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A Dinuclear Ruthenium(II) Schiff Base Complex with Dissimilar Coordination: Synthesis, Characterization, and Biological Activity

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Abstract. A dinuclear Schiff base Ru^{II} complex derived from 5-chlorosalicylaldehyde and 2-aminopyridine was synthesized. The structure of the compound was analyzed by mass spectrometry as well as IR, UV/Vis, and ^1H NMR spectroscopy, along with chemical analysis, as well as magnetic, cyclovoltammetric and conductivity measurements. Two Ru^{II} atoms are octahedrally coordinated by azomethine and pyridine nitrogen atoms from two tridentate monobasic Schiff bases and bridging phenol oxygen atoms. The formula of the complex is $[Ru_2L_2Cl_2(Et_2NH)(H_2O)]$ [L = *N*-(2-pyridyl)-5-chlorosalicylideneimine and Et_2NH = isodiethylamine]. The Ru^{II} atoms in the dinuclear neutral complex species have different coordination environments, RuN_3O_2Cl and RuN_2O_3Cl. Interaction with CT DNA showed moderate hydrophobic binding. The compound demonstrates strong activity against methicillin-resistant *Staphylococcus aureus*, methicillin-sensitive *Staphylococcus aureus*, and especially *Enterococcus faecalis*. Microbiological tests showed significant inhibition of growth and ability to kill pathogens, similar or even improved compared to reference antibiotics vancomycin.

Introduction

The design, synthesis, and applications of ruthenium complexes have attracted special attention over recent decades for many reasons. From the synthetic point of view ruthenium is intriguing due to its wide range of oxidation states and corresponding redox properties, which can be adjusted by various ligands. In typical oxidation states, +2 and +3, ruthenium as soft and borderline-soft acid prefer nitrogen donor ligands. Many ruthenium complexes are widely studied due to their significant catalytic properties.[1,2] One of the main avenues of research have focused on the anticancer activity of ruthenium complexes, which exhibit improved selectivity and reduced side effects in comparison to platinum, present in clinical practice.[3,4] The development of new drugs is a permanent and growing global challenge thus integrating different fields of science. It is important to have in mind that for few decades not any substantially new antibiotic has been found.

As a part of the efforts to synthesize ruthenium complexes with Schiff bases with potential biological activity, the synthesis, characterization, and antimicrobial activity of the dinuclear Ru^{II} complex, $[Ru_2L_2Cl_2(Et_2NH)(H_2O)]$ [L = *N*-(2-pyridyl)-5-chlorosalicylideneimine] is reported herein. To the best of our knowledge, this is the first dinuclear Ru^{II} complex with bridging Schiff bases through phenol oxygen.

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Experimental Section

Materials: Most chemicals were obtained from commercial sources and used without further purification. Tetraethylammonium perchlorate was prepared from bromide salt as described previously.[5] Fibrous *calf thymus* DNA (A_{260}/A_{280} = 1.3) was purified with phenol-chloroform-isoamyl alcohol extraction up to A_{260}/A_{280} = 1.8 ratio. DNA was precipitated as sodium salt with ethanol in 0.1 M acetate buffer (pH 4.60). Solid nucleic acid was suspended in 0.1 M Tris-HCl buffer, pH 7.40, and left one day for hydration at 4 °C before measurements. Gram-positive and Gram-negative bacteria were collected from the Microbiology Laboratory of the Institute of Public Health of Canton Sarajevo.

