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Electrochemistry and Bioactivity Relationship of Pt(IV) Complexes with Cyclohexyl-Functionalized Ethylenediamine-*N,N'*-Diacetate-Type Ligands

Ljiljana E. Mihajlović, Dalibor Stanković, Jelena Poljarević, Dragan Manojlović, Tibor J. Sabo, Sanja Grgurić-Šipka[*]

Faculty of Chemistry, University of Belgrade, Studentski trg 12-16, 11000 Belgrade, Serbia
[*]E-mail: sanjag@chem.bg.ac.rs

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Pt(IV) complexes with ethylenediamine-*N,N'*-diacetate-type ligands exhibit strong antitumor activity towards various cancer cell lines. In order to explain their complete mechanisms of action, we examined bioreductive character of eight compounds in total using cyclic voltammetry, differential pulse voltammetry and electrochemical impedance spectroscopy. Recorded voltammograms of all Pt(IV) complexes with methyl, ethyl, *n*-propyl and *n*-butyl esters of (*S,S*)-ethylenediamine-*N,N'*-di-2-(3-cyclohexyl)propanoic acid confirmed two successive one-electron transfer steps followed by the loss of the corresponding axial ligands. The redox potential values for Pt(IV) complexes were in the range of -0.92 V<Ep<-0.70 V vs. Ag/AgCl, indicating hard reduction. The obtained electrochemical data pointed to the fact that the length of alkyl substituents influences on the reduction as well as on their biological activity. In addition, the electrochemical data were correlated with the biological data but this correlation wasn't established. The reported electrochemical data well correlated with the biological results pointed to the future structural modifications among the investigated compounds and helped select the most suitable candidates for further research.

Keywords: platinum(IV), anticancer, reduction potential, voltammetry.

1. INTRODUCTION

The history of metal-based drugs started in the mid-1960s with the discovery of cisplatin [1-2] by Barnett Rosenberg. Ever since then, thousands of compounds have been synthesized [3] in order to overcome the side effects [4] of commonly used medications [5-6]. The desired structure of a biologically active molecule and lowered toxicity towards nonmalignant cells are the main characteristics which scientists try to achieve when they synthesize new anticancer drugs. The main

problem with the perfect drug is not only its synthesis but also its significant pharmacological parameters (kinetic stability, lipophilicity, reduction potential) which directly influence its mechanism of action [7-8]. Therefore, scientists make a lot of effort trying to validate structure-activity relationship [9] as one of the significant factors in the area. One step in evaluating structure-property relationship is based on reduction mechanism studies [10-11]. Reduction potential values are an unavoidable parameter in establishing the mode of action since most metallopharmaceuticals are activated by *in vivo* electron transfer [12]. Metal-based antitumor agents are delivered to the body in an inactive form known as a prodrug. There are numerous ways of improving anticancer selectivity such as drug accumulation by a suitable mediator (e.g. transferrin), photoactivation, activation by oxygen products (e.g. bleomycin), activation by pH manipulation, by aquation and a selective reduction [13].

It is commonly believed that the selective reduction is the most frequent way in which antitumor drugs manifest their biological activity. Relevant data obtained by Choi's and Hambley's research groups [10,14] indicate that Pt(IV) drugs are reduced to lower oxidation states in the presence of extra- and intracellular reducing agents, although some of Pt(IV) compounds can avoid reduction [15].

Upon Pt(IV)→Pt(II) reduction, complexes change their coordination environment by releasing both axial ligands and obtaining square planar geometry [13] eventually resulting in reaction with DNA as the main target [16]. Apart from the influence of the metal center, the nature of the ligand environment also has a great influence on cellular reduction [17]. The proper choice of corresponding axial/equatorial ligands may be a good way to tune reduction potentials of the designed compounds.

