Full Paper

Applied Organometallic Chemistry

Received: 30 January 2012 Revised: 18 March 2012 Accepted: 9 April 2012 Published online in Wiley Online Library

(wileyonlinelibrary.com) DOI 10.1002/aoc.2872

Spectroscopic, thermal and antimicrobial properties of the copper(II) complex of Schiff base derived from 2-(salicylidene) aminopyridine

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Schiff bases and their complex combinations with metallic ions represent a class of compounds with antimicrobial activity. A ligand was prepared by condensation of the salicylaldehyde with 2-aminopyridine obtaining 2-(salicylidene) aminopyridine (SB) with a high capacity for complexing Cu(II) ions. The new compound has been characterized by physical constants (melting point, solubility, stability) and the chemical structure was confirmed by elemental, spectral (IR, UV–visible, ^{1}H NMR and ^{13}C-NMR) and thermal analyses. The elemental analysis gives a coordination ratio of 1:2 metal:Schiff base. Lethal dose 50 (DL_{50}) values of new Schiff base and their complex with metallic ions were established. The antimicrobial activity of this complex was tested in comparison with the activity of the corresponding Schiff base on strains of *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Candida albicans*, and *Klebsiella*. These were compared with the activity of the reference drugs (chloramphenicol, tetracycline, ofloxacin and nystatin) on the above-mentioned strains. It has been established that all compounds tested were very active against both Gram-positive and Gram-negative bacteria. Copyright © 2012 John Wiley & Sons, Ltd.

Keywords: Schiff base; 2-(salicylidene) aminopyridine; Cu(II) complex; antimicrobial activity; toxicity

Introduction

Investigation of metal organic complexes represents one of the most active areas of material science and chemical research. Major advances have been made in these materials because of their interesting properties and potential in various applications, e.g. electrical conductivity, magnetism, host guest chemistry, ion exchange, catalysis, nonlinear optics.[1] Synthesis of new Schiff bases and their metal complexes is still the aim of many recent investigations. Schiff bases have played an important role in the development of coordination chemistry as they readily form stable complexes with most of the transition metals. They show interesting properties, e.g. their ability to reversibly bind oxygen,[2] catalytic activity in hydrogenation of olefins,[3] transfer of an amino group,[4] photochromic properties[5] and complexing ability towards toxic metals.[6]

Schiff bases continue to have an important role as ligands in metal coordination chemistry,[7] even almost a century since their discovery. These applications were generated by the possibility of formation of some complex combinations with transition metals.

Most of the metal complex model studies of Schiff base ligands containing salicylaldehyde and amino acids have focused upon the binding mode of these ligands[8,9] and only a few studies are related on their applications.

Ligands derived from substituted salicylaldimine have played an important part in revealing the preferred coordination geometries of metal complexes. Of particular interest were those involving copper(II) since they reveal surprising molecular diversity not only in coordination geometry but also in more subtle changes in the ligands. Thus complexes with four, five or six donors or with marked tetrahedral 'distortions' are accompanied by bond length changes and deviations from expected ligand geometry. So-called 'stepped' or 'umbrella' distortions are well documented, with deviations from planarity and unexpected bond lengths in the aromatic ring of salicylaldiminato (salim) groups.

Schiff bases can form a new class of drugs through mechanisms of immune potentiation and therapeutic potential. The complex combination of Schiff bases with metallic ions represents a class of compounds with the most interesting properties, both from the point of view of chemical and biological behavior. The literature data quotes the antimicrobial, anti-inflammatory and antioxidant action of the complex combinations of Schiff bases with different cations.[10,11]

Several Schiff base complexes of copper(II), such as *N*,*N*′-1, 2-phenylene-bis(salicylideneiminato)copper(II), bis(*N*-phenylpyridoxylideneiminato)copper(II), aqua(5-phosphopyridoxylidene-DL-phenylalanineato)copper(II) were reported in the literature data. No studies were found on the Cu(II) complexes of the Schiff base derived

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from 2-(salicylidene) aminopyridine.[12] Schiff bases and their complexes have a variety of biological,[13] clinical[14] and analytical[15] applications. Earlier works have shown that some drugs had increased activity when administered as metal chelates rather than as organic compounds.

