a**, **15c**, and **43a** induced

Table 2. Inhibition of proliferation of the pyrazole-fused xanthenone and benzo[*b*]xanthenone derivatives (IC_{50} values in μM[a]) and DNA binding (EC_{50} values in μM)

Compound	R_1	R_2	L1210	HT-29	MES-SA	MES-SA/D × 5	RF[e]	EC_{50} (μM[f])
24a[b,i]	$N(CH_3)_2$	NO_2	1.81 (±0.41)	7.49 (±0.31)	1.03 (±0.58)	0.63 (±0.25)	0.61	38 (±2.40)
24b[b]	$N(C_2H_5)_2$	NO_2	5.43 (±0.86)	54.10 (±2.65)	1.98 (±0.61)	1.95 (±0.44)	0.98	[g]
24c[b,i]	$N(CH_2)_4$	NO_2	3.52 (±0.35)	15.8 (±2.38)	1.13 (±0.07)	1.47 (±0.21)	1.29	[g]
24d[b,i]	$N(CH_2)_5$	NO_2	10.7 (±1.85)	41.6 (±7.99)	4.05 (±0.75)	5.38 (±1.26)	1.33	[g]
38a[b]	$N(CH_3)_2$	NO_2	1.91 (±0.64)	3.41 (±0.28)	1.30 (±0.40)	0.39 (±0.05)	0.30	[h]
38b[b]	$N(C_2H_5)_2$	NO_2	4.63 (±1.19)	1.85 (±0.21)	0.55 (±0.08)	0.30 (±0.02)	0.55	[g]
38c[b]	$N(CH_2)_4$	NO_2	1.18 (±0.27)	1.83 (±0.46)	0.57 (±0.12)	0.51 (±0.17)	0.89	[h]
38d[b]	$N(CH_2)_5$	NO_2	5.60 (±1.55)	5.15 (±0.82)	3.87 (±1.51)	1.49 (±0.10)	0.39	[g]
27a[c]	$N(CH_3)_2$	$NHCOCH_2R_1$	1.75 (±0.28)	3.41 (±0.50)	1.65 (±0.23)	1.74 (±0.19)	1.05	9.6 (±1.05)
27b[c]	$N(C_2H_5)_2$	$NHCOCH_2R_1$	5.58 (±1.20)	5.77 (±1.46)	1.87 (±0.65)	1.62 (±0.33)	0.87	[g]
27c[c]	$N(CH_2)_4$	$NHCOCH_2R_1$	1.41 (±0.30)	2.26 (±0.08)	0.59 (±0.25)	0.86 (±0.24)	1.46	12.6 (±1.53)
27d[c]	$N(CH_2)_5$	$NHCOCH_2R_1$	4.87 (±1.05)	7.22 (±1.79)	1.63 (±0.18)	4.57 (±0.51)	2.80	[g]
41a[d]	$N(CH_3)_2$	$NHCOCH_2R_1$	8.87 (±1.89)	3.74 (±0.61)	3.55 (±0.40)	1.84 (±0.21)	0.52	[h]
41b[d]	$N(C_2H_5)_2$	$NHCOCH_2R_1$	2.01 (±0.44)	1.48 (±0.15)	1.12 (±0.26)	1.28 (±0.33)	1.14	[g]
41c[d]	$N(CH_2)_4$	$NHCOCH_2R_1$	1.77 (±0.36)	0.39 (±0.11)	1.55 (±0.25)	0.96 (±0.06)	0.62	24.3 (±3.03)
41d[d]	$N(CH_2)_5$	$NHCOCH_2R_1$	1.65 (±0.18	2.23 (±0.47)	1.95 (±0.27)	1.17 (±0.19)	0.60	[g]
43a[b]	$N(CH_3)_2$	H	2.88 (±0.51)	7.84 (±1.49)	1.40 (±0.65)	1.45 (±0.57)	1.04	[h]
43b[b]	$N(C_2H_5)_2$	H	8.83 (±1.87)	9.22 (±2.53)	4.73 (±1.09)	4.53 (±0.76)	0.96	[g]
43c[b]	$N(CH_2)_4$	H	8.09 (±0.99)	10.7 (±3.25)	3.00 (±1.15)	6.20 (±1.68)	2.07	[h]
43d[b]	$N(CH_2)_5$	H	38.4 (±5.33)	12.1 (±2.87)	7.50 (±1.45)	6.90 (±0.95)	0.92	[g]
45a[b,i]	$N(CH_3)_2$	H	1.17 (±0.20)	20.9 (±9.45)	2.79 (±0.69)	2.76 (±1.08)	0.99	32.6 (±1.14)
45b[b]	$N(C_2H_5)_2$	H	6.37 (±0.75)	11.5 (±2.67)	6.55 (±0.73)	5.78 (±0.80)	0.88	[g]
45c[b,i]	$N(CH_2)_4$	H	1.79 (±0.21)	11.5 (±1.89)	1.75 (±0.16)	1.58 (±0.12)	0.90	58.6 (±6.66)
45d[b,i]	$N(CH_2)_5$	H	2.74 (±0.70)	9.92 (±3.41)	4.40 (±0.90)	3.88 (±0.35)	0.88	[g]
Mx			0.077 (±0.010)	0.020 (±0.004)	0.003 (±0.000)	0.030 (±0.020)	11.55	0.7 (±0.75)
Dx			0.080 (±0.005)	0.320 (±0.180)	0.016 (±0.008)	1.56 (±0.10)	98.25	

[a] The results represent means (±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] Dihydrochloride.
[d] Free base form.
[e] IC_{50}-resistant cells/IC_{50}-sensitive cells.
[f] The results represent means of two individual experiments (±1–10%) and are expressed as EC_{50}, the concentration of the compound that causes a 50% reduction in the fluorescence of the calf thymus DNA/ethidium bromide complex.
[g] Not tested.
[h] >100 μM.
[i] Results previously reported in Ref. 43.

Table 3. Cell-cycle phase distribution (%)[a]

Compound	G_0/G_1	S	G_2/M
14c[b]	41.70 (±2.12)	20.79 (±3.67)	37.41 (±5.51)
15a	69.02 (±4.19)	19.83 (±3.35)	11.15 (±1.35)
15c	62.38 (±3.78)	27.23 (±5.11)	10.39 (±3.22)
20a	86.91 (±7.18)	9.91 (±2.35)	3.18 (±1.12)
20c	59.86 (±4.44)	12.70 (±1.71)	27.44 (±3.50)
21a	27.01 (±4.81)	6.97 (±3.16)	66.02 (±5.19)
21c	37.72 (±2.47)	53.52 (±0.47)	8.77 (±2.94)
44c[b]	59.41 (±3.71)	32.66 (±4.12)	7.93 (±1.49)
42a	56.92 (±2.83)	29.54 (±3.54)	13.54 (±2.88)
42c	53.35 (±3.73)	25.51 (±1.96)	21.14 (±3.25)
24a[b]	30.00 (±4.90)	43.40 (±8.12)	26.60 (±5.75)
38a	46.07 (±1.78)	35.28 (±3.37)	18.65 (±0.64)
38c	60.37 (±3.88)	20.93 (±4.51)	18.70 (±2.34)
27a	81.97 (±6.14)	7.71 (±2.67)	10.32 (±3.16)
27c	45.67 (±0.47)	0.10 (±0.10)	54.23 (±0.54)
41a	29.54 (±1.76)	1.37 (±0.82)	69.09 (±3.49)
41c	27.53 (±6.83)	59.74 (±0.28)	12.73 (±4.37)
45a[b]	33.06 (±3.08)	20.87 (±4.21)	46.08 (±3.60)
45c[b]	22.63 (±1.19)	14.81 (±0.91)	62.57 (±3.88)
43a	45.28 (±6.91)	18.81 (±3.13)	35.91 (±4.77)
43c	40.10 (±4.15)	12.40 (±1.25)	47.50 (±6.66)
Control	54.70 (±4.80)	35.57 (±2.84)	9.74 (±1.96)

[a] Mean (±SD) of three independent experiments.
[b] Results previously reported in Ref. 43.

significant percentages of apoptosis, as judged by the appearance of a sub-diploid peak (12.3%, 15.7%, 27.7%, 11.4%, and 16.7%, respectively, compared to 1.3% of the untreated cells; data not shown in Table 3).

3. Conclusions

In summary, the present study deals with the synthesis of a number of new xanthenone and benzo[*b*]xanthenone amino derivatives bearing structural analogy to PZA. The compounds inhibit the proliferation of a panel of cancer cell lines, although probably not via a common mechanism, since they exhibit significant variation in their DNA binding capacity and they arrest the cells in different phases of the cell-cycle. In general, it seems that the insertion of a second basic side chain in the chromophore results in the improvement of cytotoxicity and DNA binding affinity of the derivatives. An interesting feature of this class of compounds is the lack of cross-resistance with doxorubicin against the MES-SA/D × 5 cell line, a finding that would require further studies in the future.

4. Experimental

4.1. Chemistry

All chemicals were purchased from Aldrich Chemical Co. Melting points were determined with a Büchi apparatus and are uncorrected. ^{1}H NMR spectra and 2D spectra were 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. For the ^{1}H–^{15}N GHMQC spectrum, data were acquired as 3072×400 data points with a total of 290 transients accumulated per t_1 increment. The F1 spectral window employed was set from 100 to 400 ppm and the F2 from 0 to 10 ppm correspondingly. 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 at the Microanalytical Sections of the National Hellenic Research Foundation on a Perkin-Elmer PE 240C Elemental Analyzer (Norwalk, CT) and are within ±0.4% of the theoretical values.

4.1.1. Ethyl 2-(3-chloro-4-nitrophenyloxy)benzoate (6) and ethyl 2-(5-chloro-2-nitrophenyloxy)benzoate (8). To a solution of ethyl salicylate (**3**, 33.37 g, 201 mmol) in dry DMF (50 mL), 2,4-dichloronitrobenzene (**5**, 38.4 g, 200 mmol), powdered K_2CO_3 (27.74 g, 201 mmol), and Cu_2O (2.85 g, 20.1 mmol) were added under argon and the mixture was heated at 110 °C for 8 h. The reaction mixture was then filtered hot, the filter cake was washed with CH_2Cl_2, the filtrate was concentrated in vacuo, and the residue was dissolved in CH_2Cl_2, washed with water, dried (Na_2SO_4), and evaporated to dryness. Flash chromatography on silica gel using a mixture of cyclohexane/EtOAc 100:5 as the eluent provided the title compounds **6** and **8** (23% and 64%, respectively).

Data for ethyl 2-(3-chloro-4-nitrophenyloxy)benzoate (**6**): mp 76–78 °C (Et_2O/*n*-hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.17 (t, J = 7 Hz, 3H, CH_2CH_3), 4.25 (q, J = 7 Hz, 2H, CH_2CH_3), 6.96 (d, J = 2 Hz, 1H, H-2′), 6.84 (dd, J = 9 Hz, 2 Hz, 1H, H-6′), 7.16 (d, J = 8 Hz, 1H, H-3), 7.41 (dt, J = 8 Hz, 1 Hz, 1H, H-4), 7.63 (dt, J = 8 Hz, 1 Hz, 1H, H-5), 7.98 (d, J = 9 Hz, 1H, H-5′), 8.06 (dd, J = 8 Hz, 1H, H-6); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 14.15 (CH_2CH_3), 61.45 (CH_2CH_3), 114.85 (C-6′), 118.85 (C-2′), 123.32 (C-3), 124.46 (C-1), 126.45 (C-4), 128.01 (C-5′), 129.74 (C-4′), 132.62 (C-6), 134.43 (C-5), 141.63 (C-3′), 152.86 (C-2), 162.16 (C-1′), 164.54 (CO). Anal. Calcd for $C_{15}H_{12}ClNO_5$: C, 56.00; H, 3.76; N, 4.35. Found: C, 55.87; H, 3.40; N, 4.28.

Data for ethyl 2-(5-chloro-2-nitrophenyloxy)benzoate (**8**): mp 87–89 °C (Et_2O/*n*-hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.16 (t, J = 7 Hz, 3H, CH_2CH_3), 4.23 (q, J = 7 Hz, 2H, CH_2CH_3), 6.71 (d, J = 2 Hz, 1H, H-6′), 7.11 (dd, J = 9 Hz, 2 Hz, 1H, H-4′), 7.18 (d, J = 8 Hz, 1H, H-3), 7.41 (dt, J = 8 Hz, 1 Hz, 1H, H-4), 7.63 (dt, J = 8 Hz, 1 Hz, 1H, H-5), 7.96 (d, J = 9 Hz, 1H, H-3′), 8.03 (dd, J = 8 Hz, 1H, H-6); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 13.81 (CH_2CH_3), 61.42 (CH_2CH_3), 117.65 (C-6′), 122.21 (C-4′), 122.95 (C-3), 124.16 (C-1), 126.25 (C-5), 126.92 (C-2), 132.72 (C-6), 134.34 (C-4), 138.13 (C-2′), 140.20 (C-5′), 152.76 (C-1′, C-2), 164.45 (CO). Anal. Calcd for $C_{15}H_{12}ClNO_5$: C, 56.00; H, 3.76; N, 4.35. Found: C, 55.94; H, 3.51; N, 4.41.

4.1.2. Ethyl 3-(5-chloro-2-nitrophenyloxy)-2-naphthoate (9). This compound was prepared by a procedure analogous to that of **8** starting from **4**. From the resulting mixture of the naphthoates **7** and **9** we have only isolated the desired isomer **9** (33%) by trituration in boiling ethanol. Mp 112–114 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.18 (t, J = 7 Hz, 3H, CH_2CH_3), 4.21 (q, J = 7 Hz, 2H, CH_2CH_3), 6.73 (d, J = 2 Hz, 1H, H-6′), 7.02 (dd, J = 9 Hz, 2 Hz, 1H, H-4′), 7.62 (t, J = 8 Hz, 1H, H-6), 7.64 (s, 1H, H-4), 7.68 (t, J = 8 Hz, 1H, H-7), 7.86 (d, J = 8 Hz, 1H, H-5), 8.02 (d, J = 9 Hz, 1H, H-3′), 8.03 (d, J = 8 Hz, 1H, H-8), 8.67 (s, 1H, H-1); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 13.89 (CH_2CH_3), 61.53 (CH_2CH_3), 117.73 (C-6′), 120.45 (C-4), 122.10 (C-4′), 122.76 (C-3), 126.95 (C-3′), 127.05 (C-7), 127.25 (C-5), 129.12 (C-6), 129.23 (C-8), 130.63 (C-8a), 134.82 (C-1), 135.71 (C-4a), 138.15 (C-2′), 140.22 (C-5′), 149.01 (C-3), 153.58 (C-1′), 168.53 (CO). Anal. Calcd for $C_{19}H_{14}ClNO_5$: C, 61.38; H, 3.80; N, 3.77. Found: C, 61.09; H, 3.64, N, 3.89.

4.1.3. 1-Chloro-4-nitro-9*H*-xanthen-9-one (12).[48] To a suspension of **8** (1.29 g, 4 mmol) in ethanol at room temperature was added dropwise a cold 40% NaOH solution (2 ml). The mixture was stirred for 30 min at room temperature and then poured into water and acidified with 18% HCl solution. The resulting 2-(5-chloro-2-nitrophenyloxy)benzoic acid (**10**) was filtered, air-dried, and dissolved in hot polyphosphoric acid. The mixture was heated at 110 °C for 1 h and upon cooling it was poured into ice water. The precipitate was filtered and air-dried to give crude **12**, which was purified by column chromatography (silica gel) using a mixture of CH_2Cl_2/cyclohexane 1:4–3:1 as the eluent. Yield: 81%; mp >270 °C (EtOH); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.53 (td, J = 8 Hz, 1 Hz, 1H, H-7), 7.62 (d, J = 8 Hz, 1H, H-5), 7.66 (d, J = 8 Hz, 1H, H-2), 7.90 (dt, J = 8 Hz, 1 Hz, 1H, H-6), 8.14 (d, J = 8 Hz, 1 Hz, 1H, H-8), 8.43 (d, J = 8 Hz, 1H, H-3); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 118. 35 (C-5), 120.44 (C-9a), 122.14 (C-8a), 125.76 (C-7), 126.67 (C-8), 126.74 (C-2), 130.42 (C-3), 136.51 (C-4), 136.61 (C-6), 138.87 (C-1), 150.07 (C-4a), 154.27 (C-10a), 174.29 (C-9).

4.1.4. 1-Chloro-4-nitro-12*H*-benzo[*b*]xanthen-12-one (13). This compound was prepared by a procedure analogous to that of **12** starting from **9**. The crude intermediate 3-(5-chloro-2-nitrophenyloxy)-2-naphthoic acid (**11**) was ring closed as described in the foregoing procedure. Yield: 65%; mp >270 °C (DMF); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.14 (d, J = 8 Hz, 1H, H-2), 7.51 (t, J = 8 Hz, 1H, H-9), 7.62 (t, J = 8 Hz, 1H, H-8), 7.90 (d, J = 8 Hz, 1H, H-7), 7.92 (s, 1H, H-6), 8.01 (d, J = 8 Hz, 1H, H-10), 8.12 (d, J = 8 Hz, 1H, H-3), 8.44

(s, 1H, H-11); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 114.97 (C-6), 119.97 (C-12a), 121.53 (C-11a), 126.94 (C-9), 127.46 (C-2), 127.79 (C-7), 128.33 (C-11), 130.43 (C-3, C-8), 130.61 (C-10, C-10a), 135.62 (C-6a), 136.27 (C-4), 140.16 (C-1), 150.12 (C-4a), 151.05 (C-5a), 179.35 (C-12). Anal. Calcd for $C_{17}H_8ClNO_4$: C, 62.69; H, 2.48; N, 4.30. Found: C, 62.38; H, 2.41; N, 4.05.

4.1.5. *N*,*N*-Dimethyl-*N*′-(4-nitro-9-oxo-9*H*-xanthen-1-yl)ethane-1,2-diamine (14a). A solution of **12** (271 mg, 0.983 mmol) and 2-(dimethylamino)ethylamine (793 mg, 9 mmol) in dry pyridine (6 mL) was refluxed for 90 min. After cooling, the mixture was vacuum-evaporated, extracted with ethyl acetate–water, the organic layer was dried (Na_2SO_4) and evaporated to dryness. The residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH 93:7) to provide **14a** (300 mg, 93%); mp (hydrochloride) >270 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.37 (s, 6H, 2 x CH_3), 2.69 (t, J = 7 Hz, 2H, NHCH$_2$CH_2), 3.44 (q, J = 7 Hz, 5 Hz, 2H, NHCH_2CH$_2$), 6.43 (d, J = 9 Hz, 1H, H-2), 7.41 (td, J = 8 Hz, 1 Hz, 1H, H-7), 7.60 (dd, J = 8 Hz, 1 Hz, 1H, H-5), 7.74 (dt, J = 8 Hz, 1 Hz, 1H, H-6), 8.25 (dd, J = 8 Hz, 1 Hz, 1H, H-8), 8.34 (d, J = 9 Hz, 1H, H-3), 10.74 (t, J = 5 Hz, 1H, D_2O exch, NH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 41.17 (NHCH$_2$CH$_2$), 45.77 (2× CH$_3$), 57.37 (NHCH$_2$$CH_2$), 103.24 (C-2), 105.33 (C-9a), 118.09 (C-5), 121.69 (C-8a), 125.00 (C-7), 125.84 (C-8), 126.82 (C-4), 133.60 (C-3), 135.00 (C-6), 153.32 (C-1), 154.51 (C-10a), 155.54, (C-4a), 179.10 (C-9). Anal. Calcd for $C_{17}H_{17}N_3O_4$·HCl·3/2H_2O: C, 52.24; H, 5.42; N, 10.75. Found: C, 52.43; H, 5.37; N, 10.57.

4.1.6. *N*,*N*-Diethyl-*N*′-(4-nitro-9-oxo-9*H*-xanthen-1-yl)ethane-1,2-diamine (14b). This compound was prepared by an analogous procedure as described for the preparation of **14a**. Yield: 95%; mp (hydrochloride) 241–243 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.10 (t, J = 7 Hz, 6H, 2× CH$_2$CH_3), 2.63 (q, J = 7 Hz, 4H, 2× CH_2CH$_3$), 2.80 (t, J = 7 Hz, 2H, NHCH$_2$CH_2), 3.39 (q, J = 7 Hz, 5 Hz, 2H, NHCH_2CH$_2$), 6.40 (d, J = 9 Hz, 1H, H-2), 7.38 (t, J = 8 Hz, 1H, H-7), 7.55 (d, J = 8 Hz, 1H, H-5), 7.70 (t, J = 8 Hz, 1H, H-6), 8.20 (dd, J = 8 Hz, 1 Hz, 1H, H-8), 8.29 (d, J = 8 Hz, 1H, H-3), 10.70 (t, J = 5 Hz, 1H, D_2O exch, NH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 11.35 (2× CH$_2$$CH_3$), 40.87 (NH$CH_2CH_2$), 47.08 (2× CH$_2CH_3$), 51.67 (NHCH$_2$$CH_2$), 103.21 (C-2), 105.06 (C-9a), 117.93 (C-5), 121.54 (C-8a), 124.91 (C-7), 125.70 (C-4), 125.74 (C-8), 133.37 (C-3), 134.91 (C-6), 153.44 (C-1), 154.37 (C-10a), 155.46, (C-4a), 179.01 (C-9). Anal. Calcd for $C_{19}H_{21}N_3O_4$·HCl·H_2O: C, 55.68; H, 5.90; N, 10.25. Found: C, 55.51; H, 5.98; N, 10.33.

4.1.7. 4-Nitro-1-[2-(pyrrolidin-1-yl)ethylamino]-9*H*-xanthene-9-one (14c). This compound was prepared by an analogous procedure as described for the preparation of **14a**. Yield: 95%; mp (hydrochloride) >270 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.80 (m, 4H, N(CH$_2$CH_2)$_2$), 2.59 (m, 4H, N(CH$_2$CH$_2$)$_2$), 2.80 (t, J = 7 Hz, 2H, NHCH$_2$CH_2), 3.43 (q, J = 7 Hz, 5 Hz, 2H, NHCH_2CH$_2$), 6.33 (d, J = 9 Hz, 1H, H-2), 7.33 (t, J = 8 Hz, 1H, H-7), 7.48 (d, J = 8 Hz, 1H, H-5), 7.65 (t, J = 8 Hz, 1H, H-6), 8.11 (dd, J = 8 Hz, 1 Hz, 1H, H-8), 8.21 (d, J = 9 Hz, 1H, H-3), 10.60 (t, J = 5 Hz, 1H, D_2O exch, NH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 23.52 (N(CH$_2$$CH_2$)$_2$), 42.30 (NH$CH_2CH_2$), 54.06 (N($CH_2CH_2$)$_2$), 54.07 (NHCH$_2$$CH_2$), 103.10 (C-2), 104.93 (C-9a), 117.74 (C-5), 121.40 (C-8a), 124.79 (C-7), 125.35 (C-4), 125.56 (C-8), 133.31 (C-3), 134.78 (C-6), 153.05 (C-1), 154.23 (C-10a), 155.37 (C-4a), 178.63 (C-9). Anal. Calcd for $C_{19}H_{19}N_3O_4$·HCl·1/4H_2O: C, 57.91; H, 5.18; N, 10.66. Found: C, 58.03; H, 4.97; N, 10.41.

