Potential Antitumor Agents. 19. Multiply Substituted 4′-(9-Acridinylamino)methanesulfonanilides

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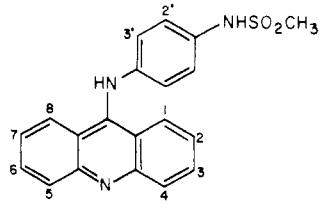
A series of 42 multiply substituted 4′-(9-acridinylamino)methanesulfonanilides has been prepared and evaluated in the L1210 system. In addition to biologic activity changes resulting from altered agent lipophilic–hydrophilic balance variants containing both acridine 4-CH_3 and 3-NH_2, $NHCOCH_3$ or NO_2 substituents have reduced activity. Variants 3,6-disubstituted, using functions of differing electronic character, have depressed activity, suggesting that there is limited site bulk tolerance. Asymmetric 3,5-disubstitution should then be the preferred pattern; the 3-NH_2-5-CH_3-3′-OCH_3 variant is the most dose potent (optimum dose qd 1–5, 1.25 mg/kg/day) of the high activity agents of this series so far prepared.

During pharmacologic tracking of ^3H-labeled samples of an AMSA [4′-(9-acridinylamino)methanesulfonanilide, **1**] congener which will receive clinical trial, it was observed that there was rapid in vivo drug decay. This decay was shown not due to metabolism but to direct chemical reaction with host thiols.[1] 9-Anilinoacridines and thiols



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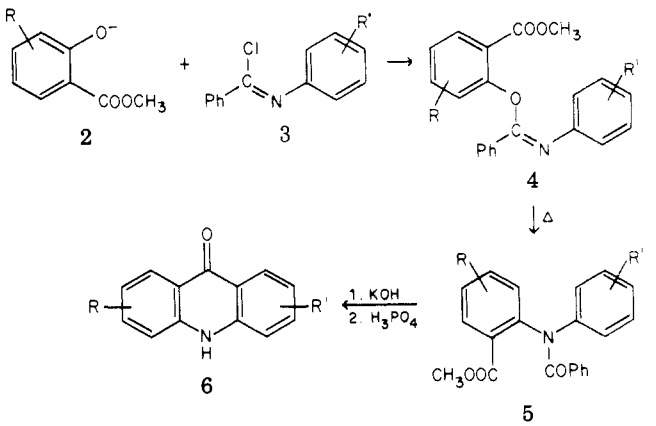
react with expulsion of the acridine side chain and production of an acridine 9-thioether which is able to react further with additional nucleophiles.[2] The logarithms of agent half-lives ($T_{1/2}$) in the presence of excess thiol were shown linearly related to Hammett's σ_p values for drug 1′-substituents.[2] Electron-donor 1′-substituents are necessary for L1210 activity[3] and are those that promote most rapid thiolysis.[2] This apparent correlation between antileukemic activity and capacity to alkylate thiol functions was destroyed when steric inhibition of thiolysis by 3′-substituents was noted.[2] Of the 3′ functions examined, those having negative σ_p values provided L1210 active drugs.[4] Thiolytic cleavage rates could be reduced to negligible proportions with a 3′-CH_3 group but full antileukemic activity was retained.[2] As effective inhibition of drug cleavage could possibly increase pharmacologic half-life, thereby increasing either antileukemic activity and/or dose potency, further agents bearing σ_p negative 3′-substituents have been synthesized for L1210 evaluation.

From our analysis of the structure–activity relationships (SAR) of acridine ring substituents and a consideration of a DNA intercalation site model, we have suggested that 3-substituents of site-lodged agents are in a hydrophobic area.[5] This model predicts that an additional hydrophobic area might then be located alongside the 6 position of the acridine nucleus. Several variants with hydrophobic 3,-6-substituents have been prepared to probe this point.

A further possibility from the site model is that the dose potency enhancing effects of 2- or 3-NH_2 groups could be a consequence of hydrogen bond formation between drug amine and site phosphodiester residues.[6] It was earlier concluded that amine-substituted variants were more hydrophilic than considered necessary for maximum activity.[5] Congeners having both NH_2 and CH_3 functions should then have lipophilic–hydrophilic balance more closely approximating the optimum figure and, if CH_3 groups are correctly located, there could be augmented drug-site binding due to hydrophobic interactions.[5] A

Scheme I



range of 3-NH_2-*x*-CH_3 variants has been synthesized and evaluated in the L1210 system.

Chemistry. There was a common step involved in the preparation of all agents listed in Table I: acid-catalyzed coupling of a 4-aminosulfonanilide component and a 9-chloroacridine. The amine component necessary for the preparation of **7** was obtained by mesylation of the requisite nitrophenetidine and a following reduction.

Reaction of 2-bromoethanol with potassium 2-acetamido-5-nitrophenate provided 2-(2-hydroxyethoxy)-4-nitroacetanilide. The further steps of O-acetylation, reduction of NO_2 function, and mesylation of the formed NH_2 group provided, after removal of masking *O*- and *N*-acetyl functions, the side-chain intermediate necessary for the synthesis of **8**.

Most 9-chloroacridine intermediates were prepared by either ring closure ($POCl_3$) of *N*-phenylanthranilic acids or by treatment of the corresponding 9(10*H*)-acridone with $POCl_3$ or $SOCl_2$–DMF.[5] Necessary *N*-phenylanthranilic acids could be generated by a Jourdan–Ullmann diphenylamine synthesis from a 2-halobenzoic acid and an aromatic amine component utilizing the experimental modifications listed earlier.[5] Yields of 4,5-disubstituted acridine derivatives by this latter route were extremely poor. Although following a multistep route an alternative *N*-phenylanthranilic acid synthesis, using the Chapman rearrangement (Scheme I),[7] provided much higher overall yields of 4,5-disubstituted acridines. Two simple modifications facilitate this synthesis: (a) the thermal rearrangement step (**4** → **5**, Scheme I), in the cases we have examined, proceeded smoothly and cleanly in refluxing Dowtherm A; (b) the extremely strenuous hydrolytic conditions necessary for removal of benzoyl protecting group in **5** can be avoided by direct ring closure (H_3PO_4 or polyphosphoric acid) of the acid resulting from saponification of the methyl ester **5**; the benzoyl group is re-

