tion of in vivo antitumor activity by Mr. M. Jones and Mrs. P. Goddard of the Chester Beatty Research Institute and Dr. H. Wood of the National Cancer Institute.

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Potential Antitumor Agents. 16. 4′-(Acridin-9-ylamino)methanesulfonanilides

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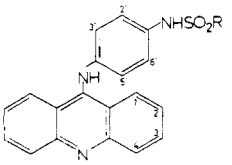
The structure–antileukemic activity (L1210) relationships for sulfonanilide ring-substituted variants of 4′-(acridin-9-ylamino)methanesulfonanilides have been investigated. Electron-donor substituents are necessary for antileukemic activity and it is suggested that high electron density at the 6′ position is associated with high activity. A 3′-OCH_3 function markedly increases (2–8-fold) potency with a variety of acceptable acridine ring substituents. Further variants with hydrophobic acridine 3-substituents have been shown to be more active than expected on the basis of overall molecular hydrophilic–lipophilic balance. There is a size limit to 3-substituents which may acceptably be as large as an iodine atom but should be smaller than an isopropyl function.

In a recent publication[1] we analyzed the structure–antileukemic activity relationships in a series of acridine ring-substituted 4′-(acridin-9-ylamino)alkanesulfonanilides **1**. The present work examines the effect on antileukemic activity (L1210) of substituents appended to the sulfonanilide ring of **1** and probes our earlier conclusion that lipophilic substituents at the 3 position of the acridine can increase activity.



1

Chemistry. There was a common reaction step involved in the preparation of the bulk of the congeners described (Table I), viz., acid-catalyzed coupling of a 9-chloroacridine with a 4-aminosulfonanilide component. Necessary aminosulfonanilide components were prepared by either of two methods. In method A a substituted 4-nitroaniline was acylated with a sulfonyl chloride and the nitro function in the resulting 4-nitrosulfonanilide reduced (Fe/H^+). Alternatively (method B), the same substituted 4-nitroaniline intermediate was first converted to the 4-nitroacetanilide, the nitro group reduced, and then the amine function so generated acylated with a sulfonyl chloride. Hydrolytic removal of the protecting acetyl function from the resulting 4-acetamidosulfonanilide then provided a 4-aminosulfonanilide having the isomeric substitution pattern to that produced by application of method A.

Isolation and further purification of the aminosulfonanilides prepared by method B were unnecessary and indeed wasteful. Direct coupling of 9-chloroacridine with acid hydrolysates of 4-acetamidosulfonanilides provided higher overall yields of product (method C).

The 2′- and 3′-amino-substituted variants **7** and **16** were prepared by reduction (Fe/H^+) of the corresponding nitro compounds **6** and **15**, respectively.

The 4″-aminobenzenesulfonanilide congeners (e.g., **25**) were obtained by mild acidic hydrolysis of the acetamido analogs **24** (method D).

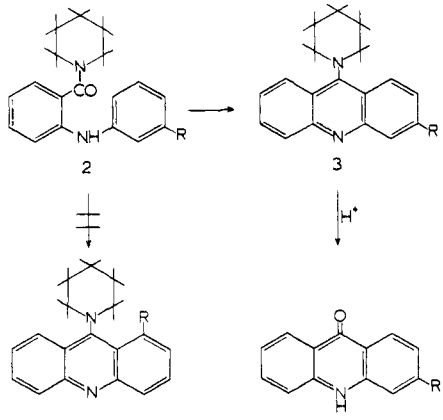
The nitrosulfonanilide required as the precursor to the 4″-methylsulfonyl analog **28** was obtained by oxidation ($KMnO_4/OH^-$) of 4-methylthiobenzenesulfon-*p*-nitroanilide.

While the 2′-azalog **21** could be readily prepared by application of method A, attempted synthesis of the 3′ isomer **22** by similar means failed. Reaction of 9-chloroacridine and 2-amino-5-nitropyridine provided 2-(acridin-9-ylamino)-5-nitropyridine and reduction of the nitro function in this molecule to amino and a following acylation with mesyl chloride provided the desired 3′-azalog **22**. Similarly, the 3′-NO_2 analog **15** was most readily prepared via 4′-(acridin-9-ylamino)-3′-nitroacetanilide in turn prepared by coupling 9-chloroacridine and 4-amino-3-nitroacetanilide. Hydrolytic removal of the acetyl function from this acetanilide and mesylation of the resulting amine provided the required **15**.

The use of a 2-halobenzoic acid and the readily available 3-trifluoromethylaniline in the Jourdan–Ullmann reaction provides *N*-(3-trifluoromethylphenyl)anthranilic acid.[2] Ring closure of this acid ($POCl_3$) provides a mixture of 1- and 3-trifluoromethyl-9-chloroacridines but the required 3 isomer, necessary for the preparation of variants **31** and **32**, is that produced in lower yield. Necessary trifluoromethyl-substituted intermediates that allow unambiguous preparation of the required 3-trifluoromethyl-9-chloroacridine in reasonable yield are relatively inaccessible. In seeking al-

ternative methods for the preparation of substantial quantities of this 9-chloro compound we considered two isolated observations which, when considered together, have led to a general method for controlling the direction of ring closure of *N*-phenylanthranilic acids to provide the 3-substituted acridine derivatives. These observations are the following. (i) Alkylamides of *N*-phenylanthranilic acid (**2**, Scheme I) will cyclize ($POCl_3$) in similar fashion to the corresponding acids but provide the corresponding 9-alkylaminoacridine **3** rather than the 9(10*H*)-acridone.[3-9] (ii) As a result of steric interaction between the 1,8 positions of the acridine ring and the alkyl groups of a 9-(*N*,*N*-dialkylamino)acridine such tertiary amines are unstable; in aqueous solution these readily cleave providing acridone and a dialkylamine.[10]

Scheme I



It was then predicted that on attempted ring closure of a dialkylamide of an *N*-(3-R-phenyl)anthranilic acid **2** (Scheme I), interaction between the 3-substituent and the alkyl groups of the amide function would provide a steric barrier to the formation of the 1-substituted acridine derivative; the often less favored 3 isomer should become the dominant product. The predicted product would be a 9-(*N*,*N*-dialkylamino)acridine but mild acid hydrolysis could be expected to provide the acridone which in turn could be transformed in essentially quantitative yield ($SOCl_2$–DMF)[1] to the desired 9-chloroacridine.

This prediction has been substantiated; ring closure of the piperidide of *N*-(3-methylphenyl)anthranilic acid (**2**, R = CH_3) proceeded smoothly providing the amine **3** (R = CH_3) which on mild acid treatment afforded 3-methylacridone in 86% yield. In the cases we have examined the isomeric 1- and 3-substituted acridones are separable by TLC so that isomer ratios and purity of products can be readily monitored. Conveniently, the reaction sequence (Scheme I) can be performed in a single flask. In contrast, direct ring closure of *N*-(3-methylphenyl)anthranilic acid is claimed to produce a mixture containing 20% of 3- and 80% of the 1-methylacridone.[2] Application of this sequence (Scheme I) to the preparation of the CF_3 analog provided pure 3-trifluoromethylacridone in 87% overall yield from the requisite *N*-phenylanthranilic acid. Direct ring closure of *N*-(3-trifluoromethylphenyl)anthranilic acid provides a mixture containing 35 and 65% of the 3- and 1-substituted acridones, respectively.[2]

In an attempt to reduce the number of synthetic steps necessary, the corresponding piperidide was used in place of 2-chlorobenzoic acid in a Jourdan-Ullmann synthesis; this attempt failed, and unreacted piperidide was recovered.

