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A Novel Schiff base ligands of isoxazole and their Co(II), Ni(II), Cu(II) and Zn(II) complexes: synthesis, spectroscopic characterization, studies on thermal behavior and biological activity.

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Abstract:

Schiff bases are excellent ligands which are synthesized from the condensation of primary amine with carbonyl group. The Co(II), Ni(II), Cu(II) and Zn(II) metal complexes have been synthesized with isoxazole Schiff bases. A tetra dentate Schiff ligand{4-[(5-methylisoxazol-3yl) Sulfonyl Benzyli dine]-N-[(E)-1-(2-Pyrrol)methylidine)amine [MSBPMA] (L_1) has been synthesized via the reaction of sulphametoxazole with stiochimetric amount of pyrrole 2-carbaldehyde in ethanol solution. Ligand {N-[(E)-1H-Imidazol-3yl)-N-[(Imino metylidine)-(3-Hydroxy-3-Isoxazol)]}[NINIHI] (L_2) was prepared by reaction of histamine with 3-hydroxy 5-carbaldehyde Isoxazole in absolute methanol and ligand N-[(3-hydroxy-3-Isoxazol) Imino] methyl 4-oxo-4H Chromine}[NHIIMC] (L_3) on the other hand, was obtained through a reaction between muscinol and Chromine 3-Carbaldehyde in hot ethanolic solution. The ligands and their metal complexes were well characterized by the elemental analysis, FT IR, ^{1}H-NMR, UV-Visible spectra, Mass spectral data and molar conductance. Thermal behaviors of all compounds were investigated from room temperature to 800^{o}C with heating rate of 10^{o}C /minute, and also biological studies, the results exhibited suitable antibacterial (Pseudomonas and Bacillus) and antifungal (Angier and R.solani) properties for ligands and metal complexes. The study has shown that the complexation of ligands to metal ion center to enhancement of antibacterial /anti fungal activity.

Key words: Schiff bases, metal chelate, histamine, muscinol, antimicrobial acvitivity.

1. Introduction

Schiff bases also known as azomenthine compounds are the product of a condensation reaction of an aldehyde and amine. Schiff bases are as important chelating ligand can be and liked to metal ions via azomethine nitrogen atom and other probable coordination groups and consequently they are able to stabilized the metal ions in various oxidation states in the various heterocyclic Schiff base having O, N and S donor atoms have been reported by several scientist [1]. The isoxazole Schiff base complex have been investigated extensively for last several decades leading to new synthesis and the wide application of such as in pharmaceutical as well as agrochemical industry, nitrogen-containing heterocyclic building blocks are of great importance to both medical and organic chemists recently, Schiff base and especially some of their coordination compounds have attracted considerable attention because of their usage in catalysis reaction such as mediated oxygenation of olefin[2], reduction of aromatic compounds [3], water photolysis [4] and etc. Tetra dentate 3-amino 5-methyl Isoxazole schiff bases with a N_2, O_2 donor atom set are known to coordinate with metal ions, and this has attracted many researchers [5]. In the present investigation we report here synthesis spectroscopic characterization studies on thermal behavior and biological activity of MSBPMA (L_1), NINIHI (L_2) and NHIIMC (L_3) ligands and their metals complexes.

2. Experimental

2.1 material and spectral measurement

All chemicals and solvent used were of analytical grade. Elemental analysis was carried out on Elementar Vario ELII.Conductance measurement of 0.001M solution of the complexes in DMSO was carried out on an equiptronic models no Eq.660A. IR spectrum was recorded on FTIR Spectrophotometer in the range of 400-4000cm^{-1}. purity of the synthesis compounds was checked by Thin-layer chromatography (TLC) glass plates are coated with silica gel 60F were used. Whereas the mass spectrum of these ligands were obtained using a VG-707Emass spectrometer the elections impact excitation was 70eV. The weight loss and thermal behavior of the sample are investigated by thermo gravimetric analysis (TGA) and differential scanning calorimetric (DSC) performed on a TA instrument Q600SDT.Sample, heated at a rate of 10^0C/min from room temp 800^0C.

