Articles

6- and 7-Substituted 2-[2′-(Dimethylamino)ethyl]-1,2-dihydro-3*H*-dibenz[*de,h*]isoquinoline-1,3-diones: Synthesis, Nucleophilic Displacements, Antitumor Activity, and Quantitative Structure−Activity Relationships

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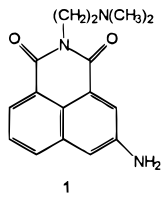
New 2-[2′-(dimethylamino)ethyl]-3*H*-dibenz[*de,h*]isoquinoline-1,3-diones with substituents at the 6- and 7-positions were prepared. Nucleophilic aromatic displacement was a key reaction in the syntheses. Ten of the new compounds were more potent than the unsubstituted compound, azonafide, in a panel of tumor cells including human melanoma and ovarian cancer and murine sensitive and MDR L1210 leukemia. They also were less cardiotoxic in cell culture. Four of these compounds were not cross-resistant with the MDR leukemia, and one of them, 6-ethoxyazonafide, was nearly as potent against solid tumor cells as leukemia cells. These compounds also had good potency against human breast, colon, and lung cancer cells, including doxorubicin and mitoxantrone resistant cell lines. Advantages of the new analogues over azonafide were less *in vivo*, but 6-ethoxyazonafide was more effective against L1210 leukemia and subcutaneous B16 melanoma in mice. Although correlations of antitumor potency in cells and physicochemical properties of substituents were not found, there were statistically significant correlations of DNA melt transition temperature (ΔT_m) with potency in solid tumor cells and sensitive and MDR resistant L1210 leukemia cells for 6-substituted azonafides and with solid tumors for 7-substituted azonafides.

The first article in this series outlined the rationale and synthesis of 1,2-dihydro-3*H*-dibenz[*de,h*]isoquinoline-1,3-diones,[1] based on our previous anthracene studies and analogy with the antitumor agent amonafide (**1**).[2,3] It also provided a structure−activity relationship (SAR) study which showed that the (dimethylamino)-ethyl group was the best side chain for substitution on N2 of the azonafide nucleus (**2**). In the second article, the side chain was held constant and the effect of position of a substituent on the nucleus was determined.[4] There was a strong influence of the nuclear position on the antitumor activity and cardiotoxicity of amino and acetylamino groups. Furthermore, 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]



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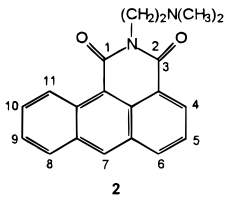

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The present article is concerned with quantitative correlations between potency against tumor cells or cardiotoxicity and a variety of substituents at fixed positions. This study has been guided partly by the ease of aromatic nucleophilic displacements at positions 6 and 7 of azonafide analogues, which have provided a significant number of substituents at these two positions. Variation in the nature of substituents has allowed the parameter set for QSAR to be expanded to include partition coefficient (π), electronegativity (σ), and size (MR).

Chemistry

Aromatic nucleophilic displacements do not occur readily on the anthracene nucleus; however, the presence of electron-withdrawing substituents greatly enhances the displacement of appropriately located leaving groups. In azonafide analogues, leaving groups *ortho* and *para* to the carbonyl groups (positions 4, 6, and 7) are labile. The ease of displacement of halogens from aromatic rings increases in the order I < Br < Cl < F because the reaction occurs in two steps: addition followed by elimination, and the addition step is usually rate determining.[5,6] As described below, fluoro-substituted intermediates were too reactive to give azonafides, except when they were not directly conjugated with the carbonyl groups. Consequently, chloroanthracenes were chosen to provide chloroazonafides which were used for the nucleophilic displacements. 1-Chloroanthracene (**3**) was converted into 6-chloroazonafide (**12**) with moderate regioselectivity, as previously described.[4] Compound **12** was the basis for the preparation of many 6-substituted analogues. Because of symmetry, there is no regioselectivity problem with 9-chloroanthracene (**7**), and it was readily converted into 7-chloroazonafide (**16**), from which 7-substituted azonafides were prepared.

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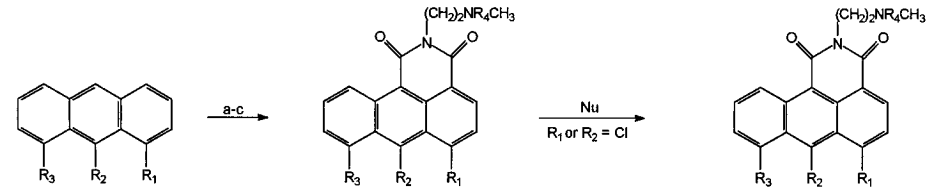
Scheme 1[a]



3: R_1 = Cl, R_2 = R_3 = H
4: R_1 = I, R_2 = R_3 = H
5: R_1 = CH_3, R_2 = R_3 = H
6: R_1 = $NHCOCH_3$, R_2 = R_3 = H
7: R_1 = R_3 = H, R_2 = Cl
8: R_1 = R_3 = H, R_2 = $NHCOCH_3$
9: R_1 = R_3 = H, R_2 = CH_3
10: R_1 = R_3 = Cl, R_2 = H
11: R_1 = R_2 = R_3 = H

12: R_1 = Cl, R_2 = R_3 = H, R_4 = CH_3
13: R_1 = I, R_2 = R_3 = H, R_4 = CH_3
14: R_1 = R_4 = CH_3, R_2 = R_3 = H
15: R_1 = $NHCOCH_3$, R_2 = R_3 = H, R_4 = CH_3
16: R_1 = R_3 = H, R_2 = Cl, R_4 = CH_3
17: R_1 = R_3 = H, R_2 = $NHCOCH_3$, R_4 = CH_3
18: R_1 = R_3 = H, R_2 = R_4 = CH_3
19: R_1 = R_3 = Cl, R_2 = H, R_4 = CH_3
20: R_1 = R_2 = H, R_3 = I, R_4 = CH_3
21: R_1 = Cl, R_2 = R_3 = R_4 = H
2: R_1 = R_2 = R_3 = H, R_4 = CH_3
d → **22**: R_1 = C_2H_5, R_2 = R_3 = H, R_4 = CH_3

23: R_1 = OCH_3, R_2 = R_3 = H, R_4 = CH_3
HBr → **24**: R_1 = OC_2H_5, R_2 = R_3 = H, R_4 = CH_3
→ **25**: R_1 = OH, R_2 = R_3 = H, R_4 = CH_3
26: R_1 = $O(CH_2)_2N(CH_3)_2$, R_2 = R_3 = H, R_4 = CH_3
27: R_1 = $O(CH_2)_2CH_3$, R_2 = R_3 = H, R_4 = CH_3
28: R_1 = R_3 = H, R_2 = OCH_3, R_4 = CH_3
HBr → **29**: R_1 = R_3 = H, R_2 = OC_2H_5, R_4 = CH_3
→ **30**: R_1 = R_3 = H, R_2 = OH, R_4 = CH_3
31: R_1 = OCH_3, R_2 = R_3 = R_4 = H
32: R_1 = SCH_3, R_2 = R_3 = H, R_4 = CH_3
33: R_1 = SC_2H_5, R_2 = R_3 = H, R_4 = CH_3
34: R_1 = R_3 = H, R_2 = SCH_3, R_4 = CH_3
35: R_1 = NH_2, R_2 = R_3 = H, R_4 = CH_3
36: R_1 = $NH(CH_2)_2N(CH_3)_2$, R_2 = R_3 = H, R_4 = CH_3
37: R_1 = $NH(CH_2)_2N(CH_3)_2$, R_2 = H, R_3 = Cl, R_4 = CH_3
38: R_1 = $NH(CH_2)_2OH$, R_2 = R_3 = H, R_4 = CH_3
39: R_1 = $N(CH_3)_2$, R_2 = R_3 = H, R_4 = CH_3
40: R_1 = R_3 = H, R_2 = $NH(CH_2)_2N(CH_3)_2$, R_4 = CH_3
41: R_1 = R_3 = H, R_2 = NH_2, R_4 = CH_3

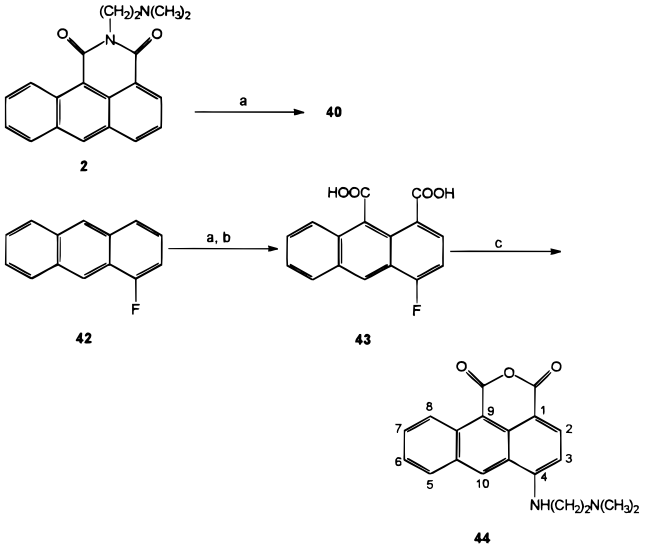
[a] Reagents: (a) $(COCl)_2$, $AlCl_3$, CS_2; (b) H_2O_2, NaOH; (c) *N*,*N*-dimethylethylenediamine or *N*-methylethylenediamine; (d) C_2H_5MgBr, THF, Nu = nucleophile (RO^-, RS^-, N_3, R_2NH).

