Scientific paper

New Synthetic Routes for Ruthenium-1,10-Phenanthroline Complexes. Tests of Cytotoxic and Antibacterial Activity of Selected Ruthenium Complexes.

Iztok Turel,[1,*] Amalija Golobič,[1] Jakob Kljun,[1] Petra Samastur,[1] Urška Batista[2] and Kristina Sepčić[3]

[1] *Faculty of Chemistry and Chemical Technology, University of Ljubljana, Večna pot 113, SI-1000 Ljubljana, Slovenia.*

[2] *Institute of Biophysics, Faculty of Medicine, University of Ljubljana, Lipičeva 2, SI-1000 Ljubljana, Slovenia*

[3] *Biotechnical Faculty, Department of Biology, University of Ljubljana, Večna pot 111, SI-1000 Ljubljana, Slovenia.*

** Corresponding author: E-mail: iztok.turel@fkkt.uni-lj.si*
Tel: +386 1 4798525

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Dedicated to the memory of Prof. Dr. Jurij V. Brenčič.

Abstract

Three novel complexes have been prepared through reactions of precursor $[(dmso)_2H][trans\text{-}RuCl_4(dmso\text{-}S)_2]$ (**P**) and 1,10-phenanthroline (phen) at different conditions. Whereas the analogs of *mer*-$[RuCl_3(dmso\text{-}S)(phen)]$ (**1**) and $[Ru(phen)_3]Cl_2 \cdot 6CH_3OH$ (**3** · $6CH_3OH$) have already been prepared by other synthetic routes before, product $(H_3O)[RuCl_4(phen)] \cdot 4H_2O$ (**2** · $4H_2O$) is unprecedented. In the latter, isolated from highly acidic medium, also the second, strongly bound dmso molecule in precursor **P** was substituted by chloride. Biological activities of **1** and previously isolated ruthenium-purine complexes ([*mer*-$RuCl_3$(dmso-*S*)(acv)(CH_3OH)] (**4**) (acv = acyclovir); [*trans*-$RuCl_4$(dmso-*S*)(guaH)] (**5**) (guaH = protonated guanine) were tested and compared. These data show that compounds **1, 4** and **5** are slightly cytotoxic against B-16 malignant melanoma cells but not against non-transformed V-79-379A cells. The results indicate that coordinated phen ligand increases the cytotoxicity of **1** in comparison to ruthenium precursor. The inability of tested compounds to induce lysis of bovine erythrocytes shows that their cytotoxic effect is not due to the membrane damage.

Keywords: Ruthenium complexes, Phenanthroline, X-ray structure, Biological activity

1. Introduction

Ruthenium-dimethylsulfoxide (dmso) complexes are useful precursors in inorganic synthesis and their chemistry has been thoroughly studied for a long time.[1,2] The products prepared through such reactions exert versatile interesting properties which are applicable for example in asymmetric synthesis[3] in design of new drugs[4,5] and other.

The most famous representative of biologically active compounds from the group is NAMI-A ([ImH][*trans*-$RuCl_4$(dmso-*S*)Im] (Im = imidazole)) which was prepared from $[(dmso)_2H][trans\text{-}RuCl_4(dmso\text{-}S)_2]$. The isolation of NAMI-A has shown that the presence of simple *N*-donor ligands on ruthenium(III) species is a requirement for finding favorable biological activity.[6] NAMI-A is not cytotoxic for cancer cells *in vitro* but shows a strong antimetastatic effect *in vivo*. This compound has undergone advanced preclinical trials, phase I clinical trials and is currently undergoing phase II clinical studies as anti-metastatic drug.[7,8] All these findings triggered extensive exploration of new ruthenium-dmso compounds that may bear a biological potential.

On the other hand, ruthenium complexes with 1,10-phenanthroline (phen) or 2,2'-bipyridine (bipy) and their derivatives are also extensively studied due to their interesting physico-chemical and biological properties.[9–15] Amongst these the most studied are tris chelate complexes. Such complexes are namely useful in elucidating chemical principles which govern the recognition of nucleic acids, in developing photochemical reagents as new diag-

nostic tools, in the design of novel chemotherapeutics[16–19] and in electron transfer mediated by the DNA double helix. Though mono- and bis-oligopyridyl ruthenium complexes have not been studied so thoroughly as tris-complexes, their useful properties as precursors or catalysts are also known.[20,21]

The aims of this work were (**i**) to study the reactivity of precursor [$(dmso)_2H$][*trans*-$RuCl_4$(dmso-*S*)$_2$] (abbreviation **P**) with 1,10-phenanthroline at different conditions, and (**ii**) to assess the biological activity of the selected products. Whereas there has been extensive research on cytotoxic ruthenium compounds, related studies on anti-viral[22] and antibacterial ruthenium compounds remain sparse.[23–26] Therefore we have decided to test not only the cytotoxicity of selected compounds (against normal and tumor cells) but also their antibacterial potential. Additionally, hemolysis of bovine erythrocytes was performed in order to check whether tested compounds can damage the cell membrane.

2. Materials and Methods

2. 1. Syntheses

The ruthenium precursor **P** and compounds **4** and **5** were prepared as reported.[1,5,27] All other chemicals and solvents were commercially available (Sigma-Aldrich, U.S.A.) and were used without prior drying or purification.

mer-[$RuCl_3$(dmso-*S*)(phen)] (1)

The precursor **P** (0.0600 g; 0.108 mmol) was dissolved in MeOH (20.0 mL) and phenanthroline (0.0214 g; 0.108 mmol) was added while stirring vigorously. The orange solution was refluxed for 3 h. Brown-red crystals appeared in the solution after five days of isothermal evaporation of the solvent in air. The crystals were filtered out, washed with diethyl ether and dried in air. Similar syntheses can be performed in other solvents and result in the formation of structurally similar solvate containing products *mer*-[$RuCl_3$(dmso-*S*)(phen)]·X (X = methanol, ethanol, acetone). Found for **1**: C, 36.11; H, 2.78; N, 6.03; Calc. for $C_{14}H_{14}Cl_3N_2OSRu$ (M_r= 465.76): C, 36.10; H, 3.03; N, 6.01). Selected IR bands (Nujol, cm^{-1}): 1636 (s), 1626 (s), 1592 (s) (phen ring C=C, C=N); 1093 (s, S=O for dmso-S); 427 (w, Ru-S). UV (MeOH) λ_{max}/nm (ε/dm^3 mol^{-1} cm^{-1}) 296 (7660); 401 (6671).

(H_3O)[$RuCl_4$(phen)]·$4H_2O$ (**2**·$4H_2O$) (2)

Compound **1** (0.010 g; 0.0215 mmol) was suspended in 10.0 mL of concentrated HCl and refluxed for 4 h to form a clear yellow solution. After several days of isothermal evaporation of the solvent in air few brownish crystals appeared in the solution. Compound **2** · $4H_2O$ is unstable in air and was only characterized by X-ray crystallography.

[$Ru(phen)_3$]Cl_2 · $6CH_3OH$ (**3** · $6CH_3OH$) (3)

The synthesis was carried out solvothermally in a Teflon container. The precursor **P** (0.0799 g, 0.144 mmol) was dissolved in MeOH (0.7 mL) to form an orange solution. Phenanthroline (0.0853 g, 0.432 mmol) was added while stirring vigorously. A dark orange suspension was put in stainless steel autoclave followed by heating at 130 °C for 24 h under autogeneous pressure. Facile loss of interstitial solvent was observed for compound **3.** Found: C, 57.08; H, 4.52; N, 9.85 Calc. for $C_{40}H_{40}Cl_2N_6O_4Cl_2Ru$: C, 57.13; H, 4.80; N, 10.0). Selected IR bands (Nujol, cm^{-1}): 1649 (s), 1633 (s), 1573 (s) (phen ring C=C, C=N). UV (MeOH) λ_{max}/nm (ε/dm^3 mol^{-1} cm^{-1}) 260 (95849) 416 (15062); 444 (15996).

2. 1. 1. Elemental Analyses

Elemental analyses were performed on a Perkin-Elmer 204C microanalyser (U.S.A.).