Physical Measurements: Elemental analysis was performed with a Perkin-Elmer 2400 Series CHNS/O Analyzer. Ruthenium content was determined by graphite furnace atomic absorption spectrometry with a Varian 240ZAA from DMSO solution.[6] Chlorine was determined by mercurimetric method. Mass spectrum was recorded with a matrix-assisted laser desorption / ionization-time-of-flight MALDI-TOF/TOF mass spectrometer (4800 Plus MALDI TOF / TOF Analyzer, Applied Biosystems Inc., Foster City, CA, USA) equipped with Nd : YAG laser, wavelength of 355 nm, with firing rate 200 Hz in positive ion reflector mode. 1600 shots per spectrum were acquired in *m*/*z* range 10–1000 Da with delay time 300 ns. Thiamine mononitrate and azithromycin were used as internal calibrants. Proton NMR spectrum was collected with a 300 MHz Bruker BioSpin GmbH instrument in –1.0 to 19 ppm range with 256 scans using $[D_6]$aceton as solvent and TMS as internal standard. Spectrum was analyzed using SpinWorks 3.1.8.1. Infrared spectra were collected with a Perkin-Elmer BX FT-IR spectrophotometer as KBr pallets in the region 4000–400 cm^{-1}. Electronic spectra were recorded with a Perkin-Elmer UV/Vis lambda 35 spectrophotometer in CH_2Cl_2 solution in the range 200–700 nm. Magnetic measurements were performed with a SQUID magnetometer MPMS-XL5 (Quantum Design) at 300 K. Cyclic voltammograms were recorded with a Autolab potentiostat / galvanostat (PGSTAT 12) electrochemical workstation using three electrode system: glassy carbon

electrode as working, Ag/AgCl as reference electrode and Pt wire as counter electrode. Recordings were performed with scan rate 0.1 V·s^{-1} and step potential 0.025 V in the range of potentials –1.0 to 0.0 V for dimethylformamide/sodium perchlorate solutions and –1.1 do –0.5 V for acetonitrile /tetraethylammonium perchlorate. The conductivity of 10^{-3} M solution of complex was measured in dimethylformamide solution on Conductivity meter Phywe.

Synthesis of Schiff Base: Schiff base, *N*-(2-pyridyl)-5-chlorosalicylideneimine, was prepared according to published procedure.[7] Warm solution of 2-aminopyridine (5 mmol, 471 mg) in absolute ethanol (10 mL) was added to solution of 5-chlorosalicylaldehyde (5 mmol, 780 mg) in absolute ethanol (10 mL). The mixture was kept at 70 °C for 2 h. The resulting solution was slowly evaporated overnight, at ambient temperature. Orange crystals were collected with yield of 60%.

Synthesis of Complex: Warm solution containing $RuCl_3 \cdot 3H_2O$ (0.5 mmol, 130 mg) in absolute ethanol (5 mL) was added to absolute ethanol solution containing Schiff base (1 mmol, 232 mg) and triethylamine (1 mmol, 0.14 mL). The reaction mixture was heated at 70 °C for 4 h changing color from brown to dark green. The volume was reduced by distillation under reduced pressure. The solution was cooled in ice-salt bath. The solid was collected, washed with small portions of cold water, ethanol and ether and dried at 60 °C. Recrystallization was performed from ethanol : dichloromethane, 1/1 v/v. Dark green solid is soluble in dimethylformamide (DMF), dimethylsulfoxide (DMSO), acetonitrile (MeCN), moderately soluble in methanol and ethanol and insoluble in water and ether.

Aqua-1κO-dichlorido-1κCl,2κCl-diethylamine-2κN-bis[*N*-(2-pyridyl-κN)-5-chloro-2-(μ-oxy-1:2$\kappa^2 O$)-benzylidenimine-κN(1-)]diruthenium(II,II): Dark green powder. Yield: 60%. $C_{28}H_{31}Cl_4N_5O_4Ru_2$: calcd. C 39.77; H 3.70; N 8.29; Cl 16.77; Ru 23.91%; found: C 39.59; H 3.00; N 8.10; Cl 17.04; Ru 23.59%. **MALDI-TOF MS**: *m/z*: 560.9712 ($[C_{24}H_{16}Cl_2N_4O_2Ru]^+$), 737.8099 ($[C_{24}H_{16}Cl_4N_4O_2Ru_2]^+$). **IR** (KBr): $\tilde{\nu}_{max}$ = 1602 vs ν_{sym}(C=N), 1289 m ν_{sym}(C–O), 1013 m δ_{bend}(C_2N) cm^{-1}. **UV/Vis** [CH_2Cl_2]: λ_{MLCT}/nm (log[ε /M^{-1} cm^{-1}]): 435 (3.97). **^{1}H NMR** (300 MHz, [D_6]acetone): δ = 10.05 [s, 2 *H*(4)], 7.81 [d, 2 *H*(3), ^4J = 2.61 Hz], 7.53–7.62 [m, 12 *H*: 2 *H*(2), 2 *H*(1), 2 *H*(5), 2 *H*(6), 2 *H*(7), 2 *H*(8)], 5.61 [s, 1 *H*(9)], 3.45 [q, 4 *H*(10), ^3J = 6.44 Hz], 2.82 [s, *H*(12)], 1.11 [t, 6 *H*(11), ^3J = 6.42 Hz] ppm.

