Original Article

Synthesis, Characterization, Biological and Photoluminescence Study of Co(II) Complexes of Fused Heterocyclic Ring Systems

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ABSTRACT

Aim: To synthesize and characterize the transition metal complexes of fused heteroccylic ring systems. **Background:** Many of the fused ring heterocyclic compounds were found to be biologically active in the literature like anti-microbial, anti-cancer, anti-inflammatory, antioxidant and so on. The present work focus on the synthesis of the biologically active compounds. **Materials and Methods:** Synthesis of the ligands and the complexes are done using the standard procedures in earlier reports. Melting points were found using open glass capillaries on a Raaga melting point apparatus and are uncorrected. The percentage of C, H, N , infra-red and UV-Visible spectra of the compounds were recorded using Elementar Vario EL III CHN analyser, Shimadzu spectrophotometer (4000-400 cm^{-1}) and Elico SL 159 UV-Vis spectrophotometer respectively. The anti-microbial activity of the compounds was carried out by well diffusion method. The anti-cancer activity of the compounds were carried out for the MCF-7 cell line (breast Cancer) using MTT assay. **Discussion and Conclusion:** Structural elucidation of newly synthesized Co(II) metal complexes of fused heterocyclic ring systems were done using various spectral techniques like FT-IR, ^{1}H-NMR, Electronic and TGA-DTA studies. The anti-microbial activity of the prepared complexes and the DNA Cleavage studies were screened for various test pathogens and explained. The photoluminescence property of the fused heterocyclic ligand and their complexes were studied and compared. Fluorescence enhancement is observed in these complexes which is contradictory to the normal Fluorescence quenching phenomena by added organic derivatives, paved way for photochemical applications.

Key words: Tetrazoloquinoxaline, Fused heterocycles, Anti-microbial, Flouresence, DNA Cleavage, Cobalt metal complexes.

INTRODUCTION

Heterocyclic compounds like pyrimidines, oxadiazoles, imidazoles, triazoles, benzothiazoles and so on play a significant part in the research of clinically active materials, also have gained significant interest among the researchers in the past years. Fused heterocyclic compounds like quinoxalinopyrimidine, pyrazoloquinoxaline. Pyrazolopyrimidines and so on were expected to have increased biological activity. Based on the literature reports with fused heterocyclic compounds having increased potency for biological applications, tetrazoloquinoxalines, derivatives starting from dichloroquinoxaline were synthesized and characterized using spectral techniques. The development of Photoluminesent materials from fused heterocyclic compounds has been reported in the earlier reports.[1-3] These important properties of the mixed ring systems created an interest in us to study some of the biological photoluminecent properties of the synthesized fused heterocyclic compounds and their Co(II) complexes.

Experimental

The detailed procedures for materials and methods and their biological activities are given in the supplementary information.

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Synthesis of ligands

Synthesis of ligand 1 (L^1): 1-substituted benzylidenehydrazino-2(tetrazolo-[1, 5-a]quinoxa lin-4-yl derivatives: The synthesis of the above mentioned ligand involves three stages and it is synthesized as reported in the literature.[1] The analytical data of the ligand is shown as follows;

m.pt: 192-194°C. IR(cm^{-1}): 3042 (Ar-CH), 3298 (-NH), 1587 (-N=N-), 1562 (>C=N), 1532 (>C-N). ^{1}H-NMR (DMSO-d^6): δ 6.96 – 7.95 (m, 8H, Ar-H), 3.87 (s, 1H, NH), 8.02 (s, 1H, -CH=N). Anal calcd (%) for $C_{15}H_{11}N_7O$ % C, 59.71; H, 3.35; N, 32.80; Found (%): C, 59.74; H, 3.38; N, 32.83.

Synthesis of ligand 2 (L^2): 2,3-dichloroquinoxaline (0.19g, 0.001mol) and 4-amino-5-pyridin-4-yl-4H-[1,2,4] triazole-3-thiol (0.19g, 0.001mol) in 50 ml of DMF were refluxed for 3 h. The product obtained was filtered and recrystallised from dichloromethane. Yield (84%), m.pt. 192-194°C, mass (m/z): 319.13. IR (KBr) cm^{-1}: 3042 (Ar-CH), 3318 (-NH), 1656 (C=N), 1533 (C-N). ^{1}H-NMR (DMSO-d^6): δ 7.35 – 8.04 (m, 8H, Ar-H), 5.03 (s, 1H, NH).Anal calcd (%) for $C_{15}H_{19}N_7S$ % C, 56.42; H, 2.84; N, 30.70; Found (%): C, 56.34; H, 2.88; N, 30.74.

