Bis(phenazine-1-carboxamides): Structure−Activity Relationships for a New Class of Dual Topoisomerase I/II-Directed Anticancer Drugs

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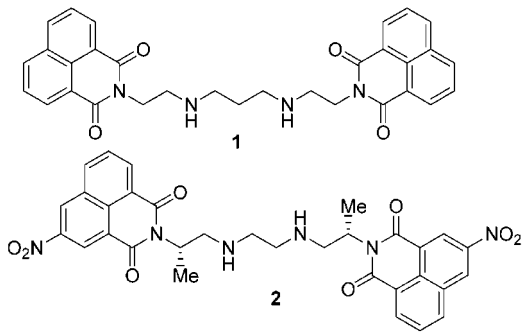
Ring-substituted bis(phenazine-1-carboxamides), linked by a $-(CH_2)_3NMe(CH_2)_3-$ chain, were prepared from the corresponding substituted phenazine-1-carboxylic acids by reaction of the intermediate imidazolides with bis(3-aminopropyl)methylamine. The compounds were evaluated for growth inhibitory activity in a panel of tumor cell lines, including P388 leukemia, Lewis lung carcinoma, and wild-type (JL_C) and mutant (JL_A and JL_D) forms of human Jurkat leukemia. The latter mutant lines are resistant to topoisomerase (topo) II targeted agents because of lower levels of the enzyme. Analogues with small, lipophilic substituents (e.g., Me, Cl) at the 9-position were the most potent inhibitors, superior to the corresponding dimeric bis(acridine-4-carboxamides) (bis-DACA analogues). Several of the compounds were preferentially (up to 2-fold) more cytotoxic toward the mutant Jurkat lines than the wild-type. To test whether this selectivity was related to topoisomerase action, the most potent of the compounds (9-methyl) was evaluated in a cell-free system. It poisoned topo I at drug concentrations of 0.25 and 0.5 μM and inhibited the catalytic activity of both topo I and topo II at concentrations of 1 and 5 μM, respectively. Results from the NCI human tumor cell line panel showed the compounds had preferential activity toward colon tumor lines (on average 9.5-fold more active in the HT29 line than in the cell line panel as a whole). Several analogues produced significant growth delays in the relatively refractory subcutaneous colon 38 tumor model in vivo. In particular, the 9-methyl compound was substantially more potent in this tumor model than the clinical dual topo I/II poison DACA (total dose 90 versus 400 mg/kg) with comparable activity. The bis(phenazine-1-carboxamides) are a new and interesting class of dual topo I/II-directed anticancer drugs.

There is current interest in dimeric analogues of lipophilic, neutral, DNA mono-intercalating agents as potential anticancer drugs. The bis(naphthalimide) LU 79953 (**1**) is a DNA bis-intercalator[1] and very potent cytotoxin, with broad-spectrum activity against a variety of human solid tumor cell lines (both in culture and as xenografts in nude mice),[2,3] and is in Phase I clinical trial.[4] The related bis(nitronaphthalimide) DMP 840 (**2**)



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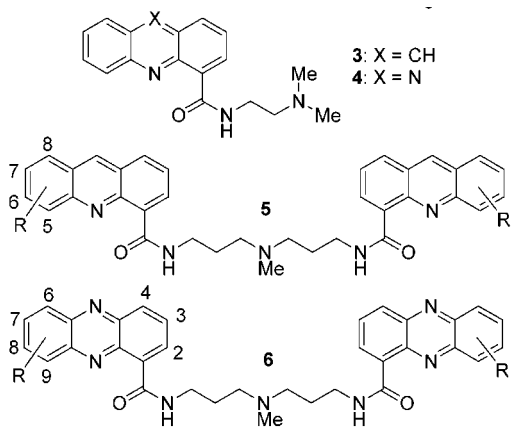
2

appears to be only a mono-intercalator[5] but shows curative activity against a variety of human solid tumor xenografts in nude mice,[6,7] and Phase I clinical trials have been reported.[8] The mode of action of these compounds is still unclear,[8,9] but they are known to be topoisomerase inhibitors.[4]

We have recently shown[10] that, of a series of tricyclic aromatic carboxamides, the bis(acridine-4-carboxamide) (**5**) and the related bis(phenazine-1-carboxamide) (**6**: R = H) showed the largest increase in potency over their monomeric counterparts (**3** and **4**). More extensive structure−activity relationship (SAR) studies of acridine-substituted analogues of **5** showed that small substituents (e.g., Me, Cl) at the 5-position provided compounds of highest cytotoxicity, with IC_{50}s as low as 2 nM against the Lewis lung carcinoma cell line.[11] Several examples produced significant growth delays in the relatively refractory subcutaneous colon 38 tumor



3: X = CH
4: X = N

5

6

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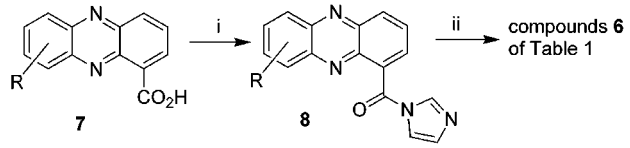
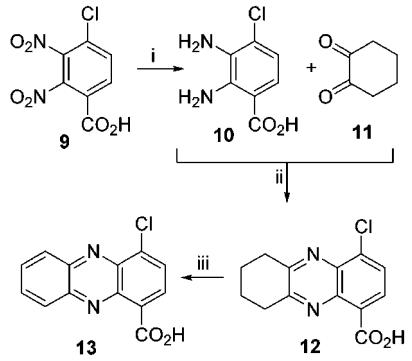
Scheme 1[a]



[a] (i) CDI/DMF/45 °C, 30 min; (ii) $[H_2N(CH_2)_3]_2NMe$/THF/0−20 °C/4 h.

Scheme 2[a]



[a] (i) Pt-C/MeOH/50 psi/1 h; (ii) MeOH/reflux/15 min; (iii) DDQ/benzene/reflux/15 h.

model in vivo at substantially lower doses than the corresponding monomer DACA (**3**).

Because the acridine chromophore in the clinical monomeric acridine-4-carboxamide DACA (**3**) undergoes rapid oxidative metabolism to the acridone by enzymes such as aldehyde oxidase,[12] and because monomeric phenazine-1-carboxamides are known[13] to be effective cytotoxins, we investigated bis(phenazine-1-carboxamide) analogues of **6** to determine substituent SAR and to see whether analogues of superior activity to the bis(acridine-4-carboxamides) could be obtained.

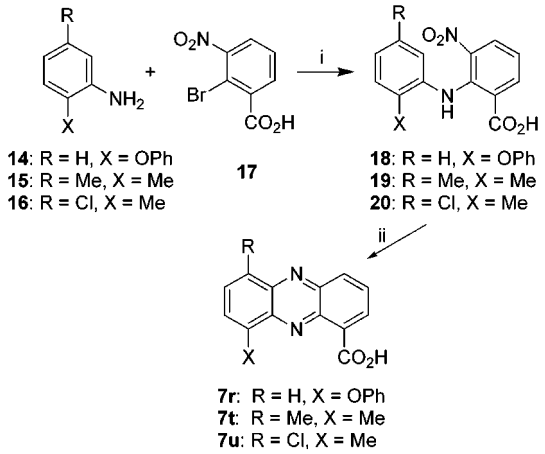
Chemistry

The bis(phenazine-1-carboxamides) (**6a**−**6w**) were prepared by activation of the acids **7** with 1,1′-carbonyldiimidazole. The resulting *N*-imidazolides (**8**) were isolated (this was necessary to obtain pure products) and immediately reacted with a stoichiometric amount of bis(3-aminopropyl)methylamine (Scheme 1). Most of the substituted phenazine-1-carboxylic acids (**7**) required were known and were prepared by reported methods.[13,14]

4-Chlorophenazine-1-carboxylic acid (**13**) was prepared from 2,3-dinitro-4-chlorobenzoic acid (**9**).[15] Reduction of this by hydrogenation over Pt/C and condensation of the resulting diamine (**10**) with cyclohexane-1,2-dione (**11**) gave the tetrahydro derivative (**12**), which was dehydrogenated with DDQ to **13** (Scheme 2) in low yield. However, reaction of this with bis(3-aminopropyl)-methylamine as above gave none of the desired bis(4-chlorophenazine) product (possibly due to concomitant displacement of the relatively labile chloro group).

The 9-phenoxy-, 6,9-dimethyl-, and 6-chloro-9-methylphenazine-1-carboxylic acids (**7r**, **7t**, and **7u**) were prepared by Ullmann-type condensation of the requisite amines (**14**−**16**) with 2-bromo-3-nitrobenzoic acid (**17**) to give the diphenylamines **18**−**20**, respectively. Reductive cyclization of these with $NaBH_4$ in refluxing aqueous sodium hydroxide or sodium ethoxide gave the

Scheme 3[a]



[a] (i) CuCl/Cu/*N*-ethylmorpholine/butane-2,3-diol/70 °C/18 h; (ii) $NaBH_4$/aq NaOH(or NaOEt/EtOH)/reflux/8 h.

required phenazine-1-carboxylic acids in moderate yields (Scheme 3). 9-(Dimethylamino)phenazine-1-carboxylic acid (**7s**) was prepared by reacting 9-fluorophenazine-1-carboxylic acid (**7p**) in 40% aqueous dimethylamine in a bomb at 100 °C for 3 h.

