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SPECTRAL CHARACTERISTICS AND CYTOSTATIC EFFECT OF Pd(II) AND Pt(II) CARBOTHIOAMIDE π-COMPLEXES ON *ALLIUM CEPA* L MERISTEM CELLS

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The spectral characteristics of the synthesized cisplatin analogs based on π-complexes of Pd(II) and Pt(II) with N-allylmorpholine-4-carbothioamide and 1-allyl-3-*tret*-butylthiourea and the mechanism of their cytostatic and antiproliferative action on meristem cells of *Allium cepa* L. are reported in this work for the first time. It is shown that the electronic absorption and diffuse reflection spectra of complexes show absorption bands corresponding to the transitions with charge transfer from a ligand to metal, dd-electron transitions and intraligand $\pi\rightarrow\pi^*$ and $n\rightarrow\pi^*$ electronic transitions with the contributions of multiple bonds of allyl moiety and carbothioamide group. In this case, the absorption bands in the ultraviolet region of the spectra of the complexes undergo a hypochromic shift with respect to the spectra of the ligands themselves, which is due to the presence of a coordination link. Analysis of the titration curves shows the possible coordination of the ligands in solution in both chelate and non-chelate ways only through the atoms of sulfur of the carbothioamide group with molar ratios M:L=1:1, 1:2 and 1:3. Theoretical quantum-chemical calculations of the IR spectra are carried out; their results are in good agreement with the experimental data of infrared spectroscopy. It is established that cisplatin analogues exhibit a specific cytostatic effect on *Allium cepa* meristem cells with high antiproliferative activity. Due to the formation of numerous cross-links of metal-containing preparations with DNA molecules and with nuclear proteins, chromatin is not compacting as normal. This results in a disrupted cell cycle, a decreased mitotic index and anomalous cell division, which are usually followed by apoptosis. Palladium-based π-complexes cause chromatin dispersion, which leads to significant abnormalities in the processes of karyokinesis, which cause the chromosomes to lose their typical structure and undergo fragmentation, mostly with loss of telomeric sites.

Keywords: π-complexes, carbothioamides, vibrational spectroscopy, anticancer activity, genotoxicity.

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Introduction

N-Allyl-substituted thioureas are the original type of ambidentate ligands. The favorable mutual spatial arrangement in their structure of N,S-nucleophilic atoms and allyl moiety creates for them the prerequisites for the formation of stable six-membered chelate metallocycles with the formation of π-bonds with ions of a number of metals belonging to «soft» Lewis acids, such as Pd(II), Pt(II), Cu(I), Ag(I), and others [1,2]. Coordination compounds of this type are of interest as catalysts for organic reactions and as potential biologically active

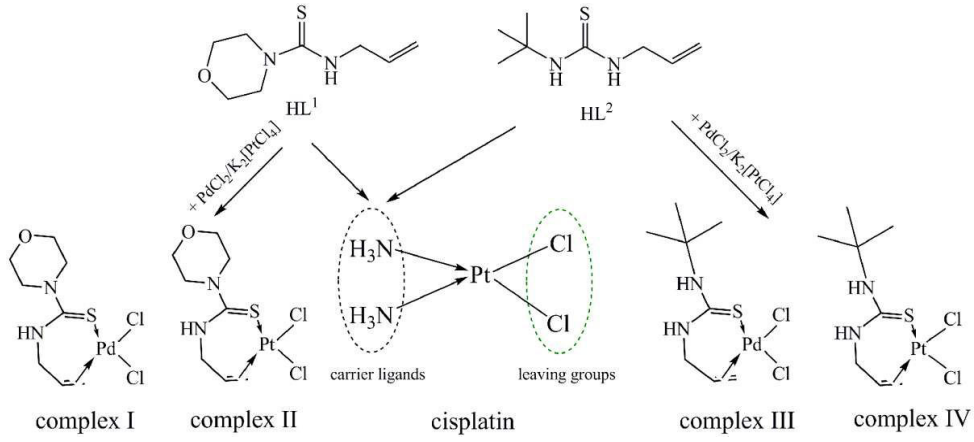

Fig. 1. Schematic molecular structures of ligands HL^1, HL^2, complexes I–IV and cisplatin

substances. However, despite the fact that π-complex compounds of transition metal ions are known for a long time, their biological activity has hardly been studied. In our previous works [1,3,4], we have shown that the synthesized π-complexes of Pd(II) and Pt(II) with N-allylthiourea derivatives (I–IV) exhibit a pronounced antitumor activity which is an order of magnitude higher than known pharmaceutical drug cisplatin (Fig. 1).

In the molecules of cisplatin and its analogues, square-planar coordination polyhedrons are formed on the one hand by «carrying» nitrogen atoms in the *cis* position and on the other hand by «leaving» group represented by chloride ions. However, unlike cisplatin, in the structure of the complexes synthesized by us, thiourea scaffold are coordinated to Pd(II) and Pt(II) ions by even «softer» donor atoms (in conformity with Pearson's classification) with high *trans*-influence (according to the effect of symbiosis in Pearson's transfusion). Consequently, the affinity of metal ions in the complexes I–IV to the «soft» sulfur atoms of other substances may decrease, which should prevent the binding them with thiol-containing substances which are present in the cytoplasm of cells. As a result, it should hinder the migration of these potential intercalators towards DNA, the main pharmaceutical target. In addition, functionally substituted thioureas are able to influence a number of other biological targets critical for the process of carcinogenesis [5], which is an additional basis for their use in the design of new anticancer agents.

In this work, using spectral methods, we studied the formation of Pd(II) and Pt(II) complex compounds with allylthioureas in solutions in order to predict the behavior of these complexes in biological media. The mechanism of their cytostatic and antiproliferative effects on meristem cells of *Allium cepa* (as the test object widely used to evaluate the genetic potential of chemical compounds) was also studied. Besides platinum, palladium was also used as a central atom to synthesize novel cisplatin analogues. It should be noted that the structural and thermodynamic similarities with platinum and its lower toxicity make palladium compounds promising objects for the design of new potential antitumor agents, as evidenced by numerous publications [6,7]. However, there are only a few reports concerning π-complexes as objects of search for new anticancer agents [8], which together with taking into account our previous results [1,3,4], makes them promising objects for research in this direction.

