Electron-Deficient DNA-Intercalating Agents as Antitumor Drugs: Aza Analogues of the Experimental Clinical Agent *N*-[2-(Dimethylamino)ethyl]acridine-4-carboxamide

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A series of azaacridine (benzonaphthyridine) analogues of the drug *N*-[2-(dimethylamino)ethyl]-acridine-4-carboxamide (DACA) (currently in clinical trial) were synthesized. These compounds showed DNA binding affinities similar to that of DACA, as determined by the fluorometric ethidium displacement assay, but were generally less potent cytotoxins against P388 leukemia *in vitro*. The only compounds showing higher cytotoxicity than DACA were analogues with nitro substituents at the (acridine) 1-position; by analogy with the 1-nitroacridine nitracrine, these compounds probably undergo reductive metabolism. The only azaacridine to show significant *in vivo* antileukemic activity was benzo[*b*][1,5]naphthyridine-6-carboxamide. A possible reason for the unexpectedly low activity of these compounds (given the wide acceptability of substituents in DACA) may be their much lower lipophilicities, which are likely to result in lower rates of cell uptake.

The anticancer drug *N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide (1; DACA, NSC 601316) emerged from a drug-development program exploring the utility of tricyclic DNA-intercalating chromophores possessing a cationic side chain attached via an electron-withdrawing function.[1] Later work showed that chromophores which were effectively uncharged at physiological pH had activity against remotely-implanted Lewis lung carcinoma *in vivo*,[2] with the acridine-4-carboxamide series showing particular utility.[3] DACA (1), the parent compound in this series, has exceptional activity against the Lewis lung[3] and colon 38 tumors.[4] It is highly effective against cell lines which express multidrug resistance mediated both by over-expression of P-glycoprotein and by alteration in the structure of the topoisomerase II enzyme[5] and is shortly to begin clinical trials.[6]

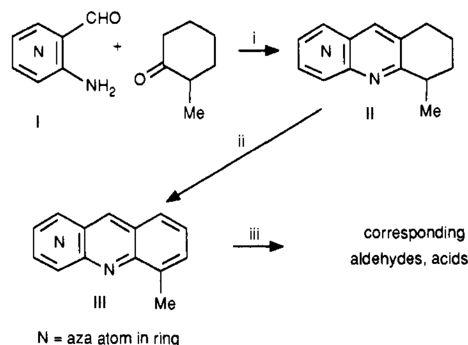
Both the nature and positioning of the carboxamide sidechain in the tricyclic carboxamides is critical, with *peri* positioning next to an electron-withdrawing atom in the central ring being required.[7] These structure–activity relationships have been related to their effects on the DNA binding kinetics of the compounds.[8] Carboxamides of related electron-deficient chromophores such as phenazines[9] also show broad-spectrum antitumor activity.

In this paper we report the preparation and evaluation of a further series of electron-deficient benzo[*b*]naphthyridine (azaacridine) analogues of the acridinecarboxamide 1, together with the corresponding oxo compounds and some nitro analogues. Previous work[10] on electron-deficient derivatives of the 9-anilinoacridine antitumor agent amsacrine 2 showed that both the benzo[*b*][1,7]- and benzo[*b*][1,8]naphthyridines (3 and 4), and the corresponding nitro compounds 5 and 6 had *in vivo* antileukemic activities comparable with that of the parent (but lower potency).

Chemistry

Most of the required precursor aldehydes and acids required for the synthesis of compounds 7–34 of Table 1 have been reported.[11-14] The benzo[*b*][1,5]-, benzo[*b*]-[1,7]-, and benzo[*b*][1,8]naphthyridines were prepared by the method shown in Scheme 1. Friedlander condensation of 2-methylcyclohexanone with the appropriate aminopyridinecarboxaldehydes (I) gave the tetrahydro intermediates (II), which were oxidized to the fully aromatic systems (III) before elaboration of the methyl group.[11,12] The benzo[*b*][1,6]naphthyridines were prepared[12] by the Pfitzinger reaction of 7-methylisatin and 1-benzyl-4-piperidinone to give 38, followed by one-pot oxidation, debenzylation, and decarboxylation to form 6-methylbenzo[*b*][1,6]naphthyridine (39) (Scheme 2).[12] 7-Nitro-5-oxo-5,10-dihydrobenzo[*b*][1,8]naphthyridine-9-carboxylic acid (36) was prepared by standard nitration

Scheme 1[a]



[a] (i) tBuOK/tBuOH/reflux/1.5 h; (ii) Pd/C/$(Ph)_2O$/reflux/4–5 h; (iii) SeO_2/chlorobenzene/reflux/ca. 10 h.

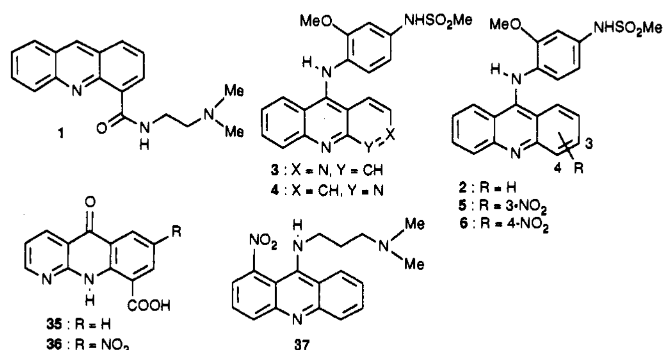


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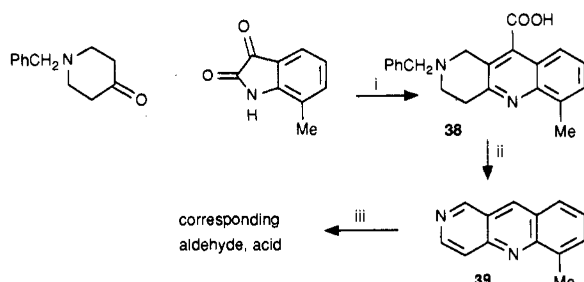
Table 1. Physicochemical and Biological Properties of Benzonaphthyridinecarboxamides and Analogues

