Design, Synthesis, and Antiproliferative Activity of Some New Pyrazole-Fused Amino Derivatives of the Pyranoxanthenone, Pyranothioxanthenone, and Pyranoacridone Ring Systems: A New Class of Cytotoxic Agents

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A series of novel pyranoxanthenones, pyranothioxanthenones, and pyranoacridones have been designed and synthesized as analogues of the acridone alkaloid acronycine, and their DNA binding and in vitro cytotoxicities have been investigated. The title compounds were derived by reaction of the corresponding 6-tosylates **5a**−**e** with 2-hydroxyethylhydrazine, followed by conversion of the free hydroxyl of the substituted ethanols **6a**−**e** to the corresponding mesylates, which were then treated with the suitably substituted secondary amines to provide the target derivatives **8**−**27**. An alternative synthetic procedure for the preparation of these types of compounds is also developed, which resulted in an improvement of the overall yield. The new compounds exhibited interesting cytotoxic activity against the murine leukemia L1210 cell line, being more active than the parent compound, and a number of them possessed cytotoxicity against some human solid tumor cell lines. Especially in the case of a colon adenocarcinoma cell line, their IC_{50} values were comparable to that of mitoxantrone. The results of this study indicate that the incorporation of an amino-substituted pyrazole ring into the acronycine chromophore, or into its isosteres, results in an improvement of the lead compound's activity, and therefore, it may be of use in the search of new anticancer agents derived from this natural product.

Introduction

The acridone alkaloid acronycine (**1**; Figure 1) has attracted much attention over the last few years, due to its broad spectrum of activity in experimental tumors, including X-5563 myeloma, S-91 melanoma, and the Ridgeway osteogenic sarcoma.[1] However, the clinical development of this agent was not successful, because of its extremely low water solubility.[2] Several structural modifications of acronycine have been reported focusing on substitutions on the acridone chromophore and the pyran moiety as well.[3] A certain number of these derivatives exhibited promising antitumor properties, with a wide spectrum of activity and an increased potency on several tumor strains in vitro and in vivo.[4] Furthermore, some pyranoxanthenones and pyranothioxanthenones (**2a,b**; Figure 1), which can be viewed as acronycine isosteres, have also demonstrated cytotoxicity comparable, or superior in some cases, to that of the parent compound.[5]

On the other hand, the anthracene-9,10-dione mitoxantrone (**3**; Figure 1) is an important anticancer agent in clinical use today, and it has gained a well-established role in the treatment of human leukemia and lymphomas, as well as in combination therapy of advanced breast and ovarian cancers.[6] Molecular modeling and molecular pharmacology studies of mitoxantrone and several structurally related compounds revealed that the mode of action of this drug is multimodal in nature, though it is considered to be an intercalating agent that exerts its action primarily

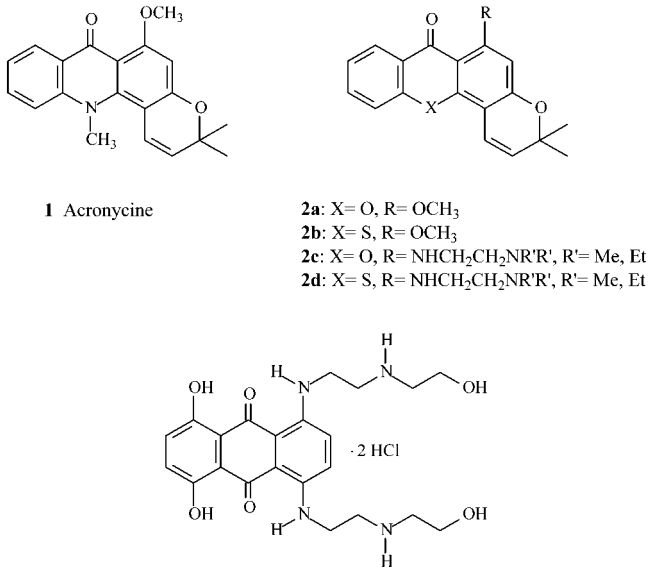

Figure 1. Structures of acronycine, mitoxantrone, pyranoxanthenones, and pyranothioxanthenones.

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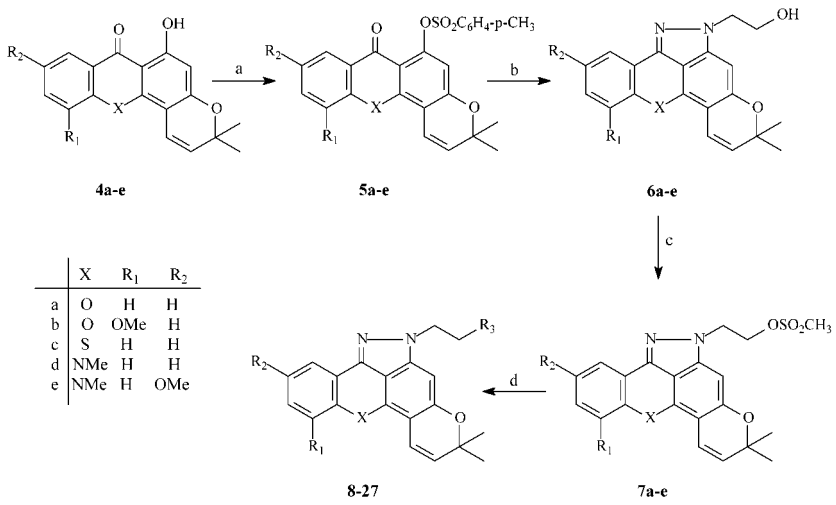
Scheme 1[a]



[a] Reagents: (a) *p*-toluenesulfonyl chloride, Na_2CO_3, Me_2CO, reflux; (b) 2-hydroxyethylhydrazine, DMSO, 150 °C; (c) methanesulfonyl chloride, Et_3N, CH_2Cl_2, rt; (d) *N*,*N*-dialkylaminoethylamine, EtOH, reflux.

through binding and interaction with DNA.[7] A great deal of research effort has been directed toward the finding of some new derivatives that might retain the remarkable anticancer activity of this agent, while reducing or eliminating its side effects, mainly cardiotoxicity and the development of resistant tumors (MDR phenotype).[8] The intensive search for active compounds led to the in corporation of a pyrazole ring fusion at the 1- and 9-positions of the tricyclic skeleton of mitoxantrone. The resulting anthrapyrazoles are endowed with the goals of significantly increasing the spectrum of antitumor activity (including solid tumors) while reducing the cardiotoxicity exhibited by the quinone chemotypes.[9] The remaining carbonyl has been replaced by sulfur or nitrogen, and some highly active molecules have also resulted.[10]

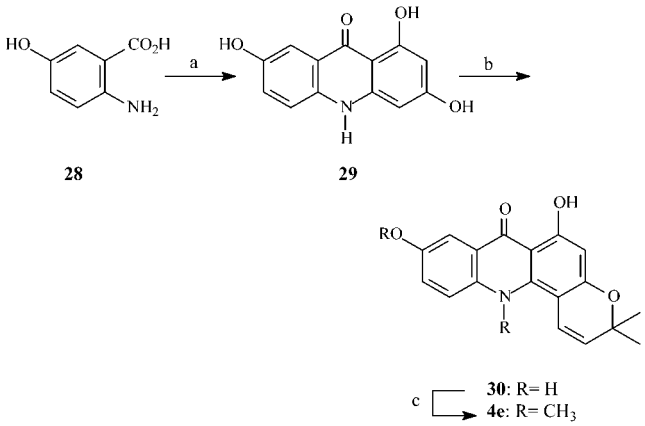
We have recently reported on the synthesis of a number of amino-substituted pyranoxanthenones and pyranothioxanthenones (**2c,d**; Figure 1) which exhibited potent cytotoxicity against the leukemia L1210 cell line, when compared to acronycine.[11] As a continuation of this study, we present here the synthesis and biological evaluation of a new class of compounds bearing structural similarity to both acronycine and the anthrapyrazoles.

The objective of this investigation was to incorporate a pyrazole ring fusion into the acridone and (thio)-xanthenone ring of the acronycine analogues to study the effect of this structural modification, commonly used in similar antitumor series, on the cytotoxic activity of the new compounds against leukemia and some solid tumors in particular.

Chemistry

The synthesis of the target compounds is outlined in Scheme 1. We used the 6-hydroxypyranoxanthenones **4a** and **4b**, [5a] the 6-hydroxypyranothioxanthenone **4c**,[5c] and the 6-hydroxypyranoacridones **4d**[12] and **4e** as starting materials. The synthesis of **4e** has not previously been reported. Thus, as depicted in Scheme 2, 5-hydroxyanthranilic acid (**28**) was first reacted with 1,3,5-trihydroxybenzene to provide 1,3,7-trihydroxyacridone (**29**), which was then converted to 9-hydroxybis-

Scheme 2[a]



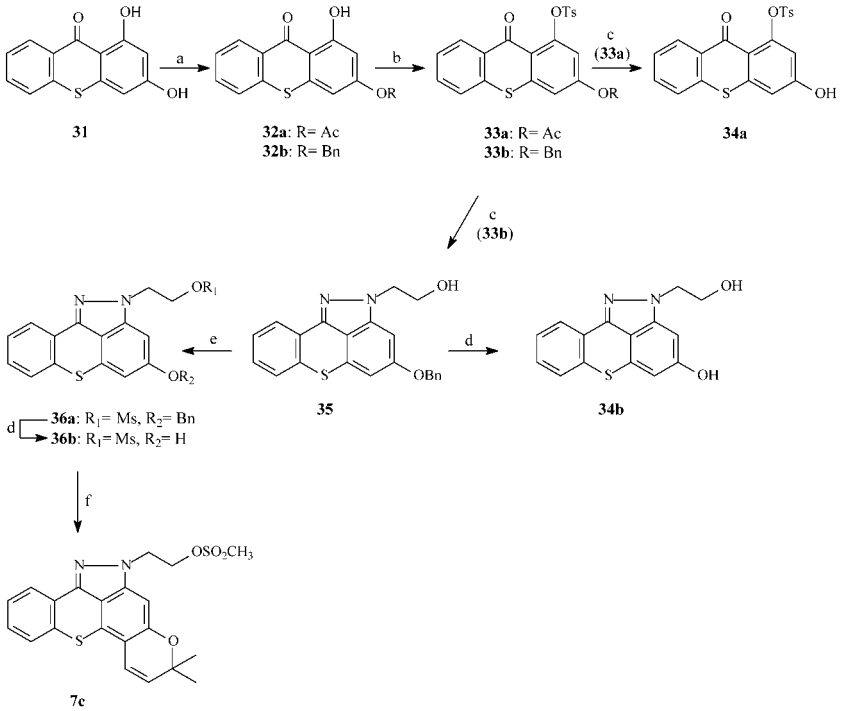
[a] Reagents: (a) 1,3,5-trihydroxybenzene, $ZnCl_2$, 1-butanol, 120 °C; (b) 3-methyl-2-butenal, pyridine, 115 °C; (c) dimethyl sulfate, K_2CO_3, Me_2CO, rt.

noracronycine (**30**) by treatment with 3-methyl-2-butenal. The 9-hydroxyl of **30** was methylated with dimethyl sulfate in the presence of potassium carbonate to provide **4e**.

The derivatives **4a**−**e** were easily converted to the corresponding tosylates **5a**−**e** via standard conditions. Treatment of the latter analogues with commercially available 2-hydroxyethylhydrazine afforded the carbinols **6a**−**e**. The structural assignment for the carbinols was confirmed using NOESY experiments. The side chain methylene, which is adjacent to the pyrazole ring, possessed NOEs with the 5-aromatic proton, but not with the 8-aromatic proton. Furthermore, the structure of the above-mentioned carbinols was also confirmed by a gradient inverse-detected long-range $^1H-^{15}N$ correlation experiment at natural abundance, where a clear cross-peak was observed between N-6 and both the adjacent side chain methylene and the aromatic H-5.

The target compounds **8**−**27** were then prepared in reasonable yields by the conversion of the carbinols **6a**−**e** to the corresponding mesylates **7a**−**e** followed by nucleophilic substitution of the readily displaced mesyl group of these compounds with the appropriately substituted secondary amines.

Scheme 3[a]



[a] Reagents: (a) (i) (for **32a**) Ac_2O, Et_3N, CH_2Cl_2, rt, (ii) (for **32b**) benzyl chloride, Na_2CO_3, NaI, Me_2CO, reflux; (b) *p*-toluenesulfonyl chloride, Na_2CO_3, Me_2CO, reflux; (c) 2-hydroxyethylhydrazine, DMSO, 150 °C; (d) BCl_3, CH_2Cl_2, 0 °C; (e) methanesulfonyl chloride, Et_3N, CH_2Cl_2, rt, (f) phenylboronic acid, 3-methyl-2-butenal, AcOH (glacial), toluene, reflux.

We have also attempted to prepare the amines **8**−**27** upon treatment of the tosylates **5a**−**e** with the corresponding 2-dialkylaminoethylhydrazines. However, none of the desired products were obtained following this procedure, possibly due to the instability of the substituted hydrazines to the high temperature and the prolonged reaction time required.

An alternative methodology for the preparation of the target amines was also developed which involves the initial formation of the pyrazole ring followed by the formation of the pyran ring. The synthetic pathway used is depicted in Scheme 3 concerning the thioxanthenone derivatives.

