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Spectral Analysis and *In vitro* Biological Activity of Cu(II) Complex with Tridentate ONO Schiff Base Ligand

Emir Horozić,[1*] Merima Ibišević,[2] Darja Husejnagić,[3] Enida Karić,[2] Nusreta Hasić,[3] and Amina Hajdarević,[1]

[1]Faculty of Technology, University of Tuzla, Urfeta Vejzagića 8, 75 000 Tuzla, B&H
[2]Faculty of Pharmacy, University of Tuzla, Urfeta Vejzagića 8, 75 000 Tuzla, B&H
[3]Faculty of Natural Sciences and Mathematics, University of Tuzla, Urfeta Vejzagića 4, 75 000 Tuzla, B&H

[*]Corresponding email: emir.horozic@untz.ba

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ABSTRACT

In this study, metal complex of Copper(II) with a Schiff base derived from 2,2-dihydroxyindane-1,3-dione and 2-aminoethanoic acid were synthesized. The product are characterized by spectral methods. The antimicrobial activity was tested on reference bacterial strains and the antioxidant capacity was analyzed by using the DPPH and FRAP methods. The spectral data indicates that the Schiff base coordinates the Copper(II) as a tridentate ONO donor ligand. The compounds showed weaker antimicrobial activity on certain tested microorganisms. *In vitro* testing of antioxidant activity showed a significant reducing ability of the complex, as well as inhibitory activity against DPPH radicals.

Keywords: imine, complexes, antioxidants, antimicrobial activity.

INTRODUCTION

Schiff bases are versatile compounds synthesized by condensation of primary amino compounds with aldehydes or ketones [1]. The mechanism of Schiff base formation is shown in Figure 1. Schiff base formation involves two steps. First, amine nitrogen acts as a nucleophile, attacking the electrophilic carbonyl carbon aldehyde or ketone. In the next step, nitrogen is deprotonated, and electrons from this N-H bond push oxygen from the carbon and form a compound with a double bond C=N, with the release of one molecule of water [2].

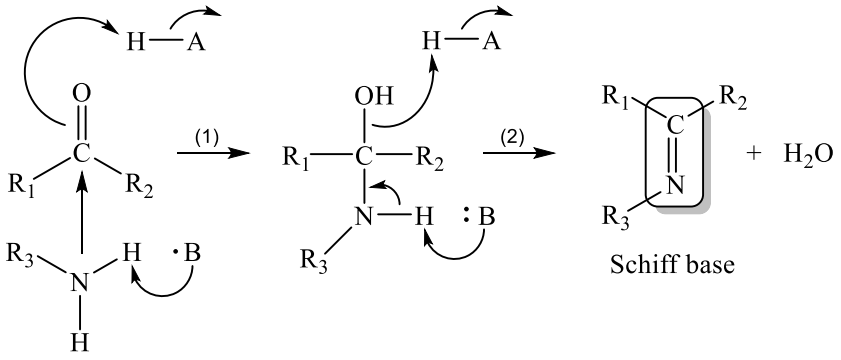

Figure 1. Mechanism of Schiff base formation [2]

Schiff bases are some of the most widely used organic compounds. They are used as pigments and dyes, catalysts, intermediates in organic synthesis, and as polymer stabilisers

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[3,4]. The high thermal of many Schiff base and their complexes were useful attributes for their application as catalysts in reactions involving at high temperatures [1]. Although the Schiff bases are known to be good chelating agents, and easily prepared and characterized, little interest has been given to their uses for analytical purposes because of two serious drawbacks, they are insoluble in aqueous solutions and they decompose easily in acidic solutions, limiting their use to basic conditions [5]. Schiff bases and its metal complexes also exhibit a wide variety of biological activities, including antifungal, antibacterial, antitumor, anti-inflammatory, trypanocidal, anti-HIV, antimalarial, and anti-urease activities [6-16]. The imine group present in these compounds is critical for their biological activities, and thus that moiety has been extensively explored for the development of new bioactive substances [6].

EXPERIMENT

All chemicals were of reagent grade, purchased from Aldrich and used without further purification.

