column with dichloromethane–ethyl acetate (9:1) gave 1.51 g of **13** as an analytically pure oil.

1-β-D-Ribofuranosyl-2,5-pyrrolidinedione (15). Method E. From Compound 2. To a solution of **2** (220 mg, 0.96 mmol) in methanol (10 mL) was added 10% palladium on activated carbon catalyst (44 mg) under a nitrogen atmosphere. The nitrogen was exchanged with hydrogen and the mixture was stirred at room temperature under a hydrogen atmosphere for 40 min. The catalyst was removed by filtration, and the filtrate was evaporated in vacuo to give colorless crystals of **15**, which melted at 113–114 °C after recrystallization from methanol–petroleum ether.

Method F. From Compound 22. To a solution of **22** (550 mg, 1.76 mmol) in methanol (17 mL) was added 6 N aqueous HCl (1.7 mL), and the solution was stirred for 6 h at 40 °C. The reaction solution was evaporated in vacuo and then dried under high vacuum to give a viscous brown oil (540 mg). Purification of the oil with preparative TLC (silica; ethyl acetate–methanol, 20:1) provided colorless crystals of **15**. The product was identical with **15** obtained from method E in terms of melting point, mixture melting point, and TLC analysis. The NMR and IR spectra of **15** from the two methods were superimposable.

1-[5-O-Acetyl-2,3-O-(1-methylethylidene)-β-D-ribofuranosyl]-3-methyl-1H-pyrrole-2,5-dione (17). Method G. At room temperature, a stirring slurry of the tosylate salt of **16**[28] (3.61 g, 10 mmol) in chloroform (50 mL) was reacted with citraconic anhydride (1.12 g, 10 mmol) and triethylamine (1.11 g, 11 mmol). The reaction mixture was stirred for 1 h, and then the volatile materials were removed under vacuum to provide a yellow oil. The oil was dissolved in acetic anhydride (20 mL), anhydrous sodium acetate (2 g) was added, and the mixture was stirred at 100 °C for 1 h. In the preparation of compound **18**, the sodium acetate was omitted. After the reaction solution cooled to room temperature, ice–water (60 mL) was added and the mixture was stirred for 1 h while cooling with an ice bath. The reaction mixture was dichloromethane extracted (30 mL × 5). The combined extracts were shaken with saturated sodium carbonate solution, followed with brine, and dried ($MgSO_4$). Solvent evaporation gave a brown oil (1.91 g), which was chromatographed on a silica gel column. Elution with dichloromethane–ethyl acetate (9:1) gave **17** (850 mg) as an oil, which gave colorless crystals after standing for several days at room temperature.

1-[5-O-Acetyl-2,3-O-(1-methylethylidene)-β-D-ribofuranosyl]-1H-pyrrole-2,5-dione (19). Method H. The procedure of Montero et al.[28] was used to prepare 2,3-(1-methylethylidene)ribofuranosylamine (**16**) as an oil in 70–90% yield from the corresponding tosylate salt.[10] A solution of **16** (1.26 g, 6.7 mmol) in ether (20 mL) was combined with maleic anhydride (0.67 g, 6.7 mmol) with stirring and cooling in an ice bath. The stirring and cooling was maintained for 1 h, during which time a white precipitate separated from solution. Evaporation of the reaction solution at reduced pressure yielded a foam, which was dissolved in acetic anhydride (20 mL) and treated with anhydrous sodium acetate (2 g). The mixture was stirred for 1 h at 100 °C, then cooled to room temperature, and ice–water (60 mL) was added with stirring and cooling with an ice bath. After 30 min, the mixture was extracted with CH_2Cl_2 (20 mL × 5). The combined extracts were washed with saturated sodium carbonate solution and brine. The dried ($MgSO_4$) dichloromethane solution was evaporated in vacuo to afford a brown oil (1.48 g), which was purified with preparative LC (elution with dichloromethane–ethyl acetate, 19:1) to give **19** (765 mg) as a colorless oil, which crystallized after standing at room temperature for several days. Continued elution of the LC column gave a second colorless oil (94 mg) which was the α-anomer (**20**).

Reaction of a slurry of **16** as the tosylate salt in chloroform with maleic anhydride in the presence of triethylamine gave, after workup as described above, 39% of **19** and 2% of **20**.

Solution Decomposition of 2–4. A solution of **2** (70 mg/mL) in sterile water (pH 6.3) containing succinimide (35 mg/mL) as an internal standard was stored in a standard NMR tube at room temperature. At regular intervals (t = 0, 24, 48, 72, and 96 h) NMR spectra were recorded, and the decrease in isoshowdomycin (**2**) concentration was determined by comparing the integrated areas of the singlet due to the succinimide methylene groups (δ 2.8) with the singlet (δ 6.9) due to the olefinic protons of **2**. The solution half-life of **2** was determined graphically from a plot of concentration vs. time. The solution decompositions of **3** and **4** were measured in a similar manner.

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(28) Montêro, J. L.; Moruzzi, A.; Oiry, J.; Imbach, J. L. *Eur. J. Med. Chem.* **1977**, *12*, 397.

