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Potential Antitumor Agents. 17. 9-Anilino-10-methylacridinium Salts

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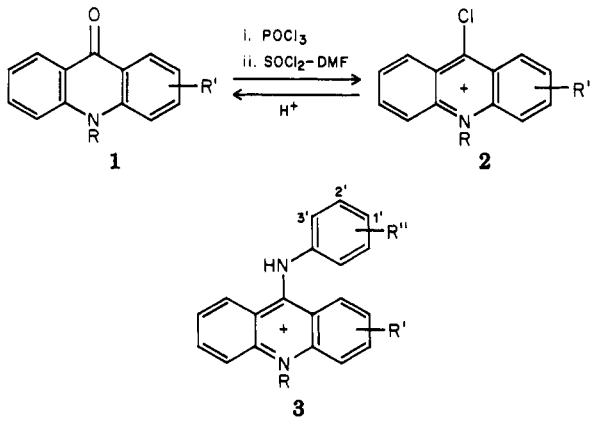
9-Anilino-10-methylacridinium salts result from reaction of substituted anilines and 9-chloro-10-methylacridinium salts in turn prepared from the 10-methyl-9(10*H*)-acridones and $SOCl_2$ or $POCl_3$. Antileukemic (L1210) activities of the quaternary salts were uniformly depressed compared to their unquaternized counterparts. If drug–solvent partition properties (log *P*) of the cations were considered, log *P*–activity relationships were similar for both base and quaternary salt series. Substituent effects on antitumor activity were similar in both series.

Our entry into the series of 9-anilinoacridines[1,2] having broad-spectrum tumor activity[3] was via earlier described bisquaternary ammonium heterocycles[4] which had a considerably narrower action spectrum. Our interest in the effect of changing drug pharmacodynamic properties on the observable tumor spectrum of action prompted an examination of quaternary analogues of the 9-anilinoacridines. Additionally, the extensive ionization of quaternary salts at physiologic pH's serves to attenuate any p*K*-dependent phenomena induced by varying acridine ring substitution in the parent series. A study of the quaternary salts might therefore illuminate the role of changing p*K* in both observed structure–activity relationships (SAR) and drug pharmacodynamic properties. This communication details synthetic methods and L1210 screening results for a series of quaternary 9-anilinoacridines.

Chemistry. In our earlier experiences with N-quaternization of heterocycles[4,5] simple pyridines reacted readily, corresponding quinoline derivatives more slowly, and certain nitroquinolines with considerable difficulty. We have now concluded that the difficulties of obtaining complete reaction and the resulting problems of product purification make direct quaternization of the 9-anilinoacridines a synthetically unattractive reaction. Invariably a mixture of the quaternary salt and the salt of unreacted acridine resulted and these have marked tendency to cocrystallize and coprecipitate. Attempts to purify crude products from quaternization by direct crystallization and either selective precipitation or solvent partition at controlled pH's were of limited value. Products can be purified by tedious chromatography. Attention has already been drawn[6] to the difficulty of purifying simple acridinium quaternary salts such as 3,6-diamino-10-methyl-

Scheme I



acridinium hydrochloride (trypaflavin); commercial preparations of this material are a mixture of quaternary and base salt.

To avoid a terminal quaternization step the required N^{10}-alkyl group was introduced at the acridone stage. Reaction of a 10-alkylacridone **2** with either $POCl_3$ or $SOCl_2$–DMF provided a 9-chloro-10-alkylacridinium salt[7,8] **3** which reacted smoothly with aromatic amines to provide the required quaternary salts **4** (Scheme I).

The tendency for acridine cocrystallization was again encountered with the *N*-alkylacridones. *N*-Methylacridone, prepared by published methods and having a literature quoted melting point,[9-11] was shown by TLC to be a cocrystallizing mixture of acridone and *N*-methylacridone. Repeated conventional crystallizations failed to provide either component of the mixture in pure form. By utilizing the cryptophenolic properties[12] of the 9(10*H*)-acridones such mixtures could be resolved. Crystallization from alcoholic KOH rapidly removed contaminating acridone and afforded TLC homogeneous *N*-methylacridone. The melting point of the pure compound was, however, not increased over that of the original impure sample having the literature quoted melting point. In a different case (2-nitro-10-methylacridone) the melting point of the product so purified (291°) was considerably higher than that previously recorded (276°).[9] Crystallization from solvent–KOH or NaOH mixtures or treatment with Claisen's cryptophenol reagent has been adopted as essential purification steps for the *N*-methylacridones described.