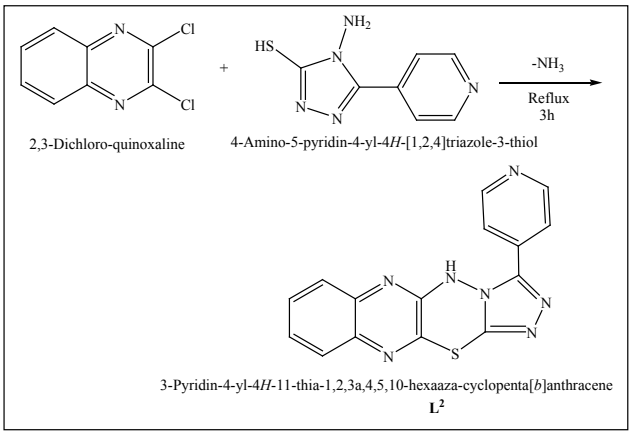
Synthesis of ligand 3 (L^3): 2,3-dichloroquinoxaline (0.19g, 0.001mol) and 4-amino-5-pyridin-4-yl-4H-[1,2,4] triazole-3-thiol (0.19g, 0.001mol) in 50 ml of DMF were refluxed for 3 h. The product obtained was filtered and recrystallised from dichloromethane. Yield (84%), m.pt. 192-194°C, mass (m/z): 319.13. IR (KBr) cm^{-1}: 3042 (Ar-CH), 3318 (-NH), 1656 (C=N), 1533 (C-N). ^{1}H-NMR (DMSO-d^6): δ 7.35 – 8.04 (m, 8H, Ar-H), 5.03 (s, 1H, NH).Anal calcd (%) for $C_{15}H_{19}N_7S$ % C, 56.42; H, 2.84; N, 30.70; Found (%): C, 56.34; H, 2.88; N, 30.74.

Synthesis of complexes

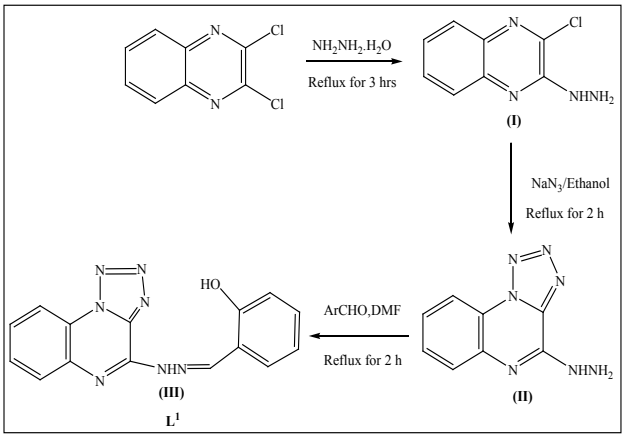
Synthesis of the complexes were done using procedures reported in our earlier reports. The product formed was an orange colour solid. It was filtered off and it was recrystallised from ethanol .Yield: 65%. m.pt: 241-244°C. Anal. calcd (%) for $C_{16}H_{15}ClCoN_7O_2$: C, 44.51; H, 3.50; N, 22.71, Found(%):C,44.17;H,3.56;N,22.45.

Synthesis of Co(II) complex 5: About 1 mmol of L^2 was dissolved in methanolic solution (20 mL) and cobaltous chloride (0. 2g, 1mmol) were added to it in a round bottom flask and refluxed for 5h. The resulting mixture was then cooled to room temperature, which resulted in the formation of dark brown coloured precipitate. It was filtered off and the solid was recrystallised from ethanol .Yield: 65%. m.pt: 241-244°C. Anal. calcd (%) for $C_{30}H_{22}Cl_2CoN_{14}S_2$: C, 46.61; H, 2.87; N, 25.38, Found (%): C, 46.57;H,2.76; N,25.51.

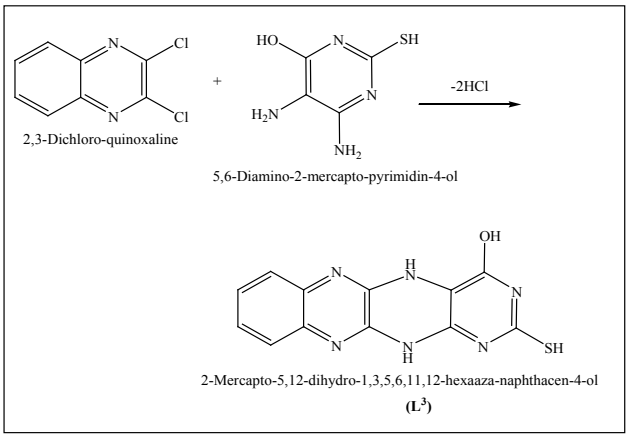
Synthesis of Co(II) complex 6: About 1 mmol of L^3 dissoved in methanol(20mL) and cobaltous chloride (0. 2g, 1mmol) were added in a round bottom flask and refluxed for 5h. The resulting mixture was then cooled to room temperature, which resulted in the formation of brown coloured precipitate. It was filtered off and the solid was recrystallised from ethanol. Yield: 65%. m.pt: 241-244°C. Anal. calcd (%) for $C_{30}H_{22}Cl_2CoN_{14}S_2$:



Scheme 2: Synthesis of ligand 2 (L^2).



Scheme 1: Synthesis of ligand 1 (L^1).



Scheme 3: Synthesis of ligand 3 (L^3).

C, 46.61; H, 2.87; N, 25.38, Found (%): C, 46.57;H,2.76; N,25.51. The Scheme of Complexes are given in SI. 1a to 1c respectively.

