equal to the product of the diameter of the dye site in millimeters and a grade of 0.5, 1, 2, 3, or 4, which was proportional to intensity of dye coloration, were assigned each injection site. The scores of a given injection site were summed for each group (n) of five or seven animals and compared to the control. The difference was expressed as percent inhibition. For compounds with an iv ED_{50} greater than 0.01 mg/kg, n was usually small and the ED_{50} was estimated from a dose–response curve fitted by eye. For compounds of greater potency, n was larger and the ED_{50} was calculated from the least-squares regression line. ED_{50} values differing by a factor of 3 or more are significantly different. The method is easily reproducible in other laboratories, as judged by the ED_{50} value of ~1 mg/kg reported by many for DSCG; we observed an ED_{50} value of 0.8, $n = 25$.

Rat Plasma Histamine Procedure. Hyperimmune antisera to chicken egg albumin (crystallized five times, Pentex) were prepared according to Orange, Valentine, and Austen[18] and 0.5 mL was injected iv into normal male Charles River CD rats 20 h before challenge. Control animals were injected with normal rat sera 20 h before challenge or were injected with hyperimmune sera but given saline in place of antigen challenge. Animals were deprived of food, but not water, after being passively sensitized. Animals were anesthetized 20 h later with diabutal, 40 mg/kg ip. Drug or saline was injected into the inferior vena cava 1 min prior to challenge with 5 mg of egg albumin. Five minutes after challenge, 3 mL of blood was withdrawn from the inferior vena cava into a syringe containing 0.3 mL of 3% sodium citrate. The histamine concentration of the plasma was determined by the method of Shore, Burkhalter, and Cohn.[19]

Guinea Pig Histamine Aerosol Procedure. Bronchodilator activity was evaluated according to the method of Van Arman, Miller, and O'Malley[20] in conscious female Reed–Willet guinea pigs (200–250 g) fasted overnight. One minute following iv administration of saline or the test drug in saline, each animal was challenged with histamine aerosol as follows: a 0.4% aqueous solution of histamine was placed in a Vaponephrine standard nebulizer (Vaponephrine Co., Edison, N.J.) and sprayed under an air pressure of 6 psi into a closed 8 × 8 × 12 in. transparent plastic container for 1 min. Immediately thereafter, the guinea pig was placed in the container. The respiratory status of the guinea pig after 1 min in the container was scored as follows: 0, normal breathing; 1, slightly deepened breathing; 2, labored breathing; 3, severely labored breathing and ataxia; 4, unconsciousness. The scores for a control group and a test group (eight animals per group) were summed and compared, and the difference was expressed as percent protection.

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Potential Antitumor Agents. 33. Quantitative Structure–Activity Relationships for Mutagenic Activity and Antitumor Activity of Substituted 4′-(9-Acridinylamino)methanesulfonanilide Derivatives

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A series of substituted 4′-(9-acridinylamino)methanesulfonanilide (AMSA) derivatives have been tested for mutagenicity using *Salmonella typhimurium* strain TA 1537 and for antitumor activity against the L1210 leukemia in mice. Two measures of mutagenic activity were determined and quantitative structure–activity relationships (QSAR) developed for them. M_{50}, the percentage of drug-induced mutant colonies observed at the concentration providing 50% inhibition of bacterial growth, is a measure of mutagenic efficiency. The lowest molar drug concentration ($1/C$) needed to induce a fixed proportion of revertants (chosen as 50 per 10^8 bacteria) is a measure of mutagenic effectiveness. The two measures of antitumor activity modeled were ILS_{max} (the percent increase in life span observed for each derivative at its LD_{10} dose), a measure of tumor cell selectivity, and $1/D_{40}$ (the dose of drug to provide an ILS of 40%), a measure of dose potency. These measures of bioactivity were intercompared and modeled in terms of a number of drug physicochemical properties. The results show that drug lipophilic/hydrophilic balance is the dominant factor in determining both mutagenic and antitumor activity, although other factors are involved. The two different types of activity can be readily separated in the AMSA drug series by appropriate choice of substituent and adjustment of overall drug lipophilic/hydrophilic balance.

The current successes of chemotherapy in the treatment of cancer have brought their attendant problems. As more patients are provided with longer periods of remission (in many cases achieving a normal life span), there is an increasing literature[1] describing the onset of drug-induced secondary cancer in patients treated with a number of chemotherapeutic drugs. Since almost all of the antitumor agents currently in use are carcinogenic to some degree,[2] the need to separate antitumor activity and carcinogenicity and provide effective but noncarcinogenic antitumor agents has become one of the major tasks facing the drug designers.

