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Synthesis and comparative evaluation of cytotoxicity in vitro of new platinum complexes with 3-amino-α-tetralonespiro-5'-hydantoin

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Abstract— **Cisplatin is one of the most successful compounds in the fight against cancer. Recently, interest was directed towards the development of cisplatin analogues which possess N-heterocyclic carrier ligands, coordinated to the cytotoxic platinum(II) moiety, instead of one or both of the am(m)ines. Hydantoins form a large group of derivatives widely applied in medicine and pharmacy, especially as anticonvulsants, antiarrhythmics, antibacterial drugs, cytotoxic agents etc. A new cis-[Pt(NH_3)LCl_2], where L is 3-amino-α-tetralonespiro-5'-hydantoin was synthesized and studied. The molecular formula of the complex was confirmed by the elemental analysis, melting point and IR spectra. The results show that the coordination of the ligand with metal ion was realized by nitrogen atom of the amine group. On the basis of the results from the physicochemical investigation, the most probable molecular structure of the platinum complex was proposed. This compound as well as previously prepared and studied Pt(II) and Pt(IV) complexes with general formulae *cis*-[PtL_2Cl_2] and *cis*-[PtL_2Cl_4], where L is the same ligand 3-amino-α-tetralonespiro-5'-hydantoin were investigated for cytotoxicity *in vitro* on HL-60 and SKW-3 human tumour cell lines. The results showed that all complexes exerted concentration dependent antiproliferative activity.**

Keywords—platinum complexes; N-heterocyclic ligands; cytotoxicicty

I. INTRODUCTION

Cisplatin is the first and one of the most successful compounds in the fight against cancer [1-2]. Following the initial examinations of the anticancer activity of cisplatin researchers began preparing a variety of platinum complexes with different ligands and testing them for antitumor activity. The final result of many studies was the emergence of the set of rules governing molecular structure that appeared to be required in order for a platinum complex to have activity [3]. These structure-activity relationships(SARs) specified that the platinum complex have square planar geometry, be charged neutral, contain two *cis*-am(m)ine carrier ligands and two *cis*-anionic leaving groups with an intermediate binding strength(e.g. Cl^-, SO_4^{2-}, citrate or oxalate) [4]. If these anionic ligands were too labile, the compounds exhibited prohibitively high level of toxicity. Recently, some new strategies to synthesis of new platinum anticancer drugs have emerged [5-8]. They are based on changing the coordinated nitrogen ligand with other donor atom or altering the leaving groups. Other strategies have been focused on changing the type of the metal center(e.g. Pd(II), Ru(III), Ga(III) complexes etc.) or applying Pt(IV) complexes that are relatively more soluble in water. Attention also has been shifted to discover "non-classical" platinum drugs that can act in a manner different from cisplatin [9]. Unconventional structures that violate the empirical SARs of platinum compounds lacking NH_3, NH_2 or NH ligands are examples of these compounds that are designed to circumvent cisplatin resistance and enhance its activity. Lately, interest was directed towards the development of cisplatin analogues that possess N-heterocyclic carrier ligands, coordinated to the cytotoxic platinum(II) moiety, through one or two am(m)ine groups.

Hydantoin derivatives possess a variety of biochemical and pharmacological properties and are used to treat many human diseases. They possess good anticonvulsant properties and depending on the nature of substitution on the hydantoin ring, a wide range of other pharmacological properties, including fungicidal, herbicidal, antitumor, anti-inflammatory, anti-HIV, hypolipidemic, antiarrhythmic, and antihypertensive activities [10,11]. Although hydantoin compounds were studied extensively because there were not many studies about their anticancer properties. Recently the cytotoxic activity of spirohydantoin derivatives was tested in ovarian and breast cancer cells [12]. It has been shown that spirohydantoin derivatives induce growth inhibition and apoptosis in leukemic cells [13].

The present study represents the synthesis, physicochemical evaluation and pharmacological investigation of new mixed am(m)ine Pt(II) complex *cis*-[$PtL(NH_3)Cl_2$]**(1),** where L is 3-amino-α-tetralonespiro-5'-hydantoin**(L1)**. The obtained complex was chemically examined in comparison with previously synthesized and published *cis*-[$Pt(L)_2Cl_2$] and *cis*-[PtL_2Cl_4] **(2-3)** complexes with the same ligand [14]. Herein we report the comparative evaluation of the cytotoxic effects of new synthesized mixed Pt(II) complex and two previously studied Pt(II) and Pt(IV) complexes with 3-amino-α-tetralonespiro-5'-hydantoin vs. the referent antineoplastic agent cisplatin on SKW-3 and HL-60 human tumor cell lines, using the standard MTT-dye reduction assay for cell viability.

