ANTIPROLIFERATIVE ACTIVITY OF GOLD(III) COMPLEXES WITH ESTERS OF CYCLOHEXYL-FUNCTIONALIZED ETHYLENEDIAMINE-N,N'-DIACETATE

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ANTIPROLIFERATIVNA AKTIVNOST ZLATO(III) KOMPLEKSA SA CIKLOHEKSIL-FUNKCIONALIZOVANIM ESTRIMA ETILENDIAMIN-N,N'-DIACETATA

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ABSTRACT

Six gold(III) complexes with esters of cyclohexyl-functionalized ethylenediamine-N,N'-diacetate, general formula $[AuCl_2\{(S,S)\text{-}R_2eddch\}]PF_6$, [(S,S)-eddch = (S,S)-ethylenediamine-N,N'-di-2-(3-cyclohexyl)propanoate, R = Me, Et, n-Pr, n-Bu, i-Bu, i-Am, **1–6**, *respectively], were tested against cancer cell lines such as human melanoma Fem-x, human colon carcinoma LS174T and non-small cell lung carcinoma A549 as well as a non-cancerous human embryonic lung fibroblasts MRC-5 using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay with the aim of assessing in vitro antitumoral activity and selectivity. All investigated complexes showed lower cytotoxicity and better or similar selectivity in comparison to cisplatin, used as reference compound. Complex $[AuCl_2\{(S,S)\text{-}(i\text{-}Am)_2eddch\}]PF_6$ (**6**) demonstrated the highest activity against Fem-x (IC_{50} = 14.98 ± 0.34 μM). Additionally, the same complex expressed 4.5 times higher selectivity than cisplatin.*

Keywords: *cytotoxicity, gold(III) complexes, R_2edda type-ligands, selectivity*

SAŽETAK

Šest kompleksa zlata(III) sa cikloheksil-funkcionalizovanim estrima etilendiamin-N,N'-diacetata, opšte formule $[AuCl_2\{(S,S)\text{-}R_2eddch\}]PF_6$, ((S,S)-eddch = (S,S)-etilendiamin-N,N'-di-2-(3-cicloheksil)propanoat, R = Me, Et, n-Pr, n-Bu, i-Bu, i-Am, 1–6), ispitivano je na humanim ćelijskim linijama malignog melanoma Fem-x, karcinoma debelog creva LS174T, karcinoma pluća A549 kao i normalnim ćelijama MRC-5 (fetalni plućni fibroblast) korišćenjem 3-(4,5-dimetiltiazol-2-yl)-2,5-difeniltetrazolium bromid (MTT) testa u cilju procene in vitro antitumorske aktivnosti i selektivnosti. Svi ispitivani kompleksi pokazali su manju citotoksičnost i bolju ili sličnu selektivnost u odnosu na cisplatinu koja je korišćena kao referentna supstanca. Kompleks 6 je pokazao najveću aktivnost sa IC_{50} (Fem-x) vrednošću od 14,98 ± 0,34 μM. Takođe, isti kompleks pokazuje 4,5 puta veću selektivnost od cisplatine.

Ključne reči: *citotoksičnost, zlato(III) kompleksi, R_2edda tip-liganada, selektivnost*

ABBREVIATIONS

A549 - Non-small cell lung carcinoma cell line
Fem-x - Human melanoma cell line
HeLa - Human cervix adenocarcinoma cell line
K562 - Human myelogenous leukemia cell line
LS174T - Human colon carcinoma cell line
MRC-5 - Non-cancerous cell line human embryonic lung fibroblast
PBMC - Human peripheral blood mononuclear cells
MTT - 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
R2edda-type ligand - *O,O'*-Dialkyl-ethylenediamine-*N,N'*-diacetate ester type ligands
R2eddch - *O,O'*-Dialkyl-(S,S)-ethylenediamine-*N,N'*-di-2-(3-cyclohexyl)propanoate ester

