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Biarylpyrimidines: a new class of ligand for high-order DNA recognition†

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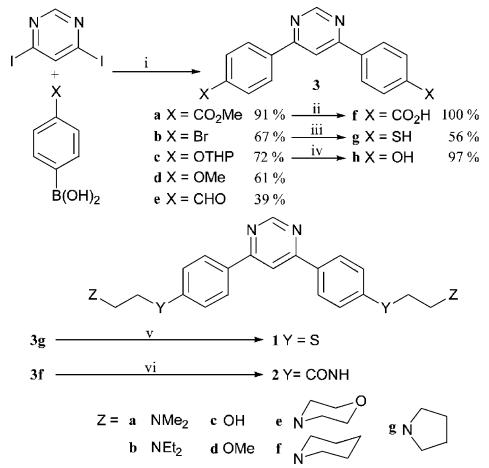
Biarylpyrimidines bearing ω-aminoalkyl substituents have been designed as ligands for high-order DNA structures: spectrophotometric, thermal and competition equilibrium dialysis assays showed that changing the functional group for substituent attachment from thioether to amide switches the structural binding preference from triplex to tetraplex DNA; the novel ligands are non-toxic and moderate inhibitors of human telomerase.

High-order (three- and four-stranded) nucleic acid secondary structures are important targets in drug design[1] and ligands with precisely-defined DNA structure selectivity have a range of potential therapeutic applications.[2] In contrast to biphenyl ring systems, 4,6-diphenylpyrimidine **1a** is planar in its crystal form and binds modestly to duplex DNA by intercalation.[3] Moreover, the width of the putative intercalating portion of the molecule (13.2 Å) is a closer match to the cross-sections of tetraplex (10.8–13.0 Å)[4] or triplex (7 or 9–12.4 Å)[5] nucleic acid structures, rather than to DNA duplexes which offer threading distances of 5.2–7.3 Å. It was also envisaged that the planar chromophore could be extended by substitution of amides for the thioether groups (i.e. **1**→**2**) and thereby further direct nucleic acid-binding preferences towards higher-order structures.

Biarylpyrimidines have hitherto been prepared by condensation of 1,3-diketones with formamide[6] or sequential aryl lithium additions to pyrimidine;[3] however, the authors' experience with Suzuki arylation of porphyrins[7] suggested that arylpyrimidines could be similarly prepared. Thus a concise biarylpyrimidine synthesis was developed that allowed the ready introduction of structural diversity (Scheme 1). 4,6-Diiodopyrimidine[8] was reacted with arylboronic acids in the presence of $Pd(Ph_3P)_4$ to afford substituted biaryl pyrimidines **3a–e** directly in up to 90% isolated yields. The amides **2** were prepared by hydrolysis of ester **3a** to acid **3f** and subsequent PyBOP-mediated coupling with the side-chains. Access to the thioethers, **1**, was *via* thiophenol **3g** obtained from nucleophilic displacement of bromoarene **3b** with NaSEt.[9] Compounds bearing basic side-chains were isolated as hydrobromide salts.‡



Scheme 1 Reagents and conditions: (i) $Pd(Ph_3P)_4/K_2CO_3$/PhMe : MeOH 9 : 1/Δ; (ii) $NaOH/H_2O$/Δ, HCl, 100%; (iii) NaSEt/DMF/Δ; (iv) AcOH : THF : H_2O 4 : 2 : 1, 45 °C, 4 h; (v) $Z(CH_2)_2Cl$/NaOH/EtOH, ≤87%, HBr; (vi) $Z(CH_2)_2NH_2$/PyBOP/Et_3N/CH_2Cl_2/RT, HBr, ≤70 %.

† Electronic supplementary information (ESI) available: experimental details of UV melting studies and example spectroscopic and analytical data. See http://www.rsc.org/suppdata/cc/b3/b301554h/

The effects of the thioethers **1a–f** and amides **2a–g** on the thermal denaturation[10] of duplex and triplex DNA were determined; ΔT_m values are presented in Table 1. The melting curve for poly(dA)·$[poly(dT)]_2$ triplex DNA in the absence of ligand showed two sequential thermal transitions: T_m^1 = 54.2 °C (triplex → duplex + single strand) and T_m^2 = 76.8 °C (melt to single strands). The thioethers **1** exhibited selective stabilisation of triplex DNA, increasing the temperature of the triplex melting transition ($\Delta T_m^1 \leq 20$ °C) whilst little affecting poly(dA)·poly(dT) duplex melting ($\Delta T_m^2 \leq 3$ °C). The presence of the charged ammonium groups in the side-chains was essential for triplex stabilisation: the hydroxyethyl (**1c**) and methoxyethyl (**1d**) derivatives showed no evidence of interaction with either triplex or duplex DNA, despite the possibilities for DNA–ligand hydrogen bonding. The corresponding amides **2c,d** showed similarly negligible effects. The extent of

Table 1 ΔT_m values (°C)[a] determined for calf thymus duplex (CT) or triplex DNA with ligands **1a–f** and **2a–g**.

