

Synthesis of Schiff Base Complexes with Cu(II) , Ni (II) and Zn (II) and their Antimicrobial Activity

Abstract

A Schiff Base resulting from the interaction of cinnamaldehyde with p-anisidine forms Bidentate complexes with Cu (II), Ni (II), and Zn (II). Elemental analysis, molar conductance, magnetic susceptibility, IR, UV-Vis and ¹HNMR studies of the Schiff Base and its Complexes have been carried. All the resulting complexes were screened for antimicrobial activity by the well diffusion technique using Dimethyl Sulphoxide (DMSO) as the solvent at 35° C and for a period of 24 Hrs. The minimum inhibitory concentration (MIC) values were calculated. All the complexes have been found to be antimicrobially active and show higher activity than the free ligand against different bacterial strains like Staphylococcus aureus, Bacillus subtilis, Escherichia coli

Keywords: Schiff Base complexes, Ligand, Antimicrobial Activity, Minimum Inhibitory Concentration (MIC), Reflux.

Introduction

Schiff Bases are the compounds containing azomethine group (HC=N-) and are condensation products of aldehydes or ketones with primary amines (aromatic or aliphatic). In Schiff Base azomethine nitrogen and other donor atoms play a vital role in coordination chemistry. Their physical and chemical properties in various fields such as preparative uses, protection and determination of aldehydes or ketones, purification of carbonyl and amino compounds, sensitive reactions have been studied by various workers^{2,3}. Schiff base complexes are potentially capable of forming stable complexes with metal ions which show excellent catalytic activity (homogenous and heterogeneous) in various reactions at higher temperature and in the presence of moisture. Metal complexes of Co (II), Cu (II) and Zn (II) ions with Schiff Bases have played role in the development of coordination chemistry, as these complexes show binding properties with DNA and cleavage properties under physiological conditions. Schiff Bases have been found to appear as reaction intermediates in a number of enzymatic reactions.

Antibacterial and analgesic activity of Schiff Base metal complexes have been found⁴. These complexes can be used in foot printing studies as sequence specific DNA binding agents, as diagnostic agents in medicinal application and for genomic research. Schiff base compounds having (-C=N) linkages is essential for biological activity and their derivatives have been found to have potent antibacterial, antifungal, antimalarial and anticancer activities⁵⁻⁷. Schiff bases, formed by the condensation of aldehydes with p-substituted aromatic amines and their complexes have a variety of applications in biological and analytical fields⁸⁻¹⁰. Some drugs show increased activity when chelated with metals rather than as simple organic compounds^{8,9}. Much work has been done on the synthesis of transition metal(III) Schiff base complexes¹¹⁻¹⁸.

In our study, we have examined the synthesis of a new type of bidentate ligand formed by the condensation of cinnamaldehyde with p-anisidine. It has been found that the resulting Schiff base coordinated with metal (II) ions in a bidentate manner through the azomethine nitrogen and the conjugated ene of cinnamaldehyde. Keeping this in view, the biological activities of these synthesized Schiff base complexes were performed on different bacterial strains like Staphylococcus aureus, Bacillus subtilis, Escherichia coli. These complexes showed higher MIC values as compared to free ligands and were found to be very useful antibacterial agents.

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Material and Methods

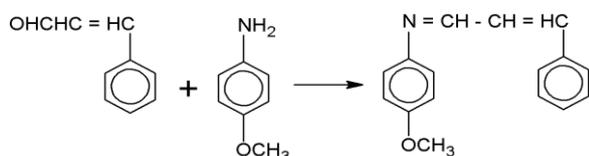
All the chemicals used in our studies, namely cinnamaldehyde, p-anisidine, Cu (II), Ni (II), and Zn (II) salts were of Himedia (AR grade).

Synthesis of Schiff base

p-anisidine (0.12 M, 1.48 g) was recrystallized and mixed with cinnamaldehyde (0.12 M, 1.53 ml) in equimolar ratio in an ethanolic medium. The mixture was stirred for 3 h and then refluxed for 2 h on a water bath in presence of few drops of piperidine as condensing agent¹⁹. The reaction mixture was cooled and the green crystals obtained were washed and separated by vacuum filtration. Yield: 75 % (mp: 80°C).

Figure A.

Reaction scheme for the formation of Schiff base.



Synthesis of complexes

Schiff base was mixed with metal (II) chloride in 2:1 ratio respectively in ethanolic medium, stirred for half an hour and then refluxed for 2 h on a water bath. The refluxed material on ice cooling gave solid product which was filtered washed with ethanol and water (9:1) and dried in dessicator.

