Potential Antitumor Agents. 30. Mutagenic Activity of Some 9-Anilinoacridines: Relationships between Structure, Mutagenic Potential, and Antileukemic Activity

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In vitro mutagenicity tests are now highly predictive for in vivo carcinogenicity. To assist development of second-generation agents of the tumor inhibitory 9-anilinoacridines, quantitative structure–mutagenicity relationships are under investigation. The carcinogenic and carcinostatic anthracyline antibiotics adriamycin and daunomycin have been included as standard agents. Employing Ames' *Salmonella typhimurium* tester strains TA 98, TA 100, and TA 1537, various measures of mutagenic activity have been evaluated. M_{50}, the percentage of drug-induced mutant colonies observed at the concentration providing 50% inhibition of the growth of the bacterial strain, proves most reproduceable. Within several homologous series of 9-anilinoacridines, increasing lipophilicity provides increased toxicity to the bacterium and decreasing mutagenic activity. Effective regression equations relating both bacterial toxicity and mutagenicity with agent lipophilic–hydrophilic balance can be derived. While bacterial toxicity is determined almost entirely by lipophilic character, mutagenicity is also markedly dependent on chemical structure. It is demonstrated that in vitro mutagenicity and in vivo antitumor activity in this drug series can be readily separated by appropriate structural modification.

As the effectiveness of clinical cancer chemotherapy improves, patients are being provided with increasingly long, symptom-free intervals. For such improvement to continue, it is essential that the chemotherapeutic agents employed are not themselves carcinogenic and capable of reinducing the disease. At present, many clinically useful agents (e.g., actinomycin, cyclophosphamide, daunomycin, and several nitrosoureas) are known animal carcinogens.[1] While possible agent carcinogenicity should be monitored, in most drug design and development programs the considerable time (40 months) and expense ($200 000) necessary to adequately test one compound[2] act as considerable deterents.

Within a large group of chemicals, of diverse structures, the correspondence between animal carcinogenicity and mutagenicity in certain bacterial strains appears greater than 90%.[3] These bacterial strains, of which those developed by Ames[4] are most widely known and used, provide rapid and inexpensive assays for monitoring mutagenic activity and, therefore, probable carcinogenicity.

One member of the experimental, broad-spectrum antitumor agents of the 9-anilinoacridine series (**22**; Table III; *m*-AMSA)[5] has progressed to the stage of phase II clinical evaluation. Second-generation analogues of this compound should now be considered, and a highly desirable design feature would be lack of carcinogenicity. However, it is not yet known if carcinogenic or mutagenic potential is an inevitable consequence of high antitumor activity or if these two types of biologic activity can be effectively separated. Quantitative measures of the antitumor (L1210) activity of more than 500 9-anilinoacridines are available,[6] and quantitative structure–activity relationships (QSAR) are under development.[7] If quantitative structure–mutagenicity relationships could also be derived for these agents, then comparison of the two sets of QSAR could allow a decision on whether mutagenicity (carcinogenicity) and antitumor activity are separable and might then also provide guidelines for the preparation of a desirable second-generation analogue.

The present publication details a preliminary study of the QSAR for mutagenic activity in certain 9-anilinoacridine congeners.

Mutagenicity Assays. The bacterial tester strains employed (Ames' TA 98, TA 100, and TA 1537) all require added histidine for satisfactory growth. Following favorable mutational events, growth on minimal medium becomes possible. Necessary mutations in the three strains, conferring ability to grow on minimal media, differ, TA 100 requiring a base-pair change while both TA 98 and TA 1537 require a frame-shift mutation.[4] In the usually employed Ames' test, agents in medium containing both bacteria and agar are poured onto a plate containing solidified minimal medium. When several of the highly colored 9-anilinoacridines were examined with this technique, it was clear from direct visual observation that the capacity of these agents to equilibrate through the layers in the plate differed markedly. The effective concentration of any agent that is in contact with the bacteria could not be readily obtained. A modified procedure was adopted in which bacterial cells were exposed to known concentrations of agent in liquid culture for a short period (1 h; ca. 1 generation time), harvested, and washed, and then samples were plated onto minimal and complete media. Additionally, these agents are susceptible to cleavage by nucleophilic species, particularly thiols,[8] resulting in loss of the 9-anilino ring. Two of the more unstable compounds (**5** and **10** in Table III) were examined to ensure that media constituents or bacterial products did not induce drug breakdown. Using ultraviolet spectroscopy, no detectable concentration change of **5** or **10** could be detected after a 1-h incubation period. It has been assumed that with the remaining, more thiolysis stable, congeners examined there is no appreciable drug destruction during incubation.

