thiol content was 0.96 mol of SH/mol. Vis: λ_{max} = 353 nm (pH 7.5). The product gave a reaction with fluorescamine, indicating the presence of a primary amino group. Reinjection into a C_{18} reversed-phase column gave a single peak (Figure 5, supplementary material).

lac–BSA–PQ and BSA–PQ. Cysteinylprimaquine was coupled to 100 mg of BSA, or lac–BSA, containing 14–15 mol of (pyridyldithio)propionate groups/mol of protein in 50 mL of 0.1 M sodium phosphate, pH 7.2, 1 mM EDTA (the buffer was purged with N_2). Aliquots of 3 mL of cysteinylprimaquine (21–22.5 μmol in 30 mL of dilute trifluoroacetic acid, purged with N_2) were added at 3-min intervals. The reaction was performed at room temperature, under N_2 and was carried on for 30 min after the last addition. The protein–drug conjugates were purified by gel filtration at 4 °C on a column of Sephadex G-25, equilibrated with PBS, purged with N_2. The protein peak was pooled, and the primaquine content was calculated from the A_{353}, using a molar extinction of 3300 M^{-1} cm^{-1}. The protein concentration was determined by the method of Lowry et al.[29] The values were corrected for the interference of primaquine (1 μg of primaquine base gives the color equivalent to 3.64 μg of BSA). The protein solutions were concentrated in an Amicon Diaflo apparatus using a UM10 filter. The protein–drug conjugates were stored as a lyophilized powder at −20 °C; shortly before use, the complex was dissolved in PBS, dialyzed, and centrifuged.

Measurement of the Stability of the Protein–Drug Conjugate in Vitro. Serum was prepared from blood of a healthy individual. Conjugate (11.6 mol, or 7.3 mol of primaquine base/mol of conjugate) was incubated at 37 °C with serum for various lengths of time (2.4 mg of conjugate/mL of serum). In a control experiment PBS was substituted for serum. To prevent any sulfhydryl–disulfide interchange during subsequent analysis, 600 μL of incubation mixture was alkylated by the addition of 75 μL of 2 M Tris-HCl, pH 8.6, and 75 μL of 1 M iodoacetic acid (neutralized with NaOH) and incubation at room temperature in the dark for 30 min. After addition of $MgCl_2$ to a final concentration of 50 mM, the alkylated incubation mixture was chromatographed on a column of Glycogel B [(aminophenyl)-boronic acid immobilized on Sepharose CL 6B; 1.5-mL bed volume in a Pasteur pipet]. The column was equilibrated with 0.25 M ammoniumacetate, pH 8.5; 50 mM $MgCl_2$, 250 mM NaCl, 2 M urea, and a modification of the procedure of Mallia et al.[31] was used. After the column was washed with 11 mL of equilibration buffer, bound material was eluted with a linear gradient of 0–0.2 M sorbitol in 100 mM Tris-HCl, pH 8.5, 50 mM EDTA, 250 mM NaCl, 2 M urea (10 mL in each gradient vessel). After 11 mL of the gradient had passed through, elution was continued with 2 M sorbitol in the same buffer. The fraction size was 0.53 mL. Purified conjugate, after dialysis against PBS and lyophilization, was analyzed for protein and primaquine as described above.

Therapeutic Studies. The procedures for growing the parasites and the isolation of sporozoites were as described by Vanderberg et al.[32] Groups of six to eight female A/J mice (about 20 g) were inoculated with 10^4 sporozoites via the tail vein. Drug, protein–drug conjugate, or protein alone, dissolved in 0.5 mL of PBS, was injected intravenously 2–3 h after inoculation. In all studies the parasitemia was assessed by examination of Giemsa-stained thin bloodsmears up to the 12th day after inoculation. In every experiment one group of animals received no drug treatment and acted as a control. Animals that did not show parasitemia by the 12th day were regarded as cured.[14] The mean causal prophylactic dose was determined from dose–response studies after probit transformation of the data.[33]

Acute Lethal Toxicity Studies. Groups of eight mice were injected intravenously with varying amounts of free primaquine or cysteinylprimaquine coupled to BSA or lac–BSA. Freshly prepared solutions in PBS were used. Controls received PBS only. The LD_{50} value was determined after probit transformation of the data.[33]

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Registry No. Cysteinylprimaquine, 102615-50-9; cystinylprimaquine, 102615-51-0; primaquine diphosphate, 63-45-6; *N*-Boc-cystine *p*-nitrophenyl ester, 51077-22-6; primaquine, 90-34-6.

Supplementary Material Available: Figures 1, 2, and 5 showing coupling of cysteinylprimaquine to lactosaminated BSA, chromatography of lac–BSA, and HPLC of the primaquine derivative, respectively (2 pages). Ordering information is given on any current masthead page.

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Potential Antitumor Agents. 47. 3′-Methylamino Analogues of Amsacrine with in Vivo Solid Tumor Activity

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Replacement of the 3′-methoxy group of the clinical antileukemic agent amsacrine (1) with a 3′-methylamino group provides a compound (3) with a broader spectrum of action, including in vivo activity against experimental solid tumors. The synthesis, physicochemical properties, and biological activity of a series of acridine-substituted analogues of 3 are described. The compounds show higher levels of DNA binding, water solubility, and in vivo solid tumor activity (Lewis lung carcinoma) than their amsacrine counterparts. However, the structure–activity relationships for acridine substitution are different, with 3,5-disubstituted 3′-methylamino compounds showing the highest activity (compared to 4,5-disubstituted amsacrine analogues).

Considerable clinical experience with the DNA-binding agent amsacrine (*m*-AMSA; 1) has shown it to be an effective drug for the treatment of leukemias and lymphomas, and it is now being employed as a component of drug

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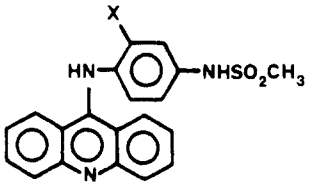
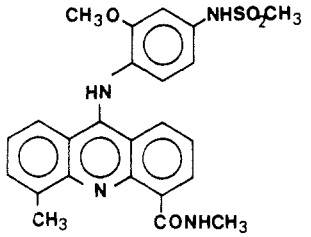
combinations used as front-line therapy for such diseases.[1,2] It also has low activity as a single agent against breast cancer,[3] but on the whole has not shown useful activity against a wide range of other solid tumors in man.[4,5]



1 X = OCH_3

3 X = $NHCH_3$

Studies on derivatives of amsacrine in our laboratory have therefore focused on the development of compounds with the potential for solid tumor activity. Following leads developed from QSAR studies[6] of in vivo antileukemic activity, new classes of disubstituted analogues of amsacrine were developed.[7,8] Many of these showed higher levels of antileukemic activity than amsacrine, especially when dosed orally,[8] and were selected for evaluation against a number of solid tumor screens, both in vitro[9] and in vivo.[10] From these studies the 4-methyl-5-*N*-(methylcarbamoyl) analogue (2; CI-921, NSC 343499) has been chosen for preclinical evaluation and toxicology[11] and for phase I clinical trials.



