Analysis of van der Waals and Electrostatic Contributions in the Interactions of Minor Groove Binding Benzimidazoles with DNA

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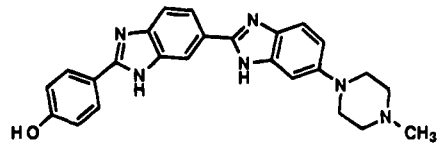
Interest in cationic benzimidazoles has been stimulated by the extensive use of Hoechst 33258 (**1**) and analogs as DNA fluorophores[1] and by the fact that bis-benzimidazoles (e.g., **2**) have potent activity against a number of microorganisms including those that lead to AIDS-related opportunistic infections.[2] Both **1** and **2** bind to DNA in the minor groove at AT-rich sequences.[2c,3] Interest in the molecular basis for specific minor groove interactions has been stimulated by theoretical studies,[4] by extensive experimental analysis including X-ray studies of complexes,[3,5] and by the need to develop a broad range of ligands with DNA recognition specificity. The latter idea led to development of "lexitropsins"[6] and to dimer recognition agents.[7] The dimer motif recognizes a sequence-dependent widening of the minor groove and is a dramatic variation on the usual model for binding of unfused aromatic cations.



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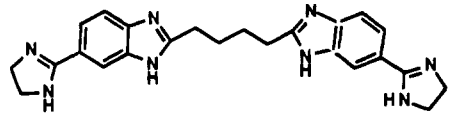

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Analysis of DNA complexes of **1**, netropsin, and analogs led to debate over the contributions of H-bonding, electrostatic, and van der Waals interactions to minor groove binding affinity and specificity. To design improved drugs that target the minor groove of DNA, it is essential to have a more detailed understanding of such interactions, but it has not been possible to experimentally determine the relative importance of these factors.[8] We have synthesized[9] and evaluated the DNA binding of a series of benzimidazoles, in which the cationic group, the number of charges, and the number of benzimidazole groups have been varied to experimentally address the importance of the factors involved in DNA complex formation.

None of the benzimidazoles show significant binding to the RNA polymer, polyApolyU, but they exhibit large ΔT_m values with polydA·polydT, indicating a strong affinity for AT-rich DNA (Table 1). From the ΔT_m values for the various cations with DNA, binding constants were calculated[11a] according to the method of Crothers[11b] with thermodynamic data of Breslauer and co-workers.[11c] The dications **10**−**12** and all bis-benzimidazoles have higher ΔT_m and K values than **3**−**9**, and their polydA·polydT complexes do not completely melt under the conditions employed. To compare the relative affinities of all compounds for AT sites in DNA, their ΔT_m values with d(CGCGAATTCGCG)$_2$ were also determined. Monocations **3**−**9** have ΔT_m values of 6.7 ± 0.6 °C with the oligomer. This surprising result suggests that neither the nature of the cationic group nor the type of oxygen substituent contributes in a crucial way to the DNA binding affinity of **3**−**9**. There is an increase in affinity for the dications **10**−**12** (ΔT_m of 16.1 ± 1.0 °C), revealing that a second charged group increases ΔT_m by ~10 °C relative to **3**−**9** and that binding of the dications is also independent of the nature of the charged group. Binding constants were also calculated from the oligomer ΔT_m values[12] and are included in Table 1.

There is no significant binding of the bis-benzimidazoles **13**−**22** to the RNA polymer, polyA·polyU, but they bind strongly to polydA·polydT with T_m values >95 °C (Table 1). The ΔT_m values observed with the oligomer and the monocations **13**−**19** are 24.2 ± 0.9 °C. The bis compounds show no significant

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(1) (a) Loontiens, F. G.; McLaughlin, L. W.; Diekmann, S.; Clegg, R. M. *Biochemistry* **1991**, *30*, 182. (b) Ellwart, J. W.; Dormer, P. *Cytometry* **1990**, *11*, 239.

