



RESEARCH ARTICLE

Synthesis, Characterization and Biological Activity of a Schiff Base Derived from 3-Ethoxy Salicylaldehyde and 2-nitro Benzoic acid and its Metal Complexes

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ABSTRACT

In the present study an intermolecular reductive Schiff base formation from nitro derivative and benzaldehydes is carried out in the presence of iron powder and dilute acid. Schiff base has been synthesized from 3-ethoxy salicylaldehyde and 2-nitro benzoic acid. Metal complexes of the Schiff base were also prepared from salts of Ni (II), Co (II) Cu (II) and Zn (II) in an alcoholic medium. The chemical structures of the Schiff-base ligand and its metal complexes were confirmed by various spectroscopic studies. On the basis of elemental and spectral studies, six-coordinated geometry was assigned to these complexes. It is suggested that this ligand acts as neutral and tridentate and coordinates to each metal atom by azomethine nitrogen and oxygen atoms of hydroxyl group of the 3-ethoxy salicylaldehyde. The free Schiff base and its complexes have been tested for their antibacterial as well as antifungal activity by using disc diffusion method and the results discussed. The experimental results suggest that Schiff base derivatives are more potent in antibacterial and antifungal activities.

KEYWORDS

Schiff base, 3-ethoxy salicylaldehyde, 2-nitro benzoic acid, Metal complexes, Antibacterial activity, Antifungal activity

INTRODUCTION

Development of non-hazardous synthetic methodologies for organic synthesis is one of the latest challenges to organic chemists. The growing concern for the environment demands the development of eco-friendly and economic processes wherein even less hazardous byproducts are not desirable.

The formation of carbon–nitrogen double bond plays important role in organic synthesis. This can be achieved by the reaction of aldehydes and amines in acidic medium which leads to synthesis of Schiff bases (imines).

Schiff bases have attracted considerable attention of organic chemists due to their significant biological activities like anticancer⁴, antitumor⁵, anti-inflammatory agents⁶, insecticidal⁷, antibacterial⁸, antituberculosis⁹, antimicrobial¹⁰, anticonvulsant¹¹ activity. The Schiff bases are also used as versatile components in nucleophilic addition with organometallic reagents¹² and in cyclo addition reactions.^{13,14}

Schiff bases are typically formed by the condensation of a primary amine and an aldehyde.

The field of Schiff base complexes has been fast developing on account of the wide variety of possible structures for the ligands depending

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upon the aldehydes and amines. Schiff bases are considered as a very important class of organic compounds, which have wide applications in many biological aspects.¹⁵

Metal complexes of Schiff bases are one of the most adaptable and thoroughly studied systems. These complexes have also applications in clinical and analytical and industrial in addition to their important roles in catalysis and organic synthesis.¹⁶

Studies of a new kind of chemotherapeutic Schiff bases are now attracting the attention of biochemists.^{17,18} Schiff base metal complexes can now be considered a widely studied subject due to their industrial and biological applications.¹⁹ Earlier work reported that some drugs showed increased activity when administered as metal complexes rather than as organic compounds.^{20,21}

In view of these facts we can clear about that Schiff base are important not only in medical chemistry, but also in organic synthetic chemistry. Schiff base perhaps are synthesized in various method.

Traditional formation of Schiff bases from nitroarene starting materials requires a two-step process in which the nitroarene is first reduced to the aniline, then isolated, and subsequently condensed with the desired carbonyl. Recently, catalytic Schiff base formation from nitroarenes and carbonyls has been reported.^{22,23}

Tandem nitroarene reduction and intramolecular Schiff base condensation to give heteroarenes has been reported using iron in aqueous media. Surprisingly, the breadth of this methodology in an intermolecular application has not been previously reported.

Herein we wish to report our findings of a tandem iron reduction of nitroarenes and subsequent condensation of arylaldehydes under mild reaction conditions.

The present aim of the work is to synthesize a Schiff base derived from 3-ethoxy salicylaldehyde and o-nitro benzoic acid and to prepare its transition metal complexes,

characterize them and study their antibacterial and antifungal activities.

EXPERIMENTAL

Chemicals

All chemicals used in this work were reagent, including the metal salts i.e. Ni(NO₃)₂.6H₂O, CuCl₂.2H₂O, ZnCl₂, CoCl₂.6H₂O, Ethanol, 3-ethoxy salicylaldehyde and 2-nitro benzoic acid, Chloroform, DMSO, CaCl₂, NH₄OH.