Treatment of 6-chloroazonafide (**12**) with sodium methoxide, sodium ethoxide, or sodium propoxide gave good to moderate yields of the corresponding 6-methoxy, 6-ethoxy, and 6-propoxy analogues **23**, **24**, and **27** (Scheme 1). Surprisingly, treatment of **12** with sodium hydroxide in ethanol gave only **24** and no 6-hydroxyazonafide (**25**), whereas treatment of 7-chloroazonafide (**16**) with sodium hydroxide in methanol gave a mixture of 7-hydroxyazonafide (**30**) and 7-methoxyazonifide (**28**). Compounds **25** and **30** were prepared cleanly and in good yield by HBr treatment of 6-ethoxyazonafide (**24**) and 7-ethoxyazonafide (**29**), respectively. The more complex 6-alkoxy derivative 6-[(dimethylamino)ethoxy]-azonafide (**26**) was prepared by treating **12** with the sodium salt of (*N*,*N*-dimethylamino)ethanol.

When **12** was heated with sodium azide in ethanol, the product was 6-aminoazonafide (**35**), as previously reported.[4] In this reaction, nitrogen is obviously eliminated from the intermediate 6-azidoazonafide and ethanol supplies hydrogens for the amino group of **35**. The acetyl derivative, **15**, of **35** was readily prepared from 1-(acetylamino)anthracene (**6**).[4] Substituted amines readily reacted with **12** to give a variety of derivatives, including $NH(CH)_2N(CH_3)_2$ (**36**), $NH(CH_2)_2OH$ (**38**), and $N(CH_3)_2$ (**39**). A small amount of **36** is formed in the synthesis of **12**. Sodium thiomethoxide and sodium thioethoxide converted **12** into the corresponding 6-methylthio analogue **32** and 6-ethylthio analogue **33**. Compound **32** gave the methylsulfonyl analogue as its *N*-oxide **45** when it was treated with hydrogen peroxide (Scheme 3). Reduction of **45** with SO_2 in ethanol then afforded the desired product **46**.

In our previous study on variations in the azonafide side chain, the (methylamino)ethyl group conferred potency nearly equal to that of the (diethylamino)ethyl group on the molecule.[1] On the basis of this observation, the 2-(methylamino)ethyl analogue, **21**, of **12** was prepared and converted into 6-methoxy analogue **31** by treatment with sodium methoxide.

Scheme 2[a]



[a] Reagents: the same as in Scheme 1.

Two 6-substituted azonafides, the iodo derivative **13** and the methyl derivative **14**, were synthesized from 1-iodoanthracene (**4**) and 1-methylanthracene (**5**) by routes parallel to the one used for the preparation of azonafide (**2**) from anthracene. The synthesis of **14** was regiospecific, whereas a small amount of a product which appeared to be the 8-iodo isomer **20** was obtained from the synthesis of **13**. Treatment of azonafide (**2**), prepared from anthracene (**11**) with ethylmagnesium bromide, gave a 27% yield of 6-ethyl derivative **22**. The corresponding reaction with methylmagnesium bromide was unsuccessful.

There are two additional examples that further define nucleophilic displacement in the anthracene nucleus. In

Table 1. Activity of 6- and 7-Substituted 2-[2′-(Dimethylamino)ethyl]-1,2-dihydro-3*H*-dibenz[*de,h*]isoquinoline-1,3-diones against Tumor Cells and Myocytes in Culture[a]

	IC_{50}, nM							
				L1210				
no.	AUCC375 melanoma[c]	OVCAR 3 ovarian[d]	solids av	sens	resist[e]	three-tumor av[f]	cardiotox	toxicity[b] ratio
2	71	57	64	7.0	7.0	45	1983	44
12	39	116	78	77	64	77	4113	53
13	416	416	416	416	416	416	43 704	105
14	141	217	179	6.8	27	122	989	8.1
15	7290	6075	6683	486	729	4617	12 880	2.8
16	514	386	450	154	206	351	10 797	31
17	730	730	730	487	487	649	>23 613	>36
18	42	28	35	34	70	35	1685	49
19	472	945	709	472	590	630	>23 613	>37
21	5333	267	2800	53	53	1884	7600	4.0
22	522	522	522	183	235	409	7833	19
23	7.8	1.3	4.6	2.6	3.9	3.9	799	205
24	19	0.8	10	8.0	6.7	9.3	1779	192
25	3133	723	1928	217	145	1357	>24 096	>18
26	31	31	31	0.2	1.3	21	5439	262
27	485	364	425	17	49	289	7758	27
28	156	130	143	117	109	134	26 042	194
29	225	175	200	63	50	154	5300	34
30	6132	1179	3656	472	212	2594	>24 096	>9
31	2159	540	1350	41	27	913	8097	8.9
32	20	8.7	14	0.37	0.52	9.7	3000	310
33	97	19	58	6.0	4.8	41	7738	189
34	1375	750	1063	6250	1225	2792	>50 000	>18
35	149	68	109	5.4	27	74	1486	20
36	11	63	37	0.04	0.04	25	4193	171
37	29	176	103	2.9	6.8	69	3226	47
38	48	19	34	6.0	48	24	1209	50
39	101	503	302	23	96	209	46 599	223
40	839	2883	1861	524	524	1415	290 634	205
41	15	12	14	5.4	6.8	10.8	1951	181
46	93	93	93	35	35	73	2315	32
doxorubicin	112	35	74	35	3884	61	10 151	166
mitoxantrone	48	5.8	27	9.7	39	21	7737	368
amonafide	2031	2180	2106	625	625	1612	48 400	30

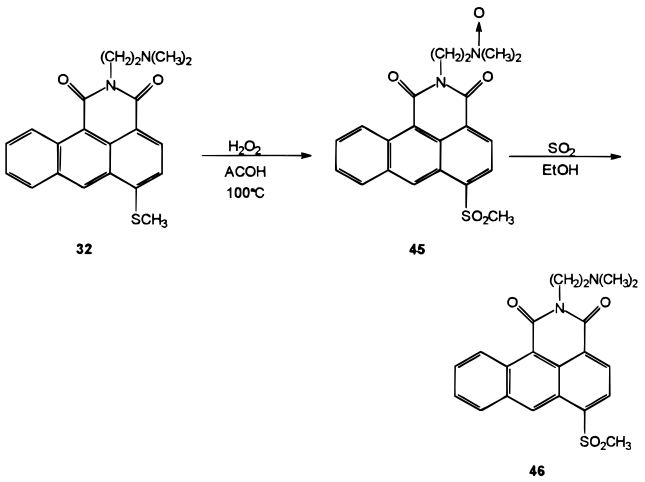
[a] The murine leukemia experiments were based on continuous drug exposure using the MTT assay (Alley, M. C.; Scudiero, D. A.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. *Cancer Res.* **1988**, *48*, 589−601). The overall standard deviation for 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 OVCAR 3 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 Cytotoxicity Assay for Anticancer-Drug Screening. *J. Natl. Cancer Inst.* **1990**, *82*, 1107−1112). Cardiotoxicity was determined by a neonatal rate 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). [b] 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%. The quotient of the IC_{50} in the myocytes was divided by the mean IC_{50} in the three tumor cell lines (from Table 2). 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). [c] A human melanoma line obtained from the University of Arizona Cancer Center. [d] A human ovarian cancer cell line obtained from the NCI. This carcinoma was resistant to all standard anticancer drugs. [e] A murine leukemia cell line. The resistant strain is multiple drug resistant. [f] An average (mean) for IC_{50} values in the two solid tumor cell lines and the sensitive L1210 leukemia cell line.

one example, 1,8-dichloroanthracene (**10**) was elaborated by the usual sequence into 4,5-dichloroanthracene-1,9-dicarboxylic acid. When this intermediate was heated with *N*,*N*-dimethylethylenediamine, the main product was 6,8-dichloroazonafide (**19**), but a small amount of 8-chloro-6-[(dimethylamino)ethyl]amino derivative **37** also was formed. There was no evidence for the 6-chloro-8-[(dimethylamino)ethyl]amino isomer. This example provides further evidence for selective nucleophilic displacement at a position conjugated with a carbonyl group. The second example resulted from an attempt to prepare 6-fluoroazonafide, which was expected to be a highly reactive intermediate for the synthesis of 6-substituted analogues. 1-Fluoroanthracene (**42**) was converted into the corresponding dicarboxylic acid **43** by the usual sequence (Scheme 2). When **43** was heated with *N*,*N*-dimethylethylenediamine, the product **44** resulted from displacement of the fluorine atom by the amine and not from imide formation with the anhydride group. Thus, the fluoride group was too reactive for use in the synthesis of analogues.