(2) (a) Bell, C. A.; Dykstra, C. C.; Naimen, N. A.; Cory, M.; Fairley, T. A.; Tidwell, R. R. *Antimicrob. Agents Chemother.* **1993**, *37*, 2668. (b) Tidwell, R. R.; Jones, S. K.; Naimen, N. A.; Berger, L. C.; Brake, W. B.; Dykstra, C. C.; Hall, J. E. *Antimicrob. Agents Chemother.* **1993**, *37*, 1713. (c) Fairley, T. A.; Tidwell, R. R.; Donkor, I.; Naimen, N. A.; Ohemeng, K.A.; Lombardy, R.; Bently, J. A.; Cory, M. *J. Med. Chem.* **1993**, *36*, 1746.

(3) (a) Zimmer, C.; Wahnert, U. *Prog. Biophys. Mol. Biol.* **1986**, *47*, 31. (b) Teng, M.; Usman, N.; Frederick, C. A.; Wang, A. H.-J. *Nucleic Acids Res.* **1988**, *16*, 2671. (c) Quintana, J. R.; Lipanov, A. A.; Dickerson, R. E. *Biochemistry* **1991**, *30*, 10294.

(4) (a) Pullman, A.; Pullman, B. *Q. Rev. Biophys.* **1981**, *14*, 289. (b) Neidle, S.; Jenkins, T. C. In *Methods in Enzymology Vol. 203, Molecular Design and Modeling: Concepts and Applications, Part B*; Langone, J. J., Ed.; Academic Press Inc.: New York, 1991; p 433. (c) Goodsell D.; Dickerson, R. E. *J. Med. Chem.* **1986**, *29*, 727.

(5) (a) Neidle, S. In *Landolt-Borstein, Nucleic Acids*; Saenger, W., Ed.; **1989**, Springer: Berlin, 1989; Vol. VII/1b, p 247. (b) Coll, M.; Fredrick, C. C.; Wang, A. H-J.; Rich, A. A. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 8385. (c) Spink, N.; Brown, D. G.; Skelly, J. V.; Neidle, S. *Nucleic Acids Res.* **1994**, *22*, 1607.

(6) (a) Lown, J. W. *Anti-Cancer Drug Des.* **1988**, *3*, 25. (b) Kopka, M. L.; Yoon, C.; Goodsell, D.; Pjura, P.; Dickerson, R. E. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 1376.

(7) (a) Mrksich, M.; Dervan, P. B. *J. Am. Chem. Soc.* **1994**, *116*, 3663. (b) Dwyer, T. J.; Geiestanger, B. H.; Mrksich, M.; Dervan, P. B.; Wemmer, D. E. *J. Am. Chem. Soc.* **1993**, *115*, 9900.

(8) (a) Tanious, F. A.; Spychala, J.; Kumar, A.; Greene, K.; Boykin, D. W.; Wilson, W. D. *J. Biomol. Struct. Dynam.* **1994**, *11*, 1063.

(9) The compounds **3**−**22**, to be reported elsewhere, were synthesized according to standard methodology. The new compounds gave analytical data (C, H, N) in agreement with calculated values and have spectroscopic data (^{1}H and ^{13}C NMR, IR, MS) in accord with the assigned structures. Physical data for the previously reported compounds were in accord with literature values.

(10) (a) T_m measurements were made in MES buffer (0.01 M 2-(*N*-morpholino)ethanesulfonic acid, 0.001 M EDTA, 0.10 M NaCl at pH 6.25) as previously described.[10b] (b) Wilson, W. D.; Ratmeyer, L.; Zhao, M.; Strekowski, L.; Boykin, D. W. *Biochemistry* **1993**, *32*, 4098.

(11) (a) The model of Figure 1 indicates that the bis-benzimidazoles have a binding site size (n) of 4−5 base pairs, while n for the mono compounds should be one base pair shorter. The shorter benzimidazoles with an n of 3 and a ΔT_m = 15 °C have a calculated $K \approx 5 \times 10^5$; for an n of 4, $K \approx 1 \times 10^6$. For the bis compounds with $n = 4$ and $\Delta T_m = 30$ °C, $K \approx 7 \times 10^6$; with $n = 5$, $K \approx 2 \times 10^7$. It is clear from these results that the benzimidazoles bind very strongly to AT sequences in DNA and that for constant conditions and DNA sequence, the K and ΔT_m values are correlated. (b) Crothers, D. M. *Biopolymers* **1971**, *10*, 2147. (c) Marky, L. A.; Curry, J.; Breslauer, K. J. *Molecular Basis of Cancer*; Alan R. Liss Inc.: New York, 1985; p 155.

