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Research Article

Structural and Biological Studies of Newly Synthesized Co(II) Coordination Complexes with Hydrazine Hydrate-based Azomethine Ligands

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ABSTRACT

This research work reports the synthesis of novel biologically active cobalt-azomethine coordination complexes $[Co(L_n)_2Cl_2]$. The azomethine ligands have been synthesized by the condensation of hydrazine hydrate with aldehydes and ketones, which was then used to synthesize novel Co(II)-azomethine coordination complexes and isolated as solid powdered products. The azomethine ligands and their biologically active Co(II)-azomethine coordination complexes $[Co(L_n)_2Cl_2]$ were characterized for molecular weight determination, elemental analysis, ^{1}H-NMR and FTIR spectroscopy. The azomethine ligands and their Co(II)-azomethine coordination complexes $[Co(L_n)_2Cl_2]$ have also been characterized for the antimicrobial activity against several bacterial and fungal species, which has been associated with the standard bactericide ciprofloxacin and fungicide ketoconazole. The outcome of the research has indicated that Co(II)-azomethine coordination complexes $[Co(L_n)_2Cl_2]$ were found to retain excess antimicrobial activity against bacterial and fungal species as compared to the free azomethine ligands.

Keywords: Azomethine ligand, Cobalt(II) Chloride, Coordination complex, Antibacterial activity, Antifungal activity.

INTRODUCTION

Azomethine ligands, a chelating ligand, have employed a dynamic role in the advancement of coordination chemistry.[1-6] Azomethine ligands ($R_2C{=}N{-}N{=}CR_2$) are nitrogenous and 2,3-diazo analogous of 1,3-butadiene and aldehyde or ketone. It can form highly stable and bioactive coordination complexes with various transition metals and has widely been used in bio-medicinal and coordination chemistry.[2,7-9] It has played a vigorous role in organic-inorganic coordination and biochemical, physiological, medicinal, and catalytic sciences.[10-13] A wide range of azomethine ligands and their transition metal complexes have been studied because these compounds have very disparate structures. It was also renowned that the existence of transition metal ions bonded to the bioactive ligands can also improve the biological and medicinal properties. Owing to the capability of performing as monodentate to a multidentate donor, these ligands are important intermediates and also recommend the prospect of developing a diverse range of bioactive transition metal-based coordination complexes.[14-16] The azomethine-transition metal coordination complexes have largely been explored in the literature owing to their exceptional biological and medicinal properties, such as antibacterial, antifungal, antiviral, antifertility and antiproliferative activity.[17-21] The azomethine-transition metal coordination complexes of Co(II), Ni(II), Cu(II), Pd(II), Zn(II) and Mn(II) have been evaluated for good antimicrobial, antituberculosis and anticancer activities.[7,8,14-16,22,23] Cobalt complexes with azomethine ligands have recently engrossed significant attention owing to their antimicrobial activities.

In this research work, the authors describe the synthesis of azomethine ligands (Fig. 1) and their newly designed Co(II)-based coordination complexes $[Co(L_n)_2Cl_2]$ (Fig. 2). The synthesized Azomethine ligands and their Co(II)-Azomethine ligand coordination complexes has been characterized for the determination of chemical structure using molecular weight determination, elemental analysis, ^{1}H-NMR and FTIR spectroscopy, and biological activity against several bacterial and fungal species. The Co(II)-azomethine coordination complexes $[Co(L_n)_2Cl_2]$ were found to retain excess antimicrobial activity against bacterial and fungal species as compared to the free azomethine ligands.

MATERIALS AND METHODS

Materials

Hydrazine hydrate, aldehydes (Benzaldehyde and furfuraldehyde), and ketones (Acetyl pyrrole and Benzophenone) were utilized for the synthesis of azomethine ligands. Cobalt(II) chloride ($CoCl_2$) was obtained and employed without further purification for the design and preparation of innovative Co(II)-azomethine coordination complexes. Methyl tetrahydrofuran (MeTHF) and ethanol were used as solvents. MeTHF, a green solvent, was dried by refluxing over sodium wire to enhance its performance. The chemicals and solvents employed in this study were of high purity and used as received. All

