Potential Antitumor Agents. 34. Quantitative Relationships between DNA Binding and Molecular Structure for 9-Anilinoacridines Substituted in the Anilino Ring

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Received May 12, 1980

In an investigation of the structure activity relationships in the 4′-(9-acridinylamino)methanesulfonanilide (AMSA) tumor inhibitory analogues, the DNA-binding properties of a series of simple 9-anilinoacridines were examined. Positional numbering as in the AMSA series has been employed. DNA binding was determined by drug competition with the fluorochrome ethidium for available sites. The decrease in fluorescence of a DNA–ethidium complex by the addition of drug is due to both drug displacement of bound ethidium and quenching of the fluorescence of bound ethidium by bound drug; measurement of both factors allows drug–DNA association constants (K) to be determined. DNA binding is augmented by 1′ or 2′ electron donor substituents, and significant correlation equations have been derived with Hammett's σ_p or σ_m constants. Group molar refractivity (MR) for 1′-substituents is an additional significant regression equation term for binding, while the values for 2′ and 3′ groups play no significant role. Most 3′-substituents decrease binding, presumably as a result of steric inhibition of entry of the acridine nucleus into intercalation sites. A 3′-$NHSO_2CH_3$ and 3′-$NHCOCH_3$ substituent confer selectivity of binding to poly[d(G-C)] and poly[d(A-T)], respectively. It is suggested that a combination of H-bond formation and stereochemical features, coupled with steric hindrance, provides the selectivity observed. Binding data are consistent with a model in which the acridine nucleus occupies an intercalation site and the noncoplanar 9-anilino ring resides in the DNA minor groove.

Structural details of certain double-stranded deoxyribonucleic acids are now known with considerable precision. Synthetic regular polymeric DNAs are readily available and, since these lack the heterogeneity of their naturally occurring counterparts, they offer considerable advantages as site models for examining fundamental interactions which may take place between bioactive drugs and their sites of action.

An extensive series of 9-anilinoacridines has been synthesized in this laboratory and shown to have a wide range of biological activity, many members possessing experimental antitumor activity.[1] Preliminary investigations have suggested that there is a relationship between drug–DNA binding ability and antitumor activity in vivo.[2] A study of the structural features influencing the DNA binding ability of the 9-anilinoacridines might then assist in the design of more effective antitumor agents.

The above fundamental and practical considerations have prompted a study of the binding of a subset of the 9-anilinoacridines to certain regular synthetic DNAs, and initial results are reported herein.

Measurement of Drug–DNA Association Constants. Fluorescence of Drug–Ethidium–DNA Mixtures: Determination of C_{50} Values. Fluorescence of ethidium is markedly enhanced when bound to DNA.[3] For example, with poly[d(A-T)] and ethidium, employing an exciting wavelength of 546 nm and monitoring emission at 595 nm, there is a 50-fold augmentation of fluorescence. Addition of a second DNA binding ligand to such DNA–ethidium complexes provides a reduction of fluorescence. The micromolar drug concentration necessary to reduce the fluorescence of initially DNA-bound ethidium by 50%, under standard assay conditions, has been defined as the C_{50} value of the drug.[4,5] In the present study, ethidium displacement assays were performed at pH 5 to ensure that the added drugs were present predominantly as the cation.

Quenching of DNA-Bound Ethidium Fluorescence. Equilibrium dialysis experiments have demonstrated that small quantities of the 3′-methoxy derivative of 14 (*m*-AMSA) are able to produce a quite marked drop in the fluorescence of DNA-bound ethidium under conditions where relatively small quantities of the latter are displaced.[6] Considerable experimentation, certain facets of which are summarized below, have led to the conclusion that many DNA-bound intercalating agents can provide a quenching of the fluorescence of DNA-bound ethidium. The stoichiometry of drug displacement in the above equilibrium dialysis experiments appears well accounted for by the neighboring site exclusion model of McGhee and von Hippel,[7] in which the effective site size for each intercalating ligand is two base pairs. From spectrophotometric titrations, both ethidium[8] and *m*-AMSA[9] individually bind to calf thymus DNA with an apparent site size of two base pairs.

m-AMSA is without effect on the weak fluorescence of ethidium itself when DNA is absent. The fluorescence quenching of DNA–ethidium complexes is not accompanied by a corresponding decrease in fluorescence lifetimes.[6] It is therefore not due to resonant energy transfer of the Förster type.[10] On theoretical grounds the latter would not be expected with any of the anilinoacridines of Table III, since there is no overlap between the fluorescence excitation spectrum of the DNA-bound ethidium and the absorption spectra of the DNA-bound forms of these acridines.

To estimate the drug–DNA association constants (K) of the 9-anilinoacridines from measurement of the drug-induced decrease in fluorescence of ethidium in the presence of DNA (C_{50} values), the extent of quenching must also be measured. The experiments summarized in Figure 1 (data gained with insufficient ethidium present to occupy all available poly[d(A-T)] sites) demonstrate

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(7) McGhee, J. D.; von Hippel, P. H. *J. Mol. Biol.* **1974**, *86*, 469.
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(10) Förster, T. *Disc. Faraday Soc.* **1959**, *27*, 7.

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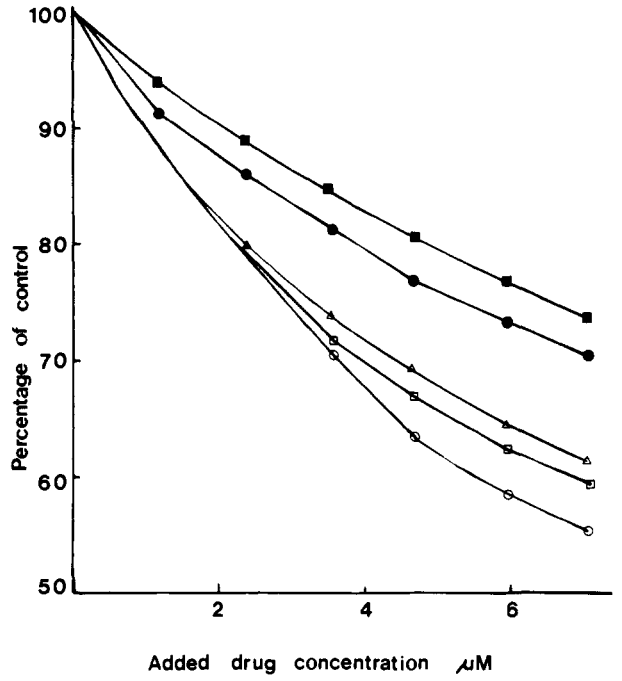

