

ANTIMICROBIAL ACTIVITY OF SOME NEW Ru(III) COMPLEXES WITH QUINOLONE DERIVATIVES

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Abstract

Two series of novel Ru(III) complexes with some quinolone derivatives previously synthesized and characterized were tested for their antimicrobial activity. The first series consists of chelate complexes with general formula $[RuL_2Cl_2]Cl \cdot nH_2O$ (L: norfloxacin (nf), $n = 4$; L: ciprofloxacin (cp), $n = 3$; L: enrofloxacin (enro), $n = 5$), while the second series is represented by complexes of general formula $RuCl_3L_2(DMSO)_m \cdot nH_2O$ (L: pipemidic acid (pip), $m = 1$, $n = 2$; L: enoxacin (enx), $m = 1$, $n = 0$; L: norfloxacin (nf), $m = 1$, $n = 1$; L: ciprofloxacin, $m = 2$, $n = 2$; L: enrofloxacin (enro), $m = 0.5$, $n = 1$; L: ofloxacin (of), $m = 1$, $n = 1$; L: levofloxacin (levof), $m = 2$, $n = 8$; DMSO: dimethylsulfoxide). The complexes were tested on *Staphylococcus aureus* methicillin resistant MRSA, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Bacillus subtilis* bacterial strains and their activity was expressed as inhibition of diameter growth (mm) and as minimum inhibitory concentration, MIC ($\mu g/mL$).

Rezumat

Două serii de complecși noi ai Ru(III) cu derivați chinolonici, sintetizați și caracterizați anterior, au fost testate pentru activitatea antimicrobiană. Prima serie constă în complecși chelați cu formula generală $[RuL_2Cl_2]Cl \cdot nH_2O$ (L: norfloxacină (nf), $n = 4$; L: ciprofloxacină (cp), $n = 3$; L: enrofloxacină (enro), $n = 5$), iar cea de a doua serie este reprezentată de complecși cu formula generală $RuCl_3L_2(DMSO)_m \cdot nH_2O$ (L: acid pipemidic (pip), $m = 1$, $n = 2$; L: enoxacină (enx), $m = 1$, $n = 0$; L: norfloxacină (nf), $m = 1$, $n = 1$; L: ciprofloxacină, $m = 2$, $n = 2$; L: enrofloxacină (enro), $m = 0.5$, $n = 1$; L: ofloxacină (of), $m = 1$, $n = 1$; L: levofloxacină (levof), $m = 2$, $n = 8$; DMSO: dimetilsulfoxid). Complecșii au fost testați pe tulpinile bacteriene *Staphylococcus aureus* metilino-rezistent MRSA, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Bacillus subtilis*, iar activitatea lor a fost exprimată ca diametrul zonelor de inhibiție (mm) și concentrația minimă inhibitoare, MIC ($\mu g/mL$).

Keywords: quinolones, Ru(III) complexes, antimicrobial activity

Introduction

Quinolones are antibacterial agents that inhibit two essential bacterial enzymes, DNA gyrase (topoisomerase II) [1] and DNA topoisomerase IV [2, 3]. Due to their specific mode of action, they are broad-spectrum antibiotics, active against gram-positive and gram-negative pathogens [4].

Many studies were focused on the capacity of quinolones to form metal chelates that exert sometimes better antimicrobial properties than the free molecules. In this regard, a norfloxacin complex with Bi(III) showed better antimicrobial activity against *S. aureus*, *E. coli* and *K. pneumoniae* compared to the free ligand [5]. A similar behaviour was reported for norfloxacin complexes with Y(III) and Pd(II), which are more active than the parent quinolone against *S. aureus*, *E. coli* and *P. aeruginosa* [6]. Some complexes of ciprofloxacin with Cu(II) [7, 8],