With each compound, a range of drug concentrations was employed in the incubation step, varying from that permitting normal bacterial survival up to one proving clearly toxic. After drug exposure and plating steps, the number of colonies growing on the complete medium (as a measure of the number of bacteria surviving) was determined (N_t). The ratio of this count to that for colonies observed in untreated controls (N_c) provided a percentage survival figure, $S = 100N_t/N_c$.

The counted mutant (revertant) colonies growing in minimal medium plates following drug exposure (M_T) were corrected for the background numbers observed on corresponding untreated control plates (M_C) to provide the number of revertant colonies attributable to drug treatment ($M_T - M_C$).

For comparison of QSAR for mutagenic activity with those for antileukemic activity, comparable response measures must be employed. The measure successfully used with the antitumor activity of these agents has been a measure of effectiveness (percentage increase in life span) at constant toxicity (LD_{10}) to the animals employed.[9,10] A corresponding index of mutagenicity would be a measure

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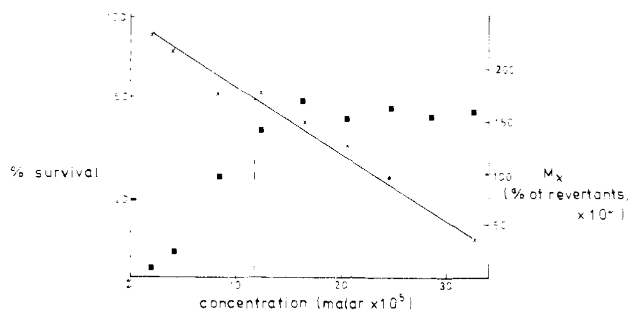


Figure 1. Dose–response relationships for toxicity and mutagenic activity of **10** (AMSA) with *Salmonella typhimurium* TA 1537: (X) percentage survival of colonies growing on complete medium; (■) percentage of revertant colonies growing on minimal medium compared to those seen in control plates at the same drug concentration $100(M_T - M_C)/N_t \times 10^4$.

of mutation frequency at a concentration providing a constant percentage survival of the bacterial strains. Scrutiny of plots of log (percentage survival) vs. drug concentration (Figure 1) suggests that the more nearly linear portion of such curves, those centering about the 50% survival figures (S_{50}), would permit most accurate interpolation of values between those concentrations actually employed. The concentrations providing 50% survival were obtained from linear correlations between the logarithm of percentage survival (S) and the corresponding doses employed (cf. Figure 1). Using the values obtained at the concentration providing 50% bacterial survival (D_{50}), the mutation frequency [the ratio of the numbers of revertant bacteria ($M_T - M_C$) to those of surviving bacteria expressed as a percentage, i.e., $100(M_T - M_C)/S_{50}$ ($=M_{50}$)] was investigated as a possible measure of mutagenicity at a constant level of toxicity to the bacterial strain. Also available from such data (Figure 1) is the maximum number of revertants observed, $(M_T - M_C)_{max}$ ($=M_{max}$), regardless of the drug concentration producing this value. Alternatively, the maximum mutation frequency, $100(M_T - M_c)/N_x$ ($=M_x$), can be examined, where N_x is the number of bacteria surviving at the concentration providing the greatest mutation frequency. The use of the number of revertants seen at a constant dose, as employed in a recent study of a series of methylchrysenes,[11] was not possible because of the wide variation in bacterial toxicity shown by the 9-anilinoacridines.

To examine the reproduceability of the response measures M_{50}, M_{max}, and M_x, three separate experiments were carried out. Each experiment used the mean colony numbers from three control and revertant plates for each dose level. Treating the data as in Figure 1, it was found that the between experiment variation appeared least with the M_{50} values (Table I). The greatest variability occurred in M_{max}, which is the only value measured in a standard Ames' test. This result suggests that the standard Ames' test procedure is unsatisfactory for measuring quantitative differences in mutagenicity.

m-AMSA (**22**; Table III) has similar broad-spectrum experimental antitumor activity to adriamycin. Both these agents are DNA intercalators, and there is demonstrable cross-resistance between these when an adriamycin resistant, P388 leukemia subline is employed.[7] It was then of interest to observe that there is an extremely dissimilar pattern of mutagenic activity when these compounds are set against the three bacterial tester strains (Table II). The two anthracycline antibiotics adriamycin and daunomycin are most active with the TA 98 strain, clearly less effective with TA 100, and not active at all with TA 1537.

