RESEARCH ARTICLE Am. J. PharmTech Res. 2014; 4(4) ISSN: 2249-3387

AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Synthesis, Characterization and Cytotoxic Activity of New Palladium Complexes with 3-Thiolanespiro-5’-Hydantoin and 4-Thio-1h-Tetrahydropyranespiro-5’-Hydantoin

Adriana GeorgievaBakalova*[1], RossenTodorov Buyukliev[1], AntoninaTzekova Nikolova[1], GeorgiTzvetanov Momekov[2]

1. Department of Chemistry, Faculty of Pharmacy, Medical University-Sofia, 2 Dunav Str., 1000 Sofia, Bulgaria,

2.Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Pharmacy, Medical University-Sofia, 2 Dunav Str., 1000 Sofia, Bulgaria

ABSTRACT

Four Pd(II) and Pd(IV) complexes with 3-thiolanespiro-5’-hydantoin and 4-thio-1H-tetrahydropyranespiro-5’-hydantoin were synthesized. Thechemical structures of newcomplexes were studied by elemental analysis, IR, ^{1}H, ^{13}C NMR spectra. The spectroscopic investigation of new palladium complexes in comparison with the metal free ligands show that the bonding of the ligands with metal ions is realized through the sulfur atom from the S-containing saturated rings. This coordination mode is in accordance with the data obtained for the platinum complexes with the same ligands. The new complexes were investigated for cytotoxicity on panel of human tumor cell lines. The tested compounds exerted concentration-dependent cytotoxic effects against some of the tumor cell lines.

Keywords: Pd complexes, cytotoxicity, S-containing saturated rings

*Corresponding Author Email: adrigebk@abv.bg

Received 14 July 2014, Accepted 25 July 2014

Please cite this article as: Bakalova AG *et al.*, Synthesis, Characterization and Cytotoxic Activity of New Palladium Complexes with 3-Thiolanespiro-5’-Hydantoin and 4-Thio-1h-Tetrahydropyranespiro-5’-Hydantoin. American Journal of PharmTech Research 2014.

INTRODUCTION

The use of metal-based compounds as therapeutic drugs dates back to 5000 years. In modern days the introduction of cisplatin in cancer chemotherapy leads to increase the interest of the metal complexes in medicinal chemistry. The major classes of metal-based anticancer drugs include platinum(II), platinum(IV), palladium(II), palladium(IV), gold(I), gold(III), ruthenium(II), ruthenium(III), bismuth(III), rhenium(I), copper(II), gallium(III) and tin(IV) compounds, some of them having been reported to demonstrate higher anticancer activity *in vitro* than cisplatin[1]. Pd(II) complexes are more reactive than respective Pt(II) species. Pd(II) complexes are used as models in the studies of ligand exchange reactions in biological systems. These studies have shown the very special properties of Pd(II) complexes, i.e. they exhibit an intense biological activity. Palladium(II) complexes are much more labile than corresponding platinum(II) complexes, the lability of central palladium(II) may be much lower because of the shielding effect [2]. The Pd(II) ions are capable of interacting with DNA thus enabling cross bindings and inhibiting its synthesis as well as inducing apoptosis [3-5]. It is possible to overcome the high labile-ligand exchange at Pd-center by designing highly thermodynamically stable and kinetically inert complexes

The palladium(II) complexes with pyrazole as ligands have been studied by Keter and they caused apoptosis of cancer cells of human and ovarian fibroma of hamsters [6]. Palladium(II) complexes with ethylenediamine and pyridine show similar effects to those characteristic for cisplatin cytostatic activity on human leukemia cell line [7]. The 2-thiosemicarbazones-Pd(II) complex with acetyl pyridine as ligand has potent antitumor properties shown *in vivo* activity against murine leukemia cells. Giovagnini*et al.* studied *in vitro* cytotoxic activity of new palladium(II) derivatives of methylsarcosinedithiocarbamate and its S-methyl ester [8]. These compounds cause the apoptosis of human leukemic promyelocytes cell line HL-60 and human squamous cervical adenocarcinoma cell line HeLa[8]. The antitumor activity of these complexes is comparable to that of cisplatin, and the value of the IC_{50} factor is even higher than in the case of cisplatin.

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, antitumour, anti-inflammatory, ant-HIV, hypolipidemic, antiarrythmyc and antihypertensive activities [9,10]. Although hydantoin compounds are studied extensively, there are not many studies about their antitumour activity. Recently, the cytotoxicity of spirohydantoin derivatives was tested in ovarian and breast cancer cells [11]. It has been shown that a spirohydantoin derivative induces growth inhibition and apoptosis in leukemic cells [12]. Former studies demonstrated that 5-arylidene-2-thiohydantoins have *in vitro*antimycobacterial activity [13].

