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Article

Synthesis, Reactivity Studies and Cytotoxicity of Two Iodidoplatinum(II) Complexes. Does Photoactivation Work?

Leticia Cubo,[1] Thalia Parro,[1] Amancio Carnero,[2] Luca Salassa,[3] Ana I. Matesanz,[1] Adoracion G. Quiroga [1]*

[1] Departamento de Química Inorgánica. Universidad Autónoma de Madrid, Spain.
[2] IBIS, Campus Hospital Universitario Virgen del Rocío, Sevilla, Spain.
[3] Donostia International Physics Center. Guipozkoa, Spain.
* Correspondence: adoracion.gomez@uam.es; Tel.: +34 914974050

Abstract:

Trans platinum complexes have been the landmark in unconventional drugs prompting the development of innovative structures that might exhibit chemical and biological profiles different to cisplatin. Iodido complexes made a new turning point in the platinum drug design field since their cytotoxicity was reevaluated and reported. In this new study, we have synthesized and evaluated diodido complexes bearing aliphatic amines and pyridines in *trans* configuration. X-ray diffraction support the structural characterization. Their cytotoxicity has been evaluated in tumor cell lines such as SAOS-2, A375, T-47D and HCT116. Moreover, we report their solution behavior and reactivity with biological models. UVA irradiation induces an increase in their reactivity towards model nucleobase 5´-GMP in early stages, and promotes the release of the pyridine ligand (spectator ligand) at longer reaction times. Density Functional calculations have been performed and the results are compared with our previous studies with other iodido derivatives.

Keywords: platinum iodido complexes; cytotoxicity; photoactivation

1. Introduction

Antitumor platinum drug design has been and still is a major project in metallodrug research. The latest reviews and the large number of contributions therein, identify new complexes design to face the known side effect problems of this type of drugs.[1] One of the latest contributions to this field has been the results observed studying the impact of the leaving group in the reactivity and cytotoxicity of the iodido complexes.[2,3]

Looking for new design, our group of research reevaluated *cis* platinum iodido complexes which reactivity turned out to be quite unexpected versus sulfur donor biomolecules.[4] The iodido groups stayed in the adducts formed with the protein cytochrome c and lysozime and the aliphatic amines acted as leaving groups.[5] This peculiar reactivity was not detected versus DNA with which they showed classical reactivity cisplatin like.

Following these results, we extended the studies to include the reactivity of *trans* diiodido diamine platinum(II) with different aliphatic amines.[6] These reactivity studies revealed a very similar profile for the *cis* and *trans* complexes upon binding to model nucleobases (DNA). The adduct formation occurs with retention of the amine spectator ligands. Additionally, *trans*-type complexes manifested a lower propensity to form adducts with peptide and a more classical reactivity, the iodido ligands release upon protein binding. Besides, these last *trans* series seemed to be affected by the size of the amine ligands showing differences in their reactivity versus S-donor models and in their cytotoxic activity.[6] The reconsideration of these iodido derivatives have shown a great impact in the field, and the trend and behavior of these complexes is being reevaluated.[7]

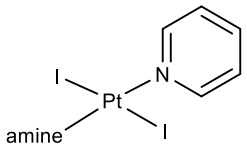
Within the design of novel metallodrugs, the use of activation therapies such as the use of UVA light offers a wide fan of possibilities for metallic complexes such as activation of reactivity [8], change in conformation [9], heterobimetallic complexes with PDT ligands [10] and singlet oxygen producing complexes.[11] Photochemical studies on a selected number of *trans* iodido complexes initially showed that irradiation induces a faster reaction with CT DNA and a higher amount of Pt bound to DNA. These complexes also reacted faster with 5′-GMP under irradiation and even showed noticeable improvements in their cytotoxic activity when treatment was combined with UVA light.[12] These results support the idea that UVA light could be used to increase the activity of the diiodido platinum complexes, and might even make it more selective, in similar way than those observed for chlorido derivatives.[8]

Within this frame, we have look at the role of the spectator ligand within the iodido complexes structure and replace one of the aliphatic amines with an aromatic planar amine, widely used in this kind of complexes with good results.[13] In particular, we prepared two *trans* configured iodido-platinum complexes with a pyridine and isopropylamine/methylamine. X-ray structure studies of both complexes complete the structural characterization. The cytotoxicity was evaluated and the possible mechanism investigated, looking for a possible photochemical activation. The interaction studies of these new complexes with a representative model biomolecules such as: model nucleobase 5′-GMP and N-Methylimidazol (MeIm) have been performed and photochemical studies on such interaction analyzed and discussed together with the DFT calculations.