Reaction of 9(10*H*)-acridone with varying methylating agents (MeI, Me_2SO_4, MeOTs, $MeOSO_2F$) in a range of solvents in the presence of different bases (K_2CO_3, KOH, CaO, CaH_2, NaH) invariably produced some *N*-methylacridone but the combination Me_2SO_4–K_2CO_3–Me_2CO provided smooth, high-yield conversion using simple techniques.

Powerful methylating agents such as methylfluorosulfonate will quaternize 9-chloroacridines in useful yield, but, to obtain completely pure end products (**4**) by this sequence, it is necessary to hydrolyze (H^+/H_2O) the 9-chloro-10-methylacridinium fluorosulfonate (**3**, X^- = FSO_3^-) to the *N*-methylacridone and purify this by the methods outlined.

Conversion of pure *N*-methylacridones to 9-chloro-10-methylacridinium salts (**3**) proceeded smoothly in most cases using either $POCl_3$ or $SOCl_2$ plus a catalytic quantity of DMF. Exceptionally, when 10-methyl-3-nitroacridone was treated with these reagents the nitro group was displaced and nitrous fumes were liberated. After hydrolysis of the reactive intermediates formed in this reaction TLC separated one compound which was identical with 3-chloro-10-methylacridone in its chromatographic behavior but there were five additional spots on the TLC sheets having typical acridone uv fluorescence. In contrast, the 2-nitro isomer reacted smoothly with $POCl_3$ to provide acridinium salt (**3**, R = 2-NO_2) which could be either coupled with substituted anilines to provide quaternary salts (**4**, R = 2-NO_2) or hydrolyzed to return 2-nitro-10-methylacridone.

Most agents (Table I) were prepared by direct coupling of substituted anilines with a 9-chloro-10-methylacridinium salt (method A). Amine-substituted variants were prepared either by demasking of acylamino precursors (method B) or reduction (Fe/H^+) of the corresponding nitro derivative (method C). Method B was the one of choice. Reduction ($SnCl_2$–HCl) of the 10-methylnitroacridones provided necessary amine procursors which were masked with either *N*-acetyl or trifluoroacetyl groups. Unmasking utilized acid hydrolysis (–NHAc) or ammonolysis (–$NHCOCF_3$).

Structure–Activity Relationships. As a measure of the relative lipophilic–hydrophilic balance of agents ΔR_m values from chromatographic data have again been used.[2] ΔR_m values and partition coefficients (*P*) in isobutyl alcohol for a series of agents provided the following correlation.

$$\log P = 1.987\ (\pm 0.193)\ \Delta R_m + 0.746\ (\pm 0.036)$$
$$s = 0.095,\ r = 0.964,\ n = 32$$

The log *P* values used in deriving this equation extended from –1.08 to 1.70, i.e., a range of 2.78. Since $\log P_{i\text{-BuOH}} = 0.71 \log P_{Oct} + 0.42$,[13] where P_{Oct} is the usual 1-octanol–water partition coefficient, the data presented can be compared with that gained using other measures of lipophilic–hydrophilic balance.

Because of their varying p*K* values movement of the acridine bases on chromatograms is very pH dependent. The migration of the more strongly basic quaternary salts is less effected by changing solvent pH. As less acid chromatographic media were used the ΔR_m values for corresponding pairs of quaternary salt and precursor base increasingly diverged. All ΔR_m values and log *P* values quoted have been measured at acid pH's to ensure that the partitioning species have been in all cases the acridinium ions. If the usual substituent additivity of log *P* values can be applied to cationic charge, then in determining ΔR_m values for drug cation a measure for the neutral drug species is also provided, i.e., ΔR_m (drug) – ΔR_m (drug·H^+) = constant.

Because of the high activity, and according central role, of 4'-(acridin-9-ylamino)methanesulfonanilide[2] we have retained the numbering of this system and all variants are discussed as if they were derivatives formed from this; i.e., numbering of the 9-anilinoacridines is as shown in **3**.

