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IN VITRO ANTIBACTERIAL ACTIVITY AND FERRIC REDUCING ANTIOXIDANT POWER OF SELECTED METAL COMPLEXES WITH SCHIFF BASES DERIVED FROM 2,2-DIHYDROXYINDANE-1,3-DIONE AND SELECTED AMINO ACIDS

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Research into the use of metal complexes as potential drugs is extremely popular and yields promising results. In this research, a group of metal complexes based on 2,2-dihydroxyindane-1,3-dione and selected amino acids (histidine and phenylalanine) was synthesized. FTIR and UV/VIS spectroscopy were used for structural characterization. Antimicrobial activity was examined by diffusion technique on reference bacterial strains (E. faecalis, S. aureus, B. subtilis, L. monocytogenes, S. enterica, E. coli and P. aeruginosa) from the ATCC collection. The reduction ability of the complex was examined by the FRAP method. The complexes showed significant inhibitory activity against gram-positive bacteria, with zones of inhibition ranging from 10-25 mm. Antioxidant capacity is also high, with FRAP values ranging from 1315.07-22038.7 μmol/L. These results indicate the potential of the synthesized complexes to be used as antibiotics and synthetic antioxidants. However, additional in vitro and in vivo studies are needed to determine their biological activity in more detail.

Keywords: metal complex, bioactivity, antibacterial agents, FRAP.

INTRODUCTION

A The chemistry of Schiff bases and their metal complexes is an important area of research due to the wide application of these compounds in medicine, pharmacy and various fields of chemistry. [1,2] A large number of synthesized metal complexes with this type of ligand showed antimicrobial [3-6], antitumor [7-10], antimalarial [11,12] and antioxidant activity [13-15]. In recent decades, little data have been published for the biological activity of complexes with Schiff bases derived from 2,2-dihydroxyindane-1,3-dione and amino acids. These complexes have shown antimicrobial [16,17] and antioxidant activity [18] which may be candidates for in vivo research. In this study, eight metal complexes with Schiff bases derived from 2,2-dihydroxyindan-1,3-dione and selected amino acids (histidine and phenylalanine) were synthesized. The antibacterial activity and reducing ability of the products were examined in vitro, with the aim of detecting their potential biological action.

MATERIAL AND METHODS

Methanol, diethyl ether, glacial acetic acid, hydrochloric acid and sodium carbonate purchased from Merck (Darmstadt, Germany). Dimethyl sulfoxide (DMSO) and 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), purchased from Sigma Chemical Co. (St. Louis, Missouri, USA). Metal salts (chlorides) and 96% ethanol were purchased from Semikem (Bosnia and Herzegovina). All chemicals used were of analytical grade and were used as received without any further purification.

Synthesis of complexes

The synthesis of the complexes was performed according to the previously 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 salt was added to the solution, and the mixture was stirred for 30 minutes. 0.01 mol of amino acid (histidine, $\mathbf{L_1}$ and phenylalanine, $\mathbf{L_2}$) was then added to the flask. The 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 Fourier-transform infrared (FTIR) and ultraviolet/visible (UV/VIS) spectroscopy. Attenuated Total Reflection (ATR) was used to record FTIR spectra. The samples were recorded in the wavelength range of 4000-525cm^{-1}, on a Nicolet iS10 FT-IR spectrophotometer. Methanolic solutions at con-

centrations of 0.01 mg/mL were used to record the UV spectra in Perkin Elmer Lambda 25 UV/VIS spectrophotometer.

Antimicrobial activity testing

Antibacterial activity were investigated by diffusion method on reference bacterial strains *E. faecalis, S. aureus, B. subtilis, L. monocytogenes, S. enterica, E. coli and P. aeruginosa*. From the microorganisms strains of overnight cultures, suspensions of 0.5 McFarland turbidity were prepared (density 10^7-10^8 CFU mL^{-1}). The strains were then placed on the surface of the nutrient substrate-Mueller-Hinton agar, dispersed in sterile Petri dishes. Substrate thickness was 4 mm. In the agar sterile drill-shaped holes were made ("wells"), into which 100 µL of metal complex solutions in concentration of 1 mg mL^{-1} were added. After the plates were left at room temperature for 15 minutes, the substance was diffused into agar and incubated at 37°C/24 h. After the incubation period, the size of inhibitory zone was measured.

