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Synthesis and biological evaluation of metal complexes with pyrazoline derivative bearing indole and naphthyl moieties

J. Joseph*, T. M. Sharmila and M.Sirajul Muneera
Department of Chemistry, Noorul Islam Centre for Higher Education, Kumaracoil-629 180, Tamil Nadu, India.
***Corresponding author: E-mail:chem_joseph@yahoo.co.in**

ABSTRACT

Novel Cu(II), Co(II), Ni(II) & Zn(II) complexes with pyrazoline derivatives containing indole and naphthyl moieties were synthesized. The pyrazoline derivative and metal complexes were elucidated using analytical and spectroscopic techniques. The molar conductance, magnetic moment and electronic spectra have assigned square planar geometry for the complexes. The antibacterial screening of ligand and its metal complexes indicated that the complexes exhibited higher inhibitory efficiency as compared to ligands. The DNA binding behavior of the complexes with CT-DNA has been investigated by electronic absorption spectral and electrochemical techniques. The DNA binding constants (K_b) suggested that the complexes interact with DNA through intercalation binding mode and also hydrogen bonding interactions with amino acid in base pairs of DNA. Therefore, the synthesized copper complexes exhibited biological accessible oxidation state Cu(II)/Cu(I) redox potential during electrolysis.

Keywords: Pyrazoline; binding; antioxidant; screening.

1. INTRODUCTION

Many therapeutic agents are "organic" in nature while other elements placed in the periodic table, particularly transition metals, offer unique role in therapeutic applications. In ancient times, the utilization of metal compounds as therapeutic drugs to treat broad range of infectious diseases. In the medicinal chemistry, the traditional medicines are dominated by carbon based organic molecules. In modern years, the metal complexes have received considerable interest as diagnostic approach towards anticancer, anti-viral, anti-inflammatory etc.

In the carbonyl reactions, the Claisen Schmidt reaction is one of the most significant and well-designed synthetic approach to build up carbon-carbon bonds. As look into the literature evidences, the organic transformations are Aldol, Knoevenagel, Henry and Michael reactions in the presence of base. The condensation reaction of aromatic aldehyde and acetophenone yielded as chalcone. It was first reported in 1881 by Schmidt and Claisen. The chalcone undergoes cyclization into pyrazoline derivative and showed variety of pharmacological application.

The structural modification could enhance solubility and stability in the biological medium and enhanced biological activities. Keeping these literature facts in mind, the present studies focused on the design, synthesis and structural elucidation of Cu(II), Ni(II), Co(II) and Zn(II) complexes with pyrazoline contain active pharmacopore indole and naphthyl moieties. Further, the sythesised metal complexes were subjected to antimicrobial and DNA binding studies.

2. EXPERIMENTAL

Material: All chemicals and solvents were analaR grade and were purchased from Merck. All supporting electrolyte solutions were prepared using analytical grade reagents. Calf thymus DNA purchased from Genie Biolab, Bangalore, India.

Preparation of ligand (L): A solution of 1-acetyl-2-hydroxynapthalene (0.01 M) and indole-3-carboxaldehyde (0.01 M) in 40 ml ethanolic NaOH was stirred for 6 hrs at RT. The solid precipitate was obtained, washed with cold water and dried. Then, it was recrystallized from ethanol. A solution of chalcone (0.01 M) and phenylhydrazine (L) (0.01 M) in the presence of NaOH was refluxed in 20 mL of hot ethanol for 8 hrs. The solution was poured into ice water which resulted into the precipitation of the ligand (L). The precipitate was filtered and recrystallized from methanol. The recrystallized ligand was dried in a vacuum.

