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Solution study under physiological conditions and cytotoxic activity of gold(III) complexes with L-histidine-containing peptides

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Abstract: Proton NMR spectroscopy and cyclic voltammetry were applied to study the stability of three gold(III) complexes with L-histidine-containing peptides, [Au(Gly–L-His–*N*,*N*′,*N*″)Cl]$NO_3 \cdot 1.25H_2O$ (**Au1**), [Au(L-Ala–L-His––*N*,*N*′,*N*″)Cl]$NO_3 \cdot 2.5H_2O$ (**Au2**) and [Au(Gly–Gly–L-His–*N*,*N*′,*N*″,*N*‴)]$Cl \cdot H_2O$ (**Au3**) under physiologically relevant conditions. It was found that tridentate coordination of Gly–L-His and L-Ala–L-His dipeptides, as well as tetradentate coordination of Gly–Gly–L-His tripeptide in **Au1**, **Au2** and **Au3** complexes, respectively, stabilized +3 oxidation state of gold and prevented its reduction to Au(I) and Au(0). No release of the coordinated peptides from Au(III) was observed under these experimental conditions. Considering the remarkable stability of the **Au1**, **Au2** and **Au3** complexes, their cytotoxic activity was evaluated by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay toward five human tumor cell lines, MCF-7 (human breast adenocarcinoma), HT-29 (human colon adenocarcinoma), HeLa (human cervix carcinoma), HL-60 (human promyelocytic leukemia), Raji (human Burkitt's lymphoma) and one human normal cell line MRC-5 (human fetal lung fibroblasts). While the cytotoxic activity of **Au1**, **Au2** and **Au3** against investigated human malignant cell lines was strongly cell line dependent, none of these complexes was cytotoxic against normal MRC-5 cell line. This study can contribute to the future development of gold(III)–peptide complexes as potential antitumor agents.

Keywords: gold(III) complexes; L-histidine-containing peptides; ^{1}H-NMR spectroscopy; cyclic voltammetry; cytotoxic activity.

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INTRODUCTION

Platinum(II) complexes are the most widely used drugs for cancer treatment.[1] However, due to the cross-resistance and severe toxicity, ongoing efforts are aimed at finding novel metal-based drugs that would exhibit comparable or even greater antitumor activity and overcome toxic-side effects produced by platinum(II) complexes.[1] In this context, during the last decades, special attention was devoted to the synthesis and biological evaluation of gold(III) complexes as potential antitumor agents.[2] The initial interest for gold(III) complexes was based on their chemical features, very similar to those of clinically approved platinum(II) complexes, such as square-planar geometry and d^8 electronic configuration of the corresponding metal ion. However, the high reduction potential and fast rate of hydrolysis of Au(III) complexes under physiologically relevant conditions hindered their possible application in medicine.[3] Despite that, stability of the Au(III) ion has been achieved by an appropriate choice of the ligands, in most cases containing nitrogen donor atoms.[4–16] Thus, different polydentate ligands, such as polyamines, bipyridine, terpyridine, phenanthroline, macrocyclic ligands (cyclam), porphyrins and dithiocarbamates, have been used and a number of mononuclear and dinuclear gold(III) complexes have been synthesized that show remarkable stability under physiologically relevant conditions.[4–16] Some of these complexes displayed *in vitro* cytotoxicity comparable or even greater than that of cisplatin toward different human tumor cell lines, but only a few have been proved to be antitumor active *in vivo*.[17–19] Peptides containing the amino acid histidine in the side chain have also been shown to be good chelating agents, being able to coordinate and stabilize the Au(III) ion.[20–22] Although the reactions of gold(III) ion with L-histidine-containing peptides have been extensively investigated,[23] only a few gold(III)–peptide complexes were synthesized and characterized by application of X-ray crystallography: those of glycyl–L-histidine (Gly–L-His), [Au(Gly–L-His–*N*,*N*′,*N*″)Cl]Cl·3H_2O,[20] [Au(Gly–L-His–*N*,*N*′,*N*″)Cl]NO_3·1.25H_2O,[21] [Au(Gly–L-His–*N*,*N*′,*N*″)Cl)]NO_3[21] and [Au(Gly–L-His–*N*,*N*′,*N*″,*N*‴)]$_4$·10H_2O,[20] that of the dipeptide L-alanyl–L-histidine (L-Ala–L-His), [Au(L-Ala–L-His–*N*,*N*′,*N*″)Cl]NO_3·2.5H_2O[21] and of the tripeptide glycyl–glycyl–L-histidine (Gly–Gly–L-His), [Au(Gly–Gly–L-His–*N*,*N*′,*N*″,*N*‴)]Cl·H_2O.[22] However, data related to the biological evaluation of the above mentioned complexes are rather scarce. Hitherto, only the gold(III) complex with Gly–L-His dipeptide has been evaluated toward A2780 human ovarian carcinoma cell line.[24] The results from this investigation showed that the Gly–L-His–Au(III) complex manifested a far higher cytotoxic activity than its Zn(II), Pd(II), Pt(II) and Co(II) analogues, proving that the Au(III) center plays a crucial role in determining pharmacological effects.[24]

The promising cytotoxic activity of the Gly–L-His–Au(III) complex encouraged further investigation of the biological activity of gold(III) complexes

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with different histidine-containing peptides. Hence, the present paper reports the synthesis, spectroscopic characterization, solution study and cytotoxic activity of three gold(III) complexes with L-histidine-containing peptides, [Au(Gly–L-His––*N*,*N*′,*N*″)Cl]NO_3·1.25H_2O (**Au1**), [Au(L-Ala–L-His–*N*,*N*′,*N*″)Cl]NO_3·2.5H_2O (**Au2**) and [Au(Gly–Gly–L-His–*N*,*N*′,*N*″,*N*‴)]Cl·H_2O (**Au3**).

