Dicationic Bis(9-methylphenazine-1-carboxamides): Relationships between Biological Activity and Linker Chain Structure for a Series of Potent Topoisomerase Targeted Anticancer Drugs

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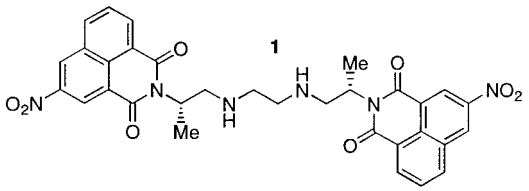
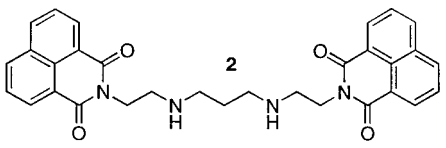
Bis(9-methylphenazine-1-carboxamides) joined by a variety of dicationic $(CH_2)_nNR(CH_2)_mNR(CH_2)_n$ linkers of varying length (carboxamide N−N distances from 11.0 to 18.4 Å) and rigidity were prepared by reaction of 9-methylphenazine-1-carboxylic acid imidazolide with the appropriate polyamines. The compounds were evaluated for growth inhibitory properties in P388 leukemia, Lewis lung carcinoma, and wild-type (JL_C) and mutant (JL_A and JL_D) forms of human Jurkat leukemia with low levels of topoisomerase II (topo II). The compounds all had IC_{50} ratios of <1 in the resistant Jurkat lines, consistent with topo II inhibition not being the primary mechanism of action. Analogues joined by an $(CH_2)_2NR(CH_2)_2NR(CH_2)_2$ linker were extremely potent cytotoxins, with selectivity toward the human cell lines, but absolute potencies declined sharply from R = H through R = Me to R = Pr and Bu. In contrast, $(CH_2)_2NR(CH_2)_3NR(CH_2)_2$ compounds showed reverse effects, with the R = Me analogue being more potent than the R = H one as well as being the most potent in the series [IC_{50} in JL_C cells 0.08 nM; superior to that for the clinical bis(naphthalimide) LU 79553]. Overall, the IC_{50}s of analogues with linker chains $(CH_2)_nNH(CH_2)_mNH(CH_2)_n$ were inversely proportional to linker length. Constraining the rigidity of the linker chain by incorporating a piperazine ring did not decrease potency significantly. A representative compound bound tightly to DNA with high selectivity for GC sites, compatible with recent work suggesting that compounds of this type place their side chains in the major groove, making specific contacts with guanine bases. Representative compounds were susceptible to transport mediated resistance, being much less effective in cells that overexpressed P-glycoprotein. Overall the results suggest these compounds have a similar mode of action, mediated primarily by poisoning of topo I (possibly with some involvement of topo II). The bis(9-methylphenazine-1-carboxamides) show very high in vitro growth inhibitory potencies compared to their monomeric analogues. Two compounds showed in vivo activity in murine colon 38 syngeneic and HT29 human colon tumor xenograft models using intraperitoneal dosing.

Compounds containing two neutral, relatively lipophilic DNA monointercalating chromophores linked by a flexible chain are of current interest as potential topoisomerase (topo) inhibitors and anticancer drugs. Most such compounds have been symmetric, employing chromophores such as naphthalimides,[1−4] benzonaphthalimides,[5] imidazoacridanones,[6,7] anthracyclinones,[8,9] acridinecarboxamides,[10] and phenazinecarboxamides,[11] but some unsymmetric compounds with dissimilar chromophores have also been reported.[12] Many of these bis compounds are much more potent cytotoxins than the corresponding monomers, and some show broad spectrum activity against a variety of human solid tumor cell lines, both in culture and as xenografts in nude mice. The bis(naphthalimide) (**1**: DMP 840) has undergone clinical trials[4] and is reported to be a topo II inhibitor in yeast.[3] The related analog LU 79553 (**2**) is highly effective against tumor xenografts in vivo[13] and is reported to bisintercalate DNA, with the side chain binding in the major groove,[14,15] and to inhibit topo II.[15]





Most of the structure−activity work with these compounds has focused on alterations in the linker chain, with relatively few changes in the terminal chromophores. In contrast, our studies on bis(acridine-4-carboxamides)[10] **3** and bis(phenazine-1-carboxamides)[11] **4** focused on evaluation of the role of chromophore substituents and showed that structure−activity rela-

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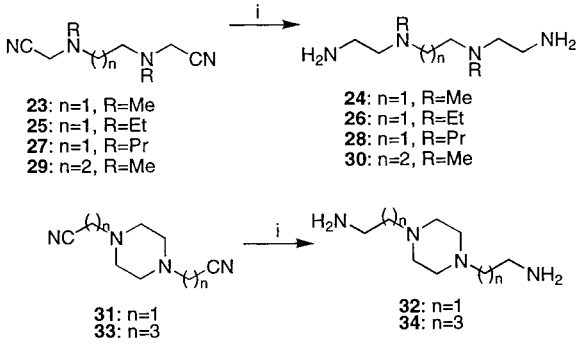
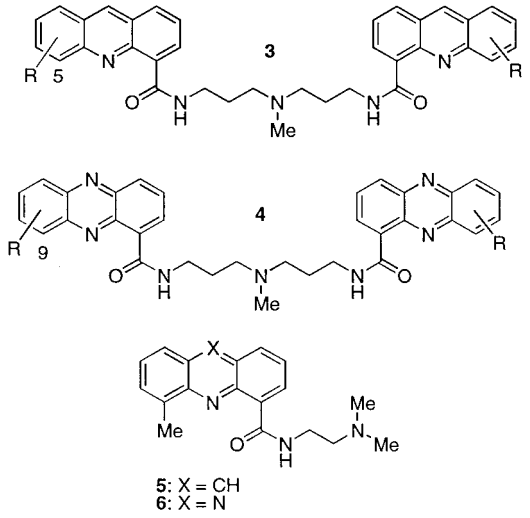
Scheme 1[a]



[a] (i) Raney Ni/H_2/dry EtOH/NH_3(g)/20 °C/5 d, or $LiAlH_4$/THF/reflux/4 h.

tionships (SAR) earlier derived for the corresponding monomers[16−18] applied to the bis compounds. Thus analogues bearing small, lipophilic substituents (particularly methyl) at the unoccupied position *peri* to the ring nitrogen [position 5 for bis(acridine-4-carboxamides) (e.g., **5**) and position 9 for bis(phenazine-1-carboxamides) (e.g., **6**)] showed the highest cytotoxicity in the monomeric series.[17,18]

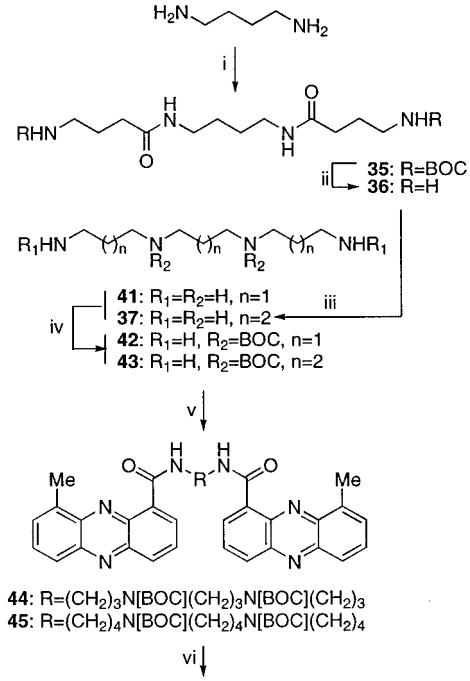


Following this identification of 9-methylphenazine as the optimal chromophore (in terms of cytotoxicity) for bis analogues, in the present paper we explore structure−activity relationships for bis(9-methylphenazine-1-carboxamides) **7**−**22**, employing a variety of dicationic linker chains where the length, rigidity, and charge density on the chain are varied.

Chemistry

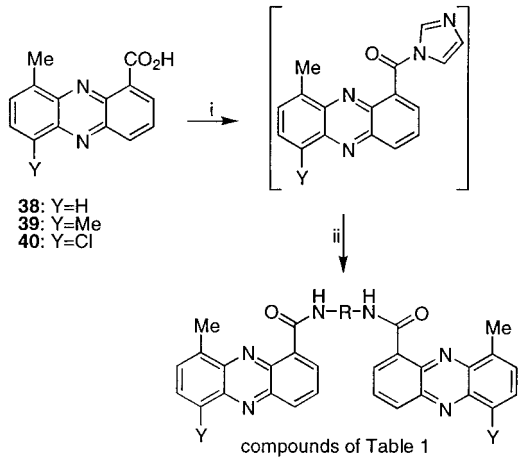
Many of the linker chains for this study were commercially available. The *N*,*N*′-dialkyl-*N*,*N*′-bis(2-aminoethyl)-1,X-diaminoalkanes (**24**, **26**, **28**, **30**) were prepared by formation of the corresponding bis(cyanomethyl) analogues (**23**, **25**, **27**, **29**, respectively) and subsequent reduction of these with either $LiAlH_4$ or Raney nickel (Scheme 1).[5,19] 1,4-Bis(2-aminoethyl)piperazine (**32**) and the known[20] 1,4-bis(aminobutyl)piperazine (**34**) were similarly prepared via the corresponding dinitriles **31** and **33**. *N*,*N*′-Bis(4-aminobutyl)-1,4-butanediamine (**37**) for compound **20** was prepared from the corresponding bisamides **35** and **36** according to the method of Brana et al. (Scheme 2).[5]

Scheme 2[a]



[a] (i) $BOCNH(CH_2)_3CO_2H$/CDI/THF; (ii) HCl(g)/MeOH, then Amberlite (OH^-) resin; (iii) BH_3−Me_2S/THF; (iv) $EtCO_2CF_3$/MeOH/N_2/20 °C/15 h, then BOC_2O/0−20 °C/3 h, then NH_4OH/20 °C/15 h; (v) **38**, CDI/DMF/50−60 °C/15 h; (vi) HCl(g)/MeOH/20 °C/5 d.

Scheme 3[a]

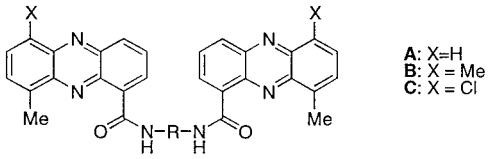


[a] (i) CDI/DMF/50−60 °C/15 h; (ii) H_2N-R-NH_2/THF/20 °C/15 h.

