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New ruthenium(II) bipyridyl complex: Synthesis, crystal structure and cytotoxicity

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Abstract: A new Ru(II) bipyridyl complex with O^4-hydrogenpyridine-2,4--dicarboxylate was synthesized and characterized by IR, NMR and mass spectrometry, X-ray diffraction analysis and elemental analysis. The electrochemical characteristics of the complex were investigated by cyclic voltammetry, revealing Ru(II)/Ru(III) electron transfer in the positive range of potentials. On the opposite potential side, multiple partially reversible peaks were dominant, representing subsequent reductions of the bulky bipyridyl moiety. The cytotoxic activity of the complex was tested in two human cancer cell lines: A549 (lung cancer) and K562 (leukemia) as well as non-tumor MRC-5 cells, by MTT assays. The IC_{50} values were > 300 and 177.63±2.28 μM for the A549 and K562 cells, respectively.

Keywords: metal complex; characterization; X-ray; redox properties; biological study.

INTRODUCTION

The search for efficient antitumor agents is a topic of prime interest in the field of medicinal chemistry.[1–3] Despite the fact that metal complexes are key--components in the treatment of some tumors, there is currently a lack of suitable drugs. Novel, more efficient and less toxic substances, capable of treating cancers are therefore considered highly desirable compounds. While the clinical success of platinum complexes is indisputable,[4,5] due to the many side effects of these

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drugs (nerve damage, hair loss and nausea) and cellular resistance, thousands of new platinum and non-platinum complexes were synthesized, in order to find a more suitable antitumor drug.[6,7] Coordination compounds allow a very diverse platform for drug design. In addition to various oxidation states of metals, metal complexes have different geometries and coordination numbers that ensure the fine-tuning of their chemical reactivity.[8,9] Ruthenium compounds are particularly favored because of their tendency to cause fewer side effects compared to platinum drugs and their physicochemical properties, which include chemical stability and structural diversity.

Ruthenium compounds have shown highly promising biological activity with the two structurally similar compounds KP1019 and NAMI-A, which are under evaluation in phase II clinical trials (KP1019 is active against primary cancers whereas NAMI-A is active against secondary tumor cells).[8,10–13]

Picolinic acid plays an important role as a component of specific enzymes and as an active agent in a number of drugs. For example, 2,4-pyridinedicarboxylic acid showed immuno-suppressive and fibro-suppressive properties,[14] while 2,4-, 2,5- and 2,6-pyridinedicarboxylic acid were included into inhibition or activation of some metalloenzymes.[15,16]

Iron(III) complexes with 2,6-pyridinedicarboxylates were synthesized and their significant role in electron transfer in some models of biological systems was documented in several papers.[17,18] In addition, these molecules were recognized as specific molecular tools in DNA cleavage tests.[19]

In the last two decades, numerous organo-ruthenium(II) complexes containing the *p*-cymene moiety and a pyridine derivative, coordinating in a monodentate or bidentate manner, were synthesized and their *in vitro* antiproliferative activity was investigated in the numerous cell lines. The obtained results indicated that these compounds exhibit moderate antitumor activity with one complicated mechanism of action, involving extra and intra-cellular processes, which is quite different compared to the classical platinum drugs.[20–22]

In this work, the synthesis and full characterization of a new ruthenium(II) bipyridyl complex, $[RuL(bpy)_2]PF_6 \cdot 0.5H_2O$ (**1**), where L represents O^4-hydrogenpyridine-2,4-dicarboxylate, are described. The antitumor potential of the synthesized compound and its electrochemical profile are also reported.

EXPERIMENTAL

Materials and measurements

All experiments were performed under atmospheric conditions with commercially available chemicals and solvents used as received. In particular, 2,4-pyridinedicarboxylic acid was purchased from Sigma–Aldrich. The starting complex, $[RuCl_2(bpy)_2]$ was synthesized according to a previously described, but slightly modified synthetic route.[23,24]

