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Oxovanadium(V) complexes with hydrazone ligands: Synthesis, characterization and biological activity

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ABSTRACT

$[VOSO_4].5H_20$ with Pyrazine-2-carboxylic acid(phenyl-thiophene-2-yl-methylene)- hydrazide (HL_1) and Pyrazine-2-carboxylicacid (phenyl-pyridin-2-yl-methylene)-hydrazide (HL_2), respectively, in methanol affords oxovanadium(V) complexes, $[VO(SO_4)L_1]$ (I) and$[VO(SO_4)L_2]$ (II). Both complexes have been characterized by elemental analysis and spectroscopic techniques (I.R, U.V, mass). The mononegative hydrazone ligands coordinate to the metal atoms through azomethine nitrogen, carbonyl oxygen and sulphur of thiophene ring (HL_1)/ nitrogen of pyridine ring(HL_2) and found to have square pyramidal geometry around each vanadium ion. Ligands and complexes were screened for in vitro antibacterial activity and antifungal activity at different concentrations against bacteria viz. Gram positive Bacillus subtilis, Micrococcus Luteus and Gram negative Pseudomonas aeruginosa, Pseudomonas mendocina and fungi Verticillum, Cladosporium, Tinospora. A distinct enrichment in biocidal activity of the ligands under similar experimental conditions was observed as a consequence of coordination with vanadium atom.

INTRODUCTION

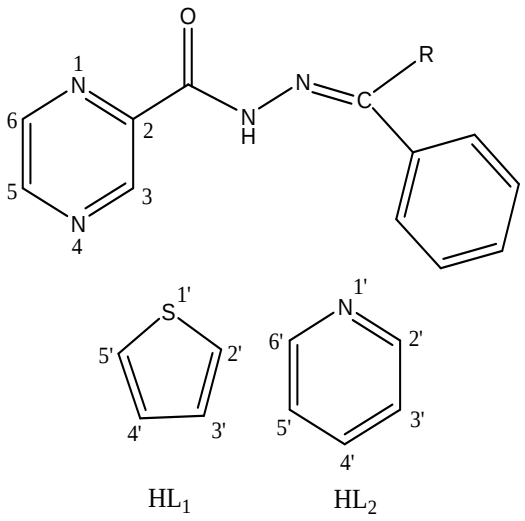
Vanadium is a biological trace element able of accessible in extensive variety of oxidation state +3, +4, and +5, which advances the utility of this element in biological systems[1]. Vanadium possesses medicinal, pharmacological and biological relevance in numerous enzymatic reactions. Current advances in catalytic and medicinal properties of vanadium complexes have enthused their design and synthesis. Vanadium (V) is used in the treatment of diabetes mellitus predates the invention of insulin [2], however, the insulin-like activity of vanadium compounds has established rising attention [3]. Vanadium ions and complexes have been verified to make use of a variety of insulin-like and anti-diabetic effects, such as enhancing glucose transport and metabolism in adipocytes, hepatocytes, and skeletal muscle, motivating glycogen synthesis and lipogenesis, or inhibition of lipolysis and protein catabolism, both in animal model systems and cell cultures [4-6]. Its biological significance is further exemplified by its assimilation in natural products and enzyme in potent inhibitor of phosphoryl transfer [7-9]. Schiff base hydrazones compounds have been extensively used as versatile ligands in coordination chemistry and Schiff base hydrazone vanadium complexes are also very attractive as model compounds for the elucidation of several biochemical processes. Transition metal complexes V=O with Schiff base chelating ligand has been reported [10-11]. In the present context, we have aimed to study the effect on biological activity of tridentate hydrazone ligands Pyrazine-2-carboxylic acid(phenyl-thiophene-2-yl-methylene)- hydrazide (HL_1) and Pyrazine-2-carboxylicacid (phenyl-pyridin-2-yl-methylene)-hydrazide (HL_2) towards oxovanadium(IV) ions.

