

Studies on metal(II) complexes of heterocyclic ligand derived from 4,6-bis(pyridylamino)-2-(carbazol-9-yl)-1,3,5-triazine and their biological evaluation

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Abstract

Tridentate chelate complexes of ML type (where M= Cu(II), Ni(II), and Co(II)) have been synthesized from triazine based ligand 4,6-bis(pyridylamino)-2-(carbazol-9yl)-1,3,5-triazine (BPACT). Microanalytical data, magnetic susceptibility measurements, IR, ¹H NMR, UV-vis and mass spectral techniques were used to characterise the structure of chelates. The electronic absorption spectra and magnetic susceptibility measurements suggest that metal complexes show square pyramidal geometry. The electrochemical behavior of copper(II) complex is studied by cyclic voltammetry. The second harmonic generation efficiency (SHG) of the ligand has been found to be higher than that of urea and KDP. The antimicrobial activity of the ligand and metal(II) complexes against the species *Basillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Candida albicans* and *Aspergillus niger* has been carried out and compared with the standard one. The ligand and its metal(II) complexes were screened for analgesic and anti-inflammatory activities *in vivo*.

Keywords: triazine derivative, metal complexes, Biological activity, Analgesic, Anti-inflammatory.

1. Introduction

Heterocyclic compounds have drawn attention due to their applications in pharmaceutical field [1]. Heterocyclic moieties serve as an integral part of a broad variety of biologically active natural products and synthetic compounds [2,3]. Transition metal complexes of pyridine derivatives possess versatile biological activities, such as antimicrobial, anticonvulsant, antitumor, cytotoxic, antioxidant, anti-inflammatory and analgesic activities [4,5]. Carbazole has been used as a functional building block in the fabrication of organic photoconductors, non-linear optical materials and photo refractive materials [6-8] due to its strong emission large band gap and interesting optical electronic properties [9,10]. Carbazole derivatives have found application in electroluminescent devices as hole transporting and emitting materials due to their excellent hole transporting ability, luminescence efficiency, thermal stability and attractive

photoelectronic properties [11-14]. N-substituted carbazole derivatives possess versatile pharmaceutical activities such as antioxidant, anti-inflammatory, antibacterial, antitumor, anticonvulsant and antidiabetic activities [15-21].

The rapid development of pathogen resistance to most of the known antibiotics is becoming a serious health problem. One possible long-term solution is the development of agents that act on unexploited bacterial targets. S-triazine derivatives have received considerable attention due to their potent biological activity such as antitubercular, anticancer, antitumor, anti-inflammatory, antiviral, antibacterial and herbicidal activities. In this perspective, we have synthesized Cu(II), Ni(II) and Co(II) complexes of 4,6-bis(pyridylamino)-2(carbazol-9yl)-1,3,5-triazine (**L**), characterized and screened for antimicrobial, anti-inflammatory and analgesic activities.

2. Experimental

2.1. Materials and methods

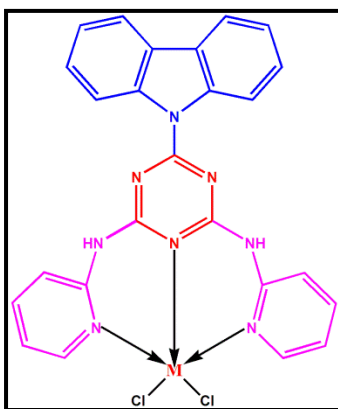
All chemicals were obtained from Aldrich Chemical & Co. and used without purification. The UV-Vis spectra of the ligand and metal complexes were recorded using a JASCO V-530 spectrophotometer. Infrared (IR) spectra in KBr discs were recorded on a SHIMADZU FT-IR 460 plus spectrophotometer at USIC, Madurai Kamaraj University, Madurai. Cyclic voltammetry measurements were carried out at room temperature in DMSO (CH Instruments, USA, voltammograph) using a three electrode cell containing a reference Ag/AgCl electrode, Pt wire auxiliary electrode and glassy carbon working electrode with tetrabutylammonium perchlorate as supporting electrolyte. The metal contents of the complexes were estimated by incinerating them to metal oxides [22] Elemental analyses were performed at SAIF, CDRI, Lucknow. ¹H-NMR spectrum was recorded in CDCl₃ using a Bruker DRX-300, 300MHz NMR spectrometer. Electron-ionization (EI) mass spectra were recorded by JEOL-GC MATE-2 at IIT, Madras-Chennai. Magnetic Susceptibility of the complexes was measured on a MSB mark 1 Sherwood, UK at Thiagarajar College, Madurai. Effective magnetic moments were calculated using the formula $\mu_{\text{eff}} = (2.828\chi_M T)^{1/2}$, where χ_M is the corrected molar susceptibility. Molar conductances of the complexes (10⁻³ mol L⁻¹) were measured in DMSO at room temperature using a Systronic conductivity bridge.

