Macedonian Journal of Chemistry and Chemical Engineering, Vol. **35**, No. 1, pp. 79–86 (2016)

MJCCA9 – 706

ISSN 1857-5552
e-ISSN 1857-5625

Received: May 19, 2015
Accepted: July 3, 2015

UDC:546.924-386:543.42
546.98-386:543.42
DOI:10.20450/mjcce.2016.729
Original scientific paper

CYTOTOXICITY OF PLATINUM(IV) AND PALLADIUM(II) COMPLEXES WITH *meso*-1,2-DIPHENYL-ETHYLENEDIAMINE-*N*,*N*'-DI-3-PROPANOIC ACID. CRYSTAL STRUCTURE OF [Pd(1,2-dpheddp)] COMPLEX

Marina Ž. Mijajlović[1], Miloš V. Nikolić[1], Verica V. Jevtić[2], Zoran R. Ratković[2], Jelena Milovanović[1], Aleksandar Arsenijević[1], Bojana Stojanović[1], Slađana B. Novaković[3], Goran A. Bogdanović[3], Srećko R. Trifunović[2], Gordana P. Radić[1*]

[1]*Faculty of Medical Sciences, University of Kragujevac, Svetozara Markovića 69, 34000 Kragujevac, Serbia*
[2]*Department of Chemistry, Faculty of Science, University of Kragujevac, Radoja Domanovića 12, 34000 Kragujevac, Serbia*
[3]*VINČA Institute of Nuclear Sciences, Laboratory of Theoretical Physics and Condensed Matter Physics, University of Belgrade, PO Box 522, 11001 Belgrade, Serbia*

vasic_gordana@yahoo.com

The syntheses of tetradentate ligand, *meso*-1,2-diphenyl-ethylenediamine-*N*,*N'*-di-3-propanoic acid (H_2-1,2-dpheddp) and corresponding platinum(IV) and palladium(II) complexes are reported here. The spectroscopically predicted structure of the obtained palladium(II) complex was confirmed by X-ray analysis. Singe crystals suitable for X-ray measurements were obtained by slow crystallization from a DMSO-water mixture. Cytotoxic effects of platinum(IV), palladium(II) complexes and *cisplatin* on the 4T1 and B16F1 cell lines were determined using the MTT colorimetric technique. The complexes showed a dose dependence on cytotoxic effect toward both cell lines. Both complexes were less active than *cis-platin*, the exception was concentrations above 62.5 μM of platinum(IV) complex in the B16F1 cell line.

Keywords: *meso*-1,2-diphenyl-ethylenediamine-*N*,*N'*-di-3-propanoic acid; platinum(IV) complex; palladium(II) complex; crystal structure; cytotoxicity

ЦИТОТОКСИЧНОСТ НА КОМПЛЕКСИТЕ НА ПЛАТИНА(IV) И ПАЛАДИУМ(II) СО *meso*-1,2-ДИФЕНИЛ-ЕТИЛЕНДИАМИН-*N*,*N*'-ДИ-3-ПРОПАНОНСКА КИСЕЛИНА. КРИСТАЛНА СТРУКТУРА НА КОМПЛЕКСОТ НА [Pd(1,2-dpheddp)]

Во овој труд е опишана синтезата на тетрадентатниот лиганд, *meso*-1,2-дифенил-етилендиамин-*N*,*N*'-ди-3-пропанонска киселина (H_2-1,2-dpheddp), со соодветните комплекси на платина(IV) и паладиум(II). Спектроскопски предвидената структура на добиениот комплекс на паладиум(II) е потврдена со рендгенска структурна анализа. Монокристалите соодветни за рендгенските испитувања се добиени со бавна кристализација од воден раствор на DMSO. Цитотоксичните ефекти на комплексите на платина(IV) и на паладиум(II) врз типови на клетки 4T1 и B16F1 се определени со MTT колориметрија и споредени со соодветните ефекти на цисплатин. Двата комплекса покажаа дозирачки зависен цитотоксичен ефект врз двата типа клетки. Двата комплекса покажаа послаба активност од онаа на цисплатин, со исклучок на повисоките концентрации (над 62,5 μM) на комплексот на платина(IV) спрема клетките B16F1.

