



Repositorio Institucional de la Universidad Autónoma de Madrid

<https://repositorio.uam.es>

Esta es la **versión de autor** del artículo publicado en:
This is an **author produced version** of a paper published in:

Dalton Transactions 41.40 (2012): 12538-12547

DOI: <http://dx.doi.org/10.1039/c2dt31199b>

Copyright: © The Royal Society of Chemistry 2012.

El acceso a la versión del editor puede requerir la suscripción del recurso

Access to the published version may require subscription

Cite this: DOI: 10.1039/c2dt31199b

www.rsc.org/dalton

PAPER

New palladium and platinum complexes with bioactive 3,5-diacetyl-1,2,4-triazol bis(4-cyclohexyl thiosemicarbazone) ligand: chemistry, antiproliferative activity and preliminary toxicity studies†

Ana I. Matesanz,^a Josefina Perles^b and Pilar Souza^{*a}

Received 4th June 2012, Accepted 21st August 2012

DOI: 10.1039/c2dt31199b

The preparation and characterization of the new 3,5-diacetyl-1,2,4-triazol bis(4-cyclohexyl thiosemicarbazone) ligand, H_5L^1 , is described. Treatment of H_5L^1 with K_2PtCl_4 gave the dinuclear complex $[\text{Pt}(\text{H}_3\text{L}^1)]_2$, **1**, but using $\text{MCl}_2(\text{PPh}_3)_2$ where $\text{M} = \text{Pd}$ or Pt , mononuclear complexes **2** and **3**, of general formula $[\text{M}(\text{H}_3\text{L}^1)\text{PPh}_3]$, were obtained. Subsequent reaction of the $[\text{Pd}(\text{H}_3\text{L}^1)\text{PPh}_3]$ complex with $\text{PdCl}_2(\text{PPh}_3)_2$ yielded a new dinuclear complex $[(\text{PPh}_3)\text{Pd}(\text{H}_2\text{L}^1)\text{PdCl}]$, **4**. All compounds have been characterized by elemental analysis and FAB^+ spectrometry and by IR and NMR spectroscopy. The molecular structures of mononuclear complexes **2** and **3** and dinuclear complex **4** have been determined by X-ray crystallography. 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 the H_5L^1 ligand and $[\text{Pt}(\text{H}_3\text{L}^1)]_2$, complex **1**, may be endowed with important cytotoxic properties since they are capable of not only circumventing cisplatin resistance in A2780cisR but also exhibit antiproliferative activity in NCI-H460. The interactions of these compounds with calf thymus DNA were investigated by UV-vis absorption and a nephrotoxic study, in LLC-PK1 cells, has also been carried out.

Introduction

The synthesis of preorganized dinuclear transition metal complexes is a field of research that has been rapidly growing for the past few years. Scaffolds to support and control a bimetallic arrangement may be provided by compartmental binucleating ligands that host two metal ions in suitable proximity.^{1–5}

Among the ligands that are capable of positioning two metal centers in close proximity, aromatic five-membered heterocycles containing two or three nitrogen atoms appear to be particularly suitable candidates. 1,2,4-Triazole-based ligands with chelating side arms in the 3- and 5-positions of the heterocycle are especially valuable for this purpose since the 1,2,4-triazole has a high tendency to span two metal ions, and the individual coordination spheres, as well as the metal–metal separations, can be tuned by appropriate alterations of the appended chelate substituents.^{6,7}

Such polydentate ligands quite often are Schiff bases (or the related reduced derivatives), obtained by condensation of appropriately designed and prepared formyl- (or keto-) and primary amine precursors.^{8–11} Thiosemicarbazones ($>\text{C}=\text{N}-\text{NH}-\text{C}(\text{S})-\text{N}<$) are an important class of Schiff base ligands that are structurally interesting, since both sulfur and nitrogen atoms may be involved in the coordination, thus invoking the *a* or *b* character of the metallic centre.^{12–15}

In this line, we have been employing the diketone heterocycle 3,5-diacetyl-1,2,4-triazole in condensation reactions with a series of thiosemicarbazides to obtain ligands containing two thiosemicarbazone moieties separated by the five-membered heterocycle ring 1,2,4-triazol which have been shown to be versatile polydentate ligands towards metal ions with square planar coordination geometry. Depending on the participation of the central ring in the coordination (monodentate N^1/N^2 versus N^1N^2 bridging mode) we could synthesize and characterize both dimers $[2 + 2]$ helicates and tetranuclear compounds probing the dinucleating tendency of these triazol/bis(thiosemicarbazone) ligands (Fig. 1).^{16–19}

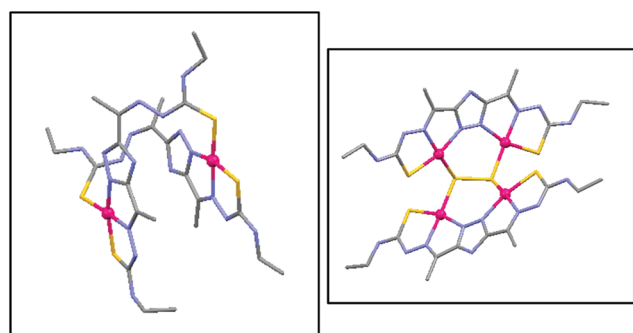
A factor of importance in the synthesis of compounds with 1,2,4-triazole is the ease of formation of the triazolone ion by deprotonation. The 1,2,4-triazolone anion can bind metal ions through different coordination modes, as is depicted in Scheme 1.

N^1N^4 Bridging is relatively rare compared with N^1N^2 bridging, this is probably because when both hydrazine-type

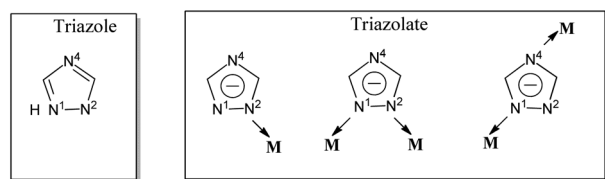
^aDepartamento de Química Inorgánica (Módulo 07), Facultad de Ciencias, c/Francisco Tomás y Valiente, 7, Universidad Autónoma de Madrid, 28049 Madrid, Spain. E-mail: pilar.souza@uam.es; Fax: (+34) 914974833; Tel: (+34) 914975146

^bServicio Interdepartamental de Apoyo a la Investigación, Facultad de Ciencias, c/Francisco Tomás y Valiente, 7, Universidad Autónoma de Madrid, 28049 Madrid, Spain

†CCDC 879640, 879641 and 879642 for complexes **2**, **3** and **4**. For crystallographic data in CIF or other electronic format see DOI: 10.1039/c2dt31199b



Q4 Fig. 1 Capped sticks representation of both dimer [2 + 2] helicate (left) and tetranuclear (right) Pd(II) complexes derived from 3,5-diacetyl-1,2,4-triazol bis(4-cyclohexylthiosemicarbazone).



Scheme 1 Schematic representation of the 1,2,4-triazole geometry and three types of coordination modes of the 1,2,4-triazolate anion.

