AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Synthesis, Spectral Characterization, Antimicrobial Screening and DNA Studies of Transition metal complexes of Cu(II), Co(II), Ni(ii) and Zn(II) with Heterocyclic Triazol based derivative

P.Saravana Bhava[1*], P.Tharmaraj[2], V.Muthuraj[3] , M. Umadevi [4]

1.Department of Chemistry, S.Vellaichamy Nadar Polytechinc College, Virudhunagar, India.
2.Department of Chemistry, Thiagarajar College, Madurai, India.
3.Research Department of Chemistry, VHNSN College, Virudhunagar, India.
4. PG & Research Department of Chemistry, Nehru Memorial College, Puthanampatti, Tiruchirappalli, Tamilnadu, India.

ABSTRACT

A new series transition metal complexes of Cu(II), Co(II), Ni(II) and Zn(II) complexes have been synthesized from 2-(4-amino-5-mercapto-4H-1,2,4-triazol-3-yl)phenol (AMPT). The nature of bonding and the geometry of the complexes have been deduced from elemental analyses, magnetic susceptibility, infrared, electronic, ^{1}H NMR, ^{13}C NMR, and EPR spectral studies. The redox behavior of copper complexes was studied by cyclic voltammetry. The second harmonic generation (SHG) efficiency was measured by Kurtz and Perry method. The ligand and their metal complexes were screened by Well diffusion method. The interaction of complexes with CT- DNA was investigated by viscosity measurement. Results suggest that all the complexes bind to DNA *via* an intercalative mode. The DNA cleavage ability of all the complexes was examined on calf thymus (CT-DNA) plasmids using gel electrophoresis experiment in presence of H_2O_2. From the results it is concluded that all the complexes cleave DNA.

Keywords: 1,2,4-Triazoles, Metal complexes, SGH, Antibacterial activity, DNA cleavage.

*Corresponding Author Email: psbhava@gmail.com
Received 18 April 2013, Accepted 13 May 2013

Please cite this article in press as: Bhava PS. *et al.*, Synthesis, Spectral Characterization, Antimicrobial Screening and DNA Studies of Transition metal complexes of Cu(II), Co(II), Ni(ii) and Zn(II) with Heterocyclic Triazol based derivative. American Journal of PharmTech Research 2013.

INTRODUCTION

Heterocyclic compounds are the largest classical division of organic chemistry and are of immense importance. Synthesis of such heterocyclic compounds is of pharmaceutical importance and a foremost task of chemists due to its vast pharmacological and industrial applications. Nitrogen and oxygen containing five member heterocyclic compounds have been used as a scaffold to synthesize numerous therapeutic molecules. The Nitrogen and Sulphur atoms play a key role in the coordination of metals at the active sites of numerous metallobiomolecules. The chemistry of 1,2,4-triazoles belong to an important class of heterocyclic compounds in medicinal chemistry and its derivatives have received considerable attention because of their effective biological importance and applications. Among these the mercapto and amino group substituted 1,2,4-triazole ring system have been reported for antimicrobial, anticancer, diuretic and hypoglycemic activities. They are known to possess significant anti-inflammatory[1], antimicrobial[2], antimyotic activity such as fluconazole, itraconazole, voriconzole[3] and anticancer like alprazolam[4], Etizolam[5]. The thione substituted triazoles are reported for a variety of biological activities such as antibacterial[6], antifungal[7], antitubercular[8], anticancer[9], antihypertensive[10], antiviral[11], antimigrane[12] and antileishmanial[13] activities. Metal complexes of 1,2,4-triazole and its derivatives often exhibit enhanced biological activities compared to the un complexed ligands[14–15]. Among the 1,2,4-triazole derivatives, the mercapto- and thionesubstituted 1,2,4-triazole ring systems have also been studied and reviewed[16, 17]. Hence there is a great demand for new compounds that can combat with resistant bacteria. With this knowledge we made an efficient attempt in synthesizing new antimicrobial compounds.

A survey of the literature reveals that, no work has been carried out on the synthesis of metal complexes with 2-(4-amino-5-mercapto-4H-1, 2, 4-triazol-3-yl) phenol. These ligand have donor sites with the N, O and S sequence and varied coordination abilities. Prompted by these observations, as part of our research program aimed at developing new biologically active nitrogen and sulphur containing heterocycle compounds were synthesis and report the substituted mercapto-1,2,4-triazole derivative were prepared. (Figure. 1).

MATERIALS AND METHODS

All chemicals were obtained from Aldrich Chemical & Co. and used without purification. The UV-Vis. spectra of the ligand and its metal complexes were recorded in DMSO using a JASCO V-530 spectrophotometer. IR spectra in KBr discs were recorded on a JASCO FT-IR 460 plus spectrophotometer at Thiagarajar College, 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 (TBAP) as supporting electrolyte. Elemental analyses were performed at SAIF, CDRI, Lucknow. ^{1}H-NMR, ^{13}C NMR spectra were recorded in $CDCl_3$ using a Bruker DRX-300, 300 MHz NMR spectrometer. EI mass spectra were recorded at IIT, Madras. EPR spectrum was recorded at SAIF, IIT, Bombay. Magnetic moments of the complexes were measured on a Magnetic Susceptibility Balance Mark 1 Sherwood UK at Thiagarajar College, Madurai. Effective magnetic moments were calculated using the formula $\mu eff = 2.828 (\chi_M T)^{1/2}$ where χ_M is the molar susceptibility. Molar conductance of the complexes (10^{-3} mol L^{-1}) was measured in DMF at room temperature using a Systronic conductivity bridge. Commercial solvents were distilled and then used for the preparation of ligands and their complexes. DNA was purchased from Bangalore Genei (India).Microanalyses (C, H and N) were performed in Carlo Erba 1108 analyzer at Sophisticated Analytical Instrument Facility (SAIF), Central Drug Research Institute (CDRI), Lucknow, India. The second harmonic generation (SHG) activity was confirmed by the Kurtz and Perry powder technique at Indian Institute of Science, Bangalore.

Synthesis of 2-(4-amino-5-mercapto-4H-1,2,4-triazole-3-yl)phenol. [AMTP] Ligand

Synthesis of 2-Hydroxy benzohydrazide

A mixture of methyl salycylate (7.7 ml, 0.06 mole) and 98% hydrazine hydrate (2.8 ml, 0.06 mole) was heated under reflux for 3 hours. On cooling, cold water (150 ml) was added to the mixture and the separated white crystalline solid was filtered, washed with cold water, dried and crystallized from water to yield 98% of 2- Hydroxy bezohydrazide.

