UDC 57.085.23:547.792:546.98:546.74

EFFECT OF Pd(II) AND Ni(II) COORDINATION COMPOUNDS WITH 4-AMINO-3-MERCAPTO-5-METHYL-1,2,4-TRIAZOLE ON THE MITOCHONDRIAL DEHYDROGENASES ACTIVITY

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*Pd(II) and Ni(II) complex compounds: $[Pd(AMMT)_2]Cl_2$ (**1**), $[Pd(AMMT)_4]Cl_2$ (**2**) and $[Ni(AMMT)_2(H_2O)_2](NO_3)_2$ (**3**) with 4-amino-3-mercapto-5-methyl-1,2,4-triazole (AMMT) have been synthesized. The spectral characteristics of **1, 2** were studied by ^1H (^{13}C) NMR and UV-Vis spectroscopy. X-ray diffraction studies established that all complexes contain the AMMT molecule, which are coordinated to the central metal ion in the thione tautomeric form. At the ratio M : L = 1 : 2 ligand is coordinated in bidentate chelate manner by the nitrogen of amino- and sulfur of mercapto group (compounds **1, 3**). But the molar ratio M : L = 1 : 4 leads to monodentate coordination of AMMT molecules only by sulfur of mercaptogroup (complex **2**). Vacant coordination sites of the metal ion are occupied by water molecules (complex **3**). The screening of complexes **1**–**3** and starting compounds [AMMT, K_2PdCl_4 (**4**), $Ni(NO_3)_2 \cdot 6H_2O$ (**5**)]by their mitochondrial dehydrogenase activity have been performed by us for the first time, resulting in established that the Pd(II) complexes (**1, 2**), Pd(II) salt (**4**) and AMMT normalize the activity of mitochondrial dehydrogenases of cancer HeLa cells, identified by MTT-test. In contrast, the Ni(II) complex (**3**) and Ni(II) salt (**5**) do not stimulate the activity of mitochondrial dehydrogenases. It has been found, that all investigated compounds do not affect on the cell cycle and the level of apoptotic cells as well as do not show a toxic effect. Thus, these results indicate that AMMT and Pd(II) complexes may be used as modifiers of mitochondrial respiration, which dysfunction is particularly evident in the tumor cells.*

K e y w o r d s: 1,2,4-triazole, Pd(II) and Ni(II) complexes, ^1H (^{13}C) NMR spectroscopy, the mitochondrial dehydrogenases activity, level of apoptotic cells.

In recent decades a considerable attention has been paid to the development of methods for the synthesis of transition metal complexes, such as platinum with bioactive organic ligands, due to their high activity and stability in physiological media and their potential as drugs [1, 2].

The complexation enhances the bioavailability of organic substances as well as metal ions improving their transport through the cell membranes. Furthermore, the coordination of organic compounds to the transition metal ions extends the range of their biological targets. The nitrogen-containing heterocycle derivatives such as 1,2,4-triazole are the imidazole structural analogs (which are the components of nucleosides) and widely used as bioactive ligands [3, 4].

The triazole ring contains three N-donor reacting sites, which are capable to coordinate transition metal ions. The insertion of additional donor functional groups (amino-, mercapto-, hydroxyl-, carboxy-, etc.) into the triazole structure allows obtaining the polydentate chelating ligand systems forming stable chelate metalocycles with transition metal ions which show antiproliferative [5] and cytostatic [6] effects. Further studies of complexation reactions with substituted 1,2,4-triazoles and the de-

velopment of methods for the synthesis of complexes on their base are the promising direction in design of new pharmaceutical drugs, including anticancer ones.

It is known that the characteristic features of tumor cells are high level of glycolysis, low level of the mitochondrial respiration and weakened Pasteur effect (the suppression of the glycolysis by the respiration) [7]. This fact is also confirmed by the suppression of the activity in transformed cells of such crucial components of the mitochondrial respiration as cytochrome C, cytochrome oxidase and succinate dehydrogenase [8–10]. Tumor cells are characterized by the development of the lactic acidosis, which is associated with alterations in the activity and isoenzyme spectrum of lactate dehydrogenase [11]. The decrease in the activity of the pyruvate dehydrogenase complex induces the accumulation of pyruvic acid. These alterations cause the tissue hypoxia, resulting redox system abnormalities [12]. Therefore, the development of methods for the synthesis of low-toxicity compounds, which could be used for the metabolism restoration in a case of malignant growth of cells, is essential nowadays. As shown below, Pd(II) and Ni(II) complexes (1–3) with 4-amino-3-mercapto-5-methyl-1,2,4 triazole (AMMT) can be attributed to such type of compounds.

It is known from the open literature, that the reaction AMMT with Pd(II) ions, depending on the reagents ratio, forms three complexes, which contain 1, 2 or 4 molecules of S-monodentate coordinated ligand [13]. The Ni(II) complex synthesized by Sen A. K. and co-workers [14] contains two molecules of ligand with the S,N-bidentate coordination of AMMT. In contrast to results obtained by Grap S. R. and co-workers [13], complex **1** was obtained by the interaction between AMMT and Pd(II) ions. This complex contains two molecules of bidentate chelate ligands coordinated to metal ion by the nitrogen of amino group and sulfur of thione group. Complexes **1** and **2** [13], **3** [14] have been used for the study of their effects on the mitochondrial dehydrogenase activity in HeLa tumor cell line in comparison with the analogous effects of inorganic salts such as K_2PdCl_4 (**4**), $Ni(NO_3)_2{\cdot}6H_2O$ (**5**) and uncoordinated AMMT.

