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Novel complexes of tris(aminomethyl)phosphanes with platinum(II): Structural, spectroscopic, DFT and biological activity studies

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ABSTRACT

Two new platinum(II) complexes with tris(aminomethyl)phosphanes: [*trans*-$PtCl_2\{P(CH_2N(CH_2CH_2)_2NCH_3)_3\}_2$] (**1Pt**) and [*trans*-$PtCl_2\{P(CH_2N(CH_2CH_2)_2O)_3\}_2$] (**2Pt**) were prepared and characterized with NMR and UV–Vis spectroscopies. Their structures were investigated by X-ray crystallography and DFT methods. TDDFT calculations were employed to interpret the electronic spectra of the complexes. Obtained results are not unequivocal, however population analysis indicate, that the character of HOMO and HOMO−1 orbitals depend strongly on the electron donoring properties of the phosphane ligand. Biological activity of **2Pt** complex, which is more stable and more soluble in polar solvents than **1Pt**, was examined *in vitro* on the Vero cell line (IC_{50} = 12.5 μM). At higher concentrations it induces apoptosis, probably due to changes of the cell cytoskeleton. Luminescence quenching studies and CD spectroscopy of interactions of **2Pt** with HSA and BSA indicate that these albumins bind the complex slightly – without altering their tertiary structures, however HSA interacts with **2Pt** noticeably stronger than BSA. It was also found that **2Pt** does not cleave supercoiled pUC18 plasmid.

1. Introduction

Metal complexes have been investigated extensively as potential chemotherapeutic agents or diagnostic agents during past few decades [1,2]. Despite this growing interest, clinically approved platinum based drugs are limited to a few examples. The oldest one – cisplatin is still a drug for the treatment of testicular and other germ-cell tumors [3], however its clinical use is limited by the diminished activity against a number of cancers, the acquired resistance developed by many tumors and severe side effects. Therefore, the new improved platinum antitumor agents are developed constantly [4]. Several new analogs of transplatin which exhibit a different spectrum of cytostatic activity including activity in tumor cells resistant to cisplatin have been described [5–9]. Such drugs exhibit a cytotoxic activity in cisplatin-sensitive cells comparable to cisplatin and considerably higher in several cisplatin-resistant tumor cells [10]. That circumvention of cisplatin-resistance might be related with the ability of these drugs to induce apoptosis [6,10–12]. There are also known several examples of active platinum complexes containing aminophosphanes [13,14], triphos [15], other phosphorus derivatives [16] and an aliphatic amine together with triphenylphosphine [17].

Herein we present the new platinum(II) chloride complexes with aliphatic, water soluble aminomethylphosphanes of $P(CH_3NR_2)$ type: $P(CH_2N(CH_2CH_2)_2NCH_3)_3$ (**1**) [18,19] and $P(CH_2N(CH_2\ CH_2)_2O)_3$ (**2**) [18–21] (Fig. 1) together with the preliminary studies of the biological properties of the complex with **2**. These ligands were chosen for present study because phosphanes derived from aminoacids [22–25] or prepared from the highly water-soluble aliphatic secondary amines [20,21], seem to be the most interesting in the terms of formation the potential conjugates with a wide range of biomolecules.

Phosphanes **1** and **2** are well soluble in water and their solutions are air-stable. Moreover, **1** and **2** show a very weak or no antimicrobial activity [18] and no toxic properties (no cytopathic effect up to *c* = 16 mM [19]) against the continuous cell line Vero recommended for chemical toxicity screening *in vitro* [26]. Therefore, their complexes can be interesting as chemotherapeutic or diagnostic agents.

2. Experimental

All reactions were carried out under a dinitrogen atmosphere using standard Schlenk techniques. Neutral red, Hoechst 33258, acridine orange (AO) and all reagents for cell culture were purchased from Sigma–Aldrich. Phosphanes **1** and **2** were synthesized as described previously [18]. K_2PtCl_4 was synthesized from K_2PtCl_6

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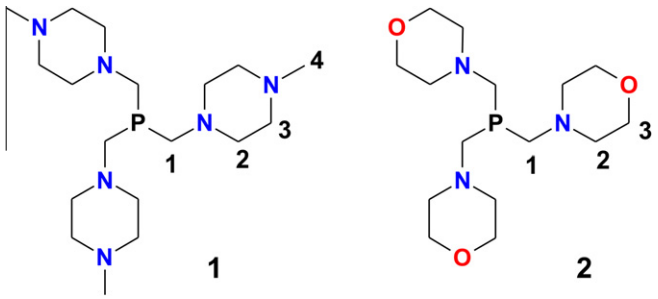

Fig. 1. The molecules of **1** and **2** with the atom numeration schemes.

