that of the *S* isomer 11. Anal. ($C_{15}H_{14}FNO_3$) C, H, N.

5-Bromo-6-fluoroquinoline (14). To a solution of 13[7] (40.0 g, 0.27 mol) in 190 mL of dichloroethane was added $AlCl_3$ (66.5 g, 0.50 mol) in small portions with stirring. The temperature of the mixture rose spontneously to approximately 55 °C and a tan precipitate formed. Bromine (78.2 g, 0.49 mol) in 20 mL of dichloroethane was added dropwise to the stirred mixture over a period of 2 h at 45–50 °C. The dark red solution was then refluxed for 3 h and poured over ice and the ice mixture made strongly basic with 50% NaOH. The mixture was extracted with 2 × 250 mL of CH_2Cl_2, and the combined extracts were washed with water. The organic phase was washed with dilute aqueous HCl and the acidic extract made basic with concentrated NH_4OH. A solid precipitated, which was filtered, washed with water, and dried to give 16.0 g (26.1%) of crude product. The crude product was recrystallized from hexane to give colorless crystals, mp 102–104 °C. The ^{13}C and proton NMR data are available as supplementary material. Anal. (C_9H_5BrFN) C, H, N.

6-Fluoro-5-methylquinoline (15). A solution of 14 (5.6 g, 0.025 mol) in THF/ether (250 mL, 1:1) in a three-neck round-bottom flask equipped with a rubber septum, thermometer, and mechanical stirrer was cooled to −78 °C in a dry ice bath. *n*-Butyllithium (22.5 mL of 2.2 M hexane solution, 0.05 mol) was added dropwise to the stirred solution at −78 °C. When addition was complete, the solution was stirred for 15 min and CH_3I (7.2 g, 0.05 mol) was added to the reaction mixture. After the mixture was stirred for 15 min, EtOH was added cautiously followed by a saturated solution of NH_4Cl. The mixture was warmed to room temperature and the organic phase evaporated to dryness in vacuo. The residue was dissolved in dilute HCl, the solution washed with CH_2Cl_2, and the aqueous phase made basic with 50% NaOH. The crude product was flash chromatographed on silica gel (Merck, grade 60, 230–400 mesh, 60 A) with 5% EtOAc/CH_2Cl_2 to give 1.1 g of product as a clear oil. This product was taken on without further purification.

6,7-Dihydro-9-fluoro-8-methyl-1-oxo-1*H*,5*H*-benzo[*ij*]-quinolizine-2-carboxylic Acid (17). Compound 15 (1.0 g, 6.2 mmol) was hydrogenated on a Parr apparatus in glacial HOAc eith 5% Pt/C as catalyst. The catalyst was filtered from the mixture and the filtrate evaporated in vacuo. The residue was mixed with H_2O and made strongly basic with 50% NaOH and the intermediate tetrahydroquinoline 16 was extracted into petroleum ether (35–60 °C). The petroleum ether was dried over $MgSO_4$ and evaporated in vacuo to give 1.0 g of crude 16. Crude 16 was converted to 17 in 37.5% yield by the same method described for 11 and 12. Several recrystallizations from DMF gave analytically pure product as colorless needles: mp 292–296 °C; NMR ($CDCl_3$) δ 8.70 (s, 1 H, C3-H), 8.00 (d, *J* = 9.3 Hz, 1 H, C10-H), 4.27 (t, *J* = 5.7 Hz, 2 H, C5-H), 3.03 (m, 2 H, C6-H), 2.38 (d, *J* = 1.5 Hz, 3 H, C8-CH_3), 2.36 (m, 2 H, C7-H). Anal. ($C_{14}H_{12}FNO_3$) C, H, N.

***N*-Tosyl-(*S*)-proline Methyl Ester (6a).** The methyl ester of *N*-tosyl-(*S*)-proline was prepared from the acid chloride[7] and methanol. Recrystallization of the product from ether/petroleum ether (35–60 °C) gave colorless crystals: mp 73–74.5 °C; $[\alpha]_D$ −98.1° (c 0.1449 g/10 mL, dichloroethane); NMR ($CDCl_3$) δ 7.74 (d, *J* = 0.1 Hz, 2 H, Tos Ar H), 7.3 (d, *J* = 8.1 Hz, 2 H, Tos Ar H), 4.3 (m, 1 H, C2-H), 3.7 (s, 3 H, OCH_3), 3.3 (m, 1 H C5-H), 2.42 (s, 3 H, Tos CH_3), 1.98 (m, 3 H, C3-H, C4-H_2), 1.75 (m, 1 H, C3-H). Anal. ($C_{13}H_{17}FNO_3$) C, H, N.

Microbiology. Solutions of tricyclic quinolone antibacterials were prepared in dimethyl sulfoxide at a concentration of 5 mg/mL. Serial 2-fold dilutions in nutrient broth were than carried out for 11 tubes of 0.5 mL final volume. The contents were further diluted to 50.0 mL with Mueller–Hinton (M–H) agar (55 °C), mixed by repeated inversion, and poured into square 100 × 15 mm dishes to harden, M–H agar was supplemented with 5% defibrinated rabbit blood to promote growth of hemophilic bacteria. The drug dilution series contained the following concentrations: 12.5, 6.2, 3.1, 1.6, 0.8, 0.4, 0.2, 0.1, 0.05, 0.025, 0.012, 0.006 μg/mL. Inocula were prepared by harvesting the growth from an overnight culture (except *Bordetella pertussis*) in sterile saline and resuspending turbidimetrically to McFarland standard 6 (2 × 10^6/mL). A uniform inoculum was deposited on the surface of prepared plates with the Steers replicator. The test conditions of incubation were 37 °C for 30 h (except *B. pertussis*). The lowest drug concentration preventing visible growth (MIC) was recorded.

Acknowledgment. We thank Thomas S. Robison of the Riker Chemical and Analytical Development group for ^{13}C and ^1H NMR verification of the structure of 5-bromo-6-fluoroquinoline.

Supplementary Material Available: Tables listing fractional coordinates, anisotropic thermal parameters, hydrogen atom coordinates, bond lengths, and bond angles (7 pages); table of structure factors for diastereomer 8 (15 pages). Ordering information is given on any current masthead page.

Potential Antitumor Agents. 51. Synthesis and Antitumor Activity of Substituted Phenazine-1-carboxamides

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Received September 3, 1986

In a further investigation of electron-deficient DNA-intercalating ligands as antitumor drugs, a series of substituted *N*-[2-(dimethylamino)ethyl]phenazine-1-carboxamides have been synthesized and evaluated. Fluorine-directed ring closure of *N*-phenyl-3-nitroanthranilic acids provided a new, unequivocal synthesis of several of the required phenazine-1-carboxylic acids, and the corresponding carboxamides were prepared and evaluated against L1210 leukemia in vitro and against P388 leukemia and Lewis lung carcinoma in vivo. Substitution on the phenazine ring was broadly tolerated, and the cytotoxicity of the resulting compounds correlated positively with the electron-withdrawing power of the substituent group. The positional effects of substituents were even more evident, with 9-substituted compounds being the most active. One derivative, *N*-[2-(dimethylamino)ethyl]-9-methoxyphenazine-1-carboxamide, had activity against Lewis lung carcinoma in mice equal to that of the best DNA-intercalating agents yet described, being capable of effecting a high-proportion cure of the advanced disease.

We have recently described[1] the synthesis and evaluation of a series of acridine-4-carboxamides (1), a new class of DNA-intercalating antitumor drugs with broad-spectrum in vivo activity against both leukemia and solid-tumor models. In this series, biological activity depends critically not only on intercalation of the acridine chromophore but also on positioning of the carboxamide side chain peri to the acridine nitrogen. Thus the acridine-1-carboxamide (3) and the anthracene-1-carboxamide (4) derivatives, which lack the nitrogen in this position, are inactive although both intercalate DNA efficiently.[2] A

(1) Atwell, G. J.; Rewcastle, G. W.; Baguley, B. C.; Denny, W. A. *J. Med. Chem.*, in press.

(2) Unpublished work, this laboratory.