Biological Studies: Spectroscopic titration of complex compound with CT DNA was performed by successive addition of 10 μL-portions of DNA into 2 mL of complex compound solution. Each addition of DNA was compensated in the blank. Titration was carried out in 0.1 M Tris-HCl buffer, pH 7.42 in the presence of 150 mM NaCl at 295 K. Antibacterial in vitro activity of Ru complex was tested against methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-sensitive *Staphylococcus aureus* (MSSA), *Enterococcus faecalis, Klebsiella pneumoniae* (wild type), *Klebsiella pneumoniae* (ESBL type), and *Pseudomonas aeruginosa*. Turbidity of bacterial suspensions was set before testing to be equivalent to 0.5 McFarland. Activity was tested by disc-agar diffusion method. Pathogens were inoculated on Mueller-Hinton agar and the holes were made by sterile Durham's tubes. Volume of 50 μL of Ru complex in DMSO solution containing 1.5 mg·mL^{-1} was inserted into drilled wells. Diameter (mm) zone of inhibitions of bacterial growth were measured after 24 h of incubation at 37 °C. Vancomycin and gentamicin were used as antibiotic controls. For extended antimicrobial study minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values were determined. Decreasing concentrations of the Ru^{II} compound were prepared in serial two fold dilution from stock solution of complex in dimethylsulfoxide (1.5 mg·mL^{-1}).

Results and Discussion

Characterization of Complex Compound

The ruthenium complex, hereinafter $[Ru_2L_2Cl_2(Et_2NH)(H_2O)]\cdot H_2O$, where L represents Schiff bases derived from 5-chlorosalicyladehyde and 2-aminopyridine (Et_2NH = diethylamine) was prepared according to Figure 1.

Deprotonation of the phenolic oxygen atom from Schiff base was carried out using triethylamine. On the basis of chemical analysis, different spectroscopic techniques, and magnetic measurements, a dinuclear Ru^{II} structure with bridging Schiff bases is proposed. In the dinuclear Ru^{II} complex species, each ruthenium atom in octahedral environment is coordinated by chloride and tridentate monobasic O,N,N Schiff base, whereas the sixth positions are occupied by different ligand, water, or diethylamine. Reduction of starting Ru^{III} might be the result of oxidative decomposition of triethylamine to acetaldehyde and methylamine, similar to decomposition with hexacyanoferrate(III).[8]

Mass and ^{1}H NMR Spectra

The mass spectrum of the complex showed isotopic distribution characteristic for ruthenium species at *m/z* value 737.8099 confirming molecular formulation of $[C_{24}H_{16}Cl_4N_4O_2Ru_2]$. ^{1}H NMR spectra prove binding of Schiff base as anionic tridentate ligand. The absence of a singlet in high frequencies region (10–12 ppm), which typically corresponds to phenolic hydrogen, indicates that *N*-2-pyridyl-5-chlorosalicylideneimine binds ruthenium by deprotonated phenolic oxygen. The singlet that appears at δ = 10.05 ppm is ascribed to azomethine hydrogen, which is less shielded after coordination through azomethine nitrogen. Typical doublet located at δ = 7.81 ppm

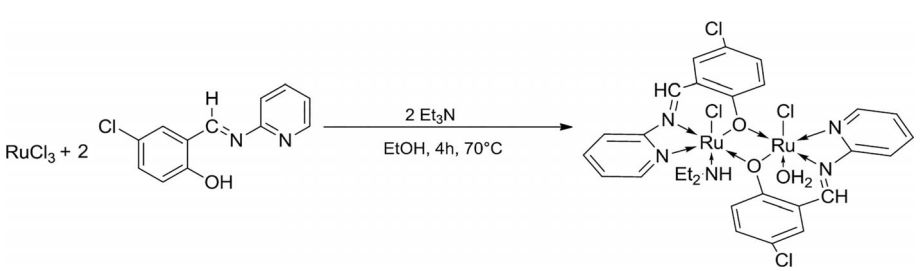

Figure 1. Synthesis of $[Ru_2L_2Cl_2(Et_2NH)(H_2O)]$.