[27]. All solvents were deaerated prior to use. Low EEO agarose was purchased from AppliChem. Bromophenol blue and glycerol were obtained from POCH. The plasmid pUC18 was purchased from Sigma.

2.1. Instrumental methods

NMR spectra were recorded at 298 K on a Bruker Avance III 300 MHz and a Bruker Avance III 600 MHz spectrometers with traces of solvent as an internal reference for ^{1}H and ^{13}C and 85% H_3PO_4 in H_2O as an external standard for ^{31}P. The splitting of proton resonances in the reported spectra is defined as s = singlet (* – strongly broadened signal), d = doublet, t = triplet and m = multiplet; atom numeration is taken from Fig. 1. Photoluminescence measurements were performed at room (298 K) temperature using a SpectraPro 750 monochromator, equipped with Hamamatsu R928 photomultiplier and a 1200 l/mm grating blazed at 500 nm. The 450 W xenon arc lamp was used as an excitation source. It was coupled with 275 mm excitation monochromator which used a 1800 l/mm grating blazed at 250 nm. Samples were excited with 285 nm line using 305 nm filter. CD spectra were recorded on a Jasco-715 spectrometer. Elemental analysis was performed with a Vario EL3 CHN analyzer. Mass spectra were recorded on a Bruker Daltonics micrOTOF-Q mass spectrometer equipped with electrospray ionization (ESI) source and operated in positive ion mode.

2.2. Syntheses of *1Pt* and *2Pt*

To a concentrated water solution of 2.50 mmol of **1** (0.9263 g) or **2** (0.8285 g) a saturated solution of 1.20 mmol (0.4981 g) of K_2PtCl_4 in water was added slowly at room temperature. Yellowish solid, which precipitated almost immediately, was filtered and washed twice with cold water. Crystals of **1Pt**·$2CH_3OH$ or **2Pt**·$2CH_3COCH_3$ for the X-ray analysis were obtained by a slow evaporation of the mixture of water with methanol or acetone, respectively.

1Pt: Yield: 68%; *Anal.* Calc. for $C_{36}H_{78}Cl_2N_{12}P_2Pt$: C, 42.94; H, 7.81; N, 16.69. Found: C, 42.90; H, 7.92; N, 16.64%. *m/z* ($CHCl_3$): 1007.50 (100%) $[M+H]^+$; 971.53 (65%) $[M-Cl]^+$; 934.55 (40%); 895.40 (30%); NMR (Bruker 300): ($CDCl_3$, 298 K): $^{31}P\{^{1}H\}$: −12.3 ($^{1}J(P-^{195}Pt)$ = 2311 Hz). ^{1}H: H^1 3.03 (s), H^2 2.78 (s*), H^3 2.36 (s*), H^4 2.22 (s), $^{13}C\{^{1}H\}$: C^1 47.7 (pseudo t, 23.1 Hz), C^2 55.5 (s*), C^3 55.3 (s), C^4 45.9 (s).

2Pt: Yield: 83%; *Anal.* Calc. for $C_{30}H_{60}Cl_2N_6O_6P_2Pt$: C, 38.79; H, 6.51; N, 9.05. Found: C, 38.77; H, 6.52; N, 9.02%. *m/z* ($CHCl_3$): 929.31 (100%) $[M+H]^+$; 892.34 (10%) $[M-Cl]^+$; NMR (Bruker 300): ($CDCl_3$, 298 K): $^{31}P\{^{1}H\}$: −8.2 ($^{1}J(P-^{195}Pt)$ = 2320 Hz). ^{1}H: H^1 3.10 (s), H^2 2.77 (s*), H^3 3.65 (s*), $^{13}C\{^{1}H\}$: C^1 48.3 (pseudo t, 23.1 Hz), C^2 55.5 (pseudo t, 2.8 Hz), C^3 66.9 (s). (toluene-d_6, 298 K): $^{31}P\{^{1}H\}$: −8.4 ($^{1}J(P-^{195}Pt)$ = 2336 Hz). ^{1}H: H^1 3.01 (s*), H^2 2.64 (m), H^3 3.54 (m), $^{13}C\{^{1}H\}$: C^1 48.8 (pseudo t, 22.8 Hz), C^2 56.1 (pseudo t, 3.1 Hz), C^3 67.0 (s). (DMSO-d_6, 298 K): $^{31}P\{^{1}H\}$: −8.3 ($^{1}J(P-^{195}Pt)$ = 2320 Hz). ^{1}H: H^1 3.03 (s*), H^2 2.68 (m), H^3 3.54 (m), $^{13}C\{^{1}H\}$: C^1 48.0 (pseudo t, 22.4 Hz), C^2 55.3 (pseudo t, 3.0 Hz), C^3 66.2 (s). NMR (Bruker 600): ($CDCl_3$, 298 K): ^{1}H: H^1 3.10 (s), H^2 2.77 (s*), H^3 3.65 (m*), $^{13}C\{^{1}H\}$: C^1 48.36 (pseudo t, 22.67 Hz), C^2 55.55 (pseudo t, 3.32 Hz), C^3 66.94 (s).

