Potential Antitumor Agents. 24. Dicationic Analogues of the 4'-(9-Acridinylamino)alkanesulfonanilides

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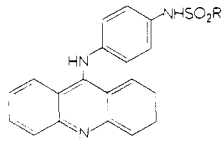
A consequence of a DNA intercalation site of action for the tumor inhibitory 4'-(9-acridinylamino)methanesulfonanilides is that correctly positioned, additional cationic functions should increase site binding. A series of 4'-(9-acridinylamino)alkanesulfonanilides bearing an additional basic function, appended terminally to the alkanesulfonamide chain [$-NHSO_2(CH_2)_nNH_3^+$; $-NHSO_2(CH_2)_nNHC(=NH_2)NH_2^+$], has been synthesized and evaluated in the L1210 leukemia system. Congeners with an unsubstituted acridine ring system had very low L1210 activity. Attenuation of agent base strength by acridine substitution with 3-NO_2 groups, or interposition of an amide function into the alkyl chain, provided highly active compounds. Alternatively, the more hydrophilic, strongly basic 3-amino-10-methyl-9-acridinylamino nucleus also provided highly active variants. From consideration of the range of available examples it is proposed that log P_0, in this drug series, alters with changing drug base strength. Both log P values and drug–site electrostatic interactions vary as alkyl chain length (n) changes; the chain length associated with highest biologic activity represents a compromise between these two factors.

The structure–activity relationships (SAR) of the experimental, broad spectrum 4'-(9-acridinylamino)alkanesulfonanilide antitumor agents (1), in the L1210 leukemia system, were examined in earlier parts of this series.[1-7] The SAR findings were shown compatible with drug intercalation into twin-helical DNA as a putative site of drug action.[1,2] It has since been shown that representative members of this drug series bind strongly to double-stranded DNA and can unwind closed circular, twin-helical, PM-2 bacteriophage DNA in a manner characteristic of intercalative drugs.[8] The DNA unwinding angle associated with drug intercalation (21°; 1, R = CH_3) approaches that of the well-characterized intercalator ethidium (reference at 26°),[9] under the same conditions, and is appreciably higher than that observed with simpler acridines not bearing the 9-anilino ring function.



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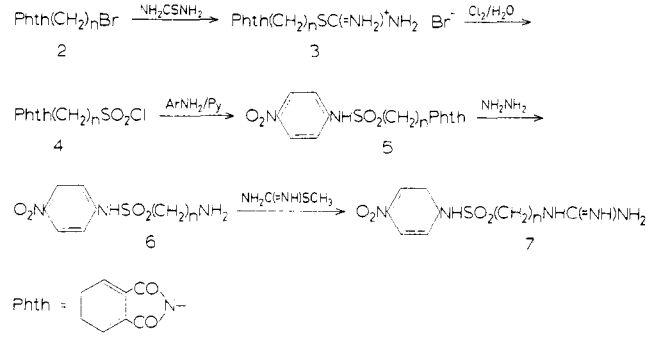
The origin of this drug series lies in earlier prepared antileukemic bisquaternary ammonium heterocycles.[10] Based on speculations as to possible sites of action for the latter, several molecular probes were prepared and screened for antitumor effectiveness.[3] Molecular manipulation[4] of the resulting tumor-active examples led ultimately to the present acridine agents.[2] Since there are two cationic charges in the progenitor drug series[10] it would appear reasonable to suggest that at least one further basic function might be acceptably appended to the singly charged acridine agents. Additionally, the drug–DNA intercalation model predicts that a multitude of anionic site charges are available for binding of additional drug basic functions and demonstrates the most likely acceptable positions of attachment for these.

The present work details the SAR, for the L1210 system, of 4'-(9-acridinylamino)alkanesulfonanilides having one additional cationic function appended terminally to the alkanesulfonamide chain.

Chemistry. Generation of the structural framework of variants of 1 employed acid-catalyzed coupling of a 4'-aminosulfonanilide component with the requisite 9-chloroacridine, as before.[1,2] Necessary alkanesulfonanilide intermediates, containing a terminal cationic function, were prepared by the route of Scheme I. Preparation of the 2-alkylisothioureas 3, from the corresponding alkyl bromides 2, provides Br^- salts which must be converted

Scheme I



by metathesis to alternatives (conveniently OAc^-) before the Cl_2 oxidation step (3 → 4); chlorinolysis of Br^- salts of 3 provides variable mixtures of sulfonyl chloride plus sulfonyl bromide.[11] The Cl_2 oxidation step may conveniently be performed by addition of an aqueous solution of $NaClO_3$ to a suspension of the isothiourea 3 in aqueous HCl.

Due to the deactivated amino group of 4-nitroanilines, the sulfonanilides 5 (Scheme I) were only obtained in moderate (47–69%) yield; subsequent work has shown that better yields (85–98%) result if more reactive amines (e.g., 4-alkanamidoanilines) are employed.

Of the few solvents which will dissolve both the amines 6 and 2-methylisothiourea sulfate, dimethylformamide proved most acceptable and provided excellent yields of the guanidines 7. All guanidine intermediates (7) were readily isolated and purified as their highly crystalline nitrate salts.

The primary aromatic amines produced by catalytic (Pd/C) or Fe(H^+) nitro group reduction of the aliphatic amines 6 and the guanidines 7 were extremely readily autoxidized and were accordingly, with minimum further manipulation, coupled with the requisite 9-chloroacridine under acid conditions. While reaction of the 9-chloroacridine with either amine group of the diamine intermediates, for example, 6 with NH_2 in place of NO_2, is theoretically possible single products resulted in which there was coupling to the aromatic amine function only. Weakly basic aromatic amines react with γ-chloro heterocycles via acid catalysis[12-14] while more strongly basic aliphatic amines, existing entirely as the cation under these conditions, fail to react. Reaction of aliphatic amines with γ-chloro heterocycles usually employs basic media[15] and can require vigorous conditions, except in phenol as solvent when facile reaction is mediated via the corresponding γ-phenoxy heterocycles.[16-18] In substantiation of the

selectivity of these couplings the phthalimide derivative **13**, prepared by phthaloylation of **10**, was identical with the product formed by nitro group reduction of **5** (n = 4) and following coupling of the formed amine with 9-chloroacridine. Attempted hydrazinolytic removal of the phthaloyl-protecting group of **13**, providing a possible alternative unambiguous route to **10**, displaced the 9-anilino function and produced 9-hydrazinoacridine as the major acridine product.