Results and Discussion

The bis(phenazines) were evaluated in a panel of cell lines in culture. They were used as the monohydrochloride salts, which were highly water-soluble. The murine P388 leukemia[16] and murine Lewis lung carcinoma[17] lines were used to provide comparison with previous data on related compounds. The three human leukemia (Jurkat) lines[18,19] were used as examples of human lines, and to provide insights into the mechanism of cytotoxicity. JL_C is the wild-type (sensitive) line, while JL_A and JL_D, developed for resistance to amsacrine and doxorubicin, respectively, are 123-fold and 110-fold resistant, respectively, to the topo II inhibitor amsacrine because of a reduced level of topo II enzyme.[18] Table 1 provides the IC_{50} values for the P388, LL, and JL_C lines, together with ratios of IC_{50} values (JL_A/JL_C and JL_D/JL_C) for the resistant Jurkat lines. Ratios of less than about 2-fold suggest a nontopo II mediated mechanism of action. DACA (**3**), a dual topo I/II inhibitor,[20] has values of 2.3 and 2.5 for these ratios, while the related monomeric phenazine (**4**) has values of 1.1 and 1.1.

The parent unsubstituted bis compound **6a** was a moderately potent cytotoxin, showing similar IC_{50} values in the cell line panel to those determined previously[10] for the related bis(acridine-4-carboxamide) **5** (e.g., IC_{50} JL_C 137 and 110 nM, respectively). A comparison of data for **6a** and the corresponding bis(phenazine-2-carboxamide) **20** (IC_{50} JL_C 2760 nM)[10] confirmed the requirement for the linker chain to be *peri* to the ring nitrogen (on the same side as the carbox-

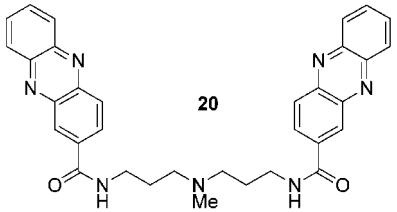
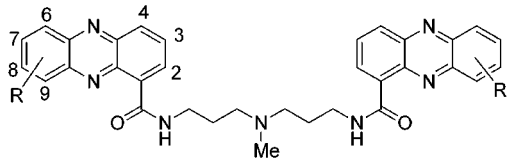


Table 1. Cytotoxicities of Bis[(phenazine-1-carboxamido)propyl]methylamines



			IC_{50} (nM)[a]			IC_{50} ratios	
no.	R	mp	P388[b]	LL[c]	JL_C[d]	JL_A/JL_C[e,f]	JL_D/JL_C[e,g]
6a	H	ref 10	520	107	173	0.5	0.7
6b	2-Cl	206−207	1.2×10^4	8370	2370	0.6	0.8
6c	3-Me	75−78	472	48	208	0.7	0.9
6d	3-Cl	169−170	204	12	74	0.6	0.8
6e	4-Me	218−220	375	26	102	0.7	0.9
6f	6-Me	228−230[h]	584	46	172	0.8	1.0
6g	6-Cl	198−200	7740	151	209	0.9	0.9
6h	7-Me	213-215[h]	623	72	280	0.8	0.9
6i	7-Cl	173.5−175	2960	83	249	0.8	0.9
6j	7-OMe	225−229[h]	600	64	195	0.9	1.0
6k	8-Me	161−162	690	108	148	0.6	0.8
6l	8-Cl	210−121.5	97	44	42	0.4	0.6
6m	8-OMe	182−186	75	24	30	0.5	0.8
6n	9-Me	262−264[h]	15	1.6	5.7	0.6	0.6
6o	9-Cl	169−171.5	39	8.8	14	0.6	0.9
6p	9-F	183−186	294	120	177	0.6	0.6
6q	9-OMe	220−222	815	160	124	0.7	1.0
6r	9-OPh	nc[i]	3450	1310	1250	1.0	1.1
6s	9-NMe_2	nc[i]	2280	310	695	0.9	0.9
6t	6,9-diMe	97−101	26	5.3	21	0.5	0.8
6u	6-Cl, 9-Me	200−202	29	5.2	9.8	0.4	0.8
6v	6,7-benzo	138−142	450	410	290	0.8	1.5
6w	8,9-benzo	214−215.5	24	3.2	9.0	0.6	0.8
5		ref 11	130	30	110	0.7	0.8

[a] IC_{50}; concentration of drug (nM) to reduce cell number to 50% of control cultures (see text). The value is the average of at least two independent determinations; the coefficients of variation were between 15 and 22%. [b] Murine P388 leukemia. [c] Murine Lewis lung carcinoma. [d] JL_C: wild-type human Jurkat leukemia. [e] Ratios of IC_{50}s in the cell lines shown. [f] JL_A: amsacrine-resistant Jurkat. [g] JL_D: doxorubicin-resistant Jurkat. [h] HCl salt. [i] Noncrystalline.

amide) as previously for both monomeric phenazine-1-carboxamides[13] and bis(acridine-4-carboxamides).[11]

The ring-substituted compounds **6b**−**6u** contain the same linker chain $[(CH_2)_3NMe(CH_2)_3]$ used in the previous bis(acridine-4-carboxamide) study,[11] selected in order to allow direct comparison of substituent effects in the two series. Because neither large or hydrophilic groups were beneficial in the bis(acridine-4-carboxamide) series, these were not prepared here. Although fewer compounds were therefore studied, substitutions were again made at every available chromophore ring position. Overall, the substituted bis(phenazine-1-carboxamides) were more cytotoxic toward the mouse carcinoma line LL than the mouse leukemia line P388 (eq 1), but they had very similar IC_{50} values for the human and mouse leukemia lines (eq 2). These general relationships are similar to those found previously[11] for substituted bis(acridine-4-carboxamides).

$$\log(IC_{50})_{LL} = 0.91(\pm 0.11)\log(IC_{50})_{P388} - 0.59(\pm 0.30) \quad (1)$$

$$n = 21 \qquad r = 0.88 \qquad s = 0.35 \qquad F = 67$$

$$\log(IC_{50})_{JLC} = 0.75(\pm 0.07)\log(IC_{50})_{P388} - 0.10(\pm 0.26) \quad (2)$$

$$n = 21 \qquad r = 0.97 \qquad s = 0.17 \qquad F = 103$$

For each position, except 2- and 4-, at least Cl and Me substituents (representative of small, lipophilic electron-withdrawing and electron-donating groups, respectively) were evaluated (note that the substituent numbering is different between the acridine and phenazine chromophores). In the 2- and 4-positions, only one analogue was made. The 2-Cl compound **6b** was much less cytotoxic than the parent, due presumably to the substituent *ortho* to the carboxamide side chain interfering with the conformation of the latter. This phenomenon was true also in both the monophenazine[13] and the bis(DACA)[11] series, and no other 2-substituted compounds were made. In the 4-series, the 4-Me compound **6e** was substantially more cytotoxic than the parent (although not as active as other Me analogues), but the corresponding 4-Cl analogue could not be prepared (see above).

In terms of absolute potency, the remainder of the specific substituent effects fell into two categories, very similar to those seen in the bis(acridine-4-carboxamides).[11] First, 9-substituents (especially small lipophilic ones) were the most beneficial, with the 9-Me analogue **6n** proving the most cytotoxic of all the compounds made (25-fold more potent than **6a** in JL_C cells), and the 6,9-diMe and 6-Cl, 9-Me analogues (**6t** and **6u**) being the next active. In contrast, substituents in the remaining positions (3-, 4-, 6-, 7-, and 8-) showed no marked effects. Compounds **6t** and **6u** show that, in 6,9-disubstituted derivatives, the 9-substituent dominates in terms of biological effects, with these compounds showing similar potencies to their corresponding 9-monosubstituted analogues. Too few compounds were made to study QSAR for local substituent steric and