Клучни зборови: *meso*-1,2-дифенил-етилендиамин-*N*,*N*'-ди-3-пропанонска киселина; платина(IV)-комплекс; паладиум(II)-комплекс; кристална структура; цитотоксичност

1. INTRODUCTION

Since the seventies, when *cisplatin* with antitumor activity was discovered by Rosenberg and coworkers [1, 2], thousands of platinum complexes have been synthesized in order to obtain a new platinum(IV) compound with improved properties in comparison to the parent drug *cisplatin* [3–5]. Platinum(IV) compounds are more inert than platinum(II) species and this property may allow oral usage. Jolley *et al*. [6] reported the synthesis and cytotoxicity of several platinum(II) and platinum(IV) complexes containing ethylenediamine derivatives, including the complex with the ethylenediamine-*N,N'*-diacetate ligand. The latter complex can be considered a variant of JM-216, having the carboxylate groups bonded to the amine ligands. The authors found that complexes of the type $[PtCl_2(edda)]$ were less active than JM-216 (JM-216 is pharmaceutical abbreviation for the *trans,cis,cis*-diacetato-dichlorido-ammino-cyclohexylammino-platinum(IV)-complex, *trans, cis, cis*-$[Pt(CH_3COO)_2Cl_2(NH_3)(c\text{-}C_6H_{11}NH_2)]$) [6].

Natile and co-workers investigated how the substitution of edda with eddp (eddp = ethylenediamine-*N,N'*-di-3-propanoate ion) influenced their biological activity and concluded that eddp-Pt(IV) had lower activity than corresponding edda-Pt(IV) complex [7]. They supposed this fact due to the different complex geometry, *s-cis* in the case of edda and *trans* in the case of eddp.

We wanted to extend the investigation of platinum complexes with ethylenediamine-*N,N'*-di-3-propanoate derivatives. The initial idea of this work was to prepare a new linear edda-like ligand, *meso*-1,2-diphenyl-ethylenediamine-*N,N'*-di-3-propanoic acid and corresponding platinum(IV) complexes in *s-cis* and/or *trans* geometries. In all of our attempts we prepared only the *s-cis* geometrical isomer, with a low solubility in water and common organic solvents.

The preparation and spectral characterization of *meso*-1,2-diphenyl-ethylenediamine-*N,N'*-di-3-propanoic acid and corresponding platinum(IV) and palladium(II) complexes were published earlier [8] using the same method as Liu [9]. In the present study, palladium(II) complex with *meso*-1,2-diphenyl-ethylenediamine-*N,N'*-di-3-propanoic acid was characterized by X-ray analysis.

The aim of this paper is to synthesize palladium(II) and platinum(IV) complexes and research their cytotoxic effects *in vitro*.

2. EXPERIMENTAL

2.1. *Materials and measurements*

The reagents were obtained commercially and used without further purification. Elemental analyses were done on a Vario III CHNOS Elemental Analyzer, Elemental Analysensysteme GmbH.

2.2. *Syntheses*

2.2.1. *Preparation of meso-1,2-diphenyl-ethylenediamine, 1,2-dphen*

The *meso*-1,2-diphenyl-ethylenediamine was prepared according to the procedure described earlier [10]. Benzaldehyde (30.00 g) was refluxed with ammonium-acetate (60.00 g) for 3 h. The reaction mixture was cooled and the product was filtered and washed with ethanol. After recrystallization from 1-butanol *N*-benzoyl-*N'*-benzylidene-*meso*-1,2-diphenyl-ethylenediamine was obtained. Hydrolysis of that compound with 70% sulfuric acid under reflux for 1 h produced *meso*-1,2-diphenyl-ethylenediamine as the basic product of hydrolysis.

