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Hydrophilic ligands derived from glucose: Synthesis, characterization and *in vitro* cytotoxic activity on cancer cells of Pt(II) complexes

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ABSTRACT

Aiming to contribute to the design of new antitumoral drugs, we synthesized new hydrophilic Pt(II) complexes of general formula [$PtCl_2$(*N*,*N*′)] containing nitrogen bidentate amine–imine and di-imine ligands derived from glucose. Some chemical properties were discussed. The X-ray molecular structure of [$PtCl_2$(α-D-glucopyranoside-methyl-6-deoxy-6(2-(methylimino)methyl)pyridine) (**D**) was reported. [$PtCl_2$(β-D-glucopyranosylimine-*N*-(2-pyridinylmethyl))] (**A**), which is well-soluble both in organic solvents and in water, was tested for cytotoxicity.

1. Introduction

A topical field of interest is the design of new molecules displaying antitumor activity. Within this ambit, platinum chemistry has been for a long time investigated, for it offers the opportunity to prepare very effective antitumor drugs [1]. The present research aims to combine two features considered relevant in the design of modern antitumor platinum compounds: the established clinical efficacy of complexes with bidentate nitrogen ligands [1], and the presence of suitable auxiliaries of natural origin, i.e. sugars, able to confer useful physical properties such as proper solubility in water, combined with increased membrane permeability.

In particular, interest for nitrogen chelates is demonstrated by literature reports, which describe several Pt(II) compounds with *N*,*N*′-diamines, *N*,*N*′-amine–imine or *N*,*N*′-di-imines ligands developed for the therapy of tumors [2].

At the same time, carbohydrates chemistry deserves growing attention within the pharmaceutical field. In fact, sugars are often convenient sources of chiral auxiliaries, and, hence, useful building blocks for drugs. In addition, carbohydrates display several functional groups, which can be easily functionalised. These features allow the synthesis of molecules with tunable properties [3]. For example, suitable protection or deprotection of the hydroxyl groups can significantly affect the solubility properties or improve the membrane permeability.

Currently, innovative drugs containing sugar residues, such as bleomicine and adriamicine [2c], are used in antitumor therapy. Advantageously, these molecules are more water-soluble than conventional medicines. Furthermore, they are well tolerated by the organism, probably because their carbohydrate fragments resembles the molecules which are naturally involved in several biological functions and which are active constituents of glycolipids, glycoproteins and nucleotides.

On the grounds of these considerations we have developed the synthesis of chelating nitrogen ligands (*N*,*N*′-amine–imine and *N*,*N*′-di-imines) derived from glucose, and of the corresponding complexes of platinum(II) [$PtCl_2$(*N*,*N*′)] for an evaluation of their *in vitro* cytotoxic activity. This strategy is expected to yield compounds more featuring than the traditional drugs based on platinum, in terms of higher activity, reduced toxicity and easier administration.

2. Experimental

2.1. General methods

Mono- and bi-dimensional NMR spectra were recorded in $CDCl_3$ ($CHCl_3$, $\delta = 7.26$; $^{13}CDCl_3$, $\delta = 77.0$), CD_3OD (CHD_2OD, $\delta = 3.30$; $^{13}CD_3OD$, $\delta = 49.05$), $(CD_3)_2SO$ [$(CHD_2)_2SO$, $\delta = 2.35$; ($^{13}CHD_2)_2SO$, $\delta = 39.50$], by using 200, 300, 400, 500 MHz spectrometers (Varian Model Gemini, Bruker).

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The following abbreviations were used for describing NMR multiplicities: s, singlet; d, doublet; t, triplet; dd, double doublet; dt, double triplet; m, multiplet; q, quartet. Chemical shift were reported in δ and coupling constants in hertz.

Specific optical rotatory powers [α] were measured with a Perkin–Elmer Polarimeter (model 141) at 298 K and 589 nm in methanol, dichloromethane and dimethylsulphoxide (c = 1.0 g per 100 mL).

The synthesis of β-D-glucosamine (**a1**) [4], phenylmethyl 2-amino-2-deoxy-4,6-O-(1-methylethylidene)-α-D-glucopyranoside (**b1**) [5], phenylmethyl 2-amino-2-deoxy-α-D-glucopyranoside (**c1**) [6], methyl 6-azido-6-deoxy-α-D-glucopyranoside (**d1**) [7], *cis*-platinum-dichlorobis(methyl sulfoxide) [8] are described in literature. 1,4-Dioxane was distilled from Na/benzophenone.

2.2. Synthesis of [$PtCl_2$(***1-Im***)] (***A***)

To a suspension of **a1** (0.215 g, 1.20 mmol) in dioxane (4 mL), py-2-aldehyde (0.115 mL, 1.20 mmol) was added. The addition of [$PtCl_2(DMSO)_2$] (0.506 g, 1.20 mmol) at 298 K afforded the product **A** as an orange precipitate. After 30 min of stirring, the mixture was centrifuged, the solid washed with dioxane (2 × 3 mL) and diethyl ether (3 × 3 mL) and dried under vacuum. Then it was further purified by column chromatography on silica gel with ethylacetate/methanol (7/3 v/v) as eluent and the product was obtained in 62% of yield (0.400 g).

^{1}H NMR, CD_3OD, 200 MHz: δ 9.53 (d, 1H, py, ^3J (Pt–H) = 36), 9.41 (s, 1H, N=CH, ^3J (Pt–H) = 92), 8.35 (t, 1H, py), 8.16 (dd, 1H, py), 7.86 (dd, 1H, py), 5.75 (d, 1H, H1, ^3J (H1–H2) = 8), 4.00–3.20 (m, 5H, H2, H3, H4, H5, H6ax, H6eq).