Potential Antitumor Agents. 35. Quantitative Relationships between Antitumor (L1210) Potency and DNA Binding for 4′-(9-Acridinylamino)methanesulfon-*m*-anisidide Analogues

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Factors influencing dose potency of 4′-(9-acridinylamino)methanesulfon-*m*-anisidide (*m*-AMSA) analogues in L1210 assays have been investigated by multiple regression analysis. The dependent variable was D_{40}, the dose to provide 40% life extension in L1210 tests. Independent variables examined were chromatographic R_m values, as a measure of agent lipophilic–hydrophilic balance; R_m^2; log K, where K is the agent–DNA association constant for poly[d(A-T)]; log $T_{1/2}$, the half-life for congener thiolytic cleavage; and agent pK_a values. A regression equation containing terms in R_m^2 and log K was derived with the latter term accepting the greater proportion of the biological variance. DNA binding, of acridine substituted *m*-AMSA variants, is the most important factor influencing dose potency. Modeling of log K for 3-substituted derivatives provided an equation in substituent $\mathcal{R}$ constants and molar refractivities (MR).

During the historical development of the field of quantitative molecular structure–biological activity relationships (QSAR), the almost inevitable lack of knowledge of the strength of interaction between the drug congeners and site of action has required that such interactions be approximated by indirect methods. Within series of substituted drug congeners, changes in drug–site interactions can sometimes be adequately modeled by employing extrathermodynamic substituent parameters. However, such treatments neglect a major contribution that might be made by QSAR methodology. Normally, the site of drug action is inferred from mode of action studies which implicate a critical enzyme, or other cellular macromolecule, as a possible target. The often formidable logistics asso-

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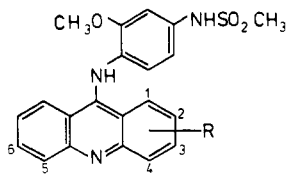
ciated with mode of action studies limits the number of drug congeners which can be so studied and frequently there is examination of a mere single agent, usually that selected for possible clinical trial. Identification of crucial sites by such methods cannot be considered unequivocal. A considerably superior approach would be to show that for a set of congeners, with widely varying levels of biological activity, QSAR methods could demonstrate a quantitative relationship between that activity and the congener association constants for interaction with the putative site.

For the tumor inhibitory agent 4′-(9-acridinylamino)-methanesulfon-*m*-anisidide (*m*-AMSA; 1, R = H), we have



1

suggested that DNA might be the site of action.[1] The recent development of a convenient technique for measuring drug–DNA association constants[1] coupled with an extensive set of biologically active *m*-AMSA congeners[2] together provide an unrivaled opportunity to investigate the above strategy for site identification.

Approach Method. From available L1210 leukemia screening data, two conventionally employed biological responses which could be used in attempted site identification are maximum increase in life span (ILS_{max}) and the molar dose providing a 40% increase in life span (D_{40}).[2] The requirement for widely varying levels of biological activity eliminates log ILS_{max} (range ca. 1.4–2.2) from consideration as a dependent variable. For the present study, log D_{40} (range 3.40–6.38; Table I) is the clearly superior variable.

An additional feature of *m*-AMSA analogues, which could possibly vitiate the results of the above study, is their ready degradation by thiols in vivo.[3,4] Differing congener susceptibilities to thiolytic cleavage might modulate levels of active drug reaching critical sites and therefore influence the D_{40} values. The rates of thiolytic cleavage of all the agents considered (Table I), in the presence of excess thiol (2-mercaptoethanol), have been measured. For use in the linear free-energy type formalism of QSAR, log (thiolytic cleavage rate) would be a suitable investigatory regression term. As measured,[2] the kinetics of thiolysis are first order and, therefore, log (rate) is linearly related to log (half-life) and the latter, as log $T_{1/2}$ values, may be conveniently employed directly in regression equations. In an earlier study,[2] with a limited number of examples, pK_a values proved a significant regression term but were highly covariant with $T_{1/2}$ figures. To examine if $T_{1/2}$ and pK_a values continued covariant within a larger data set and also which was the pertinent variable, the later have been included in the presence analysis. As before, R_m values from reversed-phase partition chromatography have been used as measures of agent lipophilic–hydrophilic balance. For a series of standard compounds, R_m and log P (1-octanol–water) values are related by eq 1.[2]

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

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

The agent association constants (K) measured with the synthetic, double-stranded DNAs poly[d(A-T)] and poly[d(G-C)], as well as calf thymus DNA, proved highly covariant; the lowest correlation coefficient (r) for linear correlations between the log K values for any two of these DNAs was 0.95. Equivalent regression equations result from the use of the log K values for any of the DNAs examined. Accordingly, association constants for binding to poly[d(A-T)] only are provided (Table I) and are those used in regression analyses.

Sigma values for the various ether derivatives **28–31**, whose additional functionality is isolated from the ether oxygen by aliphatic components, have been assumed equal to that of the stem molecule **27**. Similarly, for the 4-carboxamido variants **34–41**, the σ_m value assumed is that of the methylcarboxamide **33**. Scrutiny of the σ-responsive pK_a values for these various compounds supports these assignments.

Results

A cross correlation matrix of all measured drug properties proposed as independent variables is shown in Table II. The stepwise development summarized in Table III provided eq 2. The residuals provided in Table I were

$$\log (1/D_{40}) = 1.32\ (\pm 0.35) \log K - 2.64\ (\pm 1.14)\ R_m^2 - 2.34 \quad (2)$$

$$n = 48,\ r = 0.80,\ s = 0.44,\ F_{2,45} = 39.0$$

calculated from this equation. A pK_a term entered only at the 10% significance level.

