(Electro)chemical and antimicrobial characterization of novel Ru(II) bipyridine complexes with acetylpyridine analogs

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ABSTRACT

Three ruthenium-bipyridine complexes (1–3) carrying acetylpyridine ligand unit were synthesized in methanol via the reaction of [RuCl₂(bpy)₂] with corresponding acetylpyridine (2-, 3-, and 4-acpy). Obtained complexes were characterized by (1H and 13C) NMR and IR spectroscopy, MS spectrometry, UV–vis spectrophotometry, and cyclic voltammetry. Their structural characterization revealed bidentate coordination mode for 2-acpy while 3- and 4-acpy acted as monodentate ligands. The electrochemical profile of newly synthesized compounds was investigated by cyclic voltammetry which confirmed their electrochemical activity. Voltammetric responses within the −1.20 < Ep < 1.50 V range of potentials were summarized in two major events: Ru(II) → Ru(III) oxidation spotted at app. ΔEp = 0.65 V and successive reductions of bpy units located from −0.79 V to 0.47 V (vs. Ag/AgCl (3 M) electrode). The DNA-binding activity of the complexes was evaluated by both UV–vis spectrophotometry and cyclic voltammetry indicating DNA-intercalation with a slight contribution of electrostatic interactions. Furthermore, antimicrobial activity was tested against bacterial and fungal strains, for which moderate activity was observed. Assessment of in vitro toxicity against freshly hatched nauplii of Artemia salina as well as radical scavenging capacity was evaluated. The test compounds showed neither toxicity nor antioxidant activity.

ARTICLE HISTORY

Received 22 March 2022
Accepted 3 June 2022

KEYWORDS

Ruthenium; acetylpyridine; cyclic voltammetry; antimicrobial; UV–vis

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Supplemental data for this article is available online at https://doi.org/10.1080/00958972.2022.2090247.
1. Introduction

The versatile chemistry of ruthenium complexes involves thousands of compounds aimed for different applications related to, for example, homogenous catalysis, cancer therapy, tumor diagnosis, advanced materials, and so on [1–7]. Various architectures of complexes containing Ru provide specific biological, photophysical, optical, catalytic and electronic properties which model their final efficiency [8]. Most of the structures were designed on the basis of suitable metal-ligand framework meaning that each structural unit contributes the overall role in, for example, biological system. Over the past decade, the research interest for building polypyridyl-based species has increased mainly due to their high complexing affinity for numerous metal ions and chemical stability [9]. In particular, compounds carrying 2,2’-bipyridine (bpy) unit(s) were characterized as highly diverse photochemical and redox systems allowing various fine tuning combinations with co-ligands. As some features of Ru(II) complexes are strongly ligand-dependent, many authors emphasize the selection of an appropriate co-ligand [10]. Thus, introducing the moiety such as acetylpyridine (acpy) with replaceable acetyl substituent may functionalize the final molecule making it more attractive in terms of efficient molecular design. The acpy scaffold is well-recognized in coordination chemistry for its ability to bind the metal ion via imine nitrogen as well as through the amide oxygen [11, 12]. The literature concerning acpy type of ligand has reported complexes of group IV representatives (Fe, Co, Ni, Cu, and Zn) [12–18], Cd [16–18], and some Ru [19], Pd [20] and Pt species [21] in a dominant +2 oxidation state (Figure 1). While Cu-based compound was primarily obtained to serve as an anti-microbial agent, Ru, Pd, and Pt complexes aimed for reaching increased cytotoxicity [12, 19–21]. On the other hand, very few manuscripts reported promising antibacterial activity of Ru-bpy containing compounds but without a straightforward correlation between their affinity and antimicrobial efficiency. Yet, longing towards well-grounded structure-activity relationship remains the topic of concern.

Figure 1. Representative transition metal complexes with acpy moiety.
Electrochemical profiling of newly synthesized molecules has become unavoidable as it provides necessary data on redox behavior in biologically relevant systems. The most common analytical method used for these types of studies is cyclic voltammetry, well-known for its simple, rapid and economical features [22]. Obtained data usually provide insight into the nature of the redox process influenced by the coordination number, stereochemistry and the hard/soft character of the ligand donor atoms [23]. Due to the presence of various species in a solution, connecting coordination number with stereochemistry is scarce and the redox reaction is specified on the basis of ligand’s nature. Patterson’s and Holm’s studies reported how soft ligands demonstrate more positive $E_p$ values unlike hard ones which tend to show more negative $E_p$ values [24, 25]. Although the intensive research within the field of Ru(II) bpy complexes is well-documented in terms of synthetic procedures and characterization data, the electrochemistry of such compounds is scarce. Very first electrochemical study regarding these complexes was carried out 40 years ago comparing the first oxidation potentials (vs. SCE) for four ruthenium complexes, respectively, containing terpyridine (1.28 V), bipyridine (1.35 V), ortho-phenanthroline (1.40 V) and 2,4,6-tripyridyl-s-triazine (1.52 V) [26]. Further, van der Westhuizen’s study of substituted [Ru(bpy)$_3$]$^{2+}$ complexes pointed out the Ru(II)—Ru(III) reversible redox wave. Our interest in oxidation/reduction pathway of biologically active compounds emerged with the study of some Ru(II) bpy-based complexes promoted as antitumor agents [27]. Still, most of such compounds are designed as antibacterial species demonstrating either comparable or, in some cases, even improved activity than standard drugs.

