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SYNTHESIS, PHYSICO-CHEMICAL CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF 2-AMINOBENZIMIDAZOLE COMPLEXES WITH DIFFERENT METAL IONS

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Complexes of 2-aminobenzimidazole (L) with nitrates of cobalt(II), nickel(II), copper (II), zinc(II) and silver(I) were synthesized. The molar ratio metal:ligand in the reaction of the complex formation was 1:2. It should be noticed, that the reaction of all the metal salts yielded bis(ligand) complexes of the general formula $M(L)_2(NO_3)_2 \times nH_2O$ *(M=Co, Ni, Cu, Zn or Ag; n=0, 1, 2 or 6). The complexes were characterized by elemental analysis of the metal, molar conductivity, magnetic susceptibility measurements and IR spectra. Co(II), Ni(II) and Cu(II) complexes behave as non-electrolytes, whilst Zn(II) and Ag(I) are 1:1 electrolytes. Cu(II) complex has a square-planar stereochemistry, Ag(I) complex is linear, whilst the Co(II), Ni(II) and Zn(II) complexes have a tetrahedral configuration. In all the complexes ligand is coordinated by participation of the pyridine nitrogen of the benzimidazole ring. The antimicrobial activity of the ligand and its complexes against Pseudomonas aeruginosa, Bacillus sp., Staphylococcus aureus and Saccharomyces cerevisiae was investigated. The effect of metal on the ligand antimicrobial activity is discussed.*

KEYWORDS: Benzimidazole; complexes; cobalt(II); nickel(II); copper(II), zinc(II); silver(I); physico-chemical characterization; antimicrobial activity

INTRODUCTION

Physiological activity and commercial applications of many benzimidazole derivatives have received much attention. Benzimidazole and its derivatives have different activities as they can act as bacteriostats or bactericides, fungicides, anticarcinogens, etc (1-4). This ring system is present in numerous antiparasitic, antihelmintic and anti-inflammatory

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drugs (5). Several thousands of analogs of imidazole and benzimidazole have been synthesized and screened for pharmacological activity. Some of these compounds exhibited anti-HIV activity (6,7). The complexes of transition metals with benzimidazole and related ligands have been extensively studied as models of some important biological molecules (8-12).

We have recently reported isolation, characterization and antimicrobial activities of different metal complexes wiith benzimidazole derivatives (9-12). As an extension of our previous studies, the objective of the present work was to synthesize and study physico-chemical characteristics, as well as antimicrobial activitiy of 2-aminobenzimidazole complexes with nitrates of cobalt(II), nickel(II), copper(II), zinc(II) and silver(I). Antimicrobial activities of the ligand and its complexes were evaluated against *Pseudomonas aeruginosa, Bacillus sp., Staphylococcus aureus* and *Saccharomyces cerevisiae.*

EXPERIMENTAL

Reagents

All chemicals used to prepare the complexes were of analytical reagent grade, commercially available from different sources.

Synthesis of complexes

All the complexes were prepared following the same procedure. A solution of 5 mmol of the metal nitrate in 10 cm^3 of EtOH was added to a solution of 10 mmol of the ligand (2-aminobenzimidazole (L)) in 10 cm^3 of 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 $CaCl_2$. The yield of the complexes varied in the range of 45-50%.

Measurement methods

The metal content was determined by using a chelatometric titration with EDTA. Magnetic susceptibility measurements were made at room temperature using an MSB-MKI magnetic susceptibility balance (Sherwood Scientific Ltd., Cambridge, England). Molar conductivies of freshly prepared 1×10^{-3} mol dm^{-3} solutions (DMF) were measured on a Jenway 4010 conductivity meter. Infrared spectra (KBr pellets) were recorded on an Infrared 457 Perkin-Elmer spectrophotometer.

Antimicrobial investigations

For these investigations the filter paper disc method was applied. Each of the investigated isolates of bacteria were seeded in the tubes with nutrient broth (NB). The seeded NB (1 cm^3) were homogenized in the tubes with 9 cm^3 of melted (45°C) nutrient agar (NA). The homogeneous suspension was poured into Petri dishes.