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INTRODUCTION

Cancer is a disease of deregulated cellular behavior (1). The main processes of cancer treatment in humans are surgery, radiation, and chemotherapy. Cancer chemotherapeutic agents can often provide temporary relief of symptoms, give the patient more time and rarely cure. In recent years, a lot of research has been conducted to the synthesis of potential anticancer drugs (2–4). A successful anticancer drug should kill or inactivate cancer cells without causing excessive damage to normal cells (5, 6). Discovering of cisplatin by Rosenberg in the 1970s, platinum complexes became one of most commonly explored class of cytostatics in anticancer chemotherapy, but only a few are in worldwide clinical practice use or in clinical trials (7–10). An overview or some currently employed anticancer drugs (I–III), in clinical trials (IV, V) platinum-based compounds are illustrated in Fig.1.

The accumulation of platinum compounds in the body has deleterious effects. The two major problems associated with the use of cisplatin derivatives are the severe toxic side effects (11–15) and the intrinsic or acquired resistance manifested in various types of cancers (16). Therefore, in recent decades, a large number of new metal-based complexes have been developed and tested (17–19).

Gold complexes have recently gained a considerable attention as a class of antitumor compounds with different pharmacodynamics and kinetic properties than cisplatin with strong cell growth inhibiting effects (20, 21). Since gold(III) is isoelectronic to platinum(II) and both metals preferentially generate square planar complexes (d^8 system) a number of gold(III) complexes were synthesized and tested as an alternative to the anticancer drug cisplatin (22–27). Due to the fact that the gold(III) has high reduction potential, a range of strategies has been applied in order to obtain gold(III) complexes with sufficient stability under physiologically relevant conditions. These strategies are mostly related to appropriate ligand selection, which has been shown to be crucial in decreasing the pronounced tendency of the gold(III) metal center to be reduced to gold(I) or/and metallic gold (28). The chelation of the metallic center with multidentate ligands has shown to enhance the stability of the complex (29, 30).

The mechanisms of action of cytotoxic gold(III) complexes seem to be innovative and substantially different from that of cisplatin (31). Indeed, it is well known that DNA is a primary target for platinum(II) complexes while the gold(III) complexes act by targeting mitochondria of cancer cells (32) or by inhibiting the activities of different proteins (33, 34). Recently, it was found that thiol-containing enzymes, such as thioredoxin reductase (TrxR), can play important roles in the mechanisms of action of anticancer gold complexes (33).

Lately, we reported synthesis and characterization of gold(III) complexes with esters of cyclohexyl-functionalized ethylenediamine-*N,N'*-diacetate, general formulae $[AuCl_2\{(S,S)\text{-}R_2eddch\}]PF_6$, ((*S,S*)-eddch = (*S,S*)-ethylenediamine-*N,N'*-di-2-(3-cyclohexyl)propanoate) (Fig. 2) (22).

The *in vitro* cytotoxic evaluation of the investigated complexes against tumor cell lines: human adenocarcinoma HeLa, human myelogenous leukemia K562 and against normal stimulated and nonstimulated peripheral blood mononuclear cells PBMC, showed that the cytotoxic action of gold(III) complexes with cyclohexyl-functionalized ethylenediamine-*N,N'*-diacetate esters, (R= *i*-Bu, *i*-Am), is fairly comparable to that of cisplatin (22). Additionally, it was found that these complexes reduce to gold(I) species in two steps through short-living intermediate gold(II) (35). This is very important because it is believed that the cytotoxic activity of gold(III) complexes comes from appropriate gold(I) species produced by gold(III) reduction *in vivo* (24, 35).

Inspired by these promising results we are deeply interested in further investigations related to the *in vitro* antiproliferative activity of $[AuCl_2\{(S,S)\text{-}R_2eddch\}]PF_6$ complexes against other cancer cell lines such as human melanoma Fem-x, human colon carcinoma LS174, and non-small cell lung carcinoma A549 as well as a non-cancerous cell line human embryonic lung fibroblast MRC-5.

