Stereoelectronic Factors in the Interaction with DNA of Small Aromatic Molecules Substituted with a Short Cationic Chain: Importance of the Polarity of the Aromatic System of the Molecule†

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ABSTRACT: We have performed a quantitative analysis of the interaction with DNA of several unfused aromatic compounds synthesized in our laboratory and substituted with one or two short cationic chains. These and similar literature compounds, for which DNA binding data are available, bind with DNA by partial intercalation of the aromatic system, groove interaction of the linker chain, and groove electrostatic interactions of the terminal cationic group. Several independent quantitative and qualitative approaches show consistently that the strength of the interaction of the aromatic unit of the molecule with DNA binding sites depends on the direction and magnitude of polarity of the aromatic system. The phenomenon is explained in terms of the greatest negative potential in the DNA grooves, a concept extensively elaborated by Pullman and Pullman [cf. Lavery, R. and Pullman, B. [(1985) *J. Biomol. Struct. Dyn. 2*, 1021–1032] and references therein]. Classical, fused-ring planar intercalators do not follow the polarity–DNA affinity correlation, presumably because the intercalative forces depend more strongly on polarizability than on polarity of the aromatic system.

Since DNA is an important cellular receptor for many anticancer drugs, the design of DNA binding molecules, and studies of their interaction features, have received considerable attention. Biologically active compounds can bind reversibly with a DNA duplex in one of the grooves or by intercalation (Baguley, 1991; Denny, 1989; Wilson, 1990; Wilson & Jones, 1981). Typically, groove binding molecules are composed of several heteroaromatic rings linked together through amide or other functional groups, or directly through single bonds (Grootenhuis et al., 1990; Wilson, 1990). Unfused polyaromatic systems, depending on their structure and stacking surface, may also intercalate with DNA to form a nonclassical intercalation complex in which the intrinsic inter-ring twist of the molecule and the propeller twist (Dickerson, 1983) of the DNA base pairs can be retained (Strekowski et al., 1988a,b; Wilson et al., 1988, 1989). By contrast, the DNA propeller twist is reduced severely in a classical intercalation complex with planar fused-ring systems (Wilson, 1990). The binding strength in all these interaction modes is greatly enhanced by the presence of a cationic center in the interacting molecule. This additional increase in the binding strength is due to counterion release and interaction of a cation with the electrostatic field of the anionic backbone of DNA (Baase et al., 1984; Manning, 1978; Record et al., 1978). Interestingly, in DNA complexes with monocations or polycations, the cationic units have been found to interact with the DNA grooves (Gessner et al., 1989; Jain et al., 1989; Quigley et al., 1980; Wang et al., 1987). This finding can be explained in terms of the greatest negative potential in the grooves of the DNA duplex rather than on the backbone, despite the location of the anionic phosphates within the backbone (Burridge et al., 1987; Lavery & Pullman, 1985). A number of other theoretical studies strongly support the groove interaction model (Feuerstein et al., 1986; Strekowski et al., 1989; Zakrzewska & Pullman, 1986).

In this paper we report that the concept of the negative potential in DNA grooves (Lavery & Pullman, 1985) has important ramifications for an understanding of the interaction of cation-substituted aromatic molecules with DNA. An important feature in such molecules is a short bridging unit, which dictates the stereochemistry of the molecule in the complex, between the cationic center and the aromatic system. More specifically, the location of the cationic center in the DNA grooves allows only a limited number of orientations of the aromatic system of the molecule with respect to the local structure of DNA. We present evidence that polarity of the aromatic system is of primary importance for the overall binding strength of such molecules with DNA. The molecules, in which the positive part of the aromatic dipole can favorably interact with the negative potential of the DNA groove, show stronger binding with DNA than their analogs with the opposite polarity.

MATERIALS AND METHODS

Syntheses of bicyclic pyrimidines **1–9** (Brown et al., 1982; Strekowski, et al., 1988a), tricyclic pyrimidines **10–13** (Strekowski et al., 1991; Wilson et al., 1989), and a quinolinecarboxamide **20** (Palmer et al., 1988) have been reported. Microanalyses for all new compounds reported below were within 0.2, 0.1, and 0.1% of the theoretical values for C, H, and N, respectively.

