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On the cytotoxic activity of Pd(II) complexes of N,N-disubstituted-N′-acyl thioureas

Ana M. Plutín [a,*], Raúl Mocelo [a], Anislay Alvarez [a], Raúl Ramos [a], Eduardo E. Castellano [b], Marcia R. Cominetti [c], Angelica E. Graminha [d], Antonio G. Ferreira [d], Alzir A. Batista [d,*]

[a] Laboratorio de Síntesis Orgánica, Facultad de Química, Universidad de La Habana, La Habana, Cuba
[b] Instituto de Química de São Carlos, Universidade de São Paulo, São Carlos, SP, Brazil
[c] Departamento de Gerontologia, Universidade Federal de São Carlos, São Carlos, SP, Brazil
[d] Departamento de Química, Universidade Federal de São Carlos, São Carlos, SP, Brazil

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ABSTRACT

The rational design of anticancer drugs is one of the most promising strategies for increasing their cytotoxicity and for minimizing their toxicity. Manipulation of the structure of ligands or of complexes represents a strategy for which is possible to modify the potential mechanism of their action against the cancer cells. Here we present the cytotoxicity of some new palladium complexes and our intention is to show the importance of non-coordinated atoms of the ligands in the cytotoxicity of the complexes. New complexes of palladium (II), with general formulae $[Pd(PPh_3)_2(L)]PF_6$ or $[PdCl(PPh_3)(L)]$, where L = N,N-disubstituted-N′-acyl thioureas, were synthesized and characterized by elemental analysis, molar conductivity, melting points, IR, NMR(^1H, ^{13}C and $^{31}P\{^1H\}$) spectroscopy. The spectroscopic data are consistent with the complexes containing an O, S chelated ligand. The structures of complexes with *N,N*-dimethyl-*N′*-benzoylthiourea, *N,N*-diphenyl-*N′*-benzoylthiourea, *N,N*-diethyl-*N′*-furoylthiourea, and *N,N*-diphenyl-*N′*-furoylthiourea were determined by X-ray crystallography, confirming the coordination of the ligands with the metal through sulfur and oxygen atoms, forming distorted square-planar structures. The N,N-disubstituted-N′-acyl thioureas and their complexes were screened with respect to their antitumor cytotoxicity against DU-145 (human prostate cancer cells), MDA-MB-231 (human breast cancer cells) and their toxicity against the L929 cell line (health cell line from mouse).

1. Introduction

The success of cisplatin in cancer therapies has triggered great interest to search for more effective antitumor agents, and as a consequence many other transition metal complexes have been obtained and tested against tumor cells. The number of metal compounds in current clinical use for this purpose is very limited and concerns only the platinum complexes [1]. However, it is important to search for other therapeutic agents different from those of platinum, which are in clinical use, in order to overcome the known drawbacks caused by them. Thus, a number of drug-design strategies have been employed, mainly in the past twenty years, and literature reports palladium(II) and platinum(II) complexes of phenylacetaldehyde thiosemicarbazone that are capable to, *in vitro*, bind to DNA, and have enhanced capacity to form inter-strand cross links by comparison with cisplatin [2,3].

The acyl thiourea ligand systems are very versatile and small structural changes can be readily made that lead to very different chemical and physical properties. Chelating thiourea ligands containing N, S and O donor atoms show broad biological activity and the existence of metal ions bonded to biologically active compounds may enhance their activities, and it was shown that the mode of binding of platinum to DNA is affected by the substituent nature on the acyl thiourea [4]. Acyl thiourea derivatives have been increasingly important with a wide diversity of applications in heterocyclic chemistry, metal complexes, molecular electronics and exhibit an array of biological activities [5–9]. Some of them are employed as fungicidal, antiviral, antimicrobial [10], parasiticidal [11], antitumoral [12] and pesticidal agent [13–15]. Indeed cytotoxicity studies using HeLa cancer cell lines have demonstrated that some of these platinum acyl thioureas show cytotoxic behavior, with the antiproliferative effects being dependent on the nature/type of the substituent in the acyl thiourea ligand [16–18]. Having this in mind our research group has been interested in developing new thiosemicarbazones or similar ligands, including polydentate ones such as acyl thioureas, containing N, S and O atoms with the capacity to form transition metal complexes. It turns out that these ligands are able to bind a wide variety of metal ions in different coordination modes, forming stable complexes. In most of the ones containing acyl thioureas, which were structurally characterized, they act as bidentate O,S-monoanionic ligands [19–22]. Also, it is well known that palladium(II) complexes are potential candidates to be used as metal based therapeutic agents, since this metal has a very similar chemistry to that of platinum(II), some of whose compounds are well known as anticancer agents [23].

* Corresponding authors.
E-mail addresses: anap@fq.uh.cu (A.M. Plutín), daab@ufscar.br (A.A. Batista).

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Here we report the synthesis, crystal structures and cytotoxicity studies of new palladium(II) complexes containing PPh_3 and *N,N*-disubstituted-*N′*-acyl thioureas as ligands. The N,N-disubstituted-N′-acyl thioureas used as ligands in this work were synthesized by the procedure previously reported in literature [24]. Scheme 1 shows the pathway for the synthesis of the Pd(II) complexes, which were obtained by reacting methanolic solutions of acyl thioureas with the precursor, the dichloro(bistriphenylphosphine)palladium(II).

The coordination between acyl thiourea derivatives and the precursor was proceeded by an exchange reaction, which involved deprotonation of the acyl thioureido group of the ligands during the complexes formation [25].

2. Experimental

2.1. Material and measurements

Bis diphenylphosphine palladium(II) chloride was obtained from Strem. All reagents were purchased with reagent grade and used without further purification. Solvents were dried and used freshly distilled, unless otherwise specifically indicated.

