Synthesis and Antitumor Activity of Pyrido-Amsacrine Analogues and Related Compounds

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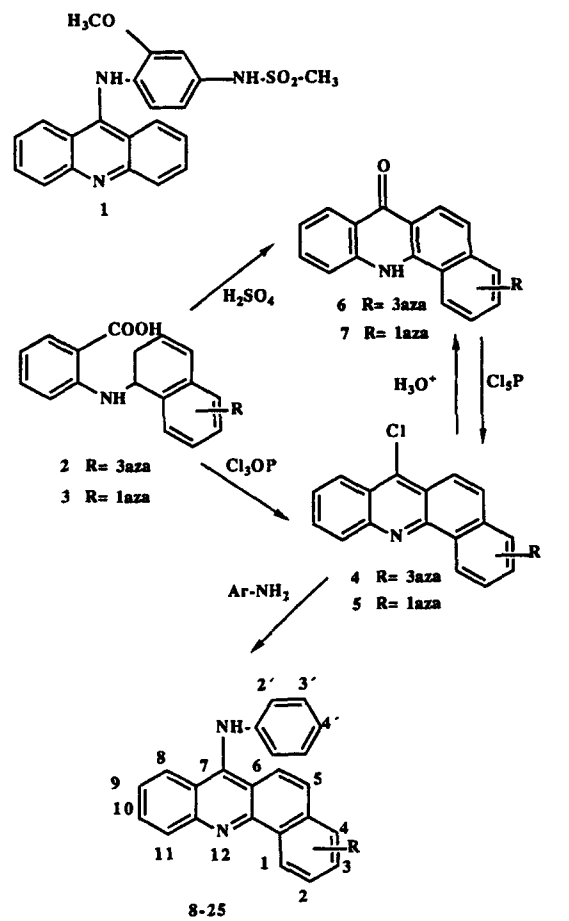
Abstract □ The pyrido derivatives of amsacrine [4'-(9-acridinylamino)methanesulfon-*m*-anisidine] were prepared and evaluated in the L1210 leukemia system. Almost all the pyrido analogues were tighter DNA-binding ligands than the corresponding amsacrine compounds. The significant inhibition of L1210 produced by pyrido-acridan-7-ones demonstrates that the anilino side chain is not essential for activity, although most of the compounds did not have improved activity compared with amsacrine.

The general class of 9-anilino acridines possesses antineoplastic activity towards various disseminated tumors.[1] These compounds fall into a general group that binds tightly to double-stranded DNA by intercalation of the acridine chromophore between the base pairs.[2] Topoisomerase II is suggested to be the primary target for these compounds, which have been shown to induce protein-associated DNA strand breaks by stabilizing a "cleavable complex" between topoisomerase II and DNA.[3] The clinically useful antileukemic agent amsacrine [4'-(9-acridinylamino)methanesulfon-*m*-anisidine; **1**] is a member of this class of antitumor agents. An extensive set of data on amsacrine and derivatives has been gathered by Denny and co-workers.[4–9] However, these anilino derivatives are rapidly metabolized in vivo to yield nontoxic and tumor-inactive products by nucleophilic attack at the C-9 position by plasma thiols, resulting in the loss of the anilino moiety, and by microsomal oxidation to the corresponding quinoneimines to yield compounds that possess a secondary amine functionality at the C-4' position.[10] To obtain antitumor compounds (not limited to linear tricyclic chromophores) with higher metabolic stability at the C-4' position, we synthesized, characterized, and evaluated the DNA binding and biological properties of some 7-anilino-pyridoacridines.

Results and Discussion

Chemistry—The required chloropyridoacridines (**4** and **5**) were synthesized by ring closure of anthranilic acids (**2** and **3**),[11] which were in turn produced by a modified Jourdan–Ullmann reaction[12] (see *Experimental Section*). When closure of anthranilic acids was effected with sulfuric acid, the 12*H*-benzo(b)(1,8)-phenanthrol-7-one (**6**) and 12*H*-benzo(b)(1,10)-phenanthrol-7-one (**7**) were obtained. These compounds can be readily converted to the chloro derivatives (**4** and **5**) by treating with phosphorous pentachloride. The chloro derivatives (**4** and **5**) coupled satisfactorily with the appropriate anilines, provided the corresponding 7-anilinobenzo(b)(1,8)- and 7-anilinobenzo(b)(1,10)phenanthrolines (**8–25**, Scheme I). The physicochemical and ^{1}H NMR data for these compounds are reported in Tables I and II.

