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Synthesis and biological activity of novel heavy metal complexes of 5-amino-1, 10-phenanthroline and 1,10-phenanthroline

Short Communication

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Abstract: Novel heavy metal complexes: Sr(5-NH_2-phen)$_4$(NO_3)(OH)(H_2O)$_2$ (**1**) (synthesized *via* a static self-assembly process) and Sn(phen)(NO_3)(OH)(H_2O) (**2**), Sn(5-NH_2-phen)(OH)(Cl)(H_2O) (**3**), Pb(5-NH_2-phen)(NO_3)$_2$(H_2O) (**4**) (obtained *via* metal competitive reactions under mild conditions) were reported. The coordination compounds were characterized by elemental analysis, FTIR-spectroscopy and FAB-mass spectrometry. Their cytotoxicity was measured by MTS-test towards human tumour (MDA-MB-231, HT-29, HeLa, HepG2) and non-tumour diploid (Lep-3) cell lines. The most pronounced cytotoxic effect on all cancer lines showed **1** and **4** at their high concentrations as well as **1** at its lower ones ($\leq 4\times10^{-2}$ mg). Therefore, strontium complex of 5-amino-o-phenanthroline (**1**) exhibited the widest antitumour spectrum activity, having no toxicity to non-tumour cells at quantities $\leq 4\times10^{-2}$ mg. The computed EC_{50} values of **1**-**4** against MDA-MB-231, HT-29, HeLa, HepG2 varied from 1.40×10^{-3} to 6.31×10^{-6} M. Towards Lep-3 substances **2**-**4** showed IC_{50} 7.52×10^{-4} - 0.44 M. Substance **1** possess $EC_{50}=1.26\times10^{-7}$ M to the non-tumour cells.

Keywords: *Metal complexes • Self-assembly • 5-amino-1,10-phenanthroline • 1,10-phenanthroline • Cytotoxic activity*

1. Introduction

Recently there has been a growing interest on the preparation and characterization of coordination compounds with various organic ligands. Some medicines and many other biologically active substances (*e.g.* enzymes, hormones, hemoglobin, chlorophyll, *etc.*) belong to this group.

One of the most promising approaches to the generation of complex supramolecular architectures is metal-driven self-assembly. They are prepared when metal-coordinated building blocks interact spontaneously with bridging units which are connected by weak non-covalent bonds. Molecules with a wide variety of topologies and shapes have been constructed in this way including boxes, microcycles, helicates and others [1,2].

However, there are almost no data on the synthesis and biological activity of complexes of 5-amino-1,10-phenanthroline (5-NH_2-phen) with alkaline earth and heavy metals, while there are some publications concerning coordination compounds of 1,10-phenanthroline (phen). Metal complexes with the following structure are described: [MeEtSn]Cl_2(phen)$_2$ [3], (phenH)[$SnFS_2$].xH_2O [4], [Pb(phen)$_2$(iso-Bu_2PS_2)$_2$] [5], [Pb_2(bta)$_2$(phen)$_2$].2H_2O, where (H_2bta) is N,N-bis[1(2) H-tetrazol-5-yl]amine [6]. Some other coordination compounds - [Ca(PDALC)$_2$](ClO_4)$_2$ and [Pb(PDALC)

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$(ClO_4)_2$], where (PDALC) is 2,9-bis(hydroxymethyl)-phen are reported as well [7]. Their crystal structures were solved by X-ray diffraction.

In our previous investigations, we reported on the synthesis, characterization and crystal structure of complexes of alkaline earth metal ions with phen. Under specific mild conditions, *via* self-assembly processes, we obtained the following complexes: $Mg(phen)_3(NO_3)_2(H_2O)_9$ [8], $[Ca(phen)_2(H_2O)_2(NO_3)]NO_3$ [9] and $Ba(phen)_2(NO_3)_2(H_2O)_2$ [10]. The X-ray diffraction analysis revealed that in the calcium complex only one of the nitrate anion is coordinated to the metal ion, while another is in the outer shell [9]. We also described the synthesis of some metal complexes of phen as well as of 5-NH_2-phen with composition: $Pd(phen)_2(H_2O)(NO_3)_2$ [11] and Pd(5-NH_2-phen)$_2(NO_3)_2$ [12]. It is noteworthy to mention the various biological activity of the resulting complexes. Coordination compounds of alkaline earth metals (Mg, Ca, Ba) with phen and their palladium(II) analogue show high antibacterial activity, especially against *Escherichia coli*. Moreover, they have no acute toxicity when administered intraperitoneally (*i.p.*) in rats at doses up to 10 mg [11]. The complex Pd(5-NH_2-phen)$_2(NO_3)_2$ possesses strong anticancer activity against a 100% lethal myeloid subcutaneous tumour in hamsters.