2. 1. 2. Spectroscopy (IR, UV-Vis)

IR spectra were recorded on a Perkin-Elmer FTIR 1720 spectrometer (U.S.A.) and on a Perkin-Elmer 2000 FTIR spectrometer (U.S.A.) in Nujol with CsI windows. The UV-VIS spectra of the solutions were recorded on a Perkin Elmer Lambda 19 spectrometer (U.S.A.).

2. 1. 3. X-ray Structural Analysis

Diffraction data for all three compounds were collected on a Nonius Kappa CCD diffractometer with graphite monochromatic Mo$K\alpha$ radiation at temperature 150 K. The data were processed using DENZO program. Structures were solved by direct methods using SIR97. Most of the positions of hydrogen atoms were obtained from the difference Fourier maps, the remaining were calculated. We employed full-matrix least-squares refinements on F magnitudes with anisotropic displacement factors for non-hydrogen atoms using Xtal3.6. The crystallographic data were deposited in the Cambridge Crystallographic Data Center and were assigned the deposition numbers CCDC 1028368–1028370 for compounds **1**–**3** respectively. References for the crystallographic software are given in the Supporting Information file.

2. 2. Biological Assays

2. 2. 1. Cells and Preparation of Ruthenium Solutions

The cells V-79-379A (diploid lung fibroblasts of Chinese hamster) and B-16 (mouse melanoma) were

grown in Eagle minimal essential medium (MEM, Gibco, Paisley, Scotland) supplemented with 10% fetal calf serum (FCS) (Sigma, Germany), penicillin (100 U/mL) and streptomycin (100 μg/mL) at 37 °C in CO_2 incubator.

The stock solutions (500 μM) of each compound were prepared in culture medium. Test solutions (1, 10, 100 μM) were made by dilution in complete medium, containing 10% FCS just before experiments.

2. 2. 2. In Vitro Cytotoxicity Assay (MTT test)

Cells were plated in 96-well microtiter plates (Costar, U.S.A.) at concentration 5000 cells/well. The ruthenium compounds at concentrations of 1, 10 and 100 μM, prepared in medium with serum, were added for 48 hours. The cytotoxicity was determined with MTT test. After the addition of 20 μL of MTT (5 mg/mL) (Sigma, Germany) to each well (100 μL) for 3 h, MTT-containing medium was carefully removed. The formazan crystals were dissolved in 100 μL dmso (Sigma, Germany) and the absorbance was measured at 570 nm using Anthos microplate reader (Anthos, Australia). Proliferation (%) was expressed as ratio between optical density of treated cells and untreated cells after 48 hours of growth. The data are presented by means with standard deviations of 3–4 independent experiments. The differences were analyzed using Student's t-test on two populations with $p < 0.05$ and $p < 0.001$ considered significant.

2. 2. 3. Determination of Hemolytic Activity

Bovine erythrocytes were centrifuged from freshly collected citrated blood and washed twice with an excess of 0.9% saline and once with 140 mM NaCl, 20 mM TRIS.HCl buffer pH 7.4. Hemolytic activity was measured by a turbidimetric method.[28] Stock solution (1 mg/mL) of tested compounds in 50 mM TRIS.HCl buffer pH 7.4 was progressively diluted by two-fold in the same buffer, and 100 μL of resulting solutions were combined with 100 μL of erythrocyte suspension. The initial absorbance of the lysing mixture at 650 nm was 0.5. The potential decrease of optical density, due to the direct membrane damage and consequent hemolysis, was recorded for 30 minutes at 650 nm using a Kinetic Microplate Reader (Dynex Technologies, U.S.A.). All experiments were performed at 25 °C, repeating every measurement three times. Marine sponge-derived polymeric alkylpyridinium salts (poly-APS) were used in the final concentration of 10 μg/mL as a positive, hemolytically active control.[29]

2. 2. 4. Determination of Antibacterial Activity

The following bacterial strains were used: *Staphylococcus aureus* EXB-V54, *Micrococcus luteus* EXB-V52, *Bacillus subtilis* EXB-V68, *Escherichia coli* EXB-V1, *Proteus vulgaris* EXB-V17, *Klebsiella pneumoniae* EXB-V12, *Pseudomonas aeruginosa* EXB-V28, and *Salmonella typhimurium* EXB-V8. The strains were obtained from the culture collections of the Chair of Molecular Genetics and Microbiology of the Biotechnical Faculty, University of Ljubljana.

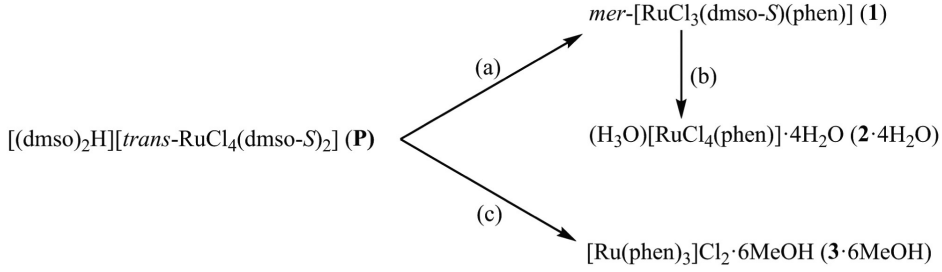
Activities were evaluated using the standard agar diffusion test.[30] Bacteria were allowed to grow overnight and their concentration was then determined. Bacterial culture was incorporated in Lauria Broth nutrient agar previously cooled to 42 °C. The final concentration of bacteria was approximately 5×10^5 colony forming units (CFU)/mL. Twenty milliliters of inoculated medium were poured into Petri dishes and kept at 4 °C until use. Circles of agar ($\Phi = 1$ cm) were cut out from the cooled medium.

For estimating minimal inhibitory concentration (MIC), the compounds were progressively diluted by five-fold in TRIS.HCl buffer pH 7.4. 100 μL of each dilution were poured into the holes cut in the inoculated medium, after which the agar plates were kept at 37 °C for 24 h. Finally, the diameters of inhibition zones were measured.

3. Results and Discussion

3. 1. Syntheses

Three novel products have been isolated during reactions between precursor $[(dmso)_2H]$ [*trans*-$RuCl_4$(dmso-*S*)$_2$] (**P**) and 1,10-phenantroline by varying experimental conditions – *mer*-[$RuCl_3$(dmso-*S*)(phen)] (**1**), $(H_3O)[RuCl_4(phen)] \cdot 4H_2O$ (**2**·$4H_2O$) and $[Ru(phen)_3]Cl_2 \cdot 6CH_3OH$ (**3** · $6CH_3OH$).



Scheme 1: Synthetic pathways to complexes **1**, **2**, and **3**. (a) **P**, 1 eq. phen, MeOH, 3 h; (b) **1**, HCl(conc.), reflux 4 h; (c) **P**, 3 eq. phen, MeOH, solvothermal 130°C, 24 h.

Product **1** was obtained by refluxing precursor **P** and phenanthroline ligand in a suitable solvent (Scheme 1, a). Phenanthroline replaced one dmso and a chloride ligand from the precursor to bind bidentately to ruthenium ion.

