



## X-Ray structure and cytotoxic activity of a picolinate ruthenium(II)–arene complex

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**Abstract:** A ruthenium(II)–arene complex with picolinic acid,  $[(\eta^6\text{-}p\text{-cymene})\text{RuCl}(\text{pico})]\cdot\text{H}_2\text{O}$ , was prepared by the reaction of  $[(\eta^6\text{-}p\text{-cymene})\text{RuCl}_2]_2$  with picolinic acid in a 1:2 molar ratio in 2-propanol. The compound was characterized by elemental analysis, and IR and NMR spectroscopy. X-ray diffraction analysis showed that the molecule adopts a “three-leg piano-stool” geometry, which is common for this type of complexes. The cytotoxic activity of the complex was tested in two human cancer cell lines HeLa (cervix) and FemX (melanoma) by MTT assay. The  $IC_{50}$  values were at 82.0 and 36.2  $\mu\text{mol dm}^{-3}$  for HeLa and FemX cells, respectively.

**Keywords:** ruthenium(II)–arene; picolinic acid; cytotoxic activity.

### INTRODUCTION

The structures and chemical properties of metal complexes of some pyridine carboxylates have been widely investigated.<sup>1–5</sup> Picolinic acid (2-pyridinecarboxylic acid) is a biologically important ligand incorporated into some enzymes, and it is an active agent in some drugs as well.<sup>6–11</sup> It is also catabolite of L-tryptophan detected in the human body.<sup>12,13</sup> Picolinates are used as dietary supplements. In particular, the chromium(III) complex reduces diabetes risk and therefore is used as a dietary supplement for obese people.<sup>14</sup> Zinc picolinate revealed an effect in the oxidant–antioxidant balance in patients with chronic obstructive pulmonary disease.<sup>15</sup> Alkaline picolinates inhibit the growth of *Escherichia coli*.<sup>16,17</sup> Platinum complexes with picolinic acid have been synthesized and screened for cytotoxic activity.<sup>18</sup>

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In recent years, ruthenium complexes have gained much attention<sup>19–23</sup> in attempts to overcome the downsides of platinum complexes. Organometallic complexes and half-sandwich complexes of Ru(II) emerged as promising scaffolds for anticancer drug design.<sup>24–35</sup> They often show aqueous solubility along with the necessary lipophilicity. The electronic system of the arene ligand stabilizes the metal in its lower oxidation state and also provides a hydrophobic face in the complex, which might enhance transport of ruthenium through cell membranes. In addition, ruthenium compounds possess good cytotoxic activity, while not notably affecting normal cells.<sup>36,37</sup> One aspect of the action of ruthenium complexes is their ability to bind with the serum proteins: transferrin and albumin.<sup>38</sup> Tumor cells are more susceptible to ruthenium complexes due to an increased demand for iron and therefore there is an increased number of transferrin receptors on their surface.<sup>39,40</sup> In addition, because ruthenium can mimic iron in binding to carrier proteins, its excess can be removed from cells, which is the reason for lower toxicity of ruthenium complexes compared to platinum complexes.<sup>39</sup>

Recently, two series of Ru(II)-arene complexes with functionalized pyridines were described of the general formulae  $[(\eta^6\text{-}p\text{-cymene})\text{Ru}(\text{XY})\text{Cl}]$  and  $[(\eta^6\text{-}p\text{-cymene})\text{Ru}(\text{X})\text{Cl}_2]$ , where XY were the mono-anionic N,O-bidentates 2,3-pyridine-, 2,4-pyridine-, 2,5-pyridine- and 2,6-pyridine-dicarboxylate, while X were monodentate ligands 3-acetylpyridine, 4-acetylpyridine, 2-amino-5-chloropyridine, isonicotinic or nicotinic acid bound to ruthenium(II) *via* the pyridine nitrogen.<sup>41</sup>

Herein the X-ray diffraction structure of  $[(\eta^6\text{-}p\text{-cymene})\text{Ru}(\text{pico})\text{Cl}]$  and its antiproliferative activity in two human cancer cell lines (cervix HeLa and melanoma Femx) are reported. Since the introduction of picolinate into a metal complex can result in enhanced activity,<sup>42,43</sup> the aim of this work was to compare the activities of the prepared complex with previously described analogue complexes.<sup>41</sup> It should be noted that the complex was previously described but its X-ray diffraction structure has not hitherto been reported.<sup>44</sup>

