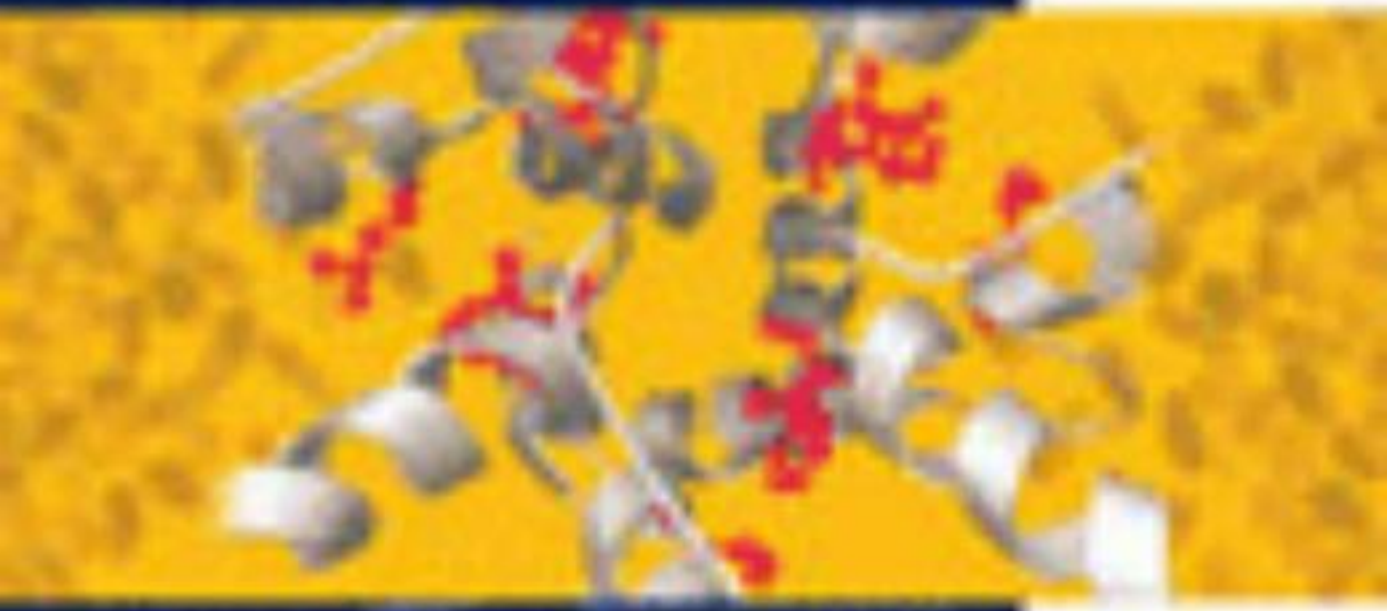
Volume 1 Number 1

Medicinal Chemistry



Cytotoxicity of gold(III) Complexes on A549 Human Lung Carcinoma Epithelial Cell Line

Miloš Arsenijević[1,*], Marija Milovanovic[1], Vladislav Volarevic[1], Ana Djeković[2], Tatjana Kanjevac[1], Nebojša Arsenijević[1] Svetlana Đukić[1] and Živadin D. Bugarčić[2]

[1]*Faculty of Medicine, University of Kragujevac, 69 Svetozara Markovica Street, 34000 Kragujevac, Serbia*

[2]*Faculty of Science, Department of Chemistry, R. Domanovica 12, P. O. Box 60, 34000 Kragujevac, Serbia*

Abstract: We have studied the kinetics of the complex formation of gold(III) complexes, $[Au(en)Cl_2]^+$ (dichlorido(ethylendiamine)aurate(III)-ion) $[Au(dach)Cl_2]$ (dichloride(1,2-diaminocyclohexane)aurate(III)-ion) and $[Au(bipy)Cl_2]^+$ (dichlorido(2,2'-bipyridyl)aurate(III)-ion) with guanosine5'-monophosphate (5'-GMP). It was shown that 5'-GMP have a high affinity for gold(III) complex, which may have important biological implications, since the interactions of Au(III) with DNA are thought to be responsible for the anti-tumor activity. The $[Au(bipy)Cl_2]^+$ complex is more reactive than $[Au(en)Cl_2]^+$ or $[Au(dach)Cl_2]^+$. The activation parameters for all studied reactions suggest an associative substitution mechanism. The cytotoxicity of gold(III) complexes was tested on A549 human lung carcinoma epithelial cell line and was evaluated by cytotoxic (MTT and LDH test) and apoptotic assays. The results showed that all tested gold(III) complexes displayed cytotoxic effect on A549 cells. Among the tested gold (III) complexes, AuBIPY showed the best cytotoxic effects.

Keywords: gold(III), complexes, DNA, kinetics, cytotoxic, A549 human lung carcinoma epithelial cell line.