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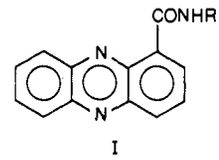
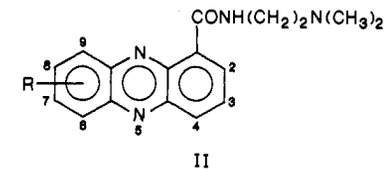
Table I. Physicochemical and Biological Properties of Phenazine Derivatives



I

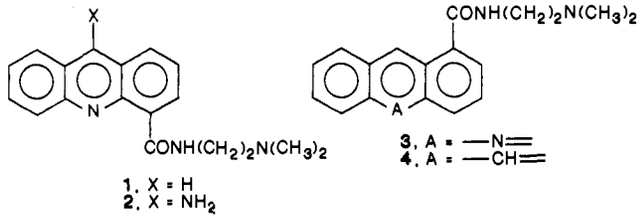


II

no.	formula	R	R_m[a]	log K[b] AT	log K[b] GC	L1210[c] IC_{50}	P388 OD[d]	P388 ILS_{max}[e]	LL OD[d]	LL ILS_{max}[e]
5	I	$(CH_2)_2N(CH_3)_2$	−0.29	6.58	6.86	1400	150	88	150	57
6	I	$(CH_2)_3N(CH_3)_2$	−0.21	6.29	6.14	1500	150	NA[f]	225	NA
7	I	$(CH_2)_4N(CH_3)_2$	−0.11	6.30	6.34	1170	150	49	225	NA
8	I	$(CH_2)_2N(Et)_2$	0.09	6.40	6.26	2100	225	43	150	NA
9	I	$(CH_2)_2NH(CH_2)_2OH$	−0.32	6.32	6.50	2000	65	NA	65	NA
10	I	$(CH_2)_2NM$[g]	−0.21	6.42	6.49	11000	225	NA	225	NA
11	II	2-OCH_3	−0.50	6.65	6.70	1620	225	33	225	43
12	II	2-Cl	−0.27	6.24	6.22	14900	100	NA	NT[h]	NT
13	II	3-CH_3	−0.15	6.48	6.55	410	225	91 (2)[i]	225	158 (5)
14	II	3-OCH_3	−0.25	6.96	7.00	830	100	22	150	NA
15	II	3-Cl	−0.06	7.08	7.22	160	150	106	150	63
16	II	4-CH_3	0.08	6.78	6.73	400	100	28	150	NA
17	II	4-OCH_3	−0.41	6.58	6.60	550	225	NA	NT	NT
18	II	6-CH_3	−0.01	6.68	6.74	820	150	58	150	NA
19	II	6-OCH_3	−0.44	6.63	6.47	5000	150	NA	NT	NT
20	II	6-Cl	−0.18	6.76	6.93	780	150	56	150	49
21	II	7-CH_3	−0.18	6.80	6.82	1500	150	29	100	NA
22	II	7-OCH_3	−0.27	6.40	6.30	4100	100	NA	150	NA
23	II	7-Cl	−0.13	6.50	7.57	1140	65	20	65	NA
24	II	8-CH_3	−0.20	6.43	6.82	630	150	60	225	81 (1)
25	II	8-OCH_3	−0.33	6.48	6.96	1050	100	39	150	NA
26	II	8-Cl	−0.20	6.87	7.48	670	100	64	100	64 (2)
27	II	9-CH_3	−0.23	6.76	6.93	42	65	45	65	62 (1)
28	II	9-OCH_3	−0.42	6.59	7.00	48	100	106	100	128 (4)
29	II	9-Cl	−0.37	7.44	8.12	11	30	61	45	124 2)
30	II	3,4-benz	0.11	7.63	7.29	170	65	84	65	NA
31	II	6,7-benz	−0.32	7.75	7.63	310	45	29	45	NA
32	II	8,9-benz	−0.05	7.12	6.70	33	30	98	45	56
33	II	5-oxide	−0.58			3000	150	81	150	51

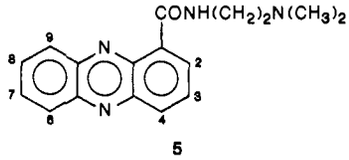
[a] R_m: chromatographic measure of drug lipophilicity, determined as detailed in ref 24. [b] log K: logarithm of association constant for binding to poly·[d(AT)] and poly·[d(AC)], determined by ethidium displacement; quenching correction was unnecessary. See ref 1 and 25. [c] IC_{50}: the nanomolar concentration of drug needed to reduce the growth of L1210 cells in culture to 50% of control values after 70 h. [d] OD: optimal dose in mg/kg per day, administered intraperitoneally as a solution in 0.1 mL of 30% v/v EtOH/water on days 1, 5, and 9 after intraperitoneal inoculation of 10^6 P388 cells or on days 5, 9, and 13 after intravenous inoculation of 10^6 LL cells. [e] ILS_{max}: the average increase in life span of treated animals over control groups of tumor-bearing untreated animals when drug is given at the optimal dose. Values of ILS greater than 20% (P388) or greater than 40% (LL) are considered statistically significant. [f] NA: no significant activity at doses up to acutely toxic ones. [g] M: morpholino. [h] NT: compound not tested. [i] Numbers in parentheses are the average number of animals (out of a group of six) that survived for 50 days (P388) or 60 days (LL).

further requirement for activity against remotely sited solid tumors appears to be a noncharged chromophore. This is thought to be related to drug distribution properties; thus the strongly basic 9-aminoacridine-4-carboxamide derivative (**2**) is active in vivo only against leukemia models,[3] in spite of very potent in vitro cytotoxicity (IC_{50} of 35 nM against L1210 leukemia).



1, X = H
2, X = NH_2

3, A = —N=
4, A = —CH=



5

Another chromophore that fulfills these fundamental physicochemical requirements is the phenazine nucleus, and *N*-[2-(dimethylamino)ethyl]phenazine-1-carboxamide (**5**) has moderate in vivo activity against both the P388 leukemia and the Lewis lung (LL) carcinoma. In this paper we extend our studies on tricyclic carboxamides with solid-tumor activity by reporting the synthesis, biological activity, and structure–activity (SAR) relationships for phenazine-monosubstituted derivatives of **5**. Following our previous strategy,[1,4] we have prepared an essentially complete set of derivatives of *N*-[2-(dimethylamino)ethyl]-phenazine-1-carboxamide (**5**) bearing CH_3, OCH_3, and Cl groups at every available phenazine position (compounds **11–29** of Table I), to enable a comparison of the SAR of these compounds with similarly substituted acridine-4-carboxamides[3] and 9-aminoacridine-4-carboxamides[4] to be carried out.

Chemistry

The amides of Table I were mostly prepared by coupling the appropriate phenazine-1-carboxylic acids and amines

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Scheme I

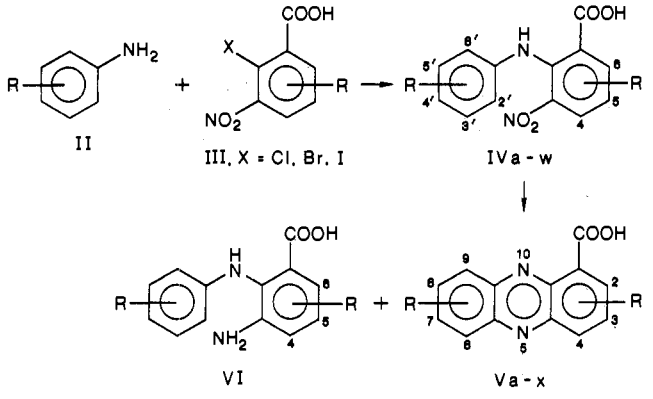

Table II. Analytical Data for the New Compounds of Table I

no.	mp, °C	formula	analyses
5	232–237	$C_{17}H_{18}N_4O\cdot HCl$	C, H, N, Cl
6	226–228	$C_{18}H_{20}N_4O\cdot HCl$	C, H, N, Cl
7	186–191	$C_{19}H_{22}N_4O\cdot HCl$	C, H, N, Cl
8	236–238	$C_{19}H_{22}N_4O\cdot HCl$	C, H, N, Cl
9	203–205	$C_{17}H_{18}N_4O_2\cdot HCl$	C, H, N, Cl
10	250–252	$C_{19}H_{20}N_4O_2\cdot HCl$	C, H, N, Cl
11	207–209	$C_{18}H_{20}N_4O_2\cdot HCl\cdot 0.25H_2O$	C, H, N, Cl
12	236–239	$C_{17}H_{17}ClN_4O\cdot HCl\cdot 0.5H_2O$	C, H, N, Cl
13	236–238	$C_{18}H_{20}N_4O\cdot HCl$	C, H, N, Cl
14	242–245 dec	$C_{18}H_{20}N_4O_2\cdot HCl$	C, H, N, Cl
15	250–253 dec	$C_{17}H_{17}ClN_4O\cdot HCl$	C, H, N, Cl
16	226 dec	$C_{18}H_{20}N_4O\cdot HCl$	C, H, N, Cl
17	238–240	$C_{18}H_{20}N_4O_2\cdot HCl$	C, H, N, Cl
18	246–248	$C_{18}H_{20}N_4O\cdot HCl$	C, H, N, Cl
19	246–248	$C_{18}H_{20}N_4O_2\cdot HCl$	C, H, N,[b] Cl
20	257–260 dec	$C_{17}H_{17}ClN_4O\cdot HCl$	C, H, N, Cl
21	225–227	$C_{18}H_{20}N_4O\cdot HCl$	C, H, N, Cl
22	224–228	$C_{18}H_{20}N_4O_2\cdot HCl$	C, H, N
23	223–225	$C_{17}H_{17}ClN_4O\cdot HCl$	C, H, N, Cl
24	251–253	$C_{18}H_{20}N_4O\cdot HCl$	C, H, N, Cl
25	229–231	$C_{18}H_{20}N_4O_2\cdot HCl\cdot 0.5H_2O$	C, H,[a] N
26	253–255	$C_{17}H_{17}ClN_4O\cdot HCl$	C, H, N, Cl
27	248–250	$C_{18}H_{20}N_4O\cdot HCl$	C, H, N, Cl
28	255 dec	$C_{18}H_{20}N_4O_2\cdot HCl$	C, H, N
29	246–249	$C_{17}H_{17}ClN_4O\cdot HCl$	C, H, N, Cl
30	254–256	$C_{21}H_{20}N_4O\cdot HCl$	C, H, N, Cl
31	238–240	$C_{21}H_{20}N_4O\cdot HCl$	C, H, N, Cl
32	227–230	$C_{21}H_{20}N_4O\cdot HCl$	C, H, N, Cl
33	215–217	$C_{17}H_{18}N_4O_2\cdot HCl$	C, H, N, Cl