Figure 1. Fluorescence (measured at 595 nm with excitation at 546 nm) of a complex of ethidium and poly[d(A-T)] (20 μM in base pairs) in the presence of various concentrations of compound 14 (Table III). The fluorescence is expressed as a percentage of the control fluorescence caused by 3 (○–○), 4 (□–□), 6.5 (△–△), 9 (●–●), or 14 μM (■–■) ethidium.

certain essential features of the quenching phenomenon. The extent of quenching can be gauged from the fluorescence decrease seen when an initial ethidium binding ratio of 0.1 molecules per DNA base pair is employed (Figure 1, lower curve) and AMSA is added at a ratio of 0.1 molecules per base pair, that is, at a level well below available binding sites. Under these conditions, fluorescent emission decreases by 18%, but the calculated displacement of ethidium (see below) is under 0.4%. Curves such as the lower one in Figure 1 were determined for each of the compounds of Table III. The percentage decrease in fluorescence seen with added drug concentrations of 2 μM and an initial ethidium binding ratio of 0.1 are provided as quenching values (Q) in Table III. There is wide variation in Q values for the compounds examined. It is important to note that the presence of DNA-bound drug does not decrease the fluorescence of bound ethidium by absorption of incident light, since the excitation wavelength employed (546 nm) is above the absorption spectrum of the drugs examined either free or bound to DNA.

Calculation of Drug–DNA Association Constants. The measurement of the decrease in fluorescence in the C_{50} assay (ethidium in excess) and the quenching assay (limited ethidium) permits the drug–DNA association constants to be derived, subject to the assumptions detailed (see Experimental Section). To ascertain if the quench-corrected method does provide reasonable values for association constants, the binding of seven acridine derivatives to calf thymus DNA was investigated employing this technique. K values for these compounds have been determined using either equilibrium dialysis[11] or, in four cases,[9] spectrophotometric titrations. The K value for ethidium (K_e), necessary for calculations employing eq 10–17 (see Experimental Section), was taken from the study of Gaugain et al.,[8] who determined the binding constant of ethidium to calf thymus DNA at a range of ionic strengths employing the treatment of McGhee and von Hippel.[7] From that work, at 0.01 M ionic strength, $K_e = 2.1 \times 10^6\ M^{-1}$, expressed in base-pair units. The K values of the acridines, obtained by the three methods of determination, were in excellent agreement (Table I).

Table I. Determination of Association Constants for Calf Thymus DNA: Comparison of Ethidium Displacement Method with Classical Methods

compd	$\log_{10}$ association constant[a]		
	ethidium displacement	equilibrium dialysis[b]	spectrophotometry[c]
14	5.62	5.76	5.50
14 (R = 3′-OMe)	5.15	5.16	5.13
14 (R = 2′-OMe)	5.54	5.61	5.50
14 (R = 2-Me)	5.29	5.15	
9-aminoacridine	5.59	5.49	5.72
9-(methylamino)-acridine	5.62	5.54	
3,6-diamino-acridine	5.90	6.06	

[a] All measurements carried out at 0.01 M ionic strength. [b] Reference 11. [c] Reference 9.

While quenching has proved to be an important factor when assaying drug–DNA interactions of intercalating agents, certain minor-groove binding drugs, for example, the antileukemic bisquaternary ammonium heterocycles,[12] show levels of quenching which are so low in relation to ethidium displacement that quenching can be acceptably ignored.

Drug–DNA Binding Geometry. Representative members of the 9-anilinoacridines have been shown by biophysical techniques to be DNA intercalating agents.[5,13] Model building suggests that intramolecular steric constraints will likely ensure that the 9-anilino function and the acridine ring system do not become coplanar. X-ray diffraction analysis has established that in crystals of 14 these respective ring planes are inclined at an angle of 77°.[14] It is then unlikely that both 9-anilino and acridine ring systems of one molecule could reside in the same DNA intercalation site. Structural similarities between 9-anilinoacridine and the well-characterized DNA-intercalator ethidium have led us to suggest that the acridine ring of the former intercalates between DNA base pairs and the appended out-of-plane 9-anilino ring is located in the minor groove of twin-helical DNAs.[15] Provided that added substituents do not introduce steric barriers to the adoption of this binding mode, essential features of this model could remain constant for a wide range of congeners. Fitting of drug models to a model of a DNA intercalation site suggests that noncharged 1′- and 2′-substituents of limited size are likely to produce the least distortion, while, for example, asymmetrically placed acridine substituents might produce quite major changes in binding geometry and, if sufficiently large, could produce changes in conformation of the DNA receptor by wedging effects. For such reasons, first investigations have concentrated on congeners substituted on the 9-anilino function alone.

Modeling of Drug–DNA Binding Energies. If drug-DNA complexes afford models for interaction of

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(15) Cain, B. F.; Seelye, R. N.; Atwell, G. J. *J. Med. Chem.* **1974**, *17*, 922.

Table II. Factors Influencing Drug Substituent-Site Interactions[a]

interaction	distance relationship	proportionality terms
ion-ion[b]	1/d	$z_1 z_2$
ion-dipole[c]	$1/d^2$	$\mu \cos \theta$
ion-induced dipole[d]	$1/d^4$	MR
dipole-dipole[e]	$1/d^6$	$\mu^2(1 + \cos^2 \phi)$
dipole-induced dipole	$1/d^6$	$MR.\mu^2$
dispersion energies[f]	$1/d^6$	$MRI_1I_2/(I_1 + I_2)$
charge transfer[g]		σ_p
hydrophobic		π

[a] Reference 19. [b] z_1 and z_2 are the magnitudes of the interacting site and drug electrostatic charges. [c] μ is substituent group dipole moment (aromatic) and θ the relative orientation of the dipole to the electrostatic charge; see ref 19. [d] MR is the molar refractivity of a drug substituent. [e] ϕ provides the relative orientation of interacting dipoles, see ref 19. [f] I_1 and I_2 are the ionization potentials of substituent and interacting site functionality, respectively. Application of the approximation of Pauling and Pressman,[20] that ionization potential has on the average a value of 14 eV, would reduce overall proportionality to MR alone. [g] Reference 21.

bioactive molecules with sites of action, it is of fundamental importance to divine the intrinsic substituent properties which modify drug–DNA binding. Possible component binding energies, and the substituent properties to which they will be responsive, are listed in Table II. Invariant factors common to all mathematical descriptiors of binding energy (for example, dielectric constant and numerical scaling factors) have been ignored.

The range of 9-anilinoacridines examined (Table III) have varying pK_a values and, to ensure that binding of drug cation alone was examined, the DNA binding assays were performed at pH 5. The pK_a values were, for solubility reasons, determined in 20% DMF–H_2O.[16] For those examples where it has also been possible to measure pK_a values in water, these values have been, on average, 0.6 unit higher than those measured in DMF–H_2O.

