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Anticancer metallopharmaceutical agents based on mixed-ligand palladium(II) complexes with dithiocarbamates and tertiary organophosphine ligands

Hizbullah Khan[a,b,c], Amin Badshah[b]*, Muhammad Said[b,f], Ghulam Murtaza[b], Jamil Ahmad[d], Bertrand J. Jean-Claude[e], Margarita Todorova[e] and Ian S. Butler[a]

Mixed-ligand palladium(II) complexes of the type [(DT)Pd(PR_3)Cl], where DT = diethyldithiocarbamate (1), dibutyldithiocarbamate (2,3), dipropyldithiocarbamate (4,5), bis(2-methoxyethyl)dithiocarbamate; PR_3 = benzyldiphenylphosphine (1,4), diphenyl-*o*-tolylphosphine (2), diphenyl-*t*-butylphosphine (3), P-chlorodiphenylphosphine (5) and triphenylphosphine (6), have been synthesized and characterized by elemental analyses and FT-IR, Raman and multinuclear NMR spectroscopy. The structures of compounds 1 and 2 were determined by single-crystal X-ray diffraction (XRD) measurements and these analyses showed that the complexes have pseudo square-planar geometry around the Pd(II) and that the dithiocarbamate ligand is bound in a bidentate fashion, while the remaining two positions are occupied by a tertiary organophosphine and a chloride ligand. The anticancer studies showed that the Pd(II) complexes are highly active against cisplatin-resistant DU145 human prostate carcinoma (HTB-81) cells with the highest activity shown by compound 6 (IC_{50} = 2.12 μM). The redox behavior and ds-DNA-denaturing ability of the complexes were studied by cyclic voltammetry and two reduction and one oxidation waves were observed. The decrease in the reduction peak currents illustrated the consumption of the mixed-ligand drug by the DNA molecule. Copyright © 2013 John Wiley & Sons, Ltd.

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Keywords: anticancer activity; palladium(II) complexes; dithiocarbamates; tertiary organophosphines; X-ray diffraction; cyclic voltammetry; DNA binding studies

Introduction

The field of metallodrugs has become a major research area for medicinal inorganic chemists, after the discovery of cisplatin (*cis*-$PtCl_2(NH_3)_2$) as a DNA-modifying agent with high anticancer activity.[1–3] Cisplatin is currently used for the treatment of tumors of the ovaries, testes, and cancers of the head and neck region.[4,5] Despite its wide spectrum of utility against tumors, cisplatin has several drawbacks, viz. neurotoxicity, nephrotoxicity, ototoxicity, gastrointestinal and bone marrow toxicity, and acquired resistance after continued treatment.[6–9] The N-7 atom of guanine in a DNA strand is generally considered to be the main target for cisplatin antitumor action.[10]

With the emergence of exciting antitumor activities of various other transition metal complexes, the focus of research has gradually been expanding beyond platinum.[11,12] Owing to the structural resemblance of palladium(II) complexes to those of platinum(II) and also because they exhibit promising antineoplastic characteristics,[6,13,14] palladium(II) complexes are natural candidates to be considered as anticancer drugs.[15,16] The pH of normal body cells is 7.4, while tumor cells generally have a lower pH value of about 6.8. Several palladium(II) complexes, e.g. $[(RO)CS_2]_2Pd]$ (R = Et, *i*-Pr, Cy), have been reported to show antineoplastic activity at pH 6.8.[15] Moreover, *trans*-L_2PdCl_2 complexes exhibit higher cytostatic activity than do their *cis*-analogues.[1] Palladium(II) complexes are 10^5 times more labile than are the analogous platinum(II) compounds,[17] and new charged species that interact with DNA are produced at an even faster rate than with the platinum complexes.[18]

A suitable carrier ligand is crucial for the success of a cytostatic drug,[19] as it plays an important role in stabilizing a specific oxidation

* *Correspondence to: A. Badshah, Department of Chemistry, Quaid-i-Azam University, Islamabad 45320, Pakistan. E-mail: aminbadshah@yahoo.com*

a *Department of Chemistry, McGill University, Montreal, Quebec, Canada H3A 2 K6*

b *Department of Chemistry, Quaid-i-Azam University, Islamabad 45320, Pakistan*

c *Department of Chemistry, University of Science and Technology, Bannu 28100, Pakistan*

d *Department of Chemistry, Government College University, Lahore 54000, Pakistan*

e *Department of Medicine, Royal Victoria Hospital, Montreal, Quebec, Canada H3A 1A1*

f *Department of Chemistry, Abdul Wali Khan University, Mardan, KPK, Pakistan*

state of the metal ion, imparting substitution inertness, modifying reactivity and lipophilicity and facilitating positive impacts on the target site.[20] Sulfur-containing ligands like thiocarbonyls and thiols are currently under investigation as chemoprotectants in platinum-based chemotherapy[21] and the aminothiol molecule $[NH_2(CH_2)_3NH(CH_2)_2SPO_3H_2]$ exhibits protective characteristics against chemo- and radiotherapy.[22] Dithiocarbamates have the capability to stabilize transition metals in a variety of oxidation states.[23] These ligands selectively remove platinum metal from the enzyme–thiol complex by nucleophilic attack of the sulfur atoms on the platinum moiety and have been evaluated for their efficacy to inhibit cisplatin-induced nephrotoxicity without undermining their antiproliferative activity.[24] Marzano and co-workers have reported a class of mixed ligands palladium(II) and platinum (II) complexes with dithiocarbamate and various amines, exhibiting greater cytotoxicity than does cisplatin,[25] very low *in vitro* and *in vivo* nephrotoxicity and zero cross-resistance with cisplatin.[26]

Stimulated by the promising earlier results of palladium(II) complexes with dithiocarbamate ligands against DU145 human prostate carcinoma (HTB-81) cells,[27] it was decided to synthesize some novel mixed-ligand palladium(II) complexes with dithiocarbamates and organophosphine ligands. In this article we report the synthesis, characterization and antitumor activities of six complexes of the general formula $[(DT)Pd(PR_3)Cl]$ (DT = diethyldithiocarbamate, dibutyldithiocarbamate, dipropyld ithiocarbamate, and bis(2-methoxyethyl)dithiocarbamate; PR_3 = benzyldiphenylphosphine, diphenyl-*o*-tolylphosphine, diphenyl-*t*-butylphosphine, chlorodiphenylphosphine and triphenylphos phine). The antitumor activities of the synthesized complexes have been evaluated by testing against DU145 human prostate carcinoma (HTB-81) cells. To further investigate the mode of action of these complexes, the interaction with DNA has been studied by cyclic voltammetry (CV).