2.2.2. *Preparation of meso-1,2-diphenyl-ethylenediamine-N,N'-di-3-propanoic acid dihydrochloride-monohydrate, H_2-1,2-dpheddp·2HCl·H_2O*

3-Chloro-propanoic acid (4.34 g, 0.04 mol) was dissolved in 5.0 ml of water on ice and carefully neutralized with cold water solution of 5.0 ml NaOH (1.60 g, 0.04 mol). *Meso*-1,2-diphenyl-ethylenediamine (4.24 g, 0.02 mol) was added to this solution. The mixture was stirred for 4 h at 90°C, and during this period 5.0 ml NaOH water solution (1.60 g, 0.04 mol) was introduced. After that, 5.6 ml of 6 mol/l HCl was added and the resulting solution was evaporated to the volume of 7.0 ml; 6.0 ml *conc.* HCl, 6.0 ml of ethanol and 6.0 ml of ether were added to the mixture. The white precipitate of *meso*-1,2-diphenyl-ethylenediamine-*N,N'*-di-3-propanoic acid dihydrochloride monohydrate, H_2-1,2-dpheddp·2HCl·H_2O was separated by filtration and refined with solution water : ethanol = 1 : 2. Yield: 4.00 g, 44.69%. *Anal*. Calcd. for H_2-1,2-dpheddp·2HCl·H_2O = $C_{20}H_{28}Cl_2N_2O_5$ (M_r = 447.344): C, 53.69; H, 6.31; N, 6.28. Found: C, 53.88; H, 6.70; N, 6.08.

2.2.3. *Preparation of s-cis-dichlorido-(*meso-*1,2-diphenyl-ethylenediamine-N,N'-di-3-propanoate)-platinum(IV), s-cis-[$PtCl_2$(1,2-dpheddp)]*

Potassium-hexachloridoplatinate(IV) (0.2000 g, 0.411 mmol) was dissolved in 10.0 ml water on a steam bath and *meso*-1,2-diphenyl-ethylenediamine-*N,N'*-di-3-propanoic acid dihydrohloride

monohydrate (0.1839 g, 0.411 mmol) was added. The reaction mixture was heated for 12 h; during this period, 10.0 ml of LiOH water solution (0.0394 g, 1.65 mmol) was added in small portions and the solution was filtered and evaporated to a small volume. The orange precipitate of *s-cis*-[$PtCl_2$(1,2-dpheddp)] was separated by filtration, washed with cold water and air-dried. Yield: 0.095 g, 37.25%. *Anal*. Calcd. for *s-cis*-[$PtCl_2$(1,2-dpheddp)] = $C_{20}H_{26}Cl_2N_2O_4Pt$ (M_r = 620.396): C, 38.72; H, 3.57; N, 4.52. Found: C, 38.38; H, 3.81; N, 4.60.

2.2.4. *Preparation of (meso-1,2-diphenyl-ethylenediamine-N,N'-di-3-propanoate) palladium(II) complex threehydrate, [Pd(1,2-dpheddp)]·3H_2O*

$K_2[PdCl_4]$ (0.2000 g, 0.613 mmol) was dissolved in 10.0 ml of water on a steam bath and *meso*-1,2-diphenyl-ethylenediamine-*N,N'*-di-3-propanoic acid dihydrochloride monohydrate, H_2-1,2-dpheddp·2HCl·H_2O (0.2742 g, 0.613 mmol) was added. The mixture was stirred for 2 h and during this period water solution of LiOH (0.059 g, 2.452 mmol in 10.0 ml of water) was introduced. The complex [Pd(1,2-dpheddp)], as a yellow precipitate, was filtered, washed with cold water and air-dried. Yield: 0.25 g, 79.11%. Anal. Calcd. for [Pd(1,2-dpheddp)] = $C_{20}H_{28}N_2O_7Pd$ (M_r = 514.864) (%): C, 46.65; H, 5.48; N, 5.44. Found: C, 46.64; H, 5.87; N, 5.31.