Because of the excellent orientational control obtained using amides of piperidine, the use of more sterically demanding dialkyl-substituted amides, which should further improve the 3 to 1 isomer ratio, has not been examined. Further examples of the use of this method will be published in due course. The method evolved may prove applicable to the synthesis of other types of fused ring systems when similar orientation problems are encountered.

Reaction of ethyl- and *tert*-butylbenzene with o-nitrobenzaldehyde, under the conditions of the Tănăsescu reaction,[11-14] afforded a simple route to 3-ethyl- and 3-*tert*-butylacridone. However, under the same reaction conditions cumene failed to provide any 3-isopropylacridone. This latter acridone was prepared via *N*-(3-isopropylphenyl)anthranilic acid; ring closure of the piperidine as described above (Scheme I) provided the required 3-substituted acridone exclusively.

Drug Research and Development, Division of Cancer Treatment, National Cancer Institute, selected **12** as NSC141549 (hydrochloride salt) and NSC156303 (methanesulfonate salt) as a candidate for clinical trial. There was thus generated a need for substantial quantities of the intermediary 4-amino-3-methoxymethanesulfonanilide. Early samples of this intermediate were prepared by a tedious and low yielding route which started with the nitration of *m*-acetanisidide and required the separation of the correct nitro isomer. A considerably superior method is to convert the commercially available 2-methoxy-4-nitroaniline to the *N*-*n*-butyranilide which is a suitably protected derivative of convenient lipophilic character for easy manipulation. The following steps of reduction (Fe/H^+), mesylation, and then acidic hydrolysis provide an overall route capable of inexpensively generating large quantities of the required aminomethoxysulfonanilide.

Biological Testing. Full details of the L1210 test procedures have been detailed earlier.[1] As noted then, high reproducibility in these screening tests requires that drugs be examined as soluble formulations. When the hydrochloride salts have been found to be too insoluble the methanesulfonate salts (see Table I) usually provided a higher order of water solubility.

Structure-Activity Relationships (SAR). The change in overall molecular lipophilic-hydrophilic balance produced by an added drug substituent is by itself likely to alter observable biologic activity; steric and electronic contributions of the substituent to biologic activity are then obscured. In earlier work a simple method for divorcing such effects was described.[1] A plot of log (increase in life span L1210) (=log ILS) vs. a measure of lipophilic-hydrophilic balance (log *P*, $\Sigma\pi$, or conveniently ΔR_m values) for members of a homologous series provides a parabolic reference curve.[1] All structure-activity comparisons are then made at isolipophilicity. From the reference curve it can be predicted what log ILS can be expected from a substituted drug variant of particular ΔR_m value. The difference between predicted and observed figures (Δ log ILS) provides a measure of substituent effects on biologic activity divorced from changes in overall molecular lipophilic-hydrophilic balance. Δ log ILS should then provide a measure of steric, electronic, and hydrophobic substituent contributions to drug action. Derived Δ log ILS figures can provide substituent constants as operationally defined by Free and Wilson in their approach to the analysis of structure-activity relationships.[15] However, by compensating for changing lipophilic-hydrophilic balance in the calculation of Δ log ILS, the major detractor from the more widespread use of the Free-Wilson approach is eliminated.

Substituent effects on drug activity have been reported as Δ log ILS in Table I. Our experience with reproducibility in L1210 assays suggests that little weight should be ascribed to Δ log ILS figures of less than ± 0.2.