Table I

No.	Substituents in 1	Mp, °C	Formula	Analyses[a]	R_m[b]	OD[c]	L1210 ILS[d]	Δ log ILS[e]
1	Parent for comparison				0.00	45	104	
7	3'-OC_2H_5	296 dec	$C_{22}H_{21}N_3O_3S\cdot HCl\cdot H_2O$	C, H, N, Cl	0.37	250	29	−0.19
8	3'-OCH_2CH_2OH	182-183	$C_{22}H_{21}N_3O_4S\cdot HCl\cdot H_2O$	C, H, N, Cl	−0.03	62.5	57	−0.39
9	3-Cl, 6-CF_3	284-286	$C_{21}H_{15}F_3ClO_2S\cdot HCl\cdot H_2O$	C, H, N, Cl	0.48	>500		
10	3-Cl, 6-CF_3, 3'-OCH_3	198-199	$C_{22}H_{17}F_3ClN_3O_3S\cdot HCl\cdot 2H_2O$	C, H, N, Cl	0.66	>500		
11	3,6-Cl_2	331 dec	$C_{20}H_{15}Cl_2N_3O_2S\cdot MsOH$[f]	C, H, N, S	0.29	>500		
12	3,6-Cl_2, 3'-OCH_3	318-319	$C_{21}H_{17}Cl_2N_3O_3S\cdot MsOH\cdot H_2O$	C, H, N, S	0.47	100	52	−>0.32
13	3,6-Br_2	>360	$C_{20}H_{15}Br_2N_3O_2S\cdot HBr$	C, H, N, Br	0.31	250		
14	3,6-Br_2, 3'-OCH_3	292 dec	$C_{21}H_{17}Br_2N_3O_3S\cdot HBr$	C, H, N, Br	0.50	200	82	−>0.51
15	3,6-$(NO_2)_2$, 3'-OCH_3	265-266	$C_{21}H_{17}N_5O_7S$[g]	C, H, N, S	0.08	45	58	−0.24
16	3-NO_2 for comparison[k]				−0.07	25	123 (2)	+0.02
17	3-NO_2, 3'-OCH_3	212-214	$C_{21}H_{18}N_4O_5S\cdot HCl$	C, H, N, Cl	0.11	4.5	84	−0.04
18	3-NO_2, 3'-CH_3	277 dec	$C_{21}H_{18}N_4O_4S\cdot HCl$	C, H, N, Cl	0.14	75	64	−0.08
19	3-NO_2, 5-CH_3	304 dec	$C_{21}H_{18}N_4O_4S\cdot HCl$	C, H, N, Cl	0.02	100	113 (2)	−0.02
20	3-NO_2, 5-CH_3, 3'-OCH_3	261-262	$C_{22}H_{20}N_4O_5S$	C, H, N, S	0.20	11	107 (1)	+0.26
21	3-NO_2, 5-CH_3, 3'-CH_3	295-297	$C_{22}H_{20}N_4O_4S\cdot HCl$	C, H, N, Cl	0.24	>500[h]	74 (1)	+0.18[i]
22	3-NO_2, 6-CH_3	>360	$C_{21}H_{18}N_4O_4S\cdot HCl$	C, H, N, Cl	0.04	>500[h]	99	−0.06[i]
23	3-NO_2, 6-CH_3, 3'-OCH_3	276-278	$C_{22}H_{20}N_4O_5S\cdot HCl\cdot H_2O$	C, H, N, Cl	0.22	50	91	+0.23
24	3-NO_2, 5,6-$(CH_3)_2$	264-265	$C_{22}H_{20}N_4O_4S$	C, H, N, S	0.09	150	103 (1)	+0.03
25	3-NO_2, 5,6-$(CH_3)_2$, 3'-OCH_3	254-255	$C_{23}H_{21}N_4O_5S$	C, H, N, S	0.27	35	92 (1)	+0.33
26	3-NO_2, 5,6-$(CH_3)_2$, 3'-CH_3	282-283	$C_{23}H_{22}N_4O_4S\cdot HCl$	C, H, N, Cl	0.32	320	57	+0.24
27	3-NO_2, 4-CH_3	200-202	$C_{21}H_{18}N_4O_4S\cdot HCl$	C, H, N, Cl	0.09	110	54	−0.25
28	3-NO_2, 4,5-$(CH_3)_2$	201-203	$C_{22}H_{20}N_4O_4S$	C, H, N, S	0.15	210	28	−0.39
29	3-NO_2, 4,5-$(CH_3)_2$, 3'-OCH_3	221-224	$C_{23}H_{22}N_4O_5S$	C, H, N, S	0.30	205	33	−0.04
30	3-NO_2, 4,6-$(CH_3)_2$	223-225	$C_{22}H_{20}N_4O_4S$	C, H, N, S	0.20	220	34	−0.24
31	3-NH_2, 3'-OCH_3	283-284	$C_{21}H_{20}N_4O_3S\cdot HCl\cdot H_2O$	C, H, N, Cl	−0.01	6	82	−0.18
32	3-NHAc, 3'-OCH_3	221-223	$C_{23}H_{22}N_4O_4S\cdot HCl\cdot 0.5H_2O$	C, H, N, Cl	0.06	24	89 (1)	−0.07
33	3-NH_2, 3'-CH_3	347 dec	$C_{21}H_{20}N_4O_2S\cdot HCl$	C, H, N, Cl	0.04	37.5	108 (2)	−0.01
34	3-NHAc, 3'-CH_3	235-237	$C_{23}H_{22}N_4O_3S\cdot HCl$	C, H, N, Cl	0.09	55	107 (2)	+0.05
35	3-NH_2, 4-CH_3	200-202	$C_{21}H_{18}N_4O_4S\cdot HCl$	C, H, N, Cl	−0.09	12.5	76	−0.27
36	3-NHAc, 4-CH_3	331 dec	$C_{23}H_{22}N_4O_3S\cdot HCl$	C, H, N, Cl	−0.04	25	54	−0.39
37	3-NH_2, 5-CH_3	228-229	$C_{21}H_{20}N_4O_2S\cdot HCl$	C, H, N, Cl	−0.11	2	103 (1)	−0.14
38	3-NHAc, 5-CH_3	327-329	$C_{23}H_{22}N_4O_3S\cdot HCl$	C, H, N, Cl	−0.06	10	117 (2)	−0.07
39	3-NH_2, 5-CH_3, 3'-OCH_3	210-212	$C_{22}H_{22}N_4O_3S\cdot HCl$	C, H, N, Cl	0.12	1.25	125 (1)	+0.16
40	3-NH_2, 5,6-$(CH_3)_2$	244-245	$C_{22}H_{22}N_4O_2S\cdot HCl$	C, H, N; Cl[j]	−0.03	5	87	−0.17
41	3-NH_2, 5,6-$(CH_3)_2$, 3'-OCH_3	228-230	$C_{23}H_{24}N_4O_3S\cdot HCl\cdot H_2O$	C, H, N, Cl	0.15	3	79	+0.03
42	3-NH_2, 4,5-$(CH_3)_2$	314-315	$C_{22}H_{22}N_4O_2S\cdot HCl$	C, H, N, Cl	−0.03	3.7	47	−0.44
43	3-NHAc, 4,5-$(CH_3)_2$	282-284	$C_{24}H_{24}N_4O_3S$	C, H, N, S	0.02	15	53	−0.35
44	4-$COOC_2H_5$	186-187	$C_{23}H_{21}N_3O_4S$	C, H, N, S	0.21	>500		−>0.35
45	4-CH_2OH	151 dec	$C_{21}H_{19}N_3O_3S\cdot HCl$	C, H, N, Cl	−0.24	50	63	−0.31
46	3-I, 5-OCH_3	225-226	$C_{21}H_{19}N_3IO_3S\cdot HCl$	C, H, N, Cl, I	0.30	50	74	+0.31
47	3-I, 5,3'-$(OCH_3)_2$	235-236	$C_{22}H_{20}N_3IO_4S$	C, H, N, I	0.47	6	63	−>0.40
48	3-I, 5-CH_3	259-261	$C_{21}H_{18}N_3IO_2S\cdot HCl$	C, H, N, Cl, I	0.35	25	76	+0.42
49	3-I, 5-CH_3, 3'-OCH_3	209-214	$C_{22}H_{20}N_3IO_3S\cdot HCl$	C, H, N, Cl, I	0.51	10	86	−>0.54
50	3-NO_2, 5,3-$(OCH_3)_2$	260-262	$C_{22}H_{21}N_4O_6S\cdot HCl$	C, H, N, Cl	0.14	25	66	−0.07

