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Potential Antitumor Agents. 14. Acridylmethanesulfonanilides

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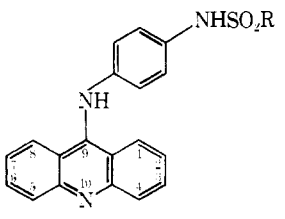
A series of 74 structural variants of 4′-(9-acridinylamino)methanesulfonanilide has been prepared and evaluated in the L1210 leukemia system. The intact acridine ring system appears essential for antileukemic activity. Lengthening the alkyl chain in either the 4′-(9-acridinylamino)-*n*-alkanesulfonanilides or 4′-(3-acetamidoacridin-9-ylamino)-*n*-alkanesulfonanilides caused a progressive decrease in antileukemic activity. Plots of ΔR_m, as a convenient measure of lipophilic–hydrophilic balance, against log biologic response for members of these homologous series provided a parabolic reference curve. Substituent effects on biologic activity were then gauged in relation to the activity predicted from the reference curve on the basis of the ΔR_m value of the variant. There appears to be bulk intolerance about positions 1(8) and 2(7) of the acridine ring, the activity of all 1- or 2-substituted variants being depressed below that expected from ΔR_m values or abolished. When allowance was made for the increase in lipophilic character due to hydrophobic 3-substituents, there was an increase in activity over that expected. It is predicted that more hydrophilic agents with hydrophobic 3-substituents may show greater activity. Analogies are drawn between the agents synthesized and the 9-phenylphenanthridinium salts; structural features as determined by X-ray crystallographic analyses are similar; both series have biologic activities in common and have the capacity to bind to and intercalate into DNA twin helices. Thirteen variants provided a proportion of indefinite survivors in early intraperitoneal (10^5) L1210 tests.

An earlier report from this laboratory demonstrated the excellent activity of 1 (R = CH_3) against the early intraperitoneal L1210 test system.[1] This communication is the first of a series in which this lead will be developed.



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Chemistry. The basic step involved in preparing all compounds listed in Table I was the coupling of the requisite γ-chloro heterocycle with a *p*-aminosulfonanilide component in acid medium (Scheme I).

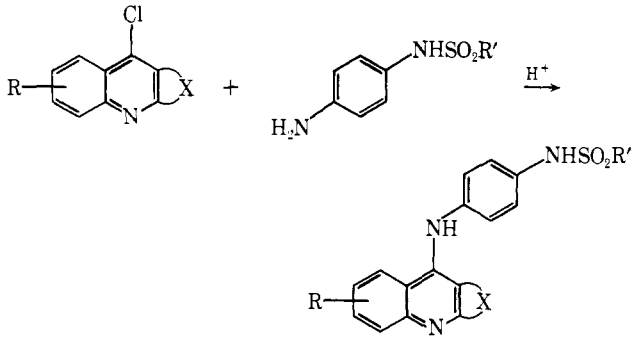
The required 9-chloroacridines were synthesized by ring closure of *N*-arylanthranilic acids which were in turn produced by an Ullmann[2] synthesis (Scheme II). A modification of the conditions used for Ullmann synthesis has considerably simplified the application of this method without diminution of yields (see Experimental Section).

Use of meta-substituted anilines in this acridine synthesis (Scheme II) produces a mixture of 1- and 3-substituted 9-chloroacridines by ring closure in the two possible directions. Separation of isomers has been avoided when possible by using unequivocal syntheses from substituted 2-chlorobenzoic acid components. A different route can sometimes avoid isomer formation; for example, 3-methylacridone is conveniently prepared by condensing 3-methylcyclohexanone and anthranilic acid to 3-methyl-1,2,3,4-tetrahydroacridone[3,4] which on dehydrogenation (Pd/C; refluxing Dowtherm A) is quantitatively converted to 3-methylacridone. This dehydrogenation method is an improvement on an earlier one (Cu/air at 360°; 11% yield).[3]

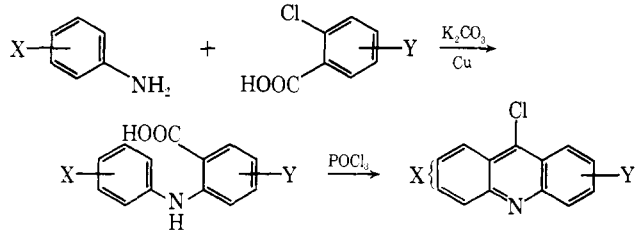
Simple 9(10*H*)-acridones on treatment with $SOCl_2$ containing catalytic quantities of DMF are converted in excellent yield to the corresponding 9-chloro compounds; more vigorous conditions previously used (refluxing $POCl_3$) can then be avoided.

In the bulk of the examples listed (Table I) coupling of the γ-chloro heterocycle and the requisite aminosulfonanilide in refluxing EtOH–H_2O (method A) provided acceptable yields (61–96%). In certain cases low yields were found to result from competing hydrolysis of the chloroacridine to acridone. Coupling in anhydrous solvents (method B) then provided acceptable yields.

Scheme I



Scheme II



For the preparation of the acetamidoacridines quoted (**57, 58–61, 63, 65, 69–73**) two alternative methods were evolved. In method C acetamidoacridones were converted to the corresponding 9-chloro compounds with $POCl_3$ and catalytic amounts of DMF in nitrobenzene at 90°. The crude acetamidochloroacridines were then condensed with the aminosulfonanilide components in NaOAc-buffered solution; unless the progressive liberation of acid during the condensation is compensated for, considerable hydrolysis of acetyl functions results. Alternatively, aminoacridines produced by Fe reduction[5] (method D) of the nitroanilinoacridines were acylated in pyridine solution with acetic acid and *o*-phenylene phosphorochloridite.[6,7] The sulfonamide function of simple sulfonanilides is not acylated by alkanoic acids in the presence of the sterically demanding *o*-phenylene phosphorochloridite. Reduction of the nitro-9-anilinoacridines (*e.g.*, **32**) to the corresponding amines did not proceed cleanly and products were difficult to purify. There was clean high-yielding conversion if reduction ($SnCl_2$–HCl) was carried out at the nitroacridone stage. The resulting aminoacridones could then be acetylated and converted to the acetamido-9-anilinoacridines in good yields (59–87%) by method C. When the same acetamidoacridine was prepared by both methods (**58, 59**) the route *via* the acetamidochloroacridine provided higher yields.

Mild acid hydrolysis of the acetamido-substituted congeners readily provided high-purity amine substituted derivatives of **1** (method G).

Biological Testing. All variants were screened at a series of doses ranging from the toxic to the inactive, doses being separated by 0.18 log dose intervals. Intraperitoneal drug administration was started 24 hr after ip L1210 implantation (10^5) and was continued daily for 5 days. Every effort was made to ensure that agents were administered as true solutions. If suspensions of insoluble salts were administered screening results were variable. This variation was most clearly seen in the optimum doses recorded when tests were repeated. In cases where the chloride or bromide salts could not be administered as true solutions (**16–18, 21, 59, 65–73**) the methanesulfonate salts often proved sufficiently soluble to provide true solutions and the screening results then obtained were acceptably reproducible. The variation in the optimum doses recorded for pairs of insoluble chloride and soluble methanesulfonate salts was most marked with the more lipophilic congeners (**66, 70–73**). Marked differences in L1210 screening results were previously noted when a solution and a suspension of an epipodophyllotoxin derivative were compared.[8]

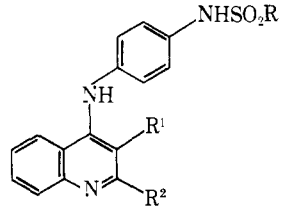
Congeners bearing nitro substituents (**30–33, 49–51, 57**) are extremely insoluble in water and no satisfactory technique for solubilizing these derivatives could be found. Life extensions recorded in Table I for these nitro compounds are a mean of results obtained from three or more tests where, in each test, dilutions of a prepared suspension were screened ensuring that the dosage range extended from the toxic to the suboptimal.

