

Supplementary information

Photocytotoxic Pt(IV) complexes as prospective anticancer agents

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All the spectra presented in this document were recorded at room temperature unless otherwise stated.

Paragraph S1. Preparation of the complexes

4'-Phenyl-2,2':6',2''-terpyridine, prepared according to reference 1:

¹H NMR (CDCl₃, 400 MHz): δ 8.76 (s, 2H, H3'); 8.74 (m, ³J_{HH} = 4.8 Hz, ⁴J_{HH} = 1.7 Hz, 2H, H6); 8.68 (m, ³J_{HH} = 7.8 Hz, ⁴J_{HH} = 1.1 Hz, 2H, H3); 7.92 (m, ³J_{HH} = 7.9 Hz, 2H, Ha); 7.89 (dd, ³J_{HH} = 7.8 Hz, ⁴J_{HH} = 1.7 Hz, 2H, H4); 7.49 (m, 3H, Hb, Hc); 7.36 (ddd, ³J_{HH} = 7.8 Hz, ³J_{HH} = 4.8 Hz, ⁴J_{HH} = 1.1 Hz, 2H, H5).

¹³C NMR (CDCl₃, 100 MHz): δ 156.4, 156.0, 150.5 (quaternary carbons); 149.2 (C6); 138.6 (quaternary carbon); 137.0 (C4); 129.2 (Cc); 129.1 (Cb); 127.5 (Ca); 124.0 (C5); 121.5 (C3); 119.1 (C3'). Anal. Calcd (%) for C₂₁H₁₅N₃: C 81.53, H 4.89, N 13.58. Found C 82.04, H 4.88, N 13.82.

[PtCl₂(COD)], prepared according to reference 2:

¹H NMR (CDCl₃, 400 MHz): 5.61 (s with ¹⁹⁵Pt satellites, ²J_{PtH} = 67 Hz, 4H) 2.71(m, 4H); 2.26 (m, 4H).

¹⁹⁵Pt NMR (CDCl₃, 86 MHz): -3339 ppm.

Anal. Calcd (%) for C₈H₁₂Cl₂Pt: C 25.68, H 3.23. Found: C 25.60, H 3.14.

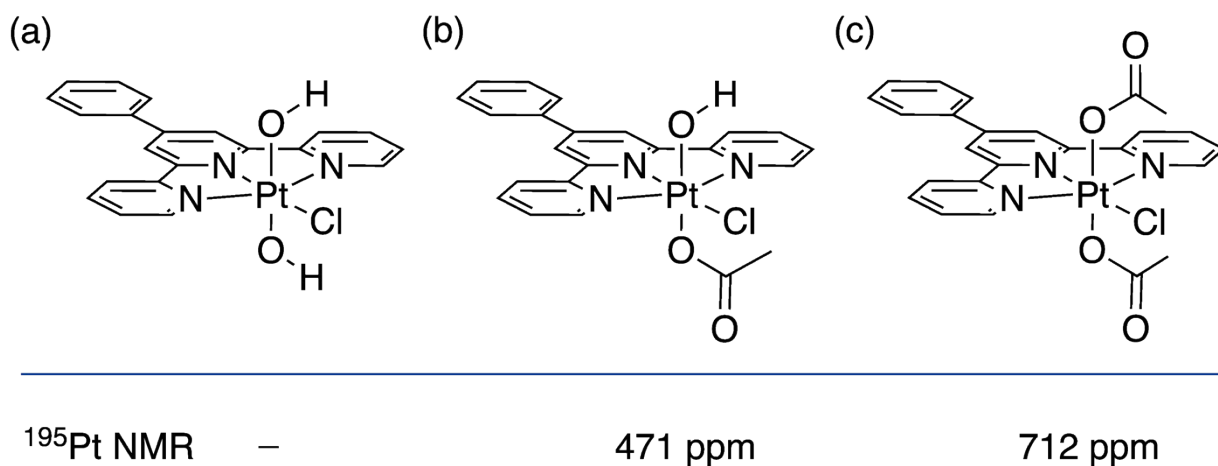


Figure S1. The possible isomers produced during the oxidation of $[\text{PtCl}(\text{phterpy})][\text{CF}_3\text{SO}_3]$ with H_2O_2 in acetic acid as solvent. The species were identified with the help of ^1H , ^{195}Pt NMR spectroscopy and according to the reported oxidation mechanism of Pt(II) with H_2O_2 ³. The ^{195}Pt NMR resonance was recorded only for the species present in a sufficiently high amount.

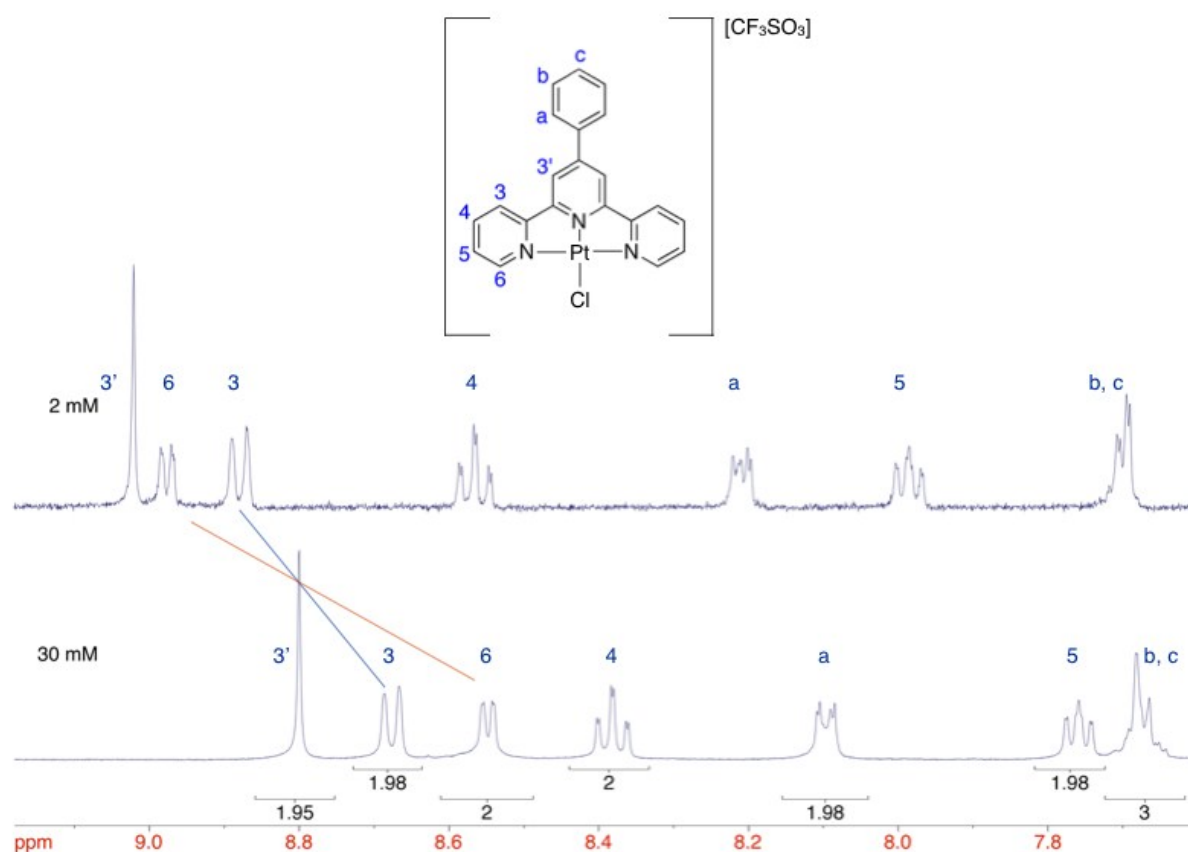
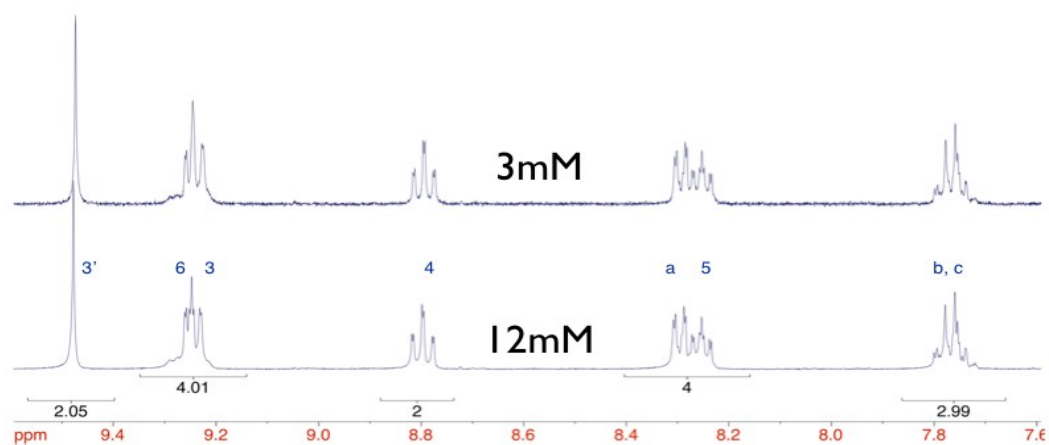


Figure S2. ^1H NMR spectrum of **1** in $\text{DMSO-}d_6$ at two different concentrations. The signals of the protons, indicated by the numbers in the picture of the compound, change their resonances according to the concentration. They are upfield shifted as the concentration increases, especially the proton H6.

(a)



(b)

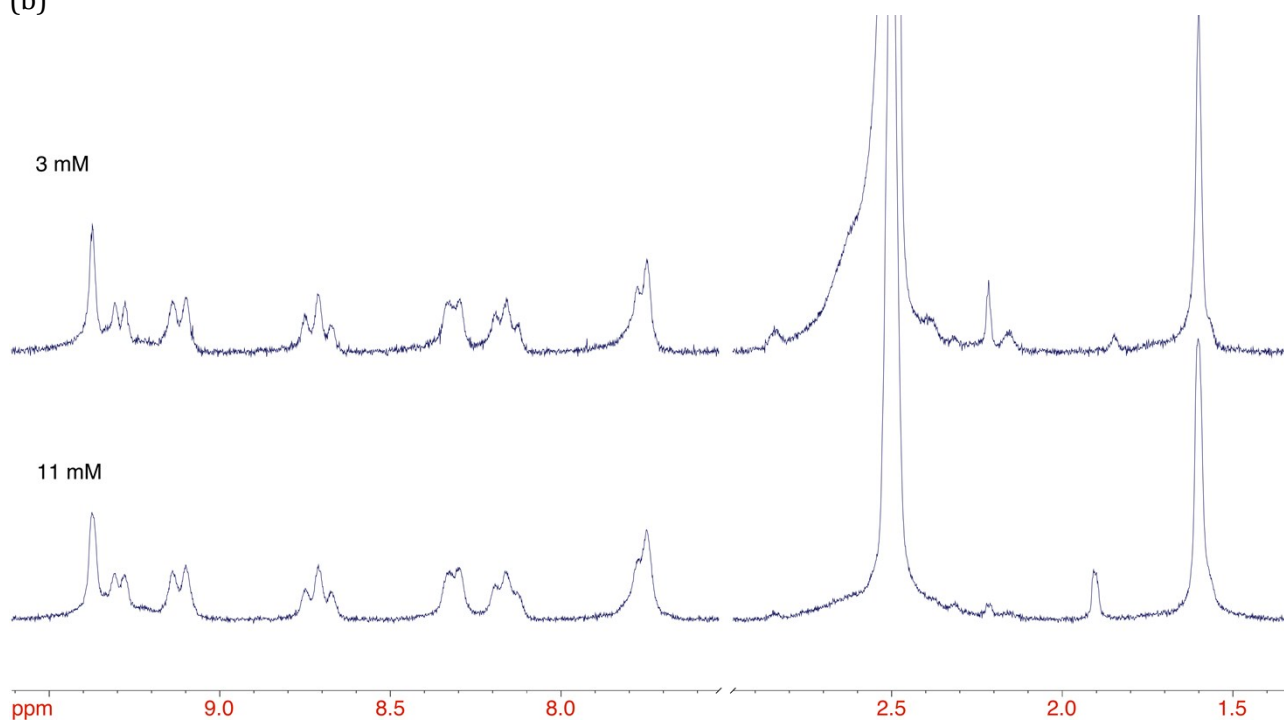


Figure S3. ¹H NMR spectrum of **2** (a) and of **3** (b) in DMSO-*d*₆ at the concentrations specified. The spectra show that the concentration dependence (shown in Figure S2 for compound **1**) is lost after the coordination of two axial chlorides or acetates, to give the octahedral Pt(IV).

Paragraph S2. Solution behavior

We studied the stability of **1** in DMSO, in order to see if there is displacement of the chloride ligand in the coordinating solvent. **1** was firstly treated with an excess of AgCF_3SO_3 in DMSO and a ^{195}Pt NMR spectroscopy analysis was carried out. A very small signal due to the species without the chloride (at -2450 ppm) was found together with the signal of the parent compound (at -2698 ppm). The chloride abstraction might only be partial because the precipitation of AgCl in DMSO, which is the driving force for the reaction, is not complete (AgCl is slightly soluble in DMSO, by less than 10 mg in 100 mL at 25 °C, data taken from Gaylord Chemical Company). Without the addition of the silver salt, only the signal of the parent compound is found in DMSO solution, so there seems to be no chloride dissociation.

Secondly, a cyclic voltammetry of **1** in DMSO showed no oxidation waves scanning towards the positive potential, indicating that all the species present in solution are stable with respect to oxidation. But when NBu_4Cl (0.5 equivalents with respect to Pt(II)) was dissolved in the system, a new oxidation process took place at 0.75 V (Figure S4). This process is clearly visible and was attributed to the oxidation $\text{Cl}^- \rightarrow 1/2\text{Cl}_2$. Therefore, even if we had a very small concentration of chlorides in solution, we would have been able to see them: since **1** in DMSO did not produce any oxidation wave, its chloride should not be dissociated.

