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Journal of Chemical and Pharmaceutical Research

ISSN No: 0975-7384
CODEN(USA): JCPRC5

J. Chem. Pharm. Res., 2011, 3(1):169-175

Synthesis, Antioxidant and Biocidal Features of Macrocyclic Schiff Bases with Oxoanadium (V) Complexes

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ABTRACT

The macrocyclic ligands are synthesized by taking etylenediamine and semicarbazide hydrochloride with acetyl acetone respectively in ethanolic medium further their Oxovanadium complexes were synthesized by taking metal salt of type VO (acac)$_2$. Ligands and complexes have been analysed for their physical properties with the help of melting point, molecular weight, conductance , TLC determination, magnetic moment, elemental analysis and electronic, IR, ^{1}HNMR spectral studies. In the present work, we report the antioxidant activity by nitric oxide scavenging assay and also the biocidal activities against various bacteria like Gram negative E.coli,Gram positive S.aureus, M.luteus and B.licheniformis of all the synthesized ligands and complexes.

Keywords: Antioxidant; Nitric oxide scavenging; Macrocyclic ligands, Oxovanadium complexes.

INTRODUCTION

Macrocyclic ligands are polydentate ligands containing their donor atoms either incorporated in or less commonly attached to a cyclic back bone. The ring should consists of at least minimum of nine atoms. The chemistry of macrocyclic complexes has received much attention and such compounds have been extensively studied in recent years. Studies on macrocyclic complexes have shown that some of them are involved in important biological processes, such as photosynthesis and dioxygen transport in addition to their catalytical properties which may lead to important industrial applications. [1, 2]

Macrocyclic complexes of transition metal ions having oxo and aza groups show some interesting properties and biological functions such as being models for metalloproteins and oxygen carrier systems. [3] There has been increasing interest in the fundamental chemistry of vanadium compounds .The presence of vanadium in biological systems, its insulin-enhancing action and anticancer activity has driven a considerable amount of research. Particular interest has been given to the study of the potential benefits of vanadium compounds as oral insulin substitutes for the treatment of diabetes. [4, 5]

Antioxidants and antibacterials [6] are important in the prevention of human diseases. Antioxidant compounds may function as free radical scavengers, which play important role in food and chemical material degradation, and significantly delay or prevent the oxidation of easily oxidable substrates. Therefore, the importance of search for antioxidants has greatly increased in the recent years[7,8]. These facts have led to a large amount research involving such systems. Our work of macrocyclic ligands involving such systems led us to describe the synthetic, antioxidant and biocidal features of oxovanadium (V) complexes.

EXPERIMENTAL SECTION

Physical Properties

All the chemicals used in present investigation were of A.R.quality. Benzil, semicarbazide hydrochloride and etylenediamine were of CDH. All the solvents used were of high purity and distilled in the laboratory before use.

Purity of the compounds was judged by using silica gel TLC plates and spots were developed exposing the slides in iodine vapor chamber. Melting points were taken in open capillaries on Sunsim electric melting point apparatus and are uncorrected. Molecular weights were determined by Rast Camphor. Conductivities measured on Equiptronics model no.EQ-660A. Magnetic moments of the compounds were determined by Guoy Balance using mercury tetraisocynato cobaltate as calibrant at room temperature.

IR spectra were recorded on Perkin-Elmer FT-IR spectrophotometer in range 4000- 500cm^{-1} using KBr pellets and ^{1}HNMR spectra in MeOD at 300 MHz using TMS as an internal standard. The ligands and complexes were analysed for C, H&N. All done at CDRI,Lucknow.

Antibacterial activities

The compounds were screened for antibacterial activities against *E.coli*(-) *S.aureus*(+) *M.luteus*(+) and *B.licheniformis* (+) (ATCC) at different concentration(100,500 and 1000ppm) following zone inhibition technique. Compounds were dissolved in DMF. Muller Hinton agar medium was allowed to set and were uniformly seeded with the bacterium to be tested. Small sterile discs (having 6mm diameter) of Whatman no.1 filter paper, impregnated with standard solution of test compounds were placed on the plates of culture medium. Plates were immediately transferred to incubator. After one day of incubation, the degree of sensitivity is determined by measuring the zone of inhibition. The IC_{50} values were also determined. [9,10]

Antioxidant activities

Nitric oxide was generated from sodium nitroprusside and measured by Griess reaction Sodium nitroprusside (5mM) in standard phosphate buffer solution was incubated with different concentration of (125,100,75,50 μg/ml) the test compounds dissolved in standard phosphate buffer (0.025M, pH 7.4) and the tubes were incubated at 25°C for 3 hr. After incubation 2 ml of 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 by using digital spectrophotometer. The experiment was performed in triplicate and % scavenging activity was calculated using the formula (%) = Ao – A_1 / Ao x100 where Ao is control absorbance and A_1 is the absorbance of the sample. The activity was compared with ascorbic acid, which was used as a standard antioxidant. Then % inhibitions were plotted against respective concentrations used and from the graph IC_{50} values were calculated. [11]

Synthesis of Macrocyclic Schiff base (EDB)

Ethanolic solutions of Etylenediamine (0.1mol, 0.06gm) and of Benzil (0.1mol, 0.21gm) in equimolar ratio were added dropwise under constant stirring in 25ml of methanol. The resulting mixture was refluxed for 22-24 hours.The solid crystals were collected and dried over $CaCl_2$ in vaccum and were recrystallised by ethanol & Petroleum ether. The colour of ligand was orange. (60% yield, mp-125°C).

