Brief Articles

Platinum(IV) Complex with Adamantylamine as Nonleaving Amine Group: Synthesis, Characterization, and in Vitro Antitumor Activity against a Panel of Cisplatin-Resistant Cancer Cell Lines

František Žák,[†] Jaroslav Turánek,*[,‡] Aleš Kroutil,[†] Petr Sova,[†] Adolf Mistr,[†] Anna Poulová,[†] Petr Mikolin,[†] Zdirad Žák,[§] Andrea Kašná,[‡] Dana Záluská,[†,‡] Jiří Neča,[‡] Lenka Šindlerová,[||] and Alois Kozubík[||]

PLIVA-Lachema a.s., Karásek 1, 621 33 Brno, Czech Republic; Department of Immunology, Veterinary Research Institute, Hudcova 70, 621 33 Brno, Czech Republic; Department of Inorganic Chemistry, Masaryk University, Kotlářska 2, 611 37 Brno, Czech Republic; and Institute of Biophysic, The Academy of Sciences of the Czech Republic, Královopolská 135, 61265 Brno, Czech Republic

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Procedure of the synthesis is described for new platinum(IV) drug LA-12 [(*OC*-6−43)-bis-(acetato)(1-adamantylamine)amminedichloroplatinum(IV)]. The X-ray diffraction analysis shows that the structure is created by molecules with octahedral arrangement of ligands around a platinum atom and contains one H_2O molecule that is not a part of the coordination sphere of platinum. This new drug is more reactive with glutathione than cisplatin and is lacking cross-resistance with cisplatin as proven on the panel of cancer cell lines.

Introduction

Clinical tests with cisplatin began in 1972, and this drug is included in the basic armament in antitumor chemotherapy. There are two major limitations of cisplatin in anticancer therapy. The first, it has serious adverse effects (nephrotoxicity, neurotoxicity, severe nausea and vomiting, ototoxicity) and second, many tumors exhibit resistance, either ab initio (e.g., non-small-cell lung cancer and colon cancer) or it is acquired during therapy (e.g., small-cell lung cancer or ovarian cancer).[1] The phenomenon of drug resistance is very complex, and little information is available on how disparate mechanisms are coordinated with each other.

The development of orally active platinum drugs led to synthesis of several compounds, some of which are in Phase II evaluation. The leading compound is Pt(IV) complex JM-216, [(*OC*-6−43)-bis(acetato)amminedichloro-(cyclohexylamine)platinum(IV)], which entered clinical testing in 1992.[1] We have synthesized a series of platinum complexes with bulky hydrophobic ligands. The platinum(IV) complex (*OC*-6−43)-bis(acetato)(1-adamantylamine)amminedichloroplatinum(IV) (Figure 1) expressed a rapid and high cytotoxic effect without cross-ressistance to cisplatin, and the mode of synthesis was acceptable with respect to industrial manufacturing. Therefore, this compound was selected for the further evaluation.

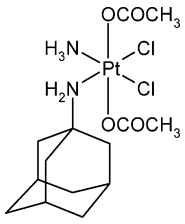

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Figure 1. Structural formula of new anticancer platinum drug **1**.

Scheme 1. Preparation of **1** (am = 1-adamantylamine)

cis-$[PtCl_2(NH_3)_2]$ + HCl → $NH_4[PtCl_3(NH_3)]$

$NH_4[PtCl_3(NH_3)]$ + Cl^- → $[PtCl_3(NH_3)]^-$ + NH_4Cl

$[PtCl_3(NH_3)]^-$ + HCl → $H[PtCl_3(NH_3)]$ + Cl^-

$H[PtCl_3(NH_3)]$ + KCl → $K[PtCl_3(NH_3)]$ + HCl

$K[PtCl_3(NH_3)]$ + am → *cis*-$[PtCl_2(am)(NH_3)]$ (**2**) + KCl

$[PtCl_2(am)(NH_3)]$ + H_2O_2 → *cis,trans,cis*-$[PtCl_2(OH)_2(am)(NH_3)]$ (**3**)

