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Photoactivatable Cell-selective Dinuclear Trans Diazido Platinum(IV) Anticancer Prodrugs

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Supporting Information

INTRODUCTION
Photoactivation is a promising effective and non-invasive chemotherapeutic strategy.1–4 Photocytotoxicity using appropriate platinum complexes has the potential to overcome some limitations of conventional cisplatin therapy, including poor pharmacokinetics, dose-limiting side effects, restricted spectrum of anticancer activity, high incidence of resistance, and lack of normal-cell discrimination.5–8 The application of oxygen-dependent photodynamic therapy (PDT) is limited owing to the low concentration of O2 in hypoxic tumours, together with the need for diffusion of O2 from adjacent tissues.9 Photoactivated chemotherapy (PACT), in contrast, is less dependent on oxygen for cytotoxicity and provides a new avenue to anticancer drug design.

Octahedral, low-spin 5d4 PtIV complexes are usually considered to be prodrugs, since they are kinetically more inert than their PtII counterparts under biological conditions.8–10 Photoactivatable PtIV prodrugs can exhibit high dark stability and potential photocytotoxicity,11–13 examples include diazido- PtIV complexes containing various non-leaving amines and axial substituents.11–20 Among them, trans, trans, trans-[Pt(N3)2(py)(pyz)] (FM-190) is highly potent,21,22 has good aqueous solubility, is relatively stable in cell culture media and towards reactions with the abundant intracellular tripeptide glutathione (GSH, γ-L-Glu-L-Cys-Gly), and can be activated by UVA, blue (420 nm) and green (500±30 nm) light, achieving low micromolar IC50 values even in cisplatin-resistant cell lines, with a high phototoxicity index. Not only does the platinum center become reactive on photoreduction, but in resistant cell lines, with a high phototoxicity index. Not only does the platinum center become reactive on photoreduction, but in resistant cell lines, with a high phototoxicity index. Not only does the platinum center become reactive on photoreduction, but in resistant cell lines, with a high phototoxicity index.

Axial ligands in diazido-PtIV complexes normally act as leaving groups upon reduction to PtIII and greatly influence the reduction potential of PtIV. Derivatisation of an axial ligand can be used to improve pharmacological properties without interfering with the ultimate mode of action of the active PtIII species or the potential production of other reactive species. Several studies have been carried out previously on the conjugation of FM-190 to functional fragments, including αβ and αβ integrin-selective RGD-
containing peptides, 24 RNA-binding guanidinosemicyanin, 25 TEMPO radical, 26 upconversion-luminescent nanoparticles 27 and G-quadruplex G-K1 borate hydrogels. 28 For the former two conjugates, the selectivity and cellular uptake toward specific cancer cells was remarkably enhanced, while for the produg with TEMPO, azidyl and TEMPO radicals were released upon irradiation with blue light (420 nm), accompanied by the formation of toxic Pt4 species, resulting in improved cytotoxicity. Upconversion-luminescent nanoparticles enable photoactivation with near-infrared light, and hydrogels allow treatment of surface cancers with minimal damage to normal tissues.

Polynuclear platinum complexes have attracted much attention owing to their unique biological properties, including coordinative binding to DNA bases and interaction of the phosphodiester linker through electrostatic and hydrogen-bonding effects. 29 The pre-association of polynuclear platinum complexes with DNA prior to coordinate bond formation can significantly affect the kinetics of cross-link formation, 30-33 which distinguishes them from their mononuclear counterparts. The trinuclear complex [{trans- PtCl(NH3)2}μ-trans-Pt(NH3)2[NH2(CH2)2NH2]+]4+ (BBR3464) underwent phase I and II human clinical trials as a promising anticancer platinum drug. 34, 36

In order to investigate structure-activity relationships related to the modification of the axial ligands of diazido-PtIV prodrugs, we have synthesized and characterized a series of dinuclear platinum(IV) complexes of the type trans, trans- [{Pt(N2py)2(OH)(OC)(OC)H2CH2}]2+ (R = -CH2CH2- (1); trans, trans-1,2-C6H4Cl2 (2); p-C6H4Cl (3); -CH2CH2CH2CH2 (4)). The x-ray crystal structures of complexes 1,2MeOH and 4 were determined. These appear to be the first photoactivatable dinuclear platinum(IV) complexes containing azides to be reported. Their photodecomposition, photochemistry and the activity of these complexes containing two platinum centers and four azides in one molecule are compared to related mononuclear diazido complexes, including the influence of the nature of the bridging ligand.

EXPERIMENTAL SECTION

Materials and instruments. O-(Benzotriazol-1-yl)-N, N', N'-tetramethyluronium tetrafluoroborate (TBTU) was purchased from Merck, Pyridine was from Fischer Scientific UK, ct-DNA, K3PcI3, Na2S, H2O2 (30%), succinic anhydride and other chemicals were from Sigma Aldrich and used without further purification.

NMR spectra were recorded on Bruker Avance III HD 400 MHz or Bruker 500 MHz spectrometers; the residual signal of the solvent was used as a reference. ESI-MS spectra were recorded on an Agilent 6130B single quadrupole detector instrument and ESI-HR-MS data were collected on a Bruker microTOF instrument at 298 K with a scan range of m/z 50-2000 for positive ions. Samples were prepared in methanol or aqueous solution. Electronic absorption spectra were recorded on a Varian Cary 300 UV-vis spectrophotometer in a quartz cuvette and referenced to neat solvent. The spectral width was 200-800 nm and the bandwidth was 1.0 nm, the scan rate was set to 600 nm/min. Analytical reverse-phase HPLC analyses were carried out on an Agilent ZORBAX Eclipse XDB-C18 column (250×4.6 mm, 5 μm, flow rate: 1 mL/min), by using linear gradients of 0.1% TFA in H2O (solvent A) and 0.1% TFA in CH3CN (solvent B). LC-MS were carried out on Bruker Amazon X connected online with a HPLC. The light sources used for photoactivation were a LzC-IICH2 photoreactor (Luzchem Research Inc.) equipped with a temperature controller and 8 Luzchem LZC-420 lamps without light filtration, and KiloArcTM broadband arc lamp monochromator supplied with the appropriate filters to cut off any unwanted light. An LED light source with λmax = 465 nm was used for in vitro growth inhibition and cell cycle assays. Platinum contents were analysed on an ICP-OES 5300DV (Perkin Elmer) or ICP-MS 7500cx (Agilent). The emission wavelengths detected for Pt were 265.945, 214.423, 299.797, 204.937 and 193.700 nm.