2 CI-921

Concomitant with this work, we have been evaluating other classes of amsacrine analogues for possible solid tumor activity. An earlier observation[12] that small electron-donating substituents on the anilino ring enhanced antitumor activity led to the development[13] of the 3′-alkylamino series, exemplified by the parent compound (3). This proved considerably more active than amsacrine against the P388 leukemia in vivo (increased life span (ILS) of 152 vs. 78%) at a similar optimal dose level.[13] The compound also showed a more favorable level of in vitro cytotoxicity toward a cultured human solid tumor line (the HCT-8 colon carcinoma[14]) in comparison to its cytotoxicity toward the mouse L1210 leukemia line. Most DNA-binding antitumor drugs are considerably less active against the solid tumor compared to the leukemia; thus, amsacrine has a ratio of IC_{50} values (concentrations of drug in nanomolar to inhibit cell growth by 50%) of 3.5 in favor of the leukemia (see Table I). For derivatives of amsacrine, lower values of this ratio, indicating a relatively higher activity toward the solid tumor cell line, have been shown to be predictive for solid tumor activity in vivo.[15,16] Amsacrine itself, with a ratio of 3.5, has only marginal activity against the advanced Lewis lung carcinoma (LL) in mice.[10] In contrast, the derivative (2; CI-921), with a ratio of 1.3, can provide a percentage of cures against the same tumor model and is also active in a number of other solid tumor systems.[11]

The 3′-methylamino derivative (3) has a ratio of 2.5, somewhat better than that of amsacrine. This, coupled with its superior P388 activity and greater water solubility[13] prompted us to explore the structure–activity relationships (SAR) for acridine substitution in this series of antitumor agents. Primary aims of this study were to see whether suitable acridine substitution could further improve the antitumor activity (especially activity against solid tumors) of the parent compound (3), as has been the case with amsacrine in the 3′-methoxy series,[11] and to see if the structure–activity relationships followed the same pattern as in the 3′-methoxy series.[6]

Chemistry. Preparation of the compounds of Table I involved acid-catalyzed coupling of the appropriate 9-chloroacridines with *N*-[4-amino-3-(methylamino)-phenyl]methanesulfonamide. The precursor nitro compound (V) has been prepared directly from *N*-(3-chloro-4-nitrophenyl)methanesulfonamide by treatment with methylamine under pressure.[13] An alternative low-pressure route to V is outlined in Scheme I. Treatment of 3-chloro-4-nitroacetanilide (I) with *N*-benzylmethylamine at 100 °C for 5 h, followed by base-catalyzed hydrolysis of the acetyl group, gave III directly. Mesylation followed by acid hydrolysis provided crystalline V. This was reduced catalytically to give the unstable amine, which was used immediately for preparation of the compounds of Table II.

Most of the 9-chloroacridines have been reported previously.[6,17] The precursor 3-methoxy-5-methylacridone for compound 32 was prepared via the Chapman route from methyl 2-hydroxy-4-methoxybenzoate. 3-Fluoro-5-methyl-9-chloroacridine and 3-fluoro-5-methoxy-9-chloroacridine were elaborated from 2-chloro-4-fluorobenzoic acid, obtained from 3-fluorochlorobenzene by

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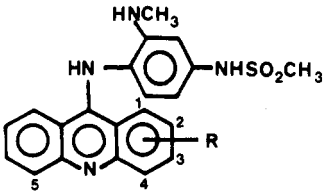
Table I. Physiochemical and Biological Data for 3'-$NHCH_3$ Analogues of Amsacrine



				in vitro IC_{50} values[c]			P388 in leukemia in vivo			Lewis lung carcinoma in vivo		
no.	R	R_m[a]	log K[b]	L1210	HCT-8	ratio	D_{50}[d]	OD[e]	ILS_{max}[f]	D_{50}[d]	OD[e]	ILS_{max}[f]
1	amsacrine	0.18	5.57	33	120	3.6	3	13.3	78	*g*	13.3	42
2	CI-921	0.34	6.40	38	49	1.3	3.5	20	200 (3)[h]	9	30	167 (3)
3	H	0.17	6.42	70	178	2.5	1.5	13.3	152 (2)	11	30	80
4	2-CH_3	0.28	5.89	550	1700	3.1	55	100	8	*g*	100	20
5	2-OCH_3	0.30	5.80	1200	1900	1.6	*g*	100	25	*g*	150	*i*
6	2-Cl	0.36	6.22	>1600	2500		*g*	100	*i*	*g*	150	*i*
7	3-CH_3	0.44	6.71	19	59	3.1	3.5	8.9	88	*g*	8.9	41
8	3-OCH_3	0.21	6.64	35	180	5.1	4.5	8.9	132 (2)	15	20	75
9	3-F	0.28	5.78	94	460	4.9	15	45	157 (2)	20	45	79
10	3-Cl	0.36	6.18	24	170	7.1	10	30	149	15	20	98
11	3-Br	0.34	6.99	38	140	3.7	5	66	136 (1)	*g*	20	49
12	3-I	0.35	6.42	29	79	2.7	10	45	70	*g*	30	*i*
13	3-NO_2	0.04	6.72	110	140	1.3	3	5.9	70	*g*	8.9	*i*
14	3-$NHCH_3$	0.18	7.53	5.8	810	140	0.2	2.6	208 (2)	*g*	2.6	*i*
15	3-$NHCOOCH_3$	0.13	7.21	76	715	9.4	1.5	13.3	135 (1)	*g*	8.9	*i*
16	3-$CONHCH_3$	−0.10	5.78	>1600	>104		30	65	62		NT[j]	
17	4-CH_3	0.19	6.60	53	79	1.5	3	13.3	157 (2)	8.9	20	139 (2)
18	4-OCH_3	0.12	6.43	130	140	1.1	5	45	170 (1)	19	45	153
19	4-F	0.05	6.91	400	690	1.7	20	45	152	60	65	55
20	4-Cl	0.10	6.87	690	740	1.1	65	100	64	80	100	59
21	4-Br	0.09	6.37	190	1800	9.5	*g*	100	35	*g*	100	*i*
22	4-$CONH_2$	−0.39	6.28	560	2800	5.0	25	45	109	*g*	30	*i*
23	4-$CONHCH_3$	0.07	6.11	56	1800	32	30	65	82	6.8	45	91
24	4-$CONHCH_2CONH_2$	−0.70	5.57	3500	>104		45	68	87	*g*	45	*i*
25	1,4-$(CH_3)_2$	0.28	6.18	>1700	2100		*g*	65	*i*		NT	
26	1-Cl, 4-$CONHCH_3$	0.04	6.75	>1600	4800		*g*	150	38		NT	
27	3,4-diCH_3	0.25	6.96	43	76	1.8	7	13.3	148	10	20	155
28	3,4-benz	0.25	7.46	189	400	2.1	45	45	50	*g*	20	*i*
29	3,5-$(CH_3)_2$	0.38	7.23	19	24	1.3	2	5.9	92	4.2	5.9	93
30	3-F, 5-CH_3	0.31	6.66	150	150	1.0	6	45	152 (4)	25	45	143 (3)
31	3-Cl, 5-CH_3	0.40	7.26	15	73	4.9	5	13.3	128 (1)	8	30	126
32	3-Br, 5-CH_3	0.38	7.27	21	49	2.3	7	13.3	122	*g*	13.3	34
33	3-OCH_3, 5-CH_3	0.31	7.16	16	29	1.8	0.3	1.8	166	4	4	50
34	3-CH_3, 5-OCH_3	0.33	6.686	65	70	1.1	5.5	13.3	122	10	20	194 (1)
35	3-F, 5-OCH_3	0.17	6.44	200	370	1.9	20	65	100	45	65	85
36	3-Cl, 5-OCH_3	0.28	6.94	120	143	1.2	20	65	236 (2)	13	45	159 (3)
37	3-Br, 5-OCH_3	0.30	6.94	41	150	3.7	9	65	174 (4)	40	65	111
38	3-Cl, 5-$CONHCH_3$	0.23	6.79	110	1900	17.37	30	45	61	*g*	30	*i*
39	4,5-$(CH_3)_2$	0.16	7.00	91	190	2.1	6	45	202 (2)	25	65	125
40	4,5-$(OCH_3)_2$	0.14	6.22	130	160	1.2	25	150	184 (2)	30	65	116 (2)
41	4-CH_3, 5-$CONHCH_3$	0.23	7.05	82	353	4.3	6	13.3	94	25	30	62
42	4-OCH_3, 5-$CONHCH_3$	0.07	6.92	320	>1000		30	65	148	*g*	65	34