In the previous study from our laboratory has investigated Pt(II), Pt(IV), Pd(II) and Pd(IV) complexes with 3,5-disubstituted hydantoins and spirohydantoins[14-18]. Some of these complexes showed higher cytotoxicity *in vitro* than cisplatin.

As a part of our drug discovery program to develop more effective palladium based anticancer drugs, here we report the antiproliferative activity of some Pd(II) and Pd(IV) complexes. The present study represents the synthesis, physicochemical evaluation and pharmacological investigation of Pd(II) and Pd(IV) complexes with 3-thiolanespiro-5'-hydantoin (**L1**) and 4-thio-1H-tetrahydropyranespiro-5'-hydantoin **(L2)**. The cytotoxic activity of the newly synthesized Pd(II) and Pd(IV) complexes was compared with those of Pt(II) and Pt(IV) complexes with the same ligands [19].

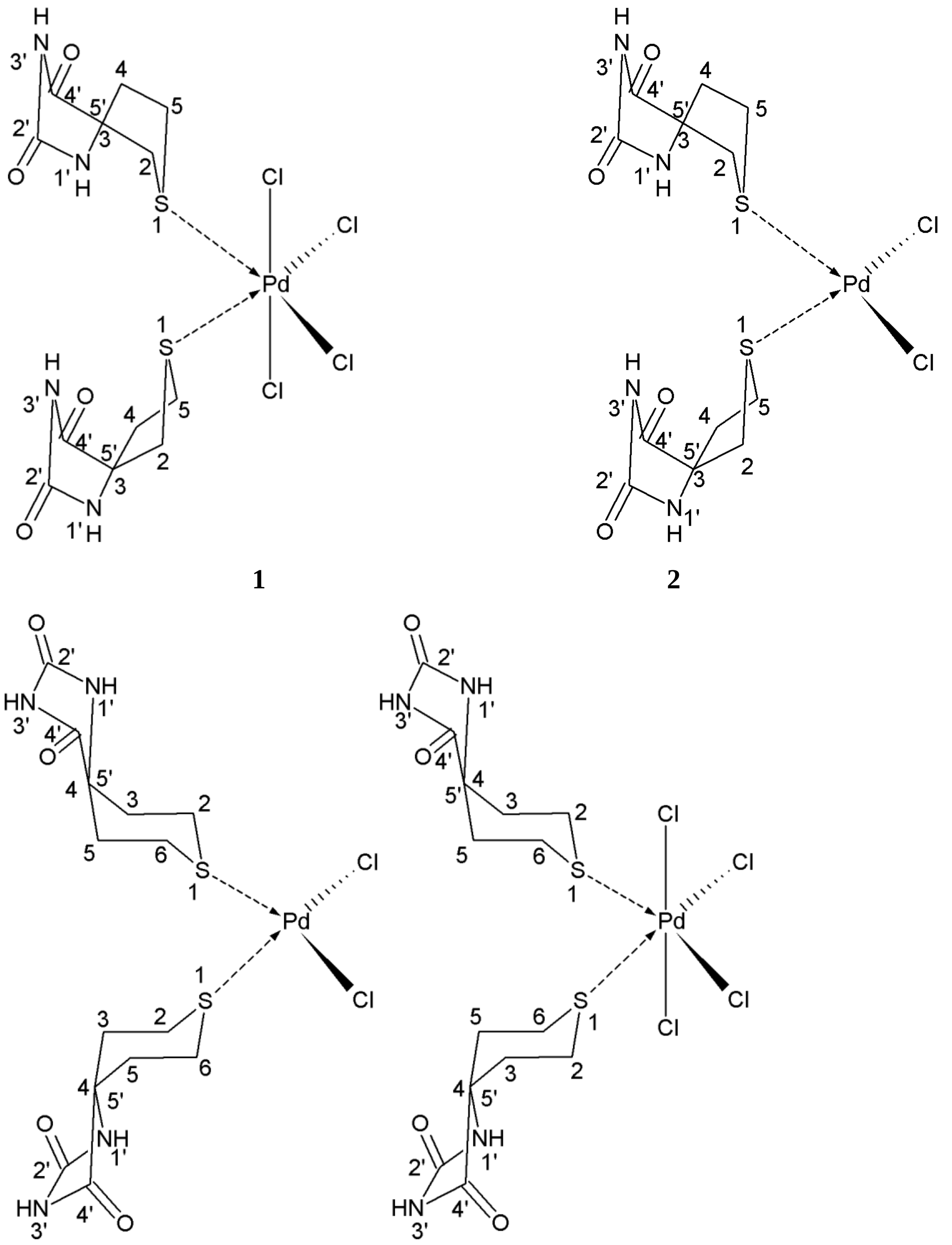
MATERIALS AND METHODS:

