ECO-FRIENDLY SYNTHESIS AND *In-vitro* ANTIMICROBIAL, ANTIOXIDANT ACTIVITIES AND COMPUTATIONAL STRATEGY OF V²⁺ COMPLEX WITH BIDENTATE SCHIFF BASE

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ABSTRACT

The Schiff base V²⁺ metal complex has been synthesized and characterized by various physicochemical, spectral and bio-potential studies viz., elemental analysis, metal estimation, molar conductance, magnetic moment and UV-Visible spectra, IR and Far-IR spectra. The Schiff base was synthesized by the condensation of Isoniazid and 4-chlorobenzaldehyde by the eco-friendly method. The elemental analysis of Schiff base and metal complex is confirmed by its stoichiometry and molecular formulae of them which is further confirmed metal estimation. Molar conductance of 10⁻³ M complex solution in acetonitrile shows the non-electrolytic nature. Magnetic moment and electronic spectral studies indicate the octahedral geometry of V²⁺ complex. The IR and Far-IR spectral data confirmed the complexing ability and metal chelates (M-N and M-O) of the metal chelate. *In-vitro* bio-potential activity (antibacterial and antifungal) of V²⁺ complex of Schiff base was assessed with various bacterial and fungal strains viz., *E. coli* and *A. Niger* by Agar well diffusion method. The DPPH free radical scavenging activity of Schiff base and its metal complex assessed by antioxidant activity was further confirmed by molecular docking studies of selected targeted protein viz., cytochrome p450.

Key Words: Isoniazid, 4-chlorobenzaldehyde, Eco-friendly, Antibacterial, Antifungal, Antioxidant, Molecular Docking.

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INTRODUCTION

Medicinal inorganic chemistry is the interface between medicine and inorganic chemistry.¹ Metal complexes used to design novel chemotherapeutic agents which can choose by their oxidation state, ligands and geometry.² Tuberculosis is the largest disease in developing and developed countries caused by mycobacterium tuberculosis (MTb). The drug developed against TB globally by multi-drug resistant tuberculosis (MDRTB) and extensively drug-resistant tuberculosis (XDRTB). Isoniazid metal-based drug is the active drug against TB.³ The Schiff bases are chelates and outstanding ligand in coordination chemistry which are having antibacterial, antifungal⁴, herbicidal, anti-inflammatory, anticancer⁵, anticonvulsant and anti-HIV agent.^{6,7,8} Isoniazid is a pro-drug which has therapeutic applications. Schiff bases are derived from Isoniazid have been synthesized by refluxing aldehyde (4-chlorobenzaldehyde) with water as a solvent. This method is a green synthetic route at room temperature. 4-chlorobenzaldehyde form Schiff base with Isoniazid by effective coordination.⁹⁻¹¹ In the present study aims to the synthesis of Schiff base metal complex of vanadium and characterized by various physicochemical, spectral and *in-vitro* bio-potential activities viz., antibacterial, antifungal and antioxidant. The computational strategy of the metal complex is also carried out by the molecular docking method.

EXPERIMENTAL

Materials

Isoniazid, 4-chlorobenzaldehyde (Alfa Aesar), VOSO_4 , CH_3OH , $\text{C}_2\text{H}_5\text{OH}$ (AnalaR grade).

Synthesis of Schiff Base

The Schiff base were synthesized by mixing Isoniazid 0.528g in 10 ml water: methanol (1:1) and 0.541 g of 4-chlorobenzaldehyde in 5 ml of ethanol and add 15 ml water as a green solvent and the mixture was stirred on 15-20 min at room temperature the precipitated white powder washed with water and dried in a desiccator.

Synthesis of V^{2+} Complex

The V^{2+} complex was prepared by mixing Schiff base 2.05g 20 ml of methanol and sodium nitrite 0.545 g in 10 ml water to 1 g of vanadylsulphate in 15 ml of water and 10 ml of water added to the mixture used as a green solvent, the mixture was stirred at 15-20 mins at room temperature, the green color precipitate filtered and dried in a desiccator, the complex is stable at room temperature.

