Antibacterial Activity of Pd(II) Complexes with Salicylaldehyde-Amino Acids Schiff Bases Ligands

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Palladium(II) complexes with Schiff bases ligands derived from salicylaldehyde and amino acids (Ala, Gly, Met, Ser, Val) have been synthesized and characterized by Fourier transform (FT)-IR, UV-Vis and 1H-NMR spectroscopy. The electrospray mass spectrometry (ES-MS) spectrometry confirms the formation of palladium(II) complexes in 1/2 (M/L) molar ratio. All the Pd(II) complexes 1, [Pd(SalAla)2]Cl2; 2, [Pd(SalGly)2]Cl2; 3, [Pd(SalMet)2]Cl2; 4, [Pd(SalSer)2]Cl2; 5, [Pd(SalVal)2]Cl2; have shown antibacterial activity against Gram-positive bacteria Staphylococcus aureus and Gram-negative bacteria Escherichia coli.

Key words salicylaldehyde-amino acid Schiff base; Pd(II) complex; antibacterial activity

As bacterial drug resistance to currently administered treatments is increasing, the need for the development of new therapeutic strategies against bacterial infection becomes more stringent. Therefore, there is an urgent demand for the development of new antimicrobial agents with improved properties such as enhanced activity, reduced toxicity and shortened duration of therapy.3,4 Schiﬀ bases have a variety of applications in chemical, biological and pharmacological ﬁelds.2,3 The bi-, tri- or tetra-dentate Schiﬀ bases with N, O as donor atoms, can be obtained by condensation of aldehydes or ketones with various amines, diamines or aminoacids.4 Schiﬀ bases have been used as organic chelating ligands in the synthesis of diverse transition metal complexes.5,6 Due to their structural and functional properties, sometimes similar with some biological systems, the coordination compounds with Schiﬀ bases are used in inorganic, organic and biological ﬁelds.7,8

Amino acids play an important role in many biochemical processes. The metal complexes with amino acids play an important role in understanding biological functions of macromolecules such as proteins in human body.9 The aminoacids form with salicylaldehyde heterodentate Schiﬀ bases that proved to possess catalytic activity10 or interact with DNA.11 During recent years, a series of aminoacids based coordination compounds have been prepared and found to be active against several Gram-positive and Gram-negative bacterial strains.12 Owing to the ability of palladium ions to form complexes, they bind to aminoacids (e.g., L-cysteine, L-cystine, L-methionine), proteins (e.g., casein, silk ﬁbroin, many enzymes), DNA or other macromolecules (e.g., vitamin B6).13 Furthermore, it is very important to fully understand the physiological functions of the metal elements from human body by studying their coordination behavior.14

Palladium compounds may interact with isolated DNA in vitro. However, with one exception, mutagenicity tests of several palladium compounds with bacterial or mammalian cells in vitro (Ames test: Salmonella typhimurium; SOS chromotest: Escherichia coli; micronucleus test: human lymphocytes) gave negative results. Also, an in vivo genotoxicity test (micronucleus test in mouse) with tetraammine palladium hydroxide carbonate gave negative results.13 Staphylococcus aureus and Escherichia coli are common bacteria affecting mammmals. Although, there are a variety of drugs available for the treatment of their infections, strains of these bacteria such as methicillin-resistant Staphylococcus aureus (MRSA), extended-spectrum beta-lactamase (ESBL) producing E. coli, and multi-resistant bacteria are now showing resistance to these drugs. The present study describes the synthesis, characterization and antibacterial activities of the next palladium(II) complexes of salicylaldehyde-amino acids (Gly, Ala, Met, Val, Ser) Schiﬀ bases: 1, [Pd(SalAla)2]Cl2; 2, [Pd(SalGly)2]Cl2; 3, [Pd(SalMet)2]Cl2; 4, [Pd(SalSer)2]Cl2; 5, [Pd(SalVal)2]Cl2. All five Pd(II) complexes have been preliminarily tested for their antibacterial activity against Gram-positive bacteria (Staphylococcus aureus) and Gram-negative bacteria (Escherichia coli). The mode of action of palladium ions and of elemental palladium is not fully clear. Complex formation of palladium ions with cellular components probably plays a basic role initially. Oxidation processes may also be involved, due to the different oxidation states of palladium.13

Experimental

All commercially available products were used without further puriﬁcation unless otherwise speciﬁed. The Fourier transform (FT)-IR spectra were recorded on a JASCO FT-IR 660 Plus spectrometer using KBr pellets. The UV-VIS spectra were recorded in EtOH solution, c=1 mm, using a Cintra 101 device. The 1H-NMR spectra were recorded on a Bruker 500 spectrometer. All chemical shifts are quoted on the δ-scale in ppm. The mass spectra were recorded on a Excalibur spectrometer using MeOH–CH2Cl2 (1:1, v/v) mixture, electrospray ionization (ESI) in the positive mode; m/z values are reported in Daltons.

A study regarding the antibacterial activity of the Pd(II) complexes was determined by diffusion method.15

General Procedure for Synthesis of Pd(II) Complexes

Salicylaldehyde (0.010 mol) in ethanol (20 mL) was added to an aqueous solution of amino acids (0.010 mol in 20 mL water). Then, an aqueous solution (10 mL) of sodium acetate (10 mL, 0.020 mol) was added. A solution of Pd(II) chloride

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(0.0085 mol) in MeCN (25 mL) was added drop wise to the reaction mixture while stirring at room temperature. The color of the solution turns from yellow to brown. If the product precipitated from solution, it was separated by filtration and successively washed with water and diethyl ether, if not, it was allowed to crystallize, and successively washed with water and diethyl ether. If necessary the product was recrystallized from MeOH (75–85% yields).