[a] H out by 0.7. [b] N out by 0.5.

by using 1,1-carbonyldiimidazole;[3,5] this procedure gave higher yields than the alternative method via the acid chlorides. For the 2-substituted compounds, however, steric hindrance made formation of the imidazolides difficult, and these compounds were prepared via their acid chlorides. The main work involved synthesis of the desired substituted phenazine-1-carboxylic acids (V). The chemistry of phenazines has been stimulated by the fact they occur as natural products,[6,7] and several routes to phenazinecarboxylic acids have been developed.[8-10] The most widely used method[10] involves Jourdan–Ullmann copper-catalyzed condensation of suitably substituted anilines (II) with 3-nitro-2-halobenzoic acids (III) to give substituted *N*-phenyl-3-nitroanthranilic acids (IV) and the reductive cyclization of these compounds with $NaBH_4$ in alkali (Scheme I). Using the anhydrous conditions previously described by us[11] (butane-2,3-diol as the solvent and *N*-ethylmorpholine as the base) but at the lower temperature of 70–80 °C results in generally good to excellent yields in the Ullmann reaction (Table IV). Only when the aniline is severely sterically hindered (e.g., 2-chloro-6-fluoroaniline) are the yields of IV signficantly lowered (Table IV). The only exception to the use of these conditions was the reaction of 2,6-dichloro-3-nitrobenzoic acid, where copper-catalyzed reaction would result in the displacement of both halogens. This reaction was performed as previously reports,[12] without a copper catalyst at 135–140 °C in neat aniline (Table IV).

Table III. Comparative Cytotoxicities of Substituted Phenazine-1-carboxamides

position	OCH_3	CH_3	Cl
A. Effect of Nature of Substituent Groups: IC_{50} Ratios			
3	1.0[a]	0.5	0.4
4	1.0	0.7	
6	1.0	0.6	0.6
7	1.0	0.4	0.3
8	1.0	0.9	0.6
9	1.0	0.9	0.2
B. Effect of Position of Substituent Group: IC_{50} Ratios			
3	1.0[b]	1.0	1.0
4	0.6	1.0	
6	6.0	2.0	5.0
7	5.0	3.7	7.1
8	1.4	1.5	4.2
9	0.05	0.10	0.07

[a] Ratio of IC_{50} values, relative to OMe derivative (1.00). [b] Ratio of IC_{50} values, relative to 3-substituted derivative (1.00).

All of the aniline derivatives were commerically available with the exception of 2,6-dimethoxyaniline, which was prepared from 6-nitroresorcinol by methylation (dimethyl sulfate) and hydrogenation (Pd/C), 2-chloro-6-fluoroaniline, which was prepared from 2-chloro-6-fluorobenzoic acid as published,[13] and 5-chloro-2-fluoroaniline, which was obtained from 5-amino-2-fluoroacetanilide[14] by sequential diazotization, reaction with CuCl, and hydrolysis.[14]

The 2,5- and 2,6-dichloro-3-nitrobenzoic acids for the preparation of 3-chloro- and 2-chlorophenazine-1-carboxylic acids, respectively, were obtained by nitration (concentrated HNO_3 in H_2SO_4) of the analogous dichlorobenzoic acids.[12] 2-Iodo-5-methyl-3-nitrobenzoic acid for the preparation of 3-methylphenazine-1-carboxylic acid was obtained from 5-methylanthranilic acid by sequential acetylation, nitration, and hydrolysis by the procedure of Cassebaum,[15] followed by diazotization and treatment with KI.[16]

Reductive cyclization of these *N*-phenyl-3-nitroanthranilic acids IV with $NaBH_4$ in sodium ethoxide has been shown[10] to give phenazine-1-carboxylic acids (V), and this method was used directly to obtain the 3-, 7-, and 9-methyl derivatives and the 6,7- and 8,9-benzo derivatives of V in good yields (Scheme I and Table V). Since halogens on phenazines are potentially susceptible to replacement by alkoxide,[6] preparation of the 2-, 3-, and 7-chloro derivatives of V was carried out by cyclization of

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Table IV. Physical Data for Substituted *N*-(Phenyl)-3-nitroanthranilic Acids (IV)[a]

compd	R	yield, %	mp, °C	lit. mp, °C	formula	analyses
IVa	H	78	196–198	195–196[b]		
IVb	4-OCH_3	50[c]	223–225		$C_{14}H_{12}N_2O_5$	C, H, N
IVc	5-CH_3	89[c]	177–179	174[d]		
IVd	5-Cl	59[e]	173–176		$C_{13}H_9ClN_2O_4$	C, H, N, Cl
IVe	6-Cl	52[e,f]	206–208	206[g]		
IVf	2′-CH_3	67	196–199		$C_{14}H_{12}N_2O_4$	C, H, N
IVg	2′-OCH_3	79	227–228	227[h]		
IVh	2′-F	67	195–197		$C_{13}H_9FN_2O_4$	C, H, N, F
IVi	2′-Cl	58	219–221	214–216[h]		
IVj	3′-CH_3	58	184–185		$C_{14}H_{12}N_2O_4$	C, H, N
IVk	3′-Cl	40	180–182	176–178[h]		
IVl	4′-CH_3	63	225–227		$C_{14}H_{12}N_2O_4$	C, H, N
IVm	4′-OCH_3	58	182–185	174–176[h]		
IVn	4′-Cl	60	200–203	170–172[h]	$C_{13}H_9ClN_2O_4$	C, H, N, Cl
IVo	2′,3′-F_2	57	171–173		$C_{13}H_8F_2N_2O_4$	C, H, N, F
IVp	2′,3′-benz[i]	35	210–212		$C_{17}H_{12}N_2O_4$	C, H, N
IVq	2′-F, 5′-CH_3	64	189–190		$C_{14}H_{11}FN_2O_4$	C, H, N, F
IVr	2′,5′-F_2	62	185–188		$C_{13}H_8F_2N_2O_4$	C, H, N, F
IVs	2′-F, 5′-Cl	31	199–201		$C_{13}H_8ClFN_2O_4$	C, H, N
IVt	2′,6′-OCH_3	42	176–178		$C_{15}H_{14}N_2O_6$	C, H, N
IVu	2′,6′-F_2	58	220–222		$C_{13}H_8F_2N_2O_4$	C, H, N, F
IVv	2′-Cl, 6′-F	12	206–208		$C_{13}H_8ClFN_2O_4$	C, H, N, Cl
IVw	3′,4′-benz[j]	62	191–193	194[k]		

[a] Reaction generally performed between anilines (II) and 2-bromo-3-nitrobenzoic acid (III) under anhydrous Ullmann conditions at 70–80 °C (see Experimental Section). [b] Reference 29. [c] Substituted 2-iodo-3-nitrobenzoic acid and aniline used. [d] Reference 36. [e] Substituted 2-chloro-3-nitrobenzoic acid and aniline used. [f] Reaction performed without copper catalyst in excess aniline at 135–140 °C. [g] Reference 12. [h] Reference 37. [i] Systematic name is *N*-1-naphthyl-3-nitroanthranilic acid. [j] Systematic name is *N*-2-naphthyl-3-nitroanthranilic acid. [k] Reference 38.

Table V. Physical Data for Substituted Phenazine-1-carboxylic Acids V

no.	R	sm[a]	method[b]	yield, %	mp, °C	lit. mp, °C	formula	analyses
Va	H	IVa	A	82	240–241	239–240[c]		
Vb	2-OCH_3		B	89	206–208	204–205[d]		
Vc	2-Cl	IVe	C	59	247–249		$C_{13}H_7ClN_2O_2$	C, H, N, Cl
Vd	3-CH_3	IVc	A	48	212–213		$C_{14}H_{10}N_2O_2$	C, H, N
Ve	3-OCH_3		B	88	242–243		$C_{14}H_{10}N_2O_3$	C, H, N
Vf	3-Cl	IVd	C	87	255–257		$C_{13}H_7ClN_2O_2$	C, H, N, Cl
Vg	4-CH_3		D	28	239–241		$C_{14}H_{10}N_2O_2$	C, H, N
Vh	4-OCH_3		D	42	250–251		$C_{14}H_{10}N_2O_3$	C, H, N
Vi	6-CH_3	IVj	A,E	44	216–217		$C_{14}H_{10}N_2O_2$	C, H, N
Vj	6-OCH_3	IVo	F	84	291–293	292–293[e]		
Vk	6-Cl	IVk	C,G	40	310 dec		$C_{13}H_7ClN_2O_2$	C, H, N, Cl
Vl	7-CH_3	IVl	A	73	260 dec		$C_{14}H_{10}N_2O_2$	C, H, N
Vm	7-OCH_3	IVm	F	70	266–268	256–258[c]		
Vn	7-Cl	IVn	C	65	274 dec		$C_{13}H_7ClN_2O_2$	C, H, N, Cl
Vo	8-CH_3	IVq	A	81	238 dec		$C_{14}H_{10}N_2O_2$	C, H, N
Vp	8-OCH_3	IVr	F	79	289–290	292–293[e]		
Vq	8-Cl	IVs	C	64	286–289		$C_{13}H_7ClN_2O_2$	C, H, N, Cl
Vr	9-CH_3	IVf	A	56	237–238	235[f]		
Vs	9-OCH_3	IVu	F	77	262–265	262–263[e]		
Vt	9-Cl	IVv	C	66	272–273		$C_{13}H_7ClN_2O_2$	C, H, N, Cl
Vu	3,4-benzo[g]		H	94	272–274	275–278[h]		
Vv	6,7-benzo[i]	IVw	A	56[j]	255–256	255[k]		
Vw	8,9-benzo[i]	IVp	A	61	243–246	256[m]		
Vx	5-oxide		I	38	222–223	223[n]		