Synthesis and characterization. Caution! Heavy metal azides can be shock-sensitive detonators. We did not encounter any problems during the work reported here, but due care and attention with appropriate precautions should be taken in their synthesis and handling. All synthesis and purifications were carried out in the dark with minimal light exposure.

General synthesis procedure for complexes 1-4. To a solution of trans, trans, trans-[Pt(N2py)2(OH)(sucinate)(py)]2+ (24.4 mg, 42.8 μmol) and TBTU (11.8 mg, 36.8 μmol) in freshly degassed anhydrous DMF (2 mL), N, N-diisopropylethylamine (DIPEA) (100 μL) was added. After stirring for 3 min, a solution of 0.5 mol equiv of corresponding diamine, DIPEA (60 μL) and DMF (1 mL) was added to the resulting mixture. The reaction mixture was stirred at overnight in a nitrogen atmosphere. After evaporation to dryness, the oily residue was collected and purified by column chromatography on aluminum oxide (5% methanol + 95% DCM).

Trans, trans, trans-[Pt(N2py)2(OH)(OC)(OC)H2CH2]2+ (1). Diamine = ethylenediamine. 1H NMR (DMSO-d6, 400 MHz); 8.81 (dd, J = 5.5 Hz, Jtrans-Pt-Htrans-Pt-H = 26.6 Hz, 8H, Hpy), 8.26 (t, J = 7.5 Hz, 4H, Hpy), 7.83 (t, J = 7.0 Hz, 8H, Hpy), 7.75 (t, J = 5.7 Hz, 2H, CONH), 3.65 (s, 2H, OH), 3.05 (s, 4H, CH2), 2.46 (t, J = 7.6 Hz, 4H, CH2), 2.22 (t, J = 7.3 Hz, 4H, CH2). 13C NMR (DMSO-d6, 125 MHz): 175.27 (COO), 172.08 (CONH), 149.83 (Cpy), 144.22 (Cpy), 126.66 (Cpy), 38.80 (CH2), 32.39 (CH2), 32.21 (CH2). ESI-MS [M+Na]+: 1189.216. Anal. Calcd: C36H34Na2O8Pt2; C 30.88, H 3.11, N 21.61. Found: C 30.43, H 3.10, N 20.69.

Trans, trans, trans-[Pt(N2py)2(OH)(OC)(OC)H2CH2]2+ (2). Diamine = trans-1,2-cyclohexanediamine. 1H NMR (DMSO-d6, 400 MHz); 8.82 (dd, J = 5.5 Hz, Jtrans-Pt=1Htrans-Pt-H = 26.5 Hz, 8H, Hpy), 8.25 (t, J = 7.5 Hz, 4H, Hpy), 7.82 (t, J = 6.9 Hz, 8H, Hpy), 7.52 (d, J = 7.3 Hz, 2H, CONH), 3.65 (s, 2H, OH), 3.47 (s, 2H, CH2-cyclohexyl), 2.48-2.33 (m, 6H, CH2), 2.26-2.10 (m, 4H, CH2), 1.74 (s, 2H, CH2-cyclohexyl), 1.62 (s, 2H, CH2-cyclohexyl), 1.18 (s, 4H, CH2-cyclohexyl). 13C NMR (DMSO-d6, 125 MHz): 175.23 (COO), 171.68 (CONH), 149.84 (Cpy), 143.28 (Cpy), 126.65 (Cpy), 55.38 (CH2-cyclohexyl), 52.50 (CH2-cyclohexyl), 49.06 (CH2-cyclohexyl), 32.74 (CH2), 32.58 (CH2), 32.10 (CH2-cyclohexyl), 24.87 (CH2-cyclohexyl). ESI-MS [M+Na]+: 1243.2631. Anal. Calcd: C36H34Na2O8Pt2; C 33.84, H 3.47, N 20.65. Found: C 33.84, H 3.48, N 19.14.

Trans, trans, trans-[Pt(N2py)2(OH)(OC)(OC)H2CH2]2+ (3). Diamine = p-phenylenediamine. 1H NMR (DMSO-d6, 400 MHz); 9.79 (2H, CONH), 8.82 (dd, J = 5.5 Hz, Jtrans-Pt=1Htrans-Pt-H = 26.7 Hz, 8H, Hpy), 8.25 (t, J = 7.3 Hz, 4H, Hpy), 7.79 (t, J = 7.1 Hz, 8H, Hpy), 7.50 (s, 4H, CH benzyl), 3.67 (2H, OH), 2.67 (t, J = 7.3 Hz, 4H, CH2), 2.33 (t, J = 7.6 Hz, 4H, CH2). 13C NMR (DMSO-d6, 125 MHz): 175.20 (COO), 170.71 (CONH), 149.83 (Cpy), 143.29 (Cpy), 135.10 (C benzyl), 126.64 (Cpy), 119.77 (CH benzyl), 33.09 (CH2), 32.13 (CH2). ESI-MS [M+Na]+: 1237.2138. Anal. Calcd: C36H34Na2O8Pt2; C 34.41, H 3.47, N 19.56. Found: C 34.35, H 3.76, N 19.96.

Trans, trans, trans-[Pt(N2py)2(OH)(OC)(OC)H2CH2]2+ (4). Diamine = 1,4-butenediamine. 1H NMR (DMSO-d6, 400 MHz); 8.82 (dd, J = 5.5 Hz, Jtrans-Pt=1Htrans-Pt-H = 26.9 Hz,
The whole cell pellets were adjusted/verified after ICP-OES metal quantification. Stock concentrations for all controls. IC\textsubscript{50} values in the corresponding cell line. Additional plates were incubated with medium alone as a negative control. After 1 h of incubation in the dark at 310 K, the cells were rinsed three times with cold phosphate-buffered saline (PBS) and harvested by trypsinisation. The number of cells in each sample was counted manually using a haemocytometer. Then the cells were centrifuged to obtain the whole cell pellet for ICP-MS analysis. All experiments were conducted in triplicate.

