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Biologically active $[Pd_2L_4]^{4+}$ quadruply-stranded helicates: stability and cytotoxicity†

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There is emerging interest in the anti-proliferative effects of metallosupramolecular systems due to the different size and shape of these metallo-architectures compared to traditional small molecule drugs. Palladium(II)-containing systems are the most abundant class of metallosupramolecular complexes, yet their biological activity has hardly been examined. Here a small series of $[Pd_2(L)_4](BF_4)_4$ quadruply-stranded, dipalladium(II) architectures were screened for their cytotoxic effects against three cancer cell lines and one non-malignant line. The helicates exhibited a range of cytotoxic properties, with the most cytotoxic complex $[Pd_2(\mathbf{hextrz})_4](BF_4)_4$ possessing low micromolar IC_{50} values against all of the cell lines tested, while the other helicates displayed moderate or no cytotoxicity. Against the MDA-MB-231 cell line, which is resistant to platinum-based drugs, $[Pd_2(\mathbf{hextrz})_4](BF_4)_4$ was 7-fold more active than cisplatin. Preliminary mechanistic studies indicate that the $[Pd_2(\mathbf{hextrz})_4](BF_4)_4$ helicate does not induce cell death in the same way as clinically used metal complexes such as cisplatin. Rather than interacting with DNA, the helicate appears to disrupt the cell membrane. These studies represent the first biological characterisation of quadruply-stranded helicate architectures, and provide insight into the design requirements for the development of biologically active and stable palladium(II)-containing metallosupramolecular architectures.

Introduction

Spurred on by the success of small molecular inorganic drugs[1] such as cisplatin[2] (*cis*-$[Pt(NH_3)_2Cl_2]$, CDDP)[3] there has been an upsurge of interest in the biological properties[4] of metallosupramolecular architectures.[5] This is driven by the hope that the different size and shape of these metallosupramolecular systems, compared to small molecule drugs, will lead to cytotoxic agents with novel biological mechanisms of action. The biological properties of a range of self-assembled architectures including metallo-macrocycles,[6] cages/prisms[7] and helicates have been examined. It is the metallo-helicates,[8] however, that have received particular attention. Building on early work from Lehn and co-workers,[9] which had shown that cationic double-helical copper(I) complexes interact strongly with DNA, Hannon and co-workers carried out a series of pioneering studies on the biological properties of the triply-stranded $[Fe_2L_3]^{4+}$ (L = *N*,*N*′-(methanediyldibenzene-4,1-diyl)bis[1-(pyridin-2-yl)methanimine]) helicate.[10] This tetracationic cylinder interacts strongly with duplex DNA, binding in the major groove,[11] but interestingly it can display alternative binding modes including non-covalent complexation at the center of three-way (Y-shaped) DNA[12] and RNA[13] junctions. The binding of $[Fe_2L_3]^{4+}$ to DNA has also been shown to induce coiling of the nucleic acid structure.[14] Additionally, Hannon and co-workers found that these tetracationic iron(II) cylinders and related ruthenium(II) systems display both anti-cancer[15] and anti-bacterial[16] properties. The biological properties of other related triply-stranded helicate systems have also been examined. These molecules have been shown to bind G-quadruplex DNA,[17] inhibit Aβ amyloid aggregation[18] and also display cytotoxic (anti-cancer[19] and anti-bacterial[19b]) properties. While it is clear from these studies that cationic helicate architectures can strongly interact with DNA, a recent report from Scott and co-workers[20] has suggested that DNA binding is not always responsible for the observed biological activity.

Palladium(II) containing systems[21] represent the most common class of metallosupramolecular architectures. However, despite considerable interest in small palladium(II)

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†Electronic supplementary information (ESI) available: The ESI contains the experimental procedures, ^1H, ^{13}C and DOSY NMR, ESMS, and crystallographic data. CCDC 1045884–1045889. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c5dt01259g

complexes,[22] the biological properties of palladium-containing metallosupramolecular architectures have hardly been examined.[6i,23]

Herein, as part of our interests in metallosupramolecular architectures[24] and the biological applications of metal complexes,[25] we screened four quadruply-stranded, dipalladium(II) architectures for their antiproliferative effects against three different cancer cell lines, and compared this activity against non-malignant cells. It is shown that the most stable dipalladium(II) helicate displays low micromolar IC_{50} values against all the tested cell lines. Furthermore, mechanistic studies indicate that the helicate does not cause cell death by interacting with DNA, rather it seems to disrupt the cell membrane. To the best of our knowledge this is the first reported cytotoxic study of quadruply-stranded helicate architectures.

