

COMPLEXES OF PALLADIUM(II) WITH 1-PHENYL-1-HYDROXYMETHYLENE BISPHOSPHONIC ACID AND THEIR ANTITUMOR ACTIVITY

O. M. KOZACHKOVA¹, N. V. TSARYK¹, V. V. TRACHEVSKYI²,
A. B. ROZHENKO³, Yu. H. SHERMOLOVICH³, O. I. GUZYR³,
N. I. SHARYKINA⁴, V. F. CHEKHUN⁵, V. I. PEKHNYO¹

¹Vernadsky Institute of General and Inorganic Chemistry,
National Academy of Sciences of Ukraine, Kyiv;

²Technical Centre of National Academy of Sciences of Ukraine, Kyiv;

³Institute of Organic Chemistry, National Academy of Sciences of Ukraine, Kyiv;

⁴SI "Institute of Pharmacology and Toxicology, National Academy
of Medical Sciences of Ukraine", Kyiv;

⁵Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology,
National Academy of Sciences of Ukraine, Kyiv;

e-mail: complex@ionc.kiev.ua

Complex formation of $K_2[PdCl_4]$ with 1-phenyl-1-hydroxymethylene bisphosphonic acid (PhHMBP, H_4L) has been studied by pH potentiometry, electron and NMR spectroscopy. It was found that in aqueous solution with physiological concentration of chlorine anions (0.15 mol/l KCl), anionic complexes of the equimolar compositions $[PdHLC_2]^{3-}$ ($\lg\beta = 24.51$ (0.3)) and $[PdLC_2]^{4-}$ ($\lg\beta = 20.74$ (0.02)) are formed. In the first coordination sphere palladium was surrounded by two oxygen atoms of two phosphonic groups of the bidentately coordinated ligand with closure of six-membered [O, O] ring, and two chlorine anions. The formation of palladium(II) equimolar complexes with PhHMBP and bidentate coordination of the ligand to the central metal cation was confirmed by ^{31}P NMR spectroscopy. Cytotoxic activity (IC_{50} based on metal content) of the synthesized Pd(II) complexes with PhHMBP against human MG-63 osteosarcoma and MCF-7 mammary tumor cells was compared with cisplatin on *in vitro* models. It was established that cytotoxic activity of the Pd complexes was lower than that of cisplatin. The acute toxicity (LD_{50} based on metal content) of solutions of Pd(II) complexes with PhHMBP was found to be lower compared to cisplatin. It was shown that the use of solutions of palladium(II) complexes with PhHMBP inhibited tumor growth in mice with sarcoma 180.

Key words: palladium(II) complexes, bisphosphonic acid, cytotoxic activity, toxicity.

Cisplatin is still one of the most famous and efficient anticancer drugs used in the clinical practice [1]. Nevertheless, searching for new compounds with antineoplastic action continues to eliminate the serious side effects, to improve the clinical efficacy and to broaden the antitumor spectrum. In particular, palladium(II) bisphosphonates are promising species for the treatment of bone cancer and metastases: complexes include a cytotoxic metal (palladium) and a bisphosphonic acid, which possesses affinity for bone tissue and can ensure targeted delivery of the cytotoxic metal to the lesion site

[2-9]. The P-C-P moiety present in bisphosphonic acids provides their active link to the bone matrix, and two side substituents determine their physicochemical and pharmacologic properties. To study the effect of the structure of complex and chemical nature of the substituents bonded to the carbon atom of bisphosphonate moiety on the biological activity, the formation of palladium(II) complexes with 1-phenyl-1-hydroxymethylene bisphosphonic acid was investigated, which, besides two phosphonic groups, contain a hydroxy group and a phenyl moiety.

The aim of the work is to determine the composition, structure, stability and biological activity of palladium (II) complexes with PhHMBP.

Materials and Methods

The following reagents were used in the work: 1-phenyl-1-hydroxymethylene bisphosphonic acid (PhHMBP, H₄L) synthesized by the procedure presented in [10], concentrated solution of pure carbonate-free potassium hydroxide, palladium black. Potassium tetrachloropalladate was prepared according to the standard scheme [11]:



The palladium content of PdCl₂ was determined by the reduction of a weighed amount of the compound in a hydrogen stream to metallic palladium [12].

The standard solution of hydrochloric acid was prepared from a titrimetric standard substance. The potassium hydroxide solution was prepared right away before using from a saturated KOH solution. The exact KOH concentration was determined by titration with the standard solution of hydrochloric acid. The exact concentration of PhHMBP was determined by titration with KOH.

Weighting was performed on a Vibra HT-120 CE balance. The measurements were made a Metrohm 827 pH lab pH meter. The device was calibrated using standard buffer solutions with pH 1.68, 6.86, 9.18 (25 °C).

The electronic absorption spectra of the solutions were recorded on a Specord M-40 spectrophotometer (range 50000-11000 cm⁻¹). The recording was performed in quartz cuvettes with l = 1 cm.

To prepare solutions for pH potentiometric and spectrophotometric studies, freshly distilled water was used.

