SANJA O. PODUNAVAC–KUZMANOVIĆ
DRAGOLJUB D. CVETKOVIĆ

Faculty of Technology, University of Novi Sad, Novi Sad

SCIENTIFIC PAPER

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ANION EFFECT ON ANTIMICROBIAL ACTIVITY OF METAL COMPLEXES WITH BENZIMIDAZOLE DERIVATIVE

Chloride and nitrate of copper(II) and zinc(II) react with 1–(4–chlorobenzoyl)–2–methylbenzimidazole (L) to give complexes of the type $ML_2A_2 \cdot nH_2O$ *(M=Cu or Zn; A=*Cl^- *or* NO_3^-*; n=0, 1 or 2). Ligand and its complexes were evaluated for their in vitro antimicrobial activity against three Gram–positive bacterial strains: Bacillus sp., Staphylococcus aureus and Sarcina lutea, one Gram–negative isolate: Pseudomonas aeruginosa and one yeast: Saccharomyces cerevisiae. The compounds were more active against Gram–positive than Gram–negative bacteria. None of the compounds were significantly effective against the yeast S. cerevisiae, except copper(II) complex with chloride anion, which moderately inhibited the yeast growth. Zinc(II) complex with chloride was found to be slightly active against Saccharomyces cerevisiae. The anion effect on antimicrobial activity of tested compounds was discussed. The minimum inhibitory concentration (MIC) was determined for the ligand and its complexes. The most active compound was copper(II) complex with chloride anion. The MIC value of this complex was 60 μg/ml against Bacillus sp. and 125 μg/ml against S. aureus and S. lutea.*

Key words: Benzimidazole, complexes, copper(II), zinc(II), antimicrobial activity, MIC.

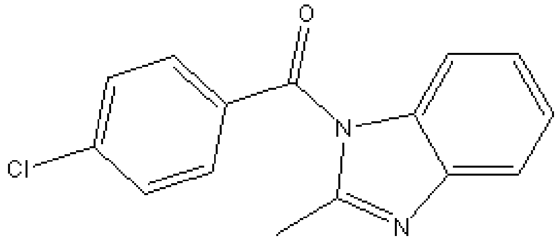
Imidazole and benzimidazole systems are presented in a large number of common therapeutic agents. They were used in organic and medicinal chemistry. Their derivatives with substituents, such as methyl and chloro in different positions, have been found to have an inhibitory effect on the growth of several yeasts and bacteria. The synthesis of benzimidazoles fused to another heterocyclic ring has attracted great attention due to their diverse use as antibacterial, antiviral, antitumorial and anti–inflammatory agents [1–7]. Various benzimidazoles are effective growth inhibitors of lactobacilli, vaccinia virus, influenza virus and HIV–virus [8,9].

The coordination chemistry of benzimidazole and its derivatives has received considerable attention because of their biological significance and interesting spectral, magnetic and structural aspects. Regarding previous observations [10–13] according to which the presence of the metal ions considerably enhance the biological activity of organic molecules, we report the antimicrobial study of copper(II) and zinc(II) complexes with 1–(4–chlorobenzoyl)–2–methylbenzimidazole. Antimicrobial activities of the ligand and its complexes were evaluated against some test microorganisms.

EXPERIMENTAL

Reagents

All chemicals used to prepare the ligand and its complexes were of an analytical reagent grade, commercially available from different sources. The ligand 1–(4–chlorobenzoyl)–2–methylbenzimidazole (L) was synthesized as described elsewhere [14].



Synthesis of complexes

All the complexes were prepared following the same procedure. The solution of 10 mmol of metal chloride or nitrate in 10 cm^3 of ethanol was added to a solution of 20 mmol of ligand. 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 ethanol and dried *in vacuo* over $CaCl_2$.

