Diazido Mixed-Amine Platinum(IV) Anticancer Complexes Activatable by Visible-Light Form Novel DNA Adducts

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Abstract: Platinum diam(m)ine complexes, such as cisplatin, are successful anticancer drugs, but suffer from problems of resistance and side-effects. Photoactivatable PtIV prodrugs offer the potential of targeted drug release and new mechanisms of action. We report the synthesis, X-ray crystallographic and spectroscopic properties of photoactivatable diazido complexes trans,trans-[Pt(N3)2(OH)2(MA)(Py)] (1; MA = methylamine, Py = pyridine) and trans,trans-[Pt(N3)2(OH)2(MA)(Tz)] (2; Tz = thiazole), and interpret their physicochemical properties by TD-DFT modelling. The orientation of the azido groups is highly dependent on H bonding and crystal packing, as shown by polymorphs 1p and 1q. Complexes 1 and 2 are stable in the dark towards hydrolysis and glutathione reduction, but undergo rapid photoreduction with UVA or blue light with minimal amine photodissociation. They are over an order of magnitude more potent towards HaCaT keratinocytes, A2780 ovarian, and OE19 oesophageal carcinoma cells than cisplatin and show particular potency towards cisplatin-resistant human ovarian cancer cells (A2780cis). Analysis of binding to calf-thymus (CT), plasmids, oligonucleotide DNA and individual nucleotides reveals that photoactivated 1 and 2 form both mono- and bifunctional DNA lesions, with preference for G and C, similar to transplatin, but with significantly larger unwinding angles and a higher percentage of interstrand cross-links, with evidence for DNA strand cross-linking further supported by a comet assay. DNA lesions of 1 and 2 on a 50 bp duplex were not recognised by HMGB1 protein, in contrast to cisplatin-type lesions. The photo-induced platination reactions of DNA by 1 and 2 show similarities with the products of the dark reactions of the PtII compounds trans-[PtCl2(MA)(Py)] (5) and trans-[PtCl2(MA)(Tz)] (6). Following photoactivation, complex 2 reacted most rapidly with CT DNA, followed by 1, whereas the dark reactions of 5 and 6 with DNA were comparatively slow. Complexes 1 and 2 can therefore give rapid potent photocytotoxicity and novel DNA lesions in cancer cells, with no activity in the absence of irradiation.

Keywords: antitumor agents · DNA binding · medicinal chemistry · photoactivity · platinum

Introduction

Platinum-based anticancer drugs (e.g., cisplatin, cis-[PtCl2(NH3)2]) are amongst the most important antitumour agents currently available in the clinic, and have proved to be highly effective towards a variety of solid tumours. However, severe side-effects and intrinsic or acquired resistance can limit the scope of their application. To overcome these drawbacks a number of new methods are being investigated, including the strategy of prodrugs. We have previously reported photoactivatable PtIV diazodihydroxido anticancer complexes (e.g., trans,trans,trans-[Pt(N3)2(OH)2(NH3)(Py)] (3) and trans,trans,trans-[Pt(N3)2(OH)2(Py)2] (4, Scheme 1) which are inert and nontoxic in a biological environment in the dark. Upon irradiation with light, these complexes can be selectively activated to become potently cytotoxic towards a number of cancer cell lines. It was demonstrated previously that replacing one or two NH3 ligands with pyridine (Py) in trans,trans,trans-[Pt(N3)2(OH)2(NH3)2] leads to higher photocytotoxicity and visible-light activation. Here we show that complexes which incorpo-
The syntheses of complexes trans-[PtCl₂(MA)(Py)] (5) and trans-[PtCl₂(MA)(Tz)] (6) were carried out by a method analogous to that of Kauffman and Cowan.[9] The PtIV diazidodihydroxido complexes trans,trans-trans-[Pt(N₃)₂(OH)₂(MA)(Py)] (1) and trans,trans,trans-[Pt(N₃)₂(OH)₂(MA)(Tz)] (2) were synthesised by oxidation of the respective trans-PtII diazido complexes.[5] Complex trans,trans-trans-[Pt(N₃)₂(OH)₂(NH)₂(Tz)] (9) has been reported previously.[9] X-ray crystal structures of 1 and 2 are depicted in Figure 1.

For complex 1, polymorphs 1p and 1q were isolated under similar conditions, but from different batches. The complexes exhibit octahedral geometry and Pt–ligand bond lengths are similar to those of reported related complexes with the nitrogen atoms of the azido ligands adopting almost linear conformations. Other structural features and crystallographic data are summarised in the Supporting Information.

**Dark stability:** Complexes 1 (trans,trans-trans-[Pt(N₃)₂(OH)₂(MA)(Py)]) and 2 (trans,trans-trans-[Pt(N₃)₂(OH)₂(MA)(Tz)]) have good aqueous solubility (>40 μmol, unbuffered) and are very stable in water and in EBSS (Earle’s balanced salt solution, pH 7.2–7.6), a biological cell culture medium, for >7 months in the dark, as monitored by ¹H NMR spectroscopy. Complexes 1 and 2 (3.9 μmol) did not react with 5'-GMP or L-ascorbic acid (2 mol equiv) in aqueous solution (initial pH adjusted to 7.4) in the dark for three days. They react with glutathione (GSH); 2% of complex 1 reacted after three days at ambient temperature.

**Spectroscopic properties and photolysis study by UV/Vis spectroscopy:** Complexes 1 and 2 exhibit similar absorption maxima at 289 nm (N₃-to-PtIV CT band, Figure 2) and the absorption tail extends up to about 500 nm (for extinction coefficients at various wavelengths, see Table 1). Absorption bands not only at 365 nm but also at 420 and 450 nm were observed and were hence utilised in the photoactivation and cytotoxicity studies. Irradiation of an aqueous solution of 1 and 2 with ss-DNA, as depicted in Figure 1, results in the formation of PtD–DNA adducts, as monitored by ¹H NMR spectroscopy. Complexes 1 and 2 (3.9 μmol) did not react with 5'-GMP or L-ascorbic acid (2 mol equiv) in aqueous solution (initial pH adjusted to 7.4) in the dark for three days. They react with glutathione (GSH); 2% of complex 1 reacted after three days at ambient temperature. The toxicities of complexes 1, 2 and also of trans,trans-trans-[Pt(N₃)₂(OH)₂(NH)₂(Tz)] (9) towards several carcinoma cell lines, including a cisplatin-resistant cancer cell line (A2780cis) in the presence of UVA/blue light are investigated. Also, the reactivity of complexes 1, 2, 5, and 6 with a 12-mer oligodeoxyribonucleotide and natural high-molecular-mass DNA, as well as the properties of their Pt–DNA adducts, are studied.

### Results

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or 2 at 365, 420 or 450 nm resulted in a decrease in intensity of absorption at 289 nm (Figure 3), indicating loss of the PtIV–N3 bond(s).

**DFT and TD-DFT calculations:** DFT and TD-DFT were employed to gain insights into the photochemistry of complexes 1, 2 and 9. Singlet excited-state transitions were calculated from the ground state geometries of the complexes (Figures S7, S9 and S11 in the Supporting Information), and are in good agreement with the experimental data (Figure 3, and absorption spectrum for 9). The presence of low-energy weak absorption bands was confirmed by calculations (Figure 2), which show that transitions with low oscillator strength are present in the 420–430 nm region. As reported for other PtIV–azido complexes, such transitions involve strongly s-antibonding LUMOs (Figure 2, inset) conferring dissociative character to the corresponding excited states. They have a mixed LMCT and d-d nature, whereas the transitions composing the main absorption in the UV region are mostly LMCT. DFT geometry optimisation of lowest-lying triplet states for 1, 2 and 9 shows that these are also dissociative and ligand release/Pt reduction can occur, as well, from the triplet manifold after intersystem crossing. A full description of the computational results is in the Supporting Information.