Instruments

The percentage compositions of C, H, and N of complexes were determined by using micro analytical methods on Perkin Elmer 24°C (USA) elemental analyzer.

Infrared spectra of the ligand and its complexes were carried out by using KBr pellets in the range (4000-400 cm⁻¹) on Perkin Elmer Infra red model 337.

The electronic absorption was carried out by using a Shimadzu UV-1601 using DMSO as solvent. TGA studies were carried on Mettler Toledo Star system in the temperature range of 0-1000°C.

The Mass spectra were recorded by ESI technique on VG AUTOSPEC mass spectrometer instrument. The ¹H and ¹³C NMR spectra were recorded on Varian Gemini Unity Spectrometer by employing TMS as internal standard.

Melting points of the ligand and decomposition temperature of complexes were determined on Polmon instrument (model No.MP-96).

The Molar conductance measurements were carried out in DMSO (10⁻³ M) using Digisun Electronic Digital conductivity meter of model: DI-909 having a dip-type cell calibrated with KCl.

The Magnetic susceptibilities of complexes were determined on Gouy balance model 7550 at 23°C. The diamagnetic corrections were made by Pascal's constant and Hg[Co(SCN)₄] was used as a calibrant.

Biological Activity

The antimicrobial tests were performed by the standard disc diffusion method. The complexes were screened for their antifungal activity against fungi viz. *Aspergillus niger* and *Fusarium oxysporum*.

These fungal species were isolated from the infected parts of the host plants i.e. potato dextrose agar. The cultures of the fungi were purified by single spore isolation technique. A concentration of 1 mg/ml of each metal complex compound in DMSO solution was prepared for testing against spore germination of each fungus.

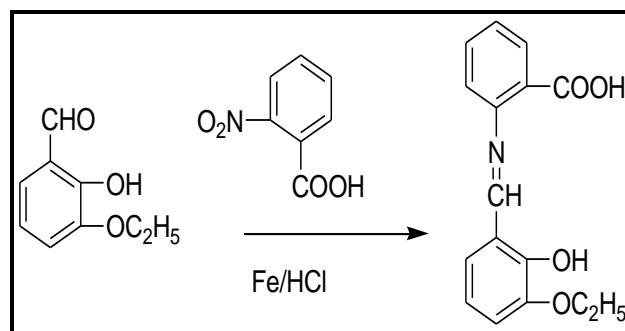
Filter paper discs of 5 mm in size, prepared by using Whatman filter paper no. 1 (sterilized in an autoclave) were saturated with 10 μ l of the metal complex compounds dissolved in DMSO solution or DMSO as negative control. The fungal culture plates were inoculated and incubated at $25 \pm 2^\circ\text{C}$ for 48 h. The plates were then observed and the diameters of the inhibition zones (in mm) were measured and tabulated.

The antibacterial activity of the complexes was studied against Gram-positive bacteria *Staphylococcus aureus* (MTCC 96) and Gram-negative bacteria *Escherichia coli* (MTCC 443).

Each of the metal complex compounds dissolved in DMSO at a concentration of 1 mg/ml was prepared. Paper discs of Whatman filter paper no. 1 were cut and sterilized in an autoclave. The paper discs were saturated with 10 μ l of the metal complex compounds dissolved in DMSO solution or DMSO as negative control and were placed aseptically in the Petri dishes containing Nutrient agar media inoculated with the above mentioned two bacteria separately. The petridishes were incubated at 37°C and the inhibition zones were recorded after 24 h of incubation.

Synthesis of Schiff base

The intermolecular reductive Schiff base formation of 3-ethoxy salicylaldehyde and 2-nitro benzoic acid in ethanol/water yields a single product according to following reaction:



Hydrochloric Acid (0.13 mL, 4.5 mmol) was added to a mixture of 2 nitro benzoic acid (1.20 gr, 0.72 mmol), 3-ethoxy salicylaldehyde (1.195g, 0.72 mmol), and iron powder (0.409 g, 7.32 mmol) in 24 mL of EtOH–H₂O (2:1 v/v) solution. The reaction was heated to 65°C for 1.5 h before being filtered while hot. The filtrate was extracted using CH₂Cl₂ (2 \times 25 mL) after which the organic layers were combined, dried over MgSO₄, filtered, and concentrated in vacuo to yield 1.69g (70%). The melting point of the resulting schiff base was found to be 142°C . The color of the product is red.