Elemental analysis was performed on an Elemental Vario EL III microanalyzer. A Nicolet 6700 FT-IR spectrometer was used for recording the infrared spectrum. The signal

intensities are reported in wavenumbers and denoted by the following abbreviations: *vs* = very strong, *s* = strong, *m* = medium and *w* = weak. An LTQ Orbitrap XL mass spectrometer (Heated ESI) was used for recording the mass spectra in acetonitrile (HPLC grade) in the positive mode. The obtained peaks were assigned and interpreted according to the dimensionless mass/charge ratio. The ^{1}H-NMR spectrum was recorded using a Bruker Avance III 500 spectrometer with TMS as the reference. For proton assignments, following abbreviations were used: (*b*)*s* = (broad) singlet, *d* = doublet, *dd* = doublet of doublets, *t* = triplet, *q* = quartet, *p* = pentet, *m* = multiplet and Ar = aromatic protons. A rough estimation of the melting points of the compound was realized using an electrothermal melting point apparatus. Analytical and spectral data of the compound are given in Supplementary material to this paper.

Synthesis of the complex

For the preparation of complex **1**, $[RuCl_2(bipy)_2]$ (100 mg, 0.21 mmol) was dissolved in ethanol (15 mL) and stirred for 20 min at 40 °C. 2,4-Pyridinedicarboxylic acid (35 mg, 0.21 mmol) was dissolved in a small volume of ethanol (5 mL) and added to the solution of the starting complex. The reaction mixture was immediately stirred under reflux for 3 h and afterwards left to cool to room temperature. After the addition of an equimolar amount of NH_4PF_6 (33.7 mg, 0.21 mmol), a dark red precipitate was isolated by filtration. The crude product was washed with a small amount of water and diethyl ether.

Single crystal X-ray diffraction

For the reported structure, X-ray intensity data were collected, at 100 K, on an Agilent Supernova dual source (Cu at zero) diffractometer equipped with an Atlas CCD detector using ω scans and CuK_α (λ = 1.54184 Å) radiation. The images were interpreted and integrated with the CrysAlisPro program (Rigaku Oxford Diffraction, 2015).[25] Using Olex2,[26] the structure was solved by direct methods using the ShelXS structure solution program and refined by full-matrix least-squares on F^2 using the ShelXL program package.[27,28] Non-hydrogen atoms were anisotropically refined and the hydrogen atoms were refined in the riding mode. Isotropic temperature factors were fixed at 1.2 times *U*(eq) of the parent atoms. The hydrogen atoms of the solvent water molecule and the O^4-hydrogenpyridine-2,4-dicarboxylate carboxyl group were located from a difference Fourier electron density map and restrained refined with isotropic temperature factors fixed at 1.5 times *U*(eq) of the parent atoms.

Electrochemical measurements

Electrochemical measurements were performed with a CHI-760B instrument at room temperature. The voltammetric measurement was performed in a three-electrode cell, which consisted of a glassy carbon electrode (Model 6.1204.300), an auxiliary platinum electrode with large surface area (model CHI221, cell top including a platinum wire counter electrode) and an Ag/AgCl reference electrode (model CHI111). For the purpose of experiments, 1.0 mM solution of the synthesized complex was prepared in DMSO and TBAP was added as a supporting electrolyte. Cyclic voltammograms for **1** were obtained at 25, 50, 100, 150, 200 and 300 mV s^{-1}.

Cytotoxicity

Reagents and cell cultures. Human alveolar basal adenocarcinoma (A549), human chronic myelogenous leukaemia (K562) and human fetal lung fibroblast (MRC-5) cell lines were maintained as a monolayer culture in the Roswell Park Memorial Institute (RPMI) 1640 nutrient medium (Sigma–Aldrich). The nutrient medium was supplemented with 10 % heat-deactivated fetal calf serum (FCS, Sigma–Aldrich), 4-(2-hydroxyethyl)piperazine-1-ethane-

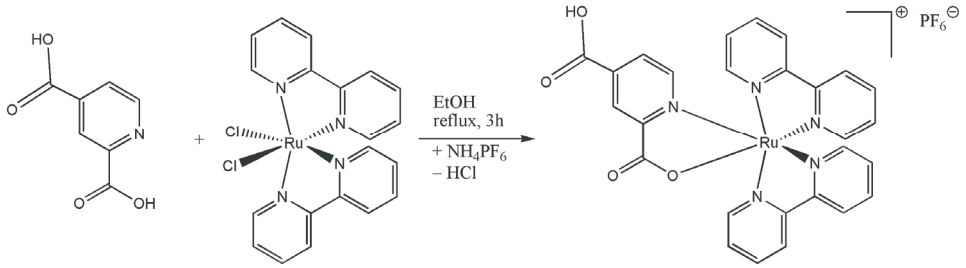
sulfonic acid (HEPES, 25 mM), penicillin (100 units mL^{-1}), streptomycin (200 μg mL^{-1}) and L-glutamine (3 mM). The cells were maintained as a monolayer culture in tissue culture flasks (Thermo Scientific Nunc™), in an incubator at 37 °C, in a humidified atmosphere composed of 5 % CO_2.