Since animal carcinogenicity testing is too costly to carry out on a routine basis, attention has focused on bacterial mutagenicity tests as suitable systems for quantitative evaluation of carcinogenic risks. There is currently a brisk discussion concerning what relevance results from such tests might have for predicting carcinogenic risks for humans.[3,4] However, these tests do measure the results of events which take place at the molecular level in similar ways in both bacterial and mammalian cells. By estimating the ability of a chemical to be active in the first stage

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(initiation) of mammalian carcinogenesis, such tests have some relevance for the evaluation of carcinogenicity. The most widely used system is the "Ames test", employing mutant strains of the bacterium *Salmonella typhimurium*.[5] Most of these strains lack the usual lipopolysaccharide permeability barrier and the normally error-free excision repair DNA enzyme system. They are thus particularly sensitive to mutagens, which cause reversion to wild-type ability to grow in the absence of histidine. The tests are rapid, sensitive, and quantifiable.

We recently began a study of the mutagenicity of a number of 9-anilinoacridines,[6] using this test to monitor the mutagenicity of congeners during drug development. This class of compounds has been studied for some time in this laboratory as potential antitumor agents, and the initial study was designed to answer a number of questions. The primary need was to quantitate the test, allowing the possible subsequent development of QSAR for mutagenic activity. This involved both experimental design and a choice of the most suitable measure of mutagenic activity, and the initial results have been reported.[6]

Just as there are different and independent measures of antitumor activity,[7,8] so different measures of mutagenic activity can be devised. Brookes et al.[9] have emphasized the importance of distinguishing between mutagenic efficiency (defined as mutagenic frequency per lethal event) and mutagenic effectiveness (defined as mutagenic frequency per does applied). For the Ames test, we have shown[6] that the most reproducible measure of mutagenic efficiency is M_{50}, the mutation frequency due to the drug at a concentration (D_{50}) providing 50% inhibition of bacterial cells grown on full medium as controls. Thus, $M_{50} = 100(M_T - M_C)/S_{50}$, where M_T, the number of revertant colonies growing on minimal medium, is corrected for the spontaneous background reversion rate, M_C, in the absence of drug and divided by the number of colonies, S_{50}, found in control plates containing histidine-enriched medium at the drug concentration (D_{50}) which causes 50% inhibition of bacterial growth. Mutagenic effectiveness is best measured at the lowest concentration of drug needed to cause a fixed proportion of revertant colonies. Such a measure, log $(1/C)$, where C is the molar concentration, has recently been employed by Hansch and co-workers[10] in developing QSAR for the mutagenic activity of a series of dialkyltriazenes. Their study was carried out using the *Salmonella* strain TA 92 and a liver microsome activation system. The dialkyltriazenes require oxidative dealkylation to be active and are not mutagenic in the TA 92 strain without activation. Our previous work[6] was carried out with strain TA 1537, which possesses a frame-shift mutation in a GC-rich region, and is particularly sensitive to acridine derivatives,[5] which have been suggested to act by stabilization of the imperfect pairing resulting from single-strand "slippages".[11,12]

The 9-anilinoacridines are active in the TA 1537 strain without activation, thus removing another potential source of variability in the results. Our initial investigation[6] was confined to five small homologous series of 9-anilinoacridines, where chain homologation was effected in the 1′ position. Evidence was available that DNA binding was not affected by chain homologation, so that the main variable altering through a homologous series was lipophilic/hydrophilic balance. A striking relationship was observed between mutagenic efficiency (log M_{50}) and lipophilic/hydrophilic balance, modeled by R_m values from partition chromatography, within the two series for which all members were active mutagens. In these cases, bacterial toxicity (D_{50}) and mutagenic efficiency appeared closely related, because both were similarly dependent on R_m. However, when results for the whole data base of compounds bearing various substituents in the acridine and anilino rings were considered, bacterial toxicity and mutagenic efficiency were essentially independent. In a similar way, in vivo toxicity (LD_{10}) and tumor cell selectivity (ILS_{max}) have been shown to be essentially independent for several series of antitumor agents, including the AMSA derivatives.[7,8,13]

In this paper, we extend the initial study[6] to include a range of 4′-(9-acridinylamino)methanesulfonanilide (AMSA) derivatives, bearing a variety of different groups in the 3 position of the acridine ring (compounds **9**–**24**), as well as the initial homologous series of AMSA derivatives (compounds **3**–**8**). A further group of AMSA compounds (**25**–**48**) bear selected substituent groups in various positions on the drug skeleton, in an attempt to probe the relative importance of position-dependent steric and electronic factors.

