# Synthesis and Biological Evaluation of a New Schiff Base and its Cu(II) Complex

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*A new Schiff base ligand, N-hydroxy-N'-salicylidene-urea was synthesized through the condensation of salicylaldehyde with hydroxyurea. The copper(II) complex of the Schiff base has been also obtained. Their structure has been proven using spectral methods such as UV-Vis, FT-IR, $^1$H-NMR and elemental analysis. The antimicrobial activity of the copper(II) complex was evaluated through comparison to the activity of the Schiff base on various bacterial strains. All tested compounds were very active against both gram-positive and gram-negative bacteria.*

*Keywords: Schiff base, coordinative complex, antimicrobial activity, UV-Vis, FT-IR and $^1$H-NMR experimental techniques*

Compounds with the azomethine group (-C=N-) in their structure have been known as Schiff bases. They are usually synthesized through condensation of primary amines with compounds with active carbonyl groups [1]. The biological activity of Schiff bases has been attracting the attention of organic chemists and medical researchers for many years. Nowadays, Schiff bases have well known representatives in the groups of anticancer [2], antimicrobial [3, 4], anti-inflammatory [5, 6], antiviral [7], analgesic [8], pesticidal [9] and antioxidant agents [10, 11].

Based on the above-mentioned applications of Schiff bases, this study presents the synthesis, physico-chemical characterization and antimicrobial effects of a new Schiff base and its Cu(II) complex.

## Experimental part

### *Materials and methods*

All chemicals and solvents were analytical reagent grade and they were supplied by Merck (Germany) and Chimopar (Romania). The melting points were determined using a Boethius apparatus without correcting the result. The IR spectra (from KBr pellets) were recorded on a FTS-135 BIO-RAD spectrometer. The UV-Vis spectra was obtained on a Hewlett-Packard 8453 UV-Vis spectrophotometer. Elemental analysis of C, H and N was carried out with an Elemental Vario Analyzer. The quantitative determination of Cu(II) was performed using the AAS-IN Carl-Zeiss-Jena spectrometer.

### *Synthesis and characterization of N-hydroxy-N'-salicylidene-urea*

0.01mol (0.76g) of hydroxyurea dissolved in 10mL of methanol was mixed with 0.01mol (1.06mL) of salicylaldehyde dissolved in 30mL of methanol and then it was refluxed for 2-4 h [12-14]. The reaction mixture was concentrated *in vacuo* and after the addition of ethyl ether, a brown solid precipitate was collected. It was washed with a 2:1 ether/ethanol mixture and then it was crystallized from a diethyl ether.

The ligand was a brown crystalline powder, stable at room temperature, insoluble in water, soluble in ethanol and methanol, very soluble in acetone and dimethylformamide (DMF). Yield 75.7%; m.p. 158-160°C. UV-VIS $\lambda_{max}$ (DMF) nm (ε, mol$^{-1}$·L cm$^{-1}$): 282(3.10), 325(3.27). FT-IR (KBr), cm$^{-1}$: $\nu_{max}$ 3390(-OH aril), 1665(C=O), 1064(C-N), 1685(C=N), 1370, 760($C_6H_5$), 1280(-OH arom). $^1$H-NMR ($CDCl_3$): δ11.31-11.45 (s, 2H, OH), 7.26-7.49 (m, 3H, H-Ar), 8.51 (s, 1H, CH=N), 5.45 (s, 1H, NH). Anal. Calcd. for $C_8H_8N_2O_3$: C, 53.34; H, 4.48; N,15.55. Found: C, 53.98; H, 4.75; N, 15.69.