The discs of filter paper (diameter 5 mm) were ranged on cool. After cooling on the formed solid medium, 2×10^{-5} dm^3 of the investigated compounds were placed with micropipette. After incubation for 24 hours in a thermostat at 25-27°C, inhibition (sterile) zone diameters (including disc) were measured and expressed in mm. Inhibition zone diameter over 5.5 mm indicates the tested compound is active against bacteria under investigation. Every test was done in three replications.

The antimicrobial activities of the investigated compounds were tested against *Pseudomonas aeruginosa, Bacillus sp., Staphylococcus aureus* and *Saccharomyces cerevisiae.* In parallel with antibacterial investigations of Co(II), Ni(II), Cu(II), Zn(II) and Ag(I) complexes, ligands were tested too, as well as the pure solvent. The concentration of each solution was 5×10^{-2} mol dm^{-3}. Commercial DMF was used to dissolve the tested samples.

RESULTS AND DISCUSSION

The elemental analysis of complexes, magnetic moments and molar conductivity data are summarized in Table 1.

Table 1. Some physical characteristics and analytical data of the complexes

Complex	Colour	μ_{eff} (μ_B)	λ_M *	Metal % Found (Calcd.)
$Co(L)_2(NO_3)_2 \times 6H_2O$	blue	4.58	13.4[a]	10.27 (10.58)
$Ni(L)_2(NO_3)_2 \times H_2O$	violet	3.35	16.2[a]	12.14 (12.58)
$Cu(L)_2(NO_3)_2$	blue	1.83	15.6[a]	13.89 (14.01)
$Zn(L)_2(NO_3)_2 \times 2H_2O$	white	diam.	86.1[a]	13.02 (13.30)
$Ag(L)_2NO_3$	light brown	diam.	88.7[b]	24.32 (24.75)

* [a]DMF, [b]methanol, 1 mmol dm^{-3} solution at 25°C; in S cm^2 mol^{-1}

The complexes were synthesized in the reaction of warm ethanolic solution of the $Me(NO_3)_2 \times nH_2O$ (M = Co(II), Ni(II), Cu(II), Zn(II) and Ag(I)) with 2-aminobenzimidazole (L) in a mole ratio 1:2. It should be noticed that the reaction of all the metal ions yielded bis(ligand) complexes. All the complexes are insoluble or sparingly soluble in common organic solvents such as alcohols or acetone, but highly soluble in dimethylformamide and dimethylsulphoxide.

The molar conductivity values of the Co(II), Ni(II) and Cu(II) complexes (Table 1) indicate that these complexes behave as non-electrolytes. The molar conductivity of Zn(II) complex in DMF solutions fall in the range of 65-90 Scm^2mol^{-1}, corresponding to a 1:1 type of electrolyte (13). It indicates that one coordinated nitrate anion in the Zn(II) complex has been replaced by solvent molecule. The molar conductivity of the Ag(I) complex in methanol coresponds to a 1:1 type of electrolyte.

Magnetic properties

An indication of the most probable stereochemistry of the synthesized Co(II) complex is given by its colour and magnetic moment. Namely, blue cobalt(II) complexes usu-

ally have a tetrahedral configuration. The magnetic value of cobalt(II) complex (Table 1) are in the expected range (μ_{eff}=4.2-4.7μ_B) for a tetrahedral stereochemistry. The room temperature effective magnetic moment for Ni(II) complex suggests tetrahedral configuration, too, whilst the μ_{eff} value for Cu (II) complex supports square-planar geometry (14). Zinc(II) and Ag(I) complexes are diamagnetic so the complex of zinc is tetrahedral, whilst silver complex is linear.

Infrared spectra

The infrared spectrum of the ligand exhibits the bands at 3450-3330 cm^{-1} and ca. 1650 cm^{-1}, assigned to ν (NH_2) and δ (NH_2) of the benzimidazole ring, respectively (15). The band appearing at about 1560 cm^{-1} may be assigned to τηε ν (C=N) vibrations. The infrared spectra of the investigated complexes are similar to those of the corresponding ligands.