INTRODUCTION

In recent years, great interest has been focused on gold(III) complexes as cytotoxic and anticancer drug [1-2]. To design a metal-based applicable anticancer drug, however, is quite challenging. Presently, platinum drugs are playing a major role in established medical treatments of cancer [3-4]. Gold(III) complexes are square-planar d^8, isoelectronic and isostructural to Pt(II) complexes. Generally speaking, gold(III) complexes are not very stable under physiological conditions because of their high reduction potential and fast hydrolysis rate. Therefore, selection of a suitable ligand to stabilize the complex becomes a foremost challenge in the design of gold(III) with one or more multidentate ligand to enhance the stability of the complex. However, Au(III) is coordinated by at list two chelating nitrogen donors which lower the reduction potential of metal center and thereby stabilize the complex. The acceptable solution stability of these gold(III) complexes [5-6], facilitated extensive pharmacological investigation, both in vitro and in vivo [7-10].

We have performed and now report here a detailed study on the complex formation kinetics of some selected gold(III) complexes, *viz.* $[Au(en)Cl_2]^+$, $[Au(dach)Cl_2]$, $[Au(bipy)Cl_2]^+$ with 5'-GMP. We choose 5'-GMP because it is the fragment of DNA, and it seems that DNA is the primary target for the gold(III) complexes. The reactions were studied in aqueous solutions at physiological pH (7.2), using stopped-flow technique.

*Address correspondence to this author at the Faculty of Medicine, University of Kragujevac, 69 Svetozara Markovica Street, 34000 Kragujevac, Serbia; Tel: +381 34 306800; Fax: +38134306800, ext.112; E-mail: arne@medf.kg.ac.rs

In addition, we evaluated and report here *in vitro* cytotoxic activity of these complexes on A549 human lung carcinoma epithelial cell line. A549 cells are i*n vitro* most usually used cancer cell line for research in the field of testing cytotoxicity and metabolism of new synthesized complexes towards human lung carcinoma epithelial cells [11].

It was envisaged that this study could throw more light on the interactions of gold(III) complexes with nitrogen-donor nucleophiles suggesting these complexes as potentaily new therapeutic agents in the treatment of lung carcinoma.

2. EXPERIMENTAL

2.1. Chemicals

The nucleophile guanosine-5'-monophosphate sodium salt hydrate, (5'-GMP) was obtained from Acros Organics. Nucleophile stock solutions were prepared shortly before use, by dissolving the chemicals in purified water. The ligands 2,2'-bipyridyl (bipy) and (1R,2R)-1,2-diaminocyclohexane (dach), ethylenediamine (en) were obtained from Acros Organics. Starting complex potassium tetrachloridoaurate(III), $K[AuCl_4]$, was purchased from ABCR GmbH & Co. KG, 98%. All the other chemicals were of the highest purity commercially available and were used without further purification. Ultra pure water was used in all experiments.

The solutions of complexes and ligands were prepared in 25 mM Hepes buffer (pH = 7.20). The reactions of bifunctional complexes were studied in the presence of 20 mM NaCl, to prevent the hydrolysis of complexes.

Cisplatin (*cis*-diamminedichloroplatinum(II), *cis*-[Pt(NH3)2Cl2]), was purchased from Sigma-Aldrich.

1875-6638/12 $58.00+.00

2.2. Synthesis of the Complexes

The complexes $[Au(en)Cl_2]Cl$ and $[Au(bipy)Cl_2]Cl$ were prepared according to the published procedure [12]. Complex $[Au(dach)Cl_2]Cl$ was synthesized starting from $KAuCl_4$. Salt (0.2 g, 0.5 mmol) was dissolved in a little amount of water and to the solution was dropped the solution obtained by dissolving (1R,2R)-1,2-diaminocyclohexane (0.057 g, 0.5 mmol) to the mixture of $MeOH/H_2O$ (1:1, v/v). The reaction was stirred for 5 h at room temperature. Obtained yellow solution was left in the darkness to evaporate. After few days formed yellow crystals were filtrated, washed with cold water and dried. Found: H, 4.91; C, 13.66; N, 2.84; Calc. for $AuC_6H_{14}N_2Cl_3$: H, 5.34; C, 13.80; N, 2.71 % .

2.3. Instrumentation

Chemical analyses were performed on a Varian III CHNOS Elemental Analyzer, Elemental Analysensysteme, GmbH. The optical density was measured using microplate multimode detector Zenyth 3100. UV-VIS spectra were recorded on Shimadzu UV 250 and Hewlett-Packard 8452A diode-array spectrophotometers with thermostated 1.00 cm quartz Suprasil cells. Kinetic measurements were carried out on an Applied Photophysics SX.18MV stopped-flow instrument coupled to an online data acquisition system. The temperature was controlled throughout all kinetic experiments to ± 0.1 K.

2.4. Kinetic Measurements

At the beginning of the investigations, spectral changes resulting from the mixing of complex and nucleophile solutions were recorded over the wavelength range 280 to 350 nm to establish a suitable wavelength at which kinetic measurements must be performed. After that, the reactions were initiated by mixing equal volumes of the complex and ligand solutions directly in the stopped-flow instruments and were followed for at least eight half-lives at determined wavelength (working wavelengths are given in Supp. Material, Tables **S1**-**S3**). All kinetic measurements were performed under *pseudo*-first-order conditions, *i.e*., at least a 10-fold excess of the entering nucleophile was used. The kinetic traces were evaluated using the OLIS KINFIT (Jeferson, GA) set of programs. Kinetic runs were fitted as double exponential function for the reactions of bifunctional $[Au(bipy)Cl_2]^+$, $[Au(dach)Cl_2]^+$, $[Au(en)Cl_2]^+$ complexes.

