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Research Paper

Synthesis, Characterization of New Copper Complexes of Thiosemicarbazone Derivatives and their Biological Activities

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Abstract: *Cu(II)complexes of ligand 2-[2-(4-chorophenylamino acetyl-N-phenyl hydrazine carbothioamide (H_2L^1) and 2-(4-methyl-chloro- phenylaminoacetyl- -N-phenyl hydrazine carbothioamide (H_2L^2) have been synthesized and characterized by using IR spectra, ^1H NMR, elemental analyses, molar conductance, UV-Visible spectra, fluorescence spectra and thermal analysis (DTA/TG). The study revealed that the ligands behave as a neutral bidentate or monobasic bidentate or tridentate and coordination takes place via NH, C=O, and C-S. The thermal stability of the copper(II) complexes was investigated by thermogravimetry (TG), differential thermal analysis (DTA) techniques. Results were correlated to their structure. The thermal study revealed that Cu(II)complex(2) shows higher thermal stability as compared with all copper complexes. DNA binding properties of the copper(II) complexes with DNA have been investigated by absorption spectra and fluorescence spectra measurements. Results also showed that Cu(II) complex(1) exhibited chelating activity when incubated with isolated DNA. According to the electophoretic mobility, complex(1) showed the highest binding affinity with DNA and the observed enhancement of fluorescence may be utilized in sensing DNA. The anti-bacterial activities have also been studied, all complexes showed good anti-bacterial activity against gram positive and gram negative bacteria.*

Keywords: Synthesis, Complexes, IR, Fluorescence, Thermal Analysis (DTA/TG), DNA.

Introduction

Thiosemicarbazone derivatives are a class of organic compounds that gained its importance from their biological activities such as antibacterial and antiviral properties. These compounds are heterocyclic sulfur and oxygen containing derivatives that have been the subjects of extensive investigation because of their use in different biological applications[1,2]. Research has been directed towards bonding modes, biological implications, structural diversity, and ion-sensing ability of these compounds[3]. The biological applications and properties of metal complexes differ from those of either ligands or metal ions, and increased (and/or) decreased biological activities of transition metal complexes like Cu(II) are reported in the literature[4-6].

The copper(II) complexes of two salicylaldehyde semicarbazones, $HOC_6H_4CH{=}N{-}NHCONR_2$ [H_2Bnz_2 (R=CH_2Ph) and H_2Bu_2 (R=Bu)] were evaluated for their DNA binding, cleavage properties by spectrophotometric DNA titration, ethidium bromide displacement assay and electrophoretic mobility shift assay. The complexes show similar DNA cleavage activity, which is reflected in the similarity of their frontier molecular orbital energies calculated by density functional theory. These results are discussed in relation to the anticancer properties of the complexes[7].

Recently, a series of copper complexes of 2-anilinophenyisothiocyanate semicarbazone has been prepared and characterized physico-chemically by elemental analysis, infrared spectroscopy (IR), Electronic spectra, magnetic moment, molar

conductance measurements and X-ray diffraction pattern before and after γ-irradiation. The antitumor activities of these compounds were investigated against solid tumor induced in mice by injection of Ehrlich Ascites Carcinoma (EAC) cell line. Results revealed that tested compounds significantly reduced the tumor size. Gamma-irradiated compounds showed potent antitumor activities when compared to that of non-irradiated compounds. In addition, tested compounds exhibited stimulatory effect on the level of catalase and superoxide dismutase activities and glutathione content in liver of tumor bearing mice, while the level of lipid peroxidation was significantly reduced. It is concluded that thiosemicarbazone complexes and ligand are considered as promising anticancer drugs candidate. Moreover, the γ-irradiation evokes the antitumor activity of the tested compounds[8].

Material and Methods

Reagent grade chemicals were used without further purification.

Synthesis of ligands

The ligand 2-[2-(4- chorophenylamino acetyl-N-phenyl hydrazine carbothioamide (H_2L^1) and 2-(4-methyl-chloro- phenylaminoacetyl--N-phenyl hydrazine carbothioamide(H_2L^2) were prepared by mixing equimolar amounts of desired hydrazide (0.01mol) in 10ml of absolute ethanol with 4-chloro phenyl isothiocyanate (0.01mol) in 10 ml of absolute ethanol [9,10] . The reaction mixture was refluxed for 3 hrs. The reaction mixture was recrystalized several times from ethanol.