To aid comparison the compounds listed in Table I are grouped in pairs; each pair comprises the parent acridine and its quaternized counterpart. In all cases the quaternary compound was less L1210 active than the parent base. Despite this quantitative difference there are common substituent effects in the two series.

(1) As the alkyl chain in the 1'-alkanesulfonamides is increased (**4**–**11**) activity rapidly decreases.[2] The lipophilic cutoff at shorter chain length (**8**) in the quaternary salts presumably reflects their overall decreased activity. As before[2] the change in activity in these homologous series, as a function of ΔR_m, can be used to unravel substituent effects.

(2) The unsubstituted 9-anilinoacridines **12** and **13** are both inactive. The ΔR_m values (cf. **12**, **8**; **11**, **13**) suggest

Table I

No.	Substituents in 3 R	Substituents in 3 R	Mp, °C	Formula	Method	Analyses[a]	R_m[b]	O.D.[c]	L1210, T/C, %[d]
4	CH_3	1′-$NHSO_2CH_3$	292–293	$C_{21}H_{20}ClN_3O_2S$	A	C, H, N, Cl	−0.19	95	184
5	H	1′-$NHSO_2CH_3$	*e*				0.00	45	207
6	CH_3	1′-$NHSO_2C_2H_5$	280–281	$C_{22}H_{22}ClN_3O_2S$	A	C, H, N, Cl	−0.01	110	166
7	H	1′-$NHSO_2C_2H_5$	*e*				0.20	150	183
8	CH_3	1′-$NHSO_2(CH_2)_2CH_3$	267–268	$C_{23}H_{24}ClN_3O_2S\cdot 2H_2O$	A	C, H, N, Cl	0.18	>500	*h*
9	H	1′-$NHSO_2(CH_2)_2CH_3$	*e*				0.39	225	134
10	CH_3	1′-$NHSO_2(CH_2)_3CH_3$	248–250	$C_{24}H_{26}ClN_3O_2S$	A	C, H, N, Cl	0.37	>500	
11	H	1′-$NHSO_2(CH_2)_3CH_3$	*e*				0.56	>500	
12	CH_3	H	248 dec	$C_{20}H_{17}ClN_2$	A	C, H, N	0.23	>500	
13	H	H	*f*				0.51	>500	
14	CH_3	1′-NH_2	273–274	$C_{20}H_{18}BrN_3$	A, B	C, H, N, Br	−0.36	75	163
15	H	1′-NH_2	*f*				−0.02	33	172
16	CH_3	2′-$NHCOCH_3$	297–298	$C_{22}H_{20}ClN_3O\cdot 0.5H_2O$	A	C, H, N, Cl	0.17	67	132
17	H	2′-$NHCOCH_3$	*f*				0.48	30	133
18	CH_3	2′-NH_2	257–259	$C_{20}H_{18}BrN_3$	B	C, H, N; Br[i]	−0.35	120	137
19	H	2′-NH_2	*f*				−0.02	40	146
20	CH_3	1′-SO_2NH_2	273 dec	$C_{20}H_{18}ClN_3O_2S$	A	C, H, N, S	−0.52	>500	
21	H	1′-SO_2NH_2	*f*				−0.33	>500	
