2-[2′-(Dimethylamino)ethyl]-1,2-dihydro-3*H*-dibenz[*de,h*]isoquinoline-1,3-diones with Substituents at Positions 4, 8, 9, 10, and 11. Synthesis, Antitumor Activity, and Quantitative Structure−Activity Relationships

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New 2-[2′-(dimethylamino)ethyl]-1,2-dihydro-3*H*-dibenz[*de*,*h*]isoquinoline-1,3-diones with substituents at the 4, 8, 9, 10, and 11 positions were synthesized. Diazonium salts prepared from aminoazonafides were key intermediates for many of the analogues. Six of the new compounds were more potent than azonafide in a panel of tumor cells including human melanoma and ovarian carcinoma and murine L1210 leukemias. Three of these compounds, the 10-OCH_3, 10-OC_2H_5, and 10-F analogues, had better ratios of cardiotoxicity to tumor-cell toxicity than azonafide. Eight compounds were not cross-resistant with MDR L1210 leukemia, and the 10-CN analogue was more potent against solid tumor cells than leukemia cells. The 9-OH, 10-CN, and 10-F analogues had high potency against both sensitive and resistant cell lines of MFX 7 breast carcinoma and WiDr colon carcinoma and sensitivity A599 lung carcinoma. Advantages of the 10-Cl, 10-NH_2, and 10-CN analogues over azonafide were apparent in P388 leukemia in mice, and the 10-CN analogue was more effective than doxorubicin in this assay. Quantitative structure−activity relationship studies revealed statistically significant correlations between DNA binding strength of 8- and 10-substituted azonafides, as measured by ΔT_m, and toxicity to tumor cells. There also were correlations between substituent size, as measured by MR, and cytotoxicity for 9- and 10-substituted azonafides and between MR and ΔT_m for 4- and 11-substituted azonafides. Lipophilicity of substituents (π) correlated with cytotoxicity for 9-, 10-, and 11-substituted azonafides. These results lend support to a model in which DNA binding strength influences cytotoxic potency, and lipophilicity increases DNA binding whereas large substituents decrease it.

Azonafide, 2-[2′-(dimethylamino)ethyl]-1,2-dihydro-3*H*-dibenz[*de*,*h*]isoquinoline-1,3-dione (**2**), is a potential antitumor agent whose structure is related to amonafide (**1**).[2,3] Previous articles in this series have discussed the synthesis and antitumor activities of azonafide analogues with structural variation in the side chain,[1] the position of substituents (amino and acetylamino) on the nucleus,[4] and the nature of different substituents at C6 and C7.[5] Quantitative structure−activity relationship (QSAR) studies revealed generally good correlations between potency against tumor cells or cardiotoxicity and DNA binding strength as measured by increases in melt transition temperatures (ΔT_m).[4,5] Synthetic methods were developed based on regioselective oxalylation of substituted anthracenes and facile nucleophilic aromatic displacements in 6- and 7-substituted azonafides.[5]

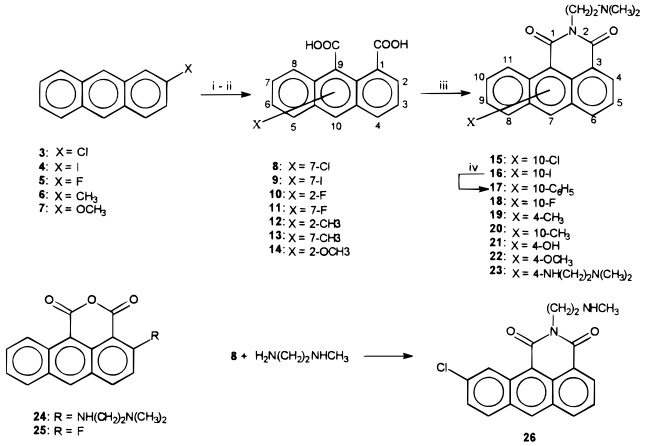
The present article is concerned with the synthesis and biological activity of azonafide analogues in which a variety of new substituents are present at nuclear positions C4, C8, C9, C10, and C11.

New analogues with C5 substituents were not prepared for this study because previous ones required long routes involving tetrahydroanthracene intermediates and separation of isomers.[4] Improved routes are being explored.

Chemistry

A variety of 4- and 10-substituted azonafides **15**, **16**, and **18**−**23** (Scheme 1) were synthesized from 2-substituted anthracenes (**3**−**7**) by way of the corresponding 2- or 7-substituted anthracene-1,9-dicarboxylic acids (**8**−**14**) following our standard route.[1] Thus, treatment of the anthracenes with oxalyl chloride and $AlCl_3$ in CS_2, followed by alkaline hydrogen peroxide, gave the dicarboxylic acids, which were converted into the azonafide analogues with *N*,*N*-dimethylethylenediamines.

Scheme 1[a]



[a] (i) $(CO)_2Cl_2$, $AlCl_3$, CS_2; (ii) 30% H_2O_2, NaOH; (iii) $H_2N{\cdot}(CH_2)_2N(CH_3)_2$; (iv) phenylboronic acid, $[P(Ph)_3]_4Pd(0)$, $NaHCO_3$, $(CH_2)_2(OCH_3)_2$, (H_2O, △).

An anomalous reaction occurred when 2-methoxyanthracene-1,9-dicarboxylic acid (**14**) was heated with

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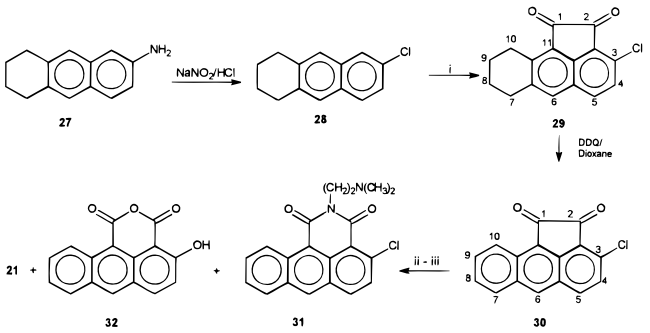
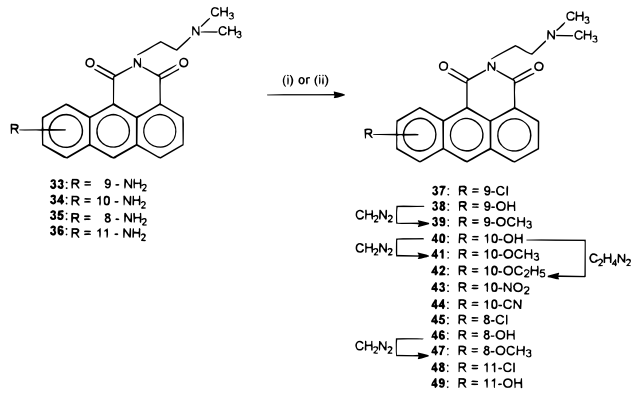
Scheme 2[a]



[a] Reagents are the same as those in Scheme 1.

Scheme 3[a]



[a] (i) (a) $NaNO_2$/HCl, 0 °C; (i) (b) $NaNO_2$/HCl, then Cu_2Cl_2; (ii) $NOHSO_4$/ACOH, 10 °C; then $NaNO_2$ or NaCN.

N,*N*-dimethylethylenediamine in dry toluene. In addition to the expected 4-methoxyazonafide (**22**), 4-hydroxyazonafide (**21**) and 4-[[(dimethylamino)ethyl]amino]-azonafide (**23**) were obtained. The yields were 2%, 45%, and 5%, respectively. The PMR spectrum of **21** showed long-distance coupling between $CONCH_2$ in the side and the 4-OH group. Deuterium exchange reduced the $CONCH_2$ from a quartet to a triplet, and irradiation of the $CONCH_2$ quartet reduced the 4-OH from a triplet to a singlet and the adjacent CH_2 group also became a singlet. Formation of **23** can be explained by displacement of the 4-methoxy group by *N*,*N*-dimethylethylenediamine. The 4-position is activated by its ortho relationship to the 3-carbonyl group. Formation of **21** probably results from reaction of **22** with the water molecules that are liberated in imide formation. Another example of nucleophilic displacement at the 4-position occurred when 4-fluoroanthracene-1,9-dicarboxylic acid anhydride (**25**) was treated with *N*,*N*-dimethylethylenediamine. In this example, the 4-fluorine was displaced without any imide formation to give **24**.

10-Phenylazonafide (**17**) was prepared in 79% yield by treating 10-iodoazonafide (**16**) with phenylboronic acid and tetrakis(triphenylphosphine)palladium(0).[6]

The methylamino analogue **26** of 10-chloroazonafide (**15**) was prepared by treating intermediate **8** with *N*-methylethylenediamine.

A route for the synthesis of 4-chloroazonafide (**31**) was based on 7-amino-1,2,3,4-tetrahydroanthracene (**27**), which had been prepared previously in eight steps from cyclohexane-1,2-dicarboxylic acid anhydride.[4] Conversion of **27** into a diazonium salt, followed by treatment with cuprous chloride in HCl, gave 2-chloro derivative **28** (Scheme 2). The oxalylation of **28** was regioselective, affording dione **29** in 50% yield. Dehydrogenation of **29** by 2,3-dicyano-5,6-dichloro-1,4-benzoquinone to furnish **30**, followed by oxidation with alkaline hydrogen peroxide, gave crude 2-chloroanthracene-1,9-decarboxylic acid, which was treated directly with *N*,*N*-dimethylethylenediamine in dry toluene. The desired product **31** was obtained in 18% yield, and the previously described anomalous product, 4-hydroxyazonafide (**21**), was obtained in 25% yield. A third product (3% yield), possibly 2-hydroxyanthracene-1,9-dicarboxylic acid anhydride (**32**) according to its NMR spectrum, was not characterized further.

