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Synthesis, structural characterization and cytotoxic activity of two new organoruthenium(II) complexes

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Abstract: Two new *p*-cymene ruthenium(II) complexes containing as additional ligands *N*-methylpiperazine ([(η^6-*p*-cymene)$RuCl_2(CH_3NH(CH_2)_4NH)$]PF_6, complex **1**) or vitamin K_3-thiosemicarbazone ([(η^6-*p*-cymene)$RuCl_2(K_3tsc)$], complex **2**) were synthesized starting from [(η^6-*p*-cymene)$_2RuCl_2]_2$ and the corresponding ligand. The complexes were characterized by elemental analysis, IR, electronic absorption and NMR spectroscopy. The X-ray crystal structure determination of complex **1** revealed "piano-stool" geometry. The differences in the cytotoxic activity of the two complexes are discussed in terms of the ligand present.

Keywords: ruthenium(II) complexes; *p*-cymene; K_3-thiosemicarbazone; *N*-methylpiperazine; cytotoxic activity.

INTRODUCTION

The field of organoruthenium complexes has been widely explored in recent years with regard to biological activity as well as catalytic activity of these complexes.[1–5] Many of these compounds are soluble in water and display cytotoxicity to cancer cells, including cisplatin-resistant cancer cells.[6,7]

Vitamin K_3, menadione, (2-methylnaphthalene-1,4-dione), is a fat-soluble vitamin and it is necessary for the production of prothrombin and five other blood clotting factors in humans. It also regulates bone calcification.[8] In addition, it was found that vitamin K_3, as well as its water-soluble derivative, menadione sodium bisulfite, has significant antitumor activity *in vitro* and *in vivo*.[9]

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On the other hand, thiosemicarbazones play an important role in research related to biological activity.[10] These ligands form complexes with metals and the influence of chelatation on antitumor activity has been very intensively investigated.[11,12] Thiosemicarbazones are the ligands of choice because thiosemicarbazones themselves exhibit antineoplastic activity. One of the most effective thiosemicarbazone is triapine (3-aminopyridine-2-carboxaldehyde thiosemicarbazone).[13,14] This compound acts as a very strong iron chelator and, consequently, inhibits ribonucleotide reductase (RR) activity.[15] The role of RR in the rate of replication of cancer cells has been well established.[16]

Hitherto, the thiosemicarbazone derivative of vitamin K_3 was used in the preparation of complexes with metals, such as Mn(II), Co(II), Ni(II), Cu(II), Zn(II) and Au(I).[17–19] It should be mentioned that the ruthenium chemistry of thiosemicarbazones has received little attention, especially concerning their potential antitumor activity. Ru(II) formed several complexes with thiosemicarbazones and some of them showed cytotoxic activity.[20–22] In addition, recently a lot of other ruthenium complexes containing the arene moiety have been synthesized and some of them have been evaluated for activity both *in vitro* and *in vivo*.[23–26] Some of the prepared complexes of the type [(η^6-arene)-RuCl(X)(Y)] showed cytotoxic activity to cisplatin-resistant cell lines. Preliminary structure–activity data showed that the hydrophobic arene group, the diamine NH group and the Cl leaving group may all play important roles in the anticancer activity of these complexes.[27]

In this paper, the characterization of two newly synthesized complexes of Ru(II) with *p*-cymene, which contain additional ligands K_3tsc or *N*-methylpiperazine, is described.

EXPERIMENTAL

Materials

Menadione sodium bisulfite (MSB), thiosemicarbazide, α-terpinene and *N*-methylpiperazine were commercially available and used without further purification. MSB thiosemicarbazone (NaK_3tsc) was prepared by treating thiosemicarbazide with menadione sodium bisulfite in an ethanol–water mixture using a published procedure.[17]

The complex $[(\eta^6\text{-}p\text{-cymene})_2RuCl_2]_2$ was prepared following a published protocol.[28]

Synthesis of the complexes

Synthesis of [(η^6-p-*cymene)RuCl*$_2$*(CH*$_3$*NH(CH*$_2$*)*$_4$*NH)]PF*$_6$ ***(1)***. A solution of *N*-methylpiperazine (0.07 ml, 0.50 mmol) in dry methanol (5.0 ml) was added to a solution of $[(\eta^6\text{-}p\text{-cymene})_2RuCl_2]_2$ (120 mg, 0.20 mmol) in dry methanol (10 ml). The resulting mixture was stirred 2 h in dark. Orange solution was then concentrated to half volume and 250 mg (1.5 mmol) of NH_4PF_6 was added. The light orange product that appeared during night was filtered off, washed with ethanol, and ether. Yield: 92.2 mg (42 %).

