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PALLADIUM(II) COMPLEXES WITH 5-METHYL-5-(4-PYRIDYL)-2,4-IMIDAZOLIDENEDIONE
Synthesis, thermogravimetric and cytotoxic investigation

Adriana Bakalova[1*], *H. Varbanov*[1], *R. Buyukliev*[2], *G. Momekov*[3] *and D. Ivanov*[1]

[1]Department of Chemistry, Faculty of Pharmacy, Medical University, Sofia, 2 Dunav Str., 1000 Sofia, Bulgaria
[2]Department of Organic Chemistry, Faculty of Pharmacy, Medical University, Sofia, 2 Dunav Str., 1000 Sofia, Bulgaria
[3]Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Pharmacy, Medical University, Sofia, 2 Dunav Str. 1000 Sofia, Bulgaria

Two new palladium(II) complexes with 5-methyl-5-(4-pyridyl)-2,4-imidazolidenedione(mpyh) were synthesized: *cis*-$[Pd(mpyh)_2Cl_2]\cdot H_2O$ and *cis*-$[Pd(mpyh)_2Br_2]\cdot 2H_2O$. The molecular formulae of the complexes were confirmed by elemental analysis, IR, ^1H NMR spectra and DTA study. The ligand is coordinated to the palladium ion with N-atom of the pyridine ring. The spectroscopic data indicate a square planar geometry with two N-pyridine atoms and two halogene anions in *cis* position. The final product of the thermal decomposition of *cis*-$[Pd(mpyh)_2Cl_2]\cdot H_2O$ is metallic Pd, whereas for *cis*-$[Pd(mpyh)_2Br_2]\cdot 2H_2O$ the residue consists of metallic Pd and C. The cytotoxic effects of the complexes were examined in vitro on some human tumor cell lines. The *cis*-$[Pd(mpyh)_2Cl_2]\cdot H_2O$ proved to be more active as compared to the *cis*-$[Pd(mpyh)_2Br_2]\cdot 2H_2O$.

Keywords: *cytotoxicity, hydantoins, Pd(II) complexes, synthesis, thermal analysis*

Introduction

The first transition metal to be used successfully as anticancer agent was platinum. It was used in the compound Cisplatin (*cis*-$[Pt(NH_3)_2Cl_2]$), and its ability to inhibit tumors was discovered by Rosenberg *et al.* in 1969 [1, 2]. Cisplatin is known as agent with strong anticancer potency [3]. It is widely used for the chemotherapy of diverse solid tumours such as head and neck, ovarian cancer, cervical cancer, urinary bladder cancer etc. [4, 5]. Despite its wide application as a therapeutic agent in chemotherapy, Cisplatin is associated with many serious side toxic effects, such as nephrotoxicity, othotoxicity, allergy, peripheral neuropathy, etc. [6].

However, later it was shown that not only platinum(II) complexes exhibited antitumor effects. Numerous planar and octahedral platinum complexes as well as compounds of other platinum group metals such as ruthenium, rhodium, aurium, titanium or palladium were characterized by cytotoxic activity [7–9]. Although platinum complexes are still among those most frequently studied in the search for new metal based drugs, the ruthenium(III) complexes also reach clinical level of trials [10]. Relatively few palladium(II) and palladium(IV) complexes have been investigated for their cytotoxic and antitumor activity [10–13].

Based on the structural analogy between Pt(II) and Pd(II) complexes, some studies on palladium compounds as suitable drugs have been carried out [14].

The present study represents the synthesis, physicochemical study and the pharmacological investigation of Pd(II) complexes with 5-methyl-5-(4-pyridyl)-2,4-imidazolidenedione *in vitro* as compared to the clinically applied drug Cisplatin and novel Pt(II) complexes with the same ligand [15].

Experimental

Materials

The ligand 5-methyl-5-(4-pyridyl)-2,4-imidazolidenedione was synthesized according previously described method [16]. $K_2[PdCl_4]$ utilized for the synthetic procedures was purchased from Fluka. All of the other chemicals were of analytical grade.

