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Complexes of platinum and palladium with 4-nitrobenzoic hydrazide: synthesis and cytotoxic activity

Research Article

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Abstract: New complexes of platinum and palladium were isolated with 4-nitrobenzoic hydrazide (4-NH) and characterized by spectroscopic techniques. Results show that the ligand is coordinated to metallic ions by the basic nitrogen of NH_2 group and have the general structure *cis*-$[M(4\text{-}NH)_2X_2]$ where M= Pt or Pd and x = Cl or I. The compound **III**, $[Pt(4\text{-}NH)_2I_2]$, was found to display cytotoxicity (IC_{50} = 0.96 μmol L^{-1}) against the K562 tumoral cell line. This complex is significantly more cytotoxic than cisplatin.

Keywords: *Platinum complexes • Palladium complexes • Hydrazones • Cytotoxic activity*

1. Introduction

Cisplatin, *cis*-$[Pt(NH_3)_2Cl_2]$, is one of the most important chemotherapeutic agents used in the treatment of a wide variety of solid tumors. Despite the important contribution of cisplatin in cancer therapy, its use presents some limitations such as development of resistance and severe side effects, such as, nephrotoxicity, ototoxicity and neurotoxicity [1,2]. These considerations have stimulated the search for new platinum complexes to be used as anticancer agents. Thus, in an attempt to reduce toxicity and widen the spectrum of activity of cisplatin and its analogous, thousands of platinum complexes have been prepared by varying the nature of the leaving groups and the carrier ligands [1-3]. The use of different types of organic ligands can modify the biological activity of the complexes and, for this reason, the use of hydrazides is very interesting.

The properties of hydrazides are of interest due to their biological activities and their use as metal extracting agents. The formation of metal complexes plays an important role in the growth of their biological activity [4-7]. Hydrazides successfully provide various active potential donor sites (C=O, NH and NH_2) and, therefore, many coordination compounds containing hydrazides have been synthesized and characterized [8-11].

Hydrazides and their metallic complexes show fungicide and antibacterial activity [12-14]. Particularly, platinum complexes with hydrazides derived from benzoic acid showed strong growth inhibitory effect in leukemia cells in vitro, not verified with the free ligands [12].

In view of the biological activities of hydrazides and their metallic compounds, this paper reports on the synthesis and characterization of new complexes. The compounds were characterized by elemental analysis, NMR, IR and UV-Vis. The cytotoxic activity of the synthesized compounds was also evaluated.

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2. Experimental procedure

2.1. Starting materials

The reagents (4-nitrobenzoic hydrazide and metal salts) are commercially available (Aldrich). All other reagents chemicals were of analytical grade, purchased from different sources, and used without further purification.

2.2. Physical measurements

Conductivity studies were carried out with a Digimed DM 31 conductivity meter using a cell of constant 1.00 cm^{-1}, spectroscopic grade dimethylformamide (Merck) (Λ_M = 1.20 μs cm^{-1}) and tetraethylammonium bromide (Λ_M = 78.39 μs cm^{-1}) as a standard.

Elemental analyses were performed at *Central Analytical* of the University of São Paulo, using a Perkin-Elmer 2400 CHN Elemental Analyser.

IR spectra were registered in KBr pellets on a Shimadzu FTIR-Irprestige-21 spectrometer.

A spectrophotometer UV-2501 PC Shimadzu was used for UV and visible absorption measurements.

NMR spectra were obtained using a Bruker Avance DPX 200 spectrometer with tetramethylsilane as an internal standard.

2.3. Synthesis of complexes

The complexes **I** and **II** were synthesized following the same general procedure, and we describe below only the synthesis of the complex **I** or $[Pt(4\text{-}NH)_2Cl_2]$. 0.2075 g of K_2PtCl_4 (0.5 mmol) previously dissolved in water was added to 5 mL of an methanolic solution of 4-nitrobenzoic hydrazide (1.0 mmol), and the mixture was stirred for 24 h. The solid formed was separated by filtration, washed with water, and dried under a vacuum. For the complex **III** or $[Pt(4\text{-}NH)_2I_2]$, a K_2PtI_4 solution was obtained by mixing K_2PtCl_4 (0.415 g in 5 mL H_20) and KI (0.67 g in 5 mL H_20) for twenty minutes. After this time, 5 mL of a methanolic solution of 4-nitrobenzoic hydrazide (1.0 mmol) was added, and the mixture was stirred for 24 h.