Reaction of 9-methylphenazine-1-carboxylic acid (**38**), 6,9-dimethylphenzazine-1-carboxylic acid (**39**), or 6-chloro-9-methylphenazine-1-carboxylic acid (**40**) with 1,1′-carbonyldiimidazole (CDI) in DMF gave the corresponding imidazolides. These were purified from excess reagent by crystallization from CH_2Cl_2/petroleum ether, then suspended in THF and treated with 0.5 equiv of the appropriate diamine (Scheme 3).

For the preparation of compounds **18** and **20**, it was found desirable to protect the secondary amines of linkers **41** and **37**, as difficulties were encountered in purification of the desired products. Thus the primary amine groups were selectively protected by reaction with ethyl trifluoroacetate, the remaining secondary amines

Table 1. Growth Inhibitory Properties of Bis(phenazine-1-carboxamides)



no.	fm	R	link[a] (Å)	IC$_{50}$ (nM)[b] P388[c]	LL[d]	JL$_C$[e]	IC$_{50}$ ratios A/C[f]	D/C[g]
5[h]				6.4	5.6	46	4.6	4.8
6				18	39	207	1.1	1.1
7[i]	A	$(CH_2)_3NMe(CH_2)_3$	9.8	15	3.3	5.6	0.6	0.6
8	A	$(CH_2)_2NH(CH_2)_2NH(CH_2)_2$	11.0	21	2.8	0.18	0.5	0.9
9	A	$(CH_2)_2NMe(CH_2)_2NMe(CH_2)_2$	11.0	33	20	5.3	0.4	0.6
10	A	$(CH_2)_2NEt(CH_2)_2NEt(CH_2)_2$	11.0	170	48	16	0.6	0.7
11	A	$(CH_2)_2NPr(CH_2)_2NPr(CH_2)_2$	11.0	250	120	46	0.5	0.8
12	A	$(CH_2)_2NH(CH_2)_3NH(CH_2)_2$	12.2	7.4	1.2	0.24	0.7	0.6
13	A	$(CH_2)_2NMe(CH_2)_3NMe(CH_2)_2$	12.2	1.6	0.3	0.08	0.4	0.8
14	A	$(CH_2)_3NH(CH_2)_2NH(CH_2)_3$	13.5	740	35	1.7	0.4	1.3
15	A	$(CH_2)_2NpipN(CH_2)_2$	10.3	2240	12	11	0.6	0.9
16	A	$(CH_2)_3NpipN(CH_2)_3$	12.5	48	0.46	1.3	0.8	0.7
17	A	$(CH_2)_4NpipN(CH_2)_4$	15.2	96	0.34	1.1	0.5	0.5
18	A	$(CH_2)_3NH(CH_2)_3NH(CH_2)_3$	14.7	430	31	1.3	0.5	1.0
19	A	$(CH_2)_3NH(CH_2)_4NH(CH_2)_3$	16.0	880	112	8.7	0.2	1.2
20	A	$(CH_2)_4NH(CH_2)_4NH(CH_2)_4$	18.4	890	290	67	0.3	0.9
21	B	$(CH_2)_2NH(CH_2)_2NH(CH_2)_2$	11.0	8.3	3.9	0.6	0.5	0.6
22	C	$(CH_2)_2NH(CH_2)_2NH(CH_2)_2$	11.0	37	10	0.6	0.4	0.8
2		LU 79553	12.3	1.5	11	0.7	0.3	0.6

[a] Length of linker chain (fully extended N−N distance between the carboxamides, see text). [b] IC$_{50}$; concentration of drug (nM) to reduce cell number to 50% of control cultures (see text). [c] P388 murine leukemia. [d] Lewis lung carcinoma. [e] Jurkat human leukemia. [f] A/C = JL$_A$/JL$_C$. [g] D/C = JL$_D$/JL$_C$. [h] Data from ref 18. [i] Data from ref 11.

were BOC-protected, and then the trifluoroacetamides were cleaved with aqueous ammonia in a one-pot procedure.[21] The BOC-protected amines (**42**, **43**) were then reacted with the 9-methylphenazine imidazolide as above, and the resulting BOC-protected intermediates (**44**, **45**) were purified before subsequent cleavage to the final products (Scheme 2).

Structure−Activity Relationships for Cellular Growth Inhibition

The structures of the bis compounds prepared by the above methods are listed in Table 1, together with their cytotoxicities (as IC$_{50}$ values) in a panel of cell lines. The selection of the cell line panel has been discussed previously.[10,18] P388 is a murine leukemia line,[16] and Lewis lung is a murine carcinoma[22] line. The three human leukemia (Jurkat) lines[23,24] provide some insight into the mechanism of cytotoxicity. JL$_C$ is the wild-type (sensitive) line, while JL$_A$ is 85-fold resistant to the topo II inhibitor amsacrine because of a reduced level of topo II enzyme. JL$_D$ is a doxorubicin-resistant line, also primarily by virtue of lower topo II levels. IC$_{50}$ values are given for the P388, LLTC, and JL$_C$ lines, together with ratios of IC$_{50}$ values against JL$_C$ and the other two Jurkat lines (ratios JL$_A$/JL$_C$ and JL$_D$/JL$_C$). Values of these ratios of less than about 2-fold suggest a likely non topo II mediated mechanism of action. The 5-methylacridine-4-carboxamide **5** was the most potent compound in the monomeric series of DACA analogues,[16] but the ratios of 4.6 and 4.8 (Table 1 and ref 18) suggest a predominantly topo II mechanism. In contrast, the 9-methylphenazine-1-carboxamide **6**, while also among the most potent monomeric phenazine analogues,[17] has ratios of only 1.1 and 1.1, respectively (Table 1), suggestive of a primarily non topo II or mixed topo I/II mechanism. While the bis(naphthalimide) **2** is reported[15] as a topo II inhibitor, the Jurkat ratios for this compound in Table 1 (0.3 and 0.6) also suggest a similar mechanism.

Compounds **7**−**20** in Table 1 explore structure−activity relationships for the linker chain in bis(9-methylphenazine-1-carboxamides). Linker chain lengths were compared by computing the carboxamide N−N distances (in the fully extended form) using the 3D module from ACD Labs. With the possible exception of **7**, all compounds had N−N distances compatible with a possible bisintercalative mode of DNA binding, by comparison with the known[14,15] bisintercalator **2**. This has an N−N distance of 12.3 Å and, as a bis(imide), probably a more rigid overall conformation than the bis(carboxamides). All of the bis compounds had IC$_{50}$ ratios in the resistant Jurkat lines of <1, consistent with a primarily non topo II mechanism of action.

As reported previously,[11] the monocationic analogue **7** is a much more potent cytotoxin than the corresponding monomer **6** (10-fold in LL, 37-fold in JL$_C$). With the shortest linker chain (an N−N distance of 9.8 Å), this compound has the least flexibility and low cationic charge density.

The dicationic compounds **8**−**11**, with $(CH_2)_2NR(CH_2)_2NR(CH_2)_2$ linkers (separation distance 11.0 Å), showed very clear SAR. The unmethylated compound **8** shows similar activity to **7** in the murine cell lines, but a sharply enhanced potency (30-fold; IC$_{50}$ 0.18 nM) in the human leukemia lines. Alkylation of the NH groups in the linker with Me, Et, or Pr groups (compounds **9**−**11**) resulted in a steady loss of potency in all cell lines as the size of the alkyl group was increased.

The closely related $(CH_2)_2NR(CH_2)_3NR(CH_2)_2$ analogues **12** and **13**, with an additional methylene unit

Table 2. Growth Inhibitory Properties of Selected Bis(phenazine-1-carboxamides) in the NCI Cell Line Panel

	GI_{50} (nM)[a]						
no.	MCF7	NCI-ADR	CAK-1	SKOV3	HT29	HCT-116	mid[b]
7	<10[c]	19	<10	220	<10	<10	20
8	<10	15	<10	<10	<10	<10	13
13	<10	<0.1	<0.1	<0.1	<10	<0.1	0.29
14	24	8030	1000	8890	27	21	524
19	74	906	8280	13600	366	335	1995

[a] GI_{50}: concentration of drug (nM) resulting in inhibition of cell growth to 50% of controls. [b] mid: the average GI_{50} value for the drug over the whole cell line panel. [c] Lowest dose tested.

between the cationic centers (separation distance 12.2 Å), showed quite different behavior. The unmethylated compound **12** showed similar cytotoxicities to **8** in the cell line panel, but in this case methylation of the NH groups to give **13** led to significant increases in potency, making it the most effective compound in all of the cell lines (e.g., an IC_{50} of 0.08 nM in JL_C, 10-fold superior to that of the clinical agent **2**). Similar SAR have been reported previously, with related pairs of both bis(naphthalimides)[2] and bis(indeno[1,2-*b*]quinoline-6-carboxamides),[25] but the reasons for this quite consistent behavior are not clear.

Compounds **14**, **18**, and **19** have progressively longer linker chains, $(CH_2)_3NH(CH_2)_nNH(CH_2)_3$, where n is 2−4. Analogues **14** and **18**, separated by 13.5 and 14.7 Å, respectively) showed comparable activity (albeit less than **8** or **12**), whereas **19** (16.0 Å) was much less effective. Compound **20**, with the longest linker chain [$(CH_2)_4NH(CH_2)_4NH(CH_2)_4$; 18.4 Å], was the least active of all the compounds studied. In fact, for the subset of compounds with linker chains $(CH_2)_xNH(CH_2)_nNH(CH_2)_x$, there is an excellent inverse correlation between potency and linker length (e.g., eq 1).

$$\log IC_{50}\ (JL_C) = 0.36\ (\pm 0.07)[\text{length}] - 4.92\ (\pm 1.1) \quad (1)$$

$$n = 5, \quad r = 0.985, \quad F = 94.7$$

A similar trend was seen with bis(indeno[1,2-*b*]quinoline-6-carboxamides), where extending the linker chain from $(CH_2)_2NH(CH_2)_3NH(CH_2)_2$ to $(CH_2)_3NH(CH_2)_3NH(CH_2)_3$ led to a 200-fold drop in potency.[25]

Compounds **15**−**17** constrain the linker chain by incorporating a piperazine ring. Analogue **15**, with the shortest and most rigid of all the dicationic linkers, has relatively low potency. There are no strictly comparable linear chain compounds to compare **16** and **17** to, but they are among the most potent derivatives in the set. These results suggest that, as shown previously with bis(benzonaphthalimides),[5] constraining the linker chain is acceptable, provided it has some minimum length and overall flexibility. On the other hand, too long and flexible a chain is disadvantageous, presumably because of unfavorable entropic effects on DNA binding.[26] Compounds **21** and **22** are analogues of **8** with an additional substituent at the 5-position. As shown previously with monocationic bis(phenazine-1-carboxamides),[11] while these substituents are acceptable they do not significantly affect cytotoxicity.

