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SYNTHESIS AND ANTIMICROBIAL EVALUATION OF DIFFERENT SCHIFF BASES AND THEIR COMPLEXES

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SYNOPSIS

N, N' - disalicylidenemethylendiamine (BBS) and 2-(salicyliden) aminopyridine (BS) have a good capacity of complexing Zn(II) and Cu(II) ions. Schiff bases and their Zn(II) and Cu(II), complexes were physico-chemically and chemically characterized through elemental analysis, UV-VIS and IR spectrum and their mass formula was determined, which confirmed the ratio of metal/ ligand combination, melting point, solubility and stability. The antimicrobial activity of these complexes was tested in comparison with the activity of the Schiff bases on the strains: *Staphylococcus aureus*, *Staphylococcus aureus* meticilin-resistant, *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Candida albicans*, *Klebsiella* and it was compared to the witnesses: Chloramphenicol, Tetracycline, Ofloxacine and Nystatin. All tested compounds were very active against both gram-positive and gram- negative bacteria.

INTRODUCTION

Schiff bases can form a new class of drugs through mechanisms of immune potentiation and therapeutic potential. The complex combinations of Schiff bases with metallic ions are a class of compounds with the most interesting properties both from the point of view of the chemical behavior and the biological one. The specialty literature quotes complex combinations of Schiff bases with different cations that may present an antimicrobial, anti-inflammatory, antioxidant action (Chohan et al., 2008; Cozzi, 2004; Santoni & Rehder, 2004).

The objectives of this work were to study:

- the physico–chemical characterization of two Schiff bases and their complexes with Zn(II) and Cu(II) ions;

- the antimicrobial activity of these complexes in comparison with that of Schiff bases on the strains: *Staphylococcus aureus ATCC 25923, Staphylococcus aureus meticilin-resistant, Bacillus cereus ATCC 14579, Bacillus subtilis ATCC6633, Escherichia coli ATCC25922, Candida albicans ATCC 10231, Klebsiella spp*. and it was compared to the witnesses: Chloramphenicol, Tetracycline, Ofloxacine and Nystatin.

By condensing salicylaldehyde with 2-aminopyrine, salicylaldehyde and ammonia in the presence of formaldehyde in methanol, at room temperature, a fine intense yellow and yellow crystalline powder was obtained after 48 hours: 2-[salicyliden]-amino-pyridine (BS) type NNO (Cascaval, 1996) and N,N'-disalicyl-idenemethylendiamine (BBS) type ONNO (Cascaval, 1998). These bases present a good capacity of ion reagent for the spectrophotometric determination of those ions, the results being the subject of some previous works (Tantaru et al., 2007; Tantaru et al., 2009).

MATERIALS AND METHOD

- 10-2 M methanolic solution BS and BBS;
- 10-2 M methanolic solutions of Cu(OAc)2 •H2O and Zn(OAc)2•2H2O;
- 1000 µg/mL BS solution in DMSO;
- 1000 µg/mL BBS solution in DMSO;
- 1000 µg/mL BS-Cu(II) solution in DMSO;
- 1000 µg/mL BBS-Zn(II) solution in DMSO;
- Bacterial strains of *Staphylococcus aureus ATCC 25923, Staphylococcus aureus meticilin-resistant, Bacillus cereus ATCC 14579, Bacillus subtilis ATCC 6633, Escherichia coli ATCC 25922, Klebsiella spp., Candida albicans ATCC 10231*;
- References substances: Chloramphenicol (C), Tetracycline (T), Ofloxacine (OF) and Nystatin (N).
- Saboureaud medium;
- Discs of sterile paper.

The melting points were determined with Boetius apparatus and are uncorrected The IR spectra were recorded on a FTS-135 BIO-RAD in KBr pellets (4000-400 cm-1 range). The electronic spectra have been obtained on a UV-VIS spectrophotometer Hewlett-Packard 8453.

To 2 mM (0,396g) Schiff Base (50mL) methanolic solution was added 1.001 mM (0.2g) Cu(OAc)2•H2O methanolic solution. The resulted solution is refluxed for 1.5 hours and then left to rest at room temperature for 48 hours. It is filtered, then left to dry on Filter Paper and in the end it is recrystallized from dimethylformamide. Light green crystalline powder is obtained.

The 1.0023 mM (0.22g) Zn(OAc)2•2H2O and 1.003 mM (0.255g) of BBS were dissolved in anhydrous methanol (50mL).The resulting solution was left for the evaporation at room temperature for 24 hours. Those crystals are washed with C2H5OH-H2O (1:1,v/v) and then they are recrystallized from ether.

The newly prepared against compounds were screened for their antibacterial activity against Gram-positive and Gram-negative bacteria. Suspensions in sterile peptone water from 24h 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 dimethylsulfoxide (DMSO) to a final 1000 µg/mL. The discs (6 mm in diameter) were impregnated with 10µL of each compounds and placed on the inoculated agar. 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 in the microorganism's growth (Brown et al., 1979; Murray et al.,1995; Tavares et al.,1997).