Preparation of the Metal Complexes

A mixture of the schiff base under investigation (0.01 mol) in 25 ml ethanol and the same amount of the same solvent of metal salt (0.01 mol) (MX₂, where M= Ni (II), Co (II), Cu (II) and Zn(II); X=Cl/NO₃/acetates) were refluxed for two hours at $70\text{--}80^\circ\text{C}$ on water bath. On cooling, colored solid product was collected by filtration and then washed several times with hot ethanol until the washing becomes colorless. The product was dried in air and stored in a desiccator over anhydrous CaCl₂ under vacuum. All the metal complexes are colored and stable to air and moisture.

RESULTS AND DISCUSSION

All the Schiff base metal complexes are stable at room temperature and are non-hygroscopic. Upon heating they decompose at high temperatures. The metal complexes are insoluble in water but are soluble in DMSO.

The analytical data and physical properties of Schiff base and metal complexes are given in table. It is clear from the data obtained;

Table 1: physico-analytical data of the Schiff base and Schiff base complexes

Compound	Empirical Formula Color	Elemental Analysis, calcd(found)%			Ω 1cm ⁻² mol ⁻¹	Melting Point	Mol Wt
		C	H	N			
Schiff base	C ₁₆ H ₅ NO ₄ red	67.34 (67.02)	5.58 (5.39)	5.04 (5.10)	--	142	285.0
Ni II complex	[NiC ₁₆ H ₂₁ NO ₈] Light green	46.69 (46.40)	5.19 (5.07)	3.45 (3.50)	5.8	>300	414.0
CoII complex	[CoC ₁₆ H ₂₁ NO ₈] Light orange	46.89 (46.78)	5.33 (5.19)	3.50 (3.46)	6.7	>300	415.0
CuII complex	[CuC ₁₆ H ₂₁ NO ₈] Dark green	46.05 (45.84)	5.14 (5.10)	3.63 (3.33)	10.8	260	418.5
ZnII complex	[ZnC ₁₆ H ₂₁ NO ₈] Off white	45.95 (45.55)	5.11 (5.00)	3.55 (3.28)	13.2	320	420.5

Table 2: Infrared spectral data of Schiff base and Schiff base metal complexes cm⁻¹

Compound	ν (C=N)	ν (C-O)	ν (C=O)	ν (M-O)	ν (M-N)	ν H ₂ O/OH
Schiff base	1622	1138	1692	--	---	3437
CoL ₂	1588	1363	1654	425	520	3430
NiL ₂	1556	1372	1586	418	562	3375
CuL ₂	1592	1327	1614	414	555	3445
ZnL ₂	1591	1326	1614	436	505	3430

the Schiff base metal complexes are in good agreement with the theoretical values calculated for 1:1 ratio.

The molar conductance of metal complexes are measured using 10⁻³ M in DMSO solvent suggests the presence of non-electrolytic nature and that no anions are present outside the coordination sphere.

Thermogram of Ni- complex indicated a total weight loss of 67% up to 1000°C, which is observed in three steps, (i) a small weight loss in the range of 40°C – 80°C which is assigned to loss of lattice water, (ii) maximum weight loss in the range of 390-450°C is attributable to the loss of coordinated water (iii) and gradual weight loss in the range of 540-1000°C can be

assigned to complete decomposition of ligand moiety around the metal ion respectively.

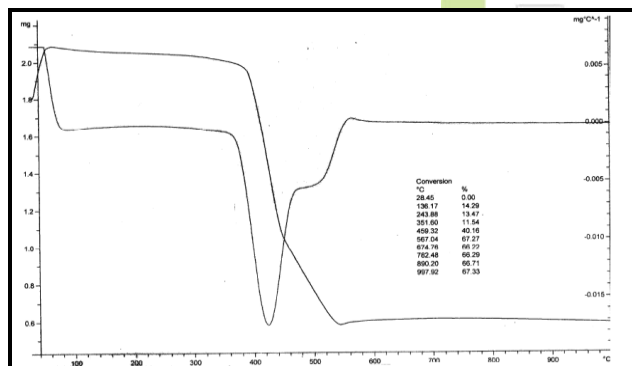
Finally the complex is converted into its metal oxide. The presence of water molecules is further confirmed by the endothermic bands observed in respective DTA curve in the temperature region where TGA curves indicate loss in weight.

The DTA curve of the nickel complex showed endothermic peaks at 80-85°C, a sharp peak at 430-435°C and 480-485°C and a small exothermic peak at 560-565°C which are attributed to phase transition accompanied by loss of water and decomposition of the metal complex.

Infrared Spectra of the Schiff Base and its Complexes

The IR spectra provide valuable information regarding the nature of functional group attached to the metal atom.

