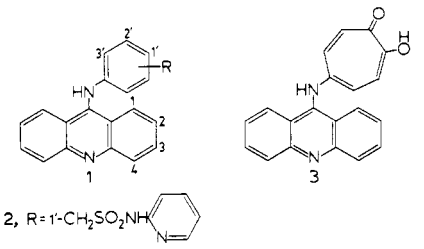
Potential Antitumor Agents. 26. Anionic Congeners of the 9-Anilinoacridines

William A. Denny, Graham J. Atwell, and Bruce F. Cain*

New Zealand Cancer Society's Experimental Chemotherapy Laboratory, Auckland, New Zealand. *Received June 2, 1977*

To investigate the possible importance of small levels of sulfonamide anion in the antileukemic (L1210) 4'-(9-acridinylamino)methanesulfonanilides, an anionic derivative was prepared, in which -COOH replaced $-NHSO_2CH_3$, and shown to have experimental antitumor activity. Analogue synthesis and evaluation show that acceptable placement of the carboxylate function on the 9-anilino ring is restricted to the 1' position. While the 1'-COOH and 1'-$(CH_2)_2$COOH analogues proved active, the intermediate acetate variant (1'-CH_2COOH) was inactive. There were marked differences in the effects of added acridine ring substituents, on biologic activity, depending on the function attached to the 9-anilino ring (1'-$NHSO_2CH_3$ or 1'-COOH). Consideration of the vector components of possible drug-binding forces, acting on a DNA-intercalated agent, suggests that there may be low-energy barriers to two-dimensional reorientation of a planar, intercalated chromophore in relation to the neighboring purine–pyrimidine base pairs. It is proposed that site forces from an added substituent could lead to modified DNA-binding orientations. Observed acridine–ring substituent effects on biologic activity would then depend on the 9-anilino ring function (1'-$NHSO_2CH_3$, 1'-COOH) employed.

Earlier papers of this series demonstrated that simple congeners of the 9-anilinoacridine (1) series bearing



electron-withdrawing 1'-functionality were devoid of experimental antileukemic (L1210) activity.[1-3] Most prepared examples demonstrating biological activity contained a 1'-electron donor substituent. To this viewpoint the exemplary activity conferred by a 1'-alkanesulfonamide moiety (e.g., 1, R = 1'-$NHSO_2CH_3$; 4, Table I) is remarkable since such substituents are quoted to be mildly electron withdrawing [σ_p(-$NHSO_2CH_3$) = 0.03].[4] However, the pK_a values of typical alkanesulfonanilides encountered in this laboratory lie in the range 8.9–9.3. At physiologic pH values there will be small proportions (1.2–3%) of sulfonanilide anion which will function as an extremely effective electron-donor component. In agreement, a congener in which ionization of the sulfonamide function is prevented by N-alkylation (5, Table I) is both less active and less dose potent. Hammett's σ values derived from physicochemical studies, when these are carried out at a nonphysiologic pH, may not serve adequately in attempted derivation of quantitative molecular structure–biologic activity relationships when the drug substituents employed are partially ionized at the pH values encountered in the biological screening system.

While partial ionization of a sulfonamide substituent could confer substantial electron-donor properties, there is also the possibility that the resulting 1'-anionic function could, through electrostatic or other interactions, itself provide additional site binding. To probe the latter possibility the simple carboxylic acid analogue (1, R = 1'-COOH; 6, Table I) was prepared and found to have relatively high experimental antitumor activity, independently substantiated by screening of the corresponding sodium salt as NSC235082. The ease of formulation of such analogues as anionic salts, and the high activity initially encountered (6), prompted the following investigation of the structure–antileukemic relationships for a series of anionic derivatives of the 9-anilinoacridines.

Chemistry. Agent synthesis involved mild acid-catalyzed coupling of the requisite 9-chloroacridine and a substituted aromatic amine, as before.[1-3] Synthetic methods for all necessary nitro-substituted precursors, to the required aromatic amines, have been earlier described in the chemical literature. Nitro-group reduction, without complicating hydrogenation of double bond in, for example, the side-chain component necessary for 33, could be conveniently carried out with Zn–NaOH.