4.1.8. 4-Nitro-1-[2-(piperidin-1-yl)ethylamino]-9*H*-xanthene-9-one (14d). This compound was prepared by an analogous procedure as described for the preparation of **14a**. Yield: 93%; mp (hydrochloride) >270 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.54 (m, 2H, 4-piperidine-H), 1.74 (m, 4H, 3,5-piperidine-H), 2.62 (m, 4H, 2,6-piperidine-H), 2.72 (t, J = 7 Hz, 2H, NHCH$_2$CH_2), 3.41 (q, J = 7 Hz, 5 Hz, 2H, NHCH_2CH$_2$), 6.41 (d, J = 9 Hz, 1H, H-2), 7.36 (t, J = 8 Hz, 1H, H-7), 7.50 (d, J = 8 Hz, 1H, H-5), 7.71 (t, J = 8 Hz, 1H, H-6), 8.18 (dd, J = 8 Hz, 1 Hz, 1H, H-8), 8.30 (d, J = 9 Hz, 1H, H-3), 10.69 (t, J = 5 Hz, 1H, D_2O exch, NH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 24.33 (4-piperidine-C), 25.94 (3,5-piperidine-C), 40.65 (NHCH$_2$CH$_2$), 54.54 (2,6-piperidine-C), 56.67 (NHCH$_2$$CH_2$), 103.32 (C-2), 105.23 (C-9a), 118.02 (C-5), 121.62 (C-8a), 124.90 (C-7), 125.47 (C-4), 125.78 (C-8), 133.46 (C-3), 134.89 (C-6), 153.31 (C-1), 154.41 (C-10a), 155.51 (C-4a), 178.89 (C-9). Anal. Calcd for $C_{20}H_{21}N_3O_4$·HCl·H_2O: C, 56.94; H, 5.73; N, 9.96. Found: C, 56.86; H, 5.69; N, 10.08.

4.1.9. *N*,*N*-Dimethyl-*N*′-(4-nitro-12-oxo-12*H*-benzo[*b*]-xanthen-1-yl)ethane-1,2-diamine (15a). This compound was prepared by an analogous procedure as described for the preparation of **14a**, starting from **13**. Yield: 88%; mp (hydrochloride) >270 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.41 (s, 6H, 2× CH$_3$), 2.73 (t, J = 6 Hz, 2H, NHCH$_2$CH_2), 3.48 (q, J = 6 Hz, 5 Hz, 2H, NHCH_2CH$_2$), 6.47 (d, J = 8 Hz, 1H, H-2), 7.52 (t, J = 8 Hz, 1H, H-9), 7.66 (t, J = 8 Hz, 1H, H-8), 7.96 (d, J = 8 Hz, 1H, H-7), 8.04 (s, 1H, H-6), 8.06 (d, J = 8 Hz, 1H, H-10), 8.36 (d, J = 8 Hz, 1H, H-3), 8.85 (s, 1H, H-11), 10.8 (t, J = 5 Hz, 1H, D_2O exch, NH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 41.12 (NHCH$_2$CH$_2$), 45.44 (2× CH$_3$), 57.33 (NHCH$_2$$CH_2$), 103.37 (C-2), 104.51 (C-12a), 114.04 (C-6), 120.83 (C-11a), 125.13 (C-4), 126.02 (C-9), 127.33 (C-7), 127.55 (C-11), 129.25 (C-8), 129.61 (C-10), 130.15 (C-10a), 133.81 (C-3), 136.60 (C-6a), 150.55 (C-5a), 155.58 (C-1), 155.62, (C-4a), 179.66 (C-12). Anal. Calcd for $C_{21}H_{19}N_3O_4$·HCl·1/2H_2O: C, 59.65; H, 5.01; N, 9.94. Found: C, 59.71; H, 4.81; N, 9.92.

4.1.10. *N*,*N*-Diethyl-*N*′-(4-nitro-12-oxo-12*H*-benzo[*b*]-xanthen-1-yl)ethane-1,2-diamine (15b). This compound was prepared by an analogous procedure as described for the preparation of **14a**, starting from **13**. Yield: 89%; mp (hydrochloride) >270 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.12 (t, J = 7 Hz, 6H, 2× CH$_2$CH_3),

2.68 (q, $J = 7$ Hz, 4H, 2× CH_2CH_3), 2.86 (t, $J = 6$ Hz, 2H, $NHCH_2CH_2$), 3.45 (q, $J = 6$ Hz, 5 Hz, 2H, $NHCH_2CH_2$), 6.43 (d, $J = 8$ Hz, 1H, H-2), 7.53 (t, $J = 8$ Hz, 1H, H-9), 7.64 (t, $J = 8$ Hz, 1H, H-8), 7.93 (d, $J = 8$ Hz, 1H, H-7), 8.02 (s, 1H, H-6), 8.04 (d, $J = 8$ Hz, 1H, H-10), 8.37 (d, $J = 8$ Hz, 1H, H-3), 8.86 (s, 1H, H-11), 10.81 (t, $J = 5$ Hz, 1H, D_2O exch, NH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 11.87 (2× CH_2CH_3), 41.56 ($NHCH_2CH_2$), 47.02 (2× CH_2CH_3), 50.88 ($NHCH_2CH_2$), 103.54 (C-2), 104.63 (C-12a), 114.04 (C-6), 120.92 (C-11a), 125.22 (C-4), 126.02 (C-9), 127.36 (C-7), 127.53 (C-11), 129.20 (C-8), 129.64 (C-10), 129.75 (C-10a), 133.76 (C-3), 136.44 (C-6a), 150.55 (C-5a), 155.60 (C-1), 155.67, (C-4a), 179.39 (C-12). Anal. Calcd for $C_{23}H_{23}N_3O_4 \cdot HCl \cdot H_2O$: C, 60.06; H, 5.70; N, 9.14. Found: C, 59.88; H, 5.74; N, 9.11.

4.1.11. 4-Nitro-1-[2-(pyrrolidin-1-yl)ethylamino]-12*H*-benzo[*b*]xanthene-12-one (15c). This compound was prepared by an analogous procedure as described for the preparation of **14a**, starting from **13**. Yield: 87%; mp (hydrochloride) >270 °C (EtOH); ^1H NMR ($CDCl_3$, 400 MHz) δ 1.92 (m, 4H, $N(CH_2CH_2)_2$), 2.62 (m, 4H, $N(CH_2CH_2)_2$), 2.88 (t, $J = 6$ Hz, 2H, $NHCH_2CH_2$), 3.49 (q, $J = 6$ Hz, 5 Hz, 2H, $NHCH_2CH_2$), 6.42 (d, $J = 8$ Hz, 1H, H-2), 7.46 (t, $J = 8$ Hz, 1H, H-9), 7.58 (t, $J = 8$ Hz, 1H, H-8), 7.90 (d, $J = 8$ Hz, 1H, H-7), 7.94 (s, 1H, H-6), 7.97 (d, $J = 8$ Hz, 1H, H-10), 8.29 (d, $J = 8$ Hz, 1H, H-3), 8.73 (s, 1H, H-11), 10.80 (t, $J = 5$ Hz, 1H, D_2O exch, NH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 23.67 ($N(CH_2CH_2)_2$), 45.53 ($NHCH_2CH_2$), 54.26 ($N(CH_2CH_2)_2$), 54.27 ($NHCH_2CH_2$), 103.37 (C-2), 104.40 (C-12a), 113.99 (C-6), 120.74 (C-11a), 125.02 (C-4), 126.01 (C-9), 127.33 (C-7), 127.41 (C-11), 129.21 (C-8), 129.59 (C-10), 130.09 (C-10a), 133.73 (C-3), 136.56 (C-6a), 150.41 (C-5a), 155.65 (C-1), 155.69, (C-4a), 179.49 (C-12). Anal. Calcd for $C_{23}H_{21}N_3O_4 \cdot HCl \cdot 1/2H_2O$: C, 61.54; H, 5.16; N, 9.36. Found: C, 61.69; H, 4.98; N, 9.28.

4.1.12. 4-Nitro-1-[2-(piperidin-1-yl)ethylamino]-12*H*-benzo[*b*]xanthene-12-one (15d). This compound was prepared by an analogous procedure as described for the preparation of **14a**, starting from **13**. Yield: 87%; mp (hydrochloride) >270 °C (EtOH); ^1H NMR ($CDCl_3$, 400 MHz) δ 1.53 (m, 2H, 4-piperidine-H), 1.71 (m, 4H, 3,5-piperidine-H), 2.55 (m, 4H, 2,6-piperidine-H), 2.76 (t, $J = 6$ Hz, 2H, $NHCH_2CH_2$), 3.51 (q, $J = 6$ Hz, 5 Hz, 2H, $NHCH_2CH_2$), 6.43 (d, $J = 8$ Hz, 1H, H-2), 7.59 (t, $J = 8$ Hz, 1H, H-9), 7.61 (t, $J = 8$ Hz, 1H, H-8), 7.91 (d, $J = 8$ Hz, 1H, H-7), 7.98 (s, 1H, H-6), 8.04 (d, $J = 8$ Hz, 1H, H-10), 8.32 (d, $J = 8$ Hz, 1H, H-3), 8.78 (s, 1H, H-11), 10.81 (t, $J = 5$ Hz, 1H, D_2O exch, NH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 24.99 (4-piperidine-C), 25.89 (3,5-piperidine-C), 40.59 ($NHCH_2CH_2$), 54.51 (2,6-piperidine-C), 56.62 ($NHCH_2CH_2$), 103.44 (C-2), 104.44 (C-12a), 113.97 (C-6), 120.78 (C-11a), 124.99 (C-4), 125.95 (C-9), 127.26 (C-7), 127.41 (C-11), 129.15 (C-8), 129.57 (C-10), 130.07 (C-10a), 133.67 (C-3), 136.53 (C-6a), 150.43 (C-5a), 155.62 (C-1), 155.68, (C-4a), 179.39 (C-12). Anal. Calcd for $C_{24}H_{23}N_3O_4 \cdot HCl \cdot 1/2H_2O$: C, 62.27; H, 5.44; N, 9.08. Found: C, 62.42; H, 5.17; N, 8.82.

4.1.13. 2-Chloro-*N*-[1-(2-dimethylaminoethylamino)-9-oxo-9*H*-xanthen-4-yl]acetamide (18a). A solution of **14a** (1.31 g, 4 mmol) in absolute EtOH (40 mL) was hydrogenated in the presence of 10% Pd/C (100 mg), under a pressure of 50 psi at room temperature for 3 h. The resulting mixture was filtered through a Celite pad and the filtrate was evaporated to dryness to result in oil corresponding to the 4-aminoderivative **16a**. Without further purification, the residue was dissolved under argon in dry THF (10 mL) and to this solution were added dropwise at 0 °C triethylamine (1.67 mL, 12 mmol) and a solution of chloracetylchloride (0.32 mL, 4 mmol) in dry THF (5 mL). The resulting solution was stirred for 10 min at 0 °C and at room temperature for another 90 min. The solvent was then evaporated to dryness and the residue was purified by column chromatography (silica gel) using a mixture of CH_2Cl_2/MeOH 8:1 as the eluent to provide compounds **18a** (1.24 g, 83%); mp >270 °C (EtOAc/*n*-hexane); ^1H NMR ($CDCl_3$, 400 MHz) δ 2.67 (s, 6H, 2× CH_3), 2.97 (t, $J = 7$ Hz, 2H, $NHCH_2CH_2$), 3.64 (q, $J = 7$ Hz, 5 Hz, 2H, $NHCH_2CH_2$), 4.29 (s, 2H, $COCH_2$), 6.47 (d, $J = 9$ Hz, 1H, H-2), 7.35 (t, $J = 8$ Hz, 1 Hz, 1H, H-7), 7.40 (d, $J = 8$ Hz, 1H, H-5), 7.67 (t, $J = 8$ Hz, 1H, H-6), 8.18 (m, 2H, H-3, H-8), 8.73 (s, 1H, D_2O exch, NHCO), 9.43 (t, $J = 5$ Hz, 1H, D_2O exch, NH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 43.04 ($COCH_2$), 43.89 ($NHCH_2CH_2$), 45.31 (2× CH_3), 56.16 ($NHCH_2CH_2$), 102.91 (C-2), 106.55 (C-9a), 112.80 (C-4), 117.10 (C-5), 121.66 (C-8a), 124.26 (C-7), 126.18 (C-8), 129.74 (C-3), 134.56 (C-6), 148.04 (C-1), 148.42 (C-4a), 154.40 (C-10a), 163.81 (NHCO), 179.39 (C-10). Anal. Calcd for $C_{19}H_{20}ClN_3O_3$: C, 61.04; H, 5.39; N, 11.24. Found: C, 61.33; H, 5.09; N, 11.39.

Spectral data for the intermediate *N*,*N*-dimethyl-*N*′-(4-amino-9-oxo-9*H*-xanthen-1-yl)ethane-1,2-diamine (**16a**): ^1H NMR (DMSO-d_6, 400 MHz) δ 2.48 (s, 6H, 2× CH_3), 2.82 (t, $J = 7$ Hz, 2H, $NHCH_2CH_2$), 3.40 (q, $J = 7$ Hz, 5 Hz, 2H, $NHCH_2CH_2$), 6.27 (d, $J = 8$ Hz, 1H, H-2), 6.98 (d, $J = 8$ Hz, 1 Hz, 1H, H-3), 7.21 (t, $J = 8$ Hz, 1 Hz, 1H, H-7), 7.29 (d, $J = 8$ Hz, 1 Hz, 1H, H-5), 7.53 (t, $J = 8$ Hz, 1 Hz, 1H, H-6), 8.11 (d, $J = 8$ Hz, 1H, H-8), 8.81 (t, $J = 5$ Hz, 1H, D_2O exch, NH).

4.1.14. 2-Chloro-*N*-[1-(2-diethylaminoethylamino)-9-oxo-9*H*-xanthen-4-yl]acetamide (18b). This compound was prepared by an analogous procedure as described for the preparation of **18a**. Yield: 87%; mp 194–196 °C (EtOAc/*n*-hexane); ^1H NMR ($CDCl_3$, 400 MHz) δ 1.10 (t, $J = 7$ Hz, 6H, 2× CH_2CH_3), 2.65 (q, $J = 7$ Hz, 4H, 2× CH_2CH_3), 2.81 (t, $J = 7$ Hz, 2H, $NHCH_2CH_2$), 3.34 (q, $J = 7$ Hz, 5 Hz, 2H, $NHCH_2CH_2$), 4.28 (s, 2H, $COCH_2$), 6.42 (d, $J = 9$ Hz, 1H, H-2), 7.29–7.44 (m, 2H, H-5, H-7), 7.65 (t, $J = 8$ Hz, 1H, H-6), 8.23 (m, 2H, H-3, H-8), 8.67 (s, 1H, D_2O exch, NHCO), 9.40 (t, $J = 5$ Hz, 1H, D_2O exch, NH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 11.68 (2× CH_2CH_3), 41.16 ($NHCH_2CH_2$), 43.11 ($COCH_2$), 47.15 (2× CH_2CH_3), 51.27 ($NHCH_2CH_2$), 103.10 (C-2), 106.26 (C-9a), 111.70 (C-4), 116.96 (C-5), 121.96 (C-8a), 124.09 (C-7), 126.33 (C-8), 129.49 (C-3), 134.23 (C-6), 148.02 (C-1), 149.01

(C-4a), 154.41, (C-10a), 163.60 (NHCO), 179.15 (C-9). Anal. Calcd for $C_{21}H_{24}ClN_3O_3$: C, 62.76; H, 6.02; N, 10.46. Found: C, 62.60; H, 5.88; N, 10.60.

Spectral data for the intermediate *N*,*N*-diethyl-*N*′-(4-amino-9-oxo-9*H*-xanthen-1-yl)ethane-1,2-diamine (**16b**): ^{1}H NMR (CD_3OD, 400 MHz) δ 1.11 (t, J = 7 Hz, 6H, 2× CH_2CH_3), 2.49 (q, J = 7 Hz, 4H, 2× CH_2CH_3), 2.73 (t, J = 7 Hz, 2H, $NHCH_2CH_2$), 3.21 (q, J = 7 Hz, 5 Hz, 2H, $NHCH_2CH_2$), 6.29 (d, J = 8 Hz, 1H, H-2), 7.04 (d, J = 8 Hz, 1H, H-3), 7.33 (t, J = 8 Hz, 1H, H-7), 7.40 (d, J = 8 Hz, 1H, H-5), 7.64 (t, J = 8 Hz, 1H, H-6), 8.16 (d, J = 8 Hz, 1H, H-8), 9.36 (t, J = 5 Hz, 1H, D_2O exch, NH).

4.1.15. 2-Chloro-*N*-[1-[2-(pyrrolidin-1-yl)ethylamino]-9-oxo-9*H*-xanthen-4-yl]acetamide (18c). This compound was prepared by an analogous procedure as described for the preparation of **18a**. Yield: 83%; mp 259–260 °C (EtOAc/*n*-hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.91 (m, 4H, $N(CH_2CH_2)_2$), 2.83 (m, 4H, $N(CH_2CH_2)_2$), 2.98 (t, J = 7 Hz, 2H, $NHCH_2CH_2$), 3.55 (q, J = 7 Hz, 5 Hz, 2H, $NHCH_2CH_2$), 4.29 (s, 2H, $COCH_2$), 6.48 (d, J = 9 Hz, 1H, H-2), 7.36 (t, J = 8 Hz, 1H, H-7), 7.40 (d, J = 8 Hz, 1H, H-5), 7.67 (t, J = 8 Hz, 1H, H-6), 8.23 (m, 2H, H-3, H-8), 8.68 (s, 1H, D_2O exch, NHCO), 9.44 (t, J = 5 Hz, 1H, D_2O exch, NH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 23.48 ($N(CH_2CH_2)_2$), 41.38 ($NHCH_2CH_2$), 43.15 ($COCH_2$), 54.26 ($NHCH_2CH_2$), 54.28 ($N(CH_2CH_2)_2$), 103.13 (C-2), 106.44 (C-9a), 112.14 (C-4), 117.10 (C-5), 121.88 (C-8a), 124.23 (C-7), 126.29 (C-8), 129.71 (C-3), 134.42 (C-6), 148.09 (C-1), 148.71 (C-4a), 154.49 (C-10a), 163.71 (NHCO), 179.37 (C-9). Anal. Calcd for $C_{21}H_{22}ClN_3O_3$: C, 63.08; H, 5.55; N, 10.51. Found: C, 63.25; H, 5.69; N, 10.32.

Spectral data for the intermediate 4-amino-1-[2-(pyrrolidin-1-yl)ethylamino]-9*H*-xanthene-9-one (**16c**): ^{1}H NMR (CD_3OD, 400 MHz) δ 1.93 (m, 4H, $N(CH_2CH_2)_2$), 2.90 (m, 4H, $N(CH_2CH_2)_2$), 3.01 (t, J = 7 Hz, 2H, $NHCH_2CH_2$), 3.57 (q, J = 7 Hz, 5 Hz, 2H, $NHCH_2CH_2$), 6.43 (d, J = 8 Hz, 1H, H-2), 7.09 (d, J = 8 Hz, 1H, H-3), 7.31 (t, J = 8 Hz, 1H, H-7), 7.41 (d, J = 8 Hz, 1H, H-5), 7.65 (t, J = 8 Hz, 1H, H-6), 8.21 (d, J = 8 Hz, 1H, H-8), 9.95 (t, J = 5 Hz, 1H, D_2O exch, NH).

4.1.16. 2-Chloro-*N*-[1-[2-(piperidin-1-yl)ethylamino]-9-oxo-9*H*-xanthen-4-yl]acetamide (18d). This compound was prepared by an analogous procedure as described for the preparation of **18a**. Yield: 82%; mp 264–266 °C (EtOAc/*n*-hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.60 (m, 2H, 4-piperidine-H), 1.88 (m, 4H, 3,5-piperidine-H), 3.06 (m, 4H, 2,6-piperidine-H), 3.10 (t, J = 7 Hz, 2H, $NHCH_2CH_2$), 3.62 (q, J = 7 Hz, 5 Hz, 2H, $NHCH_2CH_2$), 4.27 (s, 2H, $COCH_2$), 6.31 (d, J = 9 Hz, 1H, H-2), 7.16 (d, J = 8 Hz, 1H, H-5), 7.23 (t, J = 8 Hz, 1H, H-7), 7.53 (t, J = 8 Hz, 1H, H-6), 7.97 (m, 2H, H-3, H-8), 8.71 (s, 1H, D_2O exch, NHCO), 9.17 (t, J = 5 Hz, 1H, D_2O exch, NH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 23.85 (4-piperidine-C), 25.68 (3,5-piperidine-C), 39.85 ($NHCH_2CH_2$), 42.98 ($COCH_2$), 54.62 (2,6-piperidine-C), 57.25 ($NHCH_2CH_2$), 102.98 (C-2), 106.43 (C-9a), 112.88 (C-4), 117.11 (C-5), 121.62 (C-8a), 124.00 (C-7), 126.44 (C-8), 129.02 (C-3), 134.22 (C-6), 147.33 (C-1), 147.62 (C-4a), 154.11 (C-10a), 163.72 (NHCO), 179.52 (C-9). Anal. Calcd for $C_{22}H_{24}ClN_3O_3$: C, 63.84; H, 5.84; N, 10.15. Found: C, 63.71; H, 5.70; N, 9.87.

Spectral data for the intermediate 4-amino-1-[2-(piperidin-1-yl)ethylamino]-9*H*-xanthene-9-one (**16d**): ^{1}H NMR (CD_3OD, 400 MHz) δ 1.51 (m, 2H, 4-piperidine-H), 1.68 (m, 4H, 3,5-piperidine-H), 2.53 (m, 4H, 2,6-piperidine-H), 2.86 (t, J = 7 Hz, 2H, $NHCH_2CH_2$), 3.47 (q, J = 7 Hz, 5 Hz, 2H, $NHCH_2CH_2$), 6.38 (d, J = 9 Hz, 1H, H-2), 6.99 (d, J = 8 Hz, 1H, H-3), 7.38 (t, J = 8 Hz, 1H, H-7), 7.45 (d, J = 8 Hz, 1H, H-5), 7.67 (t, J = 8 Hz, 1H, H-6), 8.14 (d, J = 9 Hz, 1H, H-8), 9.01 (t, J = 5 Hz, 1H, D_2O exch, NH).

