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Antibacterial and Antioxidant Properties of Macrocyclic Schiff Bases with Vanadium(V) Complexes

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Macrocyclic Schiff bases have been synthesized by the condensation of acetyl acetone with semicarbazide hydrochloride and thiosemicarbazide in presence of methanol. Further, their oxovanadium complexes have been synthesized by using vanadium acetylacetone. The structural assignment of these compounds has been made on the basis of m.f., TLC, m.w. determination, conductivities, elemental analysis and UV, IR and ^{1}H NMR spectral data. The synthesized compounds were screened for their *in vitro* growth inhibiting activity against different strains of bacteria *viz*., *Staphylococcus aureus, Bacillus licheniformis, Escherichia coli* and *Micrococcus luteus* (ATCC) and were compared with the standard antibiotic oflaxocin. Also *in vitro* antioxidant activity of all compounds was determined by nitric acid free radical scavanging assay.

Key Words: Macrocyclic Schiff bases, Vanadium complexes, Antioxidant activities.

INTRODUCTION

The macrocyclic ligands are highly significant in bioinorganic chemistry, catalysis, extraction of metal ions from solution, *etc*[1]. Macrocyclic on complexation with transition metal ions show some interesting properties and biological functions, such as being models for metalloproteins and oxygen carrier systems[2]. Nowadays interest is focused on the synthesis of macrocyclic complexes with potential medicinal applications. Similarly macrocyclic complexes of vanadium are very useful, due to the fact that vanadium compounds are in clinical trials as a potential treatment for non-insulin dependent diabetes mellitus. Also, they are highly significant from the biological point of view[3-5]. Keeping the above facts in mind and in continuation of our research work, in the present paper we report the synthesis and characterization of vanadium(V) complexes of macrocyclic Schiff bases derived from the condensation of acetyl acetone with semicarbazide hydrochloride and thiosemicarbazide.

Antioxidant compounds reduce the action of reactive oxygen species (ROS) in damaged tissues during the recovery process. The search for new bioactive products with antioxidant activity has lead to the present study, whose aims were to investigate the antioxidant activity and also the effects of antibacterial activities.

EXPERIMENTAL

All chemicals were used of AR grade. All the solvents used were of high purity and distilled in the laboratory before use. The identification and purity of the products were checked by TLC with ethanol:water (3:1) using iodine vapours for visualization of the spots. Melting points were measured by open capillaries using Sunsim electric melting point apparatus and are uncorrected. Molecular weights were determined by Rast Camphor. Conductivities measured on Equiptronics model No. EQ-660A of 10^{-3} M solution in DMF. Electronic spectra of the compounds were recorded on a digital spectrophotometer.

IR Spectra were taken on a Perkin Elmer, FTIR spectrophotometer in range 4000-500 cm^{-1} using potassium bromide pellets. ^{1}H NMR spectra were recorded in DMSO and MeOD on Bruker Advance 400 MHz FT NMR spectrometer using TMS as an internal standard. Elemental analysis was obtained on a Vario EL III Elementar Carlo Erba 1108. All done at CDRI, Lucknow.

Synthesis of macrocyclic Schiff bases: Both macrocyclic ligands SCHA and TSCA were synthesized by taking equimolar ratios of acetylacetone in ethanol with the solutions of semicarbazide hydrochloride in hot water (neutralized by dil. NaOH) and thiosemicarbazide in ethanol, respectively and then both were added dropwise in 25 mL of ethanol under constant stirring for atleast 3 h. Precipitate was obtained which were filtered, collected and dried over $CaCl_2$ in vaccum and were recrystallised by ethanol and petroleum ether. The colour of both ligands SCHA and TSCA was ivory.