The dimethylamino intermediate necessary for preparation of **22** was conveniently prepared by Eschweiler–Clarke methylation of the primary amine **6** (n = 4).

Quaternary acridine analogues **24**, **25**, **30**, **41**, and **42** were synthesized from the corresponding N-methylacridones as detailed earlier.[5] 3-Amino quaternary salts were generated from the 3-acetamido-10-methylacridone precursor[5] and the protecting acetyl function was removed by mild acid hydrolysis before product isolation.

Side-chain components for the peptide analogues **28–32** were prepared by the active ester method and employed the 4-nitrophenyl esters of the amino acids protected as their N-phthaloyl derivatives. Reaction of these protected active esters with amine **6** (n = 2) provided N-phthaloyl derivatives of the required side-chain intermediates and following hydrazinolytic removal of the protective group provided analogues of **6** containing an amide function interposed into the alkane chain. Application of the standard elaboration sequence, using these amide variants, provided products **28–32**.

Biological Testing. All L1210 tests were performed in $C_3H \times DBA_2$ F_1 hybrid mice using a standard tumor inoculum of 10^5 cells. Agents were sufficiently soluble to be administered as aqueous solutions; dosage was by the intraperitoneal (ip) route commencing 24 h after tumor implantation and continued once daily for 5 days. Drug doses in ip L1210 tests were arranged at twofold intervals and ranged from the clearly toxic to the inactive. On retest doses were set midway between those initially employed and arranged to span the previously observed most effective dose.

Following our earlier studies, on the variation of antileukemic effectiveness with changing site of tumor inoculation,[6] those agents which have shown moderate activity (greater than 50% life extension) in ip L1210 tests have also been screened against subcutaneously (sc) implanted leukemia. In sc tests at least three dose levels, separated by 0.18 log dose intervals, were screened at one time with the median dose being the optimum initially observed in the ip tests. For acceptance of the results of such tests the highest dose must show some evidence of toxicity as gauged by either premature animal deaths before controls or shorter mean group life span in comparison with the next lowest dose employed. The lowest dose employed must be clearly suboptimal, as evidenced by shorter mean life span than the next higher dose, or nontoxic, as demonstrated by clear animal weight gain during drug treatment.

Lipophilic–Hydrophilic Balance. As before R_m values from reverse-phase chromatography have been used as a relative measure of overall molecular lipophilic–hydrophilic balance.[5] In earlier research we concluded that the species of these basic drugs which should be employed in gaining measures of lipophilic–hydrophilic balance is either the totally ionized drug or, alternatively, the neutral base.[5] If the log P change on ionization is sensibly constant among drug congeners, i.e., $\log P_{\text{neutral drug}} - \log P_{\text{cation}}$ = constant, then measures of lipophilic–hydrophilic balance for either species should prove equally acceptable. It was also pointed out then the conceptual difficulty in appreciating the physiologic pertinence of log P of neutral drug species with earlier examined bisquaternary salts,[10] as their strongly basic nature virtually eliminated the possibility of any reasonable proportion of neutral species being present at physiologic pH values. Similarly, in the present series, it is difficult to fathom the physiological significance of the lipophilic–hydrophilic balance of neutral drug species of the strongly basic guanidines (**33–46**) and particularly those examples containing two strongly basic functions (cf. **41** and **42**). Use of measures of log P for the ionized drugs has been retained and the R_m values quoted (Table I) have been obtained in sufficiently acidic media (0.05 N) that results should apply to the fully ionized molecules. The 3,6-dinitro variant (**46**) is exceptional, being very weakly basic (acridine pK_a = 3.68), and it is difficult to obtain sufficiently acidic conditions to ensure total drug ionization and yet maintain the integrity of the chromatographic media normally used. The somewhat higher than expected value for this analogue (**46**) probably reflects incomplete ionization during chromatography.

Since the agents discussed have an additional cationic function, relative to earlier examples, and measures of log P refer to cationic drug, observed R_m values are considerably lower than those found with earlier prepared monobasic acridines. R_m optima for the mono-[2] and dicharged (Table I) series should not be expected to coincide.

The increases seen in R_m values on lengthening the alkyl chain between the acridine moiety and primary amino function (**8–12**) are smaller than those observed on corresponding extension of the alkyl chain in the monocharged series [**1**, R = $(CH_2)_nCH_3$].[2] Albert[19] earlier commented on the relatively small changes in solvent partition properties of a series of mepacrine analogues where there was similar chain variation. There appears to be some shielding of the lipophilic character of the hydrocarbon chains, by the cationic functions, in these examples.

Structure–Activity Relationships. Analogues with an unmodified acridine nucleus spaced from a primary aliphatic amine function, by a range of alkane chain lengths (**8–12**), showed very low levels of antileukemic activity despite the predictions of model fitting to a DNA intercalation site that most should be acceptable. In vitro studies of drug interaction with calf thymus DNA showed that, at low ionic strength (0.01), all these agents bound more strongly, by orders of magnitude, than simpler analogues not bearing the additional basic function.[20]

In the evolutionary steps leading from the bisquaternary ammonium heterocycles[10] to the present acridine agents, we had encountered certain hybrid, tumor active, nonquaternary dicationic compounds which serve to link the two drug series.[3] In these dicationic hybrid agents there appeared to be a clear dependence on pK_a of the cationic centers; if both pK_a values were high then these agents, which were considerably more lipophilic than the precursor bisquaternary salts, proved L1210 inactive.[3] However, variants of closely similar log P, which had lower pK_a values, were convincingly tumor active.[3] These findings were interpreted at that time to imply that sufficient neutral species must be available for ready, multiple partitioning type, drug translocation to site of action. Such a viewpoint would suggest that in the present series attenuation of the pK_a of either cationic center of agents **8–12** might provide tumor-active agents and this proved to be the case. Members of the more weakly basic 3-nitroacridine analogues, of comparable chain length (**14–20**),

Table I. Physical Properties and L1210 Screening Data for Bis-cationic Acridine Congeners