## EXPERIMENTAL

### *Materials and measurements*

Picolinic acid was purchased from Acros Organics and used without further purification.  $[(\eta^6\text{-}p\text{-Cymene})\text{RuCl}_2]_2$  was prepared according to a published procedure.<sup>45</sup> Elemental analysis was realized using an Elemental Vario EL III microanalyzer. The infrared spectra were recorded on a Nicolet 6700 FT-IR spectrometer using the attenuated total reflectance (ATR) technique. The <sup>1</sup>H- and <sup>13</sup>C-NMR spectra of the ligand and the complex were recorded on a Varian Gemini 200 instrument. Chemical shifts were referenced to residual <sup>1</sup>H and <sup>13</sup>C present in deuterated dimethyl sulfoxide.

### *Synthesis of the complex*

To a warm solution of  $[(\eta^6\text{-}p\text{-cymene})\text{RuCl}_2]_2$  (0.100 g, 0.16 mmol) in 2-propanol (25 cm<sup>3</sup>) was added a solution of picolinic acid (0.046 g, 0.35 mmol) in 2-propanol (5 cm<sup>3</sup>). The

mixture was stirred at room temperature for 7 days and then kept in refrigerator until the product precipitated. The yellow-orange product was filtered off, washed with several drops of 2-propanol and then diethyl ether and dried in air. A crystal suitable for X-ray analysis was obtained by the slow evaporation of the mother liquor.

#### Crystallographic structure determination

The measurement was performed on a Bruker X8 APEXII CCD diffractometer. A single crystal was positioned at 35 mm from the detector and 941 frames were measured, each for 30 s over a 1° scan width. The data were processed using SAINT-Plus software.<sup>46</sup> The crystal data, data collection parameters and structure refinement details are given in Table I. The structure was solved by direct methods and refined by full-matrix least-squares techniques. Non-hydrogen atoms were refined with anisotropic displacement parameters. The H atoms were placed at calculated positions and refined as riding atoms in the subsequent least-squares model refinements. The isotropic thermal parameters were estimated to be 1.2 or 1.5 times (methyl groups) the values of the equivalent isotropic thermal parameters of the non-hydrogen atoms to which the hydrogen atoms were bonded. The following software programs, personal computer and tables were used: structure solution, SHELXS-97,<sup>47</sup> refinement, SHELXL-97,<sup>48</sup> molecular diagrams, ORTEP,<sup>49</sup> Pentium IV, Tables 4.2.6.8 and 6.1.1.4 for the scattering factors were taken from the literature.<sup>50</sup>

TABLE I. Crystal data and details of data collection for 1·H<sub>2</sub>O

Empirical formula	C <sub>16</sub> H <sub>20</sub> ClNO <sub>3</sub> Ru
<i>FW</i>	410.85
Space group	<i>Pn</i>
<i>a</i> / Å	8.9150(4)
<i>b</i> / Å	8.6498(4)
<i>c</i> / Å	10.6539(4)
$\beta$ / °	91.853(3)
<i>V</i> / Å <sup>3</sup>	821.12(6)
<i>Z</i>	2
$\lambda$ / Å	0.71073
$\rho_{\text{calcd}}$ / g cm <sup>-3</sup>	1.662
Crystal size, mm <sup>3</sup>	0.50×0.05×0.01
<i>T</i> / K	100
$\mu$ / mm <sup>-1</sup>	1.128
<i>R</i> <sub>1</sub> <sup>a</sup>	0.0381
<i>wR</i> <sub>2</sub> <sup>b</sup>	0.0687
GOF <sup>c</sup>	0.979

<sup>a</sup> $R_1 = \sum |F_o| - |F_c| / \sum |F_o|$ ; <sup>b</sup> $wR_2 = \{\sum [w(F_o^2 - F_c^2)^2] / \sum [w(F_o^2)^2]\}^{1/2}$ ; <sup>c</sup>GOF =  $\{\sum [w(F_o^2 - F_c^2)^2] / (n - p)\}^{1/2}$ , where *n* is the number of reflections and *p* is the total number of parameters refined.