2.3. *X-ray experiment*

The diffraction data from a selected single crystal of [Pd(1,2-dpheddp)]·3H_2O were collected at room temperature with an Oxford Diffraction Xcalibur Gemini S diffractometer equipped with CuKα radiation (λ = 1.5418 Å). Data were processed with CRYSALIS software and corrected for absorption by numerical absorption correction method based on Gaussian integration over a multifaceted crystal model [11]. Crystal structure was solved by direct methods, using SHELXS [12] and refined using the SHELXL program [12].

Hydrogen atoms belonging to water molecules were located in different Fourier maps and refined as riding, with O—H = 0.85 Å and U_{iso}(H) = 1.2U_{eq}(O). Hydrogen atoms attached to carbon and nitrogen atoms in the complex molecule were placed at geometrically calculated positions with C–H distances fixed to 0.98, 0.97 and 0.93 Å from methine, methylene and phenyl C atoms, respectively, and N–H distances fixed to 0.91 Å. The corresponding isotropic displacement parameters of the hydrogen atoms were equal to 1.2 U_{eq} of the parent C and N atoms. The PARST [13] program was used to perform geometrical calculation and the program ORTEP [14] was employed for molecular graphics. The crystallographic data are listed in Table 1.

T a b l e 1

Crystallographic data for [Pd(1,2-dpheddp)]·3H_2O

Empirical formula	$C_{20}H_{28}N_2O_7Pd$
Formula weight	514.84
Color, crystal shape	Yellow, prism
Crystal size (mm^3)	0.38 × 0.23 × 0.14
Temperature (K)	293(2)
Wavelength (Å)	1.5418
Crystal system	Orthorhombic
Space group	$Pna2_1$
Unit cell dimensions:	
a (Å)	20.8248(5)
b (Å)	8.9226(2)
c (Å)	11.2833(2)
V ($Å^3$)	2096.57(8)
Z	4
D_{calc} (Mg/m^3)	1.631
μ (mm^{-1})	7.534
θ range for data collection (°)	4.25 to 72.43
Reflections collected	4787
Independent reflections, R_{int}	2991, 0.0261
Completeness (%) to θ = 67°	100.0
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2991 / 1 / 295
Goodness-of-fit on F^2	1.057
Final R_1/wR_2 indices [$I > 2\sigma(I)$]	0.0283/ 0.0684
Absolute structure parameter [15]	–0.021(9)
Largest diff. peak and hole (e $Å^{-3}$)	0.490 –0.535

2.4. *In vitro cytotoxicity studies*

2.4.1. *Preparation of drug solutions*

Platinum complexes were dissolved in dimethylsulfoxide at a concentration of 20 mM and filtered through a 0.22 mm millipore filter. These stock solutions were diluted in culture medium immediately before use MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide was dissolved (5 mg/ml) in phosphate-buffered saline with a pH of 7.2, and filtered through the 0.22 mm millipore filter before use. All reagents were purchased from Sigma Chemicals.

2.4.2. *Cell lines*

The mouse breast tumor cell line 4T1 and mouse malignant melanoma cell line B16F1 were purchased from the American Type Culture Collection (ATCC). The cells were maintained in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), 100 IU/ml penicillin G and 100 mg/ml streptomycin.

2.4.3. *Assessment of cytotoxicity*

Cytotoxic effects of platinum(IV) and palladium(II) complexes and cisplatin on 4T1 and B16F1 cell lines were determined using the MTT colorimetric technique [16]. The cells were diluted with medium to $5\cdot10^4$ cells/ml and aliquots ($5\cdot10^3$cells/100 µl) were placed in individual wells in 96-multiplates. Approximately 24 h after cell adherence, the medium was exchanged with 100 µM of platinum(IV) and palladium(II) complexes, or cisplatin which had been serially diluted 2-fold in medium to concentrations ranging from 500 µM to 3.9 µM. Cells were incubated at 37°C in a 5% CO_2 incubator for 72 h. After incubation supernatant was removed, 15% MTT (3-(4,5)-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium-bromide) (5 mg/ml in phosphate buffered saline) was added to each well and the plates were incubated for an additional 4 h. Upon incubation the cell-free supernatants were suctioned off, and DMSO (150 µl) and glycine buffer (20 µl) were added to dissolve the crystals. The plates were shaken for 10 minutes. The optical density of each well was determined at 595 nm using microplate multimode detector Zenyth 3100. The percentage of cytotoxicity was calculated using the formula: % cytotoxicity = 100–((TS−BG0)−E/(TS−BG0)×100), where "BG0" stands for background of medium alone, "TS" for total viability/spontaneous death of untreated target cells, and "E" for experimental well.