In connection with our ongoing interest for the abovementioned structures and their interesting (electro)chemical and biological features, three Ru(II)-bpy complexes with acpy analogs were prepared and characterized. The complexes were also evaluated for their electrochemical and antibacterial activities.

2. Experimental

2.1. Materials and methods

All experiments were performed under atmospheric conditions with commercially available chemicals and solvents used as received. The starting complex [RuCl$_2$(bpy)$_2$] was synthesized according to slightly modified synthetic route [28, 29]. Infrared spectra were recorded for both ligands (2-acetylpyridine = 2-acpy; 3-acetylpyridine = 3-acpy; 4-acetylpyridine = 4-acpy) and corresponding complexes 1–3 on a Nicolet Summit FTIR spectrometer using ATR technique. Signal intensities were reported with wavenumbers according to their relative intensities as very strong (vs), strong (s), medium (m), and weak (w). Varian instrument (Agilent, USA) was used for obtaining NMR spectra with a 5 mm ATB probe using standard pulse sequences at 399.73 MHz for $^1$H and 100.52 MHz for $^{13}$C nuclei. Reported chemical shifts ($\delta$) for $^1$H and $^{13}$C spectra were referenced to residual $^1$H and $^{13}$C in deuterated dimethylsulfoxide (DMSO-d$_6$) and given in ppm relative to TMS as internal standard. The same instrument was used for $^1$H NMR stability study in DMSO-d$_6$ for all three complexes. Spectra were recorded immediately after dissolving and further recorded after 4 h, 8 h, 24 h, 48 h, and 72 h, as well as after 7 days. The mass spectra of 1–3 were obtained in acetonitrile with a LTQ
Orbitrap XL mass spectrometer. UV–visible (UV–vis) spectra were obtained in DMSO using a LLG-uniSPEC 2 spectrophotometer (for pure compounds 1–3) and a GBC Cintra 6 spectrophotometer (for DNA binding studies).

2.2. Syntheses of complexes

2.2.1. Synthesis of [RuL(bpy)2](PF6) (1)
A warm solution of 2-acpy (0.1000 g, 0.83 mmol) in methanol (1.5 mL) was added to a warm solution of [RuCl2(bpy)2] (0.1333 g, 0.28 mmol) in methanol (10.0 mL). The reaction mixture was refluxed overnight and afterwards left to cool to room temperature. After adding an equimolar amount of NH4PF6 (0.0449 g, 0.28 mmol), the mixture was stirred for an additional 3 h. The product was isolated by filtration over a celite layer and recrystallized from dichloromethane to yield a dark red microcrystalline powder. Yield: 0.0613 g, 33%. 1H NMR (400 MHz, DMSO-d6), δ (ppm): 8.83 (sext, 5H, bpy), 8.32 (d, 1H, C6acpy), 8.30–8.22 (m, 3H, bpy), 8.16–8.07 (m, 2H, C4acpy and C3acpy), 7.92 (d, 1H, bpy), 7.80 (t, 1H, bpy), 7.76 (d, 1H, bpy), 7.71–7.64 (m, 4H, C5acpy and bpy), 7.48 (dt, 2H, bpy), 3.12 (s, 3H, –CH3). 13C NMR (100 MHz, DMSO-d6), δ (ppm): 214.49 (–C¼O), 157.52 (bpy), 157.19 (bpy), 156.87 (bpy), 156.38 (bpy), 153.16 (C2), 152.52 (bpy), 152.26 (bpy), 151.94 (bpy), 151.46 (C6), 138.58 (bpy), 138.19 (bpy), 137.99 (bpy), 137.30 (C4), 132.78 (bpy), 132.54 (bpy), 128.24 (bpy), 127.86 (bpy), 127.51 (C5), 124.63 (bpy), 124.28 (bpy), 124.18 (C3), 26.50 (–CH3). IR (cm−1): 1603.8 (m, C¼O), 1578.5 (m, C¼N), 836.1 (vs, P–F), 760.7 (s, C–H), 556.5 (s, Ru–N). MS (m/z, relative abundance, %), [M–PF6]+: calculated, 535.0953; found, 534.0866, 15. UV–vis (DMSO) λmax/nm (ε/M−1 cm−1): 284 (3625), 436 (686), 510 (557).

