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Short communication

Synthesis and cytotoxic evaluation of the first *trans*-palladium(II) complex with naturally occurring alkaloid harmine

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Abstract – Treatment of *trans*-[$Pd(DMSO)_2Cl_2$] with naturally occurring alkaloid harmine, in a 1:1 molar ratio in DMSO, leads to the formation of *trans*-[Pd(DMSO)(harmine)Cl_2]. The latter was isolated from DMSO solution by addition of 0.05 M HCl solution. The complex has been characterized physicochemically and spectroscopically. It was tested for its cytotoxicity against P388, L_{1210} and K_{562} cell lines, and showed promising activity, as a first *trans*-palladium(II) complex, when compared with the reference standards cisplatin, carboplatin and 5-fluororuracil (5-FU). © Elsevier, Paris

trans-palladium(II) /harmine / complex / cytotoxicity

1. Introduction

Platinum complexes and, to a much lesser extent, palladium complexes have received considerable attention since the discovery of Rosenberg [1] that *cis*-[$Pt(NH_3)_2Cl_2$] (cisplatin) has a potent antitumour activity. A large body of platinum complexes has been tested for their antitumour activities with mixed success. Hence [$Pt(NH_3)_2\{O_2(CO)_2C\text{-}CH_2CH_2CH_2\}$] (carboplatin) and [$Pt(DACH)C_2O_4$] (oxaliplatin), DACH is (1R,2R)-1,2-cyclohexanediamine, have been discovered to have antitumour activity and they are under use clinically as second generation analogues of cisplatin [2, 3]. Current research on platinum complexes and chemotherapy was covered recently by at least two review articles [4, 5]. During the last ten years, we have been engaged in the synthesis and characterization of metal complexes and their biological evaluation, especially as anti-tumour agents. Our studies have mainly focused on the use of some naturally occurring nitrogen-containing ligands, e.g. β-carboline alkaloids [6–10]. Part of this work was presented at an international conference [11]. Ever since the discovery of antitumour activity of metal complexes, most studies have concentrated on platinum complexes rather than other metal complexes; furthermore, most work has been done on *cis*-isomers and only very rarely on *trans*-isomers [12, 13]. This was due to biological aruguments concerning DNA linkage with the complex used [14].

Because of this, and as a continuation of our intrinsic interest on such complexes, we present here the synthesis of the first *trans*-palladium(II) complex with 7-methoxy-1-methyl-9H-pyrido[3,4-b]indole (harmine), the naturally occurring β-carboline alkaloid (isolated from *Peganum harmala* seeds) and its cytotoxic evaluation against the three cell lines P388, L_{1210} and K_{562}. As far as the available literature is concerned this work is novel.

2. Chemistry

The starting material *trans*-[$Pd(DMSO)_2Cl_2$] (**1**) was prepared as described in the experimental protocols. The preparation of this complex and its reaction with harmine are summarized in *figure 1*. Complex (**2**) was isolated

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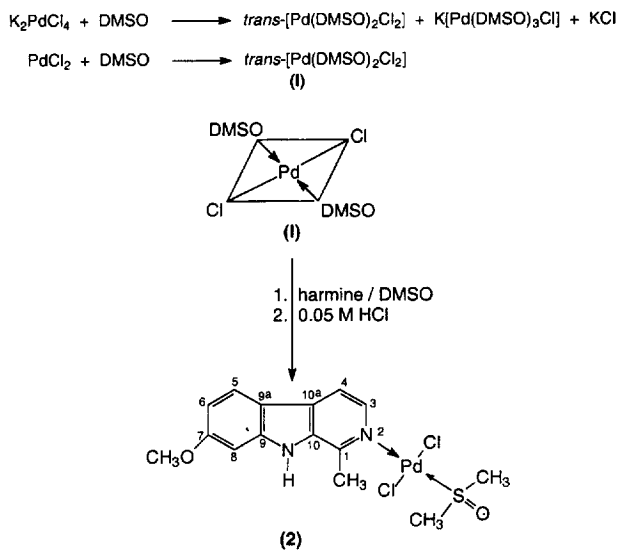

Figure 1.

from its DMSO solution and purified as described in the experimental protocols. Its physical properties are listed in *table I*.