II. MATERIALS AND METHODS

A. Methods

α-tetralonespiro-5'-hydantoin was obtained from Assoc. Prof. R. Buyukliev (Faculty of Pharmacy – Medical University – Sofia, Bulgaria). $K[Pt(NH_3)Cl_3]$ was trade product and was purchased from Aldrich – USA. All other chemicals were of analytical grade.

The newly synthesized Pt(II) complex with 3-amino-α-tetralonespiro-5'-hydantoin was characterized by elemental analysis, melting point and IR spectra. The carbon, nitrogen and hydrogen contents of the compound were determined by elemental analysis. The elemental analysis was carried out on a "EuroEA 3000 – Single, EuroVector SpA apparatus. The IR spectra were recorded on Thermo Scientific Nicolet iS10 spectrophotometer in the range of 4000-400 cm^{-1} as ATR and IFS 113 v Bruker FTIR spectrophotometer in the range of 400-150 cm^{-1} as polyethylene. Corrected melting point was determined, using a Bushi 535 apparatus.

B. Synthesis of cis-amminedichloro-3-amino-α-tetralonespiro-5'-hydantoin - (Cis-$[PtL(NH_3)Cl_2]$)

The water-ethanol solution of **L1** (0.0652 g, 0.2823 mmol) was added dropwise to the water solution of $K[Pt(NH_3)Cl_3]$ (0.1007 g, 0.2816 mmol) at constant stirring and at room temperature. The homogenous solution was stirred for 5-6 h, concentrated and cooled to 4°C. A light yellow product was obtained, which was filtered off, washed several times with ethyl ether and dried in a vacuum desiccator. The substance is soluble in DMSO. The purity is checked up by thin layer chromatography with the eluent $CH_3COOC_2H_5/C_2H_5OH$ - 2:1 and elemental analysis.

C. Pharmacology

1) Cell lines and culture conditions.

The cell lines used for the experiments were: SKW-3 or a KE-37 derivative (human T-cell leukemia, established from peripheral blood of a 61-year-old man with T-cell lymphocytic leukemia) and HL-60 (acute myeloid leukemia, established from the peripheral blood of a patient with acute promyelocyte leukemia). They have been well validated in our laboratory as a proper test system for platinum agents. The cell lines were obtained from DSMZ German Collection of Microorganisms and Cell Cultures. Their DSMZ catalogue numbers are as follows: HL-60 (ACC 3) and SKW-3 (ACC 53).

2) Cytotoxicity assessment (MTT-dye reduction assay).

The cell viability was assessed using the standard MTT-dye reduction assay as described by Mosmann [15] with minor modifications [16]. Method is based on the reduction of the yellow tetrazolium salt MTT to a violet formazan product *via* the mitochondrial succinate dehydrogenase in viable cells. Aliquots of 100 µl/well cellular suspension (at a density of $1x10^5$ exponentially growing cells/ml) were seeded in 96-well flat-bottomed microplates and after 24 h incubation at 37°C were exposed to various concentrations of the tested compounds for 72 h.

For each concentration at least 8 wells were used. After the incubation with the test compounds 10 µl MTT solution (10 mg/ml in PBS) were added to each well and the microplates were further incubated for 4 h at 37°C. Thereafter the formazan crystals formed were dissolved through addition of 100 µl/well 5% formic acid solution in 2-propanol. The MTT-formazan absorption was measured using a microprocessor-controlled ELISA reader (Labexim LMR-1) at 580 nm. Cell survival fractions were calculated as percentage of the untreated control. In addition IC_{50} values were calculated from the concentration-response curves. The experimental data were processed by means of GraphPad Prism software and were fitted to sigmoidal concentration-response curves via non-linear regression.

III. RESULTS AND DISCUSSION

A. Chemistry

The Pt(II) complex with 3-amino-α-tetralonespiro-5'-hydantoin as carrier ligand was obtained according to reported procedure with minor revision [17]. The method consists in interaction of water/ethanol solution of the ligand **(L1)** with water solution of $K[Pt(NH_3)Cl_3]$ at constant stirring. The scheme of the synthesis of the new mixed Pt(II) complex is shown in Figure 1.

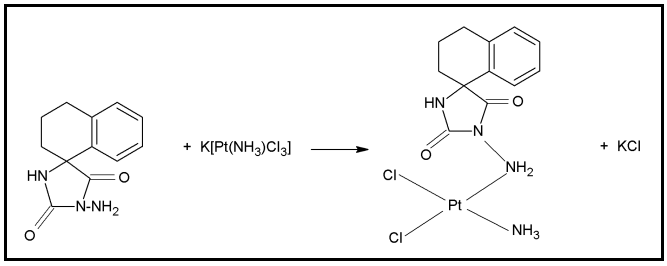

Fig. 1. Scheme of the synthesis of new Pt(II) complex **(1)**

The elemental analysis of the new Pt(II) complex was in good agreement with the following chemical formula: $[Pt(C_{12}H_{13}N_3O_2)(NH_3)Cl_2].2H_2O$**(1).** The determination of crystal water content in the complex was defined by DTA analysis. In order to evaluate the mode of coordination of the ligand to the metal ion IR spectra of the metal free ligand as well as of its Pt(II) complex were recorded.