^{13}C NMR, CD_3OD, 100 MHz: δ 172.3, 157.5, 149.9, 140.5, 129.5, 128.4, 91.8, 80.0, 77.6, 75.8, 69.1, 61.8. Optical activity: [α] (CH_3OH, 0.01 g/mL): +2.

Anal. Calc. for $C_{12}H_{16}Cl_2N_2O_5Pt$: C, 26.98; H, 3.02; N, 5.24. Found: C, 26.75; H, 3.15; N, 5.20%.

2.3. Synthesis of [$PtCl_2$(***2-Am***)] (***B***)

2.3.1. Synthesis of benzyl-2-(E-[2-pyridinyl-methylene]amino)-4,6-O-isopropylidene-2-deoxy–D-glucoside b2

To a suspension of **b1** (1.00 g, 3.23 mmol) in toluene, py-2-aldehyde (0.310 mL, 3.23 mmol) was added. The mixture was refluxed for 2 h. Then the solvent was removed under vacuum and the product obtained as an oil without further purification (1.30 g, yield 100%).

^{1}H NMR, $CDCl_3$, 200 MHz: δ 8.58 (s, 1H, CH=N), 8.38 (d, 1H, py), 8.08 (d, 1H, py), 7.4–6.60 (m, 7H, py, Ph), 4.75 (d, 1H, H1, ^3J (H1–H2) = 3.4), 4.60 (d, 1H, CH*H*Ph, ^2J = 12), 4.47 (t, 1H, H3, ^3J (H3–H2) = ^3J (H3–H4) = 8.3), 4.38 (d, 1H, C*H*HPh), 4.10 (dt, 1H, H5, ^3J (H5–H4) = ^3J (H5–H6ax) = 8.3; ^3J (H5–H6eq) = 4.1), 3.90 (dd, 1H, H6eq, ^2J (H6eq–H6ax) = 11), 3.75 (t, 1H, H6ax), 3.65 (t, 1H, H4), 3.45 (dd, 1H, H2), 1.52 (s, 3H, CH_3), 1.38 (s, 3H, CH_3).

2.3.2. Synthesis of benzyl-2-([2-pyridinyl-methyl]amino)-4,6-O-isopropylidene-2-deoxy–D-glucoside b3

To a cold solution of **b2** (1.30 g, 3.23 mmol) in methanol/toluene 1/1 v/v (12 mL), $NaBH_4$ (0.245 g, 6.54 mmol) was added at 273 K. After 24 h of stirring at 298 K, 25 mL of a saturated solution of ammonium chloride was added and the product extracted with CH_2Cl_2 (4 × 15 mL). The organic extracts were collected and dried over sodium sulfate. The solvent was removed under vacuum to get 1.10 g of the product (yield 85%).

^{1}H NMR, $CDCl_3$, 200 MHz: 8.38 (d, 1H, py), 7.20–6.40 (m, 8H, py, Ph), 4.80 (d, 1H, H1, ^3J (H1–H2) = 3.3), 4.55 (d, 1H, CH*H*Ph, ^2J = 12), 4.20 (d, 1H, C*H*HPh), 4.00–3.60 (m, 7H, H3, H4, H5, H6eq, H6ax, NCH_2), 2.75 (dd, 1H, H2, ^3J (H2–H3) = 8.3), 1.48 (s, 3H, CH_3), 1.38 (s, 3H, CH_3).

2.3.3. Synthesis of benzyl-2-([2-pyridinyl-methyl]amino)-2-deoxy–D-glucoside 2-Am

b3 (1.10 g, 2.74 mmol) was dissolved in aqueous acetic acid solution (6 mL, 80% v/v) and the mixture refluxed for 2 h. Then toluene was added and co-evaporated (3 × 2 mL). The product was washed with hexane (3 × 2 mL), diethyl ether (3×2 mL) and dried under vacuum (0.991 g, yield 86%).

The obtained product **b4** (0.991 g, 2.36 mmol) was dissolved in hot water (5 mL) and neutralized with KOH (0.230 g, 4.12 mmol). The mixture was stirred for 30 min. The product was then extracted with dichloromethane (4 × 10 mL). The organic phases were collected and dried over sodium sulfate. The solvent was removed under vacuum and the ligand **2-Am** obtained as a brown oil (0.522 g, yield 61%).

^{1}H NMR, $CDCl_3$, 300 MHz: δ 8.50 (d, 1H, py), 7.64 (t, 1H, py), 7.40–7.15 (m, 7H, py, Ph), 4.94 (d, 1H, H1, ^3J (H1–H2) = 3.3), 4.70 (t, 1H, H4, ^3J (H4–H5) = ^3J (H4–H3) = 10.7), 4.71(d, 1H, CH*H*Ph, ^2J = 12), 4.44 (d, 1H, C*H*HPh), 3.98 (q_{AB}, 2H, NCH_2, ^2J = 16.0), 3.85–3.55 (m, 4H, H3, H5, H6ax, H6eq), 2.72 (dd, 1H, H2, ^3J (H2–H3) = 10.7). ^{13}C NMR, $CDCl_3$, 75 MHz: δ 159.5, 148.9, 137.4, 136.8, 128.3, 128.0, 127.7, 122.4, 122.1, 96.5, 73.0, 71.6, 71.0, 69.3, 62.0, 52.3. Optical activity [α] (CH_3OH, 0.01 g/mL): +92.3.