On the other hand, its phen analogue - $Pd(phen)_2(H_2O)(NO_3)_2$ did not reveal any antitumour activity [12]. This led us to obtain and study the anticancer activity of other heavy metal complexes with 5-NH_2-phen. It should be noted that compounds Pd(5-NH_2-phen)$_2(NO_3)_2$ and $Pd(phen)_2(NO_3)_2(H_2O)$ did not show any histological alterations in the observed lung, liver, spleen and lymph nodes of White Wistar rats as well as any cytotoxic effect. The tests for immunological response predominantly showed stimulation of the antibody-producing B-cells [13]. Some new molecular complexes of phen with aromatic heterocycles also showed well expressed anticancer activity and lack of toxicity towards non-tumour cells [14].

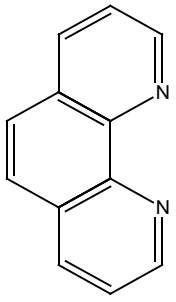
The synthesis of mixed-ligand complexes of palladium(II) with phen and 5-NO_2-phen, possessing antitumour activity, have been described [15,16]. Narla *et al*. [17] reported on anticancer agent containing vanadium and 4,7-dimethyl-phen (Me_2-phen) with the formula VO(Me_2-phen)$_2$.

Aim of the present study is the obtaining of a new coordination compound of the heavy alkaline earth metal (Sr) with a rarely used ligand 5-NH_2-phen throughout a self-assembly process. *Via* competitive reactions between heavy (Sn and Pb) and alkaline earth (Ca) metals, novel complexes of phen and 5-NH_2-phen will be synthesized. Their citotoxicity towards some human tumour cell lines also will be tested.

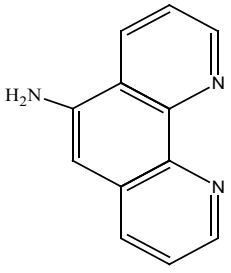
2. Experimental procedure

2.1. Chemistry

A novel coordination compound of heavy alkaline earth metal (Sr) with 5-amino-1,10-phenanthroline with a composition $Sr(5\text{-}NH_2\text{-}phen)_4(NO_3)(OH)(H_2O)_2$ (**1**) was synthesized *via* a static self-assembly process. This is possible due to weak interactions between an organic ligand and metal having s^2-electron configuration. To obtain other three new complexes: $Sn(phen)(NO_3)(OH)(H_2O)$ (**2**), $Sn(5\text{-}NH_2\text{-}phen)(OH)(Cl)(H_2O)$ (**3**) and $Pb(5\text{-}NH_2\text{-}phen)(NO_3)_2(H_2O)$ (**4**), competitive reactions between heavy metal cations ($Sn^{2+}$and Pb^{2+}), possessing a p^2-electron configuration, and Ca^{2+} in a water-alcohol solution at the presence of phen or 5-NH_2-phen were conducted.



1,10-phenanthroline (phen)



5-amino-1,10-phenanthroline (5-NH_2-phen)

The synthesized compounds were characterized by elemental analysis, FTIR-spectroscopy and FAB-mass spectrometry.

2.1.1. Chemicals and apparatus

1,10-Phenanthroline.H_2O, $Ca(NO_3)_2$•$4H_2O$, $Sr(NO_3)_2$, $SnCl_2$•$2H_2O$ and $Pb(CH_3COO)_2$•$3H_2O$ were obtained from Merck, Darmstadt. 5-Amino-1,10-phenanthroline.H_2O was prepared in our laboratory from

5-nitro-1,10-phenanthroline by reduction with hydrazine hydrate using Raney-nickel as a catalyst. All other chemicals used were of the highest available quality. Composition of the products was determined by elemental analysis on a VarioEl (Elemental analysen systeme GmbH). FAB-mass spectra were taken on a VG autospec mass spectrometer. The samples were dissolved in NBA and lactic acid. Infrared spectra were recorded on a Perkin-Elmer 1600 Series FTIR spectrophotometer in the range of 4400–450 cm^{-1}, resolution 4 cm^{-1}, in KBr pellets.