no.	struct.	X	R	mp (°C)	formula	% yield[a]	C_{50}[b]	IC_{50}[c]	OD[d]	ILS[e]
7	i	H	$CONH(CH_2)_2NMe_2$	f			15	1.7	150	NA[g]
8	1	1-NO_2	$CONH(CH_2)_2NMe_2$	262–265	$C_{18}H_{18}N_4O_4·HCl$	85	23	0.55		
9	i	2-NO_2	$CONH(CH_2)_2NMe_2$	290–291	$C_{18}H_{18}N_4O_4·HCl$	90	4.5	0.91	45	NA
10	i	5-NO_2	$CONH(CH_2)_2NMe_2$	295–296	$C_{18}H_{18}N_4O_4·HCl$	87	2.4	0.083	45	65
11	i	6-NO_2	$CONH(CH_2)_2NMe_2$	266–267	$C_{18}H_{18}N_4O_4·HCl$	80	4.1	1.2	65	NA
12	i	7-NO_2	$CONH(CH_2)_2NMe_2$	316–317	$C_{18}H_{18}N_4O_4·HCl$	88	5.9	3.5		
13	ii	H	$CONH(CH_2)_2NMe_2$	170–172	$C_{17}H_{18}N_4O_2$	78	27.7	5.5	150	NA
14	ii	H	$CH{=}NNHCOCH_2NMe_3^+$	dec	$C_{18}H_{20}ClN_5O_2·4H_2O$	93	7.3	>40		
15	ii	H	$CH{=}NNHY$[h]	>300	$C_{16}H_{14}N_6O·HBr·0.25H_2O$	70	4.0	22		
16	ii	6-NO_2	$CONH(CH_2)_2NMe_2$	133–135	$C_{17}H_{17}N_5O_4$	80	10.4	0.018	65	31
17	ii	7-NO_2	$CONH(CH_2)_2NMe_2$	213–215	$C_{17}H_{17}N_5O_4$	88	11	1.3		
18	iii	H	$CONH(CH_2)_2NMe_2$	145–147	$C_{17}H_{18}N_4O_2·0.25H_2O$	60	18.5	>40		
19	iiia			140–142	$C_{18}H_{16}N_4O_3$[i]	25	10.7	>40		
20	iv	H	$CONH(CH_2)_2NMe_2$	223–225	$C_{17}H_{18}N_4O_2$	57	7.6	>40		
21	iv	9-NO_2	$CONH(CH_2)_2NMe_2$	210–212	$C_{17}H_{17}N_5O_4$[i]	70	6.2	1.0		
22	v	H	$CONH(CH_2)_2NMe_2$	288–290	$C_{17}H_{18}N_4O_2·HCl·0.5H_2O$	71	30.7	>40		
23	v	H	$CH{=}NNHY$[h]	>320	$C_{16}H_{14}N_6·HBr$	64	9.6	>40		
1	vi	H	$CONH(CH_2)_2NMe_2$	j		–	5	0.11	65	91
24	vii	H	$CONH(CH_2)_2NMe_2$	114–116	$C_{17}H_{18}N_4O·0.5H_2O$	93	7.6	0.49	65	NA
25	vii	H	$CH{=}NNHCOCH_2NMe_3^+$	dec	$C_{18}H_{20}ClN_5O·4H_2O$	84	11.1	1.65	65	NA
26	vii	H	$CH{=}NNHY$[h]	>250	$C_{16}H_{14}N_6·HBr·2H_2O$	71	5.0	12		
27	vii	6-NO_2	$CONH(CH_2)_2NMe_2$	148–150	$C_{17}H_{17}N_5O_3·1.25H_2O$	84	2.1	0.15	20	NA
28	viii	H	$CONH(CH_2)_2NMe_2$	178–180	$C_{17}H_{18}N_4O$	95[k]	4.6	8.3		
29	viii	H	$CH{=}NNHY$[h]	257–258	$C_{16}H_{14}N_6·HBr·1.5H_2O$	74	5.9	25		
30	ix	H	$CH{=}NNHY$[h]	205–207	$C_{16}H_{14}N_6·HBr·1.5H_2O$	67	6.6	12.3		
31	ix	9-NO_2	$CONH(CH_2)_2NMe_2$	172–174	$C_{17}H_{17}N_5O_3·0.25H_2O$	79	4.4	5.0		
32	x	H	$CONH(CH_2)_2NMe_2$	157–159	$C_{17}H_{18}N_4O$	95	11.1	4.4	65	48
33	x	H	$CH{=}NNHY$[h]	>320	$C_{16}H_{14}N_6·HBr·H_2O$	78	5.4	3.3	45	NA
34	x	9-NO_2	$CONH(CH_2)_2NMe_2$	163–165	$C_{17}H_{17}N_5O_3$	89	3.1	0.085	45	NA

[a] Yield in coupling step from acid or aldehyde. [b] C_{50} is the micromolar concentration of drug needed to displace 50% of previously-bound ethidium bromide from [poly(dA-dT)][poly(dA-dT)]; ref 17. [c] IC_{50} is the micromolar concentration of drug needed to inhibit growth of P388 leukemia cells in culture to 50% of control values, after a 70-h exposure; ref 18. [d] OD is the optimal dose of drug in milligrams/kilogram/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 leukemia cells. [e] ILS is the percentage increase in lifespan of drug-treated, tumor-bearing animals compared with tumor-bearing controls, when treated at the optimal dose. Values of >20% are considered significant. [f] Reference 7. [g] Not active (ILS < 20%) at all dose levels. [h] Y = (4,5-dihydro-1*H*-imidazol-2-yl). [i] No satisfactory microanalysis. [j] Reference 3. [k] Crude yield.