It has been established that complex compounds Pd(II) **1**, **2** and AMMT are able to enter freely into cells and serve as a substrate for mitochondrial dehydrogenase, in particular for succinate dehydrogenase. Moreover these complexes are also able to enhance the functional activity of the mitochondrial dehydrogenase and improve the mitochondrial respiration in general. It allows one to consider the complex compounds based on AMMT and palladium inorganic salts as potential agents for the restoration of the metabolism under condition of the malignant growth.

Materials and Methods

^1H (^{13}C) NMR spectra were recorded on Bruker Avance DRX-500 (500 (124.75) MHz) spectrometers with dimethylsulfoxide-d_6 as a solvent in the presence of tetramethylsilane.

The crystal structure of complex **1** was determined by a single-crystal X-ray diffraction method. Measurements were performed on XCalibur 3 diffractometer (MoKα radiation, graphite monochromator, λ = 0.71073 Å) at room temperature. The structure was solved by the direct method and refined by full matrix least-square method against F^2 with the anisotropic approximation for non-hydrogen atoms using SHELXTL program package. The $PdCl_2$, K_2PdCl_4 and $Ni(NO_3)_2{\cdot}6H_2O$ (Merck, Cermany) were used for the synthesis of complex compounds.

Synthesis of AMMT and the complexes 1–3

AMMT was synthesized according to [14]. m. p. 205-206 °C. NMR spectrum: ^1H (500 MHz; d_6-DMSO), δ ppm: 2.24 s (3H, CH_3); 5.51 s (2H, NH_2); 13.39 s (1H, SH). NMR spectrum ^{13}C (125.71 MHz; d_6-DMSO), δ ppm: 10.50 (C^3); 149.21 (C^2); 165.47 (C^1). Found, %: C - 27.77; H - 4.88; N - 43.00; S - 24.52. $C_3H_6N_4S$. Calculated, %: C - 27.68; H - 4.65; N - 43.04; S - 24.63.

*The complex $[Pd(AMMT)_2]Cl_2{\times}2H_2O$ (**1**).* The $PdCl_2$ (22.2 mg, 0.125 mM) was dissolved in the mixture of 20 ml ethanol and 2 ml 2N HCl under stirring and heating. The solution of AMMT (32.5 mg, 0.25 mM) in 15 ml of ethanol was slowly added to the hot reaction mixture under constant stirring. The reaction mixture was heated at 60 °C for 1 h. The resulting orange solution was left in a dark place for crystallization. After 10 days, the orange needle-like crystals were formed. The crystals were filtered, washed with ethanol and ether and then dried at room temperature. Yield 49.7 mg (84%). $T_{dec.}$ = 143-144 °C. NMR ^1H, δ ppm: 2.24 s (3H, CH_3); 6.18 w.s. (2H, NH_2); 9.58 s (^1H, $NH_{thiazole}$). NMR ^{13}C, δ ppm: 10.48 (C^3); 149.19 (C^2); 166.49 (C^1). Found, %: C - 15.13; H - 3.52; Cl - 15.09; N - 23.73; Pd 22.58; S 13.51. $C_6H_{16}C_{l2}N_8O_2PdS_2$. Calculated, %:

C - 15.22; H - 3.40; Cl - 14.97; N - 23.65; Pd - 22.47; S - 13.54.

*The complex $[Pd(AMMT)_4]Cl_2$ (**2**).* The synthesis was carried out in accordance with [13]. 15 ml of the hot aqueous solution of K_2PdCl_4 (32.6 mg, 0.1 mM) was slowly added to 15 ml of the hot aqueous solution of AMMT (52.1 mg, 0.4 mM) with stirring. The resulting red-orange solution was left to crystallize. After 5 days, the red crystals were formed. The crystals were filtered, washed with ethanol and ether and then dried at room temperature. The yield: 57.2 mg (82%). $T_{dec.}$ = 199-200 °C. NMR ^{1}H, δ ppm: 2.29 s (3H, CH_3); 6.14 w. s (2H, NH_2); 9.49 s (1H, $NH_{thiazole}$). NMR ^{13}C, δ ppm: 10.50 (C^3); 149.18 (C^2); 165.42 (C^1). Found, %: C - 20.53; H - 3.58; Cl - 10.01; N - 32.00; Pd - 15.13; S - 18.26. $C_{12}H_{24}Cl_2N_{16}PdS_4$. Calculated, %: C - 20.65; H - 3.47; Cl - 10.16; N - 32.11; Pd - 15.25; S - 18.3. The identity of structure of synthesized complex and the compound previously obtained in accordance with [13] was confirmed by X-ray diffraction method.

*The complex $[Ni(AMMT)_2(H_2O)_2](NO_3)_2$ (**3**).* The synthesis was carried out in accordance with [14]. The hot solution of AMMT (65.1 mg, 0.5 mM) in ethanol (15 ml) was slowly added to the hot solution of $Ni(NO_3)_2 \cdot 6H_2O$ (72.7 mg, 0.25 mM in ethanol (15 ml) under constant stirring. The resulting light green solution was heated for 2 h at 60 °C and then left to crystallize in a dark place. After 5-6 days the light green crystals were formed which then were filtered, washed with ethanol ether and then dried at room temperature. The yield: 94.6 mg (79%). $T_{dec.}$ = 193-195 °C. Found, %: C - 14.92; H - 3.50; N - 29.07; Ni - 12.14; S - 13.31. $C_6H_{16}N_{10}NiO_8S_2$. Calculated, %: C - 15.04; H - 3.37; N - 29.24; Ni - 12.25; S - 13.39.