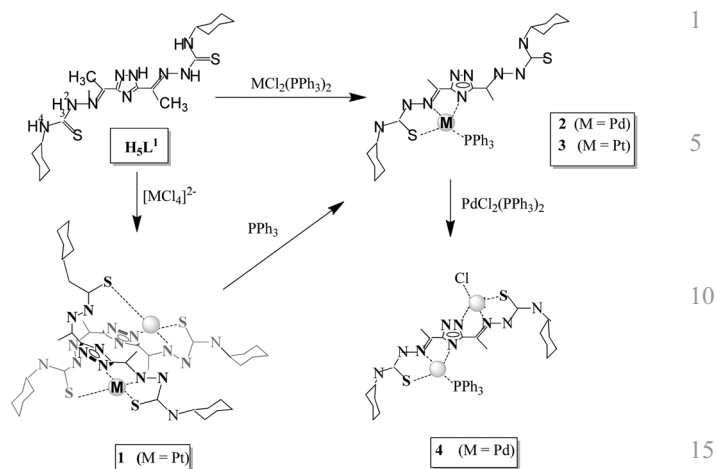
nitrogen atoms are bound to metal ions, the N^4 easily acts as a hydrogen acceptor providing additional stability. In fact, in addition to the ligand–metal interactions, hydrogen bonding plays a highly important role in the geometry of the coordination compounds of these ligands.²⁰

On the other hand many α -N-heterocyclic thiosemicarbazones have been reported to exert tumor-inhibiting effects and one of the most promising 3-aminopyridine-2-carboxaldehyde thiosemicarbazones (Triapine®) has entered several phase I and II clinical trials.^{21–23} Also, phosphines and phosphine metal containing complexes are of current interest due to their potential use as antitumor agents. Particularly, 1,2-bis(diphenylphosphino)ethane and some of its analogues have been shown to have antitumor activity against a wide range of tumors, and moreover, their activity is enhanced upon coordination to metal ions, such as gold(I).²⁴

Recently, investigations from our laboratory have led to the development of the first mononuclear Pd(II) complexes derived from 3,5-diacetyl-1,2,4-triazol bis(4-monosubstituted thiosemicarbazones) bearing triphenylphosphine as a coligand. Their excellent antiproliferative activity was demonstrated by *in vitro* experiments in various human cell lines derived from different types of solid tumours.²⁵

An X-ray structural study demonstrated that 3,5-diacetyl-1,2,4-triazol bis(4-monosubstituted thiosemicarbazone) ligands act as tridentate dianion binding Pd(II) ions *via* one $N_{\text{triazolic}}$ and N_{iminic} and S donor atoms from one thiosemicarbazone arm. Therefore some of the ligand donor atoms are still free and thus the presence of uncoordinated donor atoms and their relative dispositions in space suggested that such complexes might provide a possibility to bind to a second metal ion.

Keeping in view the above observations, it was planned to construct a series of dinuclear N^1N^4 triazol-bridged metal



Scheme 2 Structure of the 3,5-diacetyl-1,2,4-triazol bis(4-cyclohexylthiosemicarbazone) ligand and its metal complexes synthesized.

complexes based on 3,5-diacetyl-1,2,4-triazol bis(4-monosubstituted thiosemicarbazone) triphenylphosphine palladium(II) precursors. Herein, we report on the synthesis and characterization of the new 3,5-diacetyl-1,2,4-triazol bis(4-cyclohexylthiosemicarbazone) ligand together with the study of its complexation potential for Pd(II) and Pt(II) ions (Scheme 2).

The cytotoxic activity of the new compounds synthesized and cisplatin (assumed as the reference antitumor drug) against five human cancer cell lines, NCI-H460 (non-small cell lung cancer), HepG2 (hepatocellular carcinoma), MCF-7 (breast cancer), A2780 and A2780cisR (epithelial ovarian cancer), has been studied. The interaction of these compounds with calf thymus DNA (CT-DNA) was also investigated. In addition toxicity studies, on normal renal LLC-PK1 cells, have been carried out as an attempt to provide an insight into the pharmacological properties of these compounds.

The present work aims to contribute to the understanding of the structural and cytotoxic characteristics of these compounds.

Results and discussion

Synthesis and spectroscopic characterization

A new multidentate ligand, H_5L^1 , which possesses two NNS coordination sites was synthesized as a platform for metal arrangement. Complexation with $[MCl_4]^{2-}$ ions ($M = Pd$ or Pt) resulted in the formation of dimers [2 + 2] helicates, in which each triazol-bis(thiosemicarbazone) behaves as a dianionic ligand with deprotonation of one hydrazinic (2NH) and the triazol ring protons and $[NNS]$ donor set.

Since the cleavage of binuclear Pd(II) and Pt(II) complexes with tertiary phosphines is well known²⁶ we decided to examine the behavior of the dimers [2 + 2] helicates synthesized. So, subsequent treatment of both palladium and platinum dimers with PPh_3 , in 1 : 2 molar ratios, yielded the expected mononuclear complexes in which each metal atom is coordinated to a phosphine ligand. Interestingly, reaction of the H_5L^1 ligand with $MCl_2(PPh_3)_2$ salts ($M = Pd$ or Pt) in toluene, in the presence of

triethylamine base, in 1 : 1 molar ratios afforded the same mononuclear complexes of stoichiometry $[M(H_3L^1)PPh_3]$.

Once the mononuclear complexes were obtained, the presence of several potential uncoordinated donor atoms and their relative dispositions in space suggested that such complexes might provide a possibility to bind to a second metal ion. So, upon the addition of $MCl_2(PPh_3)_2$ to the $[M(H_3L^1)PPh_3]$ precursors, we have achieved the synthesis of a new dinuclear complex, in which the triazol/bis(thiosemicarbazone) ligands have been found to coordinate two metal ions as a symmetric trianionic hexadentate ligand with deprotonation of the two hydrazinic (2NH) and the triazol ring protons. It may be pointed out here that the two metal ions contain different coordination spheres [NNSP] and [NNSCI].

The compounds described in this paper were characterized by elemental analysis and FAB^+ spectrometry and by IR, 1H NMR and ^{31}P NMR spectroscopy (the significant IR vibrational bands and the 1H chemical shift values 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 shift to lower wavenumbers in the spectra of the complexes suggesting coordination of the imine nitrogen and sulfur atoms. In complexes **2**, **3** and **4**, the presence of the triphenylphosphine ligand is confirmed by the existence of the characteristic bands around 3050 and 1097 cm^{-1} for $\nu(CH)$ and $\nu(P-C)$, with no significant change when compared to the precursor $PdCl_2(PPh_3)_2$.

In the 1H NMR spectrum, the free ligand shows various peaks at low fields: $\delta \sim 15$ ppm assigned to the triazolonic proton, $\delta \sim 12$ ppm assigned to the hydrazinic protons (2NH) and

$\delta \sim 10$ ppm assigned to the thioamidic protons (4NH). In the dimeric complex **1** as well as in mononuclear complexes **2** and **3**, the absence of any signals above 15 ppm, indicative of deprotonation of the triazole ring, together with the presence of only one signal assigned to 2N hydrazinic hydrogens is consistent with the asymmetric di-deprotonation typical of 3,5-diacetyl-1,2,4-triazol bis(thiosemicarbazone) ligands. However both the signals assigned to the triazolonic proton and the hydrazinic protons were absent in the spectrum of complex **4**, thus confirming the symmetric tri-deprotonation of the ligand.

^{31}P NMR spectroscopy was used to further confirm complexation of the ligand. Palladium complexes **2** and **4** display a singlet at 32.75 and 29.00 ppm respectively for the phosphorus atom of the triphenylphosphine ligand, while the platinum derivative **3** displays typical resonance at 11.20 ppm.

Description of mononuclear crystal structures **2** and **3**

$[Pd(H_3L^1)PPh_3] \cdot EtOH$ and $[Pt(H_3L^1)PPh_3] \cdot EtOH$ were isolated as neutral compounds. The most significant parameters for these complexes are shown in Table 1.

