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Short communication

Synthesis, crystal structure and initial biological evaluation of the new enantiomerically pure chiral palladium(II) complex *trans*-bis{endo-(1*R*)-1,7,7-trimethylbicyclo[2.2.1]-heptan-2-amino}palladium(II)dichloride

Adnan S. Abu-Surrah [a,*], Talal A.K. Al-Allaf [b], Luay J. Rashan [b], Martti Klinga [c], Markku Leskelä [c]

[a] *Department of Chemistry, Hashemite University, P.O. Box 150459, Zarqa 13115, Jordan*
[b] *Applied Science University, Amman 11931, Jordan*
[c] *Department of Chemistry, Laboratory of Inorganic Chemistry, University of Helsinki, P.O. Box 55, FIN-00014, Helsinki, Finland*

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Abstract

The palladium(II) complex *trans*-bis{(*R*)-(+)-bornylamino}palladium(II) dichloride was synthesised and characterised. The solid state structure of the complex was determined by X-ray structure analysis. The compound crystallises in the monoclinic space group $P2_1$ with $a = 12.383(2)$, $b = 23.689(5)$, $c = 12.769(3)$ Å, $\beta = 93.25(3)^\circ$, and $V = 3739.6(13)$ Å^3. The complex was tested for its cytotoxicity against L_{929}, K_{562} and HeLa cell lines using the MTT assay technique. It is also tested for its anticomplementary activity using a test that detects complement proteins inhibition. These activities were compared with those of the reference standards, cisplatin, carboplatin and oxaliplatin. The significance of these results is given and discussed.

Keywords: *trans*-Palladium(II); (*R*)-(+)-Bornylamine; Ligand; Complexes; Cytotoxicity; Anticomplementary

1. Introduction

The notable analogy between the coordination chemistry of platinum(II) and palladium(II) compounds has advocated studies of Pd(II) complexes as antitumour drugs [1]. Recent advances in this field have focused on Pd(II) compounds bearing diamino [2] or mixed aminoether [3] bidentate ligands. The complexes tested up to know have been either of marginal antitumour activity or unsuccessful, probably due to their higher lability (about 10^5-fold) versus platinum analogs [4,5].

In our previous article [6] we showed that *trans*-[Pd(DMSO)(harmine)Cl_2] has a promising cytotoxic activity against P388, L_{1210} and K_{562} cell lines as a first *trans*-palladium complex with a bulky monodentate amine ligand. This was followed by our discovery that another *trans*-palladium complex, i.e. *trans*-[Pd(pyrazole)$_2Cl_2$] (pyrazole = 1,3-dimethylpyrazole) showed a significant activity against P388, HeLa and RD cell lines [7]. These and other results have been reviewed recently by us as a comparative study between *cis*- and *trans*-complexes [8].

Very recently, we have reported the anticomplementary activities of several platinum and palladium complexes in both the *cis*- and *trans*-forms and to relate these activities to their structures and their cytotoxicities [9]. In the present study, we are presenting the synthesis and molecular structure of a new enantiometrically pure, chiral *trans*-Pd(II) complex with the bulky amine ligand, *endo*-(1*R*)-1,7,7-trimethylbicyclo[2-2-1]-heptan-2-amine {(*R*)-(+)-bornyl-amine}, its cytotoxic evaluation against the three cell lines L_{929}, K_{562} and HeLa, and its anticomplementary activity. As far as we know this work is novel.

* Correspondence and reprints
E-mail address: asurrah@hu.edu.jo (A.S. Abu-Surrah).

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2. Chemistry

The complex *trans*-[Pd{(*R*)-(+)-bornylamino}$_2$Cl$_2$] (**1**) was prepared as describedin Section 4. It crystallises from acetone as yellow crystals having the empirical formula $C_{20}H_{38}Cl_2N_2Pd\cdot 2/3C_3H_6O$. Its physical properties are as follows: melting point, 230 °C (decomposition). Anal. Found: C, 50.45; H, 8.35; N, 5.48. Calc. for $C_{22}H_{42}Cl_2N_2O_{0.67}Pd$: C, 50.56; H, 8.04; N, 5.36%. Mass spectrum (EI): *m/e* (relative intensity) 447 (50.0%, $[M^+ - Cl]$), 410 (30.0%, $[M^+ - 2Cl]$).

