Biaryl polyamides as a new class of DNA quadruplex-binding ligands†

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We report a novel class of biaryl polyamides highly selective for G-quadruplex DNA, and with significant cytotoxicity in several cancer cell lines; they form planar U-shaped structures that match the surface area dimensions of a terminal G-quartet in quadruplex structures rather than the grooves of duplex DNA.

Guanine-rich nucleic acid sequences can fold into four-stranded G-quadruplex structures.[1] These structures may be adopted by telomeric DNA repeats as well as sequences in promoter and other regulatory regions within human and other genomes.[2] Stabilization of the 3′-end single-stranded overhang of telomeric DNA as G-quadruplex structures can inhibit the telomerase enzyme complex (which is expressed in most cancer cells) from synthesizing further telomeric DNA repeats, resulting in selective cell growth inhibition. The prevalence of quadruplex sequences in gene promoter regions suggests a potential role for quadruplex structures in transcription,[3] and such quadruplexes have been characterized in the promoter sequences of a number of proto-oncogenes and cancer-associated growth factors,[3] notably *c-myc*, *c-kit*, *k-ras*, *PDGF-A* and *bcl-2*. In addition, G-quadruplex structures in the 5′-untranslated regions of gene transcripts have the potential to modulate translation.[4] Therefore, small molecules that selectively bind and stabilize G-quadruplex nucleic acids are of interest as a new class of anticancer agents. The majority of quadruplex-binding molecules studied to date comprise functionalized polycyclic aromatic systems such as acridines, anthraquinones, indoloquinolines and porphyrins.[5] A number of these molecules have the ability to provide quadruplex-related biological effects, although their physico-chemical features are generally non-drug-like. More recently, a number of ligands have been reported that have non-conjugated groupings, whilst still maintaining the essential co-planarity with the G-quartet motif.[6]

We report here a novel class of acyclic biaryl‡ polyamides **1–6** which selectively bind to G-quadruplexes, and which offer synthetic versatility and significant quadruplex selectivity. Previous attempts to devise quadruplex-binding acyclic polyamides, based on a distamycin motif, have not resulted in molecules with significant selectivity over duplex DNA.[7] The molecules described here are from an 84-member solution-phase library, the design of which allowed positioning of the biaryl units at either end of the polyamide core in order to introduce ligand diversity. The biaryl polyamides have two different structural motifs. While **1–4** contain one biaryl unit, **5** and **6** are dimers of biaryl units (Fig. 1A).

Our preliminary goal was to design molecules that have no affinity for duplex DNA but have high affinity for DNA quadruplexes. We used a distamycin scaffold as a starting point and introduced biaryl building blocks in place of pyrroles to switch preference from duplex to quadruplex.

A simple linear synthetic route was devised to prepare the initial building blocks, and the points of diversity were introduced using parallel chemistry. Our synthesis was based on the application of HOBT–DIC-mediated amide coupling and microwave-assisted Suzuki coupling[8] to afford the building blocks (Scheme 1). The pyrrole carboxamide **7** was coupled to different 5-membered bromoheterocyclic acids to provide the Suzuki substrates **8** in excellent yields. The Suzuki coupling

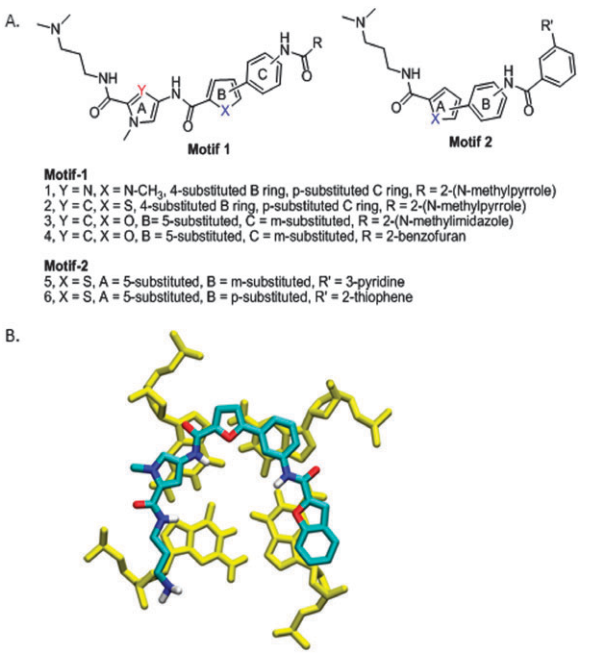

Fig. 1 **A,** Structures of biaryl polyamides. **B,** A molecular model of compound **4** (blue) bound to a terminal G-quartet (yellow) taken from a human intramolecular telomeric quadruplex–ligand complex crystal structure, showing the characteristic U shape of the biaryl polyamides.