The structures together with the atom labelling schemes are shown in Fig. 2 and 3. Both compounds are isostructural, hence displaying nearly identical cell parameters, crystallize in the triclinic $P\bar{1}$ space group with $Z = 2$ and the asymmetric units contain one molecule of the neutral complex and one ethanol molecule (for compound **2** the H atoms of the EtOH molecule were not located in the Fourier difference map). The metal ion presents a square planar geometry the bis(thiosemicarbazone) ligand

Table 1 Crystal data and structure refinement for **2**, **3** and **4** complexes

	2	3	4
Molecular formula	$C_{40}H_{46}N_9OPPdS_2$	$C_{40}H_{52}N_9OPPtS_2$	$C_{45}H_{62}ClN_9OPPd_2S_3$
Formula weight	870.35	963.07	1120.44
Temperature (K)	100(2)	100(2)	100(2)
Wavelength (\AA)	0.71073	0.71073	1.54178
Crystal system	Triclinic	Triclinic	Monoclinic
Space group	$P\bar{1}$	$P\bar{1}$	$P2(1)/n$
a (\AA)	10.7402(7)	10.769(5)	12.4247(4)
b (\AA)	11.8911(7)	12.054(5)	16.3391(5)
c (\AA)	17.4118(12)	17.499(8)	24.2489(8)
α ($^\circ$)	81.039(4)	81.09(2)	90
β ($^\circ$)	89.542(4)	89.09(2)	92.702(2)
γ ($^\circ$)	70.946(3)	70.974(19)	90
Volume (\AA^3)	2074.0(2)	2120.1(16)	4917.3(3)
Z	2	2	4
Density (calculated) ($g\ cm^{-3}$)	1.394	1.509	1.513
Absorption coefficient (mm^{-1})	0.630	3.488	8.247
$F(000)$	900	972	2300
Crystal size (mm^3)	$0.15 \times 0.10 \times 0.05$	$0.30 \times 0.34 \times 0.38$	$0.20 \times 0.15 \times 0.13$
Index ranges	$-13 \leq h \leq 13, -14 \leq k \leq 14, -21 \leq l \leq 21$	$-13 \leq h \leq 13, -15 \leq k \leq 13, -22 \leq l \leq 21$	$-14 \leq h \leq 14, -19 \leq k \leq 19, -29 \leq l \leq 29$
Reflections collected	49 659	51 305	30 211
Independent reflections	8437 [$R(int) = 0.0644$]	8787 [$R(int) = 0.0555$]	9122 [$R(int) = 0.0340$]
Data/restraints/parameters	8437/0/499	8787/0/485	9122/11/569
Goodness-of-fit on F^2	1.129	1.115	1.039
Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0432, wR_2 = 0.1129$	$R_1 = 0.0340, wR_2 = 0.1123$	$R_1 = 0.0412, wR_2 = 0.1105$
R indices (all data)	$R_1 = 0.0652, wR_2 = 0.1373$	$R_1 = 0.0462, wR_2 = 0.1584$	$R_1 = 0.0459, wR_2 = 0.1148$
Largest diff. peak and hole ($e\ \text{\AA}^{-3}$)	1.555 and -0.585	2.609 and -3.968	2.164 and -0.727

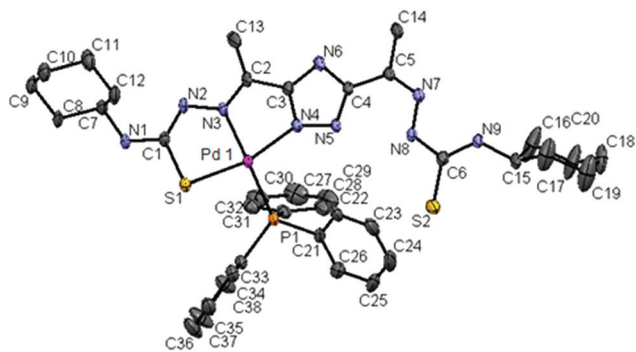


Fig. 2 Molecular structure of complex **2**, hydrogen atoms are omitted for clarity.

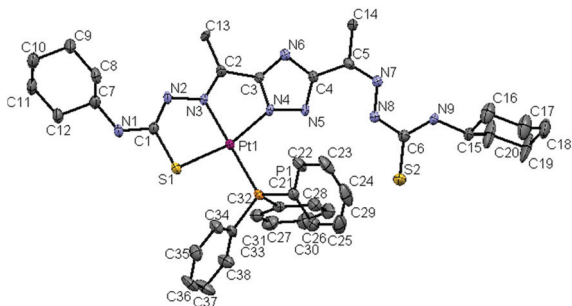


Fig. 3 Molecular structure of complex **3**, hydrogen atoms are omitted for clarity.

being attached through the $N_{\text{triazolic}}$, and the N_{iminic} and S atoms from one thiosemicarbazone arm. The fourth coordination position is occupied by a P atom from the PPh_3 coligand which is coordinated to Pd or Pt *trans* to N_{iminic} .

The bis(thiosemicarbazone) ligand is in dianionic form showing *Z, E* configuration, that is the coordinated thiosemicarbazone arm, involved in two five-membered (PdSCNN and PdNCCN or PtSCNN and PtNCCN) chelate rings, with the sulfur atom *cis* to the azomethine nitrogen atom, and the uncoordinated thiosemicarbazone arm with the S atom *trans* to the azomethine nitrogen atom. This arrangement is reinforced by intramolecular hydrogen bonds between the ^2NH of the uncoordinated thiosemicarbazone arm and one triazole nitrogen atom.

It is important to note that upon coordination, the deprotonated arm undergoes significant evolution from the thione to the thiol form which is reflected in a C–S distance of 1.786(6) Å for **2** and 1.776(5) Å for **3** while the neutral thiosemicarbazone arm presents a shorter C–S bond length [1.668(6) for **2** and 1.693(6) Å for **3**]. The C–N and N–N bond distances (Tables 2 and 3) are intermediate between formal single and double bonds, pointing to extensive delocalization over the entire 3,5-diacetyl-1,2,4-triazole bis(thiosemicarbazone) skeleton, however metal coordination provokes an important shortening of the C– $N_{\text{hydrazinic}}$ distance in the deprotonated arm as compared to the undepronated arm.

Inspection of the angles formed between the metal ion ($M = \text{Pd}^{2+}, \text{Pt}^{2+}$) 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

Table 2 Selected bond distances (Å) and angles (°) for Pd(II) complexes **2** and **4**

	2	4
S(1)–C(1)	1.774(4)	1.781(4)
S(2)–C(6)	1.681(4)	1.763(4)
C(1)–N(1)	1.338(5)	1.329(6)
C(1)–N(2)	1.307(5)	1.314(5)
C(2)–N(3)	1.280(5)	1.302(5)
C(3)–N(4)	1.338(5)	1.349(5)
C(3)–N(6)	1.333(5)	1.341(5)
C(4)–N(5)	1.339(5)	1.331(5)
C(4)–N(6)	1.354(5)	1.373(5)
C(5)–N(7)	1.288(4)	1.300(5)
C(6)–N(8)	1.354(5)	1.312(5)
C(6)–N(9)	1.315(5)	1.347(5)
C(7)–N(1)	1.456(5)	1.475(6)
N(2)–N(3)	1.372(4)	1.368(5)
N(4)–N(5)	1.349(4)	1.344(5)
N(7)–N(8)	1.377(4)	1.375(5)
Pd(1)–S(1)	2.2434(10)	2.2578(9)
Pd(1)–N(3)	2.026(3)	2.032(3)
Pd(1)–N(4)	2.031(3)	2.037(3)
Pd(1)–P(1)	2.2436(10)	2.2689(10)
N(3)–Pd(1)–N(4)	79.72(12)	79.29(13)
N(3)–Pd(1)–S(1)	84.00(9)	84.00(9)
N(3)–Pd(1)–P(1)	178.09(10)	171.40(9)
N(4)–Pd(1)–S(1)	163.71(9)	163.27(10)
N(4)–Pd(1)–P(1)	101.19(9)	97.56(10)
S(1)–Pd(1)–P(1)	95.07(4)	98.90(4)
N(6)–Pd(2)–N(7)		81.16(12)
N(6)–Pd(2)–S(2)		165.05(9)
N(6)–Pd(2)–Cl(1)		104.92(9)
N(7)–Pd(2)–S(2)		84.05(10)
N(7)–Pd(2)–Cl(1)		172.98(10)
S(2)–Pd(2)–Cl(1)		98.90(4)