EXPERIMENTAL

Reagents

Distilled water was demineralized and purified to a resistance of greater than 10 MΩ cm^{-1}. Potassium tetrachloridoaurate(III) (K[$AuCl_4$]) and deuterium oxide (99.8 %) were purchased from the Sigma–Aldrich Chemical Co. The peptides glycyl–L-histidine (Gly–L-His), L-alanyl–L-histidine (L-Ala–L-His) and glycyl–glycyl–L-histidine (Gly–Gly–L-His) were obtained from Bachem A.G. Nitric acid, sodium dihydrogen phosphate monohydrate, sodium hydrogen phosphate dihydrate and sodium chloride were obtained from Zorka Pharma. All the employed chemicals were of analytical reagent grade.

Synthesis of the gold(III) complexes

The gold(III) complexes with L-histidine-containing peptides were synthesized by modification of previously described methods.[20-22] A solution of 0.5 mmol of the corresponding peptide (106.1 mg of Gly–L-His, 113.1 mg of L-Ala–L-His and 134.6 mg of Gly–Gly–L-His) in 5.0 mL of water (pH had previously been adjusted to 2.00–2.50 by addition of 1 M HNO_3) was added slowly under stirring to the solution containing an equimolar amount of K[$AuCl_4$] (188.9 mg of K[$AuCl_4$] in 3.0 mL of water). The resulting solution was stirred in the dark in a water bath at 37 °C for at least 5 days. Any colloidal gold formed was removed by filtration and filtrate was left standing in the dark at ambient temperature to evaporate slowly. The yellow crystals of [Au(Gly–L-His–*N*,*N*′,*N*″)Cl]NO_3·1.25H_2O (**Au1**), [Au(L-Ala–L-His––*N*,*N*′,*N*″)Cl]NO_3·2.5H_2O (**Au2**) and [Au(Gly–Gly–L-His–*N*,*N*′,*N*″,*N*‴)]Cl·H_2O (**Au3**) that formed after 3–5 days were filtered off, washed with cold acetone, and dried in the dark at ambient temperature. The yield was 54 % for **Au1** (142.6 mg), 48 % for **Au2** (135.5 mg) and 44 % for **Au3** (113.9 mg). The purity of the complexes was checked by elemental microanalysis and ^{1}H-NMR spectroscopy. These data were all found to be in accordance with those reported previously for the corresponding Au(III) complexes.[21,22]

Measurements

Elemental microanalyses. Elemental microanalyses for carbon, hydrogen and nitrogen of the synthesized gold(III)–peptide complexes were performed by the Microanalytical Laboratory, Faculty of Chemistry, University of Belgrade.

*[Au(Gly–L-His–*N,N′,N″*)Cl]NO_3·1.25H_2O (**Au1**)*. Anal. Calcd. for $C_8H_{13.50}AuClN_5O_{7.25}$ (FW: 528.15): C, 18.19; H, 2.58; N, 13.26 %. Found: C, 18.45; H, 2.48; N, 13.09 %.

*[Au(L-Ala–L-His–*N,N′,N″*)Cl]NO_3·2.5H_2O (**Au2**)*. Anal. Calcd. for $C_9H_{18}AuClN_5O_{8.50}$ (FW: 564.70): C, 19.14; H, 3.21; N, 12.40 %. Found: C, 19.02; H, 3.29; N, 12.24 %.

*[Au(Gly–Gly–L-His–*N,N′,N″,N‴*)]Cl·H_2O (**Au3**)*. Anal. Calcd. for $C_{10}H_{15}AuClN_5O_5$ (FW: 517.68): C, 23.20; H, 2.92; N, 13.53 %. Found: C, 23.43; H, 2.58; N, 13.34 %.

^{1}H-NMR spectroscopy. The ^{1}H-NMR spectra of **Au1**, **Au2** and **Au3** were recorded on a Varian Gemini 2000 spectrometer (200 MHz) using 5-mm NMR tubes. Sodium trimethylsilylpropane-3-sulfonate (TSP, δ = 0.00 ppm) was used as an internal reference. All samples were prepared in 50 mM phosphate buffer at pH 7.40 in D_2O, containing 4 mM NaCl. The

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concentration of the final solution was 10 mM in each complex and the total volume was 600 μL. The NMR spectra were processed using Varian VNMR software (version 6.1, revision C). The chemical shifts are reported in parts per million (ppm).

Cyclic voltammetry. Cyclic voltammetric (CV) measurements were performed with an Autolab potentiostat (PGSTAT 302N). The working electrode for the cyclic voltammetric measurements was glassy carbon (GC) with a 3 mm inner and 9 mm outer diameter PTFE sleeve. Prior to use, the GC electrode was wet-polished on an Alpha A polishing cloth (Mark V Lab) with small particles of alumina (0.05 μm diameter). The electrode was washed twice with doubly-distilled water and then with the background electrolyte solution. The washed electrode was then placed into the voltammetric cell with supporting electrolyte solution. The reference electrode was a saturated calomel electrode (SCE) type 401 (Radiometer, Copenhagen) and the counter electrode was a platinum wire.

