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Synthesis, cytotoxicity, DNA interaction and cell cycle studies of *trans*-diiodophosphine Pt (II) complexes.

Angeles Medrano[a], Stephen M. Dennis[b], Amparo Alvarez-Valdés[a], Josefina Perles, [c] Tracey McGregor Mason*[b], Adoracion G. Quiroga*[a]

Platinum complexes, bearing aliphatic amines and phosphine ligands in *trans* configuration with iodide as leaving groups, are synthesized and characterized. The crystal structure of *trans*-PtI_2(isopropylamine)(PPh_3) is reported. The complex bearing isopropylamine is demonstrated to be the
10 best candidate as its cytotoxic activity is comparable to or better than cisplatin. A remarkably higher interaction of the complexes with DNA is reported as compared to the parent chlorido series. Cell cycle studies of the complexes in six human cell lines are performed and also compared with the previous series.

Introduction

15 The discovery of new metallodrugs that can overcome the unwanted effects produced by cisplatin (currently the most efficient metallodrug in the clinic) has been extended in the past decades to new platinum analogues with a design that goes against the *cis* configuration requirement established by
20 cisplatin.[1] The activity of *trans* platinum complexes of the form *trans*-[$PtCl_2$(L)(L')] were reported in the early nineties, demonstrating that synthetic variations in the coordinating ligands may lead to new active species[2]. Using aliphatic amines as spectator ligands, our research group was also able to
25 demonstrate that active *trans* Pt(II) complexes were achievable[3]. The combination of aliphatic amines in the *trans* geometry with other ligands has further proved that antitumor action is not only possible but also can be increased in some cases[4]: In fact, phosphine ligands with hydrophobic character have been shown
30 to increase cytotoxicity, possibly caused by the enhancement of cellular membrane transfer [5,6]. The molecular mechanism of *trans* complexes bearing aliphatic amines starts with (as with most platinum complexes) the hydrolysis reaction. Mechanistic studies in aqueous solution also revealed the importance of the type of
35 ligands bound to the platinum atom,[7] but recent studies have demonstrated that using an iodide as leaving group, particularly in the *cis* geometry[8], may have an important role in the reactivity versus some biological targets.

The enhanced cytotoxicity achieved with *trans* complexes
40 bearing phosphine ligands and aliphatic amines, along with our latest results using iodide as leaving groups, prompted us to synthesize and study the combination of these variations; the final compounds prepared are shown in Scheme 1.

The knowledge gained of tumor-associated cell cycle defects
45 have provided excellent information for the design of anticancer compounds [9, 10], but there are few studies of this type that can particularly help in the design of metallodrugs.
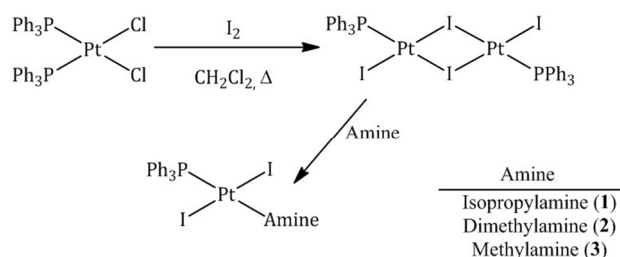
Scheme 1. General route of synthesis use to prepare the complexes.

It is known that normal cells treated with cisplatin produce cell
50 cycle arrest at the G2 phase of the cell cycle, which coincides with the induction of Nucleotide Excision Repair, the main pathway through which DNA-Pt lesions are removed[11, 12]. It has also been reported that interstrand adducts induced by some *trans* platinum compounds might provide G1 and S-phase arrest,
55 allowing only low levels of progression towards G2/M phase. DNA damage would then shunt the cell toward apoptosis and cell death from G2/M[13, 14]. However, our data have indicated that some *trans* complexes can induce a higher accumulation of cells into S phase which does not translate into differential cytotoxic
60 effects[5], providing evidence that further exploration is needed to determine if a possible differential mechanism of action indeed exists.

With these novel complexes, we are interested to know if the *trans* platinum complexes of Scheme 1 illicit the same cell cycle
65 arrest pattern as the previously reported complexes and to compare these results in terms of the cytotoxicity data previously reported [5, 13, 15].