Thin layer chromatography (TLC) was performed on 0.25 mm silica gel pre-coated plastic sheets (40/80 mm) (Polygram_ SIL G/UV254, Macherey & Nagel, Düren, Germany) using benzene/methanol (9/1) as eluent.

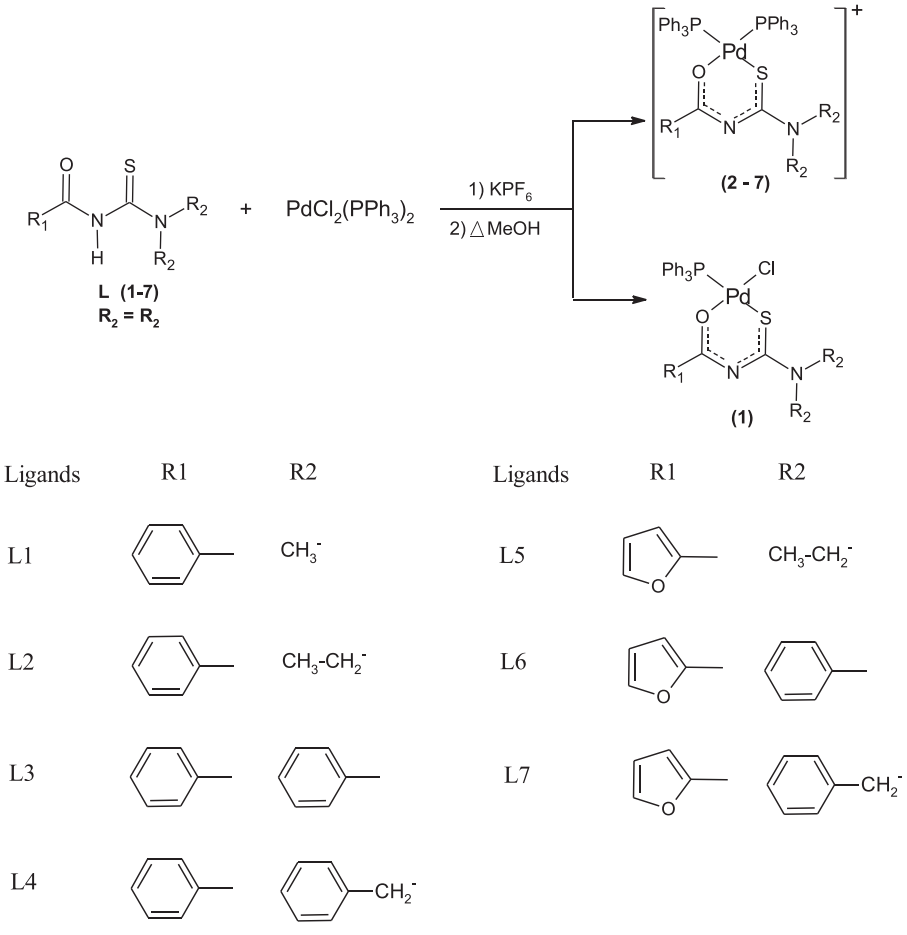
The IR spectra were recorded on a FTIR Bomem-Michelson 102 spectrometer in the 4000–200 cm^{-1} region using CsI pellets. Conductivity values were obtained using 1.0 mM solutions of complexes in CH_2Cl_2, using a Meter Lab CDM2300 instrument. The molar conductance measurements (Λ) were carried out in dichloromethane at 25 °C, using concentrations of 1.0×10^{-3} M for the complexes. ^1H, ^{31}P and ^{13}C NMR were recorded on a Bruker DRX 400 MHz, internally referenced to TMS, chemical shift (δ), multiplicity (*m*), spin–spin coupling constant (*J*), integral (*I*). $CDCl_3$ was used as solvent, unless mentioned. The ^{31}P shifts are reported in relation to H_3PO_4, 85%. Partial elemental analyses were carried out on the Department of Chemistry of the Federal University of São Carlos — UFSCar, in an instrument of CHNS staff EA 1108 of the FISONS.

2.2. Synthesis of N,N-dialkyl-N′-acyl thioureas

Detailed descriptions of syntheses and characterization of ligands have been reported elsewhere [24]. A solution of an appropriately acyl chloride (30 mmol) in acetone (50 mL) was added drop wise to a suspension of KSCN (0.01 mol) in acetone (30 mL). The mixture was stirred until a precipitate appeared (ammonium chloride), indicating the formation of the respective organic isothiocyanate. The corresponding amine (40 mmol), dissolved in acetone was added, slowly and with constant stirring to the resulting solution. The solution was cooled in an ice-water bath and the stirring was continued at room temperature during 2–9 h, until the reaction was completed (the reaction progress was monitored by TLC). The reaction mixture was then poured into 600 mL of cold water. The solid *N,N*-disubstituted-*N′*-acyl thioureas were collected by filtration and finally purified by recrystallization from ethanol. The identity of the products was confirmed by comparing their ^1H and ^{13}C NMR data with those reported in the literature [24]. The structures of the ligands are shown in Fig. 1.

2.3. Synthesis of the complexes

The complexes were obtained from direct reactions of the precursor, $[PdCl_2(PPh_3)_2]$, with the *N,N*-disubstituted-*N′*-acyl thioureas, in



Scheme 1. Pathways for the synthesis of N,N-disubstituted-N′-acyl thioureas Pd (II) complexes, and the R1 and R2 groups present in the ligands.

