Tetramethylpyridiniumporphyrazines—a new class of G-quadruplex inducing and stabilising ligands†

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3,4-Tetramethylpyridiniumporphyrazines bind strongly and selectively to human telomeric G-quadruplex DNA, inducing the formation of an antiparallel quadruplex in a process that mimics molecular chaperones.

Telomerase is a reverse transcriptase which is responsible for the synthesis of telomeres, and is upregulated in about 80–85% of human cancer cells.[1] Human telomeric DNA (*Htelo*) is composed of tandem repeats of the TTAGGG sequence with a single stranded 3′-end overhang, where the guanine-rich strand can fold into a four-stranded G-quadruplex structure. Telomeric DNA quadruplexes can form at the chromosome extremities.[2] The formation and stabilisation of intramolecular telomeric G-quadruplex structures by quadruplex binding molecules can inhibit telomerase activity *in vitro*. Thus, there is considerable interest in ligand-mediated strategies for the interference of telomere maintenance that can induce cell death.[3–5] As telomerase activity is low in human somatic cells, it is a very promising target for anticancer drug development. Furthermore, it has been demonstrated that G-quadruplex motifs are prevalent throughout the genome.[6]

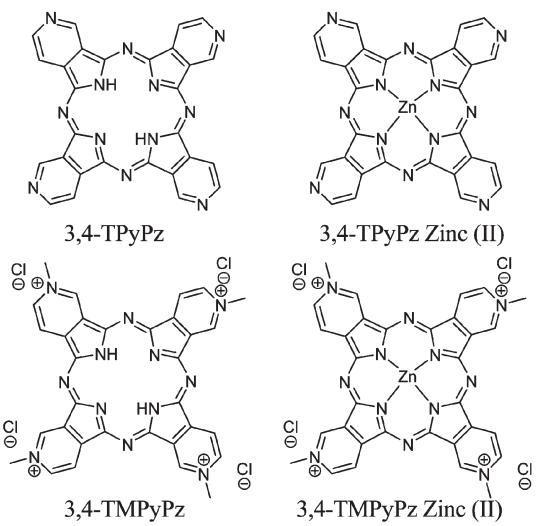

Fig. 1 Chemical structures of the 3,4-TPyPz, 3,4-TPyPz zinc(II), 3,4-TMPyPz and 3,4-TMPyPz zinc(II) porphyrazines.

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Cationic porphyrins, in particular tetramethylpyridiniumporphyrin (TMPyP4), are well known for their ability to bind to different types of G-quadruplexes and, in some cases, to facilitate G-quadruplex formation.[7–11] We now report that tetramethylpyridiniumporphyrazines (TMPyPz) bind strongly to quadruplexes, selectively inducing the antiparallel conformer.

Tetrapyridinoporphyrazines (TPyPz) are non-symmetrical phthalocyanine azo-analogues in which four pyridine moieties substitute the four benzene groups in the macrocycle periphery. They differ from porphyrins by having nitrogen atoms in the *meso* positions linking the individual pyrrole units. The pyridyl groups of the 3,4-TPyPz compounds can readily be methylated to give 3,4-TMPyPz which is water soluble. The syntheses of the 3,4-TPyPz, 3,4-TPyPz zinc(II), 3,4-TMPyPz and 3,4-TMPyPz zinc(II) porphyrazines (Fig. 1) have already been reported,[12,13] but, to the best