Selected compounds were also evaluated in the NCI cell human line panel,[27] and results for six of those lines are shown in Table 2 as GI_{50} values (the concentration of drug resulting in inhibition of cell growth to 50% of controls; equivalent to IC_{50}), together with the GI_{50}(mid) value (the average GI_{50} value for the drug over the whole 60-cell line panel). The individual cell line results are of limited value since many were not titrated down, but the overall GI_{50}(mid) values correlate reasonably well with the JL_C results in Table 1. For the less active compounds **14** and **19**, a comparison of results in the MCF7 and NCI/ADR lines (the latter overexpresses P-glycoprotein) shows they are significantly affected by P-glycoprotein mediated multidrug resistance. Four compounds (**2**, **7**, **8**, **21**) were also evaluated in HT-29 human colon and MOLT4 and U937 human leukemia lines (Table 3). All showed similar patterns of activity, with the MOLT4 line proving most sensitive.

Table 3. Growth Inhibitory Properties of Selected Bis(phenazine-1-carboxamides) in Other Tumor Cell Lines

	HT-29	MOLT4	U937	AuxB1		D3F	
no.	IC_{50}[a]	IC_{50}[a]	IC_{50}[a]	IC_{50}[a]	ratio[b]	IC_{50}[a]	ratio[b]
7	1.5	0.35	6.9	0.57	31.3	2.9	0.57
8	0.08	0.0062	0.047	3.7	47.1	4.4	0.91
21	0.15	0.067	0.52	4.5	30.3	6.5	0.77
2	0.15	0.037	0.34				

[a] IC_{50}; concentration of drug (nM) to reduce cell number to 50% of control cultures (see text). [b] Ratio IC_{50}[CHrC5]/IC_{50}[AuxB1]. Ratio IC_{50}[D3F-C10]/IC_{50}[D3F].

Mechanism of Action Studies

The binding constants of **7** and **8** to DNA (to the copolymers poly·[dA-dT] and poly·[dG-dC]) were determined at 0.01 ionic strength by the ethidium displacement method.[28] The monocationic compound **7** bound equally well to both copolymers, with binding constants of 8×10^7 and 7×10^7 M^{-1}. The dicationic compound **8** showed a similar level of binding to poly·[dA-dT] (9×10^7 M^{-1}) but bound much more strongly to poly·[dG-dC] ($K = 1.6 \times 10^9$ M^{-1}). This high GC-selectivity is consistent with reported binding models for both acridinecarboxamides[29] and the bis(naphthalimide) **2**,[15] where the side chains lie in the major groove and make specific contacts with guanine bases.

To further evaluate mechanism of action, selected compounds were evaluated in a series of cell lines, along with the bis(naphthalimide) **2** (Table 3). The CHrC5 line is derived from the AuxB1 Chinese hamster line and has highly amplified expression of the P-glycoprotein drug efflux pump.[30] The DC3F-C10 line is derived from the D3F Chinese hamster line and has a point mutation in the topo I enzyme, leading to a >60-fold resistance to camptothecin.[31] The CHrC5/AuxB1 ratios show that all the compounds are susceptible to the P-glycoprotein mediated resistance (although it should be noted that this cell line has a high level of overexpression). The low DC3F-C10/D3F ratios are consistent with previous data that all of these compounds, because they bind DNA rather than topo I protein, are not affected by this point mutation.[32]

These compounds are expected to be efficient DNA intercalating agents, and this is demonstrated for **8** in Figure 1, where using topo I to relax the supercoiled DNA before addition of **8** indicated that the compound can induce drug-dependent supercoils in the DNA, typical behavior for a DNA intercalator.[33,34]

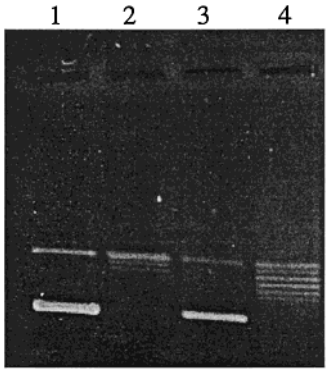


Figure 1. Agarose gel of **8** with supercoiled and topo I relaxed DNA. Lane 1: supercoiled DNA. Lane 2: relaxed DNA. Lane 3: relaxed DNA + 1.0 μM compound **8**. Lane 4: relaxed DNA + 0.1 μM compound **8**.

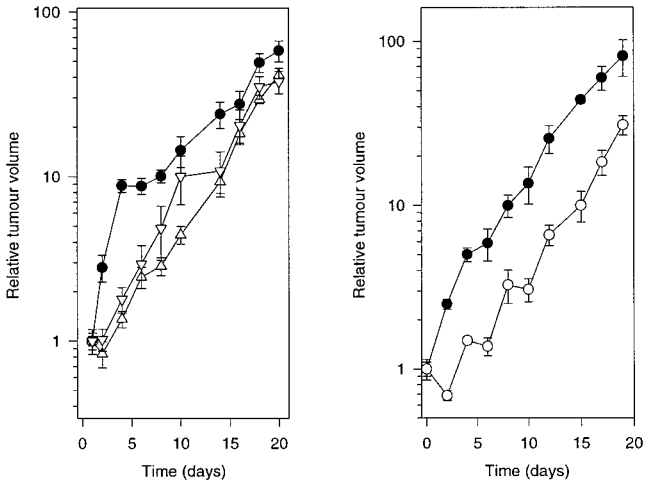


Figure 2. Effect of **8** (A) and **21** (B) on the growth of colon 38 adenocarcinoma. Panel A: Mice with subcutaneously implanted tumors of approximately 4 mm in diameter were treated with **8** at doses of 20 mg/kg (△) or 13.3 mg/kg (▽) on an intermittent dose schedule (every 4 days × 3). Symbols (●) show untreated tumor controls. Panel B: Mice were treated as above with **21** at 30 mg/kg.

Table 4. In Vivo Activity of Compounds **8** and **21**

no.	tumor	dose	growth delay (days)
8	colon 38	20	7.0
	colon 38	13.3	4.0
	colon 38	8.9	2.8
	HT29	13.3	2.4
21	colon 38	30	7.5
	colon 38	20	4.4
	colon 38	13.3	2.4
	colon 38	8.9	2.8
	HT29	20	7.8

In Vivo Studies

Compounds **8** and **21** were evaluated both in the relatively refractory subcutaneous syngeneic colon 38 tumor model in C57/Bl mice. Both compounds were active in this model (growth delays of 7 days at 20 mg/kg for **8**, and 7.5 days at 30 mg/kg for **21** (Figure 2 and Table 4)) when given intraperitoneally. This compares with a growth delay of 12 days at 30 mg/kg previously reported[11] for the less cytotoxic analogue **7** in this model. Compound **21** was also active against HT29 human colon tumor xenografts in nude mice by the intraperitoneal route, with a growth delay of 7.8 days at 20 mg/kg (Table 4). Compound **8** was relatively inactive against HT29 by this route, with a growth delay of only 2.4 days at 13.3 mg/kg. However, **8** has been reported[35] to show good activity in this model (22-day growth delay) when given intravenously (15 mg/kg, q4d × 3) and to induce complete tumor regression in the majority of animals in the H69 SCLC model.

Conclusions

The dicationic bis(9-methylphenazine-1-carboxamides) are extremely potent cytotoxins in cell culture, particularly in human cell lines, with generally greater activity toward mutant lines underexpressing topo II. There was a negative correlation between absolute IC_{50} values and linker length. This is similar to that observed for dicationic bis(indeno[1,2-*b*]quinoline-6-carboxamides)[25] but not for dicationic bis(benzonaphthalimides),[5] where in vitro potency did not alter significantly with chain length. The highly GC-selective DNA binding of **8** is consistent with the reported binding model for the related bis(naphthalimide) **2**,[15] with the linker chain lying in the major groove, making specific contacts with guanines.

The compounds all showed higher activity toward mutant Jurkat leukemia lines that underexpress topo II, and they also inhibited the action of purified topo I. This is consistent with their primary mode of action being via poisoning of topo I. However, other studies on the stabilization of cleavable complex by **8** have shown that this compound can poison both topo I and II in a purified enzyme system.[36] Studies with selected examples (including **8**) showed they had much lower effectiveness in cells with artificially elevated P-glycoprotein.

Two representative compounds (**8** and **21**) showed significant but modest antitumor effects in vivo against synegeneic colon 38 and HT29 human tumor xenografts when given intraperitoneally, indicating that their very high in vitro growth inhibitory potencies (compared to their monomeric analogues) do not necessarily translate into comparable increases in in vivo effectiveness. However, **8** does show good activity against HT29 tumors when given intravenously.[35]

Experimental Section

Chemistry. Analyses were carried out in the Microchemical Laboratory, University of Otago, Dunedin, NZ. Melting points were determined on an Electrothermal 2300 melting point apparatus. NMR spectra were obtained on a Bruker DRX-400 spectrometer and are referenced to Me_4Si for organic solutions and 3-(trimethylsilyl)propanesulfonic acid, sodium salt for D_2O solutions. Thin-layer chromatography was carried out on aluminum-backed silica gel (Merck 60 F_{254}) or alumina plates. Flash column chromatography was carried out on Merck silica gel (230−400 mesh) or alumina. Petroleum ether refers to the fraction boiling at 40−60 °C. Satisfactory high-resolution mass spectral data were obtained for the bis(phenazines), using a VG 7070 spectrometer at nominal 5000 resolution. All of the bis(phenazines) were judged to be >98% pure by reverse-phase HPLC.