2.2.1. Preparation of the ligand (L_1) {MSBPMA}

The ligand of MSBPMA {4-[(5-metylisoxazol-3yl) Sulfonyl Benzyl dine]-N-[(E)-1-(2-Pyrrol)methylidine)amine has been synthesized by refluxing the reaction mixture of ethanol solution (30ml) of sulphametoxazole (0.01mol) with ethanol solution (30ml) of pyrrole 2-carbaldelyde (0.01mol)For about 2-3.hours at 60-70^0C the resulting solution was concentrated up to 10 ml on a water bath the mixture was allowed to stand overnight. After that the colored solid product was dried under reduced pressure over anhydrous $CaCl_2$.That purity of synthesized compound was monitored by TLC using silica gel-G.

2.2.2. Preparation of the ligaad (L_2) [NINIHI].

The ligand of NINIHI{N-[(E)-1H-Imidazol-3yl)-N-[(Imino metylidine)-(3-Hydroxy-3-Isoxazol)]}has been synthesized by refluxing the reaction mixture of 3-hydroxy 5-carbaldehyde isoxazole (0.1 mol) in 20 ml methanol and adds to the histamine (0.1 mol) dissolved in 20 ml methanol than the reaction was refluxed for 4 hrs at 50-60^0C. After complete refluxation of Schiff base was separated out on removal of the solvent at room temperature. A light yellowish crystalline solid obtained and than dried over anhydrous $CaCl_2$ in vacuum.

2.2.3. Preparation of the ligands (L_3) [NHIIMC].

The ligand NHIIMC {N-[(3-hydroxy-3-Isoxazol) Imino] methyl 4-oxo-4H Chromine} was prepared by modification of the reported method [6,7]. A solution of 0.1mol muscinol (dissolved in 20 ml ethanol) was slowly added to a solution of 0.1 mole of Chromine 2-carbaldehyde (4-oxo-4H Chromine 3-carbaldehyde) (in 20 ml ethanol).After stirring the reaction mixture for 30 min, than the mixture of the above solution was refluxed for about 4-5 hrs at 60-70^0C. The resulting solution was concentrated up to 10ml on water bath. The mixture was allowed to stand overnight after that the solid product was filtrated and the recrystallized with ethanol. The purity of synthesis ligand was checked by TLC using gel-G

The above synthesized ligands were characterized by using UV-Visible, IR, ^{1}H-NMR and Mass spectrum.

2.3. General procedure for preparation of the metal complexes.

Co(II),Ni(II),Cu(II) and Zn(II) complexes were prepared by the addition 5m mol of $Co(CH_3COOH)_2.4H_2O$,$Ni(CH_3COO)_2.4H_2O$,$Cu(CH_3COO)_2.4H_2O$and $Zn(CH_3COOH)_2.2H_2O$ which where dissolved in about 20-30ml of water, into a ethanol solution of 10m mol of the ligand(1:2 molar ratio) the color of the complexes changes in a solution few minutes. The mixture was then refluxed for 2-3 hrs. At 50-60^0C the precipitated solid were filtrated from the reaction mixture. The solid were washed with methanol and them with dry ethyl ether, following by drying at 60^0C over night.

2.4. 1. Antimicrobial activity:-

The ligands and corresponding complexes were evaluated for their antibacterial activity against *bacillus* and *pseudomonas*, and antifungal activity against *Angier* and *R.solani* by the agar-well diffusion method [8]. These studied bacteria and fungi were incubated into nutrient both for 24 hrs and Malt-Extracted both for 48 hrs respectively.