4.1.17. 2-Chloro-*N*-[1-(2-dimethylaminoethylamino)-12-oxo-12*H*-benzo[*b*]xanthen-4-yl]acetamide (19a). This compound was prepared by an analogous procedure as described for the preparation of **18a**, starting from **15a**. Yield: 81%; mp 244–246 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.39 (s, 6H, 2× CH_3), 2.71 (t, J = 7 Hz, 2H, $NHCH_2CH_2$), 3.40 (q, J = 7 Hz, 2H, $NHCH_2CH_2$), 4.41 (s, 2H, $NHCOCH_2$), 6.45 (d, J = 8 Hz, 1H, H-2), 7.48 (t, J = 8 Hz, 1H, H-9), 7.62 (t, J = 8 Hz, 1H, H-8), 7.78 (s, 1H, H-6), 7.88 (d, J = 8 Hz, 1H, H-7), 8.06 (d, J = 8 Hz, 1H, H-10), 8.31 (d, J = 8 Hz, 1H, H-3), 8.82 (s, 1H, D_2O exch, NHCO), 8.84 (s, 1H, H-11), 9.58 (t, J = 5 Hz, 1H, D_2O exch, NH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 40.96 ($NHCH_2CH_2$), 45.55 ($CH_2CH_2N(CH_3)_2$), 57.85 ($NHCH_2CH_2$), 43.16 ($COCH_2$), 103.17 (C-2), 104.12 (C-12a), 112.45 (C-4), 112.53 (C-6), 121.51 (C-11a), 125.56 (C-9), 126.92 (C-7), 127.98 (C-11), 128.97 (C-8), 129.71 (C-10), 129.82 (C-10a), 130.00 (C-3), 136.35 (C-6a), 149.30 (C-4a), 149.38 (C-1), 150.86 (C-5a), 163.64 (NHCO), 179.77 (C-12). Anal. Calcd for $C_{23}H_{22}ClN_3O_3$: C, 65.17; H, 5.23; N, 9.91. Found: C, 65.34; H, 5.35; N, 9.64.

Spectral data for the intermediate *N*,*N*-dimethyl-*N*′-(4-amino-12-oxo-12*H*-benzo[*b*]xanthen-1-yl)ethane-1,2-diamine (**17a**): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.43 (s, 6H, 2× CH_3), 2.71 (t, J = 7 Hz, 2H, $NHCH_2CH_2$), 3.43 (q, J = 7 Hz, 5 Hz, 2H, $NHCH_2CH_2$), 6.34 (d, J = 8 Hz, 1H, H-2), 7.11 (d, J = 8 Hz, 1H, H-3), 7.46 (t, J = 8 Hz, 1H, H-9), 7.57 (t, J = 8 Hz, 1H, H-8), 7.78 (s, 1H, H-6), 7.85 (d, J = 8 Hz, 1H, H-7), 8.01 (d, J = 8 Hz, 1H, H-10), 8.80 (s, 1H, H-11), 9.11 (t, J = 5 Hz, 1H, D_2O exch, NH).

4.1.18. 2-Chloro-*N*-[1-(2-diethylaminoethylamino)-12-oxo-12*H*-benzo[*b*]xanthen-4-yl]acetamide (19b). This compound was prepared by an analogous procedure as described for the preparation of **18a**, starting from **15b**. Yield: 84%; mp >270 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.11 (t, J = 7 Hz, 6H, $N(CH_2CH_3)_2$), 2.70 (q, J = 7 Hz, 4H, $N(CH_2CH_3)_2$), 2.84 (t, J = 7 Hz, 2H, $NHCH_2CH_2$), 3.41 (q, J = 7 Hz, 2H, $NHCH_2CH_2$), 4.36 (s, 2H, $NHCOCH_2$), 6.47 (d, J = 8 Hz, 1H, H-2), 7.47 (t, J = 8 Hz, 1H, H-9), 7.62 (t, J = 8 Hz, 1H,

H-8), 7.79 (s, 1H, H-6), 7.89 (d, $J = 8$ Hz, 1H, H-7), 8.06 (d, $J = 8$ Hz, 1H, H-10), 8.31 (d, $J = 8$ Hz, 1H, H-3), 8.78 (s, 1H, D_2O exch, NHCO), 8.83 (s, 1H, H-11), 9.56 (t, $J = 5$ Hz, 1H, D_2O exch, NH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 11.34 ($CH_2CH_2N(CH_2CH_3)_2$), 41.27 ($CH_2CH_2N(CH_2CH_3)_2$), 43.25 ($NHCOCH_2$), 47.16 ($CH_2CH_2N(CH_2CH_3)_2$), 51.43 ($CH_2CH_2N(CH_2CH_3)_2$), 102.91 (C-2), 105.12 (C-12a), 112.47 (C-6), 112.53 (C-4), 121.40 (C-11a), 125.64 (C-9), 127.05 (C-7), 127.80 (C-11), 129.04 (C-8), 129.63 (C-10), 129.74 (C-3), 130.02 (C-10a), 136.52 (C-6a), 148.75 (C-4a), 149.10 (C-1), 150.94 (C-5a), 163.72 (NHCO), 179.66 (C-12). Anal. Calcd for $C_{25}H_{26}ClN_3O_3$: C, 66.44; H, 5.80; N, 9.30. Found: C, 66.26; H, 5.62; N, 9.24.

Spectral data for the intermediate *N*,*N*-diethyl-*N*′-(4-amino-12-oxo-12*H*-benzo[*b*]xanthen-1-yl)ethane-1,2-diamine (**17b**): ^1H NMR ($CDCl_3$, 400 MHz) δ 1.07 (t, $J = 7$ Hz, 6H, $N(CH_2CH_3)_2$), 2.54 (q, $J = 7$ Hz, 4H, $N(CH_2CH_3)_2$), 2.71 (t, $J = 6$ Hz, 2H, $NHCH_2CH_2$), 3.20 (q, $J = 6$ Hz, 5 Hz, 2H, $NHCH_2CH_2$), 6.19 (d, $J = 8$ Hz, 1H, H-2), 6.97 (d, $J = 8$ Hz, 1H, H-3), 7.33 (t, $J = 8$ Hz, 1H, H-9), 7.44 (t, $J = 8$ Hz, 1H, H-8), 7.54 (s, 1H, H-6), 7.69 (d, $J = 8$ Hz, 1H, H-7), 7.90 (d, $J = 8$ Hz, 1H, H-10), 8.64 (s, 1H, H-11), 8.94 (t, $J = 5$ Hz, 1H, D_2O exch, NH).

4.1.19. 2-Chloro-*N*-[1-[2-(pyrrolidin-1-yl)ethylamino]-12-oxo-12*H*-benzo[*b*]xanthen-4-yl]acetamide (19c). This compound was prepared by an analogous procedure as described for the preparation of **18a**, starting from **15c**. Yield: 80%; mp 251–253 °C (dec) (EtOH); ^1H NMR ($CDCl_3$, 400 MHz) δ 1.91 (m, 4H, $N(CH_2CH_2)_2$), 2.66 (m, 4H, $N(CH_2CH_2)_2$), 2.91 (t, $J = 7$ Hz, 2H, $NHCH_2CH_2$), 3.50 (q, $J = 7$ Hz, 2H, $NHCH_2CH_2$), 4.31 (s, 2H, $NHCOCH_2$), 6.50 (d, $J = 8$ Hz, 1H, H-2), 7.52 (t, $J = 8$ Hz, 1H, H-9), 7.64 (t, $J = 8$ Hz, 1H, H-8), 7.81 (s, 1H, H-6), 7.92 (d, $J = 8$ Hz, 1H, H-7), 8.06 (d, $J = 8$ Hz, 1H, H-10), 8.31 (d, $J = 8$ Hz, 1H, H-3), 8.81 (s, 1H, D_2O exch, NHCO), 8.85 (s, 1H, H-11), 9.59 (t, $J = 5$ Hz, 1H, D_2O exch, NH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 23.55 ($N(CH_2CH_2)_2$), 42.23 ($NHCH_2CH_2$), 43.16 ($COCH_2$), 54.36 ($N(CH_2CH_2)_2$), 54.70 ($NHCH_2CH_2$), 103.19 (C-2), 105.51 (C-12a), 111.83 (C-4), 112.53 (C-6), 121.48 (C-11a), 125.56 (C-9), 126.92 (C-7), 127.89 (C-11), 128.98 (C-8), 129.62 (C-10), 129.82 (C-10a), 130.02 (C-3), 136.33 (C-6a), 148.11 (C-4a), 149.30 (C-1), 150.82 (C-5a), 163.64 (NHCO), 179.66 (C-12). Anal. Calcd for $C_{25}H_{24}ClN_3O_3$: C, 66.74; H, 5.38; N, 9.34. Found: C, 66.51; H, 5.04; N, 9.61.

Spectral data for the intermediate 4-amino-1-[2-(pyrrolidin-1-yl)ethylamino]-12*H*-benzo[*b*]xanthen-12-one (**17c**): ^1H NMR ($CDCl_3$, 400 MHz) δ 1.83 (m, 4H, $N(CH_2CH_2)_2$), 2.64 (m, 4H, $N(CH_2CH_2)_2$), 2.84 (t, $J = 7$ Hz, 2H, $NHCH_2CH_2$), 3.49 (q, $J = 7$ Hz, 5 Hz, 2H, $NHCH_2CH_2$), 6.32 (d, $J = 8$ Hz, 1H, H-2), 7.06 (d, $J = 8$ Hz, 1H, H-3), 7.42 (t, $J = 8$ Hz, 1H, H-9), 7.54 (t, $J = 8$ Hz, 1H, H-8), 7.69 (s, 1H, H-6), 7.83 (d, $J = 8$ Hz, 1H, H-7), 7.98 (d, $J = 8$ Hz, 1H, H-10), 8.68 (s, 1H, H-11), 9.08 (t, $J = 5$ Hz, 1H, D_2O exch, NH).

4.1.20. 2-Chloro-*N*-[1-[2-(piperidin-1-yl)ethylamino]-12-oxo-12*H*-benzo[*b*]xanthen-4-yl]acetamide (19d). This compound was prepared by an analogous procedure as described for the preparation of **18a**, starting from **15d**. Yield: 80%; mp 256–258 °C (EtOH); ^1H NMR ($CDCl_3$, 400 MHz) δ 1.46 (m, 2H, 4-piperidine-H), 1.62 (m, 4H, 3,5-piperidine-H), 2.56 (m, 4H, 2,6-piperidine-H), 2.74 (t, $J = 7$ Hz, 2H, $NHCH_2CH_2$), 3.47 (q, $J = 7$ Hz, 5Hz, 2H, $NHCH_2CH_2$), 4.38 (s, 2H, $COCH_2$), 6.49 (d, $J = 8$ Hz, 1H, H-2), 7.49 (t, $J = 8$ Hz, 1H, H-9), 7.63 (t, $J = 8$ Hz, 1H, H-8), 7.80 (s, 1H, H-6), 7.90 (d, $J = 8$ Hz, 1H, H-7), 8.06 (d, $J = 8$ Hz, 1H, H-10), 8.30 (d, $J = 8$ Hz, 1H, H-3), 8.79 (s, 1H, NHCO), 8.84 (s, 1H, H-11), 9.59 (t, $J = 5$ Hz, 1H, D_2O exch, NH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 24.11 (4-piperidine-C), 25.27 (3,5-piperidine-C), 40.26 ($NHCH_2CH_2$), 42.97 ($NHCOCH_2$), 54.46 (2,6-piperidine-C), 56.97 ($NHCH_2CH_2$), 103.07 (C-2), 104.98 (C-12a), 112.36 (C-4), 112.46 (C-6), 121.36 (C-11a), 125.70 (C-9), 127.01 (C-7), 127.77 (C-11), 129.03 (C-8), 129.98 (C-10a), 129.67 (C-10), 130.04 (C-3), 136.47 (C-6a), 148.34 (C-4a), 148.67 (C-1), 150.80 (C-5a), 164.02 (NHCO), 179.52 (C-12). Anal. Calcd for $C_{26}H_{26}ClN_3O_3$: C, 67.31; H, 5.65; N, 9.06. Found: C, 67.00; H, 5.78; N, 9.15.

Spectral data for the intermediate 4-amino-1-[2-(piperidin-1-yl)ethylamino]-12*H*-benzo[*b*]xanthen-12-one (**17d**): ^1H NMR ($CDCl_3$, 400 MHz) δ 1.52 (m, 2H, 4-piperidine-H), 1.68 (m, 4H, 3,5-piperidine-H), 2.55 (m, 4H, 2,6-piperidine-H), 2.91 (t, $J = 7$ Hz, 2H, $NHCH_2CH_2$), 3.48 (q, $J = 7$ Hz, 5 Hz, 2H, $NHCH_2CH_2$), 6.26 (d, $J = 8$ Hz, 1H, H-2), 7.02 (d, $J = 8$ Hz, 1H, H-3), 7.39 (t, $J = 8$ Hz, 1H, H-9), 7.48 (t, $J = 8$ Hz, 1H, H-8), 7.59 (s, 1H, H-6), 7.77 (d, $J = 8$ Hz, 1H, H-7), 7.97 (d, $J = 8$ Hz, 1H, H-10), 8.68 (s, 1H, H-11), 8.98 (t, $J = 5$ Hz, 1H, D_2O exch, NH).

4.1.21. 2-Dimethylamino-*N*-[1-(2-dimethylaminoethylamino)-9-oxo-9*H*-xanthen-4-yl]acetamide (20a). To a solution of the chloride **18a** (375 mg, 1 mmol) in absolute ethanol (10 mL) was added dropwise a 5.6 M ethanolic solution of dimethylamine (720 μL, 4 mmol) 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/MeOH 8:1 as the eluent, to provide **20a** (363 mg, 95%); mp (dihydrochloride) 195–197 °C (EtOH); ^1H NMR ($CDCl_3$, 400 MHz) δ 2.41 (s, 6H, $CH_2CH_2N(CH_3)_2$), 2.49 (s, 6H, $COCH_2N(CH_3)_2$), 2.74 (t, $J = 7$ Hz, 2H, $NHCH_2CH_2$), 3.16 [s, 2H, $COCH_2N(CH_3)_2$], 3.41 (q, $J = 7$ Hz, 5 Hz, 2H, $NHCH_2CH_2$), 6.42 (d, $J = 8$ Hz, 1H, H-2), 7.29–7.35 (m, 2H, H-5, H-7), 7.65 (td, $J = 8$ Hz, 1 Hz, 1H, H-6), 8.19–8.26 (m, 2H, H-3, H-8), 9.35 (t, $J = 5$ Hz, 1H, D_2O exch, NH), 9.41 (s, 1H, D_2O exch, NHCO); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 40.43 ($NHCH_2CH_2N(CH_3)_2$), 45.17 ($CH_2CH_2N(CH_3)_2$), 46.01 ($COCH_2N(CH_3)_2$), 57.48 ($NHCH_2CH_2N(CH_3)_2$), 63.48 ($NHCOCH_2$), 102.95 (C-2), 106.41 (C-9a), 112.77 (C-4), 116.84 (C-5), 121.88 (C-8a), 123.87 (C-7), 126.22 (C-8), 130.00 (C-3), 134.08 (C-6), 148.02 (C-1), 148.20 (C-4a), 154.52

(C-10a), 168.64 (NHCO), 179.33 (C-9). Anal. Calcd for $C_{21}H_{26}N_4O_3{\cdot}2HCl{\cdot}1/2H_2O$: C, 58.80; H, 6.81; N, 13.06. Found: C, 58.52; H, 6.54; N, 13.31.

4.1.22. 2-Diethylamino-*N*-[1-(2-diethylaminoethylamino)-9-oxo-9*H*-xanthen-4-yl]acetamide (20b). This compound was prepared by an analogous procedure as described for the preparation of **20a**. Yield: 93%; mp (dihydrochloride) >270 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.05 (t, J = 7 Hz, 6H, $CH_2CH_2N(CH_2CH_3)_2$), 1.17 (t, J = 7 Hz, 6H, $COCH_2N(CH_2CH_3)_2$), 2.59 (q, J = 7 Hz, 4H, $CH_2CH_2N(CH_2CH_3)_2$), 2.71 (q, J = 7 Hz, 4H, $COCH_2N(CH_2CH_3)_2$), 2.75 (t, J = 7 Hz, 2H, $NHCH_2CH_2$), 3.21 (s, 2H, $COCH_2N(CH_2CH_3)_2$), 3.28 (q, J = 7 Hz, 5 Hz, 2H, $NHCH_2CH_2$), 6.39 (d, J = 9 Hz, 1H, H-2), 7.25–7.32 (m, 2H, H-5, H-7), 7.60 (td, J = 8 Hz, 2 Hz, 1H, H-6), 8.21 (dd, J = 8 Hz, 2 Hz, 1H, H-8), 8.37 (d, J = 9 Hz, 1H, H-3), 9.27 (t, J = 5 Hz, 1H, D_2O exch, NH), 9.77 (s, 1H, D_2O exch, NHCO); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 11.68 ($CH_2CH_2N(CH_2CH_3)_2$), 12.67 ($COCH_2N(CH_2CH_3)_2$), 41.13 ($CH_2CH_2N(CH_2CH_3)_2$), 47.01 ($CH_2CH_2N(CH_2CH_3)_2$), 48.66 ($COCH_2N(CH_2CH_3)_2$), 51.20 ($CH_2CH_2N(CH_2CH_3)_2$), 58.33 ($NHCOCH_2$), 102.91 (C-2), 106.22 (C-9a), 112.99 (C-4), 116.52 (C-5), 121.88 (C-8a), 123.18 (C-7), 126.18 (C-8), 128.90 (C-3), 133.90 (C-6), 147.28 (C-1), 148.05 (C-4a), 154.49 (C-10a), 169.67 (NHCO), 179.04 (C-9). Anal. Calcd for $C_{25}H_{34}N_4O_3{\cdot}2HCl{\cdot}H_2O$: C, 56.71; H, 7.23; N, 10.58. Found: C, 56.33; H, 7.01; N, 10.20.

4.1.23. *N*-[1-[2-(Pyrrolidin-1-yl)ethylamino]-9-oxo-9*H*-xanthen-4-yl]-2-pyrrolidin-1-yl-acetamide (20c). This compound was prepared by an analogous procedure as described for the preparation of **20a**. Yield: 96%; mp (dihydrochloride) >270 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.78 (m, 4H, $CH_2CH_2N(CH_2CH_2)_2$), 1.91 (m, 4H, $COCH_2N(CH_2CH_2)_2$), 2.60 (m, 4H, $CH_2CH_2N(CH_2CH_2)_2$), 2.79 (m, 4H, $COCH_2N(CH_2CH_2)_2$), 2.80 (t, J = 7 Hz, 2H, $NHCH_2CH_2$), 3.31 (s, 2H, $COCH_2N(CH_2CH_2)_2$), 3.37 (q, J = 7 Hz, 5 Hz, 2H, $NHCH_2CH_2$), 6.36 (d, J = 9 Hz, 1H, H-2), 7.17 (dd, J = 8 Hz, 2 Hz, 1H, H-5), 7.26 (td, J = 8 Hz, 2 Hz, 1H, H-7), 7.57 (td, J = 8 Hz, 2 Hz, 1H, H-6), 8.15 (dd, J = 8 Hz, 2 Hz, 1H, H-8), 8.25 (d, J = 9 Hz, 1H, H-3), 9.26 (t, J = 5 Hz, 1H, D_2O exch, NH), 9.46 (s, 1H, D_2O exch, NHCO); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 23.30 ($CH_2CH_2N(CH_2CH_2)_2$), 24.08 ($COCH_2N(CH_2CH_2)_2$), 41.82 ($NHCH_2CH_2N(CH_2CH_2)_2$), 54.10 ($CH_2CH_2N(CH_2CH_2)_2$), 54.28 ($COCH_2N(CH_2CH_2)_2$), 54.43 ($NHCH_2CH_2$ $N(CH_2CH_2)_2$), 59.17 ($NHCOCH_2$), 102.84 (C-2), 106.15 (C-9a), 112.84 (C-4), 116.52 (C-5), 121.73 (C-8a), 123.68 (C-7), 126.03 (C-8), 129.31 (C-3), 133.97 (C-6), 147.47 (C-1), 147.98 (C-4a), 154.34 (C-10a), 168.71 (NHCO), 179.08 (C-9). Anal. Calcd for $C_{25}H_{30}N_4O_3{\cdot}2HCl{\cdot}$ $3/4H_2O$: C, 57.64; H, 6.48; N, 10.75. Found: C, 57.50; H, 6.57; N, 10.83.

4.1.24. *N*-[1-[2-(Piperidin-1-yl)ethylamino]-9-oxo-9*H*-xanthen-4-yl]-2-piperidin-1-yl-acetamide (20d). This compound was prepared by an analogous procedure as described for the preparation of **20a**. Yield: 96%; mp (dihydrochloride) >270 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.45 [m, 2H, CH_2CH_2(4-piperidine-H)], 1.55 [m, 2H, $COCH_2$(4-piperidine-H)], 1.64 [m, 4H, CH_2CH_2(3,5-piperidine-H)], 1.74 [m, 4H, $COCH_2$(3,5-piperidine-H)], 2.54 [m, 4H, CH_2CH_2(2,6-piperidine-H)], 2.62 [m, 4H, $COCH_2$(2,6-piperidine-H)], 2.72 (t, J = 7 Hz, 2H, $NHCH_2CH_2$), 3.13 (s, 2H, $NHCOCH_2$), 3.41 (q, J = 7 Hz, 5 Hz, 2H, $NHCH_2CH_2$), 6.42 (d, J = 9 Hz, 1H, H-2), 7.29–7.34 (m, 2H, H-5, H-7), 7.64 (td, J = 8 Hz, 1.5 Hz, 1H, H-6), 8.22 (dd, J = 8 Hz, 1.5 Hz, 1H, H-8), 8.39 (d, J = 9 Hz, 1H, H-3), 9.29 (t, J = 5 Hz, 1H, D_2O exch, NH), 9.71 (s, 1H, D_2O exch, NHCO); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 23.63 [CH_2CH_2(4-piperidine-C)], 24.04 [$COCH_2$(4-piperidine-C)], 25.55 [CH_2CH_2(3,5-piperidine-C)], 26.68 [$COCH_2$(3,5-piperidine-C)], 40.14 ($NHCH_2CH_2$), 54.51 [CH_2CH_2(2,6-piperidine-C)], 54.91 [$COCH_2$(2,6-piperidine-C)], 57.12 ($NHCH_2CH_2$), 62.78 ($NHCOCH_2$), 103.13 (C-2), 106.37 (C-9a), 113.35 (C-4), 116.62 (C-5), 121.95 (C-8a), 123.86 (C-7), 126.32 (C-8), 128.93 (C-3), 134.15 (C-6), 147.27 (C-1), 147.93 (C-4a), 154.62 (C-10a), 168.63 (NHCO), 179.21 (C-9). Anal. Calcd for $C_{27}H_{34}N_4O_3{\cdot}2HCl{\cdot}$ $1/2H_2O$: C, 59.55; H, 6.85; N, 10.29. Found: C, 59.71; H, 6.50; N, 10.03.