*Complexes **1-3** and starting compounds (AMMT, K_2PdCl_4 (**4**), $Ni(NO_3)_2 \cdot 6H_2O$ (**5**)) biological activity screening.* HeLa cervical carcinoma cell line was used for the assessment of mitochondrial respiration level, cell cycle characteristic and apoptosis level in the presence of the compounds **1-5** and AMMT. In order to assess mitochondrial respiration level the cells were incubated with studied compounds for 24 hours in 96-well plates under standard culture conditions at 37 °C, 5% CO_2 and 100% humidity. Cells were grown in the RPMI-1640 medium (Sigma, USA) with addition of 10% the fetal calf serum (Sigma, USA), 2 mM L-glutamine and 40 μg/ml gentamicin. The initial cells concentration was $2.5 \cdot 10^4$ cells/ml. Cells were seeded in a volume of 100 μl. Compounds **1**–**5** and AMMT were added to cells after 4-hours of adaptation in the concentrations range $4.0 \cdot 10^{-6}$–$1.3 \cdot 10^{-4}$ M in the volume of 100 μl. Cells were cultured under standard conditions for 24 h. Cell viability was assessed by routine counting method in the Goryaev chamber by the trypan blue dye inclusion into dead cells.

The flow cytometric method was used to determine the cell cycle phase distribution and the apoptosis level [19]. For this purpose cells were seeded in 6-well plates at a concentration of $2.5 \cdot 10^4$ cells/ml in 3 ml of the total culture medium volume and incubated with compounds **1-5** and AMMT (at concentration $2.5 \cdot 10^{-5}$ M each) for 48 h at standard conditions. At least $5 \cdot 10^5$ cells were used for the sample preparation. The cells were precipitated by the centrifugation at 1000 g for 5 min. The supernatant was removed and cells were washed with the saline or 1.0 M sodium phosphate buffer (PBS, pH 7.2). Cells were resuspended in 200 μl PBS, then the 300 μl of 1.0 M citrate buffer (pH 6.8) containing 0.1% Triton X-100 was added. After 1 min, 10 μl of ribonuclease and 10 μl of propidium iodide PI (Sigma, USA) for DNA staining were added [19]. The mixture was incubated for 10 min at 37 °C in the dark and additionally for 30 min at room temperature. Then it was centrifuged at 1000 g for 10 min and the supernatant was removed. Then cells were fixed by adding 400 μl PBS with 0.4% formalin and the DNA contents were analyzed in samples. The flow-cytometric analyses were performed with FACS Calibur (Becton Dickinson, USA), which has two lasers with wavelengths of 488 and 625 nm. The narrow-band filter 585/42 nm was used for the measurement of PI fluorescence. The data analysis was performed using CellQuest and ModFit LT 2.0 (BDIS, USA) software.

The cells distribution in different cell cycle phases were assessed with flow cytometry. Cells were seeded in 6-well plates at a concentration of $2.5 \cdot 10^4$ cells/ml in 3 ml of the total culture medium volume. Cells were incubated with compounds **1-5** and AMMT (at concentration $2.5 \cdot 10^{-5}$ M each) for 48 h under standard conditions. Cells proportions in different phases of the cell cycle was measured by flow cytometry with the argon laser ($\lambda_{excitation}$ = 488 μm, $\lambda_{emission}$ = 585/40 μm, Becton Dickinson, USA) in accordance with standards for dyeing [19]. The apoptotic index was assessed by staining cells with PI, which is selectively bound with DNA intercalating sites.

The assessment of the mitochondrial dehydrogenase activity in the presence of studied compounds was performed by using the MTT assay [15]. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) is the yellow monotetrazolium salt (Fig. 1). Its reduction is the most often used method for measurements of the cell proliferation and cytotoxicity [16].

The biochemical essence of this method is based on the fact that mitochondrial dehydrogenases of living cells are capable to cleave tetrazolium rings with formation of insoluble purple crystals (formazan). The dehydrogenase reduction activity considerably depends on the intracellular concentration of NADH and NADPH, which is driven by the presence of extracellular glucose [17]. Mitochondrial succinate dehydrogenase and cytochrome C are mainly involved in the MTT reduction [18]. Thus, compounds affecting the MTT reduction rate may serve as modifiers of the mitochondrial respiration.

MTT (20 μl) was added to the culture medium 4 h before the termination of the cells incubation in order to achieve the final concentration of 0.6 mM. Formazan crystals formed after the incubation with MTT were dissolved in 100 μl of dimethylsulfoxide. The plate was analyzed on the spectrophotometer at 540 nm.

The graphical and statistical data processing (calculation of means and standard deviation, statistical analysis of the reliability, differences in values of all described experiments according to Student's *t*-test standard criteria) were performed using the software Microsoft Excel™ 2010. All experimental results are expressed as a value ± standard deviation ($M \pm m$). Values with $P \leq 0.05$ were considered as reliable.

Results and Discussion

The tendency of AMMT to the thiol-thione tautomerism and the presence of various nucleophilic reactive sites in the molecule, which cause its polydentate nature, determine the possibility to form transition metal complexes with various compositions and structures as well as the way of the ligand coordination dependence on synthesis conditions and the metal nature. The synthesis of complexes **1-3** were performed in accordance with the scheme shown in Fig. 2.

The composition and structure of all synthesized compounds were determined by the elemental analysis, X-ray diffraction study, UV-Vis spectra and nuclear magnetic resonance (NMR) on proton (^{1}H) and carbon (^{13}C) nucleus. The complex **1** is novel and the general form of the molecule **1** according to the X-ray diffraction data is represented on Fig. 3. The Complex **1** crystallizes in the triclinic crystal system, space group P-1, with following unit cell parameters: a = 8.076(19), b = 10.48(2), c = 10.639(16), α = 104.82(17), β = 99.08(18), γ = 103.3(2). Bonds C(1)-S(1)/C(4)-S(2), N(3)-N(4)/N(7)-N(8) in comparison with the original AMMT [20] are slightly elongated to 0.029/0.023 and 0.019/0.033 Å due to the coordination of sulfur and nitrogen atoms with metal ions.

