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A Photoactivated *trans*-Diammine Platinum Complex as Cytotoxic as Cisplatin

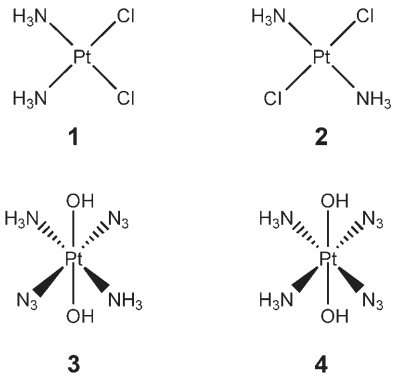
Fiona S. Mackay,[a] Julie A. Woods,[b] Harry Moseley,[b] James Ferguson,[b] Alice Dawson,[a] Simon Parsons,[a] and Peter J. Sadler*[a]

Abstract: The synthesis and X-ray structure (as the tetrahydrate) of the platinum(IV) complex *trans,trans,trans*-$[Pt(N_3)_2(OH)_2(NH_3)_2]$ **3** are described and its photochemistry and photobiology are compared with those of the *cis* isomer *cis,trans,cis*-$[Pt(N_3)_2(OH)_2(NH_3)_2]$ **4**. Complexes **4** and **3** are potential precursors of the anticancer drug cisplatin and its inactive *trans* isomer transplatin, respectively. The *trans* complex **3** is octahedral, contains almost linear azide ligands, and adopts a layer structure with extensive intermolecular hydrogen bonding. The intense azide-to-platinum(IV) charge-transfer band of complex **3** (285 nm; $\varepsilon = 19500\,M^{-1}cm^{-1}$) is more intense and bathochromically shifted relative to that of the *cis* isomer **4**. In contrast to transplatin, complex **3** rapidly formed a platinum(II) bis(5′-guanosine monophosphate) (5′-GMP) adduct when irradiated with UVA light, and did not react in the dark. Complexes **3** and **4** were non-toxic to human skin cells (keratinocytes) in the dark, but were as cytotoxic as cisplatin on irradiation for a short time (50 min). Damage to the DNA of these cells was detected by using the "comet" assay. Both *trans*- and *cis*-diammine platinum(IV) diazide complexes therefore have potential as photochemotherapeutic agents.

Keywords: antitumor agents · bioinorganic chemistry · nucleotides · photochemotherapy · platinum complexes

Introduction

It was discovered early in the search for platinum anticancer agents that although *cis*-$[Pt^{II}Cl_2(NH_3)_2]$ (**1**), the anticancer drug cisplatin, is highly toxic to cancer cells, its *trans* isomer (transplatin, **2**) is relatively non-toxic.[1] The reasons for this have been the subject of much discussion. Cisplatin forms lethal intrastrand GG cross-links on DNA,[2] whereas the geometrical arrangement of the reactive chloride sites on transplatin prevents this. Also, although monofunctional adducts of transplatin with DNA form rapidly, their evolution into bifunctional adducts occurs very slowly (>24 h).[3] Reactions of transplatin (and its monoaqua adduct) with biomolecules may also cause inactivation before it reaches DNA. Activity can be restored to *trans* complexes by substituting one or both of the ammine ligands of transplatin by an aliphatic or heterocyclic amine, or an imino ether.[4,5] Sterically demanding amines appear to reduce the extent of unwanted side reactions, as is the case for some *trans*-diamine platinum(IV) complexes, which are activated in vivo by chemical reduction.[6,7] Here we report a different approach to the design of active *trans*-diam(m)ine anticancer



[a] F. S. Mackay, Dr. A. Dawson, Dr. S. Parsons, Prof. Dr. P. J. Sadler
School of Chemistry, The University of Edinburgh
King's Buildings, West Mains Road, Edinburgh EH9 3 JJ (UK)
Fax: (+44) 131-650-6453
E-mail: P.J.Sadler@ed.ac.uk

[b] Dr. J. A. Woods, Dr. H. Moseley, Prof. Dr. J. Ferguson
Photobiology Unit, Department of Dermatology
University of Dundee
Ninewells Hospital and Medical School
Dundee DD1 9SY (UK)

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complexes. We have synthesized the inert *trans*-diammine platinum(IV) complex *trans,trans,trans*-$[Pt(N_3)_2(OH)_2(NH_3)_2]$ (**3**) and studied the effect of photoactivation on nucleotide binding and toxicity towards skin cells (human keratinocytes, HaCaT cells), in comparison with the *cis*-diammine isomer *cis,trans,cis*-$[Pt(N_3)_2(OH)_2(NH_3)_2]$ **4**, cisplatin, and transplatin. Intriguingly, the *trans*-platinum(IV) complex **3** is relatively non-toxic to cells in the dark, but as cytotoxic as cisplatin when photoactivated. Such complexes have potential as novel photoactivated chemotherapeutic agents with significant advantages over currently used photosensititizers.[8]