2.1. Crystal structure of complex *1*

Suitable crystals of the chiral Pd(II) complex *trans*-[Pd{(*R*)-(+)-bornylamino}$_2$Cl$_2$] (**1**) (Fig. 1) were obtained by crystallisation from acetone. The cell parameters and specific data collection parameters are summarised in Table 1.

Selected bond lengths and angles are given in Table 2. The palladium atom is at the center of square-planar arrangement with two chlorine atoms *trans*- to each other as clarified in Fig. 2. The compound crystallises in the monoclinic space group $P2_1$ with $a = 12.383(2)$, $b = 23.689(5)$, $c = 12.769(3)$ Å, $\beta = 93.25(3)°$, and $V = 3739.6(13)$ Å^3, $Z = 6$. Three crystallographically independent complex molecules and two acetone molecules, which were determined, are loosely connected by hydrogen bonds. In an H-bonding network most nitrogen atoms act as donors and coordinated chlorine atoms together with acetone oxygen atoms are behaving as acceptors stabilizing the solid state structure. All H-bonds are weak which can be seen from the numerical values (supplementary publication). In the solid state independent complex molecules have generally the same conformation, but owing to packing effects and H-bonding small differences exist.

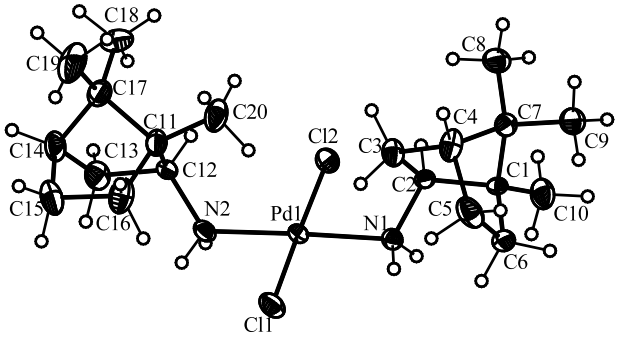

Fig. 1. One of three independent complex molecules (**1**) with atomic numbering scheme. Acetone molecules omitted. Thirty percent probability ellipsoids depicted. Hydrogen atoms on an arbitrary scale.

Table 1
Crystallographic data and parameters for data collection and refinement of complex **1**·2/3C_3H_6O

Empirical formula	$(C_{20}H_{38}Cl_2N_2Pd)\cdot 2/3(C_3H_6O)$
Formula weight	522.54
T (K)	193(2)
Crystal system	monoclinic
Space group	$P2_1$ (no. 4)
a (Å)	12.383(2)
b (Å)	23.689(5)
c (Å)	12.769(3)
α (°)	90
β (°)	93.25(3)
γ (°)	90
V (A^3)	3739.6(13)
Z	6
D_{calc} (Mg m^{-3})	1.392
Absorption coefficient (mm^{-1})	0.972
$F(000)$	1640
Crystal dimensions (mm)	0.26 × 0.24 × 0.22
θ Range (°)	2.51–25.0
Scan mode	ω–2θ
Wavelength	Mo Kα
Data collected	7075
Reflections observed (criterion)	5905 [$F > 4\sigma(F)$]
Unique data	6750 [$R_{int} = 0.0425$]
Data refined	6750
Parameters	748
R_1 [a] [$F > 4\sigma(F)$]	0.0429
wR_2 [b] (all data)	0.0980
Goodness-of-fit on F^2	1.039

[a] $R_1 = \Sigma\ ||F_o| - |F_c||/\Sigma\ |F_o|$.
[b] $wR_2 = [\Sigma\ [w(F_o^2 - F_c^2)^2]/\Sigma\ [w(F_o^2)^2]]^{1/2}$.