In the situation where an essentially fully ionized range of cationic congeners bind to an anionic site, and if it is assumed that binding geometry is the same for all analogues, then the component forces listed in Table II can be evaluated.

Ion (drug)–ion (site) and ion (drug)–dipole (site) interactions should be constant through the series. However, the influence of, for example, a 1′-substituent on the dipolar interactions of the 9-anilino ring with a DNA phosphate ion will be expected to be proportional to $\mu \cos \theta$, where μ is the group dipole moment of the 1′-substituent and θ is the angle between the dipole and the 1′–4′ axis of the ring. Regardless of approach to the calculation of dipolar interactions, there is a paucity of μ and θ values for even quite commonly employed drug substituents. However, for those groups for which μ, θ, and σ_p values are available in the literature,[17,18] there is high colinearity between σ_p and $\mu \cos \theta$ (eq 1). It is evident that the use

$$\mu \cos \theta = -3.04\ (\pm 0.61)\ \sigma_p - 0.61 \qquad (1)$$

$$n = 33,\ r = 0.87,\ s = 0.75,\ F_{1,31} = 99.5$$

of Hammett's σ constants would model dipolar interactions between the aromatic ring system to which a substituent is attached and site ion(s).

Site anionic charges may induce dipoles in added drug substituents and the binding energy changes should be proportional to the polarizability of the substituents, which are in turn proportional to molar refractivity values (MR).[19] Those energies (Table II) which vary inversely as the sixth power of the separation of interacting functionalities are likely to provide only small contributions compared to those depending on lesser distance exponents.

There is excellent communication of electronic effects from substituents on the 9-anilino ring of 1 to the acridine system. Virtually any added substituent modifies the pK_a of the acridine ring system.[22] If these electronic perturbations modified charge-transfer interactions between the acridine chromophore and elements in the DNA, then the energy changes resulting would be expected to be proportional to the σ value of the added substituent.[21]

A wide range of chemicals appear to bind to diverse macromolecules according to the logarithms of their octanol–water partition coefficients.[23] A similar situation for drug binding to DNA would provide binding changes proportional to the alterations in the logarithm of such partition coefficients produced by an added substituent X, i.e., $\log P_X - \log P_H = \pi$.

While seeking to employ more fundamental substituent parameters we were inevitably brought to the use of the extrathermodynamic parameters π, σ, and MR, which have proved of such value in QSAR studies. The data set selected for DNA binding measurements (Table III) bear substituents whose π, MR, and π_p values are satisfactorily mutually orthogonal (Table IV).

1′-Substituted Variants. To ensure a common nomenclature for the 9-anilinoacridines, positional numbering as in the 4′-(9-acridinylamino)methanesulfonanilides (AMSA series; 1 R = 1′-$NHSO_2CH_3$) has been employed. One analogue (the 3′-OCH_3 derivative of 14; cf. Table I) has been selected as an internal standard in the measurement of drug–DNA association constants (K), and in 28 determinations of the C_{50} value for this agent, over a 2-year period, the standard deviation about the mean was ±13%. The additional error in the determination of quenching values provided further observational error of ±10%, resulting in a standard deviation for $\log_{10} K$ of ±0.10. Inspection of the data in Table III indicates that in almost all cases the C_{50} values with poly[d(A-T)] are lower than those for poly[d(G-C)]. However, the degree of quenching is dependent on the polymer employed, and the differences in quenching compensate for those in the C_{50} values such that the resultant log K values for both polymers are extremely similar. The ratio of the binding constants, expressed as log $[K_{(AT)}/K_{(GC)}]$, for the 1′-variants

(16) Atwell, G. J.; Cain, B. F.; Denny, W. A. *J. Med. Chem.* **1977**, *20*, 1128.
(17) Dipole moments (μ) and their associated θ vaues are provided in the compilation of Minkin, V. I.; Osipov, O. A.; Zhdanov, Yu. A. "Dipole Moments in Organic Chemistry"; Plenum: New York, 1970; Chapter 3.
(18) σ_p and other employed extrathermodynamic parameters have been taken from the compilation of Hansch, C.; Leo, A.; Unger, S. H.; Kim, K. H.; Nikaitani, D.; Lien, E. J. *J. Med. Chem.* **1973**, *16*, 1207.
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(23) Hansch, C. In "Drug Design"; Ariens, E. J., Ed.; Academic Press: New York, 1971; Vol. 1, pp 280–284.
(24) Clark, J.; Perrin, D. D. *Q. Revs.* **1964**, *18*, 304.

(2–33) (Table III) lies in the range 0.032 ± 0.067. K values for both polymers are, within experimental error, the same: thus, the 1′-substituted 9-anilinoacridines show no selectivity in respect of binding to the two DNA polymers. Virtually identical regression equations result from employing either $K_{(AT)}$ or $K_{(GC)}$; those from the former alone are presented.

Most variance in the binding data for the 1′-analogues **2-33** is accepted by a simple equation in Hammett's σ_p (p = para) constant (eq 2).

$$\log K_{(AT)} = -0.51\ (\pm 0.10)\ \sigma_p + 6.11 \quad (2)$$

$$n = 32,\ r = 0.88,\ s = 0.13,\ F_{1,30} = 107.4$$

Addition of a term in molar refractivity (eq 3) provides

$$\log K_{(AT)} = -0.49\ (\pm 0.08)\ \sigma_p + 0.67\ (\pm 0.34)\ \mathrm{MR} + 6.00 \quad (3)$$

$$n = 32,\ r = 0.93,\ s = 0.12,\ F_{2,29} = 84.4$$

a further significant reduction in total variance. Substituent group MR values have been appropriately scaled by dividing by 100.

There is no discernible relationship between subsitutent π values and DNA binding in the 1′-substituted 9-anilinoacridine series.

2′-Substituted Variants. The ratio of the binding constants, log $[K_{(AT)}/K_{(GC)}]$, for compounds **34–43** is −0.022 ± 0.121. Again there is no significant difference in the K values, and the 2′-variants display no sequence selectivity of binding.

Provided σ_m values are used in place of the σ_p term of eq 2, an essentially similar regression equation can be derived for the 2′-variants. By employing an overall σ term (= σ_p for 1′, σ_m for 2′) both 1′- and 2′-substituents can be considered together, the resulting equation (eq 4) being extremely similar to that derived for the 1′-substituents alone (eq 2).

$$\log K_{(AT)} = -0.53\ (\pm 0.09)\ \sigma + 6.08 \quad (4)$$

$$n = 42,\ r = 0.88,\ s = 0.13,\ F_{1,40} = 133.0$$

Further inclusion of a term in MR for a 1′-substituent [$\mathrm{MR}_{(1')}$] provided a significant decrease in variance (eq 5). In contrast, the MR values for 2′-substituents absorbed no significant portion of the remaining variance. In eq 5 an MR value of 1.03 for H has been used in the case of the 2′-substituted compounds. Again, substituent π values absorbed no significant variance. For compounds **2–43**:

$$\log K_{(AT)} = -0.49\ (\pm 0.07)\ \sigma + 0.70\ (\pm 0.28)\ \mathrm{MR}_{(1')} + 6.00 \quad (5)$$

$$n = 42,\ r = 0.92,\ s = 0.10,\ F_{2,39} = 112.4$$

The residuals in Table III for compounds **2–43** have been calculated from this equation.