Preparation of Linker Chains. *N,N′*-Dimethyl-*N,N′*-bis(2-aminoethyl)-1,2-ethanediamine (24). A solution of *N,N′*-dimethyl-*N,N′*-bis(cyanomethyl)-1,2-ethanediamine[37] (**23**) (5.0 g, 100 mmol) in absolute EtOH saturated with dry ammonia was hydrogenated over Raney nickel for 5 days at 20 °C (additional Raney nickel was added as required). The

catalyst was removed by filtration through a pad of Celite, and evaporation of solvents gave essentially pure **24** (4.60 g, 88%) that was used directly: ^{1}H NMR ($CDCl_3$) δ 2.24 (s, 6 H, 2 × CH_3), 2.43 (br s, 4 H, 2 × CH_2), 2.49 (s, 2 H, 2 × CH_2), 2.73 (br s, 2 H, 2 × CH_2).

***N,N′*-Diethyl-*N,N′*-bis(2-aminoethyl)-1,2-ethanediamine (26).** Sodium *m*-bisulfite (11.5 g, 60.5 mmol) was dissolved in water (25 mL) and 40% aqueous formaldehyde (7.75 mL). The solution was boiled for 10 min and cooled to room temperature, and then *N,N′*-diethyl-1,2-ethanediamine (6.6 g, 56.8 mmol) was added with vigorous stirring. After the mixture was stirred at room temperature for 4 h, a solution of NaCN (6.25 g, 127.5 mmol) in water (12.5 mL) was added, and the mixture was stirred overnight at room temperature. The product was extracted into CH_2Cl_2 and dried (Na_2SO_4). Evaporation of solvents gave a yellow oil that was chromatographed on alumina, eluting with CH_2Cl_2, to give *N,N′*-bis(cyanomethyl)-*N,N′*-diethyl-1,2-diaminoethane (**25**) (5.63 g, 51%) as an oil: ^{1}H NMR ($CDCl_3$) δ 1.11 (t, J = 7.2 Hz, 6 H, 2 × CH_2CH_3), 2.60 (q, J = 7.2 Hz, 4 H, 2 × CH_2CH_3), 2.67 [s, 4 H, $N(CH_2)_2N$], 3.69 (s, 4 H, 2 × CH_2CN).

A solution of **25** (0.522 g, 2.69 mmol) in THF (20 mL) was added dropwise to a suspension of $LiAlH_4$ (0.47 g, 12.38 mmol) in THF (20 mL). The reaction mixture was heated under reflux for 4 h and then cooled in ice, and water (0.47 mL), 15% aqueous NaOH (0.47 mL), and water (1.41 mL) were added sequentially. The resulting granular precipitate was filtered and washed with Et_2O. The combined filtrates were evaporated under reduced pressure, and the residue was chromatographed on silica gel (eluting with $MeOH/CH_2Cl_2/NH_4OH$, 30:60:10) to give **26** (0.45 g, 85%) as an oil (lit.[38] oil): ^{1}H NMR ($CDCl_3$) δ 1.02 (q, J = 7.2 Hz, 6 H, 2 × CH_2CH_3), 1.96 (br s, 4 H, 2 × NH_2), 2.48−2.57 (m, 8 H, 4 × CH_2), 2.52 [s, 4 H, $N(CH_2)_2N$], 2.73 (t, J = 6.1 Hz, 4 H, 2 × $NCH_2CH_2NH_2$).

***N,N′*-Di-(*n*-propyl)-*N,N′*-bis(2-aminoethyl)-1,2-ethanediamine (28).** Similar reaction of *N,N′*-di-*n*-propyl-1,2-ethanediamine gave *N,N′*-bis(cyanomethyl)-*N,N′*-di-*n*-propyl-1,2-ethanediamine (**27**) (8.3 g, 66%) as an oil: ^{1}H NMR ($CDCl_3$) δ 0.92 (t, J = 7.4 Hz, 6 H, 2 × CH_3), 1.49 (sext, J = 7.4 Hz, 4 H, 2 × CH_2), 2.49 (t, J = 7.4 Hz, 4 H, 2 × CH_2), 2.66 (s, 4 H, 2 × CH_2), 3.67 (s, 4 H, 2 × CH_2). HRMS (EI) *m/z* calcd for $C_{12}H_{22}N_4$ (M^+), 222.1845; found, 222.1850.

Reduction of **27** with $LiAlH_4$ as above gave **28** (1.96 g,78%) as an oil: ^{1}H NMR ($CDCl_3$) δ 0.87 (t, J = 7.2 Hz, 6 H, 2 × CH_3), 1.47 (sext, J = 7.4 Hz, 4 H, 2 × CH_2), 2.39 (t, J = 7.5 Hz, 4 H, 2 × CH_2), 2.50 (t, J = 7.0 Hz, 4 H, 2 × CH_2), 2.52 (s, 4 H, 2 × CH_2), 2.73 (t, J = 6.0 Hz, 4 H, 2 × CH_2), 3.12 (s, 8 H, 2 × CH_2 and 2 × NH_2). HRMS (FAB) *m/z* calcd for $C_{12}H_{30}N_4$ (MH^+), 231.2549; found, 231.2551.

***N,N′*-Bis[(3- *tert*-butyloxycarbonylamino)propyl]-1,3-propanediamine (42).** To a solution of commercial *N,N′*-bis(3-aminopropyl)-1,3-propanediamine (**41**) (1.00 g, 5.32 mmol) in MeOH (20 mL) under N_2 was added ethyl trifluoroacetate (2.64 g, 18.6 mmol). This mixture was stirred for 15 h at room temperature at which point the reaction was complete by TLC. This solution was then cooled to 0 °C (ice/water) and then BOC_2O (4.64 g, 21.3 mmol) added, and the reaction was allowed to warm to room temperature with stirring. After 3 h at this temperature, concentrated aqueous NH_3 was added until the pH reached 11, and then the solution was stirred for 15 h at room temperature. The mixture was then concentrated, diluted with $CHCl_3$ (300 mL), and dried with Na_2SO_4. All solids were removed by filtration and washed thoroughly with $CHCl_3$, and the organic fractions were combined. The $CHCl_3$ was then removed under reduced pressure to yield an oil which was purified by chromatography on silica (eluting with 1% NH_4OH in $MeOH/CH_2Cl_2$ 20:80) to give *N,N′*-bis[(3- *tert*-butyloxycarbonylamino)propyl]-1,3-propanediamine (**42**) as a pale yellow oil (1.71 g, 83%): ^{1}H NMR ($CDCl_3$) δ 1.45 (br s, 18 H, 6 × CH_3), 1.52−1.88 (m, 12 H, 4 × CH_2 and 2 × NH_2), 2.69 (br t, J = 6.6 Hz, 2 H, 1 × CH_2), 3.11−3.31 (br m, 8 H, 4 × CH_2).

***N,N′*-Bis[(4-*tert*-butyloxycarbonylamino)butyl]-1,4-butanediamine (43).** According to the method of Brana et al.,[5] a mixture of *N*-BOC-4-aminobutyric acid (7.11 g, 33.1 mmol) and CDI (5.89 g, 36.4 mmol) in dry THF (100 mL) was stirred for 15 h at room temperature. A solution of 1,4-diaminobutane (1.47 g, 16.6 mmol) in dry THF (50 mL) was slowly added to the above solution of imidazolide. This mixture was allowed to stir at room temperature for 2 days. The solvent was removed under reduced pressure and the residue partitioned between 1 M Na_2CO_3 (200 mL) and $CHCl_3$ (200 mL). The $CHCl_3$ layer was then washed with water (300 mL) and 0.5 M HCl solution (200 mL) and then dried (Na_2SO_4). The $CHCl_3$ was removed under reduced pressure to give a white solid which was purified by chromatography on silica gel (2−3% MeOH in CH_2Cl_2 as eluant), giving *N,N′*-bis(4-*tert*-butyloxycarbonylamino-1-oxobutyl)-1,4-butanediamine (**35**) (2.72 g, 38%): mp (EtOAc) 118−119 °C; ^{1}H NMR [$(CH_3)_2SO$] δ 1.39−1.54 (m, 22 H, 6 × CH_3 and 2 × CH_2), 1.56 (quin, J = 7.3 Hz, 4 H, 2 × CH_2), 2.02 (t, J = 7.5 Hz, 4 H, 2 × CH_2), 2.88 (q, J = 6.6 Hz, 2 H, 2 × CH_2), 2.97−3.04 (br m, 4 H, 2 × CH_2), 6.78 [t, J = 5.4 Hz, 2 H, 2 × CONH (amide)], 7.75 [t, J = 5.5 Hz, 2 H, 2 × CONH (carbamate)]. Anal. ($C_{22}H_{42}N_4O_6$) C, H, N.

Diamide **35** (2.09 g, 4.56 mmol) in MeOH (50 mL) saturated with HCl(g) was stirred overnight at room temperature, and the solvent was then removed under reduced pressure. The residue was converted from the HCl salt to the free base by elution through a short column of Amberlite (OH^-) resin with EtOH, to give *N,N′*-bis(4-amino-1-oxobutyl)-1,4-butanediamine (**36**) as a white solid (1.18 g, 100%): mp (EtOH) 120−124 °C; ^{1}H NMR (D_2O) δ 1.51 (br s, 4 H, 2 × CH_2), 1.94 (quin, J = 7.6 Hz, 4 H, 2 × CH_2), 2.36 (t, J = 7.5 Hz, 4 H, 2 × CH_2), 3.01 (t, J = 7.8 Hz, 4 H, 2 × CH_2), 3.19 (br s, 4 H, 2 × CH_2). Anal. ($C_{12}H_{26}N_4O_2 \cdot 0.5H_2O$) C, H, N.

Reduction of **36** with $BH_3 \cdot Me_2S$ gave the known[39] polyamine *N,N′*-bis(4-aminobutyl)butane-1,4-diamine (**37**) as a crude oil. This oil was purified by derivatization as described for compound **42**, giving *N,N′*-bis[(4- *tert*-butyloxycarbonylamino)-butyl]-1,4-butanediamine (**43**) as a transparent oil, 56% over two steps from the amide; ^{1}H NMR ($CDCl_3$) δ 1.45 [s, 18 H, 2 × $C(CH_3)_3$], 1.39−1.54 (br m, 12 H, 6 × CH_2 and 2 × NH_2), 2.70 (t, J = 7.0 Hz, 4 H, 2 × CH_2NH_2), 3.17 [br s, 8 H, 2 × $CH_2N(BOC)CH_2$].

