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Interactions of Anilinoacridines with Nucleic Acids: Effects of Substituent Modifications on DNA-Binding Properties†

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ABSTRACT: Spectroscopic methods are used to probe the interactions of several anilinoacridine analogues with calf thymus DNA over a wide range of temperatures and sodium chloride concentrations. The structurally similar compounds *m*-AMSA, AMSA (both active as antitumor agents), and *o*-AMSA (inactive as an antitumor agent) have been widely studied in their abilities to bind DNA in an intercalative manner. Recent studies from this laboratory reveal distinct differences in the thermodynamic binding mechanisms between *m*-AMSA and *o*-AMSA (Wadkins & Graves, 1989), with the *m*-AMSA–DNA interaction being an enthalpy-driven process while the binding of *o*-AMSA to DNA is characterized by more positive entropy values. To further examine the physical chemical properties associated with these compounds and their correlation with antitumor activities, an in-depth investigation into the thermodynamic parameters of these compounds and structurally related anilinoacridine analogues was performed. These studies demonstrate that substituent type and position on the aniline ring of the anilinoacridines greatly influences both the affinities of these drugs in binding to DNA and dictates whether the DNA binding is an enthalpy- or entropy-driven process. The differences in thermodynamic mechanisms of binding between the two isomers along with molecular modeling studies reveal the electronic and/or steric factors resulting from the positioning of the methoxy substituent group on the anilino ring directs the DNA-binding properties through orientation of the methanesulfonamido group at the 1′ position of the aniline ring. The orientation of this substituent group may result in favorable contacts through hydrogen bonding with neighboring base pairs and ultimately influence the biological effectiveness as an antitumor agent.

Amsacrine [4′-(9-acridinylamino)methanesulfon-*m*-anisidide, *m*-AMSA][1] is a 9-anilinoacridine analogue that was developed by Cain et al. (1975). This acridine analogue has been shown to be highly effective against experimental tumors and is particularly valuable because of its lower incidence of cardiotoxicity than the anthracyclines (Hall et al., 1983;

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[1] Abbreviations: *m*-AMSA, 4′-(9-acridinylamino)methanesulfon-*m*-anisidide; *o*-AMSA, 4′-(9-acridinylamino)methanesulfon-*o*-anisidide; EDTA, ethylenediaminetetraacetic acid; bp, base pairs.

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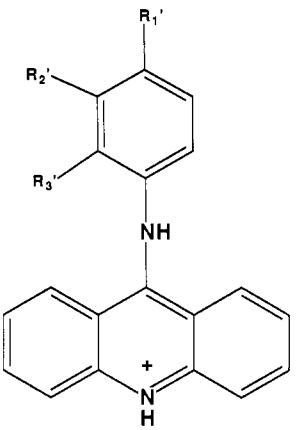
Marsoni & Wittes, 1984; Winton et al., 1983). A similar compound, 4′-(9-acridinylamino)methanesulfonanisidide (AMSA), also exhibits a high antitumor activity but requires a dosage 4 to 7 times that of *m*-AMSA to elicit a comparable biological response. Interestingly, the structural isomer 4′-(9-acridinyl-amino)methanesulfon-*o*-anisidide (*o*-AMSA) demonstrates no antitumor activity, even at 75 times the dosage of that for *m*-AMSA (Cain et al., 1975). Related compounds, *N*-phenyl- and the 2′- and 3′-methoxyanilinoacridine analogues, also show a complete absence of antitumor activity.

The molecular mechanism(s) responsible for the antineoplastic activities of *m*-AMSA are thought to arise as a result of topoisomerase II inhibition. Studies by Pommier (1984, 1985), Minford et al. (1986), Michaels et al. (1986), and Robinson and Osheroff (1990) have shown *m*-AMSA to induce the formation of protein-associated single-strand breaks in nuclear DNA probably via a ternary complex formed between the *m*-AMSA, DNA, and topoisomerase II. Although the specific mechanism remains unknown, it is thought that the presence of the drug alters the cleaved protein–DNA complex such that the religation step of the reaction is inhibited [for reviews, see D'Arpa and Liu (1989); Drlica and Franco (1988)].

Thus far, little evidence has been presented to provide a link between the chemical and biophysical properties of *m*-AMSA with its antitumor activity. Theoretical calculations by Chen and Pullman (1988) predict the binding energies for the interactions of *m*-AMSA and *o*-AMSA with DNA to be similar. Both compounds are presumed to form intercalation complexes with DNA with their bulky anilino ring substituents residing in the minor groove of the DNA preferentially at AT rather than GC sequences. In addition, studies by Wilson et al. (1981) and Denny and Wakelin (1986) showed no discernable trends to correlate the equilibrium binding and kinetic properties of several anilinoacridine analogues with DNA with antitumor activities.