Table I

No.	Substituents in 1, R = CH_3	Mp, °C	Formula	Analyses	Method	ΔR_m	OD[a]	T/C, %[b]	Log ILS[c]	Pred log ILS[d]	Δ log ILS[e]
1	Parent for comparison[f]						45	207			
4	2′-Cl	308–309	$C_{20}H_{16}ClN_3O_2S\cdot HCl$	C, H, N, Cl	A	+0.23	>500	–[g]		1.80	> –0.40
5	2′-F	302 dec	$C_{20}H_{16}FN_3O_2S\cdot HCl$	C, H, N, Cl	A	+0.07	>500	–		2.07	> –0.67
6	2′-NO_2	308–310	$C_{20}H_{16}N_4O_4S\cdot HCl$	C, H, N, S	C	+0.11	>500	–		2.01	> –0.61
7	2′-NH_2	235 dec	$C_{20}H_{18}N_4O_2\cdot HCl\cdot 1.5H_2O$	C, H, N, Cl		–0.33	50	207	2.03	2.03	0.00
8	2′-OCH_3	231–232	$C_{21}H_{19}N_3O_3S\cdot HCl$	C, H, N, Cl	A	+0.08	>500	–		2.04	> –0.64
		291–292	$C_{21}H_{19}N_3O_3S\cdot MsOH$[h]	C, H, N, S			>500	–			
9	2′-CH_3	310–311	$C_{21}H_{19}N_3O_2S\cdot HCl$	C, H, N, Cl	A	+0.22	150	127	1.43	1.83	–0.40
		299–300	$C_{21}H_{19}N_3O_2S\cdot MsOH$	C, H, N, S			100	–			
10	3′-Cl	320–321	$C_{20}H_{16}ClN_3O_2S\cdot HCl$	C, H, N, Cl	A	+0.24	>500	–		1.76	> –0.36
11	3′-F	317 dec	$C_{20}H_{16}FN_3O_2S\cdot HCl$	C, H, N, Cl	C	+0.08	>500	–		2.04	> –0.64
12	3′-OCH_3	197–199	$C_{21}H_{19}N_3O_3S\cdot HCl$	C, H, N, Cl	A, B	+0.12	12	196 (1)[i]	1.98	2.00	–0.02
		292–293	$C_{21}H_{19}N_3O_3S\cdot MsOH$	C, H, N, S			6.7	214 (2)	2.06		+0.06
13	3′-CH_3	312 dec	$C_{21}H_{19}N_3O_2S\cdot HCl$	C, H, N, Cl	C	+0.25	333	199 (1)	2.00	1.74	+0.26
		283–284	$C_{21}H_{19}N_3O_2S\cdot MsOH$	C, H, N, S			97	206	2.03		+0.29
14	3′-OH	327 dec	$C_{20}H_{17}N_3O_3S\cdot HCl$	C, H, N, S, Cl	B	–0.17	25	188	1.94	2.14	–0.20
15	3′-NO_2	262–263	$C_{20}H_{16}N_4O_4S\cdot HCl$	C, H, N, Cl		+0.03	>500	–		2.08	> –0.68
16	3′-NH_2	259 dec	$C_{20}H_{18}N_4O_2S\cdot HBr\cdot 0.5H_2O$	C, H, N, Br		–0.29	45	206	2.03	2.04	–0.01
17	3′-$NHSO_2CH_3$	275–276	$C_{21}H_{20}N_4O_4S\cdot MsOH$	C, H, N, S	A	–0.17	220	–		2.18	> –0.78
18	1′-$N(CH_3)SO_2CH_3$	269–270	$C_{21}H_{19}N_3O_2S\cdot HCl$	C, H, N, Cl	A	+0.125	120	158	1.77	1.99	–0.22
19	1′-$N(SO_2CH_3)_2$	257–258	$C_{21}H_{19}N_3O_4S\cdot HCl$	C, H, N, Cl	A	–0.03	>500	–		2.15	> –0.75
20	1′-$NHSO_2CH_2Cl$	311 dec	$C_{20}H_{16}ClN_3O_2S\cdot HCl$	C, H, N, Cl	A	+0.27	110	149	1.69	1.71	–0.02
21	2′-N=[j]	298–297	$C_{22}H_{21}N_3O_2S\cdot HCl$	C, H, N, Cl	A	–0.13	110	218	2.07	2.19	–0.12
22	3′-N=	216–218	$C_{19}H_{16}N_4O_2S\cdot HCl$	C, H, N, Cl, S[k]		–0.09	>500	–		2.17	> –0.77
23	R = C_6H_5 for comparison[f]					+0.33	37	197 (2)	1.99	1.53	+0.46
24	R = *p*-$AcNHC_6H_4$	227–229	$C_{27}H_{22}N_4O_3S\cdot HCl\cdot H_2O$	C, H, N, Cl	A	+0.26	120	175	1.88	1.72	+0.16
25	R = *p*-$H_2NC_6H_4$	309–310	$C_{25}H_{20}N_4O_2S\cdot HCl$	C, H, N, Cl	D	+0.12	7	212 (2)	2.05	2.00	+0.05
		297–299	$C_{25}H_{20}N_4O_2S\cdot MsOH$	C, H, N, S			4.5	215 (2)	2.18		+0.18
26	3-Cl, R = *p*-$AcNHC_6H_4$	313–314	$C_{27}H_{21}ClN_4O_3S\cdot HCl$	C, H, N, Cl	A	+0.43	160	167	1.83	<1.40	>0.43
27	3-Cl, R = *p*-$H_2NC_6H_4$	>360	$C_{25}H_{19}ClN_4O_2S\cdot HCl$	C, H, N, Cl	D	+0.36	7	208 (3)	2.03	1.45	+0.58
28	R = *p*-$CH_3SO_2C_6H_4$	327 dec	$C_{26}H_{21}N_3O_4S_2\cdot MsOH$	C, H, N, S	A	–0.02	340	220 (3)	2.08	2.14	+0.06
29	2′-N=*p*-$AcNHC_6H_4$	309 dec	$C_{26}H_{21}N_5O_3S\cdot HCl$	C, H, N, Cl	A	+0.09	180	164	1.81	2.03	–0.22
30	2′-N=*p*-$H_2NC_6H_4$	290 291	$C_{24}H_{19}N_5O_2S\cdot HCl$	C, H, N, Cl	D	–0.02	10	197 (2)	1.99	2.15	0.16
31	3–CF_3	296 297	$C_{21}H_{17}F_3N_3O_2S\cdot MsOH$	C, H, N, S	A	+0.41	>500	–		<1.40	
32	3′-OCH_3, 3-CF_3	166–169	$C_{22}H_{18}F_3N_3O_3S\cdot MsOH$	C, H, N, S	B	+0.53	125	163	1.80	<1.40	>0.40
33	3′-OCH_3, 3-Cl	202–203	$C_{21}H_{18}ClN_3O_3S\cdot HCl\cdot 2H_2O$	C, H, N, Cl	B	+0.37	10	172	1.86	1.41	+0.45
34	3-Cl[f]					+0.24	75	203	2.01	1.75	+0.25
35	3′-OCH_3, 3-CH_3	276–277	$C_{22}H_{21}N_3O_3S\cdot MsOH$	C, H, N, S	B	+0.29	4	188	1.94	1.64	+0.30

36	3-CH_3[f]					+0.16	25	194	1.97	1.92	+0.05
37	3′-OCH_3, 4-CH_3	202–204	$C_{22}H_{21}N_3O_3S$·HCl	C, H, N, Cl	B	+0.17	11	189	1.95	1.91	+0.04
		237–238	$C_{22}H_{21}N_3O_3S$·MsOH	C, H, N, S			6	211	2.05		+0.14
38	4-CH_3[f]					+0.04	33	213	2.06	2.09	−0.03
39	3′-CH_3, 3-CH_3	242–243	$C_{22}H_{21}N_3O_2S$·MsOH	C, H, N, S	C	+0.41	22	211	2.05	<1.40	>0.65
40	3′-CH_3, 4-CH_3	324–326	$C_{22}H_{21}N_3O_2S$·HCl	C, H, N, Cl	C	+0.29	24	191	1.86	1.64	+0.22
		198–199	$C_{22}H_{21}N_3O_2S$·MsOH	C, H, N, S			17	244	2.02		+0.38
41	3′-OCH_3, 3-Br	278–279	$C_{21}H_{18}BrN_3O_3S$·MsOH	C, H, N, Br	B	+0.39	6	219 (1)	2.08	<1.40	>0.68
42	3-I	288 dec	$C_{20}H_{16}IN_3O_2S$·HCl	C, H, N, Cl, I[l]	A	+0.31	100	228 (2)	2.11	1.62	+0.49
43	3′-OCH_3, 3-I	220 dec	$C_{21}H_{18}IN_3O_3S$·HCl	C, H, N, I[m]	B	+0.41	12.5	198	1.99	<1.40	>0.59
44	3′-OCH_3, 3-C_2H_5	188–190	$C_{23}H_{23}N_3O_3S$·HCl·H_2O	C, H, N, Cl	B	+0.45	30	218	2.07	<1.40	>0.67
45	3′-OCH_3, 3-$CH(CH_3)_2$	193 dec	$C_{24}H_{25}N_3O_3S$·HCl·$2H_2O$	C, H, N, Cl	B	+0.53	250	146	1.66	<1.40	>0.26
46	3′-OCH_3, 3-$C(CH_3)_3$	210–212	$C_{25}H_{27}N_3O_3S$·HCl	C, H, N, Cl	B	+0.61	>500	–			

[a] OD = optimum dose in mg/kg/day. [b] T/C (%) = ratio of the mean life span of leukemic animals, treated with drug at the optimum dose, to that of controls expressed as a percentage. [c] Log ILS = log (increase in life span) = log [T/C (%) − 100]. [d] Predicted from ΔR_m values of the variant and the reference curve of ΔR_m vs. log ILS obtained for members of the homologous series I, R = $(CH_2)_nCH_3$; see ref 1. [e] Δ log ILS = observed log ILS − predicted log ILS. [f] Full details provided in ref 1. [g] − signifies that T/C values were not statistically significant (<125%) at the doses employed. [h] MsOH = methanesulfonic acid. [i] Figures in parentheses are the number of animals from a group of six that survived greater than 50 days after completion of drug dosing. [j] Pyridine azalog. [k] S: calcd, 8.2; found, 7.6. [l] I: calcd, 24.1; found, 23.4, 25.2. [m] I: calcd, 22.8; found, 21.9.