**Antimicrobial Testing**

The antibacterial activity of the agents salicylaldehyde-amino acids Schiff bases ligands 1–5 has been tested using the diffusion method. Antimicrobial tests were made on standardized 24 h bacterial cultures *Staphylococcus aureus* ATCC 29213 (Gram-positive bacteria) and *Escherichia coli* ATCC 25922 (Gram-negative bacteria), obtained in nutrient broth liquid medium, after thermostating at 37°C, as described by guidelines in National Committee for Clinical Laboratory Standards (NCCLS)-approved standard document M7-A7. Antimicrobial activities of the newly synthesized chemical compounds were performed in Mueller–Hinton medium (Oxoid) at a pH of 7.3. Both cultures were brought to a 0.5 McFarland turbidity standard, which for most bacterial species corresponds to $2 \times 10^8$ colony forming unit (cfu)/mL. A build-in technique for solid media was used; thus, nutrient agar was melted and cooled to 45°C, then poured in Petri plates and homogenized with 1 mL bacterial culture obtained in nutrient broth. After the medium solidified, a well was made in the plates with sterile borer (5 mm). The test compounds (0.1 mg) was introduced into the well and plates were incubated at 37°C for 72 h. Microbial growth was determined by measuring the diameter of zone of inhibition. These zones of inhibition are the spaces in which microorganisms have not replicated due to the action of the active substance and therefore did not form bacterial colonies.

**Results and Discussion**

The complexes were synthesized by template method, Chart 1 and their structure were investigated by ESI-MS, UV-VIS, IR and NMR.

**Mass Spectra of the Pd(II) Complexes**

The mass spectrum of the complex 3, presents a molecular pattern with maximum intensity at $m/z = 739$ (Fig. 1) that is in good agreement with $[\text{M}+\text{H}]^+$ for the structure presented in Fig. 2. Similarly, all Pd(II) complexes show molecular patterns in electrospray (ES)-MS that confirm the general formula: $[\text{Pd(SalAmac)}_2]\text{Cl}_2\cdot n\text{H}_2\text{O}$ for these complexes. Thus, the mass spectra of complex 1 shows a pattern with maximum at $m/z = 600$ corresponding to $[\text{Pd(Sal Ala)}_2]\text{Cl}_2\cdot 2\text{H}_2\text{O}$ formula, the mass spectra of complex 2 shows a pattern with maximum at $m/z = 554$ for $[\text{Pd(Sal Gly)}_2]\text{Cl}_2\cdot \text{H}_2\text{O}$, complex 4 furnished a pattern with maximum at $m/z = 614$ corresponding to $[\text{Pd(Sal Ser)}_2]\text{Cl}_2\cdot \text{H}_2\text{O}$ and complex 5 showing a pattern with maximum at $m/z = 638$ for $[\text{Pd(Sal Val)}_2]\text{Cl}_2\cdot \text{H}_2\text{O}$.

**UV-VIS Spectra**

The electronic spectra of the Pd(II) complexes exhibit bands in the UV range at 275–280 nm and...
380–390 nm, Table 1. All these Pd(II) complexes show a broad d–d transition band in the region of 480–490 nm assignable to ¹B₁g  → ¹A₁g transition, typical to square planar geometry.¹⁷

**Infrared Spectra** The infrared frequencies of Pd(II) complexes are given in Table 2. All the complexes exhibit broad bands in the range of 3440–3590 cm⁻¹, which can be attributed to the presence of coordinated water molecules. The strong IR absorption band observed in the free Schiff base at around 1600–1630 cm⁻¹ is assigned to the presence of coordinated water molecules. The region of 1340–1386 cm⁻¹, bands detected around 420–460 cm⁻¹ and the –OH free groups from ligands increase the lipophilic character of the complexes.

**Antibacterial Activity** The diameter of the inhibition zones area has been measured. These inhibition zones provide important information about the putative mechanism of bacterial resistance. All the tested palladium(II) complexes show a remarkable biological activity against Gram-positive Staphylococcus aureus and Gram-negative Escherichia coli bacteria. The data are shown in Fig. 3.

The screening for the palladium chloride salts and new complexes were performed at the fixed amount of 0.1 mg. Based on the obtained values of the relative zone inhibition,¹⁴,¹⁵ 1 and 2 complexes were found to be very effective against Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* bacteria, Table 4.

The obtained metal complexes showed increased activity compared to the corresponding metal salt. The higher activity of the complexes may be due to the change in the structure of compounds, as polarity of the metal. This can be explained by partial sharing of the positive charge of the metal with the donor groups of the ligands. Chelation of the metallic ion and –OH free groups from ligands increase the lipophilic nature of the central metal atom, which favors its permeation.
more efficiently through the lipid layer of the microorganisms. Therefore, obtained complexes present remarkable biological activity.

**Conclusion**

The salicylaldehyde and amino acids form *in situ* Schiff bases that proved to be ligands for complexation of Pd(II) ion. The coordination of metallic ion to the ligands, by imino nitro-bases that proved to be ligands for complexation of Pd(II) ion.

Table 4. Inhibitions Zones Data (mm) of Palladium Complexes

| Compounds          | Zones of inhibition (mm) |
|--------------------|--------------------------|
|                    | *Staphylococcus aureus* ATCC 29213 | *Escherichia coli* ATCC 25922 |
| PdCl2/MeCN         | 6                         | 7                           |
| 1                  | 22                        | 20                          |
| 2                  | 19                        | 24                          |
| 3                  | 13                        | 14                          |
| 4                  | 13                        | 8                           |
| 5                  | 14                        | 11                          |
| Ampicillin<sup>a</sup> | 13                     | 8                           |
| Streptomycin<sup>a</sup> | 12                     | 6                           |

<sup>a</sup> Ref. 1.

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