SYNTHESIS, CHARACTERIZATION, SPECTRAL STUDIES AND ANTIMICROBIAL APPLICATION OF LIGAND N², N⁶-BIS (5-NITROTHIAZOL-2-YL) PYRIDINE 2, 6-DICARBOXAMIDE AND ITS COMPLEXES OF Cu(II), Ni(II) and Fe(III)

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ABSTRACT

The ligand N²,N⁶-bis(5-Nitrothiazol-2-yl)pyridine 2, 6-dicarboxamide ligand was synthesized by the reaction of pyridine 2,6 dicarboxylic acid and 2-amino 5-nitro thiazole. The synthesized ligand was reacted in THF with salts of metal (Cu, Ni and Fe) to form complexes. Deprotonated ligand shows tridentate nature and coordinates to metal ion to form pincer cavity. In all Complexes, metals were surrounded by three nitrogen atoms and other sites coordinated by other groups (Chloride, Acetate). All the synthesized complexes were characterized by spectroscopic techniques and physical measurements like UV Visible, ¹³C NMR, and ¹H NMR, IR spectroscopy, DSC and elemental analysis and molar conductance measurements. The ligand and its complexes were tested for antimicrobial activity against bacterial and fungal strains by determining inhibition zone, minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). The complexes showed moderate antimicrobial activity when tested against bacterial strains

Keywords: Pyridine 2, 6-dicarboxamide, Triphenyl phosphite, Metal Complexes, 2-Amino-5-nitro thiazole, Antimicrobial Activity.

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INTRODUCTION

The novel amide based ligands are most important and valuable in coordination chemistry¹⁻³ and in medicinal chemistry.⁴⁻⁵ The amide functional organic group found in naturally occurring molecules, polymer, and agrochemicals.⁶⁻⁷ Such novel type of ligands after donating a proton to form deprotonated ligand which forms a pincer cavity. The cavity formed by two Nitrogen of two amide groups and one nitrogen atom from pyridine ring so they show strong coordination bonding towards metal ions. Fiedler and co-workers have used pyridine 2, 6-dicarboxamide ligand appended with pyridine and quinolone ring to synthesized Cu(II) complex.⁸ There are so many compounds of amide group-containing Cu complexes reported in earlier.⁹⁻¹² Other ruthenium complexes were reported.¹³⁻¹⁴ Deepak and co-workers have used to synthesize ligand having appended groups thiazole, thiazoline and benzothiazole.¹⁵ This mode of coordination makes it attractive in coordination chemistry and in supramolecular structure.¹⁶⁻¹⁷ There are so many complexes synthesized and reported in the literature such as Ni (II)¹⁸⁻²⁷, Fe(III).²⁸⁻³³ In this paper we have used pyridine 2, 6-dicarboxamide scaffold with appended group thiazole derivative. Synthesized ligand (H₂L) used to synthesize complexes of Cu(II) (1), Ni(II) (2) and Fe(III) (3) (Scheme 1). These well-characterized complexes were tested for microbial activity.

EXPERIMENTAL

All the chemicals were used of analytical grade and used without purification. The ¹H and ¹³C NMR spectral studies done by using Bruker 300 MHz NMR spectrometer. ¹H NMR chemical shifts were

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reported relative to residual solvent peaks. IR spectra were recorded in KBr pellets with Perkin FT-IR spectrophotometer in the 4000–400 cm^{-1} region. Elemental analysis of synthesized compounds was estimated by the Elemental GmbH Vario EL-3 instrument. Perkin UV-Visible spectrophotometer was used for the characterization of UV spectra. The ES-Mass Spectra were recorded on Agilent Q-TOF LC-MS Mass Spectrometer. The melting points of the compounds measured by the melting point apparatus.