	CT DNA[b]	poly(dA)·$[poly(dT)]_2$[c]	
Compound	ΔT_m Duplex melt	ΔT_m^1 Triplex melt	ΔT_m^2 Duplex melt
1a	12.2	10.5	0.3
1b	10.1	20.2	1.2
1c	0.8	4.0	0.6
1d	1.3	1.7	0.2
1e	1.6	13.9	1.1
1f	9.7	17.4	2.8
2a	11.2	0.7	7.7
2b	12.5	5.7	8.1
2c	1.4	0.8	1.2
2d	0.1	0.8	0.8
2e	2.3	−1.3[d]	5.1
2f	13.9	−5.0[d]	14.2
2g	13.8	−5.3[d]	13.7

[a] $\Delta T_m = T_m$(DNA + ligand) − T_m(DNA). [b] 50 μM DNA (in base pairs), 20 μM ligand; T_m for CT DNA = 68.3 °C. [c] 50 μM triplex (in base triads), 25 μM ligand. [d] Ligand destabilises the DNA triplex; see text for T_m^1 and T_m^2 values in the absence of ligand. All data are mean values (±0.1 °C).

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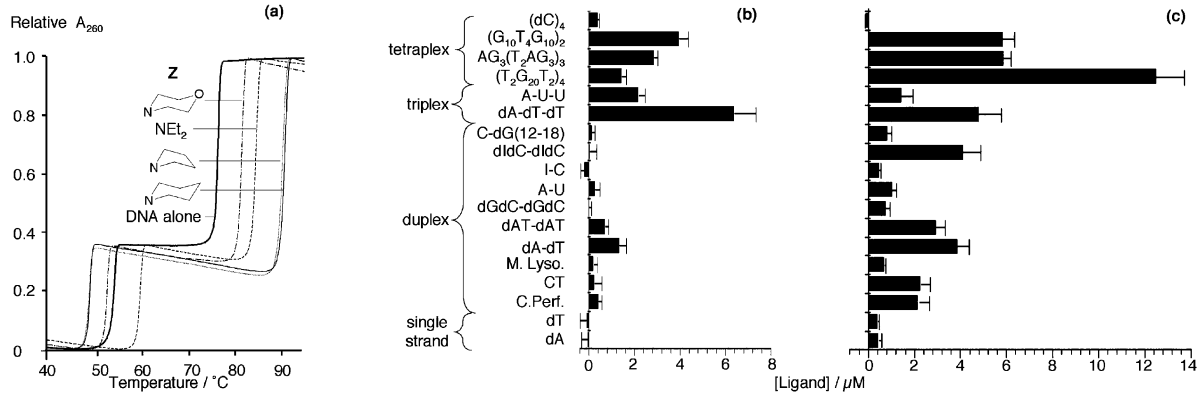

Fig. 1 (a) Thermal melting curves for poly(dA)·[poly(dT)]$_2$ triplex DNA in the absence and presence of amide ligands **2b, 2e–g** (DNA: 50 μM in base triads; ligands: 25 μM. Competition equilibrium dialysis data for compounds (b) **1b** and (c) **2b**; for a full description of the nucleic acid structures used, see reference 11.

triplex stabilisation was dependent upon the nature of the basic side-chains with the bulkier and more hydrophobic groups being more effective (e.g. piperidinyl, **1f**: ΔT_m^1 = 17.4 °C; diethylamino, **1b**: ΔT_m^1 = 20.2 °C) although no correlation with the pK_a of the basic amino group (**1a** < **1e** < **1b**) was apparent.

Minor structural alteration to the amides **2**, resulted in a significantly different pattern of ΔT_m values. The simple dialkylamino compounds, e.g. **2a**, induced a small stabilisation of poly(dA)·poly(dT) duplex ($\Delta T_m^2 \leq 8.1$ °C) with little effect on triplex melting (mostly $\Delta T_m^1 \leq 1$ °C). Spectrophotometric titration of dimethylamino compounds **1a** or **2a** with poly(dA)·poly(dT) duplex or poly(dA)·[poly(dT)]$_2$ triplex confirmed the lack of interaction of amide **2a** with the three-stranded DNA structure: hypochromicity and sharp isosbestic points were observed in all titrations except in the case of amide **2a** with triplex-form DNA. Remarkable structure-selective differences were seen for the amide ligands bearing saturated heterocyclic side chains (**2e–g**), see Figure 1 panel (a). Ligands **2f,g** showed closely similar effects: a marked stabilisation of poly(dA)·poly(dT) duplex ($\Delta T_m^2 \approx 14$ °C) accompanied by a specific destabilisation of the triplex structure ($\Delta T_m^1 \approx -5$ °C) that preserved the shape of the biphasic melting curve. An intermediate effect was observed for the morpholino derivative **2e**. The structural basis for these differential behaviours and the selective destabilisation of triplex DNA is uncertain and the subject of continuing investigations.