#### Cytotoxicity

**Cell culture.** Human cervix carcinoma cells (HeLa) and human melanoma cells (FemX) were maintained as monolayer cultures in Roswell Park Memorial Institute (RPMI) 1640 nutrient medium (Sigma Chemicals Co, USA). The RPMI 1640 nutrient medium was prepared in sterile deionized water, supplemented with penicillin (192 U ml<sup>-1</sup>), streptomycin (200 µg ml<sup>-1</sup>), 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) (25 mM), L-glutamine (3 mM) and 10 % heat-inactivated fetal calf serum (FCS) (pH 7.2). The cells were grown at 37 °C in a 5 % CO<sub>2</sub> humidified air atmosphere.

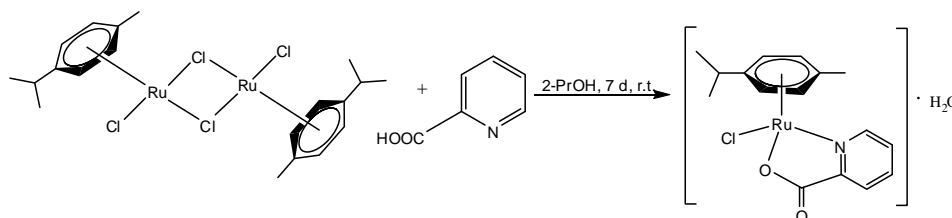
**Cytotoxicity assay.** The drug-induced cytotoxicity was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma) assay.<sup>51</sup> Cells were seeded in 96-well cell culture plates (NUNC), HeLa (2000 c/w) and FemX (2000 c/w), in culture medium and grown for 24 h. A stock solution of the complex was prepared in DMSO at a concentration of 30 mM and subsequently diluted with nutrient medium to the desired final concentrations (in the range up to 300  $\mu\text{M}$ ).

Solutions of various concentrations of the examined compound were added to the wells, except for the control wells where only the nutrient medium was added. All samples were prepared in triplicate. Nutrient medium with corresponding agent concentrations but without the target cells was used as the blank, also in triplicate. The cells were incubated with the test compound for 48 h at 37 °C, in a 5 % CO<sub>2</sub> humidified air atmosphere. After incubation, 20  $\mu\text{L}$  of MTT solution, 5 mg mL<sup>-1</sup> in phosphate buffer solution (PBS), pH 7.2, were added to each well. The samples were incubated for 4 h at 37 °C in a 5 % CO<sub>2</sub> humidified air atmosphere. Formazan crystals were dissolved in 100  $\mu\text{L}$  10 % sodium dodecyl sulfate (SDS) in 0.01 M HCl. The absorbance was recorded on an enzyme-linked immunosorbent assay (ELISA) reader after 24 h at a wavelength of 570 nm. The IC<sub>50</sub> ( $\mu\text{M}$ ) was defined as the concentration of drug producing 50 % inhibition and was determined from cell survival diagrams.

## RESULTS AND DISCUSSION

### Synthesis

The reaction of  $[(\eta^6\text{-}p\text{-cymene})\text{RuCl}_2]_2$  with picolinic acid in a 1:2 molar ratio in 2-propanol at room temperature leads to the formation of the complex  $[(\eta^6\text{-}p\text{-cymene})\text{RuCl}(\text{pico})]\cdot\text{H}_2\text{O}$  in high yield (Scheme 1). Crystals precipitated directly from the reaction mixture. The complex is soluble in water, methanol, ethanol, acetonitrile and dimethyl sulfoxide.



Scheme 1. Synthesis of the complex  $[(\eta^6\text{-}p\text{-cymene})\text{RuCl}(\text{pico})]\cdot\text{H}_2\text{O}$