4.1.25. 2-Dimethylamino-*N*-[1-(2-dimethylaminoethylamino)-12-oxo-12*H*-benzo[*b*]xanthen-4-yl]acetamide (21a). This compound was prepared by an analogous procedure as described for the preparation of **20a**, starting from **19a**. Yield: 95%; mp (dihydrochloride) 231–233 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.42 (s, 6H, $CH_2CH_2N(CH_3)_2$), 2.57 (s, 6H, $COCH_2N(CH_3)_2$), 2.74 (t, J = 7 Hz, 2H, $NHCH_2CH_2$), 3.21 [s, 2H, $COCH_2N(CH_3)_2$] 3.42 (q, J = 7 Hz, 2H, $NHCH_2CH_2$), 6.43 (d, J = 8 Hz, 1H, H-2), 7.46 (t, J = 8 Hz, 1H, H-9), 7.57 (t, J = 8 Hz, 1H, H-8), 7.62 (s, 1H, H-6), 7.84 (d, J = 8 Hz, 1H, H-7), 7.99 (d, J = 8 Hz, 1H, H-10), 8.27 (d, J = 8 Hz, 1H, H-3), 8.79 (s, 1H, H-11), 9.44 (s, 1H, D_2O exch, NHCO), 9.48 (t, J = 5 Hz, 1H, D_2O exch, NH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 40.55 ($NHCH_2CH_2N(CH_3)_2$), 45.22 ($CH_2CH_2N(CH_3)_2$), 46.09 ($COCH_2N(CH_3)_2$), 57.55 ($NHCH_2CH_2N(CH_3)_2$), 63.58 ($NHCOCH_2$), 102.99 (C-2), 105.56 (C-12a), 112.18 (C-6), 112.66 (C-4), 121.44 (C-11a), 125.28 (C-9), 126.70 (C-7), 127.74 (C-11), 128.74 (C-8), 129.58 (C-10), 129.59 (C-10a), 130.57 (C-3), 136.14 (C-6a), 148.07 (C-4a), 148.59 (C-1), 150.92 (C-5a), 168.65 (NHCO), 179.61 (C-12). Anal. Calcd for $C_{25}H_{28}N_4O_3{\cdot}2HCl{\cdot}2H_2O$: C, 55.45; H, 6.33; N, 10.35. Found: C, 55.19; H, 6.46; N, 10.52.

4.1.26. 2-Diethylamino-*N*-[1-(2-diethylaminoethylamino)-12-oxo-12*H*-benzo[*b*]xanthen-4-yl]acetamide (21b). This compound was prepared by an analogous procedure as described for the preparation of **20a**, starting from **19b**. Yield: 96%; mp (dihydrochloride) 239–241 °C (dec) (EtOH). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.14 (t, J = 7 Hz, 6H, $CH_2CH_2N(CH_2CH_3)_2$), 1.27 (t, J = 7 Hz, 6H, $COCH_2N(CH_2CH_3)_2$), 2.71 (q, J = 7 Hz, 4H, $CH_2CH_2N(CH_2CH_3)_2$), 2.80 (q, J = 7 Hz, 4H, $COCH_2N(CH_2CH_3)_2$), 2.86 (t, J = 7 Hz, 2H, $NHCH_2CH_2$), 3.29 (s, 2H, $COCH_2N(CH_2CH_3)_2$), 3.43 (q, J = 7 Hz, 2H, $NHCH_2CH_2$), 6.48 (d, J = 8 Hz, 1H, H-2), 7.49

(t, J = 8 Hz, 1H, H-9), 7.61 (t, J = 8 Hz, 1H, H-8), 7.71 (s, 1H, H-6), 7.88 (d, J = 8 Hz, 1H, H-7), 8.05 (d, J = 8 Hz, 1H, H-10), 8.44 (d, J = 8 Hz, 1H, H-3), 8.83 (s, 1H, H-11), 9.53 (t, J = 5 Hz, 1H, D_2O exch, NH), 9.98 (s, 1H, D_2O exch, NHCO); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 11.82 ($CH_2CH_2N(CH_2CH_3)_2$), 12.54 ($COCH_2N(CH_2\ CH_3)_2$), 41.49 ($CH_2CH_2N(CH_2CH_3)_2$), 47.21 ($CH_2CH_2\ N(CH_2CH_3)_2$), 47.58 ($COCH_2N$-$(CH_2CH_3)_2$), 51.56 ($CH_2CH_2N(CH_2CH_3)_2$), 58.38 ($NHCOCH_2$), 103.37 (C-2), 106.01 (C-12a), 112.38 (C-6), 112.52 (C-4), 121.67 (C-11a), 125.32 (C-9), 126.82 (C-7), 127.94 (C-11), 128.59 (C-8), 129.60 (C-10a), 129.63 (C-10), 130.15 (C-3), 136.34 (C-6a), 147.12 (C-4a), 147.64 (C-1), 151.09 (C-5a), 168.66 (NHCO), 179.79 (C-12). Anal. Calcd for $C_{29}H_{36}N_4O_3{\cdot}2HCl{\cdot}H_2O$: C, 60.10; H, 6.96; N, 9.67. Found: C, 60.42; H, 7.08; N, 9.44.

4.1.27. *N*-[1-[2-(Pyrrolidin-1-yl)ethylamino]-12-oxo-12*H*-benzo[*b*]xanthen-4-yl]-2-pyrrolidin-1-yl-acetamide (21c). This compound was prepared by an analogous procedure as described for the preparation of **20a**, starting from **19c**. Yield: 96%; mp (dihydrochloride) 198–200 °C (EtOH); ^1H NMR ($CDCl_3$, 400 MHz) δ 1.95 (m, 4H, $CH_2CH_2N(CH_2CH_2)_2$), 2.01 (m, 4H, $COCH_2N$-$(CH_2CH_2)_2$), 2.83 (m, 4H, $CH_2CH_2N(CH_2CH_2)_2$), 2.96 (m, 4H, $COCH_2N(CH_2CH_2)_2$), 3.10 (t, J = 6 Hz, 2H, $NHCH_2CH_2$), 3.41 (s, 2H, $COCH_2N(CH_2CH_2)_2$), 3.58 (q, J = 5 Hz, 2H, $NHCH_2CH_2$), 6.42 (d, J = 8 Hz, 1H, H-2), 7.35 (s, 1H, H-6), 7.40 (t, J = 8 Hz, 1H, H-9), 7.48 (t, J = 8 Hz, 1H, H-8), 7.66 (d, J = 8 Hz, 1H, H-7), 7.92 (d, J = 8 Hz, 1H, H-10), 8.21 (d, J = 8 Hz, 1H, H-3), 8.59 (s, 1H, H-11), 9.32 (t, J = 5 Hz, 1H, D_2O exch, NH), 9.51 (s, 1H, D_2O exch, NHCO); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 23.28 ($CH_2CH_2N(CH_2CH_2)_2$), 24.23 ($COCH_2N(CH_2CH_2)_2$), 40.57 ($NHCH_2CH_2N$-$(CH_2CH_2)_2$), 53.84 ($NHCH_2CH_2\ N(CH_2CH_2)_2$), 54.16 ($CH_2CH_2N(CH_2CH_2)_2$), 54.39 ($COCH_2N(CH_2CH_2)_2$), 59.17 ($NHCOCH_2$), 102.91 (C-2), 105.43 (C-12a), 111.99 (C-4), 113.13 (C-6), 121.05 (C-11a), 125.28 (C-9), 126.66 (C-7), 127.49 (C-11), 128.70 (C-8), 129.32 (C-10a), 129.40 (C-10), 130.02 (C-3), 135.98 (C-6a), 147.63 (C-4a), 147.87 (C-1), 150.55 (C-5a), 168.73 (NHCO), 179.39 (C-12). Anal. Calcd for $C_{29}H_{32}N_4O_3{\cdot}2HCl{\cdot}H_2O$: C, 60.52; H, 6.30; N, 9.74. Found: C, 60.37; H, 6.66; N, 9.91.

4.1.28. *N*-[1-[2-(Piperidin-1-yl)ethylamino]-12-oxo-12*H*-benzo[*b*]xanthen-4-yl]-2-piperidin-1-yl-acetamide (21d). This compound was prepared by an analogous procedure as described for the preparation of **20a**, starting from **19d**. Yield: 96%; mp 171–173 °C (EtOH); ^1H NMR ($CDCl_3$, 400 MHz) δ 1.45 [m, 2H, CH_2CH_2(4-piperidine-H)], 1.51 [m, 2H, $COCH_2$(4-piperidine-H)], 1.62 [m, 4H, CH_2CH_2(3,5-piperidine-H)], 1.68 [m, 4H, $COCH_2$(3,5-piperidine-H)], 2.55 [m, 4H, CH_2CH_2(2,6-piperidine-H)], 2.61 [m, 4H, $COCH_2$(2,6-piperidine-H)], 2.88 (t, J = 7 Hz, 2H, $NHCH_2CH_2$), 3.15 (s, 2H, $NHCOCH_2$), 3.44 (q, J = 7 Hz, 5 Hz, 2H, $NHCH_2CH_2$), 6.46 (d, J = Hz, 1H, H-2), 7.44 (t, J = Hz, 1H, H-9), 7.52 (t, J = Hz, 1H, H-8), 7.56 (s, 1H, H-6), 7.73 (d, J = Hz, 1H, H-7), 7.97 (d, J = Hz, 1H, H-10), 8.28 (d, J = Hz, 1H, H-3), 8.74 (s, 1H, H-11), 9.45 (t, J = 5 Hz, 1H, D_2O exch, NH), 9.95 (s, 1H, D_2O exch, NHCO); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 23.82 [CH_2CH_2(4-piperidine-C)], 24.07 [$COCH_2$(4-piperidine-C)], 25.53 [CH_2 CH_2(3,5-piperidine-C)], 26.59 [$COCH_2$(3,5-piperidine-C)], 40.32 ($NHCH_2CH_2$), 54.35 [CH_2CH_2(2,6-piperidine-C)], 54.93 [$COCH_2$(2,6-piperidine-C)], 56.83 ($NHCH_2CH_2$), 62.86 ($NHCOCH_2$), 103.11 (C-2), 105.77 (C-12a), 112.47 (C-4), 112.56 (C-6), 121.35 (C-11a), 125.31 (C-9), 126.73 (C-7), 127.62 (C-11), 128.67 (C-8), 129.54 (C-10a), 129.55 (C-10), 130.26 (C-3), 136.22 (C-6a), 147.92 (C-4a), 147.92 (C-1), 150.73 (C-5a), 169.02 (NHCO), 179.53 (C-12). Anal. Calcd for $C_{31}H_{36}N_4O_3$: C, 72.63; H, 7.08; N, 10.93. Found: C, 72.48; H, 7.31; N, 10.72.

4.1.29. 2-[5-Nitro-2*H*-benzopyrano[4,3,2-*cd*]indazol-2-yl]-1-ethanol (22). A solution of **12** (1.1 g, 4 mmol) and 2-hydroxyethylhydrazine (684 mg, 9 mmol) in dry pyridine (8 mL) was stirred at room temperature for 12 h. The reaction mixture was then diluted with CH_2Cl_2 (100 mL), washed successively with 18% HCl solution and water, dried (Na_2SO_4), and evaporated to dryness. The residue was purified by column chromatography (silica gel) using a mixture of cyclohexane/EtOAc 1:3 as the eluent, to provide compound **22** (1.06 g, 89%); mp 211–213 °C (EtOH); ^1H NMR (DMSO-d_6, 400 MHz) δ 3.93 (t, J = 7 Hz, 2H, NCH_2CH_2OH), 4.47 (t, J = 7 Hz, 2H, NCH_2CH_2OH), 7.13 (d, J = 8 Hz, 1H, H-3), 7.37 (t, J = 8 Hz, 1H, H-9), 7.46–7.58 (m, 2H, H-7, H-8), 7.86 (d, J = 8 Hz, 1H, H-10), 7.99 (d, J = 8 Hz, 1H, H-4); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 49.84 ($N–NCH_2CH_2OH$), 62.11 (NCH_2 CH_2OH), 104.52 (C-3), 116.00 (C-2b), 116.84 (C-10a), 118.84 (C-7), 122.81 (C-10), 124.82 (C-5), 126.07 (C-9), 126.34 (C-4), 131.20 (C-8), 139.06 (C-10b), 142.75 (C-2a), 146.02 (C-5a), 153.69 (C-6a). Anal. Calcd for $C_{15}H_{11}N_3O_4$: C, 60.61; H, 3.73; N, 14.14. Found: C, 60.75; H, 3.60; N, 14.00.

4.1.30. 2-[5-Nitro-2*H*-benzopyrano[4,3,2-*cd*]indazol-2-yl]-1-ethanol methanesulfonate (23). A solution of methanesulfonyl chloride (70 μL, 0.9 mmol) in dry CH_2Cl_2 (5 mL) was added dropwise at 0 °C to a suspension of triethylamine (279 μL, 2 mmol) and **22** (257 mg, 0.865 mmol) in dry CH_2Cl_2 (20 mL) and the mixture was stirred at 0 °C for 10 min and then at room temperature for 4 h. The reaction mixture was then washed with 1 N HCl and the organic layer was dried (Na_2SO_4) and concentrated to dryness. The residue was purified by column chromatography (silica gel), using a mixture of cyclohexane/EtOAc 3:1 as the eluent, to give compound **23** (304 mg, 94%). Mp 234–236 °C (EtOAc/*n*-hexane); ^1H NMR ($CDCl_3$, 400 MHz) δ 2.87 (s, 3H, SO_2CH_3), 4.67–4.75 (m, 4H, $N–NCH_2CH_2$), 6.93 (d, J = 9 Hz, 1H, H-3), 7.37 (t, J = 8 Hz, 1H, H-9), 7.52 (t, J = 8 Hz, 1H, H-8), 7.62 (d, J = 8 Hz, 1H, H-7), 7.97 (d, J = 8 Hz, 1H, H-10), 8.21 (d, J = 9 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 37.06 (SO_2CH_3) 48.15 ($N–NCH_2CH_2$), 67.32 ($N–NCH_2CH_2$), 104.29 (C-3), 115.17 (C-2b), 116.75 (C-10a), 118.95 (C-7), 122.81 (C-10), 124.25 (C-5), 126.05 (C-9), 126.19 (C-4), 131.16 (C-8), 138.95 (C-10b), 142.74 (C-2a), 145.75 (C-5a), 153.06 (C-6a). Anal. Calcd for $C_{16}H_{13}N_3O_6S$: C,

51.20; H, 3.49; N, 11.19. Found: C, 51.25; H, 3.21; N, 11.35.

4.1.31. Dimethyl-[2-[5-nitro-2*H*-benzopyrano[4,3,2-*cd*]indazol-2-yl]ethyl]amine (24a). To a solution of the mesylate **23** (375 mg, 1 mmol) in absolute ethanol (12 mL) was added dropwise a 5.6 M ethanolic solution of dimethylamine (540 μL, 3 mmol) and the resulting solution was refluxed for 10 h. After cooling, the solvent was removed under reduced pressure, and the residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH 94:6) to give **24a** (300 mg, 92%); mp (hydrochloride) >270 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.32 (s, 6H, 2× CH_3), 2.89 (t, J = 7 Hz, 2H, N–NCH$_2$CH_2), 4.44 (t, J = 7 Hz, 2H, N–NCH_2CH$_2$), 6.81 (d, J = 9 Hz, 1H, H-3), 7.29 (t, J = 8 Hz, 1H, H-9), 7.43 (t, J = 8 Hz, 1H, H-8), 7.50 (dd, J = 8 Hz, 1 Hz, 1H, H-7), 7.90 (dd, J = 8 Hz, 1 Hz, 1H, H-10), 8.06 (d, J = 9 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 45.39 (2× CH_3), 48.03 (N–N*C*H$_2$CH$_2$), 58.03 (N–NCH$_2$*C*H$_2$), 102.18 (C-3), 115.92 (C-2b), 116.96 (C-10a), 119.12 (C-7), 122.98 (C-10), 125.01 (C-5), 125.67 (C-9), 126.84 (C-4), 130.67 (C-8), 140.00 (C-10b), 142.17 (C-2a), 146.40 (C-5a), 153.60 (C-6a). Anal. Calcd for $C_{17}H_{16}N_4O_3$·HCl·2H$_2$O: C, 53.61; H, 5.56; N, 14.71. Found: C, 53.74; H, 5.75; N, 14.86.

4.1.32. Diethyl-[2-[5-nitro-2*H*-benzopyrano[4,3,2-*cd*]indazol-2-yl]ethyl]amine (24b).[48] This compound was prepared by an analogous procedure as described for the preparation of **24a**. Yield: 91%; mp (hydrochloride) 268–270 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.92 (t, J = 7 Hz, 6H, (CH$_2$CH_3)$_2$), 2.53 (q, J = 7 Hz, 4H, (CH_2CH$_3$)$_2$), 2.93 (t, J = 7 Hz, 2H, N–NCH$_2$CH_2), 4.34 (t, J = 7 Hz, 2H, N–NCH_2CH$_2$), 6.75 (d, J = 9 Hz, 1H, H-3), 7.27 (t, J = 8 Hz, 1H, H-9), 7.41 (t, J = 8 Hz, 1H, H-8), 7.48 (d, J = 8 Hz, 1H, H-7), 7.86 (d, J = 8 Hz, 1H, H-10), 8.01 (d, J = 9 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 11.85 [(CH$_2$*C*H$_3$)$_2$], 47.36 [(*C*H$_2$CH$_3$)$_2$], 48.94 (N–N*C*H$_2$CH$_2$), 52.58 (N–NCH$_2$*C*H$_2$), 102.45 (C-3), 115.65 (C-2b), 116.93 (C-10a), 119.03 (C-7), 122.85 (C-10), 124.76 (C-5), 125.57 (C-9), 126.45 (C-4), 130.53 (C-8), 139.76 (C-10b), 142.26 (C-2a), 146.26 (C-5a), 153.50 (C-6a).

4.1.33. 5-Nitro-2-[2-(pyrrolidin-1-yl)ethyl]-2*H*-benzopyrano[4,3,2-*cd*]indazole (24c). This compound was prepared by an analogous procedure as described for the preparation of **24a**. Yield: 94%; mp (hydrochloride) >270 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.75 [m, 4H, N(CH$_2$CH_2)$_2$], 2.57 [m, 4H, N(CH_2CH$_2$)$_2$], 3.03 (t, J = 7 Hz, 2H, N–NCH$_2$CH_2), 4.46 (t, J = 7 Hz, 2H, N–NCH_2CH$_2$), 6.78 (d, J = 9 Hz, 1H, H-3), 7.28 (t, J = 8 Hz, 1H, H-9), 7.42 (t, J = 8 Hz, 1H, H-8), 7.49 (d, J = 8 Hz, 1H, H-7), 7.88 (d, J = 8 Hz, 1H, H-10), 8.05 (d, J = 9 Hz, 1H, H-4). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 23.44 [N(CH$_2$*C*H$_2$)$_2$], 49.32 (N–N*C*H$_2$CH$_2$), 54.21 [N(*C*H$_2$CH$_2$)$_2$], 55.24 (N–NCH$_2$*C*H$_2$), 102.14 (C-3), 115.82 (C-2b), 116.92 (C-10a), 119.05 (C-7), 122.91 (C-10), 124.93 (C-5), 125.59 (C-9), 126.70 (C-4), 130.59 (C-8), 139.89 (C-10b), 142.06 (C-2a), 146.33 (C-5a), 153.53 (C-6a). Anal. Calcd for $C_{19}H_{18}N_4O_3$·HCl·2H$_2$O: C, 53.97; H, 5.48; N, 13.25. Found: C, 54.16; H, 5.30; N, 13.07.

4.1.34. 5-Nitro-2-[2-(piperidin-1-yl)ethyl]-2*H*-benzopyrano[4,3,2-*cd*]indazole (24d). This compound was prepared by an analogous procedure as described for the preparation of **24a**. Yield: 90%; mp (hydrochloride) >270 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.39 (m, 2H, 4-piperidine-H), 1.50 (m, 4H, 3,5-piperidine-H), 2.43 (m, 4H, 2,6-piperidine-H), 2.81 (t, J = 7 Hz, 2H, N–NCH$_2$CH_2), 4.40 (t, J = 7 Hz, 2H, N–NCH_2CH$_2$), 6.78 (d, J = 9 Hz, 1H, H-3), 7.27 (td, J = 8 Hz, 1 Hz, 1H, H-9), 7.41 (td, J = 8 Hz, 1 Hz, 1H, H-8), 7.48 (dd, J = 8 Hz, 1 Hz, 1H, H-7), 7.86 (dd, J = 8 Hz, 1 Hz, 1H, H-10), 8.04 (d, J = 9 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 24.04 (4-piperidine-C), 25.88 (3,5-piperidine-C), 48.04 (N–N*C*H$_2$CH$_2$), 54.73 (2,6-piperidine-C), 58.22 (N–NCH$_2$*C*H$_2$), 102.51 (C-3), 115.81 (C-2b), 116.99 (C-10a), 119.12 (C-7), 122.90 (C-10), 124.85 (C-5), 125.62 (C-9), 126.54 (C-4), 130.62 (C-8), 139.85 (C-10b), 142.24 (C-2a), 146.32 (C-5a), 153.56 (C-6a). Anal. Calcd for $C_{20}H_{20}N_4O_3$·HCl·H$_2$O: C, 57.35; H, 5.53; N, 13.38. Found: C, 57.03; H, 5.74; N, 13.56.

