VIP Photoactivated Prodrugs

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A Potent *Trans*-Diimine Platinum Anticancer Complex Photoactivated by Visible Light**

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Photoactivation provides a route for the selective activation of anticancer drugs within tumors, thereby avoiding damage to surrounding normal tissue. It also has the potential for new mechanisms of action and non-cross-resistance with existing therapies. Many tumors are relatively hypoxic and photoactive metal complexes in clinical use are typically less effective in low-oxygen conditions.[1,2] Thus complexes based on, for example, platinum[3,4] and rhodium[5] that offer the possibility of oxygen-independent photochemotherapy are particularly attractive.

The challenge is to design complexes which are thermally stable and can reach the target site (for example DNA) in an intact form before photoexcitation. They need to remain unreactive towards extra- and intracellular biomolecules, such as the reducing agent glutathione (GSH) present in most cells at millimolar concentrations. A further goal is to control the wavelength of photoactivation so that excitation can be achieved at different depths within tissues (longer wavelengths penetrate more deeply than short wavelengths).

Platinum complexes are particularly attractive as potential photochemotherapeutic agents because of the clinical success of the platinum(II) diam(m)ine complexes cisplatin, carboplatin, and oxaliplatin.[6] Selective photoactivation of platinum complexes in cancer cells might avoid toxic side-effects and extend their application to resistant cells and to a wider range of cancer types. Our search for suitable dark-stable photoactivatable platinum complexes has led to a focus on *trans*-dihydroxo platinum(IV) diazido complexes with *trans* diamine ligands. However to date, we have been able to generate significant cancer cell cytotoxicity only upon irradiation with short wavelengths (UVA, 365 nm), which is of limited use in clinical applications.[1] Herein we present the discovery that the novel platinum(IV) diazido complex *trans*,*trans*,*trans*-$[Pt(N_3)_2(OH)_2(py)_2]$ (**1**) is stable in solution and also towards GSH for several days and can be photoactivated with low doses, not only of UVA, but also of visible blue and green light to give potent, cytotoxic effects in a number of cell lines.

Complex **1** was synthesized on a gram scale in good yield by H_2O_2 oxidation of the *trans*-bispyridine platinum(II) intermediate, and characterized by elemental analysis, ^1H, ^{195}Pt, ^{13}C, and ^{15}N NMR and UV/Vis spectroscopy, HRMS, and X-ray crystallography. ^{15}N-Pyridyl-**1** (*trans*,*trans*,*trans*-$[Pt(N_3)_2(OH)_2(^{15}Npy)_2]$, **1a**) and doubly labeled *trans*,*trans*,*trans*-$[Pt(^{15}N_3{*})_2(OH)_2(^{15}Npy)_2]$ (**1b**) (where $^{15}N_3{*}$ = end-labeled azide, $[^{15}N{-}^{14}N{-}^{14}N]^-$; Supporting Information, Figure S1), were also synthesized to aid NMR spectroscopic studies. ^{195}Pt NMR spectra of the complexes are shown in the Supporting Information, Figures S2,S3.

In the X-ray crystallographic structure of **1** (Figure 1 and Supporting Information), octahedral platinum(IV) is coordinated to two approximately linear *trans* azido ligands (N-N-N angles 174°–176°). The angles subtended at the coordinated azido ligand N(5/2)–N(4/1)–Pt (118–121°) and the Pt–Nα (azido) bond lengths are similar to those for *trans*,*trans*,*trans*-$[Pt(N_3)_2(OH)_2(NH_3)(py)]$[4] and *trans*,*trans*,*trans*-$[Pt(N_3)_2(OH)_2(NH_3)_2]$.[7]

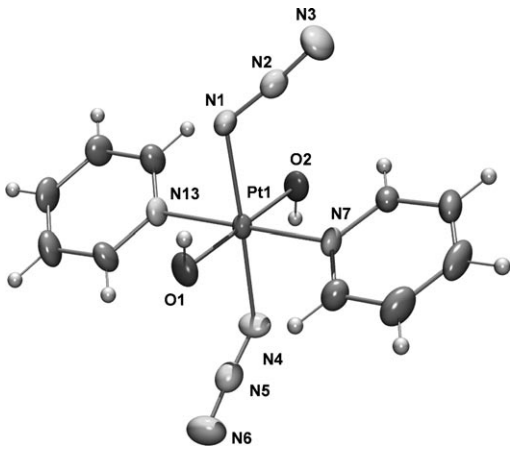

Figure 1. X-ray crystallographic structure of *trans*,*trans*,*trans*-$[Pt(N_3)_2(OH)_2(py)_2]$ (**1**) with ellipsoids set at 50% probability.[8]