This work deals with the study of the physicochemical characterization of a Schiff base derived from 2-(salicylidene) aminopyridine and its complex with Cu(II) ions. The antimicrobial activity of this complex was compared with that of Schiff base on strains of *Staphylococcus aureus* ATCC 25923, methicillin-resistant *S. aureus* (MRSA), *Bacillus cereus* ATCC 14579, *B. subtilis* ATCC6633, *Escherichia coli* ATCC25922, *Candida albicans* ATCC 10231 and *Klebsiella* spp. This activity was also compared with that of the reference drugs (chloramphenicol, tetracycline, ofloxacin and nystatin) on the above-mentioned strains.

Materials and Methods

Materials

All chemicals used in this work were reagent grade (by Merck and Chimopar Bucharest), including $Cu(OAc)_2.H_2O$, salicylaldehyde, 2-aminopyridine, DMSO and methanol. Double-distilled water was used.

Synthesis of Ligand and Complex

Synthesis of Schiff base

The Schiff base used as ligand has been prepared by condensing salicylaldehyde with 2-aminopyridine in methanol, in a 1:1 molar ratio, at room temperature. The precipitate was collected after 48 h by filtration, washed with water, ethanol–water mixture (1:1, v/v) and absolute ethanol, and recrystallized from methanol. A fine, intense yellow crystalline powder of 2-[salicylidene]-aminopyridine (SB) type NNO was obtained.[16] The product yield for the schiff base was 85%.

Synthesis of the complex

To 2 M (0.396 g) Schiff base (50 ml) methanolic solution was added 1.001 mM (0.2 g) $Cu(OAc)_2.H_2O$ methanolic solution. The resulting solution was refluxed for 1.5 h and left to rest at room temperature for 48 h; it was filtered, then left to dry on filter paper. Finally, it was recrystallized from dimethylformamide. A light-green crystalline powder was obtained. The product yield for the complex was about 65%. See Fig. 1 for structures of the ligand and complex.

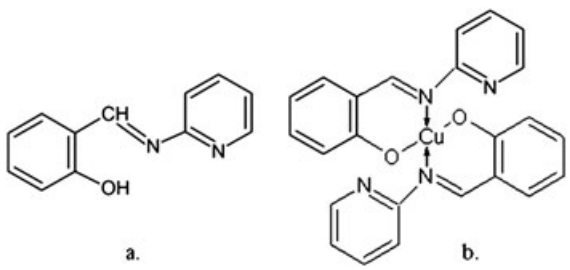

Figure 1. Structure of the ligand (a) and complex (b)

Methods

Elemental analyses

Elemental analyses (C, H, N) were carried out with an Elemental Vario EL analyzer. The quantitative determination of Cu(II) ions from the synthesized complexes was performed using an AAS- IN atomic absorption spectrometer (Carl Zeiss, Jena, Germany).

FT-IR spectroscopy

FT-IR spectra were recorded on a solid sample in a KBr pellet by means of an FT-IR DIGILAB, Scimitar Series Spectrometer (USA) with a resolution of 4 cm^{-1}. The concentration of the sample was 5 mg/500 mg^{-1} KBr. Processing of the spectra was performed using the Grams/32 program (Galactic Industry Corporation).

UV–visible spectroscopy

The electronic spectra were obtained on a Hewlett- Packard 8453 UV–visible spectrophotometer.

NMR spectroscopy

^1H and ^{13}C-NMR spectra were recorded on Bruker 400 MHz equipment, using DMSO as solvent.

Thermogravimetry

Thermogravimetric analysis (TGA) was carried out under constant nitrogen flow at a heating rate of 15°C min^{-1}, using a Mettler Toledo TGA/SDTA 851 balance. The heating scans were performed on 3–5 mg of sample, in the temperature range 25–900°C.

