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Short communication

# Cationic Pd(II) complexes acting as topoisomerase II inhibitors: Synthesis, characterization, DNA interaction and cytotoxicity

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Pd(II) 4-methyl-3-thiosemicarbazide complexes with interesting *in vitro* antitumor activity were synthesized. These compounds are unable to interact to the DNA in low concentration, but are capable of inhibiting human topoisomerase IIα and cathepsin B in the same range of concentration.

Type II topoisomerases are a class of ubiquitous enzymes that alter DNA topology by catalyzing the passing of an intact DNA double helix through a transient double-stranded break made in a second helix [1]. The topoisomerase IIα (topo IIα) isoform is considered to be the primary pharmacological target for some of the most active drugs currently available for the treatment of human malignancies, since topo IIα is highly up-regulated in transformed and cancer cells [2–5]. All topoisomerase II-targeting drugs are able to interfere with at least one step of the catalytic cycle [6]. Agents able to stabilize the covalent DNA topo IIα complex are traditionally called topo IIα poisons (e.g. doxorubicin, daunorubicin, mitoxantrone, amsacrine, and etoposide) whereas those acting on any of the other steps in the catalytic cycle are called catalytic inhibitors [6,7]. The cytotoxic effects of these agents may be mediated by the formation of an intermediate covalent DNA-topo IIα complex, which is highly effective in triggering tumor cell apoptosis [8]. There is now a growing body of evidence to support that topo IIα is highly sensitive to thiol-reactive agents such as quinones [9], selenium compounds [10], cadmium [11], thimerosal [12] and cisplatin [13]. According to Hasinoff and coworkers [13], topo IIα monomer has at least five free cysteines that could potentially react with a thiophilic metal center. Given that the electrophilic reaction of soft metals with critical sulfhydryl groups on topo IIα is thought, in part, to be responsible for its inhibition, this suggests that other metal based compounds bearing thiophillic metal can be investigated as new potential inhibitor of topo IIα.

In this sense, palladium(II) derivatives soon appeared to be excellent candidates because of its high affinity for sulfur-containing ligands [14]. An important breakthrough reported by Caires' group has revealed that the cyclopalladated $[Pd_2(S(-)C_2,N\text{-dmpa})_2(\mu\text{-dppe})Cl_2]$ {dmpa = N,N-dimethyl-1-phenethylamine; dppe = 1.2-bis(diphenylphosphino)ethane} interacts with protein thiol groups in the mitochondrial membrane [15,16]. In addition, this class of compounds has also displayed the ability to inhibit cathepsin B [17], a cysteine protease whose active site is composed of cysteine, histidine, and asparagine residues in a catalytic triad [18]. It is believed that the Cys residue in the cathepsin B catalytic site might be able to bind to Pd(II) center replacing one of its ligands (e.g. $Cl^-$, $H_2O$), while the remaining ligand moiety might establish favourable interactions with other residues in the active site cavity [19]. Surprisingly, very few studies on the topo IIα inhibition activity of Pd(II) compounds have been described so far [20].

Recently, we have synthesized cationic complexes of the type $[PdX(4\text{-PhT})(PPh_3)]X$ ($PPh_3$ = triphenylphosphine; 4-PhT = 4-phenyl-3-thiosemicarbazide; X = Cl, Br, I, SCN) and evaluated their cytotoxic effects on breast (LM3) and lung (LP07) tumor murine cells [21]. These Pd(II) compounds were more cytotoxic towards LM3 cell line than cisplatin (30.3 ± 3.7 μM), displaying $IC_{50}$ values in the range 2.79–8.84 μM. For the LP07 cells, most of these complexes exhibited cytotoxic effects similar to that of cisplatin (4.3 ± 0.4 μM).