2-[[2‴-(Dimethylamino)ethyl]thio]-6-furan-2″-yl-4-(1′-methylpyrrol-2′-yl)pyrimidine Dihydrobromide (***14*·2HBr**). Treatment of 2-chloro-6-furan-2″-yl-4-(1′-methylpyrrol-2′-yl)pyrimidine (Strekowski et al., 1990) with sodium 2-(dimethylamino)ethanethiolate and subsequent workup were conducted using a general procedure (Strekowski et al., 1991) to give **14** (85%) as an oil: ^{1}H NMR ($CDCl_3$) δ 2.32 (s, 6 H),

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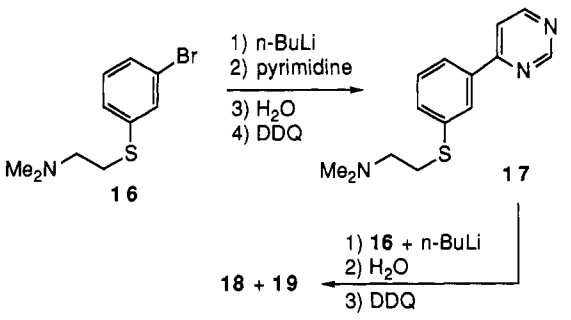
Scheme I



2.75 (m, 2 H), 3.38 (m, 2 H), 4.06 (s, 3 H), 6.20 (m, 1 H), 6.58 (m, 1 H), 6.85 (m, 2 H), 7.20 (m, 1 H), 7.44 (s, 1 H), 7.56 (m, 1 H). The dihydrobromide was obtained and crystallized by using a general procedure (Brown et al., 1982): mp 250–253 °C (dec). Anal. ($C_{17}H_{20}N_4OS \cdot 2HBr$) C, H, N.

N-[2‴-(Dimethylamino)ethyl]-6-furan-2″-yl-4-(1′-methylpyrrol-2′-yl)pyrimidin-2-amine Dihydrobromide (***15*·2HBr**). Substitution of *N,N*-dimethylethylenediamine for a thiolate in the procedure given above furnished **15** (90%) as an oil: ^{1}H NMR ($CDCl_3$) δ 2.25 (s, 6 H), 2.53 (m, 2 H), 3.55 (m, 2 H), 4.06 (s, 3 H), 5.60 (m, 1 H, exchangeable with D_2O), 6.19 (m, 1 H), 6.50 (m, 1 H), 6.80 (m, 2 H), 7.09 (m, 1 H), 7.14 (s, 1 H), 7.51 (m, 1 H). The dihydrobromide had mp 266–268 °C (dec). Anal. ($C_{17}H_{21}N_5O \cdot 2HBr$) C, H, N.

4,6-Bis[3′-[[2″-(dimethylamino)ethyl]thio]phenyl]pyrimidine Dihydrobromide Hemihydrate (***18*·2HBr·$^1/_2H_2O$**). Synthesis of **18** is outlined in Scheme I. A previously published procedure for the preparation of a 4-thiophenyl isomer of **18** was used (Wilson et al., 1988). Briefly, 3-bromobenzenethiol was treated with 2-(dimethylamino)ethyl chloride hydrochloride in the presence of sodium hydroxide. After standard workup, the resultant crude *N,N*-dimethyl-2-[(3′-bromophenyl)thio]ethylamine (**16**) was distilled on a Kugelrohr [100 °C (0.15 mmHg)] to furnish analytically pure **16** (78%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 2.26 (s, 6 H), 2.60 (m, 2 H), 3.10 (m, 2 H), 7.25 (m, 3 H), 7.51 (m, 1 H). Anal. ($C_{10}H_{14}BrNS$) C, H, N. A lithium derivative, generated in the reaction of **16** with *n*-butyllithium, was allowed to react with pyrimidine, and the resultant, substituted dihydropyrimidine was dehydrogenated by treatment with DDQ[1] to furnish *N,N*-dimethyl-2-[(3′-pyrimidin-4″-ylphenyl)thio]ethylamine (**17**; 47% after chromatography) as an oil: ^{1}H NMR ($CDCl_3$) δ 2.30 (s, 6 H), 2.60 (m, 2 H), 3.10 (m, 2 H), 7.40 (m, 2 H), 7.65 (dd, J = 5, 1.8 Hz, 1 H), 7.80 (m, 1 H), 8.06 (m, 1 H), 8.71 (d, J = 5 Hz, 1 H), 9.20 (d, J = 1.8 Hz, 1 H). Anal. ($C_{14}H_{17}N_3S$) C, H. N. Substitution of **17** for pyrimidine in the procedure described above and followed by chromatography (Wilson et al., 1988) gave **18** (50%) as an oil. The oil was treated with hydrobromic acid using a procedure published previously (Brown et al., 1982), and the resultant salt was crystallized from aqueous ethanol: mp 233–235 °C (dec); ^{1}H NMR (a phosphate buffer, pH 7.00) δ 2.87 (NMe_2), 3.36 (NCH_2), 3.44 (SCH_2), 7.74 (H4′), 7.66 (H5′), 8.03 (H6′), 8.17 (H2′), 8.29 (H5), 9.24 (H2). Anal. ($C_{24}H_{30}N_4S_2 \cdot 2HBr \cdot {}^1/_2H_2O$) C, H, N.