Normally drug toxicity, as LD_{10}, is notoriously difficult to model, but in the present series, employing the measured drug physicochemical properties used in the D_{40} analysis, a similar equation resulted (eq 3). Steps in the

$$\log (1/LD_{10}) = 1.00\ (\pm 0.29) \log K - 2.04\ (\pm 0.94)\ R_m^2 - 1.28 \quad (3)$$

$$n = 47,\ r = 0.77,\ s = 0.36,\ F_{2,44} = 33.0$$

development of this equation are provided in Table IV. One compound (**30**) had to be deleted from this analysis since the LD_{10} dose could not be reached.

Further extension to the modeling of a chemotherapeutic index for these agents [log (LD_{10}/D_{40})] then proved possible (eq 4). In this case, R_m rather than R_m^2 was entered

$$\log (LD_{10}/D_{40}) = 0.39\ (\pm 0.23) \log K - 0.39\ (\pm 0.30)\ R_m - 1.42 \quad (4)$$

$$n = 47,\ r = 0.50,\ s = 0.28,\ F_{2,44} = 7.2$$

as the significant second variable (Table V).

Modeling of Drug Physiochemical Properties. Investigation of the possible underlying features associated with covariance of log $T_{1/2}$ and pK_a values (see Table II) first utilized the subset of 3-monosubstituted *m*-AMSA analogues (**2–19**). For these compounds, pK_a is understandably highly correlated with σ_p (eq 5) and appreciably less well fit by σ_m values.

compounds **2–19**

$$pK_a = -2.41\ (\pm 0.20)\ \sigma_p + 7.42 \quad (5)$$

$$n = 18,\ r = 0.99,\ s = 0.19,\ F_{1,16} = 577.2$$

(1) Cain, B. F.; Baguley, B. C.; Denny, W. A. *J. Med. Chem.* **1978**, *21*, 658.
(2) Denny, W. A.; Atwell, G. J.; Cain, B. F. *J. Med. Chem.* **1979**, *22*, 1453; see also references quoted therein.
(3) Cain, B. F.; Wilson, W. R.; Baguley, B. C. *Mol. Pharmacol.* **1976**, *12*, 1027.
(4) Wilson, W. R.; Cain, B. F.; Baguley, B. C. *Chem.-Biol. Interact.* **1977**, *18*, 163.

Table I. Structural and Physiochemical Details for the *m*-AMSA Analogues Considered

