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Antibacterial activity of cobalt(II) complexes with some benzimidazole derivatives

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Abstract: The antibacterial activities of cobalt(II) complexes with two series of benzimidazoles were evaluated *in vitro* against three Gram-positive bacterial strains (*Bacillus cereus*, *Staphylococcus aureus*, and *Sarcina lutea*) and one Gram-negative isolate (*Pseudomonas aeruginosa*). The minimum inhibitory concentration was determined for all the complexes. The majority of the investtigated complexes displayed *in vitro* inhibitory activity against very persistent bacteria. They were found to be more active against Gram-positive than Gram-negative bacteria. It may be concluded that the antibacterial activity of the compounds is related to the cell wall structure of the tested bacteria. Comparing the inhibitory activities of the tested complexes, it was found that the 1-substituted--2-aminobenzimidazole derivatives were more active than complexes of 1-substituted-2-amino-5,6-dimethylbenzimidazoles. The effect of chemical structure on the antibacterial activity is discussed.

Keywords: benzimidazole derivatives; complexes; cobalt(II); antibacterial; *in vitro* studies.

INTRODUCTION

The benzimidazole nucleus, which is a useful structure for further research and for the development of new pharmaceutical molecules, has received a great deal of attention in the last decade. Due to their antimicrobial activities, new benzimidazoles have been synthesized and investigated for medical applications. The position and type of the substituents on the benzimidazole ring are responsible for the variety of their biological activities. Many derivatives of benzimidazole are well known as antibacterial agents.[1–7] This class of compounds has been found to show antimicrobial activities against Gram-positive and Gram-negative bacteria, primarily because of the potential bio-activity of benzimidazole--based ligands.[8–10] Hence, the incorporation of imidazole and benzimidazole nuclei is an important synthetic strategy in drug discovery.

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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 of various strains of microorganisms.[11–14] Antimicrobial activity of this class of compounds against *Helicobacter pylori*[15] and oral *Streptococci*[16] has also been reported. The synthesis of benzimidazoles fused to another heterocyclic ring has attracted widespread attention due to their diverse application as antioxidant,[17,18] antifungal,[19] antitubercular,[20] anticancer,[21,22] and antiallergic drugs.[23] Various benzimidazoles are also effective inhibitors of the growth of the HIV-virus.[24,25]

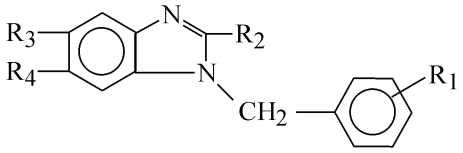
In the last period, possible therapeutical properties of metal complexes with derived benzimidazoles have also excited wide interest. It was found that the complexes of transition metal salts with benzimidazole derivatives showed greater antimicrobial activity than the ligands applied alone.[26]

The development of resistance to current antibacterial therapy continues to drive the search for more effective agents. In order to obtain more potent compounds, our previous studies[4–9] prompted us to investigate the antibacterial activity of cobalt(II) complexes containing 1-benzylbenzimidazoles against three Gram-positive bacterial strains and one Gram-negative isolate. The effect of the ligand and complex structure on the inhibitory activity of tested compounds was also examined.

EXPERIMENTAL

In the present study, the antibacterial activity of cobalt(II) complexes with the following starting ligands: 2-amino-1-(3-chlorobenzyl)benzimidazole (L^1), 2-amino-1-(3-fluorobenzyl)–benzimidazole (L^2), 2-amino-1-(3-chlorobenzyl)-5,6-dimethylbenzimidazole (L^3), 2-amino-1--(3-fluorobenzyl)-5,6-dimethylbenzimidazole (L^4) and 2-amino-5,6-dimethyl-1-(3-methylbenzyl)benzimidazole (L^5), was evaluated (Table I).

TABLE I. Structural formulae of the ligands



	R_1	R_2	R_3	R_4
		Series I		
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

All the ligands were synthesized by Vlaović *et al.* according to a procedure described earlier.[27] The cobalt(II) complexes were prepared following the same procedure described in a previous paper.[7]

Antibacterial investigations

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

The antibacterial activities of the complexes were tested by the disc-diffusion method under standard conditions using Mueller-Hinton agar medium, as described by NCCLS.[28] Each of the investigated isolates of bacteria was seeded in tubes with nutrient broth (NB). 1 cm^3 of seeded NB was 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 the cool medium. After cooling on the formed solid medium, 0.02 cm^3 of the investigated compounds (c = 1000 μg/ml) were placed by micropipette. After incubation of 24 h at 25–27 °C, the inhibition (sterile) zone diameters (including disc) were measured (in mm). An inhibition zone diameter over 8 mm indicates the tested compound is active against the microorganism. Every test was done in triplicate. Antimicrobial activities of the free ligands against the same bacteria were tested in a previous study.[8]

Minimum inhibitory concentration (*MIC*) was determined by the agar dilution method according to guidelines established by the NCCLS standard M7-A5.[29] The *MIC* is described as the lowest concentration of a compound that visibly inhibited the growth of a colony. 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 than incubated at 35 °C for 16–20 h. A control using DMF without any test complex was included for each organism. It was found that the solvent had no activity against any of the test micro-organisms.