Synthesis of Ligand (Scheme-1)

Synthesis of ligand(H_2L)

Pyridine 2, 6-dicarboxylic acid (0.668g, 0.004mol) and 2-amino 5-nitro thiazole (1.16 g, 0.008mol) were taken in 15 ml pyridine and refluxed with stirring for 30 min at 110°C. Triphenyl Phosphite (1.32 g, 0.0084mol) was added to the reaction mixture drop-wise and the reaction mixture was further stirred at 100 °C for 6 hr. After completion of the reaction, the reaction mixture was cooled below room temperature and poured into ice-cold water. Due to the pouring of the mixture, a pale yellow precipitate formed. Filter the precipitate and washed with water and dried over the air. Yield 1.39 g (76.3%). Anal. Cal for $\text{C}_{13}\text{H}_7\text{N}_7\text{O}_6\text{S}_2$ C, 37.06, H.1.67, N.23.27, S, 15.22, O, 22.78, Found: C, 37.26, H.1.76, N.23.40, S.15.30, and O, 22.70. M.P:212°C, MS (ESI- CH_3OH , H_2O , m/z): 471.95 for ligand .Found for M^+ 472.07, FT-IR Spectrum (ATR, cm^{-1}): 3520(NH), 1680(C=O) cm^{-1} . ^1H NMR spectrum (400MHz, DMSO): 8.94 (d, J = 5.1, 2H), 7.80-8.78 (m), 6.82-7.80 (m, 0n), δ 9.29 (s, 0H). ^{13}C NMR (126 MHz, DMSO) δ 151.08, 140.22, 130.80, 129.79, 126.50, 126.84, 122.35, 121.39, 72.78, 77.00.

Synthesis of Metal Complexes (Scheme-2, 3 and 4)

Synthesis of $[\text{Cu}(\text{L})(\text{CH}_3\text{COO})_2]$

To a THF (10 ml) solution of ligand(0.421g, 0.001 mmol) and $[\text{Cu}(\text{CH}_3\text{COO})_2]$ (0.181g, 0.001 mmol) dissolved in THF (10 ml) was added drop-wise with stirring. The resulting solution of ligand and metal salt formed mixture. The solution mixture stirred for 2 hr. The reaction mixture was filtered and the filtrate was left for evaporation. The bluish crystalline compound appeared after 10-12 hr. The product remains after evaporation washed with diethyl ether and dried over the air. Yield: 0.511 g (85%) M.P.245°C, MS (ESI- Methanol, m/z):541.52. FTIR spectrum ATR, cm^{-1}): 1645(C=O) cm^{-1} . ^1H NMR spectrum (400MHz, DMSO):8.94 (d, J = 5.1, 2H), 7.80-8.78 (m), 6.82-7.80 (m, 0n), δ 9.29 (s, 0H). ^{13}C NMR (126 MHz, DMSO) δ 151.08, 140.22, 130.80, 129.79, 126.50, 126.84, 122.35, 121.39, 72.78, 77.00.

Synthesis of $[\text{Ni}(\text{L})\text{Cl}_2]$

To a THF (10 ml) solution of ligand(0.252g, 0.0006 mmol) and NiCl_2 (0.149 g, 0.0006 mmol) dissolved in THF (10 ml) were added drop-wise while stirring. The resulting green solution stirred for 2 hr. The reaction mixture was filtered and the filtrate was left for evaporation. Light Green crystalline compound appeared after 12hr. This product was filtered, washed with diethyl ether and dried. Yield: 0.340g (85%). M.P:230°C. MS(ESI, CH_3CN , m/z) 506.85. FT-IR spectrum ATR,): 1641(C=O) cm^{-1} . ^1H NMR spectrum (400MHz, DMSO): 7.80-8.78 (m), 6.82-7.80 (m, 0n), δ 9.29 (s, 0H). ^{13}C NMR (126 MHz, DMSO) δ 151.08, 140.22, 130.80, 129.79, 126.50, 126.84, 122.35, 121.39, 72.78, 77.00

Synthesis of $[\text{Fe}(\text{L})\text{Cl}_3]$

To a THF (10 ml) solution of ligand(0.2105 g, 0.0005 mmol) and FeCl_3 (0.0811 g, 0.0005 mmol) dissolved in THF (10 ml) were added drop-wise while stirring. The resulting brown solution was finally stirred for 2 hr. The reaction mixture was filtered and the filtrate left for evaporation. The dark brown crystalline compound appeared after 12hr. This product was filtered, washed with diethyl ether and dried. Yield: 0.275g (94%). M.P:217°C, MS (ESI, Methanol, m/z):577.25.91, FT-IR spectrum ATR: 1645(C=O) cm^{-1} . ^1H NMR spectrum (400MHz, DMSO): 7.80-8.78 (m), 6.82-7.80 (m, 0n), δ 9.29 (s, 0H). ^{13}C NMR (126 MHz, DMSO) δ 151.08, 140.22, 130.80, 129.79, 126.50, 126.84, 122.35, 121.39, 72.78, 77.00

Scheme.1. Synthesis of Ligands 1

Scheme-2.Synthesis of Metal complexes of Cu.