Scheme 2[a]



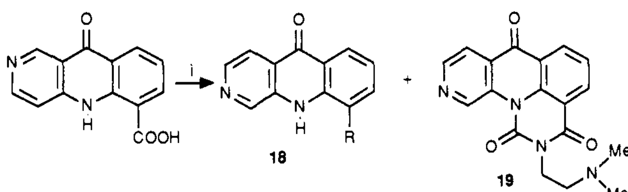
[a] (i) KOH/reflux/20 h; (ii) Pd/C/$(Ph)_2O$/reflux/4 h; (iii) SeO_2/chlorobenzene/reflux/ca. 10 h.

of 5-oxo-5,10-dihydrobenzo[*b*][1,8]naphthyridine-9-carboxylic acid (**35**).[11]

The amides were prepared from the corresponding acids via activated esters or mixed anhydrides; in most cases isobutyl chloroformate was used to form the intermediate, and reaction with *N,N*-dimethylethylenediamine gave the required amide. With some compounds, it was necessary to use an excess of the ester and diamine in order to achieve a good conversion of acid. In one instance under these conditions, a byproduct was isolated and assigned structure **19**. Formation of this compound is attributed to the excess of isobutyl chloroformate providing a means to bridge the amide and acridone nitrogens of the initially-formed **18** (Scheme 3).

Reaction of the appropriate aldehydes with 2-hydrazino-2-imidazoline hydrobromide under reflux in ethanol gave the corresponding hydrazones, while analogous reaction with (carboxymethyl)trimethylammonium chloride hydrazide gave the Girard T derivatives. The NMR spectra

Scheme 3[a]



[a] (i) Et_3N, then isobutyl chloroformate, −10 °C, then *N,N*-dimethylethylenediamine/CH_2Cl_2.

(in DMSO) of the Girard T derivatives, but not the corresponding hydrazones, showed the presence of two components in each. These were assigned as *E* and *Z* isomers, and shifts of the CH=N proton (upfield in the *Z* isomer) were in accord with the literature for hydrazones.[15,16] A significant difference was also noted in the shift of the side-chain CH_2 peak, with that for the *Z* isomer being downfield. The *Z* form was favored (2.4:1) for the [1,8]naphthyridine (**25**), while hydrogen bonding between the first side-chain nitrogen and the acridone NH is presumably responsible for the greater amount (1.4:1) of *E* isomer in the corresponding oxo derivative **14**.

For measurement of DNA binding and biological properties, those compounds not already in salt form were converted to the hydrochloride salts by crystallization of the free bases from MeOH/EtOAc/HCl.

Results and Discussion

DNA binding and biological data for the compounds studied are presented in Table 1. Compounds **24–34** are the fully-aromatic benzo[*b*]naphthyridines (azaacridines), while compounds **13–23** are the corresponding benzo[*b*]-naphthyridones (azaacridones). In the oxo series, the corresponding nitroacridone analogues (**7–12**) were also prepared, in order to compare the effects of these two series of electron-deficient heterocycles.[10]

The relative affinity of the compounds for DNA was estimated by the fluorometric ethidium displacement method,[17] using poly[d(A-T)] in 0.01 acetate buffer at pH 5. For intercalating ligands, the micromolar concentration of ligand required to displace 50% of previously-bound ethidium (the C_{50} value) is inversely proportional to the ligand–DNA association constant. In the oxo series, all four isomeric naphthyridonecarboxamides (**13, 18, 20, 22**) could be obtained. They possessed significantly lower C_{50}s than either the unsubstituted acridonecarboxamide (**7**) or the corresponding nitroacridones (**8–12**). In the fully aromatic series, only three of the parent naphthyridine-carboxamides could be obtained (**24, 28, 32**), but these showed similar levels of DNA binding to the acridine analogue (**1**; DACA). In this series the corresponding nitroacridinecarboxamides were not available. Where alternative side chains were used, DNA binding was broadly similar (e.g., **24–26**).

In vitro cytotoxicities were determined against exponentially-growing P388 cells in 96-well culture dishes, as described previously.[18] The parent compound (**1**; DACA) is moderately cytotoxic for a DNA intercalating agent, with an IC_{50} of 0.11 μM. The [1,8]naphthyridinecarboxamide (**24**) showed comparable activity (0.49 μM), but the isomeric compounds (**28, 32**) were less potent. The nitro analogues (**27, 34**) were significantly more cytotoxic than the respective parent compounds, possibly as a result of reductive metabolism. They have structures similar to that of the reductively-activated 1-nitroacridine nitracrine (**37**)[19] and would be expected to be even more easily reduced. The azaacridone (**16**) corresponding to **27** also showed very potent cytotoxicity *in vitro*.

In vivo evaluation of selected compounds (essentially those with IC_{50} values below approximately 3 μM) were carried out in mice inoculated intraperitoneally with 10^6 P388 leukemia cells. Drugs were given as solutions of the hydrochloride salts in 30% v/v aqueous ethanol on days 1, 5, and 9 after inoculation, at dose levels spaced 1.5-fold apart, covering the range from inactive to toxic. The parent compound (**1**) shows significant activity (albeit at only moderate potency), suggesting that this system can serve as a suitable prescreen for analogues. Two of the nitroacridones (**10** and **16**) showed moderate activity, but the only active acridine derivative was the benzo[*b*][1,5]-naphthyridine-6-carboxamide (**32**).

Conclusions

Previous studies[2,3] have shown that electron-deficient DNA-intercalating agents such as acridine-4-carboxamides have excellent anticancer activity, and the parent compound (**1**) is about to begin clinical trials. The azaacridine (benzonaphthyridine) derivatives studied here show similar levels of DNA binding, but in general were less potent cytotoxins *in vitro*. The only compounds with higher potency were the nitro-substituted compounds **27** and **34**, which probably undergo facile bioreductive activation. The benzo[*b*][1,5]naphthyridine-6-carboxamide (**32**) was the only azaacridine with any *in vivo* activity. These results are unexpected, since previous structure–activity studies[3] with nuclear-substituted analogues of **1** show there is a wide acceptability of substituents. A contributing factor to the relative inactivity of the benzonaphthyridines might be their much lower lipophilicities, which are likely to result in lower rates of cell uptake.

Experimental Section

Analyses indicated by symbols of the elements were within ±0.4% of theoretical. NMR spectra were obtained at 90 or 300 MHz and are referenced to Me_4Si. Thin-layer chromatography was carried out on aluminum-backed silica gel plates (Merck 60 F_{254}). Column chromatography was carried out on silica gel (Merck 230–400 mesh).