New substituents at positions 8, 9, 10, and 11 of azonafide were prepared by way of diazonium ions from the corresponding amines, which were described previously.[4] From 10-aminoazonafide (**34**) was obtained 10-hydroxyazonafide (**40**) together with some 10-chloroazonafide (**15**), 10-nitroazonafide (**43**), and 10-cyanoazonafide (**44**) (Scheme 3). Warming the diazonium ions from 9-aminoazonafide (**33**) and 8-aminoazonafide (**35**) with HCl gave in each case mixtures of the corresponding chloro and hydroxy analogues **37** and **38**, **45** and **46**, which were readily separated by preparative thin-layer chromatography. Treatment of **36** under the same conditions gave only 11-hydroxyazonafide (**49**); however, when cuprous chloride was added, a mixture of **49** and 11-chloroazonafide (**48**) was obtained. This difference in behavior between the diazonium salt of **36** and the behaviors of **33** and **35** might be caused by steric hindrance at C11 by the 1-carbonyl group. Treatment of hydroxyazonafides **38**, **40**, and **46** with diazomethane gave the corresponding methoxy derivatives **39**, **41**, and **47**. 11-Hydroxyazonafide (**49**) failed to react with diazomethane probably because of steric hindrance from the 1-carbonyl group. 10-Ethoxyazonafide (**42**) was prepared by treating **40** with diazoethane.

Biology

The potencies of new azonafide analogues are compared with those of azonafide, amonafide, doxorubicin, and mitoxantrone in Tables 1 and 2. In Table 1 the human tumor-cell lines include a melanoma and an ovarian carcinoma that is resistant to standard antitumor drugs. Murine L1210 leukemia includes a sensitive cell line and one that has multiple drug resistance based on increased levels of P-glycoproteins. The sulforhodamine[7] or the MTT[8] assay with continuous drug exposure was used, and IC_{50} values were determined. The average IC_{50} values for sensitive L1210 leukemia and the two human tumors are given as an overall index of the relative potencies of the azonafide analogues. Also listed in Table 1 are IC_{50} values for the relative cardiotoxicity of azonafide analogues as determined by the neonatal rat heart myocyte assay.[9] A toxicity ratio, determined by the quotient of IC_{50} values in the myocytes divided by the average value in three tumor lines, is included. This kind of ratio has been used previously to compare the relative therapeutic indices of anthracycline antitumor agents.[10]

As indicated in Table 1, potencies against tumor cells are highly dependent on both the nature and location of the nuclear substituent. For example, the average IC_{50} values of compounds with OH substituents vary from 10 nM for the 9-OH analogue **38** to 6596 nM for

Table 1. Activity of 2-[2′-(Dimethylamino)ethyl]-1,2-dihydro-3*H*-dibenz[*de,h*]isoquinoline-1,3-diones against Tumor Cells[a] and Myocytes[b] in Culture

		IC_{50}nM							
compd	substituent	melanoma UACC375[d]	ovarian OVCAR3[e]	solid av	L1210 sens	L1210 MDR[f]	3-tumor av[g]	cardiotox	toxicity[c] ratio
19	4-CH_3	176	122	149	54	68	117	1357	12
31	4-Cl	332	180	256	10	15	174	900	5.2
21	4-OH	7547	5500	6524	6739	14825	6595	>134952	>20
22	4-OCH_3	182	507	345	70	179	253		
23	4-$NH(CH_2)_2N(CH_3)_2$	390	715	553	210	245	439	734	1.7
45	8-Cl	77	39	58		18	45	1028	22.8
46	8-OH	270	216	243	108	67	198	2949	15
47	8-OCH_3	52	52	52	13	18	39	806	21
37	9-Cl	90	231	161	7.7	7.7	110	1542	14
38	9-OH	4.0	22	13	5.4	6.7	10	404	40.4
39	9-OCH_3	163	543	353	54	54	253	<2597	<10.3
20	10-CH_3	81	68	75	41	54	63	1004	16
17	10-C_6H_5	1858	348	1103	70	128	759		
44	10-CN	10	12	11	15	13	12	105	9
18	10-F	16	6.7	11	8.1		10	456	45.6
15	10-Cl	64	64	64	5.1	7.7	44	771	18
26	10-Cl,DM[h]	44	102	73	5.8	14.6	51	1430	28
16	10-I	624	176	400	312	52	371	4787	13
40	10-OH	1215	2126	1671	189	540	1177	>26990	>23
41	10-OCH_3	52	78	65	21	52	50	9103	182
42	10-OC_2H_5	77	77	77	25	50	60	11292	188
43	10-NO_2	18	23	21	25	25	22	801	36
48	11-Cl	2056	8997	5527	1779	617	4278	8226	1.9
49	11-OH	540	1887	1214	189	189	872	15424	18
doxorubicin		112	35	74	35	3884	61	10151	166
mitoxantrone		48	5.8	27	9.7	39	21	7737	368
amonafide (**1**)		2031	2180	2106	625	625	1612	48400	30
azonafide (**2**)		71	57	64	7.0	7.0	45	1983	44

[a] The murine leukemia experiments were based on continuous drug exposure during the MTT assay (Alley, M.C.; Scudiero, D. A.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Schoemaker, R. H.; Boyd, M. R. Feasibility of Drug Screening with Panels of Human Tumor Cell Lines Using a Microculture Tetrazolium Assay. *Cancer Res.* **1988**, *48*, 589−601. The overall standard deviation of the four cell lines used in determining antitumor activity in the MTT assay involved five concentrations per analogue tested and six determinations per drug concentration and was calculated to be 12.1% of the mean IC_{50} values (range 1.1−28.9% of the mean values). Determination of cytotoxicity against AUCC375 and OVCAR3 utilized the sulforhodamine B assay (Skehan, P.; Strong, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. New Colorimetric Assay for Anticancer-Drug Screening, *J. Natl. Cancer Inst.* **1990**, *82*, 1107−1112. [b] Cardiotoxicity was determined by a neonatal rat heart myocyte assay. In this assay, cardiotoxicity is measured by the ATP/protein ratio compared with untreated controls. The IC_{50} is the 1 h drug concentration that reduces this ratio to 50% of that in untreated control myocytes (Dorr, R. T.; Bozak, K. A.; Shipp, N. G.; Hendrix, M.; Alberts, D. S.; Ahmann, F. *In Vitro* Rat Myocyte Cardiotoxicity Model for Antitumor antibiotics Using Adenosine Triphosphate/ Protein Ratios. *Cancer Res.* **1988**, *48*, 5222−5227). For the heart cell assays, the mean standard deviation for all of the IC_{50} determinations was 13.8%. The range of standard deviations as a percent of these mean IC_{50} values was 0.8−42.7%. [c] The quotient of the IC_{50} in the myocytes divided by the mean IC_{50} in the three tumor cell lines (from this table). This ratio has been used previously to compare anthracycline antitumor agents (Dorr, R. T.; Shipp, N. G.; Lee, K. M. Comparison of Cytotoxicity in Heart Cells and Tumor Cells Exposed to DNA. Intercalating Agents *In Vitro. Anti-Cancer Drugs* **1991**, *2*, 27−33). [d] A human melanoma cell line obtained from the University of Arizona Cancer Center. [e] A human ovarian cancer cell line obtained from the NCI. [f] A murine leukemia cell line. The resistant strain is multiple drug resistant because it has increased levels of P glycoproteins. [g] An average (mean) for IC_{50} values in the two solid tumor cell lines and the sensitive L1210 leukemia cell line. [h] DM stands for demethyl.

the 4-OH analogue **21**. Furthermore, average IC_{50} values for 10-substituted azonafides vary from 10 nM for 10-F analogue **18** to 1177 nM for 10-OH analogue **40**. These results are consistent with those in our previous articles wherein the positions of substituents[4] and the nature of substituents[5] were varied separately.

The most potent compounds in Table 1, having average IC_{50} values less than 50 nM, include the following derivatives of azonafide: 8-Cl (**45**), 8-OCH_3 (**47**), 9-OH (**38**), 10-CN (**44**), 10-F (**18**), 10-NO_2 (**43**), 10-Cl (**15**), and mitoxantrone. Compounds with the best ratios of IC_{50} for cardiotoxicity to average antitumor cell potency (>150) were the following: 10-OCH_3 (**41**), 10-OC_2H_5 (**42**), doxorubicin, and mitoxantrone.

One of our goals is to develop analogues which retain potency against multidrug resistant (MDR) tumor cells. The compounds in Table 1 with potency against MDR L1210 leukemia cells greater than that against sensitive L1210 leukemia cells are **16**, **44**, **46**, and **48**. Compounds **37**, **39**, **43**, **49**, azonafide (**2**), and amonafide (**1**) have equal potency against these cell lines. Another goal is to develop analogues with good ratios of potency against cells from solid tumors to potency against leukemia cells. Compounds in Table 1 with better potency against the solid tumors (average) are 10-cyanoazonafide (**44**), 10-nitroazonafide (**43**), and 4-hydroxyazonafide (**21**). None of the positive controls had this selectivity.