Results and Discussion

We synthesized *trans,trans,trans*-$[Pt(N_3)_2(OH)_2(NH_3)_2]$ (**3**) by treatment of transplatin with $AgNO_3$ to convert it to the diaqua adduct, followed by substitution of the aqua ligands by azides, and oxidation of the resulting *trans*-diazidoplatinum(II) complex with H_2O_2. Since the second chloride ligand is difficult to remove from transplatin, an excess of azide was used in the subsequent step with a long reaction time to ensure that the reaction went to completion. X-ray crystallography of **3**·$4H_2O$ confirmed the octahedral stereochemistry and the presence of an inversion center (Figure 1a). The azide ligands are almost linear (∢N1A-N2A-N3A 174.5°) and the Pt-N1A-N2A angle is 117.2°. The azide N–N bond closest to Pt (N1A–N2A 1.210 Å) is about 0.07 Å longer than the terminal azide N–N bond (N2A–N3A 1.143 Å), which is typical for azide ligands and can be explained by the resonance structure.[9] The bond lengths and angles of **3** are comparable to those of the *cis* isomer **4**,[10] and in good agreement with literature values for related complexes.[11–14]

The crystal structure of **3** consists of layers of molecules formed through OH···N_3 and NH···OH hydrogen bonds, which connect molecules in pairs across inversion centres (Figure 1b). The layers are interleaved with water molecules which form hydrogen bonds to the NH_3 groups. Details of the hydrogen bonds are listed in Table 1.

Aqueous solutions of complex **3** exhibit an intense absorption band at 285 nm ($\varepsilon = 19500\,M^{-1}cm^{-1}$), assignable as an azide-to-Pt^{IV} (ligand-to-metal (LM)) charge-transfer (CT) band.[15] This band is shifted to longer wavelength and is more intense than that of the *cis* isomer **4** (λ_{max} 256 nm; $\varepsilon = 13305\,M^{-1}cm^{-1}$). Irradiation with UVA light ($\lambda = 365$ nm, P = 12 mW) led to a decrease in the intensity of the CT bands (Figure 2a and 2b) indicating loss of the azide ligands, probably as N_2 gas, as the formation of bubbles was observed in the solutions.

Photoinduced reactions of aqueous solutions of ^{15}N-**3** and ^{15}N-**4** were also monitored by 2D [^1H, ^{15}N] HSQC NMR spectroscopy. The $^1H/^{15}N$ NMR chemical shifts of cross-peaks are useful for the identification of the Pt oxidation state and nature of the bound ligands. For example, peaks for platinum(II) ammine complexes are expected in the region $\delta = 5.0$ to 3.0/−30 to −90 ppm, whereas peaks for platinum(IV) ammine complexes usually have shift ranges of $\delta = 7.0$ to 5.0/−10 to −60 ppm.[16] The *cis* complex **4** initially gave rise to several new Pt^{IV} products with $\delta(^1H)$, $\delta(^{15}N)$ chemical shifts in the region 4.9 to 5.3, −55 to −44 ppm (Figure 2e). Peaks corresponding to Pt^{II} complexes first appeared after 60 min of irradiation, and were still visible after 120 min (Figure 2f), although precipitates formed during the reaction. These precipitates may arise from multinuclear oxygen-bridged species, but have not been further characterized. Irradiation of **3** also resulted in the formation of new

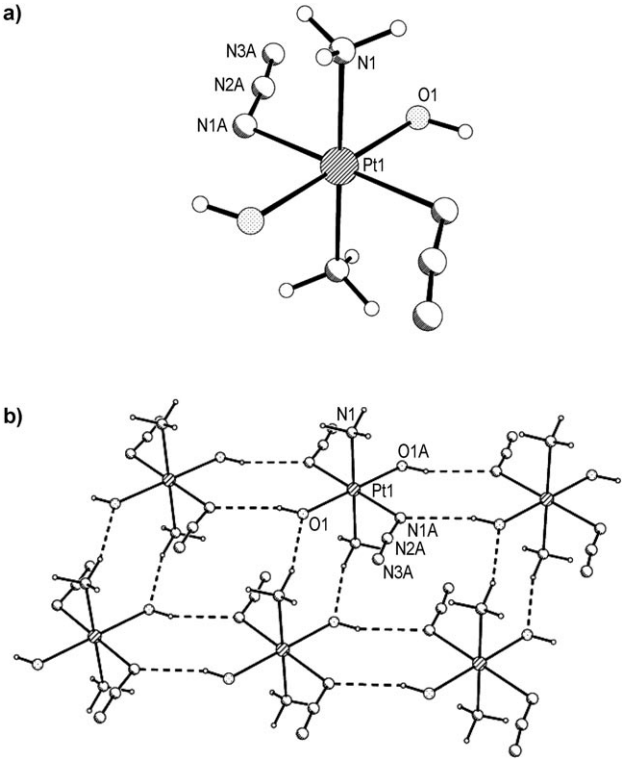

Figure 1. X-ray crystal structure of *trans, trans, trans*-$[Pt(N_3)_2(OH)_2(NH_3)_2]$ (**3**), showing a) inversion centre in the octahedral Pt^{IV} complex, and b) hydrogen bonds involved in the layering of molecules. Selected bond lengths [Å] and angles [°]: Pt1–N1 2.036(3), Pt1–N1A 2.046(3), Pt1–O1 2.006(2), N1A–N2A 1.210(4), N2A–N3A 1.143(5); N1-Pt1-N1A 91.18(12), N1A-N2A-N3A 174.5(3), N2A-N1A-Pt1 117.2(2), O1-Pt1-N1A 91.96(11).

