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Assessment of presumed small-molecule ligands of telomeric i-DNA by BioLayer Interferometry (BLI)

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Abstract: Biolayer Interferometry (BLI) and circular dichroism (CD) spectroscopies were used to investigate the interaction between previously reported i-motif DNA (i-DNA) ligands and folded or unfolded i-DNA in acidic (pH 5.5) and near-neutral (pH 6.5) conditions. We observed that although several ligands, in particular macrocyclic bis-acridine (BisA) and pyridostatin (PDS), showed good affinities for the telomeric i-motif forming sequence, none of the ligands displayed selective interactions with i-DNA structure nor was able to promote its formation.

i-Motifs of DNA (hereafter, i-DNA), known *in vitro* for nearly three decades,¹ are unusual four-stranded structures, in which cytosines are intercalated *via* a stack of hemi-protonated C–C base pairs (CH⁺:C).² Some of these DNA structures have been well characterized *in vitro* and, because i-DNA may mirror other four-stranded G-rich structures (G-quadruplexes, or G4) present in gene promoters or at telomeres, their biological relevance has been investigated.³ Several studies indicated that i-DNA formation modulates transcription,⁴ and a recent study using a specific antibody (iMab) revealed their presence in the nuclei of human cells, co-localizing with either telomeres or transcription factors.⁵ More recently, Trantirek and coll. also demonstrated, using in-cell NMR, that exogenous i-DNA are stable in the nuclei of human cells.⁶

However, our knowledge about i-DNA biology is still limited, in part due to the lack of suitable small-molecule probes to interrogate the biological roles of these structures. For example, most of our knowledge on the impact of four-stranded DNA secondary structures, such as G4, on cell metabolism resulted from the use of selective G4 ligands and the characterization of proteins that bind or modulate the formation of such structures.^{7,8} In contrast, relatively few molecules were reported to interact with i-DNA.⁹ The main issue in this regard are the strong pH-dependency, flexibility, polymorphism and complex folding behavior of i-DNA, that

introduce potential bias into screening methods. In particular, low-pH conditions used to induce the formation of i-DNA lead to the protonation of many ligands, that can strongly increase their non-specific nucleic acid binding. This latter point is particularly critical because the use of small molecules to study the biological functions of such structures is essential. To date, the following molecules including TMPyP4,¹⁰ mitoxantrone,¹¹ IMC-48,¹² [Ru(phen)₂dppz]²⁺,¹³ berberine,¹⁴ and others such as PhenDC3, BRACO-19 and PDS have been reported as putative i-DNA ligands. However, a strong controversy persists with regard to their binding mode, affinity, and selectivity for i-DNA with respect to duplex or G4-DNA forms.^{15,16} The identification and evaluation of i-DNA ligands are hampered by the lack of robust, uniform assays such as FRET-melting and FID assay that became routine in the field of G4 ligand. In fact, these methods have strong biases and provide untrustworthy results with i-DNA, as evidenced in recent publications,^{17,18} prohibiting direct comparison of the ligands described by different groups.

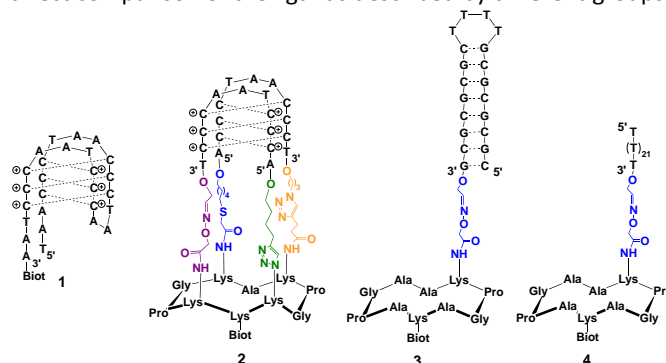


Fig. 1 Structure of the native telomeric i-motif h-telo **1**, i-motif forming DNA-peptide conjugate **2**, hairpin control **3** and single-stranded control **4**.