An upward shift (5-10 cm^{-1}) of ν(C=N) in the IR spectra of the complexes as compared to their values for the free ligand, suggests coordination through the pyridine nitrogen of 2-aminobenzimidazole (16). The bands due to ν (NH_2) and δ (NH_2) in the complexes are shifted to lower frequency. These shifts may be indicative of the presence hydrogen bonding between the $-NH_2$ group of the ligand and water molecules. The results indicate the presence of two types of nitrate: coordinated nitrate anions and free nitrate anions in synthesized complexes. The peak at 1030cm^{-1} is attributed to N-O stretching vibrations of the coordinated nitrate groups. A very strong band corresponding to NO_3 stretching of free nitrate anions is observed at 1378 cm^{-1}. The other bands in the spectrum of each complex are similar to those in the corresponding ligand spectrum except for slight shifts in their positions and changes in their intensities due to coordination.

The presented results (molar conductivities, magnetic moments and IR spectra) suggest that copper(II) complexe has a square-planar stereochemistry, silver(I) complex is linear, whilst the cobalt(II), nickel(II) and zinc(II) complexes have a tetrahedral configuration. In all the complexes (except silver complex), configurations are realized by coordination of the two organic ligand molecules through the pyridine nitrogen, nitrate anions or molecules of water. In the case of silver complex, water molecules and nitrate anions are not included in coordination.

Antimicrobial investigations

In this work, 2-aminobenzimidazole and its complexes were screened for their *in vitro* antimicrobial activity against the four microbial isolates. The antimicrobial activities of the compounds were tested by the agar disc-diffusion method. The results of these studies are summarized in Table 2.

As can be seen from Table 2, the majority of the investigated compounds displayed *in vitro* antimicrobial activity against very persistent microorganisms. The starting ligand (2-aminobenzimidazole), as well as its silver complex showed no antimicrobial activity. However, some metal ions (Co, Cu, Zn) coordinated with ligand strongly increased the general antimicrobial activity of the 2-aminobenzimidazole. In the case of gram-negative isolate *Pseudomonas aeruginosa* only copper(II) and zinc(II) complexes exhibited antibacterial activity. Gram-positive bacteria *Bacillus species* was persistent only in the case

of nickel(II) and silver(I) complexes. In the case of *Staphylococcus aureus* nickel(II) and copper(II) complexes exhibited very low inhibitory activity, whilst cobalt(II) and zinc(II) complexes were highly active against the same bacteria. Also, Ni(II) and Cu(II) complexes had no activity against yeast *Saccharomyces cerevisiae*, but Co(II) and Zn(II) complexes were very active. Of the complexes, the most active compound was the one containing zinc(II), which exhibited high inhibitory activity against all the tested isolates. Cobalt(II) complex was also highly active, except in the case of gram-negative bactera. Comparing the activities of the tested compounds, it can be seen that some of the complexes were more active than the starting ligand. In view of the structural formula of the complexes that exhibited antimicrobial activity, it can be thought that metal may play a significant role. This can be explained in terms of chelation theory, which states that a decrease in the polarizability of the metal can enhance the lipophilicity of the complexes (12).

Table 2. *In vitro* antimicrobial activity of 2-aminobenzimidazole and its complexes

Compound	Microbial isolates tested			
	P. aeruginosa	***B. species***	***S. aureus***	***S. cerevisiae***
L	+/–	∅	∅	∅
$Co(L)_2(NO_3)_2 \times 6H_2O$	∅	+++	+++	+++
$Ni(L)_2(NO_3)_2 \times H_2O$	∅	∅	+/-	∅
$Cu(L)_2(NO_3)_2$	+++	+++	+/-	∅
$Zn(L)_2(NO_3)_2 \times 2H_2O$	+++	+++	+++	+++
$Ag(L)_2NO_3$	∅	∅	∅	∅

∅ - no activity; +/– - very low inhibitory activity; + - low inhibitory activity;
+++ - high inhibitory activity