Structure–Activity Relationships. Biological activity is retained in the antimalarial acridines (quinacrine) when one aromatic ring is deleted to yield the quinoline (chloroquine),[9] but in the present series such deletion yielded an inactive species (**3**). Replacement of an acridine unit by quinoline lowers π values by -1.4.[10] To ensure that the inactivity of the quinoline analog **3** was not due to its more hydrophilic nature alone, a more lipophilic example was sought. We have previously shown the more lipophilic benzenesulfonanilide **1** (R = C_6H_5) to be convincingly active.[1] By combining quinoline and benzenesulfonanilide components we obtained a quinoline derivative **4** similar to the lead molecule in lipophilic character ($\Sigma\pi = 0.2$) but this variant also proved inactive.

Molecular models of **1** show considerable steric interaction between the 9-anilino substituent and the 1(8) positions of the acridine ring. An X-ray crystallographic analysis of **1** (R = CH_3)[11] demonstrated considerable distortion of the C(9)–N–C(4′) bond angle from normal figures and showed that the 9-anilino function was rotated so that the ring plane lay at right angles to the plane of the acridine system. If such steric features were necessary conditions for antileukemic activity then the less hindered quinolines (**3, 4**), with presumably different stereochemistry, would not be antileukemic. When 3-substituents were appended to the quinoline ring, in order to approximate the steric effects operating in the parent acridine **1**, inactive agents **5–8** resulted.

A further possible difference between the quinoline and acridine ring systems is the lack, in the former, of a substituent α to the ring nitrogen blocking ready oxidation to the nonbasic carbostyril. This blocking of oxidation has been postulated as a possible reason for the high experimental effectiveness of the 2-phenylquinolinemethanols as antimalarial agents.[12,13] An example embracing the 2-phenylquinoline system **9** proved inactive. The quoted results, coupled with the inactivity of the tetrahydroacridine **10**, make it clear that the three fused ring system is essential for activity in this series.

In his continued unraveling of the multiple variables



3, R = CH_3; $R^1 = R^2 = H$
4, R = C_6H_5; $R^1 = R^2 = H$
5, R = CH_3; $R^1 = CH_3$; $R^2 = H$
6, R = C_6H_5; $R^1 = CH_3$; $R^2 = H$
7, R = CH_3; R^1 = Cl; $R^2 = H$
8, R = C_6H_5; R^1 = Cl; $R^2 = H$
9, R = CH_3; $R^1 = H$; $R^2 = C_6H_5$
10, R = CH_3; $R^1R^2 = (CH_2)_4$

Table I

Agent	Substituents in 1[a]	Mp, °C	Formula	Analyses[b]	Method	$-\Delta R_m$[c]	OD,[d] mg/kg	L1210 T/C, %[e]	Δ log ILS[o]
1		*f*	$C_{20}H_{17}N_3SO_2 \cdot HCl$				50	218 (4)	
		296–297	$C_{20}H_{17}N_3SO_2 \cdot MsOH$[g]	C, H, N, S	A, F		45	207	
3		304 dec	$C_{16}H_{15}N_3SO_2 \cdot HCl$	C, H, N, Cl	A	−0.46	>500	*h*	
4		303–304	$C_{21}H_{17}N_3SO_2 \cdot HCl$	C, H, N, Cl	A	+0.06	250		>−0.66
5		186–187	$C_{17}H_{17}N_3SO_2 \cdot HBr$	C, H, N, Br	A	−0.32	250		>−0.42
6		293–294	$C_{22}H_{19}N_3SO_2 \cdot HCl$	C, H, N, Cl	A	+0.21	200		>−0.41
7		185–186	$C_{16}H_{15}ClN_3SO_2 \cdot HBr$	C, H, N	A	−0.23	>500		>−0.71
8		221–222	$C_{21}H_{16}ClN_3SO_2$	C, H, N, Cl	A	+0.27	>500		>−0.29
9		203–204	$C_{22}H_{19}N_3SO_2 \cdot HBr$	C, H, N, Br	A	+0.21	150		>−0.41
10		145–146	$C_{20}H_{21}N_3SO_2 \cdot TsOH$	C, H, N, S	A	+0.30	200		>−0.20
15	1-CH_3	256–257	$C_{21}H_{19}N_3SO_2 \cdot HBr$	C, H, N, Br	A	+0.13	200	134	−0.44
16	2-CH_3	307–309	$C_{21}H_{19}N_3SO_2 \cdot HCl \cdot 0.5H_2O$	C, H, N, Cl	A	+0.17	>500		>−0.50
		264–265	$C_{21}H_{19}N_3SO_2 \cdot MsOH$	C, H, N, S	F		>500		
17	3-CH_3	192–194	$C_{21}H_{19}N_3SO_2 \cdot HCl$	C, H, N, Cl	A	+0.16	25	194	+0.08
		278–279	$C_{21}H_{19}N_3SO_2 \cdot MsOH$	C, H, N, S	F		17	217	+0.10
18	4-CH_3	209–211	$C_{21}H_{19}N_3SO_2 \cdot HCl$	C, H, N, Cl	A	+0.04	50	190 (1)	−0.13
		244–246	$C_{21}H_{19}N_3SO_2 \cdot MsOH$	C, H, N, S	F		33	213	−0.03
19	4-C_2H_5	301–302	$C_{22}H_{21}N_3SO_2 \cdot HCl$	C, H, N, Cl	B	+0.20	150	153	−0.12
20	1-OCH_3	219–220	$C_{21}H_{19}N_3SO_3 \cdot HCl$	C, H, N, Cl	A	−0.16	150		>−0.75
21	2-OCH_3	323 dec	$C_{21}H_{19}N_3SO_3 \cdot HCl$	C, H, N, Cl	A	+0.03	200		>−0.69
		302–303	$C_{21}H_{19}N_3SO_3 \cdot MsOH$	C, H, N, S	F		250		
22	3-OCH_3	201–202	$C_{21}H_{19}N_3SO_3 \cdot HCl$	C, H, N, Cl	A	−0.05	35	181	−0.23
23	4-OCH_3	200–201	$C_{21}H_{19}N_3SO_3 \cdot HCl \cdot H_2O$	C, H, N, Cl	A	−0.19	75	181	−0.23
24	2-Cl	308–309	$C_{20}H_{16}ClN_3SO_2 \cdot HCl \cdot 0.5H_2O$	C, H, N, Cl	A	+0.19	200		>−0.46
25	3-Cl	304 dec	$C_{20}H_{16}ClN_3SO_2 \cdot HCl$	C, H, N, Cl	B	+0.24	75	203	+0.26
26	4-Cl	278–280	$C_{20}H_{16}ClN_3SO_2 \cdot HCl \cdot H_2O$	C, H, N, Cl	A	+0.28	250		>−0.26
27	2-F	307–308	$C_{20}H_{16}FN_3SO_2 \cdot HCl$	C, H, N, Cl	A	+0.11	250		>−0.63
28	3-F	299–300	$C_{20}H_{16}FN_3SO_2 \cdot HCl$	C, H, N, Cl	B	+0.06	200	138	−0.46
29	4-F	291–293	$C_{20}H_{16}FN_3SO_2 \cdot HCl$	C, H, N, Cl	A	+0.09	>500		>−0.63
30	1-NO_2[m]	273–274	$C_{20}H_{16}N_4SO_4 \cdot HCl$	C, H, N, Cl	B	+0.21	75		>−0.42
31	2-NO_2[m]	337–338	$C_{20}H_{16}N_4SO_4 \cdot HCl$	C, H, N, Cl	B	+0.07	250	127	−0.72
32	3-NO_2[m]	285–286	$C_{20}H_{16}N_4SO_4 \cdot HCl$	C, H, N, Cl	B	+0.18	25[n]	223 (2)[n]	+0.21
33	4-NO_2[m]	255–256	$C_{20}H_{16}N_4SO_4$	C, H, N, S	B	+0.20	>500		>−0.44
34	2-NH_2	252–253	$C_{20}H_{18}N_4SO_2 \cdot HCl \cdot 0.5H_2O$	C, H, N, Cl	D, G	−0.57	25	178	
35	3-NH_2	200–202	$C_{20}H_{18}N_4SO_2 \cdot HBr$	C, H, N, Br	D, G	−0.47	2.5	181 (1)	
36	4-NH_2	198–200	$C_{20}H_{18}N_4SO_2 \cdot HCl \cdot 1.5H_2O$	C, H, N, Cl	D	−0.14	125	187	−0.21
37	2-$N(CH_3)_2$	318 dec	$C_{22}H_{22}N_4SO_2 \cdot HCl \cdot H_2O$	C, H, Cl, N	B	+0.07	150	147	−0.38
38	3-CN	240–242	$C_{21}H_{16}N_4SO_2$	C, H, N	A	+0.26	150		>−0.30
39	3-SO_2CH_3	282 dec	$C_{21}H_{19}N_3S_2O_4 \cdot HCl$	C, H, N, S	A	+0.03	>500		>−0.69
40	3-SO_2NH_2	241 dec	$C_{20}H_{18}N_4SO_4 \cdot HCl$	C, H, N, S	A	−0.17	75		>−0.74
41	3-Br	302–303	$C_{20}H_{17}BrN_3SO_2 \cdot HCl$	C, H, N, Cl	B	+0.34	35	165	+0.31
42	3,4-(CH=CH)$_2$[i]	211–213	$C_{24}H_{19}N_3SO_2 \cdot HCl$	C, H, N, Cl	B	+0.48	300	147	>+0.27
43	3-Cl, 5-OCH_3	204–205	$C_{21}H_{18}ClN_3SO_3 \cdot HCl \cdot H_2O$	C, H, N, Cl	A	+0.07	150	155	−0.31
44	3-Cl, 6-OCH_3	271–272	$C_{21}H_{18}ClN_3SO_3 \cdot HCl$	C, H, N, Cl	B	+0.19	300	141	−0.25