no.	R	R_m [a]	pK_a [b]	σ [c]	log K [d]	$T_{1/2}$ [e]	LD_{10} [f]	log $(1/D_{40})$ [g] obsd	calcd	diff
2[h]	H	0.18	7.43	0.00	5.57	13.2	9.0	5.29	4.93	0.36
3[h]	3-$NHCOCH_3$	0.07	7.56	0.00	6.24	23.6	22.5	5.98	5.88	0.10
4[i]	3-N=[j]	−0.14	5.53	(0.78)[k]	5.09	2.01	125	4.33	4.33	0
5[i]	3-NH_2	0.06	9.92	−0.66	6.21	1285	8	5.52	5.85	−0.33
6[i]	3-$NHCOOCH_3$	0.25	7.77	−0.15	6.37	35.1	11	5.48	5.90	−0.42
7[k]	3-$NHCH_3$	0.17	10.05	−0.84	6.17	1956	1.7	6.38	5.73	0.65
8[i]	3-NO_2	0.10	5.72	0.78	5.65	1.9	4.83	5.38	5.09	0.29
9[l]	3-CH_3	0.44	7.70	−0.17	5.95	32.6	7	5.52	5.00	0.52
10[l]	3-CH_2CH_3	0.56	7.65	−0.15	5.66	29	40	4.77	4.30	0.47
11[l]	3-$CH(CH_3)_2$	0.68	7.66	−0.15	5.46	28.3	450	3.40	3.65	−0.25
12[i]	3-OCH_3	0.29	7.81	−0.27	5.83	51.3	6	5.66	5.13	0.53
13[i]	3-F	0.31	7.05	0.06	5.54	6.34	26	5.07	4.72	0.35
14[l]	3-Cl	0.32	6.84	0.23	6.06	4.6	13	5.42	5.39	0.03
15[l]	3-Br	0.34	6.84	0.23	6.29	4.4	17	5.36	5.66	−0.30
16[l]	3-I	0.41	6.77	0.18	6.35	4.5	15	5.56	5.60	0.04
17[i]	3-CN	0.06	5.94	0.66	5.65	1.83	75	4.26	5.11	−0.85
18[m]	3-$CONH_2$	−0.41	6.66	0.36	5.66	3.57	110	4.83	4.69	0.14
19[l]	3-CF_3	0.54	6.24	0.54	5.24	2.26	130	4.17	3.81	0.36
20[k]	2-NH_2	−0.15	7.37	−0.16	5.95	233	8	5.87	5.45	0.42
21[i]	2-CH_3	0.40	7.45	−0.07	5.35	32.9	300	3.69	4.30	−0.61
22[i]	2-$CH(CH_3)_2$	0.66	7.40	−0.07	5.39	49.7	480	3.58	3.62	−0.04
23[i]	2-F	0.32	6.71	0.34	5.28	10.3	90	4.73	4.36	0.37
24[i]	2-I	0.36	6.60	0.35	5.70	6.32	170	3.67	4.84	−1.17
25[m]	4-N=[j]	−0.07	6.09	(0.71)[k]	5.27	1.12	70	4.41	4.60	−0.19
26[m]	4-OCH_3	0.19	7.39	0.12	5.94	13.4	5	5.34	5.41	−0.07
27[m]	4-OCH_2CH_3	0.43	7.37	0.10	5.77	13.1	27	4.80	4.79	−0.01
28[m]	4-OCH_2CH_2OH	0.10	7.36	(0.10)	5.74	14.8	12	5.41	5.21	0.20
29[m]	4-$OCH_2CH(OH)CH_2OH$	−0.09	7.30	(0.10)	5.90	11.4	23	5.60	5.43	0.17
30[m]	4-$OCH_2CONHCH_3$	−0.02	7.35	(0.10)	5.80	10.8	>500	5.51	5.31	0.20
31[m]	4-$O(CH_2)_3CONH_2$	−0.15	7.36	(0.10)	5.74	10.9	25	5.26	5.18	−0.08
32[m]	4-$CONH_2$	−0.27	6.37	0.28	5.47	2.52	42	4.65	4.69	−0.04
33[m]	4-$CONHCH_3$	0.06	6.36	0.35	5.54	3.01	9.5	5.23	4.96	0.27
34[m]	4-$CON(CH_3)_2$	0.09	6.16	(0.35)	5.04	3.29	24	4.74	4.29	0.45
35[i]	4-CON-c-C_4H_8O[n]	−0.18	6.03	(0.35)	4.96	3.55	200	3.91	4.12	−0.21
36[m]	4-$CONH(CH_2)_3CH_3$	0.47	6.36	(0.35)	5.40	2.89	28	4.38	4.20	0.18
37[i]	4-$CONH(CH_2)_2OH$	−0.16	6.27	(0.35)	5.42	3.29	40	5.11	4.75	0.36
38[i]	4-$CONH(CH_2)_3OH$	−0.15	6.30	(0.35)	5.40	3.37	80	4.84	4.73	0.11
39[i]	4-$CONHCH_2CHOHCH_3$	0.09	6.20	(0.35)	5.34	2.94	60	5.53	4.69	0.84
40[m]	4-$CONHCH_2CHOHCH_2OH$	−0.36	6.34	(0.35)	5.26	2.84	115	4.52	4.26	0.26
41[m]	4-$CONHCH_2CONH_2$	−0.50	6.18	(0.35)	5.39	3.91	25	4.65	4.11	0.54
42[l]	4-CH_3	0.25	7.39	−0.07	6.03	11.1	8.5	5.74	5.45	0.29
43[m]	4-$(CH_2)_2CONCH_2$	−0.17	7.15	(−0.07)	5.65	8.44	13.5	5.56	5.04	0.52
44[m]	4-C_6H_5	0.45	7.02	0.06	5.60	3.20	190	3.92	4.52	−0.60
45[i]	4-F	0.21	6.33	0.34	5.65	4.28	23	5.10	5.00	0.10
46[i]	4-Cl	0.23	6.11	0.37	5.76	2.42	44	4.43	5.12	−0.69
47[i]	4-Br	0.25	6.09	0.39	5.57	2.36	80	4.50	4.85	−0.35
48[i]	4-CN	−0.03	4.87	0.56	5.01	3.78	100	4.08	4.27	−0.19
49[l]	4-NO_2	0.09	5.05	0.71	5.20	2.81	90	3.90	4.50	−0.60

[a] $R_m = \log(1/R_f - 1)$. Measure of agent lipophilic-hydrophilic balance from reverse-phase partition chromatography; see ref 12. [b] Acridine pK_a measured UV spectrophotometrically; see ref 13. [c] σ is σ_p for acridine 3-substituents and σ_m for 2- and 4-substituents. Values taken from ref 10. Values assumed by analogy are provided in parentheses. [d] K is the agent association constant measured for binding to poly[d(A-T)]; see text. [e] $T_{1/2}$ is the half-life of the agent in the presence of excess 2-mercaptoethanol under standard conditions; see ref 2. Assumed values are provided in parentheses. [f] Lethal dose for 10% of treated animals on average; obtained by the methods of ref 12. [g] D_{40} is the molar dose of agent providing a 40% increase in the life span of leukemic mice, obtained by the methods of ref 12: obsd, the figure observed; calcd, that calculated from eq 2; diff, the difference between observed and calculated. [h] Reference 2. [i] New compound; see Table VIII for details. [j] Aza homologue; —N= replacing ring —C=. σ values taken to be that of a NO_2 group. [k] Reference 7. [l] Reference 8. [m] Reference 9. [n] NcC_4H_8O used as an abbreviation for the morpholide.

Table II. Squared Correlation Matrix for Variables Examined with Log $(1/D_{40})$

	log $(1/LD_{10})$	log K	R_m^2	R_m	pK_a	log $T_{1/2}$
log $(1/D_{40})$	0.81	0.47	0.18	0.04	0.23	0.17
log $(1/LD_{10})$	1	0.44	0.18	0.01	0.21	0.16
log K		1	0.00	0.05	0.43	0.26
R_m^2			1	0.41	0.04	0.01
R_m				1	0.09	0.04
pK_a					1	0.78

Table III. Stepwise Development of Multivariable Equations for Log $(1/D_{40})$

intercept	log K	R_m^2	pK_a	R_m	r	s	$F_{1(x)}$[a]
−2.71	1.35				0.68	0.52	40.6 (46)
−2.34	1.32	−2.64			0.80	0.44	20.4 (45)
−1.88	1.04	−2.92	0.17		0.81	0.43	3.2[b] (44)
−2.29	1.10	−2.24	0.18	−0.42	0.82	0.42	1.9[b] (43)

[a] $F_{1(x)}$ is the F statistic for introduction of each single variable, with the degrees of freedom (x) in parentheses. [b] Not significant at 5% level.