3. RESULTS AND DISCUSSION

Potassium-hexachloridoplatinate(IV) and potassium-tetrachloridopalladate(II) react with *meso*-1,2-diphenyl-ethylenediamine-*N*,*N*′-di-3-propanoic acid dihydrochloride monohydrate, H_2-1,2-dpheddp·2HCl·H_2O in aqueous solution in the presence of lithium hydroxide to give the *s-cis*-dichlorido-(*meso*-1,2-diphenyl-ethylenediamine-*N*,*N*′ -di-3-propanoate)-platinum(IV), *s-cis*-[$PtCl_2$(1,2-dpheddp)] and (*meso*-1,2-diphenyl-ethylenediamine-*N*,*N*′-di-3-propanoate)palladium(II) complex, [Pd (1,2-dpheddp)], respectively.

In the case of platinum(IV) complex with two additional monodentate ligands the linear edda-type tetradentate ligands can occupy four of the sites around a central metal ion.

3.1. *Crystal and molecular structure of [Pd(1,2-dpheddp)]·3H_2O*

The crystal structure of [Pd(1,2-dpheddp)]·3H_2O was determined by single-crystal X-ray analysis. The complex molecule together with the atom-labelling scheme is presented in Figure 1. The selected geometric parameters are listed in Table 2.

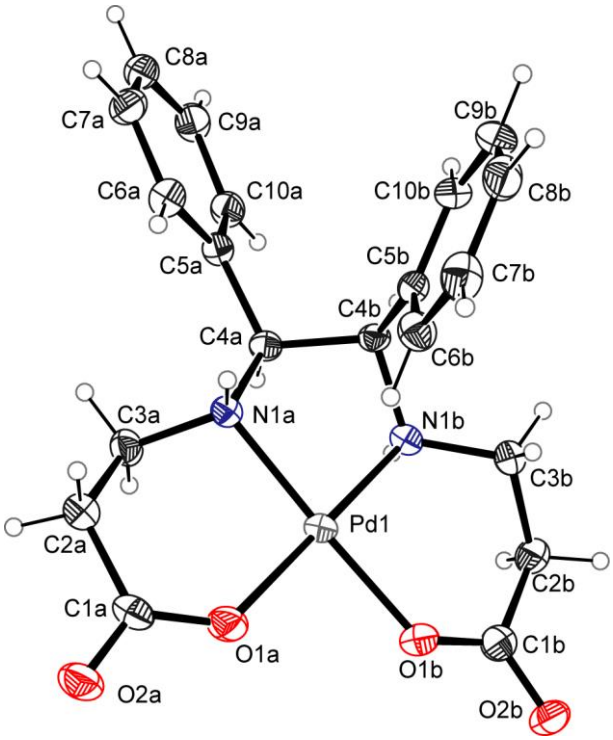

Fig. 1. Molecular structure and atom-numbering scheme of [Pd(1,2-dpheddp)]. Displacement ellipsoids are drawn at 30% probability level

The asymmetric unit consists of neutral complex molecule and three molecules of co-crystallized water. The 1,2-dpheddp ligand has two halves that are equivalent in composition and labelled in the following text as fragments A and B. The central palladium atom is placed in a deformed square-planar environment formed by two *N* and two *O* donor atoms of tetradentate eddp derivative. In this coordination, atoms Pd1, O1a, O1b, N1a and N1b deviate from the corresponding mean plane by –0.008(2), 0.053(2), –0.050(2), –0.049(2) and 0.054(2) Å, respectively. The coordination bonds belonging to A and B fragments (Table 2)

show a noticeable difference in lengths which is more prominent for two Pd–N bonds (0.04 Å). The rest of the equivalent bonds in A and B halves of the molecule agree within the standard deviation limits. The coordination angles deviate from the ideal value of 90º by 4º on average.

