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Activity on *Trypanosoma cruzi*, erythrocytes lysis and biologically relevant physicochemical properties of Pd(II) and Pt(II) complexes of thiosemicarbazones derived from 1-indanones

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ABSTRACT

American trypanosomiasis or Chagas disease, caused by the protist parasite *Trypanosoma cruzi* (*T. cruzi*), is a major health concern in Latin America. In the search for new bioactive compounds, eight Pd(II) and Pt(II) complexes of thiosemicarbazones derived from 1-indanones (HL) were evaluated as potential anti-*T. cruzi* compounds. Their unspecific cytotoxicity was determined on human erythrocytes. Two physicochemical features, lipophilicity and redox behavior, that could be potentially relevant for the biological activity of these complexes, were determined. Crystal structure of $[Pd(H\mathbf{L1})(\mathbf{L1})]Cl\cdot CH_3OH$, where H**L1** = 1-indanone thiosemicarbazone, was solved by X-ray diffraction methods. Five of the eight metal complexes showed activity against *T. cruzi* with IC_{50} values in the low micromolar range and showed significantly higher activity than the corresponding free ligands. Four of them resulted more active against the parasite than the reference antitrypanosomal drug Nifurtimox. Anti-*T. cruzi* activity and selectivity towards the parasite were both higher for the Pd(II) compounds than for the Pt(II) analogues, showing the effect of the metal center selection on the biological behavior. Among both physicochemical features tested for this series of compounds, lipophilicity and redox behavior, only the former seemed to show correlation with the antiproliferative effects observed. Metal coordination improved bioactivity but lead to an increase of mammalian cytotoxicity. Nevertheless, some of the metal complexes tested in this work still show suitable selectivity indexes and deserve further developments.

1. Introduction

American trypanosomiasis or Chagas disease, caused by the protist parasite *Trypanosoma cruzi* (*T. cruzi*), is a major health concern in Latin America. Despite the decrease in the incidence of new infections through enforcement of public health programs, e.g. vector and blood transfusion controls, it is still endemic in large areas of Central and South America. Furthermore, globalization and immigration of unknowingly infected people from Latin America has also led to several infection cases in developed countries, mainly due to lack of controls and screening in blood and organ banks and to immigrant mother to child transmission during pregnancy. The chemotherapy of this parasitic infection remains undeveloped and no effective method of immune prophylaxis is available. The treatment has been based on the old and quite unspecific nitroaromatic drugs, Nifurtimox and Benznidazole, that have significant activity only in the acute phase of the disease and cause severe side effects [1–7]. Novel bioactive compounds are needed to develop an effective and safe therapy against this "neglected" disease. Due to the lack of interest of the pharmaceutical industry in developing new drugs for its treatment, current efforts in this direction mainly rely on academic basic research.

Thiosemicarbazones have shown a wide range of bioactivities, and their chemistry and pharmacological applications have been extensively investigated [8]. Among them, some families of thiosemicarbazones have demonstrated interesting trypanosomicidal activities [9–14]. In particular, the thiosemicarbazone functionality has been included into

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compounds designed to inhibit cruzipain, a cystein protease expressed in all life cycle stages of the parasite. In the search for a pharmacological control of Chagas' disease, our group has previously designed new 5-nitrofuran derivatives and new 1-indanone derivatives containing the thiosemicarbazone moiety that have shown significant anti-*T. cruzi* activity [9,15]. In particular, many of the 1-indanones derivatives showed anti-*T. cruzi* activities of the same order of the reference trypanosomicidal drug Nifurtimox, together with low unspecific mammalian cytotoxicities [15].

The development of bioactive metal complexes is a promising approach in the search for new potential drugs. Different attempts towards developing trypanosomicidal metal-based compounds have been described [16–22]. Currently, we are successfully working on the development of metal-based potential antitrypanosomal agents [16,17,22]. Most of them are based on the metal coordination of trypanosomicidal organic ligands. The obtained metal compounds could act through dual or even multiple mechanisms of action by combining the pharmacological properties of both the ligand and the metal, or could at least lead to additive effects or to circumvention of drug resistance phenomena. The development of agents that act against different parasitic targets could diminish host toxic effects by lowering therapeutic dose. Through this approach we have exhaustively studied Pd(II), Pt(II), Ru(II) and Ru(III) coordination compounds of bioactive 5-nitrofuryl and 5-nitroacroleine containing thiosemicarbazones [22–27]. Many of the Pd and Pt compounds showed increased antitrypanosomal activity in respect to the free ligands with retention of the ligand's mechanism of action and, additionally, significant interaction with DNA, suggesting this biomolecule as a second molecular target [23–25,27,28].

