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Synthesis, Spectral Characterization, Fluorescence Studies and Antimicrobial Studies of some Novel Schiff base Complexes Derived from E-4-(2-Hydroxy-3-Methoxybenzlideneamino) -N-(thiazol-2-yl) Benzenesulfonamide

KEYWORDS: Schiff base, 2-sulphanilamidothiazole, 2-hydroxy-3-methoxybenzaldehyde, Metal complexes, Fluorescent behaviour, Antimicrobial activity.

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ABSTRACT *New Schiff base has been prepared from 2-sulphanilamidothiazole and 2-hydroxy-3-methoxy benzaldehyde in ethanolic media and then complexed with Mn(II), Co(II), Ni(II), Cu(II) and Zn(II) ions. The synthesized ligand and complexes were characterised by Elemental analysis, Molar conductance ,Magnetic susceptibility, IR, UV, 1H & 13C NMR, EPR and Fluorescence studies.The spectral data of the complexes have revealed bidentate complexing nature of the Schiff base ligand through phenolic oxygen and azomethine nitrogen atoms. The Schiff base ligand and the complexes were screened for antimicrobial activity and fluorescent behaviour. From the analytical and spectral data, the stoichiometry has been found to be 1:2 for all the complexes. An octahedral structure has been proposed. All the new complexes were found to be active against bacteria and fungi.*

INTRODUCTION

Sulpha drugs which are derivatives of a compound p-aminobenzenesulfonamide commonly known as sulfanilamide, were the first effective chemotherapeutic agents widely used for the cure of bacterial infections in humans. They are termed as sulfonamides, due to the presence of sulfonamide group ($-SO_2NH_2$). Many sulpha drugs like sulphapyridine, sulphathiazoel, sulphamethoxazole possess SO_2NH moiety as an important toxophoric function[1]. The direct use of transition metal salts as antimicrobial agents cannot be recommended as they are very toxic to host animals. It has been reported that the metal complexes showed greater activity compared with free ligands[2]. Transition metal complexes of these ligands are developed due to their chelating capability, structural flexibility, electrical as well as magnetic properties[3]. Sulpha drugs are metabolic inhibitors of folic acid in microorganisms. The drug combination of sulphamethoxazole-trimethoprim is an antimicrobial agent that is frequently used for prophyl axis to prevent pneumocystis carionii pneumonia in AIDS patients and in other immunocompromised individuals[4]. Sulfonamide derivatives are found to be anti-angiogenic, antitumour, anti-inflammatory and anti-tubercular agents [5-7]. In addition, the chemotherapeutic usefulness of sulfa drugs against infections caused by fungus paracoccidioides brasiliensis has been reported[8].

The interest in metal based sulfonamides was stimulated by the successful introduction and preparation of Ag(I) and Zn(II) sulphathiazole and sulphadiazine complexes to prevent various bacterial infections.[9]

As part of our investigation into designing new chelating agents from biologically active compounds, we are interested in synthesizing the Schiff base ligand derived from 2-sulphanilmidothiazole and 2-hydroxy-3-methoxybenzaldehyde and its metal complexes. It is aimed to characterize the complexes by analytical and spectral studies to propose a suitable structure. The complexes are subjected to biological studies also. The ligand and its metal complexes were characterized by Elemental analysis, Molar conductance and Magnetic susceptibility measurements, IR, UV, ^1H& ^{13}C NMR and ESR. The biological activities are also studied against gram positive and gram negative bacterial and fungi organisms for Schiff base ligand and its complexes. The structure of Schiff base ligand proposed in the present work is given in Figure 1