T a b l e 2

Selected bond lengths (Å) and angles (°) for [Pd(1,2-dpheddp)]. The values for two equivalent halves of the molecule are given in two columns labelled A and B.

Bond	*A*	*B*
Pd1−O1	2.004(4)	2.023(3)
Pd1−N1	2.041(4)	2.004(4)
N1−C3	1.490(5)	1.471(5)
N1−C4	1.482(5)	1.498(6)
C1−C2	1.534(7)	1.530(9)
C2−C3	1.515(7)	1.506(6)
O2−C1	1.233(6)	1.241(6)
C4−C5	1.521(6)	1.519(6)
C4a−C4b	1.537(6)	
Angle		
O1−Pd1−N1	94.98(14)	93.47(15)
C3−N1−Pd1	109.4(3)	115.5(3)
C1−O1−Pd1	128.7(3)	128.6(4)
N1−C3−C2	112.9(4)	110.6(4)
C1−C2−C3	118.7(4)	115.9(4)
O1a−Pd1−O1b	86.57(15)	
N1a−Pd1−N1b	85.12(15)	
O1a−Pd1−N1b	176.47(16)	
O1b−Pd1−N1a	177.25(14)	
N1a−C4a−C4b	108.2(4)	
N1b−C4b−C4a	102.9(3)	

According to the Cambridge Structural Database (CSD, Version 5.33 of 2012) [17], the present crystal structure is the only example of a palladium(II) complex with eddp derivative. In the case of the other metal atoms, there are two octahedral complexes of platinum(IV) [18, 19] and square-pyramidal complex of copper(II) with unmodified eddp ligand [20]. As previously observed in the complexes comprising propionate-chelates [18–21] the six-membered chelate rings of [Pd(1,2-dpheddp)]·$3H_2O$ are essentially planar in their carboxylate parts and puckered in their amino parts.

Two six-membered chelate rings in the present crystal structure exhibit some noteworthy structural differences. Regarding the mean plane defined by Pd1/O1/C1/C2 (r.m.s. of fitted atoms is 0.009 Å in both molecular halves), the atoms N1 and C3 significantly deviate *i.e.* to opposite sides of the mean plane for –0.250(8) and 0.528(9) Å in fragment A and to the same side of the mean plane for –0.153(7) and –0.801(8) Å in fragment B. The O1–C1–C2–C3 torsion angle has the values of 25.7 (8) and 34.1(6)° in the fragments A and B respectively. The significant difference is also observed between the Pd–N1–C3 angles [109.4(3) and 115.5(5)º in A and B fragments, respectively], while the equivalent bonds in two chelate rings have similar lengths (Table 2).

The five-membered ring of the complex can be described as twisted on the C4a–C4b bond. Each of the C4 atoms attaches a phenyl substituent which takes equatorial and axial position in A and B parts of the ethylenediamine ring, respectively. This is described by torsion angle Pd1–N1–C4–C5, with corresponding values of –173.0(3) and 81.8(4)º. The presence of voluminous phenyl substituents with dissimilar orientation induce the noticeable difference between N1a–C4a–C4b and N1b–C4b–C4a angles [108.2(4) and 102.9(3)º]. In the complexes comprising unmodified eddp ligand [18–20], the distribution of the angles within the ethylenediamine fragment is more consistent (from 106.3 to 108.2º, not including the chelate angle).

