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Article

Structure/Activity of Pt^{II}/*N*,*N*-Disubstituted-*N*'-acylthiourea Complexes: Anti-Tumor and Anti-*Mycobacterium tuberculosis* Activities

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The syntheses, characterization, cytotoxicity against tumor cells and anti-*Mycobacterium tuberculosis* activity assays of Pt^{II}/PPh_3/*N*,*N*-disubstituted-*N*'-acylthioureas complexes with general formulae $[Pt(PPh_3)_2(L)]PF_6$, PPh_3 = triphenylphosphine; L = *N*,*N*-disubstituted-*N*'-acylthiourea, are here reported. The complexes were characterized by elemental analysis, molar conductivity, infrared (IR), nuclear magnetic resonance (NMR) (^1H, $^{13}C\{^1H\}$ and $^{31}P\{^1H\}$) spectroscopy. The $^{31}P\{^1H\}$ NMR data are consistent with the presence of two PPh_3 ligands *cis* to each other position, and one *N*,*N*-disubstituted-*N*'-acylthiourea coordinated to the metal through O and S, in a chelate form. The structures of the complexes were determined by X-ray crystallography, forming distorted square-planar structures. The complexes were tested in human cell lines carcinomas and also screened with respect to their anti-*Mycobacterium tuberculosis* activity (H37RvATCC 27294). It was found that complexes with *N*,*N*-disubstituted-*N*'-acylthiourea containing open and small chains as R2 groups show higher cytotoxic and higher anti-*Mycobacterium tuberculosis* activity than those containing rings in this position.

Keywords: platinum(II), tumor cells, *Mycobacterium tuberculosis*

Introduction

Among the most effective agents for the treatment of cancer, there are some metallodrugs based on platinum(II). However, due to the frequent development of drug resistance, they have acquired several limitations, including their side effects.[1] This damage has led to the need for development of new metal-based anticancer drugs whose structure and mode of action differ from that of cisplatin and derivatives, aiming to improve their cytotoxicity and minimizing their side effects.[2-9] Thus, in an attempt to overcome the drawbacks of cisplatin and derivatives (severe toxicity, drug resistance and poor oral bioavailability), the development of platinum-based drugs have progressed to the newest generation of drugs, such as satraplatin, picoplatin and the multinuclear platinum complex BBR3464 (triplatin). In this context, platinum(II) complexes of the type [Pt(L)Cl(DMSO)] (L = acylthiourea ligand, R1–C(O)NHC(S)NR2; R' = aryl, NR2 = amine; DMSO = dimethylsulfoxide) were prepared by Sacht *et al.*[10-12] for their biological and chemical evaluation. The acylthiourea group, after deprotonation of the amide moiety (NHCO), can act as a bidentate chelating ligand, coordinating to platinum through the oxygen and sulfur donor atoms.

The facility of affording the replacement of the functional groups R1 and R2 (see below) to obtain a wide range of ligands and platinum(II) complexes with different physical and chemical properties, made the assessment of these compounds especially attractive.[13-15]

In previous works,[16-20] some of us synthesized and determined the structures of some ligands related to those here described, and their corresponding complexes with Co^{II}, Cu^{II}, Ni^{II}, Pd^{II} and Pt^{II} were also studied, which contain a thiourea derivative as a bidentate ligand, in different environments. In the present work, we studied the syntheses,

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characterization, cytotoxicity and anti-*Mycobacterium tuberculosis* activity of new platinum(II) complexes containing PPh_3 and *N*,*N*-disubstituted-*N*'-acylthioureas as ligands. The *N*,*N*-disubstituted-*N*'-acylthioureas used as ligands were synthesized by the procedure previously reported.[21] Scheme 1 shows the pathway for the synthesis of the Pt^{II} complexes, which were obtained by reacting methanolic solutions of acylthioureas with the precursor, dichloro-bis(triphenylphosphine)platinum(II).

The complexes were obtained by a nucleophilic substitution reaction of the two chlorido ligands from the precursor $[PtCl_2(PPh_3)_2]$, by the acylthiourea ligands. For the formation of the platinum(II) complexes the loss of the hydrogen atom of the acylthioureido group of the ligands occurs (see Scheme 1).[16]

Cytotoxic studies realized on DU-145 (human prostate tumor cells) and MDA-MB-231 (human breast tumor cells) tumor cell lines have shown that certain palladium(II) complexes with the acylthiourea ligands exhibit cytotoxicity with antiproliferative effects being dependent on the nature or the type of the substituent at the acylthiourea ligand.[20] Recently there have been efforts to design non-classical platinum-acylthiourea complexes, due to their antifungal activity and inhibitory activities against viruses. Thus, here we investigate the cytotoxicity of the complexes against MDA-MB-231 and DU-145 tumor cells, and their anti-*Mycobacterium tuberculosis* activity, with the aim of evaluating a possible influence of R1 and R2 in the cytotoxicity and in the anti-mycobacterial activity of the complexes.[8] In this work three series of *N*,*N*-disubstituted-*N*'-acylthioureas were synthesized where R1 = phenyl, furoyl group or thiophenyl group.

Experimental

Material and measurements

The dichloro-bis(triphenylphosphine)platinum(II) complex was obtained from Sigma. 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 (40/80mm) (Polygram_SIL G/UV254, Macherey& Nagel, Düren, Germany) using Caution benzene/methanol (9:1) as eluent.

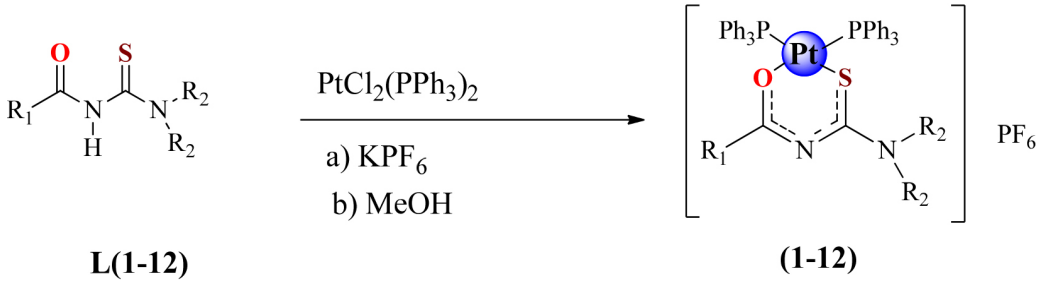
The infrared (IR) spectra of the compounds were recorded on a Fourier transform infrared (FTIR) Bomem-Michelson 102 spectrometer in the 4000-200 cm^{-1} region using CsI pellets. Conductivity values were obtained using 1.0 mM solutions of complexes in CH_2Cl_2, using a Meter Lab CDM2300 instrument. ^1H, $^{31}P\{^1H\}$ and $^{13}C\{^1H\}$ nuclear magnetic resonance (NMR) were recorded on a Bruker DRX 400 MHz, internally referenced to tetramethylsilane (TMS), chemical shift (δ), multiplicity (m), spin-spin coupling constant (*J*), integral (I). $CDCl_3$ was used as a solvent unless mentioned. The $^{31}P\{^1H\}$ shifts are reported in relation to H_3PO_4, 85%. 2D heteronuclear single quantum coherence (HSQC) NMR experiments were performed in order to unequivocally assign the C=O and C=S signals of the complexes. Partial elemental analyses were carried out by the Department of Chemistry of the Federal University of São Carlos, in an instrument of CHNS staff EA 1108 of the FISONS.

Syntheses of *N*,*N*-disubstituted-*N*'-acylthioureas

The *N*,*N*-disubstituted-*N*'-acylthiourea ligands L (**1**-**12**) used in this work were synthesized by the procedure previously reported, and the identity of the products was confirmed by comparing their ^1H and $^{13}C\{^1H\}$ NMR data with those reported in the literature.[21] The groups R1 and R2 of the ligands are shown in Figure 1.

Synthesis of the complexes

The complexes were obtained as previously described for similar Pd^{II} complexes, from direct reactions of the precursors, $[PtCl_2(PPh_3)_2]$, with the *N*,*N*-disubstituted-*N*'-acylthioureas, in methanol solutions.[20] The complexes were separated from the reaction mixtures as white crystalline solids. Filtration and further washing with



Scheme 1. Pathways for the syntheses of the $[Pt^{II}(PPh_3)_2$(*N*,*N*-disubstituted-*N*'-acylthioureato-k^2-O,S)] complexes.