2.3.1. Complex I - $[Pt(4\text{-}NH)_2Cl_2]$

Yield: 82%. Color: Brown. Anal. Calcd. for $[Pt(C_7N_3O_3H_7)_2Cl_2]$: C, 26.76; H, 2.22; N, 13.37%; Found: C, 26.74; H, 2.43; N, 13.10%. IR spectra in KBr, v (cm^{-1}): 3296, 3175, 3090, 1670, 1602, 1570, 1484, 1340, 1296, 1234, 1117, 1020, 962, 946, 868, 850, 713, 564, 505, 458, 423.

RMN de ^1H (200 MHz; DMSO-d_6): δ 7.88 (s, 2H, $-NH_2$); δ 8.08 and 8.33 (2d, 4H, HAr); δ 11.23 (s, H, -NH).

^{13}C NMR (100 MHz DMSO-*d6*): 124.3, 129.50, 137.46, 149.69 (CAr); □ δ 164.27 (C=O)

2.3.2. Complex II - $[Pd(4\text{-}NH)_2Cl_2]$

Yield: 87%. Color: Yellow. Anal. Calcd. for $[Pd(C_7N_3O_3H_7)_2Cl_2]$: C, 31.20; H, 2.59; N, 15.56%; Found: C, 30.98; H, 2.74; N, 15.26%. IR spectra in KBr, v (cm^{-1}): 3309, 3209, 3111, 3072, 1656, 1600, 1537, 1352, 1327, 1315, 1296, 1260, 1117, 1020, 962, 946, 877, 854, 715, 541, 516, 492, 426.

RMN de ^1H (200 MHz; DMSO-d_6): δ 7.08 (s, 2H, $-NH_2$); δ 8.06 and 8.29 (2d, 4H, HAr); δ 10.74 (s, H, -NH).

2.3.3. Complex III - $[Pt(4\text{-}NH)_2I_2]$

Yield: 91%. Color: Brown. Anal. Calcd. for $[Pt(C_7N_3O_3H_7)_2I_2]$: C, 20.72; H, 1.72; N, 10.35%; Found: C, 21.08; H, 1.67; N, 10.40%. IR spectra in KBr, v (cm^{-1}): 3301, 3277, 3181, 3105, 3078, 1669, 1600, 1570, 1523, 1484, 1347, 1326, 1298, 1234, 1117, 1020, 962, 946, 868, 850, 716, 537, 504, 457, 417.

2.4. Cells and culture

The K562 cell line was purchased by the Rio de Janeiro Cell Bank (number CR083 of the RJCB collection). This cell line was established from pleural effusion of a 53 year-old female with chronic myelogenous leukemia in terminal blast crisis. Cells were cultured in RPMI 1640 (Sigma Chemical Co.) medium supplemented with 10% fetal calf serum (CULTILAB, São Paulo, Brazil) at 37 °C in a humidified 5% CO_2 atmosphere. Cultures grow exponentially from 10^5 cells mL^{-1} to about 8×10^5 cells mL^{-1} in three days. Cell viability was checked by Trypan Blue exclusion. The cell number was determined by Coulter counter analysis.

For cytotoxicity assessment, 1×10^5 cells mL^{-1} were cultured for 72 hours in the absence and presence of a range of concentrations of tested compounds. The sensitivity to compound was evaluated by the concentration that inhibits cell growth by 50%, IC_{50}. Stock solutions were prepared in DMSO/H_2O using a minimum amount of DMSO.