The supporting electrolyte used to perform the cyclic voltammetric experiments was 50 mM phosphate buffer containing 4 mM NaCl at pH 7.40. Cyclic voltammograms of **Au1**, **Au2** and **Au3** were recorded immediately after dissolving of the corresponding complex in phosphate buffer, as well as after 24 and 48 h of incubation in a water bath at 37 °C. The concentration of the gold(III) complexes in the final solutions was 5×10^{-4} M. The conditions were the following: E_{begin} = –1.0 V and E_{end} = 1.5 V, as well as E_{begin} = 0.0 V and E_{end} = 1.5 V; E_{step} = = 0.003 V at a scan rate of 0.070 V s^{-1}. All experiments were repeated at least three times. The data were collected and analyzed using the Origin 8 program.

pH measurements. All pH measurements were made at ambient temperature (25 °C). The pH meter (Iskra MA 5704) was calibrated with a Fischer certified buffer solutions of pH 4.00 and 7.00. The results were not corrected for the deuterium isotope effect.

Cytotoxicity studies

Cell lines. The cell lines used in the study were MCF-7 (human breast adenocarcinoma, American Type Culture Collection, ATCC, HTB 22), HT-29 (human colon adenocarcinoma, ATCC, HTB 38), HeLa (human cervix carcinoma, ATCC, CCL 2), HL-60 (human promyelocytic leukemia, ATCC, CCL 240), Raji (human Burkitt's lymphoma, ATCC, CCL 86), and MRC-5 (human fetal lung fibroblasts, ATCC, CCL 171). The cells were grown in RPMI 1640 medium (HL-60 and Raji) or Dulbecco's modified Eagle's medium (DMEM) with 4.5 % of glucose (MCF-7, HeLa, HT-29 and MRC-5), supplemented with 10 % of fetal calf serum (FCS, NIVNS) and antibiotics: 100 IU mL^{-1} penicillin and 100 μg mL^{-1} streptomycin (ICN Galenika). All cell lines were cultured in flasks (Sarstedt, 25 cm^2) at 37 °C under a 100 % humidity atmosphere and 5 % of CO_2. The cells were sub-cultured twice a week and a single cell suspension of adherent cells was obtained using 0.25 % trypsin in EDTA (Serva). Exponentially growing cells were used throughout the assays. The cell density (number of cells per unit volume) and percentage of viable cells were determined using the dye exclusion test by trypan blue. The viability of cells used in the assay was over 95 %.

MTT assay. Cytotoxicity was evaluated by the tetrazolium colorimetric MTT assay (Sigma). The assay is based on the cleavage of the tetrazolium salt MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to formazan by mitochondrial dehydrogenases in viable cells. Gold(III) complexes, **Au1**, **Au2** and **Au3**, were dissolved in water at pH 7.00. Cells were plated into 96-well microtiter plates (Costar) in a volume of 90 μL per well, in the complete medium at the optimal seeding density of 10^4 (Raji and HL-60 cells) or 5×10^3 cells (adherent cells) per well to assure a logarithmic growth rate throughout the assay period. Tested substances at concentration ranging from 10^{-8} to 10^{-4} M were added to all wells except to the control ones. Plates were incubated at 37 °C for 48 h. Three hours before the end of

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incubation period, 10 μL of MTT solution (5 mg mL^{-1}) was added to all wells and the plates were incubated for 3 h at 37 °C, after which, the medium and MTT were removed by suction. The formazan product was then solubilized in acidified 2-propanol (0.04 M HCl was added in 2-propanol). After a few minutes at room temperature, the plates were read on a spectrophotometer plate reader (Multiscan MCC340, Labsystems) at 540/690 nm. The wells without cells containing complete medium and MTT only acted as the blank.

The cytotoxicity was expressed as a percent and calculated according the formula:

$$\% C = (1 - A_{test}/A_{control}) \times 100$$

where A_{test} is the absorbance of the treated cells and $A_{control}$ is the absorbance of the control (untreated) cells.

Data analysis. Two independent experiments were set up with quadruplicate wells for each concentration of the complex. The IC_{50} value defines the dose of the complex that inhibits cell growth by 50 %. The IC_{50} of the complexes was determined by median effect analysis.[25]

RESULTS AND DISCUSSION

Three gold(III) complexes with L-histidine-containing peptides:
[Au(Gly–L-His–*N*,*N*′,*N*″)Cl]$NO_3 \cdot 1.25H_2O$ (**Au1**),
[Au(L-Ala–L-His–*N*,*N*′,*N*″)Cl]$NO_3 \cdot 2.5H_2O$ (**Au2**) and
[Au(Gly–Gly–L-His–*N*,*N*′,*N*″,*N*‴)]$Cl \cdot H_2O$ (**Au3**) (Fig. 1),
were synthesized by modification of the previously described methods.[20–22] The square-planar geometry of these complexes was confirmed by comparison of their ^{1}H-NMR data with those previously reported in the literature.[21,22] The obtained spectroscopic data of **Au1** and **Au2** complexes are in accordance with tridentate coordination of the Gly–L-His and L-Ala–L-His dipeptides to gold(III), respectively. This coordination occurred through the amino group of the glycyl/ /alanyl residue, the deprotonated peptide nitrogen, the imidazole N3 nitrogen and a chloride ion in the fourth coordination site. However, in **Au3** complex, the tripeptide Gly–Gly–L-His acts as a tetradentate ligand coordinating to the Au(III) ion through the terminal amino group, two deprotonated peptide nitrogens, and the N3 imidazole nitrogen. The solution behaviors of the **Au1**, **Au2** and **Au3** complexes were investigated under physiologically relevant conditions (50 mM phosphate buffer containing 4 mM NaCl at pH 7.40 and 37 °C) by ^{1}H-NMR spectroscopy and cyclic voltammetry (CV). The cytotoxic activity of these complexes was evaluated toward five human tumor cell lines, MCF-7 (human breast adenocarcinoma), HT-29 (human colon adenocarcinoma), HeLa (human cervix carcinoma), HL-60 (human promyelocytic leukemia), Raji (human Burkitt's lymphoma) and one human normal cell line MRC-5 (human fetal lung fibroblasts).