Synthesis of Vanadium (V) complex with (EDB)

The complex of Vanadium (v) has been prepared by reacting an ethanolic solution of vanadium acetylacetone salt with ethanolic solution of prepared (EDB) ligand in 1:1 molar ratio. Resulting reaction mixture was refluxed on water bath for 5-6 hours. Brownish black powder was obtained & recrystallised by petroleum ether. (65% yield mp-above 200°C).

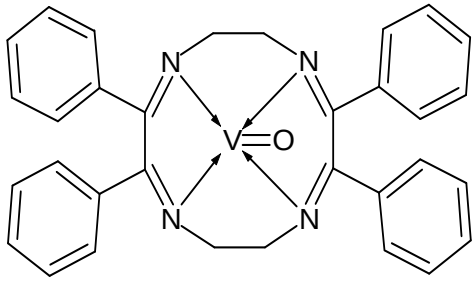

Fig 1 Oxovanadium complex of ligand (EDB)

Synthesis of Macrocyclic Schiff base (SCHB)

The solution of Semicarbazide hydrochloride (1mol, 0.55gm) in hot water (neutralized by dil.NaOH) and Benzil(1mol, 1.05gm) in ethanol taken in equimolar ratio and then both were added dropwise under constant stirring for atleast 3 hours. Precipitate was obtained which were filtered, collected and dried over $CaCl_2$ in vaccum and were recrystallised by ethanol & Petroleum ether. The colour of ligand was light yellow. (65% yield, mp-170°C).

Synthesis of Vanadium (V) complex with (SCHB)

The complex of Vanadium (v) has been prepared by reacting an ethanolic solution of vanadium acetylacetone salt with ethanolic solution of prepared (SCHB) ligand in 1:1 molar ratio. Resulting reaction mixture was refluxed on water bath for 5-6 hours. Greenish black powder was obtained & recrystallised by petroleum ether. (65% yield mp-above 200°C).

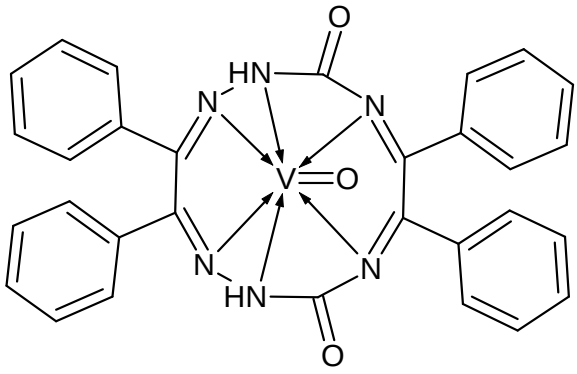

Fig 2 Oxovanadium complex of ligand (SCHB)

RESULTS AND DISCUSSION

Physical Properties

The synthesised macrocycic Schiff bases and their oxovanadium complexes are intensely coloured and soluble in methanol, ethanol, DMF& DMSO.

They are stable at room temperature and are non-hygroscopic. 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 and were found in accordance with calculated value, confirming the monomeric nature of the compounds. The values of molar conductance in DMF (10^{-3}M) were in the range 5-35ohm^{-1}cm^{2}mol^{-1} suggesting a non-electrolytic nature of the compounds.The magnetic moment value of prepared complexes revealed the existence of a diamagnetic character.

Physical data are summarized in the following table:-

Table 1: Physical datas of ligands and their Oxovanadium complexes

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Comp.	Yield in %	Colour	MP in °C	MW found (calc)	Element analysis found (calc) in %				
					C	H	N	O	V
Ligand (EDB) $C_{32}H_{28}N_4$	60	Orange	125	466 (468)	81.6 (82)	6.3 (5.9)	12.3 (11.9)	-	-
Vanadium Complex of (EDB) $C_{32}H_{28}N_4OV$	65	Brownish black	Above 200	537 (535)	71.4 (71.7)	5.7 (5.2)	9.9 (10.4)	3.3 (2.9)	10.1 (9.5)
Ligand (SCHB) $C_{30}H_{22}N_6O_2$	65	Light yellow	170	569 (570)	71.6 (72.2)	4.8 (4.4)	17.2 (16.8)	5.9 (6.4)	-
Vanadium Complex of (SCHB) $C_{30}H_{22}N_6O_3V$	65	Greenish black	Above 200	634 (637)	64.1 (63.7)	4.3 (3.8)	14.2 (14.8)	8.6 (8.4)	8.7 (9.0)

Spectral analysis

In the IR spectra of both ligands strong peak is observed in the region1590-1640cm-1 due to C=N which is assignable to the macrocyclic Schiff bases. In spectra of vanadium complexes, very sharp peak in region 970-990cm^{-1} suggests the presence of V=O bond. The band due to C=N has shifted to lower frequency in the complexes, indicating the coordination through azomethine nitrogen.