The formation constants of complexes and their equilibrium concentrations were calculated using the PSEQUAD program [13] from the data of EASs and pH of equilibrium solutions from the formulas:

$$\beta_{\text{HzL}} = [\text{H}_z\text{L}]/[\text{L}][\text{H}]^z \quad (1);$$

$$\beta = [\text{M}_x\text{L}_y\text{H}_z\text{Cl}_q]/[\text{M}]^x[\text{L}]^y[\text{H}]^z[\text{Cl}]^q \quad (2).$$

The formation constants of palladium(II) chloroqua complexes: lgβ[PdCl]⁺ = 4.47; lgβ[PdCl₂] = 7.76; lgβ[PdCl₃]⁻ = 10.17; lgβ[PdCl₄]²⁻ = 11.54 (I = 1 mol/l HClO₄ + NaClO₄, T = 25 °C) [14].

The ³¹P NMR spectra with proton decoupling (³¹P-¹H) were recorded on a Bruker AVANCE 400 spectrometer without deuterium stabilization of the resonance conditions. The value of ³¹P NMR chemical shift was determined relative to 85% H₃PO₄.

The pH potentiometric and spectrophotometric studies of complex formation in solutions with physiological concentration of chloride ions (0.15 mol/l KCl) were carried out for the systems with a constant K₂[PdCl₄] concentration (C_{Pd(II)} = 2·10⁻³ mol/l) and a variable concentration of PhHMBP, neutralized with three KOH equivalents (C_{H4L} = 4·10⁻⁴-4·10⁻³ mol/l), and for the systems K₂[PdCl₄] – PhHMBP = 1:1 and 1:2 (C_{Pd(II)} = 2·10⁻³ mol/l, C_{H4L} = 2·10⁻³, 4·10⁻³ mol/l) depending on pH. Sets of solutions with the molar ratios K₂[PdCl₄]:PhHMBP = 1:1 (C_{Pd(II)} = C_{H4L} = 0.05 mol/l) and 1:2 (C_{Pd(II)} = 0.025 mol/l, C_{H4L} = 0.05 mol/l) were investigated by ³¹P NMR spectroscopy.

All measurements were carried out a day after the preparation of solutions, the period required for the establishment of chemical equilibrium.

To carry out the studies of biological activity, solutions of a Pd(II) complex with PhHMBP (C_{Pd(II)} = 10.0 mg/ml (0.094 mol/l); C_{H4L} = 0.094 mol/l, C_{KCl} = 0.15 mol/l, pH = 4.56) were prepared. To study *in vitro* cytotoxic activity, we used MG-63 human osteosarcoma and cells of the MCF-7 line (cell line obtained from human breast cancer). The cells were cultured in a DMEM culture medium (Biowest, France) from 10% fetal calf serum (FCS) (HyClone, France) at 37 °C in a humid atmosphere in the presence of 5% CO₂. The obtained cell suspensions were placed on a 96-well plate in a concentration of 1.2–1.5·10⁴ cells/well (in a volume of 0.1 ml) and incubated for 72 h at 37 °C in the presence of 5% CO₂. The solution of palladium complex under investigation was added four or two times (100 μl each time) with two-fold dilution from a concentration of 200 μg/ml in the first wells to a concentration of 1.56 μg/ml. The wells intact cell cultures were used as a control. After the addition of a solution of palladium complexes, the test and control cultures in the plates were incubated for 72 h. The cytotoxicity of palladium(II) complexes with PhHMBP was assessed on the basis of the number of alive cells in the test and control wells (MTT test) [15]. To this end, after the completion of incubation, the supernatant with dead cells and detritus was completely removed, and 50 μl of a crystal violet solution was put in each well to fix and color the cells

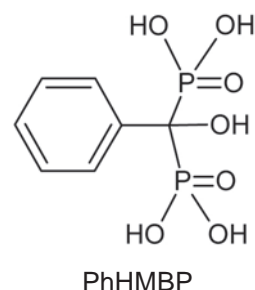
on the substrate. The plate was incubated for 10 min at room temperature. Then the dye was removed, the plate wells were washed with water and dried at room temperature. The optical density, which is proportional to the number of alive cells in the wells, was determined with a Labsystem Multiscan Plus spectrometer at an excitation wavelength of 540 nm.

The *in vivo* toxicity of Pd(II) complexes with PhHMBP was assessed by determining the half-lethal dose (LD_{50}) and studying the percentage of sarcoma 180 (S-180) growth inhibition in while mice in the case of introducing solutions of Pd(II) complexes with PhHMBP. The half-lethal dose of Pd(II) complexes with PhHMBP (LD_{50}) was determined according to [16]. The solutions were injected intraperitoneally in while mice once before feeling in different doses. The screening studies of the anticancer activity of Pd(II) complexes with PhHMBP were carried out on while nonlinear mice of the same sex with a body weight of 18–20 g on a sarcoma 180 model [17]. The number of animals in the test group was 7 and in the control group 22. The substance under investigation was injected 24 h after tumor transplantation in a dose of $1/3 LD_{50}$ daily (7 injections). The effect parameter was the percentage of tumor growth inhibition by mass. The minimum activity criterion is tumor growth inhibition of over 50%.

Results and Discussion

According to the results of a pH potentiometric titration in the pH range 2.2–11.3, PhHMBP is a tetrabasic acid (H_4L).