2.2. Synthesis of 4,6-bis(pyridylamino)-2(carbazol-9yl)-1,3,5-triazine [BPACT] (**L**)

1.88g (20mmol) of 2-aminopyridine in ethanol was refluxed with 3.15gm of 4,6-dichloro-(2-carbazole-9-yl)-1,3,5-triazine [23] and few drops of ethylene triamine for 2h. A pale yellow solid separated out on cooling, was filtered and purified by recrystallization from hot ethanol, and dried in vacuum over anhydrous calcium chloride. Yield: 74-78%

2.3. Synthesis of metal (II) complexes:

An ethanolic solution of ligand (10mmol) was stirred with anhydrous metal (II) chloride (10mmol) (Cu (II), Ni(II), Co(II)) in 20ml ethanol for 1 h. The resultant reaction mixture was reduced to one third of its volume, a colored complex separated on cooling was filtered and washed thoroughly with ethanol by recrystallization from ethanol. Yield: 68-72%



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

Proposed structure of metal(II) complexes

3. Pharmacology

3.1. Anti-inflammatory activity

The anti-inflammatory activity was studied by Carrageenan-induced rat paw edema method. Albino mice of either sex (180-200g) were selected and kept in animal house under standard laboratory conditions. The animals were divided into six groups of six animals each. Group I served as control and received 10ml/kg normal saline. Group II served as standard and received Diclofenac sodium (10mg/kg) through IP route and the remaining four groups served as test groups and received ligand and its metal(II) complexes (10 mg/kg) 30 min prior to carrageenan injection. The initial left hind paw volume of the rats was measured using a digital plethymometer. The volume of the left hind paw of the test group, standard, and control groups

were measured at 60, 240, and 360 min after carrageenan injection. Mean increase in paw volume was measured and percentage of inhibition was calculated.

$$\% \text{ Anti-inflammatory activity} = (\mathbf{Vc-Vt} / \mathbf{Vc}) \times 100$$

Where, **Vt**-mean increase in paw volume in rats treated with test compounds, **Vc**-mean increase in paw volume in control group of rats.

3.2. Analgesic activity

Analgesic activity was evaluated using acetic acid induced writhing method. After 30 min of the oral administration of test compound and standard drug, each animal was injected with 1ml of 1% v/v acetic acid solution intraperitoneally. After 10 min of acetic acid injection, the numbers of writhing in albino mice were counted for a period of 10 min. A significant reduction in the number of writhing by any treatment as compared to control animals was considered as positive analgesic response. The average number of writhes in each group of treated mice was compared with that of the control.

3.3. Biological activity

Antimicrobial activity of the compounds was tested *in vitro* by the well diffusion method against the bacteria *Staphylococcus aureus*, *Escherichia coli*, *Salmonella enterica typhi*, *Bacillus subtilis* using agar nutrient as the medium. Antifungal activity was evaluated against *Aspegillus niger* and *Candida albicans* cultured on potato dextrose agar as medium. All the bacterial strains mentioned above were incubated in Nutrient Broth (NB) at 37° C for 24 h and fungal isolates were incubated in PDA broth at 28° C for 2 to 3 days. The well each of 5 mm in diameter was made in Muller Hinton agar using cork borer. The test solution was prepared in 10⁻³ moles litre⁻¹ concentration (DMSO) and then 100 µL of the solution was transferred into each well. The plates were incubated for 24 h at 37° C and examined for clear inhibition zone around the well.