3′-Substituted Variants. On the basis of the findings with the 1′- and 2′-congeners, investigation of the importance of electronic effects with the 3′-variants would be expected to require σ_o (o = ortho) substituent values. The difficulty of deriving a generally applicable set of such constants is well recorded, and it has been concluded that σ_o values should be directly determined for the set of congeners under examination. This latter device has been applied and σ_o values have been derived from measured pK_a values. For the various 1′- and 2′-variants (**2–43**), excluding compounds **32**, **33**, and **35**, where σ values are not available, and **24**, where pK_a measurements could not be made because of ionization of the phenolic function:

$$pK_a = -1.78\ (\pm 0.14)\ \sigma + 7.26 \quad (6)$$

$$n = 38,\ r = 0.97,\ s = 0.14,\ F_{1,36} = 613.1$$

In this equation $\sigma = \sigma_p$ for 1′-substituted and σ_m for 2′-substituted compounds. The 1′- or 2′-substituted 9-anilinoacridines then afford a convenient device for deriving, respectively, the σ_p and σ_m constants of any substituent R. Corresponding σ_o values were also calculated for the 3′-substituents employed using eq 6 and the requisite pK_a values where these could be measured. Values so derived are provided in parentheses in Table I. For certain of the substituents employed, σ_o' values have previously been calculated from the pK_a values of ortho-substituted anilines (listed in ref 22). For these groups, the σ_o values derived from the 3′-substituted 9-anilinoacridines and the σ_o' values of Clark and Perrin[24] are well correlated (eq 7).

$$\sigma_o = 1.06\ (\pm 0.11)\ \sigma_o' - 0.29 \quad (7)$$

$$n = 11,\ r = 0.99,\ s = 0.055,\ F_{1,10} = 385.9$$

Inspection of the log $[K_{(AT)}/K_{(GC)}]$ ratios for the 3′-variants shows that three compounds have values at apparent variance with the remainder. These compounds and the ratios involved are as follows: **60**, −0.61; **62**, +0.38; **65**, −0.38. The average ratio for the remaining 62 compounds is 0.021 ± 0.10; the probabilities that the three outliers are significantly different from the remainder are, respectively, $p < 0.001$, $p < 0.01$, and $p < 0.01$.

Significant regression equations relating to derived σ_o values and $K_{(AT)}$ or $K_{(GC)}$ figures, with or without the exclusion of the outliers **60**, **62**, and **65**, could not be derived. The most reasonable equation obtained, in fact, resulted from the use of π values (eq 8). For compounds **44–65**:

$$\log K_{(AT)} = -0.13\ (\pm 0.07)\ \pi + 5.42 \quad (8)$$

$$n = 22,\ r = 0.66,\ s = 0.16,\ F_{1,21} = 15.0$$

However, this equation must be considered as somewhat suspect; the substituents for which Taft's steric parameter (E_s) are available show a high covariance between π and E_s values ($r = 0.87$), and for these particular compounds an essentially similar equation in E_s results:

$$\log K_{(AT)} = 0.16\ (\pm 0.11)\ E_s + 5.33 \quad (9)$$

$$n = 10,\ r = 0.71,\ s = 0.11,\ F_{1,9} = 8.1$$

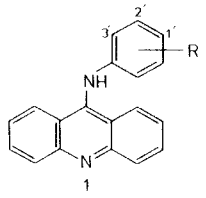
From model fitting it can be predicted that steric interactions of 3′-substituents are likely to be of prime importance (see discussion), and such would be more in line with dependence on E_s values.

Antitumor Activity. A fraction of the agents described have provided significant antileukemic activity in L1210 assays. For the sake of completeness, biological data on these materials has been included (Table III), but the small numbers involved make detailed discussion of any regression equations derived unwarranted. A later QSAR study will incorporate these materials, along with all tumor-active 9-anilinoacridine variants prepared in this laboratory.

Discussion

If the 9-anilinoacridines bind to DNAs as suggested, by intercalation of the acridine chromophore between adjacent base pairs, then the 9-anilino function, with its ring plane twisted away from that of the acridine, appears admirably suited for lodgement in the spiraling minor groove of the DNAs. In such a binding configuration a 1′-substituent could be located directly above a DNA phosphate anion. Ion (site) induced dipole (1′-substituent)

Table III. Structural Details, Physicochemical Parameters, and Biological Data for the 9-Anilinoacridines