Synthesis of complexes

Copper(II) complexes of the ligand were prepared by adding stoichiometric amounts of the copper(II)chloride, bromide, acetate and nitrate in EtOH to 2-[2-(4- chorophenylamino acetyl-N-phenyl hydrazine carbothioamide(H_2L^1) and 2-(4-methyl-chloro- phenylaminoacetyl- -N-phenyl hydrazine carbothioamide (H_2L^2) in EtOH in a 1:1 molar ratio. The reaction solution was stirred magnetically at 60°C for 3-5hrs. The resulting solids were filtered off, washed several times with EtOH and dried under vacuum over P_4O_{10}.

Physical measurements

Elemental analyses (C, H and Halogen) were performed by Microanalytical unit of the Cairo University, Egypt. IR absorption spectra were recorded using KBr discs and a Perkin-Elmer 1430 recording spectrophotometer. ^{1}H NMR spectra were recorded in d^6-DMSO using 300 MHz Varian NMR spectrometer. The electronic spectra were carried out as solution (10^{-3}M) in DMF using a Perkin- Elmer Lambda 4B spectrophotometer. The molar conductivity measurements were made in DMF solution (10^{-3}M) using a Tacussel conductometer type CD6N. The fluorescence spectra were carried out using LS 45 PerkinElmer Fluorescence Spectrometer. Magnetic susceptibilities were measured at 27°C using a modified Gouy method with Johnson Mattey balance. Thermogravimetric analysis (TGA) was carried out in air using a Schimadzu (Japan) thermal analyzer at a heating rate of 10°C min^{-1} in the temperature range 25-600 using platinum crucibles.

Biological test

Prepared Cu(II)complex was incubated with different concentrations of a purified DNA (pBSII) sample. Concentrations started from 0.1 mM to 0.001 mM DNA. All Complex-DNA samples were subjected to the same conditions of measurements of complexes and ligands alone.

Agarose gel electrophoresis of DNA and Complex-DNA samples

Agarose gel electrophoresis was carried out by preparing 1% agarose gel[11]. Electrophoresis buffer (TBE buffer) was added to the electrophoresis tank to cover the gel while DNA samples were mixed with loading buffer (6X) (0.25% bromophenol blue, 0.25% xylene cyanol, and 30% glycerol in water) on 6:1 volume bases. DNA samples 10 µl each were loaded into the agarose gel after which the electrophoresis process was carried out at 125 volts and 80 mA for about 2 hours. The DNA gel was then stained with ethidium bromide (4 drops of 10 mg/ml to approximately 100 ml tap water) for 5-10 minutes after which it was washed with water and then visualized under UV.

Antibacterial activity

Antibacterial activity (in vitro) of Cu(II) complexes(1-6) were studied against Gram positive (*Streptococcus pyogenes*) and Gram negative(*Escherichia coli)* bacteria at two concentrations (lmg/l,5mg/l) by using Broth Dilution Method [25] with some alterations, to investigate the inhibitory effect of the synthesized Cu(II) complexes (1-6) on the sensitive organisms *Streptococcus pyogenes* as Gram positive bacteria and *Escherichia coli* as Gram negative bacteria. broth medium was prepared by using Brain Heart Infusion (BHI) broth and distilled water. Test compounds in measured quantities were dissolved in DMSO which has no inhibition activity to get two different concentrations (1mg/L, 5mg/L) of compounds. The bacteria were then cultured for 24 h at 37°C in an incubator. One ml of the standard bacterial culture was used as inoculation in a nutrient broth. Growth was calculated at 650 nm using Spectrophotometer. The growth rate of different bacteria in absence as well as in presence of test compounds was performed for each concentration. Absorption measurements were accomplished by spectrophotometer after 24 and 48 h

of incubation and used to calculate the (%) inhibition. Antibacterial activity studies were carried out at Genetic Engineering and Biotechnology Research Institute, Department of Environmental Biotechnology Sadat City University, Egypt.