***N,N′*-Bis[2-(9-methylphenazine-1-carboxamido)ethyl]-1,2-ethanediamine (8): General Method of Scheme 3.** A mixture of 9-methylphenazine-1-carboxylic acid[17] (**38**) (3.59 g, 15.1 mmol) and CDI (3.66 g, 22.6 mmol) in DMF (20 mL) was stirred and heated to 50−60 °C for 2 h. Most of the DMF was removed under reduced pressure, and CH_2Cl_2 (20 mL) was added to dissolve the crude imidazolide. Addition of CH_2Cl_2/light petroleum (1:4, 200 mL) and cooling in ice gave crystalline imidazolide, which was collected, washed with cold CH_2Cl_2/light petroleum (1:4, 100 mL), and dried (3.34 g, 77%). This was dissolved in THF (30 mL), cooled in ice/salt, and treated with a solution of *N,N′*-(2-aminoethyl)-1,2-diaminoethane (0.85 g, 5.8 mmol) in THF (5 mL). The mixture was allowed to slowly warm to room temperature and stirred overnight. Most of the THF was evaporated, then water (200 mL) was added, and the mixture was stirred for 1 h. The resulting precipitate was filtered, washed with water, dried (vacuum; P_2O_5), then dissolved in MeOH/AcOH, clarified with charcoal/Celite, filtered, and basified with Et_3N to give **8** (2.5 g, 77%): mp (MeOH) 243−245 °C; ^{1}H NMR (CF_3CO_2D) δ 3.15 (s, 6 H, 2 × $ArCH_3$), 3.90 (t, J = 4.6 Hz, 4 H, 2 × CH_2NHCH_2), 4.06 (s, 4 H, $NHCH_2CH_2NH$), 4.33 (t, J = 4.6 Hz, 4 H, 2 × $CONHCH_2$), 8.30 (d, J = 5.0 Hz, 2 H, ArH), 8.42−8.48 (m, 4 H, ArH), 8.55 (t, J = 8.1 Hz, 2 H, ArH), 8.81 (d, J = 8.9 Hz, 2 H, ArH), 9.09 (d, J = 7.2 Hz, 2 H, ArH). Anal. ($C_{34}H_{34}N_8O_2$) C, H, N.

***N,N′*-Bis[2-(9-methylphenazine-1-carboxamido)ethyl]-*N,N′*-dimethyl-1,2-ethanediamine (9).** Similar reaction of **38** and **24**, followed by chromatography of the product on silica gel (eluting with a gradient of 1−6% MeOH in CH_2Cl_2), gave **9** (1.10 g, 78%): mp (CH_2Cl_2/hexane) 124−125 °C; ^{1}H NMR ($CDCl_3$) δ 2.33 (s, 6 H, 2 × NCH_3), 2.63 (br s, 4 H, $CH_3NCH_2CH_2NCH_3$), 2.74 (t, J = 6.5 Hz, 4 H, $CONHCH_2CH_2$), 2.83 (s, 6 H, 2 × $ArCH_3$), 3.73 (q, J = 6.2 Hz, 4 H, 2 × $CONHCH_2$), 7.61 (d, J = 6.7 Hz, 2 H, H-8), 7.68 (dd, J = 8.7, 6.8 Hz, 2 H, H-7), 7.89 (dd, J = 8.7, 7.2 Hz, 2 H, H-3), 7.98 (d, J = 8.7 Hz,

2 H, H-6), 8.26 (dd, J = 8.7, 1.5 Hz, 2 H, H-4), 8.89 (dd, J = 7.0, 1.5 Hz, 2 H, H-2), 10.85 (t, J = 5.2 Hz, 2 H, 2 × CONH). Anal. ($C_{36}H_{38}N_8O_2 \cdot 2HCl \cdot 0.5H_2O$) C, H, N, Cl.

***N,N′*-Bis[2-(9-methylphenazine-1-carboxamido)ethyl]-*N,N′*-diethyl-1,2-ethanediamine (10).** Similar reaction of **38** and **26** gave **10** (78%) as an oil: ^{1}H NMR ($CDCl_3$) δ 0.99 (t, J = 7.1 Hz, 6 H, 2 × CH_2CH_3), 2.64 (q, J = 7.1 Hz, 4 H, 2 × CH_2CH_3), 2.71 [s, 4 H, $N(CH_2)_2N$], 2.82 (t, J = 6.8 Hz, 4 H, 2 × $CONHCH_2CH_2$), 2.84 (s, 6 H, 2 × $ArCH_3$), 3.72 (q, J = 6.4 Hz, 4 H, 2 × CH_2), 7.63 (d, J = 6.7 Hz, 2 H, H-8), 7.70 (dd, J = 8.7, 6.6 Hz, 2 H, H-7), 7.88 (dd, J = 8.6, 7.2 Hz, 2 H, H-3), 8.01 (d, J = 8.5 Hz, 2 H, H-4), 8.28 (dd, J = 8.7, 1.7 Hz, 2 H, H-6), 8.92 (dd, J = 7.2, 1.5 Hz, 2 H, H-2), 10.91 (t, J = 5.6 Hz, 2 H, 2 × CONH). HRMS (FAB) $C_{38}H_{43}N_8O_2$ (MH^+) requires 643.3509; found, 643.3517.

***N,N′*-Bis[2-(9-methylphenazine-1-carboxamido)ethyl]-*N,N′*-di-*n*-propyl-1,2-ethanediamine (11)**. Similar reaction of **38** and **28** gave **11** (97%) as an oil: ^{1}H NMR ($CDCl_3$) δ 0.78 (t, J = 7.4 Hz, 6 H, 2 × CH_2CH_3), 1.41 (sext, J = 7.4 Hz, 4 H, 2 × CH_2CH_3), 2.49 (t, J = 7.5 Hz, 4 H, 2 × $CH_2CH_2CH_3$), 2.70 (br s, 4 H, 2 × CH_2), 2.82 (t, J = 6.5 Hz, 4 H, 2 × CH_2), 2.86 (s, 6 H, 2 × $ArCH_3$), 3.71 (q, J = 6.3 Hz, 4 H, 2 × CH_2), 7.64 (d, J = 6.8 Hz, 2 H, H-8), 7.71 (dd, J = 8.7, 6.8 Hz, 2 H, H-7), 7.89 (dd, J = 8.6, 7.2 Hz, 2 H, H-3), 8.03 (d, J = 8.4 Hz, 2 H, H-6), 8.30 (dd, J = 8.7, 1.5 Hz, 2 H, H-4), 8.93 (dd, J = 7.1, 1.6 Hz, 2 H, H-2), 10.92 (br s, 2 H, 2 × CONH). HRMS (FAB^+) m/z calcd for $C_{40}H_{47}N_8O_2$ (MH^+), 671.3822; found, 671.3801.

***N,N′*-Bis[2-(9-methylphenazine-1-carboxamido)ethyl]-1,3-propanediamine (12).** Similar reaction of **38** and *N,N′*-bis(2-aminoethyl)-1,3-propanediamine gave **12** (47%): mp (CH_2Cl_2/MeOH) 194−195 °C; ^{1}H NMR ($CDCl_3$) δ 1.73 (quin, J = 6.9 Hz, 2 H, $CH_2CH_2CH_2$), 2.79 (t, J = 6.9 Hz, 4 H, 2 × CH_2), 2.88 (s, 6 H, 2 × $ArCH_3$), 2.97 (t, J = 6.2 Hz, 4 H, 2 × CH_2), 3.75 (q, J = 6.0 Hz, 4 H, 2 × CH_2), 7.64−7.69 (m, 2 H, ArH), 7.72 (dd, J = 8.6, 6.8 Hz, 2 H, ArH), 7.93 (dd, J = 8.7, 7.2 Hz, 2 H, ArH), 8.04 (dd, J = 8.7, 0.9 Hz, 2 H, ArH), 8.33 (dd, J = 8.7, 1.5 Hz, 2 H, ArH), 8.96 (dd, J = 7.2, 1.5 Hz, 2 H, ArH) and 11.06 (br t, J = 5.3 Hz, 2 H, 2 × CONH); HRMS (FAB^+) m/z calcd for $C_{35}H_{37}N_8O_2$ 601.3039 (MH^+), found 601.3043. Anal. ($C_{35}H_{36}N_8O_2 \cdot 0.5H_2O$) C, H: N; Found 19.0; Calculated 18.4%.

***N,N′*-Bis[2-(9-methylphenazine-1-carboxamido)ethyl]-*N,N′*-dimethyl-1,3-propanediamine (13).** Reaction of *N,N′*-bis(cyanomethyl)-1,3-propanediamine (**29**) with Raney nickel in EtOH in the presence of ammonia gas gave the known[40] amine *N,N′*-bis(2-aminoethyl)- *N,N′*-dimethyl-1,3-propanediamine (**30**) in 49% yield. This amine was reacted directly with **38** as above, followed by chromatography on silica (1−2% MeOH in CH_2Cl_2 as eluant) to give **13** as a yellow foam (87%): ^{1}H NMR ($CDCl_3$) δ 1.60−1.65 (m, 2 H, $CH_2CH_2CH_2$ − obscured by H_2O), 2.18 (s, 6 H, 2 × NCH_3), 2.40 (t, J = 7.3 Hz, 4 H, 2 × CH_2), 2.57 (t, J = 6.5 Hz, 4 H, 2 × CH_2), 2.83 (s, 6 H, 2 × $ArCH_3$), 3.69 (q, J = 6.1 Hz, 2 × CH_2), 7.61−7.64 (m, 2 H, ArH), 7.66 (dd, J = 8.6, 6.8 Hz, 2 H, ArH), 7.90 (dd, J = 8.7, 7.2 Hz, 2 H, ArH), 7.97 (d, J = 7.3 Hz, 2 H, ArH), 8.28 (dd, J = 8.7, 1.5 Hz, 2 H, ArH), 8.95 (dd, J = 7.2, 1.5 Hz, 2 H, ArH), 10.82 (br t, J = 4.9 Hz, 2 H, 2 × CONH). Anal. ($C_{37}H_{40}N_8O_2$) C, H, N.