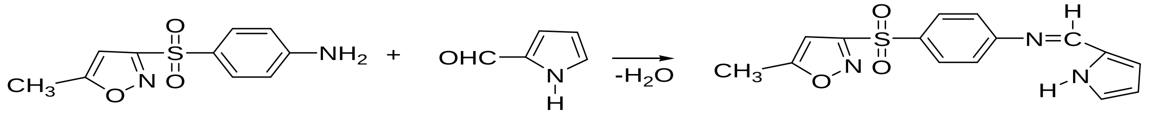
In this method, nutrient agar for bacteria and Malt-Extracted agar sterilized in a flask and cooled to 50^0C was distributed [50 ml] to sterilized Petri dishes (10cm in diameter) after injecting 0.1 ml cultures of bacteria or fungus, prepared at mentioned above and allowed to solidify the diluted plate method was used to enumerate micro organisms (10^5 cell mL-1) for 24 hrs. By using a sterilized proper tube (3mm dia) wells were drug in the culture plates. Ligands or metal complexes dissolved in DMSO were added (100 m mol) to the wells. The Petri dishes were left at 4^0C for 2 hrs and then the plates were incubated at 30^0C for bacteria 18-24hrs and 72hrs for fungi. At the end of the period inhibition zones formed on the medium evaluated as millimeters (mm) dia. The control sample were DMS) only.

2.4.2. Determination of minimal inhibitory concentration (MIC)

Nutrient and Malt-Extract are employed as basal medium for the growth of bacteria and fungi respectively. The culture medium (20 ml) was poured into Petri dishes (9mm) and maintained at 45^0C until the sample was incorporated into the agar. The sample was added as 1ml using an automatic micropipette while constantly stirring to assure a uniform distribution. Each sample was tested and 200 micro mol mL-1 in DMSO. The different bacterial strain was layered to place 30 ml over the surface of the solidification culture medium containing a sample. After the bacteria were absorbed into the agar, the plates were incubated at 30^0C for 24-48 hrs. Bacterial growth was monitored visually and the MIC was determined.

3. Results and discussion

The elemental analysis of ligands and metal complexes some physical properties are summarized in **Table-1.**The analytical data of the complexes corresponding well with general formula [M (II)(L) $_2$.$2H_2O$].the molar conductance value of all metal complexes were found to be low in 10^{-3} M DMSO solution, indicating all complexes are electrolyte.

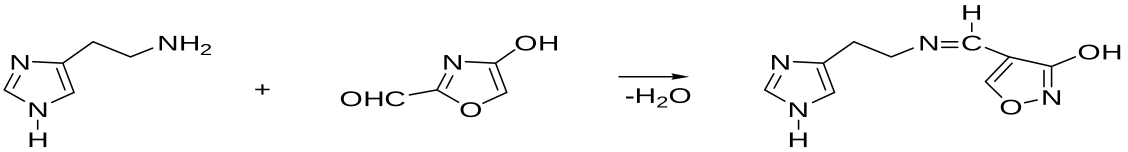


Sulphametoxazole

pyrrole 2-carbaldelyde

{4-[(5-metylisoxazol-3yl) Sulfonyl Benzyl dine]-N-[(E)-1-(2- Pyrrol)methyl dine) [MSBPMA]

Fig-1

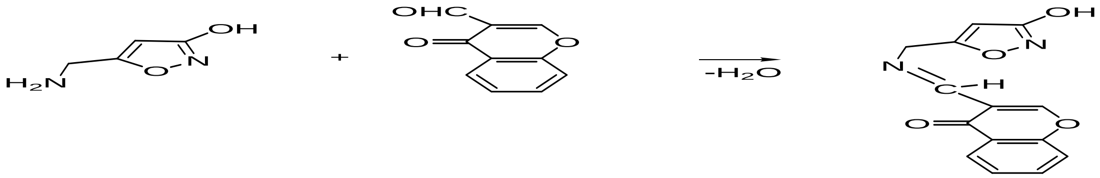


Histamine

3-hydroxy 5-carbaldehyde isoxazole

N-[(E)-1H-Imidazol-3yl)-N-[(Imino metylidine)-(3-Hydroxy-3-Isoxazol)]} [NINIHI]