The Rm values (chromatographic measures of drug lipophilicity) from reversed-phase high-performance liquid chromatography have been used as a relative measure of overall molecular lipophilic–hydrophilic balance. The observed lipophilicities of these derivatives (Table III) can be compared in some cases with the corresponding amsacrine derivatives.[13,14] As expected, the pyrido analogues proved more hydrophilic than the amsacrine derivatives.



8	R= 3aza 4'=H	**17**	R= 3aza 4'=NH-SO_2-Ph
9	R= 3aza 4'=F	**18**	R= 3aza 4'=NH-SO_2-p-Ph-CH_3
10	R= 3aza 4'=Br	**19**	R= 3aza 4'=NH-SO_2-CH_3 2'=OCH_3
11	R= 3aza 4'=OH	**20**	R= 1aza 4'=H
12	R= 3aza 4'=OCH_3	**21**	R= 1aza 4'=F
13	R= 3aza 4'=$N(CH_3)_2$	**22**	R= 1aza 4'=$N(CH_3)_2$
14	R= 3aza 4'=NH-CO-CH_3	**23**	R= 1aza 4'=NH-SO_2-CH_3
15	R= 3aza 4'=SO_2-NH_2	**24**	R= 1aza 4'=NH-SO_2-Ph
16	R= 3aza 4'=NH-SO_2-CH_3	**25**	R=1aza 4'=NH-SO_2-CH_3 2'=OCH_3

Scheme I

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Table I—Analytical Data for 4–25

Compound[a]	mp, °C[b]	Yield, %	IR Data ν, cm^{-1}
4	204–205(A)	45	1580(CC), 750(CCl)
5	188–189(A)	40	1600(CC), 740(CCl)
6	350(B)	60	3100(NH), 1620(CO)
7	350(B)	56	3150(NH), 1625(CO)
8	288–289(C)	54	3360(NH), 1600(CC)
9	283(C)	46	3400(NH), 1025(CF)
10	270–272(C)	59	3300(NH), 650(CBr)
11	311–312(C)	66	3100–3400(NH,OH)
12	226–227(D)	48	3300(NH), 1250(OC)
13	193–194(D)	42	3400(NH)
14	200(B)	48	3350(NH), 1650(CONH)
15	308–309(B)	59	3100–3360(NH), 1350(SO_2NH_2)
16	310–311(C)	62	3100–3300(NH)
17	248–249(C)	60	3100–3460(NH)
18	260–261(B)	55	3040–3400(NH)
19	284–285(B)	58	3100–3350(NH), 1210(OC)
20	308–309(C)	50	3310(NH), 1600(CC)
21	288–289(C)	50	3400(NH), 1025(CF)
22	202–203(D)	56	3350(NH)
23	310–311(C)	65	3050–3350(NH)
24	260–261(B)	52	3100–3450(NH)
25	296–297(B)	60	3100–3350(NH)

[a] Satisfactory analytical data (+0.4% for C, H, N, O, S) were reported for all new compounds listed in the table. [b] Solvent recrystallization: A, benzene; B, ethanol; C, ethyl acetate; D, chloroform.