CONCLUSIONS

The ligand, 2-aminobenzimidazole (L), formed with cobalt(II), nickel(II), copper(II), zinc(II) and silver(I) the complexes of the general formula $M(L)_2(NO_3)_2 \times nH_2O$ (M=Co, Ni, Cu, Zn or Ag; n=0, 1, 2 or 6). The complexes were synthesized and characterized by elemental analysis of the metal, molar conductivity, magnetic susceptibility measurements and IR spectra. Co(II), Ni(II) and Cu(II) complexes behave as non-electrolytes, whilst Zn(II) and Ag(I) are 1:1 electrolytes. Cu(II) complex have a square-planar stereochemistry, Ag(I) complex is linear, whilst the Co(II), Ni(II) and Zn(II) complexes have a tetrahedral configuration. In all the complexes (except silver complex), configuration is realized by coordination of the pyridine nitrogen, nitrate anions or molecules of water. In the case of silver complex, water molecules and nitrate anions are not included in coordination. The results of antimicrobial investigations indicate that the starting ligand (2-aminobenzimidazole), as well as the silver complex, showed no antimicrobial activity. However, some metal ions (Co^{2+}, Cu^{2+}, Zn^{2+}) coordinated with the ligand, strongly increased the general antimicrobial activity of the 2-aminobenzimidazole. Of the complexes, the most active compound was the one containing zinc(II), which exhibited high inhibitory activity against all the tested isolates. Cobalt(II) complex was also highly active,

except in the case of gram-negative bactera. In view, of the structural formula of the complexes that exhibit antimicrobial activity, metal moiety may play a significant role in the antimicrobial activity.

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REFERENCES

1. Kucukbay, H., Durmaz, R., Orhan, E., S. Gunal: Synthesis, Antibacterial and Antifungal Activities of Electron-rich Olefins Derived Benzimidazole Compounds. Il Farmaco **58** (2003) 431-437.
2. Garuti, L., Roberti, M., C. Cermelli: Synthesis and Antiviral Activity of Some N-Benzenesulphonyl-benzimidazoles. Bioorg. Medicinal Chem. Letter **9** (1999) 2525-2530.
3. Gata, L., Perna, F., Figura, N., Ricci, C., Holton, J., D′Anna, L., Miglioli, M., D. Vaira: Antimicrobial Activity of Esomeprazole Versus Omeprazole Against *Helicobacter pylori*. J. Antimicrob. Chemother. **51** (2003) 439-444.
4. Garuti, L., Roberti, M., C. Cermelli: Synthesis and Antiviral Activity of Some N-Benzenesulphonyl-benzimidazoles. Bioorg. Medicinal Chem. Letter **9** (1999) 2525-2530.
5. El-Masry, A.H., Fahmy, H.H., S.H. Ali Abdelwahed: Synthesis and Antimicrobial Activity of Some New Benzimidazole Derivatives. Molecules **5** (2000) 1429-1438.
6. Akbay, A., Oren, I., Temiz-Arpaci, O., Aki-Sener, E., I. Yalcin: Synthesis and HIV-1 Reverse Transcriptase Inhibitor Activity of Some 2,5,6-Substituted Benzoxazole, Benzimidazole and Oxazolo(4,5-b) pyridine Derivatives. Arzneim.-Forcsh./Drug Res. **53** (2003) 266-271.
7. Casse, C., Giannoni, F., Nguyen, V.T., Dubois, M.F., O. Bensaude: The Transcriptional Inhibitors, Actino-mycin-D and Alpha-Amanitin, Activate the HIV-1 Promoter and Favor Phosphorylation of the RNA-Polymerase Terminal Domain. J. Biol. Chem. **274** (1999) 16097-16106.
8. Gumus, F., Algul, O., Eren, G., Eroglu, H., Diril, N., Gur, S., A. Ozkul: Synthesis, Cytotoxic Activity On MCF-7 and Mutagenic Activity of Platinum(II) Complexes with 2-Substituted Benzimidazole Ligands. E. J. Med. Chem. (2003) 303-307.
9. Podunavac-Kuzmanović, S.O., Lj.S. Vojinović,: Synthesis and Physico-Chemical Characterization of Zinc(II), Nickel(II) and Cobalt(II) Complexes with 2-Phenyl-2-Imidazoline. Acta Periodica Technologica **34** (2003) 119-124.
10. Podunavac-Kuzmanović, S.O., Leovac, V.M., Ćetković, G.S., S.L. Markov: Synthesis, Physico-Chemical Characterization and Biological Activity of Copper(II) and Nickel(II) Complexes with 1-Benzoyl-2-methylbenzimidazole Derivatives. Acta Periodica Technologica **33** (2002) 151-156.

