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Article

Novel 3-(Aminomethyl)naphthoquinone Mannich Base-Platinum(IV) Complexes: Synthesis, Characterization, Electrochemical and Cytotoxic Studies

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Três novos complexos de platina(IV), *cis,cis,trans*-[Pt(**HL1-3**)$Cl_2(OH)_2$] **1b-3b** (**HL** = 2-hidroxi-3-[(R^1-amino)(piridin-2-il)metil]-1,4-naftoquinona, R^1 = *n*-butil, **HL1**; *n*-heptil, **HL2** and *n*-decil, **HL3**) foram obtidos através da oxidação dos respectivos precursores *cis*-[Pt(**HL1-3**)Cl_2] **1a-3a**. Estudos de voltametria cíclica de **1b**-**3b** em MeCN mostraram o processo redox quase reversível do íon naftoquinonato (NQO^-, i.e., L^-) e o processo irreversível atribuído à redução do par Pt^{4+}/Pt^{2+}, em potenciais aproximadamente 400 mV menos negativos que no precursor da cisplatina *cis,cis,trans*-[Pt$(NH_3)_2Cl_2(OH)_2$]. Propõe-se que este processo Pt^{4+}/Pt^{2+} seja favorecido, em **1b-3b**, por uma interação de hidrogênio entre o grupo 2-hidroxil da naftoquinona e um ligante hidroxil axial. Estudos de citotoxicidade contra quatro linhagens de células tumorais humanas mostraram que, em geral, os derivados de platina(IV) e platina(II) exibem o mesmo perfil citotóxico, além de serem mais ativos que a cisplatina. Valores de CI_{50} *in vitro* mais baixos foram observados para **2b**-**3b**, cujos ligantes possuem os maiores grupos R^1 (**HL2**-**HL3**), sendo, portanto, mais lipofílicos. Além disto, os valores semelhantes de CI_{50} dos complexos análogos de platina(II) e platina(IV) devem-se à rápida redução de **1b**-**3b** *in vitro* para gerar **1a**-**3a**.

Three novel platinum(IV) complexes *cis,cis,trans*-[Pt(**HL1-3**)$Cl_2(OH)_2$] **1b**-**3b** (**HL** = 2-hydroxy-3-[(R^1-amino)(pyridin-2-yl)methyl]-1,4-naphthoquinone, R^1 = *n*-butyl, **HL1**; *n*-heptyl, **HL2** and *n*-decyl, **HL3**) have been obtained from the oxidation of the respective precursors *cis*-[Pt(**HL1-3**)Cl_2] **1a-3a**. Cyclic voltammetry studies of **1b**-**3b** in MeCN showed the *quasi*-reversible naphthoquinonate (NQO^-, i.e., L^-) redox process and irreversible process attributed to the reduction of the Pt^{4+}/Pt^{2+} pair, at potentials about 400 mV less negative than for the cisplatin precursor *cis,cis,trans*-[Pt$(NH_3)_2Cl_2(OH)_2$]. Hydrogen bond interaction between the naphthoquinone 2-hydroxyl group and an axially coordinated hydroxide ligand in **1b**-**3b** has been proposed to favor the Pt^{4+}/Pt^{2+} reduction. The cytotoxicity studies against four human cancer cell lines have shown that in general the platinum(IV) and platinum(II) derivatives exhibit the same cytotoxic profile and are all more active than cisplatin. The lowest *in vitro* IC_{50} values have been observed for **2b**-**3b**, which bear ligands with the largest R^1 groups (**HL2**-**HL3**) being the most lipophilic. Furthermore similar IC_{50} values for platinum(II) and platinum(IV) complexes of the same ligands have been associated with rapid *in vitro* reduction of the latter complexes to afford **1a**-**3a**.

Keywords: Mannich bases, 2-hydroxy-3-(aminomethyl)-1,4-naphthoquinone, platinum(IV) complexes, Raman spectroscopy, cyclic voltammetry, cytotoxic activity

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Introduction

Cisplatin (*cis*-diaminodichloro-platinum(II)) is a well known antineoplastic agent[1] that acts mainly through DNA binding, interrupting replication and transcription processes and consequently inducing apoptosis.[2] Even though cisplatin has been currently used in cancer treatment, side effects such as nephrotoxicity and acquired resistance have limited its effectiveness and motivated the search for new Pt-based drugs able to overcome these drawbacks.[3,4]

One of the problems associated with platinum(II) complexes, including cisplatin, is their deactivation by sulfur containing species,[5] which prevents their uptake into the cell and DNA binding. As an alternative, octahedral low spin d^6 platinum(IV) complexes of the type *cis,cis,trans*-[Pt(AA')Cl_2L_2] (AA' = amine or diamine carrier ligands, L = axial ligands), which have slow ligand exchange rates, have been designed aiming to avoid side reactions in the bloodstream.[6] These complexes are believed to be activated by reduction to platinum(II) complexes *in vivo* by reductants, such as glutathione, ascorbic acid and cystein, with loss of the axial L ligands, the resulting species exerting their effects in a similar manner to cisplatin and analogous compounds.[7] The advantage of the platinum(IV) complexes is their relative inertia, compared to the platinum(II) counterparts, which allows them to be administrated orally.[7] Two factors are critical for their activation: the Pt^{4+}/Pt^{2+} reduction potential of the complex and the rate of reduction,[6] and both can be tuned by judicious choice of the carrier and axial ligands, the latter playing an important role in the pharmacokinetic properties of these complexes.[7,8] Electron withdrawing axial ligands are known to favor thermodynamically the reduction process.[8-10] Furthermore, axial and carrier ligand steric hindrance have also been found to influence both the reduction potential and rate of reduction.[8,9] Recent evidences of multiple reduction pathways for *cis,cis,trans*-[Pt(AA')Cl_2L_2] and *cis,trans*-[$PtCl_2$(mpy)(NH_3)L_2] (mpy = 2-methylpyridine) with axial L = acetate ligands have indicated that the mechanism proposed for the reduction of platinum(IV) complexes may not be as simple as commonly assumed.[6,11]