Photoreactions with 5′-GMP. 2, 4 or 8 mol equiv of guanosine 5′-monophosphate disodium salt hydrate (5′-GMP-Na\textsubscript{2}) was mixed with 30 μM complex 4 in methanol:water 5:95 v/v. The solution was incubated at 310 K for 1 h then irradiated for 1 h and analysed immediately. HPLC analysis was carried out on an Agilent ZORBAX Eclipse XDB-C18 column (250 × 4.6 mm, 5 μm, flow rate: 1 mL/min). The mobile phases for HPLC were A (0.1 % formic acid in HPLC grade H\textsubscript{2}O, volume percentage) and B (0.1 % formic acid in HPLC grade acetonitrile, volume percentage). The Pt adducts were isolated and analysed by Bruker Amazon X connected online with the HPLC.

Electron paramagnetic resonance (EPR) spectroscopy. The EPR spectra were recorded on a Bruker EMX (X-band) spectrometer at 298 K. Samples (ca. 100 μL) in aqueous solution were prepared and transferred using a plastic syringe with metal needle to a standard quality quartz tube with inner diameter of 1.0 mm and outer diameter of 2.0 mm (Wilmad LabGlass) and sealed with parafilm. Using the y-incremental sweep mode of 120 with an accumulation of 5 scans in the x dimension, Typical key EPR spectrum settings were modulation amplitude 2.0 G, microwave power 1.37 mW, 1.0 ×10\textsuperscript{5} receiver gain, conversion time 5.12 ms, time constant 5.12 ms, sweep width 200 G. The LED465E light irradiation source was mounted within the EPR magnet, supported by a foam sponge, to maintain its position throughout the EPR measurements. The distance from the tip of the irradiation light bulb to the EPR cavity was ca. 3 cm. Data were processed by Matlab R2016b with easyspin 5.1.12 through a multicomponent fit.

Flow cytometry. All flow cytometry experiments were carried out using a Becton Dickinson FACScan Flow Cytometer in the School of Life Sciences at Warwick University. Typically, cells were seeded in 6-well plates using 1.5 × 10\textsuperscript{5} cells per well. Experiments included 24 h of pre-incubation in drug-free media at 310 K in CO\textsubscript{2}: humidified atmosphere, followed by 1 h of drug exposure under the same conditions. After this, samples were irradiated for 1 h. For comparison, the dark plates were kept in the incubator for another 1 h. Supernatants were removed, cells were washed with PBS. Samples were then collected after trypsinisation, washed with PBS and stained in the dark with a mixture of propidium iodide and RNase. After 30 min staining, cell samples were washed and set up for flow cytometry reading on the red channel FL-2.

Kinetics of binding to ct-DNA. ct-DNA was mixed with complexes in 10 mM NaClO\textsubscript{4} and immediately irradiated (visible light, λ\textsubscript{max} = 455 nm) for 60 or 120 min and then kept at 310 K in
the dark. The $r_i$ value was 0.05-0.08. Aliquots were removed at various time intervals and quickly filtered using a Sephadex G-50 column to remove free (unbound) Pt. The Pt content in these DNA samples ($r_n$, defined as the number of the molecules of platinum complex coordinated per nucleotide residue) was determined by AAS and concentration of DNA by UV-vis spectroscopy.

Characterization of DNA Adducts of Photoactivated complexes by EtBr Fluorescence. $ct$-DNA was incubated with the Pt complexes under irradiation conditions (visible light, $t_{irrad} = 455$ nm for 2 h and subsequently incubated in the dark for an additional 23 h) at various $r_n$ values in 10 mM NaClO$_4$ at 310 K. Fluorescence measurements of ct-DNA modified by Pt complexes in the presence of EtBr were performed at an excitation wavelength of 546 nm, and the emitted fluorescence was analyzed at 590 nm. The fluorescence intensity was measured in NaCl (0.4 M) to avoid secondary binding of EtBr to DNA. The concentrations were 0.01 mg mL$^{-1}$ for DNA and 0.04 mg mL$^{-1}$ for EtBr, which corresponded to the saturation of all binding sites for EtBr in DNA. The fluorescence spectra were recorded by a Varian Cary Eclipse spectrophotofluorometer using a 0.5 cm quartz cell at room temperature.

Formation of interstrand cross-links. Solutions of the plasmid pUC19 linearized by EcoRI (50 μg mL$^{-1}$) were incubated with the Pt complex under irradiation conditions (for 1 h and subsequently incubated in the dark for an additional 23 h) at various $r_n$ values in NaClO$_4$ (10 mM) at 310 K. Autoradiograms were recorded of denaturing 1% agarose gel of linearized DNA which was 3'-end labeled and nonmodified or modified by FM-190, 1 and 4 photoactivated by visible light.

Transcription mapping of platinum-DNA adducts. Transcription of the (NdeI/HpaI) restriction fragment of pSP73KB supercoiled pSP73 plasmid was digested with DNase I to yield 158 and 2306 bp fragments. The 158 bp fragment was purified by 1% agarose gel electrophoresis and isolated from the gel by a Promega Wizard SV Gel cleanup system.

The crystallographic data are summarized in Table 4. The structure of complexes $^{1}$-4 was determined by X-ray crystallography. The crystals of complexes $^{1}$-4 were grown by the diffusion of diethyl ether into corresponding solutions in methanol. Complexes $^{1}$-4 crystallized in space group P4$_1$2$_1$2$_1$ with cell parameters: $a = b = c = 78.8$ Å, $\alpha = \beta = \gamma = 90^\circ$.