Results and discussion

70 **Chemistry**

The synthesis of these three novel complexes was achieved in a two-step procedure. First, the reaction of I_2 with *cis*-$[PtCl_2(PPh_3)_2]$ afforded the dinuclear complex *trans*-$[Pt_2I_2(\mu$-$I)_2(PPh_3)_2$ in a good yield. Longer reaction times did not aid in
5 the formation of the dinuclear complex; on the contrary, the isomerisation of the dinuclear complex takes place, and a mixture of *cis* and *trans* platinum dinuclear complex was detected (at -3853.5 and -4775.3 in the ^{195}PtNMR spectra). This mixture can be used as the intermediate compound as well in the synthesis of
10 the final *trans* complexes.

Using *cis*-$[PtI_2(PPh_3)_2]$ as starting material allowed an earlier detection of the dinuclear complex, but it never resulted in a major species; as the reaction proceeded it produced a complicated mixture of polynuclear complexes in which the
15 desired dinuclear complex was not visible after 24h (monitoring the reaction by ^{31}P NMR). The second step is the cleavage of the dinuclear complex by the direct reaction of isopropylamine to produce complex **1**, or by the slow addition of an equivalent amount of NaOH to an aqueous solution of the hydrochloric salt
20 to produce complexes **2** and **3.**

The characterization of these complexes, including the intermediate complexes[16, 17], was carried out by the techniques of IR and ^{195}Pt, ^{31}P, ^{1}H and ^{13}C NMR. All of the microanalytical data was consistent with the empirical formulas, and the values of
25 the coupling constant and chemical shifts of the ^{31}P and ^{195}Pt NMR were in accordance to similar compounds reported in the literature.[5, 18, 19]..

Crystal structure of complex 1

The structure of complex 1 was determined by single crystal X-
30 ray diffraction. The molecular species *trans*-$PtI_2(ipa)(PPh_3)$ displays a Pt atom located in a distorted square planar environment. The metal center is coordinated to one phosphorus, one nitrogen and two iodine atoms in a *trans* disposition. Selected bond lengths (Å) and angles (º) are summarized in the caption of
35 Figure 1.

We have found that the structure of compound 1 is one of the few examples found in the literature, in fact there is only one complex with a similar Pt-N distance in the literature[20], although in this case the geometry of the coordination environment may be
40 influenced by the interaction of the Pt(II) with another nitrogen atom from the ligand. Pt-I distances, however, fall within the range of the reported values (Pt-I=2.567(1)-2.6763(6) Å).
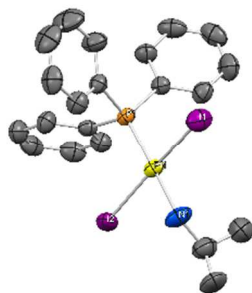

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Figure 1. Ellipsoid plot (50 % probability) of complex **1.** Hydrogen atoms have been omitted for clarity. Selected bond

lengths (Å): Pt1-I1 = 2.6255(5), Pt1-I2 = 2.5920(5), Pt1-N1 = 2.120(4), Pt1-P1 = 2.244(1); Selected bond angles (º): I1-Pt1-I2 =
50 172.33(2), I1-Pt1-P1 = 91.51(3), I1-Pt1-N1 = 87.8(2), I2-Pt1-P1 = 93.99(3), I2-Pt1-N1 = 86.5(2), N1-Pt1-P1 = 177.4(1).

Compared to the recently reported Pt^{IV} derivatives containing phosphines $PtCl_4(ipa)(PPh_3)$[21], Pt-N and Pt-P distances found in
55 compound **1** are very similar to the ones in the chlorido derivative

Table 1. Cytotoxic Potency of Pt(II) Complexes **1-3** on the Different Cell Lines Tested (IC50, μM)

Complex	A375	HCT116	HCT116-/-	T47D	SKOV 3
1	20	>100	27	>100	22
2	>100	>100	>100	>100	>100
3	>100	>100	>100	>100	>100
CDDP	51	5	47	60	73

(Pt-N=2.127(3) Å, and Pt-P=2.2396(9) Å), and longer than the ones found in analogous Pt(II) complexes included in the CSD database, in which Pt-N distances range from 2.051(4) to 2.079(7) Å, and Pt-P distances from 2.156(3) to 2.225(2) Å[17, 22,
60 23].