2.2.2. Synthesis of [RuL(bpy)2Cl](PF6) (2)
A warm solution of 3-acpy (0.1000 g, 0.83 mmol) in methanol (1.5 mL) was added to a warm solution of [RuCl2(bpy)2] (0.1333 g, 0.28 mmol) in methanol (10.0 mL). The reaction mixture was refluxed overnight and afterwards left to cool to room temperature. After adding an equimolar amount of NH4PF6 (0.0449 g, 0.28 mmol), the mixture was stirred for an additional 3 h. The product was isolated by filtration over a celite layer and recrystallized from dichloromethane to yield a dark red microcrystalline powder. Yield: 0.1921 g, 91%. 1H NMR (400 MHz, DMSO-d6), δ (ppm): 9.84 (d, 1H, bpy), 9.12 (s, 1H, C2acpy), 8.78 (d, 1H, C6acpy), 8.65 (d, 2H, bpy), 8.58 (d, 1H, bpy), 8.50 (d, 1H, bpy), 8.33 (d, 1H, C4acpy), 8.28 (d, 1H, bpy), 8.17 (q, 2H, bpy), 7.94–7.86 (m, 4H, bpy), 7.70 (t, 1H, bpy), 7.58 (d, 1H, C5acpy), 7.50 (t, 1H, bpy), 7.38 (t, 1H, bpy), 7.30 (t, 1H, bpy), 2.45 (s, 3H, –CH3). 13C NMR (100 MHz, DMSO-d6), δ (ppm): 195.86 (–C=O), 158.07 (bpy), 157.65 (bpy), 157.33 (bpy), 153.44 (C2), 152.61 (bpy), 152.35 (bpy), 151.88 (bpy), 151.65 (bpy), 149.50 (C6), 136.82 (bpy), 136.50 (bpy), 136.26 (bpy), 135.81 (bpy), 135.61 (C4), 132.95 (C3), 127.34 (bpy), 127.24 (bpy), 126.82 (bpy), 126.21 (bpy), 125.36 (bpy), 124.05 (bpy), 123.91 (C5), 123.65 (bpy), 123.18 (bpy), 26.79 (–CH3). IR (cm−1): 1690.7 (vs, C=O), 1585.9 (m, C=CH2), 836.1 (vs, P–F), 761.8 (s, C–H), 556.5 (s, Ru–N). MS (m/z, relative abundance, %), [M–PF6]+: calculated, 570.0638; found, 570.0644 [M–PF6]+, 28. UV–vis (DMSO) λmax/nm (ε/M−1 cm−1): 291 (4534), 352 (1211), 493 (1145).
2.2.3. Synthesis of [RuL(bpy)_2Cl](PF_6) (3)

A warm solution of 4-acpy (0.1000 g, 0.83 mmol) in methanol (1.5 mL) was added to a warm solution of [RuCl_2(bpy)_2] (0.1333 g, 0.28 mmol) in methanol (10.0 mL). The reaction mixture was refluxed overnight and afterwards left to cool to room temperature. After adding an equimolar amount of NH_4PF_6 (0.0449 g, 0.28 mmol), the mixture was stirred for an additional 3 h. The obtained solution was left to precipitate at 5°C for 5 days and the final product was isolated by filtration yielding a dark red microcrystalline powder. Yield: 0.1803 g, 92%.

1H NMR (400 MHz, DMSO-d_6), δ (ppm): 9.83 (d, 1H, bpy), 8.79 (t, 2H, C2_acpy and C6_acpy), 8.66 (d, 2H, bpy), 8.58 (d, 1H, bpy), 8.49 (d, 1H, bpy), 8.18 (q, 2H, bpy), 7.95–7.88 (m, 3H, bpy), 7.84 (d, 1H, bpy), 7.81 (d, 1H, bpy), 7.73–7.70 (m, 3H, C3_acpy and C5_acpy, bpy), 7.57 (d, 1H, bpy), 7.36 (t, 1H, bpy), 7.31 (t, 1H, bpy), 2.54 (s, 3H, –CH_3).

