Complexes of Pt(II) and Pd(II) with Symmetrical Bipodal N,N-bis-antipyrine-N’pyridinethioureas

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In this study, we prepared complexes of Pt(II) and Pd(II) with symmetrical bipodal N,N-bis-antipyrine-N’pyridinethioureas. All the structures of these complexes have been characterized by elemental analyses, thermal analyses, IR, UV-Vis, 1H-NMR, 13C-NMR and mass spectrometry for ligand. The biological activity of synthesized compounds was tested on human tumor cell lines.

Keywords: bipodal complexes, UV-Vis, IR-spectroscopy, biological activity

Strong affinity of N-aroyl-N’benzyl thiourea ligands for “softer” transition metals is known for a long time. More systematic studies of these ligands by the research groups of Koch [1] have confirmed the utility of these compounds as potentially selective ligands, especially for the transition metal ions. Thiourea derivatives have been the subject of interest because it has been shown that they have antitumor and antifungal bioactivities and inhibitory activities against viruses [2-6]. There are mentioned in literature [6-10] metallic macro cycles, containing 2,3 ligand molecules. It was proposed the obtaining of a metallic macrocycle formed by 2,3,6 ligand molecules, with metals Pt(II), Pd(II). This ligand has a symmetrical structure, functioning as a tetradentate ligand. The capacity of symmetrical ligands to form macro cycles with transition metals is intensely studied and ligand can form macro cycles containing up to 6 molecules [1].

Experimental part

All chemicals used for the preparation of the compounds were of reagent grade quality. The elemental microanalyses of the prepared compounds for C, H, N and S were performed on COSTECH ECS 4010 CHNSO analyzer. 1H-NMR and 13C-NMR spectra were performed on Varian Gemini 300 MHz spectrometer. The samples were dissolved in DMSO-d6 using tetra methyl silane as internal references. Thermal analysis curves (TG, T and DTA) were registered in an air current, by using a thermo gravimetric analyzer LABSYS 1200 SETARAM with samples weights between 3-15 mg, in the temperature interval 30-900°C and a heating rate of 10 K/min. Infrared spectra were recorded on an Escalibur FT-IR spectrometer, IR spectra of ligand and of the compounds have been achieved by reflexion on ATR with diamond crystal deposited onto KR55, on the domain 4000-250cm⁻¹. The UV-VIS spectra were recorded on a Jasco UV-VIS 540V spectrophotometer ranged from 200-900 nm. The mass spectra were performed using Varian mass spectrometer. Metal contents were determined with the Atomic Absorption Varian spectrometer. The biological activity experiments was performed using DMSO as solvent. Cell viability was determined by absorbance measurements at 570 nm and correlated to the ability of reducing MTT to produce formazan.

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General procedure for the synthesis of N,N-bis-antipyrine-N’pyridinethiourea

The ligand in this work were prepared according to a slightly modified procedure of Douglass and Dains [11], using commercially available reagents. The 2,6-Pyridinedicarboxylic acid chloride was used without further purification, while the amine 4-aminoantipyrine were dried over calcium chloride. The analytical grade potassium thiocyanate was dried at (105°C) immediately before use. This method consists in formation of benzoyl iso thiocyanate in a first step, followed by condensation of iso- thiocyanate as a primary (fig. 1).

To a solution containing 0.05 mol potassium thiocyanate in 15 mL freshly distilled acetone is added drop-wise an equimolar quantity of 2,6-Pyridinedicarboxylic acid chloride (0.025 mol) in anhydrous acetone. The mixture was heated under reflux for 45 min, and cooled to room temperature under nitrogen. To this stirred reaction mixture, was added drop-wise 0.05 mol of the desired amine in 20 mL acetone over 10 min, followed by heating under reflux for a further...
120 min. After cooling, the mixture was poured into 40-75 mL of cold water and the acetone, this results in the precipitation of the crude product. Recrystallisation of the dried product was from ethanol.

N,N-bis-antipyrine-N’pyridinethiourea yellow crystals, 85% yield. Found: C, 56.79; H, 4.42; N, 19.23; S, 9.77%. C₃₀H₂₄N₈O₄S₂ requires: C, 57.20; H, 5.01; N, 19.42; S, 10.12. 1H NMR (300 MHz, DMSO-d₆): δ 11.7 ppm (s, 2H, CONH), 10.9 ppm (s, 2H, CSNH), 7.2-7.6 ppm (m, 3H, Pyridin-H), 3.13 ppm (s, 6H, -NCH₃), 2.23 ppm (s, 6H, CH₂), 6.0 (s, 2H, CSNH), 7.2-7.6 ppm (m, 10H, Ar-H), 8.26-8.63 ppm (m, 3H, Pyridin-H), 3.23 ppm (s, 6H, -NCH₃), 2.29 ppm (s, 6H, CH₃), ε (λmax 390 nm) = 16 159, ε (λmax 282 nm) = 15 765 dm³ mol⁻¹ cm⁻¹.

