Chromophore-Modified Bis-Naphthalimides: Synthesis and Antitumor Activity of Bis-Dibenz[*de,h*]isoquinoline-1,3-diones

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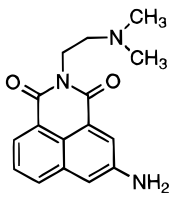
The bis-dibenz[*de,h*]isoquinoline-1,3-diones are a new series of antitumor agents that consist of two chromophores bridged by an alkylamino linker. In the present study we have explored the effect produced by the presence of two dibenz[*de,h*]isoquinoline-1,3-dione moieties with different polyamine chains on cellular cytotoxicity. Bis-dibenz[*de,h*]isoquinoline-1,3-diones with the bridge $(CH_2)_2-NH-(CH_2)_n-NH-(CH_2)_2$, where $n = 2-5$, showed optimum cytotoxicity with IC_{50}'s around 10 nM. Compound **16**, which has the $(CH_2)_2-NH-(CH_2)_3-NH-(CH_2)_2$ bridge, altered DNA mobility and topoisomerase I and II activity at approximately 5 μM. When tested in vivo, compound **16** increased the median survival time of mice implanted with M5076 with an optimum %T/C of 154% and produced cures in 50% of mice implanted with Lox melanoma.

DNA intercalators represent one of the most important drug classes in cancer therapy. These agents are characterized by the presence of flat chromophores bearing π electron-deficient polyconjugated areas bound to polar groups.[1] Their antitumor action results from DNA distortion and altered nuclear protein interaction as a consequence of reversible complex formation.[2]

In our laboratory we have discovered the antitumor potential of a series of compounds bearing a naphthalimide chromophore.[3,4] These agents bind to linear DNA, increase the length of sonicated DNA, and cause unwinding of closed circular superhelical DNA.[5] These effects are characteristic of intercalating drugs.[6] Two of the most active mononaphthalimides, amonafide (**1**) and mitonafide (**2**) have been selected for phase II



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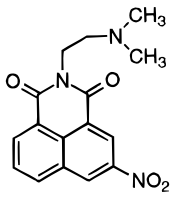

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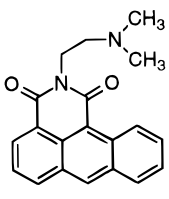

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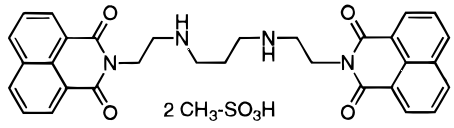
clinical trials.[7,8] Unfortunately, given on some schedules mitonafide had inappropriate central nervous system (CNS) toxicity and overall has produced limited clinical activity.[9] Amonafide has been examined more extensively in clinical trials. While some activity has been identified the effects again have been limited.[10−13] Recently approaches have been used to enhance the potency of the mononaphthalimides. One approach has been to modify the chromophore to include an anthracene moiety rather than the simpler naphthalene. These 1,2-dihydro-3*H*-dibenz[*de,h*]isoquinoline-1,3-diones include a structure unofficially named azonafide (**3**), which has shown an increase in cellular cytotoxic potency over amonafide.[14,15] A second approach that we have recently adopted is the formation of a bis-intercalator by linking two naphthalimide groups with a polyamine bridge.[16−18] When appropriately optimized this flexible linker can lead to an improved cellular cytotoxicity. For example, the bis-naphthalimides have a higher cellular cytotoxicity than amonafide or mitonafide.[16] One bis-naphthalimide, LU 79553 (**4**), has potent cellular cytotoxicity and excellent in vivo antitumor activity in several human tumor xenograft models.[19] This agent is currently in phase I of clinical development.



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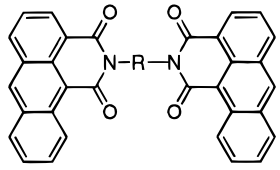

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In the present study we have explored the effect produced by the presence of two dibenz[*de,h*]isoquinoline-1,3-dione moieties with different polyamine chains (**9**−**24**) on cellular cytotoxicity. The influence on activity of a NO_2 group has also been explored.

Results and Discussion

Chemistry. The preparation of bis-dibenz[*de,h*]isoquinoline-1,3-diones **9**−**23** was carried out by reaction of the corresponding polyamine with 2 equiv of the necessary anthracene-1,9-dicarboxylic acid anhydride, using toluene as solvent, as is shown in Scheme 1. In Table 1 are shown all the bis-dibenz[*de,h*]isoquinoline-1,3-diones synthesized and their physical properties.

The anthracene-1,9-dicarboxylic acid anhydride was obtained following essentially the procedure already described.[20] Nitration of the anthracene-1,9-dicarboxy-

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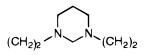
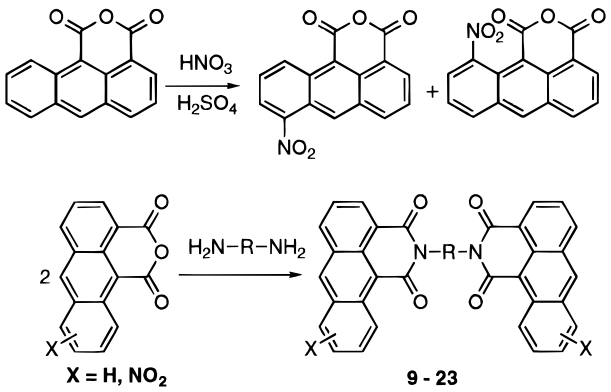
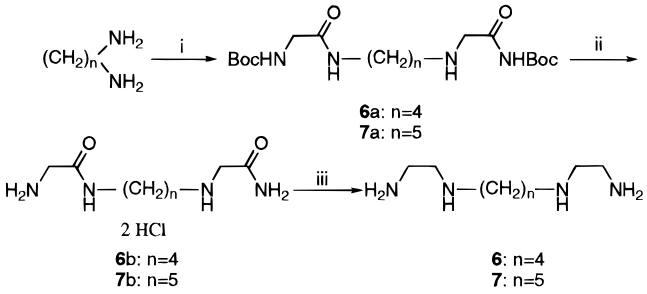
Table 1. Bis-Dibenz[*de,h*]isoquinoline-1,3-diones **9**−**24**

