Synthesis and Structure−DNA Binding Relationship Analysis of DNA Triple-Helix Specific Intercalators†

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4-[*N*-(Aminoalkyl)amino]-2-arylquinolines with conformational freedom around positions 2 and 4 of the quinoline stabilize strongly poly(dT·dA·dT) (triplex DNA) and bind weakly to poly(dA·dT) (duplex DNA). Basicity of N1 of the quinoline parallels the interaction strength of these compounds with the triple-helical DNA structure suggesting that N1 of the quinoline is protonated in the complex with the DNA triplex. The experimental results support the interaction model suggested previously (Wilson *et al. Biochemistry* **1993**, *32*, 10614).

Introduction

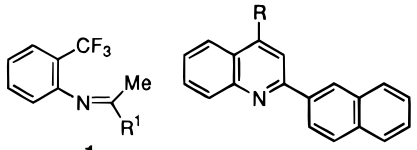
Triple-helical DNA is formed by sequence specific binding of the third strand within the major groove of duplex DNA.[1,2] The interaction is rather weak at physiological conditions but can be enhanced by complex formation of the triple DNA structure with polyamines,[2,3] fused intercalators,[2,4,5] and unfused intercalators.[6−8] The therapeutic potential of the triplex interaction and stabilization is enormous. Intense research is underway to selectively target regions of known sequences on specific genes with synthetic complementary oligonucleotides or analogs and to stabilize the resultant triplex forms as a means of inhibiting expression of the genes. An exciting approach involves intramolecular stabilization of the triplex structure by an intercalator tethered to the third strand oligonucleotide.[2,9]

We have reported recently that, in contrast to fused intercalators, the unfused quinolines **4**, **6**, **8**, and **11** (Table 1) provide highly selective stabilization of triplexes relative to duplexes.[6−8] These unfused intercalators are highly specific for T·AT sequences in the presence of duplex structure of any sequence.[7,8] Also, they are relatively nontoxic to human cells.[10]

Our modeling studies[6] have suggested intercalation of the quinoline portion between the Watson−Crick AT base pairs of the duplex in the triplex, stacking of the 2-aryl substituent with T bases of the third DNA strand, and electrostatic interaction of the protonated amino side chain with DNA phosphates in the minor groove. In order to test this model and to better understand structural requirements of the unfused quinoline intercalators for the triplex-DNA stabilization, we have synthesized an extended series of quinoline derivatives and analyzed their interaction with T·AT and AT polymers. The results are presented in this paper.

Results and Discussion

Chemistry. Condensation of 2-(trifluoromethyl)aniline with an aryl methyl ketone (R^1COMe) followed by lithium alkylamide or dialkylamide (R^2-Li)-mediated cyclization of the resultant ketimine **1** provides a convenient method for the preparation of 2,4-disubstituted quinolines.[6, 10−12] All *N*-substituted 2-arylquinolin-4-amines **4**−**18** (Table 1) were obtained by this two-step synthesis. A modification of this chemistry provides easy access to 2-aryl-4-fluoroquinolines[13] such as **2**. Compounds **19** and **20** were obtained by nucleophilic displacement of fluoride in the corresponding 4-fluoroquinolines. Finally, carboxamides **21** and **22** were synthesized from the readily available carboxylic acid **3**.[14]



1 (See Table 1 for R^1)

2: R = F
3: R = COOH

Structure−Activity Relationship (SAR) Analysis of 4−22. Quinolines **4**−**22** are substituted at position 2 with diverse aromatic groups (R^1) and contain terminal amino functions which are protonated under physiological conditions. These amino functions are linked to the quinoline system at position 4 through structural units containing amino, thio, or aminocarbonyl groups which provide a different degree of steric hindrance at position 4 and affect basicity of the ring nitrogen atom of the quinoline.

Binding of compounds **4**−**22** to nucleic acids was evaluated by thermal melting experiments. Triplex to duplex and single-strand transitions [poly(dT·dA·dT) to poly(dA·dT) and poly(dT)] at low temperature is followed by duplex denaturation transitions at higher temperature. These two types of thermal melting are well-resolved in 0.2 M NaCl buffer used in this and previous studies.[6] Compounds which cause stabilization of the triplex- or duplex-DNA structures also cause increases in the temperature of dissociation of the respective DNA structures. As can be seen from Table 1, the stabilization of the triplex-DNA structure by unfused intercalators **4**−**22** is strongly dependent on substituents at the quinoline system. Regardless of the type of substituents, however, all compounds **4**−**22** show a strong preference toward complexation with the triplex DNA

† Dedicated to the memory of Professor Jerzy L. Mokrosz, Institute of Pharmacology, Polish Academy of Sciences.