[a] R_m values were determined as in ref 12, using 4'-(9-acridinylamino)methanesulfonanilide as a standard. [b] Log K: binding constant to poly[d(A-T)], determined by ethidium bromide displacement; see ref 24. [c] ID_{50}: concentration of drug in nM to inhibit growth of murine leukemia (L1210) or human colon tumor (HCT-8) cells in culture by 50%, following a 40-h exposure. See ref 19 and 14. [d] D_{50}: dose of drug in mg/kg per day to provide an average % ILS of 50; determined from dose–response graph. [e] OD: optimal dose of drug in mg/kg per day, administered intraperitoneally as a solution in 0.1 mL of 30% v/v ethanol–water on days 1, 5, and 9 after intraperitoneal inoculation of 10^6 P388 leukemia cells, or on days 5, 9, and 13 after intravenous inoculation of 10^6 Lewis lung carcinoma cells. See ref 10. [f] ILS_{max}: the percentage increase in lifespan of drug-treated, tumor-bearing animals compared to nontreated, tumor-bearing controls when treated at the optimal dose; values above 20% for P388 and above 40% for Lewis lung are considered statistically significant. [g] An ILS of 50% could not be reached at any dose level. [h] Numbers in parentheses indicate the number of animals in a group of six that were long-term survivors (30 days for P-388, 40 days for Lewis lung). Such animals are normally considered cured. [i] Compound inactive at all dose levels up to toxic ones. [j] Compound not tested.

Friedel–Craft acylation followed by NaOBr oxidation.[18]

Results and Discussion

Table I gives data for amsacrine, the amsacrine analogue CI-921 (**2**), the 3'-methylamino derivative (**3**), and 39 acridine-substituted derivatives. Protocols for in vitro assays using both L1210 leukemia cells[19] and HCT-8 human colon carcinoma cells[9] have been published. Testing against P388 leukemia followed established protocols,[13,20] with the drug being administered intraperitoneally as a solution in 30% aqueous EtOH on days 1, 5, and 9 following intraperitoneal inoculation of 10^6 leukemia cells. For the Lewis lung carcinoma, 10^6 cells (as a single cell

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Scheme I

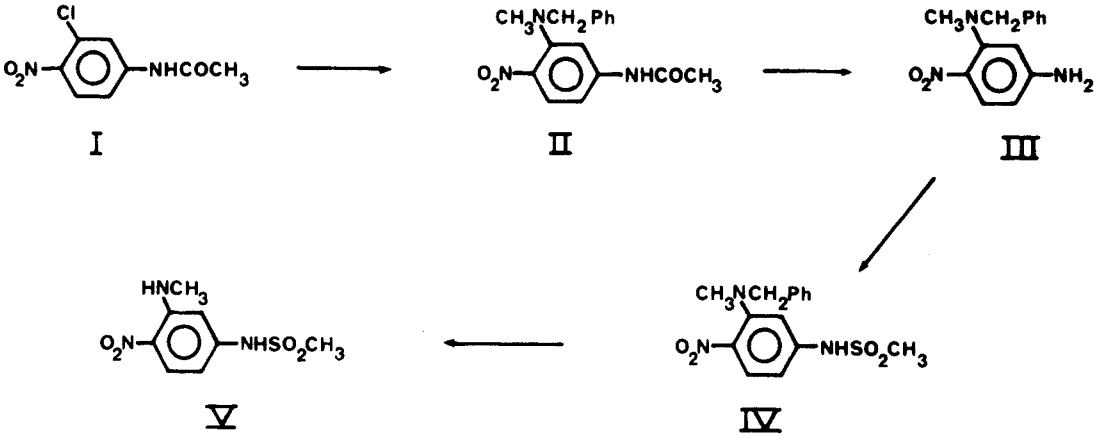

Table II. Analytical Data for the New Compounds of Table I

no.	mp, °C	formula	anal.
4	296–298	$C_{22}H_{22}N_4O_2S\cdot HCl$	C, H, N, Cl
5	288–291	$C_{22}H_{22}N_4O_3S\cdot HCl$	C, H, N, Cl
6	293–295	$C_{21}H_{19}ClN_4O_2S\cdot HCl$	C, H, N, Cl
7	295–296	$C_{22}H_{22}N_4O_2S\cdot HCl$	C, H, N, Cl
8	231–233	$C_{22}H_{22}N_4O_3S\cdot HCl$	C, H, N
9	250–255	$C_{22}H_{19}FN_4O_2S\cdot 2HCl$	C, H,[a] N, Cl
10	250–253	$C_{21}H_{19}FN_4O_2S\cdot 2HCl$	C, H, N, Cl
11	299–300	$C_{21}H_{19}BrN_4O_2S\cdot HCl$	C,[b] H, N, Cl
12	228–230	$C_{21}H_{19}IN_4O_2S\cdot 2HCl$	C,[c] H, N
13	275–276	$C_{21}H_{19}N_5O_4S\cdot HCl\cdot 0.5H_2O$	C, H, N, Cl
14	261–263	$C_{22}H_{23}N_5O_2S\cdot 2HCl$	C, H, N, Cl
15	243–245	$C_{23}H_{23}N_5O_4S\cdot HCl$	C, H, N, Cl
16	268–271	$C_{23}H_{23}N_5O_3S\cdot HCl$	C, H, N, Cl
17	240–241	$C_{22}H_{22}N_4O_2S\cdot HCl\cdot 0.5H_2O$	C, H, N, Cl
18	217–221	$C_{22}H_{22}N_4O_3S\cdot HCl$	C, H, N
19	220–225	$C_{21}H_{19}FN_4O_2S\cdot HCl\cdot 0.5H_2O$	C, H,[a] N, Cl
20	225–227	$C_{21}H_{19}ClN_4O_2S\cdot HCl$	C, H, N, Cl
21	183–187	$C_{21}H_{19}BrN_4O_2S\cdot CH_3SO_3H$	C, H, N
22	270–275	$C_{22}H_{21}N_5O_3S\cdot HCl\cdot 0.5H_2O$	C, H, N, Cl
23	242–245	$C_{23}H_{23}N_5O_3S\cdot 2HCl$	C, H, N
24	229–232	$C_{24}H_{24}N_6O_4S\cdot HCl$	C, H, N
25	235–237	$C_{23}H_{24}N_4O3_S\cdot HCl$	C, H, N, Cl
26	260–263	$C_{23}H_{22}ClN_5O_3S\cdot HCl$	C, H, N, Cl
27	227–230	$C_{23}H_{24}N_4O_2S\cdot HCl\cdot 0.25H_2O$	C, H, N, Cl
28	234–237	$C_{25}H_{22}N_4O_2S\cdot 2HCl$	C, H, N
29	261–263	$C_{23}H_{24}N_4O_2S\cdot HCl$	C, H, N, Cl
30	240–245	$C_{22}H_{21}N_4O_2S\cdot HCl$	C, H, N, Cl
31	276–278	$C_{22}H_{21}ClN4_{O2}S\cdot HCl$	C, H, N, Cl
32	274–276	$C_{22}H_{21}BrN_4O_2S\cdot HjCl\cdot 0.25H_2O$	C, H, N, Cl
33	276–277	$C_{23}H_{24}N_4O_3S\cdot HCl\cdot 0.5H_2O$	C, H, N, Cl
34	261–263	$C_{23}H_{24}N_4O_3S\cdot 2HCl$	C, H, N, Cl
35	235–238	$C_{22}H_{21}FN_4O_3S\cdot 2HCl\cdot 0.5H_2O$	C, H, N, Cl
36	274–276	$C_{22}H_{21}ClN_4O_3S\cdot HCl$	C, H, N, Cl
37	261–263	$C_{22}H_{21}BrN_4O_3S\cdot HCl$	C, H, N
38	265–270	$C_{23}H_{22}ClN_5O_3S.HCl$	C, H, N, Cl
39	279–280	$C_{23}H_{24}N_4O_2S\cdot HCl\cdot 0.5H_2O$	C, H,[d] N, Cl
40	268–272	$C_{23}H_{24}N_4O_4S\cdot HCl$	C, H, N, Cl
41	249–251	$C_{24}H_{25}N_5O_3S\cdot HCl$	C, H, N, Cl
42	279–282	$C_{24}H_{25}N_5O_4S\cdot HCl$	C, H, N, Cl