Recently, this laboratory provided a detailed comparison of the thermodynamic binding properties associated with the interaction of *m*-AMSA and *o*-AMSA with DNA (Wadkins & Graves, 1989). These studies reveal that the enthalpy and entropy contributions to the free energy of DNA binding provide a direct correlation with the biological effectiveness of these compounds as antitumor agents. The basic mechanism by which both *o*-AMSA and *m*-AMSA interact with DNA is via intercalation of the acridine chromophore between adjacent base pairs of the DNA duplex, with a resulting free energy change of approximately −6 kcal/mol. Examination of the enthalpy and entropy components of these binding energies reveals that the two structurally similar compounds bind DNA by markedly different thermodynamic mechanisms with the *m*-AMSA–DNA interaction being enthalpy driven while the *o*-AMSA interaction is predominantly entropy driven.

In an effort to resolve the biophysical properties that dictate these distinctly different binding properties, thermodynamic analyses characterizing the DNA-binding properties of the biologically active *m*-AMSA and AMSA are compared with those of selected anilinoacridine compounds of similar structure and chemical composition (Figure 1). This study provides a detailed analysis of the influence of chemical substituent modification and position on the DNA-binding properties of these compounds and their correlation with biological activities. With the use of 9-aminoacridine as a control compound, acridine analogues were designed such that the addition of chemical substituent groups (i.e., the phenyl ring, the methoxy, and methanesulfonamido groups) and their positions could be correlated with changes in thermodynamic properties in the binding of these analogues to DNA. Through a series of systematic modifications of the anilinoacridine system, we have demonstrated that the placement and type of substituent groups added to the anilino ring play a pivotal role in dictating the thermodynamic mechanism(s) by which these compounds interact with DNA. Although speculative, these studies provide evidence that the thermodynamic mechanisms by which these compounds interact with DNA may be correlated with their effectiveness as antitumor agents.



Name	R_1'	R_2'	R_3'
N-phenyl	H	H	H
2′-methoxy	H	OCH_3	H
3′-methoxy	H	H	OCH_3
AMSA	$NHSO_2CH_3$	H	H
m-AMSA	$NHSO_2CH_3$	H	OCH_3
o-AMSA	$NHSO_2CH_3$	OCH_3	H

FIGURE 1: Chemical structure of anilinoacridine analogues. R_1', R_2', and R_3' represent the 1′, 2′, and 3′ positions on the aniline ring, respectively.

Materials and Methods

Drug Preparations. *m*-AMSA (NSC-249992) was obtained from the National Cancer Institute. The purity of the *m*-AMSA was confirmed by TLC with KC_{18} (Whatman) plates and a methylene chloride–methanol–H_2O mixture (100:20:2) as the solvent. Ethidium bromide, proflavin, and 9-aminoacridine were purchased from Sigma Chemical Co. and purities determined by proton NMR spectroscopy (Bruker AC-300). The AMSA, *o*-AMSA, 3′-methoxy, 2′-methoxy, and *N*-phenyl analogues (structures are shown in Figure 1) were synthesized according to methods reported by Denny et al. (1982) and used as their hydrochloride salts. Authenticities of these compounds were confirmed by elemental analyses and NMR spectroscopy. The compounds were desiccated and stored in the dark at −20 °C until ready to be used. Molar absorptivities for the compounds are listed in Table I [Supplementary Material (see paragraph at end of paper regarding ordering information)].

Due to their low aqueous solubility, dimethyl sulfoxide (DMSO) was used to dissolve the compounds into concentrated stock solutions (~0.5 mg/mL). The DMSO–drug solutions were then diluted 1:10 in 0.01 M sodium phosphate (pH 7.0), 0.001 M disodium EDTA, and 0.1 M sodium chloride and filtered through a 0.22 μm syringe filter (Millipore). The DMSO content in all of the experiments was never higher than 1.7% (v/v). The pK_a's obtained for all compounds were determined by visible spectroscopy and are provided in Table II. The pK_a values, with the exception of the analogues

Table II: pK_a Data and Binding Constants for Amsacrine Analogues at 0.1 M NaCl

compd[a]	pK_a[b]	fraction protonated[c]	K_{obs}[d] (M^{-1})	K_b[e] (M^{-1})	n (base pairs)
m-AMSA	7.89	0.89	$(1.6 \pm 0.2) \times 10^4$	$(1.8 \pm 0.2) \times 10^4$	3.8 ± 0.3
o-AMSA	7.56	0.78	$(4.2 \pm 0.4) \times 10^4$	$(5.4 \pm 0.4) \times 10^4$	3.0 ± 0.1
AMSA	7.69	0.83	$(7.3 \pm 0.3) \times 10^4$	$(8.8 \pm 0.3) \times 10^4$	2.8 ± 0.1
3′-methoxy	8.07	0.92	$(2.3 \pm 0.1) \times 10^4$	$(2.5 \pm 0.1) \times 10^4$	1.9 ± 0.1
2′-methoxy	8.09	0.92	$(3.8 \pm 0.1) \times 10^4$	$(4.1 \pm 0.1) \times 10^4$	2.2 ± 0.1
N-phenyl	8.09	0.92	$(3.6 \pm 0.2) \times 10^4$	$(3.9 \pm 0.2) \times 10^4$	1.9 ± 0.1
9-aminoacridine	9.5	1.0	$(1.4 \pm 0.6) \times 10^5$		3.2 ± 0.1