cis,trans,cis-$[PtCl_2(OH)_2(am)(NH_3)]$ + 2 $(CH_3CO)_2O$ →

→ *cis,trans,cis*-$[PtCl_2(CH_3COO)_2(am)(NH_3)]$ (**1**) + 2 CH_3COOH

Results and Discussion

Chemistry. Scheme 1 shows the reactions involved in the synthesis of the tested substance (*OC*-6−43)-bis-(acetato)(1-adamantylamine)amminedichloroplatinum-(IV), coded as LA-12.

A complex with platinum (oxididation state II) was prepared from Cossa's salt and 1-adamantylamine. After purification, the complex was oxidized by a dilute solution of hydrogen peroxide at high temperature and consequently acetylated with acetic anhydride at ambient temperature. The final product was obtained as an

* Corresponding author: RNDr. Jaroslav Turánek, CSc., Veterinary Research Institute, Laboratory of Immunopharmacology and Immunotoxicology, Hudcova 70, 621 32 Brno, Czech Republic. Phone: + 420 5 4132 1241, Fax: + 420 5 4121 1229, e-mail: turanek@vri.cz.

[†] PLIVA-*Lachema a.s.*

[‡] Veterinary Research Institute.

[§] Masaryk University.

[||] The Academy of Sciences of the Czech Republic.

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anhydrous substance from a acetone−ether system and contains only one contaminant at a 0.25% level. The platinum complex was chromatographically pure (purity > 99.5%). The composition of **1** as determined by elemental analysis showed a good agreement between the theoretical and actual values.

The band assignment in IR spectroscopy was performed based on data from the literature[2] and is in accordance with possible vibrations of structural units in the complexes under study. The results of NMR measurements and their interpretations were compared with data from the literature regarding measurements of platinum complex JM-216.[3] It enabled the assignment of the structure *cis,trans,cis*-[$PtCl_2(CH_3COO)_2(C_{10}H_{15}NH_2)(NH_3)$] to **1**.

The structure is formed by octahedral molecules, as expected, with a water molecule outside the coordination sphere of Pt. In the crystal structure, the molecules are arranged in layers with no apparent bonding between them. However, within one layer the molecules are bonded by a system of both intra- and intermolecular hydrogen bonds via NH_2 and H_2O hydrogens. The structure of **1** can be compared with (*OC*-6−43)-bis-(acetato)ammine-dichloro(cyclohexylamine)platinum-(IV) (JM-216),[4] both having the same coordination sphere around Pt atom. While the Pt−ligand distances for both compounds are the same within 3σ, there are some differences in corresponding interatomic angles. Generally, the angles in the coordination sphere of JM-216 tend to be more even, 86.7−94.3° and 174.7−178.3° as compared with **1**, 81.5−95.2° and 172.1−178.0°. The most apparent difference is in a mutual orientation of the acetato groups. In JM-216, the angle between the best planes through both acetato groups is 76.2° while in **1** these groups are almost eclipsed, the angle being 9.9°. The ammonia nitrogen is thus almost equidistant from both carbonyl oxygens which lead to much stronger intramolecular H-bonds between ammonia hydrogens and carbonyl oxygens in our structure. Corresponding donor−acceptor distances are 2.65(1) and 2.79(1) Å as compared with those in JM-216, 2.78(3) and 2.84(3) Å, respectively.

Biological Activities. Platinum complexes are known to react readily with sulfur ligands; hence, elevated cellular glutathione (GSH) may reduce the cytotoxicities of platinum drugs. However, the evidence for the involvement of GSH- and GSH-dependent enzymes in platinum drug resistance still remains equivocal. The role of GSH in modulating Pt(IV) drug cytotoxicity has not been investigated extensively.[5]

We have found **1** much more reactive toward glutathione than cisplatin. Velocity constants for pseudo-first order are in Supporting Information. This high reactivity is unique for **1** and was not found within the set of tested platinum(II) and (IV) drugs (e.g. oxaliplatin, JM-216, **2**) (unpublished results). The reason for this high reactivity is not clear at present, but we speculate that structure of water molecules surrounding the bulky hydrophobic symmetrical adamantylamine ligand can affect the reaction with glutathione and other thiols.