Cytotoxicity

The IC_{50} values of the three *trans*-complexes were determined
65 using a standard crystal violet staining assay (Table 1). Cisplatin was included as a positive control. The methylamine (**3**) and dimethylamine (**2**) complexes were determined to be universally inefficacious, with IC_{50} values beyond the range of concentrations applied. However, the isopropylamine (**1**)
70 compound resulted in values in the same range as cisplatin for 3 of the cell lines, A375, HCT116-/- and SKOV3. The 10-fold lower cisplatin value for HCT116 wt reflects the known increased sensitivity of the cell line to cisplatin compared to the p53 null version of the cell line, HCT116(-/-)[24]. However the HCT116 wt
75 cell line showed a similar sensitivity to the isopropylamine complex compared to cisplatin. Because the IC_{50} values for the *trans* complexes did not follow the same pattern in the HCT116 and HCT116(-/-) lines as cisplatin, it suggests that the IC_{50} value of 27 μM for HCT116-/- occurred through a different mechanism
80 of action and perhaps independent of p53 status. The result for complex **1** in the lines of HCT116(-/-), A375 and SKOV3 is lower than the value for cisplatin, suggesting a dissimilar mechanism that overcomes the known resistance of SKOV3 and HCT116(-/-) towards cisplatin. This is encouraging, as ERK1/2
85 phosphorylation occurs in A375 cells, offering protection from cisplatin-mediated apoptosis[11,25,26]. In all cases it is worth pointing out that the complex with the most promise as an antitumor drug is the complex with the most bulky aliphatic amine ligand. In addition, the larger size of the PPh_3 spectator
90 ligand has been previously discussed to be responsible for increased DNA distortion[5], additionally the complex with the most promising IC_{50} value is the complex with the most bulky isopropylamine ligand.

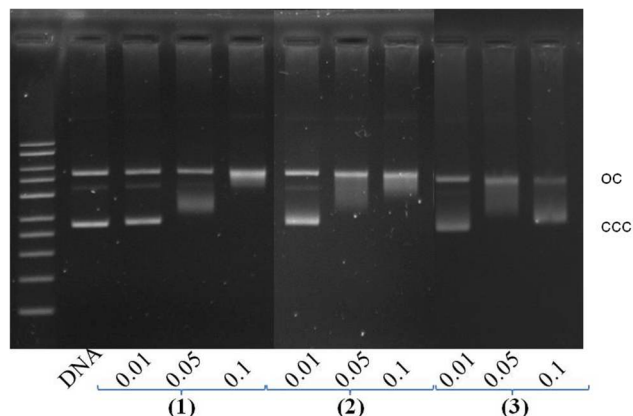## Binding studies using DNA models: 9-EtG and pBR322

Figure 2. Agarose gel electrophoresis of pBR322 plasmid treated with the *trans* Pt[II] complexes. Lane 1: Marker and lane 2: DNA of control plasmid.
5 Plasmid DNA incubated at r_i = 0.01, 0.05 and 0.1 with complexes **1** (lanes 3 to 5), complex **2** (lanes 6 to 8) and complex **3** (lanes 9 to 11). oc = open circular DNA form; ccc =covalently closed circular DNA.

We have studied the reactivity of the most active compound, **1**
with 9-ethyl guanine (9-EtG) to model the reactions with DNA
10 purine nucleobases. 9-EtG has been extensively used as a DNA
model for metallodrugs studies[27,28], including iodido complexes
bearing aliphatic amines[29], and has become a good model to
allow for the comparison with these new phosphine idodido
derivatives. In our studies, the reaction of complex **1** with 9-EtG
15 was monitored by ^{1}H NMR spectroscopy as described in the
Supplementary material and recorded at different times over 24
hours incubation at 37 °C (Figure SM-B1).
The solubility of the new iodido complexes is higher than the
chlorido derivatives[5], but in spite of this improved solubility, the
20 problem of the concentration hasn't been fully overcome, as can
be seen in the low resolution of the aromatic area of the spectra in
Figure SM-B1. When following the changes in the H8 peak of 9-
EtG, which is the most affected signal when the platination of the
base takes place at N7, we can detected a broad signal which
25 arises at ca. 8.00 ppm corresponding to the H8 resonance of any
possible new adduct. The spectra, as previously mentioned,
showed low resolution probably caused by speciation. Another
feature that supports speciation is the new signal at 6.4 ppm,
which possibly belongs to the H8 of an additional species. The
30 low concentration of the spectra does not allowed further
experiments to be recorded, but it has been reported previously
that H8 of 9-EtG can be extremely unshielded when is wedged
between bulky groups[27].
Most importantly, from the obtained spectra, we are sure that no
35 isopropylamine free ligand is shown at 0.9 ppm (methyl group
from free ipa). So the new complexes react versus DNA in a
classic manner, losing the iodido ligands. Moreover we can
clearly see that the reactivity of this complex is much slower than
the iodido reported previously[29, 30], which after only 2 hours most
40 of the 9EtG-free signal was already reacted. Complex **1**, on the
other hand, does show 9EtGfree (H8) signal as a major species,
after 24 hours.
The DNA unwinding assay is a standard assay used to determine
DNA interactions with platinum compounds. It is known that the