Solution study of the gold(III)–peptide complexes

An essential prerequisite for biological evaluation of metal complexes as cytotoxic agents is their sufficient stability in solution under physiologically relevant conditions. In order to investigate the stability of the gold(III)–peptide

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complexes within a physiological environment, **Au1**, **Au2** and **Au3** were dissolved in 50 mM phosphate buffer containing 4 mM NaCl at pH 7.40, and their ^{1}H-NMR spectra and cyclic voltammograms were recorded after dissolution, as well as after 24 and 48 h of incubation in a water bath at 37 °C.

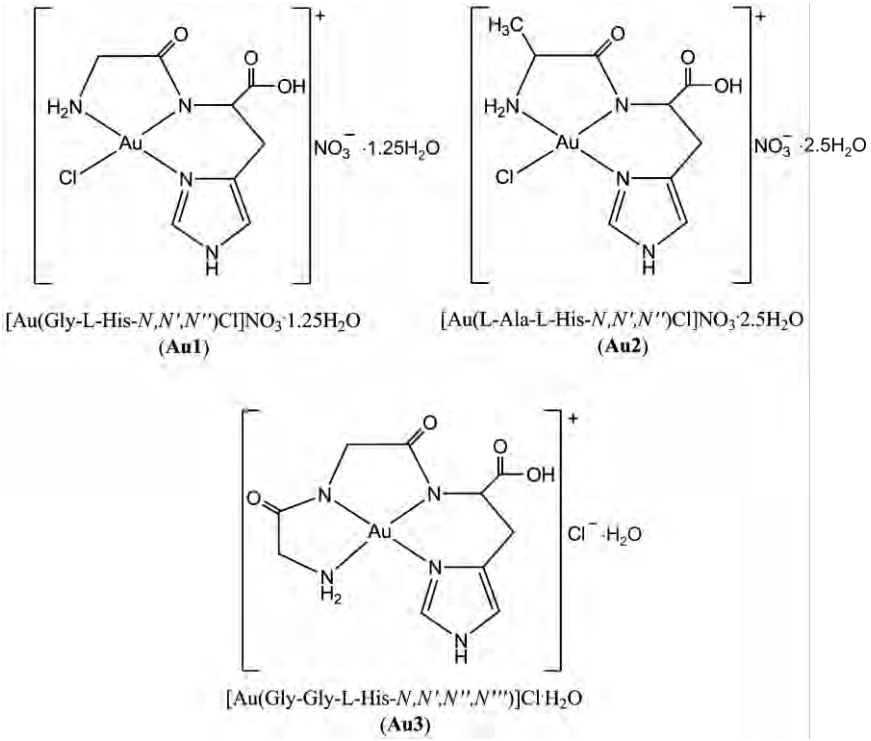

Fig. 1. Schematic drawings of the gold(III) complexes with L-histidine-containing peptides used in the present study.

^{1}H-NMR spectroscopy. When **Au1** and **Au2** complexes were dissolved in 50 mM phosphate buffer solution at pH 7.40, along with two singlets for the C2H and C5H imidazole protons of the corresponding monomeric gold(III)–peptide complex, four new resonances for these protons appeared in the ^{1}H-NMR spectrum (Table I and Fig. 2). This undoubtedly confirms that some changes in the structure of monomeric **Au1** and **Au2** complexes occurred under these experimental conditions. It is assumed that these changes resulted from the formation of the Au(III)–imidazole bridged terameric [Au(Gly–L-His–*N*,*N*′,*N*″,*N*‴)]$_4$ and [Au(L-Ala–L-His–*N*,*N*′,*N*″,*N*‴)]$_4$ complexes. The ^{1}H-NMR spectra over time showed that the intensities of the singlets due to the C2H and C5H imidazole protons of the monomeric **Au1** and **Au2** complexes decreased, while those for the observed imidazole protons of the corresponding tetrameric complexes

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enhanced. The formation of the tetrameric [Au(Gly–L-His–*N*,*N*′,*N*″,*N*‴)]$_4$ and [Au(L-Ala–L-His–*N*,*N*′,*N*″,*N*‴)]$_4$ complexes is in accord with previous finding by Messori *et al.* for the solution behavior of the Gly–L-His–Au(III) complex with chloride as the counter-anion under physiologically relevant conditions.[24] Moreover, the tetrameric [Au(Gly–L-His–*N*,*N*′,*N*″,*N*‴)]$_4$·10H_2O complex was obtained by Lippert *et al.* by dissolving the monomeric [Au(Gly–L-His–*N*,*N*′,*N*″)Cl]Cl·3H_2O complex in water and then adjusting the pH to 6.00–7.00.[20] The crystallographic results for the latter tetrameric complex[20] showed that each Au(III) ion was coordinated by three nitrogen atoms as in the monomeric complex (amino, amidate and N3 imidazole nitrogens) and in addition to the N1 nitrogen of the deprotonated imidazole of the second monomeric unit. It was found that tetramerization of the **Au2** complex was slower in comparison to the same process of **Au1** (Fig. 2). Thus, after 24 h of incubation at 37 °C, the tetramerization of the **Au1** was complete, while the tetrameric [Au(L-Ala–L-His–*N*,*N*′,*N*″,*N*‴)]$_4$ complex was obtained in a yield of 55 % during this time. The difference in the rate of the tetramerization process of **Au1** and **Au2** complexes could be attributed to the effects of the electron donating methyl group of the L-alanyl residue, which decreased the acidity of the gold(III) center.