[a] See Figure 1. [b] From optical spectroscopy. [c] At pH 7.0. [d] K_{obs} refers to the binding constant for the drug molecule to an isolated DNA-binding site (obtained at 20 °C) by titration methods and determined by the McGhee and von Hippel cooperative-ligand equation (McGhee & von Hippel, 1974). [e] K_b refers to the intrinsic binding constant after corrected for state of protonation of the drug at pH 7.0.

containing a 2′- or 3′-methoxy group, are in excellent agreement with those reported by Denny et al. (1982) in 20% DMSO upon adding an average 0.6 units, suggesting a different degree of hydration for these compounds in 20% and 1.7% DMSO.

DNA Preparations. Calf thymus DNA (sodium salt, Type I) was purchased from Sigma Chemical Co. and purified as described by Chaires et al. (1982). Briefly, the DNA was sheared by sonification for 30 min at 5 °C in the presence of bubbling nitrogen. Afterward, the DNA solutions were subjected to T_1 RNase and proteinase K (Boehringer Mannheim) digestions. The solutions were repeatedly extracted with a 1:1 mixture of chloroform and phenol and precipitated with cold absolute ethanol. After centrifugation, the DNA pellet was dissolved in sodium phosphate buffer (pH 7.0), 0.001 M disodium EDTA, and the desired concentration of sodium chloride. The DNA solutions were dialyzed against appropriate buffers and filtered through 0.45 μm syringe filters (Millipore) prior to their use. The concentrations of the DNA solutions are stated in terms of base pairs (bp) by using the molar absorptivity of ϵ_{260nm} of 13 200 M^{-1} cm^{-1}.

DNA-Binding Studies. DNA-binding isotherms for each of the AMSA derivatives were obtained by titrating measured quantities of a stock drug solution into a known volume of a calf thymus DNA solution and monitoring the resulting change in the absorbance spectrum of the drug. Data were analyzed by Scatchard plots and quantitated by using the cooperative-ligand and neighbor-exclusion models of McGhee and von Hippel (1974). Optical titrations were performed on a Varian Cary 2290 UV/visible spectrophotometer equipped with 10-cm cylindrical cells (Uvonic Instruments, Inc.). The temperatures were regulated to within ±0.1 °C with a Lambda circulating water bath. Molar absorptivity data for the free and bound drug species are provided in Table I (Supplementary Material).

Examination of the DNA-binding isotherms of the 3′-methoxy and *N*-phenyl compounds as a function of temperature resulted in relatively small changes in the slopes of the Scatchard plots, thus limiting the accuracy of the determination of the binding enthalpies with classical van't Hoff methodology. To circumvent these problems, the fixed-ratio method outlined by Chaires (1985) and Shimer et al. (1988) was used. Data were obtained by mixing a known concentration of drug and DNA and monitoring the resulting change in the absorbance of the drug. The temperature of the drug–DNA solution was then adjusted as necessary, and the change in absorbance as a function of temperature was remeasured. Linear least-squares analyses of a plots of ln K vs $1/T$ (K) were used to determine the enthalpy from the van't Hoff relationship.

Results

Influence of the pK_a on Equilibrium Binding. Accurate determination of the binding isotherms for the interactions of these anilinoacridine analogues with DNA required that the protonation states of each compound be determined (Blears & Danyluk 1967; Capomacchia et al., 1974; Jones & Wilson, 1981). These studies reveal that at pH 7 protonation of the anilinoacridine analogues ranges from 78% to 92%, depending on the type and position of substituent added to the *N*-phenyl ring as shown in Table II.

Binding constants determined from the spectroscopic data at pH 7.0 are influenced by the protonic equilibria of the drug by the equation

$$K_{obs} = \frac{K_b}{(K_a/[H^+]) + 1} \quad (1)$$

where K_{obs} is the equilibrium constant obtained from analysis of the binding data and includes both protonated and nonprotonated drug species, K_a is the acid dissociation constant for the drug, $[H^+]$ is the hydrogen ion concentration, and K_b is the binding constant determined for the protonated species to the DNA. Table II lists both the observed binding constant K_{obs}, obtained from data analysis by using the McGhee and von Hippel neighbor-exclusion model, and K_b, the binding constant after correction for the fraction of nonprotonated drug.