Table 1. Hydrogen bonding distances [Å] and angles [°] in the crystal structure of *trans, trans, trans*-$[Pt(N_3)_2(OH)_2(NH_3)_2]\cdot 4H_2O$.

D–H	*d*(D–H)	*d*(H···A)	<DHA	*d*(D···A)	A
O1–H1	0.845	2.175	156.45	2.969	N1A[a]
N1–H1A	0.910	1.978	164.97	2.867	O1[b]
N1–H1B	0.910	2.074	161.04	2.950	O1W
O1W–H1W1	0.839	2.003	175.44	2.840	O2W[c]
O1W–H2W1	0.835	2.197	163.05	3.006	O2W[d]
O2W–H1W2	0.832	1.926	168.92	2.748	O1
O2W–H2W2	0.826	2.343	129.72	2.941	O1W[e]
O2W–H2W2	0.826	2.628	144.87	3.338	O1W

Symmetry operations: [a] $x, y+1, z$. [b] $x-1, y, z$. [c] $x, y-1, z$. [d] $-x+1, -y, -z-1$. [e] $-x, -y, -z-1$.

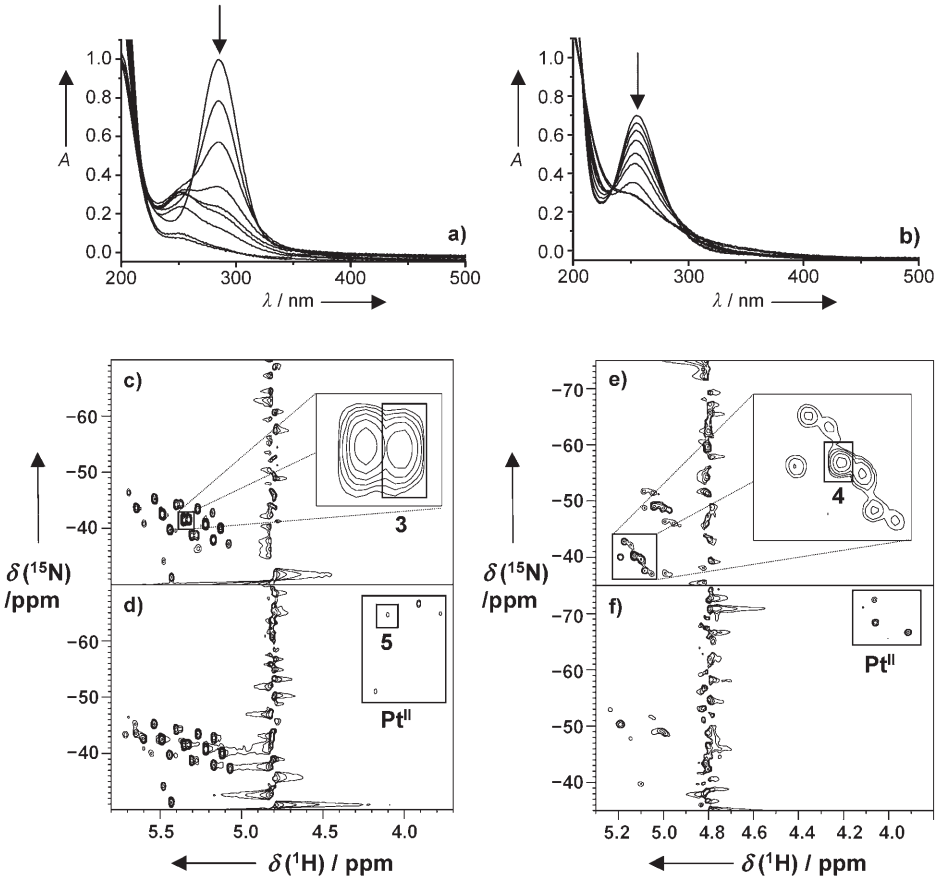

Figure 2. Photoinduced decomposition of complexes **3** and **4**. Dependence of the UV/Vis spectra of aqueous solutions of a) complex **3** and b) complex **4** on the time of irradiation with UVA light (0, 2, 5, 10, 20, 30, 50, 105, and 125 min). The arrows denote the decrease in intensity of the azide-to-platinum(IV) charge-transfer bands with time. 2D [^{1}H, ^{15}N] HSQC NMR spectra of **3** after c) 30 and d) 120 min irradiation; and **4** after e) 30 and f) 120 min irradiation.

Pt^{IV} complexes (Figure 2c), possibly isomers. Photoisomerisation of platinum(IV) am(m)ine compounds has been observed previously and utilized in synthesis.[17,18] However, these photoproducts did not disappear upon further irradiation, perhaps suggesting that they do not contain azide and are not isomers. After irradiation of a solution of **3** for 60 min, peaks for Pt^{II} species appeared, including *trans*-[Pt-($^{15}NH_3$)$_2$(OH$_2$)$_2$]$^{2+}$ (**5**; $\delta = 4.10$, -64.61 ppm).[19] After irradiation for 120 min (Figure 2d), the majority of Pt was still in the +4 oxidation state, and had not been reduced; unlike irradiation of **4**, only a very small amount of precipitation occurred.