Methods

Using Vario make EL-III Elemental analyzer to predict C, H, and N present in the complex. Metal ion present in the complex was estimated by the colorimetric method by standard procedure. Molar conductance of complex solution at 10^{-3}M concentration was measured using Systronic Conductivity Bridge. Using Lake Shore 7410 Vibrating Sample Magnetometer, the magnetic moment of V^{2+} complex was predicted at RT. The absorption spectra of the complex by DRS method were carried out using Varian make, CARY-5000 model, UV-VIS-NIR Spectrophotometer. Making KBr pellet, the IR spectra of complex compared with Schiff base and carried out Using Shimadzu, FT-IR, 8400 S Model IR spectrometer. Bruker, Germany makes 3000 Hyperion Microscope with Vertex 80 FTIR instruments used to predict Far-IR spectra. NMR spectra (^1H and ^{13}C) of Schiff base at room temperature in DMSO-d_6 were measured using the Bruker instrument.

Scheme-1: Synthesis of Schiff Base

Antimicrobial Study

Bio-potential activity of Schiff base and its V^{2+} complex was predicted by Agar disc diffusion method. The nutrient agar (NA)/potatoes dextrose agar (PDA) medium was used to grow the bacterial and fungal strains. Chloramphenicol and fluconazole are used as a standard for bacterial and fungal strain. The zones of inhibition of the tested microorganisms by the samples were measured using a millimeter scale.

In-vitro Antioxidant Activity

2 ml aliquot of DPPH methanol solution ($25\mu\text{g/ml}$) was added to 0.5 ml sample solution at different concentrations. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. Then the absorbance was measured at 517nm in a spectrophotometer. Lower absorbance of the reaction mixture indicated higher free-radical scavenging activity.

$$\text{Radical Scavenging Activity (\%)} = 100 - [(A_c - A_s) / A_c] \times 100$$

$$1500$$

Where A_C = control is the absorbance and A_S = sample is the absorbance of the reaction mixture (in the presence of sample).

Scheme-2: Synthesis of V²⁺ Complex

Statistical Analysis

Tests were carried out in triplicate for 3 separate experiments. The amount of sample needed to inhibit free radicals concentration by 50%, IC₅₀, was graphically determined by a linear regression method using Ms-Windows-based graph pad Instant (Version 3) software. Results were expressed as graphically/ mean $\pm$ standard deviation.

RESULTS AND DISCUSSION

The physical properties of the Schiff base and its metal complex viz., molecular weight, color, melting point, microanalytical data (elemental analysis and metal estimation) and molar conductance are given in Table-1. The experimental and theoretical values of elemental analysis and metal estimation confirmed the very close agreement with the stoichiometry of the metal: ligand ratio was concluded the molecular formulae of the complex. The sharp melting point of the Schiff base differs from the starting materials (Isoniazid and 4-chlorobenzaldehyde) indicating the new complex formation by dehydration. Molar conductance of 10⁻³ M metal chelates in acetonitrile solution lying in the lower value at 20 Ohm⁻¹cm²mol⁻¹ indicating the non-electrolyte (1:0 type) of the complex.¹²

UV-Visible Spectra

The UV-Visible spectrum of Schiff base exhibit two absorptions at 256 nm and 310 nm indicating the π - π^* and n - π^* transitions confirmed by the imine and carbonyl chromophore group present in the Schiff base.¹³ In the VO(II) complex shows three predominant peaks at 265 nm, 390 nm and 510 nm corresponding to three spins allowed transitions ${}^4A_{2g} \rightarrow {}^4T_{1g}(P)$, ${}^4A_{2g} \rightarrow {}^4T_{1g}$, and ${}^4A_{2g} \rightarrow {}^4T_{2g}$ respectively from the ground term ${}^4A_{2g}$ confirm the octahedral geometry of VO(II) complex which is further corroborated by μ_{eff} value at 3.59 BM.¹⁴

Table-1: Analytical Data for Schiff Base and its Complex

S.No.	Compound	MW (g/mol)	MP (°C)	Elemental Analysis						Molar Conductance (Ohm ⁻¹ cm ² mol ⁻¹)
				%C	%H	%N	%O	%Cl	%M	
1	Schiff base	259.70	180-185	60.06 (48.01)	3.85 (3.90)	16.17 (16.10)	6.16 (6.10)	13.47 (13.19)	-	20.00
2	[VO(SB) ₂ (NO ₂) ₂]	662.37	190	47.10 (48.01)	3.01 (3.90)	16.90 (16.10)	14.40 (14.80)	10.56 (10.80)	7.69 (7.10)	20.00