Certain necessary components bearing ortho-disposed amine and acid functions, e.g., 2-aminophenylacetic, 3-(2-aminophenyl)propionic, and 2-aminocinnamic acid, can undergo facile ring closure to provide lactams which do not react with 9-chloroacridines under our standard reaction conditions. As before[6] catalytic nitro group reduction ($Pd-H_2$) of the potassium salts of 2-nitrophenylacetic and 3-(2-nitrophenyl)propionic acids provided the required amino acid components, stabilized as their carboxylate salts. Similarly, reduction of 2-nitrocinnamic acid with Zn–NaOH afforded the sodium salt of 2-aminocinnamic acid.[5] At an initial reaction pH of 6.5, the carboxylate function, in these latter three amino acid salts, remains essentially fully ionized but reaction of the amine function with 9-chloroacridine proceeds and the desired products (35–37) can be obtained. Product yields in these couplings were depressed, below those seen in more usual examples, and higher than normal amounts of the by-product 9-(10*H*)-acridone were produced. Presumably these lower yields stem from competing ring closure of the amino acid components. Once formed these acid variants (35–37) appeared stable and ring closure of the carboxylic function onto the acridine 9-amino group was not detected.

Results and Discussion

As before[3] measures of molecular lipophilic–hydrophilic balance, which play a dominant role in determining the attainable level of biological selectivity,[7] employ R_m values from reversed-phase partition chromatography. Following earlier findings[3] partitioning of the agent cation has been examined and the acidity of the solvents employed (0.05 N CH_3SO_3H) should be sufficient to repress ionization of an appended carboxylate function. As the carboxylate anion may play a role in determining partition-dependent, rate-of-drug arrival at the ultimate site of action, the R_m values as measured for strictly cationic variants, and those bearing anionic functions, should not be directly compared.

The aminoacridine antibacterial agents require cationic character to display biologic activity.[8] Addition of anionic functions to 9-aminoacridine (2- or 4-COOH; 1-, 2-, 3-, or 4-OH) furnishes zwitterionic products lacking appreciable antibacterial activity.[9] In these zwitterionic products the pK_a values of both acidic and basic centers are markedly augmented above the values seen in congeners containing either the 9-amino group or the anionic function alone.[10]

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Table I. Physicochemical Properties and L1210 Screening Data for the 9-Anilinoacridines