Figure 1 is a plot of relative potency for the average of three tumors as a function of the position of substitution for azonafide analogues with hydroxy, methoxy, chloro, and amino groups. The 5-position is not included because only the amino group was incorporated there. This figure shows a similar pattern of alternating peaks and troughs for the methoxy- and chloro-substituted azonafides, with the main difference being the high potency of 6-methoxyazonafide. In contrast, the hydroxy- and amino-substituted azonafides differ significantly in patterns from each other and from the methoxy and chloro analogues. The only hydroxy compound with good potency is 9-hydroxyazonafide (**38**). A very different pattern is shown by the amino-

Table 2. Activity of Selected 2-[2′-(Dimethylamino)ethyl]-1,2-dihydro-3*H*-dibenz[*de,h*]isoquinoline-1,3-diones against Sensitive and Resistant Breast, Colon, and Lung Cancer Cells in Culture[a]

	IC_{50}, nM					
	MXF7 breast[b]			WiDr colon[c]		A549 lung
no.	sens	dox	mitox	sens	MDR	sens
19	390	330	140	60	120	34
31	50	85	60	90	190	12
21	1200	2800	1500	2220	4700	820
22	350	690	520	660	900	81
23	190	530	230	400	680	78
45	60	80	19	20	110	22
46	83	230	120	130	900	60
47	120	67	5.4	9.0	30	4.8
37	49	71	68	100	140	9.9
38	13	20	17	17	20	7.1
39	73	120	95	65	130	27
20	110	140	90	120	120	14
17	70	190	33	180	320	120
44	13	20	12	11	16	2.2
18	15	25	12	17	13	4.8
15	58	8.0	48	17	85	8.2
26	49	50	42	51	64	31
16	42	59	49	40	130	31
40	5.8	35	17	17	240	1.7
41	38	52	41	40	61	31
42	32	100	15	100	120	61
43	13	8.7	14	9.5	74	2.5
48	340	710	120	390	1000	61
49	81	200	80	100	270	40
doxorubicin[d]	28	1172	28	53	130	22
mitoxantrone[d]	8.7	72	41	8.1	488	3.1
azonafide (**2**)	18	70	20	13	94	10
amonafide (**1**)	1100	1800	1200	1100	2200	1100

[a] IC_{50} values determined in tyhe sulforhodamine B assay with a 7-day exposure period. Skehan, P.; Stoering, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesh, H.; Kenney, S.; Boyd, M. R. New Colorimetric Assay for Anticancer-Drug Screening. *J. Natl. Cancer Inst.* **1990**, *82*, 1107−1112. [b] A human mammary carcinoma including a sensitive cell line and cell lines resistant to doxorubicin and mitoxantrone. [c] A human colon carcinoma including sensitive and multidrug resistant cell lines. [d] Average of five determinations.

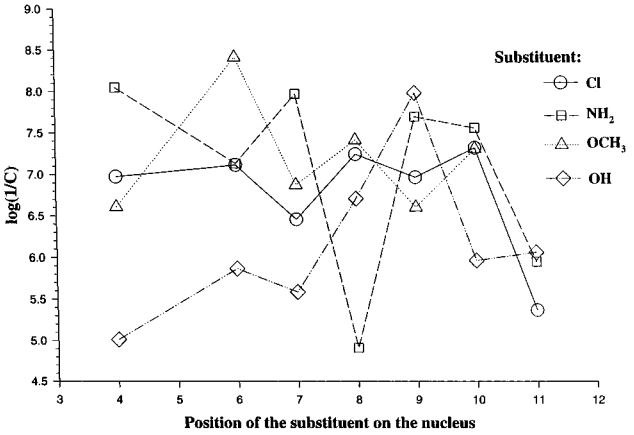


Figure 1. Plot of potency of azonafide analogues, using an average of IC_{50} values log(1/*C*) for three tumors, against the position of substituents on the nucleus.

substituted compounds,[4] which have high potency for 4-aminoazonafide and 7-aminoazonafide, but very low potency for 8-aminoazonafide. The extreme potency differences for some of the compounds in Table 1 probably reflect specific interactions including hydrogen bonding and steric hindrance between the analogues and their DNA intercalation site.

Compounds in Table 1 were tested against additional cultured solid tumor cells including MCF7 breast carcinoma (sensitive, doxorubicin resistant, and mitoxantrone resistant), WiDr colon carcinoma (sensitive and multidrug resistant), and A549 lung carcinoma. Table 2 contains the results of these screens. The most potent compounds across the spectrum of tumor cells are **18**, **38**, and **44**, which have average IC_{50} values of 15, 16, and 12 nM, respectively. Some compounds have about the same potencies against sensitive breast carcinoma cells as against the doxorubicin and mitoxantrone cell lines, indicating no cross resistance. Certain analogues, including **15**, **19**, and **47**, actually are more potent against both resistant cell lines than against the sensitive cell line. Most compounds are somewhat more potent against sensitive WiDr colon carcinoma cells than against the multidrug resistant (MDR) cell line, but **18** is more potent against the MDR cell line and **20** is equally potent in the two cell lines. One interesting difference between Tables 1 and 2 was that compounds **16**, **17**, and especially **40** were substantially more potent against the tumor cells in Table 2 than against those in Table 1.

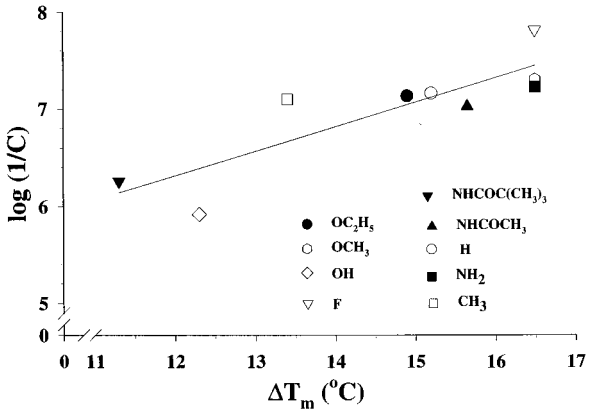


Figure 2. Linear correlation of ΔT_m with potency against melanoma cells for 10-substituted azonafides.

Table 3. Activity against Tumors in Mice[a]

	P388 leukemia	
no.	dose, mg/kg	% ILS
15	12	111
34	30	109
44	3	105
azonafide (**2**)	15	79
amonafide (**1**)	15	88
doxorubicin	4.5	72[b], 113[c]
mitoxantrone	1.6	200

[a] Conducted according to standard NCI protocols. The leukemia cells (10^6) were given ip, and the compounds were given ip in equal doses on days 1, 5, and 9. Results are expressed as the percent increase in life span (ILS) = 100 × [(life span treated − life span controls)/life span controls]. The highest dose used was 10% less than the LD_{10} for acute toxicity in the particular species of mouse. Only the ILS at the highest nontoxic dose is given in this table. [b] The positive control for compound **44**. [c] The positive control for compounds **15**, **34**, and amonafide.

Following the evaluation of activity in tumor cell cultures, azonafide and three of its analogues, **15**, **34**, and **44**, were tested against P388 leukemia in mice. As shown in Table 3, all of them were active (ILS ≥ 25%). Compounds **15** and **34** were roughly as effective as doxorubicin, whereas **44** was somewhat more effective (note the different value for the doxorubicin positive control) and azonafide and amonafide were less potent. Mitoxantrone was the most potent and effective compound in this assay.

Table 4. Data for Correlations among Cytotoxic Potency, DNA Transition Melt Temperature Increase, and Physicochemical Properties for Substituents[a]

compd	subst	ΔT_m, °C	melanoma	ovarian	L1210	cardiotox	π	σ	MR
2	H	15.2	7.15	7.24	8.16	5.70	0	0	1.03
19	4-CH_3	15.3	6.75	6.91	7.27	5.87	0.499	−0.17	5.65
21	4-OH	11.6	5.12	4.83	5.17	NA[b]	−0.328	−0.37	2.85
22	4-OCH_3	11.2	6.74	6.30	7.28	NA[b]	0.181	−0.27	7.87
23	4-$NH(CH_2)_2N(CH_3)_2$	17.8	6.53	6.24	6.90	6.13	−0.127	0.0	
31	4-Cl[c]	14.7	6.89	6.75	8.00	6.05	0.881	0.23	6.03
	4-NH_2[c]	13.2	8.02	7.92	8.27	6.36	−0.907	−0.66	5.42
	4-$NHCOCH_3$[c]	15.1	6.01	6.21	6.22	NA[b]	−0.687	0.0	14.93
	4-$NHCOC(CH_3)_3$[c]	5.3	5.06	6.18	5.66	NA[b]	0.60	0.0	28.9
35	8-NH_2[c]	10.2	4.69	5.02	5.17	NA[b]	−0.907	−0.66	5.42
45	8-Cl	13.5	7.11	7.41		5.99	0.881	0.23	6.03
46	8-OH	6.8	6.57	6.67	6.97	5.53	−0.328	−0.37	2.85
47	8-OCH_3	13.7	7.28	7.28	7.89	6.09	0.181	−0.27	7.87
	8-$NHCOCH_3$	10.1	5.92	5.70	6.31	4.92	−0.687	0.0	14.93
	8-NO_2[c]	14.3	7.42	7.30	7.30	6.19	−0.280	0.78	7.36
	8-I[d]	neg	6.08	5.68	5.68	NA[b]	1.291	0.18	13.94
33	9-NH_2[c]	14.0	7.35	7.85	8.57	6.09	−0.907	−0.66	5.42
37	9-Cl	NP	7.05	6.64	8.11	5.81	0.881	0.23	6.03
38	9-OH	16.0	8.34	7.66	8.27	6.39	−0.328	−0.37	2.85
39	9-OCH_3	10.6	6.79	6.27	7.27	NA[b]	0.181	−0.27	7.87
	9-$NHCOCH_3$[c]	12.9	7.22	7.89	6.84	6.31	−0.687	0.0	14.93
15	10-Cl	concv	7.19	7.11	8.29	6.11	0.881	0.23	6.03
16	10-I	concv	6.20	6.75	6.51	5.32	1.291	0.18	13.94
17	10-C_6H_5	0.6	5.73	6.46	7.16	NA[b]	1.96	−0.01	25.36
18	10-F	16.5	7.80	8.17	8.10	6.34	0.14	0.06	0.92
20	10-CH_3	13.4	7.09	7.17	7.39	6.00	0.499	−0.17	5.65
34	10-NH_2[c]	16.5	7.20	7.70	8.60	6.03	−0.907	−0.66	5.42
40	10-OH	12.3	5.92	5.72	6.77	NA[b]	−0.328	−0.37	2.85
41	10-OCH_3	16.5	7.28	7.11	7.68	5.04	0.181	−0.27	7.87
42	10-OC_2H_5	14.9	7.12	7.20	7.60	4.89	0.71	−0.24	12.47
43	10-NO_2	insol	7.44	7.64	7.60	6.10	−0.280	0.78	7.36
44	10-CN	concv	7.96	7.92	7.84	6.98	−0.57	0.66	6.33
	10-$NHCOCH_3$[c]	13.2	7.01	7.31	7.31	5.11	−0.687	0.0	14.93
	10-$NHCOC(CH_3)_3$[c]	11.3	6.26	6.48	6.36	4.96	0.60	0.0	
36	11-NH_2[c]	4.5	6.02	5.76	6.17	4.57	−0.907	−0.66	5.42
48	11-Cl	6.58	5.69	5.05	5.75	5.09	0.881	0.23	6.03
49	11-OH	15.68	6.27	5.72	6.72	4.81	−0.328	−0.37	2.85
	11-NO_2[c]	5.9	7.13	6.65	7.64	5.06	−0.280	0.78	7.36
	11-$NHCOCH_3$[c]	6.4	5.61	5.61	6.61	NA[b]	−0.687	0.0	14.93