The determined formation constants of PhHMBP forms of different degree of deprotonation are listed in Table 1. They were calculated from the data of two parallel titrations (each titration included more than 50 points) (according to the measurement data in two parallel experiments). The first dissociation constant value for PhHMBP (pK_1) is close to 2, which complicates its calculation from the data of pH potentiometric titration. The values of pK_2 , pK_3 and pK_4 , which correspond to the consecutive disso-



ciation of OH protons from the phosphonic groups, within the experimental error agree with the published data [18]. The geminal hydroxy group remains protonated below the pH of 13.

The diagram of the equilibrium concentration distribution of PhHMBP forms of different degree of protonation as a function of solution pH is shown in Fig. 1. It is seen from the diagram that in the range $6 \leq \text{pH} \leq 8$, $[H_2L]^{2-}$ and $[H_3L]^-$ are predominating forms.

It should be taken into account that depending on the concentration of chlorine anions in aqueous solutions of $K_2[PdCl_4]$, palladium(II) exists as chloro aqua complexes of the composition $[PdCl_{4-n} \cdot (H_2O)_n]^{n-2}$ ($n = 0-4$). In a 0.15 mol/l KCl solution with a $K_2[PdCl_4]$ concentration of $2 \cdot 10^{-3}$ mol/l, the following Pd(II) complex species are in equilibrium: $[PdCl_4]^{2-}$ ($1.51 \cdot 10^{-3}$ mol/l), $[PdCl_3(H_2O)]^-$ ($4.78 \cdot 10^{-4}$ mol/l) and $[PdCl_2(H_2O)_2]$ ($1.20 \cdot 10^{-3}$ mol/l), with the total absorption at 21650 cm^{-1} .

When a $K_2[PdCl_4]$ solution is titrated in 0.15 mol/l KCl with a solution of PhHMBP neutralized with three KOH equivalents, the maximum in the electronic absorption spectra (EASs) shifts from 21650 cm^{-1} to approx. 24800 cm^{-1} (pH 4.91-5.24) due to the formation of a palladium(II) complex with PhHMBP (Fig. 2).

Similar changes are observed in the EASs of the $K_2[PdCl_4] : \text{PhHMBP} = 1 : 1$ system within the pH range 2.59-7.04 (Fig. 3).

The formation constants of complexes of the compositions $[PdHLC_2]^{3-}$ ($\lg \beta = 24.51(0.3)$) and

Table 1. Formation constants of protonated PhHMBP forms and corresponding dissociation constants

$\lg \beta [HL]^{3-}$	$\lg \beta [H_2L]^{2-}$	$\lg \beta [H_3L]^-$	pK_4	pK_3	pK_2	Ionic strength	Literature data
10.80	17.47	20.00	10.80	6.67	2.53	0.10 mol/l KNO_3 and NaCl, 25 °C	[18]
10.77(0.02)	17.59(0.02)	20.33(0.02)	10.77	6.82	2.74	0.15 mol/l KCl, 20 °C	Our data

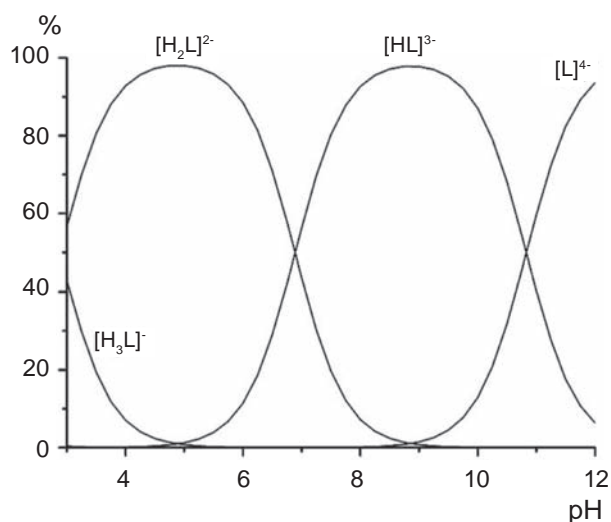


Fig. 1. Diagram of the equilibrium concentration distribution of PhHMBP forms of different degree of protonation as a function of solution pH ($C_{H4L} = 5 \cdot 10^{-3}$ mol/l)

$[PdLCl_2]^{4-}$ ($\lg\beta = 20.74(0.02)$) were calculated from spectrophotometric and pH potentiometric data.

The coordination mode of ligand to the central metal ion was established on the basis of the dependence of the position of the absorption band maximum on the composition of the first coordination sphere (chromophore) of square-planar palladium complexes [19]. In particular, in the case of bidentate coordination of ligand by two oxygen atoms of phosphonic groups the absorption band with max-

imum at ~ 24800 cm^{-1} indicates the formation of a complex with chromophore of the composition $[Pd; 2Cl; 2OPO_3]$.

The values of formation constant calculated for complexes of equimolar composition were used to construct a diagram of the equilibrium concentration distribution of palladium complexes as a function of pH (Fig. 4).

It is seen from the diagram that the anionic complex of the equimolar composition $[PdLCl_2]^{4-}$ is predominant in the solution at the physiological concentration of chlorine anions and pH close to 7.

The composition of the complexes and the mode of the coordination of ligand to the central metal ion, determined from spectrophotometric data, are corroborated by the results of investigation of the system $K_2[PdCl_4] : PhHMBP = 1 : 1$ and $1 : 2$ by the ^{31}P NMR method.