Experimental

Synthesis of complex compounds

Synthesis of complexes I–IV was performed by the method described elsewhere [1]. The IR spectra were recorded using a Specord M80 spectrometer in the frequency range of 4000–400 cm^{-1} in KBr pellets. The electronic absorption spectra and diffuse reflectance spectra were measured by a Specord M40 spectrophotometer (in the range of 50000–11000 cm^{-1}). Spectrophotometric studies of ligands HL^1, HL^2 and complexes I–IV were performed using mixture solvents DMF:ethanol (2:1). The starting solutions for titration were prepared by dissolving the exact weighed sample of $PdCl_2$ or K_2PtCl_4 in 4 mL of 2 M HCl and the carbothioamides HL^1 or HL^2 in EtOH. The solution volume was brought to 25 mL with ethanol. Herewith the concentration of metal salts (C_M) was unchanged (10^{-4} M), while the concentration of carbothioamides HL^1/HL^2 was changed from $1.67 \cdot 10^{-5}$ M to $33.33 \cdot 10^{-5}$ M.

Computational details

Geometry optimization with subsequent normal mode analysis of N-allylthioureas HL and metal

complexes [M(HL)Cl_2] were performed by means of the density functional theory. A combination of BP86 nonhybrid functional and a polarized triple-zeta (TZP) valence basis set in combination with Huzinaga's model core potentials (MCP) was employed. The initial structures were taken from the X-ray diffraction data [1]. The assignment of vibrational modes was achieved based on comparison of experimental and calculated IR spectra. Scale factor of 1.034 was used for the frequencies lower than 1600 cm^{-1} [9]. Calculations were carried out by using the GAMESS (US) program package. Computing resources were provided by the SCIT supercomputer (V.M. Glushkov Institute of Cybernetics of the NAS of Ukraine).

Allium cepa L. test

Allium cepa test is a simple, cost-effective and sensitive method for detection of chromosomal mutations. The results obtained using this test correlated highly with the results of studies on mammalian, including human cells [10].

Nuclear DNA was detected with Feulgen stain. Hydrochloric acid hydrolyzes the deoxyribose into an aldehyde which turns red-purple in reaction with fuchsin-sulfurous acid (Schiff's reagent) [11].

Microscopy and photographing of the results of cytological studies were performed by an Axio Scope A1 research class microscope using AxioVision 4.7 software (Carl Zeiss, Germany). Digital bitmap was processed in the specialized program Image-Pro Premier 9.0 (Media Cybernetics, USA). The chromatin condensation and DNA content in the interphase cells of the test culture were qualitatively evaluated using a software module for automatic pixel calculation with a histochemical reaction color palette.

Results and discussion

The data of $^1H/^{13}C$ NMR spectra and X-ray analysis of carbothioamides HL1, HL2 and their complexes as well as parameters for assessing their biological activity, such as IC50 and their DNA binding ability, were reported previously in ref. [1]. In continuation of that work, this paper reports the processes of complex formation in solutions based on UV-Vis spectra in order to establish detailed conditions of complex formation and compare the spectral characteristics of compounds with each other both in solution and in the solid state. In addition, to study the spectral characteristics of the solid state of the synthesized cisplatin analogues, we present the IR and diffuse reflection spectra.

UV-Vis and diffuse reflection spectra of carbothioamides HL^1, HL^2 and complexes I–IV based on them

The results showed that the UV-Vis spectra of both carbothioamides are similar and consist of low-intensity shoulder-type absorption bands at 33620 and 34220 cm^{-1}, which are due to intraligand $\pi\rightarrow\pi^*$ and $n\rightarrow\pi^*$ electron transitions with the contribution of the multiple bond (C=S) of the carbothioamide moiety, respectively. The observed absorption difference ($\Delta\nu$=600 cm^{-1}) is associated with the influence of the nature of the substituent in the HL^1 and HL^2 molecules (Fig. 2, Table 1) [2,12].

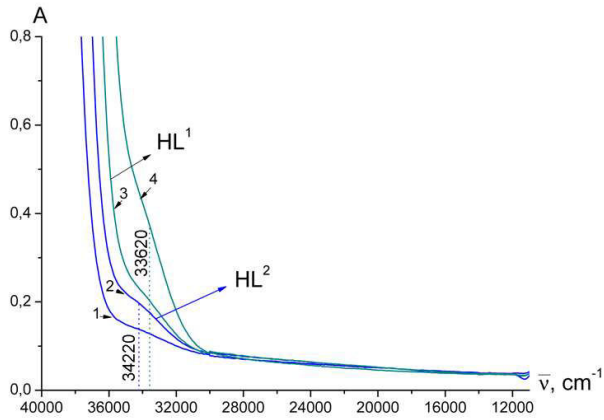

Fig. 2. UV-Vis spectra of carbothioamides HL^1, HL^2 in a mixture DMF:ethanol = 2:1 (1, 2 – $C_{(HL2)}$=1·10^{-4} M; 3, 4 – $C_{(HL1)}$=1·10^{-4} M)

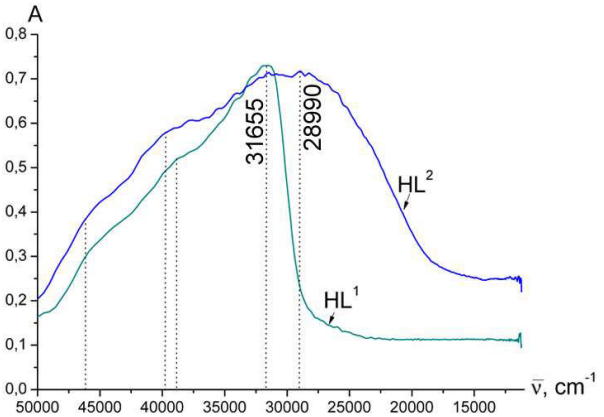

Fig. 3. Diffuse reflection spectra of carbothioamides HL^1, HL^2

Unlike the UV-Vis spectra, the diffuse reflection spectra of HL^1 and HL^2 show several maxima at 46154, 38860, 31655 and 46154, 39790, 31655, 28990 cm^{-1}, respectively. These maxima are related