2.1.2. Synthesis of $Sr(5\text{-}NH_2\text{-}phen)_4(NO_3)(OH)(H_2O)_2$ (1)

0.096 g (0.450 mmol) 5-amino-1,10-phenanthroline•H_2O, 0.355 g (1.677 mmol) $Sr(NO_3)_2$ and 2.5 mL of 96% ethanol were dissolved in 20 mL distilled water at 70°C. The solution was hot filtered, cooled to room temperature and water was added to 25 mL. The filtrate stayed in a refrigerator at 2–3°C for one month. Yellow crystals formed, filtered off, washed with cold distilled water and dried in a desiccator over P_4O_{10}. Yield: 0.052 g.

Anal. calcd. for $C_{48}H_{41}N_{13}O_6Sr$ (C, H, N): Calculated (%): C, 58.56; H, 4.16; N, 18.50. Found (%): C, 58.70; H, 4.57; N, 18.53. FTIR (cm^{-1}): 3440, 3350, 3226, 1636, 1513, 1384, 824. FAB-mass (m/z): 196 (5-NH_2-phen).H^+, 391 (5-NH_2-phen)$_2$.H^+, 413 (5-NH_2-phen)$_2$.Na^+, 476 [Sr(5-NH_2-phen)$_2$-H^+]$^+$.

2.1.3. Synthesis of $Sn(phen)(NO_3)(OH)(H_2O)$ (2)

0.386 g (1.950 mmol) 1,10-phenanthroline•H_2O, 0.519 g (2.301 mmol) $SnCl_2$•$2H_2O$, 0.590 g (2.498 mmol) $Ca(NO_3)_2$•$4H_2O$ and 10 mL of 96% ethanol were diluted in 80 mL distilled water at 70°C for 30 min. The solution was hot filtered, cooled to room temperature and water added to 100 mL. The filtrate stayed in a refrigerator at 2–3°C for 24 h. Amorphous yellow residue formed was filtered off, washed with cold distilled water, diethyl ether and dried in a desiccator over P_4O_{10}. Yield: 0.137 g.

Anal. calcd. for $C_{12}H_{11}N_3O_5Sn$ (C, H, N): Calculated (%): C, 36.32; H, 2.78; N, 10.60. Found (%): C, 36.21; H, 2.85; N, 10.54. FTIR (cm^{-1}): 3490, 3327, 1519, 1385, 824. FAB-mass (m/z): 181 (phen).H^+, 217 (phen).H^+.$2H_2O$, 235 (phen).H^+.$3H_2O$, 361 [Sn(phen)(NO_3)]$^+$, 541 [Sn(phen)$_2(NO_3)$]$^+$.

2.1.4. Synthesis of $Sn(5\text{-}NH_2\text{-}phen)(OH)(Cl)(H_2O)$ (3)

0.096 g (0.450 mmol) 5-amino-1,10-phenanthroline•H_2O, 0.148 g (0.627 mmol) $Ca(NO_3)_2$•$4H_2O$, 0.141 g (0.625 mmol) $SnCl_2$•$2H_2O$ and 2.5 mL of 96% ethanol were dissolved in 20 mL distilled water at 70°C. The solution was hot filtered, cooled to room temperature and water added to 25 mL. The filtrate stayed in a refrigerator at 2–3°C for one week. Amorphous yellow residue formed, were filtered off, washed with cold distilled water and ether, and dried in a desiccator over P_4O_{10}. Yield: 0.065 g.

Anal. calcd. for $C_{12}H_{12}N_3O_2ClSn$ (C, H, N): Calculated (%): C, 37.49; H, 3.12; N, 10.93. Found (%): C, 37.89; H, 2.72; N, 11.26. FTIR (cm^{-1}): 3404, 3324, 3225, 1638, 1516. FAB-mass (m/z): 154 $(SnCl)^+$, 196 (5-NH_2-phen).H^+, 385 Sn(5-NH_2-phen).H^+Cl_2, 391 (5-NH_2-phen)$_2$.H^+, 413 (5-NH_2-phen)$_2$.Na^+.

2.1.5. Synthesis of $Pb(5\text{-}NH_2\text{-}phen)(NO_3)_2(H_2O)$ (4)

0.096 g (0.450 mmol) 5-amino-1,10-phenanthroline•H_2O, 0.148 g (0.627 mmol) $Ca(NO_3)_2$•$4H_2O$, 0.237 g (0.625 mmol) $Pb(CH_3COO)_2$•$3H_2O$ and 2.5 mL of 96% ethanol were dissolved in 20 mL distilled water at 70°C. The mixture was hot filtered, cooled to room temperature and water added to 25 mL. The filtrate stayed in a refrigerator at 2–3°C for one week. Yellow crystals formed, were filtered off, washed with cold distilled water and dried in a desiccator over P_4O_{10}. Yield: 0.099 g.

