

SYNTHESIS, CHARACTERIZATION AND BIOACTIVITY OF SELECTED METAL COMPLEXES WITH IMINE LIGANDS

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT:

The chemistry of complex compounds containing imine ligands is attracting significant attention from researchers today. In this work, complexes of selected transition metals (Cu, Co, Ni and Fe) with imines based on ninhydrin and amino acids methionine and cysteine were synthesized. FTIR and UV/VIS spectroscopy were used for structural characterization. Antioxidant activity of the complex was analyzed by the FRAP method. The synthesized compounds showed a significant reducing ability, ranging from 221.94 to 756.30 $\mu\text{mol/L}$. *In vitro* antimicrobial activity was tested on strains from the ATCC collection. Inhibitory activity against the tested microorganisms was recorded, and the zones of inhibition ranged from 10-24 mm. Preliminary research shows that these compounds have biological potency, but more detailed *in vitro* and *in vivo* studies are required for their use.

KEYWORDS: metal complexes, imine, bioactivity, antibacterial agents, FRAP

INTRODUCTION

Schiff bases are synthetically accessible and structurally diverse compounds, typically obtained by facile condensation between an aldehyde, or a ketone with primary amines [1]. They are known in the name of Hugo Schiff who first reported the reversible acid-catalyzed condensation reaction between primary amine with carbonyl compounds [2]. These compounds are also known as imines with general structure R-CH=N-R' , where R and R' is linear or cyclic alkyl and/or aryl group which may be differently substituted [3,4]. They are most widely used organic compounds and can be coordinated with metals especially the transition metals to form Schiff base complexes [5,6]. As previously stated, Schiff bases are versatile metal complexing agents and have been known to coordinate all metals to form stable metal complexes with vast therapeutic applications [7]. Their wide spectrum of biological activities is the reason why they are very important in pharmaceutical industry. Most of them show biological activities including antibacterial, antifungal, antidiabetic, antitumor, antiproliferative, anticancer, herbicidal, and anti-inflammatory activities [8,9]. Also they show excellent catalytic

activity in various reactions and in the presence of moisture [6]. Many of them have high thermal stability and they are reported as effective candidates in homogenous and heterogeneous catalysis [10,11]. Due to excellent ligating properties and relatively facile synthesis methods the imine based ligands are widely used in transition metal coordination chemistry. These complexes are used as model molecules for biological oxygen carrier systems [12,13]. The compounds of this type can be greatly modified by introducing different substituents providing very useful model compounds for investigation of different chemical processes and its effects [14,15]. It is important to emphasize the structural similarity between Schiff bases possessing different donor atoms (N, O, S, etc.) and biologically active compounds found in natural biological systems [16,17].

MATERIALS AND METHODS

All chemicals used were of analytical grade and were used as received without any further purification.

PREPARATION OF THE METAL COMPLEXES

The synthesis of the complexes was performed according to described procedure [18]. Ninhydrin (0.01 mol) was transferred to a round bottom flask and dissolved in 25 mL of 96% ethanol, with stirring and heating. After dissolution, 0.005 mol of metal salts was added to the solution, and the mixture was stirred for 30 minutes. 0.01 mol of amino acid (methionine, cysteine) was then added to the flask and mixture was refluxed for 3 hours at 70-80 °C. The product was then filtered, washed with absolute ethanol and stored in a desiccator until analysis.

SPECTRAL CHARACTERIZATION

The products were characterized by FTIR and UV/VIS spectroscopy. Attenuated Total Reflection was used to record FTIR spectra. The samples were recorded in the wavelength range of 4000-525 cm^{-1} , on a Nicolet iS10 FT-IR spectrophotometer. The recordings were made in triplicate. Methanolic solutions at concentrations of 0.01 mg/mL were used to record the UV spectra in Perkin Elmer Lambda 25 UV/VIS spectrophotometer.