Some analytical and physical data of complex **(1)** were summarized in Table I.

TABLE I. SOME PHYSICAL PROPERTIES OF NEW PLATINUM COMPLEXES **(1), (2)** AND **(3)**

№	Compound (Empirical formula)	Color	m.p. (°C) dec.	Yield %	M_w	Elemental analysis found (Calc.)		
						%C	*%H*	*%N*
1	$[Pt(C_{12}H_{13}N_3O_2)(NH_3)Cl_2].2H_2O$	Light yellow	228	58	549.9	25.82 (26.19)	3.47 (3.64)	10.39 (10.18)
2	$[Pt(C_{12}H_{13}N_3O_2)_2Cl_2]$	yellow	238	56	727.9	39.49 (39.57)	4.22 (3.57)	11.77 (11.54)
3	$[Pt(C_{12}H_{13}N_3O_2)_2Cl_4]$	yellow	209	26	798.8	36.39 (36.05)	3.22 (3.25)	10.77 (1052)

TABLE II. SELECTED INFRARED SPECTRAL DATA OF THE 3-AMINO-A-TETRALONESPIRO-5'-HYDANTOIN AND ITS PLATINUM COMPLEXES **(1), (2)** AND **(3)**

Compounds	ν(NH_2)	ν(C=O) - hyd	δ(NH_2)	ν(M-N)	ν(M-Cl)
L=3-amino-α-tetralonespiro-5'-hydantoin	3237	1768 1717	1600	-	-
Cis-$[Pt(C_{12}H_{13}N_3O_2)(NH_3)Cl_2]$	3152	1706 1717	1634	543 523	358 337
Cis-$[Pt(C_{12}H_{13}N_3O_2)_2Cl_2]$	3164	1780 1719	1621	557 501	434 379
Cis-$[Pt(C_{12}H_{13}N_3O_2)_2Cl_4]$	3220	1779 1713	1618	562 551	351 322

B. IR spectra

The comparative analysis of the infrared spectra of the complex **(1)** and of the metal-free ligand **(L1)** revealed that the absorption band characteristic for the ν(NH_2) were blue shifted from 3237 cm^{-1} for the **(L1)** to 3152 cm–1 for the complex **(1)**. Absorption band characteristic for the δ(NH_2) was blue shifted from 1600 cm^{-1} for the ligand **(L1)** to 1634 cm^{-1} for the complex **(1)**. This indicates that the NH_2 group at N3 position from hydantoin ring participates in coordination to the metal ion in the complex. The new bands in the low-energy region at 543–523 cm^{-1} were assigned to the ν(Pt–N) stretching vibrations. The bands at 358–337 cm^{-1} were ascribed to the ν(Pt–Cl). In the spectra of the complex **(1)** two bands for ν(Pt–Cl) and ν(Pt–N) stretching vibrations were observed, implying *cis*-location of chloride and am(m)ine ligands according to Nakamoto [18]. (Table II).

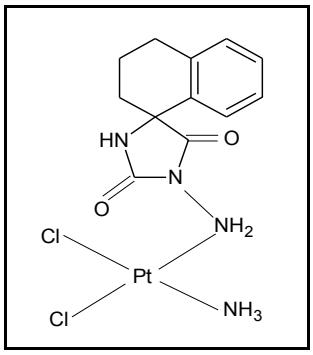

Fig. 2. Scheme of the complex **(1)**, object of the recent work

All these arguments corresponded to the previously obtained and studied Pt(II) and Pt(IV) complexes with the same ligand **(L1)**[14]. In our earlier research we have been proved that the bonding of the ligand with metal ions was realized by nitrogen atom from NH_2 group at N3 position from hydantoin ring in Pt(II) and Pt(IV) complexes with 3-amino-α-tetralonespiro-5'-hydantoin and general formulas *cis*-$[ML_2Cl_2]$ and *cis*-$[ML_2Cl4]$

On the basis of all experimental data the following chemical formulas for complex **(1)** in comparative aspect with the previously reported Pt(II) **(2)** and Pt(IV) **(3)** complexes could be proposed. (Figures 2, 3, 4).