4. Results and discussion

All the complexes were obtained as solids by the reaction of ligand with anhydrous metal salts. The elemental analysis data of the ligand and its metal (II) complexes (Table 1) are in good agreement with the calculated ones. The ligand and its metal(II) complexes are stable at room temperature and soluble in common organic solvents such as DMSO, DMF, and acetone. Based on the elemental analysis and spectral studies, the coordination geometry has been assigned and is shown in Figure 1. The molar conductance of complexes in 10⁻³ M DMSO solution is in the range 6.4–9.1 ohm⁻¹ cm² mol⁻¹, which reveals the nonelectrolytic nature of the complexes [24].

Table 1. Physical characterization, analytical, and molar conductance data of the ligand and metal(II) complexes

Compounds	Fw g/m	Elemental analyses				Calculated (found).%	$\Omega^{-1}\text{cm}^2\text{mo}^{-1}$	M.P.(°C)
		C	H	N	M			
C ₂₅ H ₁₈ N ₈ (L)	430.47	69.76 (68.71)	4.18 (3.94)	26.04 (25.68)	-	-	-	154
[Cu(L)Cl ₂] (1)	563.92	53.28 (52.62)	3.19 (2.98)	19.89 (18.84)	11.28 (10.72)	6.4		202
[Ni(L) Cl ₂](2)	560.07	53.76 (52.51)	3.22 (2.72)	20.07 (19.58)	10.52 (9.40)	9.1		254
[Co(L) Cl ₂](3)	560.30	53.66 (52.06)	3.22 (2.95)	20.03 (19.20)	10.54 (9.18)	7.8		270

4.1. Electronic spectra and magnetic moment

The electronic spectral data of the ligand and its metal (II) complexes are given in Table 2. The absorption spectrum of the ligand shows strong peak at 33003 cm^{-1} and 37037 cm^{-1} which are attributed to $\pi\text{-}\pi^*$ and $n\text{-}\pi^*$ transitions respectively. The electronic spectrum of Cu(II) complex showed three absorption bands at 12710 cm^{-1} , 15224 cm^{-1} and 18522 cm^{-1} which can be tentatively assigned to the transitions ${}^2\text{B}_1 \rightarrow {}^2\text{A}_1$ (ν_1), ${}^2\text{B}_1 \rightarrow {}^2\text{B}_2$ (ν_2), and ${}^2\text{B}_1 \rightarrow {}^2\text{E}$ (ν_3), respectively, are characteristic of square pyramidal geometry [25]. Such bands near 18656 cm^{-1} , 14815 cm^{-1} and 10799 cm^{-1} have been observed for related copper(II) complexes with square pyramidal geometry [26,27]. The copper(II) complex at 1.86 B.M. is as suggestive of a five-coordinate square pyramidal geometry [28]. The molar extinction coefficient for Cu(II) complex (ϵ) is in the range of several hundred indicating the noncentrosymmetric nature of the complex. The electronic spectrum of Ni(II) complex exhibits three d-d bands at 10006 cm^{-1} (ϵ , $181\text{ M}^{-1}\text{ cm}^{-1}$), 16472 cm^{-1} (ϵ , $210\text{ M}^{-1}\text{ cm}^{-1}$) and 23048 cm^{-1} (ϵ , $293\text{ M}^{-1}\text{ cm}^{-1}$) and is assigned to the transitions ${}^3\text{B}_1(\text{F}) \rightarrow {}^3\text{E}(\text{F})$, ${}^3\text{B}_1(\text{F}) \rightarrow {}^3\text{A}_2(\text{P})$, and ${}^3\text{B}_1(\text{F}) \rightarrow {}^3\text{E}(\text{P})$, respectively, consistent with square pyramidal environment [29]. The spectra resemble those reported for five-coordinate square pyramidal nickel(II) complexes [30,31]. The Co(II) complex displayed three absorption bands at 9808 cm^{-1} , 16418 cm^{-1} , and 20008 cm^{-1} which can be assigned to the transitions ${}^4\text{A}_2 + {}^4\text{E} \rightarrow {}^4\text{B}_1$ (ν_1), ${}^4\text{A}_2 + {}^4\text{E} \rightarrow {}^4\text{E}(\text{P})$ (ν_2), and ${}^4\text{A}_2 + {}^4\text{E} \rightarrow {}^4\text{A}_2(\text{P})$ (ν_3), respectively, which are characteristic of square pyramidal geometry[32]. Thus the electronic spectral data of the cobalt(II) complex are in good agreement with the already reported square pyramidal geometry