The observed *pseudo*-first-order rate constants, k_{obsd}, were calculated as the average value from five to eight independent kinetic runs. All experimental data are reported in Tables **S1** – **S3**, Supp. Material.

All reactions were followed at three different temperatures (288, 298 and 310 K). The temperature was controlled throughout all kinetic experiments to ± 0.1 ^oC.

2.5. Culture of Human A549 Lung Carcinoma Epithelial Cell Line

The A549 cells were purchased from American Type Culture Collection (ATCC), Manassas, VA, USA. The A549 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% FBS, 100 IU/mL penicillin G and 100 µg/mL streptomycin (Sigma-Aldrich chemical, Munich, Germany). A549 cells in passage 3 were used throughout these experiments.

2.6. Cytotoxicity Assays

Effects of gold(III) complexes on A549 cell viability were determined using MTT 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric technique and LDH (Lactate dehydragenase) assay.

2.6.1. MTT Assay

Gold(III) complexes are biologically active substances of special interest as potential anticancer agents. Effects of $[Au(en)Cl_2]^+$ $[Au(dach)Cl_2]$ and $[Au(bipy)Cl_2]^+$ complexes on cell viability were determined using MTT colorimetric technique [13]. A549 cells were diluted with medium to 4 x 10^4 cells/mL and aliquots (4 x 10^3 cells/100µL) were placed in individual wells in 96-multiplates. After 24 hours, cells were treated with selected concentrations of complexes for 3 days. Control wells were prepared by the addition of culture medium. Wells containing culture medium without cells were used as blanks. After incubation drug containing medium was discarded and replaced with serum free medium containing 15% of MTT (5mg/ml) dye. After additional 4 h of incubation 37°C in a 5% CO2 incubator, medium with MTT was removed and DMSO (150 µL) with glycine buffer (20 µL) was added to dissolve the blue formazan crystals. The plates were shaken for 10 min. The optical density of each well was determined at 595 nm. The percentage of cytotoxicity was calculated using the formula:

$$\% \text{ cytotoxicity} = 100-((TS-BG0)-E/(TS-BG0) \times 100)$$

where BG0 is for background of medium alone, TS is for total viability/spontaneous death of untreated target cells, and E is for experimental well.

2.6.2. LDH Assay

LDH assays can be performed by assessing LDH released into the media as a marker of dead cells or performing lysis LDH as a marker of remaining live cells. LDH, therefore, is the most widely used marker in cytotoxicity study. [14] Cytotoxicity of gold complexes was examined by Cytotoxicity Detection Kit (LDH) (Roche Applied Science). A549 cells were prepared and treated with complexes in the same manner as for MTT assay. Additional wells were prepared as high control, cells were treated with Triton X (1%). Cells exposed to medium were used as low controls. After treatment, supernatant (100µL) was transferred to new plate and incubated with an equivalent volume of substrate solution. After incubating the plates for 30 minutes at RT, 50 µl/well stop solution was added and data were acquired by spectrophotometry at 450 nm. The percentage of dead cells was calculated using the formula:

% of dead cells=(exp. value-low control)/(high control-low control) x 100.

2.7. Apoptosis Assay

For the detection of apoptosis, the Annexin V binding capacity of treated cells was examined by flow cytometry. At the onset of apoptosis, phosphatidylserine which is normally

$$[Au(NN)Cl_2]^+ + 5'\text{-GMP} \underset{k_{-2,f}}{\overset{k_{2,f}}{\rightleftharpoons}} [Au(NN)Cl(5'\text{-GMP})]^{2+} + Cl^-$$

$$[Au(NN)Cl(5'\text{-GMP})]^{2+} + 5'\text{-GMP} \underset{k_{-2,s}}{\overset{k_{2,s}}{\rightleftharpoons}} [Au(NN)(5'\text{-GMP})_2]^{3+} + Cl^-$$

NN = ethilendiamine, 2,2'-bipyridine or 1,2-diaminocyclohexane

Scheme 1.

found on the internal part of the plasma membrane becomes translocated to the external portion of the membrane and becomes available to bind to the annexin V-FITC. 7-AAD bind to the cellular DNA in cells where the cell membrane has been totally compromised [15]. After A549 cells reached subconfluency, medium was replaced with gold (III) complexes (500 μM). Exposed A549 cells were placed at 37oC in a 5% CO2 incubator for 24h. Cultured cells were washed twice with phosphate-buffered saline (PBS, Sigma Aldrich) and resuspended in 1x binding buffer (10x binding buffer: 0,1 M Hepes/NaOH (pH 7,4), 1,4 M NaCl, 25 mM CaCl2) at concentration 1x106 / mL. Annexin FITC and propidium iodide (PI) were added to the 100 μL of cell suspension and incubated for 15 min at room temperature (25 oC) in the dark. After incubation 400 μL of 1x binding buffer was added to each tube and stained cells were analyzed within 1 hour using FACS Calibur (BD, San Jose, USA) and Win-MDI software. Since, Annexin V FITC staining precedes the loss of membrane integrity that accompanies the later stage identified by PI, Annexin FITC positive, PI negative indicates early apoptosis, while viable cells are Annexin V FITC negative, PI negative. Cells that are late apoptosis or already dead are both Annexin V FITC and PI positive.