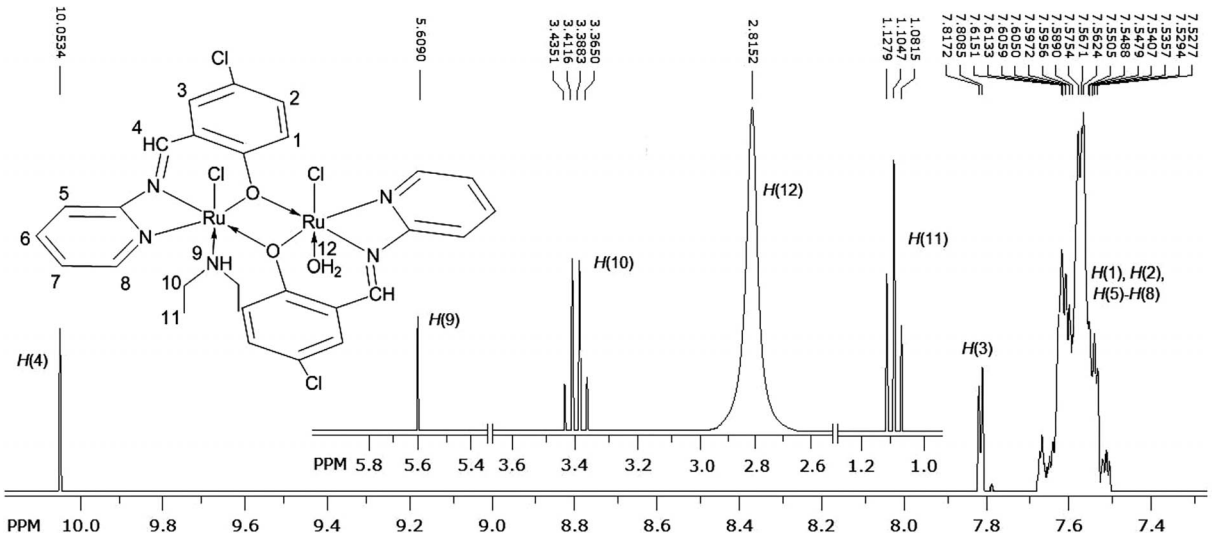

Figure 2. ^{1}H NMR spectrum of $[Ru_2L_2Cl_2(Et_2NH)(H_2O)]$ in $(CD_3)_2CO$. Inset: Proposed structure and denotation of different types of hydrogen atoms.

is ascribed to hydrogen atoms denoted as H(3) in Figure 2. The *meta* coupling constant $^4J_{H(3)H(2)}$, 2.61 Hz, confirms coupling of H(3) with H(2) atoms. The multiplet found in the spectrum of the complex in the 7.53–7.62 ppm region corresponds to coupled hydrogen atoms of aromatic rings. A singlet at δ = 5.61 ppm was found and attributed to amine hydrogen H(9) confirming coordination of diethylamine to Ru^{II}. In free diethylamine this singlet is located at lower values, around 2 ppm. A shift to higher frequencies for 3.5 ppm is a result of deshielding of amine hydrogen atoms after coordination of diethylamine to Ru^{II} atom by nitrogen. A quartet of methylene hydrogen is located at δ = 3.45 ppm in the complex compared to 2.60 ppm in the free ligand. A methyl hydrogen triplet at δ = 1.11 ppm is not affected by coordination due its distance from nitrogen atom. ^{1}H NMR spectroscopic data are in good agreement with other experimental results and strongly support the proposed structure of the complex. The ^{1}H NMR spectrum of $[Ru_2L_2Cl_2(Et_2NH)(H_2O)]\cdot H_2O$ is presented in Figure 2.