***N,N′*-Bis[3-(9-methylphenazine-1-carboxamido)propyl]-1,2-ethanediamine (14).** Similar reaction of **38** and *N,N′*-bis(3-aminopropyl)-1,2-ethanediamine gave **14** as a gum, which was converted to the dihydrochloride salt (10%): mp (MeOH) 276 °C;^{1}H NMR (HCl salt; D_2O) δ 2.07 (quin, J = 6.7 Hz, 4 H, 2 × CH_2), 2.82 (s, 6 H, 2 × $ArCH_3$), 3.17 (m, 4 H, 2 × CH_2), 3.31 (br s, 4 H, 2 × CH_2), 3.65 (t, J = 6.6 Hz, 4 H, 2 × CH_2), 7.87 (m, 4 H, ArH), 7.96−8.00 (m, 4 H, ArH), 8.27−8.30 (m, 2 H, ArH), 8.60 (d, J = 7.2 Hz, 2 H, ArH). HRMS (FAB^+) m/z calcd for $C_{36}H_{38}N_8O_2$, 615.3196 (MH^+); found, 615.3196.

***N*¹,*N*⁴-Bis[2-(9-methylphenazine-1-carboxamido)ethyl]-piperazine (15).** Reaction of *N,N′*-bis(cyanomethyl)piperazine[41] (**31**) with Raney nickel in EtOH in the presence of ammonia gas gave the known[42] amine 1,4-bis(2-aminoethyl)-piperazine (**33**) in 73% yield. This amine was reacted directly with **38** as above, followed by chromatography on alumina (1% MeOH in CH_2Cl_2 as eluant) to give **15** (77%): mp (CH_2Cl_2/*n*-hexane) 279−281 °C; ^{1}H NMR ($CDCl_3$) δ 2.58−2.65 (br s, 8 H, 4 × CH_2pip), 2.77 (t, J = 6.6, 4 H, 2 × CH_2Npip), 2.95 (s, 6 H, 2 × $ArCH_3$), 3.84 (q, J = 6.2 Hz, 4 H, 2 × CH_2NHCO), 7.74 (d, J = 6.7 Hz, 2 H, ArH), 7.80 (dd, J = 8.6, 6.8 Hz, 2 H, ArH), 7.96 (dd, J = 8.6, 7.2 Hz, 2 H, ArH), 8.13 (d, J = 8.7 Hz, 2 H, ArH), 8.39 (dd, J = 8.7, 1.5 Hz, 2 H, ArH), 8.99 (dd, J = 7.2, 1.5 Hz, 2 H, ArH), 11.03 (br t, J = 5.0 Hz, 2 H, 2 × CONH), HRMS (FAB^+) m/z calcd for $C_{36}H_{37}N_8O_2$, 613.3039 (MH^+); found, 613.3080. Anal. ($C_{36}H_{36}N_8O_2$) C, H, N.

***N*¹,*N*⁴-Bis[3-(9-methylphenazine-1-carboxamido)propyl]-piperazine (16).** Similar reaction of **38** and 1,4-bis(3-aminopropyl)piperazine gave a crude product that was dissolved in MeOH/AcOH, clarified with charcoal/Celite, filtered, and basified with Et_3N to give **16** (45%): mp (MeOH) 252−253 °C; ^{1}H NMR ($CDCl_3$) δ 1.98 (quin, J = 7.3 Hz, 4 H, 2 × $CH_2CH_2CH_2$), 2.52 (t, J = 7.4 Hz, 12 H, CH_2Npip & pipCH_2), 2.92 (s, 6 H, 2 × $ArCH_3$), 3.72 (q, J = 6.5 Hz, 4 H, 2 × CH_2NHCO), 7.75−7.82 (m, 4 H, ArH), 7.96 (dd, J = 8.7, 7.2 Hz, 2 H, H-3), 8.13 (dd, J = 8.4, 0.8 Hz, 2 H, ArH), 8.39 (dd, J = 8.7, 1.5 Hz, 2 H, ArH), 9.00 (dd, J = 7.2, 1.5 Hz, 2 H, H-2), 11.11 (t, J = 5.3 Hz, 2 H, 2 × CONH). Anal. ($C_{38}H_{40}N_8O_2 \cdot 0.5H_2O$) C, H, N.

***N*¹,*N*⁴-Bis[4-(9-methylphenazine-1-carboxamido)butyl]-piperazine (17).** Similar reaction of **38** and 1,4-bis(4-aminobutyl)piperazine[20] gave **17** (68%): mp (CH_2Cl_2/hexane) 181−182 °C; ^{1}H NMR ($CDCl_3$) δ 1.69 (quin, J = 7.8 Hz, 4 H, 2 × CH_2), 1.82 (quin, J = 7.3 Hz, 4 H, 2 × CH_2), 2.42 (t, J = 7.5 Hz, 4 H, 2 × CH_2), 2.43 (br s, 8 H, 4 × CH_2pip), 3.70 (q, J = 6.6 Hz, 4 H, 2 × CH_2NHCO), 7.73−7.81 (m, 4 H, ArH), 7.95 (dd, J = 8.6, 7.0 Hz, 2 H, H-3), 8.11 (d, J = 7.7 Hz, 2 H, ArH), 8.37 (dd, J = 8.7, 1.3 Hz, 2 H, ArH), 8.99 (dd, J = 7.1, 1.5 Hz, 2 H, H-2), 11.05 (br t, J = 5.2 Hz, 2 H, 2 × CONH). Anal. ($C_{40}H_{44}N_8O_2$) C, H, N.

***N,N′*-Bis[3-(9-methylphenazine-1-carboxamido)propyl]-1,3-propanediamine (18): Scheme 2.** Similar reaction of **42** (407 mg, 1.05 mmol) with **38** as above, followed by chromatography on silica gel (CH_2Cl_2/MeOH 99:1 as eluant), gave the BOC-protected bis-phenazine (**44**) as a crude yellow oil (570 mg, 78%): ^{1}H NMR ($CDCl_3$) δ 1.43 (br s, 18 H, 6 × CH_3), 1.80 (br s, 2 H, CH_2), 2.02 (quin, J = 7.3 Hz, 4 H, 2 × CH_2), 2.89 (s, 6 H, 2 × $ArCH_3$), 3.23 (br s, 4 H, 2 × CH_2), 3.37 (t, J = 7.0 Hz, 4 H, 2 × CH_2), 3.66 (q, J = 6.8 Hz, 4 H, 2 × CH_2), 7.70−7.78 (m, 4 H, ArH), 7.93 (t, J = 7.7 Hz, 2 H, ArH), 8.07 (d, J = 8.3 Hz, 2 H, ArH), 8.34 (d, J = 8.4 Hz, 2 H, ArH), 8.94 (br d, J = 5.8 Hz, 2 H, ArH), 11.03 (br s, 2 H, 2 × CONH).

A solution of **44** (570 mg, 0.68 mmol) in MeOH (10 mL) saturated with HCl(g) was stirred at room temperature for 5 days, when the reaction was complete by TLC. The solvent was removed under reduced pressure, and the resulting solid was then eluted through an Amberlite (OH^-) resin column to convert the material to the free base. The elution solvent (EtOH) was removed under reduced pressure, and the residue was purified by chromatography on silica (1% NH_4OH and 20% MeOH in CH_2Cl_2 as eluant) to give **18** (341 mg, 79%): mp (CH_2Cl_2/*n*-hexane) 155−160 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.69−1.79 (m, 2 H, CH_2), 2.02 (quin, J = 7.1 Hz, 4 H, 2 × CH_2), 2.76 (t, J = 6.7 Hz, 4 H, 2 × CH_2), 2.84 (t, J = 7.0 Hz, 4 H, 2 × CH_2), 2.90 (s, 6 H, $ArCH_3$), 3.77 (q, J = 6.7 Hz, 4 H, 2 × CH_2), 7.67−7.70 (m, 2 H, ArH), 7.73 (dd, J = 8.4, 6.8 Hz, 2 H, ArH), 7.92 (dd, J = 8.6, 7.2 Hz, 2 H, ArH), 8.06 (d, J = 8.5 Hz, 2 H, ArH), 8.33 (dd, J = 8.5, 1.5 Hz, 2 H, ArH), 8.96 (dd, J = 7.1, 1.4 Hz, 2 H, ArH), 11.07 (br t, J = 5.3 Hz, 2 H, 2 × CONH). HRMS (FAB^+) m/z calcd for $C_{37}H_{41}N_8O_2$, 629.3352 (MH^+); found, 629.3391. Anal. ($C_{37}H_{40}N_8O_2 \cdot H_2O$) C, H, N.

***N,N′*-Bis[3-(9-methylphenazine-1-carboxamido)propyl]-1,4-butanediamine (19)**. Similar reaction of **38** and *N,N′*-bis(3-aminopropyl)butanediamine gave **19** (73%): mp (CH_2Cl_2/hexane) 86−90.5 °C; ^{1}H NMR ($CDCl_3$) δ 1.53 (quin, J = 3.2 Hz, 4 H, 2 × CH_2), 1.97 (quin, J = 7.0 Hz, 4 H, 2 × CH_2), 2.62 (t, J = 6.2 Hz, 4 H, 2 × CH_2), 2.79 (t, J = 7.0 Hz, 4 H, 2 × CH_2), 2.88 (s, 6 H, 2 × $ArCH_3$), 3.74 (q, J = 6.6 Hz, 4 H, 2 × CH_2), 7.71−7.78 (m, 4 H, ArH), 7.93 (dd, J = 8.7, 7.2 Hz, 2 H, H-3), 8.08 (d, J = 7.9, 0.8 Hz, 2 H, ArH), 8.34 (dd, J = 8.7,

1.5 Hz, 2 H, H-4), 8.96 (dd, J = 7.1, 1.5 Hz, 2 H, H-2), 11.05 (t, J = 5.2 Hz, 2 H, 2 × CONH). Anal. ($C_{38}H_{42}N_8O_2 \cdot 1.5H_2O$) C, H, N.

***N,N′*-Bis[4-(9-methylphenazine-1-carboxamido)butyl]-butanediamine (20).** Similar reaction of **43** with **38** as above, followed by chromatography on silica gel (4% MeOH in CH_2Cl_2 as eluant) gave the BOC-protected bis-phenazine **45** as a yellow foam (80%): ^{1}H NMR ($CDCl_3$) δ 1.34−1.49 [m, 22 H, 2 × C(CH_3)$_3$ and 2 × CH_2], 1.67−1.82 (m, 8 H, 4 × CH_2), 2.90 (s, 6 H, 2 × $ArCH_3$), 3.10−3.28 (br m, 8 H, 4 × CH_2), 3.70 (q, J = 6.4 Hz, 4 H, 2 × CH_2), 7.72−7.81 (m, 4 H, ArH), 7.95 (t, J = 7.9 Hz, 2 H, ArH), 8.11 (d, J = 8.2 Hz, 2 H, ArH), 8.36 (d, J = 8.1 Hz, 2 H, ArH), 8.99 (d, J = 6.3 Hz, 2 H, ArH), 11.09 (br s, 2 H, 2 × CONH). Anal. ($C_{50}H_{62}N_8O_6 \cdot 3H_2O$) C, H, N.

