Synthesis, characterization, antibacterial and cytotoxic activity of new palladium(II) complexes with dithiocarbamate ligands: X-ray structure of bis(dibenzyl-1-S:S′-dithiocarbamat...

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Synthesis, characterization and *in vitro* cytotoxicity of palladium(II) complexes with mixed ligands. X-ray diffraction study of $C_{31}H_{36}ClNPPdS_2$

Farkhanda Shaheen[1]*, Amin Badshah[1], Marcel Gielen[2]*, Christine Gieck[3] and Dick de Vos[4]

[1]Department of Chemistry, Quaid-I-Azam University, Islamabad, Pakistan
[2]Vrije Universiteit Brussel, Faculty of Engineering, HNMR Unit, B-1050 Brussels, Belgium
[3]Università del Piemonte Orientale, 15100 Alessandria, Italy
[4]Pharmachemie BV, PO Box 552, 2003 RN Haarlem, The Netherlands

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Pd(II) complexes with organophosphines and dithiocarbamates derivatives of α-amino acids were synthesized by reacting *N*,*N*-dicyclohexyldithiocarbamate (DCHDTC, compounds 1–3) and *N*-methylcyclohexyldithiocarbamate (MCHDTC, compounds 4–6) with $(R_3P)_2PdCl_2$ (R = Ph, *o*-tolyl, Ph_2Cl) in a 1:1 molar ratio. The complexes were characterized by elemental analyses, FT-IR, multinuclear (^1H, ^{13}C and ^{31}P) NMR and single X-ray crystallography, showing that the dithiocarbamate acts as a bidentate ligand and binds to Pd(II) via two sulfur atoms, resulting in a square planar geometry around Pd(II). The cytotoxicity of compounds 2, 3 and 4 was determined *in vitro* against six human tumour cell lines, MCF7, EVSA-T, WIDR, IGROV, M19 MEL, A498 and H226. Compounds 3 and 4 showed a moderate to low cytotoxicity, whereas compound 2 exhibited a very low cytotoxicity. The results of antifungal assays showed that compounds 1–6 possess antifungal activity against *Fusarium moniliformes*, *Fusarium saolani*, *Mucor* sp., *Aspergillus niger* and *Aspergillus fumigatus*. The anti-inflammatory screening results of 1–6 are quite similar to those observed for the standard drug Declofenac at 10 mg kg^{-1}, which inhibited the odema by 74% after 4 h. Copyright © 2007 John Wiley & Sons, Ltd.

KEYWORDS: palladium(II); dithiocarbamate; crystal structure; NMR; bidentate

INTRODUCTION

Platinum and palladium drugs have played a key role in metal-based anticancer agents.[1] The Pd(II) ions are capable of interacting with DNA, enabling cross bindings and inhibiting its synthesis as well as inducing apoptosis.[2] The *trans*-configuration of two leaving groups at the square planar Pd center should not exclude their having antiproliferative or anticancer activity because some very promising antitumor agents have been found among the *trans*-Pt complexes.[3,4]

The dithiocarbamate derivatives of α-amino acids are another class which acts as a useful model to study the coordination of proteins to metallic cations.[5] The dithiocarbamate derivatives of α-amino acids, in which the NC(S)SH moiety is replaced by NC(S)SR, are able to coordinate to transition metals as terminal or as bridging ligands.[6,7] The chemistry of transition metal–sulfur clusters is now growing rapidly because of their wide range of biological and catalytic applications owing to their similarity to certain biological and industrial catalysts. Their biological as well as catalytic activities are enhanced by complexation with Pd(II).[8] The dithiocarbamate moiety chelates the palladium with a (-S:S′) coordination mode.[9,14] Several Pt and Pd complexes with dithiocarbamates and dithioesters are known to exhibit cytotoxic activity against some cancers, such as lungs cells, ovarian,[10] melanoma, colon,[11] renal, prostate[12] and breast cancer.[13]

In order to explore the scope, nature of bonding and coordination modes of cyclic ambidentate dithiocarbamate