compd	X	R	yield (%)	mp (°C)	recrystn solvent	formula	anal.	IC_{50} (μM)
9	H	$(CH_2)_2$-NH-$(CH_2)_2$	53	271	DMF−H_2O	$C_{37}H_{27}N_3O_4$	C, H, N	0.030
10	H	$(CH_2)_2$-NH-$(CH_2)_3$	78	260	DMF	$C_{37}H_{27}N_3O_4$	C, H, N	2.000
11	H	$(CH_2)_3$-NH-$(CH_2)_3$	83	184	toluene	$C_{38}H_{29}N_3O_4$	C, H, N	0.020
12	H	$(CH_2)_3$-NCH_3-$(CH_2)_3$	35	194	toluene	$C_{39}H_{32}N_3O_4$	C, H, N	0.019
13	H	$(CH_2)_4$-NH-$(CH_2)_3$	41	244	DMF	$C_{39}H_{32}N_3O_4 \cdot 0.5H_2O$	C, H, N	0.070
14	H	$(CH_2)_2$-NH-$(CH_2)_2$-NH-$(CH_2)_2$	66	203	DMF−H_2O	$C_{38}H_{30}N_4O_4$	C, H, N	0.009
15	H	$(CH_2)_2$-NCH_3-$(CH_2)_2$-NCH_3-$(CH_2)_2$	78	255	toluene	$C_{40}H_{34}N_4O_4$	C, H, N	0.075
16	H	$(CH_2)_2$-NH-$(CH_2)_3$-NH-$(CH_2)_2$	50	191	toluene	$C_{39}H_{32}N_4O_4$	C, H, N	0.004
17	H	$(CH_2)_2$-NH-$(CH_2)_4$-NH-$(CH_2)_2$	40	183	toluene	$C_{40}H_{34}N_4O_4$	C, H, N	0.041
18	H	$(CH_2)_2$-NH-$(CH_2)_5$-NH-$(CH_2)_2$	25	122	toluene	$C_{42}H_{38}N_4O_4$	C, H, N	0.009
19	H	$(CH_2)_3$-NH-$(CH_2)_2$-NH-$(CH_2)_3$	61	180	toluene	$C_{40}H_{34}N_4O_4$	C, H, N	0.095
20	H	$(CH_2)_3$-NH-$(CH_2)_3$-NH-$(CH_2)_3$	41	140	toluene	$C_{41}H_{36}N_4O_4$	C, H, N	0.090
21	H	$(CH_2)_3$-NH-$(CH_2)_4$-NH-$(CH_2)_3$	46	183	toluene	$C_{42}H_{38}N_4O_4$	C, H, N	0.200
22	8-NO_2	$(CH_2)_2$-NH-$(CH_2)_2$-NH-$(CH_2)_2$	53	340	toluene	$C_{38}H_{28}N_6O_8$	C, H, N	0.095
23	8-NO_2	$(CH_2)_2$-NH-$(CH_2)_3$-NH-$(CH_2)_2$	46	340	toluene	$C_{39}H_{30}N_6O_8 \cdot 0.5H_2O$	C, H, N	0.035
24	H		60	222	DMF−H_2O	$C_{40}H_{32}N_4O_4$	C, H, N	0.002
amonafide								3.000
mitonafide								0.600
azonafide								0.140
LU 79553								0.014

Scheme 1

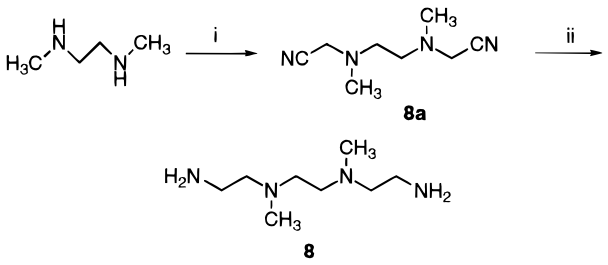


Scheme 2[a]



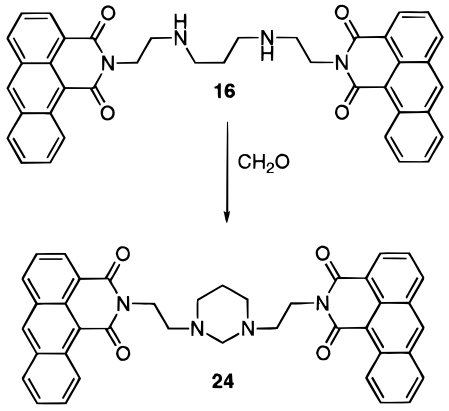
[a] Reagents: (i) *N*-Boc-glycine, CDI, CH_2Cl_2; (ii) HCl gas, MeOH; (iii) BH_3−THF, THF.

Scheme 3[a]



[a] Reagents: (i) $ClCH_2CN$, K_2CO_3, CH_3CN; (ii) H_4LiAl, diethyl ether.

