Amino-Substituted 2-[2′-(Dimethylamino)ethyl]-1,2-dihydro-3*H*-dibenz[*de,h*]isoquinoline-1,3-diones. Synthesis, Antitumor Activity, and Quantitative Structure–Activity Relationship

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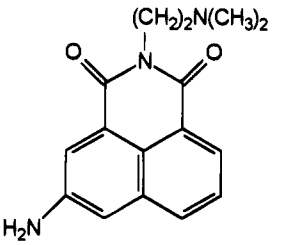
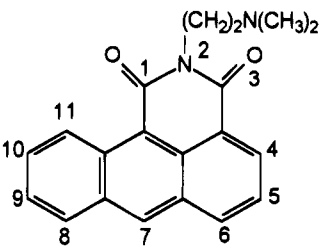
Sets of 2-[2′-(dimethylamino)ethyl]-1,2-dihydro-3*H*-dibenz[*de,h*]isoquinoline-1,3-diones with amino and actylamino groups at each of the eight positions on the anthracene nucleus were synthesized from appropriately substituted anthracenes. Their evaluation in *in vitro* antitumor and cardiotoxicity assays revealed a very strong dependence of potency on the position of substitution. Certain compounds, including the 4-, 5-, 7-, and 9-amino derivatives, showed significantly higher potency than the unsubstituted parent compound, azonafide. Among them, 7-aminoazonafide had low cardiotoxicity relative to cytotoxicity. In general, the acetylamino analogues were less potent than the amino derivatives against tumor cells and neonatal rat heart myocytes; however, 5-(acetylamino)azonafide was highly cardiotoxic. 9-Aminoazonafide was more efficacious than azonafide or amonafide against P388 leukemia in mice. Statistically significant correlations were made between the ability of amino analogues to increase the transition melt temperature (ΔT_m) of DNA and their potency against solid tumors, leukemia cells, or cardiac myocytes.

The previous article in this series outlined the rationale, synthesis, and antitumor activity of 1,2-dihydro-3*H*-dibenz[*de,h*]isoquinoline-1,3-diones with a variety of substituents at the 2-position.[1] These compounds have structural resemblance to amonafide (**1**),[2,3] but they have the anthracene chromophore rather than the naphthalene chromophore. The parent compound in the new series, unofficially named azonafide (**2**), showed significant enhancement of antitumor potency over amonafide when tested against a panel of human and murine tumor cells *in vitro*, and it had some improvement relative to amonafide in the ratio of cardiotoxicity (fetal rat heart myocytes) to the mean cytoxic potency in tumor cell lines. Azonafide also was more active against subcutaneous B16 melanoma in mice. Certain 2-substituted analogues of azonafide showed significant *in vitro* antitumor activity, but none was clearly superior to it.

The present article compares the effects on antitumor activity and cardiotoxicity produced by amino-containing substituents on the anthracene nucleus. The main objective was to prepare a set of analogues with the same substituent, the amino group, at each of the eight nuclear positions, determine their cytotoxicity and cardiotoxicity in cell culture systems, and derive qualitative and quantitative structure–activity relationships between antitumor activity and DNA-binding properties. Because the syntheses of the target compounds, called aminoazonafides, generally involved acetylamino intermediates, the complete set of (acetylamino)azonafides was prepared as a complement to the aminoazonafides. These complementary sets could also determine if the increase in toxicity resulting from metabolic acetylation of the amonafide amino group was a general effect or limited to a specific position on the nucleus. Some [(trimethylacetyl)amino]azonafides also were prepared as intermediates.



1. amonafide



2. azonafide

Chemistry

The aminoazonafides were prepared by a variety of routes, which depended on the availability of starting material and regioselectivity obtained in reactions of the anthracene chromophore (Scheme 1). Nitration of the known anthracene-1,9-dicarboxylic acid anhydride (**3**)[4] with 1 equiv of HNO_3 gave a mixture of 8-nitro derivative **4** and 5-nitro derivative **5**. This product was treated with *N,N*-dimethylethylenediamine to afford a mixture of 11-nitroazonafide (**6**) and 8-nitroazonafide (**9**), which was separated by chromatography. Catalytic hydrogenation of each of these intermediates converted them into 11-aminoazonafide (**7**) and 8-aminoazonafide (**10**). 11-Aminoazonafide was acetylated to give **8**.

When 2-(acetylamino)anthracene (**12**) (prepared by acetylation of **11**) was treated with oxalyl chloride and aluminum chloride in carbon disulfide, a mixture of three oxalyl derivatives **14**–**16** was obtained. This mixture was oxidized to the corresponding dicarboxylic acids with alkaline hydrogen peroxide and then treated with *N,N*-dimethylethylenediamine to give a mixture of (acetylamino)azonafides. Separation of this mixture by preparative TLC gave mainly 10-(acetylamino)-azonafide (**22**) and smaller amounts of 4-(acetylamino)-azonafide (**18**) and 9-(acetylamino)azonafide (**20**). Acid

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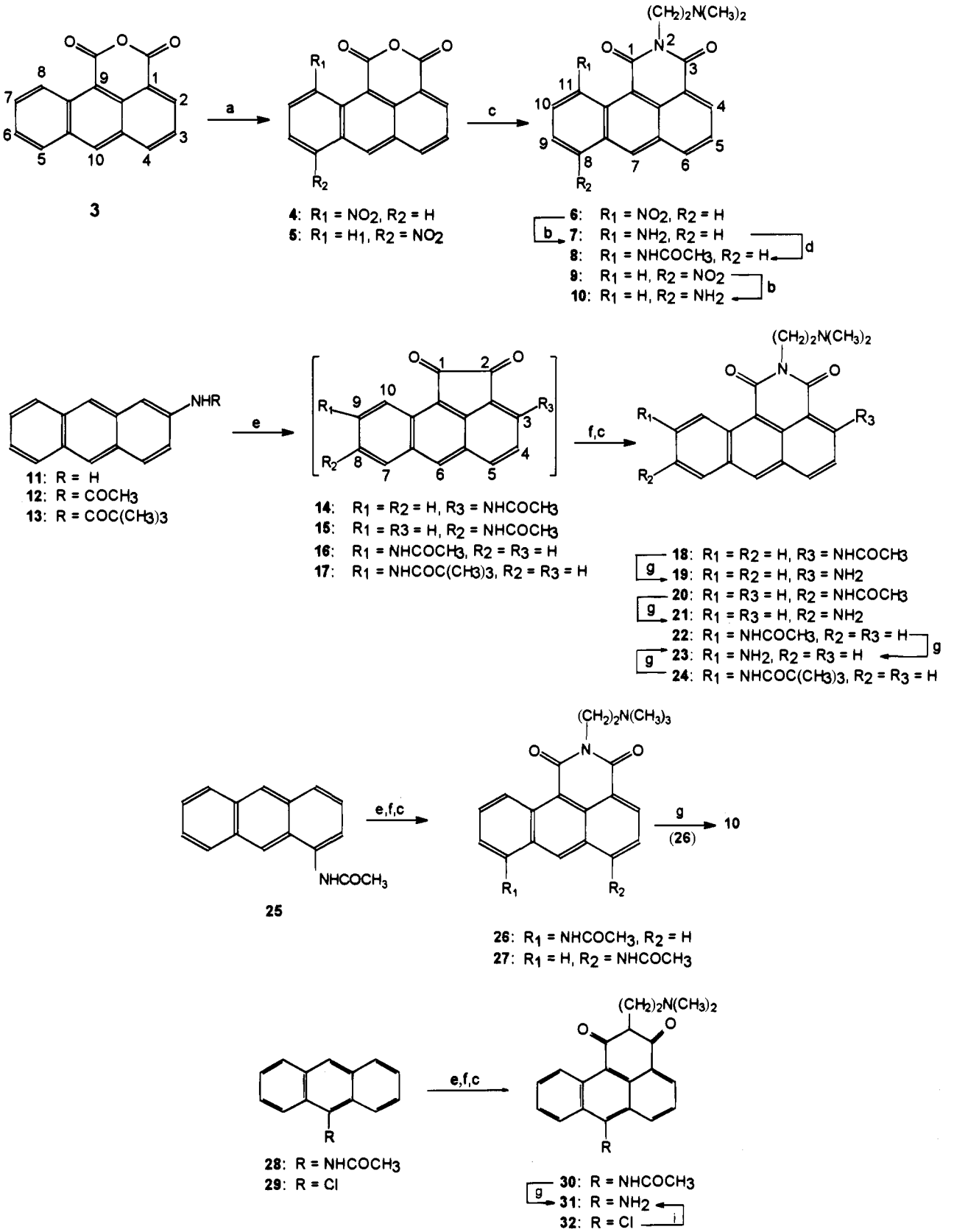
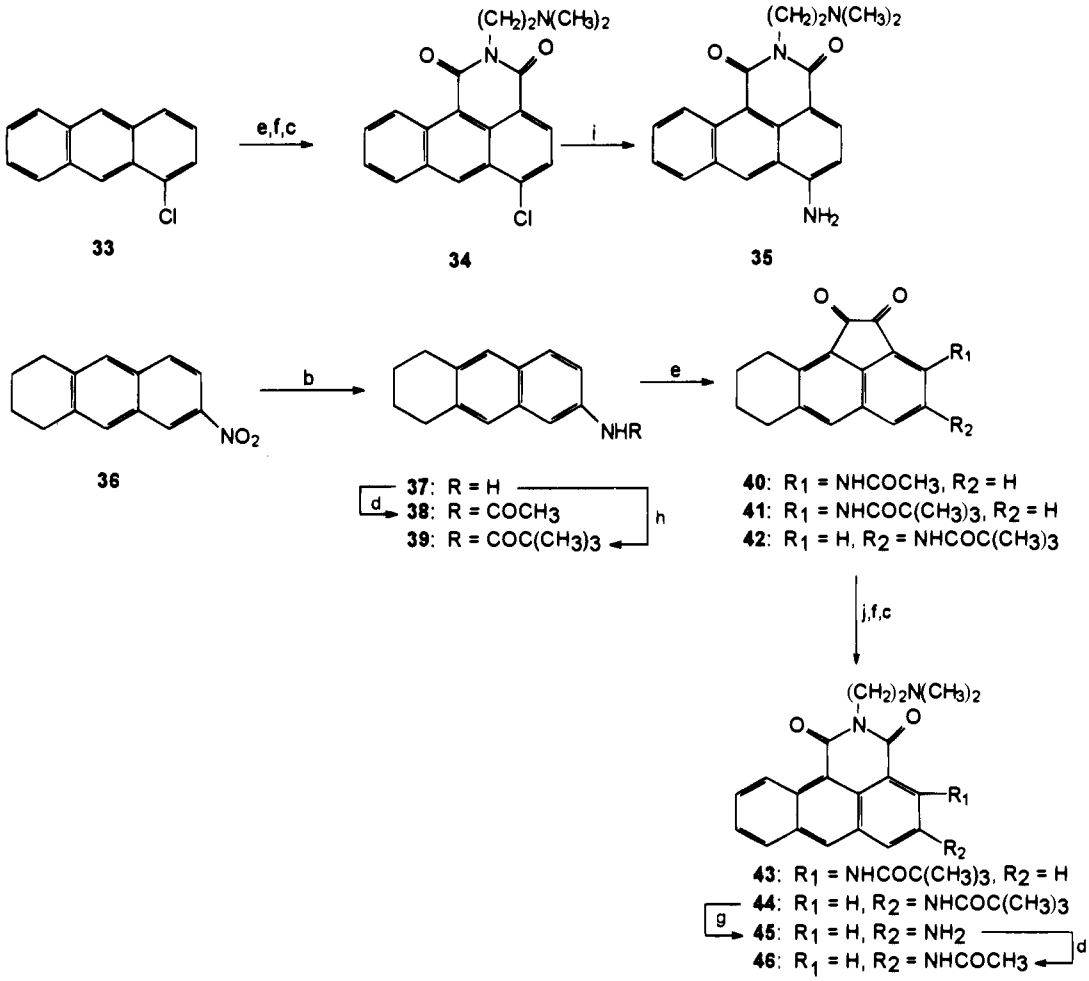
Scheme 1. Synthesis of Aminoazonafides[a]