2. Results

2.1. Synthesis and characterization

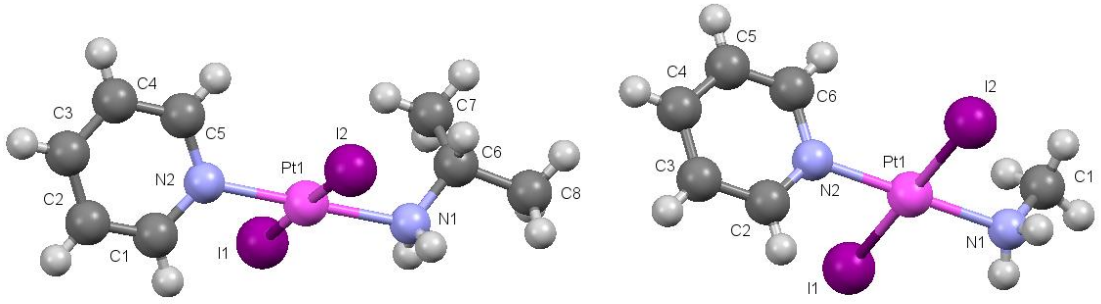
The *trans*-[PtI_2(amine)(pyridine)] complexes were prepared according to published procedures using the *cis*-[PtI_2(amine)$_2$] complexes with the corresponding aliphatic amine.[6] The structure and numbering of the complexes are depicted in Figure 1. Their characterization performed by usual techniques is nicely in agreement with the structure proposed and detailed data have been collected in the experimental section.



amine	Ccmplex
$(CH_3)_2CHNH_2$	**1**
$(CH_3)NH_2$	**2**

Figure 1. Structure and numbering of the complexes studied in this manuscript

The molecular structure of complexes **1** and **2** are shown in Figure 2. Crystal data are listed in Table S1. Selected bond lengths and angles are shown in Table 1. The platinum(II) atom has a square planar coordination geometry and is coordinated to two nitrogen atoms of the methylamine or isopropylamine and pyridine ligands and two iodido ligands in *trans*-arrangement.



(**a**) (**b**)

Figure 2. Molecular views: (**a**) complex **1** (**b**) complex **2**

Table 1. Selected distances (Å) and angles (°) in complexes **1** and **2**.

distances	Complex 1	Complex 2
Pt-I1	2.5962(9)	2.5906(11)
Pt-I2	2.5920(8)	2.59861(19)
Pt-N1	2.050(9)	2.077(13)
Pt-N2	2.014(8)	2.025(13)
angles	**Complex 1**	**Complex 2**
N2-Pt-N1	179.24(4)	179.3(5)
N1-Pt-I1	90.1(3)	90.2(4)
N1-Pt-I2	90.0(3)	89.1(4)
N2-Pt-I1	89.7(3)	90.1(4)
N2-Pt-I2	90.02(3)	90.6(4)
I1-Pt-I2	179.05(3)	177.24(4)

2.2. Cytotoxicity of trans-[PtI$_2$(amine)(py)] complexes

The cytotoxicity of complexes **1** and **2** was determined in comparison to cisplatin. The cancer cell lines selected for this study are SAOS-2 (human osteosarcoma), A375 (melanoma), T-47D (breast carcinoma), HCT116+ + (human colon carcinoma with the presence of p53), HCT116- -(human colon carcinoma in the absence of p53). IC_{50} and standard deviations values for complexes **1** and **2** are shown in Table 2.

Table **2**. Cytotoxicity of complexes **1** and **2** as compared to cisplatin in some cancer cell lines

Cell line	Complex 1	Complex 2	cisplatin
SAOS-2	32.9 (22.6)	53.7(15.8)	5.9(1.5)
A375	18.9(3.4)	27.9(5.1)	9.2(1.7)
T-47D	30.9(5.6)	45.0(2.5)	10.2(3.4)
HCT116++	18.1(3.6)	29.8(4.4)	8.3(2.7)
HCT116--	56.4(10.3)	63.4(17.3)	62.2(14.3)

2.3. Reactivity of trans-[PtI$_2$(amine)(py)] complexes with 5′-GMP and MeIm

One of the main cellular targets of metallodrugs is DNA.[14,15] There are many techniques to study the interaction with DNA, as our objective in this section is to compare the reactivity of these compounds with the data available in the references, we have used a small model of DNA, such as 5′-GMP. This model of interaction study has proved to be a good approach in many systems by monitoring the changes at its H8 signal by ^{1}H NMR.[16,17]