Anal. calcd. for $C_{12}H_{11}N_4O_7Pb$ (C, H, N): Calculated (%): C, 46.38; H, 2.89; N, 18.03. Found (%): C, 46.43; H, 2.87; N, 17.79. FTIR (cm^{-1}): 3430, 3342, 3227, 1635, 1515, 1362, 822. FAB-mass (m/z): 196 (5-NH_2-phen).H^+, 465 [Pb(5-NH_2-phen) (NO_3)]$^+$, 660 [Pb(5-NH_2-phen)$_2(NO_3)$]$^+$.

2.2. Citotoxicity assays

The cytotoxicity of the substances was measured *in vitro* using the cultivated human tumour cell lines: epithelial colorectal carcinoma HT-29, breast cancer epithelial-like morphology MDA-MB-231, epithelial cervix adenocarcinoma HeLa, hepatocellular carcinoma liver HepG2 and non-tumour diploid cell line Lep-3 (as a control). Compounds **1**, **2**, **3** and **4** were evaluated for their cytotoxicity *in vitro*, towards the following cultivated human tumour and non-tumour cell lines, using MTS-test [18]:

- Breast cancer cells, epithelial-like morphology MDA-MB-231 (American Type Culture Collection ATCC HTB-26).
- Human epithelial colorectal carcinoma HT-29 (American Type Culture Collection ATCC HTB-38).
- Epithelial cervix adenocarcinoma HeLa (American Type Culture Collection ATCC CCL-2).
- Differentiated hepatocellular liver carcinoma HepG2 (American Type Culture Collection, Rockville, MD, USA).

- Non-tumour diploid cell line Lep-3 (as a control).
- MTS - [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrasolium inner salt].

2.3. Toxicity was determined by the MTS-test

All cells were raised in culture medium DMEM supplemented with 10% fetal calf serum (FCS) and 100U penicillin, incubated with ascending 10-fold dilutions of the initial (4 mg mL^{-1}) substance solutions. Cells were seeded in 96-well flat-bottomed microplates (Orange scientific) at a concentration of 2×10^4 cells/well. At the 24th h of incubation at 37^{o}C under 5% carbon dioxide and 95% air atmosphere to each sample were added 20 µL MTS. Each amount was applied in 4 wells. Samples of cells grown in non-modified medium served as a control. The absorbance of each well at 490 nm was read by an automatic microplate reader (Absorbance Reader "Tecan" (Austria). Relative cell *via*bility, expressed as a percentage of the untreated control (100% *via*bility), was calculated for each quantity. Applied amount response curves were constructed manually for each experiment. All data points represent an average of three independent assays.

3. Statistical analysis

Statistical deviations were calculated automatically by Excel 2007 software program.

4. Results and discussion

Compound **1**, in the form of yellow crystals, was obtained through a static self-assembly process by interaction of 5-NH_2-phen and $Sr(NO_3)_2$ in water-alcohol media under mild conditions. For this process to be energetically favorable, reducing the Gibbs free energy is necessary. Thus the self-assembled structure is thermodynamically more stable than the raw, unassembled components.

Compounds **2** and **3** were produced as a result of competitive reactions between two metal cations (Sn^{2+} and Ca^{2+}) with phen or 5-NH_2-phen. As might be expected, in both synthesis, complexes containing only one metal atom (Sn) were formed. Compound **4**, containing lead, was obtained in a similar way *via* interaction between $Pb(CH_3COO)_2\bullet3H_2O$, $Ca(NO_3)_2\bullet4H_2O$ and 5-NH_2-phen under mild conditions. This is possible because tin and lead are p-elements and unlike alkaline earth metals rapidly form complexes *via* covalent bonds with the ligands used. Additional evidence for the formation of metal complexes described (**1-4**) are characteristic stretching vibrations ($\nu C\!=\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-C$) observed in their FTIR spectra. They range from 1513 cm^{-1} to 1519 cm^{-1}, while the corresponding value for o-phen is 1503 cm^{-1} and for 5-NH_2-phen is 1505 cm^{-1}. Also in the spectra of complexes **1**, **3** and **4** absorption bands typical for the organic ligand, in the range of 3324 - 3350 cm^{-1} for ν_{as} NH_2 as well as of 3225 -3227 cm^{-1} for ν_s NH_2, were clearly observed. In all spectra of the compounds, the signals for H_2O molecule stretching vibrations (3404 - 3490 cm^{-1}) were also presented. In the case of **1** and **3**, signals for OH^- anions overlaped with those for ν_{as} NH_2 groups. Two other characteristic bands in FTIR spectra of compounds **1**, **2** and **4** were those for NO_3^- anions in the ranges (1362 - 1385 cm^{-1}) and (822-824 cm^{-1}). Unfortunately, detailed structure studies of the new complexes by X-ray diffraction analysis were not possible because of the difficulties in obtaining suitable single crystals.