Spectroscopic studies of model reactions with guanosine and agarose gel mobility shift assay showed that $[PdX(4\text{-PhT})(PPh_3)]X$ complexes have a limited reactivity towards nucleobases and DNA. We therefore hypothesized that one plausible molecular target for this type of complexes might be thiol-containing molecules, such as topo IIα and cathepsin B. As a part of our continuing research into the coordination and biological chemistry of metal-based compounds [22–24], we present in this work the synthesis, characterization, DNA interaction

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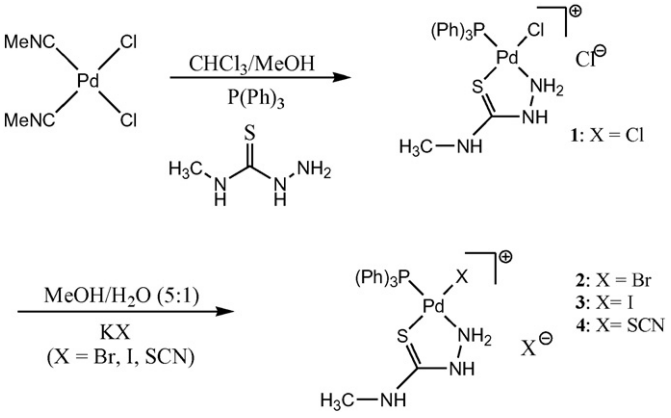


**Scheme 1.** Synthesis of complexes **1**–**4**.

studies, *in vitro* cytotoxicity and inhibitory activity evaluation (topoisomerases I and II, cathepsin B) of four cationic compounds of the type [PdX($PPh_3$)(4-MeT)]X {$PPh_3$ = triphenylphosphine; 4-MeT = 4-methyl-3-thiosemicarbazide; X = Cl(**1**), Br(**2**), I(**3**), SCN(**4**)}.

The synthesis of the [PdX(4-MeT)($PPh_3$)]X was achieved starting from bis(acetonitrile)dichloropalladium(II) (see synthetic procedures, ESI†). First, $PPh_3$ and 4-methyl-3-thiosemicarbazide displace the labile ligand acetonitrile and one of the two chloro-ions to obtain **1**. In a second step, the Cl ions are easily replaced by Br, I and SCN ions by the addition of two equivalents of their appropriate potassium salt to afford **2**–**4** (Scheme 1).

The formation of the [PdX(4-MeT)($PPh_3$)]X compounds was confirmed by elemental analysis, IR and $^1$H NMR spectroscopies and ESI/MS spectra (ESI†). Formation of the *N,S*-chelated products was proved by spectroscopic data. IR spectra show a significant shift of 30 $cm^{-1}$ to lower frequency for the $\nu$C═S band after coordination. Variation of ~4 ppm downfield of the chemical shift ($^1$H NMR) was observed for the two N2 protons after complexation. ESI/MS spectra confirmed the expected molecular mass of the compounds. After several attempts, single crystals suitable for X-ray diffraction studies were obtained for complexes **3** and **4** by vapor diffusion of diethyl ether at 4 °C into a saturated methanol. The ORTEP representations of cationic compounds **3** and **4** with the atom labeling scheme are presented in Fig. 1A and B, respectively.

Thiosemicarbazide acts as a neutral bidentate ligand leading to *cis* coordination through its S1 and N2 atoms to form a five membered ring. The remaining two coordination sites of the palladium atom are occupied by an iodide ion (**3**) or a thiocyanate group (**4**) coordinated *trans* to S1 and a triphenylphosphine ligand coordinated trans to N2. The palladium atom adopts a distorted square-planar geometry with bond lengths and angles in the usual range expected [25] [ESI†].

**Table 1**
Cytotoxicity values for complexes **1**–**4.**

| Compound | $IC_{50}$ (μM) | | |
|---|---|---|---|
| | LM3 | LP07 | MCF-7 |
| [PdCl($PPh_3$)(4-MeT)]Cl (**1**) | 5.92 ± 0.59 | 5.61 ± 1.32 | 8.78 ± 1.12 |
| [PdBr($PPh_3$)(4-MeT)]Br (**2**) | 6.05 ± 0.76 | 6.98 ± 0.71 | 9.04 ± 1.27 |
| [PdI($PPh_3$)(4-MeT)]I (**3**) | 3.22 ± 0.84 | 4.69 ± 0.14 | 9.54 ± 1.01 |
| [Pd(SCN)($PPh_3$)(4-MeT)]SCN (**4**) | 5.55 ± 0.20 | 4.83 ± 0.91 | 10.63 ± 0.40 |
| 4-MeT | >100 | >100 | >100 |
| Cisplatin | 30.3 ± 3.7 | 4.3 ± 0.4 | >50 |
| Etoposide | nd | nd | 44.87 ± 4.87 |

nd., not determined.