[a] Antitumor data is taken from Table 1. Transition melt temperatures increase for calf thymus DNA was determined at 5×10^{-5} M (base pairs) in pH 7.0 buffer solution 0.01 M is NaH_2PO_4 and 0.001 M in EDTA. Azonafide analogues were 2×10^{-4} M in the same buffer. [b] NA signifies that the compound had such low potency that the IC_{50} could not be determined. [c] Data from ref 1. [d] For synthesis, see ref 5.

QSAR

In the two preceding articles on azonafide analogues,[4,5] statistically significant correlations were found between cytotoxicity and DNA binding strength as measured by increases in transition melt temperatures (ΔT_m). For the present investigation, ΔT_m values were determined using the hydrochloride salts of azonafides and an established method.[5] The resulting ΔT_m values are listed in Table 4. Certain compounds did not afford useful ΔT_m values. Thus, 8-iodoazonafide gave a negative ΔT_m, the 10-chloro, -iodo, and -cyano analogues (**15**, **16**, and **44**) gave concave melt transition curves from which ΔT_m could not be determined, and 10-nitroazonafide (**43**) was too insoluble for ΔT_m measurement. Other data in Table 4 includes potencies against melanoma, ovarian, and sensitive L1210 leukemia cells, plus cardiotoxicity, all expressed as log(1/*C*) and calculated from the IC_{50} values in Table 1. Physicochemical properties of substituents, including lipophilicity (π), electronegativity (σ), and size (MR, the molar refractivity) also are included in Table 4. The π values of certain substituents were calculated using the MacLog P program[11] and para-substituted benzamide as a model structure, whereas σ and MR values were taken from tables by Hansch and Leo.[12]

Potential correlations among the variables listed above were examined by simple linear regression using the programs Sigmastat and Sigma Plot for Windows.[13] Table 5 lists those correlations which are statistically significant at the 95% confidence level or higher. As expected,[4,5] there are correlations between ΔT_m and cytotoxicity to the three tumor cell types, but they are significant only for 8- and 10-substituted azonafides. Correlations between π and cytotoxicity, with the most lipophilic compounds having highest potency, were found for 10-substituted azonafides in ovarian cancer and leukemia and for 11-substituted azonafides in leukemia, whereas in the correlation of π with ovarian cancer in 9-substituted azonafides, the least lipophilic compounds had the highest potency. Substituent size, as estimated by MR, gave a correlation with cytotoxicity to ovarian cancer cells for 9-substituted azonafides and with leukemia cells and melanoma cells for 10-substituted azonafides. In all cases, the smallest substituents conferred the greatest potency. Correlations also were found between MR and ΔT_m for 4- and 11-substituted azonafides, with the most potent compounds having the smallest substituents. Inspection of a computer model for the binding of azonafide to DNA[14] suggests that large 4-substituents clash with the DNA backbone. 11-

Table 5. Correlations among Cytotoxicity, DNA Transition Melt Temperature Increase, and Physicochemical Properties of Azonafide Substituents[a]

correlation	equation	n	r^2	ES_1[b]	ES_v[c]	F
	4-Substituted Azonafides					
MR and ΔT_m	$\Delta T_m = 15.3-0.28$MR	9	0.56	1.27	0.10	7.69
	8-Substituted Azonafides					
ΔT_m and melanoma[d]	$\log(I/C) = 0.91 + 0.44\Delta T_m$	6	0.78	1.52	0.12	14.3
ΔT_m and ovarian[d]	$\log(I/C) = 0.99 + 0.44\Delta T_m$	6	0.85	1.20	0.09	22.9
ΔT_m and leukemia[d]	$\log(I/C) = 0.89 + 0.45\Delta T_m$	6	0.82	1.33	0.10	13.7
π and ovarian[e]	$\log(I/C) = 6.87 + 1.30\pi$	7	0.67	0.23	0.41	10.3
π and leukemia[e]	$\log(I/C) = 7.83 + 2.58\pi$	7	0.92	0.19	0.38	46.1
MR and ovarian[f]	$\log(I/C) = 7.63-0.113$MR	7	0.59	0.38	0.04	7.2
	9-Substituted Azonafides					
π and ovarian	$\log(I/C) = 7.13-0.88\pi$	6	0.70	0.17	0.28	9.5
MR and leukemia	$\log(I/C) = 8.59-0.11$MR	6	0.69	0.30	0.04	9.0
	10-Substituted Azonafides					
ΔT_m and melanoma[g]	$\log(I/C) = 3.33 + 0.25\Delta T_m$	9	0.73	0.84	0.06	19.0
ΔT_m and ovarian[g]	$\log(I/C) = 2.88 + 0.29\Delta T_m$	9	0.65	1.20	0.08	12.8
ΔT_m and leukemia[d]	$\log(I/C) = 2.96 + 0.31\Delta T_m$	9	0.75	1.02	0.07	20.6
MR and leukemia	$\log(I/C) = 7.99 - 0.044$MR	14	0.39	0.20	0.016	7.6
MR and melanoma	$\log(I/C) = 7.42 - 0.043$MR	14	0.30	0.24	0.019	5.1
	11-Substituted Azonafides					
π and leukemia[h]	$\log(I/C) = 7.98 + 2.08\pi$	5	0.84	0.29	0.53	15.5
MR and ΔT_m[h]	$\Delta T_m = 18.1 - 1.89$MR	5	0.80	2.81	0.55	11.7

[a] Only correlations statistically significant at the 95% confidence limit are included. Certain correlations were not statistically significant unless one compound was removed. In these cases, removal was justified on the basis that they were significant outliers according to the DFFITS test. [b] The standard error for the intercept. [c] The standard error for the independent variable. [d] Without the 8-OH analogue **46**. [e] Without the 8-I analogue. [f] Without the 8-NH_2 analogue. [g] ΔT_m values could not be measured for the 10-Cl, 10-I, 10-CN, and 10-NO_2 analogues. The 10-phenyl analogue **17** was dropped. It had almost no ΔT_m and was an outlier. [h] Without the 11-Cl analogue **48**.

Substituents clash strongly with the 1-carbonyl group of azonafides, producing a warp in the chromophore that likely reduces intercalation enthalpy. No correlation was found between σ and any other variable. Although the number of data points was relatively small for some of the correlations ($n = 5-10$), it should be noted that only simple linear regression was attempted and that the equations obtained are those of straight lines.

Conclusions

The previously established data base on azonafide analogues was expanded to include 24 new compounds with substituents at the 4-, 8-, 9-, 10-, and 11-positions on the anthracene nucleus. Many of these compounds were prepared by way of diazonium salts derived from the corresponding amines. Other compounds were synthesized from appropriately substituted anthracenes by established routes.

Advances were made in developing new compounds with greater cytotoxicity than azonafide against a number of tumor cell lines in cultures, including cell lines resistant to the standard antitumor agents doxorubicin and mitoxantrone. Three compounds were tested against P388 leukemia in mice, and one of them, 10-cyanoazonafide, was more effective than doxorubicin.

As found previously with azonafide analogues, there were significant correlations of DNA binding strength, as measured by ΔT_m, and cytotoxicity to solid and leukemia cells in culture, but only for the 8- and 10-substituted azonafides. Other correlations were found for lipophilicity (π) and cytotoxicity at positions 9, 10, and 11 and for size (MR) and ΔT_m at positions 4 and 11. Although these types of correlations are not statistically significant at all positions, they lend support to a model in which the DNA binding strength (ΔT_m) of azonafides is a factor in determining cytotoxicity, and physicochemical properties including substituent size and lipophilicity influence both DNA binding and cytotoxicity. One surprising observation was that 10-phenylazonafide (**17**), which binds very weakly to DNA, has substantial potency against leukemia cells (IC_{50} = 70 nm). This finding suggests that a mechanism different than simple DNA binding might be operating. Possibly **17** binds strongly to a DNA−topoisomerase complex.

The one position on the anthracene nucleus of azonafide not adequately studied with a variety of substituents is C5. This position will be explored in future studies.

Experimental Section

Melting points were recorded on a Mel-Temp apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker 250 WM spectrometer, and absorptions are reported as downfiled from Me_4Si (δ values in ppm). Mass spectra were recorded on a Varian-MAT311 spectrometer. Elemental analyses were performed by Desert Analytics, Inc., Tucson, AZ. Preparative thin layer chromatography (PTLC) was performed on Analtech silica gel plates (20 × 20 × 0.2 cm) using the indicated solvents.