A study on the reactivity of complexes **1** and **2** with the model nucleobase 5′-GMP was carried out. The complexes under investigation were incubated at a molar ratio of complex: 5′-GMP of 1:2 as described in the experimental section and the reactivity toward this nucleobase was monitored by NMR spectroscopy. The ^{1}H NMR spectra of the interaction of complex **1** with 5′-GMP monitored from 1 h to 7 h is shown in Figure 3. When following the changes at the H8 peak signal of 5′-GMP, a new signal arises at 9.1 ppm that is assignable to a H8 nucleotide adduct which intensity increases along the first 3 hours of reaction while the H8-signal of the free nucleotide decreases. At longer reaction times, the first monoadduct intensity does not longer increase and simultaneously, a new adduct is detected at 8.2 ppm. The monitoring of the reaction provide evidence for the presence of 5′-GMP adducts in the sample. The reaction of the *trans* complex **2** with 5′-GMP was also studied and the results are similar to the reactivity described for complex **1**. However, the reactivity of compound **2** seems to be slower since the signals assigned to the adduct species are weaker in complex **2** than

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for complex **1** after 3 hours of reaction (Figure S1). Besides, smaller amount of the model nucleotide adducts are detected for complex **2** even using longer periods of time.

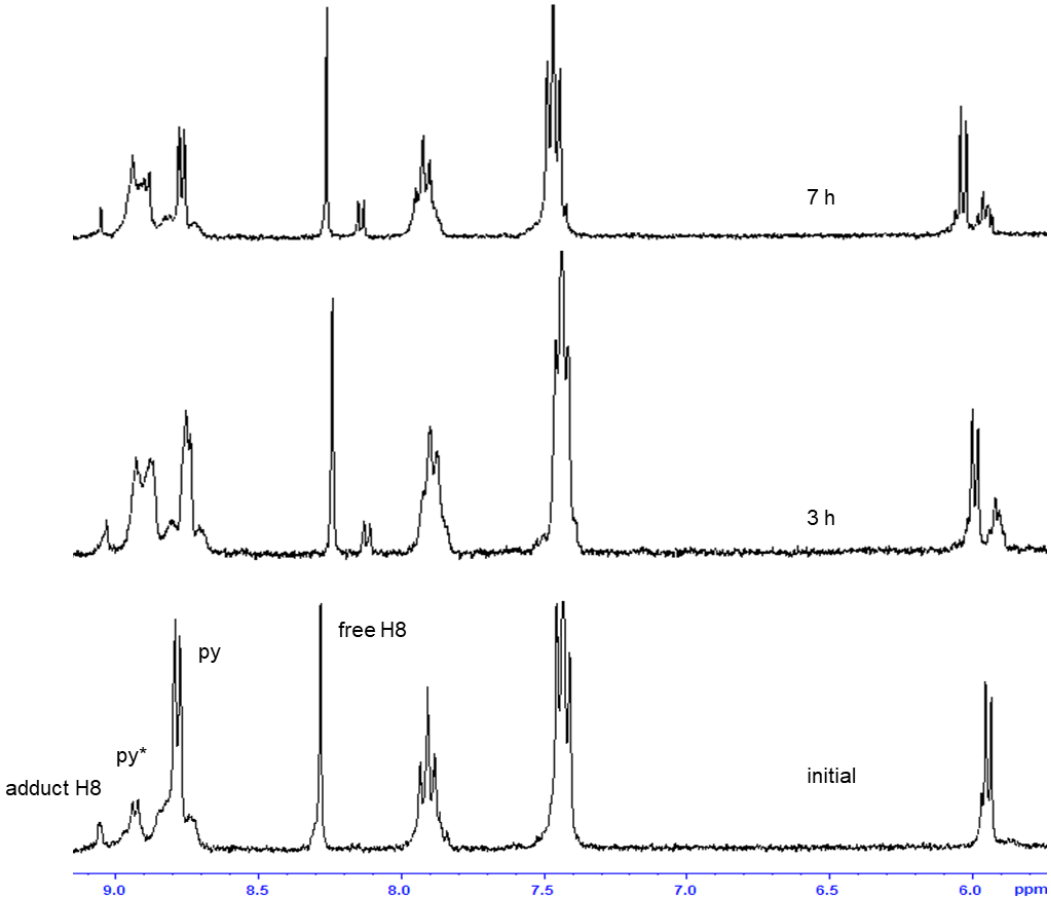


Figure 3. Progress of the reaction between complex **1** and 5′-GMP at 37 °C monitored by ^{1}H NMR showing changes in the aromatic area. Labels: free H8, free nucleotide; adduct H8, nucleotide adduct; py, pyridine ligand complex 1; py*, pyridine signal adducts.