[a] H out by 0.6%. [b] C out by 0.5%. [c] C out by 0.6%. [d] H out by 0.5%.

suspension) were inoculated intravenously, and drug treatment was intraperitoneally on days 5, 9, and 13. Delay of drug treatment until 5 days after tumor inoculation allowed the tumor foci that appear in the lung to develop to an average size of several hundred cells, reminiscent of an early solid tumor.[10] In control experiments, an average of 23 tumor foci were seen in the lungs after 9 days, a figure which agrees well with independent estimates of an average of 27 clonogenic tumor cells per 10^6.

In each case, D_{50} values are the dose of drug (in mg/kg/dose) needed to produce a 50% increase in lifespan of tumor-bearing animals compared to untreated controls and are determined from dose–response graphs by the method of ref 21. ILS values quoted are those obtained at the optimal dose (OD), which is a reasonable approximation to the LD_{10}, provided that a higher toxic dose has been given. For the P388 leukemia, ILS values of 20% or above are considered significant; for the LL the value is 40% or above.[20]

The first set of derivatives (**4–24**) are those bearing a single substituent in the acridine ring at either the 2-, 3-, or 4- position. No 1-substituted derivatives were evaluated, as previous studies of amsacrine analogues[6] have shown that 1-substituted derivatives are invariably inactive and nontoxic, presumably due to steric interference with intercalative binding to DNA.[6] The same binding model predicts little bulk tolerance at the 2-position also, but a few derivatives with small groups in the 2-position have shown significant activity in the amsacrine series, and compounds **4–6** were included for comparison. Most of the singly substituted derivatives bear groups in the 3- or 4-positions, which in the amsacrine series have provided analogues with a wide variation in in vivo antileukemic activity.[6] The derivatives **25–42** encompass a variety of substitutions, but the majority employ the 3,5-disubstitution pattern already found to be very effective in the amsacrine series.[6,7] Overall, the substituents were chosen to have a wide variation in lipophilic, electronic, and steric properties.

Lipophilicity. The observed lipophilicities of the 3′-methylamino derivatives can be compared with the corresponding amsacrine (3′-methoxy) analogues. The chromatographically determined R_m values recorded in Table I are for the drug cations at pH 1–2 and are directly comparable with those for amsacrine derivatives.[7,22] R_m values have been shown[8] to correlate closely with octanol–water log P measurements for the drug cations. Replacement of the 3′-OCH_3 group (π value −0.02)[22] with the more polar 3′-$NHCH_3$ (π value −0.47)[23] would be expected to provide more polar compounds, but the R_m values for the two parent compounds (**1** and **2**) are identical (Table I). This is true also for the monosubstituted compounds **4–24** where the 3′-methylamino derivatives have measured lipophilicities identical to those of their amsacrine analogues (amsacrine data not shown[7,22]). Despite this, the 3′-methylamino compound (**3**) is more than 4-fold more water soluble than amsacrine (0.49 mg/mL for the hydrochloride

(21) Denny, W. A.; Cain, B. F. *J. Med. Chem.* **1978**, *21*, 430.
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(23) Hansch, C.; Leo, A. J. *Substituent Constants for Correlation Analysis in Chemistry and Biology*; Wiley: New York, 1979.

Table III. Acridine Substituent Effects on DNA Binding in the Amsacrine (1) and 3'-Methylamino (3) Series of 9-Anilinoacridines

substituent	Δ log K	
	amsacrine[a]	3'-methylamino
none	log K = 5.57	log K = 6.42
1-CH_3		−0.42[b]
2-CH_3	−0.22	−0.53
2-OCH_3	−0.08	
2-Cl	−0.08	−0.20
3-CH_3	0.38	0.29
3-OCH_3	0.26	0.22
3-F	−0.03	−0.64
3-Cl	0.49	−0.24
3-Br	0.72	0.57
3-I	0.78	0.00
3-NO_2		0.06
3-$NHCH_3$		1.11
3-$NHCOOCH_3$		0.79
4-CH_3	0.46	0.18
4-OCH_3	0.37	0.01
4-Cl	0.19	0.45
4-$CONHCH_3$	−0.03	−0.31

[a] Log K values from amsacrine derivatives derived from data in ref 22. [b] Indirect calculation from compounds **17** and **25** of Table I.

salt in water at 20 °C, compared with 0.12 mg/mL for amsacrine HCl salt).

In the 3,5-disubstituted series (**29–38**) the 3'-methylamino derivatives did prove more hydrophilic, with R_m values on average 0.04 units lower than the amsacrine analogues, and for the 4,5-disubstituted compounds (**39–42**) the difference was 0.15 R_m units. This latter difference, amounting to approximately 0.3 log P units, is acceptably close to the difference in π values between the two substituent groups (0.45 units). The reasons for the smaller differences with other substitution patterns are not clear. Generally, the 3'-methylamino derivatives were appreciably more water-soluble than the amsacrine analogues.