Table 1

UV-Vis and diffuse reflection spectra data (n, cm^{-1}) of HL^1, HL^2 and complexes I–IV

Compound	Assignments in UV-Vis			Assignments in Diffuse reflection spectra		
	$\pi\rightarrow\pi^*$, $n\rightarrow\pi^*$	$(LMCT)^1$ transitions	dd	$\pi\rightarrow\pi^*$, $n\rightarrow\pi^*$	$(LMCT)^1$ transitions	dd
HL^1	33620	–	–	46154, 38860, 31655	–	–
Complex I	36890	30150, 26325	21273	46000, 38800, 29937	24882	20065
Complex II	32680		26100	46000, 38800, 29937	26100	18500
HL^2	34220	–	–	46154, 39790, 31655, 28990	–	–
Complex III	36565	34200, 28112	21100	45087, 38555, 31355	24822	21245
Complex IV	32455		25450	45087, 38555, 31355,	26112	18655

Note: [1] – (LMCT) – ligand-to-metal charge-transfer.

to intraligand $\pi\rightarrow\pi^*$ and $n\rightarrow\pi^*$ electron transitions with contributions of multiple bonds of the allyl moiety and carbothioamide group (Fig. 3, Table 1). The both spectra are characterized by the presence of a broad and low pronounced maximum at 28990 cm^{-1} in the HL^2 spectrum and $\Delta\nu_2$=930 cm^{-1}, which is related to the influence of the nature of the substituent and, as a consequence, to the different electron density distribution in the organic ligand molecule. In UV-Vis spectra, the absorption in the ultraviolet region from 50000 to 36000 cm^{-1} overlaps the absorption of solvent (DMF).

The UV-Vis spectra of complexes I–IV in a mixture DMF:ethanol=2:1 mainly consist of absorption bands responsible for ligand-to-metal charge-transfer (LMCT) and d–d electron transitions in the metal ion (Figs. 4, 5, Table 1).

The broad absorption band at 32680/32455 cm^{-1} in the spectrum of the complexes of platinum II and IV corresponds to the overlap of the intraligand electron transitions $\pi\rightarrow\pi^*$ and $n\rightarrow\pi^*$ of the carbothioamide group with the charge transfer from ligand to metal. The absorption bands of LMCT transitions and dd-electron transitions are visualized separately in the spectrum of palladium complexes I and III, which is related to the nature of the metal. In this case, the absorption bands of intraligand electronic transitions $\pi\rightarrow\pi^*$ and $n\rightarrow\pi^*$ undergo a significant hypochromic shift with respect to the ligands themselves. These changes are due to the presence of a coordination link [13].

Unlike UV-Vis spectra, the diffuse reflection spectra exhibit several highs in the curves which are related with both intraligand $\pi\rightarrow\pi^*$ and $n\rightarrow\pi^*$ electron transitions of carbothioamide group as well as LMCT and dd-electron transitions (Figs. 6, 7, Table 1).

To analyze the complex formation in ethanol solution, the dependence of the absorbance on the ligand concentration was studied (Figs. 8, 9). Unlike the UV-Vis spectra of the synthesized complexes dissolved in a mixture DMF:ethanol=2:1 (Figs. 4, 5), the UV-Vis spectra of ethanol solutions of

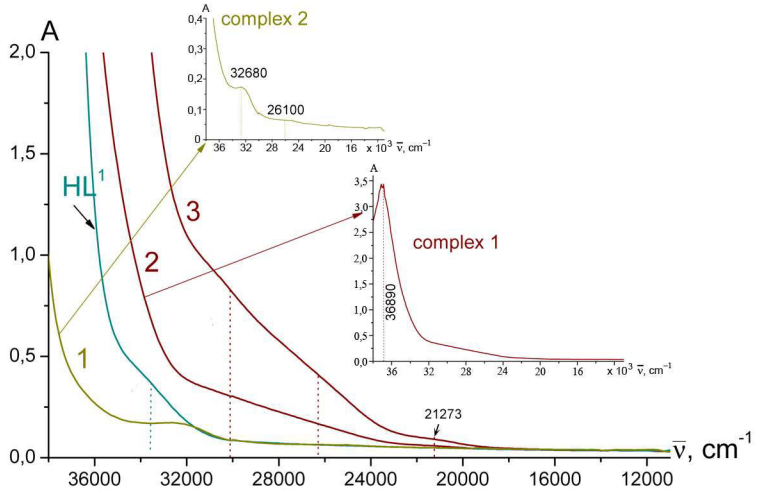

Fig. 4. UV-Vis spectra of carbothioamide HL^1 and complexes I and II in a mixture DMF:ethanol=2:1 (curves 1–3 show the spectra for different concentrations of complexes, $1\cdot10^{-3}$ and $1\cdot10^{-4}$ M)

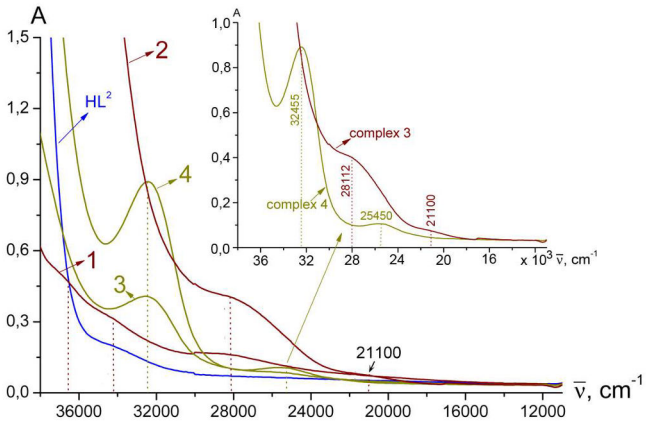

Fig. 5. UV-Vis spectra of carbothioamide HL^2 and complexes III and IV in a mixture DMF:ethanol=2:1 (curves 1–4 show the spectra for different concentrations of complexes, $1 \cdot 10^{-3}$ and $1 \cdot 10^{-4}$ M)