[a] Reagents: (a) 70% HNO_3, H_2SO_4; (b) H_2, Pd/C; (c) $H_2N(CH_2)_2N(CH_3)_2$; (d) Ac_2O, Et_3N; (e) ClCOCOCl, $AlCl_3$, CS_2; (f) H_2O_2, NaOH; (g) 38% HCl, EtOH, reflux; (h) $(CH_3)_3COCl$, Et_3N; (i) NaN_3, absolute EtOH, reflux; (j) DDQ, dioxane, reflux.

hydrolysis converted these compounds into the corresponding aminoazonafides **23**, **19**, and **21**. An unexpected phenomenon was encountered in the isolation of **22**. This compound gave a single band on TLC, but the solid obtained was only partially soluble in chloroform. The soluble fraction afforded a product that was confirmed to be **22** by its ^1H NMR spectrum and elemental analysis. A similar ^1H NMR spectrum was obtained on the insoluble fraction, except that the two methyl groups and the methylene group attached to the side-chain nitrogen were significantly deshielded. This observation suggested that the insoluble fraction was a salt, which was readily confirmed by dissolving it in water and adding base. A product with an ^1H NMR spectrum identical with that of **22** was obtained. The salt appears to involve silicic acid, although its composition has not been confirmed. We previously obtained a silicic acid salt of triethylamine from silica gel chromatography. Both fractions gave 10-aminoazonafide **23** on hydrolysis. In contrast to the behavior of **12**, 2-[(trimethylacetyl)-amino]anthracene (**13**) gave a single dione **17** when it was treated with oxalyl chloride and aluminum chloride. Conversion of **17** to azonafide analogue **24** was carried out in the usual way. Hydrolysis of **24** gave an improved synthesis of 10-aminoazonafide **23**.

1-(Acetylamino)anthracene **25** was subjected to the same set of reactions described for the elaboration of **12** into azonafide analogues. Thus, treatment with oxalyl chloride followed by oxidation and then treatment with *N,N*-dimethylethylenediamine gave a mixture that was separated into 6-acetylazonafide (**27**) and 8-acetylazonafide (**26**). Acid hydrolysis of **26** provided 8-aminoazonafide (**10**), identical in ^1H NMR spectrum with the sample obtained from reduction of **9**. 9-(Acetylamino)-anthracene (**28**) gave 7-(acetylamino)azonafide (**30**) when it was taken through the reaction sequence of acetylation with oxalyl chloride, oxidation, and amine condensation. Hydrolysis by acid gave 7-aminoazonafide (**31**). An alternative route to **31** was based on displacement by sodium azide, with loss of N_2, of chloride from 7-chloroazonafide (**32**), which was prepared from 9-chloroanthracene (**29**) by the sequence of reactions described above.

6-Aminoazonafide (**35**) was prepared by heating 6-chloroazonafide (**34**) with sodium azide in absolute ethanol. The synthesis of **34** was from 1-chloroanthracene (**33**) by the above reaction sequence. Only one isomer was obtained.

5-(Acetylamino)azonafide (**46**) was the only isomer not obtained by elaboration of 2-(acetylamino)anthracene. In order to obtain **46** and the related amine **45**, it was necessary to devise an alternative synthesis in which initial reaction by oxalyl chloride at C10 was not followed by subsequent acylation in the ring farthest from the acetylamino group (to give **15**). This goal was met by using an acylated form of 6-amino-1,2,3,4-tetrahydroanthracene (**37**), which was obtained by catalytic reduction of the known 6-nitro-1,2,3,4-tetrahydroanthracene (**36**).[5] The first attempt to prepare **45** was based on the acetyl derivative **38** of **37**. Treatment

Table 1. Antitumor Activities and Cardiotoxicity (nM) of Hydrochlorides of Azonafide Analogues IC_{50} for Human and Murine Tumor Cells and Neonatal Rat Myocytes in Culture[a]

no.	testing code, AMP-	UACC375[b] melanoma	OVCAR3[c] ovarian	solid[d] av	L1210 sensitive	L1210[e] resistant	av[f]	cardiotoxicity[g]	toxicity ratio[h]
	doxorubicin	112	35	74	35	3884	61	11064[i]	181
	mitoxantrone	48	5.8	27	5	39	20	7737[i]	387
1	amonafide	2031	2180	2106	625	625	1612	48400	30
2	1 (azonafide)	71	57	64	7.0	7.0	45	1983	44
6	2	75	226	151	23	50	108	8647	80
9	13	38	50	44	50	20	46	650	14
7	3	949	1762	1356	678	678	1130	27100	24
10	14	20325	9485	14905	6775	6775	12195	>81300	>6.7
8	52	2433	2433	2433	243	2433	1703	>22500	>13.2
26	61	1214	2002	1608	485	510	1234	12136	9.8
18	32	973	608	791	608	560	730	≫24331	≫33
20	26	61	13	37	114	170	63	487	7.7
22	27	97	49	73	49	219	65	7786	120
19	33	9.5	12	11	5.4	7.9	9.0	434	48
21	29	45	14	30	2.7	16	21	813	39
24	96	250	150	200	200	200	200	11025	55
23	30	62	20	41	2.5	2.7	28	943	34
27	82	7290	6075	6683	486	729	4617	12880	2.8
30	34	730	730	730	487	487	649	≫24331	≫37
31	35	15	12	14	5.4	6.8	11	1951	177
35	37	149	68	109	5.4	27	74	1486	20
43	86	1601	493	1047	862	172	985	>24630	>25
44	85	132	33	83	44	15	70	662	9.5
45	87	25	20	23	6.2	15	17	1724	101
46	105	73	61	67	19	73	51	177	3.5

[a] The murine leukemia experiments were based on continuous drug exposure using 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.; Shoemaker, R. H.; Boyd, N. R. Feasibility of drug screening with panels of human tumor cells using a microculture tetrazolium assay. *Cancer Res.* **1988**, *48*, 589–601). 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.; Kinney, S.; Boyd, M. R. New Colorimetric Cytotoxicity Assay for Anticancer-Drug Screening. *J. Natl. Cancer Inst.* **1990**, *82*, 1107–1112). [b] A human melanoma line obtained from the University of Arizona Cancer Center. [c] A human ovarian cancer cell line obtained from the NCI. This carcinoma is resistant to many standard anticancer agents. [d] Average IC_{50} for the melanoma and ovarian cell lines. [e] A murine leukemia cell line. The resistant strain is multiple drug resistant. [f] An average of IC_{50} values for the two solid tumors and sensitive L1210. [g] Neonatal rat heart myocyte assay, 1 h exposure. Cytotoxicity is measured by the ATP/protein concentration that reduces this ratio to 0.5 (Dorr, R. T.; Bozak, K. A.; Shipp, N. G.; Hendrix, M.; Alberts, D. S.; Ahmann, F. *In Vitro* Rat Myoctye Cardiotoxicity Model for Antitumor Antibiotics using Adenosine Triphosphate/Protein Ratios. *Cancer Res.* **1988**, 48, 5222–5227). [h] The quotient of the IC_{50} in myocytes divided by the average IC_{50} in three tumor lines (from Table 1). This ratio was 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. [i] This cardiotoxicity value was taken from the preceding reference.

of **38** with oxalyl chloride and aluminum chloride gave almost exclusively the wrong isomer **40**. Reasoning that steric hindrance at C6 would direct the first acylation to C8, we prepared 6-[(trimethylacetyl)amino]-1,2,3,4-tetrahydroanthracene (**39**) and treated it with oxalyl chloride and aluminum chloride. In this case, the desired isomer **42** was the main product, although some of the other isomer **41** was obtained. The yields of both compounds were low. These isomers were separated by chromatography on silica gel, and each one was converted into a [(trimethylacetyl)amino]azonafide (**44** and **43**, respectively) by a route involving aromatization to the anthracene with DDQ and then the usual elaboration with alkaline hydrogen peroxide followed by *N,N*-dimethylethylenediamine. Acid hydrolysis of 5-(trimethylacetyl)azonafide (**44**) gave the corresponding amine **45**, which was acetylated to give **46**.

Structures of the aminoazonafides and intermediates in their synthesis were determined by microanalysis and ^{1}H NMR spectroscopy. Assignment of the positions of substituents on the nucleus was determined by the multiplicity of peaks and by decoupling experiments in the ^{1}H NMR spectra.