### *Synthesis and characterization of Cu(II) complex*

The Cu(II) complex was synthesized using the general procedure reported previously [15,16]. 1.99g (0.01mol) of Cu $(OAc)_2 . H_2O$ dissolved in 25mL methanol was added drop wise to 1.8g (0.01mol) of ligand dissolved in advance in 25mL methanol. The mixture was stirred at room temperature for 4 hours and then it was evaporated at 90°C, until the solution darkened; sparkling black micro-crystals were filtered, washed with a mixture of ethanol-water (1:1, v/v) and then with ethyl ether. The Cu(II) complex was a green crystalline powder that was stable at room temperature, insoluble in water, ethanol, benzene or chloroform, soluble in methanol, DMSO and DMF. Yield: 65.3%; m.p. 179-180°C. UV-VIS $\lambda_{max}$ (DMF) nm ( ε, mol$^{-1}$·L cm$^{-1}$) $8.09 \cdot 10^4$, Ks = $6.91 \cdot 10^5$; solubility (mol/L): $5.08 \cdot 10^{-4}$, 355 nm, FT-IR (KBr), cm$^{-1}$: $\nu_{max}$ 1610(C=N), 1668(C=O), 1055(C-O), 1360, 1370, 740($C_6H_4$), 528(Cu(II)-N), 505(Cu(II)-O). $^1$H-NMR($CDCl_3$): 7.28-7.50 (m, 5H, H-Ar), 9.78 (s, 1H, CH=N). Anal. Calcd. for Cu[$C_8H_6N_2O_3 \cdot H_2O$]: C, 36.99; H, 3.10; N, 10.78; Cu, 24.47. Found: C, 37.06; H, 3.25; N, 10.85, Cu, 24.51.

### *Evaluation of antimicrobial activity*

The antimicrobial activity of the ligand and the copper (II) complex were evaluated against Gram-positive bacteria: *Staphylococcus aureus* ATCC 25923 *(Sa), Bacillus cereus* ATCC 14579 *(Bc), Bacillus subtilis* ATCC 6633 *(Bs),* and Gram-negative bacteria: *Escherichia coli* ATCC 25922 (Ec), *Pseudomonas aeruginosa* ATCC 9027*(Pa)*. Chloramphenicol and Ampicillin were used as reference substances.

The qualitative antimicrobial assay of the compounds was performed using the agar diffusion method according to standard accepted disk sensitivity criteria of The National Committee for Clinical Laboratory Standards [17-20].

The agar dish diffusion procedure is a method approved by the National Committee for Clinical Laboratory

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| Antimicrobial agents | Antimicrobial agent | | | |
|---|---|---|---|---|
| | Ligand | Cu(II) complex | Ampicillin | Chloramphenicol |
| Pa | 22.70 ±0.28 | 25.30±0.55 | 24±0.22 | 24±0.22 |
| Sa | 16.30±0.57 | 19.66±0.52 | 30±0.27 | 29±0.53 |
| Bc | 20.20±0.53 | 25.70±0.53 | 27±0.52 | 28±0.42 |
| Bs | 18.30±0.42 | 22.70±0.27 | 28±0.45 | 30±0.37 |
| Ec | 19.30±0.33 | 22.50±0.28 | 23±0.38 | 26±0.35 |

**Table 1**
*IN VITRO* ANTIMICROBIAL ACTIVITY OF THE LIGAND AND Cu(II) COMPLEX AGAINST GRAM-POSITIVE AND GRAM-NEGATIVE STRAINES THROUGH THE DIAMETER OF THE INHIBITION ZONE (MILLIMETERS)

Standards and was one of the first methods for evaluating the *in vitro* efficacy of antimicrobial agents. The microbiological assay is one in which the antimicrobial agent placed in a reservoir (paper disc, cylinder), diffuses directly against seeded bacteria.

A standard suspension of each reference strain was prepared from fresh overnight cultures and it was mixed with 15mL portions of molten nutrient agar in sterile Petri plates, resulting a final concentration of about $10^6$cells/mL. The plates were sliced with solid metal cylinders (6mm in diameter) and then 0.2mL samples solutions of ligand and complex and standard commercial disks of 10 μg Ampicillin and 30 μg Chloramphenicol were transferred into each well. Each microorganism was tested in triplicate and the zones of inhibition around the wells were measured after incubation at 37°C for 24 h. The diameter of the inhibition zones were evaluated as mean ± SD.

## Results and discussions

The structures of the ligand and its complex was confirmed using spectroscopic methods and elemental analysis. The UV-Vis spectrum of the ligand included a large absorption peak at 282nm that shifted to 355nm in the UV-Vis spectrum of its Cu(II) complex due to the ligand's coordination with the metallic ion.