In this context, we have previously assembled two peptide-DNA conjugates that form i-motif structures,^{19,20} with one of them, namely conjugate **2** (Fig. 1), able to fold into a

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stable i-motif at room temperature and, most importantly, at near-neutral pH.¹⁹ This stabilized mimic of the i-motif adopted by the h-telo sequence may be used to study the interaction of the i-motif with putative ligands at a more physiologically relevant pH. As a proof of concept, we report herein a comparative biolayer interferometry (BLI) study of the interaction of several of the most promising i-DNA ligands reported to date, with the native i-motif structure of telomeric DNA (h-telo **1** in Fig.1) along with the stabilized DNA-peptide conjugate **2**. BLI has recently emerged as a powerful optical technique for isothermal characterization and quantification of interactions between molecules, in particular because it does not require any microfluidics set-up and enables real-time and label-free characterization of the interactions with the determination of affinity and selectivity in a 96-well microplate format. It has already been exploited to study the interactions of ligands with G-quadruplex DNA.²¹⁻²³

The interactions with putative ligands were investigated at two distinct pH, an acidic pH of 5.5 at which both h-telo **1** and mimic **2** are folded into an i-motif structure as confirmed by CD analysis and a near-neutral pH of 6.5 under which the native telomeric sequence does not form an i-motif unlike the stabilized i-motif **2** (Fig. S20 in ESI).²⁴ Hairpin (HP) **3** and single-stranded DNA **4** were also used in this study as controls. To perform BLI analysis, biomolecular systems **1-4** were anchored on the BLI sensors through biotin-streptavidin interactions. Nine ligands (Fig. 2) including the aforementioned state-of-the-art i-motif binders and previously reported i-DNA interactors were investigated for their interaction with the DNA structures **1-4**. The acquired sensorgrams allowed for the determination of the equilibrium dissociation constants of the interaction from the responses obtained at the steady state ($\Delta\lambda$) based on a 1:1 interaction model and after fitting of the Langmuir isotherm (Table 1 and Fig. S2-S19 in ESI).

Previous studies, performed with native, non-stabilized, i-motif h-telo sequence **1** were mostly done at the acidic pH of 5.5 to ensure the formation of the i-motif. Our current data for h-telo **1** at pH 5.5 are in part concordant with these previous reports. Specifically, for mitoxantrone, we found a K_D value of 11 μM which agrees with the previously determined affinity using SPR measurements.¹¹ Likewise, PhenDC3 showed a K_D value around 2 μM which is consistent with the value of 4 μM obtained with related phenanthroline compounds using fluorometric titrations.²⁵ For TMPyP4, a previous report suggested 1 μM affinity for the human telomeric i-motif at pH 5.2, that is quite close to the K_D value of 4.8 μM observed in the present study.²⁶ It should be also noted that the techniques used for the determination of affinity (UV and CD titrations) differ from our BLI method. For berberine, a K_D value of 19.6 μM was previously reported by using fluorescence titration, while no significant signal variation was measured by BLI and the Langmuir isotherm did not converge,²⁷ thus confirming the very weak affinity of berberine for i-DNA. We were unable to measure a K_D value for $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ (racemic mixture) whereas an interaction of this complex with i-DNA was reported in the literature by using UV melting and luminescence.¹³

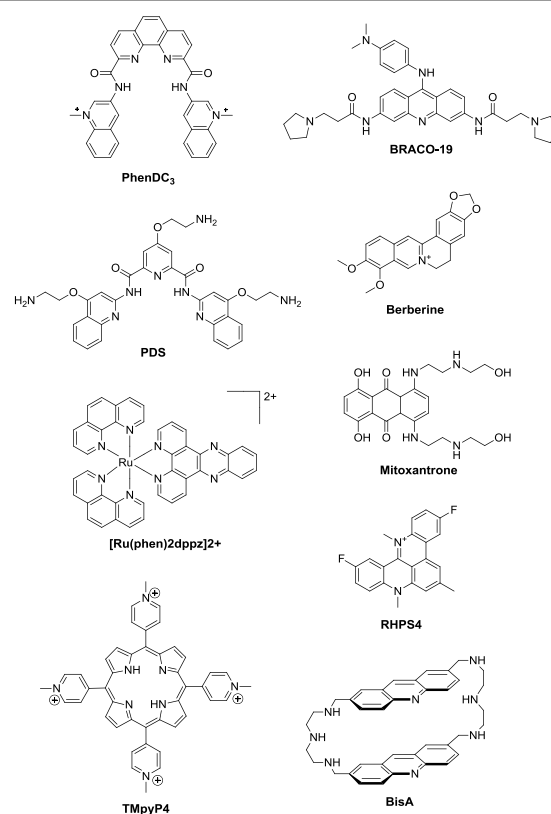


Fig. 2 Structures of the ligands investigated in the study.

