

Interactions of ruthenium coordination cubes with DNA†‡

Nicolas P. E. Barry,^a Nurul H. Abd Karim,^b Ramón Vilar^{*b} and Bruno Therrien^{*a}

The interactions of four octacationic ruthenium coordination cubes with duplex and quadruplex (telomeric and *c-myc*) DNA have been studied by FID and SPR, showing these cubes to bind strongly to tetrastranded DNA structures.

Guanine bases have the ability to self-assemble *via* hydrogen-bonding interactions between the Watson–Crick edge of one guanine and the Hoogsteen edge of its neighbour to yield tetrads. In guanine rich sequences of DNA, these tetrads can stack on top of each other giving rise to quadruplex DNA structures.¹ Recent bioinformatic studies have shown that in the human genome there are approximately 350 000 guanine-rich sequences that can potentially form quadruplex DNA structures.² Some of these sequences have been identified as potential anticancer drug targets. For example, the formation of quadruplexes in human telomeric DNA has been shown to inhibit telomerase—an enzyme over-expressed in 85–90% of cancer cells which plays an important role in cancer cell immortalization.³ On the other hand, formation of quadruplex DNA structures in the promoter region of certain oncogenes (*e.g.* *c-myc* and *c-kit*) has been shown to control transcription of these genes and as a consequence their expression.⁴

An ongoing challenge in this area is to develop molecules that can interact strongly with quadruplex DNA but weakly with duplex DNA. Achieving this selectivity is essential to realise the potential advantages of quadruplex-targeting anticancer drugs. Most quadruplex DNA stabilisers reported to date are based on planar polyaromatic compounds that interact with guanine quartets *via* π – π stacking.⁵ In addition these molecules are often substituted with positively charged groups (*e.g.* protonated amines) to increase their solubility and also electrostatic interactions with the loops and grooves of DNA. However it is often the case that planar polyaromatic molecules, not only stack on top of the guanine quartet of quadruplexes, but also intercalate in between base pairs of duplex DNA reducing their selectivity for quadruplex *vs.* duplex DNA. With the aim of reducing the unwanted interactions between quadruplex binding molecules and duplex DNA, square-based pyramidal metal complexes have been recently reported as quadruplex DNA stabilisers.⁶ The axial ligand in these complexes is proposed to reduce their ability to intercalate

in between base pairs of duplex DNA. Polymetallic complexes where the metals are positioned outside the guanine quartet have also been recently reported to increase quadruplex DNA affinity and selectivity.⁷

Herein we report a new type of quadruplex DNA stabiliser based on the octacationic ruthenium coordination cubes **1–4** (see Fig. 1). Porphyrins are known to bind strongly to quadruplex DNA; however, their selectivity is usually poor since they also bind strongly to duplex DNA. We hypothesised that by linking two porphyrin rings *via* coordination bonds, their quadruplex binding ability would be retained but their ability to intercalate in-between bases of duplex DNA would be greatly reduced. Herein we show that the ruthenium coordination cubes **1–4** bind strongly to quadruplex DNA with modest selectivity for quadruplex over duplex DNA.

Recently we used arene ruthenium complexes as building blocks for the assembly of a series of cationic octanuclear metalla-prisms, $[\text{Ru}_8(\eta^6\text{-arene})_8(\text{tpp-H}_2)_2(\text{dhbq})_4]^{8+}$ (arene = toluene, *p*-cymene, hexamethylbenzene), incorporating arene ruthenium building blocks, bridged by 2,5-dihydroxy-1,4-benzoquinonato (dhbq) ligands, and connected by two 5,10,15,20-tetra(4-pyridyl)porphyrin (tpp-H₂)⁸ or 5,10,15,20-tetra(4-pyridyl)porphyrin–Zn(II) (tpp–Zn)⁹ tetrapodal subunits. Fig. 1 shows the four complexes from this series (**1–4**) chosen to carry out DNA binding studies.