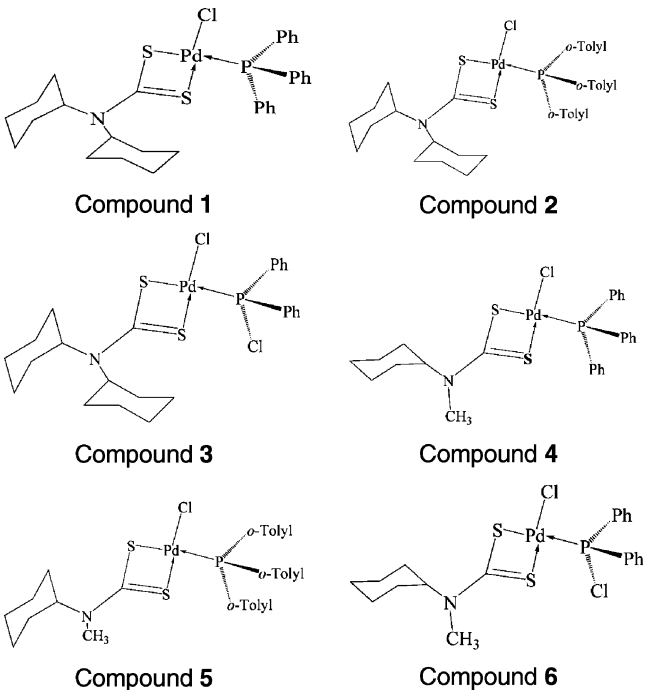
*Correspondence to: Farkhanda Shaheen, Department of Chemistry, Quaid-I-Azam University, Islamabad, Pakistan and Marcel Gielen, Vrije Universiteit Brussel, Faculty of Engineering, HNMR Unit, B-1050 Brussels, Belgium.
E-mail: fshaheenpk@yahoo.com; mgielen@vub.ac.be

and phosphorus ligands, such as chloro(4-methylpiperidine-1-dithiocarbamato-S : S′)(PPh_3)Pd(II), we synthesized a new series of Pd(II) complexes with mixed ligands of organophosphine/dithiocarbamates derivatives of α-amino acids as the continuation of our previous work.[14] In our previous work, we have studied the coordination behavior of dithiocarbamate, as an extension of this research work and in connection with our current interest in the coordination chemistry and anti-cancer activity of palladium(II) complexes with mixed ligands. We have selected the two ligands, *N*,*N*′-dicyclohexyldithiocarbamate (DCHDTC) and *N*-methyl-*N*-cyclohexyldithiocarbamate (MCHDTC), and synthesized a series of complexes, Pd(L)(PR_3)Cl (L = DCHDTC, MCHDTC; R = Ph, *o*-tolyl, $ClPh_2$), and characterized them by elemental analyses, FT-IR, multinuclear (^1H, ^{13}C and ^{31}P) NMR and single X-ray crystallography.

EXPERIMENTAL

Elemental analyses were carried out on a Fisons EA1108 CHNS-O microanalyser. Melting points were determined using a Mitamura Riken Kogyo (Japan) instrument. The IR spectra of the synthesized complexes were recorded on Nicolet 5SXC FT-IR spectrometers, using KBr disks from 4000 to 400 cm^{-1} and CsI disks from 500 to 200 cm^{-1} with Perkin-Elmer FT-IR Nexus spectrometer.

The ^1H and ^{13}C NMR spectra were recorded on a Bruker 300 MHZ spectrometer with $CDCl_3$ as a solvent and TMS as a reference operating at 300 and 75.5 MHz, respectively.



Scheme 1. Structure of **1**–**6**.

^{31}P NMR spectra were recorded on Bruker WM-300 and AM-400 spectrometers operating at 121.51 and 162.40 MHz, respectively. Spectra were run in $CDCl_3$ solution at ambient temperature and referenced to external 85% phosphoric acid with downfield shifts defined as positive.

Dicyclohexylamine and *N*-methylcyclohexylamine were purchased from Aldrich (USA) and distilled before use. All reagents were of analytical grade and used without further purification. The organic solvents were dried before use over sodium benzophenone by standard method.[15]

Synthesis of $PdCl_2(PR_3)_2$ and of the dithiocarbamate ligands

$PdCl_2(PR_3)_2$(R = Ph, *o*-tolyl, Ph_2Cl) was prepared using the literature method.[16] The dithiocarbamates RDTC [R = dicyclohexylamine (DCH) and *N*-methylcyclohexylamine (MCH)] were prepared by the reactions of secondary amines with carbon disulfide at 0 °C.[17]

General synthetic procedure of Pd(II) complexes with mixed ligands

Pd(II) complexes of mixed ligands were synthesized by suspending $PdCl_2(PR_3)_2$ (1.14 mmol) in 15 cm^3 of CH_2Cl_2 and adding to it a solution of DCHDTC/MCHDTC (1.14 mmol) in 15 cm^3 CH_2Cl_2 in a two-necked flask fitted with a reflux condenser at 40 °C. The reaction mixture was refluxed for 1 h to obtain a clear solution and then cooled to room temperature. The solvent was removed under reduced pressure. The resultant solid was recrystallized from CH_2Cl_2–*n*-hexane (3 : 1).