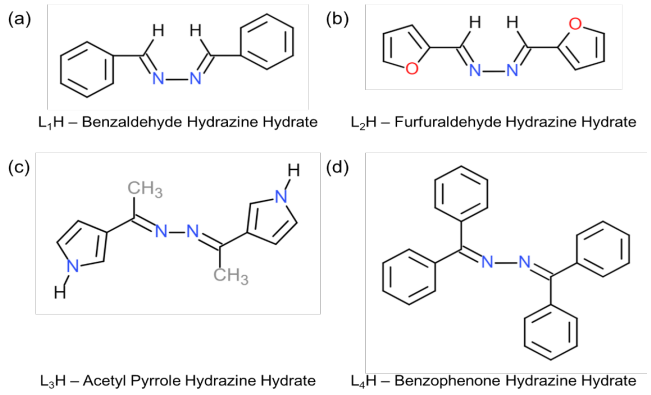

Fig. 1: Chemical structure of Azomethine ligands. (a) L_1H, (b) L_2H, (c) L_3H, and (d) L_4H

glassware was thoroughly washed, rinsed with distilled water, and dried in an oven before use.

Synthesis and Characterization of Azomethine Ligands

The typical procedure involved mixing a hot ethanolic solution of hydrazine hydrate (0.25 g; 0.005M) with a hot ethanolic solution of either benzaldehyde (1.06 g; 0.01M), furfuraldehyde (0.96 g; 0.01M), 2-acetyl pyrrole (1.09 g; 0.01M), or benzophenone (1.82 g; 0.01M) in a 1:2 molar ratio for the synthesis of the Azomethine ligands L_1H, L_2H, L_3H, and L_4H, respectively. Ethanol (80 mL) was used as the solvent, and the reaction mixtures were refluxed for 2 to 3 hours in a water bath, then allowed to cool to ambient temperature. The crystalline solid products were filtered, purified by recrystallization from the same solvent, and dried under vacuum. Molecular weight determination, elemental analysis, FTIR, and ^{1}H-NMR spectroscopy characterized the azomethine ligands. Analytical results of the azomethine ligands are summarized in Table 1.

Synthesis and Characterization of Co(II) Coordination Complexes

The synthesis of Co(II) coordination complexes $[Co(L_n)_2Cl_2]$ involved combining cobalt chloride ($CoCl_2$) and Azomethine ligands in 1: 2 molar ratio using MeTHF solvent. The resulting mixture underwent reflux for 8 to 9 hours under a fractionating column. After filtration, the product was dried under vacuum. Purification occurred through recrystallization from ethanol, repeating the process 2-3 times, followed by drying the purified product under vacuum at ambient temperature. Thin-layer chromatography (TLC) on silica gel-G adsorbent confirmed the compound's purity. Characterization of the synthesized Co(II) coordination complexes $[Co(L_n)_2Cl_2]$ involved determining the molecular weight, elemental analysis, FTIR and ^{1}H-NMR spectroscopy. Analytical results of the Co(II) coordination complexes $[Co(L_n)_2Cl_2]$ are presented in Table 1.

Characterization

Spectroscopic and analytical techniques were employed to characterize the azomethine ligands and their metal-ligand coordination compounds. Fourier-transform infrared (FTIR)

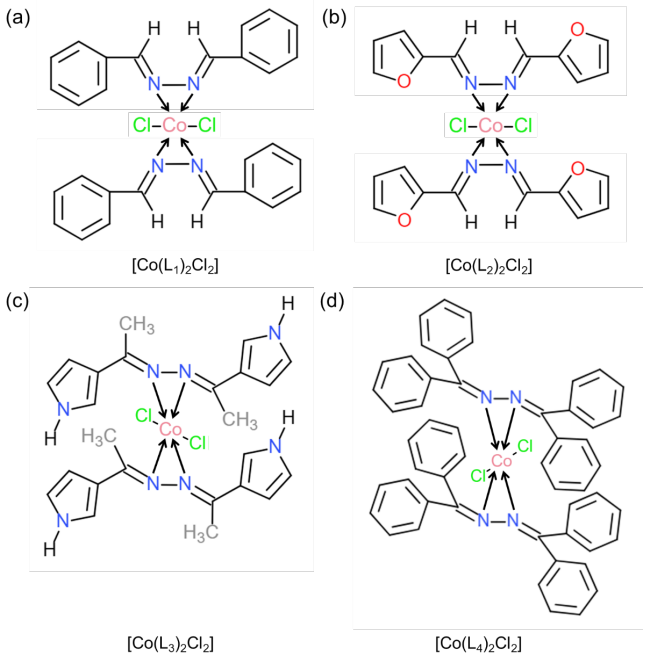

Fig. 2: Chemical structure of Co(II)-Azomethine coordination complexes. (a) $[Co(L_1)_2Cl_2]$, (b) $[Co(L_2)_2Cl_2]$, (c) $[Co(L_3)_2Cl_2]$, and (d) $[Co(L_4)_2Cl_2]$

spectra were obtained using KBr pellets on a Shimadzu FTIR spectrophotometer. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Jeol Al-300 spectrometer. Mass spectra were acquired with an XEVO G2S QTOF-YDA220 mass spectrometer. The molecular weights were determined via the Rast Camphor Method. The Elemental Analyzer estimated elemental analysis. Chlorine was quantified using the Volhard method.