**Photocytotoxicity:** The photoactivated dose-dependent inhibition of cell viability (IC50 values) for complexes trans,trans,trans-[Pt(N3)2(OH)2(MA)(Py)] (1), trans,trans,trans-[Pt(N3)2(OH)2(MA)(Tz)] (2), and trans,trans,trans-[Pt(N3)2(OH)2(NH3)(Tz)] (9) towards human cell lines are summarised in Table 2. The corresponding data for cisplatin (7), trans,trans,trans-[Pt(N3)2(OH)2(NH3)(Py)] (3)[5] and trans,trans,trans-[Pt(N3)2(OH)2(Py)2] (4)[6] are also listed for comparison.

In the absence of light, complexes 1 and 2 were not substantially cytotoxic to HaCaT human keratinocytes, cisplatin-sensitive A2780 ovarian adenocarcinoma cells, the cisplatin-resistant subline A2780cis or OE19 oesophageal adenocarcinoma cells under the experimental conditions used. Complex 9 did succeed in killing about 50% of the A2780 cells at about 187 μm (Table 2).

Upon irradiation with UVA (5 J cm⁻²; λmax = 365 nm), the cytotoxicities of complexes 1, 2 and 9 dramatically increased and were significantly greater than that of cisplatin (50–65-fold) under the experimental conditions used. Visible blue light also caused cell death in the presence of the complexes. The confidence intervals for the blue light experiments tended to be wider, and this might not be surprising given that the UVA waveband is closer to the absorption maximum of the complexes. In addition, other endogenous phototoxic chromophores, such as porphyrins, may be influenced by blue light irradiation, in vitro.

Complexes 1 and 2 were similarly photocytotoxic towards A2780 cells upon irradiation with UVA compared to previously reported complexes 3 and 4, but they were about threefold more toxic towards the cisplatin-resistant sub-line
with UVA. Care was taken during these experiments to ensure no intrusion of UV photons during the visible light treatments.

Substituting the pyridine group of 3 with the thiazole group of 9 increased UVA phototoxicity in HaCaT and A2780cis cells, and increased blue visible light phototoxicity in HaCaT cells by about four-fold. No change in phototoxicity was seen in A2780 cells, and in fact the presence of the thiazole group increased cytotoxicity to these cells. Addition of a methylamine group (2) seemed to decrease the cytotoxicity of the thiazole group without decreasing phototoxicity, and in fact may have slightly increased the phototoxicity of UVA and blue visible light. The resistance factor of 2 and 9 in the ovarian carcinoma paired cell line (A2780/ A2780cis) was 1.7 and 1.8, respectively, with overlapping confidence intervals. This compares with a resistance factor of 5.5 obtained with 3, suggesting that the thiazole-containing molecules were more effective towards the resistant cell line. The phototoxicity of UVA and blue light was also increased by 2 in the oesophageal carcinoma cells. Substituting a methylamine group (1) in place of the ammine group of 3

A2780cis. It is notable that the photocytotoxicity of complexes with visible light are not dissimilar to when irradiated with UVA. Care was taken during these experiments to ensure no intrusion of UV photons during the visible light treatments.

Figure 3. UV/Vis spectra for complexes 1 (30 µM) and 2 (60 µM) in H2O upon irradiation at 365 nm (A and D, light dose: 0, 0.7, 2.1, 4.2, 8.4 J cm⁻²), 420 nm (B and E, light dose: 0, 1.5, 4.6, 9.2, 18.4 J cm⁻²) and 450 nm (C and F, light dose: 0, 15, 45, 90, 180 J cm⁻²). Arrows indicate change in absorbance upon irradiation with light over the time of experiment.

Table 2. Phototoxicity of PtIV complexes 1, 2, and 9, in comparison with complexes 3, 4 and cisplatin (7).

| UVA | HaCaT TL03[a] | sham[a] | UVA | A2780 TL03 | sham | UVA | A2780cis TL03 | sham | UVA | OE19 TL03 | sham |
|-----|---------------|--------|-----|-----------|------|-----|--------------|------|-----|----------|------|
| 1   | 2.6 (18.3–3.8) | 14.7   | >236.3 2.3 | 6.6  | >236.3 4.4 | 13.2 | >236.3 1.7 | 10.1 | >236.3 |
| 2   | 3.5 (10.8–19.9) | 14.7   | >232.9 3.2 | 28.2 | >232.9 5.3 | 6.4  | >232.9 6.2 | 19.3 | >232.9 |
| 3   | 6.8 (2.7–4.5) | >244.4 3.1 | NT[a] | >244.4 16.9 | NT[a] | >244.4 10.0 | 32.0 | >244.4 |
| 4   | 8.5 (8.2–14.8) | >244.4 3.1 | NT[a] | >244.4 16.9 | NT[a] | >244.4 10.0 | 32.0 | >244.4 |
| 5   | 11.2 (5.4–8.6) | >244.4 3.1 | NT[a] | >244.4 16.9 | NT[a] | >244.4 10.0 | 32.0 | >244.4 |
| 6   | 14.7 (6.0–32.2) | >244.4 3.1 | NT[a] | >244.4 16.9 | NT[a] | >244.4 10.0 | 32.0 | >244.4 |
| 7   | 13.9 (124–166) | >244.4 3.1 | NT[a] | >244.4 16.9 | NT[a] | >244.4 10.0 | 32.0 | >244.4 |
| 8   | 19.3 (153–195) | >244.4 3.1 | NT[a] | >244.4 16.9 | NT[a] | >244.4 10.0 | 32.0 | >244.4 |
| 9   | 32.0 (133–172) | >244.4 3.1 | NT[a] | >244.4 16.9 | NT[a] | >244.4 10.0 | 32.0 | >244.4 |

[a] The concentration of complex that inhibited dye uptake by 50%. The lower value indicates the higher toxicity to cells. Each value is the mean of two or three independent experiments performed in triplicate. The figures in brackets are the 95% confidence intervals for the IC50 values. [b] TL03 is a blue fluorescence source (λmax=420 nm). [c] Sham irradiated samples. [d] Greater than sign indicates an IC50 value greater than the concentration range used. [e] Not tested.
increased UVA phototoxicity in HaCaT, A2780 and A2780cis cells, but there was no change in the percentage of OE19 cells surviving the treatment. In contrast, the phototoxicity of complex 1 was greater than that of complex 3, when irradiated with blue light, in both cell lines tested (HaCaT and OE19). The bis-pyridine complex (4) was generally the most UVA-photoactive of complexes tested across the panel of cell lines. Compared to 3, it was also more effective when activated with blue light. However, although the confidence interval for photoactivated 4 in A2780cis cells was wide, nevertheless the resistance factor, similar to 3, was higher than for 1 or 2.

