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Antimicrobial activity study of some binuclear complexes with Schiff bases derived from an aromatic dialdehyde and some amino acids

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Abstract:
Many metal complexes with amino acid derived Schiff bases have been synthesized and tested for their biological activity. Thus, the aim of the present study was to evaluate the antimicrobial activity of some binuclear complexes with Schiff bases derived from an aromatic dialdehyde and some amino acids by studying the influence of the tested compounds on the development of microbial biofilms on an inert substrate. The biofilms developed on inert substrate by *Klebsiella pneumoniae ESBL*$^{+}$, *Citrobacter freundii 1748, Bacillus sp., Serratia marcescens 0804, Staphylococcus aureus ATCC 25923* and *Escherichia coli 1576* strains were inhibited at concentrations of the tested compounds between 250 $\mu g \cdot mL^{-1}$ and 500 $\mu g \cdot mL^{-1}$. 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.

Keywords: amino acid derived Schiff bases , binuclear complexes, antimicrobial activity, microbial biofilms

1. INTRODUCTION

The chemistry of the metal complexes of Schiff bases containing nitrogen and other donor atoms has attracted a great deal of attention due to their stability, biological activity [1] and potential applications in many fields such as oxidation catalysis [2], electrochemistry [3] etc.

Amino acid Schiff bases and their first-row transition metal complexes were reported to exhibit fungicidal, bactericidal, antiviral and antitubercular activity [4]. The Schiff bases derived from 2-hydroxy-1-naphthaldehyde and an amino acid (glycine, alanine, phenylalanine, histidine, tryptophan) and their manganese (III) complexes show antimicrobial activity [5]. The Schiff base obtained from 2-thiophene carboxaldehyde and 2-aminobenzoic acid and its metal complexes also show antibacterial activity [6]. In addition, tryptophan, an essential amino acid in human nutrition, is an important and frequently used starting material in the chemical synthesis of a range of pharmaceuticals [7]. Some of its derivatives are potent drugs [8].

The synthesis and characterization of the Cu(II), Ni(II), Co(II), Zn(II) and Mn(II) binuclear complexes with Schiff bases derived from some amino acids (2-aminobenzoic acid, L-tryptophan) and the aromatic dialdehyde, 2,2'-(propane-1,3-diyldioxy)dibenzaldehyde, were presented in previous studies [9-11]. Moreover, it was found that all these binuclear complexes show antimicrobial activity [11-13] and the Cu(II), Ni(II), Co(II) and Zn(II) binuclear complexes with the Schiff base derived from the aromatic dialdehyde and 2-aminobenzoic acid also show antiviral and antitumor activity [14,15].

Thus, the aim of the present work was to evaluate the antimicrobial activity of all these binuclear complexes by the resistance study of the microbial biofilms developed on an inert substrate. 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.

2. MATERIALS AND METHODS

2.1. Materials

All the binuclear complexes with Schiff base ligands derived from 2,2'-(propane-1,3-diyldioxy)dibenzaldehyde and 2-aminobenzoic acid, L-tryptophan respectively, were prepared according to a procedure described

in previous studies, by template condensation of the aromatic dialdehyde, corresponding amino acid and metal salt in alcoholic medium, in a 1:2:2 molar ratio [9-11].

The Cu(II), Ni(II), Co(II) and Zn(II) binuclear complexes with Schiff base derived from aromatic dialdehyde and 2-aminobenzoic acid were also obtained by refluxing the isolated Schiff base ligand (obtained by a method also described in the same previous study) and the corresponding metal salt in a 1:2 molar ratio [10].

The Cu(II), Ni(II), Co(II) and Mn(II) binuclear complexes with Schiff base derived from an aromatic dialdehyde and L-tryptophan were prepared only by template synthesis, in alkaline medium, since any attempt to isolate the free Schiff base ligand was unsuccessful, because it is easily hydrolysed in contact with water [11].

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

The studied compunds were denoted as follows: H_2L' (**1**), $[Cu_2L'(OH)_2(H_2O)_4]$ (**2**), $[Ni_2L'(OH)_2(H_2O)_4]$ (**3**), $[Co_2L'(OH)_2(H_2O)_4]$ (**4**), $[Zn_2L'(OH)_2(H_2O)_4]$ (**5**), $[Cu_2L''(OAc)_2(H_2O)_4]$ (**6**), $[Ni_2L''(OAc)_2(H_2O)_4]$ (**7**), $[Co_2L''(OAc)_2(H_2O)_4]$ (**8**) and $[Mn_2L''(OAc)_2(H_2O)_4]$ (**9**), where L'= $C_{31}H_{24}O_6N_2$ (H_2L' is the Schiff base derived from aromatic dialdehyde and 2-aminobenzoic acid) and L''= $C_{39}H_{34}O_6N_4$ (H_2L'' is the unisolated Schiff base derived from aromatic dialdehyde and L-tryptophan).

