Antimicrobial screening of oxovanadium(IV)salophen complexes against human pathogens

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ABSTRACT: *Biological activity of synthetic compounds for potential antimicrobial activity continues to be an important strategy for the identification of new drugs. The in vitro antimicrobial activity of the synthesized oxovanadium(IV) salophen complexes (I-IV) were examined against different gram positive (Bacillus sp., Staphylococcusaureus, Staphylococcusepidermidis) and gram negative bacteriae (E. coli, Proteus sp., Citrobacter sp.) organisms and antifungal activity against fungal organisms (Candida sp. and Aspergillus sp.) by agar well diffusion method. The antibacterial activity of all the complexes against the microorganisms were assessed from the presence of inhibition zones and zone diameters. The results are compared with the standard drugs chloramphenicol for bacteria and nystatin for fungi. On the basis of the observed zone of inhibition, it was found that all the four oxovanadium(IV) salophen complexes were moderately active towards certain specific microorganisms and have the potential to be used as antimicrobial agents.*

Key Words: *Oxovanadium(IV)-salophen complex, Antimicrobial activity, Zone of inhibition*

1.INTRODUCTION

Microbial infection is a growing problem in contemporary medicine, and the use of antibiotics is inevitable. Development of resistance to antibiotics has become a major problem in recent years. A continuous increase in the number of infections caused by bacterial resistance to one or multiple class of antibiotics poses a significant threat as it may lead to treatment failure and some associated complications [1]. Despite the availability of many antibiotics and chemotherapeutics, the emergence of old and new antibiotic resistant bacterial strains in the last decade constitutes a substantial need for the development of new classes of antibacterial agents [2].

Schiff base-metal complexes are an area of increasing interest due to their biological activity. Schiff base complexes have numerous applications such as, antibacterial agents, antivirus agents, antifungal agents, used in the treatment of cancer and for other biological properties [3]. Schiff bases can coordinate a wide variety of transition metal ions in different oxidation states forming complexes with interesting properties, architectures and applications [4,5]. Several V(IV) and V(V) complexes have been found to influence the growth and metabolism of microorganisms [6,7]. One of the most interesting properties of metal complexes including those of vanadium is their antimicrobial activity [8].

Based on the global concern to public health, discovery of new antibiotics has become an important objective. Biological activity of synthetic compounds for potential antimicrobial activity continues to be an important strategy for the identification of new drugs with possible clinical values. Hence, the preliminary screening of four oxovanadium(IV) salophen complexes for their antimicrobial properties against human pathogens has been discussed.

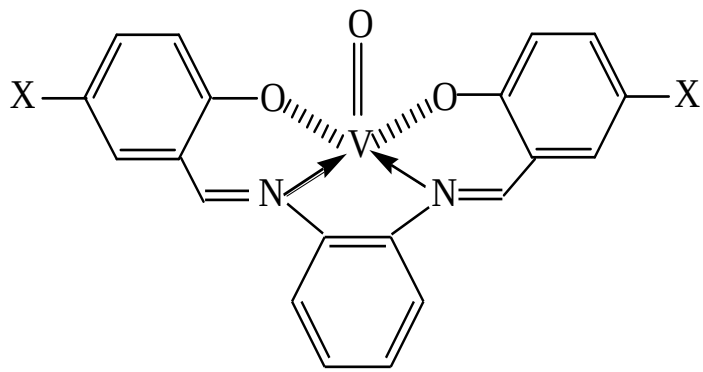
2. EXPERIMENTAL

2.1 Synthesis of ligands

The various salophen ligands required for the preparation of the oxovanadium(IV) salophen complexes were synthesized using established procedures [9]. The general procedure for the preparation of salophen ligand involves the condensation of salicylaldehyde and 1,2-benzenediamine in the ratio of 2:1 in an alcoholic medium.