TABLE I. Proton NMR chemical shifts (δ / ppm) for the monomeric **Au1**, **Au2** and **Au3** and the corresponding tetrameric complexes in 50 mM phosphate buffer containing 4 mM NaCl at pH 7.40 and 37 °C (the chemical shifts are in accordance with those previously reported for gold(III) complexes with L-histidine-containing peptides characterized by X-ray analysis[20-22])

Gold(III)–peptide complex	Imidazole protons		Gly1-CH_2	Gly2-CH_2	Alaα-CH	Alaβ-CH_3	Hisα-CH	Hisβ-CH	Hisβ′-CH
	C2H	C5H							
[Au(Gly–L-His–*N*,*N*′,*N*″)Cl]NO_3·1.25H_2O (**Au1**)	8.55	7.27	4.00	–	–	–	4.45	3.02	3.50
[Au(Gly–L-His–*N*,*N*′,*N*″,*N*‴)]$_4$	7.39; 7.27	7.13; 6.71	4.01	–	–	–	4.39	3.10	3.56
[Au(L-Ala–L-His–*N*,*N*′,*N*″)Cl]NO_3·2.5H_2O (**Au2**)	8.52	7.24	–	–	4.13	1.55	4.43	3.04	3.50
[Au(L-Ala–L-His–*N*,*N*′,*N*″,*N*‴)]$_4$	7.39; 7.20	7.10; 6.75	–	–	4.14	1.54	4.37	3.11	3.55
[Au(Gly–Gly–L-His–*N*,*N*′,*N*″,*N*‴)]Cl·H_2O (**Au3**)[a]	8.26	7.25	4.15	4.29	–	–	4.52	3.00	3.50

[a]No terameric species was observed in solution

On the other hand, only one monomeric species was observed in the buffered solution of the **Au3** complex (Table I). The singlets at 8.26 and 7.25 ppm were assigned to the C2H and C5H imidazole protons of the tetradentate coordinated Gly–Gly–L-His tripeptide, respectively. The absence of formation of Au(III)–imidazole bridged species in the case of **Au3** complex is in accordance with the

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fact that this complex has no monodentate coordinated ligand (H_2O, Cl^- or NO_3^-), like **Au1** and **Au2**, which could be replaced by the deprotonated N1 imidazole nitrogen atom of the second monomeric unit.

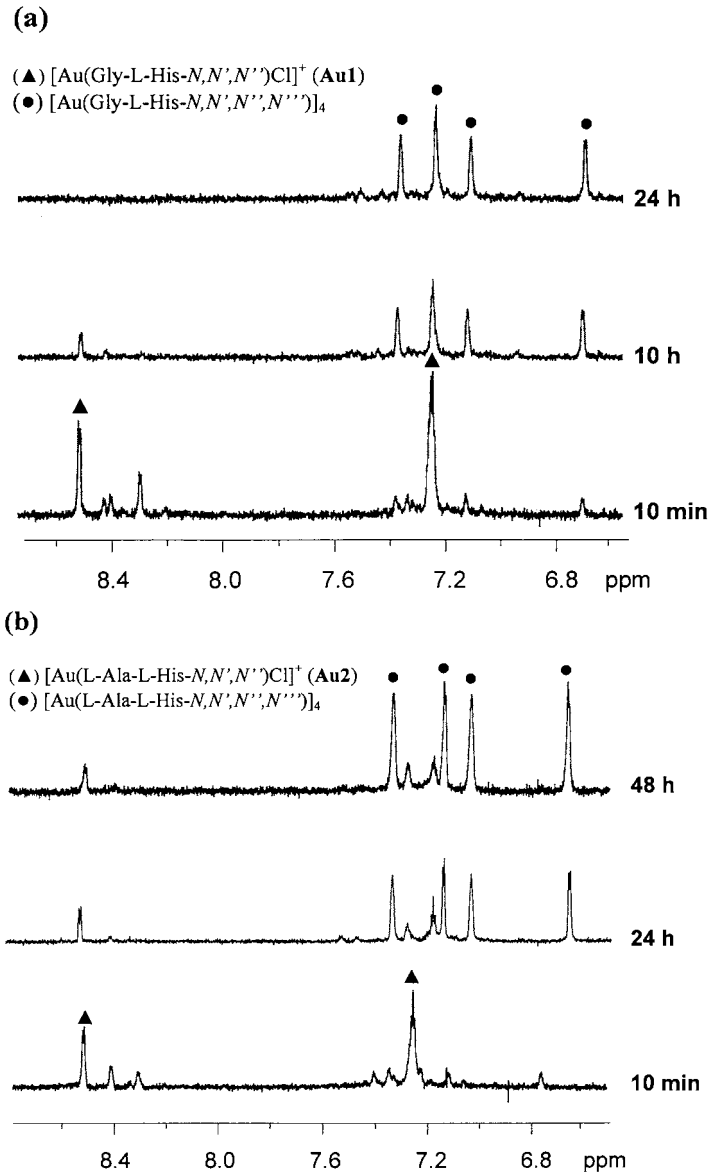

Fig. 2. Proton NMR spectra in the imidazole region (C2H and C5H protons) showing the changes in concentration of monomeric **Au1** (a) and **Au2** (b) and corresponding tetrameric complexes during time. All spectra were recorded in 50 mM phosphate buffer containing 4 mM NaCl at pH 7.40 and 37 °C.