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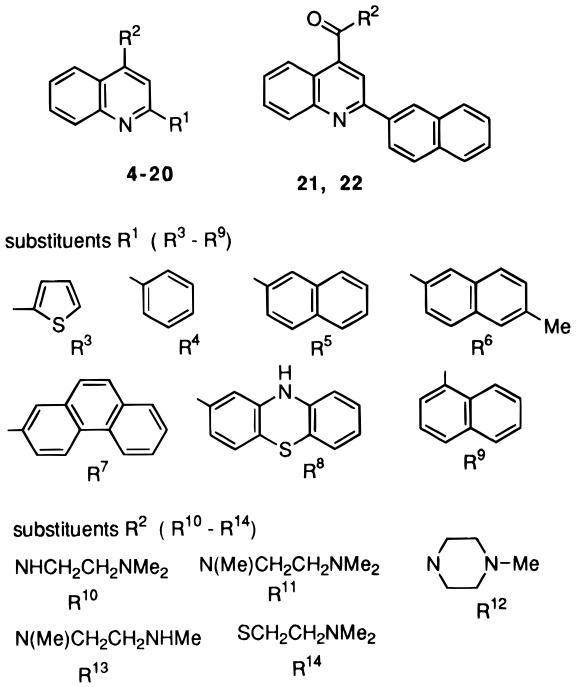
Table 1. T_m Increases for DNA Triplex and Duplex Structures[a] in the Presence of Quinoline Derivatives **4**−**22**[b]



			ΔT_m, °C		
			triplex		
no.	R^1	R^2	exptl	calcd[c]	duplex
4	R^3	R^{10}	9.4[d]	8.1	0.7
5	R^3	R^{11}	3.0	3.5	0.0
6	R^4	R^{10}	11.9[d]	9.5	0.3
7	R^4	R^{12}	3.2	4.0	0.0
8	R^5	R^{10}	35.6[d]	39.9	5.5
9	R^5	R^{13}	24.1	24.4	0.2
10	R^5	R^{12}	19.1	16.8	0.4
11	R^6	R^{10}	32.9[d]	37.6	2.2
12	R^6	R^{11}	19.0	16.3	0.4
13	R^6	R^{13}	23.0	23.0	0.5
14	R^6	R^{12}	15.5	15.8	0.4
15	R^7	R^{10}	35.3	39.5	5.2
16	R^7	R^{12}	18.6	16.6	0.3
17	R^8	R^{10}	33.1	33.6	2.4
18	R^8	R^{13}	20.8	20.5	1.5
19	R^9	R^{10}	1.9		0.1
20	R^5	R^{14}	18.6		0.2
21		R^{10}	11.7		0.1
22		R^{11}	2.1		0.0

[a] Poly(dT·dA·dT) (triplex) and poly(dA·dT) (duplex). [b] See the Experimental Section for conditions. [c] Values from the Free−Wilson analysis (eq 1). [d] Taken from ref 6.

relative to the duplex DNA. The best compounds, **8**, **11**, **15**, and **17**, are *N*-monosubstituted quinolin-4-amines with the 2-aryl substituent composed of at least two fused rings. Conformational freedom around the aryl−quinoline junction is important for efficient triplex-DNA stabilization because the 2-(1-naphthyl) derivative **19** shows negligible affinity toward the triplex. The 4-thio derivative **20** and 4-carboxamides **21** and **22** stabilize the triplex to a lesser extent than the 4-amino counterpart **8**.