Results and Discussion

Synthesis and characterization of complexes 1-4. The synthetic route for dinuclear Pt$^{II}$ complexes 1-4 is summarized in Scheme 1. The mononuclear complexes $trans$, $trans$-[Pt(N$_2$)$_2$(OH)$_2$(py)$_2]$ (FM-190) and $trans$, $trans$-[Pt(N$_2$)$_2$(OH)OC(CH$_3$)CO(OH)(py)$_2]$ (FMSA) were prepared by similar procedures to those reported.26 The two Pt$^{II}$ centers with axial succinate carboxylates were coupled through amide bond formation using bridging diamines containing ethylene (1), 1,2-cyclohexyl (2), 1,4-phenylene (3), or butyl (4) linkers. Complexes 1-4 are air-stable at ambient temperature, both in the solid state and in aqueous solution. They gave satisfactory elemental analysis and were further characterized by ESI-MS, NMR and UV/Vis spectroscopy, and for complexes 1 and 4 by x-ray crystallography. The $^1$H and $^{13}$C NMR spectra of complexes 1-4 in DMSO-$d_6$ contain the expected peaks (Figures S1-S8). In the $^1$H NMR spectra, the doublets with $^3$Pr satellites at ca. 8.82 ppm ($J = 5.5$ Hz, $^1$H$^{90}$Pt-$H = 26.6$ Hz) and the triplets at ca. 8. 26 ppm ($J = 7.5$ Hz), 7.82 ppm ($J = 7.0$ Hz) are assigned to the $H_a$, $H_b$ and $H_b$ of pyridine, respectively. The peaks at ca. 3.66 ppm are assignable to the resonances of the hydroxyl protons and the peaks at 7.52-9.79 ppm to the amide protons. The $^{13}$C NMR resonances of complexes 1-4 at ca. 149.83, 142.40 and 126.66 ppm are attributable to the $C_a$, $C_y$ and $C_b$ carbons of pyridine, respectively. The complexes all display peaks at ca. 175.27 ppm and 172.00 ppm for the carbonyl and amide carbons, respectively. The electronic absorption spectrum of complex 1 is similar to that of its mononuclear analogue $trans$, $trans$-[Pt(N$_2$)$_2$(OH)$_2$(py)$_2]$ (in water/methanol (2:3) solution, complex 1 shows a high-energy band at 262 nm ($\varepsilon = 25260$ M$^{-1}$ cm$^{-1}$) and a maximum absorption band at 300 nm (34109 M$^{-1}$ cm$^{-1}$), which are assigned to a LMCT (OH→Pt, N→Pt) character and involves Ni and OH ligands and platinum.21 This band enables the photoactivation of complex 1 using blue light. Complexes 2-4 show similar electronic absorption spectra as complex 1, except that the high-energy band (at 260 nm, $\varepsilon = 49189$ M$^{-1}$ cm$^{-1}$) of complex 3 is more intense than those of its analogues ($\varepsilon \approx 25000$ M$^{-1}$ cm$^{-1}$), owing to the absorption of the phenyl fragment.

X-ray crystallography. Crystals suitable for X-ray diffraction studies of complexes 1-4 were obtained through the diffusion of diethyl ether into corresponding solutions in methanol. The structure of complexes 1-4 were shown in Figure 1. The crystallographic data are summarized in Table S1 and selected bond distances and angles are listed in Table 1. Complex 1 crystallized in the triclinic space group P-1 with one complex and two methanol molecules in the unit cell. Complex 4 crystallized in...
Scheme 1. The synthetic route for photoactive dinuclear diazido platinum(IV) complexes 1–4.

\[
\begin{align*}
\text{Diamine, TBTU, DMF, N}_2 \\
\text{24 h, R.T.}
\end{align*}
\]

the monoclinic space group P2\textsubscript{1}/n with two molecules in the unit cell.

Complexes 1·2MeOH and 4 are neutral and consist of two mononuclear Pt\textsuperscript{IV} fragments bridged by an aliphatic linker of a diamine which forms amide bonds to the axial succinate carboxylates. The two Pt centers are symmetric with the diamine linker sitting on an inversion center. The geometries of the Pt centers are similar to their mononuclear analogue \[\text{trans, trans, trans-}[\text{Pt(N}_3]_2(\text{OH})(\text{py})_2]\]: octahedral with \[\text{N}_4\text{O}_2\] coordination. The equatorial plane is defined by four nitrogen atoms, two from the \textit{trans} pyridine molecules and two from the \textit{trans} azides. An oxygen atom from a hydroxido ligand is located in an axial position, while the other axial position is occupied by an oxygen from one of the succinate carboxylate groups.

**Figure 1.** X-ray crystal structures of 1·2MeOH (A) and 4 (B). The complexes sit on inversion centers and only the asymmetric unit is labelled. Thermal ellipsoids are drawn at the 50% probability level.

Compared with the fully symmetrical complex \textit{trans, trans, trans-}[\text{Pt(N}_3]_2(\text{OH})(\text{py})_2], complexes 1·2MeOH and 4 exhibit distortions from ideal octahedral geometry. The axial bond angles between Pt\textsuperscript{IV} and \textit{trans} donor atoms are <180° and the Pt-O (hydroxide, 1.9857(18) Å) bond is slightly shorter than Pt-O (succinate, 2.0185(18) Å), which can be attributed to the steric hindrance caused by the relatively bulky carboxylate groups. The azido ligands are almost linear with the Pt-N(α)-N(β) angles slightly smaller than 120°. The terminal azide N-N bond (1.140(4) and 1.138(4) Å) is slightly shorter than the azide N-N bond (1.211(3) and 1.220(3) Å) closest to Pt, which is typical of azide ligands due to their resonance structure. The Pt-N(pyridine) bond (2.030(2) and 2.038(2) Å) is shorter than the Pt-N(azide) bond (2.053(2) and 2.052(2) Å). The amide C=O distances are 1.240(3) and 1.236(5) Å, while those in the ester group are 1.216(3) and 1.217(4) Å, which resemble typical carbonyl groups in their mononuclear analogues.\textsuperscript{25} Weak hydrogen bonds are observed in complexes 1·2MeOH and 4 (Table S2, detailed description in the Supporting Information).