DNA Binding. The parent 3'-methylamino derivative (**3**) binds much more strongly to DNA than amsacrine, in spite of a similar pK_a for the acridine nitrogen (data not shown), as demonstrated by their respective log K values of 5.57 and 6.42 for binding to poly[d(A-T)]. These values were determined by the ability of the drugs to displace the fluorochrome ethidium bromide from DNA[24] and are an accurate reflection of relative binding constants. As expected, substitution in the 1- and 2-positions greatly reduces DNA binding, as it does in the amsacrine series[22] (Table III). In contrast, small lipophilic groups at the 3-position considerably enhance binding in both series. The exception in the amsacrine series is the 3-fluoro, which has no significant effect. In the 3'-methylamino series, the augmenting effect of 3-substituents is generally smaller, but a similar trend is seen. In the 4-position, most substituents enhance DNA binding in the amsacrine series; the low log K value for the 4-$CONHCH_3$ compound is surprising, in view of the relatively high pK_a (6.36).[8] For the 3,4-disubstituted compound **27**, the observed log K value is reasonably well approximated by simple addition of the log K values for the individual substituents (Table IV). However, such calculations for the 3,5-disubstituted compounds (using the appropriate values for 3- and 4-monosubstituted compounds) generally lead to log K values much less than those observed (Table IV). The exceptional DNA binding of these compounds, together with previous data on the superior activity of 3,5-disubstituted derivatives of amsacrine,[7] led us to concentrate primarily on this acridine disubstitution pattern in the 3'-methylamino series.

Table IV. Observed and Calculated log K Values for DNA Binding of Acridine-Disubstituted 3'-Methylamino Derivatives

compd	substitution	log K		Δ log K
		obsd	calcd	
27	3,4-$(CH_3)_2$	6.96	6.89	−0.07
28	3,4-benz	7.46		
29	3,5-$(CH_3)_2$	7.23	6.89	−0.34
30	3-F, 5-CH_3	6.66	6.00	−0.66
31	3-Cl, 5-CH_3	7.26	6.40	−0.86
32	3-Br, 5-CH_3	7.27	7.17	−0.10
33	3-OCH_3, 5-CH_3	7.16	6.82	−0.34
34	3-CH_3, 5-OCH_3	6.68	6.72	−0.04
35	3-F, 5-OCH_3	6.44	5.83	−0.61
36	3-Cl, 5-OCH_3	6.94	6.23	−0.71
37	3-Br, 5-OCH_3	6.94	7.00	0.06
38	3-Cl, 5-$CONHCH_3$	6.79	5.91	−0.88
39	4,5-$(CH_3)_2$	7.00	6.78	−0.22
40	4,5-$(OCH_3)_2$	6.22	6.44	0.22
41	4-CH_3, 5-$CONHCH_3$	7.05	6.29	−0.76
42	4-OCH_3, 5-$CONHCH_3$	6.92	6.12	−0.80

In Vitro Cytotoxicity. The in vitro cytotoxicities of the 3'-methylamino derivatives against L1210 leukemia cells show broad structure–activity relationships similar to those of HCT-8/L1210 substituted amsacrines.[25] Thus substitution at position 2 greatly reduces L1210 (and HCT-8) cytotoxicity (Table I), without significantly altering the selectivity, while the same (lipophilic) substituents (e.g., CH_3, OCH_3, halogen) at the 3-position provide a significant enhancement of L1210 cytotoxicity. However, the increase in activity of these derivatives against the HCT-8 is less marked, leading to an increase in the IC_{50} ratio in favor of the leukemia (from 2.5 for **3** to 4–7 for compounds **7–12**). This trend is dramatically enhanced in the case of 3-NH-containing substituents, with the 3-$NHCH_3$ compound (**14**) having a ratio of 140 in favor of the leukemia. The poor cytotoxicity of the 3-$CONHCH_3$ compound (**16**) against both cell lines is unexpected, considering the activity of the amsacrine analogue (IC_{50} for L1210, 290 nM).

Of the 4-substituted compounds, only the $-CH_3$, $-OCH_3$, and $CONHCH_3$ derivatives (**17**, **18**, and **23**) showed L1210 activity comparable to that of the parent compound **3**, and, of these, only the first two had a more favorable ratio (1.5 and 1.1, respectively). In particular, alteration of the methylcarboxamide side chain led to compounds with much poorer levels of absolute cytotoxicity and poorer HCT-8/L1210 ratios.

The deleterious effect of 1-substitution on cytotoxicity can be seen in the results for compounds **25** and **26**, especially the L1210 data for the 1,4-dimethyl derivative **25** (IC_{50} = 1700 nM), when compared with the 3,4-dimethyl (**27**; IC_{50} = 43 nM) and 3,5-dimethyl (**29**; IC_{50} = 19 nM) isomers.

Cytotoxicity data for the 3-substituted-5-methyl and 3-substituted-5-methoxy compounds (**29–33** and **34–37**, respectively) show that addition of a further substituent to the 4-methyl or 4-methoxy compounds (**17** and **18**) does not improve the absolute activity against the leukemia, in spite of a significant increase in DNA binding over the latter two derivatives (see above). The selectivity of these compounds for the solid tumor line is also little improved over compounds **17** and **18**, although much better than for any of the corresponding 3-substituted derivatives. The cytotoxicity of the 3-Cl, 5-$CONHCH_3$ compound (**38**) is dominated by the methylcarboxamide, showing very poor selectivity. However, this dominance is not evident in the

(24) Baguley, B. C.; Denny, W. A.; Atwell, G. J.; Cain, B. F. *J. Med. Chem.* 1981, *24*, 170.

4,5-disubstituted compounds (**39–42**), which show patterns of cytotoxicity comparable to those of the parent compound **3**.

In Vivo Antitumor Activity. All compounds were tested over a full dose range from inactive to acutely toxic, so the quoted optimal dose values (OD) are reasonable approximations to the LD_{10} and can be used as a comparative measure of drug potency (Table I). The difference between the OD and D_{50} values (D_{50}, minimum dose of drug to give a 50% increase in lifespan[20]) is a measure of the therapeutic range or the steepness of the dose–response curve for a compound. For the standard protocols used the ILS values can be used as a comparative indication of the tumor cell kill, or tumor cell selectivity, of the compounds.

The results show that 2-substitution significantly reduces P388 activity, and the 2-Cl derivative (**6**) is in fact inactive in vivo. Compounds **7–12**, bearing a lipophilic 3-substituent, have ILS values in the P388 comparable to that of the parent, but generally lower potency and a lower therapeutic range. Their activity in the solid tumor, while better than that of amsacrine, is not improved over the parent 3′-methylamino derivative (**3**). The only 3-derivative with clearly superior P388 activity is the 3-$NHCH_3$ compound, but this (together with the other 3-nitrogen-containing derivatives **13–15**) is completely inactive in the LL solid tumor system.

Of the 4-substituted derivatives (**17–24**), only the 4-CH_3 and 4-OCH_3 compounds show P388 activity comparable to the parent; these compounds, in addition, are the only monosubstituted derivatives to show clearly superior LL activity, with similar potency and therapeutic range but higher selectivity. The other 4-substituted compounds were uninteresting; even the 4-$CONHCH_3$ derivative (**23**) showed disappointingly low activity in both tumor systems, which contrasts with the high activity of the corresponding amsacrine analogue.[8]

Those disubstituted compounds bearing a 1-substituent were uninteresting, as expected. The 3,4-dimethyl compound (**27**) had moderate activity and high potency, while the more tightly DNA binding 3,4-benz analogue (**28**) was barely active. The 3,5-disubstituted compounds appear to have the best potential. The 3,5-dimethyl derivative (**29**) had only moderate activity, but was the most potent of all the LL-active compounds (optimal dose 5.9 mg/kg, compared to 30 for the parent **3**). The 3-halo-5-methyl set (**30–32**) is intriguing, with the 3-F compound **30** being probably the most active drug in the whole series (4/6 cures in the P388, 3/6 in the LL), with activity then decreasing in both tumor systems as the halogen is changed to Cl and Br. This trend is opposite to the trend in intrinsic cytotoxicity (IC_{50} values and is accompanied by an increase in potency (the OD declines 3.4-fold from fluoro to bromo). This suggests that the superior activity of the fluoro analogue is due, not to better structure-related selectivity, but to a lower mammalian toxicity, allowing a higher dose of drug to be tolerated. For the set of 3-halo-5-methoxy derivatives **35–37** the opposite trend is seen. Here the most active compound both in vitro and in vivo is the 3-bromo derivative **37** (although there is little to choose between this and the 3-chloro compound), and in vivo potency varies much less across the series.