[Pt(HL-S,O)]₂

A 0.3 mmol portion of ligand H₂L (200 mg) was dissolved in 20 mL ethanol to which 2.0 mmol (49.2 mg) sodium acetate dissolved in 15 mL water was added. A 0.3 mmol portion of K₂PtCl₄ dissolved in 10 mL water, was added drop-wise to the ligand solution over a period of ca. 60 minutes. The mixture was stirred at room temperature for further 3 h before being diluted with 40 mL water. The fine yellow-orange precipitate which formed was recovered by centrifugation and washed with ethanol.

Yellow-orange, 55% yield, m.p. 239 °C. Found: C, 43.76; H, 3.14; N, 14.82; S, 7.52; Pt, 22.94%. requires: C, 43.34; H, 2.5; N, 15.23; S, 7.20; Pt, 22.5%. FT-IR (cm⁻¹) ν (max) = 15765 dm³ mol⁻¹ cm⁻¹, ε (λmax 212 nm) = 16 512 dm³ mol⁻¹ cm⁻¹, Λ = 17 (Ω mol⁻¹ cm²)

[Pd(HL-S,O)]₂

A solution containing 0.6 mmol quantities PdCl₂ (106.2 mg, dissolved from 15 mL of water and 5 mL HCl), was added drop-wise to the ligand solution, made from 0.6 mmol equivalent of the ligand dissolved in 20 mL ethanol. The mixture was stirred at room temperature for 1 h, after which 50-100 mL of water was added.

Yellow, 65% yield, m.p. 243 °C. Found: C, 48.94; H, 3.68; N, 16.57; S, 8.42; Pd, 13.94%. requires: C, 49.01; H, 4.23; N, 16.10; S, 8.76; Pd, 14.13%. FT-IR (cm⁻¹) ν (max) = 13 248, ν (min) = 1077, ν = 1412, ν_p,-COO = 404, H NMR (300 MHz, DMSO-d₆): δ 11.3 ppm (s, 2H, CONH), 11.6 ppm (s, 2H, CSNH), 7.2-7.64 ppm (m, 3H, Pyridin-H), 3.25 ppm (s, 6H, -NCH₃), 2.36 ppm (s, 6H, CH₃), ε (λmax 390 nm) = 14 564, ε (λmax 350 nm) = 16 152 dm³ mol⁻¹ cm⁻¹, Λ = 17 (Ω mol⁻¹ cm²)

Growth inhibition assays

Cells (MCF-7) were suspended in 150 μL of media (RPMI1640; FCS 10%; L-glutamina 300mg/100mL; penicilina-streptomicina 1%; then it was added 1 μL of MTT reagent were added/well, after 24 h incubation, and incubated 3 hrs. Readings were performed at 570nm on an ELISA plate reader, with a reference at 655nm.

Cells (A2058 – Melanoma) were suspended in 150 μL of media ( RPMI 80%; FCS 20%; L-glutamine 300mg/mL; penicillin-streptomycin 1%; then it was added 1 μL of compound (1 mg of sample was dissolved in 0.5 mL DMSO), and also 1 μL of DMSO, as negative control, to each well and incubated with the test compounds for 24 h at 37°C. After the incubation period, 15 μL of 5 mg/mL MTT was added into the media and incubated for three hours at 37°C and 5% CO₂. At the end of the incubation period with MTT, the solvent analysis was added. Ten minutes later there were performed readings at 570nm, with a reference at 655nm, on an ELISA microplate reader.

Results and discussions

The ligand was synthesized in excellent yields following the method described by Douglass and Dains [11]. All spectroscopic methods and elemental analyses confirm the proposed structure of the ligand figure 1.

The ligand showed two peaks in the 3382 cm⁻¹ and 3194 cm⁻¹ regions due to the N-H stretching vibrations. The vNH stretching vibration frequencies are due to an intramolecular [12] hydrogen bond, where by the carbonyl group is connected to the imine group. Compound showed a single peak in the 1668 cm⁻¹ region, due to the C=O stretching vibration band. A strong band in the 1297 cm⁻¹ and 1061 cm⁻¹ regions is assigned to the thiocarbonyl group. IR stretching vibration. A strong band in the 1297 cm⁻¹ and 1061 cm⁻¹ regions is assigned to the thiocarbonyl group. Compound showed a single peak in the 1668 cm⁻¹ region, due to the C=O stretching vibration band. The characteristic IR bands [13] of the synthesized ligand are presented in experimental part.

The 1H-NMR data of the ligand revealed its formation by the presence of the two N-H signals for the compound, observed at 11.7 ppm and 10.9 ppm. Because of the formation of an intramolecular hydrogen bond, the NHCS imine group proton participating in the hydrogen bond appears at low field [12-13], and the NHCO group appears at high field. The mass spectrum of ligand HL showed molecular ion peak at 656 corresponding to its molecular formula [M+H⁺]⁺ (scheme 1), base peak at m/z (%)= 411 (37%), peak at 246 (100%), which confirms the suggested structure (fig.1).