The sensitivity of some human cell lines, expressed as % vitality, to different amounts of the tested substances are presented in Figs. 1-5.

The normal diploid cell line (Lep-3) (Fig. 1) showed a proliferative response (vitality above 100%) when incubated with lower substance amounts, with exception of **2**. It is worth noting that this complex showed slight toxicity (80% vitality) even at high concentration. Compound **4**, containing a lead atom, was not toxic to normal cells at all concentrations tested.

All tumour cell lines used were sensitive to the four compounds in a different way, but the general trend was that all these substances suppressed the cells' proliferation capacity. Most sensitive were HeLa, followed by MDA-MB-231 and HepG2 cells (Figs. 2,4,5). Human epithelial colorectal carcinoma HT-29 (Fig. 3) line was affected by substances tested only in their high doses. The most sensitive HeLa cells revealed almost equal inhibition by the examined substances at low quantities, but above 4×10^{-2} mg their vitality was about 20-25%. The complexes tested increased vitality of MDA-MB-231 (Fig. 2) at amounts below 4×10^{-2} mg and in comparison to HeLa it reached level up to 70-75%. From Fig. 3, one can realize that human colorectal cancer line was inhibited by all compounds only at 4 and 0.4 mg but proliferated quickly (above 100%) at their lower concentrations. HepG2 did not reach 100% vitality in all cases, but nevertheless it was not as sensitive as HeLa line (Figs. 4,5). Complex **3** was not so strongly toxic towards hepatocellular liver carcinoma like other compounds.

The lack of inhibition effect of all lower substance doses as well as of **4** and **2** (particularly at their high

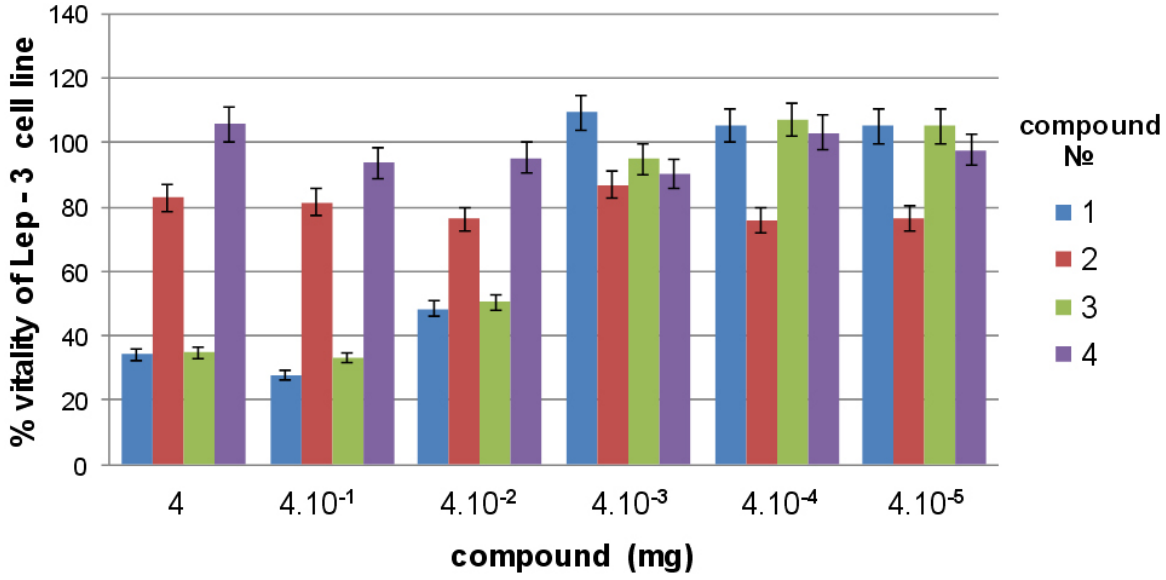


Figure 1. Vitality (%) of non-tumour human diploid cells Lep-3 (as a control), treated with substances **1- 4**.