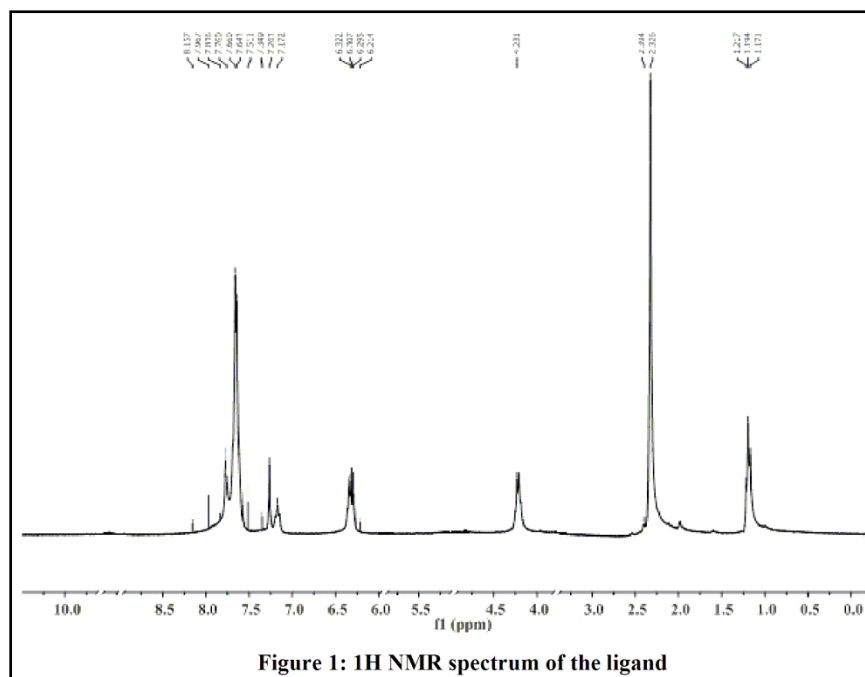
[32]. Cobalt(II) and Nickel(II) complexes have magnetic moment values 4.72 and 3.02 B.M, respectively, characteristics of square pyramidal geometry. The molar absorptivity (ϵ) of the above complexes is in the order of 10^2 M cm^{-1} indicates the geometry of the molecule.

Table 2. Electronic and magnetic susceptibility data of ligand and their metal(II) complexes

Compound	λ_{max} (cm^{-1})	Transition	Geometry	μ_{eff} (B.M)
[Cu(L)Cl ₂] (1)	12,710	$^2B_1 \rightarrow ^2A_1$	Square pyramidal	1.86
	15,224	$^2B_1 \rightarrow ^2B_2$		
	18,522	$^2B_1 \rightarrow ^2E$		
[Ni(L) Cl ₂](2)	10,006	$^3B_1(F) \rightarrow ^3E(F)$	Square pyramidal	3.01
	16,472	$^3B_1(F) \rightarrow ^3A_2(P)$		
	23,048	$^3B_1(F) \rightarrow ^3E(P)$		
[Co(L) Cl ₂](3)	9,808	$^4A_2 + ^4E \rightarrow ^4B_1$	Square pyramidal	4.72
	16,418	$^4A_2 + ^4E \rightarrow ^4E(P)$		
	20008	$^4A_2 + ^4E \rightarrow ^4A_2(P)$		

4.2. ¹H NMR Spectral data

The ¹H NMR spectrum of ligand in CDCl₃ Fig.1 shows signals consistent with the proposed structure. The spectrum of the free ligand exhibits aromatic signals in the region and the singlet at 4.3 ppm corresponds to NH proton respectively.