Complex **3** did not react with guanosine 5′-monophosphate (5′-GMP) in the dark, but on irradiation of an aqueous solution of **3** containing two molar equivalents of 5′-GMP with UVA light, 2D [^{1}H,^{15}N] HSQC NMR studies showed that about 7% of **3** had already undergone photoreduction and photosubstitution after only one minute to give a product with $\delta(^1H)$, $\delta(^{15}N)$ chemical shifts of 4.14, -60.51 ppm, respectively, characteristic of the bis-GMP adduct *trans*-[Pt($^{15}NH_3$)$_2$(5′-GMP-*N7*)$_2$]$^{2+}$ (**6**; Figure 3).[19] It was notable that photoreductions proceeded faster than in the absence of 5′-GMP. After 1 h, >75% of **3** had been converted into **6** as the major product (Figure 3e). This is remarkably fast when compared to hydrolysis rates reported for transplatin ($t_{1/2} > 2$ h).[20] Binding to N7 of 5′-GMP was confirmed by a ^{1}H NMR titration over the range pH 1–11. The H8 chemical shift change associated with the protonation of N7 of 5′-GMP (pK_a ca. 2.5) was absent for the product (Figure 4). A bis-adduct *trans*-[Pt($^{15}NH_3$)$_2$(9-ethylguanine-*N7*)$_2$] ($\delta(^1H) = 4.04$, $\delta(^{15}N) = -61.42$ ppm) also appeared as a product from the photoreaction of complex **3** with 9-ethylguanine after irradiation for only 1 min. ESI-MS gave a peak at *m*/*z* 295.3 assignable to [Pt($^{15}NH_3$)$_2$(9-ethylguanine-*N7*)$_2$]$^{2+}$ (calcd *m*/*z* 294.7).

A similar irradiation of the *cis*-diammine isomer **4** in the presence of 5′-GMP gave rise not only to the *cis*-GMP platinum(II) adduct (**7**), but also to the *trans* isomer **6**. The formation of a major product on irradiation of **3** in the presence of 5′-GMP, and absence of the range of side reactions seen on irradiation of compound **3** alone, suggest that 5′-GMP can rapidly trap reactive intermediates produced during the photoexcitation process.

Interestingly (in view of the deeper tissue penetration of red light), irradiation of solutions containing **3** and 5′-GMP with red light ($\lambda = 647.1$ nm, P = 18 mW) also gave rise to a detectable amount of *trans*-[Pt($^{15}NH_3$)$_2$(5′-GMP-*N7*)$_2$]$^{2+}$ (ca. 2% after 90 min).

These reactions of **3** with guanine derivatives are remarkable for two reasons. First, platination occurs very rapidly in comparison to reactions of transplatin, and second, bis-guanine adducts are readily formed, whereas the progression from mono- to bis-adducts is rarely observed for reactions of transplatin. The lack of ability of transplatin to form bis-G adducts is thought to be responsible for its lack of anticancer activity.[21]

Irradiation of HaCaT cells for 50 min with 5 J cm^{-2} UVA after incubation with complexes **3** and **4** for 1 h, resulted in a concentration-dependent decrease in viability. Both complexes were equally active, and as effective as cisplatin incubated under identical conditions but without irradiation (Table 2). Complexes **3** and **4** were non-cytotoxic in the ab-

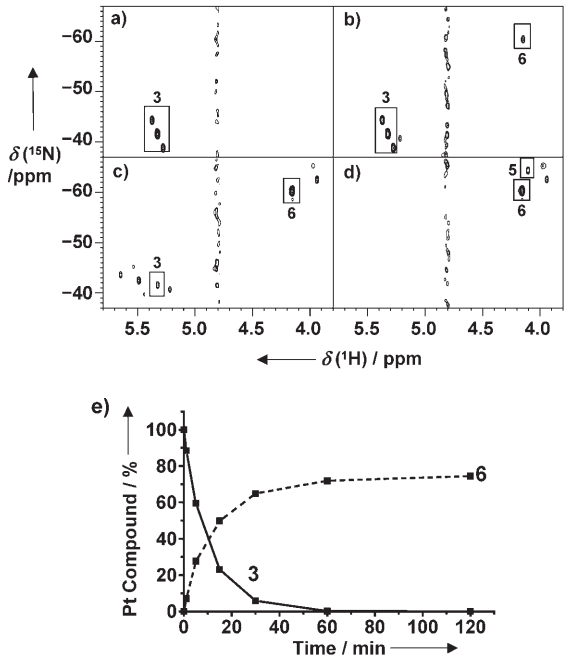

Figure 3. 2D [^{1}H, ^{15}N] HSQC NMR spectra recorded during reaction of **3** with two molar equivalents of 5′-GMP (298 K in 90% H_2O/10% D_2O) after a) 0 min, b) 1 min c) 30 min, and d) 120 min irradiation. The pH was adjusted to 5±0.2 after every irradiation. e) Decrease in concentration of Pt^{IV} complex **3** and formation of Pt^{II} product *trans*-[Pt($^{15}NH_3$)$_2$(5′-GMP-N7)$_2$]$^{2+}$ (**6**) as monitored by 1D [^{1}H] and 2D [^{1}H, ^{15}N] HSQC NMR spectroscopy.