Deprotection of **45** was carried out with MeOH/HCl(g) as above, giving **20** as a pale yellow solid (64%): mp (CH_2Cl_2/*n*-hexane) 122−126 °C; ^{1}H NMR ($CDCl_3$) δ 1.43−1.72 (m, 8 H, 4 × CH_2), 1.82 (quin, J = 7.4 Hz, 4 H, 2 × CH_2), 2.55−2.62 (m, 4 H, 2 × CH_2), 2.68 (t, J = 7.2 Hz, 4 H, 2 × CH_2), 2.95 (s, 6 H, 2 × $ArCH_3$), 3.69 (q, J = 6.6 Hz, 4 H, 2 × CH_2NHCO), 7.71−7.82 (m, 4 H, ArH), 7.96 (dd, J = 8.6, 7.1 Hz, 2 H, ArH), 8.10 (d, J = 8.2 Hz, 2 H, ArH), 8.36 (dd, J = 8.6, 1.5 Hz, 2 H, ArH), 8.99 (dd, J = 7.1, 1.6 Hz, 2 H, ArH), 11.08 (br s, 2 H, 2 × CONH), HRMS (FAB$^+$) *m/z* calcd for $C_{40}H_{47}N_8O_2$, 671.3822 (MH^+); found, 671.3815. Anal. ($C_{40}H_{46}N_8O_2 \cdot 0.5H_2O$) C, H, N.

***N,N′*-Bis[2-(6,9-dimethylphenazine-1-carboxamido)ethyl]-1,2-ethanediamine (21).** Similar reaction of 6,9-dimethylphenazine-1-carboxylic acid[11] (**39**) and *N,N′*-(2-aminoethyl)-1,2-diaminethane gave **21** (99%): mp (dihydrochloride salt from MeOH) 299 °C (dec); ^{1}H NMR (CF_3CO_2D) δ 3.06 (s, 6 H, 2 × $ArCH_3$), 3.09 (s, 6 H, 2 × $ArCH_3$), 3.87 (br s, 4 H, 2 × CH_2), 3.91 (br s, 4 H, 2 × CH_2), 4.27 (br s, 4 H, 2 × CH_2), 8.20 (d, J = 7.3 Hz, 2 H, H-7 or H-8), 8.24 (d, J = 7.3 Hz, 2 H, H-7 or H-8), 8.43 (t, J = 8.1 Hz, 2 H, H-3), 8.96 (d, J = 8.8 Hz, 2 H, H-4), 9.02 (d, J = 7.3 Hz, 2 H, H-2). Anal. ($C_{36}H_{40}Cl_2N_8O_2$) C, H, N.

***N,N′*-Bis[2-(6-chloro-9-methylphenazine-1-carboxamido)ethyl]-1,2-ethanediamine (22)**. Similar reaction of 6-chloro-9-methylphenazine-1-carboxylic acid[11] (**40**) and *N,N′*-(2-aminoethyl)-1,1-diaminoethane gave **22** as a yellow solid (6%): mp (HCl salt from MeOH/EtOAc) 301 °C (dec); ^{1}H NMR (CF_3CO_2D) δ 3.87 (br s, 4 H, 2 × CH_2NH), 4.04 (br s, 4 H, 2 × CH_2NH), 4.09 (s, 6 H, 2 × $ArCH_3$), 4.29 (br s, 4 H, 2 × CONHCH_2), 8.25 (br d, J = 7.9 Hz, 2 H, ArH), 8.44 (br d, J = 7.7 Hz, 2 H, ArH), 8.53 (br s, 2 H, ArH), 9.03 (br d, J = 8.7 Hz, 2 H, ArH) and 9.09 (br s, 2 H, ArH); HRMS (FAB$^+$) *m/z* calcd for $C_{34}H_{33}35Cl_2N_8O_2$, 655.2104 (MH^+); found, 655.2075.

Calculation of Linker Chain Lengths. These were computed (between the carboxamide nitrogens) for the fully extended forms of the linkers, using the 3D module of ACD Labs, 133 Richmond Street West, Suite 605, Toronto, Ontario, Canada M5H 2L3.

DNA Unwinding Gel Assays. These were performed essentially as described by Pommier et al.[33] Supercoiled DNA was relaxed by incubation with topo I for 15 min before the addition of **8**.

Topoisomerase Assays. Topo I assays with **8** were carried out[43] in a reaction (30 μL) containing 50 mM Tris HCl (pH 7.5), 50 mM KCl, 10 mM $MgCl_2$, 0.5 mM DTT, 0.1 mM EDTA, 30 μg/mL bovine serum albumin, and 0.125 μg of pBR322 supercoiled plasmid DNA, together with calf thymus topo I (5 units for relaxation assays and 15 units for cleavable complex formation assays). Reactions were assembled on ice with drug and topoisomerase added last, incubated at 37 °C for 30 min, and terminated by adding prewarmed 1% sodium dodecyl sulfate, followed by proteinase K treatment (50 μg/mL) for an additional 30 min. Relaxation assay samples were loaded onto 1% agarose gels in 40 mM Tris acetate buffer (pH 8.0) containing 1 mM EDTA and were electrophoresed at 1.4 V/cm for 14−16 h.

In Vitro Cytotoxicity Assays. Murine P388 leukemia cells, Lewis lung carcinoma cells (LL), and human Jurkat leukemia cells (JL_C), together with their amsacrine and doxorubicin-resistant derivatives (JL_A and JL_D, respectively), were obtained and cultured as described.[32] Growth inhibition assays were performed by culturing cells at 4.5×10^3 (P388), 10^3 (LL), and 3.75×10^3 (Jurkat lines) per well in microculture plates (150 mL per well) for 3 (P388) or 4 days in the presence of drug. Cell growth was determined by [3H]TdR uptake (P388)[44] or the sulforhodamine assay.[45] Independent assays were performed in duplicate.

In Vivo Tumor Assays. Colon 38 tumors were grown subcutaneously from 1 mm^3 fragments implanted in one flank of C57/Bl mice (anesthetized with pentobarbitone 90 mg/kg). HT-29 cells were grown in culture, and a suspension (5×10^6 cells) was implanted in each flank of nude mice. When tumors reached a diameter of approximately 4−6 mm (7−8 days), mice were divided into control and drug treatment groups (5 mice/group), with similar average tumor volumes in each group. Drugs were administered as solutions of the hydrochloride salts in distilled water and were injected intraperitoneally in a volume of 0.01 mL/g body weight, using an intermittent (q4d × 3) schedule. The mice were monitored closely, and tumor diameters were measured with callipers three times a week. Tumor volumes were calculated as $0.52 \times a^2 \times b$, where a and b are the minor and major tumor axes, and data were plotted on a semilogarithmic graph as mean tumor volumes (± SEM) versus time after treatment. The growth delay was calculated as the time taken for tumors to reach a mean volume 4-fold higher than their pretreatment volume.

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References

(1) McRipley, R. J.; Burns-Horwitz, P. E.; Czerniak, P. M.; Diamond, R. J.; Diamond, M. A.; Miller, J. L. D.; Page, F. J.; Dexter, D. L.; Chen, S. F. Efficacy of DMP 840: a novel bis-naphthalimide cytotoxic agent with human solid tumor xenograft selectivity. *Cancer Res*. **1994**, *54*, 159−164.

(2) Brana, M. F.; Castellano, J. M.; Moran, M.; Perez de Vega, M. J.; Perron, D.; Conlon, D.; Bousquet, P. F.; Romerdahl, C. A.; Robinson, S. P. Bis-naphthalimides 3: Synthesis and antitumor activity of *N,N′*-bis[2-(1,8-naphthalimido)ethyl]alkane-diamines. *Anti-Cancer Drug Des*. **1996**, *11*, 297−309.

(3) Nitiss, J. L.; Zhou, J. F.; Rose, A.; Hsiung, Y. C.; Gale, K. C.; Osheroff. N. The bis(naphthalimide) DMP-840 causes cytotoxicity by its action against eukaryotic topoisomerase II. *Biochemistry* **1998**, *37*, 3078−3085.

(4) O'Reilly, S.; Baker, S. D.; Sartorius, S.; Rowinsky, E. K.; Finizio, M.; Lubiniecki, G. M.; Grochow, L. B.; Gray, J. E.; Pieniaszek, H. J.; Donehower, R. C. A Phase I and pharmacologic study of DMP 840 administered by 24-hour infusion. *Ann. Oncol*. **1998**, *9*, 101−104.

(5) Brana, M. F.; Castellano, J. M.; Perron, D.; Maher, C.; Conlon, D.; Bosquet, P. F.; George, J.; Qian, X.-D.; Robinson, S. P. Chromophore-modified bis-naphthalimides: synthesis and antitumor activity of bis-dibenz[*de,h*]isoquinoline-1,3-diones. *J. Med. Chem*. **1997**, *40*, 449−454.

(6) Hernandez, L.; Cholody, W. M.; Hudson, E. A.; Resau, J. H.; Pauly, G.; Michejda, C. J. Mechanism of action of bisimidazoacridones, new drugs with potent, selective activity against colon cancer. *Cancer Res*. **1995**, *55*, 2338−2345.

(7) Cholody, W. M.; Hernandez, L.; Hassner, L.; Scudiero, D. A.; Djurickovic, D. B.; Michejda, C. J. Bisimidazoacridones and related compounds − new antineoplastic agents with high selectivity against colon tumors. *J. Med. Chem*. **1995**, *38*, 3043−3052.

(8) Leng, F. F.; Priebe, W.; Chaires, J. B. Ultratight DNA binding of a new bisintercalating anthracycline antibiotic. *Biochemistry* **1998**, *37*, 1743−1753.

(9) Chaires, J. B.; Leng, F. F.; Przewloka, T.; Fokt, I.; Ling, Y. H.; Perez-soler, R.; Priebe, W. Structure-based design of a new bisintercalating anthracycline antibiotic. *J. Med. Chem*. **1997**, *40*, 261−266.

