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Evidence on Antimicrobial Activity of Sodium Dichlorobis[*N*-phenyl-5-chlorosalicylideneiminato-*N,O*]ruthenate(III) against Gram-positive Bacteria

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ABSTRACT

Antimicrobial activity of a Ruthenium (III) complex with *N*-phenyl-5-chlorosalicylideneimine ligands have been studied against Gram-positive and Gram-negative pathogenic bacterial strains by using disc diffusion method. The compound showed significant *in vitro* antimicrobial activities against community acquired methicillin-resistant *Staphylococcus aureus*, hospital acquired methicillin-resistant *Staphylococcus aureus*, methicillin-sensitive *Staphylococcus aureus*, *Bacillus cereus* ATCC 11778, *Bacillus subtilis* ATCC 6633 and *Enterococcus faecalis* ATCC 29212. The minimal inhibitory concentration values of the compound were found to be in the range of 11.72–23.44 µg/mL. Additionally, minimal bactericidal concentration values and considerable sensitivity pathogenic Gram-positive microorganisms on Ru(III) compound against led to conclude the complex have prospective antibiotic properties. The compound has no activity *in vitro* against Gram-negative bacteria *Klebsiella pneumonia*, *Enterobacter* species, *Pseudomonas aeruginosa*, *Escherichia coli* ATCC 25922 and *Salmonella typhimurium* ATCC 14028.

Keywords: Antimicrobial activity, *Bacillus subtilis*, *Enterococcus faecalis*, MRSA, Ruthenium, Schiff bases.

INTRODUCTION

Metal complexes are of great importance in the treatment of many diseases of which cancer and various infections are in the focus of interest. It is intriguing that a systematic study of metal complexes as anticancer agents began with Rosenberg's examination on platinum-induced filamentous growth in *Escherichia coli* since cisplatin has become the most widely used non-organic drug in the treatment of many solid cancers [1]. Only few platinum-based compounds such as carboplatin and oxaliplatin are in clinical use, although more than 2000 platinum compounds as potential drugs never reached advanced clinical trials [2,3]. In recent decades, ruthenium complexes are particularly investigated due to their reactivity against primary cancer and metastatic lesions as potential drug candidates [4-7]. Along with anticancer drugs, the development of new antibiotics is a permanent challenge, mainly since pathogens quickly and continually are developing resistance on the drugs. The capability to react with DNA might be, at least, one common property for anticancer and antimicrobial agents. That is a reason why the antimicrobial activity of many anticancer agents is investigated. Many organic molecules exhibit activity against bacteria, while their metal complexes are known to demonstrate improved effect. *N*-substituted sulphonamides which are considered to be the most widely used antimicrobial agent with excellent activities are illustrative example; the copper complexes with sulphonamide are more reactive towards bacteria as a result of the higher liposolubility compared to free ligand [8]. Schiff bases are organic molecules derived from amines and carbonyl compounds which contain azomethine group. Considerable interest in these ligands and their metal complexes is a result of high stereo-chemical flexibility and abilities to form thermodynamically stable metal complexes. From kinetic point of view, these complexes are generally stable toward hydrolysis which is also one of the requirements for potential drugs. Many Schiff bases,

present in different natural, semi-synthetic and synthetic compounds, demonstrate their biological activity such as compounds derived from salicylaldehyde and different amines. Chlorosalicylideneimines are reported to demonstrate activity against several strains including *E. coli* and *S. Aureus* [9,10]. The nature of the substituents on salicylaldehyde ring shows the impact on the antimicrobial activity of the Schiff base [11]. As a result of the biological activity of these molecules the antimicrobial properties of their metal complexes are subject of growing interest. These metal complexes generally exhibit significant activity against Gram-positive bacteria, especially *S. aureus*. Co(II) and Zn(II) complexes with salicylideneimine ligands exhibit considerable inhibition of bacterial growth although for relatively high concentrations of metal compound in the range of 0.2-3 mg/mL [12,13]. Ru(III) compounds with tetradentate Schiff bases derived from salicylaldehyde showed two times less activity than the reference drug streptomycin at relatively high concentrations of Ru(III) of 20 mg/mL [14]. The role of metals in the antimicrobial activity of their complexes have still remained unclear, however complexes of those metals which demonstrate significant activity towards DNA also show significant antimicrobial activity.