45 binding of cisplatin introduces severe distortion in the DNA helix
in the form of bending and unwinding of negative supercoils.
In this work, supercoiled pBR322 DNA was incubated with
increasing amounts of complexes **1-3** and the amount of
unwinding at those complex: DNA ratios (r_i) was compared. All
50 three complexes caused negatively supercoiled plasmid DNA to
migrate more slowly on an agarose gel, confirming that negative
supercoils are removed as the complexes bind. Complex **1**
appears to completely relax the supercoils at an r_i of 0.1, while
the other complexes don't result in complete unwinding at these
55 concentrations. This coincides with the toxicity data that follows
showing the lack of efficaciousness of complexes **2** and **3**.
However we must be careful making these kinds of comparisons
because the active chloride parent complexes showed less
distortion under the same conditions, yet demonstrated high
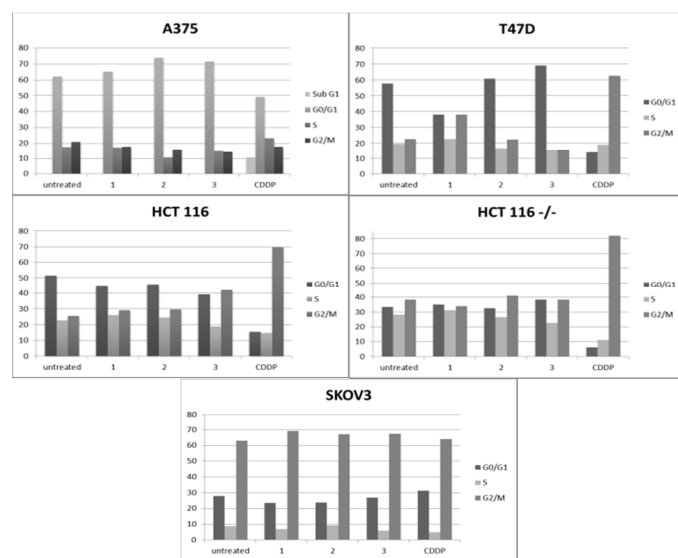60 cytotoxicity.

Cell cycle studies

Figure 3. Percent of cells at each cell cycle phase after treatment with
65 *trans*-platinum (II) complexes **1-3** and cisplatin

Once the IC_{50} values were established for the various cell lines
studied, a fixed concentration of complex was used to treat
cultures in preparation for cell cycle analysis. They were treated
exactly as cells used for IC_{50} establishment, but were fixed, PI
70 (propidium iodide) stained and analysed for DNA/cell content. In
the SKOV3 cell line, the cell cycle distribution was similar for all
complexes tested and cisplatin (Figure 3), while the *trans*
complexes showed a lower overall G0/G1 accumulation. A
similar result in SKOV3 was seen with other *trans*-dichloro Pt
75 (II) complexes with phosphine ligands prepared by this group[5].
The HCT116 wt cell line, showed a similar result. In comparison,
however, the HCT116-/- cell line treated with cisplatin showed its
typical G2/M accumulation[13,31], while none of the *trans*
complexes showed comparable accumulation. Also notable is the
80 increase in G0/G1 accumulation in A375 cells treated with *trans*
complexes relative to cisplatin. Also interestingly, this was the
only cell line with which a sub-G1 peak was noted with cisplatin
treatment.

This presence of hypodiploid DNA in the histogram is suggestive of apoptosis, but should be interpreted with caution[32]. This particular assay is incapable of definitively reporting apoptosis, however we can interpret the lack of a clear sub-G1 peak in the histogram of any of the *trans*-treated cells as indication of a clear difference in the mechanism of action from cisplatin in A375 melanoma cells.

It has been reported that longer treatment eventually results in cell cycle arrest as the cellular distribution profile changes over time, depending on repair deficiencies that may or may not exist in a particular cell line[31]. However, in our previous experience, in we have seldom been able to find information or values that could help us to detect such differences with longer treatments[15]. In this regard we are convinced that additional work can and should be done, and our plan is to use these compounds or some with similar and small structural variations to obtain more valuable information into possible differences in the mechanism of action of *trans*-PPh complexes.