Synthesis of Potassium 2-(2- Hydroxy benzoyl)hydrazinecarbodithioate

Carbon disulphide (11.4 g, 0.15 mole) was added dropwise to a solution of 2- Hydroxy bezohydrazide (15.2 g, 0.1 mole) and potassium hydroxide (8.4 g, 0.15 mole) in ethanol (250 ml), and the mixture was stirred at room temperature for 3 hours. Dry ether (200 ml) was then added to the mixture and the precipitated solid was filtered, washed with ether and dried at 65 ^{o}C to yield (75-80 %) m.p. 90 ^{o}C

Synthesis of 2-(4-amino-5-mercapto-4H-1,2,4-triazole-3-yl)phenol [AMTP]

A mixture of potassium 2-(2-hydroxybenzoyl)hydrazinecarbodithioate (13.3 g, 0.05 mmol) and hydrazine hydrate (0.05 mmol, 10 ml) was heated under reflux till hydrogen sulphide completely ceased down (1 hour) and 200 ml of water was added to the reaction mixture. Finally

the reaction mixture was neutralized with 10% HCl and allowed to stand for 3 hours. The separated crude product was filtered, washed with water, dried and then crystallized using ethanol[18, 19]. The yield obtained was 80 %, m.p. 180 ºC. The Schematic representation of the ligand is given in figure. 1.

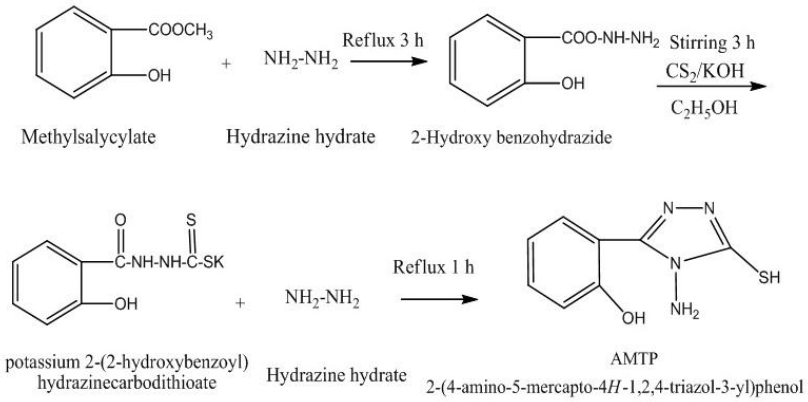

Figure 1. Synthesis of Ligand AMTP

Synthesis of metal complexes

All metal(II) complexes were obtained by stirring the AMTP (4 mmol) with metal(II) chloride (2 mmol) in ethanol (25 mL). After completion of the reaction the metal (II) complexes were filtered and concentrated to 1-2 mL. Addition of petroleum ether gives metal(II) complexes in high yield (75-80 %) m.p. 260-280 ºC. The Schematic representation of the metal(II) complexes is given in fig. 2.

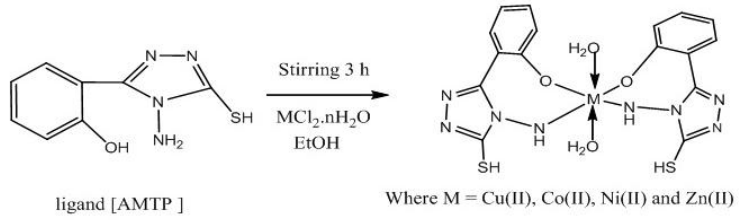

Figure. 2. Synthesis of metal(II) complexes

RESULTS AND DISCUSSION

This study aims to synthesize of AMTP and to investigate the bonding modes and geometry of the ligand with various transition metal (Cu(II), Co(II), Ni(II) and Zn(II)) which may act as a models to metalloenzymes. In this section the studies of novel new AMTP ligand and their metal complexes have been investigated. The modern spectral techniques such as IR, UV-Vis, ^{1}H NMR ^{13}C NMR, CV, EPR and Mass are used for characterization.

Elemental analysis data and physical characteristics of AMTP ligand and complexes are summarized in table. 1. All these complexes are intensively colored, air and moisture firm

amorphous solids. They are insoluble in common organic solvents and only soluble in DMF and DMSO. Molar conductance values of the soluble complexes in DMF (10^{-3}M solution at 25 °C) indicate that the complexes non electrolytic in nature. The elemental analyses data concur well with the planned formulae for the ligand and also recognized the $[ML_2].2H_2O$ composition of the metal(II) chelats.

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

Compound	Formula weight	Color	Found (Calcd) (%)					m.p. (°C)	μ eff (B.M.)	Λ_M (Ω^{-1} cm^2 mol^{-1})
			M	C	H	N	S			
$C_8H_8N_4OS$ (AMTP)	208	Colorless	--	46.04 (46.14)	3.74 (3.87)	26.83 (26.90)	15.32 (15.40)	180	---	---
[Cu(AMTP)$_2$. 2H$_2$O]	511	Grey	12.32 (12.41)	37.47 (37.53)	3.08 (3.15)	21.80 (21.88)	12.47 (12.52)	>280	1.82	17.13
[Co(AMTP)$_2$. 2H$_2$O]	507	Greenish Brown	11.57 (11.61)	37.78 (37.87)	3.09 (3.18)	22.00 (22.08)	12.56 (12.64)	>270	4.62	17.34
[Ni(AMTP)$_2$. 2H$_2$O]	506	Green	11.50 (11.57)	37.80 (37.89)	3.09 (3.18)	22.02 (22.09)	12.59 (12.64)	>275	2.94	19.26
[Zn(AMTP)$_2$. 2H$_2$O]	512	Yellow	12.68 (12.73)	37.36 (37.40)	3.08 (3.14)	21.73 (21.81)	12.39 (12.48)	>260	--	22.28

Infrared Spectral Studies:

IR spectral data of the ligand AMTP and its metal(II) complexes are shown in Table 2. In order to study the binding mode of the ligand in the metal complexes, IR spectrum of free ligand was compared with spectra of its metal complexes. The ligand AMTP exhibits tautomerism[20] as shown in the fig. 3. one can expect both ν(S-H) and ν (C=S). A medium intensity band around 2712 cm^{-1} due to ν (S-H) indicates the thiol form of the ligand. The band due to ν(C=S) in the 750 cm^{-1} of the ligand has remained unperturbed in these complexes indicating that N or S of the thiamide group is not involved in the bond formation [21]. These observations suggest the non-involvement of sulphur atom in coordination. The infrared spectra of ligand exhibits high intensity band around 1605cm^{-1} is due to ν (C=N). The band around 3286 cm^{-1} due to ν (NH_2) group in ligand is shifted to lower frequency in all complexes indicating the ligand is coordinated through Nitrogen atom of –NH_2. The band around 3434 cm^{-1} due to phenolic OH, which is observed in ligand, disappears in complexes, this indicates the ligand is coordinate to the metal ion through phenolic oxygen atom[22] of OH group via deprotonation. A broad band observed in the region 3424-3451cm^{-1} in all complexes indicates the presence of coordinated water molecule[23]. The participation of oxygen and nitrogen in coordination with the metal ion is further

supported by the new band appearance of ν (M-N) and ν (M-O) at 584-654 cm^{-1} and 459-464 cm^{-1} respectively in the far infrared region[24, 25]. The Proposed structure of metal(II) complexes is as shown in the figure.4.