Table 3 Selected bond distances (Å) and angles (°) for Pt(II) complex **3**

S(1)–C(1)	1.775(6)	N(3)–Pd(1)–N(4)	79.3(1)
S(2)–C(6)	1.692(7)	N(3)–Pd(1)–S(1)	84.0(1)
C(1)–N(1)	1.352(6)	N(3)–Pd(1)–P(1)	171.4(1)
C(1)–N(2)	1.311(8)	N(4)–Pd(1)–S(1)	163.3(1)
C(2)–N(3)	1.288(8)	N(4)–Pd(1)–P(1)	97.6(1)
C(3)–N(4)	1.340(7)	S(1)–Pd(1)–P(1)	98.90(4)
C(3)–N(6)	1.330(7)		
C(4)–N(5)	1.346(7)		
C(4)–N(6)	1.370(7)		
C(5)–N(7)	1.286(7)		
C(6)–N(8)	1.367(7)		
C(6)–N(9)	1.331(6)		
C(7)–N(1)	1.453(8)		
N(2)–N(3)	1.397(6)		
N(4)–N(5)	1.355(5)		
N(7)–N(8)	1.386(8)		
Pt(1)–S(1)	2.259(2)		
Pt(1)–N(3)	2.027(6)		
Pt(1)–N(4)	2.037(4)		
Pt(1)–P(1)	2.245(2)		

tridentate ligand as reflected in the $\text{N}(3)\text{--M}(1)\text{--N}(4)$ and $\text{N}(3)\text{--M}(1)\text{--S}(1)$ angles (less than 90°). The angles $\text{S}(1)\text{--M}(1)\text{--P}(1)$ and $\text{P}(1)\text{--Pd}(1)\text{--N}(4)$ are therefore greater than 90° .

Within each molecule, the bis(thiosemicarbazone)–metal moiety is close to planar, this disposition appears to be favored by the presence, at the not coordinated thiosemicarbazone arm,

of an intramolecular hydrogen bond between the hydrazinic hydrogen and one triazolic nitrogen atom. So the supramolecular association involves π - π stacking interactions between parallel layers of molecules. It is suggested that ethanol solvent molecules link adjacent units of the complex *via* intermolecular hydrogen bonds.

Description of dinuclear crystal structure 4

The molecular structure of the $[(\text{PPh}_3)\text{Pd}(\text{H}_2\text{L}^1)\text{PdCl}]$ complex, which crystallized with one DMSO molecule and one pentane solvent molecule in the monoclinic $P2(1)/n$ space group, together with the atom labelling scheme is shown in Fig. 4. The most characteristic feature is the linear arrangement of the two Pd(II) ions, which are bridged by the triply deprotonated ligand. Crystallographic data are shown in Table 1 with selected bond lengths and angles shown in Table 2.

The 3,5-diacetyl-1,2,4-triazol bis(4-cyclohexyl thiosemicarbazone) ligand behaves as a trianion bridging the two metal ions through the two not adjacent nitrogen atoms of the central triazole ring. So, the ligand coordinates to the Pd(1) center *via*: triazolic nitrogen atom N(4), iminic nitrogen N(3) and sulfur atom S(1); the coordination sphere is completed by the phosphorus atom from the PPh_3 coligand. The other palladium atom, Pd(2), is coordinated to the remaining triazolic nitrogen N(6), iminic nitrogen N(7) and sulfur S(2) atoms of the ligand being the fourth position occupied by a terminal chlorido ligand.

The geometry around the palladium atoms is square-planar with slight distortions from ideal values according to the observed *trans* bond angles N-Pd-S and N-Pd-P or N-Pd-Cl. This may be attributed to the restricted bite angle of the tridentate moieties.

The Pd-N and Pd-S bond distances in the range 1.9947(3)–2.151(3) Å and 2.291(10)–2.2578(9) Å, respectively, are comparable with those reported for Pd(II) thiosemicarbazone complexes.²⁷ The C-S bond lengths of 1.681(4) and 1.781(4) Å are within the normal range of a C-S single bond, indicating that the thiosemicarbazone moieties adopt the thiol tautomeric form.

The C-N and N-N bond distances are intermediate between formal single and double bonds, pointing to extensive

delocalization over the entire 3,5-diacetyl-1,2,4-triazole bis(thiosemicarbazone) skeleton.

In compound 4 intramolecular hydrogen bonding interactions are not observed and intermolecular hydrogen bonding is limited to N(1)–H(1)···O(1) with a contact distance of 2.85 Å and $\angle(\text{NHO})$ angle 62.1°.

Antiproliferative activity against tumor and normal cells

Cisplatin, *cis*-diamminedichloridoplatinum(II), and its analogs are among the most effective agents for the treatment of cancer. However its restricted clinical utility, due to the frequent development of drug resistance, the limited spectrum of tumors against which these drugs are active and severe normal tissue toxicity, has generated new areas of research, which mainly focused on the search for new compounds with low toxicity and improved therapeutic properties.^{28–32}

To assess the antitumor potential of the newly 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 (MCF-7), liver (Hep-G2) and ovarian (A2780 and A2780cisR) cancers. For comparison purposes the cytotoxicity of cisplatin was always evaluated under the same experimental conditions (Table 4). Out of an initial screening of the five compounds synthesized, two of them presented relevant antiproliferative actions, with IC_{50} values in the low μM range. The remaining compounds show, at 100 μM concentration, very low cellular growth inhibition ($<50\%$) and therefore had no evaluable cytotoxicity ($\text{IC}_{50} > 100 \mu\text{M}$).

In particular, this study identified both the free ligand H_5L^1 and the $[\text{Pt}(\text{H}_3\text{L}^1)]_2$ dimer complex, 1, as having antiproliferative activity in the low-micromolar range, against ovarian (A2780, cisplatin sensitive, and A2780cisR, cisplatin resistant) and lung (NCI-H460) cancer cells. The ligand also exhibits antiproliferative activity against breast (MCF-7) cancer cells.

Of particular relevance are the values of the resistance factor, RF (defined as IC_{50} in A2780cisR/ IC_{50} in A2780), since both compounds tested have a much better RF than cisplatin. This observation may suggest that these compounds are able to circumvent cisplatin resistance and this is probably due to a different mechanism of action. In fact, it has been demonstrated that the main molecular target of α -(*N*)-heterocyclic thiosemicarbazones is the ribonucleotide reductase (RR), one of the most complex enzymes in the cell, from a biological, structural and regulatory point of view.^{33,34}

Moreover, since one of the mechanisms of cisplatin induced nephrotoxicity is the inactivation of some enzymes because of the reaction between platinum ions and sulfur containing proteins, the compounds reported here, bearing nitrogen and sulfur atoms in their structure, could prevent the adverse reaction described.^{35,36}

In order to investigate this possible side effect, the compounds investigated and cisplatin were subsequently tested, *in vitro*, in the same μM range (1–100), on normal renal LLC-PK1 cells. As is shown in Table 5, the H_5L^1 ligand exhibits very low cellular growth inhibition ($<50\%$), at the maximum concentration of 100 μM , and therefore had no evaluable cytotoxicity ($\text{IC}_{50} > 100 \mu\text{M}$). In addition, complex 1 was demonstrated to be

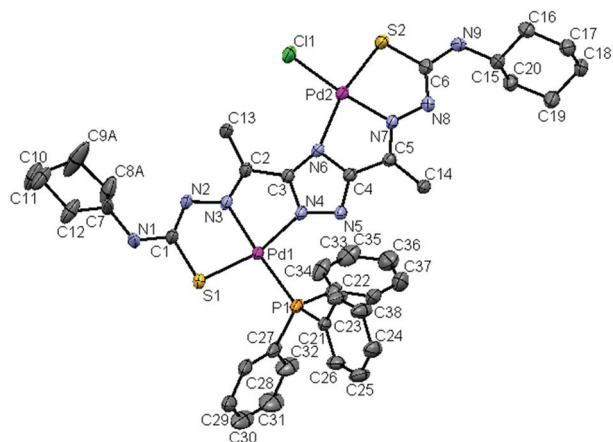


Fig. 4 Molecular structure of complex 4, hydrogen atoms are omitted for clarity.