Conclusion

Complexes with phosphine and aliphatic amines were synthesized and studied. The results showed similar cytotoxicity to cisplatin for complex **1**, and such studies can be correlated to the unwinding pattern shown versus DNA. The structural studies of complex **1** represent a good candidate with which to evaluate the differences with similar complexes made previously. The biological studies indicates that complex **1** showed the most promising results versus cancer cell lines tested and it is the best candidate for further study into the biological action of these complexes.

Experimental Methods

Chemistry

The starting material were prepared following a procedure previously described in our laboratory for *cis*-[PtCl$_2$(PPh$_3$)$_2$] and *trans*-[Pt$_2$I$_2$(μ-I)$_2$(PPh$_3$)$_2$] was synthesized as following:

A solution of I$_2$ (208.1mg, 0.82 mmol) in 6 mL of CH$_2$Cl$_2$ was added in small portions (1.5 mL) to a solution of complex cis-[PtCl$_2$(PPh$_3$)$_2$] (500 mg, 0.63mmol) in CH$_2$Cl$_2$ and heated at 50 ºC. After 6 days, the reaction was cooled down at room temperature and the resulting red solid was filtrated, washed with cool CH$_2$Cl$_2$ and vacuum dried. Red Solid. Yield: 63% Elemental analysis: calculated C$_{36}$H$_{30}$I$_4$P$_2$Pt$_2$•0.7CH$_2$Cl$_2$, C: 29.75 %, H: 2.14 %; found: C: 29.61 %, H: 2.18 %. δH(300 MHz; DMSO-d6) 7.67 (m, 6H, H$_{ortho}$), 7.52 (m, 9H, H$_{meta-para}$). δC(75.47 MHz; DMSO-d6) 134.7(C$_{ortho}$), 128.6(C$_{meta}$), 131.6(C$_{para}$). δP(300 MHz; DMSO-d6) 18.14 (JP-Pt=3561.24Hz). δPt(64.53 MHz; ; DMSO-d6; Na$_2$PtCl$_6$):-4319.22. (JPt-P=3805.18Hz). MS (ESI): 1444.72 amu [M+Na]+

Method a: trans-[PtI$_2$(amine)(PPh$_3$)] where amine: methylamine and dimethylamnie

A solution of 2 equivalents of NaOH in water was added to a mixture of *trans*-[Pt$_2$I$_2$(μ-I)$_2$(PPh$_3$)$_2$] (80 mg, 0.056 mmol) and the desired amine: methylamine hydrochloride (7.5 mg, 112 mmol), dimethylamine hydrochloride (9.12 mg, 0.112 mmol) in chloroform and stirred in darkness at room temperature for 2 days. The resulting orange solution was dried to a crude solid which was purified by redissolving in diethyl ether. The final solution was left at room temperature to slowly evaporate and the final product was isolated as an orange solid.

trans-[PtI$_2$(dma)(PPh$_3$)]: Orange solid. Yield: 71%. Elemental analysis: calculated C$_{20}$H$_{22}$NI$_2$PPt•(CH$_3$)$_2$CO: C: 33.92 %, H: 3.47%, N: 1.72%. CHN; found: C: 34.53 %, H: 3.34 %, N: 1.63 %.δ$_H$(300 MHz; acetone-d6) 7.72 (m, 6H, H$_{ortho}$), 7.42 (m, 9H, H$_{meta-para}$), 4.68 (bs, 1H, NH), 2.80 (d, 6H, CH$_3$-dma). δ$_C$(75.47 MHz; acetone-d6) 135.1(C$_{ortho}$), 127.5(C$_{meta}$),130.5(C$_{para}$), 42.2(CH$_3$-ma). δ$_P$(300 MHz; acetone-d6) -1.2 (JP-Pt=3262.22 Hz). δ$_{Pt}$(64.53 MHz; ; acetone-d6; Na$_2$PtCl$_6$) -4933.6 (JPt-P=3262.37 Hz). MS (ESI): 755.9 amu [M]$^+$

trans-[PtI$_2$(ma)(PPh$_3$)]: Orange solid. Yield: 60%. Elemental analysis: calculated C$_{19}$H$_{20}$NI$_2$PPt·0.6CHCl$_3$·0.6C$_4$H$_{10}$O: C: 30.64 % , H: 3.11 %, N: 1.62 %. CHN; found: C: 30.97 %, H: 2.72 %, N: 1.62 %. δ$_H$(300 MHz; CDCl$_3$) 7.71 (m, 6H, H$_{orto}$), 7.40 (m, 9H, H$_{meta-para}$), 2.72 (d, 3H, CH$_3$-ma, JH-H=7Hz). δ$_C$(75.47 MHz; CDCl$_3$) 135.5(C$_{orto}$), 128.6(C$_{meta}$), 131.4(C$_{para}$), 24.5(CH$_3$-ma). δ$_P$(300 MHz; CDCl$_3$) 0.9 (JP-Pt=3412.32 Hz). δ$_{Pt}$(64.53 MHz; ; CDCl$_3$; Na$_2$PtCl$_6$)-5045.5 (JPt-P= 3411.31 Hz).