Results and discussion

Synthesis and stability

The ligands (**tripy**,[27] **bntrz**[24g] and **hextrz**[24e]) and complexes ($[Pd_2(\mathbf{tripy})_4](BF_4)_4$,[26] $[Pd_2(\mathbf{bntrz})_4](BF_4)_4$,[24h] $[Pd_2(\mathbf{hextrz})_4](BF_4)_4$[24e]) were generated using previously reported procedures (Fig. 1 and ESI†). The new bistriazole ligand (**pegtrz**) and its corresponding dipalladium helicate $[Pd_2(\mathbf{pegtrz})_4](BF_4)_4$ were synthesised using the conditions[24e] previously exploited to generate the **hextrz** ligand and helicate (Fig. 1 and ESI†).

Before carrying out cytotoxicity measurements the stability of the $[Pd_2(\mathbf{L})_4](BF_4)_4$ architectures in the presence of common biological nucleophiles (chloride (Cl^-), histidine and cysteine) was determined using ^{1}H NMR competition experiments. Solutions (d_6-DMSO/D_2O, 3 : 2, 2.5 mM) of the $[Pd_2(\mathbf{L})_4](BF_4)_4$ architectures were treated with 8 equivalents of one of the nucleophiles and the course of the reaction monitored, using time-course ^{1}H NMR spectroscopy, for a period of 20 hours (Table 1 and ESI†). Under these conditions the $[Pd_2(\mathbf{tripy})_4](BF_4)_4$ cage rapidly decomposed. The half-life for the decomposition of the palladium(II)-tripyridyl complex with histidine was 5 minutes. The half-lives for the corresponding reactions with the chloride (Cl^-) and cysteine nucleophiles were less than one minute. This rapid decomposition behaviour is consistent with previous experiments using chloride and 4-dimethylaminopyridine in acetonitrile (CH_3CN) solutions.[26]

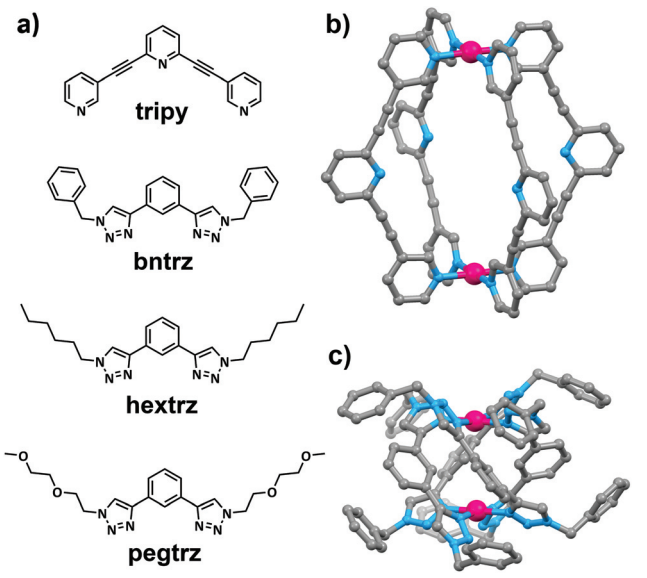

Fig. 1 (a) Chemical structures of the pyridine- (**tripy**) and triazole- (**bntrz**, **hextrz** and **pegtrz**) based ligands; the molecular structures of (b) $[Pd_2(\mathbf{tripy})_4](BF_4)_4$ [26] and (c) $[Pd_2(\mathbf{bntrz})_4](BF_4)_4$ [24h] architectures. Anions, solvent molecules and hydrogen atoms have been omitted for clarity.

Table 1 Half-lives ($t_{1/2}$) for the decomposition of the Pd_2L_4 architectures against 8 equivalents of selected biologically relevant nucleophiles (3 : 2 d_6-DMSO/D_2O, 298 K, 500 MHz)

	Nucleophile			
$[Pd_2(\mathbf{L})_4](BF_4)_4$ architecture	Chloride	Histidine	Cysteine	^{13}C δ^a
$[Pd_2(\mathbf{tripy})_4](BF_4)_4$	<1 min	5 min	<1 min	158.7
$[Pd_2(\mathbf{bntrz})_4](BF_4)_4$	>20 h	12 h	30 min	161.3
$[Pd_2(\mathbf{hextrz})_4](BF_4)_4$	>20 h	>20 h	>20 h	161.4
$[Pd_2(\mathbf{pegtrz})_4](BF_4)_4$	>20 h	12 h	20 min	161.4

[a] Chemical shift of Pd(II) probe complex carbene carbon (ESI).