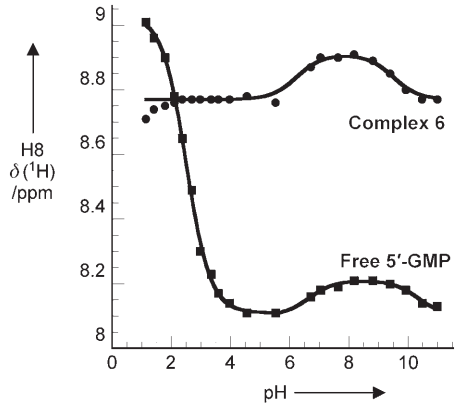

Figure 4. Variation with pH of the H8 NMR chemical shift of the major product from irradiation of **3** in the presence of 5′-GMP (**6** *trans*-[Pt($^{15}NH_3$)$_2$(5′-GMP-N7)$_2$]), in comparison with free 5′-GMP. The large increase in chemical shift below pH 4 observed for free 5′-GMP is absent for **6** indicating coordination of Pt to N7.

sence of light, and, as expected, non-irradiated transplatin was not toxic under these experimental conditions (Figure 5), but surprisingly, the combination of transplatin and UVA light decreased cell viability in a dose-dependent fashion (Table 2). Non-linear analysis of the concentration-response graphs confirms that both **3** and **4** are photoactive in cells (Figure 5). Transplatin was slightly photoactive, but much less so than either **3** or **4**.

Table 2. Toxicity of platinum complexes towards human keratinocytes in the dark (−UVA) and on irradiation with light (+UVA).

	−UVA		+UVA	
Compound	IC_{50} [μM][a]	95% Con. int[b]	IC_{50} [μM]	95% Con. int[b]
cisplatin	173	153–197	144	124–163
transplatin	>333	NA[c]	245	190–329
3	>288	NA[c]	156	141–167
4	>288	NA[c]	176	161–190

[a] IC_{50} = the concentration of complex which reduced the number of viable cells by 50%. Note the short incubation times used in these experiments (1 h followed by 50 min of irradiation). All IC_{50} values are the result of 3–4 independent experiments. [b] Con. int = confidence interval. [c] NA = not applicable.

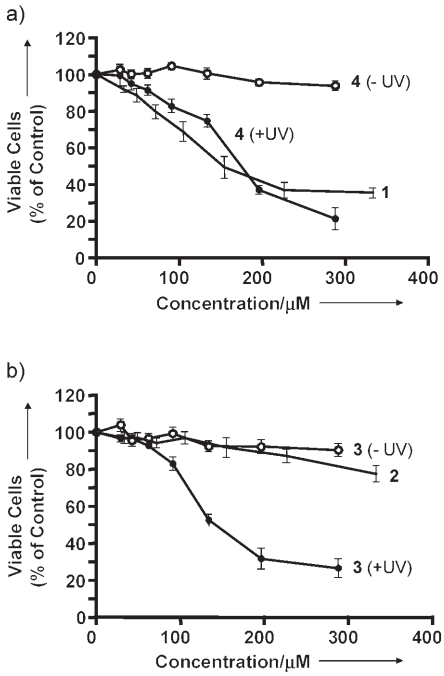

Figure 5. Phototoxicity of Pt complexes towards human HaCaT keratinocytes. a) *cis,trans,cis*-[Pt(N_3)$_2$(OH)$_2$(NH_3)$_2$] (**4**) in the light (+UV) and dark (−UV), in comparison with cisplatin (**1**), and b) *trans,trans,trans*-[Pt(N_3)$_2$(OH)$_2$(NH_3)$_2$] (**3**) in comparison with transplatin, **2**. Each data point on the graph represents the mean ±SE of 3–4 independent experiments performed in triplicate.

The intensity of the UVA irradiation (5 J cm^{-2}) used in these experiments is equivalent to about 30–60 min of exposure to sunlight. The sensitivity of HaCaT keratinocytes to this dose is similar to primary skin keratinocytes, and under our experimental conditions cell viability (+UV/−UV) at 5 J cm^{-2} was 88±2%; and at 9 J cm^{-2} was 80±5%. Acceptance criteria for the assay specify that viability should be at least 80% at 5 J cm^{-2} and not less than 50% at 9 J cm^{-2}.[22]

Since photoactivation of complexes **3** and **4** can lead to rapid binding to guanine, we investigated their ability to damage DNA in intact cells in comparison to cisplatin and transplatin using the "comet assay" (see Figure S1 in the Supporting Information). The comet assay uses single cell electrophoresis. DNA from cells which has been damaged and fragmented by nicks or breaks and has free ends and

loops can migrate away from the nucleus towards the anode and takes on the appearance of a comet when stained with a fluorescent dye, the intensity of which represents the relative amount of DNA fragmentation (see Supporting information). The comet assay showed that both **3** and **4** produced DNA cross-linking in living cells on irradiation with 5 J cm^{-2} UVA. The effects were similar to those produced by incubation of the cells with cisplatin and transplatin under identical experimental conditions but without the irradiation (Figure 6a and b). Neither **3** nor **4** was active in the absence of irradiation.

