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Structure-cytotoxicity relationship for different types of mononuclear platinum(II) complexes with 5,7-ditertbutyl-1,2,4-triazolo[1,5-*a*]pyrimidine

Iwona Łakomska [a,*], Marzena Fandzloch [a], Tadeusz, Muzioł [a], Jerzy Sitkowski [b], Joanna Wietrzyk [c]

[a] Faculty of Chemistry, Nicolaus Copernicus University, Gagarina 7, 87–100 Toruń, Poland
[b] National Institute of Medicines, Chełmska 30/34, 00–725 Warszawa, Poland
[c] Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Weigla 12, 53–114 Wrocław, Poland

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ABSTRACT

To compare the *in vitro* cytotoxicity of platinum(II) complexes with 5,7-ditertbutyl-1,2,4-triazolo[1,5-*a*]pyrimidine (dbtp), three complexes were prepared: *cis*-[$PtI_2(dbtp)_2$] **(1)**, *cis*-[$Pt(NO_3)_2(dbtp)_2$] **(2)** and *cis*-[$Pt(C_4H_4O_5)(dbtp)_2$] **(3)**. The coordination compounds have been structurally characterized by IR; ^{1}H, ^{13}C, ^{15}N, ^{195}Pt NMR and single-crystal X-ray diffraction **(1)**. Spectroscopic studies reveal the monodentate coordination of the heterocycle ligand (dbtp) *via* N(3) to platinum(II) ions. In addition, the crystal structure of **(1)** shows that the platinum(II) ion is located in nearly square-planar PtI_2N_2 environments with two heterocycle ligands (dbtp) arranged in a head-to-head orientation. The complexes have been screened for their cytotoxicity against two human cells: non-small cell lung carcinoma (A549) and breast cancer (T47D). All of the complexes demonstrated a significant antiproliferative activity against both cell lines. On the basis of these results, it is concluded that the cytotoxicity of the studied compounds against T47D follows the order: **(3)**<**(1)**<**(2)**.

1. Introduction

Cisplatin is mainly used in the treatment of ovarian, head and neck, and bladder cancer [1–4]. However, its clinical effectiveness has been limited by significant, undesirable side effects. Additionally, the induced drug resistance to cells and its low aqueous solubility has restricted the application of this drug. These clinical inconveniences in cisplatin chemotherapy prompted the design and synthesis of more effective and less toxic platinum-based anticancer drugs [5–8]. Consequently, new platinum drugs with equal or greater antitumor activity but lower toxicity have been developed by replacing the labile chloride and stable amine ligands with other leaving groups such as cyclic and acyclic amines.

In the design of compounds with antitumor activity, we have focused on modifying the pharmacokinetics of platinum(II) complexes with 5,7-ditertbutyl-1,2,4-triazolo[1,5-*a*]pyrimidine (dbtp) as a choice of non-leaving group.

Triazolopyrimidine derivatives contain fused 5-membered and 6-membered rings and thus resemble the adenine and guanine nucleobases of DNA. A variety of coordination compounds with triazolopyrimidines is already known and has been reviewed [9–19]. Our previous studies on dichlorido platinum(II) compounds with triazolopyrimidine demonstrated that the *in vitro* cytotoxicity of platinum(II) depends on the nature of the heterocyclic ligand. In addition, we confirm that the nature of the alkyl group substituent in heterocyclic ligands and the coordination sphere geometry directly influence cytotoxicity [11]. The best antitumor parameters are demonstrated by *cis*-[$PtCl_2(dbtp)_2$], *cis*-[$PtCl_2(dptp)_2$] [11] and *cis*-[$PtCl_4(dbtp)_2$] [16], which suggests that the presence of a bulky ligand (tertbutyl or phenyl group in heterocyclic ring) might be a major factor in the cytotoxicity of the platinum(II) compounds.