The analyses of UV-Vis spectra of AMMT and complexes **1-3** were carried out after their decomposition into Gaussian components (Table 1). The wide absorption band of the AMMT solution at 40383 cm^{-1} consists of three components that correspond to π→π* electron transitions (C=N) of the triazole ring [23]. It should be noted that AMMT thiolic tautomer dominates in solutions [20], as a result the π→π* electron transitions of the (C=S) group are not

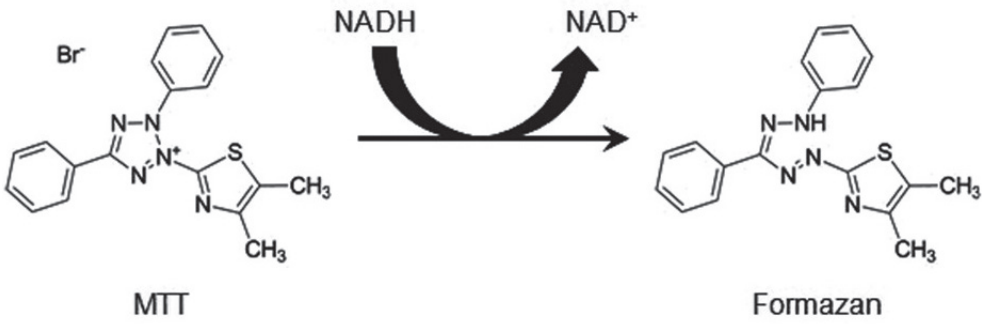

Fig. 1. Scheme of reduction of 3-(4,5-dimethyltiazole-2-yl)-2,5-diphenyltetrazole bromide to crystallized formazan

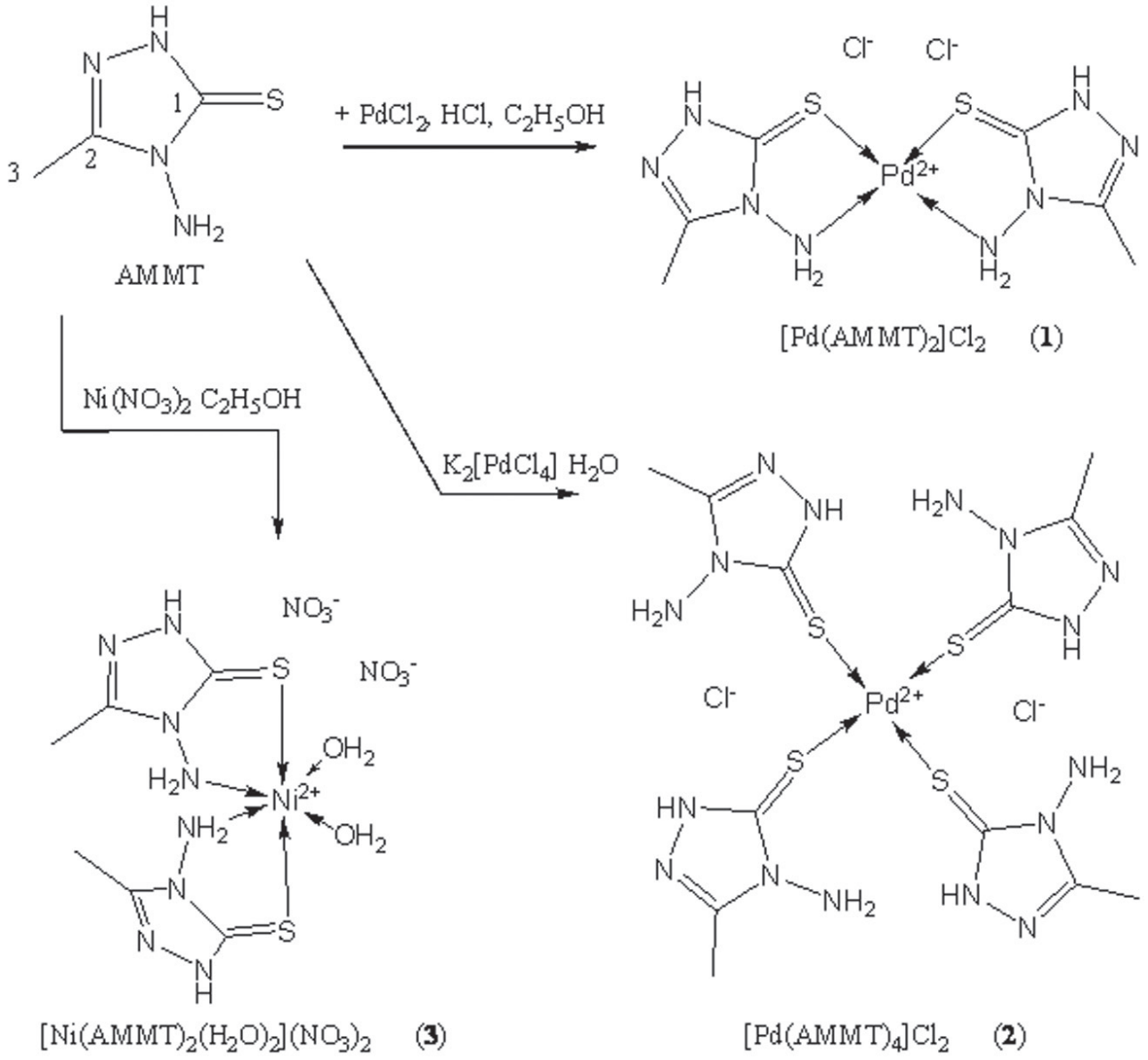

Fig. 2. Scheme of the synthesis of palladium(II) and nickel(II) complex compounds with AMMT