Table I. Mutagenicity and Toxicity of AMSA (10) Determined for *Salmonella typhimurium* TA 1537

expt	D_{50}[a]	M_{50}[b]	M_{max}[c]	M_x[d]
1	10.55	140	112	202
2	11.27	120	760	168
3	11.19	135	904	182
av (±SD)	11.00 (±0.39)	127 (±10)	928 (±182)	184 (±17)
% SE	3.5	7.9	19.6	9.2

[a] Concentration (M, $\times 10^5$) providing 50% inhibition of bacterial growth, as estimated by the methods demonstrated in Figure 1. [b] Mutation frequency (mutant colonies expressed as a percentage of the surviving bacteria) at the D_{50} ($\times 10^4$). [c] Maximum number of mutant colonies observed. [d] Maximum mutation frequency ($\times 10^4$).

Table II. Mutagenic Activity of Adriamycin, Daunomycin, AMSA, and *m*-AMSA in Various Strains of *Salmonella typhimurium*

compd	strain	D_{50}[a]	M_{50}[b]	M_{max}[c]	M_x[d]
adriamycin (3)[e]	TA 98	0.61	14	118	11
	TA 100	1.13	0.5	16	1.5
	TA 1537	1.63	0	0	0
daunomycin (4)[e]	TA 98	1.31	20	106	128
	TA 100	1.11	4.5	22	4.5
	TA 1537	0.74	0	0	0
AMSA (10)[e]	TA 98	18.01	0	0	0
	TA 100	19.4	0	7	0.7
	TA 1537	11.00	127	928	184
m-AMSA (22)[e]	TA 98	32.3	0	0	0
	TA 100	33.0	0	0	0
	TA 1537	24.9	4	38	5

[a-d] See corresponding footnotes in Table I. [e] Entry numbers in Table III.

With AMSA and *m*-AMSA, this order is virtually reversed; these agents do not observedly affect the TA 98 strain and show most strongly against TA 1537.

The maximum number of revertants (M_{max}), either in the present work (Table II) or in a standard Ames' test,[12] suggest that adriamycin and daunomycin are similar in their mutagenic activity. The M_{50} and M_x values from the modified test system used here reveal that daunomycin is a stronger mutagen than adriamycin but that mutagenic activity occurs at more cytotoxic dose levels in daunomycin.

It appears a fortunate coincidence that *m*-AMSA, as a candidate for clinical usage, is clearly less mutagenic in these test systems than the parent agent AMSA.

Chemistry. The terminal step in the preparation of all new agents involved coupling of the appropriate 9-chloroacridine (unsubstituted for compounds **6–9**, **26**, and **27**, bearing a 4-$CONH_2$ group for compounds **29–32**) with an aromatic amine. Preparation of the acridines and a detailed description of the coupling conditions have been published.[13] The side-chain amines needed for the synthesis of compounds **26**, **27**, and **32** were prepared by the general method previously given.[14]

Lipophilic–Hydrophilic Balance. Relative measures of this balance have been obtained employing R_m values from reverse-phase partition chromatography as before.[15] Calibration of this chromatographic system, using a series of compounds of known log P (n-octanol–water) values, has provided the relationship in eq 1.[7]

$$\log P = 2.00\ (\pm 0.15)\ R_m + 0.51\ (\pm 0.10) \quad (1)$$

$$n = 21;\ r = 0.99;\ s = 0.21;\ F_{1,19} = 678$$

Results and Discussion

The average number of colonies on the untreated minimal medium plates was 2.3. Of the 28 9-anilino-

acridines in this study (Table III), 20 appeared to cause more colonies than this on the minimal plates (using TA 1537), and for these all three measures of response were derived. A cross-correlation matrix between the logarithms of these measures, as the quantities which could be modeled in regression analyses, showed a high degree of correspondence (Table IV). However, from this matrix, none of the three measures appears related to drug toxicity to the bacteria (D_{50}).