Infrared and Electronic Spectra

The infrared spectrum of $[Ru_2L_2Cl_2(Et_2NH)(H_2O)]\cdot H_2O$ confirms coordination of Ru^{II} by tridentate anionic ON_2 chelating Schiff base, diethylamine, and water. The azomethine group appears in the complex at 1602 cm^{-1} compared to 1611 cm^{-1} in free *N*-(2-pyridyl)-5-chlorosalicylideneimine. Stretching vibration of C–O(H) bond, found in free Schiff base at 1278 cm^{-1}, was moved to 1289 cm^{-1} in complex species as a result of deprotonation and coordination to ruthenium. Schiff base coordination through pyridine nitrogen affected bending deformation in plane, therefore the frequency in free ligands at 452 cm^{-1} was moved to a higher value and appears at 469 cm^{-1} in the spectrum of the ruthenium complex. A new band in the spectrum of complex compound appears at 824 cm^{-1}, which is ascribed to Ru–O–Ru stretching, and confirms O-bridging character of *N*-(2-pyridyl)-5-chlorosalicylideneimine.[9]

The presence of diethylamine in the coordination sphere, attached to one of the two Ru^{II} atoms, was proven by C_2N frequency at 1013 cm^{-1} which occurs at 1037 cm^{-1} in free diethylamine. Well-resolved absorption in the complex around 3200 cm^{-1} is attributed to N–H stretching, whereas this frequency occurs at 3288 cm^{-1} in free diethylamine. Triethylamine, which was used in synthesis, has no absorption in the region of N–H stretching. Strong and broad absorption around 3435 cm^{-1} was attributed to water O–H stretching frequencies (Table 1)

In the electronic spectrum of $[Ru_2L_2Cl_2(Et_2NH)(H_2O)]\cdot H_2O$ three ligand-centered transitions of coordinated Schiff base appear in the region 236–351 nm (bands I–III). These bands are attributed to spin allowed $\pi\rightarrow\pi^*$ and $n\rightarrow\pi^*$ (charge transfer in whole ligand molecule) transitions. Bands II and III are shifted toward higher energy after coordination therefore confirming

Table 1. IR frequencies and electronic spectrum transitions for $[Ru_2L_2Cl_2(Et_2NH)(H_2O)]$ and the ligand.

IR spectrum	ν_{sym}(N–H) Complex (free ligand) /cm^{-1}	ν_{sym}(C=N)	ν_{sym}(C–O)	δ_{bening}(C_2N)	$\delta_{bending}$(Ru_2O)	$\delta_{in\ plane}$(py)
	3200 (3180)	1602 (1611)	1289 (1278)	1013 (1037)	824 (–)	469 (452)
Electronic spectrum	Band I λ /nm (log ε)	Band II	Band III	MLCT	$t_{2g}^6\rightarrow\pi^*$	
	236 (4.44)	293 (4.20)	351 (3.82)	438 (3.97)	696 (0.33)	

binding of ligand through O,N,N-donor atoms. Compared to free ligand, two additional bands were found in spectrum of complex. The absorption at 438 nm is attributed to MLCT (metal ligand charge transfer), based on the position and extinction coefficient. A very poor but well defined absorption band in the spectrum of complex at 696 nm is assigned to spin allowed $^1A_{1g} \rightarrow {}^1T_{1g}$ ($t_{2g}{}^6 \rightarrow \pi^*$) transition of Ru^{II} in strong ligand field. The electronic spectrum of $[Ru_2L_2Cl_2(Et_2NH)(H_2O)]\cdot H_2O$ is shown on Figure 3.

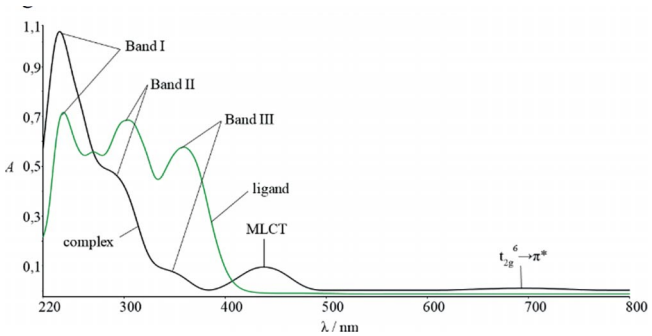

Figure 3. Electronic spectrum of $[Ru_2L_2Cl_2(Et_2NH)(H_2O)]\cdot H_2O$ (4×10^{-5} M) and L = *N*-(2-pyridyl)-5-chlorosalicylideneimine (2×10^{-5} M) in CH_2Cl_2.

