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Original Scientific Paper

Synthesis, Structure and Cytotoxicity of Platinum(IV) Complexes of 3-Aminocyclohexanespiro-5-hydantoin and 3-Aminocycloheptanespiro-5-hydantoin

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Abstract. Two new platinum(IV) complexes of 3-aminocyclohexanespiro-5-hydantoin (achsh) and 3-aminocycloheptanespiro-5-hydantoin (achpsh) were prepared and characterized. *Ab initio* calculation of the structure and the measurements of IR and NMR spectra of complexes were also performed. The complexes were evaluated for *in vitro* cytotoxicity in murine erythroleukemia (MEL) cells, clone F4N. The complexes exerted low *in vitro* toxicity, comparable with that of the corresponding platinum(II) complexes.

Keywords: platinum(IV) complexes, molecular structure, cytotoxic effect, *ab initio* calculations

INTRODUCTION

The use of *cis*-diamminedichloroplatinum(II), known as cisplatin or *cis*-DDP in cancer chemotherapy has a major impact on some tumor types, such as testicular or ovarian carcinoma.[1] However, cisplatin has two major drawbacks: 1) severe toxicity that includes nephrotoxicity, neurotoxicity and ototoxicity and 2) the acquisition or presence of resistance to the drug.[2] Because tumor resistance to cisplatin limits its efficacy, there is urgent need for new platinum complexes capable of overcoming cisplatin resistance. One of the approaches to overcome the acquired resistance to *cis*-DDP is to use Pt^{IV} complexes. There exist several Pt^{IV} complexes such as tetraplatin $Pt(dach)Cl_4$ (dach, diaminocyclohexane); iproplatin $Pt(ipa)_2(OH)_2Cl_2$ (ipa, isopropylamine) and JM216 $Pt(NH_3)(cha)(OAc)_2Cl_2$ (cha, cyclohexylamine) which recently became very promising antitumor agents.[1,3] It is widely believed that reduction to platinum(II) is essential for the anticancer activity of platinum(IV) complexes. There are a number of factors to consider when assessing the possible effects of administering the Pt^{IV} analogue of a Pt^{II} complex. The kinetic inertness of Pt^{IV} complexes offers an increased ability of the compounds to arrive the cellular targets intact. Modifying the axial ligands of platinum(IV) complexes alters the solubility of the complex and thus the ability to enter tumor cells before being reduced to yield the active platinum(II) drug.[3] Another advantage of Pt^{IV} complexes is that some of them could be orally administered. In the present work, we report the synthesis and characterization of two platinum(IV) complexes of 3-aminocyclohexanespiro-5-hydantoin and 3-aminocycloheptanespiro-5-hydantoin. The *in vitro* inhibitory effect of the complexes on the growth of murine erythroleukemia (MEL) cells, clone F4N in culture was studied.

3-Aminocyclohexanespiro-5-hydantoin and 3-Aminocycloheptanespiro-5-hydantoin contain NH_2 group with excellent coordination properties, and it has been found that the activity increases with introducing amino group in the hydantoin ring.[4] A relatively small number of platinum complexes with hydantoins as ligands have been synthesized yet.[5] The present paper is an extension of our previously reported results[6,7] for Pt^{II} complexes with above mentioned amino-ligands.

EXPERIMENTAL SECTION

Starting Materials

$K_2[PtCl_4]$ was prepared according to Spassovska *et al.*[8] The ligands, 3-aminocyclohexanespiro-5-hydantoin (achsh)

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and 3-aminocycloheptanespiro-5-hydantoin (achpsh) were synthesized from cyclohexanone and cycloheptanone to cyclohexanespiro-5-hydantoin and cycloheptanespiro-5-hydantoin respectively, following the Bucherer-Lieb reaction.[9] After that cyclohexanespiro-5-hydantoin and cycloheptanespiro-5-hydantoin were transformed into 3-aminocyclohexanespiro-5-hydantoin and 3-aminocycloheptanespiro-5-hydantoin by means of $NH_2NH_2.H_2O$.[10] The remaining reagents and solvents were AR grade products.