2.3.4. Synthesis of ***B***

To a solution of **2-Am** (0.200 g, 0.552 mmol) in dioxane (4 mL), [$PtCl_2(DMSO)_2$] (0.233 g, 0.552 mmol) was added. The mixture was stirred at 323 K for 24 h. A light brown solid precipitated and it was filtrated, washed with dioxane (3 × 5 mL) and diethyl ether (3 × 5 mL) and dried under vacuum. The complex was obtained as a yellow-orange product and exists as a couple of diastereoisomers in 5/1 ratio (240 mg, yield 69%).

^{1}H NMR, $CD_3OD/CDCl_3$ (5/1 v/v), 200 MHz, major diastereoisomer: δ 8.99 (d, 1H, py), 7.62 (t, 1H, py), 7.30–6.90 (m, 7H, py, Ph), 6.29 (d, 1H, H1, ^3J (H1–H2) = 3.2), 4.60 (d, 1H, NCH*H*, ^2J = 13), 4.50 (q_{AB}, 2H, CH_2Ph, ^2J = 16), 4.05 (dd, 1H, H3, ^3J (H3–H2) = 8.5, ^3J (H3–H4) = 10), 3.78 (d, 1H, NC*H*H), 3.70–3.30 (m, 5H, H2, H4, H5, H6ax, H6eq). Selected resonances ^{1}H NMR of the minor diastereoisomer: δ 8.92 (d, 1H, py), 5.88 (d, 1H, H1, ^3J (H1–H2) = 2.8).

^{13}C NMR, $CD_3OD/CDCl_3$ (5:1), 50 MHz: δ 168.2, 147.9, 139.4, 137.9, 129.0, 128.6, 128.4, 124.0, 121.8, 99.6, 74.3, 72.1, 70.5, 68.9, 65.7, 62.1, 56.7. Optical activity [α] (CH_3OH, 0.01 g/mL): +74.5.

Anal. Calc. for $C_{19}H_{24}Cl_2N_2O_5Pt$: C, 36.44; H, 3.86; N, 4.47. Found: C, 36.54; H, 3.78; N, 4.68%.

2.4. Synthesis of [$PtCl_2$(***2-Im***)] (***C***)

A solution of **c1** (0.130 g, 0.490 mmol) and py-2-aldehyde (0.047 mL, 0.490 mmol) in methanol (3 mL), was refluxed for 2 h. Then [$PtCl_2(DMSO)_2$] (0.207 g, 0.490 mmol) was added and the mixture was refluxed for 15 min and kept for 20 min at room temperature. The slow addition of diethyl ether afforded the complex as a brick red solid that was washed with diethyl ether and dried under vacuum (0.199 g, yield 65%).

^{1}H NMR, CD_3OD, 500 MHz: δ 9.48 (d, 1H, py, ^3J(Pt–H) = 39), 9.01 (s, 1H, N=CH, ^3J(Pt–H) = 102), 8.32 (t, 1H, py), 8.05 (d, 1H, py), 7.83 (t, 1H, py), 7.25–7.00 (m, 5H, Ph), 5.51 (d, 1H, H1, ^3J(H1–H2) = 3.4), 5.04 (dd, 1H, H2, ^3J(H2–H3) = 11), 4.71(d, 1H, CH*H*Ph, ^2J = 12), 4.38 (d, 1H, C*H*HPh), 4.29 (t, 1H, H3, ^3J (H3–H4) = 11), 3.80 (m, 1H, H6eq), 3.70 (m, 2H, H5 e H6ax), 3.47 (m, 1H, H4).

^{13}C NMR, CD_3OD, 75 MHz: 171.8, 159.1, 150.7, 141.6, 138.6, 130.1, 129.6, 129.5, 129.4, 129.0, 98.2, 74.7, 72.3, 71.8, 70.7, 70.3, 62.6.

Optical activity [α] (CH_3OH, 0.01 g/mL): +111.9.

Anal. Calc. for $C_{19}H_{22}Cl_2N_2O_5Pt$: C, 36.55; H, 3.55; N, 4.49. Found: C, 36.49; H, 3.50; N, 4.58%.

2.5. Synthesis of the complex [$PtCl_2$(**6-Im**)] (**D**)

In an ice cooled flask dimethylphenylphosphine (0.144 mL, 1.00 mmol) was dissolved in dioxane (5 mL) under nitrogen atmosphere. In a second flask **d1** (0.219 g, 1.00 mmol) was dissolved in dioxane (5 mL) and added drop by drop to the solution of the phosphine. A vigorous production of nitrogen was observed. After 2 h of stirring at room temperature py-2-aldehyde (0.096 mL, 1.00 mmol) was added and the mixture refluxed for 2 h. Then [$PtCl_2(DMSO)_2$] (0.421 g, 1.00 mmol) was added. After 20 min, the addition of 30 mL of diethyl ether afforded an orange solid that was filtrated and washed with diethylether, dried under vacuum. 0.410 g of the product was obtained (yield 75%).