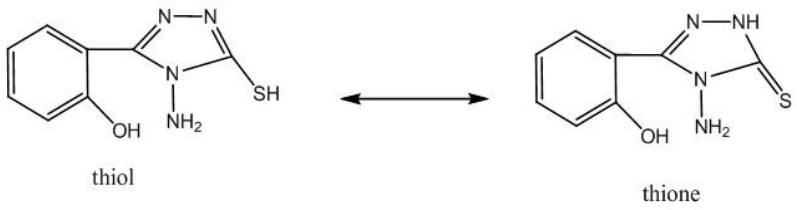

Figure. 3. Tautomerism of ligand [AMTP]

Table.2. IR spectral data (cm^{-1}) of the ligand AMTP and its metal(II)complexes

Compounds	Vibrational frequencies (cm^{-1})							
	ν(H_2O)	**ν(OH)**	**ν(S-H)**	**ν(N-H)**	**ν(NH_2)**	**ν(C=S)**	**ν(M-N)**	**ν(M-O)**
Ligand[AMTP]	---	3434	2712	2943	3286	750	---	---
[Cu (AMTP)$_2$2H_2O](1)	3424	---	2724	2926	3146	747	584	464
[Co (AMTP)$_2$2H_2O](2)	3424	---	2720	2927	3158	754	585	464
[Ni(AMTP)$_2$2H_2O](3)	3424	---	2705	2949	3160	754	583	464
[Zn(AMTP)$_2$2H_2O](4)	3451	---	2707	2947	3164	752	581	459

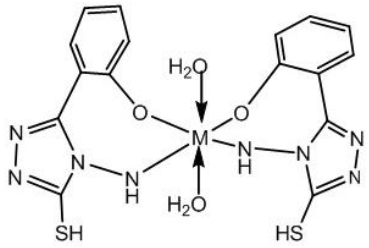

Figure . 4. Proposed structure of metal(II) complexes of AMT M = Cu(II), Co(II), Ni(II) and Zn(II)

NMR spectroscopy

The ^{1}H NMR spectral data for the 2-(4-amino-5-mercapto-4H-1,2,4-triazole-3-yl)phenol [AMTP **]** was recorded in $CDCl_3$. The spectrum of the [AMTP] showed a multiplet at 7.6–8.8 δ (m, 4H, Aromatic-H) due to aromatic ring protons, singlet at 10.6 δ due to -SH proton (s, 1H, SH), doublet at 5 δ aromatic –OH proton (s, 1H, OH), singlet at 5.7 δ due to –NH_2 protons (s, 2H, NH_2).

^{1}H NMR spectrum of Zn(II) complex

In the ^{1}H NMR spectrum of Zn(II) complex the aromatic protons have resonated in the region δ 6.9-7.5 as a multiplet. The signal due to OH at 5.35 δ in ligand, disappears in case of complexes

indicating the involvement of OH in the complexes formation via deprotonation. The NH_2 proton is shifted slightly downfield at 5.6 δ which reveals the bonding of the $–NH_2$ nitrogen to Zn(II)ion. The signal due to SH proton appears at 10.6 δ unperturbed in the complexes indicating the non-involvement of sulfur atom of SH group.

^{13}C NMR Spectra of Ligand

The ^{13}C NMR spectra data for the ligand AMTP was recorded in $CDCl_3$. The ligand shows multiplet of aromatic Carbon the δ value C: [114.92 (CH), 117.23 (CH),119.59 (CH),126.02(C), 134.04 (C)]. In triazole carbon δ 149.12 (triazole C-3), δ 158.48
(triazole C-5).

Mass spectra

The electron impact mass spectrum of ligand shows a molecular ion peak m/z at 207 (M^+) with a relative intensity 20% which is equivalent to its molecular weight and the main mass fragmentation of the ligand is given in the Figure. 5. The series of peaks in the range i.e. 69, 78, 92, 96, 121, 129, 160 and 192 etc, may be assigned to various fragments. The [Co(AMTP)$_2$.2H$_2$O] complex gives a molecular ion peak at m/z 507 (M^+) with intensity 15%. The intensities of these peaks gives an idea of the stability and abundance of the fragments. The stoichiometry $ML_2.2H_2O$ was confirmed by the mass spectra of other complexes. It is in good agreement with the micro analytical data.

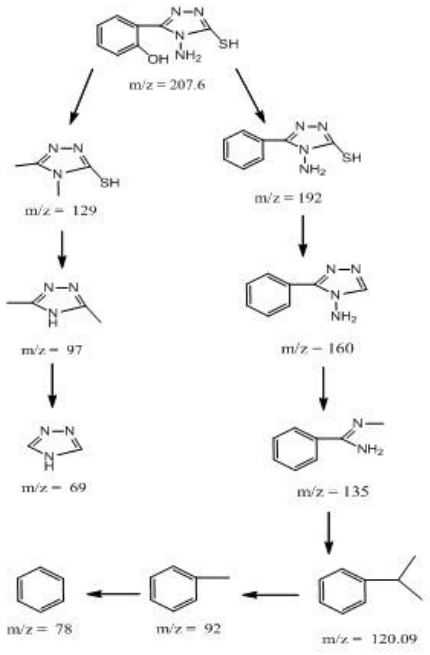

Figure. 5. Mass fragmentation pattern of (AMTP)

Electronic spectral and magnetic moment data of the metal complexes

The electronic spectral measurements were used for assigning the stereochemistry of metal ions in the complexes based on the position and number of d-d transition peaks. The bands appearing at the low energy side are attributable to n-π* transitions associated with the azomethine chromophores. The bands at higher energy arise from π-π* transitions within the phenyl rings[26]. The absorption bands of the complexes are shifted to longer wavelength region compared to those of the ligand[27]. The electronic spectrum of the Cu(II) complex (Table.3) showed one broad band at 15750 cm^{-1} in the electronic spectrum of the Cu(II) complex assigned to $^2E_g \rightarrow {}^2T_{2g}$ transition which is in conformity with octahedral geometry [28]. The magnetic moment of copper complex is 1.82 BM.

Table .3. Electronic spectral data (cm^{-1}) of the ligand [AMTP] and its metal(II)complexes

Compounds	Solvent	Frequency (cm^{-1})	Assignment	Geometry
Ligand [AMTP]	DMSO	36905	INCT	-
		35465		
[Cu (AMTP)$_2$2H$_2$O]	DMSO	15750	$^2E_g \rightarrow {}^2T_{2g}$	octahedral
[Co (AMTP)$_2$2H$_2$O]	DMSO	17701	$^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$	octahedral
		10966	$^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$	
[Ni (AMTP)$_2$2H$_2$O]	DMSO	19649	$^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$	octahedral
		11237	$^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$	
		10102	$^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$	

Cobalt(II) complex of AMTP showed two absorption bands allowed transitions at 17707 and 10966 cm^{-1}which may be assigned to $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$, $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$ transitions respectively and suggesting octahedral geometry around the cobalt ions[29, 30]. Again, the six coordinate octahedral cobalt(II) complex was confirmed from the magnetic moment value 3.1 B.M, indicative of three unpaired electrons.