						poly[d(A-T)]									
								log K^g							
no.	R	R_m[a]	pK_a[b]	MR[c]	σ[d]	C_{50}[e]	Q[f]	obsd	calcd	diff	C_{50}[e]	Q[f]	log K^g	LD_{10}[h]	ILS_{max}[i]
2[j]	1'-NO_2	0.27	5.58	7.36	0.78	23	9.5	5.48	5.69	−0.21	19	5.5	5.69	>500	*k*
3[l]	1'-SO_2CH_3	−0.08	5.81	13.49	0.72	12	6.5	5.86	5.79	0.07	14	3.5	5.88	>500	
4[j]	1'-CN	0.24	5.93	6.33	0.66	17	6.5	5.70	5.75	−0.05	15	2.5	5.86	200	
5[l]	1'-SO_2NHCH_3	−0.03	6.02	16.0	0.57	14	5	5.82	5.86	−0.04	16	2.5	5.85	>500	
6[m]	1'-SO_2NH_2	−0.47	6.11	12.28	0.57	10	5	5.96	5.84	0.12	14	3.5	5.92	>500	
7[i]	1'-$COCH_3$	0.35	6.12	11.18	0.50	12	6	5.87	5.83	0.04	14	3.5	5.89	>500	
8[j]	1'-$COOCH_3$	0.51	6.21	12.87	0.45	11	7	5.88	5.87	0.01	15	3	5.86	>500	87
9[n]	1'-$CONH_2$	−0.25	6.47	9.81	0.36	13	6	5.83	5.89	−0.06	18	2.5	5.77	140	
10[l]	1'-F	0.48	7.50	0.92	0.06	13	3.5	5.90	6.02	−0.12	16	2.5	5.85	160	
11[j]	1'-Cl	0.60	7.06	6.03	0.23	11	3.5	5.99	5.98	0.01	14	5	5.91	100	
12[j]	1'-Br	0.60	7.00	8.88	0.23	7.3	9	6.02	6.00	0.02	11	4.5	5.98	300	
13[l]	1'-I	0.59	6.90	13.94	0.18	5.0	9	6.19	6.02	0.17	8.2	5.5	6.13	90	
14[m]	1'-$NHSO_2CH_3$	0.00	7.19	18.17	0.03	4.1	19	6.15	6.12	0.03	8.9	5.5	6.27	66	131
15[m]	1'-$NHSO_2C_6H_5$	0.45	7.09	37.88	0.01	3.7	16	6.20	6.28	−0.08	5.7	5.5	6.23	60	75
16[m]	1'-H	0.43	7.46	1.03	0.00	15	3	5.86	6.01	−0.15	16	2.5	5.85	200	
17[m]	1'-$NHCOCH_3$	0.26	7.51	14.93	0.00	2.7	19	6.30	6.16	0.14	4.7	9	6.25	37	60
18[m]	1'-$NHCOOCH_3$	0.47	7.64	17.0	−0.15	2.4	18	6.36	6.20	0.16	5.3	7.5	6.23	60	75
19[j]	1'-CH_3	0.69	7.72	5.65	−0.17	7.8	10	5.99	6.11	−0.12	10	5	5.99	125	87
20[l]	1'-$NHCOC_6H_5$	0.64	7.39	34.64	−0.19	2.8	25	6.20	6.32	−0.12	5.4	12	6.14	130	35
21[o]	1'-$NHCONHCH_3$	0.26	7.77	18.0	−0.25	2.0	34	6.24	6.24	0.00	4.1	16	6.17	68	114
22[l]	1'-$NHCONHC_6H_5$	0.67	7.67	39.0	−0.25	2.0	23	6.37	6.41	0.04	3.8	11	6.30	>500	73
23[m]	1'-OCH_3	0.52	7.94	7.89	−0.27	4.4	17	6.12	6.18	−0.06	7.4	8	6.08	110	
24[m]	1'-OH	0.32	7.91	2.85	−0.37	2.9	18	6.28	6.13	0.15	5.1	8	6.21	180	80
25[p]	1'-$NH(CH_2)_5CH_3$	1.03	8.24	27.0	−0.50	1.1	38	6.43	6.42	0.08	1.6	32	6.38	90	
26[p]	1'-$NH(CH_2)_3CH_3$	0.86	8.24	24.26	−0.51	1.0	47	6.45	6.40	0.05	1.6	32	6.39	70	
27[p]	1'-$NH(CH_2)_2CH_3$	0.74	8.25	19.0	−0.55	1.0	51	6.35	6.35	0.00	2.0	31	6.28	45	
28[p]	1'-$NHCH_2CH_3$	0.51	8.30	14.98	−0.61	1.0	42	6.44	6.32	0.12	1.7	31	6.36	55	35
29[m]	1'-NH_2	−0.08	8.36	5.42	−0.66	1.4	41	6.31	6.24	0.07	2.3	31	6.23	45	69
30[m]	1'-$N(CH_3)_2$	0.66	8.46	15.55	−0.83	0.9	42	6.51	6.36	0.15	2.0	31	6.29	80	50
31[m]	1'-$NHCH_3$	0.24	8.42	10.33	−0.84	0.7	49	6.55	6.30	0.25	1.2	32	6.54	70	53
32[q]	1'-$N(CH_3)SO_2CH_3$	0.12	6.95	22.8	0.15	7.9	14	5.89	6.11	−0.22	9.9	5	6.00	190	65
33[q]	1'-$NHSO_2C_6H_4$-*p*-NH_2	−0.04	7.15	40.0	0.01	2.4	22	6.30	6.32	−0.02	4.4	10	6.26	8	110
34[l]	2'-NO_2	0.32	6.26	5.62	0.71	23	4	5.56	5.77	−0.21	27	2	5.62	>500	
35[l]	2'-aza	−0.22	6.46	(0.5)	0.61	18	4	5.75	5.81	−0.06	13	3	5.96	>500	
36[l]	2'-Cl	0.47	6.76	6.03	0.37	11	2	6.02	5.87	0.15	11	1	6.02	>500	
37[m]	2'-$NHCOCH_3$	0.35	7.16	14.93	0.21	9.7	6	5.90	5.97	−0.05	13	3	5.96	120	55
38[m]	2'-$NHSO_2CH_3$	0.02	6.85	18.17	0.20	16	6	5.73	5.89	−0.16	15	3.5	5.86	270	99
39[l]	2'-OCH_3	0.51	7.21	7.87	0.12	12	5	5.86	5.96	−0.10	14	2.5	5.91	280	
40[m]	2'-OH	0.38	7.30	2.85	0.12	7.8	9	6.00	5.98	0.02	8.2	4.5	6.10	270	
41[l]	2'-CH_3	0.69	7.52	5.65	−0.07	8.2	4	6.09	6.03	0.06	15	2	5.90	375	
42[m]	2'-NH_2	0.09	7.58	5.42	−0.16	4.9	15	6.11	6.04	0.07	8.6	7	6.01	55	50
43[m]	2'-$NHCH_3$	0.49	7.61	10.33	−0.31	3.5	23	6.11	6.05	0.06	7.0	12	6.01	250	
44[l]	3'-SO_2NH_2	−0.23	na[r]	12.28		18	5	5.67			18	2	5.77	>500	

