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Biological activity of Zn(II) complexes with Schiff bases derived from some amino acids

Research article

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Abstract
The binuclear complex compounds of Zn**(II)** with three Schiff bases derived from some amino acids and 2,2'-(propane-1,3-diyldioxy)dibenzaldehyde were tested for their biological activity – antimicrobial and antitumor activity. The results of the biological tests indicated that all the three complexes of Zn(II) show biological activity.

Keywords: biological activity, Zn(II) complexes, Schiff bases, amino acids

1. INTRODUCTION

Schiff base metal complexes have a versatile biological activity towards various kinds of pathogens and tumors and present biochemical, clinical and pharmacological properties. The main reason for the biological properties is the presence of the imine group in this compounds [1]. Amino acids, a significant class of organic compounds, contain potential donor sites such as COOH and/or NH_2 which have

good ability to coordinate with the metal ions [2]. The Schiff bases derived from amino acids, when compared with the classical Schiff bases, have a more stable and higher solubility in organic solvents and new compounds can be derived in easier coordination form owing to the conformational flexibility of their backbones [3].

Many metal complexes with amino acid derived Schiff base have been synthesized and tested for their biological activity [4, 5]. It was found that these metal complexes are more active than the free Schiff base ligand. Especially, the Zn(II) complexes show higher activity than other metal complexes.

The synthesis and characterization of the Zn(II) binuclear complexes with three Schiff bases derived from some amino acids (2-aminobenzoic acid, L-tryptophan and tyrosine) and the aromatic dialdehyde, 2,2′-(propane-1,3-diyldioxy)dibenzaldehyde, were presented in a previous study [6].

In this paper we present the results of the biological tests for these Zn(II) binuclear complexes.

2. MATERIALS AND METHODS

2.1. Materials

The binuclear complexes of Zn(II) with Schiff base ligands derived from three amino acids and 2,2′-(propane-1,3-diyldioxy)dibenzaldehyde were prepared according to a procedure described in a previous study, by condensation of the amino acid, aromatic dialdehyde and zinc salt in alcoholic medium [6].

Using this procedure, the complex $[Zn_2(C_{31}H_{24}O_6N_2)(OH)_2(H_2O)_4]$ (**1**) was obtained by refluxing the ethanolic solution of 2,2′-(propane-1,3-diyldioxy)dibenzaldehyde, 2-aminobenzoic acid and zinc chloride in a 1:2:2 molar ratio. The complexes $[Zn_2(C_{39}H_{34}O_6N_4)(OAc)_2(H_2O)_4]$ (**2**) and $[Zn_2(C_{35}H_{32}O_8N_2)(OAc)_2(H_2O)_4]$ (**3**) were synthesized by condensation of the methanolic solution of the dialdehyde, L-tryptophan / tyrosine (in alkaline medium) and zinc acetate in a 1:2:2 molar ratio. Analytical grade reagents from Sigma and Merck were used in all experiments.

The microbial strains were isolated from different clinical sources and were identified by aid of VITEK I automatic system [7].

2.2. Methods for the antimicrobial assays

The antimicrobial activity of these compounds was tested against nine bacterial strains: Gram-positive *Staphylococcus aureus ATCC 25923*, *Bacillus sp.* strains, Gram-negative *Escherichia coli 1576*, *Salmonella sp. 9246*, *Pseudomonas aeruginosa 846*, *Klebsiella pneumoniae ESBL*$^{+}$, *Citrobacter freundii 1748*, *Providencia stuartii 1116*, *Serratia marcescens 0804* strains. Microbial suspensions of density corresponding to 0.5 McFarland UI obtained from 24 h microbial cultures developed on solid media were used in the experiments. The antimicrobial activity of these compounds was tested on Mueller-Hinton agar medium [8].

The tested compounds were dissolved in dimethylformamide (DMF) and used for the antimicrobial activity screening at 10 mg/mL concentration of stock solutions.

The qualitative screening was performed by an adapted diffusimetric method (the spot method). In this purpose, Petri dishes with Mueller Hinton medium were seeded with bacterial inoculum and then the stock solutions of the tested compounds (5 μL) were added as spots. The plates were left at room temperature for 20-30 min and then incubated at 37°C for 24 h. The positive results were read as the occurrence of an inhibition zone of microbial growth around the spot [9].