Binding to DNA—Quantitative correlations have been demonstrated between drug binding affinity for DNA and both in vivo antitumor activity and in vitro cytotoxic potencies.[15,16] In addition, long residence times at specific DNA binding sites contribute to increased cytotoxic action.[17] The generally accepted model for amsacrine binding to DNA is with the acridine chromophore intercalated in the same manner as in 9-aminoacridine, between adjacent base pairs.[18] Almost all these derivatives studied bind more strongly to DNA than amsacrine (see Table III). The more lipophilic groups at the C-4′ position enhance binding in both series (3-aza and 1-aza), whereas 4–7 with no substitutient at C-7, as expected, have reduced binding to DNA.[19] This reduced binding to DNA has the advantage that nonspecific binding to other cell macromolecules, such as proteins and lipids (which correlates with chromophore size and lipophility), will also be reduced.[20]

In Vitro Growth Inhibition—The growth inhibition assays were performed on L1210 murine leukemia cells (Table III). The 7-anilino derivatives (8–25) are approximately as active as the acridanones (6 and 7). This result is in disagreement with the binding model for a majority of the tumor-active intercalating agents with acridine chromophores in which the side chain is considered essential for biological activity. Whereas the intercalative process for amsacrine concerns only the acridine chromophore, inhibition of topoisimerase II involves the anilino ring, which lodges in the minor groove with a nearly orthogonal orientation with respect to the mean plane of the acridine ring.[21] The results obtained can be explained by the presence of the additional pyridine ring. The tetracyclic chromophore may facilitate inhibition of topoisomerase II by binding more tightly to DNA and reducing the kinetics of drug–DNA dissociation. The substituted aniline ring is not required for antitumor activity in this series, and varying its structure does not induce large changes in activity.

A lack of activity at 1700 nM was observed for the chloro derivatives (4 and 5). These derivatives do not contain a hydrogen atom that is able to provide additional electrostatic binding to the target, as do the anilino group in 8–25 and the acridine nitrogen in 6 and 7. The lack of activity of the chloro derivatives 4 and 5 also shows that under physiologic conditions 4 and 5 are not hydrolyzed to the active acridanones 6 and 7, respectively. The electronic character of the 4′-substituent did not have significant effects on activity. Thus, powerful electron-donor substituents [-$N(CH_3)_2$; OCH_3] and electron-withdrawing substituents (-F; -$NH\text{-}SO_2CH_3$) gave compounds with similar activities.

These observations are quite different from those for the amsacrine series in which electron-withdrawing anilino ring substitution abolishes activity. The explanation for these observations can be related to the extension of the acridine chromophore of amsacrine; however, the structure–activity relationships are incomplete, possibly because inhibition of topoisomerase II activity depends on other structural features in addition to those that contribute to binding to DNA. The attachment of a pyridine heterocycle to the acridine ring does not alter DNA binding properties; likewise, the nitrogen atom position has no influence in this regard, with 3-aza and 1-aza derivatives sharing similar activity.

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 of the LD_{10} (dose that is lethal in 10% of doses) and can be used as a comparative measure of drug potency (Table III). The difference between OD and D_{50} (minimum dose of drug to give a 50% increase of life span) values is a measure of the therapeutic effectiveness or the steepness of the dose–response curve for a compound. The ILS (increase of lifespan) values can be used as a comparative indication of the tumor cell kill, or tumor cell selectivity, of the compounds.

The activities of the compounds when dosed intraperitoneally against L1210 leukemia vary over a considerable range. No correlation between the pharmacological activity of the compounds and their ability to bind to nucleic acids was found. None of these compounds is more active than amsacrine; however, 7-acridanone derivatives (6 and 7) appear to be slightly more potent than the others.

In summary, the fact that the several of these derivatives share antitumor activity indicates that the ring system that intercalates with DNA can contain a four-ring heterocycle, whereas the anilino chain is not an essential component required for binding to DNA. However, the amine binds electrostatically to DNA and, in general, is required to solubilize these hydrophobic heterocyclic systems.