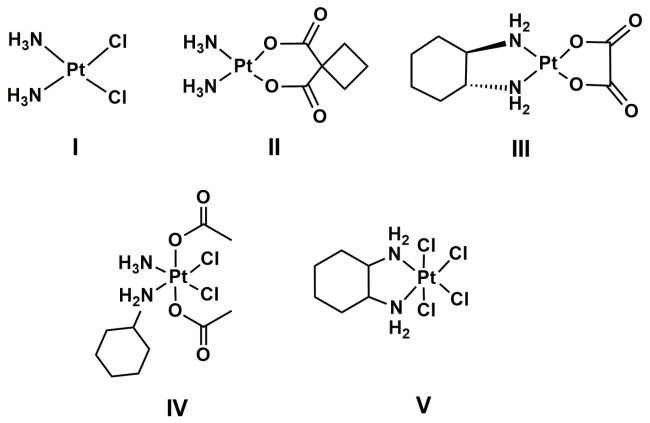
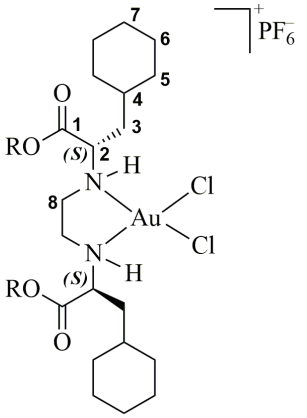


Figure 1. The structure of platinum complexes: Cisplatin (**I**); Carboplatin (**II**); Oxaliplatin (III); Satraplatin (**IV**); Tetraplatin (**V**)



R = Me, Et, *n*-Pr, *n*-Bu, *i*-Bu, *i*-Am
1-6

Figure 2. $[AuCl_2\{(S,S)\text{-}R_2eddch\}]PF_6$ complexes

MATERIALS AND METHODS

Complexes

Gold(III) complexes were synthesized according to literature procedure. Shortly, $Na[AuCl_4]$ was reacted with with an equimolar amount of corresponding ligands, methyl, ethyl, *n*-propyl, *n*-butyl, isobutyl and isoamyl esters of (*S*,*S*)-ethylenediamine-*N*,*N*'-di-2-(3-cyclohexyl)propanoic acid respectively (22). Each ligand was suspended in methanol, deprotonated with $LiOH{\cdot}H_2O$ and after stirring of 1h, a solution of $Na[AuCl_4]{\cdot}2H_2O$ in methanol was added. The desired complexes were obtained after addition of ammonium hexafluorophosphate. Purity and constitution of the obtained products were confirmed with elemental analysis, ^1H and ^{13}C NMR as well as UV/Vis spectroscopies and mass spectrometry. As examples for analytical and spectroscopic data for complex $[AuCl_2\{(S,S)\text{-}i\text{Am}_2\text{eddch}\}]PF_6$ is provided (22). Numeration of carbon atoms is shown in Fig. 2.

$[AuCl_2\{(S,S)\text{-}i\text{Am}_2\text{eddch}\}]PF_6$ (22): Yield: 66 mg, 57%. Anal. Calcd for $C_{30}H_{56}N_2O_4AuCl_2PF_6$: C, 39.09; H, 6.12; N, 3.04%. Found: C, 38.98; H, 6.13; N, 3.11%. ^1H NMR (200 MHz, $CDCl_3$): δ 0.95 (d, $(CH_3)_2CHCH_2CH_2$–OOC–, 12H; m, C^7H_2, 4H), 1.24 (m, $C^{5,6}H_2$, 8H), 1.50-1.90 (m, C^3H_2, C^4H, $C^{5,6}H_2$, $(CH_3)_2CHCH_2CH_2$–OOC– and $(CH_3)_2CHCH_2CH_2$–OOC–, 20H), 3.43 (m, C^8H_2, 4H), 3.92 (m, C^2H, 2H), 4.30 (m, $(CH_3)_2CHCH_2CH_2$–OOC–, 4H), 4.71 (s, N*H*, 2H). δ ^{13}C NMR (50 MHz, $CDCl_3$): 11.1 ($(CH_3)_2CHCH_2CH_2$–OOC–), 16.3 ($(CH_3)_2CHCH_2CH_2$–OOC–), 22.3 ($(CH_3)_2CHCH_2CH_2$–OOC–), 25.9 (C^6), 32.4 (C^4), 33.1 (C^7), 33.8 (C^5), 36.9 (C^3), 44.5 (C^8), 59.2 (C^2), 65.8 ($(CH_3)_2CHCH_2$–OOC–), 171.0 (C^1). IR (ATR): ν_{max} = 2929, 2854, 1731, 1453, 1260, 1212, 851 cm^{-1}; UV/Vis ($CHCl_3$): λ_{max} (ε, 6630 M^{-1} cm^{-1}) 320.95 nm; ESI–MS (CH_3CN), positive: m/z: 775.33 $[M]^+$, 776.33 $[M + H]^+$.