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Supporting information for this article, including details of chemicals and biochemicals, synthesis and characterization of complexes, and methods and techniques used, is available on the WWW under http://dx.doi.org/10.1002/anie.201003399.

Complex **1** is more thermally stable than either the monoammine complex *trans,trans,trans*-$[Pt(N_3)_2(OH)_2(NH_3)(py)]$ or the diammine complex *trans,trans,trans*-$[Pt(N_3)_2(OH)_2(NH_3)_2]$, and decomposes only at about 453 K. It also has good aqueous solubility (ca. 34 mM; 291 K, by ICP-OES) and is stable in Eagle's balanced salt solution (EBSS, a biological cell culture medium) for a period of six weeks at 293 K (no change in the UV/Vis spectrum). Complex **1** was detected by ESI-MS from CH_3OH/H_2O as the sodium adduct $[\mathbf{1}+Na]^+$ at *m/z* 494.0638, with $[(\mathbf{1})_2+Na]^+$ and $[(\mathbf{1})_2+H]^+$ also present. The $[\mathbf{1}+Na]^+$ complex fragmented by collision-induced dissociation through loss of neutral fragments (pyridine, N_2, $N_3^{\bullet}$) giving $[Pt(N_3)(OH)_2(py)+Na]^+$ and $[Pt(OH)_2(N)(py)+Na]^+$ as daughter ions (Supporting Information, Figure S4). Complex **1** (6 mM in D_2O) underwent only a very slow reaction when incubated with 2 mol equiv GSH in the dark at 298 K, as monitored by ^1H NMR spectroscopy. After one week, 70% of **1** remained unreacted.

Using 365 nm radiation, complex **1** was potently phototoxic towards a number of human cell lines, including keratinocytes (HaCaT), parental (A2780) and cisplatin-resistant (A2780CIS) ovarian carcinoma, oesophageal adenocarcinoma (OE19), and hepatoma (HepG2) cells (Table 1).

Table 1: IC_{50} values and phototoxic index of **1**. Data are from at least two independent experiments.

Cell type	IC_{50} [μM]			Phototoxic index (PI)[a]	
	365 nm	420 nm	sham (dark)	UV-A	visible[b]
HaCaT	1.4	9.5	>212.3	>151	>22
A2780	1.4	n.d.[c]	>212.3	>151	n.d.
A2780CIS	14.5	n.d.	>212.3	>15	n.d.
OE19	4.7	8.4	>212.3	>45	>25
HepG2	2.5	n.d.	>212.3	>85	n.d.

[a] PI values for Photofrin for comparison: HaCaT 1667, OE19 >1000; PI CPZ: HaCaT 42, A2780 54, A2780CIS 33, OE19 34, HepG2 35. [b] Blue light. [c] n.d. = not determined.

In HaCaT cells, complex **1** is an order of magnitude more potent than cisplatin when activated with a low-dose (5 J cm^{-2}) of visible light (420 nm). The IC_{50} value of 8.4 μM in OE19 cells when irradiated with visible light (Table 1) is notable; globally oesophageal cancer ranks eighth in incidence and is increasing in Western populations.[9] It is therefore a potential target for platinum photochemotherapy.

The wavelength dependence of the photodecomposition of **1** in solution correlated closely with the UV/Vis absorption spectrum (Figure 2; Supporting Information, Tables S10,S11). The phototoxicity of **1** in cells extends into the green region of the electromagnetic spectrum (500 ± 30 nm; Supporting Information, Figure S8) and is a function of at least three parameters: accumulation, the pseudo quantum yield of photodecomposition of the parent compound, and toxicity of the initial photoproducts produced at each wavelength. The efficiency of production and toxicity of secondary photoproducts and downstream reactions involving biomolecules present in the cell could also play a role.