Synthesis of vanadium(V) complexes: The complexes of vanadium(V) have been prepared by reacting an ethanolic

solution of vanadium acetylacetone salt with ethanolic solution of prepared ligands (SCHA)-(TSCA) in 1:1 molar ratio. Resulting reaction mixture was refluxed on water bath for 5-6 h. Dark coloured precipitate was obtained which was recrystallised by petroleum ether (60-80 ºC). Both the complexes are shown in Figs. 1 and 2.

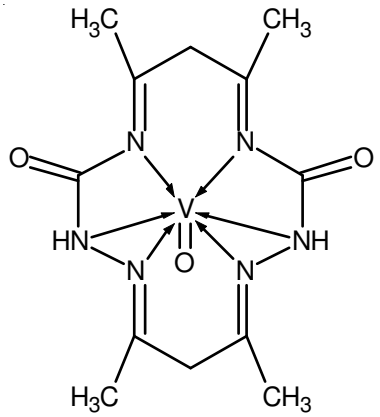

Fig. 1. Proposed structure of oxovanadium complex of ligand SCHA

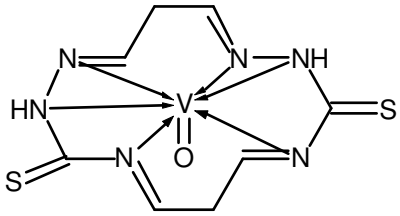

Fig. 2. Proposed structure of oxovanadium complex of ligand TSCA

Antibacterial activities: Antibacterial activities of the compounds were tested against using Muller Hinton agar medium[6]. The sterilized (autoclaved at 121 ºC for 15 min) medium (40-50 ºC) was poured into the petri dishes to give a depth of 3-4 mm and allowed to solidify. The suspension of the microorganism then streaked on plates. The paper discs impregnated with the test compounds was placed on the solidified medium. The plates were pre-incubated for 1 h at room temperature and incubated at 37 ºC for 24 h. Ofloxacin was used as standard[7,8].

Scavenging of nitric oxide: Sodium nitroprusside (5 mm) in standard phosphate buffer solution was incubated with different concentration of (125, 100, 75 and 50 µg/mL) the test extracts dissolved in standard phosphate buffer (0.025 M, pH 7.4) and the tubes were incubated at 25 ºC for 5 h. After 5 h, 2 mL of incubation solution was removed and diluted with 2 mL Griess reagent (prepared by mixing equal volume of 1 % sulphanilamide in 2 % phosphoric acid and 0.1 % naphthylethylene diamine dihydrochloride in water). The absorbance of solution formed was read at 546 nm. The experiment was performed in triplicate and % scavenging activity was calculated using the formula (%) = $A_o - A_1/A_o \times 100$ where A_o is control absorbance and A_1 is the absorbance of the sample. The activity was compared with ascorbic acid[9-11]. Then percentage inhibitions were plotted against respective concentrations used and from the graph IC_{50} values were calculated.

Statistical analysis: In all activities, the mean ± SEM were statistically calculated for each parameter using ANOVA. Statistical significance was determined by using student's *t*-test to study the differences amongst the means.

RESULTS AND DISCUSSION

The synthesized macrocyclic ligands were having ivory colour while their complexes were intensely coloured. The solubility tests for the compounds in different solvents, established their solubility in methanol, ethanol, DMF - DMSO. The electrical conductivities of 10^{-3} M solution of the complexes measured in DMF are low, with values less than 4.0 ohm^{-1} cm^2 mol^{-1} indicating non electrolytical nature of the compounds. Purity of compounds was confirmed as both ligands and complexes moves as a single spot indicating the presence of only one component. Molecular weights determined by Rast camphor method were found in accordance with calculated value, confirming the monomeric nature of the compounds. Microanalytical datas are shown in Table-1.

All the spectral data was consistent with the assigned structure of the compounds. Electronic spectra of ligand (SCHA) shows weak band at 300-360 nm attributable to π-π^*-n-π^* transition, respectively in its complex first remains unchanged while second shows blue shift and a band appear at 310 nm due to donation of lone pair of C=N group to vanadium atom. Ligand (TSCA) shows the same weak band at 310 and 365 nm while second appears at 320 nm.