RESULTS AND DISCUSSION

Structural description of the ligands

Spectral Characterization like IR, UV-visible spectra and ^{1}H-NMR has been taken for the synthesized quinoxalino derivatives. In the ^{1}H-NMR spectra of ligand L^1, a signal at δ 8. 02 ppm is characteristic of >CH=N proton. The signals appeared at δ 3.80 and δ 4.60 ppm are attributed to the >NH and the -OH protons respectively. The aromatic protons of the quinoxalino derivatives were observed in the region δ 6.96– 7.95 ppm. The ^{1}H-NMR spectrum of the ligand L^2 shows the following signals at the following ppm values; δ 7.35 – 8.04 ppm due to the aromatic protons and δ 5.03ppm due to the >NH protons. The ^{1}H-NMR spectrum of the ligand L^3 attributes the following signals at the values; δ 6.90 ppm and δ 7.10 ppm due to the aromatic protons, δ 4.40 ppm due to SH proton, δ 3.60 ppm due to NH proton and δ 13.80 ppm due to hydroxyl proton. These values confirm the structure of the ligands and their NMR spectrum are given in Figure SI. 1a to 1c for L^1, L^2 and L^3 respectively.

FT-IR spectra

The prominent FT-IR spectral information of the ligands and its metal complexes are presented in Table SI 1a. Spectrum of the ligand (L^1) shows band at 3398 cm^{-1} which is attributed to phenolic –OH group. This band also appeared in the complex 4 creating a doubt about the co-ordination of phenolic –OH with the metal but the existence of deprotonated phenolic –OH group in Co(II) complex 4 is confirmed by the shift of γ(C-O) stretching band observed at 1203 cm^{-1} in the free ligand to higher frequency at 1221 cm^{-1} proving its co-ordination. The azomethine and C=N(thiazole) vibration of the ligand at 1562 cm^{-1} and 1434 cm^{-1} was shifted to lower frequency after complexation respectively confirming the co-ordination to the metal. The band at 1587cm^{-1} remains unchanged in complexes compared to the free ligands suggesting the non-involvement of N=N. The FT-IR spectral values of ligand 2 (L^2) and its complex 5 are as follows; the –NH stretching frequency of the ligand and the complex appears around 3300cm^{-1}. The >C=N stretching frequency of the quinoxaline ring in the ligand appeared at 1680 cm^{-1} and this has been shifted to 1656 cm^{-1} proving its co-ordination to the metal. The other stretching frequencies like >N-N<, >C=N (thiazole) appears at 1442, 1527 cm^{-1} in the ligand and its Co(II) complex proving its non-involvement in co-ordination. The FT-IR spectral values of ligand (L^3) and its complex 6 are as follows; the –NH and the –OH stretching frequencies appears around 3300cm^{-1}. The >C=N stretching frequency in the ligand appears at 1674 cm^{-1} and this has been shifted to 1615 cm^{-1} proving its co-ordination to the metal. The other stretching frequencies like >C=N (pyrimidine), C=S, appears at 1530 and 1393 cm^{-1} for the ligand and 1528 and 1390 cm^{-1} in the complex and the appearance of these bands at same region without considerable shift in the complexes show the non-involvement of these groups in co-ordination.[4-6] The stretching frequencies of the ligand and the complexes are shown in Table SI 1a and the FT-IR spectra of the ligand and the complexes (1-6) are shown in Supplementary Information (SI) Figure SI. 2a to 2f respectively.

Electronic spectra

The electronic spectra of the ligand and the complexes were recorded using DMSO as the solvent. The cobalt(II) complex exhibited four bands having λ_{max} at 298, 368, 432 and 440 nm (Figure 1). The band at 328 nm assigned to charge transfer band and last three bands could be assigned for corresponding d-d transitions, due to $^4T_{1g} \rightarrow {}^4T_{2g}$, $^4T_{1g} \rightarrow {}^4A_{2g}$ and $^4T_{1g} \rightarrow {}^4T_{1g}$ which justified the octahedral geometry of Co(II) complex. The analytical, CHN analysis and metal estimation data, it seems to be tetrahedral complex and the characteristic band at 611 nm is absent due to the involvement of DMSO in the trans position making it distorted octahedral geometry in solution state.[7,8]

Thermal analysis

The simultaneous TGA/DTA analysis of the Co(II) complexes of the three ligands was studied from ambient temperature to 1000°C under nitrogen atmosphere.[9] The TGA curve of the Co(II) complex 4 exhibited a mass loss at 51.42°C which corresponds to the loss of lattice water and the chloride and the organic part of the complex is lost at two different stages at 325.66°C and 466.02°C with a total mass loss of 82.11 % (calcd.81.90 %) and leaving 17.89% CoO as residue (calcd. 16.95%). The TGA/DTA curve of the Co(II) complex 5 exhibited a mass loss at 253.60°C and 380.29°C which is attributed to the co-ordinated part of the ligand and chloride molecule. The stability of the complex till 253.60°C shows the absence of lattice and co-ordinated water molecule. The total mass loss is 86.77 % (calcd. 85.12 %) with CoO as residue. The TGA curve of the Co(II) complex 6 shows a single decomposition peak at 286.37°C for the loss of organic fragments leaving

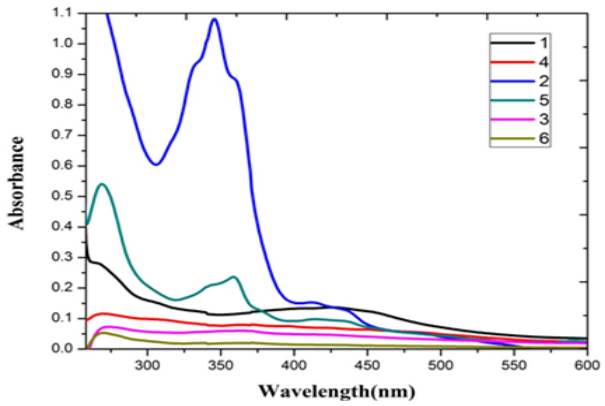


Figure 1: Electronic spectrum of the complexes.