Pd(DCHDTC)(PPh_3)Cl (1)

Pd(DCHDTC)(PPh_3)Cl was synthesized by the method described above and re-crystallized from a mixture of dichloromethane (20 cm^3) and *n*-hexane (5 cm^3). Orange crystals were obtained after one month by slow evaporation at room temperature. Data (80% yield): m.p. 238 °C. Anal. found (calcd): C, 53.51(53.5); H, 5.31(5.32); N, 2.10(2.05); P, 4.43, (4.46); S, 9.19(9.20); IR (cm^{-1}): 1481 (C-N str), 998 (-CSS), 340 (Pd-Cl str). ^1H NMR ($CDCl_3$): 4.87–5.09 (m, 10H, HC-N), 0.8–1.42 (m, 12H, -CH_2), 7.3–7.86 (m, 15H, Ph), ^{31}P NMR ($CDCl_3$): 32.8 (s).

Pd(DCHDTC)[P(*o*-tolyl)$_3$]Cl (2)

A suspension of $PdCl_2[P(o\text{-tolyl})_3]_2$ (1.14 mmol) in 20 cm^3 of CH_2Cl_2 and a solution of DCHDTC (1.14 mmol) in 15 cm^3 CH_2Cl_2 were reacted according to the above method. Orange crystals were obtained after recrystallization from a mixture of dichloromethane and *n*-hexane. Data (83% yield): m.p. 295 °C: Anal. found (calcd): C, 58.26(58.31); H, 5.83(5.85); N, 2.10(2.00); P, 4.42(4.42); S, 9.13(9.14). IR (cm^{-1}): 1492 (C-N str), 1013 (-CSS), 340 (Pd-Cl str). ^1H NMR ($CDCl_3$): 5.12–5.28 (m, 10H, HC-N), 0.7–1.41 (m, 12H, -CH_2), 2.5 (s, 3H, -CH_3), 7.2–7.56 (m, 10H, Ph), ^{31}P NMR ($CDCl_3$): 31.3 (s).

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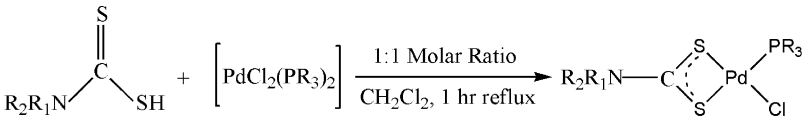

where R_1 = cyclohexyl
R_2 = cyclohexyl, methyl
R_3 = $(phenyl)_3$, $(o\text{-tolyl})_3$, $(phenyl)_2Cl$

Scheme 2. General synthetic layout.

Pd(DCHDTC)($ClPh_2P$)Cl (3)

A suspension of $PdCl_2[P(Ph)_2Cl]_2$ (1.14 mmol) in 20 cm^3 of methanol was reacted with a solution of DCHDTC (1.14 mmol) in 15 cm^3 acetone. By adopting the method above, a yellow crystalline product was obtained at room temperature in mixture of CH_2Cl_2–OEt_2 (1 : 1). Data (80% yield): m.p. 285 °C. Anal. found (calcd): C, 51.36 (51.4); H, 5.46 (5.48); N, 2.40 (2.40); P, 5.32 (5.31); S, 10.99 (10.97). IR (cm^{-1}): 1492 (C-N str), 1030 (-CSS), 340 (Pd-Cl str). ^{1}H NMR ($CDCl_3$): 4.82–5.03 (m, 10H, HC-N), 0.9–1.59 (m, 12H, -CH_2), 6.7–7.4 (m, 10H, Ph), ^{31}P NMR ($CDCl_3$): 33.1(s).

Pd(MCHDTC)(PPh_3)Cl (4)

The complex Pd(MCHDTC)(PPh_3)Cl was synthesized following the method above. Data (80% yield): m.p. 301 °C. Anal. found (calcd): C, 53.36 (53.3); H, 5.16 (5.15); N, 2.65 (2.65); P, 5.50 (5.50); S, 11.36 (11.37). IR (cm^{-1}): 1498 (C-N str), 1018 (-CSS), 340 (Pd-Cl str). ^{1}H NMR ($CDCl_3$): 4.72–4.92 (m, 5H, HC-N), 0.8–1.31 (m, 6H, -CH_2), 3.12 (s, 3H, -CH_3), 7.3–7.8 (m, 15H, Ph), ^{31}P NMR ($CDCl_3$): 28.8 (s).