The ^{1}HNMR spectras of ligands (EDB) and (SCHB) shows signal between δ7.25-7.59 and δ7.30-7.74 respectively due to aromatic ring which gets shifted downfield in their vanadium complexes.

Antibacterial activities

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 and 1000ppm). 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 shown in table 3.

Table 2: Antibacterial activities of ligands and their complexes (in mm)

Bacteria	Conc In ppm	Ligand EDB (Mean±SEM)	Complex of EDB (Mean±SEM)	Ligand SCHB (Mean±SEM)	Complex of SCHB (Mean±SEM)
E. coli(-ive)	100	16±0.418	18±0.473	16±0.473	18±0.529
	500	25±0.473	28±0.440	26±0.418	29±0.360
	1000	29±0.360	32±0.551	30±0.503	34±0.418
S.aureus(+ive)	100	14±0.305	18±0.529	15±0.503**	18±0.458
	500	24±0.529	27±0.436	24±0.557	28±0.305
	1000	28±0.416	31±0.404	29±0.529	32±0.416
M.luteus(+ive)	100	15± 0.360	18±0.569	16±0.529**	18±0.493
	500	24± 0.458	27±0.451	25±0.360	29±0.436
	1000	28±0.473	32±0.493	29±0.513	33±0.458
B.licheniforms (+ive)	100	15±0.416	17±0.458	16±0.458	17±0.473
	500	24±0.404	27±0.305	25±0.416	28±0.440
	1000	29±0.493	31±0.418	29±0.458	33±0.404

*Significance level P< 0.001, **P< 0.01. Each value represents Mean±SEM (n=3)*

Table 3: IC_{50} values of ligands and their complexes

Compound	IC_{50}values (in mg/ml) against			
	E. coli(-ive)	S.aureus(+ive)	*M.luteus* (+ive)	*B.licheniforms* (+ive)
Ligand EDB	0.39	0.46	0.45	0.45
Complex of EDB	0.21	0.23	0.23	0.28
Ligand SCHB	0.35	0.45	0.39	0.39
Complex of SCHB	0.20	0.21	0.20	0.26

Antioxidant activities

Sodium nitroprusside generate nitric oxide radical in presence of physiological buffer solution at 25°C. Nitric oxide reacted with Griess reagent and diazotization of nitrite with sulphanilamide

and subsequent coupling with napthyl ethylene diamine form color complexes. Decrease in color intensity is directly proportional to nitric oxide radical scavenging. All compounds showed considerable antioxidant activity. Ascorbic acid was used as reference standard. The % inhibition as (Mean±SEM) is shown in Table 4. The IC_{50} values were also derived which is the concentration of the compound that decreases the initial NO radical concentration by 50%. The IC_{50} values for ligands (EDB) and (SCHB) are158 ug/ml & 72ug/ml which were lowered down to 60ug/ml & 52ug/ml in their complexes respectively. However the antioxidant activity of ligand (SCHB) was more compared to ligand (EDB). This could be due to the lack of Oxygen in the structure of ligand (EDB).

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			
	50ug/ml	75ug/ml	100ug/ml	125ug/ml
Ligand EDB	38.23±0.088	41.19±0.160	43.13±0.125	46.74±0.085
Complex of EDB	53.77±0.087	55.22±0.052	57.69±0.086	59.81±0.089
Ligand SCHB	48.43±0.125	50.30±0.057	54.43±0.096	57.54±0.063
Complex of SCHB	58.94±0.083	61.40±0.071	63.52±0.063	66.35±0.078

CONCLUSION

On the basis of all structural evidences the tentative structure with possibly four coordinated oxovanadium(V) complex of ligand(EDB) can be proposed with having square planar geometry and for six coordinated oxovanadium(V) complex of ligand (SCHB), an octahedral geometry can be proposed.
From the results of antibacterial activities we can conclude that all compounds exhibited strong to moderate activity. Oxovanadium complexes have been found to be more effective then 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. Thus, these compounds may serve as good antibacterials.

All the 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 medicinal use.

Acknowledgment
We are thankful to Head, Dept. of chemistry, Government College, Ajmer for providing the research facilities, to the microbiological department of MDSU Ajmer for providing us bacteria and to CDRI, Lucknow for providing all spectral and elemental analysis.

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