Supporting information

Experimental

Instrumentation and measurements

The ^{1}H NMR spectra were recorded on a Varian INOVA Spectrometer in DMSO-D_6 operating at 300 MHz. The chemical shifts, were measured in ppm relative to TMS. Magnetic susceptibility of the palladium complex was measured at 298 K on a Faraday Balance with a field strength of 7000 G using Hg{$Co(SCN)_4$] as a calibrant. IR spectra were recorded in KBr discs in the range 4500-400 cm^{-1} on a Perkin-Elmer 1615 FTIR spectrophotometer, while the electronic spectra were recorded on a Genesys spectrophotometer in the range 200-1100 nm. Cyclic voltametric measurements were made in DMSO solvent on BAS CV-27 instrument with an X-Y recorder using Pt as working electrode against SCE and Pt wire as auxiliary electrode with tetraethylammonium perchlorate (TEAP) as the supporting electrolyte.

X-ray crystallography of complex [Pd(PQTSC)Cll].DMF

Diffraction intensity data were collected with a Bruker Smart Apex CCD diffractometer Crystal Data collection and refinement parameters are given in **Table 1**. The space group was determined from systematic absences. The structure was solved by direct methods and Fourier difference syntheses and refined by full-matrix least squares procedures on peak intensities (F^2). All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were inserted in their calculated positions. Software and atomic scattering factors[1] are contained in the SHELXTL program package.[2]

Cell Cultures:

The human breast cancer cell lines MCF-7, T47D, MDA-MB-231, BT-474, and BT-20, and the mammary epithelial cell line, MCF10A were obtained from and maintained according to the instructions provided by the American Type Culture Collection (Rockville, MD). In addition, two normal mammary epithelial (NME) cell lines having a finite life span were purchased from Clonetics (San Diego, CA). The drug-resistant MCF-7/DOX cell line was established via continuous culture of MCF-7 cells in the presence of doxorubicin as described previously.[3] Similarly, a thpasagargin -resistant MCF-7 cell line (MCF-7/TG) was established by weaning the parental MCF-7 cells through increasing concentrations (5-50 nM) of thapsagargin (Sigma Chemical Co., St Louis, MO) over a period of 3 months. All cell lines were routinely grown in RPMI 1640 medium supplemented with 10 mM HEPES buffer, 2 mM glutamine, 0.2 % Normocin (Invivogen) and 10% FCS.[4] The cells were cultured in a humidified incubator with 5% CO_2 in air and were maintained in exponential growth by twice a week passage.

Cell proliferation assays

Cell growth assays were carried out essentially according to the procedure described earlier.[3] Briefly, the cells (2000/well) were plated in 0.2 ml of the medium (RPMI 1640 with 10% FBS) in 96-well Corning plates in the presence or absence of the drug. At appropriate times, the medium was removed and cells were either counted by a hemocytomter or monitored by crystal violet staining. In preliminary experiments we determined that the crystal violet staining method to determine cell viability correlates well with the cell number determined by detachment with a trypsin solution and counting with a hemocytometer. Relative cell viability calculated by dividing optical density in the presence of the test sample by optical density in the absence of the test sample (medium) and multiplying the results by 100.

MTT assays

The number of viable cells remaining after appropriate treatment was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma Chemical Co.) assay.[5] Briefly, cells were plated (4,000 cells/well per 0.2 mL RPMI 1640 medium) in 96-well microliter plates and incubated overnight. The test agent was then added at indicated concentrations to quadruplicate wells. After 48 hrs, MTT was added to each well at a final volume of 0.5 mg/mL, and microplates were incubated at 37^0C for 3 h. After the supernatant was removed, the formazan salt resulting from the reduction of MTT was solubilized in dimethyl sulfoxide (DMSO; Sigma Chemical Co.) and the absorbance was read at 570 nm using an automatic plate reader (Molecular Devices Corporation, Sunnyvale, CA). The cell viability was extrapolated from optical density $(OD)_{570}$ values and expressed as percent survival using the following formula:

$$\% \text{ cell viability} = \frac{OD_{570} \text{ of drug treated sample} \times 100}{OD_{570} \text{ of untreated control sample}}$$

Apoptosis and DNA fragmentation

At the end of the treatment period, cells were washed and resuspended in PBS (0.5×10^6 to 1.0×10^6 cells/mL). Eighty microliter aliquots were mixed with 100 μL of permeabilization buffer (0.1% Triton X-100, 0.05 N HCl, 0.15 M NaCl) and incubated on ice for 2 min. Acridine orange solution (8 μg/mL; Polysciences, Warrington, PA) was added, and then cell fluorescence was immediately determined, using a fluorescence-activated cell scan flow cytometer (Becton Dickinson, San Jose, CA) at a 488-nm excitation of a 15-mW argon laser and filter settings for green (530 nm, DNA) and red (585 nm, RNA) fluorescence. Ten thousand events were analyzed, and the percentage of sub-G_1 (hypodiploid) apoptotic cells was calculated.