Correlation of Drug Structure with DNA-Binding Affinities. The interactions of selected anilinoacridine analogues with DNA have been examined and their binding properties correlated to their structural components. Sample binding isotherms for the interactions of these conformers with calf thymus DNA are shown in Figure 2. Both K_{obs} and K_b values are reported in Table II; however, only the values that are corrected for the nonprotonated state (K_b) are discussed.

The parent compound, 9-aminoacridine, demonstrates the highest affinity for binding DNA (data not shown), with a binding constant of 1.4×10^5 M^{-1}. Upon addition of the *N*-phenyl moiety to the acridine ring, the affinity for binding DNA is shown to decrease by a factor of 4 to 3.6×10^4 M^{-1}. Placement of the methoxy group at the 3′ position of the phenyl ring results in a substantial decrease in the DNA-binding affinity (2.3×10^4 M^{-1}), effectively 6 times lower than that of the parent 9-aminoacridine and 2 times lower than that observed for the *N*-phenylanilinoacridine analogue. A similar decrease in the DNA-binding affinity is observed for *m*-AMSA (which also has a 3′-methoxy substituent).

In contrast, placement of the method group at the 2′ position on the *N*-phenyl ring results in a DNA-binding affinity of approximately the same magnitude as observed for the *N*-phenyl analogue. Analysis of the DNA-binding affinity of *o*-AMSA (whose methoxy substituent is also located at the 2′ position) reveals a similar binding constant, indicating that placement of the methoxy group at the 2′ position neither enhances nor restricts the interactions of these compounds with DNA. Thus, influences on DNA binding due to the methoxy group appear to be dictated by the position to which this

Table III: Summary of Thermodynamic Parameters for Amsacrine Analogue–DNA Interactions

compd	$\Delta G°_b$[a] (kcal/mol)	$\Delta H°_{obs}$[b] (kcal/mol)	protonation $\Delta H°$[c] contribution (kcal/mol)	$\Delta H°_b$[d] (kcal/mol)	$\Delta S°_b$[e] (cal deg^{-1} mol^{-1})
m-AMSA	−5.7	−6.3 ± 0.2	−1.1	−5.2 ± 0.2	+1.7 ± 0.2
o-AMSA	−6.3	−5.2 ± 0.2	−2.2	−3.0 ± 0.2	+11.2 ± 0.2
AMSA	−6.6	−7.6 ± 0.2	−1.7	−5.9 ± 0.2	+2.4 ± 0.2
3′-methoxy	−5.9	−2.2 ± 0.2	−0.8	−1.4 ± 0.2	+15.4 ± 0.2
2′-methoxy	−6.2	−4.5 ± 0.2	−0.8	−3.7 ± 0.2	+8.4 ± 0.2
N-phenyl	−6.2	−3.5 ± 0.2	−0.8	−2.7 ± 0.2	+11.9 ± 0.2

[a] Calculated from K_b in Table II from the relation $\Delta G° = -RT \ln K$. [b] Values obtained from the slope of the van't Hoff plots. [c] From Christianson et al. (1976). [d] $\Delta H°$ value corrected for the protonation state of the drug at pH 7.0. [e] The entropy was evaluated from the relation $\Delta S° = -(\Delta G° - \Delta H°)/T$.

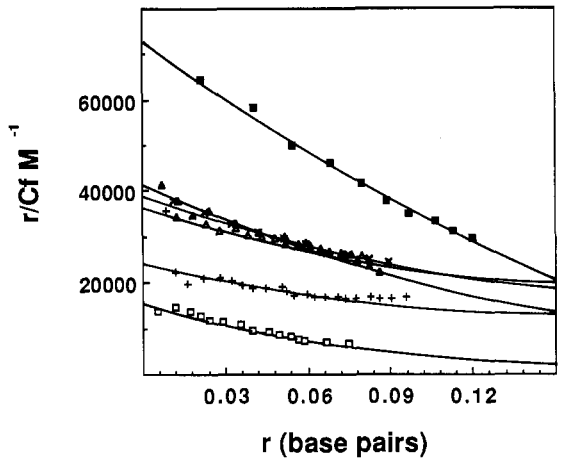


FIGURE 2: Scatchard plots for the binding of the anilinoacridine analogues to calf thymus DNA. Binding data were obtained at 20 °C in 0.01 M sodium phosphate buffer, pH 7.0, 0.001 M disodium EDTA, and 0.1 M sodium chloride. Solid lines represent best fits to the McGhee and von Hippel equation. The antitumor active compounds *m*-AMSA (□) and AMSA (■) represent the lowest and highest binding affinities of the anilinoacridines, respectively. In contrast, the binding of *o*-AMSA (▲) is shown to be intermediate to that of AMSA and *m*-AMSA, along with the *N*-phenyl (△) and 2′-methoxy (x) derivatives. The DNA-binding isotherm of the 3′-methoxy derivative is represented by (+). Parameters used to fit these data to the McGhee and von Hippel equation are provided in Table II.

substituent is added to the *N*-phenyl ring.