[a] Analyses for the indicated elements were within ±0.4% of the calculated figures. [b] $R_m = \log(1/R_f - 1)$, R_f being determined as described in the Experimental Section. [c] Optimum dose in mg/kg/day; that dose providing maximum ILS in L1210 assays; or, for L1210 inactive agents, maximum tolerated. [d] Mean increase in life span of leukemic animals, treated with the optimum drug dose, to that of nontreated controls as a percentage. Number of animals from a group of six surviving 50 days after dosing is provided in parentheses. [e] Difference between log ILS observed and that predicted from the R_m value of the variant and earlier derived parabolic relation between R_m and log ILS.[5] [f] MsOH = methanesulfonic acid. [g] When an acid component has not been specified it has been necessary to handle the agent free base to obtain satisfactory elemental analyses; see Experimental Section. [h] Maximum dose tried; optimum dose may be even greater. [i] Optimum dose not reached; these figures may therefore be low. [j] Cl: calcd, 8.0; found, 8.6. [k] See ref 5.

moved in the course of closure and the required acridone is obtained directly. No acceptable method for ring closure of the ester **5** itself could be found; ester hydrolysis was a prerequisite for ready ring closure.

Necessary 3,6-disubstituted acridone intermediates have been prepared by ring closure ($POCl_3$) of the piperidides of the requisite *N*-phenylanthranilic acids. We have earlier described in full this method for controlling the direction of ring closure to provide predominantly the 3-(6)-substituted acridone.[4]

Preparation of halogenoacridones by Sandmeyer replacement of amine functions has provided low yields and this route has only been useful for the preparation of monoiodoacridones. 3,6-Diaminoacridone is readily available from vigorous reduction ($SnCl_2$–HCl) of 2,4,-2',4'-tetranitrobenzophenone[8] but attempted Sandmeyer conversion to 3,6-dibromoacridone provided a complex mixture with less than 10% (TLC) of the desired product. Ring closure of the piperidide[4] of *N*-(3-bromophenyl)-5-bromoanthranilic acid provided excellent yields of pure 3,6-dibromoacridone. Similarly, ring closure of the piperidide of *N*-(3-nitrophenyl)-5-nitroanthranilic acid furnished 3,6-dinitroacridone rather than a mixture with the 1,6-dinitro isomer predominating as is obtained by direct $POCl_3$ cyclization of the acid.[9]

Most 3-NH_2 compounds listed were prepared by reduction (Fe–H^+)[10] of the corresponding nitro compounds. 3-Acetylamino compounds were either prepared by acetylation (*o*-phenylene phosphorochloridite–HOAc)[10] of the amine variant or by application of the standard synthesis using a 3-acetylamino-9-chloroacridine. In certain cases hydrolysis of the 3-acetylamino analogues, produced by the latter method, provided a better route to the 3-NH_2 compound.

The reaction of the ethyl ester of acridone-4-carboxylic acid with $SOCl_2$–DMF provided the corresponding 9-

chloroacridine which was elaborated to the ester **45**; ester reduction ($LiBH_4$) then provided the benzylic alcohol **46**.