The Free−Wilson analysis[15] (eq 1 and Figure 1) was conducted with a series of compounds (**4**−**18**) for which the quinoline system is substituted with groups R^1 and R^2, each present in at least two molecules. In this nonparameter analysis, for every compound of the series the values ΔT_m used in the logarithmic scale are

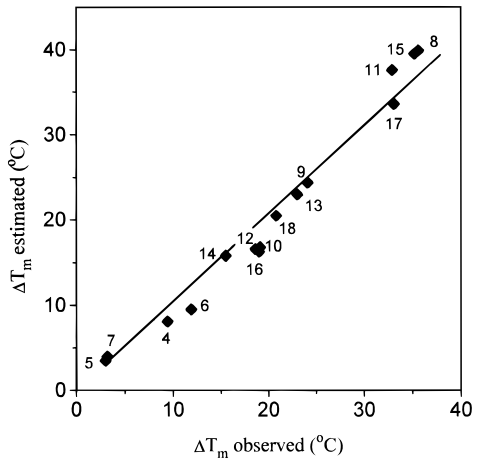


Figure 1. Free−Wilson analysis of **4**−**18** for binding with poly(dT·dA·dT).

expressed as the sum of the triplex-DNA binding contributions α of the substituents R^1 and R^2, referring to the overall average contribution (the intercept 1.22 ± 0.02 in eq 1).

$$\log \Delta T_m = 1.22\ (\pm 0.02) + \alpha_{R^1} + \alpha_{R^2} \qquad (1)$$

Compounds 4−**18**

R^1 (R^3−R^8)	α_{R^1}	R^2 (R^{10}−R^{13})	α_{R^2}
R^3	−0.50 ± 0.06	R^{10}	0.19 ± 0.02
R^4	−0.43 ± 0.06	R^{11}	−0.17 ± 0.07
R^5	0.19 ± 0.05	R^{12}	−0.18 ± 0.04
R^6	0.17 ± 0.04	R^{13}	−0.02 ± 0.05
R^7	0.19 ± 0.06		
R^8	0.12 ± 0.06		

$n = 15$, $r = 0.985$, $s = 0.09$; variance in log ΔT_m explained by the regression: 97.1%. $F = 25.3$; significance level of the *F*-test: 99.9%

This function (eq 1) shows that the partial contributions of substituents R^1 and R^2 to the overall triplex-DNA affinity of the quinoline intercalators are additive and can be quantified. The results strongly support the interaction model suggested previously.[6] In particular, the high contribution of a 2-phenothiazinyl group ($R^1 = R^8$), the central thiazine ring of which is not planar, is consistent with efficient stacking of thymine bases with a planar portion of the phenothiazinyl group (composed of one benzene ring and two heteroatoms). On the basis of the computer-derived structural model for the triplex-DNA intercalation complex,[6] it can be suggested that the planar subunit adjacent to the quinoline is involved in the stacking interaction.

In the molecular modeling work[6] it was assumed that the quinoline ring nitrogen atom is protonated in the complex. In this study the problem was addressed, in part, by measuring the pK_a of conjugated acids of selected quinolines **8**−**10**, **20**, and **21**, all containing the 2-naphthyl substituent R^1 but differing in the compound-induced increase in T_m. It appears that protonation of N1 of the quinoline increases stability of the complex with the DNA triplex because the obtained order of pK_a, **8** (7.1), **9** (6.2), **10** (5.6), **20** (4.4), and **21** (2.9), parallels the order of ΔT_m (Table 1). It should be noted, however, that steric hindrance around position 4 of the quinoline in the less active compounds may also play a direct role by destabilizing intercalation of the quinoline system with the triplex structure.

Experimental Section

General. Melting points (Pyrex capillary) are not corrected. Unless stated otherwise, ^{1}H NMR (400 MHz) and ^{13}C NMR (75 MHz) spectra were obtained at 28 °C in $CDCl_3$ for free bases and in DMSO-d_6 for hydrobromide salts with Me_4Si as an internal reference. Mass spectra (EI, 70 eV) are reported only for compounds which gave a molecular ion peak.

Synthesis of Quinolin-4-amines 4−18. Conditions for the preparation of ketimines **1** and their cyclization to quinolin-4-amines **4**, **6**−**8**, and **11** by the reaction with lithium amide reagents (R^2-Li) (see Table 1 for the substituents) have been reported previously.[6,10−12] The remaining compounds **5**, **9**, **10**, and **12**−**18** were obtained in a similar fashion and purified by silica gel chromatography (hexanes/Et_3N/EtOH, 7:2:1). Hydrobromide salts were obtained as described previously[10] and crystallized from 95% EtOH.