Table 2
Selected bond length (Å) and angle (°) ranges for three independent complex molecules, $C_{20}H_{38}Cl_2N_2Pd$ (**1**)

Bond length ranges		Angle ranges	
Pd–N	2.028(7)–2.051(7)	N–Pd–N	176.2(3)–178.4(3)
Pd–Cl	2.281(3)–2.308(2)	N–Pd–Cl	87.7(2)–92.5(2)
N–C	1.466(10)–1.510(11)	Cl–Pd–Cl	177.88(9)–178.31(9)

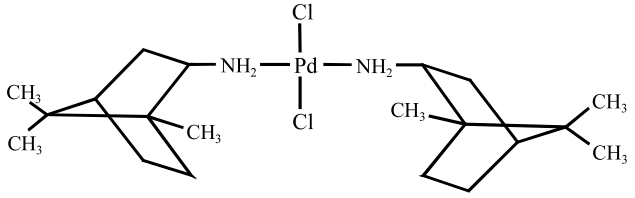

Fig. 2. Drawing of the structure of complex **1** for clarity.

3. Biological investigation and discussion

In both human cell lines K_{562} and HeLa, complex **1** showed some activity where the IC_{50} values of 55.9 and 86.1 μ(M), respectively. The activity of complex **1** against K_{562} is less potent than the three standard

Table 3
Cytotoxic activities of complex (**1**) with standardreferences against different cell lines

Compound	IC_{50} μ(M)		
	L_{929}	K_{562}	HeLa
Complex **1**	> 111.9	55.9	86.1
Cisplatin	9.33	16.3	94.3
Carboplatin	> 134.8	32.9	104.3
Oxaliplatin	15.87	0.5	71.3

references (Table 3). However, it showed more or less similar activity against HeLa cells when compared with those of the three standard references cisplatin, carboplatin and oxaliplatin where their IC_{50} values are 94.3, 104.3 and 71.3 μ(M), respectively. On the other hand, as expected, complex **1** was far less potent against the murine fibrosarcoma cell line L_{929} compared to cisplatin and oxaliplatin, but it has better activity than carboplatin (Table 3). The relatively higher coordination capability of dimethylsulphoxide (DMSO), which was used as a solvent in the cytotoxicity test, to palladium metal center compared to platinum could be in a part responsible for the lower biological activity observed for the *trans*-palladium complex **1**.

On the contrary, complex **1** showed a little inhibition when tested for its anti-complementary activity and at a concentration of 2.24 μ(M) (Table 4). Whereas, the three standard references, cisplatin, carboplatin and oxaliplatin showed inhibition at a concentration less than 0.4 μ(M) (Table 4). Thus it seems rather very difficult at this stage to correlate between the cytotoxic and anticomplementary activities of this complex. Preliminary speaking it seems that complex **1** has a higher nonspecific toxicity than cisplatin and other platinum drugs used in this study.

4. Experimental

4.1. Chemistry

4.1.1. Materials

$[Pd(PhCN)_2Cl_2]$ was prepared according to literature procedures [10]. The free ligand: *endo*-(1*R*)-1,7,7-trimethyl-bicyclo[2.2.1]heptan-2-amine{(*R*)-(+)-bornylamine} was purchased from Aldrich. Acetone was dried over $CaSO_4$. Reagent grade chemicals were used as received unless otherwise stated. The standard references, cisplatin, carboplatin and oxaliplatin were prepared in our laboratories and compared with an authentic sample.

Table 4
Anticomplementary activities

Compound	IC_{50} μ(M)
Complex **1**	2.24
Cisplatin	< 0.333
Carboplatin	< 0.270
Oxaliplatin	< 0.252

4.1.2. Physical measurements

Elemental analyses were performed at the Pharmacology Department, University of Helsinki (EA 1110 CHNS-O CE instrument). Mass spectra (EI) were acquired with a JEOL JMS-SX102 mass spectrometer.