2.3. X-ray crystallography

The data were collected at 100 K using a KM4-CCD diffractometer and graphite-monochromated Mo Kα radiation (λ = 0.71073 Å) generated from diffraction X-ray tube operated at 50 kV and 20 mA. The images were indexed, integrated, and scaled using the Oxford Diffraction data reduction package [28]. The structures were solved by direct methods using SHELXS97 and refined by the full-matrix least-squares method on all F^2 data [29]. Non-H atoms were included in the refinement, with anisotropic displacement parameters and the H atoms was included from geometry of molecules. The data were corrected for absorption [28].

2.4. Crystal/refinement data

1Pt·**2**(**CH_3OH**)≡$C_{38}H_{86}Cl_2N_{12}O_2P_2Pt$, M_r = 1071.11, triclinic, space group $P\bar{1}$ (no. 14), a = 8.636(1) Å, b = 11.332(1) Å, c = 14.622(2) Å, α = 69.92(2)°, β = 74.72(1)°, γ = 74.69(1)°, V = 1272.5(3)Å^3, D_{calc} (Z = 1) = 1.398 g/cm^3, μ_{Mo} = 2.968 mm^{-1}, specimen: 0.12 × 0.11 × 0.10 mm, T_{min} = 0.707, T_{max} = 0.743, reflections collected/unique 24709/10901 [R_{int} = 0.0490], final R indices [$I > 2\sigma(I)$] R_1 = 0.0313, wR_2 = 0.0614, R indices (all data) R_1 = 0.0327, wR_2 = 0.0618, GOF = 0.997, T = 100(2) K.

2Pt·**2**(**CH_3COCH_3**)≡$C_{36}H_{72}Cl_2N_6O_8P_2Pt$, M_r = 1044.92, monoclinic, space group $P2(1)/n$ (no. 14), a = 12.633(1) Å, b = 13.022(1) Å, c = 14.295(2) Å, β = 105.61(1)°, V = 2264.9(4) Å^3, D_{calc} (Z = 2) = 1.532 g/cm^3, μ_{Mo} = 3.339 mm^{-1}, specimen: 0.15 × 0.12 × 0.10 mm, T_{min} = 0.624, T_{max} = 0.716, reflections collected/unique 26590/4491 [R_{int} = 0.0274], final R indices [$I > 2\sigma(I)$] R_1 = 0.0168, wR_2 = 0.0383, R indices (all data) R_1 = 0.0244, wR_2 = 0.0417, GOF = 1.058, T = 100(2) K.

2.5. DFT calculations

DFT calculations for **1Pt** and **2Pt** complexes were performed using the GAUSSIAN 03 package [30]. For geometry optimizations and the single-point energy calculations we employed the Becke hybrid three parameter DFT method using the Lee, Yang and Parr correlation functional [31–33] (B3LYP). The basis sets employed for platinum atom for was the D95V with the Stuttgart/Dresden (replacing inner 60 electrons) ECP and for the rest of the atoms 6-31G(d,p) for the geometry optimization or 6-311+G(2d,p) for the single point energy calculations. The singlet ground-state structures of the compounds were optimized in the gas phase starting from corresponding X-ray geometries. Minima of energy were characterized as such by computation of the harmonic vibrational frequencies. For the complexes in the singlet ground states time-dependent density functional theory (TDDFT) [34–36] was used to calculate the singlet excited-state energies. The percentage of the atomic/ligand contributions were calculated as $[n^2/\{\mathrm{sum}(n^2)\}] \times 100\%$, where n – atomic orbital coefficients in a specific molecular orbital.

2.6. The toxicological studies

Continuous cell culture Vero derived from kidney epithelial cells of the African Green Monkey *Cercopithecus aethiops* (cat. no. ATCC CCL-37, passage 70) was maintained in 37 °C in 25 cm^2 flasks in Parker's 199 medium supplemented with 5% fetal calf serum and L-glutamine, penicillin and streptomycin (100 mg/ml). Cells were