It was already established before that one of the two *trans S*-coordinated dmso molecules in precursor X[*trans*-$RuCl_4$(dmso-*S*)$_2$] (X = protonated dmso, Na^+, NH_4^+), could easily be substituted which was explained by the large *trans* effect of this ligand.[1] However, it is much more difficult to substitute the second dmso molecule which is more tightly bound (due to backbonding contribution).[1] A nitrogen donor ligand (L) can replace the axially coordinated dmso molecule and through such synthetic procedure a wide class of derivatives with the general formula X[*trans*-$RuCl_4$(dmso-*S*)L] have been prepared. It is known that in these products stepwise chloride hydrolysis occurs under physiological conditions which is thus relevant for their *in vivo* mechanism of action.[31] Interestingly, in our studies between ruthenium precursor X[*trans*-$RuCl_4$(dmso-*S*)$_2$] and antiviral agent acyclovir, we have further realized that coordination of acyclovir trans to dmso-*S* was always accompanied by replacement of one chloride with a molecule of a hydrogen-bond donor ligand (water, alcohol).Whereas the replacement of one dmso-*S* molecule in the precursor with the N-donor ligand was expected, further replacement of one of the four chloride ions by the oxygen bearing ligand is rare.[5,27] It is worth to note that such products have been isolated only with acyclovir, which is a guanine derivative but not with other purine derivatives used (pure guanine, hypoxanthine, N^6-butyladenine theophylline, theobromine, caffeine).[27]

According to all these facts, the coordination of phen in **1** was not surprising. Various modifications of compound **1** have been prepared from different solvents (containing different solvate molecules). X-ray diffraction analysis of these products confirmed that the coordination sphere of ruthenium is the same but the solvate molecules are present in the lattice. During our studies we have also noticed that an analogue of **1**, with cocrystallized toluene molecules has already been prepared through a different synthetic procedure.[32]

All our efforts to incorporate the second phen molecule to form bis(1,10-phenantroline) ruthenium product from precursor X[*trans*-$RuCl_4$(dmso-*S*)$_2$] were not successful though the conditions (T, molar ratio, solvent etc.) have been varied. However, we have unexpectedly isolated a new compound (H_3O)[$RuCl_4$(phen)] (**2**) during this work. Again, similar as in **1,** phenanthroline replaced one dmso and one chloride ligand from the precursor (Scheme 1, b). Compounds containing a single molecule of dmso coordinated to ruthenium are quite inert to the substitution (as explained above), but obviously, under certain conditions (*e.g.* in highly acidic medium), the second dmso molecule could be replaced by the chloride. Only few crystals of this compound have grown from solution which merely allowed to determine the crystal structure.

By applying more severe (solvothermal) conditions in reaction mixture containing X[*trans*-$RuCl_4$(dmso-*S*)$_2$] and 1,10-phenanthroline we were able to substitute all ligands bonded to ruthenium in the precursor with three phen molecules (Scheme 1, c). Of course various similar products of this type have been isolated before,[17,18] but to the best of our knowledge this solvate of compound **3** – [$Ru(phen)_3$]$Cl_2 \cdot 6CH_3OH$ (**3** $\cdot$ $6CH_3OH$) was not reported up to now. The cocrystallized solvent molecules are not strongly bound. The crystals rapidly deteriorate at room temperature due to the loss of solvent and the elemental analyses of two samples both correspond to the presence of four methanol molecules. Also the reaction route described in this paper was not used for the isolation of this type of complexes before. It is to be noted that in the precursor ruthenium is in a +3 oxidation state but was obviously reduced during the reaction to +2 state. Redox reactions are not unusual under solvothermal conditions[33] and reduction of metal ion was also observed in our previous solvothermal work with copper and antibacterial ciprofloxacin.[34]

3. 2. X-ray Crystal Structure Characterization

Complex **1** adopts a distorted octahedral geometry where the ruthenium(III) ion is coordinated by three chloride ions in a *mer*- configuration, by dmso (coordinated through S) and by bidentately bonded phen (Figure 1) and is isostructural with its iridium analogue [$IrCl_3$(dmso-*S*)(phen)] reported by Scharwitz *et al.* in 2008.[35] All distances and angles around the metal center are similar to those found in compounds prepared by van der Drift *et al.*[32] and Spek *et al.*[36] The main difference between **1** and

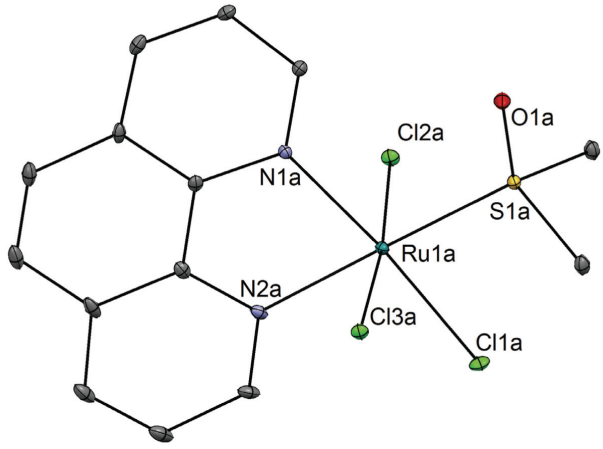


Figure 1: Ruthenium species in **1** with heteroatom labeling. The ellipsoids are represented at a 30% probability level. Only one of the two crystallographically independent molecules of **1** that are present in the asymmetric unit is displayed and hydrogen atoms are omitted for clarity. The bond lengths and angles do not differ significantly between the two molecules. Selected bond lengths (Å): Ru1a–Cl1a 2.353(2), Ru1a–Cl2a 2.351(1), Ru1a–Cl3a 2.353(1), Ru1a–S1a 2.294(1), Ru1a–N1a 2.085(5), Ru1a–N2a 2.097(5).

its analogs reported in the literature is the presence of solvent molecules (toluene[32] or chloroform[36]) in the previously known crystal structures. The conformation of complex molecules in **1** is the same as in structures containing solvent since it is stabilized by analogous C–H···O and C–H···Cl intramolecular hydrogen bonds. Due to the incorporation of solvent the packing of molecules is different but is in all three cases stabilized by C–H···Cl intermolecular hydrogen bonds and π···π and π···σ stacking interactions among phenanthroline rings.

In compound **2** · $4H_2O$ the ruthenium center is in a slightly distorted environment comprising of bidentately bonded phenanthroline and four chlorido anions (Figure 2). The bond lengths of axially coordinated chloride ions (Ru–Cl1 = 2.3483 (16), Ru–Cl2 = 2.3490 (16) Å) are slightly shorter as those of other two chloride ions (Ru–Cl3 = 2.3890 (16), Ru–Cl4 = 2.3854 (15) Å). All other distances and angles are in agreement with the literature data.[37] The charge of coordination anions $[RuCl_4(phen)]^-$ is compensated by oxonium cations. The structure contains also solvate water molecules. The lattice is stabilized by an array of hydrogen bonds (Table S5) in which the oxonium ion is the donor of three strong O–H…O hydrogen bonds to adjacent water molecules which in turn form additional, weaker hydrogen bonds among themselves and with the coordinated chlorido ligands. It is interesting to compare this structure with $H_5O_2[RuCl_4(L)] \cdot 2H_2O$, where L is dcbpy = 4,4'-dicarboxylic acid-2,2'-bypiridine[38] instead of phen. Ru–N and Ru–Cl coordination bond lengths and angles are similar to those in **2** – in both compounds the bond lengths of axially coordinated chloride ions are slightly shorter in comparison with those of other two chloride ions, which are bonded *trans* to N atoms. The counter ions are in both compounds protonated water molecules – oxonium cations. However, the crystal packing of these two compounds is different due to the fact that phen and dcbpy ligands have different sizes and dcbpy is involved in hydrogen bonding with water molecules and the oxonium cation through carboxylic groups.

In compound **3** · $6CH_3OH$ three bidentately bonded phen ligands are coordinated to ruthenium to form a propeller-shaped complex cation (Figure 3). Its charge is compensated by two chloride ions. The asymmetric unit contains also six solvent methanol molecules. The length of the Ru–N bonds [2.063(8)–2.075(9) Å] and the ligand bite angles [79.9(3)–80.3(3)°] fall within the interval of values reported in the Cambridge Structural Database for the 20 structures containing the $[Ru(phen)_3]^{2+}$ fragment ([2.025–2.093(4) Å] and [78.63–80.85°], respectively). In spite of a very similar conformation of cations their crystal packing is different in comparison with compound **3**·$6CH_3OH$ due to different kind of anions and co-crystallized solvent molecules. Moreover, compound **3** · $6CH_3OH$ is isostructural with $[Fe(phen)_3]Cl_2 \cdot 6CH_3OH$, the compound[39] which differs from **3** · $6CH_3OH$ only in the kind of central ion.