The IR spectrum of the ligand included a characteristic band at 1685cm$^{-1}$ which was due to vibration of C=N group. The shifting of that group to a lower frequency (1610cm$^{-1}$) in the spectrum of Cu(II) complex implied the coordination of the metal ion through the nitrogen atom of the azomethine group. It was expected that the coordination of nitrogen to the metal atom would reduce the electron density in the azomethine group and thus lower the C=N group absorption. The band at 1665cm$^{-1}$ attributed to the vibration of C=O group in the spectrum of the ligand also shifted to a lower frequency (1668cm$^{-1}$) in the spectrum of its Cu(II) complex, which implied that the oxygen atom of the C=O group was not linked to the metal ion. The band at 1280cm$^{-1}$ assigned to the stretching frequency of phenolic C-OH bond and the band at 3390cm$^{-1}$ both observed in the spectrum of the ligand disappeared from the spectrum of the complex.

Two new bands which were not present in the spectrum of the ligand, appeared in the spectrum of the complex at 505cm$^{-1}$ and 528cm$^{-1}$ corresponding to vibration of M-O and M-N groups. The appearance of those bands proved the involvement of N and O atoms in the complexation of Cu(II). The complex exhibited a broad and relatively intense band around 3400cm$^{-1}$ which indicated the presence of water molecules. That band corresponded to the vibration of O-H stretching. That band was accompanied by two other bands in the 700-800cm$^{-1}$ range in the spectrum of the complex. That fact suggested that the water molecules were coordinated.

The $^1$H-NMR spectrum of the complex possessed significant modifications due to the coordination process in reference to that of the ligand. The proton signal of the -OH (11.45ppm) and the -NH (5.45ppm) groups from the structure of the ligand disappeared upon complexation with Cu(II). The aromatic protons did not seem to register significant changes as a result of the coordination process.

The results of the elemental analysis of ligand and its complex were found to be in good agreement with the values that had been theoretically calculated.

The Antimicrobial activity was correlated to the ability of the compounds to diffuse through biological membranes to reach its site of action. The cylinder technique was used for testing because it was more sensitive than paper discs technique. The antimicrobial activity was estimated by measuring the diameter of the area inhibited by the Cu (II) complex when compared to that of the ligand.

Table 1 summarized the antimicrobial activity of tested compounds against Gram-positive and Gram-negative reference strains, in comparison to Ampicillin and Chloramphenicol.

The Cu(II) complex showed a higher antimicrobial action than the free ligand and that effect was evident against all reference bacteria tested. The Cu(II) complex was more active than the ligand because of the coordination involving –OH and the nitrogen atom of C=N group.

The association of the ligand with Cu(II) substantially increased the *in vitro* susceptibility of Gram-positive and Gram-negative tested bacteria. Against *Staphylococcus aureus* ATCC25923, both ligand and its Cu(II) complex exerted the lowest degree of antimicrobial activity when compared to sporulated bacteria, *Escherichia coli* and *Pseudomonas aeruginosa*.

Both tested substances showed at concentrations of 10μg/mL an antimicrobial profile similar to that of ampicillin and chloramphenicol (30 μg/disk) against *Bacillus cereus, Escherichia coli* and *Pseudomonas aeruginosa*. On the contrary, *Staphylococcus cereus* and *Bacillus subtilis* were less sensitive to these compounds than *Bacillus cereus*, *Escherichia coli* and *Pseudomonas aeruginosa*. The data of table 1 shows large inhibition zones of microbial growth. The differences in activity probably reflected the differences in the mode of action of their chemical structures against the bacterial cell.

## Conclusions

The research study reported the successful synthesis and antimicrobial activity evaluation of a new Schiff base and its Cu($^{22}$) complex. Both substances were physically and chemically characterized through elemental analysis, $^1$H-NMR, UV-Vis and IR analysis, the ratio of metal/ligand combination, the melting point and the solubility. The IR spectrum confirmed the hypothesis of the formation of

the complex by coordination of copper ions to the azomethinic nitrogen and to the phenolic oxygen.

The tested compounds were active against both gram-positive and gram-negative bacteria. The antimicrobial activity of the complex resembled that of Ampicillin and Chloramphenicol. Also, all tested bacteria revealed a lower sensitivity against the ligand than against the complex.

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