**Table 1.** Selected Bond Lengths (Å) and Bond Angles (°) for 1·2MeOH and 4.

<table>
<thead>
<tr>
<th>Bond Lengths</th>
<th>1·2MeOH</th>
<th>4</th>
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<tbody>
<tr>
<td>Pt1–O1</td>
<td>1.9857(18)</td>
<td>Pt1–O1</td>
</tr>
<tr>
<td>Pt1–N1</td>
<td>2.030(2)</td>
<td>Pt1–N1</td>
</tr>
<tr>
<td>Pt1–N7</td>
<td>2.038(2)</td>
<td>Pt1–N7</td>
</tr>
<tr>
<td>Pt1–O13</td>
<td>2.0185(18)</td>
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<td>1.211(3)</td>
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<td>N22–N21–Pt1</td>
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<td>N23–N24–Pt1</td>
<td>113.68(18)</td>
<td>N24–N25–Pt1</td>
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<tr>
<td>N25–N24–N23</td>
<td>175.6(3)</td>
<td>N26–N25–N24</td>
</tr>
</tbody>
</table>

**Figure 2.** Photochemical decomposition with blue light (420 nm) of 1 (5×10\textsuperscript{-5} M, in H\textsubscript{2}O: methanol 1:2 v/v) determined by UV/vis spectroscopy (Insert: time dependence of absorbance change at 300 nm).

**Photoactivation.** The photodecomposition of complexes 1–4 in aqueous solution was monitored by UV-vis spectroscopy at various
time intervals after irradiation with blue light (420 nm) at ambient temperature. Figure 2 shows the decrease in absorbance maximum of complex 1 at ca. 300 nm, assigned as the Ni≡Pt LMCT band. The decrease indicates the release of the azide ligands was complete within 1 h. This photodecomposition behaviour is similar to that of the mono-Pt counterpart. Similar results were obtained for the other three complexes (Figure S9). Photo-reactions of dinuclear complex 4 were investigated by LC-MS (Figure S10 and Table S3). The HPLC peak assigned to complex 4 disappeared within 10 min of irradiation with blue light (420 nm). Peaks assignable to dinuclear photoproducts increased in intensity gradually and peaked after 5 min, and then decreased over 30 min with a concomitant increase in the formation of mononuclear photoproducts \[{\text{[Pt}^2\text{py}]{(\text{OC(OH)}\text{H})_2}}^{+}\text{Na}^+\] (466.05) and \[{\text{[Pt}^2\text{(CH-CN)(N}3\text{)py}]^{+}\}^+\] (436.10). It appears that complex 4 loses one azide from both platinum centers initially, followed by the dissociation of a platinum center and the associated bridging ligand (Figure S10).

![EPR spectra of complexes](image)

**Figure 3.** Observed (black) and simulated (red) EPR spectra of complex 4 in aqueous solution with 5% DMSO showing the formation of DMPO–N• and DMPO–OH• adducts after irradiation (465 nm). The experimental trace is the accumulation of 500 scans (conversion time 5.12 ms, time constant 5.12 ms, sweep time 10.48 s for each scan) with continuous irradiation (465 nm). Parameters for simulation: DMPO–N• (g = 2.00583, αM = 14.5 mT, αH = 14.2 mT, αP = 0.31 mT); DMPO–OH• (g = 2.0058, αM = 14.5 mT, αH = 14.2 mT).

**Formation of azidyl radicals.** The formation of azidyl radicals from the photodecomposition of these PtIV complexes was monitored by EPR using DMPO as a spin trap. EPR spectra were recorded continuously for 5 mM solution of complex 4 and 40 mM DMPO in 5% DMSO/95% H2O on irradiation with blue light (465 nm, 20 mW) at 298 K. Both azidyl and hydroxyl radical adducts of DMPO (DMPO–N• and DMPO–OH•) were detected upon irradiation, as a 1 : 2 : 2 : 1 quartet of triplets and a 1 : 2 : 2 : 1 quartet, respectively (Figure 3). The simulation was carried out using the parameters listed in Figure 3, which is in accordance with reference.23

**Photocytotoxicity and cellular accumulation studies.** Upon irradiation with blue light (465 nm, 4.8 mW/cm², 1 h), complexes 1–4 exhibited photocytotoxicity towards several human cancer cell lines, including A2780, A2780cis ovarian and OE19 oesophageal cancer cells. The dose-dependent inhibition of cell viability determined by the sulforhodamine B (SRB) colorimetric assay both in the dark and after irradiation of complexes 1–4, is summarised in Table 2. In parental (A2780) and cisplatin resistant (A2780cis) ovarian carcinoma, complexes 1–4 showed no cytotoxicity in the dark. However, upon irradiation for 1 h, complexes 1–4 exhibited cytotoxicity to varying extents. Complex 4 was the most potent member of the series (IC50 = ca. 17.0 μM for ovarian cancer cells A2780 and A2780cis, and 8.8 μM for OE19) with photocytotoxic index (PI) towards cancer cells > 6 for ovarian carcinoma and 11 for oesophageal adenocarcinoma with light exposure. Complexes 1 and 3 had lower IC50 values toward the cisplatin-resistant A2780cis cell line (IC50 = 29.5 μM (1), 35.5 μM (3)) compared to the parental A2780 cell line (IC50 = 77.0 μM (1), 78.3 μM (3)), which suggests a different mechanism of action from cisplatin and the possibility of overcoming the resistance. In contrast, the mononuclear analogue FM1-190 was 10x more toxic to parental A2780 (1.4 μM) compared with cisplatin resistant A2780cis (14.5 μM) cells upon irradiation with UVA (365 nm). Complexes 1–4 displayed cytotoxicity towards OE19 both in the dark and upon irradiation. However, irradiation improved the cytotoxicity slightly for complexes 1–3, and considerably so for 4, with a photocytotoxic index (PI) > 11 (IC50 = 97.0 μM (dark), 8.8 μM (light)).

In contrast to the photocytotoxicity observed towards cancer cell lines, complexes 1–4 were relatively non-toxic toward the normal cell line MRC5, with IC50 values >100 μM, even after irradiation (Table 2). Since azidyl and hydroxyl radicals generated from platinum(IV) complexes with azides are key species involved in cell death,23 their relative non-cytotoxicity towards MRC5 may be due to the lower basal ROS level of healthy cells, with higher radical levels being needed to cause cell death.41 For comparison, cisplatin tested under the same conditions induced little cytotoxicity in all cell lines due to the short incubation time (1 h, IC50 >100 μM with or without light).