Pd(MCHDTC)[P(*o*-tolyl)$_3$]Cl (5)

The complex Pd(MCHDTC)[P(*o*-tolyl)$_3$]Cl was synthesized by adopting the above method. Data (73% yield): m.p. 245 °C. Anal. found (calcd): C, 56.42 (56.41); H, 5.66 (5.67); N, 2.24 (2.20); P, 5.02 (5.02); S, 10.38 (10.37). IR (cm^{-1}): 1493 (C-N str), 995 (-CSS), 356 (Pd-Cl str). ^{1}H NMR ($CDCl_3$): 4.83–4.97 (m, 5H, HC-N), 0.9–2.25 (m, 6H, -CH_2), 3.2 (s, 6H, -CH_3), 7.2–7.8 (m, 10H, Ph), ^{31}P NMR ($CDCl_3$): 34.2 (s).

Pd(MCHDTC)($ClPh_2P$)Cl (6)

Pd(MCHDTC)(PPh_2Cl)Cl was also prepared as described above. Data (85% yield): m.p. 265 °C. Anal. found (calcd): C, 48.21 (48.21); H, 4.84 (4.82); N, 2.81 (2.81); P, 6.22 (6.22); S, 12.86 (12.85). IR (cm^{-1}): 1513 (C-N str), 966 (-CSS), 356 (Pd-Cl str). ^{1}H NMR ($CDCl_3$): 4.51–4.91 (m 5H, HC-N), 0.8–1.12 (m, 6H, -CH_2), 3.1 (s, 3H, -CH_3), 7.4–8.6 (m, 10H, Ph), ^{31}P NMR ($CDCl_3$): 34.3(s).

In vitro cytotoxicity screenings

The test and reference compounds were dissolved to a concentration of 250 000 ng ml^{-1} in full medium, by 20-fold dilution of a stock solution which contained 1 mg compound per 200 µl. The compounds were taken into dimethylsulfoxide. Cytotoxicity was estimated by the microculture sulforhodamine B (SRB) test.[38] The human cancer cell lines examined in the present study were: A498, renal cancer; MCF-7, estrogen receptor (ER)+/progesterone receptor; (PgR) + breast cancer; EVSA-T, estrogen receptor (ER)−/progesterone receptor; (PgR)− breast cancer; H226, non-small cell lung cancer; IGROV, ovarian cancer; M19 MEL, melanoma; and WIDR, colon cancer.

The experiment was started on day 0. On day 0, 10 000 cells per well were seeded into 96-well flat-bottomed microtiter plates (Falcon 3072, DB). The plates were incubated overnight at 37 °C in 5% CO_2 to allow the cells to adhere to the bottom. On day 1, a three-fold dilution sequence of 10 steps was made in full medium, starting with the 250 000 ng ml^{-1} stock solution. Every dilution was done in quadruplicate by adding 200 µl to a column of four wells. This procedure resulted in a highest concentration of 625 000 ng ml^{-1} present in column 12. Column 2 was used for the blank. After incubation of 3 days, the plates were washed with PBS twice. Fluorescein diacetate (FDA) stock solution was diluted to 2 µg ml^{-1} with PBS and 200 µl of this solution was added to each of the control, experimental and blank wells. The plates were incubated for 30 min at 37 °C and the fluorescence generated from each well was measured at an excitation wavelength of 485 nm and an emission wavelength of 535 nm using an automated microplate reader (Labsystems Multiskan MS). Data were used for construction of concentration–response curves and determination of the ID_{50} value by use of Deltasoft 3 software. The variability of the *in vitro* cytotocicity test depends inter alia on the cell lines used and the serum applied. With the same batch of cell lines and the same batch of serum, the inter-experimental CV (coefficient of variation) is 1–11% depending on the cell line and the intra-experimental CV is 2–4%. These values may be higher with other batches of cell lines and/or serum. For further details on the *in vitro* cytotoxicity tests Boyd[18] and Keepers *et al*.[19]