Compd	Acridine substituent	R in 1	Mp, °C	Formula	Analyses	pK_a[a]	R_m	O.D.[b]	L1210 ILS, %[c] ip	L1210 ILS, %[c] sc
1		CH_3, parent for comparison purposes[d]				7.19	0.00	45	107	28
8		$(CH_2)_2NH_2$	299 dec	$C_{21}H_{20}N_4O_2S \cdot 2HCl \cdot 0.5H_2O$	C, H, N, Cl	6.62	−0.88	35	–[e]	
9		$(CH_2)_3NH_2$	308 dec	$C_{22}H_{22}N_4O_2S \cdot 2HCl$	C, H, N, Cl	6.89	−0.86	40	26	
10		$(CH_2)_4NH_2$	256 dec	$C_{23}H_{24}N_4O_2S \cdot 2HCl \cdot 1.5H_2O$	C, H, N, Cl	6.91	−0.82	25	27	
11		$(CH_2)_5NH_2$	242–243	$C_{24}H_{26}N_4O_2S \cdot 2HCl$	C, H, N, Cl	6.92	−0.76	60	–	
12		$(CH_2)_6NH_2$	282–283	$C_{25}H_{28}N_4O_2S \cdot 2HCl \cdot 0.5H_2O$	C, H, N, Cl	6.92	−0.69	65	–	
13		$(CH_2)_4$NPhth[f]	167–169	$C_{31}H_{26}N_4O_4S \cdot HCl \cdot 2H_2O$	C, H, N, Cl					
14	3-NO_2	$(CH_2)_2NH_2$	262 dec	$C_{21}H_{19}N_5O_4S \cdot 2HCl$	C, H, N, Cl	5.25	−0.95	20	31	
15	3-NO_2	$(CH_2)_3NH_2$	240–241	$C_{22}H_{21}N_5O_4S \cdot 2HCl$	C, H, N, Cl	5.33	−0.93	20	60	–
16	3-NO_2	$(CH_2)_4NH_2$	275 dec	$C_{23}H_{23}N_5O_4S \cdot 2HCl \cdot 1.5H_2O$	C, H, N, Cl	5.34	−0.91	60	102 (2)[g]	39
17	3-NO_2	$(CH_2)_5NH_2$	285 dec	$C_{24}H_{25}N_5O_4S \cdot 2HCl$	C, H, N, Cl	5.36	0.86	44	77	–
18	3-NO_2	$(CH_2)_6NH_2$	290–291	$C_{25}H_{27}N_5O_4S \cdot 2HCl$	C, H, N, Cl	5.34	0.79	2	53	–
19	3-NO_2	$(CH_2)_7NH_2$	285–287	$C_{26}H_{29}N_5O_4S \cdot 2HCl$	C, H, N, Cl	5.36	−0.66	25	26	
20	3-NO_2	$(CH_2)_8NH_2$	268 dec	$C_{27}H_{31}N_5O_4S \cdot 2HCl$	C, H, N, Cl	5.35	−0.43	30	–	
21	3-NO_2; 3′-OCH_3	$(CH_2)_4NH_2$	245 dec	$C_{24}H_{25}N_5O_5S \cdot 2HCl \cdot H_2O$	C, H, N, Cl	5.58	−0.80	4.4	49	
22	3-NO_2	$(CH_2)_4N(CH_3)_2$	275 dec	$C_{25}H_{27}N_5O_4S \cdot 2HCl \cdot 0.5H_2O$	C, H, N, Cl	5.36	−0.81	10	43	–
23	3-$NHCOCH_3$	$(CH_2)_4NH_2$	226–227	$C_{25}H_{27}N_5O_5S \cdot 2TsOH \cdot H_2O$[h]	C, H, N, S	7.16	−0.87	50	72	36
24	10-CH_3	$(CH_2)_4NH_2$	100–102	$C_{24}H_{26}N_4O_2S \cdot 2HCl$	C, H, N, Cl	6.72	−1.01	30	32	
25	3-NH_2; 10-CH_3	$(CH_2)_4NH_2$	210–212	$C_{24}H_{27}N_5O_2S \cdot 2HCl \cdot 4H_2O$	C, H, N, Cl	(9.9)[k]	−1.12	20	145 (3)	80
26	3-NO_2; 5-CH_3	$(CH_2)_4NH_2$	249 dec	$C_{24}H_{25}N_5O_4S \cdot 2HCl \cdot 0.5H_2O$	C, H, N, Cl	5.44	−0.78	200	–	
27	3-NO_2; 5,6-$(CH_3)_2$	$(CH_2)_4NH_2$	254 dec	$C_{25}H_{27}N_5O_4S \cdot 2HCl \cdot 2H_2O$	C, H, N, Cl	5.52	−0.74	20	31	
28		$(CH_2)_2NHCOCH_2NH_2$	249–251	$C_{23}H_{23}N_5O_3S \cdot 2HCl \cdot 2H_2O$	C, H, N, Cl	6.90	−0.98	50	58	
29		$(CH_2)_2NHCO(CH_2)_2NH_2$	97–98	$C_{24}H_{25}N_5O_3S \cdot 2HBr \cdot H_2O$	C, H, N, Br	6.91	−0.82	50		
30	10-CH_3	$(CH_2)_2NHCO(CH_2)_2NH_2$	98–99	$C_{25}H_{27}N_5O_3S \cdot 2HCl \cdot 3H_2O$	C, H, N, Cl	6.70	−1.13	120		
31	3-NO_2	$(CH_2)_2NHCOCH_2NH_2$	287 dec	$C_{23}H_{22}N_6O_5S \cdot 2HCl$	C, H, N, Cl	5.34	−1.08	200	212 (2)	27
32	3-NO_2	$(CH_2)_2NHCO(CH_2)_2NH_2$	288 dec	$C_{24}H_{24}N_6O_5S \cdot 2HCl$	C, H, N, Cl	5.35	−1.00	100	102	57
33	3-NO_2	$(CH_2)_2NHC(=NH)NH_2$	251 dec	$C_{22}H_{21}N_7O_4S \cdot 2HCl \cdot 1.5H_2O$	C, H, N, Cl	5.26	−0.96	50	260 (2)	74 (1)
34	3-NO_2	$(CH_2)_3NHC(=NH)NH_2$	289 dec	$C_{23}H_{23}N_7O_4S \cdot 2HCl \cdot 0.5H_2O$	C, H, N, Cl	5.34	−0.93	50	210 (1)	79 (1)
35	3-NO_2	$(CH_2)_4NHC(=NH)NH_2$	272–273	$C_{24}H_{25}N_7O_4S \cdot 2HCl \cdot 1.5H_2O$	C, H, N, Cl	5.36	−0.88	56	107	77
36	3-NO_2	$(CH_2)_5NHC(=NH)NH_2$	258 dec	$C_{25}H_{27}N_7O_4S \cdot 2HCl \cdot 0.5H_2O$	C, H, N, Cl	5.36	−0.79	75	84	39
37	3-NO_2	$(CH_2)_6NHC(=NH)NH_2$	240 dec	$C_{26}H_{29}N_7O_4S \cdot 2HCl \cdot 0.5H_2O$	C, H, N, Cl	5.35	−0.67	75	47	
38		$(CH_2)_4NHC(=NH)NH_2$	184–186	$C_{24}H_{26}N_6O_2S \cdot 2HCl$	C, H, N, Cl	6.91	−0.77	75	–	
39	3-Cl	$(CH_2)_4NHC(=NH)NH_2$	193–194	$C_{24}H_{25}ClN_6O_2S \cdot 2HCl \cdot 3H_2O$	C, H, N, Cl	6.33	−0.64	150	–	
40	10-CH_3	$(CH_2)_4NHC(=NH)NH_2$	67–68	$C_{25}H_{28}N_6O_2S \cdot 2HBr$	C, H, N, Br	6.69	−0.99	150	–	
41	3-NH_2; 10-CH_3	$(CH_2)_3NHC(=NH)NH_2$	139–140	$C_{24}H_{27}N_7O_6S \cdot 2HClO_4$	C, H, N, Cl	(9.9)[k]	−1.09	20	90 (4)	56
42	3-NH_2; 10-CH_3	$(CH_2)_4NHC(=NH)NH_2$	176–178	$C_{25}H_{29}N_7O_2S \cdot 2HBr$	C, H, N, Br	(9.9)[k]	−1.06	18	105 (4)	65 (2)
43	4,5-$(CH_3)_2$	$(CH_2)_4NHC(=NH)NH_2$	207–209	$C_{26}H_{30}N_6O_2S \cdot 2HCl \cdot 1.5H_2O$	C, H, N, Cl	6.41	−0.72	60	33	
44	3-$NHCOCH_3$; 6-NO_2	$(CH_2)_2NHC(=NH)NH_2$	241–244	$C_{24}H_{24}N_8O_5S \cdot 2HCl \cdot 0.5H_2O$	C, H, N, Cl	5.55	−1.07	25	128 (4)	118 (2)
45	3-$NHCOCH_3$; 6-NO_2	$(CH_2)_3NHC(=NH)NH_2$	240–242	$C_{25}H_{26}N_8O_5S \cdot 2HCl \cdot 0.5H_2O$	C, H, N, Cl	5.58	−1.03	25	139 (4)	110 (3)
46	3,6-$(NO_2)_2$	$(CH_2)_3NHC(=NH)NH_2$	281 dec	$C_{23}H_{22}N_8O_6S \cdot HCl$	C, H, N, Cl	3.68	0.43	>500[i]	95[j]	

