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ANTIMICROBIAL INVESTIGATIONS OF COPPER(II) COMPLEXES WITH SOME 1-BENZYLBENZIMIDAZOLE DERIVATIVES

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In the paper the antimicrobial activity of copper(II) complexes with two series of benzimidazoles was investigated. The first one was based on 1-benzyl-2-aminobenzimidazole, and the second one contained 1-benzyl-2-amino-5,6-dimethylbenzimidazole. Antibacterial activities of the complexes were evaluated *in vitro* against three gram-positive bacterial strains (*Bacillus cereus, Staphylococcus aureus*, and *Sarcina lutea*) and one gram-negative isolate (*Pseudomonas aeruginosa*). All the investigated compounds displayed antimicrobial activity against very persistent microorganisms, whilst the inhibitory activity is upper against gram-positive than gram-negative bacteria. Minimum inhibitory concentration (MIC) was determined for all the complexes. The effect of ligand and complex structure on the antimicrobial activity is discussed.

INTRODUCTION

Heterocyclic benzimidazoles and their transition metal complexes have received considerable attention from the coordination chemists, because of their well-documented biological activities.[1-7] This class of compounds have been found to show antimicrobial activities against gram-positive and gram-negative bacteria, primarly because of the potential bio-activity of benzimidazole-based ligands.[8-10] They are of wide interest because of their diverse biological activity and clinical applications so the incorporation of the imidazole and benzimidazole nuclei is an important synthetic strategy in drug discovery. Extensive biochemical and pharmacological activities have confirmed that these molecules are effective against RNA viruses and inhibit the formation of virus induced RNA polymerase, thereby preventing or retarding RNA synthesis various strains of microorganisms.[11-14] Antimicrobial activity of these class of compounds against *Helicobacter pylori*[15] and oral *Streptococci*[16] was also reported. Synthesis of benzimidazoles fused to another heterocyclic ring has attracted wide spread attention due to their diverse application as antioxidant,[17,18] antitubercular,[19] antifungal,[20] anticancer,[21,22] and antiallergic drugs.[23,24] Also, the HIV-virus is inactivated by the various benzimidazoles.[25,26]

In the last time possible therapeutical properties of the metal complexes with derived benzimidazoles have also excited wide interest. It was found that the complexes of transition metal salts with benzimidazole derivatives showed larger antimicrobial activity than the ligands applied alone.[27]

The development of resistence to current antibacterial therapy continues to drive the search for more effective agents. Following our previous studies in view of obtaining some potential biological active compounds,[4-7] the present paper reports on the antimicrobial activity of the copper(II) complexes containing 1-benzylbenzimidazoles against three gram-positive bacterial reference strains and one gram-negative reference strain.

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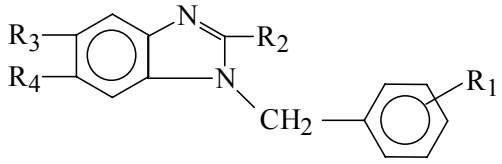
RESULTS

In the present paper we evaluated the antibacterial activity of copper(II) complexes with the following starting ligands: 1-(3-chlorobenzyl)-2-aminobenzimidazole(L^1), 1-(3-fluorobenzyl)-2-aminobenzimidazole(L^2), 1-(3-chlorobenzyl)-2-amino-5,6-dimethylbenzimidazole(L^3), 1-(3-fluorobenzyl)-2-amino-5,6-dimethylbenzimidazole(L^4) and 1-(3-methylbenzyl)-2-amino-5,6-dimethylbenzimidazole(L^5) (Table 1).

Table 1
Structural formulae of the ligands



Series I	R_1	R_2	R_3	R_4
L^1	m-Cl	NH_2	H	H
L^2	m-F	NH_2	H	H
Series II				
L^3	m-Cl	NH_2	CH_3	CH_3
L^4	m-F	NH_2	CH_3	CH_3
L^5	m-CH_3	NH_2	CH_3	CH_3

The results of antibacterial studies of the copper(II) complexes with two series of 1-benzylbenzimidazole derivatives tested by the agar disc-diffusion method are summarized in Table 2. As it can be seen from the data, all the investigated compounds displayed *in vitro* antimicrobial activity against very persistent microorganisms. They were found to be more active against gram-positive than gram-negative bacteria (*Pseudomonas aeruginosa*). In the case of gram-negative isolate only complexes of ligands from series I exhibited significant antibacterial activity. Copper(II) complexes of L^3, L^4 and L^5 were slightly or very slightly active against the *Pseudomonas aeruginosa*. Gram-positive bacteria *Bacillus cereus* was persistent in all investigated cases. Copper(II) complexes of ligands L^1 and L^2 express higher activity against this gram-positive bacteria than complexes of ligands from series II. In the case of *Staphylococcus aureus* and *Sarcina lutea* copper(II)complexes containing L^1 and L^2 were very highly or highly active, respectively. On the other hand, complexes of L^3, L^4 and L^5 were moderately active against the same bacteria.