Biological Activity

Toxicity study

Acute toxicity was estimated by oral administration of SB and SB-Cu(II), using sodium carboxymethyl cellulose (Na-CMC) 0.1% suspension as carrier, to groups of 6–10 Swiss male mice (each mouse weighing 20–25 g). Administration was made according to classical laboratory methodology.[17] The animals received food and water *ad libitum*. Three hours before testing their access to water was discontinued. Acute toxicity was evaluated, using geometrically progressing doses (ratio 2) in single administrations. Death of animals and their behavioral reactions were followed during 10 days. The testing was made in accordance with the international legislation and the internal regulations[18,19] of the University of Medicine and Pharmacy concerning experiments using lab animals.

Statistics: the interpretation of the results was made by analyzing the regression lines, and the data were submitted for ANOVA testing.

Antibacterial activity

The newly prepared compounds were screened for their antibacterial activity against Gram-positive and Gram-negative bacteria (*S. aureus* ATCC 25923, MRSA, *B. cereus* ATCC 14579, *B. subtilis* ATCC 6633, *E. coli* ATCC 25922, *Klebsiella* spp., *C. albicans* ATCC 10231) using disc diffusion assay.[20] Suspensions in sterile peptone water from 24 h cultures of microorganisms were adjusted to 0.5 McFarland. Muller–Hinton Petri dishes of 90 mm

were inoculated using these suspensions. The tested compounds were dissolved in DMSO to a final concentration of 1000 μg ml^{-1}. The discs (6 mm in diameter) were impregnated with 10 μl of each compound and placed on the inoculated peptone water. DMSO-impregnated discs were used as negative controls. Toxicity tests of the solvent, DMSO, showed that the concentrations used in antibacterial activity assays did not interfere with microorganism growth.[21]

Results and Discussion

The synthesized compounds are crystalline and non-hygroscopic. They are insoluble in water, partially soluble in ethanol, and soluble in acetone, DMF and DMSO. Composition and identity of the assembled compounds were deduced from elemental analyses, spectroscopic techniques (IR, UV–visible, NMR) and thermal studies. The analytical data of the complex indicated 1:2 metal to ligand stoichiometry. The composition of the ligand and complex (Table 1) was calculated and compared with the experimental values.

For the characterization of coordination compounds, an important study is related to the complex stability of Cu(II) with (SAP) ligand.

The stability constant was determined following the method of Harvel and Manning, and the dissociation degree according to the relations

$$K_i = \alpha^2 C/1 - \alpha; \alpha = A_m - A_s/A_m$$

where α = dissociation degree, A_m = maximum absorbance, A_s = equilibrium absorbance, and C = concentration of the solutions of Cu(II) and SB.

$\alpha = 0.1118$; $K_i = 1.407 \times 10^{-6}$; $K_s = 7.1 \times 10^5 \times \varepsilon = 1.20 \times 10^5$ mol^{-1} L cm^{-1}. Solubility = 1.09×10^{-2} mol L^{-1}.

Combination rate was established by isomolar series method (Fig. 2).

The SB-Cu(II) complex has a higher K_s than other Cu(II) complexes, which have $K_s = 4.3 \times 10^5$ and $\varepsilon = 1.08 \times 10^4$ mol^{-1} L cm^{-1}.[22] By comparing the K_i value of our complex with that of an SB-Mn (II) complex, which is 2.943×10^{-5},[23] it can be observed that the first one is more stable than the last one. From Fig. 2, the M/L ratio evidenced that every Cu ion needs two ligands.

FT-IR and UV–Visible Spectroscopy

IR spectroscopy is a powerful method for highly sensitive and selective concentration determination and identification of chemical species. The specific absorption of the substance in the 'fingerprint' region enables the distinct recognition of various chemical species and even of structural isomers.

IR spectra of SB along with its complex are displayed in Fig. 3.