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

		GI_{50} (nM)[a]						
no.	sub.	MCF7	NCI/ADR	CAK-1	SKOV-3	HT29	HCT-116	mid[b]
6a	H	120	1050	225	850	44	16	210
6b	2-Cl	4070	$>10^5$	3.4×10^4	8.8×10^4	135	285	5750
6c	3-Me	310	5730	170	1.2×10^4	130	470	725
6d	3-Cl	110	125	1170	1460	<10	18	180
6f	6-Me	480	920	370	1.2×10^4	270	63	740
6h	7-Me	550	2660	430	5840	235	455	600
6i	7-Cl	330	1140	530	1960	80	35	560
6j	7-OMe	2000	2.0×10^4	3020	9660	93	37	4170
6k	8-Me	330	1570	240	1.1×10^4	90	325	290
6l	8-Cl	<10[c]	240	62	1880	<10	<10	70
6m	8-OMe	<10	1210	82	800	<10	<10	72
6n	9-Me	<10	19	<10	220	<10	<10	20
6o	9-Cl	35	350	15	790	70	<10	93
6p	9-F	460	7450	1730	1.4×10^4	400	225	1320
6q	9-OMe	47	2.1×10^4	1420	1560	35	<10	575
6r	9-OPh	3480	1630	5360	1.1×10^4	1790	1770	3160
6s	9-NMe_2	1820	1.1×10^4	2910	1.0×10^4	530	430	2510
6t	6,9-diMe	<10	35	<10	74	<10	<10	34
6u	6-Cl, 9-Me	<10	50	<10	2030	40	<10	50

[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.

electronic effects at individual positions. While most attention was focused on 9-substituents, even here only six analogues were made. However, the results qualitatively support the observations made in the bis(acridine-4-carboxamide) series[11] that large substituents are disfavored. Overall, the substituted bis(phenazine-1-carboxamides) showed very similar cytotoxicities to the corresponding substituted bis(acridine-4-carboxamides).[11] Thus, comparing IC_{50} values in the JL_C cell line for all pairs of methyl-, methoxy-, and chloro-substituted bis(phenazines) and bis(acridines) (bisacridine data from ref 11) gave eq 3, with a slope of unity running essentially through the origin:

$$\log(IC_{50})_{JLC}(\text{phenazines}) = 0.99(\pm 0.18) \log(IC_{50})_{JLC}(\text{acridines}) - 0.01(\pm 0.28) \quad (3)$$

$$n = 17 \qquad r = 0.87 \qquad s = 0.27 \qquad F = 45$$

Most of the compounds were also evaluated in the NCI cell human line panel,[21] 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). As shown previously[11] with bis(DACA) derivatives, a comparison of the GI_{50}(mid) values and IC_{50} values in the JL_C line shows a good correlation (eq 4), indicating that the latter is a useful model for broadly ranking cytotoxicities.

$$\log(GI_{50})_{mid} = 0.97(\pm 0.10) \log(IC_{50})_{JLC} + 0.57(\pm 0.22) \quad (4)$$

$$n = 19 \qquad r = 0.92 \qquad F = 93$$

Again, similarly to the bis(DACA) compounds,[11] a comparison of results in the MCF7 and NCI/ADR lines (the latter overexpresses P-glycoprotein) shows the bis(phenazines) are moderately affected by P-glycoprotein mediated multidrug resistance. On average, the compounds were 38-fold less active in the P-glycoprotein overexpressing NCI/ADR line, but no obvious structure–activity relationships could be observed; thus the 9-OPh analogue **6r** was 2-fold more potent in the NCI/ADR line than in MCF7, but the 9-OMe compound **6q** was 445-fold less potent.

As expected, the relatively refractory solid tumor lines CAK-1 (renal) and SKOV3 (ovarian) were resistant, with GI_{50} for most of the compounds being greater than the GI_{50}(mid) values. Overall, and to an even greater extent than the bis(DACA) compounds,[11] the bis(phenazines) were preferentially active toward colon cell lines. In the representative HT29 line, selectivities [measured as GI_{50}(mid)/GI_{50}(HCT-116)] varied from 1.7-fold (for the 9-OPh analogue **6r**) to >40-fold for the 2-Cl and 7-OMe analogues **6b** and **6j**, with an average of 9.5-fold (note these are underestimated by taking IC_{50} values in Table 2 of <10 nM as 10 nM for the purpose of calculation). Selectivities for HCT-116 were similar. Compounds **6b** and **6j** in particular were relatively nonpotent in all but the colon lines.

The low IC_{50} ratios (JL_A/JL_C and JL_D/JL_C) for the 9-methyl analogue **6n** (Table 1) indicated selective toxicity toward cell lines expressing low levels of topo II. To investigate the action of this compound on topoisomerases, purified topo I and II were incubated with increasing concentrations of drug in the presence of supercoiled plasmid DNA, and the products were subjected to electrophoresis in the presence of ethidium bromide to separate closed and open circular DNA. While no evidence for poisoning of topo II was obtained, drug concentrations of 0.1 and 0.25 μM caused the appearance of nicked circular DNA, indicative of poisoning of topo I (Figure 1). Higher drug concentrations (1 μM and 5 μM) were found to inhibit the relaxation of plasmid DNA by both topo I and II, respectively (data not shown). Compound **6v** showed cytotoxic as well as cytostatic activity in the H460 human lung line[25] by clonogenic assay, causing 90% and 99% inhibition of survival at 9.0 and 15 μM, respectively.

To assess the in vivo antitumor potential of this series, the parent compound (**6a**) and three of the more potent analogues (**6m**, **6n**, and **6o**) were evaluated in the relatively refractory subcutaneous colon 38 tumor

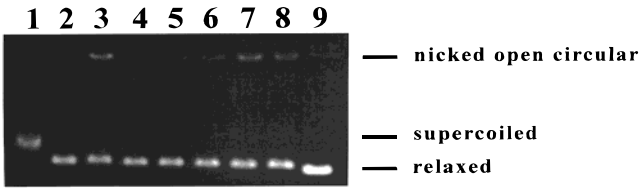


Figure 1. Topo I cleavable complex formation assays. Gel electrophoresis of supercoiled plasmid pBR322 DNA with no additions (lane 1); reacted with topo I (15 units) in the absence of drug (lane 2); topo I plus camptothecin at 100 μM (lane 3); topo I plus compound **6n** at concentrations of 2 nM (lane 4); 10 nM (lane 5); 50 nM (lane 6); 100 nM (lane 7); 250 nM (lane 8); relaxed marker pBR322 DNA (lane 9). Supercoiled and relaxed DNA run at the bottom of the gel whereas nicked open circles, which represent the formation of topoisomerase I cleavable complexes, run at the top.

Table 3. In Vivo Antitumor Activity of Selected Bis(phenazine-1-carboxamides) against Subcutaneous Colon 38 Carcinoma

no.	dosing schedule	optimal dose (mg/kg)	growth delay (days)
6a	every 4 days × 3	100 × 3	6.0
6m	single dose	100	8.0
6n	single dose	30	7.6
6n	every 4 days × 3	30 × 3	12.3
6o	single dose	100 × 3	7.0

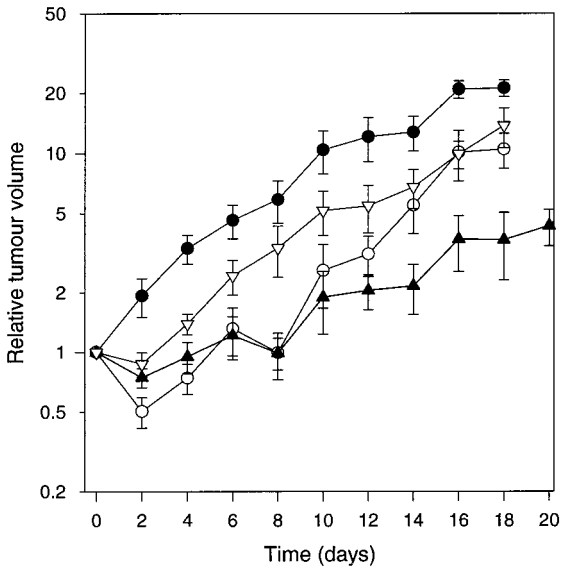


Figure 2. Growth of colon 38 adenocarcinoma in the absence of drug treatment (●) and following administration of compound **6n** (commencing at day 0 on the graph, or 8 days after tumor inoculation). The schedules tested in this experiment were single dose (30 mg/kg; ○), repeated daily dose (10 mg/kg × 7; ▽) and intermittent dose (30 mg/kg every 4 days × 3; ▲).

model. The data are shown in Table 3 and Figure 2. The most active analogue was **6n**, which showed a growth delay of 12.3 days (at 30 mg/kg using a 4 days × 3 dosing schedule). This is comparable to the activity of the clinical agent DACA in this model, which showed a growth delay of 12.5 days (at 200 mg/kg using a schedule of 200 mg/kg × 2)[22] but at a considerably lower total dose (90 versus 400 mg/kg).

Conclusions

The bis(phenazine-1-carboxamides) are potent cytotoxins in cell culture. Structure−activity relationships are broadly similar to those found previously for bis(DACA) compounds; small lipophilic substituents *peri* to the ring nitrogen (in the 9-position) are the most beneficial, with substituents in the remaining positions showing no marked effects. Results from the NCI human tumor panel showed the compounds were moderately affected by P-glycoprotein mediated multidrug resistance, but were highly preferentially active toward colon cell lines. The 9-methyl analogue **6n** is a dual topo I/II inhibitor and a topo poison. Analogues showed significant growth delays in a refractory subcutaneous colon 38 tumor model in vivo. The bis(phenazine-1-carboxamides) are an interesting class of dual topo I/II-directed anticancer drugs.