As a part of our research of Ru(III) compounds that might be potential candidates for anticancer and antimicrobial drugs we started testing of *in vitro* activity of a Ru(III) complex containing *N*-phenyl-5-chlorosalicylideneimine ligands against strains of Gram-positive and Gram-negative bacteria and herein we briefly report on the antimicrobial activity.

MATERIALS AND METHODS

Synthesis of Ru(III) complex

Sodium dichloro-bis[*N*-phenyl-5-chlorosalicylideneiminato-*N,O*]ruthenate(III), hereinafter Na[RuCl₂(N-Ph-5-Cl-salim)₂] was synthesized from RuCl₃ and freshly prepared *N*-phenyl-5-chlorosalicylideneimine according to the procedure previously reported [15]. Purity was checked by CHN elemental analysis and IR spectroscopy.

Bacterial Cultures

The pathogens were collected from the Microbiology Laboratory of the Institute of Public Health of Canton Sarajevo. Antimicrobial activity of Ru(III) complex was tested against community acquired methicillin-resistant *Staphylococcus aureus* (CAMRSA), hospital acquired methicillin-resistant *Staphylococcus aureus* (HA-MRSA), methicillin-sensitive *Staphylococcus aureus* (MSSA), *Bacillus cereus* ATCC 11778, *Bacillus subtilis* ATCC 6633, *Enterococcus faecalis* ATCC 29212, *Klebsiella pneumoniae* (ESBL strains), *Enterobacter species*, *Pseudomonas aeruginosa*, *Escherichia coli* ATCC 25922 and *Salmonella typhimurium* ATCC 14028.

Drug Susceptibility Testing

Antibacterial *in vitro* screening was performed with the disc-agar diffusion method [16,17]. Whatman 3MM chromatography paper of uniform size were impregnated by equal volume (50 µL) of DMSO solutions of Na[RuCl₂(N-Ph-5-Cl-salim)₂] and reference antibiotics vancomycin and gentamicin of the same concentrations (1.5 mg/mL) giving 75 µg/disc. Experimental and control antibiotic discs were placed in Petri dishes seeded with organism in nutrient Mueller-Hinton agar medium. Antimicrobial activity was determined after 24 hours of incubation at 37°C and was expressed in mm of diameter of zone of inhibition. As a control, discs either with dimethylsulfoxide or with ligand, *N*-phenyl-5-chlorosalicylideneimine, had no inhibitory effect under the same experimental conditions.

Minimal inhibitory concentration and minimal bactericidal concentration

Extended study of microbiological activity was performed by determining minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values. The MIC values of Ru(III) compound was determined by serial dilution technique. Decreasing concentration of the Ru(III) compound, varying from 750-5.86 µg/mL, were prepared in serial two fold dilution using stock solution of Na[RuCl₂(NPh-5-Cl-salim)₂] in DMSO (1.5 mg/mL). Into each tube containing the Ru(III) solution and nutrient broth (trypticose-soya), the bacterial suspension (10 µL) of turbidity 0.5 McF was inoculated and incubation was performed for 24 hours at 37°C. MBC values were determined by applying 10 µL from each tube (used to determine MIC) on agar plates using a calibrated loop and counting the number of bacterial colonies found after 24 hours of incubation at 37 °C. The MBC value is expressed as the lowest concentration of the complex compound that reduces visible growth of bacteria. The MIC and MBC were conducted in triplicate.