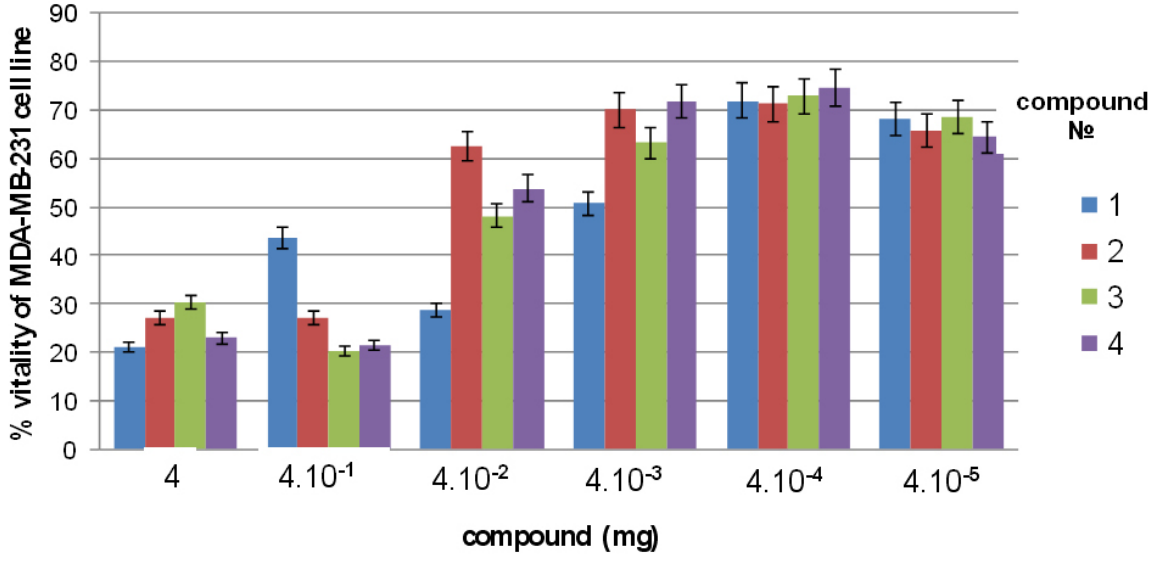


Figure 2. Vitality (%) of human breast cancer cells, epithelial-like morphology MDA-MB-231, treated with substances **1- 4**.

quantities) toward normal diploid cells (Fig. 1) is a sign that these compounds can be used potentially as non-toxic tumour inhibitors.

Comparing the effects of **2** and **3** to normal cells, it was found that in high amounts **2** was less toxic. The general trend is that **3** was a weaker tumour inhibitor against HepG2, but the activity of both compounds was comparable to other lines. The priority of **3** at low doses was a less pronounced toxicity to Lep-3, compared with **2**, probably due to the presence of electron donor amino group directly connected to the phenanthroline aromatic system at fifth place.

In summary, it can be stated that the best pronounced inhibitor activity against the four human tumour lines showed compounds **1** and **4** at their high amounts as well as **1** at its lower ones (≤ 4×10^{-2} mg). Therefore, the strontium complex of 5-amino-o-phenanthroline displays the widest anticancer activity spectrum as well as the least toxicity to non-tumour cells at low doses. This makes **1** suitable for further in-depth studies *in vivo*. These results are in good agreement with our previous studies where the complex of 5-amino-1,10-phenanthroline with Pd(II) again demonstrated a well pronounced antitumour activity. This complex displayed the longest mean survival time (2.2 times longer than the controls) in hamsters infected with cancerous (100% lethality) myeloid subcutaneous tumour [12]. In addition,

