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Research paper

BSA binding affinity, free radical scavenging capacity and antibacterial activity of new Silver(I) complexes with Schiff base ligands

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Abstract

In this work, four silver(I) complexes with Schiff bases derived from ninhydrin and selected amino acids (methionine, histidine, cysteine, and phenylalanine) were synthesized. The aim of this study is to determine the potential biological activity of these complexes. FTIR and UV/VIS spectroscopy were used for structural characterization of the products. Antioxidant activity was examined *in vitro* using DPPH method. Antimicrobial screening was performed by diffusion technique on reference bacterial strains from the ATCC collection. Interaction with bovine serum albumin (BSA) was examined using UV/VIS spectroscopy. Based on the obtained spectral data, it is assumed that all Schiff bases coordinate the Ag(I) ion as a tridentate ONO donor ligand. The antioxidant activity of the synthesized compounds is extremely high, with a range of IC_{50} values of 0.023-0.06 mg/mL. Antimicrobial screening determined the inhibitory ability of almost all complexes for concentrations of 1 and 2 mg/mL, with zones of inhibition in the range of 9-19 mm. Examination of the interaction of BSA with Ag(I) complexes revealed the same change in the absorption maximum (hyperchromic shift) in the region of about 205 nm, which indicates that the interaction of BSA and the complex results in conformational changes of BSA.

Keywords: *Metal Complexes; Bioactivity; Antioxidants; BSA Interaction.*

1. Introduction

Silver and its compounds have been used for centuries in the treatment of ulcers, burns and infections [1]. They were popular remedies for tetanus and rheumatism in the 19th century and were also used against colds and gonorrhea before the advent of antibiotics in the early 20th century [2], [3]. Due to certain disadvantages of silver-based pharmaceutical formulations, their therapeutic use is limited. This primarily refers to silver nanoparticles (AgNPs) whose application can cause structural and physiological changes to vital organs. Toxic effects of AgNPs on rat hepatocytes and neurons, as well as human lung epithelial cells, have been confirmed in *in vitro* and *in vivo* studies [4], [5]. In the last decade, the evaluation of the biological properties of silver complexes has garnered considerable attention, in particular, fostering their use as antimicrobials or anticancer agents [6]. In the last few years, the results of research on the antimicrobial and antitumor activity of silver(I) complexes with different types of ligands have been published. It is known that silver complexes can display bactericidal activity against *B. contaminans*, *S. aureus*, *S. epidermidis*, *P. aeruginosa*, *E. coli*, *P. hauseri* and fungistatic activity against *Candida* species [7], [8]. Study on novel antimicrobial agents amongst silver complexes group seems to be proved as silver(I) complexes are known to possess substantial antimicrobial activity and have been demonstrated to be less toxic to the human body than other metal complexes. In the past, silver(I) complexes did not receive much scientific attention compared to other metal complexes, although silver complexes can also demonstrate significant biological activity, such as antimicrobial and cytotoxic activity against many cancer cell lines [9]. Nevertheless, a lot of silver(I) complexes have been found to exhibit greater cytotoxic activity than cisplatin, with relatively low toxicity and greater selectivity toward cancer cells which is dependent on the type of ligand linked to the silver(I) ions [10]. Studies of the interaction of potential biologically active substances with biomolecules are the basis for monitoring their biodistribution, toxicity and mechanism of action. Because bovine serum albumin (BSA) is a structural analogue of human serum albumin (HSA), it is extremely suitable for investigating possible interactions with synthetic components *in vitro* [11]. To date, several papers have been published indicating the interaction of the silver (I) complexes with BSA [12-14].

In this paper, the results of newly synthesized silver(I) complexes with potential biological activities are presented. The aim of this study was to determine the antibacterial activity, BSA binding affinity and free radical scavenging capacity of four newly synthetized Silver(I) complexes with Schiff base ligands.

2. Materials and methods

All chemicals used were of analytical grade and were used as received without any further purification. Chemicals were purchased from Merck (Darmstadt, Germany) and Sigma Chemical Co. (St. Louis, Missouri, USA). Melting points were determined on a Krüss melting point apparatus and are uncorrected.

2.1. Preparation of the Silver(I) complexes

The synthesis of the complexes was performed according to the previously described procedure [15]. 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 silver nitrate was added to the solution, and the mixture was stirred for 30 minutes. 0.01 mol of amino acid (methionine, L_1, histidine, L_2, phenylalanine, L_3 and cysteine, L_4) 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.