Cellular accumulation is related to the molecular structure as well as the lipophilic nature of the complex and often correlates with the antiproliferative activity of metallo-drugs. The extent of cellular uptake of complexes 1–4 was investigated by treating cancer cell lines with IC50 concentrations for 1 h in the dark. The cell accumulation of Pt was then determined by ICP-MS. As shown in Table 3, complex 3 exhibited > 5x higher Pt accumulation than other complexes in all cell lines, attributable to its aromatic bridge linker and higher lipophilicity. For complexes with aliphatic linkers, 1 showed slight higher levels of Pt accumulation in all cancer cell lines compared with complexes 2 and 4. It is notable that accumulation of Pt from complex 4 in cisplatin-resistant A2780cis ovarian cancer cells is 2x that for the parental A2780 cell line, even though complex 4 is equally toxic to both cell lines. These results appear to support the ability of the dinuclear complexes to overcome resistance to cisplatin.

**Cell cycle analysis.** The effect of complexes 3 and 4 in the presence of light on the progression of the cell cycle was studied. Cells were exposed to produgs at equipotent photocytotoxic IC50 concentrations for 1 h in the dark, then irradiated for 1 h (465 nm, 4.8 mW/cm², 1 h). After 24 h recovery, cells were stained in the dark with propidium iodide in the presence of RNAse then analysed by flow cytometry. The results are summarised in Table S4 and Figure 4.

In all cancer cell lines, the populations of cells in the stages of the cycle were not affected by the complexes in the dark, but changed dramatically upon irradiation. In OE19 oesophageal cancer cells, a reduction of cells in the G0/G1phase was observed (ca. 45% to 29 %), paralleled by an accumulation of cells in the G2/M phases (ca. 36% to 57 %), suggesting that dinuclear complexes 3 and 4 inhibit progression from G2/M to G0/G1 upon irradiation.
Photoactivated complex 4 induced more significant changes in the cell cycle progression compared with complex 3 (two-tail t-test with unequal variances against control, Table S4), consistent with the higher photocytotoxicity of complex 4. Similar changes in cell cycle populations were found for A2780 and A2780cis ovarian cancer cells (Figure 4). This is in contrast to cisplatin which arrests cells in S phase, suggesting a different mechanism of action and a lack of cross-resistance between complexes 3 and 4.

**Photoinduced reactions with 5′-guanosine monophosphate were investigated.** Since the N7 site of the DNA base guanine is a preferred target for platinum amine anticancer complexes, photoinduced reactions of 5′-GMP with blue light (420 nm) at 310 K were investigated. The photoreactions were carried out by irradiating solutions of complexes with 5′-guanosine monophosphate were investigated. The photoreactions were carried out by irradiating solutions of complexes with 5′-GMP (2, 4 or 8 mol equiv.) with blue light (420 nm) at 310 K and monitored by reverse-phase HPLC (Figure S11, Table S5). The photorelease of the bridging ligand \([\text{HOOCC}(\text{CH}_2)_2\text{C}(\text{O})\text{NH}(\text{CH}_3)_2\text{NCH}(\text{O})(\text{CH}_2)_2\text{COOH}+\text{H}^+]^-\) (289.21) was confirmed by LC-MS and the amount released did not change significantly when the ratio of 5′-GMP/Pt was increased. The major photoproduct is assignable as [Pt\(^{\text{IV}}\)(CH\(_3\)CN)(py)\(_2\)(GMP-H\(^+\))]\(^+\) (756.09), which increased in proportion at higher 5′-GMP concentrations.

The decomposition of complex 4 can be detected within only 1 min of irradiation, the apparent decrease of the absorbance of 5′-GMP and the formation of [Pt\(^{\text{IV}}\)(CH\(_3\)CN)(py)\(_2\)(GMP-H\(^+\))]\(^+\) (756.09) is observed only after 5 min of irradiation. No dinuclear photoproduct with 5′-GMP was detected, which suggests that the Pt centers do not react with 5′-GMP until they are detached from the bridging ligands. The results above agree well with those previously reported for the parent complex and its succinate derivative, suggesting that the two Pt\(^{\text{IV}}\) centers have little effect on each other during the photoreactions with 5′-GMP.

**DNA binding studies in cell-free media.** To further explore the differences between mono- and dinuclear complexes, and the effect of bridge linkers, the DNA binding ability of photoinactivated dinuclear complexes 1 and 4 was investigated in comparison with mononuclear FM-190.

Ct-DNA was mixed with complexes in 10 mM NaClO\(_4\) and immediately irradiated with blue visible light (\(\lambda_{\text{max}} = 455\) nm) for 1 h then kept at 310 K in the dark. Aliquots were removed at various time intervals and quickly filtered using a Sephadex G-50 column to remove free (unbound) Pt. The Pt content in these DNA samples was determined by AAS and the concentration of DNA was determined by UV-vis to give the kinetics of Pt binding to ct-DNA. The amount of Pt bound to DNA increased with time for all complexes. No significant difference was found between the investigated complexes (Figure 5A).

The DNA intercalator EtBr was used as a fluorescent probe to characterize perturbations induced in DNA by the adducts with Pt complexes. Modification of DNA by platinum complexes under irradiation conditions resulted in a decrease of EtBr fluorescence.
Figure 4. Flow cytometry histograms showing cell cycle analysis of A2780, A2780Cis ovarian and OE19 oesophageal cancer cells. (■ Dark, ■ Irradiated) In all cases, the experiments involved 1 h of drug exposure followed by 1 h of either irradiation (465 nm) or darkness. PI = Propidium Iodide.

The decrease caused by the adducts of photoactivated 1 or 4 was double than that induced by the DNA adducts of FM-190 at equivalent $n_r$ values (Figure 5B). It was also verified that irradiation of ct-DNA by visible light in the absence of complexes for 2 h had no effect on EtBr fluorescence. The results of these experiments suggest that the conformational distortions induced in DNA by the adducts formed by the treatment of DNA with irradiated 1 and 4 are delocalized and extend over the base pairs around the platination sites to an extent which corresponds to the distortions induced in DNA by two adducts of mononuclear FM-190. Thus, these results are consistent with the decomposition of the dinuclear complexes 1 and 4 due to irradiation resulting in the release of two mononuclear fragments similar to those from FM-190.