These octacationic arene ruthenium cubes, isolated as their triflate salts, have been fully characterised by standard techniques and they have shown great stability in polar solvents such as dichloromethane, acetonitrile, water and acetone.⁸ Moreover, under conditions of electrospray mass spectrometry, these cationic cubes have shown a remarkable stability: the ESI-MS spectra showing peaks corresponding to $[\text{M} + (\text{CF}_3\text{SO}_3)_4]^{4+}$ and $[\text{M} + (\text{CF}_3\text{SO}_3)_5]^{3+}$ for all derivatives studied.^{8,9} However, in dimethylsulfoxide-*d*₆ at 40 °C, additional new sets of signals clearly attributed to the different components of the cage after decomplexation was observed by ¹H NMR spectroscopy. Therefore, to ensure the integrity of our system, the stability of two of the cubes (**1** and **2**) was further investigated under the conditions used for the DNA binding studies (*i.e.* in aqueous buffer solution, and in aqueous buffer solution + DNA). For this, the UV-visible spectra of the cubes were recorded over a period of 1 h (see Fig. S1 and S2 as examples in the ESI†). These experiments showed that, although there was a slight decrease in the λ_{max} intensities of the cubes over this period, the changes were not significant and therefore we proceeded to carry out the experiments described below.

In order to evaluate the ability of complexes **1–4** to interact with quadruplex and duplex DNA, fluorescence intercalation displacement (FID) assays were carried out.¹⁰ In this recently reported assay, thiazole orange (TO) is mixed with quadruplex DNA with which it interacts in a single-site manner and with high affinity. The fluorescence of this dye is quenched in solution,

^aInstitut de Chimie, Université de Neuchâtel, 51 Avenue de Bellevaux, CH-2000 Neuchâtel, Switzerland. E-mail: bruno.therrien@unine.ch; Tel: +41 32 7182499

^bDepartment of Chemistry, Imperial College London, London, UK SW7 2AZ. E-mail: r.vilar@imperial.ac.uk; Tel: +44 2075941967

† Electronic supplementary information (ESI) available: UV-visible spectra for complex **2** $[\text{CF}_3\text{SO}_3]_8$ under DNA binding conditions. Experimental details for FID and SPR studies. See DOI: 10.1039/b913642h

‡ Work first presented at the 10th FIGIPAS Meeting in Inorganic Chemistry, Palermo, July 1–4, 2009.