Of the 2′-substituents examined (**4–9**, Table I) only amine and methyl functions (**7, 9**) provided active congeners. In the 3′ position both the electron-donor substituents OCH_3 (**12**) and CH_3 (**13**) provided active compounds. There was marked increase (6.7-fold) in potency with the 3′-OCH_3 compound **12** in comparison to the unsubstituted parent **1**.

Earlier we demonstrated that it was essential to have electron-donor functions attached to the 9-anilino ring to obtain active agents.[16] The present work reinforces this point; all the sulfonanilide ring substituents providing active variants (Table I) are electron donor in nature. The results can be further rationalized by postulating that the 6′ position of the sulfonanilide ring contacts an electron-deficient site component. The 2′-substituents with negative σ_m values (NH_2, −0.16; CH_3, −0.07),[17] thus capable of increasing electron density at the 6′ position, are those providing active congeners. The further electron-donor substituent (OCH_3) has a positive σ_m value (0.12)[17] and when located at the 2′ position affords an inactive agent (**8**). Similarly, the 3′-OCH_3 and 3′-CH_3 groups, with negative σ_p values (−0.27 and −0.17, respectively),[17] can both increase electron density at the 6′ position and the resulting agents (**12** and **13**) show excellent activity. Logically, 3′ functions with even greater negative σ_p values (e.g., OH, NH_2; −0.37, −0.66)[17] should then provide more active agents. While the 3′-OH and 3′-NH_2 congeners (**14, 16**) are active as expected, their activity is well predicted from ΔR_m alone; there is no significant activity enhancement. It was noticeable when manipulating these compounds (**14, 16**) in the laboratory that, as substituted o-aminophenol and o-phenylenediamine derivatives, these were subject to ready autoxidation. If there was a similar in vivo susceptibility to oxidative degradation, the activity observed with these variants might be lower for this reason. If oxidative destruction of drug was facilitated by increasing electron density, then an optimum $\Sigma\sigma$ value (6′ position) would be expected for maximum antileukemic activity; lessened activity would result from either higher or lower $\Sigma\sigma$ values. While most current SAR correlation approaches consider linear relationships in σ, the circumstances described would provide a nonlinear dependence on σ.

The azalog **21** is of interest since pyridine is considered an electron-deficient heterocycle but is known to have a high electron density on the ring N atom.[18] A pyridine ring N atom might then act as an acceptable substitute for the suggested necessary 6′-electron-rich region of the sulfonanilides. Conversion to the pyridine isostere will also increase the pK_a of the sulfonamide system; in order to claim that the reasonable activity of the pyridine variant **21** supports the above views, the importance of sulfonamide pK must first be clarified. The activity of the more acidic[19] α-chloromethanesulfonanilide **20** was well predicted from ΔR_m alone. While flexible control of sulfonamide pK can be obtained by substituent variation in the corresponding benzenesulfonanilides (cf. **23–30**) the additional aromatic ring increases lipophilic character to the extent that only variants bearing hydrophilic substituents can be profitably examined. Sulfonamide pK values of 9.1 and 7.5 can be predicted[19] for congeners **25** and **28** but the activities of these are well predicted from their ΔR_m values (Table I). Similarly, the activities of the substituted p-aminobenzenesulfonanilide congeners **25, 27,** and **30** and their acetyl derivatives **24, 26,** and **29** could be predicted from their ΔR_m values and the Δ log ILS figures observed with the corresponding substituted methanesulfonanilides **34, 1,** and **21**. Anomalously the parent benzenesulfonanilide **23** appears to have greater activity than expected from its ΔR_m value; this has been confirmed on repeated testing. While no

clear-cut result on the importance of sulfonamide pK emerged from this brief study the N-methylsulfonanilide **18**, in which the sulfonamide function cannot ionize, is convincingly active. Since ionization of the sulfonamide function is not a prerequisite for antileukemic activity, we suggest that the activity of the pyridine derivate **21** lends support to our hypothesis that high electron density at or close to the 6'-sulfonanilide position is necessary for antileukemic activity. In confirmation the isomeric 3'-pyridine derivative **22**, which should have lowered electron density at the 6' position[18] in relation to the parent **1**, is inactive.

The potency enhancing properties of a 3'-OCH_3 function appeared to be general and could be observed when there were a variety of acceptable acridine substituents present (**33, 35, 37, 41,** and **43**). It was earlier suggested that hydrophobic acridine 3-substituents were protherapeutic and that examination of the 3-CF_3 analog might prove profitable.[1] The CF_3 analog was prepared (**31**) and provided no anti-L1210 activity or signs of toxicity at dosing levels up to the practical limit of 500 mg/kg. Further addition of a 3'-OCH_3 function provided a compensatory increase in potency and effective dose levels of the 3-CF_3, 3'-OCH_3 analog **32** could be reached. Note that the Δ log ILS figure for this variant is positive and provides support for our earlier expressed views.[1]

Further hydrophobic 3-substituents (Et, **44**; i-Pr, **45**; t-Bu, **46**; I, **43**) have been investigated. Details of Δ log ILS values and optimum doses for analogs bearing both a 3'-OCH_3 group and a hydrophobic 3-acridine substituent, together with pertinent extrathermodynamic substituent parameters, are collected in Table II. Most hydrophobic 3-substituents are associated with positive Δ log ILS figures. It might be suspected that the marked decrease in potency associated with a 3-CF_3 function (**32**) was due to a weakening of acridine base strength. However, σ_p differences between the 3-CH_3 (**36**) and the three halogen analogs (Cl, Br, I; **33, 41, 43**) range from 0.35 to 0.40, i.e., approaching σ_p for a CF_3 function, but there is very little difference in the optimum doses (10–17 mg/kg) of these four compounds. (While σ_p is provided as an example, consideration of σ_m or σ^+ values singly leads to the same conclusion.) Additionally, the more powerfully base weakening 3-NO_2 substituent (σ_p = 0.78) has provided analogs of high potency and activity.[1] If explanation of the observed differences in optimum doses on electronic grounds is discounted, then the potency difference between the 3-Br and 3-CF_3 analogs, the substituents of these having equivalent π values (Table II), presumably arises from steric factors. The larger CF_3 function (E_s = −1.16) provides a much less potent molecule than does Br (E_s = 0.08). Of the two other examples with bulky 3-substituents **45** (3-i-Pr) has low potency and **46** (3-t-Bu) is inactive. It appears that for good activity with high potency an acridine 3-substituent in this series should be hydrophobic and smaller than an i-Pr group (i.e., E_s > −0.47) although it may quite acceptably be as large as an iodine atom (E_s = −0.16).