[a] Compounds IVa–w identified in Table IV. [b] Methods: (A) reduction of substituted *N*-(phenyl)-3-nitroanthranilic acids IV with $NABH_4$ in EtOH containing 2 M NaOEt, (B) NaOMe on chlorophenazine acid in MeOH, (C) reduction of IV with $NaBH_4$ in 2 M NaOH solution, (D) dehydrogenation of 6,7,8,9-tetrahydrophenazine-1-carboxylic acid, (E) separation from the 8-isomer by chromatography of methyl esters, (F) reduction of IV with $NaBH_4$ in MeOH containing 2 M NaOMe, (G) separation from the 8-isomer by fractional crystallization in DMF, (H) reduction of di-*N*-oxide with sodium dithionite (ref 9), (I) oxidation of phenazine-1-carboxylic acid with H_2O_2 in AcOH (ref 38). [c] Reference 10. [d] Reference 30. [e] Reference 19. [f] Reference 39. [g] Systematic name: benzo[*a*]phenazine-6-carboxylic acid. [h] Reference 32. [i] Systematic name: benzo[*a*]phenazine-8-carboxylic acid. [j] Only one of two possible isomeric products obtained. [k] Reference 38. [l] Systematic name: benzo[*a*]phenazine-11-carboxylic acid. [m] Reference 39. [n] Reference 40.

the corresponding *N*-phenyl-3-nitroanthranilic acids IV in aqueous solution (NaOH). Alkoxide exchange can also occur with this reduction system, so the 7-OCH_3 derivative was obtained from the corresponding *N*-(4-methoxyphenyl)-3-nitroanthranilic acid by cyclization in $NaBH_4$/sodium methoxide. This susceptibility to substituent displacement was then turned to account to prepare the 2- and 3-methoxyphenazine-1-carboxylic acids from the corresponding chloro compounds by reaction with MeOH/KOH at 100 °C under pressure for 2 days.

A limitation of this synthetic route is that reduction of 3′-substituted *N*-phenyl-3-nitroanthranilic acids IV gives rise to mixtures of both 6- and 8-substituted phenazine-1-carboxylic acids V, with the 6-isomer usually predominating.[10,17–19] Thus 6-chlorophenazine-1-carboxylic acid

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was separated by fractional crystallization from the mixture obtained from *N*-(3-chlorophenyl)-3-nitroanthranilic acid, and the 6-methylphenazine-1-carboxylic acid was obtained from a similar mixture of isomers by chromatography of the phenazine methyl esters,[17] but the corresponding 8-isomers could not be prepared this way.

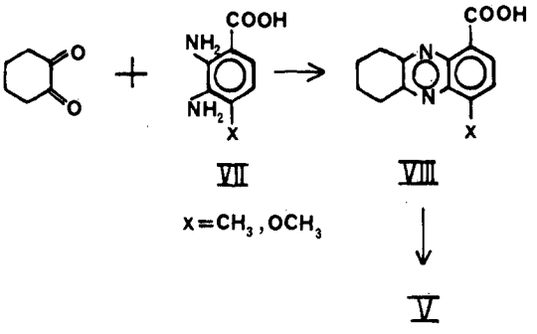
Another problem is that *N*-phenyl-3-nitroanthranilic acids IV substituted with displaceable groups (e.g., Cl, OCH_3) in the ortho (2′) position often[19,20] yield mixtures of the desired 9-substituted phenazine-1-carboxylic acids V together with the unsubstituted acid resulting from displacement of the substituent group. For the specific case of 9-methoxyphenazine-1-carboxylic acid, the use of *N*-(2,6-dimethoxyphenyl)-3-nitroanthranilic acid (IVt), which ring closed by displacement of one of the methoxy groups, gave a good yield of the desired compound. However, this modification has only limited utility for the production of 9-substituted compounds, since steric hindrance of the amine group leads to low yields in the Ullmann reaction (42% for 2,6-dimethoxyaniline but only 12% for 2-chloro-6-fluoroaniline and no reaction in the case of 2,6-dichloroaniline).

A more general solution to both the above problems was based on the observation[21] that *N*-(2-fluorophenyl)-3-nitroanthranilic acid (IVh) undergoes reductive ring closure exclusively on to the fluoro-substituted carbon atom, yielding phenazine-1-carboxylic acid in similar yield to the cyclization of unsubstituted *N*-phenyl-3-nitroanthranilic acid, with no detectable 9-fluorophenazine-1-carboxylic acid formed. Thus ring closure of *N*-(2,6-difluorophenyl)-3-nitroanthranilic acid in sodium methoxide gave the 9-methoxyphenazine-1-carboxylic acid in 77% yield, by displacement of one fluoro atom on ring closure and subsequent reaction of the 9-fluorophenazine-1-carboxylic acid formed with excess sodium methoxide. 9-Chlorophenazine-1-carboxylic acid was similarly obtained from *N*-(2-chloro-6-fluorophenyl)-3-nitroanthranilic acid when the reduction was carried out in 2 N NaOH, with the ring closure proceeding exclusively by fluorine displacement to give the desired compound as the only isolable product in 66% yield.

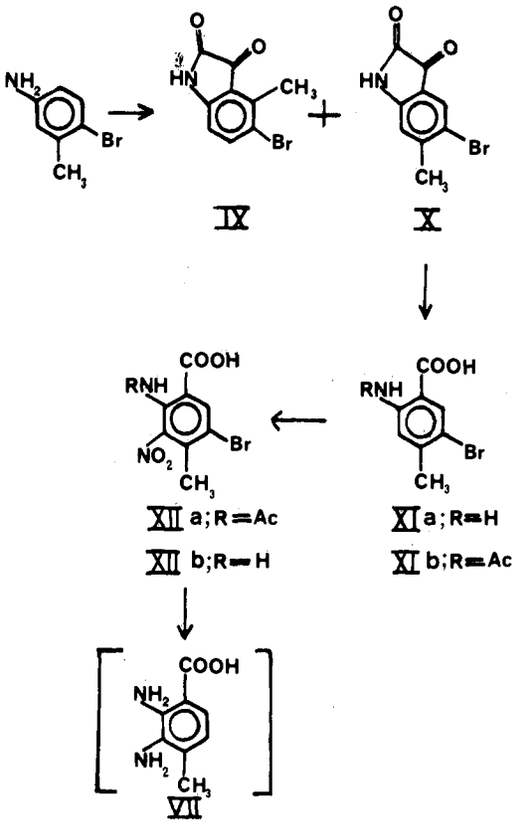
This method was also used for the unequivocal synthesis of the 8-substituted phenazine-1-carboxylic acids that are not readily obtainable by the conventional route. Ring closure of *N*-(2-fluoro-5-methylphenyl)-3-nitroanthranilic acid gave 8-methylphenazine-1-carboxylic acid in 81% yield, and the 8-chloro derivative was similarly obtained. Ring closure of the isomeric *N*-(2,3-difluorophenyl)- and *N*-(2,5-difluorophenyl)-3-nitroanthranilic acids in $NaBH_4$/sodium methoxide gave the corresponding 6- and 8-methoxyphenazine-1-carboxylic acids in over 80% yield via the intermediate (unisolated) fluorophenazines.

In most of the cyclization reactions to give V from IV, it was possible to isolate varying amounts of the unsubstituted *N*-phenyl-3-aminoanthranilic acid (VI), the product of direct reduction of the nitro group without ring formation. In the case of *N*-phenyl-3-nitro-4-methoxyanthranilic acid (IV, R = 4-OCH_3), this was the only product isolated, necessitating the use of a different method[8] for the synthesis of the 4-substituted phenazine-1-carboxylic acids (Scheme II). Condensation of the appropriate 4-substituted 2,3-diaminobenzoic acids VII with cyclohexane-1,2-dione gave good yields of the tetrahydrophenazine-1-carboxylic acids (VIII), which were dehydrogenated with Pd/C in dichlorobenzene to give the required compounds.