Equation 5 can be effectively extended to cover the 2- and 4-substituted derivatives if σ_m values for these are employed (eq 6). In this equation $\sigma = \sigma_p$ for 3-substituted and σ_m for 2- and 4-substituted variants.

$$pK_a = -2.58\ (\pm 0.25)\ \sigma + 7.31 \quad (6)$$

$$n = 48,\ r = 0.95,\ s = 0.29,\ F_{1,46} = 397.6$$

Log $T_{1/2}$ is also clearly σ dependent, and for the 3-monosubstituted compounds **2–19**:

$$\log T_{1/2} = -1.87\ (\pm 0.32)\ \sigma_p + 1.32 \quad (7)$$

$$n = 18,\ r = 0.947,\ s = 0.30,\ F_{1,16} = 133$$

This poorer correlation, in relation to that seen with pK_a (eq 5), is not due to scatter in the experimental data, since a plot of log $T_{1/2}$ vs. σ_p is a smooth curve (Figure 1). Further investigation of the thiolytic cleavage reaction (unpublished observations) shows that the critical interaction is between agent cation and thiol anion. Identical conclusions were reached by Wild and Young[5] in an examination of the thiolysis of certain 9-aminoacridine derivatives with hydrosulfide anion. Since the *m*-AMSA agents have pK_a values in the physiological range, correction for agent ionization is then warranted. Such ionization-corrected log $T_{1/2}$ values then show a straight-line relationship with σ_p values (Figure 2), and an excellent regression equation (8) can be derived.

$$\log T_{1/2} + \log \frac{[H^+]}{[H^+] + K_a} = -2.80\ (\pm 0.17)\ \sigma_p + 0.99 \quad (8)$$

$$n = 18,\ r = 0.993,\ s = 0.15,\ F_{1,16} = 1076$$

In this equation, $[H^+]/([H^+] + K_a) = \alpha$, the fraction of ionized agent.

As with the pK_a correlation, by employment of σ_m for 2- and 4-substituents this regression equation can be extended to all compounds in the data set (Table I) for which σ values can be obtained (eq 9).

$$\log T_{1/2} + \log \alpha = \pm 3.07\ (-0.08)\ \sigma + 0.96 \quad (9)$$

$$n = 48,\ r = 0.95,\ s = 0.32,\ F_{1,46} = 509$$

It may be noted that, since pK_a is a σ-dependent variable (eq 6), K_a in eq 8 may effectively be replaced by an exponential term in σ_p, a quite complex overall equation in σ then resulting. There have been hesitations about employing more complex functions of σ in QSAR derivations, but the above example suggests that these may in certain circumstances be quite justifiable.

Earlier[6] we modeled the DNA-association constants of a series of simple anilino-ring substituted 9-anilinoacridines in terms of two fundamental substituent properties, σ and MR. In continuation, we have attempted to model the association constants for the acridine-substituted analogues in the present work. The greatest range of structural types is seen within the 3-substituted set (Table I), these also being of the greatest biological interest, and attempted modeling of log K has therefore been restricted to this group. Again, σ and MR appear to be the dominant variables (Table VI).

$$\log K = -0.34\ (\pm 0.34)\ \sigma_p + 0.036\ (\pm 0.031)\ MR + 5.56 \quad (10)$$

$$n = 18,\ r = 0.68,\ s = 0.30,\ F_{2,15} = 6.5$$

However, if our drug–site model for these agents is correct, with the acridine moiety intercalated into the DNA stack and the 9-anilino function residing in the minor groove,[6] then substituent space about groups on the anilino ring or the acridine 3 position are quite different in nature. Accordingly, we felt that examination of the role played by field ($\mathcal{F}$) and resonance ($\mathcal{R}$) components of σ_p values for acridine 3-substituents, where these groups reside in the intercalation site, could be of interest. $\mathcal{R}$ values, in fact, accepted a significantly greater proportion of the variance in log K values than did σ_p (Table VI), and an $\mathcal{F}$ term failed to prove significant. Equation 11 was used to calculate the residuals in Table VII.

$$\log K = -0.71\ (\pm 0.46)\ \mathcal{R} + 0.037\ (\pm 0.026)\ MR + 5.42 \quad (11)$$

$$n = 18,\ r = 0.76,\ s = 0.27,\ F_{2,15} = 10.5$$

Table IV. Stepwise Development of Multivariable Equation for Log $(1/LD_{10})$

intercept	log K	$R_m{}^2$	pK_a	r	s	$F_{1(x)}{}^a$
−1.55	1.01			0.66	0.42	35.0 (45)
−1.28	1.00	−2.04		0.77	0.36	17.9 (44)
−0.94	0.79	−2.26	0.13	0.79	0.35	2.7[b] (43)

[a,b] See corresponding footnotes in Table III.