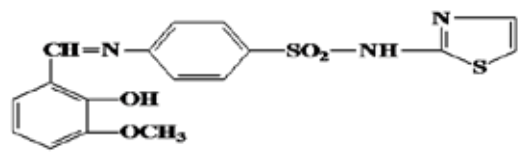

Figure 1 Structure of the ligand

EXPERIMENTAL

All the chemicals used were of analytical reagent grade (AR) and of highest purity available . Solvents were purified and dried according to the standard procedures. All metal (II) compounds were used as acetate salts. IR spectra of the complexes were recorded in KBr pellets with a Perkin Elmer RX1 FT-IR Spectrophotometer in the 4000-400cm^{-1} range. The electronic spectra were recorded in DMF on a Perkin Elmer Lambda 35 spectrophotometer in the 190-1100 nm range. The ^1H& ^{13}C NMR spectra were recorded on a Bruker 400MHz FT-PMR spectrometer (DMSO-d_6). Elemental analysis of the ligand and complexes were obtained using Elementar Vario EL CHN rapid analyser. The X-band EPR Spectrum were recorded on a Bruker ESP X-band EPR Spectrometer using powdered samples at a microwave frequency 9450MHz. Magnetic susceptibilities were measured on a Automagnetic Susceptibility meter (MSB-Auto) at room temperature. Melting points were determined using melting point apparatus (Elico) and are uncorrected. Conductivity measurements for the complexes were carried out on Elico Conductivity Bridge and a dip conductivity cell using dimethyl formamide as solvent. Fluorescence spectra of the complexes were recorded using Perkin Elmer LS 45 Spectrofluorometer.

Synthesis of Schiff base ligand :

(L) The Schiff base was prepared by the condensation of equimolar amounts of 2-sulphanilamidothiazole and 2-hydroxy-3-methoxy benzaldehyde in minimum quantity of ethanol. The resulting mixture was then refluxed on a water bath for 5 hours. A pale orange coloured solid mass separated out on cooling is filtered , washed and dried over anhydrous $CaCl_2$ in a desiccator. The purity of the ligand was checked by melting point, TLC and spectral data. The ligand is insoluble in some common organic solvents viz.acetone, benzene and soluble in polar solvents viz.DMF, DMSO.

Synthesis of metal complexes:

Metal complexes were synthesized by mixing the hot solu-

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tion of ligand (0.004 mole) in minimum quantity of dimethyl formamide and ethanolic solution of metal acetates (0.002 mole). The resulting mixture was then refluxed in a water bath for 6 hours. The complexes obtained in each case were cooled, filtered and washed with ethanol several times to remove any excess of the ligand. Finally the complexes were washed with anhydrous diethylether and dried in a desiccator.

RESULTS AND DISCUSSION

The Schiff base ligand is synthesized by using equimolar quantities of 2-sulphanilamidothiazole and 2-hydroxy-3-methoxy benzaldehyde and is complexed with metal acetates according to the following equations

$$M(CH_3COO)_2.nH_2O + 2L \xrightarrow[\text{reflux}]{\text{ethanol/DMF}} ML_{2-2H}(H_2O)_2 + 2CH_3COOH + nH_2O$$

reflux

The metal complexes derived vary in their colour. All the complexes are stable, non-hygroscopic and coloured solids. The results of the elemental analysis(Table 1) of the Schiff base and their complexes are in good agreement with those calculated for the suggested formula and agree with a 1:2 metal to ligand stoichiometry for all the complexes.

TABLE 1
Physical Characteristics and Microanalytical data of Schiff base ligand and their complexes

S.No	Ligand/ Complexes	Colour	Molecular Formula	M.P ◦C	Yield %	Elemental Analysis (%) (Calcd)found C	H	N	S	CN	Λ_m ohm^{-1}m^2 mol^{-1}
1	L	orange	$C_{17}H_{15}N_3O_4S_2$	160	75	(52.44) 52.40	(3.85) 3.80	(10.70) 10.68	(16.45) 16.43	-	-
2	$[ML_2(H_2O)_2]$	Pale yellow	$C_{34}H_{32}N_6O_{10}S_4Mn$	220	60	(47.06) 46.08	(3.69) 3.70	(9.68) 9.59	(14.76) 14.37	6	2.60
3	$[CoL_2(H_2O)_2]$	Dark brown	$C_{34}H_{32}N_6O10S_4Co$	260	65	(46.84) 46.80	(3.67) 3.67	(9.64) 9.62	(14.69) 14.68	6	2.37
4	$[NiL_2(H_2O)_2]$	Yellow	$C_{34}H_{32}N_6O_{10}S_4Ni$	270	70	(46.85) 46.84	(3.67) 3.65	(9.64) 9.62	(14.69) 14.67	6	2.94
5	$[CuL_2(H_2O)_2]$	Dark brown	$C_{34}H_{32}N_6O_{10}S_4Cu$	230	70	(46.59) 46.56	(3.65) 3.55	(9.59) 9.45	(14.61) 14.57	6	2.37
6	$[ZnL_2(H_2O)_2]$	Pale yellow	$C_{34}H_{32}N_6O_{10}S_4Zn$	275	60	(46.50) 46.48	(3.64) 3.58	(9.57) 9.54	(14.58) 14.49	6	6.02