From the high degree of covariance seen between the response measures, it would be expected that these would have similar QSAR. However, because of the greater reproduceability of M_{50} and its pertinence to response at constant toxicity, this factor was employed in regression analyses.

For the complete set of 9-anilinoacridines **5**–**32** (Table III), toxicity to the bacterium is clearly related to the lipophilic–hydrophilic balance of the agents, as provided by R_m values (eq 2).

$$\log D_{50} = -1.65\ (\pm 0.48)\ R_m + 1.56 \qquad (2)$$

$$n = 28;\ r = 0.79;\ s = 0.40;\ F_{1,25} = 43.8$$

The range of D_{50} values embraced by eq 2 extend over more than two orders of magnitude. The D_{50} values for adriamycin and daunomycin are not compatible with this equation; both are more toxic and more hydrophilic than any of the 9-anilinoacridines examined. Equation 2 was not significantly improved by inclusion of an R_m^2 term; it is clear that if there is an optimum R_m value for bacterial toxicity, it is above that of any compound examined. For the 9-anilinoacridines, it appears that bacterial toxicity is little affected by structure and the major determinant is agent lipophilic–hydrophilic balance, a phenomenon frequently noted for in vitro systems.[16] In contrast, marked variations in in vivo toxicity (LD_{10}) can be seen for compounds of similar R_m values but different structures (cf. **11** and **18**). No quantitative relationship could be derived linking D_{50} and LD_{10}.

With only two sets of homologous compounds (**5**–**9** and **10**–**15**), all members were active mutagens. For these two series, M_{50} is clearly linearly related to R_m values for compounds **5**–**9**

$$\log M_{50} = -3.33\ (\pm 2.66)\ R_m + 3.37 \qquad (3)$$

$$n = 5;\ r = 0.82;\ s = 0.82;\ F_{1,3} = 6.2$$

and for compounds **10**–**15**

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

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

Despite the different substitution patterns in these two series, eq 3 and 4 could be effectively combined.

$$\log M_{50} = -2.57\ (\pm 1.24)\ R_m + 2.60 \qquad (5)$$

$$n = 11;\ r = 0.81;\ s = 0.60;\ F_{1,9} = 16.9$$

Similarly, when D_{50} values are considered for these two series, for compounds **5**–**9**

$$\log D_{50} = -1.65\ (\pm 1.46)\ R_m + 1.62 \qquad (6)$$

$$n = 5;\ r = 0.79;\ s = 0.45;\ F_{1,3} = 5.0$$

and for compounds **10**–**15**

$$\log D_{50} = -1.36\ (\pm 0.61)\ R_m + 1.18 \qquad (7)$$

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

Again, these equations may be effectively combined:

$$\log D_{50} = -1.31\ (\pm 0.68)\ R_m + 1.27 \qquad (8)$$

$$n = 11;\ r = 0.79;\ s = 0.32;\ F_{1,9} = 14.7$$

Although the overall correlation matrix (Table IV) from all active compounds suggests no overall relationship between D_{50} and M_{50}, eq 5 and 8 do suggest that there is such a relationship for this subgroup of compounds, and this may be expressed