Scheme II



Scheme III



2,3-Diamino-4-methoxybenzoic acid for the preparation of 4-methoxyphenazine-1-carboxylic acid (Scheme II) was obtained from 3-methoxy-2-nitroaniline via the methoxynitroisatin and the resulting 4-methoxy-3-nitroanthranilic acid in moderate overall yield. Due to persistently lower yields in the formation of analogous methylnitroisatins, 2,3-diamino-4-methylbenzoic acid for the synthesis of 4-methylphenazine-1-carboxylic acid was prepared by a different route (Scheme III) from 4-bromo-3-methylaniline. In this case the isatin reaction gave two isomers, which could be readily separated by pH control,[22] the desired compound (X) being obtained in 33% yield. Oxidation gave 5-bromo-3-methylanthranilic acid (XIa) (identical with the product obtained by bromination of 4-methylanthranilic acid in AcOH), which was acetylated and nitrated to give XIIa, with the bromine blocking group directing nitration to the desired position. Hydrolysis and

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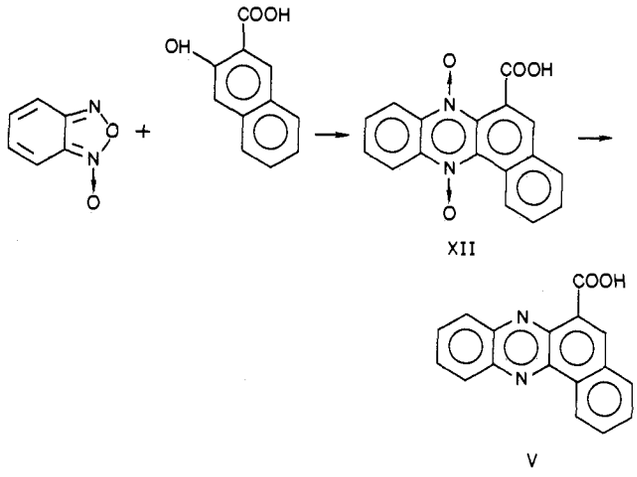
Scheme IV



exhaustive hydrogenation then gave the desired 2,3-diamino-4-methylbenzoic acid (VII, X = CH_3).

The 3,4-benzo derivative (systematically benzo[*a*]-phenazine-6-carboxylic acid) was prepared by condensation of benzofuroxan and 3-hydroxy-2-naphthoic acid by using published procedures,[9] followed by dithionite reduction of the resulting dioxide XII (Scheme IV).

Results and Discussion

Data for the parent *N*-[2-(dimethylamino)ethyl]-phenazine-1-carboxamide (**5**), five side-chain variants (**6–10**), and 19 of the possible 21 monosubstituted methyl, methoxy, and chloro compounds (**11–29**) are recorded in Tables I and II. These three substituent groups provide a reasonable variation of electronic and lipophilic properties and are of similar size; thus some idea of the steric effects can be gleaned from studies of the effect on activity of the position as opposed to the nature of the substituent groups.[4] The 3,4-benzo derivative **30** and its positional isomers **31** and **32** were also included in this study.

The pK_a of the parent compound **5** (for protonation of chromophore in aqueous solution) was determined as 0.84 by UV spectroscopy,[23] demonstrating that, for all of the phenazine compounds, the chromophore will remain uncharged under physiological conditions.

Drug lipophilicity was determined by the same method used for the acridine compounds,[1,24] namely, liquid–liquid chromatography in the presence of 0.3% methanesulfonic acid. Under these conditions the phenazine chromophore is not protonated, and the drugs run as monocations. For substitution at any particular position except C-9, the difference in lipophilicity between the Cl and OCH_3 derivatives is about 0.20 R_m unit, approximately equivalent to the 0.3 difference in π values for the substituents. However, as previously observed,[1] there are considerable positional differences in the lipophilic effects of a particular substituent, with the 2- and 6-substituted compounds being generally the most hydrophilic.

Binding of the compounds to the DNA homopolymers poly·[d(AT)] and poly·[d(GC)] was determined by ethidium displacement, using reported methods.[25] Compared with the equivalently substituted acridine-4-carboxamides[1] (the ring systems are numbered differently), the phenazine-1-carboxamides generally bind about twofold less strongly. As with the acridines, there is little selectivity for binding to either homopolymer. One trend not seen with the acridines is the tendency of the Cl-substituted compounds to bind more tightly. As expected, the three benzophenazine derivatives **30–32**, with larger aromatic chromophores, bind to DNA about 10-fold more strongly than compound **5**.

The phenazine-1-carboxamides are much less cytotoxic than the acridines. The parent compound **5** has an IC_{50} against L1210 cells of 1400 nM, compared to that of the parent acridine-4-carboxamide of 105 nM. Pairwise comparison of the substituted phenazines shows them to be less cytotoxic than the acridines by about 1 order of magnitude, although significant variation exists. The substituted compounds **11–29** showed a large range of cytotoxic potency (370-fold), even if the 2-substituted compounds are excluded. The very low potency of the 2-substituted derivatives is similar to that seen with the equivalent 3-substituted acridine-4-carboxamides[1] and is presumably due to steric interactions between these substituents and the side chain. However, the rest of the substituted phenazines show two clear patterns of cytotoxicity. For substituents at a particular position, the OCH_3 derivatives were the least potent, followed by the CH_3 compounds, with the Cl compounds being the most potent (Table IIIA), but the difference was only two- to three-fold. The largest differences in cytotoxic potency were interpositional, rather than between substituents at a particular position, as shown in Table IIIB. Compounds substituted at the 3- and 4-positions with a particular group were of similar potency, while the same group at the 6- and 8-positions led to a two- to fourfold loss of potency and was least effective at the 7-position, with a four- to eightfold decrease. However, all groups (OCH_3, CH_3, and Cl) at the 9-position provided a large increase in potency (10–20-fold).

Our attention was initially drawn to the phenazine-carboxamide series when the parent compound **5** was shown to have moderate in vivo activity against both the P388 leukemia and the LL carcinoma. Although **5** is much less cytotoxic in vitro than the parent acridine-4-carboxamide, their in vivo potencies are similar. Lengthening the side chain of **5** to give **6** and **7** results in loss of LL activity and a decrease in P388 activity. As shown before with the acridine-4-carboxamides,[1] use of the hydroxyethyl and morpholide side chains is dystherapeutic. Phenazine-4-carboxamides bearing a 2-substituent proved inactive, as was the case for the equivalent 3-substituted acridine-4-carboxamides. Substitution at every other phenazine position resulted in active compounds, in a broad SAR similar to that seen with the acridine-4-carboxamides.[1] However, in many cases this activity is only moderate, and no clear relationship with the nature of the substituent group can be seen. The only compound to show in vivo activity clearly superior to that of the parent **5** is the 3-CH_3 compound **13**, which provided a proportion of long-term survivors in both test systems, and the three 9-substituted derivatives **27–29**. Of the latter group, the 9-OCH_3 compound **28** is clearly the best and is also the most active compound so far discovered in the series. Although of comparable activity to the parent in the P388 leukemia, the compound is much more active than **5** against the remotely implanted LL carcinoma, consistently providing a high proportion of cures of this relatively resistant tumor.[26,27]

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Conclusions

The above structure–activity relationships appear to be midway between those seen previously for the acridine-4-carboxamides[1] (general tolerance of chromophore substitution) and those for the 9-aminoacridine-4-carboxamides[4] (only groups at the 5-position allowed). While groups at all phenazine positions are compatible with broad-spectrum activity, there is a clear preference for 9-substitution (equivalent to 5-substitution in the 9-aminoacridine-4-carboxamides). In addition to this positional effect, which has no obvious explanation in terms of DNA-binding interactions, the cytotoxicity of the phenazine-1-carboxamides is positively correlated with the electron-withdrawing effects of the substituent groups (Table IIIA).

In spite of relatively low DNA-binding strength and in vitro cytotoxicity relative to other DNA-intercalating antitumor drugs, the phenazine-1-carboxamides display broad-spectrum in vivo activity at acceptable dose levels. The most active compound in the series so far, the 9-methoxy derivative **28**, has activity against the Lewis lung carcinoma equal to that of any DNA-intercalating agent yet described. The broad structure–activity relationships described above provide clear guidelines for further development of the series, and this work is being pursued.

Experimental Section

Analyses indicated by element symbols were within ±0.4% of theoretical values. Analyses were carried out in the Microchemical Laboratory, University of Otago. Melting points were determined on an Electrothermal apparatus using the supplied stem-corrected thermometer and are as read.

Representative Jourdan–Ullmann Reaction: Preparation of *N*-Phenyl-3-nitroanthranilic Acid (IVa). A mixture of aniline (5.68 g, 61 mmol), 2-bromo-3-nitrobenzoic acid[28] (10 g, 41 mmol), CuCl (0.5 g), Cu powder (0.1 g), and *N*-ethylmorpholine (15 mL, 0.12 mol) in butane-2,3-diol (25 mL) was heated with stirring at 70 °C for 15 h, and the resulting solution was diluted with 0.1 M aqueous NH_4OH (200 mL) and filtered through Celite. The resulting bright orange solution was poured slowly into 2 N HCl to give *N*-phenyl-3-nitroanthranilic acid (IVa) (8.23 g, 78%), mp (benzene) 196–198 °C (lit.[29] mp 195–196 °C).

2-Chlorophenazine-1-carboxylic Acid (Vc). A solution of *N*-phenyl-6-chloro-3-nitrobenzoic acid[12] (IVe) (5.0 g, 17 mmol) and $NaBH_4$ (4.3 g, 0.1 mol) in 2 N NaOH (400 mL) was heated under reflux for 5 h. Cooling gave the Na salt of the phenazine acid, which was collected and washed with ice-cold 2 N NaOH. Acidification gave 2-chlorophenazine-1-carboxylic acid (Vc) (2.61 g, 59%), mp (MeOH) 247–249 °C. Anal. (Table V).