visualized. In contrast to AMMT, one of Gaussian components in spectra of complexes **1-3** corresponds to π→π* electron transitions of the (C=S) group of the thione tautomeric form of coordinated ligand molecules in the region of 28221–28775 cm^{-1} (Table 1). Furthermore, in **1** and **2** complexes spectra the Gaussian component at 24153 and 23835 cm^{-1} corresponds to d-d electron transitions in the Pd^{2+} ion. Thus, UV-Vis data prove the similarity in structures of complexes in solid states and in solutions.

Coordination compounds are also stable in dimethyl sulfoxide solutions, which are indicated by ^{1}H (^{13}C) NMR spectra of complexes **1** and **2**. The analysis of ^{1}H NMR of the starting AMMT proved that in solution AMMT exists as the thiolic tautomer since the spectrum contains the singlet at 13.39 ppm which corresponds to the proton signal of the SH functional group. This singlet is absent in the spectra of complexes **1** and **2**, but a wide singlet at 9.58/9.49 ppm is observed. This singlet corresponds to the protonated nitrogen atom of the triazole moiety. The wide singlet of the NH_2 group in spectra of complexes is shifted downfield by Δδ = +0.67/+0.63 ppm. There are three carbon signals in ^{13}C NMR spectra of complexes **1** and **2** in positions 1, 2, 3 at 166.49/165.42, 149.19/149.18, 10.48/10.50 ppm, respectively. The signal C(1) of the complex 1 has the largest downfield shift (Δδ = +1.02 ppm) in comparsion with free

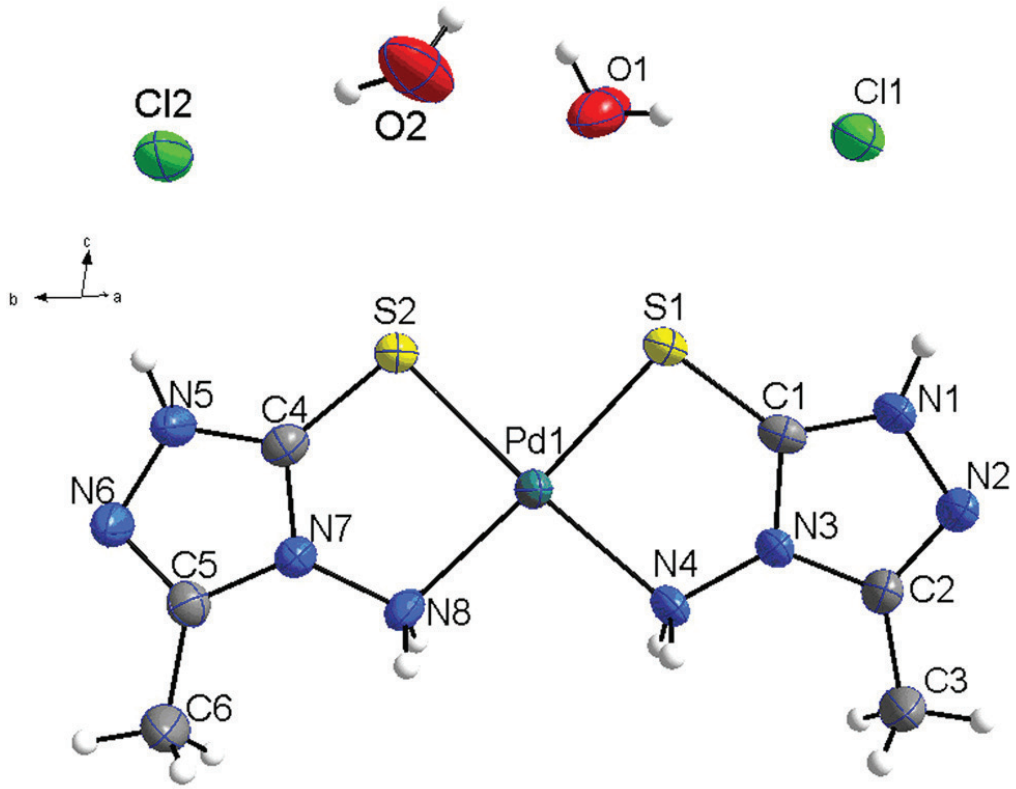

Fig. 3. Molecular structure of the complex 1 according to X-ray diffraction analysis

Table 1. UV-Vis data of AMMT and complexes 1-3 (ν, cm^{-1})

AMMT	complex **1** $\pi\rightarrow\pi^*_{\text{triazole}}$	$\Delta\nu_1$	complex **2** $\pi\rightarrow\pi^*_{\text{triazole}}$	$\Delta\nu_2$	complex **3** $\pi\rightarrow\pi^*_{\text{triazole}}$	$\Delta\nu_3$
48746	49926	+1179	0	0	48446	-300
45453	45324	-129	46732	+1279	45432	-21
42457, 40383	41257	+874	43345, 40673	+888	42709, 40540	+252, +157
39129	38843	-286	39438	+290	39042	-87
35650	35412	+238	35532	+309	35819	+169
31433	31778	+345	0	+118	31941	+508
0	28255 (C=S)	0	28775 (C=S)	0	28221 (C=S)	0
0	24153 (d-d)	0	23835 (d-d)	0	0	0

$\Delta\nu_1$–$\Delta\nu_3$ – the frequency shift value between Gaussian components of AMMT and complexes **1**, **2**, **3**

AMMT that may be related to its bidentate coordination to the central metal ion.