Synthesis of [(η^6-p-*cymene)RuCl*$_2$*(K*$_3$*tsc)] (**2**)*. A solution of K_3tsc (220 mg, 0.50 mmol) in dry methanol (5.0 ml) was added to a solution of $[(\eta^6\text{-}p\text{-cymene})_2RuCl_2]_2$ (120 mg, 0.20 mmol) in dry methanol (10 ml). The resulting mixture was stirred for 3 h. The obtained

orange solution was then concentrated to half volume and left at 4 °C overnight. The light orange product was filtered off, washed with methanol and then diethyl ether. Yield: 166 mg (67 %).

Physical measurements

Elemental analyses were carried out with an Elemental Vario EL III microanalyser. The infrared spectra were recorded on a Perkin–Elmer FTIR 31725X spectrometer using KBr pellets (4000–400 cm^{-1}). The electronic spectra were obtained using a GBC UV/Vis Cintra 40 spectrophotometer. The NMR spectra were recorded on a Varian Gemini 200 instrument. The chemical shifts for the ^1H and ^{13}C spectra are referenced to the residual ^1H and ^{13}C present in deuterated dimethyl sulfoxide.

X-ray crystal structure determination

A crystal of complex **1** suitable for an X-ray diffraction study was obtained by the slow diffusion of *n*-propanol into an aqueous solution of the complex. The data of complex **1** were collected with a CCD Oxford Xcalibur S ($\lambda(Mo_{K\alpha})$ = 0.71073 Å) using the ω and φ scan modes at 130 K. Semi-empirical correction for absorption was performed with SCALE3 ABSPACK.[29] The structure was solved by direct methods.[30] Structure refinement was performed with SHELXL-97.[31] All non-hydrogen atoms were refined anisotropically and the H atoms were located in ΔF and refined isotropically. Table I lists crystallographic details. The hydrogen atoms from the *N*-methylpiperazine ligand were placed at their calculated positions and refined using the riding model. The PF_6^- anions are disordered as demonstrated by their larger-than-normal mean-square displacement parameters. The structural disorder of the PF_6^- anions explains the relatively high *R* factors. A disordered model for PF_6 with split F-atom positions was introduced. The resultant thermal parameters were better than those without the split of the F-atom positions. Also, the split of the F-atom positions in three parts were examined. In addition, the latter refinement involved severe correlations and the resultant thermal parameters of the F-atoms were still unreasonable. Therefore, the split positions within the PF_6^- anion were introduced into the calculations and a least-squares refinement in the space group $P2_1/c$ with disordered F-atoms was preferred with the present data.

TABLE I. Crystal data, data collection and refinement parameters for **1**

Empirical formula		$C_{15}H_{27}Cl_2N_2RuF_6P$
Formula weight, g mol^{-1}		552.33
Crystal system/space group		monoclinic/$P2_1/c$
a / Å		12.2534(7)
b / Å		7.9458(16)
c / Å		14.0359(8)
β / °		111.114(6)
V / $Å^3$		2082.2(2)
Z		4
ρ / g cm^{-3}		1.762
μ(MoKα) / mm^{-1}		1.142
F(000)		1112
Scan range, °		$2.98 < \theta < 25.51$
Reciprocal lattice segments	h	$-14 \rightarrow 14$,
	k	$-15 \rightarrow 15$,
	l	$-17 \rightarrow 16$
Reflections collected		3860

Table I. Continued

Reflections independent [R_{int}]	2198 [0.078]
Data/restraints/parameters	3860/0/236
Goodness-of-fit on F^2	1.00
R_1, wR_2 [$I > 2\sigma(I)$]	0.074, 0.183
R_1, wR_2 (all data)	0.135, 0.209
Largest differences peak and hole (e Å^{-3})	3.55 and –1.19

Crystallographic data for the structural analyses of **1** have been deposited with the Cambridge Crystallographic Data Centre, CCDC-658171. Copies of this information may be obtained free of charge from The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: +44 1223-336033; E-mail: deposit@ccdc.cam.ac.uk; http//www.ccdc.cam.ac.uk).