Preparation of 10-Chloro-2-[2′-(dimethylamino)ethyl]-1,2-dihydro-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (15). To a suspension of 2 g (7.5 mmol) of 9-chloro-1,2-dihydrocyclopentano[*de*]anthracene-1,2-dione (prepared from 2-chloroanthracene (**3**) as described in ref 4) in 40 mL of dioxane and 15 mL of 2 N NaOH was added at 15 °C 12 mL of 30% hydrogen peroxide solution. The mixture was stirred at room temperature for 45 min and then diluted with 100 mL of water. Acidification with dilute H_2SO_4 gave 2.14 g (95%) of 7-chloroanthracene-19-dicarboxylic acid (**8**), crystallized from a dioxane−dimethyl sulfoxide mixture (4:1): mp 325−327 °C (probably mp of the anhydride); ^{1}H NMR (DMSO-d_6) 7.78−7.81 (d, 1, H-6), 7.91−7.96 (t, 1, H-3), 8.42−8.46 (d, 1, H-5), 8.70−8.73 (d, 2, H-2 + H-4), 9.40 (s, 1, H-10), 9.62 (s, 1, H-8). Anal. (consistent with the anhydride formula $C_{16}H_7ClO_3$) C, H, Cl.

A suspension of 1 g (3.54 mmol) of **8** in 70 mL of toluene was heated at reflux for 8 h with 360 mg (4.1 mmol) of *N*,*N*-dimethylethylenediamine. The solvent was removed under reduced pressure, and the orange residue was purified by column chromatography on silica gel with 10% methanol in

toluene to give 1.23 g (98%) of **15**, crystallized from toluene: mp 166−168 °C; ^{1}H NMR ($CDCl_3$) 2.43 (s, 6, NCH_3), 2.65−2.80 (t, 2, CH_2N), 4.32−4.47 (t, 2, $CONCH_2$), 7.43−7.60 (t, 1, H-5), 7.70−7.77 (d, 1, H-9), 7.90−7.97 (d, 1, H-8), 8.19−8.30 (d, 1, H-4), 8.64 (s, 1, H-7), 8.64−8.72 (d, 1, H-6), 9.93 (s, 1, H-11). Anal. ($C_{20}H_{17}ClN_2O_2$) C, H, Cl, N.

Preparation of 2-[2′-(Dimethylamino)ethyl]-1,2-dihydro-10-iodo-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (16). To a cold (−5 to 0 °C) stirred suspension of 1.49 g (4.6 mmol) of 2-iodoanthracene (**4**) in 50 mL of dry carbon disulfide was added 4 mL (44.3 mmol) of oxalyl chloride, followed by 2.5 g (18.7 mmol) of anhydrous aluminum chloride. The mixture was stirred at −5 to 0 °C for 6 h and then at room temperature overnight. Decomposition with dilute cold HCl gave an orange solid which was collected and digested well with 50 mL of 5% sodium hydroxide solution. The insoluble material was filtered, washed with water, and dried in air to afford 1 g (61%) of 9-iodo-1,2-dihydrocyclopentano[*de*]anthracene-1,2-dione. Alkaline hydrogen peroxide oxidation of this intermediate as described under **15** gave a 93% crude yield of 7-iodoanthracene-1,9-dicarboxylic acid (**9**) which was used directly in the next step. A suspension of 500 mg (1.28 mmol) of **9** in 30 mL of toluene was refluxed for 4 h with a solution of 124 mg (1.41 mmol) of *N*,*N*-dimethylethylenediamine in 7 mL of ethanol. The solvent was evaporated to dryness, and the residue was isolated on a silica gel column with 5% methanol in chloroform as solvent to give 485 mg (86%) of **16**: mp 190−192 °C after crystallization from toluene; ^{1}H NMR ($CDCl_3$) 2.41 (s, 6, NCH_3), 2.68−2.74 (t, 2, CH_2N), 4.35−4.40 (t, 2, $CONCH_2$), 7.65−7.72 (d over t, 2, H-5 + H-9, $J_{9,8} = 8.746$), 7.76−7.78 (dd, 1, H-8, $J_{8,9} = 8.811$, $J_{8,11} = 1.598$), 8.21−8.25 (dd, 1, H-4), 8.61 (s, 1, H-7), 8.65−8.68 (dd, 1, H-6, $J_{6,4} = 1.263$), 10.34−10.35 (t, 1, H-11, $J_{11,8} = 1.666$, $J_{11,9} = 0.853$). Anal. ($C_{20}H_{17}IN_2O_2$) C, H, N; I: calcd, 28.60; found, 28.08.

Preparation of 2-[2′-(Dimethylamino)ethyl]-1,2-dihydro-10-phenyl-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (17). A mixture of 100 mg (0.225 mmol) of **16**, 40 mL of ethylene glycol dimethyl ether, 25 mg (0.022 mmol) of tetrakis(triphenylphosphine)palladium(0), 36 mg (0.295 mmol) of phenylboric acid, and a solution of 168 mg (2 mmol) of sodium bicarbonate in 2 mL of water was heated at reflux for 24 h. The reaction mixture was concentrated to dryness, and the residue was chromatographed by PTLC on silica gel with 5% methanol in chloroform to give 70 mg (79%) of **17**, crystallized from toluene−hexanes (1:1): mp 171−173 °C; ^{1}H NMR ($CDCl_3$) 2.41 (s, 6, NCH_3), 2.71−2.76 (t, 2, CH_2N), 4.42−4.47 (t, 2, $CONCH_2$), 7.42−7.48 [m, 1, H-4′ (phenyl)], 7.52−7.58 (m, 2, H-3′ + H-5′), 7.68−7.73 (t, 1, H-5), 7.87−7.93 (m, 3, H-2′ + H-6′ + H-9), 8.14−8.17 (d, 1, H-8), 8.31−8.34 (d, 1, H-4), 8.72−8.75 (d, 1, H-6), 8.79 (s, 1, H-7), 10.31 (s, 1, H-11). Anal. ($C_{26}H_{22}N_2O_2$) C, H, N.

Preparation of 2-[2′-(Dimethylamino)ethyl]-1,2-dihydro-10-fluoro-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (18) and 2-[[2′-(Dimethylamino)ethyl]amino]anthracene-1,9-dicarboxylic Acid Anhydride (24). A mixture of 2- and 7-fluoroanthracene-1,9-dicarboxylic acids (**10** and **11**) was prepared in an overall yield (crude) of 46% from 2-fluoroanthracene (**5**) following the procedure described for **16**. Amination of the mixture with *N*,*N*-dimethylethylenediamine as described in the same procedure gave a product from which **18** was isolated in 85% yield: mp 173−175 °C after crystallization from hexanes containing least amount of toluene; ^{1}H NMR ($CDCl_3$) 2.41 (s, 6, NCH_3), 2.70−2.75 (t, 2, CH_2N), 4.39−4.44 (t, 2, $CONCH_2$), 7.38−7.45 (heptet, 1, H-9, $J_{9,8} = 9.203$, $J_{9,11} = 2.553$, $J_{9,F} = 8.377$), 7.68−7.74 (t, 1, H-5), 8.06−8.12 (dd, 1, H-8, $J_{8,9} = 9.226$, $J_{8,F} = 6.236$), 8.29−8.33 (d, 1, H-4), 8.71−8.75 (d, 1, H-6), 8.77 (s, 1, H-7), 9.64−9.70 (dd, 1, H-11, $J_{F,11} = 13.410$, $J_{11,9} = 2.581$). Anal. ($C_{20}H_{17}FN_2O_2$) C, H, F, N.

In addition to **18** another two products were isolated. The first one is **24** and was obtained in 6% yield after crystallization from a toluene−methanol mixture. It forms a semisolid at 193−197 °C with complete melting (dec) at 230 °C: ^{1}H NMR ($CDCl_3$) 2.38 (s, 6, NCH_3), 2.69−2.74 (t, 2, CH_2N), 3.57−3.64 (q, 2, $NHCH_2$), 7.13−7.16 (d, 1, H-3), 7.51−7.58 (t, 1, H-6), 7.76−7.83 (t, 1, H-7), 7.94−8.00 (t, 2, H-4 + H-5), 8.40 (s, 1, H-10), 9.64−9.67 (d, 1, H-8), 10.06−10.10 (t, 1, NH); HRMS (EI) calcd for $C_{20}H_{18}N_2O_3$ 334.13174, found M^+ 334.13083. The second product **25** was obtained in 2% yield and indicated by ^{1}H NMR to be 2-fluoroanthracene-1,9-dicarboxylic acid anhydride. No further characterization of this compound was done: ^{1}H NMR ($CDCl_3$) 7.38−7.42 (d, 1, H-3), 7.58−7.61 (t, 1, H-6), 7.78−7.82 (t, 1, H-7), 7.99−8.03 (d, 1, H-4), 8.02−8.05 (d, 1 H-5), 8.55 (s, 1, H-10), 9.61−9.65 (d, 1, H-8).

Preparation of 2-[2′-(Dimethylamino)ethyl]-1,2-dihydro-4- and -10-methyl-3*H*-dibenz[*de,h*]isoquinoline-1,3-diones (19 and 20). A mixture of 3- and 9-methyl-1,2-dihydrocyclopentano[*de*]anthracene-1,2-dione was prepared from 2-methylanthracene (**6**) as described in the literature.[15] Alkaline hydrogen peroxide oxidation of the mixture as described under **15** gave a mixture of the corresponding 2- and 7-methylanthracenedicarboxylic acids (**12** and **13**) in 99% crude yield. A suspension of 280 mg (1 mmol) of the diacid mixture in 25 mL of ethanol and 30 mL of toluene was heated at reflux overnight with 106 mg (1.2 mmol) of *N*,*N*-dimethylethylenediamine. The solvent was evaporated, and the residue was isolated by PTLC on silica gel with toluene−Et_3N (250:2). Compound **19** was obtained in 113 mg (40%) yield: crystallized from hexanes, mp 142−144 °C; ^{1}H NMR ($CDCl_3$ + DMSO-d_6) 2.53 (s, 6, NCH_3), 2.83−2.88 (t, 2, CH_2N), 3.00 (s, 3, CH_3), 4.37−4.42 (t, 2, $CONCH_2$), 7.45−7.48 (d, 1 H-5), 7.56−7.62 (t, 1, H-9), 7.73−7.80 (t, 1, H-10), 8.03−8.06 (d, 1, H-8), 8.12−8.16 (d, 1 H-6), 8.69 (s, 1, H-7), 9.88−9.92 (d, 1, H-11). Anal. ($C_{21}H_{20}N_2O_2 \cdot {}^1/_4H_2O$) C, H, N.

