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13*H*-benzo[6-7]indolo[3,2-*c*]quinolines (B[6,7]IQ): optimization of their DNA triplex-specific stabilization properties†

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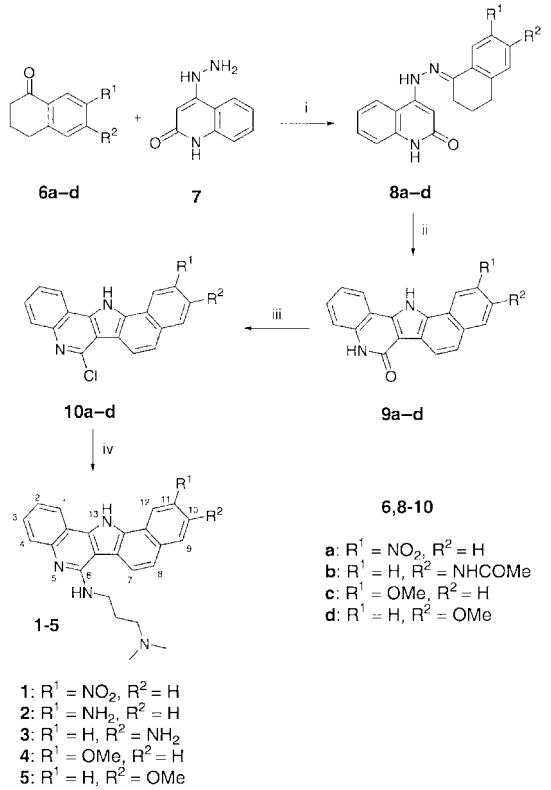
The triple helix stabilization property of 13*H*-benzo[6-7]indolo[3,2-*c*]quinoline was significantly improved by changing the electron-donor acceptor properties of the substituent at position 10 or 11.

Sequence-specific recognition of double-stranded DNA (dsDNA) by triplex-forming oligonucleotides (TFOs) offers a promising strategy to control the expression of genetic information and to develop new molecular tools.[1–3] TFOs bind to the major groove of dsDNA through formation of Hoogsteen or reverse Hoogsteen hydrogen bonds with the purines already engaged in Watson–Crick base pairs.[2] For this reason, triplex helix formation is mainly restricted to oligopyrimidine–oligopurine tracts of dsDNA. As a triple helix is generally thermodynamically less stable than a double helix, considerable efforts have been made to stabilize triple helices, and thus to extend the scope of dsDNA targeting by TFOs to shorter and therefore more numerous oligopyrimidine–oligopurine sequences. A number of triplex-specific ligands have thus been developed to promote the formation of triple helices, which would be otherwise unstable under physiological conditions.[4]

Recently, we have designed and synthesized a series of 13*H*-benzo[6-7]indolo[3,2-*c*]quinolines (B[6,7]IQ) which are pentacyclic crescent-shaped aromatic molecules.[5] Characteristic of the B[6,7]IQ compounds is their great efficacy for triplex stabilization, and weak binding towards duplexes. Possessing an extended aromatic surface, B[6,7]IQ derivatives are able to intercalate into triplexes, and to provide large π orbital overlap with the neighboring triplets at the intercalation site. In an effort to further optimize the design of an efficient triplex stabilizer, we report herein, the synthesis and DNA binding behavior of B[6,7]IQ derivatives **1**–**4**, bearing either an electron-donating or an electron-withdrawing functionality at the C–10 or C–11 center.