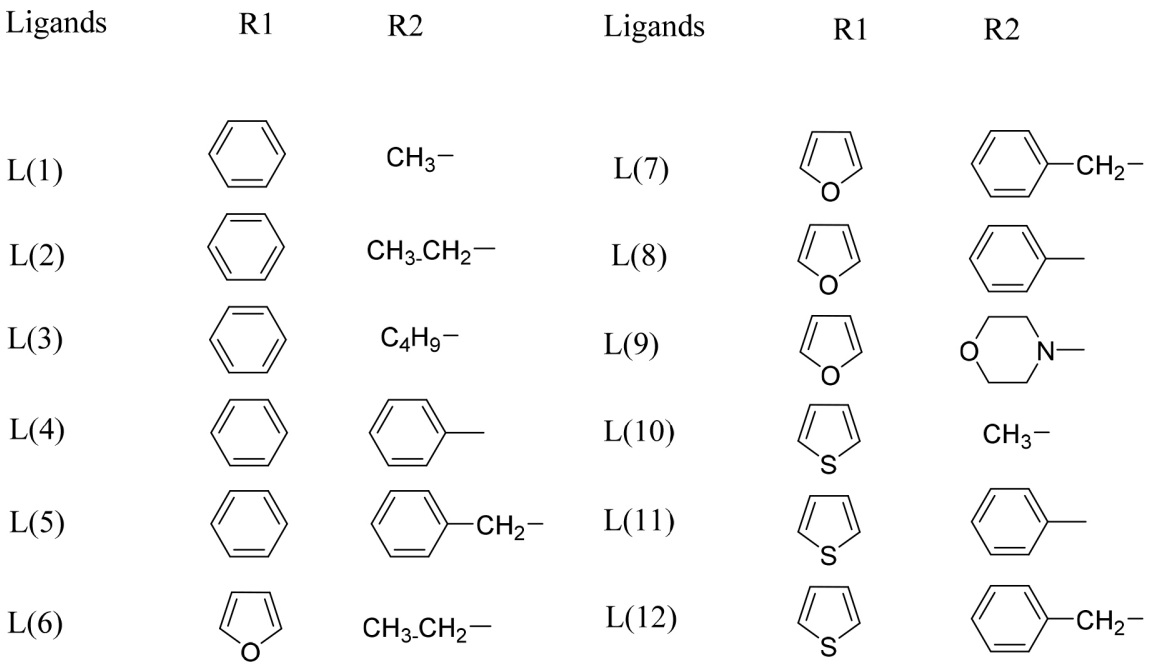


Figure 1. Structures of R1 and R2 for the *N*,*N*-disubstituted-*N*'-acylthioureas used as ligands in this work.

hot water and hot hexane were enough to afford pure compounds, in about 80% yields. Thus, the general procedure for the syntheses of the complexes is described: a solution of $[PtCl_2(PPh_3)_2]$ 1580 mg (2 mmol) in 5 mL of methanol was added dropwise to a solution of the corresponding *N*,*N*-dialkyl-*N*'-acylthiourea (2 mmol), dissolved in 30 mL of the same solvent, and 368 mg (2 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 white solids obtained were filtered off and washed, successively, with hot water and hot hexane (3 × 20 mL). The obtained compounds are stable in DMSO solutions for at least five days, as it was showed by $^{31}P\{^1H\}$ NMR experiments. After this time the spectra of the complexes were the same, when compared with those recorded using fresh solutions.

The ^1H, $^{13}C\{^1H\}$ and $^{31}P\{^1H\}$ NMR data, the elemental analyses, melting point temperature (mp) and molar conductivity (Λ_m, 1.0 × 10^{-3} M in CH_2Cl_2) for the complexes (**1**-**12**) are listed below and the other data used for the characterization of the complexes are in the text (the multiplicity of signals in the $^{13}C\{^1H\}$ NMR due the coupling C–P).

cis-[Pt(PPh_3)$_2$(*N*,*N*-Dimethyl-*N*'-benzoylthioureato-k^{2}O,S)] PF_6 (**1**)

^1H NMR (400.13 MHz, $CDCl_3$) δ 8.11-6.99 (30H atoms of PPh_3, 5H aromatic of Ph), 3.44 (3H, s, CH_3), 3.10 (3H, s, CH_3); $^{13}C\{^1H\}$ NMR (100.00 MHz, $CDCl_3$) δ 168.25 (C=O), 167.55 (C=S), 134.47, 134.53 (d, C_{meta}–PPh_3, $^3J_{C-P}$ 10.62, 10.51 Hz), 131.76, 132.26 (C_{para}–PPh_3), 131.91 (C_{para}–Ph), 129.67 ($C_{quaternary}$–Ph), 129.39 (C_{meta}–Ph), 128.67, 128.85 (d, C_{ortho}–PPh_3, $^2J_{C-P}$ 11.78, 11.30 Hz), 127.73 (C_{ortho}–Ph), 126.10, 127.13 (d, C_c-PPh_3, $^1J_{C-P}$ 68.10, 59.10 Hz), 40.78, 41.78 (CH_3); $^{31}P\{^1H\}$ NMR (161.98 MHz, CH_2Cl_2/D_2O) δ 20.83, 9.30 (d), $^2J_{P-P}$ 25.10 Hz, 30.52 (d), 11.30 (d), $^2J_{Pt-P}$ 3080 Hz, 21.08 (d), –2.53 (d); $^2J_{Pt-P}$ 3840 Hz, –144.51 (m, PF_6); IR (CsI) ν / cm^{-1} 3018 ν(CH–PPh_3), 1585 ν(C=N), 1503 ν(C=O), 844 ν(P–F), 745 ν(C=S), 695 ν(Ph_3–P–Ph_3), 549 ν(Pt–P). Anal. found (calcd.) for [$C_{46}H_{41}F_6N_2OP_3PtS$], %: C, 51.22 (51.54); H, 4.08 (3.86); N, 2.58 (2.61); S, 3.13 (2.99). mp 251-253 °C; Λ_m = 41.4 Ω^{-1} cm^2 mol^{-1}.

cis-[Pt(PPh_3)$_2$(*N*,*N*-Diethyl-*N*'-benzoylthioureato-k^{2}O,S)] PF_6 (**2**)

^1H NMR (400.13 MHz, $CDCl_3$) δ 8.13-7.00 (30H atoms of PPh_3, 5H aromatic of Ph), 3.86 (d, *J* 7.07 Hz, 2H, CH_2), 3.48 (d, *J* 7.07 Hz, 2H, CH_2), 1.62 (t, *J* 7.02, 7.02 Hz, 3H, CH_3), 1.31 (t, *J* 7.02, 7.02 Hz, 3H, CH_3); $^{13}C\{^1H\}$ NMR (100.00 MHz, $CDCl_3$) δ 168.12 (C=O), 166.12 (C=S), 134.49, 134.54 (d, C_{meta}–PPh_3, $^3J_{C-P}$ 10.54, 10.30 Hz), 131.81, 132.30 (C_{para}–PPh_3), 131.89 (C_{para}–Ph), 129.66 ($C_{quaternary}$–Ph), 129.30 (C_{meta}–Ph), 128.69, 128.86 (d, C_{ortho}–PPh_3, $^2J_{C-P}$ 11.85, 11.33 Hz), 127.78 (C_{ortho}–Ph), 125.92, 127.13 (d, $C_{quaternary}$–PPh_3, $^1J_{C-P}$ 57.69, 68.02 Hz), 46.53, 47.44 (CH_2), 12.00, 13.13 (CH_3); $^{31}P\{^1H\}$ NMR (161.98 MHz, CH_2Cl_2/D_2O) δ 20.66, 10.00 (d), $^2J_{P-P}$ 26.07 Hz, 29.90 (d), 10.22 (d), $^2J_{Pt-P}$ 3163 Hz, 21.78 (d), –2.08 (d), $^2J_{Pt-P}$ 3863 Hz, –144.51 (m, PF_6); IR (CsI) ν / cm^{-1} 3015 ν(CH–PPh_3), 1586 ν(C=N), 1497 ν(C=O), 843 ν(P–F), 749 ν(C=S), 696 ν(Ph_3–P–Ph_3),

550 ν(Pt–P). Anal. found (calcd.) for [$C_{48}H_{45}F_6N_2OP_3PtS$], %: C, 52.67 (52.41); H, 4.50 (4.12); N, 2.60 (2.55); S, 3.12 (2.91). mp 251-253 °C; Λ_m = 50.4 Ω^{-1} cm^2 mol^{-1}.

cis-[Pt(PPh_3)$_2$(*N,N*-Dibuthyl-*N'*-benzoylthioureato-k^{2}O,S)]PF_6 (**3**)

