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## Journal of Inorganic Biochemistry

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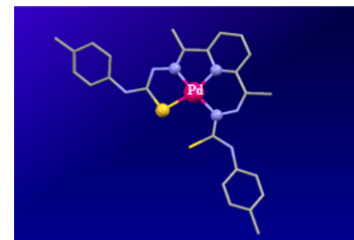
## Graphical abstract

**Palladium(II) and platinum(II) bis(thiosemicarbazone) complexes of the 2,6-diacetylpyridine series with high cytotoxic activity in cisplatin resistant A2780cisR tumor cells and reduced toxicity**

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Palladium and platinum bis(thiosemicarbazone) complexes of the 2,6-diacetylpyridine series have been synthesized and characterized. This study has identified the [PdL<sup>1</sup>], [PdL<sup>2</sup>] and [PtL<sup>2</sup>] complexes as having important antitumor activity since they are capable of circumventing cisplatin resistance in A2780cisR cells and reducing nephrotoxicity.

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## Highlights

**Palladium(II) and platinum(II) bis(thiosemicarbazone) complexes of the 2,6-diacetylpyridine series with high cytotoxic activity in cisplatin resistant A2780cisR tumor cells and reduced toxicity**

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- 2,6-Diacetylpyridine bis(<sup>4</sup>N-tolylthiosemicarbazone) palladium(II) and platinum(II) complexes
- These complexes exhibit high cytotoxic activity in MCF-7 tumor cells and moreover are able to circumvent cisplatin resistance in A2780cisR cells.
- All complexes are in vitro non-toxic against normal LLC-PK1 renal cells at the concentration range tested.



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## Regular paper

# Palladium(II) and platinum(II) bis(thiosemicarbazone) complexes of the 2,6-diacetylpyridine series with high cytotoxic activity in cisplatin resistant A2780cisR tumor cells and reduced toxicity

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## ABSTRACT

Preparation and characterization of four novel 2,6-diacetylpyridine bis(4*N*-tolyl-thiosemicarbazonato) palladium(II) and platinum(II) complexes, [PdL<sup>1-2</sup>] and [PtL<sup>1-2</sup>], are described. All compounds have been characterized by elemental analysis and by IR and NMR spectroscopy, and the crystal and molecular structures of complexes [PdL<sup>2</sup>] and [PtL<sup>2</sup>] have been determined by a single crystal X-ray diffraction. The ligands act as dianionic tetradentate donors coordinating to the metal center in a square planar geometry through the N<sub>pyridinic</sub> atom and the N<sub>iminic</sub> and the S atoms from one thiosemicarbazone arm, the fourth coordination position is occupied by the N<sub>hydrazinic</sub> of the other arm. The new compounds synthesized have been evaluated for antiproliferative activity in vitro against NCI-H460, HepG2, MCF-7, A2780 and A2780cisR human cancer cell lines. The cytotoxicity data suggest that [PdL<sup>1</sup>], [PdL<sup>2</sup>] and [PtL<sup>2</sup>] may be endowed with important antitumor properties since they are capable of not only circumventing cisplatin resistance in A2780cisR cells but also exhibiting high antiproliferative activity in breast cancer MCF-7 cells. Subsequent toxicity study, in LLC- $\Phi$ K1 cells, has also been carried out and shows that none of these compounds are in vitro toxic in the tested concentration range.

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## 1. Introduction

Platinum metallo-drugs are among the most effective agents for the treatment of cancer however its clinical utility is restricted due to the frequent development of drug resistance, the limited spectrum of tumors against which these drugs are active and severe normal tissue toxicity being the nephrotoxicity an important side effect which interferes with their therapeutic efficiency [1–5].

These disadvantages have driven the development of improved platinum-based anticancer drugs different from the traditional cisplatin structure and which could probably have different DNA-binding modes as well as exhibit different biological profiles [6–10].

In this regard and taking into account that one of the mechanisms inducing the nephrotoxicity is due to the inactivation of some enzymes because of the reaction between platinum ions and sulfur containing proteins, an active area of research focuses on the synthesis of chelate platinum(II) complexes bearing nitrogen and sulfur mixed donor atoms, which should prevent the adverse reaction described [11,12].

