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Antitumor Metallothiosemicarbazonates: Structure and Antitumor Activity of Palladium Complex of Phenanthrenequinone Thiosemicarbazone

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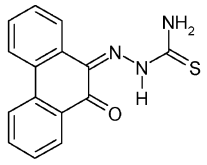
The crystal structure of the potential antitumor metal compound, viz. chloro, mono(phenanthrenequinone thiosemicarbazonato) palladium(II) dimethyl formamide solvate, is reported. The central palladium(II) atom is in a square planar environment provided by the tridentate, monoanionic thiosemicarbazone ligand and the ancillary chloride ion. The compound exhibited remarkable activity against drug-sensitive and drug-resistant breast cancer cell lines and was relatively nontoxic toward the normal mammary epithelial cells. The drug-induced killing effect against breast cancer cell lines was predominantly mediated via apoptosis, a physiologic form of cell death.

Emergence of resistance to anticancer drugs poses a major clinical challenge in successful treatment of cancer[1] since some tumor cells develop a particular phenotype, called multidrug resistance (MDR), which makes these cells resistant to other classes of anticancer agents to which the tumor cells have not been treated previously. These drugs include anthracyclins, epipodophyllotoxins, actinomycin D, and Vinca alkaloids.[2] MDR cell lines have been shown to display a complex spectrum of biochemical and cytogenetic changes such as the overexpression of p-glycoprotein,[3] increased levels of glutathione related enzymes,[4] downregulation of mono-oxygenases,[5] and altered expression of protein kinase C.[6]

It has been reported that tissue transglutaminase (tTGase), which is a protein cross-linking enzyme, is overexpressed in MDR-positive breast cancer (MCF-7)[7] and lung cancer (PC-147) cells.[8] Tissue TGase (EC 2.3.3.13) is a Ca^{2+}-dependent enzyme that catalyzes irreversible covalent cross-links between the γ-carboxamide group of the peptide-bound glutamine residue and the primary amine group of a variety of molecules.[9,10] Several studies have implicated induction and activation of tTGase in apoptosis.[11] It has been demonstrated that doxorubicin-resistant MCF-7 (MCF-7/DOX) cells exhibiting MDR phenotype have increased levels of tTGase expression.[7] Similarly MCF-7/DOX cells contain deficient intracellular calcium pools[12] and exhibit exquisite sensitivity toward calcium ionophore (A12387)-induced apoptosis, presumably due to the activation of endogenous tTGase.[13]



phenanthrenequinone thiosemicarbazone (PQTSC, **1**)

The quinonoidal compounds appended with thiosemicarbazone pharmacophore have been known to exert cytotox-

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Table 1. Crystallographic Data for [Pd(PQTS)Cl]·DMF

chemical formula	$C_{18}H_{17}ClN_4O_2PdS$
fw	495.27
space group	P2(1)/c
a	10.552(4) Å
b	5.3936(18) Å
c	33.071(11) Å
α	90°
β	90.252(8)°
γ	90°
T	295 K
wavelength	0.71073 Å
V	1882.2(11) Å^3
Z	4
D_{calcd}	1.748 Mg/m^3
abs coeff	1.260 mm^{-1}
final *R* indices [$I > 2\sigma(I)$]	R1 = 0.1566, wR2 = 0.2799
R indices (all data)	R1 = 0.1715, wR2 = 0.2859

icities toward many cancer cells.[14] Complexation of such thiosemicarbazone ligands with metal ions has been found to produce synergistic effects on the antiproliferative activities of the parent ligands.[15] In the present work, we describe synthesis and characterization of a palladium complex of phenathrenequinone-thiosemicarbazone (PQTSC, **1**) and evaluation of its antiproliferative properties in a panel of breast cancer and normal cell lines. We used the estrogen receptor-positive (ER+) MCF-7/WT, drug sensitive (MCF-7), MCF-7/TG (thapsigargin resistant MCF-7 cell line with deficient intracellular calcium pools), BT-20, and T47D as well as ER-MCF-7/DOX (doxorubicin resistant MCF-7 exhibiting MDR phenotype), MDA-MB-231 (highly metastatic), and BT-474 breast cancer cell lines. Three normal mammary epithelial cell lines, 21 NT (mortal mammary epithelial), MCF-10A (normal immortal breast epithelial), and HME (finite normal mammary epithelial), were also tested. The present study suggests that the palladium complex of **1** is a potent antineoplastic agent which has selective activity against tumor cells and is effective against drug-resistant breast cancer cell lines.

PQTSC was synthesized by the literature procedure as described earlier.[14] A mixture of K_2PdCl_4 (1 mmol, 0.32 g) and **1** (1 mmol, 0.28 g) in ethanol was refluxed on a water bath for 2 h which on cooling precipitated the metal complex. The palladium complex was washed with cold ethanol and dried in a vacuum. Dark green crystals suitable for the single crystal X-ray diffraction analysis were obtained by slow evaporation of its DMF solution (see Table 1).