3. RESULTS

3.1. Kinetic Measurements

Here we report the results of kinetic studies of some bifunctional gold(III) complexes. The substitution reactions of gold(III) complexes $[Au(en)Cl_2]^+$, $[Au(dach)Cl_2]^+$ and $[Au(bipy)Cl_2]^+$ with nucleophile 5'-GMP were investigated spectrophotometrically by following the change in absorbance at suitable wavelengths as a function of time under *pseudo*-first-order conditions. All reactions were studied at physiological (pH = 7.20) conditions in 25 mM Hepes, with the presence of NaCl, to prevent the hydrolysis of the starting complexes. All reactions were performed as a function of nucleophile concentration and temperature using stopped-flow technique.

The substitution reactions of $[Au(en)Cl_2]^+$ and $[Au(dach)Cl_2]^+$ and $[Au(bipy)Cl_2]^+$ with nucleophile 5'-GMP were studied also at physiological pH 7.20 in 25 mM Hepes with the presence of 20 mM NaCl, to prevent the hydrolysis of the complexes. The process of substitution could be described as shown in Scheme **1**.

The first step of the substitution is a reversible process, where the direct reaction nucleophile substitutes one chloride ion from the coordination sphere of the starting complex. However, due to the presence of 20 mM NaCl solvolytic step is eliminated and the reaction becomes equilibrium. The second step is also equilibrium when another molecule of nucleophile substitutes the other chloride ion.

The obtained *pseudo*-first order rate constants for the first ($k_{obsd,f}$) and the second ($k_{obsd,s}$) steps of the substitution (data are given in Tables **S1**- **S3**. Supp. Material) were plotted versus the concentrations of the entering nucleophiles. A linear dependence on the nucleophile concentration was observed for all reactions. Representative plots for the substitution reactions of $[Au(bipy)Cl_2]^+$, $[Au(dach)Cl_2]^+$ and $[Au(en)Cl_2]^+$ complexes with 5'-GMP are shown in Fig. (**2**), and in Supp. Mat., Fig. (**1S** and **2S**).

The observed *pseudo*-first-order rate constant, $k_{obsd,f}$, as a function of the total concentration of nucleophile is described by Eq. (1) and the rate constants $k_{2,f}$ and $k_{-2,f}$ can be determined from the slopes and intercepts of the plots.

$$k_{obsd,f} = k_{-2,f} + k_{2,f}[Nu] \quad (1)$$

The observed rate constants for the second reaction step can be expressed as given in Eq. 2.

$$k_{obsd,s} = k_{-2,s} + k_{2,s}[Nu] \quad (2)$$

All The rate constants for the first and the second reaction steps for the all reactions, calculated by OriginPro8, are summarized in Table **1**.

The first reaction step occurs via nucleophilic attack of N7 donor atom of purine base comprised in 5'-GMP, resulting in the formation of a product by departing one chloride ion [16-17]. The second step includes the substitution of another chloride ion from the starting complex, when 1:2 complexes is formed. However, the first and the second steps of the substitution of $[Au(bipy)Cl_2]^+$ complex are faster than in the case of $[Au(dach)Cl_2]^+$ and $[Au(en)Cl_2]^+$ complex due to the same effect of inert, bidentate, ligand as it was explained before [17].

The temperature dependence of rate constants enabled the calculation of the activation enthalpies and entropies by using Eyring equation. Activation parameters derived from these experiments are summarized in Table **1**. The entropy of activation for all studied systems is large and negative, which is in line with an associative substitution mechanism.

3.2. Gold (III) Complexes Showed Cytotoxic Effects and Induce Apoptosis of A549 Cells

All of three gold (III) complexes showed cytotoxic effect on A549 cells (Figs. **3**, **4**, **5**). The concentration increase is followed by markedly increase of apoptotic cell's percentage. The concentrations from 7.8 μM to 250 μM of all tested

Table 1. Rate Constants and Activation Parameters for the First and the Second Steps of the Substitution Reactions of $[Au(dien)Cl_2]^+$ and $[Au(en)Cl_2]^+$ Complexes with Ligand 5'-GMP at 298 K in 25 mM Hepes bufFer (pH = 7.2 and 20 mM NaCl).

