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Labile Pd-sulphur and Pt-sulphur bonds in organometallic palladium and platinum complexes [(COD)M(alkyl)(S-ligand)]$^{n+}$—A speciation study

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ABSTRACT

Reaction of various sulphur ligands L (SEt^-, SPh^-, $SC_6F_4H\text{-}4^-$, SEt_2, $StBu_2$, $SnBu_2$, DMSO, DPSO) with the precursors [(COD)M(R)Cl] (COD = 1,5-cyclooctadiene, M = Pd or Pt; R = methyl (Me) or benzyl (Bn); DMSO = dimethyl sulfoxide; DPSO = diphenyl sulfoxide) allowed isolation and characterisation of mononuclear neutral (n = 0) or cationic (n = 1) complexes $[(COD)Pt(R)(L)]^{n+}$. Reaction of L-cysteine (HCys) with [(COD)Pt(Me)Cl] under similar conditions gave the binuclear cationic complex in $[\{(COD)Pt(Me)\}_2(\mu\text{-Cys})]Cl$. Detailed NMR spectroscopy and single crystal X-ray diffraction in the case of $[(COD)Pt(Me)(SEt_2)][SbF_6]$ and $[(COD)Pt(Me)(DMSO)][SbF_6]$ reveal markedly labilised Pt–S bonds as a consequence of the highly covalent Pt–C bonds of the R coligands in these organometallic species. Cationic charge (n = 1) seems to lower the Pt–S bond strength further. Consequently, most of these complexes are not stable long-term in aqueous DMF (*N*,*N*-dimethylformamide) solutions. This made the evaluation of their antiproliferative properties towards HT-29 colon carcinoma and MCF-7 breast adenocarcinoma cell lines impossible. Only the two complexes $[(COD)Pt(R)(SC_6F_4H\text{-}4)]$ with R = Me or SC_6F_4H-4 coligands could be tested with the R = Me complex showing promising activity (in the range of cisplatin), while the R = SC_6F_4H-4 derivative is largely inactive, as were the phosphane complexes $[(dppe)Pt(SC_6F_4H\text{-}4)_2]$ (dppe = 1,2-bis(diphenylphosphino)ethane), *cis*-$[(PPh_3)_2Pt(SC_6F_4H\text{-}4)_2]$ and *cis*-$[(PPh_3)_2PtCl_2]$ which were tested for comparison. In turn, our findings might pave the way to new Pt anti-cancer drugs with largely reduced unwanted depletion of incorporated drugs and reduced side-effects from binding to S-containing biomolecules.

1. Introduction

Since the discovery of the anti-cancer drug cisplatin (*cis*-$[PtCl_2(NH_3)_2]$ by Rosenberg in the 1960s [1] enormous research activity in the field of platinum containing cytostatics has been carried out focussing mainly on two aspects. Many investigations sought to elucidate the mode of action of cisplatin. Today the main mechanisms both from a chemical (chemical transformations of the complex - ligand exchange) and from a biological/medical (uptake into the cell, DNA binding, or cell apoptosis) point of view were established [2,3], although still a number of aspects are not completely understood. Another big focus of the investigations was to develop derivatives exhibiting less toxic side effects, higher solubility, oral applicability or higher selectivity to tumour cells compared with cisplatin. One of the major drawbacks for all platinum containing anti-cancer drugs is the low uptake of the administered material into the cell. The main reason for this depletion is considered to be due to binding of the drugs to proteins in the body, especially to cysteine side chains or to the ubiquitous glutathione [2–8]. Furthermore, it has been shown, that in a variety of model organisms, toxic side effects of cisplatin can be reduced by donation of sulphur containing compounds, such as sodium thiosulfate (STS), *N*-acetyl-cysteine (NAC), methionine, sodium diethyldithiocarbamate, or glutathione (GSH). Therefore, a vast part of the side-effects is considered to be due to unwanted binding of the Pt anti-cancer drug to S-containing biomolecules hampering their biological functions [6]. It would be thus an interesting target to design Pt anti-cancer drugs with markedly reduced tendency to bind to S ligands.