Synthesis of the Complexes

The synthesis of the complex was performed according to the previously published procedure [17.18]. 0.01 mol of 2,2-dihydroxyindane-1,3-dione was dissolved in 30 ml of 96% ethanol. After complete dissolution, 0.005 mol of $CuCl_2$ x $2H_2O$ was added to the solution. The reaction mixture was refluxed for 30 minutes, after which 0.01 mol of 2-aminoethanoic acid was added. The mixture was refluxed for another 2 hours. The resulting dark brown precipitate was filtered off, washed with ethanol and stored in a desiccator.

FTIR characterization

FTIR spectra were recorded by ATR technique in the range 525-4000 cm^{-1} using a Nicolet iS10 FT-IR spectrophotometer (Thermo Fisher Scientific).

Determination of Stoichiometric Ratio

Stoichiometric ratio Cu(II):L was tested using Joe and Yones method [19]. A series of solutions with a constant concentration of Cu(II) ions of 1.35 x 10^{-4} mol/L were prepared, while ligand concentration was changed. Based on the obtained results, the stoichiometric composition of the complex and the calculated stability value of the K_{ML} were determined.

Determination of antioxidant capacity *in vitro*

2,2-diphenyl-1-picryl-hydrazyl (DPPH) method was performed according to earlier described method [20]. Different volumes of the complex solution were added to the tubes which were then made up to 2000 µL with methanol. To the solution was then added 500 µL of 0.5 mM DPPH solution and the samples were incubated for half an hour in the dark. After incubation, absorbance was measured at 517 nm with methanol as a blank. Results are expressed as IC_{50} value (mg/mL). Vitamin C was used as a control.

Ferric reducing antioxidant power (FRAP) asay was performed according to earlier described method [21]. The FRAP working solution was prepared by mixing 300 mM acetate buffer, 10 mM TPTZ (2,4,6-tri(2-pyridyl)-S-triazine) and 20 mM $FeCl_3$ solution in a 10:1:1 *v/v/v* ratio. 100 µL of the complex solution was mixed with 3000 µL of FRAP reagent and incubated for 30 minutes in a water bath at 37 °C. After incubation, absorbance was measured at 593 nm.

In vitro determination of antimicrobial activity

Antimicrobial activity was tested using agar diffusion method according to CLSI guidelines [22] with some modifications [23]. Antibacterial activitiy was investigated on reference bacterial strains: *Escherichia coli* (*E. coli*) ATCC 25922, *Enterococcus faecalis* (*E. faecalis*) ATCC 51299, *Staphylococcus aureus* (*S. aureus*) ATCC 25923, *Bacillus subtilis* (*B. subtilis*) ATCC 6633, *Listeria monocytogenes* (*L. monocytogenes*) ATCC 19118 and *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC 27853. Antifungal activity of the complex was tested on *Candida albicans* (*C. albicans*) ATCC 2091. From the microorganisms strains of overnight cultures, suspensions of 0.5 McFarland turbidity (1.5 x 10^8 CFU/ml) were prepared. In the agar were made sterile drill-shaped holes ("wells"), into which 80 µL of complex solution in concentration of 5 mg/mL were added. After the plates were left at room temperature for 15 minutes, the substance was diffused into agar, and plates were incubated at 37 ºC/24 h. After incubation, the size of the inhibition zones was measured.

RESULT AND DISCUSSION

Structure of the complexes

Figure 2 shows the reaction scheme and the proposed structure of the Cu(II) complex with a Schiff base derived from 2-aminoethanoic acid and 2,2-dihydroxyindane-1,3-dione.