Antifungal assay

The susceptibility test was performed as described by Choudary *et al*.,[20] with some modifications. The compounds were solubilized in dimethylsulfoxide and a dilution was performed in Sabouraud dextrose agar (Merck) medium with pH 5.5–5.6, containing a relatively high concentration of glucose (40%), which was prepared by mixing (SDA)

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6.5 g ml^{-1} distilled water. The contents were dissolved and dispensed as 4 ml volumes into screw capped tubes, then autoclaved at 121 °C for 15 min. A 67 µl aliquot of the compound was added to SDA to obtain a concentration of 200 µg ml^{-1}. Tubes were then allowed to solidify in slanting position at room temperature. Tubes were prepared in triplicate for each fungus species. Other media supplemented with dimethylsulfoxide and reference antifungal drugs were used as negative and positive controls, respectively. Each tube was inoculated with 4 mm diameter piece of inocula, removed from a 7-day- old culture of fungus. The tubes were incubated at 28 °C for 7 days. Cultures were examined twice weekly during the incubation. Growth in the media was determined by measuring the linear growth (mm) and growth inhibition was calculated with reference to the negative control.

$$\text{Percentage inhibition of fungal growth} = 100 - \frac{\text{Linear growth in test (mm)}}{\text{Linear growth in control (mm)}} \times 100$$

A growth control of the test strains and a susceptibility standard test using terbinafine 200 µg ml^{-1} as the reference system were performed by applying the same technique.

Anti-inflammatory activity

The rat paw odema was induced using 0.1% carrageenan (50 µl per paw), which was injected into the right-hind paw plantar surface to groups of three animals each.[21] The measurement of foot volumes was carried out using the plythesmographic method.[22] It was done by recording the rat paw volume before the drug injection at 0 h and then at 1 h intervals for 4 h. Acute carrageenan-induced inflammatory reactions were observed in the peritoneal cavity of the rats. Six groups of three animals each received one compound, i.e. **1**–**6** at 25 mg kg^{-1}. The standard drug potassium declofenac (10 mg per 5 ml) was used as a positive control. The negative control group received 0.75% CMC sodium (carboxymethylcellulose). One hour later, a volume of 50 µl per paw of carrageenan at 0.1% in distilled water was injected into the rat's peritoneal cavity. The effect was noted at 1 h intervals for 4 h after the injection of carrageenan.

Percentage oedema inhibition was calculated as:

$$\frac{\text{mean increase in paw volume(}-\text{ve control)} - \text{mean increase in paw volume (sample)}}{\text{mean increase in paw volume (}-\text{ve control)}} \times 100$$

RESULTS AND DISCUSSION

Single crystal X-ray diffraction

The X-ray diffraction study of compound **1** [Pd(DCHDTC)(PPh_3)Cl] shows that its crystal structure consists of Pd(DCHDTC)(PPh_3)Cl molecules. Single-crystal X-ray diffraction data were collected at 293 K using an Oxford Diffraction Xcalibur 2 diffractometer with MoK_α radiation. Data reduction and analytical absorption correction were performed using the CrysAlis RED software suite.[23] The structure was solved using SIR2004[24,25] and refined using SHELXL97.[26]

An ORTEP view showing the overall geometry of the complex and the atomic notation scheme is presented in Fig. 1. In the structure of complex **1**, the Pd atom is four-coordinate and exhibits a slightly distorted square-planar geometry. The dithiocarbamate acts as a bidentate ligand and is coordinated to Pd via the two S atoms.

The propeller-like PPh_3 groups interlock in order to maximize the packing in the unit cell, resulting in the formation of a supra-molecular arrangement.[26] The molecular arrangement is in fact composed of independent 3_1 helices running in the *c* direction, the center of the helix being defined by the *c* axis.

Infrared spectroscopy

The tentative assignments of IR spectra of the synthesized complexes were given according to the literature.[27,28] For all six compounds, the position of the υ(C–N) stretching mode of free dithiocarbamates ligands was shifted to higher