22	CH_3	1′-$CONH_2$	154–155	$C_{21}H_{18}ClN_3O\cdot 1.5H_2O$	A	C, H, N, Cl	−0.46	>500	
23	H	1′-$CONH_2$	*f*				−0.22	>500	
24	CH_3	2-NO_2, 1′-$NHSO_2CH_3$	291 dec	$C_{21}H_{19}ClN_4SO_4$	A	C, H, N, Cl	−0.36	>500	
25	H	2-NO_2, 1′-$NHSO_2CH_3$	*e*				−0.21	250	127
26	CH_3	2-$NHCOCH_3$, 1′-$NHSO_2CH_3$	291 dec	$C_{23}H_{23}ClN_4SO_2\cdot 1.5H_2O$	A	C, H, N, Cl	−0.36	15	134
27	H	2-$NHCOCH_3$, 1′-$NHSO_2CH_3$	*e*				−0.15	375	147
28	CH_3	2-NH_2, 1′-$NHSO_2CH_3$	274–277	$C_{21}H_{21}BrN_4O_2S$	B, C	C, H, N, Br	−0.49	5	167
29	H	2-NH_2, 1′-$NHSO_2CH_3$	*e*				−0.31	25	178
30	CH_3	3-$NHCOCH_3$, 1′-$NHSO_2CH_3$	298–299	$C_{23}H_{23}ClN_4O_3S$	A	C, H, N, Cl	−0.31	6	181 (1)[j]
31	H	3-$NHCOCH_3$, 1′-$NHSO_2CH_3$	*e*				−0.12	13	214 (2)
32	CH_3	3-NH_2, 1′-$NHSO_2CH_3$	242–243	$C_{21}H_{21}ClN_4O_2S\cdot H_2O$	B	C, H, N, Cl	−0.36	1.25	180
33	H	3-NH_2, 1′-$NHSO_2CH_3$	*e*				−0.17	2.5	181 (1)
34	CH_3	3′-OCH_3, 1′-$NHSO_2CH_3$	260–261	$C_{22}H_{22}ClN_3O_3S$	A	C, H, N, Cl	−0.03	12	172
35	H	3′-OCH_3, 1′-$NHSO_2CH_3$	*g*				0.18	12	196 (1)
36	CH_3	3-Cl, 3′-OCH_3, 1′-$NHSO_2CH_3$	186–188	$C_{22}H_{21}Cl_2N_3O_3S\cdot H_2O$	A	C, H, N, Cl	0.12	12	179
37	H	3-Cl, 3′-OCH_3, 1′-$NHSO_2CH_3$	*g*				0.31	10	172
38	CH_3	3-Cl, 3′-OCH_3, 1′-$NHSO_2C_2H_5$	236–237	$C_{23}H_{23}Cl_2N_3O_3S$	A	C, H, N, Cl	0.30	60	166
39	CH_3	3-Br, 1′-$NHSO_2CH_3$	215 dec	$C_{21}H_{19}BrClN_3O_2S$	A	C, H, N, S	−0.01	47	168
40	H	3-Br, 1′-$NHSO_2CH_3$	*e*				0.14	35	165
41	CH_3	3-Br, 3′-OCH_3, 1′-$NHSO_2CH_3$	192 dec	$C_{22}H_{21}BrClN_3O_3S$	A	C, H, N, S	0.19	12	158
42	H	3-Br, 3′-OCH_3, 1′-$NHSO_2CH_3$	*g*				0.34	6	219 (1)
43	CH_3	2-NO_2	272–274	$C_{20}H_{16}ClN_3O_2\cdot 0.5H_2O$	A	C, H, N, Cl	0.39	>500	
44	CH_3	2-$NHCOCH_3$	251–252	$C_{22}H_{22}ClN_3O\cdot H_2O$	A	C, H, N, Cl	0.14	50	126
45	CH_3	2-NH_2	123–124	$C_{20}H_{18}BrN_3$	B, C	H, N, Br; C[k]	−0.12	20	148
46	CH_3	3-$NHCOCH_3$	292–293	$C_{22}H_{20}ClN_3O$	A	C, H, N, S	0.04	22	158
47	CH_3	3-NH_2	102–104	$C_{20}H_{18}BrN_3\cdot 2H_2O$	B	C, H, N, Br	−0.06	14	162