Method b: trans-[PtI$_2$(amine)(PPh$_3$)] where amine: isopropylamine

A mixture of *trans*-[Pt$_2$I$_2$(μ-I)$_2$(PPh$_3$)$_2$] (80 mg, 0.056mmol) and isopropylamine (130μl, 1.5mmol) in chloroform was stirred in darkness at room temperature for 2 days. The resulting orange solution was dried to a crude solid which was purified by redissolving in diethyl ether. The final solution was left at room temperature to slow evaporation to isolate the final product as an orange solid.

trans-[PtI$_2$(ipa)(PPh$_3$)]: Orange solid. Yield:71%. Elemental analysis calculated for C$_{21}$H$_{24}$I$_2$NPPt•1.3C$_4$H$_{10}$O: C: 36.31%, H: 4.03%, N: 1.62% CHN found: C: 36.99%, H: 3.52%, N: 1.85%. δ$_H$(300 MHz; CDCl$_3$) 7.73 (m, 6H, H$_{ortho}$), 7.71 (m, 9H, H$_{meta-para}$), 3.84 (sp, 1H, CH-ipa), 3.51 (bs, 2H, NH$_2$), 1.38 (d, 6H, CH$_3$-ipa). δ$_C$(75.47 MHz; CDCl$_3$) 135.1(C$_{ortho}$), 132.8(C$_{ipso}$) 130.6(C$_{para}$), 127.5(C$_{meta}$), 40.0 (CH-ipa), 25.4(CH$_3$-ipa). δ$_P$(300 MHz;CDCl$_3$) 0.9 (JP-Pt=3407.28Hz). δ$_{Pt}$(64.53 MHz; ; CDCl$_3$; Na$_2$PtCl$_6$) -5004.9 (JPt-P=3407.28Hz). MS (MALDI-TOF): 771 amu [M-H]$^+$.

Biology

pBR322 binding studies and conditions.

The DNA binding studies were performed in a total volume of 20 μl. The DNA aliquots containing 8 μL of DNA-pBR322 (10 ng/mL stock) in 10 mM Tris-HCl (pH 7.6) and 1 mM EDTA were incubated with the platinum compounds at several r$_i$ values (0.001 to 0.5) using the corresponding amount of platinum from either a 5 μM or 50 μM stock solution. The samples were incubated at 37 ºC for 24 h, after which time 2 μL of a loading dye containing 50 % glycerol, 0.25 % bromophenol blue and 0.25 % xylene cyanol was added. The entire sample (20 μL) was loaded in the wells of a 0.8 % agarose gel. Electrophoresis was carried out for a period of 2.5 h at approximately 50 V. After electrophoresis, the gel was immersed in 800 mL of Millipore water containing 64 μL of a 10 mg/mL stock solution of ethidium bromide for 30 minutes to stain the DNA.

Cytotoxicity.

The following cell lines were used in this work: A375, human malignant melanoma and SKOV3 human ovarian adenocarcinoma (both from ATCC, Manassas, VA); T47D, breast duct epithelial carcinoma, HCT116, human colorectal carcinoma (both a gift from Paul Miller, Johns Hopkins University); and HCT116(-/-), (a gift from Margaret Wear, Uniformed Services University of the Health Sciences, Bethesda, MD) All cell lines were maintained in monolayers in humidified air with 5% CO_2 at 37 ºC in Dulbecco's Modified Eagle Media-α (DMEM-α) with the exception of HCT116(-/-) and T47D, which were maintained in RPMI media. All media contained 10% fetal bovine serum, and 1x streptomycin/ampicillin. To determine cytoxicity, cells were seeded at a density of 4000 cells per well in a 96-well plate. The cells were allowed to attach and recover for 48 hours and then were treated in triplicate with 0.2 mL of increasing concentrations of drug diluted in media. Compounds **1-3** were diluted from a 10 mM stock of compound freshly dissolved in DMSO; while cisplatin stock was prepared to 5 mM in PBS (phosphate buffered saline). The compound remained on the cells for 2 hours, after which the media containing drug was aspirated and replaced with fresh media. After another 24 hours, the cells were washed twice with PBS, then fixed in 10% glutaraldehyde for 15 minutes. The cells were again washed twice with PBS, then stained with 0.25 % crystal violet at 37 ºC for 15 minutes. They were then extensively washed with PBS and the remaining dye was solubilized using 0.200 mL 33 % acetic acid. The absorbance of purple color was quantified at 595 nm using a Tecan Sunrise Absorbance plate reader.