The band in region 1640-1590 cm^{-1} due to C=N which is assignable to the macrocyclic Schiff bases, appeared in both synthesized ligands. This band gets shifted to lower frequency in the complexes, indicating the coordination through azomethine nitrogen. It is found from the IR spectra of the complexes that there are wide and strong band at 996-990 cm^{-1}, which are assigned to V=O stretching vibration. The ^{1}H NMR spectral data of ligand (SCHA) shows signal at δ 2.18, δ 2.48-δ5.82-5.99 assigned to methyl, methylene and NH group, respectively. Ligand (TSCA) shows signal at δ 2.16-2.29, δ 2.50-

TABLE-1
MICROANALYTICAL DATAS OF ALL COMPOUNDS

Compound	Yield (%)	Colour	m.p. (ºC)	m.w. Found (calc) (%)	Elemental analysis (%) found (calcd.)				
					C	H	N	V	S
Ligand (SCHA) $C_{12}H_{18}N_6O_2$	60-65	Ivory	105	280 (278)	49.9 (51.7)	6.1 (6.7)	31.8 (30.2)	–	–
Vanadium complex of (SCHA) $C_{12}H_{18}N_6O_3V$	65-70	Dark green	165	345 (346)	40.8 (41.6)	6.1 (5.2)	23.8 (24.2)	14.7 (15.0)	–
Ligand (TSCA) $C_{12}H_{18}N_6S_2$	65-68	Ivory	100	312 (310)	47.2 (46.4)	5.6 (5.8)	27.4 (27.0)	–	19.62 (20.64)
Vanadium complex of (TSCA) $C_{12}H_{18}N_6S_2OV$	68-70	Reddish black	140	376 (378)	39.2 (38.0)	5.3 (4.7)	21.0 (22.2)	13.1 (13.7)	16.1 (16.9)

TABLE-2
ANTIBACTERIAL ACTIVITIES OF ALL COMPOUNDS [SIGNIFICANCE LEVEL $p < 0.001$, $*p < 0.01$ (n = 3)]

Microorganism	Conc. (ppm)	Ligand (SCHA) (mean ± SEM)	Complex of (SCHA) (mean ± SEM)	Ligand (TSCA) (mean ± SEM)	Complex of (TSCA) (mean ± SEM)
E. coli (–)	100	16 ± 0.346	18 ± 0.332	17 ± 0.346	18 ± 0.231
	500	22 ± 0.231	25 ± 0.251	23 ± 0.231	27 ± 0.346
	1000	29 ± 0.462	31 ± 0.651	29 ± 0.586	32 ± 0.115
S. aureus (+)	100	17 ± 0.152	18 ± 0.607*	16 ± 0.557*	18 ± 0.264
	500	24 ± 0. 251	27 ± 0.551	22 ± 0.651	26 ± 0.622
	1000	30 ± 0.305	32 ± 0.901	28 ± 0.603	31 ± 0.569
M.luteus (+)	100	15 ± 0.208	17 ± 0.473	16 ± 0.603*	17 ± 0.529
	500	23 ± 0.404	27 ± 0.289	22 ± 0.551	27 ± 0.473
	1000	28 ± 0.458	32 ± 0.231	28 ± 0.513	32 ± 0.416
B. licheniformis (+)	100	16 ± 0.503	18 ± 0.569	15 ± 0.436	18 ± 0.651*
	500	22 ± 0.557	26 ± 0.503	22 ± 0.473	25 ± 0.436
	1000	29 ± 0.321	32 ± 0.608	28 ± 0.751	31 ± 0.551

2.64-δ 9.13-9.55 assigned to methyl, methylene and NH group, respectively. All the signals get shifted downfield in their vanadium complexes, thus confirming the coordination.