3. Results and discussion

Three new complexes containing 4-nitrobenzoic hydrazide (4-NH) were synthesized by the slow addition of the ligand to K_2PdCl_4, K_2PtI_4 or K_2PtCl_4, previously dissolved in water. After 24 hours, the resulting compounds were isolated by simple filtration and characterized by elemental analysis, IR, UV-Vis and NMR. The chemical structures of the ligand and its platinum complex containing chloride are presented in Fig. 1.

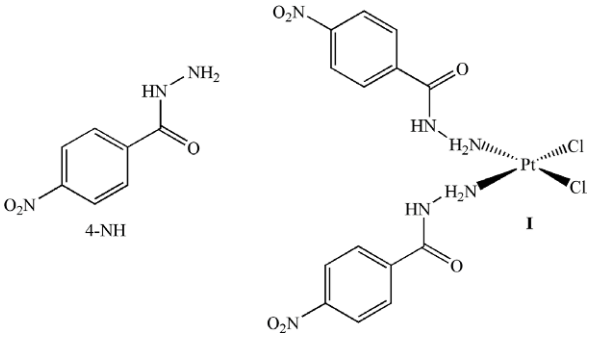

Figure 1. 4-NH and the structure proposal for complex **I**.

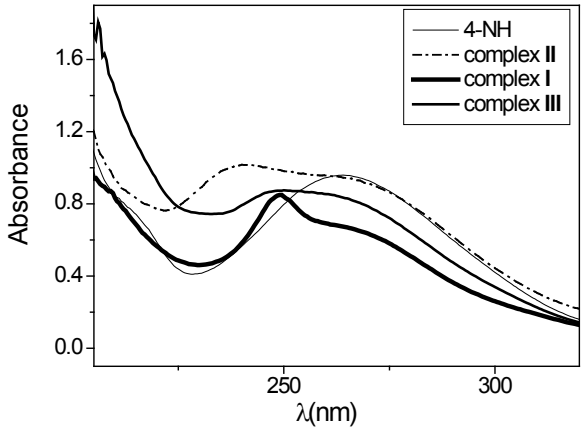

Figure 2. UV-Vis spectra of the hydrazide ligand and their complexes (acetonitrile; 1.0×10^{-5} mol L^{-1}).

The results of the elemental analysis are in accordance with the proposed structures.

The molar conductivity values of 10^{-3} mol L^{-1} solutions for all complexes were far below that of the 1:1 standard electrolyte indicating that they are not charged [15]. The same pattern was found when the complexes were dissolved in a mixture containing water/DMSO, even after 30 minutes. This result indicates that the complexes are stable in solution.

3.1. IR spectra

The IR spectrum of the free ligand (4-NH) was performed just for comparison to the corresponding complexes isolated. The characteristic absorptions in the 3330 to 3000 cm^{-1} region were observed, corresponding to νNH_2, νNH and νCH(aromatic). In addition, the spectrum of the ligand exhibited one band at 1645 cm^{-1} assigned to $\nu C{=}O$ [11].

The IR spectra of the complexes showed the absorptions corresponding to νNH around 3300 and 3100 cm^{-1}. The peak νNH_2 in the free ligand shifted towards lower wavenumbers in the complexes. Thus, it is possible to ascertain the coordination of the nitrogen of the NH_2 group to metal. The position of the νNH band remained virtually unaltered in the complexes excluding the coordination of the ligand through this group. In the IR spectra of the complexes, the $\nu C{=}O$ of the carbonyl appears almost in the same wavenumber of the ligand, therefore, we ruled out an involvement of this group in the coordination to the metallic ion.

Some of the weak bands in the range of 530-515 cm^{-1} in the spectra of the complexes could tentatively be ascribed to M-N stretchings. For the complexes **I** e **II**, two new absorptions in the region of 325 and 333 cm^{-1} may be assigned to ν(M-Cl) stretching in accordance to *cis* geometry [16].

The remaining characteristic bands and signals of the free ligand were not affected by metal coordination, and these results suggest that the ligand was bonded to the metal ions *via* the NH_2 group.

3.2. Electronic spectra

The spectra in the UV-visible regions in acetonitrile (Fig. 2) was analyzed to confirm the metal binding to the ligand.