4.3. IR spectra

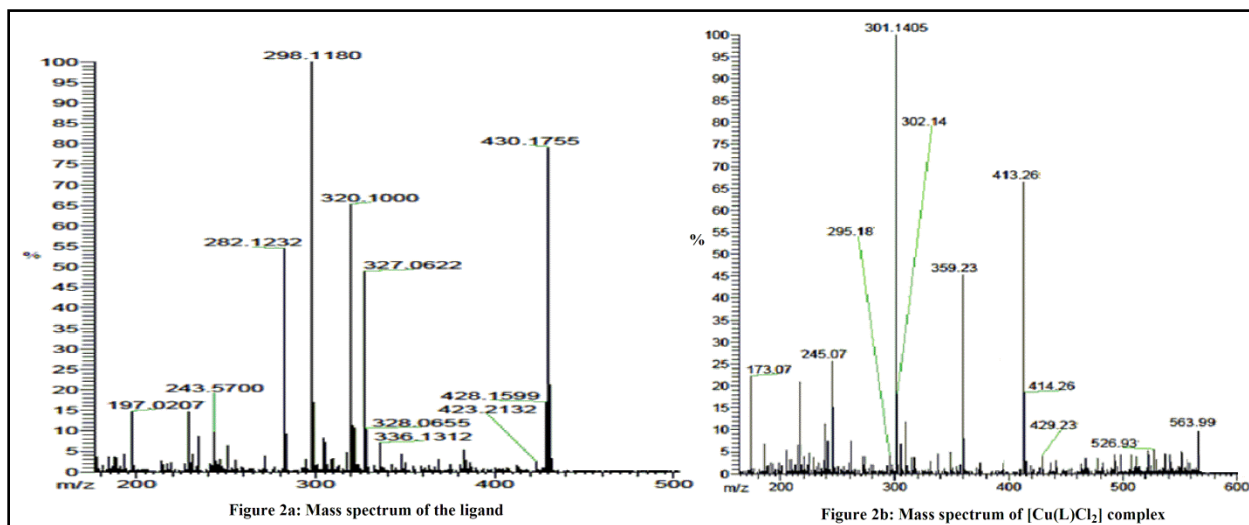
The ligand which exhibits a band at 1620 cm^{-1} is characteristic of (C=N) of pyridine ring [33]. In complexes, this strong band is shifted to $1598\text{--}1594\text{ cm}^{-1}$ region corresponding to (C=N) coordination to metal ion. The ligand shows a strong band at 825 cm^{-1} and 1488 cm^{-1} , which is characteristic of the $\nu(\text{C--N})$ and $\nu(\text{C=N})$ groups in S-triazine. The band is shifted to lower frequency of $816\text{--}810\text{ cm}^{-1}$ and $1452\text{--}1444\text{ cm}^{-1}$ upon complexation indicates that the above group [34] is one of the coordinating atoms in the ligand. The IR spectrum of the free ligand shows 2985 cm^{-1} assignable to the appearance of this mode at almost in the same frequency in all the complexes suggest that the above group is not taking part in coordination. In the far IR spectra of complexes, the weak bands appeared at $448\text{--}456\text{ cm}^{-1}$ and $340\text{--}352\text{ cm}^{-1}$ regions can be assigned to $\nu(\text{M--N})$ and $\nu(\text{M--Cl})$ vibrations [35], respectively, and confirm the coordination of metal with ligand. The IR spectrum of the ligand and its metal(II) complexes is shown in Table 3.