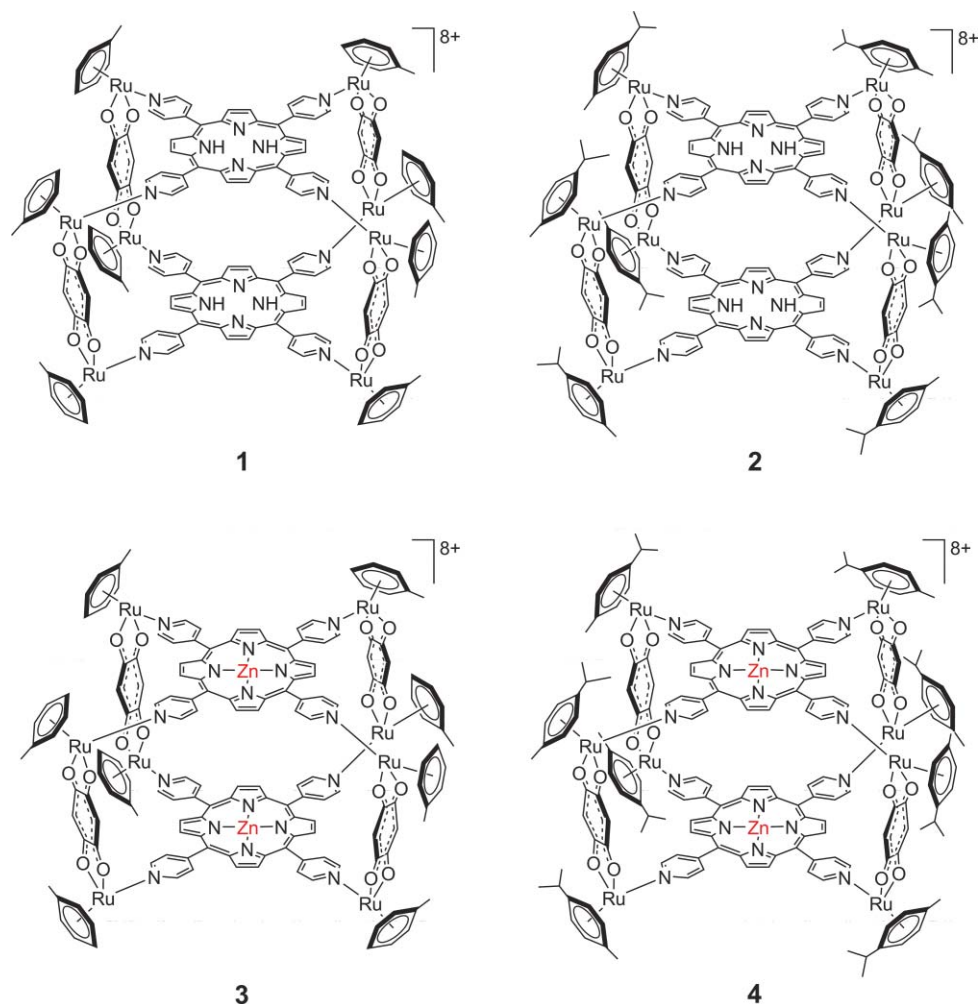


Fig. 1 Chemical structure of octa-ruthenium cubes 1–4.

however, upon interaction with quadruplex DNA, it displays up to a 3000-fold increase in its emission. Therefore, the displacement of TO by another molecule provides an approximate measure of the affinity of a compound for quadruplex (and duplex) DNA. In order to quantify the displacement, the compound's concentration at which TO fluorescence decreases by 50% (assumed to be 50% displacement of TO), is determined (G^4DC_{50}). Table 1 summarises the DC_{50} values obtained by FID for the interactions of complexes 1–4 with two different quadruplex DNA sequences (*Htelo* and *c-myc*) and one duplex DNA sequence (*ds*). From these DC_{50} values it is possible to conclude that the octanuclear ruthenium cubes are able to displace TO at very low μM concentrations, which

indicates that they interact strongly with quadruplex DNA (both telomeric and *c-myc*). The low micromolar G^4DC_{50} values ($0.15 \leq G^4DC_{50} \leq 0.70 \mu\text{M}$) are comparable to those previously found for a range of good quadruplex binders (see the extensive study by Teulade-Fichou¹⁰). Complexes 1–4 show, however, modest binding selectivity for quadruplex vs. duplex DNA (below the proposed “selectivity threshold” for a range of successful quadruplex DNA binders¹⁰). Among the four complexes, 1 shows the highest binding affinity towards quadruplex DNA (see Fig. 2 for a graphical representation of the results).

In order to analyse further the interaction between these coordination cubes and DNA, surface plasmon resonance

Table 1 ${}^{Htelo}DC_{50}$, ${}^{ds}DC_{50}$ and ${}^{cmyc}DC_{50}$ values (μM) determined using FID assay for complexes 1–4 (values are averaged over three independent measurements; experimental errors being estimated at $\pm 5\%$)

Compound	Thiazole orange displacement			Selectivity		
	${}^{Htelo}DC_{50}/\mu\text{M}$	${}^{ds}DC_{50}/\mu\text{M}$	${}^{cmyc}DC_{50}/\mu\text{M}$	${}^{ds}DC_{50}/{}^{Htelo}DC_{50}$	${}^{ds}DC_{50}/{}^{cmyc}DC_{50}$	${}^{cmyc}DC_{50}/{}^{Htelo}DC_{50}$
1	0.15	0.72	0.32	4.8	2.3	2.1
2	0.22	0.94	0.27	4.3	3.5	1.2
3	0.33	1.25	0.70	3.8	1.8	2.1
4	0.20	0.65	0.37	3.3	1.8	1.9

