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Platinum(II) carboxylato complexes containing 7-azaindoles as *N*-donor carrier ligands showed cytotoxicity against cancer cell lines

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ABSTRACT

The platinum(II) malonato (Mal) and decanoato (Dec) complexes of the general formulas [Pt(Mal)(*n*aza)$_2$] (**1**–**3**) and *cis*-[Pt(Dec)$_2$(*n*aza)$_2$] (**4**–**7**) were prepared, characterized and tested for their *in vitro* cytotoxicity against *cisplatin*-sensitive (A2780) and *cisplatin*-resistant (A2780R) human ovarian carcinoma cell lines and non-cancerous human lung fibroblasts (MRC-5); *n*aza = halogeno-derivatives of 7-azaindole. Complexes **1**–**7** effectively overcome the acquired resistance of ovarian carcinoma cells to *cisplatin*. Complexes **2** (IC_{50} = 26.6 ± 8.9 μM against A2780 and 28.9 ± 6.7 μM against A2780R), **4** (IC_{50} = 14.5 ± 0.6 μM against A2780 and 14.5 ± 3.8 μM against A2780R) and **5** (IC_{50} = 13.0 ± 1.1 μM against A2780 and 13.6 ± 4.9 μM against A2780R) indicated decreased toxicity against healthy MRC-5 cells (IC_{50} > 50.0 μM for **2** and >25.0 μM for **4** and **5**). The representative complexes **2** and **4** showed mutually different effect on the A2780 cell cycle at IC_{50} concentrations after 24 h exposure. Concretely, the complex **2** caused cell cycle arrest at G_0/G_1 phase, while **4** induced cell death by apoptosis with high population of cells in sub-G_1 cell cycle phase. The hydrolysis and interactions of the selected complexes with biomolecules (glutathione (GSH) and guanosine monophosphate (GMP)) were also studied by means of ^{1}H NMR and ESI+ mass spectra.

1. Introduction

Most of the platinum complexes, which entered the clinical trials as the perspective anticancer chemotherapy substances, involved one bidentate or two monodentate *O*-donor carboxylates in their structures [1]. This can be explained by several advantages of platinum carboxylato complexes (*e.g.* higher hydrolytic stability under physiological conditions and higher accumulation to the target cancer cells) over their dichlorido analogues. As an example regarding decanoato complexes, the following compound could be mentioned: 1*R*,2*R*-diaminocyclohexane-bis(neodecanoato)platinum(II), that is involved as the active species in the liposomal drug candidate *aroplatin*, showed higher *in vitro* and *in vivo* activity as compared with *cisplatin* against both the *cisplatin*-sensitive and -resistant human cancer cell lines [2,3]. This formulation was not cross-resistant with *cisplatin*, its application was not connected with nephrotoxicity and it effectively treated the metastatic tumours. Nevertheless, *aroplatin* was not approved for the clinical use in oncology practise, mainly due to economic reasons. Besides the mentioned neodecanoato complexes, the formulations with the platinum complexes involving different long-chain aliphatic monodentate carboxylates (*e.g.* decanoate as in the case of the presented work), were reported in the literature to date [4–6]. Among them, the nanosystem involving the decanoatoplatinum complex deal with the nanoparticles carrying the mentioned platinum prodrug together with REV1/REV3L-specific siRNAs, whose combinative biological effect enhances the cell response [6].

As for the malonato complexes, *heptaplatin*, (4*R*,5*R*)-4,5-bis(aminomethyl)-2-isopropyl-1.3-dioxolan-malonatoplatinum(II) [7], showed several advantages over *cisplatin* during the *in vitro*, *in vivo* and clinical testing — for example high stability in solution, circumventing the resistance of various tumour types to *cisplatin*, or less severe platinum therapy drawbacks (nephrotoxicity, proteinuria, neutropenia, emesis) [7,8]. It was approved for the gastric cancer chemotherapy in the Republic of Korea [9]. The biological importance of the malonato leaving group of platinum complexes was demonstrated on numerous compounds showing comparable or even higher *in vitro* anticancer activity than *cisplatin*. The first of them was the simple [Pt(NH$_3$)$_2$(Mal)] complex reported as *in vivo* effective against Sarcoma

Abbreviations: *3Br*aza, 3-bromo-7-azaindole; *3I*aza, 3-iodo-7-azaindole; *4Br*aza, 4-bromo-7-azaindole; *4Cl*aza, 4-chloro-7-azaindole; A2780, human ovarian carcinoma cell line; A2780R, human *cisplatin*-resistant ovarian carcinoma cell line; Dec, decanoato; GSH, reduced glutathione; Mal, malonato; MRC-5, non-cancerous human lung fibroblasts; *n*aza, general abbreviation for the used 7-azaindole derivatives; SD, standard deviation; SI, selectivity index.