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 analysis with diode array detection.

Preparation of Phenazine Acids. 4-Chlorophenazine-1-carboxylic Acid (13). A solution of 4-chloro-2,3-dinitrobenzoic acid (**9**)[15] (1.6 g, 6.48 mmol) in MeOH was hydrogenated over Pt/C for 30 min, and the resulting solution of crude diamino acid **10** was filtered directly into a solution of cyclohexane-1,2-dione (**11**) (1.57 g, 6.48 mmol) in MeOH (20 mL). The reaction mixture was stirred and heated to reflux for 15 min, then concentrated to half volume, cooled, and diluted with water. The resulting precipitate was collected and dissolved in CH_2Cl_2. Insoluble material was removed by filtration, and the solution was dried (Na_2SO_4) and evaporated to give a crude product (1.4 g). This was chromatographed on silica gel in CH_2Cl_2 to give 4-chloro-6,7,8,9-tetrahydrophenazine-1-carboxylic acid (**12**) (0.5 g, 33%): ^{1}H NMR ($CDCl_3$) δ 2.12 (m, 4 H, 2 × CH_2), 3.30 (m, 4 H, 2 × CH_2), 7.94 (d, J = 8.1 Hz, 1 H, H-3), 8.60 (d, J = 8.1 Hz, 1 H, H-2) 15.01 (s, 1 H, CO_2H). Anal. ($C_{13}H_{11}ClN_2O_2$) C, H, N.

A mixture of **12** (10 mg, 0.038 mmol) and DDQ (20 mg, 0.088 mmol) in benzene (5 mL) was refluxed overnight. Solvents were removed under reduced pressure, and the residue was chromatographed on silica gel, eluting with CH_2Cl_2/MeOH (99:1) to give **13** (1.2 mg, 12%): ^{1}H NMR ($CDCl_3$) δ 8.03−8.11 (m, 2 H, H-7,8), 7.72 (d, J = 8.2 Hz, 1 H, H-3), 8.32 (dd, J = 8.4, 1.8 Hz, 1 H, H-6 or H-9), 8.49 (dd, J = 7.6, 1.6 Hz, 1 H, H-6 or H-9), 8.91 (d, J = 7.7 Hz, 1 H, H-2), 15.35 (s, 1 H, CO_2H). HRMS (FAB$^+$) calcd for $C_{13}H_8{}^{35}ClN_2O_2$ 258.0919 (MH^+); found, 258.0914.

9-Phenoxyphenazine-1-carboxylic Acid (7r). A mixture of 2-aminodiphenyl ether (**14**) (15 g, 80 mmol), 2-bromo-3-nitrobenzoic acid (**17**) (10 g, 40 mmol), CuCl (1 g) in *N*-ethylmorpholine (15 mL), and butane-2,3-diol (25 mL) were stirred at 80 °C for 4 h. The cooled mixture was diluted with 0.01 N aqueous NaOH, clarified with charcoal, and filtered through Celite. The cloudy solution was seeded with 2-aminodiphenyl ether to precipitate unreacted starting material, and the resulting clear solution was acidified with dilute HCl to give *N*-(2-phenoxyphenyl)-3-nitroanthranilic acid (**18**) (13 g, 91%): mp (benzene/petroleum ether) 153−155 °C; ^{1}H NMR ($CDCl_3$) δ 6.95−7.08 (m, 8 H, ArH), 7.09−7.30 (m, 2 H, ArH), 8.11 (dd, J = 8.1, 1.7 Hz, 1 H, ArH), 8.22 (dd, J = 7.8, 1.6 Hz, 1 H, ArH), 9.69 (br s, 1 H, NH). Anal. ($C_{19}H_{14}N_2O_5$) C, H, N.

A solution of **18** (10 g, 2.85 mmol) and $NaBH_4$ (10 g) in 2 N aqueous NaOH (600 mL) was heated under reflux for 48 h. The cooled mixture was filtered, and the solid was dissolved in hot water. Acidification with concentrated HCl gave a mixture of the desired product and the amine resulting from direct reduction. The dried mixture was suspended in boiling MeOH, and sufficient Et_3N was added to just give a homogeneous solution. Acidification with AcOH then precipitated **7r** (0.93 g, 10%): mp 232−234 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 7.22−7.25 (m, 3 H, ArH), 7.45−7.50 (m, 3 H, ArH), 8.02 (dd, J = 8.8, 7.6 Hz, 1 H, ArH), 8.15−8.18 (m, 2 H, ArH), 8.59 (dd, J = 8.8, 1.4 Hz, 1 H, ArH), 8.68 (dd, J = 7.0, 1.2 Hz, 1 H, ArH), 15.02 (br s, 1 H, COOH). Anal. ($C_{19}H_{12}N_2O_3$) C, H, N. The filtrate from the original reaction mixture was acidified with concentrated HCl to give phenazine-1-carboxylic acid (resulting from displacement of the phenoxy group during ring closure).

9-(Dimethylamino)phenazine-1-carboxylic Acid (7s). A solution of 9-fluorophenazine-1-carboxylic acid[14] (**7p**) (200 mg, 0.8 mmol) in 40% aqueous Me_2NH (20 mL) was heated at 100 °C in a bomb for 3 h. The resulting intensely purple solution was diluted with water and then neutralized with AcOH. The aqueous solution was then extracted with $CHCl_3$ (3 × 50 mL) until all color was removed. The organic layer was further washed with water (1 × 150 mL) and then dried over Na_2SO_4, and the solvent was removed under reduced pressure. The resulting purple solid was dissolved in a minimal amount of CH_2Cl_2, and petroleum ether was added until crystallization occurred, giving **7s** as dark purple needles (210 mg, 95%): mp 186−187.5 °C; ^{1}H NMR ($CDCl_3$) δ 3.16 [s, 6 H, $N(CH_3)_2$], 7.26 (dd, J = 6.8, 1.8 Hz, 1 H, H-6 or H-8), 7.81−7.88 (m, 2 H, H-7 and H-8 or H-6), 8.01 (dd, J = 8.7, 7.0 Hz, 1 H, H-3), 8.48 (dd, J = 8.7, 1.2 Hz, 1 H, H-4), 8.91 (dd, J = 7.0, 1.3 Hz, 1 H, H-2). Anal. ($C_{15}H_{13}N_3O_2$) C, H, N.

6-Chloro-9-methylphenazine-1-carboxylic Acid (7u). A mixture of 5-chloro-2-methylaniline (**16**) (8.63 g, 61.0 mmol), 2-bromo-3-nitrobenzoic acid (**17**) (10.0 g, 41.0 mmol), CuCl (0.5 g), copper powder (0.1 g) in butane-2,3-diol (25 mL), and *N*-ethylmorpholine (15 mL) was stirred and heated for 18 h at 70 °C. The reaction mixture was diluted with 0.5 M NH_4OH (500 mL), then filtered through Celite. The orange filtrate was then slowly added to a stirred solution of 2 N HCl, and the resulting yellow precipitate was collected by filtration, dried, and recrystallized to give 2-[(5-chloro-2-methylphenyl)amino]-3-nitrobenzoic acid (**20**) (6.97 g, 55%): mp (EtOAc/*n*-hexane) 228−230 °C; ^{1}H NMR ($CDCl_3$) δ 2.35 (s, 3 H, CH_3), 6.79 (d, J = 2.1 Hz, 1 H, H-6′), 6.96−7.00 (m, 2 H, H-4′ and H-5), 7.15 (d, J = 8.0 Hz, 1 H, H-3′), 8.07 (dd, J = 8.1, 1.8 Hz, 1 H, H-4 or H-6), 8.24 (dd, J = 7.9, 1.7 Hz, 1 H, H-6 or H-4), 9.51 (s, 1 H, NH). Anal. ($C_{14}H_{11}ClN_2O_2$) C, H, N.

A solution of **20** (3.59 g, 11.7 mmol) and $NaBH_4$ (2.62 g, 68.8 mmol) in 2 M NaOH was heated at reflux for 8 h. The reaction mixture was then cooled and acidified with AcOH, and the resulting precipitate was collected and recrystallized to give **7u** (1.42 g, 45%): mp (acetone) 255−257 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 2.86 (s, 3 H, CH_3), 7.90 (dd, J = 7.4, 1.1 Hz, 1 H, ArH), 8.11−8.18 (m, 2 H, ArH), 8.57−8.61 (m, 2 H, ArH), 14.52 (br s, 1 H, COOH). Anal. ($C_{14}H_9ClN_2O_2$) C, H, N, Cl.