In a recent work we synthesized and characterized four $[MCl_2(HL)]$ and four [M(HL)(L)]Cl novel complexes, where M = Pd(II) or Pt(II) (Fig. 1, compounds **3**–**10**) and L = 1-indanone containing thiosemicarbazones **1** and **2** (Fig. 1) [29]. The eight metal compounds showed antiproliferative activity against the human leukemia U937 cell line. Platinum (II) complexes displayed selective apoptotic activity in U937 cells but not in peripheral blood monocytes or the human hepatocellular carcinoma HepG2 cell line used to screen for potential hepatotoxicity, suggesting that they are promising compounds with potential therapeutic application against hematological malignancies [29]. Metabolic pathways of *Trypanosoma* parasites are supposed to be similar to those present in tumor cells and other highly proliferative cells, leading in many cases to a correlation between antitrypanosomal and antitumor activities [18,30]. We extensively explored this hypothesis through the development of new metal complexes or the study of known ones that showed both, antitrypanosomal and antitumor activities [17,31–33]. Based on this hypothesis, in this work the *in vitro* antiproliferative activity on *T. cruzi* and the unspecific mammalian cytotoxicity, tested on red blood cells, of the eight previously developed Pd(II) and Pt(II) complexes of the 1-indanone containing thiosemicarbazones **1** and **2** are presented (Fig. 1). In addition, some physicochemical properties considered potentially relevant for the biological activity of these $[MCl_2(HL)]$ and [M(HL)(L)]Cl complexes were studied. Characterization of the previously reported Pd(II) and Pt(II) thiosemicarbazone compounds was expanded by the resolution of the crystal structure of complex **7**, [Pd(H**L1**)(**L1**)] $Cl \cdot CH_3OH$, by X-ray diffraction methods.

2. Experimental

2.1. Materials

Common laboratory chemicals were purchased from commercial sources and used without further purification.

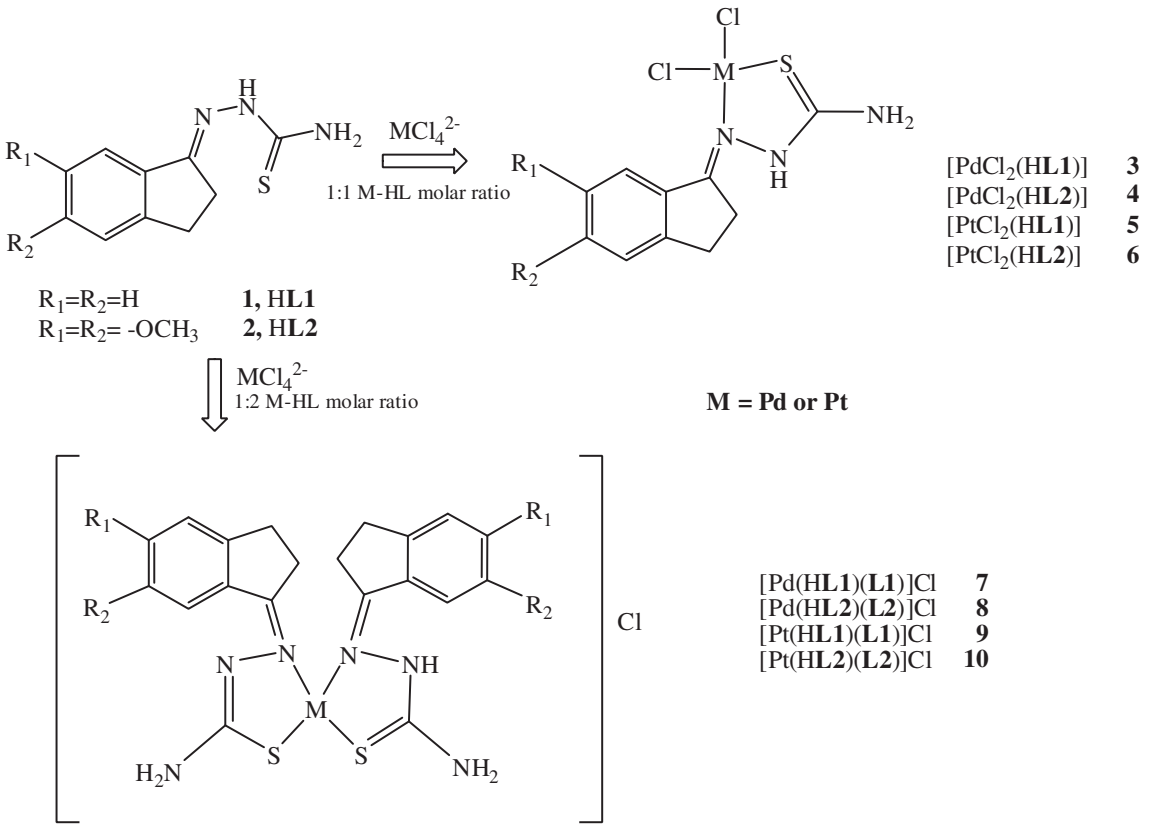

Fig. 1. Thiosemicarbazones derived from 1-indanones selected as ligands and their palladium (II) and platinum (II) complexes [29].

2.2. Synthesis of the ligands

Thiosemicarbazones derived from 1-indanones **1** and **2** (Fig. 1) were obtained from the corresponding 1-indanone and thiosemicarbazide according to the general procedure previously described and were characterized as previously reported [15,34].