No.	Substituents in 1	Mp, °C	Formula	Analyses[a]	pK_a[b]	σ_p[c]	R_m[d]	O.D.[e]	L1210[f] ILS_{max}
4	1′-$NHSO_2CH_3$	*g*			7.19	0.03	0.00	45	107
5	1′-$N(CH_3)SO_2CH_3$	*h*			6.95		0.12	120	58
6	1′-COOH	285–286	$C_{20}H_{14}N_2O_2$	C, H, N	7.17	0.00[i]	0.27	70	120
7	3′-CH_3,1′-COOH	303–305	$C_{21}H_{16}N_2O_2$	C, H, N			0.40	>500	26[j]
8	3′-CH_3,1′-$NHSO_2CH_3$	*h*					0.15	97	106
9	3′-OH,1′-COOH	313–315	$C_{20}H_{14}N_2O_3 \cdot HCl \cdot H_2O$	C, H, N, Cl			0.35	>500	
10	3′-OH,1′-$NHSO_2CH_3$	*h*					0.09	25	88
11	3′-OCH_3,1′-COOH	286–287	$C_{21}H_{15}N_2O_3 \cdot HCl$	C, H, N, Cl	7.41		0.42	260	67
12	3′-OCH_3,1′-$NHSO_2CH_3$	*h*			7.43		0.18	6.7	114 (2)
13	3-NH_2,1′-COOH	335–337	$C_{20}H_{15}N_3O_2 \cdot HCl$	C, H, N, Cl	8.90		0.10	50	
14	3-NH_2,1′-$NHSO_2CH_3$	*g*			9.80		−0.14	2.5	81 (1)
15	3-N_3,1′-COOH	>360	$C_{20}H_{13}N_5O_2$	C, H, N			0.39	100	72 (1)
16	3-N_3,1′-$NHSO_2CH_3$	*k*			7.00		−0.02	16	116 (2)
17	3-$NHCOCH_3$,1′-COOH	333–335	$C_{22}H_{17}N_3O_3 \cdot HCl$	C, H, N, Cl	7.42		0.15	>500	
18	3-$NHCOCH_3$,1′-$NHSO_2CH_3$	*g*			7.34		−0.12	13	114 (2)
19	3-NO_2,1′-COOH	186–188	$C_{20}H_{13}N_3O_4$	C, H, N			0.34	150	30
20	3-NO_2,1′-$NHSO_2CH_3$	*g*			5.52		−0.08	25	123 (2)
21	3-I,1′-COOH	208–210	$C_{20}H_{13}IN_2O_2 \cdot 0.5H_2O$	C, H, N, I			0.47	>500	74[j]
22	3-I,1′-$NHSO_2CH_3$	*h*			6.52		0.20	100	128 (2)
23	4-CH_3,1′-COOH	321–323	$C_{21}H_{16}N_2O_2 \cdot HCl \cdot 0.25H_2O$	C, H, N, Cl			0.34	65	67
24	4-CH_3,1′-$NHSO_2CH_3$	*g*			7.15		0.07	33	113
25	4-$CONH_2$,1′-COOH	183–185	$C_{21}H_{15}N_3O_3$	C, H, N			−0.19	220	
26	4-$CONH_2$,1′-$NHSO_2CH_3$	284–285	$C_{21}H_{18}N_4O_3S \cdot HCl$	C, H, N, Cl	6.12		−0.47	280	44
27	2′-COOH	>360	$C_{20}H_{14}N_2O_2 \cdot H_2O$	C, H, N	7.62		0.54	>500	
28	2′-$NHSO_2CH_3$	*l*			6.85		0.02	330	93
29	3′-COOH	266–268	$C_{20}H_{14}N_2O_2 \cdot HCl$	C, H, N	7.28		0.31	93	
30	1′-CH_2COOH	255–257	$C_{21}H_{16}N_2O_2$	C, H, N	7.92		0.23	420	
31	2′-CH_2COOH	239–241	$C_{21}H_{16}N_2O_2 \cdot HCl \cdot H_2O$	C, H, N, Cl	7.82		0.24	37	
32	2′-$(CH_2)_2$COOH	225–227	$C_{22}H_{18}N_2O_2 \cdot HCl \cdot H_2O$	C, H, N			0.42	>500	
33	2′-CH=CHCOOH	290–292	$C_{22}H_{16}N_2O_2 \cdot HCl$	C, H, N, Cl			0.40	>500	
34	2′-$(CH=CH)_2$COOH	300–303	$C_{24}H_{18}N_2O_2 \cdot HCl$	C, H, N, Cl			0.49	>500	
35	3′-CH_2COOH	229–231	$C_{21}H_{16}N_2O_2 \cdot HCl$	C, H, N, Cl	8.12		0.30	210	
36	3′-$(CH_2)_2$COOH	270–271	$C_{22}H_{18}N_2O_2 \cdot HCl$	C, H, N, Cl			0.53	165	
37	3′-CH=CHCOOH	265–266	$C_{22}H_{16}N_2O_2 \cdot HCl \cdot 0.5H_2O$	C, H, N, Cl			0.39	375	
38	1′-$(CH_2)_2$COOH	235–236	$C_{22}H_{18}N_2O_2$	C, H, N	7.91		0.48	140	172 (2)
39	1′-CH=CHCOOH	235–236	$C_{22}H_{16}N_2O_2$	C, H, N	7.17		0.30	>500	98[j]
40	3′-OCH_3,1′-CH_2COOH	247–249	$C_{22}H_{18}N_2O_3 \cdot HCl$	C, H, N, Cl			0.36	90	
41	3-$NHCH_3$,1′-CH_2COOH	282–283	$C_{22}H_{19}N_3O_3 \cdot HCl$	C, H, N, Cl			0.34	300	
42	4-$OCH_2CHOHCH_2OH$,1′-CH_2COOH	214–216	$C_{24}H_{22}N_2O_5 \cdot H_2O$	C, H, N			−0.09	>500	
43	3′-OCH_3,1′-$(CH_2)_2$COOH	225–227	$C_{23}H_{20}N_2O_3 \cdot HCl$	C, H, N, Cl			0.86	120	82