4.1.35. 2-Chloro-*N*-[2-(2-dimethylaminoethyl)-2*H*-benzopyrano[4,3,2-*cd*]indazol-5-yl]acetamide (26a). This compound was prepared by an analogous procedure as described for the preparation of **18a**, starting from **24a**. Yield: 77%; mp >270 °C (EtOAc/*n*-hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.33 (s, 6H, CH$_2$CH$_2$N(CH_3)$_2$), 2.81 (t, J = 7 Hz, 2H, N–NCH$_2$CH_2), 4.28 (s, 2H, COCH_2), 4.30 (t, J = 7 Hz, 2H, N–NCH_2CH$_2$), 6.87 (d, J = 9 Hz, 1H, H-3), 7.21 (t, J = 8 Hz, 1H, H-9), 7.27–7.35 (m, 2H, H-7, H-8), 7.83 (d, J = 8 Hz, 1H, H-10), 7.87 (d, J = 9 Hz, 1H, H-4), 8.34 (s, 1H, D_2O exch, NHCO); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 42.54 (CO*C*H$_2$), 45.54 (CH$_2$CH$_2$N(*C*H$_3$)$_2$), 48.08 (N–N*C*H$_2$CH$_2$), 58.46 (N–NCH$_2$*C*H$_2$), 101.68 (C-3), 111.99 (C-5), 116.74 (C-2b), 118.12 (C-7), 118.33 (C-10a), 123.51 (C-10), 124.50 (C-9), 126.00 (C-4), 129.54 (C-8), 137.68 (C-10b), 138.42 (C-2a), 139.21 (C-5a), 153.99 (C-6a), 163.46 (NHCO). Anal. Calcd for $C_{19}H_{19}ClN_4O_2$: C, 61.54; H, 5.16; N, 15.11. Found: C, 61.38; H, 5.00; N, 15.02.

4.1.36. 2-Chloro-*N*-[2-(2-diethylaminoethyl)-2*H*-benzopyrano[4,3,2-*cd*]indazol-5-yl]acetamide (26b). This compound was prepared by an analogous procedure as described for the preparation of **18a**, starting from **24b**. Yield: 79%; mp >270 C (EtOAc/*n*-hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.01 (t, J = 7 Hz, 6H, CH$_2$CH$_2$N(CH$_2$CH_3)$_2$), 2.59 (q, J = 7 Hz, 4H, CH$_2$CH$_2$N(CH_2CH$_3$)$_2$), 2.97 (t, J = 7 Hz, 2H, N–NCH$_2$CH_2), 4.35 (s, 2H, COCH_2), 4.40 (t, J = 7 Hz, 2H, N–NCH_2CH$_2$), 6.86 (d, J = 9 Hz, 1H, H-3), 7.19 (t, J = 8 Hz, 1H, H-9), 7.26 (d, J = 8 Hz, 1H, H-7), 7.35 (t, J = 8 Hz, 1H, H-8), 7.84 (d, J = 8 Hz, 1H, H-10), 7.88 (d, J = 9 Hz, 1H, H-4), 8.37 (s, 1H, D_2O exch, NHCO); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 11.69 (CH$_2$CH$_2$N(CH$_2$*C*H$_3$)$_2$), 42.54 (CO*C*H$_2$), 47.35 (CH$_2$CH$_2$N(*C*H$_2$CH$_3$)$_2$), 48.74 (N–N*C*H$_2$CH$_2$), 52.43 (N–NCH$_2$*C*H$_2$), 101.92 (C-3), 111.53 (C-5), 116.88 (C-2b), 118.21 (C-7),

118.24 (C-10a), 123.16 (C-10), 124.41 (C-9), 126.12 (C-4), 129.67 (C-8), 137.85 (C-10b), 138.44 (C-2a), 139.11 (C-5a), 154.26 (C-6a), 163.88 (NHCO). Anal. Calcd for $C_{21}H_{23}ClN_4O_2$: C, 63.23; H, 5.81; N, 14.05. Found: C, 63.44; H, 5.65; N, 13.94.

4.1.37. 2-Chloro-*N*-[2-(2-pyrrolidin-1-yl-ethyl)-2*H*-benzopyrano[4,3,2-*cd*]indazol-5-yl]acetamide (26c). This compound was prepared by an analogous procedure as described for the preparation of **18a**, starting from **24c**. Yield: 78%; mp >270 °C (EtOAc/*n*-hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.81 [m, 4H, N(CH_2CH_2)$_2$], 2.65 [m, 4H, N(CH_2CH_2)$_2$], 3.09 (t, J = 7 Hz, 2H, N–NCH_2CH_2), 4.29 (s, 2H, $COCH_2$), 4.53 (t, J = 7 Hz, 2H, N–NCH_2CH_2), 6.88 (d, J = 8 Hz, 1H, H-3), 7.21 (t, J = 8 Hz, 1H, H-9), 7.28–7.37 (m, 2H, H-7, H-8), 7.84 (d, J = 8 Hz, 1H, H-4), 7.89 (d, J = 8 Hz, 1H, H-10), 8.31 (s, 1H, D_2O exch, NHCO); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 23.45 [N(CH_2CH_2)$_2$], 42.93 ($COCH_2$), 50.89 (N–NCH_2CH_2), 54.25 (N–NCH_2CH_2), 54.28 [N(CH_2-CH_2)$_2$], 101.99 (C-3), 112.32 (C-5), 117.11 (C-2b), 117.88 (C-10a), 118.35 (C-7), 123.20 (C-10), 124.67 (C-9), 126.25 (C-4), 130.07 (C-8), 138.05 (C-10b), 138.12 (C-2a), 138.66 (C-5a), 153.92 (C-6a), 163.52 (NHCO). Anal. Calcd for $C_{21}H_{21}ClN_4O_2$: C, 63.55; H, 5.33; N, 14.12. Found: C, 63.61; H, 5.23; N, 14.27.

4.1.38. 2-Chloro-*N*-[2-(2-piperidin-1-yl-ethyl)-2*H*-benzopyrano[4,3,2-*cd*]indazol-5-yl]acetamide (26d). This compound was prepared by an analogous procedure as described for the preparation of **18a**, starting from **24d**. Yield: 82%; mp 221–223 °C (dec) (EtOAc/*n*-hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.45 (m, 2H, 4-piperidine-H), 1.63 (m, 4H, 3,5-piperidine-H), 2.58 (m, 4H, 2,6-piperidine-H), 2.94 (t, J = 7 Hz, 2H, N–NCH_2CH_2), 4.28 (s, 2H, $COCH_2$), 4.49 (t, J = 7 Hz, 2H, N–NCH_2CH_2), 6.85 (d, J = 9 Hz, 1H, H-3), 7.18 (t, J = 8 Hz, 1H, H-9), 7.27 (d, J = 8 Hz, 1H, H-7), 7.33 (t, J = 8 Hz, 1H, H-8), 7.81 (d, J = 8 Hz, 1H, H-10), 7.87 (d, J = 9 Hz, 1H, H-4), 8.31 (s, 1H, D_2O exch, NHCO); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 23.67 (4-piperidine-C), 25.25 (3,5-piperidine-C), 42.89 ($COCH_2$), 46.94 (N–NCH_2CH_2), 54.58 (2,6-piperidine-C), 57.74 (N–NCH_2CH_2), 101.92 (C-3), 110.89 (C-5), 116.55 (C-2b), 118.20 (C-10a), 118.24 (C-7), 123.09 (C-10), 124.56 (C-9), 125.92 (C-4), 129.89 (C-8), 137.97 (C-10b), 138.71 (C-2a), 140.40 (C-5a), 154.22 (C-6a), 164.11 (NHCO). Anal. Calcd for $C_{22}H_{23}ClN_4O_2$: C, 64.31; H, 5.64; N, 13.64. Found: C, 64.58; H, 5.71; N, 13.71.

4.1.39. 2-Dimethylamino-*N*-[2-(2-dimethylaminoethyl)-2*H*-benzopyrano[4,3,2-*cd*]indazol-5-yl]acetamide (27a). This compound was prepared by an analogous procedure as described for the preparation of **20a**, starting from **26a**. Yield: 97%; mp (dihydrochloride) 227–229 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.32 (s, 6H, $CH_2CH_2N(CH_3)_2$), 2.47 (s, 6H, $COCH_2N(CH_3)_2$), 2.84 (t, J = 7 Hz, 2H, N–NCH_2CH_2), 3.17 (s, 2H, $COCH_2$), 4.42 (t, J = 7 Hz, 2H, N–NCH_2CH_2), 6.83 (d, J = 9 Hz, 1H, H-3), 7.19 (t, J = 8 Hz, 1H, H-9), 7.27 (d, J = 8 Hz, 1H, H-7), 7.33 (t, J = 8 Hz, 1H, H-8), 7.89 (d, J = 8 Hz, 1H, H-10), 7.96 (d, J = 9 Hz, 1H, H-4), 9.52 (s, 1H, D_2O exch, NHCO); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 45.65 ($CH_2CH_2N(CH_3)_2$), 46.05 ($COCH_2N(CH_3)_2$), 48.00 (N–NCH_2CH_2), 58.55 (N–NCH_2CH_2), 63.55 ($COCH_2$), 101.48 (C-3), 112.03 (C-5), 116.80 (C-2b), 118.20 (C-7), 118.57 (C-10a), 123.16 (C-10), 124.41 (C-9), 125.96 (C-4), 129.67 (C-8), 137.79 (C-10b), 138.34 (C-2a), 139.88 (C-5a), 154.37 (C-6a), 169.03 (NHCO). Anal. Calcd for $C_{21}H_{25}N_5O_2{\cdot}2HCl{\cdot}1/2H_2O$: C, 54.67; H, 6.12; N, 15.18. Found: C, 54.44; H, 6.13; N, 15.27.

4.1.40. 2-Diethylamino-*N*-[2-(2-diethylaminoethyl)-2*H*-benzopyrano[4,3,2-*cd*]indazol-5-yl]acetamide (27b). This compound was prepared by an analogous procedure as described for the preparation of **20a**, starting from **26b**. Yield: 92%; mp (dihydrochloride) 158–160 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.00 (t, J = 7 Hz, 6H, $CH_2CH_2N(CH_2CH_3)_2$), 1.17 (t, J = 7 Hz, 6H, $COCH_2N(CH_2CH_3)_2$), 2.60 (q, J = 7 Hz, 4H, $CH_2CH_2N(CH_2CH_3)_2$), 2.68 (q, J = 7 Hz, 4H, $COCH_2N(CH_2CH_3)_2$), 2.97 (t, J = 7 Hz, 2H, N–NCH_2CH_2), 3.21 (s, 2H, $COCH_2$), 4.39 (t, J = 7 Hz, 2H, N–NCH_2CH_2), 6.82 (d, J = 9 Hz, 1H, H-3), 7.12–7.22 (m, 2H, H-7, H-9), 7.29 (t, J = 8 Hz, 1H, H-8), 7.85 (d, J = 8 Hz, 1H, H-10), 8.03 (d, J = 9 Hz, 1H, H-4), 9.52 (s, 1H, D_2O exch, NHCO); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 11.62 ($CH_2CH_2N(CH_2CH_3)_2$), 12.64 ($COCH_2N(CH_2CH_3)_2$), 47.38 ($CH_2CH_2N(CH_2CH_3)_2$), 47.97 ($COCH_2N(CH_2CH_3)_2$), 48.70 (N–NCH_2CH_2), 52.19 (N–NCH_2CH_2), 58.18 ($COCH_2$), 101.55 (C-3), 112.36 (C-5), 116.55 (C-2b), 117.94 (C-7), 118.49 (C-10a), 123.02 (C-10), 124.30 (C-9), 125.07 (C-4), 129.59 (C-8), 137.57 (C-10b), 138.08 (C-2a), 139.08 (C-5a), 154.33 (C-6a), 170.10 (NHCO). Anal. Calcd for $C_{25}H_{33}N_5O_2{\cdot}2HCl{\cdot}3/4H_2O$: C, 57.52; H, 7.05; N, 13.42. Found: C, 57.45; H, 7.31; N, 13.22.

4.1.41. *N*-[2-(2-Pyrrolidin-1-yl-ethyl)-2*H*-benzopyrano[4,3,2-*cd*]indazol-5-yl]-2-pyrrolidin-1-yl-acetamide (27c). This compound was prepared by an analogous procedure as described for the preparation of **20a**, starting from **26c**. Yield: 95%; mp (dihydrochloride) 219–221 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.75 (m, 4H, $CH_2CH_2N(CH_2CH_2)_2$), 1.91 (m, 4H, $COCH_2N(CH_2CH_2)_2$), 2.55 (m, 6H, $CH_2CH_2N(CH_2CH_2)_2$, N–NCH_2CH_2), 2.77 (m, 4H, $COCH_2N(CH_2CH_2)_2$), 3.35 (s, 2H, $COCH_2$), 4.42 (t, J = 7 Hz, 2H, N–NCH_2CH_2), 6.80 (d, J = 9 Hz, 1H, H-3), 7.13–7.20 (m, 2H, H-7, H-9), 7.29 (t, J = 8 Hz, 1H, H-8), 7.85 (d, J = 8 Hz, 1H, H-10), 7.97 (d, J = 9 Hz, 1H, H-4), 9.20 (s, 1H, D_2O exch, NHCO); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 23.41 ($CH_2CH_2N(CH_2CH_2)_2$), 24.03 ($COCH_2N(CH_2CH_2)_2$), 49.06 (N–NCH_2CH_2), 54.25 ($CH_2CH_2N(CH_2CH_2)_2$), 54.35 ($COCH_2N(CH_2CH_2)_2$, N–NCH_2CH_2), 59.25 ($COCH_2$), 101.41 (C-3), 112.18 (C-5), 116.62 (C-2b), 118.02 (C-7), 118.50 (C-10a), 123.06 (C-10), 124.31 (C-9), 125.48 (C-4), 129.56 (C-8), 137.54 (C-10b), 138.09 (C-2a), 139.45 (C-5a), 154.27 (C-6a), 169.15 (NHCO). Anal. Calcd for $C_{25}H_{29}N_5O_2{\cdot}2HCl{\cdot}H_2O$: C, 57.47; H, 6.37; N, 13.40. Found: C, 57.39; H, 6.57; N, 13.33.

4.1.42. *N*-[2-(2-Piperidin-1-yl-ethyl)-2*H*-benzopyrano [4,3,2-*cd*]indazol-5-yl]-2-piperidin-1-yl-acetamide (27d). This compound was prepared by an analogous proce-

dure as described for the preparation of **20a**, starting from **26d**. Yield: 95%; mp (dihydrochloride) 168–170 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.41 [m, 2H, CH_2CH_2(4-piperidine-H)], 1.52 [m, 2H, $COCH_2$(4-piperidine-H)], 1.62 [m, 4H, CH_2CH_2(3,5-piperidine-H)], 1.73 [m, 4H, $COCH_2$(3,5-piperidine-H)], 2.44 [m, 4H, CH_2CH_2(2,6-piperidine-H)], 2.61 [m, 4H, $COCH_2$(2,6-piperidine-H)], 2.80 (t,J = 7 Hz, 2H, N–NCH_2CH_2), 3.05 (s, 2H, $COCH_2$), 4.37 (t, J = 7 Hz, 2H, N–NCH_2CH_2), 6.79 (d, J = 9 Hz, 1H, H-3), 7.11–7.18 (m, 2H, H-7, H-9), 7.29 (t, J = 8 Hz, 1H, H-8), 7.83 (d, J = 8 Hz, 1H, H-10), 8.04 (d, J = 9 Hz, 1H, H-4), 9.44 (s, 1H, D_2O exch, NHCO); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 23.71 [CH_2CH_2(4-piperidine-C)], 24.04 [$COCH_2$(4-piperidine-C)], 25.47 [CH_2CH_2(3,5-piperidine-C)], 26.06 [$COCH_2$(3,5-piperidine-C)], 51.30 (N–NCH_2CH_2), 54.25 [CH_2CH_2(2,6-piperidine-C)], 54.40 [$COCH_2$(2,6-piperidine-C)], 57.81 (N–NCH_2CH_2), 62.15 ($COCH_2$), 101.18 (C-3), 112.03 (C-5), 116.11 (C-2b), 118.13 (C-7), 118.51 (C-10a), 122.61 (C-10), 123.93 (C-9), 124.27 (C-4), 129.19 (C-8), 136.95 (C-10b), 138.42 (C-2a), 139.02 (C-5a), 153.85 (C-6a), 168.37 (NHCO). Anal. Calcd for $C_{27}H_{33}N_5O_2{\cdot}2HCl{\cdot}1/2H_2O$: C, 59.88; H, 6.70; N, 12.93. Found: C, 59.97; H, 6.84; N, 12.81.

4.1.43. *N*-(2-Hydroxyethyl)-*N*-[2-(2-hydroxyethyl)-2*H*-benzo[*g*]benzopyrano[4,3,2-*cd*]indazol-5-yl]hydrazine (28). A suspension of **13** (623 mg, 2 mmol) and 2-hydroxyethylhydrazine (304 mg, 4 mmol) in dry DMSO (8 mL) was stirred at room temperature for 24 h. The reaction mixture was then poured into crushed ice and the precipitate was filtered, washed with water, and air-dried. The residue was purified by column chromatography (silica gel) using a mixture of cyclohexane/EtOAc 1:2 as the eluent, to provide compounds **28** (503 mg, 67%) and **29** (67 mg, 9%).

Data for *N*-(2-hydroxyethyl)-*N*-[2-(2-hydroxyethyl)-2*H*-benzo[*g*]benzopyrano[4,3,2-*cd*]indazol-5-yl]hydrazine (**28**): mp >270 °C (DMF); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.91 (q, J = 7 Hz, 2H, N–$NHCH_2CH_2$), 4.06 (q, J = 7 Hz, 2H, NH_2–NCH_2CH_2), 4.70 (t, J = 7 Hz, 2H, N–NCH_2CH_2), 4.78 (t, J = 7 Hz, 2H, NH_2–NCH_2CH_2), 5.04 (t, J = 7 Hz, 1H, D_2O exch, OH), 5.15 (t, J = 7 Hz, 1H, D_2O exch, OH), 7.39 (t, J = 8 Hz, 1H, H-10), 7.41 (s, 1H, H-7), 7.49 (t, J = 8 Hz, 1H, H-9), 7.81 (d, J = 8 Hz, 1H, H-4), 7.89 (d, J = 8 Hz, 1H, H-3), 7.91 (d, J = 8 Hz, 1H, H-8), 7.96 (d, J = 8 Hz, 1H, H-11), 9.56 (s, 1H, H-12); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 51.41 (N–NCH_2CH_2), 52.46 (NH_2–NCH_2CH_2), 58.29 (N–NCH_2CH_2), 60.51 (NH_2–NCH_2CH_2), 108.40 (C-5), 110.64 (C-3), 113.38 (C-4), 113.55 (C-7), 119.41 (C-12a), 122.78 (C-12), 123.81 (C-10), 126.21 (C-2b), 127.32 (C-8), 127.92 (C-9), 128.43 (C-11), 129.91 (C-11a), 134.42 (C-5a), 134.50 (C-7a), 141.52 (C-2a), 143.78 (C-12b), 153.93 (C-6a). Anal. Calcd for $C_{21}H_{20}N_4O_3$: C, 67.01; H, 5.36; N, 14.88. Found: C, 67.16; H, 5.70; N, 14.59.

Data for 2-[5-nitro-2*H*-benzo[*g*]benzopyrano[4,3,2-*cd*]indazol-2-yl]-1-ethanol (**29**): mp 225–227 °C (DMF–H_2O); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.22 (t, J = 7 Hz, 2H, N–NCH_2CH_2), 4.51 (t, J = 7 Hz, 2H, N–NCH_2CH_2), 7.06 (d, J = 9 Hz, 1H, H-3), 7.43–7.54 (m, 2H, H-9, H-10), 7.86 (d, J = 8 Hz, 1H, H-8), 7.90 (d, J = 8 Hz, 1H, H-11), 8.02 (s, 1H, H-7), 8.05 (d, J = 9 Hz, 1H, H-4), 8.47 (s, 1H, H-12); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 53.04 (N–NCH_2CH_2), 60.63 (N–NCH_2CH_2), 105.04 (C-3), 114.97 (C-2b), 115.84 (C-12a), 116.71 (C-7), 122.43 (C-12), 125.88 (C-5), 126.84 (C-10), 126.92 (C-4), 127.67 (C-8), 128.15 (C-9), 128.88 (C-11), 130.08 (C-11a), 134.15 (C-7a), 139.42 (C-12b), 143.22 (C-2a), 145.02 (C-5a), 150.83 (C-6a). Anal. Calcd for $C_{19}H_{13}N_3O_4$: C, 65.70; H, 3.77; N, 12.10. Found: C, 65.88; H, 3.59; N, 12.20.

4.1.44. 2,3,4,12-Tetrahydro-1*H*-benzo[*b*]xanthene-1,12-dione (31). A suspension of **30** (207 mg, 1.1 mmol) and 1,3 cyclohexanedione (112 mg, 1 mmol) in polyphosphoric acid (3 g) was heated at 150 °C for 2 h. After cooling, the reaction mixture was poured into crushed ice, the precipitate was filtered, washed with 10% Na_2CO_3 and water, and air-dried. Flash chromatography on silica gel using a mixture of cyclohexane/EtOAc 2:1 as the eluent provided **31** (169 mg, 64%); mp 259–261 °C (EtOAc/*n*-hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.18 (m, 2H, H-3), 2.61 (t, J = 6.5 Hz, 2H, H-4), 3.02 (t, J = 6.5 Hz, 2H, H-2), 7.52 (dt, J = 8.0 Hz, 1.0 Hz, 1H, H-9), 7.60 (dt, J = 8.0 Hz, 1.0 Hz, 1H, H-8), 7.81 (s, 1H, H-6), 7.86 (dd, J = 8.0 Hz, 1.0 Hz, 1H, H-7), 8.02 (dd, J = 8.0 Hz, 1.0 Hz, 1H, H-10), 8.78 (s, 1H, H-11). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 19.81 (C-3), 29.84 (C-4), 38.52 (C-2), 114.13 (C-6), 115.81 (C-12a), 123.39 (C-11a), 126.48 (C-9), 127.28 (C-7), 128.24 (C-11), 129.12 (C-8), 129.78 (C-10), 130.63 (C-10a), 135.67 (C-6a), 150.96 (C-5a), 174.52 (C-12), 179.04 (C-4a), 193.96 (C-1). Anal. Calcd for $C_{17}H_{12}O_3$: C, 77.26; H, 4.58. Found: C, 77.50; H, 4.31.

4.1.45. 1-Hydroxy-12*H*-benzo[*b*]xanthen-12-one (32). A solution of **31** (100 mg, 0.365 mmol) and DDQ (86 mg, 0.38 mmol) in dry toluene (10 mL) was refluxed for 40 min. After cooling, the solvent was removed under reduced pressure, and the residue was purified by column chromatography (silica gel, cyclohexane to cyclohexane/EtOAc 9:1); Yield: 88%; mp 210–212 °C (lit.[44] 212–214 °C).