11. Podunavac-Kuzmanović, S.O., Ćetković, G.S, Leovac, Markov, S.L., N.U., J.J. Rogan: Physico-chemical Characterization and Antibacterial Activity of Copper(II), Zinc(II) and Nickel(II) Complexes with 2-methylbenzimidazole. Acta Periodica Technologica **32** (2001) 145-149.
12. Podunavac-Kuzmanović, S.O., Leovac, V.M., Perišić-Janjić, N.U., Rogan, J., J. Balaž: Complexes of Cobalt(II), Zinc(II) and Copper(II) with Some Newly Synthesized Benzimidazole Derivatives and Their Antibacterial Activity. J. Serb. Chem. Soc. **64** (1999) 381-388.
13. W.J. Geary: The Use of Conductivity Measurements in Inorganic Solvents for the Characterization of Coordination Compounds. Coord. Chem. Rev. **7** (1971) 81-122.
14. Mabbs, F.E., D.J. Machin: Magnetism and Transition Metal Complexes. Chapman Hall, London (1973) 232.
15. K. Nakamoto: Inrfared and Raman Spectra of Inorganic and Cordination Compounds, Wiley, New York (1986) 324.
16. Arali, V.KH, Revankar, V.K., Mahale, V.B., P.J. Kulkarni: Cobalt(II), Nickel(II) and Copper(II) with 2-Substitited Benzimidazole Complexes. Transition Met. Chem. **19** (1994) 57-60.

СИНТЕЗА, ФИЗИЧКО-ХЕМИЈСКА КАРАКТЕРИЗАЦИЈА И БИОЛОШКА АКТИВНОСТ КОМПЛЕКСА 2-АМИНОБЕНЗИМИДАЗОЛА СА РАЗЛИЧИТИМ ЈОНИМА МЕТАЛА

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Синтетисани су комплекси 2-аминобензимидазола (L) са нитратима кобалта(II), никла(II), бакра(II), цинка(II) и сребра(I). Комплекси се добијају реакцијом топлих етанолних раствора лиганда са нитратима метала у молском односу 2:1, дајући комплексе типа $M(L)_2(NO_3)_2 \cdot nH_2O$ (M=Co, Ni, Cu, Zn или Ag; n=0, 1, 2 или 6). Комплекси су окарактерисани елементарном анализом (метал), магнетним и кондуктометријским мерењима и IR спектрима. Врло ниске вредности моларне проводљивости за комплексе кобалта(II), никла(II) и бакра(II) у ДМФ указују на неелектролитне карактеристике раствора комплекса. На основу вредности моларних проводљивости за комплексе цинка(II) и сребра(I) изводи се закључак да су то електролити типа 1:1. На основу вредности магнетних момената и IR спектара изолованих комплекса кобалта(II), никла(II) и цинка(II) може се претпоставити да испитивани комплекси имају тетраедарску структуру. За комплексе бакра(II) претпостав-

љена је квадратно-планарна структура, док су комплекси сребра(I) линеарни. Стереохемија свих синтетисаних комплекса (изузев комплекса сребра) реализује се монодентатном координацијом два молекула лиганда преко пиридинског атома азота и координацијом нитратна јона или молекула воде. У случају сребро(I) комп-лекса нитратни јони, као ни молекули воде не учествују у координацији. Испитана је антимикробна активност лиганда и његових комплекса на четири врсте микроорганизама: *Pseudomonas aeruginosa*, *Bacillus* sp., *Staphylococcus aureus* и *Saccharomyces cerevisiae*. Дискутован је утицај метала на повећање антимикробне активности лиганда.

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