44	3′-OCH_3,1′-CH=CHCOOH	209–210	$C_{23}H_{18}N_2O_3 \cdot HCl \cdot H_2O$	C, H, N, Cl			0.55	>500	
45	1′-OCH_2COOH	287–288	$C_{21}H_{16}N_2O_3 \cdot 1.5H_2O$	C, H, N	8.19		0.06	>500	
46	1′-$CH_2CH(NH_3^+)COO^-$	221 dec	$C_{22}H_{19}N_3O_2 \cdot HCl \cdot 0.5H_2O$	C, H, N, Cl			−0.92	160	
47	1′-$CH_2CH(NH_3^+)COO^-$,3-NO_2	>360	$C_{22}H_{18}N_4O_4 \cdot HCl \cdot 0.5H_2O$	C, H, N			−0.99	200	
48	Formula 3	293–295	$C_{20}H_{14}N_2O_2 \cdot HCl$	C, H, N, Cl			−1.07	310	
49	1′-$COOCH_3$	173–175	$C_{21}H_{16}N_2O_2 \cdot H_2O$	C, H, N	6.21	0.45	0.51	>500	87[j]
50	1′-$CONH_2$	*l*			6.47	0.36	−0.25	125	
51	1′-CH_2CONH_2	231–232	$C_{21}H_{18}N_3O_4 \cdot MsOH \cdot H_2O$[m]	C, H, N, S	7.43	0.07	−0.33	31	56
52	1′-$(CH_2)_2CONH_2$	220–222	$C_{22}H_{19}N_3O \cdot H_2O$	C, H, N	7.63		−0.17	65	94
53	1′-CH=CHCONH$_2$	213–215	$C_{22}H_{17}N_3O \cdot HCl$	C, H, N			−0.15	>500	59[j]
54	3′-OCH_3,1′-$(CH_2)_2CONH_2$	167–169	$C_{23}H_{21}N_3O_2 \cdot HCl \cdot H_2O$	C, H, N, Cl			0.13	60	65
55	3′-OCH_3,1′-CH=CHCOOH	213–215	$C_{23}H_{19}N_3O_2 \cdot HCl \cdot H_2O$	C, H, N			0.07	160	39
56	1′-SO_3H	>360	$C_{19}H_{14}N_2O_3S \cdot H_2O$	C, H, N, S	6.87	0.09[n]	−0.78	420	
57	1′-CH_2SO_3H	>360	$C_{20}H_{16}N_2O_3S \cdot 0.5H_2O$	C, H, N, S	7.64		−0.68	>500	
58	1′-$(CH_2)_2SO_3H$	>360	$C_{21}H_{18}N_2O_3S \cdot 0.5H_2O$	C, H, N	7.77		−0.60	>500	
59	3-NO_2,1′-$(CH_2)_2SO_3H$	>360	$C_{21}H_{17}N_3O_5S$	C, H, N			−0.66	>500	
60	1′-SO_2NH_2	*l*			6.11	0.57	−0.52	>500	
61	1′-$CH_2SO_2NH_2$	239–242	$C_{20}H_{17}N_3O_2S \cdot HCl$	C, H, N, Cl	7.05		−0.36	62	46
62	1′-$(CH_2)_2SO_2NH_2$	292–294	$C_{21}H_{19}N_3O_2S \cdot HCl \cdot H_2O$	C, H, N, Cl	7.43		−0.20	60	58
63	1′-R; R = 2	188–189	$C_{22}H_{19}N_3O_2 \cdot HCl \cdot 0.5H_2O$	C, H, Cl; N[o]			−0.04	280	72
64	1′-NO_2	221–223	$C_{19}H_{13}N_3O_2$	C, H, N	5.58	0.78	0.27	>500	
65	1′-CN	102–103	$C_{20}H_{13}N_3 \cdot HCl$	C, H, N, Cl	5.93	0.63	0.24	200	
66	1′-$COCH_3$	275–277	$C_{21}H_{16}N_2O \cdot HCl \cdot H_2O$	C, H, N, Cl	6.12	0.52	0.35	>500	
67	1′-Br	258–260	$C_{19}H_{13}BrN_2 \cdot HCl \cdot 0.5H_2O$	C, H, N, Br	7.00	0.23	0.60	300	
68	1′-Cl	245–247	$C_{19}H_{13}ClN_2 \cdot HCl$	C, H, N, Cl	7.06	0.23	0.57		
69	1′-$NHSO_2C_6H_5$	*l*			7.09	0.01	0.45	37	97 (2)
70	1′-H	*l*			7.46	0.00	0.42	170	
71	1′-$NHCOCH_3$	*l*			7.51	0.00	0.26	20	53
72	1′-$NHCOOCH_3$	*l*			7.64	−0.15	0.47	50	62
73	1′-CH_3	292–295	$C_{20}H_{16}N_3 \cdot HCl$	C, H, N, Cl	7.72	−0.17	0.69	125	48
74	1′-$NHCONHCH_3$	*p*			7.77	−0.25	0.08	60	112 (2)
75	1′-OCH_3	*l*			7.94	−0.27	0.52	110	
76	1′-NH_2	*l*			8.36	−0.66	−0.08	33	72
77	3-NO_2,1′-$NHSO_2CH_3$,3′-OCH_3	*q*			5.72		0.11	4.5	84
78	3,6-$(NO_2)_2$,1′-$NHSO_2CH_3$,3′-OCH_3	*r*			3.82		0.08	45	58
79	3-N=,1′-$NHSO_2CH_3$,3′-OCH_3	*q*			5.53		−0.13	90	80
80	4-N=,1′-$NHSO_2CH_3$,3′-OCH_3	*q*			6.09		−0.07	62	89