MATERIALS AND METHODS

All the chemicals used were obtained through Aldrich and used as such without any further purification. IR spectra were recorded on Shimadzu IR affinity-I 8000 FT-IR spectrometer using KBr disc having wavelength range 4000-400 cm^{-1}. ^{1}H NMR and ^{13}C NMR were recorded on Bruker Avance II 300 MHz NMR spectrometer and all chemical shifts were reported in parts per million relative to TMS as internal standard in $CDCl_3$. UV spectra were recorded on UV-VIS-NIR Varian Cary-5000 spectrometer in DMF. Molar conductance measurements of a 10^{-3} M solution of metal complexes in DMF at room temperature were carried out using a model-306 Systronics

Synthesis of the Schiff base Hydrazone

To the methanolic solution of pyrazine carboxylic acid hydrazide (0.01 mol) was added 2- benzoyl thiophene / 2-benzoyl pyridine (0.01 mol) [12], and the solution was refluxed for 5 hr. The solvent was evaporated in vacuum to half its volume and cooled to room temperature. The solid obtained was filtered and washed with methanol.



HL_1 HL_2

Structure of ligand

Pyrazine-2-carboxylic acid(phenyl-thiophene-2-yl-methylene)- hydrazide (HL_1)

Yield (75%) ; MP.: 155˚C; IR (ν_{max}, KBr, cm^{-1}): 3300 (NH str.), 1651 (C=O), 1575 (C=N), 671 (th); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 14.08 (s,1H, NH), 9.25 (s, IH, C_3-H), 8.76 (J=3.3, d, C_6-H), 8.65 (J= 3.3, d, C_5-H), 7.34 (J= 7.8, d, 1H, C_5'-H), 7.25 (J= 7.2, d, C_3'-H), 7.05 (J= 7.8, J= 2.1, dd, C_4'-H), 7.48 -7.28 (m, 5H, Ph-H); ^{13}C NMR (DMSO-d_6, 300 MHz): 161.32(C=O), 152.05(C=N), 149.52(C_2'), 148.85(C_5'), 147.91 (C_2), 147.34(C_5), 137.24(C_3), 136.84(C_6), 136.73(C_4'), 129.32-128.24(Ph-C), 126.90(C_3'); Anal Calc. For $C_{16}H_{12}N_4OS$ (308.36)(%) : C- 62.32, H- 3.92, N-18.17, S-10.40; Found (m/z= 308.21)(%): C- 62.48, H- 3.78, N-18.45, S- 10.62.

Pyrazine-2-carboxylicacid (phenyl-pyridin-2-yl-methylene)-hydrazide (HL_2)

Yield (70%); MP.:165˚C; IR (ν_{max}, KBr, cm^{-1}): 3350 cm^{-1} (NH str.), 1681 (C=O), 1580 (C=N), 693 (Py); ^{1}H NMR ($CDCl_3$-d_6, 300 MHz): δ 15.08(s,1H, NH), 9.50(s, IH, C_3-H), 8.93 (J= 3.3, d, C_6'-H), 8.77(J=2.1,d,C_6-H), 8.62(J= 2.1, d, C_5-H),7.84 (J= 7.8, d, 1H, C_3'-H), 7.67 (J= 9.0, J= 3.3, dd, C_4'-H,C_5'-H), 7.48 -7.28 (m, 5H, Ph-H); ^{13}C NMR (DMSO-d_6, 300 MHz): 162.47(C=O), 152.53(C=N), 149.20(C_2'), 148.85(C_6'), 148.18(C_2), 147.90(C_5), 137.84(C_3), 137.46(C_6), 136.94(C_4'), 135.97(C_5'), 129.63-128.45(Ph-C), 127.10(C_3'); Anal. Calc. for $C_{17}H_{13}N_5O$ (303.31) (%): C- 67.32, H- 4.32, N- 23.09; Found (m/z= 303.8) (%): C- 67. 56, H- 4.53, N- 23.52.

Synthesis of the Vanadium(V) Complexes I and II

$VOSO_4 \cdot 5H_2O$(0.01mmol) dissolved in minimum amount of water was added dropwise to a methanolic solution (10 mL) of Schiff base (0.01 mmol) with stirring, and then the mixture was refluxed and stirred for 2 hr, cooled to room temperature and then a colored solution was concentrated to yield the complex. The latter was washed with diethyl ether and dried over calcium chloride.