Fig-2

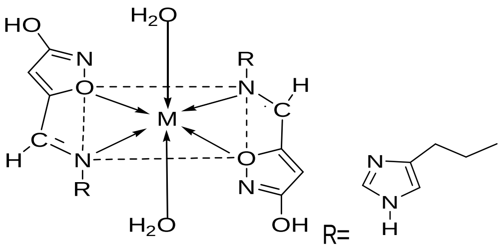


Muscinol

Chromine 2-Carbaldehyde

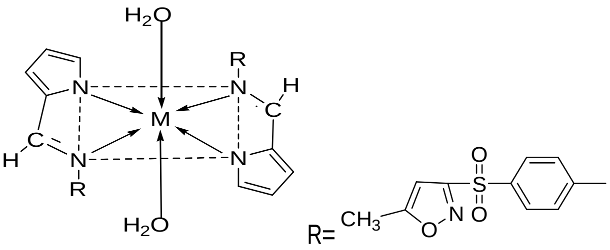
{N-[(3-hydroxy-3-Isoxazol) Imino] methyl 4-oxo-4H Chromine} [NHIIMC]

Fig-3



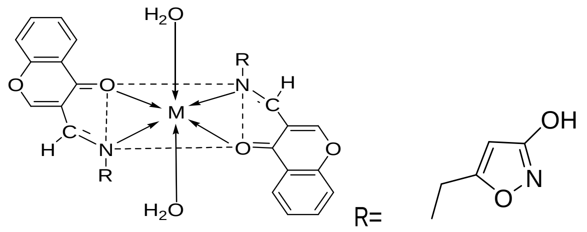
(Where M= Co (II), Ni (II), Cu (II) and Zn (II))

Fig-4.Proposed Structure of Complex M(II) $(L_1)_2.2H_2O$



(Where M= Co (II), Ni (II), Cu (II) and Zn (II))

Fig-5.Proposed Structure of Complex M(II) $(L_2)_2.2H_2O$



(Where M= Co (II), Ni (II), Cu (II) and Zn (II))

Fig-6.Proposed Structure of Complex M(II) $(L_3)_2.2H_2O$

Table-1. Analytical data and some physical properties

Compounds	Colours	yield	C% Calc(Found)	H% Calc(Found)	N% Calc(Found)	M% Calc(Found)	Melting point	MolarConO hm^{-2} Cm^{2} mole^{--1}
$C_{16}O_3N_3H_{13}$ (L_1)	Light Yellow	82%	65.08 (65.04)	4.40 (4.37)	14.23 (14.21)	_	186-188^{0}C	_
Co (II)$(L_I)_2.2H_2O$	Green	84%	56.63 (56.45)	4.42 (4.35)	12.38 (12.31)	7.66 (7.45)	210-212^{0}C	8.21
Ni (II)$(L_I)_2.2H_2O$	Yellow Green	79%	56.60 (56.43)	4.37 (4.22)	12.32 (12.15)	7.69 (7.52)	2240-243^{0}C	6.56
Cu (II)$(L_I)_2.2H_2O$	Blue	87%	56.54 (56.42)	4.39 (4.21)	12.30 (12.23)	7.71 (7.56)	254-256^{0}C	8.65
Zn (II)$(L_I)_2.2H_2O$	Yellow	80%	56.49 (56.42)	4.38 (4.21)	12.37 (12.23)	7.65 (7.42)	198-200^{0}C	7.02
$C_9O_2N_4H_{10}$ (L_2)	Light yellow	81%	60.67 (60.45)	5.61 (5.45)	31.46 (31.23)	_	105-107^{0}C	_
Co (II)$(L_2)_2.2H_2O$	Dark Green	87%	48.64 (48.43)	5.40 (5.34)	25.22 (25.14)	11.71 (11.23)	142-144^{0}C	8.65
Ni (II)$(L_2)_2.2H_2O$	Green	89%	48.60 (48.45)	5.37 (5.34)	25.26 (25.22)	11.73 (11.65)	161-165^{0}C	6.89
Cu (II)$(L_2)_2.2H_2O$	Blue	92%	48.61 (48.56)	5.39 (5.22)	25.24 (25.13)	11.75 (11.56)	173-175^{0}C	8.45
Zn (II)$(L_2)_2.2H_2O$	Light yellow	82%	48.63 (48.34)	5.35 (5.23)	25.21 (25.12)	11.77 (11.65)	126-128^{0}C	6.23
$C_{14}O_4N_2H_{10}$ (L_3)	Yellow green	83%	62.22 (62.12)	3.70 (3.34)	10.37 (10.22)	_	205-209^{0}C	_
Co (II)$(L_3)_2.2H_2O$	Green	85%	53.50 (53.34)	3.82 (3.65)	8.91 (8.87)	8.28 (8.12)	256-258^{0}C	7.46
Ni (II)$(L_3)_2.2H_2O$	Light Green	89%	53.47 (53.34)	3.84 (3.65)	8.94 (8.65)	8.29 (8.21)	267-269^{0}C	6.21
Cu (II)$(L_3)_2.2H_2O$	Blue	92%	53.45 (53.21)	3.87 (3.67)	8.93 (8.54)	8.28 (8.23)	279-284^{0}C	7.89
Zn (II)$(L_3)_2.2H_2O$	Light Yellow	84%	53.43 (53.34)	3.86 (3.76)	8.90 (8.86)	8.24 (8.23)	231-233^{0}C	6.66