6,9-Dimethylphenazine-1-carboxylic Acid (7t). Reaction of 2-bromo-3-nitrobenzoic acid (**17**) and 2,5-dimethylaniline (**15**) as above gave 2-[(2,5-dimethylphenyl)amino]-3-nitrobenzoic acid (**19**) (65%): mp (benzene/acetone) 215−217 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 2.10 (s, 3 H, CH_3), 2.23 (s, 3 H, CH_3), 6.53 (s, 1H, H-6′), 6.79 (d, J = 7.4 Hz, 1 H, H-4′), 7.02 (t, J = 8.0 Hz, 1 H, H-5), 7.11 (d, J = 7.7 Hz, 1 H, H-3′), 8.03 (dd, J = 8.1, 1.4 Hz, 1 H, H-6), 8.22 (dd, J = 7.7, 1.5 Hz, 1 H, H-4), 9.84 (br s, 1 H, NH), 13.8 (br s, 1 H, CO_2H). Anal. ($C_{15}H_{14}N_2O_4$) C, H, N.

Reductive ring closure of **19** with $NaOC_2H_5$/EtOH/$NaBH_4$ under the above conditions gave **7t** (64%): mp (MeOH) 246−247 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 2.78 (s, 3 H, CH_3), 2.83 (s, 3 H, CH_3), 7.80 (d, J = 7.0 Hz, 1 H, H-7 or H-8), 7.83 (d, J = 7.0 Hz, 1 H, H-7 or H-8), 8.12 (dd, J = 8.5, 7.2 Hz, 1 H, H-3), 8.56 (d, J = 8.7 Hz, 1 H, H-4), 8.66 (d, J = 7.0 Hz, 1 H, H-2), 15.24 (br s, 1 H, CO_2H). Anal. ($C_{15}H_{12}N_2O_2$) C, H, N.

Bis[3-(3-methylphenazine-1-carboxamido)propyl]methylamine (6c): Example of General Method of Scheme 1. A suspension of 3-methylphenazine-1-carboxylic acid (**7c**)[13] (1.02 g, 4.29 mmol) in DMF (10 mL) was treated with 1,1′-carbonyldiimidazole (1.39 g, 8.57 mmol), and the mixture was stirred at 45 °C for 30 min. After cooling, the mixture was diluted with CH_2Cl_2/petroleum ether (1:1) to complete precipitation of the crude imidazolide (**8c**), which was collected, washed with petroleum ether, dried, and immediately added to an ice-cold solution of bis(3-aminopropyl)methylamine (0.31 g, 2.15 mmol) in THF (50 mL). The mixture was stirred at 20 °C for 4 h, then volatiles were removed under reduced pressure, and the residue was partitioned between CH_2Cl_2 and aqueous 1 M Na_2CO_3. The organic layer was washed with water, dried, and evaporated, and the residue was chromatographed on alumina-90. Elution with CH_2Cl_2/MeOH (20:1), followed by crystallization from CH_2Cl_2/petroleum ether, gave bis[(3-methylphenazine-1-carboxamido)propyl]methylamine (**6c**) (1.05 g, 84% from the acid): mp (CH_2Cl_2/*n*-hexane) 75−78 °C; ^{1}H NMR ($CDCl_3$) δ 2.03 (quin, J = 7.0 Hz, 4 H, 2 × $CH_2CH_2CH_2$), 2.37 (s, 3 H, NCH_3), 2.67 (d, J = 0.9 Hz, 6 H, 2 × CH_3), 2.73 [t, J = 7.2 Hz, 4 H, $CH_2N(CH_3)CH_2$], 3.73 (q, J = 6.3 Hz, 4 H, 2 × CH_2NH), 7.62−7.70 (m, 4 H, 2 × H-7 and 2 × H-8), 7.98−8.03 (m, 6 H, 2 × H-6, 2 × H-9 and 2 × H-2 or 2 × H-4), 8.73 (d, J = 2.1 Hz, 2 H, 2 × H-4 or 2 × H-2), 10.88 (br t, J = 5.2 Hz, 2 H, 2 × CONH). HRMS (FAB^+) *m/z* calcd for $C_{35}H_{36}N_7O_2$ 586.2930 (MH^+); found, 586.2937. Anal. ($C_{35}H_{35}N_7O_2 \cdot H_2O$) C, H, N.

Bis[3-(2-chlorophenazine-1-carboxamido)propyl]methylamine (6b). From similar reaction of 2-chlorophenazine-1-carboxylic acid[13] (**7b**) (45% yield): mp (CH_2Cl_2/*n*-hexane) 206−207 °C; ^{1}H NMR ($CDCl_3$) δ 1.83 (quin, J = 6.0 Hz, 4 H, 2 × $CH_2CH_2CH_2$), 2.17 (s, 3 H, NCH_3), 2.72 [t, J = 6.1 Hz, 4 H, $CH_2N(CH_3)CH_2$], 3.67 (q, J = 6.0 Hz, 2 H, 2 × CH_2NH), 7.03 (br t, J = 5.9 Hz, 2 H, 2 × CONH), 7.47 (d, J = 9.4 Hz, 2 H, 2 × H-3 or 2 × H-4), 7.60−7.68 (m, 4 H, 2 × H-7 and 2 × H-8), 7.89 (d, J = 9.3 Hz, 2 H, 2 × H-4 or 2 × H-3), 7.91−7.97 (m, 4 H, 2 × H-6 and 2 × H-9). HRMS (FAB^+) *m/z* calcd for $C_{33}H_{30}{}^{35}Cl_2N_7O_2$ 626.1838 (MH^+); found, 626.1854. Anal. ($C_{33}H_{29}Cl_2N_7O_2$) C, H, N.

Bis[3-(3-chlorophenazine-1-carboxamido)propyl]methylamine (6d). From similar reaction of 3-chlorophenazine-1-carboxylic acid[13] (**7d**) (76% yield): mp (CH_2Cl_2/*n*-hexane) 169−170 °C; ^{1}H NMR ($CDCl_3$) δ 2.02 (quin, J = 6.9 Hz, 4 H, 2 × $CH_2CH_2CH_2$), 2.36 (s, 3 H, NCH_3), 2.72 [t, J = 7.3 Hz, 4 H, $CH_2N(CH_3)CH_2$], 3.71 (q, J = 6.3 Hz, 4 H, 2 × CH_2NH), 7.69−7.76 (m, 4 H, 2 × H-7 and 2 × H-8), 7.94−8.00 (m, 4 H, 2 × H-6 and 2 × H-9), 8.20 (d, J = 2.5 Hz, 2 H, 2 × H-4), 8.74 (d, J = 2.5 Hz, 2 H, 2 × H-2), 10.65 (br t, J = 5.2 Hz, 2 H, 2 × CONH). HRMS (FAB^+) *m/z* calcd for $C_{33}H_{30}{}^{35}Cl_2N_7O_2$ 626.1838 (MH^+); found, 626.1824. Anal. $C_{33}H_{29}Cl_2N_7O_2 \cdot H_2O$) C, H, N.

Bis[3-(4-methylphenazine-1-carboxamido)propyl]methylamine (6e). From similar reaction of 4-methylphenazine-1-carboxylic acid[13] (**7e**) (78% yield): mp (CH_2Cl_2/*n*-hexane) 218−220 °C; ^{1}H NMR ($CDCl_3$) δ 2.04 (quin, J = 7.0 Hz, 2 H, 2 × $CH_2CH_2CH_2$), 2.38 (s, 3 H, NCH_3), 2.75 [t, J = 7.3 Hz, 4 H, $CH_2N(CH_3)CH_2$], 2.90 (s, 6 H, 2 × CH_3), 3.71 (q, J = 6.3 Hz, 4 H, 2 × CH_2NH), 7.58 (ddd, J = 8.6, 6.7, 1.3 Hz, 2 H, ArH), 7.65 (ddd, J = 8.6, 6.6, 1.4 Hz, 2 H, ArH), 7.70 (dd, J = 7.2, 1.0 Hz, 2 H, ArH), 7.94 (dd, J = 8.6, 0.9 Hz, 2 H, ArH), 8.00 (d, J = 8.7 Hz, 2 H, ArH), 8.77 (d, J = 7.3 Hz, 2 H, ArH), 10.88 (br s, 2 H, 2 × CONH). HRMS (FAB^+) *m/z* calcd for $C_{35}H_{36}N_7O_2$ 586.2930 (MH^+); found, 586.2922. Anal. ($C_{35}H_{35}N_7O_2 \cdot 2.5H_2O$) C, H, N.