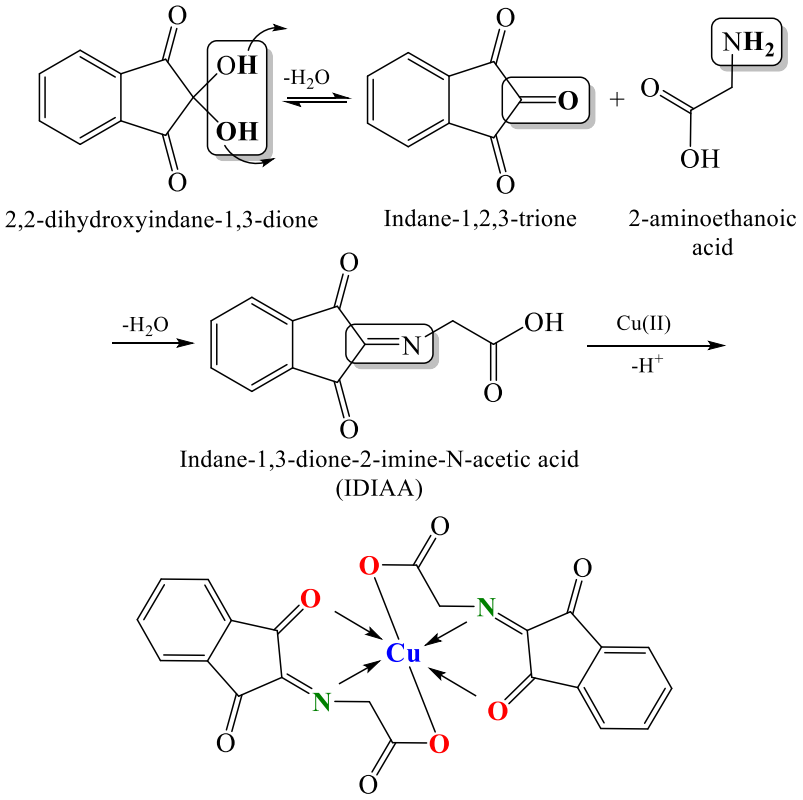


Figure 2. Reaction scheme and proposed structure of the complexes

In the presence of copper ions, 2,2-dihydroxyindane-1,3-dione and 2-aminoethanoic acid condense to form an intermediate Schiff base (indane-1,3-dione-2-imine-N-acetic acid, IDIAA). Deprotonation of IDIAA produces the anion indane-1,3-dione-2-imine-N-acetate (IDIA) which acts as a potential tridentate ONO donor ligand. The oxygen atom of the carboxylate anion, the carbonyl group of the indan part of the molecule and the nitrogen from

the newly formed imine group participate in the formation of the bond with the metal center.

Spectral characterization

Table 1 shows the spectral data obtained by FTIR spectroscopy. On the FTIR spectra of 2-aminoethanoic acid, a band of medium intensity at 3152 cm^{-1} is observed, which corresponds to NH_3^+ stretching of free primary amino acids. The carboxylate anion is characterized by strong absorption at 1577 cm^{-1} (v_{as}) and weak absorption at about 1400 cm^{-1} (v_s $C(=)O_2$ stretches). Two intense bands at about 1746 and 1712 cm^{-1} indicate the presence of carbonyl group on the FTIR spectra of 2,2-dihydroxyindane-1,3-dione. At 3296 cm^{-1} appears a medium-intensity band corresponding to the O-H bond.

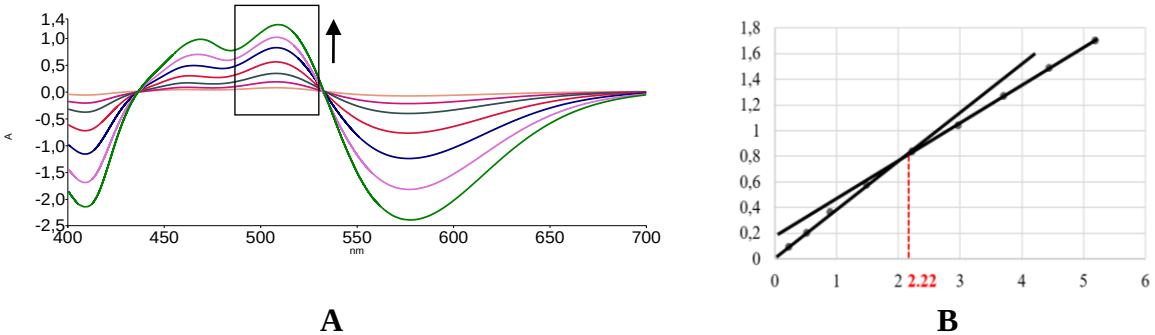
Most of the absorption bands other than those characteristic of the O-H bond were recorded on the FTIR spectra of the complex. Changes in the intensity and position of the band characteristic of the C=O bond indicate the involvement of oxygen atoms of the carbonyl group in the formation of the copper bond. The reduced intensity of the carboxylate anion band to about 1532 cm^{-1} indicates the involvement of the oxygen atom in the formation of a bond with the metal center. The band of the newly formed Cu-N bond was recorded at about 553 cm^{-1}. Based on the above results, the assumption that indane-1,3-dione-2-imine-N-acetate is coordinated by copper as a tridentate ONO ligand is confirmed.