Compound **20** was obtained in 106 mg in 38% yield: crystallized from hexanes, mp 110−112 °C; ^{1}H NMR ($CDCl_3$ + DMSO-d_6) 2.67 (s, 3, CH_3), 2.93 (s, 6, NCH_3), 3.41−3.46 (t, 2, CH_2N), 4.56−4.61 (t, 2, $CONCH_2$), 7.48−7.52 (d, 1 H-9), 7.72−7.79 (t, 1, H-5), 8.06−8.10 (d, 1, H-8), 8.43−8.47 (d, 1, H-4), 8.67−8.70 (d, 1 H-6), 8.92 (s, 1, H-7), 9.70 (s, 1, H-11). Anal. ($C_{21}H_{20}N_2O_2$) C, H, N.

Preparation of 2-[2′-(Dimethylamino)ethyl]-1,2-dihydro-4-hydroxy-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (21), 2-[2′-(Dimethylamino)ethyl]-1,2-dihydro-4- methoxy-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (22), and 2-[2′-(Dimethylamino)ethyl]-1,2-dihydro-4-[[2′-(dimethylamino)-ethyl]amino]-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (23). 2-Methoxyanthracene-1,9-dicarboxylic acid (**14**) was prepared as follows:

To a cold (−10 °C) stirred solution of 500 mg (2.4 mmol) of 2-methoxyanthracene (**5**) in 50 mL of anhydrous 1,2-dichloroethane was added 2 mL (22.2 mmol) of oxalyl chloride followed by 500 mg (3.75 mmol) of anhydrous aluminum chloride. The mixture was stirred at −10 to −5 °C for 8.5 h and then decomposed with 50 mL of dilute HCl. The organic layer was separated, and the aqueous layer was extracted with chloroform. The extract was combined with the organic layer, and the solution was dried over Na_2SO_4. The solvent was removed under reduced pressure, and the residue was chromatographed on a silica gel column with chloroform to give 226 mg of unreacted 2-methoxyanthracene and 305 mg (88% based on reacted material) of 3-methoxyaceanthrenequinone, crystallized from toluene or dioxane: mp 229−231 °C; ^{1}H NMR ($CDCl_3$) 4.28 (s, 3, OCH_3), 7.33−7.36 (d, 1, H-4), 7.53−7.59 (t, 1, H-8), 7.70−7.76 (t, 1, H-9), 7.99−8.03 (d, 1 H-7), 8.15−8.18 (t, 1, H-5), 8.56 (s, 1, H-6), 8.94−8.97 (d, 1, H-10). Anal. ($C_{17}H_{10}O_3$) H; C: calcd, 73.38; found, 72.96.

Alkaline hydrogen peroxide oxidation of 3-methoxyaceanthrenequinone as described for the preparation of **15** gave an 89% yield of 2-methoxyanthracene-1,9-dicarboxylic acid (**14**), crystallized from dimethyl sulfoxide: mp 317−320 °C (probably of the anhydride); ^{1}H NMR (DMSO-d_6) 3.97 (s, 3, OCH_3), 7.50−7.53 (t, 1, H-6), 7.58−7.61 (t, 1, H-7), 7.64−7.66 (d, 1, H-3), 8.07−8.10 (t, 2, H-4 + H-5), 8.27−8.29 (d, 1, H-8), 8.73 (s, 1, H-10). Anal. (consistent with the anhydride formula $C_{17}H_{10}O_4$) C, H.

A suspension of 485 mg (1.64 mmol) of **14** in 50 mL of toluene and 30 mL of absolute ethanol was heated at reflux for 18 h with 241 mg (2.74 mmol) of *N*,*N*-dimethylethylenediamine. The solvent was removed under reduced pressure, and the residue was isolated by PTLC on silica gel with 10% methanol in chloroform or 20% methanol in toluene to give

the following: (a) 244 mg (45%) of **21**, crystallized from methanol; mp 197−199 °C; ^{1}H NMR ($CDCl_3$) 2.36 (s, 6, NCH_3), 2.66−2.71 (t, 2, CH_2N, J_{CH_2,CH_2} = 6.48), 3.52−3.59 (q, 2, $CONCH_2$, J_{CH_2,CH_2} = 6.48, $J_{CH_2,OH}$ = 5.28), 7.06−7.10 (d, 1, H-5), 7.49−7.76 (t, 1, H-9), 7.73−7.79 (t, 1, H-10), 7.88−7.92 (d, 2, H-6 + H-8), 8.30 (s, 1, H-7), 9.59−9.62 (d, 1, H-11), 9.99−10.03 (t, 1, OH, *J* for long-range coupling with $CONCH_2$ = 5.26. Irradiation of the $CONCH_2$ quartet reduces the OH resonance to a singlet and the CH_2N also became a singlet. The OH resonance disappeared upon D_2O exchange and the $CONCH_2$ became a triplet). Anal. ($C_{20}H_{18}N_2O_3$) C, H, N. (b) 10 mg (2%) of **22**, crystallized from hexanes−toluene (4:1); mp 172−174 °C; ^{1}H NMR ($CDCl_3$) 2.43 (s, 6, NCH_3), 2.71−2.77 (t, 2, CH_2N), 4.25 (s, 3, OCH_3), 4.42−4.48 (t, 2, $CONCH_2$), 7.47−7.51 (d, 1, H-5), 7.57−7.60 (t, 1, H-9), 7.77−7.81 (t, 1, H-10), 8.00−8.04 (d, 1, H-8), 8.26−8.29 (d, 1, H-6), 8.64 (s, 1, H-7), 10.02−10.06 (d, 1, H-11). Anal. ($C_{21}H_{20}N_2O_3 \cdot {}^1/_4H_2O$) C, H, N. (c) 33 mg (5%) of **23**, crystallized from toluene−hexanes (1:3); mp 117−119 °C; ^{1}H NMR ($CDCl_3$) 2.36 (s, 6, 4-NCH_3), 2.44 (s, 6, 2-NCH_3), 2.65−2.75 (m, 4, CH_2N), 3.48−3.55 (q, 2, $NHCH_2$), 4.40−4.46 (t, 2, $CONCH_2$), 6.96−7.00 (d, 1, H-5), 7.44−7.50 (t, 1, H-9), 7.69−7.76 (t, 1, H-10), 7.76−7.80 (d, 1, H-8), 7.85−7.89 (d, 1, H-6), 8.23 (s, 1, H-7), 9.99−10.03 (d, 1, H-11), 10.97−10.99 (t, 1, NH, J_{NH,CH_2} = 5.091). Anal. ($C_{24}H_{28}N_4O_2$) C, H, N.

Preparation of 10-Chloro-2-[2′-(methylamino)ethyl]-1,2-dihydro-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (26). A mixture of 300 mg (1.062 mmol) of **8**, 95 mg (1.28 mmol) of *N*-methylethylenediamine, and 50 mL of toluene was heated at reflux for 20 h. After the mixture was cooled to room temperature, the insoluble material (111 mg) was filtered and discarded. The filtrate was evaporated to dryness, and the residue was chromatographed by PTLC in 10% methanol in chloroform to give 151 mg (42%) of **26**, crystallized from methanol: mp 176−178 °C; ^{1}H NMR ($CDCl_3$) 1.45 (br s, 1, NH), 2.52 (s, 3, CH_3), 3.02−3.06 (t, 2, CH_2N), 4.41−4.44 (t, 2, $CONCH_2$), 7.52−7.54 (d, 1, H-9), 7.70−7.73 (t, 1, H-5), 7.98−8.02 (d, 1, H-8), 8.28−8.30 (d, 1, H-4), 8.71−8.73 (s over d, 2, H-6 + H-7), 10.01 (s, 1, H-11). Anal. ($C_{19}H_{15}ClN_2O_2$) C, H, N; Cl: calcd, 10.34; found, 9.86.

Preparation of 4-Chloro-2-[2′-(dimethylamino)ethyl]-1,2-dihydro-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (31). A solution of 841 mg (12.2 mmol) of sodium nitrite in 8 mL of water was added at 0 °C over 20 min to a stirred solution of 2 g (10.2 mmol) of 7-amino-1,2,3,4-tetrahydroanthracene (**27**)[4] in 50 mL of concentrated hydrochloric acid and 10 mL of water. After being stirred at 0 °C for $^1/_2$ h, the resulting diazonium chloride solution was added at 0 °C to a solution of 4.9 g (50 mmol) of Cu_2Cl_2 in 20 mL of 28% HCl. The mixture was stirred at 0 °C for $^1/_2$ h and then at room temperature overnight. The solid that formed was collected and purified by column chromatography on silica gel with hexanes to give 1.55 g (71%) of 7-chloro-1,2,3,4-tetrahydroanthracene (**28**). A solution of 1.40 mg (6.47 mmol) of **28** in 35 mL of CS_2 was treated at 0 °C with 5 mL of oxalyl chloride. $AlCl_3$ (2.5 g, 19 mmol) was added at once to the cold (0 °C) mixture while vigorously stirring. Stirring was continued at 0 °C for 5 h and then at room temperature overnight. The mixture was decomposed with dilute cold HCl, and the yellow solid was collected and digested with 100 mL of 5% NaOH solution. The insoluble material was filtered, washed well with water, dried in air, and then chromatographed on a silica gel column with $CHCl_3$. The yellow fraction was collected to give 881 mg (50%) of 3-chloro-1,2,7,8,9,10-hexahydrocyclopentano[*de*]anthracene-1,2-dione (**29**). After crystallization from methanol it had mp 216−218 °C: ^{1}H NMR ($CDCl_3$) 1.90−1.96 (m, 4, H-8 + H-9), 3.06−3.11 (t, 2, H-7), 3.42−3.47 (t, 2, H-10), 7.51−7.54 (d, 1, H-4), 7.87 (s, 1, H-6), 7.95−7.98 (d, 1, H-5). Anal. ($C_{16}H_{11}ClO_2$) C, H, Cl, N.