However, the i-DNA sequences used for this study were not the same and the authors concluded that $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ did not stabilize short-looped sequences but were able to stabilize the relatively less stable, long-looped i-motifs. Therefore, our results further confirm the null to weak affinity of $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ for short-looped i-motif forming sequences. For Braco-19, a well-known G4 ligand, the relatively low affinity observed (4.6 μM) associated with poor selectivity against control hairpin would preclude the use of such molecule as an i-motif binder. The same conclusion can be reached for RHPS4 (also a known G4 binder) for which no binding could be determined in the range of used concentrations. Finally, BisA and PDS appeared to be the best ligands for i-DNA **1** with K_D values of 60 nM and 120 nM, respectively.

Promisingly, we found virtually no difference (within three-fold) between the affinities of the studied ligands for the native i-DNA **1** and the constrained i-DNA **2** at pH 5.5 which appears to confirm the structural similarity of both i-DNA in these acidic conditions and the absence of any detrimental effect of the cyclic peptide scaffold on the recognition properties as already reported.²⁸ Encouragingly, the affinities measured with **2** at pH 6.5 were found to be very similar to the ones measured at pH 5.5 for both i-DNA **1** and **2**. This indicates that constrained i-DNA **2** is also a potent mimic of i-motif DNA at higher pH. Surprisingly, by using **1** at pH 6.5, *i.e.*, pH under which the sequence is not expected to fold into an i-motif, we also observed affinities similar to those obtained at pH 5.5 as

well as for the interaction of the ligands with constrained i-DNA **2**.

Table 1: Thermodynamic equilibrium constant, K_D , (in μM) of the interaction of the ligands with the DNA sequences at pH 5.5 and pH 6.5. **n.d.*: non determined as K_D values determined by the fitting of the Langmuir equation were superior to the acceptable value in the studied concentration range.

Ligand	H-telo i-DNA 1		Constrained i-DNA 2		HP control 3		SS control 4	
	pH 5.5	pH 6.5	pH 5.5	pH 6.5	pH 5.5	pH 6.5	pH 5.5	pH 6.5
PhenDC3	2 ± 0.3	6 ± 0.2	3.5 ± 1	6 ± 0.4	6.8 ± 0.9	4.5 ± 0.9	2 ± 0.5	2.3 ± 0.6
PDS	0.12 ± 0.04	0.32 ± 0.02	0.26 ± 0.04	0.36 ± 0.06	1.2 ± 0.2	1.3 ± 0.1	0.12 ± 0.03	0.10 ± 0.03
TMPyP4	4.8 ± 0.6	8.7 ± 2	14 ± 2	10.7 ± 3	4.1 ± 1.6	8.8 ± 3	3.4 ± 0.3	9.8 ± 2
Braco-19	4.6 ± 0.7	11 ± 3	9.5 ± 2	8.5 ± 2	15 ± 3	18.2 ± 4	4.1 ± 0.9	4.6 ± 0.9
Berberine	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	44 ± 12	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>
[Ru(Phen) ₂ dppz] ²⁺	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	37 ± 16	54 ± 10	9.6 ± 1	18 ± 2
RHPS4	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>
BisA	0.06 ± 0.01	0.22 ± 0.06	0.13 ± 0.06	0.29 ± 0.1	2.1 ± 0.2	8.45 ± 3	0.21 ± 0.04	0.35 ± 0.08
mitoxantrone	11 ± 2	12 ± 0.9	19 ± 3	13 ± 4	72 ± 17	87 ± 21	10 ± 2	11 ± 2

Similar affinities for the interaction of the ligands with presumably folded (pH 5.5) and unfolded (pH 6.5) structures could be interpreted in multiple ways: *i*) i-DNA **1** might be partially or fully unfolded by the ligands at pH 5.5 and the resulting structure would compare to the one obtained at pH 6.5; *ii*) conversely, the interaction of the ligands with the unfolded i-DNA **1** at pH 6.5 may trigger folding into an i-motif structure such as observed at pH 5.5 and present in i-DNA **2**; *iii*) the measured affinities do not depend on the presence of folded i-motif structure but merely reflects interactions with the cytosine-containing sequences or *iv*) the interactions are due to non-specific interactions with DNA backbone.

