cis-$PtI_2(NH_3)_2$: a Reappraisal

Tiziano Marzo,[a] Serena Pillozzi,[b] Ondrej Hrabina,[c] Jana Kasparkova,[d] Viktor Brabec,[d] Annarosa Arcangeli,[b] Gianluca Bartoli,[b] Mirko Severi,[e] Alessandro Lunghi,[e] Federico Totti,[e] Chiara Gabbiani,[f] Adóracion G. Quiroga[g] and Luigi Messori*[a]

[a]MetMed, Department of Chemistry, University of Florence, Via della Lastruccia 3, 50019 Sesto Fiorentino, Italy.
[b]Department of Experimental and Clinical Medicine, University of Florence, Viale GB Morgagni 50, 50134 Firenze, Italy.
[c] Department of Biophysics, Faculty of Science, Palacky University, Slechtitelu 11, CZ-78371 Olomouc, Czech Republic.
[d] Institute of Biophysics, Academy of Sciences of the Czech Republic, v.v.i., Kralovopolska 135, CZ-61265 Brno, Czech Republic.
[e]Department of Chemistry "Ugo Schiff", University of Florence, Via della Lastruccia, 3, 50019 Sesto Fiorentino, Firenze, Italy.
[f]Department of Chemistry and Industrial Chemistry, University of Pisa via Moruzzi, 3, 56124 Pisa, Italy.
[g]Department of Inorganic Chemistry, Universidad Autonóma de Madrid C/Francisco Tomás y Valiente 7, 28049 Madrid, Spain

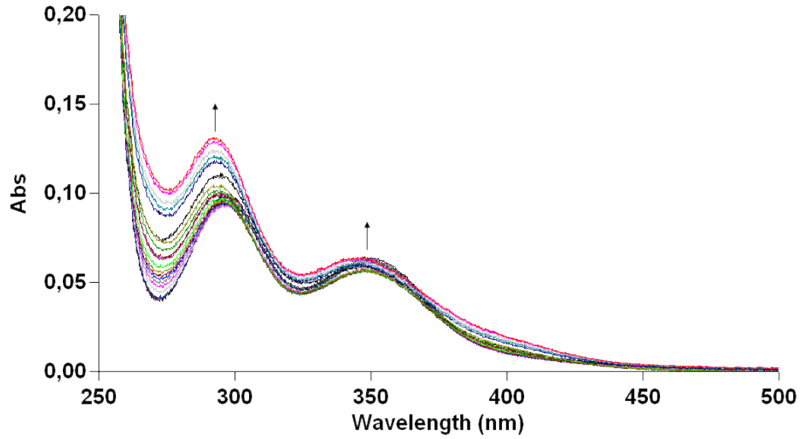

Figure S1: *UV-Vis spectrum of complex cis-$Pt(NH_3)_2I_2$ 10^{-4} M with 10^{-3} M KI in 50 mM phosphate buffer (pH=7.4) followed for 72 h*

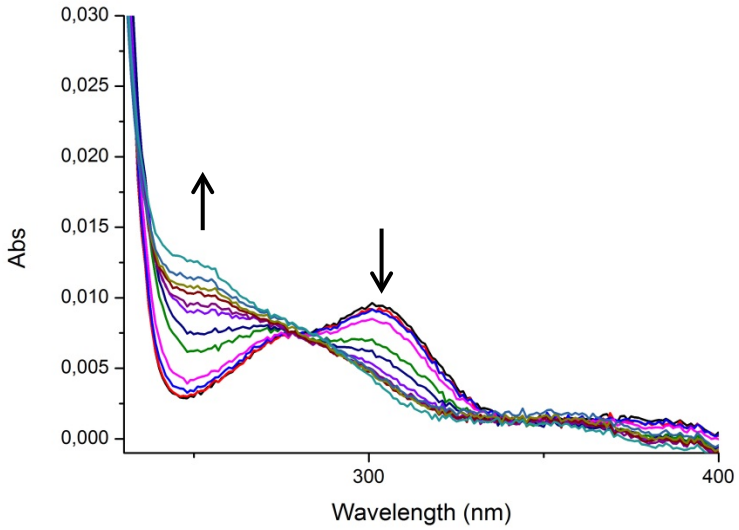

Figure S2: *Time dependent UV-Vis spectrum of cisplatin 10^{-4} M buffer phosphate 50 mM pH=7.4 recorded for 72 h at RT.*

Isosbestic points analysis: *isosbestic points of the two complexes (285 nm for cisplatin and 284 cisPtI_2), drift during the analysis, this is the evidence that allows us to hypothesize a biphasic reaction with two very close rate constants[1].*