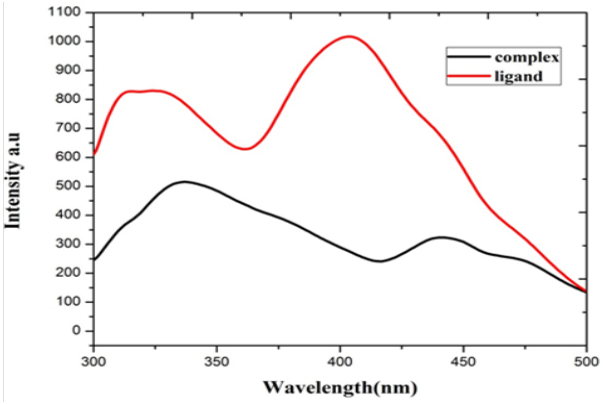


Figure 3A: Fluorescence spectra of the ligand L^1 and its Co(II) complex 4.

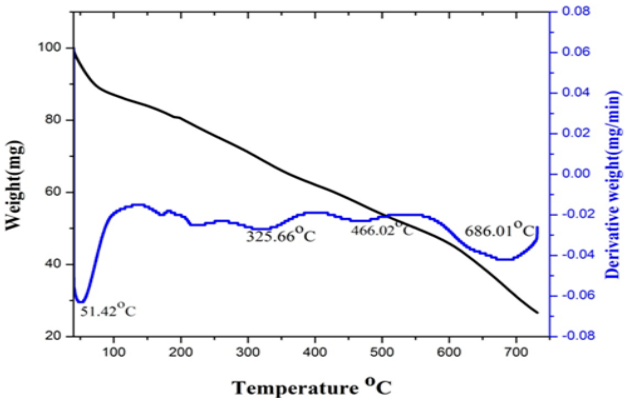


Figure 2: Thermogram of Co(II) complex 4.

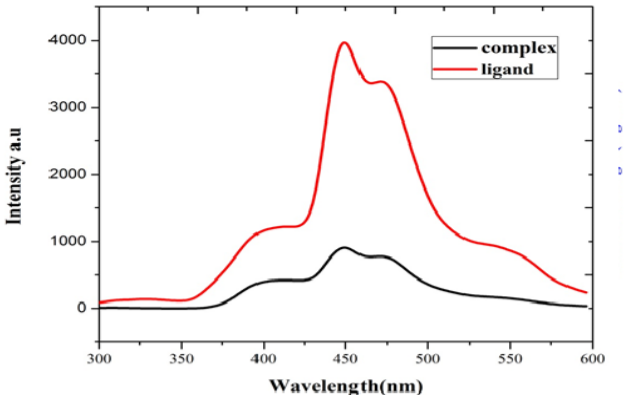


Figure 3B: Fluorescence spectra of the ligand L^2 and its Co(II) complex 5.

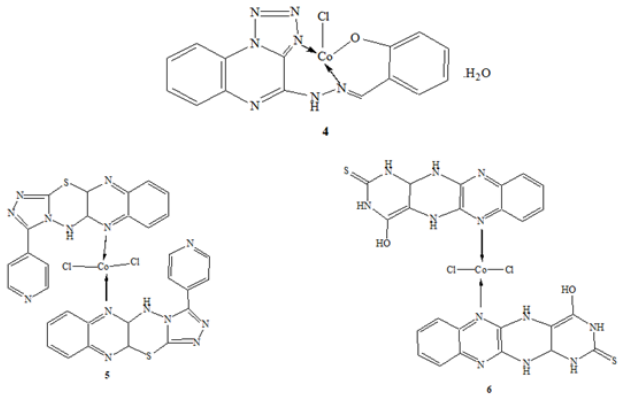


Figure 2A: Proposed geometry of the complexes.

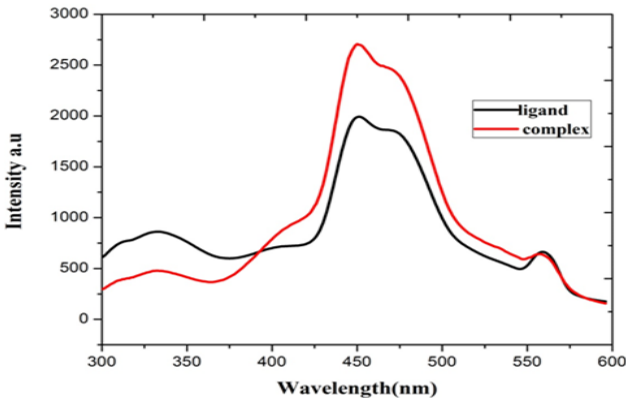


Figure 3C: Fluorescence spectra of the ligand L^3 and its Co(II) complex 6.

behind CoO as residue with 18.76 % (calcd. 12. 34 %). The thermograms of the complex 4 is given in Figure 2, SI. 3a and b respectively. The proposed geometry of the complexes is given in Figure 2a.