A solution of 871 mg (3.22 mmol) of **29** in 30 mL of anhydrous dioxane was refluxed for 96 h with 2.2 g (9.7 mmol) of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone. The mixture was cooled to room temperature and filtered. The filtrate was concentrated, and the residue was isolated on a silica gel column with chloroform to give 522 mg (64%) of 3-chloroacenanthrenequinone (**30**). Alkaline hydrogen peroxide oxidation of **30** as described under **15** gave an 82% crude yield of 2-chloroanthracene-1,9-dicarboxylic acid. A suspension of 482 mg (1.6 mmol) of the latter in 40 mL of toluene was heated at reflux for 3 h with a solution of 168 mg (1.9 mmol) of *N*,*N*-dimethylethylenediamine in 8 mL of absolute ethanol. The solvent was evaporated to dryness, and the residue was isolated by PTLC on silica gel with 2.5% methanol in chloroform to give 133 mg (25%) of **21** and 98 mg (18%) of **31**, crystallized from methanol: mp 202−204 °C; ^{1}H NMR ($CDCl_3$ + DMSO-d_6) 2.41 (s, 6, NCH_3), 2.69−2.74 (t, 2, CH_2N), 4.34−4.38 (t, 2, $CONCH_2$), 7.56−7.62 (t, 1, H-9), 7.74−7.80 (t, 1, H-10), 7.95−7.98 (d, 1, H-8), 8.13−8.14 (d, 1, H-5), 8.47−8.48 (d, 1, H-6), 8.52 (s, 1, H-7), 9.80−9.83 (d, 1 H-11). Anal. ($C_{20}H_{17}ClN_2O_2$) C, H, N, Cl.

A third compound appeared to be 3-hydroxy-1,9-anthracenedicarboxylic acid anhydride (**32**) from its ^{1}H NMR spectrum ($CDCl_3$ + DMSO-d_6): 7.47−7.50 (d, 1, H-3), 7.56−7.61 (t, 1, H-6), 7.68 (s, 1, OH), 7.76−7.82 (t, 1, H-7), 8.06−8.09 (d, 2, H-4 + H-5), 8.63 (s, 1, H-10), 9.52−9.55 (d, 1, H-8). It was not further characterized.

Preparation of 9-Chloro-2-[2′-(dimethylamino)ethyl]-1,2-dihydro-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (37) and 2-[2′-(Dimethylamino)ethyl]-1,2-dihydro-9-hydroxy-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (38). A cold (0 °C) solution 840 mg (0.58 mmol) of sodium nitrite in 2 mL of water was added to a stirred cold (0 °C) solution of 123 mg (0.37 mmol) of **33**[4] in 15 mL of concentrated HCl. The mixture was stirred at 0 °C for $1^1/_2$ h and then at room temperature overnight and at 75 °C for $^1/_2$ h. After neutralization with sodium bicarbonate the mixture was extracted with chloroform containing a little methanol. The extract was concentrated, and the residue was isolated by PTLC on silica gel with 10% methanol in chloroform. Then, 47 mg (36%) of compound **37** was obtained and crystallized from hexanes containing the least amount of toluene: mp 177−179 °C; ^{1}H NMR ($CDCl_3$) 2.40 (s, 6, NCH_3), 2.69−2.74 (t, 2, CH_2N), 4.38−4.44 (t, 2, $CONCH_2$), 7.67−7.76 (m, 2, H-5 + H-10), 8.03 (s, 1, H-8), 8.29−8.32 (d, 1, H-4), 8.67 (s, 1, H-7), 8.72−8.75 (d, 1, H-6), 9.93−9.96 (d, 1, H-11). Anal. ($C_{20}H_{17}ClN_2O_2$) C, H, N; Cl: calcd, 10.07; found, 9.66.

A second band from PTLC gave 47 mg (38%) of **38**, crystallized from a chloroform−methanol mixture (1:1): mp 249−252 °C; ^{1}H NMR ($CDCl_3$ + DMSO-d_6) 2.29 (s, 6, NCH_3), 2.58−2.62 (t, 2, CH_2N), 4.26−4.31 (t, 2, $CONCH_2$), 7.40 (s, 1, H-8), 7.46−7.50 (d, 1, H-10), 7.72−7.77 (t, 1, H-5), 8.40−8.43 (d, 1, H-4), 8.55−8.57 (d, 1, H-6), 8.82 (s, 1, H-7), 9.79−9.82 (d, 1 H-11), 10.3 (br s, 1, OH). Anal. ($C_{20}H_{18}N_2O_3$) C, H, N.

Preparation of 2-[2′-(Dimethylamino)ethyl]-1,2-dihydro-10-hydroxy-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (40). This compound was prepared in 24% yield from **34**[4] by the procedure described for the preparation of **38**. It was crystallized from a chloroform−methanol mixture: mp 224−226 °C; ^{1}H NMR ($CDCl_3$ + DMSO-d_6) 2.32 (s, 6, NCH_3), 2.60−2.63 (t, 2, CH_2N), 4.24−4.26 (t, 2, $CONCH_2$), 6.91−6.93 (d, 1, H-9), 7.56−7.59 (t, 1, H-5), 7.72−7.74 (d, 1, H-8), 7.86 (s, 1, H-7), 8.22−8.24 (d, 1, H-4), 8.39 (s, 1, H-11), 8.50−8.52 (d, 1 H-6). Anal. ($C_{20}H_{18}N_2O_3 \cdot {}^1/_2H_2O$) C, H, N.

In addition to **40**, compound **15** was isolated from the reaction product in 28% yield.

Preparation of 2-[2′-(Dimethylamino)ethyl]-1,2-dihydro-9- and -10-methoxy-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (39 and 41). A solution of 1 equiv of **38** or **40** in a chloroform−methanol mixture (1:2) was treated at 0 °C with excess (46 equiv) ethereal diazomethane. The mixture was stirred at 0−5 °C for 1 h and then stored in a refrigerator overnight. The reaction mixture was concentrated under reduced pressure, and the residue was purified by PTLC on silica gel with 10% methanol in chloroform, followed by crystallization from hexanes containing the least amount of toluene. Compound **39** was obtained in 58% yield and crystallized from hexanes containing the least amount of toluene: mp 160−161 °C; ^{1}H NMR ($CDCl_3$) 2.45 (s, 6, NCH_3), 2.77−2.81 (t, 2, CH_2N), 4.01 (s, 3, OCH_3), 4.43−4.48 (t, 2, $CONCH_2$), 7.29 (s, 1, H-8), 7.48−7.52 (d, 1, H-10), 7.68−7.73 (t, 1, H-5), 8.28−8.31 (d, 1, H-4), 8.67−8.70 (s over d, 2, H-6 + H-7), 9.91−9.95 (d, 1 H-11). Anal. ($C_{21}H_{20}N_2O_3$) C, H, N.

Compound **41** was obtained in 61% yield and crystallized from hexanes containing the least amount of toluene: mp 170−172 °C; ^{1}H NMR ($CDCl_3$) 2.46 (s, 6, NCH_3), 2.78−2.84 (t, 2, CH_2N), 4.12 (s, 3, OCH_3), 4.44−4.50 (t, 2, $CONCH_2$), 7.27−7.32 (d, 1, H-9), 7.68−7.71 (t, 1, H-5), 7.97−8.01 (d, 1, H-8), 8.31−8.34 (d, 1, H-4), 8.72 (s, 1, H-7), 8.74−8.77 (d, 1 H-6), 9.43 (s, 1, H-11). Anal. ($C_{21}H_{20}N_2O_3$) C, H, N.

Preparation of 2-[2′-(Dimethylamino)ethyl]-1,2-dihydro-10-nitro-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (43). A solution of nitrosylsulfuric acid (prepared by dissolving 165 mg (2.4 mmol) of sodium nitrite in 4 mL of cold 98% H_2SO_4) was added to a vigorously stirred cold solution of 300 mg (0.90 mmol) of **34** in 10 mL of glacial acetic acid. After addition, the mixture was stirred at 5−10 °C for 1 h. It was then diluted with excess anhydrous ether, and the precipitated diazonium sulfate was filtered and washed with a mixture of methanol and ether (1:1) and then with ether. The salt was dissolved in 10 mL of cold (5 °C) water, and the solution was added in portions with vigorous stirring to a cold (10 °C) saturated sodium nitrite solution containing 300 mg of copper powder. After addition the mixture was stirred at room temperature overnight and then diluted with water. The solid material was filtered and extracted well with hot dioxane. The extract was concentrated into a yellowish brown solid which was chromatographed on silica gel column with 5% methanol in chloroform to afford 161 mg (49%) of **43**, crystallized from toluene: mp 240−242 °C; ^{1}H NMR ($CDCl_3$ + DMSO-d_6) 2.34 (s, 6, NCH_3), 2.64−2.70 (t, 2, CH_2N), 4.33−4.38 (t, 2, $CONCH_2$), 7.90−7.97 (t, 1, H-5), 8.32−8.39 (d, 1, H-9), 8.41−8.43 (d, 1, H-8), 8.53−8.57 (d, 1, H-4), 8.72−8.75 (d, 1, H-6), 9.20 (s, 1, H-7), 10.93 (s, 1 H-11). Anal. ($C_{20}H_{17}N_3O_4$) C, H, N.