Magnetic Susceptibility, Electrochemical Properties, and Conductivity

The magnetic susceptibility provides an insight into the important characteristics of complex compounds, especially electronic state of metal atoms. Also, susceptibility can suggest the degree of metal-metal interactions in polynuclear complex species. Based on starting $RuCl_3$ used in the synthesis, a positive value of magnetic susceptibility arising from $t_{2g}{}^5$ ground electronic state for Ru^{III} is expected. In contrast, experimental magnetic susceptibility (χ_ρ) at 300 K for the ruthenium complex has a negative value -1.79×10^{-6} cm^3 g^{-1} strongly supporting the presence of diamagnetic $t_{2g}{}^6$ Ru^{II} metal centers.

The conductivity of 1 mM solution of $[Ru_2L_2Cl_2(Et_2NH)(H_2O)]\cdot H_2O$ in dimethylformamide is 20.4 μS cm^{-1}, compared to 223 μS cm^{-1} for sodium perchlorate as reference electrolyte, thus confirming non-electrolytic nature of neutral complex species.

Different ligands in coordination sphere can dramatically change redox properties of central metal atoms, which are crucial in numerous applicative fields like catalysis and especially in design of metal-based drugs. Authentic examples are $[Ru(NH_3)_5N_2]^{3+}$ and $[Ru(NH_3)_5OH]^{2+}$ having redox potentials +1.10 V and –0.08 V, respectively.[10] Cyclic voltammograms of $[Ru_2L_2Cl_2(Et_2NH)(H_2O)]\cdot H_2O$ were recorded in dimethylformamide and acetonitrile (Figure 4).

Cathodic and anodic peaks are well defined in acetonitrile due to poor solvation properties. Voltammograms show quasi-reversible one-electron Ru^{III}/Ru^{II} process based on position, ratio of cathodic and anodic peaks, and peak-to-peak separations. Electron transfer is more reversible in acetonitrile with ΔE = 0.200 V compared to significantly higher peak-to-peak separation ΔE = 0.526 V in dimethylformamide (Table 2).

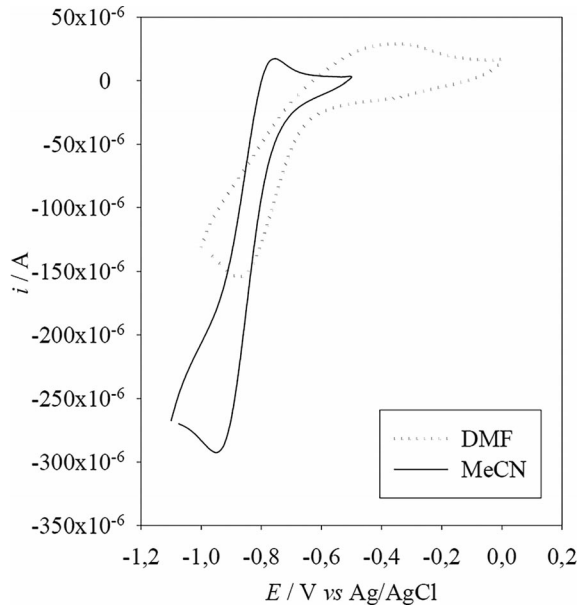

Figure 4. Cyclic voltammograms of $[Ru_2L_2Cl_2(Et_2NH)(H_2O)]\cdot H_2O$ in DMF/$NaClO_4$ and MeCN/Et_4NClO_4.

Table 2. Cyclic voltammetry for $[Ru_2L_2Cl_2(Et_2NH)(H_2O)]\cdot H_2O$.

Solvent	E_c /V	E_a /V	ΔE /V	$E_{1/2}$ /V
DMF	–0.875	–0.349	0.526	–0.612
MeCN	–0.950	–0.750	0.200	–0.850

Quite negative values of half-wave potentials $E_{1/2}$ = –0.612 V in dimethylformamide and –0.850 V in acetonitrile are ascribed to Ru^{III}/Ru^{II} redox couple. The presence of oxygen atoms in Ru^{II} complexes moves the redox potential toward more negative values as a result of modest affinity of Ru^{II} toward oxygen atoms. In the case of mononuclear Ru^{III} complex species with similar coordination core, e.g. $RuCl_2N_2O_2$ and RuN_2O_4, corresponding half wave-potentials have more negative values.[11,12] The presence of O-bridging atoms in $[Ru_2L_2Cl_2(Et_2NH)(H_2O)]\cdot H_2O$ stabilizes the complex toward oxidation compared to corresponding mononuclear species.