Bis[3-(6-methylphenazine-1-carboxamido)propyl]methylamine (6f). From similar reaction of 6-methylphenazine-1-carboxylic acid[13] (**7f**) (47% yield): mp (HCl salt from MeOH/EtOAc) 228−230 °C; ^{1}H NMR ($CDCl_3$) δ 2.06 (quin, J = 6.9 Hz, 4 H, 2 × $CH_2CH_2CH_2$), 2.39 (s, 3 H, NCH_3), 2.79 (s, 6 H, 2 × CH_3), 2.81 [t, J = 7.0 Hz, 4 H, $CH_2N(CH_3)CH_2$], 3.75 (q, J = 6.1 Hz, 4 H, 2 × CH_2NH), 7.42 (t, J = 7.8 Hz, 2 H, 2 × H-8), 7.61 (d, J = 8.8 Hz, 2 H, ArH), 7.87 (dd, J = 8.5, 7.1 Hz, 4 H, 2 × H-3 and ArH), 8.27 (dd, J = 8.7, 1.5 Hz, 2 H, 2 ×

H-4), 8.88 (dd, J = 7.0, 1.5 Hz, 2 H, 2 × H-2), 10.93 (br s, 2 H, 2 × CONH). Anal. ($C_{35}H_{35}N_7O_2$·0.5H_2O) C, H, N.

Bis[3-(6-chlorophenazine-1-carboxamido)propyl]methylamine (6g). From similar reaction of 6-chlorophenazine-1-carboxylic acid[13] (**7g**) (56% yield): mp (CH_2Cl_2/MeOH) 198−200 °C; ^{1}H NMR ($CDCl_3$) δ 2.03 (quin, J = 7.0 Hz, 4 H, 2 × $CH_2CH_2CH_2$), 2.37 (s, 3 H, NCH_3), 2.73 [t, J = 7.2 Hz, 4 H, $CH_2N(CH_3)CH_2$], 3.72 (q, J = 6.2 Hz, 4 H, CH_2NH), 7.62 (dd, J = 8.7, 7.2 Hz, 2 H, 2 × H-8), 7.74 (dd, J = 7.2, 1.2 Hz, 2 H, 2 × H-7 or 2 × H-9), 7.91 (dd, J = 8.8, 1.2 Hz, 2 H, 2 × H-9 or 2 × H-7), 7.93 (dd, J = 8.7, 7.1 Hz, 2 H, 2 × H-3), 8.39 (dd, J = 8.7, 1.6 Hz, 2 H, 2 × H-4), 8.88 (dd, J = 7.1, 1.6 Hz, 2 H, 2 × H-2), 10.59 (br t, J = 5.1 Hz, 2 H, 2 × CONH). HRMS (FAB$^+$) m/z calcd for $C_{33}H_{30}Cl_2N_7O_2$ 626.1838 (MH^+); found, 618.1840. Anal. ($C_{33}H_{29}Cl_2N_7O_2$) C, H, N, Cl.

Bis[3-(7-methylphenazine-1-carboxamido)propyl]methylamine (6h). From similar reaction of 7-methylphenazine-1-carboxylic acid (**7h**)[13] (63% yield): mp (HCl salt from MeOH/EtOAc) 213−215 °C; ^{1}H NMR ($CDCl_3$) δ 2.06 (quin, J = 6.9 Hz, 4 H, 2 × $CH_2CH_2CH_2$), 2.38 (s, 3 H, NCH_3), 2.44 (s, 6 H, 2 × CH_3), 2.79 [t, J = 7.4 Hz, 4 H, $CH_2N(CH_3)CH_2$], 3.75 (q, J = 6.2 Hz, 4 H, 2 × CH_2NH), 7.40 (dd, J = 8.9, 1.6 Hz, 2 H, 2 × H-8), 7.62 (br s, 2 H, 2 × H-6), 7.77 (d, J = 8.9 Hz, 2 H, 2 × H-9), 7.86 (dd, J = 8.5, 7.1 Hz, 2 H, 2 × H-3), 8.22 (dd, J = 8.6, 1.5 Hz, 2 H, 2 × H-4), 8.86 (dd, J = 7.2, 1.5 Hz, 2 H, 2 × H-2), 10.85 (t, J = 4.9 Hz, 2 H, 2 × CONH). Anal. ($C_{32}H_{35}N_7O_2$·2HCl·2H_2O) C, N, Cl: H; found 6.6; requires 6.0%.

Bis[3-(7-chlorophenazine-1-carboxamido)propyl]methylamine (6i). From similar reaction of 7-chlorophenazine-1-carboxylic acid (**7i**)[13] (71% yield): mp (CH_2Cl_2/MeOH) 173−175 °C; ^{1}H NMR ($CDCl_3$) δ 2.02 (quin, J = 6.9 Hz, 4 H, 2 × $CH_2CH_2CH_2$), 2.37 (s, 3 H, NCH_3), 2.73 [t, J = 7.2 Hz, 4 H, $CH_2N(CH_3)CH_2$], 3.73 (q, J = 6.2 Hz, 4 H, 2 × CH_2NH), 7.54 (dd, J = 9.3, 2.4 Hz, 2 H, H-8), 7.84 (d, J = 9.3 Hz, 2 H, 2 × H-9), 7.90 (d, J = 2.5 Hz, 2 H, 2 × H-6), 7.92 (dd, J = 8.7, 7.1 Hz, 2 H, 2 × H-3), 8.20 (dd, J = 8.7, 1.6 Hz, 2 H, 2 × H-4), 8.88 (dd, J = 7.1, 1.5 Hz, 2 H, 2 × H-2), 10.54 (br t, J = 5.1 Hz, 2 H, 2 × CONH). HRMS (FAB$^+$) m/z calcd for $C_{33}H_{30}Cl_2N_7O_4$ 626.1838 (MH^+); found, 618.1844. Anal. ($C_{33}H_{29}Cl_2N_7O_2$) C, H, N, Cl.

Bis[3-(7-methoxyphenazine-1-carboxamido)propyl]methylamine (6j). From similar reaction of 7-methoxyphenazine-1-carboxylic acid[13] (**7j**) (60% yield): mp (HCl salt from MeOH/EtOAc) 225−229 °C; ^{1}H NMR (free base in $CDCl_3$) δ 2.03 (quin, J = 6.9 Hz, 4 H, 2 × $CH_2CH_2CH_2$), 2.37 (s, 3 H, NCH_3), 2.74 [t, J = 7.2 Hz, 4 H, $CH_2N(CH_3)CH_2$], 3.73 (q, J = 6.3 Hz, 4 H, 2 × CH_2NH), 3.93 (s, 6 H, 2 × OCH_3), 7.10 (d, J = 2.7 Hz, 2 H, 2 × H-6), 7.28 (dd, J = 9.1, 3.1 Hz, 2 H, ArH), 7.78 (d, J = 9.5 Hz, 2 H, 2 × H-9), 7.83 (dd, J = 8.7, 1.5 Hz, 2 H, ArH), 8.17 (dd, J = 8.6, 1.5 Hz, 2 H, ArH), 8.81 (J = 7.2, 1.5 Hz, 2 H, ArH), 10.77 (t, J = 4.6 Hz, 2 H, 2 × CONH). Anal. ($C_{35}H_{35}N_7O_4$.2HCl·3H_2O) C, H, N.

Bis[3-(8-methylphenazine-1-carboxamido)propyl]methylamine (6k). From similar reaction of 8-methylphenazine-1-carboxylic acid[13,14] (**7k**) (76% yield): mp (CH_2Cl_2/hexane) 161−162 °C; ^{1}H NMR ($CDCl_3$) δ 2.16 (quin, J = 6.6 Hz, 4 H, 2 × $CH_2CH_2CH_2$), 2.52 (s, 9 H, NCH_3 and 2 × CH_3), 2.93 [br s, 4 H, $CH_2N(CH_3)CH_2$], 3.76 (q, J = 6.3 Hz, 4 H, 2 × CH_2NH), 7.41 (d, J = 8.6 Hz, 2 H, 2 × H-6), 7.77 (br s, 2 H, 2 × H-9), 7.86 (dd, J = 8.5, 7.1 Hz, 4 H, 2 × H-3 and 2 × H-7), 8.26 (dd, J = 8.6, 1.5 Hz, 2 H, H-4), 8.87 (dd, J = 7.7, 1.5 Hz, 2 H, H-2), 11.00 (br s, 2 H, 2 × CONH). Anal. ($C_{35}H_{35}N_7O_2$·0.5H_2O) C, H, N.

Bis[3-(8-chlorophenazine-1-carboxamido)propyl]methylamine (6l). From similar reaction of 8-chlorophenazine-1-carboxylic acid[13,14] (**7l**) (85% yield): mp (CH_2Cl_2/*n*-hexane) 210−212 °C; ^{1}H NMR ($CDCl_3$) δ 2.04 (quin, J = 7.0 Hz, 4 H, 2 × $CH_2CH_2CH_2$), 2.39 (s, 3 H, NCH_3), 2.73 [t, J = 7.2 Hz, 4 H, $CH_2N(CH_3)CH_2$], 3.74 (q, J = 6.3 Hz, 4 H, 2 × CH_2NH), 7.56 (dd, J = 9.2, 2.4 Hz, 2 H, 2 × H-7), 7.92 (dd, J = 8.7, 7.2 Hz, 2 H, 2 × H-3), 7.98 (d, J = 9.2, 2 H, 2 × H-6), 8.03 (d, J = 2.2 Hz, 2 H, 2 × H-9), 8.26 (dd, J = 8.7, 1.5 Hz, 2 H, 2 × H-4), 8.92 (dd, J = 7.2, 1.5 Hz, 2 H, 2 × H-2), 10.64 (br t, J = 5.2 Hz, 2 H, 2 × CONH). HRMS (FAB$^+$) m/z calcd for $C_{33}H_{30}{}^{35}Cl_2N_7O_2$ 626.1838 (MH^+); found, 626.1860. Anal. ($C_{33}H_{29}Cl_2N_7O_2$) C, H, N, Cl.