Cytotoxicity assays

Preparation of the drug solutions. Stock solutions of the investigated ruthenium complexes were prepared in dimethyl sulfoxide (DMSO) at a concentration of 10 mM, filtered through a 0.22 μm Millipore filter before use and diluted with nutrient medium to various working concentrations. DMSO was used due to solubility problems. The nutrient medium was RPMI 1640 medium, without phenol red, supplemented with L-glutamine (3.0 mM), streptomycin (100 μg ml^{-1}), penicillin (100 IU ml^{-1}), 10 % fetal bovine serum (FBS) and 25 mM Hepes, was adjusted to pH 7.2 with bicarbonate solution. MTT, 3-(4,5-dimethylthiazol-2-yl)--2,5-diphenyltetrazolium bromide was dissolved (5.0 mg ml^{-1}) in phosphate buffer saline, pH 7.2, and filtered through a 0.22 μm Millipore filter before use. All reagents were products of Sigma Chemicals.

Cell culture. Human cervix adenocarcinoma HeLa, malignant melanoma Fem-x and human breast carcinoma MDA-MB-361 and MDA-MB-453 cells were cultured as monolayers in the nutrient medium, while human myelogenous leukemia K562 cells were maintained as a suspension culture. The cells were grown at 37 °C in a 5.0 % CO_2 humidified air atmosphere. For the growth of MDA-MB-361 and MDA-MB-453 cells and all subsequent experiments, the complete medium was enriched with 1.11 g dm^{-3} glucose. Peripheral blood mononuclear cells (PBMC) were separated from whole heparinized blood of a healthy volunteer by Lymphoprep (Nycomed, Oslo, Norway) gradient centrifugation. The interface cells, washed three times with Haemaccel (aqueous solution supplemented with 145 mM Na^+, 5.1 mM K^+, 6.2 mM Ca^{2+}, 145 mM Cl^- and 35 g dm^{-3} gelatin polymers, pH 7.4) were counted and resuspended in nutrient medium.

Cell sensitivity analysis. HeLa (2,000 cells per well), Fem-x (2,000 cells per well), MDA-MB-361 (10,000 cells per well), and MDA-MB-453 cells (3,000 cells per well) were seeded into 96-well microtiter plates and 20 h later, after cell adherence, five different concentrations of the investigated compounds were added to the wells. The final concentrations were in the range from 12.5 to 200 μM. Only nutrient medium was added to the cells in the control wells. The investigated compounds were added to a suspension of leukemia K562 cells (3,000 cells per well) 2 h after cell seeding, in the same final concentrations as applied to the HeLa and Fem-x cells. All experiments were performed in triplicate. Nutrient medium with the corresponding concentrations of the compounds but void of cells was used as the blanks. PBMC were seeded (150,000 cells per well) into nutrient medium or into nutrient medium enriched with (5.0 μg ml^{-1}) phytohaemaglutinin (PHA, Welcome Diagnostics, England) in 96-well microtiter plates and 2 h later the investigated compounds were added to the wells, in triplicate, at five final concentrations, except to the control wells where nutrient

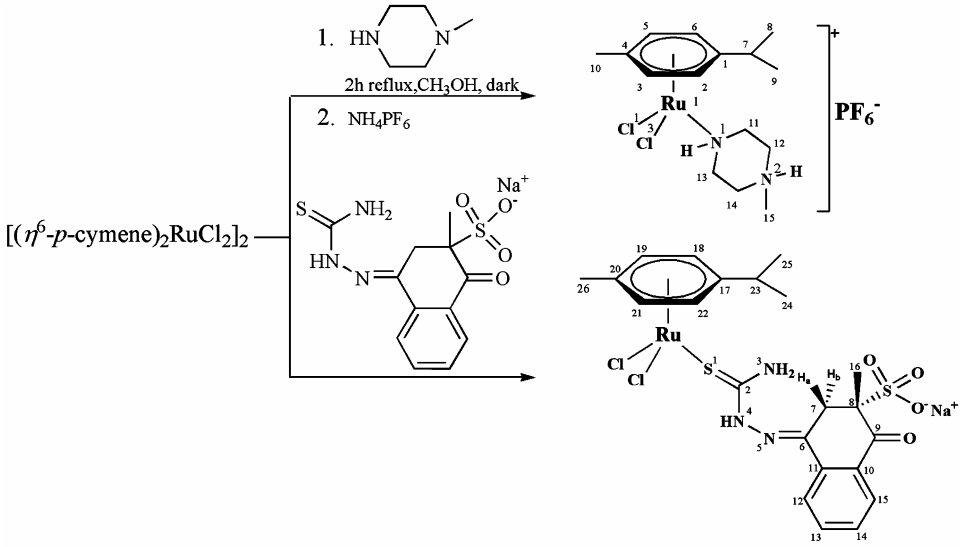
medium only was added to the cells. Nutrient medium with the corresponding concentrations of the compounds but void of cells was used as the blanks.