(12) (a) The ΔT_m values for the oligomer complexes increase significantly up to a ratio of one compound per duplex and increase only slightly at higher ratios. Binding constants for the oligomer complexes were thus calculated[11b] for one binding site per oligomer with enthalpy values from Marky et al.,[12b] and the results are included in Table 1. The value for **1** is in good agreement with the direct determination,[1a] particularly considering the temperature difference of the measurements. (b) Marky, L. A.; Blumenfeld, K. S.; Kozlowski, S.; Breslauer, K. J. *Biopolymers* **1983**, *22*, 1247.

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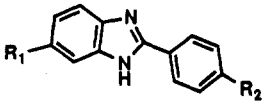
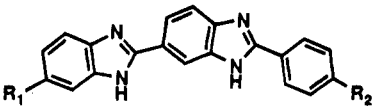
Table 1. Benzimidazole Structures and T_m Results[a]

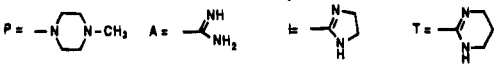


compd	R1[b]	R2[b]	ΔT_m(RNA)	ΔT_m(DNA)	$\Delta T_m/K$(oligo)[c]
3	A	OH	0.8	16.0	7.0/0.05
4	A	OCH_3	1.8	16.5	6.2/0.04
5	A	OCH_2CH_3		16.0	4.9/0.02
6	I	OH	2.2	21.0	5.6/0.03
7	I	OCH_3	1.8	20.0	7.5/0.06
8	I	OCH_2CH_3	0.2	21.6	7.0/0.05
9	T	OH	0.2	23.6	7.0/0.05
10	A	A	0.9	>29	17.1/1.0
11	I	I	2.5	>29	15.6/0.7
12	T	T	0	>29	17.1/1.0



1	P	OH	0.1	>25	17.9/1.2
13	A	OH	0	>30	23.0/5.2
14	A	OCH_3	0.2	>30	23.0/5.2
15	A	OCH_2CH_3	0	>30	23.0/5.2
16	I	OH	0	>30	23.9/6.7
17	I	OCH_3	0	>30	24.4/7.7
18	I	OCH_2CH_3	0	>30	25.8/11.2
19	T	OH	0	>30	25.4/10.0
20	A	A	0.2	>30	32.8/70.9
21	I	I	0	>30	30.1/35.1
22	T	T	0.2	>30	30.7/41.1

[a] T_m measurements were made as described.[10] RNA, DNA, and oligo samples are described in the text. [b] Key to R1 and R2:



[c] K values have been multiplied by 10^{-8}.

changes in ΔT_m with variation of the phenyl substituent or the cationic center. A second benzimidazole ring increases the ΔT_m by ~17 °C: the change on comparison of **3**–**5** with **13**–**15**, **6**–**8** with **16**–**18**, **9** with **19**, and **10**–**12** with **20**–**22** is addition of a benzimidazole, and the observed result is a 16–18 °C increase in ΔT_m for the DNA complexes (a free energy difference of ~3 kcal/mol). Replacement of the 2-phenyl-OR with a charged group (compare **3**–**9** with **10**–**12** and **13**–**19** with **20**–**21**) results in an 8–10 °C increase in T_m of the oligomer complexes under the experimental conditions (a free energy difference of ~2 kcal/mol). Clearly, the benzimidazole contacts with the DNA minor groove are more important to the binding affinity than the electrostatic interactions.

