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ANTIMICROBIAL ACTIVITY OF COBALT(II) COMPLEXES WITH 2-AMINOBENZIMIDAZOLE DERIVATIVES

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Cobalt(II) chloride reacts with 2-aminobenzimidazole derivatives to give complexes of the formula [CoL_2Cl_2], where L=2-aminobenzimidazole, 1-benzyl-2-aminobenzimidazole and 1-(4-methylbenzyl)-2-aminobenzimidazole. All the ligands and their cobalt(II) complexes were evaluated for their in vitro antimicrobial activity against Pseudomonas aeruginosa, Bacillus sp., Staphylococcus aureus, Sarcina lutea and Saccharomyces cerevisiae. The minimum inhibitory concentration (MIC) was determined for all ligands and their complexes. It was found that tested compounds were more active against gram-positive than gram-negative bacteria. None of the compounds were significantly effective against yeast Saccharomyces cerevisiae, except 2-aminobenzimidazole complex, which moderately inhibited the growth of yeast. 1-(4-methylbenzil)-2-aminobenzimidazole was found to be slightly active against Saccharomyces cerevisiae. The same ligand showed the lowest MIC value of 60 μg/ml against Pseudomonas aeruginosa, as well as 125 μg/ml, against Bacillus sp. and Sarcina lutea. The MIC value of its cobalt(II) complex was 60 μg/ml against Pseudomonas aeruginosa. Cobalt(II) complex with 1-benzyl-2-aminobenzimidazole showed the lowest MIC value of 60 μg/ml against Staphylococcus aureus. The effect of ligand and complex structure on the antimicrobial activity was discussed.

KEYWORDS: Benzimidazole; complexes; cobalt(II) ; antimicrobial activity; in vitro studies

INTRODUCTION

Benzimidazole and its derivatives are interesting heterocycles because of their presence in many various medicaments. It has been found that they possess antibacterial, antifungal, antihistaminic, cytostatic, local analgesic, hypotensive and antiinflammatory activity (1-6). It was confirmed to have a moderate in vitro anti-HIV activity (7,8). In recent years, transition metal complexes have attracted particular interest because of their poten-

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potential use in several homogeneously catalyzed processes, such as hydrogenation, hydroformylation and acetylation reactions. However, in the recent time, possible therapeutic properties of the metal complexes have been examined. It was found that the complexes of transition metals with benzimidazole derivatives showed a higher antimicrobial activitiy than the corresponding ligands (9-13).

Following our studies of the reactivity of benzimidazole derivatives with metallic halides (10-13), we evaluated the antimicrobial activity of this type of complexes in this study. We report *in vitro* antimicrobial activities of 2-amino benzimidazoles and their cobalt(II) complexes against three gram-positive bacterial strains: *Bacillus* sp.*, Staphylococcus aureus* and *Sarcina lutea,* one gram-negative isolate: *Pseudomonas aeruginosa* and yeast, *Saccharomyces cerevisiae*.

EXPERIMENTAL

Reagents

All chemicals used to prepare the ligands and complexes were of analytical reagent grade, commercially available from different sources. The ligands were synthesized as described in the literature (14).

Synthesis of complexes

All the complexes were prepared following the same procedure. A solution of 5 mmol of $CoCl_2 \times 6H_2O$ in 10 cm^3 of EtOH was added to a solution of 10 mmol of the ligands (2-aminobenzimidazole (L^1), 1-benzyl-2-aminobenzimidazole (L^2) and 1-(4-methylbenzil)-2-aminobenzimidazole (L^3)) in 10 cm^3 EtOH. The resulting mixture was boiled under reflux on a water bath for about 2 h and then cooled. The complexes were separated from the reaction mixture by filtration, washed with EtOH and dried *in vacuo* over $CaCl_2$.

Antimicrobial investigations

Antimicrobial activity was tested by the disc-diffusion method under standard conditions, using Mueller-Hinton agar medium as described by NCCLS (15). Each of the investigated isolates of bacteria was seeded in the tubes with nutrient broth (NB), and 1 cm^3 of seeded NB and homogenized was taken in tubes with 9 cm^3 of melted (45°C) nutrient agar (NA). The homogeneous 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=1000 μg/ml) were placed by micropipette. After incubation for 24 hours in thermostat at 25-27°C, inhibition (sterile) zone diameters (including disc) were measured and expressed in mm. Inhibition zone diameter over 8 mm indicates the tested compound is active against microorganisms under investigation. Every test was done in three replications. In parallel with antimicrobial investigations of the complexes, ligands were tested too.