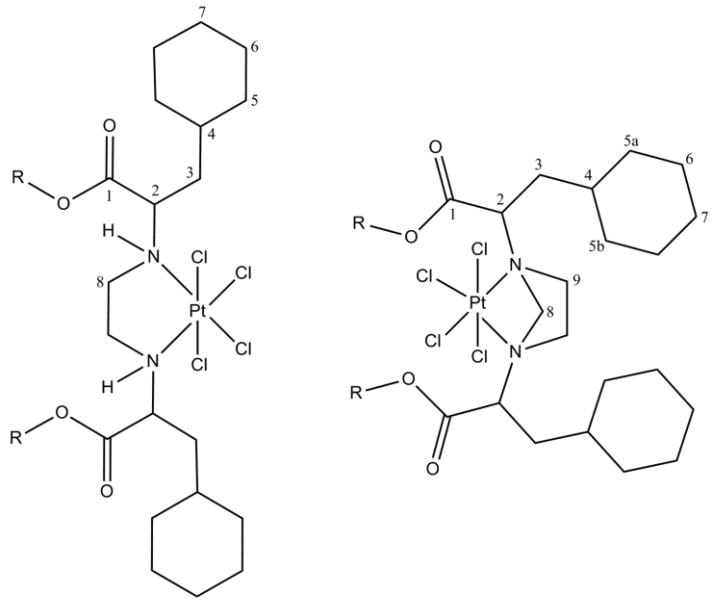

Figure 1. Structure formulas of the studied Pt(IV) complexes.

Considering all the relevant facts necessary for a complete investigation of a metal-containing drug candidate, we report the electrochemical study of series of Pt(IV) (**C1**: $[PtL_1Cl_4]$, **C2**:$[PtL_2Cl_4]$),

C3:[PtL_3Cl_4]), **C4**:[PtL_4Cl_4]), where L_1, L_2, L_3 and L_4 were methyl, ethyl, *n*-propyl and *n*-butyl esters of (*S,S*)-ethylenediamine-*N,N'*-di-2-(3-cyclohexyl)propanoic acid respectively) and a series of Pt(IV) complexes (**C5**:[PtL_5Cl_4], **C6**: [PtL_6Cl_4], **C7**:[PtL_7Cl_4], **C8**:[PtL_8Cl_4] where L_5, L_6, L_7 and L_8 were methylated methyl, ethyl, *n*-propyl and *n*-butyl esters of (*S,S*)-ethylenediamine-*N,N'*-di-2-(3-cyclohexyl)propanoic acid respectively) which show remarkable cytotoxic properties compared to cisplatin. The synthesis, characterization and biological activity of Pt(IV) complexes with the above-mentioned ligands have recently been published within the overall study in recent years [18-19]. Strong antitumor activity of the synthesized Pt(IV) complexes was demonstrative of the need for further research on structure-activity relationship. We present the determination of reduction potentials as one approach to selecting the best candidate for further investigation among different complex compounds.

2. MATERIAL AND METHODS

2.1. Reagents

Dimethyl sulfoxide (DMSO) and lithium perchlorate were purchased commercially and used without further purification. All complexes (**C1-C8**) insoluble in water were dissolved in DMSO in the concentration of 0.1 mM, followed by addition of 0.01 M $LiClO_4$ as a supporting electrolyte.

2.2. Synthesis of the complexes

Complexes **C1-C8** were synthesized and fully characterized in our recent publications [18-19].

Pt(IV) complexes **C1-C4** were synthesized in the reaction of $K_2[PtCl_6]$ with an equimolar amount of corresponding ligands, methyl, ethyl, *n*-propyl and *n*-butyl esters of (*S,S*)-ethylenediamine-*N,N'*-di-2-(3-cyclohexyl)propanoic acid respectively [18]. Solid ligand was added at 80ºC to the water solution of $K_2[PtCl_6]$ and stirred for 8 h while a solution of LiOH was added portionwise.

Pt(IV) complexes **C5-C8** were synthesized in the reaction of $K_2[PtCl_6]$ dissolved in warm water with an equimolar amount of corresponding ligands, methylated methyl, ethyl, *n*-propyl and *n*-butyl esters of (*S,S*)-ethylenediamine-*N,N'*-di-2-(3-cyclohexyl)propanoic acid respectively in dichloromethane stirred under reflux at 40°C [19].