[a] pK_a for the acridine nucleus only; determined as detailed in the Experimental Section. [b] Optimum dose, that dose in mg/kg/day, employing a daily qd 1–5 ip treatment schedule which provided greatest life extension in L1210 tests. Alternatively, for tumor inactive agents, the maximum tolerated dose. [c] Maximum percentage increase in life span (ILS) in L1210 tests at the optimum dose when tumor was implanted either intraperitoneally (ip) or subcutaneously (sc). [d] See ref 2. [e] – signifies significant ILS (>25%) was not observed at any dose employed. [f] Phth = abbreviation for phthaloyl. [g] Number of animals surviving 50 days after drug treatment, from an initial group of six, is provided in parentheses. [h] TsOH = *p*-toluenesulfonic acid. [i] Maximum dose level tried; optimum dose may be higher. [j] Limiting dose not reached; higher doses may provide greater life extension. [k] Overlapping pK_a values precluded direct measurement. Value provided is an estimate based on the measured pK_a of 4′-(3-amino-10-methyl-9-acridinylamino)-methanesulfonanilide (10.07) and the base weakening effect provided by the basic side chain.

showed excellent L1210 activity. These results contrast with the parent monocationic agents, the acridinyl and 3-nitroacridinyl variants having similar activities in ip L1210 tests.[2] Similarly, retaining the acridine nucleus but reducing the primary amine pK_a by interposition of an amide function in the alkyl chain as in the glycinamide analogue **28** (predicted primary amine pK_a = 7.97)[21] provided an active molecule while the more strongly basic β-alanyl analogue **29** (predicted amine pK_a = 9.67)[21] proved inactive. Presumably these peptide analogues are active per se since in vivo cleavage of the amide bond would liberate the L1210 inactive amine **8**. Not unexpectedly, on this thesis, when a low-basicity 3-nitroacridinyl unit was employed with these two peptide side chains both glycyl and β-alanyl congeners proved active (**29** and **30**).

The marked pK_a lowering produced by an aryl function appended to the amino group of 9-aminoacridine (pK_a = 9.6)[19] is readily apparent from the examples of Table I. The pK_a values for the 3-nitroacridinyl variants (**14–22**, **31–37**) show that these analogues would exist dominantly as a monocation at physiological pH values.