One of the strategies to enhance the cytotoxic activity of platinum(IV) complexes has been the incorporation of biologically relevant fragments to their axial positions,[12,13] since these ligands are frequently released upon reduction of the platinum(IV) center. For instance, increased cellular uptake has been achieved by tethering nanocarriers, e.g., carbon nanotubes, to axial carboxylate ligands, which prolong the circulation time of these drugs in the bloodstream.[14,15] DNA-Au-NP (NP = nanoparticles) tethered to a platinum(IV) complex *via* the carboxylate ligand were also successful carrying vehicles[16] and most recently, a platinum(IV) complex containing carboxylate axial ligands was covalently incorporated into cross-linked micelles allowing slow drug release.[17]

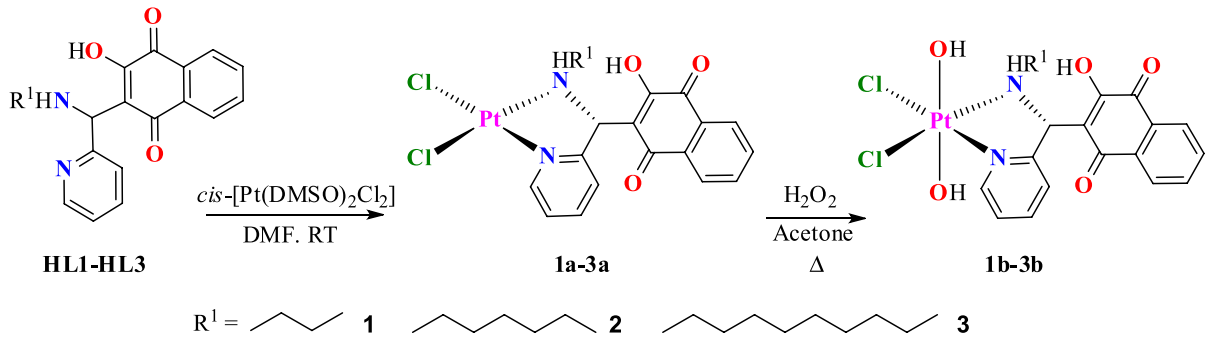
The use of ligands possessing recognized cytotoxic activity in the design of novel platinum drugs has also been described.[18] Our interest in hybrid platinum-3-(aminomethyl) naphthoquinone complexes stems from the fact that both platinum complexes and aminonaphthoquinones are active fragments, and therefore compounds with enhanced antineoplastic activity might be anticipated.[19] Cisplatin and analogous compounds exert their cytotoxicity by DNA platination, leading to apoptosis.[20] The mechanism of action of naphthoquinone derivatives is still not totally clear but it is known that they can act *via* inhibition of topoisomerases,[21] generation of reactive oxygen species (ROS) by quinone redox cycle[22] and/or bioalkylation.[23]

It was reported that hybrid platinum(II) complexes of 3-(aminomethyl)naphthoquinones, **1a**-**3a**, show moderate to high activity against selected human cancer cell lines with IC_{50} values lower than cisplatin.[24] Recently, a series of biophysical and cellular studies with these compounds have been undertaken.[25] Complexes **1a**-**3a** bind covalently to DNA model bases, inducing cellular DNA strand breaks. The same behavior has been observed *in vitro*. However, they are still less active than the pro-ligands **HL1-HL3**. For this reason, with the aim of improving the cytotoxicity of the platinum(II) compounds **1a**-**3a**, we have synthesized their platinum(IV) analogues. In this work, we describe the synthesis, characterization, electrochemical and cytotoxic studies of the novel complexes [Pt(**HL**)$Cl_2(OH)_2$] **1b-3b**, shown in Scheme 1.

Experimental

Material and methods

All chemicals were used without further purification. 2-Hydroxy-1,4-naphthoquinone, 2-pyridine-carboxaldehyde, butylamine, heptylamine, decylamine and K_2PtCl_4 were purchased from Aldrich and H_2O_2, NaOH and solvents were acquired from Vetec. **HL1-3** (orange powders),[26] *cis*-[Pt$(DMSO)_2Cl_2$],[27] **1a-3a** (yellow powders)[23] and *cis,cis,trans*-[Pt$(NH_3)_2Cl_2(OH)_2$][28] were prepared as described in the literature and their identity was confirmed by melting point measurements and ^{1}H nuclear magnetic resonance (NMR) spectra. Microanalyses were performed using a Perkin-Elmer CHN 2400 micro analyzer at Central Analítica (Instituto de Química, USP, São Paulo State, Brazil). Melting Points were obtained with a Digital Melting Point IA9100, ThermoFischer Scientific-USA apparatus and were not corrected. Infrared (IR) spectra were



Scheme 1. Synthesis of Mannich base-platinum(IV) complexes **1b-3b**.

recorded on a Nicolet FTIR Magna 760 spectrophotometer using CsI pellets and Raman experiments were carried out on a Bruker FT-Raman MultiRAM using the 1064 nm line of a Nd:YAG laser and a Ge detector operating at liquid nitrogen temperature. Electrospray ionization mass spectroscopy (ESI-MS) spectra were acquired on a quadrupole time-of-flight mass spectrometer (QTOF spectrometer) (Micromass, Manchester, U.K.) at Instituto de Química, UFRJ, Rio de Janeiro State, Brazil. The mass spectrometry (MS) parameters were set to scan from 90 to 1000 *m/z* using positive detection. The dry gas flow was set to 50.0 L min^{-1}, the dry temperature to 120 °C and the flow injection rate to 4.0 µL min^{-1}. All measurements were performed using MeCN and formic acid. Cyclic voltammetry was carried out with a BASi EPSILON potentiostat/galvanostat system at room temperature, in 5 mL of MeCN (anhydrosolv grade-Tedia Brazil) solutions of the compounds (1.0×10^{-3} mol L^{-1}) containing 0.1 mol L^{-1} *n*-Bu_4NClO_4 as the supporting electrolyte under an argon atmosphere. A conventional three-electrode cell was used to carry out these experiments: a glassy carbon electrode as the working electrode, a platinum wire as counter-electrode and Ag/AgCl (0.1 mol L^{-1} *n*-Bu_4NClO_4/MeCN) was used as the reference electrode. The scan rate was in the 0.100-0.200 V s^{-1} range and the experiments were carried out in the cathodic branch. The ferrocene/ferrocenium (FcH/FcH^+) couple was used as internal reference.[29] For the studies in the presence of $NaOH_{(aq)}$ and $HCl_{(aq)}$, the conditions were the same as those described above but for the sequential addition of 50 µL aliquots of 0.02 mol L^{-1} NaOH and 20 µL aliquots of 0.05 mol L^{-1} HCl. Pure argon gas was bubbled through the electrolytic solution to remove oxygen in all experiments.