It was shown previously\textsuperscript{43} that modification of DNA by irradiated FM-190 resulted also the formation of DNA interstrand cross-links. Therefore, we investigated the capability of irradiated 1 and 4 to form DNA interstrand cross-links as well. Plasmid pUC19 linearized by EcoRI (50 μg mL\textsuperscript{-1}) and the platinum complex were irradiated with blue light for 1 h and subsequently incubated in the dark for an additional 23 h in NaClO\textsubscript{4} (10 mM) at 310 K. The plasmid was modified to the extent corresponding to the $n_r$ values of 0.003 and 0.0006 (in the case of FM-190) or 0.00015 and 0.0003 (in the case of 1 and 4) and analyzed by agarose gel electrophoresis under denaturing conditions (Figure 5C). Interstrand crosslinked DNA appears as the top band (marked as ICLs) migrating on the gel more slowly than single-stranded DNA (contained in the bottom bands and marked as ss). The quantitative evaluation of the radioactivity associated with the individual bands showed that irradiated 1 and 4 formed two-fold amount of the interstrand crosslinks in DNA than FM-190 (the frequency of interstrand crosslinking was 12.5 ± 0.5, 24 ± 2 and 24 ± 2 for FM-190, 1 and 4, respectively) at the same level of modification (related to the complex). Hence, the results of DNA interstrand cross-linking experiments also support the hypothesis that the irradiation of the dinuclear complexes 1 and 4 leads to their decomposition resulting in the release of two molecules similar to the photoproduct from mononuclear FM-190. Our previous work suggested that the trans-[Pt(py)$_2$]$_2^{2+}$ fragment released from FM-190 can form GG interstrand DNA cross-links, including 5′-CG/5′-CG cross-links, 5′-GC/5′-GC cross-links and cross-links between G and the
complementary C. It is, therefore, reasonable to envisage that treatment of DNA by irradiated dinuclear 1 or 4 results in the formation of trans GG interstrand DNA cross-links as in the case of the treatment with mononuclear FM-190.

It has been shown that the binding of various antitumor metallo-drugs to closed circular DNA can cause partial unfolding of the DNA. This process lowers the superhelical density of plasmid DNA, which causes a decrease in the rate of migration through an agarose gel. This fact makes it possible to observe and quantify the mean value of unwinding per adduct. In the present study, we investigated the unwinding induced in negatively supercoiled pSP73KB plasmid by 1, 4 photoactivated by visible light (λ = 420 nm) for 1 h, then incubated for another 23 h at 310 K in the dark. The degree of supercoiling was monitored using electrophoresis in a native agarose gel. We investigated the effect of increasing amounts of the investigated platinum complexes photoactivated by visible light bound to a mixture of relaxed and supercoiled pSP73KB DNA on migration of these forms in a native agarose gel (shown in Figure 5D). The unwinding angle is given by Φ = −180/\( \sigma \)c, where σ is the superhelical density, and \( \sigma \)c is the value of \( \sigma \) (related to the complex) at which the supercoiled and nicked forms comigrate. The DNA unwinding angle determined for DNA modified by dinuclear 1 and 4 photoactivated by visible light was 57 ± 8°. This unwinding angle was two-fold higher than that found under identical conditions for DNA modified by mononuclear FM-190 (28 ± 4°). Thus, consistent with the results of the experiments aimed at characterization of DNA adducts by EtBr fluorescence and DNA interstrand cross-linking, the results of DNA unwinding experiments (Figure 5D) can be interpreted to mean that the consequence of the irradiation of the dinuclear complexes 1 and 4 is their decomposition resulting in the release of two molecules of mononuclear FM-190.

A parameter which makes identification of preferential DNA binding sites of platinum antitumor drugs possible can be obtained from transcription mapping experiments. In vitro RNA synthesis by RNA polymerases on DNA templates containing several types of bifunctional adducts of platinum complexes can be prematurely terminated at the level, or in the proximity, of DNA adducts. We prepared a 212-bp fragment by cutting of pSP73KB DNA by NdeI terminated at the level, or in the proximity, of DNA adducts. We investigated the unwinding induced in negatively supercoiled pSP73KB plasmid DNA by the investigated platinum complexes photoactivated by visible light (\( \lambda_{\text{max}} = 455 \) nm) in NaClO4 (10 mM) at 37 °C for 24 h. C) Formation of interstrand (intramolecular) cross-links by the investigated platinum complexes photoactivated by visible light (\( \lambda_{\text{max}} = 455 \) nm) in pUC19 plasmid linearized by EcoRI. Solutions of the linearized plasmid (50 \( \mu \)g mL\(^{-1} \)) were incubated with the platinum complex under irradiation conditions (for 1 h and subsequently incubated in the dark for an additional 23 h) at various \( r_\beta \) values in NaClO4 (10 mM) at 37 °C. Autoradiogram of denaturing 1% agarose gel of the DNA, which causes a decrease in the rate of migration through an agarose gel. We investigated the effect of increasing amounts of the investigated platinum complexes photoactivated by visible light bound to a mixture of relaxed and supercoiled pSP73KB DNA on migration of these forms in a native agarose gel (shown in Figure 5D). The unwinding angle is given by Φ = −180/\( \sigma \)c, where σ is the superhelical density, and \( \sigma \)c is the value of \( \sigma \) (related to the complex) at which the supercoiled and nicked forms comigrate. The DNA unwinding angle determined for DNA modified by dinuclear 1 and 4 photoactivated by visible light was 57 ± 8°. This unwinding angle was two-fold higher than that found under identical conditions for DNA modified by mononuclear FM-190 (28 ± 4°). Thus, consistent with the results of the experiments aimed at characterization of DNA adducts by EtBr fluorescence and DNA interstrand cross-linking, the results of DNA unwinding experiments (Figure 5D) can be interpreted to mean that the consequence of the irradiation of the dinuclear complexes 1 and 4 is their decomposition resulting in the release of two molecules of mononuclear FM-190.