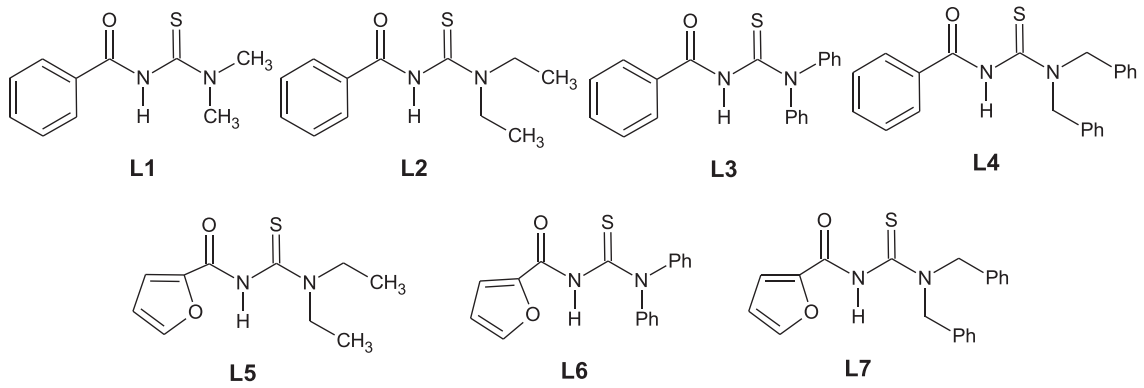


Fig. 1. Structures of the *N*,*N*-disubstituted-*N*′-acyl thioureas used as ligands in this work.

methanol solutions. The complexes were separated from the reaction mixtures as yellow crystalline solids. Filtration and further washing with hot water and hot hexane were enough to afford pure and stable compounds, in about 80% yields. Thus, the general procedure for the synthesis of the complexes is described: A solution of $[PdCl_2(PPh_3)_2]$ (1.49 mg; 0.002 mmol) in 5 mL of methanol, was added drop wise to a solution of corresponding *N*,*N*-dialkyl-N′-acyl thiourea (0.002 mmol), dissolved in 30 mL of the same solvent, and 0.368 g (0.002 mmol) of KPF_6. The reaction was heated under magnetic stirring at 80 °C, for 2 h. The reaction mixture was left in the refrigerator overnight. The yellow solids obtained were filtered off and washed, successively, with hot water and hot hexane (3 × 20 mL). The obtained compounds are stable in the air.

The ^1H and ^{13}C NMR data, the elemental analyses (calculated values in parenthesis), melting point temperature and molar conductivity (Λ_M, 1.0×10^{-3} M in CH_2Cl_2) for the complexes (**1**–**7**) are listed below and the other data used for the characterization of the complexes will be find in the text:

(1) $[PdCl(PPh_3)_2$(*N*,*N*-dimethyl-*N*′-benzoylthioureato-k^2O,S)]
NMR ^1H, ppm:. 8.07–7.95 (m, 5H, Ph), 7.78–7.22 (m, 5H), 7.21–7.13 (m, 10H), 3.78 (3H, s, CH_3), and .3.76 (3H, s, CH_3). NMR ^{13}C; ppm: 162. 68 (C═S); 161.84 (C═O); 133.87, 133.77, 133.40, 131.87, 131.76–116.34 (C – Ph), 43.85 (CH_3), 43.61 (CH_3); $^{31}P\{^1H\}$, 23.39 (s). IR: ν(C═O),1539; ν(C═S), 799; ν(C═N), 1573 cm^{-1}.
Anal. (%): Found (Calc.): C, 54.92 (55.00); H, 4.18 (4.29); N 4.58 (4.58); S, 5.13 (5.24); MP, 226–228 °C; $\Lambda_M = 1.4\ \Omega^{-1}\ cm^2\ mol^{-1}$.

(2) *cis*-$[Pd(PPh_3)_2$(*N*,*N*-diethyl-*N*′-benzoylthioureato-k^2O,S)]PF_6
NMR ^1H; ppm: 8.18–8.07 (m, 1H), 7.96–7.86 (m, 5H), 7.69–7.10 (m, 5H), 7.05–6.92 (m, 10H), 3.84 (d, $J = 7.07$ Hz, 2H, CH_2), 3.46 (d, $J = 7.07$ Hz, 2H, CH_2)1.64 (t, $J = 7.02, 7.02$ Hz, 3H, CH_3), 1.30 (t, $J = 7.02, 7.02$ Hz, 3H, CH_3); NMR ^{13}C; ppm: 170.30 (C═S); 169.86 (C═O); 136.22, 136.18, 135.89, 134.48, 133.82–126.01 (C – Ph), 47.46 (CH_2), 46.63 (CH_2), 13.13 (CH_3), 12.20 (CH_3); $^{31}P\{^1H\}$, 26.68; 38.36 (d), $^2J_{P-P} = 32.40$ Hz. IR: ν(C═O),1528; ν(C═S), 787; ν(C═N), 1576 cm^{-1}.
Anal. (%): Found (Calc.): C, 54.87 (55.01); H, 4.35 (4.49); N, 2.58 (2.77); S, 3.25 (3.17); MP, 190–193 °C; $\Lambda_M = 50.4\ \Omega^{-1}\ cm^2\ mol^{-1}$;

(3) *cis*-$[Pd(PPh_3)_2$(*N*,*N*-diphenyl-*N*′-benzoylthioureato-k^2O,S)]PF_6
NMR ^1H, ppm: 7.43–7.18 (m, 1H), 7.03 (s, 1H), 6.13 (d, $J = 1.60$ Hz, 1H), 6.13–6.01 (m, 1H), NMR ^{13}C, ppm: 174.07. (C═S); 162.92 (C═O); 150.00, 149.93, 146.60, 144.00, 143.57, 134.53–112. 11 (C – Ph). $^{31}P\{^1H\}$, 27.63; 37.71 (d), $^2J_{P-P} = 29.16$ Hz. IR: ν(C═O), 1517; ν(C═S), 784; ν(C═N), 1586 cm^{-1}.
Anal. (%): Found (Calc.): C, 60.59 (60.74); H, 4.19 (4.10); N, 2.45 (2.53); S, 2.83 (2.90); MP, 220–221 °C; $\Lambda_M = 52.7\ \Omega^{-1}\ cm^2\ mol^{-1}$.