13C NMR (100 MHz, DMSO-d_6), δ (ppm): 197.01 (–C=O), 158.79 (bpy), 157.82 (bpy), 157.60 (bpy), 157.27 (bpy), 152.57 (bpy), 152.35 (bpy), 151.79 (bpy), 151.45 (C2), 150.81 (C6), 142.00 (C4), 136.88 (bpy), 136.48 (bpy), 136.38 (bpy), 135.84 (bpy), 127.34 (bpy), 127.27 (bpy), 127.25 (bpy), 126.76 (bpy), 124.01 (bpy), 123.70 (bpy), 123.59 (bpy), 123.18 (bpy), 122.53 (C3 and C5), 121.31 (bpy), 26.79 (–CH_3). IR (cm⁻¹): 1696.7 (s, C=O), 1603.6 (m, C=N), 833.9 (vs, P–F), 556.5 (s, Ru–N).

MS (m/z, relative abundance, %), [M–PF_6]^+: calculated 570.0638; found 570.0638., 10.

UV-vis (DMSO) λ_max/nm (ε/M⁻¹ cm⁻¹): 291 (4014), 349 (706), 400 (774), 487 (1150).

2.3. Electrode preparation

Primarily, GCE was polished using alumina (0.5 and 0.3 μ) followed with rinsing with distilled water. Afterwards, the electrode was sonicated for 3 min in ethanol, rinsed with ultrapure water and used for measurements.

2.4. Electrochemical measurements

Electrochemical measurements were performed in a three electrode system where glassy carbon electrode was used as working electrode, Ag/AgCl (non-aqueous, silver wire in contact with a DMSO solution of AgNO_3 (0.01 M) and 0.1 M of the same supporting electrolyte as that employed in the cell) as the reference electrode and platinum wire as the auxiliary electrode. Experiments were conducted on a CHI 760 b electrochemical workstation (potentiostat/bipotentiostat) controlled with CHI 2.0 software. For interpretation of results Origin 8.5 software was used. Experiments were performed at ambient temperature.

2.5. Antimicrobial study

Antimicrobial activity of parent complex, ligands and synthesized complexes was tested against a panel of microorganisms including: Gram-negative bacteria Escherichia coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 9027), Proteus hauseri (ATCC 13315), Klebsiella pneumoniae (ATCC 10031), Salmonella enterica subsp. enterica serovar Enteritidis (ATCC 13076), Gram-positive bacteria Staphylococcus aureus (ATCC 6538), Bacillus subtilis (ATCC 6633), Clostridium sporogenes (ATCC 19404), Micrococcus luteus...
(ATCC 10240), *Kocuria rhizophila* (ATCC 9341), yeasts *Candida albicans* (ATCC 10231), *Saccharomyces cerevisiae* (ATCC 9763) and fungal strain *Aspergillus brasiliensis* (ATCC 16404).

Antimicrobial activity was determined using broth microdilution method according to NCCLS [30]. For antibacterial activity Mueller-Hinton broth was used, while Sabouraud dextrose broth was used for yeasts and fungi. All tested compounds were dissolved in DMSO, added in the plate and double diluted using a multichannel pipette (range of the concentration was 5000 – 10 µg/mL). Suspension of bacteria, yeasts and fungi was prepared in sterile 0.9% saline. Suspension turbidity evaluation was conducted by comparison with 0.5 McFarland’s standard. A 10 µL of diluted bacterial, yeast or spores suspension was added to each well to give a final concentration of 5 × 10^5 CFU/mL for bacteria and 5 × 10^3 CFU/mL for yeast and fungi. Erythromycin served as positive control for bacteria, while amphotericin B served as positive control for yeasts and fungi. DMSO was used as negative control.

The inoculated plates were incubated at 37°C for 24 h for bacteria and at 28°C for 48 h for the yeasts and fungi. The bacterial growth was visualized by adding 10 µL of 0.6% solution of resazurin. Minimum inhibitory concentration (MIC) was defined as the lowest concentration of the compounds that inhibited bacterial growth (pink color indicates growth and blue means inhibition of growth).

### 2.6. Brine shrimp lethality bioassay

About 40 g of commercially available mixture of salt and lyophilized eggs of the brine shrimp *Artemia salina* was added to 1 L of tap water. Air was passed through the suspension thermostated at 301 K, under illumination for 24 h. The tested substances were dissolved in DMSO. In a glass vial, filled with 1 mL of artificial sea water containing 10–20 of freshly hatched nauplii, the solutions of tested compounds were added at concentrations from 0.5 to 0.05 mg/mL. After 24 h illumination at room temperature, the number of dead and surviving nauplii were counted and statistically analyzed. LC50 was defined as a concentration of compounds that caused the death of 50% of the nauplii. All samples were done in triplicate. DMSO was inactive under applied conditions.