Preparation of *N*-[2-(Dimethylamino)ethyl]-2-nitro-9-oxo-9,10-dihydroacridine-4-carboxamide (9): Example of the General Method. A suspension of 2-nitro-9-oxo-9,10-dihydroacridine-4-carboxylic acid[14] (0.7 g, 2.5 mmol) and 1,1′-carbonyldiimidazole (0.6 g, 3.7 mmol) in dry DMF (15 mL) was warmed to 40 °C until it became homogeneous and gas evolution ceased. The mixture was cooled to 10 °C and *N,N*-dimethylethylenediamine (0.4 g, 0.4 mmol) was added. After 10 min at 20 °C, the mixture was partitioned between EtOAc and aqueous Na_2CO_3, and the organic layer was worked up to give *N*-[2-(dimethylamino)ethyl]-2-nitro-9-oxo-9,10-dihydroacridine-4-carboxamide (**9**) (0.68 g, 77%). Crystallization from MeOH/EtOAc containing a few drops of concentrated HCl gave the hydrochloride salt, mp 290–291 °C. Anal. ($C_{18}H_{18}N_4O_4$·HCl) C, H, N, Cl. Similar preparations from the requisite acids[13,14] gave the corresponding 1-, 5-, 6-, and 7-nitro analogues (**8, 10, 11, 12**) listed in Table 1.

Preparation of *N*-[2-(Dimethylamino)ethyl]-7-nitro-5-oxo-5,10-dihydrobenzo[*b*][1,8]naphthyridine-9-carboxamide (17): Example of the General Method. KNO_3 (1.2 g, 11.9 mmol) was added in portions over 30 min to a solution of 5-oxo-5,10-dihydrobenzo[*b*][1,8]naphthyridine-9-carboxylic acid (**35**)[12] (0.4 g, 1.7 mmol) in concentrated H_2SO_4 (8 mL). The mixture was stirred at room temperature for 48 h and then poured onto ice and the pH brought to 1-2 with concentrated NH_4OH. The bright yellow solid which separated was filtered off and washed with water to give 7-nitro-5-oxo-5,10-dihydrobenzo[*b*]-

[1,8]naphthyridine-9-carboxylic acid (36) (0.44 g, 86%): mp 315 °C dec ($(CH_3)_2SO$/EtOH); ^1H NMR (90 MHz, $(CD_3)_2SO$) δ 7.55 (dd, J = 8.0, 4.0 Hz, 1 H, H-3), 8.7 (dd, J = 7.0, 2.0 Hz, 1 H, H-4), 8.9 (dd, J = 4.0, 2.0 Hz, 1 H, H-2), 8.95–9.10 (2 d, J = 2 Hz, 2 H, H-6,8).

Freshly distilled Et_3N (0.5 mmol) was added to a stirred suspension of **36** (0.4 mmol) in CH_2Cl_2 (20 mL). The resulting solution was taken to <−10 °C, and a solution of isobutyl chloroformate (0.5 mmol) in CH_2Cl_2 (10 mL) was added dropwise over 30 min. After a further 30 min at this temperature, a solution of N,N-dimethylethylenediamine (0.5 mmol) in CH_2Cl_2 was slowly added, and the solution was stirred at <−5 °C for 30 min, 0 °C for 30 min, and room temperature for 1 h. A saturated solution of $NaHCO_3$ was then added. The organic layer was separated, washed with brine and water, and dried ($MgSO_4$), the solvent was evaporated, and the residue was triturated with petroleum ether (bp 60–80 °C) and filtered to give N-[2-(dimethylamino)-ethyl]-7-nitro-5-oxo-5,10-dihydrobenzo[b][1,8]naphthyridine-9-carboxamide (17) as an orange-red solid: 88%; mp 213–215 °C (toluene); ^1H NMR (300 MHz, $CDCl_3$) δ 2.4 (s, 6 H, NMe_2), 2.7 (t, J = 6 Hz, 2 H, CH_2N), 3.75 (br q, $NHCH_2$), 7.14 (dd, J = 7.7, 4.3 Hz, 1 H, H-3), 8.43 (d, J = 7.7 Hz, 1 H, H-4), 8.56 (d, J = 4.3 Hz, 1 H, H-2), 8.86 (d, J = 2.0 Hz, 1 H, H-8(6)), 9.06 (d, J = 2.0 Hz, 1 H, H-6(8)). Anal. ($C_{17}H_{17}N_5O_4$) C, H, N.

The following N-[2-(dimethylamino)ethyl]carboxamides were prepared in this manner. In addition to the NMR peaks listed below, all of these carboxamides had a common pattern for the side chain: δ 2.4 (s, 6 H, $N(CH_3)_2$), 2.7 (t, J = 6 Hz, 2 H, CH_2N), 3.75 (q, J = 6 Hz, 2 H, $NHCH_2$).

N-[2-(Dimethylamino)ethyl]benzo[b][1,8]naphthyridine-9-carboxamide (24): 93%; mp 114–116 °C [toluene/petroleum ether (bp 90–110 °C)]; ^1H NMR (90 MHz, $CDCl_3$) δ 7.45–7.8 (m, 2 H, H-3,7), 8.15 (dd, J = 8.0, 1.5 Hz, 1 H, H-6), 8.4 (dd, J = 9.0, 2.0 Hz, 1 H, H-3), 8.9 (s, 1 H, H-5), 9.05 (dd, J = 7.0, 1.5 Hz, 1 H, H-8), 9.3 (dd, J = 4.0, 2.0 Hz, 1 H, H-2). Anal. ($C_{17}H_{18}N_4O \cdot 0.5H_2O$), C, H, N.

N-[2-(Dimethylamino)ethyl]-5-oxo-5,10-dihydrobenzo[b]-[1,8]naphthyridine-9-carboxamide (13): 78%; mp 170–172 °C [toluene/petroleum ether (bp 90–110 °C)]; ^1H NMR (300 MHz, $CDCl_3$) δ 7.0–7.3 (m, 2 H, H-3,7), 7.54 (br s, 1 H, NH), 8.02 (d, J = 7.4 Hz, 1 H, H-8), 8.55 (d, J = 8 Hz, 1 H, H-6), 8.66–8.72 (m, 2 H, H-2,4). Anal. ($C_{17}H_{18}N_4O_2$) C, H, N.