Ferric reducing antioxidant power (FRAP) assay

The reducing powers of the metal complexes that reflected their antioxidant activity were determined following the protocol [19]. 3 mL of prepared FRAP reagent is mixed with 100 µL of complex solution. Absorbance at 593 nm is recorded after a 30 min incubation at 37 °C. The FRAP value was calculated from the calibration curve of iron(II) sulfate heptahydrate ($y = 0{,}001x + 0{,}0698$; $R^2 = 0{,}9997$). Results are expressed for a solution concentration of 1 mg/mL.

RESULTS AND DISCUSSION

Structure and physico-chemical characteristics of the complexes

The reaction scheme and the proposed structure of the complexes are shown in Figure 1. Based on the spectral data obtained by FTIR spectroscopy, it is concluded that the imine synthesized from amino acid and 2,2-dihydroxyindane-1,3-dione coordinates metal center as a tridentate ONO donor ligand. Nitrogen from the newly formed imine bond, the oxygen atom of the carboxyl group and the carbonyl group of the indan part of the molecule participate in the formation of the bond. The exception is the complex of Co(II) with phenylalanine where from the spectral data it is concluded that the complex contains an O-H bond (from the carboxyl group of the amino acid). Nitrogen from the imine bond and the carbonyl group of the indan part of the molecule participate in the formation of the bond with a metal center.

Table 1 shows the spectral data for the synthesized complexes, obtained by FTIR spectroscopy. On the FTIR spectra of the complex, changes in the strength and positions of the bands in relation to the parent components from which the ligand was synthesized were observed.

Table 1. Spectral data of the synthesized complexes obtained by FTIR spectroscopy

Compound	Infrared spectral data [cm^{-1}]						UV/VIS λ_{max} [nm]
	$\nu(COO^-)$	ν(O-H)	ν(C=N)	ν(C=O)	ν(C-N)	ν(M-N)	
$Fe(NinL_1)_2$	1616	-	1506	1716	1215	547	228
$Fe(NinL_2)_2$	1599	-	1540	1714	1255	544	202
$Cu(NinL_1)_2$	1604	-	1506	1715	1235	544	201
$Cu(NinL_2)_2$	1602	-	1543	1714	1259	547	230
$Co(NinL_1)_2$	1614	-	1506	1716	1213	540	202
$Co(NinL_2)_2$	1581	3501	1542	1705	1315	544	201
$Ni(NinL_1)_2$	1606	-	1538	1708	1228	544	222
$Ni(NinL_2)_2$	1604	-	1538	1708	1235	540	202

The newly formed M-N bond was recorded on the FTIR spectra of the complex at about 540 cm^{-1}. The absence of a band at about 3500 cm^{-1} (except in the case of the Co(II) complex with phenylalanine), characteristic of the O-H bond from the carboxyl group, indicates the involvement of the oxygen atom in the formation of the bond with the metal center. 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.