RESULTS

Sodium dichloro-bis[*N*-phenyl-5-chlorosalicylideneiminato-*N,O*]ruthenate(III), has been previously described as a moderate CT DNA intercalating metal complex [16] which is a reason why its antimicrobial activity was examined in the present study. Ruthenium is tightly coordinated by two chlorine atoms and two Schiff bases through azomethine nitrogen and phenolic oxygen. The structure of compound is shown in Figure 1.

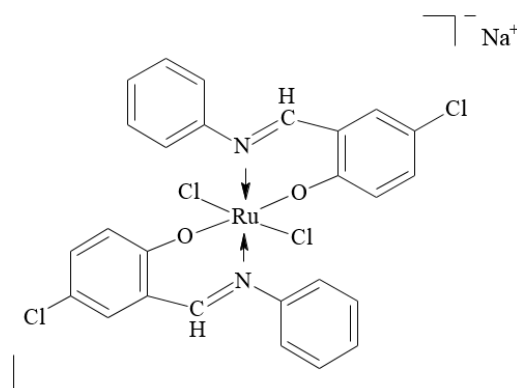


Figure 1. Structure of $\text{Na}[\text{RuCl}_2(\text{N-Ph-5-Cl-salim})_2]$

The complex is stable in air, insoluble in water, soluble in acetonitrile, DMF, acetone, ethyl alcohol, DMSO, CH_2Cl_2 . After initial dissolution in an organic solvent such as DMSO, $\text{Na}[\text{RuCl}_2(\text{N-Ph-5-Cl-salim})_2]$ shows considerable resistance to hydrolysis in spite of the presence of two chlorine atoms directly bound to ruthenium.

The initial test of antimicrobial activity was performed by disk diffusion method. The Ru(III) compound exhibits activity against Gram-positive bacteria having a fairly uniform zone of inhibition (20-21 mm) for different MRSA strains, *Bacillus cereus*, *Bacillus subtilis* and *Enterococcus faecalis*, while the reference antibiotics vancomycin and gentamicin show different zone of inhibition under the same experimental conditions. $\text{Na}[\text{RuCl}_2(\text{N-Ph-5-Cl-salim})_2]$ had no antimicrobial activity for any Gram-negative bacteria that have been tested.

For further evaluation of the antimicrobial activity of Ru (III) compound we determined the MIC and MBC values. The lowest MIC and MBC, 11.72 $\mu\text{g/mL}$ and 23.44 $\mu\text{g/mL}$ respectively, were found for CA-MRSA, *Bacillus cereus* and *Bacillus subtilis* strains, while the Ru(III) compound has demonstrated lower efficiency to methicillin-sensitive *Staphylococcus aureus*. Diameter of zone of inhibition, MIC and MBC values are given in Table 1.

Table 1. Antimicrobial activity of $\text{Na}[\text{RuCl}_2(\text{N-Ph-5-Cl-salim})_2]$

Tested microorganisms	Diameter of zone of inhibition (mm)			Ru(III) complex	
	Ru(III) comp. (75 μg /disc)	Vancomycin (75 μg /disc)	Gentamicin (75 μg /disc)	MIC ($\mu\text{g/mL}$)	MBC ($\mu\text{g/mL}$)
CA-MRSA	20	34	24	11.72	23.44
HA-MRSA	21	30	0	23.44	46.88
MSSA	20	30	24	46.88	93.75
<i>Bacillus cereus</i> ATCC 11778	21	30	36	11.72	23.44
<i>Bacillus subtilis</i> ATCC 6633	20	29	32	11.72	23.44
<i>Enterococcus faecalis</i> ATCC 29212	22	25	30	23.44	46.88
<i>Klebsiella pneumoniae</i> (ESBL strains)	0	-	23	375.00	750.00
<i>Enterobacter species</i>	0	-	20	375.00	750.00
<i>Pseudomonas aeruginosa</i>	0	-	19	375.00	750.00
<i>Escherichia coli</i> ATCC 25922	0	-	22	375.00	750.00
<i>Salmonella typhimurium</i> ATCC 14028	0	-	19	375.00	750.00

Number of surviving Gram-positive bacteria after treatment with $\text{Na}[\text{RuCl}_2(\text{NPh-5-Cl-salim})_2]$ during twenty-four hour incubation at 37 °C indicates a significant sensitivity to the Ru(III) complex. The special sensitivity to the drug shows community acquired methicillin-resistant *Staphylococcus aureus*, while Ru(III) complex has lower efficiency against methicillin-sensitive *Staphylococcus aureus* (Table 2.).