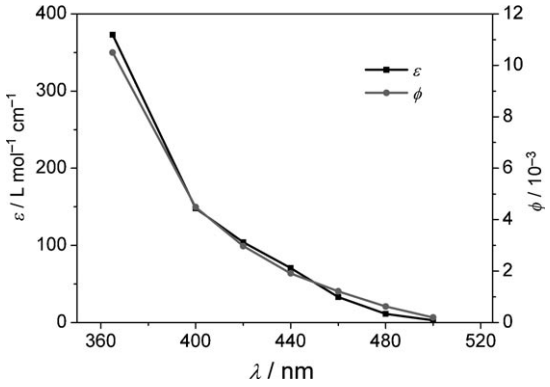

Figure 2. Wavelength dependence of the photochemical decomposition of **1** (50 μM solution in H_2O) as determined by UV/Vis spectroscopy. ■ Extinction coefficient ε; ● pseudo quantum yield ϕ [%]; ϕ was calculated using potassium ferrioxalate actinometry (see the Supporting Information).

No pyridine release from **1** was observed by ^1H NMR spectroscopy following irradiation of an aqueous solution of **1** (λ_{irr} 350, 420, or 450 nm). This behavior is in contrast to both *trans,trans,trans*-$[Pt(N_3)_2(OH)_2(NH_3)(py)]$ and *trans,trans,trans*-$[Pt(N_3)_2(OH)_2(NH_3)_2]$, which show NH_3 dissociation on irradiation.[4,7] Ammine ligand loss may be responsible for the reduced cytotoxic activity of these two derivatives compared to **1**.

DFT (restricted and unrestricted) and TDDFT calculations were used to characterize the singlet and triplet excited states of **1** (Supporting Information, Figures S5–S7 and Tables S1–S8).[10] The theoretical UV/Vis spectrum (red line, Figure 3) was obtained by calculating 32 singlet excited transitions and was then compared with the experimental spectrum (black line). Both spectra show two absorption bands in the UV region (shoulder for theoretical data), which, according to TDDFT, can be assigned to dissociative ^{1}LMCT ($N_3\rightarrow Pt$) and mixed ^{1}LMCT/^{1}IL ($OH\rightarrow Pt,N_3$; IL = interligand) transitions. Interestingly, **1** has low-intensity transitions in the blue region of the visible spectrum (414 nm; Figure 3 inset, Supporting Information, Tables S2,S3) that are disso-

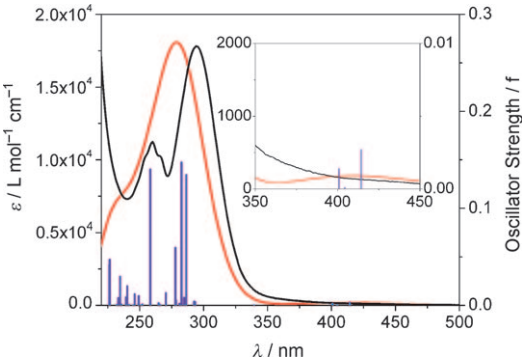

Figure 3. Calculated (red) and experimental (black) absorption spectrum of *trans,trans,trans*-$[Pt(N_3)_2(OH)_2(py)_2]$ (**1**) in H_2O. Inset: expansion of the region around 414 nm. The excited states are shown as vertical blue bars, with heights equal to the extinction coefficients. The theoretical curve was obtained using GAUSSSUM 2.2.[13]

ciative and have mixed ^{1}LMCT/^{1}IL character and involve N_3 and OH ligands and platinum. Such transitions might account for the photoactivity induced by blue light, because they have significant contributions from the LUMO orbital, which is strongly σ-antibonding towards the two Pt–N_3 bonds.[10,11] Population of this orbital upon light excitation causes Pt–N_3 bond elongation, which can eventually lead to azide release.

The presence of a heavy transition metal such as platinum can promote ultrafast intersystem crossing and formation of triplet states once the complex is excited by light. Optimization of the geometry of the lowest-lying triplet state (Supporting Information, Tables S4–S7) indicates that such a state is highly dissociative and can give rise to dissociation of the two azido ligands. The Pt–N_3 distances in the triplet state are significantly increased compared to the ground-state geometry: from 2.073 Å to 2.420 Å and from 2.074 Å to 2.628 Å.