Compared to the $[Pd_2(\mathbf{tripy})_4](BF_4)_4$ system the triazole-based helicates $[Pd_2(\mathbf{bntrz})_4](BF_4)_4$, $[Pd_2(\mathbf{hextrz})_4](BF_4)_4$ and $[Pd_2(\mathbf{pegtrz})_4](BF_4)_4$ were all considerably more stable. All of the triazole-based helicates displayed good stability against chloride (Cl^-) nucleophiles, with the half-lives for the decomposition reactions all over 20 hours. The $[Pd_2(\mathbf{bntrz})_4](BF_4)_4$ and $[Pd_2(\mathbf{pegtrz})_4](BF_4)_4$ helicates were less stable in the presence of histidine and cysteine. The half-lives for the helicate decomposition reactions with histidine were both approximately 12 hours, while a much more rapid disassembly of the $[Pd_2(\mathbf{bntrz})_4](BF_4)_4$ and $[Pd_2(\mathbf{pegtrz})_4](BF_4)_4$ helicates occurred with the sulphur-containing cysteine nucleophile ($t_{1/2}$ = 30 and 20 minutes, respectively). Interestingly, the $[Pd_2(\mathbf{hextrz})_4](BF_4)_4$ helicate was long-lived in the presence of all the nucleophiles tested ($t_{1/2}$ = >20 hours for all the nucleophiles). No cage decomposition was observed in the presence of chloride (Cl^-), and only 13% of the $[Pd_2(\mathbf{hextrz})_4](BF_4)_4$ cage was broken down when exposed either to histidine or cysteine.

It appears that both electronic and steric factors are responsible for the observed differences in the stability of the $[Pd_2(\mathbf{L})_4](BF_4)_4$ architectures. The electron donor strength of the pyridyl and triazolyl ligands was determined using the palladium(II) probe complexes reported by Huynh and co-workers (ESI†).[28] The ^{13}C carbene carbon chemical shift of these probe complexes suggested that the triazolyl ligands are all more electron-rich and therefore "better" ligands than the pyridyl system (Table 1 and ESI†). The observed higher ligand donor strength of the triazole ligands relative to the pyridyl system undoubtedly contributes to the greater stability of the triazole based helicates relative to the pyridyl cage. Inspection of the

molecular structures (Fig. 1) of the $[Pd_2(\mathbf{L})_4](BF_4)_4$ architectures indicates that steric factors also potentially help stabilize the triazole-based helicates. The $[Pd_2(\mathbf{tripy})_4](BF_4)_4$ architecture adopts a lantern shape that contains an open/accessible internal cavity. This allows the nucleophiles access to the square planar palladium(II) ions of the cage either from the "outside" face of the cage or through the central cavity of the architecture. Conversely, the helical twist of the triazole-based architectures means that there is no open central cavity in these systems. As such the nucleophiles can only attack the palladium(II) ions of the helicates from the "outside" face of the architectures. This steric effect in combination with the greater ligand donor strength of the triazole ligands leads to the higher stability of the helicates relative to the tripyridyl cage system.

The difference in the stabilities of the three triazole-based helicates is more subtle. Each system has the same helicate structure and the ^{13}C carbene chemical shift of the probe complexes suggested that the donor strength of all the triazolyl ligands was very similar (Table 1 and ESI†). Despite these structural and electronic similarities, the stability testing showed that the $[Pd_2(\mathbf{hextrz})_4](BF_4)_4$ complex was considerably more stable than the $[Pd_2(\mathbf{bntrz})_4](BF_4)_4$ and $[Pd_2(\mathbf{pegtrz})_4](BF_4)_4$ helicates against the amino acid nucleophiles histidine and cysteine. We postulate that this enhanced stability is connected to the presence of the hydrophobic hexyl substituents in the $[Pd_2(\mathbf{hextrz})_4](BF_4)_4$ complex. Presumably these hydrophobic chains aggregate together in the polar d_6-DMSO/D_2O solvent mixture used for stability studies and reduce the access of the nucleophiles to the palladium(II) ions of the helicate, thereby increasing the kinetic stability of this system relative to the other helicates.