### Analytic and spectral data

Yield: 0.1 g, 76.9 %. Anal. Calcd. for C<sub>16</sub>H<sub>20</sub>O<sub>3</sub>NRuCl ( $M_r = 410.86$ ): C, 46.77; H, 4.91; N, 3.41 %. Found: C, 46.70; H, 4.98; N, 3.39. IR (ATR, cm<sup>-1</sup>): 3536, 3467 (*m*), 3069, 2955 (*w*), 1637 (*s*), 1601 (*w*). <sup>1</sup>H-NMR (199.97 MHz, DMSO-*d*<sub>6</sub>,  $\delta$  / ppm): 1.12 (6H, *dd*, -CH(CH<sub>3</sub>)<sub>2</sub>,  $J = 2.2$  and 7 Hz), 2.15 (3H, *s*, -CH<sub>3</sub>), 2.72 (1H, *m*, -CH(CH<sub>3</sub>)<sub>2</sub>,  $J = 6.8$  Hz), 5.88 and 5.65 (4H, 2*t*, CH (arene),  $J = 4.6$  and 7.8 Hz), 7.74 (1H, *m*, H<sup>4</sup>,  $J = 7.3$  Hz), 7.79 (1H, *m*, H<sup>3</sup>), 8.09 (1H, *td*, H<sup>5</sup>,  $J = 7.5$  Hz), 9.26 (1H, *d*, H<sup>6</sup>,  $J = 5.6$  Hz). <sup>13</sup>C-NMR (50.28 MHz, DMSO-*d*<sub>6</sub>,  $\delta$  / ppm) 18.27 (CH<sub>3</sub>), 22.00 (CH(CH<sub>3</sub>)<sub>2</sub>), 30.65 (CH(CH<sub>3</sub>)<sub>2</sub>), 80.23, 81.21, 82.60,

82.78, 98.38 and 101.17 (CH (arene)), 125.59 (C3), 128.30 (C5), 139.86 (C4), 150.73 (C2), 154.01 (C6), 170.70 (C1).

### Spectroscopy

$[(\eta^6\text{-}p\text{-Cymene})\text{RuCl}(\text{pico})]\cdot\text{H}_2\text{O}$  exhibits an asymmetric stretching vibration  $\nu_{\text{as}}(\text{COO}^-)$  at  $1637\text{ cm}^{-1}$ . Picolinic acid revealed an analogous vibration of the free carboxylic group at  $1718\text{ cm}^{-1}$ . The difference in frequency is due to coordination of the ligand through one of the oxygen atoms of the carboxylic group and nitrogen atom of the pyridine ring, and is consistent with the X-ray diffraction structure.

The  $^1\text{H}$  NMR spectrum of the complex contains a characteristic pattern for the *p*-cymene moiety. A methyl group singlet is seen at 2.15 ppm, the resonance signal of  $-\text{CH}(\text{CH}_3)_2$  appears as a multiplet at 2.72 ppm and  $-\text{CH}(\text{CH}_3)_2$  as a doublet at 1.12 ppm. The resonances of the arene ring protons were found at 5.64 and 5.88 ppm. Aromatic region of the  $^1\text{H}$ -NMR spectrum of the complex also shows four resonances (7.74 (1H), 7.78 (1H), 8.09 (1H), 9.26 (1H)) of coordinated picolinate. Concerning the pyridine protons,  $\text{H}^3$  and  $\text{H}^4$  are shifted downfield by 0.3 and 0.27 ppm, respectively, while  $\text{H}^5$  and  $\text{H}^6$  are shifted upfield by 0.44 and 0.52 ppm, respectively, compared with the free ligand as a consequence of picolinate coordination to the ruthenium(II) atom.

The  $^{13}\text{C}$ -NMR spectrum displays resonances at 18.27 ppm from the methyl group attached to the cymene moiety, 22.00 ppm from  $-\text{CH}(\text{CH}_3)_2$ , while the signal at 30.65 ppm is due to the  $\text{CH}(\text{CH}_3)_2$  group. The aromatic carbons from cymene display resonances within 80.23–101.17 ppm. Five pyridine carbon resonances were observed at 125.59 (C<sup>3</sup>), 128.30 (C<sup>5</sup>), 139.86 (C<sup>4</sup>), 150.73 (C<sup>2</sup>), 154.01 (C<sup>6</sup>) and carboxylate carbon at 170.67 ppm (C<sup>1</sup>).