RESULTS AND DISCUSSIONS

Schiff bases (BS, BBS) and their complex combination were characterized from he physicochemical and chemical point of view by elemental analysis, spectrum in UV-VIS, IR and they had the formula mass determined, which confirms the radio of metal/ligand combination, melting point, solubility, stability.(Table 1,2,3).

The IR spectra of the synthesized compounds in the wavelength range 4000-400 cm-1 were measured were obtained using KBr pellets. The interpretation of the spectra attributed the main characteristic bands to the structural groups and it also followed the main changes that happened during the complexation reaction. The spectra of the complexed substances shows differences due to the position and intensity of the bands, while some absorption bands disappear or new bands appear. The IR spectra of the 2-[salicyliden] aminopyridine complex is characterized by the 1650 cm-1 band witch is due to the variations of valence of the C=N bond that is shifted from 1620 cm-1 because of the metal coordination. The 3000 cm-1 band is characteristic to the CH aliphatic group. The valence variation of the M-O and M-N groups are characterized by the 500 cm-1 and 518 cm-1 absorption bands that do not appear in the spectra of the Schiff Base. In the ligand's UV spectrum, an absorbtion maximum with 350 nm. In the spectrum of the BS-Cu(II) (1:2) reaction mixture, a shoulder appears at 315 nm, being probably due to the ligand's coordination with the metallic ion.

Table 1: Psysico-chemical characteristics of BS and BBS.

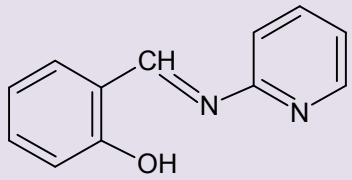
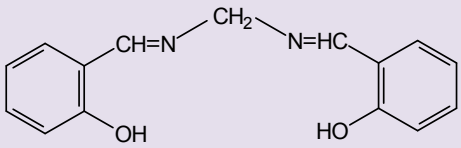
Structure	Colour and solubility	M.P. °C	Molecular formula	I.R. (KBr) cm-1
 2-[salicyliden]-amino pyridine	intense yellow-crystalline powder, insoluble in water, soluble in ethanol, methanol, very soluble in acetone and DMF	69-70	C12H10N2O	3400 3000 1620 1450
 N,N'-disalicylidenemethylendiamine	Yellow crystalline powder, insoluble in water, ethanol, benzene, CAN, CHCl3, soluble in methanol, DMSO	136-137	C15H14N2O2	3300 3600 1610

Table 2: Physico-chemical data of the complexes.

Complex	Elemental analysis					M.P. °C	I.R. (KBr) cm-1
		C	H	N	Cu, Zn		
BS-Cu(II)	Calculated	62.97	3.94	12.24	13.85	158-160	3000 500 518 1650
	Found	63.18	4.04	16.12	13.90		
BBS-Zn(II)	Calculated	56.66	3.78	8.82	20.57	242-243	3300 3600 460 525 1630
	Found	56.82	3.85	8.77	20.55		

Table 3: Psysico-chemical characteristics of the complexes.

Complex	Colour	M/L	ε L·mol-1·cm-1	Ks	S mol/L
BS-Cu(II)	light green crystalline powder	1:2	5.75 105	7.1·105	1.09·10-2
BBS-Zn (II)	light yellow crystalline powder	1:1	5.59·105	3.1·104	6.3·10-3

The BBS-Zn(II) complex is characterized by the 1630 cm-1 band witch is shifted from 1610 cm-1 as this band is influenced the most by the metallic ion coordination due to the C=N group. The higher modification, comparatively with the ligand, appears at the band from the 3300-3600 cm-1 domain, attributed to the –OH phenolic group. Lowering of the intensity of this band in spectrum of the complex form indicates involvement of the –OH group in the reaction of complexation. More than that, in the complex's spectrum there appears a peak at 525 cm-1, which may be attributed to the M-N bond, and another at 460 cm-1 attributed to the M-O bond. In the ligand's UV spectrum, two maxima occur at 255 nm and respectively, 320 nm, the suffers a bathochromic shifting at 380 nm in the complex's spectrum, which suggests the involvement of the C=N group in the reaction of coordination with Zn(II). In the spectrum of the BBS-Zn(II) (1:1) reaction mixture, a small shoulder appears at 294 nm, being probably due to the ligand's coordination with the metallic ion.

The antimicrobial activity is estimated by measuring the diameter of the area inhibited by the tested compounds: BS, BBS, BS-Cu(II), BBS-Zn(II) (Table 4).

Table 4: Antimicrobial activity of the tested compounds.