$VO(L_1)SO_4$(I)
Yield (70%); D.P >300˚C; IR (ν_{max}, KBr, cm^{-1}): 1579 (C=N), 1089 (C-O), 960(V=O), 665 (th), 518(V–N), 480(V-S), 464(V–O); UV(ν,cm^{-1})- 28010 (M→L); Anal. Calc. for $C_{16}H_{11}N_4O_6S_2V$ (469.96) (%): C- 40.86, H- 2.36, N-11.91, S- 13.63, V-10.83; Found (m/z= 470.5) (%): C- 40.96, H- 2.78, N- 12.04, S- 13.87, V- 10.54.

$VO(L_2)SO_4$(II)
Yield (70%); D.P >300˚C; IR (KBr, cm^{-1}): 1574 (C=N), 1078 (C-O), 962(V=O), 698 (py), 523(V-N), 520(V–N), 475(V–O); UV (ν,cm^{-1}): 29472 (M→L); Anal. Calc. for $C_{17}H_{12}N_5O_6SV$ (465.31) (%): C- 43.88, H- 2.60, N-15.05, S- 6.89, V- 10.95; Found (m/z= 465.8) (%): C- 43.96, H- 2.84, N- 15.23, S- 7.09, V-10.55.

Biological assay
Test microorganisms
Total seven microbial strains of bacteria and fungi were selected on the basis of their clinical importance. Two Gram-positive bacteria (*Bacillus subtilis*, *Micrococcus Luteus*) and two Gram negative bacteria (*Pseudomonas aeruginosa*, *Pseudomonas mendocin)* and three fungi (*Verticillum*, *Cladosporium*, *Tinospra*). The bacteria were subcultured on nutrient agar, whereas fungi on Sabouraud dextrose.

***in vitro* antibacterial activity**
Stock solution was prepared by dissolving compound in minimum amount of DMSO. The media was made by dissolving Nutrient agar (15g) in 1 L distilled water. The mixture was autoclaved for 15 min at 120 ˚C and then dispensed into sterilized Petri dishes, allowed to solidify and then used for inoculation. Target microorganism cultures were prepared separately in 15 ml of liquid Nutrient agar for activation. Inoculation was done with the help of micropipette with sterilized tips, 100 µl of activated strain was placed onto the surface of agar plate, spread over the whole surface and then two wells having diameter of 10 mm were dug in media. Sterilized stock solutions were used for the application in the well of inoculated agar plates. In this well of inoculated agar plates 100 µl of solution was poured and incubated at 37 ˚C for 48 hrs. Activity was determined by measuring the diameter of zone showing complete inhibition and has been expressed in mm. All these experiments were performed in triplicate.

In vitro antifungal activity
For antifungal activity, the moulds were grown on Sabouraud dextrose agar (SDA) at 25°C for 7 days and used as inoculate. 15 mL of molten SDA (45°C) was added to 100 µL volume of each compound having concentration of 100µg/mL, reconstituted in the DMSO, poured into a sterile Petri plate and allowed to solidify at room temperature. The solidified poisoned agar plates were inoculated at the centre with fungal plugs 10 mm obtained from the actively growing colony and incubated at 25°C for 7 days. The DMSO was used as the negative control, whereas *Fluconazole* was used as the positive control. The experiments were performed in triplicates. Diameter of the fungal colonies was measured and expressed as percent mycelial inhibition determined by applying the following formula.

$$\text{Inhibition of mycelial growth \%} = (d_c - d_t)/d_c \times 100$$

d_c, average diameter of fungal colony in negative control; d_t average diameter of fungal colony in experimental plates.