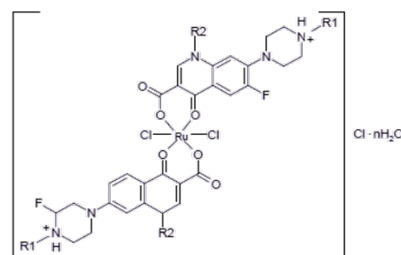
Co(II), Ni(II), Zn(II) [8] have exhibited an equal or superior activity compared to the free ligand against *S. aureus*, *B. subtilis*, *E. faecalis*, *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *S. typhimurium*. A series of enrofloxacin complexes with Cu(II) were more active on *S. aureus* [9, 10], *E. coli*, *P. aeruginosa* [9, 11], and a $MoO_2(II)$ complex was more active compared to the parent drug against these three bacterial strains [10]. In order to explain the increased biological activity of metal chelates the Overtone's concept of cell permeability and Tweedy's chelation theory were introduced. The polarity of a metal ion is reduced upon chelation, due to the partial sharing of positive charge with the donor groups of ligand and as a consequence of overlap with the ligand orbitals. Chelation increases the delocalization of π electrons over the whole chelate ring and thus increases the lipophilic nature of the central ion. This increased

in lipophilicity enhances the passage of complex through the lipid membranes and the penetration in cells [12, 13].

The antimicrobial activity of metal complexes depends on many factors: (i) the nature of the metal ion; (ii) the nature of the ligands; (iii) the chelate effect; (iv) the total charge of the complex; (iv) the nature of the ion neutralizing the ionic complex; and (vi) the nuclearity of the metal centre in the complex [10, 14-16].

In this study we have investigated the antibacterial activity of two series of novel Ru(III) complexes. The complexes from the first series, corresponding to the general formula depicted in Figure 1, display an octahedral stereochemistry with the quinolone ligand acting as bidentate chelate coordinated through carboxylic and pyridone oxygen atoms.

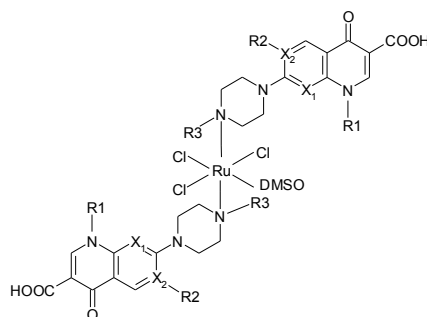
The complexes from the second series, corresponding to the general formula depicted in Figure 2, display also an octahedral stereochemistry, but the quinolone ligand acts as monodentate coordinated through N4 atom of piperazinyl ring.



Complex	R1	R2
[Ru(enro) ₂ Cl ₂]Cl·5H ₂ O (Ru-enro; enro = enrofloxacin)	C ₂ H ₅	
[Ru(cp) ₂ Cl ₂]Cl·3H ₂ O (Ru-cp; cp = ciprofloxacin)	H	
[Ru(nf) ₂ Cl ₂]Cl·4H ₂ O (Ru-nf; nf = norfloxacin)	H	C ₂ H ₅

Figure 1.

Structures of the investigated Ru(III) chelate complexes



Complex	X1	X2	R1	R2	R3
[RuCl ₃ (pip) ₂ (DMSO)]·2H ₂ O (Ru-pip-DMSO; pip = pipemidic acid)	N	N	C ₂ H ₅	-	H
[RuCl ₃ (enx) ₂ (DMSO)] (Ru-enx-DMSO; enx = enoxacin)	N	C	C ₂ H ₅	F	H
[RuCl ₃ (nf) ₂ (DMSO)]·H ₂ O (Ru-nf-DMSO; nf = norfloxacin)	CH	C	C ₂ H ₅	F	H
[RuCl ₃ (cp) ₂ (DMSO)]·DMSO·2H ₂ O (Ru-cp-DMSO; cp = ciprofloxacin)	CH	C		F	H
[RuCl ₃ (enro) ₂ (DMSO)]·H ₂ O (Ru-enro-DMSO; enro = enrofloxacin)	CH	C		F	C ₂ H ₅
[RuCl ₃ (of) ₂ (DMSO)]·H ₂ O (Ru-of-DMSO; of = ofloxacin)	CH	C		F	CH ₃
[RuCl ₃ (levof) ₂ (DMSO)]·DMSO·8H ₂ O (Ru-levof-DMSO; levof = levofloxacin)	CH	C		F	CH ₃

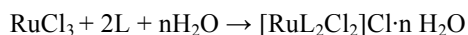
Figure 2.

Structures of the investigated Ru(III) complexes

Materials and Methods

Chemistry

The synthesis of the compounds from the first series was carried out according to the following scheme:



A quantity equivalent with 0.8 mmol ligand was suspended in water (30 mL) and 0.4 mmol of ruthenium (III) chloride monohydrate was added while stirring. The final pH of the resulting brown solution was 5. The solution was kept on the steam bath until a fivefold reduction in volume has been

achieved. The brown precipitate, which formed after the addition of an equal volume of ethanol, was collected by filtration, washed with ethanol, and dried in air [17].