(4) *cis*-$[Pd(PPh_3)_2$(*N*, *N*-dibencyl-*N*′-benzoylthioureato-k^2O,S)]PF_6
NMR ^1H, ppm: 8.43–8.32 (m, 1H), 7.80–7.40 (m, 1H), 7.10–6.97 (m, 1H), 6.76–6.02 (m, 1H), 5.29 (s, 2H, CH_2), 5.06 (s, 2H, CH_2). NMR ^{13}C; ppm: 172.54 (C═S); 170.99 (C═O); 149.96, 149.88, 146.94, 134.64, 134.41–112.06 (C – Ph), 53.93 (CH_2), 51.25 (CH_2). $^{31}P\{^1H\}$, 27.37; 37.95 (d), $^2J_{P-P} = 29.97$ Hz. IR: ν(C═O),1518; ν(C═S), 783; ν(C═N), 1576 cm^{-1}.
Anal. (%): Found (Calc.): C, 61.48 (61.35); H, 4.30 (4.35); N, 2.45 (2.47); S, 2.90 (2.82); MP, 160–162 °C; $\Lambda_M = 48.6\ \Omega^{-1}\ cm^2\ mol^{-1}$.

(5) *cis*-$[Pd(PPh_3)_2$(*N*, *N*-diethyl-*N*′-furoylthioureato-k^2O,S)]PF_6
NMR ^1H, ppm: 7.78–7.50 (m, 1H), 7.46–6.80 (3H, m, furoyl), 3.12 (2H, c, CH_2), 3.09 (2H, c, CH_2), 1.31 (3H, t, CH_3), 1.06 (3H, t, CH_3). NMR ^{13}C, ppm: 175.92. (C═S), 169.53 (C═O), 161.60, 155.11, 154.29, 153.77–150.16 (C – Ph), 148.02–111.99 (C – Ph and furan ring), 46.75 (CH_2), 46.47 (CH_2), 13.18 (CH_3) 12.07 (CH_3). $^{31}P\{^1H\}$, 26.63; 38.52 (d), $^2J_{P-P} = 32.40$ Hz. IR: ν(C═O),1511; ν(C═S), 756; ν(C═N), 1581 cm^{-1}.
Anal. (%): Found (Calc.): C, 55.09 (55.18); H, 4.30 (4.33); N, 2.95 (2.80); S, 3.15 (3.20); MP, 226–228 °C; $\Lambda_M = 59.6\ \Omega^{-1}\ cm^2\ mol^{-1}$.

(6) *cis*-$[Pd(PPh_3)_2$(*N*,*N*-diphenyl-*N*′-furoylthioureato-k^2O,S)]PF_6
NMR ^1H, ppm: 7.42–7.03 (m, 1H, Ph), 6.13–6.01 (m, 1H, the furan ring), NMR ^{13}C ppm: 174.08 (C═S); 162.93 (C═O); 150.01, 149.94, 146.60, 144.03–129.78 and 128.98–112.10 (C – Ph and furoyl ring). $^{31}P\{^1H\}$, 26.78; 38.07 (d), $^2J_{P-P} = 30.78$ Hz. IR: ν(C═O),1523; ν(C═S), 769; ν(C═N), 1574 cm^{-1}.
Anal. (%): Found (Calc.): C, 58.95 (59.11); H, 4.00 (3.95); N 2.50 (2.55); S, 2.88 (2.92); MP, 300–302 °C; $\Lambda_M = 52.8\ \Omega^{-1}\ cm^2\ mol^{-1}$.

(7) *cis*-$[Pd(PPh_3)_2$(*N*,*N*-dibenzyl-*N*′-furoylthioureato-k^2O,S)]PF_6
NMR ^1H; ppm: 7.92–6.94 (44H, m, C – Ph and furan ring), 5.29 (2H, s, CH_2), 5.10 (2H, s, CH_2). NMR ^{13}C; ppm: 173.09. (C═S), 170.99 (C═O), 135.72, 135.29, 135.22, 134.67–129.01 and 128.96–125.79 (C – Ph and furoyl ring). $^{31}P\{^1H\}$, 27.41; 37.96 (d), $^2J_{P-P} = 30.78$ Hz. IR: ν(C═O),1534; ν(C═S), 752; ν(C═N), 1581 cm^{-1}.
Anal. (%): Found (Calc.): C, 59.77 (59.77); H, 4.35 (4.21); N, 2.40 (2.49); S, 2.73 (2.85); MP, 200–202 °C; $\Lambda_M = 48.6\ \Omega^{-1}\ cm^2\ mol^{-1}$.

2.4. Crystal structure determination

Single crystals suitable for X-ray diffraction were obtained by slow evaporation of $CHCl_3$:n-hexane (3:1) solutions of the complexes **1**, **3**, **4**, **5** and **6**. Diffraction data were collected on an Enraf-Nonius Kappa-CCD diffractometer with graphite-monochromated Mo Ka radiation (λ = 0.71073 Å). The final unit cell parameters were based on all reflections. Data collections were performed using the COLLECT program [26]; integration and scaling of the reflections were performed with the HKL Denzo–Scalepack system of programs [27]. Absorption corrections were carried out using the Gaussian method [28]. The structures were solved by direct methods with SHELXS-97 [29]. The models were refined by full-matrix least-squares on F^2 by means of SHELXL-97 [30]. The projection views of the structures were prepared using ORTEP-3 for Windows [31]. Hydrogen atoms were stereochemically positioned and refined with the riding model. Data collections and experimental details

are summarized in Table 1. Relevant interatomic bond lengths and angles are listed in Table 2. Also included are the CCDC deposit numbers for supplementary crystallographic data.