AgL_1 complex: Yellow cristals, 74.7% yield, m.p.: 243-245 °C. FT-IR (ATR, ν, cm^{-1}): 1702 (C=O), 1588 (COO^-), 1526 (C=N), 711 (C-S), 530 (M-N); UV/Vis (Met-OH, λ_{max}, nm): 227.

AgL_2 complex: Dark green cristals, 51% yield, m.p.: 216-219 °C. FT-IR (ATR, ν, cm^{-1}): 1697 (C=O), 1601 (COO^-), 1538 (C=N), C-N (1249), N-H (3124), M-N (559); UV/Vis (Met-OH, λ_{max}, nm): 205.

AgL_3 complex: Brown cristals, 25.2% yield, m.p.: 168-171 °C. FT-IR (ATR, ν, cm^{-1}): 1713 (C=O), 1598 (COO^-), 1489 (C=N), 543 (M-N); UV/Vis (Met-OH, λ_{max}, nm): 203.

AgL_4 complex: Dark brown cristals, 31.4% yield, m.p.: 273-276 °C. FT-IR (ATR, ν, cm^{-1}): C=O (1704), 1590 (COO^-), 1533 (C=N), 526 (M-N); UV/Vis (Met-OH, λ_{max}, nm): 223.

2.2. 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 4000-525 cm^{-1}, on a Nicolet iS10 FT-IR spectrophotometer. Methanolic solutions at concentrations of 0.01 mg/mL were used to record the UV spectra in Perkin Elmer Lambda 25 UV/VIS spectrophotometer.

2.3. *In vitro* antibacterial activity testing

The *in vitro* antibacterial activities of the metal complexes and standard antibiotic drugs were investigated using four Gram-positive bacteria (*B. subtilis*, *S. aureus*, *L. monocytogenes* and *E. faecalis*) and three Gram-negative bacteria (*E. coli*, *S. enterica* and *P. aeruginosa*) by applying the diffusion technique. Complex solutions of a concentrations 1 and 2 mg/mL, prepared by dissolving the complex in dimethyl sulfoxide, were used for the assay. The sample volumes applied to the agar wells were 100 µL. Ciprofloxacin (conc. 1 mg/mL) were used as a control.

2.4. DPPH radical scavenging activity

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) [16]. Vitamin C was used as a control.

2.5. Determination of binding interaction of Ag(I) complexes with BSA

A 0.01 mg/mL BSA solution was prepared by dissolving BSA in phosphate-buffered saline (PBS; pH=7.4). The initial concentration of the Ag(I) complexes solution was 0.005 mg/mL. In 10 mL of BSA solution, 80 µL of the complex was added in portions (per 10 µL) and stirred on a magnetic stirrer for 1 min. The interaction of BSA with complexes was examined on a Perkin Elmer Lambda 25 UV/VIS spectrophotometer, in the range of wavelengths of 200-400 nm.

3. Results and discussion

3.1. Reaction scheme and spectral characterization of synthesized complexes

The reaction scheme and the proposed structure of the Ag(I) complexes are shown in Figure 1. Based on the obtained spectral data, it is assumed that all Schiff bases coordinate the Ag(I) ion as a tridentate ONO donor ligand. One carbonyl group from the indane part of the molecule, the oxygen atom from the carboxyl group of the amino acid and the nitrogen atom from the newly formed imine bond participate in the formation of the bond. Otherwise, ninhydrin and amino acids have been found to form a ligand with a larger number of potential donor atoms. However, previous studies of these types of ligands derived from glycine, alanine and tryptophan have shown that such ligands coordinate metal centers as tridentate ONO donor ligands [15], [17].

Silver most commonly forms complexes of linear and tetrahedral geometry [18], while complexes of higher coordination number are rare. However, the spectral data obtained from this study indicate the formation of a complex of coordination number 6. On the FTIR spectra of the synthesized complexes, a band at about 550 cm^{-1} is observed, which indicates the interaction of the metal with the nitrogen donor atom from the imine group. A doublet of the carbonyl group was observed on the FTIR spectra of ninhydrin, while a band of lower intensity was recorded on the FTIR spectra of the complex, which indicates the involvement of π electrons of one carbonyl group in the formation of a bond with the metal center. The attenuation of the band intensity on the FTIR spectra of the complex, characteristic of the carboxylate anion, also indicates the formation of a bond with the metal center.