3-thiolanespiro-5'-hydantoin**(L1)** and 4-thio-1H-tetrahydropyranespiro-5'-hydantoin**(L2)** werepreparedand were describedin our previous article. They were used as carrier ligands for the obtaining of new Pd(II) and Pd(IV) complexes. Potassium tetrachloropalladate(II) and Potassium hexachloropalladate(IV) chloride utilized for the synthetic procedures were purchased from Aldrich-USA. All other chemicals were of analytical grade.

ThePd(II) and Pd(IV) complexes were characterized by elemental analysis, IR, ^{1}H, ^{13}C NMR spectra.

The carbon, nitrogen and hydrogen contents of the compounds were determined by elemental analysis, carried out on a "EuroEA 3000 apparatus.

The IR spectra were recorded on Thermo Scientific Nicolet iS10 spectrophotometer in the range of 4000-400 as ATR and on IFS 113 v Bruker FTIR spectrophotometer in the range of 400-150 cm^{-1} in polyethylene. Intensities of reported IR bands are defined as br = broad, s = strong, m = medium, and w = weak. The ^{1}H and ^{13}C NMR spectra were registered on a Bruker WM 250 (250 MHz) spectrometer in freshly prepared DMSO-d_6 solutions. The splitting of proton resonances in the ^{1}H NMR spectra is defined as s = singlet, d = doublet, dd = doublet of doublets and m = multiplet (see Figures 1-4 for NMR numbering scheme). Corrected melting points were determined, using a Buchi 535 apparatus.



1

2

3

4

Figure 1. Chemical structures of Pd complexes (1 – 4)