Scheme 4



lic acid anhydride with 1 equiv of nitric acid in sulfuric acid yielded a mixture of 5- and 8-mononitro derivatives[15] (Scheme 1). In our hands, the more soluble 5-nitroanhydride was separated from the mixture by treatment with boiling toluene. This allowed removal of the 8-nitro isomer as an insoluble precipitate.

All the amines were commercially available, except *N*,*N*′-bis(2-aminoethyl)-1,4-butanediamine (**6**) and *N*,*N*′-bis(2-aminoethyl)-1,5-pentanediamine (**7**) used for the synthesis of compounds **17** and **18**. These compounds were initially prepared by Israel et al. from the corresponding diamine and aziridine.[21] To avoid the use of aziridine, we have now modified this synthetic procedure (Scheme 2). The central diamine segments were acylated at each end with Boc-glycine in the presence of carbonyldiimidazole, and the resulting diamide was hydrolyzed and reduced with BH_3−THF complex.

The synthesis of *N*,*N*′-bis(2-aminoethyl)-*N*,*N*′-dimethyl-1,2-ethylenediamine (**8**), used in the preparation of **15**, was performed following an analogous procedure to that described by Alcock.[22] *N*,*N*′-Dimethylethylenediamine was reacted with chloroacetonitrile, and the dinitrile **8a** formed was reduced with lithium aluminum hydride (Scheme 3).

The cyclization of **16** was accomplished by reaction of the NH groups with formaldehyde[23] yielding the acidic unstable compound **24** (Scheme 4).

Biology. The compounds were initially evaluated for in vitro cytotoxicity against the HT-29 human colon carcinoma cell line. We have previously used this cell