While the above results are in accord with our earlier views, on the role of base strength, later findings with homologous series of dialkanolamine dialkanesulfonic ester alkylating agents[22] suggested an alternative hypothesis. Attempted multiple regression analysis of the screening data for these alkylating agents was unsuccessful unless it was assumed that the optimum log P of each homologous series changed with the pK_a value of the core structure. It was found[22] than an operational log P where

$$\log P_{\text{operational}} = \log P_{\text{measured for cation}} + \log [H^+/(H^+ + K_a)] \quad (1)$$

would permit all variants to be accommodated in a single binomial regression equation. Such findings are also capable of extension to explain the acridine results described above; if strongly basic agents prove inactive because of supralipophilic character, then attenuation of pK_a values could shift the operational log P into the range where biologic activity might then be observed. The correction factor $H^+/(H^+ + K_a)$ of eq 1 provides major impact when pK_a exceeds pH by less than two units. Further increase in pK_a will then make little further change. However, on our earlier hypothesis additional increase in pK_a, by reducing further the available neutral species, would prove detrimental. The very high L1210 activity seen with members of the strongly basic guanidines **33–37** accords more with the conclusions reached from eq 1. Replacement of a 3-nitroacridinyl unit in an active guanidine example (**35**), by acridinyl (to provide **38**), again furnished a more extensively ionized and inactive congener. Attenuation of acridine pK_a by means other than a nitro group should also provide active agents but most investigated substituents which can acceptably accomplish this[2] further increase lipophilic character. For example, a 3-Cl decreases pK_a but a more lipophilic compound results (**39**) and proves inactive. This finding should be contrasted with the parental 4'-(9-acridinylamino)methanesulfonanilide series where a 3-Cl substituent provides excellent activity.[2]

Methyl groups adjacent to the polar acridine ring nitrogen atom were earlier shown not to provide the full expected increase in lipophilic character[7] and 4,5-dimethyl substitution lowers pK_a values by steric inhibition of proton addition to acridine ring nitrogen. The 4,5-$(CH_3)_2$ analogue **43** thus has similar acridine pK_a to the 3-Cl analogue **39** but is less lipophilic and does provide marginal L1210 activity.

Earlier, addition of a 5-CH_3 or 5,6-$(CH_3)_2$ to 4'-(3-nitroacridin-9-ylamino)methanesulfonanilide provided analogues showing exemplary activity against both ip and sc implanted L1210.[6] In the present series addition of the same substituents to the 3-NO_2 analogue **16** furnished congeners **26** and **27** with only marginal activity. On our present hypothesis contributing factors to these low activities are the increases in both lipophilic character and pK_a values due to the added methyl groups.

It can be further predicted from eq 1 that substituents added to the acridine nucleus of **4** which provide an overall more hydrophilic molecule, without necessarily decreasing acridine base strength, should provide more active agents and, if sufficient hydrophilic character could be conferred, even more strongly basic acridine nuclei would then be acceptable. In agreement, the more hydrophilic 3-$NHCOCH_3$ analogue **23**, while having a pK_a close to that of the precursor **10**, is significantly more active. Quaternization also provides a more hydrophilic molecule but only marginal activity results in the amine series (cf. **24** and **10**) while the more strongly basic guanidine analogue **24** is inactive. However, the yet more hydrophilic, strongly basic 3-amino quaternary salts **25**, **41**, and **42** show high activity.

Chain Length Dependence. The primary amine function of **16** and the more distal nitrogen atom of the guanidine moiety of **33** are equivalently located in relation to the 3-nitroacridine nucleus. Since these two agents appear to be the optimum members of their respective series (**14–20**, **33–37**) it might be suggested that this overlap position then locates the most favorable position for the second cationic charge. However, in isomers such as **14–20** and **33–37** the chain length associated with the most active member of each subseries could be dictated by lipophilic cutoff rather than closeness of matching of drug cation and site anion components. When charge separation is maintained as in the optimum subseries member **16**, but lipophilic character is increased in a variety of ways, agents of decreased activity result. Thus, the more lipophilic dimethylamino variant **22** is less active than **16**. The 3'-OCH_3 variant **21** was initially prepared to ascertain that this function exerts its usual dose potency increasing effect[1,7] in these dibasic agents and, as shown by the optimum doses of **21** and **16**, does so. However, the higher lipophilic character of **21** in relation to **16** is associated with lower activity. Increasing lipophilic character of **16** by ring methylation, to provide **26** and **27**, also decreases activity although acridine pK_a also changes. In contrast, the activity of the β-alanyl peptide **32** can be compared with that of the equivalent chain length analogue **18** and it is seen that the more hydrophilic chain of **32** confers greater activity at this longer chain length. The C_6 chain of **18** must then be supralipophilic and the peak activity seen with the C_4 chain isomer **16**, in the series **14–19**, may be dictated by lipophilic cutoff rather than the best matching of complementary drug–cation and site–anion. To derive quantitative SAR for these dicationic agents, delineating optimum log P, charge separation, and pK_a, as well as the important properties (steric, electronic etc.) of the substituents employed to modulate pK_a, could require the preparation and screening of a considerable cluster of compounds.

Conclusions

It is clear that, in agreement with the proposed DNA intercalation site model,[2,8] subsidiary basic functions may be acceptably appended to the series of tumor-active 4'-(9-acridinylamino)alkanesulfonanilides.

The screening results are in qualitative agreement with

the view that the optimum operational log P in this series is influenced by agent pK_a. It is difficult to embrace in one series all tumor active acridines so far prepared without such a hypothesis. Highly active examples now range from the very lipophilic, but weakly basic, 4′-(3-nitro-5,6-dimethyl-9-acridinylamino)-3′-methoxymethanesulfonanilide[7] through to the extremely hydrophilic, strongly basic guanidine examples (41 and 42) presented in this paper. The more hydrophilic of these dicationic acridines now have R_m values approaching closely those of maximally active bisquaternary salts which were the original progenitors of this drug series.[10] It is clear from the screening results presented that by preparing very hydrophilic, strongly basic, dicationic agents in this series excellent activity in usual L1210 assays can be obtained. However, for eradication of the clinically established and disseminated disease the need is not only for highly active agents but also those which can penetrate into pharmacological sanctuaries and kill every sequestered tumor cell. By preparing materials that are both extremely hydrophilic and highly charged drug distribution is proscribed and, for example, those congeners described here which are active against sc L1210 are without significant effect against intracerebrally inoculated leukemia. In our opinion it would be following the wrong course to further pursue these hydrophilic, strongly basic materials. The alternative suggested by eq 1 appears more realistic, to examine less basic agents which can, in consequence, have higher optimum lipophilic character. To achieve adequate drug–site binding, without employing conventional drug–cation site–anion interactions, requires that further consequences of a DNA intercalation site be examined.