The 3-acetate **32a** was first prepared from 1,3-dihydroxythioxanthen-9-one (**31**)[5c] and subsequently converted to the corresponding tosylate **33a**. Reaction of **33a** with 2-hydrohyethylhydrazine furnished compound **34a** through deprotection of the 3-hydroxyl, instead of the anticipated formation of compound **34b**. Consequently, the less labile 3-benzyl ether **32b** was prepared upon reaction of **31** with benzyl bromide, which, after prior conversion to the tosylate **33b**, provided the derivative **35** at reflux treatment with 2-hydrohyethylhydrazine. Removal of the benzyl group was effectively accomplished by treatment of **35** with 1 M BCl_3 in dichloromethane, since catalytic hydrogenation was proved unsuccessful. Initial attempts at formation of the pyran ring through the reaction of the derived phenol **34b** with 3-chloro-3-methyl-1-butyne in the presence of potassium carbonate, sodium iodide, and a catalytic amount of copper iodide resulted in a complex, unseparable mixture of products. We therefore first prepared the mesylate **36a**, which was then treated with 1 M BCl_3 in dichloromethane at 0 °C to provide the 4-hydroxy derivative **36b**. This was smoothly reacted with 3-methyl-2-butenal in the presence of phenylboronic acid and acetic acid to give the mesylate **7c**, which led to the desired amines **16**−**19** through nucleophilic displacement of the mesylate with the required amine, as described above. This reaction sequence resulted in a marked improvement in the overall yield (approximately by 20%) taking into account the experimental procedures toward the preparation of the pyran derivatives **4a**−**e**, while the use of some toxic reagents (*N*,*N*-diethylaniline, necessary in the first procedure) and extreme experimental conditions (e.g., high reaction temperatures) were avoided. On the other hand, it possesses the disadvantage of the use of certain expensive reagents (e.g., 3-methyl-2-butenal).

To examine the DNA-binding properties and the in vitro antineoplastic activity of these agents, the free base forms of the amines **8**−**27** were converted into their water-soluble hydrochloride or fumarate addition salts, by treatment with either hydrochloric or fumaric acid, respectively, in methanol.

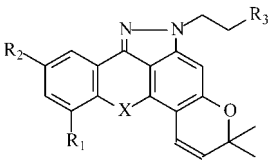
Results and Discussion

The new compounds were evaluated for their DNA-binding affinity and for their in vitro cytotoxic activity in the established model of the murine leukemia cell line L1210, as well as against several human solid tumor cell lines (colon HT-29, HCT 116, and HRT-18, lung A549, and breast MDA-MB-231).

For comparative reasons, the hydroxyethyl and mesyloxyethyl analogues **6a**−**e** and **7a**−**e** were also included in the above-mentioned assays. The results, comprising also the three reference compounds (acronycine, mitoxantrone, and ellipticine), are presented in Tables 1 and 2.

In general, the tested compounds proved to possess weak ethidium bromide displacement potency. Some of

Table 1. Ethidium Bromide Displacement Assay



compd	X	R_1	R_2	R_3	EC_{50} (μM)[a]
6a	O	H	H	OH	*d*
6b	O	OCH_3	H	OH	273.8
6c	S	H	H	OH	*d*
6d	NCH_3	H	H	OH	*d*
6e	NCH_3	H	OCH_3	OH	*d*
7a	O	H	H	OSO_2CH_3	*d*
7b	O	OCH_3	H	OSO_2CH_3	*d*
7c	S	H	H	OSO_2CH_3	>500
7d	NCH_3	H	H	OSO_2CH_3	*d*
7e	NCH_3	H	OCH_3	OSO_2CH_3	*d*
8[b]	O	H	H	$N(CH_3)_2$	431.1
9[b]	O	H	H	$N(CH_2CH_3)_2$	*d*
10[b]	O	H	H	$N(CH_2)_4$	*d*
11[c]	O	H	H	$N(CH_2)_5$	*d*
12[b]	O	OCH_3	H	$N(CH_3)_2$	149.5
13[b]	O	OCH_3	H	$N(CH_2CH_3)_2$	273.2
14[b]	O	OCH_3	H	$N(CH_2)_4$	71.7
15[c]	O	OCH_3	H	$N(CH_2)_5$	289.9
16[b]	S	H	H	$N(CH_3)_2$	>500
17[b]	S	H	H	$N(CH_2CH_3)_2$	282.3
18[b]	S	H	H	$N(CH_2)_4$	257.0
19[b]	S	H	H	$N(CH_2)_5$	334.2
20[b]	NCH_3	H	H	$N(CH_3)_2$	211.8
21[b]	NCH_3	H	H	$N(CH_2CH_3)_2$	*d*
22[b]	NCH_3	H	H	$N(CH_2)_4$	204.9
23[b]	NCH_3	H	H	$N(CH_2)_5$	175.8
24[b]	NCH_3	H	OCH_3	$N(CH_3)_2$	>500
25[b]	NCH_3	H	OCH_3	$N(CH_2CH_3)_2$	*d*
26[b]	NCH_3	H	OCH_3	$N(CH_2)_4$	*d*
27[b]	NCH_3	H	OCH_3	$N(CH_2)_5$	390.6
acronycine					270.4
mitoxantrone					3.4
ellipticine					22.2

[a] The results represent the mean 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. [b] Fumarate. [c] Hydrochloride. [d] Not tested.

them were more potent than acronycine (**14** > **12** > **23** > **22** > **20** > **18** > acronycine), but the most potent, **14**, was clearly 1 order of magnitude weaker than mitoxantrone. However, their cytotoxic activity was undoubtedly more promising. In particular, a certain number of the derivatives **6** and **7** showed strong cytotoxicity against the L1210 leukemia cell line. More precisely, the hydroxyethylacridine analogues **6d** and **6e** are, respectively, 3- and 9-fold more active than acronycine, whereas the corresponding mesylates **7d** and **7e** proved to be even more active, being, respectively, 10- and 65-fold more potent than the reference compound. Furthermore, all the evaluated amines **8**−**27** (with the exception of **16**) were more potent than acronycine against leukemia L1210 cells. The activity of these compounds was also extended against the solid tumor cell lines. Especially against colon HT-29 cells, the compounds **22**, **20**, **25**, **23**, **26**, **15**, **11**, and **27** were more active than the reference compound mitoxantrone. The other solid tumor cell lines were in general less sensitive to the above-mentioned compounds than to mitoxantrone. Nevertheless, some of the new derivatives such as **25**, **20**, and **22** exhibited a universal profile of IC_{50} values below or around 10 μM for the whole spectrum of cell lines used. In conclusion, through the structural modifications of acronycine presented here, we have achieved significant enhancement of the activity of the new compounds against the leukemia cells, concurrently extending their effectiveness against solid tumor cells.

Table 2. Inhibition of Proliferation (IC_{50} Values in μM[a])

compd	murine leukemia L1210	human lung A549	human breast MDA-MB-231	human colon HT-29	human colon HCT-116	human colon HRT-18
6a	>10	>100	100	12 (2)	28 (12)	4 (0.5)
6b	≫10	>100	>100	>100	64 (18)	>100
6c	>10	d	d	18 (5)	37 (10)	33 (12)
6d	8.7 (1.1)	>100	>100	40 (15)	52 (12)	52 (17)
6e	3 (0.4)	90 (10)	50 (7)	40 (7)	60 (19)	*d*
7a	31.9 (7.6)	>100	53 (5)	24 (2)	33 (8)	22 (8)
7b	26.7 (3.7)	*d*	*d*	*d*	*d*	*d*
7c	15 (1.5)	*d*	>100	45 (25)	44 (12)	30 (8)
7d	2.5 (0.25)	*d*	*d*	20 (5)	49 (12)	*d*
7e	0.4 (0.13)	>100	12 (0.8)	18 (6)	12 (7)	*d*
8[b]	9.8 (1.2)	32 (19)	50 (10)	18 (8)	54 (23)	50 (17)
9[b]	9.1 (0.7)	10 (3)	9 (0.5)	9 (0.5)	40 (12)	5 (0.2)
10[b]	8.3 (1.3)	27 (18)	12 (1)	9 (0.2)	42 (5)	30 (11)
11[c]	10.1 (0.9)	15 (6)	20 (3)	6.5 (0.4)	20 (6)	10 (2)
12[b]	14.6 (2.1)	50 (22)	48 (6)	9 (0.3)	51 (18)	17 (2.5)
13[b]	9.9 (0.4)	33 (12)	11 (2.5)	9 (0.2)	41 (4)	30 (14)
14[b]	10.6 (1.3)	33 (8)	11 (4)	8 (0.9)	43 (11)	60 (18)
15[c]	16.5 (2)	31 (9)	37 (7)	6 (1.1)	54 (17)	12 (2)
16[b]	37 (12)	20 (13)	8 (1)	7.5 (0.5)	50 (15)	10 (0.7)
17[b]	10.1 (1.4)	26 (11)	28 (4)	17 (3)	33 (6)	40 (15)
18[b]	6.5 (0.2)	8 (0.5)	9 (0.7)	9 (1)	30 (7)	12 (0.8)
19[b]	17.2 (4.2)	30 (5)	21 (3)	10 (1.5)	61 (22)	30 (5)
20[b]	6.9 (0.8)	10 (2)	12 (2)	3.1 (0.9)	10 (2)	12 (3)
21[b]	4.7 (0.5)	19 (7)	15 (1.3)	6 (0.8)	17 (4)	18 (10)
22[b]	7.5 (0.5)	10 (1.5)	10 (0.5)	3 (0.4)	11 (2)	24 (12)
23[b]	6.7 (0.3)	29 (3.1)	11 (0.9)	5 (1.3)	21 (6)	43 (13)
24[b]	5.9 (0.1)	41 (9)	10 (1)	9 (0.6)	30 (16)	*d*
25[b]	2 (0.2)	10 (0.2)	10 (0.8)	4 (2)	8 (0.5)	*d*
26[b]	3.1 (0.1)	8.5 (1.5)	>100	5.5 (2.5)	7 (0.4)	20 (7)
27[b]	2.7 (0.3)	10 (0.7)	50 (11)	6.5 (2.5)	11 (1.5)	40 (12)
acronycine	27 (4.1)	>100	*d*	>100	>100	>100
mitoxantrone	*d*	0.3 (0.05)	1 (0.08)	8 (3.9)	*d*	*d*
ellipticine	*d*	10 (0.2)	2.3 (0.2)	15 (5)	*d*	*d*

[a] The results represent the mean (± standard deviation) of three independent experiments and are expressed as IC_{50}, the concentration that reduced by 50% the optical density of treated cells with respect to untreated controls. [b] Fumarate. [c] Hydrochloride. [d] Not tested.

Experimental Section

All chemicals were purchased from Aldrich Chemical Co. Melting points were determined on a Büchi apparatus and are uncorrected. ^{1}H NMR spectra and 2D spectra were 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. Pulse widths were 8.55 μs for ^{1}H and 27.7 μs for the ^{15}N at powers of 0 dB and −3 dB. The F1 spectral window employed was set from 100 to 400 ppm. Pulsed field gradients, gt1−gt3, had durations of 0.8 ms. Gradient pairs were optimized as 70/30/50 for ^{15}N. 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.

1,3,7-Trihydroxyacridone (29). To a solution of 5-hydroxyanthranilic acid (**28**) (12.1 g, 78.9 mmol) in 1-butanol (50 mL) were added phloroglucinol (10.9 g, 78.9 mmol) and $ZnCl_2$ (10.8 g, 78.9 mmol). The reaction mixture was stirred for 5 h at 120 °C, and the water produced was removed with a Dean−Stark apparatus. The resulting precipitate was filtered and washed with water and CH_2Cl_2 to give **29** (9.20 g, 48%): mp 280−282 °C dec (EtOH); ^{1}H NMR (DMSO-d_6, 200 MHz) δ 5.95 (d, J = 1 Hz, 1H, H-2), 6.21 (d, J = 1 Hz, 1H, H-4), 7.23 (dd, J = 9, 2.5 Hz, 1H, H-6), 7.36 (d, J = 9 Hz, 1H, H-5), 7.44 (d, J = 2.5 Hz, 1H, H-8), 9.75 (br s, 1H, D_2O exch, NH), 10.50 (br s, 1H, D_2O exch, OH-7), 11.66 (s, 1H, D_2O exch, OH-3), 14.34 (s, 1H, D_2O exch, OH-1). Anal. ($C_{13}H_9NO_4$) C, H, N.

7,12-Dihydro-6,9-dihydroxy-3,3-dimethyl-3*H*-pyrano-[2,3-*c*]acridin-7-one (30). To a solution of **29** (200 mg, 0.82 mmol) in dry pyridine (1 mL) was added 3-methyl-2-butenal (0.2 mL, 2.1 mmol), and the reaction mixture was stirred for 1.5 h at 115 °C. Then the reagents were removed under reduced pressure (using a high-vacuum pump), and the solid residue was submitted to flash chromatography with cyclohexane/EtOAc (80/20 to 50/50) to give compound **30** (101 mg, 40%): mp > 280 °C dec (EtOH); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.41 (s, 6H, 2 *gem*-CH_3), 5.67 (d, J = 10 Hz, 1H, H-2), 5.99 (s, 1H, H-5), 7.05 (d, J = 10 Hz, 1H, H-1), 7.30 (dd, J = 9, 3 Hz, 1H, H-10), 7.48 (d, J = 3 Hz, 1H, H-8), 7.69 (d, J = 9 Hz, 1H, H-11), 9.75 (s, 1H, D_2O exch, 9-OH), 11.08 (s, 1H, D_2O exch, NH), 14.83 (s, 1H, D_2O exch, 6-OH); ^{13}C NMR (DMSO, 50 MHz) δ 27.48 (2 CH_3), 76.96 (C-3), 95.74 (C-5), 97.65 (C-12b), 103.60 (C-6a), 106.91 (C-8), 116.25 (C-1), 119.23 (C-11), 119.92 (C-7a), 124.63 (C-10), 125.32 (C-2), 134.70 (C-11a), 137.49 (C-12a), 152.56 (C-9), 158.77 (C-4a), 163.77 (C-6), 179.87 (C-7). Anal. ($C_{18}H_{15}NO_4$) C, H, N.

7,12-Dihydro-6-hydroxy-9-methoxy-3,3,12-trimethyl-3*H*-pyrano[2,3-*c*]acridin-7-one (4e). To a solution of **30** (1.13 g, 3.65 mmol) in dry acetone (20 mL) were added dimethyl sulfate (9.0 mL) and anhydrous K_2CO_3 (2.0 g). The reaction mixture was stirred for 24 h at room temperature. The resulting precipitate was filtered and washed with water to give pure **4e** (736 mg, 60%): mp 181−183 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.54 (s, 6H, 2 *gem*-CH_3), 3.92 (s, 3H, NCH_3), 3.94 (s, 3H, 9-OCH_3), 5.50 (d, J = 10 Hz, 1H, H-2), 6.27 (s, 1H, H-5), 6.57 (d, J = 10 Hz, 1H, H-1), 7.36 (dd, J = 9, 3 Hz, 1H, H-10), 7.41 (d, J = 9 Hz, 1H, H-11), 7.77 (d, J = 3 Hz, 1H, H-8), 14.80 (s, 1H, D_2O exch, 6-OH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 26.79 (2 CH_3), 43.66 (NCH_3), 55.75 (OCH_3), 76.90 (C-3), 97.54 (C-5), 100.56 (C-12b), 105.30 (C-8), 106.66 (C-6a), 117.79 (C-11), 121.58 (C-1), 122.50 (C-2, C-7a), 124.45 (C-10), 139.63 (C-11a), 144.11 (C-12a), 154.92 (C-9), 161.39 (C-4a), 165.10 (C-6), 180.39 (C-7). Anal. ($C_{20}H_{19}NO_4$) C, H, N.