45[l]	3'-$CONH_2$	0.09	na[r]	9.81		30	2	5.37		38	1	5.43	>500
46[l]	3'-aza	0.25	6.32	(0.53)	(0.5)	18	2	5.60	5.52	0.08	42	1	5.43	300
47[l]	3'-NO_2	0.33	na[r]	7.36		41	2	5.36			36	1	5.49	400
48[l]	3'-I	0.52	6.47	13.94	(0.44)	50	2	5.33	5.07	0.26	126	1	5.27	>500
49[l]	3'-Br	0.51	6.41	8.88	(0.48)	48	7	5.35	5.10	0.25	58	3.5	5.32	>500
50[l]	3'-Cl	0.50	6.55	6.03	(0.40)	44	2.5	5.39	5.24	0.15	41	1.5	5.47	>500
51[l]	3'-F	0.48	6.84	0.92	(0.24)	28	2	5.59	5.16	0.43	27	1	5.65	240
52[m]	3'-OH	0.54	7.80	2.85	(−0.30)	35	6.5	5.36	5.61	−0.25	39	3.5	5.42	>500
53[l]	3'-OCH_3	0.45	7.74	7.87	(−0.27)	24	7	5.50	5.32	0.18	34	3.5	5.47	80
54[l]	3'-OCH_2CH_3	0.80	7.85	12.47	(−0.33)	48	7	5.20	5.46	−0.26	85	2.5	5.08	90
55[l]	3'-CH_3	0.63	7.76	5.65	(−0.28)	55	2.5	5.25	5.43	−0.18	67	1.5	5.23	180
56[l]	3'-CH_2CH_3	0.56	7.68	10.30	(−0.24)	67	2	5.18	5.37	−0.19	83	1	5.15	300
57[l]	3'-$CH(CH_3)_2$	0.66	7.64	14.98	(−0.21)	91	1.5	5.08	5.23	−0.15	142	0.5	4.92	150
58[l]	3'-$C(CH_3)_3$	0.73	7.62	19.62	(−0.20)	70	0.5	5.23	5.15	0.07	100	0.5	5.09	200
59[m]	3'-NH_2	0.15	7.87	5.42	(−0.34)	7.7	23	5.76	5.77	−0.01	13	10	5.76	85
60[l]	3'-$NHSO_2CH_3$	0.09	6.86	18.17	(0.22)	33	3	5.49	5.56	−0.07	7.3	6	6.10	370
61[l]	3'-$NHCOOCH_3$	0.42	7.24	17.0	(0.01)	27	4	5.56	5.49	0.07	17	2.5	5.78	>500
62[l]	3'-$NHCOCH_3$	0.32	7.24	14.93	(0.01)	16	2	5.84	5.60	0.24	42	1	5.46	200
63[l]	3'-$COOCH_3$	0.51	6.30	12.87	(0.54)	24	9	5.47	5.23	0.24	40	3.5	5.41	
64[l]	3'-$CH_2NHSO_2CH_3$	0.09	7.17	22.8	(0.11)	25	5	5.60	5.53	0.07	46	2	5.39	>500
65[l]	3'-$N(CH_3)SO_2CH_3$	0.15	6.97	22.8	(0.16)	60	6	5.08	5.47	−0.39	37	3	5.46	300

[a] A measure of lipophilic/hydrophilic balance from partition chromatography; see ref 25. [b] Acridine ionization determined spectrophotometrically in 20% DMF-H_2O; see ref 11. [c] Group molar refractivity, from ref 18. [d] σ values from ref 18; σ_p values for compounds 2-33, σ_m values for compounds 34-43. σ_o values (in parentheses) calculated from eq 6. [e] The concentration, μM of drug to give a 50% drop in fluorescence of ethidium bound to DNA, see text. [f] Percentage quenching of fluorescence of bound ethidium by drug at an added drug/base pair ratio of 0.1; see text. [g] Association constant for drug binding to DNA; see text for determination of observed values; calculated values from eq 5. [h] Drug dose in (mg/kg)/day proving lethal to 10% of animals on average; determined by the methods of ref 25. [i] Maximum percent increase in life span in L1210 tests, determined by the methods of ref 25, or, for nontoxic material [$LD_{10} > 500$ (mg/kg)/day], the ILS observed at 500 (mg/kg)/day. [j] Reference 22. [k] Inactive compounds, ILS less than 25%, if no entry made. [l] New compounds, details in Table IV. [m] Reference 27. [n] Reference 25. [o] Reference 26. [p] Reference 28. [q] Reference 29. [r] pK_a value not able to be determined spectrophotometrically.

Table IV. Square Correlation Matrix for Physicochemical Parameters Employed[a]

	σ	MR	π
log $K_{(AT)}$	0.69	0.18	0.12
σ		0.03	0.07
MR			0.01

[a] Data for compounds 2-43 of Table III.

Table V. Analytical Details for New Compounds in Table III

no.	mp, °C	formula	anal.[a]
3	299-231	$C_{20}H_{16}N_2O_2S$	C, H, N, S
5	314-316	$C_{20}H_{17}N_3O_2S\cdot HCl$	C, H, N, Cl
10	174-175	$C_{19}H_{13}FN_2$	C, H, N, F
13	266-269	$C_{19}H_{13}IN_2\cdot HCl\cdot 0.5H_2O$	C, H, N, I
20	340 dec	$C_{26}H_{19}N_3O\cdot HCl$	C, H, N
22	303-305	$C_{26}H_{20}N_4O\cdot HCl$	C, H, N, Cl
34	186-188	$C_{19}H_{13}N_3O_2$	C, H, N
35	268-269	$C_{18}H_{13}N_3$	C, H, N
36	260-262	$C_{19}H_{13}ClN_2\cdot CH_3SO_3H$	C, H, N, S
39	207-209	$C_{20}H_{16}N_2O\cdot CH_3SO_3H$	C, H, N, S
41	295-297	$C_{20}H_{16}N_2\cdot HCl$	C, H, N, Cl
44	298-300	$C_{19}H_{15}N_3O_2S\cdot HCl\cdot 0.5H_2O$	C, H, N, Cl
45	289-291	$C_{20}H_{15}N_3O\cdot HCl$	C, H, N, Cl
46	258-259	$C_{18}H_{13}N_3\cdot HCl\cdot 0.5H_2O$	C, H, N, Cl
47	241-243	$C_{19}H_{13}N_3O_2\cdot HCl$	C, H, N, Cl
48	292-293	$C_{19}H_{13}IN_2\cdot HCl$	C, H, N, I
49	300-301	$C_{19}H_{13}BrN_2\cdot HCl$	C, H, N, Br
50	303-304	$C_{19}H_{13}ClN_2\cdot HCl$	C, H, N, Cl
51	325-326	$C_{19}H_{13}FN_2\cdot HCl$	C, H, N
53	252-254	$C_{20}H_{16}N_2O\cdot CH_3SO_3H$	C, H, N, S
54	262-264	$C_{21}H_{18}N_2O\cdot HCl$	C, H, N, Cl
55	277-280	$C_{20}H_{16}N_2\cdot HCl$	C, H, N, Cl
56	282-285	$C_{21}H_{18}N_2\cdot HCl$	C, H, N, Cl
57	280-282	$C_{22}H_{20}N_2\cdot HCl$	C, H, N, Cl
58	258-259	$C_{23}H_{22}N_2\cdot HCl$	C, H, N, Cl
60	312-314	$C_{20}H_{17}N_3O_2S\cdot HCl$	C, H, N, Cl
61	232-233	$C_{21}H_{17}N_3O_2\cdot HCl$	C, H, N, Cl
62	255-256	$C_{21}H_{17}N_3O\cdot HCl$	C, H, N, Cl
63	209-210	$C_{21}H_{16}N_2O_2\cdot HCl$	C, H, N, Cl
64	250-251	$C_{21}H_{19}N_3O_2S\cdot HCl$	C, H, N, Cl
65	270-271	$C_{21}H_{19}N_3O_2S\cdot HCl$	C, H, N, Cl

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

interactions would then be expected with binding energies predicted responsive to $MR_{(1')}$. With ion-induced dipole energies reducing as the fourth power of the distance between the interacting groups, substituents at the 2' position, these further removed from site anions, would be expected to provide a lesser binding contribution and possibly this is the reason why $MR_{(2')}$ terms accept no significant portion of variance in the binding data.