Ligand (L): Molecular formula $C_{27}H_{21}N_3O$, Mol wt. 403. Percentage yield: 68; Elemental analysis: calcd for N 10.41, H 5.26, C 80.27 Found: N 10.21, H 5.06, C 80.14.UV (nm): 352, 242 nm. FT-IR (KBr disc): 3286 (Ar O–H); 3080-3070 (Ar–H); 2968, 2899 (C–H); 1650 (C=N); 1228 (Ar C–OH). ^{1}H-NMR (δ, $CDCl_3$): 3.16 (1H, 17.2 Hz, 4-Htrans, dd, J = 4.0), 5.24 (1H, 11.8 Hz, dd, J = 4.0, 5-H), 3.56 (1H,17.4 Hz, dd, J = 11.0,4-Hcis), 6.48-7.56 (m, 15 Ar-H), 9.6 (-NH, indole moiety), 3.6 (-CH=), 11.2 (1H, -OH, s,). ^{13}C NMR (ppm, $CDCl_3$): 125.2 (C1), 125.8 (C2), 122.6 (C3), 126.8 (C4), 132.4 (C5), 116.4 (C6), 152.6 (C7), 112.0 (C8), 134.8 (C9), 128.2 (C10), 156.4 (C11), 40.8 (C12), 53.4 (C13), 135.5 (C14), 100.6 (C15), 124.6 (C16), 119.6 (C17), 120.2 (C18), 119.6 (C19), 110.5 (C20), 134.9 (C21), 144.6 (C22), 11.6 (C23), 128.4 (C24), 116.2 (C25), 128.4 (C26), 11.6 (C27). MS(m/z): 404 [M+1]. LogP Found (calculated): 6.23 (6.45).

Synthesis of complexes: A hot ethanolic solution of metal acetate (0.05 M) and solution of ligand (0.05 M) was mix together and stirred for 5 hrs. The precipitate was filtered and washed with cold methanol and hexane. The other metal complexes were prepared using similar procedure. The complexes were dried in a vacuum dessicator.

Copper complex of L: Molecular formula $C_{29}H_{25}N_3O_3Cu$. Mol wt.543. Yield: 72%; Elemental analysis: calcd; C 64.14, H 4.64, N 7.74, Cu 11.70; Found: C 63.94, H 4.40, N 7.62, Cu 11.58. UV (nm): 346, 252 & 492 nm. FT-IR (KBr): 3176 (N–H), 1157 (C–N), 1599 (C=N), 547 (M–N), 480 (M–O). MS): *m/z*: 544 [M+1]. μ_{eff}(BM) = 1.89. Λ_m (Scm^2mol^{-1}) =14.

Nickel complex of L: Molecular formula $C_{24}H_{22}N_4O_4Ni$. Mol wt. 489. Yield: 70%; Elemental analysis: calcd; C 58.94, H 4.53, N 11.45, Ni 12.00; Found: C 58.72, H 4.30, N 11.36, Ni 11.88. UV (nm): 344, 242, 540 & 739 nm. FT-IR (KBr disc): 3182 (N–H), 1164 (C–N), 1590 (C=N), 542 (M–N), 478 (M–O). ^{1}H-NMR (δ, $CDCl_3$): 3.12 (1H, dd, pyrazoline core, J = 4.1, 17.6 Hz, 4-Htrans), 3.56 (1H, dd, J = 11.8, pyrazoline core, 17.6 Hz, 4-Hcis), 5.24 (1H, dd, pyrazoline core, J = 4.1, 12.1 Hz, 5-H), 6.76-7.52 (m, 11 Ar-H), 11.2 (1H, s, -OH), 7.8-7.5 (-CH=CH-, dd, 2H), 12.6 (-NH(indole), 1H, s). MS): *m/z*: 490 [M+1]. μ_{eff}(BM) = 0. Λ_m (S cm^2mol^{-1}) = 8.

Cobalt complex of L: Molecular formula $C_{24}H_{22}N_4O_4Co$. Mol wt. 489. Yield: 60%; Elemental analysis: calcd; C 58.91, H 4.53, N 11.45, Co 12.04; Found: C 58.72, H 4.10, N 11.38, Co 11.92. UV (nm): 314, 500 & 645 nm. FT-IR (KBr disc): 3168 (N–H), 1150 (C–N), 1588 (C=N), 544 (M–N), 486 (M–O). MS: *m/z*: 490 [M+1]. μ_{eff}(BM) = 1.75. Λ_m (S cm^2mol^{-1}) =10.