A parameter which makes identification of preferential DNA binding sites of platinum antitumor drugs possible can be obtained from transcription mapping experiments. In vitro RNA synthesis by RNA polymerases on DNA templates containing several types of bifunctional adducts of platinum complexes can be prematurely terminated at the level, or in the proximity, of DNA adducts. We prepared a 212-bp fragment by cutting of pSP73KB DNA by NdeI and HpaI restriction endonucleases. A substantial part of its nucleotide sequence is shown in Figure S13B. This fragment contained a T7 RNA polymerase promotor (in the upper strand close to its 3'-end, Figure S13B). The experiments were carried out using this linear DNA fragment, modified by cisplatin, transplatin (in the dark), or 1 or 4 photoactivated by visible light. The samples were irradiated for 1 h and subsequently incubated in the dark for additional 23 h. The \( r_\beta \) values were in the range of 0.004 - 0.01 and are indicated in the legend to Figure S13A. The major stop signals for DNA modified by dinuclear 1 and 4 (Figure S13) were identical to those found previously under identical conditions for DNA modified by irradiated FM-190.

In order to obtain further information on the sequence-specificity of DNA binding in dinuclear 1 and 4 analogues of complex FM-190, a DNase I footprinting experiment was also performed (Figure S14). The details of this experiment are described in the Supporting information. The results show that the preferential binding sites of dinuclear 1 and 4 are those containing mainly G/A-rich sequences (related to the complex) at which the supercoiled and nicked forms comigrate. The DNA unwinding angle determined for DNA modified by dinuclear 1 and 4 photoactivated by visible light was 57 ± 8°. This unwinding angle was two-fold higher than that found under identical conditions for DNA modified by mononuclear FM-190 (28 ± 4°). Thus, consistent with the results of the experiments aimed at characterization of DNA adducts by EtBr fluorescence and DNA interstrand cross-linking, the results of DNA unwinding experiments (Figure 5D) can be interpreted to mean that the consequence of the irradiation of the dinuclear complexes 1 and 4 is their decomposition resulting in the release of two molecules similar to photoproducts from mononuclear FM-190.

**CONCLUSIONS**

We report the synthesis and characterization of a series of novel dinuclear photoactive platinum(IV) antitumor prodrugs trans, trans-\{\text{Ph(NH}_{3})_{2}\text{py} \text{Cl}_{2}\} \text{OC(O)CH}_{2} \text{C(O)NH}_{3}) \text{R}\) containing pyridine (py) and bridging dicarboxylate (R = -CH_{2}CH_{2}CH_{2}CH_{2} - (4)): \text{trans-1,2-C}_{2} \text{H}_{4} \text{H}_{2} - (\text{2}); \text{p-C}_{6} \text{H}_{5} - \text{CH}_{2} \text{CH}_{2} \text{CH}_{2} \text{C(O)NH}_{3}) \text{R} - \text{trans-1,2-C}_{2} \text{H}_{4} \text{H}_{2} - (\text{3); p-C}_{6} \text{H}_{5} - \text{CH}_{2} \text{CH}_{2} \text{CH}_{2} - (4)}) ligands. We compared their photoactivity with that of a mononuclear analogue FM-190. Azidyl and hydroxyl radicals and PtH\(_{2}\) species were generated during the photodecomposition of these dinuclear complexes. LC-MS
analyses of photoreactions of dinuclear complex 4 revealed the photorelease of the bridging ligand and formation of mononuclear photoproducts. In the presence of 5′-GMP, the PtIII-5′-GMP adduct ([Pt(CH2CN)(py)2(GMP-H)]+) (756.09) was formed upon irradiation. Dinuclear complexes exhibited promising photocytotoxicity upon irradiation with low-dose blue light (465 nm, 4.8 mW/cm², 1 h, 1 h) towards a series of human cancer cell lines, including A2780, A2780cis, and OE19, with high dark stability. Moreover, the dinuclear complexes were remarkably nontoxic towards normal cells (MRC5), even after irradiation. The introduction of an aromatic bridging ligand enhanced cell uptake significantly for complex 3 when compared with the complexes containing an aliphatic bridge. The inhibition of progression from G2/M to G0/G1 stages in the cell cycle induced by dinuclear complexes upon irradiation suggests that DNA is a target. The dinuclear complexes behaved in cell-free media and after photoactivation similarly to the photoactivated mononuclear complex FM-190 in terms of the kinetics of binding to ct-DNA, transcription mapping and DNase I footprinting of Pt-DNA adducts. However, dinuclear complexes after photoactivation were approximately two-fold more effective in quenching fluorescence of EtBr bound to DNA, forming DNA interstrand cross-links and unwinding DNA compared to photoactivated mono-Pt analogue FM-190. This is consistent with photodecomposition of the dinuclear complexes into two molecules of mononuclear platinum(II) species. Hence, dinuclear complexes exhibit promising dark stability, photocytotoxicity and selectivity towards cancer cells, dependent on the nature of the bridging ligand.

ASSOCIATED CONTENT
Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.inorgchem.********.

1H NMR, 13C NMR spectra for all dinuclear complexes and UV–vis spectra (PDF). X-ray crystallographic files in CIF format for complexes 1 and 4. Reactions with 5′-GMP, DNA binding and transcription mapping.

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Notes
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REFERENCES
9. Van der Veer, J. L.; Peters, A. R.; Reedijk, J. Reaction products from platinum(IV) amine compounds and 5′-GMP are mainly bis(5′-GMP) platinum (II) amine adducts. J. Inorg. Biochem. 1986, 26, 137–142.


42. Muhammad, N.; Sadia, N.; Zha, C.; Luo, C.; Guo, Z.; Wang, X. Biotin–tagged platinum(IV) complexes as targeted...


Synopsis

Novel *all-trans* dinuclear platinum(IV) complexes bridged by a dicarboxylate linker, highly stable in the dark, generate azidyl and hydroxyl radicals on irradiation with blue light. They are photocytotoxic to human cancer cells, whereas cisplatin was inactive under these conditions, and more photoactive towards cisplatin-resistant ovarian cancer cells compared to wild-type cells. Remarkably, the dinuclear complexes were relatively non-toxic toward normal human cells. Cell cycle and DNA binding experiments suggested that DNA is a target.