Photoluminescence spectra

The photoluminescence properties of the quinoxalino derivatives and their complexes ((10^{-4} M solution in DMSO) were studied at RT. Excitation and emission slit widths were set at 10 nm with a scan speed of 500 nm/min. The maxima peak was observed at 450nm in the photoluminescence spectra. The Co(II) complex of ligand 2 show the strongest quenching compared to ligand 1 as an usual change that will occur in photoluminescence spectra. As shown in Figure 3a, the ligand exhibited an emission at 410nm which shifted to 445nm upon binding to the cobalt metal ion. A decrease in the emission intensity was observed with

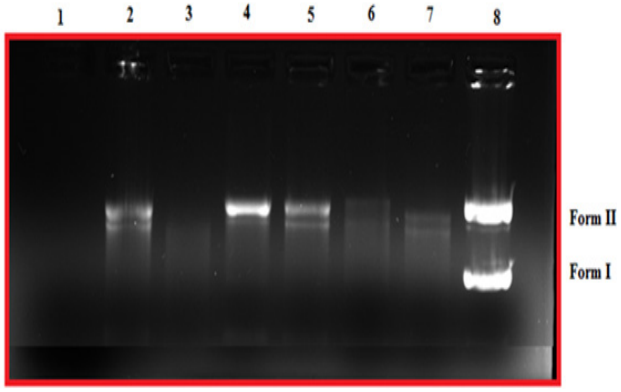


Figure 4: Chanes in the agarose gel electrophoretic pattern of pBr322 DNA indiced by H_2O_2 and Co(III) complexes, lane 1, DNA alone; lane 2, DNA + H_2O_2; lane 3, DNA(20 µM) +4 H_2O_2; lane 4, DNA(40 µM) +4 H_2O_2; lane 5, DNA(20µM)+5+H_2O_2; lane 6, DNA(40 µM) +5 H_2O_2; lane 7, DNA(20 µM) +6+H_2O_2, lane 8, DNA(40 µM) +6 H_2O_2.

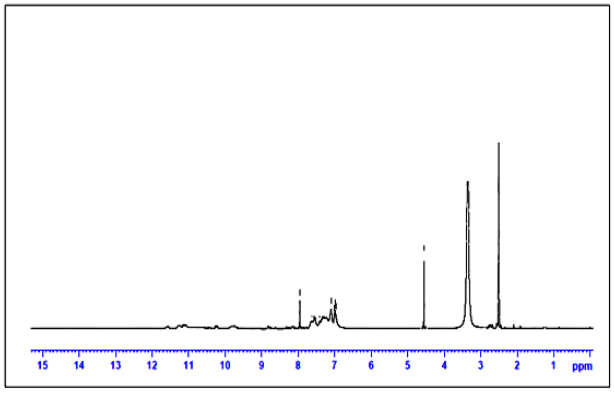


Figure S1: 1a: ^{1}H-NMR Spectrum of Ligand 1 (L^1).

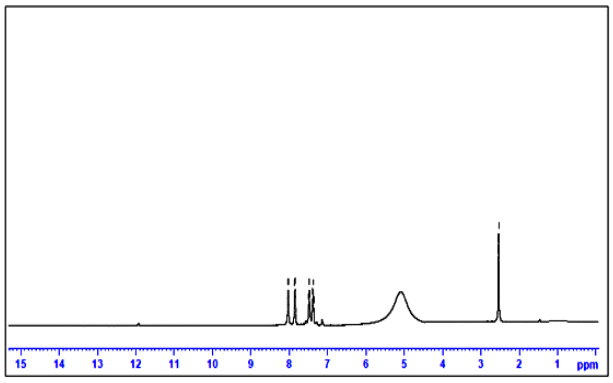


Figure S1: 1b: ^{1}H-NMR spectrum of Ligand 2 (L^2).

cobalt metal ions with ligand 1 and 2 and an increase in the emission intensity with ligand 3. Thus it is evident that the decrease in the fluorescence emission intensity is because of its complexation with Co(II) through N atoms (Figure 3A-C).[10] Quenching of flouresence is common phenomena, but in ligand 3 and its complexes,

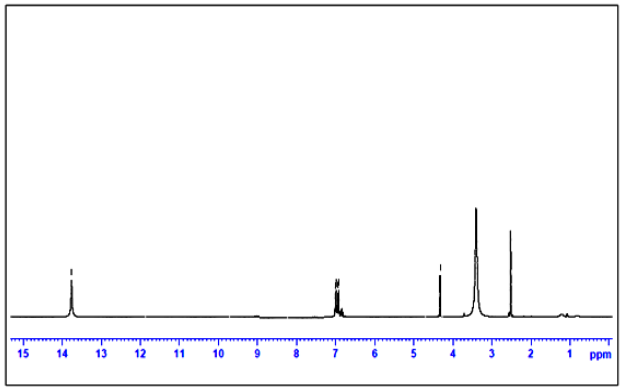


Figure S1: 1c: ^{1}H-NMR spectrum of Ligand 3 (L^3).