Thiosemicarbazones (R<sub>1</sub>R<sub>2</sub>C=N–NH–C(S)–NR<sub>3</sub>R<sub>4</sub>) are an important and versatile type of ligands due to the potential donor

atoms that they possess, among which sulfur is of paramount importance in the metal-ligand linkage. Particularly, compounds in which the thiosemicarbazone side-chain is attached in  $\alpha$  position to an N-heterocyclic ring, namely  $\alpha$ -N-heterocyclic thiosemicarbazones (N-TSCs), are strong metal chelating agents and moreover some of them have showed antineoplastic activity by themselves. It has been demonstrated that the biochemical mechanism of action involves, among others, ribonucleotide reductase (RR) inhibition and non-covalent DNA binding [13–19].

On the other hand, the pyridine ring itself is a part of many natural and synthetically prepared pharmaceuticals. In addition, the pyridine moiety plays a significant role in many biological processes like nicotinamide adenine dinucleotide phosphate NADP or vitamin B6 [20,21].

Keeping in view the above observations and as part of our systematic investigation on the coordination chemistry of thiosemicarbazone derivatives we planned to construct a series of  $\alpha$ -N-heterocyclic bis(thiosemicarbazone) platinum(II) and palladium(II) complexes bearing 2,6-diacetylpyridine as heterocyclic ring specifically using 2,6-diacetylpyridine bis(4*N*-ortho-tolylthiosemicarbazonato), H<sub>2</sub>L<sup>1</sup>, and 2,6-diacetylpyridine bis(4*N*-para-tolylthiosemicarbazonato), H<sub>2</sub>L<sup>2</sup>, ligands (Scheme 1).

Thus, this work is aimed to describe the synthesis and chemical characterization of four new 2,6-diacetylpyridine bis(4*N*-tolylthiosemicarbazonato) palladium(II) and platinum(II) complexes. The cytotoxic

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<sup>1</sup> The contributions made by this co-author were performed as part of her Research Project at Universidad Autónoma de Madrid (Erasmus Student from Universidade do Porto).



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Table 1

Crystal data and structure refinement for  $\text{H}_2\text{L}^1$ ,  $[\text{PdL}^2]$  and  $[\text{PtL}^2]$  compounds.

	$\text{H}_2\text{L}^1 \cdot \text{DMSO} \cdot 0.5\text{H}_2\text{O}$	$[\text{PdL}^2] \cdot 0.5\text{DMSO}$	$[\text{PtL}^2] \cdot \text{DMSO}$
Molecular formula	$\text{C}_{27}\text{H}_{34}\text{N}_7\text{O}_{1.5}\text{S}_3$	$\text{C}_{26}\text{H}_{28}\text{N}_7\text{O}_{0.5}\text{PdS}_{2.5}$	$\text{C}_{27}\text{H}_{31}\text{N}_7\text{OPTS}_3$
Formula weight	576.79	633.10	760.86
Temperature (K)	100 (2)	100 (2)	100 (2)
Wavelength (Å)	0.71073	0.71073	0.71073
Crystal system	Monoclinic	Monoclinic	Monoclinic
Space group	$\text{C2}/\text{c}$	$\text{P2}_1/\text{c}$	$\text{P2}_1/\text{c}$
a (Å)	35.225 (3)	14.0884 (13)	13.9098 (12)
b (Å)	7.4795 (7)	7.6059 (6)	7.4227 (7)
c (Å)	21.9895 (16)	27.227 (2)	27.182 (2)
$\alpha^\circ$	90	90	90
$\beta^\circ$	98.215 (3)	91.119 (5)	90.439 (4)
$\gamma^\circ$	90	90	90
Volume (Å <sup>3</sup> )	5734.0 (8)	2917.0 (4)	2806.4 (4)
Z	8	4	4
Density (calculated) (g/cm <sup>3</sup> )	1.336	1.442	1.801
Absorption coefficient (mm <sup>−1</sup> )	0.295	0.845	5.259
F(000)	2440	1292	1504
Crystal size (mm <sup>3</sup> )	$0.40 \times 0.15 \times 0.10$	$0.24 \times 0.04 \times 0.04$	$0.23 \times 0.02 \times 0.02$
Index ranges	$-47 \leq h \leq 47$ , $-10 \leq k \leq 10$ , $-29 \leq l \leq 29$	$-16 \leq h \leq 16$ , $-9 \leq k \leq 9$ , $-32 \leq l \leq 31$	$-16 \leq h \leq 16$ , $-8 \leq k \leq 8$ , $-31 \leq l \leq 29$
Reflections collected	35398	39446	9556
Independent reflections	7429 [R(int) = 0.0629]	5330 [R(int) = 0.1624]	4195 [R(int) = 0.062]
Data/restraints/parameters	7429/0/358	5330/1/352	4195/0/328
Goodness-of-fit on F <sup>2</sup>	1.002	0.999	1.011
Final R indices [I > 2σ(I)]	R1 = 0.0571, wR2 = 0.1598	R1 = 0.09, wR2 = 0.2526	R1 = 0.085, wR2 = 0.247
R indices (all data)	R1 = 0.1016, wR2 = 0.1970	R1 = 0.2039, wR2 = 0.3237	R1 = 0.1403, wR2 = 0.2891
Largest diff. peak and hole, e. Å <sup>−3</sup>	1.596 and −0.463	1.399 and −1.015	2.156 and −2.641