3.1. Thermal analysis:-

The heating rates were suitably controlled at 10^{0}C min^{-1} under nitrogen atmosphere, and the weight loss was measured from the ambient temperature up to 800^{0}C. the TGA curves of all metal complexes show that the initial mass loss occurring with 100-300^{0}C range is interpreted as loss of mass and hydrated water molecules during the chelate drying process and the second weight loss at around more than 300^{0}C range is due to loss of coordinated water molecules, temperature was a horizontal and the find product of decomposition at this temperature region corresponds to metal oxide. Presence of water molecule is further confirmed by the endothermic bands observed is the respective

DTA curve in the temperature region where the TGA curves loss in weight. In addition to the endothermic bands, the bands appeared at higher temperature; represent phase transition, oxidation and more decomposition of the compound.

3.2.1. ^{1}H- NMR and Mass spectrum of Ligand (L_1) [MSBPMA].

The ^{1}H-NMR spectra of the ligand (L_1) recorded in $CDCl_3$ showed the following signals.($C-CH_3$) three protons at 3.2δ (S,3H) and (N-H) proton on pyrrole ring at 9.02 δ (S,1H), and the imino proton (N=C-H) appeared at 8.72 δ (S, 1H). The Mass spectra of the ligand show molecular ion peaks which are good agreement with the expected value. The Mass spectra of the ligand (L_1), give a peak at 298 m/z which was also supported by the Nitrogen rule since the compound possesses three Nitrogen atoms.

3.2.2. ^{1}H-NMR and Mass spectrum of Ligand (L_2) [NINIHI].

In ^{1}H-NMR ($CDCl_3$) the imino proton appeared at 8.52 δ on the pyrrole ring at 9.07 δ (S,1H), (N-H) proton and hydroxyl group on isoxazole ring at 4.56δ (S,1H), (-OH). The Mass spectra of the ligand showed a molecular ion peak at 182 m/z, which was also supported by the Nitrogen rule since the compound possesses four Nitrogen atoms.

3.2.3. ^{1}H-NMR and Mass spectrum of Ligand (L_3) [NHIIMC].

The ^{1}H-NMR spectra of the Schiff base ligand is recorded in DMSO-d_6. The ^{1}H-NMR spectra of Schiff base ligand (L_3) at peaks 7.42δ and 4.35δ are singlet. Those peaks are assignable for the proton of azomethine group and hydroxyl group on isoxazole ring respectively.The Mass spectra of the ligand showed molecular ion peak which is good agreement with the expected value. The Mass spectra of ligand (L_3) give a peak at 271 m/z, which is assigned for (L+H) peak.