### X-Ray crystallography

The structure of  $[(\eta^6\text{-}p\text{-cymene})\text{RuCl}(\text{pico})]\cdot\text{H}_2\text{O}$  was confirmed by X-ray diffraction. The complex crystallized in the monoclinic space group *Pn* and has the typical “three-leg piano-stool” geometry well-documented for a large number of ruthenium(II) and osmium(II) arene complexes, and in particular, for the closely related compounds  $[(\eta^6\text{-}1,3,5\text{-Me}_3\text{C}_6\text{H}_3)\text{RuCl}(\text{pico})]^{52}$  and  $[(\eta^6\text{-}p\text{-cymene})_2\text{OsCl}(\text{pico})]^{42}$  with the  $\eta^6$   $\pi$ -bound arene ring forming the seat and the picolinate ligand bound *via* a nitrogen and one carboxylic oxygen, with one chloride ligand as the legs of the piano-stool. Selected bond lengths and angles are given in the legend to Fig. 1. The bond lengths Ru–ring centroid, Ru–Cl, Ru–O1 and Ru–N1 in  $[(\eta^6\text{-}p\text{-cymene})\text{Ru}^{\text{II}}\text{Cl}(\text{pico})]\cdot\text{H}_2\text{O}$  of 1.665(9), 2.4225(9), 2.085(2) and 2.101(3) Å, respectively, are slightly longer than similar bonds 1.652(2), 2.4048(13), 2.080(3) and 2.090(4) Å in  $[(\eta^6\text{-}p\text{-cymene})\text{Os}^{\text{II}}\text{Cl}(\text{pico})]^{42}$ . The shortening of the Ru–N and Ru–O bonds in *mer*- $[\text{Ru}^{\text{III}}(\text{pico})_3]\cdot\text{H}_2\text{O}$  (2.052(3), 2.064(3), 2.052(3)

and 2.002(3), 2.024(3), 1.996(2) Å, respectively)<sup>53</sup> is even more evident. The Ru–Cl, Ru–N1 and Ru–O1 bonds in  $[(\eta^6\text{-}1,3,5\text{-Me}_3\text{C}_6\text{H}_3)\text{RuCl}(\text{pico})]^{52}$  are at 2.420(1), 2.102(4) and 2.101(4) Å, respectively. Two hydrogen bonding interactions between the co-crystallized water molecule and **1** of the type O3–H...O2 (O3–H, 0.86, H...O2, 1.94, O3...O2, 2.78 Å, O3–H...O2, 168.5°) and O3–H...Cl1<sup>i</sup> ( $x + 0.5, -y + 1, z + 0.5$ ) (O3–H, 0.87, H...O2, 1.94, O3...Cl1<sup>i</sup>, 2.78 Å, O3–H...Cl1<sup>i</sup>, 172.6°) are evident in the crystal structure of **1**·H<sub>2</sub>O.

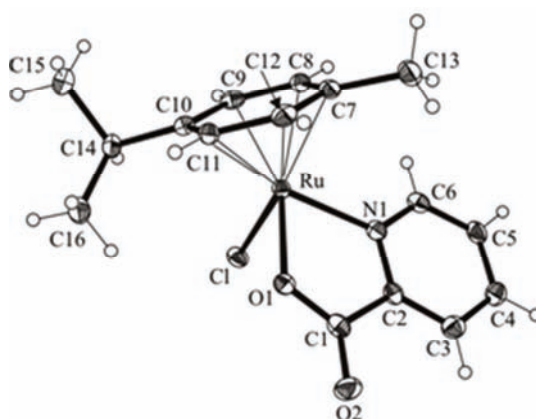


Fig. 1. ORTEP view of a molecule of **1** with atom-labeling scheme and thermal ellipsoids drawn at the 50 % probability level. Selected bond distances (Å) and angles (°): Ru–O1 2.085(2), Ru–N1 2.101(3), Ru–Cl 2.4225(9), Ru–C7 2.195(3), Ru–C8 2.186(3), Ru–C9 2.175(4), Ru–C10 2.211(4), Ru–C11 2.192(4) and Ru–C12 2.176(4), O1–Ru–N1 77.96(10).

### Cytotoxic activity

The antiproliferative activity of the prepared complex was assayed in two human cancer cell lines HeLa (cervix) and FemX (melanoma) by the MTT assay. The tumor cells were incubated for 48 h with the investigated complex. The results of these tests indicate that the complex after 48 h of incubation exhibited cytotoxic activity with  $IC_{50}$  81.97  $\mu\text{M}$  for HeLa cells and 36.23  $\mu\text{M}$  for FemX cells. These values are the mean of 2 to 3 independent experiments, whereby the standard deviations were less than 15 %. The results of representative experiments are shown in Fig. 2.