Synthesis of the platinum(IV) complexes **1b-3b**

The yellow-orange complexes **1b**-**3b** were obtained by selective oxidation of **1a**-**3a** with H_2O_2[30] (Scheme 1). For a suspension of the platinum(II) complexes **1a**-**3a** (0.8-0.95 mmol) in acetone was added 1.0 mL of H_2O_2 (10 mmol). The solution was heated under reflux for 7 h. The solvent was removed under vacuum and the residue was washed with isopropanol or petroleum ether to yield light-yellow powders, which were isolated by centrifugation and dried under vacuum.

[Pt(**HL1**)$Cl_2(OH)_2$] **1b**

From 572 mg of **1a**. Yield: 485 mg, 80%, mp 186 °C (dec.). Anal. Calc. for $C_{20}H_{22}Cl_2N_2O_5Pt.1.5H_2O$: C 36.21; H, 3.80; N, 4.22%. Found: C, 35.09; H, 3.71; N, 4.06%. ESI-MS (positive-ion mode): *m/z* 635.1082 $[M]^+$. IR (Raman) ν_{max}/cm^{-1}: 3491 (O–H/N–H); 3113, 3082 (C–$H_{(ar)}$); 2961, 2931, 2872 (C–$H_{(al)}$); 1684 (C=O); 1625, 1591 (C=C/C=N), 1555 (δCNH), 1479, 1458, 1365 (δHCH); 1274 (C–O/C–N).

[Pt(**HL2**)$Cl_2(OH)_2$] **2b**

From 580 mg of **2a**. Yield: 290 mg, 48%; mp 164 °C (dec.). Anal. Calc. for $C_{23}H_{28}Cl_2N_2O_5Pt.H_2O$: C 39.66; H 4.34; N 4.02%. Found: C 39.42; H 4.45; N 4.01%. ESI-MS (positive-ion mode): *m/z* 677.1535 $[M]^+$. IR (Raman) ν_{max}/cm^{-1}: 3473 (O–H/N–H); 3112, 3080 (C–$H_{(ar)}$); 2955, 2929, 2857 (C–$H_{(al)}$); 1686 (C=O); 1629, 1592 (C=C/C=N); 1552 (δCNH); 1479, 1458, 1364 (δHCH); 1274 (C–O/C–N); 567 (Pt-O); 346, 331 (Pt-Cl).

[Pt(**HL3**)$Cl_2(OH)_2$] **3b**

From 550 mg of **3a**. Yield: 292 mg, 51%; mp 170 °C (dec.). Anal. Calc. for $C_{26}H_{34}Cl_2N_2O_5Pt.2H_2O$: C 41.28; H 5.06; N 3.70%. Found: C 39.89; H 4.99; N 3.88%. ESI-MS (positive-ion mode): *m/z* 719.2115 $[M]^+$. IR (Raman) ν_{max}/cm^{-1}: 3483 (O–H/N-H); 3113, 3080 (C–$H_{(ar)}$); 2953, 2927, 2855 (C–$H_{(al)}$); 1686 (C=O); 1630, 1592 (C=C/C=N); 1554 (δCNH); 1479, 1458, 1365 (δHCH); 1274 (C–O/C–N); 571 (Pt-O); 347, 333 (Pt-Cl).

Biological studies

Cells and culture conditions

The human cell lines used in this work were MDA-MB-435 (melanoma), promyelocytic leukaemia (HL-60), colorectal adenocarcinoma (HCT-8) and glioma (SF-295), which were all obtained from the National Cancer Institute (Bethesda, MD, USA). The cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 1% penicillin, and 1% streptomycin at 37 °C with 5% CO_2.

Proliferation assays

For all experiments cells were plated in 96-well plates (0.7×10^5 cells/well for adherent cells or 0.3×10^5 cells/well for suspended cells). After 24 h the compounds (0.66 to 77.0 μmol L^{-1}) dissolved in 1% DMSO were added to each well using a high-throughput screening system (Biomek 3000-Beckman Coulter, Inc. Fullerton, California, USA) and the cultures were incubated for 72 h. Doxorubicin hydrochloride (Sigma Aldrich) was used as a positive control. Control groups received the same amount of DMSO. Tumor cell growth was quantified by the ability of living cells to reduce the yellow dye 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) to the purple formazan product.[31] At the end of the incubation, the plates were centrifuged and the medium was replaced with fresh medium (200 μL) containing MTT 10%. Three hours later, the plates were centrifuged (3000 rpm/10 min), the MTT formazan product was dissolved in 150 μL DMSO, and the absorbance was measured using a multiplate reader (Spectra Count, Packard, Ontario, Canada). The drug effect was quantified as the percentage of the control absorbance of the reduced dye at 595 nm.