Chemistry. The terminal step in the preparation of all the new AMSA agents (**12**, **15**, **37**, **47**, and **48**) involved coupling of the appropriate 9-chloroacridine with 4-methanesulfonamidoaniline by the methods previously described.[14] The 9-chlorocarboxamidoacridine needed for the preparation of compound **47** was synthesized from the appropriate diphenylamine dicarboxylic acid by the method published[14] for 9-chloro-4-carboxamidoacridine, required for the preparation of **48**. 1,9-Dichloroacridine for the preparation of **37** was isolated from the mixed isomers by the fractional crystallization technique given by Albert.[15] The acridinones for the preparation of **12** and **15** were prepared from 3-aminoacridinone and, respectively, methyl chloroformate and methanesulfonyl chloride.

Results and Discussion

The bacterial assay was carried out as detailed before.[6] Toxicity was recorded as D_{50}, the concentration of drug needed to kill 50% of the cells in control colonies grown on histidine-enriched medium. Mutagenic efficiency (M_{50}) was measured as defined earlier, the percentage of drug-induced revertant colonies seen at an isotoxic level (D_{50}) for each drug. Mutagenic effectiveness $(1/C)$ was the lowest molar concentration of each drug needed to provide a constant proportion of revertant colonies (chosen as 50 per 10^8 bacteria). These values are recorded in Table I. All experiments were carried out in triplicate at each concentration level, and the mean number of colonies was

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determined. Reproducibility of D_{50} and M_{50} values was essentially as seen previously,[6] while the standard error of $1/C$ determinations was about 7%. Antitumor testing was carried out as before,[16] using the intraperitoneally implanted L1210 leukemia. Values for in vivo toxicity (LD_{10}), tumor cell selectivity (ILS_{max}) and dose potency ($1/D_{40}$) were determined and recorded in Table I.

Except for six compounds which were not mutagenic enough for $1/C$ values to be determined (compounds **15**, **23**, **36**, **41**, **42**, and **47**), all three measures of biological activity, in the *Salmonella* system (D_{50}, M_{50}, and $1/C$) were measured for the compounds listed in Table I. For these 40 active compounds, the cross-correlation matrix of the activity parameters in the *Salmonella* system is shown in Table II. As observed before,[6] mutagenic efficiency (log M_{50}) is essentially independent of toxicity (log D_{50}), whereas mutagenic effectiveness [log $(1/C)$] is closely related to toxicity, even though this parameter is measured at doses well below the toxic range for each compound. It thus seemed important to attempt modeling of both of these independent measures of mutagenic activity for the AMSA derivatives. We noted before[6] that, for the homologous series of unsubstituted AMSA derivatives (**3**–**8**), bacterial toxicity was very dependent on lipophilic/hydrophilic balance (eq 1). This equation predicts well the

$$\log (D_{50}) = -1.65(\pm 0.61)R_m + 1.18 \quad (1)$$

$$n = 6,\ r = 0.91,\ s = 0.20,\ F_{1,4} = 18.6$$

bacterial toxicities of the 3-substituted compounds (**9**–**24**), and the results for these can be incorporated to give the very similar eq 2. The only exception was the 3-nitro

$$\log (D_{50}) = -1.38(\pm 0.28)R_m + 1.20 \quad (2)$$

$$n = 21,\ r = 0.91,\ s = 0.20,\ F_{1,19} = 95.4$$

compound **16**, which was much more toxic than predicted by eq 2 (log D_{50} observed, −0.27; calculated, 1.31) and which was omitted in calculation of the equation. The anomalous mammalian toxicity of the 3-nitro compound has been commented on before,[8] and it may be that reduction to toxic electrophilic species is occurring. When the remaining substituted AMSA compounds were examined, it was clear that broadly similar relationships between toxicity and R_m existed for these compounds also. Data from all the derivatives in Table I (except **16**) could be summarized in eq 3. It is interesting that eq 3 includes

$$\log (D_{50}) = -1.08(\pm 0.26)R_m + 1.22 \quad (3)$$

$$n = 45,\ r = 0.76,\ s = 0.25,\ F_{1,43} = 60.3$$

three other nitro-substituted compounds (**40**–**42**) whose bacterial toxicities are well predicted. The LD_{10} values for the in vivo toxicity of these compounds in the mouse are all over 500 mg/kg (Table I), whereas the LD_{10} for the 3-nitro compound **16** is 12.5 mg/kg.

It is apparent that the toxicity shown by the AMSA compounds toward the bacterial strain is essentially independent of drug structure for the considerable set of substituent groups examined. Although the range of lipophilic/hydrophilic balance has been extended to considerably more hydrophilic areas than before,[6] eq 1–3 were not improved by addition of a term in R_m^2; so, presumably, compounds of greater hydrophilicity would be even less toxic. Equation 3 was used to calculate the residuals for bacterial toxicity, log D_{50}, listed in Table I.