The nickel(II) complex exhibited three bands at 10102 cm^{-1} 11237 cm^{-1} and 19649 cm^{-1} which may be assigned to $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ (ν_1), $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ (ν_2) and $^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$ (ν_3) transition characteristic of octahedral geometry[31]. The magnetic moment values for these complexes are in the range 2.1 B.M which suggests an octahedral geometry[32].

Electrochemical studies

Cyclic voltammetric studies of the copper(II) complex were performed in acetonitrile solution at room temperature with tetrabutylammonium perchlorate (TBAP) as supporting electrolyte; glassy carbon as working electrode; Pt wire as auxiliary electrode and Ag/AgCl as reference electrode; scan rate 100 mVs^{-1} (-0.2 to 1.4 V) shows a well-defined redox process corresponding to the formation of Cu(II)/Cu(I) couple at E_{pa} = 0.25 V and E_{pc} = 0.4 V and is found to be quasi-reversible with ΔE_p = 0.15 V. The ratio of anodic to cathodic peak currents (I_{pc}/I_{pa} = ~1) corresponding to a simple one-electron process was also reported by R. Klement *et al* [33]. The

peak current for the complex varies with scan rate and the $\Delta E_p = (E_{pa} - E_{pc})$ values are greater than 200 mV which, indicates that the reduction process are quasi-reversible in nature and chemical changes occur with the electron transfer[34]. The cyclic voltammogram of the copper(II) complex is shown in Figure. 6.

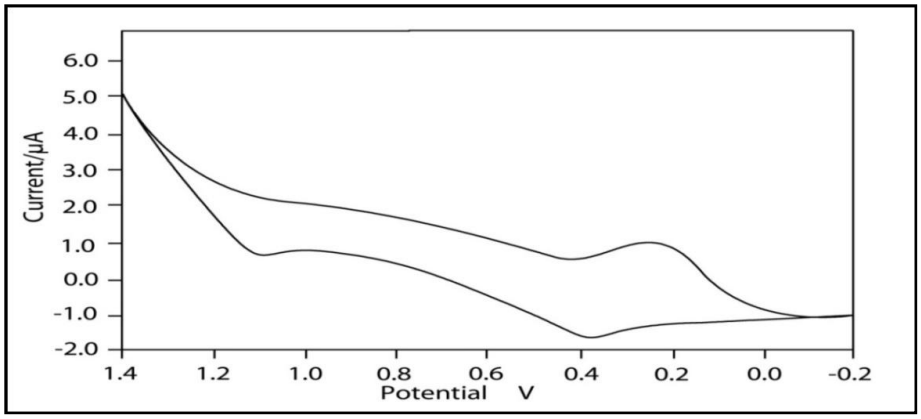

Figure. 6. Cyclic voltammogram of copper(II) complex

EPR spectra

EPR spectra of the copper complexes recorded in polycrystalline state at room temperature provide information about the coordination environment around Cu(II) in these complexe. To obtain further information about the stereochemistry and the site of the metal ligand bonding and to determine the magnetic environment in the metal complexes, EPR studies of copper(II) complexes were carried out. Powder samples were used to record X-band EPR spectra of the Cu(II) complexes and the spectra were recorded in DMSO at liquid nitrogen temperature (LNT) and at room temperature (RT). The interaction between the electron magnetic moment (µ) and the applied field is represented by the Hamiltonian:

$$H = -\mu.B = g\,\beta B.S$$

Where β is the Bohr magneton, g is referred to as g-factor and S is the effective spin including any orbital contribution. In the present case, Cu(II) complexes, measured in polycrystalline sample at room temperature, give the following values:

$$g_{\parallel}=2.6141,\ g_{\perp}=2.1289$$

The trend exhibits an auxiliary symmetric g-tensor parameters with $g_{\parallel} > g_{\perp} > 2.0036$ indicating that the copper site has a d_{x2-y2} ground state characteristic of octahedral geometry [35]. $G = g_{\parallel}-2.0036/g_{\perp}-2.0036$. The hyperfine parameters ($A_{\parallel}$ and $A_{\perp}$) have been calculated using the expression.

$$A_{\parallel} = P - (4\,\alpha^2/7) - K + (g_{\parallel} - 2.0036) + 3/7\,(g_{\perp} + 2.0036)$$

$$A_{\perp} = P + (2\,\alpha^2/7) - K + (11/14)\,(g_{\perp} - 2.0036)$$

Where P = 0.036 and K = 0.30 are the spin–orbit interaction factor and Fermi contact term, respectively. The molecular orbital coefficients namely in-plane π-bonding (β^2) and in plane σ-bonding (α^2) were calculated using the following expressions.

$$\alpha^2 = (A_\parallel/0.036) + (g_\parallel - 2.0036) + 3/7\,(g_\perp - 2.0036) + 0.04$$

$$\beta^2 = (g_\parallel - 2.0036)\,E / (-8\lambda\alpha^2)$$

Where λ is the spin-orbital coupling constant for the free ion and E is the electronic transition energy of $^2B_{1g} \rightarrow {}^2B_{2g}$. The value of α^2 (0.6603) < 1 indicates moderate covalency for the σ-bonding and the value of β^2 is > 0.5 (1.307). The α^2 values for the present complex fall in the range 0.6603 indicating the presence of appreciable in-plane covalency. The calculated value, $(g_\parallel/A_\parallel)$ > 200 cm for the complexes is an indication of its strong distortion in the structure.

The observed g_{iso} value (2.133) deviates from the free ion value 2.0036 and this suggests that the complex is appreciably covalent. The spin- orbit coupling constant (λ) for the copper ion in the complex is found to be less than of free Cu(II) ion (-828 cm^{-1}) indicative of covalency in Cu-L bonding [36].

Molar conductance

Values of molar conductance of the complexes are given in Table 1. The low values in DMF indicate that the complexes are non electrolytes.

Nonlinear Optical Studies

NLO material capable of frequency conversion is generally composed of an electron donor (D), an acceptor (A) and a conjugated π- system as a bridge providing the electronic communication between the donor and acceptor[37]. The advantages offered by organic over inorganic systems include high Electronic susceptibility through high molecular polarizability ($\chi^{(2)}$),fast response time, facile modification through standard synthesis method and relative ease of device processing. Heterocyclic five-member ring polymers (furan, pyrrole, thiophene, triazole,etc) are the most extensively studied of the poly conjugated and conductive polymers[38, 39]. Experimental measurements suggest that organic molecules containing rings (furan, pyrrole, thiophene, triazole, etc) exhibit significant nonlinear optical properties. Theoretically and experimentally a new class of second order nonlinear optical materials utilize the coupling of electron rich and electron deficient aromatic heterocyclic units to provide the charge asymmetry for nonlinear optical effect [40].