CCDC 918229, 918230 and 923814 contain the supplementary crystallographic data for compounds  $\text{H}_2\text{L}^1$ ,  $[\text{PtL}^2]$  and  $[\text{PdL}^2]$  respectively. These data can be obtained free of charge at [www.ccdc.cam.ac.uk/conts/retrieving.html](http://www.ccdc.cam.ac.uk/conts/retrieving.html) [or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44-1223/336-0333; e-mail: [deposit@ccdc.cam.ac.uk](mailto:deposit@ccdc.cam.ac.uk)].

### 2.5. In vitro antiproliferative activity

The human cancer cells A2780, A2780cisR and NCI-H460 were grown in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 2 mM L-glutamine in an atmosphere of 5% CO<sub>2</sub> at 37 °C. The human cancer cells MCF-7 were grown in EMEM (minimum essential medium, Eagle) with 2 mM L-glutamine and Earle's BSS (adjusted with 1.5 g/L NaHCO<sub>3</sub>, 0.1 mM nonessential amino acids and 1 mM sodium pyruvate) and supplemented with 10% FBS and 0.01 mg/mL bovine insulin in an atmosphere of 5% CO<sub>2</sub> at 37 °C. The human cancer cells Hep-G2 were grown in EMEM medium supplemented with 10% FBS in an atmosphere of 5% CO<sub>2</sub> at 37 °C.

Cell proliferation was evaluated by the sulforhodamine B assay. Cells were plated in 96-well sterile plates at a density of  $1.5 \cdot 10^4$  (for NCI-H460),  $1 \cdot 10^4$  (for MCF-7 and Hep-G2) or  $4 \cdot 10^3$  (for A2780 and A2780cisR) cells per well with 100 μL of medium and were then incubated for 24 h. After attachment to the culture surface the cells were incubated with various concentrations of the compounds tested freshly dissolved in DMSO (1 mg/mL) and diluted in the culture medium (DMSO final concentration 1%) for 48 h (for NCI-H460 and Hep-G2) or 96 h (for A2780, A2780cisR and MCF-7). The cells were fixed by adding 50 μL of 30% trichloroacetic acid (TCA) per well.

The plates were incubated at 4 °C for 1 h and then washed five times with distilled water. The cellular material fixed with TCA was stained with 0.4% sulforhodamine B dissolved in 1% acetic acid for 10 min. Unbound dye was removed by rinsing with 0.1% acetic acid. The protein-bound dye was extracted with 10 mM unbuffered Tris base for determination of optical density (at 515 nm) in a Tecan Ultra Evolution spectrophotometer.