Helicate cytotoxicity

To assess biological activity the cytotoxicities (as half-maximal inhibitory concentrations (IC_{50})) of the individual ligands **tripy**, **bntrz**, **hextrz** and **pegtrz**, along with their respective $[Pd(\mathbf{L})_4](BF_4)_4$ architectures, were determined against four different cell lines: A549 (lung cancer), CDDP resistant MDA-MB-231 (breast cancer),[29] DU-145 (prostate cancer), and MCF10A (immortalized non-malignant breast tissue) (Table 2).

The ligands **tripy**, **bntrz** and **pegtrz** and the $[Pd_2(\mathbf{pegtrz})_4](BF_4)_4$ helicate exhibited minimal cytotoxicity under the conditions studied (IC_{50} > 80 μM). The ligand **hextrz** and both $[Pd_2(\mathbf{tripy})_4](BF_4)_4$ and $[Pd_2(\mathbf{bntrz})_4](BF_4)_4$ helicates displayed increased cytotoxicity, with IC_{50} values ranging from 70 to 18 μM against all of the cell lines, while the $[Pd_2(\mathbf{hextrz})_4](BF_4)_4$ helicate displayed high cytotoxicity (IC_{50} = 3–8 μM against all cell lines studied). The $[Pd(\mathbf{CH_3CN})_4](BF_4)_4$ complex alone did not display any cytotoxicity (IC_{50} > 100 μM), indicating that the observed cytotoxicities were due to the helicate, rather than palladium(II) or BF_4^- administration.

There was no correlation between helicate cytotoxicity and cell line; for example $[Pd_2(\mathbf{tripy})_4](BF_4)_4$ was almost twice as potent against the A549 cell line compared to DU-145, while $[Pd_2(\mathbf{bntrz})_4](BF_4)_4$ was more potent against the DU-145 line than A549. None of the compounds displayed any selectivity towards cancerous phenotypes, with all of the helicates exhibiting approximately similar IC_{50} values against non-malignant MCF 10A cells as towards the tumour-derived cell lines. When compared, under identical conditions, to the clinically used platinum complex cisplatin (CDDP, experimentally derived IC_{50} of 41.2 ± 3.9 μM against the MDA-MB-231 cell line) $[Pd_2(\mathbf{tripy})_4](BF_4)_4$ was less potent, while $[Pd_2(\mathbf{bntrz})_4](BF_4)_4$ and $[Pd_2(\mathbf{hextrz})_4](BF_4)_4$ were 2.3 and 6.8 fold more potent, respectively.

Table 2 Half-maximal inhibitory concentrations (IC_{50}) of ligands **tripy**, **bntrz**, **hextrz** and **pegtrz** and their respective $[Pd(L)_4](BF_4)_4$ architectures at 24 h

Compound	IC_{50} (μM)			
	A549	MDA MB231	DU145	MCF10A
$[Pd_2(\mathbf{tripy})_4](BF_4)_4$	41.4 ± 3.9	56.7 ± 2.2	70.1 ± 13.8	71.4 ± 3.9
$[Pd_2(\mathbf{bntrz})_4](BF_4)_4$	55.5 ± 1.2	18.1 ± 2.7	34.1 ± 0.9	51.9 ± 0.7
$[Pd_2(\mathbf{hextrz})_4](BF_4)_4$	6.9 ± 0.9	6.0 ± 0.6	3.4 ± 0.4	8.1 ± 1.2
$[Pd_2(\mathbf{pegtrz})_4](BF_4)_4$	>100	—	—	—
tripy	95.3 ± 9.7	>100	>100	>100
bntrz	>100	>100	>100	88.6 ± 5.4
hextrz	28.5 ± 2.6	89.8 ± 10.7	28.5 ± 1.3	18.1 ± 3.1
pegtrz	>100	—	—	—

"—" signifies not determined.