Experimental Section

Equipment—Melting points are uncorrected and were determined with a Büchi capillary melting point apparatus. The IR spectra were obtained on a Perkin-Elmer 577 spectrophotometer, in KBr pellets. The ^1H NMR spectra were recorded on a Varian VXR-300 (300 MHz) spectrometer. Elemental analyses were determined on a Perkin-Elmer 2400 CHN microanalyzer. Thin-layer chromatography was carried out with UV-sensitive plates precoated with silica gel (60-F-245 Merck; 0.2 mm thick). Kieseigel 60 Merck (230–400 mesh; 0.04–0.06 mm) was used for flash chromatography according to the procedure of Still et al.[22]

Syntheses—*N*-(5-Isoquinolyl)anthranilic acid (2) and *N*-(8-quinolyl)anthranilic acid (3) were prepared in the same manner as described by Sanchez et al.[12] 7-Chloropyridoacridines (4 and 5) were prepared as described previously by Elslager and Tendick.[11]

12H-Benzo(b)(1,8)- and 12H-Benzo(b)(1,10)phenanthrol-7-ones (6 and 7)—The corresponding anthranilic acid (2 and 3, 8 g, 30 mmol) in sulfuric acid (120 mL) was heated at 100 °C for 5 h. The solution was cooled to 5 °C, and cold water (500 mL) was added very slowly to avoid rapid elevation of temperature. Strict temperature control was essential to obtain a pure product in a acceptable yield. Slightly elevated reaction temperature increased the formation of side products and decreased the total yield. The precipitated salt was filtered and dissolved in water (100 mL). The solution was neutralized with