The microbial strains were isolated from different clinical sources and were identified by aid of VITEK I automatic system [16].

2.2. 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.

The antimicrobial activity study was carried out using the following eleven bacterial strains: Gram-positive *Staphylococcus aureus ATCC 25923*, *Bacillus sp.*, *Staphylococcus epidermidis1736*, *Enterococcus faecalis ATCC 29212* strains, Gram-negative *Escherichia coli 1576*, *Salmonella sp. 9246*, *Pseudomonas aeruginosa 846*, *Klebsiella pneumoniae ESBL*$^{+}$, *Citrobacter freundii 1748*, *Providencia stuartii 1116*, *Serratia marcescens 0804* strains.

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

3.1. Results

The spectroscopic data (IR and UV-Vis electronic spectra), as well as elemental analysis, molar conductivity measurements and thermal analysis results, presented in previous studies [9-11], support the proposed general structures of the studied binuclear complex compounds (Figure 1).

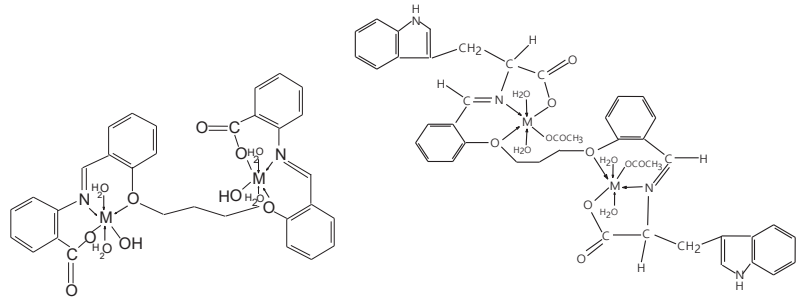

Figure 1. Proposed general structures of binuclear complexes derived from 2,2'-(propane-1,3-diyldioxy)dibenzaldehyde and 2-aminobenzoic acid (**a**), L-tryptophan (**b**), respectively

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

The biofilms developed on inert substrate by *Klebsiella pneumoniae ESBL*$^{+}$, *Citrobacter freundii 1748*, *Bacillus sp.*, *Serratia marcescens 0804*, *Staphylococcus aureus ATCC 25923* and *Escherichia coli 1576* strains were inhibited at concentrations between 250 $\mu g \cdot mL^{-1}$ and 500 $\mu g \cdot mL^{-1}$ of the tested compounds.

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 (1.95 $\mu g \cdot mL^{-1}$) of the tested compounds.

The results showed that these compounds had no inhibitory effect on adhesin synthesis in the case of the *Pseudomonas aeruginosa 846* strain.

The results also indicated 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 (15.62-250 $\mu g \cdot mL^{-1}$).

Below are presented the most suggestive graphic representations of the degree of development of the microbial biofilm on the inert substrate

formed by the tested microbial strains, in the presence of different concentrations of the studied compounds (Figures 2-6).

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.

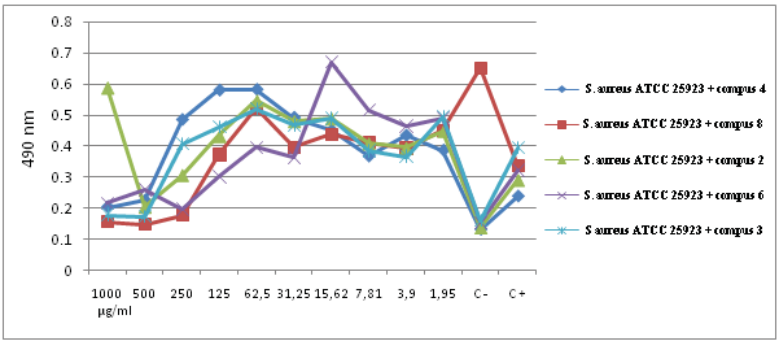

Figure 2. Graphic representation of the degree of development of the microbial biofilm on the inert substrate formed by *Staphylococcus aureus ATCC 25923* strain, in the presence of different concentrations of the compounds **2**, **3, 4, 6** and **8**