*2.3. Characterization of complexes **C3** and **C7***

C3: ESI-MS (DMSO), positive: *m/z* 453.7 $[M\text{-}PtCl_4+H]^+$, negative *m/z* 789.19 $[M]^-$, 788.19 $[M-H]^-$, 718.15 $[M\text{-}2Cl]^-$, 647.15 $[M\text{-}4Cl]^-$; ^{1}H NMR (200 MHz, [D6]DMSO): 0.92 (m, C***H***$_3$CH$_2$-, 6H, C7, 4H), 1.16 (m, C5, 4 H), 1.40 (m, C6, 4H), 1.64 (m, -C***H***$_2$-Cy; C4, 2H; C5,6, 8H; CH$_3$C***H***$_2$-, 4H), 3.50 (m, -C***H***$_2$-OOC-, 4 H), 4.16 (s, OOC-C***H***-NH-, 2 H), 4.16 (m, -NH-C***H***$_2$C***H***$_2$-NH-, 4 H), 9.78 ppm (m, N***H***, 2 H); ^{13}C NMR (50 MHz, [D6]DMSO): 10.62 (***C***H$_3$CH$_2$CH$_2$OOC-), 21.51 (CH$_3$***C***H$_2$CH$_2$OOC-), 25.68 (C6), 25.88 (C4), 31.8 (C7), 33.2 (C5), 34.44, 37.2 (C3), 57.4 (C8, C2), 67.6 (CH$_3$CH$_2$***C***H$_2$OOC-), 170.7 ppm (C1)[18].

C7: ESI-MS (methanol), positive: *m/z* 464.89 $[M\text{-}PtCl_4+H]^+$, negative *m/z* 753.23 $[M\text{-}CH_2\text{-}HCl]^-$; ^{1}H NMR (200 MHz, $CDCl_3$): 0.78-1.01 (m, C5a′, C5b′, C***H***$_3$CH$_2$CH$_2$OOC-, 10H), 1.02-1.40

(m, C7′, C6′, C4, 8H), 1.42-1.85 (m, -C***H***$_{2'}$-Cy, -C***H***$_2$-Cy, CH_3C***H***$_2CH_2OOC$-, C7, C5a, C6, C5b, 18H), 2.78-3.06 (m, -N-C***H***$_2$-C***H***$_2$-N-, 4H), 3.39 (dd, -OOC-C***H***-N-, 2H), 3.65 (s, -N-C***H***$_2$-N-, 2H), 4.07 (t, CH_3CH_2C***H***$_2$OOC-, 4H); ^{13}C NMR (50 MHz, $CDCl_3$): 10.20 (***C***$H_3CH_2CH_2OOC$-), 21.74 (CH_3***C***H_2CH_2OOC-), 25.85 (C6), 26.18 (C7), 32.80 (C5b), 33.30 (C5a), 34.09 (C4), 38.45 (-***C***H_2-Cy), 47.82 (-N-***C***H_2-***C***H_2-N-), 61.62 (CH_3CH_2***C***H_2OOC-), 65.59 (-OOC-***C***H-N-), 69.12 (-N-***C***H_2-N-), 172.85 ($CH_3CH_2CH_2OO$***C***-)[19].

2.4. Electrochemical measurements

All measurements were performed at ambient temperature using CHI-760B instrument (CHI Instruments, USA) for cyclic voltammetry, differential pulse voltammetry and electrochemical impedance spectroscopy. The voltammetric measurements were performed in a three-electrode cell which consisted of a glassy carbon electrode (Model 6.1204.300), an auxiliary platinum electrode with large surface area (Model CHI221, cell top including a platinum wire counter electrode) and an Ag/AgCl reference electrode (Model CHI111). Reduction potentials of all the compounds were determined by differential pulse voltammetry. The obtained electrochemical data correspond to the second reduction step and are shown in Table 1. Impedance measurements were performed in the potential range from 0.2 to 1.4 V at frequency of 962 Hz with applied amplitude of 0.005 mV.

2.5. Biological data

The IC_{50}(μM) values (the half maximal inhibitory concentration values) have recently been reported [18-19] and obtained from two independent experiments performing 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test for **C1-C4** and a crystal violet (CV) test for **C5-C8**. In order to correlate redox potentials with IC_{50} values, we chose two common cell lines, mouse melanoma B16 and glioblastoma U251. As seen in Table 1. all IC_{50} values for the investigated Pt(IV) complexes exhibited stronger antitumor activity compared to cisplatin.