Table 2

In vitro antimicrobial activity copper (II) complexes at a concentration of 1000μg/mL

Compound	*P.aeruginosa*	*B. cereus*	*S. aureus*	*S. lutea*
$Cu(L^1)_2Cl_2{\cdot}2H_2O$	+++	+++	++++	++++
$Cu(L^2)_2Cl_2{\cdot}H_2O$	++	+++	+++	+++
$Cu(L^3)_2Cl_2{\cdot}2H_2O$	+	++	++	++
$Cu(L^4)_2Cl_2$	+	++	++	++
$Cu(L^5)_2Cl_2{\cdot}H_2O$	+/-	++	++	++

Very highly active ++++. Highly active +++. Moderately active ++.
Slightly active +. Very slightly active +/-. Inactive ∅. Indication valid for Tables 3-6.

In the second phase, MIC of the tested compounds was performed by the agar dilution method. The results are presented in Tables 3-6. The compounds which are not shown in the table had no antibacterial activity at tested concentration.

Table 3

Antimicrobial activities of copper(II) complexes against *P.aeruginosa* at different concentration

Compound	Concentration [μg/mL]				
	750	500	250	125	60
$Cu(L^1)_2Cl_2{\cdot}2H_2O$	+++	+	+	∅	∅
$Cu(L^2)_2Cl_2{\cdot}H_2O$	++	+	∅	∅	∅
$Cu(L^3)_2Cl_2{\cdot}2H_2O$	+	∅	∅	∅	∅
$Cu(L^4)_2Cl_2$	+	∅	+	∅	∅

From the results presented in Table 3, it is seen that copper(II)complex containing L^1 was active against *Pseudomonas aeruginosa* with a MIC value of 250μg/mL, whilst $Cu(L^2)_2Cl_2{\cdot}H_2O$ was less toxic with a MIC value of 500 μg/mL. However, $Cu(L^3)_2Cl_2{\cdot}2H_2O$ and $Cu(L^4)_2Cl_2$ were same active with a MIC value of 750 μg/ml, but complex of L^5 has the low activity against the same bacteria.

Table 4

Antimicrobial activities of copper(II) complexes against *Bacillus cereus* at a different concentration

Compound	Concentration [μg/mL]				
	750	500	250	125	60
$Cu(L^1)_2Cl_2{\cdot}2H_2O$	+++	++	++	+	∅
$Cu(L^2)_2Cl_2{\cdot}H_2O$	++	++	+	+	∅
$Cu(L^3)_2Cl_2{\cdot}2H_2O$	++	+	+	∅	∅
$Cu(L^4)_2Cl_2$	++	+	+	∅	∅
$Cu(L^5)_2Cl_2{\cdot}H_2O$	++	+	∅	∅	∅

In the case of *Bacillus cereus* (Table 4) complexes containing ligands of series I were more active (MIC=125μg/mL) than complexes of second series. $Cu(L^3)_2Cl_2{\cdot}2H_2O$ was equally active as $Cu(L^4)_2Cl_2$ with higher MIC value of 250μg/mL against the same bacteria, whilst complex containing L^5 expressed MIC of 500μg/mL.

On the other hand, complexes of both series were more active against *Staphylococcus aureus* and *Sarcina lutea* than against *Bacillus cereus* (Tables 5 and 6). The complex of L^3 with a MIC value of 125μg/mL has the same activity as $Cu(L^4)_2Cl_2$, but complexes of series I were the most active and MIC of 62.5μg/mL was obtained. $Cu(L^5)_2Cl_2{\cdot}H_2O$ has the lowest activity against these two Gram-positive bacteria (MIC=250μg/mL).

Table 5

Antimicrobial activities of copper(II) complexes against *S. aureus* at a different concentration

Compound	Concentration [μg/mL]				
	750	500	250	125	60
$Cu(L^1)_2Cl_2{\cdot}2H_2O$	+++	+++	++	+	+
$Cu(L^2)_2Cl_2{\cdot}H_2O$	+++	++	++	+	+
$Cu(L^3)_2Cl_2{\cdot}2H_2O$	++	+	+	+	∅
$Cu(L^4)_2Cl_2$	++	+	+	+	∅
$Cu(L^5)_2Cl_2{\cdot}H_2O$	++	+	+	∅	∅

Table 6

Antimicrobial activities of copper(II) complexes against *S. lutea* at a different concentration