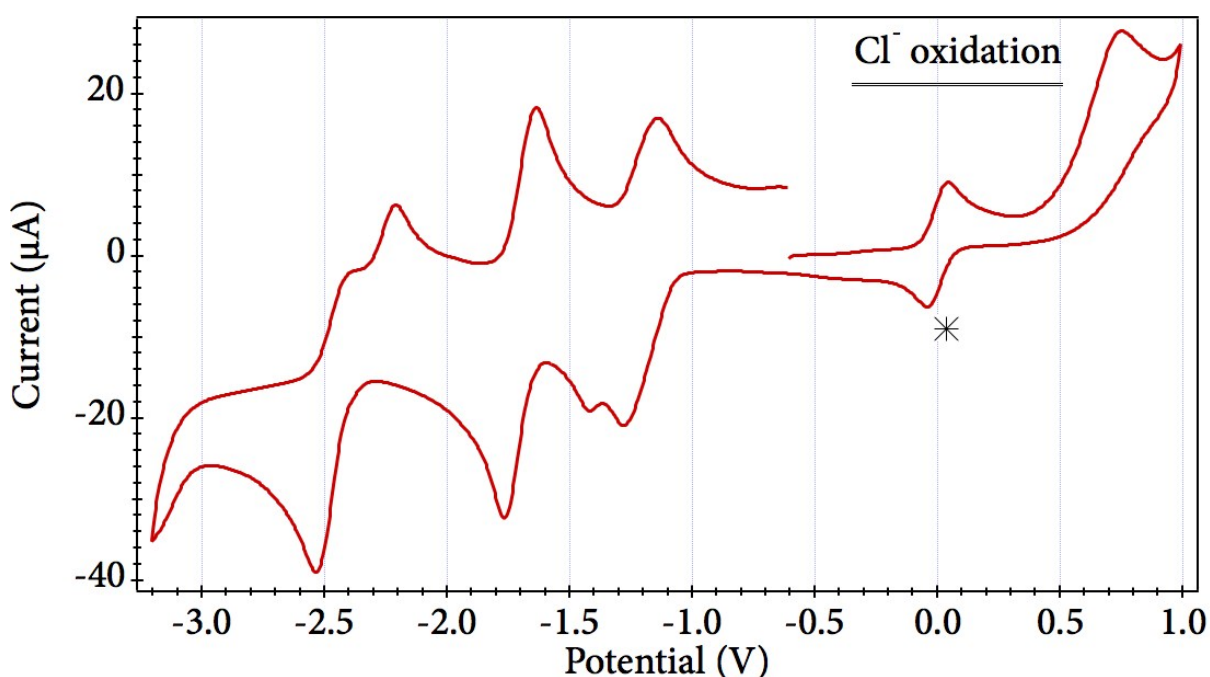


Figure S4. CV of **1** (1 mM) in DMSO with $[\text{N}^n\text{Bu}_4][\text{CF}_3\text{SO}_3]$ (0.1 M) as supporting electrolyte. The wave at 0.7 V indicates the oxidation of the chloride anions present in the solution after the addition of NBu_4Cl (0.5 equivalents). The asterisk indicates the ferrocene/ferrocenium couple.

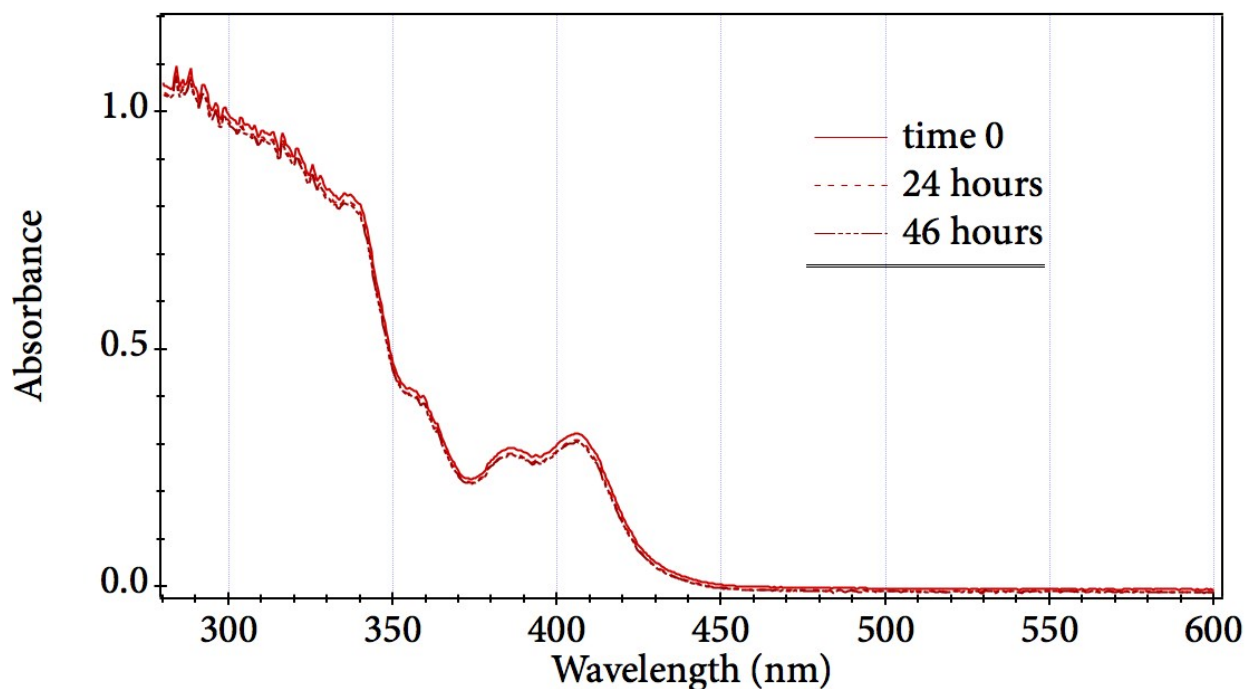


Figure S5. UV-Vis absorption spectra of **1** (5×10^{-5} M) in DMSO; the spectra were recorded every 2 hours for a total of 46 hours. The absorbance is almost unchanged after 2 days.

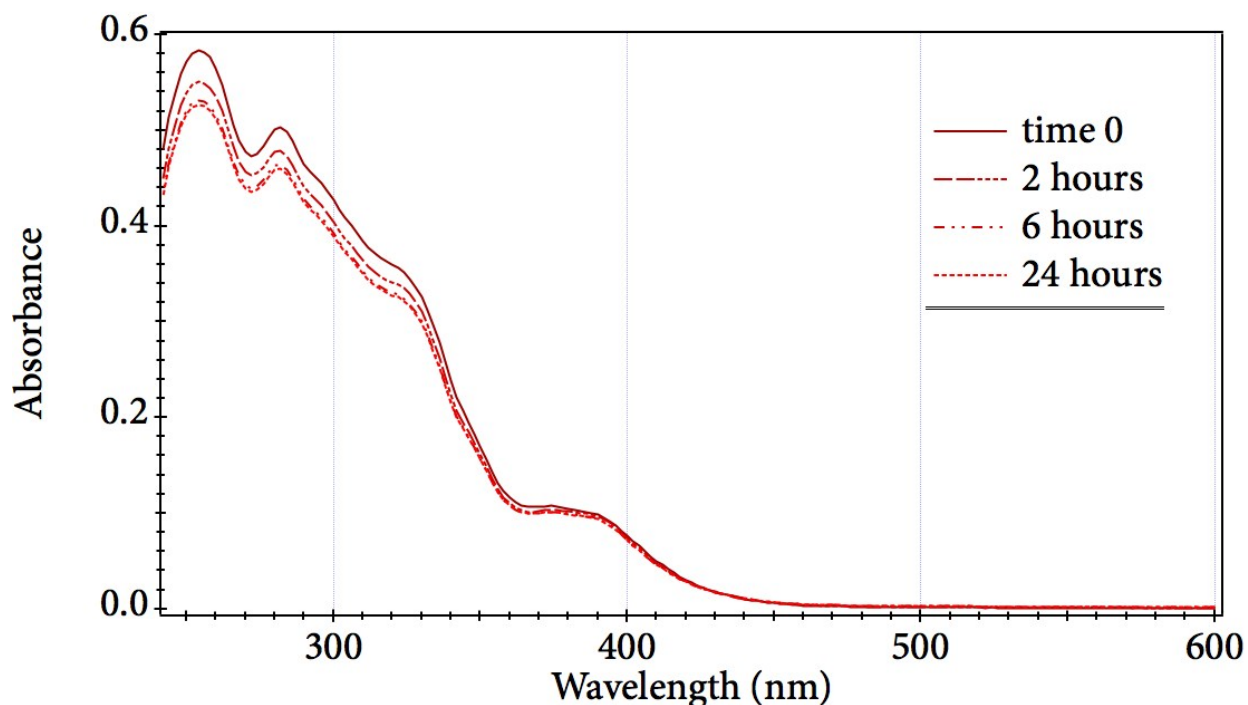


Figure S6. UV-Vis absorption spectra of **1** (2×10^{-5} M) in aqueous sodium cacodylate 0.1 M, $[Cl^-] = 0.007$ M, DMSO content less than 2 %. The pH was adjusted to 7.4 with hydrochloric acid. The spectra are recorded at the time specified in the legend.

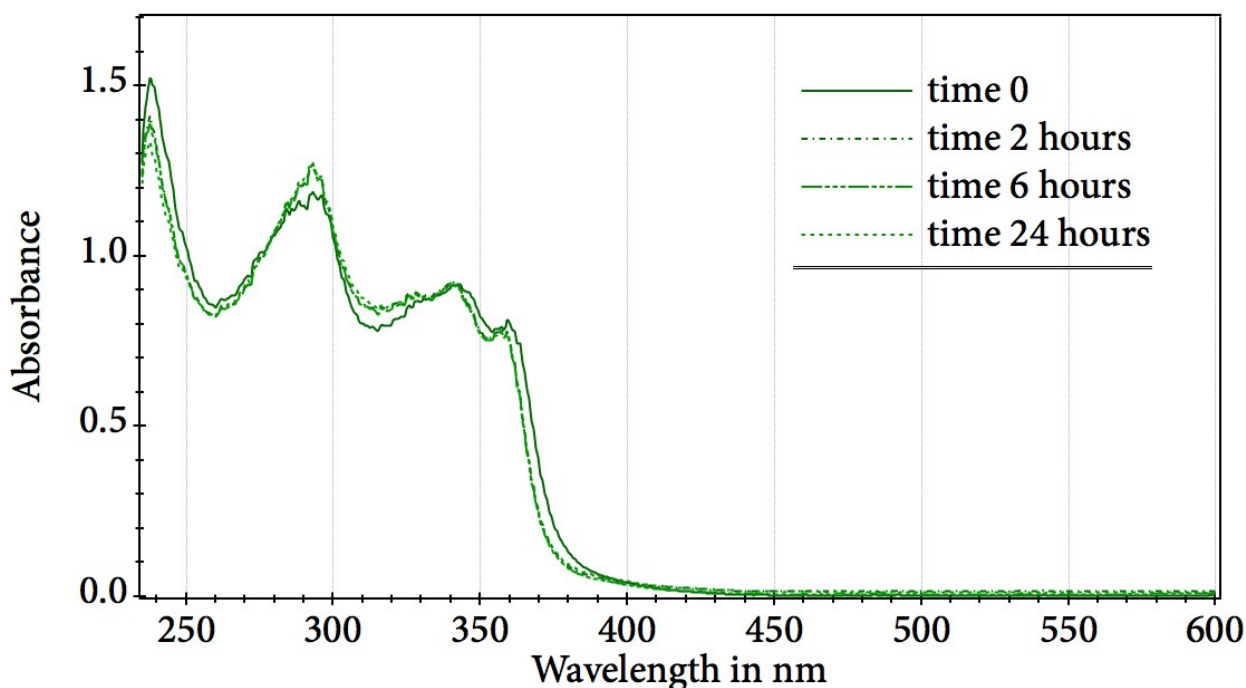


Figure S7. UV-Vis absorption spectra of **2** (4.9×10^{-5} M) in sodium cacodylate 0.1 M solution ($[\text{Cl}^-] = 0.007$ M, pH adjusted to 7.3 with hydrochloric acid and DMSO content less than 2 %). The spectra are recorded at subsequent times to see if degradation takes place. In order to be sure that the light of the spectrophotometer was not having an effect on the stability, each solution in the cuvette, after the analysis, was discarded and a mother solution, kept in the dark and prepared at time 0, was used as a replacement.

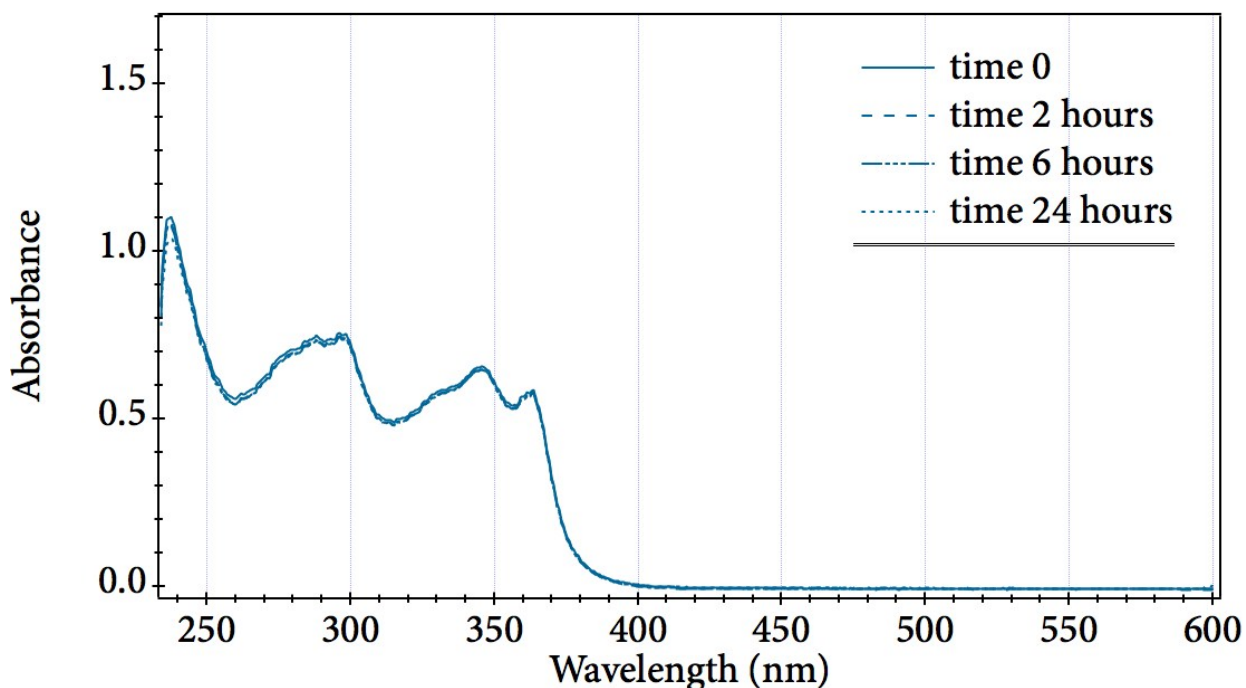


Figure S8. UV-Vis absorption spectra of **3** (4.9×10^{-5} M) in sodium cacodylate 0.1 M solution ($[\text{Cl}^-] = 0.007$ M, pH adjusted to 7.3 with hydrochloric acid and DMSO content less than 2 %). The spectra are recorded at subsequent times to see if degradation takes place. In order to be sure that the light of the spectrophotometer was not having an effect on the stability, each solution in the cuvette, after the analysis, was discarded and a mother solution, kept in the dark and prepared at time 0, was used as a

replacement.