Experimental

The experiments were carried out under the conditions of temperature and pressure specified. Complexes **1** and **2** were analyzed on a STOE IPDS image plate detector diffractometer, equipped with graphite monochromated MoKα radiation. ^1H, ^{13}C and ^{31}P NMR spectra were recorded on Mercury 300 and VNMRS500 spectrometers: ^1H NMR (300.05 or 499.89 MHz): internal standard solvent $CDCl_3$ (7.26 ppm from tetramethylsilane (TMS)) and DMSO-d_6 (2.49 ppm from TMS), internal standard TMS; ^{13}C NMR (75.44 or 125.69 MHz), internal standard TMS. The splittings of the proton and phosphorus resonances are defined as s = singlet, d = doublet, t = triplet and m = multiplet (showing a complex spectrum). FT-IR spectra were recorded on Nicolet 6700 FT-IR instrument in the range 4000–400 cm^{-1}. Raman spectra were measured on a Rensihaw In Via instrument. Elemental analyses were conducted on a LECO-183 CHNS analyzer and melting points were measured on a Stuart SMP10 apparatus. Diethylamine, dipropylamine, dibutylamine, bis(2-methoxyethyl)amine, carbon disulfide, benzyl diphenylphosphine, diphenyl-*o*-tolylphosphine, diphenyl-*t*-butyl phosphine and chlorodiphenylphosphine were purchased from Aldrich Chemical Co. and were used without further purification. Palladium(II) chloride was obtained from Fluka and used as received. All solvents were purified and dried by the reported standard methods.

Solution Phase Synthesis of Mixed-Ligand Palladium(II) Compounds (1–6)

A series of palladium(II) complexes was synthesized by reacting dithiocarbamate ligands with $(PR_3)_2PdCl_2$ in CH_2Cl_2 (Scheme 1) and proved to be stable under ambient conditions and soluble in common organic solvents.

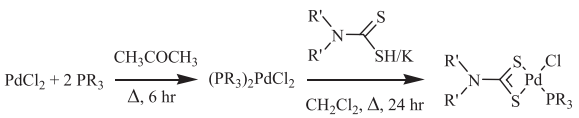
The organophosphine solution in dry acetone (2 M ratio) was added to the palladium(II) chloride (1 M ratio) solution in acidified methanol and the reaction mixture was stirred under reflux conditions for 6 h. The solid product, palladium phosphine complex, was filtered, dried at room temperature, dissolved in dichloromethane and added to the dithiocarbamic acid/sodium salt solution (1:1 M ratio). The reaction mixture was heated under reflux overnight. The solvent was removed under reduced pressure; an orange-red solid product was obtained, which was dissolved in a mixture of dichloromethane and petroleum ether (4:1) for recrystallization at room temperature. Orange-red, block-shaped crystals of **1** and **2** were obtained, while the other complexes could not be crystallized.

[Pd(diethyldithiocarbamate)(PPh_2-benzyl)Cl] (***1***)

Quantities used were 0.06 g (0.40 mmol) diethyldithiocarbamate and 0.29 g (0.40 mmol) Pd(PPh_2-benzyl)$_2Cl_2$ in 30 ml dichloromethane. Yield 0.19 g (83%) orange-red crystals; m.p. 185–186 °C. FT-IR and Raman (cm^{-1}): 3051, 2981, 1518, 907, 693, 428, 324, 234. ^1H NMR (300 MHz, $CDCl_3$, 291.9 K) δ (ppm): 7.52–7.17 (m, 15H, Ar*H*),: 4.00 (d, 2H, PCH_2, $^2J_{P-H}$ = 12 Hz), 3.69 (q, 2H, NCH_2, $^3J_{H-H}$ = 7.2 Hz), 3.53 (q, 2H, NCH_2, $^3J_{H-H}$ = 7.2 Hz), 1.25 (t, 3H, -CH_3, $^3J_{H-H}$ = 7.2 Hz), 1.15 (t, 3H, CH_3, $^3J_{H-H}$ = 7.2 Hz),. ^{13}C NMR (75.46 MHz, $CDCl_3$, 290.5 K) δ (ppm): 206.1 (*SCS*), 133.7, 131.5, 130.7, 128.5, 128.1, 127.1, 126.7, 125.6 (18C, Ar-*C*), 43.8, 43.6 (2C, NCH_2), 32.7 (PCH_2), 12.4, 12.3 (2C, CH_3), ^{31}P NMR (121.4 MHz, $CDCl_3$, 300.0 K) δ (ppm): 26.9. Anal. Calcd (%) for $C_{24}H_{27}ClNPPdS_2$: C, 50.89; H, 4.80; N, 2.47; S, 11.32. Found: C, 50.81; H, 4.76; N, 2.43; S, 11.37.

[Pd(dibutyldithiocarbamate)(PPh_2-o-tolyl)Cl] (***2***)

Quantities used were 0.08 g (0.40 mmol) dibutyldithiocarbamate and 0.29 g (0.40 mmol) Pd(PPh_2-*o*-toly)$_2Cl_2$ in 30 ml dichloromethane. Yield 0.19 g (78%) orange-red crystals; m.p. 265–266 °C. FT-IR and Raman (cm^{-1}): 3050, 2960, 1517, 1026, 693, 380, 315, 262. ^1H NMR (300 MHz, $CDCl_3$, 291.9 K) δ (ppm): 7.82–7.25 (m, 14H, Ar*H*), 3.63 (t, 2H, $CH_2CH_2CH_2CH_3$, $^3J_{H-H}$ = 7.8 Hz), 3.57 (t, 2H, $CH_2CH_2CH_2CH_3$, $^3J_{H-H}$ = 7.8 Hz), 2.66 (s, 3H, Ar-CH_3), 1.65–1.54 (m, 4H, $CH_2CH_2CH_2CH_3$), 1.45–1.25 (m, 4H, $CH_2CH_2CH_2CH_3$), 0.96 (t, 3H, $CH_2CH_2CH_2CH_3$,



PR_3 = PPh_2(benzyl) (**1**, **4**), PPh_2(*o*-tolyl) (**2**), PPh_2(*t*Bu) (**3**), PPh_2Cl (**5**), PPh_3 (**6**)

R' = ethyl (**1**), butyl (**2**, **3**), propyl (**4**, **5**), 2-methoxyethyl (**6**)

Scheme 1. Synthesis of dithiocarbamate palladium(II) complexes **1–6**.

$^{3}J_{H-H}$ = 7.5 Hz), 0.90 (t, 3H, $CH_2CH_2CH_2CH_3$, $^{3}J_{H-H}$ = 7.5 Hz). ^{13}C NMR (75.47 MHz, $CDCl_3$, 290.5 K) δ (ppm): 206.3 (SCS), 142.6, 142.3, 141.9, 138.3, 137.7, 137.2, 135.9, 133.8, 132.7, 128.5, (18C, Ar-*C*), 48.8 ($CH_2CH_2CH_2CH_3$), 47.7 ($CH_2CH_2CH_2CH_3$), 29.2 ($CH_2CH_2CH_2CH_3$), 29.1 ($CH_2CH_2CH_2CH_3$), 23.7 (Ph-CH_3), 20.1 ($CH_2CH_2CH_2CH_3$), 20.0 ($CH_2CH_2CH_2CH_3$), 13.7 ($CH_2CH_2CH_2CH_3$), 13.6 ($CH_2CH_2CH_2CH_3$), ^{31}P NMR (121.4 MHz, $CDCl_3$, 287.8 K) δ (ppm): 20.6. Anal. Calcd (%) for $C_{28}H_{35}ClNPPdS_2$: C, 54.02; H, 5.67; N, 2.25; S, 10.30. Found: C, 54.08; H, 5.66; N, 2.25; S, 10.29.