5'-GMP *First step*	$k_{2,f}/M^{-1}\,s^{-1}$	$\Delta H_2^{\neq}$/kJ mol^{-1}	$\Delta S_2^{\neq}$/J K^{-1} mol^{-1}	$k_{1,f}/s^{-1}$	$\Delta H_1^{\neq}$/kJ mol^{-1}	$\Delta S_1^{\neq}$/J K^{-1} mol^{-1}
$[Au(bipy)Cl_2]^+$	$(1.49 \pm 0.05) \cdot 10^2$	7 ± 3	-180 ±10	$(3.9 \pm 0.9) \times 10^{-2}$	86 ± 2	-7 ± 2
$[Au(dach)Cl_2]^+$	$(6.6 \pm 0.2) \cdot 10^1$	10 ± 3	-170 ±10	$(1.86 \pm 0.0.04)10^{-1}$	21 ± 2	-190 ± 20
$[Au(en)Cl_2]^+$	$(3.3 \pm 0.3) \cdot 10^1$	24 ± 3	140± 10	$(8.2 \pm 0.7) \cdot 10^{-2}$	39 ± 2	-139 ± 8
Second step	$k_{2,s}/M^{-1}\,s^{-1}$	$\Delta H_2^{\neq}$'/kJ mol^{-1}	$\Delta S_2^{\neq}$'/J K^{-1} mol^{-1}	$k_{1,s}/s^{-1}$	$\Delta H_1^{\neq}$'/kJ mol^{-1}	$\Delta S_1^{\neq}$'/J K^{-1} mol^{-1}
$Au(bipy)Cl_2]^+$	$(1.03 \pm 0.03) \cdot 10^1$	33 ± 3	-120 ± 10	$(3.9 \pm 0.1) \times 10^{-2}$	14 ± 3	-169 ± 9
$[Au(dach)Cl_2]^+$	6.2 ± 0.3	27 ± 5	140± 10	$(3.1 \pm 0.1) \times 10^{-2}$	48 ± 2	-120 ± 10
$[Au(en)Cl_2]^+$	2.5 ± 0.1	26 ± 2	-154 ± 9	$(3.1 \pm 0.04) \cdot 10^{-2}$	8 ± 4	-140± 10

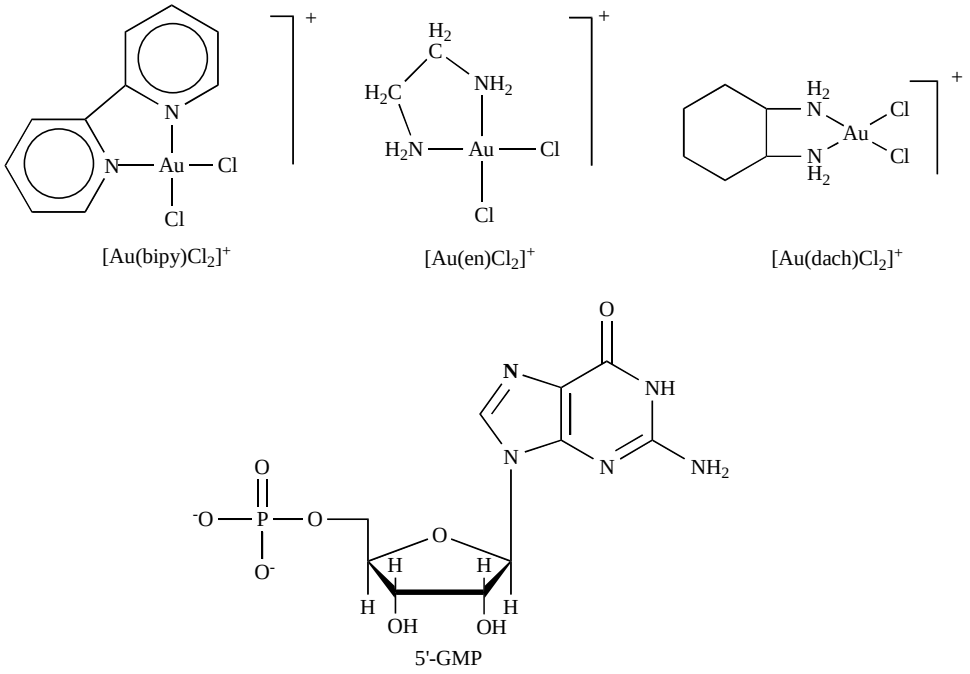


Fig. (1). Structures of the investigated complexes and nucleophile.

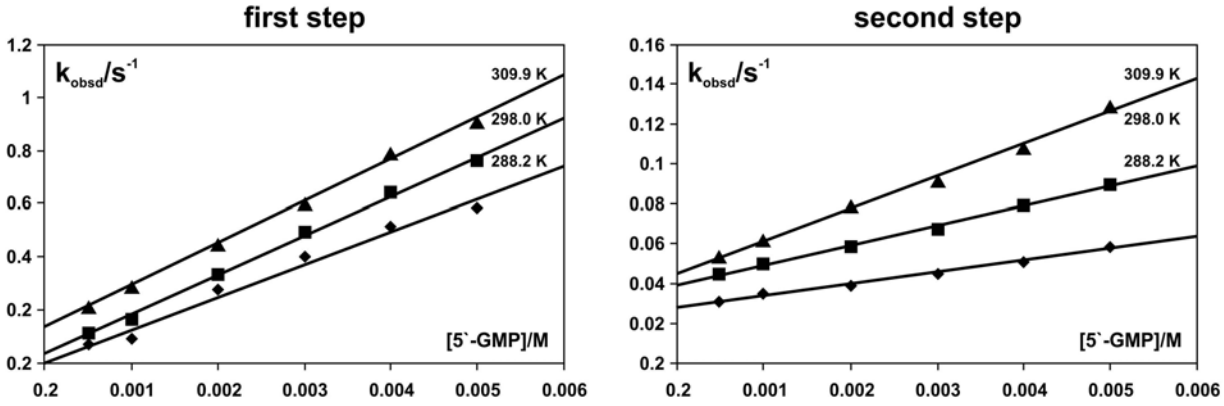


Fig. (2). *Pseudo*-first order rate constants for the first (k_{obsd1}) and the second (k_{obsd2}) steps of the substitution reactions between $[Au(bipy)Cl_2]^+$ complex and 5'-GMP as a function of ligand concentration and temperature, in 25 mM Hepes buffer (pH = 7.2) with the addition of 20 mM NaCl.