RESULTS AND DISCUSSION

Schiff base ligand has been synthesized from condensation of pyrazine-2- carboxylic hydrazide and 2-benzoyl thiophene (HL_1), 2- benzoyl pyridine (HL_2). Schiff base ligands exists in keto-enolic form, in solid state ligand exists in keto form and in solution it exists as enolic form . The ligand was chelated to metal ion in enolic form with the replacement of one hydrogen atom. Oxovandium(V) complexes were synthesized by reaction of vandium(IV) metal salt with Schiff base ligand. These complexes were nonhygroscopic and stable in the solid state at room temperature. It has been distinguished that vanadium in the metal salt is in oxidation state +4 whereas in both complexes vanadium is present in +5 oxidation state, this may be due to the oxidation by air during reaction procedure [13]. The molar conductivity measurements for $1X10^{-3}$ M solution were found to be very low, showing its nonelectrolytic nature and elemental analysis of these complexes are in satisfactory agreement with empricial formula.

IR Spectra

The IR spectra of hydrazone ligands contains a strong C=O absorption band at 1651-1681cm^{-1}and N-H absorption band at 3300-3350 cm^{-1}. Absence of υ(C=O) and υ (N-H) vibrations in the spectra of complexes indicated that hydrazone ligand has undergone deprotonation during complexation and co-ordinated to metal centre through O and N of enolic form of ligand. Confirmation of this was strengthened by existence of new stretching vibrations between1089-1078 cm^{-1} ascribed to υ(C-O) in the complexes. A Strong band in both ligands around 1575-1580 cm^{-1} attributable to the C=N stretching vibration, showed a shift (by 5-10 cm^{-1}) after complexation, indicating the coordination of azomethine nitrogen to the vanadium ion [14]. This can be explained by the donation of electrons from nitrogen to the vacant d orbital of the vanadium ion. The oxovanadium(V) complexes exhibit an additional strong absorption band near ~ 960 cm^{-1}, which may be assigned due to ν(V=O)[15]. Thiophene ring and pyridine ring show vibration at 671 cm^{-1} and 693 cm^{-1} which were shifted to higher frequencies on complexation indicated that heterocyclic sulfur of thiophene ring and nitrogen of pyridine ring was involved in coordination and this bonding through O , N and S/N further evidenced by the new bands in the spectra of the complexes appeared at 464–475 cm^{-1}, 518–523 cm^{-1} and 480 cm^{-1} assigned to the V–O, V–N and V-S stretching vibrations[16]. On the basis of these results, we concluded that the Schiff bases hydrazones in the vanadium complexes behave as monobasic tridentate molecules.

Electronic spectra and magnetic moment measurements

Complexes **I** and **II** exhibited the zero μ_{eff} value, which has been expected for the spin only value for the d^0 system. These complexes showed band observed in the region 28010–29472 cm^{-1} may be due to the charge transfer transition between dz^2 orbital of vanadium atom and $2p_x$ or $2p_y$ orbital of oxygen atom which is basis of an five coordinated square pyramidal structure for the VO(V) complexes.

Mass spectra

The LC-MS of Schiff base ligands and their complexes showed molecular ion peaks which were in agreement with their molecular formula. The molecular ion peak for the ligand HL_2 ($C_{17}H_{13}N_5O$) and its Oxovanadium(V) complex $VO(L_2)SO_4$ ($C_{17}H_{12}N_5O_6SV$) at m/z 303.2 and 465.8 respectively.

Pharmacology

In vitro antibacterial activity and antifungal activity

Schiff base and there vanadium complexes were evaluated in vitro for their antibacterial activity against bacterial strains Gram positive (*Bacillus subtilis, Micrococcus Luteus*) and Gram negative (*Pseudomonas aeruginosa, Pseudomonas mendocin),* fungal strains (*Verticillum, Cladosporium, Tinospora*) and their activity was compared to a well-known commercial antibacterial and antifungal drug *Streptomycin* and *Fluconazole*. Results of antibacterial and antifungal evaluation are summarized in Table I, II and Figure I, II.