The main IR bands and their assignments are listed in Table 2.



The IR band assignments of all metal complexes exhibit broad bands in the range of 3354 to 3423 cm^{-1} indicating the presence of coordinated water molecules.

A band at 1622 cm^{-1} in free schiff base is due to $\nu\text{C} = \text{N}$ vibration. The shifting of this group to lower frequency (1592-1556 cm^{-1}) in the metal complexes when compared to free ligand, suggests the coordination of metal ion through nitrogen atom of azomethine group. It is expected metal atom would reduce the electron density in the azomethine link and thus lower the $-\text{HC}=\text{N}$ absorption.

A band at 1692 cm^{-1} is assigned to $\nu\text{C}=\text{O}$ stretching frequency in the spectrum of free schiff base which is also shifted to lower frequency range in cm^{-1} in all the metal complexes. This indicates the involvement of oxygen atom of hydroxy group of COOH group in bonding with metal ions.

New bands, which are not present in the spectrum of ligand appeared in the spectra of complexes in the range of 505-562 cm^{-1} , corresponding to $\nu\text{M}-\text{N}$ and 414-436 cm^{-1} to $\nu\text{M}-\text{O}$ vibrations support the involvement of N and O atoms in complexation with metal ions under investigation.

The $\nu\text{C}-\text{O}$ (Phenolic) stretching frequency of ligand is seen at 1380 cm^{-1} gets shifted to a lower frequency region in the complexes in the range of 1372-1326 cm^{-1} , and this is indication of bonding through phenolic oxygen.

Magnetic Moments and Electronic Spectral Data

The electronic absorption spectra of metal complexes were recorded in DMSO in the range of 200-1000 nm.

The electronic spectrum of free schiff base shows three bands at 237 nm (42194 cm^{-1}), 270 nm (37037 cm^{-1}) and 335nm (29850 cm^{-1}) suggesting the presence of $\pi-\pi^*$ transition.

The electronic spectrum of Ni(II) complex showed three bands at 16077 cm^{-1} , 26178 cm^{-1} and 32467-37735 cm^{-1} corresponding to the transitions $3\text{A}_{2g}(\text{F}) \rightarrow 3\text{T}_{2g}(\text{v}1)$, $3\text{A}_{2g}(\text{F}) \rightarrow 3\text{T}_{1g}(\text{F}) (\text{v}2)$ and $3\text{A}_{2g}(\text{F}) \rightarrow 3\text{T}_{1g}(\text{P}) (\text{v}3)$. The magnetic moment of Ni-complex was seen at 3.32 B.M within the range of 2.5-3.5 B.M for octahedral Ni(II)complexes.

The electronic spectrum of Co(II)-complex shows bands at 16286 cm^{-1} , 27027 cm^{-1} and 32051 cm^{-1} corresponding to the transitions $4\text{T}_{1g}(\text{F}) \rightarrow 4\text{T}_{2g}(\text{F}) (\text{v}1)$, $4\text{T}_{1g}(\text{F}) \rightarrow 4\text{A}_{2g}(\text{F}) (\text{v}1)$ and $4\text{T}_{1g}(\text{F}) \rightarrow 4\text{T}_{1g}(\text{P}) (\text{v}3)$. The magnetic moment of Co(II) complex was seen at 5.01 B.M with in the expected range of 4.7-5.2B.M for octahedral geometry.

The electronic spectrum of Cu(II) complex display three prominent bands. A low intensity band at 16583cm^{-1} is assignable to $2T_2g \leftarrow 2E_g$ transition. Another high intensity band in the region $24,096\text{-}32258\text{ cm}^{-1}$ is due to symmetry forbidden ligand \rightarrow metal charge transfer and sharp bands observed at 38461cm^{-1} which is due to ligand bands.

The magnetic moment of Cu(II) complex was seen at 1.61 B.M. corresponding to one unpaired electron with a slight orbital contribution to the spin-only-value and the absence of spin-spin interactions.

On the basis of electronic spectra and magnetic susceptibility measurements, a distorted octahedral geometry around Cu(II) is suggested. The Zn(II) complex shows no d-d bands as is expected for a d^{10} system and was found to be diamagnetic in nature.

On the basis of analytical, conductance and spectral datas, Zn(II) complex was assigned an octahedral geometry.