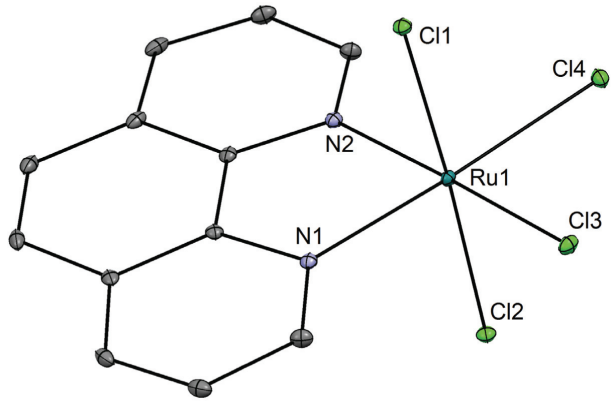


Figure 2: Anionic ruthenium species in the crystal structure of **2**·$4H_2O$ with heteroatom labeling. The ellipsoids are represented at a 30% probability level. Hydrogen atoms are omitted for clarity. Selected bond lengths (Å): Ru1–Cl1 2.348(1), Ru1–Cl2 2.349(1), Ru1–Cl3 2.389(2), Ru1–Cl4 2.385(2), Ru1–N1 2.064(5), Ru1–N2 2.056(5).

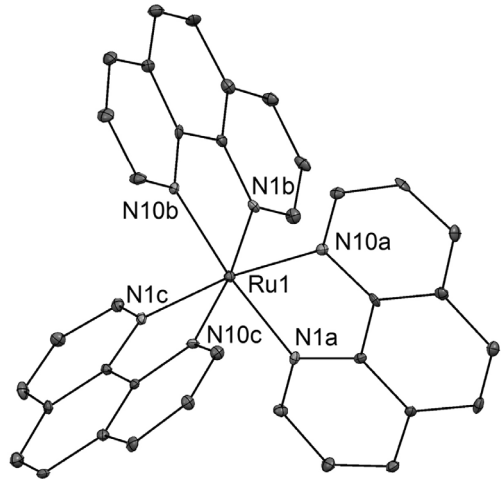


Figure 3: Cationic ruthenium species in the crystal structure of **3**·$6CH_3OH$ with heteroatom labeling. The ellipsoids are represented at a 30% probability level. Hydrogen atoms are omitted for clarity. Ru–N bond lengths are between 2.063(8) and 2.075(9) Å, phen bite angles are between 79.9(3) and 80.3(3)°.

3. 3. Biological Assays (Cytotoxicity, Hemolytic Activity, Antibacterial Tests)

The cytotoxicity of our previously isolated ruthenium-purine derivative complexes against TS/A murine adenocarcinoma cell line has been tested before.[27] It was found that these complexes showed only minor cytotoxicity which was not unexpected and was already observed for several ruthenium complexes.[31] We were interested to find if replacing the purine with phen could result in substantial change of activity which is the reason that selected previously isolated ruthenium-purine complexes have

been included in the tests herein. Ruthenium complex with acyclovir [*mer*-$RuCl_3$(acv)(dmso-*S*)(CH_3OH)] · 0.5CH_3OH (**4** · 0.5CH_3OH)[5] and ruthenium complex with guanine [*trans*-$RuCl_4$(guaH)(dmso-*S*)] · 2H_2O (**5** · 2H_2O)[27] were selected (Figure 4). Among the newly prepared complexes (**1**–**3**) only compound **1** was chosen to be tested for biological activity. The main reason to omit tests for compounds **2** and **3** is that both compounds are rather unstable in air at room temperature, which is not acceptable for potential drugs. Additionally, it has already been discovered before that $[Ru(phen)_3]^{2+}$ is not able to cross intact biological membranes.[40,41]

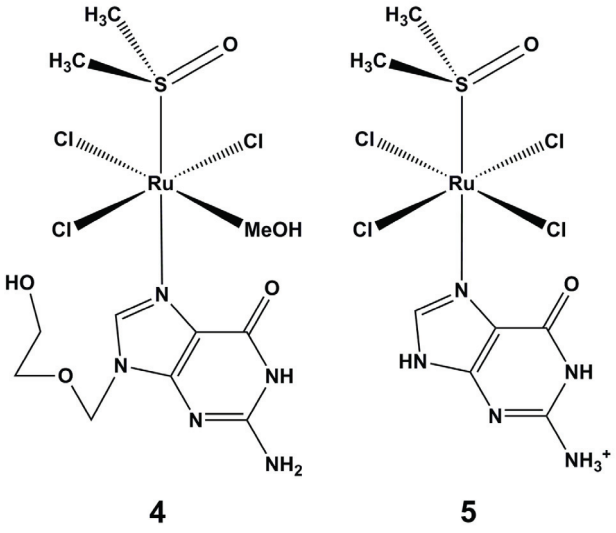


Figure 4: NAMI-type ruthenium complexes of antiviral drug acyclovir (**4**, left) and protonated nucleobase guanine (**5**, right).

Both organic ligands that are coordinated to ruthenium in complex **1**, dmso and phenanthroline respectively, exert biological effects either in free form or in form of metal complex. Bioactivities of dmso include analgesic, anti-inflammatory and cryoprotective properties.[42] It is also known that this ligand enhances the permeability of lipid membranes.[43]

1,10-Phenanthroline is important chelating agent. Due to its planar nature it was proposed it can act as a DNA intercalator. Metal complexes containing 1,10-phenanthroline are also known to bind to DNA by an intercalative mode and it is well-known that such complexes can exert interesting anticancer properties.[44] Since the discovery of Sigman and coworkers that the copper complex- $[Cu(phen)_2]^+$ efficiently cleaves DNA[45] in the presence of reducing agent and H_2O_2 several complexes of this type have been studied from this point. We were therefore very interested to find out if the simultaneous presence of phenanthroline and dmso in the coordination sphere of ruthenium might result in synergistic effect regarding biological activity.

Two different cell lines V-79-379A (normal fibroblasts of Chinese hamster) and B-16 (mouse melanoma) were used to test *in vitro* cytotoxicity of selected ruthenium complexes by MTT test. Antiproliferative activity was expressed as ratio between optical density of treated cells and control cells, as shown on Figure 5 and Figure 6. None of the tested compounds had any effect on normal V-79-379A cell line (Figure 5). A slightly selective significant inhibitory effect on B-16 malignant melanoma cell line was detected for compounds **1**, **4**, **5**, and **P**, especially at the highest tested concentration (100 μM concentration; 75.5–83.7% of control, Figure 6). Significantly reduced growth induced by phen on both V-79-379A and B-16 cell lines was observed at 10 μM and 100 μM (Figure 5 and Figure 6). Acyclovir had no effect on proliferation of both tested cell lines. Our results of cytotoxicity tests are in agreement with recently published results of Tan *et al.*[46] who tested the activity of a series of ruthenium(III) complexes (amongst other also the analogue of our compound **1**) and determined that cytotoxicity of their compounds is relatively low.

The results for compounds **4** and **5** on B-16 cell line (1 and 10 μM) were also comparable to our previous results,[27] where a TS/A murine adenocarcinoma cell line was used. It is worth to note that in present work the inhibitory effect at 100 μM concentration was less expressed.

Our results show a slight anticancer activity of the compounds **1**, **4**, **5** and **P** at the highest tested concentration (100 μM), where the growth of B-16 cells was reduced to approximately 80%, but no cytotoxic effect was found in the treatment of normal cells. There are several differences between normal and tumor cells. Apart from morphological changes (for example alternation of nuclear structure and changes of other organelles) differences exist also on molecular level (for example DNA hypomethylation,[47] protein composition etc.). Additionally, in cancer cells, showing an excessive rate of cell division, many of enzymes (*e.g.* telomerase) involved in DNA replication are overexpressed. Any of these changes could be the reason for the observed different activity of the tested compounds against cancer and normal cells.

It is interesting that the melanoma cytotoxicity of **1** is slightly increased in comparison to the ruthenium precursor (Figure 6). In contrast, in purine containing complexes **4** and **5**, the cytotoxic activity is less expressed indicating that the introduction of phen into molecule can enhance its cytotoxic activity.