Scheme.3.Synthesis of Metal Complexes of Ni

Scheme.4.Synthesis of Metal Complexes of Fe

RESULTS AND DISCUSSION

In this research paper, we reported the synthesis, and characterization of three novels Cu, Ni, and Fe complexes of ligand N², N⁶-bis (5-nitrothiazol-2-yl)pyridine-2,6-dicarboxamide. All the synthesized complexes were characterized by different spectroscopic techniques such as mass, UV-visible and IR spectroscopy, and Elemental analysis. The new ligand (H₂L) was synthesized by the reaction of pyridine 2, 6-dicarboxylic acid and 2-amino-5-nitro thiazole using pyridine as a solvent in the presence of triphenyl phosphite with stirring. The mass spectrum of ligand (fig.8) shows a molecular ion peak at m/z=472.02. The ¹H NMR showed one broad singlet for the NH at δ 9.29 in CDCl₃ solvent and the five

aromatic resonances were found which integrate into ten protons (Fig.-2). Besides these results, the Coupling between the protons is also clearly visible in spectra. Additionally, in NMR spectra the proton decoupled. ^{13}C NMR spectrum of the ligand was also simple, and showed that all carbon centers were easily identified and characterized. (Fig.-1). In ^{13}C NMR spectra, the $\text{C}=\text{O}$ carbon peak was Observed at 151.08 ppm. The FT-IR spectrum of the ligand showed two peaks, NH and CO stretching peaks. These two peaks confirm the formation of amide linkage and observed at 3375 and 1681 cm^{-1} respectively. Complexes 1-3 were synthesized the reaction of one equivalent of deprotonated ligand and one equivalent of respective salt of Cu (Cupric acetate), Ni (Nickel chloride) and Fe (Ferric chloride) in THF with stirring. The colored Complexes were formed. The color of copper complexes is dark bluish and Nickel has light green while Fe complex dark brown. The yield of these complexes was 80-85%. FT-IR spectra of ligand and complexes reveal that the formation of new functional groups during the synthesis of ligand and their metal complexes. The FT-IR spectra of complexes 1, 2, and 3 do not show the NH stretching peaks which indicates the absence of amidic proton of the amide group compared to the free ligand (Fig.-3,4 and 5) These peaks evidence strongly suggest that anionic N amide involved in the formation of coordination bond between ligand and metals. The CO stretching frequency of metal complexes 1-3 was 1645, 1641, 1645 cm^{-1} respectively. The $\nu\text{C}=\text{O}$ stretches of complexes 1to3 were compared to the ligand and metal complexes the frequency shifting towards red-shifted by 35-45 cm^{-1} than the ligand and it was concluded that the involvements of deprotonation take place. The other metal nitrogen bond stretching frequency also confirmed the metal-ligand bonding. The absorption spectra of ligands H_2L exhibits λ_{max} at 320(fig.6). The Complexes 1, 2, shows λ_{max} at 350, 348, show bathochromically shifted whereas complexes 3 exhibit spectral features at 310(fig.7). From the UV spectra, it's clear that The Cu and Ni complex exhibit two absorption peaks at 210 nm, 270 and 350 nm, 347 respectively, which is assigned to $\pi-\pi^*$, MLCT transition respectively, suggesting a tetrahedral geometry. The Fe complex shows two strong bands at 310 and 430 nm shows octahedral geometry. In the mass spectra of complexes 1, 2 and 3, the molecular ion peaks are at $m/z=541.52$, $m/z=461.98$ and $m/z=591.91$ for Cu, Ni and Fe respectively. All peaks were matched well with the theoretically calculated values, thus assured the stability of the metal complexes in solution.