3. RESULTS AND DISCUSSION

3.1. Electrochemical behavior

3.1.1. Platinum(IV) complexes, C1-C4

The observed signals in the cyclic voltammograms of Pt(IV)complex **C1** originate from the oxidation process of the corresponding ligand. As shown in Figure 1. the signal seen around 1.1 V vs. Ag/AgCl refers to the oxidation of the amino group to a nitro group. This behavior can be explained if we consider the structure of compounds **C1-C4**. The active place for oxidation (amino group) is accessible to the surface of the platinum electrode so electrochemical reaction can occur. In Figure 1. can also be observed that the increase in the square root of the scan rate leads to the increase in current intensity. Therefore, this electrochemical reaction is diffusion- controlled [20-21].

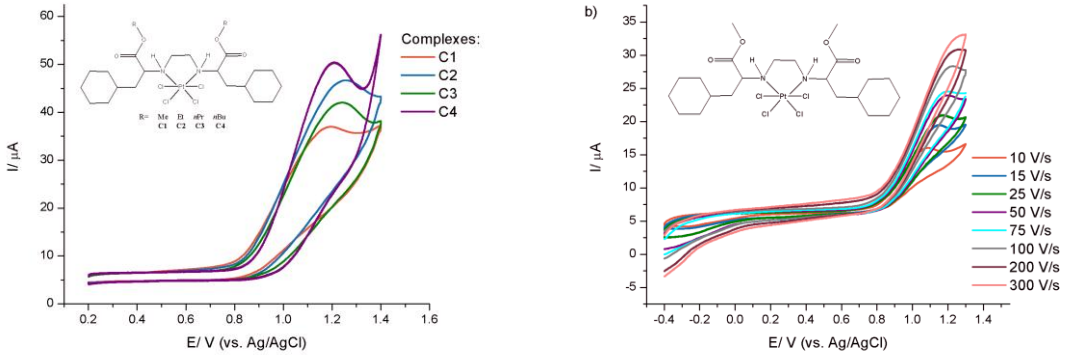

Figure 2. Cyclic voltammograms of 0.1 mM solutions of Pt(IV) complexes a) **C1-C4** and b) **C1** in DMSO with $LiClO_4$ at the scan rates of 0.010, 0.015, 0.025, 0.050, 0.075, 0.100, 0.200, 0.300 V/s using a glassy carbon working electrode.

Reduction of Pt(IV) complexes **C1-C4** presented in Figure 2. is a two-electron process followed by the loss of the corresponding axial ligands, which eventually suggests an irreversible reduction wave. Therefore, presented Ep values have kinetic and not thermodynamic character. Each wave represents transfer of one electron. The first peak represents $Pt^{IV/III}$ reduction and it is followed by our target reduction – $Pt^{III/II}$. We presented only the Ep values for the second reduction step since the reduction to Pt^{II} is expected in biological media. The spotted differences in reduction potentials are slightly shifted as the length of the alkyl chain increases from methyl (Me-) through ethyl (Et-) and *n*-propyl (*n*Pr-) to *n*-butyl(*n*Bu-) group since diffusion changes are initiated by the alkyl size modifications. On the other hand, the differences between the first and the second reduction steps remain constant.

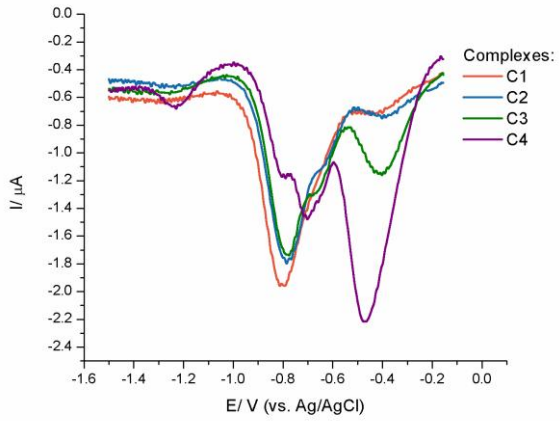

Figure 3. Differential pulse voltammograms of 0.1 mM solutions of Pt(IV) complexes **C1-C4** in DMSO with $LiClO_4$ at the scan rate of 0.10 V/s using a glassy carbon working electrode.