For a long time, it was believed that DNA is the most important target of ruthenium compounds. There is no doubt that several ruthenium complexes indeed interact with DNA, which we have also clearly confirmed for selected ruthenium-purine complexes.[48] Tan *et al.* [46] have also discovered that their ruthenium compounds with various N,N-ligands (also phen) interact with DNA and that the affinity is increased with the extension of the planar area of the N,N-ligands. Therefore they suggest that noncovalent binding of the complexes to DNA may play a more important role than covalent binding. However, it

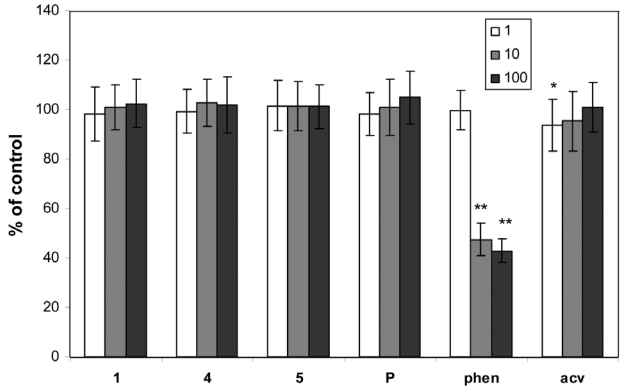


Figure 5: Effects of different concentrations (1–100 μM) of ruthenium complexes **1**, **4**, **5**, precursor **P**, and ligands **phen** and **acv** on proliferation of V-79-379A cell line expressed as% of control after 48 hours of growth. Data are derived from 3–4 independent experiments and presented as means with standard deviations. The differences were analyzed using Student's t-test on two populations; *p<0.05, ** p<0.001 vs.controls.

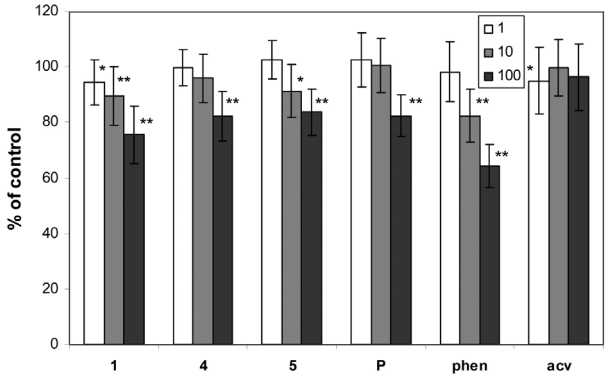


Figure 6: Effects of different oncentrations (1–100 μM) of ruthenium complexes **1**, **4**, **5**, precursor **P**, and ligands **phen** and **acv** on proliferation of B-16 cell line expressed as% of after 48 hours of growth. Data are derived from 3–4 independent experiments and presented as means with standard deviations. The differences were analyzed using Student's t-test on two populations; * p<0.05, ** p<0.001 vs. controls.

was recently suggested that the discovery of new DNA binding drugs might be less important in the future in comparison to drugs that bind other targets.[49] Even for NAMI-A and KP1019[6,50] which are perhaps the most extensively studied ruthenium compounds the mechanism of action as well as the targets crucial for activity are not completely elucidated. It was also shown that interaction of certain ruthenium(II) polypyridyl complexes with cell membrane might be crucial for their mode of action and that for such complexes enlargement of the ligands aromatic ring system results in an increase in the cellular ruthenium.[41] It is not clear how NAMI-A enters the cell, but it was hypothesized that this can be possible either by a passive diffusion and/or by an active transport.[51] Interestingly, we have recently showed that by increasing cell membrane permeability with electroporation the cytotoxicity of NAMI-A was markedly increased.[51] In order to obtain some additional data on the possible mechanism of cytotoxicity, the compounds were also tested on their ability to induce lysis of bovine red blood cells. Erythrocytes are nucleus-free cells lacking DNA, as well as all the enzymes involved in its replication. In consequence, their damage is mainly due to the disruption of the cell membrane. Erythrocytes have been extensively used as target cells for assessing the lytic potential of several membrane-active molecules and compounds.[29,52] In contrast to the cytotoxicity tests performed on nucleated cells, where slight activity was observed at high concentrations, none of the assayed compounds induced lysis of bovine erythrocytes up to the extremely high concentrations (500 μg/mL). Results of both hemolytic and cytotoxic assay indicate that the observed reduction of tumor cell growth, induced by our compounds, is not the consequence of membrane-damaging activity. One could argue that there is a possibility that ruthenium compounds do not enter the cells at all and as a consequence the interaction with DNA and (or) other intracellular components does not occur. However, in our paper[27] where also compounds **4** and **5** were studied, it was clearly confirmed that ruthenium is present inside the TS/A adenocarcinoma cells. Moreover, it was also reported that a number of Ru(II) polypyridyl complexes are transported into the cellular interior rather than associating at the membrane surface.[53]

Ruthenium compounds **P**, **4**, **5**, and acyclovir did not show any antibacterial potential up to the concentration of 1 mg/mL. Compound **1** was the only exception, and was slightly active (MIC from 250 to more than 1000 μg/mL) against different tested bacteria. The reference compound, phenanthroline, showed moderate antibacterial potential. We can conclude that the tested ruthenium compounds are not promising as potential antibacterial drugs. Table S6 showing the antibacterial potential of the tested compounds is given in Supplementary Information file.

4. Conclusions

We were able to prepare new ruthenium-phenanthroline complexes from precursor $[(dmso)_2H][trans\text{-}RuCl_4(dmso\text{-}S)_2]$ and phenanthroline. Whereas *mer*-$[RuCl_3(dmso\text{-}S)(phen)]$ (**1**) and $[Ru(phen)_3]Cl_2 \cdot 6CH_3OH$ (**3**) are only analogues of known compounds, product $(H_3O)[RuCl_4(phen)] \cdot 4H_2O$ (**2**) was not reported before. However, the synthetic routes used here are straightforward, novel and could be successfully used for the preparation of ruthenium-(N,N-ligand) complexes. Our reactions also showed that not only one weakly bonded dmso could be replaced from this precursor, but under more severe conditions, also strongly bonded dmso could be substituted.

The biological activity data show that a slightly selective significant inhibitory effect on B-16 malignant me-

lanoma cell line was detected for compounds **1**, **4**, **5**, and **P**, especially at the highest tested concentration (100 μM concentration; 75.5–83.7% of control), but have no effect on normal cell line V-79-379A. It is interesting that the tumor cytotoxicity of **1** (which contains phenanthroline as ligand) is slightly increased in comparison to the ruthenium precursor. In contrast, in purine containing complexes **4** and **5**, the cytotoxic activity is less expressed.

We have also demonstrated that the observed reduction of tumor cell growth, induced by our compounds, is not the consequence of membrane-damaging activity. Therefore further experiments are needed to reveal the biological targets and details of the mode of action of these and similar ruthenium compounds.