B[6,7]IQs **1**–**4** were obtained according to adaptations of the procedure reported for compound **5**[5] (Scheme 1). The tetralones **6a**–**d**, either commercially available, or obtainable through reported syntheses,[6] were reacted with 4-hydrazinoquinolin-2-(1*H*)-one **7** in acetic acid to form the corresponding hydrazones **8a**–**d**. Compounds **8b**–**d** were subsequently converted directly to the benzoindoloquinolones **9b**–**d** in a 'one pot' process involving thermal Fisher indolization, followed by addition of palladium/carbon (large excess) to effect aromatization of the dihydro intermediate formed. For **8a**, the treatment with Pd/C was accompanied by a further palladium-promoted reaction of the nitro group by the molecular hydrogen generated *in situ*. To circumvent this problem, compound **9a** was obtained by reaction of the isolated Fisher indolization product with a stoichiometric amount of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in DMF. Conversion of benzoindoloquinolin-6-ones **9a**–**d** to the corresponding 6-chloro derivatives **10a**–**d** was then achieved through reaction with benzyltriethylammonium chloride, *N*,*N*-diethylamine and phosphorus oxychloride.[7] These intermediates were then reacted with *N*,*N*-dimethyl-1,3-propanediamine under reflux. This latter reaction proceeded smoothly for the methoxy-substituted substrates **10c**,**d** yielding products **4** and **5** in 64 and 93% yields, respectively.[5] In a similar way the acetamido derivative **10b** was converted directly to compound **3** in 75% yield. However, for the nitro-containing substrate **10a** a mixture of both nitro (**1**) and amino-containing (**2**) substitution products (15 and 9%, respectively) was obtained. Even though the yield of this reaction was poor, it produced enough material to permit study of both compounds **1** and **2** for their ability to stabilize triple helices.

Triple helix stabilization by ligands **1**–**4** and **5**[5] was investigated by thermal denaturation experiments while monitoring the absorbance of double- or triple-helical DNA complexes at 260 nm (Fig. 1). The extent of duplex and triplex stabilization was estimated by measuring the melting temperature of the corresponding nucleic acid structures in the presence and absence of ligands **1**–**5**.



Scheme 1 Synthetic pathway and conditions of B[6,7]IQ derivatives (see text and ESI† for details): i, HOAc, room temp. 18–36 h, 79–99%; ii, for **9a**: (1) Ph_2O, reflux 4.5 h, (2) DDQ, DMF, reflux 2 h, 69%; for **9b**–**d**: Ph_2O, reflux 2–3.5 h, then 10% Pd/C, reflux 1–2 h, 44–76%; iii, $C_6H_5NEt_2$, $PhCH_2NEt_3Cl$, MeCN, $POCl_3$, reflux 24–48 h, 42–95%; iv, $NH_2(CH_2)NMe_2$, reflux 3 h, 9–93%.

† Electronic supplementary information (ESI) available: experimental. See http://www.rsc.org/suppdata/cc/b0/b001318h/

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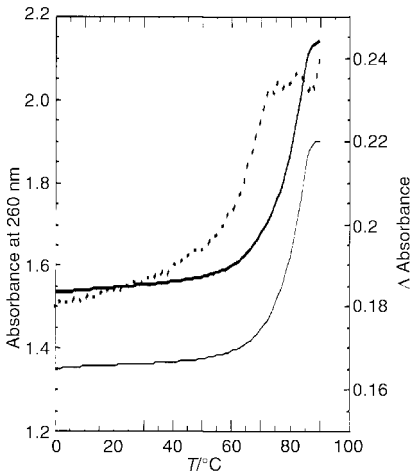

Fig. 1 Melting curves of 1.5 μM double- and triple-helices 14C3 (thin and thick lines, respectively) in the presence of 15 μM compound **5**. The triplex–duplex transition is better shown by subtracting the duplex melting curve from the triplex one (dashed thick line, scaled to the right) than by usual first derivative plot, owing to closely overlapped transitions. All thermal denaturation experiments were carried out in 10 mM sodium cacodylate buffer (pH 6.2) containing 100 mM sodium chloride.

In agreement with previously reported studies, it was determined that all five ligands, which share a common extended aromatic ring system, are able to stabilize the triplex more strongly than the duplex. Regardless of the ligand and the sequence, the differences in melting temperatures for the transition from duplex to single strands exhibited only modest variations (+13 to +19 °C). In contrast, the changes in the triplex-to-duplex melting temperatures were much higher (between +38 and +65 °C for the 14C3 triplex, or between +29 and +45 °C for the 14C5 triplex) and showed marked susceptibility to the nature of the R^1 and R^2 functional groups (Scheme 1). Thus, whereas the amino-bearing ligands **2** and **3** showed significant stabilization, with a $\Delta T_m^{3\rightarrow2}$ of +38 and +43 °C, respectively, in the presence of the 14C3 triplex sequence, and +29 and +35 °C in the presence of the 14C5 sequence, the methoxy-bearing ligands **4** and **5** induced significantly better triplex stabilization (+51 and +50 °C for the 14C3 sequence, and +39 and +41 °C for the 14C5 sequence).