Biology

The *in vitro* activities of the complete sets of amino and acetylamino derivatives of azonafide are compared with the activities of amonafide, doxorubicin, and mitoxantrone in Table 1. Compounds are listed by their numbers in Scheme 1 and by the testing code (AMP) numbers (formerly EL numbers). Human tumor cell lines include a A375 malignant melanoma and an ovarian carcinoma (OVCAR-3) that is resistant to standard anticancer drugs. Murine L1210 leukemia cells include a sensitive strain and one that has multiple drug resistance (MDR) based on increased levels of P-glycoproteins.[6] The sulforhodamine[7] or the MTT[8] assay with continuous drug exposure was used, and IC_{50} values were determined. An average of IC_{50} values for sensitive L1210 leukemia and the two human tumors is given as a crude index of the relative potencies of the azonafide analogues. Also given in Table 1 are IC_{50} values for the relative cardiotoxicity of 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, the antitumor potencies of both the amino and acetylamino analogues are highly dependent on their position on the anthracene nucleus. Thus, the average IC_{50} for aminoazonafides varies from 9.0 nM for the 4-substituent (**19**) to 12 195 nM for the

8-substituent (**10**). A similar but less extreme variation is found for (acetylamino)azonafides, among which the average IC_{50} is 51 for the 5-substituted compound **46** and 4617 for the 6-substituted compound **27**. The order for relative potencies is clearly not the same for amino and acetylamino substituents. It is 4 > 7 > 5 > 9 > 10 > 6 ≫ 11 ≫ 8 for the former and 5 > 10 > 9 ≫ 7 > 4 > 8 > 11 ≫ 6 for the latter. In both series, the 8 and 11 substituents have low potencies. Taken as a group, the aminoazonafides are more potent than the (acetylamino)azonafides. We were concerned about the especially low activity of 8-aminoazonafide **10**, together with its behavior as an outlier in all QSAR studies (see below). Consequently, it was prepared by two different routes (Scheme 1) and tested twice. The two samples were identical in TLC and NMR spectrum, and the antitumor potencies were nearly the same.

Cardiotoxicities for azonafide analogues vary widely, but the acetylamino analogues generally are less toxic than the corresponding amino analogues. The toxicity ratio (IC_{50} cardiotoxicity/IC_{50} average cytotoxicity) is a better indicator of relative toxicities. Two compounds, 5-(acetylamino)azonafide (**46**) and 6-(acetylamino)-azonafide (**27**), have very low ratios. For **27**, the low ratio is caused more by poor antitumor activity than by high cardiotoxicity. Some of the compounds have relatively high therapeutic ratios, led by 7-aminoazonafide **31** with a ratio of 177. Analogues **22** and **45** have toxicity ratios greater than 100, and eight more compounds have higher toxicity ratios than amonafide. Both mitoxantrone and doxorubicin have high toxicity ratios (387 and 181, respectively) in comparison with aminoazonafides. 7-Aminoazonafide is the only compound comparable to doxorubicin. This result is interesting in view of the known cardiotoxicity of cumulative doses of dioxorubicin; however, it might not be appropriate to extend this kind of acute cell culture comparison beyond limited sets of analogues. For example, amonafide has a very low ratio (5.5) for cardiotoxicity to cytotoxicity in human 8226 myeloma cells,[10] yet its important clinical toxicity is myelosuppression and not cardiotoxicity.[11]

One serious problem with amonafide (1) is its metabolism to the corresponding acetylamino derivative, which causes increased myelosuppression.[11] Although this assay has not been done with the compounds in Table 1, the cardiotoxicity assay in it can serve as a measure of relative toxicity for aminoazonafides and (acetylamino)azonafides. The extent to which cardiotoxicity increases or decreases when an aminoazonafide is converted into the corresponding acetylaminoazonafide is of prime interest. 5-Aminoazonafide (**45**) has an *N*-acetyl derivative (**46**) in which the cardiotoxicity is increased about 10-fold. Interestingly, **45** has the closest structural resemblance to amonafide of any azonafide analogue. The *N*-acetyl derivative **20** is about twice as cardiotoxic as 9-aminoazonafide **21**. In contrast, N-acetylation significantly decreases the cardiotoxicity of certain other compounds. Thus, 4-aminoazonafide (**19**), 7-aminoazonafide (**31**), 6-aminoazonafide (**35**), and 10-aminoazonafide (**23**) have the less toxic derivatives **18**, **30**, **27**, and **22**, respectively. The 8- and 11-substituted compounds are so inactive that similar toxicity ratios cannot be determined.

Among the (trimethylacetyl)amino derivatives of azonafide, 5-substituted analogue **44** has good overall potency (70 nM average IC50) and is 3-fold more potent against MDR L1210 cells than sensitive ones. 4-Substituted analogue **43** has low average potency (985 nM), but it is 5-fold more potent against MDR than against sensitive L1210 cells. The 10-substituted analogue **24** has moderate overall potency (200 nM) and no advantage against MDR L1210 cells. Increased lipophilicity may be a factor in the greater potency against MDR leukemia relative to sensitive leukemia for (trimethylacetyl)amino analogues **43**, **44**, and **24** compared with the corresponding amino analogues **19**, **45**, and **23**.

Table 2. Activity of Hydrochlorides of Azonafide Analogues Against WiDr Human Colon Tumor in Cell Culture[a]

compound	IC_{50} (nM) sensitive cell line	IC_{50} (nM) resistant cell line[b]
azonafide (**2**)	10	15
23	5.5	18
22	9	50
mitoxantrone	10	1400

[a] Continuous drug exposure in 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.; Shoemaker, 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). [b] Selected for resistence to mitoxantrone.

Table 3. Activity of Hydrochlorides of Azonafide Analogues Against P388 Leukemia in Mice[a]

compound	dose, mg/kg on days 1, 5, and 9	total dose	% ILS
amonafide (1)	10	30	45
	15	45	88
azonafide (**2**; EL-1)	5	15	25
	10	30	63
	15	45	79
10-aminoazonafide (**23**)	4	12	27
	8	24	73
	10	30	109
7-aminoazonafide (**31**)	8	24	31
	10	30	46
	12	36	46
doxorubicin	4.5	13.5	113
mitoxantrone	1.6	4.8	200

[a] Conducted according to standard NCI protocol. The leukemia cells were given ip. Results are expressed as the percent increase in life span (ILS) = 100 × [(life span treated − life span controls)/life span controls].

Table 2 compares the activities of 10-aminoazonafide (**23**), its *N*-acetyl derivative **22**, azonafide (**2**), and mitoxantrone against sensitive and mitoxantrone resistant WiDr human colon tumors in cell culture. The analogues show slight advantages against the sensitive tumor, but they are less effective than azonafide against the resistant tumor.

On the basis of the data in Table 1, two azonafide analogues were selected for further antitumor testing in mice. The selection criteria were high potency, low cardiotoxicity (high toxicity ratio), lack of cross resistance (equal potencies against MDR and sensitive L1210 leukemia cells), and a favorable ratio of activity in the solid tumors to sensitive L1210 leukemia. No compound satisfied all of these criteria, but 7-aminoazonafide (**31**) had an average potency of 11 nM, toxicity ratio of 177, MDR/sensitive L1210 ratio of 1.3, and ratio for the average potency to solid tumors/sensitive L210 of 2.5. For 10-aminoazonafide **23**, the corresponding values were 28, 34, 1.0, and 16.4. Table 3 gives the activities of **31** and **23** against P388 leukemia in mice, compared

Table 4. Correlation between Transition Melt Temperature Increase and Antitumor or Cardiotoxic Potentency of Azonafide Analogues[a]

compd	testing code, AMP-	subst	ΔT_m, °C[d]	cytotoxicity: IC_{50}, nM					
				solid[b]		leukemia[c]		myocytes	
				IC_{50}	log(1/C)	IC_{50}	log(1/C)	IC_{50}	log(1/C)
19	33	4-NH_2	13.2	11	7.96	5.4	8.27	4434	6.36
45	87	5-NH_2	11.4	23	7.64	6.2	8.21	1724	5.76
35	37	6-NH_2	13.8	109	6.96	5.4	8.27	1486	5.83
31	35	7-NH_2	16.0	14	7.85	5.4	8.27	1951	5.71
10	14	8-NH_2	10.2	14905	4.83	6775	5.17	>81300	
21	29	9-NH_2	14.0	30	7.52	2.7	8.57	813	6.09
23	30	10-NH_2	9.5	41	7.39	2.5	8.60	943	6.02
7	3	11-NH_2	4.5	1356	5.87	678	6.17	27100	4.57
				cytotoxicity, IC_{50} in nM					
compd	testing code, AMP-	subst	ΔT_m, °C[d]	solid[b]		leukemia[c]		cardiotox	
				IC_{50}	log(1/C)	IC_{50}	log (1/C)	IC_{50}	log(1/C)
18	32	4-NHAc	15.1	791	6.10	608	6.22	≫24331	
46	105	5-NHAc	11.6	67	7.17	19	7.72	177	6.75
27	82	6-NHAc	9.2	6683	5.17	486	6.32	12880	4.89
30	34	7-NHAc	4.9	730	6.14	487	6.32	≫24331	
26	61	8-NHAc	10.1	1608	5.79	485	6.32	12136	4.92
20	26	9-NHAc	12.9	37	7.43	114	6.94	487	6.31
22	27	10-NHAc	13.2	73	7.14	49	7.31	7786	5.11
8	52	11-NHAc	3.8	2433	5.61	243	6.61	>22500	

[a] Antitumor data is taken from Table 1. [b] An average IC_{50} for the two human solid tumor cell lines in Table 1. [c] The value for sensitive L1210 leukemia cells. [d] Transition melt temperature increase for calf thymus DNA at 5×10^{-5} M (base pairs) in pH 7.0 buffer solution 0.01 M in NaH_2PO_4 and 0.001 M in EDTA. The azonafide analogues were 2×10^{-4} M in the same buffer.

with those of amonafide, azonafide, doxorubicin, and mitoxantrone. Compound **31** is clearly the least effective analogue. There is no large difference in activity among the three compounds: **1**, **2**, and **23**, but **23** is somewhat more potent and efficaceous than the others (ILS = 109% at 10 mg/kg). Although the activity of **31** was surprisingly weak in relation to its *in vitro* activity, it was noted that the ascites fluid did not increase in mice treated with **31**. Presumably they died from central nervous system (CNS) metastasis. As expected from their clinical activity in leukemias, doxorubicin and mitoxantrone are potent in this assay.