For the homologous series of unsubstituted AMSA compounds (**3**–**8**), we had noted before that mutagenic efficiency (log M_{50}) appeared similarly related to lipophilic/hydrophilic balance (eq 4). It was suggested that

$$\log (M_{50}) = -2.61(\pm 0.96)R_m + 2.39 \quad (4)$$

$$n = 6,\ r = 0.94,\ s = 0.30,\ F_{1,4} = 29.6$$

this loss of mutagenic efficiency with increasing lipophilicity was due to increasing blockade of some toxic site, until very lipophilic compounds proved so toxic that mutagenic activity could not be demonstrated. It was thus of interest to see whether the more hydrophilic congeners in the present set of AMSA derivatives had an increased mutagenic efficiency (log M_{50}) as their bacterial toxicity dropped, a situation implied by eq 4. In fact, this was not the case. Inclusion of the M_{50} values for all the active 3-substituted AMSA compounds (except **16**) into eq 4 did not provide a significant relationship unless a parabolic term in R_m was included (eq 5). Mutagenic efficiency for

$$\log (M_{50}) = -4.27(\pm 2.02)R_m^2 + 1.67(\pm 1.40)R_m + 1.55 \quad (5)$$

$$n = 19,\ r = 0.61,\ s = 0.68,\ F_{2,16} = 4.7,\ R_m(\text{opt}) = 0.20\ (-2.25 \text{ to } 2.65)$$

these compounds is clearly more structure dependent than overall bacterial toxicity, D_{50}, and while eq 5 is significant there are clearly other factors involved. A number of parameters modeling hydrophobic, steric, and electronic effects were investigated (π, MR, σ_p), with σ_p for the 3 substituents proving the most useful (eq 6). Equation 6

$$\log (M_{50}) = -5.56(\pm 1.61)R_m^2 + 2.35(\pm 1.12)R_m + 1.06(\pm 0.79)\sigma_p + 1.57 \quad (6)$$

$$n = 19,\ r = 0.79,\ s = 0.57,\ F_{3,15} = 8.5,\ R_m(\text{opt}) = 0.21\ (-1.58 \text{ to } 2.00)$$

suggests that the greatest mutagenic efficiency is shown by those congeners with electron-withdrawing groups on the 3 position of the acridine nucleus. The reason for this dependence is not clear. For these compounds, substituent σ_p values are closely related to the pK_a of the resulting AMSA derivative. Thus, such substituents may exert an effect on overall drug partitioning properties by their effect on pK_a, and an equation of equal significance is provided by the use of pK_a as a third variable (eq 7). However,

$$\log (M_{50}) = -5.51(\pm 1.71)R_m^2 + 2.30(\pm 1.03)R_m - 0.37(\pm 0.27)pK_a + 4.20 \quad (7)$$

$$n = 19,\ r = 0.79,\ s = 0.58,\ F_{3,15} = 8.9,\ R_m(\text{opt}) = 0.21\ (-1.79 \text{ to } 2.21)$$

extension of eq 6 to the whole data base by the use of measured pK_a values for the other compounds (**25**–**48**) was not possible, suggesting that there are factors other than agent base strength involved in the determination of mutagenic efficiency. Inspection of the data in Table I suggests that all 3′ substituents significantly reduce mutagenic efficiency. We had previously noted[6] the special case where addition of a 3′-OCH_3 group to AMSA (**3**) provided the less mutagenic clinical agent *m*-AMSA (**31**).

For modeling mutagenic effectiveness, log $(1/C)$, the concentration C was chosen as the lowest molar concentration of drug to provide 50 mutant colonies per 10^8 bacteria. A similar measure of drug mutagenic effectiveness has recently been employed by Hansch and coworkers[10] for a series of dialkyltriazenes acting against *Salmonella typhimurium* strain TA 92. With this strain, still possessing the normal lipopolysaccharide permeability barrier,[17] they observed a strong dependence of mutagenic effectiveness with lipophilic/hydrophilic balance, the more

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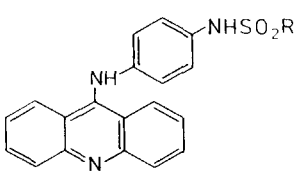
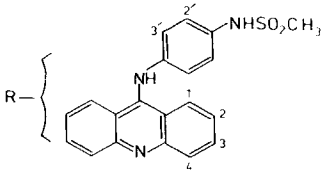
Table I. *Structural, Physicochemical, and Biologic Parameters for the 9-Anilinoacridines*