Second Harmonic Generation (SHG) Test

The second harmonic generation efficiency of AMTP sample was measured by using Kurtz–Perry powder SHG technique with potassium dihydrogen phosphate (KDP) crystal as reference

material[41]. The finely grounded sample was packed between transparent glass slides and an input energy of 2.2 mJ/ pulse was used in this particular setup. The fundamental laser beam of 1064 nm wavelength, 8 ns pulse width, with 10 Hz pulse rate was made to fall normally on the sample cell. The fundamental beam was filtered by using an IR filter. A photomultiplier tube was used as detector. The output from the sample was monochromated to collect the intensity of 532 nm component and to eliminate the fundamental radiation. The generation of the second harmonic was confirmed by the emission of green light. The SHG output efficiency for AMTP and KDP samples were found to be 195 mV and 55 mV respectively. This may be due to the presence of heterocyclic triazole nucleus. In general molecule with delocalized π-electron system can have large nonlinear polarizabilities[42] and it has been generally understood that the molecular nonlinearity can be enhanced by systems with strong donor and acceptor groups [43]. From the analysis of electronic transitions and molecular orbital involved, ligand orbital can improve the NLO properties. Thus, it is observed that SHG efficiencies of AMTP sample were found to be 3.5 times higher than that of KDP.

Table.4. The *in vitro* antimicrobial activity of the ligand AMTP and its metal(II)complexes

Compound/complexes	Zone of inhibition in mm Antibacterial Activity					Antifungal Activity
	Gram (-)ve			Gram(+)ve		
	Pseudomonas auringosa	Shigella	Chromobacterium	Streptococci	Staphlococcus aureus	Candida albicans
Ligand[AMTP]	12	12	12	11	10	11
[Cu (AMTP)$_2$2H$_2$O]	16	17	16	15	15	16
[Co (AMTP)$_2$2H$_2$O]	13	13	15	14	12	12
[Ni(AMTP)$_2$2H$_2$O]	10	13	12	18	18	15
[Zn(AMTP)$_2$2H$_2$O]	15	14	13	12	13	14
Amikain	20	22	20	22	20	---
Ketoconozole	---	---	---	---	---	20

Biological activity

The ligand AMTP and its metal(II) complexes were evaluated for antimicrobial activity by the well diffusion method[44] against the gram positive bacteria like *Streptococci, Staphlococus aureus* gram negative bacteria like *Pseudomonas, Shigella, Chromobacterium* and antifungal activity against *Candida albicans*. Amikacin and Ketoconozole were used as reference compounds for antibacterial and antifungal activities, respectively. Stock solution (10^{-3} M) was prepared by dissolving the compounds in DMSO. The diameter of the inhibition zones was measured in millimeters. Antimicrobial activities were performed in triplicate and the average was taken as the final reading. The growth of inhibition zones after incubation is shown in Table. 4.

A comparison of the Zone of inhibition value of ligand with those of the complexes indicates that the metal complexes exhibited higher antimicrobial activity than ligand. Such increased activity of the complexes can be explained based on the Overtone's concept[45] and the Tweedy chelation theory[46]. According to the Overtone's concept of cell permeability, the lipid membrane that surrounds the cell favors the passage of only lipid-soluble materials, due to which liposolubility is an important factor that controls antimicrobial activity. On chelation, the polarity of the metal ion will be reduced due to the partial sharing of positive charges with donor groups. Furthermore, it increases the delocalization of π-electrons over the whole chelate ring and enhances the lipophilicity of the complexes. This increased lipophilicity enhances the penetration of the complexes into lipid membranes and the blocking of the metal binding sites in the enzymes of microorganisms. The results obtained from the antifungal and antibacterial tests showed that all the tested complexes were more active towards bacteria than fungi. Moreover, the copper complexes were more active than the cobal, nickel and zinc (II) complexes against the tested microorganisms.

DNA BINDING

DNA is one of the most important biomacro molecules in life processes. It plays an important role in the process of storing, copying and transmitting gene messages. DNA is also a major target for drugs and some harmful chemicals, and the studies on the binding nature of these small molecules to DNA are important and fundamental issues on life science because these drugs and chemicals can significantly influence the genetic information expression and result in some diseases related to the cell proliferation and differentiation. Drug–DNA interactions can be classified into two major categories, intercalation and groove binding. Intercalation involves the insertion of a planar molecule between DNA base pairs, which results in a decrease in the DNA helical twist and lengthening of the DNA. Although intercalation has been traditionally associated with molecules containing fused bi/tricyclic ring structures, a typical intercalators with non fused rings systems may be more prevalent than previously recognized.

Viscosity Studies

Viscosity measurements at room temperature were performed using a semi-micro dilution capillary viscometer. Each experiment was performed three times and an average flow time was calculated. The data are presented as $(\eta/\eta_0)^{1/3}$ *vs.* the binding ratio, where η is the viscosity of a DNA solution in the presence of a complex and η_0 is the viscosity of DNA in solution alone.

The application of an optical photophysical technique to investigate the interactions of DNA with metal complexes generally provides clues that are needed but not sufficient by themselves

to support an intercalative binding model. Therefore, viscosity measurements were introduced to provide further support for this type of interaction between the complexes and DNA. In the absence of crystallographic structural data, hydrodynamic methods which are sensitive to the length of the DNA are known to be among the definitive and critical indicators of binding strength. Intercalation is an effect of increasing the viscosity of DNA[47, 48]. The significant increase in the viscosity of DNA that occurred upon the addition of a complex was due to intercalation, which caused the DNA bases to separate in order to the increase the effective size of the DNA, which could be the reason for the increase in the viscosity. A plot of $(\eta/\eta_0)^{1/3}$ *vs* [complex]/[DNA] (R) gives a measure of the change in viscosity.

From the Figure.7. a gradual increase in the relative viscosity was observed upon the addition of the metal complexes to the DNA solution, suggesting that the complexes mainly bind via an intercalation mode. Not only does this result give sign of an intercalative binding mode of the complex, but it is also in agreement with the pronounced hypochromism and bathochromism of the complexes in the presence of DNA.