Discussion

We have suggested that the SAR for the acridine ring substituted variants prepared earlier are compatible with drug lodgement in a DNA twin-helix intercalation site.[1] Purcell and coworkers have analyzed the SAR for certain 7-substituted antimalarial quinolines.[20] These antimalarial agents also intercalate into DNA stacks[21,22] and have a 7-substituent located in the same relative position to the ring N atom as is a 3-substituent in an acridine. By sequential regression analysis Purcell et al.[20] showed that a significant amount of the variance in the antimalarial screening data could be explained by equations employing terms in the point charge on the 7-substituent. However, this was only true if substituents larger than iodine were excluded from consideration. It was suggested that such findings reflected the stereochemical consequences of an intercalation step over large functions impeding drug entry into site. The test data for the 3-substituted acridines (Table II) show the same feature; functions more sterically demanding than an iodine atom provide considerably less potent agents.

Table II. Variants of 1 (R = CH_3) Bearing a 3'-OCH_3 Function and a 3-Hydrophobic Substituent

No.	3-Substituent	Substituent values[c] π	σ_p	E_s	OD[a]	Δ log ILS[b]
33	Cl	0.71	0.23	0.27	10	+0.45
41	Br	0.86	0.23	0.08	6	>+0.68
43	I	1.12	0.18	−0.16	12.5	>+0.59
32	CF_3	0.88	0.54	−1.16	125	>+0.40
36	CH_3	0.56	−0.17	0.00	17	+0.10
44	Et	1.02	−0.15	−0.07	30	>+0.67
45	i-Pr	1.53	−0.15	−0.47	250	>+0.26
46	t-Bu	1.98	−0.20	−1.54	>500	Inactive

[a] OD = optimum dose in mg/kg/day. [b] From Table I. [c] See ref 17.

The desirable size for a 3-substituent can be even more closely defined. The optimum doses for earlier described[1] 3-halogen substituted variants of **1** together with that of the 3-I example (Table I) are 3-F, 200 mg/kg; 3-Cl, 75 mg/kg; 3-Br, 35 mg/kg; 3-I, 100 mg/kg; i.e., the effect of these halogen substituents on potency is in the order F < Cl < Br > I. The 3'-OCH_3 congeners bearing a 3-Cl (**33**), 3-Br (**41**), and 3-I (**43**) also provide the potency order Cl < Br > I although the differences between these examples are not so marked. In reasonable agreement with our intercalation hypothesis the van der Waal's radius of a Br substituent (1.95 Å) is not dispartate with the half thickness of a planar aromatic ring system (1.85 Å).[23]

A reasonable explanation for observing maximum potency with the 3-Br derivatives would be that increasing the size of a halogen substituent provides additional hydrophobic site binding as suggested earlier.[1] Concomitantly, as the diameter of the halogen substituent exceeds the depth of the acridine ring system, entry into an intercalation site becomes increasingly difficult. The best compromise between these opposing factors is apparently reached with a 3-Br substituent.

Experimental Section

Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values. Analyses were performed by Dr. A. D. Campbell, Microchemical Laboratory, University of Otago, Dunedin, New Zealand. Melting points were determined on an Electrothermal melting point apparatus with the makers' supplied stem corrected thermometer; melting points are as read. NMR spectra were obtained on a Varian A-60 spectrometer (Me_4Si). Ir spectra (KBr) were recorded using a Beckmann 237 Infracord. Uv spectra were recorded on a Shimadzu UV-200.

To monitor the progress of reactions, purification of products, etc., TLC on SiO_2 (Merck SiO_2, F_{254}) was used. The partition chromatographic methods used in measuring ΔR_m values have been described earlier.[1]

Method A. To the requisite 4-nitroaniline in pyridine solution was slowly added a sulfonyl chloride so that the temperature of the solution remained below −5°. Products were purified as before;[1] yields of sulfonanilides ranged from 48 to 92%. Reduction (Fe/H^+)[23] of these nitro compounds provided the required aminosulfonanilides which were coupled with the requisite 9-chloroacridine by the methods earlier described in full.[1]

Method B. The 4-nitroaniline components were converted to the 4-nitroacetanilides by literature described methods and these

Table III. Methanesulfonanilides

Substituents	Mp, °C	Formula	Analyses
2-Cl, 4-NO_2	158.5–159	$C_7H_7ClN_2O_4S$	C, H, N, S
2-Cl, 4-NH_2	178–179	$C_7H_9ClN_2O_2S$	C, H, N, Cl
2-F, 4-NO_2	185–186	$C_7H_7FN_2O_4S$	C, H, N, S
2-F, 4-NH_2	177–178	$C_7H_9FN_2O_2S$	C, H, N, S
2-NO_2, 4-NH_2	184–185	$C_9H_{11}N_3O_5S$	C, H, N, S
2-OCH_3, 4-NO_2	133–134	$C_8H_{10}N_2O_5S$	C, H, N, S
2-OCH_3, 4-NH_2	193–194	$C_8N_{12}N_2O_3S$	C, H, N, S
2-CH_3, 4-NO_2	194–195	$C_8H_{10}N_2O_4S$	C, H, N, S
2-CH_3, 4-NH_2	142–143	$C_8H_{12}N_2O_2S$	C, H, N, S
3-Cl, 4-NO_2	177–178	$C_7H_7ClN_2O_4S$	C, H, N, Cl
3-Cl, 4-NH_2	129–130	$C_7H_9ClN_2O_2S$	C, H, N, Cl
3-F, 4-NHAc	185–186	$C_9H_{11}FN_2O_3S$	C, H, N, S
3-OCH_3, 4-NO_2	160–161	$C_8H_{10}N_2O_5S$	C, H, N, S
3-OCH_3, 4-NH_2	144–145	$C_8H_{12}N_2O_3S$	C, H, N, S
3-CH_3, 4-NHAc	155–156	$C_{10}H_{14}N_2O_3S$	C, H, N, S
3-$NHSO_2CH_3$, 4-NO_2	206–207	$C_8H_{11}N_6O_2S$	C, H, N, S
4-NO_2, α-Cl	159–160	$C_7H_7ClN_2O_4S$	C, H, N, Cl
4-NH_2, α-Cl	136–137	$C_7H_9ClN_2O_2S$	C, H, N, Cl
3-OH, 4-NH_2·HCl	229 dec	$C_7H_{11}ClN_2O_3S$	C, H, N, Cl, S

reduced (Fe/H^+)[23] to the 4-acetamidoanilines. Further reaction with a sulfonyl chloride as in method A produced the *p*-acetamidosulfonanilides (Table III). Removal of the masking acetyl function was by heating under reflux with 2 *N* HCl–EtOH until TLC showed reaction was complete; usually 1 hr proved sufficient. After removal of solvent and excess HCl in vacuo free amine product was precipitated by trituration of the residual hydrochloride with saturated aqueous NaOAc. Products crystallized from H_2O or EtOH–H_2O. Because of the air sensitivity of 3-hydroxy-4-aminomethanesulfonanilide it was necessary to crystallize (absolute EtOH) and store this intermediate as the hydrochloride salt (Table III). Alternatively (method C), when TLC showed that the residual hydrochloride obtained, on concentration following HCl hydrolysis, was homogeneous, then adhering traces of acid were removed by drying in vacuo over KOH pellets. Solutions of this crude hydrochloride (65% EtOH–H_2O) and a 9-chloroacridine were then mixed and coupling was completed as described earlier.[1]