As $[Pd_2(\mathbf{hextrz})_4](BF_4)_4$ displayed the lowest IC_{50} value (Table 2) and also the highest stability (Table 1), it was possible that an intact $[Pd_2(\mathbf{L})_4](BF_4)_4$ helicate structure was required for high cytotoxicity. A comparison of the ligand **hextrz** and $[Pd_2(\mathbf{hextrz})_4](BF_4)_4$ cytotoxicity data supported this hypothesis. As the palladium(II) metallocenters are coordinated by four ligands, if ligand dissociation was required for cytotoxicity then the helicate IC_{50} would be four-fold less than the IC_{50} of the individual ligands (Fig. 2). While $[Pd_2(\mathbf{hextrz})_4](BF_4)_4$ was consistently more toxic than **hextrz**, a four-fold ratio was only observed against A549 cells (Fig. 2). Additionally, the ratio of ligand to helicate toxicity varied substantially between cell lines, from approximately 2 for the MCF10A cells to 15 for the MDA-MB-231's (Fig. 2), demonstrating that there was no correlation between ligand and helicate cytotoxicity.

Time course of helicate action – a comparison with CDDP

To further identify the mechanistic basis of the high cytotoxicity displayed by $[Pd_2(\mathbf{hextrz})_4](BF_4)_4$, a time course analysis was performed to determine the time required for the onset of cytotoxicity (Fig. 3). No further difference in cytotoxicity was observed between 2 and 48 hours, indicating that by 2 hours the administration of the helicate had resulted in the rapid onset of cell death; however, after 2 hours the helicate induced no further toxicity to the surviving cells. This cytotoxic mode of cell death is distinct from classical metal complexes such as CDDP, which display increasing cytotoxicity over time.[30] CDDP

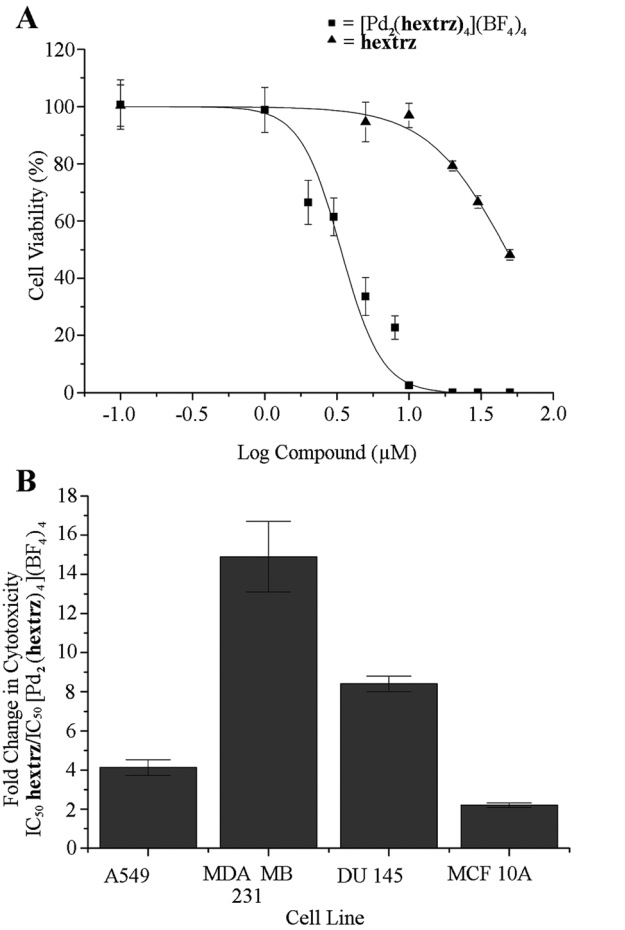

Fig. 2 $[Pd_2(\mathbf{hextrz})_4](BF_4)_4$ cytotoxicity is not due to helicate decomposition. Cells were treated with $[Pd_2(\mathbf{hextrz})_4](BF_4)_4$ or **hextrz** for 24 hours before cell viability analysis. (A) Against DU-145 cells the IC_{50} for the ligand (28.5 μM) was 8 fold higher than for the helicate (3.4 μM); if helicate toxicity was due to rapid decomposition to release the ligand, then the stoichiometry of the complex would predict a 4-fold ratio in IC_{50}. (B) A comparison of the IC_{50} ratio between ligand and helicate across the four cell lines demonstrated no correlation between helicate and ligand toxicity. Data points are expressed as means ± SEM where $n = 6$.