*[Pd(dibutyldithiocarbamate)(PPh_2-*t*-butyl)Cl]* (***3***)

Quantities used were 0.12 g (0.58 mmol) dibutyldithiocarbamate and 0.39 g (0.58 mmol) Pd(PPh_2-*t*-butyl)$_2Cl_2$ in 25 ml dichloromethane. Yield 0.29 g (83%) golden-yellow solid; m.p. 150–151 °C. FT-IR and Raman (cm^{-1}): 3074, 2953, 1531, 1095, 694, 378, 298, 259; ^{1}H NMR (300.13 MHz, $CDCl_3$, 291.9 K) δ (ppm): 8.06–7.41 (m, 10H, Ar*H*), 3.37 (t, 2H, $CH_2CH_2CH_2CH_3$, $^{3}J_{H-H}$ = 7.8 Hz), 3.58 (t, 2H, $CH_2CH_2CH_2CH_3$, $^{3}J_{H-H}$ = 7.8 Hz), 1.53 (s, 9H, C(CH_3)$_3$), 1.53–1.44 (m, 4H, $CH_2CH_2CH_2CH_3$), 1.36–1.17 (m, 4H, $CH_2CH_2CH_2CH_3$), 0.93 (t, 3H, $CH_2CH_2CH_2CH_3$, $^{3}J_{H-H}$ = 6.0 Hz), 0.83 (t, 3H, $CH_2CH_2CH_2CH_3$, $^{3}J_{H-H}$ = 6.0 Hz), ^{13}C NMR (75.46 MHz, $CDCl_3$, 290.5 K) δ (ppm): 206.3 (SCS), 135.0, 130.1, 127.6, 126.9 (12C, Ar-*C*), 48.9 ($CH_2CH_2CH_2CH_3$), 48.6 ($CH_2CH_2CH_2CH_3$), 39.2 (*C*(CH_3)$_3$), 30.0 ($CH_2CH_2CH_2CH_3$), 29.9 ($CH_2CH_2CH_2CH_3$), 28.3 (3C, C(CH_3)$_3$), 20.1 ($CH_2CH_2CH_2CH_3$), 20.0 ($CH_2CH_2CH_2CH_3$), 13.7 ($CH_2CH_2CH_2CH_3$), 13.6 ($CH_2CH_2CH_2CH_3$). ^{31}P NMR (121.4 MHz, $CDCl_3$, 300.0 K) δ (ppm) 45.1. Anal. Calcd (%) for $C_{25}H_{37}ClNPPdS_2$: C, 51.82; H, 6.52; N, 2.32; S, 10.64. Found: C, 51.78; H, 6.55; N, 2.30; S, 10.60.

[Pd(dipropyldithiocarbamate)(PPh_2-benzyl)Cl] (***4***)

Quantities used were 0.10 g (0.56 mmol) dipropyldithiocarbamate and 0.40 g (0.55 mmol) Pd(PPh_2-benzyl)$_2Cl_2$ in 30 ml dichloromethane. Yield 0.28 g (85%) golden-yellow solid; m.p. 191–192 °C. FT-IR and Raman (cm^{-1}): 3053, 2960, 1523, 1099, 692, 378, 302, 219. ^{1}H NMR (400 MHz, $CDCl_3$, 298.1 K) δ (ppm): 7.70–7.06 (m, 15H, Ar*H*), 4.00 (d, 2H, PCH_2, $^{2}J_{P-H}$ = 12 Hz);, 3.58 (t, 2H, $CH_2CH_2CH_3$, $^{3}J_{H-H}$ = 7.5 Hz) 3.42 (t, 2H, $CH_2CH_2CH_3$, $^{3}J_{H-H}$ = 7.5 Hz), 1.67–1.65 (m, 2H, $CH_2CH_2CH_3$), 1.60–1.56 (m, 2H, $CH_2CH_2CH_3$), 0.99 (t, 3H, $CH_2CH_2CH_3$, $^{3}J_{H-H}$ = 7.5 Hz), 0.92 (t, 3H, $CH_2CH_2CH_3$, $^{3}J_{H-H}$ = 7.5 Hz).^{13}C NMR (125.67 MHz, $CDCl_3$, 290.5 K) δ (ppm): 207.2 (SCS), 134.0, 131.2, 132.7, 130.6, 129.0, 128.7, 126.8, 125.6 (18C, Ar-*C*), 50.8 ($CH_2CH_2CH_3$), 50.7 ($CH_2CH_2CH_3$), 38.3 (PCH_2), 20.5 ($CH_2CH_2CH_3$), 20.4 ($CH_2CH_2CH_3$), 11.3 ($CH_2CH_2CH_3$), 11.1 ($CH_2CH_2CH_3$). ^{31}P NMR (80.9 MHz, $CDCl_3$, 300.0 K) δ (ppm): 27.9. Anal. Calcd (%) for $C_{26}H_{31}ClNPPdS_2$: C, 52.53; H, 5.26; N, 2.36; S, 10.79. Found: C, 52.48; H, 5.23; N, 2.34; S, 10.76.

[Pd(dipropyldithiocarbamate)(PPh_2Cl)Cl] (***5***)

Quantities used were 0.11 g (0.62 mmol) dipropyldithiocarbamate and 0.38 g (0.62 mmol) Pd(PPh_2Cl)$_2Cl_2$ in 30 ml dichloromethane. Yield 0.30 g (85%) golden-yellow solid; m.p. 185–186 °C. FT-IR and Raman (cm^{-1}): 3020, 2933, 1510, 1100, 690, 394, 284, 252. ^{1}H NMR (400.89 MHz, $CDCl_3$, 298.1 K) δ (ppm): 7.70–7.32 (m, 10H, Ar*H*), 3.58 (t, 2H, $CH_2CH_2CH_3$, $^{3}J_{H-H}$ = 8.0 Hz), 3.54 (t, 2H, $CH_2CH_2CH_3$, $^{3}J_{H-H}$ = 8.0 Hz), 1.81–1.76 (m, 2H, $CH_2CH_2CH_3$), 1.67–1.59 (m, 2H, $CH_2CH_2CH_3$), 0.92 (t, 3H, CH_3, $^{3}J_{H-H}$ = 7.5 Hz) 0.89 (t, 3H, $CH_2CH_2CH_3$, $^{3}J_{H-H}$ = 7.5 Hz). ^{13}C NMR (125.7 MHz, $CDCl_3$, 298.1 K) δ (ppm): 207.9 (SCS), 131.4, 130.3, 127.9, 126.8 (12 C, Ar-*C*), 54.7 ($CH_2CH_2CH_3$), 51.9 ($CH_2CH_2CH_3$), 20.5 ($CH_2CH_2CH_3$),19.3 ($CH_2CH_2CH_3$), 11.3 ($CH_2CH_2CH_3$), 11.2 ($CH_2CH_2CH_3$). ^{31}P NMR (125.7 MHz, $CDCl_3$, 298.1 K) δ (ppm): 84.4. Anal. Calcd (%) for $C_{19}H_{24}Cl_2NPPdS_2$: C, 43.45; H, 4.74; N, 2.53; S, 11.60. Found: C, 43.40; H, 4.73; N, 2.53; S, 11.63.