Table 4 *In vitro* antiproliferative activity of H_5L^1 , **1**, and cisplatin, evaluated in several human cancer cell lines

Compound	IC_{50}^a (μM)					RF $\text{IC}_{50}(\text{A2780cisR})/\text{IC}_{50}(\text{A2780})$
	A2780	A2780cisR	NCI-H460	MCF-7	Hep-G2	
H_5L^1	32 ± 1	47 ± 1	49 ± 3	54 ± 1	>100	1.46
1	6.7 ± 0.04	8.99 ± 0.02	19 ± 2	>100	>100	1.34
Cisplatin	0.54 ± 0.02	3.7 ± 0.1	3.98 ± 0.2	8.75 ± 0.35	3.23 ± 0.06	6.85

^a 50% inhibitory concentrations in the sulforhodamine B assay (48 h exposure for NCI-H460 and Hep-G2 and 96 h exposure for A2780, A2780cisR and MCF-7). The compounds were tested in 1 : 99 DMSO–PBS solutions. Values are the means \pm standard deviations obtained from at least three independent experiments.

Table 5 *In vitro* antiproliferative activity of H_5L^1 , **1**, and cisplatin, evaluated in normal LLC-PK1 renal cells

Compound	IC_{50} LLC-PK1
H_5L^1	>100
1	33 ± 1
Cisplatin	7.03 ± 0.08

much less toxic (about 5-fold) to the LLC-PK1 cells than cisplatin by comparing their IC_{50} .

DNA binding studies

To study the binding affinity between DNA and the new compounds H_5L^1 and **1**, which have a significant effect against the tested cell lines, UV absorption spectroscopy has been a very useful technique because the typical B-form DNA gives rise to a characteristic band at 260 nm in the UV region. Hyperchromism (increase in absorption of the DNA band) and hypochromism (decrease in absorption of the DNA band) are the spectral changes typical of a compound interaction with the DNA helix. Hypochromism results from contraction of the DNA helix as well as changes in its conformation, while hyperchromism results from damage to the DNA double helix structure.³⁷

In UV experiments, the spectra of CT-DNA in the presence of each compound have been recorded, by keeping constant CT-DNA concentration in diverse [CT-DNA]/[compound] mixing ratios ($R = 0.5$ – 1.5) and monitoring the change in the absorption intensity of the typical CT-DNA spectral band at 260 nm.

In the absence of CT-DNA, both compounds displayed intense absorption bands in the UV region (λ_{max} 320–350 nm) assigned to the intraligand $\pi \rightarrow \pi^*$ transition of aromatic chromophores which are also sensitive to their interaction with CT-DNA.

For both compounds, the absorption spectra display an increase in the intensity of the characteristic CT-DNA absorbance while increasing the compound concentration (Fig. 5) where the percentage of hyperchromism observed [% hyperchromism = $(A_{\text{DNA bound}} - A_{\text{DNA free}}) / A_{\text{DNA bound}}$] is about 38% for the H_5L^1 ligand and 43% for complex **1**.

These characteristics suggest noncovalent interactions such as electrostatic and surface (major or minor groove) binding along outside of the DNA helix. Intercalative DNA-binding has been excluded on the basis of the absence of hypochromism; on the other hand both the ligand and the platinum(II) complex show

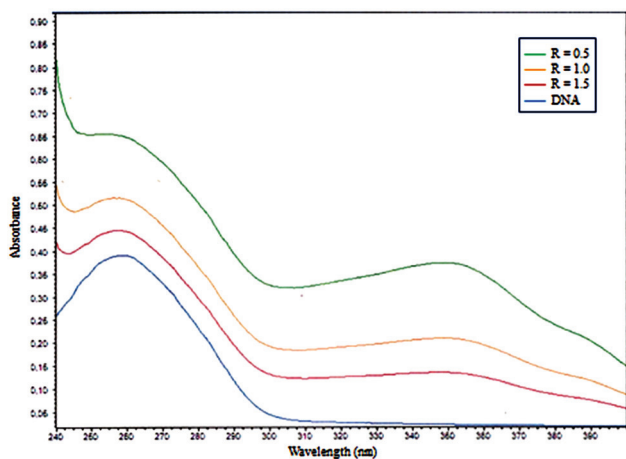
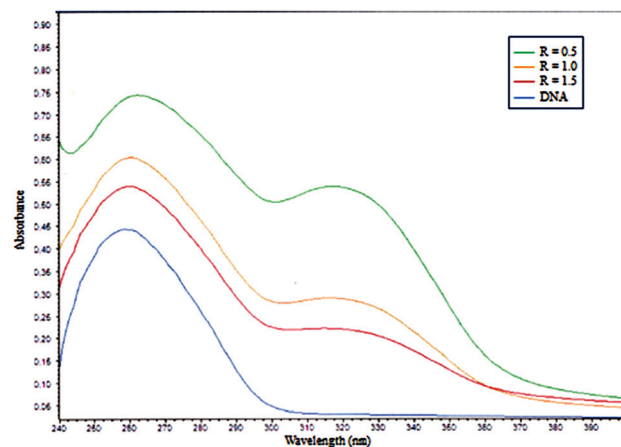


Fig. 5 UV absorption spectra of CT-DNA in the absence and presence of compounds H_5L^1 (top) and **1** (bottom). The data were collected for $[\text{CT-DNA}] = 1.25 \times 10^{-4}$ M and mixing ratio $R = 0.5$ – 1.5 (from top to bottom).

similar UV absorption profiles, therefore a covalent interaction between Pt(II) and guanine N7 is unlikely.

Experimental

Measurements

Elemental analyses were performed on a LECO CHNS-932 microanalyzer. Fast atom bombardment (FAB) mass spectra

were performed on a VG AutoSpec spectrometer (nitrobenzyl alcohol matrix). Nuclear Magnetic Resonance (NMR) spectra were recorded on a BRUKER AMX-300 spectrometer. All cited physical measurements were obtained by the Servicio Interdepartamental de Investigación (SIDI) of the Universidad Autónoma de Madrid.

Infrared spectra (KBr pellets) were recorded on a Bomem–Michelson spectrophotometer (4000–400 cm^{-1}). Electronic spectra were recorded on a Thermo Scientific Evolution 260 Bio UV-visible spectrophotometer.

Materials

Solvents were purified and dried according to standard procedures. Hydrazine hydrate, L-lactic acid, cyclohexyl isothiocyanate, palladium(II) chloride, potassium tetrachloridoplatinate(II) and triphenylphosphine were commercially available.