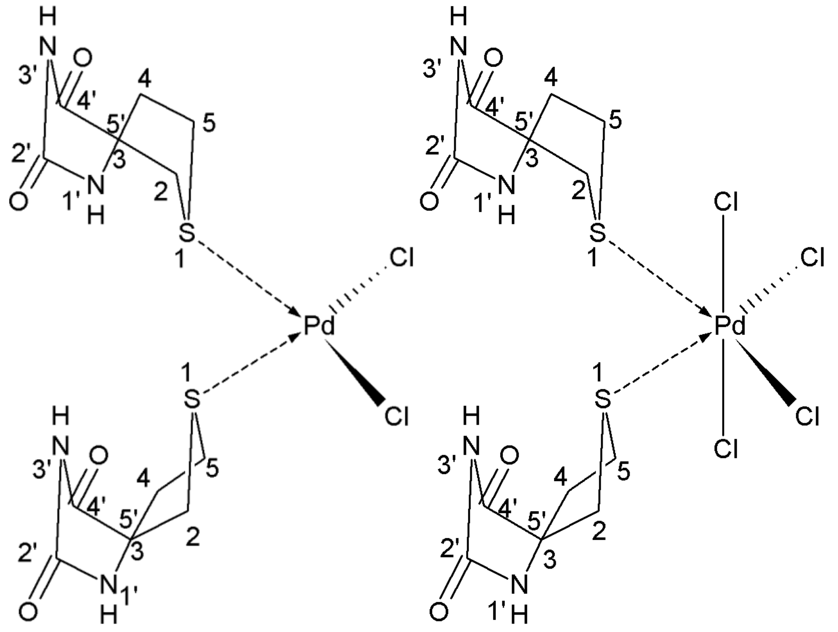


Figure 2

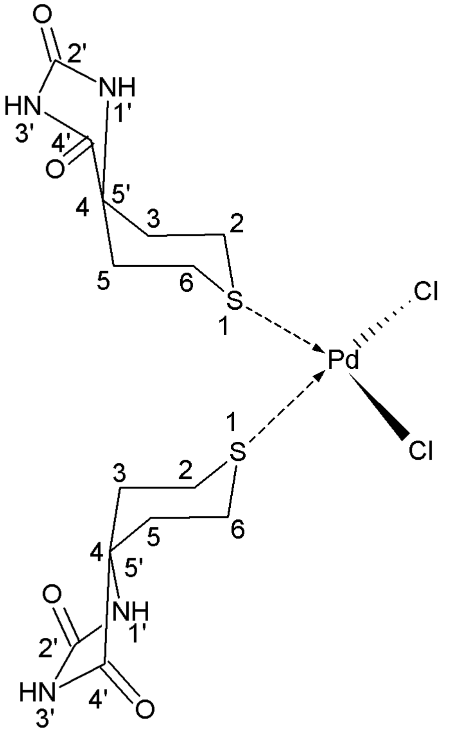


Figure 3

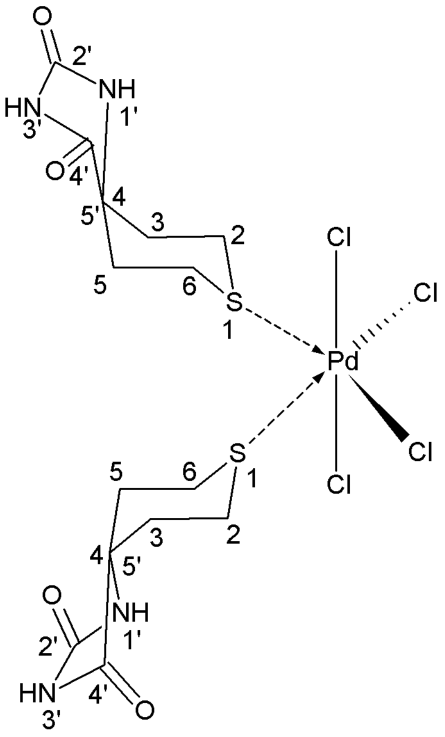


Figure 4

Pharmacology

The following cell lines were used for the experiments: (i) 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); (ii) HL-60 (acute myeloid leukemia, established from the peripheral blood of a patient with acute promyelocyte leukemia); (iii)EJ (human urinary bladder carcinoma), (iiii) LAMA-84 (human chronic myeloid leukemia, established from peripheral blood of a 29-year-old woman with chronic myeloid leukemia).

The cell culture flasks and the 96-well microplates were obtained from NUNCLON (Denmark). MTT, FCS and cisplatin were purchased from Sigma Co. The stock solutions of tested compounds (10 mM) were freshly prepared in DMSO. The serial dilutions of the tested compounds were prepared immediately before use. At the final dilutions obtained the concentrations of DMSO never exceeded 1%.

Cytotoxicity of the compounds was assessed using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] dye reduction assay as described by Mossman [20] with some modifications [21]. Exponentially growing cells were seeded in 96-well microplates (100 µl/well at a density of 3.5×10^5 cells/ml for the adherent and 1×10^5 cells/ml for the suspension cell lines) and allowed to grow for 24 h prior the exposure to the studied compounds. Cells were exposed to the tested agents for 72 h, whereby for each concentration a set of 8 separate wells was used. Every test was run in triplicate. After incubation with the tested compounds MTT solution (10 mg/ml in PBS) aliquots were added to each well. The plates were further incubated for 4 h at 37ºC and the formazan crystals formed were dissolved by adding 110 μl of 5% HCOOH in 2-propanol. The MTT-formazan absorption was measured using a multimode microplate reader (Beckman Coulter DTX880) and the results were normalized as percentage of the untreated control (set as 100% viable). The data were fitted to sigmoidal dose-response curves and the IC_{50} values were calculated using non-linear regression analysis (Curve-fir; GraphPadPrizm software for PC).

Synthesis of palladium complexes

Preparation of *cis*-dichlorido-bis(3-thiolanespiro-5'-hydantoin)palladium(II) - *cis*-$[Pd(L1)_2Cl_2]$(1)

Water solution of $K_2[PdCl_4]$ (0.1008 g, 0.3088 mmol) and ethanol/water solution of **(L1)** (0.1059 g, 0.6157 mmol) were mixed and stirred at room temperature for 7-8 hours. The precipitate was filtered off and washed with distilled water. The solid product was recrystallized from ethanol solution and dried under vacuum. The substance is soluble in DMSO, DMF and weakly soluble in water and ethanol. Anal. Calcd(%) for $[Pd(C_6H_8N_2O_2S)_2Cl_2]$: C, 27.62; H, 1.53; N, 10.74; Found(%): C, 27.24; H, 1.89; N, 11.03; IR (ATR and polyethylene) 3158 br, 3071 br, 1772 m, 1721 s, 1674 m, 1053 m, 451 w, 361 br, 316 br; ^{1}H NMR (DMSO-d_6, δ, ppm): 10.80 (s, 1H, NH-3); 8.49 (s, 1H, NH-1); 3.20-2.85 (m, 4H, CH_2(2)-S+CH_2(5)-S); 2.35-2.15 (m, 2H, CH_2(4)).