Interaction with CT DNA

DNA is almost inevitably anticipated as a main target for metal-base drugs, along with enzymes and signaling pathways. Therefore, the study of metal complexes-DNA interactions might be the first indicator of biological activity of potential drug. Metal complexes are able to bind DNA by covalent mode, or non-covalent intercalative and external groove binding mode.[13] Spectroscopic study of interaction of $[Ru_2L_2Cl_2(Et_2NH)(H_2O)]\cdot H_2O$ with CT DNA was performed by titration of fixed concentration of complex compound with increasing concentrations of *calf thymus* DNA (Table 3). The values of the binding constant, K_b were calculated on the basis of the equation:[14]

Table 3. Spectroscopic titration data of complex with CT DNA.

V_{DNA} /μL	[Complex] /10^{-5} M	[DNA]/[complex]	[DNA]/(ε_f–ε_a) /10^{-8} M^2 cm
0	6.03	0.00	–
10	6.00	0.51	2.68
20	5.97	1.01	5.59
30	5.94	1.50	6.75
40	5.91	2.00	9.53
50	5.88	2.48	12.3
60	5.85	2.97	14.6

$$\frac{[DNA]}{(\varepsilon_f - \varepsilon_a)} = \frac{[DNA]}{(\varepsilon_f - \varepsilon_b)} + \frac{1}{K_b(\varepsilon_f - \varepsilon_b)}$$

where ε_a, ε_f, and ε_b correspond to extinction coefficients for particular measurements (A_{obs}/[DNA]), free complex and completely bound form, respectively. The result of titrations in the CT band region showed hypochromic effect suggesting non-covalent binding to DNA with moderate binding constants $K_b = 3.82 \times 10^4$ M^{-1} (Figure 5). The binding constant was obtained as the ratio of the slope and intercept from graphical dependence [DNA] / ($\varepsilon_f - \varepsilon_a$) vs. [DNA] (Figure 6.) No shift of absorption maximum was observed; therefore the interaction is attributed to groove binding.

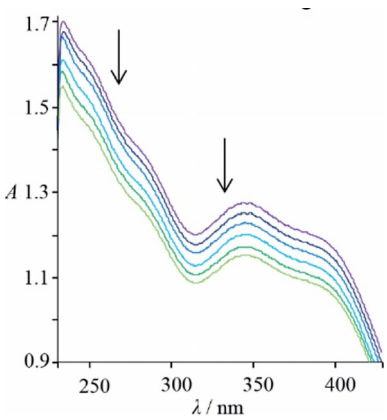

Figure 5. Spectroscopic titration of $[Ru_2L_2Cl_2(Et_2NH)(H_2O)]$ (6.03×10^{-5} M) with CT DNA (4.90×10^{-3} M, A_{260}/A_{280} = 1.81) in 0.1 M Tris-HCl buffer, pH 7.42 and 150 mM NaCl; T = 295 K; t = 5 min.

Antimicrobial Activity

Many metal complexes have been studied in the light of their antimicrobial activity over last decades especially since pathogens are permanently developing resistance on existing drugs. Despite the remarkable increase in understanding of the molecular mechanisms of antimicrobial resistance, an increase of antimicrobial-resistant bacterial species is evident. The special concern is ability of *Enterococci* and *Staphylococci* to adapt on different environments. Reports on *S. aureus*, having full resistance to vancomycin (VRSA), have urgently opened a new era in the development of bactericidal antibiotics. $[Ru_2L_2Cl_2(Et_2NH)(H_2O)]$ was tested against methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-sensitive

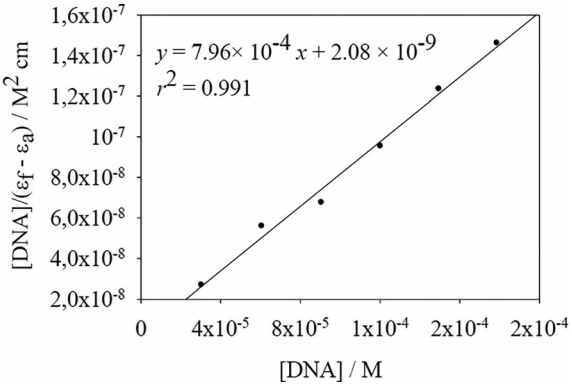

Figure 6. Graphical determination of K_b; regression is based on data from Table 3.