### 2.7. Assessment of free-radical scavenging capacity (DPPH method)

The antioxidant effect of the ligands and synthesized complexes was determined using DPPH (1,1-diphenyl-2-picrylhydrazyl) assay which measures the radical scavenging capacity [31]. The microplates were prepared by dispensing 50 µL of all tested compounds dissolved in DMSO and diluted at concentrations from 10 to 0.02 mg/mL (50 µL of pure DMSO in control). Then, 100 µL of freshly prepared methanolic DPPH solution at concentration of 6.58 × 10^-5 M was added. After incubation for 30 min at room temperature in the dark, the absorbance was measured at 517 nm. All measurements were performed in triplicate and the scavenging activity of the tested derivatives was calculated by the formula (Eq. (1)):

\[
\text{Scavenging activity (\%)} = 100 \cdot \left( \frac{A_{\text{control}} - (A_{\text{sample}} - A_0)}{A_{\text{control}}} \right)
\]
where $A_{\text{control}}$ and $A_{\text{sample}}$ refer to the absorbance of DPPH in control solution and sample, respectively, while $A_0$ refers to the absorbance of the compounds’ solutions, because of their existing color. IC$_{50}$ is defined as the concentration of antioxidant agent necessary to reduce the starting amount of DPPH by 50% and is calculated from the concentration-dependent free radical scavenging activity graph. Ascorbic acid was used as the reference compound.

3. Results and discussion

3.1. Synthesis of 1–3 and their characterization

General procedure of reported complexes included mixing previously dissolved solutions of metal precursor [RuCl$_2$(bpy)$_2$] and 3 equivalents of ligand (2-, 3-, and 4-acpy) in methanol (Scheme 1). The reaction mixture was refluxed overnight and left to cool to room temperature. After adding an equimolar amount of counterion to the solution and stirring for an additional 3 h, clear color-change from dark purple to dark red was observed. Products were isolated in a form of dark red microcrystalline powder, showing no traces of decomposition in air. Compounds 1–3 were soluble in some polar solvents (e.g. DMSO, CHCl$_3$, CH$_2$Cl$_2$, and CH$_3$CN) including water, but insoluble in non-polar.

3.1.1. IR spectroscopy

Initial assumptions on coordination modes were made on the basis of IR spectra (Supplementary Material, S1–S3) obtained for both ligands and complexes (Table 1). Typical C=O stretching vibration is located at 1690 and 1700 cm$^{-1}$ in the ligands’ spectra [32, 33]. Major dislocation of this bond to lower frequencies (app. 1600 cm$^{-1}$) is noticed for 1, suggesting bidentate bonding via acpy nitrogen and carbonyl oxygen. To the best of our knowledge, only a few studies reported this type of complexation to the metal center [16, 17]. On the other hand, carbonyl vibration for 2 and 3 is noticed in the same region (at app. 1600 to 1700 cm$^{-1}$) excluding the coordination via oxygen. Stretching pyridine vibrations (C=N and C=C) are recognized in the area of $\sim$1590 cm$^{-1}$ for free pyridines [16, 17]. The same band is located at $\sim$1580 cm$^{-1}$ for 1 and 2, and 1600 cm$^{-1}$ for 3. Shifting to lower frequencies clearly confirms coordination of the metal center through pyridine nitrogen. The most intense band in the IR spectra of the complexes at $\sim$830 cm$^{-1}$ originates from P–F vibration, essentially PF$_6^-$ and is absent in the spectra of metal precursor, [RuCl$_2$(bpy)$_2$]. A strong intensity band at
~556 cm\(^{-1}\) in the spectra of 1–3 and lacking for [RuCl\(_2\)(bpy)\(_2\)] can be assigned to Ru–N\(_{\text{acpy}}\) stretching vibration [34].

### 3.1.2. NMR spectroscopy

\(^1\)H NMR spectra of 1–3 (Supplementary Material, S4–S6) shared some common structural features, concretely signals in the aromatic area (9.85–7.28 ppm) originating from acpy and bpy protons and a typical singlet located at ~2.50 ppm attached to the protons of –CH\(_3\) group. The suggested bidentate coordination for 1 is reflected through major upfield shift of a proton in C6 position (8.32 ppm) in comparison to the ligand 2-acpy (8.72 ppm). Moreover, acpy protons in C4 and C3 positions suffered shifting of a multiplet toward higher frequencies (from region 8.01–7.93 ppm for ligand to 8.16–8.07 ppm for 1). The final spectral conformation for coordination via carbonyl oxygen is confirmed with significant –C=O shift in \(^{13}\)C NMR spectrum (Supplementary Material, S7) from 199.42 ppm for 2-acpy to 214.49 ppm for the corresponding complex 1. \(^1\)H NMR spectra of 2 and 3 display signals at relatively similar positions, but of a more complex multiplicity in comparison to the ligands 3- and 4-acpy. The same trend was observed in the \(^{13}\)C NMR spectra of 2 and 3 (Supplementary Material, S8 and S9). However, the minor change in the position of –C=O signal (e.g. from 197.35 ppm for 3-acpy to 195.86 ppm for 2) confirmed prior monodentate binding for 2 and 3. Chemical behavior of 1–3 in DMSO was investigated by monitoring \(^1\)H NMR spectra during 7 days (prior to dissolution and after 4 h, 8 h, 24 h, 48 h, and 72 h as well as after 7 days). The obtained spectra showed no traces of any structural changes after 7 days (representative spectrum of 1 is given in Supplementary Material, S10). In the MS spectra of 1–3 (Supplementary Material, S11–S13) recorded in acetonitrile, the [M–PF\(_6\)]\(^{+}\) signal was detected additionally validating the abovementioned coordination mode.

