



Mono- and binuclear Ru(II) arene complexes with (fluoro substituted) picolinic acid: Synthesis, characterization and cytotoxicity

Stefan Nikolić^a, Ljiljana E. Mihajlović-Lalić^a, Marija Vidosavljević^b, Sandra Arandelović^b, Siniša Radulović^b, Sanja Grgurić-Šipka^{c,*}

^a Innovation Center of the Faculty of Chemistry, Studentski trg 12-16, Belgrade, Serbia

^b Institute for Oncology and Radiology of Serbia, Pasterova 14, Belgrade, Serbia

^c Faculty of Chemistry, University of Belgrade, Studentski trg 12-16, Belgrade, Serbia

ARTICLE INFO

Article history:

Received 2 August 2019

Received in revised form

27 September 2019

Accepted 30 September 2019

Available online 1 October 2019

Keywords:

Ruthenium

Arene ligand

Picolinic acid

Antitumor activity

ABSTRACT

Four mono- (**1–4**) and four binuclear Ru(II) arene (**5–8**) complexes have been isolated from the reaction of $[\text{Ru}(\eta^6\text{-benzene})\text{Cl}(\mu\text{-Cl})_2]$ or $[\text{Ru}(\eta^6\text{-toluene})\text{Cl}(\mu\text{-Cl})_2]$ with 2-pyridinecarboxylic acid and 6-fluoro-2-pyridinecarboxylic acid. Their structural characterization included IR and NMR spectroscopy and MS spectrometry. The cytotoxic potential of the compounds has been tested by MTT assay in seven human cancer cell lines: alveolar basal adenocarcinoma (A549), large cell lung carcinoma (HTB177), colorectal carcinoma (HCT116), malignant melanoma (A375), prostate adenocarcinoma (PC3), breast carcinoma (MDA-MB-453), cervix adenocarcinoma (HeLa), and one human non-malignant lung fibroblast cell line (MRC-5). Mononuclear complexes **1** and **3** carrying 2-pyridinecarboxylic acid have displayed moderate antiproliferative effect toward HCT116 and HeLa, slightly better in comparison to their binuclear analogues, **5** and **7**. The highest activity and cytoselectivity has been observed **1** as it has reduced viability of HCT116 cells 1.5 times more efficiently ($\text{IC}_{50} = 27.5 \mu\text{M}$), than of the MRC-5 cells ($\text{IC}_{50} = 41.3 \mu\text{M}$). In contrast to **1** and **3**, compounds **2**, **4–8** have been found to exhibit lack of cytotoxicity or mild cytotoxicity with IC_{50} values ranging from 100 to 300 μM .

© 2019 Elsevier B.V. All rights reserved.

1. Introduction

A history of inorganic medicinal chemistry dates back to the mid-1960s when the discovery of cisplatin (Fig. 1), a powerful anti-tumor agent, occurred [1]. Although established as a milestone metallodrug, cisplatin's therapeutic value has been limited by significant side effects such as drug resistance and non-specific toxicity [2]. Intensive studies in this field expanded the series of metallodrugs from platinum to other transition metal complexes such as Ru, Au, Rh, Ir, Os, Re and even Fe and Mo (Fig. 1) [3]. Among numerous metal-ligand scaffolds, Ru(II/III) complexes singled out as the most promising alternative to platinum-based chemotherapeutics [4]. Two ruthenium derivatives, imidazolium *trans*-DMSO-imidazole-tetrachlororuthenate (NAMI-A, Fig. 1) [5] and indazolium *trans*-[tetrachlorobis(1*H*-indazole)ruthenate(III)] (KP-1019) [6] have been tested in clinical trials while the sodium analogue of KP1019, NKP-1339 [7], is set to enter the clinical trials.

The prominent Ru complexes are generally designed to enable high kinetic stability through control ligand substitution and redox reactions allowing the complex to remain intact on the way to the final target [8]. Particular emphasis is given on the functionalized η^6 -arene Ru complexes with three additional coordination sites available for introducing *N*-, *O*-, *S*- or *P*-donor species and also convenient for fine tuning of their steric and electronic properties [9]. The benefit of the Ru-arene structural flexibility is reflected through their diverse areas of implementations, e.g. catalysis [10], chemical and photochemical sensing [11], supramolecular chemistry [12] and medicinal chemistry [13]. In terms of potential clinical use, advantageous features such as good aqueous solubility and relatively inert arene ligand make them very attractive for structural optimizations aimed for improved *in vivo* potency [14]. In reactions with two-electron donor ligands mononuclear complexes of general formula $[(\eta^6\text{-arene})\text{RuCl}_2(\text{L})]$ are formed [15], while reactions with bidentate ligands result in a formation of binuclear species $[(\eta^6\text{-arene})\text{RuCl}(\text{L}-\text{L})]^+$ [16]. Reported binuclear assemblies are rare in literature although they exhibit a whole variety of relevant biological effects such as significantly high affinity of

* Corresponding author.

E-mail address: sanjag@chem.bg.ac.rs (S. Grgurić-Šipka).

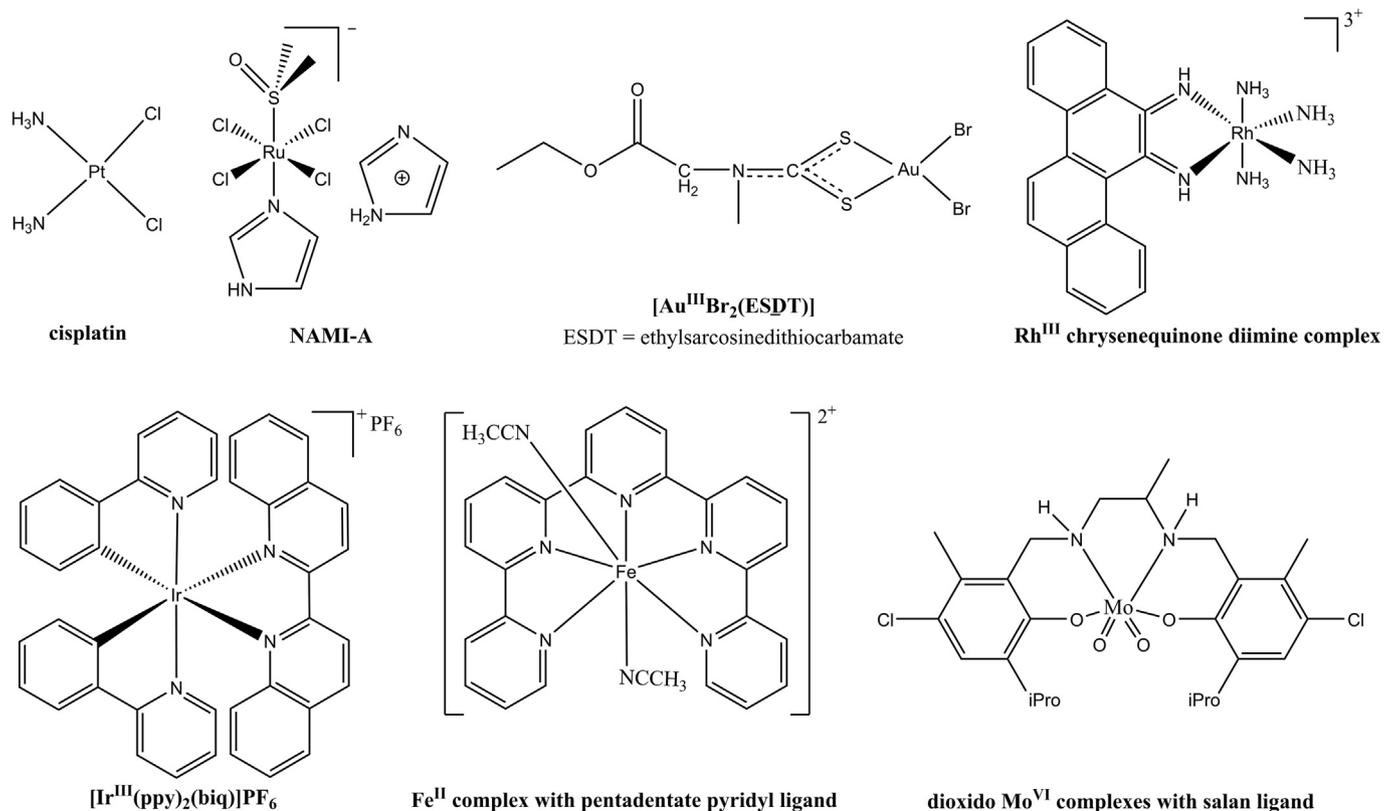
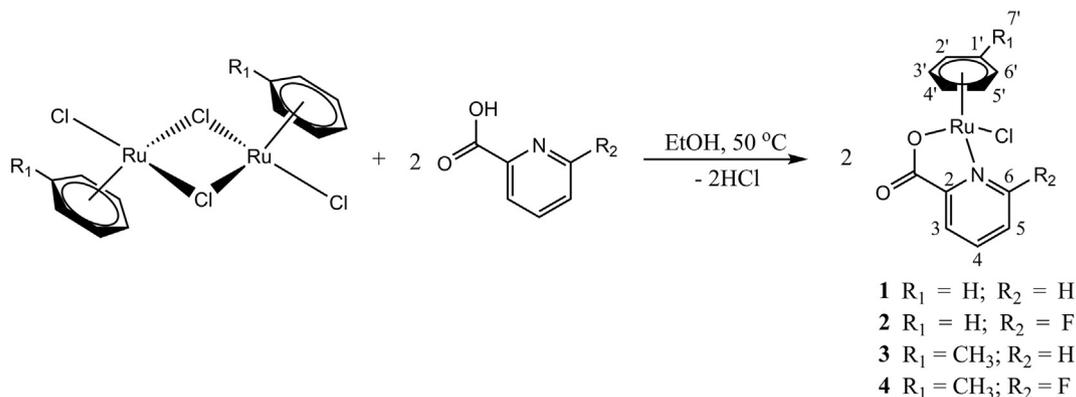