Table II—Nuclear Magnetic Resonance Chemical Shift Assignments

Compound	^{1}H NMR(δ), ppm/TMS
2[a]	6.93 (d, 1, 1CH, J = 7.7 Hz), 7.56–7.80 (m, 2, 2CH), 7.93 and 8.78 (2d, 2, 2CH, J = 6.0 Hz), 8.20–8.60 (m, 4, 4CH), 9.30 (s, 1, 1CH), 12.05 (s, 1, COOH).
3[a]	7.26 (t, 1, 1CH, J = 8.00 Hz), 7.55–7.70 (m, 3, 3CH), 8.05 (d, 1, 1CH, J = 8.00 Hz), 8.20–8.60 (m, 4, 4CH), 8.90 (d, 1, 1CH, J = 4.5 Hz), 12.00 (s, 1, COOH).
4[b]	7.67–7.86 and 8.29–8.39 (2m, 4, 4CH), 7.75 and 8.19 (2d, 2, 2CH, J = 9.4 Hz), 8.88 and 9.15 (2d, 2, 2CH, J = 4.3 Hz), 9.20 (s, 1, 1CH).
5[b]	7.60–7.85 and 8.30–8.45 (2m, 5, 5CH), 8.00 (d, 1, 1CH, J = 9.3 Hz), 7.60 (d, 1, 1CH, J = 9.5 Hz), 8.75 (d, 1, 1CH, J = 9.5 Hz), 9.10 (d, 1, 1CH, J = 5.1 Hz).
6[b]	7.41–7.85 and 8.05–8.40 (2m, 6, 6CH), 8.80 and 9.00 (2d, 2, 2CH, J = 5.0 Hz), 9.44 (s, 1, 1CH), 12.00 (s, 1, NH).
7[b]	7.35 (t, 1, 1CH, J = 4.3 Hz), 7.65–7.85 and 8.10–8.25 (m, 6, 6CH), 8.50 (d, 1 1CH, J = 8.3 Hz), 9.07 (d, 1, 1CH, J = 5.2 Hz), 12.33 (s, 1, NH).
8[b]	7.23–6.94 (m, 5, 5CH), 7.65 and 7.93 (2d, 2, 2CH, J = 7.1 Hz), 8.32 (m, 2, 2CH), 8.05 and 7.85 (2d, 2, 2CH, J = 9.5 Hz), 9.13 and 8.91 (2d, 2, 2CH, J = 5.2 Hz), 9.36 (s, 1, 1CH).
9[b]	6.92–7.07 (m, 4, 4CH), 7.65 and 7.93 (2d, 2, 2CH, J = 7.9 Hz), 8.30 (m, 2, 2CH), 8.04 and 7.88 (2d, 2, 2CH, J = 9.5 Hz), 9.12 and 8.90 (2d, 2, 2CH, J = 5.2 Hz), 9.34 (s, 1, 1CH).
10[b]	6.80–7.07 (m, 4, 4CH), 7.60 and 7.80 (2d, 2, 2CH, J = 7.6 Hz), 8.10 (m, 2, 2CH), 8.00 and 7.56 (2d, 2, 2CH, J = 9.1 Hz), 9.10 and 8.90 (2d, 2, 2CH, J = 5.5 Hz), 9.40 (s, 1, 1CH).
11[b]	6.84 and 6.75 (2d, 4, 4CH, J = 8.7 Hz), 7.52 and 7.83 (2d, 2, 2CH, J = 7.4 Hz), 8.22 and 8.28 (2d, 2, 2CH, J = 8.1 Hz), 8.03 and 7.70 (2d, 2, 2CH, J = 8.5 Hz), 9.06 and 8.82 (2d, 2, 2CH, J = 5.3 Hz), 9.12 (s, 1, 1CH), 9.20 (s, 1, NH), 9.27 (s, 1, OH).
12[b]	3.74 (s, 3, OCH_3), 6.93 (2d, 4, 4CH, J = 8.7 Hz), 7.58 and 7.88 (2d, 2, 2CH, J = 7.9 Hz), 8.27 and 8.30 (2d, 2, 2CH, J = 8.3 Hz), 8.05 and 7.76 (2d, 2, 2CH, J = 9.1 Hz), 9.10 and 8.87 (2d, 2, 2CH, J = 5.5 Hz), 9.31 (s, 1, 1CH), 9.24 (s, 1, NH).
13[b]	2.85 (s, 6, NMe_2), 6.90 and 6.68 (2d, 4, 4CH, J = 8.6 Hz), 7.50 and 7.82 (2d, 2, 2CH, J = 7.7 Hz), 8.20 and 8.30 (2d, 2, 2CH, J = 8.5 Hz), 8.05 and 7.68 (2d, 2, 2CH, J = 8.7 Hz), 9.06 and 8.82 (2d, 2, 2CH, J = 5.3 Hz), 9.12 (m, 2, CH, NH).
14[b]	2.15 (s, 3, $COCH_3$), 7.62 and 7.10 (2d, 4, 4CH, J = 8.7 Hz), 7.64 and 7.97 (2d, 2, 2CH, J = 7.3 Hz), 8.35 and 8.47 (2d, 2, 2CH, J = 8.1 Hz), 8.10 and 7.84 (2d, 2, 2CH, J = 8.6 Hz), 9.27 and 8.93 (2d, 2, 2CH, J = 5.7 Hz), 9.41 (s, 1, 1CH), 10.09 (s, 1, NH).
