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

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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-RuCl4(dmso-S)2] (P) and 1,10-phenanthroline (phen) at different conditions. Whereas the analogs of mer-[RuCl3(dmso-S)(phen)] (1) and [Ru(phen)]Cl2·6CH3OH (3·6CH3OH) have already been prepared by other synthetic routes before, product (H3O)[Ru-Cl4(phen)]·4H2O (2·4H2O) 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-RuCl4(dmso-S)(acv)(CH3OH)] (4) (acv = acyclovir); [trans-RuCl4(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 synthesis3 in design of new drugs4,5 and other.

The most famous representative of biologically active compounds from the group is NAMI-A ([ImH][trans-RuCl4(dmso-S)Im] (Im = imidazole)) which was prepared from [(dmso)2H][trans-RuCl4(dmso-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 drug7,8. 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\textsuperscript{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.\textsuperscript{20,21}

The aims of this work were (i) to study the reactivity of precursor \([\text{[dmso]}_2\text{H}][\text{trans-RuCl}_3(\text{dmsos})_2]\) (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\textsuperscript{22} and antibacterial ruthenium compounds remain sparse.\textsuperscript{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.\textsuperscript{15,27} All other chemicals and solvents were commercially available (Sigma-Aldrich, U.S.A.) and were used without prior drying or purification.

\[
\text{mer-[RuCl}_4(\text{dmsos})(\text{phen})]\] (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. The crystals were dissolved in MeOH (0.7 mL) to form an orange solution. After several days of isothermal evaporation of the solvent in air few brownish crystals appeared in the solution. Compound \(2\cdot4\text{H}_2\text{O}\) is unstable in air and was only characterized by X-ray crystallography.

\[
[Ru(\text{phen})]_2\text{Cl}_2 \cdot 6\text{CH}_3\text{OH} (3 \cdot 6\text{CH}_3\text{OH}) \] (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 \(\text{C}_{49}\text{H}_{40}\text{Cl}_2\text{N}_8\text{O}_4\text{Cl}_2\text{Ru}: \text{C}, 57.13; \text{H}, 4.80; \text{N}, 10.0\). Selected IR bands (Nujol, \(\text{cm}^{-1}\)) 1649 (s), 1633 (s), 1573 (s) (phen ring \(\text{C}=\text{C}, \text{C}=\text{N}\)), UV(MeOH) \(\lambda_{\text{max}}/\text{nm} (\epsilon/\text{dm}^3 \text{mol}^{-1} \text{cm}^{-1})\) 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 se-
rum (FCS) (Sigma, Germany), penicillin (100 U/mL) and streptomycin (100 μg/mL) at 37 °C in CO2 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 dis-
solved in 100 μL dmsos (Sigma, Germany) and the absorb-
ance was measured at 570 nm using Anthos microplate reader (Anthos, Australia). Proliferation (%) was expres-
sed as ratio between optical density of treated cells and untreated cells after 48 hours of growth. The data are pre-
tated by means with standard deviations of 3–4 indepen-
dent 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. Stock solution (1 mg/mL) of tested compounds in 50 mM TRIS.HCl buffer pH 7.4 was progressively diluted by five-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 da-
mage and consequent hemolysis, was recorded for 30 min-
tures at 650 nm using a Kinetic Microplate Reader (Dynex Technologies, U.S.A.). All experiments were per-
formed at 25 °C, repeating every measurement three ti-
mes. Marine sponge-derived polymeric alkylpyridinium salts (poly-APS) were used in the final concentration of 10 μg/mL as a positive, hemolytically active control.

2. 2. 4. Determination of Antibacterial Activity

The following bacterial strains were used: Staphylo-
coccus 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 Salmonel-
la 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. 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 × 10^7 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 (Φ = 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. 100μL of each dilu-
tion were poured into the holes cut in the inoculated me-
dium, 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-Ru-
Cl_4(dmso-S)_2] (P) and 1,10-phenanthroline by varying ex-
perimental conditions – mer-[RuCl_3(dmso-S)(phen)] (1),
(H_2O)[RuCl_4(phen)] · 4H_2O (2-4H_2O) and [Ru(phen)_3]Cl_2-6CH_3OH (3-6CH_3OH).