Despite the substantial number of analogues prepared by displacement of chlorine from **12**, some displacements were unsuccessful. In particular, we could find no conditions suitable for displacement by cyanide.

Synthesis of 7-substituted azonafide analogues followed from routes essentially parallel to those used for the 6-substituted analogues. 7-Chloroazonafide (**16**) was prepared from 9-chloroanthracene (**7**) as previously described. Treatment with sodium methoxide and sodium ethoxide gave analogues **28** and **29**, respectively. 7-Aminoazonafide (**41**) was prepared by treatment of **16**

Scheme 3



with sodium azide or by synthesis from 9-(acetylamino)-anthracene (**8**) by way of **17**. Both methods were described previously.[4] Nucleophilic displacement converted **16** into its 7-[(dimethylamino)ethyl]amino derivative **40**, and nucleophilic displacement by sodium thiomethoxide gave 7-methylthio derivative **34**. 7-Methylazonafide (**18**) was made from 9-methylanthracene (**9**) by the usual route.

Biology

In vitro activities for the 6- and 7-substituted azonafide analogues are compared with those of doxorubicin, mitoxantrone, and amonafide in Table 1. Compounds are listed by their numbers in the schemes. Human tumor cell lines include a melanoma and an ovarian carcinoma that is resistant to standard anticancer drugs. Murine L1210 includes a sensitive strain and one that has multiple drug resistance (MDR) 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 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, potencies against tumor cells by both the 6- and 7-substituted azonafide analogues are highly dependent on the nature of the substituent. Average potencies in three tumor cell types vary from 3.9 nM for 6-methoxyazonafide (**23**) to 4617 nM for 6-(acetylamino)azonafide (**15**). For some substituents (Cl, OCH_3, $N(CH_2)_2N(CH_3)_2$, and SCH_3), the 6-position is more active than the 7-position, whereas for other substituents (NH_2, $NHCOCH_3$, and CH_3) the 7-position is more active. Compounds with 6-substituents and the (methylamino)ethyl side chain (**21** and **31**) were much less active than the corresponding compounds (**12** and **23**) with the (dimethylamino)ethyl side chain; consequently, no further compounds of the former type were prepared.

Table 2. Activity of Selected 6- and 7-Substituted 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			WiDr colon		A549 lung
no.	sens	dox	mitox	sens	resist	sens
2	18	70	20	13	94	10
12	25	120	50	70	350	19
13	11	30	10	65	620	10
23	14	270	19	17	33	2.2
24	11	18	14	17	63	2.9
26	3.9	8.9	3.9	2.4	10	0.58
27	80	170	110	110	270	19
28	150	180	270	180	260	91
29	23	200	110	170	33	56
32	10	12	44	12	64	4.2
33	20	75	33		110	4.5
36	1.0	14	1.9	1.7	14	0.43
39	68	130	79	90	130	23
41	20	71	80	18	100	7.0
doxorubicin[b]	28	1172	28	53	130	22
mitoxantrone[b]	8.7	72	41	8.1	488	3.1

[a] IC_{50} values were determined in the sulforhodamine B assay with a 7-day exposure period (Skehan, P.; Stoering, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. New Colorimetric Cytotoxicity Assay for Anticancer-Drug Screening. *J. Natl. Cancer Inst.* **1990**, *82*, 1107−1112). [b] Average of five determinations.

The most potent compounds in Table 1, having average IC_{50} values <50 nM, include the following derivatives of azonafide: 7-CH_3 (**18**), 6-OCH_3 (**23**), 6-OC_2H_5 (**24**), 6-$O(CH_2)_2N(CH_3)_2$ (**26**), 6-$NH(CH_2)_2OH$ (**38**), 6-$NH(CH_2)_2N(CH_3)_2$ (**36**), 7-NH_2 (**41**), 6-SCH_3 (**32**), and 6-SC_2H_5 (**33**), as well as mitoxantrone. Among these compounds, **23** had the greatest average potency against tumor cells (3.9 nM). Compounds with the best ratios of IC_{50} for cardiotoxicity to average antitumor cell potency (>150) were **23, 24, 26, 28, 32, 33, 36, 39**−**41,** doxorubicin, and mitoxantrone. Among these compounds, **32** and mitoxantrone had toxicity ratios of >300.

One of our goals is to develop analogues which retain potency against MDR tumor cells.[11] The compounds in Table 1 with greater potency against MDR L1210 leukemia cells than sensitive L1210 leukemia cells are **12, 24, 25, 28**−**30, 33, 34**, and *N*-demethyl analogue **31**. Compounds equal in potency to MDR and sensitive L1210 leukemia cells include azonafide (**2**), **13, 17, 21, 36, 40**, and **46**. Another goal was to develop analogues with good ratios of potency against solid tumor cells to potency against leukemia. Although in general azonafide analogues are more potent against sensitive L1210 leukemia cells than the average of the two solid tumor cells in Table 1, the 7-SCH_3 analogue **34** was more active against the solid tumor cells. Compounds equal or nearly equal (IC_{50} ratio solid/tumor average: L1210 ≤ 1.25) included **12, 13, 18, 24**, and **28**. Compound **36** had a high solid/L1210 ratio because of its extremely low IC_{50} value against L1210 leukemia (40 pM).

Fourteen of the more active 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 MDR), and A549 lung carcinoma. The results are given in Table 2. The most potent compounds across the spectrum of tumor cells are **26** and **36**, which have an average IC_{50} of about 5

Table 3. Activity against Tumors in Mice[a]

	P388 leukemia		L1210 leukemia		sc B16 melanoma	
no.	dose, mg/kg	% ILS	dose, mg/kg	% ILS	dose, mg/kg	% TGI
2	15	79	15	72	15	74
23			8	86		
24	5	55	8	100	6	>100[b]
26			8	NA[c]		
36	3	44			3	66
14	15	NA				
amonafide	15	88				
doxorubicin	4.5	113	5	210	4	120
mitoxantrone	1.6	200	1.6	150	3.2	130

[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. B16 melanoma cells (10^6) were injected subcutaneously into C57/vBL male mice, and the compounds were given ip in equal doses on days 1, 5, and 9. Tumor growth was measured by calipers using the widest perpendicular widths of palpable subcutaneous tumor as the end point. These widths were converted into an estimated tumor mass according to the formula $L(\text{mm}) \times W^2(\text{mm})/2 = \text{mg}$. Tumor masses were not allowed to grow beyond 750 mg for humane reasons. Percent tumor growth inhibition (TGI) is calculated by the equation % TGI = 100 × (days to 750 mg tumor) × [(wt control tumor − wt tumor)/wt control tumor]. [b] No palpable tumor. [c] NA means not active at the highest dose tested (% ILS < 25).

nM. Both of these analogues have a basic amino group in the 6-substituent as well as in the side chain. Other compounds with good potencies are **24** and **32**. They all have moderate sized substituents with π-values in the range −0.73 to 1.12. The 6-OCH_3 analogue **23** has good potency for all tumor cell lines except for doxorubicin resistant breast carcinoma. Potencies of the best azonafide analogues are higher than those of doxorubicin and mitoxantrone, and there is only limited cross-resistance.

Following the determination of potent activity in tumor cell cultures, azonafide and five of its analogues were tested against three different tumors in mice: P388 leukemia, L1210 leukemia, and subcutaneous B16 melanoma. As shown in Table 3, azonafide and its 6-OC_2H_5 analogue **24** were active (ILS ≥ 25%) against all three tumors. The 6-OCH_3 analogue **23** was not tested against P388 leukemia, but it was active against L1210 leukemia, with an ILS of 86%. Surprisingly, the 6-$O(CH_2)_2N(CH_3)_2$ analogue **26** was inactive against L1210 leukemia, despite its high potency *in vitro*. The 6-$NH(CH_2)_2N(CH_3)_2$ analogue **36** was active against P388 leukemia, but the 6-CH_3 analogue **14** was inactive. Azonafide and its 6-OC_2H_5 and 6-$NH(CH_2)_2N(CH_3)_2$ analogues were effective against subcutaneous B16 melanoma. The 6-OC_2H_5 analogue reduced this tumor to the point where it was not palpable at a dose of 6 mg/kg given on days 1, 5, and 9; however, it was toxic at higher doses. The positive controls, doxorubicin and mitoxantrone, had good activity against all three tumors as expected.