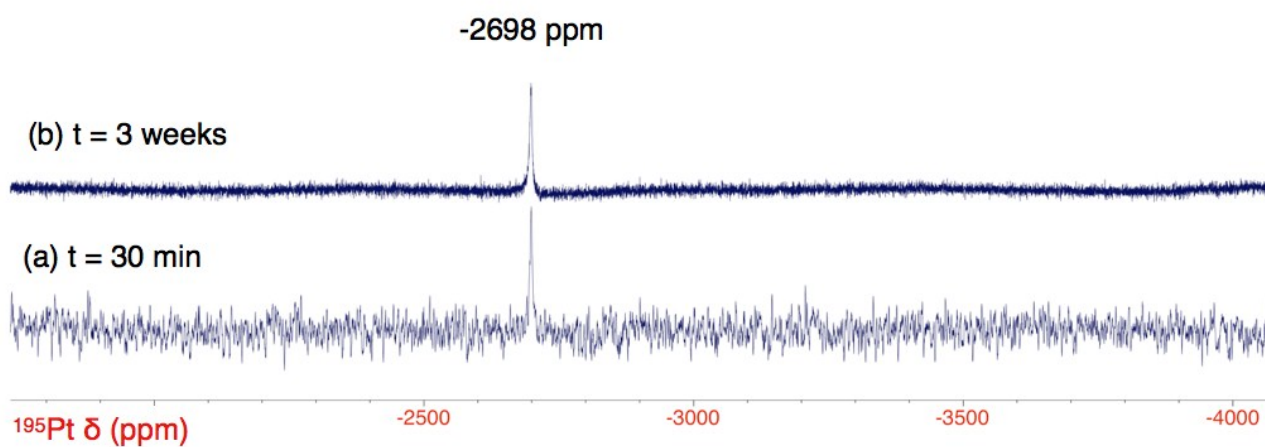


Figure S9. ^{195}Pt NMR of **1** (30 mM) in $\text{DMSO-}d_6$. The spectrum (a) was recorded 30 minutes after the preparation of the sample, while (b) was recorded 3 weeks later. The resonance observed did not change, which means that the coordination sphere of the platinum center is unchanged.

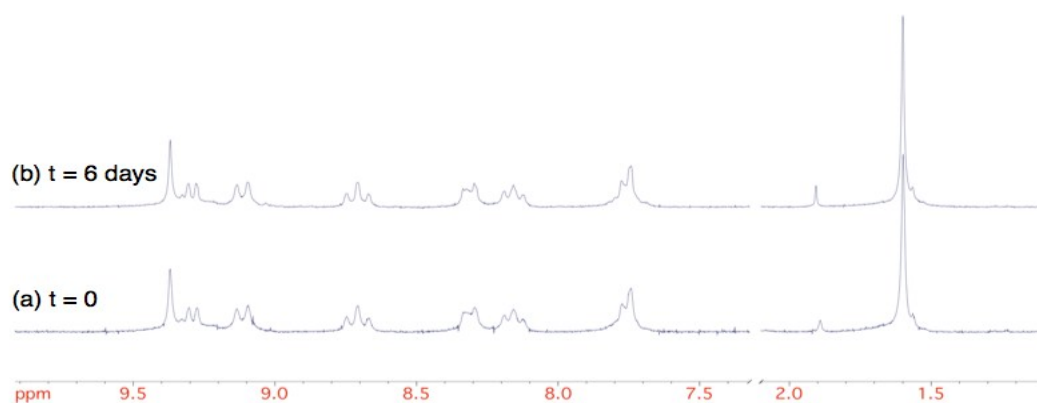


Figure S10. ^1H NMR resonances of **3** in $\text{DMSO-}d_6$. The spectrum (a) was recorded right after the preparation of the solution, while (b) was recorded after 6 days. The signals do not change their resonances with time, indicating that the Pt(IV) species is stable in the dark in DMSO .

Table S1. The Partition coefficient octanol/water, measured by UV-Vis determination.

<i>Compound</i>	<i>LogP</i>
1	-0.6
2	-0.5
3	-1.5
Cisplatin	-2.4 ^a

^a taken from reference 4

Paragraph S3. Irradiation studies

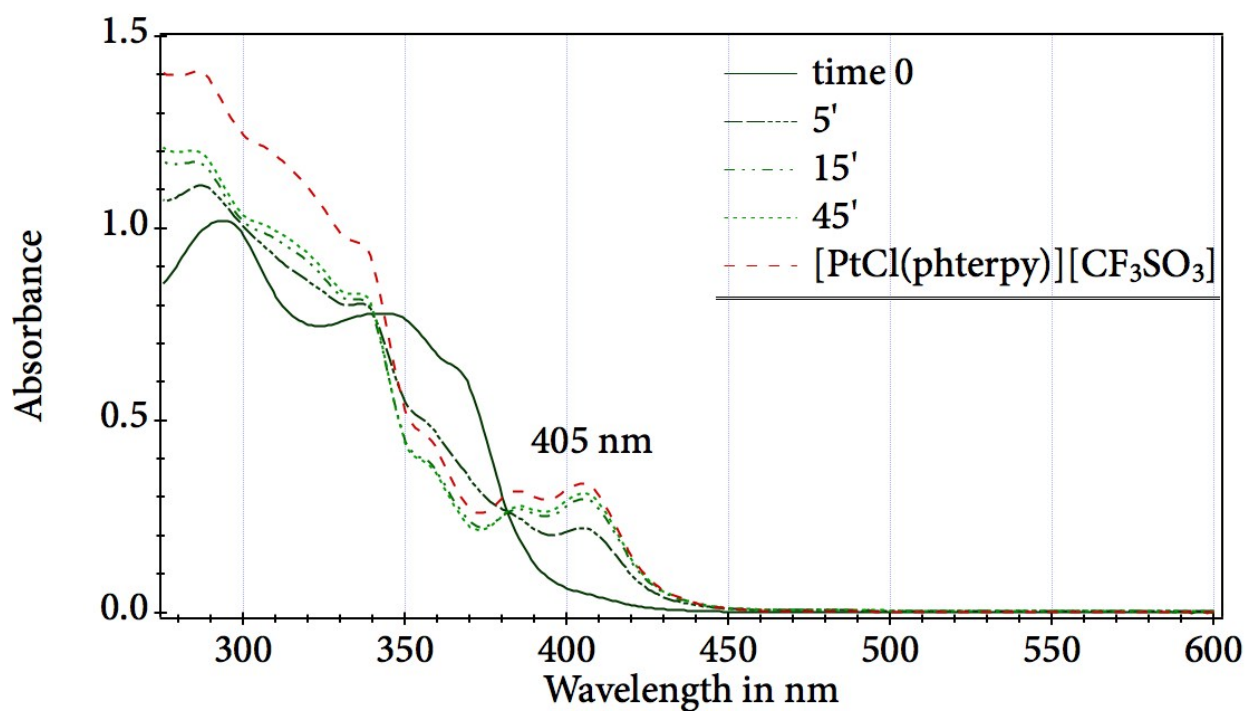


Figure S11. UV-Vis absorption spectra of **2** (4.9×10^{-5} M) in DMSO; the spectra were recorded after irradiation with a UV lamp operating at 365 nm and the irradiation time is specified in the legend. Compound **1** (4.9×10^{-5} M) is also included in the spectrum.

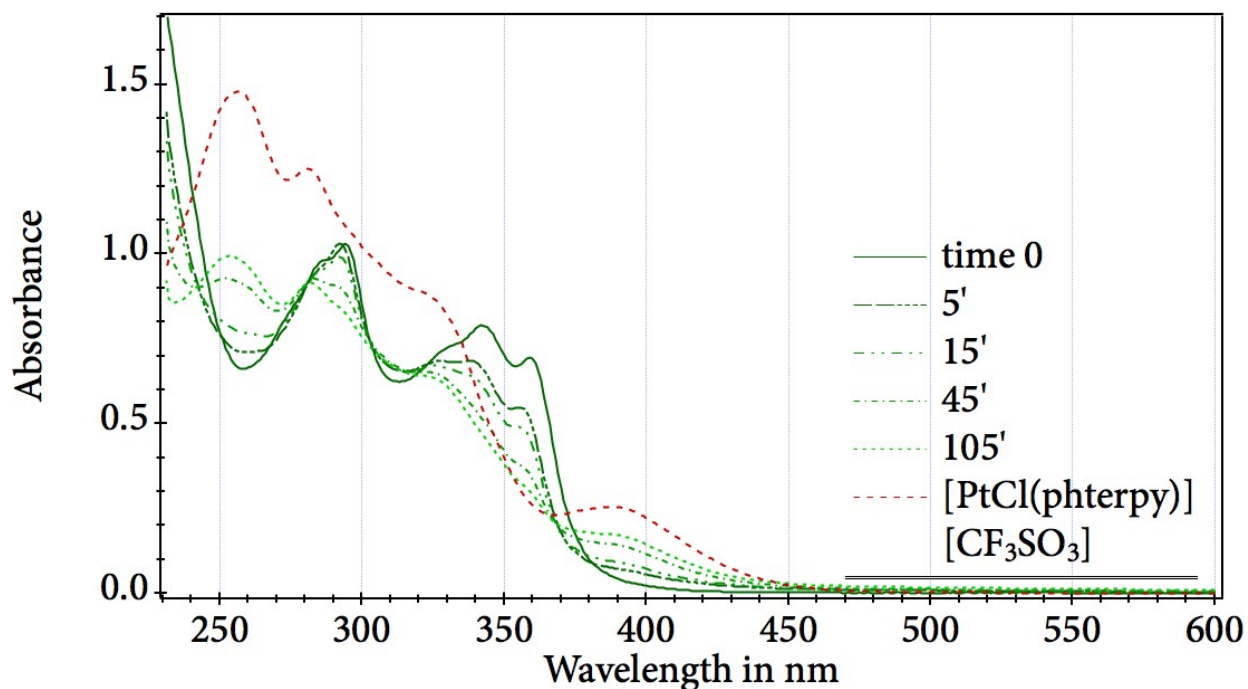


Figure S12. UV-Vis absorption spectra of **2** (4.9×10^{-5} M) in sodium cacodylate 0.1 M solution with $[\text{Cl}^-] = 0.007$ M and pH adjusted to 7.4 with hydrochloric acid. The spectra are recorded after irradiation with a UV lamp operating at 365 nm at the time specified in the legend. Compound **1** (conc. 5×10^{-5} M) is also included in the spectrum.

Table S2. The time constants (reciprocal of the rate constants) are reported for **2** and **3**, together with their standard deviation, as determined after the UV-Vis studies. All values in s.

<i>Time constant (s)</i>	2	3
DMSO	350 ± 60	280 ± 20
Buffer	1800 ± 200	660 ± 90

This behaviour might be explained as follows: **2** and **3** display a different HOMO-LUMO energy gap, which is an indirect measure of the reduction potential reported in the electrochemical studies. The UV light used for our studies could be more effective in reducing **3** because it fits the energy gap better than in the case of **2**. This is only a speculative reasoning, as no DFT studies have been carried out. We cannot compare the time constants obtained here with the literature values, because the reaction order of the photoreaction is dependent upon concentration.⁵ However, we could compare the values of the time constants for **2** and **3** obtained in the two solvents, as the conditions were very similar.

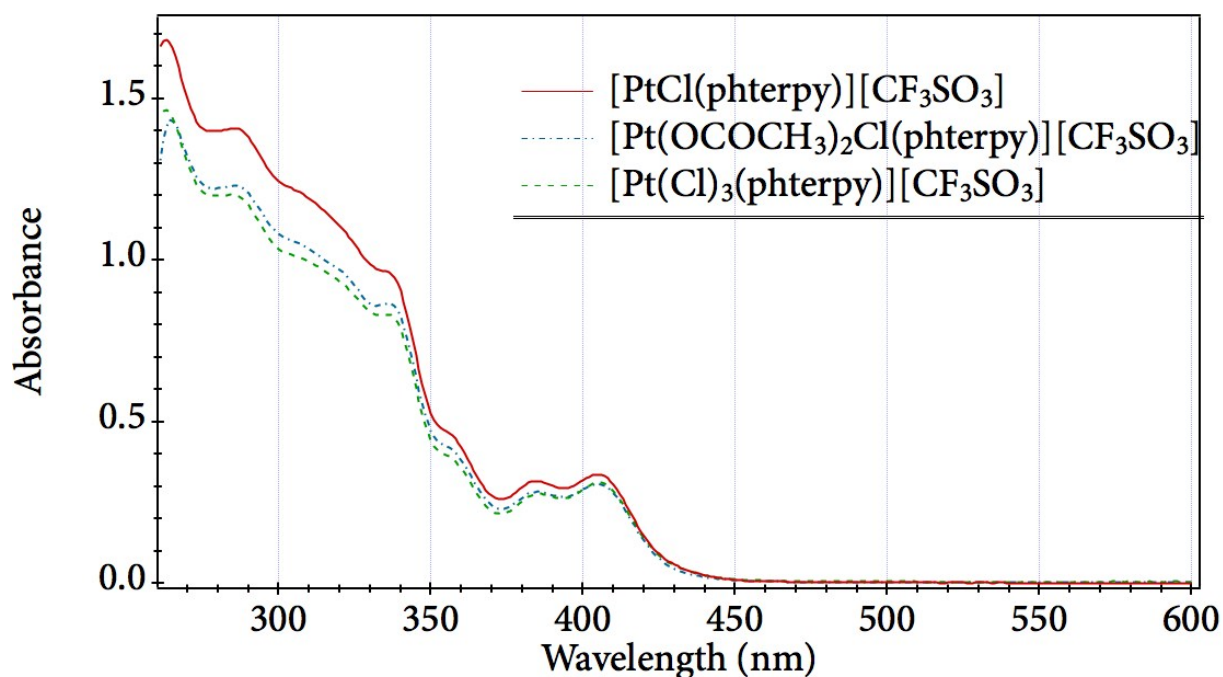


Figure S13. The irradiated profiles of **3** and **2** in DMSO (at 365 nm for 45 minutes); the spectrum of **1** is overlaid as a comparison.