The formation of crosslinks correlated closely with loss of cell viability for cisplatin and **3** (correlation coefficient $R2 = 0.911$ and 0.974, respectively). The correlation for **4** was not as good ($R2 = 0.58$) when the entire dose range was analyzed, but this may be accounted for by the lack of change in the inhibition of DNA migration between the two highest doses. Transplatin was not cytotoxic despite forming crosslinks.

As a control, chlorpromazine, a photoactive compound that does not produce DNA crosslinks on photoactivation, was also tested under identical conditions. This compound gave rise to an enhancement of H_2O_2-induced DNA migration rather than the decrease induced by crosslinking agents.

We also examined whether the compounds cleaved DNA (i.e. were clastogenic) during photoactivation by measuring strand-breaks and alkali labile lesions. Figure 6c and d shows that the photoactivated complexes were not clastogenic, except at the highest concentration of 288 μM, which corresponded to cell viabilities of 21.4% ± 5.9 (**3**) and 26.6% ± 5.1 (**4**). Therefore the DNA strand breaks observed at high doses of **3** and **4** are likely to result from the cell death processes rather than be caused directly by the compounds.

These data suggest that the greater toxicity of cisplatin compared to transplatin is due to the type of DNA adduct formed (in agreement with published data).[5] Evidently this is also the case for complex **3**. The mechanism of toxicity of **3** in HaCaT cells differs markedly from that of transplatin. Evidently complex **3** is not simply a prodrug for transplatin. We have previously shown that **4** can form GG adducts similar to those produced by cisplatin on plasmid DNA.[23]

Conclusion

The photoactivatable complexes **3** and **4** have potential for use in photochemotherapy, in which a chemical is activated

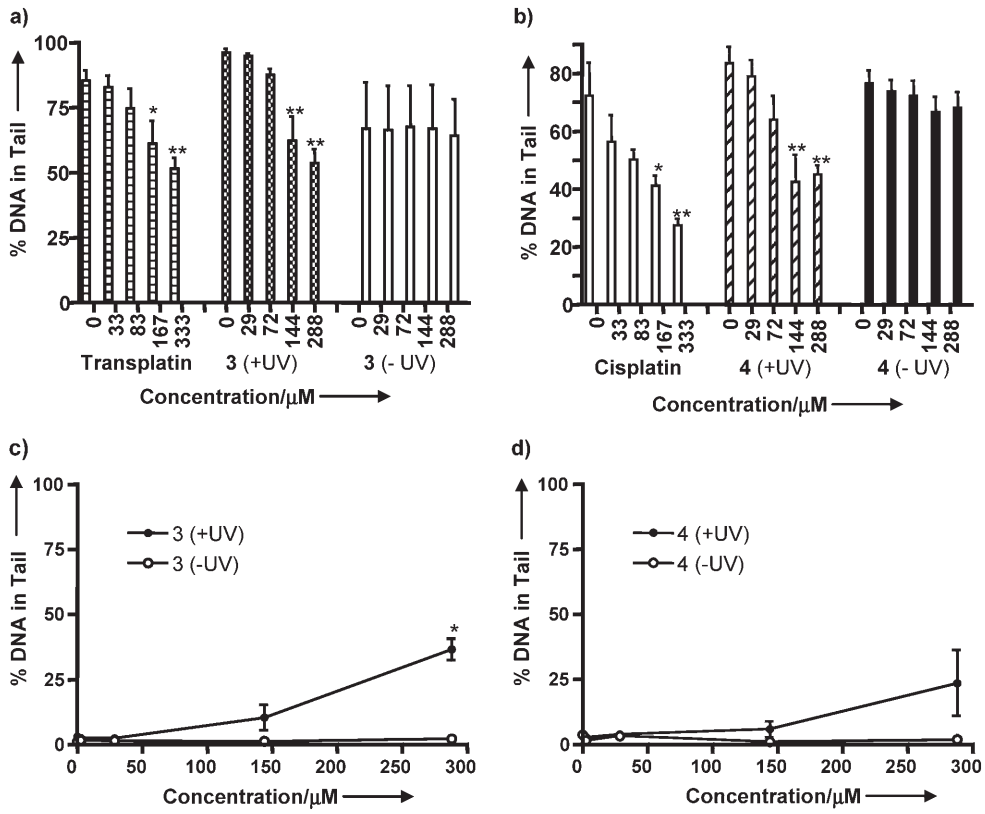

Figure 6. Single-cell gel electrophoresis data (comet assay). a) and b) the production of crosslinked DNA in human HaCaT keratinocyte cells treated with complex **3** in the light (+UV) and dark (−UV) in comparison to transplatin, and complex **4** in comparison to cisplatin. The cells were treated with H_2O_2 which causes DNA fragmentation so that in the absence of crosslinking a large comet tail appears (size set at 100%). Crosslinking reduces the amount of DNA fragmentation and hence reduces the size of the tail (see Figure S2 in the Supporting Information). Maximum DNA damage in the presence of H_2O_2 was 89.7 ± 4.0%. c) and d) Generation of DNA strand-breaks and alkali-labile lesions (no H_2O_2 treatment). The results represent the mean ± SE of 2–4 independent experiments performed in duplicate. The baseline level of alkaline labile damage in non-irradiated cells was 2.5 ± 0.4%, and in UVA-only irradiated cells was 3.1 ± 0.2%. *P < 0.05, **P < 0.01; significantly different from untreated control.