Addition of the methanesulfonamido group to the 1′ position of the phenyl ring results in an enhancement in the DNA-binding affinity. In comparing the *N*-phenyl derivative with AMSA, an increase from 3.6×10^4 M^{-1} (as observed for the *N*-phenyl analogue) to 7.4×10^4 M^{-1} for AMSA was observed. The *o*-AMSA (1′-methanesulfonamido-2′-methoxy) and *m*-AMSA (1′-methanesulfonamido-3′-methoxy) exhibit drastically different binding affinities when examined under identical conditions. Positioning the substituent groups ortho with respect to each other results in a more favorable binding affinity (5.2×10^4 M^{-1}) as compared to the meta position (1.8×10^4 M^{-1}). Interestingly, the two compounds that demonstrate antitumor activity, AMSA and *m*-AMSA, lie at opposite extremes of DNA-binding affinities, with the AMSA binding with the highest affinity as compared to *m*-AMSA, which exhibits the lowest DNA binding affinity. The equilibrium constant of *o*-AMSA lies between these two extremes, with approximately the same affinity as was observed for the *N*-phenyl and 2′-methoxy analogues. No significant enhancement in the binding constant is observed for *o*-AMSA over that of the *N*-phenyl derivative, suggesting that the methanesulfonamido group of *o*-AMSA may be restricted from making additional contacts with the DNA. In addition, the 2′-methoxy group does not hinder DNA binding as evidenced by comparing of the binding affinities of the 2′-methoxy analogue and *o*-AMSA with the *N*-phenyl derivative.

Thermodynamic Parameters of the Anilinoacridine Analogues. DNA-binding enthalpies and entropies were estimated by van't Hoff analysis for the series of anilinoacridines as well as the parent 9-aminoacridine. Data were obtained by classical titration methods in the form of Scatchard plots at various temperatures (fit to the McGhee and von Hipple equation) and by the fixed-ratio method described by Chaires (1986) and Shimer et al. (1988). Values obtained by both methods were identical and are reported in Table III. Data were collected over a range of *r* values (0.01–0.2) and found to be independent of the fractional saturation of the DNA.

The $\Delta G°$ values for the interactions of the parent 9-aminoacridine and amsacrine analogues with DNA are comparable, ranging from −5.6 to −6.5 kcal/mol. However, the binding enthalpies for the anilinoacridine derivatives span a wide range of values with the parent 9-aminoacridine exhibiting the largest $\Delta H°$ at −9.2 kcal/mol. The interactions of AMSA and *m*-AMSA to DNA are characterized by $\Delta H°$ values of −5.9 and −5.2 kcal/mol, respectively. In contrast, the biologically inactive *N*-phenyl, 2′- and 3′-methoxy, and *o*-AMSA analogues exhibit significantly lower $\Delta H°$ values, ranging from −3.0 to −1.4 kcal/mol, approximately half the magnitudes observed for AMSA and *m*-AMSA. Placement of the *N*-phenyl ring on the acridine chromophore results in a positive increase of ~6 kcal/mole in the $\Delta H°$ to −2.7 kcal/mol. Addition of the methoxy group to the 3′ position on the *N*-phenyl ring further hinders the binding of the drug to DNA, with the binding enthalpy of the 3′-methoxy derivative being approximately 1 kcal/mol more positive than that of the *N*-phenyl derivative. Similarly, the difference in binding enthalpies between *m*-AMSA and AMSA (addition of the 3′-methoxy group) is approximately 1 kcal/mol more positive.

Examination of the thermodynamic profiles shown in Table III reveals the nearly identical manner in which the *N*-phenyl, 2′-methoxy and *o*-AMSA derivatives bind DNA, which suggests that the 2′-methoxy group does not directly influence the interaction of these compounds with DNA. This is illustrated by comparing the binding profiles of AMSA, *m*-AMSA, and *o*-AMSA. For the two antitumor agents (*m*-AMSA and AMSA), both bearing the sulfonamido moiety, the enthalpies of binding are very similar at −5.2 to −5.9 kcal/mol. However, the *o*-AMSA–DNA complex is characterized by an enthalpy of −3.0 kcal/mol, which is 2–3 kcal/mole less favorable, demonstrating marked differences in the DNA-binding properties between the antitumor active *m*-AMSA and the inactive structural isomer *o*-AMSA.