45	3-Cl, 7-OCH_3	322–323	$C_{21}H_{18}ClN_3SO_3 \cdot HCl$	C, H, N, Cl	B	+0.11	>500		>−0.60
46	3,6-$(OCH_3)_2$	293 dec	$C_{22}H_{21}N_3SO_4 \cdot HCl \cdot 0.5H_2O$	C, H, N, Cl	A	−0.10	60	141	−0.54
47	3,4-$(OCH_3)_2$	183 dec	$C_{22}H_{21}N_3SO_4 \cdot HCl \cdot 0.5H_2O$	C, H, N, Cl	A	0.0	>500		>−0.74
48	3,4-$(CH_3)_2$	214–216	$C_{22}H_{21}N_3SO_2 \cdot HCl$	C, H, N, S, Cl	A	+0.18	50	191 (1)	+0.07
49	3-NO_2, 5-OCH_3[m]	288–289	$C_{21}H_{16}N_4SO_5 \cdot HCl$	C, H, N, Cl	B	−0.05	250[n]	181[n]	−0.23
50	3-NO_2, 6-OCH_3[m]	282–283	$C_{21}H_{16}N_4SO_5 \cdot HCl$	C, H, N, Cl	B	+0.13	250[n]	151[n]	−0.29
51	3-NO_2, 7-OCH_3[m]	292–293	$C_{21}H_{18}N_4SO_5 \cdot HCl$	C, H, N, Cl	B	+0.21	400		>−0.42
52	3-NH_2, 5-OCH_3	226–228	$C_{21}H_{20}N_4SO_3 \cdot HCl$	C, H, N, Cl	D	−0.68	2.5	168	
53	3-NH_2, 6-OCH_3	282 dec	$C_{21}H_{20}N_4SO_3 \cdot HCl$	C, H, N, Cl	D	−0.53	80	172 (1)	
54	3-NH_2, 7-OCH_3	328–329	$C_{21}H_{20}N_4SO_3 \cdot HCl$	C, H, N, Cl	D	−0.67	150		
55	3-NH_2, 6-NO_2[m]	360	$C_{20}H_{17}N_5SO_4 \cdot HCl$	C, H, N, Cl	G	−0.19	10[n]	199 (1)[n]	−0.15
56	3,6-$(NH_2)_2$	200–202	$C_{20}H_{19}N_5SO_2 \cdot HI \cdot H_2O$	C, H, N, I	D, G	−1.12	1	141	
57	3-NHAc, 6-NO_2[m]	246–247	$C_{22}H_{19}N_5SO_5 \cdot HCl$	C, H, N, Cl	C	−0.06	12[n]	224 (2)[n]	−0.01
58	2-NHAc	310 dec	$C_{22}H_{20}N_4SO_3 \cdot MsOH$	C, H, N, S	C, E	−0.25	375	147	−0.48
59	3-NHAc	334 dec	$C_{22}H_{20}N_4SO_3 \cdot HCl \cdot 1.5H_2O$	C, H, N; S[i]	C, E	−0.22	35	226 (2)	−0.02
		268–269	$C_{22}H_{20}N_4SO_3 \cdot MsOH$	C, H, N, S	F		13	214 (2)	−0.06
60	4-NHAc	292–293	$C_{22}H_{20}N_4SO_3$	C, H, N, S	C	+0.07	>500		>−0.65
61	2,7-$(NHAc)_2$	252–254	$C_{24}H_{23}N_5SO_4 \cdot HBr$	C, H, N, Br	C	−0.34	>500		
62	2,7-$(NH_2)_2$	335 dec	$C_{20}H_{19}N_5SO_2 \cdot HBr$	C, H, N	G	−0.99	2	133	
63	2,6-$(NHAc)_2$	344–345	$C_{24}H_{23}N_5SO_4 \cdot HBr$	C, H, N	C	−0.46	>500		
64	2,6-$(NH_2)_2$	313–314	$C_{20}H_{19}N_5SO_2 \cdot HBr$	C, H, N, Br	G	−1.01	25	158 (1)	
65	3,6-$(NHAc)_2$	273 dec	$C_{24}H_{23}N_5SO_4 \cdot HCl$	C, H, N, Cl	C	−0.39	165	193 (1)	
		277–278	$C_{24}H_{23}N_5SO_4 \cdot MsOH$	C, H, N; S[k]	F		27	201 (2)	
66	R = CH_3CH_2	298–299	$C_{21}H_{19}N_3SO_2 \cdot HCl$	C, H, N, Cl	A	+0.16	150	183	0.0
		300–301	$C_{21}H_{19}N_3SO_2 \cdot MsOH$	C, H, N, S	F		55	171	−0.07
67	R = $CH_3(CH_2)_2$	282–283	$C_{22}H_{21}N_3SO_2 \cdot HCl \cdot 0.5H_2O$	C, H, N, Cl	B	+0.32	225	134	−0.01
		301–302	$C_{22}H_{21}N_3SO_2 \cdot MsOH$	C, H, N, S	F		250	129	−0.08
68	R = $CH_3(CH_2)_3$	297–298	$C_{23}H_{23}N_3SO_2 \cdot HCl$	C, H, N, Cl	B	+0.48	260		
		265–266	$C_{23}H_{23}N_3SO_2 \cdot MsOH$	C, H. N, S	F		>500		
69	3-NHAc, R = CH_3CH_2	327–328	$C_{23}H_{22}N_4SO_3 \cdot HCl$	C, H, N, Cl	C	−0.07	80	244 (1)	+0.02
		194–196	$C_{23}H_{22}N_4SO_3 \cdot MsOH \cdot 2H_2O$	C, H, N, S	F		27	273	+0.10
70	3-NHAc, R = $CH_3(CH_2)_2$	302 dec	$C_{24}H_{24}N_4SO_3 \cdot HCl$	C, H, N, Cl	C	+0.01	255	205	0.0
		310–311	$C_{24}H_{24}N_4SO_3 \cdot MsOH$	C, H, N, S	F		26	197	+0.03
71	3-NHAc, R = $CH_3(CH_2)_3$	321 dec	$C_{25}H_{26}N_4SO_3 \cdot HCl$	C, H, N, S	C	+0.26	60	149	−0.01
		308–309	$C_{25}H_{26}N_4SO_3 \cdot MsOH$	C, H, N, S	F		25	143	−0.07
72	3-NHAc, R = $CH_3(CH_2)_4$	304–305	$C_{26}H_{28}N_4SO_3 \cdot HBr$	C, H, N; Br[l]	C	+0.42	250	127	
		281–282	$C_{26}H_{28}N_4SO_3 \cdot MsOH$	C, H, N, S	F		37		
73	3-NHAc, R = $CH_3(CH_2)_5$	289 dec	$C_{27}H_{30}N_4SO_3 \cdot HBr$	C, H, N, Br	C	+0.58	500		
		273–274	$C_{27}H_{30}N_4SO_3 \cdot MsOH$	C, H, N, S	F		50		
74	3,4,6-$(CH_3)_3$	209–211	$C_{23}H_{23}N_3SO_2 \cdot HCl$	C, H, N, Cl	B	+0.22	50	225	+0.30
		288–289	$C_{23}H_{23}N_3SO_2 \cdot MsOH$	C, H, N, S	F		35	238	+0.34