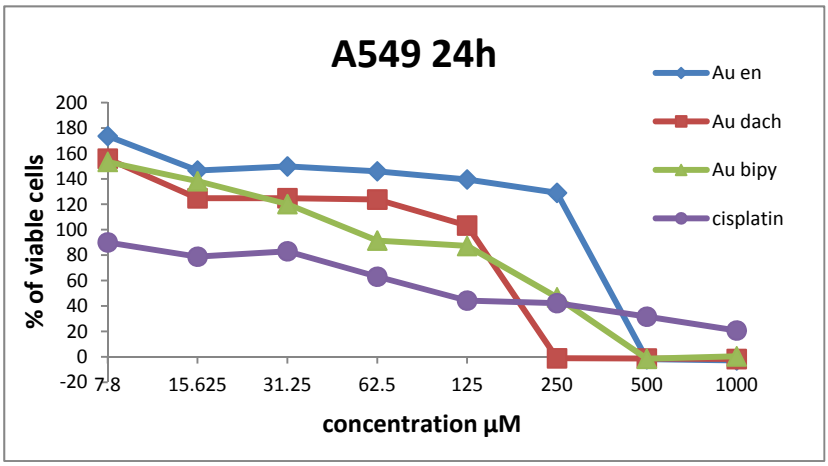


Fig. (3). The results of MTT cytotoxic assay.

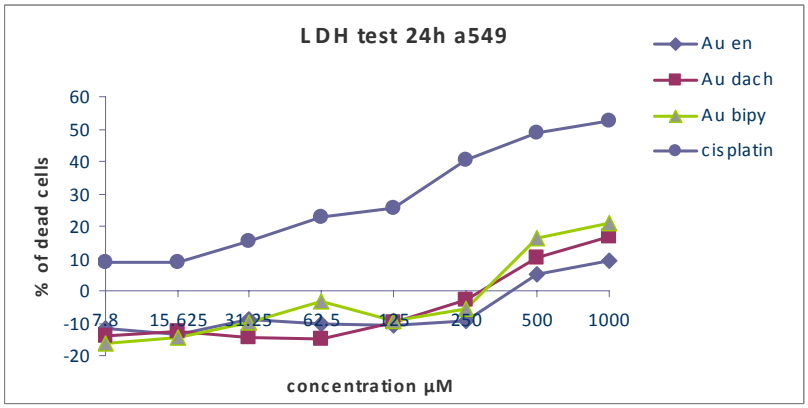


Fig. (4). The results of LDH cytotoxic assay.

complexes showed similar and low cytotoxic effect. However, gold complexes differ at concentration of 250 µM. $[Au(dach)Cl_2]^+$ complex show high cytotoxicity with almost 100% of dead cells and $[Au(bipy)Cl_2]^+$ had low cytotoxic effect (40% dead cells). Interestingly, A549 cells proliferated 24 h after treatment with $[Au(en)Cl_2]^+$ complex (at concentration 250 µM). All three gold (III) complexes show 100% of cytotoxicity at the concentration of 500 µM (Fig. **3**).

Results obtained from LDH assay correlate with MTT results (Fig. **4**). Apoptotic assay showed that most of dead cells were in the stage of early apoptosis (Fig. **5**).

Flow cytometry results have shown that cisplatin had 54.77% cells in the early apoptosis, $[Au(en)Cl_2]^+$ 7.16%, $[Au(dach)Cl_2]^+$ 21.19% and $[Au(bipy)Cl_2]^+$ 60,92 %.

This indicates that $[Au(bipy)Cl_2]^+$ shows higher cytotoxic effect on A549 cells after 24hrs of exposure in comparison to the cisplatin. Also it is shown that at the concentration of 250 µM both cisplatin and $[Au(bipy)Cl_2]^+$ show same level of of cytotoxicity measured through MTT assay.

4. DISCUSSION

Here we, for the first time, demonstrate the cytotoxic effects of newly synthesized $[Au(bipy)Cl_2]^+$ $[Au(dach)Cl_2]^+$ $[Au(en)Cl_2]^+$ gold (III) complexes on A549 human lung carcinoma epithelial cell line. Our results showed that all tested gold(III) complexes displayed cytotoxic effect on A549 cells (Fig. **3**,**4**,**5**). The concentration decrease was followed by markedly decrease of apoptotic cell's percentage. At the highest and at the lowest concentrations (500 µM *vs*. 7,8 µM) all tested complexes showed similar cytotoxic effects. However, at the concentration of 250 µM for which we suppose that could be used *in vivo*, $[Au(bipy)Cl_2]^+$ complex showed the best cytotoxic effects among tested gold (III) complexes and similar cytotoxicity compared to cisplatinum that we used as control. At concentration 250 µM, only 24 hours after treatment with $[Au(bipy)Cl_2]^+$ almost all A549 cells were dead (Fig. **3**).