QSAR

Based on the assumption that antitumor potency is dependent on DNA binding strength, correlation was attempted using increases in DNA transition melt temperatures (ΔT_m) produced by azonafide analogues as the measure of binding strength. This approach should give a simple relationship within the sets of aminoazonafides and acetylaminoazonafides because other factors such as the partition coefficient should be nearly constant throughout the set. Table 4 gives the calf thymus ΔT_m values for the two sets, together with appropriate data on antitumor potencies against solid tumors (average), sensitive L1210 leukemia, and cardiotoxicity derived from Table 1. 8-Aminoazonafide (**10**) was not used in the correlations because it is a significant outlier. Analysis of data for the set of amines using the program Sigma Plot for Windows[12] fitted the data points with the best curve and then established statistical parameters including r^2 (amount of variance accounted for) and standard error. Results shown were statistically significant at the 95% confidence limit. In all three cases, quadratic equations gave the best fit. For the solid tumors, the derived equation was $\log(1/C) = 3.785 + 0.566\Delta T_m - 0.021(\Delta T_m)^2$ ($r^2 = 0.758$, SE = 0.433, $F = 6.272$). The equation for L1210 leukemia was $\log(1/C) = 3.805 + 0.627\Delta T_m - 0.021(\Delta T_m)^2$ ($r^2 = 0.963$, SE = 0.198, $F = 52.466$), and for cardiotoxicity the equation was $\log(1/C) = 2.687 + 0.498\Delta T_m - 0.019(\Delta T_m)^2$ ($r^2 = 0.939$, SE = 0.241, $F = 15.026$). These equations are plotted in Figure 1. The decrease in potency in going from leukemia to solid tumors to cardiotoxicity is apparent and expected. There is a small increase in the ratio of solid tumor potency to cardiotoxicity as potency increases and an increase of about 1 log in the ratio of leukemia potency to cardiotoxicity as potency increases.

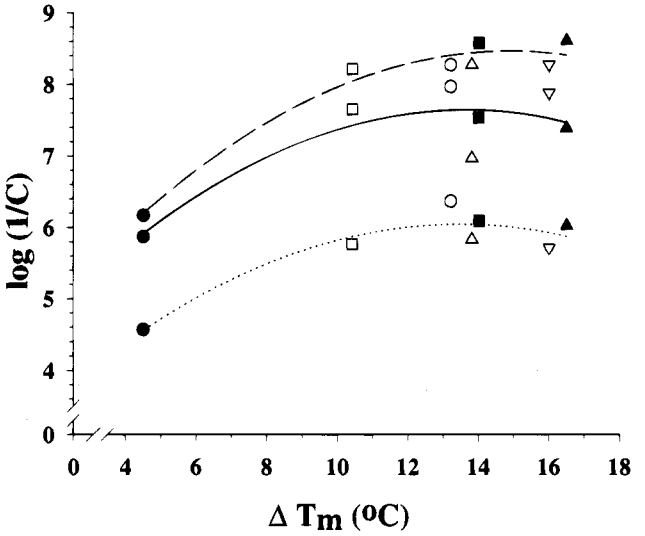


Figure 1. Correlation between ΔT_m and antitumor or cardiotoxic potency for aminoazonafides: (—) solid tumor, (— — —) leukemia, (- - -) cardiotoxicity. Position of NH_2: (●) 11, (□), 5, (○), 4, (△) 6, (■) 9, (▽) 7, (▲) 10.

Correlations between antitumor potency and ΔT_m also were examined for the acetylaminoazonafides. The data are shown in Table 4. The contrast to the aminoazonafides, (acetylamino)azonafides did not afford statistically significant correlations between ΔT_m and cytotoxicity to either solid tumor or leukemia cells. The data are plotted in Figure 2, but curves are not drawn. Quadratic equations gave the "best" fits, but statistical

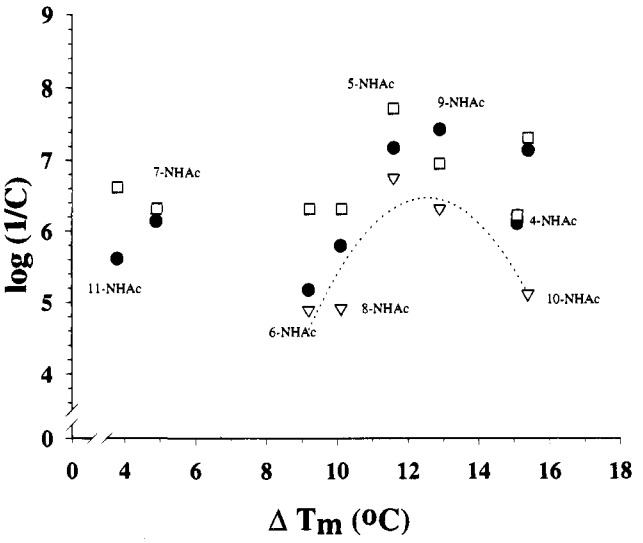


Figure 2. Correlation between ΔT_m and antitumor or cardiotoxic potency for (acetylamino)azonafides: (- -) cardiotoxicity.

parameters for the solid tumors were $r^2 = 0.325$, SE = 0.805, and $F = 1.206$, and they were $r^2 = 0.124$, SE = 0.615, and $F = 0.354$ for L1210 leukemia.

Only five compounds could be used for the correlation between ΔT_m and cardiotoxicity, because (acetylamino)-azonafides **8**, **18**, and **30** were so nontoxic that IC_{50} values could not be determined. These five analogues gave a statistically significant quadratic equation, $\log(1/C) = 19.589 + 4.155\Delta T_m - 0.166(\Delta T_m)^2$, with $r^2 = 0.804$, SE = 0.546, and $F = 4.11$.

Conclusions

Synthetic routes were developed to produce all of the possible aminoazonafides and their acetylamino derivatives. Some of these routes were efficient and gave single isomers, as with the 6-, 7-, and 10-substituted analogues. The other compounds were obtained in small amounts or by extended schemes. Five compounds had greater average antitumor potencies than azonafide. They included the 4-, 5-, 7-, 9-, and 10-amino derivatives. The (acetylamino)azonafides were generally less cytotoxic and cardiotoxic than the corresponding amino compounds, although 5-(acetylamino)azonafide (**46**), the compound most resembling N-acetylated amonafide, was highly cardiotoxic. 7-Aminoazonafide (**31**) had the best ratio of IC_{50} for cardiotoxicity to cytotoxicity, 4 times that of azonafide; it did not show cross resistance with MDR leukemia; and it had a ratio of 2.2:1 for solid tumor to leukemia potency. It is the best candidate for future development among the aminoamonafides. The next best candidate is 4-aminoazonafide (**19**), which had all of the same desirable properties as **31**, except that it showed moderate cardiotoxicity. The 5-amino analogue **45** had good overall activity and low cardiotoxicity, but the toxicity of its *N*-acetyl derivative makes it undesirable. Both the 9- and 10-amino analogues had good potency, but they showed cross resistance in MDR leukemia and unfavorable ratios of solid tumor to leukemia potency. The 10-amino analogue (**23**) had moderate cardiotoxicity and 9-amino analogue (**21**) was moderately cardiotoxic, but its *N*-acetyl derivative **20** was even more toxic.

The most important conclusion from the QSAR study is that it is possible to increase antitumor potency and ΔT_m for aminoazonafides, which agrees with other evidence that DNA binding is fundamental to the mode of action of azonafide analogues. A study in progress, featuring many different types of substituents at a single nuclear position, is addressing the effects of other factors, such as partition coefficient and electronegativity, on antitumor potency and cardiotoxicity.

Experimental Section

Melting points were recorded on a Mel-Temp melting point apparatus and are uncorrected. ^1H NMR spectra were recorded on a Bruker 250 WM spectrometer, and absorptions are reported as downfield from Me_4Si. Some of the spectra showed small meta and para couplings. The coupling constants are listed in the compound descriptions. The usual ortho coupling constants for aromatic rings are not given in detail. 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 preformed on Analtech silica gel plates (20 × 20 × 0.2 cm) using the indicated solvents. All compounds were converted into hydrochlorides for biological assay by treatment with excess concentrated HCl in methanol followed by evaporation.

2-[2′-(Dimethylamino)ethyl]-1,2-dihydro-11-nitro-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (6) and 2-[2′-(Dimethylamino)ethyl]-1,2-dihydro-8-nitro-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (9). A stirred solution of 416 mg (1.68 mmol) of anthracene-1,9-dicarboxylic acid anhydride (**3**)[1,4] in 25 mL of concentrated H_2SO_4 was treated at −10 to −12 °C with a solution of 155 mg of 70% nitric acid (1.7 mmol) in 1 mL of concentrated H_2SO_4. Stirring was continued for 15 min after the addition was complete, and then the mixture was poured into ice water. The resulting yellow precipitate, a mixture of isomeric mononitro derivatives (**4** and **5**), was washed well with water and dried in air. It was used directly in the next step.

A suspension of 510 mg (1.95 mmol) of the mononitro derivatives in 50 mL of dry toluene was treated with a solution of 206 mg (2.35 mmol) of *N,N*-dimethylethylenediamine in 15 mL of dry ethanol. The mixture was heated at reflux temperature for 4 h, during which time an amber solution formed. After evaporation of the solvent, the solid residue was separated into its components by chromatography on a silica gel column using $CHCl_3$−acetone (1:1) as solvent. Concentration of the first yellow fraction gave 247 mg (35%) of **6**, which formed yellow flakes with mp 238−240 °C after crystallization from toluene: ^1H NMR (DMSO-d_6) δ 2.99 (s, 6, CH_3), 3.52−3.57 (t, 2, NCH_2), 4.49−4.53 (t, 2, $CONCH_2$), 7.79−7.86 (t, 1, H5), 7.92−7.98 (t, 1, H9), 8.47−8.50 (d, 1, H4), 8.59−8.67 [d over d (appears as t), 2, H6 + H8)], 8.75−8.77 (d, 1, H10), 9.32 (s, 1, H7). Anal. ($C_{20}H_{17}N_3O_4$) C, H, N.