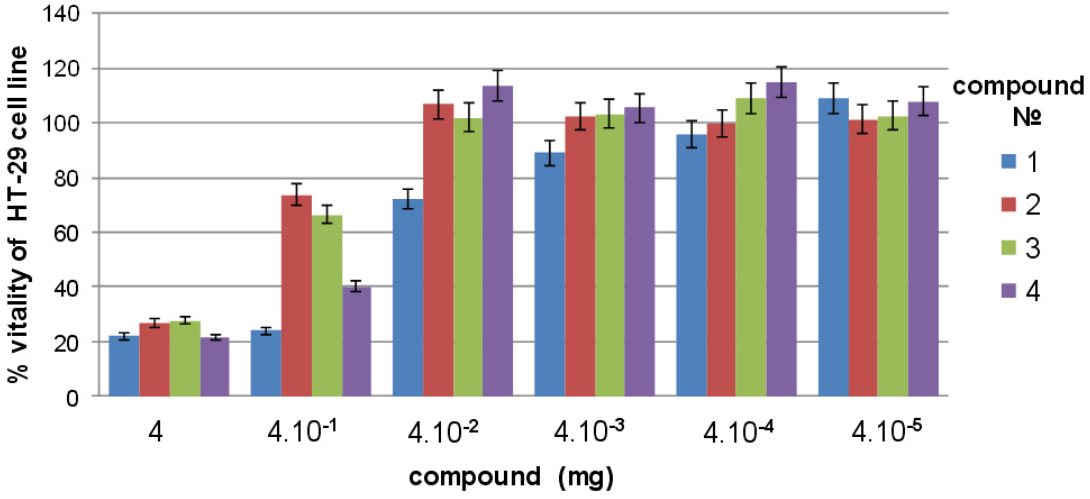


Figure 3. Vitality (%) of human epithelial colorectal carcinoma HT-29, treated with substances **1- 4**.

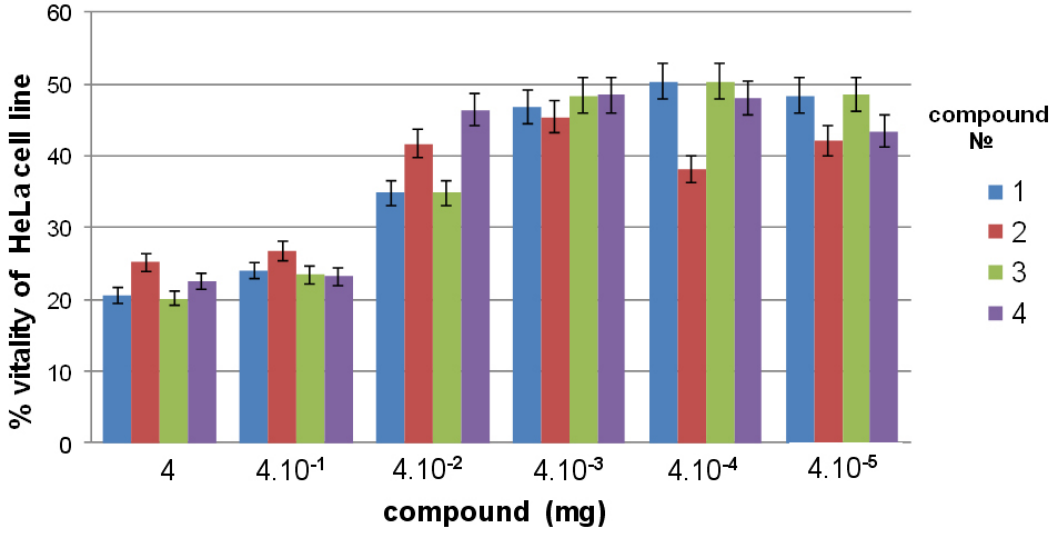


Figure 4. Vitality (%) of human epithelial cervix adenocarcinoma HeLa, treated with substances **1- 4**.