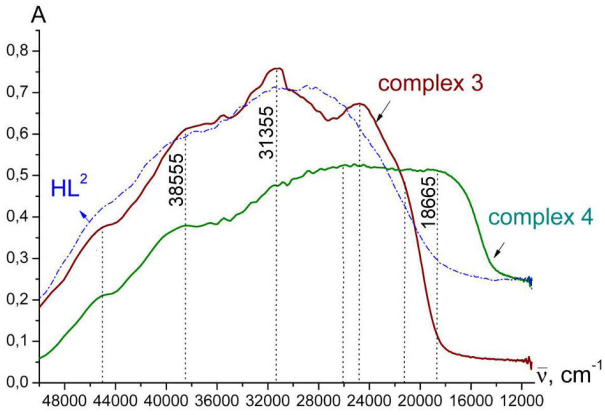

Fig. 6. Diffuse reflection spectra of HL^2 and complexes III and IV

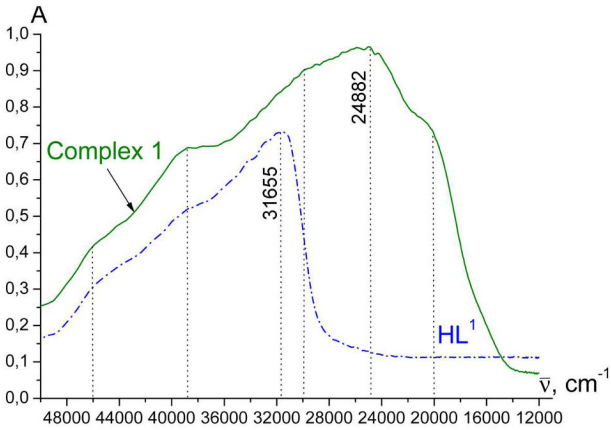

Fig. 7. Diffuse reflection spectra of HL^1 and complex I

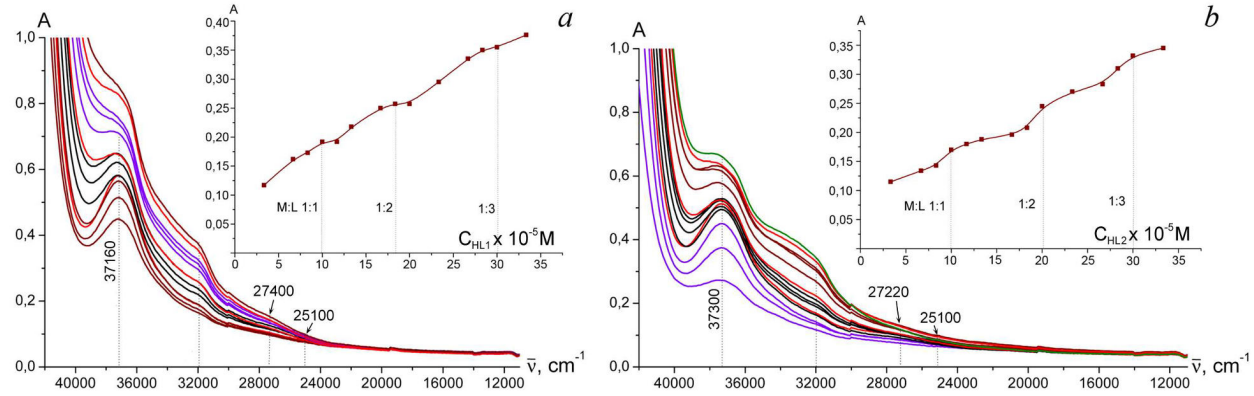

Fig. 8. The UV-Vis spectra and titration curves of complexes I (a), III (b)

complexes (obtained «in situ») contain the absorption bands of intraligand $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ electron transitions (at 37160/38200, 36600/37300/38370, 36600, 31950/32700/32000/32500), LMCT (at 27400/27700/27220/27700/) and dd (at 25100/25700/25100/25900) cm^{-1}. These absorption bands show a hypochromic shift relative to the previous spectra, which is caused by the different nature of the solvent. The results showed that the titration curves have slightly pronounced inflections at the ratio of the components M:L=1:1, 1:2 and 1:3, indicating possible coordination of the ligands in

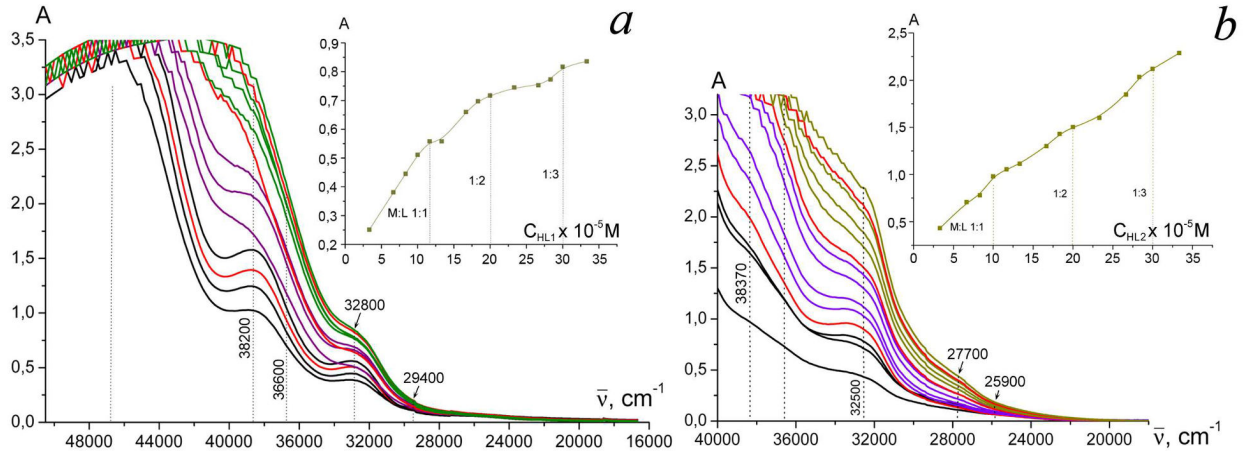

Fig. 9. The UV-Vis spectra and titration curves of complexes II (a), IV (b)