Fig. 1. Metal based representatives with antitumor effect.

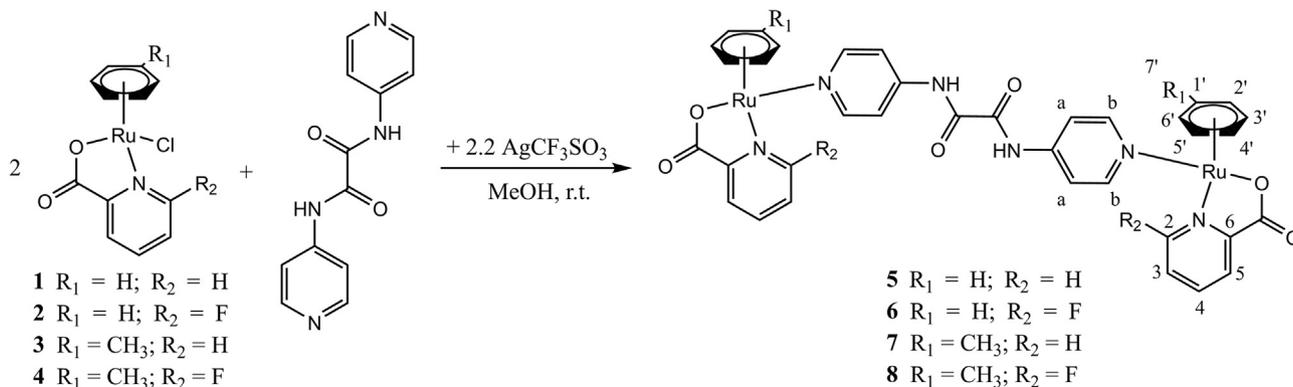


Scheme 1. Synthesis of 1–4 complexes.

binding to DNA [17] or promising cytotoxic effects in human cancer cells gained through cooperatively acting of both Ru centers [18]. Moreover, the appealing structural diversity of Ru complexes can be also functionalized by the effective synergism of both ligands, arene and chelating moiety [19]. In that sense, the literature suggests a rational use of bioinspired molecules with specific roles in certain biological systems. One of the commonly used chelating agent is picolinic acid, a naturally occurring product of tryptophan degradation found in various biological mediums including blood serum, human milk and pancreatic juice [20]. With two different ligand atoms, pyridinecarboxylic acid represents desirable ligand model whose transition metal complexes (e.g. Pt(II), Fe(II), Co(II), Al(III)) have been widely investigated [21–23]. Among them, Cr(III) picolinate derivatives are the most recognizable due to their massive

use as nutrition additives [24]. Numerous studies investigated the biological activity of this type of complexes regarding their potential antitumor and antituberculosis activity [25].

Starting from 2011 Grgurić-Sipka and co-workers explored various sets of Ru-arene complexes with 2-pyridinecarboxylic (picolinic) acid and its halogen (fluoro-, chloro-, bromo-) or methyl-substituted derivatives and reported their cytotoxic profile [26]. In order to further contribute the field regarding this interesting model systems, eight new Ru(II)-arene (benzene and toluene) complexes bearing picolinic acid and 6-fluoropicolinic acid were prepared. The first set of compounds included the preparation of mononuclear Ru(II) complexes whose structure was further optimized by reacting them with a suitable linker, *N,N'*-di(4-pyridinyl)ethanediamide (bpo). The obtained binuclear



Scheme 2. Synthesis of the binuclear complexes, **5–8**.

assemblies were primarily inspired with arene ruthenium metalla-rectangles highly potent towards human ovarian cancer cells with a pronounced selectivity for cancer over healthy cells [27]. The novel compounds were spectroscopically characterized when the coordination mode of *N,O* ligand was confirmed, identically like in earlier studies. The cytotoxic activity of the reported compounds was investigated by the colorimetric MTT assay in a panel of one human non-malignant cell line (MRC-5), and seven human cancer cell lines (A549, HTB177, PC3, A375, HeLa, HCT116, MDA-MB-453). The obtained results were discussed in terms of comparison between cytotoxic potential of newly synthesized compounds and published Ru(II) picolinato complexes [26] and the examination of the structure-activity correlations between mononuclear species and their corresponding binuclear analogues.

2. Results and discussion

2.1. Preparation and characterization of 1–4

The reaction of $[Ru(\eta^6\text{-benzene})Cl(\mu\text{-Cl})_2]$ or $[Ru(\eta^6\text{-toluene})Cl(\mu\text{-Cl})_2]$ with picolinic acid or 6-fluoropicolinic acid in a 1:2 M ratio in ethanol gives rise to **1–4** with more than 70% yields (Scheme 1). The complexes are soluble in DMSO, acetone, chloroform, dichloromethane, and methanol, and insoluble in water, petroleum ether and diethyl ether.

The IR spectra of **1–4** point out the typically intensive C=O vibration observed in the area from 1670 to 1640 cm^{-1} . The significant difference between wavenumbers for C=O vibration in the spectra of free ligands (ca. 1720 cm^{-1}) occurs due to the coordination of the ruthenium(II) center via carboxylic oxygen [26a]. The complexes also display stretching bands at ca. 1600 cm^{-1} assigned to the C=N vibration.

In ^1H NMR spectra of **1** and **3** (Figs. S1 and S3, ESI) with picolinic acid, the significant shift of the pyridine protons is observed due to the coordination via pyridine nitrogen (C3, C4 and C6 are shifted downfield while C5 is upfield). Additional conformation of the proposed coordination via carboxylic oxygen is noticed in a significant shift of COO^- group from 166.22 ppm in free picolinic acid to ca. 170 ppm in ^{13}C NMR spectra of **1** and **3**. ^1H NMR spectra of **2** and **4** (Figs. S2 and S4, ESI) bearing 6-fluoropicolinic acid display quartets for C4 which are shifted downfield in a comparison to the free ligand while resonance of C3 located at ca. 7.74 ppm is shifted upfield. Methyl groups from toluene moiety are dislocated downfield with minor shifts around 0.05 ppm. ^{13}C NMR spectra confirmed coordination via carboxylic oxygen as the COO^- signal is shifted from ca. 164 ppm to ca. 170 ppm.