Molar Conductance and Magnetic Susceptibility Measurements:

The observed molar conductances of all the complexes in 10^{-3}M DMF solution are found within the range of 2.0-9.0 ohm^{-1}cm^{2}mol^{-1} showing their non-electrolytic nature. The magnetic data for Co ^{II} and Ni ^{II} complexes is consistent with octahedral geometry around the metal ion for both the complexes. The magnetic moment value of 1.92 BM for Cu (II) complex lies in the range expected for d^9 system which contains one unpaired electron with octahedral geometry [10]. Zn (II) Complexes are found to be diamagnetic as expected. The observed magnetic moment value of 5.85BM for the Mn (II) complex suggests the octahedral geometry.

Infrared Spectra:

The structurally significant IR bands for free ligand and its complexes have been reported in Table 2. Schiff base showed a strong absorption band at 1562 cm^{-1} characteristic of ν (C=N) whereas the broad band at 3433 cm^{-1} characteristic of hydrogen bonded ν(O-H) stretching vibration[12]. The azomethine ν(>C=N) band at 1562 cm^{-1} in Schiff base is shifted to higher frequency in Mn(II),Co(II),Ni(II),Cu(II), and Zn(II) by 55,65,56,44and48cm^{-1}respectively which indicated the co-ordination of azomethine nitrogen on complexation[13] .The shifting of phenolic (OH) at 3433 cm^{-1} in all the complexes suggests the coordination of phenolic xygen after deprotonation[14]. The linkage with oxygen atom is further supported by the appearance of a band in the region around 420-464 cm^{-1} which may be assigned to ν (M-O)[15]. A further evidence of the coordination of the N atom of the Schiff base with the metal atom was shown by the appearance of a new weak frequency band at 512-578cm^{-1} assigned to the metal nitrogen ν(M-N)[16]. These new bands were observed only in the spectra of the metal complexes and not in Schiff base which confirmed the participation of the donor groups. The band in the ligand and in the complexes almost remain unchanged indicating that this –SO_2 group is not participating in coordination. This is confirmed by the unchanged ν(S-N) and ν(C-S) modes appearing around 941cm^{-1} and 860cm^{-1} respectively. The ring nitrogen (=N-) of the Schiff base does not take part in coordination, supported by unchanged band around 1250 cm^{-1}.

TABLE 2
IR and Electronic spectral data

Ligand/ Complexes	IR spectral data , cm^{-1} γ(O-H)	γ(C=N)	γ(M-N)	γ(M-O)	Electronic spectral data, cm^{-1}
L	3433	1562	-	-	42,105 and 39,521
$[ML_2(H_2O)_2]$	3371	1617	567	470	34,866
$[CoL_2(H_2O)_2]$	3441	1627	556	472	37,717 and 36,523
$[NiL_2(H_2O)_2]$	3443	1618	546	466	36,358
$[CuL_2(H_2O)_2]$	3442	1606	550	476	38,201 and 34,607
$[ZnL_2(H_2O)_2]$	3366	1610	551	466	34,140 and 33,612

^{1}H &^{13}C NMR Spectra:

The ^{1}H NMR Spectra of Schiff base and its complexes were recorded in DMSO (d_6). The azomethine proton (-CH=N-) in Schiff base appeared at δ = 8.9 ppm has been shifted to downfield in metal complexes. This confirms the coordination by azomethine nitrogen [17]. The aromatic protons in Schiff base appeared in the range at δ 6.55 to 7.86 ppm and metal complexes in the range δ 6.39 to 8.61 ppm. The disappearance of phenolic –OH proton signal at δ 12.76 ppm confirms the coordination by phenolic oxygen to metal ion[18]. The ^{13}C-nmr spectral data (imine at δ 165.1 ppm, aromatic C-OH at δ 123.83-127.85 ppm) for ligand supports the proposed structure.