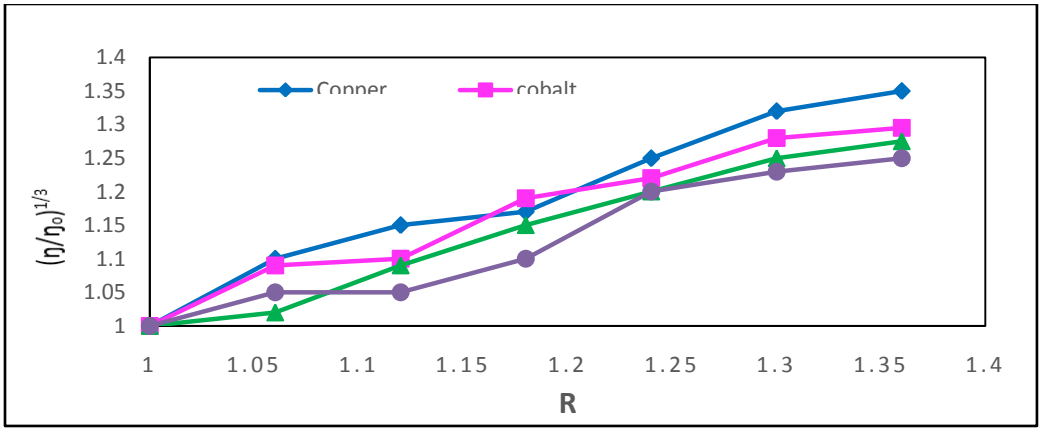

Figure.7.Effect of increasing amounts of [Cu(L3)$_2$.2H$_2$O] (♦),[Co(L3)$_2$.2H$_2$O](■), [Ni(L3)$_2$.2H$_2$O](▼) [Zn(L3)$_2$.2H$_2$O] (●), on the viscosity of DNA. R = [complex]/[DNA].

DNA cleavage studies

The oxidative cleavage activity of the complexes of AMTP was studied by gel electrophoresis using calf thymus DNA (15 µl) in Tris-HCl buffer (pH = 7.0). Selected CT- DNA cleavage activity of the gel diagram is shown in Fig. 8. The gel to conduct electrophoresis with such systems including DNA alone, DNA+H$_2$O$_2$+M(II), (M = Cu(II), Co(II), Ni(II) and Zn(II) complexes, were prepared under the same condition and kept at 2 h in order to eliminate the influence of the reaction speed. The cleavage activity of the complexes was carried out for 2 h exposure. The cleavage efficiency of the complexes compared with that of the control is due to their efficient DNA-binding ability.

In the present study, the CT- DNA gel electrophoresis experiment was carried out at 35 °C using our synthesized complexes in presence of H_2O_2 as an oxidant. It was found that, all complexes exhibit nuclease activity in presence of H_2O_2.

The general oxidative mechanisms proposed to account for DNA cleavage by hydroxyl radicals *via.* abstraction of a hydrogen atom from sugar units and predict the release of specific residues arising from transformed sugars, depending on the position from which the hydrogen atom is removed[49]. The cleavage is inhibited by the free radical scavengers implying that hydroxyl radical or peroxy derivatives mediate the cleavage reaction. From Fig. 8. it is evident that all the complexes cleave DNA more efficiently in the presence of an oxidant (H_2O_2). This may be attributed to the formation of hydroxyl free radicals. The production of a hydroxyl radical due to the reaction between the metal complex and oxidant may be explained as shown below:

$(Ligand)Cu^{2+} + H_2O_2 \rightarrow (Ligand)Cu^{3+} + OH^{\bullet} + OH^{-}$

The $OH^{\bullet}$ free radicals participate in the oxidation of the deoxyribose moiety,

followed by hydrolytic cleavage of a sugar phosphate backbone. All the complexes showed pronounced nuclease activity in the presence of the oxidant H_2O_2, which may be due to the increased production of hydroxyl radicals.

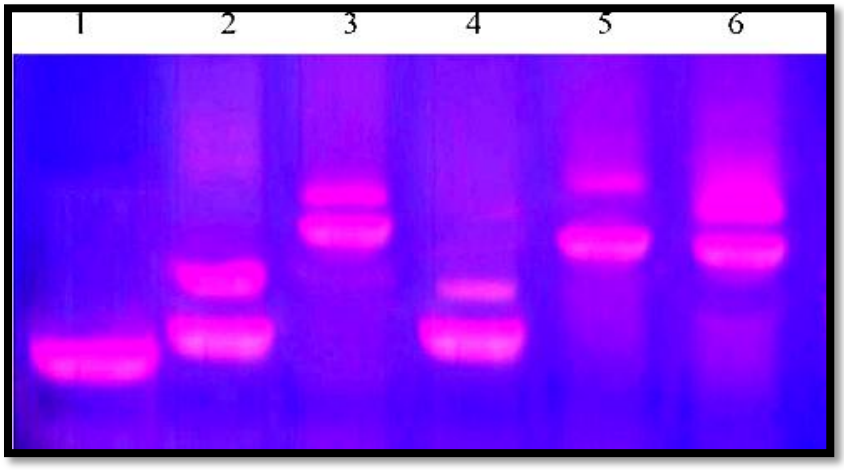

Figure. 8 Changes in the gel electrophoretic pattern of CT DNA induced by H_2O_2 and Cu(II), Co(II), Ni(II) and Zn(II) complex.

Lane 1: CT DNA alone
Lane 2: CT DNA + Cu(II) complex
Lane 3: CT DNA + Co(II) complex
Lane 4: CT DNA + Ni(II) complex
Lane 5: CT DNA + Cd(II) complex
Lane 6: CT DNA + Zn(II) complex

Control experiment using DNA alone does not show any significant cleavage of CT DNA even on longer exposure time. Hence, we conclude that the copper(II), cobalt(II), nickel(II) and

zinc(II) complexes cleaves DNA as compared with control DNA in the presence of H_2O_2. Further, the gel diagram indicates the presence of radical cleavage[50].

CONCLUSIONS:

Based on the above observations of the elemental analysis, molar conductivity, UV-Vis., magnetic moment IR and ^1H NMR spectral data it is possible to determine the type of coordination of the ligands in their metal complexes. The results of these studies suggested a Octahedral geometry for all the complexes. From the results of cyclic voltammetry it is shown that copper(II) complex exhibit quasi-reversible cyclic voltammetric responses in acetonitrile solution corresponding to the Cu(II)/Cu(I) redox process.. The EPR parameters of copper(II) complex indicate that the complex has octahedral geometry. The analytical data show the presence of one metal ion per two ligand molecules and suggest a mononuclear structure for the complexes. The SHG efficiency was measured using Kurtz and Perry method and is found to be about 1.2 times that of the standard KDP crystal. These results show that AMTP is a NLO material with possible applications for frequency conversion. In addition the binding modes of CT-DNA to the metal complexes are also studied by viscosity measurements method. The metal complexes can interact with CT-DNA through intercalative binding mode. Chemical nuclease activity of these metal complexes with CT- DNA was investigated by gel electrophoresis. From the results, it is found that the metal complexes cleaved DNA efficiently in the presence of H_2O_2 as compared to the control DNA. The *in vitro* biological activity of the AMTP and its metal(II) complexes indicate that the complexes have higher antimicrobial activity than the free ligand.