3,3-Dimethyl-6-[[(4-methylphenyl)sulfonyl]oxy])-3*H*,-7*H*-pyrano[2,3-*c*]xanthen-7-one (5a). To a solution of 3,3-dimethyl-6-hydroxy-3*H*,7*H*-pyrano[2,3-*c*]xanthen-7-one (**4a**; 1.32 g, 4.5 mmol)[5a] in dry acetone (20 mL) were added under argon *p*-toluenesulfonyl chloride (1.7 g, 9 mmol) and anhydrous sodium carbonate (1.9 g, 18 mmol), and the mixture was refluxed for 4 h. The mixture was then vacuum-evaporated and extracted with CH_2Cl_2–water, the combined extracts were dried (Na_2SO_4) and vacuum-evaporated, and the residue was purified by column chromatography, using CH_2Cl_2 as the eluent, to give compound **5a** (1.79 g, 89%): mp 158−160 °C (Et_2O−*n*-hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.42 (s, 6H, 2 *gem*-CH_3), 2.35 (s, 3H, 4′-CH_3), 5.66 (d, J = 10.5 Hz, 1H, H-2), 6.55 (s, 1H, H-5), 6.81 (d, J = 10.5 Hz, 1H, H-1), 7.30 (d, J = 8 Hz, 2H, H-3′, H-5′), 7.46 (m, 3H, H-9, H-10, H-11), 7.91 (d, J = 8 Hz, 2H, H-2′, H-6′), 8.16 (dd, J = 8, 2 Hz, 1H, H-8). Anal. ($C_{25}H_{20}O_6S$) C, H.

3,3-Dimethyl-11-methoxy-6-[[(4-methylphenyl)sulfonyl]-oxy]-3*H*,7*H*-pyrano[2,3-*c*]xanthen-7-one (5b). This compound was prepared by a procedure analogous to that of **5a**, starting from **4b**:[5a] yield 91%; mp 154−156 °C (Et_2O); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.52 (s, 6H, 2 *gem*-CH_3), 2.43 (s, 3H, 4′-CH_3), 3.98 (s, 3H, OCH_3), 5.72 (d, J = 10 Hz, 1H, H-2), 6.68 (s, 1H, H-5), 6.97 (d, J = 10 Hz, 1H, H-1), 7.16 (dd, J = 8, 1.2 Hz, 1H, H-10), 7.24 (t, J = 8 Hz, 1H, H-9), 7.33 (d, J = 8 Hz, 2H, H-3′, H-5′), 7.79 (dd, J = 8, 1.2 Hz, 1H, H-8), 7.99 (d, J = 8 Hz, 2H, H-2′, H-6′). Anal. ($C_{26}H_{22}O_7S$) C, H.

3,3-Dimethyl-6-[[(4-methylphenyl)sulfonyl]oxy])-3*H*,-7*H*-pyrano[2,3-*c*]thioxanthen-7-one (5c). This compound was prepared by a procedure analogous to that of **5a**, starting from **4c**:[5c] yield 96%; mp 187−189 °C (Et_2O); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.45 (s, 6H, 2 *gem*-CH_3), 2.30 (s, 3H, 4′-CH_3), 5.78 (d, J = 10 Hz, 1H, H-2), 6.74 (s, 1H, H-5), 6.61 (d, J = 10 Hz, 1H, H-1), 7.31 (d, J = 8 Hz, 2H, H-3′, H-5′), 7.49 (m, 3H, H-9, H-10, H-11), 7.82 (d, J = 8 Hz, 2H, H-2′, H-6′), 8.25 (dd, J = 8, 1 Hz, 1H, H-8). Anal. ($C_{25}H_{20}O_5S_2$) C, H.

3,3,12-Trimethyl-6-[[(4-methylphenyl)sulfonyl]oxy])-3*H*,7*H*-pyrano[2,3-*c*]acridin-7-one (5d). This compound was prepared by a procedure analogous to that of **5a**, starting from **4d**:[12] yield 89%; mp 198−201 °C (Et_2O−*n*-pentane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.40 (s, 3H, *gem*-CH_3), 1.49 (s, 3H, *gem*-CH_3), 2.39 (s, 3H, 4′-CH_3), 3.78 (s, 3H, NCH_3), 5.58 (d, J = 10 Hz, 1H, H-2), 6.53 (d, J = 10 Hz, 1H, H-1), 6.57 (s, 1H, H-5), 7.20 (t, J = 8 Hz, 1H, H-9), 7.29 (d, J = 8 Hz, 2H, H-3′, H-5′), 7.32 (d, J = 8 Hz, 1H, H-11), 7.61 (td, J = 8, 1.5 Hz, 1H, H-10), 7.97 (d, J = 8 Hz, 2H, H-2′, H-6′), 8.27 (dd, J = 8, 1.5 Hz, 1H, H-8). Anal. ($C_{26}H_{23}NO_5S$) C, H, N.

9-Methoxy-3,3,12-trimethyl-6-[[(4-methylphenyl)sulfonyl]oxy]-3*H*,7*H*-pyrano[2,3-*c*]acridin-7-one (5e). This compound was prepared by a procedure analogous to that of **5a**, starting from **4e**: yield 67%; mp 188−191 °C (Et_2O−*n*-pentane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.53 (s, 6H, 2 *gem*-CH_3), 2.45 (s, 3H, 4′-CH_3), 3.82 (s, 3H, NCH_3), 3.92 (s, 3H, 9-OCH_3), 5.60 (d, J = 10 Hz, 1H, H-2), 6.51 (s, 1H, H-5), 6.56 (d, J = 10 Hz, 1H, H-1), 7.27 (dd, J = 9, 3 Hz, 1H, H-10), 7.33 (d, J = 9 Hz, 1H, H-11), 7.36 (d, J = 8 Hz, 2H, H-3′, H-5′), 7.80 (d, J = 3 Hz, 1H, H-8), 8.03 (d, J = 8 Hz, 2H, H-2′, H-6′). Anal. ($C_{27}H_{25}NO_6S$) C, H, N.

2-[6,9-Dihydro-9,9-dimethyl(1)benzopyrano[4,3,2-*c*,*d*]-pyrano[3,2-*f*]indazol -6-yl]-1-ethanol (6a). To a solution of **5a** (1.93 g, 4.31 mmol) in dry DMSO (25 mL) was added 2-hydroxyethylhydrazine (1.5 mL, 22 mmol), and the mixture was heated, under argon, at 150 °C for 90 min. Upon cooling, the mixture was poured into water and extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4, and the solvent was vacuum-evaporated. The residue was purified by column chromatography, using a mixture of cyclohexane/EtOAc (60/40 to 30/70) as the eluent, to give compound **6a** (835 mg, 58%): mp 180−182 °C (EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.40 (s, 6H, 2 *gem*-CH_3), 3.35 (t, J = 4 Hz, 1H, D_2O exch, OH), 4.08 (q, J = 5, 4 Hz, 2H, NCH_2CH_2), 4.32 (t, J = 5 Hz, 2H, NCH_2CH_2), 5.61 (d, J = 10 Hz, 1H, H-10), 6.21 (s, 1H, H-7), 6.69 (d, J = 10 Hz, 1H, H-11), 7.21−7.35 (m, 3H, H-1, H-2, H-3), 8.05 (dd, J = 8, ∼0 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 27.78 (2 *gem*-CH_3), 51.05 (NCH_2CH_2), 62.01 (NCH_2CH_2), 76.83 (C-9), 88.84 (C-7), 100.39 (C-11a), 111.62 (C-11c), 115.52 (C-11), 118.07 (C-4a), 118.26 (C-1), 123.14 (C-4), 124.28 (C-3), 127.89 (C-10), 129.93 (C-2), 138.83 (C-4b), 140.31 (C-6a), 144.51 (C-7a), 154.74 (C-12a), 156.88 (C-11b). Anal. ($C_{20}H_{18}N_2O_3$) C, H, N.

2-[6,9-Dihydro-9,9-dimethyl-1-methoxy(1)benzopyrano-[4,3,2-*c*,*d*]pyrano[3,2-*f*]indazol-6-yl]-1-ethanol (6b). This compound was prepared by a procedure analogous to that of **6a**: yield 59%; mp 217−218 °C (EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.40 (s, 6H, 2 *gem*-CH_3), 3.25 (t, J = 5.5 Hz, 1H, D_2O exch, OH), 3.95 (s, 3H, OCH_3), 4.13 (q, J = 5.5, 5 Hz, 2H, NCH_2CH_2), 4.32 (t, J = 5 Hz, 2H, NCH_2CH_2), 5.65 (d, J = 10 Hz, 1H, H-10), 6.28 (s, 1H, H-7), 6.80 (d, J = 10 Hz, 1H, H-11), 6.95 (dd, J = 8, 1.5 Hz, 1H, H-2), 7.12 (t, J = 8 Hz, 1H, H-3), 7.47 (dd, J = 8, 1.5 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 27.58 (2 *gem*-CH_3), 51.05 (NCH_2CH_2), 56.33 (OCH_3), 62.01 (NCH_2CH_2), 76.73 (C-9), 89.13 (C-7), 100.65 (C-11a), 112.00 (C-11c), 112.88 (C-2), 114.99 (C-4), 115.88 (C-11), 118.99 (C-4a), 123.99 (C-3), 127.79 (C-10), 139.02 (C-4b), 140.55 (C-6a), 144.14 (C-1), 144.19 (C-7a), 144.41 (C-12a), 156.81 (C-11b). Anal. ($C_{21}H_{20}N_2O_4$) C, H, N.

2-[6,9-Dihydro-9,9-dimethyl(1)benzothiopyrano[4,3,2-*c*,*d*]pyrano[3,2-*f*]indazol-6-yl]-1-ethanol (6c). This compound was prepared by a procedure analogous to that of **6a**:

yield 62%; mp 167−169 °C (EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.40 (s, 3H, *gem*-CH_3), 1.51 (s, 3H, *gem*-CH_3), 3.40 (t, J = 4.5 Hz, 1H, D_2O exch, OH), 4.13 (q, J = 5, 4.5 Hz, 2H, NCH_2CH_2), 4.20 (t, J = 5 Hz, 2H, NCH_2CH_2), 5.72 (d, J = 10 Hz, 1H, H-10), 6.31 (s, 1H, H-7), 6.35 (d, J = 10 Hz, 1H, H-11), 7.15−7.38 (m, 3H, H-1, H-2, H-3), 7.95 (dd, J = 8, ∼0 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 27.78 (2 *gem*-CH_3), 50.58 (NCH_2CH_2), 61.84 (NCH_2CH_2), 76.59 (C-9), 91.07 (C-7), 109.70 (C-11a), 115.82 (C-11c), 117.70 (C-11), 123.91 (C-4), 125.42 (C-4a), 125.78 (C-11b), 126.58 (C-1), 126.80 (C-3), 128.45 (C-2), 129.25 (C-10), 133.20 (C-12a), 140.65 (C-6a), 141.81 (C-4b), 155.06 (C-7a). Anal. ($C_{20}H_{18}N_2O_2S$) C, H, N.

2-[6,12-Dihydro-9,9,12-trimethyl-9*H*-pyrano[2,3-*c*]pyrazolo[3,4,5-*m*,*n*]acridin-6-yl]-1-ethanol (6d). This compound was prepared by a procedure analogous to that of **6a**: yield 73%; mp 223−225 °C (EtOAc−*n*-hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.47 (s, 6H, 2 *gem*-CH_3), 3.10 (t, J = 4 Hz, 1H, D_2O exch, OH), 3.72 (s, 3H, NCH_3), 4.07 (q, J = 4.5, 4 Hz, 2H, NCH_2CH_2), 4.26 (t, J = 4.5 Hz, 2H, NCH_2CH_2), 5.55 (d, J = 10 Hz, 1H, H-10), 6.17 (s, 1H, H-7), 6.72 (d, J = 10 Hz, 1H, H-11), 7.10 (t, J = 8 Hz, 1H, H-3), 7.16 (d, J = 8 Hz, 1H, H-1), 7.37 (td, J = 8, 1.5 Hz, 1H, H-2), 7.91 (dd, J = 8, 1.5 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 26.78 (2 *gem*-CH_3), 40.57 (NCH_3), 51.37 (NCH_2CH_2), 60.16 (NCH_2CH_2), 74.68 (C-9), 87.14 (C-7), 98.50 (C-11a), 113.35 (C-11c), 116.76 (C-1), 118.60 (C-4a), 120.89 (C-11), 121.91 (C-4, C-3), 124.74 (C-10), 129.33 (C-2), 136.06 (C-11b), 139.85 (C-4b), 140.33 (C-6a), 143.89 (C-12a), 156.87 (C-7a). Anal. ($C_{21}H_{21}N_3O_2$) C, H, N.