Preparation of cis-dichloro-bis(5-methyl-5-(4-pyridyl)-2,4-imidazolidenedionepalladium(II) monohydrate – *cis*-$[Pd(mpyh)_2Cl_2]\cdot H_2O$

Two water solutions of $K_2[PdCl_4]$ and 5-methyl-5-(4-pyridyl)-2,4-imidazolidenedione(mpyh) were prepared for the synthesis of the complex *cis*-$[Pd(mpyh)_2Cl_2]\cdot H_2O$ **(1)**. The solution of the ligand (0.1164 g, 0.6094 mmol) was added dropwise to the water solution of $K_2[PdCl_4]$ (0.1005 g, 0.3079 mmol)

* Author for correspondence: adrigebk@abv.bg

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Akadémiai Kiadó, Budapest, Hungary
Springer, Dordrecht, The Netherlands

at constant stirring and after the addition of the ligand the homogenous solution was stirred for 1–2 h. A bright-yellow product was obtained, which was filtered, washed several times with water and dried in a vacuum desiccator. The substance is soluble in DMSO and weakly soluble in water and ethanol. The purity is checked up by thin layer chromatography. Yield: ca. 82%, m.p.: >320°C (dec.).

^{1}HNMR (DMSO-d6): 11.08 (NH-3), 8.78 (2H, py), 8.59 (NH-1), 7.65 (2H, py), 1.68 ppm (3H, s).

Preparation of cis-dibromo-bis(5-methyl-5-(4-pyridyl)-2,4-imidazolidenedione)palladium(II) dihydrate – *cis*-$[Pd(mpyh)_2Br_2]\cdot 2H_2O$

The complex *cis*-$[Pd(mpyh)_2Br_2]\cdot 2H_2O$ **(2)** was prepared according to a reported procedure [17]. 0.1007 g $K_2[PdCl_4]$ (0.3085 mmol) was mixed with a saturated solution of potassium bromide (in excess) (0.1906 g) and heated on a water bath for 5 min, thus $K_2[PdCl_4]$ was quantitatively converted into a solution of $K_2[PdBr_4]$. To this mixture 0.1173 g (0.6141 mmol) of the ligand were added. The solution was stirred for 1 h. Brown crystals were obtained and filtered. The complex is weakly soluble in DMSO. The purity is checked up by thin layer chromatography. Yield. ca. 97%, m.p.: >290°C (dec.).

^{1}HNMR (DMSO-d6): 11.08 (NH-3), 8.84 (2H, py), 8.77 (NH-1), 7.63 (2H, py), 1.68 ppm (3H, s).

Instrumentation

The carbon, nitrogen and hydrogen content of the compounds were determined by elemental analysis. The elemental analysis was carried out on an Elementar Analysensysteme GmbH VarioEl. The results were within ±0.5% of the theoretical values.

The IR spectra were recorded on IR IFS 113 v. Bruker FTIR spectrophotometer in the range of 4000–400 cm^{-1} as KBr tablets and on FTIR-GX PerkinElmer in the range of 400–220 cm^{-1} as CsI tablets. The ^{1}H-NMR spectra were registered on a Bruker WM 250 (250 MHz) spectrometer in DMSO-d6. Corrected melting points were determined using a Bushi 535 apparatus. Thermal analysis were performed on a C.MOM thermal analyzer (Budapest, Hungary) with a simultaneous DTA-TG module using the following conditions: sample mass 50 mg, heating range 20–900°C (293–1173 K), heating rate 5°C min^{-1}, air atmosphere.

In vitro assays; chemicals, solutions and other materials

The cell culture flasks and the 96-well microplates were obtained from NUNCLON (Denmark). MTT, FCS and Cisplatin was purchased from Sigma Co. The stock solutions of tested compounds (20 mM) were freshly prepared in DMSO, and stored at 4°C, protected from light for a maximum period of 1 week. The serial dilutions of the tested compounds were prepared immediately before use. At the final dilutions obtained the concentrations of DMSO never exceeded 1%.

Cell lines and culture conditions

The cell line HL-60 (acute promyelocyte leukemia) was supplied from DSMZ GmbH, Germany. SKW-3 human T-cell leukemia was supplied from DSMZ GmbH, Germany and established from peripheral blood of 61-year-old man with T-cell lymphocytic leukemia.