Table 2. Selected IR and ^{1}H-NMR Data of Compounds **9**−**24**

compd	IR (KBr, ν_{max}, cm^{-1})	^{1}H-NMR δ (ppm)
9	1670, 1640, 1560, 1430	(CF_3CO_2D) 4.07 (br s, 4H), 4.81 (br s, 4H), 7.28 (t, 2H, J = 8 Hz), 7.55 (t, 2H, J = 8 Hz), 7.76 (t, 2H, J = 8 Hz), 8.00 (d, 2H, J = 8 Hz), 8.40 (d, 2H, J = 8 Hz), 8.63−8.68 (m, 4H), 8.96 (d, 2H, J = 8 Hz)
10	1680, 1640, 1560, 1430	(CF_3CO_2D) 2.60 (m, 2H), 3.55 (m, 2H), 3.95 (m, 2H), 4.52 (m, 2H), 4.91 (m, 2H), 7.32−7.41 (m, 1H), 7.46−7.54 (m, 1H), 7.60 (t, 1H, J = 8 Hz), 7.68−7.75 (m, 1H), 7.81−7.92 (m, 2H), 8.00 (d, 1H, J = 8 Hz), 8.17 (d, 1H, J = 8 Hz), 8.39 (d, 2H, J = 8 Hz), 8.53 (d, 1H, J = 8 Hz), 8.75 (s, 1H), 8.84−8.88 (m, 1H), 8.90 (s, 1H), 9.25 (d, 1H, J = 8 Hz), 9.58 (d, 1H, J = 8 Hz)
11	1680, 1640, 1560, 1430	(CF_3CO_2D) 2.57 (t, 4H, J = 7 Hz), 3.52 (t, 4H, J = 2 Hz), 4.62 (t, 4H, J = 6 Hz), 7.53 (t, 2H, J = 7 Hz), 7.70−7.85 (m, 4H), 8.00 (d, 2H, J = 8 Hz), 8.46 (d, 2H, J = 8 Hz), 8.80 (m, 4H), 9.54 (d, 2H, J = 8 Hz)
12	1690, 1640, 1560, 1430	(CF_3CO_2D) 2.59 (m, 4H), 3.29 (s, 3H), 3.55 (m, 2H), 3.71 (m, 2H), 4.54 (t, 4H, J = 7 Hz), 7.44 (t, 2H, J = 8 Hz), 7.65−7.83 (m, 4H), 7.94 (d, 2H, J = 8 Hz), 8.44 (d, 2H, J = 8 Hz), 8.76 (m, 4H), 9.47 (d, 2H, J = 8 Hz)
13	1680, 1630, 1550, 1430	(CF_3CO_2D) 2.13 (br s, 4H), 2.53 (br s, 2H), 3.51 (m, 4H), 4.46 (m, 2H), 4.50 (m, 2H), 7.12 (t, 1H, J = 8 Hz), 7.43−7.77 (m, 6H), 7.93 (d, 1H, J = 8 Hz), 8.24 (d, 2H, J = 8 Hz), 8.39 (m, 2H), 8.61 (d, 1H, J = 7 Hz), 8.70 (m, 2H), 9.26 (d, 1H, J = 9 Hz), 9.45 (d, 1H, J = 9 Hz)
14	1685, 1650, 1580, 1470	($CDCl_3$) 2.99 (s, 4H), 3.11 (t, 4H, J = 6 Hz), 4.33 (t, 4H, J = 6 Hz), 7.47 (m, 4H), 7.65 (m, 2H), 7.82 (d, 2H, J = 8 Hz), 8.07 (d, 2H, J = 8 Hz), 8.45 (s, 2H), 8.49 (dd, 2H, J = 8, 1 Hz), 9.70 (d, 2H, J = 8 Hz)
15	1680, 1640, 1550, 1420	(CF_3CO_2D) 3.64 (s, 6H), 3.95−4.36 (m, 6H), 4.81 (m, 6H), 7.14 (t, 2H, J = 7.9 Hz), 7.47−7.71 (m, 6H), 8.30 (d, 2H, J = 8 Hz), 8.47 (s, 2H), 8.51 (d, 2H, J = 7 Hz), 9.03 (d, 2H, J = 9 Hz)
16	1680, 1645, 1560, 1430	($CDCl_3$) 1.84 (q, 2H, J = 6 Hz), 2.74 (br s, 2H, interchange with D_2O), 2.89 (t, 4H, J = 6 Hz), 3.02 (t, 4H, J = 6 Hz), 4.31 (t, 4H, J = 6 Hz) 7.54 (m, 4H), 7.73 (m, 2H), 7.89 (d, 2H, J = 8 Hz), 8.11 (d, 2H, J = 8 Hz), 8.51 (s, 2H), 8.57 (d, 2H, J = 8 Hz), 9.78 (d, 2H, J = 8 Hz)
17	1680, 1640, 1560, 1430	($CDCl_3$) 1.58 (br s, 4H), 2.76 (m, 4H), 3.06 (t, 4H, J = 6 Hz), 4.38 (t, 4H, J = 6 Hz), 7.49−7.69 (m, 4H), 7.73−7.78 (m, 2H), 7.95 (d, 2H, J = 8 Hz), 8.19 (d, 2H, J = 8 Hz), 8.62 (m, 4H), 9.83 (d, 2H, J = 8 Hz)
18	1680, 1650, 1560, 1440	($CDCl_3$) 1.50 (m, 8H), 2.68 (t, 4H, J = 7 Hz), 3.02 (t, 4H, J = 7 Hz), 4.39 (t, 4H, J = 7 Hz), 7.53−7.85 (m, 6H), 8.06 (d, 2H, J = 8 Hz), 8.30 (dd, 2H, J = 8, 1.6 Hz), 8.71 (dd, 2H, J = 7.2, 1.2 Hz), 8.75 (s, 2H), 9.96 (d, 2H, J = 8 Hz)
19	1680, 1640, 1560, 1430	($CDCl_3$) 1.63 (br s, 2H), 2.04 (m, 4H), 2.78 (m, 8H), 4.32 (t, 4H, J = 7 Hz), 7.53−7.82 (m, 6H), 8.03 (d, 2H, J = 8 Hz), 8.27 (dd, 2H, J = 8, 1 Hz), 8.69−8.72 (m, 4H), 9.96 (d, 2H, J = 8 Hz)
20	1680, 1640, 1560, 1430	($CDCl_3$) 1.76 (m, 4H), 2.06 (m, 4H), 2.77 (m, 8H), 4.33 (t, 4H, J = 7 Hz), 4.39 (t, 4H, J = 7 Hz), 7.46−7.76 (m, 6H), 7.95 (d, 2H, J = 8 Hz), 8.22 (dd, 2H, J = 8, 1.6 Hz), 8.62−8.66 (m, 4H), 9.88 (dd, 2H, J = 8, 1 Hz)
21	1680, 1640, 1560, 1430	($CDCl_3$) 1.67 (br s, 4H), 2.06 (t, 4H, J = 6 Hz), 2.79 (m, 10H), 4.27 (t, 4H, J = 7 Hz), 7.56 (m, 4H), 7.74 (m, 2H), 7.92 (d, 2H, J = 8 Hz), 8.15 (d, 2H, J = 8 Hz), 8.55 (s, 2H), 8.59 (dd, 2H, J = 8, 1 Hz), 9.82 (d, 2H, J = 8 Hz)
22	1690, 1650, 1520, 1340	(CF_3CO_2D) 3.93 (s, 8H), 4.82 (s, 4H), 7.82−7.91 (m, 4H), 8.35 (d, 2H, J = 7.4 Hz), 8.52 (d, 2H, J = 8 Hz), 8.83 (d, 2H, J = 7 Hz), 9.59 (s, 2H), 10.08 (d, 2H, J = 9.2 Hz)
23	1690, 1650, 1540, 1350	($CDCl_3$) 1.75 (c, 2H), 2.80 (t, 4H, J = 6.6 Hz), 3.01 (t, 4H, J = 6.4 Hz), 4.33 (t, 4H, J = 6.6 Hz), 7.76−7.85 (m, 4H), 8.25 (dd, 2H, J = 7.2, 1 Hz), 8.35 (d, 2H, J = 7.6 Hz), 8.76 (dd, 2H, J = 7, 1.2 Hz), 9.41 (s, 2H), 10.35 (d, 2H, J = 7 Hz)
24	1670, 1640, 1550, 1420	($CDCl_3$) 1.89 (t, 2H, J = 6 Hz), 2.81 (t, 8H, J = 8 Hz), 3.40 (s, 2H), 4.32 (t, 4H, J = 6 Hz), 7.17−7.37 (m, 6H), 7.53 (d, 2H, J = 7.6 Hz), 7.73 (dd, 2H, J = 8.4, 1.2 Hz), 7.87 (dd, 2H, J = 7, 1 Hz), 8.08 (s, 2H), 9.28 (d, 2H, J = 9 Hz)

line to guide bis-naphthalimide development.[16−18] IC_{50} values were compared with those of the monomeric compounds amonafide, mitonafide, and azonafide as well as the bis-naphthalimide LU 79553 (see Table 1). The bis-dibenz[*de*,*h*]isoquinoline-1,3-diones **9**−**24** had, in general, improved cytotoxic activity over the parental monomers. In optimized compounds this represented up to a 70-fold increase in activity over azonafide.