2,4-Bis[3′-[[2″-(dimethylamino)ethyl]thio]phenyl]pyrimidine Trihydrobromide (***19*·3HBr**). Chromatographic separation of the final mixture described above also gave pyrimidine **19** (16%) as an oil. The trihydrobromide was obtained by using a standard procedure (Brown et al., 1982) and crystallized from aqueous ethanol: mp 207–209 °C (dec); ^{1}H NMR (a phosphate buffer, pH 7.00) δ 2.87 (NMe_2), 3.37 (NCH_2), 3.44 (SCH_2), 7.65 (H5′ of ring A), 7.66 (H5′ of ring C), 7.73 (H4′ of rings A and C), 7.92 (H5), 8.13 (H6′ of ring A), 8.26 (H2′ of ring A), 8.28 (H6′ of ring C), 8.38 (H2′ of ring C), 8.93 (H6). Anal. ($C_{24}H_{30}N_4S_2 \cdot 3HBr$) C, H. N.

N-[2′-(Dimethylamino)ethyl]naphthalene-1-carboxamide (***21***). This compound was prepared from 1-naphthoic acid and *N,N*-ethylenediamine by using a general procedure described previously (Rewcastle et al., 1987) and purified by crystallization from hexanes/toluene: mp 73–75 °C; ^{1}H NMR ($CDCl_3$) δ 2.22 (s, 6 H), 2.50 (m, 2 H), 3.55 (m, 2 H), 6.60 (m, 1 H, exchangeable with D_2O), 7.20–8.40 (m, 7 H). Anal. ($C_{15}H_{18}N_2O$) C, H, N.

N-[2′-(Dimethylamino)ethyl]quinoline-4-carboxamide (***22***). This compound was prepared from quinoline-4-carboxylic acid as described above and crystallized from toluene: mp 81–83 °C; ^{1}H NMR ($CDCl_3$) δ 2.21 (s, 6 H), 2.46 (m, 2 H), 3.50 (m, 2 H), 6.80 (m, 1 H, exchangeable with D_2O) 7.26 (d, J = 4 Hz, 1 H), 7.35–8.25 (m, 4 H), 8.70 (d, J = 4 Hz, 1 H). Anal. ($C_{14}H_{17}N_3O$) C, H, N.

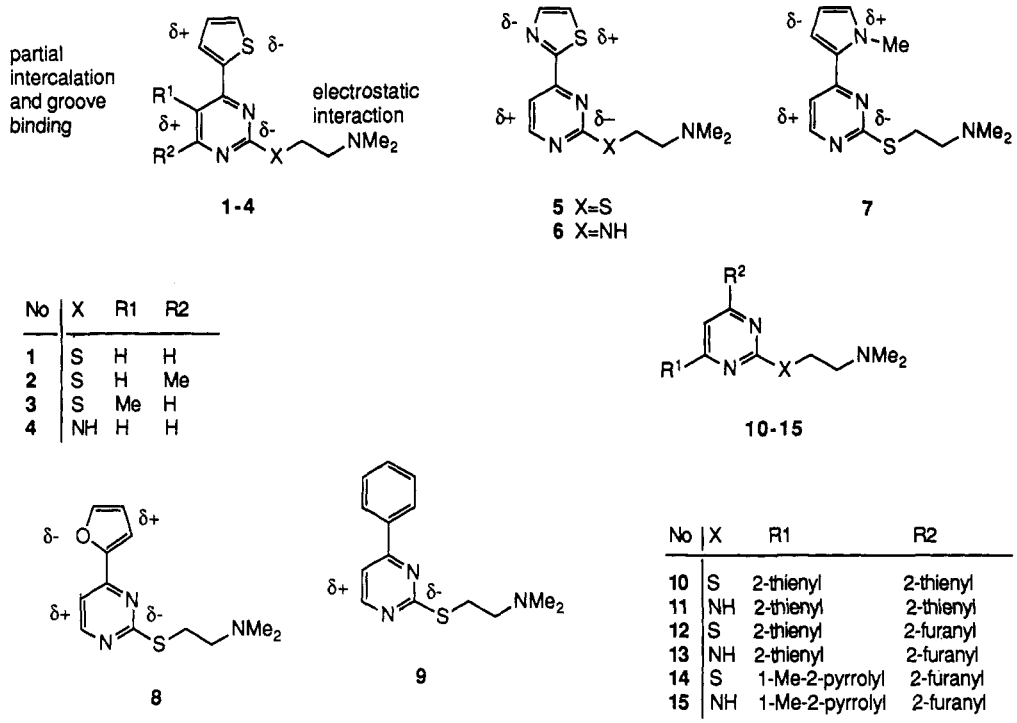
DNA Samples. Sonicated calf thymus DNA (Worthington Biochemical) was used in the NMR experiments (200 ± 50 base pairs), viscometric titrations, and spectrophotometric binding measurements (800 ± 100 base pairs). The DNA samples were purified from residual proteins and characterized as previously described (Wilson et al., 1985a,b). Plasmid pBR322 for the viscometric titrations was obtained from *Escherichia coli* strain K336 grown in Luria-Bertani media with 25 μg L^{-1} ampicillin and amplified with 100 mg L^{-1} chloramphenicol (Hillen et al., 1981). After the usual workup (Garger et al., 1983), the plasmid (concentration of 3×10^{-3} M DNA bases) was obtained by HPLC on a Nucleogen-DEAE 4000-7 column as previously described (Strekowski et al., 1988b).