It should be noted that the phototoxicity assay is not a proliferation assay. The cytotoxicity of cisplatin using a conventional, constant challenge proliferation (MTT) assay was determined, and gave IC50 values of 0.5 μM for A2780 cells and 11.0 μM for A2780cis cells (these data are comparable with those in Table 3 for cisplatin obtained using the SRB assay).

To better understand the high photocytotoxicity of complexes 1 and 2 we investigated their photochemical reactivity towards both 5′-guanosine monophosphate (5′-GMP) and DNA.

**Photoreactions with 5′-guanosine monophosphate:** The photodecomposition characteristics of complexes 1 and 2 in aqueous solution were similar to previously reported PtIV azido complexes,[11] and are summarised in the Supporting Information. Since guanine is a preferred target for DNA platination of PtIV complexes, such as cisplatin,[12] photochemical reactions of complexes 1 and 2 with 5′-GMP in aqueous solution were investigated.

Complexes 1 and 2 (3.9 mM) did not react with 5′-GMP (2 mol equiv) in water (initial pH adjusted to 7.4) in the absence of light over a period of three days, as judged by 1H NMR spectroscopy. 1H NMR spectroscopic signals at δ = 8.72, 8.30, 7.84 and 2.42 ppm were assigned to H1a, H2a, H4a of the Py ligand and CH2 of the MA ligand, respectively, and δ = 8.12 ppm to H6 of free 5′-GMP (Figure 4).

During the photoreaction of complex 1 (3.9 mM in D2O) with 5′-GMP (2 mol equiv) upon irradiation at 450 nm, the yellow colour of the solution grew deeper, and gas bubbles formed. No precipitate was observed. After irradiation for 1 h, the signals for complex 1 nearly disappeared and a new major product was formed with δ = 2.25 and 8.86 ppm, assignable to CH2 of MA and H6 of Pt coordinated 5′-GMP in (SP-4-2)-[Pt(N3)(MA)(Py)(5′-GMP)] (1b). The same chemical shifts were observed for an authentic sample of 1b. According to the 1H NMR spectra, the reaction between complex 1 and 5′-GMP had almost finished after irradiation for 1 h, and longer exposure to light caused no obvious change to the major product but only the decomposition of the side products. Little MA was released (1% after 1 h and 4% after 3 h of irradiation), as monitored by 1H NMR spectroscopy (δ(CH3) = 2.59 ppm). The chemical shift of MA was confirmed by spiking the irradiated NMR sample with free MA. Similar results were obtained for reaction of complex 2 with 5′-GMP, but the reaction took only about 15 min to reach completion, forming the mono-GMP/N3− adduct. Details are given in the Supporting Information.

HPLC-ESI-MS analysis was used to examine the products of the photochemical reactions of complexes 1 or 2 with 5′-GMP. An aqueous solution of complex 1 (0.5 mM) and 5′-GMP (1.0 mM) in H2O (initial pH adjusted to 7.4) was irradiated at 450 nm for 1 h at ambient temperature (298 K). The chromatogram is shown in Figure 5A and all the major peaks were identified by ESI-MS. The peak with a retention time (tR) = 3.46 min was assigned as 5′-GMP, the peak at tR = 11.10 min as trans-[Pt(5′-GMP)],2−[H1] (1a) and at tR = 11.47 min as (SP-4-2)-[Pt(N3)(MA)(Py)(5′-GMP)]−[H] (1b). The same retention times were obtained for the synthetic samples of the same complexes. The MS isotope distributions agree well with their corresponding simulations (Figure S15 in the Supporting Information).

This result indicated that one azido ligand and two PtIV bound hydroxyl groups were released during irradiation. The azido ligand is likely to be released as an azidyl radical[13] with a further electron being donated by a hydroxide ligand resulting in reduction of PtIV to PtIII and formation of PtIII 5′-GMP complexes. Small amounts of complex 1 also lost the second azido ligand to form a bis-GMP adduct. The ratio of integrals of peaks in the chromatogram 1a/1b is 11.89. The irradiation at 450 nm of the reaction mixture was then continued for another 2 h, and the HPLC chromatogram is shown in Figure 5B. The difference between the two chromatograms (Figure 5A and B) is very small; the intensi-
ty of the peak for \(1a\) increased slightly. The ratio of \(1a/1b\) increased to 15:85. This result suggests that the mono-GMP/N\(^3\)/C0 adduct can slowly lose N\(^3\)/C0 and form the bis-GMP adduct. Then this resulting reaction mixture was irradiated with UVA for 1 h and again analysed by HPLC-ESI-MS (Figure 5C). In this chromatogram, the signal corresponding to \(1b\) had completely disappeared, and the signal for \(1a\) increased by 36% compared to that in Figure 5B, relative to the signal of 5'-GMP. Therefore, \(1b\) is not stable to irradiation with UVA, and is transformed into the new species \(1a\) with the loss of the N\(^3\)/C0 (Scheme 3) and other unidentified species.

When complex 1 and 5'-GMP were irradiated with UVA for 60 min, \(1a\) was the only assignable product; the chromatogram is shown in Figure 5D. This result suggests that, as shown in Scheme 3, irradiation with UVA induced loss of two hydroxido ligands and two azido ligands and Pt binds to two 5'-GMP molecules to form \(1a\), meanwhile the Pt\(^{IV}\) was reduced to Pt\(^{II}\). By contrast, irradiation at 450 nm released two hydroxido ligands but only one azido ligand, and Pt binds to one 5'-GMP to form \(1b\). Thereafter, the irradiation with UVA can transform a small portion of \(1b\) to \(1a\). The photoreaction of complex 2 with 5'-GMP followed a course very similar to that of complex 1, as indicated by HPLC and MS (summarised in the Supporting Information). This result is consistent with the observations by LC-MS/MS reported previously of the loss of one hydroxido and one azido ligand upon photoreduction of compound 3, the ammine analogue of 1.\(^{14}\)

The products from photoreaction of complexes 1 or 2 with 5'-GMP were also monitored by \(^{195}\)Pt NMR spectroscopy and the chemical shifts were assigned by comparison with related complexes.\(^{13}\) A solution of complex 1 (3.9 mM) and 5'-GMP (7.8 mM) in D\(_2\)O (initial pH adjusted to 7.4) was irradiated at 450 nm in an NMR tube and the \(^{195}\)Pt NMR spectrum was recorded at various time intervals (Figure 6A–C). Before irradiation, there was only one signal at \(d = 892\) ppm for complex 1. During the first 30 min of irradiation, the peak decreased in intensity and a new signal (\(d = 2328\) ppm) in Pt\(^{II}\) region appeared, which was assigned as \(1b\). The assignment of this new signal was confirmed by a synthesised sample of \(1b\). The signal for complex 1 completely disappeared after irradiation of the sample for 1 h, whereas the signal of \(1b\) was still present. The NMR spectrum did not change much over the following 2 h of irradiation (data not shown). This photoproduct was quite stable and was not re-oxidised to Pt\(^{IV}\) species after one week of storage in the dark at ambient temperature. Similar \(^{195}\)Pt NMR spectro-
scopic experiments were performed for complex 2 (Figure 6D and E). The peak for complex 2 (δ = 907 ppm) completely disappeared after only 15 min of irradiation with 450 nm light. A new signal appeared (δ = −2296 ppm), which was assigned as 2b (SP-4-3)-[Pt(N₃)(MA)(Tz)](5'-GMP−H)], confirmed by the ¹⁹⁷Pt NMR spectrum of a synthesised sample.