ANTIMICROBIAL ACTIVITY IN VITRO

Antimicrobial activities were investigated by diffusion method for reference bacterial strains *E. coli* (EC), *E. faecalis* (EF), *S. aureus* (SA), *B. subtilis* (BS), *L. monocytogenes* (LM) and *P. Aeruginosa* (PA). Solutions of the complex were prepared by dissolving the synthesized compounds in dimethyl sulfoxide. In the agar sterile drill-shaped holes were made ("wells") into which 100 μL of complex solutions of concentration 1 mg/mL were added. After the plates were left at room temperature for 15 min, the substance was diffused into agar, incubated at 37°C for 24 h.

FERRIC REDUCING ANTIOXIDANT POWER ASSAY (FRAP)

To prepare the calibration curve, solutions of $\text{FeSO}_4 \times 7\text{H}_2\text{O}$ was prepared in the concentration range of 200-1000 mmol/L. The volume of 3.0 mL FRAP reagent was measured in five tubes and 0.1 mL standard solutions were added there too. The absorbance was measured with regard to a blank sample (3 mL FRAP reagent and 0.1 mL of water). In each tube, 0.2 mL of metal complexes (concentration 0.10 mg/mL) and 6.0 mL of FRAP reagent were added. The samples were incubated in an aqueous bath for 30 minutes at 37°C, and the absorbance was measured at 593 nm with regard to a blank sample

(6.0 mL FRAP reagent and 0.2 mL DMSO). Measurements were performed in triplicate.

RESULTS AND DISCUSSION

STRUCTURE OF THE COMPLEXES AND SPECTRAL ANALYSIS

The reaction scheme and the proposed structure of the complex are shown in Figure 1. The imine ligand synthesized from ninhydrin and amino acids coordinates metal centers as a tridentate ONO donor ligand. The oxygen atom of the carbonyl group of ninhydrin, the nitrogen from the imine bond and the oxygen atom from the carboxyl group of the amino acid part of the molecule participate in the formation of the bond. Exceptions are Co(II) and Ni(II) complexes with methionine in which the imine coordinates metal ions as a bidentate NO-donor ligand. In these two complexes, the oxygen atom of the carboxyl group of the amino acid does not participate in the formation of the bond.

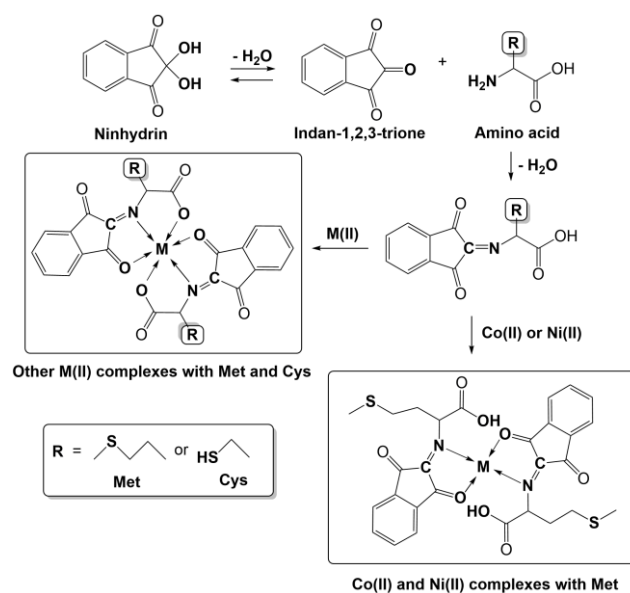


Figure 1. Reaction scheme and proposed structure of the metal complexes

Spectral data for the synthesized complexes, obtained by FTIR spectroscopy are shown in Table 1. At about 540 cm^{-1} on the FTIR spectrum was recorded a newly formed M-N bond. The absence of a band in the interval from 3000 cm^{-1} to 3500 cm^{-1} (except in the case of the Co(II) and Ni(II) complex with methionine) which is characteristic of the O-H bond from the carboxyl group, indicates the participation of oxygen atoms in the formation of the bond with the metal center. The FTIR spectra of ninhydrin and amino acids shows two strong absorption bands in the region around 1700 cm^{-1}

corresponding to the carbonyl group. Changes in the appearance of the bands in the area of about 1700 cm^{-1} also indicate the involvement of the carbonyl group in the formation of the complex. In the metal

chelates, the band characteristic of the azomethine group is shifted to $1574\text{-}1520\text{ cm}^{-1}$ suggesting the coordination of the azomethine nitrogen atoms to the metal.