[a] Analyses for the indicated elements were within ±0.4% of the calculated figures. [b] $R_m = \log(1/R_f - 1)$ from reversed phase chromatography; see the Experimental Section. [c] Optimum dose; dose in mg/kg/day providing maximum T/C in L1210 assays when doses were spaced at 0.18 log dose intervals and treatment was qd 1–5 ip. Tumor inoculum 10^5 ip. [d] T/C = ratio of life spans of treated and control groups in L1210 assays at the optimum dose. [e] Reference 2. [f] Reference 1. [g] Reference 14. [h] – signifies no significant life extension (T/C < 125%) was seen in L1210 assays. [i] Br: calcd, 21.0; found, 19.4. [j] Figures in parentheses are the mean number of 50-day survivors at the optimum dose in L1210 tests for a group of six animals. [k] C: calcd, 63.2; found, 62.6.

that this inactivity may be due to excessive lipophilic character.

(3) Addition of π^-,σ^- substituents to the parent 9-anilinoacridines at either 1' or 2' positions provided active agents (**14–19**).[2]

(4) Addition of π^-,σ^+ substituents to the 9-anilino ring at the 1' position provides congeners which have ΔR_m values in the necessary range but these are inactive (**20–23**).[2]

(5) A 1'-$NHSO_2CH_3$ group confers high activity (**4, 5**).[2]

(6) While an appended 3'-OCH_3 function appears to have considerable lipophilic character its presence confers high dosage potency (cf. **4, 34; 5, 35**).[14]

(7) Congeners bearing hydrophobic acridine 3-substituents are more active than expected from their ΔR_m values.[2] Certain 3-halogen substituted quaternary salts show appreciable activity but are more lipophilic than the propanesulfonanilide **8** which is inactive because of excessive lipophilic character (cf. **8, 38; 8, 41**).

An alternative method of increasing drug hydrophilic character, appending the substituents employed in (3) to the acridine nucleus, also provides active agents (**44–47**). Addition of the same π^- groups to the already hydrophilic methanesulfonanilides (to provide **26–33**) does not further increase activity; the optimum ΔR_m value should then be close to that of the parent methanesulfonanilide **4**. The equivalent decision was reached earlier with the acridine bases.[2]

Discussion

Provided the quaternary acridines described are active per se, and are not biologically active only after in vivo N-10 dealkylation to provide the free bases, the following arguments apply.

If both drug series (quaternary salts and bases) are to be accommodated on a single lipophilicity scale, the respective ΔR_m optima should coincide on this scale. ΔR_m values for corresponding pairs of quaternary salt and base (cf. **4, 5**), and therefore the ΔR_m optima, converge at low pH's. This forms the basis of our earlier conclusion that the ΔR_m values of cationic drug species should be employed.[2] Determining cationic ΔR_m values also provides a relative measure of partition properties of the free bases. It is therefore difficult to decide whether important in vivo partition processes are dictated by ionized or neutral drug species. However, the physical significance of log P (neutral drug) for quaternary salts in biological systems at physiological pH's could be questioned.

Quaternization increases base strength in γ-amino heterocycles to such levels (1,4-dihydro-4-imino-1-methylpyridine, pK_a = 12.5; 1,4-dihydro-4-imino-1-methylquinoline, pK_a = 12.4)[15] that usually encountered substituent effects on pK are relatively minor in comparison. Accordingly, inactivity due to 1'-δ^+ substituents (**20–23**) is not because of transmission of electron withdrawal resulting in lower acridine pK.

The possibility that 3-halogen functions augment activity in the parent series by attenuating acridine pK can also be discounted since the same augmenting effect is seen in the quaternary salts (**36, 38, 39, 41**). Our preferred explanation is that such functions augment drug–site binding by hydrophobic interactions.[2]

In contrast, introduction of 2- or 3-amino groups into either the parent sulfonanilide series (**29, 33**) or the quaternary salts (**28, 32**) markedly increases dose potency but there is no significant activity enhancement. In the parent series increase in base strength by addition of a 3-NH_2 function could be proposed to explain the enhancement (ΔpK_a in 9-aminoacridine = +1.5)[16] but this argument cannot be applied to the quaternary salts; quaternization of the parent anilino compounds will increase base strength but does not increase potency (**4, 5**). Also, addition of a 3-amino group to the strongly basic quaternary salts still provides an increase in dose potency (**4, 32**). A 2-NH_2 group also provides increases in potency (**4, 28; 5, 29**) but is considerably less base strengthening (ΔpK_a in 9-aminoacridine = +0.4).[16] The potency-enhancing effects of these amino groups do not appear directly related to their effects on basic pK.

We have suggested that drug analogues may bind to DNA twin helices following an intercalation step.[2] In such a site, provided the positioning of the acridine nucleus remains the same for all congeners, a polar 3-NH_2 group must reside in an area which we have suggested is hydrophobic in nature. However, adjacent to this hydrophobic area, which is within the overlap of the pyrimidine–purine base pairs, are the phosphodiester groups of the deoxyribose chains. From model fitting hydrogen bond formation between acridine 2- or better 3-NH_2 groups and phosphate oxygens appears feasible. Members of the isomeric angularly fused phenanthridine system are also known to intercalate into DNA.[17] Models demonstrate that, when intercalated, the amine groups of ethidium (3,8-diamino-5-ethyl-6-phenylphenanthridinium) are similarly site located to those of the acridines discussed. It has already been suggested that the amine functions of ethidium hydrogen bond to adjacent phosphodiester groups.[17] An alternative explanation of Lerman's observation[21] of the unreactivity toward nitrous acid of the amine groups of DNA intercalated proflavin (3,6-diaminoacridine) would be that these were hydrogen bonded rather than just sterically shielded by adjacent base pairs.