Two peaks with the potential values of -0.47 V vs. Ag/AgCl and -0.78 V vs. Ag/AgCl can be spotted using differential pulse voltammetry. As the surface of the first peak increases and the surface

of the second peak decreases, the difference between the intensities of the signals can be observed due to the different alkyl chain length and due to the specific structure of the active glassy carbon surface.

3.1.2. Platinum(IV) complexes, C5-C8

$Pt^{IV/II}$ reduction of complexes **C5-C8** is also a two-electron process with Ep values shifted towards a more negative scale compared to compounds **C1-C4**. The main reason for this behavior could be found in the steric hindrances of the platinum center. As it can be seen from the structures, the metal center is obstructed and surrounded by an additional methylene bridge, which makes the reduction slower. As expected, this assumption is explicitly verified since the Ep values of compounds **C5-C8** are lower than Ep values of compounds **C1-C4**.

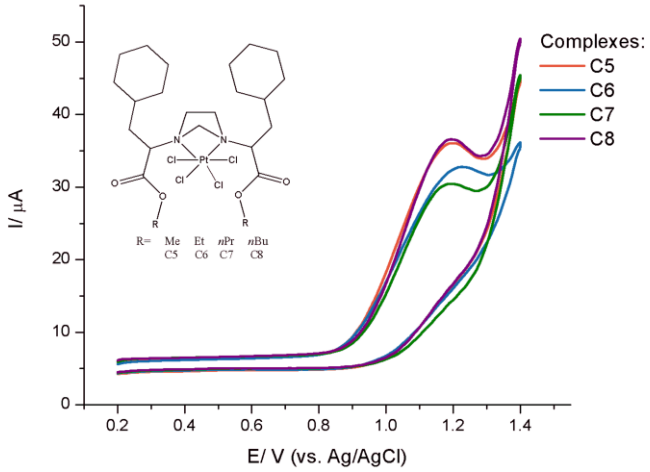

Figure 4. Cyclic voltammograms of 0.1 mM solutions of Pt(IV) complexes **C5-C8** in DMSO with $LiClO_4$.

The cathodic reduction potentials clearly depend on the type of ligands as it is shown in the previous part. The obtained values of electrode potentials (-924 mV<Ep<-701 mV vs. Ag/AgCl) are in the value range of analog Pt(IV) complexes (-705, -846 mV vs. Ag/AgCl) with R_2eddp type of ligands (R=Et, *n*-Pr; eddp=ethylenediamine-*N*,*N*'-di-3-propionate) [22].

3.1.3. Electrochemical impedance spectroscopy

Electrochemical impedance for the $Pt^{(IV)/(II)}$ couple were investigated using electrochemical impedance spectroscopy (EIS) and corresponding spectra are shown in Figure 5. For all complexes, **C1-C8**, a semicircle around 0.9V is observed followed by almost straight line with a slope around 45º in lower potential region. In this way, we confirmed assumed indication of controlled electrochemical reaction and diffusion step.

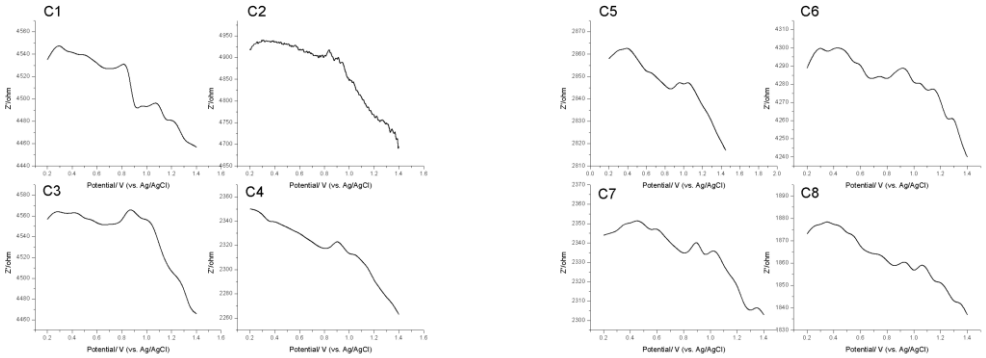

Figure 5. Impedance spectra Pt(IV) complexes **C1-C8** in DMSO with $LiClO_4$.