QSAR

In the preceding article on azonafides,[4] which dealt with amino substituents at all positions on the nucleus, a significant correlation was found between potency against tumor cells and DNA transition melt temperatures (ΔT_m). This correlation was investigated in the present article for the 6- and 7-substituted azonafides, together with possible correlations of potency against tumor cells (log I/*C*, where *C* is the IC_{50}) with physicochemical properties of the substituents including π, σ_p, and MR (molar refractivity, a measure of size). Potencies against tumor cells were calculated from IC_{50} values in Table 1 for an average of the solid tumor cells, sensitive L1210 leukemia, resistant L1210 leukemia, and cardiotoxicity. Table 4 gives all of the data used in the QSAR study. Compounds not included in Table 4 are **21** and **31**, which have different side chains, **26**, **36**, **37**, and **40**, which have a second basic aliphatic amino group and exist partially as dications at pH 7, and **19** and **20**, which have 8-substituents. Correlations were examined separately for 6- and 7-substituted analogues because a strong dependence of potency against tumor cells on position of substitution had been demonstrated previously.[4]

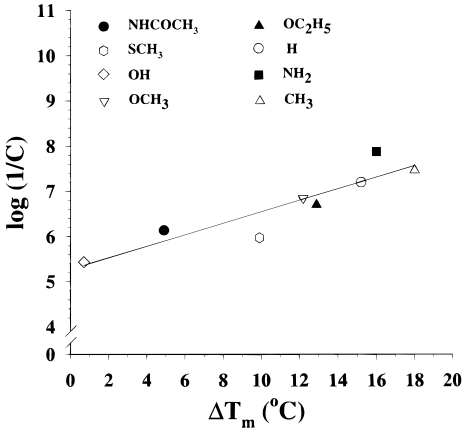


Figure 1. Linear correlation of ΔT_m with potency against solid tumors for 7-substituted azonafides.

The data on 7-substituted azonafides in Table 4 were analyzed using the program Sigmastat and Sigma Plot for Windows.[12] Data points were fitted with the best straight line or curve, and statistical parameters including r^2 (amount of variance accounted for), standard error (SE), and the *F* test were determined. All statistics were valid at the 95% confidence level unless stated otherwise. The 7-chloro substituent could not be used in correlations involving ΔT_m because the concave nature of the melt transition curve prevented the determination of ΔT_m. For the remaining eight compounds, there was a significant linear correlation of average potency against the solid tumor cells with ΔT_m. The equation was $\log(1/C) = 5.27 + 0.127\Delta T_m$ (Figure 1, $r^2 = 0.839$, SE = 0.35, $F = 31.2$).[13] Significant linear correlations were not found for ΔT_m with potency against sensitive L1210 leukemia, MDR L1210 leukemia, or cardiotoxicity at the 95% confidence level, although all of these correlations were significant at the 90% confidence level.

For the 6-substituted azonafides, the 6-SCH_3 substituent was not used in correlations involving ΔT_m because its transition melt curve was concave and ΔT_m could not be measured. It was used in all other correlations. The remaining 15 6-substituted azonafides gave a statistically significant linear correlation between ΔT_m and the average potency against solid tumor cells, but r^2 was only 0.298 ($\log(1/C) = 5.53 + 0.105\Delta T_m$; $r^2 =$

Table 4. Correlations among Antitumor or Cardiotoxic Potency of Azonafide Analogues, Transition Melt Temperature Increase, and Physicochemical Properties[a]

			log(*I*/*C*)						
				leukemia[c]					
compd	substituent	ΔT_m, °C[d]	solid[b]	sens	resist	cardiotox	π	σ	MR
2	6- or 7-H	15.2	7.19	8.15	8.15	5.70	0	0	1.03
12	6-Cl	12.3	7.11	7.11	7.19	5.39	0.88	0.23	6.03
13	6-I	18.3	6.38	6.38	6.38	4.36	1.29	0.18	19.43
23	6-OCH_3	15.8	8.34	8.59	8.41	6.10	0.18	−0.27	7.87
24	6-OC_2H_5	17.5	8.00	8.10	8.17	5.75	0.71	−0.24	12.47
27	6-OC_3H_7	14.6	6.37	7.77	7.31	5.11	1.24	−0.25	17.06
25	6-OH	1.29	5.72	6.66	6.84		−0.33	−0.37	2.85
35	6-NH_2	13.8	6.97	8.27	7.57	5.83	−0.91	−0.66	5.42
39	6-$N(CH_3)_2$	12.8	6.52	7.64	7.02	4.33	0.39	−0.83	15.55
15	6-$NHCOCH_3$	8.1	5.18	6.31	6.14	4.89	−0.69	0	14.93
38	6-$NH(CH_2)_2OH$	9.2	7.48	8.21	7.32	5.92	−0.92	−0.51	
32	6-SCH_3	CC[e]	7.84	9.43	9.30	5.52	0.75	0	13.82
33	6-SC_2H_5	10.5	7.24	8.22	8.32	5.11	1.28	0.03	18.42
46	6-SO_2CH_3	13.5	7.03	7.46	7.24	5.64	0.53	0.72	13.49
14	6-CH_3	14.4	6.75	8.17	7.57	6.01	0.50	−0.17	5.65
22	6-C_2H_5	10.0	6.28	6.74	6.63	5.11	1.03	−0.15	10.30
16	7-Cl	CC[e]	6.35	6.81	6.69	4.97	0.88	0.23	6.03
28	7-OCH_3	12.2	6.85	6.93	6.96	4.58	0.18	−0.27	7.87
30	7-OH	0.70	5.44	6.33	6.67		−0.33	−0.37	2.85
29	7-OC_2H_5	12.9	6.70	7.20	7.30	5.28	0.71	−0.24	12.47
17	7-$NHCOCH_3$	4.9	6.14	6.31	6.30		−0.69	0	14.93
41	7-NH_2	16.0	7.87	8.27	8.17	5.71	−0.91	−0.66	5.42
34	7-SCH_3	9.9	5.97	5.20	5.91		0.75	0	13.82
18	7-CH_3	18.0	7.46	7.47	7.16	5.77	0.50	−0.17	5.65

[a] Antitumor data is taken from Table 1. Parameters for the physicochemical properties σ and MR are taken from Hansch, C.; Leo, A. *Substituent Constants for Correlation Analysis in Chemistry and Biology*; Wiley-Interscience: New York, 1979; π values were calculated using C log *P* software and as *para* substituents on benzamide. [b] Calculated from the average IC_{50} for the two human solid tumor lines. [c] Calculated from the IC_{50} for sensitive and resistant 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. [e] The curve for determining ΔT_m was concave.

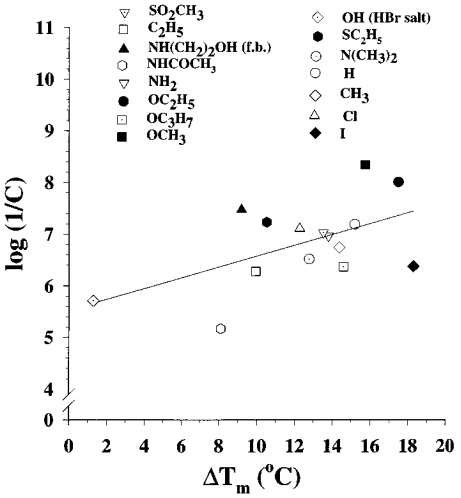


Figure 2. Correlation of ΔT_m with potency against solid tumors for 6-substituted azonafides.

0.298, SE = 0.709, F = 5.52, Figure 2). Statistically significant correlations between ΔT_m and either sensitive or MDR L1210 leukemias were not obtained; however, deletion of 6-iodoazonafide (**13**) gave correlations at the 95% confidence level. Compound **13** is clearly an outlier according to the DFFITS test for both sensitive and MDR L1210 leukemia. The equations were $\log(1/C) = 6.29 + 0.12\Delta T_m$ ($r^2 = 0.44$, SE = 0.55, $F = 9.34$) and $\log(1/C) = 6.22 + 0.10\Delta T_m$ ($r^2 = 0.37$, SE = 0.55, F = 7.14), respectively. No correlation was obtained for ΔT_m and cardiotoxicity.

It is expected that substituent physicochemical properties should influence the DNA-binding abilities of compounds and that the effects might be expressed in both ΔT_m and potency against tumor cells. To examine these possibilities, correlations of the properties π (contribution of substituent to partition coefficient), σ (electron-withdrawing power of substituent), and MR (size of substituent) with ΔT_m and with potencies against tumor cells and cardiotoxicity were explored for both the 6- and 7-substituted azonafides. Unfortunately, the data points were sufficiently scattered that only two correlations were found at the 95% confidence level. Cardiotoxicity was correlated with MR for 6-substituted azonafides at the 99% confidence level. The equation was $\log(1/C) = 6.15 - 0.07$MR ($n = 7$, $r^2 = 0.52$, F = 11.72), which shows that larger substituents decrease cardiotoxicity. A correlation of ΔT_m with MR for 7-substituted azonafides with the 7-OH analogue removed according to the DFFITS test (Figure 3) gave the equation $\Delta T_m = 18.7 - 0.68$MR ($n = 7$, $r^2 = 0.64$, F = 8.98), indicating that larger substituents decrease DNA-binding strength.