An earlier claim[24] to have prepared α-chloro-4-nitromethanesulfonanilide in 16% yield quotes this to be a yellow crystalline product of mp 143–146°; details of elementary analyses of this product are not available to us. All 4-nitromethanesulfonanilides we have prepared are uniformly very pale in color and from the higher melting point we have recorded, mp 159–160°, in conjunction with necessary analytical and spectral data for our product (Table III) leads us to suggest that the earlier worker may have recovered small amounts of 4-nitroaniline (mp 146–147°) from his reaction.

Many of the required 4-aminoacetanilides have been recorded in the literature but no details could be found for **4-amino-2-fluoroacetanilide**, mp 142–143° [Anal. ($C_8H_9FN_2O$) C, H, N], and **2-acetoxy-4-aminoacetanilide**, mp 223–225° [Anal. ($C_{10}H_{12}N_2O_3$) C, H, N].

4'-(Acridin-9-ylamino)-3'-nitroacetanilide. A solution of 4-amino-3-nitroacetanilide (0.026 mol) in *N*-methylpyrrolidine (20 ml) and C_6H_6 was heated to reflux under a water entrainment head to remove traces of water. To the dried solution were added 9-chloroacridine (0.026 mol) and, when this had dissolved, methanesulfonic acid (0.02 ml) following which benzene was distilled until the internal temperature reached 155°. After 20 min at this temperature the solution was cooled and C_6H_6 (200 ml) added. The precipitated solid was collected, washed with C_6H_6 and Me_2CO, dried, and dissolved in hot 80% EtOH–H_2O (190 ml). Addition of 1 equiv of $KHCO_3$, then cooling, afforded the crystalline base as orange-red prisms (8.24 g, 86%). Further crystallization was from DMF–EtOH–H_2O or large volumes of EtOH: mp 300–301°. Anal. ($C_{21}H_{16}N_4O_3$) C, H, N.

4'-(Acridin-9-ylamino)-3'-nitroaniline. A solution of the corresponding acetanilide (0.023 mol) in EtOH (220 ml) and concentrated HCl (55 ml) was boiled under reflux conditions for exactly 75 min. The clear solution was cooled and NH_4OH added to neutralize the bulk of the HCl; then saturated aqueous NaOAc was stirred in until a pH of 6 resulted. After thorough cooling the monohydrochloride salt was collected and crystallized twice from EtOH–H_2O–NH_4Cl,[1] pure product being obtained as maroon needles: mp 284° dec (79% yield). Anal. ($C_{19}H_{15}ClN_4O_2$) C, H, N, Cl.

4'-(Acridin-9-ylamino)-3'-nitromethanesulfonanilide (15). A solution of 4'-(acridin-9-ylamino)-3'-nitroaniline (0.012 ml) in hot Py (50 ml) was cooled to −5° and mesyl chloride (0.0126 mol) was added dropwise so that this temperature was maintained. After overnight refrigeration the solution was heated on the steam bath for 30 min and as much Py as possible removed in vacuo. Shaking the residue with water (75 ml) provided an amorphous solid which was collected and then stirred into 0.05 *N* NaOH (200 ml) at room temperature. The resulting solution was clarified and product precipitated from the filtrate by acidifying with HCl. The hydrochloride salt was recrystallized by solution in 50% EtOH–H_2O containing concentrated HCl (0.1 ml), clarifying, and adding hot saturated aqueous NaCl to the boiling solution until crystallization commenced. Slow cooling provided pure product as orange prisms; see **15**, Table I.

2-(Acridin-9-ylamino)-5-nitropyridine. 9-Chloroacridine (0.01 mol) and 2-amino-5-nitropyridine (0.012 mol) were dissolved in anhydrous *N*-methylpyrrolidine (3 ml) and the mixture was heated on the steam bath until a clear solution resulted. A catalytic amount of methanesulfonic acid (0.2 ml of a 10% solution in *N*-methylpyrrolidone) was added and heating continued for 1 hr. Crude product was precipitated by adding petroleum ether (50 ml) and then C_6H_6 portionwise until the two solvent layers became miscible. The precipitated hydrochloride was dissolved by shaking with CH_2Cl_2 (1500 ml) and excess NH_4OH. The dried (Na_2SO_4) CH_2Cl_2 extracts were concentrated until product started to crystallize. A further crystallization from CH_2Cl_2 provided pure product: mp 259–261° (57% yield). Anal. ($C_{18}H_{12}N_4O_2$) C, H, N.

2-(Acridin-9-ylamino)-5-aminopyridine. The above nitropyridine derivative was reduced with Fe/H^+ in the usual manner.[23] Product monohydrochloride was purified by several crystallizations from EtOH–H_2O–NH_4Cl. Pure product (67% yield) had mp 253–254°. Anal. ($C_{18}H_{15}ClN_4$) C, H, N, Cl.

2-(Acridin-9-ylamino)-5-methanesulfonamidopyridine (22). To a suspension of the preceding monohydrochloride (0.02 mol) in Py (35 ml) triethylamine (0.022 mol) was added and the whole mixture was warmed and stirred until the salt dissolved. Methanesulfonyl chloride (0.021 mol) was then stirred in so that the temperature remained below −5°. After 24 hr at this temperature the suspension was heated on the steam bath for 30 min and then concentrated in vacuo as far as possible. The gummy residue was suspended in water and stirred while HOAc was added until a pH of 6 was reached; saturation with solid NaCl in the hot solution then precipitated crude product monohydrochloride. The collected salt was stirred with 0.1 *N* NaOH (50 ml), the bulk dissolving. Clarification, acidification (HOAc), and then saturation with NaCl returned product. Crystallization from EtOH–H_2O–NH_4Cl provided pure compound as red needles; see **22**, Table I.

2-(3-Methylanilino)benzpiperidide (2, R = CH_3). To a stirred suspension of *N*-(3-methylphenyl)anthranilic acid (0.132 mol) in C_6H_6 (80 ml) Py (0.0137 mol) was added, a clear solution resulting. To this solution was added at 5° freshly distilled $SOCl_2$ (0.0157 mol) and 30 min later piperidine (0.0471 mol) was added in one portion. The reaction mixture was allowed to stand at room temperature for 6 hr; then the benzene solution was washed successively with H_2O, 2 *N* Na_2CO_3, 2 *N* HCl, and H_2O. The dried (Na_2SO_4) solution was evaporated, the residue dissolved in petroleum ether, and the solution clarified by filtration through a charcoal–Celite pad and concentrated to 22 ml. After addition of seed crystals the solution was refrigerated for 24 hr. Pure product was obtained as colorless prisms (4.55 g, 87% yield) of mp 79.5–80°. Anal. ($C_{19}H_{22}N_2O$) C, H, N.