Results and Discussion

The ligand 2-[2-(4- chorophenylamino acetyl-N-phenyl hydrazine carbothioamide(H_2L^1) and 2-(4-methyl-chloro-phenylaminoacetyl -N-phenyl hydrazine carbothioamide (H_2L^2) were confirmed by elemental analysis (Table 1), infrared (Table 2) and ^{1}H NMR spectroscopy. The stiochiometric of the isolated complexes of thiosemicarbazones are shown in table 1. Copper complexes(3 and 6) of the neutral ligands are formed with acetate and bromide. Copper complex of the monobasic ligand is formed with complexes (2, 4 and 5). The reaction of the ligands (H_2L^1 and H_2L^2) with different salts of Cu(II)chloride, bromide, acetate and nitrate produce complex formulae $[Cu(H_2L^1)(HL^1)Cl(H_2O)]$,$Cu(HL^1)Br(H_2O)$, $Cu(H_2L^1)(OAc)_2$, $Cu(HL^1)(NO_3)$, $Cu(HL^2)Cl(H_2O)$, $Cu(H_2L^2)Br_2$ and $[Cu(H_2L^2)(HL^2)X]$, where X= OAc (7) or NO_3(8). These air stable Cu(II)complexes are non-hygroscopic, partially soluble in most organic solvents, but freely soluble in DMF and DMSO. Values of molar conductivities in DMF (10^{-3}M) solution (Table 1) show that the complexes are non-electrolytes, indicating coordination of the ligand anions [12].

^{1}H NMR spectra

The ^{1}H NMR spectra of ligands(H_2L^1 and H_2L^2) in chloroform,which produce more information concerning intra molecular hydrogen bonding was not possible due to their low solubility, so they have recorded as d^6-DMSO solution. The resonance for the amido N(4)H attached to phenyl group is located in the 9.7-9.8 ppm spectral region, including that hydrogen bonding with d^6-DMSO does not occur, in agreement with previous results [13,14] . The ^{1}H signals due to the hydrazido group for N(1)H occurs at 10.2-10.4 ppm indicating the involvement of the hydrogen through intermolecular hydrogen bonding with the carbonyl oxygen of – C-NH group. The other hydrazido group N(2)H appears at 9.6 ppm . A singlet signal at.3.7, 2.3 ppm and multiplet at 7.5- 7.7 ppm are attributed to the protons CH_2, CH_3(for H_2L^2) and aryl groups, respectively.

Table 1: Elemental analyses and molar conductivities for ligands (H_2L^1, H_2L^2) and their Cu(II) complexes

No.	Compound	Colour Yield (%)	Mol. Wt.	Found (Calc.) %			
				C	H	Halogen	Λ_M
	H_2L^1	Pale brown (65)	334.5	53.6(53.8)	4.6(4.8)		—
1	$[Cu(H_2L^1)(HL^1)Cl(H_2O)]$	Green (65)	785	45.5(45.8)	4.1(4.0)	9.1(9.0)	45
2	$Cu(HL^1)Br(H_2O)$	Green (60)	496	36.7(36.3)	3.4(3.4)	16.3(16.1)	30
3	$Cu(H_2L^1)(OAc)_2$	Dark green (70)	516	44.1(43.8)	4.2(4.3)	-	21
4	$Cu(HL^1)(NO_3)$	Green (60)	459	36.3(36.7)	3.4(3.4)	-	19
	H_2L^2	Pale brown (60)	348.5	55.2(55.1)	5.2(5.2)	-	-
5	$Cu(HL^2)Cl(H_2O)$	Green (70)	465	41.4(41.3)	3.8(4.0)	7.5(7.6)	19
6	$Cu(H_2L^2)Br_2$	Green (70)	571.8	33.5(33.6)	3.4(3.1)	27.9(28.0)	35
7	$[Cu(H_2L^2)(HL^2)(OAc)]$	Dark green (65)	818.5	49.9(49.8)	4.5(4.6)	-	34
8	$[Cu(H_2L^2)(HL^2)NO_3]$	Dark green (70)	821.5	46.7(46.8)	4.3(4.26)	-	36