Zinc complex of L: Molecular formula $C_{24}H_{22}N_4O_4Zn$. Mol wt. 496. Yield: 72%; Elemental analysis: calcd; C 58.14, H 4.47, N 11.30, Zn 13.19; Found: C 57.96, H 4.22, N 11.08, Zn 12.95. UV (nm): 330, 245, 372 nm. FT-IR (KBr disc): 3160 (N–H), 1150 (C–N), 1582 (C=N), 536 (M–N), 476 (M–O). ^{1}H-NMR (δ, $CDCl_3$): 3.24 (1H, dd, pyrazoline core, J = 4.1, 17.6 Hz, 4-Htrans), 3.72 (1H, dd, pyrazoline core, J = 11.8, 17.6 Hz, 4-Hcis), 5.56 (1H, dd, J = 4.1, pyrazoline core, 12.1 Hz, 5-H), 6.92-7.74 (m, 11 Ar-H), 10.6 (1H, s, -OH), 7.9-7.7 (-CH=CH-, dd, 2H), 11.8 (-NH(indole), 1H, s). MS: *m/z*: 497 [M+1]. μ_{eff}(BM) = 0. Λ_m (S cm^2mol^{-1}) = 6.

DNA Binding Studies: The binding interactions between metal complexes and DNA were studied using electrochemical and electronic absorption methods by using different concentrations of CT-DNA by Joseph et al [8].

Antimicrobial activities: The *in vitro* antimicrobial activities of title compounds were evaluated against the bacterial species.

3. RESULTS AND DISCUSSION

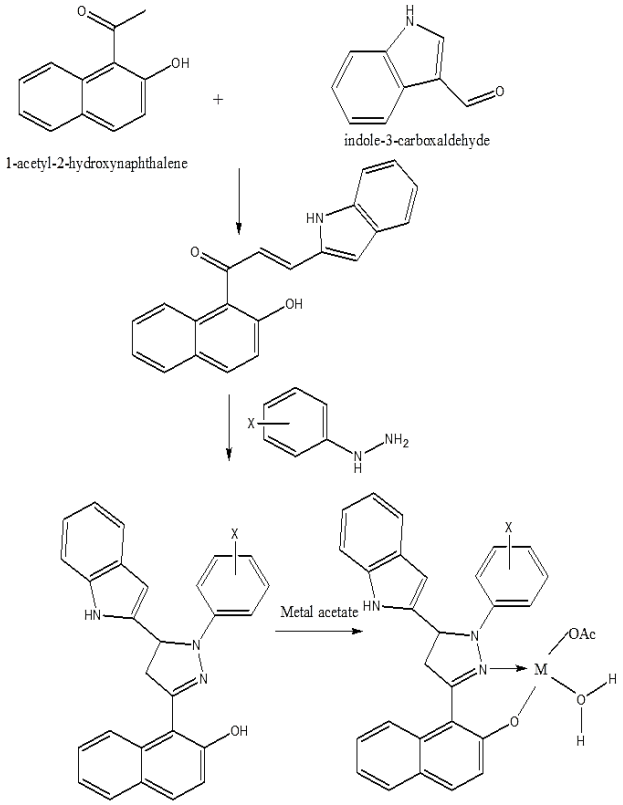
The preparation of pyrazoline derivative and its metal complexes are outlined in scheme 1. The chalcones were prepared by the Claisen-Schmidt condensation of 1-acetyl-2-hydroxynaphathalene and indole-3-carboxaldehyde in the presence of basic medium. The obtained chalcones undergo cyclization with phenylhydrazine (L) under the basic medium leads to the formation of pyrazoline derivative bearing indole and naphthyl moieties. It was purified by column chromatographic technique. The pyrazoline derivative was used as ligand to prepare metal (II) complexes [ML(OAc)(H_2O)] (Scheme 1).

All the prepared complexes were stable at room temperature (confirmed by TG measurements). The formation ligand and its metal complexes were checked by comparing the TLC with the starting materials and single spot was obtained. They were non-hygroscopic solids and insoluble in ethanol and water but soluble in DMSO. The analytical data of the ligands and their metal (II) complexes were presented in the experimental section. They are well agreed with the theoretical values within the experimental error.