**Geometry optimisation calculations of the lowest-lying triplet states:** It was observed that complex 2 reacted with 5'-GMP (Figure 6) and DNA (Figure 7) faster than complex 1 upon irradiation with blue light. Photodecomposition of complexes 1, 2 (and 9) can be directly promoted by light excitation and population of the dissociative singlet excited states previously described. Since population of triplet states can be also responsible for the photochemistry, we performed geometry optimisation calculations of the lowest-lying triplet states for 1, 2 and 9. Interestingly, upon intersystem crossing and triplet formation all complexes display a distorted geometry where the two Pt–azido bonds are significantly lengthened. In particular, complex 1 shows an increase in the Pt–N(N₃) distances of 0.32 and 0.47 Å, whereas in the case of 2 and 9 one Pt–azide bond is elongated by 0.23 Å and the other is highly elongated (released) with Pt–N(N₃) distances of >3.70 Å (Supporting Information Table S13). These results explain the higher photoreactivity in solution observed for 2.

Formation of Pt¹⁺ species agrees with the results obtained by DFT calculations on the lowest-lying triplet states of 1 and 2. As demonstrated previously,[¹⁶] such states play a fundamental role in the photochemistry of d⁶ metal complexes. Complexes 1 and 2 have a highly distorted lowest-lying triplet geometry, showing an elongated Pt–N(azide) distance and a reduced positive charge on the Pt centre, consistent with azide release and reduction from PtIV to Pt¹⁺.

**Photoreactions with 5'-CMP or 5'-AMP:** The photoreactions of complexes 1 or 2 with 5'-cytidine monophosphate or 5'-adenosine monophosphate upon irradiation at 450 nm for 60 min were also examined by LC-MS. Products of the type [Pt(N₃)(MA)(Py/Tz)(5'-AMP/5'-CMP)] were observed (Figure S18 in the Supporting Information).

**Photo-induced binding with DNA oligonucleotide:** A self-complementary 12-mer DNA, d(TATGGTACCATA), (ss-DNA I) was selected for study since it contains a GG sequence, which is usually the preferred binding site for cis-di(am)m(ine platinum drugs).[¹⁷] A solution containing complex 1 (trans,trans,trans-[Pt(N₃)₂(OH)₂(MA)(Py)], 100 μM) with ss-DNA I in a 1:1 mol ratio (initial pH adjusted to 7.4) was irradiated for 1 h with 450 nm light at 298 K, and the products were characterised by ESI-HR-MS (Figure 8). The major species in the reaction mixture was unreacted ss-

![Figure 7. Kinetics of the binding of photoactivated: A) 1, and B) 2 (420 nm light for 30 min and subsequently incubated in the dark); and C) 5, and D) 6 (in the dark) to calf thymus DNA in NaClO₄ (10 mM) at 310 K, as determined by differential pulse polarographic assays.](image)

![Figure 8. ESI-HR-MS (negative mode) spectrum for the photoreaction of complex 1 (100 μM) with ss-DNA I (1:1, in H₂O, initial pH adjusted to 7.4, 450 nm, 1 h, 298 K). Species are also present as nNa⁺ adducts. The m/z values for 1 were used for internal linear calibration.](image)
DNA I. A series of platinated products was also observed, including a monofunctional platinum–DNA adduct with one N3 group remaining on Pt, \([\text{I}+\text{Pt(N3)(MA)(Py)}]\). The MS signals detected are listed and assigned in Table S15 in the Supporting Information. A series of weak signals corresponding to the loss of the second N3 group was also found, assignable as \([\text{I}+\text{Pt(MA)(Py)}]^-\) (Figure S19 and Table S16 in the Supporting Information). The signals assignable to a bifunctional DNA adduct \([\text{I}+\text{Pt(MA)(Py)}]^-\) and \([\text{I}+\text{Pt(MA)(Py)}]+\text{Pt(MA)(Py)}(\text{N3})^-\) were also found. The sample was stored in the dark at ambient temperature for 100 days after irradiation and analysed by ESI-HR-MS again (Figure S20 in the Supporting Information).

The monofunctional adduct \([\text{I}+\text{Pt(N3)(MA)(Py)}]\) was still present. It is notable that the intensity of the signals corresponding to possible bifunctional DNA intrastrand CLs \([\text{I}+\text{Pt(MA)(Py)}]^-\) increased, even higher in intensity than for the monofunctional DNA adduct. This suggests that platinum binding to DNA is very stable, and that the monofunctional adducts can lose the N3 and be converted to bifunctional adducts slowly in aqueous solution. The isotopic distributions of the observed DNA adducts \([\text{I}+\text{Pt(N3)(MA)(Py)}]\) and \([\text{I}+\text{Pt(MA)(Py)}]+\text{Pt(MA)(Py)}(\text{N3})^-\) agree with these assignments (Figure S21 in the Supporting Information). The reaction of complex 2 and ss-DNA I (2:1) was also monitored by ESI-HR-MS. Similar adducts were formed, but a higher amount of bifunctional Pt–DNA adduct was formed, as shown in the Supporting Information.

These results suggested that complexes 1 \((\text{trans,trans,trans-}[^\text{Pt(N3)(OH)}(\text{MA})(\text{Py})])\) and 2 \((\text{trans,trans-}[^\text{Pt(N3)(OH)}(\text{MA})(\text{Tz})])\) can rapidly form DNA adducts upon irradiation with blue light, and that although the major products are monofunctional adducts, bifunctional adducts are also formed (Scheme 2). Higher levels of possible bifunctional adducts were formed in the photoreaction with ss-DNA than with nucleoside monophosphates (Figures 5 and 6). Complex 2 appeared to generate more bifunctional adducts than complex 1. The binding site was not identified; it is possible that 1,3-intrastrand CLs, such as GXA, AXA or CXA or longer intrastrand CLs, are formed.

Cytotoxicity of platinum(II) precursors: To investigate the relationship between the PtIV diazidohydroxido complexes and their PtII dichlorido analogues \(\text{trans-}[^\text{PtCl2(AM)(Am2)}]\) (where Am = amine), the cytotoxicities of complexes \(\text{trans-}[^\text{PtCl2(AM)(Py)}]\) (5) and \(\text{trans-}[^\text{PtCl2(AM)(Tz)}]\) (6) towards A2780 and its cisplatin resistant sub-line A2780cis were determined. The toxicity was not photoinduced, as the complexes are not photoactive. The data and comparison with cisplatin (7), transplatin (8) and some published \(\text{trans-PtIV}\) compounds are listed in Table 3. Complexes 5 and 6 are highly cytotoxic towards A2780 and A2780cis cells compared to the reported \(\text{trans-PtIV}\) compounds.[18–22] Complex 5 is about three-times more cytotoxic than complex 6 towards A2780 cells. More importantly, 5 and 6 are highly potent towards cisplatin-resistant cell lines; the IC50 values for A2780 and A2780cis cells are very similar (resistance factor ≈1). These results support the phototoxicity data.