Conclusions

Nucleophilic aromatic displacements occurred readily at the 6- and 7-positions of 1,2-dihydro-3*H*-dibenz[*de*,*h*]-isoquinoline-1,3-diones and provided a variety of novel substituents at these positions. By using these reactions and synthesizing other compounds from 1- and 9-substituted anthracenes, 18 new 6-substituted and 6 new 7-substituted azonafide analogues were prepared.

Potencies of the new azonafide analogues against tumor cells were highly dependent on the nature of their substituents; however, statistically significant correlations between cytotoxicity and physicochemical properties of the substituents were not obtained. Correlations were found for ΔT_m with solid tumors for 7-substituted azonafides, and statistically significant but poor cor-

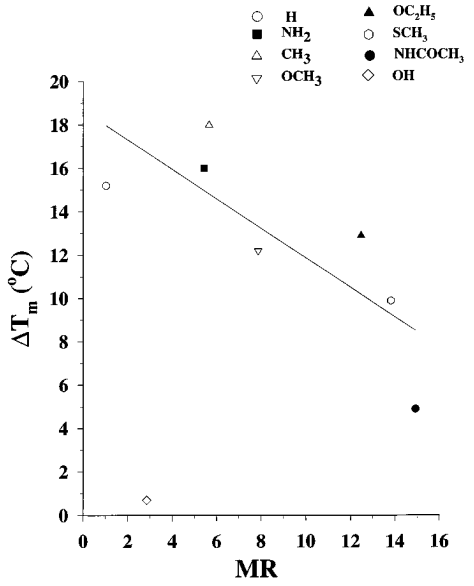


Figure 3. Correlation of ΔT_m with MR for 7-substituted azonafides. The azonafide with an OH substituent was not used in determining the straight line.

relations were found for solid tumors and sensitive and resistant L1210 leukemias with 6-substituted azonafides. These results indicate that the strength of drug binding to DNA (or DNA−topoisomerase complexes) might be the limiting factor for determining potency against tumor cells and that other factors such as cell uptake of the drug are less likely to be important. Larger substituents may decrease DNA-binding strength and biological potency, although only one correlation for each property was statistically significant.

Significant advances were made in developing new analogues with better cytotoxic properties than azonafide (**2**), as shown in Table 1. There were eight compounds (**18, 23, 24, 26, 32, 33, 36,** and **38**), in addition to previously reported **41**, with greater average potency than azonafide. All of them had superior therapeutic indices, as measured by the IC_{50} for cardiotoxicity divided by the IC_{50} for average antitumor potency, and seven of them had very high ratios (>170). Three of them (**24, 33,** and **36**) showed no cross-resistance with MDR L1210 leukemia. Although no compound was as potent against solid tumor cells as it was against sensitive L1210 leukemia, **24** was nearly as potent (10 vs 8 nM). It appears to be the best candidate for further development.

Further testing in a panel of human breast, colon, and lung tumor cells (Table 2) gave relative potencies that were in general agreement with the results in Table 1. Thus, the most potent compounds were **23, 24, 26,** and **36**. They showed good activity against doxorubicin and mitoxantrone resistant MXF7 breast cancer cells and resistant WiDr colon cancer cells.

The advantages of the new analogues over azonafide were less apparent against murine tumors *in vivo*. Compounds **26** and **14** were inactive against L1210 and P388 leukemias, respectively, and **36** was relatively toxic. The best compound appears to be 6-ethoxyazonafide (**24**), which has significant activity against L1210 leukemia and subcutaneous B16 melanoma in mice. It is not highly effective against P388 leukemia, but this factor may not be detrimental because many compounds with high potency against P388 leukemia are strongly myelosuppressive.

Experimental Section

Melting points were recorded on a Mel-Temp melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker 250 WM or JEOL FX90Q spectrometer, and absorptions are reported as downfield 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. The syntheses of compounds **2**, **12**, **15**−**17**, **35**, and **41** were reported previously.[4]

Method A: General Procedure for the Preparation of 6-Iodo- (13), 6-Methyl- (14), 7-Methyl- (18), and 6,8-Dichloro- (19) Azonafides (Scheme 1). 4-Iodo-, 4-methyl-, 10-methyl-, and 4,5-dichloroanthracene-1,9-dicarboxylic acids were prepared in an overall yields of 51%, 14%, 44%, and 65%, respectively, from the corresponding 1-iodo- (**4**), 1-methyl- (**5**), 9-methyl- (**9**), and 1,8-dichloro- (**10**) anthracenes using the procedure described in ref 4 for the preparation of 4-chloroanthracene-1,9-dicarboxylic acid. The dicarboxylic acids were used as crude materials in the preparation of the title compounds. Thus a suspension of the diacid in a toluene−absolute ethanol mixture (3:1) was heated at reflux overnight with 1.2 equiv (1.04 equiv in the case of **19**) of *N*,*N*-dimethylethylenediamine. The solvent was removed in vacuum, and the product was isolated from the residue by column chromatography on silica gel with 10% methanol in chloroform.

2-[2′-(Dimethylamino)ethyl]-1,2-dihydro-6-iodo-3*H*-dibenz[*de*,*h*]isoquinoline-1,3-dione (13): obtained in 89% yield, crystallized from ether, mp 155−157 °C; ^{1}H NMR ($CDCl_3$) δ 2.39 (s, 6, CH_3), 2.68−2.73 (t, 2, CH_2N), 4.36−4.42 (t, 2, $CONCH_2$), 7.61−7.67 (t, 1, H-9), 7.79−7.86 (t, 1, H-10), 8.10−8.13 (d, 1, H-8), 8.29−8.30 (d, 2, H-4 + H-5), 8.92 (s, 1, H-7), 9.89−9.92 (d, 1, H-11). Anal. ($C_{20}H_{17}IN_2O_2 \cdot {}^1/_4H_2O$) C, H, N, I.

In addition to **13**, a small amount (2%) of a compound which appeared to be 2-[2′-(dimethylamino)ethyl]-1,2-dihydro-8-iodo-3*H*-dibenz[*de*,*h*]isoquinoline-1,3-dione (**20**) according to its ^{1}H NMR spectrum was isolated: mp of hydrochloride salt above 360 °C; ^{1}H NMR ($CDCl_3$), δ 2.41 (s, 6, CH_3), 2.73−2.78 (t, 2, CH_2N), 4.41−4.47 (t, 2, $CONCH_2$), 7.52−7.55 (d, 1, H-9), 7.62−7.68 (t, 1, H-5), 7.85−7.88 (t, 1, H-10), 8.08−8.11 (d, 1, H-4), 8.53−8.56 (d, 1, H-6), 9.40 (s, 1, H-7), 10.02−10.05 (d, 1, H-11). We were unable to obtain a satisfactory HRMS.

2-[2′-(Dimethylamino)ethyl]-1,2-dihydro-6-methyl-3*H*-dibenz[*de*,*h*]isoquinoline-1,3-dione (14): obtained in 93% yield, mp 144−146 °C after crystallization from hexanes; ^{1}H NMR ($CDCl_3$) δ 2.40 (s, 6, CH_3), 2.66−2.72 (t, 2, CH_2N), 2.85 (s, 3, CH_3), 4.33−4.38 (t, 2, $CONCH_2$), 7.41−7.44 (d, 1, H-5), 7.52−7.58 (t, 1, H-9), 7.70−7.77 (t, 1, H-10), 7.96−8.00 (d, 1, H-8), 8.48−8.51 (d, 1, H-4), 8.70 (s, 1, H-7), 9.83−9.87 (d, 1, H-11). Anal. ($C_{21}H_{20}N_2O_2 \cdot {}^1/_4H_2O$) C, H, N.

2-[2′-(Dimethylamino)ethyl]-1,2-dihydro-7-methyl-3*H*-dibenz[*de*,*h*]isoquinoline-1,3-dione (18): obtained in 95% yield, after crystallization from toluene−hexanes (1:3), mp 155−157 °C; ^{1}H NMR (90 MHz, $CDCl_3$) δ 2.41 (s, 6, CH_3), 2.63−2.80 (t, 2, CH_2N), 3.06 (s, 3, CH_3), 4.30−4.44 (t, 2, $CONCH_2$), 7.45−7.80 (m, 3, H-5 + H-9 + H-10), 8.20−8.28 (d, 1, H-8), 8.45−8.53 (d, 1, H-4), 8.59−8.65 (d, 1, H-6), 9.89−9.99 (d, 1, H-11). Anal. ($C_{21}H_{20}N_2O_2$) C, H, N.

6,8-Dichloro-2-[2′-(dimethylamino)ethyl]-1,2-dihydro-3*H*-dibenz[*de*,*h*]isoquinoine-1,3-dione (19): obtained in 72% yield, crystallized from toluene, mp 209−211 °C; ^{1}H NMR ($CDCl_3$) δ 2.39 (s, 6, CH_3), 2.69−2.77 (t, 2, CH_2N), 4.37−4.40 (t, 2, $CONCH_2$), 7.67−7.73 (m, 2, H-9 + H-10), 7.80−7.82 (d, 1, H-5), 8.58−8.60 (d, 1, H-4), 9.58 (s, 1, H-7), 9.88 −9.90 (d, 1, H-11). Anal. ($C_{20}H_{16}Cl_2N_2O_2$) C, H, N, Cl.