ACKNOWLEDGMENTS

The authors thank the Management of Thiagarajar College, Madurai, and the Management of S.Vellaichamy Nadar Polytechnic College, Virudhunagar. P.S one of the author thanks to Directorate of Technical Education, Chennai. for permitting him to do his Ph.D. The authors thanks to SAIF, IIT, Bombay and CDRI, (SAIF) Lucknow for providing analytical facilities.

REFERENCE

1. Mathew V, Keshavayya J, Vaidya V.P. Giles D. Studies on synthesis and pharmacological activities of 3,6-disubstituted-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazoles and their dihydro analogues. Eur J Med Chem 2004; 42: 823 -840
2. S. Jantova, D. Hudecova, S. Stankovsky, K. Spirkova, Antimicrobial effects of new 1-(1,2,4-triazol-1-yl)-acetanilides, 1994 ; 39: 152-154

3. Raafat M, Shaker. The chemistry of mercapto- and thione- substituted 1,2,4-triazoles and their utility in heterocyclic synthesis Arkivoc 2006 (ix) 59-112
4. Narashima Murthy, Raghuram Rao M, Narahari Sastry G, Aromatase Inhibitors: A New Paradigm in Breast Cancer Treatment Curr. Med. Chem. - Anti-Cancer Agents, 2004, 4.
5. Brodie A.M, Schwarzel W.C, Shaikh A.A, Brodie H.J The Effect of an Aromatase Inhibitor, 4-Hydroxy-4-Androstene-3,17-Dione, on Estrogen Dependent Processes in Reproduction and Breast Cancer. Endocrinol 1977; 100:1684 -88
6. Jyoti Sharmaa, Shamim Ahmadb, M. Shamsher Alama, Bioactive Triazoles: A potential review, J Chem Pharm Research, 2012; 4(12):5157-5164
7. Anita Srivastava, Nizamuddin. Synthesis and fungicidal activity of some triazole derivatives Indian J Heterocyclic Chem. 2005; 14: 221- 224,
8. Mahendra Shiradkar, Gorentla, Venkatesa Suresh Kumar, Varaprasad, Dasari, Suresh Tatikonda, Kalyanchakra-varthy Akula, Rachit Shah. Clubbed triazoles: A novel approach to antitubercular drugs EurJ Med Chem. 2007; 42: 807-816,
9. Shivarama Holla B. Veerendra. B, Shivananda .M.K, Boja Poojary, Synthesis characterization and anticancer activity studies on some Mannich bases derived from 1,2,4-triazoles; Eur. J. Med Chem. 2003; 38, 759–767
10. Neslihan Demirbas . N, Sengül Alpay Karaoglu, Ahmet Demirbas, Kemal Sancak, Synthesis and antimicrobial activities of some new 1-(5-phenylamino-[1,3,4]thiadiazol-2-yl)methyl-5-oxo-[1,2,4]triazole and 1-(4-phenyl-5-thioxo-[1,2,4]triazol-3-yl)methyl-5-oxo- [1,2,4]triazole derivatives. Eur J Med Chem, 2004; 39 : 793–804
11. J. T. Witkowski , Roland K. Robins , Robert W. Sidwell , Lionel N. Simon, Design, synthesis, and broad spectrum antiviral activity of 1-.beta.-D-ribofuranosyl-1,2,4-triazole-3-carboxamide and related nucleosides, J. Med. Chem., 1972; *15* (11) : 1150–1154
12. Hart C, Drug for Migraine in: Modern Drug discovery, American Chemical Society, 1999.
13. Vishal Patil, William Guerrant, Po C. Chen, Berkley Gryder, Derek B. Benicewicz, Shabana I. Khan, Babu L. Tekwani, Adegboyega K. Oyelere, Antimalarial and antileishmanial activities of histone deacetylase inhibitors with triazole-linked cap group. Bioorganic & Medicinal Chemistry, 2010 ; 18 : 415–425
14. Chohan Z.H, Sumrra S.H, Youssoufi M.H,. Hadda T.B, Metal based biologically active compounds: design, synthesis, and antibacterial/antifungal/cytotoxic properties of triazole-derived Schiff bases and their oxovanadium(IV) complexes. Eur. J. Med. Chem, 2010; 45:2739,

15. Avaji P.G, Patil S.A, Synthesis, spectral characterization and microbiological studies of Co(II), Ni(II) and Cu(II) complexes with some novel 20-membered macrocyclic hydrazino-1,2,4-triazole Schiff bases J. Enzyme Inhib. Med Chem, 2009; 24: 140,
16. Collin X, Sauleau A, J. Coulon, 1,2,4-Triazolo mercapto and aminonitriles as potent antifungal Bioorg. Med. Chem. Lett. 2003; 13: 260,
17. Ali T.E, Utility of thiocarbohydrazide in heterocyclic synthesis J. Sulfur Chem. 2009; 30: 611.
18. Syed Azhar Nizami, Gurumurthy M, Swarup jyoti Chattarjee, Debashisha Panda, Synthesis, characterization & antimicrobial activity of some substituted 1,2,3-triazoles. J.Ad. Pharma Research. 2010; 1: 26-35,
19. ShobhaRani S, BairiAgaiah, Sarangapani M, Antioxidant and DNA binding study 3,3'-(5,5'-methylene bis (3-mercapto -4h-1,2,4-triazole-5,4-diyl)bis(azan-1-yl-1-ylidene) diindolin-2-ones In. J. Pharmacy&Technology, 2010; 2: 366-374,
20. N. Shalini, V. Sharma, synthesis, characterisation and biological aspects of Iron(III) and Cobalt(III) complexes with schiff bases derived from substituted mercaptotriazole, Rev. Roum. Chim.2011, 56(3), 189-196
21. Geary W.J, The use of conductivity measurements in organic solvents for the characterization of coordination compounds. Coord. Chem. Rev. 1971; 7: 81
22. Vidyavati reddy**,** N. Patil,Tukaram reddy, S. D. Angadi, Synthesis, Characterization and Biological Activities of Cu(II), Co(II), Ni(II), Mn(II) and Fe(III) Complexes with Schiff Base Derived from 3-(4-Chlorophenoxymethyl)-4-amino-5-mercapto-1,2,4-triazole, E-J Che. 2008; 5: 529-538
23. Gangadhar B. Bagihalli, Prakash Gouda Avaji, Sangamesh A. Patil, Prema S. Badami, Synthesis, spectral characterization, in vitro antibacterial, antifungal and cytotoxic activities of Co(II), Ni(II) and Cu(II) complexes with 1,2,4-triazole Schiff bases, Eur. J Med Chem, 2008; 43: 2639-2649
24. Bagihallia.G.B,. Patila S.A, Badami P.S, Synthesis, Physicochemical Investigation and Biological Studies of Zinc(II) Complexes with 1,2,4-Triazole Schiff Bases, J. Iran. Chem. Soc, 2009; 6: 259-270.
25. Fabretii A C, Grancini G C and Peyronet G, Rao C N R. Chemical Applications of Infrared Spectroscopy; Academic press Spectrochim. Acta, 1985, 26A, 698. 44.
26. Holman T.R, Hendrich M.P, Que L, EPR Studies of a Dinickel Complex in Its II,II and 11,111 Oxidation States Inorg. Chem.1992; 31: 937.