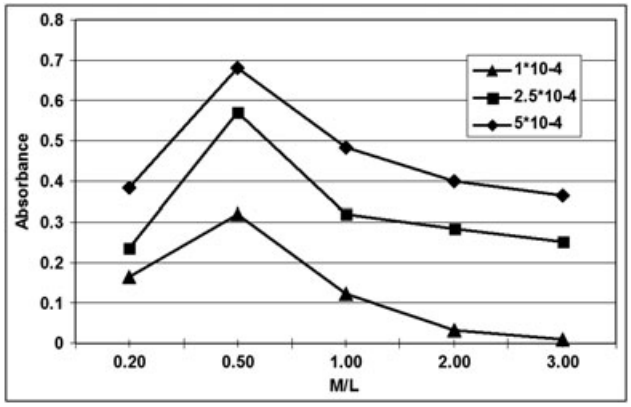

Figure 2. Adsorbance versus molar ratio M/L for SB/Cu(II) complex

The observed bands in the SB-Cu(II) complex spectrum can be classified into those originating from the ligand and those arising from the bonds formed between metal ions and the coordinating sites. The infrared spectrum of the ligand shows a broad band between 3200 and 3450 cm^{-1}, which can be attributed to the stretching vibration of the phenolic OH (3436 cm^{-1}) and to

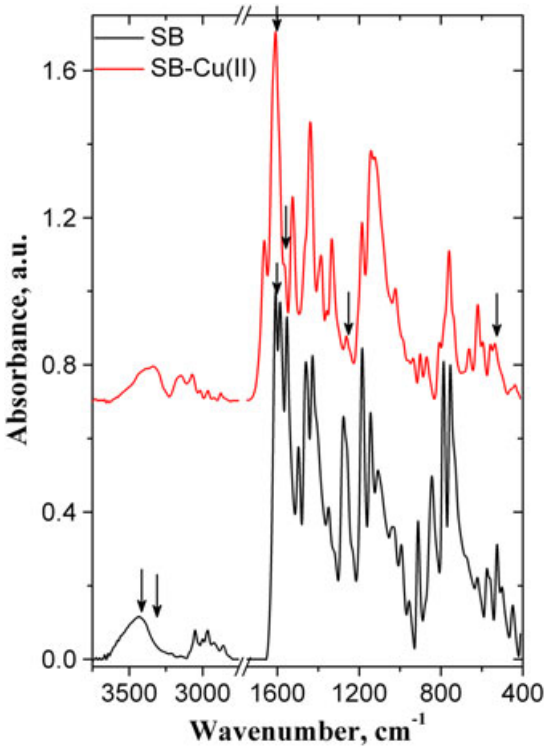

Figure 3. FT-IR spectra of SB and SB-Cu(II) complex

Table 1. Analytical and physical data of ligand and its Cu (II) complex

Sample	Color	Elemental analysis								M.p. (°C)
		C		H		N		Cu		
		Exp.	Calc.	Exp.	Calc.	Exp.	Calc.	Exp.	Calc.	
SB ($C_{12}H_{10}N_2O$)	Intense yellow crystalline powder	78.5	78.3	5.7	5.4	8.0	7.6	—	—	69
SB-Cu(II) ($C_{24}H_{18}N_4O_2Cu$)	Light-green crystalline powder	63.2	63.0	4.0	3.9	12.4	12.2	13.9	13.8	160

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the stretching vibrations of H-bonded OH groups (3325 cm^{-1}). The first band decreases and is shifted to lower wavenumbers in the SB-Cu(II) complex, suggesting the involvement of phenolic OH groups in coordination. In the Schiff base complex spectrum a new band at 1663 cm^{-1} was evidenced, which can be assigned to physical bonded water molecule stretching vibrations. Also, one can observe a shifting of the bands, corresponding to aromatic CH stretching vibrations, to higher wavenumbers.

