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Antimicrobial activity study of some binuclear complexes of Cu(II), Ni(II) and Co(II) with a new Schiff base (1,3-bis[*ortho*-(2-carboxy-phenyliminomethyl)-phenoxy]propane)

Research article

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Abstract
In this work, the antimicrobial activity of some complexes of Cu(II), Ni(II) and Co(II) with a new Schiff base (1,3-bis[*ortho*-(2-carboxy-phenyliminomethyl)-phenoxy]propane) tested on five microbial strains, is presented. The antimicrobial activity of the tested compounds was evaluated from a quantitative point of view, as well as the resistance of the microbial biofilms developed on an inert substrate.

Keywords: new Schiff base, binuclear complexes, antimicrobial activity, microbial biofilms

1. INTRODUCTION

A large number of Schiff bases and their complexes has been studied for their interesting and important properties, e.g. biological activity [1], catalytic activity in hydrogenation of olefins [2] and transfer

of an amino group [3],photochromic properties [4], complexing ability towards some toxic metals [5].

Many metal complexes with amino acid derived Schiff base have been synthesized and tested for their biological activity [6, 7]. For example, the Schiff base, obtained from 2-thiophene carboxaldehyde and 2-aminobenzoic acid and its metal complexes, show antibacterial activity [8]. Salicylidene anthranilic acid possesses antiulcer activity and the complexation with copper shows an increase in antiulcer activity [9].

Moreover, it was found that the Zn(II) complexes with Schiff bases derived from the aromatic dialdehyde, 2,2'-(propane-1,3-diyldioxy)dibenzaldehyde and some amino acids, show antimicrobial and antitumor activity [10].

The synthesis and characterization of the Cu(II), Ni(II) and Co(II) binuclear complexes with the new Schiff base (1,3-bis[*ortho*-(2-carboxy-phenyliminomethyl)-phenoxy]propane) derived from 2-aminobenzoic acid (anthranilic acid) and the aromatic dialdehyde, 2,2'-(propane-1,3-diyldioxy)dibenzaldehyde, were presented in a previous study [11].

Thus, the aim of the present study was to evaluate the supposed antimicrobial activity of these binuclear complexes.

2. MATERIALS AND METHODS

2.1. Materials

The binuclear complexes of Cu(II), Ni(II) and Co(II) with the new Schiff base ligand derived from 2-aminobenzoic acid and 2,2'-(propane-1,3-diyldioxy)dibenzaldehyde were prepared using two methods described in a previous study [11]. So, according to the first method, the complexes were obtained by template condensation of 2-aminobenzoic acid, aromatic dialdehyde and the corresponding metal salt in alcoholic medium [11]. The second method consists in refluxing of the ethanolic solutions of the isolated Schiff base (obtained by a method also described in the same previous study) sodium salt with the metal salts, in a 1:2 molar ratio [11].

The prepared complexes are of $[M_2L(OH)_2(H_2O)_4]$ type, where M= Cu(II), Ni(II) or Co(II) and L= $C_{31}H_{24}O_6N_2$.

Analytical grade reagents from Sigma and Merck were used in all experiments.

The microbial strains were isolated from different clinical sources and were identified by aid of VITEK I automatic system [12].

2.2. Methods for the qualitative and quantitative antimicrobial assays

The antimicrobial activity of these compounds was tested against five bacterial strains: *Enterococcus faecalis ATCC 29212*, *Citrobacter freundii 1748, Salmonella sp. 9246, Staphylococcus epidermidis 1736* and *Escherichia coli ESBL + 1576*. Microbial suspensions of density corresponding to 0.5 McFarland UI obtained from 24 h microbial cultures developed on solid media were used in the experiments. The antimicrobial activity of these compounds was tested on Mueller-Hinton agar medium [13].

The tested compounds were dissolved in dimethylformamide (DMF) and used for the antimicrobial activity screening at 10 mg/mL concentration of stock solutions.

The qualitative screening was performed by an adapted diffusimetric method (the spot method). In this purpose, Petri dishes with Mueller Hinton medium were seeded with bacterial inoculum and then the stock solutions of the tested compounds (5 μL) were added as spots. The plates were left at room temperature for 20-30 min and then incubated at 37°C for 24 h. The positive results were read as the occurrence of an inhibition zone of microbial growth around the spot [14].

The quantitative assay of the antimicrobial activity was performed by binary micro dilution method, in liquid medium, distributed in 96 multi-well plates, in order to establish the minimum inhibitory concentration (MIC) [15]. In this purpose, serial binary dilutions of the tested compounds were performed in a 100 μL volume of liquid medium and each well was seeded with 20 μL of microbial inoculum of 0.5 McFarland UI density. The plates were incubated for 24 h at 37°C, and MICs were recorded in each case as the minimum concentration of the compound, which inhibited the visible growth of

the tested microorganism [16].