**Table 3. Cytotoxicity**[a] for \(\text{trans-}[^\text{PtCl2(AM)(Am2)}]\) complexes towards the A2780 cell line in comparison with their dichlorido PtII analogues.

| Complex | Am1 | Am2 | IC50 [μM][b] | Resistance factor |
|---------|-----|-----|-------------|-----------------|
| 5       | MA  | Py  | 2.4 ± 0.7   | 3.0 ± 0.7       | 1.25            |
| 6       | MA  | Tz  | 6.9 ± 2.3   | 6.8 ± 0.9       | 1.0             |
| 7[4]    | NH3 (cis) | NH3 (cis) | 1.5 ± 0.3 | 10.5 ± 0.3 | 7.0 |
| 8       | NH3 | > 200 | –            | –               | –               |

[a] Ambient light conditions. [b] Data for 5–8 are the means of two independent experiments performed in triplicate. [c] This work. [d] Cisplatin.
Characterisation of DNA adducts: The experiments aimed at the characterisation of DNA adducts of photoactivated 1 or 2, and of 5 or 6 in the dark were conducted employing thiourea as a probe of DNA monofunctional adducts of trans-platinum(II) compounds[24] (for details, see the Supporting Information). After 24 h, 55±(±10), 48±(±2), 66±(±1) and 64±(±3)% of the total adducts of platinum complexes 1, 2, 5 and 6, respectively, were displaced from DNA by thiourea (Figure 10). Thus, only 45, 52, 34 and 36% of DNA adducts formed by 1 or 2 (photoactivated) and 5 or 6 (in the dark), respectively, had evolved to bifunctional lesions after this time interval.

**Interstrand cross-links:** In this experiment, interstrand cross-linking efficiency of photoactivated 1 or 2, and of 5 or 6 in the dark was investigated using pSP73KB plasmid DNA. The DNA samples were treated with complexes 5 or 6 in the dark or with 1 or 2 under irradiation conditions and then were analysed by agarose gel electrophoresis under denaturing conditions. The interstrand cross-linked DNA appears in the autoradiogram as the top bands (Figure 11), which migrate more slowly than the single-strand DNA (the bottom bands). The frequencies of interstrand cross-links formed by photoactivated 1 or 2, and 5 and 6 (in the dark) were 23±(±5), 19±(±3), 9±(±3) and 16±(±2)% respectively.

**Comet assay:** Cross-links were also studied in HaCaT and OE19 cells using the comet assay immediately following photolysis (Figure S24 in the Supporting Information). Photoactivation of 1 with blue light, or 2 with UVa or blue light, increased DNA cross-linking as measured by the assay. The comet assay data strongly support the phototoxicity trends (Table 2) and the data reported in Figures 10 and 11. Unirradiated complexes 1 and 2 did not significantly affect DNA cross-linking in the assay.
Unwinding of negatively supercoiled DNA: The unwinding induced in negatively supercoiled pUC19 plasmid DNA by treatment with photoactivated 1 or 2 or with 5 or 6 in the dark was measured. The degree of supercoiling was monitored using electrophoresis in native agarose gels.

HMGB1 protein recognition: The interactions of high mobility group protein B1 (HMGB1) with a 50 bp duplex DNA adducts formed by 1 or 2 under irradiation conditions and 5 or 6 in the dark were investigated using gel mobility shift assay. HMGB1 protein exhibited no detectable binding to the duplexes modified by photoactivated 1 or 2 and 5 or 6 in the dark as well as to the unmodified 50 bp duplex, whereas the duplex containing the adducts of cisplatin was strongly bound by HMGB1 (Figure 13). This indicates that complexes 1, 2, 5 and 6 exhibit a different mechanism of antitumor activity from that of cisplatin.

Discussion

Stability of non-leaving groups: Previous reports have examined the photoproducts of PtIV diazido complexes. Considerable loss of NH3 was observed during irradiation of trans,trans-[Pt(N3)2(OH)2(NH3)]2+ and cis,trans-[Pt(N3)2(OH)2(NH3)]2+. On the other hand, the loss of Py from PtIV complexes, such as trans,trans-[Pt(N3)2(OH)2(Py)]2+ (4), was very low. It is widely accepted that the non-leaving groups, also called “carrier ligands”, play a very important role in the anticancer activity. These ligands may affect the distortion of damaged DNA and enzyme recognition in DNA transcription and repair synthesis. Taking cisplatin as an example, the loss of ammine may partly account for the deactivation and resistance to the drug in cancer cells. It was reported that when a pyrimidine or other bulky group is present in the cis position of the DNA binding site, the rotation range of the Pt-carrier ligand fragment is significantly restricted and hence the translocation is sterically blocked. Therefore, the retention of MA in complexes 1 (trans,trans-[Pt(N3)2(OH)2(MA)(Py)]) and 2 (trans,trans-[Pt(N3)2(OH)2(MA)(Tz)]) following irradiation suggests that it would also remain coordinated to Pt when the complex binds to cellular DNA. The high stability of Pt–MA bonds may therefore contribute to their potent photocytotoxicity.

Photoreaction with nucleobases and DNA: Complexes 1 (trans,trans-[Pt(N3)2(OH)2(MA)(Py)]) and 2 (trans,trans-[Pt(N3)2(OH)2(MA)(Tz)]) can bind to 5’-GMP or DNA rapidly upon irradiation with light (Figures 5–7). In comparison, the binding of cisplatin to 5’-GMP at 310 K takes 7–8 h to reach 50%. The faster rate of binding of PtIV diazidohydroxido complexes induced by photoactiva-
tion leads to multiple advantages. First, efficient binding to DNA could lower the extent of reaction with cellular thiols (with irradiation restricted to tumour tissue) and so reduce side reactions. Second, the duration of the therapeutic treatment is short so that the discomfort and side-effects of the light dose to the patient can be minimised.

Cisplatin-like complexes usually bind to N7 atoms of guanine and adenine in double-stranded DNA, forming intrastrand cross-links.[30] Guanines are also the most important nine and adenine in double-stranded DNA, forming intrastrand cross-links.[30a] As may be expected from the geometry of the non-leaving (amine) groups, the DNA binding properties of 1-4 (upon irradiation) and 5 or 6 (in the dark) are more similar to transplatin than cisplatin, with affinity for both guanine and cytosine, although binding studies of 1 and 2 with adenine do demonstrate adenine binding to be possible in the absence of competition (Figure S18 in the Supporting Information).

Formation of mono- and bifunctional adducts with DNA: Cisplatin forms predominately bifunctional intrastrand cross-links with DNA, namely (dGpG) 1.2-intrastrand (60–65% of all adducts) and (dApG) 1.2-intrastrand (20–25%) cross-links,[34] which are believed to be the major cause of cell death. In contrast, for trans-PtIV anticancer complexes, monofunctional adducts and a small amount of 1,3-intrastrand cross-links are usually formed,[35] which may lower the level of DNA transcription and repair synthesis, limiting the development of resistance. Also, monofunctional PtIV anticancer complexes, such as pyrplatin,[36] have been found to potentely suppress the growth of cancer cells by inhibiting RNA polymerase II and nucleotide excision repair. For the platinum complexes 1, 2, 5 and 6 in this work, not only intrastrand cross-links, but also interstrand cross-links and monofunctional DNA adducts were formed.

Impact of DNA lesions: The extent and nature of DNA adducts provide useful information for explaining differences in activity. The trans-Pt complexes 1, 2, 5 and 6 gave rise to higher unwinding angles than cisplatin and transplatin (Table 4). This feature is similar to the recently reported unwinding angle for 4[36] Although 1, 2, 5 and 6 form fewer bifunctional DNA adducts than cisplatin, they produce more interstrand cross-links than cisplatin. This is consistent with the previous report that replacing NH2 ligands of transplatin with heterocyclic imines (e.g., Py and Tz) produces more interstrand cross-links.[34] Complexes 1 and 2 generated a significantly higher amount of interstrand cross-links than all Pt complexes listed in Table 4.