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6. References

1. E. Alessio, G. Balducci, M. Calligaris, G. Costa, W. M. Attia, G. Mestroni, *Inorg. Chem.*, **1991,** *30,* 609–618. http://dx.doi.org/10.1021/ic00004a005
2. E. Iengo, E. Zangrando, R. Minatel, E. Alessio, *J. Am. Chem. Soc.*, **2002,** *124,* 1003–1013. http://dx.doi.org/10.1021/ja016162s
3. F. Pezet, J. C. Daran, I. Sasaki, H. Ait-Haddou, G. G. A. Balavoine, *Organometallics*, **2000,** *19,* 4008–4015. http://dx.doi.org/10.1021/om000255l
4. G. Sava, I. Capozzi, K. Clerici, G. Gagliardi, E. Alessio, G. Mestroni, *Clin. Exp. Metastasis*, **1998,** *16,* 371–379. http://dx.doi.org/10.1023/A:1006521715400
5. I. Turel, M. Pečanac, A. Golobič, E. Alessio, B. Serli, *Eur. J. Inorg. Chem.*, **2002,** 1928–1931. http://dx.doi.org/10.1002/1099-0682(200208)2002:8<1928::AID-EJIC1928>3.0.CO;2-A
6. G. Sava, E. Alessio, A. Bergamo, G. Mestroni, in M. J. Clarke, P. J. Sadler (Eds.): *Topics in Biological Inorganic Chemistry, vol. 1*, Springer, Berlin, Germany, **1999**, pp. 143–170.
7. E. Alessio, ed., *Bioinorganic Medicinal Chemistry*, Wiley–VCH Verlag & Co. KGaA, Weinheim, Germany, **2011.**
8. A. A. Hostetter, M. L. Miranda, V. J. DeRose, K. L. M. Holman, *J. Biol. Inorg. Chem.*, **2011,** *16,* 1177–1185. http://dx.doi.org/10.1007/s00775-011-0806-7
9. E. A. P. Armstrong, R. T. Brown, M. S. Sekwale, N. C. Fletcher, X. Q. Gong, P. Hu, *Inorg. Chem.*, **2004,** *43,* 1714–1722. http://dx.doi.org/10.1021/ic0350281
10. R. T. Brown, N. C. Fletcher, M. Nieuwenhuyzen, T. E. Keyes, *Inorg. Chim. Acta*, **2005,** *358,* 1079–1088. http://dx.doi.org/10.1016/j.ica.2004.10.010
11. J. Slinker, D. Bernards, P. L. Houston, H. D. Abruna, S. Bernhard, G. G. Malliaras, *Chem. Comm.*, **2003,** *19,* 2392–2399. http://dx.doi.org/10.1039/b304265k
12. M. Zhou, G. P. Robertson, J. Roovers, *Inorg. Chem.*, **2005,** *44,* 8317–8325. http://dx.doi.org/10.1021/ic0510112
13. F. R. Keene, *Coord. Chem. Rev.*, **1997,** *166,* 121–159. http://dx.doi.org/10.1016/S0010-8545(97)00006-4
14. M. Ziegler, A. von Zelewsky, *Coord. Chem. Rev.*, **1998,** *177,* 257–300. http://dx.doi.org/10.1016/S0010-8545(98)00186-6
15. K. Traven, M. Sinreih, J. Stojan, S. Seršen, J. Kljun, J. Bezenšek, B. Stanovnik, I. Turel, T. Lanišnik Rižner, *Chem.-Biol. Interactions*, **2015,** in press, doi: 10.1016/j.cbi.2014.1011.1005.
16. K. E. Erkkila, D. T. Odom, J. K. Barton, *Chem. Rev.*, **1999,** *99,* 2777–2795. http://dx.doi.org/10.1021/cr9804341
17. B. M. Goldstein, J. K. Barton, H. M. Berman, *Inorg. Chem.*, **1986,** *25,* 842–847. http://dx.doi.org/10.1021/ic00226a024
18. M. Vrabel, M. Hocek, L. Havran, M. Fojta, I. Votruba, B. Klepetarova, R. Pohl, L. Rulisek, L. Zendlova, P. Hobza, I. H. Shih, E. Mabery, R. Mackman, *Eur. J. Inorg. Chem.*, **2007,** *12,* 1752–1769. http://dx.doi.org/10.1002/ejic.200700030
19. P. Lincoln, A. Broo, B. Norden, *J. Am. Chem. Soc.*, **1996,** *118,* 2644–2653. http://dx.doi.org/10.1021/ja953363l
20. R. S. Srivastava, F. R. Fronczek, *Inorg. Chim. Acta*, **2005,** *358,* 854–857. http://dx.doi.org/10.1016/j.ica.2004.09.040
21. B. Wu, C. Janiak, *ZAAC*, **2005,** *631,* 17–18.
22. E. L. M. Wong, R. W. Y. Sun, N. P. Y. Chung, C. L. S. Lin, N. Y. Zhu, C. M. Che, *J. Am. Chem. Soc.*, **2006,** *128,* 4938–4939. http://dx.doi.org/10.1021/ja057883k
23. R. Karvembu, K. Natarajan, *Polyhedron*, **2002,** *21,* 1721–1727. http://dx.doi.org/10.1016/S0277-5387(02)01038-0
24. R. Hudej, J. Kljun, W. Kandioller, U. Repnik, B. Turk, C. G. Hartinger, B. K. Keppler, D. Miklavčič, I. Turel, *Organometallics*, **2012,** *31,* 5867–5874. http://dx.doi.org/10.1021/om300424w
25. P. L. Lam, G. L. Lu, K. M. Hon, K. W. Lee, C. L. Ho, X. Wang, J. C. O. Tang, K. H. Lam, R. S. M. Wong, S. H. L. Kok, Z. X. Bian, H. Li, K. K. H. Lee, R. Gambari, C. H. Chui, W. Y. Wong, *Dalton Trans.*, **2014,** *43,* 3949–3957. http://dx.doi.org/10.1039/c3dt52879k
26. S. N. Shukla, P. Gaur, H. Kaur, R. S. Srivastava, *Acta Chim. Slov.*, **2011,** *58,* 542–548.
27. I. Turel, M. Pečanac, A. Golobič, E. Alessio, B. Serli, A. Bergamo, G. Sava, *J. Inorg. Biochem.*, **2004,** *98,* 393–401. http://dx.doi.org/10.1016/j.jinorgbio.2003.12.001
28. K. Sepčić, S. Berne, C. Potrich, T. Turk, P. Maček, G. Menestrina, *Eur. J. Biochem.*, **2003,** *270,* 1199–1210. http://dx.doi.org/10.1046/j.1432-1033.2003.03480.x
29. P. Malovrh, K. Sepčić, T. Turk, P. Maček, *Comp. Biochem. Phisiol. C*, **1999,** *124,* 221–226.
30. S. Magaldi, S. Mata-Essayag, C. Hartung de Capriles, C. Perez, M. T. Colella, C. Olaizola, Y. Ontiveros, *J. Infect. Dis.*, **2004,** *8,* 39–45.