Table-I *in vitro* antibacterial activity of ligands and their complexes

		Zone of Inhibition(mm)															
		Gram +ve								Gram –ve							
		B. subtilis				*M. Luteus*				*P.aeruginosa*				*P. mendocina*			
Sr. No	Compounds	25	50	100	200	25	50	100	200	25	50	100	200	25	50	100	200
1	HL_1	11	13	14	17	13	14	16	19	10	12	14	16	12	13	15	19
2	HL_2	9	12	13	15	11	13	15	18	8	10	11	13	10	11	13	18
3	$VO(L_1)SO_4$	17	18	20	21	16	18	21	21	15	17	19	21	17	18	20	23
4	$VO(L_2)SO_4$	13	15	16	19	13	15	17	18	11	13	14	17	13	15	16	18
5	Streptomycin	19	21	25	26	19	20	23	25	20	23	25	28	22	25	27	30

Table-II *in vitro* antifungal activity of ligands and their complexes

Sr. No	Compounds	mycelial growth inhibition %											
		Verticillum				*Cladosporium*				*Tinospora*			
		25	50	100	200	25	50	100	200	25	50	100	200
1	HL_1	54.6	55.3	56.5	57.4	50.1	52.3	53.4	54.3	53.2	54.1	55.5	56.4
2	HL_2	52.1	53.2	54.3	56.2	50.8	51.6	52.5	53.4	56.7	57.2	58.3	59.9
3	$VO(L_1)SO_4$	65.3	66.2	67.8	68.2	64.2	65.3	67.9	69.2	60.2	61.4	63.7	65.3
4	$VO(L_2)SO_4$	60.2	61.2	62.6	64.3	66.2	67.9	69.7	69.9	56.4	58.2	59.6	60.2
5	Fluconazole	76.4	78.2	80.5	81.2	76.3	77.4	78.3	79.7	77.2	79.2	79.6	80.2