Buffers. All viscometric titrations and spectrophotometric binding measurements were conducted in a PIPES buffer: 10 mM PIPES and 1.0 mM EDTA, pH 7.00. For NMR experiments in D_2O, a phosphate buffer was used: 15 mM NaH_2PO_4, 0.1 mM EDTA, and 0.10 M NaCl, pH 7.00.

*Structures of **1–15** and **18–22** at pH 7.* The pK_a of a dimethylamino side chain in all these compounds is greater than or equal to 8.6 (Atwell et al., 1984, 1987), so that the amino substituent is practically fully protonated at pH 7. The pK_a values for the ring nitrogen atoms were estimated to be <4.5 (Brown, 1985; Newkome & Paudler, 1982; Perrin et al., 1981; Young, 1975) in all cases studied. The ring nitrogens in **1–15** and **18–22**, thus, are not protonated at pH 7. The suggested structures were confirmed by analysis of proton NMR spectra of these compounds taken at pH 7 and under extremal conditions in 0.01 N DCl and 0.01 N NaOD in D_2O solutions. The formation of a cation resulted in large downfield shifts (>1 ppm) for signals of protons adjacent to the cationic center.

Hydrodynamic and NMR Methods. Procedures for viscometric titrations (Jones et al., 1980), spectrophotometric binding measurements (Wilson et al., 1985a,b), and NMR studies (Wilson et al., 1989) conducted on a Varian VXR-400 spectrometer have been presented. The ethidium-induced unwinding angle of 26° was taken as a reference value for the experiments with a superhelical DNA sample (Wang, 1974). The unwinding experiments were conducted at a range of

[1] Abbreviations: DDQ, 2,3-dichloro-5,6-dicyanobenzoquinone; EDTA, ethylenediaminetetraacetic acid; NOE, nuclear Overhauser effect; PIPES, piperazine-*N,N*′-bis(2-ethanesulfonic acid); QSAR, quantitative structure–activity analysis relationship.



No	X	R1	R2
1	S	H	H
2	S	H	Me
3	S	Me	H
4	NH	H	H

No	X	R1	R2
10	S	2-thienyl	2-thienyl
11	NH	2-thienyl	2-thienyl
12	S	2-thienyl	2-furanyl
13	NH	2-thienyl	2-furanyl
14	S	1-Me-2-pyrrolyl	2-furanyl
15	NH	1-Me-2-pyrrolyl	2-furanyl

FIGURE 1: Structures of unfused pyrimidine derivatives **1–15**. Stable orientations for bicyclic compounds **1–8** and polarities of individual rings in these conformations are shown. The DNA interaction model is illustrated with **1–4**.

DNA concentrations, and the maximum viscosity changes were plotted by the Vinograd method (Révet et al., 1971).

QSAR Analyses. Classical, linear regression analyses were conducted to correlate the DNA equilibrium binding constants K with calculated dipole moments μ (Strekowski et al., 1988a) and valence molecular connectivity indexes of the first order $^1\chi^v$ (Kier & Hall, 1976, 1986). Since the pyrimidines under study contain diverse substituents, a Free–Wilson approach of de novo model (Free & Wilson, 1964; Kubinyi, 1988) was also applied to the analysis of the binding constants K:

$$\log K_i = \beta + \sum_j \alpha_{jk} X_{jk} \qquad (1)$$

In this nonparameter model, for every compound of the series the binding constant values K_i used in the logarithmic scale are expressed as the sum of the binding constant contributions α_{jk} of the substituents R_k in each position j (j = 2, 4, and/or 6), referring to the overall average β. In eq 1, X_{jk} has a value of 1 when the substituent is present in the position j; otherwise its value is zero (Kubinyi, 1988). The formalism of the Free–Wilson method calls for a treatment of the otherwise identical positions 4 and 6 at the pyrimidine ring as nonequivalent when substituents at these positions including a hydrogen atom are different. This procedure doubles formally the number of unsymmetrically substituted pyrimidines in the analysis. On the other hand, a 4,6-disubstituted pyrimidine containing two identical substituents is treated as one compound.