[a]R in **1** is CH_3 unless otherwise stated. [b]Analyses for the elements noted have been within ±0.4% of the calculated figures. [c]$R_m = \log (1/R_f - 1)$. [d]Optimum dose; that dose, in mg/kg/day, producing maximum T/C under the standard test conditions or, for inactive compounds, the maximum tolerated dose. [e]T/C values are calculated from median life survivals and are the mean of at least two assays. Figures in parentheses are the mean number of 50-day survivors at the optimum dose for a group of six animals. [f]For comparison purposes, see ref 1. [g]MsOH = methanesulfonic acid. [h](−) signifies $T/C > 1.25$ was not obtained at tolerated doses. [i]Benz[*c*]acridine. [j]S: calcd, 6.60; found, 7.2. [k]S: calcd, 11.2; found, 10.6. [l]Br: calcd, 14.3; found, 14.8. [m]Screened as a suspension. [n]Variable; mean figures from multiple assays. [o]Difference between log ILS predicted from ΔR_m of the variant using Figure 1 and the log ILS observed.

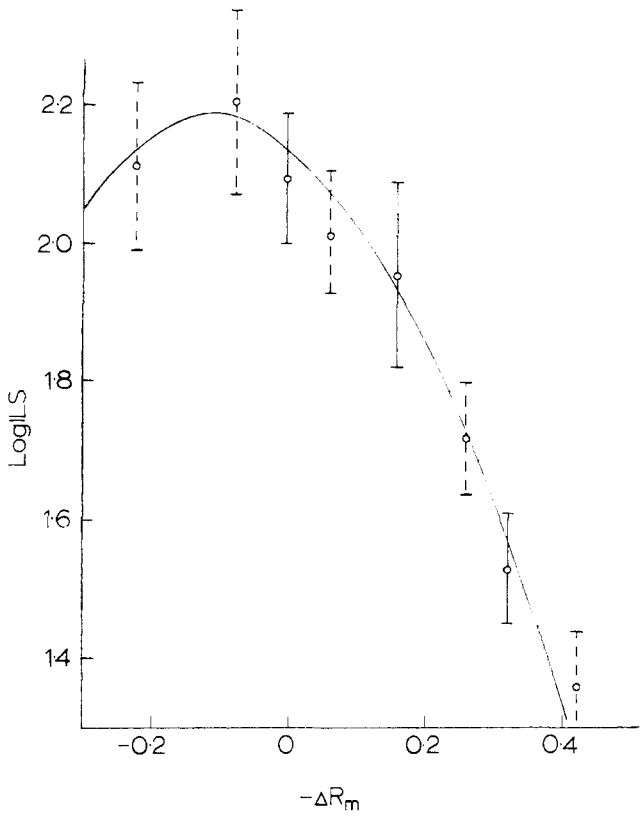


Figure 1. Ordinate: log (increase in median life span (L1210) at the optimum dose) = log ILS = log [T/C (%) − 100]. The vertical lines show the standard deviation in log ILS about the mean figure (circled). Abscissa: $-\Delta R_m$ = change in R_m value [log ($1/R_f$ − 1)] relative to the parent compound I, R = CH_3. (– – O – –) values for members of the homologous 4′-(3-acetamidoacridin-9-ylamino)-*n*-alkanesulfonanilides **59** and **69–73** (Table I). (——O——) values for the homologous 4′-(9-acridinylamino)-*n*-alkanesulfonanilides 1 and **66–68**.

operating when drugs interact with biologic systems Hansch[14] has developed powerful methods, the critical feature of his analyses being that effects due to changing lipophilic character and those due to steric, electronic, and other factors are separated.

From the many examples of successful correlations listed by Hansch[14,15] a working guide for drug development can be formulated.

"Before ascribing the changes in biologic activity produced by an added substituent to steric or electronic effects of that substituent it is necessary to be able to compensate for the changes in biologic activity resulting from the alterations in molecular lipophilic–hydrophilic balance due to that added substituent."

In earlier work[16,17] we briefly discussed a new approach for separating the effects due to the contribution of substituents to molecular lipophilic–hydrophilic balance from those due to other factors. This approach requires that a homologous series be prepared, an alkyl chain being appended to the drug at a position where there is acceptable evidence for considerable bulk tolerance. A plot of the biologic activity of the members of the resulting homologous series *vs.* a measure of partition properties ($\Sigma\pi$; log P; $-\Delta R_m$) then provides a standard curve. The biologic activity of any congeneric agent bearing an added substituent can then be compared with the standard curve at the partition value ($\Sigma\pi$; log P; $-\Delta R_m$) for the variant. The extent to which the measured biologic activity deviates from that predicted from the reference curve then provides a relative measure of the steric, electronic, and hydrophobic binding contributions of the added substituent. Using this scheme, biologic test data from each variant synthesized provide information which can be immediately utilized in attempting to predict more interesting areas for investigation. Highly active examples of a drug series might emerge more quickly by using this technique in place of retrospective correlation methods.