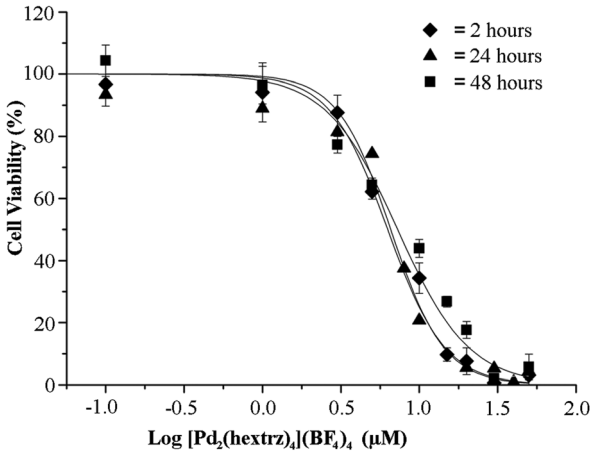

Fig. 3 Cell death caused by $[Pd_2(\mathbf{hextrz})_4](BF_4)_4$ occurs within 2 hours post administration. A549 cells were treated with 0–50 μM $[Pd_2(\mathbf{hextrz})_4](BF_4)_4$ for 2, 24 or 48 hours, and cell viability assessed. There was no significant difference in IC_{50} between time points. Data are expressed as means ± SEM ($n = 6$).

toxicity is a result of DNA binding, causing errors in the cell replication process which results in the initiation of programmed cell death *via* apoptosis. As CDDP toxicity is dependent upon the replication status of individual cells, it selectively kills cells as they undergo cell division, resulting in a staggered cell death over several days.[31] In contrast, the rapid onset of cell death with $[Pd_2(\mathbf{hextrz})_4](BF_4)_4$ provides evidence that the helicate is killing cells by a mechanism unrelated to DNA damage.

$[Pd_2(\mathbf{hextrz})_4](BF_4)_4$ treatment causes a rapid loss of cell membrane integrity

We next aimed to identify the potential biological targets of the $[Pd_2(\mathbf{hextrz})_4](BF_4)_4$ helicate. As rapid cell death is often associated with a compromised membrane integrity, we evaluated whether helicate induced cell death was attributable to membrane damage. For these studies we measured the release of the intracellular enzyme lactate dehydrogenase (LDH) into the extracellular medium. Extracellular levels of LDH are usually present at low concentrations, attributable to a small population of cells undergoing spontaneous death in culture systems.[32] However, these extracellular LDH levels rapidly increase if the cell membrane is disrupted, due to the release of intracellular enzyme into the extracellular space. The assay therefore provides a convenient method for rapidly assessing membrane integrity.[32]

Consistent with a mechanism involving membrane damage, a time and concentration dependent increase in extracellular LDH levels was observed immediately following $[Pd_2(\mathbf{hextrz})_4](BF_4)_4$ administration (Fig. 4). At a relatively high concentration

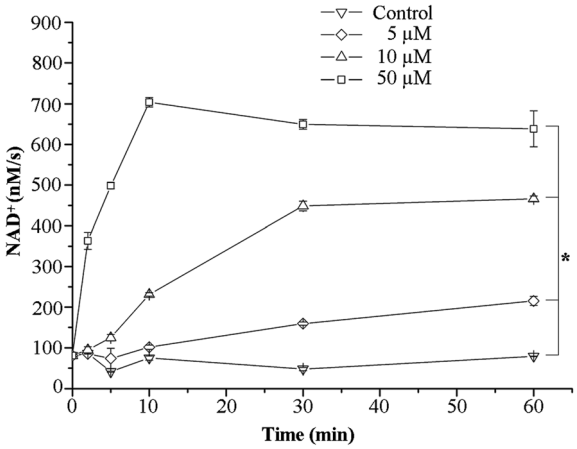

Fig. 4 $[Pd_2(\mathbf{hextrz})_4](BF_4)_4$ causes rapid disruption of the cell membrane. A549 cells were treated with $[Pd_2(\mathbf{hextrz})_4](BF_4)_4$ and the change in extracellular LDH levels calculated by measuring enzyme activity. Data represents extracellular LDH activity at each time point and are expressed as means ± SEM ($n = 3$). * = $p < 0.05$.

of 50 μM (7 fold above the IC_{50}), a rapid elevation in LDH activity was observed, which peaked at 10 minutes. However, at lower concentrations of 5 and 10 μM, continuous increases in LDH release were observable at 10, 30 and 60 minutes, indicating that around the IC_{50} concentration membrane damage was a continual process that occurred over a sustained time period (Fig. 4).