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180 on mice in the fundamental work of Cleare and Hoeschele [10], later shown as *in vitro* cytotoxic against A2780 human ovarian carcinoma cells [11], which has been followed by complexes containing various aliphatic (*e.g.* 2-hydroxy-1,3-propanediamine) or heterocyclic (*e.g.* 1,2,4-triazolo[1,5-*a*]pyrimidine derivatives) *N*-donor ligands. For example, the complex with the 2-hydroxy-1,3-propanediamine carrier ligand showed higher *in vitro* cytotoxicity against lung (A549, A549/ATCC), gastric (SGC-7901) and prostrate (LNCaP) human cancer cell lines as compared with *carboplatin* [12]. Lorenzo et al. [13] reported the complexes with variously substituted 1,2-bis(aminomethyl)carbobicyclic ligands and concluded the malonato complexes as the most *in vitro* biologically effective ones against human leukaemia (HL-60) cancer cell line. Another example reported by Varbanov et al. [14] showed higher *in vivo* activity on mice with both leukaemia (L1210; 75% of mice were cured in the group treated with 30 mg/kg dose) and colon carcinoma (CT-26; final tumour weigh of 0.12 g was markedly lower as compared with 0.27 g for the control group and 0.18 g for the group treated by *oxaliplatin*) as compared with *oxaliplatin*, while being less toxic to the tested animals. Lakomska et al. [15] prepared a series of three malonatoplatinum(II) complexes containing 1,2,4-triazolo[1,5-*a*]pyrimidine derivatives as *N*-donor carrier ligands, with the most effective representative exceeding *in vitro* cytotoxicity of *cisplatin* against lung A549, breast T47D and 4T1, melanoma B16, bladder HCV-39T, leukaemia HL-60 and colorectal HT-29 cell lines. Moreover, a series of dichlorido, oxalato, malonato and cyclobutane-1,1′-dicarboxylato complexes containing (1*R*,2*R*)-*N*1,*N*2-dibutyl-1,2-diaminocyclohexane was tested against human hepatocellular carcinoma (HepG-2), gastric carcinoma (SGC-7901), non-small-cell lung cancer (A549) and colorectal cancer (HCT-116) cell lines, showing negligible cytotoxicity, except for those with the cyclobutane-1,1′-dicarboxylato ligand [16]. Other malonatoplatinum complexes, whose *in vitro* cytotoxicity against A2780 cells was tested [17–20], are discussed below within the text.

From the mechanistic point of view, anticancer platinum(II) carboxylato complexes, such as clinically-used *carboplatin* or *oxaliplatin*, hydrolyse under physiological conditions and covalently interact with intracellular biomolecules, preferentially DNA nucleobases and sulphur-containing biomolecules [21,22]. In other words, mechanism of action of clinically used platinum carboxylato complexes is based on the formation of adduct with nuclear DNA, thus it is similar to *cisplatin*. Similarly, hydrolytic activation and formation of covalent DNA adducts of malonato and decanoato platinum(II) complexes have been reported in the literature in connection with their possible mechanism of action [23,24].

We report a series of platinum(II) complexes containing the halogeno derivatives of 7-azaindole and differing in the type of *O*-donor carboxylate, concretely malonate (**1**–**3**) and decanoate (**4**–**7**). As in the case of the recently reported platinum(II) dichlorido [25–29], oxalato [25,26,30] and cyclobutane-1,1-dicarboxylato (Cbdc) [31] complexes containing the mentioned type of the *N*-donor carrier ligands, we studied *in vitro* cytotoxicity against the A2780 cells and the A2780R *cisplatin*-resistant human ovarian carcinoma cells and interactions with relevant biomolecules glutathione (GSH) and guanosine monophosphate (GMP). In order to extend the research of the platinum complexes containing 7-azaindoles, we focused on the studies of hydrophobicity (log*P*) and especially on the evaluation of toxicity of the studied complexes against non-cancerous MRC-5 human lung fibroblast cell line. Finally, we studied an impact of the selected representatives on cell cycle of A2780 and analysed the differences over clinically used platinum-based anticancer drug *cisplatin*.