Evaluation of the polyamine bridge indicated that compounds with the bridge Z−NH−Y, where Z and Y were equal alkyl chains (**9** and **11**), were more potent cytotoxic agents than those where Z and Y were different alkyl chains (**10** and **13**). Furthermore, methylation of the basic nitrogen in compound **11** was tolerated and resulted in no loss of cytotoxic potency (compound **12**). Compounds with two nitrogen atoms in the bridge had better activity if the alkyl chain between the aminic-imidic nitrogens was ethylene (**14** and **16**−**18**) rather than propylene (**19**−**21**). The distance between the basic nitrogens, however, did not appear to influence cytotoxicity from two to five methylenes. *N*,*N*′-Dimethylation of the potent cytotoxic agent **14**, to give compound **15**, resulted in approximately a 10-fold loss of cytotoxic potency. Similarly the 8-nitro substitution of **14** and **16** gave compounds **22** and **23**, respectively, 10-fold less active. In order to confer a certain rigidity to the bridge with respect to **16**, compound **24** was synthesized. These compounds had similar cytotoxic activity.

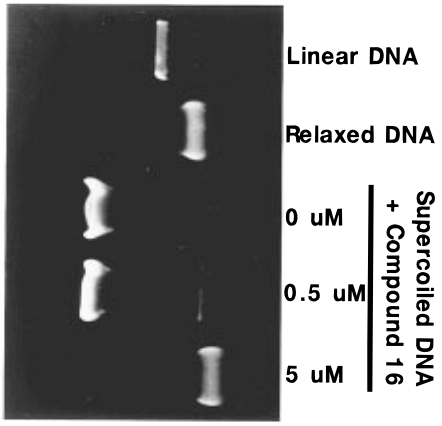


Figure 1. Effect of compound **16** on the mobility of supercoiled DNA. Incubation of compound **16** with pBR322-supercoiled DNA reduced its mobility on a 1% agarose gel to that of relaxed DNA.

Compound **16** was selected for additional testing. This compound altered DNA mobility on agarose gel at 5 μM (see Figure 1) and inhibited topoisomerase II at 5 μM (see Figure 2) and topoisomerase I at 5 μM (see Figure 3). In mice implanted ip with M5076 murine sarcoma, compound **16** produced a moderate increase in life span (see Table 3). In contrast, in a separate study the bis-naphthalimide LU 79553 (**4**) was inactive in this tumor model. Compound **16** had good activity against sc implanted Lox melanoma (see Figure 4) with some tumor cures.

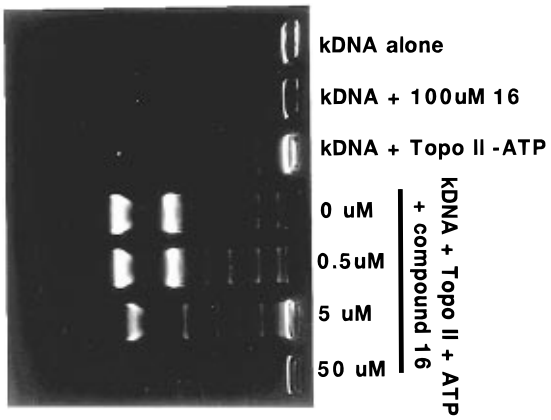


Figure 2. Inhibition of topoisomerase II decatenation of kDNA by compound **16**. kDNA was decatenated by incubating with topoisomerase II and ATP. This effect was inhibited in a concentration-related manner by compound **16**.

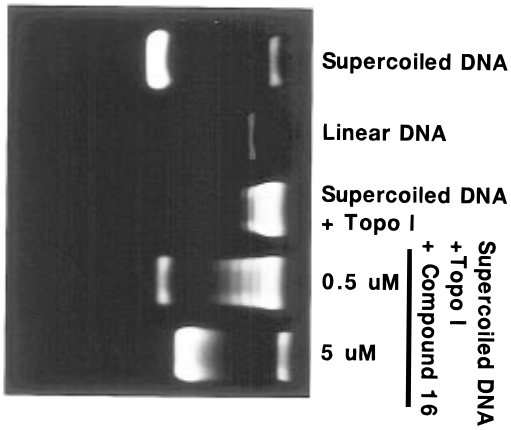


Figure 3. Inhibition of the relaxation activity of topoisomerase I by compound **16**. Supercoiled DNA was relaxed by incubating with topoisomerase I. This effect was inhibited in a concentration-related manner by compound **16**.

Table 3. Effect of Compound **16** and LU 79553 on the Life Span of C57B1/6 Mice Implanted ip with M5076

agent	dose[a] (mg/kg)	MST[b] (days)	%T/C
control		23.5	100
LU 79553	7.5	21	89
	5.0	24	102
	3.3	21.5	91
	2.2	21.5	91
control		22	100
16	1.8	31.2	143
	1.2	34	155
	0.8	31.5	143

[a] Administered ip on days 1, 5, and 9. [b] Median survival time.