In the EI-MS spectra of **1–4** the $[M^+]$ signal was detected. **1** and **2** fragmented in the same manner giving rise to $[M\text{-PhH}]^+$ ion while **3** and **4** pointed out on $[M\text{-CO}_2]^+$ fragments.

2.2. Preparation and characterization of 5–8

The reaction of two equivalents of previously synthesized complex (**1**, **2**, **3**, and **4**) with an equivalent of *N,N'*-di(4-pyridinyl)ethanediamide in methanol and the presence of AgCF_3SO_3 afforded **5–8** with a good yield (Scheme 2). The complexes are soluble in DMSO, acetone, chloroform, dichloromethane, and methanol, and insoluble in water, petroleum ether and diethyl ether.

^1H NMR spectra of **5–8** (Fig. S5–8, ESI) were assigned due to the shifts of the NH protons and pyridine protons from ethanediamide connector. Due to the formation of Ru assemblies, NH protons marked obvious dislocation from 11.27 ppm to ca. 11.60 ppm. C_a protons are also shifted downfield while C_b protons are slightly shifted upfield.

In the EI-MS spectra of **5** and **6** the $[M^+]$ and $[M\text{-PhH}]^+$ signals were detected, while **7** and **8** suffered total fragmentation.

2.3. Chemical behavior of the Ru(II)-arenes in DMSO

Numerous studies concerning the stability of the Ru(II)-arene type of complexes were performed in order to predict their fate under the physiological conditions [28]. As water and DMSO are commonly used solvents for biological assay experiments, chemical behavior is usually followed by ^1H NMR spectral changes in deuterated DMSO, as a function of time and later on compared to precursor compounds.

The stability of reported complexes **1–8** were found to be completely stable in DMSO after 72 h long monitoring (representative spectra of **1** and **5** shown as Figs. S9 and S10 in ESI). Since all NMR spectra showed no traces of the ligand dissociation or any other kind of decomposition, it is concluded that this type of Ru(II)-arenes surprisingly remains intact in DMSO.

2.4. Results of MTT assay

2.4.1. Cytotoxicity analysis

The cytotoxicity of the eight reported complexes **1–8** was investigated by the colorimetric MTT assay, in a panel of one human non-malignant cell line (MRC-5), and seven human cancer cell lines (A549, HTB177, PC3, A375, HeLa, HCT116, MDA-MB-453). Results obtained after 72 h of continuous drug action, are presented as IC_{50} values (μM), in Table 1. Well-known chemotherapeutic agent

cisplatin was also tested as reference compound.

Mononuclear Ru(II) complexes **1** and **3** carrying picolinic acid, displayed moderate antiproliferative effect particularly toward colorectal carcinoma (HCT116) and cervix adenocarcinoma cells (HeLa) (Fig. 2). The highest activity and cytoselectivity was observed for mononuclear complex **1** toward HCT116 cells: it was capable of reducing viability of HCT116 cells 1.5 times more efficiently ($IC_{50} = 27.5 \mu M$), than of the MRC-5 cells ($IC_{50} = 41.3 \mu M$). In a contrast to these mononuclear species, their binuclear analogues, **5** and **7** did not exhibit much of improvement in antiproliferative activity or selectivity toward cancer cells as the IC_{50} values exceeded $100 \mu M$. Moreover, both mono- and binuclear compounds bearing 6-fluoro substituent were found to exhibit lack of cytotoxicity or mild cytotoxicity resulting in IC_{50} values from 100 to $300 \mu M$.

Design and synthesis of polynuclear complexes are challenging comparing to the synthesis of mononuclear complexes, regarding the optimization of structure-activity correlations. It is more complicated to predict the effect of stereochemistry of polynuclear metal complexes on their biological properties *in vitro*, and their reactivity toward biomolecules, such as proteins or nucleic acids, in physiological environment. One successful example of polynuclear metal complex, which reached clinical trials, is trinuclear platinum complex BBR3464 which exhibits different mechanism of action comparing to cisplatin, and is active in human tumor xenografts resistant or poorly responsive to cisplatin, as well as in p53 mutant carcinoma cells [29]. BR 3464 also produces high levels of DNA lesions in the cell, different from cisplatin. However, literature on polynuclear ruthenium complexes and their *in vitro* antitumor potential is rare. Some previous studies indicated that there was no direct correlation between the number of metal centers and cytotoxicity of the metal complexes, since polynuclear metal core can affect compound solubility, its ability to pass through the cell membrane or to slow or interfere with binding dynamics in the cell [30,31]. In our case, synthesized binuclear Ru(II) analogues **5–8** did not show positive effect in terms of their cytotoxic activity, (IC_{50} values were in the wide range of concentrations up to $300 \mu M$, or above). For the most of compounds cytotoxicity against non-tumor (MRC-5) cell line, was found slightly higher compared to the tumor cells, with selectivity index (SI), comparable to cisplatin in tested cell lines, ranging from 0.2 to 1.5 (Table TS1, ESI). The highest SI values were obtained in cervical (HeLa) and colorectal (HCT116) carcinoma cells, however, compounds with SI value < 2 are considered to give general toxicity, i.e., it can also cause cytotoxicity on normal cells. Factors responsible for moderate tumor cells response to binuclear ruthenium complexes may also account to mechanisms associated with multidrug resistance, or elevated cellular glutathione (GSH) content [32]. Tested binuclear complexes may be also able to form different interactions with biomolecules,

in comparing to cisplatin, such as cross-links (protein-protein or DNA-protein), though further study is needed, in order to precisely determine their capacity to accumulate in the cell, intracellular drug targets and the mechanism of cell response. Surprisingly, mononuclear Ru(II) complexes, **1** and **3**, displayed moderate antiproliferative effect toward HCT116 and HeLa, significantly stronger in comparison to their binuclear analogues, **5** and **7**. Our previous study [26b] on mononuclear Ru(II) arene complexes carrying picolinato ligand, indicated the ability of this type of complexes to exhibit inhibitory effect on cell adhesion, migration and angiogenesis, regardless of their antiproliferative effect. On the basis of these considerations, further *in vitro* and *in vivo* antitumor study is needed in order to determine whether these novel mono- or polynuclear Ru(II) compounds could potentially affect cellular migratory or invasive properties.

3. Experimental

3.1. Materials and methods

All manipulations were performed under atmospheric conditions with commercially available chemicals and solvents used as received. Picolinic acid (L_1) and 6-fluoropicolinic acid (L_2) were purchased from Acros Organics. Starting complexes, $[Ru(\eta^6\text{-benzene})Cl(\mu\text{-Cl})_2]$ and $[Ru(\eta^6\text{-toluene})Cl(\mu\text{-Cl})_2]$ were synthesized according to published procedure [26f,33]. The preparation of *N,N'*-di(4-pyridinyl)ethanediamide followed the literature procedure as well [34].

Elemental analyses (C, H and N) were carried out on Elemental Vario EL III microanalyzer (for **2**, **6** and **7**) and Carlo Erba micro-analyser (for **3**). The infrared spectra were recorded on a Nicolet 6700 FT-IR spectrometer using ATR technique. The signal intensities are reported in wavenumbers and denoted by the following abbreviations: vs = very strong, s = strong, m = medium and w = weak. The NMR (1H and ^{13}C) spectra were recorded on a Bruker Avance III 500 instrument in $DMSO-d_6$ with TMS as the reference. The mass spectra of the mononuclear complexes were obtained with an Agilent Technologies 5975C inert XL MSD instrument using the direct insertion technique while the spectra for binuclear complexes were recorded with ultra performance liquid chromatography and Electrospray ionization mass spectrometry (UPLC-MS) by using an Acquit system from Gilson and a Waters X-Bridge BEH130. Spectral data of **1–8** are given in Supplementary material (ESI) to this paper.