Concentration of the second yellow fraction gave 181 mg (26%) of **9**, which formed amber cubes with mp 210−212 °C after crystallization from toluene−hexanes (1:1): ^1H NMR ($CDCl_3$) δ 2.40 (s, 6, CH_3), 2.70−2.77 (t, 2, NCH_2), 4.39−4.45 (t, 2, $CONCH_2$), 7.77−7.86 (m, 2, H5 + H10), 8.27−8.30 (d, 1, H4), 8.35−8.39 (d, 1, H6), 8.75−8.80 (d, 1, H9), 9.42 (s, 1, H7), 10.34−10.38 (d, 1, H11). Anal. ($C_{20}H_{17}N_3O_4$) C, H, N.

11-Amino-2-[2′-(dimethylamino)ethyl]-1,2-dihydro-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (7). A solution of 300 mg of **6** in 300 mL of absolute EtOH was treated with 50 mg of 10% Pd−C and shaken with H_2 at 50 psi for 5 h. The mixture was filtered, and the filtrate was concentrated to give a solid that was purified by preparative TLC on silica gel with $CHCl_3$−MeOH (9:1) as solvent. Concentration of the purple band gave 171 mg (62%) of **7**, which formed dark violet crystals with mp 189−191 °C after crystallization from toluene: ^1H NMR (DMSO-d_6) δ 2.94 (s, 6, CH_3), 3.63−3.70 (t, 2, NCH_2), 4.50−4.54 (t, 2, $CONCH_2$), 7.81−7.87 (t, 1, H9), 7.93−8.02 (d over t, 2, H5 + H10), 8.37−8.41 (d, 1, H8), 8.68−8.75 (d over d, 2, H4 + H6), 9.41 (s, 1, H7) 10.30−10.50 (broad, 2, NH_2). Anal. ($C_{20}H_{19}N_3O_2 \cdot {}^1/_4H_2O$) H, N; C: calcd, 71.11; found, 71.53.

8-Amino-2-[2′-(dimethylamino)ethyl]-1,2-dihydro-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (10). This compound

was prepared by the procedure described for **7**. From 150 mg of **9** was obtained 37 mg (27%) of **10** as blue crystals with mp 290–292 °C after purification by preparative TLC (silica gel, $CHCl_3$–MeOH (9:1) followed by crystallization from toluene: ^1H NMR (DMSO-d_6) δ 2.24 (s, 6, CH_3), 2.56–2.61 (t, 2, NCH_2), 4.26–4.31 (t, 2, $CONHCH_2$), 5.89 (s, 2, NH_2), 7.14–7.17 (d, 1, H9), 7.50–7.56 (t, 1, H10), 7.61–7.64 (d, 1, H4), 7.77–7.84 (t, 1, H5), 8.52–8.55 (d, 1 H6), 8.56–8.59 (d, 1, H11), 9.10 (s, 1, H7). Anal. ($C_{20}H_{19}N_3O_2 \cdot {}^1/_4H_2O$) C, H, N.

An alternative preparation of **10** by hydrolysis of *N*-acetyl derivative **26** (see below) was as follows: A mixture of 18 mg of **26**, 1 mL of 37% HCl, and 10 mL of absolute EtOH was refluxed for 4 h. The solvent was evaporated to dryness, and the residue was dissolved in methanol, neutralized with solid sodium bicarbonate, and filtered. The filtrate was evaporated to dryness, and the residue was purified by preparative TLC on silica gel with toluene–methanol (8.5:1.5) as solvent to give 14 mg (88%) of product identical in TLC and ^1H NMR spectrum with the material described above.

11-(Acetylamino)-2-[2′-(dimethylamino)ethyl]-1,2-dihydro-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (8). A solution of 66 mg (2 mmol) of **7** in 5 mL of dry tetrahydrofuran was treated with 45.4 mg of acetic anhydride and 4 drops of triethylamine. After the mixture was stirred at room temperature for 15 h, another 90 mg of acetic anhydride and 12 drops of triethylamine were added, and the mixture was heated at reflux for 24 h. It was then concentrated, and the residue was treated with 20 mL of warm water and then allowed to stand for a few hours. The water was removed in vacuum, and the residue was purified by preparative TLC on silica gel with chloroform–methanol (9:1) as solvent. This procedure gave 18 mg of unreacted **7** and 30 mg (56% based on reacted **7**) of **8** as a yellow solid with mp 156–158 °C after crystallization from Et_2O: ^1H NMR ($CDCl_3$) δ 2.24 (s, 3, $COCH_3$), 2.47 (s, 6, NCH_3), 2.82–2.87 (t, 2, CH_2N), 4.46–4.51 (t, 2, $CONCH_2$), 7.67–7.75 (m, 2, H5 + H9), 7.90–8.00 (d, 1, H10), 8.15–8.18 (d, 1, H8), 8.32–8.35 (d, 1, H4), 8.74–8.76 (d, 1, H6), 8.85 (s, 1, H7), 10.13 (s, 1, NH). Anal. ($C_{22}H_{21}N_3O_3 \cdot {}^1/_4H_2O$) C, H, N.

4-(Acetylamino-2-[2′-(dimethylamino)ethyl]-1,2-dihydro-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (18), 9-(Acetylamino)-2-[2′-(dimethylamino)ethyl]-1,2-dihydro-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (20), and 10-(Acetylamino)-2-[2′-(dimethylamino)ethyl]-1,2-dihydro-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (22). 2-(Acetylamino)-anthracene (**12**) was prepared by stirring a solution of 1 equiv of 2-aminoanthracene (**11**) and 1.5 equiv of Ac_2O for 3 h at room temperature. The crude product (2.9 g, 97% yield) was suspended in 35 mL of CS_2 cooled to 0 °C and then treated with 7 mL of oxalyl chloride. Anhydrous $AlCl_3$ (2.5 g) was added to the stirred mixture while maintaining the temperature at 0 °C. After 2 h, another 35 mL of CS_2 and 2.5 g of $AlCl_3$ were added to the reaction mixture, and stirring was continued at 0 °C for another 2 h and then at room temperature overnight. The mixture was decomposed with cold dilute HCl, and the brown solid that formed was collected and stirred well with 100 mL of 5% NaOH solution. The insoluble material was filtered, washed well with water, and dried in air to give 1.25 g (35%) of a mixture of 3-, 8-, and 9-(acetylamino)-1,2-dihydrocyclopentano[*de*]anthracene-1,2-dione (**14**, **15**, and **16**, respectively) which was used directly in the next step.

A suspension of the mixture of **14**, **15**, and **16** in 25 mL of *p*-dioxane and 8 mL of 2 N NaOH was treated at 15 °C with 8 mL of 30% H_2O_2. The mixture was stirred 45 min at room temperature, diluted with 50 mL of water, and filtered. The brown filtrate was acidified with dilute H_2SO_4, and the brick-red solid that formed was collected, washed well with water, and dried in air to give 1.1 g (80%) of a mixture of 2-, 6-, and 7-(acetylamino)anthracene-1,9-dicarboxylic acids which was used directly in the next step.

A suspension of 500 mg (1.55 mmol) of the (acetylamino)-anthracene-1,9-dicarboxylic acids in 50 mL of toluene was treated with 160 mg (1.82 mmol) of *N,N*-dimethylethylenediamine in 10 mL of ethanol, and the mixture was heated at reflux for 15 h. The solvent was evaporated to dryness, and the residue was fractionated by column chromatography on silica gel using toluene–methanol (8:2) and then chloroform–methanol (8:2) and finally chloroform–methanol (1:1). The first fraction gave a yellow solid which was rechromatographed by preparative TLC on silica gel with chloroform–methanol–triethylamine (19:1:0.15) as solvent to give 14 mg (2.4%) of **18**, crystallized into orange needles from MeOH, mp 202–204 °C: ^1H NMR ($CDCl_3$) δ 2.40 (s, 3, CH_3CO), 2.42 (s, 6, NCH_3), 2.68–2.74 (t, 2, CH_2N), 4.37–4.42 (t, 2-$CONCH_2$), 7.53–7.59 (t, 1, H9), 7.74–7.81 (t, 1, H10), 7.96–7.99 (d, 1, H8), 8.08–8.12 (d, 1, H5), 8.51 (s, 1, H7), 8.99–9.03 (d, 1, H6), 9.91–9.94 (d, 1, H11), 13.32 (s, 1, NH). Anal. ($C_{22}H_{21}N_3O_3 \cdot {}^1/_4H_2O$) C, H, N.

The second fraction gave 118 mg (20%) of **20**, crystallized from toluene containing the least amount of methanol into yellow crystals of melting point 249–252 °C; ^1H NMR ($CDCl_3$ + DMSO-d_6 + CF_3COOD) δ 2.34 (s, 3, CH_3CO), 3.12 (s, 6, NCH_3), 3.62–3.65 (t, 2, CH_2N), 4.62–4.65 (t, 2, $CONCH_2$), 7.66–7.70 (d, 1, H10), 7.72–7.76 (t, 1, H5), 8.33–8.36 (d, 1, H4), 8.43 (s, 1, H8), 8.63–8.66 (d, 1, H6), 8.70 (s, 1, H7), 9.07 (s, 1, NH), 9.77–9.81 (d, 1, H11). Anal. ($C_{22}H_{21}H_3O_3 \cdot H_2O$) C, H, N.

The third fraction gave 339 mg of a two-component mixture. The solid from this fraction was digested well with chloroform and the insoluble component (28 mg), presumed to be the silicic acid salt of 10-(acetylamino)-2-[2′-(dimethylamino)ethyl]-1,2-dihydro-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (**22**), was filtered and crystallized from toluene into red crystals with mp above 360 °C: ^1H NMR (DMSO-d_6) δ 2.23 (s, 3, $COCH_3$), 2.78 (s, 6, NCH_3), 3.27–3.29 (t, 2, CH_2N), 4.44–4.48 (t, 2, $CONCH_2$), 7.78–7.84 (t, 1, H5), 8.06–8.11 (d, 1, H9), 8.20–8.24 (d, 1, H8), 8.55–8.58 (d, 1, H4), 8.61–8.63 (d, 1, H6), 9.08 (s, 1, H7), 10.12 (s, 1, H11), 10.84 (s, 1, NH). Evaporation of the chloroform filtrate gave 308 mg (52%) of **22** which crystallized from toluene as orange crystals with mp 197–199 °C: ^1H NMR ($CDCl_3$) δ 2.28 (s, 3, $COCH_3$), 2.59 (s, 6, NCH_3), 3.06–3.11 (t, 2, CH_2N), 4.35–4.40 (t, 2-$CONCH_2$), 7.08–7.12 (d, 1, H9), 7.50–7.54 (t, 1, H5), 7.75–7.81 (d, 1, H8), 7.91–7.93 (s, over d, 2, H4 + H7), 8.46–8.49 (d, 1, H6), 8.79 (s, 1, H11), 9.23 (s, 1, NH). Anal. ($C_{22}H_{21}N_3O_3 \cdot {}^1/_2H_2O$) C, H, N.