Preparation of the Complexes

The procedures for preparation of Pt^{IV} complexes of achsh and achpsh were analogous to those described by Hambley *et al.*[11] The Pt^{IV} complexes were synthesized by oxidation of Pt^{II} complexes $[Pt(achsh)_2Cl_2]$ and $[Pt(achpsh)_2Cl_2]$ with 30 % hydrogen peroxide.

$[Pt(achsh)_2Cl_2(OH)_2]$ (**1**). To a suspension of 0.170 *g* (0.27 mmol) of $[Pt(achsh)_2Cl_2]$ in 20 ml acetone were added 0.5 ml of 30 % H_2O_2. The reaction mixture was refluxed for 20 min after that the solution was filtered. The solvent was removed under vacuum and the re-sulting oil was precipitated with anhydrous diethyl ether. The precipitated product was removed from the suspension by filtration and vacuum dried. Yield: 0.05 g (28 %).

Anal. Calcd. mass fractions of elements, *w*/%, for $C_{16}H_{28}N_6Cl_2O_6Pt$ ($M_r = 666.41$) are: C 28.84, H 4.23, N 12.61, Cl 10.64, Pt 29.27; Found: C 28.40, H 3.78, N 12.15, Cl 10.87, Pt 29.63.

$[Pt(achpsh)_2Cl_2(OH)_2]$ (**2**). To a suspension of 0.200 *g* (0.30 mmol) of $[Pt(achpsh)_2Cl_2]$ in 30 ml acetone 0.7 ml of 30 % H_2O_2 were added. The reaction mixture was refluxed for 30 min and then the solution was filtered. The solvent was removed under vacuum and the resulting oil was precipitated with anhydrous diethyl ether. The precipitated product was removed from the suspension by filtration and vacuum dried. Yield: 0.05 g (24 %).

Anal. Calcd. mass fractions of elements, *w*/%, for $C_{18}H_{32}N_6Cl_2O_6Pt$ ($M_r = 694.46$) are: C 31.13, H 4.64, N 12.10, Cl 10.21, Pt 28.09; Found: C 31.60, H 4.81, N 11.66, Cl 10.62, Pt 28.58.

Analyses and Physical Measurements

The elemental analyses were performed by routine microanalytical techniques (Department of Chemistry, Sofia University). ^{1}H NMR spectra were registered on a Bruker WM 250 spectrometer at 250 MHz in DMSO-d_6 solution using TMS as internal standard. Infrared spectra were recorded on a Bruker IFS-113V spectrophotometer in CsI disks (4000–150 cm^{-1}).

Quantum-chemical Calculations

The *ab initio* calculations were carried out using the GAMESS program package.[12] The structure optimizations for the $[Pt(achsh)_2Cl_2(OH)_2]$ and $[Pt(achsh)_2Cl_2]$ complexes were performed at the RHF level without any symmetry constrains. The mean gradient threshold was 0.0001 hartree/Bohr. The calculations were carried out with the Stewens-Basch-Krauss-Jasien effective core potentials (ECP) and their concomitant basis sets for all the atoms (ECP-31G).[13,14] The vibrational analysis of the obtained structures was carried out. The calculated wavenumbers of all normal modes were scaled by a factor of 0.91.

Incubation with Drugs and *In Vitro* Cytotoxicity Test

MEL cells, clone F4N (virus-transformed erythroid precursor cells)[15] were cultured in Dulbecco's modified Eagle medium (Gibco, Grand Island, NY) supplemented with 10 % calf serum, under 5 % CO_2 atmosphere at 37 °C, and passed every day at a concentration of 5×10^5 cells/ml.

The complexes were dissolved immediately before use in DMSO to obtain stock solutions of different concentrations. Each of these solutions was used at 1 % concentration in the experiments with F4N cells. The final concentration of DMSO in the medium did not affect cell growth.

Exponentially growing cells (5×10^5 cells/ml) were incubated in triplicate with increasing concentrations of the test complexes in 96-well microtiter plates. After 24, 48 and 72 h of drug treatment, the cells were counted hemocytometrically. The number of dead cells was determined by staining with trypan blue. The mean of triplicate determinations of three independent experiments was calculated. The 50 % inhibitory dose (IC_{50}) was defined as drug concentration that reduces the number of living cells by 50 %.