MTT cytotoxicity assay. The drug-induced cytotoxicity was determined using the 3-(4,5--dimethylthiazol-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma–Aldrich) assay.[29] Cells were seeded into 96-well cell culture plates (Thermo Scientific Nunc™), in number of 5000 (K562), 7000 (MRC-5) and 8000 cells per well (A549), in 100 μL of culture medium. After 24 h of growth, the cells were exposed to the serial dilutions of the tested agent. Stock solutions were prepared immediately prior to use by dissolving in dimethyl sulfoxide (DMSO), so that the DMSO content did not exceed 1 vol. %. The antiproliferative effect of the complex was evaluated in a range of concentrations up to 300 μM, for 72 h of continuous drug action. After the treatment, 20 μl of MTT solution, 5 mg mL^{-1} in phosphate buffer solution (PBS), pH 7.2, was added to each well. The samples were incubated for 4 h at 37 °C with 5 % CO_2 under a humidified atmosphere. Formazan crystals were dissolved in 100 μL of 10 % sodium dodecyl sulfate (SDS). The absorbance was recorded at a wavelength of 570 nm using a microplate reader (ThermoLabsystems Multiskan EX 200e240 V) after 24 h The IC_{50} value (μM) was defined as the concentration of the drug that produced 50 % inhibition of cell survival, and was determined based on cell survival diagrams.

RESULTS AND DISCUSSION

Synthesis

The main subject of the study was the synthesis and full characterization of a new ruthenium(II) bipyridyl complex with 2,4-pyridinedicarboxylic acid (Scheme 1). The obtained compound was air stable and showed no traces of decomposition.



Scheme 1. Synthesis of the $[RuL(bpy)_2]PF_6$ complex.

Spectroscopy

The IR spectrum of the synthesized complex generally revealed an asymmetric stretching vibration located around 1605 cm^{-1} that originated from the coordinated carboxylate group. The coordination of the metal center *via* oxygen is suggested by comparison to the band of the free carboxylic group at ≈1700 cm^{-1} in the spectrum of the ligand. The intensive band found at 840 cm^{-1} was

assigned to C–H stretching modes. In the ESI-MS spectrum of the complex recorded in acetonitrile, the [M^+–PF_6^-] signal was detected.

In the ^{1}H-NMR spectrum of the complex, all the aromatic protons were detected in the range of 7.39–8.84 ppm, which belong to aromatic protons originating from the pyridine and bipyridine moieties. Carboxylic protons were not detected because the co-ligand was coordinated to ruthenium through the oxygen atom of the carboxylic group in position 2 of the pyridine ring, while the carboxylic group in position 4 was deprotonated in DMSO.

Crystal data for compound

Compound **1** crystallized in the centro-symmetric space group $P2_1/n$, with one [RuL(bpy)$_2$]PF_6 complex in the asymmetric unit, together with one water solvent molecule (Fig. 1). The Ru(II) ion is octahedrally coordinated by four nitrogen atoms from the two bipyridine ligands, and one nitrogen and one oxygen atom from the O^4-hydrogenpyridine-2,4-dicarboxylate. In fact, while the coordinating carboxyl group in position 2 of the 2,4-dicarboxylic acid ligand is deprotonated, the other carboxyl group in position 4 is clearly protonated. The Ru(II)–N_{bpy} distances are in the range of 2.031(4)–2.055(4) Å, while the Ru(II)–N_L and Ru(II)–O_L distances are 2.049(3) Å and 2.090(3) Å, respectively. The N–Ru–N/O angles vary between 79.07(13)° and 99.05(13)°.