The quantitative assay of the antimicrobial activity was performed by binary micro dilution method, in liquid medium, distributed in 96 multi-well plates, in order to establish the minimum inhibitory concentration (MIC) [10]. In this purpose, serial binary dilutions of the tested compounds were performed in a 100 μL volume of liquid medium and each well was seeded with 20 μL of microbial inoculum of 0.5 McFarland UI density. The plates were incubated for 24 h at 37°C, and MICs were recorded in each case as the minimum concentration of the compound, which inhibited the visible growth of the tested microorganism [11].

2.3. Method for the cytotoxic tests

Evaluation of the cytotoxicity of these compounds was performed on HeLa cells, human cancer cells, using the MTT assay, at 24h and 48h incubation times and at different concentrations of the complex combinations (10, 50, 100, 200 $\mu g \cdot mL^{-1}$).

HeLa cells are the oldest human cancer cells most commonly used in scientific research [12].

The viability assay most commonly used throughout the world is the MTT assay, first described by Tim Mosmann in 1983 [13]. This colorimetric assay uses reduction of a yellow tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, or MTT) to measure cellular metabolic activity as a proxy for cell viability. Viable cells contain NAD(P)H-dependent oxidoreductase enzymes which reduce the MTT reagent to formazan, an insoluble crystalline product with a deep purple color. Formazan crystals are then dissolved using a solubilizing solution and absorbance is measured at 500-600 nm using a plate-reader (Absorbance Reader Tecan). Below is a brief description of the protocol steps:

1. Place 1000-100000 cells per well in a 96-well plate and incubate with the appropriate stimulus for the desired time (24, 48 hours).
2. Remove incubation medium and wash cells with PBS.
3. Add MTT in the incubation medium and made up to a final concentration of 0.5 $mg \cdot mL^{-1}$.
4. Incubate for 30 minutes to 4 hours at 37°C, until intracellular purple formazan crystals are visible under microscope.
5. Remove MTT and add solubilizing solution (DMSO, acidified isopropanol or SDS) and triturate.
6. Incubate at room temperature or 37°C for 30 minutes to 2 hours, until cells have been lysed and purple crystals have been dissolved.
7. Measure absorbance at 570 nm.

The absorbance reading of the blank (well containing incubation medium only) must be subtracted from those of all samples. Absorbance readings of test samples must then be divided by those of the control

(untreated cells) and multiplied by 100 to give percentage of cell viability or proliferation.

Absorbance values greater than the control indicate cell proliferation, while lower values suggest cell death or inhibition of proliferation.

3. RESULTS AND DISCUSSION

The spectroscopic data (IR and UV-Vis electronic spectra), as well as elemental analysis, molar conductivity measurements and thermal analysis results, presented in a previous study [6], support the proposed general structures of the studied compounds (Figure 1).