Table 1. Comparative biological and electrochemical data

Compound	R-substituent[a]	$IC_{50}/\mu M$[b]		Ep/ mV[c]
		U251	B16	
cisplatin	-	11.5 [18]	67.0 [18]	-
C1	Me-	2.5 [18]	4.5 [18]	-798
C2	Et-	1.9 [18]	3.4 [18]	-781
C3	*n*-Pr	2.2 [18]	3.1 [18]	-776
C4	*n*-Bu	7.4 [18]	8.3 [18]	-701
cisplatin	-	20 [19]	94.3 [19]	-
C5	Me-	17.5 [19]	21.3 [19]	-816
C6	Et-	2.9 [19]	13.8 [19]	-900
C7	*n*-Pr	12.5 [19]	9.1 [19]	-884
C8	*n*-Bu	11.8 [19]	10.2 [19]	-924

[a] Me-, Et-, *n*-Pr, *n*-Bu are methyl, ethyl, *n*-propyl and *n*-butyl alkyl groups.

[b] IC_{50} is the in vitro cytotoxic activity against U251 (glioblastoma) and B16 (mouse melanoma) cell lines expressed in μM.

[c] Ep are electrodic potential values determined using differential potential voltammetry.

3.2. Correlation between redox potentials and biological activity

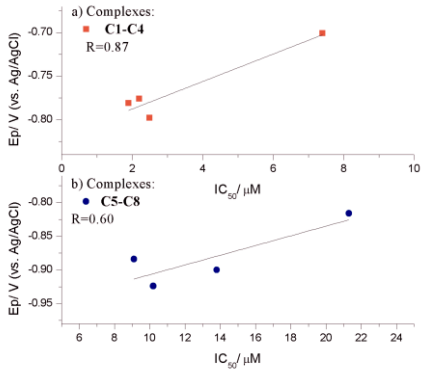

Figure 6. Correlation between reduction potentials and cytotoxicity towards **a)** U251 cell line for **C1-C4** complexes, **b)** B16 cell line for **C5-C8** complexes

The mechanism of drug action is still the subject of many researches [15,23] which mainly focus on how drugs enter the cell. It is commonly believed that bioreduction of Pt(IV) complexes leads to their more active Pt(II) analogs [5]. Although many pharmacological parameters (lipophilicity, solubility, diffusion) have a great influence on biological activity, there is no clear relationship between them [24]. Hence, we did not aim at obtaining a strict correlation between IC_{50} and Ep values, but only at showing an alternative way of finding the best candidate for further researches in this field.

In our study, a distinctive correlation between IC_{50} and Ep values for Pt(IV) complexes (**C1-C8**) wasn't observed and this fact was in agreement with literature data for amine type of ligands [25].

IC_{50} and Ep values are integrated in Table 1. in order to highlight our final remarks. As can be seen from Table 1. the most active Pt(IV) complexes towards U251 and B16 cell lines are those with bulkier equatorial ligands, **C2**, **C3, C6** and **C7**. Among these four compounds, **C6** and **C7** have the lowest Ep values and because of this reason are not suitable for further investigations. Therefore, the represented correlation directs further investigations towards **C3** complex which is the most active one and easiest to reduce.

4. CONCLUSIONS

The relationship between electrochemical behavior and cytotoxicity of eight Pt(IV) complexes coordinated to edda-type ligands was investigated. Although the clear correlation between redox potentials and IC_{50} values was not established, it was noted that bulkiness of equatorial ligands influences both IC_{50} and Ep values. This study also indicated that steric hindrance of the metal center can have a negative influence on redox potentials resulting in more negative reduction potentials. Hence, we can claim that **C3** among investigated complexes is the best drug candidate due to its extraordinary cytotoxic activity and satisfactory electrochemical properties.

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