2-[6,12-Dihydro-3-methoxy-9,9,12-trimethyl-9*H*-pyrano-[2,3-*c*]pyrazolo[3,4,5-*m*,*n*]acridin-6-yl]-1-ethanol (6e). This compound was prepared by a procedure analogous to that of **6a**: yield 65%; mp 186−188 °C (EtOAc−*n*-hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.49 (s, 6H, 2 *gem*-CH_3), 2.95 (t, J = 5.1 Hz, 1H, D_2O exch, OH), 3.71 (s, 3H, NCH_3), 3.90 (s, 3H, 9-OCH_3), 4.09 (q, J = 5.1, 4.5 Hz, 2H, NCH_2CH_2), 4.27 (t, J = 4.5 Hz, 2H, NCH_2CH_2), 5.55 (d, J = 10 Hz, 1H, H-10), 6.15 (s, 1H, H-7), 6.72 (d, J = 10 Hz, 1H, H-11), 6.97 (dd, J = 9, 3 Hz, 1H, H-2), 7.11 (d, J = 9 Hz, 1H, H-1), 7.40 (d, J = 3 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 26.94 (2 *gem*-CH_3), 40.83 (NCH_3), 50.54 (NCH_2CH_2), 55.72 (OCH_3), 62.08 (NCH_2CH_2), 75.09 (C-9), 85.78 (C-7), 98.72 (C-11a), 105.34 (C-4), 114.16 (C-11c), 117.13 (C-2), 117.39 (C-1), 119.34 (C-4a), 120.99 (C-11), 124.41 (C-10), 135.00 (C-6a), 138.49 (C-11b), 140.73 (C-12a), 141.57 (C-4b), 154.70 (C-3), 157.97 (C-7a). Anal. ($C_{22}H_{23}N_3O_3$) C, H, N.

2-[6,9-Dihydro-9,9-dimethyl(1)benzopyrano[4,3,2-*c*,*d*]-pyrano[3,2-*f*]indazol-6-yl]-1-ethanol Methanesulfonate (7a). Methanesulfonyl chloride (658 μL, 8.5 mmol) was added to a stirred suspension of **6a** (882 mg, 2.64 mmol) and triethylamine (1.35 mL, 9.7 mmol) in dry dichloromethane (40 mL) with cooling to 0 °C. The cooling bath was removed, and the mixture was stirred at room temperature for 4 h. The reaction mixture was partitioned between dichloromethane and 1 N sodium hydroxide, and the organic phase was washed with HCl (10%), water, and brine, then dried (Na_2SO_4), and concentrated to dryness. The residue was purified by flash chromatography eluting with a 70/30 to 50/50 cyclohexane/EtOAc mixture to afford compound **7a** (1.02 g, 94%); mp 155−157 °C (EtOAc−*n*-pentane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.42 (s, 6H, 2 *gem*-CH_3), 2.76 (s, 3H, SO_2CH_3), 4.45 (t, J = 5 Hz, 2H, NCH_2CH_2), 4.61 (t, J = 5 Hz, 2H, NCH_2CH_2), 5.60 (d, J = 10 Hz, 1H, H-10), 6.28 (s, 1H, H-7), 6.66 (d, J = 10 Hz, 1H, H-11), 7.12−7.31 (m, 3H, H-1, H-2, H-3), 8.05 (dd, J = 8, ∼0 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 27.64 (2 *gem*-CH_3), 37.24 (SO_2CH_3), 48.13 (NCH_2CH_2), 67.68 (NCH_2CH_2), 76.76 (C-9), 88.98 (C-7), 100.53 (C-11a), 111.64 (C-11c), 115.33 (C-11), 117.90 (C-4a), 118.26 (C-1), 122.97 (C-4), 124.23 (C-3), 128.04 (C-10), 130.00 (C-2), 139.24 (C-4b), 140.55 (C-6a), 144.47 (C-7a), 154.65 (C-12a), 156.93 (C-11b). Anal. ($C_{21}H_{20}N_2O_5S$) C, H, N.

2-[6,9-Dihydro-9,9-dimethyl-1-methoxy(1)benzopyrano-[4,3,2-*c*,*d*]pyrano[3,2-*f*]indazol-6-yl]-1-ethanol Methanesulfonate (7b). This compound was prepared by a procedure analogous to that of **7a**: yield 97%; mp 174−176 °C (EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.42 (s, 6H, 2 *gem*-CH_3), 2.74 (s, 3H, SO_2CH_3), 3.95 (s, 3H, OCH_3), 4.45 (t, J = 5 Hz, 2H, NCH_2CH_2), 4.64 (t, J = 5 Hz, 2H, NCH_2CH_2), 5.62 (d, J = 10 Hz, 1H, H-10), 6.30 (s, 1H, H-7), 6.79 (d, J = 10 Hz, 1H, H-11), 6.95 (dd, J = 8, ∼0.5 Hz, 1H, H-2), 7.10 (t, J = 8 Hz, 1H, H-3), 7.44 (dd, J = 8, 0.5 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 27.59 (2 *gem*-CH_3), 37.27 (SO_2CH_3), 48.16 (NCH_2CH_2), 56.24 (OCH_3), 67.73 (NCH_2CH_2), 76.83 (C-9), 89.06 (C-7), 100.84 (C-11a), 111.76 (C-11c), 112.95 (C-2), 114.79 (C-4), 115.64 (C-11), 118.60 (C-4a), 124.01 (C-3), 127.96 (C-10), 139.34 (C-4b), 140.60 (C-6a), 144.05 (C-7a), 144.34 (C-12a), 149.14 (C-1), 157.05 (C-11b). Anal. ($C_{22}H_{22}N_2O_6S$) C, H, N.

2-[6,9-Dihydro-9,9-dimethyl(1)benzothiopyrano[4,3,2-*c*,*d*]pyrano[3,2-*f*]indazol-6-yl]-1-ethanol Methanesulfonate (7c).

Method A. This compound was prepared according to the procedure reported for **7a**: yield 95%.

Method B. A mixture of **36b** (56 mg, 0.154 mmol), phenylboronic acid (20 mg, 0.164 mmol), 3-methyl-2-butenal (14.7 μL, 0.152 mmol), and glacial acetic acid (3.9 mL) in toluene (30 mL) was heated under reflux for 2 h with azeotropic removal of water using a Dean−Stark trap. After cooling, the reaction mixture was concentrated and the residue was purified by column chromatography, using a mixture of dichloromethane/EtOAc (95/5) as the eluent, to give **7c** (21.5 mg, 33%):

mp 153−155 °C (Et_2O); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.40 (s, 6H, 2 *gem*-CH_3), 2.78 (s, 3H, SO_2CH_3), 4.41 (t, J = 5 Hz, 2H, NCH_2CH_2), 4.62 (t, J = 5 Hz, 2H, NCH_2CH_2), 5.67 (d, J = 10 Hz, 1H, H-10), 6.32 (d, J = 10 Hz, 1H, H-11), 6.33 (s, 1H, H-7), 7.18−7.30 (m, 3H, H-1, H-2, H-3), 7.98 (dd, J = 8, ∼0 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 27.72 (2 *gem*-CH_3), 37.30 (SO_2CH_3), 47.82 (NCH_2CH_2), 67.70 (NCH_2CH_2), 76.56 (C-9), 91.18 (C-7), 109.80 (C-11a), 115.67 (C-11c), 117.56 (C-11), 123.82 (C-4), 125.15 (C-4a), 125.37 (C-11b), 126.60 (C-1), 126.78 (C-3), 128.51 (C-2), 129.40 (C-10), 133.19 (C-12a), 140.99 (C-6a), 142.23 (C-4b), 155.11 (C-7a). Anal. ($C_{21}H_{20}N_2O_4S_2$) C, H, N.

2-[6,12-Dihydro-9,9,12-trimethyl-9*H*-pyrano[2,3-*c*]pyrazolo[3,4,5-*m*,*n*]acridin-6-yl]-1-ethanol Methanesulfonate (7d). This compound was prepared by a procedure analogous to that of **7a**: yield 95%; mp 182−185 °C (EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.45 (s, 6H, 2 *gem*-CH_3), 3.66 (s, 3H, NCH_3), 3.74 (s, 3H, SO_2CH_3), 4.43 (t, J = 4.5 Hz, 2H, NCH_2CH_2), 4.64 (t, J = 4.5 Hz, 2H, NCH_2CH_2), 5.53 (d, J = 10 Hz, 1H, H-10), 6.19 (s, 1H, H-7), 6.77 (d, J = 10 Hz, 1H, H-11), 7.09 (t, J = 8 Hz, 1H, H-3), 7.11 (d, J = 8 Hz, 1H, H-1), 7.33 (td, J = 8, 1.5 Hz, 1H, H-2), 7.88 (dd, J = 8, 1.5 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 26.86 (2 *gem*-CH_3), 20.74 (SO_2CH_3), 40.72 (NCH_3), 47.82 (NCH_2CH_2), 67.85 (NCH_2CH_2), 75.05 (C-9), 86.55 (C-7), 99.38 (C-11a), 113.79 (C-11c), 116.10 (C-1), 118.68 (C-4a), 120.77 (C-11), 121.91 (C-3), 122.61 (C-4), 125.04 (C-10), 129.41 (C-2), 136.54 (C-11b), 140.95 (C-6a), 141.94 (C-4b), 144.30 (C-12a), 157.97 (C-7a). Anal. ($C_{22}H_{23}N_3O_4S$) C, H, N.

2-[6,12-Dihydro-3-methoxy-9,9,12-trimethyl-9*H*-pyrano-[2,3-*c*]pyrazolo[3,4,5-*m*,*n*]acridin-6-yl]-1-ethanol Methanesulfonate (7e). This compound was prepared by a procedure analogous to that of **7a**: yield 56%; mp 169−170 °C (EtOAc−*n*-pentane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.46 (s, 6H, 2 *gem*-CH_3), 2.78 (s, 3H, SO_2CH_3), 3.64 (s, 3H, NCH_3), 3.86 (s, 3H, 9-OCH_3), 4.44 (t, J = 4.5 Hz, 2H, NCH_2CH_2), 4.67 (t, J = 4.5 Hz, 2H, NCH_2CH_2), 5.52 (d, J = 10 Hz, 1H, H-10), 6.18 (s, 1H, H-7), 6.68 (d, J = 10 Hz, 1H, H-11), 6.93 (dd, J = 9, 3 Hz, 1H, H-2), 7.05 (d, J = 9 Hz, 1H, H-1), 7.37 (d, J = 3 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 26.84 (2 *gem*-CH_3), 37.16 (SO_2CH_3), 40.62 (NCH_3), 47.78 (NCH_2CH_2), 55.61 (OCH_3), 67.77 (NCH_2CH_2), 75.01 (C-9), 85.93 (C-7), 98.86 (C-11a), 105.15 (C-4), 113.96 (C-11c), 117.02 (C-2), 117.38 (C-1), 119.11 (C-4a), 120.84 (C-11), 124.48 (C-10), 136.46 (C-6a), 138.33 (C-11b), 141.02 (C-12a), 141.93 (C-4b), 154.58 (C-3), 157.99 (C-7a). Anal. ($C_{23}H_{25}N_3O_5S$) C, H, N.

General Procedure for the Preparation of the Amines 8−27. The appropriate *N*,*N*-dialkylaminoethylamine (10 equiv) was added to a stirred solution of compounds **7a**−**e** in

anhydrous ethanol. The mixture was stirred at reflux temperature for 12−18 h. Then the mixture was vacuum-evaporated, and the residue was purified by column chromatography, using a mixture of EtOAc/MeOH (99/1 to 80/20) as the eluent.

Data for Dimethyl-[2-[6,9-dihydro-9,9-dimethylpyrano-[3,2-*f*](1)benzopyrano[4,3,2-*c,d*]indazol-6-yl]ethyl]-amine (8): yield 96%; mp (fumarate) 202−204 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.44 (s, 6H, 2 *gem*-CH_3), 2.31 [s, 6H, $N(CH_3)_2$], 2.81 [t, J = 7 Hz, 2H, $NCH_2CH_2N(CH_3)_2$], 4.30 [t, J = 7 Hz, 2H, $NCH_2CH_2N(CH_3)_2$], 5.60 (d, J = 10 Hz, 1H, H-10), 6.27 (s, 1H, H-7), 6.71 (d, J = 10 Hz, 1H, H-11), 7.11−7.35 (m, 3H, H-1, H-2, H-3), 7.82 (dd, J = 8, 1 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 27.76 (2 *gem*-CH_3), 45.66 [$N(CH_3)_2$], 47.62 [$NCH_2CH_2N(CH_3)_2$], 58.32 [$NCH_2CH_2N(CH_3)_2$], 76.76 (C-9), 88.91 (C-7), 100.19 (C-11a), 113.58 (C-11c), 115.60 (C-11), 118.21 (C-1), 118.50 (C-4a), 123.06 (C-4), 124.18 (C-3), 127.69 (C-10), 129.64 (C-2), 138.42 (C-4b), 139.90 (C-6a), 144.56 (C-7a), 154.70 (C-12a), 156.54 (C-11b). Anal. ($C_{22}H_{23}N_3O_2 \cdot C_4H_4O_4 \cdot H_2O$) C, H, N.

Data for Diethyl-[2-[6,9-dihydro-9,9-dimethylpyrano-[3,2-*f*](1)benzopyrano[4,3,2-*c,d*] indazol-6-yl]ethyl]amine (9): yield 97%; mp (fumarate) 164−165 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.00 [t, J = 7 Hz, 6H, $N(CH_2CH_3)_2$], 1.43 (s, 6H, 2 *gem*-CH_3), 2.60 [q, J = 7 Hz, 4H, $N(CH_2CH_3)_2$], 2.91 [t, J = 7 Hz, 2H, $NCH_2CH_2N(CH_2CH_3)_2$], 4.25 [t, J = 7 Hz, 2H, $NCH_2CH_2N(CH_2CH_3)_2$], 5.62 (d, J = 10 Hz, 1H, H-10), 6.26 (s, 1H, H-7), 6.73 (d, J = 10 Hz, 1H, H-11), 7.10−7.33 (m, 3H, H-1, H-2, H-3), 7.84 (dd, J = 7, 1 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 11.99 [$N(CH_2CH_3)_2$], 27.68 (2 *gem*-CH_3), 47.50 [$N(CH_2CH_3)_2$], 48.01 [$NCH_2CH_2N(CH_2CH_3)_2$], 52.21 [$NCH_2CH_2N(CH_2CH_3)_2$], 76.69 (C-9), 89.06 (C-7), 100.09 (C-11a), 111.93 (C-11c), 115.62 (C-11), 118.19 (C-1), 118.50 (C-4a), 123.02 (C-4), 124.16 (C-3), 127.67 (C-10), 129.56 (C-2), 138.25 (C-4b), 139.92 (C-6a), 144.60 (C-7a), 154.67 (C-12a), 156.42 (C-11b). Anal. ($C_{24}H_{27}N_3O_2 \cdot C_4H_4O_4 \cdot {}^3/_2H_2O$) C, H, N.