The two phenyl rings in [Pd(1,2-dpheddp)]·$3H_2O$ are mutually inclined, forming the dihedral angle of 56.8(2)º. Although C4–C5 single bonds allow for free rotation, the axially positioned phenyl ring takes specific orientation with C6b-H6b group clearly directed towards Pd atom. The torsion angle Pd1–C4b–C5b–C6b has the value of –1.34(5)º, while H6b…Pd1 distance of 2.63 Å is significantly shorter than the sum of corresponding Van der Waals radii (3.50 Å).

In general, the molecules of co-crystallized water have the most important role in the extensive, three-dimensional hydrogen bonding network of [Pd(1,2-dpheddp)]·$3H_2O$ (Table 3). Each complex unit interacts with six-molecules of co-crystallized water, where the water molecules serve either as donors toward the carboxylate O atoms or as acceptors engaging the N–H groups. The crystal structure is also stabilized by means of weak C–H…O and C–H…π.

Table 3

Hydrogen bonds and C−H... π interactions (Å, °)

D−H…A	D−H	D…A	H…A	D−H…A
N1a−H1a...O1w^{i}	0.91	3.003(5)	2.12	163
N1b−H1b...O2w^{i}	0.91	2.824(7)	1.94	163
O1w −H2w...O2a^{ii}	0.85	2.760(6)	1.98	153
O2w−H3w…O3w^{i}	0.85	2.796(7)	1.95	171
O2w−H4w…O2b^{iii}	0.85	3.010(6)	2.19	160
O2w−H4w…O1b^{iii}	0.85	3.186(7)	2.49	139
O3w−H6w…O2b^{iv}	0.85	2.918(9)	2.13	155
O3w−H5w…O1a^{iii}	0.85	3.113(7)	2.33	154
C−H… π	C−H	H… Cg	H…Perp	C−H…Cg
C2a−H2a1...Cg2^{v}	0.97	3.33	3.10	116
C3b−H3b1...Cg1^{iv}	0.97	2.97	2.97	126
C8a−H8a...Cg2^{vi}	0.93	3.07	2.99	121

Cg1 and Cg2 are centroids of phenyl rings in A and B fragments, respectively. Symmetry codes:
(i) x, y, z; (ii) –x+1,–y, z–1/2; (iii) –x+3/2, y–1/2, z–1/2; (iv) x, y–1, z; (v) –x+1, –y, z–1/2; (vi) x, y+1, z.

3.2. *In vitro cytotoxic activity*

In order to investigate the *in vitro* cytotoxic potential of platinum and palladium complexes, various concentrations of complexes were added to target cells and the cell viability was determined after 72 h by MTT assay. The complexes showed a dose-dependence on cytotoxic effect toward both cell lines (Fig. 2 and Fig. 3). Platinum(IV) complex showed a higher cytotoxic effect compared to palladium complex on both cell lines. Both complexes were less active than cisplatin, except at concentrations of the platinum complex above 62.5 μM on the B16F1 cell line. Taking into consideration standard deviations, IC_{50} values for the B16F1 cell line were approximately the same (Pt – 223.38±25.12, Pd – 157.51±14.09, *cisplatin* – 186.63±28.11).

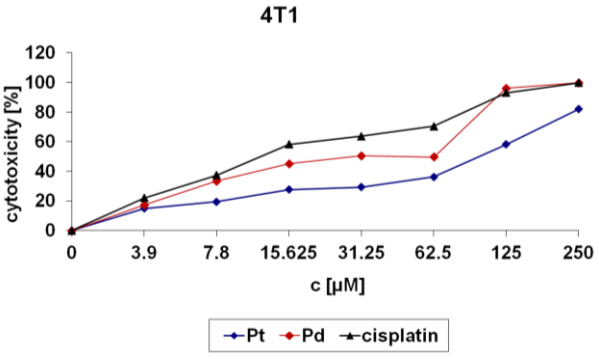

Fig. 2. Cytotoxicity of investigated complexes and cisplatin on 4T1 cells. Cytotoxicity was determined by MTT assay after 72 hours exposure to selected complexes. Data are presented as the means from three independent experiments.