^{1}H NMR (400.13 MHz, $CDCl_3$) δ 8.17-7.01 (30H atoms of PPh_3, 5H aromatic of Ph), 3.78 (d, *J* 7.06 Hz, 2H, CH_2), 3.38 (2H, q, CH_2), 1.68-1.15 (8H, m, CH_2), 0.96 (6H, t, –CH_3, *J* 7.1 Hz); ^{13}C{^{1}H} NMR (100.00 MHz, $CDCl_3$) δ 168.00 (C=O), 166.32 (C=S), 134.43, 134.54 (d, C_{meta}–PPh_3, $^3J_{C-P}$ 10.60, 10.42 Hz), 131.84, 132.30 (C_{para}–PPh_3), 131.91 (C_{para}–Ph), 129.30 (C_{meta}–Ph), 128.69, 128.86 (d, C_{ortho}–PPh_3, $^2J_{C-P}$ 12.13, 11.46 Hz), 127.77 (C_{ortho}–Ph), 126.08, 127.08 (d, $C_{quaternary}$–PPh_3, $^1J_{C-P}$ 66.86, 57.53 Hz), 51.93, 53.00 (CH_2), 29.07, 29.94 (CH_2), 20.10, 20.18 (CH_2), 13.86, 13.89 (CH_3); ^{31}P{^{1}H} NMR (161.98 MHz, CH_2Cl_2/D_2O) δ 20.72, 9.66 (d), $^2J_{P-P}$ 25.9 Hz, 30.20 (d), 11.09 (d), $^2J_{Pt-P}$ 3126 Hz, 21.59 (d), –2.10 (d), $^2J_{Pt-P}$ 3871 Hz, –144.51 (m, PF_6); IR (CsI) ν / cm^{-1} 3019 ν(CHPPh_3), 1576 ν(C=N), 1487 ν(C=O), 845 ν(P–F), 749 ν(C=S), 694 ν(Ph_3–P–Ph_3), 551 ν(Pt–P). Anal. found (calcd.) for [$C_{52}H_{53}F_6N_2OP_3PtS$], %: C, 54.17 (54.03); H, 4.30 (4.62); N, 2.50 (2.42); S, 2.62 (2.77). mp 198-203 °C; Λ_m = 50.4 Ω^{-1} cm^2 mol^{-1}.

cis-[Pt(PPh_3)$_2$(*N,N*-diphenyl-*N'*-benzoylthioureato-k^{2}O,S)]PF_6 (**4**)

^{1}H NMR (400.13 MHz, $CDCl_3$) δ 7.48-6.66 (30H atoms of PPh_3, 15H aromatic of Ph); ^{13}C{^{1}H} NMR (100.00 MHz, $CDCl_3$) δ 169.19 (C=O), 167.84 (C=S), 141.53, 142.58 (C_c–Ph–R_1), 132.74, 133.02 (d, C_{meta}–PPh_3, $^3J_{C-P}$ 10.35, 10.78 Hz), 131.04 (C_{para}–Ph–R_1), 130.38, 130.88 (C_{para}–PPh_3), 129.50 ($C_{quaternary}$–Ph–R_1), 127.77, 128.39 (C_{meta}–Ph–R_2), 128.07 (C_{meta}–Ph–R_1), 127.09, 127.41 (d, C_{ortho}–PPh_3, $^2J_{C-P}$ 11.68, 10.95 Hz), 125.84, 126.44 (C_{ortho}–Ph–R_2), 126.29 (C_{ortho}–Ph–R_1), 126.17 (C_{para}–Ph–R_2), 124.02, 125.51 (d, $C_{quaternary}$–PPh_3, $^1J_{C-P}$ 68.04, 57.12 Hz); ^{31}P{^{1}H} NMR (161.98 MHz, CH_2Cl_2/D_2O) δ 20.66, 9.25 (d), $^2J_{P-P}$ 25.91 Hz, 30.66 (d), 11.26 (d), $^2J_{Pt-P}$ 3135 Hz, 21.38 (d), –2.94 (d), $^2J_{Pt-P}$ 3793 Hz, –144.51 (m, PF_6); IR (CsI) ν / cm^{-1} 3019 ν(CHPPh_3), 1586 ν(C=N), 1481 ν(C=O), 842 ν(P–F), 748 ν(C=S), 696 ν(Ph_3–P–Ph_3), 550 ν(Pt–P). Anal. found (calcd.) for [$C_{56}H_{45}F_6N_2OP_3PtS$], %: C, 56.59 (56.24); H, 4.01 (3.79); N, 2.10 (2.34); S, 2.80 (2.68). mp 224-230 °C; Λ_m = 52.7 Ω^{-1} cm^2 mol^{-1}.

cis-[Pt(PPh_3)$_2$(*N,N*-Dibenzyl-*N'*-benzoylthioureato-k^{2}O,S)]PF_6 (**5**)

^{1}H NMR (400.13 MHz, $CDCl_3$) δ 7.63-6.02 (30H atoms of PPh_3, 5H aromatic of Ph, 10H aromatic of Bz), 5.60 (s, 2H, CH_2), 5.59 (s, 2H, CH_2); ^{13}C{^{1}H} NMR (100.00 MHz, $CDCl_3$) δ 169.58 (C=O), 167.81 (C=S), 134.50 (d, C_{meta}–PPh_3, $^3J_{C-P}$ 10.79 Hz), 131.84, 132.30 (C_{para}–PPh_3), 132.21 (C_{para}–Ph), 130.78 ($C_{quaternary}$–Ph), 130.55 ($C_{quaternary}$–Bz), 129.75 (C_{para}–Bz), 129.45 (C_{meta}–Ph), 129.03, 129.15 (C_{meta}–Bz), 128.72, 128.92 (d, C_{ortho}–PPh_3, $^2J_{C-P}$ 11.44 Hz), 128.43, 128.54 (C_{ortho}–Bz), 127.83 (C_{ortho}–Ph), 126.04, 126.94 (d, $C_{quaternary}$–PPh_3, $^1J_{C-P}$ 68.47, 58.25 Hz), 52.00, 54.22 (CH_2); ^{31}P{^{1}H} NMR (161.98 MHz, CH_2Cl_2/D_2O) δ 20.65, 9.11 (d), $^2J_{P-P}$ 25.02 Hz, 30.30 (d), 11.00 (d), $^2J_{Pt-P}$ 3122 Hz, 21.00 (d), –2.73 (d), $^2J_{Pt-P}$ 3843 Hz, –144.51 (m, PF_6); IR (CsI) ν / cm^{-1} 3018 ν(CHPPh_3), 1585 ν(C=N), 1493 ν(C=O), 842 ν(P–F), 749 ν(C=S), 696, ν(Ph_3–P–Ph_3), 548 ν(Pt–P). Anal. found (calcd.) for [$C_{58}H_{49}F_6N_2OP_3PtS$], %: C, 56.48 (56.91); H, 4.25 (4.03); N, 2.50 (2.29); S, 2.59 (2.62). mp 167-172 °C; Λ_m = 48.6 Ω^{-1} cm^2 mol^{-1}.

cis-[Pt(PPh_3)$_2$(*N,N*-Diethyl-*N'*-furoylthioureato-k^{2}O,S)]PF_6 (**6**)

^{1}H NMR (400.13 MHz, $CDCl_3$) δ 7.75-6.54 (30H atoms of PPh_3, 3H aromatic of furan ring), 4.68 (2H, c, CH_2), 4.50 (2H, c, CH_2), 3.61 (3H, t, CH_3), 3.08 (3H, t, CH_3); ^{13}C{^{1}H} NMR (100.00 MHz, $CDCl_3$) δ 168.74 (C=O), 159.80 (C=S), 146.37 ($C_{quaternary}$–Fur), 145.34 (C–O–Fur), 134.54 (d, C_{meta}–PPh_3, $^3J_{C-P}$ 11.16 Hz), 131.51 (C_{para}–PPh_3), 129.14 (d, $C_{quaternary}$–PPh_3, $^1J_{C-P}$ 62.04 Hz), 128.51 (d, C_{ortho}–PPh_3, $^2J_{C-P}$ 11.16 Hz), 112.79 (C–Fur), 61.55 (CH_2), 59.30 (CH_3); ^{31}P{^{1}H} NMR (161.98 MHz, CH_2Cl_2/D_2O) δ 20.35, 9.83 (d), $^2J_{P-P}$ 24.29 Hz, 29.91 (d), 11.02 (d), $^2J_{Pt-P}$ 3067 Hz, 21.14 (d), –2.72 (d), $^2J_{Pt-P}$ 3846 Hz, –144.51 (m, PF_6); IR (CsI) ν / cm^{-1} 3018 ν(CHPPh_3), 1586 ν(C=N), 1509 ν(C=O), 842 ν(P–F), 756 ν(C=S), 696 ν(Ph_3–P–Ph_3), 552 ν(Pt–P). Anal. found (calcd.) for [$C_{46}H_{43}F_6N_2O_2P_3PtS$], %: C, 50.39 (50.69); H, 4.28 (3.98); N, 2.50 (2.57); S, 3.16 (2.94). mp 230-238 °C; Λ_m = 59.6 Ω^{-1} cm^2 mol^{-1}.

cis-[Pt(PPh_3)$_2$(*N,N*-Dibenzyl-*N'*-furoylthioureato-k^{2}O,S)]PF_6 (**7**)