Preparation of *cis*-tetrachlorido-bis(3-thiolanespiro-5'-hydantoin)palladium(IV) - *cis*-$[Pd(L1)_2Cl_4]$(2)

Water solution of $K_2[PdCl_6]$ (0.1029 g, 0.2591 mmol) was added dropwise to ethanol/water solution of **(L1)** (0.0861 g, 0.5006 mmol) at constant stirring for 8 hours. The obtained precipitate was filtered off and washed with distilled water. The solid phase is recrystallized from ethanol solution and dried under vacuum. The substance is soluble in DMSO, DMF and weakly soluble in water and ethanol. Anal. Calcd(%) for $[Pd(C_6H_8N_2O_2S)_2Cl_4]$: C, 24.32; H, 2.70; N, 9.45; Found(%): C, 24.67; H, 2.85; N, 9.58; IR (ATR and polyethylene): 3189 br, 3074 br, 1774 m, 1728 s, 1652 m, 1054 w, 450 w, 374 br, 316 br; ^{1}H NMR (DMSO-d_6, δ, ppm): 10.85 (s,1H, N(3)-H), 8.46 (s,1H, N(1)-H), 3.15-2.85 (m, 4H, CH_2(2)-S +CH_2(5)-S), 2.20-2.00(m, 2H, CH_2(4)).

Preparation of *cis*-dichlorido-bis(4-thio-1H-tetrahydropyranespiro-5'-hydantoin)palladium(II) - *cis*-$[Pd(L2)_2Cl_2].H_2O$(3)

Water solution of $K_2[PdCl_4]$ (0.1006 g, 0.3082 mmol) and ethanol solution of **(L2)** (0.1150 g, 0.6183 mmol) were mixed and stirred at room temperature for 8-9 hours. The precipitate was filtered off, washed with several portions of distilled water and dried under vacuum. The substance is soluble in DMSO, DMF and weakly soluble in water. Anal. Calcd(%) for $[Pd(C_7H_{10}N_2O_2S)_2Cl_2].H_2O$: C, 28.70; H, 4.10; N, 9.57; Found(%): C, 28.82; H, 3.99; N, 9.13; IR (ATR and polyethylene): 3257 br, 1768 m, 1730 s, 1650 m, 1063 m, 430 w, 367 br, 318 br; ^{1}H NMR (DMSO-d_6, δ, ppm): 10.80 (s, 1H, NH-3); 8.50 (s, 1H, NH-1); 3.20-2.85 (m, 2H, CH_2(2)(a) + CH_2(6)(a)); 2.41-2.33 (m, 2H, CH_2(2)(e) + CH_2(6)(e)); 2.09-1.95 (m, 2H, CH_2(3)(a) + CH_2(5)(a)); 1.90-1.79 (m, 2H, CH_2(3)(e) + CH_2(5)(e)). ^{13}C-NMR (DMSO-d_6, δ, ppm): 177.1 (C=O – 4’); 156.0 (C=O – 2’); 60.5 (C-5’); 32.7 (C(2) + C(6); 27.0 (C(3) + C(5)).

Preparation of *cis*-tetrachlorido-bis(4-thio-1H-tetrahydropyranespiro-5’-hydantoin)palladium(IV) - *cis*-[Pd(L2)$_2$Cl$_4$](4)

Water solution of $K_2[PdCl_6]$ (0.1012 g, 0.2548 mmol) was added dropwise to ethanol solution of **(L2)** (0.0949 g, 0.5102 mmol) at room temperature for 9 hours. The precipitate was filtered off and washed with several portions of distilled water. The solid product is recrystallized from ethanol solution and dried under vacuum. The substance is soluble in DMSO, DMF and weakly soluble in water. Anal. Calcd(%) for $[Pd(C_7H_{10}N_2O_2S)_2Cl_4]$: C, 27.09; H, 3.22; N, 9.03; Found(%): C, 26.78; H, 3.51; N, 9.43; IR (ATR and polyethylene): 3256 br, 3000 br, 1746 m, 1726 s, 1063 m, 431 w, 371 br, 320 br; ^{1}H NMR (DMSO-d_6, δ, ppm): 10.80(s,1H, N(3)-H), 8.50(s,1H, N(1)-H), 3.10-2.96 (m, 2H, CH_2(2)(a) + CH_2(6)(a)); 2.41-2.34 (m, 2H, CH_2(2)(e) + CH_2(6)(e)); 2.27-2.09 (m, 2H, CH_2(3)(a) + CH_2(5)(a)); 1.90-1.77 (m, 2H, CH_2(3)(e) + CH_2(5)(e)).^{13}C-NMR (DMSO-d_6, δ, ppm): 177.1 (C=O – 4’); 156.0 (C=O – 2’); 59.7 (C-5’); 32.6 (C(2) + C(6)); 27.5 (C(3) + C(5)).