2.3. Synthesis of the metal compounds

Palladium and platinum complexes of the formulae $[MCl_2(HL)]$ (M = Pt or Pd) (Fig. 1, compounds **3**–**6**) were synthesized by ligand substitution on $Na_2[PdCl_4]$ or $K_2[PtCl_4]$, using a 1:1 metal to ligand molar ratio as previously reported [29]. [M(HL)(L)]Cl complexes (Fig. 1, compounds **7**–**10**) were synthesized by ligand substitution on the same precursors, but using a 1:2 metal to ligand molar ratio [29].

2.4. Crystallographic study

Crystals of [Pd(H**L1**)(**L1**)]Cl·CH_3OH suitable for X-ray diffraction studies were obtained from the synthesis solution. Data were collected at 125(2) K by using graphite monochromated Mo Kα radiation (λ = 0.710 73 Å) in a Bruker SMART APEX II CCD X-ray diffractometer. Structure resolution and refinement were performed using ShelX [35]. Details are included in Table 1. Those H atoms not found in Fourier maps were included from models and constrained as riding on their bound atoms. This crystal structure has been deposited as CCDC 826369.

2.5. Physicochemical properties of the compounds

Some physicochemical properties of the compounds that could be biologically relevant were studied.

2.5.1. Stability in solution

Stability of the complexes was tested in DMSO and DMF (dimethyl formamide) solutions for 5 days by conductivity measurements [36].

2.5.2. Cyclic voltammetry studies

Cyclic voltammetry measurements were performed under nitrogen atmosphere at room temperature using an Epsilon electrochemical analyzer. All the experiments were carried out in 1 mM DMSO solutions (DMSO, spectroscopic grade) of the compounds containing 0.1 M tetrabutylammonium hexafluorophosphate ($TBAPF_6$) as supporting electrolyte. A conventional three electrode cell was used with a hanging drop mercury electrode (BAS controlled growth mercury electrode) as working electrode, a Pt wire as the auxiliary electrode and a Ag/Ag^+(0.01 M in DMSO/ 0.1 M $TBAPF_6$) as the reference electrode.

Table 1
Crystal data and refinement details of *cis*-(1-indanone-thiosemicarbazonato) (1-indanone thiosemicarbazone)palladium(II) chloride-methanol (1:1).

Empirical formula	$C_{21}H_{25}ClN_6OPdS_2$
Crystal color	Red
Formula weight	583.47
Crystal System	Monoclinic
Space group	C 2/c
Temperature K	125(2)
Wavelength (Å)	0.71073
a (Å)	25.396(6)
b (Å)	17.041(4)
c (Å)	11.096(3)
β (º)	106.986(3)
Volume ($Å^3$)	4593.1(1.8)
Z, density (mg/mm^3)	4, 1.638
Absorption coefficient	1.113
Crystal size (mm)	0.29 × 0.07 × 0.05
θ range data collection	1.46, 28.28
Limiting índices	−33,33/−22,22/−14,14
Data collected /unique	29631, 5693
Max, min. transmission	0.84/0.95
Refinement method	F^2
Refined data/parameters	4602/373
Goodness-of-fit on F^2	1.029
Final R, Rw [I > 2sigma(I)]	0.0302/0.0765

2.5.3. Lipophilicity

Experimental logarithms of capacity factor (log *k*) were calculated using a Waters Alliance® HPLC equipment (Separation Module e2695, PDA Detector 2998) with a Xbridge column C8 5.0 μm, 4.6 × 150 mm (Waters Corp., Milford, MA, USA). Stock solutions of each compound (3.5 μg/ml in DMSO) were injected (10 μl) and a mobile phase composed of acetonitrile-buffer phosphate pH 7.0 (29 mM) in different volume ratios (30:70, 35:65, 40:60, 45:55 and 50:50) was pumped at a flow rate of 1.0 ml min^{-1} and the PDA Detector was set at 254 nm. Logarithms of capacity factor (log *k*) were calculated as follows:

$$\log k = \log[(t_r - t_0)/t_0]$$

t_r and t_0 being the retention time and the dead time, respectively.

A curve of log *k* vs the percentage of acetonitrile (%) in the mobile phase was built and log k_{water} (log k_w) values were extrapolated at 0% acetonitrile [37–39].

2.6. Biological evaluation

2.6.1. In vitro antitrypanosomal activity

T. cruzi epimastigotes (Tulahuen 2 strain) were grown at 28 °C in an axenic medium (BHI-Tryptose) as previously described complemented with 5% fetal calf serum [23–25]. Cells were harvested in the late log phase, re-suspended in fresh medium, counted in Neubauer's chamber and placed in 24-well plates (2×10^6/mL). Cell growth was measured as the absorbance of the culture at 590 nm, which was proved to be proportional to the number of cells. Before inoculation, the media were supplemented with the indicated amount of the studied compound from a stock solution in DMSO. The final concentration of DMSO in the culture media never exceeded 1% and the control was run in the presence of 1% DMSO and in the absence of any compound. No effect on epimastigotes growth was observed by the presence of up to 1% DMSO in the culture media. Nifurtimox was used as the reference trypanosomicidal drug. The percentage of growth inhibition was calculated as follows $\{1 - [(Ap - A_0p)/(Ac - A_0c)]\} \times 100$, where $Ap = A_{590}$ of the culture containing the studied compound at day 5; $A_0p = A_{590}$ of the culture containing the studied compound right after addition of the inocula (day 0); $Ac = A_{590}$ of the culture in the absence of any compound (control) at day 5; $A_0c = A_{590}$ in the absence of the compound at day 0. To determine IC_{50} values, parasite growth was followed in the absence (control) and presence of increasing concentrations of the corresponding compound. The IC_{50} values were determined as the drug concentrations required to reduce by half the absorbance of that of the control (without compound).