In our previous report on the properties and structures of platinum(II) and palladium(II) complexes of β-carboline alkaloids, we have shown that monomer or dimer species may be formed [6, 15]. In the present work, the monomer *trans*-[Pd(DMSO)(harmine)Cl_2] (**2**) was formed by displacement of one DMSO from the starting complex (**1**) by harmine, as shown by analyses and spectral data (*table I*). The coordination of harmine with Pd in complex (**2**) most probably takes place via N-pyridine, as this lone pair of electrons is more available for coordination than that of N-indole, due to geometrical reasons. In NMR experiments in DMSO-d_6 solution, the δ (N–H) of free harmine (11.4 ppm) remains almost unchanged upon coordination with Pd (11.9 ppm), while the chemical shifts of the carbons adjacent to N-pyridine, i.e. C-1 (141.9 ppm) and C-3 (141.2 ppm) for free harmine were found to shift markedly to a lower field, i.e. 143.7 and 142.9 ppm, respectively, upon coordination with Pd.

Furthermore, complex (**2**), and the starting material (**1**), both have the *trans*-configuration with DMSO coordinated with Pd via sulphur, as shown by IR spectral data (*table I*); the ν (S=O) band appearing at 1130 cm^{-1} was assigned clearly to Pd–S coordination. The clear single absorption band appearing at 328 cm^{-1} due to ν (Pd–Cl) for complex (**2**), was assigned to a *trans*-complex. If it were a *cis*-complex, two very well defined bands should appear [6–10, 16]. Additional support to the *trans*-configuration of complex (**2**) is the single band appearing at 425 cm^{-1} which was assigned to ν (Pd–S). These data compare with those of complex (**1**), i.e. 1123, 360 and 420 cm^{-1} for ν (S=O), ν (Pd–Cl) and ν (Pd–S), respectively, indicating that the *trans*-configuration is retained in complex (**2**) (*figure 1*).

3. Biological investigation and discussion

The cytotoxicity of complex (**2**) was performed using P388, L_{1210} and K_{562} cells and the results, in terms of IC_{50}, were compared with those of the reference standards cisplatin, carboplatin and 5-fluorouracil. The results are summarized in *table II*.

Table I. Physical properties and analyses of complex (**2**).

Method of analysis	Results
Colour	Yellow–orange
M.p. (°C)	190–192 with decomposition
IR (cm^{-1}) [a]	ν (N–H) = 3294 s; ν (C–H) = 2927, 3013 m; ν (C=C) = 1624 s; ν (C=N) = 1573 m; ν (S=O) = 1130 s; ν (Pd–Cl) = 328 m; ν (Pd–S) = 425 m
Elemental analysis	Found (%): C = 38.4, H = 3.8, N = 6.1; calculated (%) for $C_{15}H_{18}N_2O_2Cl_2SPd$: C = 38.5, H = 3.85, N = 6.0
Mass spectrum [b]	*m/z* = 467, 433, 400, 293, 213, 140, 105, 90
^1H NMR [c]	δ (CH_3) = 3.3 s (3H); δ (CH_3O) = 3.9 s (3H); δ (NH) = 11.9 b (1H); δ (HC-3) = 8.7 d (1H); δ (HC-4) = 8.3 d; δ (HC-5) = 6.9 [d]; δ (HC-6) = 8.0 d (each 1H, *J* = 6 Hz); δ (HC-8) = 7.1b (1H)
^{13}C NMR [c]	δ (C-1) = 143.7, δ (C-3) = 142.9, δ (C-4) = 113.6, δ (C-5) = 114.5, δ (C-6) = 111.3, δ (C-7) = 161.9, δ (C-8) = 95.0, δ (C-9) = 140.0, δ (C-9a) = 135.7, δ (C-10) = 129.0, δ (C-10a) = 124.0, δ (CH_3) = 22.5, δ (CH_3O) = 56.0

[a] Selected IR bands; s: strong, m: medium bands; [b] FAB technique used with cold inlet, using 3-NBA as a solvent; molecular ion *m/z* = 467.4; [c] downfield from internal TMS at room temperature, using DMSO-d_6 as a solvent; s: singlet, d: doublet, b: broad; [d] this is a poorly resolved doublet of doublet signal.