RESULTS AND DISCUSSION

The complexes are dark yellow coloured solids, stable at normal conditions, but decomposing upon heating. They are soluble in DMF and DMSO, and slightly soluble in water.

Infrared spectra

The IR spectral data of the ligand and complexes are collected in Table 1. The absorption bands in the range of 4000–600 cm^{-1} belong to the absorptions of the organic ligand. The absorption bands in the far IR below 600 cm^{-1} characterize the Pt–OH bonding as well as the ligand-metal bonding. The bands in the

Table 1. IR data for the ligands and complexes

No	Compound	Frequency[(a)], ν/cm^{-1}						
		ν(NH$_2$) ν(NH)	Amide I δ(NH$_2$) ω(NH$_2$)	ν_{as}(CH$_2$) ν_s(CH$_2$) δ(CH$_2$)	Amide II	ν(OH)	ν(Pt-OH) ν(Pt-N)	ν(Pt-Cl)
	achsh	3320 s	1610 s	2960 s	1536 m	—	—	—
		3200 s	1343 w	2890 m				
			1322 w	1452 w				
1	[Pt(achsh)$_2$Cl$_2$(OH)$_2$]	3295 s	1634 s	2938 s	1539 m	3529 s (3619, 3622)	551 sh (561, 574)	348 sh (347)
		3149 s	1342 m	2860 m			512 sh (522, 512)	341 m (343)
			1299 m	1454 m				
	achpsh	3302 m	1646 m	2931 m	1520 w	—	—	—
		3210 m	1325 w	2862 w				
			1307 w	1460 w				
2	[Pt(achpsh)$_2$Cl$_2$(OH)$_2$]	3292 s	1631 m	2985 m	1525 w	3532 s	590 sh	338 s
		3165 sh	1338 w	2858 m			519 sh	331 sh
			1303 w	1450 w				

*Abbreviations: s, strong; m, medium; w, weak; sh, shoulder. *Ab initio* calculated data are given in brackets (asym, sym)

range of absorptions of the N–H stretching vibrations in the spectra of complexes were shifted (about 10–30 cm^{-1}) to lower frequency as compared to those of the free ligand. This is indicative for coordination through the NH_2 group.[16] The existence of two absorption bands for the Pt–Cl stretching vibrations (in the range of 331–348 cm^{-1}) in the far IR spectra is an indication of the *cis*-configuration of the complexes.[6,17,18] The weak bands in the range of 519–512 cm^{-1} in the spectra of the complexes could be ascribed to Pt–N stretching vibrations.[17,18,19] The presence of absorption bands in 551 cm^{-1} and 590 cm^{-1} respectively is due to the Pt–O stretching vibrations[17,18] and it is indicative of the oxidation to Pt^{IV}. Ab initio calculated characteristic vibrations for Pt–O, Pt–N and Pt–Cl bonds of the compound shown in Figure 2 are in good agreement with experimental data listed in Table 1.

^{1}H NMR spectra

The ^{1}H NMR spectral data for freshly prepared DMSO-d$_6$ solutions of the ligands and complexes are presented in Table 2. In the spectra of the 3-aminocyclohexanespiro-5-hydantoin and 3-aminocycloheptanespiro-5-hydantoin were observed complicated multiplets in the range of 1.21–1.83 ppm due to the methylene protons at the cycloalkane rings. Signals for NH_2 groups were registered at 4.62 ppm and 4.64 ppm. For the NH groups signals were found at 8.50 ppm and 8.40 ppm, respectively. In the spectra of the complexes, the signals of the NH_2 protons were significantly shifted downfields (average 2.83 ppm) in comparison to the free ligand.