The infrared spectra of the ligands(H_2L^1, H_2L^2) and copper(II) complexes

Selected IR spectral bands for two ligands (H_2L^1, H_2L^2) and copper complexes are given in table 2. The IR spectrum of the free ligands are characterized mainly by two strong bands at 1670- 1680 cm^{-1} and 765-760 cm^{-1} assigned to ν(C=O) and ν(C=S) vibrations. The absence of any bands above 3500 cm^{-1} or the region 2600-2550 cm^{-1} due to the bands of ν(OH) and ν(SH), respectively and the lack of any signals in the NMR spectra of the free ligands due to the protons of the-OH or SH, confirms that the ligands exist entirely in the keto form. The three bands at 3345-3330, 3305-3270 and 3325 -3240 cm^{-1} in the spectra of the ligands are assigned to ν(N(4)-H), ν(N(2)-H) and ν(N(1)-H), while the ν(N-N)[15, 16] at 925 cm^{-1} as medium sharp band. Also the bands at 1500, 1440 and 1280 cm^{-1} may be due to ν(N-C=S). These bands are assigned as coupled modes consisting principally of ν(NH) and ν(CN). The IR spectra of the complexes (1, 3, 6, 7 and 8) show that the bands corresponding to ν(C=O) and (N-(2)H) shift to lower frequency as compared to free ligand. These low shift, may be ascribed to the coordination of the (C=O) and N(2)H groups to the copper ion. Also the IR spectra for complex(4) reveals that the bands corresponding to ν(C=O) shifts to lower frequency at 1595cm^{-1} and new bands appear at 1530cm^{-1} and 685cm^{-1}, assigned to ν(N=N) and ν(S-C).

While in complexes (2 and 5) show that new bands appear at 1525-1550 cm^{-1} and 690-670 cm^{-1} assigned to ν(C=N) and ν(S-C), indicating that the ligand behaves as monobasic bidentate coordinating via ν(C=N) and ν(S-C). Vibrations at 543-470 cm^{-1}, 480-395 cm^{-1} assigned to ν(Cu-O) and ν(Cu-N)[17,18] . New bands appear at 325-315 cm^{-1} and 340-360 cm^{-1} in Complexes (1,2,5 and 6) assigned to ν(Cu–halo) and ν(Cu-S) in complexes(2,4 and 5) respectively. Whereas in complexes (1, 2, 5) appear broad peaks at 4420–3447cm^{-1} indicating coordinated water.

Table 2: Infrared spectral bands (cm^{-1}) for ligands (H_2L^1, H_2L^2) and their copper (II) complexes

No	Compound	ν(N4-H)	ν (N2-H)	ν (N1-H)	ν (C=O)	ν (C=S)	ν (Cu-O)	ν (Cu-N)	ν (Cu-S)
	H_2L	3345	3305	3235	1670	765	-	-	-
1	$[Cu(H_2L^1)(HL^1)Cl(H_2O)]$	3290	3185	3100	1602	758	485	395	-
2	$Cu(HL^1)Br(H_2O)$	3285	3240	3120	1665	690	-	445	340
3	$Cu(H_2L^1)(OAc)_2$	3240	3165	3100	1595	760	470	440	-
4	$Cu(HL^1)(NO_3)$	3292	3210	3140	1595	685	543	480	345
	H_2L^2	3330	3270	3240	1680	760	-	-	-
5	$Cu(HL^2)Cl(H_2O)$	3287	3188	3133	1642	670	-	460	360
6	$Cu(H_2L^2)Br_2$	3277	3190	3127	1641	735	496	472	-
7	$[Cu(H_2L^2)(HL^2)(OAc)]$	3260	3200	3160	1640	735	480	410	-
8	$[Cu(H_2L^2)(HL^2)NO_3]$	3290	3220	3140	1640	725	495	415	-

Electronic absorption spectra and magnetic moment

The electronic spectral bands of the copper (II) complexes as well as the spectra of the ligand in solution DMF are shown in Table 3, (Fig.1).The π - π* transition band is observed at 32850- 32050cm^{-1} for ligands (H_2L^1 and H_2L^2). Compared to the free ligand, in the copper(II) complexes, this band is shifted to longer wave length (Red shift) which is consistent with an increase in the degree of Pi-cloud conjugation [19]. The electronic spectra of copper (II) complex display one band at the 15560 cm^{-1} range due to the $^2B_2g \rightarrow {}^2A_{2g}$ transition with a square planar geometry[20] , the band at 14560, 15380 and 15870 cm^{-1}, assigned to d-d transition. While for complex (1) falls within the range that is normally observed for octahedral geometry. While the complexes (1, 7 and 8) are octahedral geometry, the bands at about 25000cm^{-1} in the spectra of copper complexes may be assigned to L→Cu charge transfer transition and the more intense band at 30000 cm^{-1} in copper complexes may be due to the coincidence of charge transfer, d→ π* and intra ligand n→ π* transitions. The magnetic moment values for Cu(II) complexes (Table 3) lie in range observed for copper(II) complexes with one unpaired spin electron, 1.65-1.8 B.M [21].