[Pd(bis(2-methoxyethyl)-dithiocarbamate)(PPh_3)Cl] (***6***)

Quantities used were 0.14 g (0.57 mmol) bis(2-methoxyethyl)-dithiocarbamate and 0.40 g (0.57 mmol) Pd(PPh_3)$_2Cl_2$ in 30 ml dichloromethane. Yield 0.28 g (80%) golden-yellow solid; m.p. 165–166 °C. FT-IR and Raman (cm^{-1}): 3053, 2929, 1524, 1094, 690, 380, 279, 203; ^{1}H NMR (400.89 MHz, $CDCl_3$, 298.1 K) δ (ppm): 7.65–7.38 (m, 15H, Ar*H*), 4.00 (t, 2H, $CH_2CH_2OCH_3$, $^{3}J_{H-H}$ = 7.6 Hz), 3.85 (t, 2H, $CH_2CH_2OCH_3$, $^{3}J_{H-H}$ = 7.6 Hz), 3.64 (t, 2H, $CH_2CH_2OCH_3$, $^{3}J_{H-H}$ = 7.6 Hz), 3.52 (t, 2H, $CH_2CH_2OCH_3$, $^{3}J_{H-H}$ = 7.6 Hz), 3.33 (s, 3H, $CH_2CH_2OCH_3$), 3.30 (s, 3H, $CH_2CH_2OCH_3$). ^{13}C NMR (125.7 MHz, $CDCl_3$, 298.1 K) δ (ppm): 206.1 (SCS), 134.2, 132.1, 128.5, 127.6 (18C, Ar-*C*), 70.1 ($CH_2CH_2OCH_3$), 70.0 ($CH_2CH_2OCH_3$), 60.1 ($CH_2CH_2OCH_3$), 59.9 ($CH_2CH_2OCH_3$), 50.4 ($CH_2CH_2OCH_3$), 50.3 ($CH_2CH_2OCH_3$). ^{31}P NMR (125.7 MHz, $CDCl_3$, 298.1 K) δ (ppm): 30.6. Anal. Calcd (%) for $C_{25}H_{29}ClNO_2PPdS_2$: C, 49.02; H, 4.77; N, 2.29; S, 10.47. Found: C, 49.10; H, 4.75; N, 2.28; S, 10.44.

X-Ray Diffraction Studies

Suitable orange-red crystals of complexes **1** and **2** were obtained by dissolving the product in a mixture of dichloromethane and petroleum ether (4:1, v/v), and dichloromethane and *n*-hexane (4:1, v/v) respectively. The solvents were slowly evaporated at room temperature in an open atmosphere and orange-red crystals were obtained. The block crystals of **1** and **2** were mounted on glass fiber using epoxy glue. Measurements were made at 293(2) K on a STOE IPDS image plate detector diffractometer, equipped with graphite monochromated MoKα radiation. The program used for retrieving cell parameters, data collection and data integration was STOE X-AREA.[28] Multiscan absorption corrections were performed using SADABS.[29] The structures were solved and refined using SHELXS-97 and SHELXL-97[30] and all non-H atoms were refined anisotropically, with the hydrogen atoms placed at idealized positions. Various crystallographic parameters of the two crystals are shown in Table 4.

Antineoplastic Assay

DU145 human prostate carcinoma (HTB-81) cells were obtained from the American Type Culture Collection (ATCC catalogue number). The cells were maintained in Roswell Park Memorial Institute (RPMI-1640) medium (Wisent Inc., St Bruno, Canada) and were supplemented with 10% fetal bovine serum, 10 mM HEPES, 2 mM L-glutamine and 100 g ml^{-1} penicillin/streptomycin (GibcoBRL, Gaithersburg, MD, USA). All assays cells were plated 24 h before drug treatment. 50 mmol stock concentrations of the compounds were prepared in DMSO. Nine serial dilutions of the compounds were used to treat the cells and the final concentration of DMSO on cells did not exceed 0.05%. In the growth inhibition assay, DU145 prostate cancer cells were plated at 5000 cells per well in 96-well flat-bottom microtiter plates (Costar®, Corning®, NY, USA). After 24 h incubation, cells were exposed to different concentrations of each compound continuously for 4 days. The remaining live cells were fixed using 50 µl cold trichloroacetic acid (50%) for 60 min at 4 °C, washed with water, stained with 0.4% sulforhodamine B (SRB) for 4 h at room temperature, rinsed with 1% acetic acid and allowed to dry overnight. The resulting colored residue was dissolved in 200 µl Tris base (10 mM, pH 10.0) and optical density was recorded at 490 nm using a microplate reader ELx808 (BioTek Instruments). The results were analyzed by Graph Pad Prism (Graph Pad Software, Inc., San Diego, CA, USA) and the sigmoidal dose–response curve was used to

determine 50% cell growth inhibitory concentration (IC_{50}). The growth inhibition assay was performed once in triplicate.

Cyclic Voltammetric Analysis

Cyclic voltammetric measurements were carried out using an Eco Chemie Autolab PGSTAT 302 potentiostat/galvanostat (Utrecht, Netherlands) along with the software GPES 4.9. All the experimentation was made in a double-walled electrochemical cell (model K-64 PARC) and conventional three-electrode system, using SCE as reference electrode, a thin Pt wire as a counter electrode and bare glassy carbon of 0.071 cm^2 area as working electrode. The glassy carbon electrode surface was polished before each measurement. All the measurements were performed in DMSO (99.5%, LAB-SCAN/Analytical) using 0.1 M tetrabutylammonium perchlorate (TBAP ≥ 98%, Fluka) at 25 ± 1 °C under argon atmosphere. Sample concentration was kept at 1 mM in each sample.

Electrochemical studies were made to assess the antitumor activity of the synthesized complexes. Cyclic voltammograms of the six complexes were performed in the absence and presence of DNA (obtained from chicken blood) in DMSO.