### 3.1.3. UV–vis spectrophotometry

The UV–vis spectra of 1–3 (Supplementary Material, S14) share two common absorptions in the UV region at ~290 nm and visible region, observed in the area from 400 to 600 nm as a broad, overlapped band. Based on the literature data, the sharp UV band can be assigned to ligand-centered π–π transitions followed by additional intraligand absorptions between 315 and 425 nm [35, 36]. On the other hand, the band located in the visible region may be attributed to MLCT transitions as previously reported for some Ru(II)-bpy complexes [36].

### 3.2. Electrochemical study

The electrochemical behavior of 1–3, in concentration of 1 mg/mL, was studied by cyclic voltammetry using GC electrode in 0.1 M of tetrabutylammonium perchlorate in DMSO as supporting electrolyte, at the constant scan rate of 50 mV s\(^{-1}\). Figure 2 illustrates cyclic voltammograms (CVs) recorded in a potential range –1.00 < Ep < 1.50 V followed by summarized data in Table 2.

Due to the different coordination manner (bidentate binding for 1 and monodentate for 2 and 3), the overall redox behavior of 1 differs in comparison to 2 and 3. The CV of 1 is distinguishable by four oxidative peaks, one noted in the area of negative electrode
potentials at \( /C_0 \) 0.68 V assigned to bpy oxidation. The following signals are located in the range of positive potentials, for example, one irreversible wave located at 0.50 V followed by two reversible and one irreversible peak(s). One of the reversible couples at 0.63/0.57 V is described as Ru(II)→Ru(III) electron transfer. The obtained data is in agreement with previous studies on similar Ru-picolinate complex ([Ru(bpy)2(pic)](PF6), where Hpic is 2-picolinic acid) with the same coordination manner, which revealed the same process at 0.60/0.55 V [37]. The reduction area is characterized by two well-resolved signals, one reversible redox couple at \(-/C_0 \) 0.79 V and one irreversible at \(-/C_0 \) 0.43 V. The redox couple \(-/C_0 \) 0.68/0.79 V can be assigned to the bpy reduction as abovementioned study reported similar redox behavior [31]. On the other hand, CVs of 2 and 3 share some common features such as two oxidation couples at 0.72 V and 1.11 V for 2 and 0.66 and 1.10 V for 3. Compared to the literature data where Ru is coordinated only via pyridine nitrogen, one oxidation couple at 0.70 for 2 and 0.66 for 3 can be assigned to Ru(II)→Ru(III) oxidation [31]. The ease of oxidation follows the trend 1 (0.50 V), 3 (0.66 V) and 2 (0.70 V). In the reductive scan, only one irreversible peak is spotted at \(-/C_0 \) 0.71 V for 2 and \(-/C_0 \) 0.48 V for 3, assigned to the reduction of the auxiliary bpy ligands. Furthermore, the appearance of one irreversible peak at 0.45 V for 2 and 0.47 V for 3 is noted and may be attributed to the second reduction of bpy unit.

### Table 2. Electrochemical data for 1–3.

| Complex | \( \text{Ea} \) | \( \text{Ec} \) | \( \text{Ea} \) | \( \text{Ec} \) | \( \text{Ea} \) | \( \text{Ec} \) | \( \text{Ea} \) | \( \text{Ec} \) | \( \text{Ea} \) | \( \text{Ec} \) |
|---------|---|---|---|---|---|---|---|---|---|---|
| 1       | -0.68 | -0.79 | -0.43 | 0.50 | -0.63 | 0.57 | 1.04 | 0.96 | 1.32 | -  |
| 2       | 0.70  | 0.69  | 1.11  | 0.87 | -0.45 | -0.70 | -  | -  | -  | -  |
| 3       | 0.66  | 0.59  | 1.10  | 0.82 | -0.46 | -0.48 | -  | -  | -  | -  |

Figure 2. CVs of 0.1 mM 1–3 solutions in DMSO in the \(-1.00 < \text{Ep} < 1.50 \text{ V}\) range of potentials at GC electrode; polarization rate 50 mV s\(^{-1}\); supporting electrolyte tetrabutylammonium perchlorate.