Table II. Cytotoxic activities of complex (**2**) with standard references against different tumour cell lines.

Compound	IC_{50} μ (M)		
	P388	L_{1210}	K_{562}
(**2**)	0.385	0.385	0.364
Cisplatin	0.500	0.833	20.0
Carboplatin	> 27.0	> 27.0	> 27.0
5-FU	1.15	1.15	Not tested

From a first glance at these results, it appears that complex (**2**) shows a very significant potency in vitro against the three cell lines. It is better than that of cisplatin, 5-FU and much better than carboplatin. Further biological evaluations are required to confirm these results in animal models and continuing biological work on complex (**2**) is in progress in our laboratories.

4. Experimental protocols

Melting points (°C, uncorrected) were recorded on Stuart Scientific apparatus (UK). ^1H and ^{13}C NMR spectra were recorded at the University of Jordan, Amman, Jordan, on a Bruker-DPX300 MHz spectrometer, using DMSO-d_6 as a solvent with TMS as an internal standard. IR spectra were recorded on a Nicolet (Impact 400) FT-IR spectrometer, using KBr discs in the range $4000 - 400\ cm^{-1}$ or Cs discs in the range $4000 - 200\ cm^{-1}$ on SP2000 Pye Unicam spectrophotometer. Microanalytical data (C,H,N) were performed at the Atlantic Microlab Inc., Norcross, Georgia, 30091 (USA). The mass spectrum of complex (**2**) was recorded at Sussex University Brighton,UK, using FAB technique with cold inlet and 3-nitrobenzoylalcohol (3-NBA) as solvent. Starting materials K_2PdCl_4, $PdCl_2$ and harmine were purchased from Fluka and used without further purification. The tumour cells P388, L_{1210} and K_{562} were kindly obtained from Dr. Rees, Hallamshire Hospital, Sheffield, UK.

4.1. Chemistry

4.1.1. trans-[Pd(DMSO)$_2$Cl$_2$] (1)

This complex can be prepared as described by Price et al. [16], but the following method is superior. The salt $PdCl_2$ (2.0 g, 11.3 mmol) was suspended in DMSO (10 mL) and the suspension was heated at ca. 90 °C for 2 h until complete dissolution was achieved. The reaction mixture was filtered and the filtrate was cooled to room temperature and treated with HCl solution (0.05 M) until complete precipitation. The orange solid thus formed was filtered and washed with small portions of cold water, cold ethanol and ether, and dried under vacuum for several hours. The yield was 3.5 g (93%).

4.1.2. trans-[Pd(DMSO)(harmine)Cl$_2$] (2)

The complex *trans*-$[Pd(DMSO)_2Cl_2]$ (1.5 g, 4.5 mmol) was dissolved in DMSO (20 mL) with gentle heating and harmine (0.96 g, 4.5 mmol) was added gradually with vigorous stirring. The clear yellow–orange solution thus formed was allowed to stand at ca. 60 °C for ca. 30 min to ensure complete reaction. After cooling the solution to room temperature, cold HCl solution (0.05 M) was added drop-wise until complete precipitation. The solid product was collected by filtration, washed with small portions of water, acetone and ether and dried under vacuum at 80 °C for several hours. The yield was 1.8 g (80%).

4.2. Cytotoxicity assay

The cells P388, L_{1210} and K_{562} (2×10^4 cells/well) were cultured in corning disposable 96-well plates with 96-well plates containing 100 μL of RPMI-1640 medium supplemented with 5% fetal calf cerum [17, 18]. Various complex (**2**) concentrations (0.1, 1.0 and 10.0 μg/mL) in 10% DMSO were added to the cultures at day one after seeding. At day three, 20 μL of MTT solution (5 mg/mL) per well was added to each culture medium. After a further 4 h of incubation, 100 μL of 10% SDS–0.01 N HCl solution was added to each well and the formozan crystals in each well were dissolved by stirring with a pipette. The optical density measurements were made by using a microplate reader (Tohso MPR-A4i) with the two wavelength systems (550 and 700 nm). In all of these experiments, three replicate wells were used to determine each point.

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