To quantify the heterogeneous electron transfer kinetics, the effect of scan rate was also studied and diffusion coefficients were calculated to estimate the heterogeneous rate constants. Voltammetric behavior of all compounds was studied on the glassy carbon electrode at various scan rates (50–1000 mV s^{-1}). The Randles–Sevcik equation for an irreversible process was employed to plot i_p vs. square root of scan rate and to determine the diffusion coefficient (D°) values:[31]

$$i_p = \left(2.99 \times 10^5\right) n(\alpha n)^{1/2} AC(Dv)^{1/2} \quad (1)$$

where α is transfer coefficient, n is number of electrons transferred, A (cm^2) is area of working electrode, v (V s^{-1}) is scan rate and C (mol cm^{-3}) is bulk concentration of the sample. $\alpha n = 47.7/(E_P - E_{P/2})$ was used.[32] To calculate the standard heterogeneous rate constants, Gileadi's method was used.[33] From the shift in peak potential value while changing the scan rate, the critical scan rate was obtained, which was subsequently used in the following equation to obtain k_{sh}:

$$\log k_{sh} = -0.48\alpha + 0.52 + \log\left[\frac{\alpha n F v_c D^\circ}{2.3RT}\right] \quad (2)$$

To investigate the antitumor activity of the complexes, cyclic voltammetric measurements were made, varying the DNA concentration from 10 to 200 nM to the constant concentration of the complex (5 mM).

Results and Discussion

Spectroscopic Characterization

FT-IR and Raman spectra of the complexes showed that the main vibrational stretching modes were located at 3074–3020 (aromatic C-H), 2981–2929 (aliphatic C-H), 1531–1510 (C=N), 428–378 (Pd-S), 324–279 (Pd-Cl) and 262–203 cm^{-1} (Pd-P), while SCS_{asym} and SCS_{sym} bending modes were observed at 1100–1026 and 694–690 cm^{-1}, respectively. The appearance of a Pd-S band in the far-IR region confirms the complexation of Pd atom with dithiocarbamate moiety. The C-N stretching mode observed at about 1520 cm^{-1} indicates the intermediate nature of the carbon–nitrogen bond, which is further confirmed by X-ray crystallography (see below). The IR bands normally observed for a C-N and a C=N bond are in the 1250–1350 and 1690–1640 cm^{-1} regions, respectively.[34,35] The appearance of a single IR-active bending mode in the 1100–1026 cm^{-1} region ascertain that the dithiocarbamate species is symmetrically bonded to Pd in a bidentate fashion. The disappearance of the S-H stretching mode in the 2500–2700 cm^{-1} region in the metal complexes is another indication of bond formation between the palladium atom and the dithiocarbamate ligand.

There are no conspicuous differences in the ^{1}H NMR spectra of the complexes and the precursor ligands. The aromatic protons have signals in the range of 8.06–7.06 ppm, as reported in the literature.[36] An interesting feature of the ^{1}H NMR spectra is the asymmetry in the alkyl groups attached to the nitrogen atom of the dithiocarbamate ligand, caused by the restricted rotation around the C=N bond, which is known to have an energy barrier of 65–95 kJ mol^{-1}.[37] ^{13}C NMR spectra of the complexes show the presence of all the carbon atoms in the compounds. The SCS carbon atom has a signal in the 206.1–207.9 ppm region. The slight upfield displacement of the SCS signal in complexes demonstrates the coordination between the Pd atom and dithiocarbamate ligand, which may be attributed to the higher electron density on the SCS carbon after complexation. Like the protons of the dithiocarbamate alkyl groups, the carbon atoms of the alkyl groups are also asymmetric in nature. ^{31}P NMR spectra show a single resonance for the phosphorus atom, which is generally observed at about 25–30 ppm downfield in the complexes, in comparison with the precursor organophosphines.

Structural Study of Compounds **1** and **2**

The ORTEP representations of compounds **1** and **2**, together with selected bond distances and bond angles, are shown in Figs 1 and 2, respectively. Compound **1** crystallizes in a triclinic crystal system (P − 1), whereas compound **2** crystallizes in a monoclinic system (P2(1)/*n*). Both complexes display similar pseudo square-planar geometry with the dithiocarbamate ligand, in bidentate fashion, occupying two adjacent coordination sites, while a chloride and the organophosphine ligand are bonded to the two remaining sites. The largest distortion from the normal geometry arises from the bidentate ligand [S(1)-Pd-S(2) of 75.56(3)° and

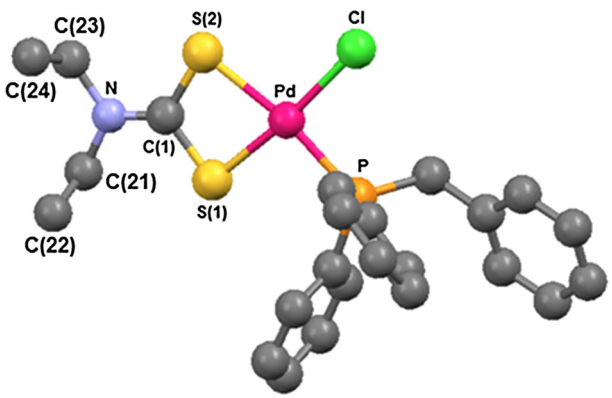


Figure 1. Ball and stick diagram (50% probability) of compound **1**. Hydrogen atoms have been omitted for clarity. Selected bond lengths (Å) and angles (°): Pd-P 2.2685(9); Pd-Cl 2.3436(8); Pd-S(1) 2.2720(8); Pd-S(2) 2.357(1); S(1)-C(1) 1.728(4); S(2)-C(1) 1.714(3); C(1)-N 1.314(5); P-Pd-Cl 90.86(3); P-Pd-S(2) 170.96(3); S(2)-Pd-S(1) 75.56(3); Cl-Pd-S(1) 173.48(3) ; S(2)-Pd-Cl 98.06(3); P-Pd-S(1) 75.56(3); C(1)-N-C(21) 120.8(3); C(1)-N-C (23) 118.5(3); C(21)-N-C(23) 118.5(3) and S(1)-C(1)-S(2) 111.0(2).

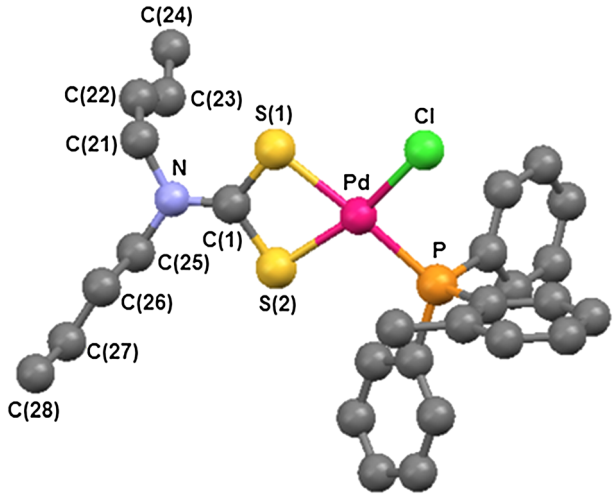


Figure 2. Ball and stick diagram (50% probability) of compound **2**. Hydrogen atoms have been omitted for clarity. Selected bond lengths (Å) and angles (°): Pd-P 2.298(1); Pd-Cl 2.321(1); Pd-S(1) 2.319(1); Pd-S(2) 2.286(1); S(1)-C(1) 1.707(4); S(2)-C(1) 1.716(4); C(1)-N 1.313(5); P-Pd-Cl 92.94(4); P-Pd-S(2) 99.99(3); S(2)-Pd-S(1) 75.25(4); Cl-Pd-S(1) 92.07(4) ; S(2)-Pd-Cl 166.60 (4); P-Pd-S(1) 173.87(4); C(1)-N-C(21) 120.3(4); C(1)-N-C(25) 122.0(3); C(21)-N-C(25) 117.7(4) and S(1)-C(1)-S(2) 110.4(2).