Thus, a number of platinum complexes have been studied in view of their binding abilities to sulphur ligands of biological relevance. Already early in the 1970s the reactivity of cysteine, methionine or glutathione towards $PtCl_4^{2-}$ [9–17] or cisplatin and other established platinum anti-cancer drugs [18–29] was studied. Here various species have been identified containing one or several of these ligands.

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Some were oligonuclear containing bridging sulphur ligands, as e.g. the species [(N^N)Pt(μ-GS)$_2$Pt(N^N)] and [(N^N)Pt(μ-GS)Pt(N^N)] (N^N: en = 1,2-diaminoethane or 1,2-DACH = 1,2-diaminocycohexane; GS^- = deprotonated glutathione) which were observed upon reaction of [(N^N)PtCl$_2$] with GSH (glutathione) or GSSG (glutathione disulfide) [25,30]. For a more defined approach to assess the binding strength of various sulphur ligands (with or without biological relevance), complex scaffolds $[(terpy)Pt]^{2+}$ (terpy = 2,2′:6,2″-terpyridine) [16,23,31–38] and $[(dien)Pt]^{2+}$ (dien = diethylene-triamine) [22,36–40] with only one coordination site, have been frequently studied, in the latter case also the Pd derivatives [36–38,41,42].

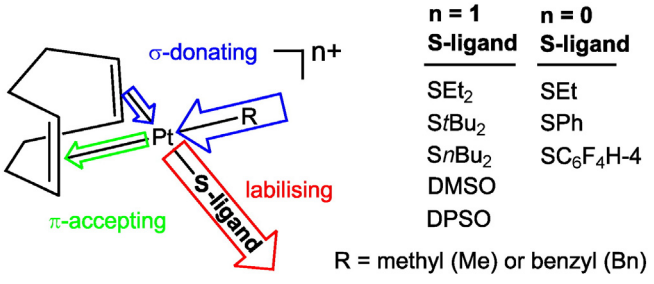
Recently, we have demonstrated the suitability of the [(COD)M(R)] (M = Pt or Pd; R = alkyl) fragment to coordinate various ligands [43–51]. For the platinum derivatives multinuclear NMR spectroscopy in addition to the determination of molecular structures has allowed an excellent insight into the binding between platinum and the introduced ligands [43–46,49–51]. From the ^{1}H–^{195}Pt coupling constant of the *trans*-oriented olefin proton the bond strength could be quantified quite reliably. In the same way other nuclei such as ^{31}P in Pt phosphane complexes or ^{15}N in Pt amine complexes have been used to determine the binding strength of ligands [52–59].

For the palladium analogues [(COD)Pd(Me)] [45,46,48] only bonding distances from molecular structure determinations give such insight. However, it became also evident, that bond distances are not only the result of metal to ligand binding, but is also influenced by crystal packing effects.

In this study we thus used the [(COD)M(R)] (COD = 1,5-cyclooctadiene; M = Pt or Pd, R = Me = methyl or Bn = benzyl) model system to assess the binding strength of various sulphur-containing ligands towards platinum and palladium. The motivation to start this study comes from the fact that organometallic derivatives of the [(COD)Pt(R)X] motif have shown marked antiproliferative activity with X = Cl, alkynyl, or alkyl [43–46]. Recently, we added the thiolate ligand SC$_6$F$_4$H-4 [50] to this series on which we report herein. Scheme 1 summarises the compounds used in this study.

Also, we reacted [(COD)Pt(Me)Cl] with L-cysteine (HCys) and were able to isolate and characterise the binuclear complex [(μ-Cys){(COD)Pt(Me)}$_2$]Cl.

Through use of ^{1}H NMR spectroscopy and mass spectrometry, these studies provide valuable information about the species present in solutions of these potential drugs, including complexes formed by dissociation or rearrangement. To an extent our results redress the relative lack of speciation studies on platinum [5,18,22,23,26,60–66], palladium [41,42] and other metal drugs [66], apart from cisplatin and related established drugs which have been extensively studied [2–8,18,22,23,26,63–66]. However, there have been recent advances in studies of organogold drugs by NMR spectroscopy [66], mass spectrometry [67], and other gold complexes by RIXS (resonant inelastic X-ray scattering) [68], a technique developed for in situ study of metallodrugs and their interaction with biomolecules [69].