#### 3.3. Complexation study

Cyclic voltammetry is a convenient analytical method whose application among others involves exploring complexation reactions [38]. In this regard, solution of the starting
Ru complex [RuCl₂(bpy)₂] was titrated with the solution of ligand (2-, 3- or 4-acpy), resulting in CV for 1 in Figure 3 (CVs for 2 and 3 are given in Supplementary Material, S15 and S16). The initial concentration of [RuCl₂(bpy)₂] was 1 mM; two successive additions of 0.05 mM of each ligand were performed. In comparison to the CV curve of [RuCl₂(bpy)₂], the obtained voltammograms for all complexes clearly demonstrated a significant decrease of both oxidative and reductive peaks upon complexation [39]. Under oxidative conditions, the anodic peak of 1-3 in the area 0.3 < Ep < 0.85 V is shifted negatively in comparison to the signal of [RuCl₂(bpy)₂] (e.g. from 0.67 V for [RuCl₂(bpy)₂] to 0.53 V for 1). The reductive scan reveals similar trend, with a dominant irreversible cathodic peak of a more negative peak current for 1 and 2. Thus, the complexation reaction of the ligand with the metal center is identified by change in the peak current and peak position of acpy analogs in the presence of metal ion.

3.4. DNA binding studies

3.4.1. UV–vis spectrophotometry

The DNA-binding activity of newly synthesized Ru complexes 1–3 was evaluated using UV–vis spectra (Figure 4). Generally, binding of metal complexes with calf-thymus (CT) DNA results in hyperchromism and hypochromism where the former suggests the rupture of the secondary structure of DNA, while the latter is due to the interaction between an aromatic chromophore of the complexes and the base pairs of DNA. The degree of hypochromism is proportional to the intensity of intercalative interactions. The studies were performed by the addition of four different concentrations of the complexes (125, 250, 375, and 500 ppm) in the DNA solution at a concentration of 500 ppm. Spectra were recorded after 5 min of incubation period. In the case of all three tested ligands, during their successive addition in the solution containing DNA adsorption spectra of the DNA slightly shifted bathochromic (red-shift) followed with

Figure 3. CVs of 0.1 mM of metal precursor and 1 solutions in DMSO in the −1.00 < Ep < 1.00 V range of potentials at GC electrode; polarization rate 50 mV s⁻¹; supporting electrolyte tetrabutylammonium perchlorate.
hypochromic effect. Both phenomena clearly indicated strong interaction of the DNA with the complexes in solution. Furthermore, observed shifts indicate that the intercalative nature of binding in the system DNA-complexes is dominant, due to an obvious active stacking interaction between the aromatic chromophore and the base pairs of DNA. To further investigate the nature of the complex-DNA binding, cyclic voltammetry was employed.

3.4.2. Cyclic voltammetry

The method is considered as the most convenient analytical technique for evaluating kinetic, analytical, thermodynamic, and mechanistic profile of drug candidates [40]. Upon complex-DNA interaction, the voltammetric response is recognized as variations in peak potential/current and is used to interpret the mode of DNA binding activity, such as intercalative, groove binding, and electrostatic interactions. Overall, the peak potential shift towards more negative values correlates with electrostatic interaction while more positive changes indicate intercalative binding [41].

The binding modes were investigated by successive addition of the DNA (25 mg/mL) in the complex solutions at concentration of 1 mg/mL (Figure 5). As can be seen for all complexes, shifts of the oxidation peak potentials toward more positive values are an indication of the intercalative mode of interaction with DNA [42]. However, the position of the first cathodic peak tends to shift to more negative values. This can be interpreted as the negligible contribution of the electrostatic mode for DNA-complex (1–3) interactions. Moreover, the obtained results are in coherence with previously mentioned DNA binding results acquired by UV–vis technique.

The interaction studies were used to determine binding constants between tested complexes and dsDNA. Constants were calculated using a procedure suggested by Deepa et al. [43], based on the intercept value of the plot of log \((1/DNA)\) versus log \((I/I_0 - I)\) from the following equation:

$$\log \left(1/|DNA|\right) = \log K + \log \left(I/I_0 - I\right)$$  \hspace{1cm} (2)

where \(K\) is the binding constant, and \(I_0\) and \(I\) are peak currents before and after 5 min of complex/DNA incubation. Obtained values are summarized in Table 3. These results indicate that 1 and 3 have highest potential to bind to DNA and can be excellent

Figure 4. UV–vis spectra of (a) 1–3 in DMSO-water; (b) pure DNA and DNA after addition of 1 in different ratios in DMSO-water.
platforms for further synthetic steps, with the aim of finding the optimal ruthenium complex with the greatest potential for clinical use.