Experimental Section

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

To monitor the progress of reactions, purification of products, etc., TLC on SiO_2 (Merck SiO_2, F_{254}) was used. The partition chromatographic methods used in measuring R_m values have been described earlier.[5]

Ionization Constants. Acridine pK_a values were determined UV spectrophotometrically using a Shimadzu UV-200 instrument having jacketed cell holders coupled to a circulating, thermostated water bath maintained at 25 °C. Ionization constants were determined in 20% DMF–buffer (v/v) mixtures and acetate, HEPES, and glycine buffers of 0.005 ionic strength were employed. While the dicationic agents of Table I are sufficiently soluble that pK_a values could be determined in aqueous solution there are several hundred further acridine variants[1-7] which are not sufficiently water soluble to treat in this manner. To provide strictly comparable measures of pK_a all determinations have been carried out in 20% DMF–buffer mixtures as solvent. Examination of the solvent induced pK_a shifts for simple variants (e.g., 1, R = CH_3) provided by varying proportions of MeOH, 2-methoxyethanol, and DMF showed that there were considerably smaller differences between pK_a values measured in H_2O and 20% DMF mixtures in comparison with comparable levels of the other solvents. Additionally, agents were appreciably more soluble in DMF–H_2O than in the alternate solvent–H_2O combinations; with the exception of the extremely insoluble 4′-(3,6-dinitro-9-acridinylamino)methanesulfonanilide[7] all other agents have proved sufficiently soluble in 20% DMF–H_2O for UV spectrophotometric pK_a determination. As gauged by multiple determinations, quoted pK_a values can be reproduced within a range of ±0.03 units.

With most agents of the type examined in this publication sulfonamide pK_a (range 8.5–9.2) is sufficiently divorced from that of the acridine nucleus (Table I) that the base strength of the latter can be measured directly by spectrophotometric means.[23] With more basic acridine analogues necessary reiterative computer separation of the two pK_a values employed the program of Albert and Sergeant.[23]

2-(3-Phthalimidopropyl)isothiuronium Acetate (3, n = 3). N-(3-Bromopropyl)phthalimide, 26.8 g (0.1 mol), and thiourea, 15.2 g (0.2 mol), were heated to boiling in absolute EtOH (3.1 mL/g of thiourea), a homogeneous solution rapidly resulting. After ca. 3 h of boiling product suddenly crystallized. After thorough cooling the isothiuronium bromide was collected, washed with a little chilled EtOH and C_6H_6, and dried: mp 227 °C dec [lit.[11] mp 228 °C dec; 30.8 g (82%)]. This product was dissolved in H_2O (150 mL) containing HOAc (1.5 mL) by heating, the solution clarified, and hot saturated aqueous NaOAc added to the hot filtrate until the acetate salt started to crystallize. When thoroughly cold the crystals were collected and dried in vacuo: mp 153 °C dec [lit.[11] mp 153.5 °C dec; 25.8 g (89%)].

In similar preparations employing the more lipophilic, longer alkyl chain N-(ω-bromoalkyl)phthalimides, the isothiuronium bromide did not spontaneously crystallize from the reaction mixture and, on the basis of TLC monitoring, reflux times of 8 h were used. The increased EtOH solubility of the isothiuronium bromides, with lengthening alkyl chain, also necessitated addition of C_6H_6 to the completed reaction mixture to ensure good product recovery.

3-Phthalimidopropanesulfonyl Chloride (4, n = 3). The aforementioned isothiuronium acetate salt, 25.2 g (0.071 mol), was suspended in 12 N HCl (100 mL) and the whole mixture stirred vigorously in an ice–water cooling bath while a solution of $NaClO_3$, 10 g (0.094 mol), in H_2O (20 mL) was added in dropwise fashion so that the temperature was maintained at 10–15 °C. At the tail end of this addition excess $NaClO_3$ provided Cl_2 and there was resulting mild frothing of the reaction mixture. When addition was complete the mixture was stirred for a further 30 min and the crystalline acid chloride collected on a sintered-glass filter, washed with a little ice–H_2O, and sucked as dry as possible. The product was dissolved in EtOH-free $CHCl_3$ (200 mL) by stirring; the clarified solution was washed with a little ice–H_2O, dried ($CaCl_2$), and evaporated. The resulting crystalline residue was recrystallized from C_6H_6 and pure product was obtained as colorless needles of mp 86–87 °C [lit.[11] mp 87 °C; 12.7 g (62%)].

Physical constants for employed N-(ω-bromoalkyl)phthalimides,[11,24-29] 2-(ω-phthalimidoalkyl)isothiouronium salts,[11,30] and ω-phthalimidoalkanesulfonyl chlorides[11,30-32] have been adequately described.

ω-Phthalimido-4′-nitroalkanesulfonanilides 5. For the purposes of example preparation of 4-phthalimido-4′-nitrobutanesulfon-m-anisidide, required as an intermediate for preparation of **21**, is detailed. A solution of 3-methoxy-4-nitroaniline, 9.35 g (0.06 mol), in pyridine (27.5 mL) was stirred in an ice–salt bath while 4-phthalimidobutanesulfonyl chloride (0.05 M) was added portionwise so the temperature remained below −5 °C. When addition was complete the mixture was stirred in the cooling bath until all acid chloride had dissolved and then stored in a refrigerator for 12 h. The mixture was then heated on a steam bath until homogeneous and then for 1 h further and as much excess pyridine as possible removed in vacuo. To the resulting brown gum boiling MeOH (75 mL) was added and the mixture boiled and stirred until a smooth paste of crystalline product resulted. After thorough cooling the crystals were collected, washed with cold MeOH (2 × 20 mL) and much H_2O, and dried. Crystallization from DMF provided pure product as very pale yellow needles of mp 192–194 °C; 13.4 g (62%). Anal. ($C_{19}H_{19}N_3O_7S$) C, H, N, S. The products of Table II were prepared in equivalent manner employing the requisite sulfonyl chloride and 4-nitroaniline.