^{1}H NMR, DMSO-*d6*, 500 MHz: δ 9.36 (d, 1H, py), 9.09 (s, 1H, N=CH, ^3J (Pt–H) = 98.0), 8.37 (t, 1H, py), 8.18 (d, 1H, py), 7.90 (t, 1H, py), 5.10 (d, 1H, OH, ^3J = 4.9), 4.90 (br, 1H, OH), 4.80 (br, 1H, OH), 4.47 (d, 1H, H1, ^3J = 3.8), 3.98 (t, 1H, H3, ^3J (H3–H4) = ^3J (H3–H2) = 10), 3.63 (t, 1H, H4, ^3J (H4–H5) = 10), 3.55 (s, 3H, OMe), 3.20 (m, 1H, H5), 3.05 (m, 1H, H2).

^{13}C NMR, DMSO, 75 MHz: 172.4, 156.5, 149.0, 140.7, 129.1, 128.3, 99.6, 73.2, 71.9, 68.1, 66.3, 60.8, 54.2.

Optical activity [α] (DMSO, 0.07 g/mL): +129.

Anal. Calc. for $C_{13}H_{18}Cl_2N_2O_5Pt$: C, 28.48; H, 3.31; N, 5.11. Found: C, 28.31; H, 3.27; N, 4.25%.

2.6. Structure determination

Yellow crystals suitable for X-ray diffraction were obtained by slow evaporation from a methanol solution of the complex at 298 K. Data collection was performed at room temperature on a Bruker–Nonius kappa CCD diffractometer (Mo Kα radiation, CCD rotation images, thick slices, φ scans + ω scans to fill the asymmetric unit). Cell parameters were determined from 37 reflections in the range $3.056° \leqslant \theta \leqslant 16.527°$. Semiempirical absorbtion correction (multi-scan SADABS) [9] was applied. The structures were solved by direct methods (SIR 97 package) [10] and refined by the full matrix least-squares method (SHELXL program of SHELX97 package) [11] on F^2 against all independent measured reflections, using anisotropic thermal parameters for all non-hydrogen atoms. All H atoms, except for the hydroxyl protons, were positioned geometrically. The hydroxyl H atoms were located in a difference Fourier map due to the influence on their position of hydrogen bond with the water crystallization molecules. The coordinates of these H atoms were refined, with an isotropic displacement parameter. In the final Fourier a small electron density (0.71 e A^{-3}) was found close to Pt1 atom (1.15 Å). Crystal data and details of the data collection are reported in Table 1.

2.7. Materials and methods for the cytotoxic activity

The MTT assay, already described by Mosmann [12], is a cytotoxicity test based on the metabolic reduction of a soluble tetrazolium salt (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) (Sigma) by a mitochondrial enzyme of cultured cells into an insoluble coloured formazan product. After harvesting, the cells were counted and diluted appropriately with culture medium; 100 μL containing 3000 (HeLa) or 5000 (MCF-7) cells were seeded in each well of a 96-well microtiter plate (Corning). After 24 h of incubation, cisplatin and its analogue were administered to each well in appropriate concentrations (from 1 to 1000 μM). The toxicity of these compounds was tested for 24, 48, and 72 h. At the end of each incubation, MTT was added at a final concentration of 500 μg/mL. Both Pt(II) complexes and MTT were dissolved in PBS (phosphate buffered saline). After 4 h of incubation, the amount of formazan was spectrophotometrically measured at 550 nm wavelength in 2-propanol. Optical density was used to calculate cell growth inhibition, as% with respect to the control.

For the statistical analysis of the data the Bonferroni–Dunn test was used and a p value <0.05 was considered significant. All the results are the mean of three, separately performed, experiments.

Table 1
Crystal, collection and refinement data for **D**.

Temperature (K)	298
Chemical formula	$C_{13}H_{18}Cl_2N_2O_5Pt{\cdot}2H_2O$
Wavelength (Å)	0.71069
Crystal system	monoclinic
Space group	*P*21
a (Å)	6.054(3)
b (Å)	6.849(4)
c (Å)	22.854(4)
β (°)	96.19(2)
V (Å^3)	942.1(3)
Z, D_x (g/cm^3)	2, 2.060
μ (mm^{-1})	7.767
θ Range (°)	3.11–25.00
Reflections collected	8771
Unique observed reflections	3030 (R_{int} = 0.0621)
Data/parameters	3030/228
R_1, wR_2 [$I > 2\sigma(I)$]	R_1 = 0.0373, wR_2 = 0.0739
R_1, wR_2 (all data)	R_1 = 0.0523, wR_2 = 0.0796
Largest differences in peak and hole (e Å^{-3})	0.710

$R_1 = \frac{\sum ||F_o|-|F_c||}{\sum |F_o|}$; $wR_2 = \left\{\frac{\sum [w(F_o^2-F_c^2)^2]}{\sum [w(F_o^2)^2]}\right\}^{\frac{1}{2}}$.

3. Result and discussion

Four new Pt(II) complexes having a *N*,*N*′-di-imine or a *N*,*N*′-amino–imine ligand derived from glucose were synthesized and characterized (Fig. 1) [13].