4.1.3. X-ray structure determinations for complex *1*

Crystal data of complex **1** was collected on a Rigaku AFC7S single-crystal diffractometer at 193(2) K using graphite monochromatised Mo Kα radiation (0.71073 Å). The intensities were corrected for Lp effects [11]. An experimental absorption correction (ψ-scan) was performed [12]. The structure was solved by direct methods (SHELX-97) [13]. All non-hydrogen atoms were refined anisotropically and hydrogen atoms isotropically on calculated positions. Final calculations were performed with SHELX-97 and SHELXTL/PC [14] program systems.

4.1.4. *trans*-[Pd{(*R*)-(+)-bornylamino}$_2$Cl$_2$] (*1*)

A filtered solution of the ligand (0.88 g, 5.74 mmol) in C_3H_6O (50 mL) was added to a solution of $[Pd(PhCN)_2Cl_2]$ (2.0 g, 5.21 mmol) in C_3H_6O (50 mL) with continuous stirring. Upon addition, a yellow solid was formed. After 5 h stirring, the precipitate was filtered, washed with C_3H_6O (2 × 5 mL), Et_2O (2 × 10 mL) and dried in vacuum. Yield: 2.1 g (83%). Yellow crystals suitable for X-ray analysis were formed by crystallisation from C_3H_6O.

4.2. Biology

4.2.1. Cell lines and culture

The human tumour cell lines K_{562} (myelogenous leukaemia) and HeLa (cervical carcinoma) and the murine fibrosarcoma L_{929} were used in the present study. Approximately, 3×10^6 cell of each of these cell lines were plated in T-75 plastic culture flasks in 10 mL of RPMI 1640 medium supplemented with 10% fetal calf serum (ICN-Flow Laboratories, UK), L-glutamine and antibiotics (140 μg mL^{-1} of streptomycin and 140 U mL^{-1} of penicillin), and incubated at 37 °C in an atmosphere of 5% CO_2 and 95% air.

4.2.2. Cytotoxicity tests

The MTT assay (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) was utilised to determine concentration-dependent inhibition of cell growth. Briefly this assay utilised MTT to quantify the translucence of the treated cells. Three different dilutions were

used from the complex; these are 0.5, 5 and 50 μg mL^{-1}. Three standard references (cisplatin, carboplatin and oxaliplatin) were also used and again three serial dilutions from these references were prepared (0.5, 5 and 50 μg mL^{-1}). All these compounds were dissolved in 10% DMSO prior to dilution in RPMI-1640 tissue culture medium. Three separate sets of controls containing the solvent (10% DMSO) were used in each plate. All the experiments were performed in triplicate. The IC_{50} μ(M) was calculated according to probit test.

4.2.3. Anticomplementary test

The inhibition of complementary activity was determined as described by Shahat et al. [15]. The tested Pd complex was purified according to established analytical methods. The complex and the standard references were dissolved in 10% DMSO and a serial concentrations (10, 1.0, 0.1, 0.001, 0.0001 μg mL^{-1}) were prepared. The assay was performed in a v-well microtiter plates. Rabbit complement (C901Virion/serion Immunodiagnostic GmbH) and hemolysing *anti*-sheep erythrocyte serum (C902Virion/serion Immunodiagnostic GmbH) was used as recommended by the manufacturer. 50 mL of the complement solution (diluted 1:50) was added to 50 μL of each sample concentration. After incubation at 37 °C for 30 min, 50 μL of a suspension of sensitised sheep erythrocytes were added to each well. Hemolysis was observed optically after an incubation at 37 °C for 60 min. Controls consisted of sensitised sheep erythrocytes incubated in buffer+DMSO (no hemolysis), with working solution complement (100% hemolysis), with 1:2 and 1:3 diluted working solution complement (partial hemolysis). Data were obtained as the results for duplicated samples. The IC_{50} values were calculated using the probit test.

5. Supplementary material

Crystallographic data for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Center, CCDC No. 182760. Copies of the data may be obtained on application to The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: +44-1223-336-033; e-mail: deposit@ccdc.cam.ac.uk or www: http://www.ccdc.cam.ac.uk).

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