Antitumor Evaluation. We tested cytotoxicity of the new platinum complex **1** on a panel of cisplatin resistant cancer cell lines to find boundaries of cisplatin cross-resistance. Results and used cell lines are summarized in the Table 1. The cytotoxic effect of **1** is rapid and strong in comparison with cisplatin. Only lung large cell carcinoma cell line CORL23/CTR, which is cisplatin-resistant and slow-growing, demonstrated relatively a higher degree of resistance toward **1**, even if lower than toward cisplatin. High cytotoxic effect against leukaemic, melanoma, and colorectal cancer cell lines is promising for the perspective application in therapy of corresponding tumors.

Table 1. Cytotoxicity of Pt Complexes against Cisplatin-Resistant Tumor Cell Lines (24 h continual exposure)

	IC_{50} (μM)	
cancer cell line	cisplatin	**1**
chronic myelogenous leukaeamia K562	>80	3
chronic myelogenous leukaemia KG-1	48	2
acute myelogenous leukaemia ML-2	>80	1
mouse mellanoma B16	>80	6
colon cancer HT-29N	>80	12
colon cancer HT29	50	8
colon cancer HCT116	>80	9
lung carcinoma A427	63	6
breast carcinoma HBL100	63	6
breast carcinoma MCF-7	71	8
lung carcinoma CORL23/CTR	>80	25
ovarian carcinoma A2780	4	4
ovarian carcinoma A2780/cis	40	3
ovarian carcinoma A2780/cis90	>80	7

Ovarian epithelial cancer cell line A2780 is a well described model for studies of the cisplatin resistance. The resistant sublines, prepared by gradual treatment with cisplatin, are available, and some mechanisms of resistance are described. **1** has proven also very low cross-resistance with cisplatin in both A2780/cis and A2780/cis90 sublines. In the case of A2780/cis90 subline we have seen some increase in IC_{50} from 4 μM to 7 μM, but this is not a dramatic change when compared to cisplatin, where a 13-fold increase of IC_{50} was observed (Table 1). There was no cisplatin cross-resistance with intermediate resistant A2780/cis. Interestingly, we have found substantial cross-resistance on the A2780 and A2780/cis90 pair of cell lines for JM-216 platinum(IV) complex (unpublished result) and cross-resistance was published also for another new generation platinum-(II) complex ZD0473 [(*SP*-4−3)-amminedichloro(2-methylpyridine)platinum(II)] (Phase II 2002) on the A2780 and A2780/cis pair.[6]

We have also compared the effect of various exposure times on IC_{50}, because various protocols are used by researchers for estimating IC_{50}. Longer exposure time (72 or 96 h) is giving lower IC_{50}, but this value is inevitably affected by exhaustion of nutrients and growth factors from the medium. Short exposure time (24 h) can underestimate the cytostatic effect in slow-growing cell lines or cell lines with longer period required for induction of the executive phase of apoptosis. The sensitive A2780 cell line exerted a sudden drop of IC_{50} for both tested drugs after 48 h incubation, and prolongation of incubation time to 72 h did not affect IC_{50} significantly. It might be linked with reaching confluency after 48 h of incubation. In comparison with A2780 cells the A2780/cis cells were slower growing and no sudden decrease in IC_{50} was observed. Cytostatic effect of LA-12 on the A2780/cis cell line was increasing during exposure time but remained in the same order of magnitude.