In this work the quality of all correlations is characterized by a regression coefficient r, a standard deviation s, and by an F_x ratio at the given probability level x.

RESULTS

Pyrimidines 1–15. This series was chosen for initial studies because of the wealth of DNA binding data available. All compounds are monocationic at pH 7 with a protonated terminal amino group. Detailed proton NMR NOE studies (Strekowski et al., 1986a,b, 1988a,b; Wilson et al., 1989) and X-ray crystallographic analysis (Strekowski et al., 1988b) showed low torsional angles for the heteroarylpyrimidine systems. More importantly, the bicyclic systems of **1–4**, **7**, and **8** and the corresponding bicyclic subunits of **10–15** exist in solution with a single preferred orientation of the aromatic rings, and this orientation is retained in the complex with DNA. The same studies strongly suggest that **5** and **6** have the orientations shown in Figure 1 both free in solution and complexed with DNA. On the basis of detailed hydrodynamic, proton NMR, and phosphorus NMR studies, it was concluded that **1–13** intercalate with DNA, although the interaction of several derivatives with DNA is rather weak (Strekowski et al., 1988a), and a nonclassical intercalation model was proposed for such unfused compounds (Strekowski et al., 1988b) as already mentioned.

With newly synthesized compounds **14** and **15**, the maximum reduced specific viscosity ratios η/η_0 for calf thymus DNA are 1.6 and 1.4, respectively, typically seen for unfused aromatic intercalators (Strekowski et al., 1988a). Upon addition of DNA aromatic protons on these compounds have upfield shifts in the range 0.5–0.7 ppm. Complex formation resulted in an approximately 0.2 ppm upfield shift in the DNA imino proton signals, and an approximately 0.2 ppm downfield shift in the DNA phosphorus NMR signal, also typically seen with unfused aromatic intercalators. The DNA binding constants are given in Table I.

Bicyclic pyrimidines **1–3**, **5**, and **7–9** were investigated previously for their ability to enhance (amplify) bleomycin-mediated degradation of DNA (Strekowski et al., 1988a). A qualitative correlation between dipole moments μ of these compounds and their effect on the bleomycin-mediated chemistry was obtained. These results also paralleled the corresponding DNA binding constants K. Thienylpyrimidine **3** has significant polarity with a positive part of the dipole at the bicyclic system facing away from the side chain and into the intercalation site in the complex (Figure 1), is the best bleomycin amplifier of the series, and has the highest DNA binding constant. An opposite example is a furanylpyrimidine **8** which has a lower dipole moment, a negligible effect on

Table I: Calculated and Experimental Data for Equations 2–4

compd	dipole moments[a] μ (D)	connectivity indexes[b] $^1\chi^v$	DNA binding constants K (M^{-1}) obsd (log K)	calcd (log K) eq 2	eq 3	eq 4
1	6.27	7.265	4.272	4.416	4.195	4.198
2	6.29	7.686	4.394	4.422	4.449	
3	6.68	7.682	4.681	4.528	4.446	
4	3.09	6.326	3.643		3.629	3.723
5	4.28	7.125	3.966	3.875	4.110	
6	0.87	6.250	3.591		3.583	
7	4.85	6.874	4.004	4.030	3.959	3.888
8	3.10	6.385	3.544	3.554	3.664	3.654
9	4.85	6.901	3.934	3.957	3.975	
10		9.630	5.470		5.620	5.682
11		8.836	5.199		5.142	5.208
12		8.831	5.217		5.139	5.139
13		7.956	4.813		4.611	4.664
14		8.440	4.777		4.903	4.829
15		7.566	4.290		4.376	4.354

[a] Calculated values for biaromatic systems in conformations shown in Figure 1, Strekowski et al. (1988a). [b] Calculated values, Kier and Hall (1986).

bleomycin, and a low DNA binding constant. A more quantitative analysis with eq 2 shows a strong correlation between dipole moments for compounds of this series and their DNA affinities

$$\log K = 2.711\ (\pm 0.173) + 0.272\ (\pm 0.033)\mu \qquad (2)$$

$$n = 7\ (\mathbf{1\text{–}3, 5, 7\text{–}9})\ \ r = 0.966\ \ s = 0.104\ \ F_{0.001} = 69.7$$

Compounds with an amino linkage between the cationic side chain and the pyrimidine (**4** and **6**) do not follow this function. Nevertheless, as can be seen from Table I, the dipole moments and binding constants of the amino compounds are in the same general order as observed with the thio derivatives. All these results strongly suggest that the polarity of the aromatic systems of these compounds is important for their DNA binding strength.