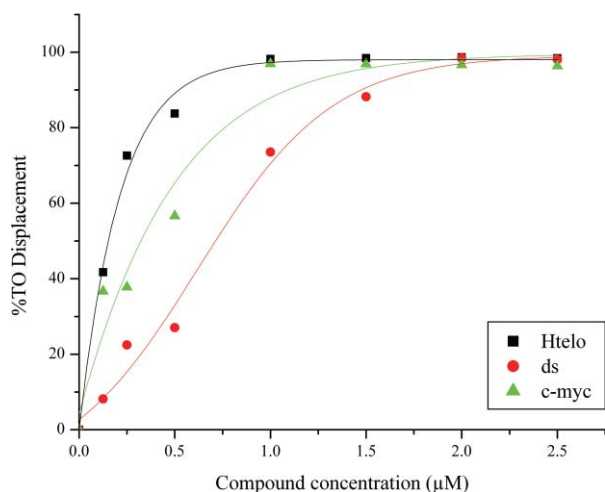


Fig. 2 Graphical representation of the TO displacement from *c-myc* (▲) and *Htelo* (■) quadruplexes and *ds* (●) duplex DNA upon increasing concentration of complex **1** from 0.125 to 2.5 μM .

(SPR) studies¹¹ were carried out on two of the complexes (**1** and **3**). The following three different 5'-biotin-labelled DNA sequences were immobilised on a sensor chip *via* biotin-streptavidin interactions: the 22-mer human telomeric quadruplex 5'-biotin-AGGG(TTAGGG)₃, the 22-mer CG-rich hairpin duplex 5'-biotin-TT(CG)₄TTTT(CG)₄, and the 36-mer *c-myc* quadruplex DNA 5'-biotin-ATG-CAT-GCG-GGG-AGG-GTG-GGG-AGG-GTG-GGG-AAG-GTG-GGG. The binding experiments were carried out under salt/buffer conditions that are suitable for DNA quadruplex formation (*i.e.* HBS-EP buffer from BIAcore supplemented with 0.2 M KCl). A range of concentrations of the corresponding complex were investigated by injecting the sample simultaneously over the three different sequences of immobilised DNA and the blank reference (flow rate of 20 $\mu\text{L min}^{-1}$; running time 5 min). Sensorgrams for the concentration-dependent binding of complexes **1** and **3** with DNA were obtained (see ESI for details[†]) from which binding constants were calculated (see Table 2).

The SPR data is consistent with complexes **1** and **3** binding to quadruplex DNA (both *Htelo* and *c-myc*) *via* a two-binding-site model. The binding constant to one of the binding sites is in the order of 10^6 while the other is in the order of 10^5 M^{-1} . In addition, the SPR results indicate that these complexes bind more strongly (one order of magnitude) to quadruplex DNA over duplex

Table 2 Association constants between complexes **1** and **3**, and DNA as determined by SPR

Compound	K_a/M^{-1a} <i>Htelo</i> quadruplex	K_a/M^{-1b} CG-rich duplex	K_a/M^{-1a} <i>c-myc</i> quadruplex
1	1.2×10^6 6.6×10^5	2.3×10^5	7.9×10^5 4.2×10^5
3	1.3×10^6 1.3×10^5	2.8×10^5	1.1×10^6 1.7×10^5

^a Binding curves were fitted using a non-equivalent two-site binding model. This yielded two different binding constants for the interactions between the cubes and quadruplex DNA. ^b Binding curves for duplex DNA were fitted using an equivalent two-site binding model.

DNA, which is consistent with the FID results. The selectivity of the coordination cubes for quadruplex DNA over duplex DNA is not as high as initially envisaged. This is probably due to the non-specific electrostatic interactions between the highly charged cubes and DNA. We are currently investigating modifications to this family of complexes to improve further the selectivity (*e.g.* by reducing the electrostatic charges).