Antimicrobial investigations

Antimicrobial activities of ligand and its complexes were evaluated against three Gram–positive bacterial strains (*Bacillus* sp., *Staphylococcus aureus* and *Sarcina lutea*), one Gram–negative isolate (*Pseudomonas aeruginosa*) and one yeast (*Saccharomyces cerevisiae*). The activity was tested by a disc–diffusion method, under standard conditions, using Mueller–Hinton agar medium as described by NCCLS [15]. Each of the investigated bacterial isolates was seeded in the tubes with nutrient broth (NB). Seeded NB (1 cm^3) was taken and homogenized with 9 cm^3 of melted (45°C) nutrient

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Authors address: S.O. Podunavac–Kuzmanović, Faculty of Technology, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia
E–mail: sanya@uns.ns.ac.yu

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agar in tubes (NA). The homogenous suspension was poured out in to Petri dishes. The discs of filter paper (diameter 5 mm) were ranged on the cool medium. After cooling 2·10–5 dm^3 of the investigated compound (γ=1000 μg/cm^3) were placed by micropipette on the solid medium. After incubation of 24 hours, in a thermostat at 25–27°C, the inhibition (sterile) zone diameters (including disc) were measured. The inhibition zone diameter over 8 mm indicated that the tested compound was active against microorganisms being investigated. Each test was done in triplicate and an average inhibitions zone of diameter was presented. Both the complexes and ligands were tested. A minimum inhibitory concentration (MIC) was assessed by the agar dilution method according to the NCCLS standard M7–A5 [16]. MIC was described as the lowest concentration of the compound that visibly inhibited the colony 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/cm^3 in two–fold dilution steps. The inoculated plates were then incubated at 35°C for 16–20 hours.

RESULTS AND DISCUSSION

The antimicrobial activity of 1–(4–chlorobenzoyl)–2–methylbenzimidazole and its copper(II) and zinc(II) complexes was first tested by the disc diffusion method against Gram–positive and Gram–negative bacteria and a yeast. The results of these studies are summarized in Table 1.

As it can be seen from Table 1, the majority of the investigated compounds displayed *in vitro* antimicrobial activity against very persistent microorganisms. Data clearly show that tested compounds were more active against Gram–positive bacteria than Gram–negative *P. aeruginosa*. None of the compounds were significantly effective against *S. cerevisiae*, except $CuL_2Cl_2\cdot 2H_2O$, which moderately inhibited the growth of yeast. ZnL_2Cl_2 complex was found to be slightly active against *S. cerevisiae*.

MIC of the compounds tested are presented in Tables 2–5. The starting ligand was active against all tested Gram positive bacteria with a MIC value of 250 μg/cm^3, as well as in the case of gram negative *P. aeruginosa* with a MIC value of 500 μg/ml the complex containing copper(II) with chloride anion was more active against *S. aureus* and *S. lutea* (MIC = 125 μg/cm^3), as well as *Bacillus* sp (MIC = 60 μg/cm^3). That complex was equally active against *P. aeruginosa* as ligand. On the other hand, copper(II) complex with nitrate anion was less active than ligand in the case of Gram–positive bacteria (MIC = 500 μg/cm^3) *P. aeruginosa* (MIC = 750 μg/cm^3).

The complex containing zinc(II) with chloride anion was equaly active as its ligand against all tested bacteria. The MIC value of this complex against *P. aeruginosa* was 500 μg/cm^3. This complex was more toxic against the Gram–positive bacteria, the MIC value being 250 μg/cm^3. In the case of zinc(II) complex with nitrate anion, the MIC value against *S. aureus* and *S. lutea* was 750 μg/cm^3 and 500 μg/cm^3 against *Bacillus* sp. The same complex was less active than its starting ligand against *P. aeruginosa* with the MIC value of 750 μg/c,^{3}.

Comparing the activities of the complexes containing different anions, the complexes with chloride

Table 1. In vitro antimicrobial activity of ligands and its complexes at a concentration of 1000 μg/cm^3

Compound	Diameter of inhibition zone (mm)				
	P. aeruginosa	*Bacillus* sp.	*S. aureus*	*S. lutea*	*S. cerevisiae*
L	26.67	32.33	32.00	32.00	Ø
$CuL_2Cl_2\cdot 2H_2O$	28.00	34.33	33.33	33.00	19.67
$CuL_2(NO_3)_2\cdot H_2O$	18.33	28.33	27.67	27.00	Ø
ZnL_2Cl_2	28.67	32.00	31.00	31.67	14.33
$ZnL_2(NO_3)_2$	17.67	27.00	18.30	17.67	Ø