1



2

no.	type	R	R_m[a]	σ_p	pK_a[b]	log (D_{50})[c] obsd	log (D_{50})[c] calcd[i]	log (D_{50})[c] diff	log (M_{50})[d] obsd	log (M_{50})[d] calcd[j]	log (M_{50})[d] diff	log ($1/C$)[e] obsd	log ($1/C$)[e] calcd[k]	log ($1/C$)[e] diff	LD_{10}[f]	log ILS_{max}[g] obsd	log ILS_{max}[g] calcd	log ILS_{max}[g] diff	log ($1/D_{40}$)[h] obsd	log ($1/D_{40}$)[h] calcd	log ($1/D_{40}$)[h] diff
3[l]	1	CH_3	0.00	0.00	7.19	1.04	1.22	−0.17	2.10	1.56	0.54	4.29	4.28	0.01	66	2.12	2.07	0.05	4.43	4.83	−0.40
4[l]	1	CH_2CH_3	0.25	0.00	7.19	0.91	0.94	−0.03	2.00	1.31	0.19	4.62	4.56	−0.04	330	1.99	2.01	−0.02	4.14	4.63	−0.49
5[l]	1	$(CH_2)_2CH_3$	0.43	0.00	7.20	0.70	0.75	−0.05	1.67	1.56	0.11	4.62	4.76	−0.14	350	1.91	1.91	0.00	3.78	4.26	−0.48
6[l]	1	$(CH_2)_3CH_3$	0.56	0.00	7.18	0.69	0.61	0.08	0.78	1.15	−0.37	4.92	4.91	0.01	350	1.82	1.81	0.01	3.69	3.87	−0.18
7[m]	1	$(CH_2)_4CH_3$	0.66	0.00	7.19	0.19	0.50	−0.31	0.60	0.69	−0.09	5.12	5.02	0.10	70	1.74	1.71	0.03	3.96	3.49	0.47
8[m]	1	$(CH_2)_5CH_3$	0.75	0.00	7.17	−0.03	0.40	−0.43	0.30	0.20	0.10	5.35	5.12	0.23	120	1.54	1.60	−0.06	nr[n]		
9[o]	2	3-NH_2	−0.18	−0.66	9.80	1.64	1.41	0.23	0.70	0.27	0.43	3.68	4.04	−0.36	2.5	2.13	2.04	0.09	5.76	6.17	−0.41
10[p]	2	3-$NHCH_3$	−0.01	−0.84	9.30	1.48	1.23	0.25	0.70	0.66	0.04	3.61	4.25	−0.64	7.5	1.89	2.07	−0.17	6.57	6.00	0.57
11[o]	2	3-$NHCOCH_3$	−0.12	0.00	7.34	1.45	1.36	0.10	−0.22	1.21	−1.43	3.95	4.12	−0.17	19	2.16	2.05	0.11	5.81	4.87	0.94
12[q]	2	3-$NHCOOCH_3$	−0.07	−0.15	7.48	1.36	1.29	0.07	0.20	1.22	−1.08	4.07	4.20	−0.13	170	2.00	2.06	−0.06	4.70	4.97	−0.27
13[r]	2	3-N_3	0.20	0.15	7.00	0.77	1.00	−0.23	2.13	1.98	0.15	4.80	4.50	0.30	24	2.33	2.03	0.30	5.04	4.60	0.44
14[r]	2	3-$N_3(CH_3)_2$	0.22	0.15	7.13	0.85	0.98	−0.13	1.46	1.98	−0.52	4.49	4.52	−0.03	11	2.14	2.03	0.11	5.72	4.64	1.08
15[q]	2	3-$NHSO_2CH_3$	−0.26	0.03	na[a]	1.80	1.50	0.30	ia[t]	0.53		nr	3.94		130	2.17	2.01	0.16	4.70	*z*	
16[o]	2	3-NO_2	−0.08	0.78	5.52	−0.27	1.31	−1.58	−0.85[u]	2.39	−1.54	5.39[u]	4.16	1.23	12.5	1.84[u]	2.06	−0.22	5.91[u]	3.87	2.04
17[v]	2	3-aza	−0.35	(0.78)	5.32	1.50	1.59	−0.09	1.20	0.90	0.30	3.86	3.84	0.02	>500	ia			nr		
18[o]	2	3-CH_3	0.24	−0.17	7.49	1.12	0.96	0.16	2.15	1.64	0.51	4.66	4.55	0.11	30	1.93	2.02	−0.09	4.97	4.82	0.15
19[o]	2	3-OCH_3	0.10	−0.27	7.57	1.21	1.11	0.10	2.04	1.47	0.57	4.45	4.38	0.07	40	2.03	2.06	−0.03	5.20	5.01	0.19
20[o]	2	3-Cl	0.14	0.23	6.57	1.12	1.06	0.06	2.28	2.04	0.24	4.64	4.43	0.21	120	1.98	2.05	−0.07	4.22	4.42	−0.20
21[o]	2	3-Br	0.16	0.23	6.56	0.67	1.04	−0.37	2.41	2.05	0.36	4.60	4.45	0.15	33	1.85	2.04	−0.19	4.52	4.00	0.12
22[w]	2	3-I	0.20	0.18	6.52	0.53	1.00	−0.47	1.71	2.01	−0.30	4.72	4.50	0.22	35	1.88	2.03	−0.15	4.94	4.33	0.61
23[o]	2	3-CN	−0.15	0.66	5.75	1.63	1.38	0.25	ia	1.75		nr	4.08		250	ia			nr		
24[o]	2	3-SO_2CH_3	−0.39	0.72	5.65	1.58	1.64	−0.06	0.78	0.58	0.20	3.64	3.84	−0.20	>500	ia			nr		
25[w]	2	2'-CH_3	0.09		7.08	1.53	1.12	0.41	2.30			4.44	4.37	0.07	200	1.51[u]			nr		
26[w]	2	3'-CH_3	0.15		7.38	1.38	1.05	0.33	0.98			3.88	4.44	−0.56	110	1.81[u]			3.77	4.86	−1.09
27[o]	2	1-CH_3	0.19		6.43	1.10	1.01	0.09	0.40			4.21	4.49	−0.28	nt[x]	nt[x]			nt[x]		
28[o]	2	2-CH_3	0.19		7.23	1.04	1.01	0.03	1.23			4.31	4.49	−0.18	130	1.48[u]			nr		
29[o]	2	4-CH_3	0.07		7.15	1.07	1.14	−0.07	2.10			4.60	4.35	0.25	45	1.91[u]			4.52	4.74	−0.27
30[w]	2	2'-OCH_3	0.03		7.03	1.61	1.18	0.43	2.71			3.79	4.30	−0.51	110	1.34[u]			nr		
31[w]	2	3'-OCH_3	0.18		7.43	1.40	1.02	0.37	2.13			4.70	4.48	0.22	9	2.06[u]			5.24	4.86	0.37
32[o]	2	1-OCH_3	−0.05		na	1.32	1.27	0.05	2.27			4.64	4.20	0.44	200	ia			nr		
33[o]	2	2-OCH_3	−0.04		6.87	1.16	1.26	−0.10	2.02			4.64	4.21	0.43	>500	ia			nr		
34[o]	2	4-OCH_3	0.01		7.15	1.41	1.21	0.20	2.31			4.37	4.27	0.10	120	2.08[u]			4.46	4.81	−0.35
35[w]	2	2'-Cl	0.07		6.44	1.44	1.14	0.30	2.09			4.38	4.36	0.03	>500	ia			nr		
36[w]	2	3'-Cl	0.24		6.34	0.81	0.96	−0.15	−0.52			nr	4.55		>500	ia			nr		
37[q]	2	1-Cl	0.01		5.86	1.53	1.21	0.32	−0.22			3.94	4.29	−0.35	nt	nt			nt		