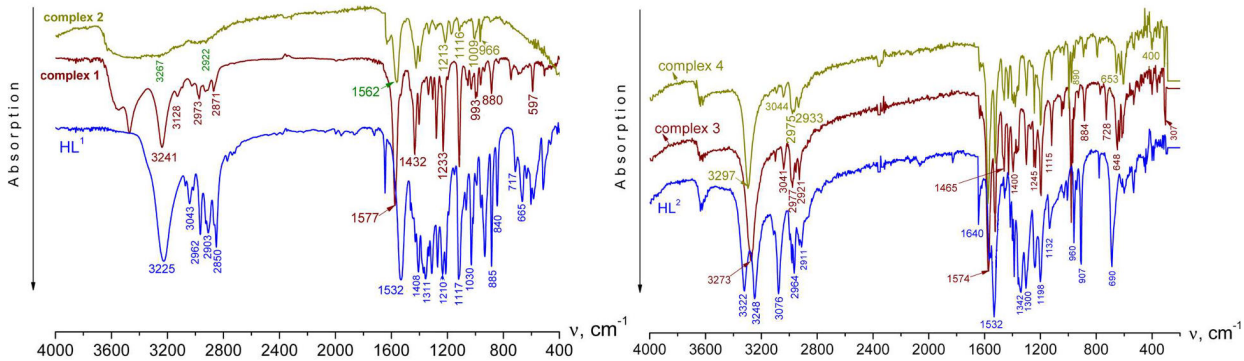

Fig. 10. IR spectra of carbothioamides HL^1, HL^2 and complexes I–IV

solution in both helate and non-chelate ways only through the atoms of sulfur of the carbothioamide group similar to the compounds studied previously [14]. However, complexes with such coordination were not obtained in the solid state, which can be caused by the strong *trans*-influence of the allyl moiety that destabilizes the bond with the «soft» atom in the *trans*-position of coordination polyhedron.

IR spectra of carbothioamides HL^1, HL^2 and complexes I–IV based on them

The assignment of the bands in the IR spectra of allylthioureas and their metal complexes (Fig. 10) was performed at BP86/MCP-TZP level of theory (Tables 2–4). In the high-frequency region of the IR spectra of HL^1 and HL^2, the absorption bands referring to stretching vibrations of ν(NH), $\nu_{as}(CH_2)_{morph}/\nu_{as}(CH_3)_{\textit{tert}\text{-but}}$, $\nu_s(CH_2)_{morph}/\nu_s(CH_3)_{\textit{tert}\text{-but}}$ and $n(CH)_{allyl}$ are present. In the IR spectra of complexes, these bands are shifted to higher frequencies by $\Delta\nu$=+16/42/25/49 cm^{-1} (ν(NH)), +85/45 cm^{-1} ($\nu_{as}(CH_2)_{morph}/\nu_{as}(CH_3)_{\textit{tert}\text{-but}}$) and 11/20 cm^{-1} ($\nu(CH)_{allyl}$), which is caused by the involvement of carbothioamide and allyl moieties in the formation of a coordination bond with a metal ion. In this case, the stretching vibrations of two NH groups in the spectrum of free HL^2 ligand are manifested by two absorption bands, and they merge in the complexes into one intense rather broad absorption band with an offset of 25–50 cm^{-1} (Fig. 10). The corresponding high-frequency shift ($\Delta\nu$=45/30/11/16 cm^{-1}) is also subjected to stretching and bending vibrations $\nu_{as}(NCN)+\delta(NH)$ (Tables 3 and 4). At the same time, the vibrations ν(CS) undergo a low-frequency shift by 72/37 cm^{-1} (Table 4), which is characteristic of the chelate coordination of the carbothioamide group with participation C=S in the formation of a metal cycle.

Cytostatic effect of carbotioamid Pd(II) and Pt(II) π-complexes on the meristem cells of Allium cepa L. compared to cisplatin

Many antitumor agents either directly interact with DNA (doxorubicin, cisplatin, etc.) or with the mitotic apparatus (for example, vinblastine blocks the tubulin and arrests the cellular division in metaphase), leading to various changes and

Table 2

Experimental and calculated frequencies of the main absorption bands in the IR spectra of HL^1 and HL^2 (cm^{-1})

HL^1		HL^2		Assignment (HL^1/HL^2)*
Experimental	Calculated	Experimental	Calculated	
3225	3505	3322, 3248	3493	ν(NH)/ν(NH)
3043	3018	3076	3042	$\nu_{as}(CH_2)_{morph}/\nu_{as}(CH_3)_{tert\text{-}but}$
2962, 2903, 2850	2936, 2915, 2902	2985, 2964, 2926, 2911	2959	$\nu_s(CH_2)$, $\nu(CH)_{allyl}/\nu_s(CH_3)$, $\nu(CH)_{allyl}$
1647	1650	1640	1656	ν(C=C)/ν(C=C)
1532	1534	1563, 1532	1535	$\nu_{as}(NCN)+\delta(NH)/\nu(NCN)+\delta(NH)+\delta_{as}(CH_3)$
1470, 1432	–	1454, 1420	1439	$\delta_s(CH_3)$
1408	1385	1388	1395	$\nu_{as}(SCN)+\omega(CH_2)/\nu_{as}(NCN)+\omega(CH_2)$
1355	1339	1342	1338	$\nu_s(NCN)+\nu(CS)+t(CH_2)/\nu_{as}(NCN)+\delta(NH)+\omega(CH_2)$
1311, 1270	1275	1300, 1237	1300, 1233, 1251	$\nu_{as}(NCN)+t(CH_2)/\nu_s(NCN)+\nu(CS)+\delta(NH)+t(CH_2)$
1236, 1210	1229	1200	1229	$\nu_{as}(NCN)+\nu(CS)+t(CH_2)/\nu(N–C(CH_3)_3)+\nu_s(NCN)+\nu(CS)$
1117	1114	1132	1164	$\nu_{as}(COC)_{morph}/\nu(N–CH_2)+\nu_s(NCN)+\nu(CS)$
1030	1050	1033	1055	$\nu_s(COC)+\nu_s(CNC)_{morph}+\nu(CN)/\nu(CN)+\rho(CH_3)+t(=CH_2)$
961, 935	981, 949	960	968	$\nu(CC)+\nu(CS)$; $\omega(=CH_2)/\nu(N–C(CH_3)_3)+\rho(CH_3)$
885, 840	899	907	949	$\omega(=CH_2)$
717, 665, 603	648, 616	690, 602	680, 597	$\nu_s(COC)+\nu(CS)+\nu_s(CNC)_{morph}/\nu(CS)+\delta(CNC)+t(=CH_2)$
517, 461	–	538, 460	548, 443	$\pi(S–C–N_2)/\pi(NH)$

Note: * ν – stretching, δ – in-plane bending, t – twisting, ω – wagging, ρ – rocking, π – out-of-plane bending as – asymmetric, and s – symmetric.