ω-Amino-4′-nitroalkanesulfonanilides 6. For the purposes of illustration preparation of 4-amino-4′-nitrobutanesulfon-m-anisidide is provided. To a vigorously stirred suspension of the above described precursor phthaloyl derivative, 10 g (0.023 mol), in boiling EtOH (75 mL) was added hydrazine hydrate (98%, 1.76 mL, 0.046 M) in one portion, a clear yellow solution resulting in ca. 3 min. Shortly afterward 1,4-phthalazinedione started to crystallize from the solution. After 30 min of further stirring and boiling HOAc (10 mL) was added and as much EtOH as possible removed in vacuo. The remaining solids were extracted with successive quantities of boiling 0.5 N HOAc (60 and then 2 × 30

Table II. ω-Phthalimido-4'-nitroalkanesulfonanilides 5

Alkane chain	Mp, °C	Yield, %	Formula[a]
$(CH_2)_2$	214–215	68	$C_{16}H_{13}N_3O_6S$
$(CH_2)_3$	231–232	69	$C_{17}H_{15}N_3O_6S$
$(CH_2)_4$	191–193	62	$C_{18}H_{17}N_3O_6S$
$(CH_2)_5$	174–175	57	$C_{19}H_{19}N_3O_6S$
$(CH_2)_6$	169–170	59	$C_{20}H_{21}N_3O_6S$
$(CH_2)_7$	150–152	43	$C_{21}H_{23}N_3O_6S$
$(CH_2)_8$	145–146	47	$C_{22}H_{25}N_3O_6S$

[a] All compounds analyzed satisfactorily for C, H, N, and S.

Table III. ω-Amino-4'-nitroalkanesulfonanilides 6

Alkane chain	Mp, °C	Yield, %	Formula[a]
$(CH_2)_2$	225–226	92	$C_8H_{11}N_3O_4S \cdot H_2O$
$(CH_2)_3$	230–231	93	$C_9H_{13}N_3O_4S$
$(CH_2)_4$	232–233	89	$C_{10}H_{15}N_3O_4S$
$(CH_2)_5$	233–234	84	$C_{11}H_{17}N_3O_4S$
$(CH_2)_6$	201–202	86	$C_{12}H_{19}N_3O_4S$
$(CH_2)_7$	207–209	83	$C_{13}H_{21}N_3O_4S$
$(CH_2)_8$	156–157	79	$C_{14}H_{23}N_3O_4S \cdot 0.5H_2O$

[a] All compounds provided satisfactory analyses for C, H, N, and S.

mL) and the clarified extracts were evaporated to dryness in vacuo yielding a thick colorless gum. After solution in the minimum quantity of boiling H_2O and cooling, excess NH_3 (pH >10) was added. Excess NH_3 was then removed by heating the solution in a steam bath under vacuum with swirling until the yellow amine suddenly crystallized from solution. After thorough cooling the base was collected, washed well with H_2O, and dried. While most amine intermediates of this type could be readily crystallized from EtOH–H_2O, this particular compound was conveniently purified by crystallization of the bromide salt from boiling H_2O. Pure product hydrobromide was obtained as pale yellow needles of mp 216 °C dec; 7.27 g (82%). Anal. ($C_{11}H_{17}N_3O_5S \cdot HBr$) C, H, N, Br.

Equivalent reaction conditions applied to the intermediates listed in Table II provided the following amines.

ω-(4-Nitrophenylsulfamoyl)alkylguanidinium Nitrate Salts 7. The requisite amine of Table III (0.05 mol), 2-methylisothiuronium sulfate (0.05 mol), and $CaCO_3$ powder (0.06 mol) were suspended in DMF (40 mL) and the mixture was stirred and heated at 120 °C (internal) for 30 min. DMF was removed in vacuo on the steam bath, HOAc (6 mL) was added, and volatiles were again removed in vacuo. The residue was extracted with boiling H_2O to completion, the extracts were evaporated as far as possible, and the residue was dissolved in the smallest possible volume of hot H_2O. Solid $NaNO_3$ was then added portionwise to the hot solution until the nitrate salt started to crystallize. Thorough cooling then provided the highly crystalline nitrate salts. Recrystallization was from H_2O–$NaNO_3$. See Table IV.

2-(2-Phthalimidoethanamido)-4'-nitroethanesulfonanilide. A suspension of *N*-phthaloylglycine 4-nitrophenyl ester, 4.60 g (14.6 mol), and 2-amino-4'-nitroethanesulfonanilide, 3.42 g (13.9 mol), in DMF (10 mL) was stirred at room temperature until homogeneous and then allowed to stand overnight. After removal of solvent in vacuo the gummy residue was triturated with 10% aqueous $KHCO_3$ and the resulting crystalline solid collected and well washed with H_2O. Recrystallization from HOAc–H_2O provided pure compound as pale yellow needles of mp 212–212.5 °C; 5.6 g (93%). Anal. ($C_{18}H_{16}N_4O_7S$) C, H, N, S.

Similar interaction of the same initial amine and 4-nitrophenyl 3-phthalimidopropionate provided **2-(3-phthalimidopropionamido)-4'-nitroethanesulfonanilide** in 92% yield: mp 208–209 °C. Anal. ($C_{19}H_{18}N_4O_7S$) C, H, N, S.

2-(2-Aminoethanamido)-4'-nitroethanesulfonanilide. The requisite phthaloyl derivative, 5 g (11.6 mmol), was moistened with EtOH (7.5 mL), then 25 mL of H_2O was added, and the suspension stirred vigorously while 98% hydrazine hydrate (34.8 mmol) was added. Hydrazine is sufficiently basic to ionize the nitrosulfonanilide moiety and a clear yellow solution rapidly resulted. After 1 h of stirring HOAc (5 mL) was added and the precipitated 1,4-phthalazinedione removed. The clarified solution was evaporated in vacuo at 30 °C and the resultant gum dissolved in 5 mL of warm H_2O. NH_3 was added to pH >10 and water pump vacuum applied at 30 °C until product amine crystallized. Recrystallization from small volumes of H_2O provided pure amine as yellow needles of mp 208–209 °C; 2.6 g (76%). Anal. ($C_{10}H_{14}N_4O_5S$) C, H, N, S.

Table IV. ω-(4-Nitrophenylsulfamoyl)alkylguanidinium Nitrate Salts 7

Alkane chain	Mp, °C	Yield, %	Formula[a]
$(CH_2)_2$	203–204	87	$C_9H_{14}N_6O_7S$
$(CH_2)_3$	226 dec	86	$C_{10}H_{16}N_6O_7S$
$(CH_2)_4$	202 dec	81	$C_{11}H_{18}N_6O_7S$
$(CH_2)_5$	173 dec	82	$C_{12}H_{20}N_6O_7S$
$(CH_2)_6$	141–142	78	$C_{13}H_{22}N_6O_7S$

[a] All compounds provided satisfactory analyses for C, H, N, and S.