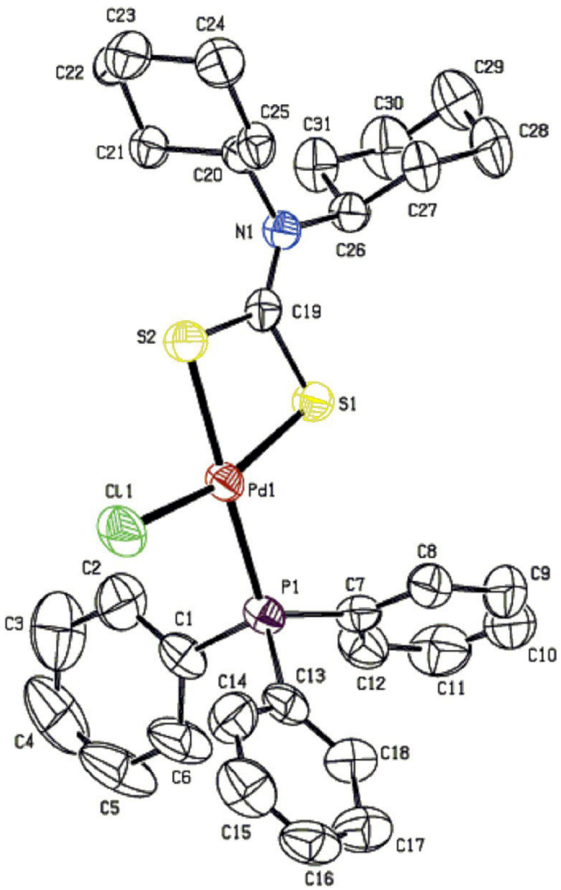

Figure 1. ORTEP diagram of compound **1** [PdCl(DCHDTC)(PPh_3)], with atomic numbering scheme. This figure is available in colour online at www.interscience.wiley.com/AOC.

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energy from 1486 to 1550 cm^{-1} after complexation with Pd(II).[29,30] The υ(C–N) stretching vibration appears in the region 1487–1550 cm^{-1} for both ligands and its complexes, which indicates the non-involvement of the C–N bond in the formation of the metal complexes due to hindrance of sulfur atoms and owing to the increased double bond character in the CS group, caused by electron delocalization towards the metal center.[6,31] The absence of a strong υ(S–H) band at 2550–2800 cm^{-1}, which was present in the free ligand, is indicative of complete metallation of the ligand in the final product. A strong new υ(Pd–Cl) band appeared at 340–348 cm^{-1} for complexes **1, 4** and**6**, at 356 and at 358 cm^{-1} for complexes **2** and**5**, and was absent in the free ligands. The strong band at ~1240 cm^{-1} in the IR spectra of both the free ligands and metal complexes appears to be due to a combination of υ(C–N) and υ(C–S); another strong band at ~1250 cm^{-1} is due to the mixing of υ(C–S) and $\upsilon(C–N)_{asym.}$[32]

A characteristic band of the PPh_3 ligand was observed around 1443 cm^{-1} and assigned to the symmetric and asymmetric stretching and bending of P–Ph bonds, respectively.[33] A single CSS stretching band is observed at (940–1099) cm^{-1} due to disulfur chelation attributed the band of υ_{asym} CSS and υ_{sym} CSS at 1020 and 950 cm^{-1}.[34] The –CSS moiety that is usually coupled to other vibrations and is very sensitive to the environment of this group, allows us to distinguish between the monodentate and bidentate dithiocarbamate coordinations.[35] In the case of a monodentate dithiocarbamate ligand, a doublet arises around 950–990 cm^{-1} due to non-equivalence of C–S stretching vibrations, whereas in the case of a bidentate ligand, only one band appears in the investigated region, which is indicative of a symmetrically bound dithiocarbamate moiety.[36] In the present series of Pd(II) complexes, only one strong band was observed in the region 1000–1099 cm^{-1}, which indicates the υ(SCS) vibrational mode and suggests a bidentate symmetrical behavior of the dithiocarbamate moiety.[37]

NMR studies

The ^{1}H NMR spectra of all compounds were identified by intensity and multiplicity patterns and the total number of protons calculated from the integration curve was in agreement with the expected molecular composition. The proton resonances of the phenyl group of the tertiary phosphine of all compounds appeared as complex patterns in the range 7.02–7.37 ppm. The spectra of the complexes showed significant differences compared with the spectra of the free ligands. The SH proton for non-coordinated organosulfur derivatives of *N*,*N*-dicyclohexyldithiocarbamate and *N*-methyl-*N*-cyclohexyldithiocarbamate appeared at $\delta = 5.48$ ppm, and the absence of that proton indicated the complexation of Pd(II) with dithiocarbamate ligands via S atoms, which was a consequence of the strong delocalization of electrons in the –CSS′ moiety mentioned above. Such a behavior resulted in a rotation barrier[27,28] of ~56 kJ mol^{-1} that forces the dithiocarbamate group into a planar configuration.[38] In the reported Pd(II) complexes **1–6**, there were significant downfield chemical shifts of the cyclohexyl proton (range 0.07–5.18 ppm) that also support the complexation and this shift being due to steric interactions/coordination between dicyclohexyldithiocarbamate ligand and the bulky $PdCl_2PPh_3$ group.[39]