Preparation of drug solutions

The stock solutions of the investigated gold(III) complexes were prepared freshly in DMSO (Sigma-Aldrich, St. Louis, MO, USA) at the concentrations of 1 mM, and immediately diluted by nutrient medium to various working concentrations. Nutrient medium was RPMI-1640 (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS; Biochrom AG, Berlin, Germany) and 1% penicillin/streptomycin (Sigma-Aldrich St. Louis, MO, USA).

Cell lines

Human melanoma Fem-x, human colon carcinoma LS174T, non-small cell lung carcinoma A549 cell lines, and non-cancerous human embryonic lung fibroblasts MRC-5 were grown in nutrient medium.

Determination of cell survival

Target cells Fem-x (5000 cells/well), LS174T (7000 cells/well), A549 (5000 cells/well), and non-cancerous MRC-5 (5000 cells/well) were seeded into the wells of a 96-well flat-bottomed microtitre plate. After 24 h, different working concentrations of investigated compounds were added to the wells, except for the controls, where only the complete medium was added. The final concentration range used in the experiments was 1–200 μM (gold(III) complexes: 12.50, 25, 50, 100, and 200). Cisplatin was used as the positive control, and the final concentrations were 2.08, 4.17, 8.33, 16.67, and 33.3 μM. The final concentration of DMSO never exceeded 0.5%, which is a non-toxic concentration for the cells. Culture medium with corresponding concentrations of investigated compounds, but without cells, was used as blank. The cultures were incubated for 72 h, and the effects of the investigated compounds on cancer cell survival were determined using the microculture tetrazolium test (MTT), according to Mosmann (36) with modification by Ohno and Abe (37). Briefly, 20 mL of MTT solution (5 mg/mL of phosphate-buffered saline, PBS) was added to each well. Samples were incubated for additional 4 h at 37 °C in a humidified atmosphere of 5% CO_2 (*v*/*v*). Afterward, 100 mL of 100 g/L sodium dodecyl sulfate (SDS) was added in order to extract the insoluble formazan, which represents the product of the conversion of the MTT dye by viable cells. The number of viable cells in each well is proportional to the intensity of the absorbance (A) of light, which was measured in an enzyme-linked immunosorbent assay (ELISA) plate reader at 570 nm, 24 h later. IC_{50} values (±SD) were calculated using four-parameter logistic function and presented as mean from three independent experiments. IC_{50} is defined as the concentration of an agent inhibiting cell survival by 50% compared to the vehicle-treated control. As positive control cisplatin was used. All experiments were performed in triplicate.

RESULTS

The cytotoxic activity of the six investigated gold(III) complexes was evaluated in comparison with cisplatin against three different cell lines: human melanoma Fem-x, human colon cancer LS174T, and non-small cell lung carcinoma A549, as well as normal non-cancerous cell line MRC-5. The results of *in vitro* cytotoxic activity are expressed as IC_{50} and presented in Table 1. The representative graphs showing the action of various concentrations of investigated complexes on Fem-x, LS174T, A549, and non-cancerous MRC-5 cell survival, determined by MTT test, upon 72 h of continuous agent action are shown in Fig. 3. Additionally, selectivity indices are presented in Table 2.

DISCUSSION

The cytotoxic action of all investigated gold(III) complexes (Table 1), was the most pronounced against human melanoma Fem-x cells and showed the significant cytotoxicity. In comparison, tested complexes show the moderate cytotoxicity on the A549 and LS174T cells that does not differ significantly. The order of the sensitivity of the examined malignant cell lines toward gold(III) complexes was: Fem-x > LS174T > A549.