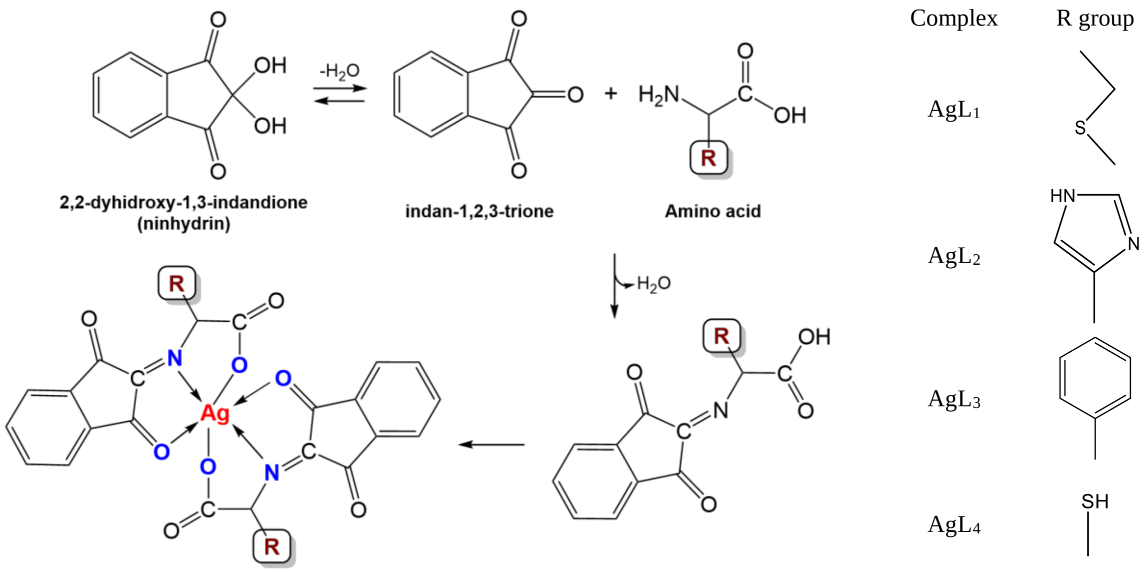
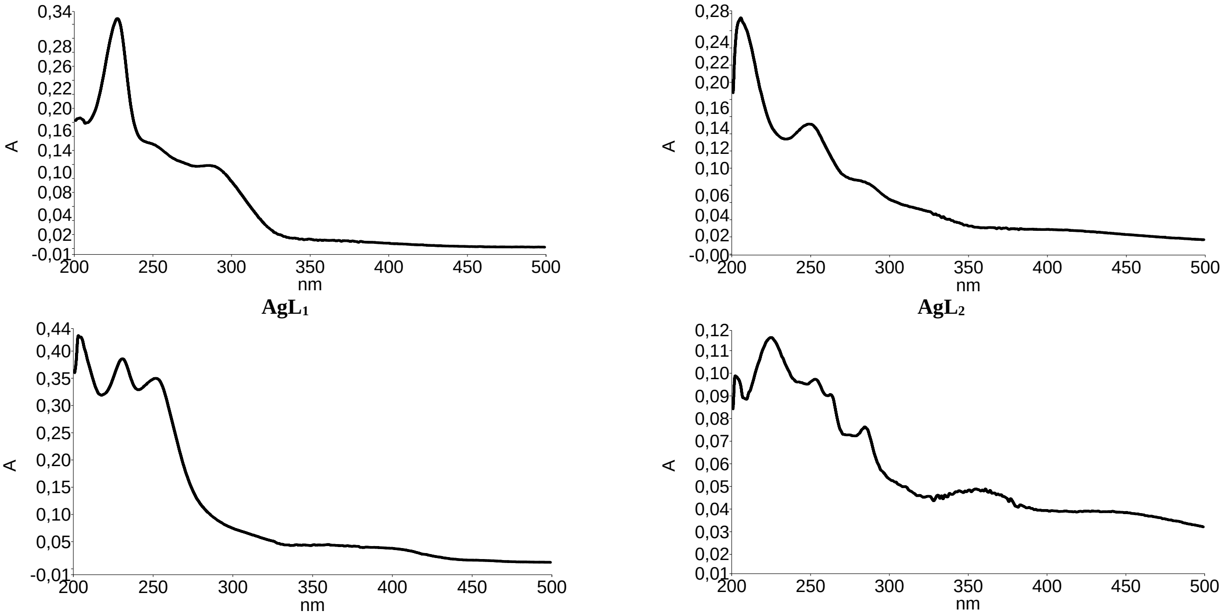


Fig. 1: Reaction Scheme and Proposed Structure of the Ag(I) Complexes.