In the ^{13}C NMR spectra, the number of the signals found corresponds with the presence of magnetically non-equivalent carbon atoms, which were assigned by comparison with literature values.[38] The resonance of the C═S carbon at δ (192.6–196.6 ppm) in the free ligands was shifted downfield to 204.5–207.6 ppm due to a strong de-shielding effect on this particular carbon atom after complexation. This downfield shift also supports the metal ion coordination with the ligand through the sulfur atoms.

^{31}P NMR spectra were run in $CDCl_3$ solutions at ambient temperature and referenced to external standard 85%

Table 1. Crystal data for $Pd(DCHDTC)(PPh_3)Cl$

Empirical formula	$C_{31}H_{37}ClNPPdS_2$
Formula weight	660.56
Temperature	293(2) K
Wavelength	0.71073 Å
Crystal system	Trigonal
Space group	$P3_1$
Unit cell dimensions	$a = 16.262(1)$ Å
	$c = 10.198(1)$ Å
Volume	2335.5(4) $Å^3$
Z	3
Density (calculated)	1.409 mg m^{-3}
Absorption coefficient	0.888 mm^{-1}
F(000)	1020
Crystal size	0.1 × 0.1 × 0.1 mm^3
Theta range for data collection	4.25–26.37°.
Index ranges	$-20 \le h \le 20$, $-20 \le k \le 20$, $-12 \le l \le 12$
Reflections collected	17684
Independent reflections	6340 [R(int) = 0.0590]
Completeness to $\theta = 25.31°$	99.7%
Absorption correction	Analytical
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	6340/1/334
Goodness-of-fit on F^2	1.007
Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0400$, $wR_2 = 0.0708$
R indices (all data)	$R_1 = 0.0647$, $wR_2 = 0.0774$
Largest difference peak and hole	0.470 and −0.270 e $Å^{-3}$

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phosphoric acid with downfield shifts defined as positive. In the complexes **1–6**, the low-field resonance was due to the interaction of phosphorus with the metal center Pd(II), and the *trans*-influence of the S-bonded to dithiocarbamate ligand (phosphorus atom trans to sulfur atom), while the highfield resonance was generally observed in the free phosphorus ligand.[38] The ^{31}P NMR spectra of all complexes displayed a singlet at δ 28.4–34.3 ppm.

In vitro cytotoxicity screenings

The cytotoxicity of compounds **2, 3** and **4** was determined *in vitro* by applying seven well-characterized human tumor cell lines (MCF7, EVSA-T, WIDR, IGROV, M19 MEL, A498 and H226) and the microculture sulforhodamine B (SRB) test. The results are given in Table 2. The ID_{50} values of six reference compounds were also determined and are given in Table 3.

Antifungal activity

The results of antifungal assay showed that the compounds **1–6** possess antifungal activity against the *Fusarium moniliformes, Fusarium saolani, Mucor* sp., *Aspergillus niger* and *Aspergillus fumigatus* (Table 4). The antifungal activity of compound **1** for *Fusarium moniliformes* at the concentration of 200 μg ml^{-1} inhibited the growth by 78%, compound **2–6** showed a growth effect between 52 and 68% inhibition. The growth inhibition of all compounds against *Fusarium moniliformes* was significant, while, against *Mucor* sp., the activity of all compounds was not significant. Compounds **1–6** showed moderate activity against *Aspergillus niger* and *Aspergillus fumigatus.*

Table 2. ID_{50} values (ng ml^{-1}) of compounds **2–4** *in vitro* using SRB as the cell viability test

	Cell line						
Test compound	A498	EVSA-T	H226	IGROV	M19	MCF-7	WIDR
2	25487	12571	22474	10835	16912	29928	41675
3	2670	1314	2532	1139	2531	2370	3226
4	3559	2903	7282	2641	3126	3290	4980

Table 3. ID_{50} values (ng ml^{-1}) of doxorubicin (DOX), cisplatin (CPT), 5-fluorouracil (5-FU), methotrexate (MTX), etoposide (ETO) and taxol (TAX)

	Cell line						
Test compound	A498	EVSA-T	H226	IGROV	M19	MCF-7	WIDR
DOX	90	8	199	60	16	10	11
CPT	2253	422	3269	169	558	699	967
5-FU	143	475	340	297	442	750	225
MTX	37	5	2287	7	23	18	<3.2
ETO	1314	317	3934	580	505	2594	150
TAX	<3.2	<3.2	<3.2	<3.2	<3.2	<3.2	<3.2

Compounds **3** and **4** showed mostly a moderate to low cytotoxicity (ID_{50} 2500–20 000 ng ml^{-1}). Compound **2** showed mostly a very low cytotoxicity (ID_{50} > 20 000 ng ml^{-1}).