Preparation of 10-Cyano-2-[2′-(dimethylamino)ethyl]-1,2-dihydro-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (44). A solution of nitrosylsulfuric acid (prepared by dissolving 0.55 g (7.97 mmol) of sodium nitrite in 15 mL of cold 98% sulfuric acid) was added into portions to a vigorously stirred cold (10 °C) solution of 1 g (3 mmol) of **34** in 50 mL of glacial acetic acid. After addition the mixture was stirred for 15 min, and then excess anhydrous ether was added with vigorous stirring. The precipitated diazonium salt was filtered, washed well with a mixture of Et_2O−MeOH (1:1) and then dissolved in 10 mL of water, and the solution was cooled to 0 °C. A solution of 32.5 g (0.65 mmol) of sodium cyanide in 50 mL of water was added to a stirred suspension of 4.9 g (0.5 mmol) of cuprous chloride in 100 mL of water. The resulting solution of cuprous cyanide was cooled to 0 °C. The diazonium salt solution was added into portions at 0 °C to the cuprous cyanide solution with stirring. The mixture was stirred at 0 °C for $^1/_2$ h and then at room temperature overnight and filtered. The precipitate was washed with water and extracted with chloroform. After the mixture was dried over anhydrous Na_2SO_4, the extract was concentrated and the residue was chromatographed by PTLC on silica gel with 5% methanol in chloroform to give 0.6 g (58%) of **44**, crystallized from toluene: mp 223−224 °C; ^{1}H NMR ($CDCl_3$) 2.40 (s, 6, NCH_3), 2.71−2.77 (t, 2, CH_2N), 4.41−4.46 (t, 2, $CONCH_2$), 7.70−7.74 (d, 1, H-9), 7.80−7.87 (t, 1, H-5), 8.18−8.22 (d, 1, H-8), 8.38−8.41 (d, 1, H-4), 8.80−8.82 (d, 1, H-6), 8.88 (s, 1, H-7), 10.53 (s, 1 H-11); IR (KBr disk) 2220 cm^{-1} (CN str). Anal. ($C_{21}H_{17}N_3O_2$) C, N; H: calcd, 5.19; found, 4.56.

Preparation of 2-[2′-(Dimethylamino)ethyl]-1,2-dihydro-10-ethoxy-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (42). This compound was prepared in 60% yield from **40** and diazoethane following the procedure described for the preparation of **39** and **41**. The compound crystallized from hexanes: mp 161−162 °C; ^{1}H NMR ($CDCl_3$) 1.55−1.59 (t, 3, CH_3), 2.41 (s, 6, NCH_3), 2.69−2.74 (t, 2, CH_2N), 4.32−4.39 (q, 2, CH_2), 4.40−4.45 (t, 2, $CONCH_2$), 7.25−7.29 (dd, 1, H-9, $J_{9,11}$ = 2.44), 7.62−7.67 (t, 1, H-5), 7.94−7.97 (d, 1, H-8), 8.26−8.29 (d, 1, H-4), 8.65 (s, 1, H-7), 8.70−8.73 (d, 1, H-6), 9.37−9.38 (d, 1, H-11, $J_{11,9}$ = 2.44). Anal. ($C_{22}H_{22}N_2O_3$) C, H, N.

Preparation of 8-Chloro-2-[2′-(dimethylamino)ethyl]-1,2-dihydro-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (45) and 8-Hydroxy-2-[2′-(dimethylamino)ethyl]-1,2-dihydro-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (46). These two compounds were prepared by treating 8-aminoazonafide (**35**)[4] with sodium nitrite and 37% HCl according to the procedure described for preparation of **37** and **38**. Compound **45**, obtained in 23% yield, had mp 115−117 °C after crystallization from CH_3OH: ^{1}H NMR ($CDCl_3$) 2.40 (s, 6, NCH_3), 2.70−2.76 (t, 2, CH_2N), 4.39−4.45 (t, 2, $CONCH_2$), 7.63−7.77 (m, 3, H-5 + H-9 + H-10), 8.32−8.36 (d, 1, H-4), 8.70−8.74 (d, 1, H-6), 9.21 (s, 1, H-7), 9.88−9.93 (d, 1-H-11). Anal. ($C_{20}H_{17}ClN_2O_2$) C, H, N; Cl: calcd, 10.07; found, 9.08.

Compound **46**, obtained in 13% yield, had mp 222−224 °C after crystallization from toluene containing the least amount of CH_3OH: ^{1}H NMR ($CDCl_3$) 2.66 (s, 6, NCH_3), 3.12−3.14 (t, 2, CH_2N), 4.57−4.61 (t, 2, $CONCH_2$), 6.36−6.39 (d, 1, H-9), 7.17−7.26 (t, 1, H-10), 7.38−7.41 (d, 1, H-4), 7.51 (s, 1, H-7), 7.55−7.61 (t, 1, H-5), 8.55−8.58 (d, 1, H-11), 8.78−8.81 (d, 1, H-6). Anal. ($C_{20}H_{18}N_2O_3\cdot H_2O$) C; H: calcd, 5.68; found, 5.11; N: calcd, 7.9; found, 7.42.

Preparation of 2-[2′-(Dimethylamino)ethyl]-1,2-dihydro-8-methoxy-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (47). A solution of 25 mg (0.075 mmol) of **46** in 50 mL of anhydrous CH_3OH and 5 mL of $CHCl_3$ was treated at 0 °C with a solution of 143 mg (3.4 mmol) of diazomethane in 10 mL of anhydrous ether. The mixture was stirred at 0 °C for 1 h and then refrigerated for 48 h. The solvent was evaporated, and the residual solid was purified by PTLC on silica gel with 5% CH_3OH in $CHCl_3$ as solvent to give 8 mg (31% yield) of **47**, which had mp 165−167 °C after crystallization from hexanes:[1] HNMR ($CDCl_3$) 2.41 (s, 6, NCH_3), 2.70−2.75 (t, 2, CH_2N), 4.11 (s, 3, OCH_3), 4.40−4.46 (t, 2, $CONCH_2$), 6.87−6.90 (d, 1, H-9), 7.67−7.75 (m, 2, H-5 + H-10), 8.34−8.38 (d, 1, H-4), 8.72−8.76 (d, 1, H-6), 9.32 (s, 1, H-7), 9.52−9.56 (d, 1, H-11); HRMS (EI) calcd for $C_{21}H_{20}N_2O_3$ 348.1478, found M^+ 348.1471. Anal. ($C_{21}H_{20}N_2O_3\cdot H_2O$) N; C: calcd, 68.85; found, 69.27; H: calcd, 6.01; found, 5.52.

Preparation of 11-Chloro-2-[2′-(dimethylamino)ethyl]-1,2-dihydro-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (48). A mixture of 2-[2′-(dimethylamino)ethyl]-11-nitro-1,2-dihydro-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione and 2-[2′-(dimethylamino)ethyl]-8-nitro-1,2-dihydro-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione was prepared as described previously.[4] Catalytic hydrogenation of this mixture as described previously for the individual components[4] gave a mixture of the corresponding 8-amino and 11-amino derivatives **35** and **36**, which was used directly in the next step. To a cold (0 °C) stirred solution of 857 mg (2.57 mmol) of the amino derivatives in 28 mL of 4% HCl was added in portions a solution of 260 mg (3.77 mmol) of sodium nitrite. The resulting solution was stirred at 0 °C for 2 h and then added to a solution of 2.97 g (30 mmol) of freshly prepared Cu_2Cl_2 in 27 mL of 11% HCl at room temperature. The mixture was stirred overnight and then at 70 °C for 1 h, neutralized with $NaHCO_3$, and extracted with $CHCl_3$. This extract was concentrated, and the solid residue was separated by PTLC on silica gel with $CHCl_3$−acetone (1:1) as solvent. The first yellow band gave 200 mg (42% yield) of **48**, which had mp 214−216 °C after crystallization from hexanes−toluene (1:1): ^{1}H NMR ($CDCl_3$) 2.39 (s, 6, NCH_3), 2.75−2.81 (t, 2, CH_2N), 4.40−4.45 (t, 2, $CONCH_2$), 7.52−7.58 (t, 1, H-9), 7.71−7.77 (t, 1, H-5), 7.84−7.88 (d, 1, H-10), 8.02−8.05 (d, 1, H-8), 8.29−8.32 (d, 1, H-4), 8.67−8.70 (d, 1, H-6), 8.75 (s, 1, H-7). Anal. ($C_{20}H_{17}ClN_2O_2$) C, H, N; Cl: calcd, 10.07; found, 9.16.

From other bands on the PTLC plate, 12 mg of **49** (see next preparation), 31 mg of **45**, and 33 mg of unreacted **35** were obtained.

Preparation of 2-[2′-(Dimethylamino)ethyl]-1,2-dihydro-11-hydroxy-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (49). This compound was prepared in 60% yield from **36**,[4] sodium nitrite, and 37% HCl following the procedure described for the preparation of **37** and **38**. It had mp 132−133 °C after crystallization from hexanes containing the least amount of toluene: ^{1}H NMR ($CDCl_3$) 2.38 (s, 6, NCH_3), 2.69−2.75 (t, 2, CH_2N), 4.42−4.47 (t, 2, $CONCH_2$), 7.32−7.36 (d, 1, H-10), 7.52−7.71 (m, 3, H-5 + H-8 + H-9), 8.24−8.28 (d, 1, H-4), 8.71−8.74 (d, 1, H-6), 8.77 (s, 1, H-7), 12.07 (s, 1, OH). Anal. ($C_{20}H_{18}N_2O_3$) C, H, N.

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