Compound	Concentration [μg/mL]				
	750	500	250	125	60
$Cu(L^1)_2Cl_2 \cdot 2H_2O$	+++	+++	++	+	+
$Cu(L^2)_2Cl_2 \cdot H_2O$	+++	++	++	+	+
$Cu(L^3)_2Cl_2 \cdot 2H_2O$	++	+	+	+	∅
$Cu(L^4)_2Cl_2$	++	+	+	+	∅
$Cu(L^5)_2Cl_2 \cdot H_2O$	++	+	+	∅	∅

DISCUSSION

By comparing the activities of the tested complexes it was found that 1-substituted-2-aminobenzimidazole derivatives (L^1, L^2) formed the copper(II)complexes which were more active than complexes of 1-substituted-2-amino-5,6-dimethylbenzimidazoles (L^3, L^4, L^5). Consequently, it is suggested that an amino group as a substituent at the position 2 enhances the general antimicrobial activity of benzimidazole. At the same time, methyl groups at the 5 or 6 position decreases the general antibacterial activity of the relevant benzimidazoles. Also, antimicrobial results shows that if the benzimidazole nucleus was substituted with a 3-chlorobenzyl group at the N1 atom, the antimicrobial activity was increased.

The differences found in the activities of the copper(II) complexes and the non-complexed ligands obtained in our previous investigations,[28] suggest that the coordinated Cu(II) may play a significant role in the antimicrobial potency. A possible explanation may be offers by the chelation theory stating a relationship between decreasing polarizability of the metal and increasing lipophilicity of the complexes. This property is now seen as an important parameter related to membrane permeation in biological system. Many of the processes of drug disposition depend on the ability or inability to cross membranes and hence there is a high correlation with measures of lipophilicity. Moreover, many of the proteins involved in drug disposition have hydrophobic binding sites further adding to the importance of lipophilicity. The latter might promote antimicrobial activity.

Moreover, the results of this study revealing that the compounds tested displayed higher activity against the gram-positive than the gram-negative one bacteria, likely point to the relevance of the structure of the bacterial cell wall in the antimicrobial potency of the substances. It is prospective because the cell wall is essential to the survival of many bacteria and some antibiotics are able to kill bacteria by inhibiting a step in the synthesis of peptydoglycan. Gram-positive bacteria possess a thick cell wall containing many layers of peptidoglycan and teichoic acids, but in contrast, Gram-negative bacteria have a relatively thin cell wall consisting of a few layers of peptidoglycan surrounded by a second lipid membrane containing lipopolysaccharides and lipoproteins. These differences in cell wall structure can produce differences in antibacterial susceptibility and some antibiotics can kill only Gram-positive bacteria and is ineffective against Gram-negative Pathogens.[29]

EXPERIMENTAL

All the ligands were synthesized by Vlaović *et al.* according to a procedure described earlier.[30] Copper(II) complexes were prepared following the same procedure described in our previous paper. [7]

Antibacterial investigations

All the copper(II) complexes were tested for their *in vitr*o growth inhibitory activity against *Bacillus cereus* ATCC 10876*, Staphylococcus aureus* ATCC 25923, *Sarcina lutea* ATCC 9341 and *Pseudomonas aeruginosa* ATCC 27853.

Antimicrobial activities of the complexes were evaluated by the disc-diffusion method under standard conditions using Mueller-Hinton agar medium as described by NCCLS.[29] Each of the investigated isolates of bacteria was seeded in the tubes with nutrient broth (NB). It was taken 1 cm^3 of seeded NB and homogenized in tubes with 9 cm^3 of melted (45°C) nutrient agar (NA). The homogenous suspension was poured out in Petri dishes. The discs of filter paper (diameter 5 mm) were ranged on cool medium. After cooling on formed solid medium, $2 \cdot 10^{-5} dm^3$ of the investigated compounds (γ=1000μg/mL) were placed by micropipette. After incubation of 24 hours in thermostat at 25-27°C, inhibition (sterile) zone diameters (including disc) were measured (in mm). Inhibition zone diameter more than 8 mm indicates the tested compound is active against microorganisms. Every test was done in three replications. Antimicrobial activities of the free ligands against the same bacteria were tested in our previous studies.[31]

Minimum inhibitory concentration (MIC) was performed by the agar dilution method according to guidelines established by the NCCLS standard M7-A5.[32] MIC was described as the lowest concentration of the compound that visibly inhibited colony`s growth. Stock solutions of the compounds were prepared in dimethylformamide (DMF). Further dilutions were performed with distilled water. The concentration range of the compounds tested was between 60-750μg/mL in two-fold dilution steps. The inoculated plates were than incubated at 35 ^{o}C for 16-20h. A control using DMF without any test complex was included for each organisms. It was determined that the solvent had no activity against any of the test microorganisms.

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