Determination of target cell survival. Cell survival was determined by the MTT test according to the method of Mosmann[32] modified by Ohno and Abe[33] and Drakulić *et al*.,[34] 72 h after drug addition. The concentration IC_{50} of the investigated compounds which diminished the survival of the target cells by 50 % was assessed from graphs of cell survival *vs.* concentration of the investigated compound.

RESULTS AND DISCUSSION

Synthesis of the complexes

The neutral [(η^6-*p*-cymene)$RuCl_2(K_3tsc)$] (**2**) and cationic [(η^6-*p*-cymene)-$RuCl_2(CH_3NH(CH_2)_4NH)]PF_6$ (**1**) organoruthenium complexes were obtained by reaction of K_3tsc and *N*-methylpiperazine, respectively, with [(η^6-*p*-cymene)$_2$-$RuCl_2]_2$ at ambient temperature (Scheme 1). Complex **1** is well soluble in water, ethanol (methanol) and DMSO, whereas complex **2** is less soluble in water and ethanol.



Scheme 1. Preparation of complexes **1** and **2**.

Thiosemicarbazones usually coordinate with a metal ion either in the neutral thione form or, after deprotonation, in the N,S ligand chelating mode. The diversity of coordination can be further increased by the introduction of an additional donor site into the thiosemicarbazone through the donor atom of carbonyl compounds.[11]

*X-ray diffraction study of **1***

The selected bond and angle parameters are listed in Table II. The molecule adopts the usual three-legged "piano-stool" arrangement generally found in

[M(η^6-arene)L_3] units (Fig. 1). Complex **1** is the first structurally characterized mononuclear complex of the [Ru(η^6-*p*-cymene)Cl_2L] type with the Ru atom bonded directly to a heterocyclic amine.

TABLE II. Selected bond lengths (Å) and angles (°) for complex **1**

Ru1–Cg[a]	1.674(5)	Ru1–Cl2	2.401(3)
Ru1–Cl1	2.420(2)	Ru1–N1	2.161(8)
Cg–Ru1–Cl1	127.15(16)	Cg–Ru1–Cl2	127.29(17)
Cg–Ru1–N1	135.0(2)	Cl1–Ru1–Cl2	87.63(9)
Cl1–Ru1–N1	82.2(2)	Cl2–Ru1–N1	80.6(2)

[a]The abbreviation Cg is the centroid of the η^6-arene ligand

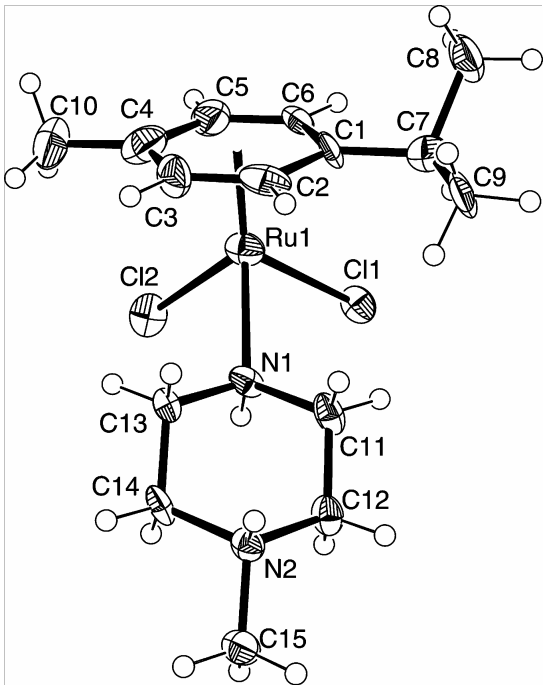


Fig. 1. ORTEP presentation of [(η^6-*p*-cymene)$RuCl_2(CH_3NH(CH_2)_4NH)]^+$ cation in crystals of **1**. Displacement ellipsoids are plotted at the 50 % probability level and H atoms are shown as small spheres of arbitrary radii.