Electronic absorption spectra and fluorescence spectra of copper complex with DNA

The UV spectra data of the ligand has an intensive band at 242 nm. It follows from the literature that band at 242 nm is related to the π - π* transition absorbance. But in the copper complex(1), the band is shifted to 265 and 305 nm, respectively.

Table 3: Solution DMF electronic spectra (cm^{-1}) for ligands and their Cu (II) complexes

Compounds	Intraligand and charge transfer			d-d bands	μ_{eff} (B.M)
H_2L^1	32850		-	-	—
$[Cu(H_2L^1)(HL^1)Cl(H_2O)]$	31950		25610	15550	1.78
$Cu(HL^1)Br(H_2O)$	31950		26320	14560	1.76
$Cu(H_2L^1)(OAc)_2$	30960		24720	15380	1.80
$Cu(HL^1)(NO_3)$	32050		25970	15870	1.72
H_2L^2	32050				-
$Cu(HL^2)Cl(H_2O)$	32080	30140	25640	15702	1.68
$Cu(H_2L^2)Br_2$	31950	30305	25508	15500	1.77
$[Cu(H_2L^2)(HL^2)(OAc)]$	32255	30245	25635	14915	1.65
$[Cu(H_2L^2)(HL^2)NO_3]$	31552	30175	25000	15385	1.67

These results suggest that the complex has formed. From the previously mentioned data, the possible structure of the complex(1) is shown in Scheme 1. The interaction of the complex(1) with DNA was investigated using absorption spectra. It is a general observation that a red shift and hypochromism in the absorption spectra accompany the binding of intercalative molecules to DNA. The change in spectra battern is related to the strength of binding and the spectra for intercalates are more perturbed than those for groove binders [22] . The absorption spectra of the complex(1) in the absence and presence of DNA are given in Figure 1. In the presence of DNA, the absorption band of the Cu(II) complex(1) was shown at about 260 nm with a shoulder at 316 nm. The results suggest an intimate association of the complex(1) with DNA and it is also likely that this complex binds to the helix by intercalation.

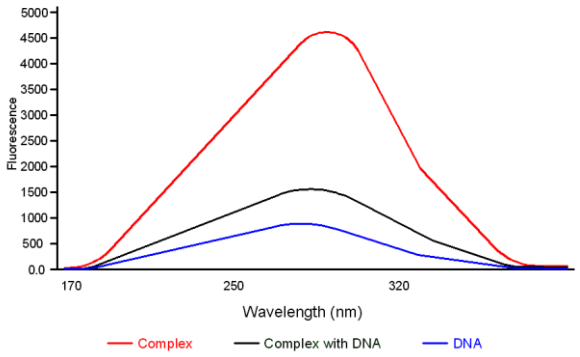


Figure 1: Fluorescence spectra of complex(1) and DNA in pH 5.5. Red: 20 µM of complex(1), Green: 20 µM Complex(1) with DNA, Blue: 20 µM DNA

This intercalation is strongly suggested to ease biochemical reactions associated with the double helix replication and DNA polymerase activity, protein synthesis and all other vital processes of transcription and translation. It also suggested that this kind of chemical bonding may result in different types of mutations (this point is under research).

The fluorescence study of the complex(1) is shown in Figure 1. Fixed amounts (20µM) of the complex(1) alone and the complex with DNA were measured at an excitation wavelength ranging from 200 nm to 350 nm. Cu(II) complex(1) exhibited a maximum emission intensity at 259 nm. The peak intensity of the complex(1) with DNA was triple the intensity of the complex peak alone. Fluorescence data provided a strong evidence for the previously discussed data of UV spectra of the interaction of the formed complex with the double helix DNA molecule.