Biologic Testing. DBA_2/C_3H F_1 hybrid mice received inocula of 10^5 L1210 cells intraperitoneally (ip) and ip drug treatment started 24 h later and continued daily for 5 days. Doses at logarithmic intervals (0.3) were screened and ranged from the clearly toxic to the L1210 inactive. On retest doses were arranged midway between those initially employed and were selected to span the initially observed optimum dose. Full details of the testing methods were provided earlier when attention was drawn to the need for soluble agent formulations to ensure high reproduceability in L1210 assays.[5] The simple expedient of preparing methanesulfonic acid salts has often provided sufficiently soluble formulations. Unfortunately, no generally satisfactory method for dissolving the extremely insoluble, weakly basic, 3-nitro analogues (**16–30, 50**) has been found. These nitro congeners have been administered to animals as homogenized suspensions in aqueous methylcellulose. After preliminary screening to approximate necessary dosage levels, a series of dilutions of a prepared drug suspension was examined in one group of tests ensuring that the doses employed covered the range from the clearly toxic to the suboptimal. With these insoluble nitro compounds it was often found that different optimum doses were found on retest although the maximum life extensions observed would normally be within satisfactory limits. Certain of the more potent nitro derivatives (**20** and **25**) could be administered to animals as true solutions in 35% Me_2SO–H_2O. Percentage increase in mean life span (ILS) in L1210 tests observed with such solutions was somewhat lower (typically 93 and 81%, respectively) and the drugs appeared slightly more toxic (optimum doses 4.7 and 22 mg/kg, respectively). Through all the discussion of the biologic activity of these nitro compounds, it should be recalled that these insoluble compounds have been screened as suspensions. Factors such as rate of dissolution may be playing a difficultly assessable role in the life extensions measured.

Structure–Activity Relationships. As before, substituent effects on overall molecular lipophilic–hydrophilic balance have been compensated for by making all biologic activity comparisons under equilipophilic conditions.[5] As a measure of biologic activity log ILS has been used. R_m values from reversed phase chromatography[6] have been used as a convenient measure of lipophilic–hydrophilic balance. A plot of log ILS vs. R_m for the homologous 4′-(9-acridinylamino)alkanesulfonanilides has provided a reference curve.[5] Any substituted variant has been considered in relation to this curve; at the R_m value of the variant the deviation of the measured biologic activity from that predicted by the curve (Δ log ILS) provides a cumulative measure of other substituent factors (steric, electronic, hydrophobic, etc.) influencing biologic activity. Substituent effects are reported as Δ log ILS values in Table I. Our experiences with reproduceability in L1210 assays have shown that if the log ILS figures for two variants differ by less than 0.2 (i.e., Δ log ILS), they should not be considered to have significantly different activities.

A 3′-OCH_3 function appended to **1** increases dose potency 6.7-fold[11] (optimum dose 6.7 mg/kg/day) and lengthens $T_{1/2}$ in thiolytic rate assays by a factor of two.[2] This rate inhibition appears of steric origin[2] and a larger 3′ function might then further slow thiolysis. However, the sterically more demanding 3′-OC_2H_5 group provided a low-potency, less active variant (**7**). While the 3′-CH_3 function increases the $T_{1/2}$ for thiolysis 59-fold,[2] the resulting agent is relatively nonpotent (optimum dose 97 mg/kg/day) although fully L1210 active (ILS 106%).[11] It could be hypothesized that site functionality interacting with these 3′-CH_3 and 3′-OCH_3 groups had polar nature thereby tending to better accept the more polar methoxyl function. The polar but sterically demanding 3′-(2-hydroxyethyl ether) **8** had no better activity than the ethyl ether **7**. Our DNA intercalation site model predicts that any 3′ function should be relatively small in size and probably polar in nature.[5] From previous SAR analyses we can add the additional requirement that such functionality shall be electron donor in character but there may be an upper limit to the electron enrichment possible.[11] These various requirements markedly restrict the number of permissible 3′ functions.

From the further 3′-substituted examples quoted, it can be concluded that when a 3′-OCH_3 has been added to an agent the new variant has proved at least as potent as the precursor but usually has been considerably more potent (**11, 12; 13, 14; 16, 17; 19, 20; 22, 23; 24, 25; 28, 29; 37, 39; 40, 41; 46, 47; 48, 49**). In contrast, a 3′-CH_3 group has invariably decreased potency (**16, 18; 19, 21; 24, 26; 31, 33; 32, 34**).

We earlier delineated the necessary steric bulk of single hydrophobic 3-substituents and showed that functions larger than iodine were unfavorable.[11] In the derivatives with both 3- and 6-hydrophobic substituents (**9–15**) an extension of earlier observed effects is seen; combination of a CF_3 group with an additional Cl function provided inactive agents (**9, 10**). Only the symmetrical dihalogen variants bearing a 3′-OCH_3 have shown activity (**12, 14**), the greatest activity being observed with the Br_2 analogue **14**. A 3-Br substituent was earlier selected as the most acceptable halogen among the monosubstituted variants.[11] The 3,6-$(NO_2)_2$ variant **15** has low activity but it is interesting to observe that some antileukemic efficacy is retained in such a low pK analogue.[12]

Appended methyl groups provided position-dependent effects. With an existing 3-substituent present (e.g., 3-NO_2, **16, 17**) addition of a 6-CH_3 (to yield **22, 23**) decreased dosage potency to a greater extent than did a 5-CH_3 group (**19, 20**). As the corresponding 5,6-$(CH_3)_2$ variants (**25, 26**) have optimum doses intermediate between those of the 5- and 6-monomethyl variants, such effects are unlikely to be due to changes in lipophilic character alone. In contrast, a 4-CH_3 group, adjacent to the nitro function, produced a clear drop in activity in relation to the isomeric methyl-substituted congeners (cf. **27, 19; 29, 25; 30, 24**). Essentially similar results are seen with a $-NH_2$ or $-NHCOCH_3$ in place of the 3-NO_2 group; a 4-CH_3 function depresses activity in comparison with isomeric methyl congeners (cf. **35, 37; 36, 38; 42, 40**). The combination of 3-NH_2, 5-CH_3, and 3′-OCH_3 substituents (**39**) provided activity as high as observed with past members of this series but this compound is the most dose potent (optimum dose 1.25 mg/kg) of those having comparable levels of activity.