2.2 Synthesis of oxovanadium(IV) salophen complexes (I - IV)

The synthesis of oxovanadium(IV)-salophen complexes was accomplished by the procedure slightly different from that reported in the literature [10]. To a hot methanolic solution (50 cc) of vanadyl sulfate ($VOSO_4.5H_2O$; 0.25 g, 1 mm), appropriate salophen ligand (1 mm) was added with stirring. The mixture was refluxed for one hour and cooled to room temperature. The green crystals separated were filtered, washed with diethyl ether and dried. Recrystallisation was carried out from pure hot methanol [11]. All the four oxovanadium(IV) salophen complexes (Figure 1) prepared were characterised by their UV-vis absorption , IR and mass spectra.(Table 1)



I : X = H ; **II :** X = OMe ; **III :** X = Me ; **IV :** X = Cl

Figure .1. Structure of oxovanadium(IV) salophen complexes.

Table 1. Absorption maxima (λ_{max},nm), IR frequency (ν, cm^{-1}) and m/z values of oxovanadium(IV) salophen complexes.

Salophen complex	λ_{max} (nm)	ν (cm^{-1})	ESI-MS
Salophen **(I)**	241, 314, 396	1538, 1151, 987	381
5,5′-OMe_2 salophen **(II)**	250, 330, 436	1598, 1221, 967	441
5,5′-Me_2 salophen **(III)**	243, 318, 412	1621, 1256, 980	409
5,5′-Cl_2 salophen **(IV)**	247, 311, 407	1610, 1284, 970	449

The solutions of oxovanadium(IV)-salophen complexes were prepared in acetonitrile and found to be stable for a prolonged period of time. The UV-visible absorption spectra of the V(IV) complexes (Fig.2.) is recorded in acetonitrile at room temperature. The λ_{max}values in the range of 200-480 nm (Table 1) are similar to the values reported in the literature for salophen complexes [9,12]. The strong high energy absorption bands at 300-350 nm are assigned to the ligand centered (LC) л-л* transition and low energy absorption band at 400-480 nm due to spin allowed ligand to metal charge transfer (LMCT) transition from л*(dimine) → dл(V) [10].

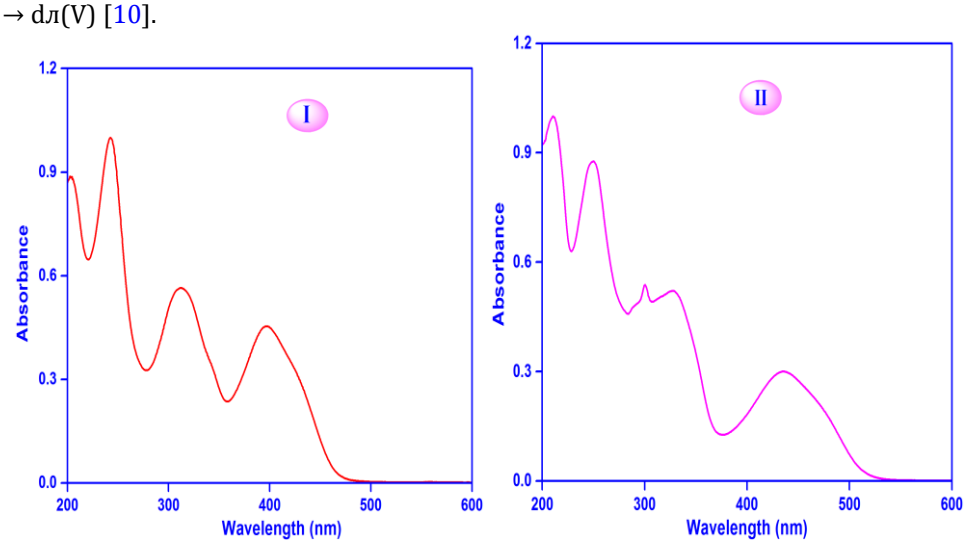


Figure 2. Absorption spectra of oxovanadium(IV) salophen complexes (**I and II**) in acetonitrile medium

IR Prestige-21 FT IR-8400s (Shimadzu Corporation, Japan) spectrometer was used to record the FT-IR spectral data of the metal complexes. Table 1 and Figure 3 reveal that the sharp and strong bands observed in the range of 1621 - 1538 cm^{-1} is due to $\nu_{(C=N)}$ of the azomethine group [13]. The bands due to $\nu_{(C-O)}$ and $\nu_{(V-O)}$ occurred at 1284 - 1151 cm^{-1} and 987 - 967 cm^{-1} respectively [14]. The ESI-MS spectra of the complexes (Figure 4) were recorded on Thermo Scientific Extractive Mass Spectrometer and the m/z values obtained are similar to the formula weight (Table 1).