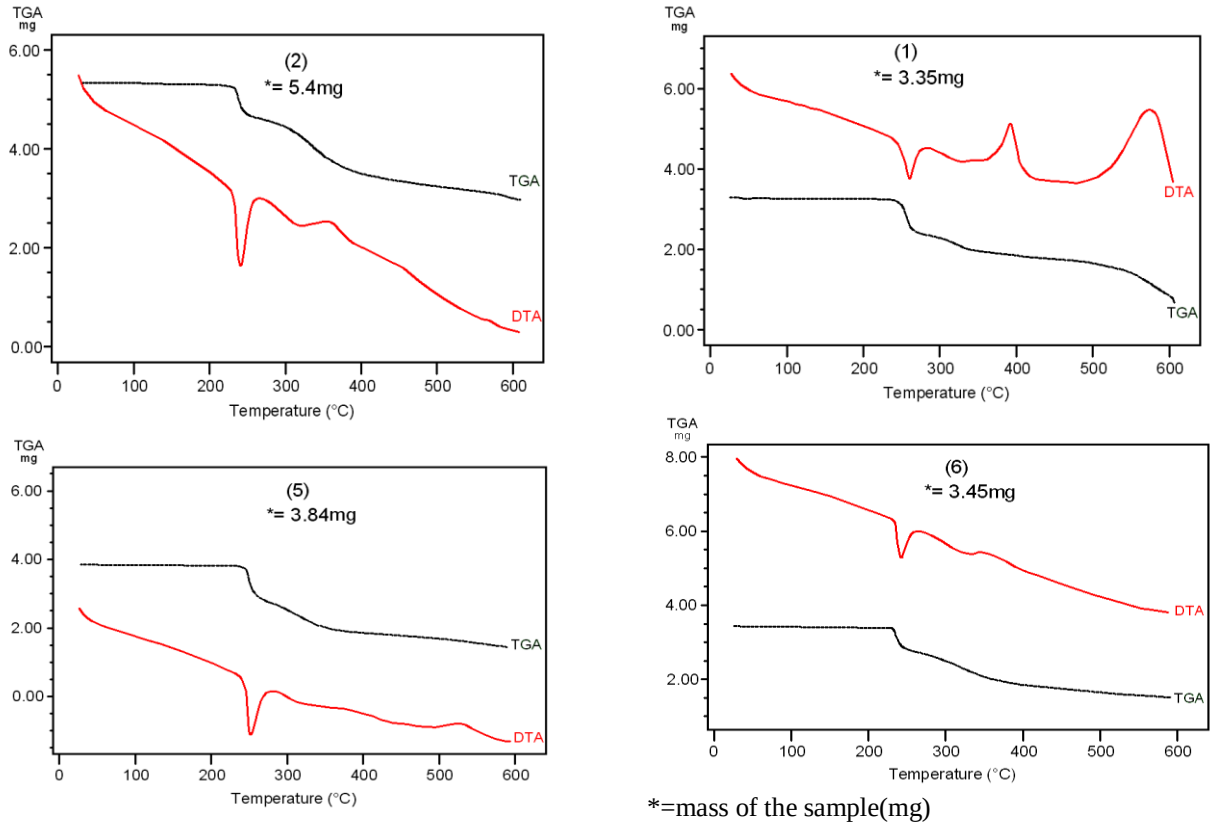
Thermal analysis (TG/DTG)

The thermal properties of copper(II) complexes were investigated by thermogravimetric analysis (TGA) and differential thermogravimetry (DTG), under nitrogen atmosphere from 30 to 600◦C, important data are summarized in Table 4. DTA and TGA curves of copper(II) complexes(1-6) (Figure 2 and Table 4), show three broad exthothermic peaks in the temperature range of 259–319°C, 362–408°C, 501–597°C in complex(1), 238–300°C, 316–362°C, 405–472°C in complex (2), 266–328°C, 364–465°C, 549–603°C indicated by TG weight loss (calc./ found. 27.3/27.3%, 10.6/10.8%, 6.4/6.7%, Table 4), (calc./ found. 12.3/12.4%, 16.8/16.90%, 37.8/38.0% and 5.4/5.1%) in complexes (1-3) respectively. While in complex (4) two exothermic peaks appear at (263-293°C, 362- 432°C) assigned to the loss of ($0.5L+CH_2$-NH, NH) which is in good agreement with the calculated value [23, 24]. On the other hand the DTA and DTG curves in complexes (5) and (6) show endothermic peak and three exothermic peaks , appear at 263-310°C, 329-429°C and 449-551°C by weight loss(calc.//found 30.2 / 29.9% and 16.6 /16.5% in complex (5), also in complex(6) exhibits one endothermic peak and two exothermic peaks of DTA curve at 213-241°C, 241-313°C and 323 -395°C by TGA weight loss (Calc./found18.6/18.8%, 16.8/17.1% and. 4.5/4.1%). At higher temperature in all complexes a part of organic decompose (Table 4) and the complexes are thermally stable but complex (2) is higher thermally stability as compared to all complexes.