In vitro studies

The present study describes a comparative evaluation of the cytotoxic effects of the metal free ligands **L1** and **L2**, the newly synthesized Pd(II) and Pd(IV) complexes vs. the referent antineoplastic agent cisplatin on a panel of human tumor cell lines, using the standard MTT-dye reduction assay for cell viability. The complexes exerted cytotoxic effects after 72 h continuous exposure, whereby the individual chemosensitivity varied among the different cell lines, as evidenced by the IC_{50} values, summarized in Table 2. The complex **(1)** proved to be more active as compared to the ligand **L1** on HL-60 and LAMA-84 cell lines. The cytotoxicity of the ligand **L2** was comparable with those of the complexes **(3)** and **(4)** on SKW-3 cell line. The cytotoxic effects of the ligands **L1** and **L2** were similar to the effects of the complexes **(1), (2), (3), (4)** respectively. The tested palladium compounds displayed cytotoxic effects in a concentration dependent manner. The antiproliferative activity of the palladium complexes in comparison with those of the platinum complexes with the same ligands was similar [19]. This proved that palladium complexes have analogous cytotoxicity to platinum compounds with the same ligands.

RESULTS AND DISCUSSION:

Chemistry

The obtaining of the ligands 3-thiolanespiro-5’-hydantoin**(L1)** and 4-thio-1H-tetrahydropyranespiro-5’-hydantoin**(L2)** was prepared by the Bucherer-Berg method and was described in our previous article [19]. Their Pd(II) and Pd(IV) complexes were synthesized using procedures which are described in the section Materials and Methods.

The elemental analysis for thenew Pd(II) and Pd(IV) complexes were in good agreement with the following molecular formulae:$[Pd(C_6H_8N_2O_2S)_2Cl_2]$**(1),** $[Pd(C_6H_8N_2O_2S)_2Cl_4]$**(2),** $[Pd(C_7H_{10}N_2O_2S)_2Cl_2].H_2O$ **(3)** and $[Pd(C_7H_{10}N_2O_2S)_2Cl_4]$ **(4)**. Thedetermination of crystal water content in the complexes **(3)** was defined by DTA analysis. In order to evaluate the mode of coordination of the ligands to the metal ions, IR, ^1H and ^{13}C NMR spectra of the metal free ligands as well as of their Pd(II) and Pd(IV) complexes were recorded.

Table 1 Physical properties of new palladium complexes

Compound code	Mol. Formula	Mol. Weight	Melting point (°C)	Yield (%)
1	$Pd(C_6H_8N_2O_2S)_2Cl_2$	521.7	243°C (dec.)	74
2	$Pd(C_6H_8N_2O_2S)_2Cl_4$	592.6	238°C (dec.)	18
3	$Pd(C_7H_{10}N_2O_2S)_2Cl_2].H_2O$	549.7	300°C (dec.)	80
4	$Pd(C_7H_{10}N_2O_2S)_2Cl_4$	620.6	291°C (dec.)	50

Table 2. Cytotoxicity of the Pd(II) and Pd(IV) complexes with 3-thiolanespiro-5’-hydantoin and 4-thio-1H-tetrahydropyranespiro-5’-hydantoin(1-4) in comparison to cisplatin in four human tumour cell lines

Cell line	SKW-3[a]	**IC_{50} values(μM)**	EJ[c]	LAMA-84[d]
Compound		HL-60[b]		
L1	114.0	202.8	115.2	174.5
1	>200	147.5	128.9	75.8
2	151.0	292.2	106.2	150.8
L2	92.6	180.9	143.5	101.1
3	111.3	114.4	127.2	182.1
4	147.9	109.2	152.7	178.4
Cisplatin	11.4	8.7	10.2	16.9

[a]T-cell leukemia; [b]acute myeloid leukemia, [c]urinary bladder carcinoma, [d]human chronic myeloid

IR spectra

Shifting of the signals corresponding to ν(C-S) bond from 1030 cm^{-1} in the metal free **L1** to 1054 and 1053 cm^{-1} in the complexes **(1)** and **(2)** respectively is observed. In the case of **L2** this values are as follows: 1013 cm^{-1} for **L2** and 1063 and 1063 cm^{-1} for complexes **(3)** and **(4)** respectively. In the IR spectra of the complexes **(1-4)** the new bands at 451, 450, 430 and 431 cm^{-1} are appeared.

