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Original scientific paper

Antibacterial evaluation of some benzimidazole derivatives and their zinc(II) complexes

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Abstract: Zinc(II) chloride was reacted with some 1-benzylbenzimidazole derivatives (L) to give complexes of the formula ZnL_2Cl_2. All the ligands and their zinc(II) complexes were evaluated for their *in vitro* antibacterial activity against *Pseudomonas aeruginosa, Bacillus cereus, Staphylococcus aureus* and *Sarcina lutea*. The majority of the investigated compounds displayed *in vitro* antimicrobial activity against very persistent microorganisms. It was found that all the tested compounds were more active against gram-positive than gram-negative bacteria. The minimum inhibitory concentration (MIC) was determined for all ligands and their complexes. The effect of the structure of the ligands and complexes on the antimicrobial activity is discussed. The complexes were found to be more toxic than the ligands.

Keywords: benzimidazole derivatives, complexes, zinc(II), antibacterial activity, *in vitro* studies.

INTRODUCTION

Benzimidazoles are remarkably effective compounds both with respect to their bacteria inhibitory activity and their favourable selectivity ratio. Extensive biochemical and pharmacological studies have confirmed that these molecules are effective against various strains of microorganisms.[1,2] Some benzimidazole compounds inhibit the biosynthesis of ergosterol, required in the cell membrane of fungi. They have antibacterial, antifungal, and antiviral activity.[3–6] This ring system is present in numerous antiparasitic and antitumoral drugs.[7,8] The benzimidazole structure is part of the nucleotide portion of vitamine B_{12} and the nucleus of some drugs, such as proton pump inhibitors and anthelmintic agents. Proton pump inhibitors (PPIs) are substituted benzimidazole derivatives that selectively and irreversibly inhibit the gastric hydrogen–potassium adenosine triphosphatase (H^+K^+-ATPase) pump mechanism. The antimicrobial activity of this class of compounds was investigated against *Helicobacter pylori*[9] and against oral streptococci.[10] Also, benzimidazole and its derivatives are of considerable importance because of their antihistaminic, cytostatic,

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local analgesic, hypotensive and anti-inflammatory activity.[11] Benzimidazole was confirmed to have moderate *in vitro* anti-HIV effect.[12]

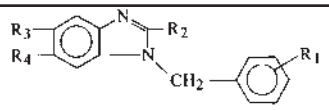
In last decades, heterocyclic benzimidazoles, their derivatives and transition metal complexes have received considerable attention in coordination chemistry, because of their well-documented biological activities. It was found that such complexes showed larger antimicrobial activities than the free ligands.[13,14] The success with these compounds stimulated the search for new biologically active derivatives.

Following studies concering the reactivity of benzimidazole derivatives with various metal ions,[15–17] the objective of the present study was to investigate the antimicrobial activity of this type of compounds. The *in vitro* antibacterial activities of 1-benzylbenzimidazole derivatives and their zinc(II) complexes are reported herein.

EXPERIMENTAL

In the present study the antibacterial activity of zinc(II) complexes and 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) and 1-(3-fluorobenzyl)-2-amino-5,6-dimethylbenzimidazole (L^4) (Table I) were evaluated.

TABLE I. 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

All the ligands were synthesized by Vlaović *et al.* according to a procedure described earlier.[18] The zinc(II) complexes were prepared following the same procedure as described in a previous paper.[15]

Antibacterial investigations

Antimicrobial activities of the ligands and their complexes were evaluated against three gram positive bacterial strains: *Bacillus cereus* (ATCC 10876), *Staphylococcus aureus* (ATCC 25923) and *Sarcina lutea* (ATCC 9341) and one gram negative isolate: *Pseudomonas aeruginosa* (ATCC 27853). The activity was tested by the disc-diffusion method under standard conditions using Mueller–Hinton agar medium, as described by NCCLS.[19] Each of the investigated isolates of bacteria was seeded in tubes with nutrient broth (NB). The seeded NB (1 cm^3) was homogenized in tubes with 9 cm^3 of melted (45 °C) nutrient agar (NA). The homogenous suspension was poured into Petri dishes. Discs of filter paper (diameter 5 mm) were ranged on the cool medium. After cooling, 2×10^{-5} dm^3 of the investigated compounds (γ = 1000 μg/ml) were placed by micropipette on the formed solid medium. After incubation for 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 that the tested compound is active against microorganisms. Every test was done in three replications. Parallel with the antimicrobial investigations of the complexes, the free ligands were also tested.

The determination of the minimum inhibitory concentration (MIC) was performed by the agar dilution method according to guidelines established by the NCCLS standard M7-A5.[20] The MIC was described as the lowest concentration of the 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 then incubated at 35 °C for 16–20 h.

RESULTS AND DISCUSSION

The antibacterial activity of the 1-benzylbenzimidazole derivatives and their zinc(II) complexes was first tested by the agar disc diffusion method against gram-positive and gram-negative bacteria. The results of these studies are summarized in Table II.