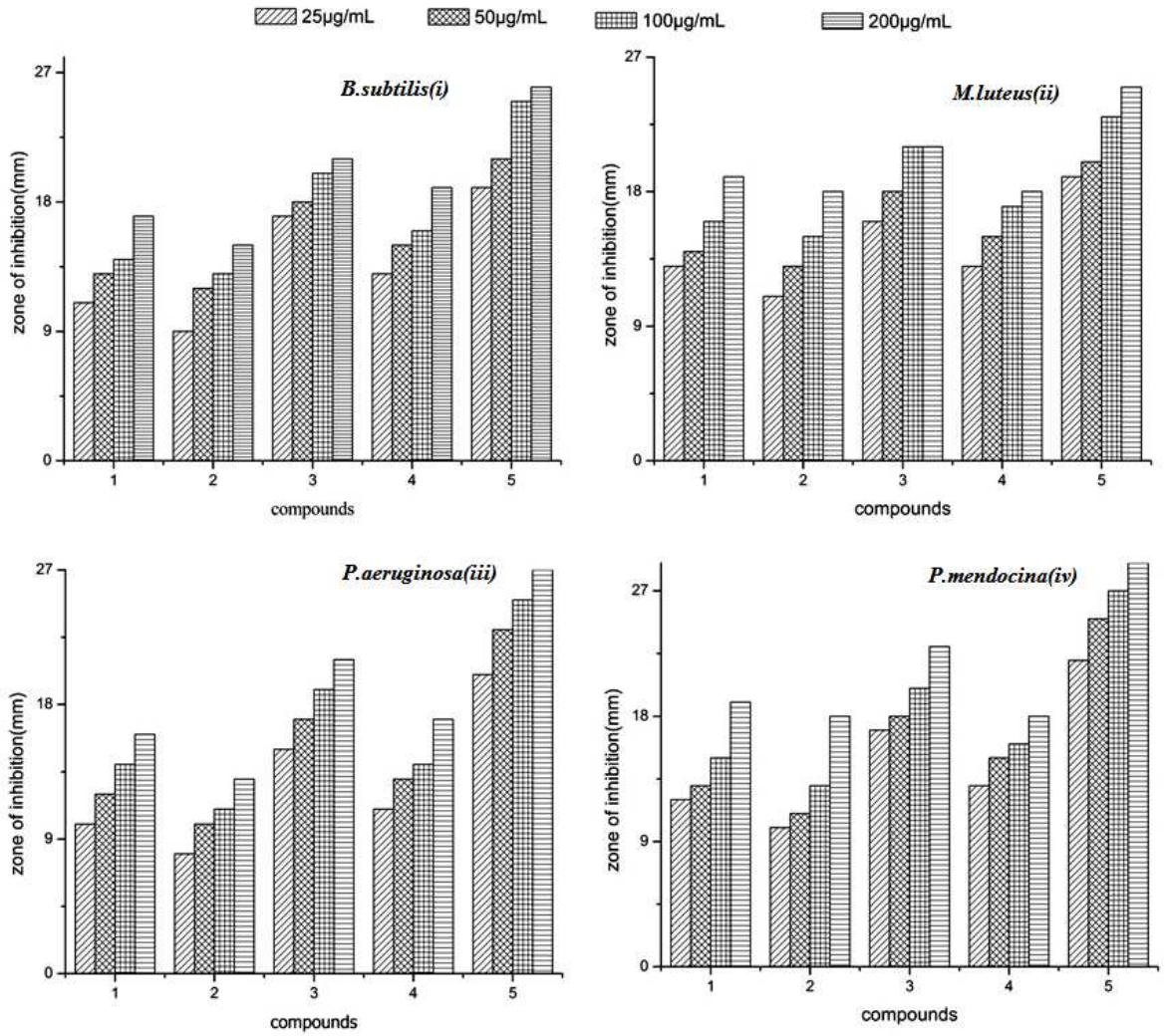


Figure I: Antibacterial activity data of ligands and their vanadium complexes (1 to 5 as in Table-I)

The antibacterial studies suggested that the Schiff bases HL_1 and HL_2 have shown marginal activity but after complexation with metal it was found that antimicrobial activity gets increased. This has been explained on the basis of Chelation theory [17]. On chelation, polarity of the metal ion is condensed to a greater extent due the overlapping of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Moreover, delocalization of the π-electrons over the whole chelate ring is increased and lipophilicity of the complexes is enhanced. The increased lipophilicity enhances the penetration of the complexes into the lipid membranes and blocks the metal binding sites in the enzymes of microorganisms. These complexes also perturb the respiration process of the cell and thus block the synthesis of proteins, which restricts additional growth of the organism.

Furthermore, the mode of action of the compounds may engross the formation of a hydrogen bond through the azomethine nitrogen atom with the active centers of cell constituents, resulting in obstruction with the normal cell process.

On the basis of zone of the inhibition produced against the test microorganism complex I showed maximum zone of inhibition this is due to thiophene ring present in ligand HL_1.