Thus, based on the aforesaid elemental analyses, IR, ^{1}H NMR, UV-Visible spectra, molar conductivity measurements, and thermal studies, the proposed structures shown in Scheme1 are confirmed.

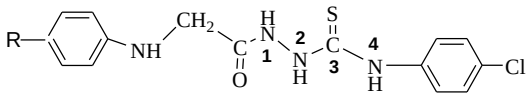
Table 4: Thermal data of copper(II) complexes

No.	Complex	DTG/°C	TG/°C	Mass loss% Cal. (F.)	Leaving species
1	$[Cu(H_2L)(HL)Cl(H_2O)]$	259- 319 362 408 501-597	215 -284 393 -365 446 -501	27.3(27.4) 10.6(10.8) 6.4(6.7)	(H_2O +0.5L + CH_2NH (0.25 L) (NHCl)
2	Cu (HL)Br(H_2O)	238 -307 316- 362 405 -472	214 - 259 296 -394	12.3(12.4) 16.8(17.0)	(H_2O CH_2NHNH) (0.25L)
3	Cu(H_2L)(OAc)$_2$	266-328 364 -465 549 -603	223 -316 401-504	37.8(38.0) 5.4(5.2)	(0.5 L +NHCH) (NHCH)
4	Cu(HL)(NO_3)	263- 293 362-432	196 -334 376- 484	42.7(43.5) 3.3(3.6)	(0.5L + CH_2NH) NH
5	Cu (HL^2)Cl(H_2O)	240-263 263-310 329-429 449-551	210-283 295-370 at 600	30.2(29.9) 16.6(16.5)	(H_2O+ Cl +.0.25L) (C_6H_5) Thermal stability
6	Cu(H_2L^2)Br_2	213-241 241-313 323 -395	213 -264 287- 367 450-525	18.6(18.8) 16.8(17.1) 4.5(4.1)	(HBr +C_2H_2) (HBr +NH_2) (C_2H_2)

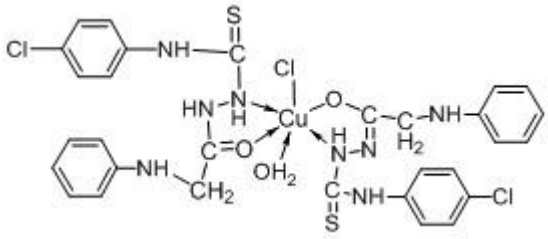


*=mass of the sample(mg)

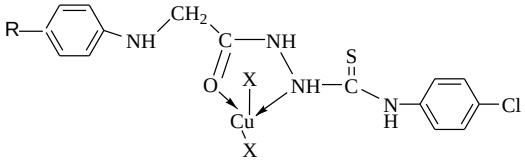
Figure 2: TG/DTG curves of copper (II) complexes (1,2,5 and 6)



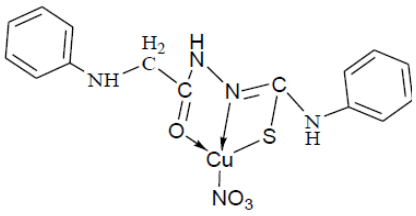
R	Compound
H	H_2L^1
CH_3	H_2L^2



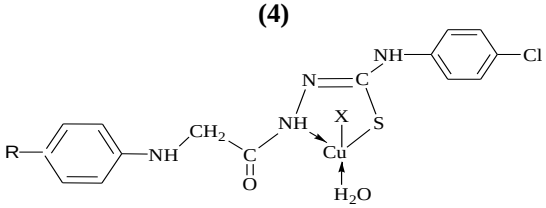
(1)



R	X	Complexes
H	OAc	(3)
CH_3	Br	(6)



(4)



R	X	Complexes
H	Br	(2)
CH_3	Cl	(5)

Scheme 1: Chemical structure of ligand and their copper complexes

Gel electrophoresis of different concentrations of DNA coupled with complex(1) is shown in Figure 4. The gel showed a similar battern for all concentrations assuming that the combination between DNA and complex(1) is formed even in small concentrations of DNA (0.001 mM)

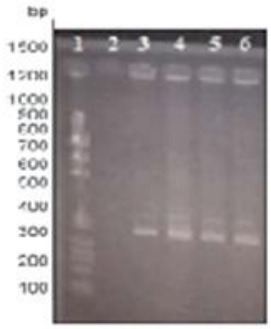


Figure 3: DNA gel electrophoresis after incubation with copper complex (1) marker, (2) control DNA (3) 0.1 mM DNA+complex 1, (4) 0.01 mM DNA+complex 1 , (5) 0.001 mM DNA+complex 1, (6) DNA + DMSO