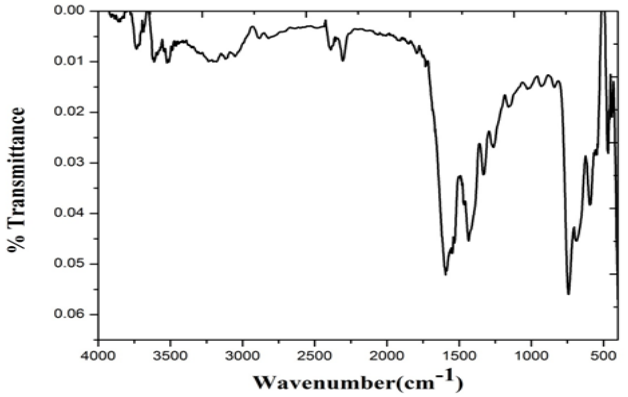


Figure S1: 2a: FT-IR spectrum of ligand 1 (L^1).

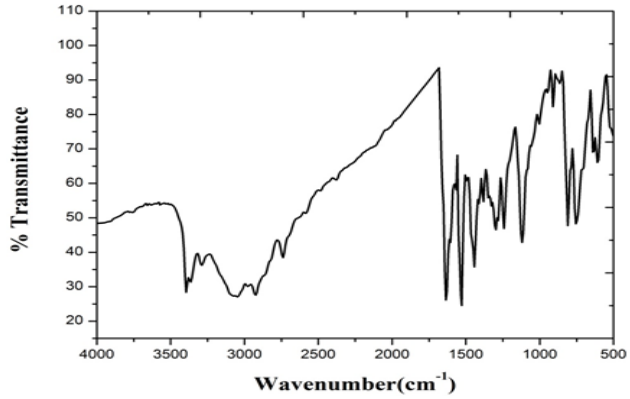


Figure S1: 2b: FT-IR spectrum of ligand 2 (L^2).

enhancement of fluorescence has taken place which pave the way to photochemical applications.

Pharmacology

Anti-microbial activity: The ligands and the Co(II) complexes were prepared and tested for their *in vitro* antimicrobial activity against six strains of microbes, which are *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae, Aspergillus niger* and *Candida albicans*. It was observed that the synthesized

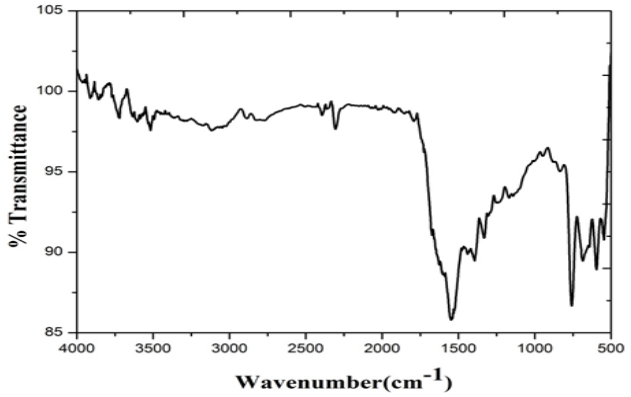


Figure S1: 2c: FT-IR spectrum of ligand 3 (L^3).

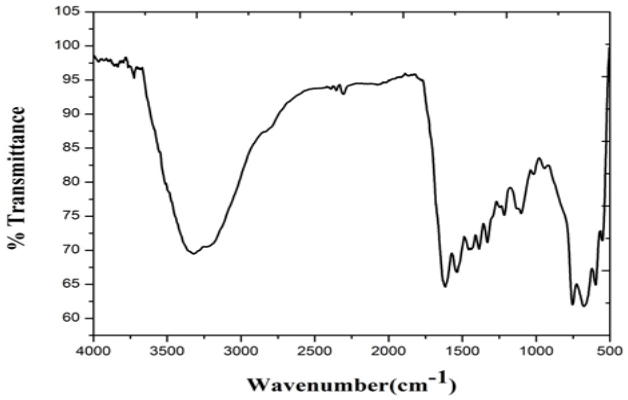


Figure S1: 2f: FT-IR spectrum of Co(II) complex 6.

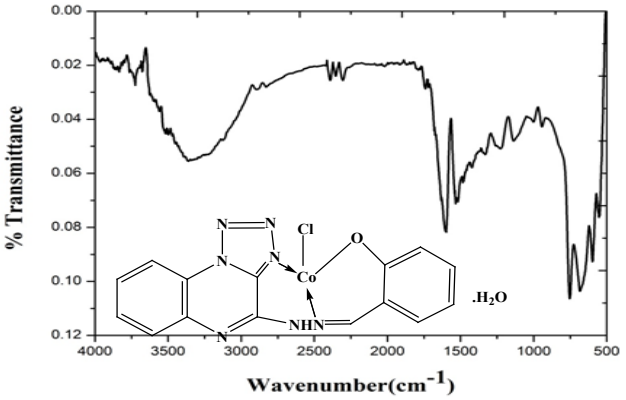


Figure S1: 2d: FT-IR spectrum of Co(II) complex 4.