An interesting feature of the thermodynamic profiles are the additive natures of the methoxy and methanesulfonamido functional groups upon addition to phenyl ring at the 1′ and 3′ positions, as illustrated in Figure 3. Addition of the methoxy group to the 3′ position on the *N*-phenyl ring (i.e., *N*-phenyl to 3′-methoxy derivative and AMSA to *m*-AMSA

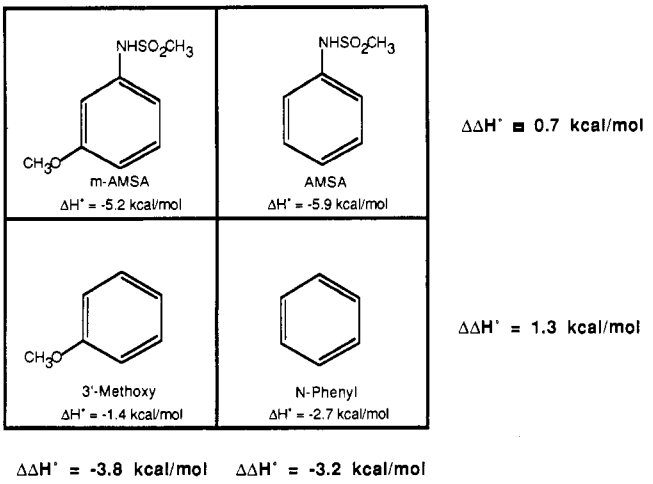


FIGURE 3: Effects of substituent addition (methoxy or methanesulfonamido groups) on the binding enthalpies of selected anilinoacridine anlogues. Comparisons from right to left depict the addition of the methoxy substituent group at the 3′ position (AMSA to *m*-AMSA and the *N*-phenyl to 3′-methoxy derivative. Analysis from bottom to top offers comparisons of addition of the methanesulfonamido group to the 1′ position (i.e., 3′-methoxy to *m*-AMSA and the *N*-phenyl to AMSA derivative). Differences in the binding enthalpies resulting from substituent addition are represented by $\Delta\Delta H°$. Addition of the methoxy group results in a change in the binding enthalpy of approximately +1 kcal/mol, while addition of the methanesulfonamido group to the 1′ position facilitates the binding enthalpy by −3.5 kcal/mol.

derivative) consistently results in a positive increase in the binding enthalpy of approximately 1 kcal/mol. In contrast, this additive effect is not observed for addition of the methoxy group to the 2′ position. Addition of the 2′-methoxy to the *N*-phenyl analogue results in a 1 kcal/mol increase in the binding enthalpy. Comparison of AMSA with *o*-AMSA (again an addition of the 2′-methoxy group) reveals a 3 kcal/mol difference in binding enthalpies. Comparisons of the $\Delta H°$ values for 3′-methoxy versus 2′-methoxyanilinoacridines as well as comparisons of *m*-AMSA versus *o*-AMSA both demonstrate a 2 kcal/mol more favorable binding enthalpy for the 2′-methoxy analogues over their 3′-methoxy analogue counterparts.

Addition of the methanesulfonamido group to the 1′ position results in more negative binding enthalpies by approximately 3 kcal/mol (comparison of the *N*-phenyl analogue with AMSA and the 3′-methoxy analogue with *m*-AMSA). The additive correspondence between functional group substitutions and binding enthalpies suggests that both *m*-AMSA and AMSA bind DNA in a similar manner and indicates both the substituent type and position on the *N*-phenyl ring is highly influential in directing specific DNA-binding properties. Interestingly, this effect is not observed in comparing the binding enthalpies of the 2′-methoxy derivative with *o*-AMSA. Addition of the 1′-methanesulfonamido group to the 2′-methoxy derivative results in less than 1 kcal/mol enhancement in the binding enthalpy, again suggesting that placement of the methoxy group at the 2′ position may disallow contacts between the 1′-methanesulfonamido group of *o*-AMSA and DNA.

The biologically inactive 2′-methoxy, and 3′-methoxy, *N*-phenyl, and *o*-AMSA derivatives exhibit large positive entropy values (ranging from 8 to 15 eu). Positive entropy contributions are expected from hydrophobic forces associated with the transfer of the drug molecule from the solvent to the intercalation site and with the resulting disruption of the hydration layers of the DNA and/or drug due to the bulky *N*-phenyl (substituted) side chain. In contrast, relatively small

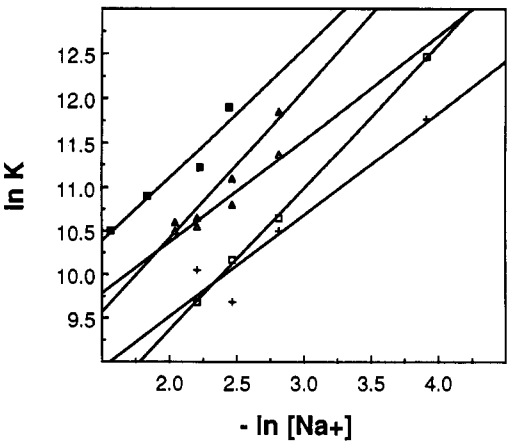


FIGURE 4: Dependence of the intrinsic equilibrium binding constant of the anilinoacridine analogues binding to calf thymus DNA of the ionic strength expressed as $[Na^+]$. Solid lines represent linear least-squares fits to the data for AMSA (■), *m*-AMSA (□), *o*-AMSA (▲), *N*-phenyl (Δ), and 3′-methoxy (+) analogues.