[a] Analyses for the indicated elements were within ±0.4% of the calculated figures for the formula provided except where noted. [b] pK_a value for the acridine ring system alone. Measured UV spectrophotometrically, see ref 20. [c] Hammett's σ_p constants for the 1′-R group only. Taken from the compilation of ref 4. [d] Measure of lipophilic–hydrophilic balance of agent cation utilizing reversed-phase chromatography; see ref 3. [e] Optimum dose in mg/kg/day in L1210 tests. Inoculum 10^5 cells ip, treatment qd 1–5 ip. [f] Maximum percentage increase in life span (ILS) in L1210 tests seen at the optimum dose. ILS = T/C% − 100. The number of animals surviving 50 days after completion of dosing, from a group of six, is provided in parentheses. [g] Reference 3. [h] Reference 2. [i] For carboxylate anion $-COO^-$. [j] Maximum tolerated dose not reached; higher doses may provide greater life extension. [k] Reference 22. [l] Reference 1. [m] MsOH = methanesulfonic acid. [n] For sulfoxylate anion $-SO_3^-$. [o] N: calcd, 11.7; found, 11.05. [p] Reference 6. [q] Reference 22. [r] Reference 11.

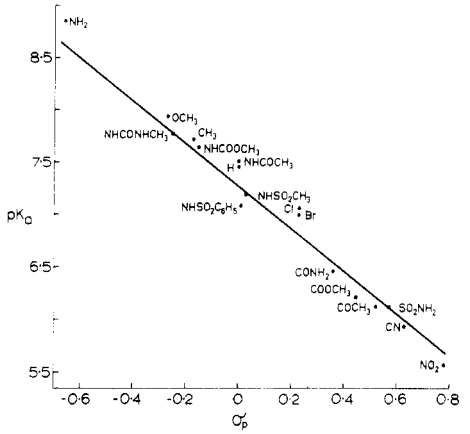

Figure 1. Relationship between the ionization constants of the 9-(*p*-R-anilino)acridines and Hammett's σ_p values for the function R.

In contrast, addition of a 1′-carboxylic acid function to 9-anilinoacridine provides a biologically active agent (**6**) and the measured acidic and basic pK_a values (4.20 and 7.17, respectively) for this compound are not augmented over the expected values. Grouped at the base of Table I are a series of analogues (**64–76**) prepared to investigate the role of electronic effects of 1′-R groups on acridine pK_a and biologic activity. There is excellent electronic communication between such groups and the acridine ring; changes in acridine base strength are of similar magnitude if a substituent is attached either at the 1′ position or directly to the acridine ring system. Acridine pK_a values in the 9-(1′-R-anilino)acridines are linearly related to the σ_p values of group R (Figure 1) as shown in eq 1. In eq

$$pK_a = -2.03\ (\pm 0.22)\ \sigma_p + 7.27\ (\pm 0.08) \qquad (1)$$
$$n = 17,\ r = 0.98,\ s = 0.165,\ F_{1,15} = 173.1$$

1, n is the number of data points, r the correlation coefficient, s the standard deviation of the correlation, and F is the F statistic. Confidence limits (95%) for the coefficient and constant term are provided in parentheses. The carboxylate residue of **6** should exist predominantly as the anion at pH values where acridine ionization alters and the acridine base strength predicted from eq 1, for an analogue with a 1′-COO^- function ($\sigma_p = 0.00$),[4] would be 7.27 (found 7.17). If the σ_p value for an un-ionized –COOH function (0.45) was operative, then a pK_a for **6** of 6.36 would be expected. When ionization of the carboxylate residue is prevented, as in the ester **49** and the amide **50**, pK_a values of 6.21 and 6.27 are observed (predicted from eq 1, 6.36 and 6.54, respectively). Even when the basic character of the acridine nucleus in an anionic variant is increased, by addition of a 3-NH_2 group as in **13**, the pK_a values observed (acridine, 8.90; –COOH, 4.36) are not augmented above those seen with the simpler 3-amino-9-anilinoacridine (9.12) and benzoic acid (4.21). Alternatively, when a more acidic anionic function is appended (–SO_3^-, **56**) the pK_a observed for the acridine (6.87) is not augmented above that predicted from eq 1 (7.09). Clearly, cationic and anionic functions are sufficiently divorced, in these derivatives, not to provide the base strength augmentation seen in the simpler 9-aminoacridine derivatives.

In the alkanesulfonanilide congeners addition of a 3′-OCH_3 group, in the presence of a range of varying acridine substituents, provided valuable increases in dose potency without significant decreases in levels of biologic effectiveness (cf. **4** and **12**).[2,3,11] In contrast, addition of this same function to the 1′-COOH analogue (**6**), to furnish **11**, provided a less dose potent and less active agent. Similarly, alternate 3′ electron-donor substituents formerly found acceptable in the alkanesulfonanilide series[2] (3′-CH_3, **8**; 3′-OH, **10**) provided marked drops in activity and dose potency in the 1′-carboxylate series (3′-CH_3, **7**; 3′-OH, **9**).