Antibacterial Test

The *in-vitro* antibacterial activity of the azomethine ligands and their Co(II) coordination complexes $[Co(L_n)_2Cl_2]$ was evaluated against gram-positive (*Bacillus thuringiensis*; *B. thuringiensis*) and gram-negative (*Escherichia coli*; *E. coli*) bacterial strains using the agar well diffusion method at different concentrations (100, 200 and 300 ppm). The compounds were dissolved in 100% dimethyl sulfoxide (DMSO) to a concentration of 10 mg/mL. Nutrient agar was melted, cooled to 40 to 45°C, and inoculated with a standardized bacterial suspension before being poured into sterile petri dishes to solidify. The plates were incubated overnight at 35 ± 2°C. The antimicrobial activity was assessed by measuring the zones of inhibition around each well.

Control wells with pure DMSO solvent were included. The results were compared to the standard antibiotic ciprofloxacin (Table 1). The diameters of the inhibition zones were measured to the nearest mm using an antibiotic zone reader. All experiments were repeated at least twice to minimize experimental error, and the mean values were reported. The antimicrobial spectrum was determined for each bacterial strain based on the zone sizes.

Antifungal Test

The antifungal activity of the azomethine ligands and their Co(II) coordination complexes $[Co(L_n)_2Cl_2]$ was investigated against fungal species such as *Aspergillus flavus* and *Aspergillus niger* using the agar well

Table 1: Analytical results of azomethine ligands and their Co(II)-azomethine coordination complexes $[Co(L_n)_2Cl_2]$

S. No.	Compound	Molecular Formula	Mol.Wt. Found (Calc.)	Elemental Analysis Found (Calc.) (%)					
				C	H	N	O	Co	Cl
1.	L_1H	$C_{14}H_{12}N_2$	208.35 (208.10)	81.81 (80.74)	6.27 (5.81)	14.01 (13.45)	-	-	-
2.	L_2H	$C_{10}H_8N_2O_2$	187.52 (188.06)	63.47 (63.82)	4.32 (4.28)	15.00 (14.89)	17.23 (17.00)	-	-
3.	L_3H	$C_{12}H_{14}N_4$	213.96 (214.12)	67.24 (67.27)	6.47 (6.59)	26.03 (26.15)	-	-	-
4.	L_4H	$C_{24}H_{20}N_2$	359.98 (360.16)	86.25 (86.64)	5.93 (5.59)	7.90 (7.77)	-	-	-
5.	$[Co(L_1)_2Cl_2]$	$C_{28}H_{24}N_4CoCl_2$	544.79 (546.36)	59.88 (61.55)	4.40 (4.43)	10.00 (10.25)	-	10.78 (10.79)	12.56 (12.98)
6.	$[Co(L_2)_2Cl_2]$	$C_{20}H_{16}N_4O_4\ CoCl_2$	506.02 (506.20)	46.58 (47.46)	3.20 (3.19)	11.09 (11.07)	12.12 (12.64)	10.84 (11.64)	13.80 (14.01)
7.	$[Co(L_3)_2Cl_2]$	$C_{24}H_{28}N_8\ CoCl_2$	557.50 (558.38)	51.01 (51.63)	5.00 (5.05)	19.16 (20.07)	-	10.25 (10.55)	12.37 (12.70)
8.	$[Co(L_4)_2Cl_2]$	$C_{48}H_{40}N_4\ CoCl_2$	848.98 (850.75)	72.56 (73.41)	4.58 (4.74)	6.75 (6.59)	-	6.68 (6.93)	8.13 (8.33)

diffusion method. The fungi were sub-cultured on potato dextrose agar (PDA) medium. The agar was poured into sterilized Petri dishes and allowed to solidify. Fungal spores were then inoculated onto the agar surface using an inoculation needle. The plates were incubated at 25 ± 2°C for 2 to 4 days. After incubation, the bioactivity was determined by measuring the diameter of the inhibition zones in mm (Table 2).