The distance between the metal and the arene centroid, 1.674(5) Å is very similar to that observed in other ruthenium(II) complexes of the [Ru(η^6-*p*-cymene)Cl_2L] class (L/bond distance = dimethylamine/1.666; *sec*-butylamine/1.663–1.664; *p*-toluidine/1.637 Å).[35–37] Similarly to the mentioned complexes, the angles between the "legs" of the stool are slightly less than 90°, due to steric hindrance of the arene ligand. The Ru–N distance (2.161(8) Å) is comparable with the same bond distance in [$RuCl_2$(η^6-*p*-cymene)($NH(CH_3)_2$)] (2.165 Å), but it is significantly longer than those found in compounds of the same type,[36,37] indicating a relatively weaker bond.

An interesting feature of this structure is the protonation of both N atoms of the *N*-methylpiperazine ring, probably originating from NH_4PF_6.

Characterization of the complexes

The analytic and spectroscopic data of the synthesized complexes are as follows.

*[(*η^6-p-*cymene)RuCl$_2$(CH$_3$NH(CH$_2$)$_4$NH)]PF$_6$ (**1**).* Anal. Calcd. for $C_{15}H_{27}Cl_2N_2PF_6Ru$: C, 32.62; H, 4.93; N, 5.07. Found: C, 32.82; H, 4.93; N, 4.94. IR (KBr, cm^{-1}): 3331–3435, 1630, 1467, 841. ^{1}H-NMR (199.97 MHz, DMSO-d_6, δ, ppm): 8.14 (1H, *s*, N**H**), 5.79, 5.51 (4H, *m*, η^6–C$_6$**H**$_4$), 3.06 (4H, *t*, HN(C**H**$_2$)$_2$), 2.86 (4H, *m*, CH$_3$N(C**H**$_2$)$_2$), 2.30 (3H, *s*, NC**H**$_3$), 2.20 − 2.00 (4H, *m*, C**H**(CH$_3$)$_2$, η^6–C$_6$H$_4$–C**H**$_3$), 1.20 (6H, *d*, CH(C**H**$_3$)$_2$). ^{13}C-NMR (50 MHz, DMSO-d_6, δ, ppm): 106.60 (C-1), 100.31 (C-4), 86.58 (C-3,5), 85.73 (C-2,6), 51.46 (C-12,14), 45.60 (C-11,13), 43.14 (C-15), 30.18 (C-7), 21.70 (C-8,9), 18.07 (C-10). Electronic spectrum (H$_2$O) [λ_{max} / nm (ε_{max} / 10^3 dm^3 mol^{-1} cm^{-1})]: 386 (*sh*) (0.4), 306 (*sh*) (0.8), 255 (1.8).