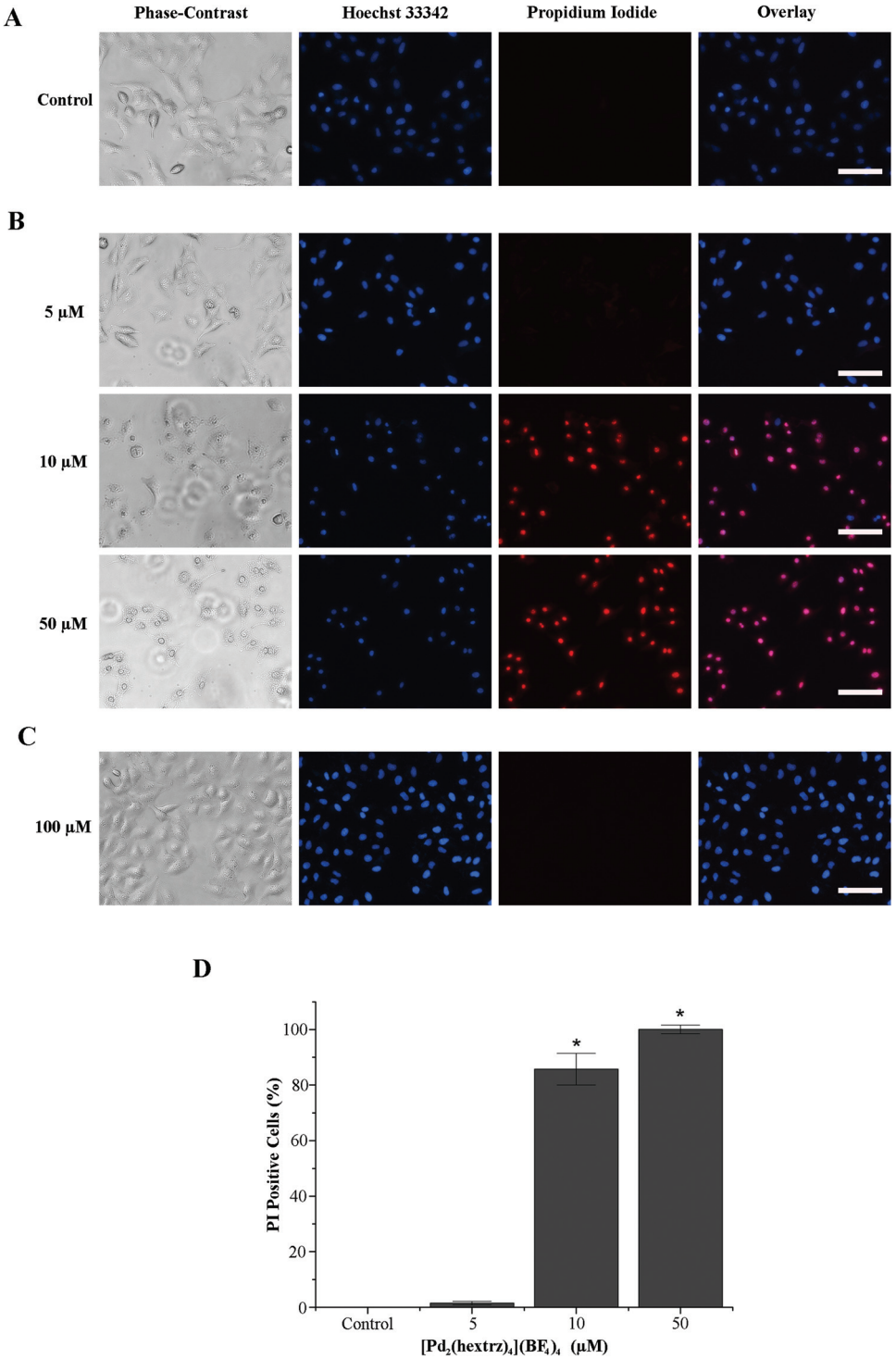

Fig. 5 $[Pd_2(\mathbf{hextrz})_4](BF_4)_4$ induces rapid membrane damage. A549 cells were treated with helicate or ligand for 10 min and then double stained with Hoechst 33342 and propidium iodide. (A) Control cells (0.15% DMSO). (B) Cells treated with 5–50 μM of $[Pd_2(\mathbf{hextrz})_4](BF_4)_4$. (C) Cells treated with **hextrz**. The representative images were acquired with a 20× objective, scale bars (white) indicate 50 μm. (D) The percentage of propidium iodide (PI) positive cells were calculated from fluorescence images obtained for each treatment condition (n = 3), data is expressed as means ± SEM. * = $p < 0.05$ as compared to control.