2.3. Method for the resistance study of the microbial biofilms developed on an inert substrate

Bacteria possess binding molecules, generically called adhesins, which are able to bind stereospecifically with the receptors on the host cell membrane, in a manner analogous to the antigen-antibody or lectin-sugar interaction. The interaction of most adhesins with the surface receptors of the sensitive cell is specific and selective. Adherence ensures the colonization of certain sites in the body, the multiplication of bacteria, the synthesis of toxins and the development of the inflammatory defense reaction.

Most bacteria have a net negative charge on their surface, but they also have limited electropositive areas, as well as hydrophobic molecules [17]. The presence of groups with opposite charges and hydrophobic molecules ensures the interaction of the bacterial cell with the surface of the epithelial cell.

Below is a brief description of the protocol steps taken to study the influence of the tested compounds on the development of microbial biofilms on an inert substrate:

1. The microbial cells were cultivated in 96-well plates with nutrient broth and in the presence of the tested compounds (after reading the MIC values), they were incubated at 37°C for 24 hours. The plates were emptied and washed twice with A.F.S.;
2. Fixation for 5 minutes of adherent cells with 100 μL 80% methanol. The methanol solution was removed by swirling;
3. Staining of adhered cells with 1% crystal violet alkaline solution (100 μL/well) for 15 minutes. The coloring solution was removed, then the plates were washed under running tap water;
4. The microbial biofilms formed on the plastic plates were resuspended in 33% acetic acid (by bubbling), and the intensity of the colored suspension was evaluated by

measuring the absorbance at 490 nm using a plate-reader (Absorbance Reader Tecan).

3. RESULTS AND DISCUSSION

The spectroscopic data, as well as elemental analysis, molar conductivity measurements and thermal analysis results, presented in a previous study [11], support the proposed general structures of the studied compounds (Figure 1).

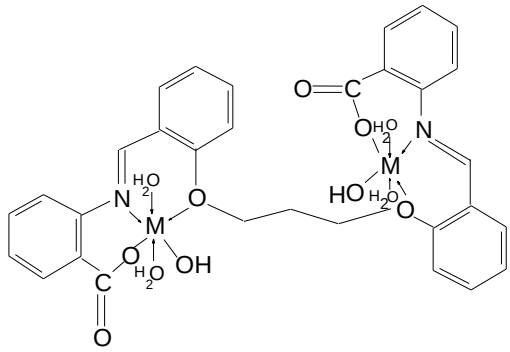

Figure 1. The general structures for the metal complexes with the new Schiff base ligand (where M = Cu(II), Ni(II) or Co(II))

3.1. Qualitative and quantitative screening of the antimicrobial activity

The qualitative method used for the screening of the antimicrobial activity of the tested compounds indicated only very low diameters of growth inhibition around the spots, so that the diameters of the inhibition zones were not measured. These results could be due to the low diffusion rates of the tested compounds in solid Mueller Hinton medium.

The quantitative assay results for the antimicrobial activity of the studied compounds, expressed as MIC values, between 1000 and 31.25 $\mu g \cdot mL^{-1}$, are presented in the Table 1.

A MIC value superior to 250 μg·mL^{-1} was considered as corresponding to a low, between 250 μg·mL^{-1} and 125 μg·mL^{-1} to a moderate and under 60.50 μg·mL^{-1} to a good antimicrobial activity [18].

The most active compound, considering both the intensity of the antimicrobial activity and the microbial spectrum proved to be the compound **2**, which showed good activity against *Salmonella sp. 9246* strain (MIC = 31.25 μg·mL^{-1}), being active against all tested microbial strains. The complex **2** also presents a moderate antimicrobial activity, superior to DMF solvent, against *Citrobacter freundii 1748* and *Escherichia coli ESBL + 1576* strains and a low antimicrobial activity, similar to that of the DMF solvent, against *Enterococcus faecalis ATCC 29212* and *Staphylococcus epidermidis 1736* strains.

Table 1. Antimicrobial activity expressed as MIC (μg·mL^{-1})

Compound	MIC/μg·mL^{-1}				
	E. faecalis ATCC 29212	*Citrobacter freundii 1748*	*Salmonella sp. 9246*	*S. epidermidis 1736*	*Escherichia coli 1576*
H_2L (**1**)	-	250	-	-	-
$[Cu_2L(OH)_2(H_2O)_4]$ (**2**)	500	250	31.25	1000	125
$[Ni_2L(OH)_2(H_2O)_4]$ (**3**)	-	-	500	-	500
$[Cu_2L(OH)_2(H_2O)_4]$ (**4**)	500	-	31.25	-	125
DMF	500	500	500	1000	250

- = no inhibition

It was found that the compound **3** presented a low antimicrobial activity against *Salmonella sp. 9246* and *Escherichia coli ESBL + 1576* strains and was inactive against three microbial strains: *Enterococcus faecalis ATCC 29212*, *Citrobacter freundii 1748* and *Staphylococcus epidermidis 1736*.