The ^{31}P NMR spectra (Fig. 5, a) of a set of solutions with the ratio $K_2[PdCl_4] : PhHMBP = 1 : 1$

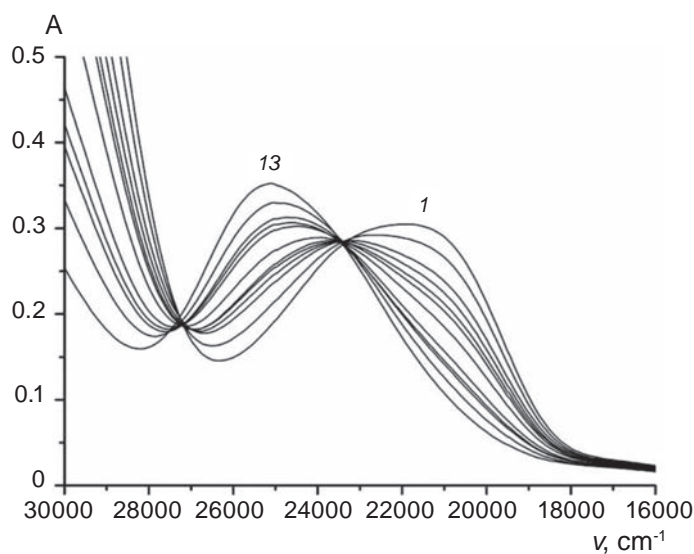
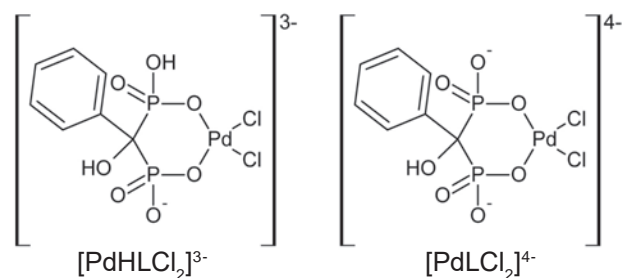


Fig. 2. EASs of the $K_2[PdCl_4] - PhHMBP$ system ($C_{Pd(II)} = 2 \cdot 10^{-3}$ mol/l, $C_{H4L} = 4 \cdot 10^{-4} - 3.2 \cdot 10^{-3}$ mol/l, pH: 1 - 4.91; 2 - 4.88; 3 - 4.86; 4 - 4.86; 5 - 4.87; 6 - 4.80; 7 - 4.92; 8 - 4.95; 9 - 4.97; 10 - 4.99; 11 - 5.05; 12 - 5.17; 13 - 5.24)

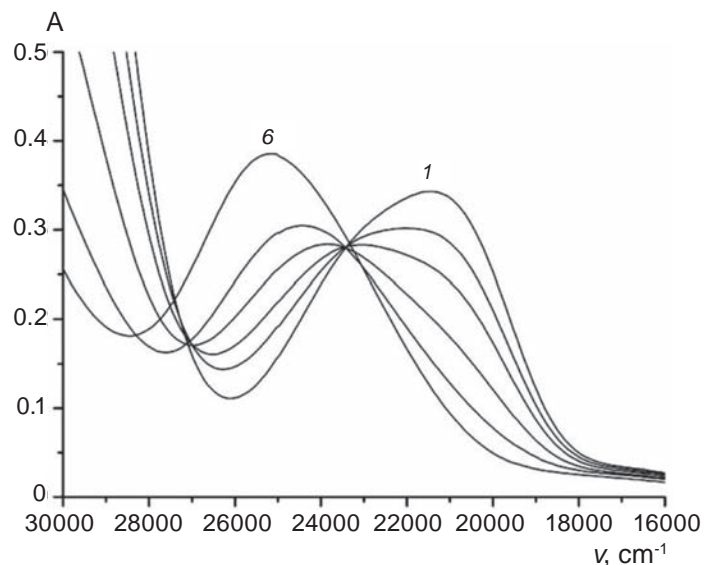


Fig. 3. EASs of the $K_2[PdCl_4] : PhHMBP = 1 : 1$ system ($C_{Pd(II)} = C_{H4L} = 2 \cdot 10^{-3}$ mol/l, pH: 1 – 2.59, 2 – 4.36, 3 – 4.57, 4 – 4.85, 5 – 5.35, 6 – 7.04)

exhibit a redistribution between the integral intensities of the signals of the phosphonic groups of free PhHMBP ($\delta^{31}P$ 15.97-15.51 ppm) and coordinated ligand ($\delta^{31}P$ 38.79-38.36 ppm) by increasing pH from 1.50 to 4.92. The position of the signals of phosphorus nuclei in the range 38.79-38.36 ppm corresponds to the formation of a complex with PhHMBP bidentately coordinated to the central metal ion. At pH 4.92, PhHMBP is almost completely involved into a complex with palladium (II).

The ^{31}P NMR spectra of solutions of the system with the ratio $K_2[PdCl_4] : PhHMBP = 1 : 2$ exhibit signals with similar values of chemical shifts (Fig. 5, b). By increasing pH, the integral intensity of the signals of coordinated phosphonic groups increases and is approximately equal to that of the signal of free acid. This evolution in the spectra of the $K_2[PdCl_4] : PhHMBP = 1 : 2$ system indicates the formation of the complexes only of equimolar composition, in which ligand is bidentately coordinated to palladium(II) by two oxygen atoms of two phosphonic groups.