Table 1. Infrared spectral data for reactants and complex

Compound	Wavelength [cm^{-1}]						
	COO^-	O-H	C=O	C=N	C-N	N-H	M-N
2-aminoethanoic acid	1577	-	-	-	1330	3152	-
2,2-dihydroxyindane-1,3-dione	-	3296	1712	-	-	-	-
Cu(II) complex	1532	-	1620	1481	1284	-	554

Stoichiometric Ratio

The spectra of the complex used to determine the stoichiometric ratio and absorbance vs. molar ratio (M/L) graph are shown in Figure 3. From the obtained data, the calculated stability constant of the complex K_{ML2} is 1.83 x 10^7. Absorption spectrum of the complex is characterized by a wide absorption band at 510 nm as a result of the d → d transition.

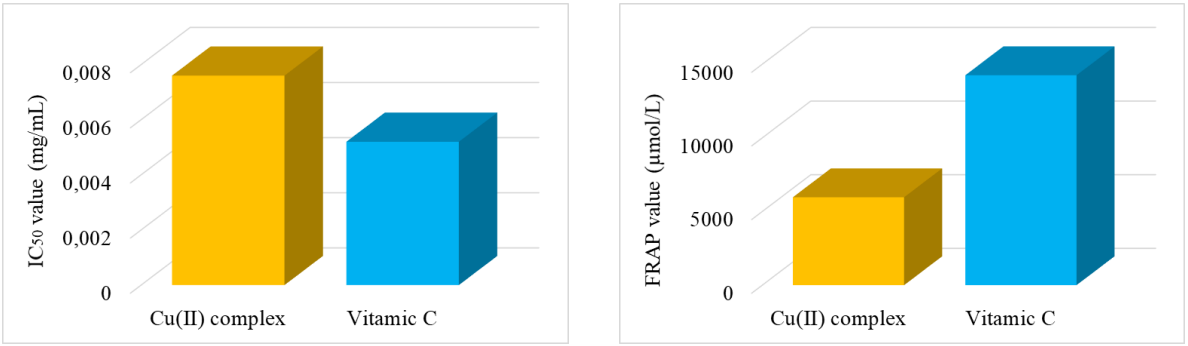


A **B**

Figure 3. Spectra of the complex used to determine the stoichiometric ratio **(A)** and absorbance vs. molar ratio (M/L) graph **(B)**

In vitro antioxidant activity

In vitro testing of antioxidant activity showed a significant reducing ability of the complex, as well as inhibitory activity against DPPH radicals. The results of antioxidant capacity are shown graphically in Figure 4. The complex is characterized by significant reducing ability (FRAP value 5967.5 µmol/L) and inhibitory activity (IC_{50} = 0.007 mg/mL). However, these values are slightly lower compared to Vitamin C which has an FRAP value of 14250 µmol/L. The IC_{50} value for vitamin C is 0.0052 mg/mL. The weaker antioxidant activity of the complex in relation to vitamin C can be explained by the absence of O-H and N-H bonds within the molecule of the complex, which in most cases are responsible for the high antioxidant capacity.



A **B**

Figure 4. Results of antioxidant capacity obtained by DPPH **(A)** and FRAP method **(B)**

In vitro antimicrobial activity

The antimicrobial activity of the complex is extremely weak on the tested bacterial strains. The complex showed good antibacterial activity against *S. aureus*, with a zone of inhibition of 16 mm. Antifungal activity is better, with a zone of inhibition of 18 mm. The control antibiotic Ciprofloxacin and the antifungal Nystatin showed significantly better efficacy, with zones of inhibition greater than 20 mm.

CONCLUSION

Indane-1,3-dione-2-imine-N-acetate (IDIA) coordinates the copper ion as a tridentate ONO donor ligand in a molar ratio of 1:2 (M:L). The complex showed weak antimicrobial activity. However, the reducing ability and inhibitory activity against free radicals indicate the potential application of this compound in biological research.

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