***N*-[2-(Dimethylamino)ethyl]-*N*-methyl-2-(2-thienyl)-quinolin-4-amine (5):** yield 64%; an oil; ^{1}H NMR δ 2.26 (s, 6 H), 2.66 (t, J = 7 Hz, 2 H), 3.05 (s, 3 H), 3.42 (t, J = 7 Hz, 2 H), 7.12 (t, J = 4 Hz, 1 H), 7.24 (s, 1 H), 7.38 (t, J = 8 Hz, 1 H), 7.41 (d, J = 4 Hz, 1 H), 7.60 (t, J = 8 Hz, 1 H), 7.68 (d, J = 4 Hz, 1 H), 8.02 (2d, J = 8 Hz, 2 H); ^{13}C NMR δ 41.1, 45.9, 54.4, 57.2, 105.1, 122.7, 123.9, 124.4, 125.2, 127.7, 127.9, 129.1, 129.8, 146.1, 149.8, 152.4, 157.7; MS m/z 58 (100), 253 (30), 311 (6, M^+). **5**·2HBr·H_2O: mp 289−290 °C. Anal. ($C_{18}H_{21}N_3S$·2HBr·H_2O) C, H, N.

***N*-[2-(Methylamino)ethyl]-*N*-methyl-2-(2-naphthyl)quinolin-4-amine (9):** yield 40%; an oil; ^{1}H NMR δ 2.50 (s, 3 H), 3.00 (t, J = 6 Hz, 2 H), 3.10 (s, 3 H), 3.52 (t, J = 6 Hz, 2 H), 7.48 (t, J = 8 Hz, 1 H), 7.50 (s, 1 H), 7.54 (m, 2 H), 7.70 (t, J = 8 Hz, 1 H), 7.90 (m, 1 H), 8.00 (m, 2 H), 8.20 (m, 2 H), 8.33 (d, J = 8 Hz, 1 H), 8.59 (s, 1 H). **9**·2HBr·1.5H_2O: mp 267−268 °C. Anal. ($C_{23}H_{23}N_3$·2HBr·1.5H_2O) C, H, N.

4-(4-Methylpiperazino)-2-(2-naphthyl)quinoline (10): yield 59%; an oil; ^{1}H NMR δ 2.32 (s, 3 H), 2.63 (t, J = 6 Hz, 4 H), 3.25 (t, J = 6 Hz, 4 H), 7.33 (s, 1 H), 7.40 (m, 3 H), 7.56 (t, J = 8 Hz, 1 H), 7.78 (m, 1 H), 7.90 (m, 3 H), 8.07 (d, J = 8 Hz, 1 H), 8.20 (d, J = 8 Hz, 1 H), 8.44 (s, 1 H). **10**·2HBr·3H_2O: mp 279−280 °C. Anal. ($C_{24}H_{23}N_3$·2HBr·3H_2O) C, H, N.

***N*-[2-(Dimethylamino)ethyl]-*N*-methyl-2-(6-methyl-2-naphthyl)quinolin-4-amine (12):** yield 37%; an oil; ^{1}H NMR δ 2.30 (s, 6 H), 2.54 (s, 3 H), 2.72 (t, J = 7 Hz, 2 H), 3.11 (s, 3 H), 3.48 (t, J = 7 Hz, 2 H), 7.36 (d, J = 8 Hz, 1 H), 7.45 (s, 1 H), 7.46 (t, J = 8 Hz, 1 H), 7.67 (m, 2 H), 7.89 (2d, J = 8 Hz, 2 H), 8.10 (d, J = 8 Hz, 1 H), 8.15 (d, J = 8 Hz, 1 H), 8.26 (d, J = 8 Hz, 1 H), 8.51 (s, 1 H); ^{13}C NMR δ 22.2, 41.4, 48.2, 49.9, 56.1, 108.2, 123.1, 124.3, 124.4, 124.7, 124.8, 125.1, 127.3, 128.1, 128.3, 128.7, 129.6, 131.3, 131.4, 136.4, 137.0, 150.5, 158.8, 158.9; MS m/z 58 (100), 311 (20), 369 (2, M^+). **12**·2HBr·0.5H_2O: mp 281−283 °C. Anal. ($C_{25}H_{27}N_3$·2HBr·0.5H_2O) C, H, N.