Its ^{1}H NMR spectrum shows absence of the singlet at 14.4 ppm due to the hydrazinic NH group observed in the parent ligand indicating enolization of the thiosemicarbazide side chain and subsequent coordination through the thiolate form. The phenanthrenequinone ring protons can be seen as multiplets between 7.4 and 8.7 ppm which are practically unaffected by the metal complexation. Presence of two singlets for the two NH_2 protons at 10.04 and 10.16 ppm suggests that the free rotation around the C-N bond is blocked due to its partial

double bond character.[6] The separation between these two peaks in the spectrum of the palladium complex is narrowed down to 0.12 ppm indicating lowering of bond order as a consequence of the thiolate complexation.[7] Although these protons are not directly involved in metal coordination their signals tend to be shielded due to inductive effects.

The electronic spectrum of **1** in DMSO solvent exhibits intense bands in the range 34000-27000 cm^{-1} that are assigned to imine and thioamide n $\rightarrow\pi^*$ transitions respectively.[8] These bands shift to lower energies upon metal complexations.[9] The S $\rightarrow$ M (II) charge transfer band in the present compound has been assigned to the absorption found in the region 27100-33000 cm^{-1} as suggested by Lever.[10]

The CV profile of phenanthrenequinone exhibits two reversible peaks centered at –0.71 and –1.51V attributed to the quinone$\rightarrow$semiquinone and semiquinone$\rightarrow$catechol redox couples respectively.[11] After derivatisation with the thiosemicarbazone side chain reversible Q$\rightarrow$SQ redox couple shows a shift towards negative potential –0.91 V while undergoing the loss of SQ$\rightarrow$CAT couple. The irreversible reduction peak observed at -1.67 V for **1** is due to the reduction of the azomethine double bond in the thiosemicarbazide side chain and is observed for many aromatic thiosemicarbazonates.[12] The additional oxidation peaks at –1.17 V and –1.57 V are probably due to the coupled chemical oxidation reactions.[13]

Reference:

(1) Chromer, D. T.; Waber, J. T. *International Tables for X-ray Chrystallography:* Kynoch Press, Vol. 1, Birmingham, 1974.

(2) Bruker Analytical X-Ray Division, Madison, WI 2000

(3) a) Chen, J. S. K.; Agarwal, N.; Mehta, K.; *Breast Cancer Res. Treat.* **2002**, *71*, 237-247; b) Chen, J. S. K.;Konopleva, M.; Andreeff, M.;Multani, A.; Pathak, S.; Mehta, K.; *J. Cell Physiol*. 2004, *200*, 223-234

(4) Devarajan, E.; Chen, J.; Multani, A. S.; Pathak, S.; Sahin, A. A.; Mehta, K. *Int. J. Oncol*. **2002**, *20*, 913-920.

(5) Sugarman, B. J.; Aggarwal, B. B.; Hass, P. E.; Figari, I. S. *Science* **1985**, *230,* 943-945.

(6) Lobana, T. S.; Sánchez, A.; Casas, J. S.; Castiñeiras, A.; Sordo, J.; García, M. S.; López, E. M. *J. Chem. Soc., Dalton Trans*. **1997**, 4289-4300.

(7) Casas, J. S.; Castaño, M. V.; Rodríguez-Argüelles, M. C.; Sánchez, A.; Sordo, J. *J. Chem. Soc., Dalton Trans*. **1993**, 1253-1259.

(8) (a) Colthup, N. B.; Day, L. H.; Wiberly, S. E. *Introduction to Infrared and Raman Spectroscopy*: Academic Press, New York 1964, 311. (b) Kanoongo, N.; Singh, R.; Tandon, J. P. *Bull. Chem. Soc. Jpn*. **1989**, *62*, 1385-1387.

(9) West, D. X.; Carlson, C. S.; Galloway, C. P.; Liberta, A. E.; Daniels, C. R. *Transition Met. Chem*. **1990**, *15*, 91-95.

(10) Lever, A. B. P. *Inorganic Electronic Spectroscopy*: 2nd Ed., Elsevier Amsterdam, 1984, 320.

(11) Dutta, S.; Peng, S. M.; Bhattacharya, S. *Inorg. Chem*. **2000,** *39***,** 2231-2234.

(12) Murugkar, A.; Unnikrishanan, B.; Padhye, S.; Bhonde, R.; Teat, S.; Triantafillou, E.; Sinn, E. *Metal Based Drugs* **1999,** *6*, 177-182.

(13) Sonawane, P.; Kumbhar, A.; Padhye, S. B.; Butcher, R. J. *Transition Met. Chem.* **1994**, *19*, 277-282.