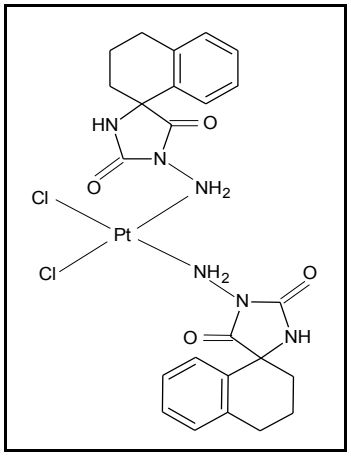

Fig. 3. Chemical formula of the complex **(2)** with ligand **L1**

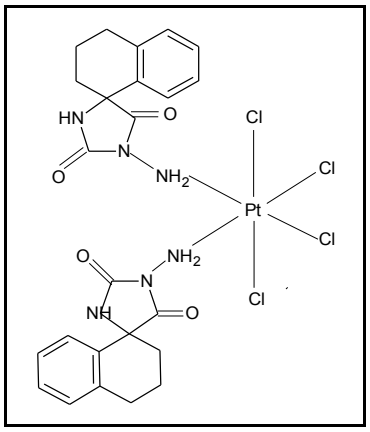

Fig. 4. Chemical structure of the complex **(3)**

C. In vitro cytotoxicity

In comparison of the cytotoxic effects of the mixed am(m)ine Pt(II) complex **(1)** with those of complexes **(2)** and **(3)** it could be seen that the newly synthesized complex **(1)** was more active than the other Pt(II) and Pt(IV) complexes on SKW-3 cell line. The IC_{50} value of the complex **(1)** was 35.5 μM while the IC_{50} values for the complexes **(2)** and **(3**) were 174.8 and 188.4 on SKW-3 cell line. (Figure 5). The complex **(1)** has very close cytotoxic effect to the cisplatin on the same cell line SKW-3. It could be explained by the fact that complex **(1)** was the cisplatin analogue, where one ammine group was replacement by one planar N-heterocyclic amine ligand. According to the empirical structure-activity relationships a necessary condition for an active platinum complex was the *cis*-configuration of the two am(m)ine groups and two *cis*-anionic leaving groups such as Cl^-, SO_4^{2-}, citrate or oxalate. This shows that the Pt(II) complex from the type *cis*-$[Pt(NH_3)LCl_2]$ was more active than the Pt(II) complexes from the types *cis*-$[PtL_2Cl_2]$ and *cis*-$[PtL_2Cl_4]$. On HL-60 cell line the cytotoxicity of the newly synthesized complex **(1)** is similar to those of the previously studied platinum complexes **(2)** and **(3)**. But, all platinum complexes **(1), (2)** and **(3)** were less active than the referent platinum drug cisplatin on the same HL-60 cell line. (Table III).

TABLE III. CYTOTOXICITY OF THE NEW PLATINUM COMPLEXES **(1)**, **(2)**, **(3)** AND REFERENT DRUG CISPLATIN

IC_{50} values (μM)		
Compounds	***SKW-3***[a]	***HL-60***[b]
Complex **(1)**	35.5	103.9
Complex **(2)**	174.8	not detected
Complex **(3)**	188.4	101.4
Cisplatin	11.4	8.7

[a] T-cell leukemia;

[b] Acute myeloid leukemia

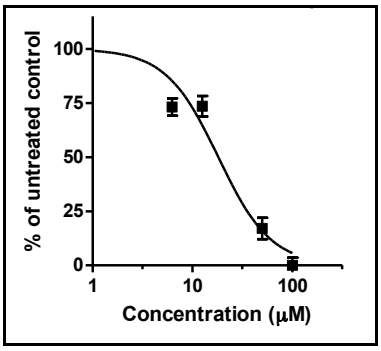

Fig. 5. Cytotoxic effect of the complex **(1)** on SKW-3 cell line

CONCLUSION

One new Pt(II) complex with formula *cis*-$[Pt(NH_3)LCl_2]$, where L is 3-amino-α-tetralonespiro-5'-hydantoin was synthesized and investigated. The molecular formula of the complex was confirmed by elemental analysis, melting point and IR spectroscopy. The coordination mode of the ligand with metal ion was demonstrated after the comparison of the complex **(1)** with the complexes **(2)** and **(3)** with the same ligand, but with different general formulas *cis*-$[PtL_2Cl_2]$ and *cis*-$[PtL_2Cl_4]$. In all platinum complexes the ligand 3-amino-α-tetralonespiro-5'-hydantoin coordinated by platinum ions through the nitrogen atom from NH_2 group at N3 position in the hydantoin ring. The tested compounds **(1), (2)** and **(3)** exerted concentration dependent cytotoxic activity on SKW-3 and HL-60 human tumour cell lines. The most significant cytotoxicity was observed for the complex **(1)**, which inhibited the viability of tested cells at low micromolar concentrations and IC_{50} value was very close to that of cisplatin.

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