Bis[3-(8-methoxyphenazine-1-carboxamido)propyl]methylamine (6m). From similar reaction of 8-methoxyphenazine-1-carboxylic acid[13,14] (**7m**) (99% yield): mp (CH_2Cl_2/hexane) 182−186 °C; ^{1}H NMR ($CDCl_3$) δ 1.92 (quin, J = 6.4 Hz, 2 × $CH_2CH_2CH_2$), 2.30 (s, 3 H, NCH_3), 2.71 [m, 4 H, $CH_2N(CH_3)CH_2$], 3.60 (q, J = 6.1 Hz, 4 H, 2 × CH_2NH), 3.85 (s, 6 H, 2 × OCH_3), 7.06 (s, 2 H, 2 × H-9), 7.19 (dd, J = 9.4, 2.4 Hz, 2 H, 2 × H-7), 7.69 (d, J = 9.4 Hz, 2 H, 2 × H-6), 7.80 (dd, J = 8.7, 7.2 Hz, 2 H, 2 × H-3), 8.11 (dd, J = 8.5, 1.4 Hz, 2 H, 2 × H-4), 8.48 (J = 7.1, 1.5 Hz, 2 H, 2 × H-2), 10.39 (t, J = 5.4 Hz, 2 H, 2 × CONH). Anal. ($C_{35}H_{35}N_7O_4$) C, H, N.

Bis[3-(9-methylphenazine-1-carboxamido)propyl]methylamine (6n). From similar reaction of 9-methylphenazine-1-carboxylic acid[13] (**7n**) (82% yield): mp (HCl salt from MeOH/EtOAc) 262−264 °C; ^{1}H NMR ($CDCl_3$) δ 1.99 (quin, J = 7.3 Hz, 4 H, 2 × $CH_2CH_2CH_2$), 2.32 (s, 3 H, NCH_3), 2.60 [t, J = 7.4 Hz, 4 H, $CH_2N(CH_3)CH_2$], 2.79 (s, 6 H, 2 × CH_3), 3.70 (q, J = 6.7 Hz, 4 H, 2 × CH_2NH), 7.56 (d, J = 6.7 Hz, 2 H, 2 × H-8), 7.65 (dd, J = 8.7, 7.2 Hz, 2 H, 2 × H-7), 7.89 (dd, J = 8.7, 7.2 Hz, 2 H, 2 × H-3), 7.97 (d, J = 8.6 Hz, 2 H, 2 × H-6), 8.27 (dd, J = 8.7, 1.5 Hz, 2 H, 2 × H-4), 8.93 (dd, J = 7.2, 1.5 Hz, 2 H, 2 × H-2), 10.94 (br s, 2 H, 2 × CONH). Anal. ($C_{35}H_{35}N_7O_2$.HCl) C, N, Cl; H, found 6.4, calcd 5.9%.

Bis[3-(9-chlorophenazine-1-carboxamido)propyl]methylamine (6o). From similar reaction of 9-chlorophenazine-1-carboxylic acid[13] (**7o**) (86% yield): mp (CH_2Cl_2/MeOH) 169−171.5 °C; ^{1}H NMR ($CDCl_3$) δ 2.01 (quin, J = 7.3 Hz, 4 H, 2 × $CH_2CH_2CH_2$), 2.32 (s, 3 H, NCH_3), 2.62 [t, J = 7.4 Hz, 4 H, $CH_2N(CH_3)CH_2$], 3.70 (q, J = 6.2 Hz, 4 H, 2 × CH_2NH), 7.64 (dd, J = 8.8, 7.4 Hz, 2 H, 2 × H-7), 7.80 (dd, J = 7.2, 1.0 Hz, 2 H, 2 × H-6 or 2 × H-8), 7.95 (dd, J = 8.7, 7.2 Hz, 2 H, H-3), 8.01 (dd, J = 8.7, 1.0 Hz, 2 H, 2 × H-8 or 2 × H-6), 8.27 (dd, J = 8.7, 1.5 Hz, 2 H, 2 × H-4), 8.99 (dd, J = 7.2, 1.5 Hz, 2 H, H-2), 10.94 (br t, J = 5.0 Hz, 2 H, 2 × CONH). HRMS (FAB$^+$) m/z calcd for $C_{33}H_{30}Cl_2N_7O_2$ 626.1838 (MH^+); found, 618.1848. Anal. ($C_{33}H_{29}Cl_2N_7O_2$) C, H, N.

Bis[3-(9-fluorophenazine-1-carboxamido)propyl]methylamine (6p). From similar reaction of 9-fluorophenazine-1-carboxylic acid[14] (**7p**) (87% yield): mp (CH_2Cl_2/MeOH) 186−187 °C; ^{1}H NMR ($CDCl_3$) δ 2.02 (quin, J = 7.1 Hz, 4 H, 2 × $CH_2CH_2CH_2$), 2.36 (s, 3 H, NCH_3), 2.72 [t, J = 7.4 Hz, 4 H, $CH_2N(CH_3)CH_2$], 3.73 (q, J = 6.1 Hz, 4 H, 2 × CH_2NH), 7.30−7.35 (m, 2 H, 2 × H-7 or 2 × H-8), 7.54−7.60 (m, 2 H, 2 × H-8 or 2 × H-7), 7.84 (d, J = 9.0 Hz, 2 H, 2 × H-6), 7.94 (dd, J = 8.7, 7.0 Hz, 2 H, 2 × H-3), 8.25 (dd, J = 8.7, 1.5 Hz, 2 H, 2 × H-4), 8.95 (dd, J = 7.0, 1.5 Hz, 2 H, H-2), 10.94 (br t, J = 5.0 Hz, 2 H, 2 × CONH). HRMS (FAB$^+$) m/z calcd for $C_{33}H_{30}F_2N_7O_2$ 594.2429 (MH^+); found, 594.2403. Anal. ($C_{33}H_{29}F_2N_7O_2$·0.5H_2O) C, H, N.

Bis[3-(9-methoxyphenazine-1-carboxamido)propyl]methylamine (6q). From similar reaction of 9-methoxyphenazine-1-carboxylic acid[13,14] (**7q**) (86% yield): mp (CH_2Cl_2/MeOH) 220−222 °C; ^{1}H NMR ($CDCl_3$) δ 2.02 (quin, J = 7.2 Hz, 4 H, $CH_2CH_2CH_2$), 2.39 (s, 3 H, NCH_3), 2.73 [t, J = 7.6 Hz, 4 H, $CH_2N(CH_3)CH_2$], 3.66 (q, J = 6.0 Hz, 4 H, CH_2NH), 3.90 (s, 6 H, 2 × OCH_3), 6.60 (dd, J = 6.7, 1.9 Hz, 2 H, 2 × H-6 or 2 × H-8), 7.32−7.38 (m, 4 H, 2 × H-7 and 2 × H-8 or 2 × H-6), 7.84 (dd, J = 8.7, 7.2 Hz, 2 H, 2 × H-3), 8.11 (dd, J = 8.7, 1.5 Hz, 2 H, 2 × H-4), 8.83 (dd, J = 7.1, 1.5 Hz, 2 H, 2 × H-2), 11.12 (br t, J = 4.7 Hz, 2 H, 2 × CONH). HRMS (FAB$^+$) m/z calcd for $C_{35}H_{36}N_7O_4$ 618.2829 (MH^+); found, 618.2847. Anal. ($C_{35}H_{35}N_7O_4$) C, H, N.

Bis[3-(9-phenoxyphenazine-1-carboxamido)propyl]methylamine (6r). From similar reaction of 9-phenoxyphenazine-1-carboxylic acid (**7r**) (51% yield): as an orange oil; ^{1}H NMR ($CDCl_3$) δ 1.71 (quin, J = 7.3 Hz, 4 H, 2 × $CH_2CH_2CH_2$), 1.97 (s, 3 H, NCH_3), 2.31 [t, J = 7.3 Hz, 4 H, $CH_2N(CH_3)CH_2$], 3.43 (q, J = 6.4 Hz, 4 H, 2 × CH_2NH), 7.11−7.14 (m, 6 H, 2 × H-2′, 2 × H-6′ and 2 × H-6 or 2 × H-8), 7.18 (t, J = 7.5 Hz, 2 H, 2 × H-4′), 7.39 (t, J = 7.5 Hz, 4 H, 2 × H-3′ and 2 × H-5′), 7.69 (dd, J = 8.7, 7.6 Hz, 2 H, 2 × H-7), 7.89 (m, 4 H, 2 × H-3

and 2 × H-8 or 2 × H-6), 8.26 (dd, J = 8.7, 1.5 Hz, 2 H, 2 × H-4), 8.90 (dd, J = 7.1, 1.5 Hz, 2 H, 2 × H-2), 10.98 (br t, J = 5.2 Hz, 2 H, 2 × CONH). HRMS (FAB^+) m/z calcd for $C_{45}H_{40}N_7O_4$ 742.3142 (MH^+); found, 742.3147.