In addition to **19**, a small amount (0.7%) of 8-chloro-2-[2′-(dimethylamino)ethyl]-1,2-dihydro-6-[2′-(dimethylamino)ethyl]-3*H*-dibenz[*de*,*h*]isoquinoline-1,3-dione (**37**) was obtained (cf. method D).

Method B: General Procedure for the Preparation of 6- and 7-Alkoxyazonafides 23, 24, and 26−29 (Scheme 1). A solution of 2−2.5 equiv of the desired alkoxide in the corresponding alcohol was added to a suspension of 1 equiv of 6- or 7-chloro-2-[2′-(dimethylamino)ethyl]-1,2-dihydro-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (**12** or **16**) in the same alcohol. The mixture was stirred at room temperature for 24 h (in the case of **28** and **29**) or heated at reflux for 3 h (**23**, **24**, and **27**) or at 100−120 °C for 15 min (**26**). The alcohol was removed on a rotary evaporator, and the products were isolated from the residue by PTLC on silica gel with toluene−methanol (9:1 for **23**, **24**, and **28**, 8:2 for **26**) or chloroform−methanol (19:1 for **27** and **29**). The ^{1}H NMR spectra of compounds **23** and **28** are given completely. Other analogues show similar spectra except for 6- and 7-substituents for which the chemical shifts are listed below. All spectra were taken in $CDCl_3$.

2-[2′-(Dimethylamino)ethyl]-1,2-dihydro-6-methoxy-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (23): obtained in 67% yield, crystallized from toluene−hexanes (1:4), mp 192−193 °C; ^{1}H NMR δ 2.41 (s, 6, NCH_3), 2.69−2.75 (t, 2, CH_2N), 4.15 (s, 3, OCH_3), 4.37−4.43 (t, 2, $CONCH_2$), 6.86−6.89 (d, 1, H-5), 7.55−7.61 (t, 1, H-9), 7.76−7.82 (t, 1, H-10), 8.03−8.07 (d, 1, H-8), 8.59−8.62 (d, 1, H-4), 9.06 (s, 1, H-7), 9.92−9.96 (d, 1, H-11). Anal. ($C_{21}H_{22}N_2O_3\cdot H_2O$) C, H, N.

2-[2′-(Dimethylamino)ethyl]-1,2-dihydro-6-ethoxy-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (24): obtained in 60% yield, crystallized from hexanes, mp 140−141 °C; ^{1}H NMR δ 1.64−1.70 (t, 3, CH_3), 4.32−4.43 (m, 4, OCH_2 + $CONCH_2$). Anal. ($C_{22}H_{22}N_2O_3$) C, H, N.

2-[2′-(Dimethylamino)ethyl]-1,2-dihydro-6-[2-(dimethylamino)ethoxy]-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (26): obtained in 80% yield, crystallized from hexanes, mp 140−142 °C; ^{1}H NMR δ 2.46 (s, 6, CH_3), 2.98−3.03 (t, 2, CH_2 N), 4.38−4.45 (m, 4, OCH_2 + $CONCH_2$). Anal. ($C_{24}H_{27}N_3O_3$) C, H, N.

2-[2′-(Dimethylamino)ethyl]-1,2-dihydro-6-propoxy-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (27): obtained in 38% yield, mp 153−155 °C after crystallization from hexanes containing the least amount of toluene; ^{1}H NMR δ 1.19−1.24 (t, 3, CH_3), 2.04−2.11 (sext, 2, CH_2), 4.23−4.28 (t, 2, OCH_2). Anal. ($C_{23}H_{24}N_2O_3$) C, H, N.

2-[2′-(Dimethylamino)ethyl]-1,2-dihydro-7-methoxy-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (28): obtained in 87% yield, crystallized from toluene−hexanes (1:1), mp 147−149 °C; ^{1}H NMR (90 MHz) δ 2.40 (s, 6, NCH_3), 2.62−2.73 (t, 2, CH_2N), 4.22 (s, 3, OCH_3), 4.33−4.48 (t, 2, $CONCH_2$), 7.50−8.85 (m, 3, H_5 + H-9 + 10-H), 8.36−8.44 (d, 1, H-8), 8.56−8.65 (d, 1, H-4), 8.65−8.74 (d, 1, H-6), 9.95−10.05 (d, 1, H-11). Anal. ($C_{21}H_{20}N_2O_3$) C, H, N.

2-[2′-(Dimethylamino)ethyl]-1,2-dihydro-7-ethoxy-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (29): obtained in 70% yield, crystallized from hexanes, mp 114−115 °C; ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ 1.65−1.70 (t, 3, CH_3), 4.34−4.45 (m, 4, OCH_2 + $CONCH_2$). Anal. ($C_{22}H_{22}N_2O_2\cdot$HCl) C, H, Cl; N: calcd, 7.02; found, 6.28.

Method C: General Procedure for the Preparation of 6- and 7-(Alkylthio)azonafides 32−34 (Scheme 1). A solution of 1 equiv of 6- or 7-chloro-2-[2′-(dimethylamino)ethyl]-1,2-dihydro-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (**12** or **16**) in anhydrous tetrahydrofuran was treated with 1.2 equiv (for **32** and **34**) or 2.5 equivs (for **33**) of the corresponding thioalkoxide as solid. The mixture was heated at reflux for 0.5 h in the case of **34**, 1 h for **32**, or 18 h for **33**. The solvent was evaporated to dryness, and the residue was purified by crystallization to give **32** or by PTLC on silica gel with 10% methanol in toluene for **34** or 5% methanol in $CHCl_3$ for **33**.

2-[2′-(Dimethylamino)ethyl]-1,2-dihydro-6-(methylthio)-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (32): obtained in 58% yield after crystallization from toluene−hexanes (1:3), mp 185−187 °C; ^{1}H NMR ($CDCl_3$) δ 2.41 (s, 6, NCH_3), 2.70−2.74 (s over t, 5, CH_2N +SCH_3), 4.39−4.44 (t, 2, $CONCH_2$), 7.33−7.35 (d, 1, H-5), 7.60−7.65 (t, 1, H-9), 7.79−7.85 (t, 1, H-10), 8.08−8.11 (d, 1, H-8), 8.56−8.58 (d, 1, H-4), 9.05 (s, 1, H-7), 9.95−9.98 (d, 1, H-11). Anal. ($C_{21}H_{20}N_2O_2S$) C, H, N, S.

2-[2′-(Dimethylamino)ethyl]-1,2-dihydro-6-(ethylthio)-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (33): obtained in 56% yield, after crystallization from hexanes containing the least amount of toluene, mp 162−163 °C; ^{1}H NMR ($CDCl_3$) δ 1.51−1.56 (t, 1, CH_3), 2.40 (s, 6, NCH_3), 2.69−2.74 (t, 2, CH_2N), 3.20−3.28 (q, 2, CH_2S), 4.39−4.44 (t, 2, $CONCH_2$), 7.43−7.46 (d, 1, H-5), 7.60−7.65 (t, 1, H-9), 7.79−7.85 (t, 1, H-10), 8.09−8.12 (d, 1, H-8), 8.56−8.59 (d, 1, H-4), 9.13 (s, 1, H-7), 9.95−9.99 (d, 1, H-11). Anal. ($C_{22}H_{22}N_2O_2S$) C, H, N, S.

2-[2′-(Dimethylamino)ethyl]-1,2-dihydro-7-(methylthio)-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (34): obtained in 66% yield, crystallized from hexanes containing the least amount of toluene, mp 135−137 °C; ^{1}H NMR (90 MHz, $CDCl_3$) δ 2.28 (s, 6, NCH_3), 2.35 (s, 3, SCH_3), 2.56−2.73 (t, 2, CH_2N), 4.24−4.42 (t, 2, $CONCH_2$), 7.53−7.81 (m, 3, H-5 + H-9 + H-10), 8.63−8.71 (d, 1, H-8), 8.93−9.03 (d, 1, H-4), 9.17−9.27 (d, 1, H-6), 9.89−9.99 (d, 1, H-11). Anal. ($C_{21}H_{20}N_2O_2S$) C, H, N, S.

Method D: General Procedure for the Preparation of 6- and 7-Amino Derivatives of Azonafide (36−40) (Scheme 1). A solution of each of **12**, **16**, or **19** in 2-propanol (for **36** and **38**), a 1:1 mixture of toluene−ethanol (for **37** and **40**), or absolute ethanol (for **39**) was treated with excess of the appropriate amine (2 equiv for **38** and 12 equiv for **36**, **37**, and **40**). In the case of **39**, the solution was saturated with dimethylamine gas. The mixture was heated at reflux, and the course of the reaction was monitored by TLC. In all cases the reaction never proceeded to completion and a small amount of unreacted starting material was always left. At the point of maximum accumulation of the product, refluxing was stopped and the solvent was evaporated to dryness. The product was isolated from the residue by PTLC on silica gel with chloroform−methanol (9:1) (**36** and **39**) or by column chromatography on silica gel with the same solvent (**37**) or with chloroform−methanol (9:1 and then 8:2) (**40**). Compound **38** was isolated from the residue by column chromatography on neutral alumina with chloroform−methanol (9.5:0.5 and then 8:2) as solvent systems.