(10) Gamage, S. A.; Spicer, J. A.; Atwell, G. J.; Finlay, G. J.; Baguley, B. C.; Denny, W. A. Structure−activity relationships for substituted bis(acridine-4-carboxamides): a new class of anticancer agents. *J. Med. Chem*. **1999**, *42*, 2383−2393.

(11) Spicer, J. A.; Gamage, S. A.; Rewcastle, G. W.; Finlay, G. J.; Baguley, B. C.; Denny, W. A. Bis(phenazine-1-carboxamides): structure−activity relationships for a new class of dual topoisomerase I/II-directed anticancer drugs. *J. Med. Chem.* **2000**, *43*, 1350−1358.
(12) Cherney, R. J.; Swartz, S. G.; Patten, A. D.; Akamike, E.; Sun, J. H.; Kaltenbach, R. F.; Seitz, S. P.; Behrens, C. H.; Getahun, Z.; Trainor, G. L.; Vavala, M.; Kirshenbaum, M. R.; Papp, L. M.; Stafford, M. P.; Czerniak, P. M.; Diamond, R. J.; McRipley, R. J.; Page, R. J.; Gross, J. L. The synthesis and antitumor evaluation of unsymmetrical bis-imides. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 163−168.
(13) Bousquet, P. F.; Brana, M. F.; Conlon, D.; Fitzgerald, K. M.; Perron, D.; Cocchiaro, C.; Miller, R.; Moran, M.; George, J.; Qian, X. D. Preclinical evaluation of LU 79553: a novel bis-naphthalimide with potent antitumor activity. *Cancer Res.* **1995**, *55*, 1176−1180.
(14) Bailly, C.; Brana, M.; Waring, M. J. Sequence-selective intercalation of antitumour bis-naphthalimides into DNA. Evidence for an approach via the major groove. *Eur. J. Biochem.* **1996**, *240*, 195−208.
(15) Gallego, J.; Reid, B. R. Solution structure and dynamics of a complex between DNA and the antitumor bisnaphthalimide LU-79553: Intercalated ring flipping on the millisecond time scale. *Biochemistry* **1999**, *38*, 15104−15115.
(16) Atwell, G. J.; Rewcastle, G. W.; Baguley, B. C.; Denny, W. A. Potential Antitumor Agents. 50. In vivo solid tumor activity of derivatives of *N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide. *J. Med. Chem.* **1987**, *30*, 664−669.
(17) Rewcastle, G. W.; Denny, W. A.; Baguley, B. C. Potential Antitumor Agents. 51. Synthesis and antitumor activity of phenazine-1-carboxamides. *J. Med. Chem.* **1987**, *30*, 843−851.
(18) Spicer, J. A.; Gamage, S. A.; Atwell, G. J.; Finlay, G. F.; Baguley, B. C.; Denny, W. A. Structure−activity relationships for acridine-substituted analogues of the mixed topo I/II inhibitor *N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide (DACA). *J. Med. Chem.* **1997**, *40*, 1919−1929.
(19) Ravikumar, V. T. A Convenient Large Scale Synthesis of N-BOC-Ethylenediamine. *Synth. Commun.* **1994**, *24*, 1767−1772.
(20) Zhou, L.-M.; He, X.-H.; Li, G.; de Costa, B. R.; Skolnick, P. Synthesis of *N*,*N*′-substituted piperazine and homopiperazine derivatives with polyamine-like actions at *N*-methyl-D-aspartate receptors. *J. Med. Chem.* **1995**, *38*, 4891−4896.
(21) Geall, A. J. and Blagbrough, I. S. Homologation of polyamines in the synthesis of lipospermine conjugates and related lipoplexes. *Tetrahedron Lett.* **1998**, *39*, 443−446.
(22) Wilkoff, L. J.; Dulmadge, E.; Chopra, D. P. Viability of cultured Lewis lung cell populations exposed to β-retinoic acid (40753). *Proc. Soc. Exp. Biol. Med.* **1980**, *163*, 233−236.
(23) Finlay, G. J.; Baguley, B. C.; Snow, K.; Judd, W. Multiple patterns of resistance of human leukemia cell sublines to analogs of amsacrine. *J. Natl. Cancer Inst.* **1990**, *82*, 662−667.
(24) Finlay, G. J.; Holdaway, K. M.; Baguley, B. C. Comparison of the effects of genistein and amsacrine on leukemia cell proliferation. *Oncol. Res.* **1994**, *6*, 33−37.
(25) Deady, L. W.; Desneves, J.; Kaye, A. J.; Finlay, G. J.; Baguley, B. C.; Denny, W. A. Synthesis and antitumor activity of some indeno[1,2-*b*]quinoline-based bis-carboxamides. *Bioorg. Med. Chem.* **2000**, *8*, 977−984.
(26) Wakelin, L. P. G. Polyfunctional DNA intercalating agents. *Med. Res. Rev.* **1986**, *6*, 275−340.
(27) Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley J.; Cronise, P.; Vaigro-Wolff, A.; Gray-Goodrich, M.; Campbell, H.; Mayo, J.; Boyd, M. Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines. *J. Natl. Cancer Inst.* **1991**, *83*, 757−766.
(28) Baguley, B. C.; Denny, W. A.; Atwell, G. J.; Cain, B. F. Potential Antitumor Agents. Part 34. Quantitative relationships between DNA binding and molecular structure for 9-anilinoacridines substituted in the anilino ring. *J. Med. Chem.* **1981**, *24*, 170−177.
(29) Adams, A.; Guss, M.; Collyer, C.; Denny, W. A.; Wakelin, L. P. G. Crystal structure of the topoisomerase II poison 9-amino-[*N*-(2-dimethylamino)ethyl]acridine-4-carboxamide bound to the DNA hexanucleotide d(CGTAGC)$_2$. *Biochemistry* **1999**, *38*, 9221−9233.
(30) Kartner, N.; Riordan, J. R.; Ling, V. Cell surface P-glycoprotein associated with multidrug resistance in mammalian cell lines. *Science* **1983**, *221*, 1285−1288.
(31) Tanizawa, A.; Pommier, Y. 1992: Topoisomerase-I alteration in a camptothecin-resistant cell line derived from Chinese hamster DC3F cells in culture. *Cancer Res.* **1992**, *52*, 1848−1854.
(32) Finlay, G. J.; Riou, J.-F.; Baguley, B. C. From amsacrine to DACA (*N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide): selectivity for topoisomerases I and II among acridine derivatives. *Eur. J. Cancer* **1996**, *32A*, 708−714.
(33) Pommier, Y.; Covey, J. M.; Kerrigan, D.; Markovits, J.; Pham, R. DNA unwinding and inhibition of mouse leukemia L1210 DNA topoisomerase I by intercalators. *Nucleic Acids Res.* **1987**, *15*, 6713−6731.
(34) Pommier, Y.; Covey, J.; Kerrigan, D.; Mattes, W.; Markovits, J.; Kohn, K. W. Role of DNA intercalation in the inhibition of purified mouse leukemia (L1210) DNA topoisomerase II by 9-aminoacridines. *Biochem. Pharmacol.* **1987**, *36*, 3477−3486.
(35) Stewart. A. J.; Mistry, P.; Dangerfield, W.; Bootle, D.; Baker, M.; Kofler, B.; Okiji, S.; Baguley, B. C.; Denny W. A.; Charlton, P. Antitumour activity of XR5944, a novel and potent topoisomerase poison. *Anticancer Drugs*, in press.
(36) Stewart, A. J.; Dangerfield, W.; Kofler, B.; Baguley, B. C.; Denny, W. A.; Charlton, P. Comparative activity of a series of joint topoisomerase I/II directed drugs: cytotoxicity, DNA binding and poisoning of topoisomerase I and II. *Proc. Am. Assoc. Cancer Res.* **2000**, *41*, 212 (abstract 1352).
(37) Alcock, N. W.; Moore, P.; Omar, H. A. A.; Reader, C. J. Nickel(II), copper(II) and zinc(II) complexes of penta-azamacrocyclic ligands. Crystal and molecular structure of 3,6,9,12-tetramethyl-3,6,9,12, 18-penta-azabicyclo[12.3.1]octadeca-1(18),14,16-trienezinc(II) perchlorate. *J. Chem Soc., Dalton Trans.* **1987**, 2643−2648.
(38) Sakiyama, H.; Tokuyama, K.; Matsumura, Y.; Okawa, H. Macrocyclic tetranuclear manganese (II) complexes of a new 'dimer of dimers' type: synthesis, structure and magnetism. *J. Chem. Soc., Dalton Trans.* **1993**, 2329−2334.
(39) Samejima, K.; Takeda, Y.; Kawase, M.; Okada, M.; Kyogoku, Y. Synthesis of ^{15}N enriched polyamines. *Chem. Pharm. Bull.* **1984**, *32*, 3428−3435.
(40) Brana, M. F.; de Vega, M. J. P.; Perron, D.; Bousquet, D. F.; Robinson, S. P. Benzimidazo[1,2-c]quinazoline dimers as potential antitumour agents. *J. Heterocycl. Chem.* **1997**, *34*, 807−812.
(41) Tsuge, O.; Mataka, S.; Tashiro, M.; Mashiba, F. Studies of *N*-sulfinyl compounds I. reaction of *N*-sulfinylanilines with ethylene carbonate. *Bull. Chem. Soc. Jpn.* **1967**, *40*, 2706−08.
(42) Hancock, R. D.; Ngwenya, M. P.; Evers, A.; Wade, P. W.; Boeyens, J. C. A.; Dobson, S. M. Open-chain polyamine ligands with more rigid double connecting bridges. study of their metal ion selectivities by molecular mechanics calculation, crystallography, and thermodynamics. *Inorg. Chem.* **1990**, *29*, 264−270.
(43) Bridewell. D. J. A.; Finlay, G. J.; Baguley, B. C. Mechanism of cytotoxicity of *N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide and of its 7-chloro derivative: the roles of topoisomerase I and II. *Cancer Chemother. Pharmacol.* **1999**, *43*, 302−308.
(44) Marshall, E. S.; Finlay. G. J.; Matthews, J. H. L.; Shaw, J. H. F.; Nixon, J.; Baguley, B. C. Microculture-based chemosensitivity testing: a feasibility study comparing freshly explanted melanoma cells with human melanoma cell lines. *J. Natl. Cancer Inst.* **1992**, *84*, 340−345.
(45) Skehan, P.; Storeng. 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.

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