Table3. IR spectral data of the ligand and its metal(II) complexes

Compound	$\nu(\text{C=N})$ pyridine ring	$\nu(\text{C=N})$ triazine ring	$\nu(\text{C=N})$ triazine	$\nu(\text{M-Cl})$	$\nu(\text{M-N})$
Ligand (L)	1620	1488	825	-	
[Cu(L)Cl ₂] (1)	1598	1452	814	340	448
[Ni(L) Cl ₂](2)	1602	1444	816	346	486
[Co(L) Cl ₂](3)	1594	1450	810	352	460

4.4. Mass spectra

The mass spectrum of the ligand(L) and Cu(II) complex are given in Fig.2(a,b). The molecular ion peak for L was observed at 430 m/z , whereas molecular ion peaks of Cu(II) complex was observed at 563 m/z which corresponds to $[\text{ML}_2\text{Cl}_2]$.



4.5. Electrochemical studies

Cyclic voltammogram of copper(II) complex (Fig.3) displays a pair of oxidation-reduction peaks at -0.578V and 1.2V. The redox peak potential separation (ΔE_p) of 556 mV suggests that the Cu(II)/Cu(I) redox couple was irreversible in nature [36]. The ratio of anodic to cathodic peak currents ($I_{pc}/I_{pa} \approx 1$) corresponding to a simple one electron process. The irreversibility observed for the reductive response of the complex may be due to a short-lived reduced state of the metal(II) ion or due to the oxidative degradation of the ligand [37].

4.6. NLO Property

The second harmonic generation (SHG) efficiency of the ligand shows much higher efficiency than KDP and urea. In general, molecule with delocalized π -electron system can have large nonlinear polarizabilities and it has been generally understood that the molecular non linearity can be enhanced by systems with strong donor and acceptor groups [38]. The second harmonic generation efficiency clearly reveals that the ligand possess 0.6 times better activity than that of urea and 2.2 times more active than KDP.

4.7. Anti-inflammatory activity

Anti inflammatory data of 4,6-bis(pyridylamino)-2-(carbazol-9-yl)-1,3,5-triazine ligand and its metal complexes were evaluated by carrageenan-induced rat paw edema method [39] and the results are shown in Table 4 and Figure 4. Injections of carrageenan into the rat paw produces inflammation resulting from metabolism of arachidonic acid. From the observation, it is found that the newly synthesized 4,6-bis(pyridylamino)-2-(carbazol-9-yl)-1,3,5-triazine ligand and its metal(II) complexes possess higher anti inflammatory activity compared to the standard. Among

all the metal complexes the % inhibition of Cu(II) complex was significant and comparable with the standard drug dichlofenac. The enhanced anti-inflammatory activity of the complexes may be explained on the basis of enhanced penetrations through cellular membranes due to complexation reported by Stefancich et al [40].