Compounds **1-3** are soluble in water, which allowed us to compare their action on metabolism of HeLa cells with the action of original compounds of AMMT and inorganic metal salts such as K_2PdCl_4 (**4**), Ni $(NO_3)_2\cdot 6H_2O$ (**5**) *in vitro*.

*The screening of complex compounds **1-3**, AMMT and metal salts **4**, **5** with regard to HeLa cells in the MTT assay.* Obtained data allow to suggest that the incubation of HeLa cells with compounds **1-5** and AMMT *in vitro* in a certain way affect their metabolism. In particular, MTT assay results showed (Fig. 4), that the compound **1** increases mitochondrial dehydrogenases activities in the concentration range from 0.032 to 0.062 mM. A similar effect for the compound **2** at concentrations 0.008, 0.032, 0.062, 0.125 mM was observed. However,

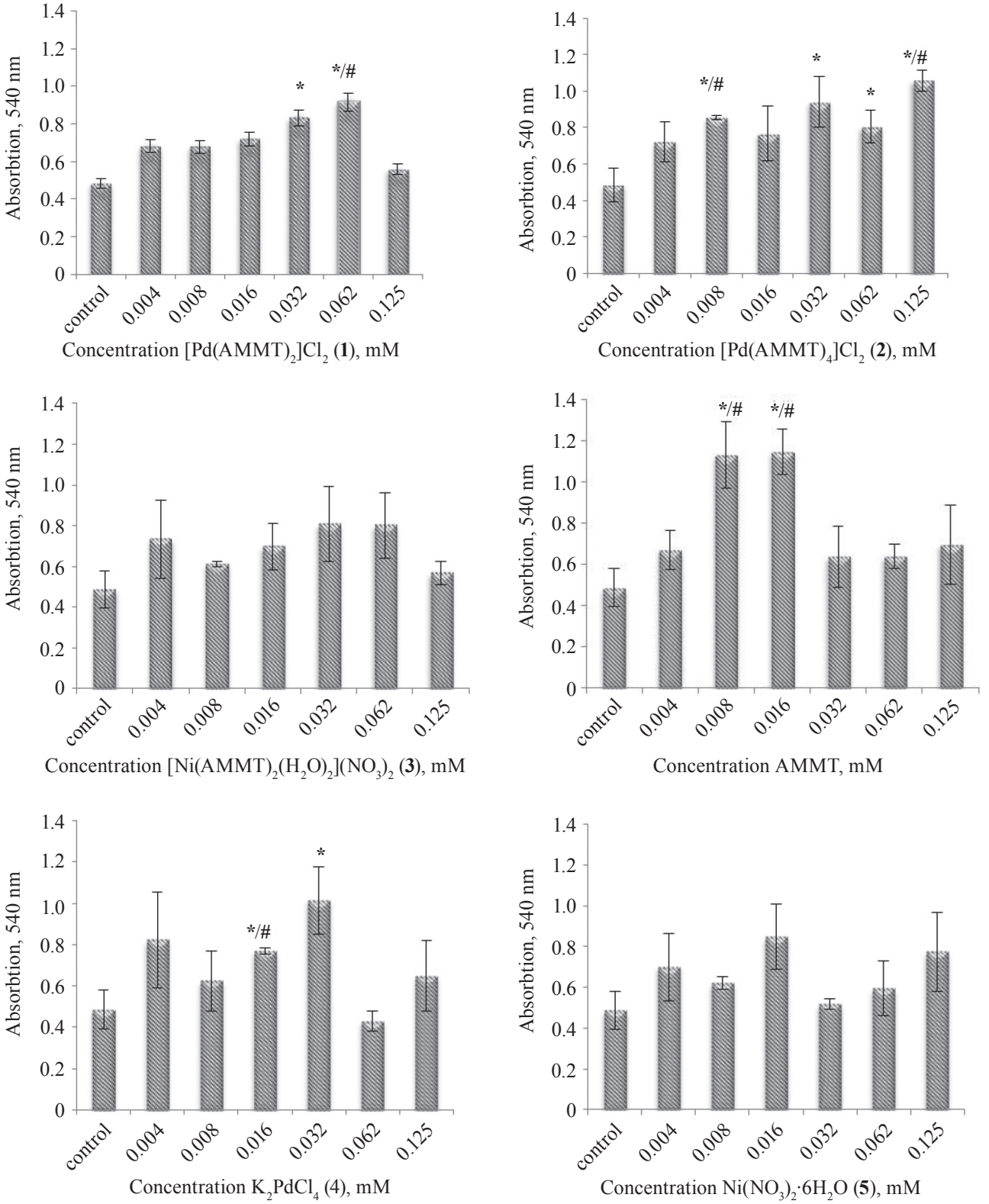

*Fig. 4. Screening of the complexes 1-3 and starting compounds AMMT, 4, 5 in regards to HeLa cells in the MTT-assay; statistical significance with reference to control * P < 0.05; # P < 0.01*

the relationship between the increase/decrease of the dehydrogenase activity and the concentration of compounds was not found. AMMT activated mitochondrial dehydrogenase in the concentration range of 0.008–0.016 mM. The concentration range for palladium inorganic salt **4** was 0.016-0.032 mM. It should be noted that cells which were incubated with compounds **3** and **5** and control cells did not show statistically significant discrepancies. Thus, the complex compound **3** and nickel inorganic salt **5** (which was used for the synthesis of complex compound **3**) do not exhibit the biological activity, as well as the toxic effect.