Treatment of the presumed silicic acid salt with dilute NaOH gave a solid which had an identical R_f on TLC, in the system described above, as the main product.

2-[2′-(Dimethylamino)ethyl]-10-[(trimethylacetyl)amino]-1,2-dihydro-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (24). 9-[(Trimethylacetyl)amino]-1,2-dihydrocyclopentano[*de*]-anthracene-1,2-dione (**17**) was obtained in 76% yield as the sole isomer from the Friedel–Crafts reaction of 2-[(trimethylacetyl)amino]anthracene (**13**) and oxalyl chloride following the procedure described for (**14**–**16**). Oxidation of the product with 30% hydrogen peroxide using the previous procedure gave 6-[(trimethylacetyl)amino]anthracene-1,9-dicarboxylic acid in 81% yield. A suspension of 1.095 g of the diacid in 65 mL of toluene was treated with a solution of 317 mg of *N,N*-dimethylethylenediamine in 15 mL of ethanol, and the mixture was heated under reflux for 1.5 h. The solvent was removed under reduced pressure, and the residue was purified by PTLC on silica gel with toluene–methanol (9:1) to give 0.734 g (59%) of **24** as orange crystals with melting point 203–205 °C after crystallization from hexane containing the least amount of toluene: ^1H NMR ($CDCl_3$) δ 1.43 (s, 9, CH_3), 2.41 (s, 6, CH_3N), 2.69–2.74 (t, 2, CH_2N), 4.38–4.44 (t, 2, $CONCH_2$), 7.62–7.68 (t, 1, H5), 7.79–8.01 (s over d, 2, NH + H9), 8.22–8.26 (dd, $J_{4,6}$ = 1.024, 1, H4), 8.38–8.43 (dd, $J_{8,11}$ = 2.076, 1, H8), 8.63 (s, 1, H7), 8.68–8.72 (dd, $J_{6,4}$ = 1.29, 1, H6), 9.67 (d, $J_{11,8}$ = 2.044, 1, H11). Anal. ($C_{25}H_{27}N_3O_3$) C, H, N.

4-Amino-2-[2′-(dimethylamino)ethyl]-1,2-dihydro-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (19). A mixture of 15 mg (0.04 mmol) of **18**, 25 mL of ethanol, and 2.5 mL of 38% HCl was heated under reflux for 3 h and then concentrated. The residue was dissolved in methanol, and the solution was made slightly alkaline with methanolic KOH. The resulting solution was concentrated under reduced pressure at 25 °C, and the residue was purified by preparative TLC on silica gel with toluene–methanol (8:2) as solvent. This procedure gave 7 mg (53%) of **19** as yellow solid with mp 207–209 °C: ^1H NMR

($CDCl_3$) δ 2.45 (s, 6, NCH_3), 2.76–2.81 (t, 2, CH_2N), 4.43–4.49 (t, 2, $CONCH_2$), 6.27 (broad s, 1, NH), 6.74–6.77 (d, 1, H5), 7.52–7.55 (t, 1, H9), 7.60–7.64 (d, 1, H6), 7.74–7.81 (t, 1, H10), 7.89–7.92 (d, 1, H8), 8.17 (s, 1, H7), 10.03 (broad s, 1, NH, H-bonded), 10.03–10.07 (d, 1, H11). Anal. ($C_{20}H_{19}N_3O_2 \cdot {}^1/_2H_2O$) C, H, N.

9-Amino-2-[2′-(dimethylamino)ethyl]-1,2-dihydro-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (21). This compound was prepared by the procedure described for **19**. From 195 mg of **20** was obtained 85 mg (49%) of **21** as brick-red solid that had mp 185–187 °C after crystallization from toluene containing the least amount of methanol: ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 2.34 (s, 6, NCH_3), 2.61–2.67 (t, 2, CH_2N), 4.30–4.35 (t, 2, $CONCH_2$), 5.34 (s, 2, NH_2), 7.11 (s, 1, H8), 7.37–7.42 (d, 1, H10), 7.62–7.68 (t, 1, H5), 8.25–8.29 (d, 1, H4), 8.49–8.52 (s over d, 2, H6 + H7), 9.70–9.74 (d, 1, H11). Anal. ($C_{20}H_{19}N_3O_2$) C, H, N.

10-Amino-2-[2′-(dimethylamino)ethyl]-1,2-dihydro-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (23). This compound was prepared in 76% yield from **22** or **24** by the procedure described for **19**. It crystallized from toluene as violet needles with mp 193–195 °C: ^1H NMR ($CDCl_3$) δ 2.46 (s, 6, CH_3), 2.79–2.84 (t, 2, CH_2N), 4.42–4.47 (t, 2, $CONCH_2$), 4.79 (s, broad, 2, NH_2), 6.87–6.90 (dd, $J_{9,11}$ = 2.148, 1, H9), 7.53–7.59 (t, 1, H5), 7.70–7.74 (d, 1, H8), 8.16–8.20 (dd, 1, H4), 8.43 (s, 1, H7), 8.66–8.69 (dd, 1, H6), 9.04–9.05 (d, $J_{11,9}$ = 1.986, 1, H11). Anal. ($C_{20}H_{19}N_3O_2 \cdot {}^1/_4H_2O$) C, H, N.

8-(Acetylamino)-2-[2′-(dimethylamino)ethyl]-1,2-dihydro-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (26) and 6-(Acetylamino)-2-[2′-(dimethylamino)ethyl]-1,2-dihydro-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (27). A mixture of 4- and 5-(acetylamino)anthracene-1,9-dicarboxylic acids was prepared in an overall combined yield of 41% from 1-(acetylamino)anthracene (**25**) by the procedure described under the preparation of **18**, **20**, and **22**. A suspension of 2.27 g (7 mmol) of this mixture in 100 mL of toluene was heated at reflux overnight with a solution of 0.74 g (8.4 mmol) of *N,N*-dimethylethylamine in 10 mL of ethanol. Evaporation of the solvent gave 2.6 g (98% crude) of a reddish-brown solid. A sample of the solid (120 mg) was resolved into two components by preparative TLC on silica gel with chloroform–acetone–triethylamine (50:50:1.5) as solvent. The first band gave 18 mg (15%) of **27** as red solid with mp 253–255 °C after crystallization from methanol–ether: ^1H NMR (DMSO-d_6) δ 2.32 (s, 3, CH_3CO), 2.82 (s, 6, NCH_3), 3.34–3.44 (t, 2, CH_2N), 4.43–4.48 (t, 2, $CONCH_2$), 7.86–7.92 (m, 3, H5 + H9 + H10), 8.66–8.69 (d, 2, H4 + H8), 9.46 (s, 1, H7), 9.71–9.75 (dd, 1, H11), 10.45 (s, 1, NH). Anal. ($C_{22}H_2N_3O_3 \cdot H_2O$) C, N; H: calcd, 5.85; found, 5.09.

The second band gave 52 mg (43%) of **26**, which had mp 245–250 °C after crystallization from methanol–ether: ^1H NMR (DMSO-d_6) δ 2.32 (s, 3, CH_3CO), 2.43 (s, 6, NCH_3), 2.80–2.84 (t, 2, CH_2N), 4.29–4.34 (t, 2, $CONCH_2$), 7.84–7.90 (m, 3, H5 + H9 + H10), 8.61–8.66 (m, 2, H4 + H6), 9.39 (s, 1, H7), 9.70–9.74 (dd appears as t, 1, H11), 10.40 (s, 1, NH). Anal. ($C_{22}H_{21}N_3O_3 \cdot {}^1/_4H_2O$) C, H, N.

7-(Acetylamino)-2-[2′-(dimethylamino)ethyl]-1,2-dihydro-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (30). 9-(Acetylamino)anthracene (**28**) was prepared from 9-nitroanthracene by the known method.[13] To a cold (0 °C) stirred suspension of 1.67 g (7.08 mmol) of **28** in 15 mL of anhydrous CS_2 was added 4 mL (44.3 mmol) of oxalyl chloride followed by 2 g (15.3 mmol) of anhydrous $AlCl_3$. After 2 h another 2 g of $AlCl_3$ and 20 mL of CS_2 were added. Stirring was continued at 0 °C for 2 h and then at room temperature overnight. The mixture was decomposed with cold HCl, and the yellow precipitate that formed was collected and digested well with 70 mL of 5% NaOH. The insoluble solid (0.58 g) was filtered and suspended in a mixture of 50 mL of dioxane and 4 mL of 2 N NaOH. This suspension was cooled to 0 °C, treated with 4 mL of 30% H_2O_2, and stirred for 45 min at room temperature. It was then diluted with 100 mL of water and acidified with dilute H_2SO_4 to give 319 mg (49%) of crude 10-(acetylamino)anthracene-1,9-dicarboxylic acid, which was used directly in the next step.

A suspension of 319 mg (0.99 mmol) of the above product in 25 mL of toluene was treated with 132 mg (1.5 mmol) of *N,N*-dimethylethylenediamine in 10 mL of ethanol. The mixture was heated at reflux for 18 h and then cooled to room temperature and filtered. The filtrate was concentrated to dryness, and the residual solid was purified by column chromatography on silica gel with toluene–methanol (8:2) as solvent to give 130 mg (35%) of **30** as yellow solid that had mp 267–269 °C after crystallization from toluene: ^1H NMR (DMSO-d_6) δ 2.38 (s, 6, NCH_3), 2.45 (s, 3, $COCH_3$), 2.66–2.72 (t, 2, CH_2N), 4.36–4.41 (t, 2, $CONCH_2$), 7.53–7.59 (t, 1, H9), 7.62–7.68 (t, 1, H5), 7.71–7.78 (t, 1, H10), 8.15–8.19 (d, 1, H8), 8.34–8.38 (d, 1, H4), 8.63–8.65 (d, 1, H6), 9.93–9.97 (d, 1, H11), 10.34 (s, 1, NH). Anal. ($C_{22}H_2N_3O_3$) C, H. N.