In essentially similar fashion were prepared **2-chlorobenzpiperidide**, colorless needles from petroleum ether (87% yield), mp 50–51° [Anal. ($C_{12}H_{14}ClNO$) C, H, N, Cl], and **2-(3-trifluoromethylanilino)benzpiperidide**, colorless plates from C_6H_6–petroleum ether (91% yield), mp 115.5–116° [Anal. ($C_{19}H_{19}F_3N_2O$) C, H, N].

1-(3-Methylacridin-9-yl)piperidine (3, R = CH_3). To a solution of the corresponding piperidide (0.017 mol) in C_6H_6 (40 ml) was added $POCl_3$ (10 ml) and this mixture was heated on a steam bath for 2 hr and then evaporated in vacuo. Repeated crystallizations of the residue from EtOH–5% aqueous NH_4Br (avoiding temperatures above 60°) provided product hydrobromide as orange needles, mp 279° dec. Anal. ($C_{19}H_{21}BrN_2$) C, H, N, Br. This salt in

HOAc–concentrated HCl (4:1) mixtures at steam bath temperature was shown by TLC to be progressively cleaved to 3-methylacridone, reaction being complete within 45 min. A further ring-closure reaction was carried out using the same quantities of reactants, the residue obtained following removal of C_6H_6 and excess $POCl_3$ was dissolved in HOAc (80 ml) plus concentrated HCl (20 ml), and the solution heated on the steam bath for 45 min and then evaporated in vacuo. Crude methylacridone precipitated on shaking with dilute NH_4OH and a single crystallization from EtOH gave 3-methylacridone (3.05 g, 86% yield) of mp 337–338° which was not depressed on admixture with authentic material.[1] On TLC (Merck SiO_2, F_{254}; $CHCl_3$ solvent) the product could not be distinguished from authentic 3-methylacridone (R_f 0.15) obtained by $POCl_3$ ring closure of *N*-(3-methylphenyl)anthranilic acid and separation of the two isomers produced.[25] Pure 1-methylacridone was easily separable under these TLC conditions (R_f 0.22).

3-Trifluoromethylacridone was prepared by an essentially similar procedure. A single crystallization of the reaction product (DMF–H_2O) provided pure acridone in 96% yield: mp >360° (lit.[2] mp >360°). TLC (SiO_2, $CHCl_3$) showed this material to be homogeneous and not separable from authentic 3-trifluoromethylacridone (R_f 0.16); no 1-trifluoromethylacridone (R_f 0.12) could be detected.

4-Methylthiobenzenesulfon-*p*-nitroanilide was prepared from the known 4-methylthiobenzenesulfonylchloride[16] and *p*-nitroaniline in Py solution and had mp 146–147°. Anal. ($C_{13}H_{12}N_2O_4S_2$) C, H, N, S.

4-Methylsulfonylbenzenesulfon-*p*-nitroanilide. $KMnO_4$ (12.4 mmol) was stirred into a solution of 4-methylthiobenzenesulfon-*p*-nitroanilide (7.55 mmol) in water (25 ml) containing KOH (0.45 g). The solution was heated on a steam bath for 30 min and boiled for 30 min; then MnO_2 was removed by filtration through a Celite pad. The hot filtrate was diluted with EtOH (40 ml); acidification with concentrated HCl then precipitated crystalline sulfone. Crystallization from DMF–H_2O provided product as colorless prisms (2.53 g, 94% yield), mp 283° dec. Anal. ($C_{13}H_{12}N_2O_6S_2$) C, H, N, S.

Reduction of this compound with Fe/H^+ provided **4-(4-methylsulfonylbenzenesulfonamido)aniline** of mp 134–135°. Anal. ($C_{13}H_{14}N_2O_4S_2$) C, H, N, S.

***N*-(3-Isopropylphenyl)anthranilic Acid.** A heterogenous mixture of 3-isopropylaniline (0.095 mol), o-chlorobenzoic acid (0.1 mol), K_2CO_3 (0.12 mol), Cu powder (0.2 g), Cu_2O (0.2 g), and 2-ethoxyethanol (10 ml) was heated under reflux conditions in an oil bath for 2 hr. The cooled reaction mixture was removed from the flask by boiling with water (200 ml) until only traces of Cu oxides remained undissolved; decolorizing charcoal was then added and the solution clarified through a Celite pad. Acidification of the filtrate (HCl) precipitated crude product as an oil that slowly crystallized over several hours. The collected crystals were dissolved in Me_2CO (100 ml) by warming, the solution was cooled, and product precipitated in crystalline form by the slow addition of H_2O (400 ml). A liberal quantity of decolorizing charcoal was stirred into the solution obtained by dissolving this material in 5% Na_2CO_3 (200 ml); after 10 min of stirring the solution was filtered through a Celite pad and compound recovered by acidification. Several crystallizations from ligroine (bp 100–120°), conveniently using a Soxhlet extractor, provided the pure acid as pale yellow needles (62% yield): mp 117–118°. Anal. ($C_{16}H_{17}NO_2$) C, H, N.

3-Isopropylacridone. To an ice-cooled solution of the preceding phenylanthranilic acid (0.031 mol) in benzene (20 ml) containing Py (0.032 mol) was added in one portion $SOCl_2$ (0.043 mol). The mixture was stirred for 10 min and then evaporated as far as possible in vacuo at room temperature. To the residue was added piperidine (0.1 mol) and triethylamine (0.1 mol) and after a further 10 min C_6H_6 (50 ml) and H_2O (50 ml). The separated benzene layer was washed successively with 2 *N* HCl, 10% $KHCO_3$, and H_2O, then dried (Na_2SO_4), and evaporated. $POCl_3$ (15 ml) was added to the residue of homogeneous (TLC) but noncrystalline piperidide and the mixture heated on a steam bath for 1 hr. After removal of excess $POCl_3$ in vacuo the syrupy mixture was cooled, HOAc (80 ml) and concentrated HCl (20 ml) were added, and the whole mixture was heated at 100° for 1 hr. After removal of solvents in vacuo product was extracted with $CHCl_3$, the extracts being washed with 2 *N* HCl, 2 *N* Na_2CO_3, and H_2O and dried (Na_2SO_4) before evaporation. The thick residue on boiling with C_6H_6 (30 ml) crystallized. The crystals were dissolved in $CHCl_3$ and the solution was filtered through a short column of SiO_2 to remove a polar impurity. Multiple crystallizations from $CHCl_3$–toluene and then EtOH–H_2O provided pure acridone as yellow needles (3.1 g, 42% yield): mp 213–214°. Anal. ($C_{16}H_{15}NO$) C, H, N.