*[(*η^6-p-*cymene)RuCl$_2$(K$_3$tsc)] (**2**).* Anal. Calcd. for $C_{22}H_{26}Cl_2N_3NaO_4S_2Ru$: C, 40.31; H, 4.00; N, 6.41; S, 9.80. Found: C, 40.41; H, 4.12; N, 6.92; S, 10.35; IR (KBr, cm^{-1}): 3363–3437, 1682, 1619, 1224, 1031, 762. ^{1}H-NMR (199.97 MHz, DMSO-d_6, δ, ppm): 10.88 (1H, *s*, N^4**H**), 9.70, 9.06 (2H, *s*, N^3**H**$_2$), 8.52 (1H, *d*, Ph**H**, tsc), 7.86 (1H, *d*, Ph**H**, tsc), 7.57 (2H, *q*, Ph**H**, tsc), 5.79, 6.28 (4H, *m*, η^6–C$_6$**H**$_4$), 3.80 (1H, *d*, **H**a, tsc), 2.87 (1H, *t*, **H**b, tsc), 2.15 (4H, *m*, C**H**(CH$_3$)$_2$, η^6–C$_6$H$_4$–C**H**$_3$), 1.51 (3H, *s*, C**H**$_3$, tsc), 1.23 (6H, *s*, CH(C**H**$_3$)$_2$). ^{13}C-NMR (50 MHz, DMSO-d_6, δ, ppm): 194.94 (C-2), 179.21 (C-9), 144.70 (C-6), 138.19 (C-10), 133.05 (C-12), 132.96 (C-15), 129.17 (C-11), 126.42 (C-14), 124.88 (C-13), 107.98 (C-17), 102.32 (C-20), 85.67 (C-19,21), 83.60 (C-18,22), 65.50 (C-8), 56.25 (C-17), 35.27 (C-7), 30.52 (C-23), 22.26 (C-26), 21.33 (C-16), 18.76 (C-24), 17.98 (C-25). Electronic spectrum (H$_2$O/EtOH, 50/50) [λ_{max} / nm (ε_{max} / 10^3 dm^3 mol^{-1} cm^{-1})]: 275 (*sh*) (15.3), 244 (19.4), 223 (19.3).

NMR spectra

The ^{1}H-NMR spectra of both complexes displayed characteristic resonances which can be attributed to the coordination of *p*-cymene, as well as thiosemicarbazone, or *N*-methylpiperazine. In both complexes the signals corresponding to the phenyl ring of cymene moiety are at δ 5.79 and 5.51 (**1**) and at 6.26 and 5.79 (**2**) ppm. The signals which can be assigned to the two methyl groups of the isopropyl groups are at 1.20 (**1**) and 1.23 (**2**) ppm. The methyl group of the *p*-cymene moiety displays a signal at around δ 2.10 ppm for both complexes. The signals assigned to the CH group of the isopropyl group were centered at δ 2.10 (**1**) and 2.15 (**2**) ppm. Hydrogen resonances due to the CH$_2$ groups of the piperazine ring are located at 2.72–2.97 ppm, appearing as a triplet of triplets ((C**H**$_2$)$_2$NCH$_3$), whereas those attached to the NH part of the heterocycle appear as a triplet centered at 3.06 ppm. The resonance confirming the protonation of the piperazine nitrogen is located at 8.14 ppm. In complex **2**, the signals corresponding to the phenyl ring of the coordinated K$_3$tsc are at δ 7.57 (*q*), 7.86 (*d*) and

8.52 (*d*). Two resonances at 3.80 and 2.87 ppm were assigned to the two diastereotropic protons of the CH_2 group of the thiosemicarbazone moiety. A signal that can be assigned to the methyl group of thiosemicarbazone is at δ 1.51 (*s*) ppm. Two resonances at 9.06 and 9.70 ppm were assigned to the two protons of the N^3H_2 chain, whereas the proton of the azomethine nitrogen appears at 11.88 ppm. The ^{13}C-NMR spectrum of complex **1** shows upfield shifts (51.46 (C-2,6), 45.60 (C-3,5), 43.14 (C-7)) for the piperazine part of the molecule comparing to free piperazine (56.29 (C-2,6), 46.75 (C-3,5) and 45.67 (C-7)), due to coordination *via* the unsubstituted N-4 nitrogen. In the spectrum of complex **2**, a slight upfield shift of C-2 was observed as a consequence of *S*-metallation, while no remarkable shifts of the other carbon atoms were observed.

Infrared spectra

The spectrum of the free ligand, K_3tsc, was compared with those of the complexes to confirm its coordination to metal ion. The ν(CS) vibrations suffer a negative shift from 1278 (**L**) to 1232 cm^{-1} (**2**). Also, the ν(CN) vibration of the C^2–N^4 bond is slightly shifted to lower frequencies due to the partial multiplicity of this bond after coordination. The peaks at 1690 cm^{-1} in the spectrum of the free ligand due to ν(C=O) vibrations and at 1639 cm^{-1} due to ν(CN) vibrations of C^6–N^5 remained unchanged in the complex, confirming the non-coordination of oxygen and the azomethine nitrogen to the metal ion.