To further investigate the effect of the ligands, we used CD analysis to gauge the folding status of i-DNA **1** in presence of each ligand (5 molar equivalents)²⁹ at both pH 5.5 and 6.5. At pH 5.5 (Fig. S21 in ESI) we observed that all the compounds induced a hypochromic effect of the band at 288 nm that was particularly marked in the case of Braco-19, PDS, PhenDC3 and Mitoxantrone. This agrees with the observations of Randazzo and *coll.* and could suggest that the interaction with some ligands induces partial unfolding of the i-motif structure.³⁰ Nevertheless, the i-motif appears to remain the preponderant structure in presence of the ligands at pH 5.5 ruling out the first hypothesis. Inversely, the CD spectra obtained at pH 6.5 (Fig. S22 in ESI) demonstrate that no ligand could efficiently promote the folding of the unstructured i-DNA **1** sequence into a well-defined i-motif structure, as the spectra remain unchanged after addition of the ligands, ruling out the second hypothesis. Finally, we recorded CD spectra at intermediate conditions (pH 6.2, *i.e.*, close to p*H*_T) at which i-DNA **1** was found in an equilibrium between folded and unfolded forms, hypothesizing that in these case even small effects of the ligands would manifest by shifting the equilibrium towards either i-motif or single-stranded forms (Fig. S23 in ESI). In these experiments, the ratio of CD signals at 285 nm (maximum of i-motif form) and 275 nm (maximum of the single-stranded form), was used as a metric of ligand-induced effect. The results (Fig. 3) showed that, among the tested compounds, PDS and Braco-19 (at 2 or 5 molar equiv.), BisA (at 5 molar equiv.) as well as PhenDC3 (at 2 molar equiv. only) seemingly increased the $\theta_{285\text{ nm}}/\theta_{275\text{ nm}}$ ratio suggesting an induction of i-motif form. However, a close inspection of CD spectra (Fig. S23 in ESI) revealed that in all these cases,

addition of the ligand reduced the intensity of the signal at 275 nm without concomitant increase of the i-motif peak.

In all other cases, the addition of ligands clearly shifted the equilibrium towards the single-stranded form (Fig. 3 and S23), which might suggest that those ligands may bind to single-stranded DNA. To further explore these hypotheses, we performed BLI analysis of the interactions between single strand poly T control **4** (Fig. 1) and all ligands at both pH 5.5 and 6.5 (Table 1). Surprisingly, at both pH, K_D values similar to the ones obtained with i-motif forming DNA **1** and **2** were measured for most of the ligands further suggesting strong interaction of the ligands with the single-stranded oligonucleotide. Thus, the binding constants observed with the substrates **1** and **2** insinuate the absence of specific recognition of i-motif structural features. Interestingly, the inspection of CD spectra recorded in equilibrium conditions (pH 6.2, Fig. S23) revealed the presence of induced CD (iCD) bands in the region of ligand absorption in the case of PhenDC3, [Ru(phen)₂dppz]²⁺ and Braco-19. As iCD is typically considered as a solid evidence of ligand–DNA interaction, we additionally recorded iCD spectra of these ligands at pH 5.5 and 7.3, in order to elucidate whether these signals arise from binding of ligands to i-DNA or unfolded DNA, respectively (Fig. S24). In the case of PhenDC3, iCD signal observed at pH 6.2 matched the one observed at pH 7.3, giving evidence of binding to unfolded h-telo. Conversely, in the case of Braco-19, iCD signal observed at pH 6.2 was similar the one observed at pH 5.5, speaking in favor of interaction of this ligand with folded i-DNA. Finally, in the case [Ru(phen)₂dppz]²⁺, no iCD was observed either at pH 5.5 or at pH 7.3, giving evidence of a complex binding behavior.