The involvement of a deprotonated phenolic moiety in the SB-Cu(II) complex is confirmed by the shifting of ν(C-O) stretching band (observed at 1276 cm^{-1} in the ligand) to a lower frequency with 14 cm^{-1}. This shifting suggests the weakening of C-O bonds and formation of Cu-O bonds. The free Schiff base ligand showed strong bands at 1610 cm^{-1} and 1586 cm^{-1}, which are characteristic of the azomethine (-HC N) groups. Coordination of the Schiff base to the metal through the nitrogen atom is expected to reduce electron density in the azomethine link and shift to a lower frequency of ν(C-N) absorption. This phenomenon was observed, proving the coordination of the azomethine nitrogen to metal ions. Coordination of the azomethine nitrogen is further supported by the appearance of bands in the range of 536 cm^{-1} due to ν(Cu-N).

The electronic spectra of the free ligand in DMF solution, recorded in the 250–800 nm region, exhibit bands in the range 250–280 and 350 nm, which can be assigned to the π→π* and n→π* transitions, respectively, of the azomethine group. The spectrum of SB-Cu(II) showed three absorption bands. The first one, at 340–350 nm, can be assigned to the O-Cu charge-transfer transition. The band situated at 420–430 nm can be attributed to the N-Cu charge-transfer transition, being overlapped by the π*→π or n→π transitions of the ligand. A new band observed as a shoulder in the spectrum at 540–550 nm can be assigned to the d→d transition of the divalent copper(II) with square-planar geometry.[24]

NMR Spectroscopy

The ^{1}H NMR spectrum of the ligand and the SB-Cu(II) complex were recorded to confirm the binding of the Schiff base to the metal ions. The spectrum of the complex showed a peak at 9.52 ppm, which has been assigned to the azomethine proton (-HC N). The position of the azomethine signal in the complex is downfield in comparison with that of the free ligand, suggesting deshielding of the azomethine proton due to its coordination to metal ions through the azomethine nitrogen. The 6.70–7.90 ppm region was assigned to chemical shifts for hydrogen of the ligand symmetrical aromatic ring and peaks in the 6.2–6.7 ppm region were assigned to the chemical shift of the pyridine hydrogen.[25] In the ^{1}H NMR spectrum of the Schiff base, the phenolic OH signal at 13.99 ppm is evidenced. This signal disappeared in the spectrum of the complex, indicating deprotonation of phenolic proton and confirming coordination through phenolic oxygen. A weak peak in the 4.5–5.2 ppm region, characteristic of the physical bonded water molecule, is observed in the spectrum of the complex.[25]

^{13}C NMR of SB shows signals at 164.41 ppm assigned to C7 carbon, 160.8 ppm assigned to C1 carbon, 157.47 ppm assigned to C12 carbon, 148.95 ppm assigned to C8 carbon, 138.98 ppm assigned to C6 carbon, 134.03 ppm assigned to C10 carbon, 133.19 ppm assigned to C3 carbon, 122.88 ppm assigned to C4 carbon, 119.68 ppm assigned to C11 carbon, 119.3 ppm assigned to C5 carbon, 118.98 ppm assigned to C2 carbon and 116.67 ppm assigned to C9 carbon (Fig. 4).

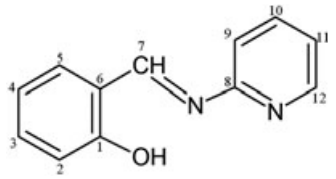

Figure 4. Numbering of carbon atoms in SB

In the sample SB-Cu(II) spectrum the bands are broad, indicating complex formation, especially because this is only partially soluble in DMSO and has paramagnetic properties.

Thermogravimetry

The thermal analysis of SB and its metal complex were recorded in a nitrogen atmosphere from 25 to 900°C using a TGA instrument. Based on the thermograms, decomposition stages, temperature ranges, decomposition product as well as weight loss percentages were evaluated.

The TG/DTG curves are given in Fig. 5 and the characteristic thermogravimetric data are summarized in Table 2.