All the ligands present two nitrogen atoms in position useful to chelate the metal. One of these functions lies on a sugar ring derived from D-glucose, while the other one belongs to a pyridine moiety. More precisely, **1-Im**, **2-Im**, **2-Am** and **6-Im** display the imino or amino function respectively in position 1, 2 and 6 of the

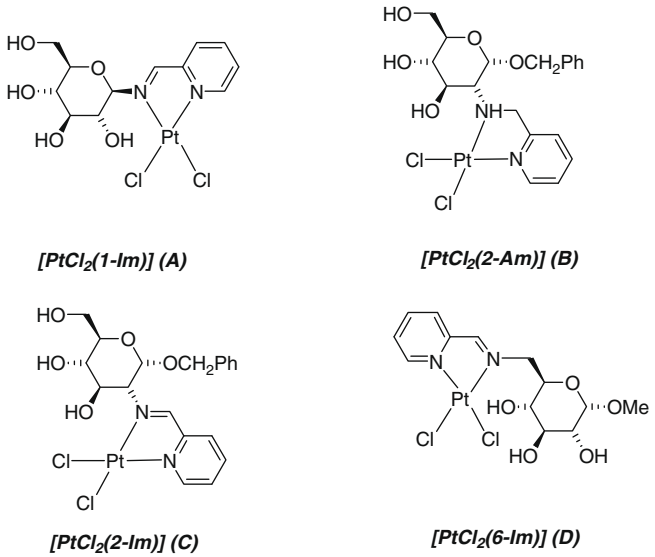


Fig. 1. [$PtCl_2$(*N*,*N*′)] complexes.

sugar ring. In all cases, the presence of free hydroxyl functions is useful to enhance the solubility in water of the corresponding complexes.

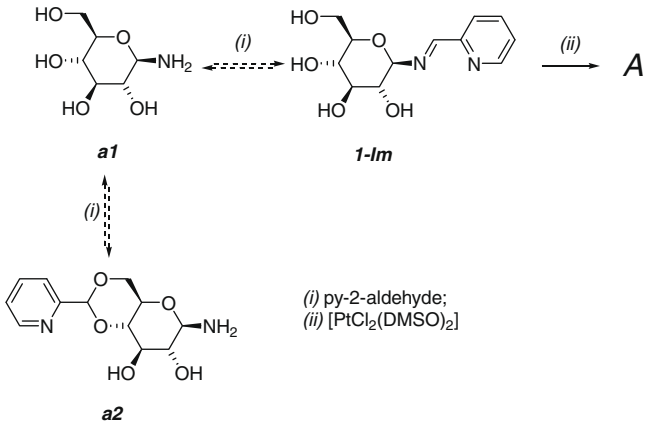
The complexes were obtained by reaction of the appropriate *N,N′* ligand with $[PtCl_2(DMSO)_2]$. All the novel compounds were fully characterized by mono and bi-dimensional NMR spectroscopy and optical measurements. The crystal structure of the complex **D** was determined. The synthetic pathway for ligands and complexes is described in Section 3.1. Cytotoxic activity of complex **A** was also discussed in Section 3.2.

3.1. Synthesis and characterization

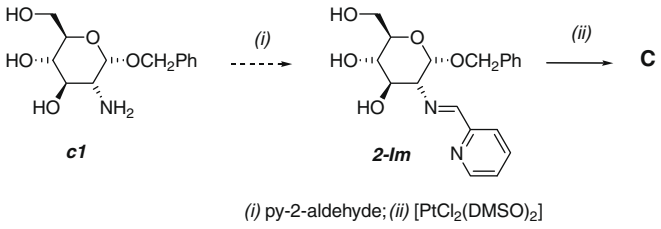
Complex **A** was obtained *in situ* by adding $[PtCl_2(DMSO)_2]$ to the reaction mixture in 1,4-dioxane of 1-amino-β-D-glucopyranoside (**a1**) with a stoichiometric amount of 2-pyridine-carboxaldehyde (Scheme 1). This procedure was necessary because the reaction of the aldehyde with the amino-sugar leads not only to the desidered **1-Im** ligand [14], but also to acetal **a2** (evidenced by the presence of a singlet at 5.53 ppm in the ^{1}H NMR spectrum of the crude reaction mixture). Therefore the addition of Pt(II) substrate contributed to shift the equilibrium toward the desidered product **A**. The ^{1}H NMR spectrum of the isolated compound showed a significant downfield shift for the imine proton upon coordination of the ligand to the metal center (δ 8.53 and 9.36 ppm for the ligand and the complex, respectively). The coupling constant to ^{195}Pt of the imine proton, in transoid position to the metal, is significantly larger than that of the CH=N-pyridine proton (J (Pt–H) = 92 Hz and 36 Hz, respectively), as already noted for pyridinecarboxyaldimines containing aromatic groups [2d]. Complex **A** showed high solubility in water (10 mg/mL) and in organic solvents (i.e. 15 mg/mL in methanol and in chloroform). In virtue of these features, the *in vitro* cytotoxic activity of this complex was evaluated (Section 3.2).

Ligand **2-Am** was obtained according to Scheme 2. The amine-sugar **b1** was condensed with 2-pyridine-carboxaldehyde; the resulting imine (**b2**) was reduced with sodium borohydride (**b3**), then deprotected in positions 4, 6 (**b4**) and neutralized (**2-Am**). The isolated ligand was reacted with the Pt(II) substrate, affording the desired product **B**. The complexation reaction was performed in 1,4 dioxane at 323 K, since the reaction was slower than the precedent one (ii, Scheme 1). In these conditions, the complex was obtained as a diasteroisomeric mixture in 5:1 ratio, as evidenced by ^{1}H NMR spectrum, being different for the configuration of the amino-nitrogen coordinated to the metal. Also complex **B** is soluble in water (4 mg/mL) as well as in organic halogenated solvents.