In summary, we investigated the affinities of nine state-of-the-art small-molecule binders of unusual nucleic acid structures, previously reported as i-motif targeting agents. BLI analysis performed with folded (at pH 5.5 and 6.5) and unfolded (at pH 6.5) i-motif derived from the human telomeric sequence uncovered massive binding promiscuity. Indeed, even though some of the ligands (BisA, PDS) demonstrated good affinities for the i-motif forming C-rich sequence and selectivity over hairpin DNA, none of the ligands were shown to discriminate between folded and unfolded i-motif structure,

nor shift the equilibrium towards the folded i-motif as confirmed by CD.

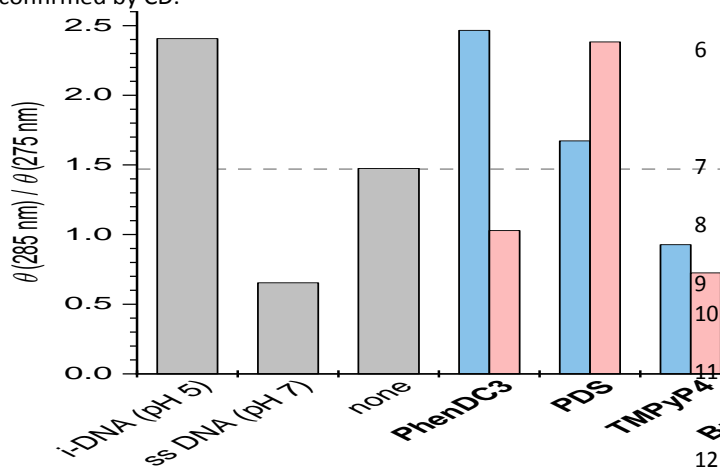


Fig. 3 Ratio of CD intensity at 285 nm (i-DNA) and 275 nm (single-stranded form) of solutions of h-telo DNA **1** at pH 6.2 in the absence and in the presence of 2 (blue bars) and 5 (pink bars) molar equiv. of ligands.

Importantly most of the studied ligands showed comparable affinity to single-strand DNA not able of forming i-motifs. Even though the analysis was not performed at physiological pH under which ligands physical properties may vary, this result still further shed light on the discrepancy that surrounds the endeavor to identify specific i-motif ligands. In this context, the use of constrained i-DNA that ensures an i-motif folding represents an interesting alternative to identify unambiguous (*i.e.*, affine and specific) i-DNA-interacting ligands.

Author Contributions

H.B., M.M, A.D., J.B.: investigation; A.G., B.E.: resources, writing, review & editing; T.L: supervision, writing, review & editing; J.D.: Formal analysis, supervision, writing, review & editing and E.D.: supervision, funding acquisition, writing, review & editing.

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Notes and references

- 1 K. Gehring, J.-L. Mergny, M. Guéron, *Nature*, 1993, **363**, 561.
- 2 J.-L. Mergny, D. Sen, *Chem. Rev.*, 2019, **119**, 6290.
- 3 H. Abou Assi, M. Garavis, M. J. Damha, *Nucleic Acids Res.*, 2018, **46**, 8038.
- 4 S. Kendrick, H.-J. Kang, M. P. Alam, M. M. Madathil, P. Agrawal, V. Gokhale, D. Yang, S. M. Hecht, L. H. Hurley, *J. Am. Chem. Soc.*, 2014, **136**, 4161.