2.6.2. Unspecific mammalian cytotoxicity: red blood cell lysis assay

Human blood collected in sodium citrate solution (3.8%) was centrifuged at 1500 rpm for 10 min at 4 °C. The plasma supernatant was removed and the erythrocytes were suspended in ice cold PBS (phosphate buffered saline). The cells were again centrifuged at 1500 rpm for 10 min at 4 °C. This procedure was repeated two more times to ensure the removal of any released hemoglobin. Once the supernatant was removed after the last wash, the cells were suspended in PBS to get a 2% w/v red blood cell solution. A volume of 400 μL of studied compounds, in PBS (final concentration 50, 100 and 200 μM), negative control (solution of PBS), or Amphotericin B (final concentration 1.5 μM) were added to 400 μL of the 2% w/v

red blood cell solution in ten microcentrifuge tubes for each concentration and incubated for 24 h at 37 °C. Complete hemolysis was attained using neat water yielding the 100% control value (positive control). After incubation, the tubes were centrifuged and the supernatants were transferred to new tubes. The release of hemoglobin was determined by spectrophotometric analysis of the supernatant at 405 nm. Results were expressed as percentage of the total amount of hemoglobin released by action of the compounds. This percentage is calculated using the equation: Hemolysis percentage (%) = $[(A_1 - A_0)/A_1$ water$] \times 100$, where A_1 is the absorbance at 405 nm of the test sample at t = 24 h, A_0 is the absorbance at 405 nm of the test sample at t = 0 h, and A_1 water is the absorbance at 405 nm of the positive control (water) at t = 24 h. The experiments were done by quintuplicate [14,40].

3. Results and discussion

3.1. Crystal structure of [Pd(HL1)(L1)]Cl·CH_3OH

Characterization of the previously reported Pd(II) and Pt(II) thiosemicarbazone compounds was expanded in this work by solving the crystal structure of [Pd(H**L1**)(**L1**)]Cl·CH_3OH (Fig. 2).

The molecular structure of the title compound resembles the one recently published by us, *cis*-[Pt(H**L1**)(**L1**)]Cl·$2CH_3OH$ [29], which has a Pt atom instead of the current Pd center. The coordination polyhedron is similar to that of the Pt complex. Only one methanol solvent of crystallization is found in the title compound; the C(methanol) atom lies on the 2-fold axis and its bound O(methanol) generates a false image [distance O(methanol) − O′(methanol) = 1.226(7) Å]. Such unrealistic separation makes us to conclude that the 2-fold axis is pseudo-symmetric.

There are some differences between the Pt and Pd complexes, as indicated by the smaller *trans* angles N-Pd-S [168.42(6)° and 169.81(6)°] compared to N-Pt-S [173.2(2)° and 175.5(2)°]. The Pd – S bonds in the title compound are different (Table 2), similarly to previously reported for the Pt complex [29]. The Pd – N bond distances are significantly different, 2.064(2) Å and 2.118(2) Å; this feature was not seen in the Pt complex because of its higher standard deviations that made Pt – N bond lengths indistinguishable. In the Pd complex the packing shows π–π stacking between different molecules, with C5--C9′ = 3.594(4) Å being the shortest separation; this feature is not found in the Pt complex. H-bonds are found between Cl and 3 N atom [Cl – N3 = 3.207(3) Å, [Cl – N7 = 3.142(2) Å, Cl′ – N7′ = 3.172(3) Å], related features were also seen in the Pt complex [29]. Further comparison between both complexes is seen in Fig. 3, showing the best fit of overlay between both molecules.

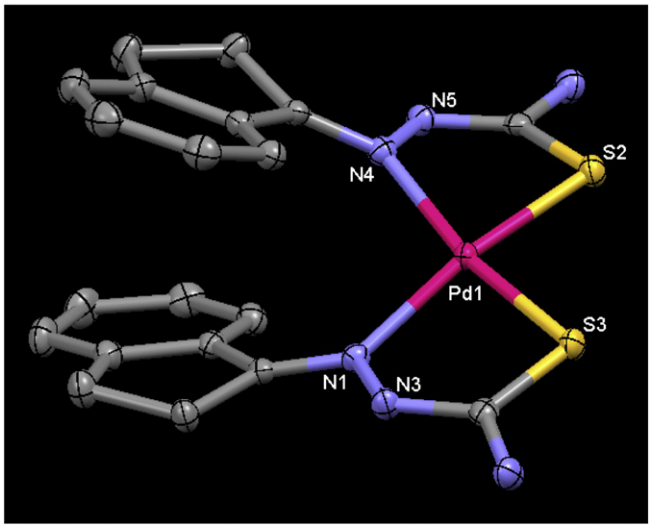

Fig. 2. Ortep drawing of [Pd(H**L1**)(**L1**)]Cl·CH_3OH with H, methanol and Cl atoms omitted.