The compound **4** showed a good antimicrobial activity, similar to that of the complex **2**, against *Salmonella sp. 9246* strain, a moderate antimicrobial activity and a low antimicrobial activity, similar to that of the compound **2**, against *Escherichia coli ESBL + 1576* and *Enterococcus faecalis ATCC 29212* strains, respectively. This complex was inactive

against *Citrobacter freundii 1748* and *Staphylococcus epidermidis 1736* strains, similar to the compound **3**.

It must be noticed that the compound **1** (the Schiff base ligand) was inactive against four of the five tested microbial strains and this compound also presented a moderate antimicrobial activity, similar to that of the complex **2**, against *Citrobacter freundii 1748* strain.

From these results of the biological evaluation it was concluded that the antimicrobial activity of the tested compounds follow the order: compound **2** > compound **4** > compound **3** ≅ compound **1** and that, in general, biological activity increases through complexation.

The lowest MIC values, 31.25 $\mu g \cdot mL^{-1}$ and 125 $\mu g \cdot mL^{-1}$ for the compounds **2** and **4**, were obtained for *Salmonella sp. 9246* and *Escherichia coli ESBL + 1576* strains, respectively.

The tested compounds showed the lowest antimicrobial activity against *Staphylococcus epidermidis 1736* strain.

Below are presented the graphical representations of the MIC values of the studied compounds and of the DMF solvent against two of the tested microbial strains.

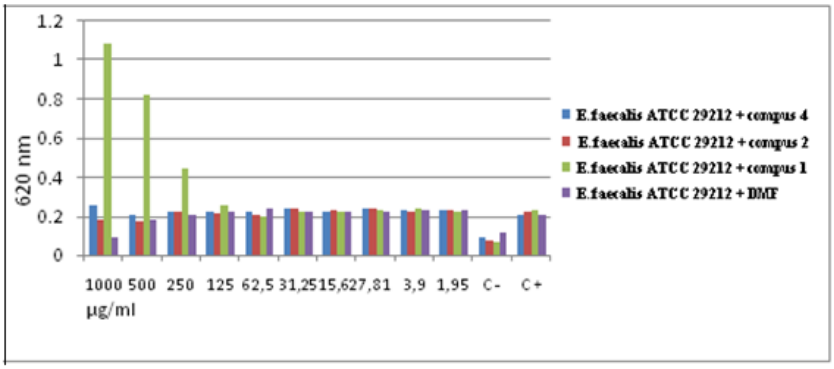

Figure 2. Graphic representation of MIC values of compounds **1**, **2**, **4** and DMF solvent tested against the *Enterococcus faecalis ATCC 29212* strain

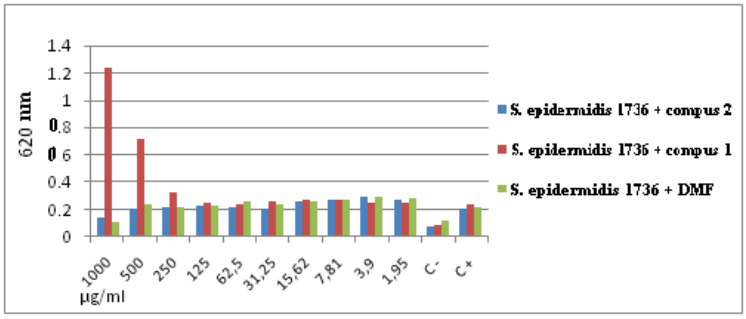

Figure 3. Graphic representation of MIC values of compounds **1**, **2** and DMF solvent tested against the *Staphylococcus epidermidis 1736* strain

3.2. The study of the resistance of microbial biofilms developed on inert substrate to the tested compounds

The biofilms developed on inert substrate by *Citrobacter freundii 1748* and *Escherichia coli ESBL + 1576* strains were inhibited at concentrations between 125 $\mu g \cdot mL^{-1}$ and 500 $\mu g \cdot mL^{-1}$.

The solvent used (DMF) showed an effect of inhibiting the adhesion of microbial cells to the inert substrate, this effect being lower than that of the tested compounds.

The studied compounds showed a pronounced biofilm inhibition effect on inert substrate in the case of the *Salmonella sp. 9246* strain, the inhibition effect being observed up to very low concentrations of the tested compounds.