Scheme 1. Suggested mass fragmentation of ligand
In 1H-NMR spectrum of Pt (II) complex the signal of ligand from 11.7 ppm disappeared, which suggests that ligand functions in a deprotonated form. The signal of CSNH proton is strongly deshielded at 11.3 ppm compared to ligand, where it appeared as a singlet at 10.9 ppm. The aromatic protons are slightly shielded compared to the free ligand, as an effect of different orientation after complexation.

In 13C-NMR spectrum Pt (II) complex there were observed slightly chemical displacements: δC=S shielding with 3.5 ppm compared to the ligand value, which suggests that this group participates at coordination. In case of δC=O group there was observed a chemical displacement with 1.06 ppm higher than ligand value. All carbon atoms of the pyridine ring presented deshieldings, with values between 0.6-2.02 ppm, due to spatial rearrangement in complex. NMR spectrum of Pd complex presents also the disappearance of CONH proton, due to the ligand deprotonation after complexation. The proton of CSNH group presented slightly modifications compared to the free ligand. Slightly deshieldings appeared also for the other protons of the complex compared to the free ligand. By analyzing the 13C-NMR spectrum it was observed that the palladium ion linkage to O and S atoms has as effect the shielding with 2.83 ppm of C=S group and with 1.35 ppm for C=O group. Slightly deshieldings appeared at the aromatic carbon atoms, due to spatial rearrangement of the ligand molecule, after complexation.

Electronic spectra

Information about complex combinations geometry was obtained from electronic spectra. Electronic spectra were registered by diffuse reflexion on matrix of magnesium oxide.

The geometry of the synthesized complex combinations was also confirmed by the electronic spectra. It is known from specialty literature that Pt(II), Pd(II), with a d⁸ electronical structure form complex combinations with a plan square symmetry.

Assignements of the absorption bands were compared to literature data. [14] Correlation of bands observed in electronic spectra of Pt (II) complex combination with ligand N,N-bis-antipyrine-N'-pyridinethiourea to those of [PtCl₄]²⁻ and [PtBr₄]²⁻ in ultraviolet and visible domain, allows the following assignments.

The absorption band from 39756 cm⁻¹ was assigned to an intra ligand transition and the absorption band from 30303 cm⁻¹ was assigned to transition 1A₁g → 1B₁g. The absorption band of complex combinations from 25608 cm⁻¹ was assigned to transition 1A₁g → 3A₂g [14-15, 22].

Correspondence of these bands to those of platinum (II) halides leads to conclusion that the symmetry of the studied complex combinations is plan square and it belongs to D₄h group of symmetry[14].
Thermal analysis

Paladium (II) complex were assigned to transitions approximately 250-253°C. The first endothermal figure 3). The complexes combination is very stable (until thermally decomposed in two well defined steps (table 2, the spectrum.

The absorption band from 40983cm⁻¹ was assigned to approximations (II) complex it was observed the existance of a quasi reversible couple PtII (II) complex it was observed the existance of a quasi reversible couple PtII, figure 2 when scanning potential increased: 0.250 V - 1.250 V.

Cyclic voltammetry studies

Redox properties of complex combination was examined by cyclic voltammetry, with DMSO in presence of TBABF₄, the reference electrode was Ag/AgCl, the working electrode was made of platinum, having a diameter of 3 mm and a platinum electrode was used as counter electrode, too; the scanning rate was of 50 mV/s. The experimental data are presented in table 1. In case of Pt (II) complex it was observed the existance of a quasi reversible couple Pt⁰ → Pt⁶, figure 2 when scanning potential increased: 0.250 V → 1.250 V.

Thermal analysis

In conformity with TG and DTA curves, the compound is thermally decomposed in two well defined steps (table 2, figure 3). The complexes combination is very stable (until approximately 250-253°C). The first endothermal decomposition step consists in partial degradation of ligand, with the elimination of a pyridine molecule per ligand molecule. It follows the organic component burning. The non unitary process is followed by a strong exothermal effect [20,21].

Biological assays

The antiproliferative activity of compounds was evaluated by means of an in vitro assay in human tumour A2058 MELANOMA cell line – serie 2 and MCF-7 (Human breast adenocarcinoma cell line) – serie 1. The values shown in figure 4 indicate that both of the complexes exhibit cytotoxicity.

The cytotoxic assays showed the moderate activity of the complex on the viability of cells melanoma and MCF-7 (Human breast adenocarcinoma cell line) in vitro. The data show that after incubation for 24 h the complex caused about 62.45% of cell death for [Pt(HL-S,O)]₂⁺ complex, 51.80% for [Pd(HL-S,O)]₂⁺

Conclusions

We have synthesized and characterized a series of platinum(II) and palladium(II) complexes with N,N-bis-antipyrene-N'-pyridinethiourea. Cytostatic activity of the complexes against A2058 melanoma tumor cells increases in the series: 1 > 2 > L. In case of MCF-7 Human breast adenocarcinoma cell line the best cytostatic activity was observed for all complexes.

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References