3.2. Synthesis of the mononuclear Ru(II) complexes, **1–4**

The precursor, $[Ru(\eta^6\text{-benzene})Cl(\mu\text{-Cl})_2]/[Ru(\eta^6\text{-toluene})Cl(\mu\text{-Cl})_2]$ (0.10 mmol, 1 eq) was dissolved in ethanol (4 mL) and stirred

Table 1
Results of MTT assay, presented in terms of IC_{50} concentrations (μM) for tested compounds, obtained after 72 h of continuous action. Results are presented as an average (\pm SEM), of two independent experiments, each consisting of three replicates, and sample means were compared to corresponding non-treated controls.

$IC_{50} \pm SEM$ (μM) ^a								
Compound	MRC5	A549	HTB177	PC3	A375	HeLa	HCT116	MDA453
1	41.3 ± 5.4	140.9 ± 15	160.2 ± 14.1	133 ± 7.7	76.6 ± 4.9	38.6 ± 1.7	27.5 ± 2.2	68.2 ± 3.9
2	163.5 ± 13.5	>300	>300	>300	248.2 ± 8.2	145.5 ± 6.2	236.5 ± 9.5	215.6 ± 11.5
3	34 ± 2.7	110.1 ± 4.2	83 ± 2.3	140.8 ± 0.9	119.6 ± 5.3	51 ± 5.3	54.1 ± 4.3	87.5 ± 6.6
4	147 ± 10.2	>300	>300	>300	>300	148.3 ± 6.3	224.9 ± 9.4	198.9 ± 22.1
5	71.5 ± 5.6	221.5 ± 5.1	257.7 ± 12.9	242.3 ± 8.9	221.5 ± 11.1	103.7 ± 11.2	131.9 ± 9.2	126.5 ± 6.8
6	107 ± 11.6	249.9 ± 22.7	>300	240.7 ± 7.4	240.5 ± 8.7	138.1 ± 9.8	215.3 ± 12.9	181.6 ± 7.9
7	79.7 ± 1.5	161.8 ± 20.9	207.4 ± 13.4	268 ± 7	185.4 ± 12.8	100.9 ± 3.4	139.9 ± 7.1	132.4 ± 6.3
8	110.2 ± 11	218.3 ± 17.6	256.4 ± 14.9	239.2 ± 13.7	222.9 ± 15.6	136.1 ± 9.4	268.6 ± 27.8	149.5 ± 10.3
Cisplatin	5.5 ± 0.7	6.1 ± 1.9	5.7 ± 0.9	7.5 ± 0.4	2.2 ± 0.2	1.3 ± 0.4	5.3 ± 0.5	4 ± 0.4

^a $>$ indicates that IC_{50} value was not obtained in the tested range of concentrations.

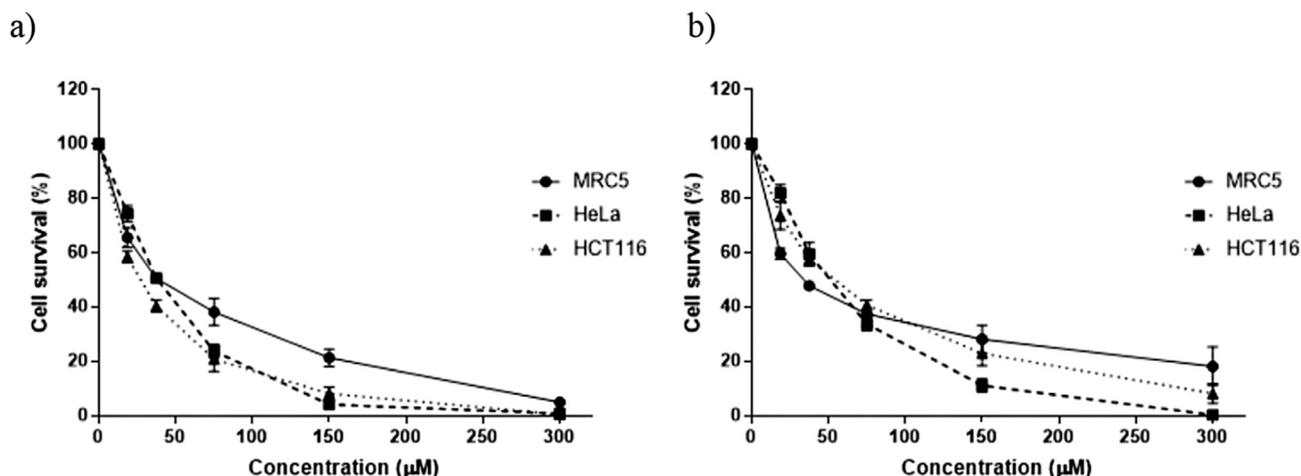


Fig. 2. Representative cell survival curves obtained in three tumor cell lines: MRC5, HeLa and HCT116, after 72 h of action of complex: a) **1** and b) **3**.

for 5 min at 50 °C. Corresponding ligand (picolinic acid/6-fluoropicolinic acid) (0.20 mmol, 2 eqs), previously dissolved in a small volume of ethanol (2 mL) was added to the ethanol solution of the starting complex. The reaction mixture was stirred for 3 h and afterwards was left stirring overnight at room temperature. Formed orange precipitate was isolated by filtration and washed with a small amount of cold diethylether (Scheme 1).

3.2.1. $[Ru(\eta^6\text{-benzene})(L_1)Cl]$ (**1**)

Yield: 74%. 1H NMR (500 MHz, DMSO- d_6), δ (ppm): 9.38 (d, 1H, C6, $J = 5.25$ Hz), 8.10 (t, 1H, C3, $J = 7.55$ and 7.50 Hz), 7.77 (d, 1H, C4, $J = 7.60$ Hz), 7.74 (t, 1H, C5, $J = 6.75$ and 6.05 Hz), 5.94 (s, 6H, C1'–6'). ^{13}C NMR (126 MHz, DMSO- d_6), δ (ppm): 170.44, 154.15, 150.37, 139.76, 127.98, 125.40, 87.50, 83.30. IR (cm^{-1}): 3058.1 (s), 1663.0 (vs), 1603.7 (m), 1471.1 (w), 1434.5 (m), 1335.8 (vs), 1287.0 (m), 1162.3 (w), 843.7 (m), 763.0 (m), 684.7 (m), 454.3 (w). EI-MS (m/z , (relative abundance, %)): 336.938 $[M]^+$, 257.938 $[M-PhH]^+$.

3.2.2. $[Ru(\eta^6\text{-benzene})(L_2)Cl]$ (**2**)

Yield: 72%. 1H NMR (500 MHz, DMSO- d_6), δ (ppm): 8.28 (q, 1H, C5, $J = 7.30$, 7.75 and 7.45 Hz), 7.77–7.73 (m, 2H, C3 and 4), 6.00 (s, 6H, C1'–6'). ^{13}C NMR (126 MHz, DMSO- d_6), δ (ppm): 169.39, 164.11, 162.08, 149.14, 145.27 and 145.20, 128.27, 122.13, 114.08, 87.68, 82.98. IR (cm^{-1}): 3535.5 (w), 3070.9 (s), 3041.7 (s), 1653.9 (vs), 1607.9 (vs), 1569.7 (s), 1466.8 (s), 1436.7 (vs), 1352.1 (vs), 1284.6 (vs), 1151.4 (w), 1020.4 (w), 840.9 (vs), 811.8 (s), 779.5 (vs), 683.3 (w), 619.6 (w), 439.2 (w). EI-MS (m/z , (relative abundance, %)): 354.972 $[M]^+$, 310.965, 276.092 $[M-PhH]^+$. Anal. Calc. for $C_{12}H_9ClFNO_2Ru$ (354.73): C, 40.63; H, 2.56; N, 3.95, Found: C, 40.15; H, 2.98; N, 3.65.