The synthesis of the compounds from the second series was performed according to the following scheme:



A DMSO solution of ligand and RuCl_3 in a 2:1 molar ratio was heated under reflux for 6 h. The solution was turned into dark-brown. After cooling, a solution 2 M of NaCl has been added in order to obtain the solid product. The brown residue was filtered off and washed several times with distilled water and dried in air [18, 19].

All complexes obtained are brown substances, insoluble in water, soluble in dimethylsulfoxide and dimethylformamide.

Microbiological study

The qualitative screening of the susceptibility spectra of different microbial strains to the complexes was performed by adapted diffusion techniques: paper filter disk impregnation with the tested substances solutions, the disposal of tested solutions in agar wells and the spotting of tested solutions on microbial inoculums seeded medium, while the quantitative assay for the establishing of the minimal inhibitory concentration (MIC, $\mu\text{g/mL}$) value was based on liquid medium serial dilutions. The *in vitro* biological screening effects were tested against a bacterial inoculum (1.5×10^8 CFU/cm³) represented by *Staphylococcus aureus* 1263 MRSA, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* IC 134202,

Staphylococcus aureus ATCC 25923 and *Bacillus subtilis* ATCC 6633. The microbial strains were identified by aid of VITEK I automatic system. For the MIC assay, stock solutions (1 mg/mL) were prepared by dissolving the compounds in DMSO and serial binary dilutions were performed in nutrient broth distributed in 96-multiwell plates, further inoculated with a standard inoculum of bacterial strains. The plates were incubated at 37°C for 24 h. The minimal inhibitory concentration was read by wells observation: in the first wells containing high concentrations of compounds the culture growth was not visible, the microbial cells being killed or inhibited by the tested compound. At lower concentrations of the tested compounds, the microbial culture became visible. The lowest concentration, which inhibited the visible microbial growth, was considered the MIC value for the tested compound.

In the next wells, including the standard culture growth control wells, the medium become muddy as a result of the microbial growth. In the sterility control wells series the medium had to remain clear. From the last well without any visible microbial growth and from the first one with a microbial growth, Gram stained smears were performed for the results confirmation.

Results and Discussion

The data of the qualitative screening of the susceptibility spectra of different microbial strains to the complexes from series 1 are represented in Figure 3.

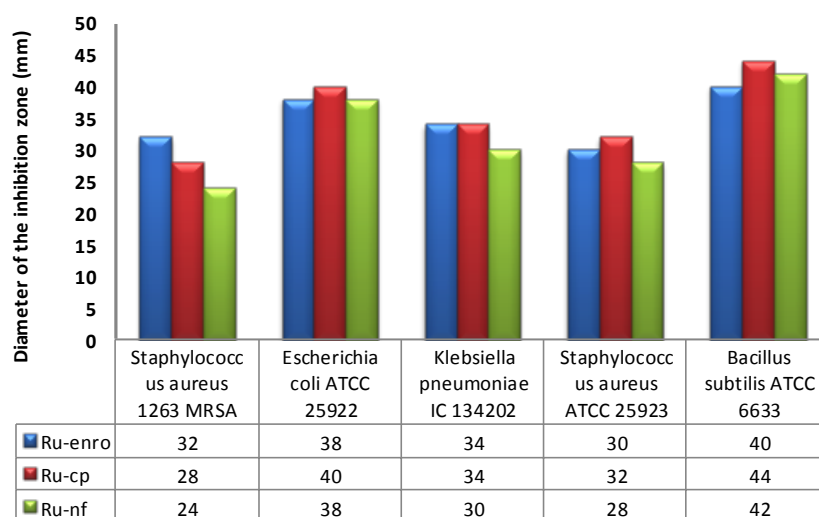


Figure 3.

Results of the qualitative assay (expressed as diameter of the inhibition zone, mm) of the antimicrobial activity of the Ru(III) chelate complexes

The results of the qualitative screening show that the Ru-enro complex is the most active on *S. aureus* MRSA, whereas the Ru-cp complex is the most active

on *E. coli*, *K. pneumoniae*, *S. aureus* and *B. subtilis*, but there are small differences among the activities of the three complexes.