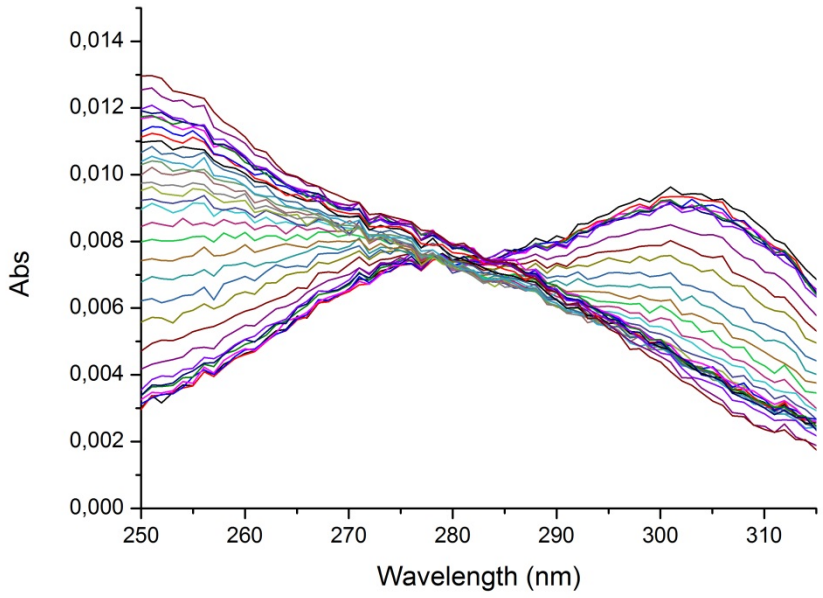

Figure S3: *Detail on isosbestic point for time dependent UV-Vis spectrum of cisplatin 10^{-4}M buffer phosphate 50 mM pH=7.4 recorded for 72 h at RT.*

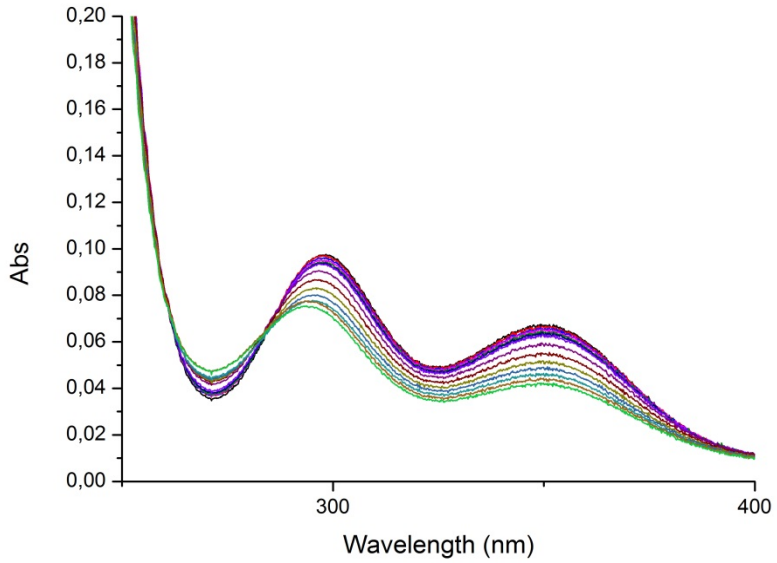

Figure S4: *Detail on isosbestic point for time dependent UV-Vis spectrum of cisPtI_2 10^{-4}M buffer phosphate 50 mM pH=7.4 recorded for 72 h at RT.*

Final products analysis: *We have considered as final UV-Vis spectrum the spectral profile recorded after 72 h, since after this time there are no evidences for changes in absorbance within the investigated range (200-800 nm). For both complexes, there are evidences of existence of the same species characterised by two absorption bands at 285 and 350 nm, as result from deconvoluted spectral profiles. The relative differences existing between DFT calculation and experimental spectral profiles may be assignable to various equilibria determined by pH value. Acid dissociation constants for coordinated water molecules in products cis-$[PtCl(H_2O)(NH_3)]^+$, cis-$[Pt(H_2O)_2(NH_3)_2]^{2+}$ and cis-*

$[Pt(H_2O)(OH)(NH_3)_2]^+$ are 6.41; 5.37; 7.21 and using buffered solution at pH=7.4 it is reasonable to hypothesise a series of equilibria that produce different species (i.e. hydroxo species) in different ratio [2]. In a similar manner same process are likely involved in the hydrolysis of $cisPtI_2$.