38[o]	2	2-Cl	0.16	6.42	0.87	1.04	−0.17	0.30	4.60	4.45	0.15	200	ia	nr			
39[o]	2	4-Cl	0.08	5.92	1.19	1.13	0.06	0.48	4.06	4.36	−0.30	>500	ia	nr			
40[y]	2	2′-NO_2	0.11	na	1.09	1.10	−0.01	1.18	4.54	4.39	0.15	>500	ia	nr			
41[w]	2	3′-NO_2	0.06	4.86	1.54	1.15	0.39	ia	nr	4.33		>500	ia	nr			
42[o]	2	2-NO_2	−0.21	5.42	1.56	1.44	0.12	−0.40	nr	4.00		>500	ia	nr			
43[y]	2	2′-NH_2	−0.33	na	1.10	1.52	−0.42	1.56	4.38	3.92	0.46	70	2.04[u]	4.92	z		
44[w]	2	3′-NH_2	−0.32	7.65	1.50	1.56	−0.06	0.85	3.55	3.92	−0.37	50	2.14[u]	4.66	4.77	−0.11	
45[o]	2	2-NH_2	−0.32	7.15	1.32	1.56	−0.24	0.85	3.78	3.92	−0.14	35	1.60[u]	4.08	4.49	−0.41	
46[o]	2	4-NH_2	−0.19	na	1.12	1.42	−0.30	1.45	4.10	4.06	0.04	125	2.00[u]	3.96	z		
47[q]	2	2-$CONH_2$	−0.45	6.34	1.61	1.70	−0.09	−0.30	nr	3.72		>500	ia	nr			
48[q]	2	4-$CONH_2$	−0.47	6.12	1.13	1.72	−0.59	1.66	4.60	3.75	0.85	450	1.70[u]	3.27	3.56	−0.29	