To investigate the extent to which LDH release was a diagnostic indicator of the viability of the entire cell population, we next conducted double labelling experiments with the dyes Hoechst 33342 and propidium iodide. Hoechst 33342 is a membrane permeable dye used for cell counting as it effectively labels all cells. In contrast, propidium iodide is a cell membrane impermeable dye, which is only taken up by non-viable cells with compromised cell membrane integrity. In combination with Hoechst 33342 it can therefore be used to distinguish between dead and viable cells within a heterogenous population.[33] In A549 cells this double label analysis confirmed that, by 10 minutes, a 10 μM concentration of $[Pd_2(\mathbf{hextrz})_4](BF_4)_4$ (slightly above the 24 hour IC_{50} of 7 μM) was sufficient to have killed >85% of the cell population, while a 50 μM concentration induced 100% cell death (Fig. 5). The phase contrast image demonstrated that $[Pd_2(\mathbf{hextrz})_4](BF_4)_4$ induced cell death was accompanied by a rounding of the cells, a characteristic feature of cell damage that can accompany necrosis[34] (Fig. 5). In contrast 5 μM $[Pd_2(\mathbf{hextrz})_4](BF_4)_4$ (slightly below the 24 hour IC_{50}) only induced 1.5% cell death at 10 min, although rounding of the cell population was observable in the phase contrast image, indicating that cell death processes had potentially been initiated in a greater number of cells. Therefore a small switch in helicate concentration, from slightly below to slightly above the IC_{50} value, could result in the sudden onset of cell death. The mechanism underlying this remarkably rapid switch in activity is currently unknown. In comparison, administration of the **hextrz** ligand alone, even at 100 μM (4-fold higher than the 24 hour IC_{50}), resulted in no cell death at 10 min, confirming that the helicate and its cognate ligand induced cytotoxicity *via* distinct molecular mechanisms (Fig. 5).

Conclusions

Herein the biological activities of four quadruply-stranded dipalladium architectures were studied. The palladium(II)-based helicates exhibited a range of cytotoxic properties, with the most cytotoxic complex $[Pd_2(\mathbf{hextrz})_4](BF_4)_4$ possessing low micromolar IC_{50} values against all of the cell lines tested, while the other helicates displayed moderate or no cytotoxicity. Stability studies indicated that the cytotoxic effect of the dipalladium architectures correlated well with the stability of these systems in the presence of biological nucleophiles, suggesting that it is intact helicate that is responsible for the biological activity.

Disappointingly, the $[Pd_2(\mathbf{hextrz})_4](BF_4)_4$ helicate displayed no selectivity towards cancerous phenotypes; the compound exhibited approximately similar IC_{50} values against non-malignant MCF 10A cells as towards the tumour-derived cell lines. Against the MDA-MB-231 cell line, which is resistant to platinum-based drugs, $[Pd_2(\mathbf{hextrz})_4](BF_4)_4$ was 7-fold more active than cisplatin and, when compared to the IC_{50} values against the other cell lines, there was no evidence of resistance. This suggests that the helicate does not induce cell death in the same way as clinically used metal complexes such as cisplatin. Preliminary mechanistic investigations with the $[Pd_2(\mathbf{hextrz})_4](BF_4)_4$ helicate revealed that it induced cell death within minutes, accompanied by a loss of cellular membrane integrity. While these observations are consistent with the helicate acting as a metallo-detergent,[35] there are several potential alternative mechanisms of membrane damage, including pore formation[36] and changes to the fluidity of the lipid bilayer.[37] With the current data the exact cause of the membrane damage is not clear. Because the cytotoxic mechanism of the helicate was loss of cellular membrane integrity, the compound exhibited no selectivity towards cancer cells compared to non-malignant cells. This lack of specificity towards cancer cells prevents the immediate application of this complex as an anti-cancer agent.

However, the current work suggests that suitably designed stable palladium(II)-containing metallosupramolecular architectures (especially quadruply-stranded helicates) can display biological activity. As such we are now examining other palladium(II)-containing architectures and quadruply-stranded helicates containing other metals ions in search of more selective anti-cancer agents.

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