2.5. Cell culture assay

In vitro cytotoxicity assays on cultured human tumor cell lines still represent the standard method for the initial screening of antitumoral agents. Thus, as a first step in assessing their pharmacological properties, the new palladium(II) complexes were assayed against human breast tumor cell lines MDA-MB-231 (ATCC: HTB-26), DU-145 (ATCC: HTB-81) and against the L929 cell line (ATCC: CCL 1). The cells MDA-MB-231 and L929 routinely maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) for cell DU-145 were maintained in RPMI-1640 supplemented with 10% FBS, at 37 ºC in a humidified 5% CO_2 atmosphere. After reaching confluence, the cells were detached by trypsinization and counted. For the cytotoxicity assay, 1.0×10^4 cells·well^{-1} were seeded in 200 μL of complete medium in 96-well assay microplates. The plates were incubated at 37 ºC in 5% CO_2 for 24 h to allow cell adhesion. All tested compounds were dissolved in sterile DMSO (stock solution with maximum concentration of 20 mmol L^{-1}) and diluted to 20; 10; 5.0; 2,5; 0.62; 0.15; 0.039 mmol L^{-1}. From each of these dilute samples 2 μL aliquots were added to 200 μL medium giving a final concentration of DMSO of approximately 1% and a final concentration of the complex diluted about 100 times. Cells were exposed to the compounds during a 48 h-period. Cell respiration, as an indicator of cell viability, was determined by the mitochondrial-dependent reduction of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to formazan [32]. MTT solution (0.5 mg/mL) was added to the cell cultures and incubated for 3 h, after which 100 μL of isopropanol was added to dissolve the precipitated formazan crystals. The conversion of MTT to formazan by metabolically viable cells was monitored in an automated microplate reader at 540 nm. The percent cell viability was calculated by dividing the average absorbance of the cells treated with the test compounds by that of the control; % cell viability was plotted against drug concentration (logarithmic scale) to determine the IC_{50} (drug concentration at which 50% of the cells are viable in relation to the control), with the error estimated from the average of 3 trials.

Table 2
Selected bond lengths (Å) and angles (°) for the complexes (**1**), (**3**), (**5**–**6**).

	(**1**)	(**3**)	(**4**)	(**5**)	(**6**)
Bond lengths					
Pd – O1	2.081 (2)	2.052 (5)	2.076 (2)	2.018 (5)	2.069 (2)
Pd – P1	2.2509 (8)	2.2571 (19)	2.2580 (10)	2.2612 (18)	2.2611 (8)
Pd – P2	–	2.3516 (19)	2.3403 (11)	2.3512 (19)	2.3389 (8)
Pd – S	2.2474 (10)	2.3026 (18)	2.3108 (11)	2.2945 (19)	2.3067 (8)
Pd – Cl	2.3269 (9)	–	–	–	–
S – C	1.733 (4)	1.755 (8)	1.748 (4)	1.727 (8)	1.746 (3)
C1 – O1	1.262 (4)	1.248 (9)	1.268 (4)	1.267 (8)	1.244 (4)
N2 – C	1.327 (5)	1.300 (9)	1.333 (5)	1.344 (9)	1.322 (4)
N2 – C1	1.316 (5)	1.314 (10)	1.320 (5)	1.315 (9)	1.310 (5)
N1 – C	1.330 (5)	1.349 (9)	1.344 (4)	1.334 (8)	1.348 (4)
Angles (°)					
O1Pd – S	91.30 (7)	91.19 (15)	93.12 (7)	91.55 (15)	91.52 (8)
O1 – Pd – P1	175.94 (7)	175.79 (18)	176.77 (8)	177.75 (15)	175.69 (8)
O1 – Pd – P2	–	81.96 (15)	82.15 (7)	83.35 (15)	81.95 (8)
S – Pd – P2	–	169.70 (7)	175.06 (3)	174.73 (7)	169.62 (3)
S – Pd – P1	92.52 (4)	90.54 (7)	89.07 (4)	89.85 (7)	90.29 (3)
P2 – Pd – P1	–	96.82 (7)	95.72 (4)	95.29 (7)	96.79 (3)

3. Results and discussion

All the synthesized palladium(II) complexes are of yellow color, and their elemental analyses, melting point temperatures, and molar conductivities were listed in the experimental section. These data suggest the formation of [Pd(PPh_3)(Cl)(L)] for complex (**1**) and [Pd(PPh_3)$_2$(L)]PF_6 for complexes (**2**–**7**), in which L represents the anionic ligand formed upon deprotonation, when the N,N-disubstituted-N′-acyl thioureas coordinate to the metal center.

The most useful infrared spectral bands for determining the ligands mode of coordination are given in the experimental section. The IR spectra of the ligands reveal broad, strong absorption bands in the range of 3053–3267 cm^{-1}, due to NH stretching vibrations. As expected, these N – H bands present in the free ligands are absent in the IR spectra of the complexes. On the other hand, the ν(C═N) band, present in the complexes at 1573–1586 cm^{-1}, is absent in the free ligands, suggesting the formation of the heterocyclics, in which the nitrogen changes from sp^3 to sp^2 hybridization when coordinating to the metal, as shown in

Table 1
Crystal data and refinement parameters for (**1**), (**3**), (**4**), (**5**) and (**6**) **c**omplexes.