N-[2-(Dimethylamino)ethyl]-6-nitrobenzo[b][1,8]naphthyridine-9-carboxamide (27): 84%; mp 148–150 °C [toluene/petroleum ether (bp 90–110 °C)]; ^1H NMR (90 MHz, $CDCl_3$) δ 7.65 (dd, J = 8.0, 4.0 Hz, 1 H, H-3), 8.5 (2 d, J = 8 Hz, 2 H, H-4,8), 9.1 (d, J = 8 Hz, 1 H, H-7), 9.4 (m, 1 H, H-2), 9.8 (s, 1 H, H-5). Anal. ($C_{17}H_{17}N_5O_3 \cdot 1.25H_2O$) C, H, N.

N-[2-(Dimethylamino)ethyl]-6-nitro-5-oxo-5,10-dihydrobenzo[b][1,8]naphthyridine-9-carboxamide (16): 80%; mp 133–135 °C (benzene); ^1H NMR (90 MHz, $CDCl_3$) δ 7.0–7.3 (m, 2 H, H-3,8), 7.95 (d, J = 9 Hz, 1 H, H-7), 8.55 (dd, J = 9.0, 2.0 Hz, 1 H, H-4), 8.7 (dd, J = 4.0, 2.0 Hz, 1 H, H-2). Anal. ($C_{17}H_{17}N_5O_4$) C, H, N.

N-[2-(Dimethylamino)ethyl]benzo[b][1,5]naphthyridine-6-carboxamide (32): 95%; mp 157–159 °C [petroleum ether (bp 90–110 °C)]; ^1H NMR (90 MHz, $CDCl_3$) δ 7.5–7.75 (m, 2 H, H-3,8), 8.15 (d, J = 9 Hz, 1 H, H-8), 8.45 (d, J = 7 Hz, 1 H, H-7), 8.85–9.1 (m, 3 H, H-2,4,10). Anal. ($C_{17}H_{18}N_4O$) C, H, N.

N-[2-(Dimethylamino)ethyl]-9-nitrobenzo[b][1,5]naphthyridine-6-carboxamide (34): 89%; mp 163–165 °C [petroleum ether (bp 90–110 °C)]; ^1H NMR (90 MHz, $CDCl_3$) δ 7.75 (dd, J = 9.0, 3.0 Hz, 1 H, H-3), 8.3–8.6 (m, 2 H, H-4,7), 8.95 (d, J = 8 Hz, 1 H, H-8), 9.1 (dd, J = 3.0, 1.5 Hz, 1 H, H-2), 9.8 (s, 1 H, H-10). Anal. ($C_{17}H_{17}N_5O_3$) C, H, N.

N-[2-(Dimethylamino)ethyl]-10-oxo-5,10-dihydrobenzo-[b][1,5]naphthyridine-6-carboxamide (22). This compound separated from the CH_2Cl_2 reaction solution as the hydrochloride salt in 71% yield: mp 288–290 °C (aqueous EtOH); ^1H NMR (300 MHz, $(CD_3)_2SO/D_2O$) δ 3.19 (s, 6 H, $N(CH_3)_2$), 3.65 and 3.8 (2 br s, 2 × 2 H, $(CH_2)_2$), 7.10 (t, J = 8 Hz, 1 H, H-8), 7.75 (dd, J = 8.0, 4.0 Hz, 1 H, H-3), 7.85–8.0 (m, 2 H, H-4,9(7)), 8.3 (d, J = 7.8 Hz, 2 H, H-7(9)), 8.73 (d, J = 4 Hz, 1 H, H-2). Anal. ($C_{17}H_{19}ClN_4O_2 \cdot 0.5H_2O$) C, H, N.

N-[2-(Dimethylamino)ethyl]benzo[b][1,7]naphthyridine-9-carboxamide (28). The crude product was obtained in 95% yield, but could not be purified by recrystallization, or by way of a perchlorate salt. Chromatography [silica; $CHCl_3$/MeOH (1:1)] gave pure product: mp 178–180 °C [petroleum ether (bp 90–110 °C)]; ^1H NMR (300 MHz, $CDCl_3$) δ 7.75 (t, J = 8.1 Hz, 1 H, H-7), 7.81 (d, J = 5.8 Hz, 1 H, H-4), 8.13 (dd, J = 8.3, 0.9 Hz, 1 H, H-6), 8.60 (d, J = 5.8 Hz, 1 H, H-3), 8.85 (s, 1 H, H-5), 9.00 (dd, J = 7.0, 0.9 Hz, 1 H, H-8), 9.36 (br s, 1 H, NH), 9.71 (s, 1 H, H-1). Anal. ($C_{17}H_{18}N_4O$) C, H, N.

N-[2-(Dimethylamino)ethyl]-10-oxo-5,10-dihydrobenzo-[b][1,6]naphthyridine-6-carboxamide (20). Only a low yield was obtained under the standard conditions but doubling the mole ratios of Et_3N, isobutyl chloroformate, and N,N-dimethylethylenediamine gave a 57% yield: mp 223–225 °C (toluene); ^1H NMR (300 MHz, $CDCl_3$) δ 7.15–7.30 (m, 2 H, H-4,8), 7.50 (br s, 1 H, NH), 7.97 (d, J = 7.2 Hz, 1 H, H-7), 8.55–8.65 (m, 2 H, H-3,9), 9.52 (s, 1 H, H-1). Anal. Found: C, 66.3; H, 5.8; N, 17.5. $C_{17}H_{18}N_4O_2$ requires: C, 65.8; H, 5.8; N, 18.1. The compound failed to give acceptable microanalysis figures due to retention of some recrystallizing solvent even after drying (detected by NMR).

N-[2-(Dimethylamino)ethyl]-9-nitro-10-oxo-5,10-dihydrobenzo[b][1,6]naphthyridine-6-carboxamide (21). This was prepared using double the normal reagent ratios. The residue obtained after removing the CH_2Cl_2 was dissolved in dilute HCl (pH 3–4), and the acid solution was extracted with $CHCl_3$. The aqueous layer was basified with dilute NH_4OH, and the product separated as an orange solid (70%): mp 210–212 °C (toluene); ^1H NMR (300 MHz, $(CD_3)_2SO$) δ 7.42 (d, J = 8.0 Hz, 1 H, H-7), 7.61 (d, J = 6.0 Hz, 1 H, H-4), 8.39 (d, J = 8.0 Hz, 1 H, H-8), 8.52 (d, J = 6.0 Hz, 1 H, H-3), 9.19 (s, 1 H, H-1), 11.1 (v br s, 1 H, NH). Satisfactory microanalysis figures could not be obtained.