Conclusions

The cytotoxic potency of azonafide on the HT-29 cell line in the present study was similar to that previously reported by Sami.[14] Our findings also supported the previous suggestion that azonafide was more potent than amonafide and indicated a increased potency over mitonafide. Appropriate polyamine linkers bridging the imidic nitrogens of two dibenz[*de,h*]isoquinoline-1,3-dione chromophores, however, were capable of increasing the cytotoxic potency of azonafide further. This result is comparable to our previous findings that the presence of two chromophores of naphthalimide can result in agents with cellular cytotoxic activity greater than that of amonafide and mitonafide.[16] Interestingly

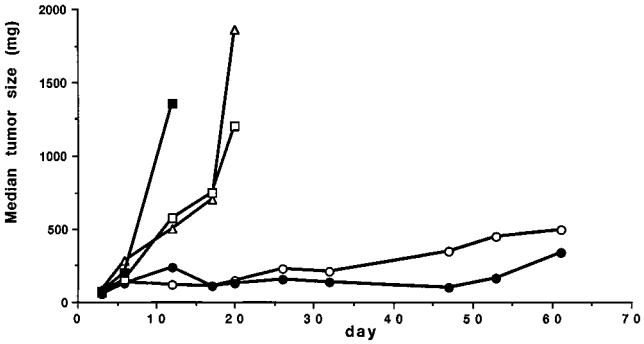


Figure 4. Effect of compound **16** on Lox melanoma growth in athymic mice. Following sc implantation of tumor, mice were treated as control (■) or iv with compound **16** at 2 mg/kg (○) or 1 mg/kg (△) on days 3−7 or at 5 mg/kg (●) or 2.5 mg/kg (□) on days 3, 7, and 11. The highest dose with each treatment schedule produced 3/6 animals that were tumor free on day 60 and considered cured.

nonsubstituted bis-naphthalimides with a $(CH_2)_2-NH-Z-NH-(CH_2)_2$ bridge need Z to be an alkyl chain of at least three methylene groups for optimum cellular cytotoxicity.[18] In the present series of bis-dibenz[*de,h*]-isoquinoline-1,3-diones, optimum activity was achieved even if Z was as short as ethylene. This is comparable to our earlier results with 3-nitro-substituted bis-naphthalimides.[18] We previously speculated that the 3-nitro may influence the orientation of the chromophore during DNA intercalation compared to the nonsubstituted form. This may also be true of the 1,2-benzo substitution in the dibenz[*de,h*]isoquinoline-1,3-diones. Compound **16** was demonstrated to bind DNA and alter its gel mobility and to inhibit topoisomerase activity. These effects are comparable to our previous findings for LU 79553.[19] This compound has the same polyamine bridge as **16** and similar in vitro activity. The in vivo activity of LU 79553 against a number of human tumor xenografts has been impressive and prompted clinical development. Compound **16** had antitumor activity against Lox melanoma comparable to effects we have reported for LU 79553 (Figure 4). In the M5076 murine tumor model, however, compound **16** produced a moderate increase in life span whereas LU 79553 was inactive. These studies suggest compound **16** and LU 79553 may have some differences in their antitumor profile.

Experimental Section

Melting points were determined using a Büchi 535 and an Electrothermal capillary melting point apparatus and are uncorrected. ^{1}H-NMR spectra at 200 MHz were recorded on a Bruker AC-200 spectrometer, with $SiMe_4$ as internal standard. IR spectra were determined with a Perkin Elmer 1310 spectrophotometer. Analyses indicated by the symbols of the elements or functions were within ±0.4% of theoretical values.

***N,N′*-Bis[[(*tert*-butoxycarbonyl)amino]acetyl]-1,4-diaminobutane (6a).** A solution of *N*-(*tert*-butoxycarbonyl)-glycine (10 g, 57 mmol) in 100 mL of dichloromethane was treated at 0 °C with 1,1′-carbonyldiimidazole (9.3 g, 57 mmol). After the mixture was stirred in an ice bath for 1.5 h, 1,4-diaminobutane (2.5 g, 28 mmol) in 50 mL of dichloromethane was added and stirred for an additional 1 h at 0 °C. After keeping at room temperature overnight, the solution was washed with a saturated solution of Na_2CO_3 (2 × 100 mL) followed by water (2 × 50 mL). This was dried on anhydrous magnesium sulfate and filtered, the solvent was evaporated and the residue was crystallized from ethyl acetate to give the title compound **6a** (5.5 g, 74%) as a white solid: mp 145 °C;

IR (KBR) 3380, 3300, 1690, 1650, 1530, 1170 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 1.36 (s, 22H, $6CH_3$, $2CH_2$), 3.03 (m, 4H, $2CH_2$), 3.46 (d, 4H, J = 6.2 Hz, $2CH_2$), 6.89 (t, 2H, J = 6 Hz, 2NH), 7.73 (t, 2H, J = 5.3 Hz, 2NH).

***N,N′*-Bis(aminoacetyl)-1,4-diaminobutane Dihydrochloride (6b).** A solution of **6a** (7 g, 17 mmol) in 200 mL of dry ethanol, at 0 °C, was saturated with hydrochloric acid (gas). After 1 h at 0 °C, the mixture was stirred at room temperature overnight. The solvent was evaporated, and the residue was washed with diethyl ether and dried, to give the title compound **6b** (4.7 g, 98%) as a white solid: mp 240 °C; IR (KBR) 3500−2500, 1650, 1600, 1460, 1120, 1100 cm^{-1}; ^{1}H-NMR (D_2O) δ 1.33 (m, 4H, $2CH_2$), 3.04 (m, 4H, $2CH_2$), 3.58 (s, 4H, $2CH_2$).

***N,N′*-Bis(2-aminoethyl)-1,4-diaminobutane Tetrahydrochloride (6c). 6b** (4.5 g, 16 mmol) in 150 mL of anhydrous tetrahydrofuran was added to 350 mL of 1 M borane−THF complex solution. This mixture was refluxed for 24 h and cooled to room temperature, and then 200 mL of methanol was added slowly and refluxed for an additional 15 h. After filtration, the solvents were evaporated and the residue dissolved in 50 mL of methanol. After the mixture cooled in an ice bath, 4 mL of concentrated hydrochloric acid was added. This mixture was treated with diethyl ether, and the solid formed was filtered to give the title compound **6c** (3.0 g, 57%) as a hygroscopic solid.