Equivalent steps from the requisite phthaloyl derivative provided **2-(3-aminopropionamido)-4'-nitroethanesulfonanilide** as yellow needles from hot H_2O of mp 210–211 °C (78%). Anal. ($C_{11}H_{16}N_4O_5S \cdot 2H_2O$) C, H, N, S.

4-Dimethylamino-4'-nitrobutanesulfonanilide. A mixture of 4-amino-4'-nitrobutanesulfonanilide, 13.65 g (0.05 mol), H_2O (3 mL), 98% formic acid (50 mL), and 35% aqueous formalin (13.3 mL, 0.155 M) was heated on a steam bath until there was no further observable liberation of CO_2 and then for a further 2 h longer. The solution was evaporated in vacuo and the residue thoroughly dried in vacuo. Ac_2O (20 mL) was then added and the mixture heated at 100 °C until homogeneous and then for 1 h further. Volatiles were removed in vacuo, a little H_2O was added, and the mixture was reevaporated. After shaking the residue with boiling H_2O (75 mL) the resulting solution was clarified and remaining nonbasic materials were removed in Et_2O. The aqueous layer was stripped of traces of Et_2O by application of vacuum and then rendered alkaline (pH >10.5) with NaOH. The yellow solution resulting from formation of the Na^+ salt of the nitrosulfonanilide moiety was clarified and CO_2 passed through the solution until the yellow color was essentially discharged. The precipitated oil was removed in EtOAc and the extracts were dried (Na_2SO_4) and evaporated. Trituration of the yellow gum with EtOAc–petroleum ether provided seed crystals and the whole mixture was then crystallized from EtOAc–petroleum ether, the product amine being obtained as yellow prisms of mp 189–190 °C; 9.35 g (62%). Anal. ($C_{12}H_{19}N_3O_4S$) C, H, N, S.

Conditions for preparation and handling of the required 9-(10*H*)-acridones, 9-chloroacridines, and the ultimate agents, as in Table I, have been adequately described.[1-7] Reduction of the nitroamines 6 (10% Pd/C, EtOH–H_2O, 45 psi of H_2, 25 °C) proceeded uneventfully with H_2 uptake stopping at the theoretical figure. The product amines are very readily autoxidized and quickly provide purple oxidation products in the presence of air unless their solutions are maintained at an acid pH. Accordingly, when filtering catalyst from reduction media 1 mol equiv of 1 N HCl was first added to the receiving flask. In the case of the intermediates used for preparation of the peptide analogues **28–32**, because of the possibility of amide cleavage by strongly acidic media, HOAc (twofold excess) was employed in place of HCl.

While the nitroguanidines 7 could also be reduced catalytically, overreduction, involving the guanidine function, sometimes occurred and provided nonhomogeneous products. $Fe(H^+)$ reductions as detailed before[33] uneventfully provided homogeneous products. As above, final products from Fe reductions were filtered into an equivalent quantity of HCl to prevent autoxidation. Purity of the reduced products, from both nitroamines and nitroguanidines, can conveniently be assayed by TLC on SiO_2 using the top phase of *n*-BuOH–HOAc–H_2O (5:1:4 v/v).

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Synthesis and Biological Action of Two Glucocorticoid Alkylating Agents[1]

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Two alkylating glucocorticoids have been synthesized in order to test the possibility of alkylating glucocorticoid receptors. The title compounds are 9α-fluoro-11β,16α,17α,21-tetrahydroxypregna-1,4-diene-3,20-dione 21-[bis(2-chloroethyl)carbamate] 16,17-acetonide (I) and 11β,17α,21-trihydroxypregn-4-ene-3,20-dione 21-[bis(2-chloroethyl)carbamate] (II), prepared from triamcinolone acetonide and cortisol, respectively, through the reaction of the C-21 hydroxyl group with phosgene and di-2-chloroethylamine in the presence of triethylamine. Both compounds are biologically active as inhibitors of the growth of cultured mouse fibroblasts and are able to compete for the specific binding of radiolabeled triamcinolone acetonide to the L929 cell receptor. The bis(2-chloroethyl)carbamate moiety is capable of reacting with nucleophilic groups as evidenced by the colorimetric reaction with 4-(*p*-nitrobenzyl)pyridine. Both the interaction with the receptor and inhibition of cell growth by these two glucocorticoids are reversible.

Several steroid derivatives with attached alkylating moieties have been found to be active against selected animal tumor systems. These include the cholesterol derivative, phenesterin[2] (NSC-104469), and two estradiol derivatives, estradiol mustard[3] (NSC-112259) and estracyt[4] (NSC-89199). A glucocorticoid alkylating agent has also been synthesized and demonstrated to be active against the L1210 murine leukemia model.[5] This compound, Leo 1031 (NSC-134087), is a chlorambucil ester of prednisolone at the 21 position that has recently been administered to a few patients with chronic lymphocytic leukemia[6] and lymphocytic lymphoma.[7] The rationale behind the development of these compounds was based upon two objectives. First, it was hoped that the passage of the alkylating agent across cell membranes might be facilitated by linking it to a more lipophilic moiety, like a steroid. Second, in the case of the sex hormone and glucocorticoid derivatives, it was hoped that two cytotoxic actions might be achieved—one resulting from hormone–receptor interaction and the second being due to a nitrogen mustard effect. The two actions could be produced by the intact compounds, or hydrolysis of the linkage between the steroid and alkylating moieties as a result of cellular esterase activity could yield two species with different cytotoxic mechanisms.

We have prepared two compounds with which to test the possibility of specifically alkylating the receptor molecule for glucocorticoids. The rationale for the compounds synthesized was as follows. In order to obtain specific alkylation it was considered optimal to utilize a steroid of very high affinity which would bind tightly to the receptor and permit us to rapidly eliminate the unbound compound without significant dissociation of the steroid–receptor complex. Accordingly, we have synthesized an alkylating derivative of the very potent glucocorticoid triamcinolone acetonide.[8] It was also thought preferable to utilize a relatively slow-reacting alkylating moiety. This might permit us to eliminate the unbound