Electronic Spectra:

The electronic spectra and magnetic moment of the ligand and its metal complexes are listed in Table 2. Electronic spectrum of the ligand shows two high intensity bands at 39,521cm^{-1} and 42,105cm^{-1} indicate $\pi \rightarrow \pi^*$ and $n \rightarrow n^*$ transitions respectively of the ligand moiety[19]. The electronic spectrum of the Mn(II) complex shows a band at 34866 cm^{-1} assignable to $^6A_{1g} \rightarrow {}^4E_{g(D)}$ [20]. The electronic spectra of Co(II) complex displays bands at 37,717 and 36523 cm^{-1}. The first band corresponds to intra ligand transition of the organic moiety and the latter corresponds to $^4T_{1g}$ (F) $\rightarrow$ $^4T_{1g}$(P) suggesting octahedral geometry of this complex[21]. Ni(II) complex shows absorption band at 36358 cm^{-1} which is due to

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$^3A_{2g} \rightarrow {}^3T_{2g}$ .The Cu(II) complex displays two bands at 38201 and 34,607cm^{-1}. The first band is attributed to intra ligand transition and the second band corresponds $^2B_{1g} \rightarrow {}^2E_g$ [22]. Zn(II) complex displays high intensity bands at 34140 and 33612 cm^{-1}. This may be due to Ligand → Metal charge transfer spectra[23]. The probable structure of complexes proposed in the present work is given in Figure 2.

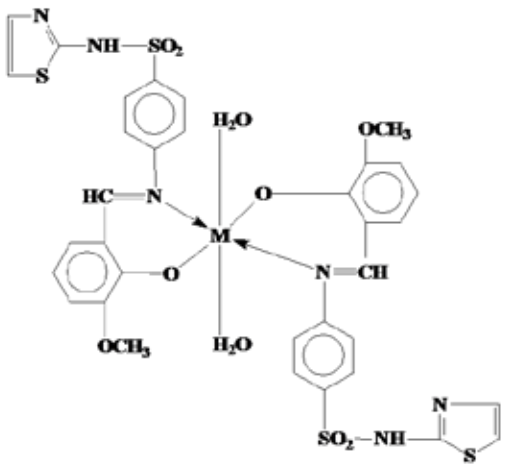

Figure 2 Structure of the Complex

EPR Spectra:

A powder ESR spectrum of Cu(II) complex were recorded at room temperature at 9780MHz. Both parallel and perpendicular features of Cu are resolved in the spectra, which are characteristic of axial symmetry. The g value of Cu(II) complex is found to be 2.0095 confirms the presence of unpaired electron in the d_{x2-y2} orbital of Cu(II).The g value are very close to those reported for a number of distorted Cu(II) complex. Moreover, the observed g value is less than 2.3, suggests the covalent nature of metal-ligand bonds in the complex. The lines of this type usually observed are either due to the intermolecular spin exchange, which may broaden the lines or to the occupancy of the unpaired electron in the degenerate orbital. The nature and pattern of the EPR spectra (Figure 3) suggests an almost octahedral environment[24] around the Cu(II) complex.

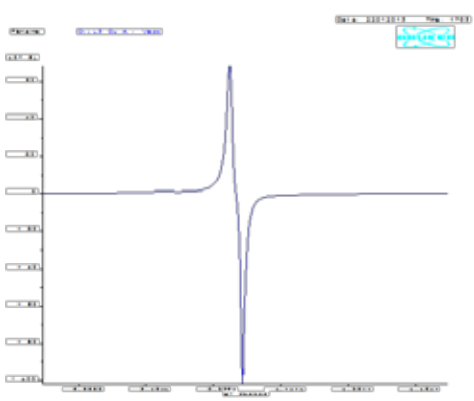

Figure 3 EPR Spectra for Cu(II) complex

Fluorescence Spectra:

The Photoluminescence properties of the azo Schiff base ligand and their complexes were studied at room temperature for 10^{-4} M solution for all compounds in DMSO solution. Excitation and emission slit widths were set at 10nm with a scan speed of 500nm/min. The excitiation spectra of the ligand show a maximum at 253nm and show an emission peak at 513nm. Generally azo Schiff base systems exhibit fluorescence due to intraligand $\pi \rightarrow \pi^*$ transitions. The fluorescent data are summarized in Table 3.