***N,N′*-Bis(2-aminoethyl)-1,4-diaminobutane (6). 6c** (3g, 10 mmol) was added to a freshly prepared solution of sodium ethoxide in ethanol [sodium (0.92 g, 40 mmol) in 75 mL of ethanol]. After the mixture stirred overnight at room temperature, the sodium chloride was removed by filtration and the solvent evaporated. The title compound **6** (0.7 g, 43%) was isolated by Kugelrohr distillation as a colorless oil: bp 160 °C/0.2 mmHg; IR (neat) 3250, 2920, 2800, 1590, 1470, 1130 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.50 (m, 10H, $2CH_2$, $2NH_2$, 2NH), 2.70 (m, 12H, $6CH_2$).

***N,N′*-Bis[[(*tert*-butoxycarbonyl)amino]acetyl]-1,5-diaminopentane (7a).** The procedure described for **6a** was followed, using 1,5-diaminopentane (2.8 g, 27 mmol). The title compound **7a** (8.4 g, 73% crude yield) was obtained as a colorless oil: IR (neat) 3390, 3320, 1680, 1640, 1530, 1160 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.33 (m, 2H, CH_2), 1.44 (s, 18H, $6CH_3$), 1.50 (m, 4H, $2CH_2$), 3.22 (m, 4H, $2CH_2$), 3.80 (d, 4H, J = 6 Hz, $2CH_2$), 5.64 (m, 2H, 2NH), 6.70 (m, 2H, 2NH).

***N,N′*-Bis(aminoacetyl)-1,5-diaminopentane Dihydrochloride (7b).** The procedure described above for **6b** was followed, using **7a** (8.4 g, 20 mmol). The title compound **7b** (5.4 g, 93%) was obtained as a white solid: mp 215 °C; IR (KBR) 3500−2500, 1650, 1560, 1490, 1280 cm^{-1}; ^{1}H-NMR (D_2O) δ 1.14 (m, 2H, CH_2), 1.31 (m, 4H, $2CH_2$), 3.01 (t, 4H, J = 7 Hz, $2CH_2$), 4.59 (s, 4H, $2CH_2$).

***N,N′*-Bis(2-aminoethyl)-1,5-diaminopentane Tetrahydrochloride (7c).** The procedure described above for **6c** was followed, using **7b** (5.3 g, 18 mmol). The title compound **7c** (3.0 g, 50%) was obtained as a white solid: mp 242 °C (ethanol−diethyl ether).

***N,N′*-Bis(2-aminoethyl)-1,5-diaminopentane (7).** The procedure described above for **6** was followed, using **7c** (2.0 g, 5.9 mmol). The title compound **7** (0.26 g, 23%) was obtained as a colorless oil: bp 185 °C/ 0.1 mmHg; IR (neat) 3300, 2900, 1590, 1450, 1120 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.50 (m, 12H, $3CH_2$, $2NH_2$, 2NH), 2.10 (m, 12H, $6CH_2$).

***N,N′*-Bis(cyanomethyl)-*N,N′*-dimethyl-1,2-diaminoethane (8a).** Chloroacetonitrile (20 g, 255 mmol) in 250 mL of acetonitrile was added to *N,N′*-dimethyl-1,2-diaminoethane (11 g, 125 mmol) and anhydrous potassium carbonate (35 g, 255 mmol) in acetonitrile (250 mL). The reaction mixture was stirred at room temperature overnight. The solid was removed by filtration and the solvent evaporated. The residue was crystallized from methanol, to give the title compound **8a** (12 g, 57%) as a white solid: mp 77−78 °C (lit.[22] mp 79−80 °C); IR (KBR) 2840, 2780, 2210, 1450, 1030 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.40 (s, 6H, $2CH_3$), 2.62 (s, 4H, $2CH_2$), 3.62 (s, 4H, $2CH_2$).

***N,N′*-Bis(2-aminoethyl)-*N,N′*-dimethyl-1,2-diaminoethane (8). 8a** (3 g, 18 mmol) in tetrahydrofuran (50 mL) was added slowly to a suspension of lithium aluminum hydride (3 g, 78 mmol) in tetrahydrofuran (100 mL). The reaction mixture was refluxed for 3 h and then cooled. Water was carefully added to decompose the excess hydride followed by 100 mL of diethyl ether. The organic layer was separated and concentrated; then the title compound **8** (2.5 g, 79%) was isolated as a colorless oil by Kugelrohr distillation: bp 115 °C/0.3 mmHg (lit.[22] bp 94−96 °C/0.7 mmHg); IR (neat) 3330, 2940, 2760, 1600, 1450, 1030 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.46 (br s, 4H, $2NH_2$), 2.19 (s, 6H, $2CH_3$), 2.37 (t, 4H, J = 6 Hz, $2CH_2$), 2.43 (s, 4H, $2CH_2$), 2.71 (t, 4H, J = 6 Hz, $2CH_2$).

General Procedure for the Preparation of Bis(1,2-dihydro-3*H*-dibenz[*de,h*]isoquinoline-1,3-diones) 9−23. To a mixture of the anthracene-1,9-dicarboxylic acid anhydride (6 mmol) in 40 mL of toluene was added the corresponding polyamine (3 mmol) dissolved in 10 mL of toluene. The suspension was heated under reflux for 4 h and then cooled to room temperature. The solid was filtered, washed with toluene, dried, and crystallized from the appropriate solvent to yield a yellow solid. Crystallization solvents were toluene dimethylformamide and mixtures of dimethylformamide and water. See Tables 1 and 2.

