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Synthesis, Characterization, Structural Interpretation, Biological Activity and DNA Cleavage Studies of 3-acyl 2-(2'hydroxy-5-X phenyl) Benzothiazoline Cu (II) Complexes

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ABSTRACT: *Benzothiazolines and other compounds containing –NC_6H_4S- are reported to have biological activity. The ligand 3-acyl 2-(2'hydroxy-5-X phenyl)benzothiazoline (X=H, Cl, NO_2, OCH_3) forms complexes [Cu(L-H)] which have been characterised by various spectro-analytival techniques such as elemental analyses, magnetic moments, molar conductance, electronic, mass, ESR, TGA and IR spectral measurements. Room temperature ESR spectra of Cu(II) complexes inferred 'g' values characteristic of square planar geometry and square pyramidal geometry. The QSAR and molecular properties of title compounds were computed by employing HyperChem 7.5 tools. The title compounds and their corresponding Cu(II) complexes were assayed by the agar disk diffusion method for antibacterial and antifungal action against* E. coli, *S. marcescens, P. aeruginosa, S. aureus, B. Subtilis and C. Albicans respectively. The DNA cleavage studies on PBR322 DNA indicated the hydrolytic cleavage by Cu (II)-AHNPBT, wherein super coiled DNA (form I) is converted into relaxed and linear DNA (form II & form III).*

Keywords: *Benzothiazoline, Cu (II) complexes, DNA Cleavage studies, ESR studies*

I. INTRODUCTION

The coordination chemistry of nitrogen-oxygen donor ligands is an interesting area of research. Many studies on benzothiazoline have been made because of their structural interest. Namely, they contain two different heteroatoms linked by one carbon. Benzothiazolines and other compounds containing –NC_6H_4S- are reported to have biological activity [1]. A large number of benzothiazolines have been prepared by the reactions of aldehyde and ketones with 2-aminothiophenol[2-4]. Benzothiazolines constitute an important class of bidentate as well as multidentate ligands[5-7].The use of these Lewis base functionalized ligands can be effective in increasing the coordination number of the central metal atom at the expense of the benzothiazoline ring to the corresponding Schiff base derivatives, leading to the greater stability of the resulting compounds.

Keeping this in view, we have synthesised new complexes of Cu(II) with 3-acyl 2-(2'hydroxy-5-X phenyl)benzothiazoline (I), X=H, Cl, NO_2, OCH_3 as ligand (Fig. 1) and described and discussed a preliminary investigation of their structure.

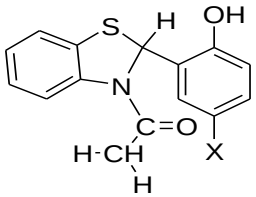

Figure 1

(Ia), AHPBT, X=H
(Ib), AHCPBT, X=Cl
(Ic), AHMPBT, X=OCH_3
(Id), AHNPBT, X=NO_2

II. EXPERIMENTAL

2.1. Chemicals

Starting materials are commercial reagents, 2-aminothiophenol from Alfa Aesar Lancaster, 5-X salicylaldehydes (where X= H, Cl, NO_2, OCH_3) from Sigma Aldrich and Copper Chloride from SD`s fine chemicals. All the chemicals and solvents used were dried and purified by standard methods. The moisture was excluded from the glass apparatus using $CaCl_2$. Experiments were performed at room temperature.

2.2. Instrumentation

The MS data was collected on Agilent Single Quad Mass Spectrometer. The IR Spectra (KBr) was recorded on a Bruker Optics Tensor-27 FTIR. Molar conductivities of the complexes were measured in DMSO at room temperature using Digison conductivity instrument.

The thermal studies were carried out in a dynamic nitrogen atmosphere (20 ml min-1) with a heating rate of (10^oC min-1) using a Shimadzu TGA-50H. Electronic spectra were recorded using a UV-Vis 2450 Schimadzu spectrophotometer. ESR spectra were registered on a Varian E112 type spectrophotometer. The measurements were done in the L band in air atmosphere at room temperature. The melting points were determined with Polmon apparatus (Model No.MP-90).

2.3. Synthesis of the ligand

The 3-acyl-2-(2'-hydroxy-5-X phenyl) benzothiazoline was formed by condensation of 2-aminothiophenol with 5-X salicylaldehyde (where X=H, Cl, NO_2 and OCH_3) in equimolar ratio in a polar solvent. Acylation of the 3-amino group was achieved by using acetic anhydride[8]. The resultant 3-acyl-2-(2'-hydroxy-5-X phenyl) benzothiazoline is recrystallized using suitable solvents.

2.4. Synthesis of the complexes

A mixture of title compound (0.005 mol) and anhydrous $CuCl_2$ (0.005 mol or 0.01 mol) in methanol medium was refluxed on water bath for 8-10 hours at 70-80 °C by adjusting pH in the range 6.0-7.0 to enable complex formation. The precipitated complex was filtered off, washed with water, hot ethanol and dried in vacuum at room temperature. The product was dried in air and stored in a desiccator over anhydrous $CaCl_2$ under vacuum.

III. RESULTS AND DISCUSSION

The new copper complexes synthesized in the present investigation have melting points higher than 300^0C. The elemental analyses data along with some physical properties of the complexes are reported (Table 1). All the metal complexes are coloured and stable to air and moisture. They are soluble in DMF and DMSO, but insoluble in other organic solvents.The molar conductivities of the complexes in DMSO were found to be 3.0-5.0 $\Omega^{-1}cm^2mol^{-1}$(Table 1) suggesting their non-electrolytic nature.

Table 1: Mass Spectral data, Molar conductivity and Magnetic moment values

No.	Compound	Molecular Formula	Colour	Mass Spectral Data	$\wedge$ $\Omega^{-1}cm^2mol^{-1}$	μ_{eff} (BM)
(1)	**Cu-AHPBT**	Cu-$C_{15}H_{13}NO_2S$ Cl.H_2O	Light Brown	391	3.2	2.13
(2)	**Cu-AHCPBT**	Cu-$C_{15}H_{13}NO_2ClS$ Cl. H_2O	Brown	423	3.1	1.81
(3)	**Cu-AHMPBT**	Cu-$C_{16}H_{15}NO_3SCl.(H_2O)_2$	Dark Brown	435	3.4	1.60
(4)	**Cu-AHNPBT**	Cu-$C_{15}H_{12}N_2O_4SCl.H_2O$	Yellowish Brown	432	3.5	1.63

3.1. Infrared Spectra

The comparative IR spectral study of the ligands AHPBT, AHCPBT, AHMPBT and AHNPBT and their Cu(II) complexes reveals the coordination mode of the ligand during the complex formation. The IR spectrum of the ligands (Table 2) shows bands at 3130-3226 cm^{-1} due to the presence of OH group, which is absent in complexes and a small broad peak is observed in the region 3323-3446cm-1 indicating the participation of –OH group in complex formation. The C=O stretching vibration is also shifted to the lower frequencies. The nature of the metal-ligand bonding is confirmed by the newly formed bands at ~ 550 cm^{-1} and ~ 470 cm^{-1} in the spectra of complexes(Fig. 2-5), which is tentatively assigned to Cu-O and Cu-N vibrations [9-11].

Table 2: IR bands and their assignments for 3-acyl 2-(2'hydroxy-5-X phenyl) benzothiazoline (Ia-d) and its Cu complexes (1)-(4).