4.1.46. 1-[(4-Methylphenyl)sulfonyl]oxy-12*H*-benzo[*b*]-xanthen-12-one (33). To a solution of **32** (524 mg, 2 mmol) in dry acetone (20 ml) were added under argon 4-toluenesulfonyl chloride (419 mg, 2.2 mmol) and anhydrous sodium carbonate (233 mg, 2.2 mmol), and the mixture was refluxed for 3.5 h. The solvent was then vacuum-evaporated, the residue was diluted with CH_2Cl_2 and extracted with water, the organic layer was dried (Na_2SO_4) and vacuum-evaporated. The residue was purified by column chromatography, using a mixture of cyclohexane/EtOAc 3:1 as the eluent, to give compound **33** as an oil (782 mg, 94%); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.38 (s, 3H, 4′-CH_3), 7.08 (d, J = 9.0 Hz, 1H, H-4), 7.32 (d, J = 8 Hz, 2H, H-3′, H-5′), 7.41 (d, J = 9 Hz, 1H, H-2) 7.47 (t, J = 8 Hz, 1H,

H-9), 7.55–7.64 (m, 2H, H-3, H-8), 7.79 (s, 1H, H-6), 7.86 (d, $J = 8$ Hz, 1H, H-7), 7.96 (d, $J = 8$ Hz, 2H, H-2′, H-6′), 8.01 (d, $J = 8$ Hz, 1H, H-10), 8.81 (s, 1H, H-11). Anal. Calcd for $C_{24}H_{16}O_5S$: C, 69.22; H, 3.87. Found: C, 69.04; H, 3.79.

4.1.47. 2-(2*H*-Benzo[*g*]benzopyrano[4,3,2-*cd*]indazol-2-yl)-1-ethanol (34). A solution of **33** (170 mg, 0.41 mmol) and 2-hydroxyethylhydrazine (154 μL, 2.05 mmol) in dry pyridine (6 mL) was refluxed for 9 h. After cooling, the reaction mixture was diluted with CH_2Cl_2 (100 mL), washed successively with a 18% HCl solution and water, dried (Na_2SO_4), and evaporated to dryness. The residue was purified by column chromatography (silica gel) using a mixture of cyclohexane/EtOAc 4:1 as the eluent, to provide compound **34** (109 mg, 88%); mp 222–224 °C (EtOH); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 4.06 (t, $J = 7$ Hz, 2H, N–NCH$_2$CH_2), 4.44 (t, $J = 7$ Hz, 2H, N–NCH_2CH$_2$), 6.56 (d, $J = 8$ Hz, 1H, H-5), 6.92 (d, $J = 8$ Hz, 1H, H-3), 7.33–7.48 (m, 3H, H-4, H-9, H-10), 7.66 (s, 1H, H-7), 7.77 (d, $J = 8$ Hz, 1H, H-8), 7.85 (d, $J = 8$ Hz, 1H, H-11), 8.33 (s, 1H, H-12); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 49.77 (N–N*C*H$_2$CH$_2$), 62.26 (NHCH$_2$*C*H$_2$), 100.77 (C-5), 101.84 (C-3), 114.26 (C-7), 115.91 (C-2b), 118.64 (C-12a), 121.89 (C-12), 125.54 (C-10), 126.94 (C-9), 127.22 (C-8), 128.11 (C-11), 130.21 (C-4), 130.42 (C-11a), 134.27 (C-7a), 137.84 (C-12b), 141.06 (C-2a), 149.56 (C-5a), 152.34 (C-6a). Anal. Calcd for $C_{19}H_{14}N_2O_2$: C, 75.48; H, 4.67; N, 9.27. Found: C, 75.30; H, 4.55; N, 9.16.

4.1.48. 2-(2*H*-Benzo[*g*]benzopyrano[4,3,2-*cd*]indazol-2-yl)-1-ethanol methanesulfonate (35). This compound was prepared by a procedure analogous to that of **23**. Yield: 93%; mp 199–201 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.86 (s, 3H, CH_3), 4.64 (t, $J = 7$ Hz, 2H, N–NCH_2CH$_2$), 4.71 (t, $J = 7$ Hz, 2H, N–NCH$_2$CH_2), 6.61 (d, $J = 8$ Hz, 1H, H-5), 6.94 (d, $J = 8$ Hz, 1H, H-3), 7.36–7.50 (m, 3H, H-4, H-9, H-10), 7.69 (s, 1H, H-7), 7.76 (d, $J = 8$ Hz, 1H, H-8), 7.88 (d, $J = 8$ Hz, 1H, H-11), 8.37 (s, 1H, H-12); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 38.43 (CH_3), 48.52 (N–N*C*H$_2$CH$_2$), 66.69 (NHCH$_2$*C*H$_2$), 100.86 (C-5), 101.98 (C-3), 114.22 (C-7), 116.01 (C-2b), 118.54 (C-12a), 121.96 (C-12), 125.53 (C-10), 127.00 (C-9), 127.32 (C-8), 128.01 (C-11), 130.09 (C-4), 130.43 (C-11a), 134.17 (C-7a), 137.84 (C-12b), 141.56 (C-2a), 149.62 (C-5a), 152.43 (C-6a). Anal. Calcd for $C_{20}H_{16}N_2O_4S$: C, 63.15; H, 4.24; N, 7.36. Found: C, 63.33; H, 4.00; N, 7.04.

4.1.49. 2-[5-Nitro-2*H*-benzo[*g*]benzopyrano[4,3,2-*cd*]indazol-2-yl]-1-ethanol methanesulfonate (36). A solution of fuming nitric acid (126 μL, 2 mmol) in glacial acetic acid (1 mL) was added dropwise to a solution of **35** (783 mg, 1.9 mmol) in glacial acetic acid (5 mL) at 5 °C and the mixture was stirred at this temperature for 5 min, followed by 30 min of stirring at room temperature. The reaction mixture was then poured into crushed ice and the precipitate was filtered, washed with water and 5% Na_2CO_3, and air-dried. The residue was purified by column chromatography (silica gel), using a mixture of cyclohexane/EtOAc 3:1 as the eluent, to give compounds **36** (492 mg, 61%) and **37** (129 mg, 16%).

Data for 2-[5-nitro-2*H*-benzo[*g*]benzopyrano[4,3,2-*cd*]indazol-2-yl]-1-ethanol Methanesulfonate (**36**). Mp 213–215 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.76 (s, 3H, CH_3), 4.62 (t, $J = 7$ Hz, 2H, N–NCH_2CH$_2$), 4.72 (t, $J = 7$ Hz, 2H, N–NCH$_2$CH_2), 6.92 (d, $J = 9$ Hz, 1H, H-3), 7.45–7.54 (m, 2H, H-9, H-10), 7.84 (d, $J = 8$ Hz, 1H, H-8), 7.89 (d, $J = 8$ Hz, 1H, H-11), 8.03 (s, 1H, H-7), 8.17 (d, $J = 9$ Hz, 1H, H-4), 8.46 (s, 1H, H-12); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 38.16 (CH_3), 48.64 (N–N*C*H$_2$CH$_2$), 67.12 (N–NCH$_2$*C*H$_2$), 104.64 (C-3), 114.94 (C-2b), 115.92 (C-12a), 116.00 (C-7), 122.12 (C-12), 125.43 (C-5), 126.28 (C-10), 126.96 (C-4), 127.65 (C-8), 127.69 (C-9), 128.01 (C-11), 130.66 (C-11a), 133.98 (C-7a), 139.74 (C-12b), 142.73 (C-2a), 145.62 (C-5a), 150.83 (C-6a). Anal. Calcd for $C_{20}H_{15}N_3O_6S$: C, 56.47; H, 3.55; N, 9.88. Found: C, 56.76; H, 3.69; N, 9.98.

Data for 2-[3-nitro-2*H*-benzo[*g*]benzopyrano[4,3,2-*cd*]indazol-2-yl]-1-ethanol Methanesulfonate (**37**). Mp 227–229 °C (EtOH); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.11 (s, 2H, CH_3), 4.65 (t, $J = 7$ Hz, 2H, N–NCH$_2$CH_2), 5.09 (t, $J = 7$ Hz, 2H, N–NCH_2CH$_2$), 6.83 (d, $J = 8$ Hz, 1H, H-5), 7.43–7.53 (m, 2H, H-9, H-10), 7.84 (d, $J = 8$ Hz, 1H, H-8), 7.86 (s, 1H, H-7), 7.99 (d, $J = 8$ Hz, 1H, H-11), 8.27 (d, $J = 8$ Hz, 1H, H-4), 8.41 (s, 1H, H-12); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 37.08 (CH_3), 52.24 (N–N*C*H$_2$CH$_2$), 69.80 (NHCH$_2$*C*H$_2$), 103.84 (C-5), 114.97 (C-7), 115.47 (C-12a), 117.65 (C-2b), 122.89 (C-12), 126.27 (C-10), 127.71 (C-8), 128.14 (C-9), 128.55 (C-3), 128.83 (C-11), 130.64 (C-11a), 131.75 (C-4), 132.97 (C-2a), 134.17 (C-7a), 138.51 (C-12b), 150.48 (C-6a), 154.30 (C-5a). Anal. Calcd for $C_{20}H_{15}N_3O_6S$: C, 56.47; H, 3.55; N, 9.88. Found: C, 56.29; H, 3.74; N, 9.66.

4.1.50. Dimethyl-[2-(5-nitro-2*H*-benzo[*g*]benzopyrano [4,3,2-*cd*]indazol-2-yl)ethyl]amine (38a). This compound was prepared by an analogous procedure as described for the preparation of **24a**, starting from **36**. Yield: 94%; mp (hydrochloride) 200–202 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.32 (s, 3H, N(CH$_3$)$_2$), 4.43 (t, $J = 7$ Hz, 2H, N–NCH$_2$CH_2), 2.91 (t, $J = 7$ Hz, 2H, N–NCH$_2$CH_2), 6.91 (d, $J = 9$ Hz, 1H, H-3), 7.43–7.55 (m, 2H, H-9, H-10), 7.85 (d, $J = 8$ Hz, 1H, H-8), 7.89 (d, $J = 8$ Hz, 1H, H-11), 8.01 (s, 1H, H-7), 8.15 (d, $J = 9$ Hz, 1H, H-4), 8.45 (s, 1H, H-12). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 45.53 (CH$_2$CH$_2$N(*C*H$_3$)$_2$), 47.99 (N–N*C*H$_2$CH$_2$), 58.56 (N–NCH$_2$*C*H$_2$), 102.51 (C-3), 114.89 (C-2b), 115.87 (C-7), 116.11 (C-12a), 122.03 (C-12), 125.30 (C-5), 126.29 (C-10), 126.96 (C-4), 127.61 (C-8), 127.66 (C-9), 127.95 (C-11), 130.60 (C-11a), 133.95 (C-7a), 139.88 (C-12b), 142.11 (C-2a), 145.82 (C-5a), 150.54 (C-6a). Anal. Calcd for $C_{21}H_{18}N_4O_3 \cdot HCl \cdot H_2O$: C, 58.81; H, 4.94; N, 13.06. Found: C, 58.62; H, 4.77; N, 13.33.

4.1.51. Diethyl-[2-(5-nitro-2*H*-benzo[*g*]benzopyrano[4,3,2-*cd*]indazol-2-yl)ethyl]amine (38b). This compound was prepared by an analogous procedure as described for the preparation of **24a**, starting from **36**. Yield: 88%; mp (hydrochloride) >270 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.96 (t, $J = 7$ Hz, 6H, (CH$_2$CH_3)$_2$), 2.58 (q, $J = 7$ Hz, 4H, (CH_2CH$_3$)$_2$), 3.01 (t, $J = 7$ Hz, 2H,

N–NCH$_2$CH$_2$), 4.45 (t, J = 7 Hz, 2H, N–NCH$_2$CH$_2$), 6.89 (d, J = 9 Hz, 1H, H-3), 7.44–7.55 (m, 2H, H-9, H-10), 7.83 (d, J = 8 Hz, 1H, H-8), 7.88 (d, J = 8 Hz, 1H, H-11), 7.98 (s, 1H, H-7), 8.14 (d, J = 9 Hz, 1H, H-4), 8.43 (s, 1H, H-12); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 12.04 [(CH$_2$CH$_3$)$_2$], 46.86 [(CH$_2$CH$_3$)$_2$], 48.54 (N–NCH$_2$CH$_2$), 52.59 (N–NCH$_2$CH$_2$), 102.54 (C-3), 114.90 (C-2b), 115.91 (C-7), 116.06 (C-12a), 121.95 (C-12), 125.26 (C-5), 126.27 (C-10), 127.14 (C-4), 127.59 (C-8), 127.62 (C-9), 127.96 (C-11), 130.63 (C-11a), 133.91 (C-7a), 139.93 (C-12b), 142.03 (C-2a), 145.77 (C-5a), 150.63 (C-6a). Anal. Calcd for $C_{23}H_{22}N_4O_3$·HCl·H$_2$O: C, 60.46; H, 5.51; N, 12.26. Found: C, 60.00; H, 5.54; N, 12.05.

4.1.52. 5-Nitro-2-[2-(pyrrolidin-1-yl)ethyl]-2*H*-benzo[*g*]-benzopyrano[4,3,2-*cd*]indazole (38c). This compound was prepared by an analogous procedure as described for the preparation of **24a**, starting from **36**. Yield: 91%; mp (hydrochloride) 180–182 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.85 [m, 4H, N(CH$_2$CH$_2$)$_2$], 2.68 [m, 4H, N(CH$_2$CH$_2$)$_2$], 3.17 (t, J = 7 Hz, 2H, N–NCH$_2$CH$_2$), 4.56 (t, J = 7 Hz, 2H, N–NCH$_2$CH$_2$), 6.91 (d, J = 9 Hz, 1H, H-3), 7.41–7.57 (m, 2H, H-9, H-10), 7.84 (d, J = 8 Hz, 1H, H-8), 7.89 (d, J = 8 Hz, 1H, H-11), 8.01 (s, 1H, H-7), 8.16 (d, J = 9 Hz, 1H, H-4), 8.46 (s, 1H, H-12); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 23.48 [N(CH$_2$CH$_2$)$_2$], 48.16 (N–NCH$_2$CH$_2$), 54.23 [N(CH$_2$CH$_2$)$_2$], 54.83 (N–NCH$_2$CH$_2$), 102.58 (C-3), 114.97 (C-2b), 115.56 (C-7), 115.96 (C-12a), 122.12 (C-12), 125.39 (C-5), 126.27 (C-10), 126.81 (C-4), 127.59 (C-9), 127.60 (C-8), 127.93 (C-11), 130.61 (C-11a), 133.96 (C-7a), 140.06 (C-12b), 142.15 (C-2a), 145.90 (C-5a), 150.57 (C-6a). Anal. Calcd for $C_{23}H_{20}N_4O_3$·HCl·H$_2$O: C, 60.72; H, 5.10; N, 12.32. Found: C, 60.69; H, 5.42; N, 12.58.

4.1.53. 5-Nitro-2-[2-(piperidin-1-yl)ethyl]-2*H*-benzo[*g*]-benzopyrano[4,3,2-*cd*]indazole (38d). This compound was prepared by an analogous procedure as described for the preparation of **24a**, starting from **36**. Yield: 86%; mp (hydrochloride) >270 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.43 (m, 2H, 4-piperidine-H), 1.55 (m, 4H, 3,5-piperidine-H), 2.49 (m, 4H, 2,6-piperidine-H), 2.91 (t, J = 7 Hz, 2H, N–NCH$_2$CH$_2$), 4.45 (t, J = 7 Hz, 2H, N–NCH$_2$CH$_2$, 6.94 (d, J = 9 Hz, 1H, H-3), 7.48–7.59 (m, 2H, H-9, H-10), 7.88 (d, J = 8 Hz, 1H, H-8), 7.92 (d, J = 8 Hz, 1H, H-11), 8.05 (s, 1H, H-7), 8.19 (d, J = 9 Hz, 1H, H-4), 8.49 (s, 1H, H-12); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 24.53 (4-piperidine-C), 26.10 (3,5-piperidine-C), 48.39 (N–NCH$_2$CH$_2$), 54.89 (2,6-piperidine-C), 58.15 (N–NCH$_2$CH$_2$), 102.59 (C-3), 114.87 (C-2b), 116.09 (C-7), 116.24 (C-12a), 122.16 (C-12), 125.28 (C-5), 126.32 (C-10), 126.90 (C-4), 127.64 (C-8), 127.74 (C-9), 127.98 (C-11), 130.58 (C-11a), 134.02 (C-7a), 139.64 (C-12b), 141.93 (C-2a), 145.68 (C-5a), 150.46 (C-6a). Anal. Calcd for $C_{24}H_{22}N_4O_3$·HCl·H$_2$O: C, 61.47; H, 5.37; N, 11.95. Found: C, 61.54; H, 5.25; N, 12.01.

4.1.54. 2-Chloro-*N*-[2-(2-dimethylaminoethyl)-2*H*-benzo-[*g*]benzopyrano[4,3,2-*cd*]indazol-5-yl]acetamide (40a). This compound was prepared by an analogous procedure as described for the preparation of **18a**, starting from **38a**. Yield: 83%; mp 193–195 °C (dec), (EtOAc/*n*-hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.31 (s, 6H, CH$_2$CH$_2$N(CH$_3$)$_2$), 2.82 (t, J = 7 Hz, 2H, N–NCH$_2$CH$_2$), 4.30 (s, 2H, COCH$_2$), 4.31 (t, J = 7 Hz, 2H, N–NCH$_2$CH$_2$), 6.83 (d, J = 9 Hz, 1H, H-3), 7.37–7.49 (m, 2H, H-9, H-10), 7.57 (s, 1H, H-7), 7.69 (d, J = 8 Hz, 1H, H-8), 7.76 (d, J = 8 Hz, 1H, H-11), 7.84 (d, J = 9 Hz, 1H, H-4), 8.27 (s, 1H, H-12), 8.35 (s, 1H, D$_2$O exch, NH) ^{13}C NMR ($CDCl_3$, 50 MHz) δ 43.05 (COCH$_2$), 45.56 (CH$_2$CH$_2$N(CH$_3$)$_2$), 48.06 (N–NCH$_2$CH$_2$), 58.46 (N–NCH$_2$CH$_2$), 102.01 (C-3), 112.94 (C-5), 114.03 (C-7), 116.04 (C-2b), 118.49 (C-12a), 122.03 (C-12), 125.48 (C-10), 125.57 (C-4), 127.12 (C-8), 127.11 (C-9), 128.13 (C-11), 130.66 (C-11a), 133.99 (C-7a), 137.48 (C-2a), 138.17 (C-5a), 138.99 (C-12b), 152.04 (C-6a), 163.66 (CONH). Anal. Calcd for $C_{23}H_{21}ClN_4O_2$. Calcd: C: 65.63, H: 5.03, N: 13.31. Found: C: 65.39, H: 4.88, N: 12.97.

4.1.55. 2-Chloro-*N*-[2-(2-diethylaminoethyl)-2*H*-benzo-[*g*]benzopyrano[4,3,2-*cd*]indazol-5-yl]acetamide (40b). This compound was prepared by an analogous procedure as described for the preparation of **18a**, starting from **38b**. Yield: 79%; mp 174–176 °C (EtOAc/*n*-hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.96 (t, J = 7 Hz, 6H, (CH$_2$CH$_3$)$_2$), 2.59 (q, J = 7 Hz, 4H, (CH$_2$CH$_3$)$_2$), 2.97 (t, J = 7 Hz, 2H, N–NCH$_2$CH$_2$), 4.22 (s, 2H, COCH$_2$), 4.39 (t, J = 7 Hz, 2H, N–NCH$_2$CH$_2$), 6.79 (d, J = 9 Hz, 1H, H-3), 7.36–7.47 (m, 2H, H-9, H-10), 7.56 (s, 1H, H-7), 7.65 (d, J = 8 Hz, 1H, H-8), 7.74 (d, J = 8 Hz, 1H, H-11), 7.80 (d, J = 9 Hz, 1H, H-4), 8.24 (s, 1H, H-12), 8.33 (s, 1H, D$_2$O exch, NH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 11.73 (CH$_2$CH$_2$N(CH$_2$CH$_3$)$_2$), 43.17 (COCH$_2$), 47.36 (CH$_2$CH$_2$N(CH$_2$CH$_3$)$_2$), 48.73 (N–NCH$_2$CH$_2$), 52.28 (N–NCH$_2$CH$_2$), 101.87 (C–3), 113.11 (C-5), 114.05 (C-7), 115.97 (C-2b), 118.61 (C-12a), 121.14 (C-12), 124.88 (C-10), 125.76 (C-4), 127.04 (C-8, C-9), 128.09 (C-11), 130.62 (C-11a), 134.05 (C-7a), 137.48 (C-2a), 138.11 (C-5a), 139.26 (C-12b), 151.93 (C-6a), 163.82 (CONH). Anal. Calcd for $C_{25}H_{25}ClN_4O_2$. Calcd: C: 66.88, H: 5.61, N: 12.48. Found: C: 66.72, H: 5.85, N: 12.10.

4.1.56. 2-Chloro-*N*-[2-(2-pyrrolidin-1-yl-ethyl)-2*H*-benzo-[*g*]benzopyrano[4,3,2-*cd*]indazol-5-yl]acetamide (40c). This compound was prepared by an analogous procedure as described for the preparation of **18a**, starting from **38c**. Yield: 79%; mp 185–187 °C (dec), (EtOAc/*n*-hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.77 [m, 4H, N(CH$_2$CH$_2$)$_2$], 2.58 [m, 4H, N(CH$_2$CH$_2$)$_2$], 3.03 (t, J = 7 Hz, 2H, N–NCH$_2$CH$_2$), 4.28 (s, 2H, COCH$_2$), 4.47 (t, J = 7 Hz, 2H, N–NCH$_2$CH$_2$), 6.85 (d, J = 9 Hz, 1H, H-3), 7.35–7.47 (m, 2H, H-9, H-10), 7.62 (s, 1H, H-7), 7.71 (d, J = 8 Hz, 1H, H-8), 7.80 (d, J = 8 Hz, 1H, H-11), 7.86 (d, J = 9 Hz, 1H, H-4), 8.31 (s, 1H, H-12), 8.36 (s, 1H, D$_2$O exch, NH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 22.67 (CH$_2$CH$_2$N(CH$_2$CH$_2$)$_2$), 42.86 (COCH$_2$), 49.25 (N–NCH$_2$CH$_2$), 54.25 (CH$_2$CH$_2$N(CH$_2$CH$_2$)$_2$), 55.36 (N–NCH$_2$CH$_2$), 102.03 (C-3), 113.11 (C-5), 113.99 (C-7), 115.98 (C-2b), 118.46 (C-12a), 122.07 (C-12), 125.43 (C-10), 125.78 (C-4), 127.05 (C-8, C-9), 128.05 (C-11), 130.61 (C-11a), 133.90 (C-7a), 137.43 (C-2a), 138.11

(C-5a), 138.68 (C-12b), 152.02 (C-6a), 164.36 (CONH). Anal. Calcd for $C_{25}H_{23}ClN_4O_2$. Calcd: C: 67.19, H: 5.19, N: 12.54. Found: C: 67.36, H: 5.07, N: 12.63.