Simple 4′-(9-acridinylamino)alkanesulfonanilides (e.g., **4**) are typical DNA-intercalating agents[12] and the structure–activity relationships of this subgroup of the 9-anilinoacridines can be rationalized in terms of such an intercalation site of action.[2] Steric interactions of acridine 1,8 positions and the 9-anilino ring force adoption of an out-of-plane conformation by the latter, x-ray crystallographic analysis of **4** showing an angle of 77° between the plane of the 9-anilino ring and that of the acridine ring system.[13] The well-characterized DNA-intercalator ethidium (3,8-diamino-5-ethyl-6-phenylphenanthridinium),[14,15] also possessing experimental antitumor properties,[16,17] has the 6-phenyl ring similarly displaced from the phenanthridine ring plane by 83°.[18] When the acridine and phenanthridine ring systems of **4** and ethidium intercalate between the purine–pyrimidine base pairs of twin-helical DNA, the out-of-plane position of the appended 9-anilino and 6-phenyl rings should dictate that these will remain outside the confines of the narrow, slot-like intercalation site. In simple ethidium–dinucleotide complexes x-ray crystallographic analysis demonstrates that the 6-phenyl group is so located.[19] When a 9-anilinoacridine is lodged in such a site, the 9-anilino ring need not occupy a single position but could be located within a considerable volume of space. Alternatively, the out-of-plane anilino ring could protrude into the DNA major groove, rather than the minor as earlier suggested,[2] providing a further range of possible locations for this group. Forces acting between the pyrimidine–purine base pairs and the sandwiched acridine ring system are, in the main, at right angles to the acridine ring plane so that there may be only small energy barriers to translational movement of the acridine within the confines of the base-pair sandwich. It would appear feasible for the 9-anilino function to reside in many of the possible alternate locations with the acridine ring system accommodating by two-dimensional slippage between the adjacent base pairs. However, once the location for the 9-anilino ring is specified, by binding interactions between ring substituents and peripheral site functionality, or possibly minimization of electrostatic repulsions (e.g., $-PO_2^{-}$ and $-COO^-$), then there is much less freedom in the positioning of the intercalated acridine. If different substituents were attached to the 9-anilino ring, then the most favored site location for this ring could vary. As the location of the anilino ring changes so must the relative positioning of the intercalated acridine ring and, as a consequence, substituent space adjacent to both the acridine and the 9-anilino rings would also change. Grouped in pairs in Table I (entries **6–28**) are a series of substituted acridine variants, one pair member bearing a 1′-$NHSO_2CH_3$ and the other a 1′-COOH function. There appears a marked difference between the effects of added acridine substituents, on antileukemic activity, depending on the anilino ring function employed, and this may be a consequence of the adoption of different site-binding orientations as the side-chain functionality is altered. A consequence of these findings is that the structure–activity relationships found with the 1′-$NHSO_2R$ congeners will not be directly transferable to the 1′-COOH analogues and those for the latter should be independently investigated.

Activity dependence on placement of carboxylate function is suggested by inactivity of the 2′ (**27**) and 3′ (**29**)

isomers and the acetate analogue **30**. While several of the 2′-alkanoic acids (**31–34**) could adopt conformations in which the carboxylate function resided in a similar relative position to that of a 1′-COOH, none of these proved active. In contrast, extension of the alkyl chain in the inactive acetate analogue **30**, to furnish the propionate **38**, restored antileukemic activity and the corresponding unsaturated acid **39** was likewise active. Several further acetate analogues (**40–42**), admittedly utilizing acridine substituents found acceptable in the alkanesulfonanilide series,[2,7] also proved inactive. Attempting to increase dose potency of the propionate **38** and acrylate **37**, by appending 3′-OCH_3 groups to provide **43** and **44**, again proved unsuccessful and in one case provided an inactive analogue (**44**).

From experiences in the alkanesulfonanilide series, it could be conjectured that increasing the electron-donor properties of the 1′-substituent might augment activity. Replacing a methylene group of the active propionate analogue **38** by an oxygen atom, to provide the more electron-donating, isosteric **45**, furnished an inactive analogue.

Subsidiary basic functions linked terminally to a 1′-alkanesulfonamide substituent earlier provided highly active compounds.[20] Discovering drug analogues which will apparently tolerate both cationic and anionic functions in the same general substituent space is a relatively rare phenomena. Combination of both cationic and anionic side chain components in one molecule provided highly polar, zwitterionic, inactive analogues (**46**, **47**).

Tropolones have pK_a values very similar to carboxylic acids and, in the example shown (**48**), provide location of anionic charge very similar to the 1′-COOH analogue **6**. For unknown reasons the tropolone **48** proved much more hydrophilic than **6** and was found to be inactive against the L1210 leukemia.