The data of the quantitative screening of the susceptibility spectra of different microbial strains to the complexes from the series 1, expressed as minimal

inhibitory concentration (MIC, $\mu\text{g/mL}$), are represented in Figure 4.

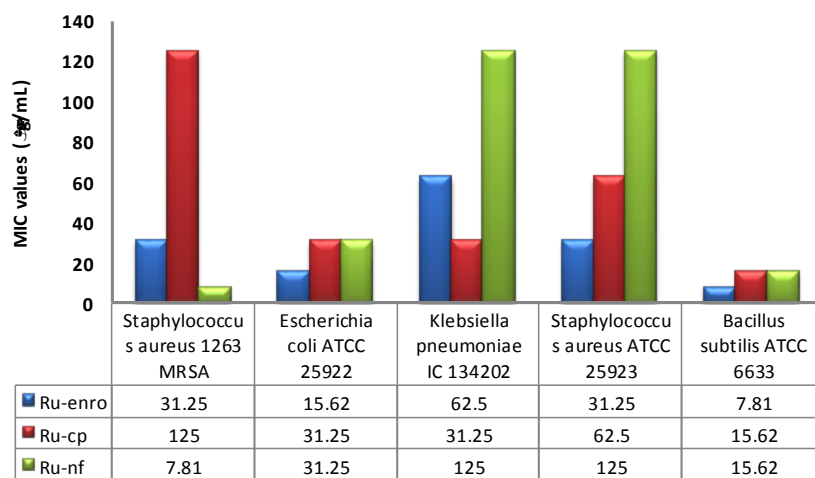


Figure 4.

Results of the quantitative assay (expressed as minimal inhibitory concentration-MIC, $\mu\text{g/mL}$) of the antimicrobial activity of the Ru(III) chelate complexes

The quantitative screening differentiated the antimicrobial activity of the tested complexes. Ru-nf is the most active complex on *S. aureus* MRSA, Ru-enro is the most active on *E. coli*, *S. aureus*, *B. subtilis*, whereas Ru-cp is the complex with the highest activity on *K. pneumoniae*.

The data of the qualitative screening of the susceptibility spectra of different microbial strains to the complexes from series 2 are represented in Figure 5, and the data of quantitative screening to these complexes are represented in Figure 6.

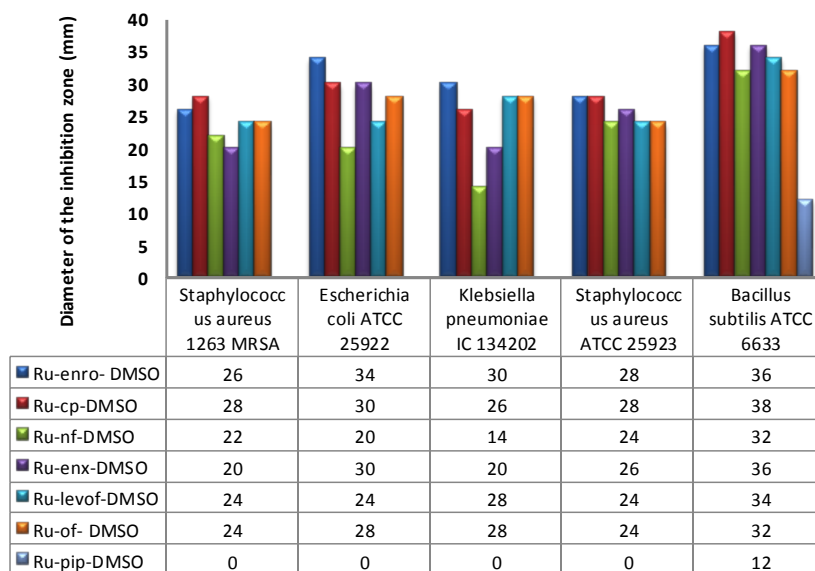
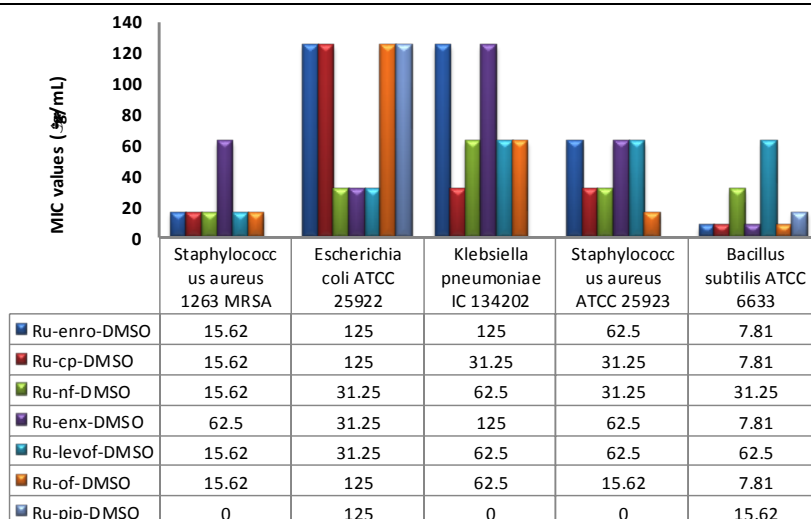