Table V. Stepwise Development of Multivariable Equation for Log (LD_{10}/D_{40})

intercept	log K	pK_a	R_m	r	s	$F_{1(x)}{}^a$
1.11	−0.33			0.37	0.30	7.2
1.42	−0.39		0.39	0.50	0.28	6.4
1.33	−0.32	−0.05	0.41	0.51	0.28	0.6[b]

[a,b] See corresponding footnotes in Table III.

Table VI. Squared Correlation Matrix for Variables in Analysis of Log K for 3-Substituted *m*-AMSA Subset

	log K	σ_p	$\mathcal{F}$	$\mathcal{R}$	MR	π
log $(1/D_{40})$	0.55	0.24	0.03	0.41	0.02	0.17
log K	1	0.27	0.06	0.38	0.33	0.05
σ_p		1	0.71	0.80	0.08	0.02
$\mathcal{F}$			1	0.26	0.09	0.00
$\mathcal{R}$				1	0.04	0.05
MR					1	0.00

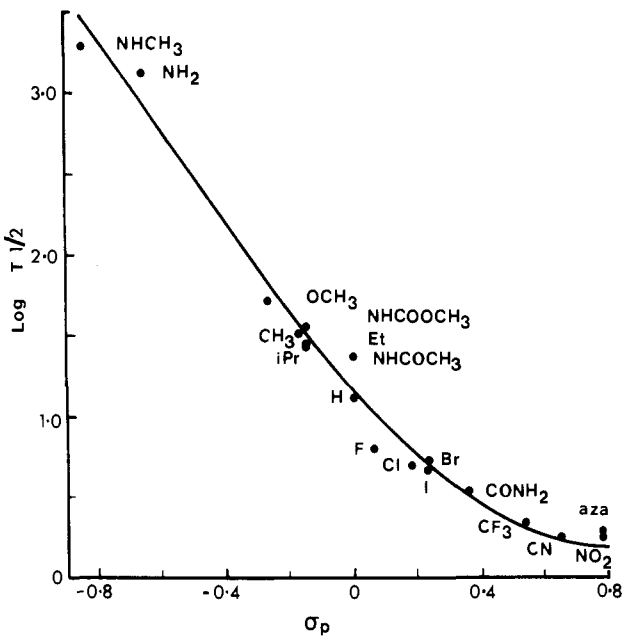


Figure 1. Relationship between agent half-lives for thiolytic cleavage ($T_{1/2}$) and Hammett's σ_p constants.

(5) Wild, F.; Young, J. M. *J. Chem. Soc.* **1965**, 7261.
(6) Part 34: Baguley, B. C.; Denny, W. A.; Atwell, G. J.; Cain, B. F. *J. Med. Chem.* **1981**, *24*, 170.

Table VII. Extrathermodynamic Parameters[a] Employed for Modeling Drug-DNA Association Constants (K) of 3-Substituted m-AMSA Analogues

no.	3-substit in 1	$\mathcal{F}$	$\mathcal{R}$	MR	π	log K[b] obsd	log K[b] calcd	log K[b] diff
2	H	0.00	0.00	1.03	0.00	5.57	5.46	0.11
3	$NHCOCH_3$	0.28	−0.26	14.93	−0.97	6.24	6.16	0.08
4	N=	0.67	0.16	0.50	−1.34	5.09	5.32	−0.13
5	NH_2	0.02	−0.68	5.42	−1.23	6.21	6.10	0.11
6	$NHCOOCH_3$	0.14	−0.28	15.53	−0.33	6.37	6.19	0.18
7	$NHCH_3$	−0.11	−0.74	10.33	−0.47	6.17	6.33	−0.16
8	NO_2	0.67	0.16	7.36	−0.28	5.65	5.58	0.07
9	CH_3	−0.04	−0.13	5.65	0.56	5.95	5.72	0.23
10	CH_2CH_3	−0.05	−0.10	10.30	1.02	5.66	5.87	−0.21
11	$CH(CH_3)_2$	−0.06	−0.08	14.96	1.53	5.46	6.03	−0.57
12	OCH_3	0.26	−0.51	7.87	−0.02	5.83	6.07	−0.24
13	F	0.43	−0.34	0.92	0.14	5.54	5.70	−0.16
14	Cl	0.41	−0.15	6.03	0.71	6.06	5.75	0.31
15	Br	0.44	−0.17	8.88	0.86	6.29	5.87	0.42
16	I	0.40	−0.19	13.94	1.12	6.35	6.07	0.28
17	CN	0.51	0.19	6.33	−0.57	5.65	5.52	0.13
18	$CONH_2$	0.24	0.14	9.81	−1.49	5.66	5.68	−0.02
19	CF_3	0.38	0.19	5.02	0.88	5.24	5.47	−0.23

[a] Parameter values have been taken from the compilation of ref 10. The σ_p values employed are provided in Table I. [b] Calculated values obtained from eq 11.