This study showed that in complex **2**, K_3tsc acts as a neutral monodentate ligand, which is coordinated to the metal ion *via* the sulfur atom.

UV/Vis spectra

The complexes exhibit intense absorption in the visible region which can be ascribed to high intensity metal-to-ligand charge transfer bands which interfere with the expected d–d transitions, as is observed in low-spin d^6 ruthenium(II) complexes.[38] In general, complexes **1** and **2** exhibit two well resolved bands. The bands at higher wavelengths can be ascribed to a Ru(4dπ) → π*(NH) MLCT transition, whereas the bands at lower wavelengths are attributable to intraligand transitions or a combination of MLCT bands and intraligand transitions.

Cytotoxicity assays

The *in vitro* cytotoxicity of complexes **1** and **2** was determined by an MTT-based assay. The complexes were tested for cytotoxic activity on tumor cell lines: human adenocarcinoma HeLa, human myelogenous leukemia K562, human malignant melanoma Fem-x, human breast carcinoma MDA-MB-361 and MDA-MB-453 cells and on normal immunocompetent cells, *i.e.*, on human peripheral blood mononuclear PBMC cells non-stimulated and stimulated for proliferation with phytohaemaglutinin (PHA). The data are given in Table III and Fig. 2.

TABLE III. IC_{50} (μM) for 72 h of action of the investigated compounds on various tumor cells and on non-stimulated PBMC and PBMC stimulated with PHA, determined by the MTT test

Tumor cell	Compound	
	1	**2**
HeLa	>200	62.50±3.54
K562	>200	71.26±4.22
Fem-x	>200	152.10±5.18
MDA-MB-361	>200	101.84±6.54
MDA-MB-453	>200	62.46±5.98
PBMC-PHA	Not detected	61.10±1.12
PBMC+PHA	Not detected	40.38±1.12