^{1}H NMR (400.13 MHz, $CDCl_3$) δ 7.75-5.99 (30H atoms of PPh_3, 3H aromatic of furan ring), 5.06 (2H, s, CH_2), 4.59 (2H, s, CH_2); ^{13}C{^{1}H} NMR (100.00 MHz, $CDCl_3$) δ 168.73 (C=O), 160.96 (C=S), 149.44 ($C_{quaternary}$–Fur), 146.32 (C–O–Fur), 135.64 (C_{para}–Bz), 134.33, 134.47 (d, C_{meta}–PPh_3, $^3J_{C-P}$ 11.21, 10.31 Hz), 131.86, 132.34 (C_{para}–PPh_3), 131.57, 132.22 ($C_{quaternary}$–Bz), 128.68, 128.86 (d, C_{ortho}–PPh_3, $^2J_{C-P}$ 12.98, 10.75 Hz), 128.18, 128.56 (C_{meta}–Bz), 127.29, 127.92 (C_{ortho}–Bz), 125.64, 126.90 (d, $C_{quaternary}$–PPh_3, $^1J_{C-P}$ 68.40, 58.97 Hz), 112.26, 118.16 (C–Fur), 52.76, 53.87 (CH_2); ^{31}P{^{1}H} NMR (161.98 MHz, CH_2Cl_2/D_2O) δ 20.35, 9.30 (d), $^2J_{P-P}$ 24.29 Hz, 29.91 (d), 11.02 (d), $^2J_{Pt-P}$ 3846 Hz, 21.14 (d), –2.72 (d), $^2J_{Pt-P}$ 3846 Hz, –144.51 (m, PF_6^-); IR (CsI) ν / cm^{-1} 3018 ν(CHPPh_3), 1574 ν(C=N),

1509 ν(C=O), 842 ν(P–F), 749 ν(C=S), 695 ν(Ph_3–P–Ph_3), 551 ν(Pt–P). Anal. found (calcd.) for [$C_{56}H_{47}F_6N_2O_2P_3PtS$], %: C, 55.77 (55.40); H, 4.15 (3.90); N, 2.91 (2.31); S, 2.74 (2.64). mp 204-208 °C; Λ_M = 48.6 Ω^{-1} cm^2 mol^{-1}.

cis-[$Pt(PPh_3)_2$(*N,N*-Diphenyl-*N'*-furoylthioureato-k^2O,S)]PF_6 (**8**)

^{1}H NMR (400.13 MHz, $CDCl_3$) δ 7.92-5.86 (30H atoms of PPh_3, 10H atoms of Ph and 3H aromatic of furan ring); $^{13}C\{^1H\}$ NMR (100.00 MHz, $CDCl_3$) δ 169.92 (C=O), 160.60 (C=S), 148.83 ($C_{quaternary}$–Fur), 147.79 (C–O–Fur), 142.91, 143.86 ($C_{quaternary}$–Ph), 134.05, 134.19 (d, C_{meta}–PPh_3, $^3J_{C-P}$ 11.02, 10.94 Hz), 131.56, 132.28 (C_{para}–PPh_3), 128.94, 129.69 (C_{meta}–Ph), 128.52, 128.66 (d, C_{ortho}–PPh_3, $^2J_{C-P}$ 12.87, 12.15 Hz), 127.22, 127.79 (C_{ortho}–Ph), 127.46 (C_{para}–Ph), 125.23, 126.58 (d, $C_{quaternary}$–PPh_3, $^1J_{C-P}$ 67.87, 57.69 Hz), 112.37, 118.36 (C–Fur); $^{31}P\{^1H\}$ NMR (161.98 MHz, CH_2Cl_2/D_2O) δ 20.24, 9.21 (d), $^2J_{P-P}$ 24.29 Hz, 29.73 (d), 10.92 (d), $^2J_{Pt-P}$ 3046 Hz, 21.28 (d), –2.70 (d), $^2J_{Pt-P}$ 3844 Hz, –144.51 (m, PF_6^-); IR (CsI) ν / cm^{-1} 3018 ν($CHPPh_3$), 1574 ν(C=N), 1523 ν(C=O), 769 ν(C=S), 842 ν(P–F), 693 ν(Ph_3–P–Ph_3), 549 ν(Pt–P). Anal. found (calcd.) for [$C_{54}H_{43}F_6N_2O_2P_3PtS$], %: C, 54.95 (54.69); H, 4.01 (3.65); N 2.19 (2.36); S, 2.89 (2.70). mp 265-268 °C; Λ_m = 52.8 Ω^{-1} cm^2 mol^{-1}.

cis-[$Pt(PPh_3)_2$(*N,N*-Morpholine-*N'*-furoylthioureato-k^2O,S)]PF_6 (**9**)

^{1}H NMR (400.13 MHz, $CDCl_3$) δ 7.40-6.98 (30H atoms of PPh_3, 3H aromatic of furan ring), 4.22 (t, 4H, CH_2), 3.80 (t, 4H, CH_2), 3.71 (bs, 8H, CH_2); $^{13}C\{^1H\}$ NMR (100.00 MHz, $CDCl_3$) δ 169.63 (C=O), 167.76 (C=S), 135.14 ($C_{quaternary}$–Fur), 134.49 (d, C_{meta}–PPh_3, $^3J_{C-P}$ 10.61 Hz), 131.86, 132.33 (C_{para}–PPh_3), 132.23 (C–O–Fur), 127.84, 129.45 (C–Fur), 128.73, 128.92 (d, C_{ortho}–PPh_3, $^2J_{C-P}$ 11.67, 11.20 Hz), 125.99, 126.90 (d, $C_{quaternary}$–PPh_3, $^1J_{C-P}$ 67.37, 58.58 Hz), 66.08, 66.37 (CH_2–O), 47.58, 49.46 (CH_2–N) of the *N*-morpholyn; $^{31}P\{^1H\}$ NMR (161.98 MHz, CH_2Cl_2/D_2O) δ 21.03, 8.66 (d), $^2J_{P-P}$ 24.29 Hz, 30.72 (d), 11.36 (d), $^2J_{Pt-P}$ 3122 Hz, 20.64 (d), –3.08 (d), $^2J_{Pt-P}$ 3838 Hz, –144.51 (m, PF_6^-); IR (CsI) ν / cm^{-1} 3017 ν($CHPPh_3$), 1586 ν(C=N), 1481 ν(C=O), 842 ν(P–F), 761 ν(C=S), 697 ν(Ph_3–P–Ph_3), 549 ν(Pt–P). Anal. found (calcd.) for [$C_{50}H_{49}F_6N_4O_4P_3PtS$], %: C, 50.48 (49.88); H, 4.30 (4.10); N, 4.45 (4.65); S, 2.90 (2.66). mp 242-248 °C; Λ_M = 47.6 Ω^{-1} cm^2 mol^{-1}.

cis-[$Pt(PPh_3)_2$(*N,N*-Dimethyl-*N'*-thiophenylthioureato-k^2O,S)]PF_6 (**10**)

^{1}H NMR (400.13 MHz, $CDCl_3$) δ 7.82-6.41 (30H atoms of PPh_3, 3H aromatic of thiophene), 0.96 (6H, s, CH_3); $^{13}C\{^1H\}$ NMR (100.00 MHz, $CDCl_3$) δ 166.67 (C=O), 164.12 (C=S), 141.12 ($C_{quaternary}$–Th), 134.44, 134.53 (d, C_{meta}–PPh_3, $^3J_{C-P}$ 10.17 Hz), 132.40 (C–S–Th), 127.73, 132.21 (C–Th), 131.76, 132.29 (C_{para}–PPh_3), 128.70, 128.86 (d, C_{ortho}–PPh_3, $^2J_{C-P}$ 12.19, 11.38 Hz), 125.92, 127.12 (d, $C_{quaternary}$–PPh_3, $^1J_{C-P}$ 68.51, 58.06 Hz), 40.75, 41.58 (CH_3); $^{31}P\{^1H\}$ NMR (161.98 MHz, CH_2Cl_2/D_2O) δ 20.72, 9.80 (d), $^2J_{P-P}$ 25.92 Hz, 29.76 (d), 10.92 (d), $^2J_{Pt-P}$ 3050 Hz, 21.39 (d), –2.47 (d); $^2J_{Pt-P}$ 3863 Hz, –144.51 (m, PF_6^-); IR (CsI) ν / cm^{-1} 3017 ν($CHPPh_3$), 1496 ν(C=N), 1415 ν(C=O), 842 ν(P–F), 749 ν(C=S), 694 ν(Ph_3–P–Ph_3), 549 ν(Pt–P). Anal. found (calcd.) for [$C_{44}H_{39}F_6N_2OP_3PtS_2$], %: C, 49.01 (49.03); H, 3.45 (3.65); N, 2.55 (2.60); S, 6.25 (5.95). mp 251-253 °C; Λ_m = 46.6 Ω^{-1} cm^2 mol^{-1}.

cis-[$Pt(PPh_3)_2$(*N,N*-Diphenyl-*N'*-thiophenylthioureato-k^2O,S)]PF_6 (**11**)