The replacement of methyl by ethyl group in ester moiety (**1** → **2**) led to increased cytotoxic activity of investigated complexes against LS174T and A549 cell lines, while the cytotoxic activity against Fem-x and non-malignant cells slightly decreased. The substitution of ethyl with *n*-propyl group (**2** → **3**) in gold(III) complexes decreased antiproliferative action against all examined cell lines except MRC-5 cells. Further substitution of *n*-propyl with *n*-butyl (**3** → **4**) led to enhancement of cytotoxic action against Fem-x cells, whereas in all other cases complexes showed lower activity. Complex with isobutyl ester moiety, **5**, have shown similar cytotoxicity in comparison to **4** against Fem-x, LS174T, and MRC-5 cell lines. On the other hand, the same complex exhibited a stronger antiproliferative effect against A549 cells. Interestingly, complex **6** expressed the highest activity against Fem-x, but it was found to be the least effective against LS174T and A549 cell lines. Generally, all examined complexes exhibited several times weaker activity than the reference compound cisplatin. Herein examined gold(III) complexes have shown lower activity against Fem-x, LS174T and A549 than on human cervix adenocarcinoma HeLa and human myelogenous leukemia K562 tumor cell lines, reported previously (22). Furthermore, it is important to note that the investigated complexes showed less toxic action against non-cancerous MRC-5 cells than on rested and stimulated normal immu-

Table 1. Concentrations of compounds that induced a 50% decrease in Fem-x, LS174T, A549, and MRC-5 cell survival rate [expressed as IC_{50} (μM)]. The cells were incubated with the compounds for 72 h

compounds	IC_{50} [μM]			
	Fem-x	LS174T	A549	MRC-5
1	23.67 ± 2.44	42.38 ± 2.41	64.91 ± 0.58	194.25 ± 3.73
2	25.16 ± 2.75	37.69 ± 0.38	45.71 ± 1.22	>200
3	28.28 ± 1.24	48.71 ± 0.96	57.86 ± 1.74	182.51 ± 2.66
4	19.77 ± 1.95	55.39 ± 1.28	68.32 ± 0.39	>200
5	21.36 ± 1.62	48.56 ± 0.86	52.18 ±1.54	188.49 ± 3.28
6	14.98 ± 0.34	72.54 ± 2.41	75.22 ±0.63	>200
cisplatin	4.82 ± 0.35	4.27 ± 0.57	10.92 ± 1.38	14.11 ± 0.72

Table 2. Selectivity indices

compounds	IC_{50} (MRC-5)/IC_{50} (tumor cell line)		
	Fem-x	LS174T	A549
1	8.21 ± 0.86	4.58 ± 0.28	2.99 ± 0.06
2	> 7.95	> 5.31	> 4.38
3	6.45 ± 0.38	3.75 ± 0.09	3.15 ± 0.11
4	> 10.12	> 3.61	> 2.93
5	8.82 ± 0.69	3.88 ± 0.10	3.61 ±0.12
6	> 13.35	> 2.76	> 2.66
cisplatin	2.93 ± 0.26	3.30 ± 0.47	1.29 ± 0.18