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Finally, from the above ^{1}H-NMR investigation, it could be concluded that **Au1, Au2** and **Au3** complexes are quite stable in solution with no release of the coordinated peptide from Au(III) under physiological conditions.

Cyclic voltammetry. When the cyclic voltammograms of **Au1**, **Au2** and **Au3** complexes were recorded at a GC electrode in 50 mM phosphate buffer containing 4 mM NaCl at pH 7.40, in potential region 0.0–1.5 V, no characteristic voltammetric peaks were observed (Fig. 3). These measurements were repeated every 2 h and no change in the cyclic voltammograms for the investigated gold(III) complexes were observed during 48 h. The cyclic voltammograms of these three gold(III)–peptide complexes were completely identical and, consequently, only the cyclic voltammogram of the **Au1** complex is shown in Fig. 3. For comparison, the cyclic voltammogram of the $[AuCl_4]^-$ complex is given in the same figure. In contrast to the investigated gold(III)–peptide complexes, the $[AuCl_4]^-$ complex displayed a distinct cathodic peak at 0.32 V, corresponding to gold(III) reduction ($[AuCl_4]^- + 3e^- \rightarrow Au(0) + 4Cl^-$).[26] On the reverse sweep, a definite oxidation wave at 1.02 V corresponding to metallic gold oxidation was observed: $Au(0) + 4Cl^- \rightarrow [AuCl_4]^- + 3e^-$.[26] The inactive nature of gold(III) metal center was also previously found for [Au(*para*-Y-TPP)]Cl complexes (TPP is *meso*-tetraphenylporphyrin and Y = Cl, Br, H, Me, MeO)[27] and gold(III)

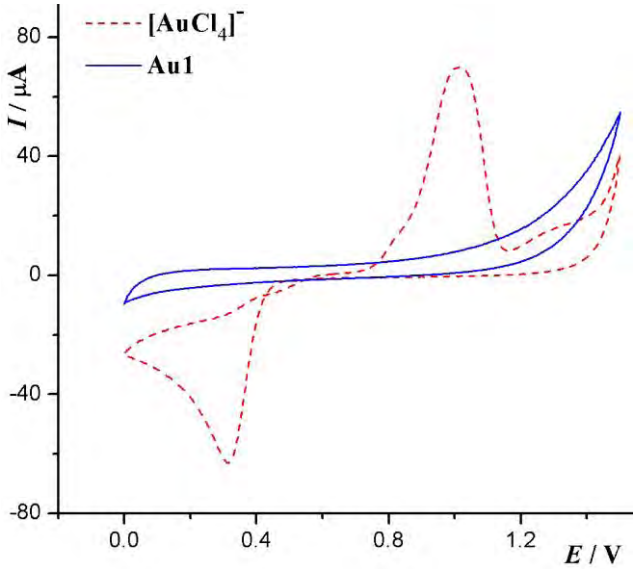

Fig. 3. Cyclic voltammogram of the [Au(Gly–L-His–*N*,*N*′,*N*″)Cl]$^+$ (**Au1**) complex recorded at a GC electrode in 50 mM phosphate buffer containing 4 mM NaCl as background electrolyte at pH 7.40 and 37 °C, E_{begin} = 0.0 V, E_{end} = 1.5 V, E_{step} = 0.003 V at a scan rate of 0.070 V s^{-1}. For comparison, the cyclic voltammogram of the $[AuCl_4]^-$ complex recorded at a GC electrode in 10 mM HCl containing 40 mM NaCl is shown.[26]

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complexes containing various tridentate cyclometalating ligands derived from 6-phenyl-2,2′-bipyridine and alkynyl ligands.[28]

Based on the obtained results, it could be concluded that the gold remained in the +3-oxidation state in the **Au1**, **Au2** and **Au3** complexes under physiologically relevant conditions. Owing to the electron-donating ability and the chelate effect of the peptide moiety,[29] tridentate coordination of Gly–L-His and L-Ala–L-His dipeptides, as well as tetradentate coordination of Gly–Gly–L-His tripeptide contributed to the complete stabilization of the +3-oxidation state of gold against reduction.

Cytotoxic activity of the gold(III)–peptide complexes

In the second part of this study, the cytotoxic activity of the **Au1, Au2** and **Au3** complexes was evaluated against human leukemia (HL-60), lymphoma (Raji), three different human carcinoma cell lines (MCF-7, breast adenocarcinoma, HT-29, colon adenocarcinoma, and HeLa, cervix carcinoma) and one human normal cell line (MRC-5, human fetal lung fibroblasts). The experiments were performed by the tetrazolium MTT assay and cells were exposed for 48 h. The IC_{50} values (μM) of the **Au1**, **Au2** and **Au3** complexes are given in Table II, while the cytotoxic curves from MTT assay showing sensitivity of HeLa and HL-60 cells as percentage of cytotoxicity for 48 h in the presence of increasing concentrations of gold(III)–peptide complexes are presented in Fig. 4.