Compound	(**1**)	(**3**)	(**4**)	(**5**)	(**6**)
Empirical formula	$C_{28}H_{26}ClN_2OPPdS$	$C_{56}H_{45}F_6N_2OP_3PdS$	$C_{58}H_{49}F_6N_2OP_3PdS$	$C_{46}H_{43}F_6N_2O_2P_3PdS$	$C_{54}H_{43}F_6N_2O_2P_3PdS$
Formula weight	611.39	1107.31	1135.36	1001.19	1097.27
Crystal system	Monoclinic	Triclinic	Monoclinic	Monoclinic	Triclinic
Space group	P21/c	P-1	$P2_1/n$	P21/a	P-1
a (Å)	14.3350 (5)	11.2530 (7)	15.7849 (8)	11.2500 (4)	11.2080 (2)
b (Å)	11.1240 (5)	11.7780 (8)	13.2553 (7)	29.0220 (16)	11.89.00 (2)
c (Å)	18.1440 (5)	20.7010 (14)	25.2766 (11)	14.1490 (7)	20.5390 (3)
β (°)	110.608 (2)	89.442 (4)	102.264 (3)	92.472 (3)	87.7960 (10)
V (Å^3)	2708.15 (17)	2559.4 (3)	5168.0 (4)	4615.3 (4)	2554.94 (7)
Z	4	2	4	4	2
D. calc (mg/m^3)	1.500	1.437	1.459	1.441	1.426
Absorption coefficient (mm^{-1})	0.944	0.561	0.557	0.615	0.562
F(000)	1240	1128	2320	2040	1016
θ range for data collection (°)	2.89 to 26.00	2.61 to 24.82	2.78 to 26.00	2.75 to 22.49	2.62 to 26.00
Index ranges	$-17 \le h \le 17$ $-13 \le k \le 10$ $-22 \le i \le 22$	$-12 \le h \le 13$ $-13 \le k \le 13$ $-23 \le l \le 18$	$-17 \le h \le 19$ $-15 \le k \le 16$ $-29 \le l \le 31$	$-10 \le h \le 12$ $-29 \le k \le 31$ $-15 \le l \le 13$	$-13 \le h \le 12$ $-14 \le k \le 14$ $-25 \le l \le 25$
Reflections collected	16636	16727	37665	21137	45097
Independent reflections (Rint)	5140 (0.0760)	7823 (0.1018)	9539 (0.1124)	6001 (0.1218)	9513 (0.980)
Goodness-of-fit on F^2	1.058	1.090	0.945	0.981	1.059
Final R indices [I > 2σ (I)]	R1 = 0.0429, wR2 = 0.1070	R1 = 0.0806, wR2 = 0.2093	R1 = 0.0506, wR2 = 0.1113	R1 = 0.0578, wR2 = 0.1171	R1 = 0.051, wR2 = 0.1624
Largest diff. peak and hole (eÅ)$^{-3}$	0.343 and −0.669	1.008 and −1.107	0.821 and −0.921	0.430 and −0.395	0.998 and −0.736
CCDC deposit number	842052	842053	931560	842056	842055

Scheme 1. The coordination of the metal to the carbonyl group decreases the C═O stretching vibration frequency by ca. 180 cm^{-1}, when compared with the free ligand, in agreement with the literature [33]. Thus, considering that the free ligands have their ν(C═O) bands at about 1680 cm^{-1}, it is reasonable to assign the bands in the range 1511–1539 cm^{-1} to the coordinated C═O group. The absorptions at 815–878 cm^{-1} in the spectra of free bases, N,N-disubstituted-N′-acyl thioureas, attributed to the ν(C═S) stretching vibrations, shift to the 752–799 cm^{-1} range in the complexes spectra. This substantial change suggests deprotonation of the ligands, indicating coordination through the sulfur atom with a formally C–S single bond [34–37]. The absorptions at about 470 cm^{-1} in the IR spectra of the complexes can be assigned to the M–O vibration mode [38,39] and the assignment of the Pd–S stretching vibration bands at about 330 cm^{-1} are in accordance to the reported by Orysyk et al. for palladium(II) complexes with 1-allyl-3-(2-pyridyl)thioureas [40]. Thus, from the IR data it is possible to suggest that the N,N-disubstituted-N′-acyl thioureas are attached to the metal through the oxygen and sulfur chelating system. In the case of complex (**1**) a chloride ion occupies one coordination position and the fourth coordination site of the metal is occupied by a triphenylphosphine, while for the other complexes (**2**–**7**) there are two coordinated triphenylphosphines. The molar conductivity of complex (**1**) shows that it is neutral, while complexes (**2**–**7**) are anionic species [41].

The molecular structures of the complexes were also investigated by NMR (^{1}H, ^{13}C and $^{31}P\{^{1}H\}$) spectroscopy and a comparative analysis on the basis of the spectroscopic data corresponding to both, free and coordinated ligands with the metallic ion was carried out.

The ^{1}H NMR data for the complexes (**1**–**7**) are given in the experimental section. The ^{1}H NMR integrations and signal multiplicities are consistent with the proposed and with the X-ray structures. The ^{1}H NMR spectra of the free ligands show basically three sets of well separated signals corresponding to their R1, R2 substituent and to the NH proton. The signals of the NH protons appear as broad singlets in the region between 8.35 and 8.80 ppm [24]. The absence of the N–H proton after the coordination of the ligands to the metal is associated with an increase in electron density at the C–N bond upon complexation [42,43]. A slight downfield shift was also observed in the aromatic