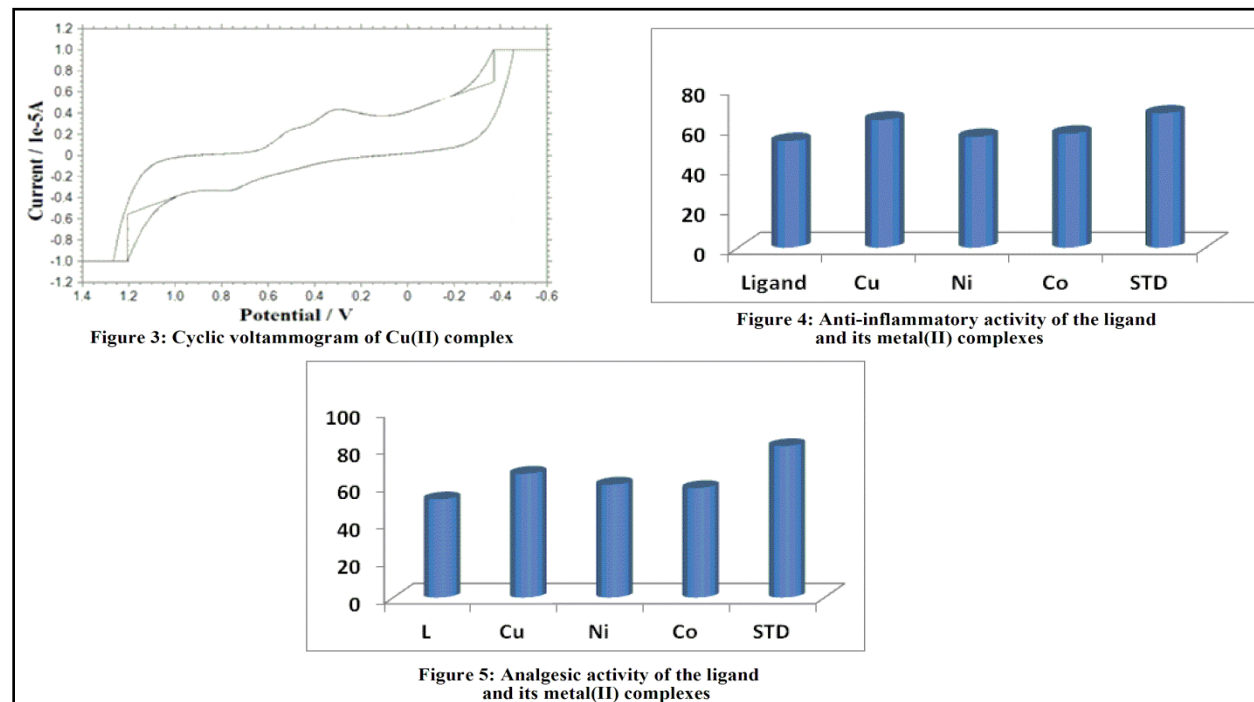
Table 4. Anti inflammatory activity of the ligand and its metal(II) complexes

Compounds	Dose mg/kg	paw volume (ml)	% inhibition of Paw edema
Control	10	5.92±0.94	-
Phenytion Sodium	10	1.70±0.48	67.30 ^{*a}
Ligand (L)	10	2.06±0.40	53.42 ^{*a}
[Cu(L)Cl ₂] (1)	10	1.96±0.52	64.12 ^{*a}
[Ni(L) Cl ₂](2)	10	1.98±0.60	55.46 ^{*a}
[Co(L) Cl ₂](3)	10	1.92±0.54	57.07 ^{*a}

Data are given as mean ± S.E.M

Data analyzed by one-way ANOVA followed by Newman's keuls multiple range tests.

^{*a} Values were significantly different from normal control at P< 0.01.



4.8. Analgesic activity

The results of acetic acid induced writhing tests are given in Table 5 and Figure 5. The data showed that all the tested compounds exhibited analgesic activity in the range of 52-66% reduction of writhing, respectively, after 1h of treatment of tested compounds compared to 81%

obtained with standard. The result revealed that ligand and its metal(II) complexes possess significant activity compared to the standard.

Table 5. Analgesic activity of the ligand and its metal(II) complexes

Compound	Dose mg/kg	Duration of writhing (minutes)	% inhibition
Control	10	35.0±2.8	-
Diclofenac Sodium	10	8.2±0.50	81.08**
Ligand (L)	10	18.4±1.60	52.60**
[Cu(L)Cl ₂] (1)	10	12.20±2.60	66.30**
[Ni(L) Cl ₂](2)	10	15.2±2.40	60.48**
[Co(L) Cl ₂](3)	10	16.66±0.66	58.64**

Data are given as mean±SEM

Data analyzed by one-way ANOVA followed by Newman's keuls multiple range tests.