Cell Cycle Analysis

To conduct cell cycle analysis, all cell lines were seeded at a density of 75,000 cells per well in a 12-well plate. The cells were allowed to attach and recover for 24 hours and then were treated with 1.0 mL of 50 or 10 μM drug diluted in media. Compounds **1-3** were diluted from 100 mM stock of compound freshly dissolved in DMSO; while cisplatin was administered at 50 μM concentration and was diluted from a stock that was prepared to 5 mM in PBS. The compound remained on the cells for 48 hours, after which the media containing drug was aspirated and replaced with fresh media. After treatment, the cells were washed and trypsinized to detach them from the plate. The cells were suspended in 1 ml fresh media and transferred to a polystyrene culture tube. The cells were pelleted at 450 x g for 5 minutes in a swing bucket centrifuge and washed twice with 1 mL of PBS. After pelleting, the washed cells they were resuspended in 0.25 mL PBS to which 2.25 mL ice cold 70 % ethanol was added slowly with vortexing. This suspension remained at -20 ºC until cell sorting. Just prior to cell cycle analysis, the cells were pelleted at 450 x g for 5 minutes, washed with 1 mL PBS and pelleted again. To the cell pellet, 0.2 mL of Guava Cell Cycle Reagent (Millipore) was added, and the cells were allowed to incubate at room temperature for 30 minutes in the dark. The samples were analysed for cell cycle distribution using a Guava Easy Cyte Flow Cytometer and Guava CytoSoft Analysis software.

Notes and references

a Department of Inorganic Chemistry. Universidad Autónoma de Madrid C/Francisco Tomas y Valiente 7.M7. 28049 Madrid.Spain. Fax: (+) 34914974850 Tel:(+)34914974050. e-mail: adoracion.gomez@uam.es
b Department of Chemistry. Stevenson University. 1525 Greenspring Valley Rd, Stevenson, MD 21153, U.S.A.e-mail: tmason@stevenson.edu
c SIDI. Universidad Autónoma de Madrid.28049 Madrid

† Supporting information for this article is available on the WWW under http://www.chemmedchem.org or from the author. Crystallografic data and CIF, and diffraction data also has been registered in the CCDC with the Nº 982662/

1. N. J. Wheate, S. Walker, G. E. Craig and R. Oun, *Dalton Trans*, 2010, **39**, 8113-8127.
2. M. S. Aris and F. P. Nicholas, *Eur J Inorg Chem*, 2009, 1293-1302.
3. A. G. Quiroga, *J Inorg Biochem*, 2012, **114**, 106-112.
4. U. Kalinowska-Lis, J. Ochocki and K. Matlawska-Wasowska, *Coor Chem Rev*, 2008, **252**, 1328-1345.
5. F. J. Ramos-Lima, A. G. Quiroga, B. Garcia-Serrelde, F. Blanco, A. Carnero and C. Navarro-Ranninger, *J Med Chem*, 2007, **50**, 2194-2199.
6. F. J. Ramos-Lima, A. G. Quiroga, J. M. Perez, M. Font-Bardia, X. Solans and C. Navarro-Ranninger, *Eur. J. Inorg. Chem*, 2003, 1591-1598.
7. L. Cubo, A. G. Quiroga, J. Zhang, D. S. Thomas, A. Carnero, C. Navarro-Ranninger and S. J. Berners-Price, *Dalton Trans*, 2009, 3457-3466.
8. L. Messori, L. Cubo, C. Gabbiani, A. Alvarez-Valdes, E. Michelucci, G. Pieraccini, C. Rios-Luci, L. G. Leon, J. M. Padron, C. Navarro-Ranninger, A. Casini and A. G. Quiroga, *Inorg Chem*, 2012, **51**, 1717-1726.
9. A. Carnero, *Brit J Cancer*, 2002, **87**, 129-133.
10. M. Malumbres and M. Barbacid, *Nat Rev Cancer*, 2009, **9**, 153-166.
11. Z. H. Siddik, *Oncogene*, 2003, **22**, 7265-7279.
12. C. M. Sorenson, M. A. Barry and A. Eastman, *J Natl Cancer I*, 1990, **82**, 749-755.
13. A. Boccarelli, D. Giordano, G. Natile and M. Coluccia, *Biochem Pharmacol*, 2006, **72**, 280-292.
14. T. Servidei, C. Ferlini, A. Riccardi, D. Meco, G. Scambia, G. Segni, C. Manzotti and R. Riccardi, *Eur J Cancer*, 2001, **37**, 930-938.
15. F. J. Ramos-Lima, V. Moneo, A. G. Quiroga, A. Carnero and C. Navarro-Ranninger, *Eur J Med Chem*, 2010, **45**, 134-141.
16. A. N. Biswas, V. Bagchi, P. Das and P. Bandyopadhyay, *Acta Crystallogr E*, 2007, **63**, M2836-U1913.
17. E. S. Tabei, H. Samouei and M. Rashidi, *Dalton Trans*, 2011, **40**, 11385-11388.
18. W. Henderson and S. R. Alley, *Inorg Chim Acta*, 2001, **322**, 106-112.
19. G. Guerrero, P. H. Mutin, F. Dahan and A. Vioux, *J Organomet Chem*, 2002, **649**, 113-120.