27. Karaoglu K, Baran T, Serbest K, Mustafa Er, Degirmencioglu I, Two Novel Macroacyclic Schiff Bases Containing bis-N_2O_2 Donor Set and Their Binuclear Complexes: Synthesis, Spectroscopic and Magnetic Properties, J. Mol. Struct. 2009; 922**:** 39.
28. Mishra A.P, Purwar H, Rajendra K, Jain, Gupta S.K, Microwave Synthesis, Spectral, Thermal and Antimicrobial Studies of Some Co(II), Ni(II) and Cu(II) Complexes Containing 2-Aminothiazole Moiety E-Journal of Chemistry, 2012; 9(4): 1655-1666
29. Manonmani J, Thirumurugan R, Kandaswamy M, Kuppayee M, Raj S.S.S, Ponnuswamy M.N, Shanmugam G, Synthesis of mononuclear nickel(II) and copper(II) complexes using compartmental ligands: X-ray and electrochemical studies Fun. H.K. Polyhedron, 2000; 19: 2011
30. Kalanithiy M, Rajarajan M, and Tharmaraj P. Synthesis, spectral, and biological studies of transition metal chelates of N-[1-(3-aminopropyl)imidazole]salicylaldimine. Journal of Coordination Chemistry 2011; 64: 842–850.
31. Lever A.B.P, Inorganic Electronic Spectroscopy, Elsevier, Amsterdam, 1984.
32. Zhu D.R, Song Y, Xu Y, Zhang Y, Raj S.S.S, Fun H.K, You X.Z, Syntheses, Crystal Structures, and Properties of the Novel Co(II) and Ni(II) Complexes with 4-(p-methylphenyl)-3,5-bis(pyridin-2-yl)-1,2,4-triazole. Polyhedran, 2000; 19: 2019
33. Klement R, Stock F, Elias H, Paulus H, Pelikan P, Valko M, Mazur M, Copper(II) complexes with derivatives of salen and tetrahydrosalen: a spectroscopic, electrochemical and structural study Polyhedron 1999; 18: 3617.
34. Raman. N, Muthuraj. V, Ravichandran. S, Kulandaisamy. A, Synthesis, characterization and electrochemical behaviour of Cu(II), Co(II), Ni(II) and Zn(II) complexes derived from acetylacetone and *p*-anisidine and their antimicrobial activity, Proc. Indian Acad. Sci. (Chem. Sci.), 2003; 3 :161–167
35. Speir G, Csihony J, Whalen A.M, Pierpont C**.**G, Studies on Aerobic Reactions of Ammonia/3,5-Di-*tert*-butylcatechol Schiff-Base Condensation Products with Cu, Cu(I), and Cu(II). Strong Cu(II)-Radical Ferromagnetic Exchange and Observations on a Unique N-N Coupling Reaction Inorg. Chem.1996; 35: 3519.
36. Raman. N, Ravichandran.S, Thangaraja. S, Copper(II), cobalt(II), nickel(II) and zinc(II) complexes of Schiff base derived from benzil-2,4-dinitrophenylhydrazone with aniline, J. Chem. Sci, 2004; 4: 215–219.
37. Figgis B.N, Lewis J, Modern Coordination Chemistry, Willey Interscience, New York 1960.

38. Khan O, Molecular Magnetism, New York, VCH, 1993.
39. Naseema K, Vijayalakshmi Rao, Sujith K.V, Balakrishna Kalluraya, Crystal growth and characterization of an NLO organic crystal: N′-[(Z)-(4-methylphenyl)methylidene]-4-nitrobenzohydrazide Curr. App.Physics 2010; 10: 1236-1241
40. Mari Carmen Ruiz Delgado, Victor Herna´ndez, Juan T. Lo´pez Navarrete, Shoji Tanaka, Yoshiro Yamashita, Combined Spectroscopic and Theoretical Study of Narrow Band Gap Heterocyclic Co-Oligomers Containing Alternating Aromatic Donor and O-Quinoid Acceptor Units , J. Phys. Chem. B 2004; 108: 2516-2526
41. Skotheim T.A, Hand book of conducting Polymers, Marcel Dekker:New York.1986;1,2
42. Hui Pan, Xuling Gao, Yue Zhang,' and Paras Prasad .N: A New Class of Heterocyclic Compounds for Nonlinear Optics Chem mater.1995; 7: 816-821
43. Kurtz S. K, Perry J. J, A Powder Technique for the Evaluation of Nonlinear Optical Materials J. Appl. Phys. 1968; 39: 3798-3813**,**
44. Irobi, O, Moo-Young M and Anderson WA Antimicrobial activity of Annatto (Bixa orellana extract) .Int J Pharm 1996 ;34: 87-90.
45. Anjaneyulu .Y, Murthy .L.N, Prabhakara Rao .R Preparation, characterisation and antimicrobial activity studies on some ternary complexes of Cu(II) with diethythiocarbamate and 8- hydroxyquinolines (1986): Synth.React.Inorg.Met org.Ehem. 16(2), 257
46. Mishra L, Singh V. K, Co(ll), Ni(ll) and Cu(lI) and Zn(lI) complexes with Schiff bases derived from 2-aminobenzimidazoles and pyrazolycarboxaldehyde Indian J. Chem. 1993; 32A: 446,
47. Sobha .S, Mahalakshmi .R, Raman. N, Studies on DNA binding behaviour of biologically active transition metal complexes of new tetradentate N_2O_2 donor Schiff bases: Inhibitory activity against bacteria, Spectrochimica Acta, 2012; 92: 175– 183
48. Liu J.G, Ye B.H, Li H, Zhen Q.X, Ji L.N, Fu Y.H, Polypyridyl ruthenium (II) complexes containing intramolecular hydrogen-bond ligand: syntheses, characterization, and DNA-binding properties J. Inorg. Biochem. 1999;76: 265.
49. Thomas A.M, Naik A.D, Nethaji M, Chakravarty A.R. Photo-induced DNA cleavage activity of ternary (N-salicylidene-L-methioninato)copper(II) complexes of phenanthroline bases Indian J. Chem, 2004; 43A: 691
50. Uma V, Kanthimathi M, Subramanian J, Nair B.U. A new dinuclear biphenylene bridged copper(II) complex: DNA cleavage under hydrolytic conditions. Biochim. Biophys. Acta, 2006; 814: 1760