***N*-[2-(Methylamino)ethyl]-*N*-methyl-2-(6-methyl-2-naphthyl)quinolin-4-amine (13):** yield 42%; an oil; ^{1}H NMR δ 1.78 (br s, exchangeable with D_2O, 1 H), 2.44 (s, 3 H), 2.51 (s, 3 H), 2.93 (t, J = 6 Hz, 2 H), 3.03 (s, 3 H), 3.45 (t, J = 6 Hz, 2 H), 7.34 (d, J = 8 Hz, 1 H), 7.43 (s, 1 H), 7.45 (t, J = 8 Hz, 1 H), 7.63 (m, 2 H), 7.85 (2d, J = 8 Hz, 2 H), 8.15 (2t, J = 8 Hz, 2 H), 8.26 (d, J = 8 Hz, 1 H), 8.50 (s, 1 H); ^{13}C NMR δ 22.5, 42.0, 49.1, 49.6, 56.0, 109.0, 123.3, 124.3, 124.4, 124.7, 124.9, 125.2, 127.4, 128.1, 128.5, 128.8, 129.7, 131.4, 131.5, 136.6, 137.1, 150.5, 158.7, 158.8. **13**·2HBr·0.5H_2O: mp 279−282 °C. Anal. ($C_{24}H_{25}N_3$·2HBr·0.5H_2O) C, H, N.

2-(6-Methyl-2-naphthyl)-4-(4-methylpiperazino)quinoline (14): yield 36%; an oil; ^{1}H NMR δ 2.45 (s, 3 H), 2.54 (s, 3 H), 2.76 (t, J = 5 Hz, 4 H), 3.38 (t, J = 5 Hz, 4 H), 7.36 (d, J = 8 Hz, 1 H), 7.45 (s, 1 H), 7.47 (t, J = 8 Hz, 1 H), 7.67 (m, 2 H), 7.89 (2d, J = 8 Hz, 2 H), 8.03 (d, J = 8 Hz, 1 H), 8.16 (d, J = 8 Hz, 1 H), 8.26 (d, J = 8 Hz, 1 H), 8.51 (s, 1 H); ^{13}C NMR δ 21.7, 46.0, 52.0, 55.0, 106.7, 122.3, 123.4, 124.8, 125.1, 126.5, 126.6, 127.6, 128.4, 128.5, 129.0, 130.2, 131.6, 133.9, 136.2, 136.6, 149.6, 157.3, 157.8; MS m/z 70 (100), 367 (10, M^+). **14**·2HBr·2.5H_2O: mp >280 °C dec. Anal. ($C_{25}H_{25}N_3$·2HBr·2.5H_2O) C, H, N.

***N*-[2-(Dimethylamino)ethyl]-2-(2-phenanthryl)quinolin-4-amine (15):** yield 45%; an oil; ^{1}H NMR (a salt) δ 2.94 (s, 6 H), 3.57 (m, 2 H), 4.16 (m, 2 H), 7.42 (s, 1 H), 7.79 (m, 3 H), 8.06 (m, 4 H), 8.21 (d, J = 8 Hz, 1 H), 8.38 (dd, J = 8, 2 Hz, 1 H), 8.63 (d, J = 8 Hz, 1 H), 8.81 (d, J = 2 Hz, 1 H), 9.00 (d, J = 8 Hz, 1 H), 9.15 (d, J = 8 Hz, 1 H), 9.27 (br s, 1 H), 9.70 (br s, 1 H); ^{13}C NMR (a salt) δ 27.6, 49.0, 49.5, 110.1, 122.4, 122.9, 123.1, 124.1, 124.4, 124.5, 124.7, 124.9, 125.1, 127.4, 128.2, 128.5, 128.9, 129.0, 129.1, 129.7, 131.1, 131.6, 137.3, 137.9, 157.9, 158.9. **15**·2HBr·3H_2O: mp 306−308 °C. Anal. ($C_{27}H_{25}N_3$·2HBr·3H_2O) C, H, N.

4-(4-Methylpiperazino)-2-(2-phenanthryl)quinoline (16): yield 30%; an oil; ^{1}H NMR δ 2.43 (s, 3 H), 2.74 (m, 4 H), 3.37 (m, 4 H), 7.47 (s and m, 2 H), 7.65 (m, 3 H), 7.76 (d, J = 8 Hz, 1 H), 7.88 (m, 2 H), 8.03 (d, J = 8 Hz, 1 H), 8.20 (d, J = 8 Hz, 1 H), 8.42 (dd, J = 8, 2 Hz, 1 H), 8.62 (d, J = 2 Hz, 1 H), 8.72 (d, J = 8 Hz, 1 H), 8.79 (d, J = 8 Hz, 1 H). **16**·2HBr·3H_2O: mp >225 °C dec. Anal. ($C_{28}H_{25}N_3$·2HBr·3H_2O) C, H, N.