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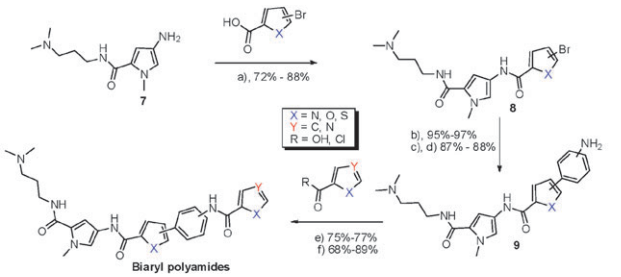

a) 4/5-bromoheterocyclic acid, DIC, HOBt, RT, 16 h; b) 4-aminoboronic acid, $(PPh_3)_4Pd$, K_2CO_3, Ethanol : Tolune: Water - 9:3:1, MW, 12-23 Mins; c) 3-nitroboronic acid, $(PPh_3)_4Pd$, K_2CO_3, Ethanol : Tolune: Water - 9:3:1, MW, 8-15 Mins; d) H_2, Pd/C, 4 h; e) heterocyclic carbonyl chloride, Dry DMF, RT, 2 h; f) heterocyclic carboxylic acid, DIC, HOBT, RT, 16 h

Scheme 1 Key synthetic steps for the quadruplex-targeting biaryl polyamides (Motif-1).

reaction between **8** and the aminoboronic/nitroboronic acids proceeded well under microwave conditions with high yields over two steps. The resulting analogues **9** were subjected to coupling reactions with either commercially available heterocyclic carbonyl chlorides or carboxylic acids to give the Motif-1 biaryl polyamides in excellent yields (see ESI† for synthesis of Motif-2 compounds).

The intermediates and each library member contained a 3,3-dimethylaminopropyl side chain which assisted in the purification of these compounds by the "catch and release" method using sulfonic acid-based SCX resins.

Assessment of the G-quadruplex interaction of **1–6** was initially carried out using a FRET-based melting assay.[9] Three different intramolecular DNA G-quadruplex sequences were used: human telomeric G-quadruplex (HT4, *FAM*-d(G_3[$TTAG_3$]$_3$)-*TAMRA*) and two different c-kit promoter G-quadruplexes (c-kit1, *FAM*-d(G_3AG_3CGCT-$G_3AG_2AG_3$)-*TAMRA* and c-kit2, *FAM*-d(G_3CG_3CGCGA-G_3AG_4)-*TAMRA*). A DNA duplex hairpin sequence [*FAM*-$(TA)_2GC(TA)_2T_6(TA)_2GC(TA)_2$-*TAMRA*] was used as a control. Together, the melting data from both G-quadruplex and duplex sequences enabled assessment of the selectivity of the ligands for different G-quadruplex sequences and selectivity for G-quadruplex *versus* duplex DNA. The minor groove binding polyamide distamycin, with established DNA duplex stabilizing ability,[7] was used as a control ligand.

The data in Table 1 show that the biaryl polyamide compounds **1–6** show significant selectivity towards G-quadruplex compared to duplex DNA, and the best molecule in the series, **3**, has no significant action on duplex DNA at this concentration. The selectivity becomes more evident when compared to distamycin, which has only very modest quadruplex affinity and a high preference for duplex DNA.

Table 1 DNA G-quadruplex and duplex stabilization by compounds **1–6** and distamycin (Dist) in the FRET melting experiments. Melting data are also available at higher ligand concentrations (ESI†)

	ΔT_m at 1 μM conc./°C			
Ligand	HT4	c-kit1	c-kit2	Duplex
1	8.8	6.1	3.2	0.4
2	2.4	2.5	3.5	0.2
3	11.8	6.0	5.6	0.0
4	6.8	4.8	4.7	0.3
5	5.9	4.3	2.4	0.1
6	1.0	4.4	4.6	0.0
Dist	0.6	0.3	0.4	11.5

Compounds **1**, **3**, **4** and **5** show greater G-quadruplex stabilizing ability with the telomeric HT4 quadruplex compared to the two c-kit ones. Compound **2** has a weak though comparable stabilizing ability for all three quadruplexes, whereas compound **6** shows distinctly superior stabilizing ability for the two c-kit quadruplexes. Compound **3** has the greatest selectivity in the series for the telomeric HT4 quadruplex, notably accompanied by no detectable duplex stabilization. The difference between compounds **3** and **4**, which have high chemical structural similarity, is likely to be due to the greater polarizability of the *N*-methyl imidazole group in the former compared to the benzofuran group in the latter, which would result in more effective π-stacking with a terminal G-quartet in these quadruplexes. Compounds **5** and **6** are biaryl dimers with the identical first biaryl units having distinct *meta vs. para* orientations. The ΔT_m data show a clear preference of **5** for the telomeric quadruplex compared to **6**. Surprisingly, the affinity of **6** for the c-kit quadruplexes is not diminished, suggesting that this ligand is sensitive to the structural differences between these three quadruplexes.