No.	Compound	$\upsilon_{C=O}$	υ_{C-X}	$\upsilon_{OH/H2O}$	$\upsilon_{Cu-O/Cu-N}$
(Ia)	**AHPBT**	1635	-	3211	-
(1)	**Cu-AHPBT**	1608	-	3446	551/472
(Ib)	**AHCPBT**	1620	740	3165	-
(2)	**Cu-AHCPBT**	1604	742	3433	570/450
(Ic)	**AHMPBT**	1631	1423	3226	-
(3)	**Cu-AHMPBT**	1620	1453	3323	543/470
(Id)	**AHNPBT**	1689	1334	3130	-
(4)	**Cu-AHNPBT**	1605	1328	3338	561/462

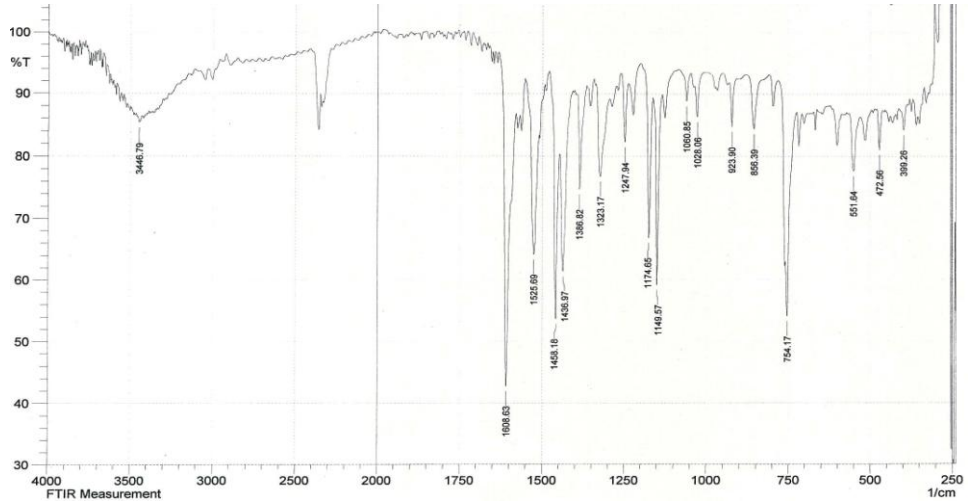

Figure 2: IR Spectrum of Cu-AHPBT

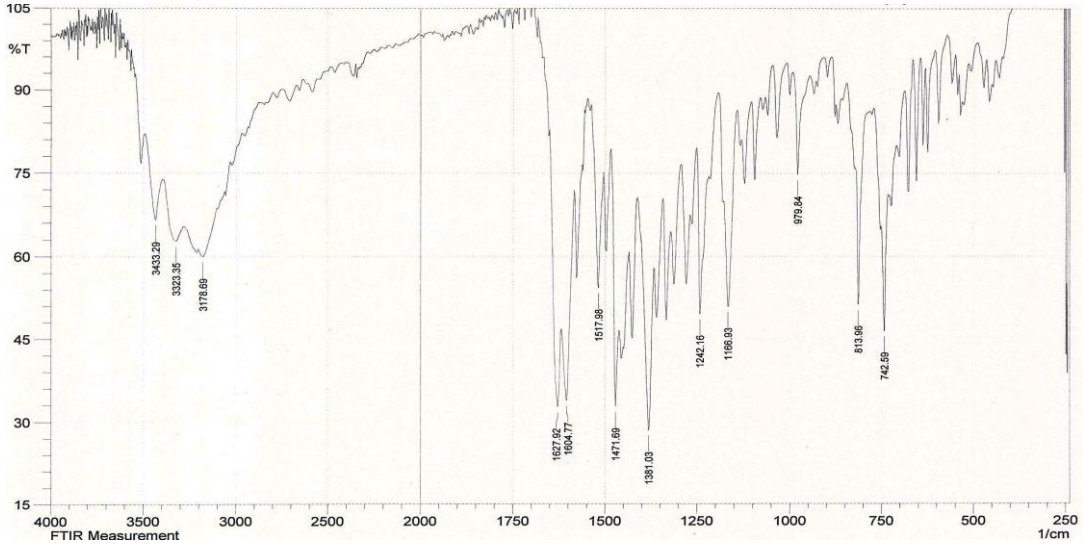

Figure 3: IR Spectrum of Cu-AHCPBT

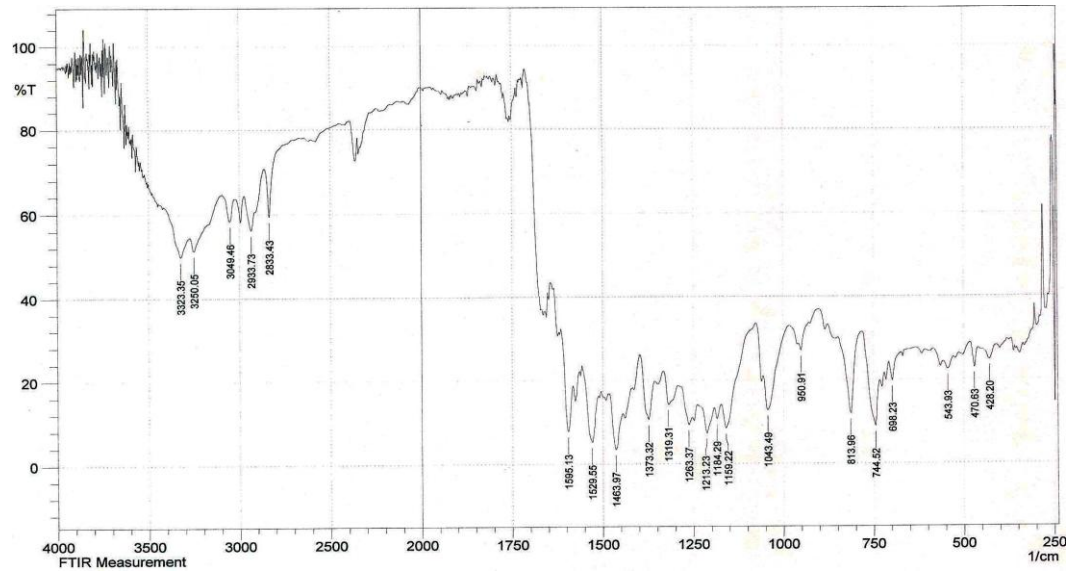

Figure 4: IR Spectrum of Cu-AHMPBT

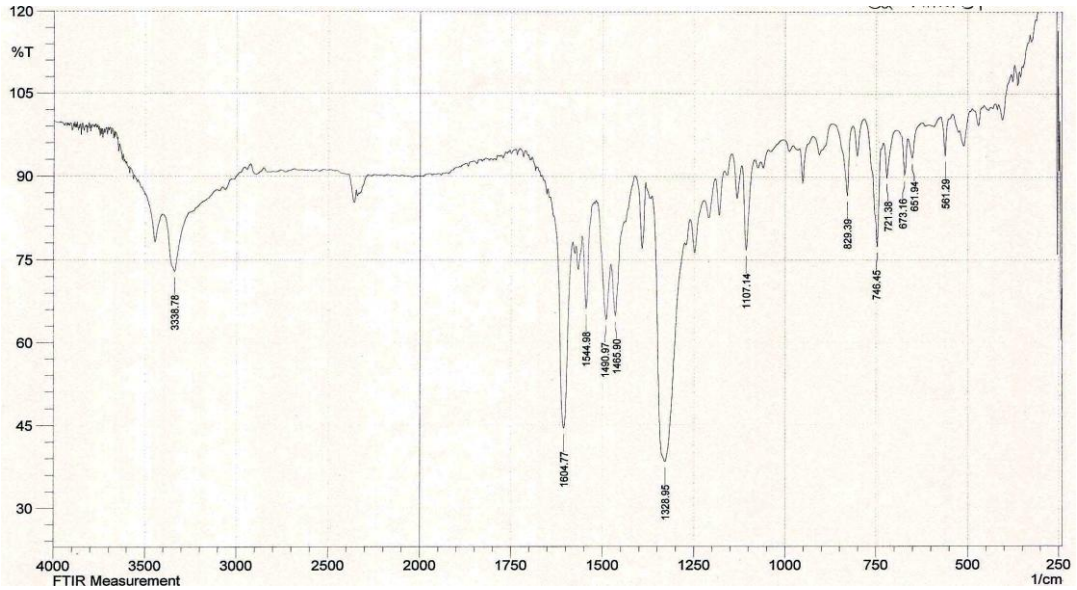

Figure 5: IR Spectrum of Cu-AHNPBT

3.2. Electronic spectra

The electronic spectra of Cu (II) complexes are compared with those of the ligands (Fig. 6-9). Two bands appeared at 38610-39840 cm^{-1} and 28735-30487 cm^{-1}, which can be assigned to π-π* and n-π* transitions, respectively, in all the ligands. The electronic spectra of