Synthesis of compounds

3,5-Diacetyl-1,2,4-triazol bis(4-cyclohexylthiosemicarbazone) ligand, H_3L^1 . An ethanolic solution of hydrazine hydrate (0.250 g, 5 mmol) was added dropwise with constant stirring to an ethanolic solution of cyclohexyl isothiocyanate (0.706 g, 5 mmol). The reaction mixture was stirred for one more hour and then the white product 4-cyclohexylthiosemicarbazide formed was filtered, washed with cold ethanol and diethyl ether, dried *in vacuo* and recrystallized from ethanol. A methanolic solution of the 4-cyclohexylthiosemicarbazide (0.346 g, 2 mmol) was then refluxed with 3,5-diacetyl-1,2,4-triazol (0.153 g, 1 mmol), which was prepared as described in the bibliography,³⁸ for 5 h and then was left to stand to ambient temperature. The solution was reduced to half volume and the pale yellow solid formed was filtered, washed with cold ethanol and diethyl ether and dried *in vacuo*.

Yield (55%), mp 205 °C. Elemental analysis found, C, 51.70; H, 7.20; N, 26.95; S, 13.50; $\text{C}_{20}\text{H}_{33}\text{N}_9\text{S}_2$ requires C, 51.85; H, 7.15; N, 27.20; S, 13.80%. MS (FAB⁺ with *m*NBA matrix) m/z 464.3 (100%) for $[\text{C}_{20}\text{H}_{33}\text{N}_9\text{S}_2 + \text{H}]^+$. IR (KBr pellets): n/cm^{-1} 3323 (s, NH-triazol), 3226 and 3099 (w, ²NH and ⁴NH), 1547 (s, CN), 853 (w, CS-thioamide IV band). ¹H NMR (300.14 MHz, DMSO-*d*₆): δ (ppm) 15.29 (s, NH-triazol, 1H), 12.57 and 10.75 (s, ²NH, 1H), 8.37, 8.34 and 8.20, 8.17 (d, ⁴NH, 1H), 4.30 (s, CH-cyclohexyl, 2H), 2.38 and 2.37 (s, CH₃-triazol, 3H), 1.91 (m, CH₂ equatorial-cyclohexyl, 10H), 1.35 (m, CH₂ axial-cyclohexyl, 10H).

[M(H₃L¹)]₂. Dinuclear [2 + 2] dimer complexes were obtained by reacting a methanol suspension of the 3,5-diacetyl-1,2,4-triazol bis(4-cyclohexylthiosemicarbazone) ligand with a methanolic solution of lithium tetrachloridopalladate(II) (0.140 g, 0.5 mmol) or a water solution of potassium tetrachloridoplatinate(II) (0.207 g, 0.5 mmol). The reaction mixture was stirred for 5 h at room temperature and the resulting orange solid obtained filtered, washed with methanol and diethyl ether, and dried *in vacuo*. The difficulty experienced in crystallizing the palladium complex because of its lower solubility resulted in less satisfactory analyses. For M = Pt the crude mass was crystallized from ca. 20 mL of DMSO to give pure [Pt(H₃L¹)]₂, **1**.

Yield (32%), mp 228 °C (decomposes). Elemental analysis found, C, 29.70; H, 5.70; N, 15.90; S, 8.20; $\text{C}_{40}\text{H}_{62}\text{N}_{18}\text{S}_4\text{Pt}_2 \cdot 16\text{H}_2\text{O}$ requires C, 30.00; H, 5.90; N, 15.70; S, 8.00%. MS (FAB⁺ with *m*NBA matrix) m/z 1313.4 for $[\{\text{Pt}(\text{H}_3\text{L}^1)\}_2 + \text{H}]^+$. IR (KBr pellets): n/cm^{-1} 3245 (w, ⁴NH), 2929, 2853 (s, CH), 1549 (s, CN). ¹H NMR (300.14 MHz, DMSO-*d*₆): δ (ppm) 12.30 (s, ²NH, 1H), 9.28–8.80, 8.50–7.90 (m, ⁴NH, 2H), 3.95 (s, CH-cyclohexyl, 2H), 2.37 (s, CH₃-triazol, 6H), 1.90–1.75 (m, CH₂ equatorial-cyclohexyl, 10H), 1.40–1.20 (m, CH₂ axial-cyclohexyl, 10H).

Attempts to grow crystals of [Pt(H₃L¹)]₂ for X-ray analysis were unsuccessful.

[Pd(H₃L¹)PPh₃], **2.** This complex was obtained by reaction of PdCl₂(PPh₃)₂ metallic salt (0.351 g, 0.5 mmol), prepared by a previously described procedure,³⁹ with the 3,5-diacetyl-1,2,4-triazol bis(4-cyclohexylthiosemicarbazone) ligand (0.232 g, 0.5 mmol) in toluene, in the presence of Et₃N, in 1 : 1 molar ratios. The reaction mixture was stirred for 2 h at room temperature. The resulting orange solution was filtered and left to stand at ambient temperature for two days. The yellow-orange microcrystalline solid formed was filtered, washed several times with hot water, recrystallized from ethanol and finally dried *in vacuo*.

Yield (73%), mp 176 °C. Elemental analysis found, C, 55.30; H, 5.60; N, 14.90; S, 7.55; $\text{C}_{38}\text{H}_{46}\text{N}_9\text{S}_2\text{PPd}$ requires C, 55.00; H, 5.55; N, 15.20; S, 7.70%. MS (FAB⁺ with *m*NBA matrix) m/z 830.4 for $[\text{Pd}(\text{H}_3\text{L}^1)\text{PPh}_3 + \text{H}]^+$. IR (KBr pellets): n/cm^{-1} 3213 (s, NH); 2927, 2851 (s, CH), 1523 (s, CN), 859 (w, CS-thioamide IV band). ¹H NMR (300.14 MHz, DMSO-*d*₆): δ (ppm) 12.70 (s, ²NH, 1H); 8.30–8.25 (d, ⁴NH, 2H), 8.05–7.80 (m, aromatic protons, 15H); 4.45 and 4.40 (s, CH-cyclohexyl, 1H), 2.90 and 2.75 (s, CH₃-triazol, 3H), 1.90 (m, CH₂ equatorial-cyclohexyl, 10H), 1.50 (m, CH₂ axial-cyclohexyl, 10H). ³¹P NMR (121.50 MHz, CDCl₃): δ (ppm) 32.75 (PPh₃).

Crystallization in ethanol allowed us to isolate single orange crystals, which were studied by X-ray diffraction techniques.

[Pt(H₃L¹)PPh₃], **3.** This complex was obtained by two different routes.

Method 1: using the same procedure as for compound **2**, namely by reaction of PtCl₂(PPh₃)₂ metallic salt (0.395 g, 0.5 mmol), prepared by a previously described procedure,⁴⁰ with the 3,5-diacetyl-1,2,4-triazol bis(4-cyclohexylthiosemicarbazone) ligand (0.232 g, 0.5 mmol) in toluene, in the presence of Et₃N, in 1 : 1 molar ratios. The reaction mixture was stirred for 2 h at room temperature. The resulting orange solution was filtered and left to stand at ambient temperature for two days. The yellow-orange microcrystalline solid formed was filtered, washed several times with hot water, recrystallized from ethanol and finally dried *in vacuo*.

Yield (70%), mp 187 °C. Elemental analysis found, C, 49.90; H, 5.50; N, 13.60; S, 6.50; $\text{C}_{38}\text{H}_{46}\text{N}_9\text{S}_2\text{PPT}$ requires C, 49.70; H, 5.00; N, 13.70; S, 7.00%. MS (FAB⁺ with *m*NBA matrix) m/z 918.5 for $[\text{Pt}(\text{H}_3\text{L}^1)\text{PPh}_3]^+$. IR (KBr pellets): n/cm^{-1} 3248, 3181 (s, NH); 2924, 2848 (s, CH), 1513 (s, CN), 856 (w, CS-thioamide IV band). ¹H NMR (300.14 MHz, DMSO-*d*₆): δ (ppm) 12.20 (s, ²NH, 1H); 7.92–7.89 (d, ⁴NH, 2H), 7.60–7.45 (m, aromatic protons, 15H); 4.33–4.29, 4.10 (m, CH-cyclohexyl, 2H), 3.30 and 2.5 (s, CH₃-triazol, 3H), 1.85, 1.70 (m, CH₂ equatorial-

cyclohexyl, 10H), 1.40–1.20 (m, CH₂ axial-cyclohexyl, 10H).
³¹P NMR (121.50 MHz, CDCl₃): δ (ppm) 11.20 (PPh₃).