N-[2-(Dimethylamino)ethyl]-5-oxo-5,10-dihydrobenzo[b]-[1,7]naphthyridine-9-carboxamide (18). 18 was prepared using double the normal reagent ratios. Chromatography of the crude product on alumina, and elution with $CHCl_3$ gave firstly N-[2-(dimethylamino)ethyl]-1H,7H-[1,7]naphthyridino[3,1-ij]-quinazoline-1,3,7(2H)-trione (19): R_f = 0.6; mp 140–142 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 2.32 (s, 6 H, $N(CH_3)_2$), 2.71 and 4.34 (2 t, 4 H, CH_2CH_2), 7.60 (t, J = 7.7 Hz, 1 H, H-3), 8.15 (d, J = 5.0 Hz, 1 H, H-8), 8.5–8.65 (m, 2 H, H-4,6), 8.70 (d, J = 5.0 Hz, 1 H, H-9), 10.24 (s, 1 H, H-11); ESMS 337 (MH^+). Further elution with $CHCl_3$ gave N-[2-(dimethylamino)ethyl]-9-oxo-9,10-dihydro-6-azaacridine-4-carboxamide (18): R_f = 0.2; mp 145–147 °C [toluene/petroleum ether (bp 90–110 °C)]; ^1H NMR (300 MHz, $CDCl_3$) δ 7.27 (t, J = 7.7 Hz, 1 H, H-7), 7.38 (br s, 1 H, NH), 7.98 (d, J = 7.3 Hz, 1 H, H-8), 8.14 (d, J = 5.1 Hz, 1 H, H-4), 8.46 (d, J = 5.1 Hz, 1 H, H-3), 8.59 (d, J = 7.9 Hz, 1 H, H-6), 8.95 (s, 1 H, H-1). Anal. ($C_{17}H_{18}N_4O_2 \cdot 0.25H_2O$) C, H, N.

N-[2-(Dimethylamino)ethyl]-9-nitrobenzo[b][1,6]naphthyridine-6-carboxamide (31). The acid and Et_3N were dissolved in DMF and treated sequentially with isobutyl chloroformate and N,N-dimethylethylenediamine in CH_2Cl_2 solution as usual. All solvents were taken off under reduced pressure, and the residue was partitioned between saturated Na_2CO_3 solution and $CHCl_3$. The organic extract gave the product (79%): mp 172–174 °C [petroleum ether (bp 90–110 °C)]; ^1H NMR (300 MHz, $CDCl_3$) δ 8.03 (d, J = 6.0 Hz, 1 H, H-4), 8.51 (d, J = 7.9 Hz, 1 H, H-7), 8.89 (d, J = 6.0 Hz, 1 H, H-3), 9.13 (d, J = 7.9 Hz, 1 H, H-8), 9.38 (br s, 1 H, NH), 9.65 (s, 1 H, H-1), 9.91 (s, 1 H, H-10). Anal. ($C_{17}H_{17}N_5O_3 \cdot 0.25H_2O$) C, H, N.

Preparation of Benzo[b][1,8]naphthyridine-9-carboxaldehyde (4,5-Dihydro-1H-imidazol-2-yl)hydrazone Hydrobromide (26): Example of the General Method for Hydrazone Synthesis. Equimolar amounts of benzo[b][1,8]-naphthyridine-9-carboxaldehyde[11] and 2-hydrazino-2-imidazoline hydrobromide were heated under reflux in EtOH solution until NMR monitoring of the solution or supernatant showed complete disappearance of the CHO signal (4 h). The solution was cooled to room temperature, when a solid separated. This was collected by filtration and recrystallized from EtOH/EtOAc to give benzo-[b][1,8]naphthyridine-9-carboxaldehyde (4,5-dihydro-1H-imidazol-2-yl)hydrazone hydrobromide (26) (71%): mp >250 °C dec; ^1H NMR (90 MHz, $(CD_3)_2SO$) δ 3.8 (s, 4 H, $(CH_2)_2$), 7.55–7.9 (m, 2 H, H-3,7), 8.35 (d, J = 8 Hz, 2 H, H-4(6)(8)), 8.55–8.8 (d

and br s, J = 8 Hz, 3 H, H-6,8(4) and NH), 9.2–9.4 (m and s, 2 H, H-2,5), 9.75 (s, 1 H, CH=N). Anal. ($C_{16}H_{15}BrN_6{\cdot}2H_2O$) C, H, N.

The following were also prepared in this way:

5-Oxo-5,10-dihydrobenzo[*b*][1,8]naphthyridine-9-carboxaldehyde (4,5-dihydro-1*H*-imidazol-2-yl)hydrazone hydrobromide (15) (24 h, 70%): mp >300 °C dec (EtOAc/EtOH); ^{1}H NMR (90 MHz, $(CD_3)_2SO$) δ 3.8 (s, 4 H, $(CH_2)_2$), 7.3–7.55 (m, 2 H, H-3,7), 8.1 (d, J = 8 Hz, 1 H, H-8), 8.35 (d, J = 8 Hz, 1 H, H-6), 8.6 (dd, J = 8.0, 2.0 Hz, 1 H, H-4), 8.65–8.9 (m, 2 H, H-2 and NH), 8.7 (s, 1 H, CH=N), 11.7 (br s, 1 H, NH). Anal. ($C_{16}H_{15}BrN_6O{\cdot}0.25H_2O$) C, H, N.