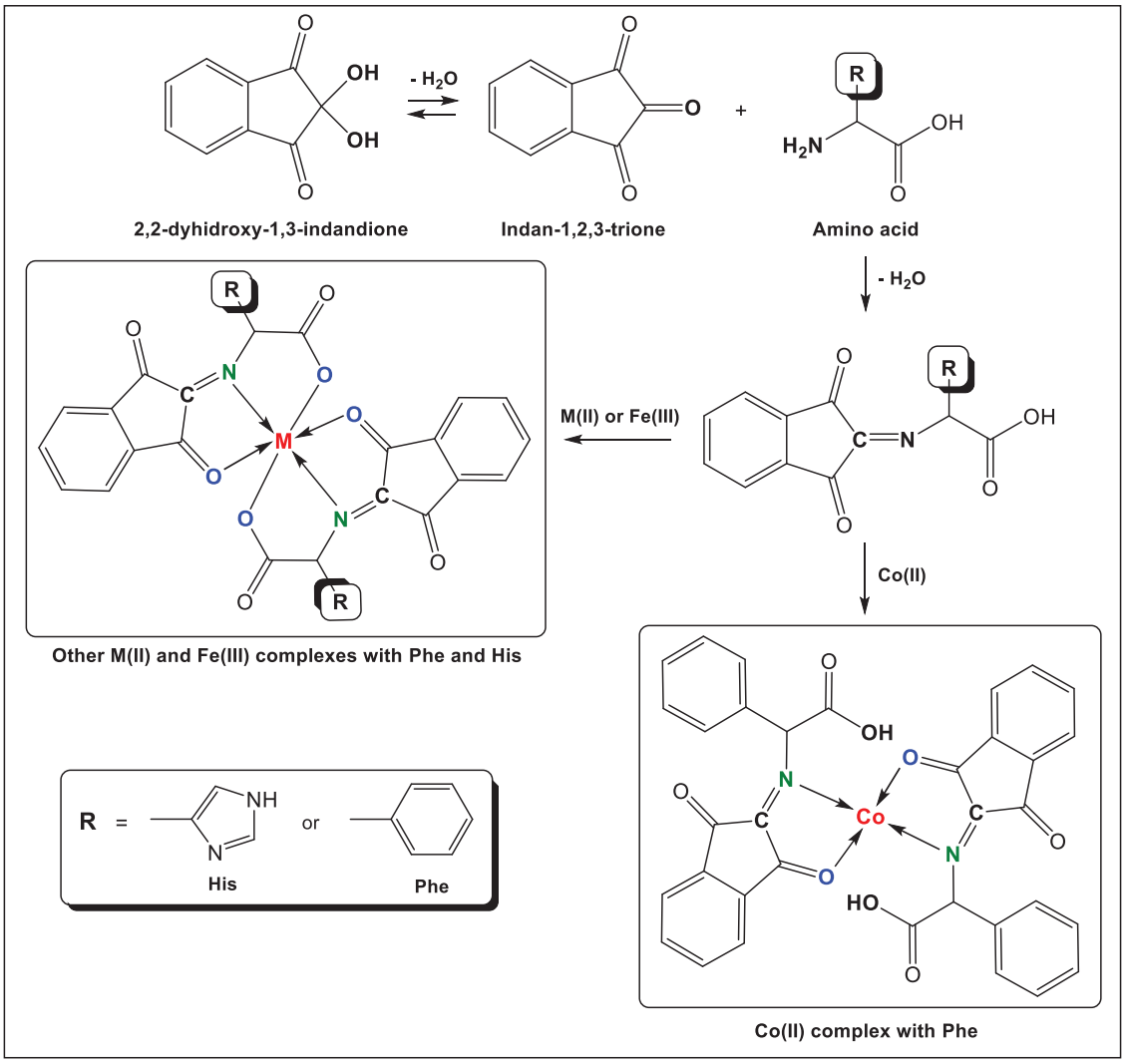

Figure 1. Reaction scheme and proposed structure of the metal complexes

The results of the solubility tests of the synthesized compounds are shown in Table 2.

The synthesized compounds are soluble in dimethyl sulfoxide and methanol, and slightly less solubility is

Table 2. Solubility of synthesized compounds in different solvents

Compound	Yield (%)	Color	Solubility in solvent				
			Methanol	Diethyl ether	Chloroform	DMSO	Water
$Fe(NinL_1)_2$	48	black	+	-	-	+	+/-
$Fe(NinL_2)_2$	31	brown	+	+	+	+	-
$Cu(NinL_1)_2$	72	dark brown	+	+	+	+	+/-
$Cu(NinL_2)_2$	14	brown	+	+	+	+	-
$Co(NinL_1)_2$	61	black	+	+/-	+/-	+	+
$Co(NinL_2)_2$	38	dark red	+	+	+	+	+/-
$Ni(NinL_1)_2$	35	black	+	+/-	+	+	+/-
$Ni(NinL_2)_2$	44	black	+	+/-	+	+	-

Legend: *(+) - soluble; (+/-) - partially soluble; (-) - insoluble*

in chloroform and diethyl ether. The weakest solubility is in water, in which all complexes with histidine are completely or partially soluble. In general, the solubility of a drug is one of the important parameters for achieving its desired concentration in the systemic circulation for the desired (expected) pharmacological response. Low solubility of drugs in water is a problem in their application and bioavailability [20].