complexes showed low energy bands at 10437-16420 cm^{-1} and a strong high energy band at 23310-28328 cm-1. The low energy band in the position may be assigned to the transitions dx^2-y^2→dyz; dz^2; dxy[12-13]. The strong high energy band is assigned to metal→ligand charge transfer. The observed magnetic moment values (Table 1) for the complexes also support for the same.

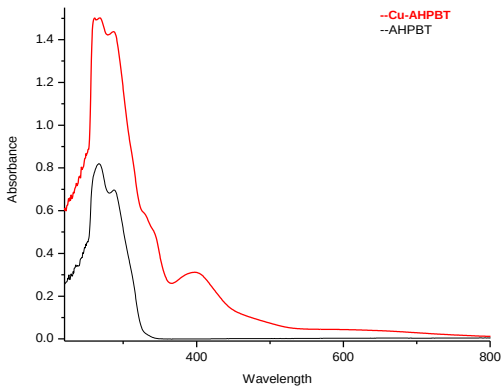

Figure 6: UV Spectrum of Cu-AHPBT

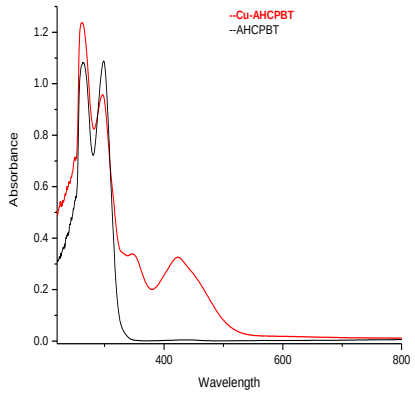

Figure 7: UV Spectrum of Cu-AHCPBT

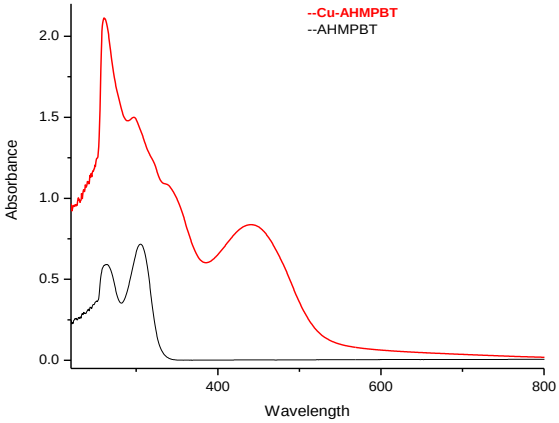

Figure 8: UV Spectrum of Cu-AHMPBT

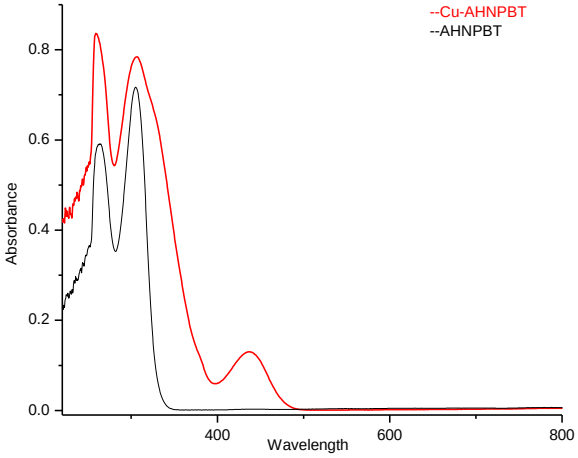

Figure 9: UV Spectrum of Cu-AHNPBT

3.3. Mass Spectra

The mass spectra of the metal complexes are presented in (Fig.10-13) and the analysed data is presented in (Table 1). All the complexes show m/z peaks which are in accordance with the expected mass. The mass spectra confirm that metal complexes are formed in 1:1 ratio.