It should be noted that the ^{31}P NMR spectra of solutions with $pH < 3$ exhibit low-intensity signals in the $\delta^{31}P$ range 24.3–23.5 ppm, which probably correspond to a complex with PhHMBP monodentately coordinated to palladium(II) by phosphonic group oxygen atom. This was not established by electron spectroscopy and pH potentiometry ($C = 2 \cdot 10^{-3}$ mol/l).

The ^{31}P NMR spectrum of bidentately coordinated PhHMBP consists of three signals with close δ values. As we have previously shown, the observed nonequivalence of the phosphorus nuclei in the coordinated ligand may be both due to the conformation isomerism of the formed complexes and a result of forming an intraligand hydrogen bond [20].

The *in vivo* anticancer activity of a palladium(II) complex with PhHMBP was determined on an MG-63 and MCF-7 cell line models (IC_{50} 21

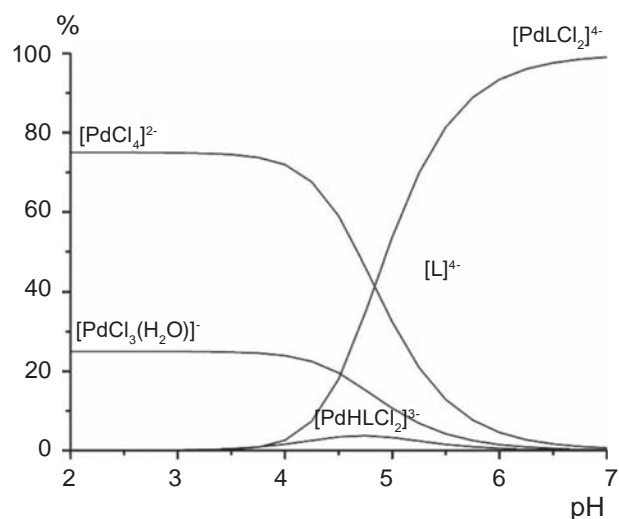


Fig. 4. Distribution diagram of the equilibrium concentration of complexes with different compositions as a function of pH in system $K_2[PdCl_4] : PhHMBP = 1 : 1$ ($C_{Pd(II)} = C_{H4L} = 2 \cdot 10^{-3}$ mol/l)