In SAR the hydrophobic binding component due to a substituent is considered proportional to that substituent's π value.[18] The negative π value for a primary aromatic amine substituent (−1.23)[18] suggests that a loss of site binding would result if such functionality intruded into a hydrophobic site area. With our site model there would be drug-binding enhancement due to H-bond formation between drug–NH_2 and site phosphodiester and this would be opposed by the loss in site binding due to intrusion of the polar NH_2 into a hydrophobic site area. It is observed that there is no clear increase in biological effectiveness with the amine-substituted variants although such agents are more potent. We have not yet encountered acceptable functionality which could possibly utilize the hydrophobic site area and act as a H-bond donor component.

The protherapeutic effect of both amine and halogen substituents in acridine agents has been described, e.g., in the antitrypanosomal trypaflavin[19] (3,6-diamino-10-methylacridinium) and the antimalarial quinacrine.[20] Both these agents are said to intercalate into twin helical DNA.[21,22] It can be speculated that both agents could act at the same ultimate site but with log P_0 for the agent–biological test system interaction dictating whether hydrophilic or lipophilic substituents would appear most acceptable. We have drawn attention to the very hydrophilic nature of trypanosomal agents such as the diamidines, ethidium and trypaflavin.[4] In contrast, the intercalating antimalarial agents, exemplified by quinacrine, are highly lipophilic.[23] Early structure–activity studies leading to these agents were performed before there was a real appreciation of the importance of lipophilic–hydrophilic balance. If these agents had close to optimum properties (log P_0) then, on transposition of NH_2 and Cl substituents, biologic activity would decay as dictated by a log P curve for a $\Sigma\pi$ change of 2.02. A drastic change

in observed biologic activity could be expected. In the present series of acridines the optimum log P_0 lies close to the parent methanesulfonanilide[2] and the NH_2 and Cl derivatives are approximately equispaced either side of the maximum. Both variants lie in the observable biologic activity–log P range and the individual effects of the substituents can be gauged. Consideration of the changes in substituent effects on biologic activity that would result if log P_0 were displaced to more or less hydrophilic areas generated the above viewpoint.

Using parameters from current SAR approaches[18] it is possible to propose molecular probes to check the validity of this viewpoint. For example, a trypaflavin analogue in which an NH_2 group has been replaced by Cl and overall molecular lipophilic–hydrophilic balance then adjusted by addition of acceptable hydrophilic (log P ca. −2) functionality could provide a new antitrypanosomal agent. The reverse substituent transposition in quinacrine and compensatory upward adjustment of lipophilic character might provide a novel structured antimalarial.

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.

Chromatography. Reverse phase chromatography used the top phase of a mixture of glycerol (250 ml), *i*-BuOH (250 ml), and 1-pentanol (250 ml) plus methanesulfonic acid (2.7 ml) as developing solvent. The mixture of solvents was shaken thoroughly for 0.5 h and then allowed to settle for 48 h before separation. Prelegended Reidel de Hahn cellulose DC cards were impregnated by immersion in a 30% methanolic solution of the bottom phase of the solvent mixture. After rapid draining excess solvent was removed from the sheets by blotting between filter paper and they were then dried at room temperature in a fume hood for 1 h. Agents were applied as solutions in 65% Me_2CO–H_2O and sheets were air-dried for a further 15 min. Ascending development was for 24 h. 4'-(Acridine-9-ylamino)methanesulfonanilide (**5**, Table I, R_m = −0.08) was included as standard on all sheets and ΔR_m values are quoted in reference to this compound.

Partition coefficients were measured in *i*-BuOH–0.01 N HCl. Agents were dissolved in the bottom phase of this mixture to provide 2×10^{-4} M solutions and the uv spectrum was recorded. After shaking with sufficient top phase to remove at least half of the dissolved agent remaining, drug concentration in the bottom phase was measured by uv spectrophotometry.