Another feature of the DNA adducts by 1, 2, 5 and 6 is that they were not recognised by HMG-domain proteins, as is also the case for transplatin lesions. The 1.2-GG intrastrand cross-link induced by cisplatin in DNA attracts HMGB1,[34] which is considered to be related to the anticancer activity of cisplatin.[35] Therefore, this feature again gives evidence for the different DNA binding modes of 1, 2, 5 and 6 compared to cisplatin and may contribute to their low cross resistance.

Anticancer activity of PtIV diazido complexes and their corresponding PtII analogues: The photocytotoxicity assay for complexes trans,trans-[Pt(N3)2(OH)2(MA)(Py)] (1), trans,trans-[Pt(N3)2(OH)2(MA)(Tz)] (2) and trans,trans-[Pt(N3)2(OH)2(NH2)(Tz)] (9) is designed to provide a very short contact time between the complexes and the cells (1 h) followed by a low dose of light (<1 h sunlight exposure in summer at midday in the UK). This is different from the method used for trans-[PtCl2(MA)(Py)] (5) and trans-[PtCl2(MA)(Tz)] (6) [typically incubation with cells for 24 h]. Therefore, a direct comparison of IC50 values between 1, 2, 5 and 6 cannot be made. Complexes 1 and 2 accumulate in cells rapidly and exhibit high cytotoxicity during a short irradiation time. In contrast, cisplatin showed low toxicity under the same experimental conditions. Complexes 5 and 6 are also very cytotoxic towards A2780 and A2780cis cells. Their IC50 values are among the lowest observed for trans-[PtCl2(Am1)(Am2)] complexes (Table 3).

Azido and hydroxido ligands can dissociate from trans,trans-[Pt(N3)2(OH)2(NH2)(Py)] complexes upon irradiation with light, and Am1 and Am2 remain as non-leaving groups.[39] The current work reveals the similarities between 1 and 5, and between 2 and 6 (Table 4). Although other processes, such as cellular accumulation, may play roles in the anticancer activity of complexes 1 and 2, the PtIV diazidohydroxido complexes can act to a certain extent as prodrugs for their corresponding trans-PtII dichlorido complexes 5 and 6. However, complexes 1 and 2 are more soluble in aqueous solution and allow targeting of tumour tissue with directed light whereas their PtII precursors do not.

Circumventing cisplatin resistance: It is notable that 1, 2, 5 and 6 exhibit lower cross-resistance to

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Table 4. Summary of the results of DNA binding experiments for trans,trans-[Pt(N3)2(OH)2(MA)(Py)] (1), trans,trans-[Pt(N3)2(OH)2(MA)(Tz)] (2), trans-[PtCl2(MA)(Py)] (5) and trans-[PtCl2(MA)(Tz)] (6) obtained in this work.[a]

| Pt bound after 24 h [%] | Bifunctional adducts after 24 h [%] | Interstrand cross-link [%] | Unwinding angle [°] | Sequence specificity | HMGB1 protein recognition |
|------------------------|-----------------------------------|--------------------------|--------------------|---------------------|-----------------------|
| 1[a]                   | 67 ± 5                            | 45 ± 10                  | 23 ± 5             | 22 ± 3              | G, C                  | no                    |
| 2[a]                   | 74 ± 3                            | 52 ± 2                   | 19 ± 3             | 20 ± 3              | G, C                  | no                    |
| 3[a]                   | 87 ± 3                            | 84–87 %                  | 6                  | –                   | G, C                  | –                     |
| 4[a]                   | 55 ± 6                            | 63                    | 12 ± 3             | 27 ± 3              | G, C                  | –                     |
| 5[a]                   | 80 ± 5                            | 34 ± 1                  | 9 ± 3              | 19 ± 5              | G, C                  | no                    |
| 6[a]                   | 63 ± 5                            | 36 ± 2                  | 16 ± 2             | 23 ± 2              | G, C                  | no                    |
| 7[a]                   | 100[a,b]                          | 97–99[a,b]              | 6[30a]             | 13[b]               | OG                    | yes[a]                |
| 8[a]                   | 90[b]                             | 90[b]                   | 12[b]              | 9[b]                | G, C, C[a,b]          | no[a]                 |

[a] A comparison with cisplatin (7), transplatin (8), trans,trans-[Pt(N3)2(OH)2(NH2)(Py)] (8) and trans,trans-[Pt(N3)2(OH)2(Py)] (4), taken from literature, is also included. [b] This work. [c] Photoactivated at 420 nm, 30 min irradiation plus incubation in the dark up to 24 h. [d] Photoactivated with UVA continuously for 5 h. [e] Photoactivated with UVA, 30 min irradiation plus incubation in the dark up to 24 h. [f] In the dark.

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cisplatin towards A2780cis cell lines than the corresponding complexes with similar structures (Tables 2 and 3). A2780cis is an acquired cisplatin-resistant sub-line derived from A2780 ovarian carcinoma cells. The mechanism of resistance may involve reduced accumulation or increased cytoplasmic detoxification that cause insufficient platinum to reach and bind to the target DNA or increased DNA repair/tolerance of platinum-DNA adducts that lead to cell survival.[39] The dark stability and rapid photochemical kinetics of complexes 1 and 2 substantially lower the chance of detoxification by cellular thiols. The formation of DNA adducts is one of the key determinants of cytotoxicity,[12b] and the fundamentally different DNA manner of binding for 1, 2, 5 and 6 compared to cisplatin may explain the activity towards A2780cis cells.

Conclusion

The platinum(IV) diazidodihydroxido complexes with methylethylene and pyridine/thiazole as amine ligands, trans-trans-trans-[Pt(N3)2(OH)2(MA)(Py)] (1), trans-trans-trans-[Pt(N3)2(OH)2(MA)(Tz)] (2) and trans-trans-trans-[Pt(N3)2(OH)2(NH)2(Tz)] (9) have been synthesised and their activity as photoactivatable anticancer prodrugs has been determined. As highlighted by computation, their high photoreactivity stems from the presence of dissociative LMCT/d-d excited states, which can be populated with UV and visible light. They exhibit potent activity towards A2780 cisplatin-resistant ovarian cancer cells upon irradiation with UVA or blue light. When irradiated with blue light (420 nm), they were also highly cytotoxic towards the A2780, OE19 and HaCaT cell lines. These results suggest that trans-mixed-amine PtIV complexes are promoting candidates for use in the cancer photochemotherapy of thin-walled organs. The PtIV analogues of 1 and 2, trans-[PtCl2(MA)(Py)] (5) and trans-[PtCl2(MA)(Tz)] (6), respectively, are also highly cytotoxic towards A2780 cancer cells, with IC50 values in the range and resistance factors close to 1 (i.e., lack cross-resistance to cisplatin). Upon irradiation, complexes 1 and 2 exhibited significantly faster binding to 5'-GMP and DNA than their trans-PtIII precursors or cisplatin. Compounds 1 and 2 bind to DNA in a manner substantially different from that of cisplatin and we suggest that this could account for their activity towards the A2780cis cisplatin-resistant cell line.