[a] Relative measure of lipophilic/hydrophilic balance from partition chromatography; see ref 6. [b] pK_{aa}, acridine ionization constant determined by UV spectophotometrically in 20% DMF–H_2O; see ref 20. [c] Concentration ($M \times 10^5$) to cause 50% inhibition of bacterial growth. [d] Mutation frequency at D_{50} ($\times 10^4$); see text. [e] C, molar concentration of drug to provide 50 revertant colonies per 10^8 bacteria. [f] LD_{10}, lethal dose for 10% of animals on average, in (mg/kg)/day, qd 1–5; see ref 20. [g] ILS_{max}, percentage increase in life span in L1210 assays at the LD_{10} dose; calculated values are from eq 11. [h] D_{40}, drug dose in (mg/kg)/day to provide an ILS of 40%; calculated values are from eq 12. [i] Log (D_{50}) values calculated using eq 3. [j] Log (M_{50}) values calculated using eq 6. [k] Log (1/C) values calculated using eq 10. [l] Reference 21. [m] Reference 16. [n] nr, particular level of biological response required not reached before drug toxicity supervened. [o] Reference 21. [p] Reference 22. [q] New compound; see Table III for physical and analytical data. [r] Reference 23. [s] na, pK_a could not be determined due to competing sulfonamide ionization. [t] Compound inactive in the appropriate test system; for M_{50}, number of revertant colonies not significantly above background at the D_{50} concentration; for ILS_{max}, value not above 25% at the LD_{10}. [u] Biological activity value not used in derivation of appropriate equation. [v] Reference 24. [w] Reference 25. [x] nt, insufficient compound available for in vivo testing. [y] Reference 26. [z] Calculated value not determinable, as no pK_a available.

Table II. Correlation Matrix (r Matrix) for Measures of Activity of AMSA Derivatives in the *Salmonella typhimurium* Strain TA 1537 System

	log M_{50}	log (1/C)
log D_{50}	0.05	−0.82
log M_{50}		0.28

lipophilic compounds being the more potent. The TA 1537 strain used in the present study lacks the lipopolysaccharide permeability barrier,[17] but even so, lipophilic/hydrophilic balance is of paramount importance in determing mutagenic effectiveness of the AMSA compounds. For the homologous series of unsubstituted AMSA derivatives, where R_m is the only parameter altering significantly, eq 8 can be determined. For the 3-substituted

$$\log (1/C) = 1.37(\pm 0.37)R_m + 4.20 \quad (8)$$

$$n = 6,\ r = 0.96,\ s = 0.12,\ F_{1,4} = 53.8$$

AMSA compounds **9**–**24** (except **16**), a similar close relationship exists between mutagenic effectiveness and lipophilic/hydrophilic balance, and the results for these compounds can be incorporated into eq 9. The positive

$$\log (1/C) = 1.54(\pm 0.29)R_m + 4.19 \quad (9)$$

$$n = 19,\ r = 0.93,\ s = 0.21,\ F_{1,17} = 107.7$$

coefficient for the term in lipophilic/hydrophilic balance indicates that the more lipophilic AMSA derivatives are the more effective mutagens. A similar conclusion was reached by Hansch and co-workers[10] in their study of the dialkyltriazenes. In their study, a term in σ^+ for the substituents on the benzene ring of the alkyltriazene was able to be fitted to the data. The authors speculated that this parameter described the relative ease of microsomal oxidation of these compounds, which are nonmutagenic without such activation. For the AMSA compounds, where no activation is needed with strain TA 1537, agent lipophilic/hydrophilic balance is the only parameter required to adequately characterize their mutagenic effectiveness over quite a wide range of parameter values.

Even when measures of mutagenic effectiveness from all the active compounds in Table I are considered, including those compounds (**25**–**48**) with substituents in all of the available nuclear anilinoacridine positions, the relationship described in eq 9 holds reasonably well (eq 10).

$$\log (1/C) = 1.12(\pm 0.36)R_m + 4.28 \quad (10)$$

$$n = 39,\ r = 0.72,\ s = 0.30,\ F_{1,36} = 36.1$$

This equation was used to calculate the residuals for log (1/C) in Table I. No improvement could be effected in eq 9 and 10 by the use of R_m^2 as an additional variable or by the use of parameters (π, MR, σ) describing substituent hydrophobic, steric, or electronic properties. The range in mutagenic effectiveness covered by these compounds is over 100-fold. Although this is only a small part of the observed[18] millionfold range in mutagenic effectiveness observed for organic chemicals, the agents considered here are closely related ones. More importantly, they are all active per se, without the additional range of mutagenic effectiveness liable to be built in by a varying degree of microsomal activation.