The dystherapeutic effects of a 4-CH_3 adjacent to a 3-NO_2 might reflect steric interactions with resulting tilting of the nitro group out of the acridine plane thereby hindering a DNA intercalation step.[11] This argument would appear less reasonable with the smaller 3-NH_2 function. The thioxanthone lucanthone[13] has a CH_3 group similarly located on a tricyclic ring system to a 4(5)-CH_3 group of the acridines. If it were necessary in the acridines for an in vivo oxidative activation as in the lucanthone–hycanthone series ($CH_3 \rightarrow CH_2OH$), then an adjacent 3-nitrogen function might sterically inhibit this step. Similarly, the observed differences between 5- and 6-CH_3

Table II. 3(6)-Acridine-Substituted Variants of 1

3,6-Disubstituted	OD[a]	ILS[b]	3-Substituted	OD	ILS
3,6-$(OCH_3)_2$[c]	60	41	3-OCH_3	35.0	81
3,6-Cl_2[d]	>500	–[e]	3-Cl[c]	75	103
3,6-Cl_2-3'-OCH_3[d]	100	52	3-Cl-3'-OCH_3[f]	10	72
3,6-Br_2[d]	250	–[e]	3-Br[c]	35	65
3,6-Br_2-3'-OCH_3[d]	200	82	3-Br-3'-OCH_3[f]	6	119 (1)
3,6-NO_2-3'-OCH_3[d]	45	58	3-NO_2-3'-OCH_3[d]	4.5	84
3-Cl-6-OCH_3[c]	300	41			
3-NO_2-6-OCH_3[c]	250	51	3-NO_2[c]	25	123 (2)
3-NO_2-6-CH_3[d]	>500	99	3-CH_3[c]	17	117

[a] Optimum dose in mg/kg/day. [b] ILS, percentage increase in life span in standard L1210 tests. Number of 50-day survivors from a group of six animals is given in parentheses. [c] Reference 5. [d] Table I. [e] –, inactive. [f] Reference 11.

substituents (cf. **19**, **22**; **20**, **23**) might reflect a more exposed location of the 5-CH_3 to enzymic attack. However, a 4-CH_2OH variant (**45**) prepared to mimic hycanthone had a very low order of activity.

An alternative explanation for the methyl group positional dependence observed is that site asymmetry, either electronic and/or steric, is best matched by a dissymmetrically substituted drug. The following points support this view. There is no clear distinction between the antileukemic activity of the 3- and 4-CH_3 congeners.[5] Distinction between methyl isomers is only possible when there is an additional substituent. Inspection of the screening data for certain 3,6-disubstituted variants (Table II) shows that these are generally decreased in either activity, potency, or both in comparison with the 3-monosubstituted compounds. Since both σ_p negative (OCH_3, CH_3) and σ_p positive (NO_2, halogens) functions are included in these disubstituted examples (Table II), it appears unlikely that the effects observed are due to, for example, cancellation of dipoles or other electronic effects. These effects may then have a steric basis. The acridine nucleus of these drugs at site would then have to be closely proscribed. 1- or 2- (7- or 8-) substituents are sterically unfavorable.[5] If the interference described above between 3-nitrogen containing substituents and a further 4 function is general, then any substituent additional to an existing 3 function can only be acceptably placed in the 5 or 6 positions. The unfavorable interactions demonstrated on 3,6-disubstitution (Table II) leaves only one position for that additional group, i.e., at the 5 position. The preferred substitution should then be the asymmetric 3,5 pattern. Investigated functions proving acceptable at the 5(4) position have been so far limited (CH_3, OCH_3).[5] Several variants utilizing a 3-I as a hydrophobic substituent and bearing either a 5-CH_3 or 5-OCH_3 are listed in Table I (**46–49**). These compounds have good potency, particularly when they are compared with the 3,6-disubstituted examples of Table II, and the large positive Δ log ILS figures (Table I) suggest that there is considerable activity enhancement. However, to fully assess the potential of such 3,5-disubstituted variants (**46–49**) methods for attaching acceptable hydrophilic functionality must be found so that the overall molecular lipophilic–hydrophilic balance can be adjusted closer to optimum figures.[5]

Experimental Section

Where analyses are indicated only by symbols of the elements, analytical results for these elements were within ±0.4% of the theoretical values. Analyses were performed by Dr. A. D. Campbell, Microchemical Laboratory, University of Otago, Dunedin, New Zealand. The hydrochlorides and hydrobromides of the agents listed in Table I tend to lose HCl and HBr on drying at elevated temperatures in vacuo, this being particularly noticeable with the weakly basic nitroacridyl compounds. Accordingly, samples for analysis have been dried in vacuo at room temperature over P_2O_5. Even though dried under these mild conditions, certain very weakly basic analogues gave elemental analyses showing extensive loss of anion; in these cases (**15**, **20**, **24**, **25**, **28–30**, **43**, **44**, **47**) the free base, prepared as before,[5] was purified and analyzed for the elements. Melting points were determined on an Electrothermal melting point apparatus with the makers' supplied stem corrected thermometer; melting points are as read.

Chromatography. Reversed phase chromatography conditions for determining R_m values were those described earlier;[6] 4'-(9-acridinylamino)methanesulfonanilide (1) was included as standard on all sheets and R_m values are quoted in reference to this compound. TLC on SiO_2 (Merck SiO_2 F_{254}) was used to monitor the progress of reactions, purification of products, etc.

***N*-(3-Bromophenyl)-4-bromoanthranilic Acid.** A heterogeneous mixture of 2-chloro-4-bromobenzoic acid (8.3 g, 0.035 mol), 3-bromoaniline (6.15 g, 0.0355 mol), anhydrous K_2CO_3 (5.9 g, 0.043 mol), 2-ethoxyethanol (12 ml), and powdered catalytic Cu (0.1 g) was heated under reflux conditions in an oil bath at 150° for 2 h. After cooling below 100° H_2O (100ml) was added and the mixture boiled with stirring until all salts had dissolved. Acidification (HCl) precipitated crude product which was well washed with boiling water and then dissolved in 100 ml of H_2O and Na_2CO_3 (5 g) by boiling. Generous quantities of decolorizing charcoal were added to the solution, the whole mixture was stirred for 0.5 h and clarified through a Celite pad, and product was recovered by acidification. Solution in Me_2CO (40 ml) and reprecipitation by addition of 4 vol of H_2O removed traces of starting 2-chloro-4-bromobenzoic acid. Crystallization from EtOH–H_2O and then small volumes of HOAc–H_2O provided pure product as pale yellow needles: mp 193–194° (6.7 g, 52% yield). Anal. ($C_{13}H_9Br_2NO_2$) C, H, N, Br.