Figure 2 shows the electronic spectra of the synthesized complexes. All products are absorbed in the UV region and are characterized by several bands in the range of 200-290 nm. All complexes have absorption maximum in the range of 203-260 nm which corresponding to π→π* transitions, due to the presence of double bonds in the ligand molecule. In the AgL_1, AgL_2 and AgL_4 complexes, bands of lower intensity in the range of 280-300 nm corresponding to n→π* transitions were recorded, and are a consequence of the presence of heteroatoms with unshared electron pair.



AgL_1 **AgL_2**

AgL_3 **AgL_4**

Fig. 2: Electronic Spectra of Synthesized Complexes.

3.2. Antibacterial activity

Table 1 shows the results of antibacterial activity of the synthesized complexes obtained by the diffusion technique. Testing was performed for concentrations of 1 and 2 mg/mL. All complexes showed greater antibacterial activity against gram-positive bacteria compared to gram-negative bacteria. The generally weaker antibacterial activity of synthesized complexes against gram-negative bacteria is explained by the presence of an outer membrane constructed of lipopolysaccharides (LPS) that represent a barrier to metal complexes [19]. AgL_1 and AgL_3 have a less polar structure, which facilitates their passage through the cell membranes of bacteria, and which was reflected in the better antimicrobial effect of these complexes. Molecules that can readily cross cell membranes are frequently needed in biological research and drug development. Due to the more polar structure, complexes with histidine (AgL_2) and cysteine (AgL_4) have weaker antibacterial activity against gram-negative bacteria due polar molecules have much more difficulty crossing the cell membranes. Complexes with polar structure can easily interact with the outer face of the membrane, but they have trouble passing through its hydrophobic core. The explanation for better antibacterial activity could be in ability of metal complexes to form hydrogen bonds with the active centers of cellular components, which leads to the disruption of normal cellular processes. Compared with the control antibiotic (Ciprofloxacin), the synthesized Ag(I) complexes showed a weaker antibacterial effect. The control antibiotic showed a better inhibitory effect, with zones of inhibition greater than 20 mm in the case of all bacterial strains. Due to the emergence of resistance to commercial antibiotics, it is necessary to examine the antibacterial potential of new, more adequate substitutes, with less toxic effects. Also, the use of these and similar complexes with confirmed antibacterial activity does not necessarily have to be directed to human use, but also as a disinfectant.

Table 1: Antibacterial Activity of Synthesized Complexes

Compound	Inhibition zone [mm]													
	BS		EC		EF		PA		SE		SA		LM	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2
AgL$_1$	11	14	12	12	12	13	14	15	10	13	14	15	11	12
AgL$_2$	12	12	11	11	13	15	11	11	9	9	10	12	10	11
AgL$_3$	11	13	10	10	14	19	13	13	9	10	15	18	12	14
AgL$_4$	-	11	11	11	10	13	12	14	-	10	10	10	-	11

* Legend: 1 - conc. 1 mg/mL; 2 - conc. 2 mg/mL; BS - *B. subtilis*; EC - *E. coli*; EF - *E. faecalis*; PA - *P. aeruginosa*; SE - *S. enterica*; SA - *S. aureus*; LM - *L. monocytogenes*.