The valence molecular connectivity indexes $^1\chi^v$ encode efficiently the additive and constitutive nature of complex molecules or substituents, including their basic stereochemical and electronic properties (Kier & Hall, 1986). In particular, the indexes parallel the lipophilic character of substituents and molecules (Kaliszan, 1987; Mokrosz et al., 1992), which affects DNA binding. The $^1\chi^v$ indexes of various groups correlate well with their electronegativities (Kier & Hall, 1981), and the indexes $-^1\chi^v$ permits quantitative evaluation of the polarity of the compound directly from its structural formula (Kier, 1981). In light of these data and the conclusion reached from the dipole moments analysis, the expected quantitative correlation between DNA affinities of all pyrimidines **1–15** and their calculated descriptors $^1\chi^v$ was obtained:

$$\log K = -0.186\ (\pm 0.250) + 0.603\ (\pm 0.033)^1\chi^v \qquad (3)$$

$$n = 15\ (\mathbf{1\text{–}15})\ \ r = 0.981\ \ s = 0.124\ \ F_{0.001} = 338$$

The Free–Wilson analysis (eq 4) was conducted with compounds **1**, **4**, **7**, **8**, and **10–15** for which the substituents at the pyrimidine ring are present in both the bicyclic and tricyclic series of compounds:

$$\log K = 4.43\ (\pm 0.03) + \alpha(R^2) + \alpha(R^4) + \alpha(R^6) \qquad (4)$$

$$n = 18\ (\mathbf{1, 4, 7, 8, 10\text{–}15})\ \ r = 0.986\ \ s = 0.138\ \ F_{0.01} = 48.9$$

R^2	$\alpha(R^2)$	R^4/R^6	$\alpha(R^4)/\alpha(R^6)$
$SCH_2CH_2NMe_2$	+0.18(±0.03)	2-thienyl	+0.53 (±0.05)
$NHCH_2CH_2NMe_2$	−0.29(±0.02)	1-Me-2-pyrrolyl	+0.22 (±0.08)
		2-furanyl	−0.01 (±0.06)

This statistically significant correlation clearly shows that the substituent effects on DNA binding strength for a closely related series of compounds are additive. It also agrees well with the suggested importance of the stereoelectronic effect in intercalator–DNA interactions as discussed above. In particular, the different effects for thienyl and furanyl substituents, as obtained from the analysis of eq 2 with a limited number of compounds, are now more strongly supported.

*Pyrimidines **18** and **19**.* These compounds are dicationic at pH 7, due to protonation of the two dimethylamino groups. The maximum reduced specific viscosity ratio of 1.6 with **18** and 1.7 with **19** for sonicated calf thymus DNA and unwinding results of supercoiled DNA, 11° for **18** and 14° for **19**, are consistent with classification of these compounds as nonclassical intercalators. The DNA equilibrium binding constants are 4.2×10^5 and 4.3×10^5 M^{-1} for **18** and **19**, respectively.

Insight into the stereochemistries of DNA complexes with **18** and **19** was obtained from NMR studies (Figure 2). The chemical shift changes on addition of DNA are consistent with electrostatic binding of the terminal (protonated) dimethylamino groups, groove binding of the dimethylene chains, and interaction of the aromatic systems of both molecules with DNA base pairs. The DNA-induced upfield shifts for the aromatic protons are lower than typically observed for intercalation in a classical sense (Wilson et al., 1992). The chemical shift changes for the two phenyl substituents, A and C, at the pyrimidine B of **18** are identical, as expected for a fast reversible interaction of a symmetrical molecule with DNA. On the other hand, the shift changes for the 4-phenyl group A are greater than those for the 2-phenyl group C in compound **19**. These results are consistent with a preferential sandwiching of a bicyclic system A–B of **19** with DNA base pairs. A nonclassical partial intercalation complex of the A–B unit can be suggested. On the basis of the NMR experiments, the alternative interaction modes involving either partial intercalation of the B–C unit or the partial intercalation of a tricyclic system A–B–C of **19** are less favored. The suggested

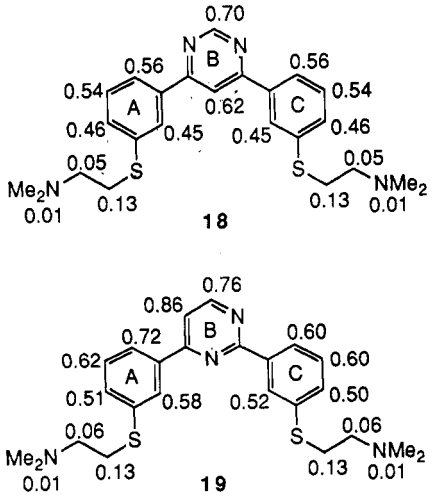
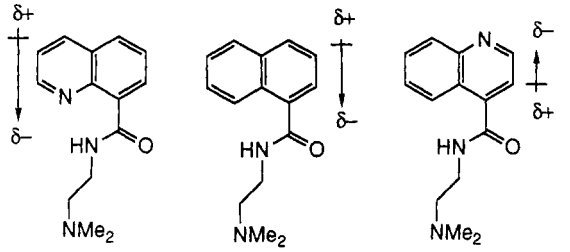


FIGURE 2: Structures of unfused pyrimidine derivatives **18** and **19** and DNA-induced upfield shifts (ppm) for protons at the molar ratio 0.3 of compound per DNA base pair. The chemical shifts in the proton NMR spectra of **18** and **19** in the absence of DNA are given in Materials and Methods.