Results and Discussion

Syntheses and general characterization

The yellow-orange platinum(IV) complexes *cis,cis,trans*-[Pt(**HL1-3**)$Cl_2(OH)_2$] **1b-3b** were obtained by oxidation of the respective square planar platinum(II) complexes *cis*-[Pt(**HL1-3**)Cl_2] **1a-3a**[24] with 30% H_2O_2 in acetone, under reflux.[30] These compounds are stable in the solid state. They are insoluble in water, but soluble in polar aprotic solvents, such as acetone, MeCN, DMSO and DMF.

Elemental analyses provided the expected chemical formulae, [Pt(**HL1-3**)$Cl_2(OH)_2$], also supported by the ESI mass spectra which exhibited the molecular ion peaks for **1b-3b** (Figures S1-S3). A broad band around 3450 cm^{-1} in the infrared spectra of all compounds (Figures S4-S7) confirms the presence of water, as suggested by the elemental analyses data.

Differently from complexes **1a-3a**,[24] single crystals of **1b-3b** suitable for X-ray diffraction studies could not be grown under a variety of conditions, however, IR and Raman spectroscopic studies are in accord with the structure proposed for complexes *cis*,*trans*-dichloridodihydroxide **1b-3b**, illustrated in Scheme 1, with two axially coordinated hydroxide ligands.

IR and Raman spectra of the platinum(IV) complexes are similar in terms of wavenumber values (Figures S4-S9), indicating the absence of a symmetry center in these compounds, as expected. Unfortunately, the Raman spectrum of **1b** was seriously disturbed by fluorescence and thus is not presented. Special attention has been paid to the region characterized by metal-ligand vibrations, which is essential to distinguish between square planar platinum(II) and octahedral platinum(IV) complexes. Thus, far-IR and Raman spectra of **HL1-3** were initially acquired. As exemplified by the spectra of **HL2** (Figure S4), they exhibit a spectral window between 300 and 350 cm^{-1}, besides a very weak intensity Raman band around 580 cm^{-1}. The Pt–Cl stretching modes are frequently observed in the former region, whereas the Pt–O stretches appear near the latter.[32-34] These features were used to analyze and compare the Raman spectra of the platinum(II) and platinum(IV) complexes.

Two very well resolved bands can be observed at 321 and 334 cm^{-1} for complex **2a**, and at 331 and 346 cm^{-1} for complex **2b** (Figure 1). These bands have been assigned to the symmetric and asymmetric Cl–Pt–Cl stretches, respectively. It is worth stressing that the bands observed for **2b** are upshifted by ca. 10 cm^{-1}, as compared to **2a**. Our result finds support in the work published by Giandomenico *et al*., who have interpreted such a shift as being due to full oxidation of the platinum atom.[32] However, the range usually reported for Pt–Cl stretches is larger than 10 cm^{-1}, so that the trend observed herein is not sufficient to distinguish between the two complexes. On the other hand, the 567 cm^{-1} band in the spectrum of **2b** is a determining factor for such distinction since it is not present in the spectrum of **2a** and has been commonly assigned to the Pt^{4+}–O vibration. One can also observe, in Figure 1, that the new band is slightly disturbed by another one at 584 cm^{-1}, which is due to the aromatic ring breathing mode of the **HL2**. It is possible that the asymmetric O–Pt^{4+}–O stretching vibration is either hidden under this band envelope, or is not Raman-active. This latter possibility seems pertinent if one recognizes the existence of a linear O–Pt–O moiety, as illustrated in Scheme 1. Unfortunately, the 1035 cm^{-1} band, attributed to the Pt–O–H bending mode[34] is blurred by another band associated to the ligand.

The Pt–N (amine) stretching modes are typically observed near the Pt–O vibrations.[35] Indeed, the mixture of these modes has been also considered by Miller *et al.*[34] with respect to the band at 567 cm^{-1}. Although the Pt–N vibration has not been observed, coordination to the metal through the N atom was indirectly accompanied in this work by the spectral changes exhibited at the 1523 cm^{-1} band, which is ascribed to the C–N–H bending mode of the ligand.[24] It is upshifted by ca. 10 cm^{-1} for platinum(II) complexes and ca. 30 cm^{-1} for platinum(IV) complexes, suggesting that the Pt^{4+} ion significantly affects the electronic density of this oscillator. Information on the Pt–N (pyridine) vibration could not be obtained due to the overlapping of bands at 230 and 260 cm^{-1}, and 1700-1580 cm^{-1}.

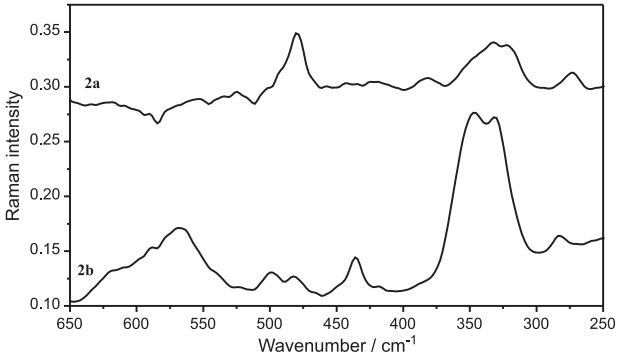


Figure 1. Raman spectra of the complexes **2a** and **2b**.

Electrochemical studies

The biological activity of platinum(IV) complexes depends on their being reduced to platinum(II) species and for this reason the redox potential of those species is believed to be an important parameter in their cytotoxic activity.[7]

Because generation of ROS is one of the possible mechanisms of action of naphthoquinones their electrochemical behavior has been studied thoroughly.[36-38] Cyclic voltammetry studies have shown that 2-hydroxy-1,4-naphthoquinone derivatives undergo two main redox processes in aprotic medium, the first one corresponding to an irreversible (**Ic/Ia**), and the second one to a *quasi*-reversible processes (**IIc/IIa**). Other peaks and shoulders in the cyclic voltammograms (CVs) of this class of compounds have been associated to concentration dependent processes due to the presence of the hydroxyl proton: (*i*) self-protonation reactions (intramolecular proton transfer) and (*ii*) hydrogen-bonding which stabilizes electron generated intermediates.[39,40] Indeed in alkaline medium (in MeCN and in the presence of *n*-Bu_4NOH),[36] only one reversible redox couple (**IIc/IIa**) at more negative potential relative to the original first one has been observed and attributed to the bielectronic instead of double-redox process of the naphthoquinone.