The activity of the 3-Cl, 5-$CONHCH_3$ compound **38** is dominated by the methylcarbamoyl group, with a ratio of 17.3 against the HCT-8 solid tumor and no activity in the LL in vivo.

With the exception of the moderately active 4,5-dimethoxy compound **40**, the 4,5-disubstituted derivatives were disappointing. This was particularly so for the 4-CH_3, 5-$CONHCH_3$ analogue **41**, the equivalent in the 3′-methylamino series of CI-921 (**2**). The acridine substitution pattern that proved optimal in the amsacrine series[11] is actually dystherapeutic here, providing a compound with lower levels of in vivo activity in both tumor systems than the parent **3**.

Conclusions

In the broadest sense, the qualitative SAR for the in vivo antileukemic activity of the 3′-methylamino compounds follow the pattern determined previously[6,22] for the acridine-substituted amsacrines. Groups at the 2-position greatly reduce activity, while most groups (especially small lipophilic ones) at the 3- or 4-positions retain or enhance activity. Although too few examples are available to consider detailed QSAR studies of in vivo antitumor activity, drug D_{50} values (the dose of drug needed to elicit a 50% ILS in vivo, converted from the mg/kg values recorded in Table I into molar values) can be quantitatively related to their intrinsic cytotoxicity (IC_{50} values against L1210 leukemia cells in culture in nanomolar) and their lipophilicities (R_m values) (eq 1; cf. also eq 1 of ref 25 relating to amsacrine analogues).

$$\log (1/D_{50}[P388]) = 1.08(\pm 0.16) \times \log (1/IC_{50}[L1210]) - 1.17(\pm 0.41)R_m + 7.07 \quad (1)$$

$$n = 34 \qquad r = 0.80 \qquad s = 0.36$$

In contrast, the fit of the data to the IC_{50} values for the HCT-8 human colon line is much poorer (eq 2).

$$\log (1/D_{50}[P388]) = 0.52(\pm 0.14) \log (1/IC_{50}[HCT\text{-}8]) + 6.04 \quad (2)$$

$$n = 34 \qquad r = 0.54 \qquad s = 0.50$$

Equations 1 and 2 encompass all of the P388-active compounds of Table I. Thus, the in vitro leukemia screen is the better predictor for in vivo antileukemic activity.

For the subset of LL-active compounds, the in vivo potency data for this solid tumor are conversely fitted better by the IC_{50} values for the HCT-8 solid tumor than by the L1210 values.

$$\log (1/D_{50}[LL]) = 0.72(\pm 0.07) \log (1/IC_{50}[HCT\text{-}8]) + 6.03 \quad (3)$$

$$n = 22 \qquad r = 0.92 \qquad s = 0.14$$

$$\log (1/D_{50}[LL]) = 0.44(\pm 0.10) \times \log (1/D_{50}[L1210]) + 1.41(\pm 0.41)R_m + 1.97 \quad (4)$$

$$n = 22 \qquad r = 0.86 \qquad s = 0.19$$

The dataset for eq 3 and 4 are all the LL-active compounds of Table I. Although the correlation coefficient for eq 4 is only slightly inferior to that for eq 3, most of the variance in the data was taken up by the R_m term (which was not significant in eq 3). For these compounds, the HCT-8 data is a slightly better predictor of in vivo solid tumor activity than the L1210 leukemia screen, but not sufficiently superior to be worth the extra effort involved in the culture of human cell lines.

The relevance of the HCT-8 data can be seen more clearly in examining ILS values for the compounds. Although in vivo potency of a drug (D_{50}) is important, it is usually closely related to acute toxicity (approximated by OD)[6] (Table I). A more important parameter for drug

(25) Baguley, B. C.; Cain, B. F. *Mol. Pharmacol.* **1982**, *22*, 486.

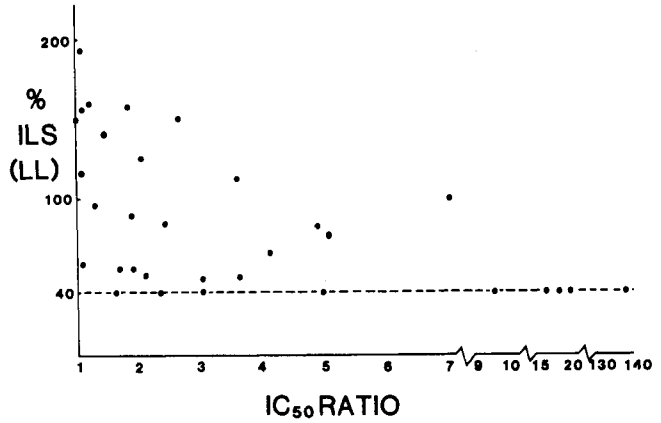

Figure 1. Relationship of in vivo antitumor activity (% ILS) of 3'-methylamino analogues of amsacrine to the ratio of their in vitro cytotoxicities for Lewis lung carcinoma.

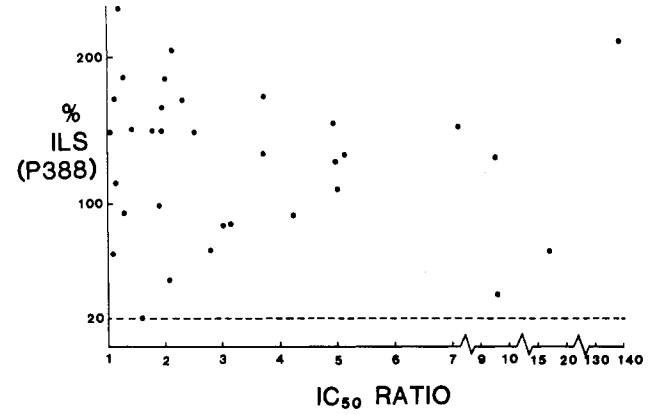

Figure 2. Relationship of in vivo antitumor activity (% ILS) of 3'-methylamino analogues of amsacrine to the ratio of their in vitro cytotoxicities for P388 leukemia.

evaluation is the maximum achievable % ILS. In tumor models such as the P388 and LL, where tumor growth fraction is essentially unity throughout the course of the disease and a fixed number of tumor cells causes death, ILS_{max} is a measure of tumor cell kill and thus tumor cell selectivity. It is usually unrelated to toxicity (see Table I).

For amsacrine analogues, a low ratio of cytotoxicities in the HCT-8/L1210 culture systems (i.e., a relatively higher cytotoxicity toward the solid tumor cell line) is predictive for selectivity (measured by ILS_{max}) against the LL solid tumor in vivo.[9] Quantitative modeling of ILS_{max} values is usually difficult due to the small absolute range of the parameter,[6] but Figure 1 does indicate a positive relationship between low values of the IC_{50} ratio and high selectivity against the LL in vivo. In contrast (Figure 2), no such relationship exists between IC_{50} ratio and ILS_{max} values against the P388 leukemia.