Mass Spectra

The Ligand [L] shows a molecular ion peak at m/z 286, which corresponds to [L+H] peak as the calculated m/z being 285. The mass spectra of Ni(II) complex shows a molecular ion peak at $m/z = 414$ and Co(II) complex shows a peak at $m/z = 415$ which corresponds to molecular weight of the respective compounds while that of Cu(II) complex shows $m/z=420$ and Zn (II) complex at $m/z=422.5$ which corresponds $[M^{+1}]$ and $[M^{+2}]$ peaks respectively. These peaks support to the structure of the complexes and confirm the stoichiometry of metal chelates as ML type.

¹H -NMR Spectra Data

The ¹H NMR spectrum of the ligand in CDCl_3 shows the following signals given in Table 5. The phenyl multiplet is seen at 6.5 - 7.3 δ and the azomethine proton is seen at 7.6 δ (singlet). The peak at 10.8 δ is attributed to phenolic OH group present in the ligand. The peak at 1.5 δ , which is a triplet is assigned to CH_3 group of ethoxy substituent on the benzene ring while

peak at 4.1 δ , which is a quartet, is attributed to $-\text{CH}_2$ protons of the ethoxy substituent

Antibacterial Activity

The *invitro* antibacterial activity of the ligand and its complexes have been carried out against the gram -ve *Escherischia.Coli* and gram positive *staphylococcus aureus* using disc diffusion method by taking DMSO as solvent.

A comparative study of the growth inhibition zone values of schiff base and its complexes indicate that metal complexes exhibit higher antibacterial activity than the free ligand.

This is probably due the greater lipophilic nature of the complexes. Such increased activity of the metal chelates can be explained on the basis of Overtone's concept and Tweedy's chelation theory. According to Overtone's concept of cell permeability, the lipid membrane that surrounds the cell favors the passage of only lipid soluble materials due to which liposolubility is considered to be an important factor that controls the antimicrobial activity.

On chelation, the polarity of the metal ion will be reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of positive charge of metal ion with donor groups.^{29,30} Further, it increases the delocalization of the π electrons over the whole chelate ring and enhances the lipophilicity of the complex. This increased lipophilicity enhances the penetration of the complexes into lipid membrane and thus blocks the metal binding sites on enzymes of microorganisms. These metal complexes also disturb the respiration process of the cell and thus block the synthesis of proteins, which restricts further growth of the organism

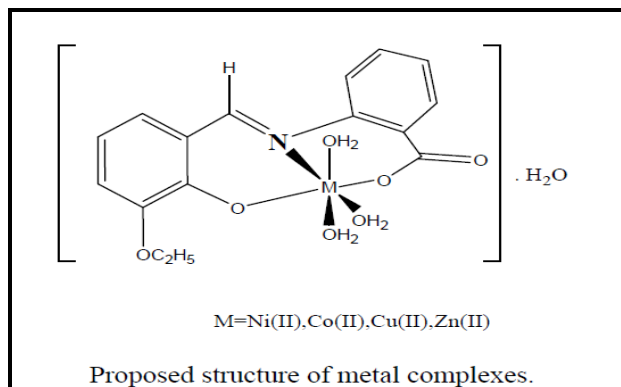
The results indicate that copper complex shows highest activity against *E.Coli* while cobalt complex shows maximum inhibition zone against *S.aureus* at 1 mg/ml.

Antifungal Activity

The antifungal activities of the ligand and its metal complexes were tested against seven-day old cultures of *Aspergillus niger* and *Fusarium*

oxysporum using disc diffusion method. The results show that the metal complexes were more active than the free ligand and can be explained by Overtone's concept and Tweedy's chelation theory.

The experimental results of the compounds are expressed as inhibition zone diameter (in mm). Copper complex shows the highest activity (i.e. 14 mm against *A. niger* and 22 mm against *Fusarium oxysporum* 1 mg/ml).



CONCLUSION

Schiff base have been prepared by a simple and environmentally friendly reductive imination procedure. This process tolerates various functional groups and often proceeds quantitatively with no need for purification. This methodology uses only Fe powder in acidic EtOH/H₂O as a reducing agent for nitro derivatives which upon reduction spontaneously condense with an aldehyde in situ.

The Schiff base ligand and its metal complexes of Ni(II), Co(II) Cu(II) and Zn(II) have been structurally characterized. The analytical data show that the metal ligand stoichiometry in all these complexes is 1:1. All the complexes are non-electrolytes in DMSO solvent. Based on analytical, molar conductance, magnetic and spectral data all these metal complexes are assigned in octahedral geometry. Biological studies of the synthesized Schiff base metal complexes are showed better activity compared to free Schiff base.

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