Table1. Spectral data of the synthesized complexes obtained by FTIR and UV/VIS spectroscopy

Compound	Infrared spectral data [cm^{-1}]						UV/VIS λ_{max} [nm]
	$\nu(\text{COO}^-)$	$\nu(\text{O-H})$	$\nu(\text{C=N})$	$\nu(\text{C=O})$	$\nu(\text{C-N})$	$\nu(\text{M-N})$	
Fe(NinMet) ₂	1612	-	1540	-	1202	567	226
Fe(NinCys) ₂	1596	-	1540	1705	1200	551	206
Cu(NinMet) ₂	1536	-	1559	1684	1162	534	208
Cu(NinCys) ₂	-	-	1540	1700	1189	556	235
Co(NinMet) ₂	-	3150	1521	-	1167	560	218
Co(NinCys) ₂	-	-	1558	1705	1197	568	211
Ni(NinMet) ₂	-	3200	1520	1716	1201	560	212
Ni(NinCys) ₂	-	-	1574	1708	1236	546	222

ANTIMICROBIAL ACTIVITY

The results of antimicrobial activity of the synthesized complexes are shown in Table 2. In general, the greatest inhibition on the tested bacterial strains had the nickel complex containing cysteine. Then, significant antimicrobial activity show complexes Co(II) with cysteine and Cu(II) with

methionine. All synthesized complexes in the study of antimicrobial activity showed its complete absence against *E.coli* and *P. aeruginosa*. In all three cases, they are gram-negative bacteria that have an outer membrane made of lipopolysaccharide (LPS) that is a barrier to metal complexes, which explains the absence of their antimicrobial activity.

Table2. Results of antimicrobial activity of the synthesized complexes

Compound	Inhibition zone [mm]					
	LM	EC	EF	BS	SA	PA
Co(NinMet) ₂	19	-	-	-	-	-
Co(NinCys) ₂	21	-	15	13	20	-
Cu(NinMet) ₂	18	-	12	24	13	-
Cu(NinCys) ₂	-	-	13	10	11	-
Ni(NinMet) ₂	-	-	-	11	-	-
Ni(NinCys) ₂	23	-	22	16	23	-
Fe(NinMet) ₂	20	-	-	-	-	-
Fe(NinCys) ₂	11	-	12	-	18	-

Antioxidant activity

The results of the antioxidant activity of the synthesized complexes obtained by FRAP method are shown in Table 3. Based on the obtained FRAP values (for concentration 0.1 mg/mL), it can be concluded that compounds containing methionine have a higher reduction potential. Observing the FRAP values of compounds containing different metals conclusion is that complexes with Co(II) generally have higher FRAP values in both cases, when they contain methionine (which is slightly

higher) and when they containcysteine. The two complexes differ in structure since spectral analysis showed that the complex with methionine has an uninvolved oxygen atom of the O-H group (from the carboxyl part) and thus a different structure compared to the complex with cysteine. FRAP values of complexes containing Fe(II) range from $221.94\text{ }\mu\text{mol/L}$ with cysteine, which is the lowest FRAP value of the tested compounds, to $736.20\text{ }\mu\text{mol/L}$ with methionine as one of the highest FRAP values.

Table 3. Results of antioxidant activity of the synthesized complexes

Sample	FRAP value [$\mu\text{mol/L}$]
Co(NinMet) ₂	660.16
Co(NinCys) ₂	482.50
Cu(NinMet) ₂	399.09
Cu(NinCys) ₂	756.30
Ni(NinMet) ₂	590.47
Ni(NinCys) ₂	283.92
Fe(NinMet) ₂	736.20
Fe(NinCys) ₂	221.94

CONCLUSION

The synthesized metal complexes with methionine and cysteine showed significant antimicrobial and antioxidant activity *in vitro*. These compounds are interesting for further and more detailed research. Their proven bioactivity offers the possibility of application in biological systems and drugs.

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