The coordination sphere is planar distorted as the sum of the four bond angles at the metal center is 361.5°, instead of the expected 360°. This distortion is imposed by the *cis* arrangement, which induces hindrance between the ligand aromatic moieties so that the N(4) – Pd – N(1) bond angle, 102.91(8)°, is much wider than the other *cis* angles in the coordination sphere, Table 2. The equivalent N – Pt – N bond angle is smaller, 99.8(4)°, suggesting a greater distortion in the title compound.

3.2. Physicochemical properties of the compounds

The compounds were stable in DMSO or DMF (dimethylformamide) solution for at least the 5-days evaluation period. No significant conductivity changes could be detected.

3.2.1. Cyclic voltammetry results

Owing to its potential relationship with biological activity, the redox behavior of the ligands **1** and **2** and the eight Pt and Pd complexes were studied by cyclic voltammetry at different scan rates (v) in DMSO solutions. Even though compounds [M(HL)(L)]Cl were also investigated they were not included in the current electrochemical study because they exhibited an erratic voltammetric pattern. Their electrochemical response changed with time and no reproducible curves were obtained. Adsorption processes on the electrode surface of the initial electroactive complex and/or new electroactive substances generated after the reduction path could be responsible of the observed behavior. The voltammetric data for the complexes $[MCl_2(HL)]$ and the corresponding ligands are presented in Table 3.

The electrochemical response of both free ligands, **1** and **2**, are qualitatively similar. The cyclic voltammograms (CVs) of **1** at different v, in the potential range where it is electroactive, is presented in Fig. 4. The compound shows one reduction/oxidation process (Ic/Ia) at −0.28 V/−0.16 V, respectively. With increasing scan rates, the response is characterized by a shift of Epc and Epa towards more difficult reactions, a small decrease of $ipc/v^{1/2}$ and an increase in ΔEp (Epc − Epa), which is consistent with a quasi-reversible one-electron reduction of the compound. When the cathode potential limit is shifted to more negative values no further reductions are detected (see inset Fig. 5). The irreversible pre-wave between 0.00 V and −0.20 V can be attributed to an adsorption process on the electrode surface [41].

Fig. 5 shows the voltammetric behavior of [$PdCl_2$(H**L1**)], **3**, and [$PtCl_2$(H**L1**)], **5**, complexes in the potential range 0.0 and −1.95 V. As can be seen, three subsequent reductions are evident for compound **5** between 0.0 V and −1.0 V. In contrast, complex **3** exhibits only two reductions in the same potential range. On the return scan two oxidations are observed which are found at similar potentials in both complexes. The first couple, Ic/Ia, is assigned to the reduction and the oxidation processes centered on the coordinated H**L1** ligand. This couple has analogous redox behavior to those described earlier for the free ligand and appears at slightly less negative potential as a consequence of coordination.

The redox behavior of the second couple, IIc/IIa, which appears at similar potential values in both complexes depends of the nature of the metal center (see below). A cathodic reduction peak IIIc at more

Table 2
Selected bond distances and bond angles of the title compound.

Pd-N(1)	2.118(2)	Pd-S(3)	2.283(7)
Pd-N(4)	2.064(2)	Pd-S(2)	2.255(8)
N(1)-Pd-S(3)	84.85(6)	N(4)-Pd-S(2)	81.53(6)
N(4)-Pd-N(1)	102.91(8)	S(3)-Pd-S(2)	92.24(2)
N(4)-Pd-S(3)	168.42(6)	N(1)-Pd-S(2)	169.81(6)
Pd-S(3)-C(21)	97.51(9)	Pd-S(2)-C(20)	94.35(8)
Pd-N(4)-N(5)	116.2(1)	Pd-N(1)-N(3)	111.1(1)

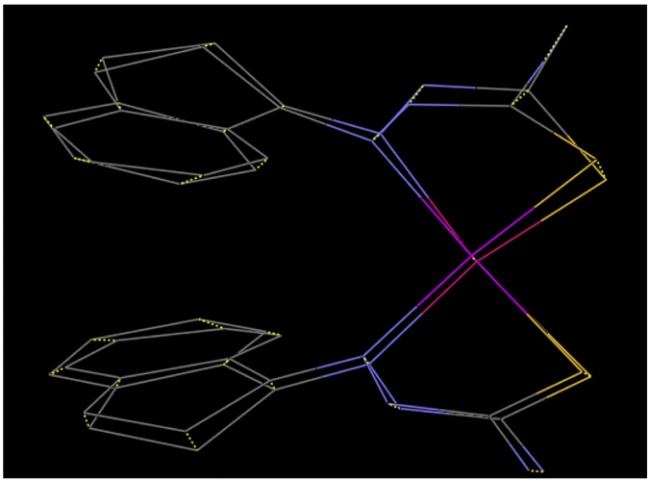

Fig. 3. Overlay of the cationic moiety of the title compound and its equivalent Pt complex.

negative potential, which is clearly observed only for complex **5**, gives no reverse signal on the subsequent anodic scan. This peak remains irreversible at all scan rates investigated and shifts towards more negative potential as *v* increases.