Attempts have been made at correlating cytotoxicity (IC_{50} or LD_{50} values) with the first or second redox $E_{1/2}$ processes of naphthoquinone derivatives.[41] Correlations between LD_{50} values and the second $E_{1/2}$ process were drawn recently for a series of 2-hydroxy-1,4-naphthoquinones.[39]

Because of the very limited aqueous solubility of platinum(II) complexes **1a-3a** and platinum(IV) complexes **1b-3b** the CVs were obtained in MeCN solutions (1.0×10^{-3} mol L^{-1}) containing 0.1 mol L^{-1} *n*-Bu_4NClO_4 as the supporting electrolyte, under argon atmosphere. The cyclic voltammetry of *cis,cis,trans*-$[Pt(NH_3)_2Cl_2(OH)_2]$ (A) was also performed in MeCN (Figure S10) in order to evaluate the effect of the naphthoquinone coordination on the Pt^{4+}/Pt^{2+} redox process. Electrochemical data are summarized in Table 1.

Table 1. Electrochemical data obtained for **1a-3a**, **1b-3b** and *cis,cis,trans*-$[Pt(NH_3)_2Cl_2(OH)_2]$ (**A**) at a scan rate of 100 mV s^{-1} in MeCN + 0.1 mol L^{-1} *n*-Bu_4NClO_4, at 25 °C

E_p / V	Compound						
	1a	**2a**	**3a**	**1b**	**2b**	**3b**	**A**
Ep_{Ic}	–0.67	–0.67	–0.69	–	–	–	–
Ep_{Ia}	–0.30	–0.31	–0.25	–	–	–	–
$Ep_{Ic'}$	broad	broad	broad	–	–	–	–
$Ep_{Ia'}$	–0.96	–0.96	–0.96	–	–	–	–
Ep_{IIc}	–1.58	–1.58	–1.58	–1.65	–1.66	–1.65	–
Ep_{IIa}	–1.36	–1.36	–1.37	–1.47	–1.51	–1.49	–
Ep_{IIIc}	–	–	–	–1.15	–1.18	–1.18	–1.60
ΔEp_I	0.37	0.37	0.44	–	–	–	–
$\Delta Ep_{I'}$	–	–	–	–	–	–	–
ΔEp_{II}	0.22	0.22	0.21	0.18	0.15	0.16	–

Data from voltammetric experiments in a cathodic scan; potential values are reported *vs.* FcH^+/FcH.

The CVs of the platinum(II) complexes (Figures 2a and S11-S13) exhibit two main redox pairs, **Ic/Ia** and **IIc/IIa**, which correspond to irreversible and *quasi*-reversible processes, respectively. The first reduction process is related to the formation of the seminaphthoquinone ($NQOH^-$, i.e., **HL^--Ic/Ia**) and the second, to the electron reduction of the respective anion (NQO^-, i.e., **L^--IIc/IIa**). This indicates that the naphthoquinone ligands **HL1-3** are indeed protonated. The two additional shoulders (**Ic'** and **Ia'**) due to self-protonation reactions and/or hydrogen bonding processes are also observed.[39,40]

In the CVs of all three platinum(IV) complexes **1b-3b** (Figures 2b and S14-S16), an irreversible peak (**IIIc**) attributed to the reduction of Pt^{4+}/Pt^{2+} is observed,[8,9] which is absent in the CVs of the analogous platinum(II) complexes **1a-3a** (Figure 2a). Unexpectedly only one couple of peaks (**IIc** and **IIa**) is observed, which has been attributed to the *quasi*-reversible naphthoquinonate (NQO^-, i.e., L^-) redox process.[36,39] The absence of the expected two redox processes present in the CVs of the analogous **1a-3a** platinum(II) complexes could be explained by the formation of a hydrogen bonded species,[42,43] involving the naphthoquinone 2-hydroxyl group and an axially coordinated hydroxide ligand (Scheme 2), which would shift the first naphthoquinone reduction process to negative potential. Platinum(IV) reduction with concomitant loss of the axial hydroxide ligands would lead to the deprotonation of the coordinated Mannich bases **HL1-3**, as illustrated in Scheme 2 to yield [Pt(**$L1^-$-3^-**)Cl_2].

The result of the cyclic voltammetry experiment of **2b** under the same conditions, except for the addition