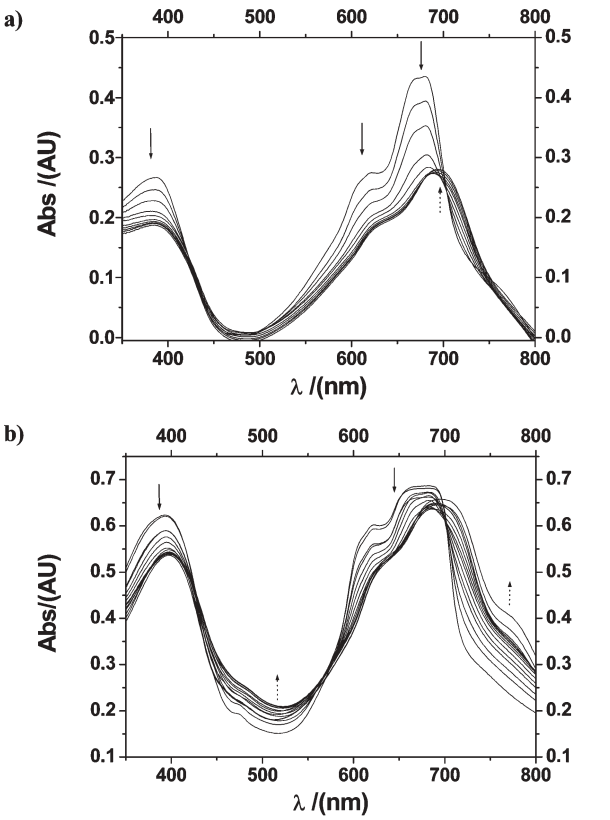

Fig. 2 (a) UV-Vis titration spectra of *Htelo* into a solution of TMPyPz; (b) UV-Vis titration spectra of *Htelo* into a solution of TMPyPz zinc(II). Both experiments were performed at 20 °C and in a 50 mM TRIS-HCl (pH 7.4), 150 mM KCl buffer.

of our knowledge, no TMPyPz-G-quadruplex binding properties have been reported.

The UV-Vis titration spectra of both 3,4-TMPyPz and 3,4-TMPyPz zinc(II) porphyrazines with annealed *Htelo* in a 50 mM TRIS-HCl (pH 7.4), 150 mM KCl buffer (Fig. 2) show a red-shift of the respective Soret bands and a decrease in the hypochromicity; these observations are often taken to indicate a specific mode of binding and strong stacking interactions with G-quadruplex DNA.[14,15]

The UV-Vis titration results were converted into Scatchard plots (*vide in* ESI†) and dissociation constants were determined by linear fitting. 3,4-TMPyPz binds strongly to the *Htelo* G-quadruplex, showing a dissociation constant (K_D) of 0.2 ± 0.02 μM, whilst 3,4-TMPyPz zinc(II) porphyrazine presents weaker binding and a K_D of 1.0 ± 0.7 μM (Table 1). The binding stoichiometry extracted from the Scatchard plot and confirmed by a Job plot (*vide in* ESI†) is 1 : 1 for 3,4-TMPyPz; however we find that the *Htelo* G-quadruplex binds four molecules of 3,4-TMPyPz zinc(II).

Table 1 Dissociation constants (K_D) of 3,4-TMPyPz and 3,4-TMPyPz zinc(II) to quadruplex DNA

	K_D *Htelo*/μM	
Porphyrazines	UV-Vis	SPR
3,4-TMPyPz	0.20 ± 0.02	0.17 ± 0.02
3,4-TMPyPz Zn	1.0 ± 0.7	0.40 ± 0.2