Thus, the broad utility of replacing the 3'-OCH_3 group of amsacrine with the more strongly electron donating 3'-$NHCH_3$ group is established. The resulting compounds **3–42** generally exhibit higher levels of DNA binding, water solubility, and solid tumor activity than their amsacrine counterparts. However, the two series are not identical and the optimal acridine substitution patterns are quite different. That of the amsacrine series is represented by compound **2** (CI-921), now in clinical evaluation. In contrast, the most active 3'-methylamino compounds are the 3-fluoro-5-methyl and 3-halo-5-methoxy derivatives **30**, **36**, and **37**, together with the 4-methyl compound **17**. These compounds are undergoing evaluation in a number of other solid tumor systems to gauge their potential for further development.

Experimental Section

Where analyses are indicated only by the symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. Analyses were performed by Dr. A. D. Campbell, Microchemical Laboratory, University of Otago, Dunedin, New Zealand. Melting points were determined on an Electrothermal melting point apparatus with the maker's supplied stem-corrected thermometer. Melting points are as read.

To monitor the progress of reactions, purification of products, etc., TLC on SiO_2 (Merck SiO_2, F_{254}) was used. The most convenient solvents were the top phase of *n*-BuOH–HOAc–H_2O (5:1:4, v/v) and $CHCl_3$ containing 10% MeOH.

3-(*N*-Benzylmethylamino)-4-nitroaniline (III). 3-Chloro-4-nitroacetanilide (I, 6.7 g, 31 mmol) was dissolved in dried (CaH_2) *N*-benzylmethylamine (15 mL) in a flask equipped with a $CaCl_2$ drying tube. The resulting solution was heated at 100 °C in a water bath for 5 h and diluted with 50 mL of EtOH. A solution of 5 g of KOH in 30 mL of water was added, and the mixture was heated under reflux for 30 min, when complete loss of the acetyl group was observed by TLC ($CHCl_3$–MeOH, 9:1). The EtOH was removed in vacuo and, after being acidified with dilute AcOH, the residue was extracted with EtOAc. The organic layer was washed successiveldy with dilute AcOH, water, dilute $KHCO_3$ solution, and brine and was dried over Na_2SO_4. Removal of the solvent in vacuo gave 7.8 g (98%) of the crude amine (III) as an orange oil, which was crystallized from methanol: mp 80–81 °C. Anal. ($C_{14}H_{15}N_3O_2$) C, H, N. If the corresponding bromo compound is used, the above reaction time can be reduced to 2 h.

***N*-(3-Methylamino-4-nitrophenyl)methanesulfonamide (V).** The above amine (2.57 g, 10 mmol) was dissolved in the minimum volume of pyridine, and after being cooled to 0 °C the solution was treated with 0.95 mL (1.2 equiv) of methanesulfonyl chloride. The mixture was allowed to warm slowly to room temperature, and after the reaction was complete (1–3 h) the excess reagent was quenched by the addition of a small volume of water. The solvent was removed in vacuo, and the residue was dissolved in EtOH–water–concentrated HCl (50 mL, 25:15:10) and heated under reflux for 1 h to effect debenzylation.

The EtOH was removed in vacuo, and the residue was dissolved in cold dilute NaOH solution. An insoluble oil was removed by treatment with charcoal/celite, and, after filtration, the orange solution was acidified with dilute AcOH acid to give a yellow solid, which was collected by filtration and washed well with deionized water. Recrystallization from aqueous EtOH gave 1.9 g (78%) of the nitrosulfonamide (V) as either orange prisms or yellow needles depending upon crystallization conditions: mp 191–193 °C (lit.[13] mp 191–193 °C).

9-Chloroacridines. The substituted 9-chloroacridines were prepared from the appropriate acridones by reaction with $SOCl_2$ as previously described.[11]

3-Methoxy-5-methyl-9-oxo-9,10-dihydroacridine. This was prepared from methyl 2-hydroxy-4-methoxybenzoate and *N*-(2-methylphenyl)benzimidoyl chloride. The crude imidoyl chloride[22] (0.1 mol) in DMF (25 mL) was added dropwise at 0 °C to a stirred solution of the Na salt of methyl 2-hydroxy-4-methoxybenzoate (0.1 mol) in toluene (50 mL). After 2 h at 20 °C and 1 h at 100 °C the solvents were removed and the residue was partitioned between light petroleum (200 mL) and 5% aqueous Et_3N (200 mL). Evaporation of the organic layer and recrystallization of the residue from methanol gave 2-methoxycarbonyl-5-methoxyphenyl *N*-(2-methylphenyl)benzimidate (20.4 g, 54%): mp 117–118 °C. Anal. ($C_{23}H_{21}NO_4$) C, H, N.

This compound (0.6 mol) was rearranged by heating in Dowtherm A under reflux for 2 h to give methyl *N*-benzoyl-*N*-(2-methylphenyl)-4-methoxyanthranilate (85% yield) as colorless plates from benzene–petroleum ether: mp 125–126 °C. Anal. ($C_{23}H_{21}NO_4$) C, H, N. Hydrolysis with KOH, followed by acid precipitation, gave the corresponding acid, which was heated in PPA at 160 °C for 1 h to provide 3-methoxy-5-methyl-9-oxo-9,10-dihydroacridine: mp 266–268 °C. Anal. ($C_{15}H_{13}NO_2$) C, H, N.

***N*-(2-Methoxyphenyl)-4-fluoroanthranilic Acid.** 2-Chloro-4-fluorobenzoic acid (5 g, 29 mmol; prepared by the method

of ref 18), 2-methoxyaniline (4 g, 32 mmol), and K_2CO_3 (4 g, 29 mmol) were stirred together in 2-ethoxyethanol (10 mL) for 2 h at 140 °C with a catalytic amount of Cu. The cooled mixture was diluted with water and acidified to pH 2. The resulting black solid was dissolved in dilute NH_4OH (100 mL) and decolorized with charcoal/celite. EtOH (50 mL) was added, followed by AcOH to pH 5, giving a solid, which was crystallized from diisopropyl ether as needles: mp 193–196 °C (3.6 g, 48%). Anal. C, H, N.

Similar reaction conditions using 2-methylaniline gave *N*-(2-methylphenyl)-4-fluoroanthranilic acid in 56% yield: mp (diisopropyl ether) 184–186 °C. Anal. C, H, N.

General Preparation of the Compounds of Table I. A solution of the above nitrocompound (0.3 mol in MeOH) was hydrogenated over palladium/C until hydrogen uptake ceased (15 min). The resultant solution of the *o*-phenylenediamine derivative was added to a methanolic solution of 9-chloroacridine (0.95 equiv) and the coupling reaction initiated with a drop of HCl. The solution was then concentrated to small volume, and EtOAc was added until crystallization of the hydrochloride salt of the product began. Details are recorded in Table I.