TABLE II. *In vitro* antimicrobial activity of the ligands and their complexes at a concentration of 1000 μg/ml

Compound	*P. aeruginosa*	*B. cereus*	*S. aureus*	*S. lutea*
L^1	++	++	+++	+++
$Zn(L^1)_2Cl_2$	+++	+++	++++	++++
L^2	+	++	++	++
$Zn(L^2)_2Cl_2$	++	+++	+++	+++
L^3	Ø	+	+	+
$Zn(L^3)_3Cl_2$	+/–	++	++	++
L^4	Ø	+	+	+
$Zn(L^4)_2Cl_2$	+/–	++	++	++

Very highly active ++++. Highly active +++. Moderately active ++. Slightly active +. Very slightly active +/–. Inactive Ø. Indication valid for Tables III–VI.

As can be seen from the data, the majority of 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 the gram-negative isolate, only ligands L^1 and L^2, as well as their zinc(II) complexes exhibited antibacterial activity. Zinc(II) complexes of L^3 and L^4 were very slightly active against *P. aeruginosa*. The main physiological difference between gram-positive and gram-negative bacteria is the structure of their cell wall. The outer layer of the outer membrane of gram-negative bacteria is composed primarily of lipopolysaccharide molecules and forms a hydrophilic permeability barrier, providing protection against the effect of highly hydrophobic drugs.[21,22] This may explain the low sensitivity of *P. aeruginosa* to the more lipophilic L^3 and L^4, as well as their zinc complexes.

The gram-positive bacteria *Bacillus cereus* was persistent in all investigated cases. Ligands L^1 and L^2 were more active than L^3 and L^4, and their complexes expressed higher activity against this gram positive bacteria than the starting ligands.

In the case of *Staphylococcus aureus* and *Sarcina lutea*, ligand L^2 exhibited middle inhibitory activity, whilst its zinc(II) complex was highly active against the same bacteria. The zinc(II) complex containing L^1 was very highly active against *Staphylococcus aureus* and *Sarcina lutea*, but its starting ligand was less active. On the other hand, both ligands L^3 and L^4 were slightly active against the same bacteria, but their complexes were more toxic.

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

TABLE III. Antimicrobial activities of the ligands and their complexes against *P. aeruginosa* at different concentrations

Compound	Concentration/μg ml^{-1}				
	750	500	250	125	60
L^1	++	+	+	Ø	Ø
$Zn(L^1)_2Cl_2$	+++	++	+	+	Ø
L^2	+	+	Ø	Ø	Ø
$Zn(L^2)_2Cl_2$	++	++	+	Ø	Ø

From the results presented in Table III, it can be seen that the zinc(II) complex containing L^1 was active against *P. aeruginosa* with a MIC value of 125 μg/ml, but its starting ligand was less toxic, with a MIC value of 250 μg/ml. However, ligand L^2 was less active than L^1 with a MIC value of 500 μg/ml, but its complex has the same activity as ligand L^1 with a MIC value of 250 μg/ml.

TABLE IV. Antimicrobial activities of the ligands and their complexes against *B. cereus* at different concentrations

Compound	Concentration/μg ml^{-1}				
	750	500	250	125	60
L^1	++	++	+	Ø	Ø
$Zn(L^1)_2Cl_2$	+++	++	++	+	Ø
L^2	++	+	+	Ø	Ø
$Zn(L^2)_2Cl_2$	++	++	+	+	Ø
L^3	+	Ø	Ø	Ø	Ø
$Zn(L^3)_2Cl_2$	++	+	Ø	Ø	Ø
L^4	+	Ø	Ø	Ø	Ø
$Zn(L^4)_2Cl_2$	++	+	Ø	Ø	Ø

In the case of *B. cereus*, the complexes containing zinc(II) were more active than the starting ligands L^1 and L^2, with a MIC value of 125 μg/ml. Ligand L^1 was

equally active as L^2 with a higher MIC value of 250 μg/ml against the same bacteria. However, ligands L^3 and L^4 expressed MIC of 750 μg/ml, but their complexes were more toxic (the MIC values were 500 μg/ml).

TABLE V. Antimicrobial activities of the ligands and their complexes against *S. aureus* at different concentrations

Compound	Concentration/μg ml^{-1}				
	750	500	250	125	60
L^1	+++	++	+	+	Ø
$Zn(L^1)_2Cl_2$	+++	+++	++	+	+
L^2	++	++	+	+	Ø
$Zn(L^2)_2Cl_2$	+++	++	++	+	+
L^3	+	+	Ø	Ø	Ø
$Zn(L^3)_2Cl_2$	++	+	+	Ø	Ø
L^4	++	+	Ø	Ø	Ø
$Zn(L^4)_2Cl_2$	++	+	+	Ø	Ø

On the other hand, ligands L^1 and L^2, as well as their zinc(II) complex were more active against *S. aureus* and *S. lutea* than against *B. cereus*. The starting ligand L^1, with a MIC value of 125 μg/ml, had the same activity as L^2, but their complexes were the most active and a MIC of 60 μg/ml was obtained.