3.3. Antioxidant activity

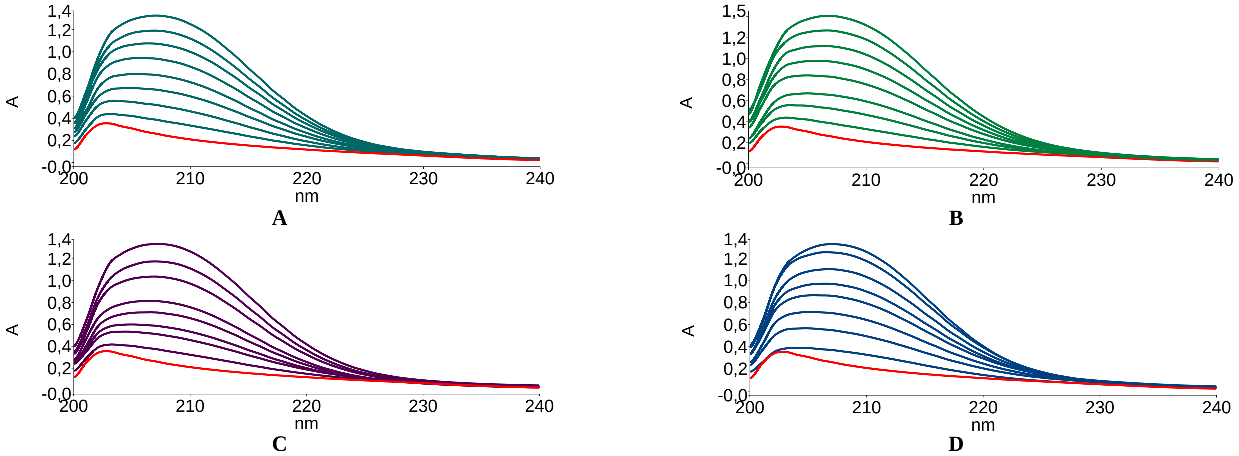
The results of the antioxidant capacity of the synthesized complexes are shown in Table 2. The highest ability to inhibit DPPH radicals have the complexes AgL$_2$ and AgL$_4$, with the amino acids histidine and cysteine in their structure. AgL$_3$ has the weakest inhibitory effect with an IC$_{50}$ value of 0.062 mg/mL. Compared with the IC$_{50}$ value of ascorbic acid (0.03 mg/mL) used as a control, the AgL$_2$ and AgL$_4$ complexes have a higher inhibitory capacity. An explanation could be in ability of the free radical to remove hydrogen atoms from the complex molecule. Since AgL$_2$ and AgL$_4$ contain R-groups that have N-H and S-H bond in their structure, it is assumed that the DPPH radical binds hydrogen atoms to itself thus becoming stable and the molecule of the complex becomes a radical. In this mechanism, the dissociation energy of the N-H or S-H bond is an important parameter in the assessment of antioxidant activity [20]. In recent years, the antioxidant capacities of several Ag(I) complexes have been investigated. Significant antioxidant capacity has been reported for silver(I) complexes with benzimidazolium salts [21], silver(I) complexes containing V-shaped bis-benzimidazole ligands [22] and silver(I) complexes containing bis (2-benzimidazolyl) aniline derivatives [23]. However, their activity needs to be further examined with other *in vitro* techniques and also *in vivo*, in order to define their possible application and potential toxic effects.

Table 2: Antioxidant Activity of Synthesized Complexes

Compound	IC$_{50}$ value [mg/mL]
AgL$_1$	0.04
AgL$_2$	0.02
AgL$_3$	0.06
AgL$_4$	0.02
Vitamic C	0.03

3.4. Interaction with BSA

Figure 3 shows an UV spectra of BSA in the absence (red line) and presence of certain concentrations of Ag(I) complexes. Change in the conformation of BSA and formation of the complex after the interaction with Ag(I) complexes is effectively studied by UV/Vis spectral measurements. All complexes showed identical behavior when interacting with BSA with almost identical changes in intensity and displacement of the absorption maximum. The band of lower intensity at about 274 nm corresponds to the π→π* transition of aromatic amino acids (phenylalanine, tryptophan, tyrosine). The absorption maximum recorded at 201 nm maintains the BSA framework conformation [24]. Partial additions of 10 µL of the 0.005 mg/mL complex solution show an increase in the intensity of the BSA absorption maximum (hyperchromic shift) with a slight shift towards longer wavelengths (batochromic shift).



A B C D

Fig. 3: UV/VIS Spectra of BSA in the Absence (Red Line) and Presence of Different Concentrations of: (A) Agl$_1$; (B) Agl$_2$; (C) Agl$_3$ and (D) Agl$_4$ Complexes.

4. Conclusion

Newly synthesized silver(I) complexes with Schiff bases derived from ninhydrin and selected amino acids showed significant antimicrobial and antioxidant activity. Silver complexes with methionine and phenylalanine, labeled as AgL$_1$ and AgL$_3$ complexes respectively, and showed the largest bactericide potency. Antioxidant activity experiments show that two newly synthetized silver complexes (AgL$_2$ and AgL$_4$) possess significant antioxidant activity against hydroxyl radicals, with more efficient hydroxy radical scavenging compared with vitamin C. The interaction of the silver(I) complexes with BSA results in conformational changes in BSA. Antibacterial and antioxidant properties of newly synthetized silver(I) complexes, coupled with their interaction with BSA, suggest that thay may have significant action incorporating anti-inflammatory capability. These complexes are currently under intense examination in our laboratories in an attempt to further elucidate their mechanisms of action and to explore their potential anticancer activities.

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