Infrared spectra in the solid state were carried out in the 4000- 500 cm^{-1} range on a Nicolet 520-FTIR spectrophotometer (UB), and on a Perkin Elmer-337 spectrophotometer (UNR), using both the KBr pellets technique. The ^1H NMR spectra in CDCl_3 were performed on a Bruker Unity-300 spectrometer (UB) and on a Bruker AC-200 E (UNR) at 25 °C. UV-Vis spectra of all the complexes were recorded in DMSO on a Shimadzu UV-1601 spectrophotometer. Elemental analysis was determined using an Elementar Vario EL at the Regional Sophisticated Instr

umentation Centre, Punjab University, Chandigarh. Magnetic susceptibility measurements of the complexes in the solid state were determined by a Gouy balance at room temperature using mercury (II) tetrathiocyanatocobaltate (II) as the calibrant. The conductivity of the ethanolic solutions was measured at room temperature on a Horiba B-173 Conductivity Meter. These measurements suggested a nonelectrolytic nature for complexes.

Biological activity

All the complexes were investigated for their biological activity. MIC (lowest concentration of antibacterial agent showing complete inhibition of growth) of the compounds against various bacterial strains were performed. The in vitro biological screening effects of the investigated complexes were tested against the bacteria *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* by the well diffusion method using agar nutrient as the medium. Different solutions were prepared by dissolving the compounds in DMSO solution in the plate containing agar medium inoculated with bacteria, a well was made and filled with the test solution. The plates were incubated for 24 h. and at the temperature of 35°C. As the test solution diffused during this period, the growth of the inoculated bacteria was immensely affected.

Results and discussion

Elemental analysis, physical properties and magnetic susceptibility data of the complexes are given in Table A the general formula of all complexes is $\text{M}(\text{CINPA})_2$ the elemental analysis corresponds to the above mentioned general formula. The complexes show square-planar geometry around the central metal ions as is evident from the magnetic susceptibility values. The neutral nature of chelates is supported by very low conductance values.

		ELEMENTAL ANALYSIS				
		Calculated (Found) (%)				
Compound	Colour	M	C	H	N	μ_{eff} (BM)
CINPA	Green	—	80.98(80.87)	6.37(6.27)	5.91(5.68)	—
$\text{Cu}(\text{CINPA})_2$	Red .Green	11.55 (11.85)	71.69(70.89)	5.27(4.99)	5.23(5.01)	1.50
$\text{Ni}(\text{CINPA})_2$	Light Green	10.98(11.05)	72.34(71.99)	5.31(5.18)	5.27(4.97)	—
$\text{Zn}(\text{CINPA})_2$	Colourless	12.03(12.15)	71.45(71.16)	5.25(4.97)	5.21(4.99)	—

Table A Physical properties, Elemental analysis and magnetic susceptibility data of the complexes

IR spectra

The IR spectrum of the ligand indicates few important bands especially at 1623 cm^{-1} (for C=N bond) which shifts to 1640 cm^{-1} in the spectrum of the complex. This indicates the involvement of C=N (azomethine) nitrogen atom to the metal centre. The IR spectra of ligand shows the absence of bands in 1700-1638 cm^{-1} due to ν C=O stretching vibrations in combinations with the presence of two strong bands of almost equal intensity in the region of 1623-1597 cm^{-1} attributable to stretching vibrations of ν C=N indicates condensation of NH_2 group of amine with the $>\text{C}=\text{O}$

group of aldehydes leading to the formation of C=N linkage which is again proved by the absence of any band in the region 3550-3000 cm^{-1} assigned to stretching vibrations of NH_2 group of amine. The moderately intense band at 1630 cm^{-1} is consistent with the olefinic C=C stretching vibration. The absorptions at 1610, 1580, 1500 and 1450 are ring vibrations of benzene system. The absorptions at 750 cm^{-1} and 690 cm^{-1} indicates monosubstituted benzene ring. The metal complexes show new bands in the regions 480– 430 cm^{-1} and 400–330 cm^{-1} which are due to the formation of M–N and M–O bonds respectively (Table B).

Table B IR spectra of Metal Complexes.