2. Experimental

2.1. Materials

The chemicals $K_2[PtCl_4]$, KI, 3-bromo-7-azaindole (*3Br*aza), 3-iodo-7-azaindole (*3I*aza), 4-chloro-7-azaindole (*4Cl*aza) and 4-bromo-7-azaindole (*4Br*aza), malonic acid (H_2Mal), sodium decanoate (NaDec), NaOH, $AgNO_3$, reduced glutathione (GSH), guanosine 5′-monophosphate disodium salt hydrate (GMP), *cisplatin* and solvents (*N*,*N*′-dimethylformamide (DMF), chloroform, acetone, methanol, diethyl ether, *n*-octanol, DMF-d_7, D_2O) were purchased from Sigma-Aldrich (Prague, Czech Republic) and Acros Organics (Pardubice, Czech Republic).

2.2. Methods

^{1}H, ^{13}C and ^{195}Pt NMR spectra and ^{1}H—^{1}H gs-COSY, ^{1}H—^{13}C gs-HMQC and ^{1}H—^{13}C gs-HMBC two dimensional correlation experiments (gs = gradient selected, COSY = correlation spectroscopy, HMQC = heteronuclear multiple quantum coherence, HMBC = heteronuclear multiple bond coherence) of the DMF-d_7 solutions were measured at 300 K on a Varian 400 device at 400.00 MHz (^{1}H), 100.58 MHz (^{13}C) and 86.00 MHz (^{195}Pt). ^{1}H and ^{13}C spectra were calibrated against the residual DMF-d_6 ^{1}H NMR (8.03, 2.92 and 2.75 ppm) and ^{13}C NMR (163.15, 34.89 and 29.76 ppm) signals. ^{195}Pt spectra were adjusted against $K_2[PtCl_6]$ in D_2O found at 0 ppm. ^{1}H—^{15} N gs-HMBC two dimensional correlation experiments were carried out at natural abundance and calibrated against the residual signals of the solvent (8.03 ppm for ^{1}H and 104.7 ppm for ^{15} N) only for the representative complexes **3** and **5**. The splitting of proton resonances in the reported ^{1}H spectra is defined as s = singlet, d = doublet, t = triplet, br = broad band, and m = multiplet. Electrospray ionization (ESI) mass spectra (in both the positive (ESI+) and negative (ESI–) ionization modes) were acquired on the methanol solutions of the studied complexes using LCQ Fleet Ion Trap mass spectrometer (Thermo Scientific; QualBrowser software, version 2.0.7). A combustion analysis (C, H, N) was carried out by Flash 2000 CHNS Elemental Analyzer (Thermo Scientific). Infrared spectra (400–4000 cm^{-1} region) were acquired using the ATR technique on a Nexus 670 FT-IR (Thermo Nicolet).

2.3. Synthesis

Stoichiometric amount of $AgNO_3$ was added into the solution of Na_2Mal (prepared *in situ* from malonic acid dissolved in a minimum volume of distilled water and neutralized by 1 M NaOH) or NaDec. The mixtures were stirred in the dark at room temperature for 10 min. The products (Ag_2Mal and AgDec) were collected, washed by distilled water (2 × 5 mL), methanol (2 × 5 mL) and diethyl ether (2 × 5 mL), dried under a vacuum and stored in the fridge.

$K_2[PtCl_4]$ (0.5 mmol) was dissolved in a minimum volume of water and five molar equivalents of KI were poured in. The reaction mixture was stirred at room temperature for 90 min to get the solution of $K_2[PtI_4]$, to which the methanolic solution of the appropriate 7-azaindole derivative (1.0 mmol; *3Br*aza for **1** and **4**, *3I*aza for **2** and **5**, *4Br*aza for **3** and **7**, and *4Cl*aza for **6**) was added. The resulting reaction mixture was stirred at the ambient temperature overnight. The yellow solid of *cis*-[PtI_2(*n*aza)$_2$] was collected by filtration and washed with distilled water (2 × 5 mL), methanol (2 × 2 mL) and diethyl ether (2 × 5 mL), and dried under a vacuum; *n*aza = halogeno-derivatives of 7-azaindole.

A mixture of *cis*-[PtI_2(*n*aza)$_2$] (0.2 mmol) with Ag_2Mal (0.2 mmol) or AgDec (0.4 mmol) was stirred at the ambient temperature in a minimum volume of chloroform in the dark for 48 h (under aluminium foil). The precipitate (AgI) was filtered off and washed with chloroform (2 × 5 mL). The combined chloroform solutions were evaporated to dryness. The obtained products of [Pt(Mal)(*n*aza)$_2$] (**1**–**3**) or *cis*-[Pt(Dec)$_2$(*n*aza)$_2$] (**4**–**7**) (Fig. 1) were suspended in diethyl ether, collected by filtration and dried under vacuum. For the results of elemental analysis (C, H, N), ^{1}H, ^{13}C and ^{15}N NMR spectroscopy, ESI mass spectrometry and IR spectroscopy, see Supporting Information.

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