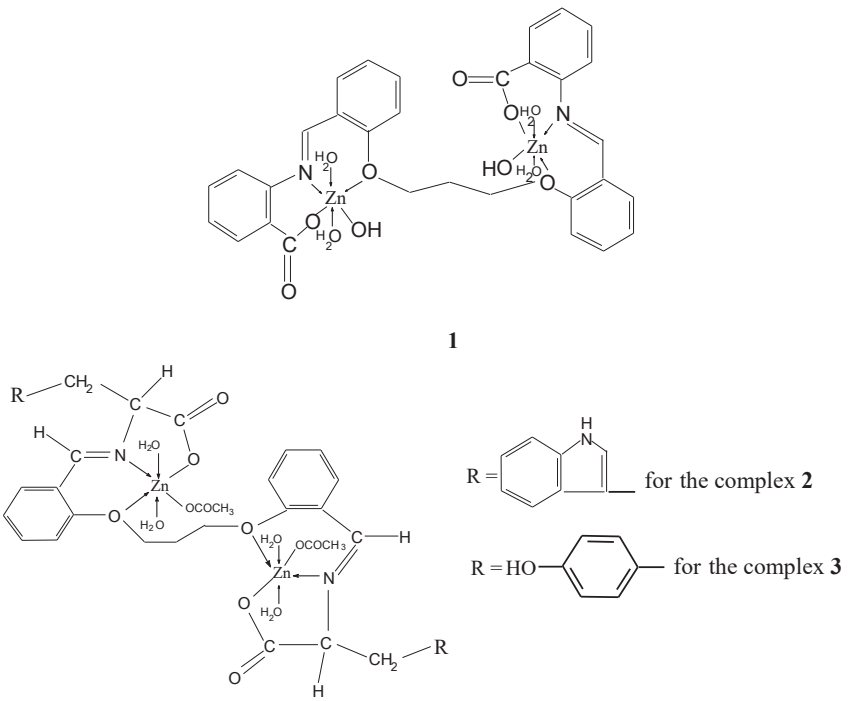

Figure 1. Proposed general structures of Zn(II) complexes derived from 2,2′-(propane-1,3-diyldioxy)dibenzaldehyde and 2-aminobenzoic acid (**1**), L-tryptophan (**2**) and tyrosine (**3**), respectively

3.1. Antimicrobial activity

The qualitative method used for the screening of the antimicrobial activity of the tested compounds indicated only very low diameters of growth inhibition around the spots, so we did not proceed to measure the diameters of the inhibition zones. These results could be due to the low diffusion rates of the tested compounds in solid Mueller Hinton medium.

The quantitative assay results for the antimicrobial activity of the studied compounds are presented in the Table 1.

Table 1. Antimicrobial activity expressed as MIC (μg·mL^{-1})

Compound	MIC/μg·mL^{-1}								
	Escherichia coli	*K. pneumoniae*	*Citrobacter freundii*	*Salmonella sp.*	*Providencia stuartii*	*P. aeruginosa*	*S. marcescens*	*S. aureus*	*Bacillus sp.*
1	125	250	250	125	500	1000	15.62	-	250
2	250	500	500	-	-	1000	125	-	-
3	125	500	250	500	500	-	60.50	-	250
DMF	250	500	500	500	500	1000	125	500	500

- = no inhibition

These results show that all the tested complexes exhibited an antimicrobial activity expressed as MIC values, which ranged between 1000 and 15.62 μg·mL^{-1}. A MIC value superior to 250 μg·mL^{-1} was considered as corresponding to a low, between 250 μg·mL^{-1} and 125 μg·mL^{-1} to a moderate and under 60.50 μg·mL^{-1} to a good antimicrobial activity [12].

The most active compound, considering both the intensity of the antimicrobial activity and the microbial spectrum proved to be the complex **1**, which showed good activity against *S. marcescens* strain (MIC = 15.62 μg·mL^{-1}), being active against eight of the nine tested microbial strains. This complex also presents a moderate antimicrobial

activity, superior to DMF solvent, against *E. coli*, *K. pneumoniae*, *C. freundii*, *Salmonella sp*. and *Bacillus sp.* strains and a low antimicrobial activity, similar to that of the DMF solvent, against *P. stuartii* and *P. aeruginosa* strains.

We have found that all the tested complexes were inactive against *S. aureus* strain.

The complex **3** showed a good antimicrobial activity, inferior to that of the complex **1** against *S. marcescens*, a moderate antimicrobial activity, similar to that of the complex **1** and superior to DMF solvent, against *E. coli*, *C. freundii* and *Bacillus sp.* strains. This complex exhibited a low antimicrobial activity, similar to that of the DMF solvent and less than the complex **1** against *K. pneumoniae* and *Salmonella sp.*, being inactive against two microbial strains: *P. aeruginosa* and *S. aureus*.

It must be noticed that the complex **1** and the complex **3** presented a low antimicrobial activity, similar to that of the DMF solvent against *P. stuartii*, while the complex **2** was inactive against this bacterium.

It can also be noticed that the complex **1** and the complex **2** showed a very low antimicrobial activity, similar to that of the DMF solvent, against *P. aeruginosa*, while the complex **3** was inactive against this bacterium.