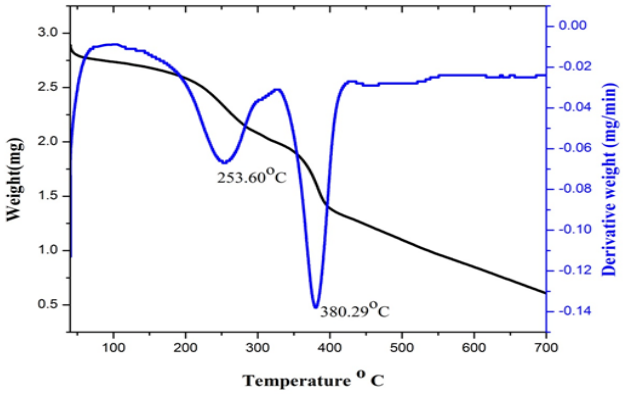


Figure S1: 3a: Thermogram of Co(II) complex 5.

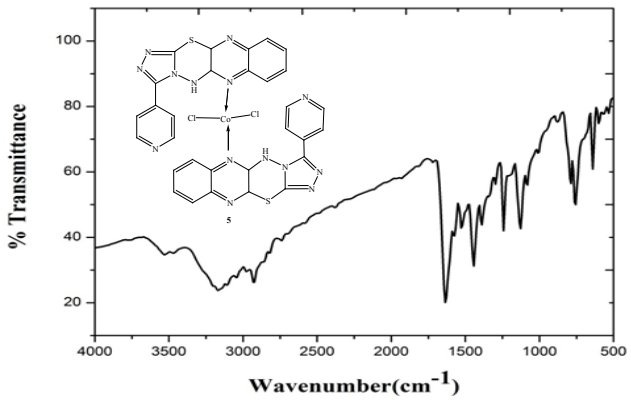


Figure S1: 2e: FT-IR spectrum of Co(II) complex 5.

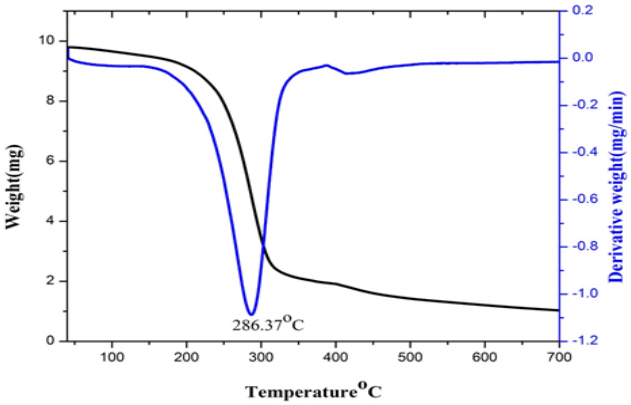
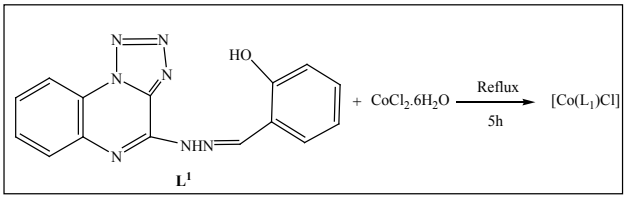


Figure S1: 3b: Thermogram of Co(II) complex 6.

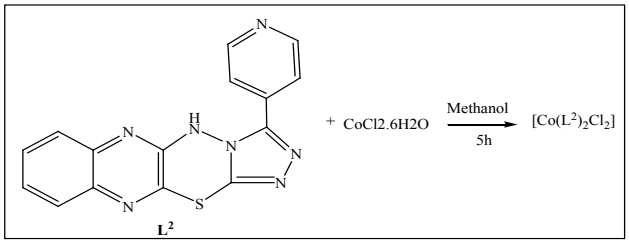
compounds showed very good antimicrobial properties. The compounds $[Co(L_2)_2Cl_2]$ and $[Co(L_3)_2Cl_2]$ were excellently equipotent against the microbial strain, *S. aureus* and moderately active against other test organisms. $[Co(L_2)_2Cl_2]$ and $[Co(L_3)_2Cl_2]$ showed optimum equipotent activity against *P.aeroginosa* and *K.pneumonia*. $[Co(L_3)_2Cl_2]$ show pronounced activity against *A. niger* and significant activity against *C. albicans*. $[Co(L^1)Cl]$ is mild active against the strains and the ligand is less active when compared to that of the complexes. Thus the presence of more heteroatoms and metal ion seems to be of great significance for antimicrobial efficacy.[11,12] The MIC values of the ligand and complexes are given in Table SI. 2.



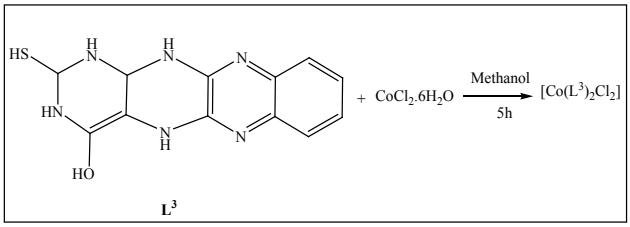
Scheme S1: 1a: Synthesis of Co(II) complex 4.

In-vitro antimicrobial activity

The antibacterial activity of the compounds were tested against the bacterial species *Pseudomonas aeroginosa, Aeromonas hydrophila, Thiobacillus thidurance, Serratia marcescens, Acinetobater baumauii, Aspergillus niger and Candida tropicalis* by the well diffusion method using agar nutrient medium. The test organisms were grown on nutrient agar for antibacterial and potato dextrose for anti-fungal in petri plates. The plates were incubated for 24 and 72 h for bacteria and fungi respectively. Then, the test solutions were diffused and the growth of the inoculated micro-organisms was affected. The inhibition zone was developed, at which the concentration of the samples was noted. DMF is used as the negative control and *Amikacin* and *Ciprofloxacin* were used as positive standards for antibacterial and *Nystatin* for anti-fungal activities. The minimum inhibitory concentration was determined by serial dilution technique.