The earlier examined 1′-$CONH_2$ variant (**50**)[7] proved inactive and this was ascribed to the high electron withdrawal [σ_p(-$CONH_2$) = 0.36] by this substituent. Attenuating electron withdrawal, by interposing a methylene group between the 9-anilino ring and amide [σ_p(-CH_2CONH_2) = 0.07], provides a biologically active compound (**51**). While the active carboxylate **6** provides an inactive amide (**50**), the situation is reversed with the acetic acid analogue **30**, which is inactive but provides an active amide (**51**). Similarly, amides of the propionate and acrylate systems (**52**, **53**), as well as their 3′-OCH_3 derivatives (**54**, **55**), proved active.

Substitution of -SO_3H for 1′-COOH, as in **56**, provided an inactive analogue. As isolation of the electron withdrawal of the -SO_3H (-SO_3^- at physiological pH values for which σ_p = 0.09) by interposed alkyl chains did not provide active analogues (**57**, **58**), such inactivity cannot be entirely ascribed to electronic effects. However, equivalent isolation of the electron withdrawal of a 1′-SO_2NH_2 (σ_p = 0.57) (**60**) did provide active congeners (**61**, **62**). While the pK_a values of aliphatic sulfonamides, such as **61** and **62**, are low, the sulfonamides of heterocyclic amines (cf. **63**) are considerably stronger acids and provide appreciable anion at physiological pH values. With the example of the antibacterial sulfonamides, and **63** as lead, it should prove possible to modulate agent lipophilic–hydrophilic balance, levels of protein binding, anionic pK_a values, plasma half-life, etc., by variation of the heteroaromatic amine employed—if so desired.

Of the agents employed in generating Figure 1 all L1210 active examples have pK_a values greater than 7. This observation suggests a relationship between agent pK_a, or, equivalently, σ_p for the 1′-R group (eq 1), and biologic activity. However, acridine substituents, in particular -NO_2 groups (**77**, **78**), markedly decrease pK_a but provide highly active materials.[7,11,21] Use of -NO_2 groups for base strength control in a biologic situation is equivocal since in vivo reduction could provide a variety of more basic materials. The demonstration that the aza analogues (cf. **79**, **80**), as nonclassical isosteres of the nitro derivatives, have comparable low pK_a values and are tumor active eliminates the latter criticism.[21] In this series of agents high acridine pK_a (>7) is therefore not a necessary condition for antitumor activity. Of the two parameters pK_a and σ_p, the latter must then be considered a dominant factor influencing biologic activity. In contradiction, there is not a clear increase in activity as more electron-donating substituents are employed (cf. progression **69–76**). Also, certain neutral and mildly electron-withdrawing functions (-$NHCOCH_3$, **71**; -$NHSO_2R$, **4** and **69**) have provided good L1210 activity as have other groups of similar dipolar character (-NHCOOR, **72**; -NHCONHR, **74**) although the latter have somewhat greater σ_p values.

Employing DNA as a putative site of action[2] it can be shown that a 1′-$NHCOCH_3$ substituent increases the binding constant to calf thymus DNA, in relation to the unsubstituted 9-anilinoacridine (**70**), by 2.5-fold and similarly a 1′-$NHSO_2CH_3$ augments binding by 2.2-fold.[23] The similar pK_a values for these three derivatives (**70**, 7.46; **71**, 7.51; **4**, 7.19) show that differences in both DNA binding and L1210 activity are unlikely due to altering basicities. Electron donation by the 1′-substituents of **71** and **4**, on the evidence of their pK_a values (eq 1), must be minimal. It must then be concluded that there is a local interaction of both the 1′-$NHCOCH_3$ and 1′-$NHSO_2CH_3$ groups with some DNA functionality. The nature of the forces involved in such augmented binding is not readily discerned from a study of the available extrathermodynamic parameters for the substituents so far employed. Certainly such studies will not be aided if different site-binding orientations are adopted as the substitution pattern alters. Indeed, as the 1′-substituent changes, in the examples portrayed in Figure 1, and side-chain DNA binding or repulsive forces also alter, each variant might well adopt a different minimum energy orientation. On this thesis a necessary condition for antitumor activity might then be adoption of an acceptable site-binding orientation.

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. Melting points were determined on an Electrothermal melting point apparatus with the makers' supplied stem corrected thermometer; melting points are as read. NMR spectra were obtained on a Varian A-60 spectrometer (Me_4Si). IR spectra (KBr) were recorded using a Beckmann 237 Infracord. UV spectra were recorded on a Shimadzu UV-200.