Method 2. Triphenylphosphine (0.007 g, 0.04 mmol) and triethylamine base (1 mL) were added to a suspension of compound **1** (0.026 g, 0.02 mmol) in toluene (20 mL) to give a clear solution, which was stirred at room temperature for 12 h. The orange solid that precipitated was filtered, washed with hot water and Et₂O and dried *in vacuo*.

Crystallization in ethanol allowed us to isolate single red crystals, which were studied by X-ray diffraction techniques.

[(PPh₃)Pd(H₂L¹)PdCl], **4.** To a solution of complex **2** (0.15 g, 0.2 mmol) in toluene was added PdCl₂(PPh₃)₂ metallic salt (0.09 g, 0.02 mmol), in the presence of Et₃N. The reaction mixture was stirred for 2 h at room temperature. The resulting orange solution was filtered and left to stand at ambient temperature for two days. The yellow-orange microcrystalline solid formed was filtered, washed several times with hot water, recrystallized from ethanol and finally dried *in vacuo*.

Yield (32%), mp 128 °C. Elemental analysis found, C, 59.10; H, 5.30; N, 8.05; S, 4.10; C₃₈H₄₄CIN₉S₂PPd₂·2PPh₃ requires C, 59.40; H, 4.95; N, 8.45; S, 4.30%. MS (FAB⁺ with *m*NBA matrix) *m/z* 936.3 for [(M – Cl) + H]⁺. IR (KBr pellets): *n*/cm⁻¹ 3213 (s, NH); 2927, 2851 (s, CH), 1523 (s, CN), 859 (w, CS-thioamide IV band). ¹H NMR (300.141 MHz, DMSO-*d*₆): δ (ppm) 12.70 (s, ²NH); 8.30–8.25 (d, ⁴NH), 8.05–7.80 (m, aromatic protons); 4.45, 4.40 (s, CH-cyclohexyl), 2.90 and 2.75 (s, CH₃-triazol), 1.90 (m, CH₂ equatorial-cyclohexyl), 1.50 (m, CH₂ axial-cyclohexyl). ³¹P NMR (121.50 MHz, CDCl₃): δ (ppm) 29.00 (PPh₃).

Single orange crystals, suitable for single crystal X-ray diffraction analysis, were grown by slow evaporation from a dimethyl-sulfoxide–pentane (3 : 1) solution.

Crystallography

Data were collected on a Bruker X8 APEX II CCD (compounds **2**, **3** and **4**). Crystallographic data and selected interatomic distances and angles are listed in Table 1. For all compounds, the software package SHELXTL was used for space group determination, structure solution, and refinement.⁴¹ The structures were solved by direct methods, completed with difference Fourier syntheses, and refined with anisotropic displacement parameters.

All of the compounds here presented crystallize with solvent molecules: compound **2** contains a disordered ethanol molecule occupying two alternative positions. Compound **3** contains an ethanol molecule, and compound **4** crystallizes with one partially disordered DMSO molecule and one *n*-pentane molecule.

In vitro antiproliferative activity

The human cancer cells A2780, A2780cisR and NCI-H460 were grown in an RPMI-1640 medium supplemented with 10% foetal bovine serum (FBS) and 2 mM L-glutamine in an atmosphere of 5% CO₂ at 37 °C. The human cancer cells MCF-7 were grown in an EMEM medium with 2 mM L-glutamine and Earle's BBS (adjusted with 1.5 g L⁻¹ NaHCO₃, 0.1 mM nonessential amino acids and 1 mM sodium pyruvate) and supplemented with 10% FBS and 0.01 mg mL⁻¹ bovine insulin in an atmosphere of 5%

CO₂ at 37 °C. The human cancer cells Hep-G2 were grown in an EMEM medium supplemented with 10% FBS in an atmosphere of 5% CO₂ 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 × 10⁴ (for NCI-H460), 1 × 10⁴ (for MCF-7 and Hep-G2) or 4 × 10³ (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⁻¹) 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⁻¹ of sodium bicarbonate in an atmosphere of 5% CO₂ 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 × 10⁴ 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⁻¹) 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 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 effects of complexes 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*_{max} is the maximal effect, IC₅₀ 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 2.01, 1996 software.⁴²

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.

DNA-binding experiments

CT-DNA stock solution was prepared by dissolving the lyophilized sodium salt in Tris-buffer (NaCl 50 mM, Tris-HCl 5 mM, pH was adjusted to 7.3 with NaOH 0.5 M) by stirring at 4 °C for 2 days. The CT-DNA solution was standardized spectrophotometrically⁴³ by using its known molar absorption coefficient at 260 nm ($6600 \text{ M}^{-1} \text{ cm}^{-1}$). The ratio of UV absorbance at 260 and 280 nm, A_{260}/A_{280} , was *ca.* 1.9, indicating that the DNA was sufficiently free of protein. Stock solutions were kept frozen until the day of the experiment.

Concentrated stock solutions ($5 \times 10^{-3} \text{ M}$) of both the ligand and complex **1** were prepared by dissolving each one of the compounds in DMSO. From these stock solutions, for all experiments the desired concentration of the compound was achieved by dilution with Tris-buffer (NaCl 50 mM, Tris-HCl 5 mM, pH was adjusted to 7.3 with NaOH 0.5 M) to give homogeneous solutions with DMSO contents of less than 2.5%.

In order to prepare the adducts with double-stranded CT-DNA, to a mixture of a fixed amount of CT-DNA ($1.25 \times 10^{-4} \text{ M}$) in Tris buffer were added predetermined quantities of the desired compound solution to achieve different *R* values ($R = [\text{CT-DNA}]/[\text{compound}]$). The mixtures were incubated for 10 min at 37 °C, and their UV-vis absorption spectra were recorded, at room temperature, in the wavelength interval of 240–400 nm.

Conclusions

In summary, a new family of Pt(II) and Pd(II) compounds, **1–4**, containing the 3,5-diacetyl-3,5-triazol bis(4-cyclohexyl thiosemicarbazone) ligand, **H₅L¹**, as a platform for metal arrangement, has been successfully prepared and characterized.

This study has identified both the free ligand **H₅L¹** and the Pt(II) dimer complex **1** as having promising antiproliferative activity since they are capable of not only circumventing cisplatin resistance in A2780cisR cells but they also exhibit antiproliferative activity against lung NCI-H460 cancer cells. The ligand also exhibits antiproliferative activity against breast (MCF-7) cancer cells. Moreover, it is important to note that these two compounds exhibit, *in vitro*, very low renal toxicity with respect to cisplatin.

Taking into account that these compounds are active in A2780cisR cisplatin resistant cells and, moreover, both ligands and platinum(II) complexes do efficiently interact with CT-DNA by inducing structural changes on DNA secondary structure different from those induced by cisplatin, it is most likely that part of the biochemical mechanism of action of these bis(thiosemicarbazone) compounds involves groove binding. The compounds synthesized here are neutral, aromatic and hydrophobic molecules and therefore should tend to accumulate within the lipophilic DNA grooves.

The studies reported here constitute a good example of how small changes in molecular structure lead to profound differences in biological activity. In our opinion, the conjunction of serendipity and rational design (probability) plays a significant role in cancer drug discovery due to the existence of several very specific factors to be considered.