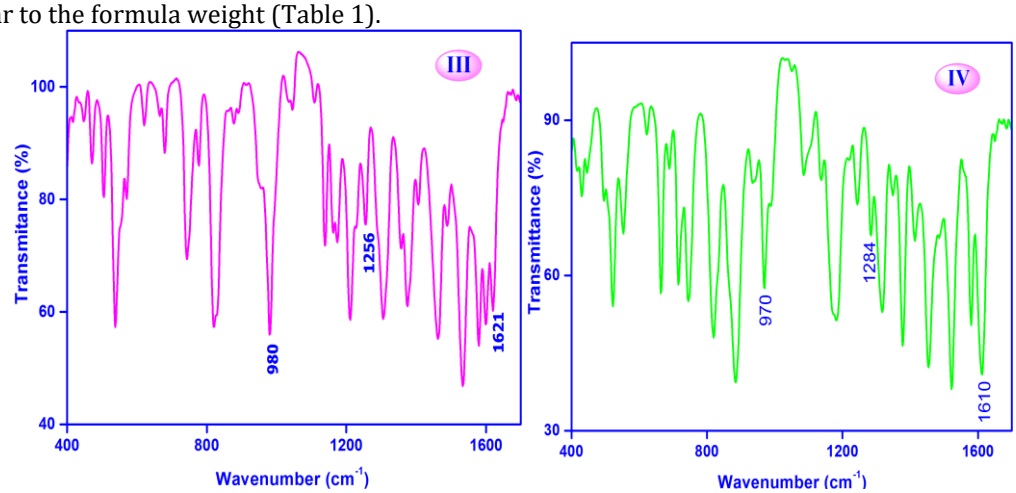


Figure 3. FT-IR spectra of complexes **(III and IV)**

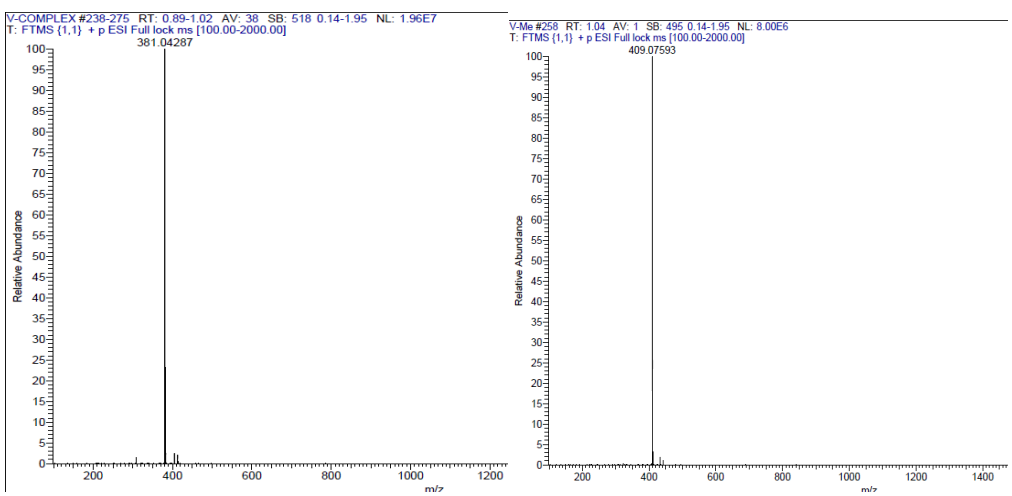


Figure 4. ESI-Mass spectra of complexes(**I and III**).

The CV measurements for the oxovanadium(IV) salophen complexes were done using a Cascade four-probe station connected to Agilent B1500A and B2912A parameter analyzers. The redox behaviour of oxovanadium(IV) salophen complexes was recorded in CH_3CN medium (Figure 5) and the reduction potential values obtained (Table 2) were consistent with the reported values [10].