**Values were considered significant at P< 0.01.

4.9. Antimicrobial study

The ligand and its metal (II) complexes were evaluated by well-diffusion method. The ligand and their metal complexes were tested for antimicrobial activity against the bacteria *Staphylococcus aureus* and *Bacillus subtilis* as gram-positive bacteria, *Salmonella typhi* and *Escherichia coli* as gram-negative bacteria and antifungal activity against the fungi *Candida albicans* and *Aspergillus niger*. *Amikacin* and *ketokonazole* were used as reference for antibacterial and antifungal studies Table 6 and Figure 6.

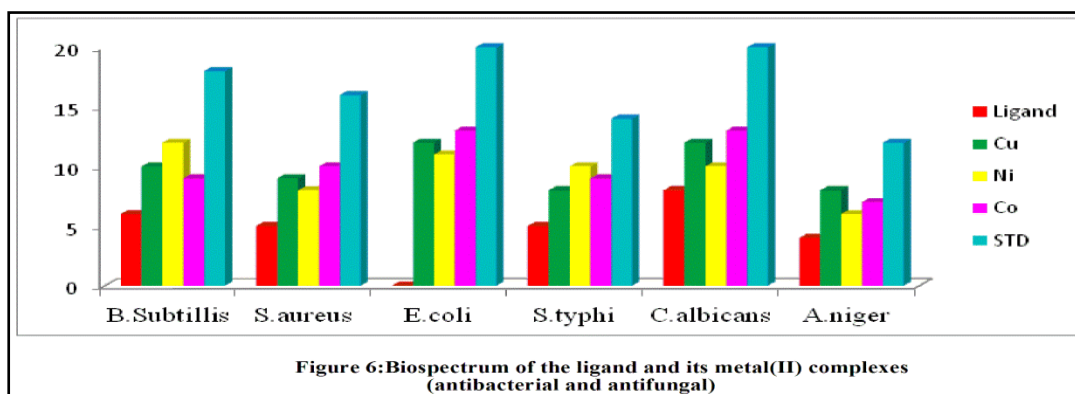
Antimicrobial activity studies were performed in triplicate and the average was taken as the final reading. All the tested compounds showed a remarkable biological activity against different types of bacteria and fungi species. *In vitro* antifungal activity data of the ligand and its metal(II) complexes against tested organisms displayed significant activity with wide range of variation. It is found that all the derived compounds exhibited higher activity against all the organisms but still lower than the standard. *In vivo* antibacterial activity data reveals that all the synthesized compounds displayed moderate to significant activity in comparison to standards. Ligand is inactive towards *E.coli* but the metal complexes show higher activity against the same organism compared to standard. In general, the increased activity of the metal (II) complexes over the ligand can be explained on the basis of Overton's concept and Chelation theory.

Table 6. Antimicrobial activity data of the ligand and their metal (II) complexes

Zone of inhibition (mm)*

Compounds	B.subtillis	S.aureus	E.coli	S. typhi	C.albicans	A.niger
Ligand (L)	06	05	R	05	08	04
[Cu(L)Cl ₂] (1)	10	09	12	08	12	8
[Ni(L) Cl ₂](2)	12	08	11	10	10	6
[Co(L) Cl ₂](3)	09	10	13	09	13	7
Amikacin	18	16	20	14	-	-
Ketokonazole	-	-	-	-	20	12

*The test was done using 10^{-3} M concentration of synthesized compounds by well diffusion technique. The values are mean of three replications.



Conclusion

The synthesized tridentate ligand undergoes coordination through triazine ring nitrogen and pyridine ring nitrogen with metal(II) ions. The analytical and spectral studies confirm that all the metal(II) complexes possess square pyramidal geometry. Electrochemical study of the copper(II) complex implies the redox reaction is one electron transfer with irreversible character. The synthesized ligand found to exhibit appreciable nonlinear property in comparison with KDP and urea. Hence, the compounds can be utilized for further NLO investigations. The ligand and its metal(II) complexes were potentially active towards all microbial strains. The bacterial studies of all the metal complexes against *E. coli* show promising results. Such increased activity of the metal complex is of tremendous importance in the pharmaceutical field. Synthesized compound also possess significant anti-inflammatory and analgesic activities.

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