All experiments were performed in duplicate or triplicate to minimize experimental error, and the mean values were reported. Ketoconazole was used as the standard antifungal control. The percentage inhibition was calculated using the equation 100(C-T)/C, where C and T are the diameters of the fungal colonies on the control and test plates, respectively. The antifungal spectrum was evaluated based on the zone of inhibition sizes.

RESULTS AND DISCUSSION

The Co(II)-Azomethine coordination complexes $[Co(L_n)_2Cl_2]$ (Fig. 2) were synthesized by the chemical reaction of $CoCl_2$ with a bidentate Azomethine ligand in 1: 2 molar ratio using MeTHF solvent medium. The synthesized ligands and their newly designed Co(II)-Azomethine coordination complexes $[Co(L_n)_2Cl_2]$ were characterized for the determination of chemical structure using molecular weight determination, elemental analysis, ^{1}H-NMR and FTIR spectroscopy and also tested for the antibacterial and antifungal activity. The Co(II)-Azomethine coordination complexes $[Co(L_n)_2Cl_2]$ were found to possess improved biological activity against bacterial species such as *B. thuringiensis* and *E. coli*, and fungal species such as *A. flavus* and *A. niger* as compared to its free ligands. After 72 hours of incubation, bioactivities of the Co(II)-Azomethine coordination complexes $[Co(L_n)_2Cl_2]$ were measured as the diameter of the inhibition zone (in mm). All experimental setups were made in duplicate or triplicate by means of more accurate results. The obtained results of these studies were compared with the standard bactericide ciprofloxacin and fungicide Ketokenazole. The inhibition was calculated by the equation 100(C – T)/C in percentage, where C and T are the diameters of the fungus colony in the control plate and test plate, respectively.

FTIR Spectra

For chemical structural identification, the FTIR spectra of azomethine ligands and their Co(II)-azomethine coordination complexes $[Co(L_n)_2Cl_2]$ have been recorded. In the FTIR spectra, the absorption bands of C=N stretching frequency of the azomethine ligands appear in the range of 1620 to 1633 cm^{-1}. The presence of azo form in the azomethine ligands is strongly recommended by the absence of any absorption band between 3250-3400 cm^{-1} due to C-NH. The strong absorption band due to >C=N is slightly shifted on coordination complexation, indicating the bonding of azo nitrogen with Co(II). The shifting towards lower frequency can be explained by the donation of nitrogen electrons to empty d-orbital of Co(II) ion. The upward shifting of the ligand's N-N absorption band on coordination complexation indicates that azomethine nitrogen is

Table 2: FT-IR spectral data of azomethine ligands and their Co(II)-azomethine coordination complexes $[Co(L_n)_2Cl_2]$.

S. No.	Compound	FTIR band (cm^{-1})		
		v(C=N)	**v**(N–N)	**v**(Co←N)
1	L_1H	1622	930	-
2	L_2H	1633	937	-
3	L_3H	1620	925	-
4	L_4H	1620	955	-
5	$[Co(L_1)_2Cl_2]$	1607	965	501
6	$[Co(L_2)_2Cl_2]$	1610	978	498
7	$[Co(L_3)_2Cl_2]$	1605	970	510
8	$[Co(L_4)_2Cl_2]$	1611	971	508

Table 3: Antibacterial activity of azomethine ligands and their Co(II)-azomethine coordination complexes $[Co(L_n)_2Cl_2]$

S. No.	Compound	*B. thuringiensis (IZ)*			*E. coli (IZ)*		
		200	*400*	*600*	*200*	*400*	*600*
1	L_1H	12	12	14	10	12	13
2	L_2H	14	14	16	12	14	14
3	L_3H	10	12	16	8	11	14
4	L_4H	11	13	13	10	12	15
5	$[Co(L_1)_2Cl_2]$	17	17	20	17	18	20
6	$[Co(L_2)_2Cl_2]$	18	20	23	18	22	24
7	$[Co(L_3)_2Cl_2]$	14	14	16	13	14	16
8	$[Co(L_4)_2Cl_2]$	14	13	15	14	15	18
9	Ciprofloxacin	30	33	34	32	36	35

Note: IZ = Inhibition zone in mm; Concentration of compound in ppm.