Cells were cultured routinely in a controlled environment: 37°C in 5% CO_2 humidified atmosphere. They were maintained in RPMI 1640 growth medium supplemented with 2 mM L-glutamine and 10% fetal calf serum. HL-60 and SKW-3 cells were subcultured twice weekly to maintain continuous logarithmic growth.

Pharmacology

Cytotoxicity assay

Cell survival was evaluated by using the MTT-dye reduction assay, which is based on the ability of viable cells to metabolize a yellow tetrazolium salt to a violet formazan product that can be detected colorimetrically. The assay was carried out as previously described [18] with minor modifications [19]. In brief, exponentially growing cells were plated in 96-well sterile plates at a density of 10^4 cells/well in 100 μL of medium and were incubated for 24 h. Thereafter the tested compounds were applied at concentrations ranging from 0.195 to 200 μM. After 72 h continuous exposure 10 μL aliquots from a 5 mg mL^{-1} MTT solution were added to each well and the plates were further incubated for 4 h at 37°C in a humidified 5% CO_2 atmosphere. The formazan crystals yielded were solubilized by addition of HCOOH (5%) acidified DMSO. The MTT-formazan absorbance was read on a microprocessor controlled multiplate reader (Labexim LMR-1).

Results and discussion

On the basis of the data from the elemental analysis for the new complexes the following formulae can be derived: *cis*-$[Pd(C_9H_9N_3O_2)_2Cl_2]\cdot H_2O$ **(1)** and *cis*-$[Pd(C_9H_9N_3O_2)_2Br_2]\cdot 2H_2O$ **(2)**, where $C_9H_9N_3O_2$

Table 1 Chemical analysis of the complexes **(1)** and **(2)**

Compound	C/%		H/%		N/%		Pd/%	
	theor.	exp.	theor.	exp.	theor.	exp.	theor.	exp.
Complex **(1)**	37.42	37.56	3.46	3.95	14.55	14.50	18.43	18.03
Complex **(2)**	31.57	31.33	2.92	2.98	12.28	11.87	15.55	15.16

is 5-methyl-5-(4-pyridyl)-2,4-imidazolidenedione as a ligand (Table 1). Calculated data are in good agreement with experimental values. In order to evaluate the mode of coordination of the ligands to the metal ion, the IR-spectra of the pure ligands as well as of their Pd(II) complexes were recorded.

FTIR spectra

The assignment of the coordination mode of the ligand with the palladium ion was done on the basis of IR-spectra of mpyh, of the Pd(II) complexes with mpyh, literature data for Pd(II) complexes with pyridine derivatives [20, 21] as well as of our earlier statements on platinum complexes with the same ligand and with other N-containing heterocyclic ligands [15, 22, 23].

The comparative analysis of the IR-spectra of the complexes **(1)**, **(2)** (Table 2) and of the free ligand revealed that the absorption bands characteristic for the stretching vibrations of –C=N– from the pyridine ring were shifted towards the higher frequencies – from 1605.3 cm^{-1} in the spectrum of the ligand to 1653.7 and 1652.4 cm^{-1} in the spectra of the complexes respectively. The other characteristic bands of the free ligand in the region 1414–1006 cm^{-1}, related to pyridine ring shift to higher frequencies upon complexation likewise. This fact suggests that the nitrogen atom from the pyridine ring participates in the coordination to the palladium ion [24, 25].

The far IR spectra of the complexes show ligand vibrations as well as new bands originating from Pd-Cl and Pd-Br stretching. In the IR spectra of the Pd(II) complexes exhibited two new bands in the low-energy region at 330–290 cm^{-1} which can be assigned to Pd-Cl of **(1)** and Pd-Br of **(2)** stretching vibrations, because they are in the range reported for other palladium complexes e.g.: *cis*-$[Pd(py)_2Cl_2]$ (342, 333 cm^{-1}), *cis*-$[Pd(Him)_2Cl_2]$ (339, 335 cm^{-1}), etc. (*py*=pyridine, *Him*=imidazole) [26–28]. The presence of these bands assigned to Pd-Cl and Pd-Br stretching vibrations indicate coordination of the palladium(II) via Cl^- and Br^- in *cis* geometry.

The bands related to the stretching vibrations of the two carbonyl groups at 1776.2 and 1730.2 cm^{-1} of the hydantoin ring remained unchanged in the complexes. This fact is evidence that these groups are not involved in the complex formation.