In addition, apoptotic assay showed high percentage of early apoptotic cells after treatment with $[Au(bipy)Cl_2]^+$ (60,92 %) compared with cisplatin (54.77%) and other tested gold (III) complexes ($[Au(dach)Cl_2]^+$ 21.19% and $[Au(en)Cl_2]^+$ 7.16%) suggesting apoptosis as the main mechanism of $[Au(bipy)Cl_2]^+$ cytotoxicity (Fig. **5**).

The activation parameters for all studied reactions suggest an associative substitution mechanism. As previously described, the first reaction step occurs *via* nucleophilic attack of N7 donor atom of purine base comprised in 5'-

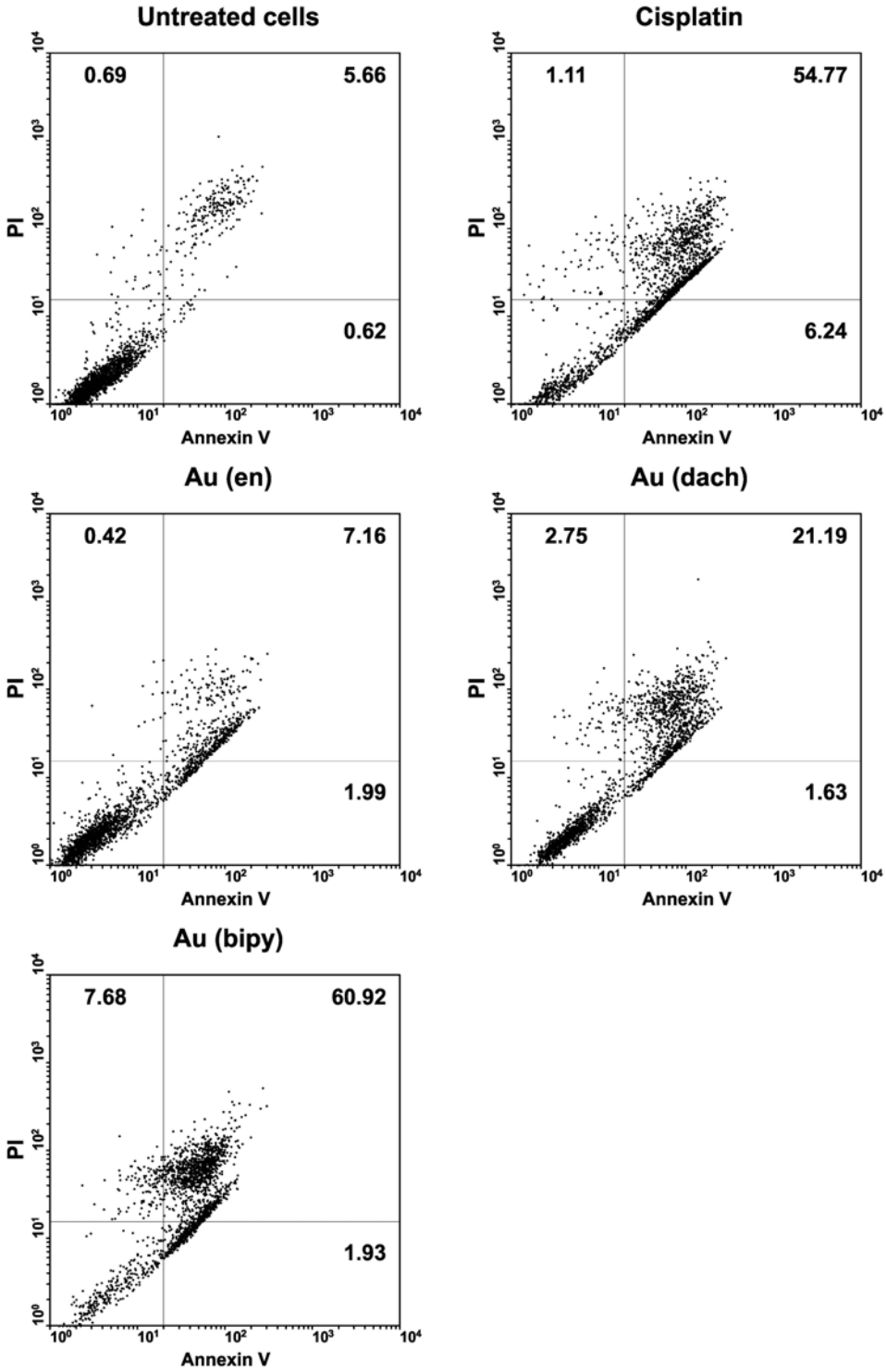


Fig. (5). The results of Apoptotic assay.

GMP, resulting in the formation of a product by departing one chloride ion [16-17]. The second step includes the substitution of another chloride ion from the starting complex, when 1:2 complexes is formed. Both the first and the second steps of the substitution of $[Au(bipy)Cl_2]^+$ complex are faster than in the case of $[Au(dach)Cl_2]^+$ and $[Au(en)Cl_2]^+$ complexes suggesting better efficacy of $[Au(bipy)Cl_2]^+$ complex.