### 3.5. Antimicrobial study

The antimicrobial activity of parent Ru(II) complex, [RuCl₂(bpy)₂], ligands (2-, 3- and 4-acpy) and synthesized complexes 1–3 against a panel of selected microorganisms was determined by microdilution method. Results are given in Tables 4 and 5.

Based on the obtained results, it can be noticed that all acpy ligands have negligible antibacterial activity, while the activity of the parent Ru(II) complex, [RuCl₂(bpy)₂], is moderate (Table 4). However, 1–3 showed better activity against most of the tested bacteria, although they did not show selectivity towards a particular bacterial strain. Against bacterial cells *P. hauseri*, *S. aureus*, *B. subtilis* and *C. sporogenes*, 1 showed approximately ten times lower activity than control antibiotic erythromycin. Complex 3 showed the same behavior towards *P. aeruginosa*, *B. subtilis* and *C. sporogenes*, and 2 against *C. sporogenes*. For comparison, the minimum inhibitory concentration of [Ru(bpy)₂(Met)](PF₆)₂ against *S. aureus* and *E. coli* was 0.147 mM and 0.586 mM, respectively, while against *P. aeruginosa* complex did not possess antibacterial activity [44]. The MIC of [Ru(bpy)₂(clbzpy)(Cl)](PF₆) against *S. aureus* was 0.615 mM, and there is also no activity towards *P. aeruginosa* [45]. The newly synthesized Ru(II) bpy complexes with acpy analogs showed similar activity against *E. coli*, but they also showed activity against *P. aeruginosa*. Such encouraging results are good motivation for the design and synthesis of new ruthenium complexes in search of more active antibacterial agents.

In terms of antifungal activity, the synthesized complexes also showed generally better activity than both the parent Ru(II) complex and the ligands, which justifies their synthesis (Table 5). A slight selectivity of 1–3 towards *A. brasiliensis* and *S. cerevisiae* was observed but, in general, these compounds do not manifest a significant antifungal activity.
3.6. In vitro toxicity and antioxidant properties

Assessment of in vitro toxicity against freshly hatched nauplii of *Artemia salina* as well as radical scavenging capacity were evaluated. The obtained results are summarized in Table 6. Compared to potassium dichromate as a control, the toxicity of all tested compounds was significantly lower. Low toxicity, together with promising antibacterial activity, classifies ruthenium complexes as compounds with good bactericidal potential.

Based on the results for radical scavenging capacity, it can be concluded that all tested compounds did not possess antioxidant effect. This result can be explained by the electrochemical properties of the examined complexes. Namely, according to electrochemical properties of the Ru-complexes, where the oxidation of Ru(II)→Ru(III) occurs at a relatively high potential (+0.63 V), the high value of the electrode potential indicates that Ru(II) is a weak reducing agent, unable to reduce DPPH radical.
4. Conclusion

Three ruthenium(II)-bipyridine complexes (1–3) with acpy type of ligand (2-, 3-, and 4-acetylpyridine) were synthesized and characterized by common instrumental techniques. Their electrochemical behavior was studied by cyclic voltammetry, revealing interesting oxidation/reduction pathway. All compounds share a common feature in terms of their oxidation, demonstrating reversible oxidation peak assigned to Ru(II)→Ru(III) electron transfer, located at ΔEp = 0.65 V. On the basis of IR, NMR, MS, and UV–vis spectra, the bidentate bonding via acpy nitrogen and carbonyl oxygen is proposed for 1 with 2-acpy, while 2 and 3 with 3- and 4-acpy moiety demonstrated monodentate coordination mode. 1 is more active in terms of the oxidation as it showed four waves in comparison to two anodic peaks of 2 and 3. On the other hand, the reductive scan of 2 and 3 showed rich redox chemistry demonstrated through four cathodic waves in contrast to two waves noticed for 1. Cyclic voltammetry was additionally used for investigating complexation reactions, clearly confirming metal-ligand bonding due to the major decrease of both oxidative and reductive peaks. The complexes were further screened for DNA binding interactions by CV and UV–vis, both indicating a predominant electrostatic intercalation. Antimicrobial study of novel compounds characterized them as agents of moderate activity followed by low toxicity.

Funding

This work has been supported by Ministry of Education, Science and Technological Development of Republic of Serbia, contract numbers: 451-03-68/2022-14/200288, 451-03-68/2022-14/200168 and 451-03-68/2022-14/200026.

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