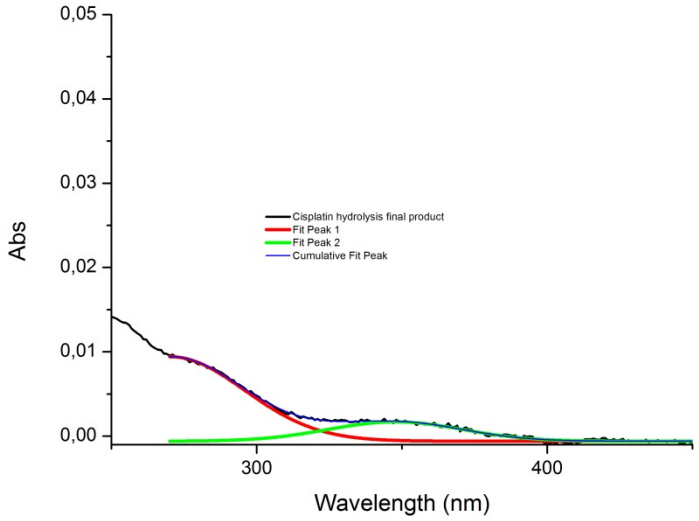

Figure S5: *Detail on deconvolution of final product spectral profile for time dependent UV-Vis spectrum of cisplatin 10^{-4}M buffer phosphate 50 mM pH=7.4 recorded for 72 h at RT.*

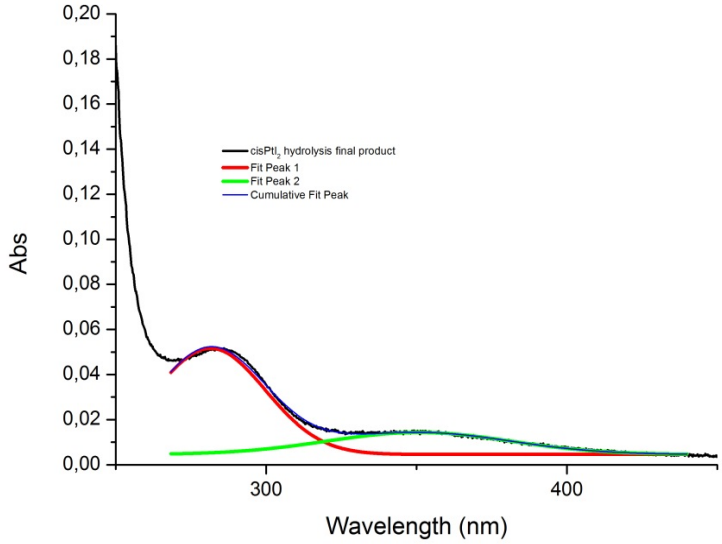

Figure S6: *Detail on deconvolution of final product spectral profile for time dependent UV-Vis spectrum of $cisPtI_2$ 10^{-4}M buffer phosphate 50 mM pH=7.4 recorded for 72 h at RT.*

Pseudo first-order rate constant calculation. *According to Miller et al. [1], it is possible to analyse these experimental time dependent spectral profiles treating the hydrolysis process as a pseudo-first order reaction. Calculations were done plotting the variation of absorbance as function of time for both $cisPtI_2$ and cisplatin respectively monitoring the maxima at 350 and 305 nm.*

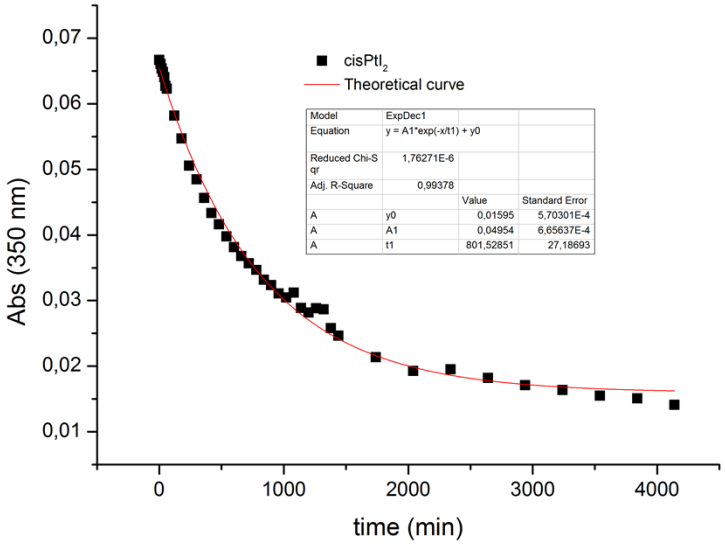

Figure S7: *Experimental kinetic traces and calculated theoretical curve for the hydrolysis of cisPtI$_2$. k_{obs} were calculated fitting with a single exponential function.*