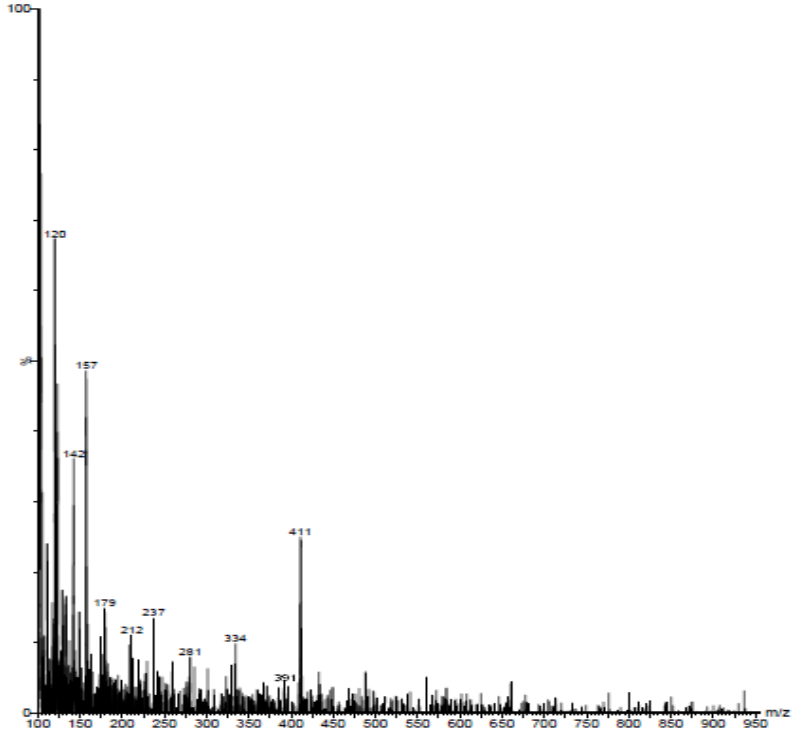

Figure 10: Mass Spectrum of Cu-AHPBT

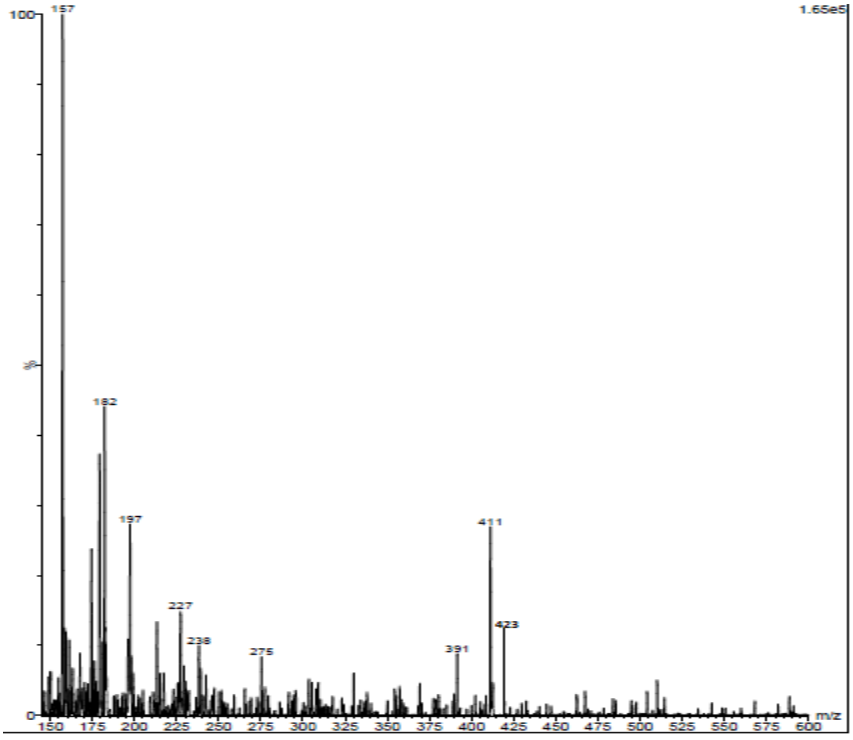

Figure 11: Mass Spectrum of Cu-AHCPBT

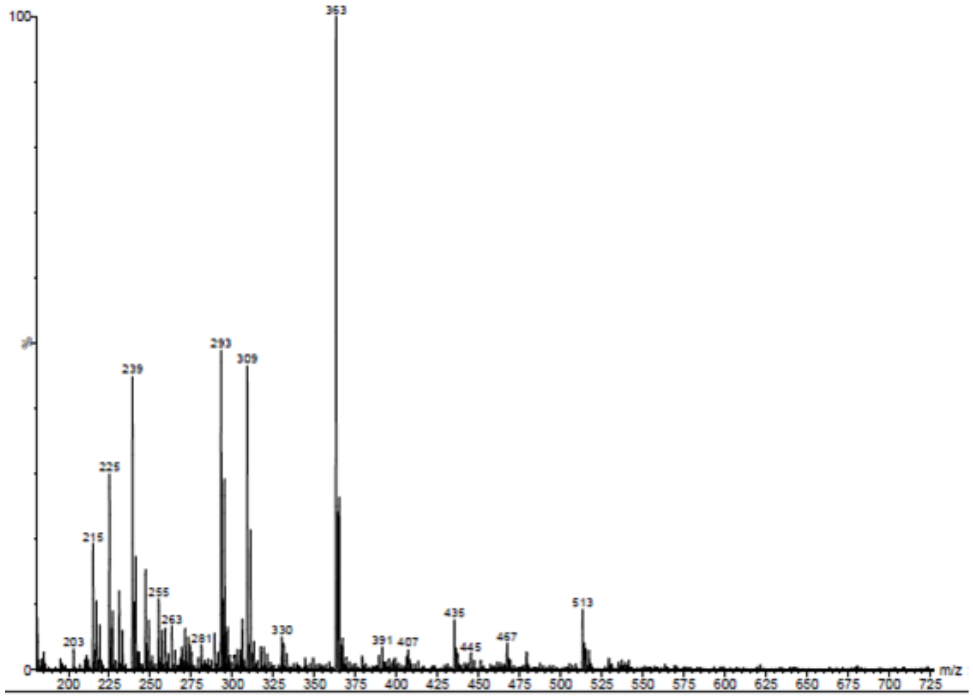

Figure 12: Mass Spectrum of Cu-AHMPBT

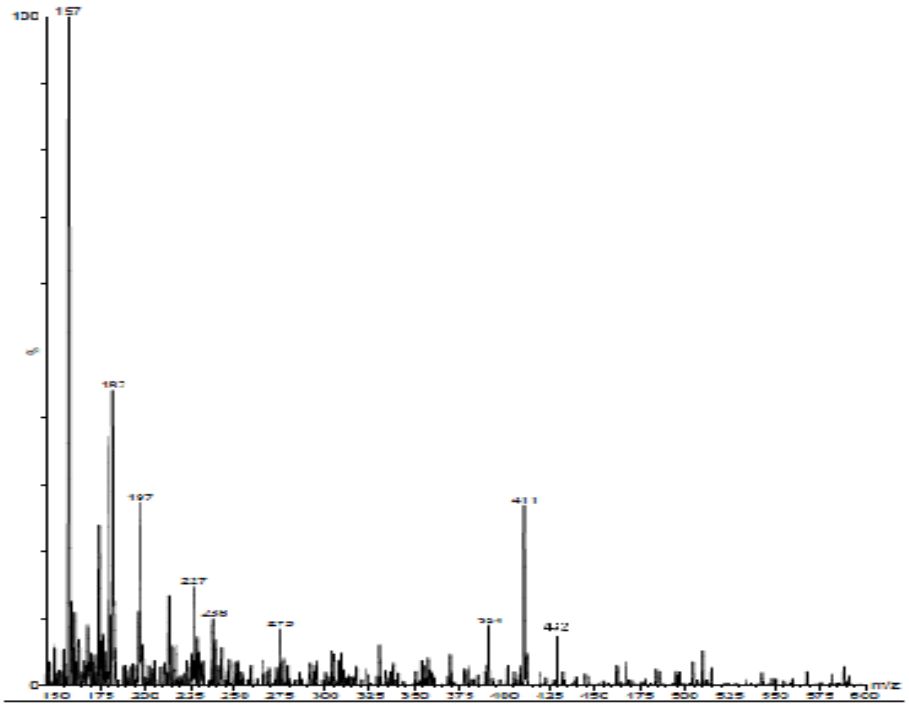

Figure 13: Mass Spectrum of Cu-AHNPBT

3.4. EDS Analysis

The EDS analysis of all the ligands and their Cu-complexes (Fig. 14-17) shows that the elemental composition is in agreement with the calculated values. The data confirms the 1:1 composition of the metal complexes.