Figure 5.

Results of the qualitative assay (expressed as diameter of the inhibition zone, mm) of the antimicrobial activity of the Ru(III) complexes

The results of qualitative assay highlight that Ru-cp-DMSO is the most active on *S. aureus*, *S. aureus* MRSA and *B. subtilis*, while Ru-enro-DMSO is the most active on *E. coli* and *K. pneumoniae* bacterial

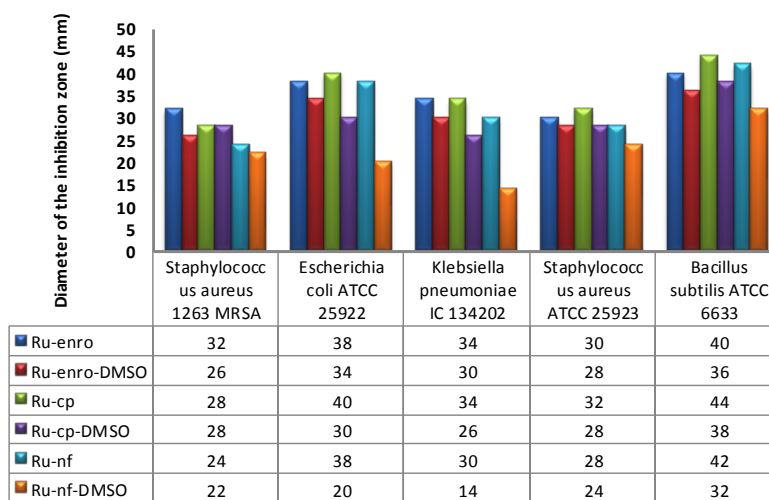
strains. Contrariwise, Ru-pip-DMSO is inactive against all the strains tested, excepting a little activity on *B. subtilis*.

**Figure 6.**

Results of the quantitative assay (expressed as minimal inhibitory concentration-MIC, $\mu\text{g/mL}$) of the antimicrobial activity of the Ru(III) complexes

By analysing the results of the qualitative assay can be observed that the complexes are more active on *B. subtilis*, followed by *S. aureus* MRSA. It worth noticed the good activity of Ru-of-DMSO on *S. aureus*. Another aspect worth considering is the influence of the coordination mode of the ligand, which is different in the case of the two series of complexes. The comparative analysis of quantitative data of pairs

of complexes belonging to the two series (Figure 7) argue for a better activity of chelate complexes, consistent with the Overtone's concept of cell permeability and Tweedy's chelation theory [20]. Unfortunately, the data of the quantitative assay are not conclusive, and this aspect could be caused by the differences in solubility of the complexes from the two series.

**Figure 7.**

Comparative representation of the antimicrobial activity (qualitative assay, expressed as diameter of the inhibition zone, mm) of the pairs of Ru(III) complexes from the two series

Conclusions

All tested compounds, excepting for Ru-pip-DMSO complex, exhibited antimicrobial activity, with large inhibition zones. From the first series of complexes, Ru-nf is the most active complex on *S. aureus* MRSA, Ru-enro complex was the most active on *E. coli*, *S. aureus* and *B. subtilis*, whereas Ru-cp complex had the best activity on *K. pneumoniae*. From the

second series of complexes, the activity of Ru-of-DMSO on *S. aureus* is remarkable.

The most susceptible strain was *B. subtilis*, followed by *S. aureus*, demonstrating the specific interaction of the tested compounds with the microbial cellular targets and their superior inhibitory activity against the Gram-positive bacterial strains.

Aknowledgement

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