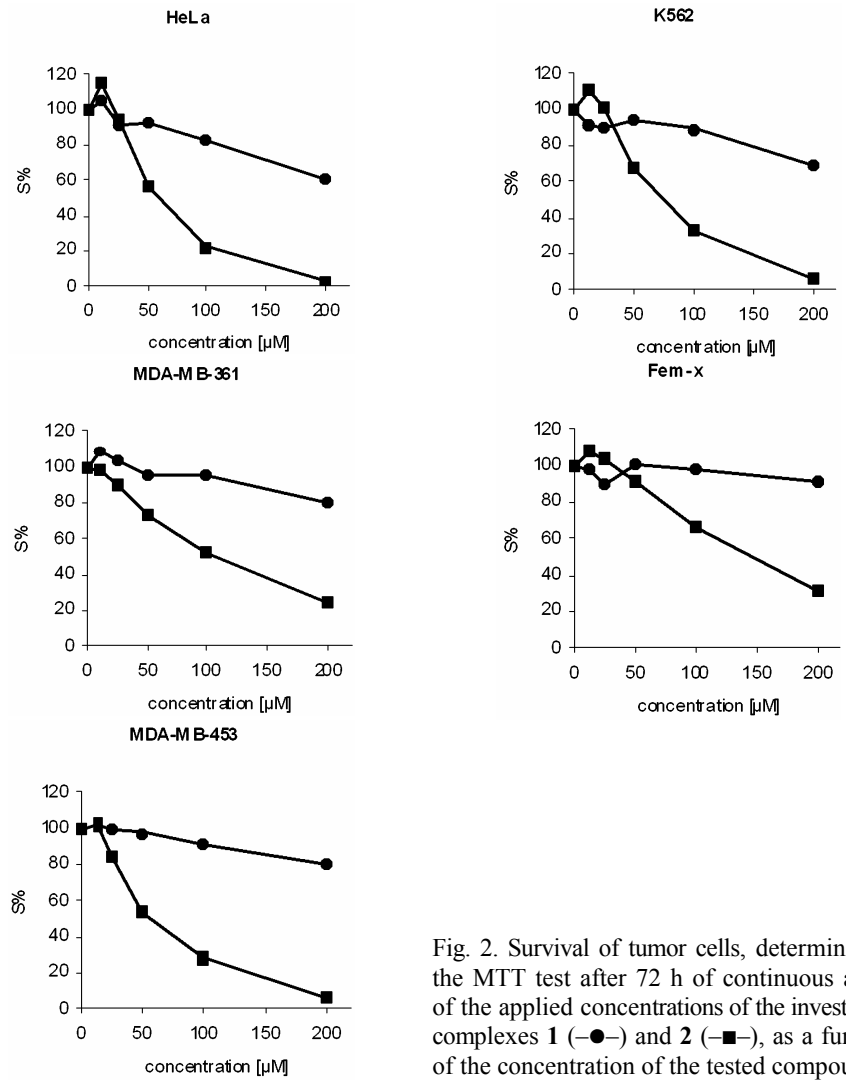


Fig. 2. Survival of tumor cells, determined by the MTT test after 72 h of continuous action of the applied concentrations of the investigated complexes **1** (–●–) and **2** (–■–), as a function of the concentration of the tested compounds.

Complex **1** exerted low activity against all the selected cell lines ($IC_{50} > 200$ μM, Table III and Fig. 2) as determined by the MTT test. This result is consistent with the characteristics of ruthenium compounds which are generally less cytotoxic than platinum compounds. One of two ruthenium complexes that is under clinical investigations, *trans*-[HInd][$RuCl_4(Ind)_2$] (Ind = indazole; KP1019) is only moderately cytotoxic to cancer cells,[39] whereas the other complex, *trans*-[HIm]-[$RuCl_4$(DMSO)(Im)] (Im = imidazole; NAMI-A) is relatively non-toxic to primary cancer cells, but exhibits antimetastatic activity.[40]

Complex **2** showed low to moderate activity with $IC_{50} = 152.10$ μM for Fem-x and $IC_{50} = 101.84$ μM for the slowly proliferating MDA-MB-361 cells. Against HeLa, K562 and the rapidly proliferating MDA-MB-453 cell lines, compound **2** exerted a moderate dose dependent antiproliferative action, with IC_{50} values of 62.50, 71.26 and 62.46 μM, respectively. Complex **2** was more active against the normal control PBMC, with $IC_{50} = 61.10$ μM for non-stimulated resting PBMC and $IC_{50} = 40.38$ μM for PBMC stimulated by PHA. Results from this work are in accordance with many publications which deal with antitumor activity of metal complexes of thiosemicarbazones[13,14,22] and of antileukemia potency of thiosemicarbazones.[41,42] In addition, this compound acted toxically, especially to PBMC which were stimulated for proliferation. This indicates that the mentioned complex might also have a capability for the suppression of autoimmune diseases. Further investigations in this direction need to be performed.

CONCLUSIONS

Two new organoruthenium complexes were synthesized and characterized. The results from this work showed that the ruthenium complex containing vitamin K_3-thiosemicarbazone as the organic part exerted its maximal cytotoxic activity against immunocompetent cells stimulated for proliferation. The higher activity of this complex compared with the complex containing *N*-methylpiperazine is probably related with the presence of vitamin K_3-thiosemicarbazone, itself active. In addition, the obtained data indicate that complex **2** could be further studied regarding its promising action for the control not only of malignant diseases, but also for the control of autoimmune diseases.

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И З В О Д

СИНТЕЗА, СТРУКТУРНА КАРАКТЕРИЗАЦИЈА И ЦИТОТОКСИЧНА АКТИВНОСТ ДВА НОВА ОРГАНОРУТЕНИЈУМ(II) КОМПЛЕКСА

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Синтетисана су два нова *p*-цимен-рутенијум(II) комплекса који садрже као додатне лиганде *N*-метилпиперазин ([(η^6-*p*-cimen)$RuCl_2(CH_3NH(CH_2)_4NH)$]PF_6, комплекс **1**) и витамин K_3-тиосемикарбазон ([(η^6-*p*-cimen)$RuCl_2(K_3tsc)$], комплекс **2**). Оба нова комплекса добијена су полазећи од [(η^6-*p*-cimen)$_2RuCl_2]_2$ комплекса и одговарајућег лиганда. Комплекси су окарактерисани елементалном анализом, ИЦ, електронско-апсорпционом и НМР спектроскопијом. Рендгено–структурна анализа комплекса **1** показала је „piano-stool“ геометрију. У зависности од присутног лиганда дискутована је разлика у цитотоксичној активности ова два добијена комплекса.

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