$$\log M_{50} = 1.73\ (\pm 0.54)\ \log D_{50} + 0.23 \qquad (9)$$

$$n = 11;\ r = 0.91;\ s = 0.43;\ F_{1,9} = 41.2$$

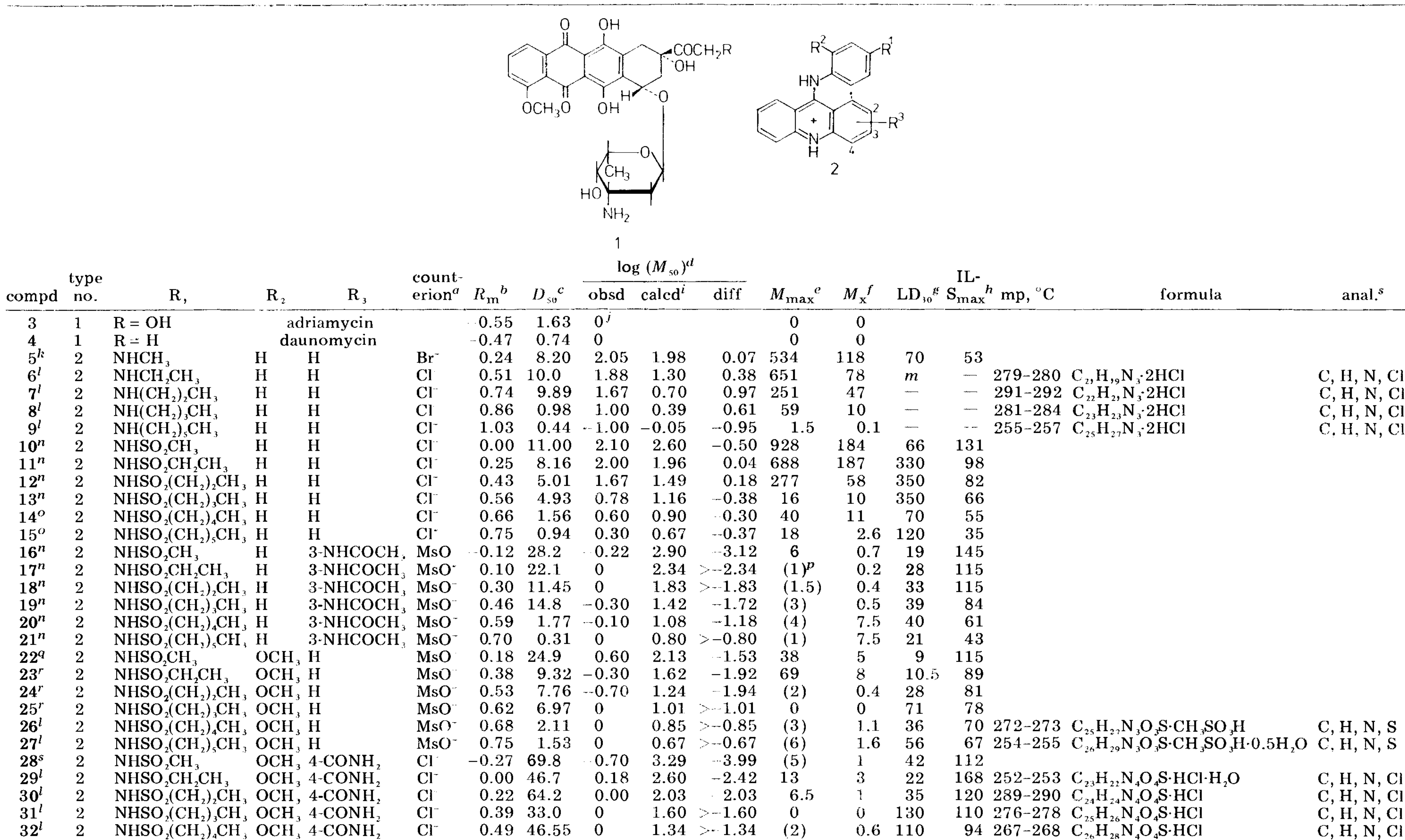
Inspection of the data base suggests that this apparent discrepancy may result from D_{50} being essentially independent of structure, whereas M_{50}, for all compounds, clearly is not.

The reduction in mutagenic activity seen as R_m increases (eq 5) could result from (1) the usual inability of more lipophilic compounds to reach remote sites; (2) increasing blockade of a site resulting in toxicity to the bacteria; and (3) reduced ability to fit to critical site features as an alkyl chain is lengthened (R_1 in **2**), thereby producing the changes seen in R_m values. For the homologous series examined, there is evidence that increasing the length of the hydrocarbon chain (R_1 in formula **2**), to effect changes in R_m, has little influence on the DNA binding of the compounds. Measured C_{50} values (those micromolar drug concentrations necessary to displace 50% of DNA-bound ethidium)[17] for the 9-anilinoacridines vary markedly with acridine structure but are essentially constant for a given structure as the alkyl chain R is lengthened.[7] A similar phenomenon has been noted with numerous homologous series of bisquaternary ammonium heterocycles. If DNA is assumed to be the site of mutagenic activity, then the site fit, as measured by C_{50} values, does not alter as R_m values increase within the homologous series **5**–**9** and **10**–**15**. Further, the *S. typhimurium* strains employed have been carefully selected for permeability.[4] The number of revertants observed with the more lipophilic and more toxic members of series **5**–**9** and **10**–**15** can be quite reasonably predicted from the toxic dose (D_{50}) of these expressed in molar terms.