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 and the top phase of a mixture of *n*-BuOH–HOAc–H_2O (5:1:4) as solvents.

10-Methyl-9(10*H*)-acridones. A 9(10*H*)-acridone (0.1 M) was suspended in anhydrous Me_2CO (2 l.), freshly dried K_2CO_3 (0.2 M) and Me_2SO_4 (0.11 M) were added, and the mixture was boiled under reflux while stirring sufficiently vigorously to keep K_2CO_3 in suspension. The initially insoluble acridone slowly dissolves as the reaction proceeds. After 2 h TLC demonstrates the extent of reaction and, as gauged by relative spot densities of initial acridone and product, further K_2CO_3 and Me_2SO_4, in the mol ratio of 2:1, are added and heating and stirring continued for a further 2 h. If an appreciable quantity of acridone is still observed on TLC plates run at this time a third quantity of reagents should be added and heating continued. When only traces of acridone can be observed on TLC the acetone solution is clarified and the remaining solids are washed to completion with boiling acetone. On evaporation of solvent the crude *N*-methylacridones crystallize. Traces of starting acridone were removed by crystallizing from MeOH–H_2O or EtOH–H_2O mixtures containing greater than 60% of the alcohol and sufficient KOH to provide a 1 N solution. With the very insoluble nitro variants it is necessary to digest on a steam bath with Claisen's cryptophenol reagent to remove unchanged acridone or, more effectively, to stir DMF solutions of the crude *N*-methylacridone into excess Claisen's alkali and then collect the separated solid. All traces of starting acridone should be removed before conventional crystallization is attempted. The acridones are more insoluble than their *N*-methyl counterparts and can increase in relative proportion over multiple crystallizations. Yields of pure products were in the range 62–87%.

Table II. 10-Methyl-9(10*H*)-acridones

Substituents	Mp, °C	Formula	Analyses
2-NO_2[a]	291–291.5	$C_{14}H_{10}N_2O_3$	C, H, N
2-NH_2[b]	212–213	$C_{14}H_{12}N_2O$	C, H, N
2-$NHCOCH_3$	325–326	$C_{16}H_{14}N_2O_2$	C, H, N
3-NO_2	219–220	$C_{14}H_{10}N_2O_3$	C, H, N
3-NH_2	295–296	$C_{14}H_{12}N_2O$	C, H, N
3-$NHCOCH_3$	>360	$C_{16}H_{14}N_2O_2$	C, H, N
3-$NHCOCF_3$	>360	$C_{16}H_{11}F_3N_2O_2$	C, H, N
3-Cl	175–176	$C_{14}H_{10}ClNO$	C, H, N, Cl
3-Br	161–162	$C_{14}H_{10}BrNO$	C, H, N, Br

[a] Lit.[7] mp 276°. [b] Lit.[7] mp 205°.

The difficulty of purifying reasonable quantities of the extremely insoluble 2-nitro-9(10*H*)-acridone together with the excellent solubility of the readily purified 2-nitro-9-chloroacridine makes the following alternative preparation valuable. To a solution of 2-nitro-9-chloroacridine (0.2 M) in anhydrous C_6H_6 (350 ml), well protected from moisture, was added freshly distilled methylfluorosulfonate (0.6 M) and the solution boiled gently for 6 h; 9-chloro-10-methyl-2-nitroacridinium fluorosulfonate slowly separated. The salt was collected from the well cooled solution, washed with C_6H_6, dried in vacuo, and then suspended in 2 N HCl. After 2 h of heating on a steam bath the heterogeneous mixture was cooled and basified (NH_4OH) and crude product collected. Traces of 2-nitroacridone were removed as described above and product was crystallized from DMF.

Preparation of Amino-10-methylacridones. 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 nitro-10-methylacridone 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 separate and coat unreacted starting material. When the vigorous reaction abated the mixture was boiled under reflux for 1.5 h. On thorough cooling crude product hydrochloride separated. The collected hydrochlorides were dissolved in hot 0.1 N HCl and the solutions clarified, and 12 N HCl was added to the hot solution until crystallization commenced; cooling then provided pure crystalline amino-10-methylacridone 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 amines. The free amines are readily autoxidized and become discolored in air. The compounds should be stored in vacuo or converted to the stable hydrochlorides or acetyl derivatives.