Paragraph S4. Electrochemistry

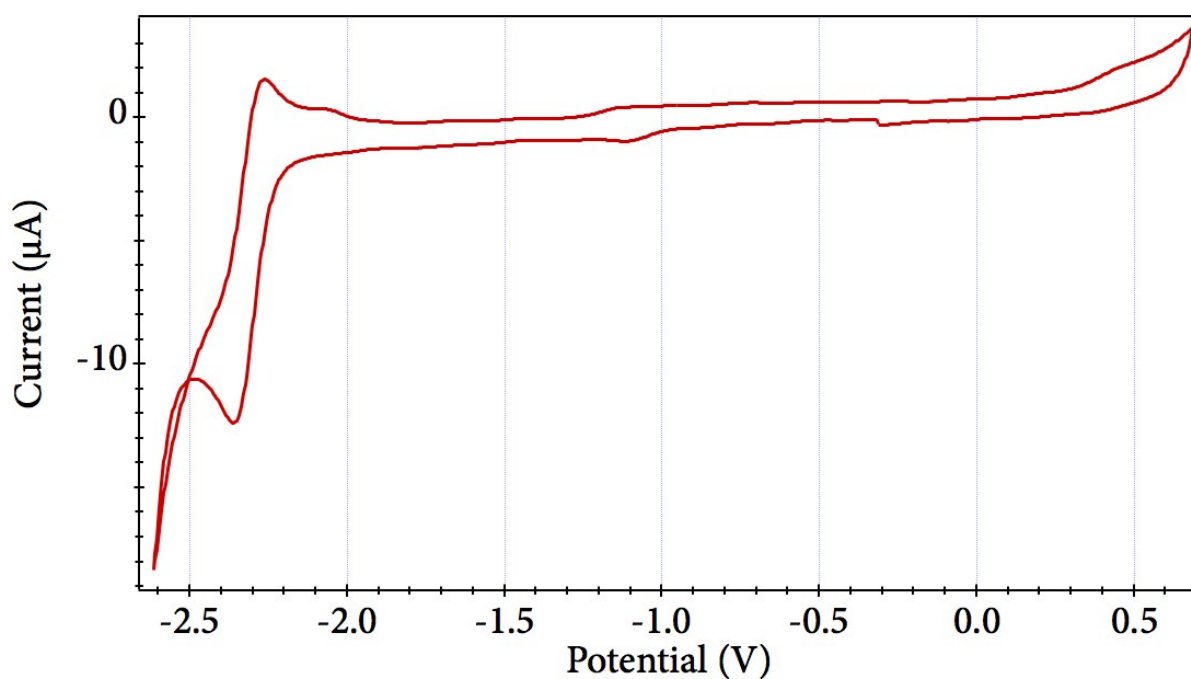


Figure S14. CV of the ligand phterpy (1 mM) in DMSO; $[N^nBu_4][CF_3SO_3]$ (0.1 M) as supporting electrolyte.

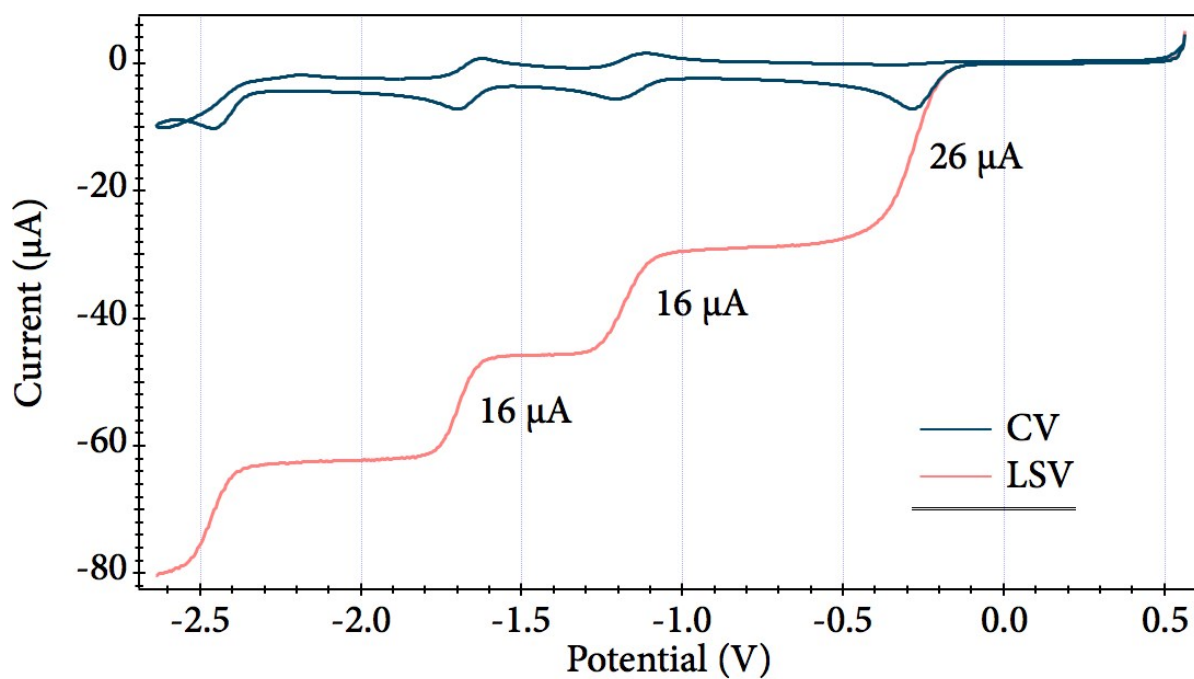


Figure S15. CV and LSV of **2** (1 mM) in DMSO; $[N^nBu_4][CF_3SO_3]$ (0.1 M) as supporting electrolyte.

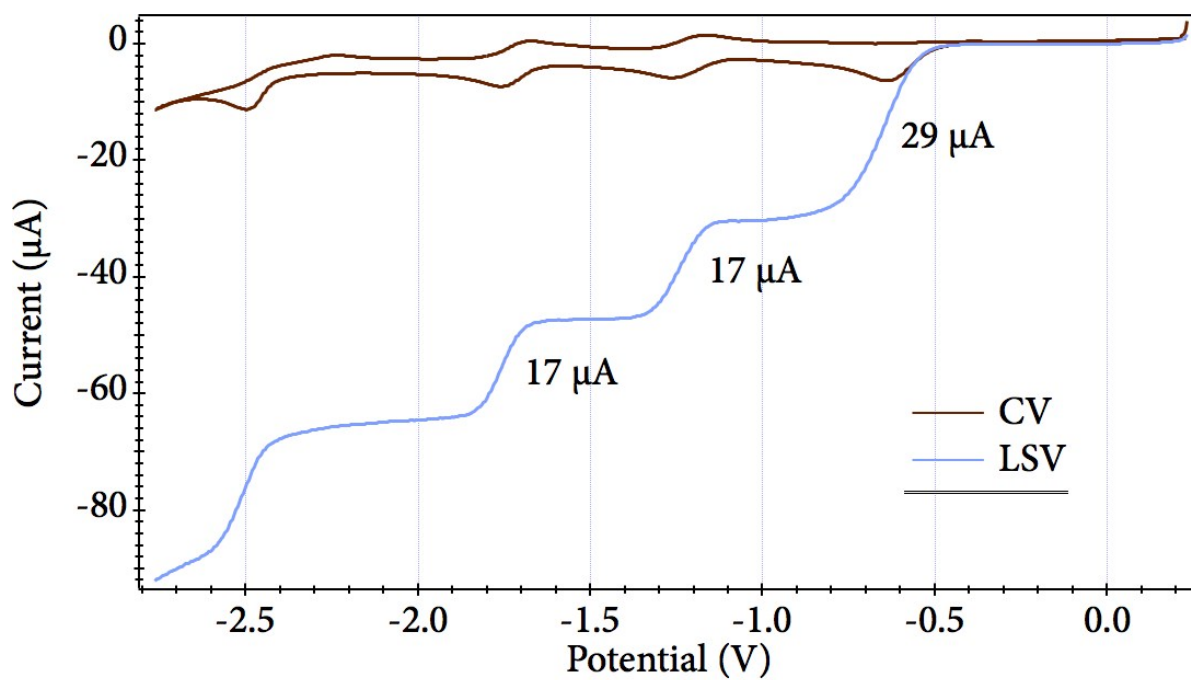


Figure S16. CV and LSV of **3** (1 mM) in DMSO; $[N^nBu_4][CF_3SO_3]$ (0.1 M) as supporting electrolyte.

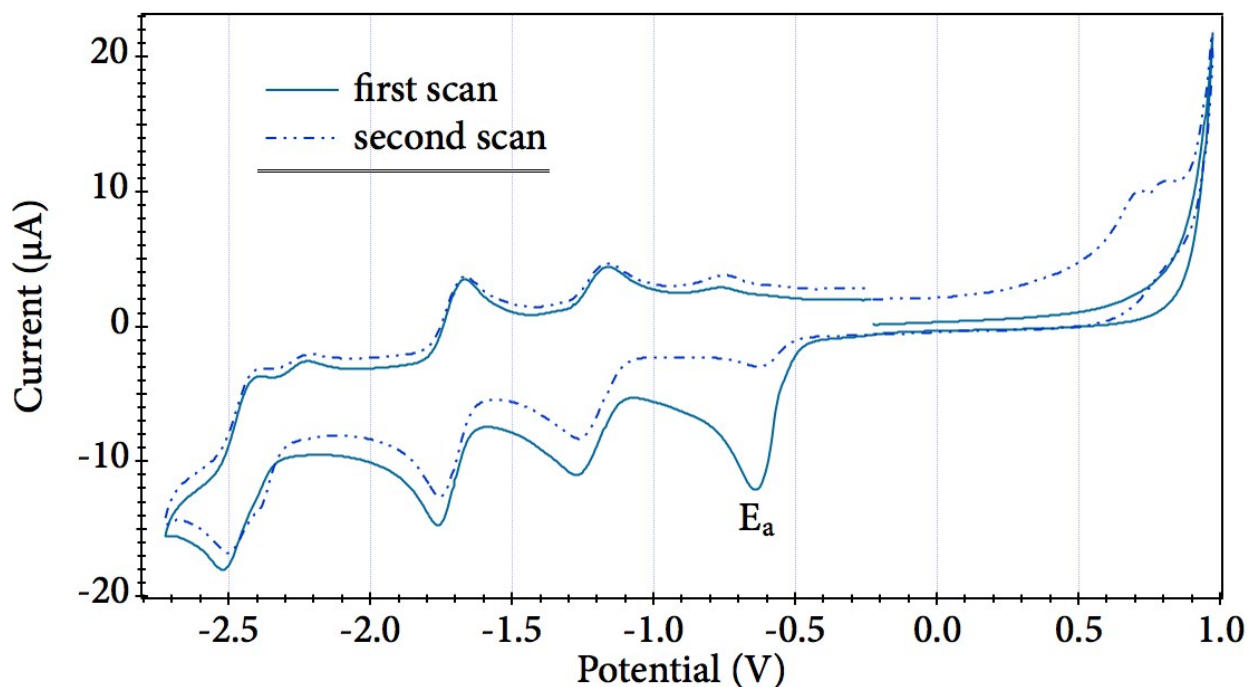


Figure S17. CV of **3** (1 mM) in DMSO; 2 scans were performed consecutively and the process at -0.64 V (E_a) is almost disappeared during the second cycle.

2 and **3** exhibited the unexpected behavior highlighted in Figure S17 when two CV scans were performed one just after the other: in the second scan the peak of the Pt(IV) reduction (E_a) disappeared almost completely, while the other peaks kept their height and position. But, upon short mixing of the solution, the Pt(IV) reduction peak was restored. Most likely, after the Pt(IV) reduction at the electrode surface, the freshly generated square planar Pt(II) species arrange in aggregates that have a smaller diffusion coefficient with respect to the single molecule.⁶ These aggregates, in turn, prevent the diffusion of Pt(IV) species from the bulk of the solution to the electrode surface. In this way, in the second cycle of the CV, only the three reductions of the Pt(II) complex are observable and E_a is not.

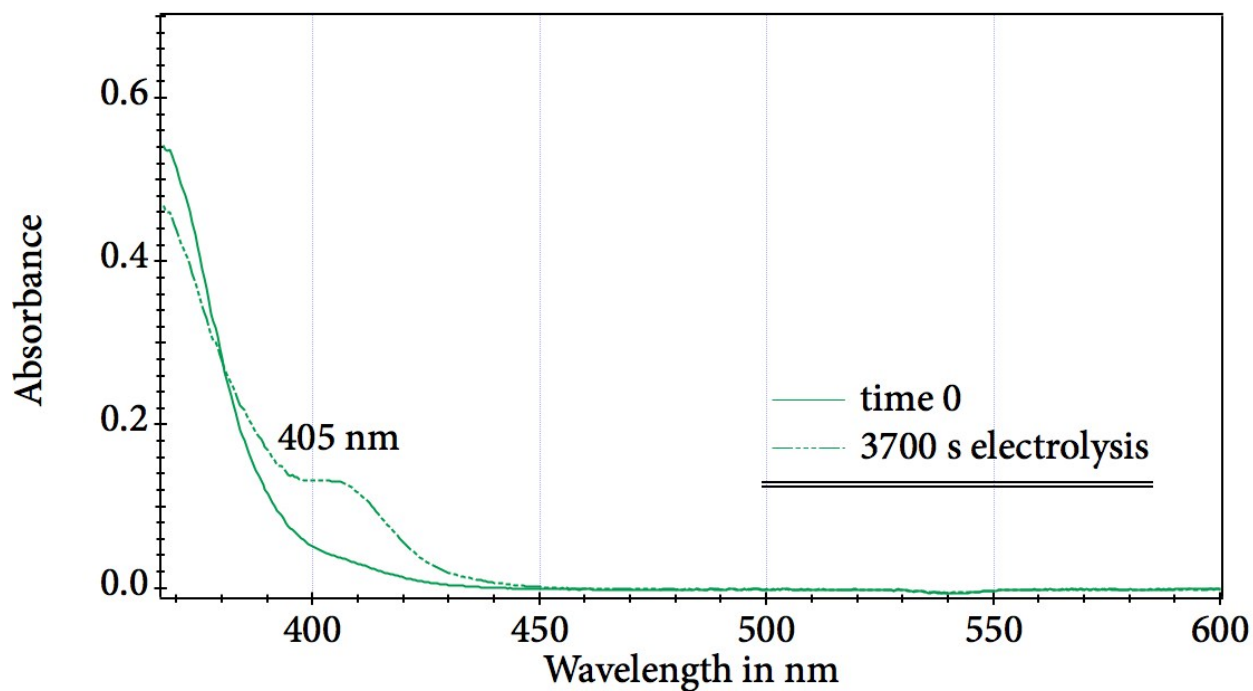


Figure S18. UV-Vis spectra of **2** before (time 0) and after the electrolysis (3700 s) at -0.5 V. The absorption at 405 nm is due to the Pt(II) species that formed as a consequence of the electrolysis.