None of the presently investigated gold(III)–peptide complexes was cytotoxic against normal fetal lung fibroblasts (MRC-5), whereas cisplatin (*cis*-$[PtCl_2(NH_3)_2]$) was very toxic to these cells (Table II). Breast (MCF-7) and colon (HT-29) adenocarcinoma cells were moderately sensitive only to the **Au1** complex (Table II). In comparison with previously reported literature data,[6] the **Au1** complex (IC_{50} = 19.68±0.23 μM) was found to be more active toward the MCF-7 cell line than $[Au(bipy^{dmb}\text{-}H)(OH)][PF_6]$ (IC_{50} = 35.30±8.80 μM) and $K[Au(pz^{Ph}\text{-}H)Cl_3]$ (IC_{50} = 33.70±2.15 μM), but less active than $[Au(bipy^{dmb}\text{-}H)(2,6\text{-xylidine-}H)][PF_6]$ (IC_{50} = 5.20±0.40 μM), where $bipy^{dmb}$ is 6-(1,1-dimethylbenzyl)-2,2′-bipyridine and pz^{Ph} is 1-phenylpyrazole. Furthermore, the **Au1** complex (IC_{50} = 14.70±1.36 μM) was more active toward HT-29 cells than $[AuX_2(damp)]$ (damp is 2-[(dimethylamino)methyl]phenyl, and X = = chloride, thiocyanate, acetate, oxalate and malonate).[30]

All the investigated gold(III)–peptide complexes displayed cytotoxic activity toward cervix carcinoma (HeLa) cells (Table II and Fig. 4a). The HeLa cells were highly sensitive to the **Au3** complex, and moderately sensitive to the **Au1** and **Au2**. Thus, the **Au3** complex (IC_{50} = 0.0045±0.0002 μM) was found to be 3.5×10^4, 7.8×10^4 and 4.5×10^2-fold more active than **Au1** (IC_{50} = 15.90±1.69 μM), **Au2** (IC_{50} = 35.14±1.08 μM) and cisplatin (IC_{50} = 2.02±0.12 μM), respectively. Moreover, the **Au3** complex manifested a far higher cytotoxic activity toward HeLa cell line than previously reported gold(III) complexes con-

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taining dithiocarbamate ligands, [Au(dmdt)X_2] and [Au(esdt)]X_2] (dmdt is *N*,*N*--dimethyldithiocarbamate, esdt is ethyl *N*-(dithiacarboxy)-*N*-methylglycinate, and X is chloride or bromide), with IC_{50} values falling in the range from 2.10 to 8.20 μM.[9]

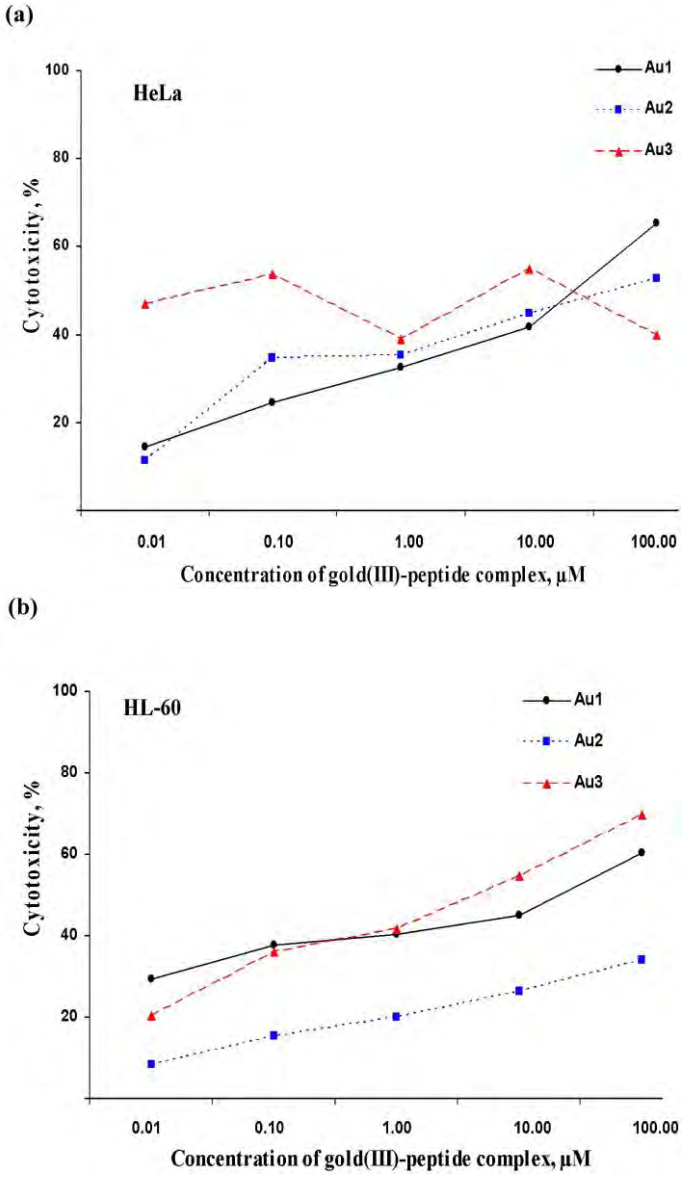

Fig. 4. Sensitivity of HeLa (a) and HL-60 (b) cells to the **Au1**, **Au2** and **Au3** complexes in the MTT assay; the results are presented as percentage of cytotoxicity and represent mean values of two independent assays, each performed in quadruplicate.