These stretching vibrations can be attached to the ν(Pd-S) coordinative bonds. New bands at 374–316 cm^{-1} were assigned to the ν(Pd–Cl) stretching vibrations. In the IR spectra of complexes **(1–4)** two bands for ν(Pd–Cl) stretching vibrations were observed, implying *cis*-location of chloride ligands [22].

The bands related to the stretching vibrations of the two carbonyl groups in the metal-free ligands did not shift upon coordination of **L1** and **L2** to Pd(II) and Pd(IV) ions, indicating that the C=O groups were not involved in binding to the metals.

NMR spectra

In the ^1H NMR spectra of freshly prepared DMSO-d_6 solutions of the Pd(II) and Pd(IV) complexes with 3-thiolanespiro-5'-hydantoin **(1,2)**, the signals of the protons for $CH_2(2)$ and $CH_2(5)$ are in relatively wide range – between 3.20 and 2.85 ppm. This is due to the fact that S-containing ring is fixed and sulfur atom is bonding with palladium ions.

In the ^1H NMR spectra of the Pd(II) and Pd(IV) complexes with 4-thio-1H-tetrahydropyranespiro-5'-hydantoin **(3,4)** the signals of the protons for $CH_2(2)$ and $CH_2(6)$ are in more wide range than previous complexes – between 3.20 and 2.33 ppm. In this case the ring is fixed because sulfur is bonded with palladium ions; axial and equatorial hydrogens are easily discernment.

In the ^{13}C NMR spectra of the Pd(II) and Pd(IV) complexes with 4-thio-1H-tetrahydropyranespiro-5'-hydantoin **(3,4)**,carbon atoms C(2) and C(6) directly connected to the sulfur. They give one signal due to the symmetry of the cyclic ring. This signal is at 32.7 ppm for complex **(3)** and 32.6 ppm for complex **(4)**.

^1H and ^{13}C NMR spectra of the complexes show little differences between metal free ligands and newly synthesized Pd(II) and Pd(IV) complexes. These results show that sulfur atom has relatively low polarization when is included in the complex.

CONCLUSION

Four complexes of Pd(II) and PdIV) with 3-thiolanespiro-5'-hydantoin and 4-thio-1H-tetrahydropyranespiro-5'- hydantoin were synthesized. The molecular formulae of the palladium complexes were determined by elemental analysis, IR, ^1H and ^{13}C NMR spectra. In all complexes the coordination mode of the ligands to the metal ions is realized through the sulphur atom from the cyclic ring. The complexes were tested for antiproliferative activity *in vitro* on panel of human tumor cell lines. The tested compounds exerted concentration-dependent cytotoxic effects against some of the tumor cell lines.

REFERENCES:

1. Galanski M, Arion V, Jakupec M, Keppler B. Recent developments in the field of tumor-inhibing metal complexes. Curr Pharmaceut Design, 2003; 9: 25: 2078-2089.
2. Daghriri H, Huq F, Beale P. Studies on activities, cell up take and DNA binding of four multinuclear complexes of the form: [{*trans*-PtCl$(NH_3)_2$}$_2$μ-{*trans*-Pd$(NH_3)_2$-$(H_2N(CH_2)_nNH_2)_2$}]Cl_4 where n = 4–7, J Inorg Chem 2004; 98: 1722-1733.
3. Quoriga A, Perez J, Montero E, West D, Alonso C, Navaro-Raninger C. Synthesis and characterization of Pd(II) and Pt(II) complexes of *p*-isopropylbenzaldehyde N-protected thiosemicarbazones. Cytotoxic activity against *ras*-transformed cells, J Inorg Biochem 1999; 75: 293-301.
4. Matesanz A, Perez J, Navaro P, Moreno J, Colacio E, Souza P, Synthesis and characterization of novel palladium(II) complexes of bis(thiosemicarbazone). Structure, cytotoxic activity and DNA binding of Pd(II)-benzyl bis(thiosemicarbazonate), J Inorg Biochem 1999; 76: 29-37.
5. Kovala-Demertzi D, Domopoulou A, Demertzis M, Valle G, Papageorgiou A, Palladium(II) complexes of 2-acetylpyridine N(4)-methyl, N(4)-ethyl and N(4)-phenyl-thiosemicarbazones. Crystal structure of chloro(2-acetylpyridine N(4)-methylthiosemicarbazonato) palladium(II). Synthesis, spectral studies, *in vitro* and *in vivo*antitumour activity, J Inorg Biochem 1997; 68: 147-155.
6. Keter FK. Pyrazole and pyrazolyl palladium(II) and platinum(II) complexes: synthesis and in vitro evaluation as anticancer agents, A thesis submitted in partial fulfillment of the requirments for the degree of masters in science in the Department of Chemistry. University of the Western Cape. November. 2004.
7. Pranczk J, Jacewicz D, Wyrzykowski D, Chmurzyński L. Platinum(II) and Palladium(II) Complex Compounds as Anticancer Drugs. Methods of Cytotoxicity Determination, Curr Pharmaceut Analysis. 2014; 20: 2-9.
8. Giovagnini L, Ronconi L, Aldinucci D, Lorenzon D, Sitran S, Fregona D. Synthesis, characterization and comparative in vitro cytotoxicity studies of platinum(II), palladium(II) and gold(III) methylsarcozinedithiocarbamate complexes, J Med Chem 2005; 48: 1588-1595.
9. Thenmozhiyal J, Wong P, Chui W, Anticonvulsant activity of phenylmethylenehydantoins: a structure-activity relationship study. J Med Chem 2004; 47: 1527-1535.