Antimicrobial agents	Diameter of inhibition zone (mm)							
	BBS-Zn(II)	BBS	BS-Cu(II)	BS	C 30 µg	T 30 µg	OF 5 µg	N 100µg
B. cereus	38	24	37	27	39	32	35	-
B. subtilis	26	18	25	25	38	30	30	-
S. aureus	42	36	34	24	26	33	30	-
S. aureus meticilin-resistant	40	36	34	20	25	28	25	-
K. spp	45	20	32	28	25	18	20	-
E. coli	30	18	28	22	22	28	28	-
C. albicans	40	40	40	40	-	-	-	26

These results can be attributed to the structure of the tested compounds seemed to be the principal factor influencing the antibacterial activity. This property is certainly correlated with the ability of a compound to diffuse through the biological membranes to reach its site of action.

Regarding the ligands, we have noticed a good action as compared to the Chloramphenicol, Tetracycline and Ofloxacine on *Staphylococcus aureus, Staphylococcus aureus meticilin-resistant, Escherichia coli* and *Klebsiella spp*. The best activity is noticed on *Candida albicans*. The BBS is more active than BS as

bidentate ligand with coordination involving the –OH and the nitrogen atom of C=N group. The BBS and BS does not have a good antimicrobial activity on Bacillus cereus and Bacillus subtilis. The BBS-Zn(II) and BS-Cu(II) are efficient against *Bacillus cereus, Staphylococcus aureus, Staphylococcus aureus meticilin-resistant, Escherichia coli, Klebsiella spp*. The best activity is noticed against *Candida albicans*. The cation involved in the complexes intensifies the antibacterial activity.

CONCLUSIONS

The research study reports the successful synthesis and antimicrobial activity of new Schiff bases complexes with Zn(II) and Cu(II) ions. Schiff bases and their Zn(II) and Cu(II), complexes were physically and chemically characterized through elemental analysis, UV - VIS and IR spectrum and their mass formula was determined, which confirmed the ratio of metal/ligand combination, melting point, solubility and stability.

The antimicrobial activity of these complexes was tested in comparison with the activity of the Schiff bases on the strains: *Staphylococcus aureus, Staphylococcus aureus meticilin - resistant, Bacillus cereus, Bacillus subtilis, Escherichia coli, Candida albicans, Klebsiella* and it was compared to the witnesses: Chloramphenicol, Tetracycline, Ofloxacine and Nystatin.

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 bases BS, BBS, and of certain complex combinations with metallic cations proves the fact that the BSS as well as some of its complees manifested an antimicrobial action comparable with Chloramphenicol, Tetracycline, Ofloxacine and Nystatin. The structure of the complexs and their generators determine an intensification of the antimicrobial activity.

REFERENCES:

BROWN, D.F.J. & BLOWERS, R. 1979: Disc method of sensitivity testing and semiquantitative methods in Laboratory methods in Antimicrobial chemotherapy. In: Reeves, Ds., Phillips, I. & Wise R. (Eds.) - *Churchill Livingstone*, 8-23.

CAŞCAVAL AL. Romania Patent no 89338/1998, C.A., 106, 66901h.

CAŞCAVAL, AL. Romania Patent no 106403/1996.

CHOHAN, Z.H., ARIF, M. & RASHID, A. 2008: Cooper (II) and Zinc (II) metal-based salicyl-, furanyl-, thienyl- and pyrrolyl- derived ONNO, NNNO, ONNS & NNNSS donor

asymmetrically mixed schiff–bases with antibacterial and antifungal potentials. - *Journal of enzyme inhibition and medicinal chemistry,* 23 (6): 785-796.

COZZI, P.C. 2004: Metal–Salen Schiff base complexes in catalysis: Practical aspects. - *Chemical Society Reviews*, 33: 410-421.

MURRAY, P.R., BARON, E.J., PFALLER, M.A., TENOVER, F.C. & YOLKE, R.H. 1995: Manual of Clinical Microbiology. - 6th Ed., *American Society of Microbiology*. 1506 pp.

SANTONI, G. & REHDER, D. 2004: Structural models for the reduced form of vanadate-dependent peroxidases: vanadyl complexes with bidentate chiral Schiff base ligands. - *Journal of Inorganic Biochemistry*, 98: 758-764.

TANTARU, G., BOSANCEANU, A., GUDRUMAN, A.D. & VASILESCU, M. 2009: Validation of U(VI) spectrophotometric assay with 2-(salicyliden) aminopyridine reagent: analytical applications. (Meeting abstract published in journal supplement) - *European Journal of Drug Metabolism and Pharmacokinetics*, 34: 87.

TANTARU, G., VIERIU, M., APOSTU, M. & STAN, M. 2007: Combinații complexe ale Cr(III) cu liganzi de tip bis baze-Schiff. - *Revista Medico-Chirurgicale*, 3(2): 399-403.

TAVARES, L.C., PENNA, T.C.V. & AMARAL, A.T. 1997: Synthesis and biological activity of nifuroxazide and analogs. - *Bollettino chimico farmaceutico*, 136(3): 244-249.

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