Within the subset of compounds examined there appears no clue which allows adequate distinction between the possibilities that the observed σ responsiveness of binding energies, for 1'- and 2'-substituents, results from ion (site)–dipole (drug) interactions, charge-transfer interactions of the intercalated acridine chromophore with adjacent base pairs, or some combination of these effects.

Although a quite reasonable range of π values is embraced within the 1' and 2' derivatives, it is intriguing that no binding dependence on this parameter can be discerned. This is in marked contrast with the demonstrated π dependent drug binding to a wide range of macromolecular substrates.[23]

Model fitting suggests that steric interactions will dominate the DNA binding of the 3'-substituted congeners. Firstly, intramolecular contacts could alter the degree of twist between the acridine and 9-anilino ring planes and possibly alter the C–N bond angles about the 9-amino function. Electronic transmission from the 3'-substituent

to the acridine ring is then likely to be altered. Secondly, such substituents must inevitably come into close contact with the central purine–pyrimidine stack, when any drug variants intercalate their acridine nuclei into any DNA. That contact might contribute in a positive fashion to overall binding, but these groups limit the entry of the acridine chromophore into the DNA stack and may force adoption of an acridine orientation which is quite different from that adopted by the unsubstituted parent agent. It is noteworthy that most 3′-substituted variants bind less strongly than their congeners bearing the same substituent in either the 1′ or 2′ position. The exact conformation that the intercalated acridine chromophore adopts will be highly dependent on the size of the 3′-substituent and the particular orientation if this latter group is not centrosymmetric. With site-binding orientations likely changing from variant to variant it would be unrealistic to expect high-grade correlation equations, and the most that might be hoped for would be demonstration of the dominance of steric effects.

Three 3′-variants appear capable of distinguishing the composition of DNA binding substrates as shown by the ratios log $[K_{(AT)}/K_{(GC)}]$. These three variants, with their $\log_{10}$ binding ratios in parentheses, are **60** (−0.61), **62** (+0.38), and **65** (−0.38). As these agents all have the same 9-aminoacridine moiety, the observed site selectivity is unlikely to result from preferential distinction of the planar faces of the purine–pyrimidine base pairs comprising the different intercalation sites in the AT and GC polymers. The distinction must then result from interaction of the 3′-functionality with that of the DNA polymer which lies exterior to the intercalation site. The out-of-plane 9-anilino ring of **60** could possibly reside in the major or minor groove of a DNA. Base-pair functionality available in the major groove cannot be readily distinguished by simple H-bonding groups of drugs, since donor (cytosine 4-NH_2; adenine 6-NH_2) and acceptor (guanine 6-O; thymine 4-O) groups are common to both the AT and GC polymers. Binding distinction in the major groove would have to stem from steric interactions with the unique functionality there present, that is, the 5-CH_3 group of thymine. However, a range of drug analogues bearing nonpolar 3′-substituents of varying size (**55**–**58**) have failed to provide effective distinction of the AT and GC polymers. In contrast, in the minor groove of the DNA there is a distinguishing H-bond donor function, the 2-NH_2 group of guanine. Suitably located drug H-bond acceptor functions might then provide distinction of GC-containing polymers from their AT counterparts. Hydrogen bond formation from an oxygen atom of the 3′-$NHSO_2CH_3$ of **60** to a guanine 2-NH_2 group would provide such distinction, and from molecular models this appears highly reasonable. If such is the explanation for the fourfold better distinction of the GC polymer by **60**, then the 9-anilino function of the latter can only reside in the minor groove of the DNA, where the 2-NH_2 group of guanine is located, in agreement with our previously postulated site model. The *N*-methyl derivative of **60** (**65**) can still distinguish the GC polymer to a significant extent, despite the added steric demand imposed by the methyl group.

Model building has employed a cis $NHSO_2$ configuration, as shown in X-ray crystallographic data of sulfonamides.[14] In the cis form, the size of the SO_2 function appears sufficient to make it unlikely that H bonds could be formed between the neighboring sulfonamide NH and the C-2 oxygen atom of either a cytosine or thymine residue. In contrast, a 3′-$NHCOCH_3$ function, in the usually accepted trans configuration, has the steric bulk of the oxygen atom on the reverse side from the NH group, and this might then form a H bond with either cytosine or thymine O-2. However, in GC base pairs the cytosine O-2 is already H bonded to the 2-NH_2 of guanine, and this amino group would provide a steric barrier to the entry of the CH_3 group of a 3′-$NHCOCH_3$ function. Such interactions should confer on **62** binding selectivity toward the AT polymer as observed. In contrast, in a methyl carbamate moiety, as in **61**, there is a cis configuration of NH and CO groups, and once more the steric effects of the carbonyl oxygen atom would provide a barrier to H-bond formation by the carbamate NH group. It might be expected that **61** could show selectivity of binding to GC polymers, by virtue of the C=O H-bond acceptor group. Models demonstrate that there are subtle distinctions in bond angles and distances involved with a $NHSO_2CH_3$ and $NHCOOCH_3$ group which influences the approach of their oxygen atoms to the guanine 2-NH_2, and it is difficult to satisfactorily align the requisite groups of a carbamate and a guanine residue.

The substituents $NHSO_2CH_3$ and $NHCOCH_3$, when added to the 3′ position of a 9-anilinoacridine, confer selectivity of binding toward the alternative DNA base pairs. Experience with polyinteracalating drugs[5] then prompts the suggestion that it may be possible to synthesize drugs which will better distinguish a particular sequence in DNA. Hopefully, such DNA sequence-selective agents might provide greater biological selectivity than the chemotherapeutic intercalating agents presently employed in the clinic.

Experimental Section

Analyses were performed 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 stem-corrected thermometer and are as read. UV spectra were recorded on a Shimadzu UV-200. To monitor the progress of reactions and the purity of products, TLC on SiO_2 (Merck SiO_2, F_{254}) was used. The methods of measurement of agent R_m and pK_a values have been described in full earlier.[16,25]

All new compounds in Table III were prepared by coupling of 9-chloroacridine with the appropriate substituted aniline under mild acid catalysis by the general methods evolved earlier,[26] all necessary substituted anilines have previously been reported in the literature.