The normal cells (LLC-PK1) were grown in 199 medium supplemented with 3% FBS and 1.5 g/L of sodium bicarbonate in an atmosphere of 5% CO<sub>2</sub> at 37 °C. Cell proliferation was evaluated by the sulforhodamine B assay. Cells were plated in 96-well sterile plates at a density of  $1 \cdot 10^4$  cells per well with 100 μL of medium and were then incubated for 24 h. After attachment to the culture surface the cells were incubated with various concentrations of the compounds tested freshly dissolved in DMSO (1 mg/mL) and diluted in the culture medium (DMSO final concentration 1%) for 48 h at 37 °C. The cells were fixed by adding 50 μL of 30% TCA per well. The plates were treated as described above for cancer cells.

The effects of compounds were expressed as corrected percentage inhibition values according to the following equation:

$$\% \text{ inhibition} = [1 - (T/C)] \times 100$$

where T is the mean absorbance of the treated cells and C the mean absorbance in the controls.

The inhibitory potential of compounds was measured by calculating concentration–percentage inhibition curves, these curves were adjusted to the following equation:

$$E = E_{\text{max}} / [1 + (\text{IC}_{50}/C)^n]$$

where E is the percentage inhibition observed,  $E_{\text{max}}$  is the maximal effects,  $\text{IC}_{50}$  is the concentration that inhibits 50% of maximal growth, C is the concentration of compounds tested and n is the slope of the semi-logarithmic dose–response sigmoid curves. This non-linear fitting was performed using GraphPad Prism software [25].

For comparison purposes, the antiproliferative activity of cisplatin was evaluated under the same experimental conditions. All compounds were tested in two independent studies with triplicate points. These experiments were carried out at the Unidad de Evaluación de Actividades Farmacológicas de Compuestos Químicos (USEF), Universidad de Santiago de Compostela.



### 3. Results and discussion

#### 3.1. Synthesis and spectroscopic characterization

Reaction of 2,6-diacetylpyridine bis(<sup>4</sup>N-tolylthiosemicarbazone) ligands with equimolar amount of  $MCl_2(PPh_3)_2$ , where  $M = Pd(II)$  or  $Pt(II)$ , led to the isolation of the neutral mononuclear complexes  $[PdL^{1-2}]$  and  $[PtL^{1-2}]$  in which the corresponding bis(thiosemicarbazone) behaves as dianionic ligand with deprotonation of hydrazinic ( $^2NH$ ) protons and [NNNS] donor set.

The new palladium(II) and platinum(II) complexes obtained are stable to air and moisture and were characterized by elemental analysis and  $FAB^+$  spectrometry and spectroscopic studies (selected IR and UV–VIS bands are listed in the Experimental section).

The infrared spectral bands most useful for determining the mode of coordination of the ligands are the  $\nu(C=N)$  iminic and  $\nu(C=S)$  thioamide IV vibrations. These bands are shifted to lower wavenumbers in the spectra of the complexes suggesting coordination of the imine nitrogen and sulfur atoms. The pyridine nitrogen also appears to be involved in coordination based on the in-plane and out-of-plane ring deformation bands which show shift to upper wavenumbers and fairly low intensity [26].

The electronic absorption spectra of  $[PdL^2]$  and  $[PtL^2]$  complexes exhibit three intense bands in the region 250–485 nm, the two most energetic can be ascribed to ligand-centered  $n \rightarrow \pi^*$  and  $\pi \rightarrow \pi^*$  transitions while the third is assigned to a combination of ligand to metal (LMCT) and metal to ligand charge transfer (MLCT) transitions. However for  $[PdL^1]$  and  $[PtL^1]$  complexes no transitions that could be assigned to an  $n \rightarrow \pi^*$  type was observed in the spectra, probably this band is covered with the more intense  $\pi \rightarrow \pi^*$  [27].

#### 3.2. Description of the crystal structures

Good quality crystals suitable for single crystal X-ray diffraction analysis were obtained for  $H_2L^1$ ,  $[PdL^2]$  and  $[PtL^2]$  compounds by recrystallization in dimethyl sulfoxide.