Acetamido-10-methylacridones were prepared by solution of the corresponding amino compound in the minimum volume of AcOH possible and adding to the hot (90°) solution Ac_2O (1.5 mol/mol of NH_2 group). After an initial vigorous reaction the acetamido compounds crystallized from the solution in essentially pure form as gauged by TLC. Recrystallization was from DMF (see Table II).

10-Methyl-3-trifluoroacetamidoacridone was prepared by the action of trifluoroacetic anhydride on a solution of the amine component in Py solution as usual.

9-Anilino-10-methylacridinium Salts. Method A. A sample of a 10-methylacridone (0.01 M) was converted to a 9-chloro-10-methylacridinium salt by treatment either with excess $POCl_3$ on the steam bath for 30 min or with excess $SOCl_2$ containing DMF (0.02 ml) at reflux for 10 min after complete solution. Removal of excess reagents in vacuo, addition of dry C_6H_6, and revaporation provided usable product. The crude products from the use of $POCl_3$ were mixed salts and rarely crystallized satisfactorily. The use of $SOCl_2$ invariably provided the highly crystalline chloride salts and this was therefore the method of

choice. The hygroscopic products are readily cleaved by water returning starting 10-methylacridones and are best prepared immediately before they are required and reacted further in the flask of preparation. The flask containing crystalline 9-chloro compound was fitted with a stopper bearing a tap and the air evacuated, and then the flask and contents were cooled to −15°. A solution of the requisite aniline component (0.0105 M) in a quantity of EtOH or EtOH–H_2O, sufficient to maintain solution at low temperature, was cooled to −15°. The cooled amine solution was then added to the freshly opened cold flask containing the 9-chloro component and the mixture stirred vigorously as the whole equilibrated to room temperature. When a clear solution resulted reaction was completed by brief (10 min) boiling and then solvent was removed in vacuo. The residue was dissolved in water, by heating if necessary, and clarified from traces of 10-methylacridone, and crude product precipitated by addition of NH_4Cl or NH_4Br as dictated by trial experiments. Recrystallization was from H_2O or EtOH–H_2O with addition of a salt of the necessary anion. The anion used is obvious from the formulas quoted in Table I. The highly colored (orange to purple) quaternary salts are, as with earlier examples,[5] often extensively hydrated and tend to separate from solution as gels. By crystallization from solutions containing high proportions of EtOH and relatively high levels of an inorganic salt of the necessary anion gel formation is minimized.

Method B. Acetylamino variants prepared by application of method A 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 that hydrolysis was complete (ca. 45 min). After evaporation in vacuo the residue was 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. Recrystallization was as above.

Alternatively, the trifluoroacetyl group was removed by solution in EtOH–H_2O (65%) and addition of sufficient NH_4OH to provide a 4 N solution. When TLC monitoring showed reaction was complete, the solution was evaporated and the residue dissolved in hot 1 N HOAc, and the product precipitated by addition of the Na salt of the requisite anion.

Method C. To a stirred solution of the nitroquaternary salt (2 g) in boiling EtOH–H_2O (65%, 80 ml) Fe powder (10 g) was added followed by 1 ml of a solution of $FeCl_3$ (32.5 g) in H_2O (100 ml). Heating and stirring were continued for a further 30 min. After addition of $CaCO_3$ powder (2 g) and an additional 20 min of boiling, the solution was clarified (Celite) and the residue well extracted with boiling 65% EtOH–H_2O. To the residue obtained on evaporating the extracts was added $CaCO_3$ (0.1 g) and the whole mixture was extracted with successive quantities of boiling water. Saturation of the extracts with solid NaCl precipitated the product chloride. Products were recrystallized as before but multiple crystallizations were necessary to remove minor products produced during the reduction step.

Biological Testing. The 10^5 L1210 cells were inoculated intraperitoneally into 18.5–22.5 g of C_3H/DBA_2 F_1 hybrids on day 1; drug treatment was initiated 24 h later and animals received a daily dose for a total of 5 days. All drug dosage was by the intraperitoneal route and an animal dose was suspended or dissolved in 0.2 ml of water. Mean survivals were calculated in the usual way. The drug doses employed for treating different animal groups were spaced at 0.18 log dose intervals. Dose levels ranged from the clearly toxic to the inactive. 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.

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