Data for 6,9-Dihydro-9,9-dimethyl-6-(2-pyrrolidin-1-ylethyl)pyrano[3,2-*f*](1)benzopyrano[4,3,2-*c,d*]indazole (10): yield 96%; mp (fumarate) 171−173 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.46 (s, 6H, 2 *gem*-CH_3), 1.76 (m, 4H, 3,4-pyrrolidine-H), 2.57 (m, 4H, 2,5-pyrrolidine-H), 2.97 [t, J = 7 Hz, 2H, $NCH_2CH_2N(CH_2)_4$], 4.33 [t, J = 7 Hz, 2H, $NCH_2CH_2N(CH_2)_4$], 5.60 (d, J = 10 Hz, 1H, H-10), 6.23 (s, 1H, H-7), 6.70 (d, J = 10 Hz, 1H, H-11), 7.11−7.32 (m, 3H, H-1, H-2, H-3), 7.78 (dd, J = 8, 1.5 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 23.47 (3,4-pyrrolidine-C), 27.69 (2 *gem*-CH_3), 48.01 [$NCH_2CH_2N(CH_2)_4$], 54.27 (2,5-pyrrolidine-H), 55.11 [$NCH_2CH_2N(CH_2)_4$], 76.71 (C-9), 89.00 (C-7), 100.17 (C-11a), 111.93 (C-11c), 115.56 (C-11), 118.19 (C-1), 118.44 (C-4a), 123.03 (C-4), 124.16 (C-3), 127.73 (C-10), 129.62 (C-2), 138.37 (C-4b), 139.87 (C-6a), 144.46 (C-7a), 154.67 (C-12a), 156.52 (C-11b). Anal. ($C_{24}H_{25}N_3O_2 \cdot C_4H_4O_4 \cdot {}^3/_4H_2O$) C, H, N.

Data for 6,9-Dihydro-9,9-dimethyl-6-(2-piperidin-1-ylethyl)pyrano[3,2-*f*](1)benzopyrano[4,3,2-*c,d*]indazole (11): yield 95%; mp (hydrochloride) 268−270 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.43 (s, 6H, 2 *gem*-CH_3), 1.54 (m, 6H, 3,4,5-piperidine-H), 2.47 (m, 4H, 2,6-piperidine-H), 2.80 [t, J = 7 Hz, 2H, $NCH_2CH_2N(CH_2)_5$], 4.31 [t, J = 7 Hz, 2H, $NCH_2CH_2N(CH_2)_5$], 5.61 (d, J = 10 Hz, 1H, H-10), 6.27 (s, 1H, H-7), 6.71 (d, J = 10 Hz, 1H, H-11), 7.10−7.32 (m, 3H, H-1, H-2, H-3), 7.83 (dd, J = 7, 1 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 24.17 (4-piperidine-C), 25.91 (3,5-piperidine-C), 27.71 (2 *gem*-CH_3), 47.26 [$NCH_2CH_2N(CH_2)_5$], 54.73 (2,6-piperidine-C), 58.06 [$NCH_2CH_2N(CH_2)_5$], 76.71 (C-9), 89.13 (C-7), 100.14 (C-11a), 111.08 (C-11c), 115.64 (C-11), 118.21 (C-1), 118.50 (C-4a), 123.04 (C-4), 124.18 (C-3), 127.72 (C-10), 129.61 (C-2), 138.30 (C-4b), 139.92 (C-6a), 144.50 (C-7a), 154.70 (C-12a), 156.44 (C-11b). Anal. ($C_{25}H_{27}N_3O_2 \cdot HCl \cdot 2H_2O$) C, H, N.

Data for Dimethyl-[2-[6,9-dihydro-1-methoxy-9,9-dimethylpyrano[3,2-*f*](1)benzopyrano[4,3,2-*c,d*]indazol-6-yl]ethyl]-amine (12): yield 97%; mp (fumarate) 182-184 °C dec (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.42 (s, 6H, 2 *gem*-CH_3), 2.32 [s, 6H, $N(CH_3)_2$], 2.82 [t, J = 7 Hz, 2H, $NCH_2CH_2N(CH_3)_2$], 3.95 (s, 3H, OCH_3), 4.31 [t, J = 7 Hz, 2H, $NCH_2CH_2N(CH_3)_2$], 5.60 (d, J = 10 Hz, 1H, H-10), 6.27 (s, 1H, H-7), 6.78 (d, J = 10 Hz, 1H, H-11), 6.91 (dd, J = 8, 2 Hz, 1H, H-2), 7.11 (t, J = 8 Hz, 1H, H-3), 7.43 (dd, J = 8, 2 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 27.64 (2 *gem*-CH_3), 46.92 [$NCH_2CH_2N(CH_3)_2$], 54.54 [$N(CH_3)_2$], 56.31 (OCH_3), 57.74 [$NCH_2CH_2N(CH_3)_2$], 76.73 (C-9), 89.15 (C-7), 100.48 (C-11a), 111.98 (C-11c), 112.73 (C-2), 114.96 (C-4), 115.88 (C-11), 119.11 (C-4a), 123.91 (C-3), 127.65 (C-10), 138.42 (C-4b), 139.90 (C-6a), 144.27 (C-7a), 144.30 (C-12a), 149.09 (C-1), 156.61 (C-11b). Anal. ($C_{23}H_{25}N_3O_3 \cdot C_4H_4O_4 \cdot {}^3/_2H_2O$) C, H, N.

Data for Diethyl-[2-[6,9-dihydro-1-methoxy-9,9-dimethylpyrano[3,2-*f*](1)benzopyrano[4,3,2-*c,d*]indazol-6-yl]ethyl]-amine (13): yield 97%; mp (fumarate) 174-176 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.01 [t, J = 7 Hz, 6H, $N(CH_2CH_3)_2$], 1.43 (s, 6H, 2 *gem*-CH_3), 2.63 [q, J = 7 Hz, 4H, $N(CH_2CH_3)_2$], 2.93 [t, J = 7 Hz, 2H, $NCH_2CH_2N(CH_2CH_3)_2$], 3.96 (s, 3H, OCH_3), 4.29 [t, J = 7 Hz, 2H, $NCH_2CH_2N(CH_2CH_3)_2$], 5.62 (d, J = 10 Hz, 1H, H-10), 6.30 (s, 1H, H-7), 6.80 (d, J = 10 Hz, 1H, H-11), 6.95 (dd, J = 8, 2 Hz, 1H, H-2), 7.12 (t, J = 8 Hz, 1H, H-3), 7.46 (dd, J = 8, 2 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 11.73 [$N(CH_2CH_3)_2$], 27.60 (2 *gem*-CH_3), 47.38 [$N(CH_2CH_3)_2$], 47.74 [$NCH_2CH_2N(CH_2CH_3)_2$], 52.01 [$NCH_2CH_2N(CH_2CH_3)_2$], 56.31 (OCH_3), 76.74 (C-9), 89.09 (C-7), 100.41 (C-11a), 111.99 (C-11c), 112.69 (C-2), 114.97 (C-4), 115.88 (C-11), 119.19 (C-4a), 123.90 (C-3), 127.61 (C-10), 138.38 (C-4b), 139.92 (C-6a), 144.26 (C-7a), 144.33 (C-12a), 149.11 (C-1), 156.53 (C-11b). Anal. ($C_{25}H_{29}N_3O_3 \cdot C_4H_4O_4 \cdot H_2O$) C, H, N.

Data for 6,9-Dihydro-1-methoxy-9,9-dimethyl-6-(2-pyrrolidin-1-yl-ethyl)pyrano[3,2-*f*](1)benzopyrano[4,3,2-*c,d*]-indazole (14): yield 95%; mp (fumarate) 228-230 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.47 (s, 6H, 2 *gem*-CH_3), 1.79 (m, 4H, 3,4-pyrrolidine-H), 2.66 (m, 4H, 2,5-pyrrolidine-H), 3.07 [t, J = 7 Hz, 2H, $NCH_2CH_2N(CH_2)_4$], 3.97 (s, 3H, OCH_3), 4.39 [t, J = 7 Hz, 2H, $NCH_2CH_2N(CH_2)_4$], 5.61 (d, J = 10 Hz, 1H, H-10), 6.30 (s, 1H, H-7), 6.79 (d, J = 10 Hz, 1H, H-11), 6.92 (dd, J = 8, 2 Hz, 1H, H-2), 7.11 (t, J = 8 Hz, 1H, H-3), 7.43 (dd, J = 8, 2 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 23.49 (3,4-pyrrolidine-C), 27.68 (2 *gem*-CH_3), 48.23 [$NCH_2CH_2N(CH_2)_4$], 54.14 (2,5-pyrrolidine-C), 54.89 [$NCH_2CH_2N(CH_2)_4$], 56.33 (OCH_3), 76.80 (C-9), 89.14 (C-7), 100.57 (C-11a), 111.90 (C-11c), 112.79 (C-2), 114.12 (C-4), 115.85 (C-11), 119.12 (C-4a), 123.92 (C-3), 127.71 (C-10), 138.57 (C-4b), 139.95 (C-6a), 144.25 (C-7a), 144.27 (C-12a), 149.12 (C-1), 156.71 (C-11b). Anal. ($C_{25}H_{27}N_3O_3 \cdot C_4H_4O_4 \cdot {}^3/_2H_2O$) C, H, N.

Data for 6,9-Dihydro-1-methoxy-9,9-dimethyl-6-(2-piperidin-1-ylethyl)pyrano[3,2-*f*](1)benzopyrano[4,3,2-*c,d*]-indazole (15): yield 96%; mp (hydrochloride) 175 °C dec (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.44 (s, 6H, 2 *gem*-CH_3), 1.62 (m, 6H, 3,4,5-piperidine-H), 2.49 (m, 4H, 2,6-piperidine-H), 2.87 [t, J = 7 Hz, 2H, $NCH_2CH_2N(CH_2)_5$], 3.98 (s, 3H, OCH_3), 4.33 [t, J = 7 Hz, 2H, $NCH_2CH_2N(CH_2)_5$], 5.61 (d, J = 10 Hz, 1H, H-10), 6.28 (s, 1H, H-7), 6.78 (d, J = 10 Hz, 1H, H-11), 6.90 (dd, J = 8, 2 Hz, 1H, H-2), 7.10 (t, J = 8 Hz, 1H, H-3), 7.42 (dd, J = 8, 2 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 23.95 (4-piperidine-C), 25.55 (3,5-piperidine-C), 27.61 (2 *gem*-CH_3), 46.87 [$NCH_2CH_2N(CH_2)_5$], 54.51 (2,6-piperidine-C), 56.26 (OCH_3), 57.69 [$NCH_2CH_2N(CH_2)_5$], 76.71 (C-9), 89.13 (C-7), 100.46 (C-11a), 111.95 (C-11c), 112.68 (C-2), 114.94 (C-4), 115.86 (C-11), 119.09 (C-4a), 123.89 (C-3), 127.62 (C-10), 138.37 (C-4b), 139.87 (C-6a), 144.21 (C-7a), 144.22 (C-12a), 149.07 (C-1), 156.59 (C-11b). Anal. ($C_{26}H_{29}N_3O_3 \cdot HCl \cdot 2H_2O$) C, H, N.

Data for Dimethyl-[2-[6,9-dihydro-9,9-dimethyl(1)benzothiopyrano[4,3,2-*c,d*]pyrano[3,2-*f*]indazol-6-yl]ethyl]amine (16): yield 93%; mp (fumarate) 204−206 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.42 (s, 6H, 2 *gem*-CH_3), 2.31 [s, 6H, $N(CH_3)_2$], 2.69 [t, J = 7 Hz, 2H, $NCH_2CH_2N(CH_3)_2$], 4.28 [t, J = 7 Hz, 2H, $NCH_2CH_2N(CH_3)_2$], 5.67 (d, J = 10 Hz, 1H, H-10), 6.32 (s, 1H, H-7), 6.34 (d, J = 10 Hz, 1H, H-11), 7.23−7.32 (m, 3H, H-1, H-2, H-3), 8.03 (dd, J = 7, 2 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 27.75 (2 *gem*-CH_3), 45.61 [$N(CH_3)_2$], 47.31 [NCH_2CH_2 $N(CH_3)_2$], 58.08 [NCH_2CH_2 $N(CH_3)_2$], 76.48 (C-9), 91.13 (C-7), 109.45 (C-11a), 115.94 (C-11c), 117.79 (C-11), 123.85 (C-4), 125.34 (C-4a), 125.85 (C-11b), 126.52 (C-1), 126.70

(C-3), 128.16 (C-2), 129.01 (C-10), 133.01 (C-12a), 140.14 (C-6a), 141.38 (C-4b), 154.72 (C-7a). Anal. ($C_{22}H_{23}N_3OS \cdot C_4H_4O_4 \cdot {}^1/_4H_2O$) C, H, N.

Data for Diethyl-[2-[6,9-dihydro-9,9-dimethyl(1)benzothiopyrano[4,3,2-*c*,*d*]pyrano[3,2-*f*]indazol-6-yl]ethyl]amine (17): yield 95%; mp (fumarate) 163−165 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.95 [t, J = 7 Hz, 6H, $N(CH_2CH_3)_2$], 1.45 (s, 6H, 2 *gem*-CH_3), 2.58 [q, J = 7 Hz, 4H, $N(CH_2CH_3)_2$], 2.92 [t, J = 7 Hz, 2H, $NCH_2CH_2N(CH_2CH_3)_2$], 4.22 [t, J = 7 Hz, 2H, $NCH_2CH_2N(CH_2CH_3)_2$], 5.63 (d, J = 10 Hz, 1H, H-10), 6.32 (s, 1H, H-7), 6.35 (d, J = 10 Hz, 1H, H-11), 7.13−7.30 (m, 3H, H-1, H-2, H-3), 8.05 (dd, J = 8, 2 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 11.93 [$N(CH_2CH_3)_2$], 27.72 (2 *gem*-CH_3), 47.49 [$N(CH_2CH_3)_2$], 47.67 [$NCH_2CH_2N(CH_2CH_3)_2$], 52.04 [$NCH_2CH_2N(CH_2CH_3)_2$], 76.38 (C-9), 91.34 (C-7), 109.42 (C-11a), 115.91 (C-11c), 117.82 (C-11), 123.82 (C-4), 125.22 (C-4a), 125.94 (C-11b), 126.52 (C-1), 126.70 (C-3), 128.13 (C-2), 129.04 (C-10), 133.01 (C-12a), 140.22 (C-6a), 141.29 (C-4b), 154.66 (C-7a). Anal. ($C_{24}H_{27}N_3OS \cdot C_4H_4O_4 \cdot {}^5/_4H_2O$) C, H, N.