From the dose profiles for mutagenic activity (cf. Figure 1) for more hydrophilic and less toxic examples of these series, the level of mutants expected if the D_{50} concentration observed for the more lipophilic examples had been employed is in good agreement with those actually observed. A reasonable hypothesis would then be that the compounds of these series can reach, and act at, sites involved with mutagenic activity in comparable fashion. The increases seen in bacterial toxicity, as R_m values increase, reduce permissible drug concentrations until ultimately, with highly lipophilic examples, such a low dose must be employed that no mutagenic activity can be demonstrated. A consequence of this viewpoint would be that the Ames' tests may provide a misleading indication of carcinogenic potential for very lipophilic compounds. Such materials may display high bacterial toxicity and, consequently, low levels of mutagenic activity, but in animals possessing quite different sites which provide toxicity, carcinogenicity might be retained. Mutagenic and antitumor activity for members of the series **5**–**15** can be readily separated by the simple expedient of manipulating agent lipophilic–hydrophilic balance. However, it is not clear whether this is an adequate method of designing out in vivo carcinogenic activity.

M_{50} values suggest that there is no mutagenic activity in the more lipophilic members of the *m*-AMSA series (**22**–**27**). However, M_x values reveal that the drugs are

Table III. Mutagenic Activity of 9-Anilinoacridines in *S. typhimurium* TA 1537



compd	type no.	R_1	R_2	R_3	counterion[a]	R_m[b]	D_{50}[c]	log (M_{50})[d] obsd	log (M_{50})[d] calcd[i]	log (M_{50})[d] diff	M_{max}[e]	M_x[f]	LD_{10}[g]	ILS_{max}[h]	mp, °C	formula	anal.[s]
3	1	R = OH	adriamycin			-0.55	1.63	0[j]			0	0					
4	1	R = H	daunomycin			-0.47	0.74	0			0	0					
5[k]	2	$NHCH_3$	H	H	Br^-	0.24	8.20	2.05	1.98	0.07	534	118	70	53			
6[l]	2	$NHCH_2CH_3$	H	H	Cl^-	0.51	10.0	1.88	1.30	0.38	651	78	*m*	—	279–280	$C_{21}H_{19}N_3 \cdot 2HCl$	C, H, N, Cl
7[l]	2	$NH(CH_2)_2CH_3$	H	H	Cl^-	0.74	9.89	1.67	0.70	0.97	251	47	—	—	291–292	$C_{22}H_{21}N_3 \cdot 2HCl$	C, H, N, Cl
8[l]	2	$NH(CH_2)_3CH_3$	H	H	Cl^-	0.86	0.98	1.00	0.39	0.61	59	10	—	—	281–284	$C_{23}H_{23}N_3 \cdot 2HCl$	C, H, N, Cl
9[l]	2	$NH(CH_2)_5CH_3$	H	H	Cl^-	1.03	0.44	-1.00	-0.05	-0.95	1.5	0.1	—	—	255–257	$C_{25}H_{27}N_3 \cdot 2HCl$	C, H, N, Cl
10[n]	2	$NHSO_2CH_3$	H	H	Cl^-	0.00	11.00	2.10	2.60	-0.50	928	184	66	131			
11[n]	2	$NHSO_2CH_2CH_3$	H	H	Cl^-	0.25	8.16	2.00	1.96	0.04	688	187	330	98			
12[n]	2	$NHSO_2(CH_2)_2CH_3$	H	H	Cl^-	0.43	5.01	1.67	1.49	0.18	277	58	350	82			
13[n]	2	$NHSO_2(CH_2)_3CH_3$	H	H	Cl^-	0.56	4.93	0.78	1.16	-0.38	16	10	350	66			
14[o]	2	$NHSO_2(CH_2)_4CH_3$	H	H	Cl^-	0.66	1.56	0.60	0.90	-0.30	40	11	70	55			