20 21 22

FIGURE 3: Structures of weak binding compounds **20–22**. The arrows illustrate qualitatively polarizations of the aromatic systems.

interaction model of **19** with DNA is fully consistent with the concept that the polarity or dipole moment of a cation-substituted aromatic molecule is an important factor in its DNA interactions.

Fused Compounds 20–22. The maximum reduced specific viscosity ratios induced in DNA by these monocationic compounds were 1.29 for **20**, 1.08 for **21**, and 1.06 for **22**. Although the value for **20** suggests intercalation, this compound does not unwind supercoiled DNA (Atwell et al., 1988b). The DNA equilibrium binding constants for all three compounds are low: 7500 M^{-1} for **20**, 2500 M^{-1} for **21** and 600 M^{-1} for **22**. The relative polarities of the aromatic systems of these compounds can be predicted directly from their structures, as for **18** and **19** discussed above. The results obtained from analysis of the electronic effects of a ring nitrogen atom and an amide substituent (Perrin et al., 1981) are given in Figure 3. The quinoline **20** has the largest dipole, which is also oriented favorably for the interaction with a negative surface potential of a DNA groove. The dipole of a naphthalene **21** is smaller but still oriented favorably for the interaction. By contrast, a small, partial negative charge can be expected at the putative interaction site of a quinoline **22**. On the basis of this simple analysis, the order of the DNA interaction strengths **20** > **21** > **22** is predicted as observed even though the compounds bind weakly with DNA.

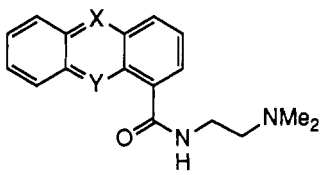
DISCUSSION

The eqs 2–4 constitute a unique and successful quantitative analysis of structure with DNA binding data. Three independent approaches were used, and all produced statistically significant correlations with the same result. All three functions show that the polarity of the unfused aromatic system of a nonclassical intercalator is one of the major factors that influences binding with DNA. For series of small, approximately isosteric molecules, such as nonclassical bicyclic intercalators **1–9**, their tricyclic analogs **10–15**, isomeric nonclassical intercalators **18** and **19**, and weak binding compounds **20–22**, the polarity can be used to predict the relative DNA affinities within the given series, and it is clear that stereoelectronic effects are important factors in binding.

Often it is difficult to separate the steric and stereoelectronic effects, and the analysis of thio-substituted pyrimidines (eq 2) and their amino-substituted analogs **4** and **6** is illustrative. In spite of similar van der Waals spheres for the S and NH linkages (Pauling, 1960) in these molecules, the stereochemistries are different. The sulfur atom is weakly conjugated with the pyrimidine ring, and the thioether linkage has a low energy barrier to rotation, which is not restricted in the NMR time scale at 400 MHz even at −100 °C. By contrast, the nitrogen atom is strongly conjugated with the pyrimidine, and this results in a low-energy coplanar conformation of the alkylamino–pyrimidine unit with restricted rotation of the alkylamino substituents as well as increased electron density and decreased polarity of the aromatic system (Strekowski et al., 1988a). The energy barrier toward rotation is approximately 11 kcal mol^{-1} (Harden et al., unpublished results). Preliminary molecular modeling results are consistent with the nonclassical DNA intercalation model for the thio-substituted pyrimidine **1** and its amino analog **4**. In the computer-derived DNA complexes for both compounds, for either major or minor groove interaction of the side chain, the biaromatic system is partially inserted between DNA base pairs and is partially located in a groove and the linker chain and the protonated terminal dimethylamino group are located in the groove. An important result is that both the (alkylthio)pyrimidine moiety of **1** and the (alkylamino)pyrimidine moiety of **4** are twisted from coplanarity. While the twist of (alkylthio)pyrimidine **1** has a negligible effect on the complex stability, the deviation from coplanar low-energy conformation of the (alkylamino)pyrimidine **4** should have a stronger destabilizing effect on the interaction with DNA, as observed. In agreement with this analysis, eq 1, which describes pure stereoelectronic factors, is not valid for (alkylamino)pyrimidines. However, the stereoelectronic factor can be seen for **4** and **6** with the same stereochemical features in their (alkylamino)pyrimidine moiety. Moreover, the DNA interaction of all thio and amino derivatives **1–15** is successfully described by functions 3 and 4, which include both stereochemical and stereoelectronic factors.