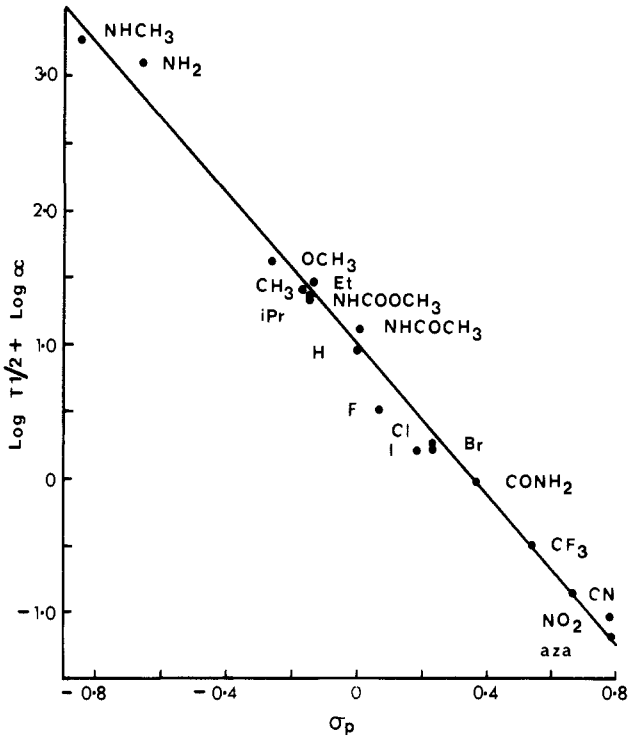


Figure 2. Relationship between $T_{1/2}$ values corrected for the effects of partial ionization and Hammett's σ_p constants. Fraction ionized = $\alpha = [H^+]/([H^+] + K_a)$.

The lack of dependence on $\mathcal{F}$ values contrasts with the earlier analysis of DNA binding of anilino-ring substituted variants[6] and the regression equations derived for pK_a and log $T_{1/2}$ in this work (eq 5 and 8). In these three cases both $\mathcal{R}$ and $\mathcal{F}$ terms enter into regression equations with closely similar coefficients, strongly suggestive of σ_p dependence, and fail to provide significant improvement over the use of this parameter aone.

Regression equation terms in π, hopefully modeling hydrophobic contacts in the intercalation site, could not be demonstrated to be of significance. Unfortunately, the lack of an adequate range of E_s values, for the groups involved, prevented simple examination of the steric role of 3-substituents, presumed important within the close confines of a DNA intercalation site.

Discussion

The demonstration of a quantitative relationship between agent–DNA association constants and dose potency, for acridine-ring substituted m-AMSA variants, provides strong supportive evidence that DNA is the site of action of these agents.

There is a clear dependence on R_m^2, while R_m is not significantly accepted into regression equations. This results from the optimum R_m value being close to zero.

For equations dealing with both D_{40} and LD_{10}, the pK_a values and half-lives for agent thiolytic cleavage ($T_{1/2}$) play no significant role. It is not known whether the high collinearity between pK_a, log $T_{1/2}$, log K, and σ could mask a possible dependence of D_{40} or LD_{10} on pK_a, log $T_{1/2}$, or σ additional to that of log K.

Animal toxicity, as provided by LD_{10} values, appears responsive to the same factors as D_{40}. However, the successful modeling of the chemotherapeutic index LD_{10}/D_{40} (eq 4) shows that as agent–DNA binding increases, selectivity toward the tumor population increases more rapidly than does toxicity. The negative dependence on R_m suggests that hydrophilic agents are likely to prove more effective.

By our proposed site model,[6] the 9-anilino ring of the AMSA agents lies in the minor groove of DNA, with the 1′-substituent located above a phosphodiester group and acridine 3-substituents lying within the confines of the intercalation site. It is then intriguing to find that attempted modeling of drug–DNA association constants clearly demonstrates the differences in substituent space about these two positions, with binding shown responsive[6] to σ_p for 1′- but to $\mathcal{R}$ values for 3-substituents.

The location of 1′-substituents and the 9-anilino ring to which they are attached, in close proximity to site anions, will likely ensure that site anion–substituent dipole interactions will play a significant role.[6] We earlier illustrated that the component dipole vectors ($\mu \cos \theta$) of 1′-substituents through the 1′–4′ axis of the 9-anilino function are linearly related to the corresponding σ_p values.[6] A binding dependence on σ_p values for 1′-substituents would then be in good accord with the physical realities of our site model. In contrast, the sandwich-like nature of intercalation complexes appears admirably suited for extensive orbital overlap of the intercalated chromophore and the purine–pyrimidine base pairs. It could be hypothesized

Table VIII. Analytical Details for Previously Unreported Compounds in Table I

no.	mp, °C	formula	anal.[a]
6	240–242	$C_{23}H_{22}N_4O_5S\cdot HCl\cdot 0.5H_2O$	C, H, N, Cl
12	209–210	$C_{22}H_{21}N_3O_4S\cdot HCl$	C, H, N, Cl
13	286–288	$C_{21}H_{18}FN_3O_3S\cdot HCl\cdot H_2O$	C, H, N, Cl
17	200–203	$C_{22}H_{18}N_4O_3S\cdot HCl\cdot H_2O$	C, H, N, Cl
21	276–278	$C_{22}H_{21}N_3O_3S\cdot HCl$	C, H, N, Cl
22	280–281	$C_{24}H_{25}N_3O_3S\cdot HCl$	C, H, N, Cl
23	221–223	$C_{21}H_{18}FN_3O_3S\cdot HCl$	C, H, N
24	288–290	$C_{21}H_{18}IN_3O_3S\cdot HCl$	C, H, N, I
35	194–196	$C_{26}H_{26}N_4O_5S\cdot HBr$	C, H, N, Br
37	282–283	$C_{24}H_{24}N_4O_5S\cdot HCl$	C, H, N, Cl
38	252–253	$C_{25}H_{26}N_4O_5S\cdot HCl$	C, H, N, Cl
39	212–215	$C_{25}H_{26}N_4O_5S\cdot HCl\cdot H_2O$	C, H, N, Cl
45	295–296	$C_{21}H_{18}FN_3O_3S\cdot HCl\cdot 0.5H_2O$	C, H, N
46	268–269	$C_{21}H_{18}ClN_3O_3S\cdot HCl\cdot H_2O$	C, H, N, Cl
47	263–265	$C_{21}H_{18}BrN_3O_3S\cdot HCl\cdot H_2O$	C, H, N, Br
48	256–257	$C_{22}H_{18}N_4O_3S\cdot HCl\cdot 0.5H_2O$	C, H, N, Cl