^{1}H NMR (400.13 MHz, $CDCl_3$) δ 7.66-6.12 (30H atoms of PPh_3, 10H aromatic of Ph and 3H aromatic of thiophene ring); $^{13}C\{^1H\}$ NMR (100.00 MHz, $CDCl_3$) δ 169.64 (C=O), 164.69 (C=S), 140.23 ($C_{quaternary}$–Th), 135.05 (C–S–Th), 134.04, 134.23 (d, C_{meta}–PPh_3, $^3J_{C-P}$ 11.00, 10.64 Hz), 132.48 (C_{para}–Ph), 131.67, 132.31 (C_{para}–PPh_3), 129.71 ($C_{quaternary}$–Ph), 129.10 (C_{meta}–Ph), 128.52, 128.78 (d, C_{ortho}–PPh_3, $^2J_{C-P}$ 11.23, 10.74 Hz), 127.98 (C_{ortho}–Ph), 127.35, 127.73 (C–Th), 125.21, 126.72 (d, $C_{quaternary}$–PPh_3, $^1J_{C-P}$ 68.41, 57.40 Hz); $^{31}P\{^1H\}$ NMR (161.98 MHz, CH_2Cl_2/D_2O) δ 20.33 (d), 9.43 (d), $^2J_{P-P}$ 24.29 Hz, 29.76 (d), 10.92 (d), $^2J_{Pt-P}$ 3051 Hz, 21.37 (d), –2.28 (d), $^2J_{Pt-P}$ 3863 Hz, –144.51 (m, PF_6^-); IR (CsI) ν / cm^{-1} 3020 ν($CHPPh_3$), 1483 ν(C=N), 1423 ν(C=O), 842 ν(P–F), 745 ν(C=S), 696 ν(Ph_3–P–Ph_3), 551 ν(Pt–P). Anal. found (calcd.) for [$C_{54}H_{43}F_6N_2OP_3PtS_2$], %: C, 53.82 (53.96); H, 3.55 (3.61); N, 2.30 (2.33); S, 6.13 (5.33). mp 232-238 °C; Λ_m = 45.6 Ω^{-1} cm^2 mol^{-1}.

cis-[$Pt(PPh_3)_2$(*N,N*-Dibenzyl-*N'*-thiophenylthioureato-k^2O,S)]PF_6 (**12**)

^{1}H NMR (400.13 MHz, $CDCl_3$) δ 7.92-6.46 (30H atoms of PPh_3, 10H aromatic of Ph and 3H aromatic of thiophene ring), 5.25 (s, 2H, CH_2), 4.75 (s, 2H, CH_2); $^{13}C\{^1H\}$ NMR (100.00 MHz, $CDCl_3$) δ 168.48 (C=O), 165.46 (C=S), 140.90 ($C_{quaternary}$–Th), 135.18 (C–S–Th), 134.36, 134.52 (d, C_{meta}–PPh_3, $^3J_{C-P}$ 10.92, 10.43 Hz), 132.87, 133.24 (C–Th), 132.81 (C_{para}–Bz), 131.97, 132.41 (C_{para}–PPh_3), 130.44, 129.41 ($C_{quaternary}$–Bz), 128.91 (d, C_{ortho}–PPh_3, $^2J_{C-P}$ 13.56 Hz), 128.69 (C_{meta}–Bz), 127.35 (C_{ortho}–Bz), 125.65, 126.95 (d, $C_{quaternary}$–PPh_3, $^1J_{C-P}$ 68.16, 58.06 Hz); $^{31}P\{^1H\}$ NMR (161.98 MHz, CH_2Cl_2/D_2O) δ 20.65, 9.11 (d), $^2J_{P-P}$ 25.02 Hz, 30.72 (d), 11.22 (d), $^2J_{Pt-P}$ 3095 Hz, 20.60 (d), –3.06 (d), $^2J_{Pt-P}$ 3866 Hz, –144.51 (m, PF_6^-); IR (CsI) ν / cm^{-1} 3018 ν($CHPPh_3$), 1585 ν(C–N), 1493 ν(C–O), 843 ν(P–F), 749 ν(C–S), 696 ν(Ph_3–P–Ph_3),

549 ν(Pt–P). Anal. found (calcd.) for [$C_{56}H_{47}F_6N_2OP_3PtS_2$], %: C, 56.48 (54.68); H, 4.00 (3.85); N, 2.15 (2.28); S, 5.40 (5.21). mp 177-179 °C; Λ_m = 48.6 Ω^{-1} cm^2 mol^{-1}.

Crystal structure determination

Single crystals suitable for X-ray diffraction were obtained by slow evaporation of $CHCl_3$:*n*-hexane (3:1) solutions of some of the complexes (**3**, **6**, **9** and **10**). Diffraction data were collected on an Enraf-Nonius Kappa-CCD diffractometer with graphite-monochromated MoKα radiation (λ = 0.71073 Å). The final unit cell parameters were based on all reflections. Data collections were performed using the COLLECT program;[22] integration and scaling of the reflections were performed with the HKL Denzo-Scalepack system of programs.[23] Absorption corrections were carried out using the Gaussian method.[24] The structures were solved by direct methods with SHELXS-97.[25] The models were refined by full-matrix least-squares on F^2 by means of SHELXL-97.[26] The projection views of the structures were prepared using ORTEP-3 for Windows.[27] 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.

Cell culture assay

In vitro cytotoxicity assays on cultured human tumor cell lines still represent the standard method for the initial screening of antitumor agents. Thus, as a first step in assessing the pharmacological properties of the new platinum(II) complexes, they were assayed against human breast tumor cell line MDA-MB-231 (ATCC: HTB-26), human prostate tumor cells DU-145 (ATCC: HTB-81) and against the L929 non-tumor cell line (ATCC: CCL 1). The cells MDA-MB-231 and L929 were routinely maintained in Dulbecco's modified Eagle's medium

Table 1. Crystal data and refinement parameters for complexes **3**, **6**, **8** and **10**

Compound	**3**	**6**	**8**	**10**
Empirical formula	$C_{52}H_{53}F_6N_2OP_3Pt\ S$	$C_{46}H_{44}F_6N_2O_2P_3Pt\ S$	$C_{54}\ H_{43}F_6N_2O_2P_3\ Pt\ S$	$C_{44}H_{39}F_6N_2OP_3Pt\ S_2$
Formula weight	1156.02	1090.89	1185.96	1077.89
Crystal system	monoclinic	monoclinic	triclinic	monoclinic
Space group	$P2_1/a$	$P2_1/a$	P-1	$P2_1/n$
a / Å	11.21870(10)	10.9930(2)	12.9584(4)	10.8928(3)
b / Å	29.5337(3)	28.6741(5)	13.7427(4)	22.5806(7)
c / Å	15.1327(2)	13.8772(3)	15.0167(4)	17.8166(3)
α / degree	90	90	95.431(2)	90
β / degree	90.9120(10)	93.7550(10)	109.262(2)	98.368
γ / degree	90	90	92.980(2)	90
V / Å^3	5013.28(9)	4364.90(14)	2503.32(13)	4335.62(19)
Z	4	4	2	4
Density calcd. / (mg m^{-3})	1.532	1.660	1. 573	1.651
Absorption coefficient / mm^{-1}	2.998	3.44	3. 006	3.506
F(000)	2320	2172	1180	2136
θ range for data collection / degree	2.633 to 25.999	2.54 to 26.00	2.81 to 26	2.74 to 26.00
Index ranges	–13 ≤ h ≤ 13	–13 ≤ h ≤ 9	–15 ≤ h ≤ 15	–12 ≤ h ≤ 13
	–36 ≤ k ≤ 36	–35 ≤ k ≤ 34	–16 ≤ k ≤ 16	–26 ≤ k ≤ 27
	–18 ≤ l ≤ 18	–17 ≤ l ≤ 16	–18≤ l ≤ 17	–21 ≤ l ≤ 18
Reflections collected	56096	34901	27368	28072
Independent reflections (R(int))	9770 [R(int) = 0.0940]	8530 [R(int) = 0.0606]	9802 [R(int) = 0.044]	8317 [R(int) = 0.0606]
Goodness-of-fit on F^2	1.100	1.001	1.022	1.001
Final R indices	R1 = 0.0437	R1 = 0.0540	R1 = 0.0394	R1 = 0.0470
[I > 2σ(I)]	wR2 = 0.1128	wR2 = 0.1377	wR2 = 0.0960	wR2 = 0.1217
Largest diff. peak and hole / eÅ^{-3}	1.865 and –1.209	2.339 and –2.030	2.196 and –1.032	1.058 and –1.060

Table 2. Selected bond lengths and angles for the complexes **3**, **6**, **8** and **10**