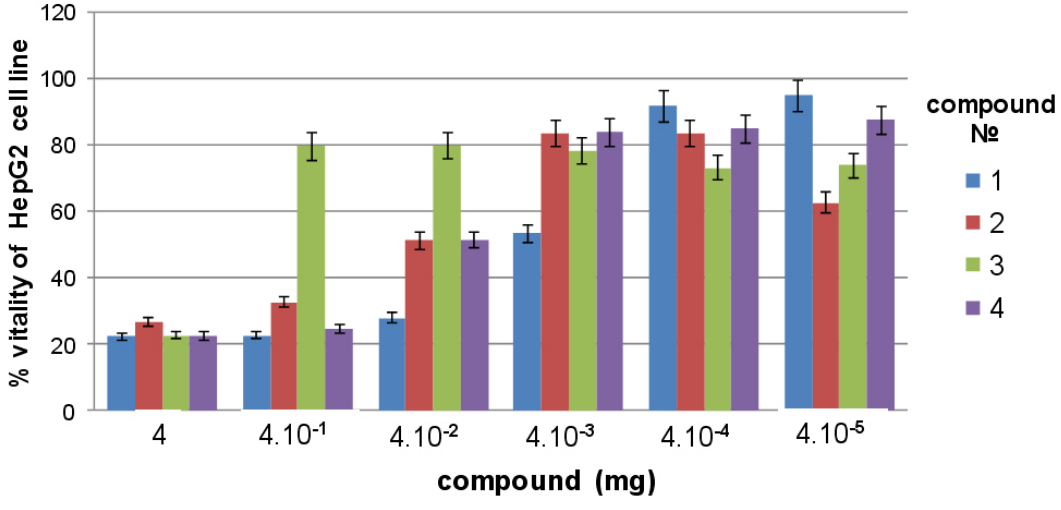


Figure 5. Vitality (%) of human differentiated hepatocellular liver carcinoma HepG2, treated with substances **1- 4**.

Table 1. *In vitro* proliferative effects of **1 - 4** towards MDA-MB-231, HT-29, HeLa, HepG2 and Lep-3 cells.

Compound	EC_{50} ± SE (M) MDA-MB-231	HT-29	HeLa	Hep G2	Lep-3
1	6.31 e-6 ± 0.404	6.24 e-5 ± 0.545	4.01 e-5 ± 0.234	3.12 e-6 ± 0.594	1.27 e-7 ± 0.457
2	4.23 e-5 ± 0.361	1.14 e-3 ± 0.524	6.50 e-4 ± 0.158	7.61 e-5 ± 0.407	ND
3	1.36 e-4 ± 0.390	1.40 e-3 ± 0.658	2.76 e-4 ± 0.219	1.76 e-4 ± 0.387	ND
4	6.44 e-5 ± 0.416	7.68 e-4 ± 0.701	2.67 e-4 ± 0.217	6.00 e-3 ± 0.483	ND

N.D. - not determined

Table 2. *In vitro* suppresion of Lep-3 cells.

Compound	IC_{50} ± SE (M)
2	4.44e-1 ± 0.133
3	6.88e-5 ± 0.465
4	7.52e-4 ± 0.328

$Pd(5\text{-}NH_2\text{-phen})_2(NO_3)_2$ showed good stimulation of the antibody-producing B-cells and of all lymphocytes as well as did not show any histological alterations and cytotoxic effect on White Wistar rats [13].

Having in mind the MTS proliferation assay is based on the fact that MTS tetrazolium compound is bioreduced by cells into a colored formazan product, soluble in the tissue culture medium and this conversion is accomplished by NADPH or NADH, produced by dehydrogenase in metabolically active cells [19], we undertook the presented investigation. The bigger release amount of formazan indicates to a higher vitality of the cells (proliferation). The low vitality demonstrates a cytotoxic influence of the experimental compounds.

The calculated EC_{50} values of **1**-**4** against MDA-MB-231, HT-29, HeLa and HepG2 varied from 1.40×10^{-3} to 6.31×10^{-6} M (Table 1). Substances **2**-**4** showed IC_{50} against Lep-3 from 7.52×10^{-4} to 0.44 M (Table 2). Compound **1** possess $EC_{50}=1.26\times10^{-7}$ M to the normal non-tumour human cells.

5. Conclusions

A new metal complex $Sr(5\text{-}NH_2\text{-phen})_4(NO_3)(OH)(H_2O)_2$ (**1**) was synthesized *via* a static self-assembly process. Three novel coordination compounds: $Sn(phen)(NO_3)(OH)(H_2O)$ (**2**), $Sn(5\text{-}NH_2\text{-phen})(OH)(Cl)(H_2O)$ (**3**) and $Pb(5\text{-}NH_2\text{-phen})(NO_3)_2(H_2O)$ (**4**) were obtained *via* metal competitive reactions in a water-alcohol solution under mild conditions.

The cytotoxicity of complexes **1**- **4** was measured using some cultivated human tumour cell lines (MDA-MB-231, HT-29, HeLa and HepG2).

As it was expected all substances showed different activities depending on the cell line and amount of the compound tested. The best pronounced cytotoxic effect on all cancer lines showed **1** and **4** at their high doses as well as **1** at its lower ones ($\leq 4\times10^{-2}$ mg). Therefore, the strontium complex of 5-amino-o-phenanthroline (**1**) exhibited the widest antitumour activity spectrum although having no toxicity towards non-tumour cells at quantities $\leq 4\times10^{-2}$ mg.

Calculated EC_{50} values of **1**-**4** against all tumour cell lines used varied from 1.40×10^{-3} to 6.31×10^{-6} M.

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