	K_{obs} (s^{-1})	$t_{1/2}$ (min)
Cisplatin	**$4,45*10^{-5}$**	**259,4**
cisPtI$_2$	**$2,08*10^{-5}$**	**555,58**

Table S1: *K_{obs} and $t_{1/2}$ values determined fitting with a single exponential function.*

ESI-MS experiment: *cis-Pt(NH$_3$)$_2$I$_2$ was solubilized in ammonium acetate buffer pH= 4.5, and ESI MS spectra were recorded by direct introduction at 5 µl/min flow rate in an Orbitrap high-resolution mass spectrometer (Thermo, San Jose, CA, USA), equipped with a conventional ESI source. The working conditions were the following: spray voltage 3.1 kV, capillary voltage 45 V, capillary temperature 220°C, tube lens voltage 230 V. The sheath and the auxiliary gases were set, respectively, at 17 (arbitrary units) and 1 (arbitrary units). For acquisition, Xcalibur 2.0. software (Thermo) was used and monoisotopic and average deconvoluted masses were obtained by using the integrated Xtract tool. For spectrum acquisition a nominal resolution (at m/z 400) of 100,000 was used.*

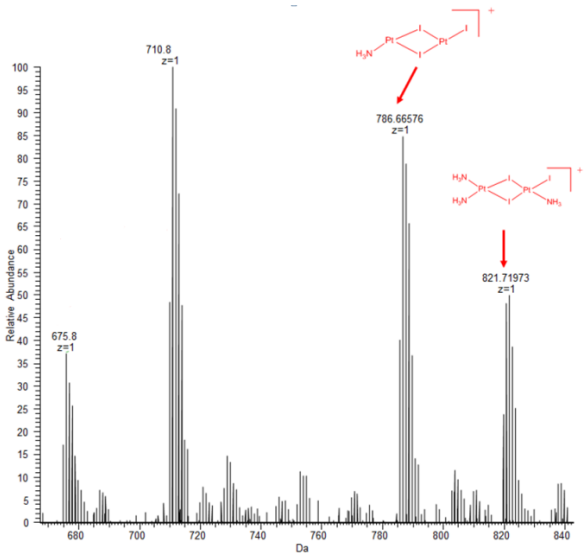

Figure S8: *ESI-MS spectrum of complex cis-Pt(NH$_3$)$_2$I$_2$ 10^{-4} M in 20 mM ammonium acetate buffer (pH=4.5)*

DNA interstrand cross-linking in the linear pUC19 DNA (linearized with EcoRI restriction enzyme). In order to quantitate the interstrand cross-linking efficiency of *cis-PtI$_2$(NH$_3$)$_2$* the above reported linearized plasmid DNA was 3′-end-labeled and modified by the *cis-PtI$_2$(NH$_3$)* or cisplatin at various r_b values. The samples were analyzed for the interstrand cross-links (CLs) by agarose gel electrophoresis under denaturing conditions.[3] Upon electrophoresis, 3′-end labeled strands of the linear fragment containing no interstrand CLs migrate as a 2686-base single strand, whereas the interstrand cross-linked strands migrate more slowly as a higher molecular mass species (Figure S9). The intensity of the more slowly migrating band increased with the growing level of the modification. The radioactivity associated with the individual bands in each lane was measured to obtain estimates of the fraction of noncross-linked or cross-linked DNA under each condition. The frequency of interstrand CLs was calculated using the Poisson distribution from the fraction of interstrand cross-linked DNA in combination with the r_b values and the fragment size.[4]

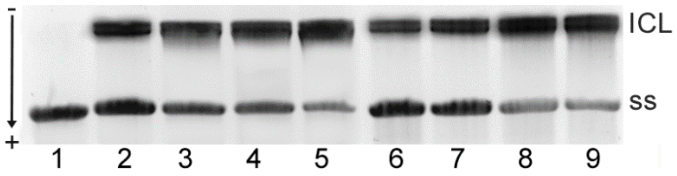

Figure S9: *Formation of interstrand cross-links. Interstrand cross-linking by cis-PtI$_2$(NH$_3$)$_2$ and cisplatin in the linear pUC19 plasmid DNA. Autoradiogram of denaturing 1% agarose gels of linearized DNA which was 3'-end labeled. The interstrand cross-linked DNA appears as the top bands migrating on the gel more slowly than the single-stranded DNA (contained in the bottom bands). Lanes: 1, control, unplatinated DNA; 2-5, DNA modified by cisplatin; 6-9, DNA modified by cis-PtI$_2$(NH$_3$)$_2$. r_b values: 0.0003 (lanes 2 and 6); 0.0005 (lanes 3 and 7); 0.0007(lanes 4 and 8); 0.001 (lanes 5 and 9).*