The test data obtained with the homologous series 1 (R = Me, Et, *n*-Pr, *n*-Bu; **66–68**) illustrate the method. As before[18] we have used partition chromatographic data as a convenient[19] relative measure of log P and such data are presented as $-\Delta R_m$ values in Table I; the excellent additivity of these values is apparent on inspection. If desired $\Sigma\pi$ values can be used, identical conclusions are reached. Certain of the ΔR_m values, obtained from compounds with 4-substituents (**18, 23, 36**), were considerably different from those obtained when the same substituents were located at the 2 or 3 positions (**16, 17, 21, 22, 34, 35**). Presumably this difference results from the perturbing effect of the neighboring charged N-10 atom of the acridine on the 4-substituents, adjacent polar functions being known to alter π values.[20] A plot of log increase in life span in L1210 tests [log ILS, log (T/C (%) − 100)] against $-\Delta R_m$ for the homologous series (Figure 1) shows a rapid cutoff in biologic effectiveness with increasing lipophilic character. That this cutoff is due to changes in physical properties and not to the lengthening alkyl chain extending into an area of bulk intolerance is shown by the examination of the second series (**59, 69–73**) where a 3-NHAc function has been appended (Figure 1). An acetamido function serves as a convenient device for lowering log P (π = −0.97)[10] without appreciably altering the electron distribution in the 9-aminoacridine system (σ_p = 0;[21] ΔpK_a = 0[22]). In the more hydrophilic 3-NHAc series the alkyl sulfonamide chain can be extended past the point of cutoff seen in the more lipophilic parent series. The L1210 activity shown by the two sets of homologs (Figure 1) corresponds with ΔR_m values and not alkyl chain dimensions. Since more hydrophilic congeners than those considered when constructing Figure 1 appear less active [for example, the 3,6-diacetamido- (**65**) and various amine substituted variants 34–36, 56, **64**] the peak seen in this curve is probably correctly placed and the optimum $-\Delta R_m$ for this series therefore lies between −0.22 and 0.0.

Discussion of all substituted derivatives in Table I is then taken in reference to the standard curve (Figure 1). The log ILS produced by a substituted variant was compared with that of the standard curve at the ΔR_m value recorded for the variant; the displacement of the observed value from the curve (Δ log ILS) should then provide a relative measure of the steric, electronic, and other site effects due to the substituent. Biologic data from *in vivo* screening systems are notoriously imprecise and from our accumulated experiences with reproducibility in L1210 assays we would suggest that when predicted and measured values are separated by less than 0.2 log ILS unit these are not significantly different.

Evaluating the data of Table I by the method described above we concluded that there is a lack of bulk tolerance about positions 1 and 2 of the acridine ring when derivatives of **1** enter into an essential interaction step. It is assumed that this argument extends equally to the equivalent 7 and 8 positions, otherwise simple ring inversion would relieve such steric interactions. The activity of all 1- or 2-substituted derivatives is depressed (**15, 31, 34, 58, 62**) below that predicted from ΔR_m values or abolished (**16, 20, 21, 24, 27, 30, 45, 51, 54, 63**).

The 3-substituents appreciably increasing activity (NO_2, Cl, and Br; **25, 32, 41**) are both σ and π positive.[19]

In contrast $\pi-$, $\sigma+$ functions (SO_2CH_3, SO_2NH_2, CN; **38–40**) abolish activity. The results from these two contrasting substituent sets led us to hypothesize that 3-substituents might bind hydrophobically to site components. If hydrophobic bonding were an important factor then it could be predicted that a 3-methyl group should augment activity but in fact only a slight and not significant Δ log ILS value had been observed (**17**). The obvious tactic of augmenting possible hydrophobic bonding by preparing a polymethyl derivative provided a molecule then showing a significant positive Δ log ILS value (**74**). The activity of the benz[c]acridine derivative **42** is considerably higher than expected from its ΔR_m value and this might also be due to enhanced binding through hydrophobic interactions.

Since aromatic methoxyl functions have little effect on partition coefficients ($\pi = -0.02$)[19] it might be predicted from the above arguments that a 3-OMe function would produce little change in activity. While a single appended 3-OMe group appeared to produce a slight decrease in activity (**22**) the cumulative effects of two such groups provided a significant depression in activity (**46**). Note also that the augmenting effect of a 3-Cl substituent (**25**) can be negated by an additional OMe function (**44**). For the overall π value of a methoxyl function to be −0.02 the oxygen atom of the function must have considerable hydrophilic character ($\pi = -0.52$) to counterbalance the hydrophobic nature of the methyl group component ($\pi = 0.50$).[19] The hydrophilic oxygen atoms of the methoxy functions in analogs **22**, **44**, and **46** would then reside in a site area that we have suggested is hydrophobic in nature; a reduction in activity would be expected. The methyl groups of the methoxyl functions, being further removed from the acridine nucleus by the spacing oxygen atoms, reside in areas that have not yet been effectively probed.

When site lodged, 3-monosubstituted acridine derivatives may have the substituent lying in either of two areas, *i.e.*, alongside the 3 or equivalent 6 positions. If either of these areas were polar in nature then $\pi-$ substituents should prove acceptable. The increase in activity seen with the variant bearing both 3- and 6-methyl groups (**74**) and the significant decrease produced by 3,6-dimethoxyl substitution (**46**) suggest that both site areas about the 3 and 6 positions of the acridine are hydrophobic in nature.

There are clearly additional site effects operative with the 4(5)-substituted derivatives since, with the exception of a methly group (**18**), any appended function produces a significant decrease in activity (**23**, **26**, **33**, **36**, **60**). Any substituent, adjacent to the charged N-10 atom of the acridine system, can provide a steric constraint to the approach of an anionic (site?) component. The substituent effects observed might then result from hydrophobic, steric, and electronic effects acting in concert. The limited number of examples available and the lack of precision in the biologic data do not permit effective examination of the importance of these various effects. However, as a guide for future synthetic work it appears that only 4-H or possibly 4-CH_3 substituents need be further considered.

Regardless of our speculations as to the significance of substituent effects at the molecular level the method of structure–activity analysis evolved provides a clear directive for future work. From the data provided in Table I and Figure 1 we conclude that attention should be directed to examples substituted in the acridine ring at the 3(6) positions with Br, Cl, NO_2, or CH_3. Additional functions such as CF_3 should clearly be investigated. A suitable point for attachment of acceptable hydrophilic functionality must be found so that the overall lipophilic–hydrophilic balance of the agents can be adjusted into the optimum range (−0.22 to 0.0 ΔR_m).

Discussion

The methods used in the structure–activity analysis show that when lipophilic contributions of substituents are compensated for, 3-NO_2, 3-Cl, and 3-Br substituents augment activity. In view of the reported importance of base strength for the biologic activity of the acridines[22] such findings are surprising since these substituents all decrease pK, markedly so in the case of the 3-nitro congeners ($\Delta pK = -2.1$).[22] Despite their low base strength the 3-nitro-9-aminoacridines are reported as having intriguing biologic activity demonstrating high antibacterial activity *in vitro*[23] and *in vivo*[24–26] and antitrypanosomal[26] and antiviral activity.[27,28] Examples of these earlier prepared agents might profitably be examined for antitumor properties.