The molecular structure of  $H_2L^1$  free ligand together with the atomic numbering scheme is shown in Fig. 1. Crystallographic data is shown in Table 1 and selected bond lengths are listed in Table 2.

The crystal structure of  $H_2L^1$  consists of discrete  $H_2L^1 \cdot DMSO \cdot 0.5H_2O$  molecules with the two thiosemicarbazone arms adopting a *syn*-open arrangement in order to minimize unfavorable electronic interactions between the two tolyl groups. Therefore to achieve the coordinative disposition found in  $d^8$  metal complexes, *syn*-close conformation, a rearrangement of the molecule structure, by twisting around the C–C bonds adjacent to the pyridine ring, is necessary [28].

On the other hand the sulfur atom S(1) and the imine nitrogen atom N(6) are in *trans* position with respect to the C(16)–N(5) bond and the

**Table 2**

Selected bond distances (Å) for  $H_2L^1$ .

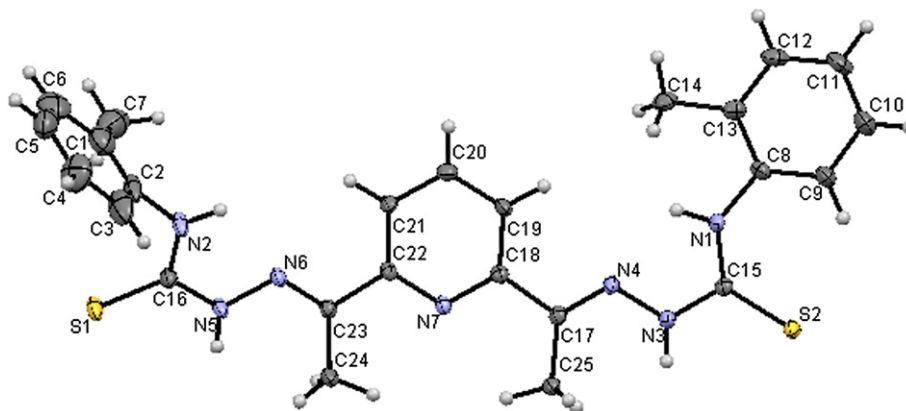
	$H_2L^1$	
S(1)–C(16)	1.678 (3)	t2.4
S(2)–C(15)	1.683 (3)	t2.5
C(2)–N(2)	1.427 (4)	t2.6
C(8)–N(1)	1.421 (3)	t2.7
C(15)–N(1)	1.344 (3)	t2.8
C(15)–N(3)	1.369 (3)	t2.9
C(16)–N(2)	1.333 (3)	t2.10
C(16)–N(5)	1.362 (4)	t2.11
C(17)–N(4)	1.290 (3)	t2.12
C(23)–N(6)	1.293 (4)	t2.13
N(3)–N(4)	1.374 (3)	t2.14
N(5)–N(6)	1.371 (3)	t2.15

same configuration is observed for S(2) and N(4) with respect to the C(15)–N(3) bond, therefore the ligand exists in *E* configuration which is often observed in thiosemicarbazones [29]. The  $C=N_{\text{imine}}$  bond distances of 1.290(3) and 1.293(4) are in conformity with a formal  $C=N$  double bond and the  $C=S$  bond distances of 1.678(3) and 1.683(3) Å, are very close to a formal  $C=S$  double bond length, these facts confirm the existence of the thiosemicarbazone groups in the thione form, in the solid state. However, the N(3)–N(4), N(3)–C(15), C(15)–N(1) and N(5)–N(6), N(5)–C(16), and C(16)–N(2) bond distances are intermediate between the ideal values of corresponding single and double bonds which is indicative of some electron delocalization along the thiosemicarbazide side chains.