3.2.3. $[Ru(\eta^6\text{-toluene})(L_1)Cl]$ (**3**)

Yield: 94%. 1H NMR (500 MHz, DMSO- d_6), δ (ppm): 9.32 (d, 1H, C6, $J = 5.25$ Hz), 8.08 (t, 1H, C3, $J = 7.60$ and 7.65 Hz), 7.77 (d, 1H, C4, $J = 7.65$ Hz), 7.73 (t, 1H, C5, $J = 6.10$ and 6.00 Hz), 6.01 (q, 2H, C3' and C5', $J = 5.25$, 4.80 and 5.10 Hz), 5.72 (t, 1H, C4', $J = 5.55$ and 5.50 Hz), 5.64–5.60 (m, 2H, C2' and C6'), 2.16 (s, 3H, C7'). ^{13}C NMR (126 MHz, DMSO- d_6), δ (ppm): 170.39, 154.04, 150.76, 139.61, 127.94, 125.35, 101.03, 87.95, 86.34, 79.41, 78.78, 77.03, 39.52, 18.40. IR (cm^{-1}): 3102.5 (w), 3049.0 (m), 1643.7 (vs), 1600.8 (s), 1443.3 (w), 1343.9 (vs), 1290.2 (w), 1168.7 (w), 1060.2 (w), 1026.8 (w), 858.0 (m), 780.6 (m), 701.6 (w), 455.8 (w). EI-MS (m/z , (relative abundance, %)): 351.056 $[M]^+$, 315.035 $[M-CO_2]^+$. Anal. Calc. for $C_{13}H_{12}ClNO_2Ru$ (350.76): C, 44.52; H, 3.45; N, 3.99, Found: C, 44.05; H, 3.30; N, 4.17.

3.2.4. $[Ru(\eta^6\text{-toluene})(L_2)Cl]$ (**4**)

Yield: 85%. 1H NMR (500 MHz, DMSO- d_6), δ (ppm): 8.27 (q, 1H, C5, $J = 7.40$, 7.50 and 7.35 Hz), 7.74 (d, 2H, C3 and 4, $J = 8.30$ Hz), 6.14–6.09 (m, 2H, C3' and 5'), 5.75–5.63 (m, 3H, C2', 4' and 6'), 2.19 (s, 3H, C7'). ^{13}C NMR (126 MHz, DMSO- d_6), δ (ppm): 169.36, 164.11, 162.07, 149.75, 145.09 and 145.01, 122.37, 114.06, 113.82, 102.20, 87.44, 85.76, 79.24, 77.85, 76.39, 18.56. IR (cm^{-1}): 3667.6 (w), 3076.1 (s), 3052.0 (vs), 1657.8 (vs), 1611.0 (s), 1568.5 (m), 1518.7 (m), 1467.0 (m), 1446.4 (vs), 1377.1 (m), 1343.6 (s), 1272.1 (s), 1182.8 (w), 1151.1 (m), 1067.7 (m), 1029.1 (m), 860.8 (s), 812.7 (m), 773.2 (s), 681.4 (w), 629.0 (w), 487.5 (w).

EI-MS (m/z , (relative abundance, %)): 368.939 $[M]^+$, 333.082 $[M-Cl]^+$, 289.035 $[M-Cl-CO_2]^+$.

3.3. Synthesis of the binuclear Ru(II) complexes, 5–8

Previously synthesized complex (**1–4**) (0.24 mmol, 2 eqs) was dissolved in methanol and $AgCF_3SO_3$ (0.26 mmol, 2.2 eqs) was added. The reaction mixture was left stirring in the following 3 h at room temperature. The obtained precipitate was filtered off while the solution was used to further react with N,N' -di(4-pyridinyl)ethanediamide (0.12 mmol, 1 Eq). The reaction mixture was left stirring overnight at room temperature. The obtained solution was evaporated to the half of its volume, and diethylether was slowly added dropwise. Orange-red precipitate was isolated and rinsed with cold ethanol and diethylether (Scheme 2).

3.3.1. $[Ru(\eta^6\text{-benzene})(L_1)(bpo)Cl]$ (**5**)

Yield: 81%. 1H NMR (500 MHz, DMSO- d_6), δ (ppm): 11.57 (s, 2H, NH), 9.64 (d, 2H, C6, $J = 5.35$ Hz), 8.46 (d, 4H, C_b, $J = 6.40$ Hz), 8.16 (t, 1H, C3, $J = 7.40$ and 7.55 Hz), 7.91 (d, 4H, C_a, $J = 5.85$ Hz), 7.75 (d, 1H, C_x, $J = 7.55$ Hz), 6.13 (s, 12H, C1'–6'). ^{13}C NMR (126 MHz, DMSO- d_6), δ (ppm): 170.32, 158.64, 154.78, 153.57, 148.95, 146.91, 140.95, 129.52, 126.25, 121.95, 119.39, 115.89, 85.69, 39.52. IR (cm^{-1}): 3501.2 (w), 3229.9 (m), 3077.1 (m), 1703.1 (s), 1667.6 (vs), 1611.9 (s), 1584.2 (s), 1510.8 (vs), 1430.1 (s), 1338.3 (s), 1259.8 (vs), 1157.3 (vs), 1028.6 (s), 837.8 (m), 773.3 (m), 637.1 (s), 556.8 (w), 517.7 (w), 455.8 (w). EI-MS (m/z , (relative abundance, %)): 842.321 $[M]^+$, 764.223 $[M-PhH]^+$.

3.3.2. $[Ru(\eta^6\text{-benzene})(L_2)(bpo)Cl]$ (**6**)

Yield: 80%. 1H NMR (500 MHz, DMSO- d_6), δ (ppm): 11.61 (s, 2H, NH), 8.38 (d, 4H, C_b, $J = 6.70$ Hz), 8.33 (q, 2H, C4, $J = 7.45$, 7.65 and 7.30 Hz), 7.95 (d, 4H, C_a, $J = 6.90$ Hz), 7.92 (d, 2H, C3, $J = 8.35$ Hz),

7.73 (d, 2H, C5, $J = 7.25$ Hz), 6.21 (s, 12H, C1'–6'), ^{13}C NMR (126 MHz, DMSO- d_6), δ (ppm): 169.38, 164.80, 162.75, 158.68, 153.80, 147.71, 147.00, 146.33 and 146.26, 123.36, 116.17, 85.45, 39.52. IR (cm^{-1}): 3483.2 (w), 3243.3 (m), 3081.9 (m), 1708.4 (m), 1659.2 (m), 1612.8 (s), 1581.9 (s), 1507.1 (vs), 1427.0 (s), 1333.9 (m), 1257.0 (vs), 1152.9 (s), 1028.3 (s), 837.2 (m), 636.1 (m), 555.7 (w), 517.2 (w). EI-MS (m/z , (relative abundance, %)): 879.017 $[\text{M}]^+$, 800.983 $[\text{M}-\text{PhH}]^+$. Anal. Calc. for $\text{C}_{37}\text{H}_{31}\text{F}_2\text{N}_6\text{O}_6\text{Ru}_2$ (895.83): C, 49.61; H, 3.49; N, 9.38. Found: C, 49.38; H, 3.90; N, 8.94.