RESULTS AND DISCUSSION

The results of the antibacterial studies of the cobalt(II) complexes with the two series of 1-benzylbenzimidazole derivatives tested by the agar disc-diffusion method are summarized in Table II.

TABLE II. *In vitro* antibacterial activity of the complexes at a concentration of 1000 μg/ml

Complex	Inhibition zone diameter, mm			
	Pseudomonas aeruginosa	*Bacillus cereus*	*Staphylococcus aureus*	*Sarcina lutea*
$Co(L^1)_2Cl_2$	22	26	26	29
$Co(L^2)_2Cl_2$	17	24	24	25
$Co(L^3)_2Cl_2$	9	17	17	18
$Co(L^4)_2Cl_2$	8	16	17	17
$Co(L^5)_2Cl_2$	5	16	17	16

It is evident that the majority of the investigated compounds displayed *in vitro* antimicrobial activity against very persistent micro-organisms. The investigated complexes were found to be more active against Gram-positive than Gram-negative bacteria (*P. aeruginosa*). In the case of Gram-negative isolate, only com-

plexes of ligands from Series I exhibited significant antibacterial activity. The cobalt(II) complexes of L^3, L^4 and L^5 were slightly active against *P. aeruginosa*. In the case of *B. cereus* and *S. aureus*, the cobalt(II) complexes of ligands L^1 and L^2 also expressed higher activity than the complexes of ligands from Series II. The Gram-positive bacterium *S. lutea* was persistent in all investigated cases. The cobalt(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.

In the second phase, the *MIC* of the tested compounds was determined by the agar dilution method. The results are presented in Tables III–VI. The compounds which are not shown in the tables had no antibacterial activity at the tested concentration.

TABLE III. Antibacterial activities of the complexes against *P. aeruginosa* at different concentrations (inhibition zone diameter in mm)

Complex	*MIC* / μg ml^{-1}				
	750	500	250	125	62.5
$Co(L^1)_2Cl_2$	18	8	5	0	0
$Co(L^2)_2Cl_2$	15	6	0	0	0
$Co(L^3)_2Cl_2$	5	0	0	0	0
$Co(L^4)_2Cl_2$	5	0	0	0	0
$Co(L^5)_2Cl_2$	0	0	0	0	0

From the results presented in Table III, it is seen that cobalt(II) complex containing L^1 was active against *P. aeruginosa* with a *MIC* value of 250 μg/ml, whilst $Co(L^2)_2Cl_2$ was less toxic with a *MIC* value of 500 μg/ml. However, $Co(L^3)_2Cl_2$ and $Co(L^4)_2Cl_2$ had the same activity with a *MIC* value of 750 μg/ml, but the complex of L^5 had a low activity against the same bacteria.

TABLE IV. Antibacterial activities of the complexes against *B. cereus* at different concentrations (inhibition zone diameter in mm)

Complex	*c* / μg ml^{-1}				
	750	500	250	125	62.5
$Co(L^1)_2Cl_2$	20	16	10	5	0
$Co(L^2)_2Cl_2$	19	14	8	3	0
$Co(L^3)_2Cl_2$	14	9	4	0	0
$Co(L^4)_2Cl_2$	12	7	4	0	0
$Co(L^5)_2Cl_2$	11	5	0	0	0

In the case of *B. cereus* and *S. aureus* (Tables IV and V, respectively), the complexes containing ligands of Series I were more active (*MIC* = 125 μg/ml) than the complexes of second series. $Co(L^3)_2Cl_2$ had the same activity as $Co(L^4)_2Cl_2$ with a high *MIC* value of 250 μg/ml against the same bacteria, whilst the complex containing L^5 expressed a *MIC* value of 500 μg/ml.

TABLE V. Antibacterial activities of the complexes against *S. aureus* at different concentrations (inhibition zone diameter in mm)

Complex	c / μg ml^{-1}				
	750	500	250	125	62.5
$Co(L^1)_2Cl_2$	20	15	9	4	0
$Co(L^2)_2Cl_2$	19	13	8	3	0
$Co(L^3)_2Cl_2$	14	9	5	0	0
$Co(L^4)_2Cl_2$	13	9	4	0	0
$Co(L^5)_2Cl_2$	12	6	0	0	0

On the other hand, the complexes of both series were more active against *S. lutea* (Table VI). The complex of L^3 with a *MIC* value of 125 μg/ml had the same activity as $Co(L^4)_2Cl_2$ but the complexes of Series I were more active and a *MIC* value of 62.5 μg/ml was obtained. $Co(L^5)_2Cl_2$ had the lowest activity against this Gram-positive bacterium (*MIC* = 250 μg/ml).