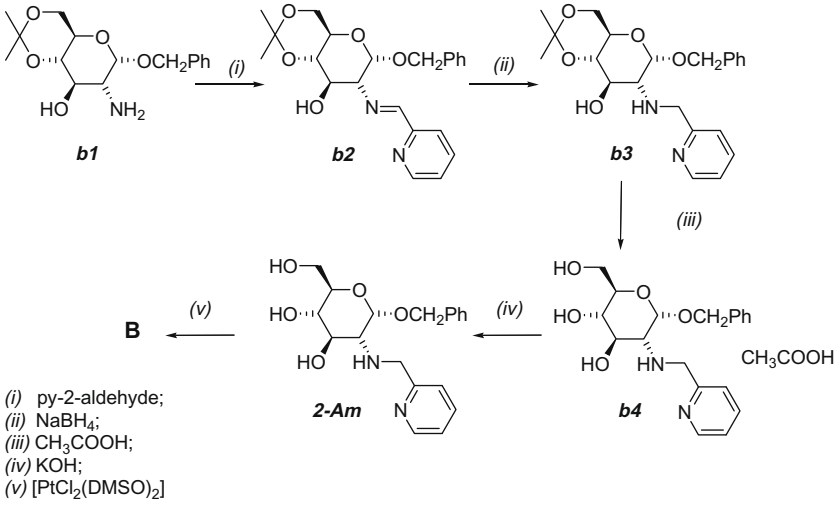
Similarly to **1-Im**, isolation of **2-Im** upon reaction of **c1** with 2-pyridine-carboxaldehyde (Scheme 3) was prevented by the possible formation of the acetal in 4, 6 position. For this reason complex **C** was obtained by adding the platinum precursor *in situ*. The ^{1}H NMR spectrum showed the downfield shift of the coordinated imine proton respect to the corresponding proton of the free ligand (δ 9.02 and 8.38 ppm, respectively) and the coupling to ^{195}Pt of the imine and pyridine protons in transoid and cisoid positions to the



Scheme 1. Synthetic pathway for **A**.



Scheme 3. Synthetic pathway for **C**.



Scheme 2. Synthetic pathway for **B**.

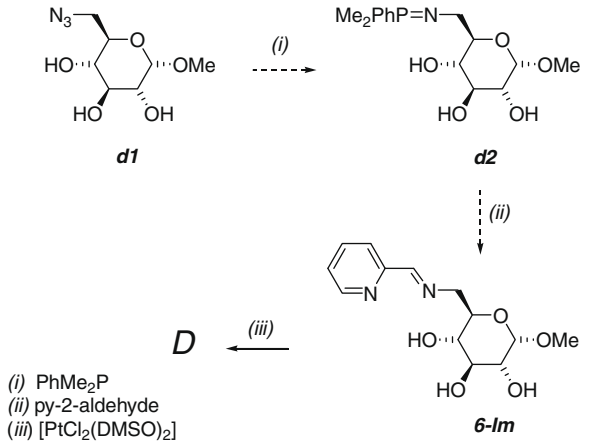
metal, respectively (^3J (Pt–H) = 101.6 and 38.8 Hz). Complex **C** shows good solubility in methanol, but poor and slow dissolution in water.

Finally, the synthesis of **D** was accomplished according to Scheme 4. Azide **d1** was reacted with dimethyl phenylphosphine affording the imine-phosphorane **d2.** Adding *in situ* the aldehyde and, 2 h later the Pt(II) substrate, afforded complex **D**.

This compound shows good solubility in acetonitrile, low in methanol, and poor in water and in organic halogenated solvents. By slow evaporation from a methanol solution of the complex at room temperature, single crystals suitable for X-ray characterization were obtained. Molecular structure is reported in Fig. 2. Complex **D** crystallizes in monoclinic system (space group *P*21). In the unit cell, for each complex molecule, two crystallization water molecules, bound through hydrogen bonds to the hydroxyl groups of the sugar ring are present. The geometry at metal centres is typical square planar, defined by two *cis* chloride atoms and two nitrogen atoms of the imine-pyridine ligand, even if a slight deviation from the regular coordination (N2–Pt1–N1 = 80.0(5)°) is observed, probably due to steric constraint of the nitrogen chelate. The pyridine ring is almost coplanar to the coordination plane (angle between the mean planes is 7.3(6)°). Pt–N e Pt–Cl bond distances are typical for similar imine-pyridine Pt(II) complexes [2d]. The sugar ring adopts the expected chair conformation and its mean plane is almost perpendicular to the coordination plane of the metal (angle between the mean planes is 70.3(6)°; Pt1–N2–C7–C8 = 82.6(1)°), arrangement previously reported for a palladium complex containing a similar ligand [15].

3.2. Cytotoxic activity

Complex **A** was investigated by checking its *in vitro* cytotoxic effect on two cellular lines with respect to cisplatin: HeLa (derived from human tumoral endometrium) and MCF-7 cells (from human breast carcinoma), sensitive and resistant to cisplatin, respectively. The new complex and cisplatin were administered at different concentrations (1, 10, 100, 200, 500, and 1000 μM) and their cytotoxicity was measured at different incubation times (24, 48, and 72 h) performing the MTT test.



Scheme 4. Synthetic pathway for **D**.