Analysis of Mulliken and NBO charges[12] of the ground- and triplet-state geometries shows a significant loss in negative charge from the azido ligands in the lowest-lying triplet state and a decrease of positive charge on platinum (Supporting Information, Table S8), thus accounting for the reductive elimination of azides (see below; as observed by ^{1}H and ^{195}Pt NMR and ESI-MS).

The nature of the photoproducts produced when **1** was irradiated in the presence of 5′-GMP was investigated, as the DNA base guanine is a preferred target for platinum amine species. The photochemical reaction of **1** (9 mM) with 2 mol equiv 5′-GMP upon irradiation (λ_{irr} 350, 420, 450, or 455 nm) in H_2O/D_2O or D_2O was monitored by ESI-MS and ^{1}H and ^{195}Pt NMR spectroscopy. In general, irradiation caused the solution to lighten in color (from an intense yellow) with concomitant formation of gas bubbles and a yellow precipitate. Irradiation (λ_{irr} 420 nm, 8 mW cm^{-2}) in D_2O led to the rapid decrease in the concentration of **1** (δ^{195}Pt 964 ppm) to give the major new species **A** (δ^{195}Pt = −2212 ppm) and **B** (δ^{195}Pt = −2288 ppm; Figure 4), as well as to the minor platinum(IV) species **D** (δ^{195}Pt = 874 ppm) assignable as $[Pt(N_3/OH)(OH)_2(5'\text{-GMP})(py)_2]^+$. Following irradiation, product **A** slowly oxidized to **D** over the course of a few days, whereas the concentration of **B** and unreacted **1** did not change. By analogy with the photochemical activity of *trans*,*trans*,*trans*-$[Pt(N_3)_2(OH)_2(NH_3)(py)]$,[4] complex **A** can be reasonably assigned as *trans*-$[Pt(N_3)(py)_2(5'\text{-GMP})]^+$ and **B** as *trans*-$[Pt(py)_2(5'\text{-GMP})_2]^{2+}$ (the charge on the GMP is ignored in the formulae). The identity of **B** was confirmed by synthesis. The retention of the pyridine ligands is again notable, suggesting that if such reactions occur on DNA, then the presence of these ligands will influence the subsequent recognition of adducts by repair enzymes and other proteins and contribute to the potency of the complex.

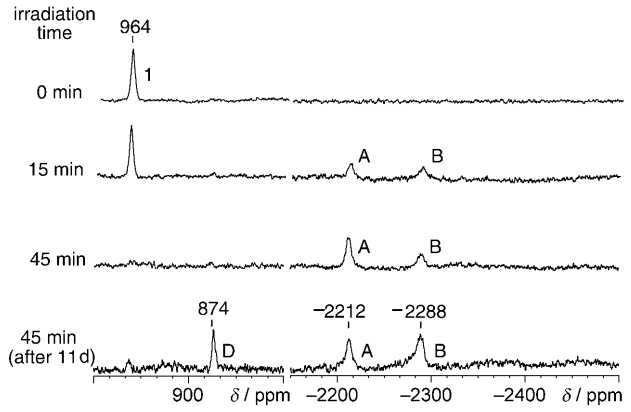

Figure 4. ^{195}Pt NMR spectra (129 MHz, 298 K) of a 9 mM D_2O solution of complex **1** and 2 mol equiv of 5′-GMP, showing the conversion into photoproducts **A** and **B** with irradiation (420 nm, 8 mW cm^{-2}) and the increase in species **D** following irradiation.