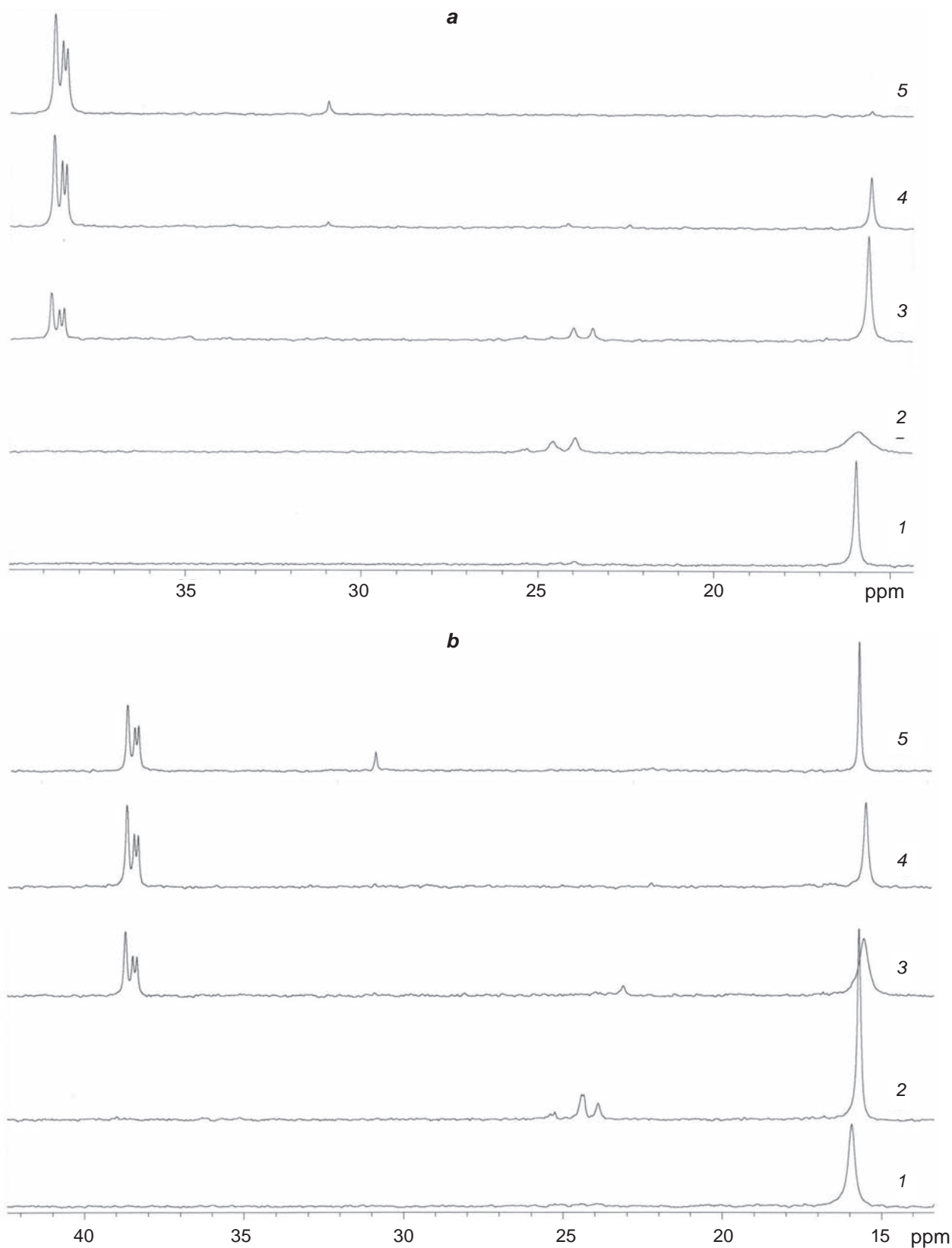


Fig. 5. ^{31}P NMR spectra of the $\text{K}_2[\text{PdCl}_4] - \text{PhHMBP}$ systems: **a** - $C_{\text{Pd(II)}} = C_{\text{H4L}} = 0.05 \text{ mol/l}$, pH: 1 - 1.49, 2 - 2.22, 3 - 3.04, 4 - 3.82, 5 - 4.92, 6 - 5.80; **b** - $C_{\text{Pd(II)}} = 0.025 \text{ mol/l}$, $C_{\text{H4L}} = 0.05 \text{ mol/l}$, pH: 1 - 1.51, 2 - 2.48, 3 - 3.56, 4 - 3.97, 5 - 6.32

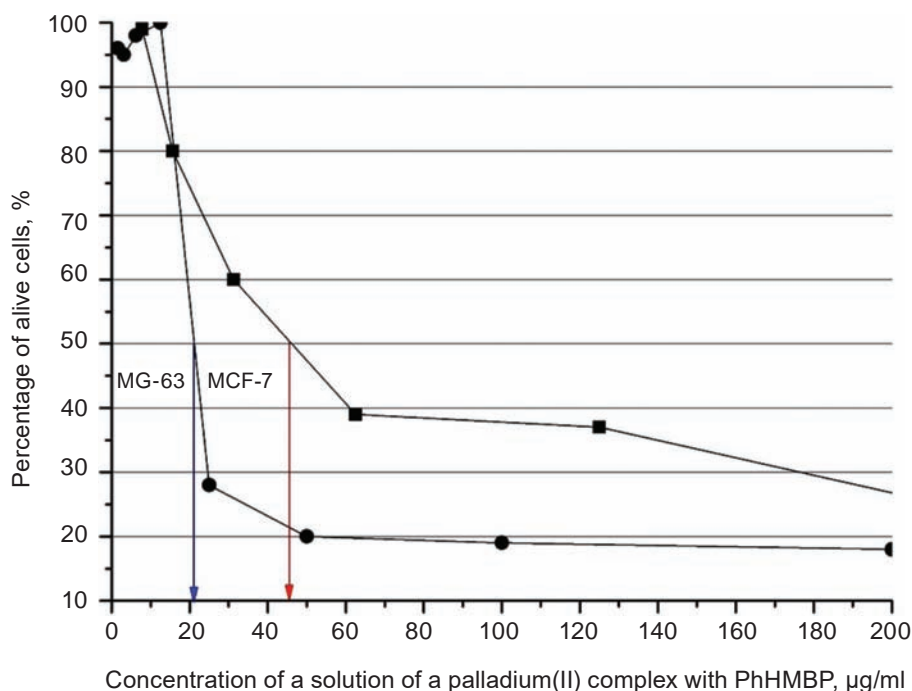


Fig. 6. Concentration-dependent effects of Pd (II) complexes with PhHMBP on the percentage of alive cells line MG63 and MCF-7

Table 2. Anticancer activity of solutions of palladium (II) complexes with PhHMBP on a sarcoma 180 model

System	Ninth day	
	Mass of tumors, g	Tumor growth inhibition, %
Pd(II) complex with PhHMBP	0.395 ± 0.097	59.0
Control	0.964 ± 0.106	–

and 41.7 µg/ml (based on palladium content), respectively, Fig. 6). The found values are lower than those found for cisplatin (2.2 and 1 µg/ml (based on palladium content), respectively).

The acute toxicity of solutions of palladium(II) complexes with PhHMBP ($LD_{50} = 41$ mg/kg (based on palladium content)) is lower than that for cisplatin, for which LD_{50} is found to be 8.42 mg/kg (based on palladium content).

When determining the antineoplastic action of solutions of palladium(II) complexes with PhHMBP

on sarcoma 180 bearing mice, the found percentage of tumor growth inhibition is over 50% (minimum activity criterion) (Table 2).

Depending on pH, the PhHMBP ligand forms in water solutions with the physiological concentration of chlorine anions (0.15 mol/l) the anionic complexes of the equimolar compositions, $[PdHCl_2]^{3-}$ and $[PdLCl_2]^{4-}$, as indicated by pH potentiometry, electron and NMR spectroscopy. The first coordination sphere involves two oxygen atoms of two phosphonic groups of ligand with closure of the six-membered [O, O] ring and two chlorine anions.

The solutions of palladium(II) complexes with PhHMBP demonstrates moderate *in vitro* cytotoxic activity (IC_{50}) against MG-63 and MCF-7 tumor cells that does not exceed that of cisplatin. At the same time, solutions of palladium(II) complexes with PhHMBP exhibit lower toxicity as compared with cisplatin. They also provide inhibition for sarcoma 180 growth in mice.