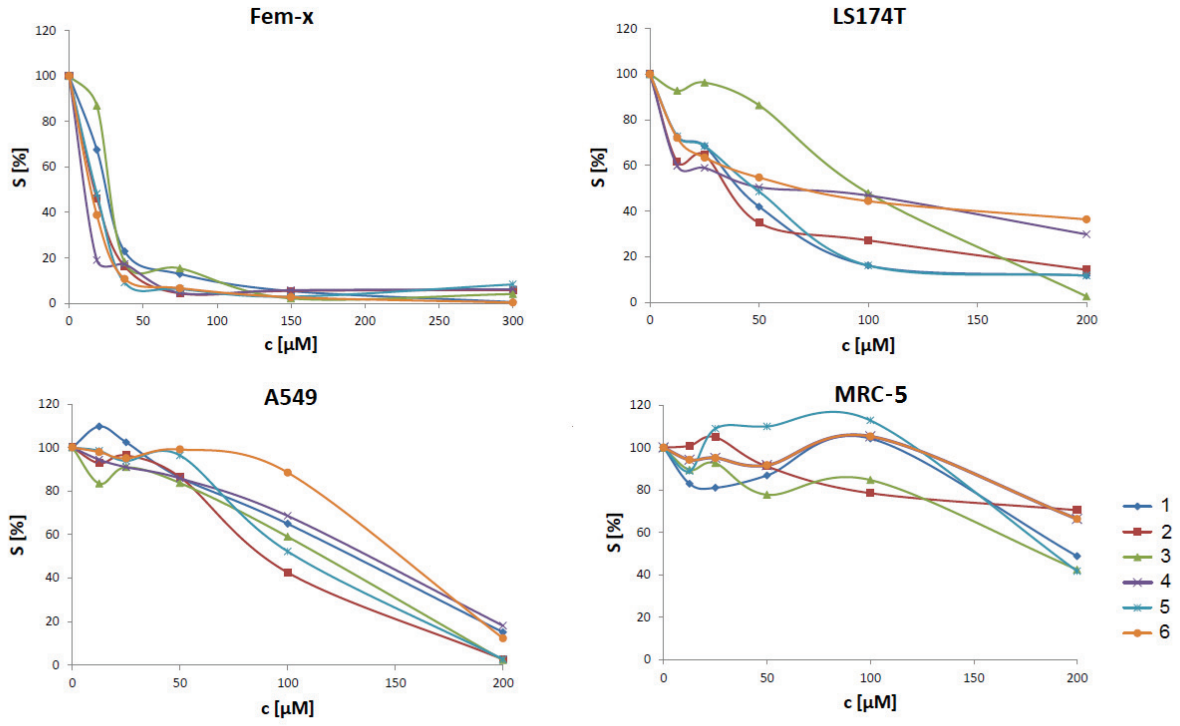


Figure 3. The survival of Fem-x, LS174T, A549 and MRC-5 cells incubated for 72 h with different concentrations of investigated gold(III) complexes (MTT assay)

nocompetent human peripheral blood mononuclear cells (PBMC) (22). This data point out that investigations concerning anticancer agents development require studies in diverse types of normal cells.

Additionally, on adherent cell lines complexes **1–6** exhibit significantly lower antitumor activity than on the nonadherent leukemic K562 cells (22). On the other hand, according to the rapidly dividing HeLa and Fem-x cells all complexes show a very similar, somewhat greater cytotoxicity (22).

Against the non-cancerous lung fibroblasts (MRC-5) all tested complexes were significantly less toxic than cisplatin. As can be seen from selectivity indices (Table 2) the selectivity of these gold(III) complexes is greater or similar than cisplatin as a reference drug. All complexes demonstrated the lowest selectivity indices on A549 cell lines, however 2–3 times higher than that of cisplatin. Complexes **1** and **2** showed the highest selectivity toward LS174T cells, even more than cisplatin, while for the other complexes it was similar to cisplatin. Considering selectivity of all complexes it was greatest toward Fem-x tumor cells. It should be noted that complex **6** shows more than 4.5 times greater selectivity on Fem-x than cisplatin and therefore it is a very promising candidate for further investigation.

CONCLUSION

The presented results showed that complex **6**, from all investigated gold(III) complexes, with isoamyl moiety in the κ^2-*N,N'* coordinated bidenatate ester ligand, exhibited the highest antitumor activity against Fem-x cell line (IC_{50} = 14.98±0.34 μM), from three cell lines tested. *In vitro* results indicate that these agents are cell type specific, for instance from the all tested tumor cell lines they exhibit the highest antitumor activity against leukemic K562 cells. Moreover, their toxicity is also cell type specific, they are less toxic against non-cancerous MRC-5 cells than on rested or stimulated PBMC. Additionally, the selectivity of these gold(III) complexes is similar or several times greater (up to 4.5 times) than cisplatin as a reference drug.

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Conflicts Of Interest

The authors declare no conflict of interest.

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