In line with the obtained results, we suppose that $[Au(en)Cl_2]^+$ complex could be a good candidate for future pharmacological evaluation as new therapeutic agent in the pre-clinical studies for the treatment of lung carcinoma.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge financial support from the Ministry of Science and Technological Development of the Republic of Serbia, project No. 172011 and project No. ON 175069.

DISCLOSURE

Part of information included in this article has been previously published in Journal of Inorganic Biochemistry Volume 104, Issue 9, September 2010, Pages 944-949.

SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's web site along with the published article.

REFERENCES

[1] Messori, L.; Marcon, G. Gold complexes as antitumor agents. *Met. Ions Biol. Syst*., **2004**, *42*, 385-424.

[2] Dyson, PJ.; Sava, G. Metal-based antitumour drugs in the post genomic era. *Dalton Trans*., **2006**, *16*, 1929-1933.

[3] Wang, D.; Lippard, SJ. Cellular processing of platinum anticancer drugs. *Nat. Rev. Drug Discov*., **2005**, *4*, 307-320.

[4] Reedijk, J. New clues for platinum antitumor chemistry: kinetically controlled metal binding to DNA. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 3611-3616.

[5] Giovagnini, L.; Ronconi, L.; Aldinucci, D.; Lorenzon, D.; Sitran, S.; Fregona, D. Synthesis, characterization, and comparative in vitro cytotoxicity studies of platinum(II), palladium(II), and gold(III) methylsarcosinedithiocarbamate complexes. *J. Med. Chem*., **2005**, *48*, 1588-1595.

[6] Che, CM.; Sun, RW.; Yu, WY.; Ko, CB.; Zhu, N.; Sun, H. Gold(III) porphyrins as a new class of anticancer drugs: cytotoxicity, DNA binding and induction of apoptosis in human cervix epitheloid cancer cells. *Chem. Comm*., **2003**, *14*, 1718-1719.

[7] Casini, A.; Hartinger, C.; Gabbiani, C.; Mini, E.; Dyson, PJ.; Keppler, BK.; Messori, L. Gold(III) compounds as anticancer agents: relevance of gold-protein interactions for their mechanism of action. *J. Inorg. Biochem.*, **2008**, *102*, 564-575.

[8] Tiekink, ER. Anti-cancer potential of gold complexes. *Inflammopharmacology*, **2008**, *16*, 138-142.

[9] Garza-Ortiz, A.; den Dulk, H.; Brouwer, J.; Kooijman, H.; Spek, AL.; Reedijk, J. The synthesis, chemical and biological properties of dichlorido(azpy)gold(III) chloride (azpy=2-(phenylazo)pyridine) and the gold-induced conversion of the azpy ligand to the chloride of the novel tricyclic pyrido[2,1-c][1,2,4]benzotriazin-11-ium cation. *J. Inorg. Bioch*., **2007**, *101*, 1922-1930.

[10] Casini, A.; Kelter, G.; Gabbiani, C.; Cinellu, MA.; Minghetti, G.; Fregona, D.; Fiebig, HH.; Messori, L. Chemistry, antiproliferative properties, tumor selectivity, and molecular mechanisms of novel gold(III) compounds for cancer treatment: a systematic study. *J. Biol. Inorg. Chem.*, **2009**, *14*, 1139-1149.

[11] Zhu, S.; Gorski, W.; Powell, DR.; Walmsley, JA. Synthesis, structures, and electrochemistry of gold(III) ethylenediamine complexes and interactions with guanosine 5'-monophosphate. *Inorg. Chem*. **2006**, *45*, 2688-2694.

[12] Sykes, AG. *Advances in Inorganic and Bioinorganic Reaction Mechanisms.* Academic Press, New York, **1986**.

[13] Giard, DJ.; Aaronson, SA.; Todaro, GJ.; Arnstein, P.; Kersey, JH.; Dosik, H.; Parks, WP. *In vitro* cultivation of human tumors: establishment of cell lines derived from a series of solid tumors. *J. Natl. Cancer Inst*., **1973**, *51*, 1417-1423.

[14] Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Meth*., **1983**, *65*, 55-63.

[15] Arechabala, B.; Coiffard, C.; Rivalland, P.; Coiffard, L.J.; de Roeck-Holtzhauer, Y. Comparison of cytotoxicity of various surfactants tested on normal human fibroblast cultures using the neutral red test, MTT assay and LDH release. *J. Appl. Toxicol.*, **1999**, *19*, 163-165.

[16] Shounan, Y.; Feng, X.; O'Connell, PJ. Apoptosis detection by annexin V binding: a novel method for the quantitation of cell-mediated cytotoxicity. *J. Immunol. Methods*, **1998**, *217*, 61-70.

[17] Milovanović, M.; Djeković, A.; Volarević, V.; Petrović, B.; Arsenijević, N.; Bugarčić, ZD. Ligand substitution reactions and cytotoxic properties of [Au(L)Cl2](+) and [AuCl2(DMSO)2]+ complexes (L=ethylenediamine and S-methyl-l-cysteine). *J. Inorg. Biochem*., **2010**, *104*, 944-949.

Received: May 24, 2011 Revised: June 14, 2011 Accepted: November 04, 2011