[a] Analyses for the indicated elements were within ±0.4% of the theoretical values for the formula provided.

that acridine 3-substituents contribute to DNA binding by resonance ($\mathcal{R}$) effects which modulate pertinent molecular orbital energies.

Experimental Section

Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values for the formula quoted. 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 marker's 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. R_m values were determined by the partition chromatographic methods detailed in ref 12 and are the mean of at least four determinations.

Agent–DNA association constants were determined by the ethidium-displacement technique exactly as described earlier.[6]

$T_{1/2}$ and pK_a values were measured in 20% aqueous DMF solution employing the conditions described in full earlier.[2,13]

Preparation of the new agents listed in Table I followed the general procedures detailed earlier.[8,11]

2-[(4-Isopropylphenyl)amino]benzoic acid was prepared by the modified Jourdan–Ullmann conditions evolved earlier[8] employing 4-isopropylaniline and 2-chlorobenzoic acid. Workup as before[8] afforded the desired product in 41% yield as pale-yellow needles from HOAc–H_2O, mp 185–186 °C. Anal. ($C_{16}H_{17}NO_2$) C, H, N.

2-Isopropyl-9(10*H*)-acridone was prepared by polyphosphate ester ring closure of the aforementioned product as before.[8,11] Reaction at 95 °C for 1 h provided clean conversion to the acridone, pure product being obtained as yellow needles from HOAc: 87% yield; mp 261–263 °C. Anal. ($C_{16}H_{15}NO$) C, H, N.

The 4-carboxamido variants **35** and **37–39** were prepared from the previously described[9] 4′-[9-[4-[(4-nitrophenoxy)carbonyl]-acridinyl]amino]methanesulfon-*m*-anisidide by suspension of the latter in DMF (4 mL/g) and then addition to the stirred mixture of the necessary amine component (1.05 molar equiv) followed by Et_3N (1.05 molar equiv). Homogeneous solutions rapidly resulted, and when TLC monitoring demonstrated complete conversion the mixture was acidified with a slight excess of HOAc followed by slow addition of 20% aqueous NaCl (20% aqueous NaBr for **35**) until turbid. Once crystallization had initiated, excess of the salt solution was added to complete separation. Recrystallization was as before[8,11] to provide the products listed in Table I.

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(7) Atwell, G. J.; Cain, B. F.; Denny, W. A. *J. Med. Chem.* **1977**, *20*, 520.
(8) Cain, B. F.; Atwell, G. J.; Denny, W. A. *J. Med. Chem.* **1975**, *18*, 1110.
(9) Cain, B. F., Atwell, G. J.; Denny, W. A. *J. Med. Chem.* **1977**, *20*, 987.
(10) Hansch, C.; Leo, A.; Unger, S. H.; Kim, K. H.; Nikaitani, D.; Lien, E. J. *J. Med. Chem.* **1973**, *16*, 1207.
(11) Cain, B. F.; Atwell, G. J.; Denny, W. A. *J. Med. Chem.* **1976**, *19*, 772.
(12) Denny, W. A.; Cain, B. F. *J. Med. Chem.* **1978**, *21*, 430.
(13) Atwell, G. J.; Cain, B. F.; Denny, W. A. *J. Med. Chem.* **1977**, *20*, 1128.

Structure–Activity Relationship in Cinnamamides. 3.[1] Synthesis and Anticonvulsant Activity Evaluation of Some Derivatives of (*E*)- and (*Z*)-*m*-(Trifluoromethyl)cinnamamide

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The (*E*)- and (*Z*)-*m*-(trifluoromethyl)-α,β-dimethylcinnamamides and some of their *N*-alkyl derivatives were prepared and pharmacologically tested as anticonvulsant agents in order to verify if a ring substituent, like the *m*-CF_3 group, different from a halogen but possessing the same electronic effect could lead to equally active compounds. Some (*E*)-*m*-(trifluoromethyl)-α-methyl- and -non-methyl-substituted-cinnamamides were also prepared and tested. In the α,β-dimethyl series the results show that the *m*-CF_3 group leads to products more active than the ones unsubstituted on the phenyl ring but still less active than the *p*-halogen-substituted compounds previously studied. In the α-methyl and non-methyl-substituted series, the trend shows the *m*-CF_3 group being able to produce less toxic and, in some cases, more active products than the previously studied amides.

A previous paper in this series[2] showed that a series of (*E*)- and (*Z*)-*N*-alkyl-α,β-dimethylcinnamamides exhibited a clear activity on the central nervous system. The *E* isomer revealed CNS-depressant and anticonvulsant ac-

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