Data for 6,9-Dihydro-9,9-dimethyl-6-(2-pyrrolidin-1-ylethyl)(1)benzothiopyrano[4,3,2-*c*,*d*]pyrano[3,2-*f*]indazole (18): yield 95%; mp (hydrochloride) 235−237 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.43 (s, 6H, 2 *gem*-CH_3), 1.77 (m, 4H, 3,4-pyrrolidine-H), 2.59 (m, 4H, 2,5-pyrrolidine-H), 2.98 [t, J = 7 Hz, 2H, $NCH_2CH_2N(CH_2)_4$], 4.32 [t, J = 7 Hz, 2H, $NCH_2CH_2N(CH_2)_4$], 5.64 (d, J = 10 Hz, 1H, H-10), 6.34 (d, J = 10 Hz, 1H, H-11), 6.35 (s, 1H, H-7), 7.10−7.27 (m, 3H, H-1, H-2, H-3), 8.03 (dd, J = 7, 2 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 23.42 (3,4-pyrrolidine-C), 27.75 (2 *gem*-CH_3), 47.80 [$NCH_2CH_2N(CH_2)_4$], 53.95 (2,5-pyrrolidine-C), 54.56 [$NCH_2CH_2N(CH_2)_4$], 76.48 (C-9), 91.28 (C-7), 109.50 (C-11a), 115.88 (C-11c), 117.73 (C-11), 123.85 (C-4), 125.22 (C-4a), 125,79 (C-11b), 126.56 (C-1), 126.70 (C-3), 128.21 (C-2), 129.13 (C-10), 133.04 (C-12a), 140.23 (C-6a), 141.50 (C-4b), 154.81 (C-7a). Anal. ($C_{24}H_{25}N_3OS \cdot HCl \cdot {}^1/_2H_2O$) C, H, N.

Data for 6,9-Dihydro-9,9-dimethyl-6-(2-piperidin-1-ylethyl)(1)benzothiopyrano[4,3,2-*c*,*d*]pyrano[3,2-*f*]indazole (19): yield 97%; mp (fumarate) 172−174 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.43 (s, 6H, 2 *gem*-CH_3), 1.56 (m, 6H, 3,4,5-piperidine-H), 2.46 (m, 4H, 2,6-piperidine-H), 2.79 [t, J = 7 Hz, 2H, $NCH_2CH_2N(CH_2)_5$], 4.29 [t, J = 7 Hz, 2H, $NCH_2CH_2N(CH_2)_5$], 5.64 (d, J = 10 Hz, 1H, H-10), 6.39 (s, 1H, H-7), 6.41 (d, J = 10 Hz, 1H, H-11), 7.16−7.26 (m, 3H, H-1, H-2, H-3), 8.02 (dd, J = 7, 1 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 24.19 (4-piperidine-C), 25.94 (3,5-piperidine-C), 27.73 (2 *gem*-CH_3), 47.04 [$NCH_2CH_2N(CH_2)_5$], 54.71 (2,6-piperidine-C), 57.86 [$NCH_2CH_2N(CH_2)_5$], 76.83 (C-9), 91.41 (C-7), 109.46 (C-11a), 115.96 (C-11c), 117.85 (C-11), 123.84 (C-4), 125.20 (C-4a), 125.95 (C-11b), 126.56 (C-1), 126.73 (C-3), 128.16 (C-2), 129.08 (C-10), 131.01 (C-12a), 140.21 (C-6a), 141.31 (C-4b), 154.67 (C-7a). Anal. ($C_{25}H_{27}N_3OS \cdot C_4H_4O_4 \cdot {}^5/_2H_2O$) C, H, N.

Data for Dimethyl-[2-[6,12-dihydro-9,9,12-trimethyl-9*H*-pyrano[2,3-*c*]pyrazolo[3,4,5-*m*,*n*]acridin-6-yl]ethyl]amine (20): yield 29%; mp (hydrochloride) 184−186 °C dec (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.44 (s, 6H, 2 *gem*-CH_3), 2.31 [s, 6H, $N(CH_3)_2$], 2.82 [t, J = 4.5 Hz, 2H, $NCH_2CH_2N(CH_3)_2$], 3.69 (s, 3H, NCH_3), 4.29 [t, J = 4.5 Hz, 2H, $NCH_2CH_2N(CH_3)_2$], 5.51 (d, J = 10 Hz, 1H, H-10), 6.17 (s, 1H, H-7), 6.70 (d, J = 10 Hz, 1H, H-11), 7.07 (t, J = 8 Hz, 1H, H-3), 7.11 (d, J = 8 Hz, 1H, H-1), 7.33 (td, J = 8, 1.5 Hz, 1H, H-2), 7.91 (dd, J = 8, 1.5 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 26.98 (2 *gem*-CH_3), 40.83 (NCH_3), 45.36 [$N(CH_3)_2$], 46.94 [$NCH_2CH_2N(CH_3)_2$], 57.89 [$NCH_2CH_2N(CH_3)_2$], 75.09 (C-9), 86.48 (C-7), 99.12 (C-11a), 114.05 (C-11c), 116.03 (C-1), 119.29 (C-4a), 120.96 (C-11), 121.87 (C-3), 122.68 (C-4), 124.74 (C-10), 129.11 (C-2), 136.83 (C-11b), 139.96 (C-6a), 141.17 (C-4b), 144.33 (C-12a), 157.64 (C-7a). Anal. ($C_{23}H_{26}N_4O \cdot HCl \cdot H_2O$) C, H, N.

Data for Diethyl-[2-[6,12-dihydro-9,9,12-trimethyl-9*H*-pyrano[2,3-*c*]pyrazolo[3,4,5-*m*,*n*]acridin-6-yl]ethyl]amine (21): yield 83%; mp (hydrochloride) 193−195 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.03 [t, J = 4 Hz, 6H, $N(CH_2CH_3)_2$], 1.44 (s, 6H, 2 *gem*-CH_3), 2.60 [q, J = 4 Hz, 4H, $N(CH_2CH_3)_2$], 2.92 [t, J = 4.5 Hz, 2H, $NCH_2CH_2N(CH_2CH_3)_2$], 3.64 (s, 3H, NCH_3), 4.23 [t, J = 4.5 Hz, 2H, $NCH_2CH_2N(CH_2CH_3)_2$], 5.51 (d, J = 10 Hz, 1H, H-10), 6.14 (s, 1H, H-7), 6.78 (d, J = 10 Hz, 1H, H-11), 7.06 (t, J = 8 Hz, 1H, H-3), 7.09 (d, J = 8 Hz, 1H, H-1), 7.31 (td, J = 8, 1.5 Hz, 1H, H-2), 7.91 (dd, J = 8, 1.5 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 12.05 [$N(CH_2CH_3)_2$], 26.97 (2 *gem*-CH_3), 40.83 (NCH_3), 47.55 [$NCH_2CH_2N(CH_2CH_3)_2$, $N(CH_2CH_3)_2$], 52.11 [$NCH_2CH_2N(CH_2CH_3)_2$], 75.04 (C-9), 86.61 (C-7), 99.00 (C-11a), 114.10 (C-11c), 116.01 (C-1), 119.29 (C-4a), 121.05 (C-11), 121.82 (C-3), 122.63 (C-4), 124.69 (C-10), 128.99 (C-2), 136.81 (C-11b), 140.12 (C-6a), 140.96 (C-4b), 144.31 (C-12a), 157.47 (C-7a). Anal. ($C_{25}H_{30}N_4O \cdot HCl \cdot {}^3/_2H_2O$) C, H, N.

Data for 6,12-Dihydro-9,9,12-trimethyl-6-(2-pyrrolidin-1-ylethyl)-9*H*-pyrano[2,3-*c*]pyrazolo[3,4,5-*m*,*n*]acridine (22): yield 97%; mp (hydrochloride) 230−232 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.48 (s, 6H, 2 *gem*-CH_3), 1.79 (m, 4H, 3,4-pyrrolidine-H), 2.60 (m, 4H, 2,5-pyrrolidine-H), 2.99 [t, J = 4.5 Hz, 2H, $NCH_2CH_2N(CH_2)_4$], 3.68 (s, 3H, NCH_3), 4.34 [t, J = 4.5 Hz, 2H, $NCH_2CH_2N(CH_2)_4$], 5.51 (d, J = 10 Hz, 1H, H-10), 6.20 (s, 1H, H-7), 6.70 (d, J = 10 Hz, 1H, H-11), 7.08 (t, J = 8 Hz, 1H, H-3), 7.11 (d, J = 8 Hz, 1H, H-1), 7.32 (td, J = 8, 1.5 Hz, 1H, H-2), 7.91 (dd, J = 8, 1.5 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 22.68 (3,4-pyrrolidine-H), 26.94 (2 *gem*-CH_3), 40.83 (NCH_3), 48.48 [$NCH_2CH_2N(CH_2)_4$], 54.32 (2,5-pyrrolidine-H), 55.09 [$NCH_2CH_2N(CH_2)_4$], 75.02 (C-9), 86.63 (C-7), 99.01 (C-11a), 114.12 (C-11c), 116.00 (C-1), 119.23 (C-4a), 120.99 (C-11), 121.84 (C-3), 122.68 (C-4), 124.70 (C-10), 129.00 (C-2), 136.83 (C-11b), 140.10 (C-6a), 140.99 (C-4b), 144.33 (C-12a), 157.53 (C-7a). Anal. ($C_{25}H_{28}N_4O \cdot HCl \cdot {}^1/_2H_2O$) C, H, N.

Data for 6,12-Dihydro-9,9,12-trimethyl-6-(2-piperidin-1-ylethyl)-9*H*-pyrano[2,3-*c*]pyrazolo[3,4,5-*m*,*n*]acridine (23): yield 68%; mp (hydrochloride) 204−206 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.46 (t, J = 3 Hz, 2H, 4-piperidine-H), 1.49 (6H, s, 2 *gem*-CH_3), 1.63 (m, 4H, 3,5-piperidine-H), 2.53 (t, J = 3 Hz, 4H, 2,6-piperidine-H), 2.86 [t, J = 4.5 Hz, 2H, $NCH_2CH_2N(CH_2)_5$], 3.73 (s, 3H, NCH_3), 4.35 [t, J = 4.5 Hz, 2H, $NCH_2CH_2N(CH_2)_5$], 5.56 (d, J = 10 Hz, 1H, H-10), 6.21 (s, 1H, H-7), 6.73 (d, J = 10 Hz, 1H, H-11), 7.12 (t, J = 8 Hz, 1H, H-3), 7.17 (d, J = 8 Hz, 1H, H-1), 7.37 (td, J = 8, 1.5 Hz, 1H, H-2), 7.96 (dd, J = 8, 1.5 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 23.93 (4-piperidine-C), 25.55 (3,5-piperidine-C), 26.95 (2 *gem*-CH_3), 40.84 (NCH_3), 46.50 [$NCH_2CH_2N(CH_2)_5$], 54.55 (2,6piperidine-C), 57.60 [$NCH_2CH_2N(CH_2)_5$], 75.02 (C-9), 86.70 (C-7), 99.09 (C-11a), 114.08 (C-11c), 116.03 (C-1), 119.15 (C-4a), 120.95 (C-11), 121.83 (C-3), 122.64 (C-4), 124.77 (C-10), 129.07 (C-2), 136.75 (C-11b), 140.10 (C-6a), 141.05 (C-4b), 144.32 (C-12a), 157.55 (C-7a). Anal. ($C_{26}H_{30}N_4O \cdot HCl \cdot H_2O$) C, H, N.

Data for Dimethyl-[2-[6,12-dihydro-3-methoxy-9,9,12-trimethyl-9*H*-pyrano[2,3-*c*]pyrazolo[3,4,5-*m*,*n*]acridin-6-yl]ethyl]amine (24): yield 50%; mp (hydrochloride) 181−184 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.46 (s, 6H, 2 *gem*-CH_3), 2.31 (s, 6H, $N(CH_3)_2$), 2.82 [t, J = 4.5 Hz, 2H, $NCH_2CH_2N(CH_3)_2$], 3.68 (s, 3H, NCH_3), 3.86 (s, 3H, 9-OCH_3), 4.30 [t, J = 4.5 Hz, 2H, $NCH_2CH_2N(CH_3)_2$], 5.50 (d, J = 10 Hz, 1H, H-10), 6.15 (s, 1H, H-7), 6.69 (d, J = 10 Hz, 1H, H-11), 6.92 (dd, J = 9, 3 Hz, 1H, H-2), 7.08 (d, J = 9 Hz, 1H, H-1), 7.42 (d, J = 3 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 26.94 (2 *gem*-CH_3), 40.90 (NCH_3), 45.53 [$N(CH_3)_2$], 47.19 [$NCH_2CH_2N(CH_3)_2$], 55.76 (OCH_3), 58.03 [$NCH_2CH_2N(CH_3)_2$], 75.09 (C-9), 85.89 (C-7), 98.61 (C-11a), 105.34 (C-4), 113.93 (C-11c), 116.99 (C-2), 117.39 (C-1), 119.67 (C-4a), 121.03 (C-11), 124.26 (C-10), 136.91 (C-6a), 138.49 (C-11b), 140.25 (C-12a), 141.21 (C-4b), 154.70 (C-3), 157.71 (C-7a). Anal. ($C_{24}H_{28}N_4O_2 \cdot HCl \cdot {}^3/_4H_2O$) C, H, N.