15[b]	7.14 (s, 2, SO_2NH_2), 7.63 and 6.85 (2d, 4, 4CH, J = 8.5 Hz), 7.71 and 7.96 (2d, 2, 2CH, J = 7.7 Hz), 8.25 and 8.38 (2d, 2, 2CH, J = 8.3 Hz), 8.37 and 8.24 (2d, 2, 2CH, J = 8.6 Hz), 9.13 and 8.90 (2d, 2, 2CH, J = 5.7 Hz), 9.35 (s, 1, 1CH), 9.70 (s, 1, NH).
16[b]	2.92 (s, 1, SO_2CH_3), 6.84 and 7.08 (2d, 4, 4CH, J = 8.9 Hz), 7.61 and 7.88 (2d, 2, 2CH, J = 7.9 Hz), 8.27 (d, 2, 2CH, J = 8.5 Hz), 8.01 and 7.82 (2d, 2, 2CH, J = 9.3 Hz), 9.08 and 8.85 (2d, 2, 2CH, J = 5.6 Hz), 9.29 (s, 1, NH), 9.30 (s, 1, 1CH), 9.39 (s, 1, NH).
17[b]	6.98 and 6.85 (2d, 4, 4CH, J = 8.7 Hz), 7.35–7.80 (m, 7, 7CH), 7.94 (m, 2, 2CH), 8.33 and 8.22 (2d, 2, 2CH, J = 8.3 Hz), 9.16 and 8.91 (2d, 2, 2CH, J = 5.5 Hz), 9.38 (s, 1, 1CH), 10.06 (s, 1, NH).
18[b]	2.36 (s, 3, CH_3), 6.93 and 6.67 (2d, 4, 4CH, J = 8.4 Hz), 7.60 and 7.37 (2d, 4, 4CH, J = 8.1 Hz), 7.59 and 7.88 (2d, 2, 2CH, J = 7.9 Hz), 8.20 and 8.27 (2d, 2 2CH, J = 8.7 Hz), 7.92 and 7.76 (2d, 2, 2CH, J = 8.7 Hz), 8.86 and 9.08 (2d, 2, 2CH, J = 5.3 Hz), 9.27 (s, 1, 1CH), 9.30 (s, 1, NH), 9.88 (s, 1, NH).
19[b]	2.99 (s, 3, SO_2CH_3), 3.77 (s, 3, OCH_3), 6.59 and 6.66 (2d, 2, 2CH, J = 8.7 Hz), 6.70 (s, 1, 1CH), 7.57 and 7.88 (2d, 2, 2CH, J = 7.5 Hz), 8.26 (m, 2, 2CH), 8.65 (s, 1, NH), 7.77 and 8.02 (2d, 2 2CH, J = 9.5 Hz), 8.87 and 9.30 (2d, 2, 2CH, J = 4.3 Hz), 9.10 (s, 1, 1CH), 9.53 (s, 1, 1NH).
20[b]	6.80–6.90 (m, 6, 5CH, NH), 7.20–8.30 (m, 5, 5CH), 7.65 and 8.00 (2d, 2, 2CH, J = 9.5 Hz), 9.00 and 9.20 (2d, 2, 2CH, J = 5.4 Hz).
21[b]	6.70–6.80 (m, 5, 4CH, NH), 7.00–8.15 (m, 5, 5CH), 7.75 and 8.00 (2d, 2, 2CH, J = 9.1 Hz), 9.10 and 9.23 (2d, 2, 2CH, J = 5.8 Hz).
22[b]	2.75 (s, 6, $2CH_3$), 6.70 and 6.90 (2d, 4, 4CH, J = 8.9 Hz), 7.60–8.20 (m, 5, 5CH), 7.50 and 7.82 (2d, 2, 2CH, J = 8.5 Hz), 9.06 and 9.12 (2d, 2, 2CH, J = 5.3 Hz), 9.14 (s, 1, NH).
23[b]	2.90 (s, 3, SO_2CH_3), 6.80 and 7.08 (2d, 4, 4CH, J = 8.9 Hz), 7.50–8.01 (m, 5, 5CH), 7.85 and 8.01 (2d, 2, 2CH, J = 9.3 Hz), 9.10 and 9.20 (2d, 2, 2CH, J = 5.6 Hz), 9.29 (s, 1, NH), 9.39 (s, 1, NH).
24[b]	6.80 and 6.90 (2d, 4, 4CH, J = 8.7 Hz), 7.35–7.80 (m, 7, 7CH), 7.20–8.10 (m, 3, 3CH), 8.20 and 8.85 (2d, 2, 2CH, J = 9.3 Hz), 9.07 and 9.30 (2d, 2, 2CH, J = 5.8 Hz), 10.00 (s, 1, 1NH).
25[b]	2.90 (s, 3, SO_2CH_3), 3.55 (s, 3, OCH_3), 6.50–6.70 (m, 3, 3CH), 6.90–8.00 (m, 5, 5CH), 8.10 and 8.35 (2d, 2, 2CH, J = 9.5 Hz), 9.10 and 9.30 (2d, 2, 2CH, J = 4.5 Hz).