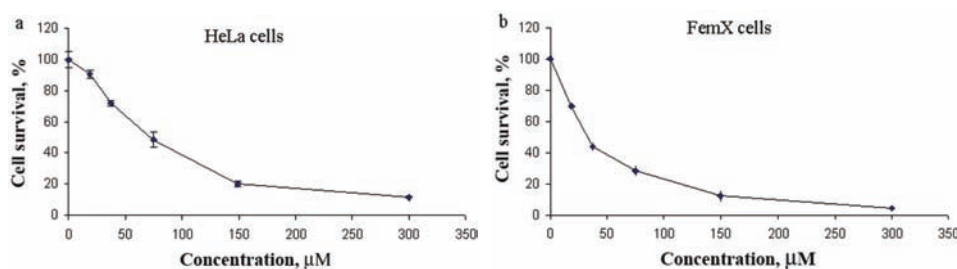


Fig. 2. Diagram of (a) HeLa and (b) FemX cells survival after 48 h of continual agent action. Data are representative for one out of two to three separate experiments with standard deviation.

## CONCLUSIONS

In this paper, the synthesis and characterization of the organoruthenium complex,  $[(\eta^6-p\text{-cymene})\text{RuCl}(\text{pico})]\cdot\text{H}_2\text{O}$  is described. Although in a previous work, structurally related complexes were found to have limited antiproliferative activity in tumor cells, the complex reported herein exhibits much higher cytotoxicity in cervix HeLa and melanoma FemX human cancer cell lines. This implies that the presence of picolinate coordinated to a metal center had a notable effect on cytotoxic activity. This makes this new ruthenium complex of interest for further investigation.

## SUPPLEMENTARY MATERIAL

Crystallographic data for **1** has been deposited with the Cambridge Crystallographic Data Center as supplementary publication No. CCDC 775760. Copies of the data can be obtained free of charge on application to The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336 033; e-mail: deposit@ccdc.cam.ac.uk).

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## ИЗВОД

РЕНДГЕНСКА СТРУКТУРНА АНАЛИЗА И ЦИТОТОКСИЧНА АКТИВНОСТ  
ПИКОЛИНАТО РУТЕНИЈУМ(II)-АРЕНСКОГ КОМПЛЕКСА

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Рутенијум(II)-аренски комплекс са пиколинском киселином  $[(\eta^6-p\text{-цимен})\text{RuCl}(\text{пиколинато})]\cdot\text{H}_2\text{O}$  синтетисан је у реакцији  $[(\eta^6-p\text{-цимен})\text{RuCl}_2]_2$  комплекса са пиколинском киселином у молском односу 1:2 у изопропанолу. Једињење је окарактерисано елементалном анализом, ИЦ и NMR спектроскопијом. Анализа дифракцијом X-зрацима показала је да молекул има тзв. “three-leg piano-stool” геометрију која је карактеристична за овај тип комплекса. Цитотоксична активност комплекса је одређена на две хумане туморске ћелијске линије, HeLa (грлића материце) и FemX (меланома), МТТ тестом. IC<sub>50</sub> вредности су биле 82,0 и 36,2  $\mu\text{mol dm}^{-3}$  за HeLa и FemX ћелије, редом.

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## REFERENCES

1. O. Jøns, E.S. Johansen, *Inorg. Chim. Acta* **151** (1988) 129
2. S.C. Dixit, R. Sharan, R.N. Kapoor, *Inorg. Chim. Acta* **109** (1989) 113
3. E. Libby, R.J. Webb, W.E. Streib, K. Folting, J.C. Huffman, D.N. Hendrickson, G. Christou, *Inorg. Chem.* **28** (1989) 4037
4. D.M. Streamns, W.H. Armstrong, *Inorg. Chem.* **31** (1992) 5178
5. W. Li, M.M. Olmstead, D. Miggins, R.H. Fish, *Inorg. Chem.* **35** (1996) 51
6. H. Ding, L.K. Olson, J.A. Caruso, *Spectrochim. Acta B* **51** (1996) 1801