Data for Diethyl-[2-[6,12-dihydro-3-methoxy-9,9,12-trimethyl-9*H*-pyrano[2,3-*c*]pyrazolo[3,4,5-*m*,*n*]acridin-6-yl]ethyl]amine (25): yield 72%; mp (hydrochloride) 176−178 °C dec (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.09 [t, J = 4 Hz, 6H, $N(CH_2CH_3)_2$], 1.50 (s, 6H, 2 *gem*-CH_3), 2.68 [q, J = 4 Hz, 4H, $N(CH_2CH_3)_2$], 2.99 [t, J = 4.5 Hz, 2H, $NCH_2CH_2N(CH_2CH_3)_2$], 3.71 (s, 3H, NCH_3), 3.91 (s, 3H, 9-OCH_3), 4.30 [t, J = 4.5 Hz, 2H, $NCH_2CH_2N(CH_2CH_3)_2$], 5.53 (d, J = 10 Hz, 1H,

H-10), 6.20 (s, 1H, H-7), 6.73 (d, J = 10 Hz, 1H, H-11), 6.96 (dd, J = 9, 3 Hz, 1H, H-2), 7.11 (d, J = 9 Hz, 1H, H-1), 7.46 (d, J = 3 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 11.99 [$N(CH_2CH_3)_2$], 26.95 (2 *gem*-CH_3), 40.87 (NCH_3), 47.53 [$N(CH_2CH_3)_2$, $NCH_2CH_2N(CH_2CH_3)_2$], 52.08 [$NCH_2CH_2N(CH_2CH_3)_2$], 55.76 (OCH_3), 75.01 (C-9), 86.00 (C-7), 98.53 (C-11a), 105.26 (C-4), 110.88 (C-11c), 116.83 (C-2), 117.35 (C-1), 119.81 (C-4a), 121.10 (C-11), 124.26 (C-10), 136.86 (C-6a), 138.48 (C-11b), 140.25 (C-12a), 141.09 (C-4b), 154.69 (C-3), 157.59 (C-7a). Anal. ($C_{26}H_{32}N_4O_2 \cdot HCl \cdot {}^1/_2H_2O$) C, H, N.

Data for 6,12-Dihydro-3-methoxy-9,9,12-trimethyl-6-(2-pyrrolidin-1-ylethyl)-9*H*-pyrano[2,3-*c*]pyrazolo[3,4,5-*m*,*n*]-acridine (26): yield 53%; mp (hydrochloride) 179−182 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.48 (s, 6H, 2 *gem*-CH_3), 1.79 (m, 4H, 3,4-pyrrolidine-H), 2.60 (m, 4H, 2,5-pyrrolidine-H), 2.99 [t, J = 4.5 Hz, 2H, $NCH_2CH_2N(CH_2)_4$], 3.69 (s, 3H, NCH_3), 3.88 (s, 3H, 9-OCH_3), 4.34 [t, J = 4.5 Hz, 2H, $NCH_2CH_2N(CH_2)_4$], 5.50 (d, J = 10 Hz, 1H, H-10), 6.16 (s, 1H, H-7), 6.69 (d, J = 10 Hz, 1H, H-11), 6.92 (dd, J = 9, 3 Hz, 1H, H-2), 7.08 (d, J = 9 Hz, 1H, H-1), 7.42 (d, J = 3 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 23.45 (3,4-pyrrolidine-C), 26.94 (2 *gem*-CH_3), 40.87 (NCH_3), 47.49 [$NCH_2CH_2N(CH_2)_4$], 54.14 (2,5-pyrrolidine-C), 54.58 [$NCH_2CH_2N(CH_2)_4$], 55.72 (OCH_3), 75.05 (C-9), 86.19 (C-7), 98.79 (C-11a), 105.30 (C-4), 113.86 (C-11c), 116.99 (C-2), 117.43 (C-1), 119.60 (C-4a), 120.92 (C-11), 124.49 (C-10), 136.76 (C-6a), 138.52 (C-11b), 140.44 (C-12a), 141.50 (C-4b), 154.70 (C-3), 157.89 (C-7a). Anal. ($C_{26}H_{30}N_4O_2 \cdot HCl \cdot H_2O$) C, H, N.

Data for 6,12-Dihydro-3-methoxy-9,9,12-trimethyl-6-(2-piperidin-1-ylethyl)-9*H*-pyrano[2,3-*c*]pyrazolo[3,4,5-*m*,*n*]-acridine (27): yield 68%; mp (hydrochloride) 224−226 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.41 (t, J = 3 Hz, 2H, 4-piperidine-H), 1.47 (s, 6H, 2 *gem*-CH_3), 1.58 (t, J = 3 Hz, 4H, 3,5-piperidine-H), 2.50 (t, J = 3 Hz, 4H, 2,6-piperidine-H), 2.81 [t, J = 4.5 Hz, 2H, $NCH_2CH_2N(CH_2)_5$], 3.66 (s, 3H, NCH_3), 3.86 (s, 3H, 9-OCH_3), 4.31 [t, J = 4.5 Hz, 2H, $NCH_2CH_2N(CH_2)_5$], 5.50 (d, J = 10 Hz, 1H, H-10), 6.14 (s, 1H, H-7), 6.68 (d, J = 10 Hz, 1H, H-11), 6.91 (dd, J = 9, 3 Hz, 1H, H-2), 7.05 (d, J = 9 Hz, 1H, H-1), 7.41 (d, J = 3 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 24.18 (4-piperidine-C), 25.87 (3,5-piperidine-C), 26.94 (2 *gem*-CH_3), 40.87 (NCH_3), 46.90 [$NCH_2CH_2N(CH_2)_5$], 54.69 (2,6-piperidine-C), 55.72 (OCH_3), 57.89 [$NCH_2CH_2N(CH_2)_5$], 75.02 (C-9), 86.08 (C-7), 98.54 (C-11a), 105.23 (C-4), 113.97 (C-11c), 116.84 (C-2), 117.35 (C-1), 119.78 (C-4a), 121.07 (C-11), 124.26 (C-10), 136.83 (C-6a), 138.49 (C-11b), 140.25 (C-12a), 141.06 (C-4b), 154.66 (C-3), 157.56 (C-7a). Anal. ($C_{27}H_{32}N_4O_2 \cdot HCl \cdot {}^1/_4H_2O$) C, H, N.

3-Acetyloxy-1-hydroxy-9*H*-thioxanthen-9-one (32a). Acetic anhydride (284 mg, 3.0 mmol) was added to a stirred solution of 1,3-dihydroxy-9*H*-thioxanthen-9-one (**31**; 690 mg, 2.83 mmol)[5c] and a catalytic amount of triethylamine in dry dichloromethane. The mixture was stirred at room temperature for 4 h and then partitioned between dichloromethane and 1 N HCl. The organic phase was washed with water and brine, dried (Na_2SO_4), and concentrated to dryness. The residue was purified by flash chromatography eluting with a 65/35 cyclohexane/CH_2Cl_2 mixture to afford **32a** (760 mg, 94%): mp 146 °C (EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.31 (s, 3H, $COCH_3$), 6.47 (d, J = 1 Hz, 1H, H-2), 6.80 (d, J = 1 Hz, 1H, H-4), 7.51−7.78 (m, 3H, H-5, H-6, H-7), 8.41 (dd, J = 8, ∼0 Hz, 1H, H-8), 14.39 (s, 1H, D_2O exch, OH-1). Anal. ($C_{15}H_{10}O_4S$) C, H.

3-Benzyloxy-1-hydroxy-9*H*-thioxanthen-9-one (32b). To a solution of **31** (2 g, 8.2 mmol)[5c] in dry acetone (20 mL) were added, under argon, benzyl chloride (0.98 mL, 8.4 mmol), anhydrous sodium carbonate (1.71 g), and anhydrous sodium iodide (1.43 g), and the mixture was stirred at reflux temperature for 20 h. The solvent was then vacuum-evaporated, the residue was extracted with CH_2Cl_2–water, and the organic phase was dried (Na_2SO_4) and vacuum-evaporated. The residue was purified by column chromatography, using a mixture of cyclohexane/EtOAc (80/20) as the eluent, to give **32b** (2.57 g, 94%): mp 158−160 °C (EtOAc−Et_2O); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.16 (s, 2H, $CH_2C_6H_5$), 6.54 (d, J = 1 Hz, 1H, H-2), 6.67 (d, J = 1 Hz, 1H, H-4), 7.33−7.54 (m, 7H, H-5, H-6, $CH_2C_6H_5$), 7.61 (td, J = 8, ∼0 Hz, 1H, H-7), 8.56 (dd, J = 8, ∼0 Hz, 1H, H-8). Anal. ($C_{20}H_{14}O_3S$) C, H.

3-Acetyloxy-1-[[(4-methylphenyl)sulfonyl]oxy]-9*H*-thioxanthen-9-one (33a). To a solution of **32a** (1.14 g, 3.98 mmol) in dry acetone (30 mL) were added, under argon, *p*-toluenesulfonyl chloride (0.78 g, 4.1 mmol) and anhydrous sodium carbonate (0.64 g, 6 mmol), and the mixture was refluxed for 40 h. The solvent was then vacuum-evaporated, and the residue was partitioned between EtOAc and NaOH 10%. The organic phase was washed with water and brine, dried (Na_2SO_4), and concentrated to dryness. The residue was purified by flash chromatography eluting with a 70/30 cyclohexane/CH_2Cl_2 mixture to afford **33a** (1.63 g, 93%): mp 172−173 °C (EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.28 (s, 3H, $COCH_3$), 2.35 (s, 3H, CH_3), 6.88 (d, J = 2 Hz, 1H, H-4), 6.92 (d, J = 2 Hz, 1H, H-2), 7.18 (d, J = 8 Hz, 2H, 3,5-*p*-toluenesulfonyl-H), 7.36−7.41 (m, 2H, H-5, H-6), 7.46 (td, 1H, J = 8, ∼0 Hz, H-7), 7.88 (d, J = 8 Hz, 2H, 2,6-*p*-toluenesulfonyl-H), 8.32 (dd, 1H, J = 8, ∼0 Hz, H-8). Anal. ($C_{22}H_{16}O_6S_2$) C, H.

3-Benzyloxy-1-[[(4-methylphenyl)sulfonyl]oxy]-9*H*-thioxanthen-9-one (33b). This compound was prepared by a procedure analogous to that of **33a**: yield 94%; mp 208−210 °C (EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.37 (s, 3H, CH_3), 5.11 (s, 2H, $CH_2C_6H_5$), 6.92 (d, J = 2 Hz, 1H, H-4), 7.00 (d, J = 2 Hz, 1H, H-2), 7.24 (d, J = 8 Hz, 2H, 3,5-*p*-toluenesulfonyl-H), 7.36−7.44 (m, 7H, H-5, H-6, $CH_2C_6H_5$), 7.54 (td, J = 8, ∼0 Hz, 1H, H-7), 7.91 (d, J = 8 Hz, 2H, 2,6-*p*-toluenesulfonyl-H), 8.32 (dd, J = 8, ∼0 Hz, 1H, H-8). Anal. ($C_{27}H_{20}O_5S_2$) C, H.

2-[4-Benzyloxy-2*H*-(1)benzothiopyrano[4,3,2-*c*,*d*]indazol-2-yl]-1-ethanol (35). To a solution of **33b** (1.5 g, 18 mmol) in dry DMSO (20 mL) was added 2-hydroxyethylhydrazine (1.22 mL, 18 mmol), and the mixture was heated, under argon, at 150 °C for 3 h. Upon cooling, the mixture was poured into water and extracted with CH_2Cl_2, the organic phase was dried (Na_2SO_4), and the solvent was vacuum-evaporated. The residue was purified by column chromatography, using a mixture of cyclohexane/EtOAc (60/40 to 40/60) as the eluent, to give **35** (0.89 g, 78%): mp 142−144 °C (EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.28 (t, 1H, D_2O exch, OH), 4.11 (t, J = 5 Hz, 2H, NCH_2CH_2), 4.32 (t, J = 5 Hz, 2H, NCH_2CH_2), 5.09 (s, 2H, $CH_2C_6H_5$), 6.42 (d, J = 0.5 Hz, 1H, H-5), 6.56 (d, J = 0.5 Hz, 1H, H-3), 7.25−7.48 (m, 8H, H-7, H-8, H-9, $CH_2C_6H_5$), 8.32 (dd, J = 8, ∼0 Hz, 1H, H-10). Anal. ($C_{22}H_{18}N_2O_2S$) C, H, N.

1-[[(4-Methylphenyl)sulfonyl]oxy]-3-hydroxy-9*H*-thioxanthen-9-one (34a). This compound was prepared by a procedure analogous to that of **35**, starting from **33a**: yield 76%; mp 182 °C (EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.38 (s, 3H, CH_3), 6.78 (d, J = 2 Hz, 1H, H-4), 6.90 (d, 1H, J = 2 Hz, H-2), 7.20 (d, J = 8 Hz, 2H, 3,5-*p*-toluenesulfonyl-H), 7.36−7.44 (m, 2H, H-5, H-6), 7.55 (td, J = 8, ∼0 Hz, 1H, H-7), 7.89 (d, J = 8 Hz, 2H, 2,6-*p*-toluenesulfonyl-H), 8.32 (dd, 1H, J = 8, ∼0 Hz, H-8), 11.05 (s, 1H, D_2O exch, OH). Anal. ($C_{20}H_{14}O_5S_2$) C, H.

2-[4-Hydroxy-2*H*-(1)benzothiopyrano[4,3,2-*c*,*d*]indazol-2-yl]-1-ethanol (34b). Boron trichloride (1.5 mL, 1 M in dichloromethane) was added dropwise under argon to a stirred solution of **35** (140 mg, 0.374 mmol) in dry dichloromethane (20 mL) with cooling to 0 °C. After 45 min, the cooling bath was removed, and ethanol (1.5 mL) was added dropwise. The reaction mixture was concentrated under reduced pressure, and the residue was purified by flash chromatography eluting with a 30/70 cyclohexane/EtOAc mixture to afford **34b** (82 mg, 77%): mp 223−225 °C dec (EtOH); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.71 (t, J = 5 Hz, 2H, NCH_2CH_2), 4.22 (t, J = 5 Hz, 2H, NCH_2CH_2), 6.39 (d, J = 0.3 Hz, 1H, H-5), 6.41 (d, J = 0.3 Hz, 1H, H-3), 7.27−7.31 (m, 2H, H-7, H-8), 7.36 (td, J = 8, ∼0 Hz, 1H, H-9), 7.89 (dd, 1H, J = 8, ∼0 Hz, H-10). Anal. ($C_{15}H_{12}N_2O_2S$) C, H, N.