4.1.57. 2-Chloro-*N*-[2-(2-piperidin-1-yl-ethyl)-2*H*-benzo-[*g*]benzopyrano[4,3,2-*cd*]indazol-5-yl]acetamide (40d). This compound was prepared by an analogous procedure as described for the preparation of **18a**, starting from **38d**. Yield: 78%; mp 172–174 °C (dec), (EtOAc/*n*-hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.43 (m, 2H, 4-piperidine-H), 1.58 (m, 4H, 3,5-piperidine-H), 2.50 (m, 4H, 2,6-piperidine-H), 2.86 (t, J = 7 Hz, 2H, N–NCH$_2$CH_2), 4.29 (s, 2H, COCH$_2$), 4.42 (t, J = 7 Hz, 2H, N–NCH_2CH$_2$, 6.81 (d, J = 9 Hz, 1H, H-3), 7.38–7.50 (m, 2H, H-9, H-10), 7.56 (s, 1H, H-7), 7.68 (d, J = 8 Hz, 1H, H-8), 7.78 (d, J = 8 Hz, 1H, H-11), 7.83 (d, J = 9 Hz, 1H, H-4), 8.26 (s, 1H, H-12), 8.37 (s, 1H, D_2O exch, NH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 24.22 [CH$_2$CH$_2$(4-piperidine-C)], 25.57 [CH$_2$CH$_2$(3,5-piperidine-C)], 43.17 (CO*C*H$_2$), 47.33 (N–N*C*H$_2$CH$_2$), 54.63 [CH$_2$CH$_2$(3,5-piperidine-C)], 58.07 (N–NCH$_2$*C*H$_2$), 102.03 (C-3), 113.05 (C-5), 114.07 (C-7), 116.09 (C-2b), 118.52 (C-12a), 122.11 (C-12), 125.29 (C-10), 125.64 (C-4), 127.18 (C-9), 127.26 (C-8), 128.13 (C-11), 130.67 (C-11a), 134.00 (C-7a), 137.46 (C-2a), 138.19 (C-5a), 139.29 (C-12b), 152.12 (C-6a), 163.87 (CONH). Anal. Calcd for $C_{26}H_{25}ClN_4O_2$. Calcd: C: 67.75, H: 5.47, N: 12.15. Found: C: 67.51, H: 5.31, N: 12.04.

4.1.58. 2-Dimethylamino-*N*-[2-(2-dimethylaminoethyl)-2*H*-benzo[*g*]benzopyrano[4,3,2-*cd*]indazol-5-yl]acetamide (41a). This compound was prepared by an analogous procedure as described for the preparation of **20a**, starting from **40a**. Yield: 97%; mp 166–168 °C (EtOAc/*n*-hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.33 (s, 6H, CH$_2$CH$_2$N(CH_3)$_2$), 2.50 (s, 6H, COCH$_2$N(CH_3)$_2$), 2.88 (t, J = 7 Hz, 2H, N–NCH$_2$CH_2), 3.18 (s, 2H, COCH$_2$), 4.47 (t, J = 7 Hz, 2H, N–NCH_2CH$_2$), 6.87 (d, J = 9 Hz, 1H, H-3), 7.35–7.51 (m, 2H, H-9, H-10), 7.65 (s, 1H, H-7), 7.74 (d, J = 8 Hz, 1H, H-8), 7.82 (d, J = 8 Hz, 1H, H-11), 8.01 (d, J = 9 Hz, 1H, H-4), 8.35 (s, 1H, H-12), 9.20 (s, 1H, D_2O exch, NH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 45.61 (CH$_2$CH$_2$N (*C*H$_3$)$_2$), 46.12 (COCH$_2$N(*C*H$_3$)$_2$), 48.03 (N–N*C*H$_2$ CH$_2$), 58.58 (N–NCH$_2$*C*H$_2$), 63.58 (CO*C*H$_2$), 101.81 (C-3), 112.69 (C-5), 114.09 (C-7), 116.00 (C-2b), 118.43 (C-12a), 121.99 (C-12), 125.56 (C-10), 125.81 (C-4), 127.06 (C-8, C-9), 128.06 (C-11), 130.59 (C-11a), 133.97 (C-7a), 137.54 (C-2a), 138.20 (C-5a), 139.19 (C-12b), 151.95 (C-6a), 169.08 (CONH). Anal. Calcd for $C_{25}H_{27}N_5O_2$. Calcd: C: 69.91, H: 6.34, N: 16.30. Found: C: 69.79, H: 6.56, N: 16.47.

4.1.59. 2-Diethylamino-*N*-[2-(2-diethylaminoethyl)-2*H*-benzo[*g*]benzopyrano[4,3,2-*cd*]indazol-5-yl]acetamide (41b). This compound was prepared by an analogous procedure as described for the preparation of **20a**, starting from **40b**. Yield: 95%; mp 129–131 °C (EtOAc/*n*-hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.01 (t, J = 7 Hz, 6H, CH$_2$CH$_2$N(CH$_2$CH_3)$_2$), 1.24 (t, J = 7 Hz, 6H, COCH$_2$N(CH$_2$CH_3)$_2$), 2.59 (q, J = 7 Hz, 4H, CH$_2$CH$_2$N(CH_2CH$_3$)$_2$), 2.76 (q, J = 7 Hz, 4H, COCH$_2$N(CH_2CH$_3$)$_2$), 2.98 (t, J = 7 Hz, 2H, N–NCH$_2$CH_2), 3.26 (s, 2H, COCH$_2$), 4.42 (t, J = 7 Hz, 2H, N–NCH_2CH$_2$), 6.89 (d, J = 9 Hz, 1H, H-3), 7.37–7.49 (m, 2H, H-9, H-10), 7.59 (s, 1H, H-7), 7.75 (d, J = 8 Hz, 1H, H-8), 7.83 (d, J = 8 Hz, 1H, H-11), 8.14 (d, J = 9 Hz, 1H, H-4),8.37(s,1H, H-12), 9.65(s, 1H, D_2O exch, NH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 12.02 (CH$_2$CH$_2$-N(CH$_2$*C*H$_3$)$_2$), 12.75 (COCH$_2$N (CH$_2$*C*H$_3$) $_2$),47.59(CH$_2$CH$_2$N(*C*H$_2$CH$_3$)$_2$), 49.75 (COCH$_2$N(*C*H$_2$-CH$_3$)$_2$), 48.88 (N–N*C*H$_2$CH$_2$), 52.63 (N–NCH$_2$-*C*H$_2$), 58.29 (CO*C*H$_2$), 101.99 (C-3), 113.06 (C-5), 113.87 (C-7), 115.89 (C-2b), 118.57 (C-12a), 121.92 (C-12), 124.93 (C-10), 125.56 (C-4), 127.03 (C-8, C-9), 128.02 (C-11), 130.59 (C-11a), 133.94 (C-7a), 137.25 (C-2a), 138.09 (C-5a), 139.50 (C-12b), 152.02 (C-6a), 170.22 (CONH). Anal. Calcd for $C_{29}H_{35}N_5O_2$: C, 71.73; H, 7.26; N, 14.42. Found: C, 71.64; H, 7.48; N, 14.49.

4.1.60. *N*-[2-(2-Pyrrolidin-1-yl-ethyl)-2*H*-benzo[*g*]benzopyrano[4,3,2-*cd*]indazol-5-yl]-2-pyrrolidin-1-yl-acetamide (41c). This compound was prepared by an analogous procedure as described for the preparation of **20a**, starting from **40c**. Yield: 94%; mp 143–145 °C (EtOAc/*n*-hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.79 (m, 4H, CH$_2$CH$_2$N(CH$_2$CH_2)$_2$), 1.98 (m, 4H, COCH$_2$N(CH$_2$CH_2)$_2$), 2.60 (m, 4H, CH$_2$CH$_2$-N(CH_2CH$_2$)$_2$), 2.83 (m, 4H, COCH$_2$N(CH_2CH$_2$)$_2$), 3.05 (t,J = 7 Hz, 2H, N–NCH$_2$CH_2), 3.40 (s, 2H, COCH$_2$), 4.51 (t, J = 7 Hz, 2H, N–NCH_2CH$_2$), 6.89 (d, J = 9 Hz, 1H, H-3), 7.38–7.51 (m, 2H, H-9, H-10), 7.58 (s, 1H, H-7), 7.75 (d, J = 8 Hz, 1H, H-8), 7.84 (d, J = 8 Hz, 1H, H-11), 8.06 (d, J = 9 Hz, 1H, H-4), 8.36 (s, 1H, H-12), 9.30 (s, 1H, D_2O exch, NH). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 23.52 (CH$_2$CH$_2$N(CH$_2$*C*H$_2$)$_2$), 24.18 (COCH$_2$N(CH$_2$*C*H$_2$)$_2$), 49.32 (N–N*C*H$_2$CH$_2$), 54.36 (CH$_2$CH$_2$N(*C*H$_2$CH$_2$)$_2$), 54.54 (COCH$_2$N-(*C*H$_2$CH$_2$)$_2$), 55.53 (N–NCH$_2$*C*H$_2$), 59.47 (CO*C*H$_2$), 101.85 (C-3), 112.80 (C-5), 113.94 (C-7), 116.08 (C-2b), 118.51 (C-12a), 121.96 (C-12), 125.56 (C-4, C-10), 127.03 (C-8, C-9), 128.02 (C-11), 130.55 (C-11a), 133.94 (C-7a), 137.36 (C-2a), 138.13 (C-5a), 138.97 (C-12b), 151.95 (C-6a), 169.33 (CONH). Anal. Calcd for $C_{29}H_{31}N_5O_2$. C: 72.33, H: 6.49, N: 14.54. Found: C: 72.59, H: 6.23, N: 14.72.

4.1.61. *N*-[2-(2-Piperidin-1-yl-ethyl)-2*H*-benzo[*g*]benzopyrano[4,3,2-*cd*]indazol-5-yl]-2-piperidin-1-yl-acetamide (41d). This compound was prepared by an analogous procedure as described for the preparation of **20a**, starting from **40d**. Yield: 95%; mp 192–194 °C (EtOAc/*n*-hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.45 [m, 2H, CH$_2$CH$_2$(4-piperidine-H)], 1.61 [m, 6H, CH$_2$CH$_2$(3,5-piperidine-H), COCH$_2$(4-piperidine-H)], 1.80 [m, 4H, COCH$_2$(3,5-piperidine-H)], 2.56 [m, 4H, CH$_2$CH$_2$(2,6-piperidine-H)], 2.66 [m, 4H, COCH$_2$(2,6-piperidine-H), 2.95 (t,J = 7 Hz, 2H, N–NCH$_2$CH_2), 3.17 (s, 2H, COCH$_2$), 4.50 (t, J = 7 Hz, 2H, N–NCH_2CH$_2$), 6.89 (d, J = 9 Hz, 1H, H-3), 7.36–7.48 (m, 2H, H-9, H-10), 7.54 (s, 1H, H-7), 7.74 (d, J = 8 Hz, 1H, H-8), 7.80 (d, J = 8 Hz, 1H, H-11), 8.12 (d, J = 9 Hz, 1H, H-4), 8.32 (s, 1H, H-12), 9.53 (s, 1H, D_2O exch, NH); ^{13}C NMR

($CDCl_3$, 50 MHz) δ 24.14 [CH_2CH_2(4-piperidine-C)], 25.65 [CH_2CH_2(3,5-piperidine-C), $COCH_2$(4-piperidine-C)], 26.86 [$COCH_2$(3,5-piperidine-C)], 47.44 (N–NCH_2CH_2), 54.72 [CH_2CH_2(3,5-piperidine-C)], 55.23 [$COCH_2$(3,5-piperidine-C)], 58.10 (N–NCH$_2$*C*H$_2$), 62.95 (CO*C*H$_2$), 102.28 (C-3), 113.45 (C-5), 114.18 (C-7), 116.17 (C-2b), 118.67 (C-12a), 122.27 (C-12), 125.32 (C-10), 125.87 (C-4), 127.34 (C-9), 127.42 (C-8), 128.30 (C-11), 130.83 (C-11a), 134.21 (C-7a), 137.71 (C-2a), 138.26 (C-5a), 138.77 (C-12b), 152.26 (C-6a), 169.31 (CONH). Anal. Calcd for $C_{31}H_{35}N_5O_2$. C: 73.06, H: 6.92, N: 13.74. Found: C: 72.88, H: 6.99, N: 13.96.

4.1.62. *N*,*N*-Dimethyl-*N*′-(12-oxo-12*H*-benzo[*b*]xanthen-1-yl)ethane-1,2-diamine (42a). This compound was prepared by an analogous procedure as described for the preparation of **14a**, starting from **33**. Yield: 88%; mp (hydrochloride) 259 °C (dec), (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.38 (s, 6H, 2× CH_3), 2.71 (t, J = 7 Hz, 2H, NHCH$_2$C*H*$_2$), 3.40 (q, J = 7 Hz, 5 Hz, 2H, NHC*H*$_2$CH$_2$), 6.43 (d, J = 8 Hz, 1H, H-4), 6.58 (d, J = 8 Hz, 1H, H-2), 7.42–7.51 (m, 2H, H-3, H-8), 7.56 (t, J = 8 Hz, 1H, H-9), 7.76 (s, 1H, H-6), 7.87 (d, J = 8 Hz, 1H, H-7), 8.02 (d, J = 8 Hz, 1H, H-10), 8.84 (s, 1H, H-11), 9.72 (t, J = 5 Hz, 1H, D_2O exch, NH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 40.87 (NH*C*H$_2$CH$_2$), 45.46 (2× CH_3), 57.82 (NHCH$_2$*C*H$_2$), 102.29 (C-2), 103.61 (C-4), 106.11 (C-12a), 112.55 (C-6), 121.68 (C-11a), 126.03 (C-9), 127.12 (C-7), 128.33 (C-11), 128.99 (C-8), 129.05 (C-10a), 129.64 (C-10), 136.59 (C-3), 136.85 (C-6a), 151.52 (C-5a), 151.66 (C-1), 158.31 (C-4a), 180.18 (C-12). Anal. Calcd for $C_{21}H_{20}N_2O_2$·HCl·2H_2O: C, 62.29; H, 6.22; N, 6.92. Found: C, 52.23; H, 6.63; N, 8.92.

4.1.63. *N*,*N*-Diethyl-*N*′-(12-oxo-12*H*-benzo[*b*]xanthen-1-yl)ethane-1,2-diamine (42b). This compound was prepared by an analogous procedure as described for the preparation of **14a**, starting from **33**. Yield: 90%; mp (hydrochloride) 251–253 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.13 (t, J = 7 Hz, 6H, (CH$_2$C*H*$_3$)$_2$), 2.67 (q, J = 7 Hz, 4H, (C*H*$_2$CH$_3$)$_2$), 2.83 (t, J = 7 Hz, 2H, NHCH$_2$C*H*$_2$), 3.35 (q, J = 7 Hz, 5 Hz, 2H, NHC*H*$_2$CH$_2$), 6.39 (d, J = 8 Hz, 1H, H-4), 6.52 (d, J = 8 Hz, 1H, H-2), 7.39–7.45 (m, 2H, H-3, H-8), 7.53 (t, J = 8 Hz, 1H, H-9), 7.68 (s, 1H, H-6), 7.81 (d, J = 8 Hz, 1H, H-7), 7.98 (d, J = 8 Hz, 1H, H-10), 8.77 (s, 1H, H-11), 9.64 (t, J = 5 Hz, 1H, D_2O exch, NH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 12.05 [(CH$_2$*C*H$_3$)$_2$], 41.14 (NH*C*H$_2$CH$_2$), 47.12 [(*C*H$_2$CH$_3$)$_2$], 51.14 (NHCH$_2$*C*H$_2$), 102.34 (C-2), 103.55 (C-4), 106.10 (C-12a), 112.68 (C-6), 122.01 (C-11a), 125.80 (C-9), 127.10 (C-7), 127.90 (C-11), 129.04 (C-8), 129.32 (C-10a), 129.60 (C-10), 136.67 (C-3), 136.91 (C-6a), 151.60 (C-1), 151.88 (C-5a), 157.73 (C-4a), 180.34 (C-12). Anal. Calcd for $C_{23}H_{24}N_2O_2$·HCl·1/2H_2O: C, 68.05; H, 6.46; N, 6.90. Found: C, 68.27; H, 6.32; N, 7.07.

4.1.64. 1-[2-(Pyrrolidin-1-yl)ethylamino]-12*H*-benzo[*b*]-xanthene-12-one (42c). This compound was prepared by an analogous procedure as described for the preparation of **14a**, starting from **33**. Yield: 88%; mp (hydrochloride) 242–244 °C (EtOH). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.89 (m, 4H, N(CH$_2$C*H*$_2$)$_2$), 2.79 (m, 4H, N(C*H*$_2$CH$_2$)$_2$), 2.96 (t, J = 7 Hz, 2H, NHCH$_2$C*H*$_2$), 3.49 (q, J = 7 Hz, 5 Hz, 2H, NHC*H*$_2$CH$_2$), 6.41 (d, J = 8 Hz, 1H, H-4), 6.54 (d, J = 8 Hz, 1H, H-2), 7.39–7.47 (m, 2H, H-3, H-8), 7.54 (t, J = 8 Hz, 1H, H-9), 7.70 (s, 1H, H-6), 7.81 (d, J = 8 Hz, 1H, H-7), 7.97 (d, J = 8 Hz, 1H, H-10), 8.74 (s, 1H, H-11), 9.67 (t, J = 5 Hz, 1H, D_2O exch, NH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 29.86 (N(CH$_2$*C*H$_2$)$_2$), 41.71 (NH*C*H$_2$CH$_2$), 54.36 (N(*C*H$_2$CH$_2$)$_2$), 54.54 (NHCH$_2$*C*H$_2$), 102.40 (C-2), 103.58 (C-4), 106.22 (C-12a), 112.51 (C-6), 121.74 (C-11a), 125.08 (C-9), 126.88 (C-7), 127.43 (C-11), 128.61 (C-8), 128.81 (C-10a), 129.56 (C-10), 136.55 (C-3), 136.44 (C-6a), 151.65 (C-1), 151.80 (C-5a), 158.31 (C-4a), 180.18 (C-12). Anal. Calcd for $C_{23}H_{22}N_2O_2$·HCl·1/2H_2O: C, 68.39; H, 5.99; N, 6.94. Found: C, 52.37; H, 4.97; N, 5.46.

4.1.65. 1-[2-(Piperidin-1-yl)ethylamino]-12*H*-benzo[*b*]-xanthene-12-one (42d). This compound was prepared by an analogous procedure as described for the preparation of **14a**, starting from **33**. Yield: 91%; mp (hydrochloride) 248–250 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.48 (m, 2H, 4-piperidine-H), 1.66 (m, 4H, 3,5-piperidine-H), 2.55 (m, 4H, 2,6-piperidine-H), 2.75 (t, J = 6 Hz, 2H, NHCH$_2$C*H*$_2$), 3.43 (q, J = 7 Hz, 5 Hz, 2H, NHC*H*$_2$CH$_2$), 6.40 (d, J = 8 Hz, 1H, H-4), 6.54 (d, J = 8 Hz, 1H, H-2), 7.37–7.48 (m, 2H, H-3, H-8), 7.55 (t, J = 8 Hz, 1H, H-9), 7.72 (s, 1H, H-6), 7.81 (d, J = 8 Hz, 1H, H-7), 7.99 (d, J = 8 Hz, 1H, H-10), 8.78 (s, 1H, H-11), 9.65 (t, J = 5 Hz, 1H, D_2O exch, NH). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 24.22 (4-piperidine-C), 25.77 (3,5-piperidine-C), 40.39 (NH*C*H$_2$CH$_2$), 54.65 (2,6-piperidine-C), 57.37 (NHCH$_2$*C*H$_2$), 102.14 (C-2), 103.68 (C-4), 106.31 (C-12a), 112.50 (C-6), 122.03 (C-11a), 125.07 (C-9), 126.87 (C-7), 127.50 (C-11), 128.56 (C-8), 128.94 (C-10a), 129.60 (C-10), 136.47 (C-3), 136.66 (C-6a), 151.84 (C-1), 151.90 (C-5a), 158.37 (C-4a), 181.02 (C-12). Anal. Calcd for $C_{24}H_{24}N_2O_2$·HCl·H_2O: C, 67.52; H, 6.37; N, 6.56. Found: C, 67.33; H, 6.71; N, 6.51.

4.1.66. Dimethyl-[2-[2*H*-benzo[*g*]benzopyrano[4,3,2-*cd*]-indazol-2-yl]ethyl]amine (43a). This compound was prepared by an analogous procedure as described for the preparation of **24a**, starting from **35**. Yield: 95%; mp (hydrochloride) 269–271 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.23 [s, 6H, N(CH$_3$)$_2$], 2.90 (t, J = 7 Hz, 2H, N–NCH$_2$C*H*$_2$), 4.48 (t, J = 7 Hz, 2H, N–NC*H*$_2$CH$_2$), 6.57 (d, J = 8 Hz, 1H, H-5), 6.88 (d, J = 8 Hz, 1H, H-3), 7.30 (t, J = 8 Hz, 1H, H-4), 7.39 (t, J = 8 Hz, 1H, H-9), 7.44 (t, J = 8 Hz, 1H, H-10), 7.63 (s, 1H, H-7), 7.73 (d, J = 8 Hz, 1H, H-8), 7.82 (d, J = 8 Hz, 1H, H-11), 8.37 (s, 1H, H-12); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 45.78 (2× CH_3), 48.13 (N–N*C*H$_2$CH$_2$), 58.75 (N–NCH$_2$*C*H$_2$), 101.02 (C-5), 102.05 (C-3), 114.14 (C-7), 116.09 (C-2b), 118.62 (C-12a), 122.00 (C-12), 125.46 (C-10), 127.04 (C-9), 127.30 (C-8), 128.11 (C-11), 130.31 (C-4), 130.49 (C-11a), 134.24 (C-7a), 137.92 (C-12b), 141.01 (C-2a), 149.72 (C-5a), 152.36 (C-6a). Anal. Calcd for $C_{21}H_{19}N_3O$·HCl·3/4H_2O: C, 66.48; H, 5.71; N, 11.08. Found: C, 66.09; H, 5.48; N, 11.32.