Benzo[*b*][1,5]naphthyridine-6-carboxaldehyde (4,5-dihydro-1*H*-imidazol-2-yl)hydrazone hydrobromide (33) (3 h, 78%): mp >320 °C dec (1-butanol); ^{1}H NMR (300 MHz, $(CD_3)_2SO$) δ 3.9 (s, 4 H, $(CH_2)_2$), 7.90 (t, J = 7.6 Hz, 1 H, H-8), 8.00 (d, J = 8.8, 3.7 Hz, 1 H, H-3), 8.52 (d, J = 8.3 Hz, 1 H, H-9), 8.59 (d, J = 8.8 Hz, 1 H, H-4), 8.8 (d, J = 6.9 Hz, 1 H, H-7), 8.90 (v br s, 1 H, NH), 9.25 (d, J = 3.7 Hz, 1 H, H-2), 9.4 (s and br s, 2 H, H-10 and NH), 9.70 (s, 1 H, CH=N). Anal. ($C_{16}H_{15}BrN_6{\cdot}H_2O$) C, H, N.

10-Oxo-5,10-dihydrobenzo[*b*][1,5]naphthyridine-6-carboxaldehyde (4,5-dihydro-1*H*-imidazol-2-yl)hydrazone hydrobromide (23) (12 h, 64%): mp >320 °C dec (AcOH/EtOH/water); ^{1}H NMR (300 MHz, $(CD_3)_2SO$) δ 3.9 (s, 4 H, $(CH_2)_2$), 7.5 (t, J = 8 Hz, 1 H, H-8), 7.91 (dd, J = 8.4, 4.0 Hz, 1 H, H-3), 8.2 (d, J = 7.5 Hz, 1 H, H-7), 8.27 (d, J = 8.4 Hz, 1 H, H-4), 8.51 (d, J = 7.8 Hz, 1 H, H-9), 8.74 (s, 1 H, CH=N), 8.8 (d, J = 4.0 Hz, 1 H, H-2), 9.0 and 11.7 (2 br s, 2 H, NH). Anal. ($C_{16}H_{15}BrN_6O$) H, N (a satisfactory C analysis could not be obtained).

Benzo[*b*][1,7]naphthyridine-9-carboxaldehyde (4,5-dihydro-1*H*-imidazol-2-yl)hydrazone hydrobromide (29) (3 h, 74%): mp 257–258 °C (EtOH); ^{1}H NMR (300 MHz, $(CD_3)_2SO$) δ 3.78 (s, 4 H, $(CH_2)_2$), 7.84 (t, J = 7.8 Hz, 1 H, H-7), 8.08 (d, J = 5.9 Hz, 1 H, H-4), 8.35 (d, J = 7.9 Hz, 1 H, H-6), 8.59 (d, J = 5.9 Hz, 1 H, H-3), 8.68 (d, J = 6.7 Hz, 1 H, H-8), 8.83 (v br s, 1 H, NH), 9.28 (s, 1 H, H-5), 9.58 (s, 2 H, H-1 and CH=N). Anal. ($C_{16}H_{15}BrN_6{\cdot}1.5H_2O$) C, H, N.

Benzo[*b*][1,6]naphthyridine-6-carboxaldehyde (4,5-dihydro-1*H*-imidazol-2-yl)hydrazone hydrobromide (30) (3 h, 67%): mp 205–207 °C (EtOH/*i*Pr$_2$O); ^{1}H NMR (300 MHz, $(CD_3)_2SO$) δ 3.80 (s, 4 H, $(CH_2)_2$), 7.46 (t, J = 7.8 Hz, 1 H, H-8), 7.69 (d, J = 6.3 Hz, 1 H, H-4), 8.02 (d, J = 8.0 Hz, 1 H, H-9), 8.29 (d, J = 6.7 Hz, 1 H, H-7), 8.43 (d, J = 6.3 Hz, 1 H, H-3), 8.99 (s, 1 H, H-100, 9.11 (s, 1 H, H-1), 9.29 (s, 1 H, CH=N). Anal. ($C_{16}H_{15}BrN_6{\cdot}1.5H_2O$) C, H, N.

Preparation of Benzo[*b*][1,8]naphthyridine-9-carboxaldehyde Girard T Chloride (25): Example of the General Method for the Preparation of Girard T Analogues. Equimolar amounts of benzo[*b*][1,8]naphthyridine-9-carboxaldehyde[11] and (carboxymethyl)trimethylammonium chloride hydrazide were heated under reflux in EtOH solution for 2 h. Cooling of the mixture gave a solid, which was collected and recrystallized from EtOH to give benzo[*b*][1,8]naphthyridine-9-carboxaldehyde Girard T chloride (25): 84%; mp >300 °C dec (EtOAc/AcOH); ^{1}H NMR (300 MHz, $(CD_3)_2SO$) δ 3.46 (s, 9 H, $N(CH_3)_3$), 4.50 (s, CH_2[*E*]), 5.03 (s, CH_2[*Z*]), 7.79 (dd, J = 8.4, 3.9 Hz, 1 H, H-3), 7.93 (t, J = 7.3 Hz, 1 H, H-7), 8.48 (d, J = 8.5 Hz, 1 H, H-6), 8.65 (d, J = 7.1 Hz, 1 H, H-8), 8.80 (dd, J = 8.4, 1.7 Hz, 1 H, H-4), 9.41 (dd, J = 3.9, 1.9 Hz, 1 H, H-2), 9.46 (s, 1 H, H-5), 9.63 (s, CH=N[*Z*]), 9.85 (s, CH=N[*E*]), 11.85 (s, NH [*Z*:*E* ratio = 2.4]). Anal. ($C_{18}H_{20}ClN_5O{\cdot}4H_2O$) C, H, N.

Also prepared in this way (after heating for 10 h) was 5-oxo-5,10-dihydrobenzo[*b*][1,8]naphthyridine-9-carboxaldehyde Girard T chloride (14): 93%; mp >300 °C dec (EtOAc/AcOH); ^{1}H NMR (300 MHz, $(CD_3)_2SO$) δ 3.43 (s, 9 H, $N(CH_3)_3$), 4.52 (s, CH_2[*E*]), 5.21 (s, CH_2[*Z*]), 7.5–7.61 (m, 2 H, H-3,7), 8.18 (d, J = 5.9 Hz, 1 H, H-8), 8.45 (d, J = 6.6 Hz, 1 H, H-6), 8.58 (s, CH=N-[*Z*]), 8.7–8.77 (m, 1 H, H-4), 8.78 (s, CH=N[*E*]), 8.96 (m, 1 H, H-2), 11.50 (s, NH [*E*:*Z* ratio = 1.4]). Anal. ($C_{18}H_{20}ClN_5O_2{\cdot}4H_2O$) C, H, N.