Table 4: Antifungal activity of azomethine ligands and their Co(II)-azomethine coordination complexes $[Co(L_n)_2Cl_2]$

S. No.	Compound	*A. flavus (IZ)*			*A. niger (IZ)*		
		200	*400*	*600*	*200*	*400*	*600*
1	L_1H	10	10	11	9	9	10
2	L_2H	10	9	12	10	10	12
3	L_3H	8	8	10	8	9	9
4	L_4H	7	9	9	8	10	11
5	$[Co(L_1)_2Cl_2]$	18	20	20	17	20	22
6	$[Co(L_2)_2Cl_2]$	16	19	22	16	18	21
7	$[Co(L_3)_2Cl_2]$	13	13	13	14	12	15
8	$[Co(L_4)_2Cl_2]$	13	15	15	12	12	14
9	Ketoconazole	27	29	29	28	32	31

Note: IZ = Inhibition zone in mm; Concentration of compound in ppm.

involved in coordination with Co(II). The coordination complexation is further supported by the presence of a new moderate intensity absorption band between 498 to 510 cm^{-1} in the coordination complexes may be allocated to (Co←N) absorption band.

^{1}H-NMR Spectra

For chemical structural identification, the ^{1}H-NMR spectra of azomethine ligands and their Co(II)-azomethine coordination complexes $[Co(L_n)_2Cl_2]$ have been recorded in DMSO-d_6. The ^{1}H-NMR spectra exhibit a broad peak at δ 8.40–8.62 ppm due to –CH=N group and it moves downfield (δ 8.68–8.82 ppm) in the $[Co(L_n)_2Cl_2]$ coordination complexes due to complexation of >C=N to theCo(II) metal atom.

Antibacterial Activity

The azomethine ligands and their Co(II)-azomethine coordination complexes $[Co(L_n)_2Cl_2]$ were then characterized for the antibacterial activity against gram-positive (*Bacillus thuringiensis*) and gram-negative (*Escherichia coli*) bacterial strains. These activities were determined by using the agar well diffusion method. Antibacterial activities of representative azomethine ligands and their Co(II)-azomethine coordination complexes $[Co(L_n)_2Cl_2]$ have been screened and reported in Table 3. All test cultures were streaked on nutrient agar medium and incubated overnight at 30°C. The results of these studies showed that there is a considerable increase in the toxicity of the Co(II)-azomethine coordination complexes $[Co(L_n)_2Cl_2]$ as compared to the free azomethine ligands. The results of these studies were compared with the standard commercial control antibiotic, ciprofloxacin. It was found that the activity increased with increasing concentration of metal-ligand complexes. The result further shows that Co(II)-azomethine coordination complexes $[Co(L_n)_2Cl_2]$ were more active than the free ligands, which indicates that metalation increases the biological activity of ligands.

Antifungal Activity

The antifungal activity was investigated *in-vitro* against *A. flavus* and *A. niger* fungi by using agar well diffusion method. The fungi were sub-cultured on potato dextrose agar (PDA) medium and incubated at 32°C for 72 hours. Here, ketoconazole was used as a reference compound for antifungal activities. It was found that the activity increased with increasing concentration of metal-ligand complexes. The result further shows that Co(II)-azomethine coordination

complexes $[Co(L_n)_2Cl_2]$ were more active than the free azomethine ligands, which indicates that metalation increases the biological activity of ligands (Table 4).

CONCLUSION

In the research work, the authors report the synthesis of novel biologically active cobalt-azomethine coordination complexes $[Co(L_n)_2Cl_2]$ by the use of azomethine ligands and cobalt chloride ($CoCl_2$). The azomethine ligands have been synthesized by the condensation of hydrazine hydrate with aldehydes and ketones, which was then used to synthesize novel Co(II) azomethine coordination complexes $[Co(L_n)_2Cl_2]$. The azomethine ligands and their biologically active Co(II)-azomethine coordination complexes $[Co(L_n)_2Cl_2]$ were characterized for molecular weight determination, elemental analysis, ^{1}H-NMR and FTIR spectroscopy. The azomethine ligands and their Co(II)-azomethine coordination complexes $[Co(L_n)_2Cl_2]$ have also been characterized for the antimicrobial activity against several bacterial and fungal species, which has been associated with the standard bactericide ciprofloxacin and fungicide ketoconazole. The outcome of the research has indicated that Co(II)-azomethine coordination complexes $[Co(L_n)_2Cl_2]$ were found to retain excess antimicrobial activity against bacterial and fungal species as compared to the free azomethine ligands. Therefore, the complexes showed good toxicity against bacteria and fungi.

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CONFLICT OF INTEREST

None declared.

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None declared.

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