The UV-visible absorption spectrum of the ligand 4-NH exhibits one band centered at 264 nm. The electronic spectra of all the complexes exhibited two or three peaks in the ultraviolet region. The splitting observed in the spectral of the complexes is consistent with the coordination of the ligand to the metal. The bands below 300 nm are assignable to intraligand $\pi\rightarrow\pi^*$ or $n\rightarrow\pi^*$ transitions. For complex **III**, the band maximum around 380 nm was tentatively assigned to metal-to-ligand charge-transfer based on bands previously observed for similar complexes [17].

3.3. NMR spectra

The ^1H NMR spectra of the complexes containing chloride were recorded in d_6-DMSO. In the spectrum of the free 4-NH, the NH_2 protons appear as a singlet at δ 4.65. This signal has been shifted downfield in the complexes (δ 7.88 for complex **I** and δ 7.08 for **II**) indicating the coordination of the NH_2 nitrogen to metals platinum and palladium. All the complexes show a singlet near δ 11.23 ppm due to the NH proton, suggesting a neutral nature of the ligand [11]. The signals of the NH protons were much less affected when compared to the group NH_2 protons excluding the participation of this group in the coordination. The resonances related to the aromatic protons were not affected by the addition of the metal ion. This pattern confirms that binding occurs in the NH_2 group.

Complex **I** is very soluble in DMSO, therefore, its ^{13}C NMR spectrum was realized. The signal at δ 164 is attributable to the carbonyl group. This signal remained virtually unaltered in the complex excluding

Table 1. IC_{50} values for the complexes and free ligand.

Compound	IC_{50}[a] (μM ± s.d.)
4-NH	10.5 ± 0.52
$[Pt(4\text{-}NH)_2Cl_2]$	3.8 ± 0.19
$[Pd(4\text{-}NH)_2Cl_2]$	77.0 ± 3.90
$[Pt(4\text{-}NH)_2I_2]$	0.96 ± 0.04
Cisplatin	4.7 ± 0.3[b]

[a]*IC_{50} is the concentration required to inhibit 50% of cell growth, after 3 days of incubation. The values are the mean of triplicate determinations.*
[b]*Value from [25].*

the coordination of the ligand through the C=O group. Signals in the δ□124 to 165 regions, due to the aromatic carbons were observed.

Thus, considering the spectral results, we propose the coordination of metals ions *via* NH_2 group. The same coordination mode here proposed was observed in previous works for some platinum complexes containing hydrazides as ligands [12,18-21].

3.4. Cytotoxic studies

The cytotoxic efficacy of the ligand 4-NH and their complexes was examined on K562 cells (Table 1). The result obtained for cisplatin was included for comparison. All compounds inhibit the growth of K562 cells with IC_{50} values between 0.96 and 77.0 μM. Concerning the effect of the metallic ions, the following order of increasing activity was established: palladium complex < platinum complexes. As expected, the platinum complexes tested were found more potent than the palladium analog and this finding is consistent with other studies conducted [22-24].

The most important result is that Pt(II) coordination to 4-NH improves the cytotoxic activity in the K562 cells, since, the activity of complex **III** is 10-fold higher than of the corresponding free ligand and 5-fold higher than cisplatin. The complex **I** too was more active than hydrazide free and cisplatin. These findings are very important and make these compounds candidates for further studies.

4. Conclusion

Three new complexes containing hydrazides were prepared and characterized, and the spectroscopic techniques showed that the ligand is coordinated to platinum or palladium by the basic nitrogen of NH_2 group and have the general structure *cis*-$[M(4\text{-}NH)_2X_2]$ where M= Pt or Pd and x = Cl or I.

The cytotoxic activity of the ligand 4-NH and its complexes was examined on K562 cells. As a whole, the complexes of platinum are more cytotoxic than the free ligand and, among the complexes examined; the complex **III** exhibited the most promising anticancer activity against K562 cells. In direct comparison with cisplatin, the cytotoxic activity of platinum complexes containing 4-NH ligand is significantly higher, therefore mechanistic studies and cytotoxicity of these complexes in other cell lines will be investigated in the future.

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