TABLE 3
Fluorescence Characteristic of ligand and its complexes

Complexes	Excitation Wavelength λ_{max}(nm)	Fluorescence wavelength λ_{max}(nm)	Quantum Yield
L	253	513	0.49
$[MnL_2(H_2O)_2]$	284	285	0.99
$[CoL_2(H_2O)_2]$	273	274	0.99
$[NiL_2(H_2O)_2]$	285	289	0.98
$[CuL_2(H_2O)_2]$	287	288	0.99
$[ZnL_2(H_2O)_2]$	290	291	0.99

Antibacterial activity:

The newly synthesized Schiff base ligand and its metal complexes were screened invitro for their antibacterial activity against two gram positive bacteria staphylococcus aureus(NCLM 2079), Klebsiella aerogenes (NCLM 2098) and two gram negative E.coli (NCLM 2065), pseudomonas aeruginosa (NCLM 2036) bacterial strains by disc diffusion technique as reported in our previous paper[25]. DMSO was used as a negative control whereas Ciprofloxacin was used as positive control.

Antifungal activity:

The newly synthesized Schiff base ligand and its metal complexes were screened invitro for their antibacterial activity against two fungi aspergillus niger (NCLM 105) and Mucor (NCLM 108). DMSO was used as a negative control whereas Nystatin was used as positive control.

All the bacterial and fungus cultures were procured from National Chemical Laboratory, Pune and subcultured on nutrient agar. Filter paper discs of diameter 6mm were used and the diameters of zones of inhibition formed around each disc after incubating for a period of 72 hours at 25-30°C were recorded. All the new complexes showed a remarkable biological activity against bacteria and fungus (Table 4). The activity order of the synthesized compounds can be represented as Cu(II) complex>Mn(II) complex>Co(II) complex>Zn(II) complex>Ni(II) complex >ligand

TABLE 4
Antimicrobial Activity of Schiff base ligand and complexes

Antimicrobial activity of the ligand and complexes	Staphylococcus aureus	Klebsiella aerogenes	E.coli	Pseudomonas aeruginosa	Mucor	Aspergillus niger
Ligand (L)	++	+++	+++	+++	+++	+++
$[MnL_2(H_2O)_2]$	++	+++	+++	+++	+++	+++
$[CoL_2(H_2O)_2]$	++	+++	+++	+++	+++	+++
$[NiL_2(H_2O)_2]$	++	+++	+++	+++	+++	+++
$[CuL_2(H_2O)_2]$	+++	+++	+++	+++	+++	+++
$[ZnL_2(H_2O)_2]$	+++	+++	+++	+++	+++_	+++

Standard= ciprofloxacin 5 g/ disc for bacteria ; Nystatin= 100 units/disc for fungi.

Highly active = +++ (inhibition zone > 15mm) ; Moderatively active = ++ (inhibition zone> 10mm) ; slightly active = + (inhibition zone >5mm); Inactive = -- (inhibition zone <5mm)The higher activity of the metal complexes is

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due to effect of metal ions on the normal cell membrane. From the results it is clear that the metal complexes are found to have more antimicrobial activity than the parent ligand[26].

Conclusion :

The coordination ability of the newly synthesized Schiff base has been proved in complexation reaction with Mn(II), Co(II), Ni(II), Cu(II) and Zn(II) ions. IR, UV, ^{1}H NMR and magnetic measurements of the ligand and its complexes confirms the suggested coordination of the ligand through azomethine linkage. Based on these facts, an octahedral structure has been proposed for all complexes. The process of chelation dominantly affects the biological activity of the complexes that are potent against pathogens. In general, all the synthesized complexes can serve as potential photoactive materials, as indicated from their characteristic fluorescence properties.

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