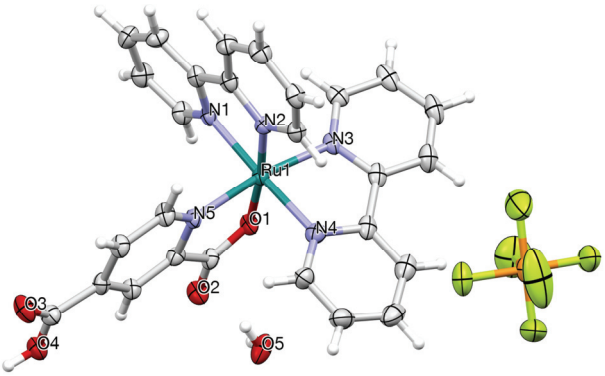


Fig. 1. Asymmetric unit of the crystal structure of **1**, consisting of one [RuL(bpy)$_2$]PF_6 complex and one water solvent molecule, with atom-labeling scheme of the heteroatoms (except for PF_6^-). Thermal displacement ellipsoids are drawn at the 50 % probability level.

In the crystal packing, hydrogen bonds are formed between the O^4-hydrogenpyridine-2,4-dicarboxylate groups and the water solvent molecule. Each water molecule forms a hydrogen bond with the deprotonated carboxylic group (O5(–H5A)···O2 = 2.692(4) Å) and with the protonated carboxylic groups of two symmetry-equivalent Ru(II) complexes (O5(–H5B)···O3^i = 2.822(4) Å and O5···(H4–)O4^{ii} = 2.552(4) Å; symmetry codes: *i*) 1/2+*x*, 3/2–y, 1/2+*z*; *ii*) 3/2–*x*,

–1/2+*y*, 1/2–*z*), connecting four Ru(II) complexes over an inversion center (Fig. 2). Furthermore, multiple π–π stacking interactions were observed between the aromatic bipyridine rings and the O^4-hydrogenpyridine-2,4-dicarboxylate rings (ring centroid–centroid distances between 4.218(2) and 5.665(2) Å).

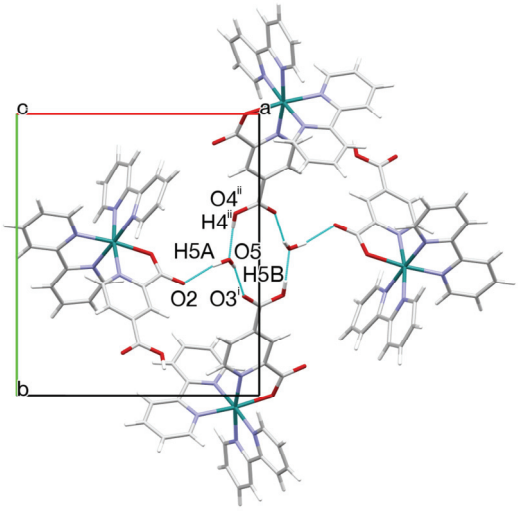


Fig. 2. Part of the crystal packing of the structure of **1**, along the *c*-axis, showing hydrogen bonds between the water solvent molecules and the O^4-hydrogenpyridine-2,4-dicarboxylate groups, with the atom-labeling scheme of the specific atoms involved. Symmetry codes: *i*) 1/2+*x*, 3/2–*y*, 1/2+*z*; *ii*) 3/2–*x*, –1/2+*y*, 1/2–*z*.

Electrochemistry

The electrochemical character of the complex was studied by cyclic voltammetry in DMSO at different scan rates (25, 50, 100, 150, 200 and 300 mV s^{-1}) in the $-2.50 < E < 1.00$ V potential range (Fig. 3). The recorded voltammograms show a reversible wave at ≈0.30 V *vs*. Ag/AgCl, which could be readily assigned to the Ru(II)/(III) redox couple. The calculated ΔE_p values tend to slightly increase with scan rate (from 100 to 210 mV), indicating the partially reversible nature of the redox process. In the region of negative potentials ($-2.35 < E < -1.15$ V), multiple partially reversible peaks could be observed. This type of reductive activity could be assigned to the subsequent reductions of the bipyridyl moiety. In comparison to the literature data,[30–32] the novel Ru(II) complex shows no exceptions concerning its electrochemical behavior.

Cytotoxic activity

The antiproliferative activity of the prepared complex was assayed in two human cancer cell lines (A549, K562) and non-tumor MRC-5 cells, by the MTT assay. The tumor cells were incubated for 72 h with the investigated complex.