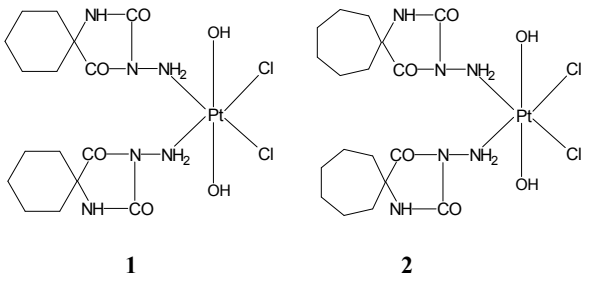
Table 2. ^{1}H NMR spectral data for the ligands and the complexes

No	Compound	δ/ppm[(a)]		
		CH$_2$	NH$_2$	NH
	achsh	1.21-1.66 m	4.62 s	8.50 s
1	[Pt(achsh)$_2$Cl$_2$(OH)$_2$]	1.41-1.82 m	7.64	8.75
	achpsh	1.54-1.83 m	4.64 s	8.40 s
2	[Pt(achpsh)$_2$Cl$_2$(OH)$_2$]	1.54-1.97 m	7.27	8.69

*Abbreviations: s, singlet; m, multiplet.

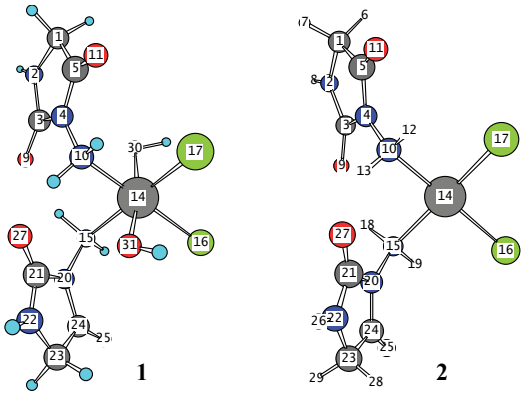
The signals of NH protons were much less affected (average 0.27 ppm). In our previous work with Pt^{II} complexes,[16] analogous effects have been described. A downfield shift of the same order has been registered for the proton signals of coordinated NH_2 groups in the spectra of a number of Pt^{II} amine complexes.[21] That is a reason to consider that in our complexes the amino-ligands coordinate to platinum via the NH_2 group. The signals of NH_2 and NH protons of the studied complexes were accompanied by some weaker peaks. These additional signals can be explained by solvolysis products of the complexes in DMSO.[22] In the spectra of the platinum complexes no significant shift of the signals due to the cyclohexane residue was registered. Similar IR and ^{1}H NMR spectral data have been reported in our previous papers for platinum complexes of differently substituted cycloalkanecarboxylic acid hydrazides.[6, 7, 16]

The elemental analysis and spectroscopic data confirm the structures of the complexes shown in Figure 1.



1 **2**

Figure 1. Chemical structures of the complexes. $[Pt(achsh)_2Cl_2(OH)_2]$, cis-trans-cis-bis(3-aminocyclohexane-spiro-5-hydantoin)-(dihydroxo)dichloroplatinum(IV) (**1**), $[Pt(achpsh)_2Cl_2(OH)_2]$, cis-trans-cis-bis(3-aminocycloheptane-spiro-5-hydantoin)-(dihydroxo)dichloroplatinum(IV) (**2**).



1 **2**

Figure 2. Ab initio calculated structure of the complexes $[Pt(achsh)_2Cl_2(OH)_2]$ (**1**) and $[Pt(achsh)_2Cl_2]$ (**2**). For simplicity cyclohexane moiety is not presented.

Table 3. Important *ab initio* calculated bond lengths/Å and bond angles/deg for the complexes $[Pt(achsh)_2Cl_2(OH)_2]$ (**1**) and $[Pt(achsh)_2Cl_2]$ (**2**), shown in Figure 2

Parameter	**1**	**2**	Experimental[a]
Bond			
Pt–Cl	2.352	2.377	2.315[b] (2.312[c])
Pt–N	2.118	2.152	2.065[b] (2.060[c])
Pt–O	1.999		1.987[b]
O–H	0.965		
Bond angle			
Cl–Pt–N	88.3	84.9	90.7[c]
Cl–Pt–Cl	91.8	95.4	89.8[c]
N–Pt–N	91.7	95.0	89.5[c]
Cl–Pt–O	93.3		