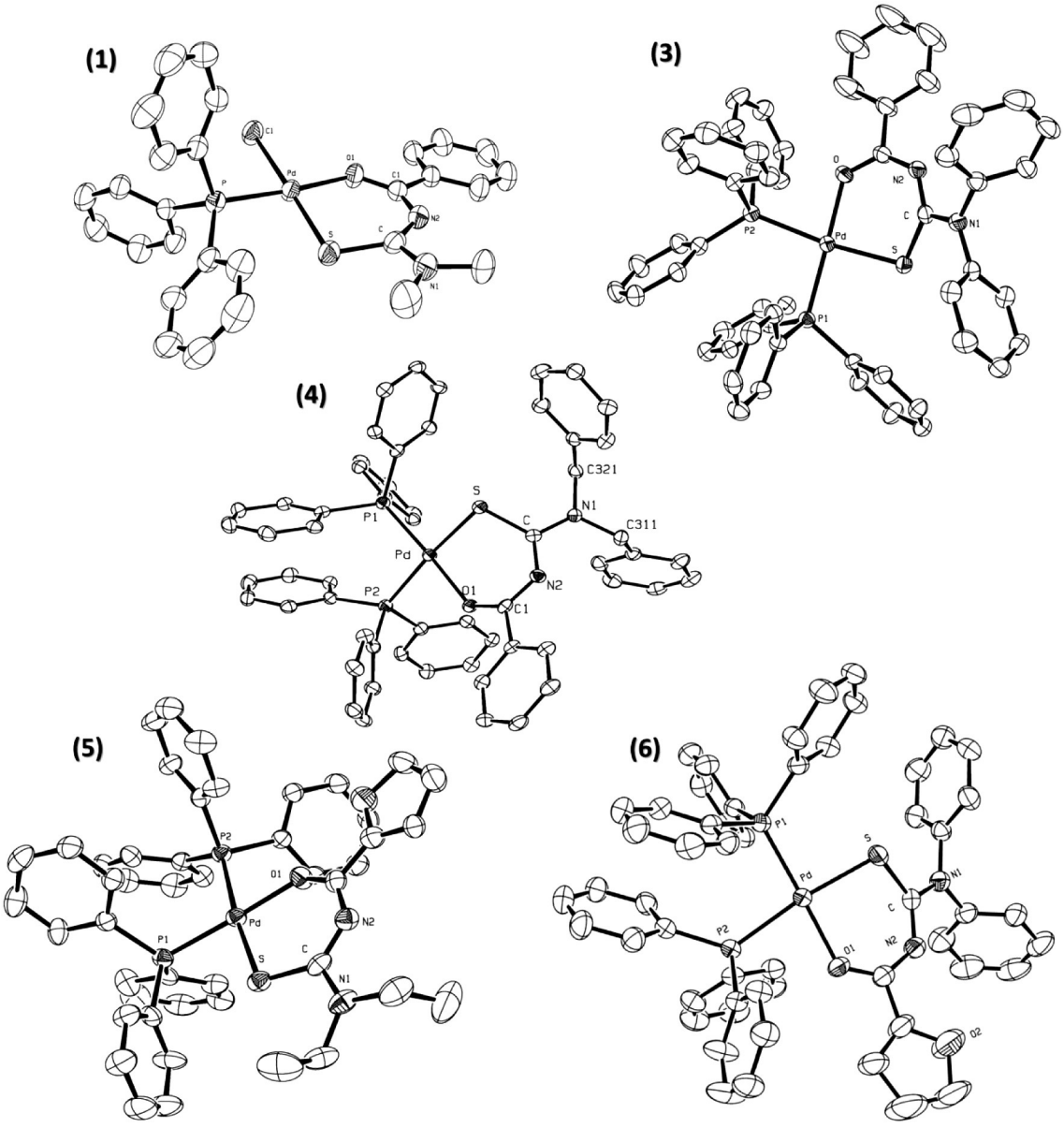


Fig. 2. ORTEP view of (**1**), (**3**), (**4**), (**5**), and (**6**) complexes showing 50% probability ellipsoids except for (**4**) which is shown at a 30% level. Hydrogen atoms, the $(PF_6)^-$ anion and some labels of the ligands are omitted for clarity.

Table 3
Cytotoxic effect of (L1–L7) ligands and the (1–7) complexes against DU-145, MDA-MB-231 and L929 cell line, after 48 h of incubation.

Complexes	IC_{50} (μmol L^{-1})		
	DU-145	MDA-MB-231	L929
(**1**)	4.25 ± 0.92	2.15 ± 0.97	<0.8
(**2**)	7.02 ± 0.81	1.12 ± 0.52	<0.8
(**3**)	>200	>200	>200
(**4**)	>200	>200	>200
(**5**)	31.85 ± 1.41	<0.8	>200
(**6**)	68.97 ± 1.64	>200	48.70 ± 13.07
(**7**)	47.85 ± 1.15	>200	37.12 ± 9.87
(L1)	>200	>200	79.11 ± 22.12
(L2)	>200	>200	95.71 ± 30.49
(L3)	>200	>200	>200
(L4)	>200	>200	68.02 ± 1.95
(L5)	>200	>200	>200
(L6)	71.36 ± 1.50	>200	>200
(L7)	45.57 ± 1.51	>200	82.85 ± 25.88
PPh_3	>200	>200	>200
Cisplatin	2.00 ± 0.47	2.43 ± 0.20	16.53 ± 2.38
$[PdCl_2(PPh_3)_2]$	>200	>200	>200

protons and in the protons of the nitrogen substituents in the coordinated ligands, when compared to the chemical shift of the free ligands. The aromatic protons appear as a complex pattern in the region δ 8.82–6.50 ppm. In the ^{13}C NMR spectra the chemical shifts of the C═S carbon atoms of the ligands moved up field after their coordination to the metal for all complexes studied, but in the case of the C═O group, this tendency was not observed for all complexes. The explanation for this is the fact that with the deprotonation of the secondary amide and with the formation of a "N = C–S–Ru" species, the sulfur atoms, and consequently their neighbor carbon atoms, became more shielded in all complexes, something that does not happen with the carbon from the carbonyl group, in the same extension. The $^{31}P\{^1H\}$ NMR spectrum of the precursor $[PdCl_2(PPh_3)_2]$ in CH_2Cl_2 solution shows a singlet peak for phosphorous atoms at 23.83 ppm. In the spectra of complex (**1**) the singlet peak is shifted to 23.39 ppm, in conformity with the presence of only one phosphorus species for the complex, in solution. For the other complexes two doublets arise, at about 27 and 38 ppm, indicating the presence of two magnetically different phosphorus atoms coordinated to the palladium(II) ion. Thus, considering that for complex (**1**) the phosphorus atoms is *trans* to the oxygen atom from the acyl thiourea ligand and that its chemical shifts is 23.39 ppm, it is reasonable to assign the $^{31}P\{^1H\}$ chemical shifts of the other complexes (**2**–**7**) at about 27 ppm also to the phosphorus *trans* to the oxygen atoms and the chemical shifts at about 38 ppm to the phosphorus *trans* to the sulfur atoms from the ligands. Also in the region of −138–158 ppm a multiple signal of the PF_6^- functioning as outer-sphere anions for the complexes obtained is observed, except for complex (**1**), indicating that this complex is neutral, in accordance to the molar conductivity measurements for these complexes (see experimental section). These data are consistent with those obtained for some palladium(II) thiosemicarbazones complexes [35].