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Registry No. 1, 51264-14-3; **2**, 80841-47-0; **3**, 88412-78-6; **4**, 102940-94-3; **4**·HCl, 102941-07-1; **5**, 102976-72-7; **5**·HCl, 102941-08-2; **6**, 102940-95-4; **6**·HCl, 102941-09-3; **7**, 88914-09-4; **7**·HCl, 88913-48-8; **8**, 88914-10-7; **8**·HCl, 88913-49-9; **9**, 88914-11-8; **9**·2HCl, 88913-50-2; **10**, 88914-12-9; **10**·2HCl, 88913-51-3; **11**, 88914-13-0; **11**·HCl, 88913-52-4; **12**, 88914-14-1; **12**·2HCl, 88913-53-5; **13**, 88914-15-2; **13**·HCl, 88913-54-6; **14**, 88914-16-3; **14**·2HCl, 102941-10-6; **15**, 88914-17-4; **15**·HCl, 88913-56-8; **16**, 102940-96-5; **16**·HCl, 102941-11-7; **17**, 88914-18-5; **17**·HCl, 88913-57-9; **18**, 88914-19-6; **18**·HCl, 88913-58-0; **19**, 102940-97-6; **19**·HCl, 102941-12-8; **20**, 102940-98-7; **20**·HCl, 102941-13-9; **21**, 88913-59-1; **21**·CH_3SO_3H, 88913-60-4; **22**, 102940-99-8; **22**·HCl, 102941-14-0; **23**, 88914-20-9; **23**·2HCl, 88913-61-5; **24**, 102941-00-4; **24**·HCl, 102941-15-1; **25**, 102976-73-8; **25**·HCl, 102976-74-9; **26**, 102941-01-5; **26**·HCl, 102941-16-2; **27**, 102941-02-6; **27**·HCl, 102941-17-3; **28**, 102941-03-7; **28**·2HCl, 102941-18-4; **29**, 88914-21-0; **29**·HCl, 88913-62-6; **30**, 88914-23-2; **30**·HCl, 88913-64-8; **31**, 88914-24-3; **31**·HCl, 88913-65-9; **32**, 88914-26-5; **32**·HCl, 88913-67-1; **33**, 88914-22-1; **33**·HCl, 88913-63-7; **34**, 88914-27-6; **34**·2HCl, 102941-19-5; **35**, 102941-04-8; **35**·2HCl, 102941-20-8; **36**, 88914-25-4; **36**·HCl, 102941-21-9; **37**, 102941-05-9; **37**·HCl, 102941-22-0; **38**, 88914-28-7; **38**·HCl, 88913-69-3; **39**, 88914-30-1; **39**·HCl, 88913-71-7; **40**, 88914-31-2; **40**·HCl, 88913-72-8; **41**, 88914-29-8; **41**·HCl, 88913-70-6; **42**, 102941-06-0; **42**·HCl, 102941-23-1; I, 712-33-4; II, 102940-79-4; III, 88914-75-4; IV, 88914-76-5; V, 88413-07-4; 3-bromo-4-nitroacetanilide, 19230-47-8; *N*-(2-methylphenyl)benzimidoyl chloride, 19053-49-7; methyl 2-hydroxy-4-methoxybenzoate sodium salt, 102940-80-7; 2-methoxycarbonyl-5-methoxyphenyl *N*-(2-methylphenyl)benzimidate, 102940-81-8; methyl *N*-benzoyl-*N*-(2-methylphenyl)-4-methoxyanthranilate, 102940-82-9; *N*-benzoyl-*N*-(2-methylphenyl)-4-methoxyanthranilic acid, 102940-83-0; 3-methoxy-5-methyl-9-oxo-9,10-dihydroacridine, 102940-84-1; *N*-(2-methoxyphenyl)-4-fluoroanthranilic acid, 102940-85-2; 2-chloro-3-fluorobenzoic acid, 102940-86-3; 2-methoxyaniline, 90-04-0; 2-methylaniline, 95-53-4; *N*-(2-methylphenyl)-4-fluoroanthranilic acid, 102940-87-4; *N*-[(2-aminophenylamino)-3-methylamino]methanesulfonamide, 102940-88-5; 9-chloroacridine, 1207-69-8; 9-chloro-*N*,5-dimethyl-4-acridinecarboxamide, 88915-00-8; 9-chloro-2-methylacridine, 16492-09-4; 9-chloro-2-methoxyacridine, 16492-13-0; 2,9-dichloroacridine, 1019-14-3; 9-chloro-3-methylacridine, 16492-10-7; 9-chloro-3-methoxyacridine, 16492-14-1; 9-chloro-3-fluoroacridine, 2377-16-4; 3,9-dichloroacridine, 35547-70-7; 3-bromo-9-chloroacridine, 35547-72-9; 9-chloro-3-iodoacridine, 88914-90-3; 9-chloro-3-nitroacridine, 1744-91-8; 9-chloro-*N*-methyl-3-acridinamine, 88914-91-4; 9-chloro-3-(methoxycarbonylamino)acridine, 88914-92-5; 9-chloro-*N*-methyl-3-acridinecarboxamide, 102940-89-6; 9-chloro-4-methylacridine, 16492-11-8; 9-chloro-4-methoxyacridine, 16492-15-2; 9-chloro-4-fluoroacridine, 3829-32-1; 4,9-dichloroacridine, 10166-44-6; 4-bromo-9-chloroacridine, 4357-59-9; 9-chloro-4-acridinecarboxamide, 63178-96-1; 9-chloro-*N*-methyl-4-acridinecarboxamide, 63178-97-2; 9-chloro-*N*-(aminocarbonylmethyl)-4-acridinecarboxamide, 102940-90-9; 9-chloro-1,4-dimethylacridine, 21193-42-0; 1,9-dichloro-*N*-methyl-4-acridinecarboxamide, 102940-91-0; 9-chloro-3,4-dimethylacridine, 6514-58-5; 7-chlorobenz[*c*]acridine, 102940-92-1; 9-chloro-3,5-dimethylacridine, 88914-93-6; 9-chloro-3-fluoro-5-methylacridine, 88914-95-8; 3,9-dichloro-5-methylacridine, 88914-96-9; 3-bromo-9-chloro-5-methylacridine, 88914-98-1; 9-chloro-3-methoxy-5-methylacridine, 88914-94-7; 9-chloro-3-methyl-5-methoxyacridine, 88914-99-2; 9-chloro-3-fluoro-5-methoxyacridine, 102940-93-2; 3,9-dichloro-5-methoxyacridine, 88914-97-0; 3-bromo-9-chloro-5-methoxyacridine, 6534-56-1; 3,9-dichloro-*N*-methyl-5-acridinecarboxamide, 86187-39-5; 9-chloro-4,5-dimethylacridine, 63345-58-4; 9-chloro-4,5-dimethoxyacridine, 89784-84-9; 9-chloro-*N*,4-dimethyl-5-acridinecarboxamide, 88915-00-8; 9-chloro-4-methoxy-*N*-methyl-5-acridinecarboxamide, 88377-34-8; 1-methyl-9-[[2-(methylamino)-4-[(methylsulfonyl)amino]phenyl]amino]-acridine, 102941-24-2; 1-methylamsacrine, 102941-25-3; 2-methylamsacrine, 79453-36-4; 2-methoxyamsacrine, 90125-88-5; 2-chloroamsacrine, 102941-26-4; 3-methylamsacrine, 53478-40-3; 3-methoxyamsacrine, 79453-41-1; 3-fluoroamsacrine, 79453-42-2; 3-chloroamsacrine, 58658-30-3; 3-bromoamsacrine, 57164-79-1; 3-iodoamsacrine, 76708-40-2; 4-methylamsacrine, 51963-57-6; 4-methoxyamsacrine, 76708-48-0; 4-chloroamsacrine, 79453-50-2; 4-(methylaminocarbonyl)amsacrine, 76708-55-9.