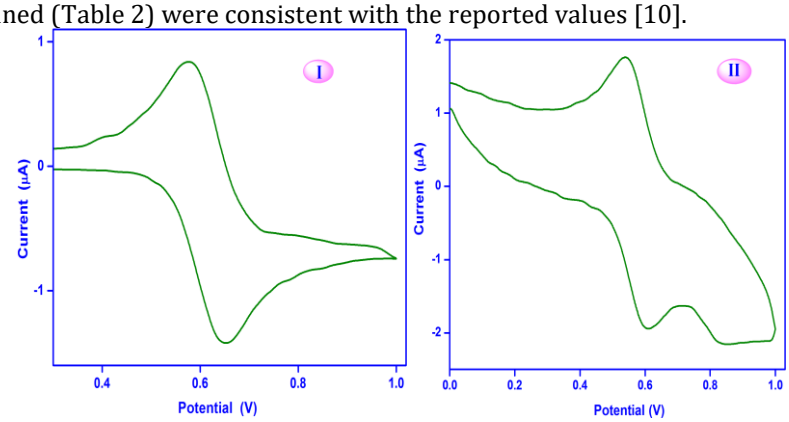


Figure 5. Cyclic voltammograms of complexes (**I** and **II**) in acetonitrile

Table 2. Reduction potential ofoxovanadium(IV) salophen complexes

Complex	E_{red} (V) Observed	Reported*
I	0.63	0.68
II	0.57	0.56
III	0.61	0.60
IV	0.75	0.75

Evalues were measured with reference to Ag /AgCl electrode

2.3 Microorganisms

The antibacterial and antifungal activity of the four oxovanadium(IV) salophen complexes have been carried out by agar well diffusion method by employing 24 hrs old cultures of Escherichia coli, Proteus sp., Citrobacter sp., Bacillus sp., Staphylococcus aureus, Staphylococcusepidermidis, 48 hrs culture of Candida sp. and fresh spores suspension of Aspergillus sp.

2.4 Antimicrobial activity

Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts [15]. The antimicrobials are allowed to diffuse out into the agar medium and interact in a plate freshly seeded with the test pathogenic microorganisms. All the complexes were tested separately for each organism using Muller Hinton Agar for bacteria and Sabouraud Dextrose Agar for Candida fungal sp. and Antimycotic Sensitivity Test Agar for Aspergillus fungal sp.

The medium was sterilized by autoclaving at 121 ^{o}C for 15 minutes. About 25 to 30 ml of autoclaved media was aseptically poured in 90 mm sterile Petri dish under laminar air flow unit and allowed to solidify. The agar plate surface was inoculated by spreading a volume of each microbial inoculum over the entire agar surface separately. Then 100 µl suspension of each respective strain of bacteria and fungi was transferred aseptically on to the agar surface of each petri plate and swabbed using sterile cotton to make a lawn of culture. Then a well of 6 mm diameter was punched aseptically with a sterile stainless-steelcork borer and a volume of 20 µL of the oxovanadium standards (10 µg) were added into 6 mm diameter well aseptically. The standard antibiotic Chloramphenicol (50 µg/well) was used as a positive control for antibacterial activity, whereas Nystatin (50 µg/well) was used as a positive control for antifungal activity. Separate negative controls were tested by using methanol, the solvent which was used for the dissolution of oxovanadium complexes. These antibacterial assay plates were incubated at 35 ± 2^{o}C for 24 to 48 hrs, antifungal assay plates were incubated at 25 ± 2 ^{o}C for 72 hrs. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested. After incubation, the resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth.

3.RESULTS AND DISCUSSION

A series of oxovanadium(IV) complexes with a class of triazole Schiff bases have been subjected to invitro antibacterial and antifungal studies. It has been found that the simple Schiff bases showed weaker to significant activity against one or more bacterial and fungal strains and in most of the cases higher activity was exhibited upon coordination with vanadium(IV) metal complexes [16]. Sobhaet al. [17] explored the activity of Cu(II), Ni(II) and Zn(II) Schiff base complexes against various bacteria like S. aureus, P. aeruginosa, E. coli, S. epidermidis and Klebsiella pneumonia by the diffusion agar technique and reported that the complexes were more potent bactericides than the free Schiff bases.