Analytical and spectroscopic data indicate that the palladium complex possesses 1:1 metal to ligand stoichiometry. Appending one of the quinone carbonyls in phenanthrenequinone with thiosemicarbazide side chain produces two additional bands at 3265 and 3413 cm^{-1} in the IR spectrum of **1** due to the asymmetric and symmetric stretches of the terminal amino group.[16] A strong imine absorption at 1629 cm^{-1} confirms the formation of the thiosemicarbazone compound. The presence of hydrazinic NH band at 1315 cm^{-1} and concomitant absence of ν(S−H) band at 2100 cm^{-1} indicate that in the solid state **1** is present as the thione tautomer.[17] The disappearance of the C═S stretch at 1147 cm^{-1} and displacement of the C═N stretching vibration at 1629 cm^{-1} to lower wavenumber (1527 cm^{-1}) indicate that **1** behaves as a tridentate, monoanionic moiety forming five-membered chelate rings around the central metal through a donor atom set comprising the quinone carbonyl oxygen, imine nitrogen, and thiolate sulfur, respectively.[18] In the metal−ligand frequency region, bands at 357 and 395 cm^{-1} are assigned to ν(Pd−S) and ν(Pd−N) linkages.[19−23]

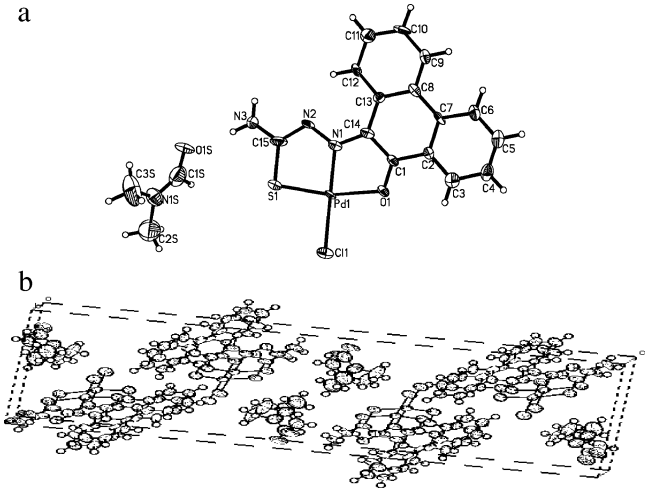

Figure 1. (a) Molecular diagram with atom numbering for [Pd(PQTSC)-Cl]·DMF and (b) its packing diagram.

The palladium complex is found to be diamagnetic in agreement with the planar geometry assigned to it. Electronic spectrum of this complex shows strong absorptions in the visible region between 15 000 and 18 000 cm^{-1} due to $^1A_{1g} \rightarrow {}^1A_{2g}$ transition while more intense bands are observed between 19 500 and 22 000 cm^{-1} which are a combination of S → Pd(II) and $^1A_{1g} \rightarrow {}^1B_{1g}$ transitions.

Figure 1a shows an ORTEP plot of the palladium(II) complex with the atom numbering scheme. The structure of the complex [Pd(PQTSC)Cl]·DMF reveals a slightly distorted square planar palladium compound whose coordination sphere consists of a tridentate, uninegative thiosemicarbazonate ligand in its thiolate form and the chlorine atom Cl-(1). The parent ligand **1** is in its *Z*-isomeric form with respect to C(14)−N(1) double bond, and the thiocarbonyl sulfur and

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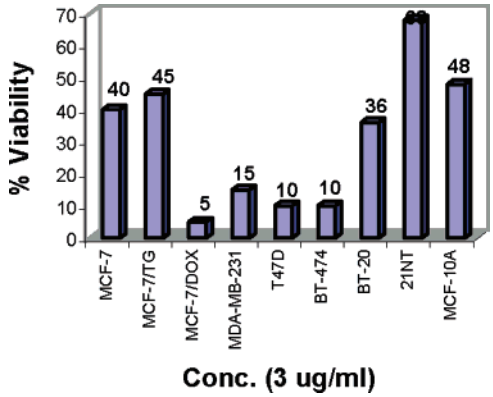

Figure 2. Effect of 3 μg/mL of the complex on breast cancer cells viability. All determinations are expressed as percentage of the control (untreated cells).