Preparation of *N,N′*-Bis[2-(1,2-dihydro-1,3-dioxo-3*H*-dibenz[*de,h*]isoquinolin-2-yl)ethyl]perhydropyrimidine (24). To a suspension of **16** (0.5 g, 0.4 mmol) in ethanol (75 mL) was added formaldehyde (0.5 mL, 35% in water) in ethanol (25 mL). The mixture was heated at reflux temperature for 4 h. After cooling the precipitated solid was filtered, washed with water, and crystallized from DMF−H_2O to give the title compound **24** (0.320 g, 60%) as a yellow solid: mp 222 °C.

DNA Mobility Assay. The interaction of the bis-dibenz[*de,h*]isoquinoline-1,3-diones with DNA was assessed on the basis of altered agarose gel mobility of supercoiled DNA pBR322. Supercoiled pBR322 (680 ng) was mixed with drug and the 20 μL final volume incubated at 37 °C for 30 min. Following the addition of 4 μL of loading buffer (5% sarkosyl, 0.0025% bromophenol blue, 25% glycerol), the sample was loaded onto a 1% agarose gel. The gel was run at 6 V/cm for 2.5 h in TAE buffer (40 mM Tris, 20 mM sodium acetate, 1 mM EDTA-Na_2, pH 8.5) and then stained with ethidium bromide and photographed under UV illumination using Polaroid instant film, type 55.

Topoisomerase II Decatenation Assay. Inhibition of the decatenation activity of topoisomerase II was measured by the method of Sahai and Kaplan.[24] Briefly, 450 ng of *C. fasciculata* kDNA was incubated with 6 units of purified human topoisomerase II in 20 μL of reaction buffer (0.05 M Tris HCl, pH 8.0, 0.12 M KCl, 0.01 M $MgCl_2$, 0.5 mM ATP, 0.5 mM DTT) for 30 min at 37 °C in the presence or absence of drug. Following the addition of 4 μL of stop buffer (5% sarkosyl, 0.0025% bromophenol blue, 25% glycerol), proteinase K was added to a final concentration of 50 μg/mL and the mixture incubated at 37 °C for 1 h. This reaction mixture was then loaded on an 1% agarose gel containing 0.5 μg/mL ethidium bromide and run for 3.5 h at 5 V/cm in TAE buffer. After destaining, the gel was photographed under UV illumination.

Topoisomerase I Relaxation Assay. Inhibition of DNA relaxation by topoisomerase I was measured by the method outlined by Chen.[25] Calf thymus topoisomerase I (4 units) was mixed with supercoiled DNA pBR322 (680 ng) in the presence or absence of drug and the 20 mL final volume incubated at 37 °C for 30 min. Following the addition of 128 μL of stop solution (containing 0.3% SDS, 75% EtOH, 10 mM $MgCl_2$, 30 mM sodium acetate, pH 5.2, to remove intercalated drug), the mixture was microfuged for 10 min and the pellet washed twice with 200 μL of 70% EtOH. The pellet was air-dried, resolubilized in 20 μL of TE buffer plus 4 μL of gel loading buffer, and run on a 1% agarose gel. Gels were stained with ethidium bromide and photographed under UV illumination.

Cytotoxicity Assay. The cytotoxicity of the bis-dibenz[*de,h*]isoquinoline-1,3-diones was measured using a standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Human colon carcinoma cell line HT-29 was obtained from American Type Culture Collection and cultured in the recommended media. Exponentially growing cells were plated at 3000/well into 96-well plates in 150 μL of complete

DMEM media containing 10% FBS. Cells were allowed to attach for 24 h before the addition of a serial (1:4) dilution of drug in 50 μL of fresh media. After 72 h of incubation at 37 °C, 5% CO_2, MTT (50 μL, 3 mg/mL in PBS) was added to each well and the plates were incubated for 4 h. Formazan crystals formed by MTT metabolism were solubilized by the addition of 50 μL of 25% SDS (pH 2) to each well and incubating overnight. The cellular metabolism of MTT was then quantified by reading the absorbance of the solubilized product at 550 nm with a 96-well plate reader. IC_{50} values were calculated as the concentration of drug needed to inhibit cell growth to 50% of controls.

In Vivo Antitumor Evaluation. M5076 murine sarcoma was kindly provided by Don Dykes (Southern Research Institute, Birmingham, AL). Cells (10^6) were implanted ip in C57Bl/6 mice. Antitumor activity was determined on the basis of median survival time (MST) of treated (T) mice compared to controls (C) expressed as a percentage (%T/C). An agent was considered active if it produced a %T/C of >125%. Lox melanoma was obtained from the tumor repository of the National Cancer Institute (Bethesda, MD) and grown in female athymic Ncr-nude mice (Taconic Farms, Germantown, NY). Tumor was implanted sc and passaged as tumor fragments (50 mg) dissected from the non-necrotic portion of a donor tumor. Tumor size was determined by caliper measurement and weight estimated according to a published method.[26]

For cytotoxicity testing the free bases of compounds were dissolved in DMSO and then diluted into media to give a final DMSO concentration that did not interfere with the assay (<1%). Compound **16** was converted into the dimethanesulfonate salt and solubilized in buffer for DNA and topoisomerase assays and water for in vivo testing. LU 79553 was used as the dimethanesulfonate salt and solubilized in water for in vivo testing.

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