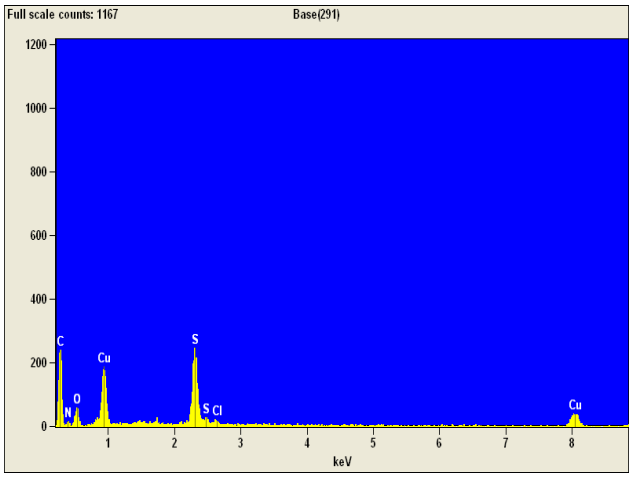

Figure 14: EDX image of Cu-AHPBT

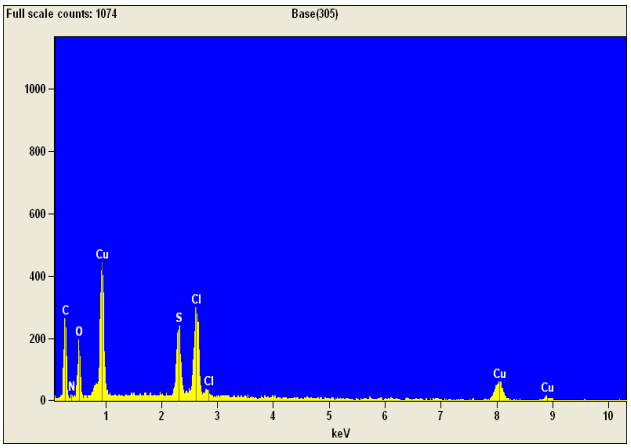

Figure 15: EDX image of Cu-AHCPBT

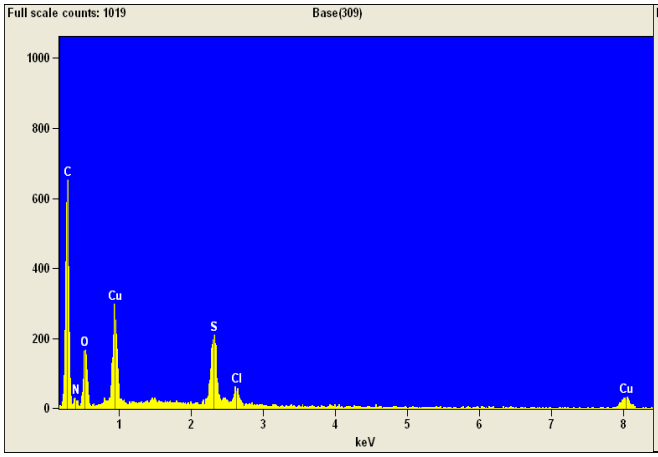

Figure 16: EDX image of Cu-AHMPBT

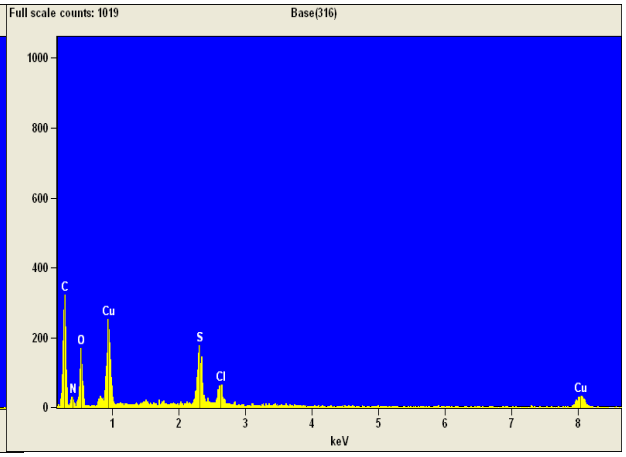

Figure 17: EDX image of Cu-AHNPBT

3.5. ESR Studies

In general the ESR spectral data of a metal complex provides not only information of metal ion geometry but also metal ligand bond covalency. The L band ESR spectra recorded at RT for the copper complexes in the present investigation (Fig.18-21) show well resolved peaks indicating hyperfine interactions. In all copper complexes under study the g value is nearly isotropic (Table 3). The hyperfine components are well resolved in Cu-AHBT and Cu- AHNBT complexes while in Cu-AHCPBT and Cu- AHMPBT systems the resolution of hyperfine components is relatively less.

Table 3: g values of Cu-Complexes

Complex	'g' factor
Cu-AHPBT	1.8677
Cu-AHCPBT	2.0016
Cu-AHNPBT	1.8678
Cu-AHMPBT	2.0711

The analysis of intensities of peaks is useful in predicting the covalent character in complexes. The copper nuclear spin being 3/2 , and one copper nuclei (n = 1) interaction through Fermi contact term with electron spin would split two spin energy levels into each four hyperfine components (m_S = +½, m_I = +3/2, +1/2, -1/2, -3/2 and m_S =- ½, -3/2 , -1/2+, +1/2, 3/2 and as per selection rules ($\Delta m_S = \pm 1$ and $\Delta m_I = 0$) the four transition are allowed.

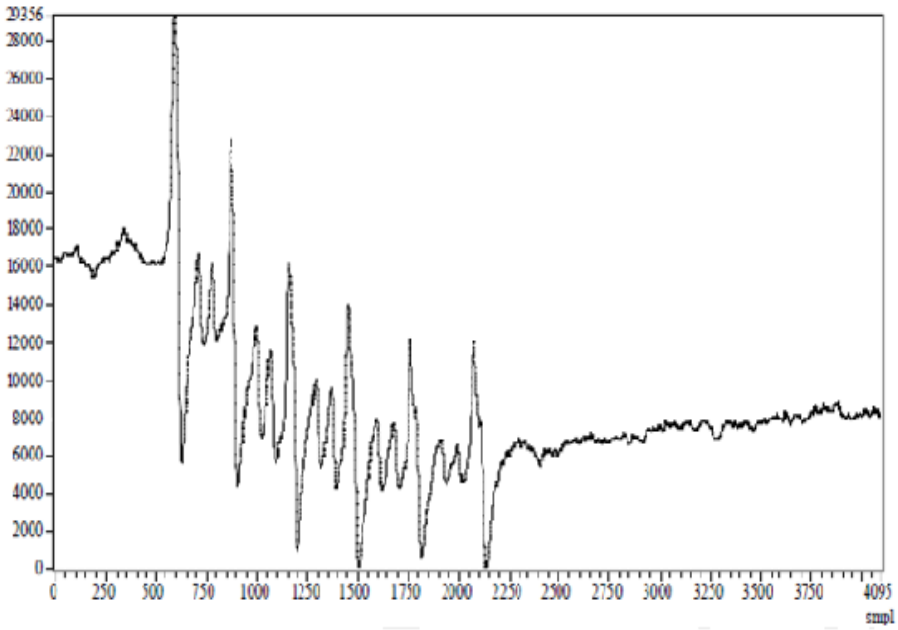

Figure 18: ESR Spectra of Cu-AHPBT

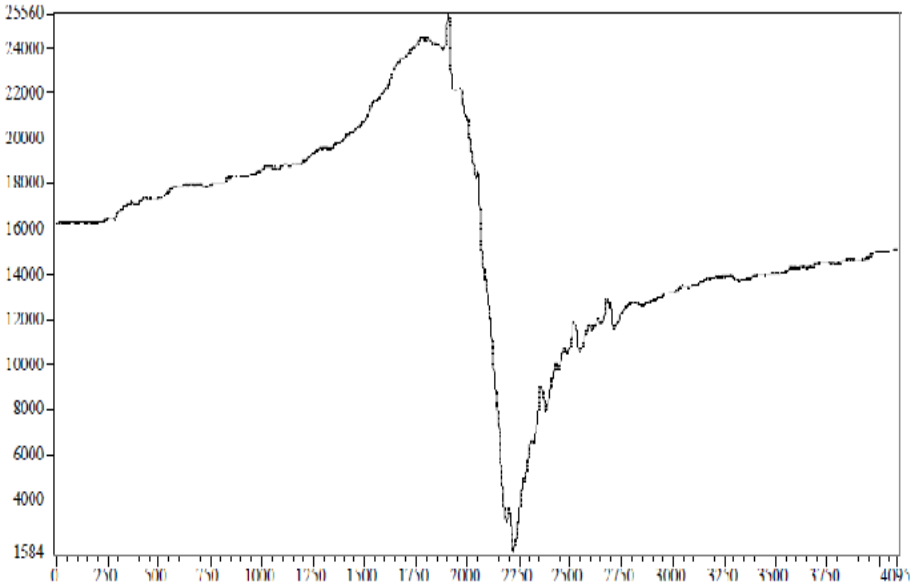

Figure 19: ESR Spectra of Cu-AHCPBT

Further splitting due to super hyperfine interactions with nuclear spin of nitrogen (I_N =1) of thiazoline ring and hydrogen (I_H=1/2) of chiral ring carbon would result a total of twenty four lines with equal intensities. While in the spectra of copper complexes, the total number of peaks recorded is eighteen wherein six peaks have double intensity. This observation is ascribable to overlap of peaks resulting in six double intensity peaks (1:2:1, 1:2:1, 1:2:1, 1:2:1, 1:2:1, 1:2:1). Analysis of intensities of peaks thus corresponds to expected number of super hyperfine components.