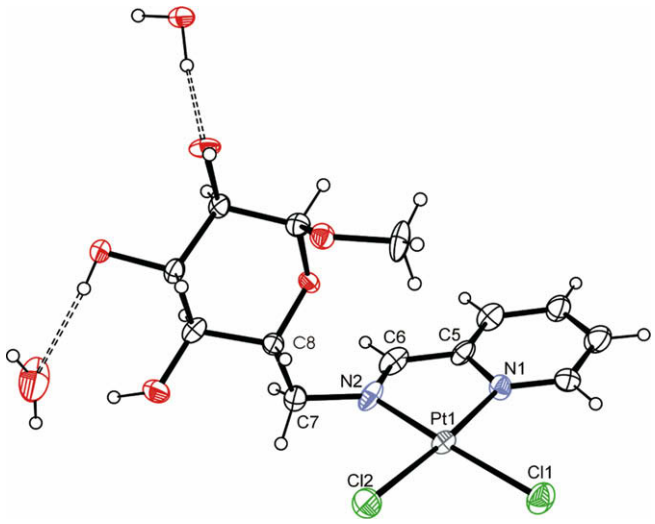


Fig. 2. ORTEP-3 view of the complex **D**. Thermal ellipsoids are shown at 30% probability level. Selected bond distances and angles (Å, °): Pt1–Cl1 = 2.294(3), Pt1–Cl2 = 2.296(3), Pt1–N1 = 2.006(9), Pt1–N2 = 2.002(8), N1–C5 = 1.38(1), C5–C6 = 1.42(2), N2–C6 = 1.27(2), N2–Pt1–N1 = 80.0(5), N2–Pt1–Cl2 = 95.7(4), Cl2–Pt1–Cl1 = 90.6(1), N1–Pt1–Cl1 = 93.8(3), N1–C5–C6–N2 = 2.3(2), Pt1–N2–C7–C8 = 82.6(1).

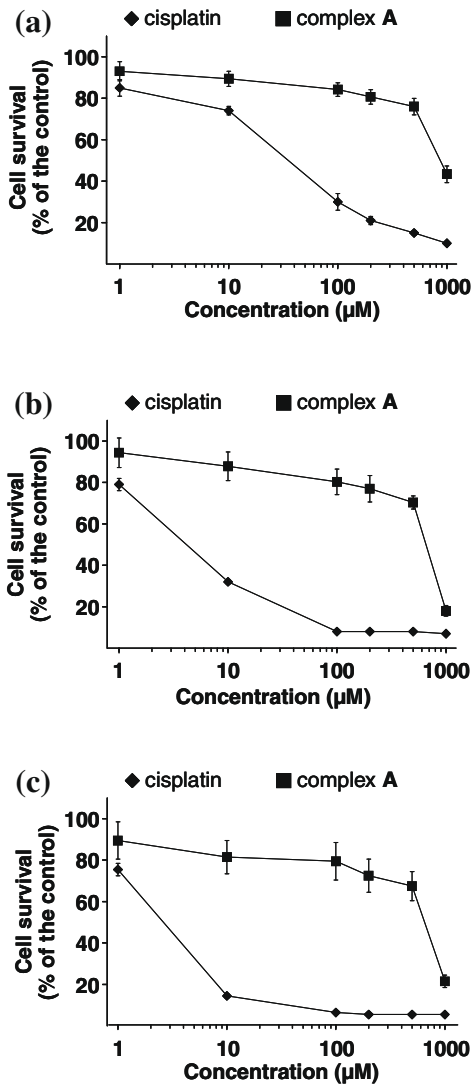


Fig. 3. The sensitivity of HeLa cells to Pt(II) complexes. Cells were treated with increasing concentrations of complex **A** and cisplatin; viable cell number was determined after 24 h (a), 48 h (b), and 72 h (c) of incubation by MTT assay, respectively. The data are the means ± SD of three different experiments run in eight replicates and are presented as percent of control.

After 24 h incubation on HeLa cells complex **A** showed a much lower cytotoxic effect with respect to cisplatin, reaching an IC_{50} value (concentration required for 50% growth inhibition) of 893.9 ± 35.8 μM whereas cisplatin gave the same cytotoxic effect at 59.1 ± 1.8 μM (Fig. 3a and Table 2). The new platinum compound exhibited a negligible cytotoxicity in the 1–500 μM concentration range whereas 100 μM cisplatin already resulted in a 70% depletion of viable cells. The highest *in vitro* cytotoxicity was obtained at the 1000 μM dose: 43% of cell survival for complex **A** and only 10% for cisplatin (Fig. 3a).

Table 2
The IC_{50} values (concentration required for 50% growth inhibition of cell cultures) of complex **A** and cisplatin on HeLa and MCF-7 cells, calculated after 24, 48, and 72 h of incubation. The data are the means ± SD of three different experiments.

	24 h	48 h	72 h	
Cisplatin	59.1 ± 1.8	6.6 ± 0.1	4.7 ± 0.1	HeLa
Complex **A**	893.9 ± 35.8	692.3 ± 20.8	684.8 ± 34.2	
Cisplatin	833.4 ± 33.3	41.9 ± 1.3	9.7 ± 0.5	MCF-7
Complex **A**	>1000	800.0 ± 24.0	719.6 ± 54.0	