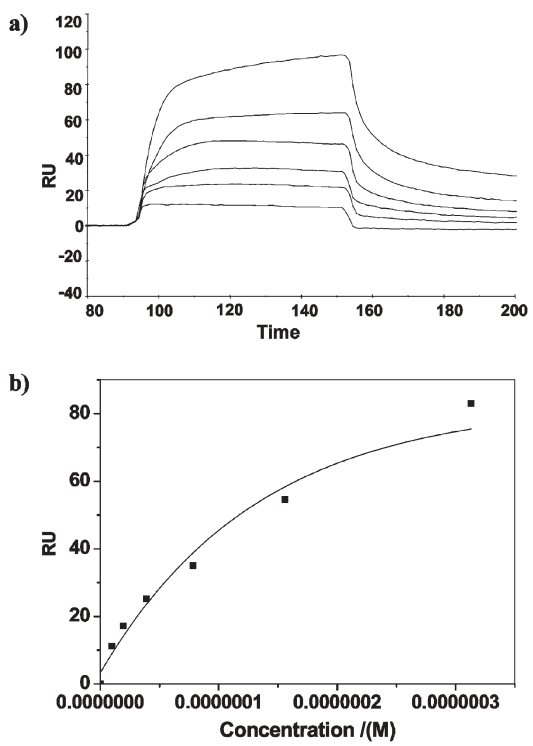

Fig. 3 (a) Sensogram overlay obtained for 6 different concentrations of 3,4-TMPyPz (313, 156, 78, 39, 19, 9.8 nM, top to bottom) binding to the *Htelo* quadruplex; (b) 3,4-TMPyPz binding curve with G-quadruplex obtained using the BIAeval V3.0.2 software (BIAcore AB, Sweden, 1994). The units of RU are seconds. The SPR experiments were carried out in 50 mM TRIS-HCl pH 7.4, 100 mM KCl using a streptavidin functionalised chip on a BIAcore 3000 SPR biosensor, as described previously.[21]

TMPyP4 also shows high binding stoichiometries but, to the best of our knowledge, no structural rationalisation has yet been presented.[16] According to results obtained by Haq *et al.*, using similar experimental conditions, the TMPyP4 porphyrin shows a K_D of 14 μM,[16] which is almost two orders of magnitude weaker than the observed binding for the porphyrazines.

The binding of the two cationic porphyrazines to duplex (5-biotin-[GGCATAGTGCGTGGGCGTTAGC]-3 hybridised with its complementary sequence) and *Htelo* G-quadruplex (5-biotin-[GTTA(GGGTTA)$_4$GG]-3) was also investigated using surface plasmon resonance (SPR). An example of a SPR sensogram is shown in Fig. 3.

The dissociation constants obtained from the SPR experiments with the *Htelo* quadruplex are similar to those obtained by UV-vis spectroscopy (Table 1). The 3,4-TMPyPz shows a K_D of 0.17 ± 0.02 μM, while the 3,4-TMPyPz zinc(II) presents a K_D of 0.40 ± 0.2 μM. The porphyrazines do not show any significant binding affinities against duplex DNA at a concentration up to 5 μM, which represents a lower limit of 30 for the binding specificity for quadruplex over duplex.‡ This is a very important improvement in relation to TMPyP4, which does not present any quadruplex/duplex selectivity.

The stronger binding of the 3,4-TMPyPz, relative to TMPyP4, with intramolecular G-quadruplexes, may be attributed to the more extended porphyrazine π-system which might completely overlap the four guanines of the G-tetrad.