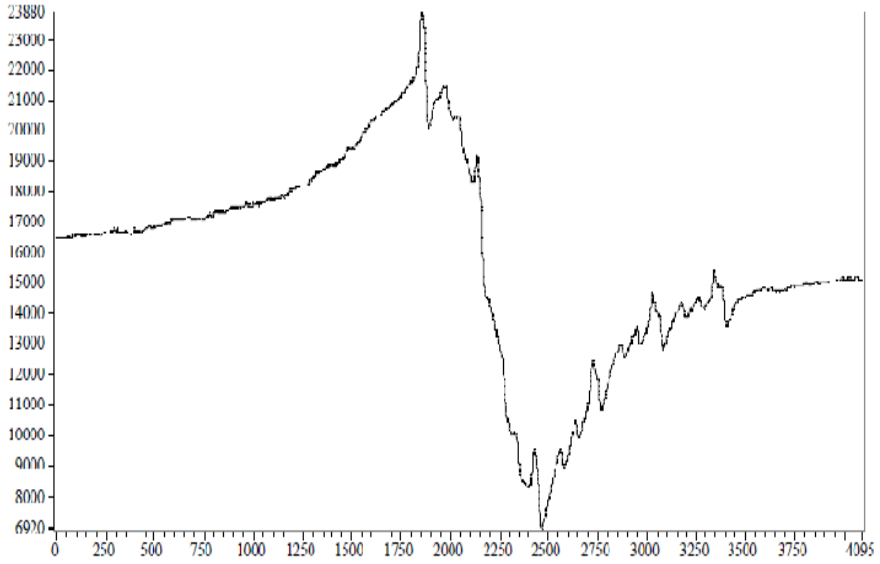

Figure 20: ESR spectra of Cu-AHMPBT

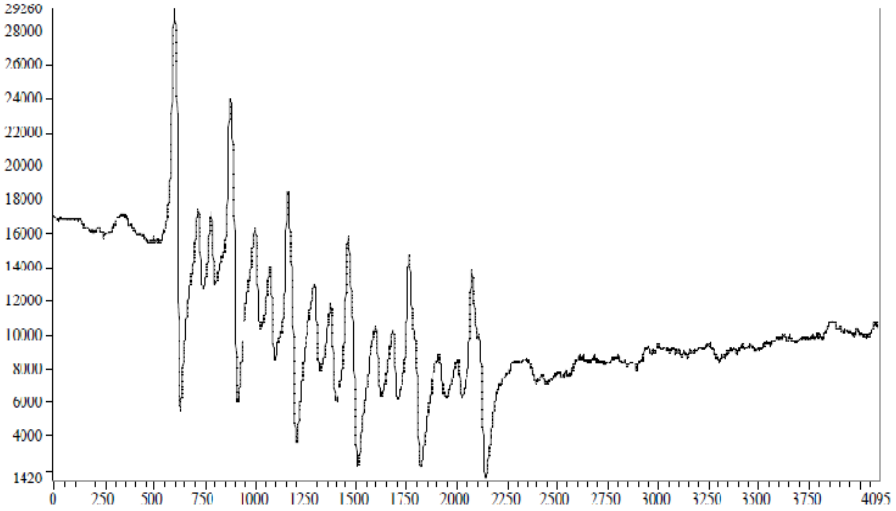

Figure 21: ESR spectra of Cu-AHNPBT

Thus the results of ESR spectra are more informative and are in supportive of delocalization of unpaired electron in complexes as electron spends some time on ligand moiety which would be possible only with metal ligand overlap of orbitals accounting for covalent character in all complexes of present investigation.

3.6. Thermo gravimetric Analysis of Cu-AHPBT, Cu-AHCPBT, Cu-AHMPBT and Cu-AHNPBT

The TGA curves of all the copper complexes (Fig. 22-25) exhibited weight loss in the range of 200 - 300^{0}C, indicating the loss of coordinated water. The gradual loss of weight in subsequent steps from 400 to 500 ^{0} C corresponds to the partial decomposition of complex. The percentage of final residue remaining above 900^0 C indicates the formation of thermally stable residue.