7. G.S. Morris, K.A. Guindry, M. Hegsted, D.L. Hasten, *Nutr. Res.* **15** (1995) 1045
8. N.E. Chakov, R.A. Collins, J.B. Vincent, *Polyhedron* **18** (1999) 2891
9. Y. Liang, L.K. Noda, O. Sala, *J. Mol. Struct.* **554** (2000) 271
10. D.M. Stearns, S.M. Silveira, K.K. Wolf, A.M. Luke, *Mutat. Res.* **513** (2002) 135
11. D.D. Hepburn, J.M. Burney, S.A. Woski, J.B. Vincent, *Polyhedron* **2** (2003) 455
12. M. Hidalgo, S.G. Eckhardt, *J. Natl. Cancer Inst.* **93** (2001) 178
13. S. Cai, K. Sato, T. Shimizu, S. Yamabe, M. Hiraki, C. Sano, H. Tamioka, *J. Antimicrob. Chemother.* **57** (2006) 85
14. J. R. Komorowski, D. Greenberg, V. Juturu, *Toxicol. In Vitro* **22** (2008) 819
15. G. Kirkil, M. Hamdi Muz, D. Seckin, K. Sahin, O. Kucuk, *Respiro. Med.* **102** (2008) 840
16. P. Koczoń, J. Piekut, M. Borawska, R. Świslocka, W. Lewandowski, *Spectrochim. Acta A* **61** (2005) 819
17. P. Koczoń, J. Piekut, M. Borawska, R. Świslocka, W. Lewandowski, *Anal. Bioanal. Chem.* **384** (2006) 302
18. R. Song, K. M. Kim, Y. S. Sohn, *Inorg. Chim. Acta* **292** (1999) 238
19. S. Grguric-Sipka, C. R. Kowol, S.-M. Valiahdi, R. Eichinger, M. A. Jakupec, A. Roller, S. Shova, V. B. Arion, B. K. Keppler, *Eur. J. Inorg. Chem.* (2007) 2870
20. C. R. Kowol, R. Eichinger, M. A. Jakupec, M. Galanski, V. B. Arion, B. K. Keppler, *J. Inorg. Biochem.* **101** (2007) 1946
21. W. F. Schmid, S. Zorbas-Seifried, R. O. John, V. B. Arion, M. A. Jakupec, A. Roller, M. Galanski, I. Chiorescu, H. Zorbas, B. K. Keppler, *Inorg. Chem.* **46** (2007) 3645
22. I. Bratsos, G. Birarda, S. Jedner, E. Zangrando, E. Alessio, *Dalton Trans.* (2007) 4048
23. C. Streu, P. J. Carroll, R. K. Kohli, E. Meggers, *J. Organomet. Chem.* **693** (2008) 551
24. W. F. Schmid, R. O. John, V. B. Arion, M. A. Jakupec, B. K. Keppler, *Organometallics* **26** (2007) 6643
25. W. F. Schmid, R. O. John, G. Mühlgassner, P. Hefeter, M. A. Jakupec, M. Galanski, W. Berger, V. B. Arion, B. K. Keppler, *J. Med. Chem.* **50** (2007) 6343
26. R. Schuecker, R. O. John, M. A. Jakupec, V. B. Arion, B. K. Keppler, *Organometallics* **27** (2008) 6587
27. L. K. Filak, G. Mühlgassner, M. A. Jakupec, P. Hefeter, W. Berger, V. B. Arion, B. K. Keppler, *J. Biol. Inorg. Chem.* **15** (2010) 903
28. S. M. Guichard, R. Else, E. Reid, B. Zeitlin, R. Aird, M. Muir, M. Dodds, H. Fiebig, P. J. Sadler, D. I. Jodrell, *Biochem. Pharm.* **71** (2006) 408
29. T. Bugarcic, A. Habtemariam, J. Stepankova, P. Heringova, J. Kasparikova, R. J. Deeth, R. D. L. Johnstone, A. Prescimone, A. Parkin, S. Parsons, V. Brabec, P. J. Sadler, *Inorg. Chem.* **47** (2008) 11470
30. T. Bugarcic, A. Habtemariam, R. J. Deeth, F. P. A. Fabbiani, S. Parsons, P. J. Sadler, *Inorg. Chem.* **48** (2009) 9444
31. T. Bugarcic, O. Nováková, A. Halámková, L. Zerzánková, O. Vrána, J. Kašpárková, A. Habtemariam, S. Parsons, P. J. Sadler, V. Brabec, *J. Med. Chem.* **51** (2008) 5310
32. M. Gras, B. Therrien, G. Süß-Fink, P. Štěpnička, A. K. Renfrew, P. J. Dyson, *J. Org. Chem.* **693** (2008) 3419
33. M. Auzias, J. Gueniat, B. Therrien, G. Süß-Fink, A. K. Renfrew, P. J. Dyson, *J. Org. Chem.* **694** (2009) 855
34. C. Sclaro, C. G. Hartinger, C. S. Allardyce, B. K. Keppler, P. J. Dyson, *J. Inorg. Biochem.* **102** (2008) 1743