Similar procedures were used to prepare 3-chloro-, 7-chloro-, 8-chloro-, and 9-chlorophenazine-1-carboxylic acids by reductive cyclization of *N*-phenyl-5-chloro-, *N*-(4-chlorophenyl)-, *N*-(5-chlorophenyl)-, and *N*-(2-chloro-6-fluorophenyl)-3-nitroanthranilic acids, respectively (see Tables IV and V).

2-Methoxyphenazine-1-carboxylic Acid (Vb). A solution of 2-chlorophenazine-1-carboxylic acid (Vc) (2 g, 7.73 mmol) in MeOH (350 mL) containing KOH (39.2 g, 0.7 mol) was heated at 100 °C in a pressure vessel for 2 days. The solvent was then removed under vacuum, and the residue was dissolved in water, filtered, and acidified with 2 N HCl to give 2-methoxyphenazine-1-carboxylic acid (Vb) (1.74 g, 89%), mp 206–208 °C (lit.[30] mp 204–205 °C). 3-Methoxyphenazine-1-carboxylic acid was similarly prepared in 88% yield from 3-chlorophenazine-1-carboxylic acid. 2-Methoxyphenazine-1-carboxylic acid can also be prepared from 2-hydroxyphenazine-1-carboxylic acid, 5,10-dioxide[31] by sequential methylation (CH_2N_2), hydrolysis (NaOH), and reduction (sodium dithionite), but the former procedure was found to be superior.

3-Methylphenazine-1-carboxylic Acid (Vd). A solution of *N*-phenyl-5-methyl-3-nitroanilic acid (IVc; 12.0 g, 44 mmol) and $NaBH_4$ (6 g, 0.16 mol) in EtOH (500 mL) containing Na (23 g, 1 mol) was heated under reflux until no starting material remained (1 h). The mixture was diluted with water, and the EtOH was allowed to boil off. The solution was then filtered and slowly acidified with concentrated HCl to give a crude product, which was dried and dissolved in MeOH/Et_3N. The solution was treated with charcoal and filtered through Celite, and the filtrate was acidified with AcOH and boiled down until crystallization commenced. The product was collected and washed with cold MeOH to give 3-methylphenazine-1-carboxylic acid (Vd; 5.7 g, 48%), mp (MeOH) 212–213 °C. Anal. (Table V). Concentration of the mother liquors from the above crystallization gave *N*-phenyl-3-amino-5-methylanthranilic acid (VI, R = 5-CH_3), mp 203–205 °C, the product of direct reduction. Anal. ($C_{14}H_{14}N_2O_2$) C, H, N.

A similar procedure was used to prepare 7-methyl-, 8-methyl-, and 9-methylphenazine-1-carboxylic acids, as well as the parent phenazine-1-carboxylic acid and its 6,7- and 8,9-benzo drivatives (systematically benzo[*a*]phenazine-8-carboxylic acid (Vv) and benzo[*a*]phenazine-11-carboxylic acid (Vw), respectively; Table V).

4-Methoxyphenazine-1-carboxylic Acid (Vh). (i) Attempted Preparation by the Method of Scheme I. A suspension of 3-methoxy-2-nitroaniline (13.6 g, 81 mmol) (prepared from 3-methoxy-2-nitrobenzoic acid) in 500 mL of water containing chloral hydrate (26.8 g, 0.16 mol), hydroxylamine sulfate (26.5 g, 0.16 mol), and sodium sulfate (150 g, 1.06 mol) was heated at 60–80 °C with stirring for 8 h. After cooling, the precipitate was collected and extracted with 2 N NaOH for 2 h at 20 °C, and the deep red filtrate from this extraction was acidified with 2 N HCl to give 3-methoxy-2-nitroisonitrosoacetanilide (9.81 g, 51%), mp (aqueous MeOH) 147–149 °C. Anal. ($C_9H_9N_3O_5$) C, H, N.

The above compound (9.8 g, 41 mmol) was finely powdered and added slowly to stirred 80% H_2SO_4 at 55–60 °C. After 15 min the solution was cooled and poured onto ice to give 6-methoxy-7-nitroisatin (8.29 g, 91%), mp (MeOH) 235 °C dec. Anal. ($C_9H_9N_2O_5$) C, H, N.

A stirred suspension of the above isatin (17.8 g, 80 mmol) in 2 N NaOH (500 mL) was treated with 27% H_2O_2 (15 mL), and after 15 min the solution was acidified with AcOH to precipiate 4-methoxy-3-nitroanthranilic acid (15.2 g, 90%), mp (aqueous MeOH) 222–224 °C. Anal. ($C_8H_8N_2O_5$) C, H, N.

A solution of the above anthranilic acid (16.5 g, 77.8 mmol) in concentrated H_2SO_4 (90 mL) was combined with a solution of $NaNO_2$ (8.5 g, 0.12 mol) in concentrated H_2SO_4 (60 mL) at 0 °C, and the stirred mixture was slowly diluted with 85% phosphoric acid (150 mL) at a rate that maintained the temperature between 10 °C. After a further 2 h at 5–10 °C, urea (10 g) was added, and when gas evolution ceased, the solution was poured onto ice (500 g) and filtered. A solution of KI (20 g, 0.12 mol) was added to the filtrate, and the mixture was heated for 30 min in a boiling water bath. After cooling, the precipitate was collected to give 2-iodo-4-methoxy-3-nitrobenzoic acid (22.4 g, 89%), mp (benzene) 278–280 °C. Anal. ($C_8H_5INO_5$) C, H, N, I.

Use of this product in the Ullmann reaction then gave *N*-phenyl-4-methoxy-3-nitrobenzoic acid (Vb) in 50% yield (Table IV). However, reduction of this compound with $NaBH_4$ in MeOH/MeONa gave none of the desired 4-methoxyphenazine-1-carboxylic acid, but only the product of direct reduction, *N*-phenyl-3-amino-4-methoxyanthranilic acid (VI, R = 4-OCH_3), mp (benzene–MeOH) 203–205 °C. Anal. ($C_{14}H_{14}N_2O_3$) C, H, N.

(ii) By the Method of Scheme II. A solution of 4-methoxy-3-nitroanthranilic acid (5.37 g, 25.3 mmol) in dilute NaOH (100 mL) was hydrogenated over Pd/C, filtered, and acidified with AcOH. The resulting solution of 2,3-diamino-4-methoxybenzoic acid (VII, X = OCH_3) was combined immediately with a solution of cyclohexane-1,2-dione (2.78 g, 23 mmol) in MeOH (200 mL). The mixture was refluxed for 15 min, and the MeOH was then allowed to boil off until the volume was reduced to half. The

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cooled solution was diluted with water, and the resulting precipitate was collected and chromatographed on SiO_2 in CH_2Cl_2 to give 4-methoxy-6,7,8,9-tetrahydrophenazine-1-carboxylic acid (VIII, X = OCH_3) (3.9 g, 66%), mp (MeOH) 189–190 °C. Anal. ($C_{14}H_{14}N_2O_2$) C, H, N.

A solution of this compound (3.85 g, 14.9 mmol) in 1,2-dichlorobenzene (25 mL) containing 3 g of 5% Pd/C was heated under reflux for 2 days. The solvent was removed under vacuum and the residue was dissolved in 2 N NH_4OH, filtered, and acidified with 2 N HCl to give a crude product. This was dissolved in MeOH/Et_3N and acidified with AcOH to give 4-methoxyphenazine-1-carboxylic acid (Vh) (1.6 g, 42%), mp 250–251 °C. Anal. (Table V).

4-Methylphenazine-1-carboxylic Acid (Vg). A mixture of 4-bromo-3-methylaniline (145.8 g, 0.78 mol), chloral hydrate (140 g, 0.85 mol), hydroxylamine sulfate (200 g, 1.22 mol), sodium sulfate (900 g, 6.34 mol), and concentrated HCl (100 mL) in water (3 L) was heated at 80 °C with stirring for 8 h. After cooling, crude 4-bromo-3-methylisonitrosoacetanilide was collected as a light brown solid (161.2 g, 80%). This compound (155 g, 0.6 mol) was finely ground and added slowly to a stirred mixture of concentrated H_2SO_4 (400 mL) and water (140 mL), with the temperature held at 50–60 °C. After addition, the temperature was raised to 80 °C for 15 min, and the mixture was cooled and then poured onto ice. The resulting solid was collected and extracted with 2 N NaOH (2 L) for 2 h, and the filtrate from this extraction was acidified with AcOH to give a red-orange solid, which was collected and washed well with water. The NaOH extraction was repeated twice more on this solid to eventually give 5-bromo-4-methylisatin (IX) (54 g, 37%), mp (MeOH) 235–237 °C. Anal. ($C_9H_6BrNO_2$) C, H, N. The combined filtrates and washings from above were pooled and acidified further with concentrated HCl to give the desired 5-bromo-6-methylisatin (X) (47.8 g, 33%), mp (MeOH) 266–269 °C. Anal. ($C_9H_6BrNO_2$) C, H, N, Br.