[a]X-ray data: [b] Ref. 20, [c] Ref. 21

Table 4. Cytotoxicity of platinum complexes in F4N cells

No	Compound	IC_{50}[a]/μmol L^{-1}		
		24 h[b]	48 h[b]	72 h[b]
	achsh		(c)	
1	$[Pt(achsh)_2Cl_2(OH)_2]$	260	247	211
	$[Pt(achsh)_2Cl_2]$[e]	(d)	(c)	(c)
	achpsh		(c)	
2	$[Pt(achpsh)_2Cl_2(OH)_2]$	237	159	133
	$[Pt(achpsh)_2Cl_2]$[f]	(c)	280	(c)
	Cisplatin	5.8	4.9	3.9

[a] Drug concentration reducing the number of living cells by 50 %.
[b] Time of drug treatment.
[c] No inhibition of cell growth at concentrations up to 200 μmol L^{-1}.
[d] 25 % inhibition of cell growth at concentration of 100 μmol L^{-1}.
[e] Ref. 6.
[f] Ref. 7.
Values are mean of triplicate determinations in two independent experiments.

Quantum-chemical Data

The *ab initio* calculated structure of the complexes $[Pt(achsh)_2Cl_2(OH)_2]$ and $[Pt(achsh)_2Cl_2]$ are shown in Figure 2, and the most important bond lengths and bond angles are listed in Table 3. The cyclohexane moiety does not lead to changes of the structure of the complex.[6] For that reason we consider theoretically 3-amino-hydantoin as ligand in the complexes $[Pt(achsh)_2Cl_2(OH)_2]$ and $[Pt(achsh)_2Cl_2]$.

The Pt–Cl and Pt–N bonds in $[Pt(achsh)_2Cl_2]$ are in agreement with the available experimental data,[23] (Table 3). The *ab initio* calculated Pt–Cl and Pt–N bonds are 0.065 Å and 0.092 Å longer, respectively, than experimental values presented in the Table. In the Pt^{IV} complex the Pt–Cl bond shortens by 0.025 Å and Pt–N bond becomes shorter by 0.034 Å than respective bonds in the Pt^{II} complex (Table 3).

The square planar coordination geometry around the Pt atom consists of two cis chlorine atoms and two nitrogen atoms from amino group of hydantoin molecule. The first hydantoin fragment is situated over the plain of the complex and the second – under the plain.

Cytotoxic Effect

The growth-inhibitory effect of the ligands achsh and achpsh and their two platinum(IV) complexes on F4N cells in culture was examined. The results, as expressed by IC_{50} values for different times of drug exposure, are presented in Table 4. The complexes were low inhibitory towards F4N cells, resembling the activity of the corresponding Pt^{II} complexes, as reported earlier.[6,7] Up to the concentration of 100 μmol L^{-1} they exhibited rather cytostatic than cytotoxic effect. Nevertheless,

complex **2** proved to be more active than complex **1**, reducing upon 72 h incubation the number of living cells by more than 90 %. The ligands did not show any inhibitory activity up to the concentration of 200 μmol L^{-1}.

We assume that in the cell, Pt^{IV} complexes undergo a rapid reduction to the respective Pt^{II} compounds thus displaying the reduced activity of the latter complexes. It would be of interest to synthesize and study *trans*-Pt^{IV} complexes with 3-aminocyclo-hexanespiro-5-hydantoin and 3-aminocycloheptanespiro-5-hydantoin as well as Pt^{IV} complexes containing mixed-amino (NH_3 and 3-aminocycloalkanespiro-5-hydantoin) ligands. Recent data indicate that such compounds could exert retarded *in vivo* reduction and increased activity against cis-platin-resistant tumor cells.[24]

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SAŽETAK

Sinteza, struktura i citotoksičnost kompleksa platine(IV) s 3-Aminocikloheksanspiro-5-hidantoinom i 3-Aminocikloheptanspiro-5-hidantoinom

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Pripravljena su i opisana dva nova kompleksa platine(IV) s 3-aminocikloheksanspiro-5-hidantoinom (achsh) i 3-aminocikloheptankspiro-5-hidantoinom (achpsh). Također su obavljeni i *Ab initio* proračuni strukture, kao i mjerenja IR i NMR spektara kompleksa. Citotoksičnost kompleksa procjenjena je *in vitro* na stanicama eritroleukemije murine (MEL), klon F4N. Kompleksi ispoljavaju nisku *in vitro* toksičnost, usporedivu s onom odgovarajućih kompleksa platine(II).