КОМПЛЕКСНІ СПОЛУКИ ПАЛАДІЮ(II) З 1-ФЕНІЛ- 1-ГІДРОКСИМЕТИЛЕН- БІСФОСФОНОВОЮ КИСЛОТОЮ ТА ЇХ ПРОТИПУХЛИННА АКТИВНІСТЬ

O. M. Kozachkova¹, H. B. Царик¹,
B. B. Трачевський², O. B. Роженко³,
Ю. Г. Шермолович³, O. I. Гузир³,
H. I. Шарикіна⁴, B. Ф. Чехун⁵, B. I. Пехньо¹

¹Інститут загальної та неорганічної хімії
ім. В. І. Вернадського НАН України, Київ;

²Технічний центр НАН України, Київ;

³Інститут органічної хімії НАН України, Київ;

⁴ДУ «Інститут фармакології та
токсикології НАМН України», Київ;

⁵Інститут експериментальної патології,
онкології і радіобіології ім. Р. Є. Кавецького
НАН України, Київ;

e-mail: complex@ionc.kiev.ua

Методами рН-потенціометрії, електронної та ЯМР спектроскопії досліджено комплексоутворення $K_2[PdCl_4]$ з 1-феніл-1-гідроксиметилєнбісфосфоновою кислотою (PhHMBP, H_4L). Встановлено, що у водному розчині з фізіологічною концентрацією аніонів хлору (0, 15 моль/л KCl) в залежності від рН утворюються аніонні комплекси еквімолярного складу $[PdHLC_2]^{3-}$ ($\lg\beta = 24,51(0,3)$) та $[PdLC_2]^{4-}$ ($\lg\beta = 20,74(0,02)$). Внутрішня координаційна сфера Pd сформована двома атомами кисню двох фосфонових груп бідентатно координованого ліганду при замиканні шестичленного [O, O] циклу та двома аніонами хлору. Методом спектроскопії ЯМР ^{31}P підтверджено утворення комплексів Pd(II) еквімолярного складу з бідентатно координованою до центрального іона металу PhHMBP двома атомами кисню фосфонових груп. На моделях *in vitro* порівняно цитотоксичну активність (IC_{50} за вмістом металу) розчинів комплексів Pd(II) з PhHMBP та цисплатину по відношенню до клітин остеосаркоми MG-63 та пухлини молочної залози MCF-7 людини. Встановлено, що розчини комплексів Pd(II) з PhHMBP виявляли меншу цитотоксичну активність порівняно з цисплатином. Параметри гострої токсичності (LD_{50} за вмістом металу) розчинів комплексів Pd(II) з PhHMBP вказували на їхню меншу токсичність порівняно із цисплатином. Показано, що застосування розчинів

комплексів Pd(II) із PhHMBP обумовлювало гальмування пухлинного росту в мишей із саркомою-180.

Ключові слова: комплекси паладію(II), бісфосфонові кислоти, цитотоксична активність, токсичність.

КОМПЛЕКСНЫЕ СОЕДИНЕНИЯ ПАЛЛАДИЯ(II) С 1-ФЕНИЛ- 1-ГИДРОКСИМЕТИЛЕН- БИСФОСФОНОВОЙ КИСЛОТОЙ И ИХ ПРОТИВООПУХОЛЕВАЯ АКТИВНОСТЬ

A. H. Kozachkova¹, H. B. Царик¹,
B. B. Трачевский², A. B. Роженко³,
Ю. Г. Шермолович³, A. И. Гузырь³,
H. И. Шарыкина⁴, B. Ф. Чехун⁵,
B. И. Пехньо¹

¹Інститут общей и неорганической химии
им. В. И. Вернадского НАН Украины, Киев;

²Технический центр НАН Украины, Киев;

³Інститут органической химии
НАН Украины, Киев;

⁴ГУ «Інститут фармакологии и
токсикологии НАМН Украины», Киев;

⁵Інститут экспериментальной
патологии, онкологии и радиобиологии
им. Р. Е. Кавецкого НАН Украины, Киев;
e-mail: complex@ionc.kiev.ua

Методами рН-потенциометрии, електронной и ЯМР спектроскопии исследовано комплексообразование $K_2[PdCl_4]$ с 1-феніл-1-гідроксиметилєнбісфосфоновой кислотой (PhHMBP, H_4L). Установлено, что в водном растворе с физиологической концентрацией анионов хлора (0, 15 моль/л KCl) в зависимости от рН образуются анионные комплексы эквиволярного состава $[PdHLC_2]^{3-}$ ($\lg\beta = 24,51(0,3)$) и $[PdLC_2]^{4-}$ ($\lg\beta = 20,74(0,02)$). Внутренняя координационная сфера Pd сформирована двумя атомами кислорода двух фосфоновых групп бідентатно координированного лиганда при замикании шестичленного [O, O] цикла и двумя анионами хлора. Методом спектроскопии ЯМР ^{31}P подтверждено образование комплексов Pd(II) эквиволярного состава с бідентатно координированной к центральному металлу PhHMBP двумя атомами кислорода фосфоновых групп. На моделях *in vitro* сравнили цитотоксическую активность (IC_{50} по содержанию металла) рас-

творов комплексов Pd(II) с PhНМВР и цисплатина по отношению к клеткам остеосаркомы MG-63 и опухоли молочной железы MCF-7 человека. Установлено, что растворы комплексов Pd(II) с PhНМВР проявляли меньшую цитотоксическую активность по сравнению с цисплатином. Параметры острой токсичности (LD_{50} по содержанию металла) растворов комплексов Pd(II) с PhНМВР указывали на их меньшую токсичность по сравнению с цисплатином. Показано, что применение растворов комплексов Pd(II) с PhНМВР обуславливало торможение опухолевого роста у мышей с саркомой-180.