3.3.3. $[\text{Ru}(\eta^6\text{-toluene})(\text{L}_1)(\text{bpo})\text{Cl}]$ (**7**)

Yield: 64%. ^1H NMR (500 MHz, DMSO- d_6), δ (ppm): 11.57 (s, 2H, NH), 9.59 (d, 2H, C6, $J = 4.10$ Hz), 8.45 (d, 4H, C_b, $J = 6.05$ Hz), 8.15 (t, 2H, C3, $J = 7.30$ and 7.45 Hz), 7.93 (d, 4H+ 2H, C_a and C4, $J = 6.15$ Hz), 7.75 (d, 2H, C5, $J = 7.55$ Hz), 6.31 (t, 2H, C1'–6', $J = 5.15$ and 5.40 Hz), 6.16 (t, 2H, C1'–6', $J = 5.50$ and 5.40 Hz), 5.93 (t, 2H, C4', $J = 5.40$ and 5.30 Hz), 5.81 (t, 4H, C2' and C6', $J = 7.25$ and 6.70 Hz), 2.07 (s, 6H, CH₃). ^{13}C NMR (126 MHz, DMSO- d_6), δ (ppm): 170.25, 158.66, 154.71, 149.44, 146.91, 140.86, 129.51, 126.25, 115.93, 105.11, 90.83, 80.25 and 80.14, 78.40, 39.52, 17.98. IR (cm^{-1}): 3412.1 (w), 3253.2 (w), 3081.6 (w), 1705.6 (m), 1656.8 (vs), 1610.6 (s), 1582.6 (s), 1509.7 (vs), 1427.1 (m), 1338.6 (s), 1257.2 (vs), 1153.8 (s), 1027.0 (s), 836.5 (m), 773.9 (w), 635.4 (m), 553.8 (w), 515.4 (w). Anal. Calc. for $\text{C}_{40}\text{H}_{42}\text{N}_6\text{O}_6\text{Ru}_2$ (904.95): C, 53.09; H, 4.68; N, 9.29. Found: C, 52.73; H, 4.21; N, 9.63.

3.3.4. $[\text{Ru}(\eta^6\text{-toluene})(\text{L}_2)(\text{bpo})\text{Cl}]$ (**8**)

Yield: 64%. ^1H NMR (500 MHz, DMSO- d_6), δ (ppm): 11.61 (s, 2H, NH), 8.36 (d, 4H, C_b, $J = 6.95$ Hz), 8.32 (q, 2H, C4, $J = 7.35$, 7.95 and 7.30 Hz), 7.96 (d, 4H, C_a, $J = 7.10$ Hz), 7.91 (d, 2H, C3, $J = 8.30$ Hz), 7.73 (d, 2H, C5, $J = 7.30$ Hz), 6.47 (t, 2H, CX toluene, $J = 5.55$ and 5.75 Hz), 6.24 (t, 2H, CX toluene, $J = 5.90$ and 5.85 Hz), 5.98 (t, 2H, CX toluene, $J = 5.80$ and 5.75 Hz), 5.89 (t, 2H, CX toluene, $J = 6.30$ and 5.95 Hz), 5.78 (d, 2H, CX toluene, $J = 5.80$ Hz), 2.09 (s, 6H, CH₃). ^{13}C NMR (126 MHz, DMSO- d_6), δ (ppm): 169.27, 162.66, 158.70, 153.77, 148.25, 146.97, 146.21 and 146.13, 123.39, 116.19, 106.64, 90.94, 90.80, 79.57, 78.83, 77.58, 39.52, 18.12. IR (cm^{-1}): 3499.4 (w), 3223.8 (w), 3065.4 (w), 1706.8 (s), 1659.5 (vs), 1613.2 (vs), 1581.9 (s), 1508.2 (vs), 1427.5 (s), 1358.4 (m), 1334.8 (m), 1273.4 (vs), 1152.8 (s), 1073.2 (w), 1030.2 (s), 871.1 (w), 837.1 (m), 783.5 (w), 637.5 (m), 557.3 (w), 517.7 (w).

3.4. Biology tests

3.4.1. Cell lines and culture conditions

Seven human cancer cell lines: alveolar basal adenocarcinoma (A549), large cell lung carcinoma (HTB177), colorectal carcinoma (HCT116), malignant melanoma (A375), prostate adenocarcinoma (PC3), breast carcinoma (MDA-MB-453), cervix adenocarcinoma (HeLa), and one human non-malignant lung fibroblast cell line (MRC-5), were used for the examination of cytotoxic effects. All cell lines were maintained as flat monolayer culture in the RPMI 1640 nutrient medium (Sigma-Aldrich, Cat. No. R8755), supplemented with 10% of heat-inactivated fetal calf serum (FCS) (pH 7.2), penicillin (100 U/mL), streptomycin (200 $\mu\text{g}/\text{mL}$), 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES) (25 mM) and L-glutamine (3 mM). Cell cultures were kept in humidified atmosphere containing 5% CO_2 before and during incubation with investigated agents.

3.4.2. Cytotoxicity analysis

For determination of cell viability in cultures, we used the mitochondrial-dependent reduction of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) to formazan as described elsewhere [35,36]. Cells were seeded into 96-

well culture plates (Thermo Scientific Nunc™) at cell densities of 5000 c/w (MRC-5, A549, HTB177, HCT116), 3000 c/w (PC3, A375, MDA-MB-453) or 2000 c/w (HeLa) in 100 μL of cell culture medium, and left overnight. Eight tested agents were dissolved in DMSO to the stock concentration of 30 mM immediately before experiment, whereas further dilutions were made in the culture medium, so that the final concentration of DMSO never exceeded 1% (v/v). Triplicate wells were treated with varying concentrations of tested compounds (18.75 μM , 37.5 μM , 75 μM , 150 μM and 300 μM), in the final volume of 150 μL cell culture medium, per well. Cisplatin (cis-diamminedichloridoplatinum(II), CDDP) was used in this study as the reference compound. After 72 h of continuous incubation at 37 °C in 5% CO_2 humidified atmosphere, 20 μL of MTT solution (Sigma-Aldrich), was added to each well (5 mg/mL). The culture plates were incubated for the next 4 h at 37 °C, and finally 100 μL of 10% sodium dodecyl sulfate (SDS) was added to dissolve formed formazan crystals. Absorbances were measured after 24 h at a wavelength of 570 nm on a microplate reader (Thermo Labsystems Multiscan EX 200–240 V). The modification in cell viability was expressed as percentage of viability in treated samples compared to untreated control samples (taken as 100%), corrected for values obtained for wells without plated cells. The IC_{50} values (concentration of investigated compound that cause 50% decrease in the number of viable cells in a treated cell population compared to a non-treated control), were determined from the cell survival diagrams.

4. Conclusions

New mono- and binuclear ruthenium(II) complexes with picolinic acid and its fluoro derivative were synthesized and characterized by various methods (IR, NMR, MS). Four mononuclear Ru compounds were obtained reacting $[\text{Ru}(\eta^6\text{-benzene})\text{Cl}(\mu\text{-Cl})_2]$ or $[\text{Ru}(\eta^6\text{-toluene})\text{Cl}(\mu\text{-Cl})_2]$ with the ligand (picolinic acid and 6-fluoropicolinic acid). The coordination mode was typical for this type of ligands, via pyridine nitrogen and carboxylic oxygen. Furthermore, the novel compounds were used as precursor complexes for obtaining their corresponding binuclear analogues with N,N' -di(4-pyridinyl)ethanediamide as a suitable connector. However, *in vitro* cytotoxicity of binuclear assemblies has surprisingly revealed the lack of cytotoxicity or mild cytotoxicity. On the other hand, precursor compounds, namely mononuclear benzene and toluene complexes bearing picolinic acid demonstrated moderate antiproliferative effect particularly toward HCT116 and HeLa. In a comparison to the compounds already reported in literature [26a,26f], these complexes displayed improved activity and selectivity. Therefore further investigations regarding their binding modes, sites, and affinities will be the topic of our interest.

Declaration of competing interest

There are no conflicts to declare.

Acknowledgements

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (grant numbers 172035 and 41026).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jorganchem.2019.120966>.