3.3. IR Spectrums of metal complexes:

3.3.1. M (II) $(MSBPMA)_2.2H_2O$, IR spectrums

The IR spectrum of M (II) $(MSBPMA)_2.2H_2O$ as summarized in **Table-2** shows the following features. A strong band appeared at 1612cm^{-1} in (L_1), this band can be assigned to **ν**(C=N) stretching vibration[9]. In these complexes the vibration band **ν** (C=N) is shifted by 10-20cm^{-1} to higher or lower wave numbers. These indicated the possibility of coordination of the amino nitrogen to metal ion. The ring skeletal vibrations **ν** (C=C) were consistent in all complexes, the bands **ν**(S=O) stretching, at 1521-1528cm^{-1} and another band also **ν**(C-H) stretching at range 3006-3014cm^{-1} unaffected by complexation.Which indicative of involvement of the pyrrole ring in the complexation also a band at 3215cm^{-1} attributed to **ν**(N-H) stretching in the ligand (L_1) disappeared in its metal complexes [10]. In the low frequency region the bands observed in the complexes in the region 557-562cm^{-1} was attributed to **ν** (M-N). All the data suggested that the metal was bonded to the pyrrole ring Nitrogen and the imino Nitrogen [11].

3.3.2. M(II) $(NINIHI)_2.2H_2O$, IR spectrums

The IR spectrum of L_2[NINIHI] as summarized in the **Table-2** shows the following features. The ligand showed strong band 1616 cm^{-1} due to **ν** (C=N) which is assignable to the Schiff base which appeared in the synthesized ligand. This band gets shifted by 10-20 cm^{-1} to lower or higher wave numbers in complexation. These indicating the co-ordination through azomenthine nitrogen to metal ion. The band **ν**(C-H) is unaffected by complexation. Which indicated of involvement of the isoxazole ring **ν** (C-O-N)) band vibration frequently 1220 cm^{-1} is shifted by 10-20 cm^{-1} to higher or lower wave numbers. These indicated the possibility of coordinating the isoxazole Oxygen to metal ion. The low frequency region, the bands are observed in the complexes in the region 460-476cm^{-1} was attributed to **ν** (M-O) and in the region 559-564cm^{-1} was attributed to **ν** (M-N). All the IR data suggested that the metal ion was bonded to the ligand L_2 (NINIHI) through the isoxazole ring Oxygen and the imino Nitrogen[12].

3.3.3. M(II) $(NHIIMC)_2.2H_2O$, IR spectrums

The IR spectrum of the ligand NHIIMC metal complexes Showed (Fig-6) the band observed of 1600cm^{-1} region in the ligand is assigned to the azomenthine group **ν**(C = N) was Shift to this band in metal complexes towards higher or lower frequencies region. There indicated the possibility of coordination of the imino nitrogen to metal ion. the ring skeletal vibrations **ν** (C-O-C) and free **ν** (OH) bands are not unaffected by complexation .In the low frequency region the bands are observed in the complexes in the region559-567cm^{-1}was attributed to **ν**(M- N) and the region 467-475cm^{-1} was attributed to ν (M-O). All the IR data suggested that the metal is bonded to the Schiff base through Oxygen and imino Nitrogen.

All the Co(II), Ni(II),Cu(II) and Zn (II) complexes showed aboard difference band at 3440-3480 cm^{-1} and another band at770-796cm^{-1} at lower frequency region indicating the presence of water molecules in the co-ordination sphere. This fact is also supported by the results of elemental analysis [13].

Table-2: IR Absorption frequencies of liands and metal complexes.

compounds	**ν** (C=N)	**ν** (C=N)	**ν** (N-H)	**ν** (M-O)	**ν** (M-N)	**ν** (C=C)	**ν** (S=0)	Water coordinated
[MSBPMA] (L_1)	1612	3010	3215	–		1522	1140	–
Co (II)(L_1)$_2$.2H_2O	1621	3003	–	–	562	1527	1142	792
Ni (II)(L_1)$_2$.2H_2O	1625	3012	–	–	557	1529	1145	781
Cu (II)(L_1)$_2$.2H_2O	1630	3006	–	–	560	1521	1140	786
Zn (II)(L_1)$_2$.2H_2O	1605	3014	–	–	561	1528	1141	789
[NINIHI] (L_2)	1616	3024	3217	–	–	–	–	–
Co (II)(L_2)$_2$.2H_2O	1624	3021	3222	466	559	–	–	780
Ni (II)(L_2)$_2$.2H_2O	1624	3016	3219	472	564	–	–	787
Cu (II)(L_2)$_2$.2H_2O	1632	3017	3226	474	562	–	–	782
Zn (II)(L_2)$_2$.2H_2O	1610	3010	3220	476	560	–	–	779
[NHIIMC] (L_3)	1600	2982	–	–	–	1523	–	–
Co (II)(L_3)$_2$.2H_2O	1612	2992	–	471	567	1521	–	794
Ni (II)(L_3)$_2$.2H_2O	1621	2999	–	475	561	1538	–	789
Cu (II)(L_3)$_2$.2H_2O	1625	3012	–	467	565	1522	–	782
Zn (II)(L_3)$_2$.2H_2O	1592	3014	–	473	559	1529	–	786