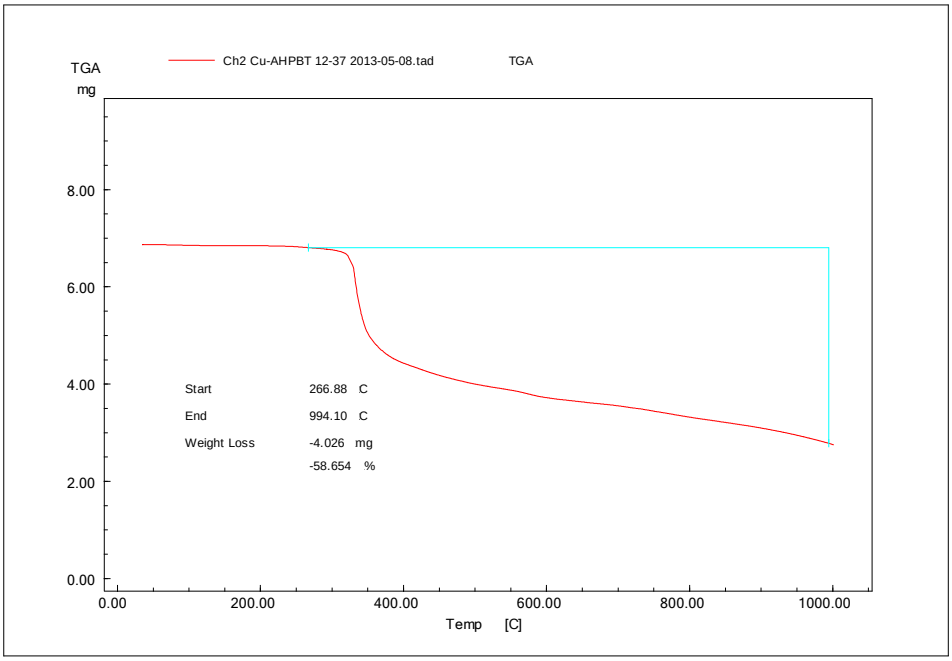

Figure 22: TGA Curve of Cu-AHPBT

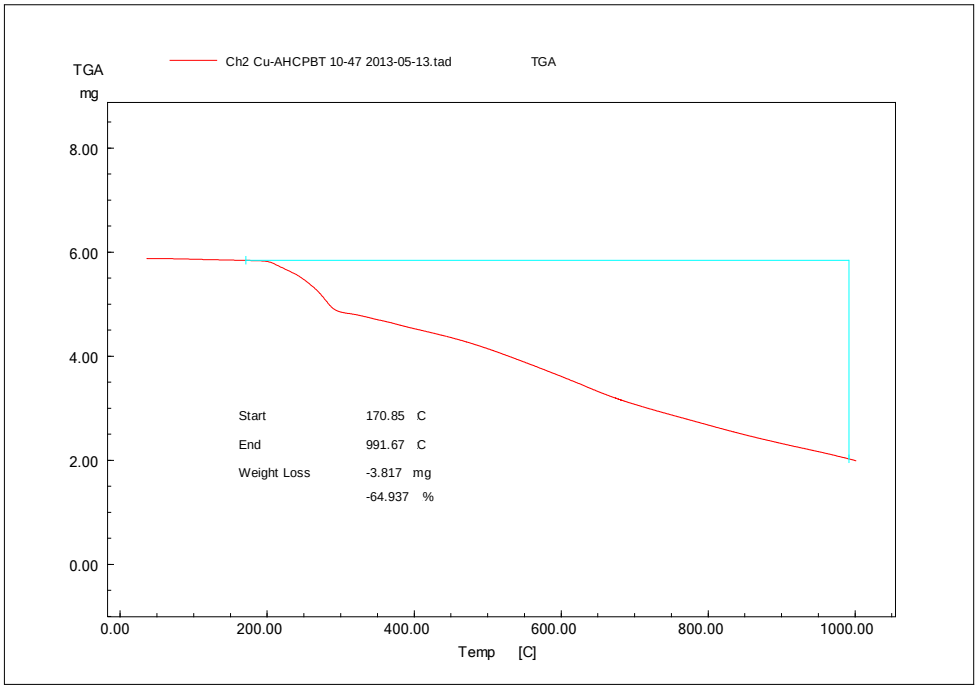

Figure 23: TGA Curve of Cu-AHCPBT

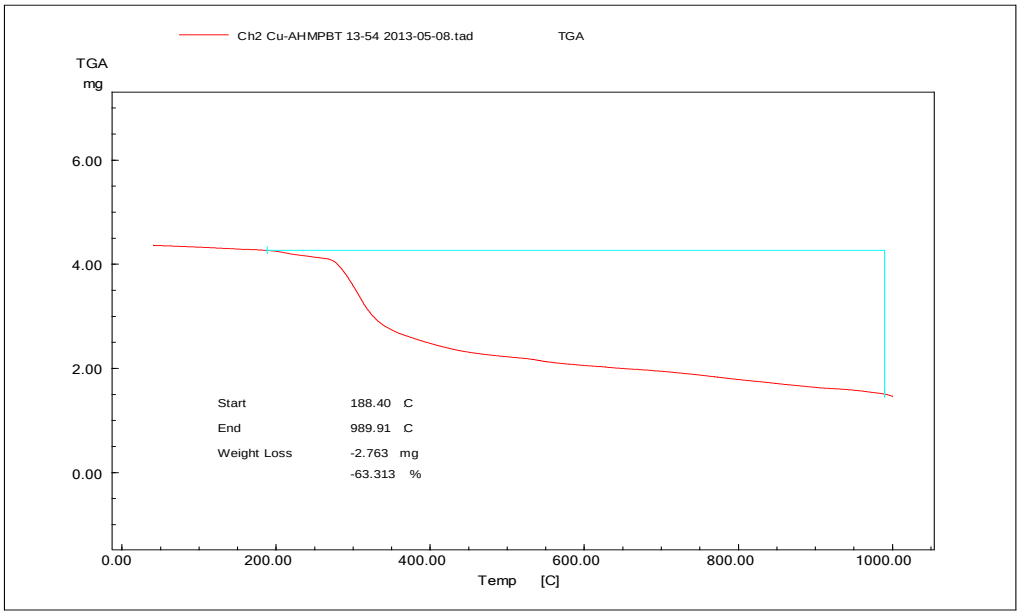

Figure 24: TGA Curve of Cu-AHMPBT

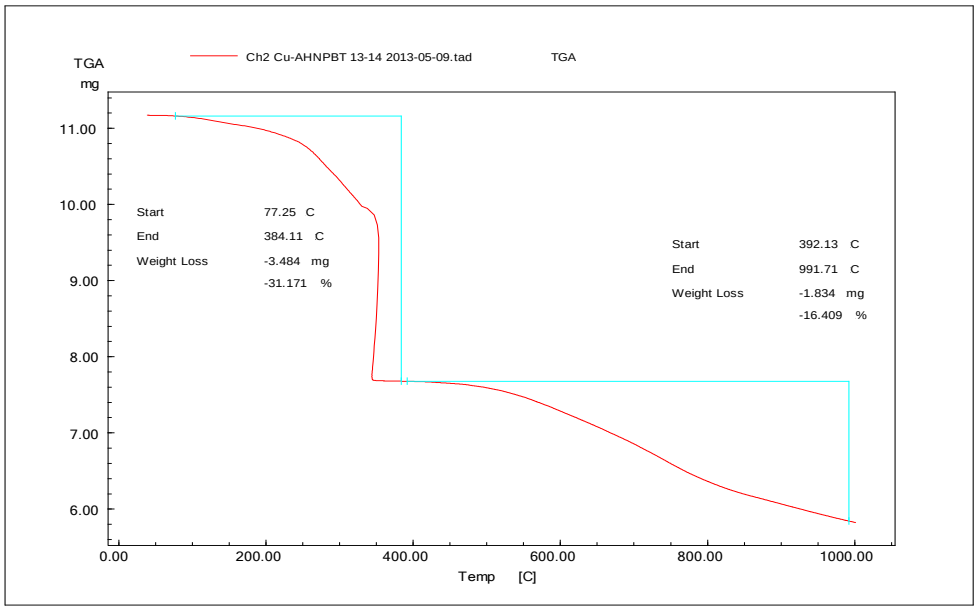

Figure 25: TGA Curve of Cu-AHNPBT

A survey of literature reveals that aryl benzothiazolines when interact with metal ions to form solid complexes, various structural changes occur in ligands [14-17]. The studies on 2-(2'-hydroxy) phenyl benzothiazoline complexes revealed that in presence of Cu(II) metal ion the cleavage of thiazoline ring, subsequent schiff base formation through the transfer of proton from nitrogen to sulphur and then dissociation of same proton from sulphur and second proton from hydroxyl group of phenyl ring occurred enabling its copper complex formation[18]. In present study as the ring cleavage is hindered by acetyl group at ring nitrogen, in presence of metal ions under the conditions of metal complex formation, only one proton dissociation occurs and the benzothiazoline ring remain as such.

FT-IR spectrum and the ESR studies reveal that the ligand is coordinated to the metal centre via the N of thiazoline ring and O of phenyl ring upon deprotonation of the H from O-H leading to the possible formation of 6-membered ring. Based on the above data the following structure can be tentatively assigned to the complexes (Fig. 26).

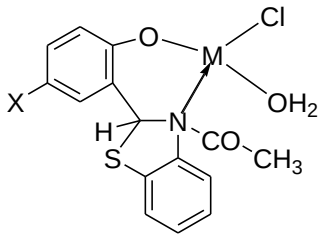

Figure 26

3.7 QSAR Studies

In the present investigation the QSAR parameters were computed employing HyperChem 7.5 software. QSAR properties like surface area, volume, hydration energy, log P, refractivity, polarisability and mass were determined by single point method (Table 4). QSAR properties enable to estimate a variety of molecular descriptors. The QSAR data analysis represents an attempt to relate structural descriptors of compounds with their physicochemical properties and biological activities.