^{1}H NMR spectra

In the ^{1}H NMR spectra of complexes **(1)** and **(2)** the signals of the protons for H-2 and H-6 from the pyridine ring were shifted from 8.56 ppm in the spectrum of the ligand to 8.78 and 8.84 ppm in the spectra of the complexes respectively. The differences between the chemical shifts of the protons of the ligand and these of the corresponding complexes are shown as Δδ (in ppm). The shifts are notice- able – Δδ=0.22 and 0.28 ppm. The signals of the H-3 and H-5 protons from the pyridine ring were shifted from 7.48 ppm in the ligand to 7.65 and 7.63 ppm in the complexes. The chemical shifts are Δδ=0.17 and 0.15 ppm and are less than those registered for the nearer to nitrogen atom protons of the pyridine ring. This shows that the most probable bounding of the ligand with the palladium ion in the complexes is realized through the nitrogen atom from the pyridine ring, similarly as it was found for platinum complexes with the identical ligand [15]. The signals for the protons at the nitrogen atoms from the hydantoin ring were not shifted. This fact indicates that these atoms are not involved in the complex formation to the palladium.

On the basis of the results from the physicochemical investigations, the following, most probable schematic structures of the Pd(II) complexes with mpyh could be proposed (Fig. 1).

Table 2 IR data for the free ligand and for the complexes **(1)** and **(2)**

Compound	ν(NH)	ν(–C=N)	ν(C=O)	ν(Pd-Cl)	ν(Pd-Br)
Ligand (mpyh)	3203.2 3114.2	1605.3	1776.2 1730.2		
Complex **(1)**	3440.6 3277.4	1653.7 1611.1	1770.3 1726.1	327.8 305.6	
Complex **(2)**	3189.4 3062.4	1652.4 1616.1	1783.1 1734.9		303.4 209.8

Thermogravimetric study

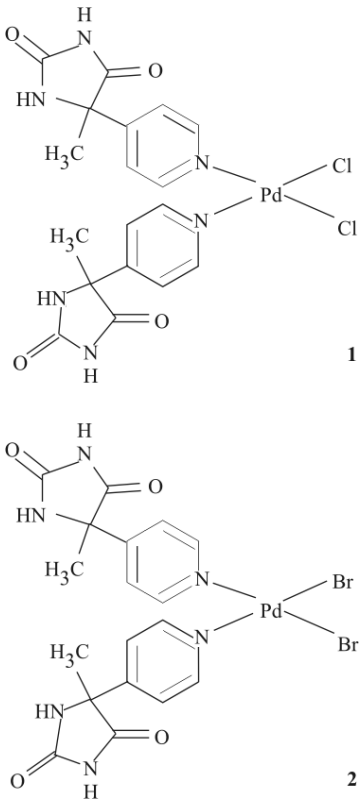

Fig. 1 Schematic structures of the investigated Pd(II) complexes *cis*-[Pd(mpyh)$_2$Cl$_2$]·H$_2$O **(1)** and *cis*-[Pd(mpyh)$_2$Br$_2$]·2H$_2$O **(2)**

Thermogravimetric analysis is a very valuable method with which to study the thermal decompositions of solid substances, such as complex compounds [29–32]. The curves obtained depict the decrease in sample mass with linear increase in treatment temperature.

Complex **(1)** is stable up to T=60°C (333 K). (Fig. 2). After this temperature decomposition in two stages begins. In the TG curve at T=100°C (373 K) mass loss Δm=9.8% is registered (theoretical mass loss Δm=9.3%) which corresponds to the disconnection of one molecule crystal water and one chloride ion [33]. This is confirmed from the endothermic effect in the DTA curve at the same temperature. Endothermic effect at T=320°C (593 K) in the DTA curve, which corresponds to an undeviating part from the TG curve, includes further decomposition of the complex. This suggests disconnection of the second chloride ion and initial destroying of the two organic ligands leading to palladium. At the beginning of these processes the experimental mass loss Δm=23.0% (theoretical mass loss Δm=23.0%). The final product of the decomposition process is metallic Pd (Δm=81.0%) [34].