75.25(4)° for **1** and **2** respectively], which causes the *trans* S(2)-Pd-P and S(1)-Pd-Cl angles to be 170.96(3)° and 173.48(3)° for **1**, and S(2)-Pd-Cl and S(1)-Pd-P angles to be 166.60(4)° and 173.87 (4)° for **2**, respectively, i.e. smaller than the expected value of 180°. The asymmetry observed in the Pd-S distances is typical for square-planar systems and reflects the *trans* influence of the organophosphine ligands. In both complexes, the Pd-S bonds *trans* to the organophosphine ligand are longer (2.357 (1) Å and 2.319(1) Å for **1** and **2**, respectively) than are the Pd-S bonds *trans* to the chloride (2.2720(8) and 2.286(1) Å for **1** and **2**, respectively). The larger divergence in the Pd-S bond lengths for compound **1** (ΔPd-S = 0.085 Å) than compound **2** (ΔPd-S = 0.033 Å) reflects the better donating capability of the (PPh_2-benzyl) group compared to the (PPh_2-*o*-tolyl) group. Both S-C distances in **1** and **2** fall between 1.728(4)–1.714(3) Å (**1**) and 1.716(4)–1.707(4) Å (**2**) and are intermediate between normal C-S (1.82 Å) and C=S (1.60 Å) distances.[38] Similarly, the C(1)-N bond lengths (1.314(5) for **1** and 1.313(5) for **2**) are significantly shorter than is a normal C-N bond (1.47 Å) and longer than a C=N bond (1.28 Å).[39] These bond values clearly demonstrate the resonance phenomenon in the SCS moiety, as reported in the previous section. This resonance phenomenon is not uncommon in dithiocarbamate complexes, e.g. $[Ni\{S_2CN(CH_2CH_2NEt)_2\}_2]$,[23] $[Ni(S_2CNC_4H_8NH_2)(dppp)]$ $(BF_4)_2$.[40]

Anticancer Activity

The mixed-ligand Pd(II) complexes were tested for antitumor activity against DU145 human prostate carcinoma (HTB-81) cells. All the complexes tested, were found to be highly active against these cells. The 50% inhibitory concentrations (IC_{50}) of compounds **1–6** are listed in Table 1. All the observed values are lower than are the literature values reported for the standard drug cisplatin.[41–43] Compound **6** is the most active, with an IC_{50} of 2.12 μM, while compound **5** has the lowest antitumor activity (IC_{50} 21.7 μM). The higher cytotoxicity of compound **6** may be ascribed to the presence of oxygen species in the dithiocarbamate ligand, which has the potential to form a hydrogen bond with the DNA bases. The lower cytotoxicity of compound **5** may be attributed to the weak Pd-P bond, due the presence of strong withdrawing chlorine and phenyl groups in the organophosphine moiety. As a result, the complex has a higher tendency to dissociate and react with other groups like glutathione, cysteines and methionines in the cell before reaching the target DNA.[44] The second lowest activity of compound **3** (IC_{50} 9.52 μM) may derive from the presence of a bulky tertiary butyl group, which renders movement of the complex to the target DNA difficult. The activities of compounds **1** (3.67 μM) and **4** (4.57 μM) are close to each other; both of these complexes have a similar benzyldiphenylphosphine ligand and their dithiocarbamate ligands differ by only one methylene group (Fig. 3).

Table 1. IC_{50} (μM) values of mixed ligand Pd(II) complexes against DU145 human prostate carcinoma (HTB-81) cells

Compound	1	3	4	5	6
IC_{50}	3.67	9.52	4.57	21.7	2.12

Cyclic Voltammetric Analysis

Voltammetric measurements were carried out to study the redox behavior of the compounds and also to investigate the possible interaction mechanisms with ds-DNA obtained from chicken blood. Representative cyclic voltammograms are presented in Fig. 4 and the corresponding reduction potential data for the compounds are tabulated in Table 2. The voltammograms represent one oxidation wave and two reduction waves sufficiently apart from each other, with a difference of 0.430–0.573 V, indicating the independent electroreductions of the compounds. The first reduction (with no corresponding oxidation) is irreversible, while the presence of an oxidation wave for the second reduction reveals its reversibility. The electrochemical irreversibility of the first electron-transfer process shows the instability of the reduced species, which in turn points to its high reactivity. The oxidation peak in the reverse scan indicates the stability of the electroreduced species generated in the second electron transfer reaction. The small current values for the first reduction (1.457–3.685 μA) illustrate the relatively slow electron transfer in comparison to the second one (current values 2.398–5.373 μA). The appearance of two cathodic waves and one anodic wave in the present work, conducted in DMSO, indicates the favorable reorganization and involvement of the solvent molecules to affect the electron transfer process. The late reduction, as indicated by a sufficiently negative reduction potential, suggests the difficult acceptability of the incoming electron by the reaction center and could be attributed to the electron-donating nature of the phosphine and dithiocarbamate ligands. The ΔE_{P2} values in Table 2 portray the quasi-reversible behavior of the second electron-transfer process.

To further probe the nature of the electron transfer process, the scan rate was varied from 20 to 1000 mV s^{-1}. The effect of scan rate is shown in Fig. 5 and corresponding data in Tables 2 and 3. It was observed that as the scan rate is increased there is a systematic negative potential shift in the reduction waves with the expected current increase, while the anodic peak shifts anodically. These observations indicate the irreversibility of the electrochemical charge transfer process; however, the shift is not large enough to justify a totally irreversible process. To investigate whether the process is adsorption controlled or diffusion