When similar experiments were repeated under irradiation, the reactivity of complex **1** with 5′-GMP showed some important differences compared to the non-irradiated sample. In the first hour of irradiation, the monoadduct reported is formed in a higher amount (integrals shows a higher formation), but after 1 h the spectra shows a new signal at 8.6 ppm (Figure 4) corresponding to free pyridine and its intensity increases along the time. We can also observed new species clearly in the high-field region of the spectra. The signals corresponding to the isopropylamine group appear also to split into several sets.

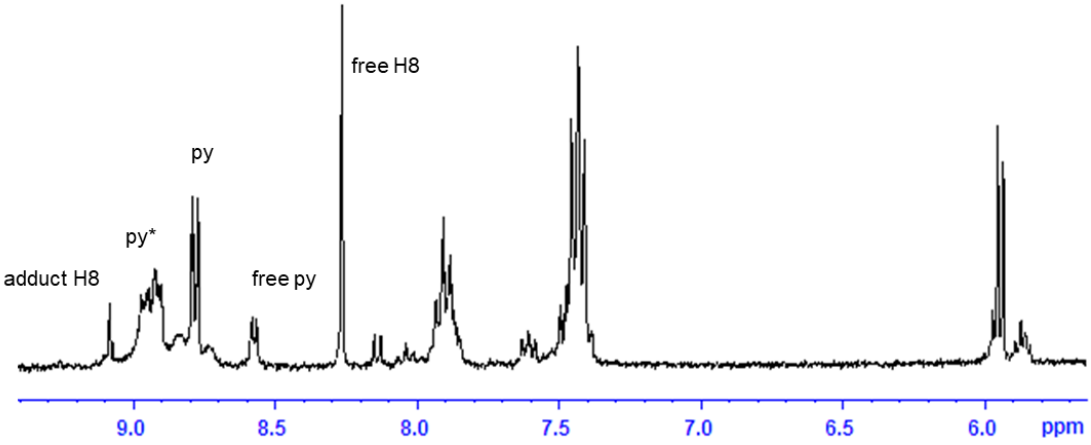


Figure 4. ^{1}H NMR spectrum of the reaction between complex **1** and 5′-GMP showing changes in the aromatic and aliphatic area after 1 h under irradiation at 37 °C. Labels: free H8 , nucleotide free; adduct H8, nucleotide adduct; py, pyridine ligand 1; free py, free pyridine; py*, pyridine signal adduct.

We extended our NMR study of the complexes' coordination to the heterocyclic ligand N-methylImidazole, MeIm. This model can provide information about the possibilities of the complex to bind into a lopsided configuration like those presented by biological molecules such as proteins or DNA.[18] Moreover its structure is different to 5-GMP (already used) but similar to those presented by the proteins at, for example, histidine's sites. The results are collected in Figure 5. The reactivity at 37 °C (Figure 5a) with this model affords the monoadduct within the first hour, becoming almost a major product only after 3 h. The speciation is clearly formed quickly and the starting material has fully reacted after 7 h. The photoactivation of the sample (Figure 5b) enhances the reactivity but more importantly, produces the release of the pyridine ligand like in its reaction with 5′-GMP.