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TABLE II. *In vitro* growth inhibition (IC_{50} / μM) of human normal (MRC-5) and human tumor cell lines (MCF-7, HT-29, HeLa, HL-60 and Raji) by **Au1**, **Au2** and **Au3** after a 48 h complex exposure. Cisplatin (*cis*-$[PtCl_2(NH_3)_2]$) is reported as a reference complex

Complex	IC_{50}±*SD* / μM					
	MRC-5[a]	MCF-7	HT-29	HeLa	HL-60	Raji
Au1	>100.0	19.68±0.23	14.70±1.36	15.90±1.69	11.93±1.02	3.30±0.02
Au2	>100.0	>100.0	>100.0	35.14±1.08	>100.0	>100.0
Au3	>100.0	92.31±7.78	>100.0	0.0045±0.0002	2.98±0.12	12.04±1.35
Cisplatin	0.48±0.02	1.56±0.26	18.6±2.32	2.02±0.12	10.31±2.54	2.25±0.10

[a]MRC-5 is human fetal lung fibroblasts, MCF-7 is human breast adenocarcinoma; HT-29 is human colon adenocarcinoma; HeLa is human cervix carcinoma; HL-60 is human promyelocytic leukemia; Raji is human Burkitt's lymphoma

The **Au1** and **Au3** complexes produced strong inhibition of the *in vitro* growth of hematopoietic cell lines, HL-60 and Raji (Table II and Fig. 4b). The **Au3** complex was 4-fold more active against HL-60 (IC_{50} = 2.98±0.12 μM) in comparison to the Raji cells (IC_{50} = 12.04±1.35 μM), while **Au1** was 3.6-fold more active against Raji cells (IC_{50} = 3.30±0.02 μM) in comparison to HL-60 cells (IC_{50} = 11.93±1.02 μM) (Table II). However, the gold(III)–peptide complexes were less active toward HL-60 cells than $[Au(dmdt)X_2]$ and $[Au(esdt)]X_2$].[9]

CONCLUSIONS

Based on the above results, it could be concluded that the tridentate coordination of Gly–L-His and L-Ala–L-His dipeptides, as well as the tetradentate coordination of the Gly–Gly–L-His tripeptide in the **Au1, Au2** and **Au3** complexes, respectively, stabilized Au(III) ion and prevented its reduction to Au(I) and Au(0) under physiologically relevant conditions. Moreover, no release of the coordinated peptides from Au(III) was observed under these experimental conditions. Given the appreciable stability of **Au1, Au2** and **Au3** complexes under physiological conditions, their cytotoxic activity toward one human normal and five human tumor cell lines was evaluated. The obtained results show that the cytotoxic activity of the gold(III)–peptide complexes is strongly cell line dependent. None of the gold(III)–peptide complexes was cytotoxic against normal fetal lung fibroblasts (MRC-5). The **Au1** complex was active against all the investigated human malignant cell lines. On the other hand, **Au2** and **Au3** complexes show different cytotoxic activity regarding the type of cell line. The **Au3** complex manifested a far higher cytotoxic activity toward the HeLa cell line than cisplatin, making this complex a good candidate for further pharmacological evaluation and *in vivo* testing. The results presented in this article could contribute to the development of new gold(III) complexes as potential antitumor agents and could be important in relation to the severe toxicity of gold-based drugs.

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ИЗВОД

ИСПИТИВАЊЕ ПОНАШАЊА У РАСТВОРУ ПРИ ФИЗИОЛОШКИМ УСЛОВИМА И ЦИТОТОКСИЧНА АКТИВНОСТ КОМПЛЕКСА ЗЛАТА(III) СА ПЕПТИДИМА КОЈИ САДРЖЕ L-ХИСТИДИН

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Применом ^{1}H-NMR спектроскопије и цикличне волтаметрије испитивано је понашање у раствору при физиолошким условима комплекса злата(III) са пептидима који садрже аминокиселину L-хистидин, [Au(Gly–L-His–*N*,*N*′,*N*″)Cl]$NO_3 \cdot 1.25H_2O$ (**Au1**), [Au(L-Ala–L-His–*N*,*N*′,*N*″)Cl]$NO_3 \cdot 2.5H_2O$ (**Au2**) и [Au(Gly–Gly–L-His–*N*,*N*′,*N*″,*N*‴)]$Cl \cdot H_2O$ (**Au3**). На основу добијених спектроскопских и електрохемијских података, закључено је да тридентатна координација Gly–L-His и L-Ala–L-His дипептида у **Au1** и **Au2** комплексима, као и тетрадентатна координација Gly–Gly–L-His трипептида у **Au3** комплексу, стабилизује +3 оксидационо стање злата и спречава његову редукцију до Au(I) и елементарног злата, Au(0). Имајући у виду значајну стабилност **Au1**, **Au2** и **Au3** комплекса у раствору при физиолошким условима, применом MTT теста, испитивана је њихова цитотоксична активност на пет туморских ћелијских линија, MCF-7 (хумани карцином дојке), HT-29 (хумани карцином колона), HeLa (хумани карцином грлића материце), HL-60 (хумана промијелоцитна леукемија), Raji (хумани Беркитов лимфом) и на једној здравој ћелијској линији MRC-5 (хумани фибропласти плућа). Нађено је да цитотоксична активност **Au1**, **Au2** и **Au3** комплекса зависи од врсте туморске ћелијске линије и да ниједан комплекс злата(III) није активан према здравој MRC-5 ћелијској линији. Ови резултати су од значаја за синтезу нових комплекса злата(III) који се потенцијално могу применити као антитуморски агенси.

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