- 5 M. Zeraati, D. B. Langley, P. Schofield, A. L. Moye, R. Rouet, W. E. Hughes, T. M. Bryan, M. E. Dinger, D. Christ, *Nat. Chem.*, 2018, **10**, 631.
- 6 S. Dzatko, M. Krafcikova, R. Hansel-Hertsch, T. Fessi, R. Fiala, T. Loja, D. Krafcik, J.-L. Mergny, S. Foldynova-Trantirkova, L. Trantirek, *Angew. Chem. Int. Ed. Engl.*, 2018, **57**, 2165.
- 7 J. Carvalho, J.-L. Mergny, G.-F. Salgado, J.-A. Queiroz, C. Cruz, *Trends Mol. Med.*, 2020, **26**, 848.
- 8 R. Hansel-Hertsch, M. Di Antonio, S. Balasubramanian, *Nat. Rev. Mol. Cell. Biol.*, 2017, **18**, 279.
- 9 S. L. Brown, S. Kendrick, *Pharmaceuticals*, 2021, **14**, 96.
- 10 L. Martino, B. Pagano, I. Fotticchia, S. Neidle, C. Giancola, *J. Phys. Chem. B*, 2009, **113**, 14779.
- 11 E. P. Wright, H. A. Day, A. M. Ibrahim, J. Kumar, L. J. E. Boswell, C. Fuguin, S. M. Stevenson, K. Peters, Z. A. E. Waller, *Scientific Reports*, 2016, **6**, 39456.
- 12 H.-J. Kang, S. Kendrick, S. M. Hecht, L. H. Hurley, *J. Am. Chem. Soc.*, 2014, **136**, 4172.
- 13 B. J. Pages, S. P. Gurung, K. McQuaid, J. P. Hall, C. J. Cardin, J. A. Brazier, *Front. Chem.*, 2019, **7**, 744.
- 14 L. Xu, S. Hong, N. Sun, K. Wang, L. Zhou, L. Jia, R. Pei, *Chem. Commun.*, 2016, **52**, 179.
- 15 H.A. Day, P. Pavlos, Z. A. E. Waller, *Bioorg. Med. Chem.*, 2014, **22**, 4407.
- 16 S. S. Masoud, K. Nagasawa, *Chem. Pharm. Bull.*, 2018, **66**, 1091.
- 17 V. B. Tsvetkov, A. V. Turaev, N. A. Petrunina, D. M. Melnik, Y. M. Khodarovich, G. E. Pozmogova, T. S. Zatsepin, A. M. Varizhuk, A. V. Aralov, *Analyst*, 2021, **146**, 4436.
- 18 P. Alberti, J. Ren, M.-P. Teulade-Fichou, L. Guittat, J.-F. Riou, J. B. Chaires, C. Helene, J.-P. Vigneron, J.-M. Lehn, J.-L. Mergny, *J. Biomol. Struct. Dyn.* 2001, **19**, 505.
- 19 A. Devaux, L. Bonnat, T. Laverigne, E. Defrancq, *Org. Biomol. Chem.*, 2020, **18**, 6394.
- 20 R. Bonnet, P. Murat, N. Spinelli, E. Defrancq, *Chem. Commun.*, 2012, **48**, 5992.
- 21 L. Bonnat, M. Dautriche, T. Saidi, J. Revol-Cavalier, J. Dejeu, E. Defrancq, T. Laverigne, *Org. Biomol. Chem.*, 2019, **17**, 8726.
- 22 M. Gillard, J. Weynand, H. Bonnet, F. Loiseau, A. Decottignies, J. Dejeu, E. Defrancq, B. Elias, *Chem. Eur. J.*, 2020, **26**, 13849.
- 23 J. Weynand, H. Bonnet, F. Loiseau, J.-L. Ravanat, J. Dejeu, E. Defrancq, B. Elias, *Chem. Eur. J.*, 2019, **25**, 12730.
- 24 The positive band observed at 288 nm and the negative at 260 nm are characteristic of an i-motif folding topology: K. Guo, V. Gokhale, L. H. Hurley, D. Sun, *Nucleic Acids Res.* 2008, **36**, 4598.
- 25 L. Wang, Y. Wu, T. Chen, C. Wei, *Int. J. Biol. Macromol.* 2013, **52**, 1.
- 26 S. Fernandez, R. Eritja, A. Avino, J. Jaumot, R. Gargallo, *Int. J. Biol. Macromol.*, 2011, **49**, 729.
- 27 R. Gargallo, A. Avino, R. Eritja, P. Jarosova, S. Mazzini, L. Scaglioni, P. Taborsky, *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.*, 2021, **248**, 119185.
- 28 P. Murat, R. Bonnet, A. Van der Heyden, N. Spinelli, P. Labbe, D. Monchaud, M.-P. Teulade-Fichou, P. Dumy, E. Defrancq, *Chem. Eur. J.* 2010, **16**, 6106.
- 29 Due to the absence of measured affinity in the BLI analysis, berberine, [Ru(Phen)₂dppz]²⁺ and RHPS4 ligands were not studied by CD.

- 30 A. Pagano, N. Iaccarino, M. A. S. Abdelhamid, D. Brancaccio, E. U. Garzarella, A. Di Porzio, E. Novellino, Z. A. E. Waller, B. Pagano, J. Amato, A. Randazzo, *Front. Chem.*, 2018, **6**, 1.