References

- [1] (a) B. Rosenberg, L. Van Camp, T. Krigas, Inhibition of cell division in *Escherichia coli* by electrolysis products from a platinum electrode, *Nature* 205 (1965) 698–699;
(b) B. Lippert, *Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug*, Wiley-VCH, Weinheim, 1999.
- [2] R. Oun, Y.E. Moussa, N.J. Wheate, The side effects of platinum-based chemotherapy drugs: a review for chemists, *Dalton Trans.* 47 (2018) 6645–6653.
- [3] (a) A. Bergamo, G. Sava, Ruthenium anticancer compounds: myths and realities of the emerging metal-based drugs, *Dalton Trans.* 40 (2011) 7817–7823;
(b) S. Thota, D.A. Rodrigues, D.C. Crans, E.J. Barreiro, Ru(II) compounds: next-generation anticancer metallotherapeutics? *J. Med. Chem.* 61 (2018) 5805–5821;
(c) S. Medici, M. Peana, V.M. Nurchi, J.I. Lachowicz, G. Crisponi, M.A. Zoroddu, Noble metals in medicine: latest advances, *Coord. Chem. Rev.* 284 (2015) 329–350;
(d) W.A. Wani, U. Baig, S. Shreaz, R.A. Shiekh, P.F. Iqbal, E. Jameel, A. Ahmad, S.H. Mohd-Setapar, Md Mushtaque, L.T. Hun, Recent advances in iron complexes as potential anticancer agents, *New J. Chem.* 40 (2016) 1063–1090;
(e) S. Roy, M. Mohanty, S. Pasayat, S. Majumder, K. Senthilguru, I. Banerjee, M. Reichelt, H. Reuter, E. Sinn, R. Dinda, Synthesis, structure and cytotoxicity of a series of Dioxidomolybdenum(VI) complexes featuring Salan ligands, *J. Inorg. Biochem.* 172 (2017) 110–121.
- [4] S.M. Meier-Menches, C. Gerner, W. Berger, C.G. Hartinger, B.K. Keppler, Structure–activity relationships for ruthenium and osmium anticancer agents – towards clinical development, *Chem. Soc. Rev.* 47 (2018) 909–928.
- [5] E. Alessio, Thirty years of the drug candidate NAMI-A and the myths in the field of ruthenium anticancer compounds: a personal perspective, *Eur. J. Inorg. Chem.* (2017) 1549–1560.
- [6] C.G. Hartinger, M.A. Jakupec, S. Zorbas-Seifried, M. Groessel, A. Egger, W. Berger, H. Zorbas, P.J. Dyson, B.K. Keppler, KP1019, a new redox-active anticancer agent – preclinical development and results of a clinical phase I study in tumor patients, *Chem. Biodivers.* 5 (2008) 2140–2155.
- [7] P.-S. Kuhn, V. Pichler, A. Roller, M. Hejl, M.A. Jakupec, W. Kandioller, B.K. Keppler, Improved reaction conditions for the synthesis of new NKP-1339 derivatives and preliminary investigations on their anticancer potential, *Dalton Trans.* 44 (2015) 659–668.
- [8] P. Zhang, P.J. Sadler, Advances in the design of organometallic anticancer complexes, *J. Organomet. Chem.* 839 (2017) 5–14.
- [9] (a) B. Therrien, Functionalised η^6 -arene ruthenium complexes, *Coord. Chem. Rev.* 253 (2009) 493–519;
(b) W.H. Ang, A. Casini, G. Sava, P.J. Dyson, Organometallic ruthenium-based antitumor compounds with novel modes of action, *J. Organomet. Chem.* 696 (2011) 989–998.
- [10] (a) A.L. Noffke, A. Habtemariam, A.M. Pizarro, P.J. Sadler, Designing organometallic compounds for catalysis and therapy, *Chem. Commun.* 48 (2012) 5219–5246;
(b) J. Václavík, P. Kačer, M. Kuzma, L. Červený, Opportunities offered by chiral η^6 -arene/*N*-Arylsulfonyldiamine-Ru^{II} catalysts in the asymmetric transfer hydrogenation of ketones and imines, *Molecules* 16 (2011) 5460–5495.
- [11] A.K. Singh, D.S. Pandey, Q. Xu, P. Braunstein, Recent advances in supramolecular and biological aspects of arene ruthenium(II) complexes, *Coord. Chem. Rev.* 270–271 (2014) 31–56.
- [12] (a) A. Mishra, S. Ravikumar, Y.H. Song, N.S. Prabhu, H. Kim, S.H. Hong, S. Cheon, J. Noh, K.-W. Chi, A new arene–Ru based supramolecular coordination complex for efficient binding and selective sensing of green fluorescent protein, *Dalton Trans.* 43 (2014) 6032–6040;
(b) A. Mishra, S.C. Kang, K.-W. Chi, Coordination-driven self-assembly of arene–ruthenium compounds, *Eur. J. Inorg. Chem.* (2013) 5222–5232.
- [13] T. Lazarević, A. Rilak, Ž.D. Bugarčić, Platinum, palladium, gold and ruthenium complexes as anticancer agents: current clinical uses, cytotoxicity studies and future perspectives, *Eur. J. Med. Chem.* 142 (2017) 8–31.
- [14] S. Movassaghi, M. Hanif, H.U. Holtkamp, T. Söhnel, S.M.F. Jamieson, C.G. Hartinger, Making organoruthenium complexes of 8-hydroxyquinolines more hydrophilic: impact of a novel L-phenylalanine-derived arene ligand on the biological activity, *Dalton Trans.* 47 (2018) 2192–2201.
- [15] A.K. Singh, P. Kumar, M. Yadav, D.S. Pandey, Synthesis, characterisation and theoretical studies on some piano-stool ruthenium and rhodium complexes containing substituted phenyl imidazole ligands, *J. Organomet. Chem.* 695 (2010) 567–573.
- [16] M. Yadav, A.K. Singh, B. Maiti, D.S. Pandey, Heteroleptic arene ruthenium complexes based on meso-substituted dipyrins: synthesis, structure, reactivity, and electrochemical studies, *Inorg. Chem.* 48 (2009) 7593–7603.
- [17] B. Önfelt, L. Göstring, P. Lincoln, B. Nordén, A. Önfelt, Cell studies of the DNA bis-intercalator $\Delta\text{-}\Delta[\mu\text{-C4}(\text{cpdppz})_2\text{-}(\text{phen})_4\text{Ru}_2]^{4+}$: toxic effects and properties as a light emitting DNA probe in V79 Chinese hamster cells, *Mutagenesis* 17 (2002) 317–320.
- [18] (a) M.G. Mendoza-Ferri, C.G. Hartinger, M.A. Mendoza, M. Groessel, A.E. Egger, R.E. Eichinger, J.B. Mangrum, N.P. Farrell, M. Maruszak, P.J. Bednarski, Transferring the concept of multinuclearity to ruthenium complexes for improvement of anticancer activity, *J. Med. Chem.* 52 (2009) 916–925;
(b) M.-G. Mendoza-Ferri, C.G. Hartinger, R.E. Eichinger, N. Stolyarova, K. Severin, M.A. Jakupec, A.A. Nazarov, B.K. Keppler, Influence of the spacer length on the *in vitro* anticancer activity of dinuclear ruthenium-arene compounds, *Organometallics* 27 (2008) 2405–2407.
- [19] L. Zeng, P. Gupta, Y. Chen, E. Wang, L. Ji, H. Chao, Z.-S. Chen, The development of anticancer ruthenium(II) complexes: from single molecule compounds to nanomaterials, *Chem. Soc. Rev.* 46 (2017) 5771–5804.
- [20] (a) R.S. Grant, S.E. Coggan, G.A. Smythe, The physiological action of picolinic acid in the human brain, *Int. J. Tryptophan Res.* (2009) 71–79;