Antimicrobial activity: All the compounds were evaluated for their antibacterial activity *in vitro* by using zone inhibition technique against *E.coli* (-) *S.aureus* (+) *M. luteus* (+) and *B. licheniformis* (+) at different concentration (100, 500-1000 ppm). Experiments were repeated three times and the results were expressed as (mean ± SEM) values in Table-2. The results obtained were compared with the standard drug ofloxacin. The IC_{50} values are also shown in Table-3.

TABLE-3
IC_{50} VALUES FOR ANTIBACTERIAL ACTIVITIES

Compound	IC_{50} values (mg/mL) against			
	E. coli (–)	*S. aureus* (+)	*M. luteus* (+)	*B. licheniforms* (+)
Ligand (SCHA)	0.51	0.42	0.50	0.51
Complex of (SCHA)	0.30	0.23	0.28	0.36
Ligand (TSCA)	0.50	0.60	0.60	0.58
Complex of (TSCA)	0.23	0.25	0.28	0.30

All the compounds showed antimicrobial activity against all the types of bacteria tested. The IC_{50} values for all compounds was found to be in the range of 0.23-0.60 mg/mL. Ligand (SCHA) was found to be most susceptible to *S. aureus* as its IC_{50} value was 0.42 mg/mL. Ligand (TSCA) was most susceptible to *E. coli* with having 0.50 mg/mL IC_{50} value. Complexes of these ligands showed the most potent activity against all the bacteria.

Antioxidant activity: All compounds showed significant free radical scavenging action against nitric oxide (NO) induced release of free radicals at different concentration 125, 100, 75, 50 µg/mL. Ascorbic acid was used as reference standard. The percentage inhibition as (mean ± SEM) is shown in Table-4.

All the compounds also showed strong antioxidant activity as determined by nitric oxide scavenging method. The IC_{50} values for ligands (SCHA)-(TSCA) are 144-170 µg/mL which were lowered down to 72-162 µg/mL in their complexes, respectively as shown in Table-5. It indicates that the compounds are effective antioxidants. However the antioxidant activity of ligand (SCHA) was more compared to ligand (TSCA). This could be due to the lack of oxygen in the structure of ligand (TSCA).

Conclusion

From the results of antibacterial effect, it is concluded that all compounds exhibited strong to moderate activity. Oxovanadium complexes have been found to be more effective than their precursor macrocyclic ligands as the process of chelation dominantly affects the overall biological behaviour of the compounds also the zone of inhibition increases with the concentration. All compounds showed varying antioxidant (free radical scavenging) activities when compared to ascorbic acid. The results suggest that the antioxidant activity of these compounds may contribute to their claimed antioxidant property and may lead to chemical entities with potential for clinical use.

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TABLE-4
IN VITRO FREE RADICAL SCAVENGING EFFECT OF ALL COMPOUNDS BY NITRIC OXIDE SCAVENGING METHOD [SIGNIFICANCE LEVEL ($p < 0.001$) (n = 3)]

Compounds	Scavenging (%) (mean ± SEM) of triplicates			
	50 (µg/mL)	75 (µg/mL)	100 (µg/mL)	125 (µg/mL)
Ligand SCHA	42.52 ± 0.029	45.55 ± 0.088	46.36 ± 0.152	48.71 ± 0.115
Complex of SCHA	50.62 ± 0.057	53.85 ± 0.036	54.68 ± 0.037	58.69 ± 0.057
Ligand TSCA	28.21 ± 0.085	31.23 ± 0.176	33.65 ± 0.200	39.67 ± 0.085
Complex of TSCA	33.19 ± 0.038	35.96 ± 0.023	37.69 ± 0.094	42.68 ± 0.092

TABLE-5
IC_{50} VALUES FOR ANTIOXIDANT ACTIVITIES OF ALL COMPOUNDS

Compounds	IC_{50} value (µg/mL)
Ligand SCHA	144
Complex of SCHA	72
Ligand TSCA	170
Complex of TSCA	162

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