20. F. P. Fanizzi, L. Maresca, G. Natile, M. Lanfranchi, A. Tiripicchio and G. Pacchioni, *JCS Chem Comm*, 1992, 333-335.

21. M. A. Medrano, A. Alvarez-Valdes, J. Perles, J. Lloret-Fillol, S. Munoz-Galvan, A. Carnero, C. Navarro-Ranninger and A. G. Quiroga, *Chem Commun*, 2013, **49**, 4806-4808.

22. M. H. Thibault, B. E. G. Lucier, R. W. Schurko and F. G. Fontaine, *Dalton Trans*, 2009, 7701-7716.

23. H. A. Ankersmit, B. H. Loken, H. Kooijman, A. L. Spek, K. Vrieze and G. vanKoten, *Inorg Chim Acta*, 1996, **252**, 141-155.

24. S. Y. Sharp, C. F. O'Neill, P. Rogers, F. E. Boxall and L. R. Kelland, *Eur J Cancer*, 2002, **38**, 2309-2315.

25. A. Mirmohammadsadegh, R. Mota, A. Gustrau, M. Hassan, S. Nambiar, A. Marini, H. Bojar, A. Tannapfel and U. R. Hengge, *J Invest Dermatol*, 2007, **127**, 2207-2215.

26. D. Wang and S. J. Lippard, *Nat Rev Drug Discov*, 2005, **4**, 307-320.

27. A. C. G. Hotze, E. P. L. van der Geer, S. E. Caspers, H. Kooijman, A. L. Spek, J. G. Haasnoot and J. Reedijk, *Inorg Chem*, 2004, **43**, 4935-4943.

28. Aldrik H. Velders, Adoracion G. Quiroga, Jaap G. Haasnoot and J. Reedijk, *Eur. J. Inorg. Chem.*, 2003, **2003**, 713-719.

29. L. Messori, L. Cubo, C. Gabbiani, A. Alvarez-Valdes, E. Michelucci, G. Pieraccini, C. Ríos-Luci, L. G. León, J. M. Padrón, C. Navarro-Ranninger, A. Casini and A. G. Quiroga, *Inorg Chem*, 2012, **51**, 1717-1726.

30. T. Parro, M. A. Medrano, L. Cubo, S. Muñoz-Galván, A. Carnero, C. Navarro-Ranninger and A. G. Quiroga, *J. Inorg. Biochem.*, 2013.

31. V. Beljanski, L. G. Marzilli and P. W. Doetsch, *Mol Pharmacol*, 2004, **65**, 1496-1506.

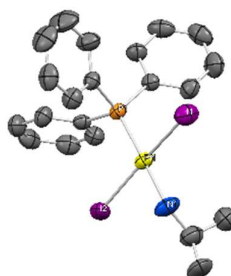32. M. G. Ormengod, *Leukemia* 1998, **12**, 1013-1025.

Entry for the Table of Contents

Diiodido phosphine Pt complexes

Angeles Medrano, Stephen M. Dennis, Amparo Alvarez-Valdés, Josefina Perles, Tracey McGregor Mason*, Adoracion G. Quiroga*

Platinum complexes bearing aliphatic amines and phosphine ligands in *trans* configuration with iodide as leaving groups

Diiodido phosphine Pt complexes were synthesized and their biological activity studied. The complex with isopropylamine was the best candidate.