IR-Spectra

The IR spectrum of bidentate Schiff base shows secondary amine N-H stretching frequency and imine H-C=N at 3456 cm⁻¹ and 1667 cm⁻¹, in complex these frequencies shifted to 3454 cm⁻¹ and 1668 cm⁻¹ respectively indicating the formation of Schiff base and coordination to the metal ion through a nitrogen atom. The aromatic pyridine ring C=C, C=O, symmetric C=N and asymmetric C=N exhibited at 1486 cm⁻¹, 1592 cm⁻¹ and 1357 cm⁻¹ respectively present in the Schiff base which are shifted to higher frequencies in complex, another aromatic ring C-H also present at 3016 cm⁻¹, which is also shifted to a lower frequency in the complex.¹⁵ From the change of frequencies in the complex on comparing Schiff base is confirming effective coordination of ligand to metal ions through its donor site ('O' and 'N'). In the ligand, more electronegative atoms present and also affect the stretching frequencies of the complex. The additional mixed anionic ligand nitrite ion shows the stretching frequencies at the lower region by skeletal mode at 1407 cm⁻¹, 1299 cm⁻¹ and 1085 cm⁻¹ corresponding to the symmetric O-N=O, asymmetric O-N=O and N-O respectively in which the symmetric stretching frequency changed very little while asymmetric very large due to coordination of nitrite ion to the metal through an oxygen atom.

The metal chelates atom ability was found from the Far-IR spectral data, the M-N and M-O coordination of Schiff base at 514 cm⁻¹ and 494 cm⁻¹ confirm the coordination of metal ion through imine nitrogen and carbonyl oxygen atom of secondary amine. The ambidentate nitrite ion coordinates to the metal ion through the oxygen atom is also confirmed by the frequency at 362 cm⁻¹.¹⁶

NMR Spectra (¹H and ¹³C)

The ¹H-NMR spectrum of the free Schiff base was recorded in DMSO-d₆ at room temperature and the relative peaks at 7.44 to 7.55 ppm, 7.64 to 7.66 ppm, 8.46 ppm, 8.09 ppm, 7.22-7.83 ppm, 8.73-8.80 shows the H₁, H₂, H₃, N-H₄, H₅ and H₆ respectively (Fig.-1) confirm the formation, Schiff.¹⁷ The ¹³C-NMR spec 135.39 ppm, 128.92 ppm, 129.39 ppm, 133.32 ppm, 144.10 ppm, 162.29 ppm, 140.77 ppm, 122.01 to 123.61 ppm, and 149-98 to 150.72 ppm, C₁, C₂, C₃, C₄, C₅, C₆, C₇, C₈, and C₉ (Fig.-2) respectively also confirm the formation of Schiff base by dehydration of condensation by Isoniazid and 4-chlorobenzaldehyde.¹⁸

Bio-potential Activity

The main motto of the synthesis of biologically active compounds is to inhibit the microorganisms without harmful to other biological cells. In-vitro antibacterial and antifungal activities of Schiff base and complex tested against *E. Coli* (bacterium), *A. Niger* (Fungus) by Agar disc diffusion method, the MIC (Fig.-3) values are predicted the results indicate the metal complex was found to have higher bio-potential comparing Schiff base due to reducing of polarity on complexation because of the partial sharing of positive charge with ligand and delocalization of π electrons in the chelates ring. The complexing ability favors the permeation which enhanced the rate of uptake of metal ions on the surface of the cell wall of microorganisms and also the metal ion disturbs the respiratory function which is blocking the protein synthesis and deactivate the enzyme.^{19, 20}

Antioxidant Activity

Antioxidant activity of Schiff base and its metal complex was carried out DPPH free radical scavenging method. The metal complex exhibits a strong electron-donating capacity than the ascorbic acid (Table-2). The IC₅₀ value of Schiff base and complex at 48.08 and 44.11 μ M/ml show higher activity against free radical DPPH than the ascorbic acid.