In view of the recorded binding of acridines to, and intercalation into, DNA duohelices[29–31] it is not surprising to find that active examples of the agents described in this paper bind to and intercalate into DNA stacks.† The agents bind more strongly to DNA than does 9-aminoacridine under comparable conditions.†

If a step equivalent to DNA intercalation was essential in order that the described acridines show antileukemic activity, then the observed substituent effects on activity could be explained, provided the assumption was made that the acridines entered the polynucleotide stack (N-10 of the acridine leading) *via* the minor groove. Possibly the acridine could lodge in the DNA minor groove as a site of residence[32] until intercalation became possible when the DNA coils unwound. To accommodate the depth of the acridine ring system it is necessary that the helix partially uncoil. Molecular models‡ demonstrate a pronounced steric role for the 9-methanesulfonanilide component when binding to such a site is considered. The ring plane of the 9 function is orthogonal to that of the acridine system[11] and the limits of the appended ring (6.2 Å)§ considerably exceed the depth of the fused ring system (3.4 Å). The 9 function could then prevent complete passage of the acridine between the purine–pyrimidine base pairs and restrict possible orientations of the intercalated ring to the neighboring base pairs. In such an environment the 9-sulfonanilide ring could contact and possibly bind to the peripheral functionality of the purine–pyrimidine base pairs (adenine 3-N; thymine and cytosine 2-O; guanine 2-NH_2). If the 9-anilino ring did bind to purine and/or pyrimidine functions then the 1′-methanesulfonamide component would be in juxtaposition to a deoxyribose phosphodiester linkage. Such positioning of the 9-sulfonanilide ring firmly orients the acridine ring between the neighboring base pairs. In such an orientation the sugar–phosphate strands of the A and B chains are within 0.6 Å of the 2- and 7-H atoms of the acridine. The demonstrated lack of bulk tolerance about the 2 and 7 positions of the acridine would be expected.

Both the stacking interactions of base pairs and the binding of intercalated molecules to purine–pyrimidine pairs have been suggested to have considerable hydrophobic character.[33] If the agents bound as postulated, then neighboring base pairs overlap the intercalated fused ring system at both the 3 and 6 positions. Additional lipophilic 3- and/or 6-acridine substituents could then contact and interact hydrophobically with the areas of overlap.

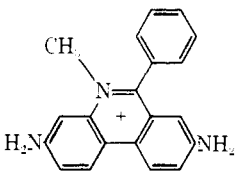
†Unpublished observations of Dr. B. C. Baguley and W. H. Wilson, this laboratory.

‡Courtald models, space filling models developed jointly by Courtalds, Maidenhead, U. K., and Griffin and George, Ltd., Wembley, U. K.

§Van der Waals envelope taken from the models.

A further result of the steric effect of the 9-sulfonanilide component is that there is proscribed access to the protonated N-10 acridine atom which remains sandwiched between adjacent base pairs. Consequently, anion access to the charged center is restricted. Any 4(5) acridine substituent would further constrain approach of an anion. From molecular models of the putative site it does not appear that such an anion would be a site component. If increasing steric hindrance to approach of an extraneous anion necessitated volume reduction of that ion, *e.g.*, by removal of hydration shell, then the necessary energy for this process must produce an equivalent compensatory loss in drug-binding energy.

Examples of the isomeric three fused ring phenanthridine system (*e.g.*, dimidium, **12**) are also known to intercalate into DNA helices.[34,35] It is interesting to note that an X-ray crystallographic analysis of dimidium (**12**)[36] showed that the 9-phenyl group lay at right angles to the plane of the fused ring, as observed with the 9-anilino ring in the acridines **1** (R = CH_3) discussed earlier. If molecular models of a 9-phenylphenanthridinium salt **12** and a 9-anilinoacridine (**1**) are superposed so that the 9-phenyl and 9-anilino functions are aligned, then there is a remarkable correspondence of the areas of ring overlap of the phenanthridine and acridine systems. The major difference between the two models is that the charged nitrogen atoms are located on opposite edges of the three fused ring systems. When tested in our standard L1210 screening test dimidium (**12**), at the optimum dose of 27.5 mg/kg/day, provided T/C values of 140%.



12

Simple phenanthridinium salts lacking a 9-phenyl group have moderate antitrypanosomal and antibacterial activity.[37] Appending the 9-phenyl function markedly enhances biologic activity.[37] It was earlier recorded that simple acridines such as proflavine (3,6-diaminoacridine) and trypaflavine (3,6-diamino-10-methylacridinium chloride) have high experimental antitrypanosomal and antibacterial activity.[38,39] It appears a remarkable omission on the part of medicinal chemists that no suitable acridines bearing an additional nonfused aromatic ring, approximating to the essential 9-phenyl function in the phenanthridinium salts, have been listed as candidate chemotherapeutic agents. If the 9-anilinoacridines described in this paper are, in fact, examples of such agents the biologic data suggest that they may be more potent than the corresponding phenanthridinium compounds and therefore worthy of test in more diverse systems.

Experimental Section

Where analyses are indicated only by symbols of the elements, analytical results obtained for these 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. The hydrochlorides and hydrobromides of the agents listed in Table I tend to lose HCl or HBr on drying at elevated temperatures *in vacuo*, this being particularly noticeable with the weakly basic nitroacridyl compounds. Accordingly, samples for analysis have been dried *in vacuo* at room temperature over P_2O_5. Melting points were determined on an Electrothermal melting point apparatus with the makers' supplied stem corrected thermometer; melting points are as read.

Chromatography. To ensure that the weakly basic nitroacridyl congeners remained as the cation during solvent development it was necessary to use an acid developing solvent and this was used for the bulk of the compounds examined. The top phase of a mixture of *n*-BuOH–0.1 *N* HCl (1:1) was used. The chloride ion concentration in this solvent mixture is sufficient to swamp the effect of other anions applied with the agents. The same R_f values were recorded if agents were applied as chloride, bromide, or methanesulfonate salts.

For amine-substituted variants, bearing a further ionizable function, the acid developing solvent had to be avoided and the top phase of a mixture of *n*-BuOH–0.1 *N* NH_4Cl (1:1) was used instead. The relative ΔR_m values of congeners not bearing amine or strongly base weakening functions were not changed significantly by interchanging the two solvent systems.

Reidel de Hahn cellulose DC cards were used as medium; plates were equilibrated for 12 hr with the lower phase before development. All plates had **1** (R = CH_3) as an internal standard and ΔR_m values were calculated in reference to that compound.

To monitor the progress of reactions, purification of products, etc., tlc on SiO_2 (Merck SiO_2 F_{254}) was used with $CHCl_3$–MeOH as solvent.