the imine nitrogen atoms in the side chain are *trans* to each other. On complexation with palladium the ligand assumes the *E*-isomeric form out of the coordination plane with respect to the C(14)−N(1) bond. The dihedral angle between the quinone ring and the plane defined by the five-membered chelate ring Pd(1)−S(1)−C(15)−N(2)−N(1) is 2.7° while the one between the quinone ring and the plane defined by Pd(1)−O(1)−C(1)−C(14)−N(1) is 1.1°, respectively. The angles between adjacent atoms in the coordination sphere are close to the expected value of 90° with the distortions being most noticeable in the thiosemicarbazone ligand. The distortion from the ideal geometry is also indicated by the fact that the angles N(1)−Pd(1)−O(1) and N(1)−Pd(1)−S(1) are less than 90° while O(1)−Pd(1)−Cl(1) and S(1)−Pd(1)−Cl(1) angles are greater than 90°. The S(1)−C(15) bond length, (1.722 Å) and the C(15)−N(2) length (1.35(3) Å) are consistent with enhanced single and double bond characters, respectively. The intramolecular hydrogen boundings were found between the thioamide nitrogens and oxygen atoms of dimethyl formamide, N(3)−H(3B)···O(1S) (2.01 Å). Dimeric units having $\pi-\pi$ interactions as shown in Figure 1b characterize the crystal packing of the complex.

The CV profile of the palladium complex was studied in DMSO solvent. Upon palladium complexation, the Q → SQ reduction peak is shifted toward more positive potential (−1.20 V) indicating facile reduction of the bound quinone species. The palladium complex shows reversible peaks at −0.35 and −0.89 V attributed to the Pd(II)/Pd(I) and Pd(I)/Pd(0) redox couples.

The effect on cell growth for [Pd(PQTS)Cl]·DMF was studied by culturing the cells in medium alone for 24 h, followed by 72 h treatment with 3 μg/mL concentrations. The viable cells remaining at the end of treatment period were determined by MTT assay and calculated as % of control, treated with vehicle alone (DMSO) under similar conditions. The palladium complex was consistently more active than any other metal conjugate analogue studied earlier,[14] especially against MCF-7/DOX cells that exhibit a high level of resistance against conventional chemotherapeutic agents. Various breast cancer and normal cell lines

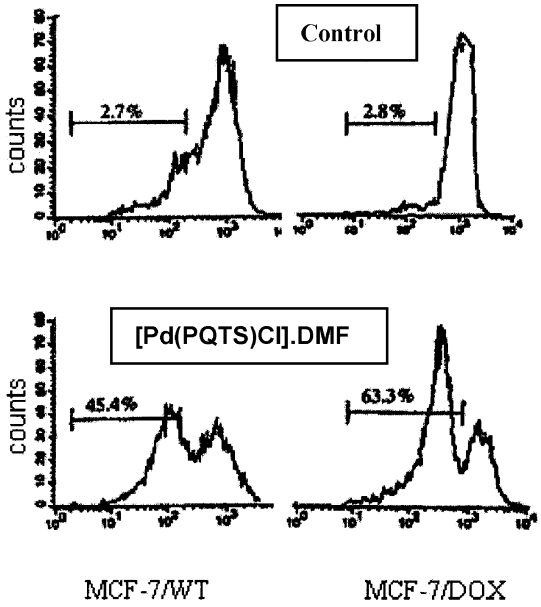

Figure 3. Untreated and drug treated MCF-7 cells were subjected to flow cytometry analysis, as described. Plot showing DNA content of cells following 48 h of incubation in medium alone (top panel) or medium containing the complex (bottom panel). Cells that exhibit the sub-G1 DNA levels are considered apoptotic.

were also tested against the palladium complex. Normal cells were relatively resistant to the toxic effects of the palladium compound indicating that its growth inhibitory effect against tumor cell lines was specific. Interestingly, the compound was more effective in arresting the growth of multidrug resistant cells than the wild-type MCF-7 cells (Figure 2) suggesting that it could be used advantageously in treating the resistant tumor cells.

Untreated and palladium compound treated MCF-7/WT and MCF-7/DOX cells were subjected to flow cytometric analysis to determine the mechanism of drug-induced cell death. Figure 3 shows the DNA content of the cells incubated for 72 h in medium alone or medium containing the drug (3 μg/mL). The analysis revealed significant degree of apoptosis (accumulation in sub-G1 phase) in both cell lines in response to the treatment with palladium compound. The palladium complex was exquisitely potent in this regard, and the MCF-7/DOX cells were much more sensitive to drug-induced apoptosis. The MCF-7 control cells under similar conditions showed no appreciable apoptosis.

Supporting Information Available: Experimental details (instrumentation and measurements, X-ray crystallography, cell culture, cell proliferation assays, MTT assay, apoptosis and DNA fragmentation, ^{1}H NMR) and bond lengths and angles (CIF format). This material is available free of charge via the Internet at http://pubs.acs.org. Crystallographic data have been deposited with the Cambridge Crystallographic Data Center, 12 Union Road, Cambridge CB2 1EZ, U.K. (Fax: + 44-1223-336-033. E-mail: deposit@cdc.cam.ac.uk.) and are available on request quoting the deposition number CCDC 240826.

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