To evaluate the molecular basis of the above observations, we have determined the crystal structure of **16** complexed with the dodecamer used in the T_m experiments.[13] The DNA adopts a B-conformation, and electron density in the minor groove AT region fits the structure of **16** with unambiguous orientation and position (Figure 1 and in supplementary material Figure 1S). There are three hydrogen bonds from benzimidazoles of **16** to the edges of the bases, two (2.7 and 3.1 Å) to O2 of successive thymines, and one (2.9 Å) to N3 of an adenine (supplementary material Figure 2S). The Hoechst 33258 dodecamer complex has four such H bonds.[3c] The imidazoline and hydroxy groups do not form H bonds in the **16** complex. There are a number of close van der Waals contacts between the ligand and the floor and walls of the minor groove. The DNA groove is ~0.8 Å narrower in the region of the imidazoline of **16** than in the Hoechst complex, reflecting the decreased thickness of the imidazoline compared to the piperazine ring in Hoechst. Clearly the imidazoline of **16** and related derivatives makes good contacts with the groove and causes little perturbation of the DNA structure; the compounds have fewer H bonds but better van der Waals interactions than Hoechst 33258, and they have higher T_m values. The hydroxy of **16** was replaced in the model with a cationic imidazoline to mimic **21**.[14] The added group forms van der Waals contacts with the walls of the groove but no H bonds. Compound **6** (ΔT_m 5.6) can be converted to **11** (ΔT_m 15.6; hydroxy to imidazoline) or to **16** (ΔT_m 23.9; added benzimidazole). In the **6** → **11** conversion, additional van der Waals and, particularly, electrostatic interactions are obtained, while in the **6** → **16** conversion, more extensive van der Waals and H-bonding interactions are added. Such results with the new compounds of Table 1 as well as those of Hoechst 33258 clearly show that, in the context of the DNA minor groove, interactions of the compounds with the walls of the groove are the most important contributions to benzimidazole derivative binding.

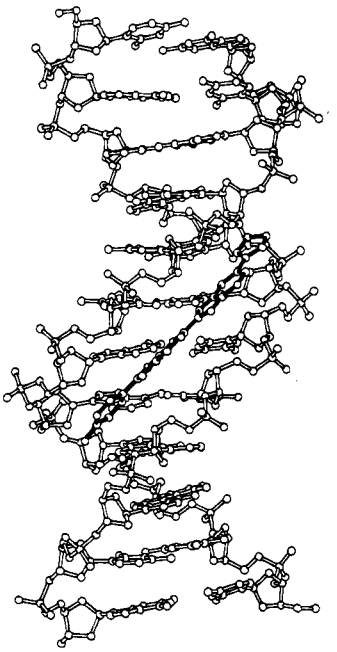


Figure 1. Model for the structure of the **16**–d(CGCGAATTCGCG)$_2$ complex.[13] The bonds of **16** are black and those of DNA are white. The view is into the minor groove with the phenyl at the bottom and the 5′ ends of the DNA chains at the upper left and lower right.

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Supplementary Material Available: Figure 1S, showing stereoviews of the **16**–d(CGCGAATTCGCG)$_2$ complex, and Figure 2S, showing the hydrogen-bonding interactions in a Hoechst 33258 complex and in the **16** complex (4 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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(13) Crystals of the **16**–d(CGCGAATTCGCG)$_2$ complex were grown according to the sitting drop method from a 14 μL droplet containing **16**, spermine, magnesium chloride, and 35% w/w 2-methylpentane-2,4-diol buffered at pH 7. X-ray diffraction data were collected on a Siemens-Xentronics multiwire area detector, resulting in 4365 unique observed reflections to a resolution of 2.0 Å and a merging R factor of 3.1%, for a crystal of unit cell dimensions a = 24.59, b = 40.44, and c = 65.76 Å and space group $P2_12_12_1$. The structure has been refined to a current R factor of 19.7% with 90 water molecules and a magnesium ion included. Coordinates and structure factors will be deposited in the Brookhaven Databank.

(14) The OH group in the oligomer–**16** complex was replaced (Sybyl) by an imidazoline identical to the one on the benzimidazole. The phenyl–imidazoline bond was rotated within the minor groove limits to evaluate possible H-bonding interactions with the DNA bases.