Bond length / Å	**3**	**6**	**8**	**10**
Pt–O	2.051(3)	2.061(4)	2.065(4)	2.058(4)
Pt–P(2)	2.3235(11)	2.3138(12)	2.3154(14)	2.3121(14)
Pt–S	2.2950(11)	2.3081(14)	2.3051(14)	2.3180(15)
Pt–P(1)	2.2458(12)	2.2406(14)	2.2435(15)	2.2399(14)
S–C(1)	1.725(5)	1.736(7)	1.746(7)	1.731(6)
O–C(2)	1.282(6)	1.254(6)	1.275(7)	1.280(7)
N(1)–C(2)	1.301(6)	1.330(8)	1.288(8)	1.304(7)
N(1)–C(1)	1.360(6)	1.331(7)	1.361(8)	1.346(7)
N(2)–C1	1.335(5)	1.344(7)	1.335(7)	1.324(7)
Bond angle / degree	**3**	**6**	**8**	**10**
O–Pt–S	91.62(9)	90.87(10)	92.11(12)	92.06(11)
O–Pt–P(1)	177.81(11)	177.80(13)	177.40(11)	173.40(13)
O–Pt–P(2)	83.28(9)	83.62(10)	82.99(12)	81.78(11)
P(2)–Pt–S	173.86(4)	173.05(5)	173.86 (4)	165.89(6)
S–Pt–P(1)	89.69(4)	88.80(5)	89.69(4)	89.55(5)
P(2)–Pt–P(1)	95.51(4)	96.87(5)	95.51(4)	98.01(5)

(DMEM) supplemented with 10% fetal bovine serum (FBS); the DU-145 cells were maintained in RPMI-1640 supplemented with 10% FBS, at 37 °C in a humidified 5% CO_2 atmosphere. After reaching confluence, the cells were detached by trypsinization and counted. For the cytotoxicity assay, 1.5×10^4 cells $well^{-1}$ were seeded in 200 µL of complete medium in 96-well assay microplates. The plates were incubated at 37 °C 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; 25; 0.62; 0.15 and 0.039 mmol L^{-1}. From each of these diluted samples, 1 µL aliquots were added to 200 µL medium giving a final concentration of approximately 0.5% of DMSO and a final concentration of the complex diluted approximately 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-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide] to formazan.[28] MTT solution (0.5 mg mL^{-1}) 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 percentage of cell viability was calculated by dividing the average absorbance of the cells treated with the test compounds by that of the control; then cell viability percentage 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.

Anti-mycobacterial activity assay

The anti-*Mycobacterium tuberculosis* activity of the compounds was determined by the REMA (resazurin microtiter assay) method.[29] Stock solutions of the tested compounds were prepared in DMSO and diluted in Middlebrook 7H9 broth (Difco) supplemented with oleic acid, albumin, dextrose and catalase (OADC), performed by Precision XS (Biotek®) to obtain the final drug concentration range of 0.09-25 µg mL^{-1}. Isoniazid was dissolved in distilled water and rifampicin in DMSO, and both were used as standard drugs. A suspension of MTB H37Rv ATCC 27294 was cultured in Middlebrook 7H9 broth supplemented with OADC and 0.05% Tween 80. The cultures were frozen at −80 °C in aliquots. After two days the CFU *per* mL (colony formation unit *per* mL) of an aliquot was determined. The concentrations were adjusted by 5×10^5 CFU *per* mL and 100 µL of the inoculum were added to each well of a 96-well microplate together with 100 µL of the compounds. Samples were set up in triplicate. The plates were incubated for 7 days at 37 °C. Resazurin (solubilized in water) was added (30 µL of 0.01%). The fluorescence of the wells was read after 24 h with a Cytation 3 (Biotek®). The MIC (minimum inhibitory concentration) was defined as the lowest concentration resulting in 90% inhibition of MTB growth.

Results and Discussion

Twelve platinum(II) complexes with general formula *cis*-$[Pt(PPh_3)_2$(*N*,*N*-disubstituted-*N*'-acylthioureas)$]PF_6$ were synthesized and characterized by elemental analyses, melting point temperatures (mp), and molar conductivities.

The data from the infrared spectra shown in the Experimental section, suggest the formation of $[Pt(PPh_3)_2(Ln)]PF_6$ complexes (**1**-**12**), where L is an anionic ligand, formed by the deprotonation of the *N*,*N*-disubstituted-*N*'-acylthiourea during its coordination to the platinum. Thus, typical NH stretching vibrations in the IR spectra of the free ligands in the range of 3050-3260 cm^{-1} as broad and strong absorptions, disappears after their coordinations to the metal, while the ν(C–N) bands, present in the complexes at 1574-1586 cm^{-1}, are absent in the free ligands, suggesting the formation of heterocyclics, according to Scheme 1. Also, comparing the band of the C=O group present in the free ligands with the infrared spectra of the complexes, there is a decreasing

of the νC–O stretching vibration frequency, which is in agreement with the literature.[30] Thus, it is reasonable to assign the bands in the range 1415-1523 cm^{-1} to the coordinated C–O group, since the ν(C=O) bands in the free ligands are at about 1680 cm^{-1}. The absorptions at 815-878 cm^{-1} in the spectra of the free bases, *N*,*N*-disubstituted-*N*'-acylthioureas, attributed to the ν(C–S) stretching vibrations, shift to the 745-769 cm^{-1} range in the complexes spectra. The infrared spectra show 3015-3019 νC–H (PPh_3), 842-845 ν(P–F), 694-697 νP–Ph (Ph_3–P–Ph_3), 531-552 ν(Pt–P).

This change suggests deprotonation of the ligands, indicating their coordination to the metal through the sulfur atom with a formally carbon-sulfur single bond.[31-34] The absorptions at about 471 cm^{-1} in the IR spectra of the complexes can be assigned to the Pt–O vibration mode and the assignment of the Pt–S stretching vibration bands at about 360 cm^{-1} are in accordance to the reported in the literature.[35-37] Thus, from the IR data it is possible to suggest that the *N*,*N*-disubstituted-*N*'-acylthioureas are attached to the metal through the oxygen and sulfur, in a chelating form.

The type of the *N*,*N*-dialkyl-acylthiourea ligands used in this work are reported in the literature,[38] and it was found that for a series of hydrophilic *N*,*N*-dialkyl-*N*-aroylthioureas, the acid dissociation constants, pK_a(NH) have been found to range from 7.5 to 10.9 in water-dioxane mixtures.

In the complexes (**1**-**12**) there are two coordinated triphenylphosphine ligands, in *cis* position, according to the $^{31}P\{^1H\}$ NMR data, and their molar conductivities show that the complexes are cationic species.[39]

The NMR (^1H and $^{13}C\{^1H\}$) spectroscopy was used to speculate about the molecular structures of the complexes, and a comparative analysis on the basis of the spectroscopic data corresponding to both, free and coordinated ligands with the metallic ion, was performed. The ^1H NMR data for the complexes (**1**-**12**) are given in the Experimental section. The ^1H NMR spectra of the free ligands show basically three sets of well-separated signals corresponding to their R1, R2 substituents and to the NH proton. The signals of the NH protons appear as a broad singlet in the region between 8.55 and 8.80 ppm,[21] and after the coordination of the ligands to the metal, these signals disappear.[40,41] The aromatic and the nitrogen substituents protons in the coordinated ligands were slightly downfield shifted when compared to the chemical shift of the free ligands. The aromatic protons appear as a complex pattern in the region δ 8.17-5.74 ppm (given in Figure 2 and Figures S1-S8 of the Supplementary Information) in comparison to similar compounds previously reported in the literature.[42]