The metal(II) complexes dissolved in DMSO solutions. The molar conductivities of 10^{-3}M solution at room temperature were measured. The lower molar conductivity values of metal complexes were found in the range of (6-16) Scm^2mol^{-1} suggested that the non-electrolyte nature of complexes. The chemical test also confirmed that the there is no acetate ions in the outside coordination sphere.

FTIR spectra: The IR spectra of metal complexes are compared with the parent ligand to determine the coordination sites involved in chelation with metal ion. There are some characteristic peaks in the spectrum of the free ligand and its metal complexes are given in the experimental part. In the present study, the condensation of 1-acetyl-2-hydroxynaphthalene with indole-3-carboxaldehyde leads to the formation of chalcone. Furthermore, the presence of (C=N) and (C-N) stretching frequencies at 1588 and 1256 cm^{-1} in the IR spectrum of the ligand (experimental section) was confirmed the cyclization of chalcone with phenylhydrazine to form the pyrazoline derivatives as ligand (L).

The IR spectra of the complexes showed a strong band in the region 1654–1646 cm^{-1} which corresponds to the ν(C=N) stretch and shift of this band (22–40 cm^{-1}) to lower frequency indicates the involvement of azomethine nitrogen in coordination. The pyrazoline derivative showed a sharp band in the region 3300–3430 cm^{-1} due to the ν(NH) stretch of indole moiety. A band at 1362 cm^{-1} was assigned to the vibration frequency of the phenolic C–O group. In the complexes, the band assigned to the vibration frequency of the phenolic C–O group undergoes positive shifts, indicating that the pyrazoline is bonded to the metallic ions through the phenolic oxygen atoms.



Scheme.1.Synthetic outline of metal complexes with pyrazoline derivative
Where M = Cu(II), Ni(II), Co(II) & Zn(II);X = -H (L)

The IR spectra of complexes showed new absorption band in the region 3300-3500 cm^{-1} indicating that the coordinated water molecules. In addition, the band at 848 cm^{-1} in the IR spectra of metal(II) complexes suggested that water molecule is coordinated to metal ions. Therefore, the proposed coordination sites are azomethine nitrogen and phenolic oxygen atoms, respectively. The synthesized ligand is coordinated to metal ions through bidentate manner. The IR spectra of all the complexes showed two absorption bands in the far infrared region 432-448 cm^{-1} and 474-516 cm^{-1}, which are assignable to υ(M-O) and υ(M-N) vibrations, respectively. Further, the υ(asym) and υ(sym) vibrational bands for acetate in the complex were appeared at 1510 and 1400 cm^{-1}. The difference is ~110 cm^{-1} indicates that acetate ion bound as monodentate fashion. The IR spectrum of ligand (L) and its copper complex are given in figures 1 & 2.

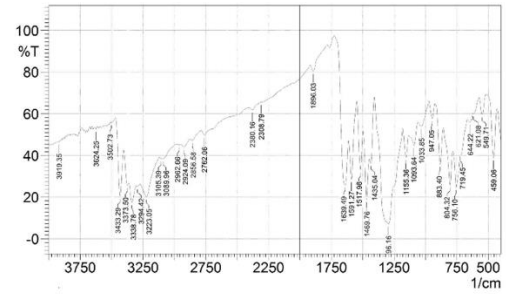


Figure.1.IR spectrum of ligand (L)