Thus, studied complexes **1**, **2**, as well as AMMT are able to enter into cells like MTT, moreover, they can compete with MTT, acting as substrates for mitochondrial dehydrogenases. Taking into consideration, that the MTT concentration was 0.6 mM (time of incubation in the presence of MTT was 4 hours), and studied compounds have been screened in much smaller ranges of concentrations for 24 hours, it is obvious that the compounds **1**, **2**, as well as AMMT and palladium inorganic salt **5** penetrate into the cell without damage to the cell membrane. They are also accelerating the energy transfer, specifically affecting the mitochondrial respiration rate. The compound **3** and nickel inorganic salt **5** do not show such an activity in the presence of MTT but cytotoxic effect also was not observed. It should be noted that unlike MTT the studied compounds did not stain cells.

As it is indicated earlier, MTT is a substrate for succinate dehydrogenase (SDH), which is involved in the electron transport chain to molecular oxygen. Using of the SDH inhibitors has showed that the inhibition of the activity of this enzyme leads to the decrease of the MTT reduction rate [16]. The SDH, which participates both in the tricarboxylic acid cycle and respiratory chain, plays an important role in the mitochondria function as well as in cell functions in general. The SDH catalyzes the reversible succinic acid (succinate) oxidation to fumaric acid in the citric acid cycle. The oxidation of 1 succinate equivalent leads to the formation of 2 adenosine triphosphate (ATP) equivalents. Thus, electrons are transferred from SDH into respiratory chain to coenzyme Q. Mitochondrial SDH of mammals is composed of four subunits: two hydrophilic and two hydrophobic. The first two subunits: A (flavoprotein) and B (iron-sulfur protein) are hydrophilic. The subunit A contains the covalently bonded flavin adenine dinucleotide (FAD), which acts as a cofactor of the succinate dehydrogenase reaction. The subunit B contains three iron-sulfur clusters [2Fe-2S], [4Fe-4S] and [3Fe-4S]. The other two subunits: C and D, are hydrophobic and membrane anchored [21]. It should be noted that, although genes which are responsible for the SDH subunits expression are "housekeeping genes", mutations in these genes cause the malignant tumor. Thus, mutations in subunits B, C and D may cause paragliomas or pheochromocytoma. Mutations in the subunit A may cause leiomyoma, leiomyosarcoma or renal-cell carcinoma [22].

It is known that the respiratory chain enzymes and the Krebs cycle dehydrogenases determine the rate of the aerobic oxidation, which is the main pathway of the energy generation. The complex compounds synthesized in this work, such as **1**, **2** and AMMT, can be involved in these metabolic conversions.

Effect of synthesized compounds on the mitotic cycle and apoptosis level in the HeLa cell line. The effect of these compounds on the mitotic cycle and apoptosis level of HeLa cell line was the next step of our study. The obtained results have showed that the synthesized complex compounds **1-3** as well as AMMT and inorganic salts of palladium **4** and nickel **5** do not affect the mitotic cycle since the number of cells in phases G_0/G_1, G_2/M, S, $G_2/M+S$ of the cell cycle in the presence of studied compounds (Table 2) do not differ from control cells.

The studied compounds did not affect the apoptotic cells level (Fig. 3) and did not cause cytotoxic/cytostatic effects, so their activity apparently is aimed at the regulation of the cellular metabolism. The studied compounds in their effects are similar to MTT which was used in work for their screening. Some of them, in particular **1**, **2** and **4**, exhibit synergy with AMMT. They enhance the mitochondrial SDH activity, however do not stain cells, in contrast to MTT. Compounds **3** and **5** were inactive against a background of the MTT.

It was interesting that palladium salt **4** showed an activating and inducing effect on mitochondrial dehydrogenases. It may indicate its role as an inorganic catalyst. The synthesized palladium complex compounds **1** and **2** demonstrated a greater activity. The compound **2** showed the effect within a wide range of concentrations. AMMT, which was used as a starting substance for their synthesis, demonstrated higher activity in comparison with the synthesized complexes, but within a little smaller range of concentrations. It should also be noted that nickel salt **5**

did not lead to changes in the mitochondrial respiration activity. The similar effects observed with the complex **3** confirm that the compound **5** may block the AMMT biological activity.

Thus, AMMT and palladium inorganic salt **4** can be used for the synthesis of biologically active complex compounds, including those, which may be involved in the regulation of cell metabolism.

It is known that MTT can be reduced by other non-mitochondrial dehydrogenases, such as N-ethylmaleimid (NEM) sensitive flavin oxidase [16]. It is obvious that studied compounds act by similar mechanisms. The decrease in activities of these enzymes leads to the decrease of the tissue respiration rate and to the decrease of the energy supply to an organism.

Three AMMT complex compounds with Pd^{2+} (**1**, **2**) and Ni^{2+} (**3**) ions have been synthesized in this study. The single-crystal X-ray diffraction method showed that in presented complexes AMMT exists in thione tautomeric form as monodentate or bidentate ligand with the S- or S,N-donor coordination to the central metal ion. The synthesized water-soluble complex compounds were proposed as potential biologically active agents.