The present electrochemical study provides a basis for concluding that the two well defined peaks IIc and IIIc for the platinum complex are consistent with two consecutive one electron reductions of the metal center, Pt(II)/Pt(I) and Pt(I)/Pt(0), respectively. The monovalent species is reoxidized to Pt(II) on the reverse scan at the potential of peak IIa. The peak to peak separation of the couple IIc/IIa increases with the scan rate in the expected way for a slow heterogeneous electron transfer (quasi-reversible behavior) [41]. This slow electron transfer may be indicative of geometric changes in the coordination sphere of the complex. The absence of a reoxidation peak IIIa would suggest that the zerovalent metal complex has a short half-life. Thus, after the second reduction path, the Pt(0) compound probably undergoes a fast follow-up homogeneous chemical reaction which would be responsible of the apparent irreversibility of peak IIIc. The current contributions at more negative potentials (−1.25 V and −1.43 V) were not analyzed in detail but they could be tentatively related to adsorption and exchange reactions of the reduction products with the mercury electrode. In contrast, the palladium complex **3** shows only one well defined peak (IIc) associated with the reduction of the metal center. On the return scan, the reduced species is oxidized at the potential of peak IIa. For this compound the cathodic current IIc is about twice of that obtained with compound **5**. Therefore, it is possible to assume that either both reductions occur simultaneously at the potential of peak IIc or they occur at very close potential values with generation of the Pd(0) complex. The peak potential separation, $\Delta Ep = Epc(IIc) - Epa(IIa)$, is higher than expected for a one reversible two electron charge transfer (28.5 mV at 25 °C) suggesting the involvement of two independent one-electron steps at close potential values ($\Delta E°$).

Table 3
Voltammetric peak potential data for reduction and oxidation of the different compounds in DMSO solution.

Compound	Peak (I)		Peak (II)		Peak (III)
	Epc(Ic)/V	Epa(Ia)/V	Epc(IIc)/V	Epa(IIa)/V	Epc(IIIa)/V
1, H**L1**	−0.28	−0.16			
5, [$PtCl_2$(H**L1**)]	−0.22	−0.15	−0.43	−0.36	−0.68
3, [$PdCl_2$(H**L1**)]	−0.23	−0.15	−0.46	−0.36	–
2, H**L2**	−0.31	−0.15			
6, [$PtCl_2$(H**L2**)]	−0.21	−0.14	−0.41	−0.34	−0.68
4, [$PdCl_2$(H**L2**)]	−0.20	−0.15	−0.43	−0.36	–

Scan rate 0.25 V/s, Epc = cathodic peak potential, Epa = anodic peak potential (values in V vs Ag/Ag^+ reference electrode).

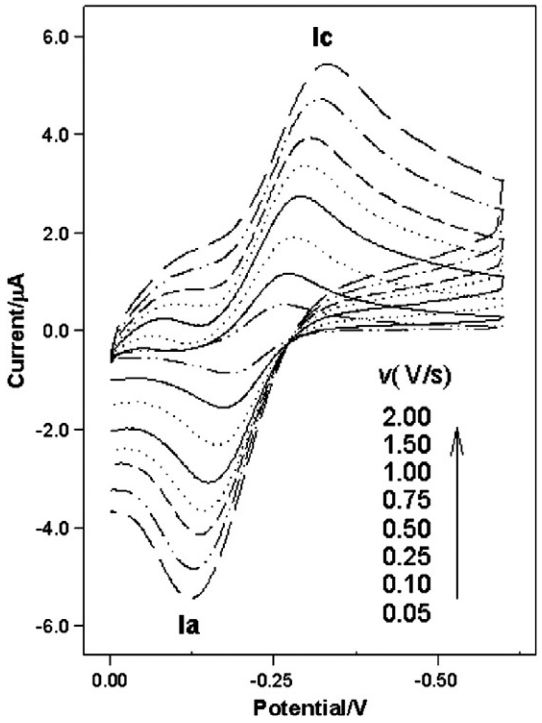

Fig. 4. Cyclic voltammograms of compound **1** in DMSO solution obtained at different *v* in the potential range between 0.0 V and −0.75 V.

With increasing *v*, the Epc and Epa peak potentials are shifted towards more negative and positive values, respectively. The electrochemical behavior suggests that the reduction/oxidation processes associated with the metallic center (IIc/IIa) occur by an ErEq mechanism, where the first electron transfer, at $E°_1$, is reversible (fast) and the second, at $E°_2$, is quasi-reversible (slow electrode kinetics). As the cathodic or anodic waves do not split when the scan rate increases, the second one-electron reduction step appears to be easier than the first one ($E°_2 > E°_1$). Thus, when the Pd(I) species is formed it is readily reduced to Pd(0) because the reaction is occurring at more negative potentials than $E°_2$ [41]. The generated Pd(0) is stable on the voltammetric time scale, as can be deduced from the current peak ratio ipc/ipa, which is about one at all scan rates investigated.