Table 4. Anti-fungal activity of palladium(II) complexes. Terbinafine, used as control, inhibited all fungi at 200 μg ml^{-1}

	Growth effect, % inhibition (*fungus*)				
Compounds	*F. moniliformes*	*F. solani*	*Mucor sp.*	*A. niger*	*A. fumigatus*
1	77.78	48	25	33.33	33.33
2	61.11	40	15	44.44	45.38
3	55.56	32	0	44.44	55.64
4	68.89	40	0	48.89	53.08
5	65.56	48	20	42.22	40.25
6	52.68	50	10	51.11	58.00
Linear length in −ve control	45	25	100	90	39

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Table 5. Acute anti-inflammatory activity of palladium(II) compounds on carrageenan-induced rat paw odema

Sample name	Percentage odema inhibition at time (h)			
	First hour	Second hour	Third hour	Fourth hour
Standard drug	2.32 ± 4.52	3.49 ± 4.32	68.70 ± 3.05	74.14 ± 2.72
1	16.974 ± 2.54	15.11 ± 5.06	25.19 ± 20.78	20.41 ± 8.35
2	16.27 ± 3.12	16.27 ± 8.72	24.42 ± 3.43	16.32 ± 1.02
3	27.90 ± 5.51	34.88 ± 8.13	69.77 ± 2.64	93.87 ± 2.35
4	14.65 ± 2.51	12.7 ± 10.13	50.38 ± 3.32	73.46 ± 3.53
5	20.84 ± 5.23	30.91 ± 6.32	68.67 ± 2.62	87.46 ± 2.25
6	18.93 ± 3.21	24.42 ± 3.41	73.12 ± 2.74	92.87 ± 2.34

Values are mean ± SEM; $n = 3$ in each group.

Anti-inflammatory screening

These results of administrated compounds are quite similar to the one observed for Declofenac (standard drug) at 10 mg kg^{-1}, which inhibited the edema by 74% after 4 h. Ueno *et al*.[41] found that the injection of carrageenan into the rat paw induces the liberation of bradykinin, which later induces the biosynthesis of prostaglandin and other autacoids, which are responsible for the formation of the inflammatory exudate.[41] Besides, in the carrageenan-induced rat paw odema model, the production of prostanoids has been through the serum expression of COX-2 by a positive feedback mechanism.[42] Therefore, it is suggested that the mechanism of action of compounds may be related to prostaglandin synthesis inhibition, as described for the anti-inflammatory mechanism of potassium delofenac in the inhibition of the inflammatory process induced by carrageenan.[43]

CONCLUSIONS

In this study we report the synthesis, characterization and structural properties of Pd(II) complexes containing two new mixed phosphine/dithiocarbamate ligands. The spectroscopic results suggested that the palladium complexes exhibit a square-planar geometry. The −NCSS moiety coordinates to the metal atom in a bidentate symmetrical mode. Furthermore the results of the X-ray crystal structure determination of one representative compound, **1**, show the coordination modes of the ligands around Pd(II) and result in a slightly distorted square planar geometry. The palladium compounds are less active than cisplatin. This might be related to a molecular configuration that differs from that of cisplatin. The compounds studied might be starting points to prepare more cytotoxic palladium derivatives. Another option is to develop an even less cytotoxic compound with antibacterial activity. The compounds showed both anti-inflammatory and anti-fungal activities, similar to those observed for non-steroidal drugs. The compounds exhibited a significant activity in the early phases of inflammation. It is suggested that the mechanism of action of the compounds might be associated with the inhibition of prostaglandin synthesis, as observed for most non-steroidal drugs. The anti-inflammatory activity of palladium(II) complexes was highly potent as compared with declofenac.

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Supplementary data

The crystallographic data for compound **1** have been deposited with the Cambridge Crystallographic Data Centre CCDC (12 Union Road, Cambridge, CB2 1EZ, UK) as CCDC number 607163 and are available on request.

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