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Symmetric bis-benzimidazoles: new sequence-selective DNA-binding molecules

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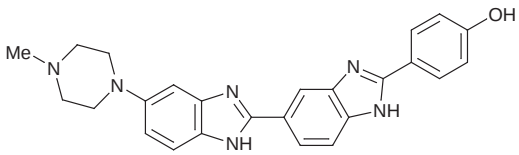
A series of *bis*-benzimidazole compounds with a head-to-head orientation have been designed as sequence-specific DNA binders; crystallographic analysis of oligonucleotide complexes has been combined with DNase I footprinting to confirm that the predicted optimal site for the core bis-benzimidazole motif is the four-base-pair sequence 5′-AATT; this sequence specificity results in inhibition of transcription at A/T sites and may be responsible for the cytotoxic and antitumour effects shown by these head-to-head bis-benzimidazoles.

The minor groove of duplex DNA is the site of non-covalent interaction of a large number of anticancer drugs, antibiotics and antiviral agents,[1] which are believed to exert their action by competing with transcription factors[2] or architectural proteins,[3] such as E2F, TATA-box binding proteins or DNA topoisomerase I/II. The molecular basis of DNA recognition for a number of drugs in this super-family (notably distamycin, netropsin, berenil, pentamidine and Hoechst 33258) has been extensively studied, by crystallographic,[4] NMR[5] and footprinting methods.[6] These studies have shown that their AT preferences are a consequence of, in particular, (i) the sequence-dependent narrow width of the minor groove of B-form DNA, resulting in stabilisation by van der Waals interactions with the walls of the groove,[7] and (ii) their ability to form specific H-bonds with donor and acceptor atoms on the minor groove edge of A : T base pairs. These factors have been utilised in the design of molecules with altered and extended recognition properties, some of which are capable of sequence-specific gene regulation.[8]



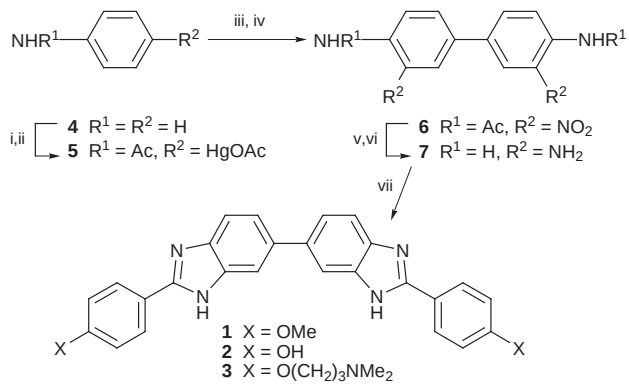
Hoechst 33258

We have previously reported crystallographic analyses of a number of oligonucleotide complexes with the head-to-tail bis-benzimidazole compound Hoechst 33258, and several of its derivatives,[9] including a tris-benzimidazole with an extended recognition site.[10] These have shown that each benzimidazole subunit interacts with two A : T base pairs by means of a pair of cross-strand H-bonds. The head-to-tail arrangement forces the site for each successive subunit to overlap the previous one by one base-pair (bp), so that each benzimidazole group in Hoechst 33258 and other head-to-tail analogues effectively recognises 1.5 A : T bps.

Molecular modelling, based on these structural studies, has suggested to us that the novel symmetric head-to-head benzimidazole arrangement could also effectively bind in the minor groove. This arrangement would extend the size of the *bis*-benzimidazole recognition site from three (in Hoechst 33258 and analogues) to four consecutive A:T bps, with distinctive cross-strand H-bonding involving each base pair. This arrangement thus extends the effective recognition of each benzimidazole sub-unit to two A:T bps. The modelling used the structures of the self-complementary duplex sequences d(CGCGAATTCGCG) and d(CGCAAATTTGCG), as found in several relevant drug complexes.[9,10] It suggested that the central 5'-AATT sequence would be an optimal site for the head-to-head motif and for maintenance of helical register with all four consecutive bps without necessitating significant DNA conformational change. An initial range of head-to-head bis-benzimidazoles have now been synthesised, and is reported here.