2-[2′-(Dimethylamino)ethyl]-1,2-dihydro-6-[[2′-(dimethylamino)ethyl]amino]-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (36): obtained in 67% yield (96% based on reacted material), crystallized from toluene, mp 191−193 °C; ^{1}H NMR ($CDCl_3$) δ 2.37 (s, 6, 6-NCH_3), 2.42 (s, 6, 2-NCH_3), 2.69−2.78 (m, 4, CH_2N), 3.33−3.39 (q, 2, $NHCH_2$), 4.35−4.41 (t, 2, $CONCH_2$), 6.40−6.43 (d, 1, H-5), 6.50−6.52 (t, 1, NH), 7.47−7.53 (t, 1, H-9), 7.67−7.74 (t, 1, H-10), 7.94−7.97 (d, 1, H-8), 8.45−8.48 (s over d, 2, H-4 + H-7), 9.86−9.89 (d, 1, H-11). Anal. ($C_{24}H_{28}N_4O_2\cdot{}^1/_4H_2O$) C, H, N.

8-Chloro-2-[2′-(dimethylamino)ethyl]-1,2-dihydro-6-[[2′-(dimethylamino)ethyl]amino]-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (37): obtained in 71% yield (93% based on reacted material), crystallized from toluene, mp 206−208 °C; ^{1}H NMR ($CDCl_3$) δ 2.40 (s, 6, 2-NCH_3), 2.41 (s, 6, 6-NCH_3), 2.68−2.71 (t, 2, 2-CH_2N), 2.78−2.81 (t, 2, 6-CH_2N), 3.39−3.43 (q, 2, $NHCH_2$), 4.37−4.40 (t, 2, $CONCH_2$), 6.52−6.54 (d, 1, H-5), 6.73−6.75 (t, 1, NH), 7.58−7.60 (m, 2, H-9 + H-10), 8.55−8.57 (d, 1, H-4), 9.03 (s, 1, H-7), 9.91−9.93 (t, $J_{11,9}$ = 5.07 Hz, 1, H-11). Anal. ($C_{24}H_{27}ClN_4O_2\cdot{}^1/_4H_2O$) C, H, N, Cl.

2-[2′-(Dimethylamino)ethyl]-1,2-dihydro-6-[(2′-hydroxyethyl)amino]-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (38): obtained in 49% yield, crystallized from toluene−hexanes, mp 228−230 °C; ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ 2.43 (s, 6, CH_3), 2.70−2.76 (t, 2, CH_2N), 3.53−3.63 (q, 3, $NHCH_2$ + OH), 3.97−4.01 (t, 2, CH_2OH), 4.33−4.39 (t, 2, $CONCH_2$), 6.51−6.54 (d, 1, H-5), 7.40−7.50 (t, 1, NH), 7.52−7.55 (t, 1, H-9), 7.71−7.76 (t, 1, H-10), 8.00−8.03 (d, 1, H-8), 8.41−8.45 (d, 1, H-4), 9.12 (s, 1, H-7), 9.81−9.97 (d, 1, H-11). Anal. ($C_{22}H_{23}N_3O_3\cdot{}^1/_2H_2O$) C, H, N.

6-(Dimethylamino)-2-[2′-(dimethylamino)ethyl]-1,2-dihydro-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (39): obtained in 70% yield (82% based on reacted material), crystallized from hexanes into brick red needles, mp 128−130 °C; ^{1}H NMR ($CDCl_3$) δ 2.41 (s, 6, 2-NCH_3), 2.69−2.74 (t, 2, CH_2N), 3.19 (s, 6, 6-NCH_3), 4.39−4.44 (t, 2, $CONCH_2$), 7.02−7.05 (d, 1, H-5), 7.57−7.62 (t, 1, H-9), 7.78−7.83 (t, 1, H-10), 8.08−8.10 (d, 1, H-8), 8.60−8.63 (d, 1, H-4), 9.02 (s, 1, H-7), 9.98−10.01 (d, 1, H-11). Anal. ($C_{22}H_{23}N_3O_2\cdot{}^3/_4H_2O$) C, H, N.

2-[2′-(Dimethylamino)ethyl]-1,2-dihydro-7-[[2′-(dimethylamino)ethyl]amino]-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (40): obtained in 57% yield (62% based on reacted material), crystallized from toluene−hexanes (1:4), mp 113−114 °C; ^{1}H NMR (DMSO-d_6) δ 2.16 (s, 6, 7-NCH_3), 2.24 (s, 6, 2-NCH_3), 2.50−2.55 (t, 2, 2-CH_2N), 2.59−2.64 (t, 2, 7-CH_2N), 3.89−3.94 (t, 2, $NHCH_2$), 4.16−4.22 (t, 2, $CONCH_2$), 7.46−7.52 (t, 1, H-9), 7.52−7.58 (t, 1, H-5), 7.71−7.76 (t, 2, H-10 + NH), 8.37−8.40 (d, 1, H-8), 8.52−8.55 (d, 1, H-4), 8.70−8.74 (d, 1, H-6), 9.82−9.86 (d, 1, H-11). Anal. ($C_{24}H_{28}N_4O_2$) C, H, N.

This compound was also obtained by the procedure indicated in Scheme 2 as follows: A mixture of 0.7 g (2.2 mmol) of azonafide (**2**) and 20 mL of *N*,*N*-dimethylethylenediamine was heated at reflux under nitrogen for 3 h. The excess amine was removed under reduced pressure, and the residue was purified by column chromatography on silica gel with chloroform−methanol (9:1 and then 8:2) to give 176 mg of unreacted **2** and 265 mg of **40** (30%, or 40% based on reacted material).

Preparation of 2-[2′-(Dimethylamino)ethyl]-1,2-dihydro-6-ethyl-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (22). A solution of 500 mg (1.57 mmol) of 2-[2′-(dimethylamino)-ethyl]-1,2-dihydro-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (azonafide, **2**) in 30 mL of dry tetrahydrofuran was treated with 4 mL of a 2 M solution of ethylmagnesium bromide in tetrahydrofuran (Aldrich Chemical Co.). The mixture was stirred at room temperature overnight and then poured into saturated ammonium chloride solution. The two layers were separated, the aqueous layer was extracted with chloroform, and the organic layers were combined, dried over Na_2SO_4, and concentrated on a rotary evaporator. The residue was purified by PTLC on silica gel with acetone−toluene (2:8) as solvent. This procedure gave 94 mg of starting material, 268 mg of a brown oil containing three components, and, in the least polar fraction, 118 mg (27%) of **22**: mp 148−150 °C, after crystallization from hexanes−toluene; ^{1}H NMR ($CDCl_3$) δ 1.37−1.43 (t, 3, CH_3), 2.42 (s, 6, NCH_3), 2.69−2.75 (t, 2, NCH_2), 3.44−3.53 (q, 2, CH_2), 4.39−4.44 (t, 2, $CONCH_2$), 7.46−7.49 (d, 1, H-5), 7.53−7.59 (t, 1, H-9), 7.72−7.79 (t, 1, H-10), 7.98−8.02 (d, 1, H-8), 8.10−8.13 (d, 1, H-4), 8.61 (s, 1, H-7), 9.93−9.97 (d, 1, H-11). Anal. ($C_{22}H_{22}N_2O_2$) C, H, N.

Preparation of 6-Chloro-1,2-dihydro-2-[2′-(methylamino)ethyl]-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (21). A mixture of 200 mg (0.71 mmol) of 4-chloroanthracene-1,9-dicarboxylic acid,[4] 64 mg (0.86 mmol) of *N*-methylethylenediamine, 84 mg of 37% hydrochloric acid, and 150 mL of absolute ethanol was heated at reflux for 24 h. The precipitate (170 mg, 64%) was filtered. It consisted of the hydrochloride salt of **21** which was crystallized from methyl sulfoxide: mp 293−295 °C; ^{1}H NMR (DMSO-d_6) δ 2.56 (s, 3, CH_3), 3.26−3.30 (t, 2, CH_2N), 4.36−4.40 (t, 2, $CONCH_2$), 7.72−7.77 (t, 1, H-9), 7.89−7.94 (t, 1, H-10), 7.98−8.00 (d, 1, H-5), 8.36−8.39 (d, 1, H-8), 8.44−8.47 (d, 1, H-4), 9.24 (s, 1, H-7), 9.71−9.74 (d, 1, H-11). Anal. ($C_{19}H_{15}ClN_2O_2$·HCl) C, H, N, Cl.