CINPA	C=N (1623 cm ⁻¹)	C=C (1630 cm ⁻¹) (Stretching)	-----
M (CINPA) 2	C=N (1640 cm ⁻¹)	C=C (1633 cm ⁻¹) (Stretching)	M-N (480– 430 cm ⁻¹) M-O (400– 330 cm ⁻¹)

¹H NMR spectra

In the deuterated chloroform, the ¹H NMR spectrum of the ligand in CDCl₃ shows signals at 6.78(m,1H), 7.48-7.60(m,6H), 5.8(m,1H), 3.71(s,1H), 6.89-6.57(4H, unsymmetrical pattern). The peak at 9.73 (d) 3.4(s) due to -CHO and NH₂ groups is missing in the spectrum.

Electronic absorption spectra

The electronic absorption spectra of the Schiff base and its M (II) complexes were recorded at room temperature using DMSO as the solvent. The free ligand shows the band at 25,708 cm⁻¹ which is assigned as an intra-ligand charge transfer band (ILCT) in its electronic spectra. The square-planar geometry of the Cu (II) and Ni (II) complexes is proved by the appearance of d-d band in the region 21,878 cm⁻¹ due to 2 B_{1g} → 2A_{1g} transition and at 20,008 cm⁻¹ assigned to 1A_{1g} → 1B_{1g} transition respectively.

UV Visible spectra of the base shows the peak at 228 nm which is due to presence of C=N group and two large peaks at 294 and 390 which are due to the conjugation of C=C with C=N.

Molar conductance

The molar conductance of complexes in Dimethyl Formamide (DMF) and DMSO at 10⁻³ molar dilution shows non electrolytic nature²⁰ of the Complexes.

Magnetic susceptibility measurements

The magnetic moment of 1.52 BM which is lower than the spin only value proves the presence of one unpaired electron in the Cu (II) complex. Lower magnetic moments have been observed by many workers also^{21,22}. It is due to mixing of orbital angular momentum from excited state via spin-orbit coupling. The magnetic moment of Ni (II) complex is zero which again indicates the square-planar structure²³⁻²⁵ of the complex. The orbital contribution is completely quenched. There is anti ferromagnetic exchange coupling. A slight lowering may also be attributed to the presence of lower symmetry components and distortion from the idealized symmetry.

Biological activity

The antibacterial activity of the compounds [Cu-L], [Ni-L] and [Zn-L] was determined on the basis of Minimum Inhibitory concentration (MIC) values. All the compounds were active against both the Gram (+ive) as well as Gram (-ive) bacteria. Well diffusion method²⁷ was utilized to carry out the antibacterial activity^{28,29} of the ligand and its complexes against bacteria like S. aureus, E. coli and B. subtilis using agar medium as the course of nutrient. These studies reveal the higher activity of metal chelates³⁰ in comparison to free ligands which can be explained on

the basis of Overtone's concept and chelation theory³¹. Liposolubility is an important factor that controls antibacterial/antimicrobial activity because the lipid membrane that surrounds the cell favours the passage of only lipid soluble matter and this is the Overtone's concept of cell permeability. Due to the overlap of the ligand orbital with the metal orbitals and the slight sharing of positive charge of the metal ion with the donor groups on chelation, the polarity of the metal ion is reduced to a greater extent. Chelation further increases the delocalization of π-electrons over the whole chelate ring and enhances the lipophilicity of the metal complex. Thus metal binding sites on the enzymes of bacteria get blocked³² because of the increased lipophilicity which in turn enhances the penetration of the metal complexes into lipid membranes (Table C).

Table C

Antibacterial Activity (In Vitro) of Metal-Ligand Complexes against different Bacterial Strains.

COMPOUND	Bacterial Strain / ug ml ⁻¹		
	E.coli	B.Subtilis	S.Aureus
Ligand (L)	16	95	79
[Cu (L)]	14	67	38
[Ni (L)]	09	07	10
[Zn (L)]	13	16	19

Conclusions

The Schiff base synthesized by the condensation of cinnamaldehyde and p-anisidine and its complexes with Cu (II), Ni (II), and Zn (II) species were characterized by electrochemical and spectral data and magnetic studies. The resulting complexes have been found to have antimicrobial activities against both the Gram (+ive) as well as Gram (-ive) bacterias (Staphylococcus aureus, Bacillus subtilis and Escherichia coli). The order of activity is Ni-L > Zn-L > Cu-L. Ligand as well as M-L coplexes are more active against Gram (-ive) bacteria as compared to Gram (+ive) bacteria. The different structure and composition of the cell envelopes of the bacteria is the cause of different ability/activity.

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