Table IV: Ionic Strength Dependence of Binding Constants at 20 °C for Amsacrine Analogues

compd	$[Na^+]$ (mol/L)	K_{obs} (M^{-1})	Z^a	Z^{*b}
m-AMSA	0.11	1.6×10^4	1.8	1.5
	0.085	2.6×10^4		
	0.06	4.2×10^4		
	0.02	2.6×10^5		
o-AMSA	0.13	4.0×10^4	1.9	1.6
	0.11	4.2×1014		
	0.085	6.6×10^4		
	0.06	1.4×10^5		
AMSA	0.21	3.7×10^4	1.6	1.2
	0.16	5.6×10^4		
	0.11	7.3×10^4		
	0.085	1.5×10^5		
3′-methoxy	0.11	2.3×10^4	1.3	1.0
	0.085	1.6×10^4		
	0.06	3.6×10^4		
	0.02	1.3×10^5		
N-phenyl	0.13	3.6×10^4	1.3	1.0
	0.11	3.8×1014		
	0.085	4.9×10^4		
	0.06	8.7×10^4		

[a] Effective electric charge as defined by Record et al. (1978). [b] Effective electric charge as defined by Wilson and Lopp (1979).

entropy values ($\Delta S°$ of ~1–2 eu) are observed for the interactions of *m*-AMSA and AMSA with DNA, indicative of additional contacts between the DNA and these drugs.

Effect of Ionic Strength on Binding. The dependence of the DNA-binding constants of the anilinoacridine analogues on the ionic strength is shown in Figure 4. The slopes of the lines are determined from a linear least-squares analysis. These slopes are described by Record et al. (1978) as

$$\frac{d \ln K}{d \ln [Na^+]} = -Z\psi \qquad (2)$$

where Z is the effective electric charge on the ligand and ψ is the fraction of counterions associated with each DNA phosphate. For double-stranded DNA $\psi = 0.88$. The resultant values for Z are given in Table IV. The effective charges determined in this manner represent the number of counterions released upon binding of the drug.

Wilson and Lopp (1979) derived a similar equation that also accounts for changes in the DNA structure upon intercalation

of the drug. This is given as

$$\frac{d \ln K}{d \ln [Na^+]} = -2n(\psi - \psi^*) - Z\psi^* \quad (3)$$

Where ψ is described above, n is the number of neighboring sites excluded upon ligand binding, and ψ^* is the fraction of counterion per phosphate and in the intercalated DNA–drug complex. With average n value of 2.0 and $\psi^* = 0.82$, values of Z have been calculated and entered in Table IV and are referred to as Z^*. All of the anilinoacridine analogues examined (including AMSA and *m*-AMSA) exhibit Z^* values approximately equal to 1, indicating similar charges and binding modes.

Discussion

The present studies provide unique insight into the effects of substituent modification and position on the biophysical properties associated with the interactions of related anilinoacridines with DNA. These studies reveal that although the amsacrine series of molecules are similar in structural composition, their thermodynamic modes of interaction with DNA are markedly different and are mediated by both the addition and position of the methanesulfonamido and methoxy substituent groups to the *N*-phenyl ring. Addition of the phenyl ring to the exocyclic nitrogen of 9-aminoacridine greatly reduces the ability of the *N*-phenyl derivative to bind to DNA. This decrease is likely due to steric interference by the phenyl ring inhibiting the full insertion of the acridine moiety into the DNA double helix, as proposed from molecular modeling studies of the *m*-AMSA–d(TACGTA)$_2$ complex by Abraham et al. (1988) and computational studies by Pullman and Chen (1988). Further destabilizing effects are introduced with the addition of the methoxy group to the 3′ position of the phenyl ring. The presence of the 3′-methoxy group may result in the disruption of the charge density of the acridine ring system, as has been proposed by Denny et al. (1983). However, a more plausible explanation is that the presence of this group at the 3′ position results in additional steric constraints imposed on the molecule through a direct influence of the orientation of the *N*-phenyl ring with respect to the acridine chromophore. In contrast, addition of the methoxy group to the 2′ position of the phenyl ring does not alter the DNA-binding affinity from that of the parent *N*-phenyl derivative.

The presence of the 1′-methanesulfonamido group is shown to enhance the DNA-binding affinity of the anilinoacridine analogues as observed in the comparison of the binding affinities of the *N*-phenyl analogue and AMSA. However, upon addition of a methoxy group to either the 2′ or 3′ positions of the 1′-methanesulfonamido derivative, substantial decreases DNA-binding affinities are observed with the 3′-methoxy derivative being more effective at inducing this reduction.