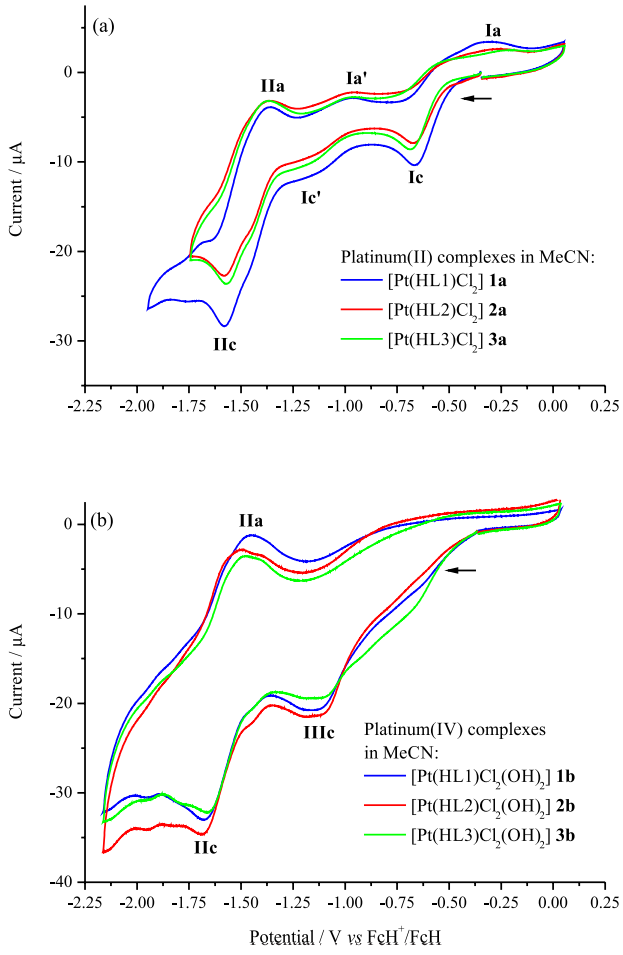
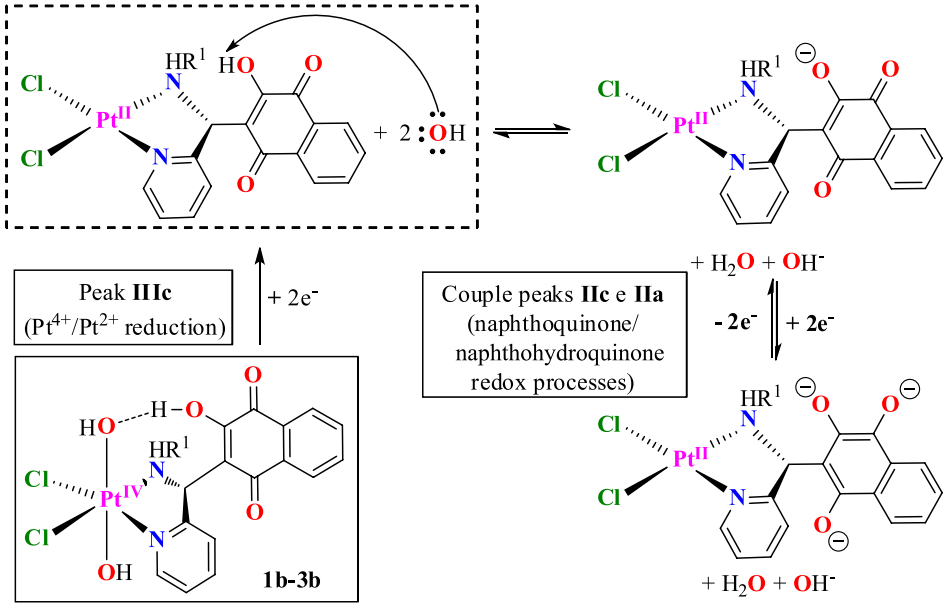


Figure 2. Cyclic voltammograms of **1a-3a** (a) and **1b-3b** (b) obtained with a glassy carbon electrode (3 mm) in 0.1 mol L^{-1} *n*-$BuNClO_4$/MeCN. The potential scan was initiated in the cathodic direction. The cathodic (**Ic**, **Ic'**, **IIc** and **IIIc**) and anodic (**Ia**, **Ia'** and **IIa**) peaks are indicated.



Scheme 2. Possible path for the redox processes of [Pt(**HL1-3**)$Cl_2(OH)_2$] (R^1 = *n*-Bu, **1b**; *n*-heptyl, **2b**; *n*-decyl) and proposed intramolecular hydrogen bond interaction in **1b-3b**.

of 3.0 equiv. of HCl, evidences two redox processes (**Ic/Ia** and **IIc/IIa**, Figure S17), thus confirming that the hydroxide ion is indeed responsible for the deprotonation of the 2-hydroxynaphthoquinones. The cathodic potential values (Ep_{Ic} = −0.67 and Ep_{IIc} = −1.60 V) are the same as those observed for the analogous platinum(II) complex **2a** (Table 1), however the anodic potentials were strongly affected by the presence of the acid: Ep_{Ia} = −0.06 V (**2a**, Ep_{Ia} = −0.31 V) and Ep_{IIa} could not be clearly assigned (Figure S17). The Pt^{4+}/Pt^{2+} irreversible reduction process (Ep_{IIIc}) is still observed at −1.22 V

The Pt^{4+}/Pt^{2+} reduction values for **1b-3b** (Table 1, Ep_{IIIc} = −1.15, −1.18 and −1.18 V *vs* FcH^+/FcH, respectively), in MeCN, are much less negative than for *cis,cis,trans*-$[Pt(NH_3)_2Cl_2(OH)_2]$ (**A**), which has been measured in the same solvent (−1.60 V *vs* FcH^+/FcH), thus showing that coordination of the Mannich bases favors the Pt^{4+}/Pt^{2+} reduction, as compared to amine ligands. The π-acceptor character of the **HL1-HL3** pyridyl group and the steric hindrance of these ligands certainty contribute to this behavior.[8,9] Moreover, the intramolecular hydrogen bond interaction in **1b-3b** proposed above would destabilize the Pt-OH bond, thus making reduction of **1b-3b** easier than for complex **A**. As expected, increasing the size of the R^1 group in the **HL1-HL3** ligands (R^1 = *n*-butyl, *n*-heptyl, *n*-decyl, respectively) does not cause appreciable shifts in the Pt^{4+}/Pt^{2+} reduction potentials of complexes **1b-3b**.

To evaluate whether coordination affects the electrochemical behavior of the pro-ligands, cyclic voltammetry of **HL1-HL3** was performed in MeCN (Figure S18 and Table 2) and compared with the CVs of **1a-3a**, whose naphthoquinone ligand is protonated.

The CVs of **HL1-HL3** in pure MeCN (Figure S18, Table 2) are similar to those previously described for analogous Mannich bases in DMF.[40] Comparison of these data (Table 2) with those obtained for **1a-3a** (Figure 2a, Table 1) indicates that upon coordination to platinum(II) the **HL1-HL3** reduction processes occur at more positive potentials (positive shifts of about 600 mV are observed for the first redox pair **Ic/Ia**), but are less reversible. It seems reasonable to suggest that intramolecular O-H···NHR[1](CHR) hydrogen-bonding occurs in the free ligands, which presumably allows for electron density transfer to the quinone enone system.[42,43] This process would be hindered by **HL1-HL3** coordination to platinum(II), with the consequent positive shift of the reduction potentials, as observed.