The results showed that, although the studied compounds had no inhibitory effect on microbial growth in the case of *Enterococcus faecalis ATCC 29212* and *Staphylococcus epidermidis 1736* strains, they exerted an antibiofilm effect.

Below are presented the graphic representations of the degree of development of the microbial biofilm on the inert substrate formed by two of the tested microbial strains, in the presence of different concentrations of the studied compounds.

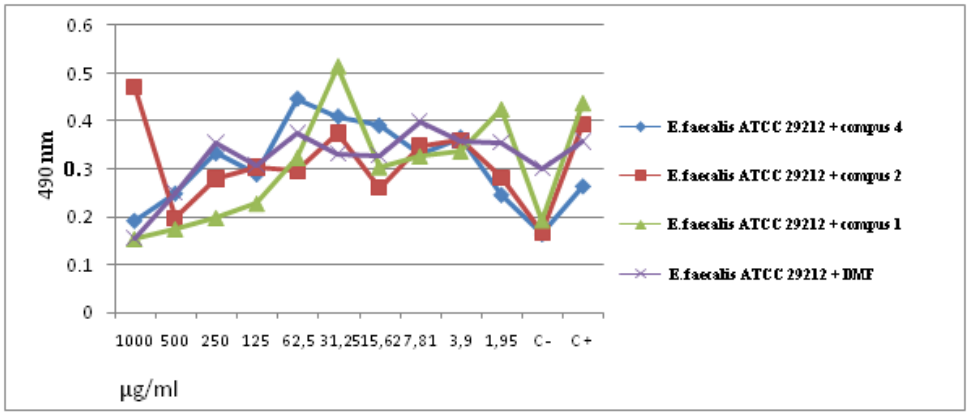

Figure 4. Graphic representation of the degree of development of the microbial biofilm on the inert substrate formed by *Enterococcus faecalis ATCC 29212* strain, in the presence of different concentrations of the compounds **1**, **2** and **4**

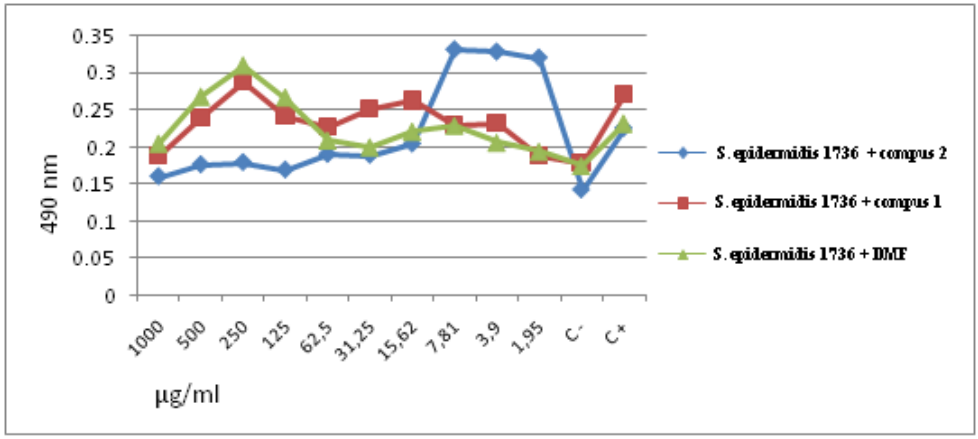

Figure 5. Graphic representation of the degree of development of the microbial biofilm on the inert substrate formed by *Staphylococcus epidermidis 1736* strain, in the presence of different concentrations of the compounds **1** and **2**

4. CONCLUSION

In this study, the Cu(II), Ni(II) and Co(II) binuclear complexes with a new Schiff base derived from 2-aminobenzoic acid and the aromatic

dialdehyde, 2,2′-(propane-1,3-diyldioxy)dibenzaldehyde, were tested for their antimicrobial activity.

From the results of the biological evaluation it was concluded that the antimicrobial activity of the tested compounds follow the order: compound **2** > compound **4** > compound **3** ≅ compound **1** and that, in general, biological activity increases through complexation. Therefore, the most active compound, considering both the intensity of the antimicrobial activity and the microbial spectrum proved to be the compound **2**, which showed good activity against *Salmonella sp. 9246* strain (MIC = 31.25 $\mu g \cdot mL^{-1}$), being active against all tested microbial strains.

The results of determining the influence of the tested compounds on the development of microbial biofilms showed that they have an inhibitory effect on microbial adhesion.

The importance of these findings lies in the fact that these compounds could be considered for the further development of novel antimicrobial drugs used for the treatment of some common diseases caused by these bacterial strains.

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