![Scheme 1: Synthetic pathways to complexes 1, 2, and 3.](image)

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Product I was obtained by refluxing precursor P and phenanthrolineligand in a suitable solvent (Scheme 1, a). Phenanthroline replaced one dmsoso 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 dmsoso molecules in precursor X[trans-RuCl4(dmso-S)] (X = protonated dmsoso, Na+, NH4+), 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 dmsoso molecule which is more tightly bound (due to backbonding contribution).1 A nitrogen donor ligand (L) can replace the axially coordinated dmsoso molecule and through such synthetic procedure a wide class of derivatives with the general formula X[trans-RuCl4(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-RuCl4(dmso-S)] and antiviral agent acyclovir, we have further realized that coordination of acyclovir trans to dmsoso-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 dmsoso-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, N6-butyladene, theophylline, theobromine, caffeine).27

According to all these facts, the coordination of phen in I was not surprising. Various modifications of compound I 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 I, 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-phenanthroline) ruthenium product from precursor X[trans-RuCl4(dmso-S)] were not successful though the conditions (T, molar ratio, solvent etc.) have been varied. However, we have unexpectedly isolated a new compound ([H2O]RuCl4(phen)) (2) during this work. Again, similar as in I, phenanthroline replaced one dmsoso and one chloride ligand from the precursor (Scheme 1, b). Compounds containing a single molecule of dmsoso 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 dmsoso 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-RuCl4(dmso-S)] 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)2]Cl2 · 6CH3OH (3 · 6CH3OH) 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 conditions33 and reduction of metal ion was also observed in our previous solvotheral work with copper and antibacterial ciprofloxacin.34

### 3.2. X-ray Crystal Structure Characterization

Complex I adopts a distorted octahedral geometry where the ruthenium(III) ion is coordinated by three chloride ions in a mer- configuration, by dmsoso (coordinated through S) and by bidentately bonded phen (Figure 1) and is isostructural with its iridium analogue [IrCl3(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 I and

![Figure 1: Ruthenium species in I with heteroatom labeling. The ellipsoids are represented at a 30% probability level. Only one of the two crystallographically independent molecules of I 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–C11a 2.353(2), Ru1a–C12a 2.351(1), Ru1a–C13a 2.353(1), Ru1a–S1a 2.294(1), Ru1a–N1a 2.085(5), Ru1a–N2a 2.097(5).](image-url)

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its analogs reported in the literature is the presence of solvent molecules (toluene or chloroform) 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 intramolecular hydrogen bonds and π···π and π···σ stacking interactions among phenanthroline rings.

In compound 2 · 4H2O 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 [RuCl4(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 H5O2[RuCl4(L)] · 2H2O, where L is dcbpy = 4,4’-dicarboxylic acid-2,2’-bypiridine 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 · 6CH3OH 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 [Rut(phen)]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 3·6CH3OH due to different kind of anions and co-crystallized solvent molecules. Moreover, compound 3 · 6CH3OH is isostructural with [Fe(phen)3Cl2 · 6CH3OH, the compound39 which differs from 3 · 6CH3OH only in the kind of central ion.

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 \([\text{mer-RuCl_3(acv)(dmso-S)(CH_3OH)}] \cdot 0.5\text{CH}_3\text{OH}\) (4 \cdot 0.5\text{CH}_3\text{OH})^27 and ruthenium complex with guanine \([\text{trans-RuCl_3(guaH)(dmso-S)}] \cdot 2\text{H}_2\text{O} (5 \cdot 2\text{H}_2\text{O})^27\) 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 \(\text{[Ru(phen)_3]^2+}\) 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).](image)

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- \(\text{[Cu(phen)_3]^+}\) efficiently cleaves DNA\(^{45}\) in the presence of reducing agent and H\(_2\)O\(_2\) 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,\(^{37}\) 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.\(^{45}\) 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 non-covalent binding of the complexes to DNA may play a more important role than covalent binding. However, it
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. Even for binding drugs might be less important in the future in was recently suggested that the discovery of new DNA was hypothesized that this can be possible either by a passive diffusion and/or by an active transport. 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.

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-RuCl₄(dmso-5)], and phenanthroline. Whereas mer-[RuCl₄(dmso-phencyphen)] (1) and [Ru(phen)₂]Cl₂·6CH₃OH (3) are only analogues of known compounds, product (H₂O)[RuCl₃(phen)]·4H₂O (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

Z reakcijo med rutenijevim prekurzorjem [(dmso)_2H][trans-RuCl_5(dmso)_3] (P) in 1,10-fenantrolinom (phen) pri različnih pogojev smo pripravili tri nove koordinacijske spojine. Analogna kompleksa spojina mer-[RuCl_3(dmso)-phen](1) in [Ru(phen)]_2Cl_2·6CH_3OH (3·6CH_3OH) sta že poznana, vendar so bili za njuno sintezo uporabljeni drugačni postopki. Kompleks 2 · 4H_2O s strukturno formulo (H_2O)[RuCl_3(phen)] · 4H_2O pa je povsem nova spojina. Izoliran je bil izoliran na dinah. Vezava liganda phen je torej povečala toksičnost rutenijeve zvrste. Eksperimenti na govejih eritrocitih so pokazali, da toksičnost ni posledica poškodb celične membrane.