[a] Solvent, $CDCl_3$. [b] Solvent, Me_2SO-d_6.

30% NaOH, and the precipitate was washed with water (50 mL) and collected. Recrystallization from ethanol gave a yellow microcrystalline product.

7-Anilinobenzo(b)(1,8)- and 7-Anilinobenzo(b)(1,10)phenanthrolines (8–25): General Procedure—A solution of the corresponding 7-chloro derivative (4 and 5, 12 mmol) and the appropriate aniline (12 mmol), in the minimum amount of anhydrous ethanol to effect solution at reflux, and methanesulfonic acid (1.2 g, 12 mmol) was added, and the mixture was heated under reflux with stirring. The solution was cooled to 0 °C and the precipitate was washed with water and neutralized with 30% NaOH. The product thus obtained was a mixture of the corresponding acridanone (6 and 7) and the desired product (8–25), which was separated from 6 or 7 by flash chromatography, with ethyl acetate as solvent, on silica gel columns.

Drug–DNA Binding—Poly(d(AT).d(AT)) was purchased from Sigma Chemical and used without purification. The binding constant (log K) to poly(dAT) was determined by ethidium bromide displacement.[23]

Biological Testing—Cell culture murine L1210 leukemia cells were maintained in logarithmic growth as suspension cultures in RPMI-1640 medium (GIBCO) containing 10% fetal calf serum. Cells were grown in 25-cm^2 tissue culture flasks in a total volume of 10 mL in a water-saturated atmosphere containing 5% CO_2 at 37 °C.

In Vivo Antitumor Activity—L1210 leukemia cells (10^5 cells) were inoculated intraperitoneally into 18.5–22.5-g $C_3HDBA_2F_1$ hybrids on day 1; drug treatment was initiated 24 h later and continued for 5 days. All drug dosage was by the intraperitoneal route, and an animal dose was suspended or dissolved in 0.2 mL of water. Groups of six animals per dose level were used and there was

Table III—Physical and Biological Data for Pyridoacridines

Compound	Rm[a]	Log k^b	IC_{50}[c]	In Vivo		
				OD[d]	ILS_{max}[e]	D_{50}[f]
1	0.18	5.66	40	12	96	3.5 (3.42)[g]
4	0.32	5.42	1700	500	—[h]	—[i]
5	0.29	5.30	1700	500	—[h]	—[i]
6	0.09	5.46	66	75	162	35 (2.14)
7	0.13	5.49	59	75	149	45 (1.66)
8	0.29	7.41	150	220	70	120 (1.83)
9	0.36	6.89	85	95	120	65 (1.46)
10	0.56	7.42	96	90	116	60 (1.50)
11	0.16	6.40	136	125	100	55 (2.27)
12	0.27	7.36	125	180	88	80 (2.25)
13	0.28	7.12	170	240	64	120 (2.00)
14	−0.10	6.05	8.50	3.80	55	150 (2.53)
15	−0.35	5.80	120	180	105	100 (1.80)
16	−0.16	7.12	122	125	86	65 (1.92)
17	0.40	7.26	180	2.40	60	115 (2.08)
18	0.46	7.19	196	180	90	80 (2.25)
19[j]	0.10	6.42	100	120	80	45 (2.66)
20	0.36	6.55	146	125	95	60 (2.08)
21	0.24	6.96	90	100	115	65 (1.53)
22	0.29	6.82	176	180	78	90 (2.00)
23	−0.14	5.80	129	220	60	100 (2.20)
24	0.42	6.55	199	220	72	80 (2.75)
25[j]	0.08	6.06	96	120	126	85 (1.41)

[a] Rm values were determined as in ref 24 with amsacrine as a standard. [b] Binding constant to poly(d(AT)) determined by ethidium bromide displacement (see ref 14). [c] Concentration of drug (nM) to inhibit in vitro growth of murine leukemia (L 1210) cells in culture by 50% after 40 h of exposure. [d] Optimal dose of drug (mg/kg) per day, administered intraperitoneally as a suspension or solution in 0.2 mL of 30% ethanol/water (v/v) on days 1.5 and 9 after intraperitoneal inoculation of 10^5 L 1210 leukemia cells. [e] Percentage increase of lifespan of drug-treated tumor-bearing control when treated at the optimal dose. [f] Expressed as μg/kg/day. [g] Numbers in parentheses indicate the ratio OD/D_{50}. [h] Compound inactive at all dose levels up to toxic ones. [i] An ILS of 50% could not be reached at any dose level. [j] Biological testing for this compound has been reported by Denny and Baguley.[8]

one control group for every six test groups. Drug doses were separated by 0.18 log dose intervals and ranged from the clearly toxic to the inactive.

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