15[o]	2	$NHSO_2(CH_2)_5CH_3$	H	H	Cl^-	0.75	0.94	0.30	0.67	-0.37	18	2.6	120	35			
16[n]	2	$NHSO_2CH_3$	H	3-$NHCOCH_3$	MsO^-	-0.12	28.2	-0.22	2.90	-3.12	6	0.7	19	145			
17[n]	2	$NHSO_2CH_2CH_3$	H	3-$NHCOCH_3$	MsO^-	0.10	22.1	0	2.34	>-2.34	(1)[p]	0.2	28	115			
18[n]	2	$NHSO_2(CH_2)_2CH_3$	H	3-$NHCOCH_3$	MsO^-	0.30	11.45	0	1.83	>-1.83	(1.5)	0.4	33	115			
19[n]	2	$NHSO_2(CH_2)_3CH_3$	H	3-$NHCOCH_3$	MsO^-	0.46	14.8	-0.30	1.42	-1.72	(3)	0.5	39	84			
20[n]	2	$NHSO_2(CH_2)_4CH_3$	H	3-$NHCOCH_3$	MsO^-	0.59	1.77	-0.10	1.08	-1.18	(4)	7.5	40	61			
21[n]	2	$NHSO_2(CH_2)_5CH_3$	H	3-$NHCOCH_3$	MsO^-	0.70	0.31	0	0.80	>-0.80	(1)	7.5	21	43			
22[q]	2	$NHSO_2CH_3$	OCH_3	H	MsO^-	0.18	24.9	0.60	2.13	-1.53	38	5	9	115			
23[r]	2	$NHSO_2CH_2CH_3$	OCH_3	H	MsO^-	0.38	9.32	-0.30	1.62	-1.92	69	8	10.5	89			
24[r]	2	$NHSO_2(CH_2)_2CH_3$	OCH_3	H	MsO^-	0.53	7.76	-0.70	1.24	-1.94	(2)	0.4	28	81			
25[r]	2	$NHSO_2(CH_2)_3CH_3$	OCH_3	H	MsO^-	0.62	6.97	0	1.01	>-1.01	0	0	71	78			
26[l]	2	$NHSO_2(CH_2)_4CH_3$	OCH_3	H	MsO^-	0.68	2.11	0	0.85	>-0.85	(3)	1.1	36	70	272–273	$C_{25}H_{27}N_3O_3S \cdot CH_3SO_3H$	C, H, N, S
27[l]	2	$NHSO_2(CH_2)_5CH_3$	OCH_3	H	MsO^-	0.75	1.53	0	0.67	>-0.67	(6)	1.6	56	67	254–255	$C_{26}H_{29}N_3O_3S \cdot CH_3SO_3H \cdot 0.5H_2O$	C, H, N, S
28[s]	2	$NHSO_2CH_3$	OCH_3	4-$CONH_2$	Cl^-	-0.27	69.8	-0.70	3.29	-3.99	(5)	1	42	112			
29[l]	2	$NHSO_2CH_2CH_3$	OCH_3	4-$CONH_2$	Cl^-	0.00	46.7	0.18	2.60	-2.42	13	3	22	168	252–253	$C_{23}H_{22}N_4O_4S \cdot HCl \cdot H_2O$	C, H, N, Cl
30[l]	2	$NHSO_2(CH_2)_2CH_3$	OCH_3	4-$CONH_2$	Cl^-	0.22	64.2	0.00	2.03	-2.03	6.5	1	35	120	289–290	$C_{24}H_{24}N_4O_4S \cdot HCl$	C, H, N, Cl
31[l]	2	$NHSO_2(CH_2)_3CH_3$	OCH_3	4-$CONH_2$	Cl^-	0.39	33.0	0	1.60	>-1.60	0	0	130	110	276–278	$C_{25}H_{26}N_4O_4S \cdot HCl$	C, H, N, Cl
32[l]	2	$NHSO_2(CH_2)_4CH_3$	OCH_3	4-$CONH_2$	Cl^-	0.49	46.55	0	1.34	>-1.34	(2)	0.6	110	94	267–268	$C_{26}H_{28}N_4O_4S \cdot HCl$	C, H, N, Cl

[a] MsO = methanesulfonate. [b] Relative measure of lipophilic–hydrophilic balance from partition chromatography; see Experimental Section. [c] Concentration (M, × 10^5) to cause 50% inhibition of bacterial growth. [d] Mutation frequency at the D_{50} (× 10^4). [e] Maximum number of drug-induced revertant colonies found. [f] Maximum mutation frequency (× 10^4). [g] LD_{10}, lethal dose for 10% of animals on average, in mg/kg each day, qd 1–5 (see ref 6). [h] ILS_{max}, percentage increase in life span in L1210 assays at the LD_{10} dose (see ref 19). [i] Calculated using eq 5. [j] No revertant colonies detected at the D_{50}. [k] Ref 20. [l] New compounds. [m] No antitumor data available. [n] Ref 14. [o] Ref 19. [p] Numbers of revertant colonies not significantly different from background; see text. [q] Ref 21. [r] Ref 22. [s] Ref 13. [s] Elemental analysis for the elements indicated gave results within ±0.4% of those required by the indicated formula.