Table.3. In vitro antimicrobial activity of oxovanadium(IV) salophen complexes (**I-IV**).

S. No.	**Organism**	**Zone of inhibition (mm)**				
		Positive control	**I**	**II**	**III**	**IV**
1.	E. coli	17.0*	10.0	9.0	8.0	6.0
2.	Proteus sp.	20.0*	6.0	6.0	6.0	12.0
3.	Citrobacter sp.	22.0*	8.0	15.0	8.0	8.0
4.	Bacillus sp.	15.0*	11.0	10.0	6.0	9.0
5.	S. aureus	26.0*	11.0	9.0	6.0	8.0
6.	S. epidermidis	26.0*	12.0	6.0	13.0	8.0
7.	Candida sp.	23.0#	12.0	8.0	8.0	6.0
8.	Aspergillus sp.	23.0#	11.0	6.0	6.0	6.0

*Chloramphenicol; #Nystatin

Antimicrobial studies carried out by Patil et al. [18] revealed that the activity of the ligand enhanced on complexation but to a lesser extent when compared to the standard used. The higher antibacterial activity of the VO(IV) complexes is attributed to the chelation effect, which further increases the delocalization of π-electrons over the whole chelate ring and enhances the lipophilicity of the complexes which again enhances the penetration of complexes into lipid membrane and blocks the metal binding sites on enzymes of micro organisms. Raman et al. [19] investigated the in vitro antimicrobial activity of Schiff base ligands of 4-aminoantipyrine derivatives of N_2O_2 type and the corresponding copper, vanadyl, nickel and zinc complexes against different bacteria and fungi species. The minimum inhibitory concentration (MIC) values indicated that the metal complexes exhibit higher antimicrobial activity than the free ligand. The increased activity of the complexes was explained on the basis of Overtones concept and Tweedy's chelation theory.

The in vitro antimicrobial screening results of all the synthesized oxovanadium(IV) salophen complexes (**I-IV**) against different gram positive (Bacillus sp., Staphylococcusaureus and Staphylococcusepidermidis) and gram negative bacteriae (E. coli, Proteus sp., Citrobacter sp.) and antifungal activity against fungal microorganisms (Candida sp. and Aspergillus sp.) are given in Table 3.

On the basis of the observed zone of inhibition, it is found that all the four oxovanadium(IV) salophen complexes are moderately active towards certain specific microorganisms. Oxovanadium complex **II** shows significant effect of antimicrobial activity (15 mm zone of inhibition) against the bacteria Citrobacter sp. which potentially causes the disease of urinary tract infections. Complex **III**also shows 13 mm zone of inhibition against S. epidermidis. Complex **IV** is active against Proteus sp. and complex **I** is found to be moderately active towards most of the microorganisms under study.The antimicrobial activity of the complexes (**I - IV**) against the various microorganisms is represented in Figure.6.