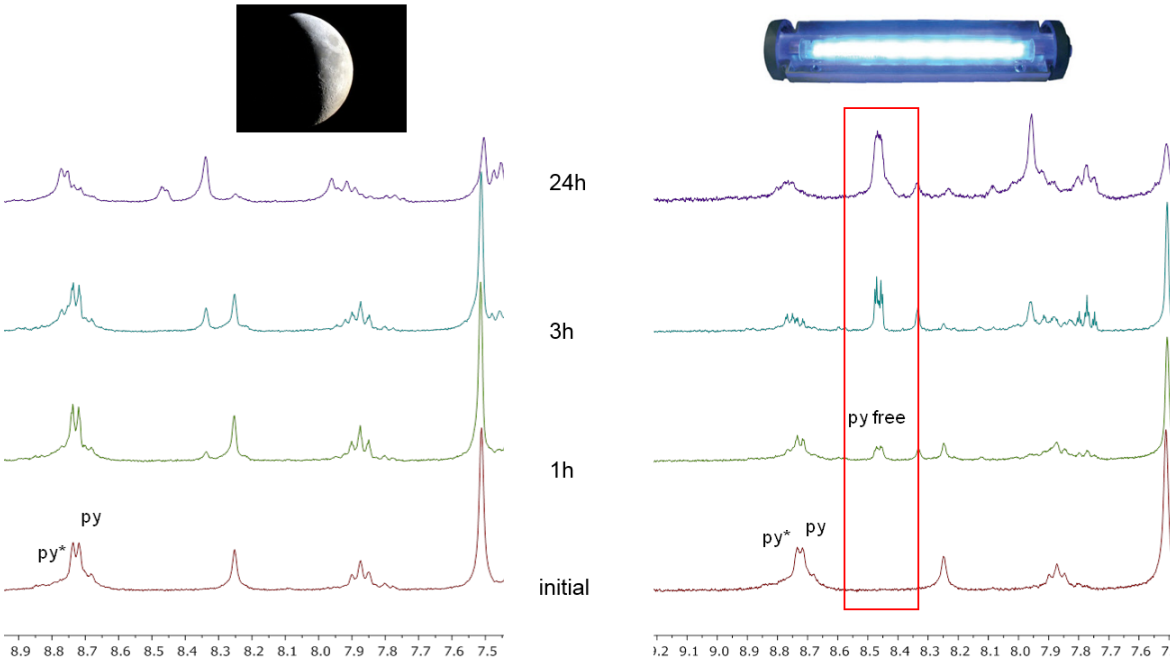
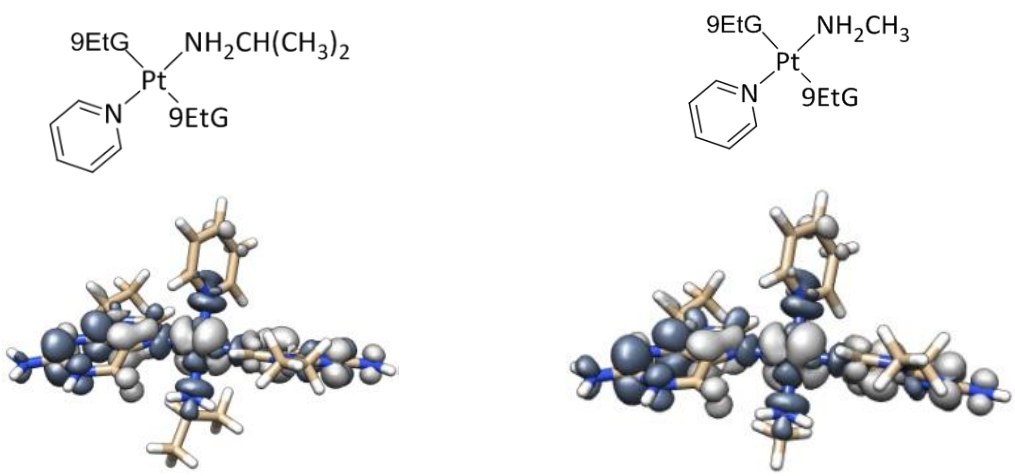


Figure 5. Progress of the reaction between complex **1** and MeIm monitored by ^{1}H NMR showing changes in the aromatic area. Labels: py, pyridine ligand complex 1; py*, pyridine signal adducts. a) reaction at 37 °C and b) reaction at 37 °C and irradiating

2.4. DFT Calculations

In order to gain more insight into the photochemistry of the diiodido Pt(II) complexes, we ran a set of Density Functional Theory (DFT) and time-dependent DFT (TD-DFT) calculations on **1** and **2** and their 9-EtG adducts. The DNA basis mimic, 9-EtG, was employed instead of 5′-GMP to ease the computational work. After geometry optimization (Table S2), we evaluated the single-singlet transitions on the complexes and gauged their excited-state chemistry (Table S3 and table S4). Consistently with experimental results, the parent complexes **1** and **2** display electronic transition that are mostly dissociative towards the iodido ligands (Table S3). The calculated absorption spectrum of the mono 9-EtG adducts of the two complexes displays a band at ca. 350 nm, which can be excited under UVA excitation (Table S5). Both mono and bis 9-EtG adducts present dissociative transition towards the pyridine ligand as shown in the electron difference density maps (EDDMs) of Figure 6 and in Table S6. A summary of the most relevant bond distances for **1** and **2** and their 9Et-G derivatives are reported in Table S7.



(a) (b)

Figure 6. Selected TD-DFT singlet-singlet transitions (transition 2, S67) and corresponding electron difference density maps (EDDMs) for the bis 9-EtG adducts of complexes **1** (**a**) and 2(**b**) in water at the CAM-B3LYP/LANL08/6-31G** level. In the EDDMs gray indicates a decrease in electron density, while blue-gray indicates an increase.