Table IV. Correlation Matrix (r Matrix) for Biologic Parameters

	log M_{50}	log M_{max}	log M_X
log D_{50}	0.20	0.24	0.21
log M_{50}		0.94	0.94
log M_X			0.95

mutagenic at doses which kill more than 50% of the cells.

Table III shows that there are factors other than lipophilic–hydrophilic balance affecting mutagenic activity. Comparing compound 11 of the parent AMSA series to approximately equilipophilic members of the other three series (**18**, **22**, and **30**) it is apparent that, although all have similar bacterial toxicities as required by eq 2, their mutagenic activities are quite different; the latter compounds show essentially no mutagenic activity in the TA 1537. Spot tests confirmed that these drugs also showed no significant mutagenic activity toward TA 98 or TA 100. The two highly mutagenic series of compounds **5–9** and **10–15** are the only ones bearing single substitution at the 1′ position, and the activities of these are so similar they can be successfully modeled together in terms of R_m (eq 5). Substitution of either the 9-anilino ring of the AMSA compounds **10–15** to provide the *m*-AMSA series **22–27**, the acridine ring furnishing **16–21**, or substitution of both ring systems to provide **28–32** leads to essentially nonmutagenic compounds. Since all of these variants retain antitumor activity, it is clear that antitumor and mutagenic activity can be separated by appropriate structural modification. Whether the types of mutational events visualized by use of the Ames' bacterial tester strains are all-embracing and cover all pertinent events, which can possibly lead to cancer induction, is not known with certainty. Examination of QSAR, for the agents examined, employing a more diverse range of mutagenicity screening systems is clearly desirable.

The different types of substitution employed with the compounds considered herein are too small in number to develop QSAR embracing substituent effects. A more diverse group of congeners is under study to examine if effective quantitation of substituent effects on mutagenicity is possible.

Experimental Section

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

To monitor the progress of reactions, purification of products, etc., TLC on SiO_2 (Merck SiO_2, F_{254}) was used. R_m values for all agents have been determined employing the reverse-phase system previously described;[15] all figures quoted are the mean of at least three determinations.

***N*-(4-Butanamido-3-methoxyphenyl)pentanesulfonamide** was prepared from equimolar quantities of *N*-(4-amino-2-methoxyphenyl)butanamide[14] and pentanesulfonyl chloride in Py solution as for the preparation of the corresponding methanesulfonamide.[14] The product was crystallized from aqueous EtOH as needles, mp 114–114.5 °C; 78% yield. Anal. ($C_{16}H_{26}N_2O_4S$) C, H, N, S. The corresponding *hexanesulfonamide* [mp 119–119.5 °C; 82% yield. Anal. ($C_{17}H_{28}N_2O_4S$) C, H, N, S] was prepared in a similar fashion.

Assays for Mutagenicity. The bacterial strains were kindly furnished by Dr. B. N. Ames (Biochemistry Department, University of California, Berkeley) and stock cultures were stored at -80 °C. Fresh cultures for mutagenesis testing were obtained by scraping a sterile loop over the surface of the frozen culture, inoculating 10 mL of L broth,[18] and shaking overnight. Cultures not used immediately in the morning were stored in the refrigerator during the day. For each experiment, a series of flasks, each containing the appropriate amount of drug dissolved in 50% ethanol and 0.5 mL of fresh bacterial culture and L broth to give a total volume of 10 mL, were incubated on a shaking water bath at 37 °C for 1 h. Cells were collected by centrifugation and aspiration of the supernatant and then resuspended in 10 mL of physiological saline. Then, 100-μL samples were plated immediately onto each of three minimal plates[4] containing 1 mM histidine and biotin. Other 100-μL samples were diluted 1 in 10^4 and plated, again in triplicate, onto L broth plates. Plates were incubated at 37 °C for 2 days and the colonies counted by standard procedures.

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