Ключевые слова: комплексы палладия(II), бисфосфоновые кислоты, цитотоксическая активность, токсичность.

References

1. Abu-Surrah AS, Kettunen M. Platinum group antitumor chemistry: design and development of new anticancer drugs complementary to cisplatin. *Curr Med Chem.* 2006; 13(11): 1337-1357.
2. Kapdi AR, Fairlamb IJ. Anti-cancer palladium complexes: a focus on PdX₂L₂, palladacycles and related complexes. *Chem Soc Rev.* 2014; 43(13): 4751-4777.
3. Sharma NK, Ameta RK, Singh M. Biological Impact of Pd (II) Complexes: Synthesis, Spectral Characterization, In Vitro Anticancer, CT-DNA Binding, and Antioxidant Activities. *Int J Med Chem.* 2016; 2016: 9245619.
4. Keswani T, Chowdhury S, Mukherjee S., Bhattacharyya A. Palladium(II) complex induces apoptosis through ROS-mediated mitochondrial pathway in human lung adenocarcinoma cell line (A549). *Curr Sci.* 2014; 107(10): 1711-1719.
5. Abu-Surrah AS, Al-Sa'doni HH, Abdalla MY. Palladium-based chemotherapeutic agents: Routes toward complexes with good antitumor activity. *Cancer Therapy.* 2008; 6: 1-10.
6. Zhang S, Gangal G, Uludağ H. 'Magic bullets' for bone diseases: progress in rational design of bone-seeking medicinal agents. *Chem Soc Rev.* 2007; 36(3): 507-531.
7. Zolotukhina MM, Krutikov VI, Lavrent'ev AN. Derivatives of diphosphonic acids: synthesis and biological activity. *Russ Chem Rev.* 1993; 62(7): 647-659.
8. Margiotta N, Ostuni R, Teoli D, Morpurgo M, Realdon N, Palazzo B, Natile G. Bisphosphonate complexation and calcium doping in silica xerogels as a combined strategy for local and controlled release of active platinum antitumor compounds. *Dalton Trans.* 2007; (29): 3131-3139.
9. Puthraya KH, Srivastava TS, Amonkar AJ, Adwankar MK, Chitnis MP. Some mixed-ligand palladium(II) complexes of 2,2'-bipyridine and amino acids as potential anticancer agents. *J Inorg Biochem.* 1985; 25(3): 207-215.
10. Lecouvey M, Mallard I, Bailly T, Burgada R, Leroux Y. A mild and efficient one-pot synthesis of 1-hydroxymethylene-1, 1-bisphosphonic acids. Preparation of new tripod ligands. *Tetrahedron Lett.* 2001; 42(48): 8475-8478.
11. Chernyaev I. Synthesis of Complex Compounds of Platinum Group Metals. Moscow: Nauka, 1964. 266 p. (In Russian).
12. Ginzburg I, Ezerskaya NA, Prokof'eva IV, Fedorenko NV, Shlenskaya VI, Relskii NK. Analytical Chemistry of Platinum Metals. Moscow: Nauka, 1972. 613 p. (In Russian).
13. Zekany L, Nagypal I. Computational methods for the determination of formation constants. New York: Plenum Press, 1985; 291-353 p.
14. Elding LI. Palladium(II) halide complexes. I. Stabilities and spectra of palladium(II) chloro and bromo aqua complexes. *Inorg Chim Acta.* 1972; 6: 647-651.
15. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods.* 1983; 65(1-2): 55-63.
16. Prozorovsky VB. Tabular Express Method of determining the average effective measures of influence on biological objects. *Toxicol Rev.* 1998; 1: 28-32. (In Russian).
17. Stephanov OV. Pre-clinical study of medicine. Kyiv: Avicenna, 2001. 528 p. (In Ukrainian).
18. Gumienna-Kontecka E, Silvagni R, Lipinski R, Lecouvey M, Marincola FC, Crisponi G, Nurchi VM, Leroux Y, Kozlowski H. Bisphosphonate chelating agents: complexation of Fe(III) and Al(III) by 1-phenyl-1-hydroxymethylene bisphosphonate and its analogues. *Inorg Chim Acta.* 2002; 339: 111-118.

19. Kozachkova AN, Tsaryk NV, Kostromina NA, Pekhnyo VI. Cis-diaminodichloropalladium(II) and hydroethylenediphosphonic acid interaction. *Ukr Khim Zhurn.* 2007; 73(3): 15-19. (In Ukrainian).
20. Kozachkova AN, Dudko AV, Tsaryk NV, Trachevskii VV, Rozhenko AB, Pekhnyo VI. Different Ways of Coordination for Aminoalkyldiphosphonic Acids in Palladium(II) Complexes. *Rus J Coord Chem.* 2011; 37(12): 941-948.

Received 20.12.2016