35. S. Grgurić-Šipka, M. M. Alshtewi Al. Arbi, D. Jeremić, G. N. Kaluderović, S. Gomez-Ruiz, Ž. Žižak, Z. Juranić, T. J. Sabo, *J. Serb. Chem. Soc.* **73** (2008) 619
36. V. Rajendiran, M. Murali, E. Suresh, S. Sinha, K. Somasundaram, M. Palaniandavar, *Dalton Trans.* (2008) 148
37. V. Djinovic, M. Momcilovic, S. Grguric-Sipka, V. Trajkovic, S. M. Mostarica, D. Miljkovic, T. Sabo, *J. Inorg. Biochem.* **98** (2004) 2168
38. A. Bergamo, L. Messori, F. Piccioli, M. Cocchietto, G. Sava, *Invest. New Drug.* **21** (2003) 401
39. A. R. Timerbaev, C. G. Hartinger, S. S. Aleksenko, B. K. Keppler, *Chem. Rev.* **106** (2006) 2224
40. W. H. Ang, P. J. Dyson, *Eur. J. Inorg. Chem.* **20** (2006) 8153
41. S. Grgurić-Šipka, I. Ivanović, G. Rakić, N. Todorović, N. Gligorijević, S. Radulović, V. B. Arion, B. K. Keppler, Ž. Lj. Tešić, *Eur. J. Med. Chem.* **45** (2010) 1051
42. A. F. A. Peacock, S. Parsons, P. J. Sadler, *J. Am. Chem. Soc.* **129** (2007) 3348
43. S. H. van Rijt, A. F. A. Peacock, R. D. L. Johnstone, S. Parsons, P. J. Sadler, *Inorg. Chem.* **48** (2009) 1753
44. D. Camm, A. El-Sokkary, A. L. Gott, P. G. Stockley, T. Belyaeva, P. C. McGowan, *Dalton Trans.* (2009) 10914
45. S. B. Jensen, S. J. Rodger, M. D. Spicer, *J. Organomet. Chem.* **556** (1998) 151
46. *SAINT-Plus*, version 7.56a, Bruker AXS Inc., Madison, WI, 2008
47. G. M. Sheldrick, *SHELXS-97, Program for Crystal Structure Solution*, University Göttingen, Göttingen, 1997
48. G. M. Sheldrick, *SHELXL-97, Program for Crystal Structure Refinement*, University Göttingen, Göttingen, 1997
49. G. K. Johnson, *Report ORNL-5138*, Oak Ridge National Laboratory, Oak Ridge, TN, 1976
50. *International Tables for X-Ray Crystallography*, Vol. C, A. J. C. Wilson, Ed., Kluwer Academic Press, Dordrecht, 1992, Tables 4.2.6.8 and 6.1.1.4.
51. R. Surpino, in *Methods in Molecular Biology, in Vitro Toxicity Testing Protocols*, S. O'Hare, C.K. Atterwill, Eds., Humana Press, New York, 1995, p. 137
52. L. Carter, D. L. Davies, J. Fawcett, D. R. Russell, *Polyhedron* **12** (1993) 1599
53. M. C. Barrel, R. Jimenez-Aparicio, E. C. Royer, M. J. Sancedo, F. A. Urbanos, E. Gutierrez-Pueblo, C. Ruiz-Valero, *J. Chem. Soc. Dalton Trans.* (1991) 1609.