Experimental Section

Synthesis and characterisation: Caution! Although no problems were encountered during this work, heavy metal azides are known to be shock and light sensitive, therefore it is essential that any Pt azido compound is handled with care, and all syntheses were carried out under controlled (dim) lighting conditions.

cis-[PtCl2(MA)]. This was synthesised following the method reported previously for cisplatin.[30]

trans-[PtCl2(MA)(Py)] (5). cis-[PtCl2(MA)] (0.33 g, 1 mmol) was suspended in H2O (9 mL), and then pyridine (240 mg, 3 mmol) was added. The reaction was heated to 353 K with stirring and kept at this temperature for 90 min. The solution was allowed to cool to ambient temperature and H2 (32 s, 1 mL) was added and stirred at 368 K for 65 h. The flask was cooled on ice and the product was filtered, washed successively with minimal cold H2O, ethanol, and diethyl ether and then dried under vacuum. The yellow product was further purified by recrystallization from HCl (0.1 m). Yield: 0.306 g (81.3%); 1H NMR (400 MHz, [D]acetone): δ = 8.83 (dd, 3H, J(H2) = 7 Hz; J(H3) = 32 Hz; 2H, H2); 7.98 (t, 3H, J(H2) = 7 Hz, 1H, H1); 7.45 (t, 3H, J(H2) = 7 Hz, 2H, H3); 4.31 (br, 2H, NH2); 2.49 ppm (td, 3H, J(H2) = 6 Hz; J(H3) = 35 Hz, 3H, CH3); 13C NMR (150.9 MHz, [D]acetone): δ = 154.1 (C3), 139.3 (C2), 126.2 (C4), 33.7 ppm (CH3); 31Pt NMR (129.4 MHz, [D]acetone): δ = 2085.3 ppm; ESI-MS: m/z (%): 304.1 [M+2-CH3]+; elemental analysis calcd (%) for C19H12N8O2Pt: C 19.26, H 2.60, N 7.37.

trans-[Pt(N3)2(MA)(Py)] (0.188 g, 0.55 mmol) was suspended in H2O (50 mL). AgNO3 (0.17 g, 1.0 mmol) was added and the solution was stirred at 323 K for 16 h. AgCl precipitate was filtered off through Celite followed by an inorganic membrane filter (Sartorius, Minisart, 0.2 μm). NaN3 (0.65 g, 10 mmol) was added and the solution was filtered off using a Buchner funnel, washed with minimal ice cold H2O, EtOH, and then dried under vacuum. Yield: 0.153 g (78.7%); 1H NMR (400 MHz, [D]acetone): δ = 8.79 (dd, 3H, J(H2) = 7 Hz; 3J(H2) = 34 Hz, 2H, H2); 8.08 (t, 3H, J(H3) = 7 Hz, 1H, H3); 7.61 (t, 3H, J(H2) = 7 Hz; H2, H3); 4.43 (br, 2H, NH2); 15.80 ppm (3H, CH3); 13C NMR (150.9 MHz, [D]acetone): δ = 135.3 (C3), 139.9 (C2), 127.3 (C4), 33.2 ppm (CH3); 31Pt NMR (129.4 MHz, [D]acetone): δ = -2202.4 ppm; ESI-MS: m/z (%): 412.1 [M+4-Na]+.

trans-trans-[Pt(N3)2(OH)2(MA)(Py)] (1): trans-[PtCl2(MA)(Py)] (39 mg, 0.1 mmol) was suspended in H2O (20 mL, 30%) and stirred at 323 K for 1 h. The volume of the solution was reduced on a rotary evaporator to about 4 mL, to give a bright yellow solution (1.2 g). It was important to maintain the temperature below 323 K. Water (50 mL) was added and the solution was lyophilised. Crystals suitable for X-ray analysis were obtained by crystallisation from water–ethanol with ether diffusion. Polymorphs 1α and 1β were isolated from different batches under similar conditions. The product was filtered off (Buchner), washed with minimal ether and dried under vacuum. Yield: 21 mg (50%); 1H NMR (400 MHz, 90% D2O): 8.05 (t, 3H, J(H2) = 7 Hz, 1H, H1); 7.84 (t, 3H, J(H2) = 7 Hz, 2H, H2); 6.65 (br, 2H, NH2); 2.42 ppm (td, 3H, J(H2) = 6 Hz; J(H3) = 35 Hz, 3H, CH3); 13C NMR (100.6 MHz, D2O, dioxane): δ = 149.60 (C3), 142.77 (C4), 127.62 (3J(H2) Pt = 25 Hz; C5); 31Pt NMR (129.4 MHz, D2O): δ = 868.66 ppm; 195Pt NMR: (1H, 1H) = 289 nm (16200 L mol-1 cm-1) (H2O); ESI-MS: m/z (%): 440.0 [M+Na]+; elemental analysis calcd (%) for C19H12N8O2Pt: C 17.02, H 2.86, N 26.47; found: C 16.57, H 2.65, N 23.87. Azido complexes often give rather large deviations than normal in the nitrogen analysis, and the purity was determined by an HPLC analysis as >96%. For HPLC conditions, see the Supporting Information.

trans-[PtCl2(MA)(Tz)] (6): cis-[PtCl2(MA)] (0.33 g, 1 mmol) was suspended in H2O (9 mL), then thiazole (255 mg, 3 mmol) was added. The reaction was heated to 353 K and stirred for 2 h. The solution was allowed to cool to ambient temperature and H2 (12 mL, 2124 mL mmol) was added. The solution was heated at 368 K and stirred overnight (>16 h). The solution was cooled on ice and the yellow product was filtered off, washed successively with minimal cold H2O, ethanol, and diethyl ether, and then dried under vacuum. The product was recrystallized from the minimum amount of HCl (0.1 m). Yield: 0.280 g (73.5%); 1H NMR (400 MHz, [D]acetone): δ = 9.05 (dd, 3H, J(H2) = 2.4 Hz; J(H3) = 26 Hz, 1H, J(H3)); 8.26 (dd, 3H, J(H3) = 2.4 Hz; J(H3) = 15 Hz, 1H, H1); 8.03 ppm (td, 3H, J(H2) = 15 Hz, 3H, CH3); 13C NMR (150.9 MHz, [D]acetone): δ = 157.8 (C3), 144.7 (C4), 1209 (C5), 33.6 ppm (CH3); 31Pt NMR (129.4 MHz, [D]acetone): δ = -2111.6 ppm;
trans-[Pt(N3)2(MA)(Tz)] (2): trans-[Pt(N3)2(MA)(Tz)] (20 mg, 0.05 mmol) was suspended in H2O (10 mL, 30%) and stirred at 90% H2O/10% D2O, dioxane, pH 7.0. Yield: 0.182 g (92%); 1H NMR (400 MHz, [D6]acetone): δ = 9.44 (dd (1H4, 1H5), J195Pt, 1H) = 2.4 Hz, J195Pt, 2H) = 28 Hz, 1H, 1H); 8.27 (dd (1H4, 1H5), J195Pt, 2H) = 3.6 Hz, J195Pt, 2H) = 4 Hz, 3H, CH2); 13C NMR (150.9 MHz, [D6]acetone): δ = 158.0 (C), 144.3 (C), 122.7 (C), 33.4 ppm (CH3). ESI-MS: m/z (%) = 417.9 [M + Na]+.