In essentially similar fashion were prepared *N*-(2-methyl-3-nitrophenyl)-4-methylanthranilic acid [mp 230–231° (37% yield). Anal. ($C_{15}H_{14}N_2O_4$) C, H, N], *N*-(2-methylphenyl)-4-nitroanthranilic acid [mp 239–240° (62% yield). Anal. ($C_{14}H_{12}N_2O_4$) C, H, N], *N*-(2,3-dimethylphenyl)-4-nitroanthranilic acid [mp 249–250° (58% yield). Anal. ($C_{15}H_{14}N_2O_4$) C, H, N], and *N*-(2-methyl-3-nitrophenyl)anthranilic acid [mp 232–233° (27% yield). Anal. ($C_{14}H_{12}N_2O_4$) C, H, N].

Most of the above *N*-arylanthranilic acids on treatment with $POCl_3$ as before[5] provide unequivocally a single substituted 9-chloroacridine. In cases, such as *N*-(3-methylphenyl)-4-nitroanthranilic acid, where it is possible to obtain mixed isomers by $POCl_3$ treatment, recourse to the piperidide ring closure described earlier[4] provided the required 3-substituted acridone. In this example the piperidide, prepared as before,[4] had mp 107–108°. Anal. ($C_{19}H_{21}N_3O_3$) C, H, N. The piperidides have in many cases inconveniently low melting points and, provided TLC has shown the neutral product to be homogeneous, the following $POCl_3$ ring closure can be applied without further purification of these intermediates.

N-Arylanthranilic acids can also be readily converted to the acridones with polyphosphoric acid (100–160°) or polyphosphate ester (steam bath temperature). Alternatively, the 9-chloroacridines resulting from $POCl_3$ treatment can be hydrolyzed (HCl–HOAc) to provide the acridone. The latter route was used to generate 3-nitro-4-methyl-9(10*H*)-acridone: mp 315–316°. Anal. ($C_{14}H_{10}N_2O_3$) C, H, N. Nitro group reduction ($SnCl_2$–HCl) as before[5] then provided 3-amino-4-methyl-9(10*H*)-acridone: mp 332–333°. Anal. ($C_{14}H_{12}N_2O$) C, H, N. Acetylation of this compound furnished 3-acetylamino-4-methyl-9(10*H*)-acridone.

Anal. ($C_{16}H_{14}N_2O_2 \cdot 0.5H_2O$) C, H, N.

Synthesis of *N*-Arylanthranilic Acids by Chapman Rearrangement. For purposes of example the preparation of 4,5-dimethyl-9(10*H*)-acridone is detailed. Troublesome condensation products encountered in usual preparations of methyl 2-hydroxy-6-methylbenzoate, required as intermediate, are avoided by the following procedure. A thick suspension of commercial *o*-cresotinic acid (40 g, 0.26 mol) in $SOCl_2$ (40 ml, 0.56 mol) containing DMF (0.1 ml) was stirred in an ice–water bath for 24 h, the suspension slowly thinning to provide a clear solution. Excess $SOCl_2$ was removed in vacuo at 25° and the residue added to MeOH (150 ml) precooled to −15°. After 30 min of stirring the solution was concentrated as far as possible on the steam bath and poured into ice–water and ester was removed by extraction into petroleum ether. The petroleum ether solution on shaking with 10% $KHCO_3$ deposited solid by-products which were removed by filtration. After further washing with $KHCO_3$ and H_2O and drying (Na_2SO_4), the petroleum ether was removed on the water bath and residual ester distilled in vacuo: bp 127° (18 mm); 36.8 g (84% yield).

2-Methoxycarbonyl-6-methylphenyl *N*-(2-Methylphenyl)benzimidate. To a suspension of NaH (20.3 g, 50% dispersion in oil) in ice-cooled DMF (50 ml) methyl *o*-cresotinate (63.8 g, 0.38 mol) was added as permitted by the vigor of reaction. When all ester had been added the heterogeneous suspension was allowed to stand at room temperature overnight. *N*-(2-Methylphenyl)benzimidoyl chloride was prepared by solution of *N*-(2-methylphenyl)benzamide (80.5 g, 0.38 mol) in $SOCl_2$ (100 ml, 1.4 mol) containing DMF (0.6 ml) and reaction at room temperature for 24 h. After removal of excess $SOCl_2$ in vacuo, C_6H_6 (75 ml) was added and the solution reevaporated. Following further addition of toluene (75 ml) the mixture was stripped at water pump vacuum in an oil bath at 160°. The remaining crude imidoyl chloride was dissolved in C_6H_6 (100 ml) and added in dropwise fashion to the cooled sodium *o*-cresotinate suspension so that the temperature remained below 5°. The suspended salts in the mixture gradually changed crystalline form as reaction produced the more granular NaCl. When reaction appeared essentially complete the mixture was heated on a steam bath for 1 h and cooled well and HOAc (10 ml), then H_2O (200 ml), and petroleum ether (400 ml) were added. After storage at 0° for 24 h the crystalline imidoyl ester was collected and washed well with water and a little petroleum ether. Crystallization from small volumes of MeOH was slow and required several days of refrigeration for good recovery. The massive crystals of product had mp 96.5–97° (98.6 g, 71% yield) (lit.[14] mp 97°).

Methyl *N*-Benzoyl-*N*-(2-methylphenyl)-3-methylanthranilate. A solution of the imidoyl ester (77.6 g, 0.56 mol) in Dowtherm A (60 ml) was brought to the boil and a few drops of solvent was distilled from the flask to expel traces of moisture. TLC monitoring showed the reaction to be complete after 2 h of boiling. To the cooled solution petroleum ether (250 ml) was added followed by seed crystals. After 12 h at −15° the crystals were collected and washed with petroleum ether. Crystallization from MeOH provided the product as colorless prisms: mp 143.5–144° (72.7 g, 93.5% yield) (lit.[14] mp 145°).

***N*-Benzoyl-*N*-(2-methylphenyl)-3-methylanthranilic Acid.** The corresponding methyl ester (70 g, 0.195 mol) was added to a solution of KOH (24.5 g, 0.445 mol) in H_2O (33 ml) and MeOH (190 ml), and the suspension was boiled until solution was complete then for a further hour. After cooling and addition of H_2O (600 ml) product was precipitated with HCl, collected, and washed well with H_2O. The moist solid was suspended in 150 ml of C_6H_6 and boiled under a Dean and Stark water entrainment head. Crude product dissolved and as last traces of water were removed from the mixture, product suddenly crystallized as a stable benzene solvate. After thorough cooling the crystals were collected, washed with petroleum ether, and then dried in vacuo at room temperature: yield 67.3 g (82%); mp 128° with effervescence. Anal. ($C_{22}H_{19}NO_3 \cdot C_6H_6$) C, H, N.