Table 2: Number of surviving bacterial colonies for different concentrations of $\text{Na}[\text{RuCl}_2(\text{N-Ph-5-Cl-salim})_2]$

Gram-positive bacteria	Concentration of $\text{Na}[\text{RuCl}_2(\text{N-Ph-5-Cl-salim})_2]$ ($\mu\text{g/mL}$)				
	5.86	11.72	23.44	46.88	93.75
	Number of surviving bacterial colonies				
CA-MRSA	35	6	0	0	0
HA-MRSA	100	18	4	0	0
MSSA	200	75	23	6	0
<i>Enterococcus faecalis</i> ATCC 2921	80	25	3	0	0

DISCUSSION

Many metal complexes that have the ability to bind DNA show antimicrobial activity. DNA molecule, which is especially important for the functioning of biological systems, is considered that might be a primary target for both anticancer and antimicrobial agents. DNA is able to bind metal complexes by covalent and noncovalent bonds. Covalent binding usually needs the presence of easily leaving groups in metal complex and activation by hydrolysis. Non-covalently mode of interaction can occur by inserting extended planar aromatic systems from ligands between base pairs of DNA, which is known as intercalation. Moreover, metal complexes might be non-covalently bound in major or minor groove of DNA that might be enhanced by hydrogen bonding, electrostatic and hydrophobic interactions. Ruthenium complexes prefer major grooves that are rich with guanine-cytosine bases. Recently, Bolhuis et al. have been reported on antimicrobial activity of ruthenium-based intercalators $[\text{Ru}(\text{phen})_2\text{dpq}]^{2+}$, $[\text{Ru}(\text{bpy})_2\text{dpqC}]^{2+}$, $[\text{Ru}(2,9\text{-Me}_2\text{phen})_2\text{dppz}]^{2+}$, the compounds based on intercalative phenanthroline and bipyridine type of molecules, and have shown the antimicrobial activity correlates with intercalating capability of ligands, but not unavoidably with the intercalative ability of metal complexes [18].

The studied complex, Sodium dichlorobis[*N*-phenyl-5-chlorosalicylideneiminato-*N,O*] ruthenate(III) is described as a moderate CT DNA intercalating agent with binding constant K_b of order 10^4 M^{-1} . By comparing the MIC values of $[\text{Ru}(\text{phen})_2\text{dpq}]^{2+}$, $[\text{Ru}(\text{bpy})_2\text{dpqC}]^{2+}$, $[\text{Ru}(2,9\text{-Me}_2\text{phen})_2\text{dppz}]^{2+}$ with MIC values of titled complex, we found that, although weaker intercalating DNA agent, $\text{Na}[\text{RuCl}_2(\text{N-Ph-5-Cl-salim})_2]$ demonstrates comparable or even improved antimicrobial activity in comparison with $[\text{Ru}(\text{phen})_2\text{dpq}]^{2+}$ and $[\text{Ru}(\text{bpy})_2\text{dpqC}]^{2+}$. MIC values of $[\text{Ru}(\text{phen})_2\text{dpq}]^{2+}$ and $[\text{Ru}(\text{bpy})_2\text{dpqC}]^{2+}$ against some MRSA strains are in the range 16-64 mg/L and for *B. subtilis* 64 mg/L [11], while the MIC values of $\text{Na}[\text{RuCl}_2(\text{N-Ph-5-Cl-salim})_2]$ against different MRSA strains are in the range 11.72-46.88 mg/L. On this basis we find that in the case of $\text{Na}[\text{RuCl}_2(\text{N-Ph-5-Cl-salim})_2]$ DNA is not the only or the most important target for antimicrobial activity since this activity might also be the result of binding to other targets such as proteins or ribosomes.