by (usually) UV irradiation, for clinical treatment of benign hyperproliferative disorders such as psoriasis. A derivative of photochemotherapy known as photodynamic therapy (PDT), uses visible light, oxygen and a photosensitizing chemical to treat tumours of the lung, brain and liver, and routinely in dermatology to treat Bowen's disease and basal cell carcinoma.[24] It is also being trialed in Barret's oesophagus and was recently approved for use in age-related macular degeneration. Advantages of using light-activated chemotherapeutics include their inertness, and lack of toxicity to cells in the dark. This means that many of the unpleasant side effects of conventional chemotherapy (nausea, hair loss etc.) are avoided, as only the photosensitizing chemical present in the lesion is irradiated and therefore activated to the cytotoxic form.

Platinum diazide complexes may circumvent some of the current limitations of PDT. The platinum complexes do not require oxygen, which is often lacking in bulky, necrotic or poorly vascularised tumours, and may not give rise to general photosensitivity after administration. After administration of systemic photosensitizing agents, patients can be photosensitive for eight weeks. On the basis of the present work, it is apparent that *trans* diam(m)ino platinum(IV) diazide complexes are worthy of further exploration as photochemotherapeutic agents. Complex **3** has a much higher aqueous solubility (>20 mM) than the *cis* isomer **4**, its LMCT band is more intense and shifted to longer wavelength, and when photoactivated is able to form DNA lesions inaccessible to transplatin.

Experimental Section

Materials: $K_2[PtCl_4]$ was purchased from Alfa-Johnson Matthey plc., KI, NaN_3, KOH, NaCl and HCl from Fisher, $AgNO_3$, $^{15}NH_4Cl$, and D_2O from Aldrich, 9-ethylguanine from Sigma, NH_4Cl from BDH, H_2O_2 (30%) from Prolabo, 5′-GMP from Acros and $HClO_4$ from Fisons. Cell culture chemicals were supplied by Sigma-Aldrich unless otherwise stated, and sterile plastics by Costar (Cambridge, UK).

Syntheses

^{15}N-1: ^{15}N-**1** was prepared by the Dhara method[25] using $^{15}NH_4Cl$ as the source of ^{15}N.

Complex 2: Complex **2** was synthesized by an adaption of the method of Kauffman and Cowan.[26]

^{15}N-2: ^{15}N-**2** was prepared by refluxing a suspension of ^{15}N-**1** (0.19 mmol) in water containing $^{15}NH_4Cl$ (1.9 mmol) pH 11 (adjusted with 2 M KOH). After 24 h, the pH was readjusted from 7 to 11 and refluxing continued until a clear, colorless solution was obtained (ca. 6 h). The solvent was removed, HCl (5.66 mmol, 2.5 mL) added, and the solution refluxed for four days, then cooled, placed on ice for 2 h, filtered, and the product washed with water, ethanol and ether. Yield: 81.8%. ^{1}H NMR (90% H_2O/10% D_2O): $\delta = 3.60$ ppm, $^1J(^{15}N, ^1H) = 72.0$ Hz; 2D [^{1}H, ^{15}N] HSQC NMR: $\delta(^1H,^{15}N) = 3.60$, −66.95 ppm, $^1J(^{195}Pt,^{15}N) = 277$ Hz, $^2J(^{195}Pt,^1H) = 52.5$ Hz.

Warning! Transition-metal ammine azides have been known to detonate easily. We encountered no problems in this study, but these materials should be handled with extreme caution.

^{15}N-3: ^{15}N-**3** was synthesized by adding $AgNO_3$ (0.129 mmol) to a suspension of ^{15}N-**2** (0.066 mmol) in H_2O (7 mL). After the mixture had been stirred in the dark at 333 K for 48 h, the AgCl precipitate was removed by filtration. NaN_3 (0.086 g) was added to the clear solution which was then stirred in the dark for 24 h. Addition of H_2O_2 (30%, 2.64 mmol) followed by stirring for a further 6 h resulted in a cloudy yellow solution. After addition of H_2O (30 mL), the insoluble AgN_3 was filtered off. Crystals of **^{15}N-3**, obtained after concentration of the solution and cooling to 277 K, were filtered off, washed with water, ethanol and diethyl ether. Yield: 56.2%. ^{1}H NMR (90% H_2O/10% D_2O): $\delta = 5.32$ ppm, $^1J(^{15}N, ^1H) = 72.0$ Hz; 2D [^{1}H, ^{15}N] HSQC NMR: $\delta(^1H,^{15}N) = 5.32$, −41.65 ppm, $^1J(^{195}Pt,^{15}N) = 282$ Hz, $^2J(^{195}Pt,^1H) = 47.5$ Hz. Crystals of **3**·4H_2O suitable for X-ray diffraction were grown from water at 277 K.