Scheme S1: 1b: Synthesis of Co(II) complex 5.



Scheme S1: 1c. Synthesis of Co(II) complex 6.

Table S1. 1a. IR stretching frequencies of the ligand and the Co(II) complexes in cm^{-1}.			
Ligand 1 amd its Co(II) complex			
	C=N (imine)	**C=N(thiazole)**	**N=N**
L^1	1562	1434	1587
$[Co(L^1)Cl]$	1530	1421	1590
Ligand 2 and its Co(II) complex			
	C=N	N-N	**C=N(thiazole)**
L^2	1680	1442	1527
$[Co(L_2)_2Cl_2]$	1656	1442	1527
Ligand 3 and its Co(II) complex			
	C=N(pyrimidine)	**C=N(quinoxaline)**	**C=S**
L^3	1530	1674	1393
$[Co(L_3)_2Cl_2]$	1528	1615	1390

Table S1: 2. *In-vitro* antimicrobial screening data of the ligand and its Co(II) complexes (MIC in µg/ml).						
Compound	***E. coli***	***P. aeroginosa***	***S. aureus***	***K. pneumonia***	***A. niger***	***C. albicans***
L^1	>500	125	125	250	250	>500
L^2	>500	62.5	62.5	125	125	>500
L^3	>500	62.5	62.5	125	125	>500
$[Co(L^1)Cl]$	250	16.12	8.00	16.12	31.25	125
$[Co(L_2)_2Cl_2]$	125	8.00	4.00	8.00	31.25	62.5
$[Co(L_3)_2Cl_2]$	62.5	8.00	4.00	8.00	16.12	31.25
Ofloxacin	2.00	2.00	2.00	2.00	-	-
Griseofulvin	-	-	-	-	8.00	8.00

DNA Cleavage activity: The synthesized Co(III) complexes has been subjected to nuclease activity in the presence of radical scavengers. There are two possible mechanisms known to play a role in the cleavage by metallonucleases: hydrolytic and oxidative. The mechanism of which is explained in our earlier reports.[13] To elucidate the mechanism of DNA strand scission by complexes 4-6 (hydrolytic or oxidative), cleavage reactions were carried out in the presence of hydrogen peroxide.[14] The results are presented in Figure 4. The lane 1 and 2 showing studies with DNA alone revealed no change in nuclease activity. From the lanes 3 to 8 the Co(III)complexes with two different concentrations 20μM and 40μM. There is no cleavage activity ie neither complex can completely convert super-coiled DNA (I) to single nicked (II) with concentrations upto 40 μM. For both complex 4 and 5, there is no such cleavage at 10μM. At 20 μM, there is minimal amounts of form II formed for the complexes. The Co(III) complex 6, at 10μM there is only very less amounts of form II formed, but at 20 μM, the complexes show both form I and form II. Overall, these results suggest that complexes 4-6 utilize an oxidative mechanism to cleave DNA.

CONCLUSION

Co(II) complexes of fused heterocyclic compounds has been synthesized and characterized using various spectral techniques like FT-IR, electronic spectra, NMR, TGA techniques. From the results of the spectral data, a tetrahedral geometry has been suggested for the complexes. The emission properties of the ligand and the complexes has been studied and compared. In complexes 4 and 6, quenching has taken place and in complex 5, the intensity of the complex has increased. These results indicate the photoluminescent properties of these compounds and these find a great deal of potential for numerous optical and electronic applications. The *in-vitro* antimicrobial activity of the ligand and the complexes has been compared, the complexes are found to be more active than that of the ligand. The DNA cleavage activity of the complexes has been studied and found to follow non-oxidative mechanisms. These results prove that these complexes are biologically active and can be aimed for the future research for pharmaceutical applications.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

DMF: Dimethyl Formamide; **DMSO:** Dimethylsulphoxide; **RT:** Room Temperature; **MIC:** Minimum Inhibitory Concentration.

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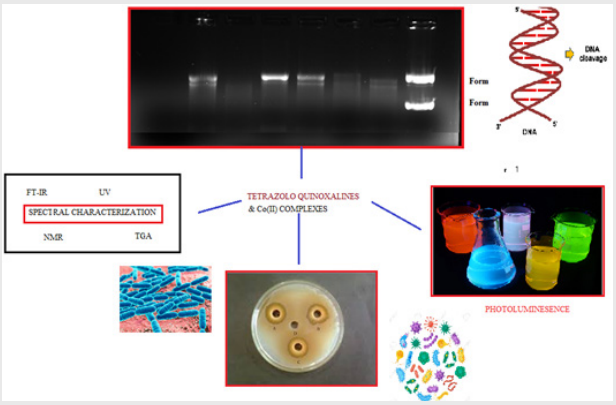
PICTORIAL ABSTRACT



SUMMARY

- Structural characterization of synthesized Co(II) metal complexes of biologically active fused heterocyclic ring systems
- Biological and Photoluminesence Properties were explored.

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