Table 3

Experimental and calculated frequencies of the main absorption bands in the IR spectra of $Pd(HL^1)Cl_2$ and $Pt(HL^1)Cl_2$ (cm^{-1})

$Pd(HL^1)Cl_2$		$Pt(HL^1)Cl_2$		Assignment
Experimental	Calculated	Experimental	Calculated	
3241	3504	3267	3507	ν(NH)
3128, 3027	3024	3088, 3024	3065, 3034	$\nu_{as}(CH_2)$
2973, 2920, 2871	2936	2965, 2922, 2880	2954, 2903	$\nu_s(CH_2)$
1577	1554	1562	1559	$\nu_{as}(NCN)+\delta(NH)$
1432	1442	1432	1448	$\nu_s(NCN)+\delta(NH)$
1400	1415	1400	1419	$\nu_s(NCN)+\nu(CS)+\omega(CH_2)$
1336	1365	1335	1351	$\nu_s(NCN)+\nu(CS)+\omega(CH_2)$
1277	1298	1290	1288	$\nu_{as}(NCN)+\nu(CN)+t(CH_2)$
1233	1241	1213	1236	$\nu_s(NCN)+\nu(CS)+t(CH_2)$
1110	1115	1116	1116	$\nu_{as}(COC+CNC)_{morph}$
1058	1078	1064	1075	$\nu_{as}(COC)_{morph}+\rho(CH_2)$
1033	1043	1045	1048	$\nu(CN)+\delta(COC, CNC)_{morph}$
993	1002	1009	987	$\nu(CN)+\nu(CS)+\rho(CH_2)$
965	979	968	949	$t(=CH_2)$
880	891	890	886	$\nu_s(COC+CNC)_{morph}+\nu(CS)+\nu(CC)$
750	755	–	–	$t(=CH_2)$
597	599	591	604	$\pi(S–C–N_2)+\delta(COC)_{mor}$

Table 4

Experimental and calculated frequencies of the main absorption bands in the IR spectra of $Pd(HL^2)Cl_2$ and $Pt(HL^2)Cl_2$ (cm^{-1})

$Pd(HL^2)Cl_2$		$Pt(HL^2)Cl_2$		Assignment
Experimental	Calculated	Experimental	Calculated	
3273	3509	3297	3505	ν(NH)
3099, 3041	3049	3094, 3044	3050	$\nu_{as}(CH_3)$
2977, 2951, 2921	2960	2975, 2933	2960	$\nu_s(CH_3)$
1574	1581	1579	1583	$\nu_{as}(NCN)+\delta(NH)$
1530	1539	1529	1545	$\nu_s(NCN)+\nu(CS)+\delta(NH)+\delta_{as}(CH_3)$
1465	1476	1460	1495	$\delta(NH)+\delta(CH_2)$
1433	1447	1433	1434	$\nu_{as}(NCN)+\delta(NH)+\delta_s(CH_3)$
1400	1388	1395	1397	$\nu_{as}(NCN)+\delta(NH)+\delta_s(CH_3)+\omega(CH_2)$
1365	1332	1363	1339	$\nu_s(NCN)+\nu(CS)+\delta(NH)+t(CH_2)$
1246	1246	1245	1257	$\nu_s(NCN)+\nu(CS)+\nu(CC)+t(CH_2)$
1200	1219	1200	1220	$\nu_s(NCN)+\nu(CS)+\nu(CN)+\rho(CH_3)$
1115	1138	1115	1133	$\nu(CS)+\nu(CN)+\delta(NCN)$
981, 970	995, 981, 975	1002, 975	1012	$\nu(CS)+\delta(NCN)+\omega(=CH_2)+\rho(CH_2)$
960	979	957	979	$\rho(CH_3)$
884	898	890	899	$\nu(CS)+\delta(NCN)$
–	–	796	816	$\nu(CS)+t(=CH_2)$
728	741	–	–	$\nu(CS)+t(=CH_2)+\rho(CH_2)$
648	675	653	679	$\nu(CS)+\delta_{ring}$
615	640	610	626	$\pi(S–C–N_2)$
535	540	539	556	$\pi(NH)+\delta_{ring}$

disruptions in daughter cells. The genetic damage manifests as small spherical particles outside the nuclear membrane in the form of micronucleus and/or chromosomal abnormalities in cells.

The effect of cytostatic agents on the interphase cells of apical meristems was evaluated by the state of chromatin. Staining DNA by Feulgen reaction prevents the nonspecific coloring and allows analyzing the concentration and state of DNA by light and fluorescent microscopy. *Allium cepa* cells have 16 chromosomes (2n=16) which are easily stained.

The intensity of specific DNA staining is inversely related to the degree of condensation. The compactly folded heterochromatin of interphase nuclei is transcriptionally inactive and is located predominantly at the periphery of the nucleus. The less condensed chromatin (euchromatin) is transcriptionally active. It is the ratio of hetero- and euchromatin in nuclei that creates the effect of diffuse staining of DNA in nuclei.

Thus, we observed the specific action of studied complex compounds on the chromatin. Palladium complexes I and III induced a relatively increasing ratio of condensed chromatin in interphase cells. That points to the partial inactivation of euchromatin in chromosomes causing certain transcription processes in cells to slow down or stop altogether. Considering that the apical meristem cells of *Allium cepa* were exposed to compounds for four hours and the average time of mitosis in that culture is two to three hours, most cells were exposed to the cytostatic effect in interphase.