The photoactivated reaction of **1** with 5′-GMP (λ_{irr} 420 nm) was also monitored by ^{1}H–^{15}N HMBC NMR spectroscopy, using the ^{15}N-labeled complex **1a** (3 mM in 10 % D_2O/90 % H_2O). Intriguingly, the ^{15}N NMR chemical shifts of the pyridine ligands in the platinum(II) photoproducts **A** (δ^{15}Npy = 174 ppm) and **B** (δ^{15}Npy = 171 ppm) are only slightly shifted downfield compared to the platinum(IV) complex **1a** (δ = 167 ppm; Supporting Information, Figure S9). The pyridine ligand ^{15}N NMR chemical shift is thus much less diagnostic of the oxidation state of platinum compared to Pt–$^{15}NH_3$ systems; for example, for $H_3{}^{15}$N–Pt–O the ^{15}N range is typically δ = −75 to −90 ppm for platinum(II) and δ = −47 to −54 ppm for platinum(IV).[14] This finding confirms that aromatic imines have a major influence on the electronic distribution in platinum complexes, which may contribute to their enhanced biological affects. The ESI-MS one month after irradiation at 420 nm was dominated by a signal at *m*/*z* 794.1232, which is in agreement with the proposed identity of **D** as $[Pt(OH)_2(N_3)(5'\text{-GMP})(^{15}Npy)_2]^+$ (calcd. *m*/*z* 794.1158; Supporting Information, Figure S10 B).

We used a comet assay (see the Supporting Information) to show that **1** could photosensitize DNA–DNA or DNA–protein crosslinks in human cells, given that the major photoproduct (**A**) in the model reaction described above was a monosubstituted 5′-GMP-Pt^{II} species. (Supporting Information, Figure S11 A–C,E). Non-photoactivated **1** did not produce detectable DNA reactivity in the assay (Supporting Information, Figure S11 A–C). Moreover, in contrast to cisplatin, photoactivated **1** did not result in fragmented or condensed nuclei (Supporting Information, Figure S12), suggesting a mechanism or response to treatment different from that of cisplatin.

In conclusion, we have synthesized a novel *trans* diimine diazido platinum(IV) complex, *trans*,*trans*,*trans*-$[Pt(N_3)_2(OH)_2(py)_2]$ (**1**), that can be photoactivated over a range of wavelengths. The complex is potently cytotoxic towards cancer cells using low doses of visible light. The wavelength-dependent photochemical decomposition in solution correlates well with the electronic absorption spectrum. The presence of planar σ-donor/π-acceptor pyridine ligands in **1** clearly has a critical effect not only on the photoactivation pathways but also on the biological activity. The pyridines appear to remain strongly bound to platinum, even after photoactivation. Planar ligands in platinum(II) species have been shown to reduce the rate of DNA binding,[15] and *trans* complexes offer the possibility of forming DNA–protein as well as DNA–DNA crosslinks.[16] *Trans* platinum(II) complexes themselves have attracted increasing attention since

the discovery that complexes such as *trans*-[$PtCl_2${E-HN=C-$(OCH_3)CH_3$}$_2$] can exhibit higher cancer cell cytotoxicity than their *cis* isomers.[17] Sterically hindered complexes, such as *trans*-[$PtCl_2$-(4-picoline)(piperidine)] and *trans*-[$PtCl_2$(4-picoline)(piperizine)]HCl, are cytotoxic towards cancer cells and are also distinct from cisplatin in their mechanism of action.[18] Isopropylamine *trans* Pt complexes have also shown appreciable cytotoxicity.[19] DFT calculations suggest that low-intensity transitions in the blue region of the visible spectrum are dissociative, have mixed ^{1}LMCT/^{1}IL character involving N_3 and OH ligands and platinum, and can account for the photoactivity induced by blue light. Both platinum(II) and platinum(IV) photoproducts may be involved in the biological activity. Importantly, the pyridine ligands remain strongly bound on photoactivation, and mono- and bis-guanine adducts are readily formed with release of the azido ligands. Photoactivated **1** did not produce fragmented or condensed nuclei in contrast to cisplatin, suggesting that the nature of any DNA lesions, or the response to them, is different from cisplatin. A recent study has revealed that the pyridine ligand in $[PtCl(NH_3)(py)]^+$ may be responsible for stalling RNA polymerase II during transcription providing a basis for a different mechanism of action for platinum–pyridine complexes compared to cisplatin.[20] It seems clear that the molecular pharmacology of the photoactivatable excited-state platinum(IV) complex **1** is quite distinct from that of ground-state platinum(IV) complexes, which are currently in clinical development.[21] Novel complexes such as **1** show promise for treating cancerous or pre-cancerous conditions with visible light (especially thin-walled organs such as the bladder and oesophagus), with the cytotoxic effect potentially acting through platination of cellular components such as DNA and/or proteins. The preclinical development of complex **1** is currently being explored.

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