*In vitro* cytotoxic activity of ligand 4-MeT and complexes **1**–**4** was determined towards LM3 (murine mammary adenocarcinoma), LP07 (murine lung adenocarcinoma) and MCF-7 (human breast adenocarcinoma) cell lines by means of the colorimetric MTT assay. The corresponding $IC_{50}$ values are listed in Table 1.

Our data for the LM3 cell line show that all the Pd(II) compounds are more cytotoxic than cisplatin, exhibiting $IC_{50}$ values in the range 3.22–6.05 μM. Complex **3** was *ca.* 9.5 times more active than the reference drug. The complexes **1**–**4** displayed remarkable cytotoxic levels over the 7.66–11.03 μM concentration range, being ca. 5-fold more potent than etoposide against MCF-7 tumor cells. Cisplatin showed no drug response at concentrations <50 μM. Concerning the cytotoxic activity on LP07 cells, compounds **1**–**4** showed interesting cytotoxicity levels (4.69–6.98 μM), values similar to cisplatin (3.9–4.7 μM).

The interaction between complexes **1**–**4** and purine bases was evaluated employing guanosine (Gua) as a model system. Initially such interaction has been carried out directly in water media. However, complexes **1**–**4** did not react with guanosine in this solvent. In addition, all attempts to remove coordinated halide/pseudohalide by using $AgNO_3$ in water were unsuccessful. Aiming at generating more reactive and soluble complexes capable to readily interact with guanosine, we have employed the $AgNO_3$/dmf method [26] to afford *in situ* reactive and soluble solvento/nitrato Pd(II) complexes. The abstraction of anionic groups was performed by the addition of 2 equiv. of $AgNO_3$ in dmf leading to the precipitation of silver halide and silver thiocyanate, then filtered through a Millipore filter. The resulting solution was combined with Gua (2.0 equiv.) and the guanosine binding was confirmed by $^1$H NMR spectroscopy in [$D_4$]MeOD (after solvent removal) and mass spectrometry (ESI†). The observed

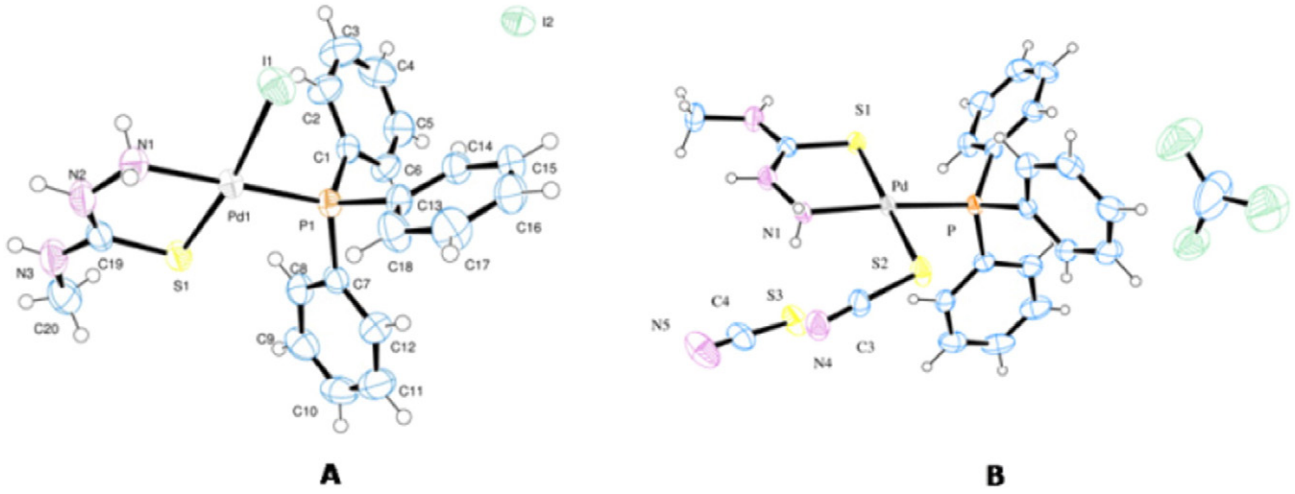


**Fig. 1.** Structures of **3** (A) and **4** (B).