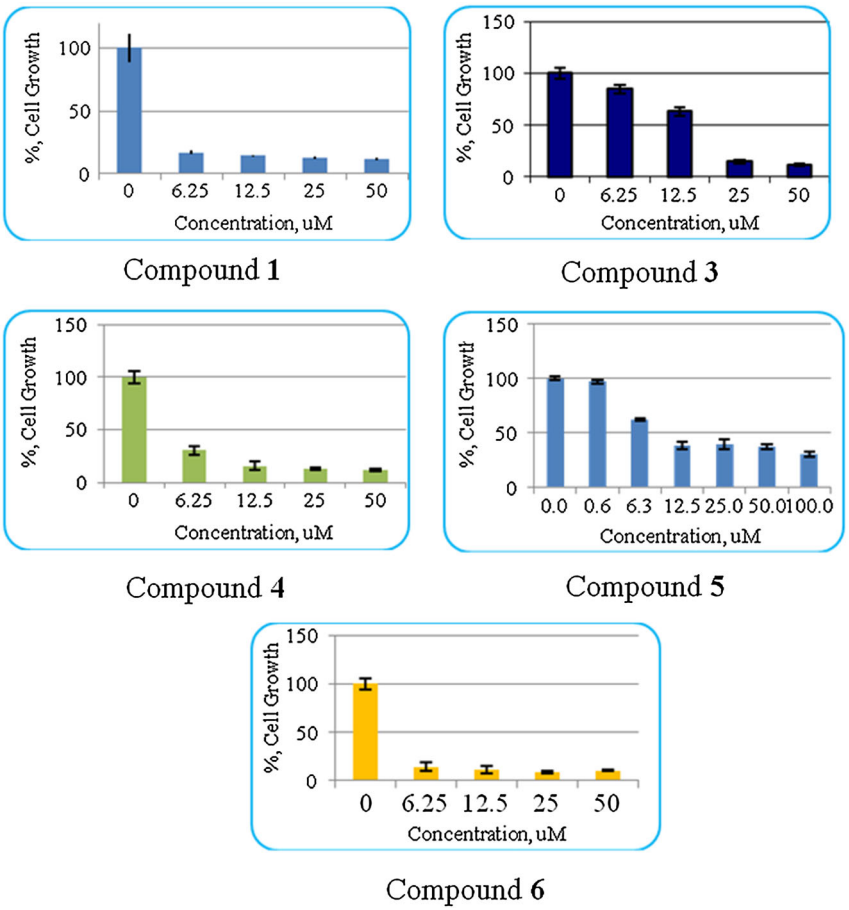


Figure 3. Cell growth of DU145 human prostate carcinoma (HTB-81) cells at various concentrations of Pd(II) complexes.

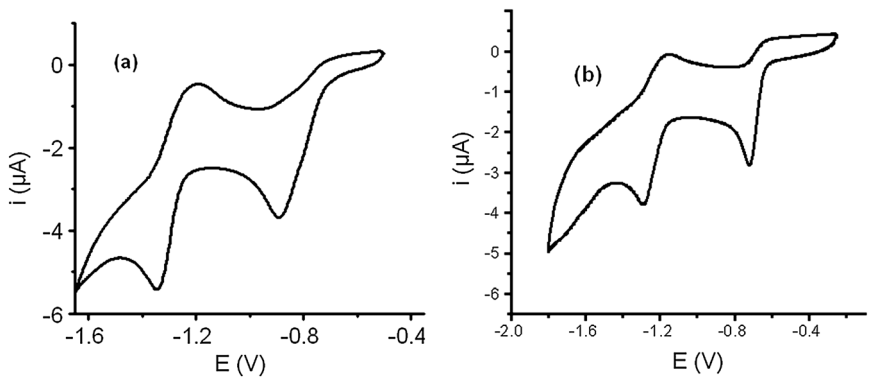


Figure 4. Representative cyclic voltammograms: (a) compound **1** and (b) compound **6** for 1 mM concentration in DMSO, in the presence of tetra-*n*-butylammonium perchlorate (TBAP), 0.1 M, at 25 ± 1 °C on glassy carbon vs. SCE at 20 mV s^{-1}.

Table 2. Reduction and oxidation potential data of the Pd(II) complexes on glassy carbon electrode vs. SCE at 25 ± 1 °C at 20 mV s^{-1} scan rate

Compound	Peak 1	Peak 2		
	$^{*}E_{P1c}$ (V)	E_{P2c} (V)	E_{P2a} (V)	ΔE_{P2} (V)
1	−0.888	−1.345	−1.250	0.095
2	−0.860	−1.357	−1.254	0.103
3	−0.903	−1.388	−1.291	0.097
4	−0.920	−1.374	−1.264	0.110
5	−0.953	−1.383	−1.283	0.100
6	−0.718	−1.291	−1.181	0.110

controlled the peak current value (i_p) was plotted against the square root of the scan rate ($v^{1/2}$) in the presence and absence of DNA, using the Randles–Sevcik equation[31] for an irreversible process. The diffusion coefficient value (D^o) of the electron-transfer process was determined from the Randles–Sevcik plot and thus, subsequently, the heterogeneous rate constant were determined employing Gileadi's method of critical scan rate.[33] The D^o values obtained suggest that the overall process is mainly diffusion controlled but charge transfer is also affecting the rate of overall electrochemical reaction. The k_{sh} values are of a magnitude which falls in the range reported for intermediate kinetic processes depicting a quasi-reversible process.[45]

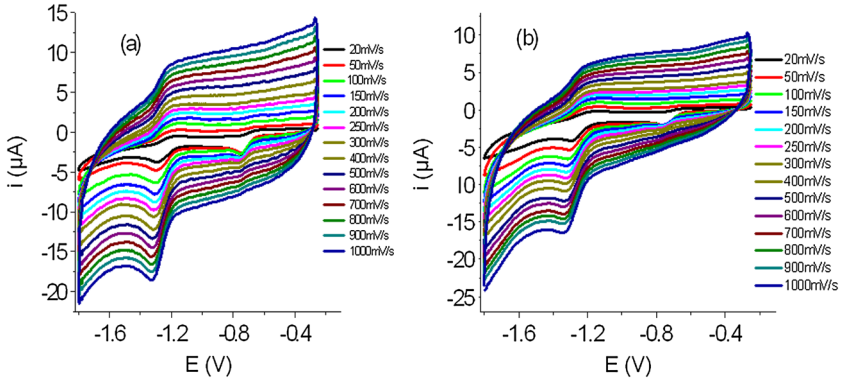


Figure 5. Effect of scan rate on compound **6**, for 1 mM concentration: (a) pure complex, (b) in the presence of DNA, in DMSO + TBAP (tetra-*n*-butyl ammonium perchlorate) 0.1 M at 25 ± 1 °C on glassy carbon vs. SCE.

Table 3. Values of diffusion coefficient (D^o) and heterogeneous rate constant (k_{sh}) for Pd(II) complexes from the second reduction peak in the absence and presence of DNA

Compound	$D^o \times 10^6$ cm^2 s^{-1}		$k_{sh} \times 10^3$ cm s^{-1}	
	Before DNA addition	After DNA addition	Before DNA addition	After DNA addition
1	1.61	0.96	4.39	3.68
2	1.06	0.79	4.28	3.67
3	1.68	0.97	5.32	3.70
4	1.62	1.03	4.63	4.00
5	1.37	0.93	4.72	3.49
6	1.32	0.84	4.28	3.53