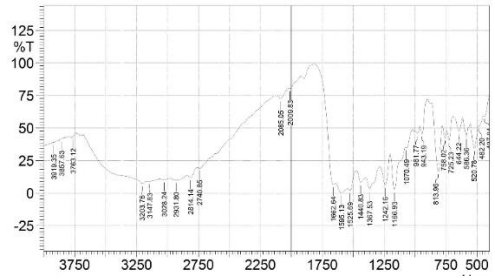


Figure.2.IR spectrum of copper complex of L2

Electronic Spectral features: The electronic spectra of free ligands showed two bands at 267 and 303 nm which are attributed to π–π* and the n–π* transitions, respectively. These bands were shifted in the electronic spectra of the complexes. The shift in absorption band and intensity changes in the spectra of the metal complexes are indicated that the chelation which increases the conjugation and delocalization of electron in the ring system. The observed results indicated that the ligand was coordinated to Co(II), Ni(II), Cu(II) and Zn(II) ions. Further, there is a shift in absorption band in the metal complexes with same ligands due to electronegativity of the different metal ions. The Cu(II) complex shows broad bands around 500 nm which may be assigned to $^2B_{1g} \rightarrow {}^2A_{1g}$ transition and suggested to square geometry around the Cu(II) ion. The zinc complex showed the bands in the region 250–386 nm and may be assigned to intraligand charge transfer transitions. Based on the spectral features the configuration of the zinc (II) ion has assigned to a square planar geometry. The electronic spectrum of the nickel complex of ligand showed a band appeared at 536 nm which is attributed to $^1A_{1g} \rightarrow {}^1A_{2g}$ transition. These transition and magnetic moment (μ_{eff} = 0) suggested a square-planar geometry of the complex. In the case of Co(II) complex of L, square planar Co(II) complex exhibited two bands around 645 and 500 nm which corresponds to the transitions $^2A_{2g} \rightarrow {}^2B_{1g}$ and $^2A_{1g} \rightarrow {}^2E_g$, respectively and its magnetic moment value is 2.18 BM. The electronic transition and magnetic moment value corresponds to square planar geometry.

ESR Spectra: The EPR spectrum of Cu(II) complexes were recorded at RT and LNT. They were exhibited four peaks with an axial symmetry (g_{II} = 2.224, 2.236, $g_{\perp}$ = 2.084, 2.120, A_{II}=156, 148 G) which is associated with square planar coordination around copper(II) ions. In order to quantify the degree of distortion of the Cu(II) complex, the f factor (g||/A||) was obtained from the ESR spectrum. Diaz et al. had studied and summarized a good correlation between 'f' factor (g_{II} / A_{II}, where A_{II} is expressed in cm^{-1}) and SOD activity of copper(II) complexes. The f factor is considered as an index of tetrahedral distortion, its value ranges between 105 and 135 for square planar complexes, depending on the nature of the coordinated atoms in the ligands. In the presence of a tetrahedrally distorted structure, the values are larger. The g/A value is 142 supports the square planar structure for Cu(II) complex. The 'f' value for Cu, Zn SOD is 160 cm, indicating a tetrahedral distortion from square planar geometry and is one of the features that enhance the catalytic activity of the enzyme. From the above EPR data the f values for copper complexes were determined to be 142 & 150 (g_{II}/A_{II}). Therefore, the synthesized Cu(II) complexes exhibiting appreciable square planar distortion is expected to show high SOD-like activity.

FAB Mass spectra: The FAB mass spectra of ligand and its metal complexes were recorded and compared their stoichiometry compositions. The ligand shows a molecular ion peak was observed at 404 m/z. The [CuL(OAc)(H_2O)] complex shows the molecular ion peak at m/z 544. This molecular ion further by the loss of water & acetate ion gave a fragment ion peak at m/z 526 & 467 and undergo demetallation to form the species $[L]^+$ gave fragment ion peak at m/z 404.

DNA binding experiments:

Cyclic Voltammetric Studies: Cyclic voltometric technique is the versatile technique useful for the DNA binding ability of electro active species. In the absence of DNA, the cyclic voltammogram of [CuL(OAc)(H_2O)] exhibited cathodic and anodic peaks was observed at -0.340 V and -0.260 V which are assigned to Cu(II)/Cu(I) conversion. In the presence of CT-DNA, there is a decreases in the peak current and shift in electrode potential was shown in the Fig.3. This is probably due to diffusion of the equilibrium mixture of free and DNA-bound metal complex to the electrode surface, in which the peak potentials both E_{pa} and E_{pc} as well as $E_{1/2}$ have shifted to negative potential. The changes in current and electrode potential indicated that metal complexes interact with CT-DNA through partial intercalative mode. The copper complex was observed that higher binding affinity than other metal complexes.