3. Discussion

The complexes were designed based on the positive results achieved with previous complexes of nonconventional structure and using iodidos, aliphatic amines and pyridines as ligands. The synthesis was performed following the published procedure, with lower yield than usual. Many attempts for varying concentrations, temperature or longer reaction time did not improve the results. The characterization by usual techniques was in accordance with the X-Ray study and allowed a detailed knowledge of the structural features. The mentioned structures are compared with those of published Pt complexes with aliphatic amines, iodido and pyridine ligands. The Pt- N (aliphatic amine) [19,20], Pt-I [21,22] and Pt-N (pyridine) [23,24] distances fall in the typical ranges. The interactions between the methyl groups and the iodido ligands results in significant strain in the complexes. This is reflected in the opening of the Pt–N–C angles to 116–119 °, which is nearer to the tetrahedral geometry than expected. Cytotoxicity was evaluated versus SAOS-2 human osteosarcoma, A375 melanoma, T-47D breast cancer, SF-268 glioblastoma, NCI-H460 lung cancer and colorectal carcinoma cell lines HCT116, and matched p53-deficient HCT116 (-/-). The antiproliferative effects are found to be moderate, with IC_{50} values generally being in the micromolar range, and differences between the cytotoxic potencies of **1** and **2** are comparatively small. Substitution of one aliphatic amine seems to decrease cytotoxic potency; the steric demand could be responsible for these poor specific antitumor effects. We believe that irradiation of the complexes will produce the enhancement of the antitumoral activity, as discussed in the following results.

- The interaction of the complexes with 5'-GMP shows the formation of the expected DNA adduct after hydrolysis of the iodido complexes as reported for similar compounds with no release of the spectator ligands.
- However, once we irradiate the sample, we can only observed a promotion of the reactivity in the first hour, and then the release of the pyridine ligand becomes clear at longer reaction times and irradiation.
- This release of the spectator ligand enhance by irradiation donors has not been observed studying *trans* isomers with iodido ligands versus DNA[25], but only with *cis* isomers and versus proteins. [5] In addition, the results when the compound **1** and **2** interactions with MeIm are evaluated and they are very similar to the results with DNA.
- Our interpretation of the data is that after irradiation, the complex **1** and **2** reactivity is enhanced forming actives species, but at longer reaction times, the compound losses the pyridine affording

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new adducts and species which are more similar to those from cytotoxic compounds reported before than their original aquaspecies/ adducts.

- DFT calculations justify the quicker formation of the bis/monoadduct (Figure 6) along the first hour, but once the irradiation applies for longer periods of time, the pyridine is released and the compound is no longer the structure proposed but similar to those bearing only aliphatic amines.[12]

The potential photoactivation of these poor pharmacological complexes is then not simple. At short term the irradiation will afford the DNA adducts but the most affective photoactivation will take place at long term, as the species will be the same then the most active iodido series reported previously (Figure 7).

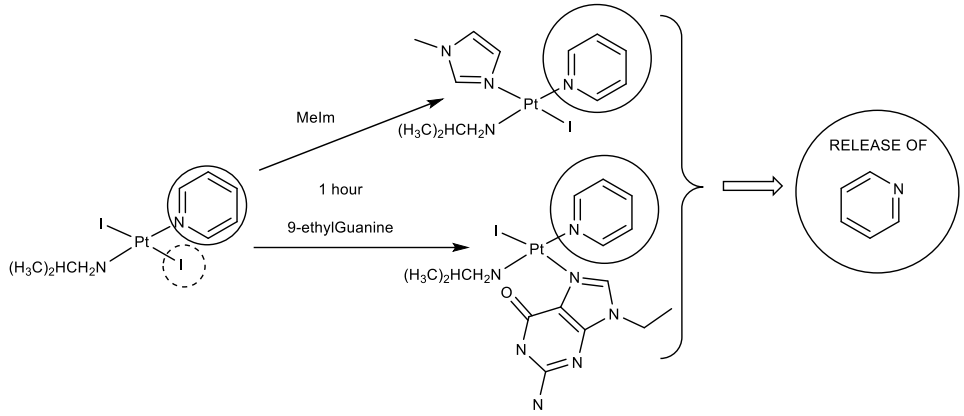

Figure 7. Reactivity versus 9-EtG and MeIm proposed in the discussion.