The CVs of the deprotonated species $\mathbf{L1^-}$-$\mathbf{L3^-}$, generated *in situ* by adding 1.6 equiv. of 0.02 mol L^{-1} $NaOH_{(aq)}$ (i.e., 400 μL of water)[44,45] to the MeCN solutions of **HL1-HL3** (Figure S19), were measured and compared with the data obtained for the platinum(IV) complexes **1b-3b** measured in MeCN in the presence of the same amount of water (Figure 3, Table 2).

The CVs of the deprotonated Mannich bases $\mathbf{L1^-}$-$\mathbf{L3^-}$ show the expected *quasi*-reversible pair of peaks (**IIc** and **IIa**) related to the formation of NQO^- (i.e., $\mathbf{L^-}$).[45,44] This pair of peaks undergoes slight positive shift upon coordination, in complexes **1b-3b** (Figure 3b, Table 2), under similar conditions, i.e., in the presence of the same amount of water, which also lead to broadening of the complexes' CVs, probably due to decrease in solubility. The irreversible peak (**IIIc**) attributed to the reduction of Pt^{4+}/Pt^{2+} is also positively shifted (70-100 mV) in the presence of water (Figures 2b, 3b and Tables 1, 2). These data indicate that water also stabilizes the coordinated reduced aminonaphthoquinone species.

Cytotoxic assays

The cytotoxicity of the platinum(IV) complexes **1b-3b** has been investigated against four tumor cell lines: MDA-MB-435 (melanoma), HL-60 (promyelocytic

Table 2. Electrochemical data for **HL1-HL3** (a), $\mathbf{L1^-}$-$\mathbf{L3^-}$ (**HL1-HL3** + 1.6 equivalent of aqueous NaOH 0.02 mol L^{-1}) (b) and **1b-3b** in the presence of water (c), obtained in MeCN + 0.1 mol L^{-1} *n*-Bu_4NClO_4 at a scan rate of 100 mV s^{-1}, at 25 °C.

E_p / V	Compound								
	HL1[(a)]	**HL2**[(a)]	**HL3**[(a)]	$\mathbf{L1^-}$[(b)]	$\mathbf{L2^-}$[(b)]	$\mathbf{L3^-}$[(b)]	**1b**[(c)]	**2b**[(c)]	**3b**[(c)]
Ep_{Ic}	−1.22	−1.24	−1.21	–	–	–	–	–	–
Ep_{IIc}	−1.78	−1.81	−1.81	−1.45	−1.46	−1.45	−1.36	−1.39	−1.40
Ep_{Ia}	−0.95	−0.96	−0.99	–	–	–	–	–	–
Ep_{IIa}	−1.66	−1.68	−1.68	−1.31	−1.32	−1.33	−1.27	−1.24	−1.24
Ep_{IIIc}	–	–	–	–	–	–	broad	−1.11	−1.09
ΔEp_I	0.27	0.28	0.22	–	–	–	–	–	–
ΔEp_{II}	0.12	0.13	0.13	0.14	0.14	0.12	0.10	0.13	0.15

Data from voltammetric experiments in a cathodic scan; potential values are reported *vs.* FcH^+/FcH.

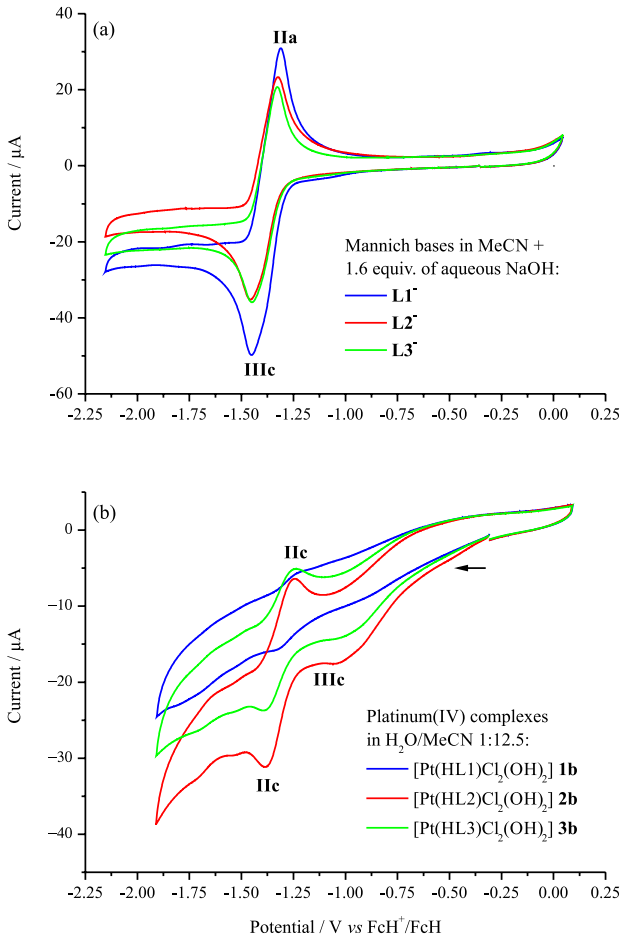


Figure 3. Cyclic voltammograms obtained with a glassy carbon electrode (3 mm) in 0.1 mol L^{-1} *n*-$BuNClO_4$/MeCN of **HL1-HL3** + 1.6 equivalent 0.02 mol L^{-1} $NaOH_{(aq)}$ (400 µL of water) (a), **1b-3b** + 400 µL of water (b). The potential scan was initiated in the cathodic direction. The cathodic (**IIc** and **IIIc**) and anodic (**IIa**) peaks are indicated.

leukaemia), HCT-8 (colorectal adenocarcinoma) and SF-295 (glioblastoma). The experimental data are presented in Table 3, together with the data previously reported for complexes **1a**-**3a** and **HL1**-**HL3** for comparison.