The highly convergent synthesis of these bis-benzimidazoles (Scheme 1) involves the condensation between the requisite 4-substitituted aryl aldehyde and 3,3′,4,4′-tetraaminobiphenyl **7**. The latter is commercially available, but is of variable quality, and we preferred to prepare it by the route shown. Thus, **1** and **2** were easily obtained from 4-methoxybenzaldehyde and 4-hydroxybenzaldehyde, respectively, while **3** was obtained from 4-(3-dimethylaminopropoxy)benzaldehyde prepared from 4-hydroxybenzaldehyde and 3-dimethylaminopropanol using a Mitsunobu reaction. In each instance the aryl aldehyde and 3,3′,4,4′-tetraaminobiphenyl were heated in nitrobenzene at 150 °C for 8–10 h to effect the condensation, and the yields of bis-benzimidazoles were typically 25–40% on the 10 mmol scale.

Crystallographic analyses have been undertaken on (i) the bis(hydroxyphenyl) compound **2** co-crystallised with the oligonucleotide sequence d(CGCGAATTCGCG), and (ii) its Me_2N derivative **3** with the sequence d(CGCAAATTTGCG). Both crystal structures[11] show a B-form DNA double helix with ligand bound in the A/T region of the minor groove [Fig. 1(*a*)]. They show H-bonding between all four central A : T bps and the benzimidazole subunits [Fig. 1(*b*)], in full accord with the modelling predictions. Each inner-facing ring nitrogen atom of



Scheme 1 *Reagents and conditions*: i, Ac_2O, AcOH; ii, $Hg(OAc)_2$, AcOH, $HClO_4$, *ca.* 20% (2 steps); iii, Cu, $PdCl_2$, Py; iv, HNO_3, AcOH, *ca.* 50% (2 steps); v, H_2SO_4; vi, Raney Ni, H_2, *ca.* 70% for 2 steps; vii, 4-XC_6H_4CHO [X+MeO (**1**), OH (**2**) or $Me_2N(CH_2)_3O$ (**3**)], $PhNO_2$, 150 °C.

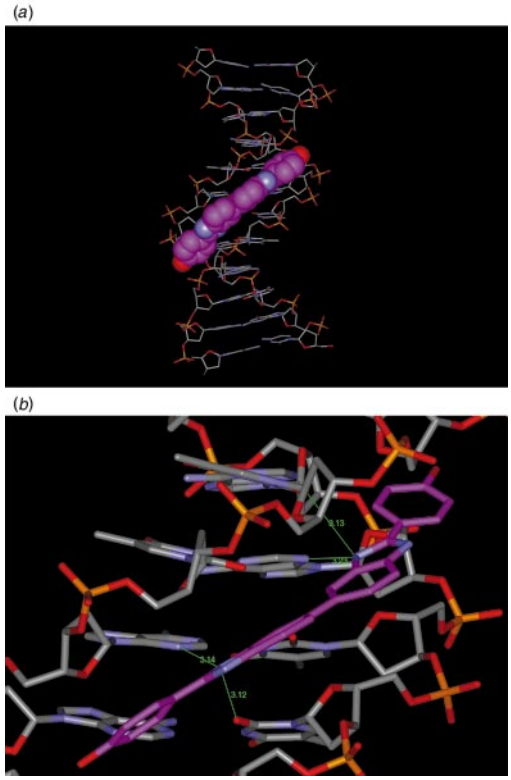


Fig. 1 Computer plots of the crystal structure of the complex of **2** with d(CGCGAATTCGCG): (*a*) shows the ligand, in space-filling mode, centrally bound in the minor groove of the 12-mer double helix, while (*b*) shows a detailed view of **2** and the H-bond contacts to A : T bp edges.