On the other hand, the lability of the Pt-X bond is crucial for the delivery of platinum to DNA without prior deactivation by other biological nucleophiles. An extremely labile bond will most likely lead either to deactivation before arrival at the tumor site or to toxicity owing to the accumulation of platinum in healthy tissues. An inert leaving group will reduce the level of DNA-bound platinum, with a high proportion of the compound passing through the body intact [20]. In the original formulation of platinum anticancer drug structure-activity relationships, chloride is the preferred leaving group. Iodide is also useful as a leaving group in the cationic and neutral class of compounds. Often available as a result of synthetic convenience, iodide is expected to depart slowly from the platinum coordination sphere, leading to more sluggish DNA modification [20].

Recently, attention has been focused on using bidentate hydroxydicarboxylate ligands as leaving groups in the quest for low-toxicity platinum anticancer drugs. Although dicarboxylatoplatinum complexes, which are rather kinetically inert, are not expected to have cytotoxic activity as strong as that of halogeno species, they merit study because they are also expected to be less toxic, especially

* Corresponding author.
E-mail address: dziubek@umk.pl (I. Łakomska).

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to kidney tissue. Platinum(II) complexes with these ligands have very promising properties, such as good water solubility, low susceptibility to hydrolysis and low reactivity with glutathione [21].

In this paper we have focused on the following studies:

i. synthesis and spectroscopic studies (IR, multinuclear NMR, X-ray) of *cis*-[$PtI_2(dbtp)_2$] **(1)**, *cis*-[$Pt(NO_3)_2(dbtp)_2$] **(2)**, *cis*-[$Pt(C_4H_4O_5)(dbtp)_2$] **(3)**;
ii. the determination of IC_{50};
iii. correlation of the *in vitro* cytotoxicity of platinum(II) complexes with different type of leaving group.

2. Experimental

2.1. Chemistry

2.1.1. Materials

KI, $AgNO_3$ and analytical-grade solvents were purchased from POCh Gliwice (Poland), whereas L(−)-malic acid was purchased from Fluka AG. 3-amino-1,2,4-triazole (98%) and 2,2,6,6-tetramethyl 3,5-heptanedione (98%) were purchased from Aldrich.

K_2PtCl_4 was prepared from metallic Pt by known procedures [22]. 5,7-ditertbutyl-1,2,4-triazolo[1,5-*a*]pyrimidines were prepared according to the method of Bülow and Haas [23] by the reaction of 3-amino-1,2,4-triazole with 2,2,6,6-tetramethyl-3,5-heptanedione [24]. Disilver malate ($Ag_2C_4H_4O_5$) was prepared by the reaction of Ag_2CO_3 with L(−)-malic acid by a published method [21].

2.1.2. Instrumentation

The contents of C, H, and N were determined by elemental semimicroanalysis. IR spectra were measured with a Perkin-Elmer Spectrum-2000 FT-IR spectrometer, using KBr (400–4000 cm^{-1}) and polyethylene discs (100–400 cm^{-1}). NMR spectra were recorded at 298 K in dmf-d_7 solutions on a Varian INOVA 500 spectrometer, operated at 499.8, 125.7, 50.6, and 107.4 MHz for ^1H, ^{13}C, ^{15}N and ^{195}Pt, respectively. The ^{15}N NMR spectra were recorded in the inverse gated decoupling mode using a 30° flip angle, a spectral range of 18 kHz, an acquisition time of 0.2 s, a relaxation delay of 5 s, and a collection of 60 k data points. The ^1H-{^{15}N} correlation spectra (g-HMQC; HMQC — Heteronuclear Multiple Quantum Coherence) were optimized for a coupling constant of 8 or 1 Hz with the following experimental conditions: an acquisition time of 0.3 s, spectral windows of 5000 (F2) and 15,000 (f1) Hz, 4096 data points, 512 time increments (zero filled to 2048), a 1.4 s relaxation delay and 64 transients per increment.

2.1.3. Preparation of platinum(II) complexes

The procedures of the multi-step synthesis of platinum(II) complexes **(1–3)** are depicted (Scheme 1) and described in details below.