Staphylococcus aureus (MSSA), *Enterococcus faecalis, Klebsiella pneumoniae* (wild type), *Klebsiella pneumoniae* (ESBL type), and *Pseudomonas aeruginosa*.

However the complex showed activity only against Gram-positive bacteria. Zone of inhibition for MRSA and MSSA strains of *Staphylococcus aureus* (24 and 20 mm, respectively) are close to vancomycin as a reference antibiotic, while activity against *Enterococcus faecalis* exceeds vancomycin zone inhibition. For further evaluation of antimicrobial activity, MIC and MBC values were determined. MRSA and MSSA were very sensitive to the tested complex with low MIC and MBC values (1.46 and 2.92 μg·mL^{-1}, respectively). It is interesting that $[Ru_2L_2Cl_2(Et_2NH)(H_2O)]\cdot H_2O$ has the same MIC value as vancomycin (0.73 μg·mL^{-1}) in the case of *Enterococcus faecalis*, whereas the MBC for tested ruthenium drug (0.36 μg·mL^{-1}) demonstrate much stronger bactericidal activity compared to vancomycin (0.73 μg·mL^{-1}).

The lack of the activity against Gram-negative bacteria suggests that the activity of this complex is probably based on inhibition of cell wall synthesis of tested pathogens. The difference in structure and composition of cell wall for Gram-positive bacteria, having thick murine layer of peptidoglycan, and Gram-negative bacteria with thin peptidoglycan layer covered by outer lipid membrane, seems to be responsible for different activity of the tested Ru complex. In Gram-positive bacteria the murine layers of peptidoglycans can hinder the passage of hydrophobic compounds due to sugars and amino acids, or can be chemically changed by drugs resulting in inhibition of cell wall synthesis. Since tested Ru complex with Schiff bases contain (i) two chlorides, which are able to hydrolyze and release two positions on ruthenium for further binding especially O- and N-donor group, and (ii) moderate hydrophobic aromatic part of Schiff bases, it is reasonable to assume that chemical changes of unprotected peptidoglycan layer for *S. aureus* and *E. faecalis* occur. The inhibitory effect of tested complex was found to be bactericidal, meaning that agent caused the death of pathogens (MBC/MIC ratio = 1–2).[15] Results based on disc diffusion method for studied complex and reference antibiotic vancomycin against Gram-positive bacterial strains, hospital acquired methicillin-resistant *Staphylococcus aureus* (HA-MRSA), methicillin-sensitive *Staphylococcus aureus*

Table 4. Antibacterial activity of complexes against MSSA, MRSA, and *Enterococcus faecalis*.

Compound	Diameter of zone of inhibition /mm			MIC μg mL^{-1} / MBC μg mL^{-1}		
	MSSA	MRSA	*Enterococcus faecalis*	MSSA	MRSA	*Enterococcus faecalis*
Complex	20	24	29	2.92 / 2.92	1.46 / 1.46	0.73 / 0.36
Vancomycin	28	28	28	0.73 / 0.73	0.730.73	0.73 / 0.73

(MSSA), and *Enterococcus faecalis* ATCC 29212 are given in Table 4.

Conclusions

A dinuclear Ru^{II} complex with *N*-(2-pyridyl)-5-chlorosalicylideneimine was synthesized. The starting Ru^{III} is reduced with triethylamine, similar to oxidative decomposition of triethylamine to diethylamine with hexacyanoferrate(III). Two ruthenium atoms are octahedrally coordinated by a different sixth ligand. The complex shows moderate hydrophobic binding to CT DNA and very strong antimicrobial activity against *Staphylococcus aureus* and *Enterococcus faecalis* with very low values of minimum inhibitory and minimum bactericidal concentration, less than 3 mg·L^{-1}. A significant result is the improved bactericidal activity of the tested complex against *Enterococcus faecalis* compared to vancomycin, a reference drug. Quite low MIC and MBC values mark this complex as a superior candidate as potential metal-based drug for advanced study.

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