Table 4: QSAR Parameters and Molecular Properties

Property	AHBPT	AHBCPT	AHBNPT	AHBMPT
Surface area(A^2)	337.98	380.83	412.32	405.54
Surface area grid(A2)	454.18	476.07	494.81	497.66
Volume(A^3)	756.4	795.82	819.72	832.08
Hydration Energy(Kcal/mol)	-4.42	-8.16	-11.6	-7.99
Log p	5.21	6.33	5.76	5.56
Refractivity(A^3)	76.7	77.97	80.49,	79.63
Polarisability(A^3)	32.39	33.77	33.68	34.31
Mass(amu)	271.33	305.78	316.33	301.36
PKa	9.06	8.52	8.33	8.72
Total Energy(Kcal/mole)	-67076	-74111	-84030	-77324
Dipole Moment (D)	4.477	1.964	7.041	2.844

3.8 Biological Studies

3.8.1 Antibacterial and Antifungal Activity

The potential antimicrobial activity of the ligands and their new complexes towards five standard bacterial strains (*E. coli, S. marcescens, P. aeruginosa, S. aureus, B. Subtilis)* and one fungal strain (Candida Albicans) was investigated.

Qualitative determination of antimicrobial activity was done using the disk diffusion method [19-21]. A loop full of microorganism was inoculated in 10 ml nutrient medium (NM) and incubated for 12-16 hours at 37 $\pm$ 1°C. Later, 200 µL of the overnight culture was transferred into fresh 10 ml NM and grown up to mid logarithm phase (0.4 - 0.5_{620}). The microorganisms were then washed twice with sterile saline pH 7.4 and resuspended in 1 ml of PBS (phosphate buffer saline) to concentration of 1-2 X 10^9 CFU/ml. Subsequently, bacterial load (1-2 X 10^7 CFU) was added to 5 ml agar NM and spread in a petriplate. In order to add the antimicrobial compound, a circular hole was punched in the agar plate. After that, the 10 µl of antimicrobial test compound was placed into the well and plates were incubated for 24 h at 37 $\pm$ 1°C in inverted position. The inhibition zones around each disc was measured by using Hiantibiotic zone scale™ (Himedia, India) in mm and compared with the controls after 24 hour zone of incubation. Ampicillin was used as a standard drug for antibacterial activity and Candida was used as a standard drug for antifungal activity.

Table 5: Antimicrobial activity of the ligands and their Cu-complexes

Sample	**Description**	**Bacteria**					**Fungus**
		Gram negative type			**Gram positive type**		
		Escherichia coli	***Serratia marcescens***	***Pseudomonas aeruginosa***	***Staphylococcus aureus***	***Bacillus subtilis***	***Candida Albicans***
AHPBT	Test Sample	13.00	14.50	0.00	9.00	13.00	13
AHCPBT	Test Sample	14.50	16.50	0.00	15.00	15.50	17.5
AHMPBT	Test Sample	9.00	8.50	0.00	0.00	10.50	5.5
AHNPBT	Test Sample	7.00	8.00	0.00	0.00	8.50	5.5
Cu-AHPBT	Test Sample	3.00	4.50	0.00	2.00	3.50	2.50
Cu-AHCPBT	Test Sample	7.00	9.50	0.00	8.00	9.50	2
Cu-AHNPBT	Test Sample	7.50	8.00	5.50	9.00	9.00	3
Ampicillin	Standard	15.50	16.00	4.50	8.00	18.00	0.00
Candida	Standard	0.00	0.00	0.00	0.00	0.00	7

From the data presented (Table 5), it may be seen that the title compounds and their copper complexes when screened against bacteria and fungus, showed varying activities. It is evident from the data that AHPBT and AHCPBT showed antibacterial activity equivalent to Ampicillin standard, except that these are inactive with ***Pseudomonas aeruginosa***. The antifungal activity of AHPBT and AHCPBT is almost two times higher compared with Candida standard. Among the title compounds, the AHCPBT showed pronounced antimicrobial and antifungal activity which can be ascribable to the presence of chloro group in it. This can also be correlated with relatively high Log p value (Table 4) which relates to high lipophilicity, and low dipole moment value which envisages the less polarity of compound. The copper complex of AHNPBT showed activity against all selected microorganism mentioned above, despite the fact that AHNPBT being inactive with ***Pseudomonas aeruginosa***. The copper complexes of AHPBT and AHCPBT also exhibited positive antibacterial and antifungal activity.

3.9 DNA Cleavage Studies

Agarose gel electrophoresis is used for the DNA cleavage studies. Agarose gel electrophoresis is a successful method to cleave super coiled DNA in to Nicked Circular and linear DNA forms. For the hydrolytic cleavage of DNA, super coiled (SC) plasmid DNA is a central substrate. For the DNA cleavage analysis a potency of compounds are quantitatively evaluated on super coiled plasmid PBR322 in the absence of oxidizing or reducing agents.

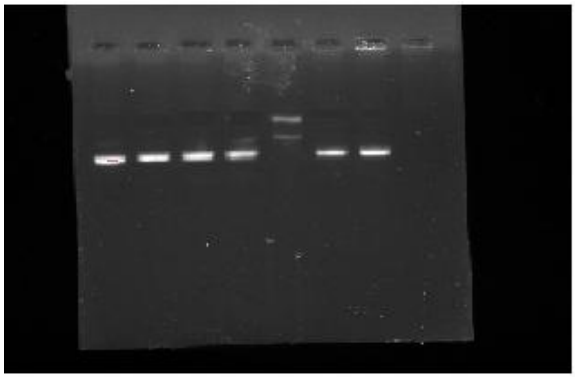

Figure 27: DNA cleavage activity of ligands and its Cu-complexes

Lane 1: DNA marker (1µl+4 µl Tris – HCl Buffer) Lane 2: DNA (1 µl+4 µl Tris – HCl Buffer) + AHPBT (5 µl of 2 mg/ml) Lane 3: DNA (1 µl+4 µl Tris – HCl Buffer) + Cu (II)-AHPBT (5 µl of 2 mg/ml) Lane 4: DNA (1 µl+4 µl Tris – HCl Buffer) + AHNPBT (5 µl of 2 mg/ml) Lane 5: DNA (1 µl+4 µl Tris – HCl Buffer) + Cu(II)-AHNPBT (5 µl of 2 mg/ml) Lane 6: DNA (1 µl+4 µl Tris – HCl Buffer) + AHCPBT (5 µl of 2 mg/ml) Lane 7: DNA (1 µl+4 µl Tris – HCl Buffer) + Cu(II)-AHCPBT (5 µl of 2 mg/ml) The ligands AHPBT, AHCPBT, AHNPBT and their Cu(II) metal complexes have been tested on DNA cleavage studies. The results revealed that only Cu (II)-AHNPBT is most efficient DNA cleaver as it has converted super coiled DNA (form I) into relaxed and linear DNA(form II & form III) (Fig. 27).

IV. CONCLUSION

New Cu(II) complexes were synthesized, characterized and structurally elucidated by the data obtained from various spectro-analytical techniques. Quantum mechanical calculations were also carried out on the ligands using 7.5 HyperChem. The studies on antimicrobial activity inferred that copper complexes of AHPBT and AHCPBT showed both antibacterial and antifungal activity. Gel-Electrophoresis mobility assay method to study DNA cleavage inferred that Cu (II)-AHNPBT is more effective in the cleavage of plasmid PBR-322 DNA.

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