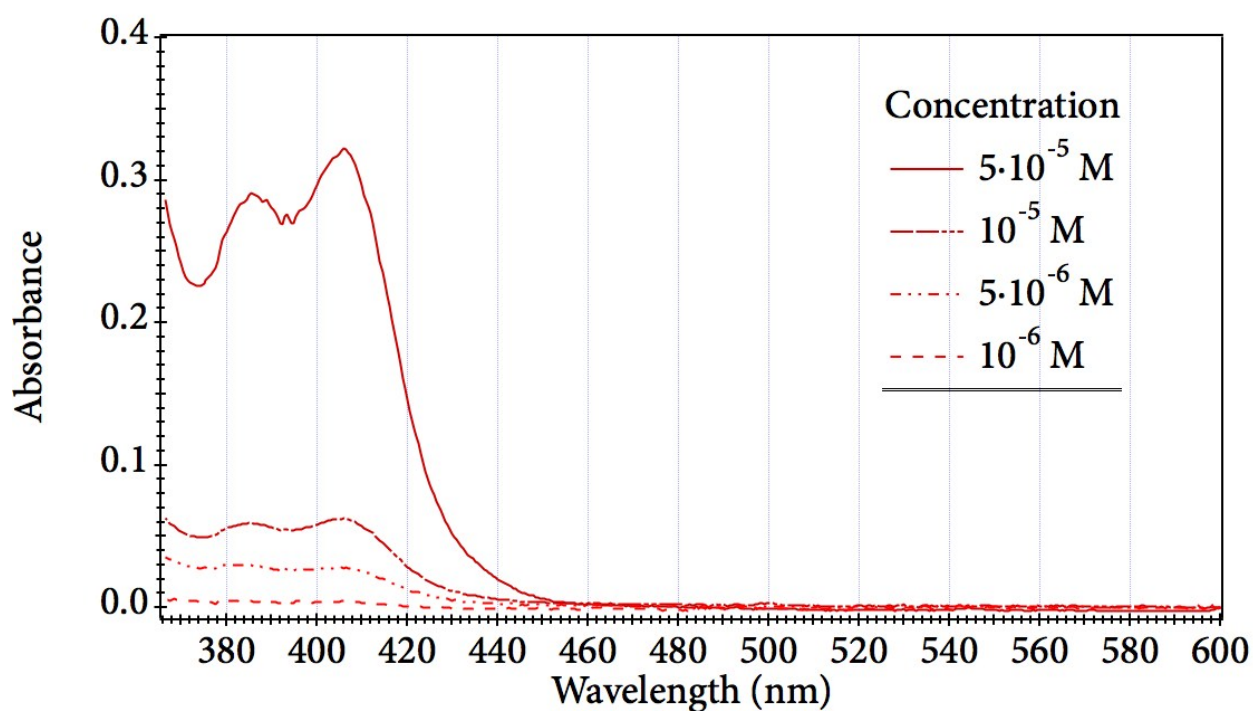


Figure S19. UV-Vis spectra of **1** in DMSO at different concentrations.

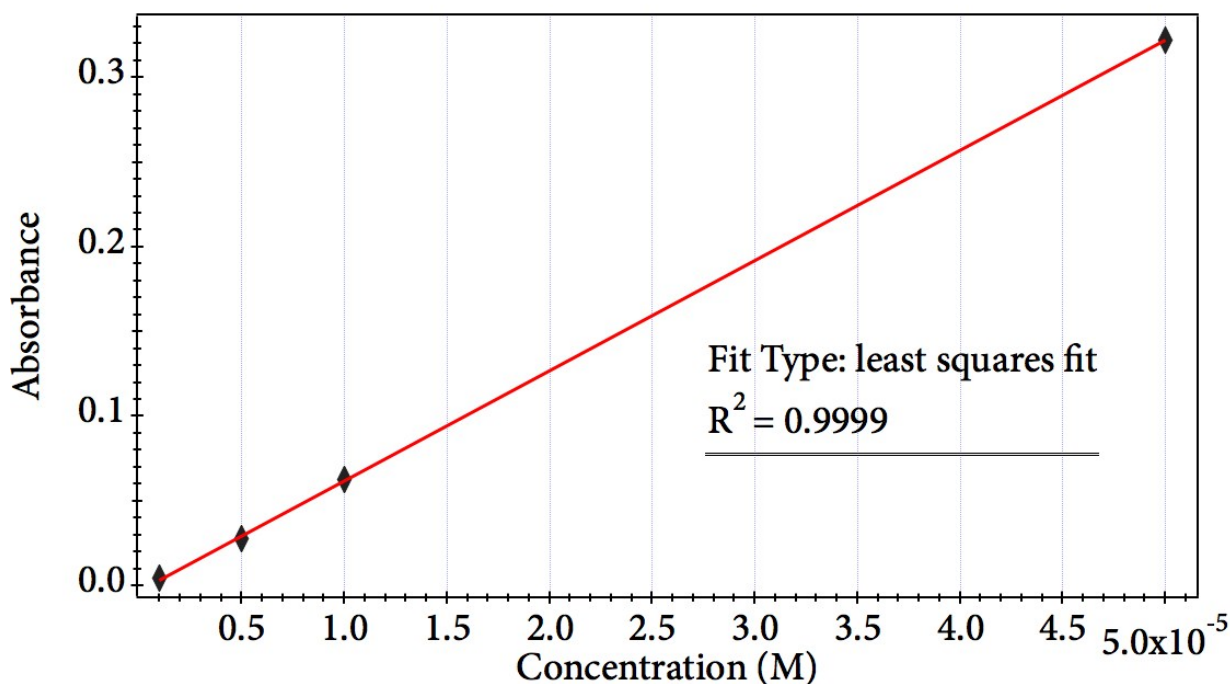


Figure S20. Calibration curve of **1** in DMSO. The ordinate values are the absorbance maxima of the spectra showed in Figure S19 at 405 nm.

Nature of the peak E_a for compound **3**.

An electrochemical cell containing **3** in DMSO was irradiated (firstly at the ambient light and then with a UV lamp at 365 nm for several minutes). The photoreaction was followed recording the CV and LSV at the glassy-carbon rotating disk electrode during the irradiation. The decrease of the limiting current of the irreversible reduction at -0.64 V (E_a) and the color change of the solution from very pale yellow to yellow were the only changes observable at the end of irradiation (Figure S21). On the other hand, the reduction waves at E_1 , E_2 and E_3 (for the values of the potential see Table 1 in the main text) kept their position and height, demonstrating that E_a is due to the reduction $\text{Pt(IV)} \rightarrow \text{Pt(II)}$ and that only one Pt(II) product was formed via photochemical reduction of **3**.

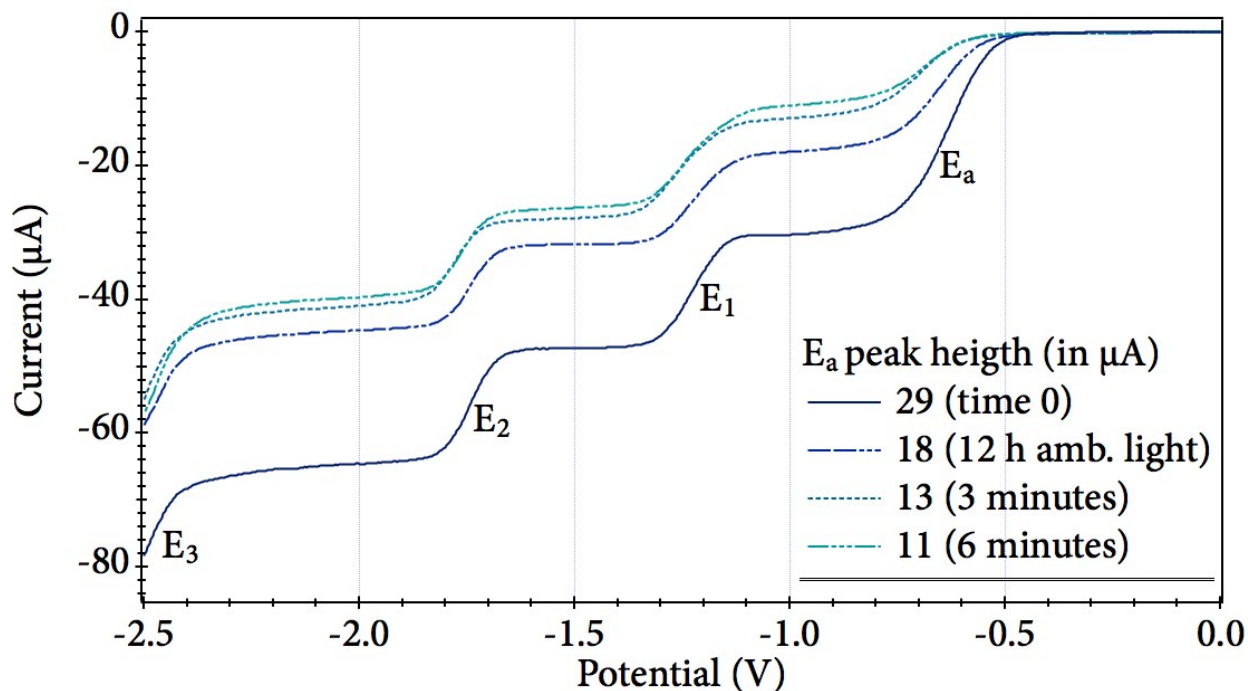


Figure S21. LSV of **3** (1 mM) in DMSO showing the decrease in height of the process happening at -0.64 V (E_a). The first decrease (29 to 18 μA) was obtained leaving the solution to ambient light for a total of 12 hours. Then the solution was deliberately irradiated with UVA light (365 nm) for 3 minutes and again for other 3 minutes. The other peaks, E_1 , E_2 and E_3 did not change.

Paragraph S5. Cellular studies

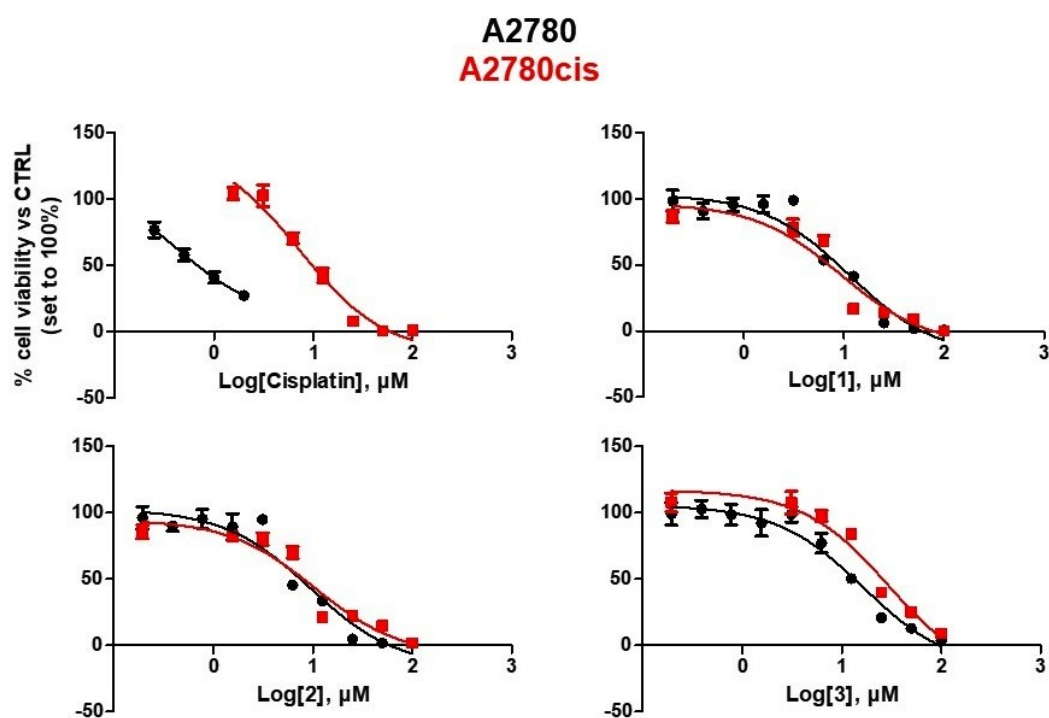
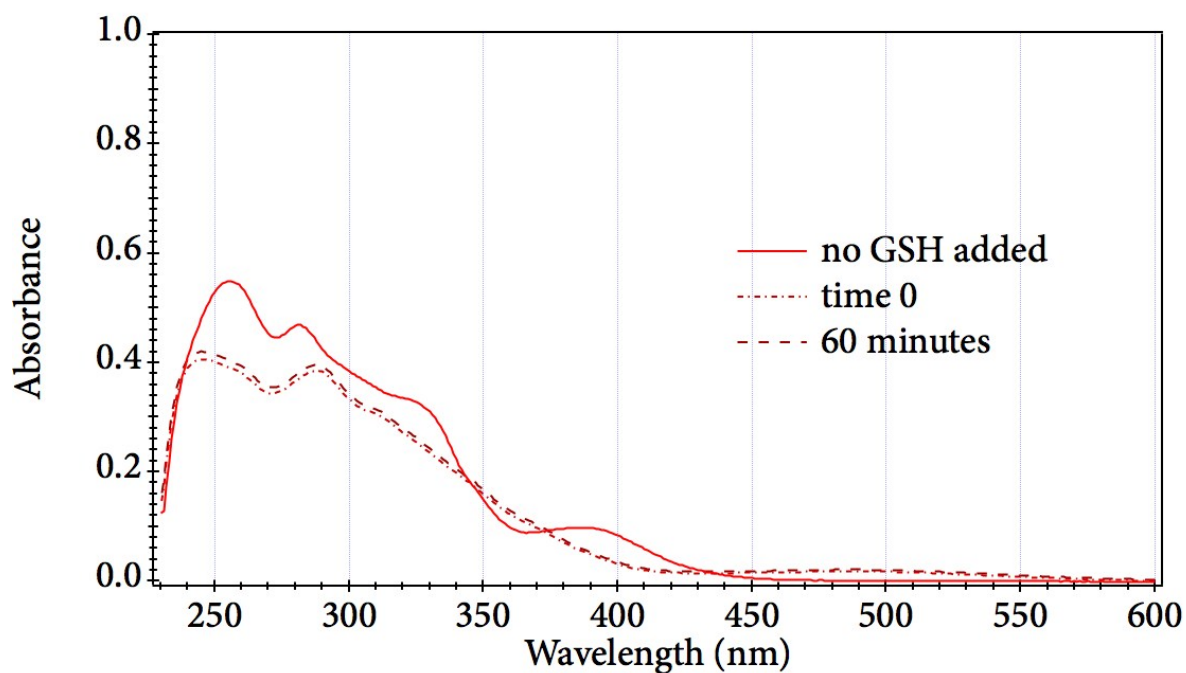


Figure S22. Sigmoidal curves reporting the cell viability as a function of the concentration of the complexes; cisplatin was tested as reference. The values of IC_{50} (reported in Table 2) were measured after 72 hours of incubation in the dark, as reported in the materials and methods section.