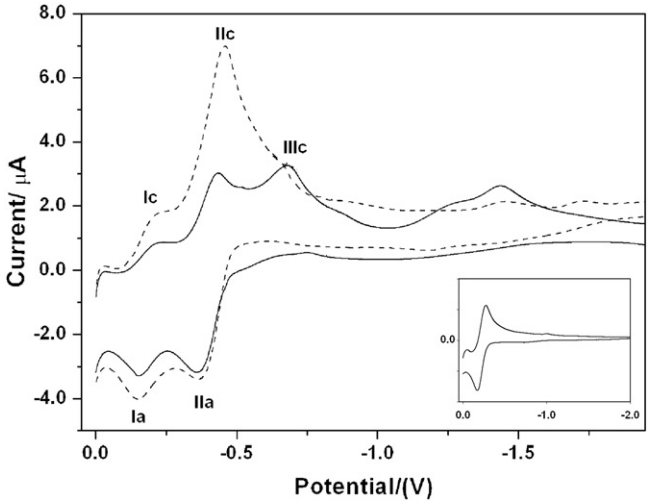

Fig. 5. Cyclic voltammograms of compounds **5**, solid line, and **3**, dashed line, obtained at *v* = 0.25 V/s between 0.0 V and −2.0 V. Inset of the figure: CV of H**L1** in the same potential range.

Finally, for [$PtCl_2$(HL2)], **6**, and [$PdCl_2$(HL2)], **4**, the main electrode processes are similar to those described for compounds **3** and **5**. Notwithstanding, some differences were detected; for these compounds additional sharp peaks were observed at different potentials as a consequence of adsorption processes on the electrode surface (data not shown).

The similar reduction potentials observed for both free ligands, **1** and **2**, suggest that the inclusion of the OCH_3 groups on the benzene ring of H**L2** does not affect the energetic of the electron transfer. Furthermore, the similar electrochemical response obtained for both ligands suggests that their different bioactivity cannot be ascribed to their redox behavior. In addition, it was not possible to infer a clear correlation between reduction potentials of the complexes and their anti-*T. cruzi* activity. Although the process associated with the reduction of the coordinated ligands occurs at slightly less negative potentials than those of the H**L1** and H**L2** free species, this shifting should not allow to expect significant differences between the redox behavior of the complexes and the free ligands in biological media.

3.2.2. Lipophilicity results

Lipophilicity (Table 4) was experimentally determined in order to study the change of ligands' physicochemical properties upon complexation to Pd(II) or Pt(II) and their potential relationship with the exhibited activity.

Log k_{water} values of the complexes were compared to those previously described for the free ligands (**1** and **2**). In all cases, the lipophilicity of the complexes (**3**–**10**) is higher than that of the corresponding ligands, thus indicating that the lipophilicity of the ligands is increased upon complexation with Pd(II) and Pt(II). Moreover as expected, complexes (**3**, **5**, **7** and **9**) derived from ligand **1** resulted more lipophilic than those derived from ligand **2** (**4**, **6**, **8** and **10**). In addition, with the exception of complexes **4** and **6**, Pd(II) complexes were more lipophilic than the Pt(II) analogous compounds.

3.3. Biological evaluation

3.3.1. In vitro antitrypanosomal activity

The metal compounds were evaluated against the epimastigote form of *T. cruzi*, Tulahuen 2 strain (Table 5). The occurrence of the epimastigote form of *T. cruzi* as an obligate mammalian intracellular stage has been reevaluated and confirmed [42–44]. Furthermore, it should be noted that a good correlation between antiproliferative epimastigote activity and *in vivo* anti-*T. cruzi* activity was observed with several compounds [45–49].

In a first stage the compounds were assayed at 25.0 μM concentration and their ability to inhibit the growth of the parasite was evaluated by comparison with untreated controls on day 5. The 50% inhibitory concentration (IC_{50}) was determined for those compounds showing activity using Nifurtimox as reference trypanosomicidal drug. Results are shown in Table 5. Compounds **3**, **4**, **7**, **8** and **9** showed IC_{50} values in the low micromolar range, four of them resulted more active than Nifurtimox and all of them showed significantly higher activity than the corresponding free ligands. All Pd(II) compounds showed high trypanosomicidal activity, but with the exception of compound **9** the Pt(II) ones showed low activity on *T. cruzi*.

There seems to be a correlation between lipophilicity and anti-*T. cruzi* activity of these series of compounds. On one hand, the metal complexes are more lipophilic than their free ligands and in most of the cases they are also more active. H**L1** complexes are more lipophilic and also more active than H**L2** analogous metal complexes (**3**>**4**, **5**>**6**, **7**>**8**, **9**>**10**). All the Pd(II) complexes are more active than the Pt(II) analogues showing most of them higher lipophilicity than the Pt(II) analogous complexes (with the exception of complexes **4** and **6**).