10. Dylag T, Zygmunt M, Maciag D, Handzlik J, Bednarski M, Filipek B, Kieć-Kononowicz K, Synthesis and evaluation of *in vivo* activity of diphenylhydantoins basic derivatives. Eur J Med Chem 2004; 39: 1013-1027.
11. Rajik Z, Zorc B, Raic-Malic S, Easter K, Kralj M, Pavelic K, Balzarini J, De Clercq E, Mintas M, Hydantoin derivatives of L- and D-amino acids: synthesis and evaluation of their antiviral and antitumoral activity. Molecules. 2006; 11: 837-846.
12. Kavitha C, Nambiar M, Kumar C, Choudhary B, Muniyappa K, Rangappa K, Raghavan S, Novel derivatives of spirohydantoin induce growth inhibition followed by appoptosis in leukemia cells. Biochem Pharmacol. 2009; 77: 348-363.
13. Kieć-Kononowicz K, Szymańska E, Antimycobacterial activity of 5-arylidene derivatives of hydantoin. Farmaco. 2002; 57: 909-916.
14. Varbanov H, Bakalova A, Buyukliev R, Momekov G, Baykushev R, Synthesis of palladium(II) Complexes with 3-amino-5-methyl-5-(4-pyridyl)-hydantoin. Cytotoxic and Antimicrobial Investigations and Comparison with their Platinum Analogues, Trans Met Chem. 2010; 35: 457-461.
15. Bakalova A, Varbanov H, Buyukliev R, Momekov G, Ivanov D, Doytchinova I, Platinum Complexes with 5-methyl-5(4-pyridyl)hydantoin and its 3-methyl derivatives. Synthesis and Cytotoxic Activity. Quantitative Structure-Activity Relationships. Arch Pharm Chem Life Sci. 2011; 11: 209–216.
16. Bakalova A,Buyukliev R, Ivanova Z, Momekov G, Ivanov D,Platinum(IV) complexes with some derivatives of 5-methyl-5-(4-pyridyl)hydantoins. Synthesis, Study and comparative pharmacological investigation, Drug Research. 2013; 63: 420-423**.**
17. Bakalova A, Varbanov H, Buyukliev R, Momekov G, Ivanov D, Palladium(II) Complexes with 5-methyl-5-(4-pyridyl)-2,4-imidazolidenedione. Synthesis, Thermogravimetric and Cytotoxic Investigation, J Therm Anal Calorim. 2009; 95: 241-246.
18. Bakalova A, Varbanov H, Buyukliev R, Momekov G, Ferdinandov D, Konstantinov S,Ivanov D, Synthesis, Characterization and Biological Activity of Pt(II) and Pt(IV) Complexes with 5-methyl-5(4-pyridyl)-2,4-imidazolidenedione, Eur J Med Chem. 2008; 43: 958-965.
19. Bakalova A, Buyukliev R, Momekov G.Pt(II) and Pt(IV) Complexes with 3-thiolanespiro-5’-hydantoin and 4-thio-1H-tetrahydropyranespiro-5’-hydantoin. Synthesis, Characterization, DFT calculations and Cytotoxic Investigation. Arch. Pharm 2014; (in press).

20. Mosmann T, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, J Immunol Methods.1983; 65: 55-63.
21. Konstantinov S, Eibl H, Berger M. BCR-ABL influences the antileukaemic efficacy of alkylphosphocholines. Br J Haematol.1999; 107:365-374.
22. K. Nakaloto in Infrared and Raman Spectra of Inorganic and Coordination Compounds 3 Rev. Ed. New York, John Wiley & Sons Inc. 1978, 197-206.