Fig.-1: ^{13}C NMR Spectra of Ligand Fig.-2: ^1H NMR Spectra of Ligand Fig.-3: IR Spectra of Fe^{+3} Complex Fig.-4: IR Spectra of Ni^{+2} Complex

Fig.-5: IR Spectra of Cu⁺² Complex

Fig.-6: UV Spectra of Ligand

Fig.-7: UV Spectra of Ligand with Metal Complexes

Fig.-8: Mass spectra of Ligand

Antimicrobial Activity

In this study, the synthesized ligand and metal complexes used for the screening of antimicrobial activity against bacteria *Staphylococcus aureus*, *E. coli* and *Salmonella typhi* and fungi *Candida albicans*, *Aspergillus Niger* and *Cladosporis*. This type of study carried out by agar diffusion method which is the standard method. The stock solution of 1mg/mL prepared by using and dissolving 10 mg in 10 mL of each compound which was used for testing. After getting the desired concentration 30µg/ml, 60µg/ml and 120µg/ml diluted to the required quantity. Microbes culture prepared in agar in Petridis and incubated at 37 °C for 24-30 hr. Standard drugs used for comparison were also incubated in the same condition.

The antimicrobial screening suggests that pyridine-2,6- dicarboxamide based ligand and its metal complexes were found potentially active against bacteria and fungi. They showed enhance activity due to the chelate formation tendency of amide based ligands. In the antibacterial screening it showed that the ligand was found biologically active against *Staphylococcus aureus*, *E. coli* and *Salmonella typhi* while the metal complexes of Cu, Ni and Fe showed more potential activity as compared to the ligand. The activity of metal complexes against bacteria was almost similar to standard drug *Gentamycin*. For antifungal activity the standard drug used as *Nystatin*. The metal complexes of Cu, Ni and Fe showed antifungal activity nearer to a standard drug taken. All data were given in Table-1.

Table-1: Antimicrobial Activity and Minimum Inhibitor Concentration of Ligand and Metal Complexes

S. No.	Compounds	Conc. µg/ml	Bacteria (% Inhibition)			Fungi (% Inhibition)		
			<i>Staphylococcus</i>	<i>E.coli</i>	<i>S.Typhi</i>	<i>C.albicans</i>	<i>A.niger</i>	<i>Cladosporis</i>
1	H ₂ L(Ligand)	30	19.14	7.50	28.29	86.11	52.24	48.56
		60	25.52	26.23	47.13	93.25	78.12	75.29
		120	42.2	105.2	62.15	95.17	85.27	89.20
2	Cu-L	30	6.70	29.8	47.65	87.22	59.39	75.27

	Complex	60	12.30	71.42	66.24	92.27	61.01	84.19
		120	19.60	88.32	85.65	97.23	71.15	90.25
3	Ni-L Complex	30	11.60	35.23	48.29	85.25	55.15	77.31
		60	27.69	68.22	71.32	92.29	67.39	80.17
		120	34.75	88.33	84.29	94.26	76.16	93.29
4	Fe-L Complex	30	19.35	47.65	58.56	74.39	49.21	45.35
		60	25.20	75.62	67.22	76.23	78.15	67.19
		120	28.30	85.24	82.39	85.17	78.28	74.22
5	Gentamycin	30	6.89	88.24	61.9	-	-	-
		60	9.10	88.37	74.25	-	-	-
		120	17.20	88.37	84.95	-	-	-
6	Nystatin	30	-	-	-	84.31	92.3	88.09
		60	-	-	-	94.1	94.87	92.85
		120	-	-	-	96.08	97.43	97.61

CONCLUSION

The newly synthesized pyridine-2, 6- dicarboxamide based ligand act as a tridentate ligand. Ligand has three nitrogen donor atoms one from pyridine ring and two from amide group after deprotonation. The ligand forms a heterocyclic pincer cavity in which metal ion coordinated by donor atom and form metal complexes. The synthesized metal complexes bonding and structures confirmed by different spectroscopic studies and physical measurement techniques. The FT-IR, NMR and Mass spectra reveal their formation and confirmation about the structure of compounds. Elemental analysis gave data about the confirmation of elemental compositions. From the application point of view, the antimicrobial studies showed the behavior and potential activity towards microbes.

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