The TG curves showed that the thermal decomposition of the ligand takes place in two steps, indicating that the different groups lead to a decrease in stability. There was no evidence of weight loss before 100°C, indicating absence of water in the SB. The mass loss within the 100–350°C temperature range indicates the decomposition of the fully organic part and may be attributed to the loss of N_2 and aliphatic groups.

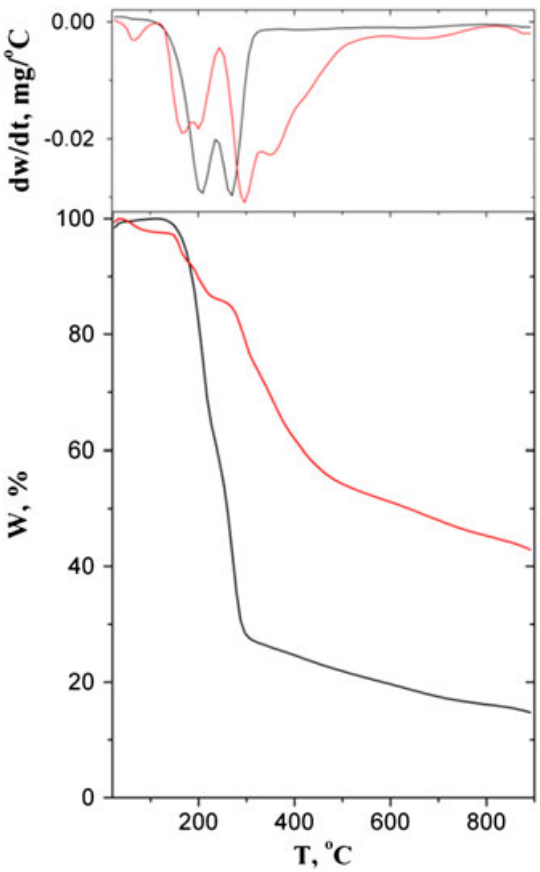

Figure 5. TG/DTG curves of SB (black line) and SB-Cu (II) complex (red line)

Table 2. Thermogravimetric data of SB and SB Cu(II) complex

	Sample	SB	SB-Cu (II)
Step I	$T_{onset} - T_{peak} - T_{endset}$ (°C)	—	41.1 – ***63.4*** – 120.6
	ΔW (%)	—	2.3
Step II	$T_{onset} - T_{peak} - T_{endset}$ (°C)	—	120.6 – ***165.2*** – 187.3
	ΔW (%)	—	6.4
Step III	$T_{onset} - T_{peak} - T_{endset}$ (°C)	100 – ***201.6*** – 237.9	187.3 – ***200.2*** – 243.8
	ΔW (%)	39.7	5.1
Step IV	$T_{onset} - T_{peak} - T_{endset}$ (°C)	237.9 – ***263.8*** – 350.5	243.8 – ***290.0*** – ***347.7 infl***-567.0
	ΔW (%)	34.6	34.2
Step V	$T_{onset} - T_{peak} - T_{endset}$ (°C)	—	597.7 – ***672.2*** – 794.1
	ΔW (%)	—	6.6
Residue		25.7	45.4

ΔW, weight loss.

The thermogram of Cu(II) complex exhibits several thermal events. The first weight loss of 2.31% in the 40–110°C temperature range and second weight loss of 6.45% in the 120–180°C temperature range corresponded to the loss the physical bonded water molecules. A rapid weight loss was observed after 180°C, indicating the decomposition of coordinated ligand. The broad band obtained after heating the complex above 570°C corresponds to the formation of stable CuO.

Toxicological Study

For SB and SB-Cu(II) complex (Fig. 6) several doses were used (100, 200,400 and 800 mg kg^{-1} b.w.). The 800 mg kg^{-1} dose induced sudden death due to convulsive phenomena. From analysis of the regression lines the LD_{50} for SB = 115.39 ± 88.16 mg kg^{-1} b.w. ($Y = 0.174 + 1.728X$, $R = 0.669$) and for SB-Cu(II) = 620.62 ± 250.45 mg kg^{-1} b.w.($Y = 1.185 + 1.850X$, $R = 0.901$), which indicates a low toxicity for SB and a very low toxicity for SB-Cu(II). For SB and SB-Cu(II) the use of doses higher than LD_{50} will induce central nervous phenomena which create significant discomfort.