In summary, we have shown by FID and SPR that complexes **1–4** bind strongly to telomeric and *c-myc* DNA. However, opposite to our initial expectations their selectivity for quadruplex over duplex DNA is very modest. This is likely to be a consequence of the octacationic nature of the cubes, which will increase their non-specific binding to DNA *via* electrostatic interactions. SPR suggests that the complexes studied (**1** and **3**) bind distinctively to two non-equivalent binding sites on the quadruplex DNA sequences—which is consistent with previously reported observations for planar polyaromatic molecules.¹² These ruthenium octacationic coordination cubes provide an interesting new family of complexes to stabilise quadruplex DNA. We are currently developing analogous systems with reduced charge with the aim of reducing non-specific binding and hence improving selectivity.

Acknowledgements

A generous loan of ruthenium chloride hydrate from the Johnson Matthey Research Centre is gratefully acknowledged. N. H. A. K. thanks the Malaysian Government for a studentship.

Notes and references

- Quadruplex Nucleic Acids*, ed. S. Neidle and S. Balasubramanian, RSC Publishing, Cambridge, UK, 2006.
- (a) J. L. Huppert, *Biochimie*, 2008, **90**, 1140; (b) J. L. Huppert and S. Balasubramanian, *Nucleic Acids Res.*, 2006, **35**, 406.
- (a) S. Neidle and G. N. Parkinson, *Biochimie*, 2008, **90**, 1184; (b) T.-M. Ou, Y.-J. Lu, J.-H. Tan, Z.-S. Huang, K.-Y. Wong and L.-Q. Gu, *ChemMedChem*, 2008, **3**, 690–713; (c) A. De Cian, L. Lacroix, C. Douarre, N. Temime-Smaali, C. Trentesaux, J. F. Riou and J. L. Mergny, *Biochimie*, 2008, **90**, 131.
- (a) L. H. Hurley, *Nat. Rev. Cancer*, 2002, **2**, 188; (b) A. Siddiqui-Jain, C. L. Grand Cory, D. J. Bearss and L. H. Hurley, *Proc. Natl. Acad. Sci. U. S. A.*, 2002, **99**, 11593; (c) Y. Qin and L. H. Hurley, *Biochimie*, 2008, **90**, 1149; (d) M. Bejugam, S. Sewitz, P. S. Shirude, R. Rodriguez, R. Shahid and S. Balasubramanian, *J. Am. Chem. Soc.*, 2007, **129**, 12926.
- (a) A. Arola and R. Vilar, *Curr. Top. Med. Chem.*, 2008, **8**, 1405; (b) S. Neidle and G. N. Parkinson, *Biochimie*, 2008, **90**, 1184; (c) D. Monchaud and M. P. Teulade-Fichou, *Org. Biomol. Chem.*, 2008, **6**, 627.
- (a) A. Arola-Arnal, J. Benet-Buchholz, S. Neidle and R. Vilar, *Inorg. Chem.*, 2008, **47**, 11910; (b) H. Bertrand, D. Monchaud, A. De Cian, R. Guillot, J.-L. Mergny and M.-P. Teulade-Fichou, *Org. Biomol. Chem.*, 2007, **5**, 2555.
- (a) R. Kieptyka, P. Englebienne, J. Fakhoury, C. Autexier, N. Moitessier and H. F. Sleiman, *J. Am. Chem. Soc.*, 2008, **130**, 10040; (b) C. Rajput, R. Rutkaite, L. Swanson, I. Haq and J. A. Thomas, *Chem.–Eur. J.*, 2006, **12**, 4611.
- N. P. E. Barry, P. Govindaswamy, J. Furrer, G. Süss-Fink and B. Therrien, *Inorg. Chem. Commun.*, 2008, **11**, 1300.
- N. P. E. Barry, M. Austeri, J. Lacour and B. Therrien, *Organometallics*, 2009, DOI: 10.1021/om900461s.
- D. Monchaud, C. Allain, H. Bertrand, N. Smargiasso, F. Rosu, V. Gabelica, A. De Cian, J. L. Mergny and M. P. Teulade-Fichou, *Biochimie*, 2008, **90**, 1207.
- J. E. Redman, *Methods Enzymol.*, 2007, **43**, 302.
- I. M. Dixon, F. Lopez, J. P. Estève, A. M. Tejera, M. A. Blasco, G. Pratviel and B. Meunier, *ChemBioChem*, 2005, **6**, 123.