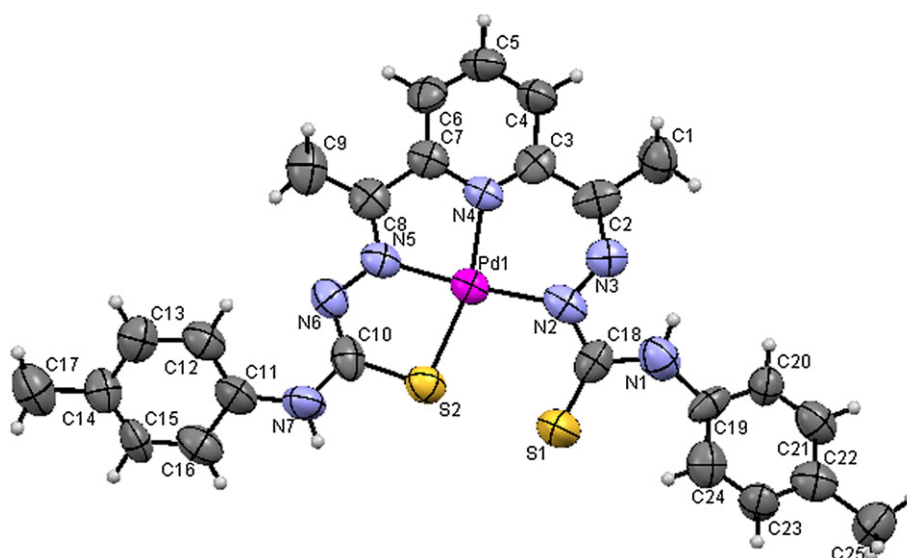
Supramolecular association involves the water of crystallization molecule, which forms two hydrogen bonds as hydrogen donor (with sulfur atoms of two DMSO as acceptors) and two more as acceptor (acting the N(3) atoms of two different ligand molecules as donor). The packing (Fig. S1, Supporting Information) suggests weak by  $\pi$ – $\pi$  stacking interactions (C–C distance about 4.1 Å) between molecules via the terminal phenyl rings.

$[PdL^2] \cdot 0.5DMSO$  and  $[PtL^2] \cdot DMSO$  were isolated as neutral compounds. The most significant parameters for these complexes are shown in Table 1. The structures together with the atom labeling schemes are shown in Fig. 2 and Fig. S2 (Supporting Information). Both compounds are isostructural hence displaying nearly identical cell parameters and crystallize in the monoclinic  $P2_1/c$  space group with  $Z = 4$ .

The metal ion presents a square planar geometry being the bis(thiosemicarbazone) ligand attached through the  $N_{\text{pyridinic}}$  atom, the  $N_{\text{imine}}$  and the S atoms from one thiosemicarbazone arm and being the fourth coordination position occupied by the  $N_{\text{hydrazinic}}$  of the other thiosemicarbazone arm generating two typical five membered ( $PdSCNN$  and  $PdNCCN$  or  $PtSCNN$  and  $PtNCCN$ ) and one six membered ( $PdNNCCN$  or  $PtNNCCN$ ) chelate rings. Coordination by



**Fig. 1.** Molecular structure of  $H_2L^1$  ligand.

Fig. 2. Molecular structure of  $[\text{PdL}^2]$  complex.

$\text{N}_{\text{hydrazinic}}$  instead of  $\text{N}_{\text{imino}}$ , although uncommon, has been found in the bibliography for some  $\text{d}^8$  bis(thiosemicarbazone) complexes [22,30–32].

It is important to note that the two thiosemicarbazone moieties coordinate in a different fashion and so upon coordination the bidentate- $\text{N}^{\wedge}\text{S}$  arm undergoes significant evolution from the thione to the thiol form which is reflected in C–S distance of 1.735(15) for  $[\text{PdL}^2]\cdot\text{DMSO}$  and 1.80(2) for  $[\text{PtL}^2]\cdot\text{DMSO}$  while the monodentate- $\text{N}_{\text{hydrazinic}}$  thiosemicarbazone arm presents a shorter C–S bond length [1.641(16) for  $[\text{PdL}^2]\cdot\text{DMSO}$  and 1.68(3) Å for  $[\text{PtL}^2]\cdot\text{DMSO}$ ]. On the other hand The C–N and N–N bond distances, listed in Table 3, are intermediate between formal single and double bonds, pointing to extensive delocalization over the entire 2,6-diacetylpyridine bis(thiosemicarbazone) skeleton [33].