Based on these results the **H₅L¹** ligand and the platinum dimer complex **1**, reported here, represent a valuable lead in the development of new anticancer chemotherapeutic agents capable of improving antitumor activity and reducing nephrotoxicity.

Acknowledgements

We are grateful to Ministerio de Economía y Competitividad, Instituto de Salud Carlos III (PI080525 and PI1100659), of Spain for financial support.

Notes and references

- 1 P. A. Vigato, V. Peruzzo and S. Tamburini, *Coord. Chem. Rev.*, 2012, **256**, 953–1114.
- 2 J. Klingele, S. Dechert and F. Meyer, *Coord. Chem. Rev.*, 2009, **253**, 2698–2741.
- 3 A. Prokofieva, A. I. Prikhod'ko, E. A. Enyedy, E. Farkas, W. Maringgele, S. Demeshko, S. Dechert and F. Meyer, *Inorg. Chem.*, 2007, **46**, 4298–4307.
- 4 C. Belle and J. Pierre, *Eur. J. Inorg. Chem.*, 2003, **23**, 4137–4146.
- 5 L. A. Oro, M. A. Ciriano, J. J. Pérez-Torrente and B. E. Villarroya, *Coord. Chem. Rev.*, 1999, **193–195**, 941–975.
- 6 J. G. Haasnoot, *Coord. Chem. Rev.*, 2000, **200–202**, 131–185.
- 7 A. Eisenwiener, M. Neuburger and T. A. Kaden, *Dalton Trans.*, 2007, 218–233.
- 8 F. Zhang and L. Shen, *J. Chem. Crystallogr.*, 2010, **40**, 681–685.
- 9 P. A. Vigato and S. Tamburini, *Coord. Chem. Rev.*, 2008, **252**, 1871–1995.
- 10 T. S. Lobana, G. Bawa and R. J. Butcher, *Inorg. Chem.*, 2008, **47**, 1488–1495.
- 11 R. Pedrido, A. M. Gonzalez-Noya, M. J. Romero, M. Martínez-Calvo, M. Vazquez Lopez, E. Gómez-Forneas, G. Zaragoza and M. Bermejo, *Dalton Trans.*, 2008, 6776–6787.
- 12 D. X. West, S. Padhye and P. B. Sonawane, *Struct. Bonding (Berlin)*, 1991, **76**, 1–49.
- 13 J. S. Casas, M. S. García-Tasende and J. Sordo, *Coord. Chem. Rev.*, 2000, **209**, 197–261.
- 14 T. S. Lobana, R. Sharma, G. Bawa and S. Khanna, *Coord. Chem. Rev.*, 2009, **253**, 977–1055.
- 15 A. Gómez and C. Navarro, *Coord. Chem. Rev.*, 2004, **248**, 119–133.
- 16 A. I. Matesanz, C. Pastor and P. Souza, *Inorg. Chem. Commun.*, 2007, **10**, 97–100.
- 17 A. I. Matesanz and P. Souza, *J. Inorg. Biochem.*, 2007, **101**, 245–253.
- 18 A. I. Matesanz, C. Joie and P. Souza, *Dalton Trans.*, 2010, **39**, 7059–7065.
- 19 A. I. Matesanz, C. Hernández, A. Rodríguez and P. Souza, *Dalton Trans.*, 2011, **40**, 5738–5745.
- 20 K. Liu, W. Shi and P. Cheng, *Dalton Trans.*, 2011, **40**, 8475–8475.
- 21 M. C. Liu, T. S. Lin and A. C. Sartorelli, *J. Med. Chem.*, 1992, **35**, 3672–3677; M. Liu, T. Lin and A. C. Sartorelli, *Prog. Med. Chem.*, 1995, **32**, 1–35.
- 22 R. A. Finch, M. C. Liu, A. H. Cory, J. G. Cory and A. C. Sartorelli, *Adv. Enzyme Regul.*, 1999, **39**, 3–12; R. A. Finch, M. C. Liu, S. P. Grill, S. P. W. C. Rose, R. Loomis, K. M. Vasquez, Y. C. Cheng and A. C. Sartorelli, *Biochem. Pharmacol.*, 2000, **59**, 983–991.
- 23 A. B. Alvero, W. Chen, A. C. Sartorelli, P. Schwartz, T. Rutherford and G. Mor, *J. Soc. Gynecol. Invest.*, 2006, **13**, 145–152.
- 24 O. M. Ni Dhubhghaill and P. J. Sadler, in *Metal Complexes in Cancer Chemotherapy*, ed. B. K. Keppler, VCH, Weinheim, 1993, p. 221.
- 25 A. I. Matesanz, C. Hernández, A. Rodríguez and P. Souza, *J. Inorg. Biochem.*, 2011, **105**, 1613–1622.
- 26 V. K. Jain and L. Jain, *Coord. Chem. Rev.*, 2005, **249**, 3075–3197.

- 1 27 P. Souza, A. I. Matesanz and C. Pastor, *Inorg. Chem. Commun.*, 2002, **5**, 344–346. 1
- 28 N. J. Wheate, S. Walker, G. E. Craig and R. Oun, *Dalton Trans.*, 2010, **39**, 8113–8127.
- 29 E. Wong and C. M. Giandomenico, *Chem. Rev.*, 1999, **99**, 2451–2466.
- 5 30 Z. Guo and P. J. Sadler, *Angew. Chem., Int. Ed.*, 1999, **38**, 1512–1531.
- 31 M. S. Razzaque, *Nephrol. Dial. Transplant.*, 2007, 1–5. 5
- 32 M. Okuda, K. Masaki, S. Fukatsu, Y. Hashimoto and K. Inui, *Biochem. Pharmacol.*, 2000, **59**, 195–201.
- 33 P. V. Bernhardt, P. C. Sharpe, M. Islam, D. B. Lovejoy, D. S. Kalinowski and D. R. Richardson, *J. Med. Chem.*, 2009, **52**, 407–415.
- 10 34 C. R. Kowol, R. Berger, R. Eichinger, A. Roller, M. A. Jakupec, P. P. Schmidt, V. B. Arion and B. K. Keppler, *J. Med. Chem.*, 2007, **50**, 1254–1265. 10
- 35 C. Marzano, A. Trevisan, L. Giovagnini and D. Fregona, *Toxicol. in Vitro*, 2002, **16**, 413–419.
- 36 J. S. Casas, E. E. Castellano, J. Ellena, M. S. García-Tasende, M. L. Pérez-Parallé, A. Sánchez, A. Sánchez-González, J. Sordo and A. Touceda, *J. Inorg. Biochem.*, 2008, **102**, 33–45. 1
- 37 F. Arjmadnd and M. Aziz, *Eur. J. Med. Chem.*, 2009, **44**, 834–844.
- 38 C. D. Brandt, J. A. Kitchen, U. Beckmann, N. G. White, G. B. Jameson and S. Brooker, *Supramol. Chem.*, 2007, **19**, 17–27. 5
- 39 W. L. Steffen and G. J. Palenik, *Inorg. Chem.*, 1976, **15**, 2432–2439.
- 40 W. P. Griffith, in *Inorganic Experiments*, ed. J. D. Woolins, Wiley VCH, Weinheim, 3rd edn, 2010, pp. 145–148.
- 41 *SHELXTL-NT version 6.12, Structure Determination Package*, Bruker-Nonius AXS, Madison, Wisconsin, USA, 2001.
- 42 *GraphPad Prism, version 2.01*, GraphPad Software, Inc., San Diego, CA, 1996. 10
- 43 M. E. Reichmann, S. A. Rice, C. A. Thomas and P. Doty, *J. Am. Chem. Soc.*, 1954, **76**, 3047–3053. 15