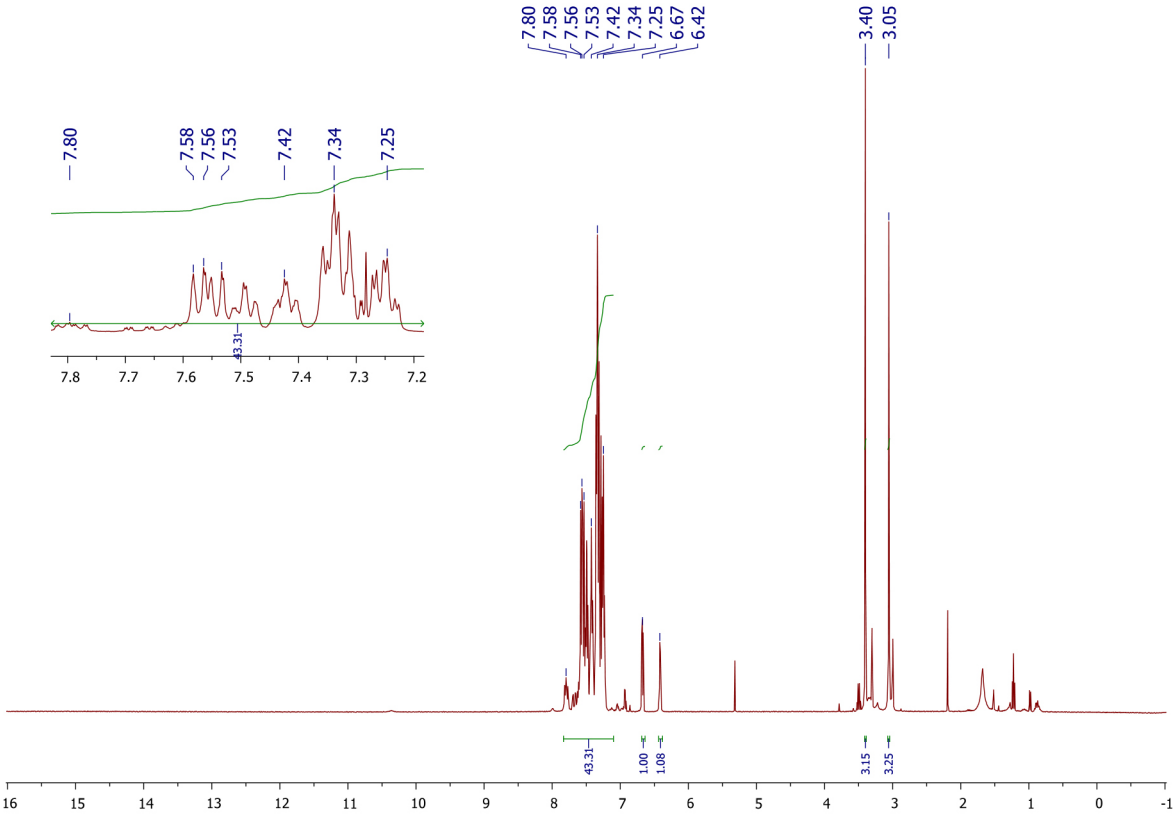


Figure 2. ^1H NMR (400 MHz, $CDCl_3$) spectrum of *cis*-[$Pt(PPh_3)_2$(*N*,*N*-dimethyl-*N*'-thiophenylthioureato-k^2O,S)]PF_6 (**10**).

Interestingly, heteronuclear multiple bond coherence (HMBC) NMR experiments showed that the chemical shifts of the carbon bonded to the sulfur atom in the coordinated ligands are at higher field than the carbon bonded to the oxygen atom, unlike the observed for the free ligands, where carbon from the C=O group is at higher field than the carbon bonded to the sulfur atom (see Figure 3).[21]

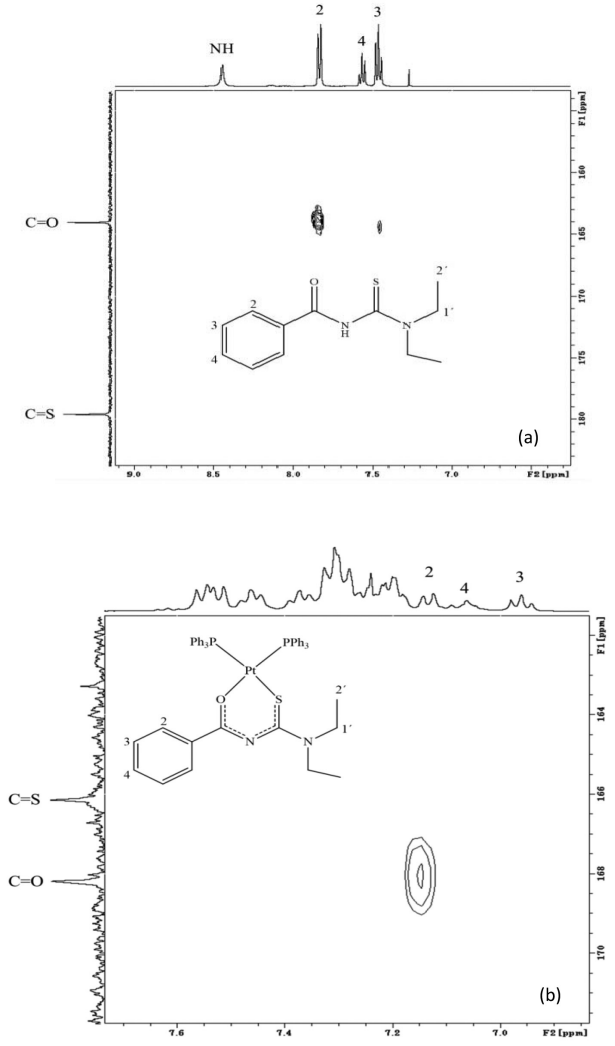


Figure 3. ^{1}H,^{13}C HMBC experiments (400 MHz, $CDCl_3$) of (a) ligand *N*,*N*-diethyl-*N*'-benzoylthiourea; (b) complex *cis*-[$Pt(PPh_3)_2$(*N*,*N*-diethyl-*N*'-benzoylthioureato-k^2O,S)]PF_6.

This, probably, is due to the delocalization of the electron density towards the sulfur atom after the deprotonation of the secondary amide of the ligand.

The precursor [$PtCl_2(PPh_3)_2$], in CH_2Cl_2 solution, shows a singlet peak for phosphorous atoms, at 13.72 ppm, in its $^{31}P\{^{1}H\}$ NMR spectrum, therefore the complexes here reported show two doublets, at about 21.08 and 9.83 ppm, indicating the presence of two magnetically different phosphorus atoms coordinated to the platinum(II) ion, and the four satellite signals of the platinum(II). Thus, in this case it is possible to assign the more shielded chemical shift to the phosphorus atom *trans* to the sulfur, since this atom is a better donor than the oxygen, and more likely the chloride ligand. The multiple signals of the PF_6^- is in the region –(138-158) ppm (Figure 4 and Figures S12-S22 of the Supplementary Information) in comparison to similar compounds previously reported in the literature.[43]

The structures of complexes **3**, **6**, **8** and **10**, were determined by X-ray diffraction analysis. Their ORTEP views are in Figure 5 and the selected bond lengths (Å) and angles (°) for the complexes are listed in Table 2. The X-ray structures of the complexes confirmed that the *N*,*N*-disubstituted-*N*'-acylthioureas are coordinated to the central ion Pt^{II} as bidentate ligands, by the oxygen and sulfur atoms, and there are two PPh_3 ligands, which are also in the *cis* fashion, as previously suggested by IR spectroscopy. In all complexes, the Pt^{II} ion is nearly planar, in a fourfold environment. For the complexes synthesized in this work, the C–S bond distance is 1.734 Å (average), longer than the C–S bond distance of neutral species (1.661-1.676 Å).[16,21,44,45] For free *N*,*N*-disubstituted-*N*'-acylthioureas, the C–O bonds lengths of 1.214-1.215 Å indicate double-bond character, whereas the C–N bonds, as single one, are at about 1.373-1.412 Å.[44,45] As a result of acylthiourea coordination to the metal, the bond lengths present significant C–S and C–O lengthening and C–N shortening (see Figure 5, Table 2), which is an evidence of resonance effect, as above mentioned in the $^{13}C\{^{1}H\}$ NMR experiment discussion, as a consequence of the deprotonation of the nitrogen atom N–H, which is between the carbonyl and thiocarbonyl groups.

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.[45]

Table 3 lists the ligand and complex concentrations that produce 50% of growth inhibition (IC_{50}, μmol L^{-1}) against DU-145 (human prostate tumor cells), MDA-MB-231 (human breast tumor cells) and against the L929 cell line (mouse healthy cell line). The new platinum(II) complexes, the free ligands, and the precursor [$PtCl_2(PPh_3)_2$] were tested against the tumor cells and L929 cells. For comparison, the cytotoxicity of cisplatin and of [$PtCl_2(PPh_3)_2$] was 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. As can be seen from Table 3, for all compounds, the IC_{50} are very high against the L929 (non-tumor cell line), indicative of selectivity towards tumor cells.

Overall, the complexes are more active against the MDA-MB 231 tumor cells, and the most promising complexes