Inspection of the angles formed between the metal ion ( $\text{M} = \text{Pd}^{2+}$ ,  $\text{Pt}^{2+}$ ) and the coordinated atoms shows that the metal is contained within a slightly distorted square-planar environment. The distortion is caused by the restricted bite angle of the N(4), N(5), S(2) donor set as reflected in the S(2)–M(1)–N(5) and N(4)–M(1)–N(5) angles (less than  $90^\circ$ ). The angles N(4)–M(1)–N(2) and N(2)–M(1)–S(2) are therefore greater than  $90^\circ$ .

Table 3

Selected bond distances (Å) and angles ( $^\circ$ ) for  $[\text{PdL}^2]$  and  $[\text{PtL}^2]$ .

	$[\text{PdL}^2]$	$[\text{PtL}^2]$
S(1)–C(18)	1.641 (16)	1.68 (3)
S(2)–C(10)	1.735 (15)	1.80 (2)
C(2)–N(3)	1.275 (17)	1.24 (3)
C(8)–N(5)	1.333 (17)	1.37 (3)
C(10)–N(6)	1.328 (18)	1.26 (3)
C(10)–N(7)	1.335 (18)	1.36 (3)
C(11)–N(7)	1.46 (2)	1.47 (3)
C(18)–N(1)	1.400 (18)	1.34 (3)
C(18)–N(2)	1.366 (17)	1.43 (3)
C(19)–N(1)	1.436 (17)	1.49 (3)
M–S(2)	2.304 (4)	2.307 (6)
M–N(2)	2.062 (12)	1.99 (2)
M–N(4)	2.017 (10)	2.04 (2)
M–N(5)	1.976 (12)	1.98 (2)
N(2)–M–N(4)	91.9 (5)	90.3 (8)
N(2)–M–N(5)	173.9 (5)	173.2 (7)
N(4)–M–N(5)	82.1 (5)	83.0 (8)
N(2)–M–S(2)	104.0 (3)	104.4 (5)
N(4)–M–S(2)	164.0 (4)	165.3 (6)
N(5)–M–S(2)	82.0 (4)	82.4 (5)

In both compounds the crystal structure is stabilized by inter-molecular hydrogen interaction involving the N(7) atom of the bidentate thiosemicarbazone arm and the oxygen atom of DMSO solvent molecule.

### 3.3. Antiproliferative activity against tumor and normal cells

To assess the antitumor potential of the synthesized compounds, its antiproliferative activity (in powder solid form) was tested in vitro against a panel of human cancer cell lines containing examples of lung (NCI-H460), breast (MCF7), liver (Hep-G2) and ovarian (A2780 and A2780cisR) cancers. For comparison purposes, the cytotoxicity of cisplatin was always evaluated under the same experimental conditions.

The results indicate that the two free ligands,  $\text{H}_2\text{L}^1$  and  $\text{H}_2\text{L}^2$ , and platinum complex  $[\text{PtL}^1]$  showed at 100  $\mu\text{M}$  concentration, very low cellular growth inhibition (<50%) and therefore had no evaluable cytotoxicity ( $\text{IC}_{50} > 100 \mu\text{M}$ ). However  $[\text{PdL}^1]$ ,  $[\text{PdL}^2]$  and  $[\text{PtL}^2]$  complexes displayed high antiproliferative activity against epithelial ovarian (A2780, cisplatin sensitive, and A2780cisR, cisplatin resistant) and breast (MCF-7) cancer cells (Table 4). It is remarkable to note that palladium complex  $[\text{PdL}^1]$  exhibited better cytotoxic effects against MCF-7 and A2780cisR cells than cisplatin by comparing their  $\text{IC}_{50}$ .

The A2780cisR cell line encompasses all of the known major mechanisms of resistance to cisplatin: reduced drug transport, enhanced DNA repair/tolerance, and elevated GSH levels. The ability of

Table 4

In vitro antiproliferative activity of the bis(thiosemicarbazone) compounds and cisplatin, evaluated in human MCF-7 (breast cancer), A2780 and A2780cisR (epithelial ovarian cancer) cell lines.