(b) S. Cai, K. Sato, T. Shimizu, S. Yamabe, M. Hiraki, C. Sano, H. Tomioka, Antimicrobial activity of picolinic acid against extracellular and intracellular *Mycobacterium avium* complex and its combined activity with clarithromycin, rifampicin and fluoroquinolones, *J. Antimicrob. Chemother.* 57 (2006) 85–93.
- [21] R. Song, K.M. Kim, Y.S. Sohn, Synthesis and properties of (diamine)platinum(II) complexes of pyridine carboxylate isomers and their antitumor activity, *Inorg. Chim. Acta* 292 (1999) 238–243.
- [22] V.A. Kawade, A.A. Kumbhar, A.S. Kumbhar, C. Nather, A. Erxleben, U.B. Sonawane, R.R. Joshi, Mixed ligand cobalt(II) picolinate complexes: synthesis, characterization, DNA binding and photocleavage, *Dalton Trans.* 40 (2011) 639–650.
- [23] T.L. Feng, J. M Tsangaris, A. R Barron, Complexes of aluminium(III) with picolinic and pipecolinic acids: an ²⁷Al-NMR investigation, *Monatshfte Chem.* 121 (1990) 113–118.
- [24] J. Chai, Y. Liu, J. Dong, B. Liu, B. Yang, Synthesis, structure, chemical and bioactivity behavior of eight chromium(III) picolinate derivatives Cr(R-pic)₃, *Inorg. Chim. Acta* 466 (2017) 151–159.
- [25] (a) É. Sija, C.G. Hartinger, B.K. Keppler, T. Kiss, É.A. Enyedy, Solution equilibrium studies of anticancer ruthenium(II)- η^6 -*p*-cymene complexes of pyridinecarboxylic acids, *Polyhedron* 67 (2014) 51–58;
(b) F.R. Pavan, G.V. Poelhsitz, M.I.F. Barbosa, S.R.A. Leite, A.A. Batista, J. Ellena, L.S. Sato, S.G. Franzblau, Virtudes Moreno, D. Gambino, C.Q.F. Leite, Ruthenium(II) phosphine/diimine/picolinate complexes: inorganic compounds as agents against tuberculosis, *Eur. J. Med. Chem.* 46 (2011) 5099–5107.
- [26] (a) I. Ivanović, S. Grgurić-Šipka, N. Gligorićević, S. Radulović, A. Roller, Ž. Lj. Tešić, B.K. Keppler, X-Ray structure and cytotoxic activity of a picolinate ruthenium(II)-arene complex, *J. Serb. Chem. Soc.* 76 (2011) 53–61;
(b) N. Gligorićević, S. Arandelović, L. Filipović, K. Jakovljević, R. Janković, S. Grgurić-Šipka, I. Ivanović, S. Radulović, Ž.Lj. Tešić, Picolinate ruthenium(II)-arene complex with *in vitro* antiproliferative and antimetastatic properties: comparison to a series of ruthenium(II)-arene complexes with similar structure, *J. Inorg. Biochem.* 108 (2012) 53–61;
(c) I. Ivanović, K.K. Jovanović, N. Gligorićević, S. Radulović, V.B. Arion, K.S.A.M. Sheweshein, Ž.Lj. Tešić, S. Grgurić-Šipka, Ruthenium(II)arene complexes with substituted picolinate ligands: synthesis, structure, spectroscopic properties and antiproliferative activity, *J. Organomet. Chem.* 749 (2014) 343–349;
(d) A.A. Baroud, LjE. Mihajlović-Lalić, N. Gligorićević, S. Arandelović, D. Stanković, S. Radulović, K. Van Hecke, A. Savić, S. Grgurić-Šipka, Ruthenium(II) bipyridine complexes: from synthesis and crystal structures to electrochemical and cytotoxicity investigation, *J. Coord. Chem.* 70 (2017) 831–847;
(e) A.A. Baroud, LjE. Mihajlović-Lalić, D. Stanković, M. Kajzerberger, K. van Hecke, S. Grgurić-Šipka, A. Savić, New ruthenium(II) bipyridyl complex: synthesis, crystal structure and cytotoxicity, *J. Serb. Chem. Soc.* 82 (2017) 267–275;
(f) J.M. Poljarević, G.T. Gál, N.V. May, G. Spengler, O. Dömötör, A.R. Savić, S. Grgurić-Šipka, É.A. Enyedy, Comparative solution equilibrium and structural studies of half-sandwich ruthenium(II)(η^6 -toluene) complexes of picolinate derivatives, *J. Inorg. Biochem.* 181 (2018) 74–85.
- [27] G. Gupta, P. Nowak-Sliwiska, N. Herrero, P.J. Dyson, B. Therrien, Increasing the selectivity of biologically active tetranuclear arene ruthenium assemblies, *J. Organomet. Chem.* 796 (2015) 59–64.
- [28] (a) J.P.G. Tabares, R.L.S.R. Santos, J.L. Cassiano, M.H. Zaim, J. Honorato, A.A. Batista, S.F. Teixeira, A.K. Ferreira, R.B. Viana, S.Q. Martínez, A.C. Stabile, D. de Oliveira Silva, A Ru(II)-*p*-cymene compound bearing naproxen-pyridineamide. Synthesis, spectroscopic studies, computational analysis and *in vitro* anticancer activity against lung cells compared to Ru(II)-*p*-cymene-naproxen and the corresponding drug ligands, *Inorg. Chim. Acta* 489 (2019) 27–38;
(b) M. Patra, T. Joshi, V. Pierroz, K. Ingram, M. Kaiser, S. Ferrari, B. Spingler, J. Keiser, G. Gasser, DMSO-mediated ligand dissociation: renaissance for biological activity of *N*-heterocyclic-[Ru(η^6 -arene)Cl₂] drug candidates, *Chem. Eur. J.* 19 (2013) 14768–14772.
- [29] G. Pratesi, P. Perego, D. Polizzi, S.C. Righetti, R. Supino, C. Caserini, C. Manzotti, F.C. Giuliani, G. Pezzoni, S. Tognella, S. Spinelli, N. Farrell, F. Zunino, A novel charged trinuclear platinum complex effective against cisplatin-resistant tumours: hypersensitivity of p53-mutant human tumour xenografts, *Br. J. Canc.* 80 (1999) 1912–1919.
- [30] A. Valente, M.H. Garcia, Anion-recognition studies of a Re(I)-based square containing the dipyrindyl-amide ligand, *Inorganics* 2 (2014) 96–114.
- [31] S.Q. Zhang, L.H. Gao, H. Zhao, K.Z. Wang, Recent progress in polynuclear ruthenium complex-based DNA binders/structural probes and anticancer agents, *Curr. Med. Chem.* 26 (2018) 1–18.
- [32] M.Y. Abdalla, Glutathione as potential target for cancer therapy; more or less is good? *Jordan J. Biol. Sci.* 4 (2011) 119–124.

- [33] R.A. Zelonka, M.C. Baird, Benzene complexes of ruthenium(II), *Can. J. Chem.* 50 (1972) 3063–3072.
- [34] B.-C. Tzeng, Y.-F. Chen, C.-C. Wu, C.-C. Hu, Y.-T. Chang, C.-K. Chen, Anion-recognition studies of a Re(I)-based square containing the dipyriddy-amide ligand, *New J. Chem.* 31 (2007) 202–209.
- [35] M. Pavlović, S. Nikolić, N. Gligorijević, B. Dojčinović, S. Arandelović, S. Grgurić-Šipka, S. Radulović, New organoruthenium compounds with pyrido[2',3':5,6]pyrazino[2,3-f][1, 10]phenanthroline: synthesis, characterization, cytotoxicity, and investigation of mechanism of action, *J. Biol. Inorg. Chem.* 24 (2019) 297–310.
- [36] R. Supino, in: S. O' Hare, C.K. Atterwill (Eds.), *MTT Assays in Vitro Toxicity Testing Protocols*, vol. 43, Humana Press, New Jersey, 1995, pp. 137–149.