Complex 4: Complex **4** was synthesized as described previously.[10]

X-ray crystallography: Diffraction data were collected with $Mo_{K\alpha}$ radiation on a Bruker Smart Apex CCD diffractometer equipped with an Oxford Cryosystems low-temperature device operating at 150 K. Data were corrected for absorption using the SADABS[27] procedure, and the structure was solved by direct methods (SHELXS).[28] The structure was refined against F^2 using all data (SHELXL).[29] All non-hydrogen atoms were modelled with anisotropic displacement parameters. Hydrogen atoms were located in Fourier maps; the NH_3 group was refined according to the Sheldrick rotating rigid group model, restraints were applied to the lengths of the OH bonds.

Crystal data for **3**·4H_2O: $H_{16}N_8O_6Pt$, triclinic, space group $P\bar{1}$, $a = 5.4038(5)$, $b = 5.8538(5)$, $c = 9.0518(8)$ Å, $\alpha = 87.8130(10)$, $\beta = 78.7900(10)$, $\gamma = 72.4560(10)°$, $V = 267.74(4)$ Å^3, $Z = 1$ (the Pt complex occupies an inversion centre), $\rho_{calcd} = 2.600$ Mg m^{-3}. The final conventional R factor [$R1$, based on $|F|$ and 1226 data with $F > 4\sigma(F)$] was 0.0157, and w$R2$ (based on F^2 and all 1226 unique data to $\theta_{max} = 28.6°$) was 0.0384. The final ΔF synthesis extremes were +1.62 and −1.56 eÅ^{-3}. CCDC-288954 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

NMR spectroscopy: 1D [^{1}H] and 2D [^{1}H, ^{15}N] HSQC NMR spectra were recorded on a Bruker DMX 500 NMR spectrometer (^{1}H: 500.13 MHz, ^{15}N: 50.7 MHz) using dioxane (3.764 ppm) as the internal $\delta(^1H)$ standard. All $\delta(^{15}N)$ values were referenced externally to $^{15}NH_4^+$ at $\delta = 0$ ppm. Spectra were acquired at 298 K, and processed using Xwinnmr (version 3.6, Bruker UK Limited) software. pH values were measured with an Orion 710 A pH meter equipped with a chloride-free microcombination electrode (Aldrich) calibrated with Aldrich standard buffers (pH 4, 7 and 10), and were adjusted with $HClO_4$ (0.1 M).

UV/Vis spectroscopy: UV/Vis spectra were recorded on a Varian Cary 300 UV-visible spectrophotometer using 1 cm path-length quartz cuvettes (3 mL). All spectra were measured in H_2O. Data were processed with Microcal Origin 5.0.

Irradiations: Chemical samples were irradiated using a Model VL-215 L UV lamp supplied by Merck Eurolab, operating at 365 nm, or with an argon–krypton ion laser (Coherent Innova 70C Spectrum) equipped with a fibre optic (FT-600-UMT, ∅ 600 μm; Elliot Scientific Ltd.). The power delivered from the fibre optic was measured by using a Fieldmate Powermeter (Coherent, OP2-VIS head).

For cell irradiation, 2×6ft Cosmolux RA Plus (Cosmedico), 15500/100W light sources were used, each filtered to attenuate UVB/UVC wavelengths (see Figure S1 in the Supporting Information). Irradiance was measured with a Waldmann PUVA meter, calibrated to the source using a double grating Spectroradiometer (Bentham, UK).

Photoreactions: Photoreactions of aqueous solutions of **3** and **4** after irradiation at 365 nm (P = 12 mW) were followed by recording UV/Vis spectra (0.05 mM) or 1D [^{1}H] and 2D [^{1}H, ^{15}N] HSQC NMR spectra (3 mM 90% H_2O/10% D_2O). For the latter, the pH was adjusted to 5 with 0.1 M $HClO_4$, and re-adjusted to 5±0.2 after each irradiation to ensure slow exchange of NH protons (on Pt^{IV}).

Reactions of **3** and **4** (3 mM in 90% H_2O/10% D_2O) with 2 molar equivalents of 5′-GMP were followed by 1D [^{1}H] and 2D [^{1}H, ^{15}N] HSQC NMR spectroscopy. Irradiation was carried out at 365 nm (P = 12 mW) and 1D and 2D spectra recorded at various time points. The pH was re-adjusted to 5±0.2 after each irradiation (to ensure slow NH exchange on Pt^{IV}). Similar spectra were obtained when reactions were carried out at pH 7

and adjusted to pH 5 just before recording NMR spectra. The photoreaction of **3** with 5′-GMP using red light was carried out with an argon-krypton ion laser as the light source ($\lambda = 647.1$ nm, P = 18 mW).

Cell experiments: HaCaT cells were incubated with test compounds in Earle's Balanced Salt Solution (EBSS) for 1 h at 310 K/5 % CO_2, and then irradiated for 50 min with 5 J cm^{-2} glass filtered UVA (1.77 mW cm^{-2}; λ_{max} 365 nm). Full details of the cell experiments including the comet assays are in the Supporting Information.

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