The bulk of the required 4(1*H*)-quinolones, 9(10*H*)-acridones, and derived γ-chloro compounds has been adequately described in the literature. Extensive experience with the Jourdan–Ullmann reaction for preparing *N*-arylanthranilic acids has shown that the ethylene glycol monoalkyl ethers have considerable advantages as solvents. A range of such ethers with varying boiling points is commercially available, the solubility of the components in these solvents accelerates reaction; water miscibility simplifies workup. The majority of the *N*-arylanthranilic acids required as intermediates in this work were readily prepared by interaction of the requisite 2-chlorobenzoic acid and substituted aniline in 2-ethoxyethanol. An excellent guide to purification of these acids is provided by Albert.[40]

9-Chloroacridines were prepared either from the *N*-arylanthranilic acid with $POCl_3$[41] or from the 9(10*H*)-acridone with $SOCl_2$–DMF. Acridones were suspended in $SOCl_2$ (5 ml/g), DMF (0.01 mol) was added, and the heterogenous mixture was heated at 80° until a clear solution resulted and then for 0.5 hr longer. After removal of $SOCl_2$ *in vacuo* the crystalline solid was dissolved or suspended in $CHCl_3$ and then stirred into chopped ice plus excess NH_4OH. The 9-chloroacridine was removed with $CHCl_3$; the extracts were washed with 2 *N* NH_4OH, dried (Na_2SO_4), and evaporated. After crystallization from anhydrous solvents the chloroacridines were used without delay; samples can be stored for limited periods in an evacuated desiccator over KOH pellets.

Method A. The γ-chloro heterocycle (0.1 mol) and *p*-aminosulfonanilide component (0.105 mol) were refluxed with sufficient EtOH–H_2O (2:1 v/v) to provide a clear solution. In all such coupling reactions it is essential that components are completely dissolved before adding the acid catalyst. After addition of HCl (12 *N*, 0.1 mol) boiling was continued for 45 min. In many cases product crystallized from the reaction mixture; more commonly EtOH was removed *in vacuo* and product salted out with NaCl or NaBr. Considerable difficulty was experienced when recrystallizing the products from aqueous media because of gel formation. Gel formation can be combated by crystallizing from small volumes of solution, keeping the proportion of organic solvent and concentration of anion high and using slow cooling rates after crystal nuclei have been obtained. The 9-anilinoacridines listed in Table I are highly colored, colors ranged from orange to deep red depending on the substituents present and the degree of hydration.

Method B. Essentially the same conditions were used as in method A except that an anhydrous solvent (either EtOH, 2-ethoxyethanol, or phenol) was used and methanesulfonic acid was used as catalyst. The 9-anilinoacridine salts were invariably insoluble in the anhydrous media and crystallized during the course of the reaction.

Method C. For purposes of example the conditions used with 3-acetamido-6-nitroacridone are quoted. $POCl_3$ (3 ml) was added to a suspension of 3-acetamido-6-nitroacridone (1.5 g) in nitrobenzene (15 ml) and the whole mixture heated to 90°. The acridone passed rapidly into solution and after *ca.* 20 min heating a phosphorus containing salt started to crystallize from the orange-red solution. After 45 min of heating the crystalline complex was collected from the cooled mixture, washed with a little $C_6H_5NO_2$ and then petroleum ether, and dried *in vacuo*. The dry complex was stirred into cold excess NH_4OH and after 0.5 hr of stirring the crystalline 9-chloro compound collected. The product was coupled with *p*-aminomethanesulfonanilide essentially as in

method A. To prevent hydrolysis of acetamido functions it is essential to add NaOAc (1-mol proportion) after the reaction has initiated as indicated by the color of the solution changing from yellow to red.

Method D. The conditions used for Fe reduction have been adequately described before.[5]

Method E. A well-dried (P_2O_5, vacuum) sample of the free base of an aminoanilinoacridine (0.1 mol) was dissolved in Py (15 ml/g) by warming and cooled to 0°, and o-phenylene phosphorochloridite[6,7] (0.16 mol) was added portionwise followed by AcOH (0.15 mol). Product slowly crystallized from the stirred solution over several hours.

Method F. The chloride or bromide salt of a 9-anilinoacridine (0.1 mol) was dissolved in the minimum volume of boiling EtOH–H_2O (2:1 v/v) and to this solution was added $KHCO_3$ (0.102 mol); then EtOH was removed *in vacuo* until either the free base crystallized or samples on refrigeration provided crystalline material. The well-washed (H_2O) and dried crystalline base was suspended in anhydrous MeOH (5 ml/g), $MeSO_3H$ (0.105 mol) was added, and the suspension was stirred until solution resulted. Crude methanesulfonate salt was precipitated by addition of excess C_6H_6 and petroleum ether, collected, washed well with C_6H_6, and dried. The methanesulfonate salts were recrystallized from *n*-BuOH–MeOH, EtOH, MeOH, or aqueous methanesulfonic acid.

Method G. Acetamido-9-anilinoacridines were hydrolyzed by dissolving in boiling EtOH–H_2O (2:1 v/v) adding 12 *N* HCl to a final concentration of 2 *N*, and then heating under reflux until tlc demonstrated hydrolysis was complete (*ca.* 45 min). After evaporation *in vacuo* to a red gum the crude products were dissolved in warm H_2O and NaOAc was added until the pH was slightly greater than 7; addition of solid NaCl or NaBr to the stirred solution then precipitated product as a monosalt.

***p*-Nitro-*n*-alkanesulfonanilides.** *p*-Nitroaniline (0.1 mol) was dissolved in Py (50 ml) by warming and the solution cooled rapidly with vigorous stirring to −15°. The requisite alkanesulfonyl chloride (0.105 mol) was added dropwise so that the temperature remained below −5°. The homogeneous solution was stored in a refrigerator overnight and then concentrated *in vacuo* on a steam bath. Water (100 ml) was added followed by sufficient HCl to neutralize the remaining Py. When solid, crude product was collected from the well-cooled mixture, washed well with ice water, and then dissolved in 1 *N* NaOH (200 ml) by vigorous stirring. Filtration then removed traces of di-N-acylated compound; the quantity of this by-product increases if the temperature is allowed to rise in the initial stages of the acylation. Product was precipitated from the clarified NaOH solution with HCl and then crystallized from EtOH–H_2O or MeOH–H_2O. Yields of pure product were in the range 47–84%.

Nitro compounds were reduced to the amines with Fe–H^+ as described earlier.[5] All sulfonanilides listed in Table II were analyzed for C, H, N, and S.

2-Chloro-4-methylsulfonylbenzoic Acid. 2-Chloro-4-methylthiotoluene (0.16 mol) was suspended in H_2O (500 ml) and $KMnO_4$ (0.55 mol) was added in 10-g portions to the well-stirred mixture as the previous portion was decolorized. The heterogeneous mixture was finally boiled until no trace of unchanged permanganate remained. The MnO_2 was filtered off and the clear aqueous solution evaporated *in vacuo* to 100 ml after the addition of AcOH (23 ml). Adjusting the pH of the concentrate to 2 with HCl precipitated crude product. Several recrystallizations from H_2O provided pure product as colorless plates: mp 198–199°; 18.3 g (49%). *Anal.* ($C_8H_7ClSO_4$) C, H, Cl, S.

***N*-Phenyl-4-methylsulfonylanthranilic Acid.** A heterogeneous mixture of 2-chloro-4-methylsulfonylbenzoic acid (0.025 mol), anhydrous K_2CO_3 (0.025 mol), aniline (0.05 mol), Cu powder (0.05 g), Cu_2O (0.05 g), and 2-ethoxyethanol (10 ml) was heated under reflux for 3 hr. After cooling the black mixture was acidified with HCl and diluted with H_2O. The resulting solid, after washing well with H_2O, was dissolved in H_2O (100 ml) containing Na_2CO_3 (4 g) by boiling; the solution was stirred with decolorizing charcoal (1 g) for 5 min and filtered through a Celite pad, and crude acid precipitated with HCl in the hot mixture. Repeated crystallization from EtOH–H_2O provided pure product as elongated yellow plates: mp 237–238°; 5.5 g (74%). *Anal.* ($C_{14}H_{13}NSO_4$) C, H, N, S.