A suspension of the above 5-bromo-6-methylisatin (X) (44.3 g, 0.18 mol) was stirred in 3% NaOH (2.5 L) and 27% H_2O_2 (100 mL) was added. As soon as the mixture was homogeneous it was filtered through Celite and acidified with AcOH to give 5-bromo-4-methylanthranilic acid (XIa) (36.5 g, 86%), mp (aqueous MeOH) 224–226 °C. Anal. ($C_8H_9BrNO_2$) C, H, N, Br. This compound was identical with that obtained by bromination (Br_2/AcOH) of 4-methylanthranilic acid.

A solution of the above anthranilic acid (XIa) (30 g, 0.13 mmol) in AcOH (350 mL) at 100 °C was treated with Ac_2O (20 mL, 0.21 mmol), and the mixture was kept at 100 °C for a further 15 min. Water (20 mL) was then added to hydrolyze the excess Ac_2O, and after 5 min the solution was diluted with water to give 2-(acetylamino)-5-bromo-4-methylbenzoic acid (XIb) (34.0 g, 94%), mp (MeOH) 226–228 °C. Anal. ($C_{10}H_{10}BrNO_3$) C, H, N.

The above compound (XIb) (33 g, 0.12 mol) was finely powdered and added slowly to stirred fuming HNO_3 (100 mL) at 0–5 °C. After 15 min the solution was poured onto ice and the precipitate was collected and washed well with water to give 2-(acetylamino)-5-bromo-4-methyl-3-nitrobenzoic acid (XIIa) (24.8 g, 64%), mp (MeOH) 222–223 °C. Anal. ($C_{10}H_9BrN_2O_4$) C, H, N.

A stirred suspension of the above compound (XIIa) (23.5 g, 74 mmol) in 50% H_2SO_4 (200 mL) was heated at 100 °C for 5 h, cooled, and diluted with water to give 5-bromo-4-methyl-3-nitroanthranilic acid (XIIb) (16.7 g, 71%), mp (MeOH) 269–271 °C. Anal. ($C_8H_7BrN_2O_4$) C, H, N, Br.

A solution of the above anthranilic acid (XIIb) (7.6 g, 27.6 mmol) in dilute NaOH (100 mL) was hydrogenated over Pd/C, filtered, and acidified with AcOH. The resulting solution of 2,3-diamino-4-methylbenzoic acid (VII) was immediately combined with a solution of cyclohexane-1,2-dione (3.0 g, 24.8 mmol) in MeOH (200 mL), and the mixture was heated under reflux for 15 min. The MeOH was then allowed to boil off until the crude produce crystallized out (5.56 g, 92%). A sample was crystallized from MeOH to give pure 4-methyl-6,7,8,9-tetrahydrophenazine-1-carboxylic acid (VIII, X = CH_3) mp 189–190 °C. Anal. ($C_{14}H_{14}N_2O_2$) C, H, N.

A solution of the above crude tetrahydrophenazine (5.05 g, 21 mmol) in 1,2-dichlorobenzene (25 mL) containing 4 g of 5% Pd/C was heated under reflux for 3 days. The solvent was removed under vacuum, and the residue was extracted with 2 N NH_4OH, filtered, and acidified with 2N HCl to give the crude phenazine. This was converted to the methyl ester ($SOCl_2$/MeOH) and chromatographed on SiO_2 (CH_2Cl_2) to give methyl 4-methylphenazine-1-carboxylate (1.47 g, 28%), mp (MeOH) 151–152 °C. Anal. ($C_{15}H_{12}N_2O_2$) C, H, N. Hydrolysis of this with aqueous NaOH/EtOH gave 4-methylphenazine-1-carboxylic acid (Vg), mp (MeOH) 239–241 °C. Anal. (Table V).

6-Methylphenazine-1-carboxylic Acid (Vi). Reduction of *N*-(3-methylphenyl)-3-nitroanthranilic acid (IVj) (5 g, 17 mmol) with $NaBH_4$ (5 g, 0.13 mol) in 2 N NaOEt (400 mL) gave a mixture of 6- and 8-methylphenazine-1-carboxylic acids, which were separated by chromatography of their methyl esters on SiO_2 as reported.[17] The major component isolated was methyl 6-methylphenazine-1-carboxylate (1.95 g, 44%), mp (MeOH) 150–151 °C (lit.[17] mp 151 °C). Hydrolysis with NaOH/EtOH/H_2O gave 6-methylphenazine-1-carboxylic acid (Vi), mp (MeOH) 239–241 °C. Anal. (Table V).

6-Methoxyphenazine-1-carboxylic Acid (Vj). A solution of *N*-(2,3-difluorophenyl)-3-nitroanthranilic acid (IVo) (4.0 g, 13.6 mmol) and $NaBH_4$ (5g, 0.13 mmol) in MeOH (400 mL) containing dissolved Na (18.4 g, 0.8 mol) was heated under reflux for 5 h, after which a further 5 g of $NaBH_4$ was added. After a further 6 h under reflux, the mixture was diluted with water until homogeneous, and the MeOH was allowed to boil off. The resulting aqueous solution was filtered and acidified with 2 N HCl to give a precipitate, which was dissolved in MeOH/Et_3N. Acidfication with AcOH gave 6-methoxyphenazine-1-carboxylic acid (Vj) (2.91 g, 84%), mp 291–293 °C (lit.[19] mp 292–293 °C). No trace of 6-fluorophenazine-1-carboxylic acid was seen, showing that complete displacement of fluorine had occurred.

A similar procedure was used to prepare 7-methoxy-, 8-methoxy-, and 9-methoxyphenazine-1-carboxylic acids from *N*-(4-methoxyphenyl)-*N*-(2,5-difluorophenyl)-, and *N*-(2,6-difluorophenyl)-3-nitroanthranilic acids, respectively.

6-Chlorophenazine-1-carboxylic Acid (Vk). Reduction of *N*-(3-chlorophenyl)-3-nitroanthranilic acid (IVk) (5.0 g, 16 mmol) with $NaBH_4$ (5 g, 0.13 mol) in 2 N NaOH solution (400 mL) gave a mixture of 6- and 8-chlorophenazine-1-carboxylic acids, with the former predominating. Two recrystallizations from DMF gave 6-chlorophenazine-1-carboxylic acid (Vk) (1.65 g, 40%), mp (EtOH) 310 °C dec. Anal. (Table V).

9-Methoxyphenazine-1-carboxylic Acid (Vs). (i) Reduction of *N*-(2-methoxyphenyl)-3-nitroanthranilic acid (IVg) with $NaBH_4$ in MeOH/MeONa gave a mixture of the desired product and phenazine-1-carboxylic acid (Va), the latter due to ring closure on to the substituted carbon and present in smaller amount. Two recrystallizations from DMF gave 9-methoxyphenazine-1-carboxylic acid (Vs) in 33% yield, mp (MeOH) 262–265 °C (lit.[19] mp 262–263 °C).

(ii) Reduction of *N*-(2,6-dimethoxyphenyl)-3-nitroanthranilic acid (IVt) as above gave 9-methoxyphenazine-1-carboxylic acid (Vs) in 69% yield.

(iii) Reduction of *N*-(2,6-difluorophenyl)-3-nitroanthranilic acid (IVv) as above gave 9-methoxyphenazine-1-carboxylic acid (Vs) in 77% yield. This latter procedure is the one of choice, since it is unequivocal and gives the highest overall yield of product.

9-Chlorophenazine-1-carboxylic Acid (Vt). (i) Reduction of *N*-(2-chlorophenyl)-3-nitroanthranilic acid (IVi) (10 g, 34.2 mmol) with $NaBH_4$ (8 g, 0.21 mmol) in 2 N NaOH (800 mL) gave a mixture of the desired product and phenazine-1-carboxylic acid (Va). Two recrystallizations from DMF gave pure 9-chlorophenazine-1-carboxylic acid (Vt) (1.85 g, 21%), mp (EtOH) 272–273 °C. Anal. (Table V).

(ii) Reduction of *N*-(2-chloro-6-fluorophenyl)-3-nitroanthranilic acid (IVv) as above gave 9-chlorophenazine-1-carboxylic acid (Vt) as the sole isolated product in 66% yield. Although this gives the best yield in the cyclization, it does not provide the greatest overall yield, due to the poor yield of *N*-(2-chloro-6-fluorophenyl)-3-nitroanthranilic acid (IVv) in the Ullmann reaction (12%). This low yield is due to steric hindrance of the aniline and is even more pronounced in the case of 2,6-dichloroaniline, where none of the desired product was obtained.

Benzo[*a*]phenazine-6-carboxylic Acid (Vu). Reaction of 3-hydroxy-2-naphthoic acid with benzofuroxan by the published procedure[9] gave benzo[*a*]phenazine-6-carboxylic acid 7,12-dioxide (XII). A solution of this compound (1.7 g, 5.55 mmol) in 0.5 N

NaOH (250 mL) was combined with a solution of sodium dithionite (4.8 g, 28 mmol) in water (100 mL), and the mixture was heated to 100 °C for 15 min. On cooling the solution to 0 °C, crystals of the Na salt of the product were obtained, and these were collected, washed with brine, redissolved in hot water, and acidified with AcOH to give benzo[*a*]phenazine-6-carboxylic acid (Vu) (1.43 g, 94%), mp 272–274 °C (lit.[32] mp 275–278 °C).