3-Ethylacridone. To a solution of o-nitrobenzaldehyde (0.05 mol) in ethylbenzene (0.1 mol) concentrated H_2SO_4 (5.3 ml) was slowly added while stirring vigorously. After 4 hr $NaNO_2$ (0.15 g) was stirred into the solution and further H_2SO_4 (10 ml) added. After 24 hr of stirring at room temperature, H_2O (200 ml) was added and excess ethylbenzene removed by steam distillation. The H_2O-insoluble residue was triturated with aqueous NH_4OH and extracted with boiling MeOH (600 ml), and the extracts were distilled until crystallization commenced. The collected crystals were washed with C_6H_6 and dried (3.18 g, 28% based on o-nitrobenzaldehyde taken). TLC show this acridone to be contaminated with traces of several other components which can be removed by conversion to the known[26] 3-ethyl-9-chloroacridine with $SOCl_2$–DMF[1] and crystallization from small volumes of petroleum ether: pale yellow needles; mp 52–52.5° (lit.[26] mp 51–52°).

3-*tert*-Butylacridone. To a stirred solution of o-nitrobenzaldehyde (0.075 mol) in *tert*-butylbenzene (0.17 mol) anhydrous *p*-toluenesulfonic acid (0.17 mol) was added. After 30 min of stirring $NaNO_2$ (0.2g) was added and the mixture stirred for 2 days at room temperature. Following addition of water (250 ml) excess *tert*-butylbenzene was removed by steam distillation. The black water-insoluble residue was triturated with NH_4OH until solid. This solid was dissolved in boiling MeOH (200 ml) and a liberal quantity of decolorizing charcoal was stirred into the solution. After clarification (Celite) an equal volume of H_2O precipitated crude acridone. This material can be shown by TLC to be contaminated with several impurities and it is difficult to obtain pure acridone by direct crystallization. Conversion to the corresponding 9-chloroacridine ($SOCl_2$–DMF)[1] provided a product which could be freed of impurities by crystallization from petroleum ether. Mild acid hydrolysis then returned 3-*tert*-butylacridone which was homogeneous to TLC criteria. Crystallization from 70% MeOH–H_2O provided pale yellow needles, mp 292–293° (2.26 g, 12% yield based on o-nitrobenzaldehyde). Anal. ($C_{17}H_{17}NO$) C, H, N.

Biological Testing. 10^5 L1210 cells were inoculated intraperitoneally into 18.5–22.5 g C_3H/DBA_2 F_1 hybrids on day 1; drug treatment was initiated 24 hr later and continued for 5 days. All drug dosage was by the intraperitoneal route and an animal dose was suspended or dissolved in 0.2 ml of water. Groups of six animals per dose level were used and there was one control group for every six tests. Drug doses were separated by 0.18 log dose intervals and ranged from the clearly toxic to the inactive.

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Antiallergic Activity of 2-Phenyl-8-azapurin-6-ones

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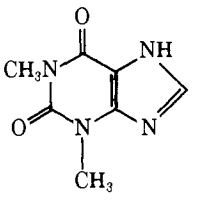
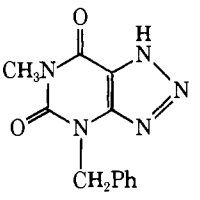
The synthesis and antiallergic activity in the rat passive cutaneous anaphylactic reaction of a series of 2-phenyl-8-azapurin-6-ones are described. Early in the investigation, a linear free-energy equation was established in which the activity was related to the size and hydrogen bonding capacity of the ortho substituent in the phenyl ring. This relationship was used to provide guidance and limits for subsequent work leading to 2-o-propoxyphenyl-8-azapurin-6-one which is 40 times more potent than disodium cromoglycate. It is suggested that good antiallergic activity in this series is associated with coplanarity of the phenyl group with the azapurin-6-one which would be favored by a high degree of hydrogen bonding.

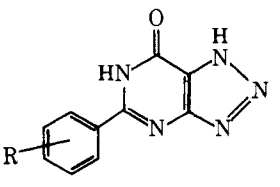
Methylxanthines, such as theophylline (I) and caffeine, inhibit the antigen-induced release of histamine and of a slow-reacting substance of anaphylaxis (SRS-A) from passively sensitized human lung and human basophilic leucocytes.[1,2] In this context the methylxanthines are much less potent than disodium cromoglycate and we have been engaged in progressive modification of the xanthine molecule in the hope of increasing potency and reducing side effects. We found that the introduction of a nitrogen atom into the 8 position, to give 8-azaxanthines, increased potency, particularly if the compounds contained a bulky substituent in the 3 position, e.g., II.[3] Further modifications have now been carried out and in the present paper we describe the synthesis and structure-activity relationships of a series of 2-phenyl-8-azapurin-6-ones (III).



I



II



III

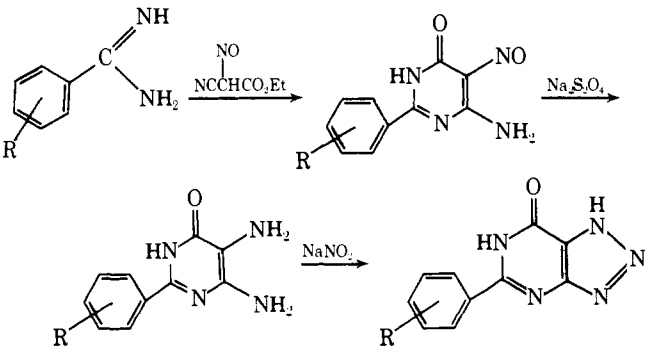
Chemistry. Most of the azapurinones were prepared from the appropriate benzamidine by the route indicated in Scheme I and are listed in Table I.

The hydroxy compound **6** was prepared by hydrogenolysis of the benzyloxy compound **19** and the amino derivative **24** by hydrolysis of the sulfonamide **29**.

The required amidines and their precursors were synthesized by standard procedures as indicated in the Experimental Section.

Many of the syntheses were carried out without purification of the intermediate, and only those compounds which have been characterized are included in the Experimental Section.

Scheme I



Biology. In screening this series, the determination of ID_{50}'s in the rat passive cutaneous anaphylactic (PCA) reaction was impracticable because the variability of response would have necessitated the use of large numbers of animals to achieve meaningful results. However, direct comparison with disodium cromoglycate (DSG) for their ability just to cause 100% inhibition of the rat PCA reaction following iv administration was satisfactory and reproducible to within ±25%.[3] The relative activities are given in Table I.

Discussion

When it was realized that the 2-aryl-8-azapurin-6-ones had interesting antiallergic properties, an extensive program of synthesis and biological screening was undertaken. We recognized that prompt application of extrathermodynamic correlation techniques,[4] if successful, could provide guidance to the direction of synthetic effort and reduce the time necessary to arrive at the most active members of the series.

Accordingly, when results were available on the first ten compounds (**1**, **2**, **4**, **7**, **14**, **19**, **30**, **31**, **38**, and **40**), correlations with partition (π), electronic (F, R), and steric parameters (MR, E_s) were sought but no significant relationships were revealed. Our work on the effect of substituents in the 8-azaxanthine system[3] led us to consider that bulky sub-