TABLE VI. Antimicrobial activities of the ligands and their complexes against *S. lutea* at different concentrations

Compound	Concentration/μg ml^{-1}				
	750	500	250	125	60
L^1	+++	++	+	+	Ø
$Zn(L^1)_2Cl_2$	+++	+++	++	+	+
L^2	++	++	+	+	Ø
$Zn(L^2)_2Cl_2$	+++	++	++	+	+
L^3	+	+	Ø	Ø	Ø
$Zn(L^3)_2Cl_2$	++	+	+	Ø	Ø
L^4	++	+	Ø	Ø	Ø
$Zn(L^4)_2Cl_2$	++	+	+	Ø	Ø

In the case of the same microorganisms, the zinc(II) complexes of L^3 and L^4 were more active than the starting ligands. The MIC values of $Zn(L^3)_2Cl_2$ and $Zn(L^4)_2Cl_2$ were 250 μg/ml, whilst L^3 and L^4 were active with a MIC value of 500 μg/ml against *S. aureus* and *S. lutea*.

Comparing the activities of the tested ligands, it was found that l-substituted-2-aminobenzimidazole derivatives (L^1, L^2) were more active than l-substi-

tuted-2-amino-5,6-dimethylbenzimidazoles (L^3, L^4). 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. At the same time, methyl groups at the 5 or 6 position decreased the general antibacterial activity of the relevant benzimidazoles. This is in agreement with previous results.[15] Also, the antimicrobial results show that ligand L^1 is more active than ligand L^2, which indicates that if the benzimidazole nucleus was substituted with a 3-chlorobenzyl group at the N1 atom, the antimicrobial activity was increased.

By comparing the antimicrobial activity of the ligands and their complexes, it was found that the complexes were more effective against all the bacteria. This fact suggests that coordinated zinc may play a significant role for antimicrobial activity. This can be explained in terms of the chelation theory, which states that a decrease in the polarizability of the metal can change the lipophilicity of the complexes. It is well known that this property is responsible for the transport and distribution of drugs in a biological system.

Considering the structures of the compounds which exhibited antimicrobial activity, the substituted ligands and metal moiety may play a role in antimicrobial activity. From the results which indicated that tested compounds were more active against gram-positive than gram-negative bacteria, it may be concluded that the antimicrobial activity of the compounds is related to the structure of the cell wall of the bacteria.

CONCLUSION

Zinc(II) chloride was reacted with some 1-benzylbenzimidazole derivatives (L) to give complexes of the formula ZnL_2Cl_2, where L = 1-(3-chlorobenzyl)-2-aminobenzimidazole, 1-(3-fluorobenzyl)-2-aminobenzimidazole, 1-(3-chlorobenzyl)-2-amino-5,6-dimethylbenzimidazole and 1-(3-fluorobenzyl)-2-amino-5,6-dimethylbenzimidazole. All the ligands and their zinc(II) complexes displayed *in vitro* antimicrobial activity against very persistent microorganisms: *Pseudomonas aeruginosa, Bacillus cereus, Staphylococcus aureus* and *Sarcina lutea*. The tested compounds were more active against gram-positive bacteria than against the gram-negative *P. aeruginosa*. It may be concluded that the antimicrobial activity of the compounds is related to the structure of the cell wall of the bacteria.

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 decrease the general antibacterial activity of the relevant benzimidazoles. Also, if the benzimidazole nucleus was substituted with a 3-chlorobenzyl group at the N1 atom, the antimicrobial activity was increased. The zinc(II) complexes were more effective than the starting ligands against all the tested bacteria. This can be explained in terms of the chelation theory, which states that a decrease in the polarizability of the metal can change the lipophilicity of the

complexes. Consideration of the structures of the compounds which exhibit antimicrobial activity indicates that the substituted ligands and the metal moiety may play a role in the antimicrobial activity.

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ИЗВОД

АНТИБАКТЕРИЈСКО ИСПИТИВАЊЕ НЕКИХ НОВОСИНТЕТИСАНИХ ДЕРИВАТА БЕНЗИМИДАЗОЛА И ЊИХОВИХ ЦИНК(II)-КОМПЛЕКСА

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Хлорид цинка(II) реагује са дериватима 1-бензилбензимидазола (L) дајући комплексе опште формуле ZnL_2Cl_2. Испитана је антимикробна активност лиганада и њихових комплекса са цинком(II) на *Pseudomonas aeruginosa, Bacillus cereus, Staphylococcus aureus* и *Sarcina lutea.* Већина испитиваних лиганада и њихових комплекса показују *in vitro* антимикробну активност према веома отпорним микроорганизмима. Сва тестирана једињења показују већу активност према грам-позитивним него према грам-негативним бактеријама. За све лиганде и њихове комплексе одређена је минимална инхибиторна концентрација (MIC) и продискутован је утицај структуре лиганда и комплекса на њихову антимикробну активност. Нађено је да комплекси показују већу биолошку активност од самих лиганда.

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