TABLE VI. Antibacterial activities of the complexes against *S. lutea* at different concentrations (inhibition zone diameter in mm)

Complex	c / μg ml^{-1}				
	750	500	250	125	62.5
$Co(L^1)_2Cl_2$	25	20	16	8	5
$Co(L^2)_2Cl_2$	21	17	14	7	3
$Co(L^3)_2Cl_2$	14	10	7	3	0
$Co(L^4)_2Cl_2$	13	9	5	3	0
$Co(L^5)_2Cl_2$	11	7	3	0	0

Comparing the activities of the tested complexes, it was found that the 1-substituted-2-aminobenzimidazole derivatives (L^1, L^2) formed cobalt(II) complexes which were more active than the complexes of the 1-substituted-2-amino-5,6-dimethylbenzimidazoles (L^3, L^4, L^5). It can be concluded that the basic antibacterial activity of the benzimidazoles was produced by the presence of an amino group at position 2 of the benzimidazole ring. Simultaneously, methyl groups at the 5 or 6 positions decreased the general antibacterial activity of the relevant benzimidazoles. Also, the antibacterial results show that if the benzimidazole nucleus was substituted with a 3-chlorobenzyl group at the N1 atom, the antibacterial activity was increased.

By comparing the antimicrobial activity of the ligands[8] and their complexes, it was found that the complexes were more effective against all bacteria. This is the result of the coordinated cobalt which plays a significant role for the antibacterial activity. The chelation theory explains that a decrease in the polarizability of the metal can change the lipophilicity or hydrophobicity of complexes. These properties are now seen as important parameters related to membrane permeation in biological systems. 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.

By consideration of the structures of compounds that exhibit antimicrobial activity, it can be concluded that substituted ligands and the metal moiety may play a role in determining the antibacterial activity. From the results which indicated that the tested compounds were more active against Gram-positive than Gram-negative bacteria, it may be concluded that the inhibitory activity of the studied compounds is related to the cell wall structure of the bacteria. This is possible because the cell wall is essential to the survival of bacteria and some antibiotics are able to kill bacteria by inhibiting a step in the synthesis of peptidoglycan. 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.[30]

CONCLUSIONS

The antibacterial activity of cobalt(II) complexes with two series of 1-benzylbenzimidazole derivatives was tested against very persistent microorganisms: *Pseudomonas aeruginosa*, *Bacillus cereus*, *Staphylococcus aureus* and *Sarcina lutea*. All the complexes displayed *in vitro* inhibitory activity, but the 1-substituted-2-aminobenzimidazole derivatives formed cobalt(II) complexes which were more active than the complexes of the 1-substituted-2-amino-5,6-dimethylbenzimidazoles. The basic antibacterial activity of the benzimidazoles was produced by the presence of an amino group at position 2 of the benzimidazole ring. Methyl groups at the 5 or 6 position decreased the general antibacterial activity of the relevant benzimidazole. Also, the results indicated that the tested complexes were more active against Gram-positive than Gram-negative bacteria. It may be concluded that the antibacterial activity of the compounds is related to the cell wall structure of the bacteria. This is possible 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 peptidoglycan.

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И З В О Д

АНТИБАКТЕРИЈСКА АКТИВНОСТ КОБАЛТ(II)-КОМПЛЕКСА СА НЕКИМ ДЕРИВАТИМА БЕНЗИМИДАЗОЛА

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Испитана је *in vitro* антибактеријска активност кобалт(II) комплекса са две серије бензимидазола према три грам-позитивне бактерије (*Bacillus cereus*, *Staphylococcus aureus* и *Sarcina lutea*) и једној грам-негативној бактерији (*Pseudomonas aeruginosa)*. За све комплексе одређена је минимална инхибиторна концентрација. Већина испитиваних комплекса показала је *in vitro* инхибиторну активност према веома отпорним бактеријама. Утврђено је да испитивани комплекси показују већу активност према грам-позитивним него према грам-негативној бактерији, што указује на то да антибактеријска активност једињења зависи од грађе ћелијског зида. Поређењем инхибиторне активности тестираних комплекса дошло се до закључка да су комплекси деривата 1-супституисаних-2-аминобензимидазола активнији од деривата 1-супституисаних-2-амино-5,6-диметилбензимидазола. Продискутован је утицај хемијске структуре на антибактеријску активност.

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