No inhibition Ø

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

Compound	Diameter of inhibition zone (mm)				
	Concentration (μg/ml)				
	750	500	250	125	60
L	22.33	17.67	Ø	Ø	Ø
$CuL_2Cl_2\cdot 2H_2O$	23.67	16.33	Ø	Ø	Ø
$CuL_2(NO_3)_2\cdot H_2O$	14.67	Ø	Ø	Ø	Ø
ZnL_2Cl_2	24.00	14.33	Ø	Ø	Ø
$ZnL_2(NO_3)_2$	12.33	Ø	Ø	Ø	Ø

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

Compound	Diameter of inhibition zone (mm)				
	Concentration (μg/ml)				
	750	500	250	125	60
L	29.67	21.67	12.33	Ø	Ø
$CuL_2Cl_2\cdot 2H_2O$	32.00	26.00	19.67	12.00	+
$CuL_2(NO_3)_2\cdot H_2O$	24.00	16.33	Ø	Ø	Ø
ZnL_2Cl_2	26.67	19.33	12.67	Ø	Ø
$ZnL_2(NO_3)_2$	24.00	16.33	Ø	Ø	Ø

Table 4. Antimicrobial activities of ligands and their complexes against S. aureus at a different concentration

Compound	Diameter of inhibition zone (mm)				
	Concentration (μg/ml)				
	750	500	250	125	60
L	29.67	22.33	14.33	Ø	Ø
$CuL_2Cl_2\cdot 2H_2O$	30.00	24.00	16.33	12.00	Ø
$CuL_2(NO_3)_2\cdot H_2O$	21.67	14.33	Ø	Ø	Ø
ZnL_2Cl_2	23.33	16.67	12.33	Ø	Ø
$ZnL_2(NO_3)_2$	14.33	Ø	Ø	Ø	Ø

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

Compound	Diameter of inhibition zone (mm)				
	Concentration (μg/ml)				
	750	500	250	125	60
L	26.00	17.33	11.00	Ø	Ø
$CuL_2Cl_2\cdot 2H_2O$	29.00	22.00	16.67	11.33	Ø
$CuL_2(NO_3)_2\cdot H_2O$	22.33	14.00	Ø	Ø	Ø
ZnL_2Cl_2	27.00	22.00	14.00	Ø	Ø
$ZnL_2(NO_3)_2$	11.00	Ø	Ø	Ø	Ø

anion were found to be more active against all the bacteria than those containing nitrate. This meant that nitrate anion reduced the general antimicrobial activity of the relevant copper(II) and zinc(II) complexes with 1–(4–chlorobenzoyl)–2–methylbenzimidazole.

Compared to the activities of the tested ligand only $CuL_2Cl_2\cdot 2H_2O$ of the tested complexes were more active against Gram–positive bacteria, but equally active against Gram–negative *P. aeruginosa*. The results presented in Tables 2–5 indicated that the complexes containing copper(II) were more active than zinc(II) complexes. In our opinion, coordinated metal(II) may play a significant role in antimicrobial activity. This can be explained by the chelation theory according to which a decrease in the polarizability of the metal can enhance the lipophilicity of the complexes.

CONCLUSIONS

Chloride and nitrate of copper(II) and zinc(II) reacts with 1–(4–chlorobenzoyl)–2–methylbenzimidazole(L) to give complexes of type $ML_2A_2\cdot nH_2O$ (M=Cu or Zn; A=Cl^- or NO_3^-; n=0, 1 or 2). All the complexes and their starting ligand were evaluated for their in vitro antimicrobial activity against *P. aeruginosa*, *Bacillus* sp., *S. aureus*, *S. lutea* and *S. cerevisiae*. None of the compounds were significantly effective against yeast *S. cerevisiae*, except for copper(II) complex with chloride anion, which moderately inhibited the yeast growth. Zinc(II) complex with chloride was slightly active against *S. cerevisiae*. Comparing the activities of the tested complexes contain different anions, complexes with chloride anion were found to be more active than those containing nitrate. These results lead to the conclusion that nitrate anion reduced the general antimicrobial activity of the relevant copper(II) and zinc(II) complexes with 1–(4–chlorobenzoyl)– 2–methylbenzimidazole.

The complexes containing copper(II) were more active than zinc(II) complexes, meaning that. Coordinated metal(II) might play a significant role in antimicrobial activity. This can be explained by the chelation theory, according to which a decrease in the polarizability of the metal can enhance the lipophilicity of the complexes.

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