Conclusions

In summary, the synthesis of new platinum(IV) drug **1** [(*OC*-6−43)-bis(acetato)(1-adamantylamine)amminedichloroplatinum(IV)] with bulky hydrophobic ligand was developed. This new drug demonstrated high cytotoxic effect in cisplatin-resistant cancer cell lines and no cross-resistance with cisplatin was seen. Studies on physical−chemical features of **1** and their relations to high cytotoxicity toward cancer cell lines are central to our further investigations of platinum complexes with bulky hydrophobic ligands. Experiments are running to find cellular target and pathways leading the cancer cells to apoptosis after the exposure to this drug.

Experimental Section

Synthesis of Platinum Complexes. Synthesis of (*OC*-6−43)-Bis(acetato)(1-adamantylamine)amminedichloroplatinum(IV) (1). The Cossa's salt was prepared from cisplatin by method described by Oksannen.[7,8]

The solution (8.72 g, 57.65 mmol) of 1-adamantylamine in 80 mL of ethanol was added under stirring to the filtered solution (20.0 g i.e., 54.56 mmol) of Cossa's salt. All operations with free amine were performed in inert atmosphere (predominantly nitrogeneous). After 5 h of stirring at 50 °C and cooling of the reaction mixture to laboratory temperature, the insoluble fraction was separated, washed with ethanol, and dried. The filter cake was mixed with 200 mL of dimethylformaminde (DMF) and then filtered. The crude product was precipitated from a yellow-orange filtrate with 760 mL of 0.5 M HCl, separated, and washed with diluted HCl of the above concentration and ethanol. After air-drying, the crude product was precipitated from the mentioned system DMF/0.5 M HCl as described above. The yield following drying in a vacuum dryer was 15.0 g (63.3%) of (*SP*-4−3)−(1-adamantylamine)-amminedichloroplatinum(II) (**2**). Anal. ($C_{10}H_{20}Cl_2N_2Pt$). Then 14.0 g (32.24 mmol) of **2** was resuspended in 54.5 mL of water, and 40 mL of 30% H_2O_2 was added within 50 min. The system was maintained at a reaction temperature of 80 °C for another 50 min. After cooling to laboratory temperature, the crude product was separated, washed with water, and dried in a vacuum-dryer. This procedure was followed by extraction of impurities to DMF, separation of the solid fraction, and washing of the filter cake with DMF and acetone. After being dried in a vacuum dryer, 12.0 g (79.5%) of (*OC*-6−43)−(1-adamantylamine)amminedichlorodihydroxoplatinum(IV) (**3**) (anal. ($C_{10}H_{22}Cl_2N_2O_2Pt$)) was obtained. Then 10.8 g (23.06 mmol) of **3** was resuspended in 85 mL of acetic anhydride at laboratory temperature. The reaction mixture was stirred at dark for 48 h, and then the solid fraction was separated and washed with acetic anhydride and diethyl ether. After being dried in a vacuum dryer until a constant weight, 9.7 g (76.1%) of crude product **1** was obtained with 98.5% purity according to HPLC. Purification consisted of the product extraction with methanol followed by recrystallization. It was performed by precipitation from an acetone−ether system with a yield 80%. Anal. ($C_{14}H_{26}Cl_2N_2O_4Pt$) C, H, N, Cl. IR (ν, cm^{-1}) 3450 (br), 3300 (w), 3175 (m), 3095 (m, br), 2900 (s), 2850 (w), 1660 (s), 1600 (s), 1370 (m), 1305 (s), 1280 (s), 707 (m). ^1H NMR (MeOD) δ 2.05 (s, CH_3, 6H), 2.04−2.03 (br, CH_2, 6H), 1.74−1.67 (m, CH_2, 6H), 2.08 (br, CH, 3H); ^{13}C NMR (MeOD) δ 182.91 (C=O), 23.42 (CH_3), 59.10 (CNH_2), 42.07 (CH_2), 36.94 (CH_2), 31.13 (CH).

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Supporting Information Available: X-ray analysis of Pt-(IV) complex, methods of in vitro anticancer evaluation, and inhibition curves. This material is available free of charge via the Internet at http://pubs.acs.org.

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