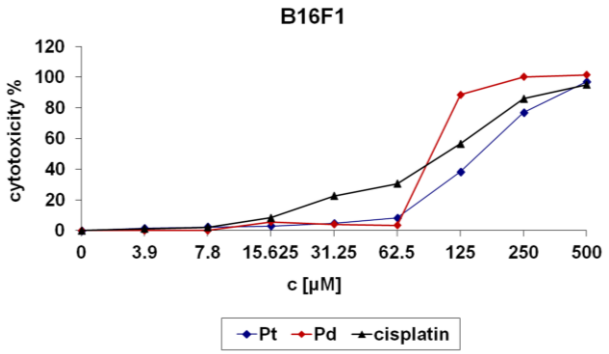

Fig. 3. Cytotoxicity of investigated complexes and cisplatin on B16F1 cells. Cytotoxicity was determined by MTT assay after 72 hours exposure to selected complexes. Data are presented as the means from three independent experiments.

The platinum complex showed a significantly higher cytotoxic effect on 4T1 cells (IC_{50} – 144.69±55.3) in comparison to the palladium complex (IC_{50} – 31.94±13.38) and *cisplatin* (IC_{50} – 17.34±2.25) (Fig. 4 and Fig. 5).

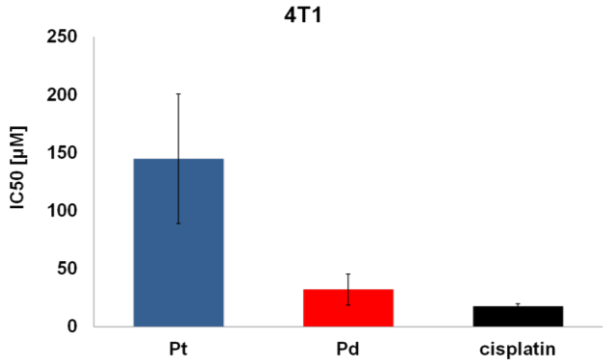

Fig. 4. IC_{50} (μM) for the 72 h of action of investigated compounds on 4T1 cells, determined by MTT assay

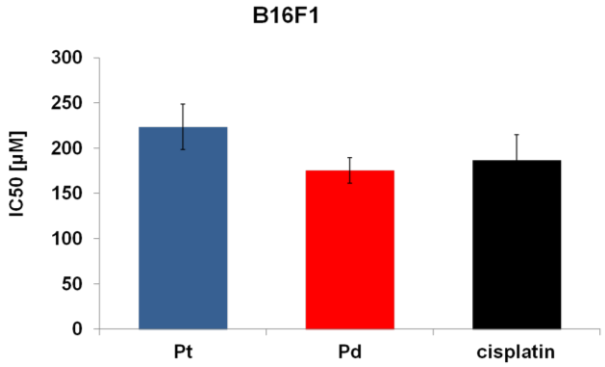

Fig. 5. IC_{50} (μM) for the 72 h of action of investigated compounds on B16F1 cells, determined by MTT assay

4. CONCLUSION

In this paper, we reported the cytotoxicity of platinum(IV) and palladium(II) complexes with *meso*-1,2-diphenyl-ethylenediamine-*N,N'*-di-3-propanoic acid and the crystal structure of the [Pd(1,2-dpheddp)] complex. Palladium(II) complex was crystallized in the form of the trihydrate. The cytotoxic effects of platinum(IV), palladium(II) complexes and cisplatin on 4T1 and B16F1 cell lines were determined using the MTT colorimetric technique. In general, investigated complexes were less active than *cisplatin*, except for the concentrations of platinum(IV) complex above 62.5 μM on the B16F1 cell line.

Acknowledgments. This work was supported by Ministry of Education, Science and Technological Development of Republic of Serbia, Projects No 172016, 172034, 172035, ON175069, ON175071, ON175103.

Appendix A. Supplementary data CCDC 930631 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223 336 033; or e-mail: deposit@ccdc.cam.ac.uk.

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