7-Chloro-2-[2′-(dimethylamino)ethyl]-1,2-dihydro-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (32). 10-Chloroanthracene-1,9-dicarboxylic acid was prepared in an overall yield of 57% from 9-chloroanthracene (**29**) by the procedure described under the preparation of **30**. It crystallized from 1,4-dioxane into yellow needles of melting point 269–271 °C. A suspension of 875 mg (2.91 mmol) of the diacid in 50 mL of dry toluene was treated with 295 mg (3.35 mmol) of *N,N*-dimethylethylenediamine, and the mixture was heated under reflux for 8 h. The solvent was removed under reduced pressure, and the residue was chromatographed on a silica gel column with toluene–methanol (8:2) as solvent to give a product which was rechromatographed by PTLC on silica gel with toluene–methanol (9:1) as solvent to afford 713 mg (69%) of **32**, crystallized from hexanes–toluene (4:1): mp 169–171 °C; ^1H NMR ($CDCl_3$) δ 2.4 (s, 6, NCH_3), 2.65–2.80 (t, 2, CH_2N), 4.34–4.50 (t, 2, $CONCH_2$), 7.55–7.88 (m, 3, H5 + H9 + H10), 8.50–8.62 (d, 1, H8), 8.70–8.75 (d, 1, H4), 8.75–8.80 (d, 1, H6), 9.98–10.00 (d, 1, H11). Anal. ($C_{20}H_{17}ClN_2O_2$) C, H, N.

7-Amino-2-[2′-(dimethylamino)ethyl]-1,2-dihydro-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (31). Method A. This compound was prepared by the procedure described for **19**, except that the ratio of ethanol to 38% HCl was 5:1. From 59 mg of **30** was obtained, after purification by PTLC and crystallization from toluene containing the least amount of methanol, 18 mg (34%) of **31** as dark pink crystals with mp 266–268 °C: ^1H NMR (DMSO-d_6) δ 2.25 (s, 6, NCH_3), 2.49–2.57 (t, 2, CH_2N), 4.22–4.27 (t, 2, $CONCH_2$), 7.48–7.53 (t, 1, H9), 7.58–7.63 (t, 1, H5), 7.76–7.82 (t, 1, H10), 8.57–8.63 (t, 2, H4 + H8), 8.70 (s, 2, NH_2), 8.93–8.97 (d, 1, H6), 9.94–9.99 (d, 1, H11). Anal. ($C_{20}H_{19}N_3O_2 \cdot 2^3/_4H_2O$) C, N; H: calcd, 6.40; found, 5.54.

Method B. A mixture of 100 mg of 7-chloroazonafide (**32**), 80 mg of sodium azide, and 200 mL of absolute ethanol was heated at reflux overnight. The solvent was removed under reduced pressure, and the residue left was purified by PTLC on silica gel with $CHCl_3$–MeOH (8:2) to give 20 mg of unreacted **32** and 59 mg of **31** (78% based on reacted material).

6-Chloro-2-[2′-(dimethylamino)ethyl]-1,2-dihydro-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (34). To a cold (0 °C) stirred mixture of 2 g (9.41 mmol) of 1-chloroanthracene (**33**) and 4.5 mL of oxalyl chloride in 20 mL of CS_2 was added 2 g of anhydrous $AlCl_3$. After 2 h, additional CS_2 (20 mL) and $AlCl_3$ (1.5 g) were added, and stirring was continued for 2 h at 0 °C and then overnight at room temperature. Dilute HCl was added, and the orange precipitate that formed was collected by filtration, washed with water, and then digested well with 100 mL of 5% NaOH. The insoluble solids were collected, washed with water, and dried in air to give 1.61 g (64%) of 6-chloro-1,2-dihydrocyclopentano[*de*]anthracene-1,2-dione. A 1.52 g portion of this intermediate was suspended in 10 mL of 2 N NaOH and 50 mL of *p*-dioxane at 15 °C and treated at 15 °C with 8.5 mL of 30% hydrogen peroxide solution with shaking. The resulting mixture was stirred for 45 min at room temperature, diluted with 100 mL of water, and acidified with dilute H_2SO_4. The precipitate was collected, washed with water, and dried in air to give 1.53 g (90%) of crude 4-chloroanthracene-1,9-dicarboxylic acid.

A suspension of 500 mg (1.66 mmol) of the dicarboxylic acid in 30 mL of toluene containing 150 mg (1.7 mmol) of *N,N*-dimethylethylenediamine and 51 mL of ethanol was heated at reflux for 4 h. The solvent was removed under reduced pressure, and the residue was purified by column chromatography on silica gel with chloroform–methanol (9:1) as solvent.

This procedure gave 503 mg (86%) of **34** as yellow solid that had mp 160–162 °C after crystallization from hexane containing the least amount of methanol: ^{1}H NMR ($CDCl_3$) δ 2.39 (s, 6, NCH_3), 2.70–2.73 (t, 2, CH_2N), 4.40–4.43 (t, 2, $CONCH_2$), 7.66–7.69 (t, 1, H9), 7.79–7.81 (d, 1, H5), 7.85–7.88 (t, 1, H10), 8.16–8.18 (d, 1, H8), 8.61–8.63 (d, 1, H4), 9.21 (s, 1, H7), 9.98–10.00 (d, 1, H11). Anal. ($C_{20}H_{17}ClN_2O_2$) C, H, N.

6-Amino-2-[2′-(dimethylamino)ethyl]-1,2-dihydro-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (35). A mixture of 50 mg (0.14 mmol) of **34** and 10 mg (0.15 mmol) of sodium azide in 15 mL of absolute ethanol was heated at reflux for 48 h. The solvent was removed under reduced pressure, and the residue was purified by preparative TLC on silica gel with chloroform–methanol (8:2) as solvent. This procedure gave 16.1 mg of unreacted **34** and 23 mg (72% based on reacted material) of **35** as a pink solid that had mp 225–227 °C after recrystallization from toluene: ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ 2.27 (s, 6, NCH_3), 2.52–2.58 (t, 2, CH_2N), 4.22–4.27 (t, 2, $CONCH_2$), 6.77–6.81 (d, 1, H5), 7.55–7.81 (t, 1, H9), 7.75–7.82 (t, 1, H10), 8.08–8.11 (d, 1, H8), 8.22 (s, 2, NH_2), 8.35–8.38 (d, 1, H4), 9.39 (s, 1, H7), 9.91–9.94 (d, 1, H11). Anal. ($C_{20}H_{19}N_3O_2 \cdot H_2O$) C, N; H: calcd, 5.97; found, 5.44.

When DMF was used as solvent and the reflux time was 20 min, **35** was obtained in 76% yield (based on reacted material).

2-[2′-(Dimethylamino)ethyl]-4-[(trimethylacetyl)amino]-1,2-dihydro-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (43) and 2-[2′-(Dimethylamino)ethyl]-5-[(trimethylacetyl)amino]-1,2-dihydro-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (44). A mixture of 4 g (17.6 mmol) of 1,2,3,4-tetrahydro-7-nitroanthracene (**36**),[5] 0.5 g of 10% palladium on carbon, and 200 mL of methanol was shaken with hydrogen at 50 psi for 5 h. The catalyst was removed by filtration, and the filtrate was concentrated to give 3.4 g (97%) of crude 7-amino-1,2,3,4-tetrahydroanthracene (**37**). Without further purification, 3.2 g (16.2 mmol) of this product was dissolved in 50 mL of dry THF and treated with 2.7 g (26.7 mmol) of triethylamine followed by 3.0 g (24.9 mmol) of trimethylacetyl chloride. The resulting solution was stirred overnight at room temperature and concentrated under reduced pressure, and the residue was triturated with warm water. The solids were washed with water and dried in air to give 4.52 g (99%) of 6-[(trimethylacetyl)amino]-1,2,3,4-tetrahydroanthracene (**39**), which had mp 202–204 °C after crystallization from methanol: ^{1}H NMR ($CDCl_3$) δ 1.35 (s, 9, CH_3), 1.81–1.87 (m, 4, H2 + H3), 2.9–2.97 (m, 4, H2 + H3), 7.31–7.35 (dd, $J_{7,5}$ = 2.196, 1, H7), 7.45 (broad s, 3, H9 + H10 + NH), 7.62–7.65 (d, 1, H8), 8.09–8.10 (d, $J_{5,7}$ = 2.176, 1, H5). Anal. ($C_{19}H_{23}NO$) C, H, N.

To a cold (−5 °C) vigorously stirred solution of 4.52 g (16.1 mmol) of **39** in 220 mL of CS_2 was added 15 mL (170.8 mmol) of oxalyl chloride followed by 6 g (45 mmol) of aluminum chloride. The mixture was stirred vigorously for 6 h at −5 to 0 °C and then overnight at room temperature. It was decomposed with 250 mL of cold dilute HCl, and the precipitate that formed was collected by filtration. Removal of CS_2 from the filtrate by evaporation gave a solid residue that was combined with the precipitate. The combined solids were stirred 30 min with 100 mL of 5% NaOH and filtered. The insoluble solid (2.1 g) was chromatographed on a silica gel column with chloroform as solvent to give two yellow fractions. The first fraction afforded 232 mg (4.3%) of 1,2,7,8,9,10-hexahydro-3-[(trimethylacetyl)amino]cyclopentano[*de*]-anthracene-1,2-dione (**41**), which had mp 276–278 °C after crystallization from ethanol–dioxane (3:1): ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 9, CH_3), 1.88–1.43 (m, 4, H8 + H9), 3.03–3.10 (m, 2, H4), 3.40–3.47 (m, 2, H10), 7.57 (s, 1, H6), 7.97–8.00 (d, 1, H4), 8.75–8.79 (d, 1, H5), 9.92 (s, 1, NH). Anal. ($C_{21}H_{21}NO_3 \cdot {}^3/_4H_2O$) C, H, N.