31. G. Sava, E. Alessio, A. Bergamo, G. Mestroni, in *Topics in biological inorganic chemistry*, eds. M. J. Clarke, P. J. Sadler, Springer Verlag, Berlin, **1999,** pp. 143–169.
32. R. C. van der Drift, J. W. Sprengers, E. Bouwman, W. P. Mul, H. Kooijman, A. L. Spek, E. Drent, *Eur. J. Inorg. Chem.*, **2002,** *8,* 2147–2155. http://dx.doi.org/10.1002/1099-0682(200208)2002:8<2147::AID-EJIC2147>3.0.CO;2-L
33. X. M. Chen, M. L. Tong, *Acc. Chem. Res.*, **2007,** *40,* 162–170. http://dx.doi.org/10.1021/ar068084p
34. P. Drevenšek, T. Zupančič, B. Pihlar, R. Jerala, U. Kolitsch, A. Plaper, I. Turel, *J. Inorg. Biochem.*, **2005**, *99,* 432–442. http://dx.doi.org/10.1016/j.jinorgbio.2004.10.018
35. M. A. Scharwitz, I. Ott, R. Gust, A. Kromm, W. S. Sheldrick, *J. Inorg. Biochem.*, **2008,** *102,* 1623–1630. http://dx.doi.org/10.1016/j.jinorgbio.2008.03.001
36. A. Spek, D. M. Tooke, A. Garza-Ortiz, J. Reedijk, *Acta Crystallogr. Sect. E.-Struct Rep. Online*, **2005,** *61,* M2428–M2430. http://dx.doi.org/10.1107/S1600536805034756
37. A. Guy Orpen, L. Brammer, F. H. Allen, O. Kennard, D. G. Watson, R. Taylor, *J. Chem. Soc. Dalton Trans.*, **1989,** *12,* S1–S83. http://dx.doi.org/10.1039/dt98900000s1
38. E. Eskelinen, P. Da Costa, M. Haukka, *J. Electroanal. Chem.*, **2005,** *579,* 257–265. http://dx.doi.org/10.1016/j.jelechem.2005.02.014
39. L. A. Plaza, K. Baranowska, B. Becker, *Acta Cryst., Sect. E*, **2007,** *63,* M1537–M1539. http://dx.doi.org/10.1107/S1600536807019873
40. J. W. Dobrucki, *J. Photochem. Photobiol. B*, **2001,** *65,* 136–144. http://dx.doi.org/10.1016/S1011-1344(01)00257-3
41. U. Schatzschneider, J. Niesel, I. Ott, R. Gust, H. Alborzinia, S. Wolfl, *ChemMedChem*, **2008,** *3,* 1104–1109. http://dx.doi.org/10.1002/cmdc.200800039
42. in *The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals, 13th ed.*, eds. S. Budavari, M. J. O'Neal, A. Smith, P. E. Heckelman, Merck & Co., Whitehouse Station, NJ, **2001,** p. 3285.
43. R. Notman, M. Noro, B. O'Malley, J. Anwar, *J. Am. Chem. Soc.*, **2006,** *128,* 13982–13983. http://dx.doi.org/10.1021/ja063363t
44. H. Sakurai, H. Tamura, K. Okatani, *Biochem. Biophys Res. Comm.*, **1995,** *206,* 133–137. http://dx.doi.org/10.1006/bbrc.1995.1019
45. D. M. Perrin, A. Mazumder, D. S. Sigman, Chem. Rev., **1993,** *96,* 2295–2316.
46. C. P. Tan, J. Liu, L. M. Chen, S. Shi, L. N. Ji, *J. Inorg. Biochem.*, **2008,** *102,* 1644–1653. http://dx.doi.org/10.1016/j.jinorgbio.2008.03.005
47. M. Ehrlich, *Epigenomics*, **2009,** *1,* 239–259. http://dx.doi.org/10.2217/epi.09.33
48. A. Garcia-Raso, J. J. Fiol, A. Tasada, M. J. Prieto, V. Moreno, I. Mata, E. Molins, T. Bunic, A. Golobič, I. Turel, *Inorg. Chem. Commun.*, **2005,** *8,* 800–804. http://dx.doi.org/10.1016/j.inoche.2005.05.023
49. P. J. Dyson, G. Sava, *Dalton Trans.*, **2006,** 1929–1933. http://dx.doi.org/10.1039/b601840h
50. C. G. Hartinger, S. Zorbas-Seifried, M. A. Jakupec, B. Kynast, H. Zorbas, B. K. Keppler, *J. Inorg. Biochem.*, **2006,** *100,* 891–904. http://dx.doi.org/10.1016/j.jinorgbio.2006.02.013
51. A. Biček, I. Turel, M. Kandušer, D. Miklavčič, *Bioelectrochemistry*, **2007,** *71,* 113–117. http://dx.doi.org/10.1016/j.bioelechem.2007.05.002
52. K. Sepčić, S. Berne, C. Potrich, T. Turk, P. Maček, G. Menestrina, *Eur. J. Biochem.*, **2003,** *270,* 1199–1210. http://dx.doi.org/10.1046/j.1432-1033.2003.03480.x
53. C. A. Puckett, J. K. Barton, *J. Am. Chem. Soc.*, **2007,** *129,* 46–47. http://dx.doi.org/10.1021/ja0677564

Povzetek

Z reakcijo med rutenijevim prekurzorjem $[(dmso)_2H][trans\text{-}RuCl_4(dmso\text{-}S)_2]$ (**P**) in 1,10-fenantrolinom (phen) pri različnih pogojih smo pripravili tri nove koordinacijske spojine. Analogna kompleksa spojin *mer*-$[RuCl_3(dmso\text{-}S)(phen)]$ (**1**) in $[Ru(phen)_3]Cl_2 \cdot 6CH_3OH$ (**3**·$6CH_3OH$) sta že poznana, vendar so bili za njuno sintezo uporabljeni drugačni postopki. Kompleks **2** · $4H_2O$ s strukturno formulo $(H_3O)[RuCl_4(phen)] \cdot 4H_2O$ pa je povsem nova spojina. Izoliran je bil z močno kisle raztopine, kar je omogočilo substitucijo tudi zadnjega dmso liganda iz prekurzorja **P** s kloridom ligandom. Preučili smo tudi biološke aktivnosti novih kompleksov ter predhodno objavljenih kompleksov s purinskimi derivati ([*mer*-$RuCl_3$(dmso-*S*)(acv)(CH_3OH)] (**4**) (acv = aciklovir); [*trans*-$RuCl_4$(dmso-*S*)(guaH)] (**5**) (guaH = protonirani gvanin). Spojine **1**, **4** in **5** so šibko toksične za celično linijo B-16 (maligni melanom), ne pa tudi za V-79-379A celično linijo. Vezava liganda phen je torej povečala toksičnost rutenijeve zvrsti. Eksperimenti na govejih eritrocitih so pokazali, da toksičnost ni posledica poškodb celične membrane.

New Synthetic Routes for Ruthenium-1,10-Phenanthroline Complexes. Tests of Cytotoxic and Antibacterial Activity of Selected Ruthenium Complexes.

Iztok Turel[a*], Amalija Golobič[a], Jakob Kljun[a], Petra Samastur[a], Urška Batista[b], Kristina Sepčić[c]

Dedicated to the memory of Prof. Dr. Jurij V. Brenčič.

[a] Faculty of Chemistry and Chemical Technology, University of Ljubljana, Večna pot 113, SI-1000 Ljubljana, Slovenia.
[b] Institute of Biophysics, Faculty of Medicine, University of Ljubljana, Lipičeva 2, 1000 Ljubljana, Slovenia
[c] Biotechnical Faculty, Department of Biology, University of Ljubljana, Večna pot 111, 1000 Ljubljana, Slovenia.
*To whom correspondence should be addressed: Tel: +386 1 4798525; E-mail: iztok.turel@fkkt.uni-lj.si

Diffraction data for all three compounds were collected on a Nonius Kappa CCD diffractometer with graphite monochromated Mo*Kα* radiation at temperature 150 K. The data were processed using DENZO[1] program. Structures were solved by direct methods using SIR97[2]. Most of the positions of hydrogen atoms were obtained from the difference Fourier maps, the remaining were calculated. We employed full-matrix least-squares refinements on F magnitudes with anisotropic displacement factors for non-hydrogen atoms using Xtal3.6[3]. The exception were C and O atoms of solvate methanol molecules in **3**, which are slightly disordered and are thus refined with isotropic displacement parameter. The position of hydroxyl H atoms of these molecules were not determined. One water molecule (O5W) in **1** is disordered over two positions O5W1 and O5W2. These two maxima were also refined with isotropic displacement parameter and the corresponding H atoms positions were not determined. The parameters of hydrogen atoms were not refined. In the final cycle of the refinement we used 3939, 7201 and 6147 reflections and 217, 397 and 454 parameters for compounds **1**, **2**·4H_2O and **3**·6MeOH, respectively. The final *R* values were 0.033, 0.030 and 0.068 and R_W values were 0.030, 0.030 and 0.045 for **1**, **2**·4H_2O and **3**·6MeOH respectively. The crystallographic data were deposited in the Cambridge Crystallographic Data Center and were assigned the deposition numbers CCDC 1028368–1028370 for compounds **1**–**3** respectively.

References

1. Z. Otwinowski, W. Minor, Methods Enzymol. 276 (1997) 307.

2. Altomare, M.C. Burla, M. Camalli, G. Cascarano, C. Giacovazzo, A. Guagliardi, A.G.G. Moliterni, G. Polidori, R. Spagna, J. Appl. Cryst. 32 (1999) 115.
3. Hall, S.R., du Boulay, D.J. & Olthof-Hazekamp, R. (**1999**). Eds. Xtal3.6 System . University of Western Australia.