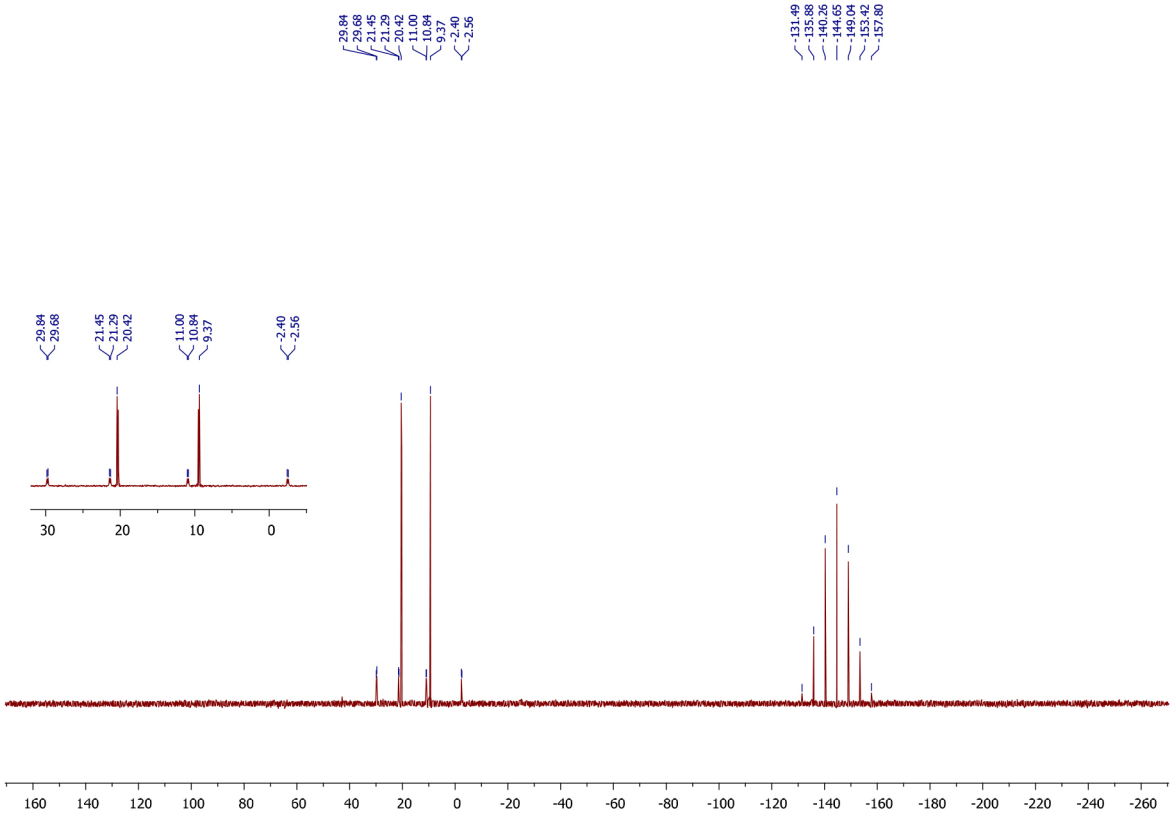


Figure 4. $^{31}P\{^{1}H\}$ (400 MHz, $CDCl_3$) spectrum of *cis*-[$Pt(PPh_3)_2$(*N*,*N*-dimethyl-*N*'-thiophenylthioureato-k^2O,S)]PF_6 (**10**).

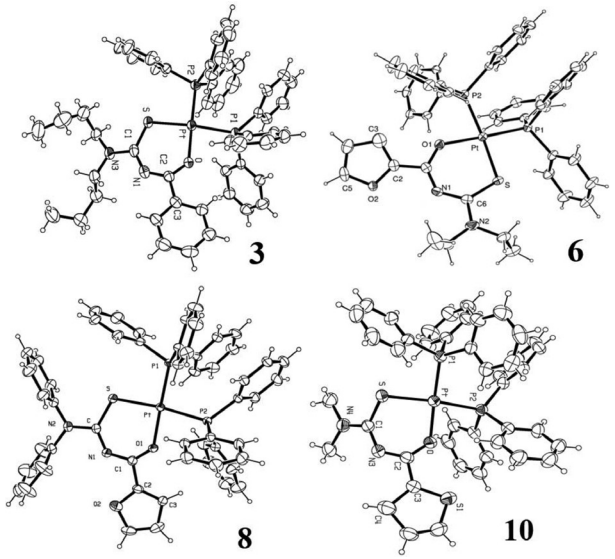


Figure 5. ORTEP view of complexes **3**, **6**, **8** and **10** showing 50% probability ellipsoids. The $(PF_6)^-$ anion is omitted for clarity.

are **1** (*N*,*N*-dimethyl-*N*'-benzoylthiourea), **2** (*N*,*N*-diethyl-*N*'-benzoylthiourea), **6** (*N*,*N*-diethyl-*N*'-furoylthiourea), and **10** (*N*,*N*-dimethyl-*N*'-thiophenylthiourea). It is interesting to observe that the ligands in these complexes are those of open chain as R2 groups when compared with other complexes. Thus, it may be that is the steric factor that better define the cytotoxicity of the complexes, mainly against the MDA-MB-231 tumor cells line. Thus, the size of the R2 group in the ligands plays a important role in the activity of the complexes. In this case, probably, the low volume of these complexes can facilitate their entrance in the cell, making them more active than the other ones.

The compounds were also investigated for their *in vitro* anti-mycobacterial activity against *Mycobacterial tuberculosis* H37Rv strains by the MABA methodology. The MICs found for the platinum complexes, free ligands and ethambutol are shown in Table 4.

Also, as can be seen from these data, the complexes **1**, **2**, **6** and **10** present lower values of MICs supporting the above correlation of structure/activity for these complexes. Work is ongoing in our laboratory, with new *N*,*N*-disubstituted-*N*'-acylthioureas, to check this hypothesis.

According to the anti-mycobacterial activity assays, compounds **1** and **10** exhibited promising activity, with MIC values of 2.81 and 2.78 μM, respectively. The results indicate that the complex **1** and **10**, which have methyl groups (R2 groups), have stronger *in vitro* activity than that of ethambutol (MIC 5.62 μM), which is clinically used as a first-line drug in several schemes of conventional tuberculosis treatment. Complexes **2** and **3** (MIC of 8.13 and 7.87 μM, respectively) were also active, but to a lesser extent. Thus,

Table 3. IC_{50} values of complexes **1**-**12** in DU-145, MDA-MB-231 and L929 cell line, after 48 h of incubation

Complex[a]	IC_{50} / (μmol L^{-1})			SI[b]	SI[c]
	DU-145	MDA-MB-231	L929		
1	0.92 ± 0.17	2.92 ± 0.22	> 100	> 108.70	> 34.24
2	14.12 ± 0.94	1.36 ± 0.05	> 100	> 7.08	> 73.53
3	9.02 ± 0.81	3.42 ± 2.52	> 100	> 11.09	> 29.24
4	> 100	> 100	> 100	–	–
5	> 200	> 200	> 200	–	–
6	31.02 ± 1.04	1.24 ± 0.24	> 100	> 3.22	> 80.64
7	70.87 ± 1.64	> 200	55.70 ± 15.07	> 0.79	< 0.28
8	32.05 ± 1.41	> 50	> 100	> 3.12	–
9	25.05 ± 2.45	> 50	> 100	> 3.99	–
10	61.03 ± 9.23	1.00 ± 0.80	> 100	> 1.64	> 100
11	> 100	> 100	> 100	–	–
12	> 100	> 100	> 100	–	–
Cisplatin	2.00 ± 0.20	2.43 ± 0.20	16.53 ± 2.38	> 8.26	> 6.80
$[PtCl_2(PPh_3)_2]$	> 100	> 100	> 100	–	–

[a]The IC_{50} for the ligands Ln are > 100 μmol L^{-1} in all three cases; [b]$IC50_{L929}/IC50_{DU\text{-}14}$; [c]$IC50_{L929}/IC50_{MDA\text{-}MB\text{-}231}$. SI: selective index.

Table 4. MIC values of anti-mycobacterial activity of platinum complexes and reference drug

Complex[a]	MIC / (μg mL^{-1})	MIC / (μmol L^{-1})
1	2.58 ± 0.14	2.81 ± 0.15
2	7.70 ± 0.20	8.13 ± 0.21
3	9.10 ± 0.40	7.87 ± 0.34
4	17.46 ± 0.63	16.74 ± 0.60
5	18.6 ± 0.35	17.43 ± 0.32
6	6.40 ± 0.63	5.86 ± 0.57
7	> 25	> 23.43
8	12.02 ± 0 65	10.13 ± 0.54
9	13.50 ± 0.64	14.20 ± 0.67
10	3.00 ± 0.20	2.78 ± 0.18
11	11.60 ± 0.50	11.05 ± 0.47
12	> 25	> 23.20
$[PtCl_2(PPh_3)_2]$	> 25	> 47.32
Reference drug ethambutol	1.02	5.62

[a]All ligands Ln present MIC > 25 μg mL^{-1}.

MIC values for free ligands (25 μg mL^{-1}) were several times higher than those observed for the respective complexes.

Conclusions

A novel series of Pt^{II} complexes with *N*,*N*-disubstituted-*N*'-acylthiourea as bidentate ligands was here synthesized and characterized. The X-ray crystallographic characterization of the Pt^{II} complexes with *N*,*N*-disubstituted-*N*'-acylthioureas as bidentate ligands shows that these ligands coordinate with the metal through the oxygen and sulfur atoms. In this work, there are three series of complexes: for the first one, the complexes **1**-**5**, R1 is the phenyl group; for the second it is the furoyl group (**6**-**9**); and for the other one, it is the thiophenyl group (**10**-**12**). In general, the complexes present better results for MDA-MB-231 tumor cells than for DU-145 tumor cells, and in this case the most promising complexes are those for which R2 groups are open and have small chains, suggesting that this is due to their low steric hindrance, which allows the complex penetrate easier to the cells, acting more effectively. Additionally, antimicrobial activity assays of the new complexes provided evidence that the complexes **1** and **10** are potential agents against mycobacterial infections, specifically against *M. tuberculosis* H37Rv.

Supplementary Information

Supplementary data (NMR spectra) are available free of charge at http://jbcs.sbq.org.br as PDF file.

Supplementary crystallographic data for the complexes (CCDC 1412820, 1412821, 1412823 and 1412822 for complexes **3**, **6**, **8** and **10**, 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.

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