Table 1 Cytotoxicity of *bis*-benzimidazole compounds, for 96 h exposure in a panel of four ovarian cell lines (as $IC_{50}/\mu M$)

Compound	A2780	A2780-Pt^R	CH1	SKOV-3
1	13.5	5.1	3.8	16.5
2	4.3	2.7	1.05	16
3	0.235	0.115	0.24	0.375
Hoechst 33258	12.0	9.5	26.5	> 100

a benzimidazole ring H-bonds to a thymine O2 atom of one bp and an adenine N3 atom on the opposite strand of an adjacent one. It is notable that the absence of formal cationic charge in **2**, does not affect its sequence selectivity, suggesting that the role of charge is to modulate affinity rather than to determine sequence selectivity.

These preferences have also been observed in DNase I footprinting studies with a natural DNA fragment, which have shown that **2** and **3** both interact preferentially with some A/T sites. Compound **3** binds to AT sites between 7–10-fold better than **2**, and has an especially high affinity for the 5′-AATT site (30 nM). Quantitative footprinting data indicated that the order of preference is 5′-AATT > 5′-ATAT > 5′-TAAT > 5′-TATA ~ 5′-TTAA, similar to that previous determined for Hoechst 33258. The increased affinity of **3** relative to **2** does not affect its sequence selectivity. This enhanced binding strength may be a consequence of its cationic charge or its increased DNA site size, since the crystal structure of the complex with $d(CGCAAATTTGCG)_2$ shows it to cover 6 bps. The ability of the head-to-head bis-benzimidazoles to discriminate between the 136 possible tetranucleotide sequences may be further enhanced by flanking sequence preferences. These remain to be systematically explored. Whilst this paper was in preparation, a report has appeared which describes compound **2** and its interactions with tRNA.[12]

These new bis-benzimidazoles have been examined for potential cytotoxic effects in a group of ovarian carcinoma cell lines. Table 1 shows that all compounds are cytotoxic at the μM level, with activity significantly greater than that shown by Hoechst 33258. The Me_2N derivative **3** is over 10-fold more active than the others in three out of the four lines. **3** also shows significant activity in several ovarian lines in the NCI 60-cell line panel (to be published). The compound with least *in vitro* activity, the hydrophobic dimethoxy derivative **1**, has been examined in an *in vivo* tumour model with the ADJ/PC6 plasmacytoma, where it showed 44% tumour inhibition with ip administration. Compound **3** has been examined in an *in vitro* transcription assay. It effectively inhibits transcription at a number of A/T sites, consistent with the above data. In addition, compound **3** inhibited transcription at least 10-fold more efficiently than compound **2**, consistent with its increased cytoxicity. We speculate that the activity of **3** *in vitro* and *in vivo* may be related to its potent ability to specifically inhibit transcription at a small sub-set of A/T sites. The relatively low affinity of compound **3** for 5′-TATA sites suggests that it is less likely to block TATA-box binding proteins at the start of transcription.

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11 Compound **2** was co-crystallised with d(CGCGAATTCGCG) (from Oswell DNA Service, Southampton) using the hanging-drop method at 286 K. Elongated prismatic crystals were obtained from a 1.5 mM solution of 1 in 50% w/w MPD, in a droplet containing 10 mM $MgCl_2$, 60 mM KCl, 0.75 mM DNA and 6 mM spermine, equilibrated against 30% w/w MPD. The crystals are orthorhombic, space group $P2_12_12_1$, with cell dimensions a = 25.46(2), b = 39.96(4) and c = 65.66(7) Å. Intensity data were collected from a flash-frozen crystal maintained at 100 K with a RAXIS-4 image plate detector, using Cu-Kα radiation from a rotating-anode generator and mirror-focussing optics. A total of 6380 unique reflections were obtained after merging Friedel equivalents (R_{merge} = 0.044) to a resolution of 1.8 Å. The structure was solved using molecular replacement, and refined with the SHELX-97 package. The final model included 174 water molecules, and had an R of 0.229 for 6157 reflections with $F > 4\sigma(F)$, and 23.2% for all data to 1.8 Å. R_{free} was 25.1%. Coordinates and reflection data have been deposited at the Nucleic Acid Database as entry no. dd0013. See http://www.rsc.org/suppdata/cc/1999/929/ for a 3D image in .pdb format.
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