Antibacterial activities

Cu(II) complexes(1-6) were screened for anti-bacterial activity against *S. pyogenes* as Gram positive bacteria and *E. coli* as Gram negative by Broth Dilution Method (Figures 4 and 5). The results indicate that the complexes activities are found to follow the order of complexes **3** > **2** > **6** > **4** > **5** > **1** for *S. pyogenes* (5gm/L) and less activities for the other concentration (1mg/L) against same microorganisms under identical experimental conditions (Table 5) and the complexes activities are found to follow the order of complexes **2** > **5**> **6** > **4** > **3** > **1** for *E. Coli* (5mg/L) and less activities for the lower concentration (1mg/L).

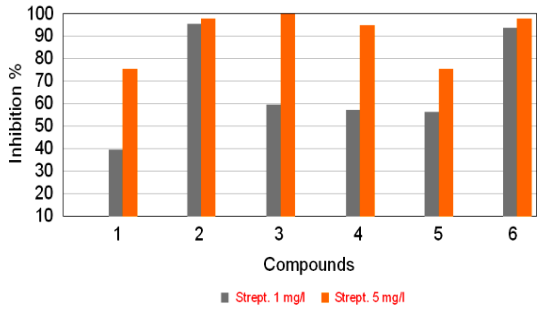


Figure 4: In vitro antibacterial activities of copper complexes(1-6) against *Streptococcus pyogene*

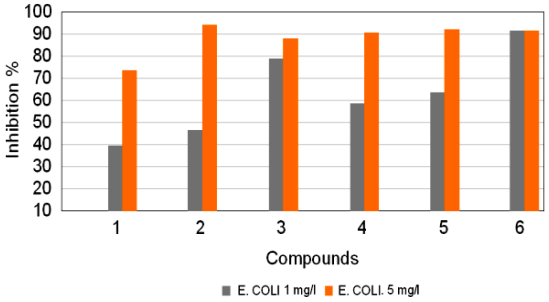


Figure 5: In vitro antibacterial activities of copper complexes(1-6) against *E. coli*

Table 5: Inhibition (%) of copper complexes against *S. pyogenes* and *E. coli* bacteria

No.	Compound	Inhibition (%)			
		S. pyogenes		*E. coli*	
		1mg/mL	5mg/mL	1mg/mL	5mg/mL
1	$[Cu(H_2L^1)(HL^1)Cl(H_2O)]$	39.42	75.96	42.0	72.73
2	$Cu(HL^1)Br(H_2O)$	95.76	98.26	46.49	95.83
3	$Cu(H_2L^1)(OAc)_2$	60.0	99.8	82.11	88.67
4	$Cu(HL^1)(NO_3)$	57.88	95.0	54.09	90.3
5	$Cu(HL^2)Cl(H_2O)$	56.92	76.34	64.50	92.67
6	$Cu(H_2L^2)Br_2$	93.84	97.88	92.10	92.10

Conclusion

In this work, synthesis and characterization of ligands and its copper(II) complexes (1-8) are reported. The analytical and physicochemical analyses confirmed the composition and the structure of the newly obtained compounds. The results obtained can be summarized as follows:

1. The new compound behaves as a neutral bidentate ligand when reacts with Cu(II) acetate, bromide(3,6), mono basicbidentate or tridentate in complex (2,5 or 4).
2. Mononuclear complexes are formed. They adopt distorted square planar configurations with the bromide, acetate and nitrate. However, complexes (1, 7 and 8) prefers distorted octahedral geometry.

3. DNA binding properties of the copper(II) complexes with DNA have been investigated by absorption spectra and fluorescence spectra measurements. All results reveal that the copper(II) complexes bind to CT-DNA via groove binding mode.

Complexation between the copper complex and the anionic DNA molecules exhibits to stiffen the backbones of the former leading to a green color in its fluorescence emission. Results showed that this type of complex(1) may be directed towards DNA binding therapeutic applications, copper complexes(1-6) are very active against the tested pathogens bacteria (*Streptococcus pyogenes* gram positive and *Escherichia coli* gram negative), these means that the copper complexes have medical importance.

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