Concentration of the second fraction gave 525 mg of 1,2,7,8,9,10-hexahydro-3-[(trimethylacetyl)amino]cyclopentano-[*de*]anthracene-1,2-dione (**42**), which contained a small amount (ca. 10%) of **41**. Further purification by crystallization from ethanol–dioxane (3:1) gave material with mp 337–339 °C: ^{1}H NMR (DMSO-d_6) δ 1.30 (s, 9, CH_3), 1.81–1.83 (m, 4, H8 + H9), 3.01–3.07 (m, 2, H7), 3.24–3.29 (m, 2 H, H10), 7.92 (s, 1, H6), 8.152–8.158 (d, $J_{3,5}$ = 1.591, 1, H3), 8.507–8.514 (d, $J_{5,3}$ = 1.642, 1, H5), 9.68 (s, 1, NH).

A mixture of 405 mg (1.21 mmol) of ca. 90% pure **42** and 1.0 g (4.40 mmol) of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone in 50 mL of dry *p*-dioxane was heated under reflux for 108 h, cooled to room temperature, and filtered. The filtrate was concentrated under reduced pressure, and the residue (400 mg, 99.9%) of 1,2-dihydro-4-[(trimethylacetyl)amino]cyclopentano-[*de*]anthracene-1,2-dione and its isomer was dissolved in 10 mL of *p*-dioxane. This solution was cooled to 15 °C, stirred, and treated with 4 mL of 2 N NaOH and 3 mL of 30% hydrogen peroxide. It was then stirred 1 h at room temperature, diluted with 30 mL of water, and acidified with dilute H_2SO_4. The yellow precipitate of 3-[(trimethylacetyl)amino]anthracene-1,9-dicarboxylic acid (220 mg) containing a small amount of 2-[(trimethylacetyl)amino]anthracene-1,9-dicarboxylic acid was used directly in the next step.

A suspension of 220 mg (0.6 mmol) of the above dicarboxylic acid in 50 mL of toluene was treated with a solution of 84 mg (0.95 mmol) of *N,N*-dimethylethylenediamine in 20 mL of absolute ethanol. The mixture was heated at reflux overnight and concentrated under reduced pressure, and the solid residue was purified by column chromatography on silica gel with chloroform–methanol (19:1) as solvent. Concentration of the first yellow fraction gave 15 mg (6%) of **43**, which had mp 216–218 °C after crystallization from methanol: ^{1}H NMR ($CDCl_3$) δ 1.46 (s, 9, CH_3), 2.41 (s, 6, NCH_3), 2.69–2.75 (t, 2, CH_2N), 4.43–4.49 (t, 2, $CONH_2$), 7.57–7.63 (t, 1, H9), 7.79–7.85 (t, 1, H10), 8.04–8.08 (d, 1, H5), 8.20–8.24 (d, 1, H8), 8.65 (s, 1, H7), 9.15–9.19 (d, 1, H6), 10.00–10.04 (d, 1, H11), 13.65 (s, 1, NH). Anal. ($C_{25}H_{27}N_3O_3 \cdot {}^1/_4H_2O$) C, H, N.

Concentration of the second fraction gave 107 mg (43%) of **44** as orange solid with mp 158–161 °C after crystallization from toluene–hexanes: ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9, CH_3), 2.40 (s, 6, NCH_3), 2.68–2.73 (t, 2, CH_2N), 4.36–4.41 (t, 2, $CONCH_2$), 7.54–7.60 (t, 1, H9), 7.69–7.76 (t, 1, H10), 7.85 (s, 1, NH), 7.97–8.00 (d, 1, H8), 8.28–8.29 (d, $J_{4,6}$ = 2.298, 1, H4), 8.58 (s, 1, H7), 8.98–8.99 (d, $J_{6,4}$ = 2.250, 1, H6), 9.79–9.83 (d, 1, H11); HRMS (EI) *m/z* 417.1965 ($C_{25}H_{27}N_3O_3$ requires 417.2052).

5-Amino-2-[2′-(dimethylamino)ethyl]-1,2-dihydro-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (45). A mixture of 25 mg of **44**, 50 mL of ethanol, and 3 mL of 38% HCl was heated at reflux for 24 h and then concentrated under reduced pressure. The residual solid was dissolved in methanol, and the solution was made slightly alkaline with methanolic KOH and then concentrated under reduced pressure at 25 °C. The residue was purified by PTLC on silica gel with chloroform–methanol (19:1) as solvent to give 10.5 mg of starting material and 11 mg (95%) of **45** (based on reacted material) as a brick-red solid which contained 0.22 mol of trimethylacetic acid according to integration of the NMR spectrum (peak at 1.25 ppm). It gave a hydrochloride salt with no definite melting point: ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ 1.25 (s, 2, $(CH_3)_3C$), 2.36 (s, 6, NCH_3), 2.63–2.69 (t, 2, CH_2N), 4.31–4.37 (t, 2, $CONCH_2$), 5.38 (s, 2, NH_2), 7.36–7.37 (d, $J_{4,6}$ = 2.376, 1, H4), 7.48–7.55 (t, 1, H9), 7.60–7.67 (t, 1, H10), 7.75 (s, OH of $(CH_3)_3CCO_2H$), 7.96–7.99 (d, 1, H8), 8.30–8.31 (d, $J_{6,4}$ = 2.447, 1, H6), 8.48 (s, 1, H7), 9.78–9.82 (d, 1, H11); HRMS (EI) 333.1468 ($C_{20}H_{19}N_3O_2 \cdot HCl$ requires 333.1477).

5-(Acetylamino)-2-[2′-(dimethylamino)ethyl]-1,2-dihydro-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (46). A mixture of 25 mg of **45**, 113 mg of acetic anhydride, and 130 mg of triethylamine in 30 mL of dry tetrahydrofuran was heated at reflux overnight. The solvent was removed under reduced pressure, and the residue was purified by PTLC on silica gel with chloroform–methanol (9:1) as solvent to give 20 mg (71%) of **46**, which gave a hydrochloride salt with no definite melting point: ^{1}H NMR ($CDCl_3$) δ 2.18 (s, 3, CH_3CO), 2.59 (s, 6, NCH_3), 2.92–2.97 (t, 2, CH_2N), 4.28–4.33 (t, 2, $CONCH_2$), 7.40–7.46 (t, 1, H9), 7.56–7.63 (t, 1, H10), 7.68–7.72 (d, 1, H8), 8.07–8.08 (s over d, 2, H4 + H7), 8.26–8.27 (d, $J_{6,4}$ = 1.894, 1, H6), 9.35 (s, 1, NH), 9.54–9.58 (d, 1, H11). Anal. ($C_{22}H_{21}N_3O_3 \cdot HCl \cdot 1{}^1/_4H_2O$) C, H; N: calcd, 9.70; found, 9.17.

Microculture Tetrazolium Assay. This assay is based on reductive cleavage of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium (MTT) bromide to a colored formazan compound as an indicator of cell viability.[8] Tumor cells were

plated at 50 000/well onto 96-well microliter plates (Costar, Cambridge, MA). On day two, drugs dissolved initially in DMSO (J. T. Baker, analytical grade) and then diluted serially with phosphate buffered saline (pH 7.4) were added at concentrations of $10^{1}-10^{-5}$ μg/mL in half-log gradations. Final concentrations of DMSO did not exceed 0.1%. The plates were incubated at 37 °C with 5% CO_2, 95% air, and 100% relative humidity for 6 days.

After the 6-day exposure period, 50 μL of a 2 mg/mL MTT solution was added to each of the wells, and the plates were incubated an additional 4 h. The medium was then aspirated, and the formazan product was solubilized by DMSO (100 μL/well). The intensity of the color, which is proportional to viable cell numbers, was quantitated by absorbance at 570 nm on an automated microculture plate reader (Biomek 1000, Beckman Instruments). Test results were calibrated in percent control absorbance from untreated tumor cells. Each drug concentration was tested in 6 wells and the IC_{50} values were averaged. The results are given in Tables 2 and 1.

Sulforhodamine B Assay. This assay is based on the spectrophotometric determination of sulforhodamine B (SRB), a pink aminoxanthine dye, bound to cellular protein.[7] The plating of tumor cells, addition of drugs, and incubation was the same as described in the MTT assay. After the 8-day exposure period, the medium was aspirated and phosphate-buffered saline (PBS) was added. The cells were fixed by gently layering 50 μL of 10% trichloroacetic acid (TCA) on top of the growth medium in each well. The cultures were incubated at 4 °C for 1 h and then washed several times with tap water. Plates were air-dired, and background optical densities were measured in wells incubated with growth medium without cells. TCA-fixed cells were stained for 30 min with 0.4% (wt/vol) SRB dissolved in 1% acetic acid, then the SRB was removed, and the cultures were quickly rinsed four times with 1% acetic acid. After the cultures were dried in air, the bound dye was solubilized with 10 mM unbuffered Tris base (pH 10.5) for 5 min on a shaker. The OD at 564 nm was read on an automated microculture plate reader (Biomek 1000, Beckman Instruments). Protein content was determined by references to a calibration curve constructed with bovine serum albumin used as a standard. Each drug concentration was tested in six wells and the IC_{50} values were averaged. The results are given in Tables 2 and 1.

Antitumor Assays in Mice. The assays for P388 leukemia and L1210 leukemia in mice were conducted as specified in the standard NCI protocols.[14] Freshly harvested tumor cells (10^6 cells) were injected ip into 10 adult DBA/2J male mice on day 0, and the test compound was given ip on days 1, 5, and 9. The control group of 10 mice was given 10^6 tumor cells ip and injected with saline on the scheduled days. Results are expressed as the percent increase in lifespan (ILS) = 100 × (lifespan treated − lifespan controls)/lifespan controls, using median values for the groups of 10 mice.

Transition Melt Temperatures. The buffer for these experiments was ion-exchange water containing 0.01 M NaH_2PO_4 and 0.001 M EDTA with the pH tuned to 7.0 with NaOH solution. DNA solution was made by dissolving calf thymus DNA in buffer and adjusting the final concentration to about $5 \times 5\ 10^{-5}$ M. This solution was made freshly before each measurement. An appropriate amount of each compound in the same buffer was added to give a ratio of 5:1 for DNA base pairs to compound. With buffer in the reference cuvette, the sample cuvette was heated from 25 °C to 100−105 °C at 0.8 °C/min, using a Perkin-Elmer Lambda 3A spectrophotometer with heated cell and temperature programmer, and a PE R100A recorder.

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Supplementary Material Available: ^1H NMR spectra of compounds **44** and **45** (4 pages). Ordering information is given on any current masthead page.

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