***N*-[2-(Dimethylamino)ethyl]-2-(2-phenothiazinyl)quinolin-4-amine (17):** yield 25%; an oil; ^{1}H NMR (a salt) δ 2.92 (s, 6 H), 3.50 (m, 2 H), 4.03 (m, 2 H), 6.80 (m, 2 H), 6.96 (d, J = 8 Hz, 1 H), 7.04 (t, J = 8 Hz, 1 H), 7.11 (s, 1 H), 7.23 (s, 1 H), 7.24 (d, J = 8 Hz, 1 H), 7.40 (d, J = 8 Hz, 1 H), 7.77 (t, J = 8 Hz, 1 H), 8.01 (t, J = 8 Hz, 1 H), 8.09 (d, J = 8 Hz, 1 H), 8.54 (d, J = 8 Hz, 1 H), 8.99 (s, 1 H), 9.20 (br s, 1 H), 9.60 (br s, 1 H). **17**·2HBr·1.5H_2O: mp >200 °C dec. Anal. ($C_{25}H_{24}N_4S$·2HBr·1.5H_2O) C, H, N.

***N*-[2-(Methylamino)ethyl]-*N*-methyl-2-(2-phenothiazinyl)quinolin-4-amine (18):** yield 39%; an oil; ^{1}H NMR (a salt) δ 2.67 (s, 3 H), 3.43 (m, 2 H), 3.55 (s, 3 H), 4.11 (m, 2 H), 6.80 (m, 2 H), 6.96 (d, J = 8 Hz, 1 H), 7.04 (t, J = 8 Hz, 1 H), 7.21 (s, 1 H), 7.22 (d, J = 8 Hz, 1 H), 7.28 (br s, 1 H), 7.41 (d, J = 8 Hz, 1 H), 7.70 (t, J = 8 Hz, 1 H), 7.99 (t, J = 8 Hz, 1 H), 8.13 (d, J = 8 Hz, 1 H), 8.40 (d, J = 8 Hz, 1 H), 8.66 (br s, 2 H), 9.02 (s, 1 H). **18**·2HBr: mp >200 °C dec. Anal. ($C_{25}H_{24}N_4S$·2HBr) C, H, N.

Synthesis of Quinolines 19 and 20. Compound **19** has been obtained previously by the reaction of 4-fluoro-2-(1-naphthyl)quinoline with *N*,*N*-dimethylethylenediamine.[13] A similar treatment of **2** with sodium 2-(dimethylamino)ethanethiolate in EtOH at 23 °C for 48 h gave **20** which was purified by silica gel chromatography with hexanes/Et_3N/EtOH (7:2:1) as an eluent.

4-[[2-(Dimethylamino)ethyl]thio]-2-(2-naphthyl)quinoline (20): yield 40%; an oil; ^{1}H NMR δ 2.39 (s, 6 H), 2.80 (t, J = 7 Hz, 2 H), 3.36 (t, J = 7 Hz, 2 H), 7.54 (m, 3 H), 7.74 (t, J = 8 Hz, 1 H), 7.83 (s, 1 H), 7.90 (m, 1 H), 8.00 (m, 2 H), 8.18 (2d, J = 8 Hz, 2 H), 8.32 (d, J = 8 Hz, 1 H), 8.57 (s, 1 H). **20**·2HBr·1.5H_2O: mp >250 °C dec. Anal. ($C_{23}H_{22}N_2S$·2HBr·1.5H_2O) C, H, N.

Synthesis of Quinoline-4-carboxamides 21 and 22. Condensation of **3**[14] with *N*,*N*-dimethylethylenediamine or *N*,*N*,*N*′-trimethylethylenediamine in the presence of carbonyldiimidazole was conducted by using a general procedure.[16]

***N*-[2-(Dimethylamino)ethyl]-2-(2-naphthyl)quinoline-4-carboxamide (21):** yield 80%; mp 158−160 °C (from EtOAc/Et_2O); ^{1}H NMR δ 2.37 (s, 6 H), 2.75 (t, J = 6 Hz, 2 H), 3.74 (m, 2 H), 7.34 (br s, 1 H), 7.55 (m, 3 H), 7.76 (t, J = 8 Hz, 1 H), 7.89 (m, 1 H), 8.02 (m, 2 H), 8.23 (m, 3 H), 8.40 (d, J = 8 Hz, 1 H), 8.71 (s, 1 H); ^{13}C NMR δ 37.4, 45.1, 57.6, 116.8, 123.4, 124.7, 125.0, 126.4, 126.9, 127.1, 127.2, 127.7, 128.7, 128.9, 130.1, 130.2, 133.4, 134.0, 136.2, 143.1, 148.8, 156.6, 167.6. Anal. ($C_{24}H_{23}N_3O$) C, H, N.