Drug–DNA Binding. C_{50} values of drugs were determined employing the requisite DNA (1 μM in nucleotides) in 0.01 M ionic strength buffer (9.3 mM NaCl, 2 mM NaOAc buffer, pH 5, plus 0.1 mM EDTA) containing 1.26 μM ethidium as described previously.[4,5] Q values for quenching were determined in the same buffer by the same methods but employed relative DNA (40 μM in nucleotides) and ethidium (2 μM) concentrations such that there was minimal ethidium displacement and high drug-induced quenching (see Figure 1).

Mathematical Basis for the Determination of DNA Association Constants. Fluorescence decrease in the C_{50} assay results from both displacement of ethidium and quenching of the fluorescence of that still bound. When the added drug concentration is sufficient to provide a 50% decrease in fluorescence

$$\frac{E_b}{E_b^0}(100 - Q_x) = 50 \qquad (10)$$

where E_b^0 and E_b are the concentrations of DNA-bound ethidium

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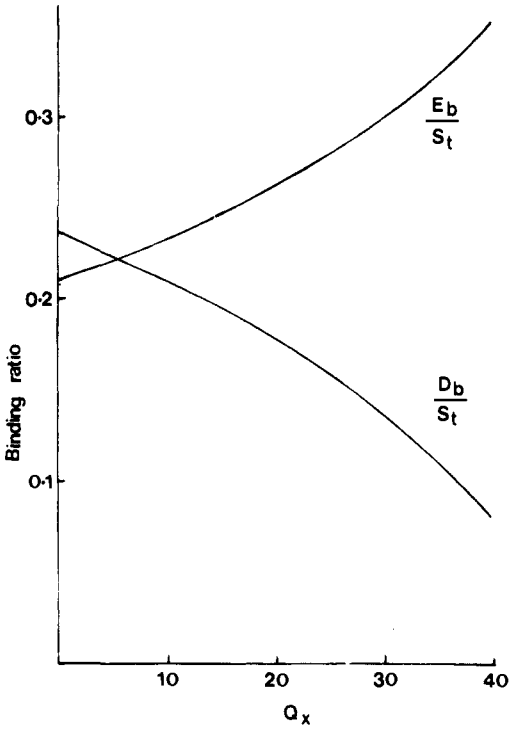

Figure 2. Relationship between ethidium binding ratio (E_b/S_t), drug binding ratio (D_b/S_t), and percentage quenching of the fluorescence of DNA-bound ethidium (Q_x) to give a resultant fluorescence (corrected for non-DNA-bound ethidium) of 50% of that in the absence of drug. The DNA (poly[d(A-T)]) concentration was 0.5 μM (base pairs), and the curves were calculated from eq 10–17 in the text.

before and after binding of drug at the DNA-bound concentration of D_b. Q_x is the percentage quenching of ethidium fluorescence at a drug binding ratio of D_b/S_t, where S_t is the total concentration of potential DNA sites in base pairs.

The association constants for drug (K) and ethidium (K_e) can be represented by the eq 11 and 12, assuming competitive binding

$$K = \frac{D_b}{D_f S_f} \tag{11}$$

$$K_e = \frac{E_b}{E_f S_f} \tag{12}$$

for the same sites. In these expressions D_f and E_f are the free concentrations of drug and ethidium, and S_f is the effective free DNA site concentration. If the total, drug plus ethidium, binding ratio is represented by ν then:

$$\nu = \frac{D_b + E_b}{S_t} \tag{13}$$

Provided all intercalation sites in the copolymeric DNA are equivalent, then S_f is provided by the treatment of McGhee and von Hippel,[7] assuming a DNA site size of two base pairs.

$$\frac{S_f}{S_t} = \frac{(1 - 2\nu)^2}{(1 - \nu)} \tag{14}$$

Solution of eq 10 to 14, putting $K_e = 9.5 \times 10^6\ M^{-1}$ and $S_t = 0.5$ μM, provides the ethidium and drug binding ratios (E_b/S_t and D_b/S_t) as functions of the percentage quenching, Q_x (Figure 2).

Elimination of S_f from eq 11 and 12 provides eq 15. Provided

$$K = \frac{K_e D_b E_f}{E_b D_f} \tag{15}$$

Q_x is known, D_b/E_b is available from Figure 2 and, coupled with the input concentrations of ethidium and drug, permits the drug–DNA association constant to be calculated.

Q_x is most simply derived from the measured quenching value Q by first calculating K on the basis that all added drug in the quenching assays is DNA bound. The actual amount of drug bound in the quenching assay can then be approximated by application of eq 16 resulting from rearrangement of eq 11.

$$\frac{D_b}{D_t} = \frac{S_f K}{(1 + S_f K)} \tag{16}$$

Employment of the first calculated K value then allows correction for unbound drug in the quenching assay, and a new relationship between D_b/S_t and Q_x can be derived, providing in turn a new estimate of K. On reiteration of this procedure, there is rapid convergence affording a self-consistent value for K.

The above treatment assumes that the quenching values determined at low ethidium binding ratios are applicable when higher ethidium inputs are employed. K values can also be calculated from quenching assays in which higher initial ethidium levels (Figure 1) have been used. In this case, it is important to correct for free ethidium levels, which can be calculated from eq 17. K values so derived have been within 5% of those resulting

$$\frac{E_b}{E_t} = \frac{S_f K_e}{(1 + S_f K_e)} \tag{17}$$

when the quenching assays employed an ethidium binding ratio of 0.1. K values in Table III were, accordingly, all derived from Q values determined at an ethidium binding ratio of 0.1. Additional support for this particular assumption is that the curves utilized to derive C_{50} values[4,5] can be extremely well simulated from the equations provided and the values of K and Q_x derived.

The alternating copolymeric DNAs employed, e.g., poly[d(A-T)], have two distinct possible intercalation sites. The binding constants for ethidium to these polymers have been derived by assuming that the two sites are equivalent. If, in the extreme case, only one of the two classes of sites can be occupied, eq 14 reduces to eq 18. Use of this equation would reduce the earlier estimate[2]

$$\frac{S_f}{S_t} = 1 - 2\nu \tag{18}$$

of K_e by about 10% and would also slightly change the relationships shown in Figure 2. The real situation may be between the conditions shown by eq 14 and 18, but trial calculations show that the alternative binding model provides surprisingly similar K values.

The K values of Table III actually refer to the binding of drugs to a complex of poly[d(A-T)] and ethidium, rather than to the DNA alone. If negative or positive cooperativity of binding was encountered, the measured K values could diverge from values measured for drug–DNA interaction alone.

Acknowledgment. The authors are indebted to Mrs. E.-M. Falkenhaug for performing the many DNA binding assays and to C. West and capable assistants for performance of the biological tests. This work was supported by the Auckland Division of the Cancer Society of New Zealand (Inc.) and in part by the Medical Research Council of New Zealand.