Molecular modelling has been used[10] to rationalize these results in the absence of structural data. Several distinct polymorphic forms of the native human intramolecular quadruplex have been identified,[11] and it is not possible on the basis of the biophysical data alone to define which is the most appropriate one to use as a starting point. Instead we have used a human quadruplex–ligand complex crystal structure,[12] focussing on maximizing ligand–G-quartet overlap by means of a docking approach. The low-energy conformations of the biaryl polyamides form planar U-shaped structures (Fig. 1B) that match the surface area dimensions of a terminal G-quartet but are too large to be accommodated within the major or minor grooves of duplex DNA. The inability of these molecules to bind to duplex DNA was further confirmed by DNAse I footprinting experiments against Hex A and Hex B sequences where they failed to provide any footprints at concentrations up to >100 μM (ESI†).

Circular dichroism (CD) can be used to indicate whether ligands bind to, and perhaps retain the overall topology of, a quadruplex nucleic acid. CD titrations of **1–6** were conducted for the telomeric quadruplex sequence in the presence of 100 mM KCl. Ligands **1–6** do not have any chiral centres and so are CD inactive. The CD spectrum of the native sequence showed the presence of mixed parallel and anti-parallel structures with positive and negative peaks around 295 nm and 240 nm, respectively. The spectrum showed significant changes on adding just one equivalent of ligand **3**. We observed a concentration-dependent enhancement of the positive peak at 295 nm, appearance of a negative peak around 260 nm, and disappearance of the negative peak around 240 nm at the concentrations used in the study. Titrations of **4** resulted in some concentration-dependent changes in the CD spectra, although it is not possible in the absence of other data to assign particular topologies from them (Fig. 2A and B).

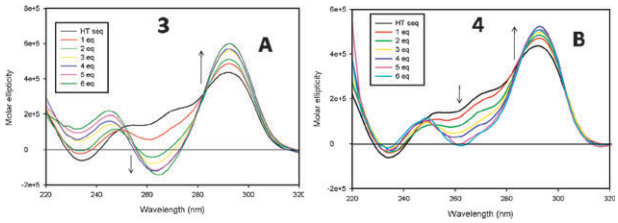

Fig. 2 CD spectra of a 5 μM solution of G-quadruplex **HT4** in Tris buffer (pH 7.4) with 0–6 equivalents of ligands **3** (A) or **4** (B).

Table 2 IC_{50} (μM) values of the biaryl polyamides **1–6** against three different cancer cell lines and a normal cell line (WI38) using the sulforhodamine B assay

Ligand	MCF7	A549	HT29	WI38
1	9.4	5.9	2.5	11.8
2	2.1	2.0	0.4	9.2
3	12.8	3.3	2.1	25.4
4	>50	17.0	9.2	13.2
5	3.6	2.6	0.6	5.1
6	2.1	2.6	2.5	11.6

The short-term cell growth inhibitory activity of the biaryl ligands was evaluated using the sulforhodamine B (SRB) assay for four different cancer cell lines and a normal human lung fibroblast cell line (WI38) (Table 2). The ligands generally showed greater cell growth inhibition against human colorectal carcinoma (HT29) and human lung carcinoma (A549) lines. The aim when targeting cancer cells is to achieve selectivity over normal cells. Although cell culture data can only be indicative compared to *in vivo* results, the data here do show that some ligands, in some cell lines, have significant selectivity. Compound **3** has the best overall profile, especially for the A549 and HT29 cell lines. This compound is also the most effective at stabilizing a human telomeric quadruplex (Table 1), although further studies will be required to unequivocally demonstrate that it is targeting telomere maintenance in tumour cells.

In summary, we report a new class of G-quadruplex ligands with high selectivity over duplex DNA. The ligands described here can be used as a template to attempt to generate more-potent molecules while retaining this selectivity, as well as for manipulating their selectivity to favour a particular type of quadruplex. We note that there is high potential for diversity in the terminal heterocyclic group, which should also be amenable to optimization to enhance drug-like characteristics.

Notes and references

‡ The term biaryl refers to two linked aromatic rings which can be carbocyclic or heterocyclic, or a combination of both.

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