In *vitro* antimicrobial activity

The results of antibacterial activity are shown in Tables 3 and 4. In general, the greatest inhibition on the tested bacterial strains had the iron complex containing histidine and the nickel complex containing phenylalanine. Other complexes also show significant antibacterial activity with zones of inhibition ranging from 10 to 25 mm. The compounds have the most pronounced action against *Staphylococcus aureus* and *Listeria monocytogenes*. The complete absence of inhibitory activity was observed in all gram-negative bacteria. This can be explained by the presence of an outer membrane in gram-negative bacteria, which is constructed of lipopolysaccharide (LPS) and is an obstacle to metal complexes [21]. The control antibiotic Ciprofloxacin showed inhibitory activity similar to or greater than the synthesized complexes, with zones of inhibition greater than 20 mm.

Table 3. Results of antibacterial activity of histidine-containing complexes

Microorganism	ATCC	Inhibition zone [mm]			
		$Fe(NinL_1)_2$	$Cu(NinL_1)_2$	$Co(NinL_1)_2$	$Ni(NinL_1)_2$
Listeria monocytogenes	19118	23	15	20	19
Enterococcus faecalis	51299	20	11	16	19
Bacillus subtilis	6633	12	10	11	15
Staphylococcus aureus	25923	25	-	20	23
Escherichia coli	25922	-	-	-	-
Pseudomonas aeruginosa	27853	-	-	-	-
Salmonella enterica	13076	-	-	-	-

Table 4. Results of antibacterial activity of phenylalanine-containing complexes

Microorganism	ATCC	Inhibition zone [mm]			
		$Fe(NinL_2)_2$	$Cu(NinL_2)_2$	$Co(NinL_2)_2$	$Ni(NinL_2)_2$
Listeria monocytogenes	19118	20	-	15	24
Enterococcus faecalis	51299	17	13	-	23
Bacillus subtilis	6633	11	10	10	19
Staphylococcus aureus	25923	21	15	14	25
Escherichia coli	25922	-	-	-	-
Pseudomonas aeruginosa	27853	-	-	-	-
Salmonella enterica	13076	-	-	-	-

Ferric reducing antioxidant power

Table 5 shows the results of antioxidant capacity obtained by the FRAP method. All synthesized complexes showed a high reduction potential ranging from 1315.07 to 22038.7 μmol/L. Comparing the obtained results with the structures of the complex, it was determined that the compounds containing histidine have a higher reduction potential. However, this is not the case with cobalt complexes. The cobalt complex with histidine has weaker antioxidant activity in contrast to the complex with phenylalanine for which the greatest reduction potential has been determined. The two complexes differ in structure since spectral analysis showed that the complex with phenylalanine has an uninvolved oxygen atom of the O-H group (from the carboxyl part) and thus a different structure compared to the complex with histidine. Based on that, it is assume that the free hydroxyl group significantly affects the increase of antioxidant capacity. Compared with ascorbic acid used as a control, all synthesized compounds except the Co(II) complex with phenylalanine have a poorer reducing effect.

Table 5. FRAP values of synthesized complexes

Compound	FRAP value [μmol/L]
$Fe(NinL_1)_2$	3362.22
$Fe(NinL_2)_2$	1315.07
$Cu(NinL_1)_2$	3327.55
$Cu(NinL_2)_2$	2451.11
$Co(NinL_1)_2$	4944.44
$Co(NinL_2)_2$	22038.7
$Ni(NinL_1)_2$	5104.44
$Ni(NinL_2)_2$	3276.85
Ascorbic acid	14250.0

CONCLUSION

The synthesized complexes of selected biogenic elements based on 2,2-dihydroxyindane-1,3-dione and aminoacids histidine and phenylalanine, showed significant antibacterial and antioxidant activity in vitro. Therefore, they represent potential biologically active agents that should be investigated in more detail in vitro and in vivo. One of the shortcomings identified through this research is poorer water solubility, which would affect their bioavailability in biological systems.

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