DNA Binding. The relative affinity of the compounds for DNA was estimated by the fluorometric ethidium displacement method,[17] using poly[d(A-T)] in 0.01 SHE buffer at pH 7. For intercalating ligands, the micromolar concentration of ligand required to displace 50% of previously-bound ethidium (the C_{50} value) is inversely proportional to the ligand–DNA association constant.[17]

Biological Evaluation. *In vitro* cytotoxicities were determined against exponentially-growing P388 cells in 96-well culture dishes, as described previously.[18] *In vivo* evaluations with selected compounds were carried out in mice inoculated intraperitoneally with 10^6 P388 leukemia cells. Drugs were given as solutions of the hydrochloride salts in 30% v/v aqueous ethanol on days 1, 5, and 9 after inoculation, at dose levels spaced 1.5-fold apart covering the range from inactive to toxic.

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References

(1) Atwell, G. J.; Cain, B. F.; Baguley, B. C.; Finlay, G. J.; Denny, W. A. Potential Antitumor Agents. 43. Synthesis and biological activity of dibasic 9-aminoacridine-4-carboxamides: a new class of antitumor agent. *J. Med. Chem.* 1984, *27*, 1481–1485.
(2) Denny, W. A.; Atwell, G. J.; Rewcastle, G. W.; Baguley, B. C. Potential Antitumor Agents. 49. 5-Substituted derivatives of N-[2-(dimethylamino)ethyl]-9-aminoacridine-4-carboxamide with in vivo solid tumor activity. *J. Med. Chem.* 1987, *30*, 658–663.
(3) Atwell, G. J.; Rewcastle, G. W.; Baguley, B. C.; Denny, W. A. Potential Antitumor Agents. 50. In vivo solid tumor activity of derivatives of N-[2-(dimethylamino)ethyl]acridine-4-carboxamide. *J. Med. Chem.* 1987, *30*, 664–669.
(4) Unpublished work, this laboratory.
(5) Finlay, G. J.; Marshall, E. S.; Matthews, J. H. L.; Paull, K. D.; Baguley, B. C. *In vitro* assessment of N-[2-(dimethylamino)ethyl]-acridine-4-carboxamide (DACA), a DNA intercalating antitumor drug with reduced sensitivity to multidrug resistance. *Cancer Chemother. Pharmacol.* 1993, *31*, 401–406.
(6) Haldane, A.; Finlay, G. J.; Gavin, J. B.; Baguley, B. C. Unusual dynamics of killing of cultured Lewis lung cells by the DNA-intercalating antitumour agent N-[2-(dimethylamino)ethyl]acridine-4-carboxamide. *Cancer Chemother. Pharmacol.* 1992, *29*, 475–479.
(7) Palmer, B. D.; Rewcastle, G. W.; Baguley, B. C.; Denny, W. A. Potential Antitumor Agents. 54. Chromophore requirements for in vivo antitumor activity among the general class of tricyclic carboxamides. *J. Med. Chem.* 1988, *31*, 707–712.
(8) Wakelin, L. P. G.; Atwell, G. J.; Rewcastle, G. W.; Denny, W. A. Relationships between DNA binding kinetics and biological activity for the 9-aminoacridine-4-carboxamide class of antitumor agents. *J. Med. Chem.* 1987, *30*, 855–862.
(9) Rewcastle, G. W.; Denny, W. A.; Baguley, B. C. Potential Antitumor Agents. 51. Synthesis and antitumor activity of phenazine-1-carboxamides. *J. Med. Chem.* 1987, *30*, 843–851.
(10) Denny, W. A.; Atwell, G. J.; Cain, B. F. Potential Antitumor Agents. Part 25. Azalogues of the 4′-(9-acridinylamino)-methanesulfonanilides. *J. Med. Chem.* 1977, *30*, 1242–1246.
(11) Chen, Q.; Deady, L. W. Synthesis of some benzo[*b*][1,8] and [1,5]-naphthyridines. *J. Heterocycl. Chem.* 1992, *29*, 1197–1201.
(12) Chen, Q.; Deady, L. W. *Synthesis* of some benzo[*b*][1,6] and [1,7]-naphthyridines. *Aust. J. Chem.* 1993, *46*, 987–993.
(13) Rewcastle, G. W.; Denny, W. A. The synthesis of substituted 9-oxoacridan-4 (or -5)-carboxylic acids. Part 2. The use of 2-iodoisophthalic acid in the Jourdan-Ullmann reaction. *Synthesis* 1985, *217*–220.
(14) Rewcastle, G. W.; Denny, W. A. The synthesis of substituted 9-oxoacridan-4 (or -5)-carboxylic acids. Part 3. The reaction of methyl anthranilates with diphenyliodonium-2-carboxylates. *Synthesis* 1985, 220–222.
(15) Karabatsos, G. J.; Taller, R. A. Structural studies by nuclear magnetic resonance. V. Phenylhydrazones. *J. Am. Chem. Soc.* 1963, *85*, 3624–3629.
(16) Bell, C. F.; Mortimore, G. R. ^{1}H NMR spectra of E and Z pyridine-2-carbaldehyde, 2′-pyridylhydrazone and related compounds. *Org. Magn. Reson.* 1975, *7*, 512–517.
(17) Baguley, B. C.; Falkenhaug, E.-M. The interaction of ethidium with synthetic double-stranded polynucleotides at low ionic strength. *Nucleic Acids Res.* 1975, *5*, 161–171.
(18) Finlay, G. J.; Wilson, W. R.; Baguley, B. C. Comparison of the in vitro activity of cytotoxic drugs towards human carcinoma and leukemia cell lines. *Eur. J. Cancer Clin. Oncol.* 1986, *22*, 655–662.
(19) Wilson, W. R.; Anderson, R. F.; Denny, W. A. Hypoxia-selective antitumor agents. 1. Relationships between structure, redox properties and hypoxia-selective cytotoxicity for 4-substituted derivatives of nitracrine. *J. Med. Chem.* 1989, *32*, 23–30.