To monitor the progress of reactions, purification of products, etc., TLC on SiO_2 (Merck SiO_2, F_{254}) was used. For the products listed in Table I the most convenient solvents are the top phase of *n*-BuOH–HOAc–H_2O (5:1:4, v/v) and $CHCl_3$ containing 2–8% MeOH. The partition chromatographic methods used in measuring R_m values have been described earlier.[3]

Ionization constants were determined by UV spectrophotometry employing the methods described in full earlier.[20]

Standard Coupling Procedure. The substituted aniline component (10.5 mM) and the requisite 9-chloroacridine (10 mM) were dissolved in an appropriate volume of EtOH or *i*-PrOH by heating and stirring. In the preparation of variants containing carboxylic acid functions, use of EtOH as solvent provides small amounts of the ethyl ester of product as contaminant and, in such

cases, *i*-PrOH is preferred. To the resulting homogeneous solution concentrated HCl (1 mM) was added and the reaction mixture heated under reflux conditions for varying times. Using 9-chloroacridine a reaction time of 15 min was sufficient. With 9-chloroacridines containing electron-withdrawing functionality ($-NO_2$, $-CONH_2$), reaction was more rapid (6–9 min). When the acridine bears electron-donating substituents, longer reaction times must be employed and time for termination is best judged by TLC monitoring. Depending on solubility, products may crystallize directly from the boiling reaction mixture, following refrigeration, or organic solvent may have to be removed and Cl ion concentration increased by addition of HCl or NaCl to depress solubility of product hydrochloride. Recrystallization employed H_2O or MeOH, EtOH, *i*-PrOH, sometimes with addition of H_2O, or MeOH–EtOAc.

Hydrochloride salts of less strongly basic analogues tend to lose HCl on drying, particularly at elevated temperatures, and acceptable elemental analysis figures are then difficult to obtain. In such cases analysis of the free base has provided satisfactory values. The free bases may be conveniently obtained by solution of the hydrochloride salts in an aqueous–alcohol (MeOH, EtOH, *i*-PrOH) mixture, addition of the theoretical quantity of $KHCO_3$, and removal of the alcohol in vacuo until the base crystallizes. Recrystallization employed toluene or MeOH, EtOH, *i*-PrOH plus H_2O.

Sodium 4-(9-Acridinylamino)benzoate (NSC235082). Under the standard coupling conditions, employing 4-aminobenzoic acid and 9-chloroacridine, 4-(9-acridinylamino)benzoic acid hydrochloride of high purity separated directly from the boiling reaction mixture. A sample (16.0 g, 0.046 M) of hydrochloride so prepared was suspended in H_2O (400 mL) at 80 °C, Na_2CO_3 (5.0 g, 0.047 M) was added, and the whole mixture was stirred until homogeneous. NaCl (10.0 g) was dissolved in the hot, clarified solution with stirring; then the mixture cooled slowly, finally at 10 °C for 3 h. The Na salt was collected, sucked as dry as possible, and then dried in vacuo. The salt was dissolved in warm *i*-PrOH (100 mL), the solution clarified, and EtOAc (400 mL) added. Cooling provided pure Na salt as silky orange needles of mp >360 °C (13.0 g, 84%).

Modified Coupling Procedure. Preparation of 31-33. The potassium salts of 2-aminophenylacetic and 3-(2-aminophenyl)propionic acids were prepared, as before,[6] by hydrogenation (10% Pd/C; 45 psi of H_2, 25 °C) of H_2O solutions of the K^+ salts of the requisite nitro acids. Sodium 2-aminocinnamate was prepared by Zn–NaOH reduction[5] of 2-nitrocinnamic acid. The alkali salts were dissolved in the minimum volume of boiling 65% EtOH–H_2O. A solution of 9-chloroacridine (10 mM) in the same solvent was added and the pH of the mixture adjusted to 6.5 by dropwise addition of HOAc. As this pH is approached initiation of reaction is heralded by the very pale yellow color of the starting 9-chloroacridine changing to the usual deep orange-red of the coupled products. Under these conditions reaction was complete after 20 min of boiling. To remove the relatively large quantities of 9(10*H*)-acridone produced, the reaction mixtures were evaporated to dryness and the residual solids extracted with boiling H_2O (20 mL/g). On cooling of the clarified solutions product hydrochlorides crystallized. Further purification was as before.

Methods for generation, purification, and handling of necessary substituted 9-chloroacridines have been adequately detailed earlier.[2,7,11]

Biological Testing. The 10^5 L1210 cells were inoculated intraperitoneally into 18.5–22.5-g C_3H/DBA_2 F_1 hybrid mice. Ip drug treatment started 24 h later and continued once daily for 5 days. Detailed description of the test procedures, dose levels employed, etc., has been described in full earlier.[1-3]

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