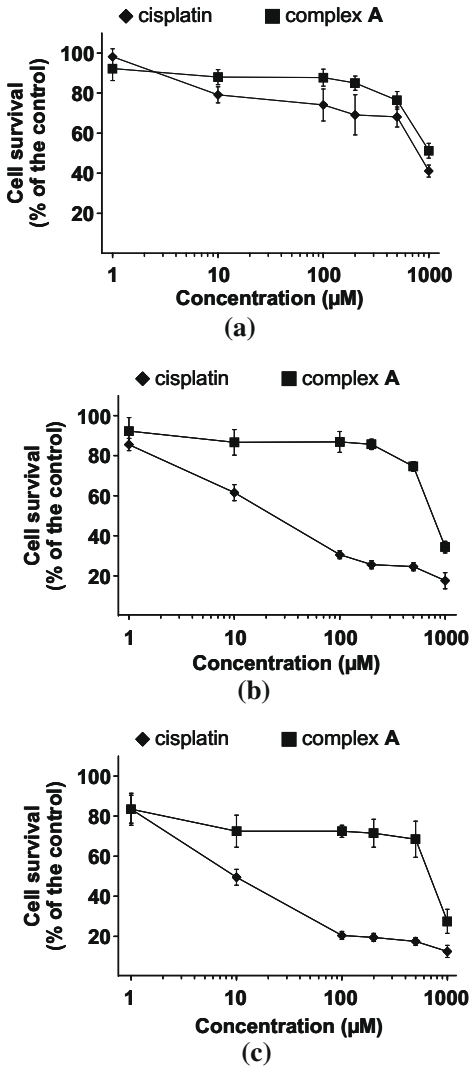


Fig. 4. The sensitivity of MCF-7 cells to Pt(II) complexes. Cells were treated with increasing concentrations of complex **A** and cisplatin; viable cell number was determined after 24 h (a), 48 h (b), and 72 h (c) of incubation by MTT assay. The data are the means ± SD of three different experiments run in eight replicates and are presented as percent of control.

The different biological effect of the two molecules was also observed at longer incubation times (48 and 72 h), especially in the 1–500 μM concentration range, giving a lower cytotoxicity for complex **A** with respect to cisplatin (IC_{50} values at 48 and 72 h were 692.3 ± 20.8 μM and 684.8 ± 34.2 μM for the new synthesized compound; 6.6 ± 0.1 μM and 4.7 ± 0.1 μM for cisplatin). At the highest tested dose (1000 μM) cell survival reached 18% and 21% for complex **A** and only 7% and 5% for cisplatin, after 48 and 72 h, respectively (Fig. 3b and c, and Table 2).

The biological assays on MCF-7 cells showed after 24 h in the 1–500 μM concentration range a very low and comparable cytotoxic effect for both the new synthesized Pt complex and cisplatin, although complex **A** IC_{50} value was significantly higher (a value >1000 μM for the new complex and 833.4 ± 33.3 μM for cisplatin, Fig. 4a and Table 2).

At longer incubation times (48 and 72 h) the differences between the new complex and cisplatin became more evident. In the 1–500 μM concentration range the complex **A** exhibited negligible toxicity, whereas 100 μM cisplatin already resulted in a 70% and 80% depletion of viable cells, after 48 and 72 h, respectively (IC_{50} values at 48 and 72 h were 800.0 ± 24.0 μM and 719.6 ± 54.0 μM for the new synthesized compound and 41.9 ± 1.3 μM and 9.7 ± 0.5 μM for cisplatin, Fig. 4b and c, and Table 2).

In conclusion the complex **A** was characterized by higher cytotoxicity on HeLa cells with respect to MCF-7 cells, as observed for cisplatin although with a much lower effectiveness. Indeed the IC_{50} values measured on HeLa cell cultures were all significantly lower than the corresponding IC_{50} values measured on MCF-7 cells both for the new compound and cisplatin, but new complex IC_{50} values were much higher with respect to cisplatin IC_{50} (Table 2). This result was confirmed by the time-course analysis of the cytotoxic effect of the complex **A** and cisplatin at the highest tested dose (1000 μM, Fig. 5) where for HeLa cells the new complex caused ~60% depletion after 24 h and ~80% after 48 and 72 h whereas cell survival was negligible at every incubation time for cisplatin. The administration of 1000 μM to MCF-7 cells caused depletion of the cell cultures ~50% after 24 h, ~65% after 48 h and ~75% after 72 h in the case of the complex **A** and ~60% after 24 h, ~85% after 48 h and ~90% after 72 h in the case of cisplatin.

4. Conclusion

New square–planar Pt(II) complexes containing ligands derived from glucose were prepared and fully characterized. Complex **A** displayed higher cytotoxicity on HeLa cells with respect to MCF-

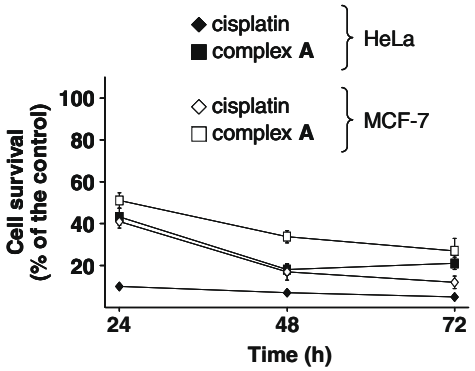


Fig. 5. Time course of the cytotoxic effect of complex **A** and cisplatin, administered at 1000 μM concentration, on HeLa and MCF-7 cells. The data are the means ± SD of three different experiments run in eight replicates and are presented as percent of control.

7 cells, as observed for cisplatin, although with a much lower effectiveness at low concentration. Despite this preliminary result, in our opinion this typology of fairly water-soluble [$PtCl_2$(*N*,*N*′)] compounds with chelating nitrogen ligands derived from glucose, is worthy of further investigation, aiming to disclose possible alternative benefits, such as minor toxicity side effects or easier administration with respect to other widely used platinum drugs.

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Appendix A. Supplementary material

CCDC 725069 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ica.2009.11.031.

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