Antibacterial Activity

Antimicrobial activity is estimated by measuring the diameter of the area inhibited by the tested compounds (Table 3). These results, attributed to the structure of the tested compounds, seemed to be the principal factor influencing antibacterial activity. This property is certainly correlated with the ability of a compound to diffuse through the biological membranes and reach its site of action.

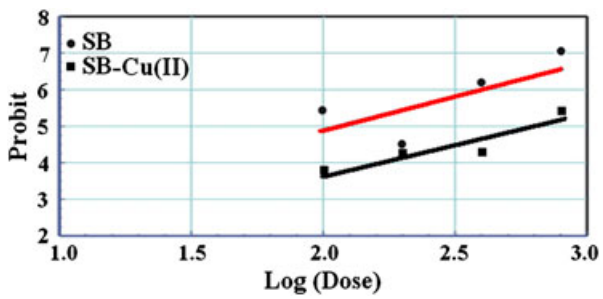

Figure 6. Regression line for SB and SB-Cu(II)

Table 3. Antimicrobial activity of the tested compounds[a]

Antimicrobial agents	SB	SB-Cu(II)	C 30 μg	T 30 μg	OF 5 μg	N 100 μg
B. cereus	27	37	39	32	35	—
B. subtilis	25	25	38	30	30	—
S. aureus	24	34	26	33	30	—
MRSA	20	34	25	28	25	—
Klebsiella spp.	28	32	25	18	20	—
E. coli	22	28	22	28	28	—
C. albicans	40	40	—	—	—	26

[a]C, chloramphenicol; T, tetracycline; OF, ofloxacin; N, nystatin.

Regarding the ligand, we have noticed a efficient action of chloramphenicol, tetracycline and ofloxacin on *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella* spp. The SB- Cu(II) complex is more active than SB as a bidentate ligand with coordination involving the -OH and the nitrogen atom of C N groups. SB does not have a effective activity in relation to *B. cereus* and *B. subtilis*. SB-Cu(II) is efficient against *B. cereus*, *S. aureus*, MRSA, *E. coli* and *Klebsiella* spp. The strongest activity is noticed in relation to *C. albicans*. The cations involved in the complex intensify the action compared to Schiff base.

Conclusions

This research reports the successful synthesis and antimicrobial activity of new Schiff bases complexes with Cu(II) ions. Schiff base and its Cu(II) complex were physicochemically and chemically characterized through elemental analyses, FT-IR, ^{1}H and $_{13}C$ NMR and UV–visible spectroscopy. These confirmed the ratio of metal/ligand combination, melting point, solubility and stability of the SB-Cu(II) complex.

The antimicrobial activity of this complex was tested in comparison with the activity of the Schiff base on the following strains: *S. aureus*, MRSA, *B. cereus*, *B. subtilis*, *E. coli*, *C. albicans* and *Klebsiella* spp. This activity was also compared with the activity of the reference drugs (chloramphenicol, tetracycline, ofloxacin and nystatin) on the above-mentioned strains.

All tested compounds were very active against both Gram-positive and Gram-negative bacteria.

The comparative study of the antimicrobial activity of a new Schiff base and of complex combination with metallic cations proves the fact that both manifested an antimicrobial action

comparable with the reference drugs chloramphenicol, tetracycline, ofloxacin and nystatin. However, the complex is more active than the Schiff base, due to the complex generator and structure.

Acknowledgments

M. C. Popescu acknowledges the financial support of the European Social Fund 'Cristofor I. Simionescu' Postdoctoral Fellowship Programme (ID POSDRU/89/1.5/S/55216), Sectoral Operational Programme Human Resources Development 2007–2013.

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