The complex **2** exhibited a moderate antimicrobial activity, less than the complex **1** and the complex **3**, similar to that of the DMF solvent against *E. coli* and *S. marcescens* strains. This complex presented a low antimicrobial activity, similar to that of the DMF solvent against *K. pneumoniae*, *C. freundii* and *P. aeruginosa* strains, being inactive against four microbial strains.

From the results of the biological evaluation we conclude that the antimicrobial activity of the tested complexes follow the order: complex **1** > complex **3** > complex **2**.

The lowest MIC values (15.62 $\mu g \cdot mL^{-1}$ for complex **1**, 60.50 $\mu g \cdot mL^{-1}$ for complex **3** and 125 $\mu g \cdot mL^{-1}$ for complex **2**) were obtained for *S. marcescens* strain.

The importance of these findings lies in the fact that these complexes could be considered for the further development of novel antimicrobial drugs used for the treatment of some common diseases

caused by these bacterial strains [14].

3.2. Antitumor activity

The cytotoxicity test results for the studied compounds, using the MTT assay [13], are presented in the Table 2.

The analysis of these results showed that the complex **1** and the complex **3** have the highest cytostatic activity. These complexes also exhibited the most pronounced antimicrobial activity.

Table 2. The results of the cytotoxicity assessment of the tested compounds

Compound	Concentration/ $\mu g \cdot mL^{-1}$	Cell viability/ % of the control	
		24 h	48 h
1	10	96.33 +/- 0.96	98.36 +/- 2.73
	50	91.80 +/-1.82	91.22 +/- 2.87
	100	79.55 +/- 0.65	76.90 +/- 7.68
	200	**2.64 +/- 0.04**	**1.57 +/- 0.01**
2	10	99.25 +/- 0.15	98.55 +/- 2.33
	50	95.25 +/- 0.02	93.75 +/- 2.55
	100	84.34 +/- 0.01	82.43 +/- 3.33
	200	65.44 +/- 0.02	55.23 +/- 0.05
3	10	97.15 +/- 0.01	96.55 +/- 2.54
	50	93.45 +/- 0.03	92.25 +/- 3.34
	100	82.33 +/- 0.05	80.51 +/- 0.15
	200	47.35 +/- 0.05	36.25 +/- 3.50

It must be noticed that viability of HeLa cells in the presence of complex **1** at a concentration of 200 $\mu g \cdot mL^{-1}$ and 24 hours incubation time was 2.64+/-0.04, while at 48 hours incubation time attained 1.57+/-0.01.

These results indicate that the complex **1** has the highest cytostatic activity.

4. CONCLUSION

In this study, the Zn(II) binuclear complexes with three Schiff bases derived from some amino acids (2-aminobenzoic acid, L-tryptophan and tyrosine) and the aromatic dialdehyde, 2,2'-(propane-1,3-diyldioxy)dibenzaldehyde, were tested for their biological activity.

From the results of the biological evaluation we conclude that both the antimicrobial activity and the antitumor activity of the tested complexes follow the order: complex **1**>complex **3**>complex **2**. Therefore, the most active compound was the complex **1** which showed good activity against Gram-negative *S. marcescens* strain, also proving its potential use as a broad spectrum antimicrobial agent. Furthermore, the viability of HeLa cells in the presence of this compound exhibited the lowest values.

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Effects of exogenous gibberellin supply on certain enzyme activities in wheat caryopses during germination

Research article

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Abstract
Wheat caryopses germinated on media supplied with gibberellic acid (GA_3), at concentrations ranging from 10 μmol·L^{-1} to 100 μmol·L^{-1}, presented increased enzyme activities of amylases, acid phosphatase and peroxidase compared to their counterparts without added GA_3. Promotion of seed germination and plant growth was observed consecutive to exogenous GA_3 exposure, with the maximum effect at 20 μmol·L^{-1}.

Keywords: wheat caryopses, gibberellic acid, germination, enzyme activities

1. INTRODUCTION

Gibberellins are phytohormones that regulate essential aspects of plants growth and development. Knowledge on their role in determining plant's height had a major impact on cereal production during the `Green Revolution` [1]. Gibberellins are also involved in the