Figure

re S23. UV-Vis spectrum of **1** (2×10^{-5} M) in buffered sodium cacodylate 0.1 M solution ($[\text{Cl}^-] = 7 \times 10^{-3}$ M, pH adjusted to 7.3, DMSO content less than 2 %) with the addition of 30 equivalents of GSH (0.5 mM).

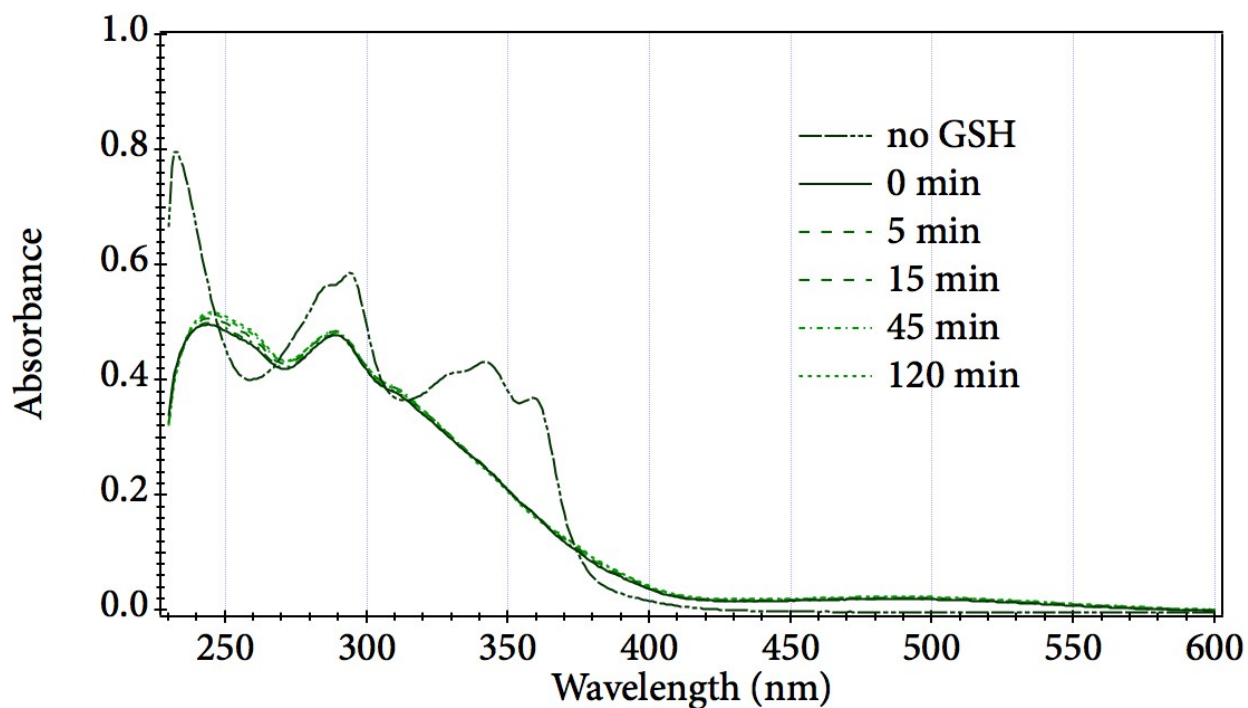


Figure S24. UV-Vis spectrum of **2** (2×10^{-5} M) in buffered sodium cacodylate 0.1 M solution ($[\text{Cl}^-] = 7 \times 10^{-3}$ M, pH adjusted to 7.3, DMSO content less than 2 %) with the addition of 30 equivalents of GSH (0.5 mM).

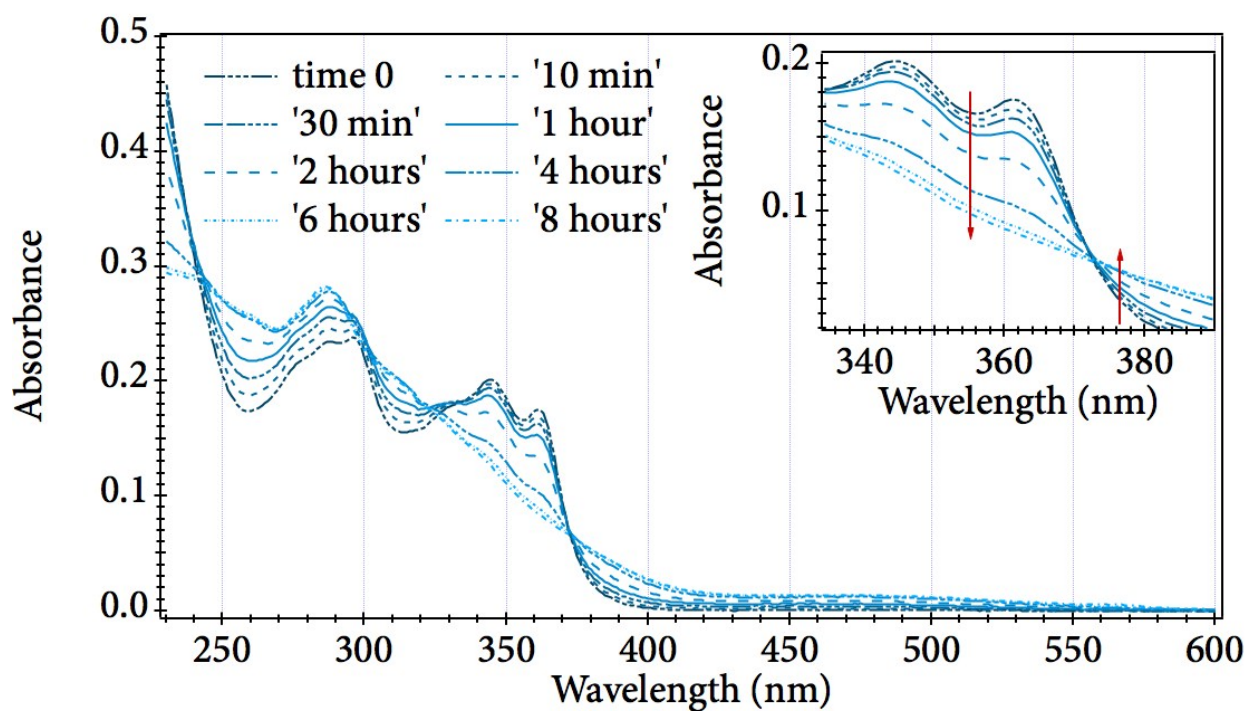


Figure S25. UV-Vis spectrum of **3** (1×10^{-5} M) in phosphate buffer (0.05 M, pH adjusted to 7.0,) with more than 400 equivalents of GSH (5 mM), recorded at 37 °C. The phosphate buffer has been used in place of cacodylate for this experiment in order to exclude the interference due to the reduction of arsenic by GSH.

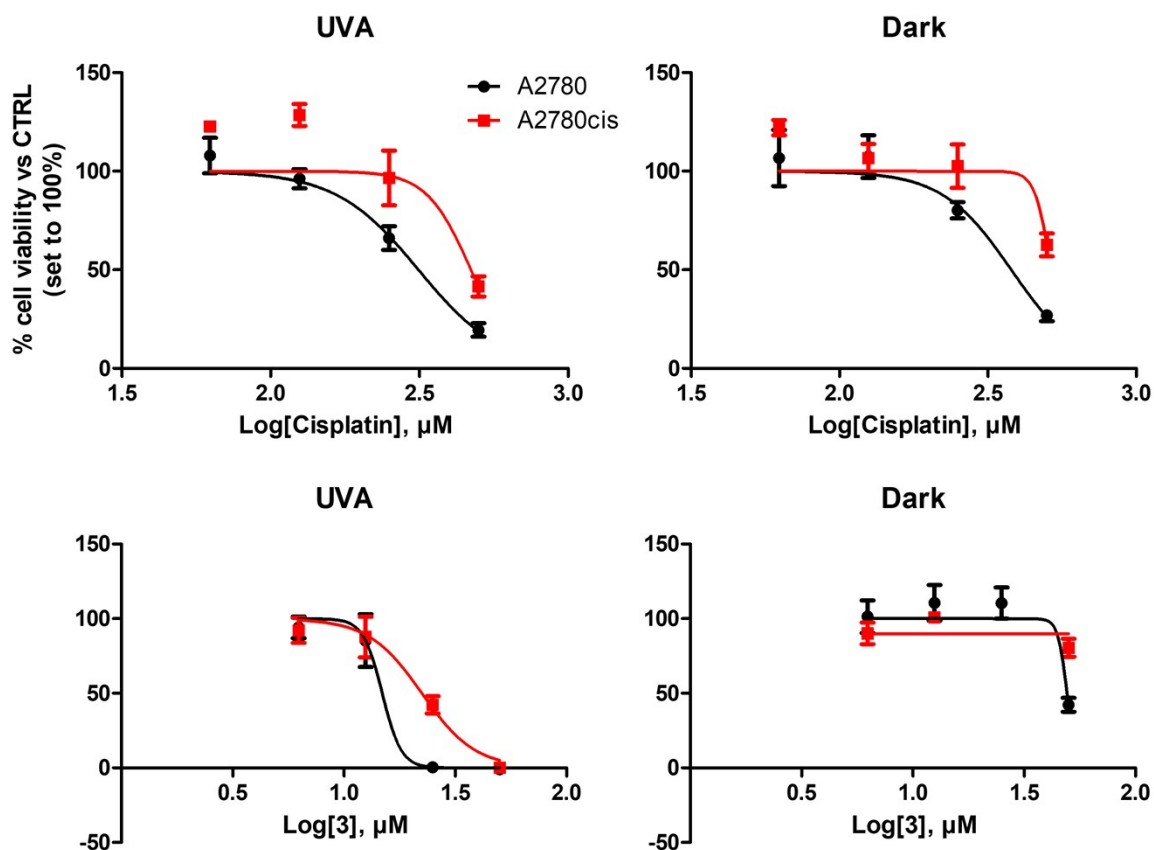


Figure S26. Sigmoidal curves showing the photoactivation of **3** and cisplatin with 6 minutes of 365 nm (UVA) light exposure, showing a dose dependent activity in A2780 and A2780cis cell lines. The IC_{50} values are reported in Table 3 in the main text.

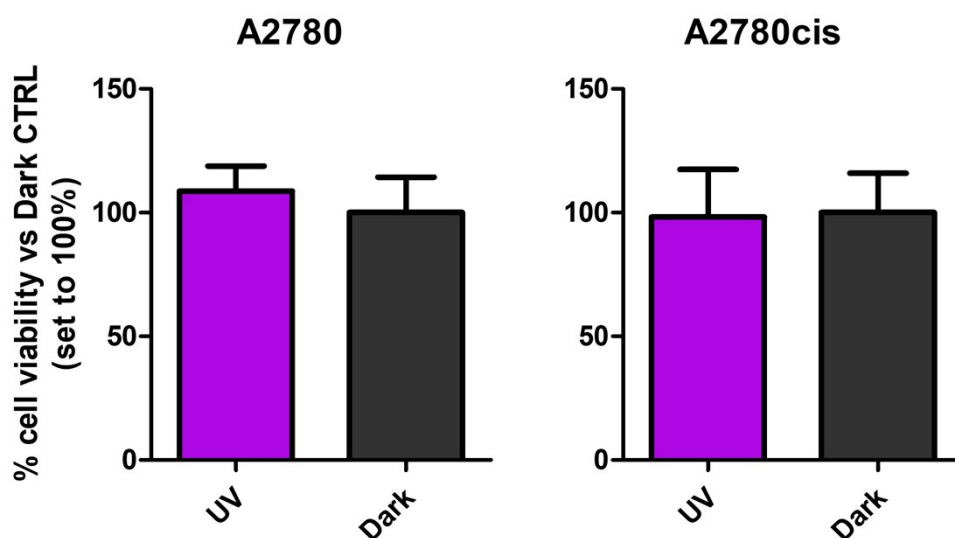


Figure S27. The column graph shows the comparison of cell viability between controls (only A2780 and A2780cis cells) exposed to UV irradiation (365 nm) and in the dark. There are no statistically significant differences.

Table S3. Platinum level measured with ICP-AES. The A2780 ovarian cell lines were incubated with **3** and cisplatin for 1 hour at a concentration of 50 μ M.

<i>Cell lines</i>	<i>Compound</i>	<i>Pt level (μg / cell)</i>
A2780	Cisplatin	1×10^{-8} μ g
A2780	3	1×10^{-8} μ g

Studies with ODN2.

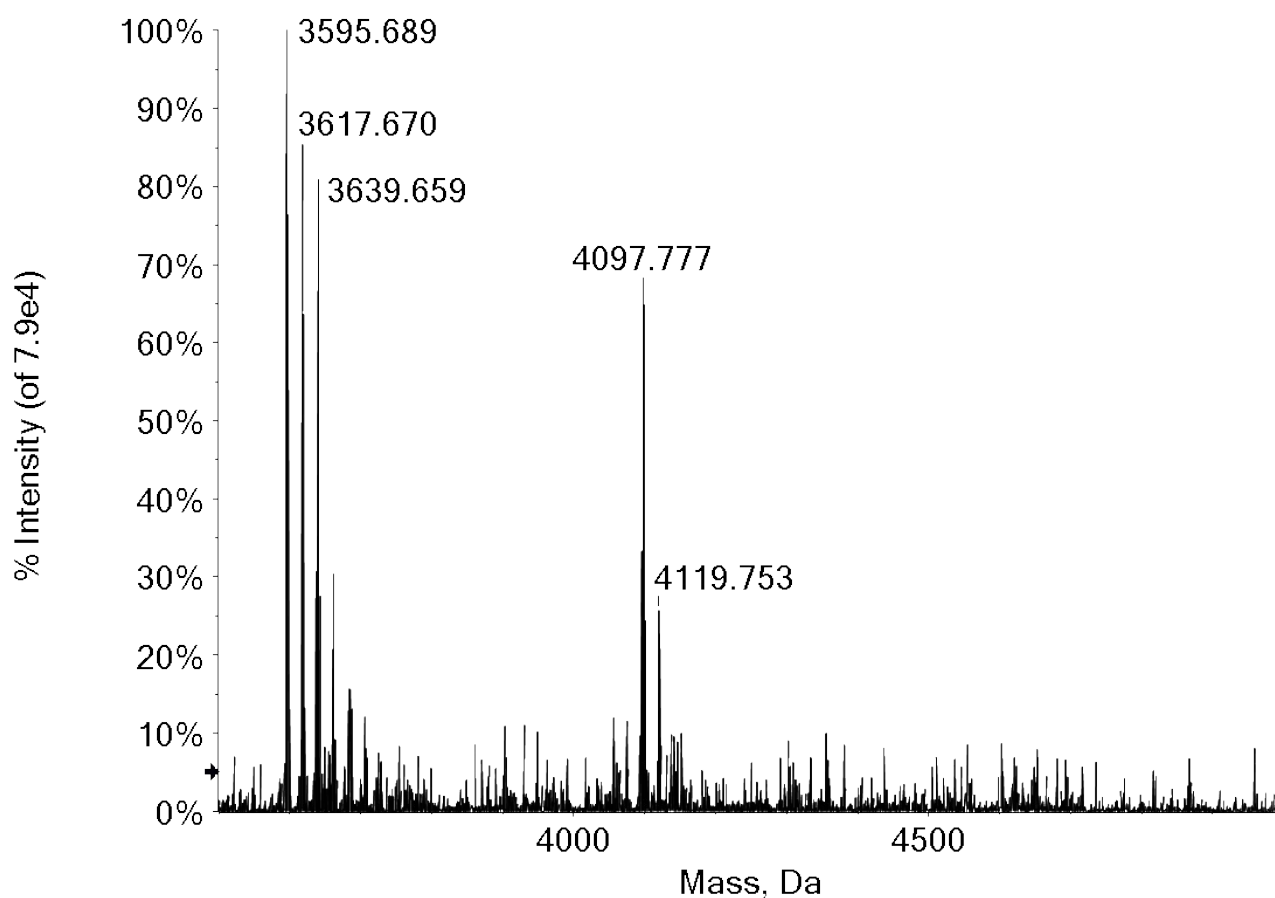


Figure S28. Deconvoluted ESI mass spectrum of **2** incubated for 72 h in the dark with ODN2 10^{-4} M (1:1 metal to oligonucleotide molar ratio) at 25 °C in MilliQ water.