DNA unwinding. Native agarose electrophoresis is used to determine the unwinding induced in negatively supercoiled plasmid DNA by monitoring the degree of supercoiling.[5] A compound that unwinds the DNA duplex reduces the number of supercoils in closed circular DNA. This decrease causes a decrease in the rate of migration through agarose gel, which makes it possible to observe and quantify the mean value of unwinding per adduct. Figure S10 shows electrophoresis gels from experiments in which different amount of *cis*-[PtI$_2$(NH$_3$)$_2$] was bound to a mixture of relaxed and negatively supercoiled pSP73KB. Interestingly, both complexes increased the mobility of the relaxed form similarly as does cisplatin, whose bifunctional binding to DNA shortens and condenses the DNA helix.[6,7] The mean unwinding angle is given by $\Phi = -18\sigma/r_b(c)$, where σ is the superhelical density and $r_b(c)$ is the value of r_b at which the supercoiled and nicked forms comigrate.[5] Under the present experimental conditions, the value for σ of plasmid DNA used in these experiments was calculated to be -0.072 on the basis of the data of cisplatin for which the $r_b(c)$ was determined in this study and $\Phi = 13°$ was assumed.[5]

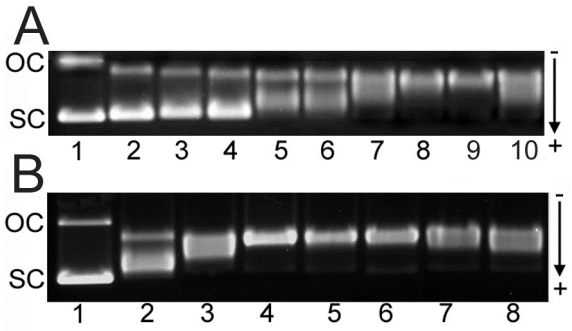

Figure S10: *Unwinding of negatively supercoiled pSP73KB plasmid DNA by platinum complexes. The top bands (oc) correspond to nicked plasmid and the bottom bands (sc) correspond to the closed, negatively supercoiled plasmid. A) Cisplatin. Lanes: 1, control, nonmodified DNA, 2–10 rb = 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09,0.11, respectively. B) cis-PtI2(NH3)2. Lanes: 1, control, nonmodified DNA, 2–8 rb = 0.05, 0.07, 0.09, 0.1, 0.12, 0.14, 0.16, respectively.*

References

[1] Miller, S.E., House, D.A. (1989**)** The hydrolysis products of cis-Dichlorodiammineplatinum(II) 2. The kinetics of formation and anation of the cis-Diamminedi(aqua)platinum(II) Cation. *ICA, 166, 189-197.*

[2] The chemistry of Cisplatin in Aqueous solution (in Platinum-based drugs in cancer therapy, Humana Press Inc., Totowa, NJ),2000, S.J. Berners-Price, T.G. Appleton, pp. 3-35.

[3]Brabec, V., and Leng, M. (1993) DNA interstrand cross-links of trans-diamminedichloroplatinum(II) are preferentially formed between guanine and complementary cytosine residues. *Proc. Natl. Acad. Sci. USA*, *90*, 5345-5349.

[4]Farrell, N., Qu, Y., Feng, L., and Van Houten, B. (1990) Comparison of chemical reactivity, cytotoxicity, interstrand cross-linking and DNA sequence specificity of bis(platinum) complexes containing monodentate or bidentate coordination spheres with their monomeric analogues. *Biochemistry*, *29*, 9522-953.

[5] Keck, M. V., and Lippard, S. J. (1992) Unwinding of supercoiled DNA by platinum ethidium and related complexes. *J. Am. Chem. Soc.*, *114*, 3386-3390.

[6] Cohen, G. L., Bauer, W. R., Barton, J. K., and Lippard, S. J. (1979) Binding of cis and trans dichlorodiammineplatinum(II) to DNA: Evidence for unwinding and shortening of the double helix. *Science*, *203*, 1014-1016.

[7] Scovell, W. M., and Collart, F. (1985) Unwinding of supercoiled DNA by cis- and trans-diamminedichloro- platinum(II): influence of the torsional strain on DNA unwinding. *Nucleic Acids Res.*, *13*, 2881-2895.