The results of these tests indicated that the complex after 72 h of incubation exhibited no cytotoxic activity with IC_{50} > 300 μM for A549, and a moderate activity with 177.63±2.28 μM for K562 (Table I). Moreover, the investigated complex did not show cytotoxic activity towards non-tumor MRC-5 cells. These values are the mean of 2 to 3 independent experiments, whereby the standard deviations were less than 15 %.

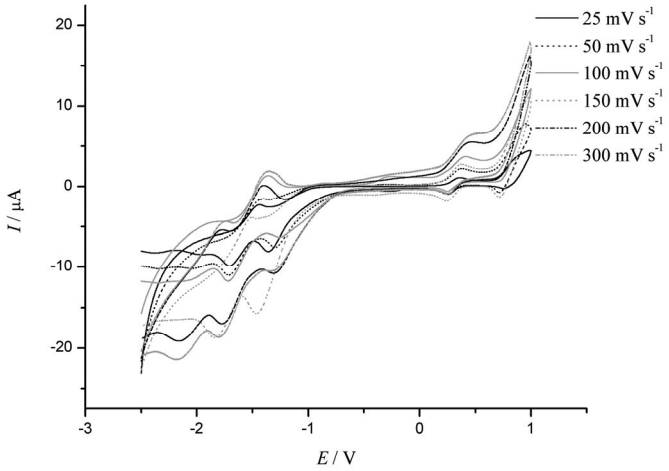


Fig. 3. The cyclic voltammograms for **1** recorded in DMSO (0.1 mM TBAP) at a glassy carbon electrode for scan rates 25, 50, 100, 150, 200 and 300 mV s^{-1}.

TABLE I. Cytotoxicity of the tested agent in terms of IC_{50} values (μM) obtained by the MTT assay for 72 h of continuous drug action; > 300 denotes that an IC_{50} value was not obtained in the range of concentrations tested up to 300 μM

Compound	A549	K562	MRC-5
1	> 300	177.63±2.28	> 300

CONCLUSIONS

The complex $[RuL(bpy)_2]PF_6 \cdot 0.5H_2O$, where L is O^4-hydrogenpyridine-2,4--dicarboxylate, was characterized by means of ^{1}H-NMR, elemental analysis, ESI-MS, IR and single-crystal X-ray analysis. The bidentate ligands are coordinated in the *cis* position, yielding a complex of octahedral geometry. In addition, the electrochemical properties of the synthesized complex were investigated and the obtained results indicated that its electrochemical behavior was in accordance with literature data for Ru(II) complexes. The cytotoxic studies showed that the synthesized complex exhibited moderate biological activity towards human cancer cell K562, which may be due to the unfavorable ligand dissociation kinetics and off-target reactivity, when once in solution. Further research on this

topic will be based on the investigation of cytotoxic activity of this compound on numerous cancer cell lines and determination of its mode of action.

SUPPLEMENTARY MATERIAL

CCDC 1524673 contains the supplementary crystallographic data for this paper and it can be obtained free of charge *via* www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44-1223-336033; or deposit@ccdc.cam.ac.uk).

Analytical and spectral data of the synthesized compound are available electronically at the pages of journal website: http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

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ИЗВОД

НОВИ РУТЕНИЈУМ(II) БИПИРИДИНСКИ КОМПЛЕКС: СИНТЕЗА, КАРАКТЕРИЗАЦИЈА И ЦИТОТОКСИЧНОСТ

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Нови рутенијум(II) бипиридински комплекс са O^4-хидроген-пиридин-2,4-дикарбоксилатом је синтетисан и окарактерисан помоћу IC и NMR спектроскопије, масене спектрометрије и рендгенске и елементалне анализе. Електрохемијски карактер комплекса је испитан цикличном волтаметријом указивајући на Ru(II)/Ru(III) трансфер електрона у опсегу позитивних потенцијала. Насупрот томе, у опсегу негативних потенцијала запажени су вишеструки реверзибилни пикови који представљају сукцесивне редукције разгранатог бипиридилског дела. Цитотоксична активност комплекса испитивана је на две хумане ћелијске линије канцера: A549 (канцер плућа) и K562 (леукемија) као и на нетуморској ћелијској линији MRC-5, MTT тестом. Добијене IC_{50} вредности су > 300 и 177,63±2,28 μM за A549 и K562 ћелије, редом.

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