Minimum inhibitory concentration was determined by the agar dilution method according to guidelines established by the NCCLS standard M7-A5 (16). MIC was descri-

bed 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 60-750 μg/ml in two-fold dilution steps. The inoculated plates were then incubated at 35°C for 16-20 h.

RESULTS AND DISCUSSION

The antimicrobial activity of the 2-aminobenzimidazole derivatives and their cobalt (II) complexes was tested first by the agar disc-diffusion method against gram-positive and gram-negative bacteria and yeast. The results of these studies are summarized in Table 1. From the data given in Table 1 it is clear that the tested compounds were more active against gram-positive bacteria than against gram-negative *Pseudomonas aeruginosa*. It may be concluded that the antimicrobial activity of the compounds is related to cell wall structure of the bacteria.

Table 1. In vitro antimicrobial activity of ligands and their complexes at a concentration of 1000 μg/ml

Compound	*P. aeruginosa*	*Bacillus* sp.	*S. aureus*	*S. lutea*	*S. cerevisiae*
L^1	+	∅	∅	∅	∅
$[Co(L^1)_2Cl_2]$	∅	+++	+++	+++	++
L^2	+	++	++	++	∅
$[Co(L^2)_2Cl_2]$	+++	+++	+++	+++	∅
L^3	++	++++	++++	++++	+
$[Co(L^3)_2Cl_2]$	+++	+++	+++	+++	∅

Very highly active ++++, Highly active +++, Moderately active ++, Slightly active +, Inactive ∅

None of the compounds were found to be significantly effective against yeast *Saccharomyces cerevisiae*, except for $[Co(L^1)_2Cl_2]$, which moderately inhibited the growth of yeast. 1-(4-methylbenzil)-2-aminobenzimidazole (L^3) was found to be slightly active against *Saccharomyces cerevisiae.*

Table 2. Antimicrobial activities of ligands and their complexes against *Pseudomonas aeruginosa* at different concentrations

Compound	Concentration (μg/ml)				
	750	500	250	125	60
L^2	+	∅	∅	∅	∅
$[Co(L^2)_2Cl_2]$	++	+	+	∅	∅
L^3	+++	++	++	++	+
$[Co(L^2)_2Cl_2]$	+++	++	++	++	+

Table 3. Antimicrobial activities of ligands and their complexes against *Bacillus* sp. at different concentrations

Compound	Concentration (μg/ml)				
	750	500	250	125	60
$[Co(L^1)_2Cl_2]$	++	+	+	∅	∅
L^2	+	+	∅	∅	∅
$[Co(L^2)_2Cl_2]$	++	+	+	∅	∅
L^3	++	++	+	+	∅
$[Co(L^2)_2Cl_2]$	++	++	+	∅	∅

Table 4. Antimicrobial activities of ligands and their complexes against *Staphylococcus aureus* at different concentrations

Compound	Concentration (μg/ml)				
	750	500	250	125	60
$[Co(L^1)_2Cl_2]$	++	++	+	∅	∅
L^2	+	∅	∅	∅	∅
$[Co(L^2)_2Cl_2]$	++	++	++	++	+
L^3	+++	++	++	+	∅
$[Co(L^2)_2Cl_2]$	++	++	++	∅	∅

Table 5. Antimicrobial activities of ligands and their complexes against *Sarcina lutea* at different concentrations

Compound	Concentration (μg/ml)				
	750	500	250	125	60
$[Co(L^1)_2Cl_2]$	++	+	+	∅	∅
L^2	+	+	∅	∅	∅
$[Co(L^2)_2Cl_2]$	++	+	+	∅	∅
L^3	++	++	+	+	∅
$[Co(L^2)_2Cl_2]$	++	++	+	∅	∅

The results of MIC determination by the agar dilution method are presented in Tables 2-5. The compounds not shown in the table had no antibacterial activity at the tested concentration.

The results presented in Tables 2-5 indicate that the complex containing cobalt(II) was more active than the starting ligand L^1 against *Bacillus* sp*., Staphylococcus aureus* and *Sarcina lutea* with a MIC value of 250 μg/ml, but less active against *Pseudomonas aeruginosa*. On the other hand, the cobaltl(II) complex was more active than the starting ligand L^2, with a MIC value of 250 μg/ml, and equally active as ligand L^3 with a MIC value of 60 μg/ml against *Pseudomonas aeruginosa*. In the cases of other bacteria the complex was more active than its ligand L^2, but less active than ligand L^3. MIC value of

$[Co(L^2)_2Cl_2]$ was 250 μg/ml, against *Bacillus* sp. and *Sarcina lutea,* as wel as 60μg/ml against *Staphylococcus aureus.* The complex containing L^3 were active against *Bacillus* sp*., Sarcina lutea* and *Staphylococcus aureus* with a MIC value of 250μg/ml, but its ligand was more toxic, with a MIC value of 125 μg/ml, against the same bacteria. Comparing the activities of the tested ligands and their complexes, it was found that if the benzimidazole nucleus was substituted with a 4-methylbenzyl group at the N1 atom, the antimicrobial activity increased (14). Considering of the structural formula of the compounds that exhibited antimicrobial activity, it can be concluded that substituted ligands and cobalt moiety may play a role in the antimicrobial activity. The antimicrobial potentials of the investigated benzimidazole derivatives are similar to those of different benzimidazoles that were determined in the previous studies (2-4, 10-14).