4,5-Dimethyl-9(10*H*)-acridone. P_2O_5 (110 g) was dissolved in H_3PO_4 (100 ml, *d* 1.75) by warming on a steam bath for 30 min. To the cooled solution a sample (39 g) of the above *N*-benzoylanthranilic acid benzene solvate was added and the mixture heated in an oil bath. Benzene (6.3 g, 81% of theory) distilled from the reaction flask as the temperature increased. After 30 min at 200° (internal) the mixture was cooled and stirred into excess H_2O. The collected solid was boiled with H_2O (200 ml) containing Na_2CO_3 (20 g) for 2 min; the insoluble fraction was collected, washed well with H_2O, and dried. This material was dissolved in boiling $CHCl_3$ (500 ml) and, after addition of decolorizing charcoal, clarified through a Celite pad. To the hot filtrate ligroine (500 ml) was added and the whole mixture distilled until crystallization commenced. After thorough cooling the crystals were collected, washed with petroleum ether, and dried. A further crystallization from HOAc–H_2O provided TLC homogeneous product: mp 233.5–234° (17.4 g, 84% yield). Anal. ($C_{15}H_{13}NO$) C, H, N. Quoted melting points for this compound prepared by two different routes are 230°[15] and 232–233°.[16]

By essentially similar methods were prepared 2-methoxycarbonyl-6-methylphenyl *N*-(2-methyl-3-nitrophenyl)benzimidate [mp 104–105°. Anal. ($C_{23}H_{20}N_2O_5$) C, H, N], methyl *N*-benzoyl-*N*-(2-methyl-3-nitrophenyl)-3-methylanthranilate [mp 177–179°. Anal. ($C_{23}H_{20}N_2O_5$) C, H, N], and 3-nitro-4,5-dimethyl-9(10*H*)-acridone [mp 292–293°. Anal. ($C_{15}H_{12}N_2O_3$) C, H, N]. Reduction of this nitro compound as before[5] provided 3-amino-4,5-dimethyl-9(10*H*)-acridone: mp 270–271°. Anal. ($C_{15}H_{14}N_2O$) C, H, N. Acetylation[5] of this aminoacridone provided 3-acetylamino-4,5-dimethyl-9(10*H*)-acridone: mp 315–316°. Anal. ($C_{17}H_{16}N_2O_2$) C, H, N.

4-Ethoxycarbonyl-9(10*H*)-acridone. Acridone-4-carboxylic acid (10 g, 0.042 mol) was suspended in $SOCl_2$ (50 ml, 0.7 mol), DMF (0.3 ml) added, and the suspension boiled under reflux conditions. After 15 min a clear solution resulted and the reaction was worked up after a further 30 min of heating. Following removal of excess $SOCl_2$ in vacuo the residual chloro compound was dissolved in EtOH (75 ml), the solution boiled for 5 min, and then H_2O (0.8 ml) added. The solution was heated on the water bath for 1 h, H_2O was added to the crystallization point, and then the whole mixture was well cooled. The collected crystals were well washed with 10% $KHCO_3$ and H_2O and then recrystallized from EtOH. Pure ester was obtained as yellow needles: mp 162–163° (9.3 g, 84% yield). Anal. ($C_{16}H_{13}NO_3$) C, H, N. Further reaction of this compound with $SOCl_2$ provided the corresponding, labile 9-chloroacridine which was immediately allowed to react with 4-aminomethanesulfonanilide in absolute EtOH solution as before.[5] Product hydrochloride crystallized directly from the reaction mixture in pure condition. The salt was converted, as before,[5] to the free base which was crystallized from EtOH–H_2O to yield pure **44** (Table I).

4′-(4-Hydroxymethyl-9-acridinylamino)methanesulfonanilide (45). A suspension of $NaBH_4$ (1.05 g) and anhydrous LiBr (2.15 g) in diglyme (10 ml, dried over CaH_2) was stirred for 30 min and then **44** (2 g) was added in small portions. After heating on a steam bath for 1 h as much solvent as possible was removed in vacuo. H_2O (10 ml) was cautiously added and then crude product extracted with EtOAc. To the yellow oil resulting on evaporation of the dried (Na_2SO_4) EtOAc extracts were added successively HOAc (1 ml), EtOH (2 ml), and then 20% NaCl–H_2O until the solution became turbid; on cooling in ice and scratching, product slowly crystallized. The collected product was boiled with H_2O (125 ml), the resulting solution was clarified and cooled to 50°, and NaCl (12.5 g) was stirred in. Thorough cooling returned crystalline product. The crystals were dissolved in H_2O (30 ml) plus EtOH (15 ml), then NaCl (2 g) was dissolved in the hot solution, and the whole mixture was quickly filtered. Slow cooling of the filtrate then provided TLC homogeneous **45** as pale yellow needles (1.32 g, 67% yield).

3-Amino-5-methoxy-9(10*H*)-acridone. To a suspension of $SnCl_2 \cdot 2H_2O$ (109 g) in concentrated HCl (110 ml) and HOAc (110 ml) was added 4-methoxy-6-nitroacridone (37.7 g). The mixture was gently warmed with swirling until the reaction initiated; then it was removed from the heat. When reaction abated the mixture was heated to boiling for 15 min, product hydrochloride starting to crystallize in the latter stages. After slight cooling concentrated HCl (75 ml) was added and then the whole mixture was cooled to −15° for several hours. The crystalline hydrochloride salt was collected on a sinter and washed with ice-cold 4 N HCl (2 × 40 ml). The crystals were boiled with H_2O (100 ml) and filtered back through the sinter; the lowered Cl^- concentration in the crystals then permits easy solution in a further quantity (100 ml) of boiling H_2O. The combined, clarified, H_2O solutions were made 2 N in

HCl and refrigerated overnight. The orange crystals of product hydrochloride were collected and dried in vacuo over KOH pellets. The dried crystalline product was dissolved in the minimum volume of boiling H_2O and filtered into a solution of NaOH (100 g) in H_2O (800 ml). After cooling product was collected, well washed with H_2O, dried, and then crystallized from DMF–H_2O. Pure product was obtained as orange needles: mp 280–281° (25.9 g, 77% yield). Anal. ($C_{14}H_{12}N_2O_2$) C, H, N.