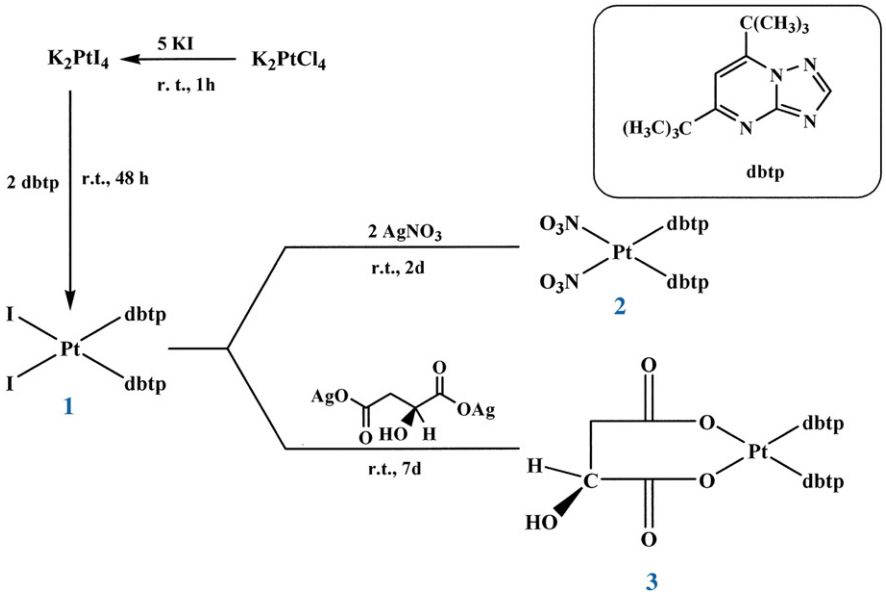
cis-[$PtI_2(dbtp)_2$] **(1)**. To a solution of K_2PtCl_4 (0.051 g; 0.12 mmol) in 10 mL of water, KI (0.103 g; 0.62 mmol) in 10 mL of water was added, and the reaction mixture was stirred in the dark at room temperature (r.t.). After 1 h, two equivalents of dbtp (0.057 g; 0.25 mmol) in 10 mL of ethanol was added to the resulting K_2PtI_4. The reaction mixture was then stirred under the same conditions for an additional 48 hours. The yellow precipitate was filtered, washed with water, ethanol, and diethyl ether and dried in a vacuum over P_4O_{10}. The yield 0.101 g (89%). Anal. found: C, 34.4; H, 4.6%. Calc for $C_{26}H_{40}I_2N_8Pt$: C, 34.2; H, 4.4%.

cis-[$Pt(NO_3)_2(dbtp)_2$] (**2**). A suspension of *cis*-[$PtI_2(dbtp)_2$] (0.070 g; 0.08 mmol) in 15 mL of acetone was treated with $AgNO_3$ (0.025 g; 0.15 mmol) in 10 mL of acetone. The reaction mixture was stirred at r.t. for 2 days. The resulting silver iodide was removed by filtration. Slow evaporation of the solvent gave a yellow precipitate which was dried in a vacuum over P_4O_{10}. The yield 0.056 g (93%). Anal. found: C, 40.1; H, 5.9%. Calc. for $C_{26}H_{40}N_{10}O_6Pt$: C, 39.9; H, 5.5%.

cis-[$Pt(C_4H_4O_5)(dbtp)_2$] **(3)**. A suspension of *cis*-[$PtI_2(dbtp)_2$] (0.060 g; 0.07 mmol) in 20 mL of acetone was treated with a stoichiometric amount of $Ag_2C_4H_4O_5$ (0.023 g; 0.07 mmol) in 10 mL of acetone. The reaction mixture was stirred in the dark at r.t. for 7 days. The resulting silver iodide was removed by filtration. Slow evaporation of the solvent gave a yellow precipitate which was dried in a vacuum over P_4O_{10}. The yield 0.048 g (92%). Anal. found: C, 45.3; H, 5.9%. Calc. for $C_{30}H_{44}N_8O_5Pt$: C, 45.5; H, 5.6%.

2.1.4. X-ray structure determination

The X-ray diffraction data for *cis*-[$PtI_2(dbtp)_2$] (**1**) were collected at room temperature for a single crystal ($0.31\times0.12\times0.08$ mm) using Oxford Diffraction KM4 CCD diffractometers, with MoKα



Scheme 1. Synthesis of platinum(II) complexes with dbtp **(1–3)**.