3.4. Electronic Spectral data of metal complexes:-

The electronic spectra of metal complexes were recorded in DMSO in the range 4,000 cm^{-1}-40,000cm^{-1}. The electronic spectra of Co(II) complexes showed bands around 9,951-13,564cm^{-1}; 17,641-20,412 cm^{-1} and 25,524-29,861 cm^{-1} assigned to the electronic transition $^4T_{1g}$ (F) → $^4T_{2g}$ ($\mathbf{v}_1$), $^4T_{1g}$ →$^4A_{2g}$ (F) ($\mathbf{v}_2$) and ^4T_1g →$^4T_{1g}$ (p) ($\mathbf{v}_3$) respectively. These transitions are in fair agreement with the predicted values of octahedral geometry[14]. The electronic spectra of Ni(II) complexes showed three bands around 9,867-13,242cm^{-1}, 15,421-18600cm^{-1} and 25,641-28,074cm^{-1} assigned to the electronic transition $^3A_{2g}$(F) → $^3T_{2g}$ (F) , $^3A_{2g}$ → $^3T_{1g}$ (F) and $^3A_{2g}$(F) → $^3T_{1g}$ (F) respectively these transitions of those complexes are suggesting octahedral geometry[15]. The spectra of Cu(II) complexes showed two bands are in the visible region 12,561-14,249 cm^{-1} and 18,963-22,469cm^{-1} are assigned to $^2B_{1g}$ → $^2A_{1g}$ and $2B_{1g}$ → $2E_{1g}$ transitions respectively those electronic bands are suggesting square planar geometry of the Cu(II) complexes[16]. The present Zn (II) complexes show no d-d bands as expects for a d^{10} system. Those Zn (II) complexes have been found to the diamagnetic in nature.

On the basic of the physical and spectra data of the complexes discussed above, one can assume that the metal ions are bonded to the Schiff base via the Oxygen and Nitrogen as illustrated in **Fig.4-6**

3.5. Antimicrobial activity:

The new ligands and metal complexes were evacuated. For antibacterial activity against *pseudomonas*, *Bacillus* and in antifungal activity against *A.ngie*r and *R.solani* by using agar-well diffusion. The complexes are activity than the ligands. The copper complexes are much more activity than other metal complexes showed in microbial activity against all microorganism and most complexes have antifungal effect except Zn (II) metal complexes. The Cu (II) complexes are showed the best activity against *A-niger* and *R.Solani*

All complexes show antibacterial activities against *pseudomonas* and *Bacillus* inhibition zone results were dependants on the type of ligands and the position of cheltion with metal ion[17]. Antimicrobial activities are also reported as minimum inhibitory concentration (MIC) values, defined as the lowest concentration of an antimicrobial that visibly inhibits growth of the bacteria after over night incubation. Minimum inhibitory concentration (MIC) of 50.00µ mol mL^{-1} where observed in all microorganism tested. The varation in the effective in different complexes against different organism depend either on different in the permeability of the cell of the microbes [18].

4. Conclusion

The result of this investigation supports the suggested structure of the metal complexes. Octahedral and square planar geometry were suggested for all complexes, the Schiff base ligands were found to be biologically active and their metal complexes display enhanced antimicrobial activity against one or more strains, chelating tends to the ligands act as more powerful and potent bactericidal agent.

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