The cells, which entered the process of mitosis before the penetration of cytostatics into the cytoplasm, were affected differently. For example, the mitotic tissue index did not decrease significantly in the first hours after exposure to cisplatin. Functional disorders of karyokinesis were detected only in the duration of the phases (Fig. 11). Cisplatin (V) abnormally increased the duration of prophase and telophase. In the condensed state, the intensity of damage of DNA molecules is significantly reduced. However, the ability of cytostatics to interact with proteins of the cytoskeleton still leads to the disruption of the spatial orientation of chromosomes in metaphase. Under the action of cisplatin and its analogue (II), cells in the meta- and anaphase stages almost did not occur. Under normal conditions, the latter are the shortest in the course of the mitotic cycle, being energy consuming and requiring the proper arrangement of cellular structures. Increasing

entropy complicates the transition of cells from the prometaphase to metaphase and further to anaphase.

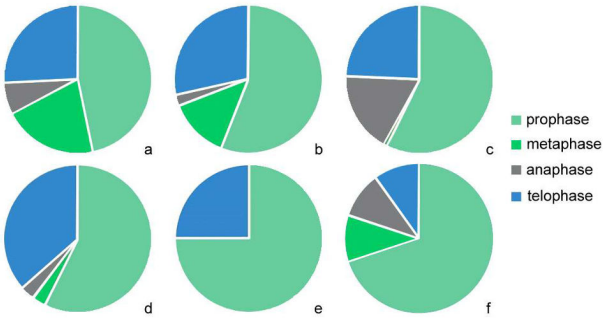

Fig. 11. Effect of cisplatin and its analogues on the duration of phases of mitosis (Allium test): a – control, b–f – compounds I (b), II (c), III (d), V (e), IV (f)

It is known that complex compounds of platinum group [6–8] and some other metals, such as rhenium [15], are capable of forming coordination bonds with the nitrogenous bases of DNA, which allows scientists to develop novel cytostatics. Numerical bonds between molecules cause chromatin dispersion. Dispersion and disruption of heterochromatin under the action of palladium-based preparations was detected in cells during mitosis (Fig. 12).

In anaphase, bridges are formed in the divergence of chromosomes due to the numerical cross-links between the telomeric regions of the daughter chromatids (Fig. 12,a, 12,c). Chromosomes lose their typical morphology, becoming homogeneous masses of proteins and DNA molecules. The proteins of division spindle contract and the chromatin loses its compact supercoiling structure, breaking into fragments and remaining in the form of thin filaments at the division equator. A similar process was observed in cells, in which chromosomes diverged to form a mitotic plate characteristic of k-mitoses. The heterochromatin is separated on the surface of chromosomes, which is evidence of disruption of its ordered structure (Fig. 12,b).

Similar but less pronounced signs of structural abnormalities of chromosomes were found in cells under the action of cisplatin (Fig. 12,e). Using fluorescence microscopy to study prometaphase cells, we found an increase in the fluorescence brightness along the contour of chromosome sections.

Under the influence of compound II, increased condensation of chromatin was observed in the interphase nuclei near the nuclear membrane. The compaction of cytoplasmic proteins was also revealed,

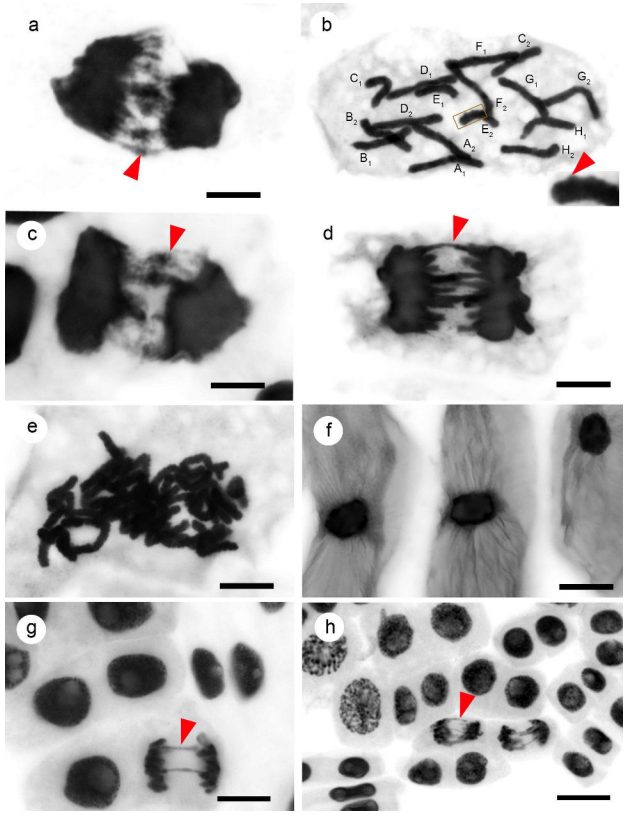

Fig. 12. Disruption of mitosis in *Allium cepa* cells: a, d – formation of numerous bridges with fragmentation of chromosomes at anaphase; b, c – dispersion of chromatin on the surface of chromosomes; d, e – dispersion of the surface of chromosomes and disruption of their spatial orientation in protoplast under the action of cisplatin; f – condensation and dispersion of chromatin in interphase nuclei under the action of platinum-containing cytostatics; arrows indicate signs of chromatin dispersion under the influence of palladium-containing compounds I (a, b) and III (c), formation of bridges under the action of compound IV (g–h); DNA staining by cold hydrolysis according to Feuglen; (inverted image); scale bars: a–e – 10 μm, f–g – 20 μm, h – 30 μm

which was accompanied by an increase in the fluorescence intensity in the red region of the spectrum (605–620 nm) (Fig. 12,f).

The last of the investigated compounds caused minor disturbances in the division of cells that were associated with the formation of numerous bridges (Fig. 12,g, 12,h). The anaphase chromosomes can rupture when they diverge with the formation of micronuclei and consequent loss of a large amount of hereditary information and cell death. However, complex IV had a less pronounced mutagenic effect compared to all other studied preparations. The number of cells with chromosome morphology disorders and anomalies of mitosis did not exceed

0.48−1.0%.