Fig.-1: ¹H-NMR Spectrum of (E)-N-(4-chlorobenzylidene)isonicotinohydrazide

Fig.-2: ¹³C-NMR Spectrum of (E)-N-(4-chlorobenzylidene)isonicotinohydrazide

Fig.-3: Antibacterial Disc of *E. coli* and *A. Niger*

Table-2: DPHH Activity of Schiff Base and V²⁺ Complex

Samples	% of Inhibitions				IC ₅₀ value (μg/ml)
	20 (μg/ml)	40 (μg/ml)	60 (μg/ml)	80 (μg/ml)	
Schiff base	19.54±1.36	40.90±2.86	65.01±4.55	82.72±5.79	48.08
Vanadium complex	21.36±1.49	46.37±3.24	73.18±5.12	84.54±5.91	44.11

Fig.-4: Antioxidant Activity of Schiff Base and Standard

Fig.-5: Antioxidant Activity of Vanadium(II) Complex and Standard

The result also concludes that the Schiff base and complex exhibit antiradical activity which favors the development of therapeutic agent (Fig.-4 and 5) used for the treatment of many chronic diseases viz., cardiovascular, cancer and neurodegenerative.²¹

Molecular Docking Studies

Molecular docking study was conducted using Auto Dock vina.²² The grid points in the X, Y and Z axes have been set. The grid core was positioned in the pocket core of the binding site. Protein and ligands have been translated to pdb, qt formats. Default docking algorithms have been set following the appropriate docking protocol. Individual docking procedures have been performed for each ligand-protein complex. The findings have been ranked in the order of rising docking energies. The lowest binding energy of each cluster was considered representative.²³ Docked complexes were further analyzed by using Pymol visualization.²⁴ To examine the binding affinity of newly synthesized Schiff base and Vanadium complex compounds with the antioxidant target enzyme cytochrome p450 (PDB code: 1og5), molecular docking studies have been carried out. With all amino acids inside the active site, the intermolecular interactions of the effectively docked conformations have been established. The binding energy and hydrogen bond information of both compounds with cytochrome p450 have been seen in Table-3.

Both compounds displayed good binding energy value with the target enzyme. The compound V^{2+} complex demonstrated greater antioxidant behavior than the compound Schiff base, depending on the scoring criteria. The synthesized compound KM showed the highest binding energy as -8 kcal/mol but the KML showed the binding energy of -5 kcal/mol. The VO(II) complex also formed the four-hydrogen bond interaction with cytochrome p450 through the amino acids' residues SER-343, ASP-349, GLU-400 and PHE-419 with a distance of 2.5, 2.5, 2.0 and 2.1. The compound Schiff base formed only one hydrogen bond interaction through GLU-400 at distance 2.5 Å (Fig.-6) but both the compounds interacted with GLU-400. So, this amino acid residue may play a key role in the antioxidant role of cytochrome p450 enzyme. It was confirmed that these two compounds may possess activity against the selected antioxidant enzyme

cytochrome p450 enzyme. Further experimentally analysis is needed to confirm the antioxidant activity of these two compounds.

Fig.-6: Molecular Docking of Schiff Base and VO(II) Complex against Cytochrome p450

Table-3: Molecular Docking Results of Synthesized Compounds with CP-450

S. No.	Compound Name	Binding Energy Kcal/mol	Details of Hydrogen Bond Interaction	Hydrogen Bond Distance Å°
1	Schiff Base	-8	SER-343	2.5
			ASP-349	2.5
			GLU-400	2.0
			PHE-419	2.1
2	VO(II) Complex	-5.2	GLU-400	2.5

CONCLUSION

The Schiff base and V^{2+} complex was synthesized by the green route method. They are characterized by various physicochemical, spectral and biological methods. The molecular formula, non-electrolytic, neutral and monomeric nature of the complex confirmed by molar conductance, magnetic moment and microanalytical data. Octahedral geometry and complexing ability confirmed by its IR, Far-IR and NMR spectral data. The complex is biologically active against tested microorganisms and they show antioxidant activity. The molecular docking study was also confirmed by the antioxidant activity of the complex have more potent than the standard.

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