The importance of the stereoelectronic effect in DNA complex formation is illustrated by analysis of the stereochemistry of the tricyclic compound **19**. The 4-phenylpyrimidine system, such as A–B of **19**, is known to have an equilibrium torsional angle between planes of the rings of about 25° (Wilson et al., 1988, 1989). The deviation from coplanarity is due to weakly repulsive interactions between the nitrogen electron pair of the pyrimidine B and the ortho hydrogen atom of the phenyl A, and much stronger repulsive interactions between two ortho hydrogen atoms. By contrast, the only weakly repulsive nitrogen–hydrogen interactions in the B–C system of **19** result in a lower torsional angle of B–C than that for A–B. On the basis of the stereochemical factor only, the formation of a nonclassical DNA intercalation complex with a low torsional angle for the partially intercalated bicyclic system should be favored for the B–C unit of **19**. More specifically, deviation from the equilibrium conformation of this unit to that with a low torsional angle in the complex with DNA would be more facile than the deviation of the more twisted unit A–B. As shown by the NMR chemical shifts, this is not the case. Again, this result is consistent with the suggestion that the observed preferential interaction of the A–B system of **19** with DNA is due to a favorable stereoelectronic effect in this bicyclic unit.

Comparison of results with pyrimidines **18** and **19** strongly supports the importance of stereoelectronic effects in interactions of unfused aromatic compounds with the negative potentials in the DNA grooves. Without dipole moment computation it could be safely predicted that the pyrimidine ring of **19** is favorably polarized for interaction with DNA, while the unfavorable polarization of **18** should inhibit the stereoelectronic component of the interaction. Although the binding constants and hydrodynamic properties of **18** and **19** complexes with DNA are similar, the NMR results show that significantly different complexes are formed by the two compounds that are strongly influenced by their stereoelectronic properties. In a nonclassical intercalation complex, the unfused ring system is only partially stacked with DNA base pairs and partially occupies a groove location. An



23 X = CH, Y = N
24 X = Y = CH
25 X = N, Y = CH

FIGURE 4: Examples of classical DNA intercalators **23–25** synthesized and studied by Denny and co-workers.

intermediate in the formation of such an intercalation complex is a groove–external complex that is associated with DNA primarily by electrostatic interactions. The partially stacked final complex formed is thus primarily governed by the kinetics of the process, which depend on the molecular polarity and electrostatic interactions with the negative potentials in the DNA grooves. The favorable stereoelectronic properties of **19** lead to faster formation of the complex, relative to **18**, but the same interactions also lead to faster dissociation of **19** to the groove binding intermediate state. The observed equilibrium properties of **18** and **19** are thus similar, but the structure of the complex of **19** with DNA is governed by its kinetics of formation and the stereoelectronic properties of the compound. The quantitative correlation observed for the binding constants of **1–15** in their interaction with DNA can be explained in terms of the interaction of the molecular dipole with the negative electrostatic potential of the grooves of DNA. These unfused aromatic compounds undergo partial insertion with DNA to form much weaker complexes relative to the tricyclic unfused systems, **18** and **19**. Most importantly, the intercalation complexes of **1–15** are in a fast exchange with the external complexes with DNA, especially at low ionic strengths, and evidence has been accumulating that intercalation and groove binding modes of the unfused cationic molecules should be viewed as two comparable-depth potential wells on a continuous energy surface (Wilson et al., 1988, 1991). These observations suggest that it is the groove binding interactions of **1–15** that account for the quantitative correlations between their binding constants and molecular properties. Consistent with this explanation are the results obtained with fused bicyclic compounds **20–22** for which intercalation must be quite weak or nonexistent.

Fused-ring classical intercalators were not investigated in this work because a large amount of DNA binding data for intercalators such as **23–25** (Figure 4) has been presented recently by Denny and co-workers (Atwell et al., 1987, 1988a,b; Rewcastle et al., 1987). The acridine **23** and the anthracene **24** are benzo-fused homologs of groove binding quinoline **20** and naphthalene **21**, respectively. In spite of similar stereoelectronic effects in the two pairs, **20**/**23** and **21**/**24**, the DNA affinities of the tricyclic derivatives do not follow the binding order observed for their bicyclic analogs. Tricyclic derivatives **23–25** all bind strongly to DNA by intercalation with very similar binding constants (Atwell et al., 1988a). These results demonstrate that the stereoelectronic effect, as observed with nonclassical intercalators, is not dominant in the formation of classical intercalation complexes. Consistent with this conclusion is a well-established fact that the strength of intercalative forces with DNA base pairs is a function of polarizability of a planar polyaromatic ring system (Müller & Crothers, 1975) rather than its polarity.

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