Preparation of 1,2-Dihydro-6-methoxy-2-[2′-(methylamino)ethyl]-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (31). A mixture of 65 mg (0.173 mmol) of the hydrochloride salt of **21**, 31 mg (0.574 mmol) of freshly prepared sodium methoxide, and 35 mL of absolute methanol was heated at reflux for 9 h. The solvent was evaporated to dryness, and the residue was analyzed by PTLC on silica gel with 15% methanol in chloroform to give 5 mg of unreacted **21** and 53 mg (92% or 99% based on reacted material) of **31**, crystallized from toluene−hexanes (1:1): mp 173−175 °C; ^{1}H NMR ($CDCl_3$) δ 1.50−1.65 (br, 1, NH), 2.53 (s, 3, CH_3), 3.00−3.05 (t, 2, CH_2N), 4.17 (s, 3, OCH_3), 4.40−4.44 (t, 2, $CONCH_2$), 6.88−6.91 (d, 1, H-5), 7.57−7.62 (t, 1, H-9), 7.77−7.83 (t, 1, H-10), 8.05−8.08 (d, 1, H-8), 8.62−8.65 (d, 1, H-4), 9.10 (s, 1, H-7), 9.93−9.97 (d, 1, H-11). Anal. ($C_{20}H_{18}N_2O_3$) C, H, N.

Preparation of the Hydrobromide Salt of 2-[2′-(Dimethylamino)ethyl]-1,2-dihydro-6-hydroxy-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (25). A mixture of 833 mg (2.3 mmol) of **24**, 35 mL of glacial acetic acid, and 45 mL of 48% HBr was heated under reflux for 24 h and then allowed to stand at room temperature overnight. The resulting yellow crystalline product was filtered, washed with methanol−ether (1:1) and then with ether, and dried in air to give 812 mg (85%) of the HBr salt of **25**: mp 292−294 °C; ^{1}H NMR (DMSO-d_6) δ 2.95 (s, 6, CH_3), 3.45−3.52 (t, 2, CH_2N), 4.45−4.49 (t, 2, $CONCH_2$), 7.15−7.18 (d, 1, H-5), 7.69−7.74 (t, 1, H-9), 7.90−7.95 (t, 1, H-10), 8.40−8.43 (d, 1, H-8), 8.57−8.60 (d, 1, H-4), 9.04−9.26 (br s, 1, OH), 9.48 (s, 1, H-7), 9.88−9.91 (d, 1, H-11). Anal. ($C_{20}H_{18}N_2O_3$·HBr) C, H, Br, N.

Preparation of the Hydrobromide Salt of 2-[2′-(Dimethylamino)ethyl]-1,2-dihydro-7-hydroxy-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (30). This compound was prepared by the procedure described for **25** except that the reflux time was 14 h. It was obtained in 81% yield after recrystallization from methanol: mp 261−263 °C; ^{1}H NMR (DMSO-d_6)[14] δ 2.94 (s, 3, CH_3), 2.96 (s, 3, CH_3), 3.47- 3.53 (q, 2, CH_2N), 4.45−4.49 (t, 2, $CONCH_2$), 7.56−7.60 (t, 1, H-9), 7.68−7.72 (t, 1, H-5), 7.82−7.86 (t, 1, H-10), 8.63−8.66 (d, 2, H-4 + H-8), 8.92−8.95 (d, 1, H-6), 9.10−9.22 (br, 1, OH), 9.89−9.92 (d, 1, H-11). Anal. ($C_{20}H_{18}N_2O_3$·HBr) C, H, N; Br: calcd, 18.86; found, 18.14.

Preparation of 4-[[2′-(Dimethylamino)ethyl]amino]anthracene-1,9-dicarboxylic Acid Anhydride (44) (Scheme 2). 4-Fluoroanthracene-1,9-dicarboxylic acid (**43**) was prepared in an overall yield of 52% from 1-fluoroanthracene (**42**) following the procedure described in ref 4. The dicarboxylic acid was used as a crude material in the next step. A solution of 196 mg (0.69 mmol) of the diacid in 80 mL of toluene−absolute ethanol mixture (4:1) was heated at reflux for 14 h with 65 mg (0.74 mmol) of *N*,*N*-dimethylethylenediamine. The solvent was removed under reduced pressure, and the residue was absorbed on a silica gel column. Elution with 5% methanol in chloroform gave in the second fraction (pink) 32 mg (14%) of **44**, crystallized from chloroform−methanol (1:1): mp 267−275 °C dec; ^{1}H NMR (DMSO-d_6) δ 2.27 (s, 6, CH_3), 2.63−2.69 (t, 2, CH_2N), 3.56−3.64 (q, 2, $NHCH_2$), 6.78−6.81 (d, 1, H-3), 7.68−7.74 (t, 1, H-6), 7.89−7.96 (t, 1, H-7), 8.16−8.19 (d, 1, H-5), 8.37−8.40 (d, 1, H-2), 8.43−8.48 (t, 1, NH), 9.50−9.53 (s over d, 2, H-8 + H-10). Anal. ($C_{20}H_{18}N_2O_3$·$^1/_4H_2O$) C, H, N.

Preparation of 2-[2′-(Dimethylamino)ethyl]-1,2-dihydro-6-(methylsulfonyl)-3*H*-dibenz[*de,h*]isoquinoline-1,3-dione (46) (Scheme 3). A solution of 155 mg (0.43 mmol) of **32** in 7 mL of glacial acetic acid was treated with 0.2 mL of 30% hydrogen peroxide. The mixture was heated on a steam bath for 0.5 h. The solvent was evaporated under reduced pressure, and the residue was isolated on a neutral alumina column with a solvent gradient of chloroform−methanol (9:1 → 8:2 → 7:3) to give 140 mg (80%) of 2-[2′-((dimethylamino *N*-oxide)ethyl]-1,2-dihydro-6-(methylsulfonyl)-3*H*-dibenz[*de,h*]-isoquinoline-1,3-dione (**45**), crystallized from chloroform containing a few drops of methanol: mp 192−194 °C; ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ 3.46 (s, 9, $ONCH_3$ + SO_2CH_3), 3.92−3.95 (t, 2, CH_2NO), 4.72−4.76 (t, 2, $CONCH_2$), 7.48−7.52 (t, 1, H-9), 7.64−7.70 (t, 1, H-10), 8.09−8.12 (d, 1, H-8), 8.43−8.45 (d, 1, H-5), 8.66−8.69 (d, 1, H-4), 9.55 (s, 1, H-7), 9.62−9.65 (d, 1, H-11). This product was used directly in the next step.

A suspension of 120 mg (0.29 mmol) of **45** in 100 mL of absolute ethanol was saturated with SO_2 gas, and the mixture was stirred at room temperature for 20 h. The solvent was evaporated under reduced pressure, and the residue was purified by PTLC on silica gel with 10% methanol in chloroform to give 71 mg (62%) of **46**, crystallized from methanol: mp 240−242 °C; ^{1}H NMR ($CDCl_3$) δ 2.39 (s, 6, NCH_3), 2.71−2.76 (t, 2, CH_2N), 3.33 (s, 3, SO_2CH_3), 4.41−4.45 (t, 2, $CONCH_2$), 7.71−7.75 (t, 1, H-9), 7.88−7.93 (t, 1, H-10), 8.20−8.23 (d, 1, H-8), 8.55−8.58 (d, 1, H-5), 8.81−8.83 (d, 1, H-4), 9.75 (s, 1, H-7), 9.96−9.99 (d, 1, H-11). Anal. ($C_{21}H_{20}N_2O_4S$) C, H, N, S.

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 2, 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 concen-

trations 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 for 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 six 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.[9] 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-dried, 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% (w/v) 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 and L1210 leukemias in mice were conducted as specified in the standard NCI protocols.[15] 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 life span (ILS) = 100 × [(life span treated − life span controls)/life span 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 5×10^{-5} M. This solution was made fresh 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 and compound in the reference cuvette, the sample cuvette was heated from 25 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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(13) Data for the two types of solid tumor cells were averaged to make the QSAR results more general. When QSARs were determined for the individual cell types, the results did not differ significantly from those obtained for the average. For example, with 7-substituted azonafides and OVCAR 3 cells, the equation was $\log(1/C) = 5.58 + 0.110\Delta T_m$ ($r^2 = 0.792$, $F = 22.8$), and for AUCC375 melanoma cells, the equation was $\log(1/C) = 5.13 + 0.133\Delta T_m$ ($r^2 = 0.834$, $F = 30.1$), which are close to the results for the average of the cell types, $\log(1/C) = 5.27 + 0.127\Delta T_m$ ($r^2 = 0.839$, $F = 31.2$). Similar results were found for correlations between ΔT_m and $\log(1/C)$ for solid tumors with the 6-substituted azonafides.

(14) In this ^{1}H NMR spectrum, the $N(CH_3)_2$ group was split into two peaks and the CH_2N peak appeared as a quartet rather than the expected triplet. This phenomenon was not observed for the corresponding 6-OH isomer. A possible explanation is that the structure of **30** exists as the tautomeric anthrone with carbonyl group at C7 and hydroxyl group at C1. The OH group could make small couplings with protons on the side chain substituents.

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