The nitro-containing ligand **1** shows unprecedented triplex-promoting properties with a $\Delta T_m^{3\rightarrow2}$ of +65 and +45 °C in the presence of the 14C3 and 14C5 sequences, respectively. Furthermore, this ligand shows only modest affinity for double-helical structures (the $\Delta T_m^{2\rightarrow1}$ for the 14C3 and 14C5 sequences are +15 and +16 °C, respectively: Table 1). Thus, compound **1** presents a significantly greater specificity for triplexes as compared to duplexes.

To rationalize the trends that we observed, at least two parameters need to be taken into account: (i) the electron withdrawing capacity of the substituent group at position 11: triplex stabilization increases in the order NH_2 < OMe < NO_2, and (ii) the position of the substituent : substitution at position 10 appears to be more stabilizing than at position 11 (at least for the 14C5 sequence).

According to molecular models,[5] substitution at position 10 or 11 occurs in a region which is supposed to interact with Hoogsteen bases in base triplets. As a consequence, these substitutions should have a smaller effect on duplex than on triplex stabilization. The complete set of data is reported in Table 1.

The observations reported herein show that a significant part of the binding energy for intercalation of B[6,7]IQ derivatives into a triplex can be ascribed to the electron-donor acceptor (EDA) properties of the substituent at position 10 or 11. This leads us to the conclusion that further improvements in the design of triplex-stabilizers could take advantage of the fact that electron-withdrawing groups provide triplex-specific intercalators with both improved efficiency and selectivity. Polynitrated or polycyanated structures which not only expand the hyper-conjugated surface, but also increase the electron-withdrawing capabilities of intercalator might represent interesting candidates to explore for their triplex stabilizing properties. Synthetic studies toward such molecules are currently in progress.

Table 1 Sequences of the triple helices studied in this work (14C3 and 14C5) and the increased melting temperatures of triple- and double-helices ($\Delta T_m^{3\rightarrow2}$ and $\Delta T_m^{2\rightarrow1}$, respectively) in the presence of the B[6,7]IQ derivatives (see the legend of Fig. 1 for experimental conditions). The oligopyrimidine–oligopurine sequence for triplex formation in the double-stranded DNA fragment is in bold letters. In the absence of ligands, the $T_m^{3\rightarrow2}$ of the triplexes 14C3 and 14C5 are 16 and 17 °C, respectively, whereas the $T_m^{2\rightarrow1}$ of the corresponding duplexes are 62 and 63 °C, respectively. The accuracy of T_m values is *ca.* ±1 °C

14C3

5′ CTATCAGCTCAA**TCTTTTTTCTTCTT**AACTCGTATC 3′
3′ GATAGTCGAGTT**AGAAAAAAGAAGAA**TTGAGCATAG 5′
3′ **TCTTTTTTCTTCTT** 5′

14C5

5′ CTATCAGCTCAA**TCTTTCCTCTTCTT**AACTCGTATC 3′
3′ GATAGTCGAGTT**AGAAAGGAGAAGAA**TTGAGCATAG 5′
3′ **TCTTTCCTCTTCTT** 5′

	Triplex			
	14C3		14C5	
Compound	$\Delta T_m^{3\rightarrow2}$	$\Delta T_m^{2\rightarrow1}$	$\Delta T_m^{3\rightarrow2}$	$\Delta T_m^{2\rightarrow1}$
1	+65	+15	+45	+16
2	+38	+13	+29	+16
3	+43	+14	+35	+15
4	+51	+16	+39	+18
5	+50	+18	+41	+19

Notes and references

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