Further information concerning the DNA-binding mechanism(s) of the anilinoacridine analogues is provided by examination of the enthalpy and entropy components of the binding data. The 2′-methoxy, 3′-methoxy, *N*-phenyl, and *o*-AMSA derivatives are characterized by low binding enthalpies (ΔH° values ranging from −1 to −3 kcal/mol) and high entropies of binding (ΔS° values of approximately +12 eu). In contrast, the favorable free energies for the binding of the biologically active *m*-AMSA and AMSA to DNA are derived largely from the contributions of a large negative enthalpy with ΔH° values of −5 kcal/mol with relatively small entropy values (1–2 eu).

The thermodynamic contributions of binding by selected functional groups appear to be quantitative. Addition of the phenyl ring to the exocyclic nitrogen adds approximately 6 kcal/mol to the binding enthalpy for the *N*-phenyl derivative binding to DNA, consistent with the model in which the amasacrine analogues cannot fully intercalate the acridine chromophore into the DNA duplex. Addition of a methoxy substituent in the 3′ position of the phenyl ring further increases the binding enthalpy by approximately 1 kcal/mol (comparing the *N*-phenyl analogue with the 3′-methoxy analogue and *m*-AMSA and AMSA). The presence of the methanesulfonamido group in the 1′ position of the phenyl ring contributes about −4 kcal/mol to the binding enthalpy. The additive nature of the substituent additions suggest that the derivatives bind in a similar manner to the DNA. The *o*-AMSA derivative does not reflect the full contribution from the presence of the 1′-methanesulfonamido group and exhibits a ΔH° value of only −3.0 kcal/mol. In analyzing the change in ΔH° contributions mediated by the position of the methoxy group (2′ versus 3′), all cases consistently demonstrated that compounds with 2′-methoxy groups bind DNA with an enthalpy 2 kcal/mol more favorable than compounds with a methoxy group located at the 3′ position. This enthalpy value for *o*-AMSA is very similar to that obtained for the 2′-methoxy derivative (−3.7 kcal/mol) an the *N*-phenyl derivative (−2.7 kcal/mol), suggesting that a substituent in the 2′ position does not interact to any extent with the DNA but instead exerts it effect on the 1′-methanesulonamide moiety.

Several molecular interactions must be considered when examining the thermodynamic components associated with the intercalative binding of these compounds to DNA. Among these are the van der Waals stacking interactions between the acridine ring of these compounds with the adjacent base pairs of the DNA duplex contributing both negative enthalpies and entropies of binding, displacement of bound water from the DNA's minor groove would provide an additional positive entropic term, and the potential for additional hydrogen-bonding contacts between the drug and the DNA, which would contribute approximately −10 eu (Eftink & Biltonen, 1980). Assuming the intercalative geometry is similar for the structurally related anilinoacridine–DNA complexes, the differences in the binding enthalpies and entropies of AMSA and *m*-AMSA with the other biologically inactive analogues provide key insight to the differences in the thermodynamic binding modes of these compounds. AMSA and *m*-AMSA exhibit entropy values that are approximately 10 eu lower in magnitude than the *N*-phenyl, 2′- and 3′-methoxy derivatives, which suggests that an additional contact (i.e., hydrogen bond) may be formed between AMSA or *m*-AMSA and DNA that is not possible for the other anilinoacridine analogues. This hypothesis is supported by the −2 to −3 kcal/mol more negative binding enthalpies observed for the AMSA and *m*-AMSA interaction with DNA.

These data support the model outlined by Denny et al. (1983) in which the acridine chromophore is intercalated into the double helix of DNA and the phenyl ring resides in the minor groove. The data presented here demonstrate that the four anilinoacridine analogues that are ineffective as tumor agents are characterized by large positive entropies, while the two antitumor drugs (AMSA and *m*-AMSA) are characterized by small binding entropies, suggesting the complexes formed by AMSA and *m*-AMSA are more constrained than complexes formed by the other anilinoacridine analogues. This observation provide insight into the biophysical mechanisms by which AMSA and *m*-AMSA interact with DNA. Systematic analyses of substituent types and positions on DNA-binding properties demonstrate significant differences between

the interactions of the potent antitumor agent *m*-AMSA with its biologically inactive constitutional isomer *o*-AMSA. Binding of both compounds to DNA has been demonstrated to be via intercalation. However, these studies reveal the binding of *m*-AMSA to DNA to be enthalpy driven, in contrast to the large entropy component of the *o*-AMSA–DNA interaction. Subsequent experiments are underway in this laboratory to determine whether these thermodynamic differences may be correlated with topoisomerase II activities and formation of the ternary complex.

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SUPPLEMENTARY MATERIAL AVAILABLE

Table I giving the molar extinction coefficients for the free and bound species of the anilinoacridine analogues shown in Figure 1 (1 page). Ordering information is given on any current masthead page.

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