4.1.67. Diethyl-[2-[2*H*-benzo[*g*]benzopyrano[4,3,2-*cd*]indazol-2-yl]ethyl]amine (43b). This compound was prepared by an analogous procedure as described for the preparation of **24a**, starting from **35**. Yield: 96%; mp (hydrochloride) 258–260 °C (EtOH). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.02 (t, J = 7 Hz, 6H, $(CH_2CH_3)_2$), 2.60 (q, J = 7 Hz, 4H, $(CH_2CH_3)_2$), 3.00 (t, J = 7 Hz, 2H, N–NCH_2CH_2), 4.44 (t, J = 7 Hz, 2H, N–NCH_2CH_2), 6.57 (d, J = 8 Hz, 1H, H-5), 6.88 (d, J = 8 Hz, 1H, H-3), 7.29 (t, J = 8 Hz, 1H, H-4), 7.39 (t, J = 8 Hz, 1H, H-9), 7.44 (t, J = 8 Hz, 1H, H-10), 7.63 (s, 1H, H-7), 7.73 (d, J = 8 Hz, 1H, H-8), 7.82 (d, J = 8 Hz, 1H, H-11), 8.37 (s, 1H, H-12); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 11.94 [$(CH_2CH_3)_2$], 47.56 [$(CH_2CH_3)_2$], 48.44 (N–NCH_2CH_2), 52.59 (N–NCH_2CH_2), 100.78 (C-5), 102.14 (C-3), 114.01 (C-7), 115.89 (C-2b), 118.57 (C-12a), 121.81 (C-12), 125.37 (C-10), 126.92 (C-9), 127.17 (C-8), 127.98 (C-11), 130.04 (C-4), 130.37 (C-11a), 134.12 (C-7a), 137.69 (C-12b), 140.96 (C-2a), 149.56 (C-5a), 152.28 (C-6a). Anal. Calcd for $C_{23}H_{23}N_3O{\cdot}HCl{\cdot}2H_2O$: C, 64.25; H, 6.56; N, 9.77. Found: C, 64.75; H, 5.90; N, 9.70.

4.1.68. 2-[2-(Pyrrolidin-1-yl)ethyl]-2*H*-benzo[*g*]benzopyrano[4,3,2-*cd*]indazole (43c). This compound was prepared by an analogous procedure as described for the preparation of **24a**, starting from **35**. Yield: 95%; mp (hydrochloride) >270 °C (EtOH). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.82 [m, 4H, $N(CH_2CH_2)_2$], 2.66 [m, 4H, $N(CH_2CH_2)_2$], 3.12 (t, J = 7 Hz, 2H, N–NCH_2CH_2), 4.56 (t, J = 7 Hz, 2H, N–NCH_2CH_2), 6.60 (d, J = 8 Hz, 1H, H-5), 6.92 (d, J = 8 Hz, 1H, H-3), 7.31 (t, J = 8 Hz, 1H, H-4), 7.41 (t, J = 8 Hz, 1H, H-9), 7.46 (t, J = 8 Hz, 1H, H-10), 7.65 (s, 1H, H-7), 7.75 (d, J = 8 Hz, 1H, H-8), 7.85 (d, J = 8 Hz, 1H, H-11), 8.39 (s, 1H, H-12); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 23.48 [$N(CH_2CH_2)_2$], 48.95 (N–NCH_2CH_2), 54.36 [$N(CH_2CH_2)_2$], 55.46 (N–NCH_2CH_2), 100.93 (C-5), 102.03 (C-3), 114.05 (C-7), 115.93 (C-2b), 118.46 (C-12a), 121.88 (C-12), 125.37 (C-10), 126.95 (C-9), 127.17 (C-8), 127.98 (C-11), 130.23 (C-4), 130.37 (C-11a), 134.12 (C-7a), 137.83 (C-12b), 140.89 (C-2a), 149.56 (C-5a), 152.24 (C-6a). Anal. Calcd for $C_{23}H_{21}N_3O{\cdot}HCl{\cdot}2H_2O$: C, 64.55; H, 6.12; N, 9.82. Found: C, 64.81; H, 6.34; N, 9.53.

4.1.69. 2-[2-(Piperidin-1-yl)ethyl]-2*H*-benzo[*g*]benzopyrano[4,3,2-*cd*]indazole (43d). This compound was prepared by an analogous procedure as described for the preparation of **24a**, starting from **35**. Yield: 97%; mp (hydrochloride) >270 °C (EtOH). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.45 (m, 2H, 4-piperidine-H), 1.60 (m, 4H, 3,5-piperidine-H), 2.53 (m, 4H, 2,6-piperidine-H), 2.92 (t, J = 7 Hz, 2H, N–NCH_2CH_2), 4.51 (t, J = 7 Hz, 2H, N–NCH_2CH_2), 6.56 (d, J = 8 Hz, 1H, H-5), 6.89 (d, J = 8 Hz, 1H, H-3), 7.29 (t, J = 8 Hz, 1H, H-4), 7.39 (t, J = 8 Hz, 1H, H-9), 7.42 (t, J = 8 Hz, 1H, H-10), 7.62 (s, 1H, H-7), 7.72 (d, J = 8 Hz, 1H, H-8), 7.83 (d, J = 8 Hz, 1H, H-11), 8.35 (s, 1H, H-12); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 24.13 (4-piperidine-C), 25.89 (3,5-piperidine-C), 47.58 (N–NCH_2CH_2), 54.82 (2,6-piperidine-C), 58.31 (N–NCH_2CH_2), 100.87 (C-5), 102.19 (C-3), 114.03 (C-7), 115.94 (C-2b), 118.51 (C-12a), 121.82 (C-12), 125.83 (C-10), 126.93 (C-9), 127.19 (C-8), 128.00 (C-11), 130.09 (C-4), 130.38 (C-11a), 134.13 (C-7a), 137.73 (C-12b), 140.93 (C-2a), 149.53 (C-5a), 152.25 (C-6a). Anal. Calcd for $C_{24}H_{23}N_3O{\cdot}HCl{\cdot}4H_2O$: C, 60.31; H, 6.75; N, 8.79. Found: C, 60.97; H, 5.57; N, 8.78.

4.1.70. *N*,*N*-Dimethyl-*N*′-(9-oxo-9*H*-xanthen-1-yl)ethane-1,2-diamine (44a). This compound was prepared by an analogous procedure as described for the preparation of **14a**, starting from the corresponding tosylate.[49–51] Yield: 80%; mp (hydrochloride) >270 C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.33 (s, 6H, 2× CH_3), 2.66 (t, J = 7 Hz, 2H, $NHCH_2CH_2$), 3.33 (q, J = 7 Hz, 5 Hz, 2H, $NHCH_2CH_2$), 6.38 (d, J = 8 Hz, 1H, H-4), 6.54 (d, J = 8 Hz, 1H, H-2), 7.28 (t, J = 8 Hz, 1H, H-7), 7.33 (d, J = 8 Hz, 1H, H-5), 7.42 (t, J = 8 Hz, 1H, H-3), 7.61 (t, J = 8 Hz, 1H, H-6), 8.22 (d, J = 8 Hz, 1H, H-8), 9.57 (t, J = 5 Hz, 1H, D_2O exch, NH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 40.98 ($NHCH_2CH_2$), 45.54 (2× CH_3), 57.85 ($NHCH_2CH_2$), 102.07 (C-2), 103.47 (C-4), 106.81 (C-9a), 117.10 (C-5), 122.07 (C-8a), 123.31 (C-7), 126.03 (C-8), 133.98 (C-6), 135.92 (C-3), 151.62 (C-1), 155.26 (C-10a), 158.01 (C-4a), 179.74 (C-9). Anal. Calcd for $C_{17}H_{18}N_2O_2{\cdot}HCl{\cdot}H_2O$: C, 60.62; H, 6.28; N, 8.32. Found: C, 60.44; H, 6.52; N, 8.51.

4.1.71. *N*,*N*-Diethyl-*N*′-(9-oxo-9*H*-xanthen-1-yl)ethane-1,2-diamine (44b). This compound was prepared by an analogous procedure as described for the preparation of **14a**, starting from the corresponding tosylate. Yield: 81%; mp (hydrochloride) 201–203 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.10 (t, J = 7 Hz, 6H, $(CH_2CH_3)_2$), 2.67 (q, J = 7 Hz, 4H, $(CH_2CH_3)_2$), 2.82 (t, J = 7 Hz, 2H, $NHCH_2CH_2$), 3.36 (q, J = 7 Hz, 5 Hz, 2H, $NHCH_2CH_2$), 6.39 (d, J = 8 Hz, 1H, H-4), 6.52 (d, J = 8 Hz, 1H, H-2), 7.27 (t, J = 8 Hz, 1H, H-7), 7.32 (d, J = 8 Hz, 1H, H-5), 7.40 (t, J = 8 Hz, 1H, H-3), 7.59 (t, J = 8 Hz, 1H, H-6), 8.20 (d, J = 8 Hz, 1H, H-8), 9.53 (t, J = 5 Hz, 1H, D_2O exch, NH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 11.35 [$(CH_2CH_3)_2$], 40.87 ($NHCH_2CH_2$), 47.08 [$(CH_2CH_3)_2$], 51.09 ($NHCH_2CH_2$), 102.11 (C-2), 103.50 (C-4), 106.77 (C-9a), 117.03 (C-5), 122.06 (C-8a), 123.24 (C-7), 125.92 (C-8), 133.90 (C-6), 135.85 (C-3), 151.51 (C-1), 155.18 (C-10a), 157.94 (C-4a), 179.59 (C-9). Anal. Calcd for $C_{19}H_{22}N_2O_2{\cdot}HCl{\cdot}H_2O$: C, 62.54; H, 6.91; N, 7.68. Found: C, 62.33; H, 6.68; N, 7.43.

4.1.72. 1-[2-(Pyrrolidin-1-yl)ethylamino]-9*H*-xanthene-9-one (44c). This compound was prepared by an analogous procedure as described for the preparation of **14a**, starting from the corresponding tosylate. Yield: 80%; mp (hydrochloride) 256–258 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.88 (m, 4H, $N(CH_2CH_2)_2$), 2.77 (m, 4H, $N(CH_2CH_2)_2$), 2.94 (t, J = 7 Hz, 2H, $NHCH_2CH_2$), 3.52 (q, J = 7 Hz, 5 Hz, 2H, $NHCH_2CH_2$), 6.46 (d, J = 8 Hz, 1H, H-4), 6.56 (d, J = 8 Hz, 1H, H-2), 7.30 (t, J = 8 Hz, 1H, H-7), 7.36 (d, J = 8 Hz, 1H, H-5), 7.44 (t, J = 8 Hz, 1H, H-3), 7.62 (t, J = 8 Hz, 1H, H-6), 8.21 (d, J = 8 Hz, 1H, H-8), 9.58 (t, J = 5 Hz, 1H, D_2O exch, NH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 23.44 ($N(CH_2CH_2)_2$), 41.64 ($NHCH_2CH_2$), 54.32 ($N(CH_2CH_2)_2$), 54.51

(NHCH$_2$*C*H$_2$), 102.47 (C-2), 103.61 (C-4), 106.92 (C-9a), 117.21 (C-5), 121.99 (C-8a), 123.43 (C-7), 126.00 (C-8), 134.12 (C-6), 136.11 (C-3), 151.47 (C-1), 155.33 (C-10a), 158.05 (C-4a), 179.92 (C-9). Anal. Calcd for $C_{19}H_{20}N_2O_2$·HCl·1/2H$_2$O: C, 64.49; H, 6.27; N, 7.92. Found: C, 64.68; H, 6.45; N, 8.04.

4.1.73. 1-[2-(Piperidin-1-yl)ethylamino]-9*H*-xanthene-9-one (44d). This compound was prepared by an analogous procedure as described for the preparation of **14a**, starting from the corresponding tosylate. Yield: 83%; mp (hydrochloride) 241 °C (dec) C (EtOH); ^{1}H NMR (CDCl$_3$, 400 MHz) δ 1.47 (m, 2H, 4-piperidine-H), 1.66 (m, 4H, 3,5-piperidine-H), 2.54 (m, 4H, 2,6-piperidine-H), 2.73 (t, J = 7 Hz, 2H, NHCH$_2$C*H*$_2$), 3.41 (q, J = 7 Hz, 5 Hz, 2H, NHC*H*$_2$CH$_2$), 6.41 (d, J = 8 Hz, 1H, H-4), 6.54 (d, J = 8 Hz, 1H, H-2), 7.28 (t, J = 8 Hz, 1H, H-7), 7.34 (d, J = 8 Hz, 1H, H-5), 7.41 (t, J = 8 Hz, 1H, H-3), 7.61 (t, J = 8 Hz, 1H, H-6), 8.21 (d, J = 8 Hz, 1H, H-8), 9.54 (t, J = 5 Hz, 1H, D$_2$O exch, NH); ^{13}C NMR (CDCl$_3$, 50 MHz) δ 24.11 (4-piperidine-C), 25.65 (3,5-piperidine-C), 40.32 (NH*C*H$_2$CH$_2$), 54.58 (2,6-piperidine-C), 57.22 (NHCH$_2$*C*H$_2$), 102.14 (C-2), 103.58 (C-4), 106.85 (C-9a), 117.10 (C-5), 122.03 (C-8a), 123.32 (C-7), 126.00 (C-8), 133.97 (C-6), 135.92 (C-3), 151.54 (C-1), 155.26 (C-10a), 158.01 (C-4a), 179.70 (C-9). Anal. Calcd for $C_{20}H_{22}N_2O_2$·HCl·3/4H$_2$O: C, 64.51; H, 6.63; N, 7.52. Found: C, 64.87; H, 6.59; N, 7.86.

4.1.74. Dimethyl-[2-[2*H*-benzopyrano[4,3,2-*cd*]indazol-2-yl]ethyl]amine (45a). This compound was prepared by an analogous procedure as described for the preparation of **24a**, starting from the corresponding mesylate. Yield: 91%; mp (hydrochloride) 233–235 °C (EtOH); ^{1}H NMR (CDCl$_3$, 400 MHz) δ 2.77 [s, 6H, N(CH$_3$)$_2$], 3.61 (t, J = 7 Hz, 2H, N–NCH$_2$C*H*$_2$), 4.81 (t, J = 7 Hz, 2H, N–NC*H*$_2$CH$_2$), 6.53 (d, J = 8 Hz, 1H, H-5), 7.03 (d, J = 8 Hz, 1H, H-3), 7.17 (t, J = 8 Hz, 1H, H-9), 7.25 (d, J = 8 Hz, 1H, H-7), 7.29–7.37 (m, 2H, H-4, H-8), 7.83 (d, J = 8 Hz, 1H, H-10); ^{13}C NMR (CDCl$_3$, 50 MHz) δ 39.15 (2× CH$_3$), 44.84 (N–N*C*H$_2$CH$_2$), 56.53 (N–NCH$_2$*C*H$_2$), 101.22 (C-5), 101.88 (C-3), 116.55 (C-2b), 117.72 (C-10a), 118.38 (C-7), 123.05 (C-10), 124.12 (C-9), 130.33 (C-4), 131.17 (C-8), 139.30 (C-10b), 140.99 (C-2a), 149.77 (C-5a), 154.70 (C-6a). Anal. Calcd for $C_{17}H_{17}N_3O$·HCl·H$_2$O. C: 61.17, H: 6.04, N: 12.59. Found (%): C: 60.92, H: 6.13, N: 12.74.

4.1.75. Diethyl-[2-[2*H*-benzopyrano[4,3,2-*cd*]indazol-2-yl]ethyl]amine (45b). This compound was prepared by an analogous procedure as described for the preparation of **24a**, starting from the corresponding mesylate. Yield: 96%; mp (hydrochloride) 257–259 °C (EtOH); ^{1}H NMR (CDCl$_3$, 400 MHz) δ 1.02 (t, J = 7 Hz, 6H, (CH$_2$C*H*$_3$)$_2$), 2.60 (q, J = 7 Hz, 4H, (C*H*$_2$CH$_3$)$_2$), 2.96 (t, J = 7 Hz, 2H, N–NCH$_2$C*H*$_2$), 4.39 (t, J = 7 Hz, 2H, N–NC*H*$_2$CH$_2$), 6.48 (d, J = 8 Hz, 1H, H-5), 6.82 (d, J = 8 Hz, 1H, H-3), 7.17 (t, J = 8 Hz, 1H, H-9), 7.25 (d, J = 8 Hz, 1H, H-7), 7.27–7.31 (m, 2H, H-4, H-8), 7.90 (d, J = 8 Hz, 1H, H-10); ^{13}C NMR (CDCl$_3$, 50 MHz) δ 12.06 [(CH$_2$*C*H$_3$)$_2$], 47.56 [(*C*H$_2$CH$_3$)$_2$], 48.37 (N–N*C*H$_2$CH$_2$), 52.52 (N–NCH$_2$*C*H$_2$), 100.08 (C-5), 101.81 (C-3), 116.69 (C-2b), 118.24 (C-7), 118.53 (C-10a), 122.98 (C-10), 124.00 (C-9), 129.70 (C-4), 130.11 (C-8), 137.94 (C-10b), 141.01 (C-2a), 149.96 (C-5a), 154.70 (C-6a). Anal. Calcd for $C_{19}H_{21}N_3O$·HCl·2H$_2$O: C, 60.07; H, 6.90; N, 11.06. Found: C, 60.35; H, 6.78; N, 11.31.

4.1.76. 2-[2-(Pyrrolidin-1-yl)ethyl]-2*H*-benzopyrano-[4,3,2-*cd*]indazole (45c). This compound was prepared by an analogous procedure as described for the preparation of **24a**, starting from the corresponding mesylate. Yield: 93%; mp (hydrochloride) 234–236 °C (EtOH); ^{1}H NMR (CDCl$_3$, 400 MHz) δ 1.78 [m, 4H, N(CH$_2$C*H*$_2$)$_2$], 2.60 [m, 4H, N(C*H*$_2$CH$_2$)$_2$], 3.03 (t, J = 7 Hz, 2H, N–NCH$_2$C*H*$_2$), 4.47 (t, J = 7 Hz, 2H, N–NC*H*$_2$CH$_2$), 6.49 (d, J = 8 Hz, 1H, H-5), 6.83 (d, J = 8 Hz, 1H, H-3), 7.17 (t, J = 8 Hz, 1H, H-9), 7.23–7.35 (m, 3H, H-4, H-7, H-8), 7.89 (d, J = 8 Hz, 1H, H-10); ^{13}C NMR (CDCl$_3$, 50 MHz) δ 23.49 [N(CH$_2$*C*H$_2$)$_2$], 49.03 (N–N*C*H$_2$CH$_2$), 54.36 [N(*C*H$_2$CH$_2$)$_2$], 55.43 (N–NCH$_2$*C*H$_2$), 100.23 (C-5), 101.66 (C-3), 116.69 (C-2b), 118.24 (C-7), 118.46 (C-10a), 123.02 (C-10), 124.01 (C-9), 129.78 (C-4), 130.22 (C-8), 138.05 (C-10b), 140.88 (C-2a), 149.96 (C-5a), 154.71 (C-6a). Anal. Calcd for $C_{19}H_{19}N_3O$·HCl·2H$_2$O: C, 60.39; H, 6.40; N, 11.12. Found: C, 60.21; H, 6.73; N, 11.26.

4.1.77. 2-[2-(Piperidin-1-yl)ethyl]-2*H*-benzopyrano[4,3,2-*cd*]indazole (45d). This compound was prepared by an analogous procedure as described for the preparation of **24a**, starting from the corresponding mesylate. Yield: 94%; mp (hydrochloride) >270 °C (EtOH); ^{1}H NMR (CDCl$_3$, 400 MHz) δ 1.43 (m, 2H, 4-piperidine-H), 1.58 (m, 4H, 3,5-piperidine-H), 2.49 (m, 4H, 2,6-piperidine-H), 2.85 (t, J = 7 Hz, 2H, N–NCH$_2$C*H*$_2$), 4.45 (t, J = 7 Hz, 2H, N–NC*H*$_2$CH$_2$), 6.49 (d, J = 8 Hz, 1H, H-5), 6.83 (d, J = 8 Hz, 1H, H-3), 7.17 (t, J = 8 Hz, 1H, H-9), 7.57–7.35 (m, 3H, H-4, H-7, H-8), 7.89 (d, J = 8 Hz, 1H, H-10); ^{13}C NMR (CDCl$_3$, 50 MHz) δ 24.18 (4-piperidine-C), 25.94 (3,5-piperidine-C), 47.52 (N–N*C*H$_2$CH$_2$), 54.80 (2,6-piperidine-C), 58.29 (N–NCH$_2$*C*H$_2$), 100.16 (C-5), 101.85 (C-3), 116.70 (C-2b), 118.21 (C-7), 118.46 (C-10a), 122.98 (C-10), 124.01 (C-9), 129.75 (C-4), 130.11 (C-8), 137.94 (C-10b), 140.96 (C-2a), 149.93 (C-5a), 154.67 (C-6a). Anal. Calcd for $C_{20}H_{21}N_3O$·HCl·H$_2$O: C, 64.25; H, 6.47; N, 11.24. Found: C, 64.33; H, 6.32; N, 11.00.

4.2. Biology

4.2.1. 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[46] subline MES-SA/D × 5 (European Collection of Cell Cultures, Salisbury, U.K.). 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/D × 5 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 approximately 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 means 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 (RF) was calculated as the ratio between the IC_{50} of MES-SA/D × 5 cells and the IC_{50} of MES-SA cells.

4.2.2. DNA binding assay. An ethidium bromide displacement assay was used to determine intercalation potency.[42,52] The test compounds were added to a 5 mM Tris–HCl and 0.5 mM EDTA buffer (pH 8) containing 1 μg/mL calf thymus DNA (sodium salt) and 1 μg/mL ethidium bromide (all from Sigma, St. Louis, MO, USA) and fluorescence emission was counted at 600 nm after excitation at 525 nm, using a Fluostar Galaxy microplate reader (BMG Labtechologies, Offenburg, Germany). Results were expressed as EC_{50}, the concentration of the compound that causes a 50% reduction in the fluorescence of the calf thymus DNA/ethidium bromide complex.

4.2.3. Cell-cycle analysis. Cell-cycle analysis was performed following incubation of exponentially growing MES-SA cells with the test substances (5 μM) 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, USA) flow cytometer using the ModFit software (Verity Software House, Topsham, ME, USA).

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