Scheme 1. Representation of the complexes used in this study.

2. Experimental

2.1. General

All preparations were carried out in a dry argon atmosphere using Schlenk techniques. Solvents (CH_2Cl_2, THF, toluene, diethyl ether and MeCN) were dried using a MBRAUN MB SPS-800 solvent purification system.

2.2. Instruments

NMR spectra were recorded on a Bruker Avance II 300 MHz spectrometer (^{1}H: 300.13 MHz, ^{13}C: 75.47 MHz) using a triple resonance ^{1}H,^{19}F, BB inverse probehead. The unambiguous assignment of the ^{1}H and ^{13}C resonances was obtained from ^{1}H TOCSY, ^{1}H COSY, ^{1}H NOESY, gradient selected ^{1}H, ^{13}C HSQC and HMBC experiments. All 2D NMR experiments were performed using standard pulse sequences from the Bruker pulse program library. Chemical shifts were relative to TMS for ^{1}H and ^{13}C. The spectral analyses were performed by the *Bruker TopSpin 2* software. Elemental analyses were carried out using a Hekatech CHNS EuroEA 3000 Analyzer. EI-MS spectra were measured with a Finnigan MAT 900S. Simulations were performed using ISOPRO 3.0. UV/Vis absorption spectra were recorded with a Varian Cary50 Scan spectrophotometer.

2.3. Crystal structure determinations

Data collections were performed at $T = 293(2)$ K on a *STOE IPDS I* diffractometer with Mo-Kα radiation (λ = 0.71073 Å) employing ω-2θ scan technique. The structures were solved by direct methods using the SHELXTL package [70] or SHELX-97 and WinGX [71] and refinement was carried out with SHELXL97 employing full-matrix least-squares methods on F^2 [72] with $F_0^2 \geq 2\sigma(F_0^2)$ with the results shown in Table 3 (and Supporting Information). All non-hydrogen atoms were treated anisotropically; hydrogen atoms were included by using appropriate riding models.

2.4. Evaluation of antiproliferative effects

The antiproliferative effects of the compounds were determined following an established procedure [73]. In short, cells were suspended in cell culture medium (HT-29: 2850 cells/mL, MCF-7: 10,000 cells/mL), and 100 μL aliquots thereof were plated in 96 well plates and incubated at 37 °C: 5% CO_2 for 48 h (HT-29) or 72 h (MCF-7). Stock solutions of the compounds in dimethylformamide (DMF) were freshly prepared and diluted with cell culture medium to the desired concentrations (final DMF concentration: 0.1% v/v). DMF was used as a replacement for the commonly used dimethyl sulfoxide (DMSO) due to its similar polarity. DMSO should not be used as a solvent for platinum complexes because of possible ligand replacement reactions. The medium in the plates was replaced with medium containing the compounds in graded concentrations (six replicates). After further incubation for 72 h (HT-29) or 96 h (MCF-7) the cell biomass was determined by crystal violet staining and the IC_{50} values were determined as those concentrations causing 50% inhibition of cell proliferation. Results were calculated from two independent experiments.

2.5. Reagents

The complexes [(COD)Pd(Me)Cl] [45,74], [(COD)Pt(Me)Cl] [45,75], [(COD)Pd(Bn)Cl] [45], and [(COD)Pt(Bn)Cl] (Bn = benzyl) [45] were synthesised according to literature procedures. The complexes [(COD)Pt(SC$_6$F$_4$H-4)$_2$], [(COD)Pt(Me)(SC$_6$F$_4$H-4)], [(dppe)Pt(SC$_6$F$_4$H-4)$_2$] (dppe = 1,2-bis(diphenylphosphino)ethane), *cis*-[(PPh$_3$)$_2$Pt(SC$_6$F$_4$H-4)$_2$], and *cis*-[(PPh$_3$)$_2$PtCl$_2$] were synthesised as recently

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