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A. E. Jamieson, S. L. Sondervan, Chem. Rev. 2007, 107, 652–662; d) H.-C. Tai, Y. Zhao, N. J. Farrer, A. E. Anastasi, G. Clarkson, P. J. Sadler, R. J. Deeth, Chem. Eur. J. 2012, 18, 10630–10642.

A. E. Farr, L. Ronconi, P. J. Sadler, Chem. Eur. J. 2009, 15, 1588–1596; b) L. Ronconi, P. J. Sadler, Dalton Trans. 2011, 40, 262–268.

A. J. Butler, L. Ronconi, P. J. Sadler, J. Med. Chem. 2012, 55, 3542–3551.

A. S. Still, P. G. A. Kumar, J. R. Aldrich-Wight, W.S. Price, Chem. Soc. Rev. 2007, 36, 685–688; b) P. S. Pregosin, Coord. Chem. Rev. 1982, 44, 247–291.

A. M. V. Lekieux, S. Záliš, Coord. Chem. Rev. 2007, 251, 258–287; b) L. Salassa, C. Garino, G. Salassa, R. Gobetto, C. Nervi, J. Am. Chem. Soc. 2008, 130, 5950–5957; c) L. Salassa, C. Garino, G. Salassa, C. Nervi, R. Gobetto, C. Lamberti, D. Gianolio, R. Bizzarri, P. J. Sadler, Inorg. Chem. 2009, 48, 1469–1481; d) L. Salassa, T. Ruus, C. Garino, A. M. Pizarro, F. Bardelli, D. Gianolio, A. Westendorf, P. J. Sadler, C. Lamberti, R. Gobetto, P. J. Sadler, Organometallics 2010, 29, 6703–6710.

J. Vinje, J. A. Parkinson, P. J. Sadler, T. Brown, E. Sletten, Chem. Eur. J. 2003, 9, 1620–1630.

R. Prokop, J. Kasparova, O. Novakova, V. Marini, A. M. a. Pizarro, F. S. Mackay, A. W. Goodwin, P. J. Sadler, Proc. Natl. Acad. Sci. USA 2007, 104, 20743–20748.

N. J. Farrer, J. A. Woods, L. Salassa, Y. Zhao, K. S. Robinson, G. Clarkson, F. S. Mackay, P. J. Sadler, Angew. Chem. 2010, 122, 9089–9092; Angew. Chem. Int. Ed. 2010, 49, 8905–8908.

N. J. Farrer, J. A. Woods, V. P. Munk, F. S. Mackay, P. J. Sadler, Chem. Res. Toxicol. 2010, 23, 413–421; b) A. F. Westendorf, J. A. Woods, K. Kropis, N. J. Farrer, L. Salassa, K. Robinson, V. Apple-yard, K. Murray, R. Grümert, A. M. Thompson, P. J. Sadler, P. J. Bednarski, Mol. Cancer Ther. 2012, 11, 1894–1904.

B. W. Hamers, W. H. Gispen, J. Peijt, Eur. J. Cancer Clin. Oncol. 1991, 27, 372–376; b) X. Yao, K. Panichpial, N. Kurtzman, K. Nagent, Am. J. Med. Sci. 2007, 334, 115–124.

J. A. Kasparkova, V. Marini, Y. Najajreh, D. Gibson, V. Brabec, P. J. Sadler, Biochem. Pharmacol. 2004, 67, 1097–1109.

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[1] a) P. J. O’Dwyer, J. P. Stevenson, S. W. Johnson, in Cisplatin: Chemistry and Biochemistry of A Leading Anticancer Drug (Ed.: B. Lipsett), Helvetica Chimica Acta, Zürich, 1999, pp. 29–69; b) B. W. Harper, A. M. Krause-Heuer, M. P. Grant, M. Manohar, K. B. Garbutcheon-Singh, J. R. Aldrich-Wight, Chem. Eur. J. 2010, 16, 7064–7077.

[2] a) F. P. T. Hamers, W. H. Gispen, J. Peijt, Eur. J. Cancer Clin. Oncol. 1991, 27, 372–376; b) X. Yao, K. Panichpial, N. Kurtzman, K. Nagent, Am. J. Med. Sci. 2007, 334, 115–124.
Visible-Light Activatable Diazido Mixed-Amine PtIV Complexes

[23] J. Pracharova, L. Zerzankova, J. Stepankova, O. Novakova, N. J. Farrer, P. J. Sadler, V. Brabec, J. Kasparkova, Chem. Res. Toxicol. 2012, 25, 1099–1111.

[24] a) K. Stuhlíková, J. Kaňápáková, O. Nováková, A. Martínez, V. Moreno, V. Brabec, FEBS J. 2006, 272, 301–314; b) A. Eastman, M. A. Barry, Biochemistry 1997, 26, 3303–3307.

[25] M. V. Keck, S. J. Lippard, J. Am. Chem. Soc. 1992, 114, 3386–3390.

[26] M. Coluccia, G. Natile, Anti-Cancer Agents Med. Chem. 2007, 7, 111–123.

[27] J. K.-C. Lau, D. V. Deubel, Chem. Eur. J. 2005, 11, 2849–2855.

[28] D. Wang, G. Zhu, X. Huang, S. J. Lippard, Proc. Natl. Acad. Sci. USA 2010, 107, 9584–9589.

[29] A. Zenker, M. Galanski, T. L. Bereuter, B. K. Keppler, W. Lindner, J. Chromatogr. B Biomed. Sci. 2000, 745, 211–219.

[30] a) J. P. Caradonna, S. J. Lippard, M. J. Gait, M. Singh, J. Am. Chem. Soc. 1982, 104, 5793–5795; b) A. Eastman, Biochemistry 1983, 22, 3927–3933; c) A. M. J. Fichtinger-Schepman, J. L. Van der Veer, J. H. J. Den Hartog, P. H. M. Lohman, J. Reedijk, Biochemistry 1985, 24, 707–713.

[31] V. Brabec, J. Kasparkova, O. Vrana, O. Novakova, J. W. Cox, Y. Qu, N. Farrell, Biochemistry 1999, 38, 6781–6790.

[32] a) T. Suchánková, M. Vojtisková, J. Reedijk, V. Brabec, J. Kasparková, J. Biol. Inorg. Chem. 2009, 14, 75–87; b) V. Brabec, M. Leng, Proc. Natl. Acad. Sci. USA 1993, 90, 5345–5349.

[33] J. Kasparkova, O. Novakova, N. Farrell, V. Brabec, Biochemistry 2003, 42, 792–800.

[34] U. M. Ohndorf, M. A. Rould, Q. He, C. O. Pabo, S. J. Lippard, Nature 1999, 399, 708–712.

[35] V. Brabec, Prog. Nucleic Acid Res. Mol. Biol. 2002, 71, 1–68.

[36] D. P. Bancroft, C. A. Lepre, S. J. Lippard, J. Am. Chem. Soc. 1990, 112, 6860–6871.

[37] M. A. Lemaire, A. Schwartz, A. R. Rahmouni, M. Leng, Proc. Natl. Acad. Sci. USA 1991, 88, 1982–1985.

[38] R. Guddneppanavar, J. R. Choudhury, A. R. Kheradi, B. D. Steen, G. Saluta, G. L. Kucera, C. S. Day, U. Bierbach, J. Med. Chem. 2007, 50, 2259–2263.

[39] S. C. Dhara, Indian J. Chem. 1970, 8, 193.

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