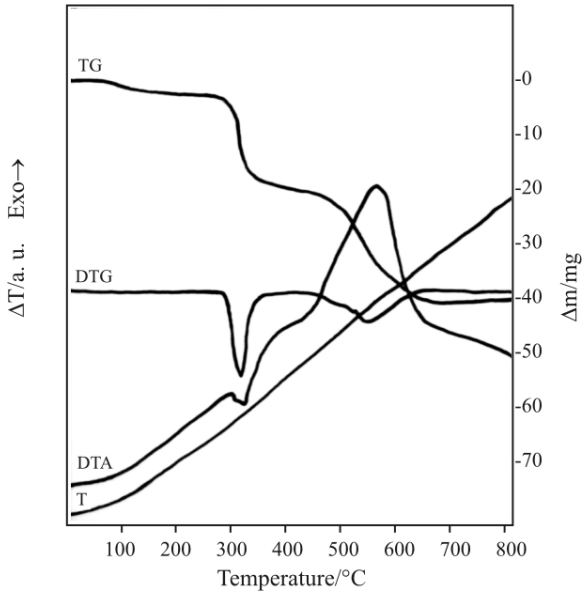

Fig. 2 TG, DTG and DTA curves of *cis*-[Pd(mpyh)$_2$Cl$_2$]·H$_2$O

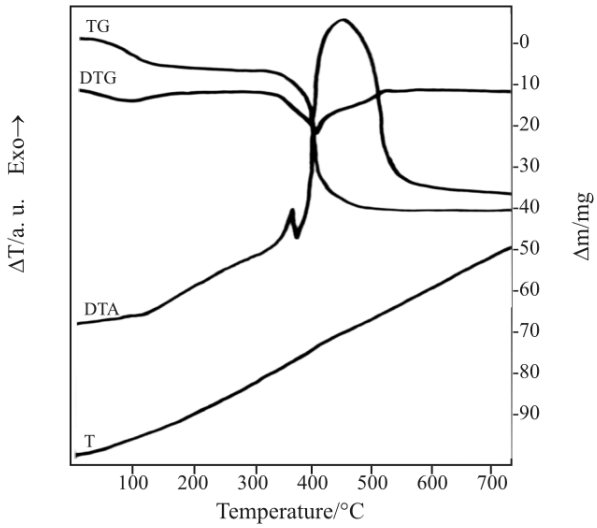

Fig. 3 TG, DTG and DTA curves of *cis*-[Pd(mpyh)$_2$Br$_2$]·2H$_2$O

Complex **(2)** is stable up to T=80°C (353 K). (Fig. 3). In the TG curve at this temperature a mass loss Δm=4.9% is registered (theoretical mass loss Δm=5.3%) which corresponds to the disconnection of two molecules crystal water. After this temperature begins gradual decomposition. In the DTA curve endothermic effect with maximum at T=330°C (603 K) is observed. It corresponds to the mass loss in the TG curve Δm=30.0%, which most probably due to the loss of one bromide ion [29]. The theoretical calculated mass loss is Δm=29.6%. After this temperature decomposition of the two organic ligands takes place.

After the T=330°C (603 K) in the DTA curve one pronounced exothermic effect at T=580°C (853 K) follows, which probably is a result from the

formation of new compounds. The final residue consists of Pd and carbon as from the TG curve a mass loss of 82.0% is registered. This statement is in accordance with the results obtained about other Pd(II) complexes [34].

Cytotoxic effect

The cytotoxic activity of the tested palladium complexes and the referent cytotoxic drug *cis*-DDP was evaluated by the MTT-dye reduction assay against the human acute promyelocyte leukemia HL-60 and the T-cell leukemia SKW-3. Evident from the concentration-response curves for HL-60 summarized in Fig. 4. The referent drug induced strong, concentration-dependent reduction of cell viability leading to almost total eradication of malignant cells at concentrations exceeding 50 μM (IC_{50}=8.7 μM). The newly synthesized palladium complexes exerted far less pronounced cytotoxicity failing to reach 50% reduction of cell viability up to concentration 200 μM. In order to allow comparative assessment of their potency IC_{20} values were calculated, as the concentrations reducing the cell viability by 20%. The dichloro analogue (IC_{20} =65.5 μM) proved to be more active as compared to the dibromo-complex (IC_{20}=152.7 μM). The IC_{20} value of Cisplatin was 3.33 μM.