The biological activity of the complexes **1**-**3** in comparison with the activity of starting AMMT, as well as inorganic salts K_2PdCl_4, $Ni(NO_3)_2{\cdot}6H_2O$ were studied. It was shown that the biologically active AMMT and its complexes with Pd^{2+} ions can be used as agents that induce the mitochondrial respiration. Nickel nitrate blocks the AMMT biological activity, however the synthesized on its base complex **3** does not affect cell metabolism, cell cycle and apoptosis. Palladium complexes **1**, **2** induce the mitochondrial dehydrogenases activity and do not affect the cell cycle and apoptosis level, moreover the compound **2** exhibits the biological effect in a much wider range of concentrations, in comparison with the compound **1**.

ВПЛИВ КООРДИНАЦІЙНИХ СПОЛУК Pd(II) І Ni(II) ІЗ 4-АМІНО-3-МЕРКАПТО-5-МЕТИЛ-1,2,4-ТРИАЗОЛОМ НА АКТИВНІСТЬ МІТОХОНДРІАЛЬНИХ ДЕГІДРОГЕНАЗ

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Синтезовано комплекси Pd(II) і Ni(II): $[Pd(AMMT)_2]Cl_2$ (**1**), $[Pd(AMMT)_4]Cl_2$ (**2**), $[Ni(AMMT)_2(H_2O)_2](NO_3)_2$ (**3**) з 4-аміно-3-меркапто-5-метил-1,2,4-триазолом (AMMT). Досліджено ЯМР ^1H (^{13}C) та ЕСП спектральні характеристики сполук **1**, **2**. Методом РСА встановлено, що в усіх комплексах молекула AMMT координована до центрального іона металу в тіонній таутомерній формі. При співвідношенні M : L = 1 : 2 ліганд координується хелатним способом атомами азоту аміно- та сірки меркаптогрупи (сполуки **1**, **3**), а при співвідношенні M : L = 1 : 4 – монодентатно, тільки атомом сірки меркаптогрупи (комплекс **2**). Вакантні координаційні місця в оточенні металу займають молекули води (комплекс **3**). Скринінг на активність мітохондріальних дегідрогеназ цільових продуктів **1**–**3** та вихідних сполук [AMMT, K_2PdCl_4 (**4**), $Ni(NO_3)_2{\cdot}6H_2O$ (**5**)] проведено нами вперше, внаслідок чого встановлено, що комплекси Pd(II) (**1**, **2**), сіль Pd(II) (**4**) та AMMT

нормалізують активність мітохондріальних дегідрогеназ пухлинних клітин лінії Hela, виявлених у МТТ-тесті. На відміну від них, комплекс Ni(II) (**3**) та сіль Ni(II) (**5**) не стимулюють активність мітохондріальних дегідрогеназ. Загальними властивостями всіх досліджуваних сполук є відсутність впливу на клітинний цикл та рівень апоптичних клітин, а також відсутність токсичного ефекту. Таким чином, одержані результати вказують на те, що AMMT і комплекси Pd(II) можуть використовуватися як модифікатори мітохондріального дихання, дисфункція якого особливо виражена у пухлинних клітинах.

Ключові слова: 1,2,4-триазоли, комплекси Pd(II) та Ni(II), ЯМР ^{1}H (^{13}C) спектроскопія, активність мітохондріальних дегідрогеназ, апоптоз клітин.

ВЛИЯНИЕ КООРДИНАЦИОННЫХ СОЕДИНЕНИЙ Pd(II) И Ni(II) С 4-АМИНО-3-МЕРКАПТО-5-МЕТИЛ-1,2,4-ТРИАЗОЛОМ НА АКТИВНОСТЬ МИТОХОНДРИАЛЬНЫХ ДЕГИДРОГЕНАЗ

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Синтезированы комплексы Pd(II) и Ni(II): $[Pd(AMMT)_2]Cl_2$ (**1**), $[Pd(AMMT)_4]Cl_2$ (**2**), $[Ni(AMMT)_2(H_2O)_2](NO_3)_2$ (**3**) с 4-амино-3-меркапто-5-метил-1,2,4-триазолом (AMMT). Исследованы ЯМР ^{1}H (^{13}C) спектральные характеристики соединений **1**, **2**. Методом РСА установлено, что во всех комплексах молекула AMMT координирована к центральному иону металла в тионной таутомерной форме. При соотношении M : L = 1 : 2 лиганд координируется хелатным способом атомами N амино- и S меркаптогруппы (соединения **1**, **3**), а при соотношении M : L = 1 : 4 координация AMMT осуществляется монодентатно только атомом S меркаптогруппы (комплекс **2**). Вакантные координационные места в окружении металла занимают молекулы воды (комплекс **3**). Скрининг на активность митохондриальных дегидрогеназ соединений **1**–**3** и исходных компонентов [AMMT, K_2PdCl_4 (**4**), $Ni(NO_3)_2{\cdot}6H_2O$ (**5**)] проведен нами впервые, в результате чего установлено, что комплексы Pd(II) (**1**, **2**), соль Pd(II) (**4**) и AMMT нормализуют активность митохондриальных дегидрогеназ опухолевых клеток линии Hela, выявленных в МТТ-тесте. В отличие от них, комплекс Ni(II) (**3**) и соль Ni(II) (**5**) не стимулируют активность митохондриальных дегидрогеназ. Общими свойствами всех исследуемых соединений является то, что они не влияют на клеточный цикл, уровень апоптических клеток и не проявляют токсический эффект. Таким образом, полученные результаты указывают на то, что AMMT и комплексы Pd(II) могут использоваться в роли модификаторов митохондриального дыхания, дисфункция которого особенно выражена в опухолевых клетках.

Ключевые слова: 1,2,4-триазолы, комплексы Pd(II) и Ni(II), ЯМР ^{1}H (^{13}C) спектроскопия, активность митохондриальных дегидрогеназ, апоптоз клеток.

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Received 18.07.2014