Conclusions

In order to predict the behavior of π-complex compounds I−IV in biological media, their state in solutions was studied by spectral methods. It is shown that the electronic absorption and diffuse reflection spectra of complexes consist of absorption bands corresponding to the transitions with charge transfer from the ligand to the metal, dd-electron transitions and intraligand $\pi\rightarrow\pi^*$ and $n\rightarrow\pi^*$ electronic transitions with the contributions of multiple bonds of allyl moiety and carbothioamide group. Analysis of the titration curves showed the possible coordination of the ligands in solution in both chelate and non-chelate ways only through the atoms of sulfur of the carbothioamide group with molar ratio M:L=1:1, 1:2 and 1:3. The complexes with such coordination are not obtained in solid state, which can be caused by the strong trans-influence of the allyl moiety that destabilizes the bond with the «soft» atom in the *trans* position. Theoretical quantum-chemical calculations of the IR spectra were also carried out. Their results are in good agreement with the experimental data of infrared spectroscopy.

It was found that cisplatin and its platinum and palladium-based analogues I−IV exert a specific cytostatic effect on *Allium cepa* meristem cells with high antiproliferative activity. Due to the formation of numerous cross-links of metal-containing preparations with DNA molecules and with nuclear proteins, chromatin is not compacting as normal. This leads to a disrupted cell cycle, a decreased mitotic index and anomalous cell division, which are usually followed by apoptosis.

Palladium-based complexes cause chromatin dispersion, which leads to significant abnormalities in the processes of karyokinesis. This causes the chromosomes to lose their typical structure and undergo fragmentation, mostly with loss of telomeric sites.

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СПЕКТРАЛЬНІ ХАРАКТЕРИСТИКИ ТА ЦИТОСТАТИЧНИЙ ВПЛИВ КАРБОТІОАМІДНИХ π-КОМПЛЕКСІВ Pd(II) І Pt(II) НА КЛІТИНИ МЕРИСТЕМ *ALLIUM CEPA* L

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*В роботі надано оригінальні результати спектральних характеристик та вперше досліджено механізм цитостатичної та антипроліферативної дії синтезованих аналогів цисплатину на основі π-комплексів Pd(II) і Pt(II) з N-алілморфолін-4-карботіоамідом та 1-аліл-3-*трет*-бутилтіосечовиною на клітини меристем* Allium cepa *L. Показано, що електронні спектри поглинання та спектри дифузного відбиття комплексів складаються зі смуг поглинання, які відповідають переходам з перенесенням заряду з ліганду на метал, dd-електронним переходам та внутрішньолігандним $\pi\rightarrow\pi^*$, $n\rightarrow\pi^*$ електронним переходам з вкладами кратних зв'язків алільного фрагменту та карботіоамідного угрупування. При цьому смуги поглинання в ультрафіолетовій ділянці спектрів комплексів зазнають гіпсохромного зсуву за відношенням до спектрів самих лігандів, що пов'язано з наявністю координаційного зв'язку. Побудовані криві титрування мають слабко виражені перегини при співвідношенні компонентів M:L=1:1, 1:2 та 1:3, що свідчить про можливу координацію лігандів в розчині як хетатним, так і нехелатним способом тільки через атоми сірки карботіоамідної групи. В роботі проведено також і теоретичні квантово-хімічні розрахунки ІЧ-спектрів, що знаходяться в хорошій відповідності з експериментальними даними інфрачервоної спектроскопії. Встановлено, що досліджувані комплекси виявляють специфічну цитостатичну дію на клітини меристем Allium cepa з високою проліферативною активністю. Внаслідок утворення чисельних зшивок металовмісних препаратів з молекулами ДНК, а також ядерними білками унеможливлюється нормальна компактизація хроматину. Це призводить до порушення клітинного циклу, зменшення мітотичного індексу і аномалій поділу клітин, що у подальшому зазвичай завершуються апоптозом. π-комплекси паладію викликають диспергування хроматину, що призводить до значних порушень у процесах каріокінезу, внаслідок яких хромосоми втрачають типову структуру, піддаються фрагментації, частіше з втратою теломерних ділянок.*

Ключові слова: π-комплекси, карботіоаміди, коливальна спектроскопія, протипухлинна активність, генотоксичність.

SPECTRAL CHARACTERISTICS AND CYTOSTATIC EFFECT OF Pd(II) AND Pt(II) CARBOTHIOAMIDE π-COMPLEXES ON *ALLIUM CEPA* L MERISTEM CELLS

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*The spectral characteristics of the synthesized cisplatin analogs based on π-complexes of Pd(II) and Pt(II) with N-allylmorpholine-4-carbothioamide and 1-allyl-3-*tret*-butylthiourea and the mechanism of their cytostatic and antiproliferative action on meristem cells of Allium cepa L. are reported in this work for the first time. It is shown that the electronic absorption and diffuse reflection spectra of complexes show absorption bands corresponding to the transitions with charge transfer from a ligand to metal, dd-electron transitions and intraligand $\pi\rightarrow\pi^*$ and $n\rightarrow\pi^*$ electronic transitions with the contributions of multiple bonds of allyl moiety and carbothioamide group. In this case, the absorption bands in the ultraviolet region of the spectra of the complexes undergo a hypochromic shift with respect to the spectra of the ligands themselves, which is due to the presence of a coordination link. Analysis of the titration curves shows the possible coordination of the ligands in solution in both chelate and non-chelate ways only through the atoms of sulfur of the carbothioamide group with molar ratios M:L=1:1, 1:2 and 1:3. Theoretical quantum-chemical calculations of the IR spectra are carried out; their results are in good agreement with the experimental data of infrared spectroscopy. It is established that cisplatin analogues exhibit a specific cytostatic effect on Allium cepa meristem cells with high antiproliferative activity. Due to the formation of numerous cross-links of metal-containing preparations with DNA molecules and with nuclear proteins, chromatin is not compacting as normal. This results in a disrupted cell cycle, a decreased mitotic index and anomalous cell division, which are usually followed by apoptosis. Palladium-based π-complexes cause chromatin dispersion, which leads to significant abnormalities in the processes of karyokinesis, which cause the chromosomes to lose their typical structure and undergo fragmentation, mostly with loss of telomeric sites.*

Keywords: π-complexes; carbothioamides; vibrational spectroscopy; anticancer activity; genotoxicity.

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