Table 4
Log k_{water} values of the metal complexes and the corresponding ligands.

Compound	Log k_{water}[a]
1	1.95 [b]
2	1.79[b]
3, [$PdCl_2$(H**L1**)]	2.77 ± 0.11
4, [$PdCl_2$(H**L2**)]	2.14 ± 0.06
5, [$PtCl_2$(H**L1**)]	2.27 ± 0.07
6, [$PtCl_2$(H**L2**)]	2.25 ± 0.04
7, [Pd(H**L1**)(**L1**)]Cl·CH_3OH	2.78 ± 0.14
8, [Pd(H**L2**)(**L2**)]Cl·$2H_2O$	2.15 ± 0.07
9, [Pt(H**L1**)(**L1**)]Cl·$2CH_3OH$	2.68 ± 0.11
10, [Pt(H**L2**)(**L2**)]Cl·$2H_2O$	2.12 ± 0.06

[a] Results expressed as mean ± SEM.
[b] Taken from reference [39].

3.3.2. Unspecific mammalian cytotoxicity

Compounds were evaluated in terms of the non-specific cytotoxicity using human erythrocytes as mammalian cell model [14,15,40] Amphotericin B was used as reference drug due to its recognized hemolytic effects. Metal compounds were evaluated at 25 μM and compared with the parasite growth inhibition at 25 μM (Table 5). The metal compounds possess quite different unspecific mammalian cytotoxicity with percentages of erythrocytes lysis between 10.1 and 100.0 at 25 μM. The selectivity indexes (SI), defined as the ratio between percentage of parasite growth inhibition at 25 μM (PGI) and percentage of hemolysis at the same dose, have values of the same order than Amphotericin B but lower than those of the free ligands **1** and **2**. Interestingly, the Pd(II) compounds depict a higher selectivity towards the parasite than the Pt(II) analogues, showing the importance of the nature of the metal center (compare Pd-complexes **3** or **4** to Pt-complexes **5** or **6**, and Pd-complex **8** to Pt-complex **10**).

4. Conclusions

Five of the eight metal complexes showed IC_{50} values against *T. cruzi* in the low micromolar range, four of them resulted more active than the reference drug Nifurtimox and all of them showed significantly higher activity than the corresponding free ligands. Although these results support the hypothesis that antitumor compounds usually show some degree of antitrypanosomal activity, in this case no clear correlation could be detected. For instance, most of the Pt complexes that had previously

Table 5
In vitro evaluation of the Pd(II) and Pt(II) 1-indanone derived thiosemicarbazone complexes. Free ligands are included for comparison.

Compound	PGI (25 μM)[a]	IC_{50} (μM)[b]	% hemolysis[c] (25 μM)	SI[d]
1	80.8	18.6[f]	0.2[f]	404.0[f]
2	19.0	>50[f]	0.1[f]	>190.0[f]
3, [$PdCl_2$(H**L1**)]	100	1.6	36.6	2.7
4, [$PdCl_2$(H**L2**)]	100	3.0	42.6	2.4
5, [$PtCl_2$(H**L1**)]	50	25.0	100.0	0.2
6, [$PtCl_2$(H**L2**)]	0.0	>25	75.5	0
7, [Pd(H**L1**)(**L1**)] Cl·CH_3OH	100	0.47	57.1	1.8
8, [Pd(H**L2**)(**L2**)] Cl·$2H_20$	100	2.3	10.5	9.5
9, [Pt(H**L1**)(**L1**)] Cl·$2CH_3OH$	89	8.7	10.1	8.8
10, [Pt(H**L2**)(**L2**)] Cl·$2H_20$	27.8	>25	19.0	1.4
Nifurtimox	100.0	7.7	–	–
Amphotericin B[e]	100.0[e]	0.152[e]	100.0[e]	1.0[e]

[a] PGI: percentage of parasite growth inhibition at 25 μM. All the values are the mean of three different experiments.
[b] IC_{50}: concentration that produces 50% reduction in parasite growth. All the values are the mean of three different experiments.
[c] % hemolysis: percentage of erythrocytes lysis at 25 μM dose.
[d] SI: selectivity index: ratio between PGI and % hemolysis both at 25 μM.
[e] Taken from reference [14].
[f] Taken from reference [15].

shown interesting *in vitro* anti-leukemia properties did not show significant tripanosomicidal activity.

Those complexes bearing good antitrypanosomal activity showed lower selectivity towards trypanosomes in respect to erythrocytes than the free thiosemicarbazone ligands demonstrating that in this case metal coordination improves bioactivity but leads to deleterious effects on mammalian cytotoxicity. Nevertheless, some of the metal complexes tested in this work still show suitable selectivity indexes and deserve further developments.

Anti-*T. cruzi* activity and selectivity towards the parasite were both higher for the Pd(II) compounds than for the Pt(II) analogues, showing the effect of the metal center selection on the biological behavior.

Among both physicochemical features tested for this series of compounds, lipophilicity and redox behavior, only the former seemed to show correlation with the antiproliferative effects observed.

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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.2012.08.024.

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