2-[4-Benzyloxy-2*H*-(1)benzothiopyrano[4,3,2-*c*,*d*]indazol-2-yl]-1-ethanol Methanesulfonate (36a). Methanesulfonyl chloride (773 μL, 10 mmol) was added to a stirred solution of **35** (1.16 g, 3.1 mmol) and triethylamine (1.58 mL, 11.3 mmol) in dry dichloromethane (60 mL) with cooling to 0 °C.

After 30 min, the cooling bath was removed, and the mixture was stirred at room temperature for 4 h. The reaction mixture was partitioned between dichloromethane and 1 N sodium hydroxide. The organic phase was washed with HCl (10%), water, and brine, then dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography eluting with a 50/50 cyclohexane/EtOAc mixture to afford compound **36a** (1.3 g, 93%): mp 153−155 °C (Et_2O−*n*-pentane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.73 (s, 3H, SO_2CH_3), 4.42 (t, J = 6 Hz, 2H, NCH_2CH_2), 4.63 (t, J = 6 Hz, 2H, NCH_2CH_2), 5.11 (s, 2H, $CH_2C_6H_5$), 6.41 (d, J = 0.5 Hz, 1H, H-5), 6.55 (d, J = 0.5 Hz, 1H, H-3), 7.25−7.48 (m, 8H, H-7, H-8, H-9, $CH_2C_6H_5$), 8.30 (dd, J = 8, 0.2 Hz, 1H, H-10). Anal. ($C_{23}H_{20}N_2O_4S_2$) C, H, N.

2-[4-Hydroxy-2*H*-(1)benzothiopyrano[4,3,2-*c*,*d*]indazol-2-yl]-1-ethanol Methanesulfonate (36b). This compound was prepared by a procedure analogous to that of **34b**: yield 74%; mp 186−188 °C (EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.75 (s, 3H, SO_2CH_3), 4.40 (t, J = 6 Hz, 2H, NCH_2CH_2), 4.59 (t, J = 6 Hz, 2H, NCH_2CH_2), 6.40 (d, J = 0.2 Hz, 1H, H-5), 6.41 (d, J = 0.2 Hz, 1H, H-3), 7.2−7.3 (m, 2H, H-7, H-8), 7.30 (td, J = 8, 0.1 Hz, 1H, H-9), 7.91 (dd, J = 8, 0.1 Hz, 1H, H-10), 10.95 (s, 1H, D_2O exch, OH). Anal. ($C_{16}H_{14}N_2O_4S_2$) C, H, N.

General Procedure for the Preparation of Fumarates. To a stirred solution of the amine in anhydrous ethanol was added a slight excess (5%) of fumaric acid. The resulting solution was stirred at reflux temperature for 18−43 h and then allowed to cool at room temperature. The solid was collected by filtration, washed with absolute ethanol and diethyl ether, and dried under vacuum (yield 62−80%).

DNA-Binding Assay. An ethidium bromide displacement assay was used to determine DNA-binding potency.[13] Briefly, the new compounds were added to a 5 mM Tris−HCl−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), and fluorescence emission was counted at 600 nm after excitation at 525 nm. The results represent the mean of two individual experiments 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.

Cell Culture and Assessment of Cytotoxicity. The new compounds were tested for their cytotoxic activity on the following human solid tumor cell lines: mammary adenocarcinoma MDA-MB-231 (American Type Culture Collection, Rockville, MD) and lung carcinoma A549, colorectal adenocarcinoma HT-29, colorectal carcinoma HCT 116, and ileocecal colorectal adenocarcinoma HRT-18 (European Collection of Cell Cultures, Salisbury, U.K.). Furthermore, the compounds were tested for cytotoxicity on the murine leukemia cell line L1210, provided by the NCI (Frederick, MD). All human cell lines were cultured in Dulbecco's minimal essential medium 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, and the cells were routinely subcultured using a trypsin 0.25%−EDTA 0.02% solution. L1210 cells were cultured in RPMI 1640 medium (Gibco BRL, Paisley, U.K.) supplemented with antibiotics and serum (see above), as well as with 10 mM HEPES buffer (pH 7.4). The cytotoxicity assay was performed by a modification of the MTT method.[14] Briefly, the cells were plated at a density of approximately 5000 cells/well in 96-well flat-bottomed microplates, and after 24 h the fractions to be tested were added, appropriately diluted with DMSO. After a 48 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). Ellipticine, acronycine, and mitoxantrone were included in the experiments as positive controls. The results represent the mean of three independent experiments and are expressed as IC_{50}, the concentration that reduced by 50% the optical density of treated cells with respect to untreated controls.

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References

(1) (a) Svodova, G. H.; Poore, G. H.; Simpson, P. J.; Boder, G. B. Alcaloids of Acronychia baueri Schott I. *Int. J. Pharm. Sci.* **1966**, *55*, 758−768. (b) Dorr, R. T.; Liddil, J. D.; Von Hoff, D. D.; Soble, M.; Osborne, C. K. Antitumor activity and murine pharmacokinetics of parenteral acronycine. *Cancer Res.* **1989**, *49*, 340−344. (c) Tillequin, F.; Michel, S.; Skaltsounis, A.-L. Acronycine-type alkaloids: Chemistry and biology. In *Alcaloids*: *Chemical and Biological Perspectives*; Pelletier, S. W., Ed.; Elsevier: Amsterdam, 1998; Vol. 12, pp 1−102.

(2) Scarffe, J. H.; Beaumont, A. R.; Crowther, D. Phase I-II evaluation of acronycine in patiens with multiple myeloma. *Cancer Treat. Rep*. **1983**, *67*, 93−94.

(3) Su, T.-L.; Watanabe, K. A. Anticancer acridone alkaloids. In *Studies in Natural Products Chemistry*, Atta-ur-Rahmann, Ed.; Elsevier: Amsterdam, 1993; Vol. 13, pp 347−382.

(4) (a) Shieh, H.-L.; Pezzuto, J. M.; Cordell, G. A. Evaluation and cytotoxic mechanisms mediated by the broad-spectrum antitumor alkaloid acronycine and selected semisynthetic derivatives. *Chem.-Biol. Interact.* **1992**, *81*, 35−55. (b) Elombri, A.; Mitaku, S.; Michel, S.; Skaltsounis, A.-L.; Tillequin, F.; Koch, M.; Pierre, A.; Guilbaud, N.; Leonce, S.; Kraus-Berthier, L.; Rolland, Y.; Atassi, G. Synthesis and cytotoxic and antitumor activity of esters in the 1,2-dihydroxy-1,2-dihydroacronycine series. *J. Med. Chem*. **1996**, *39*, 4762−4766. (c) Costes, N.; Le Deit, H.; Michel, S.; Tillequin, F.; Koch, M.; Pfeiffer, B.; Renard, P.; Leonce, S.; Guilbaud, N.; Kraus-Berthier, L.; Pierre, A.; Atassi, G. Synthesis and cytotoxic and antitumor activity of benzo[*b*]pyrano[3,2-*h*]-acridin-7-one analogues of acronycine. *J. Med. Chem*. **2000**, *43*, 2395−2402.

(5) (a) Ghirtis, K.; Pouli, N.; Marakos, P.; Skaltsounis, A.-L.; Leonce, S.; Gaignard, D. H.; Atassi, G. Synthesis and conformational analysis of some new pyrano[2,3-*c*]xanthen-7-one and pyrano-[3,2-*b*]xanthen-6-one derivatives with cytotoxic activity. *Heterocycles* **2000**, *53*, 93−106. (b) Ghirtis, K.; Pouli, N.; Marakos, P.; Skaltsounis, A.-L.; Leonce, S.; Atassi, G.; Gaignard, D. H. Design and synthesis of some new pyranoxanthenones with cytotoxic activity. *J. Heterocycl. Chem*. **2001**, *38*, 147−152. (c) Kostakis, I. K.; Pouli, N.; Marakos, P.; Mikros, E.; Skaltsounis, A.-L.; Leonce, S.; Atassi, G.; Renard, P. Synthesis, cytotoxic activity, NMR study and stereochemical effects of some new pyrano[3,2-*b*]thioxanthen-6-ones and pyrano[2,3-*c*]thioxanthen-7-ones. *Bioorg. Med. Chem*. **2001**, *9*, 2793−2802.

(6) Faulds, D.; Balfour, J. A.; Chrisp P.; Langtry H. D. Mitoxantrone, a review of its pharmacodynamic and pharmacokinetic properties and therapeutic potential in the treatment of cancer. *Drugs* **1991**, *41*, 400−449.

(7) (a) Rosenberg, L. S.; Carvlin, M. J.; Krugh, T. R. The antitumor agent mitoxantrone binds cooperatively to DNA: Evidence for heterogeneity in DNA conformation. *Biochemistry* **1985**, *24*, 4028−4085 (b) Islam, S. A.; Neidle, S.; Gandecha, B. M.; Partridge, M.; Paterson, L. H.; Brown, J. R. Comparative computer graphics and solution studies of the DNA interaction of substituted anthraquinones based on doxorubicin and mitoxantrone. *J. Med. Chem*. **1985**, *28*, 857−854. (c) Collier, D. A.; Neidle, S. Synthesis, molecular modeling, DNA binding, and antitumor properties of some substituted amidoanthraquinones. *J. Med. Chem*. **1988**, *31*, 847−857. (d) Denny, W. A.; Wakelin, L. P. G. Kinetics of the binding of mitoxantrone, ametantrone and analogs to DNA: Relationship with binding mode and antitumor activity. *Anti-Cancer Drug Des.* **1990**, *5*, 189−200.

(8) (a) Krapcho, A. P.; Maresch, M. E.; Hacker, M. P.; Hazelhurst, L. A.; Menta, E.; Oliva, A.; Spinelli, S.; Beggiolin, G.; Giuliani, F. C.; Pezzoni, G.; Tognella, S. Anthracene-9,10-diones and aza bioisosteres as antitumor agents. *Curr. Med. Chem*. **1995**, *2*, 803−824. (b) Hazlehurst, L. A.; Krapcho, A. P.; Hacker, M. P. Correlation of DNA reactivity and cytotoxicity of a new class of anticancer agents: aza-anthracenediones. *Cancer Lett*. **1995**, *91*, 115−124.

(9) (a) Cheng, C. C.; Zee-Cheng, R. K. Y. The design, synthesis and development of a new class of potent antineoplastic anthraquinones. In *Progress in Medicinal Chemisty*; Ellis, G. P., West, G. P., Eds.; Elsevier Science Publishers, B.V.: New York, 1989; pp 83−118. (b) Krapcho, A. P.; Menta, E.; Oliva, A.; Di Domenico,

R.; Fiocchi, L.; Maresch, M. E.; Gallagher, G. E.; Hacker, M. P.; Beggiolin, G.; Giuliani, F. C.; Pezzoni, G.; Spinelli, S. Synthesis and antitumor evaluation of 2,5-disubstituted-indazolo[4,3-*gh*]-isoquinolin-6(2*H*)-ones (9-aza-anthrapyrazoles). *J. Med. Chem.* **1998**, *41*, 5429−5444. (c) Judson, I. R. Anthrapyrazoles: True successors to the anthracyclines? *Anti-Cancer Drugs* **1991**, *2*, 223−231.

(10) (a) Showalter, H. D.; Angelo, M. M.; Berman, E. M.; Kanter, G. D.; Ortwine, D. F.; Ross-Kesten, S. G.; Sercel, A. D.; Turner, W. R.; Werbel, L. M.; Worth, D. F.; Elslager, E. F.; Leopold, W. R.; Shillis, J. L. Benzothiopyranoindazoles, a new class of chromophore modified anthracenedione anticancer agents. Synthesis and activity against murine leukemia. *J. Med. Chem.* **1988**, *31*, 1527−1539. (b) Capps, D. B.; Dunbar, J.; Kesten, S. R.; Shillis, J.; Werbel, L. M.; Plowman, J.; Ward, D. L. 2-(Aminoalkyl)-5-nitropyrazolo[3,4,5-*kl*]acridines, a new class of anticancer agents. *J. Med. Chem.* **1992**, *35*, 4770−4778. (c) Wentland, M. P.; Perni, R. B.; Huang, J. I.; Powles, R. G.; Aldous, S. C.; Klingbeil, K. M.; Peverly, D. A.; Robinson, R. G.; Corbett, T. H.; Jones, J. L.; Rake, J. B.; Coughlin, S. A. Cyclic variations of SR 233377 (WIN 33377) and effects on antitumor activity. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1345−1350. (d) Antonini, I.; Polucci, P.; Magnano, A.; Martelli, S. Synthesis, antitumor cytotoxicity, and DNA-binding of novel *N*-5,2-Di(*ω*-aminoalkyl)-2,6-dihydropyrazolo-[3,4,5-*kl*]acridine-5carboxamides. *J. Med. Chem.* **2001**, *44*, 3329−3333.

(11) Kostakis, I. K.; Ghirtis, K.; Pouli, N.; Marakos, P.; Skaltsounis, A. L.; Leonce, S.; Gaignard, D. H.; Atassi, G. Synthesis and cytotoxic activity of 2-dialkylaminoethylamino substituted xanthenone and thioxanthenone derivatives. *Il Farmaco* **2000**, *55*, 455−460.

(12) Hlubucek, J. R.; Ritchie, E.; Taylor, W. C. Synthesis of acronycine. *Aust. J. Chem.* **1970**, *23*, 1881−1889.

(13) Morgan, A. R.; Lee, J. S.; Pulleyblank, D. S.; Murray, N. L.; Evans, D. H. Ethidium fluorescence assays. Part 1. Physicochemical studies. *Nucleic Acids Res.* **1979**, *7*, 547−569.

(14) (a) Denizot, F.; Lang, R. Rapid colorimetric assay for cell growth and survival: Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. *J. Immunol. Methods* **1986**, *89*, 271−277. (b) Magiatis, P.; Pratsinis, H.; Kalpoutzakis, E.; Konstantinidou, A.; Davaris, P.; Skaltsounis, A.-L. Hydrolyzable tannins, the active constituents of three greek Cytinus taxa against several tumor cell lines. *Biol. Pharm. Bull.* **2001**, *24*, 707−709.

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