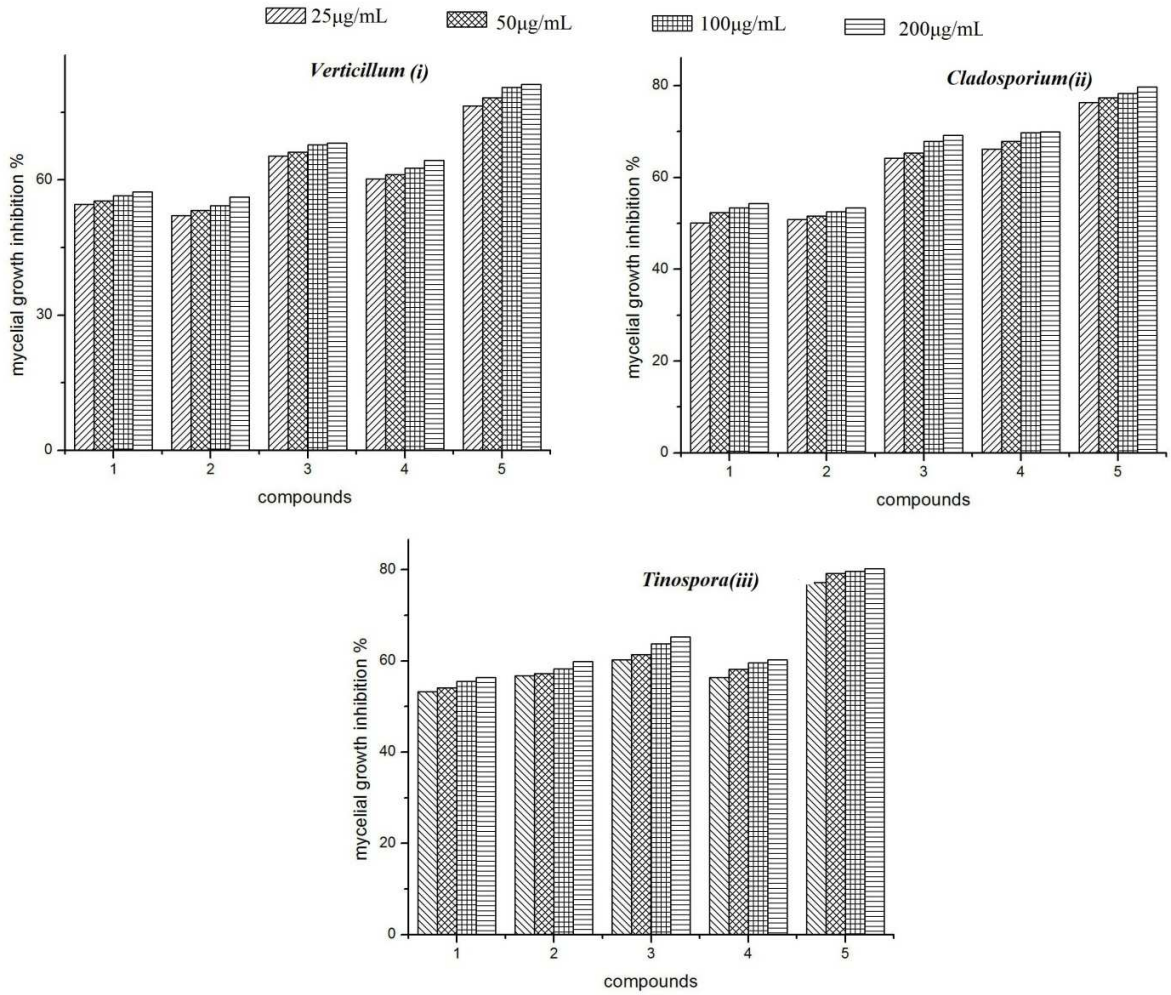
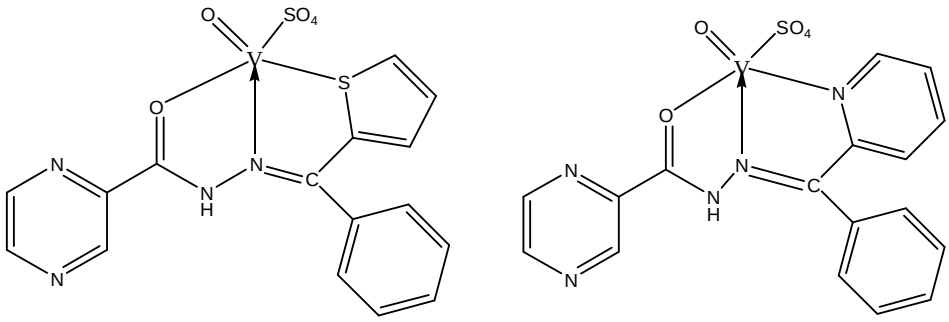


Figure II: Antifungal activity data of ligands and their vanadium complexes (1 to 5 as in Table-II)

CONCLUSION

Schiff base hydrazones and their vanadium metal complexes have been synthesized. Schiff base hydrazone ligands binds to vanadium atoms through azomethine nitrogen, carbonyl oxygen and sulphur of thiophene ring / nitrogen of pyridine ring forming five membered chelate rings. Square pyramidal geometry have been proposed for Oxovandium (V) complexes with the help of various physicochemical studies.



Proposed structure for $VO(L_1)SO_4$(I) and $VO(L_2)SO_4$(II)

The free ligands and their metal complexes showed antimicrobial activity and the complexes are found to be more potent than the free ligands and their toxicity has increased as per the increase in Concentration.

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