Bis[3-(9-dimethylamino)phenazine-1-carboxamido)-propyl]methylamine (6s). From similar reaction of 9-(dimethylamino)phenazine-1-carboxylic acid (**7s**) (78% yield) as a red-purple oil: ^{1}H NMR ($CDCl_3$) δ 1.95 (quin, J = 7.2 Hz, 4 H, 2 × $CH_2CH_2CH_2$), 2.29 (s, 3 H, NCH_3), 2.57 [t, J = 7.3 Hz, 4 H, $CH_2N(CH_3)CH_2$], 3.05 [s, 12 H, 2 × $N(CH_3)_2$], 3.68 (q, J = 6.5 Hz, 4 H, 2 × CH_2NH), 7.07 (dd, J = 7.2, 1.3 Hz, 2 H, 2 × H-6 or 2 × H-8), 7.65 (dd, J = 8.7, 7.3 Hz, 2 H, 2 × H-7), 7.70 (dd, J = 8.7, 1.3 Hz, 2 H, 2 × H-8 or 2 × H-6), 7.90 (dd, J = 8.6, 7.1 Hz, 2 H, 2 × H-3), 8.27 (dd, J = 8.6, 1.4 Hz, 2 H, 2 × H-4), 8.87 (dd, J = 7.1, 1.4 Hz, 2 H, 2 × H-2), 10.99 (br t, J = 5.1 Hz, 2 H, 2 × CONH). HRMS (FAB^+) m/z calcd for $C_{37}H_{42}N_9O_2$ 644.3461 (MH^+); found, 644.3485.

Bis[3-(6,9-dimethylphenazine-1-carboxamido)propyl]-methylamine (6t). From similar reaction of 6,9-dimethylphenazine-1-carboxylic acid (**7t**) (53% yield): mp (CH_2Cl_2/n-hexane) 97−101 °C; ^{1}H NMR ($CDCl_3$) δ 2.02−2.08 (br m, 4 H, 2 × $CH_2CH_2CH_2$), 2.34 (s, 3 H, NCH_3), 2.60−2.68 [br m, 4 H, $CH_2N(CH_3)CH_2$], 2.68 (s, 6 H, 2 × $ArCH_3$), 2.78 (s, 6 H, 2 × $ArCH_3$), 3.70 (q, J = 6.6 Hz, 4 H, 2 × CH_2NH), 7.32−7.40 (m, 4 H, 2 × H-7 and 2 × H-8), 7.86 (dd, J = 8.6, 7.2 Hz, 2 H, 2 × H-3), 8.28 (dd, J = 8.7, 1.5 Hz, 2 H, 2 × H-4), 8.90 (dd, J = 7.1, 1.5 Hz, 2 H, 2 × H-2), 11.00 (br s, 2 H, 2 × CONH). HRMS (FAB^+) m/z calcd for $C_{37}H_{40}N_7O_2$ 614.3243 (MH^+); found, 614.3237. Anal. ($C_{37}H_{39}N_7O_2 \cdot 0.5H_2O$) C, H, N.

Bis[3-(6-chloro-9-methylphenazine-1-carboxamido)-propyl]methylamine (6u). From similar reaction of 6-chloro-9-methylphenazine-1-carboxylic acid (**7u**) (84% yield): mp (CH_2Cl_2/n-hexane) 200−202 °C; ^{1}H NMR ($CDCl_3$) δ 1.97 (quin, J = 7.2 Hz, 4 H, 2 × $CH_2CH_2CH_2$), 2.31 (s, 3 H, NCH_3), 2.59 [t, J = 7.1 Hz, 4 H, $CH_2N(CH_3)CH_2$], 2.76 (s, 6 H, 2 × CH_3), 3.69 (q, J = 6.7 Hz, 4 H, 2 × CH_2NH), 7.50 (dd, J = 7.6, 1.0 Hz, 2 H, 2 × H-8), 7.78 (d, J = 7.5 Hz, 2 H, 2 × H-7), 7.93 (dd, J = 8.7, 7.2 Hz, 2 H, 2 × H-3), 8.41 (dd, J = 8.7, 1.5 Hz, 2 H, 2 × H-2), 8.94 (dd, J = 7.1, 1.5 Hz, 2 H, 2 × H-4), 10.72 (br s, 2 H, 2 × CONH). HRMS (FAB^+) m/z calcd for $C_{35}H_{34}Cl_2N_7O_2$ 654.2151 (MH^+); found, 654.2159. Anal. ($C_{35}H_{33}Cl_2N_7O_2 \cdot 0.5H_2O$) C, H, N.

Bis[3-(6,7-benzophenazine-1-carboxamido)propyl]methylamine (6v). From similar reaction of 6,7-benzophenazine-1-carboxylic acid[13] (**7v**) (66% yield): mp (CH_2Cl_2/hexane) 138−142 °C; ^{1}H NMR ($CDCl_3$) δ 2.08 (quin, J = 6.8 Hz, 4 H, 2 × $CH_2CH_2CH_2$), 2.41 (s, 3 H, NCH_3), 2.84 [t, J = 7.3 Hz, 4 H, $CH_2N(CH_3)CH_2$], 3.78 (q, J = 6.1 Hz, 4 H, 2 × CH_2NH), 7.48 (d, J = 9.2 Hz, 2 H, ArH), 7.59−7.76 (m, 10 H, ArH), 8.01 (dd, J = 8.4, 1.5 Hz, 2 H, ArH), 8.73 (dd, J = 7.3, 1.5 Hz, 2 H, ArH), 9.02 (d, J = 7.7 Hz, 2 H, ArH), 10.84 (br t, J = 5.0 Hz, 2 H, 2 × CONH). Anal. ($C_{41}H_{35}N_7O_2$) C, H, N.

Bis[3-(8,9-benzophenazine-1-carboxamido)propyl]methylamine (6w). From similar reaction of 8,9-benzophenazine-1-carboxylic acid[13] (**7w**) (77% yield): mp (CH_2Cl_2/hexane) 214−215.5 °C; ^{1}H NMR ($CDCl_3$) δ 2.10 (quin, J = 7.1 Hz, 4 H, 2 × $CH_2CH_2CH_2$), 2.43 (s, 3 H, NCH_3), 2.80 [t, J = 7.4 Hz, 4 H, $CH_2N(CH_3)CH_2$], 3.70 (q, J = 6.3 Hz, 4 H, 2 × CH_2NH), 7.18−7.31 (m, 2 H, ArH), 7.36 (t, J = 7.2 Hz, 2 H, ArH), 7.48−7.57 (m, 6 H, ArH), 7.92 (dd, J = 8.5, 7.2 Hz, 2 H, ArH), 8.28 (dd, J = 8.5, 1.5 Hz, 2 H, ArH), 8.61 (d, J = 8.0 Hz, 2 H, ArH), 8.87 (dd, J = 7.3, 1.5 Hz, 2 H, ArH), 10.52 (br s, 2 H, 2 × CONH). Anal. ($C_{41}H_{35}N_7O_2 \cdot 0.5H_2O$) C, H, N.

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.[17,20] 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)[23] or the sulforhodamine assay.[24] Independent assays were performed in duplicate, and coefficients of variation were 18% (P388), 22% (LL), 16% (JLC), 16% (JL_A), and 16%(JL_D).

Topoisomerase Assays. Topo I assays were carried out[25] 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 pBR322 supercoiled plasmid DNA, together with calf thymus topo I (5 units for relaxation assays and 15 units for cleavable complex formation assays). Topo II assays were carried out in a reaction (20 μL) in relaxation and cleavage buffers supplied by the manufacturer (TopoGEN, Inc), pRYG supercoiled plasmid DNA (0.125 μg), and purified human topo II (10 units for cleavage assays and 4 units for relaxation assays). Reactions were assembled on ice with drug and topoisomerase added last, incubated at 37 °C for 30 min, and terminated by adding pre-warmed 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 Vivo Colon 38 Tumor Assay. Colon 38 tumors were grown subcutaneously from 1 mm^3 fragments implanted in one flank of mice (anesthetized with pentobarbitone 90 mg/kg). When tumors reached a diameter of approximately 4 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 in a volume of 0.01 mL/g body weight in two equal injections administered 1 h apart. The mice were monitored closely, and tumor diameters were measured with callipers three times a week. Tumor volumes were calculated as 0.52 × a^2 × 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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