	$\text{IC}_{50} \pm \text{SD} (\mu\text{M})$			RF
	A2780	A2780cisR	MCF-7	$\text{IC}_{50}(\text{A2780cisR})/\text{IC}_{50}(\text{A2780})$
$\text{H}_2\text{L}^1$	> 100	> 100	> 100	
$\text{H}_2\text{L}^2$	> 100	> 100	> 100	
$[\text{PdL}^1]$	$5.97 \pm 0.06$	$5.80 \pm 0.49$	$4.41 \pm 0.45$	0.97
$[\text{PtL}^1]$	> 100	> 100	> 100	
$[\text{PdL}^2]$	$21 \pm 1$	$22 \pm 1$	$48 \pm 2$	0.86
$[\text{PtL}^2]$	$20 \pm 2$	$18 \pm 1$	$44 \pm 2$	1.1
Cisplatin	$0.83 \pm 0.02$	$7.21 \pm 0.13$	$8.75 \pm 0.35$	8.68



**Table 5**

In vitro antiproliferative activity of the bis(thiosemicarbazone) compounds and cisplatin, evaluated in normal LLC-PK1 renal cells.

	LLC-PK1	
	%Inhibition (100 $\mu$ M)	IC <sub>50</sub> $\pm$ SD ( $\mu$ M)
H <sub>2</sub> L <sup>1</sup>	2	>100
H <sub>2</sub> L <sup>2</sup>	1	>100
[PdL <sup>1</sup> ]	20	>100
[PtL <sup>1</sup> ]	2	>100
[PdL <sup>2</sup> ]	1	>100
[PtL <sup>2</sup> ]	23	>100
Cisplatin	90	6.86 $\pm$ 0.10

[PdL<sup>1</sup>], [PdL<sup>2</sup>] and [PtL<sup>2</sup>] complexes to circumvent cisplatin-acquired resistance was confirmed from the resistance factor values, RF (defined as IC<sub>50</sub> in A2780cisR/IC<sub>50</sub> in A2780) since all of them have a much better RF than cisplatin. An RF of <2 was considered to denote non-cross-resistance and therefore the three compounds tested are able to circumvent cisplatin resistance [34,35].

These observations, on the ability of 2,6-diacetylpyridine bis(<sup>4</sup>N-tolyl-tolylthiosemicarbazone) complexes to overcome cisplatin resistance in A2780cisR cells, agree with our previous studies of bis(<sup>4</sup>N-substituted thiosemicarbazones) derived from 3,5-diacetyl-1,2,4-triazol heterocyclic ring [36–38].

Platinum base therapies are frequently associated with cumulative and irreversible toxicities such as **nephropathy**. Therefore, in order to investigate this possible side effect, the compounds investigated and cisplatin were subsequently tested, in vitro, on normal renal LLC-PK1 cells in the same  $\mu$ M range (1–100). As showed in Table 5, all compounds showed at the maximum concentration tested very low cellular growth inhibition (<50%) and therefore had **no** evaluable cytotoxicity (IC<sub>50</sub> > 100  $\mu$ M) in this normal cell line. This means that none of these compounds are toxic, in vitro, in the tested concentration range.

#### 4. Conclusions

A new family of Pt(II) and Pd(II) bis(thiosemicarbazone) compounds incorporating the 2,6-diacetylpyridine heterocyclic ring has been successfully prepared and characterized.

This study has identified [PdL<sup>1</sup>], [PdL<sup>2</sup>] and [PtL<sup>2</sup>] complexes as having high antiproliferative activity since they are capable of not only **circumventing** cisplatin resistance in A2780cisR cells but also **exhibiting** high antiproliferative activity against breast (MCF-7) cancer cells. Moreover, it is important to note that these compounds exhibit very low nephrotoxicity with respect to cisplatin which confirmed that these complexes are very specific on cancer cells.

In conclusion, this study indicates that the newly synthesized complexes **valuably lead to** the development of new anticancer chemotherapeutic agents capable of retaining their activity in the human ovarian carcinoma cell line resistant to cisplatin A2780cisR.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.jinorgbio.2013.04.005>.

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