Although the group of AMSA derivatives in Table I was not selected with a view to modeling measures of in vivo

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antitumor activity (it contains many tumor-inactive examples), it is worthwhile noting that the main features of the QSAR already observed for the acridine-substituted AMSA derivatives[8] are present. Thus, modeling of tumor selectivity (log ILS_{max}) shows the parabolic dependence of this activity on lipophilic/hydrophilic balance for the homologous series of unsubstituted derivatives and the 3-substituted compounds (eq 11). This relationship could

$$\log (ILS_{max}) = -0.83(\pm 0.38)R_m^2 + 2.07 \quad (11)$$

$$n = 18,\ r = 0.73,\ s = 0.12,\ F_{1,16} = 18.6,\ R_m(\text{opt}) = 0.0$$

not be extended to the whole data base, those compounds possessing anilino ring substitution being poorly fitted. In contrast, dose potency [log $(1/D_{40})$] was not well modeled by terms in R_m alone. For the whole data base of active compounds, modeling of this parameter was best achieved using terms in R_m and pK_a, a similar result to that noted before[8] for a larger series of *m*-AMSA derivatives (eq 12).

$$\log (1/D_{40}) = -3.06(\pm 2.14)R_m^2 + 0.55(\pm 0.28)pK_a + 0.85 \quad (12)$$

$$n = 23,\ r = 0.76,\ s = 0.54,\ F_{2,20} = 13.6,\ R_m(\text{opt}) = 0.0$$

Conclusions

The above results clearly reinforce our earlier conclusions that, for the tumor-active but mutagenic 9-anilinoacridines, separation of the two classes of bioactivity is possible by simple manipulation of agent lipophilic/hydrophilic balance. They offer the additional information that strongly basic compounds prove to be less active mutagens (eq 7) but, at the same time, more dose-potent antitumor agents (eq 12). However, as we also noted before,[6] such answers may be deceptively simple. The results suggest that both mutagenic efficiency and mutagenic effectiveness increase with increasing lipophilicity up to a certain point (an R_m value of about 0.20, equivalent[6] to a log *P* value of about 1.0). After that, increasing toxicity to the bacteria reduces mutagenic efficiency, although mutagenic effectiveness continues to increase until the compounds are so toxic that no mutagenic effects can be observed at nontoxic doses. In animals, which possess quite different sites determing toxicity, does levels high enough to demonstrate carcinogenic effects might be permitted, even for very lipophilic compounds.

Furthermore, although more hydrophilic compounds are both less mutagenic and more active against the L1210 leukemia, recent work[19] has suggested that ideal lipophilicity for compounds to be active against solid tumors is considerably higher than the optimum for the leukemias. Since the solid tumors currently present the greatest clinical problem, new agents may possess lipophilicity values in the range for mutagenic activity. However, important as the influence of agent lipophilic/hydrophilic balance is in determining the mutagenic activity of the 9-anilinoacridines, there is sufficient variation in both classes of bioactivity with structure to permit the intelligent design of new congeners which will be at the same time less active mutagens and more active antitumor agents.

Experimental Section

Chemistry. Where analyses are indicated only by symbols for the elements, results obtained for those elements were within ±0.4% of the theoretical values. Analyses were carried out by Dr. A. D. Campbell, Microchemical Laboratory, University of Otago, Dunedin, New Zealand. Melting points were determined on an Electrothermal apparatus with the maker's supplied stem-corrected thermometer and are as read. To monitor the progress of reactions and the purity of products, TLC on SiO_2 (Merck SiO_2, F_{254}) was used, R_m values were determined as described in ref 6 and are the mean of at least four determinations.

3-(Methanesulfonamido)-9(10*H*)-acridinone was prepared by the dropwise addition of methanesulfonyl chloride (25 mM) to a well-stirred solution of 3-amino-9(10*H*)-acridinone (23.8 mM) in pyridine (60 mL) at 0 °C. The solution was kept at 20 °C for 3 h, and the volatiles were removed in vacuo at 40 °C. The residue was triturated with 2 N HCl, the resulting red solid was collected and dissolved in 2 N aqueous NaOH, the pH was adjusted to 8, and the solution was clarified with charcoal. Acidification to pH 2 precipitated the acridinone, which was recrystallized from EtOH as needles: mp 300–301 °C; yield 63%. Anal. ($C_{14}H_{12}N_2O_3S \cdot 0.5H_2O$) C, H, N. Similar exposure of 3-aminoacridinone to methyl chloroformate gave the **methyl carbamate**, which recrystallized from aqueous EtOH as needles: mp 310–312 °C; yield 79%. Anal. ($C_{15}H_{12}N_2O_3$) C, H, N.

Assays for mutagenicity and antitumor activity were carried out as detailed in ref 6 and 20.

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