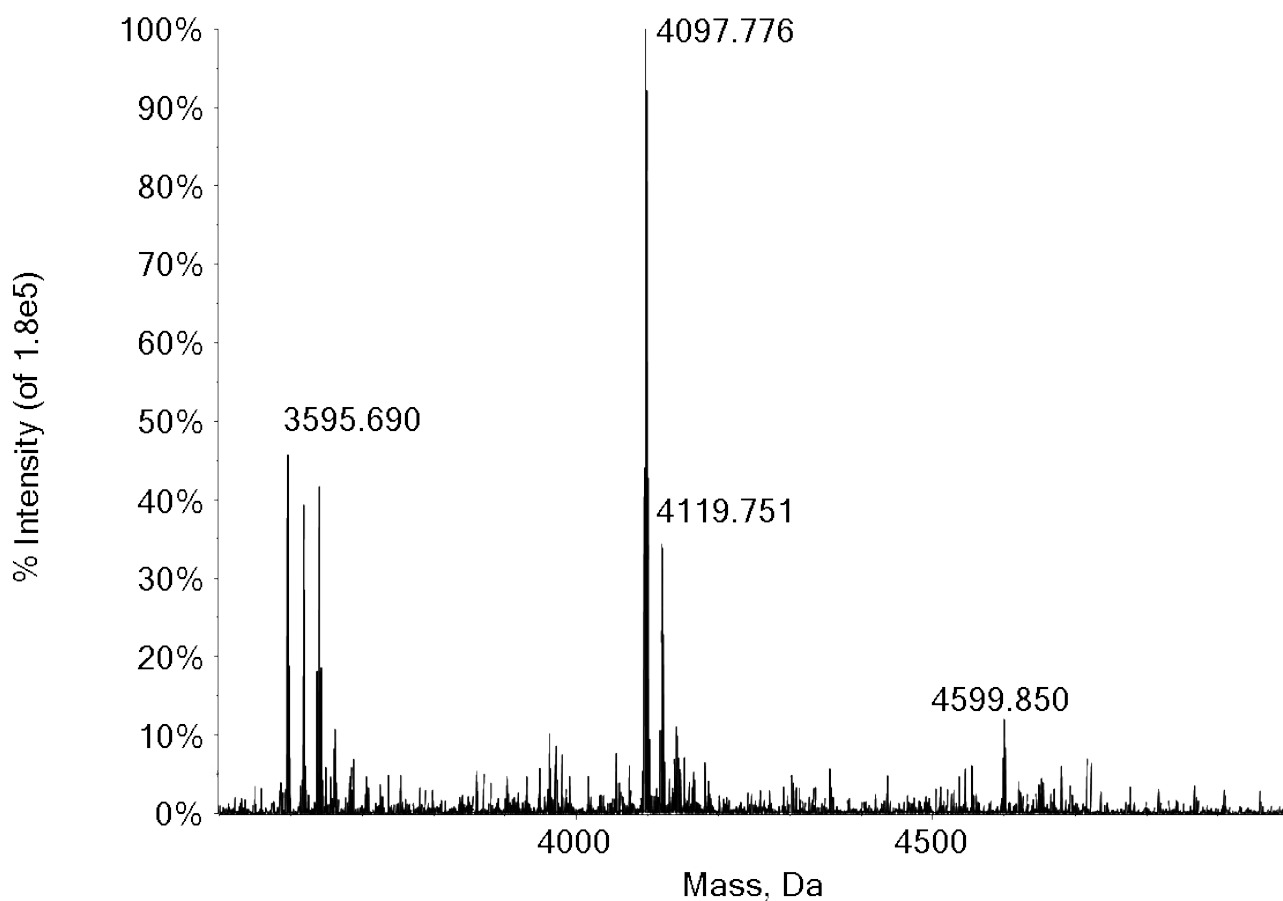


Figure S29. Deconvoluted ESI mass spectrum of **2** and ODN2, firstly irradiated at 365 nm for 5 min, then incubated for 72 h in the dark at 25 °C in MilliQ water. ODN2 10^{-4} M (1:1 metal to oligonucleotide molar ratio)

Studies with RNase A.

We mention the fact that RNase A displays three signals in the spectrum, at 13681.7, 13779.5 and 13878.0 Da; they differ from the original peak (of pure RNase, with the lowest molecular weight) by 98 and 196 Da, due to monophosphate RNase A and to diphosphate RNase A, respectively. These species are well known in literature and depend upon the preparation and purification methods for RNase A.⁷

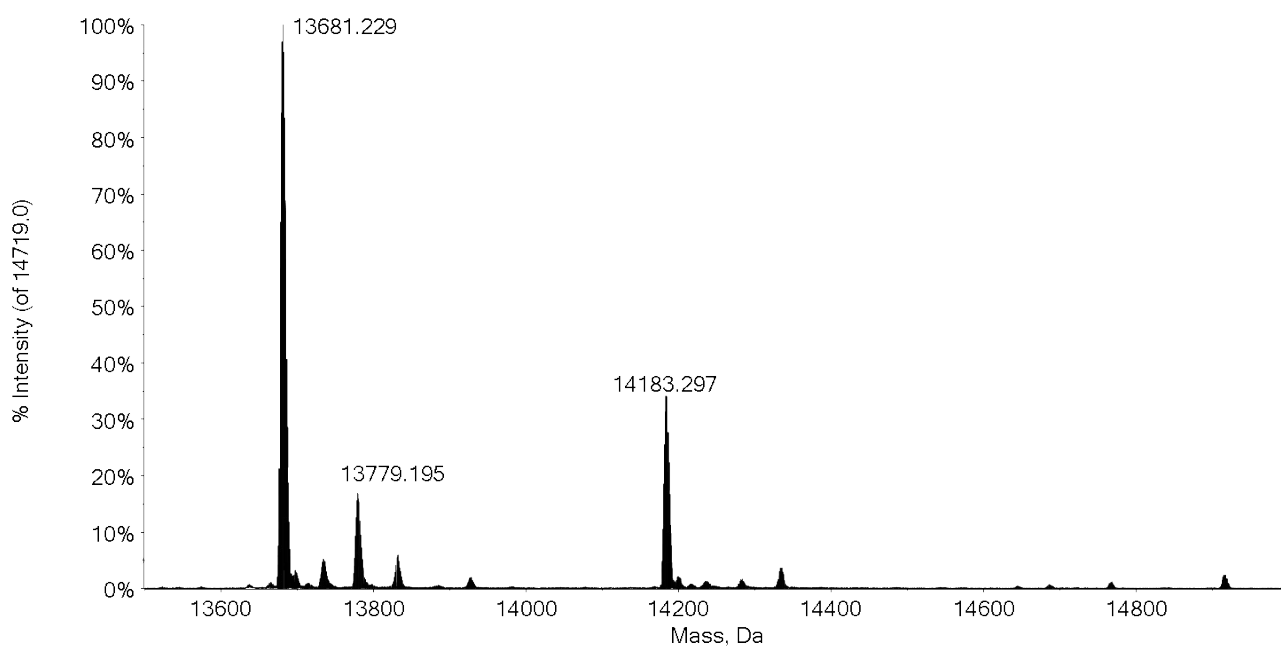


Figure S30. Deconvoluted ESI mass spectrum of **1** and RNase A. Both the macromolecule and the platinum complex have the same concentration of 10^{-4} M, total incubation time: 72 hours at ambient light. The signal at 14183.297 Da is relative to the adduct RNaseA plus the fragment $[\text{Pt}(\text{phterpy})]^{2+}$.

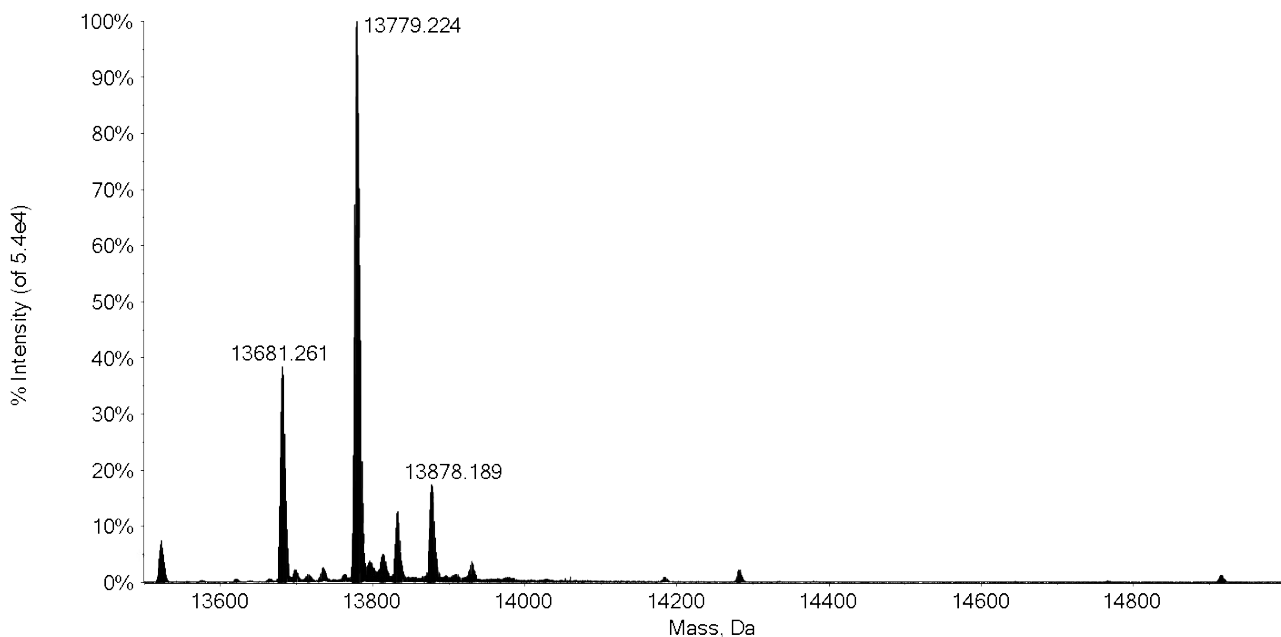


Figure S31. Deconvoluted ESI mass spectrum of **2** and RNase A. Both the macromolecule and the platinum complex have the same concentration of 10^{-4} M, total incubation time: 72 hours in the dark.

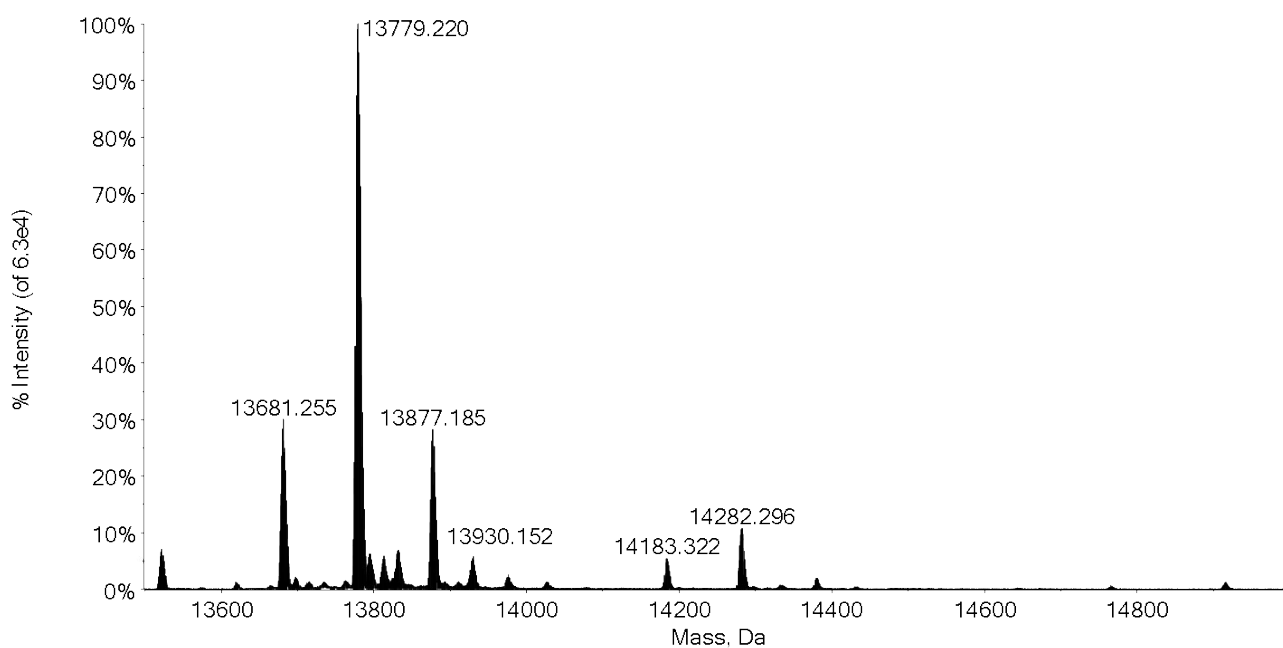


Figure S32. Deconvoluted ESI mass spectrum of **2** and RNase A. The mixture was irradiated at 365 nm for 5 minutes and then kept in the dark for the total incubation time, 72 hours. Protein to complex ratio, 1:1, concentration 10^{-4} M.

