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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.