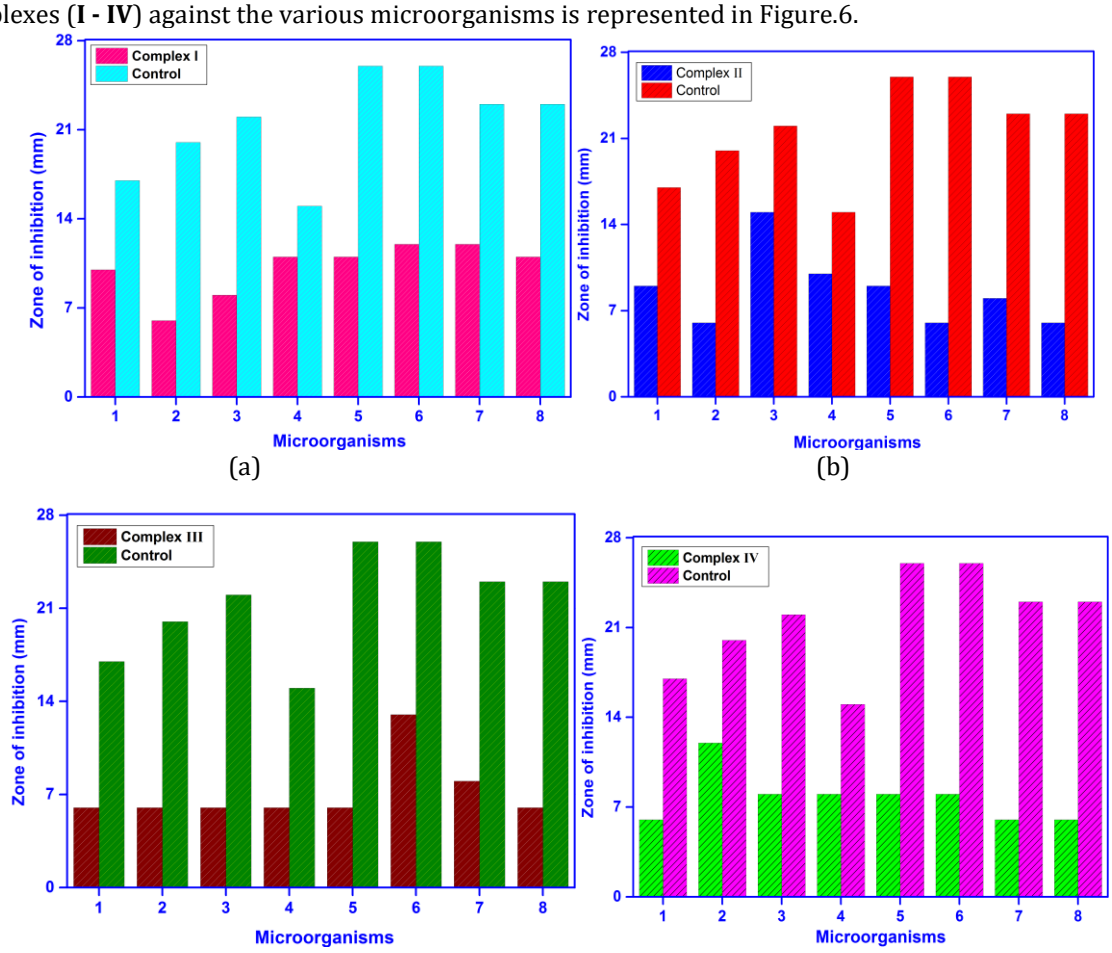


Figure. 6. Antimicrobial activities of oxovanadium(IV) salophen complexes, (a) **I**; (b) **II**; (c) **III** and (d) **IV** against microorganisms.

1- E. coli; **2** - Proteussp.; **3** - Citrobactersp.; **4** - Bacillussp.;
5 - S. aureus; **6** - S. epidermidis; **7** - Candidasp.; **8** - Aspergillussp.

The results given in Table 3 are compared with the zone of inhibition of standard drugs chloramphenicol for bacteria and nystatin for fungi. The results are comparable with the studies carried out by Hoogenkamp*et al.* [20] and Persoon*et al.* [21].In the present study, the presence of substituent in the complex has not changed the antibacterial activity remarkably. The antimicrobial activity of the metal complexes has been explained on the basis ofOvertone's concept and Chelation theory. According to Overtone's concept of cell permeability, lipo-solubility is an important factor that governs the antimicrobial activity of antibacterial agents. In metal complexes chelation reduces the polarity of the metal ions to a greater extent due to the overlap of the ligand orbitals and partial sharing of positive charge of metal atom with donor groups. Besides, it increases the delocalization of π-electrons over the whole chelate ring and increases the lipophilicity of the complexes. This increased lipophilicity enhances the penetration of the complexes into the lipid membrane and increases their antimicrobial activities.

4. CONCLUSION

The present study clearly reveals that oxovanadium(IV) salophen complex and its derivatives have moderate antimicrobial activity against certain gram positive, gram negative bacteria and fungal pathogens and thereby have the potential to be used as antimicrobial agents.

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