***N*-[2-(Dimethylamino)ethyl]-*N*-methyl-2-(2-naphthyl)-quinoline-4-carboxamide (22):** yield 61% after silica gel chromatography (EtOAc/MeOH, 4:1) and Kugelrohr distillation (150 °C/0.2 mmHg); an oil; ^{1}H NMR ($CDCl_3$, 28 °C) indicated the presence of two stable conformers in the ratio of 3:2 [major conformer δ 2.41 (s, NMe_2), 2.88 (s, NMe); minor conformer δ 1.91 (s, NMe_2), 3.30 (s, NMe)]; ^{13}C NMR ($CDCl_3$, 28 °C) gave 20 resonances for trigonal carbons and 8 signals for tetrahedral carbons; ^{13}C NMR (DMSO-d_6, 130 °C) δ 35.5, 44.3, 47.8, 56.0, 115.0, 122.5, 123.9, 124.2, 125.6, 126.1, 126.2, 126.3, 126.8, 127.5, 128.0, 129.0, 129.4, 132.6, 133.1, 135.3, 143.7, 147.4, 155.3, 166.9. Anal. ($C_{25}H_{25}N_3O$) C, H, N.

Nucleic Acid Samples. Poly(dA) and poly(dT) were purified and characterized as previously described.[6] The stock solutions were in a PIPES buffer containing 0.01 M piperazine-*N*,*N*′-bis(2-ethanesulfonic acid), 0.001 M EDTA, and 0.2 M NaCl and adjusted to pH 7.0. The DNA triplex and duplex structures were generated by addition of the DNA stock solutions to the PIPES buffer.[6]

T_m Determinations. Thermal melting curves for DNA and complexes were obtained under the previously described conditions.[6] UV−vis spectra were determined on a Varian Cary 4 spectrophotometer interfaced to a Dell/486 microcomputer. Absorption changes at 260 or 284 nm were followed as a function of temperature, and T_m values were determined from first derivative plots after the data were transferred to a Macintosh computer. The free duplex, poly(dA·dT), had a T_m of 74.1 °C, and the triplex transition was at 43.8 °C for poly(dT·dA·dT). Compounds **4**−**22** were compared by the increase in T_m [$\Delta T_m = T_m$(complex) − T_m(free DNA)] they produced in the PIPES buffer at a molar ratio of 0.3 of compound to nucleic acid bases (saturating amounts of the compound). Ratios greater than 0.3 did not affect the T_m values. The estimated errors in the ΔT_m values are ±0.5 °C.

pK_a Determinations. A solution in nanopure water of a dihydrobromide salt of **8**−**10**, **20**, or free base **21** (0.01 mM), NaCl (4 mM), and EDTA (0.1 mM) in a 1-cm UV cell was titrated with either HCl or NaOH. After each addition of the acid or the base, the pH was measured on an Accumet 910 pH meter using an Accumet microprobe glass electrode with an Ag/HCl reference, and the UV−vis spectrum (220−450 nm) was taken on a Cary 4 spectrophotometer. The total increase in volume at the end of titration did not exceed 1%. For each compound the absorption spectra were determined in a range of pH values at least 2 orders of magnitude from the pK_a value, and at least two measurements per pH unit were made. Large spectral shifts with excellent isosbestic points in the spectra were obtained in all cases studied. The absorption data for each measurement were overlayed, and the absorption of the wavelength of greatest change in the spectra was extracted, normalized, and plotted as a function of pH. The pK_a value for the protonated N1 atom of the quinoline of each compound was then obtained from the midpoint of changes in optical behavior. The second higher value for each of the terminal amino functions in **8**−**10**, **20**, and **21** (pK_a > 8) cannot be determined by the spectrophotometric titration.

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