Phenazine-1-carboxylic Acid 5-Oxide (Vx). A solution of phenazine-1-carboxylic acid (Va) (2.5 g, 11.2 mmol) in glacial acetic acid (250 mL) was diluted with 30% H_2O_2 (50 mL) and the resulting solution was stirred at 55–60 °C for 24 h before being diluted with water (500 mL) and cooled. The orange-yellow crystals were collected and dried to give phenazine-1-carboxylic acid 5-oxide (Vx) (1.01 g, 38%), mp (aqueous AcOH) 222–223 °C (lit.[33] mp 223 °C).

Preparation of the Compounds of Table I: General Examples. (i) Using 1,1-Carbonyldiimidazole. A suspension of 9-methoxyphenazine-1-carboxylic acid (Vs) (5 g, 20 mmol) in dry DMF (50 mL) was treated with 1,1-carbonyldiimidazole (6.5 g, 40 mmol). After 10 min at 20 °C gas evolution had ceased, and excess *N,N*-dimethylethylenediamine (6 mL) was added. After a further 5 min the solvent was removed under reduced pressure, and the residue was partitioned between CH_2Cl_2 and water. The organic layer was washed with water, dried, and evaporated to give the crude product. This was dissolved in the minimum amount of MeOH, 1.1 equiv of concentrated HCl was added, and EtOAc was added slowly to the boiling solution until crystallization began, to yield *N*-[2-(dimethylamino)ethyl]-9-methoxyphenazine-1-carboxamide hydrochloride (**28**) (6 g, 83%), mp 254–256 °C (Table I).

(ii) Using Thionyl Chloride. A solution of 2-methoxyphenazine-1-carboxylic acid (Vc) (1.5 g, 5.9 mmol) in $SOCl_2$ (50 mL) was heated under reflux for 30 min, and the excess $SOCl_2$ was removed under reduced pressure. A cold (5 °C) solution of excess *N,N*-dimethylethylenediamine (4 g) in dry CH_2Cl_2 (200 mL) was added rapidly, and the mixture was stirred at 10 °C until homogeneous. The solution was washed twice with water and then extracted with 2 N HCl. The aqueous layer was basified with concentrated NH_4OH and extracted into CH_2Cl_2. Drying and removal of solvent gave the crude product, which was converted to the hydrochloride as detailed above to give *N*-[2-(dimethylamino)ethyl]-2-methoxyphenazine-1-carboxamide hydrochloride (**11**) (0.87 g, 41%), mp 207–209 °C (Table I). A similar procedure was used to prepare the 2-chloro derivative (**12**, but in this case some displacement of the 2-chloro group by the side chain occurred, and the product was purified by chromatography of the free base on SiO_2, eluting with CH_2Cl_2/MeOH mixtures.

Biological Testing. Cell culture methods[34] and in vivo testing protocols[35] have been described in detail previously.

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Registry No. **5**, 103942-97-8; **5**·HCl, 103942-53-6; **6**, 103942-98-9; **6**·HCl, 103942-54-7; **7**, 103942-99-0; **7**·HCl, 103942-55-8; **8**, 103943-00-6; **8**·HCl, 103942-56-9; **9**, 103943-01-7; **9**·HCl, 103942-57-0; **10**, 106975-95-5; **10**·HCl, 106976-31-2; **11**, 106975-96-6; **11**·HCl, 106976-32-3; **12**, 106975-97-7; **12**·HCl, 106976-33-4; **13**, 103943-04-0; **13**·HCl, 103942-60-5; **14**, 103943-05-1; **14**·HCl, 103942-61-6; **15**, 103943-06-2; **15**·HCl, 103942-62-7; **16**, 106975-98-8; **16**·HCl, 106976-34-5; **17**, 106975-99-9; **17**·HCl, 106976-35-6; **18**, 103943-07-3; **18**·HCl, 103942-63-8; **19**, 106976-00-5; **19**·HCl, 106976-36-7; **20**, 103943-08-4; **20**·HCl, 103942-64-9; **21**, 103943-09-5; **21**·HCl, 103942-65-0; **22**, 106976-01-6; **22**·HCl, 106976-37-8; **23**, 103943-10-8; **23**·HCl, 103942-66-1; **24**, 106976-02-7; **24**·HCl, 106976-38-9; **25**, 103943-11-9; **25**·HCl, 103942-67-2; **26**, 103943-12-0; **26**·HCl, 103942-68-3; **27**, 103967-39-1; **27**·HCl, 103942-69-4; **28**, 103943-13-1; **28**·HCl, 103942-70-7; **29**, 103943-14-2; **29**·HCl, 103942-71-8; **30**, 103943-18-6; **30**·HCl, 103942-75-2; **31**, 103943-19-7; **31**·HCl, 103942-76-3; **32**, 103943-20-0; **32**·HCl, 103942-77-4; **33**, 103943-02-8; **33**·HCl, 103942-58-1; II (R = H), 62-53-3; II (R = 2-CH_3), 95-53-4; II (R = 2-OCH_3), 90-04-0; II (R = 2-F), 348-54-9; II (R = 2-Cl), 95-51-2; II (R = 3-CH_3), 108-44-1; II (R = 3-Cl), 108-42-9; II (R = 4-CH_3), 106-49-0; II (R = 4-OCH_3), 104-94-9; II (R = 4-Cl), 106-47-8; II (R = 2,3-F_2), 4519-40-8; II (R = 2,3-benz), 134-32-7; II (R = 2-F, 5-CH_3), 452-84-6; II (R = 2,5-F_2), 367-30-6; II (R = 2-F, 5-Cl), 2106-05-0; II (R = 2,6-OCH_3), 2734-70-5; II (R = 2,6-F_2), 5509-65-9; II (R = 2-Cl, 6-F), 363-51-9; II (R = 3,4-benz), 91-59-8; III (X = NH_2, R = 4-OCH_3), 106976-18-5; III (X = R = 2,5-Cl_2), 88-86-8; III (X = R = 2,6-Cl_2), 55775-97-8; III (X = Br, R = H), 573-54-6; III (X = I, R = OMe-4), 106976-12-9; III (X = I, R = 5-CH_3), 90414-60-1; IVa, 54420-95-0; IVb, 106976-03-8; IVc, 103942-83-2; IVd, 103942-94-5; IVe, 55776-07-3; IVf, 106976-04-9; IVg, 38120-51-3; IVh, 106976-05-0; IVi, 38120-54-6; IVj, 103942-95-6; IVk, 38120-55-7; IVl, 103942-96-7; IVm, 38120-53-5; IVn, 38120-56-8; IVo, 106976-06-1; IVp, 106976-07-2; IVq, 106976-08-3; IVr, 106976-09-4; IVs, 103942-81-0; IVt, 103942-80-9; IVu, 106976-10-7; IVv, 106976-11-8; IVw, 106987-79-5; Va, 2538-68-3; Vb, 13392-01-3; Vc, 106976-13-0; Vd, 103942-84-3; Ve, 73113-65-2; Vf, 103942-85-4; Vg, 106976-14-1; Vh, 73113-66-3; Vi, 103942-86-5; Vj, 32464-65-6; Vk, 103942-87-6; Vl, 103942-88-7; Vm, 32464-64-5; Vn, 103942-92-3; Vo, 106976-15-2; Vp, 32464-66-7; Vq, 103942-82-1; Vr, 58718-46-0; Vs, 23531-25-1; Vt, 103942-93-4; Vu, 4190-99-2; Vv, 103942-89-8; Vw, 103942-90-1; Vx, 27210-90-8; VI (R = 5-CH_3), 106976-16-3; VI (R = 4-OCH_3), 106976-19-6; VII (X = OCH_3), 106976-20-9; VII (X = CH_3), 34545-25-0; VIII (X = OCH_3), 106976-21-0; VIII (X = CH_3), 106976-21-0; VIII (X = CH_3), 106976-28-7; IX, 106976-22-1; X, 106976-23-2; XIa, 106976-24-3; XIb, 106976-25-4; XII, 26859-19-8; XIIa, 106976-26-5; XIIb, 106976-27-6; 2,4-$(OH)_2C_6H_3NO_2$, 3163-07-3; 2,5-$Cl_2C_6H_3CO_2H$, 50-79-3; 2,6-$Cl_2C_6H_3CO_2H$, 50-30-6; $(CH_3)_2N(CH_2)_2NH_2$, 108-00-9; 5-amino-2-fluoroacetanilide, 75001-47-7; 5-methylanthranilic acid, 2941-78-8; 3-methoxy-2-nitroaniline, 16554-47-5; 3-methoxy-2-nitroisonitrosoacetanilide, 106987-80-8; 6-methoxy-7-nitroisatin, 106976-17-4; cyclohexane-1,2-dione, 765-87-7; 4-bromo-3-methylaniline, 6933-10-4; 4-bromo-3-methylisonitrosoacetanilide, 106987-81-9; 4-methylphenazine-1-carboxylic acid methyl ester, 106976-29-8; methyl 6-methylphenazine-1-carboxylate, 106976-30-1; 4-methylanthranilic acid, 2305-36-4.

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