The structures of complexes (**1**), (**3**), (**4**), (**5**) and (**6**) were determined by X-ray diffraction analysis. Their ORTEP views are in Fig. 2 and selected bond lengths (Å) and angles (°) for the complexes are listed in Table 2. In the structures of the complexes the *N,N*-disubstituted-*N′*-acyl thioureas adopt a *cis* conformation, bounded to the central ion Pd(II) by the oxygen and sulfur atoms, and with two PPh_3 ligands, which are also in the *cis* fashion, except for the complex (**1**), which has just one triphenylphosphine in its structure. In all complexes the Pd(II) ion is nearly planar, fourfold environment. The thione C═S bond in the coordinated anionic ligands becomes formally a single bond making it longer (average = 1.7418 Å) than the C═S bond of the neutral moieties [24,25], and the N(2) – C bond (average 1.3252 Å) (Fig. 2, Table 2), which becomes a double bond in the anionic moieties, is typical for C═N distances [44]. The N(1) – C distance, 1.341 Å (average), is anionic and in the amine form. Thus the metal is coordinated to the negatively charged organic molecules, which act as bidentate ligands, through oxygen (average distance Pd – O = 2.059 Å) and sulfur (average distance Pd – S = 2.2924 Å) atoms. The Pd – S average distance is close to the ones found for other palladium(II) thiosemicarbazones complexes [36]. The remaining binding sites are occupied by triphenylphosphines (average distance Pd–P1 = 2.2577 Å and Pd–P2 = 2.3455 Å), or by the chloride in the case of complex (**1**), where the distance Pd–Cl is 2.3269(9) Å, which is close to those reported in the literature [36]. The distances for the C – S, C – N and C – O bonds in the chelate rings, listed in Table 2, are the characteristic of single and double bond lengths, respectively [44]. The C – O bond distances are slightly sensitive to the coordination of the ligand to the metal. In the case of the bis-triphenylphosphine-*N,N*-dietyl-*N′*-furoilthioureato-k^2O, S the C – O distance for the free ligand is 1.226(3) Å (average for two independent molecules per asymmetric unit), and, 1.2605(4) Å, after its coordination to the metal [25].

Table 3 lists the ligands and complex concentrations that produce 50% of growth inhibition (IC_{50}, μM) against DU-145 (human prostate cancer cells), MDA-MB-231 (human breast cancer cells) and against the L929 cell line (health cell line from mouse). The seven new palladium(II) complexes, the free ligands and the precursor $[PdCl_2(PPh_3)_2]$, were tested against the tumor cells and L929 cells. For comparison the cytotoxicity of cisplatin and of $[PdCl_2(PPh_3)_2]$ were also evaluated under the same experimental conditions. The IC_{50} values, calculated from the dose-survival curves generated by the MTT assays obtained after drug treatment, are shown in Table 3. It is worth pointing out that the most promising complex is (**5**), with the *N*, *N*-diethyl-*N′*-furoylthiourea ligand, which had very low IC_{50} and was selective for the MDA-MB-231 cells. Additionally, its IC_{50} against the L929 cell is very high. It is interesting to observe that the smaller volume of ligand in complex (**5**), when compared with the other complexes, may be the steric factor affecting the enhanced cytotoxicity of the compound. In this case the low steric effect of the complex can facilitate its contact with the DNA molecule, while the presence of the oxygen atom in the furoyl thiourea group allows its interaction through hydrogen bonding. These preliminary results are very encouraging in that they support our hypothesis that the non-bonded atoms present in the ligands can play an important role in the biological activity of the complex. We are observing this behavior for a series of ruthenium and platinum complexes and detailed studies are currently underway in our laboratory to investigate their biological activity and to try to establish a structure–activity relationship.

4. Conclusions

The preparation of a novel series of Pd(II) complexes with *N,N*-disubstituted-*N′*-acyl thiourea bidentate ligands is here reported. The X-ray crystallographic characterization shows that these ligands coordinate with the metal through the oxygen and sulfur atoms. The complexes exhibit a slightly distorted square-planar geometry corresponding to stoichiometry metal:ligand in 1:1 ratio. In this work there are two series of complexes: for the first one, the complexes (**1**–**4**), R1 is the phenyl group and for the other one it is the furoyl one (**5**–**7**). The most promising complex as antitumor agent against the MDA-MB-231 from the two series here reported is the one where R1 is the furoyl group and R2 is some of the ethyl groups (**5**), probably due to the lower steric hindrance caused by these R2 groups in the contact of the complex with the DNA molecule. Additionally, the furoyl oxygen atom allows the electrostatic interaction of the complex with the biological molecule.

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Appendix A. Supplementary data

Supplementary crystallographic data for complexes (**1**), (**3**), (**4**), (**5**) and (**6**) (CCDC 842052, 842053, 931560, 842056, 842055, respectively) can be obtained free of charge *via* 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 336 033; or e-mail: deposit@ccdc.cam.ac.uk. Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.poly.2012.02.008.

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