2-Chloro-4-sulfamoylbenzoic Acid. Chlorination of *p*-toluenesulfonyl chloride[42] provided 3-chloro-4-methylbenzenesulfonyl chloride, mp 36° (lit.[42] 37°). Ammonia treatment of the sulfonyl chloride afforded the sulfamide, mp 141° (lit.[42] 137°). Oxidation of an NaOH solution of the sulfamide with $KMnO_4$ produced the required acid, mp 213–214° (lit.[43] 198–199°). *Anal.* ($C_7H_6ClNSO_4$) C, H, N, Cl.

Table II

Alkyl chain	Formula	Mp, °C
	p-Nitro-*n*-alkanesulfonanilides	
Propyl	$C_9H_{12}N_2SO_4$	164.5–165
Pentyl	$C_{11}H_{16}N_2SO_4$	89–99
Hexyl	$C_{12}H_{18}N_2SO_4$	98.5–99
	p-Amino-*n*-alkanesulfonanilides	
Ethyl	$C_8H_{12}N_2SO_2$	86–87
Propyl	$C_9H_{14}N_2SO_2$	113–113.5
Butyl	$C_{10}H_{16}N_2SO_2$	70.5–71
Pentyl	$C_{11}H_{18}N_2SO_2$	105.5–106
Hexyl	$C_{12}H_{20}N_2SO_2$	78–79

***N*-Phenyl-4-sulfamoylanthranilic Acid.** 2-Chloro-4-sulfamoylbenzoic acid (0.035 mol), anhydrous K_2CO_3 (0.038 mol), aniline (0.07 mol), Cu powder (0.1 g), Cu_2O (0.1 g), and 2-ethoxyethanol (10 ml) were heated together under reflux for 3 hr. Acidification (HCl) and dilution with H_2O precipitated crude product. The acid was dissolved in H_2O (50 ml) containing Na_2CO_3 (3.75 g) by warming, the solution stirred with charcoal (1 g) for 0.5 hr and then filtered through a Celite pad, and product precipitated with HCl. Several crystallizations from EtOH–H_2O (60%) provided pure product as yellow needles: mp 242.5–243°; 5.6 g (54%). *Anal.* ($C_{14}H_{13}NSO_4$) C, H, N, S.

Preparation of Aminoacridones. A hot (60°) solution of $SnCl_2 \cdot 2H_2O$ (3.5 mol/mol of nitro group) in 12 *N* HCl–AcOH (1:1 v/v) (2 ml/g of $SnCl_2 \cdot 2H_2O$) was added in one portion to the finely powdered nitroacridone contained in a large flask. The initial vigor of the reaction was moderated by cooling in tap water, care being taken not to cool excessively otherwise product hydrochloride will sometimes separate and coat unreacted starting material. It is essential that at some stage in the reaction a clear solution is obtained. When the vigorous reaction abated the mixture was boiled under reflux for 1.5 hr. In cases where product had started to crystallize during the reaction, cooling, and addition of further HCl, provided crude product. If a clear solution resulted at the end of the heating period the solution was concentrated *in vacuo* to half volume and an equal volume of 12 *N* HCl was added; cooling then provided crystalline product. The crystalline material in all cases proved to be amine hydrochloride and not the stannic chloride. The collected hydrochlorides were dissolved in hot 0.1 *N* HCl and clarified and 12 *N* HCl was added to the hot solution until crystallization commenced; cooling then provided pure crystalline aminoacridone hydrochlorides. To obtain the free bases the hydrochlorides were dissolved in hot excess EtOH–H_2O (2:1 v/v); the solution was basified (NH_3) and rapidly filtered. EtOH was removed from the hot filtrate until crystallization commenced; cooling then provided pure samples of the aminoacridones. The aminoacridones have been prepared in the past by a variety of methods and constants are well recorded.[41-46] The free aminoacridones readily autoxidize and become discolored in air. The compounds should be stored *in vacuo* or converted to the stable hydrochlorides or acetyl derivatives.

Acetamidoacridones were readily prepared by a variety of procedures but the most convenient method was to dissolve the aminoacridone in the minimum volume of AcOH possible and add to the hot (90°) solution Ac_2O (1.5 mol/mol of NH_2 group). After an initial vigorous reaction the acetamidoacridones crystallized from the solution in essentially pure form as gauged by tlc. The acetamidoacridones show little solubility in usual solvents but could be recrystallized from DMF, Py, $C_6H_5NO_2$, or *N*-methylpyrrolidone. Tlc provided a superior index of purity to the melting points, most acetamidoacridones melting at greater than 360° with decomposition.[44-49]

Biological Testing. The 10^5 L1210 cells were inoculated intraperitoneally into 18.5–22.5-g C_3H/DBA_2 F_1 hybrids on day 1; drug treatment was initiated 24 hr 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. Median survivals were calculated in the usual way. Groups of six animals per dose level were used and there was one control group for every five tests. Compounds which under these test conditions were not given *T/C* values greater than 125% have been classed as negative and this is recorded in the requisite column in Table I.

Acknowledgments. We are grateful to the Misses B. Boreham, C. West, and N. Price for technical assistance in the performance of the many biological tests. This work was supported by the Auckland Division, Cancer Society of New Zealand, Inc., and in part by a grant from the Medical Research Council of New Zealand.

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Potential Antitumor Agents. 15. Bisquaternary Salts

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A series of 37 amine-substituted variants of bisquaternary salts of 4-[*p*-[*p*-(4-pyridylamino)phenylcarbamoyl]anilino]quinoline was prepared and evaluated against the L1210 system. Lipophilic–hydrophilic balance of the agents was adjusted with the *N*-alkyl quaternary function. The variants differed in (a) the terminal basic function, the N^1-alkyl-4-pyridylamino component being interchanged with either N^1-alkyl-5-(2,4-diaminopyrimidinyl) or amidinohydrazone; (b) the number and (c) the positioning of the primary amine substituents; (d) reversal of the central amide bond; and (e) the *N*-alkyl quaternary function. Six of the variants prepared provided 100-day survivors when administered intraperitoneally to mice bearing either intraperitoneally or subcutaneously implanted L1210 cells (10^5).

We have demonstrated that our earlier prepared symmetrical bisquaternary salts [*e.g.*, 3,3′-[terephthaloylbis(imino-*p*-phenylenecarbonylimino)] bis[1-ethylpyridinium *p*-toluenesulfonate]] showed marked chronic toxicity, any L1210 active dose proving lethal.[1] However, certain primary amine substituted variants of these agents could produce numbers of indefinite survivors in early intraperitoneal L1210 tests.[2] The role of the amine substituents in these latter variants could be to either attenuate chronic toxicity or increase drug selectivity toward the leukemic population. Our later demonstration that asymmetric bisquaternary salts of generic formula I were much less chronically toxic[1] then raised the possibility that amine substitution might further increase the antileukemic activity of this generic type. We have already shown that such agents bearing a 6- or 7-amino group on the quinoline ring will furnish a percentage of 100-day survivors in early intraperitoneal L1210 tests.[1] The present communication details the preparation and antileukemic (L1210) activity of isomeric mono- and diamine substituted variants of I.

Chemistry. The general synthetic method is detailed in Scheme I. Nitro groups were used as precursors to the required amine substituents, the nitro functions being ulti-