The human T-cell leukemia SKW-3 proved to be more sensitive to the novel compounds. The concentration-response curves for SKW-3 summarized in Fig. 5. The IC_{50} values obtained were 89.00 μM for the dichloro-complex and 160.22 μM for the dibromo analogue respectively. The referent antineoplastic drug induced strong inhibitory activity with an IC_{50} value of 11.78 μM.

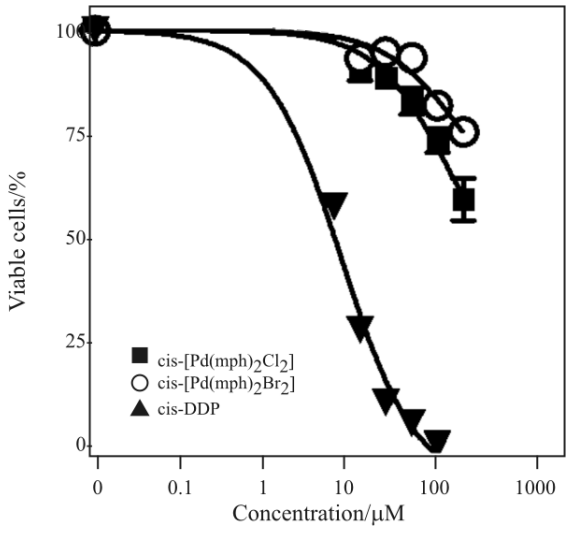

Fig. 4 Cytotoxic effects of the newly synthesized complexes *cis*-[Pd(mpyh)$_2$Cl$_2$], *cis*-[Pd(mpyh)$_2$Br$_2$] and *cis*-DDP *vs.* the human promyelocyte leukemia HL-60

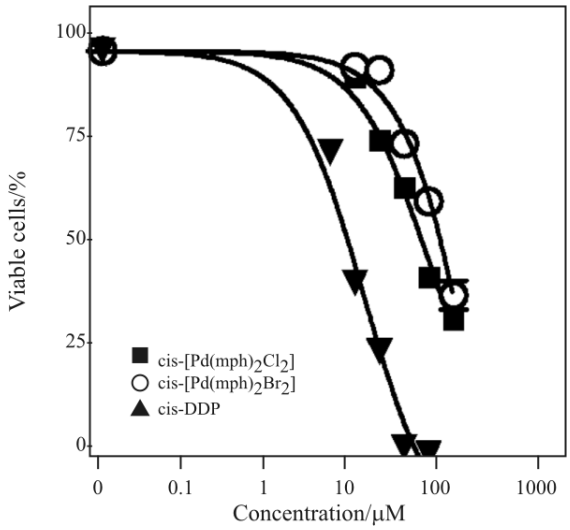

Fig. 5 Cytotoxic effects of the newly synthesized complexes *cis*-[Pd(mpyh)$_2$Cl$_2$], *cis*-[Pd(mpyh)$_2$Br$_2$] and *cis*-DDP *vs.* the human T-cell leukemia SKW-3

Statistical analysis

Student's t-test performed using commercially available software with $p<0.05$ taken as significance level. The cytotoxicity experiments were performed using eight wells per concentration. The MTT-data processing – non linear fitting (sigmoidal dose-response curves) and calculation of IC_{50} and IC_{20} values were performed using Graph Pad Prizm software.

Conclusions

Two new Pd(II) complexes with 5-methyl-5-(4-pyridyl)-2,4-imidazolidenedione and various halogen anions were synthesized. The molecular formulae of the complexes were confirmed by elemental, spectral analysis as IR, ^{1}H-spectra and DTA analysis. A NMR study indicates that the palladium is coordinated *via* N-atom of the pyridine ring. The infrared spectra in the far region of metal-ligand bond stretching frequencies indicates a square-planar *cis* geometry for Pd(II) – halogene ions in the complexes. The thermal decomposition of these complexes occurs in two consecutive steps and the final decomposition product was Pd for complex **(1)** and Pd and carbon for complex **(2)**. The dichloro analogue proved to be more active as compared to the dibromo-complex.

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