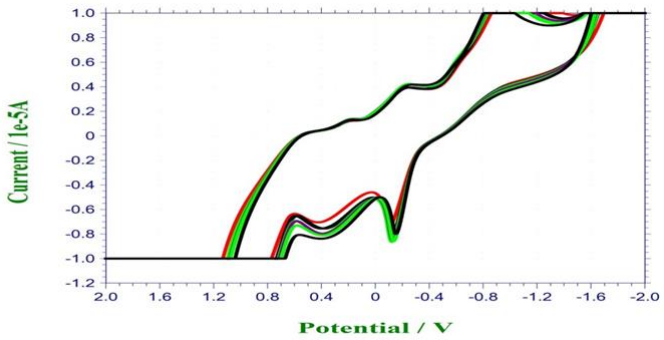


Fig.3.Cyclic voltammogram of [CuL2(OAc)(H2O)] in the presence and absence of DNA

Absorption spectral titrations: The electronic absorption spectrum of copper complex of ligand in the absence and presence of CT-DNA was recorded. In the case of copper complexes, there is a change in absorbance and shift in wavelength in the visible absorption region corresponds to the interaction of copper ion with DNA. In the absence

of DNA, the complex showed an absorption band at 332 nm. In the presence of DNA, the absorption band exhibited that the observed hypochromism of 20.80% with a blue shift of 6 nm. This spectral characteristics indicated that the copper complex interact with CT-DNA through intercalation binding mode. Among the metal complexes, the copper complex showed higher hypochromicity than other complexes indicated that the binding strength of the copper(II) complex is stronger than that of other metal ion.

Antimicrobial Activity: The parent pyrazoline deivatives and the metal complexes were evaluated for inhibition growth of Gram positive (*E. coli, B. subtilis and S. aureus*) and Gram negative bacterial (*P. aeruginosa and K. pneumoniae*) strains. Further, the MIC values were determined by serial dilution technique (Table 1). The antimicrobial activities were compared with chloramphenicol as standard antibiotic. As per the literature evidences, the DMSO is recommended vehicle to carry bioactive substance into specific sites in the tissues and its concentration 0.10% used in the present investigations. The order of antimicrobial activtities of metal complexes is shown as follows: CuL > NiL > CoL > ZnL > L

Table.1.Minimum Inhibitory Concentration of ligands and their metal complexes (µg/mL)

Compounds	MIC values (µg/mL)				
	E. coli	***S. aureus***	***K. pneumonae***	***S. Typhii***	***P. mirabilis***
L	72	84	96	90	82
Cobalt complex of L	22	26	28	32	30
Nickel complex of L	18	24	22	20	26
Copper complex of L	16	14	20	22	18
Zinc complex of L	26	28	34	38	40
Chloramphenicol	08	12	6	12	14

The metal complexes exhibited higher antimicrobial activity than parent pyrazoline analogs. Generally, the complexation may enhance liphophilic character of tested compounds. Further, the chelation decreases the polar character of metal ion because of the different donor atoms present in the pyrazoline derivatives. This process may increase the lipophilicity of metal complexes and enhanced membrane penetration. The electron transfer behavior of metal ion in the complexes may be also add to the higher antimicrobial activity. The higher activity of the copper(II) complex with respect to the nickel(II) complex might be attributed to the higher lipophilicity and lower charge density of the former as compared to the latter. The past few decades, researchers have witnessed a tremendous growth in antimicrobial studies aimed at identifying potential compounds of synergistic effect with conventional drugs. The synthesized Cu, Ni, Co and Zn complexes have 13 π bonds whereas chloramphenicol has 5 π bonds, it is indicated that the 13 π bonds present in ligand may be contributing to its potent synergistic effects and enhance antimicrobial efficiency.

4. CONCLUSION

In the present investigations, bioactive pyrazoline derivatives and their metal complexes were synthesized and characterized. On the basis spectral and analytical techniques, a square planar geometry was assigned for the metal complexes. Further, the biological studies of metal complexes were performed and obtained significant results. Therefore, the copper complexes of pyrazoline derivatives higher biological activity and may be used as therapeutic agents after clinical trials.

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