As observed for the pro-ligands (**HL1-HL3**) and the platinum(II) complexes (**1a**-**3a**)[24] (Table 3), the size of the side chain (R^1) plays an important role in the cytotoxicity of the platinum(IV) complexes, as indicated by the much lower activity of **1b** (R^1 = *n*-Bu) when compared with its longer carbon chain analogues **2b** (R^1 = *n*-hexyl) and **3b** (R^1 = *n*-decyl). Our recent accumulation studies[25] confirm that increase of the carbon chain length of R^1 causes significant enhancement of cellular accumulation of complexes **1a-3a**, consequently contributing to the highest cytotoxicity being exhibited by the *n*-heptyl and *n*-decyl derivatives.

As discussed previously, the increase of the R^1 carbon chain length from **1b** to **3b** does not alter significantly the Pt^{4+}/Pt^{2+} reduction potential, which means that the differences observed in the cytotoxicity of these complexes are not influenced by the platinum(IV) reduction in the cells.

One of the potential advantages of the platinum(IV) pro-drugs, when compared with the platinum(II) compounds, is their relative inertness, which prevents deactivation of the drug by undesirable substitution reactions with plasma proteins.[14,15,46,47] This allows a greater proportion of the drug to reach its target intact before undergoing reduction, and may result in increased cytotoxicity.

In this work, we have observed that the platinum(IV) complexes **1b**-**3b** were only slightly more active than their platinum(II) counterparts **1a**-**3a**, except in the case of **3b**, that was almost twice as active as its precursor **3a** against MDA-MB-435 (IC_{50} = 5.5 and 9.0 µmol L^{-1}, respectively)

Table 3. IC_{50} values in µmol L^{-1} of **HL1**-**HL3**, **1a**-**3a** and **1b**-**3b**, after 72 h incubation

Compound	MDA-MB-435	HL-60	HCT-8	SF-295
HL1	> 40[a]	> 40[a]	12.0 (9.2-15.7)[a]	9.0 (7.7-11.9)[a]
HL2	> 40[a]	1.9 (1.3-2.6)[a]	1.6 (1.3-1.9)[a]	1.1 (1.0-1.3)[a]
HL3	23.6 (19.2-28.6)[a]	3.8 (2.8-5.3)[a]	1.7 (1.4-1.9)[a]	1.7 (1.2-1.9)[a]
1a	19.7 (17.9-25.6)[a]	7.1 (3.8-17.9)[a]	21.0 (17.9-24.9)[a]	17.4 (13.9-21.9)[a]
2a	6.4 (5.1-7.8)[a]	5.7 (3.3-10.2)[a]	11.5 (10.1-13.3)[a]	7.9 (7.1-8.7)[a]
3a	9.0 (7.4-11.1)[a]	12.5 (9.5-16.6)[a]	15.9 (14.0-18.2)[a]	11.8 (10.0-14.0)[a]
1b	21.7 (7.8-59.5)	N.D.	20.5 (17.7-23.7)	17.4 (12.0-25.1)
2b	8.8 (7.4-10.3)	N.D.	10.3 (6.4-16.5)	6.6 (3.1-13.8)
3b	5.5 (5.0-6.2)	6.0 (4.4-8.1)	9.2 (7.6-11.1)	10.6 (9.3-12.1)
Cisplatin	15.3 (11.3-20.7)[a]	28.6 (21.3-39.0)[a]	12.3 (9.3-16.7)[a]	26.7 (17.3-41.0)[a]
Doxorubicin	0.86 (0.62-1.2)[a]	0.04 (0.02-0.04)[a]	0.07 (0.05-0.09)[a]	0.07 (0.05-0.09)[a]

[a]data from ref 24, N.D. = not determined. Data are presented with 95% of confidence interval for MDA-MB-435 (melanoma), HL-60 (promyelocytic leukaemia), HCT-8 (colonrectal adenocarcinoma) and SF-295 (glioblastoma). Doxorubicin hydrochloride was used as a positive control. Experiments were performed in triplicate.

and also more active than **3a** against HL-60 and HCT-8 tumor cell lines. Thus, the very similar cytotoxic profile exhibited by the complexes studied in this work may indicate that **1b**-**3b** undergo rapid *in vitro* reduction. It is accepted[46] that the rate of reduction depends on both the rate of electron transfer from the reductant to the platinum(IV) center and on how easily the two Pt–ligand bonds are broken. In the cases of **1b**-**3b**, one of the Pt–OH bonds would be weakened by formation of the hydrogen bonded species proposed above (Scheme 2). Also, fast electron transfer to the platinum(IV) atom has been postulated to occur in complexes containing axial hydroxide ligands, which are capable of forming an inner sphere bridge with the reductant (ascorbate). Thus, both factors would contribute to the formation of platinum(II) derivatives **1a**-**3a** outside the cell.

Conclusions

Spectroscopic and electrochemical studies have been successfully used to structurally characterize three novel aminomethylnaphthoquinone *cis,trans*-dichlorido-dihydroxide platinum(IV) complexes, **1b**-**3b**. The cytotoxicity results have indicated that the hydroxide ligands in the axial position of these platinum(IV) complexes have not led to an appreciable improvement of the cytotoxicity compared to the platinum(II) precursors **1a**-**3a**, probably due to fast reduction of the platinum(IV). A possible intramolecular H–bond between the OH^- axial ligand and the hydroxyl group at position 2 of the naphthoquinone ring may lead to the weakening of the Pt^{4+}–OH bond, thus favoring the reduction. Aiming to improve the cytotoxicity of these aminomethylnaphthoquinone platinum(IV) complexes, the synthesis of novel analogues of **1b-3b** containing different axial ligands capable of tuning the reduction potentials of the platinum(IV) is underway.

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Supplementary Information

Supplementary data are available free of charge at http://jbcs.sbq.org.br as PDF file.

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