4. Materials and Methods

Complexes *cis*-$PtI_2(amine)_2$ (where amine = methylamine or isopropylamine) were synthesized as described in the previous communications based in reported procedures. [12] Characterization was in agreement with our previous data. Chemical starting materials: K_2PtCl_4, amines, pyridine and biological molecules were purchased from VWR.

4.1. Method for the synthesis of trans-[PtI_2(amine)(pyridine)].

cis-$PtI_2(amine)_2$ (500 mg) was suspended in water and 20 equivalents of pyridine were added to the solution and heated at reflux temperature for 3-6 hours. The solution was filtered over celite and concentrated at high temperature (100 °C) until detection of an orange solid which was allowed to stand overnight at 4 °C until complete precipitation. Then the solid was filtered off, washed with warm water, and vacuum dried overnight at 60 °C in a drying oven. Afterwards, recrystallization in chloroform/ether was required. Monocrystals were achieved by slow evaporation of a chloroform solution.

trans-[PtI_2(ma)(py)] **1** (orange solid). Yield: 15%. NMR (acetone-d_6): δ (1H): 2.57 (t, J=6.5 Hz, 3H, CH_3-ma), 4.20 (b.s., 2H, NH_2) 7.40 (dd, J=7.1, 1.51 Hz, 2H, CH-py) 7.88 (td, J= 7.1, 1.9 Hz, 1H, CH-py), 8.84 (dd, J=6.6, 1.84 Hz, 2H, CH-py).

trans-[PtI_2(ipa)(py)] **2** (orange solid). Yield: 18%. NMR (acetone-d_6): δ (1H): 1.33 (d, J=6.46 Hz, 6H, $2CH_3$-ipa), 3.50 (sept, J=6.7 Hz, 1H, CH-ipa) 7.36 (dd, J= 6.6, 1.5 Hz, 2H, CH-py) 7.83 (td, J=7.7, 1.7 Hz, 1H, CH-py) 8.81(dd, J=6.6, 1.8 Hz, 2H, CH-py).

4.2. Nuclear magnetic resonance spectroscopy

1D ^{1}H NMR spectra were recorded on a 300 MHz Bruker Advance III HD and 500 MHz DRX spectrometers. Solutions were prepared in acetone-d_6 for the characterization spectra and in a mixture of acetone-d_6 and D_2O in a ratio 2:1 for the interaction studies with the 5′-GMP and MeIm

with a final concentration [Pt]=6.5 mM. ^{1}H chemical shifts were internally referenced to sodium 3-(trimethylsilyl)propionate (TSP).

4.3. Irradiation

The light source used in the photoactivation experiments was a Photoreactor LZC-ICH2 from Luzchem (Canada) fitted with UVA lamps (2 mW/cm^2, λ = 365 nm). The temperature in the light chamber during irradiation was kept at 37 °C, and the sample preparation was performed as describe in the section 4.2.

4.4.X-ray diffraction

The structural features of the new complexes **1** and **2** were unambiguously proven by X-ray diffraction. Data collection was performed on a Bruker Kappa Apex II (X8 APEX) area detector X-ray diffractometer using a graphite-monochromated Mo radiation. Crystal data and the structure refinement parameters are listed in TableS1. The CCDD numbers are: 913250 and 913249.

4.4 Cytotoxicity

SAOS-2 human osteosarcoma, A375 melanoma, T-47D breast cancer, SF-268 glioblastoma, NCI-H460 lung cancer were purchased from ATCC. Colorectal carcinoma cell lines HCT116, and matched p53-deficient HCT116 (-/-) were a kind gift of Bert Vogelstein (John-Hopkins University, Baltimore, MD). These cell lines were cultured with DMEN (A375 and NCI-H460) or RPMI (HCT116) (Sigma) with 10% fetal bovine serum, 2 mM L glutamine, 100 U/mL penicillin, 100 ◎g/mL streptomycin in humidified air with 5% CO_2 at 37 °C.

The cytotoxicity was performed in Dr. Amancio Carnero's laboratory, following the methodology briefly described. The compounds were tested in 96-well plates. Cells growing in a flask were harvested just before they became confluent, counted using a haemocytometer and diluted with media adjusting the concentration to the required number of cells per 0.2 mL (volume for each well). The cells were then seeded in the 96-well plates at a density depending of the cell size, between 1000 and 4000 cells/well. The cells were then left to plate down and grow for 24 hours before adding the drugs. The compounds were weighed out and diluted with PBS to reach 10 mM solutions. From here a "mother plate" with serial dilutions was prepared at 200X the final concentration in the culture. Each mother plate was stored at -20 °C until further use. The appropriate volume of the compound solution (usually 2 μL) was added automatically (Beckman FX 96 tip) to media to make it up to the final concentration for each drug. The media was removed from the cells and replaced with 0.2 mL of the media dosed with drug. Each concentration was assayed in triplicate. A set of control wells is left on each plate, containing media without drug. A second set of control wells were left on each plate, containing media with the same concentration of PBS. Another control set was obtained with the cells untreated just before adding the drugs (seeding control, number of cells starting the culture). Cells were exposed to the drugs for 96 hours and then washed twice with phosphate buffered saline before being fixed with 10% glutaraldehyde. Cells were washed twice and fixed with crystal violet 0.5% during 30 minutes, extensively washed and the absorbance was measured at 595 nm.