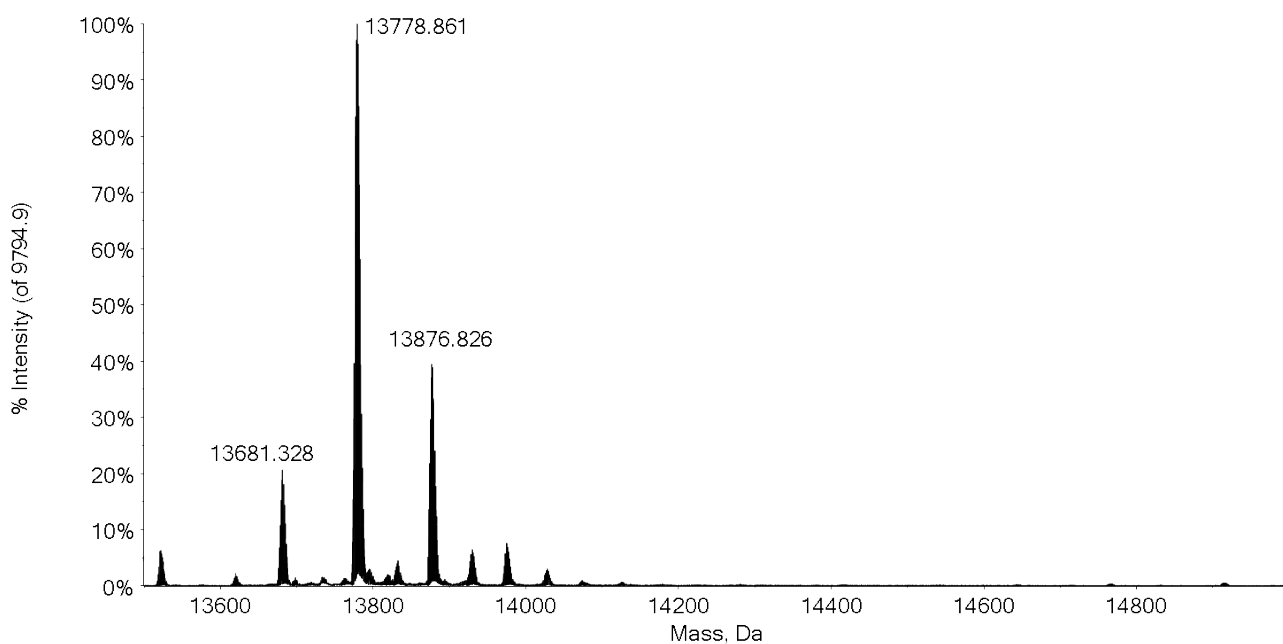


Figure S33. Deconvoluted ESI mass spectrum of **3** and RNase A. Both the macromolecule and the platinum complex have the same concentration of 10^{-4} M, total incubation time: 72 hours in the dark.

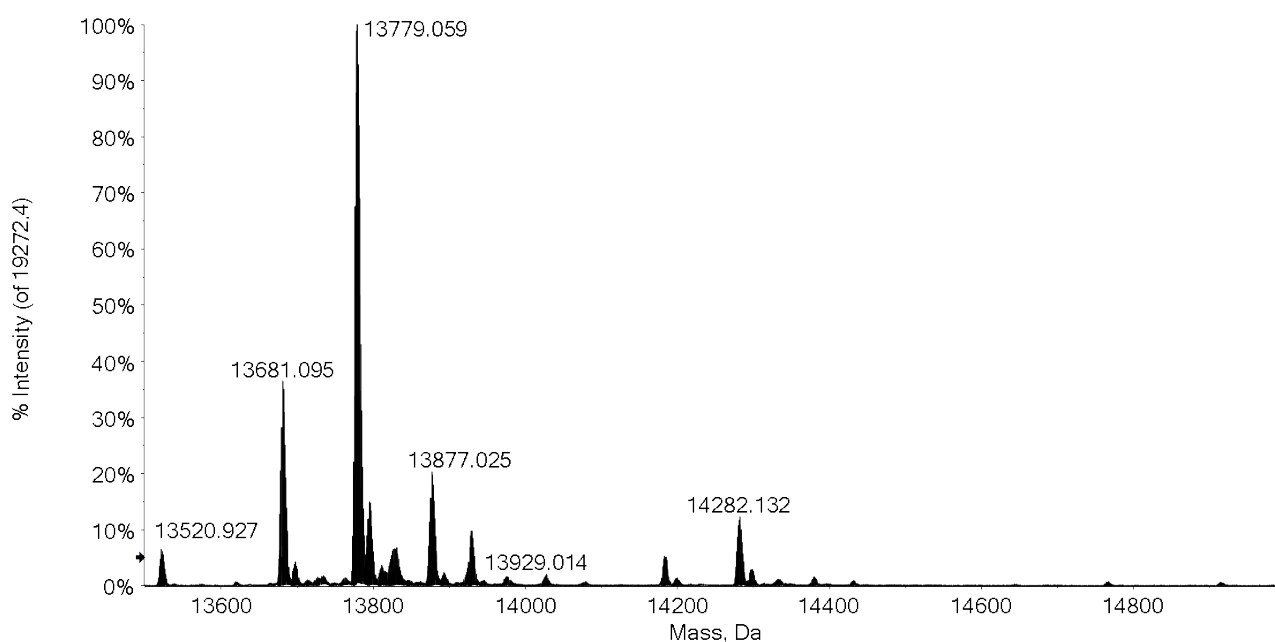


Figure S34. Deconvoluted ESI mass spectrum of **3** and RNase A. The mixture was irradiated at 365 nm for 5 minutes and then kept in the dark for the total incubation time, 72 hours. Protein to complex ratio, 1:1, concentration 10^{-4} M.

Paragraph S7. DNA interaction

Isotherm at 37 °C

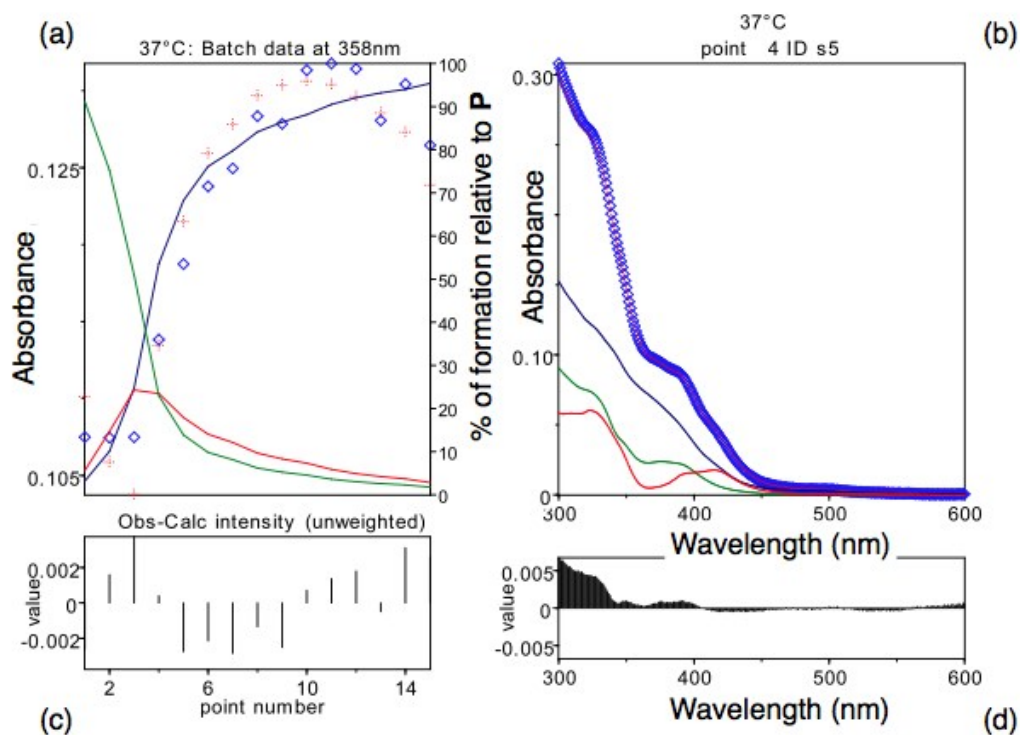


Figure S35. Example of HypSpec analysis of the interaction **1-P** at 37.0°C. (a): absorbance data at 358 nm (blue diamonds = experimental, red cross = calculated) and composition of the system as the

titration proceeds; the species **1** (green) decreases while the species **1P** (blue) increases, the red profile is **1₂P**. (b): the contributions of the different species to the overall absorption profile (blue diamonds = experimental, red cross = calculated). (c) and (d) give the error bars.

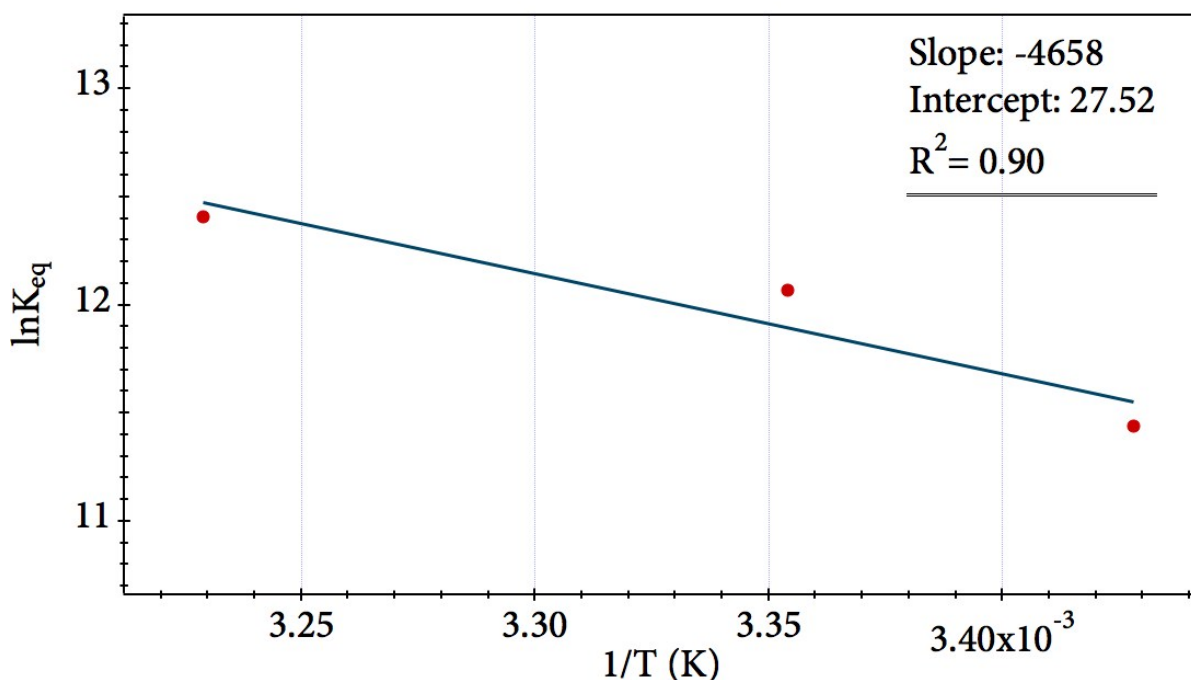


Figure S36. The plot obtained with the Van't Hoff equation for compound **1**, in which the K_{eq} are reported as a function of the reciprocal of temperature. The negative slope indicates that the reaction is endothermic.

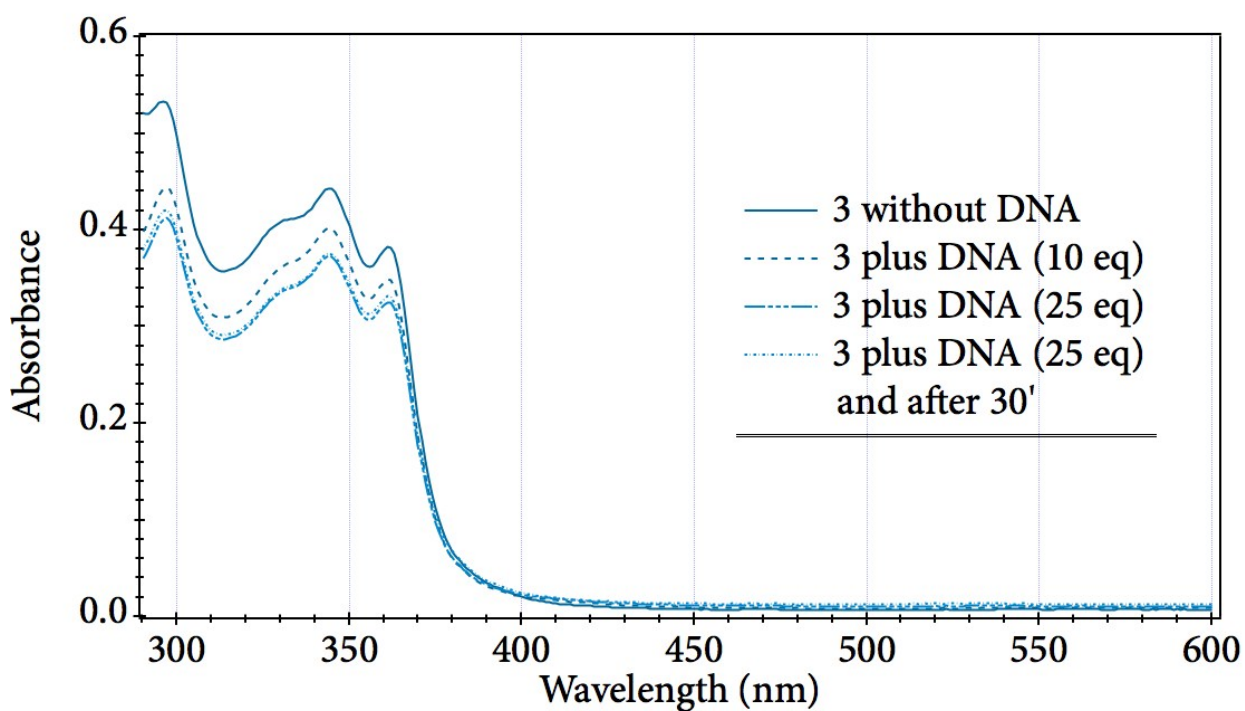


Figure S37. UV-Vis spectrum of **3** (2×10^{-5} M) in buffered sodium cacodylate 0.1 M solution (pH adjusted to 7.0, DMSO content less than 2%) with the addition of CT-DNA.

References

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