The structure preferences and relative binding affinities were further investigated by competitive equilibrium dialysis.[11] Figure 1 panels (b) and (c) show data obtained for compounds **1b** and **2b**. These results broadly confirmed that neither ligand family had a strong affinity for any nucleic acid duplex; as anticipated, preferential interactions with high-order structures were evident. In agreement with the thermal and spectrophotometric data, thioether **1b** showed a strong preference for triplex DNA (27-fold greater than for the natural duplex, CT DNA). Significantly less ligand bound to triplex RNA, indicating that at least part of the bound ligand (probably the pendant side chains) must align in the grooves of the DNA triplex—an accommodation thwarted by the 2′-hydroxyl groups in the all-RNA structure.[5] In contrast, the amide **2b** bound more strongly with tetraplex DNA structures (Figure 1, panel (c)) but was less discriminating in its recognition profile. Uncharged compounds **1c,d** and **2c,d** showed greatly reduced affinity for nucleic acids but the structure preferences generally resembled those of their dimethylamino counterparts. In both series, variation of the simple dialkyamino groups only influenced binding affinity (e.g. Z = NMe_2 > NEt_2) whilst the overall selectivity was unaffected. However, compounds bearing saturated heterocyclic side-chains, showed distinctly different patterns of binding preferences, in agreement with their anomalous thermal melting behaviour.

In conclusion, substituted biarylpyrimidines present an intriguing platform for the design of ligands to recognise high-order nucleic acid structures. Furthermore, a structural switch has been devised which alters binding preference for triplex or tetraplex DNA forms. No compounds examined showed appreciable cytotoxicity in a range of human tumour cell lines ($IC_{50} \geq 100$ μM), consistent with only weak affinity for duplex-form DNA. As expected for tetraplex-binding ligands, moderate inhibition of human telomerase was found ($IC_{50} \geq 10$ μM).§

Notes and references

‡ Satisfactory spectroscopic and analytical data were obtained for all compounds. See ESI.†

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1 T. C. Jenkins, *Current Med. Chem.*, 2000, **7**, 99–115; H. Arthanari and P. H. Bolton, *Chem. Biol.*, 2001, **8**, 221–230; J.-L. Mergny and C. Hélène, *Nature Med.*, 1998, **4**, 1366–1367; C. L. Grand, H. Han, R. M. Munoz, S. Weitman, D. D. Von Hoff, L. H. Hurley and D. J. Bearss, *Mol. Cancer Ther.*, 2002, **1**, 565–573.

2 T. de Lange and T. Jacks, *Cell*, 1999, **96**, 273–275; E. H. Blackburn, *Nature*, 1991, **350**, 569–573; S. S. Smith, A. Laayoun, R. G. Lingeman, D. J. Baker and J. Ridley, *J. Mol. Biol.*, 1994, **243**, 143–150; K. Usdin and K. J. Woodford, *Nucleic Acids Res.*, 1995, **23**, 4202–4209; A. Siddiqui-Jain, C. L. Grand, D. J. Bearss and L. H. Hurley, *Proc. Nat. Acad. Sci. USA*, 2002, **99**, 11593–11598.

3 W. D. Wilson, L. Strekowski, F. A. Tanious, R. A. Watson, J. L. Mokrosz, A. Strekowska, G. D. Webster and S. Neidle, *J. Am. Chem. Soc.*, 1988, **110**, 8292–8299.

4 Y. Wang and D. J. Patel, *Structure*, 1993, **1**, 263–282.

5 A. N. Lane and T. C. Jenkins, *Current Org. Chem.*, 2001, **5**, 845–870; V. N. Soyfer and V. N. Potaman, *Triple-helical Nucleic Acids*, Springer-Verlag, 1996.

6 H. Bredereck, R. Gompper and G. Morlock, *Chem. Ber.*, 1957, **90**, 942–951; C. G. Herbert, R. G. Bass, K. A. Watson and J. W. Connell, *Macromol.*, 1996, **29**, 7709–7716.

7 D.-F. Shi, R. T. Wheelhouse, D. Sun and L. H. Hurley, *J. Med. Chem.*, 2001, **44**, 4509–4523; D.-F. Shi and R. T. Wheelhouse, *Tetrahedron Lett.*, 2002, **43**, 9341–9342.

8 E. Boucher, M. Simard and J. D. Wuest, *J. Org. Chem.*, 1995, **60**, 1408–1412.

9 L. Testaferri, M. Tiecco, M. Tingoli and D. Chianelli, *Synthesis Commun.*, 1983, 751–755; L. Testaferri, M. Tingoli and M. Tiecco, *Tetrahedron Lett.*, 1980, **21**, 3099–3100.

10 A. W. McConnaughie and T. C. Jenkins, *J. Med. Chem.*, 1995, **38**, 3488–3501; I. Haq, J. E. Ladbury, B. Z. Chowdhry and T. C. Jenkins, *J. Am. Chem. Soc.*, 1996, **118**, 10693–10701.

11 J. S. Ren and J. B. Chaires, *Biochemistry*, 1999, **38**, 16067–16075; J. S. Ren and J. B. Chaires, *J. Am. Chem. Soc.*, 2000, **122**, 424–425; J. S. Ren and J. B. Chaires, *Meth. Enzymol.*, 2001, **340**, 99–108.