4.5. DFT Calculations

All calculations on complexes **1** and **2** and their 9-EtG adducts, were performed with the Gaussian 09 (G09) program [26] employing the DFT and TD-DFT [27,28] methods. Basis sets, ECPs for Pt and I and functionals were benchmarked (not shown) and the best combination in terms of performance and computational demand was the PBE1PBE:LANL08/6-31G** [29] for geometry optimization and CAM-B3LYP/LANL08/6-31G** [30] for electronic transition calculations (see below). The PCM solvent model [31] was adopted in all DFT and TD-DFT calculations with water as solvent. The nature of all stationary points was confirmed by normal mode analysis.

Thirty-two singlet excited states with the corresponding oscillator strengths were determined for the complexes at the ground-state geometry by TD-DFT. Theoretical UV-Vis spectra were obtained using GAUSSSUM 2.2. [32]

Molecular graphics images were produced using the UCSF Chimera package from the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco (supported by NIH P41 RR001081). [33]

5. Conclusions

We have presented two new diiodido complexes with aliphatic amines (ipa, **1** and ma, **2**) in *trans* to a pyridine ligand. Although the cytotoxic activity of the complexes was not as encouraging as cisplatin and other similar compounds, their reactivity in the presence of UVA and upon binding to model biological molecules light revealed a new interesting profile. We observed that irradiation at 365 nm enhances the DNA adduct formation at early stages (up to 1h). Our photoactivation experiments suggest that longer irradiation times produces the release of the pyridine ligand and the subsequence generation of species that have previously proved to be active and interact with biomolecular targets.

Since this is the first time that we have observed the release of the spectator ligand in *trans*-type complexes, this result provides evidences that the aromatic nature of the amine ligand plays an important role in the photochemistry of *trans* diiodido platinum complexes and also that they can be considered as potential photoactivable prodrug compounds following two different pathways.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Table S1. Crystal Data for complex 1 and 2; Figure S1. Progress of the reaction between complex 2 and 5′-GMP at 37ºC monitored by 1H NMR showing changes in the aromatic area. Labels: free H8, free nucleotide; adduct H8, nucleotide adduct; py, pyridine ligand complex 1; py*, pyridine signal adducts.; Table S2. Selected bond distances (Å) for complexes 1 and 2 optimized with the DFT method at the PBE1PBE:LANL08/6-31G** level using the PCM solvent (water) model. Table S3. Experimental and theoretical absorption spectra for 1 and 2 in water at the CAM-B3LYP/LANL08/6-31G** level. Table S4. Selected TD-DFT singlet-singlet transitions and corresponding electron difference density maps (EDDMs) for 1 and 2 in water at the CAM-B3LYP/LANL08/6-31G** level. Table S5. Experimental and theoretical absorption spectra for mono and bis 9-EtG adducts of complexes 1 and 2 in water at the CAM-B3LYP/LANL08/6-31G** level. Table S7. Selected bond distances (Å) for the mono and bis 9-EtG adducts of complexes 1 and 2 optimized with the DFT method at the PBE1PBE:LANL08/6-31G** level using the PCM solvent (water) model.

Author Contributions:

Conceptualization: L.C. and A.G.Q; Software, L. S.; Validation: L. C.; A. C., L.S., A.G.Q and A. I. M.; Formal Analysis and Data Curation: A.G. Q., A. I. M., L. S., A. C.; Investigation: L. C., T. P, A.G.Q., A C., L.S., and A. I. M.; Resources: A.G. Q, A. C., L. S.; Writing-Original Draft Preparation: A.G. Q.; Writing-Review & Editing, A.G. Q., A. I. M., L. S.,L. C. **;** Visualization: A.G. Q. and L.C.; Supervision, A.G.Q. and A. I. M.; Project Administration: A. G.Q.; Funding Acquisition: A.G. Q.

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Conflicts of Interest: The authors declare no conflict of interest.

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