3-Iodo-5-methoxy-9(10*H*)-acridone. A sample of the aforementioned aminoacridone (10 g, 0.042 mol) was dissolved in H_2O (150 ml) and 12 N HCl (7 ml) by boiling and stirring. The vigorously stirred solution was rapidly cooled until amine hydrochloride started to crystallize when 12 N HCl (7 ml) was added in one portion to precipitate the salt in a finely divided condition. The amine hydrochloride was diazotized as usual by addition of $NaNO_2$ (3.05 g, 0.044 mol) in H_2O (10 ml) at 0–5°. When nitrite addition was complete stirring was continued for 15 min and then traces of insoluble material were removed by filtration. One drop of 1-octanol was added to the filtrate to control foaming; then a solution of KI (8.4 g, 0.05 mol) in H_2O (25 ml) was added so that the temperature remained below 5°. The reaction mixture was finally heated on the steam bath for 1 h and cooled well, and the solids were collected. Adhering I_2 was removed by washing with a little sodium bisulfite solution. The dried solids were suspended in $SOCl_2$ (40 ml), one drop of DMF was added, and the solution was boiled for 15 min. The gum obtained on evaporation was dissolved in a little CH_2Cl_2 and this solution poured onto chopped ice and excess NH_4OH. Crude 9-chloroacridine was extracted with CH_2Cl_2 (2 l.). The solid obtained from the CH_2Cl_2 was extracted with boiling ligroine (2 l.) and the extract evaporated to dryness. The resulting solid was dissolved in boiling EtOH (200 ml), HCl was added to provide a 1 N solution, and the whole mixture was boiled for 1 h. Evaporation and treatment with NH_4OH provided crude iodoacridone. Multiple crystallizations from DMF–EtOH–H_2O mixtures finally provided homogeneous product as yellow crystals: mp 234–236° (5.7 g, 37% yield). Anal. ($C_{14}H_{10}INO_2$) C, H, N, I.

3-Iodo-5-methyl-9(10*H*)-acridone was prepared from 3-amino-5-methylacridone by equivalent experimental procedures. The once crystallized (DMF–EtOH) acridone (71% yield) was shown by TLC to contain several minor components. Conversion to the 9-chloroacridine ($SOCl_2$–DMF) and multiple crystallizations from petroleum ether provided TLC homogeneous product which on acid hydrolysis, as before,[4] returned pure acridone which crystallized from 2-ethoxyethanol in pale yellow prisms: mp >360°. Anal. ($C_{14}H_{10}INO$) C, H, I, N.

3,6-Dinitro-9(10*H*)-acridone was prepared via the piperidide ring closure[4] of the known[9] *N*-(3-nitrophenyl)-4-nitroanthranilic acid. The difficultly soluble acridone could be crystallized from large volumes of DMF–EtOH being obtained as dark orange prisms of high (>360°) melting point; for characterization the derived 3,6-dinitro-9-chloroacridine was prepared and after crystallization from C_6H_6 had mp 242–243° (61% yield) (lit.[9] mp 247–248°). TLC showed this compound to be free of the isomeric 1,6-dinitro-9-chloroacridine which is the major product from direct $POCl_3$ ring closure of the *N*-arylanthranilic acid.[9]

2-(2-Hydroxyethoxy)-4-nitroacetanilide. A mixture of 2-hydroxy-4-nitroacetanilide (11.0 g, 56 mmol), 2-bromoethanol (8.06 g, 64 mmol), anhydrous K_2CO_3 (8.90 g, 64 mmol), and KI (150 mg) in DMF (25 ml) was stirred and heated at 100° for 1 h. Further 2-bromoethanol (8.06 g) and K_2CO_3 (8.90 g) were added and heating was continued for 2 h. Solids were removed by filtration and the filtrate was concentrated to dryness. Crude product crystallized when the residue was shaken with dilute NH_4OH. Crystallization twice from 30% EtOH–H_2O provided pure product as colorless needles (9.95 g, 74% yield): mp 167–171°. Anal. ($C_{10}H_{12}N_2O_5$) C, H, N.

2-(2-Acetyloxyethoxy)-4-nitroacetanilide. The above product was boiled with excess Ac_2O for 90 min and then concentrated in vacuo, and the residue was crystallized from EtOAc: mp 139.5–140.5° (92% yield). Anal. ($C_{12}H_{14}N_2O_6$) C, H, N.

2-(2-Acetyloxyethoxy)-4-aminoacetanilide. The corresponding nitro compound was reduced over a 10% Pd/C catalyst in EtOH solution. Recrystallization from C_6H_6 provided the amine as colorless prisms: mp 120–121° (94% yield). Anal. ($C_{12}H_{16}N_2O_4$) C, H, N.

3-(2-Acetyloxyethoxy)-4-acetylaminomethanesulfonanilide. A stirred solution of the aforementioned amine (3.4 g, 13.5 mmol) in Py (16 ml) was treated with methanesulfonyl chloride (1.15 ml, 14.8 mmol) in dropwise fashion so that the temperature remained below 0°. After a further 2 h at room temperature Py was removed in vacuo on the steam bath, the residue shaken with H_2O (25 ml), and the whole mixture refrigerated for 12 h. The collected solid was dissolved in cold 2 N NH_4OH (20 ml) by stirring, decolorizing charcoal added, and the solution clarified (Celite) and neutralized with HOAc. The crystals which slowly separated on refrigeration were washed with a little ice–H_2O and crystallized from C_6H_6 providing product as colorless prisms: mp 126–127° (3.61 g, 81%). Anal. ($C_{13}H_{18}N_2SO_6$) C, H, N, S.

Experimental conditions for the preparation and manipulation of 9-chloroacridines, 4'-(9-acridinylimino)methanesulfonanilides, etc., and the details of the L1210 screening procedures have been detailed earlier.[4-6]

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