Table S1: Crystallographic data for compounds **1**, **2**·4H_2O and **3**·6MeOH**.**

	1	**2**·4H_2O	**3**·6MeOH
Empirical formula	$C_{14}H_{14}Cl_3N_2ORuS$	$C_{12}H_{19}Cl_4N_2O_5Ru$	$C_{42}H_{48}Cl_2\ N_6O_6Ru$
M_w	465.76	514.16	904.858
T, K	150(2)	150(2)	150(2)
Crystal system	*triclinic*	*triclinic*	*orthorhombic*
Space group	*P-1*	*P-1*	*Pbca*
a, Å	8.8592(1)	8.1409(2)	18.5607(3)
b, Å	11.7145(2)	9.2715(2)	18.6235(3)
c, Å	16.7731(3)	13.0919(3)	23.7095(5)
α, deg.	92.5915(7)	86.061(1)	90.000
β, deg.	103.1136(7)	77.551(1)	90.000
γ, deg.	93.3115(7)	78.652(1)	90.000
V, $Å^3$	1689.46(5)	945.70(4)	8195.5(3)
Z	4	2	8
D_{calc}, g/cm^3	1.831	1.806	1.466
μ, mm^{-1}	1.527	1.418	0.568
F(000)	924	514	3672
Crystal size, mm	0.44×0.16×0.14	0.175×0.125×0.10	0.30×0.25×0.20
Color	red	brown	red
Data collected / unique	24614 / 7691	16369 / 4297	30130 / 12959
R_{int}	0.031	0.039	0.10
Restraints / parameters	0 / 397	0 / 217	0 / 454
S	1.218	1.089	1.100
R_1, wR_2 [I>2σ(I)]	0.030 / 0.030	0.033 / 0.030	0.068 / 0.045
R_1, wR_2 (all data)	0.043 / 0.056	0.058 / 0.067	0.197 / 0.085
Largest diff. peak / hole (e $Å^{-3}$)	0.749 / –0.962	0.792 / –0.614	1.857 / –3.293*

*The largest diff. peak and hole in **3** is located in close proximity to the central ruthenium ion

Table S2: Selected geometric parameters (Å, °) in **1**.

Ru1a–Cl1a	2.3528(14)	Ru1b–N2b	2.093(5)
Ru1a–Cl2a	2.3512(13)	Ru1b–Cl3b	2.3350(12)
Ru1a–Cl3a	2.3346(12)	Ru1b–Cl1b	2.3333(13)
Ru1a–S1a	2.2935(12)	Ru1b–Cl2b	2.3461(13)
Ru1a–N1a	2.086(4)	Ru1b–S1b	2.3114(13)
Ru1a–N2a	2.097(5)	Ru1b–N1b	2.102(5)
Cl1a–Ru1a–Cl2a	91.97(5)	Cl1b–Ru1b–N1b	85.79(13)
Cl1a–Ru1a–Cl3a	92.93(5)	Cl1b–Ru1b–N2b	88.21(13)
Cl1a–Ru1a–S1a	86.62(5)	Cl2b–Ru1b–Cl3b	95.18(5)
Cl1a–Ru1a–N1a	174.11(13)	Cl2b–Ru1b–S1b	89.61(5)
Cl1a–Ru1a–N2a	94.74(13)	Cl2b–Ru1b–N1b	86.93(13)
Cl2a–Ru1a–Cl3a	174.42(5)	Cl2b–Ru1b–N2b	89.04(13)
Cl2a–Ru1a–S1a	92.39(4)	Cl3b–Ru1b–S1b	87.22(4)
Cl2a–Ru1a–N1a	86.83(11)	Cl3b–Ru1b–N1b	172.19(13)
Cl2a–Ru1a–N2a	92.18(13)	Cl3b–Ru1b–N2b	93.34(14)
Cl3a–Ru1a–S1a	90.55(4)	S1b–Ru1b–N1b	100.33(13)
Cl3a–Ru1a–N1a	88.03(11)	S1b–Ru1b–N2b	178.58(13)
Cl3a–Ru1a–N2a	84.77(13)	N1b–Ru1b–N2b	79.16(18)
S1a–Ru1a–N1a	99.19(12)	Cl1b–Ru1b–Cl3b	91.85(4)
S1a–Ru1a–N2a	175.18(14)	Cl1b–Ru1b–S1b	93.08(5)
N1a–Ru1a–N2a	79.56(17)	Cl1b–Ru1b–Cl2b	172.59(5)

Table S3: Selected geometric parameters (Å, °) in **2**·4H_2O.

Ru–Cl1	2.3483 (16)	Ru–N2	2.056 (5)
Ru–Cl2	2.3490 (16)	N1–C10	1.377 (7)
Ru–Cl3	2.3890 (16)	N1–C2	1.330 (8)
Ru–Cl4	2.3854 (15)	N2–C9	1.331 (8)
Ru–N1	2.064 (5)	N2–C12	1.365 (8)
Cl1–Ru–Cl2	177.28 (5)	Cl2–Ru–N2	91.46 (14)
Cl1–Ru–Cl3	88.89 (6)	Cl3–Ru–Cl4	89.60 (6)
Cl1–Ru–Cl4	91.17 (5)	Cl3–Ru–N1	95.66 (14)
Cl1–Ru–N1	89.81 (14)	Cl3–Ru–N2	174.78 (15)
Cl1–Ru–N2	87.85 (14)	Cl4–Ru–N1	174.67 (14)
Cl2–Ru–Cl3	91.61 (6)	Cl4–Ru–N2	94.54 (15)
Cl2–Ru–Cl4	91.51 (5)	N1–Ru–N2	80.26 (19)
Cl2–Ru–N1	87.48 (14)		

Table S4: Selected geometric parameters (Å, °) in **3**·6MeOH.

Ru–N1a	2.063(8)	Ru–N10a	2.075(9)
Ru–N1b	2.071(9)	Ru–N10b	2.065(9)
Ru–N1c	2.067(7)	Ru–N10c	2.066(9)
N1a–Ru–N1b	96.6(3)	N1a–Ru–N10c	88.2(3)
N1a–Ru–N1c	93.0(3)	N1b–Ru–N1c	94.9(3)
N1a–Ru–N10a	80.3(4)	N1b–Ru–N10a	90.9(3)
N1a–Ru–N10b	173.9(3)	N1b–Ru–N10b	80.3(3)

Table S5: Hydrogen bond contact distances and angles in compound **2**·4H_2O. O5w1 and O5w2 atoms have 50% occupancy.

Donor (D)	Acceptor (A)	D...A (Å)	D–H...A (°)
O1w	$O3w^{1-x,1-y,2-z}$	2.642(7)	165
O1w	O4w	2.432(7)	174
O1w	$O5w1^{-x,2-y,2-z}$	2.610(10)	174
O1w	$O5w2^{-x,2-y,2-z}$	2.467(16)	154
O2w	$Cl4^{-x,2-y,2-z}$	3.222(5)	163
O2w	$Cl3^{x,1+y,z}$	3.420(4)	162
O3w	$Cl3^{1+x,y,z}$	3.202(5)	164
O3w	Cl2	3.108(5)	166
O4w	O3w	2.737(7)	178
O4w	$O2w^{1-x,2-y,2-z}$	2.725(7)	175
O5w1	O2w	2.791(12)	–
O5w1	Cl1	3.130(15)	–
O5w2	O2w	2.711(17)	–
O5w2	Cl1	3.186(11)	–

Table S6: Minimal inhibitory concentrations (MIC) of the tested compounds against selected bacteria. n.i. = no inhibition.

MIC (ug/ml)						
Microorganism	**1**	**4**	**5**	**P**	**phen**	**Acv**
Klebsiella pneumoniae	800	>1000	>1000	>1000	60	>1000
Staphylococcus aureus	250	>1000	>1000	>1000	20	>1000
Escherichia coli	500	>1000	>1000	>1000	75	>1000
Salmonella typhimurium	1000	>1000	>1000	>1000	50	>1000
Proteus vulgaris	900	>1000	>1000	>1000	35	>1000
Pseudomonas aeruginosa	>1000	>1000	>1000	>1000	>1000	>1000
Micrococcus luteus	>1000	>1000	>1000	>1000	250	>1000
Bacillus subtilis	>1000	>1000	>1000	>1000	250	>1000