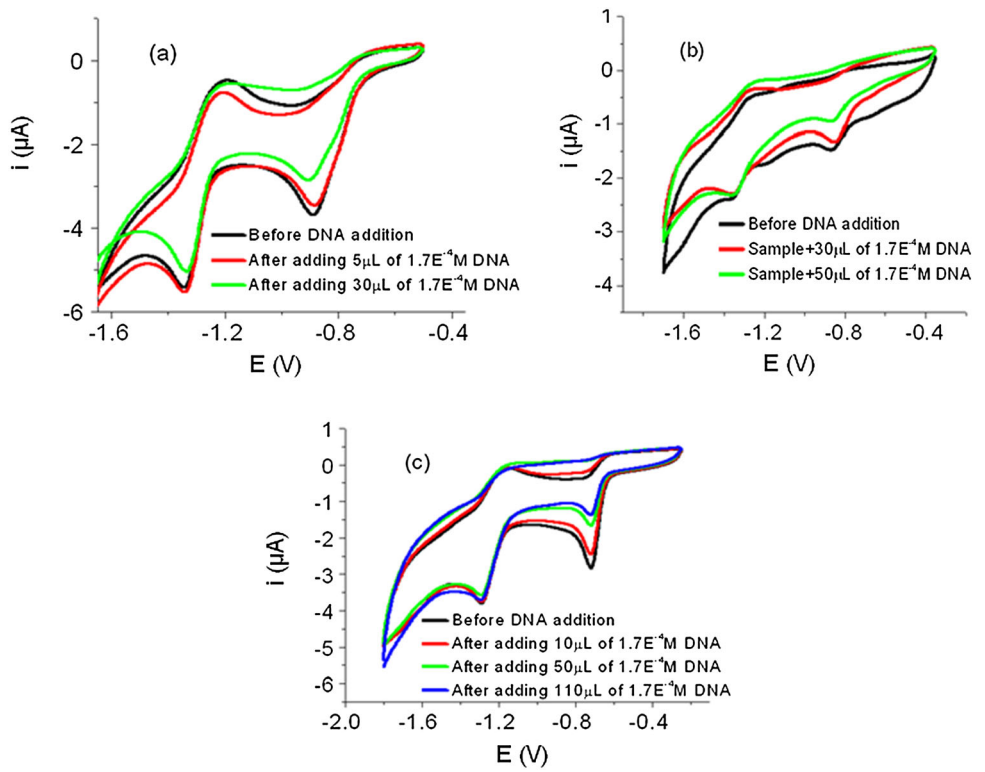


Figure 6. Cyclic voltammograms depicting the effect of DNA concentration on the complexes; (a) compound **1**, (b) compound **2** and (c) compound **6**, having 1 mM constant concentration in DMSO + 0.1 M TBAP at 25 °C on glassy carbon vs. SCE at 20 mV s^{-1} scan rate.

Table 4. Crystallographic data

	1	2
Formula	$C_{24}H_{27}ClNPPdS_2$	$C_{28}H_{35}ClNPPdS_2$
FW	566.45	622.56
Crystal system	Triclinic	Monoclinic
Space group	(P − 1)	P2(1)/*n*
a (Å)	10.9056(3)	9.3010(2)
b (Å)	11.2073(3)	20.0610(5)
c (Å)	12.9279(3)	15.9196(4)
α, β, γ (°)	66.4820(10), 89.1410(10), 62.4860(10)	90.00, 95.9120(10), 90.00
V (Å^3)	1256.79(6)	2954.60(12)
Z	2	4
Cell measurement temperature	293(2) K	296(2) K
Diffraction radiation type	MoKα	MoKα
F_{000}	576.0	1280
Goodness-of-fit on F^2	1.131	0.994
R indices (all data)	*R*1 = 0.0234, *wR*2 = 0.0893	*R*1 = 0.0589, *wR*2 = 0.1010
Reflections collected	26 944	34 381
Max. 2*θ*	56.73	56.69

Interaction with DNA

To explore the denaturing ability of the complexes, cyclic voltammetric measurements were carried out for varying DNA concentrations (20–2000 nM) against a constant concentration of the test compound (1 mM). The behavior of compounds **1**, **2** and **6** are presented in Fig. 6.

Similar responses were observed in all cases and these can be generalized into three major outcomes: (i) the systematic addition of DNA caused a significant decrease in the peak current of the first reduction; (ii) the effect on the current decrease in the second reduction remained either very small or practically constant; (iii) the anodic wave remained unaffected. The systematic decrease in the reduction peak current(s) can be attributed to the consumption of the test compound by DNA, which becomes denatured, as reported in the literature.[45] The complexes contain electron-donating ligands that make the Pd(II) center more electronegative and also reactive.[46] Upon DNA addition a complex–DNA adduct is formed and the process is linearly dependent on concentration. The same situation obtains in the case of testing the complexes against the cancer cell line. The major decrease was found in the first reduction, which corresponds to an irreversible process, and remained irreversible after the DNA addition. The overall process corresponds to an E_iC_i type mechanism, i.e. irreversible electron transfer followed by an irreversible chemical reaction, as expected. In a few cases, a very small negative shift in the potential value indicates a possible electrostatic interaction between the positive metal center and the negative oxygen atoms of the phosphate groups in DNA.[47]

To support the above qualitative hypothesis about the complex–DNA interaction, the process was studied from another point of view which includes the calculation of diffusion coefficients (D^o) of the electron-transfer process before and after DNA addition. The data obtained are collected together in Table 3. The smaller values of diffusion coefficient in the presence of DNA implies that the complex–DNA adduct formed diffuses at a slower rate than does the pure complex. Hence a decrease in the diffusion coefficient exhibits the effect of complex–DNA adduct formation on the electron transfer process. The corresponding heterogeneous rate constant data also showed the slowness of the charge transfer process across the electrode solution interface for the DNA-bound complexes.

Conclusion

Six new palladium(II) complexes with dithiocarbamates and phosphine ligands have been successfully synthesized and characterized by various spectroscopic techniques. The crystal structures of **1** and **2** illustrate the attachment of dithiocarbamate in a *cis* fashion. The geometry around the palladium moiety is pseudo square-planar with the Pd-S bond *trans* to the phosphine being longer than Pd-S bond *trans* to the chloride atom. All the compounds synthesized have shown excellent to good antitumor activity against cisplatin-resistant DU145 human prostate carcinoma (HTB-81) cells lines, with the highest activity shown by compound **6** (IC_{50} 2.12 μM). Cyclic voltammograms show the two independent electroreductions of the compounds. The first electron transfer is an irreversible process, while transfer of the second electron is a quasi-reversible process. The decrease in peak current with no shift in peak potential upon addition of DNA in all the cases describes the consumption of the complexes by DNA. The successive decrease in diffusion constant (D^o) upon addition of DNA also describes the interaction of synthesized compounds with DNA.

Supplementary Material

The crystallographic data for the structural analyses of compounds **1** and **2** (Table 4) have been deposited with the Cambridge Crystallographic Data Centre, CCDC No. for **1** is CCDC 833826 and for **2** is CCDC 833827. A copy of this information may be obtained free of charge from the Director, CCDC, 12 Union Road, Cambridge, CBZ 1EZ, UK (fax: +44 1223 336 033; e-mail: deposit@ccdc.cam.ac.uk or www: http://www.ccdc.cam.ac.uk).

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