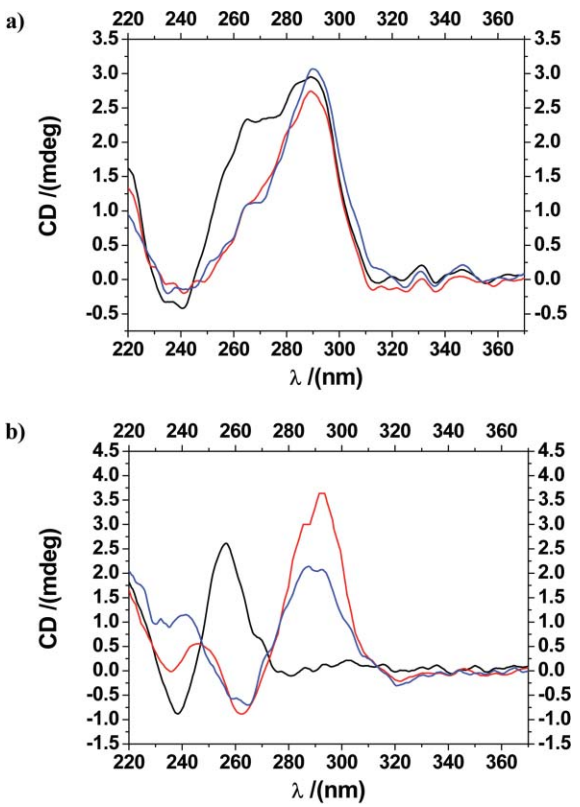

Fig. 4 (a) CD spectra of 10 μM of annealed *Htelo* DNA in a 50 mM TRIS (pH 7.4), 150 mM KCl buffer (black), in the presence of 12 equiv. of 3,4-TMPyPz (red) and of 12 equiv. of 3,4-TMPyPz zinc(II) (blue), at 20 °C. (b) CD spectra of 10 μM of non-annealed *Htelo* DNA in a 50 mM TRIS-HCl (pH 7.4) buffer (black), in the presence of 9 equiv. of 3,4-TMPyPz (red) and of 9 equiv. of 3,4-TMPyPz zinc(II) (blue), at 20 °C.

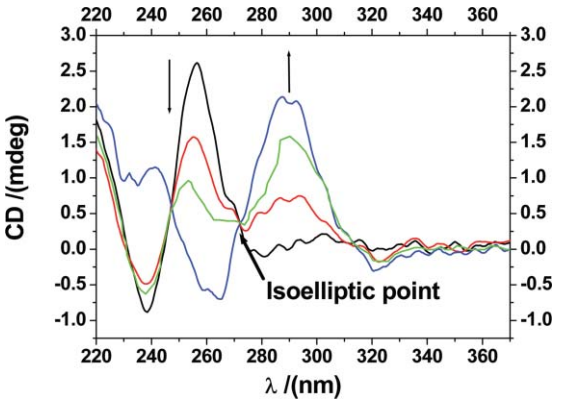

Fig. 5 CD spectra of 10 μM of non-annealed *Htelo* in a K^+-free buffer (black), in the presence of 1.2 equiv. (red), 5 equiv. (green) and 9 equiv. (blue) of the 3,4-TMPyPz zinc(II) porphyrazine.

The presence of K^+ ions induces and stabilises the parallel and antiparallel conformations of the human telomeric G-quadruplex.[17–20] CD spectroscopic titrations on 10 μM of annealed DNA indicate that both 3,4-TMPyPz and 3,4-TMPyPz zinc(II) porphyrazines preferentially induce the formation of the antiparallel G-quadruplex conformation (Fig. 4a); the characteristic antiparallel positive peak at around 295 nm is amplified,[17] while the peak at 265 nm, characteristic of the parallel conformation, is suppressed. The binding of both porphyrazines to the G-quadruplex also increases its thermal stability (*vide in* ESI†).

This strong binding and selectivity raised the possibility that the porphyrazines could induce the formation of a G-quadruplex from non-annealed *Htelo* DNA in a K^+-free buffer. Indeed, we found that the addition of 3,4-TMPyPz or 3,4-TMPyPz zinc(II) induces the development of a positive peak near 295 nm (antiparallel conformation), at the expense of the 255 nm peak (Fig. 4b) and that this appears to be a fast transition (less than min). A clear isoelliptic point at 272 nm (Fig. 5) suggests a clean transition to the antiparallel quadruplex upon addition of the porphyrazines.

It has been demonstrated that 3,4-TMPyPz binds strongly to the G-quadruplex in a 1 : 1 ratio, whilst the 3,4-TMPyPz zinc(II) shows weaker binding and different stoichiometry, probably due to aggregation. As these porphyrazines can be prepared in good yields,[12,13] bind strongly to G-quadruplex DNA and are able to recognise and induce a particular G-quadruplex conformation, they are promising candidates currently being investigated in quadruplex-related chemical binding studies.

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Notes and references

‡ We were unable to assess any binding experiments for concentrations above 5 μM, since this ligand shows non-specific binding with duplex DNA at higher concentrations.

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