It is known that antimicrobial agents can inhibit cell wall synthesis, proteins and nucleic acids syntheses. Regardless of the possible targets and mechanisms of the antimicrobial action, the drug diffusion through the cell wall is usually major barrier for potential drug activity into the cell. Lipid membranes of bacteria, formed of peptidoglycan polymer, favors diffusion of lipid-soluble materials, therefore the liposolubility is an important factor in the control of antimicrobial activity. Although two chlorine atoms directly bound to Ru(III) in the structure increase the polarity of the $\text{Na}[\text{RuCl}_2(\text{N-Ph-5-Cl-salim})_2]$ species by reducing liposolubility, this complex has demonstrated activity against Gram-positive bacteria strains *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus* and *Enterococcus faecalis*. Number of survival bacteria-concentration of complex curve presents a similar convergence of CA-MRSA, HA-MRSA and *E. faecalis* strains toward zero for concentrations of $\text{Na}[\text{RuCl}_2(\text{N-Ph-5-Cl-salim})_2]$ just above 10 $\mu\text{g/mL}$ (Figure 2.).

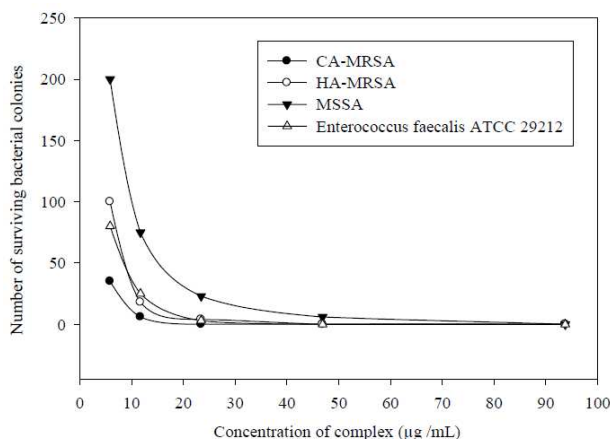


Figure 2: Number of surviving bacterial colonies vs. $\text{Na}[\text{RuCl}_2(\text{N-Ph-5-Cl-salim})_2]$ concentration

CONCLUSION

Ruthenium complexes are known to exhibit DNA binding ability that is the main reason for their testing as potential anticancer and antimicrobial drugs. The synthetic Sodium dichlorobis[*N*-phenyl-5-chlorosalicylideneiminato-*N,O*]ruthenate(III), which has moderate capability to non-covalently bind CT DNA, has been shown in the present work significant *in vitro* antimicrobial activity against Gram-positive bacteria strains *Staphylococcus aureus*,

Bacillus subtilis, *Bacillus cereus* and *Enterococcus faecalis*. The compound especially showed promising activity against community acquired methicillin-resistant *Staphylococcus aureus* with MIC value of 11.72 mg/L. The low MIC values, also for other strains of Gram-positive bacteria and degree of zone of inhibition, which reach values of up to 70% of reference antibiotics vancomycin and gentamicin, recommend Sodium dichlorobis[*N*-phenyl-5-chlorosalicylideneiminato-*N,O*]ruthenate(III) for further testing and redesigning as a prospective antibiotic of narrow spectrum for treatment of infectious caused by *S. aureus*, *B. subtilis*, *B. cereus* and *E. faecalis*. The redesigning specifically refers to the nature of axial ligands and substituents on salicylaldehyde ring which are known to might affect the activity of an antimicrobial agent. On the basis of presented results our attention will be also focused on the antimicrobial activity of the related Ru(III) complexes with Schiff bases that are being prepared in our laboratory.

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