CONCLUSIONS

Cobalt(II) chloride reacts with 2-aminobenzimidazole derivatives to give complexes of the formula $[CoL_2Cl_2]$, where L^1=2-aminobenzimidazole, L^2=1-benzyl-2-aminobenzimidazole and L^3=1-(4-methylbenzyl)-2-aminobenzimidazole. All the ligands and their cobalt(II) complexes were evaluated for their *in vitro* antimicrobial activity against *Pseudomonas aeruginosa*, *Bacillus* sp., *Staphylococcus aureus*, *Sarcina lutea* and *Saccharomyces cerevisiae*. It was found that the tested compounds were more active against gram-positive than gram-negative bacteria. None of the compounds were significantly effective against yeast *Saccharomyces cerevisiae*, except for the 2-aminobenzimidazole complex, which moderately inhibited the growth of yeast. 1-(4-methylbenzil)-2-aminobenzimidazole was found to be slightly active against *Saccharomyces cerevisiae*. The complex was more active than the starting ligand L^1 against *Bacillus* sp*., Staphylococcus aureus* and *Sarcina lutea* with a MIC value of 250 μg/ml. In the case of *Pseudomonas aeruginosa* the complex was less active than its ligand L^1, more active than ligand L^2,with a MIC value of 250 μg/ml and equally active as ligand L^3, with a MIC value of 60 μg/ml. MIC value of L^2 complex was 250μg/ml, against *Bacillus* sp. and *Sarcina lutea,* and 60 μg/ml against *Staphylococcus aureus.* The complex containing L^3 was active against *Bacillus* sp*., Sarcina lutea* and *Staphylococcus aureus*, with a MIC value of 250 μg/ml, but its ligand was more toxic, with a MIC value of 125 μg/ml against the same bacteria. Comparing the activities of the tested ligands and their complexes, it was found that if the benzimidazole nucleus was substituted with a 4-methylbenzyl group at the N1 atom, the antimicrobial activity increased. On considering the structural formula of the compounds that exhibited antimicrobial activity, it can be concluded that substituted ligands and cobalt moiety may play a role in the antimicrobial activity.

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АНТИМИКРОБНА АКТИВНОСТ КОБАЛТ(II) КОМПЛЕКСА СА ДЕРИВАТИМА 2-АМИНОБЕНЗИМИДАЗОЛА

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Кобалт(II)-хлорид реагује са дериватима 2-аминобензимидазола дајући комплексе типа $[CoL_2Cl_2]$ (L=2-аминобензимидазол, 1-бензил-2-аминобензимидазол и 1-(4-метилбензил)-2-аминобензимидазол). Испитана је антимикробна активност лиганада и њихових комплекса са кобалтом на *Pseudomonas aeruginosa*, *Bacillus* sp., *Staphylococcus aureus*, *Sarcina lutea* и *Saccharomyces cerevisiae*. За све лиганде и њихове комплексе одређена је минимална инхибиторна концентрација (МИК). Испитивана једињења показују већу инхибиторну активност према грам-позитивним, него према грам-негативним бактеријама. Ниједно од испитиваних једињења, изузев 2-аминобензимидазола, не показује значајнију активност на раст квасца *Saccharomyces cerevisiae.* 1-(4-метилбензил)-2-аминобензимидазол показује веома слаб инхибиторни ефекат на квасац. За *Pseudomonas aeruginosa* најнижу МИК вредност имају 1-(4-метилбензил)-2-аминобензимидазол и његов комплекс и она износи 60 μg/ml. МИК за *Bacillus* sp.и *Sarcina lutea* је такође најнижа за 1-(4-метилбензил)-2-аминобензимидазол и износи 125 μg/ml. За бактерију *Staphylococcus aureus* најнижа МИК је 60 μg/ml и карактеристична је за комплекс кобалта(II) са 1-бензил-2-аминобензимидазолом. Дискутован је утицај структуре лиганда и комплекса на њихову антимикробну активност.

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