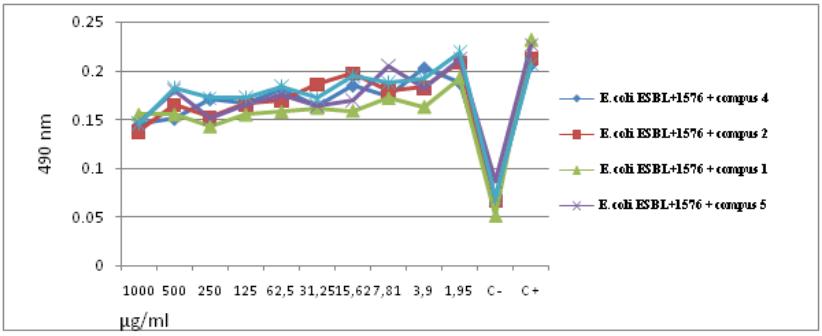

Figure 3. Graphic representation of the degree of development of the microbial biofilm on the inert substrate formed by *Escherichia coli 1576* strain, in the presence of different concentrations of the compounds **1**, **2, 4** and **5**

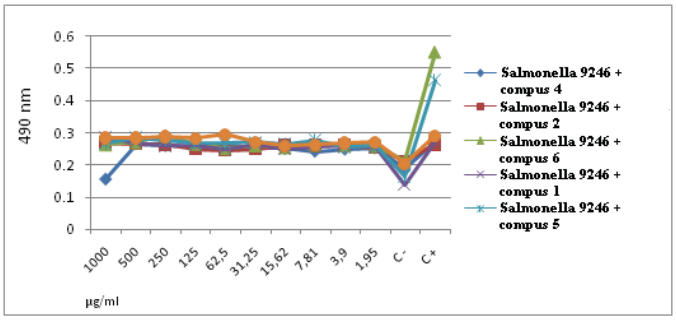

Figure 4. Graphic representation of the degree of development of the microbial biofilm on the inert substrate formed by *Salmonella sp. 9246* strain, in the presence of different concentrations of the compounds **1**, **2, 4, 5** and **6**

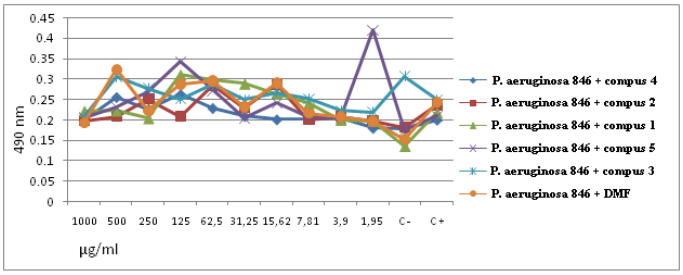

Figure 5. Graphic representation of the degree of development of the microbial biofilm on the inert substrate formed by *Pseudomonas aeruginosa 846* strain, in the presence of different concentrations of the compounds **1**, **2, 3, 4** and **5**

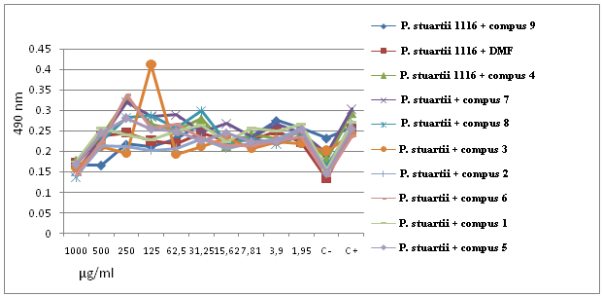

Figure 6. Graphic representation of the degree of development of the microbial biofilm on the inert substrate formed by *Providencia stuartii 1116* strain, in the presence of different concentrations of the compounds **1**, **2, 3, 4, 5, 6, 7, 8** and **9**

3.2. Discussion

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 and thus confirm their antimicrobial activity studied in previous works [11-13].

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.

4. CONCLUSION

In this study was evaluated the antimicrobial activity of some binuclear complexes with Schiff bases derived from an aromatic dialdehyde and some amino acids by studying the influence of the tested compounds on the development of microbial biofilms on an inert substrate.

The biofilms developed on inert substrate by *Klebsiella pneumoniae ESBL*$^{+}$, *Citrobacter freundii 1748*, *Bacillus sp.*, *Serratia marcescens 0804*, *Staphylococcus aureus ATCC 25923* and *Escherichia coli 1576* strains were inhibited at concentrations between 250 $\mu g \cdot mL^{-1}$ and 500 $\mu g \cdot mL^{-1}$ of the tested compounds. The results also indicated 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 (15.62-250 $\mu g \cdot mL^{-1}$).

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.

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