Synthesis, Characterization of Cu(I) Complex of Thiosemicarbazone Ligand and Antibacterial Activity of Cu(I) Complex.

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ABSTRACT

Novel complex of Cu(I) of ligand 2-(Anilinoacetyl)-*N*-phenylhydrazine-1-carbothioamide (H_2L) has been synthesized and characterized by ^{1}HNMR, IR, elemental analyses, molar conductance, UV-visible spectra, magnetic susceptibility measurements and thermogravimetric analysis (TGA/DTG). The result confirmed that the ligand behaved as neutral bidentate, coordination take place via carbonyl oxygen(C=O) and N(2)H groups. Copper complex is more thermally stable than free ligand. Copper(I) complexes is mononuclear, adopt octahedral geometry. The ligand and complex have been tested for their inhibitory effect on the growth of bacteria against gram-positive (Streptococcus pyogenes) and gram-negative (Escherichia coli). The results proved that the copper complex has less potent antibacterial as compared to the ligand.

Key words: Complex, IR, Thermal analysis, Antibacterial.

INTRODUCTION

Thiosemicarbazone derivatives are of special importance because of their versatile biological and pharmacological activities. Also Thiosemicarbazone derivatives have found application in drug development for the treatment of central nervous system disorders, of bacterial infection, as well as analgesic and antiallergic agents. Thiosemicarbazones are potent intermediates for the synthesis of pharmaceutical and bioactive materials and thus, they are used extensively in the field of medicinal chemistry. Moreover, thiosemicarbazones have found their way into almost every branch of chemistry; commercially they are used as dyes, photographic films, plastic and in textile industry (Tada *et al.,* 2011).

Thiosemicarbazones and their metal complexes have a wide range of biological properties. Because of this, a large number of organic and metal-organic compounds derived from thiosemicarbazone have been the subject of most structural and medicinal studies. Some of the detected biological activities of the thiosemicarbazones are antibacterial (Rodriguez-Arguelles *et al.,* 2005), antifungal (Chohan *et al.,* 2005), antiarthritic (Missbach *et al.,* 1996), antimalarial (Walcourt *et al*., 2004), antiamebic (Bharti *et al.*, 2003), antitumor (Ainscough *et al*., 1998; Sau *et al.*, 2003) antiviral (Varadinova *et al.*, 2001), and especially anti-HIV activity (Bal *et al.,* 2005).

Recently , a series of new novel complexes of copper(I) and mercury(II)

complexes with 4-aminoantipyrine, semicarbazide, and thiosemicarbazide ligands [(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl) carbonohydrazonoyl dicyanide (HL^1), 2-cyano-*N*-(1,5-dimethyl-3-oxo-2-phenyl-2, 3-dihydro-1*H*-pyrazol-4-yl)-2-[(*E*)- (3-methylphenyl) diazenyl] acetamide (H_2L^2), (Z)-2-(2-((E)-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl methylene hydrazinyl)-2-oxo-N-phenylacetohydrazonoylcyanide (HL^3), 2-(anilinoacetyl)-*N*-phenylhydrazine-1-carbothioamide (H_2L^4), 2-(anilinoacetyl)-*N*-(3-methylphenyl)hydrazine-1-carbothioamide (H_2L^5), and 2-anilino-*N*′-[(*E*)-(2-hydroxyphenyl) methylidene]acetohydrazide (H_2L^6)] have been prepared and characterized by physical and spectral data, including microanalysis, IR and UV-visible spectra, conductivity measurements, and thermal analyses (DTG/TG). The ligands H_2L^2 and HL^3 produced dinuclear complexes. Thermal studies revealed that the copper(I) complexes are thermally more stable than mercury(II) complexes. The copper complexes exhibited potent inhibitory effect on the MCF7 human breast carcinoma cell line, as compared to mercury(II) complexes (Aly *et al*., 2017).

The goal of the present work was to prepare copper(I) complex with 2-(anilinoacetyl)-*N*-phenylhydrazine-1-carbothioamide (H_2L) characterized by physical and spectral data, including microanalysis, IR and UV-visible spectroscopy, conductivity measurements, thermal analyses (TG/ DTG), and the antibacterial activity of ligand and Cu(I) complex against two pathogenic bacteria (*Streptococcus pyogenes* as gram-positive bacteria and *Escherichia coli* as gram-negative bacteria) were investigated.

MATERIALS AND METHODES

Material

All the chemicals and solvents of Sigm Aldrich/CDH/Rankem/Merck were Analar grade and used without any further purification. All the reactions were carried out under normal atmospheric conditions.

Synthesis of Ligand

The ligand of 2-(anilinoacetyl)-*N*-phenylhydrazine-1-carbothioamide (H_2L) was prepared by mixing (0.01 mol) of desired hydrazide with (0.01 mol) of phenyl isothiocyanate in 15 ml of absolute ethanol. The reaction mixture was refluxed for 6 hrs. The reaction mixture was recrystallized several times from ethanol.

Synthesis of Copper(I) Complex

Copper(I) complex of the ligand 2-(anilinoacetyl)-*N*-phenylhydrazine-1-carbothioamide was prepared by adding stoichiometric amount of the CuI in EtOH to a hot solution of (H_2L) in EtOH in a 1:1 molar ratio. The reaction solution was stirred magnetically at for certain about 6hrs. The resulting solids were filtered off, washed several times with EtOH and dried under vacuum over P_4O_{10} .

Measurements

Elemental analyses (C, H and N) were performed by Microanalytical unit of the Cairo University, Egypt. Copper(I) and Iodide were estimated using standard methods (El-Boraey and El-Gammal 2015).

IR absorption spectra were recorded using KBr discs and a Perkin-Elmer 1430 recording spectrophotometer.

^{1}HNMR spectra were recorded in d6-DMSO using 300 MHz Varian NMR spectrometer. The electronic spectra were carried out as solution (10^{-3} M) in DMF using a Perkin Elmer Lambada 4B spectrophotometer. The molar conductivity measurements were made in DMF solution (10^{-3}M) using a Tacussel conductometer type CD6N.

Thermal analyses were determined thermal behavior of the ligand and its complex has been studied using TG/DTAG measurements. The TGA/DTA curves of ligand and its complexes were recorded in nitrogen atmosphere from room temperature up to 800c.

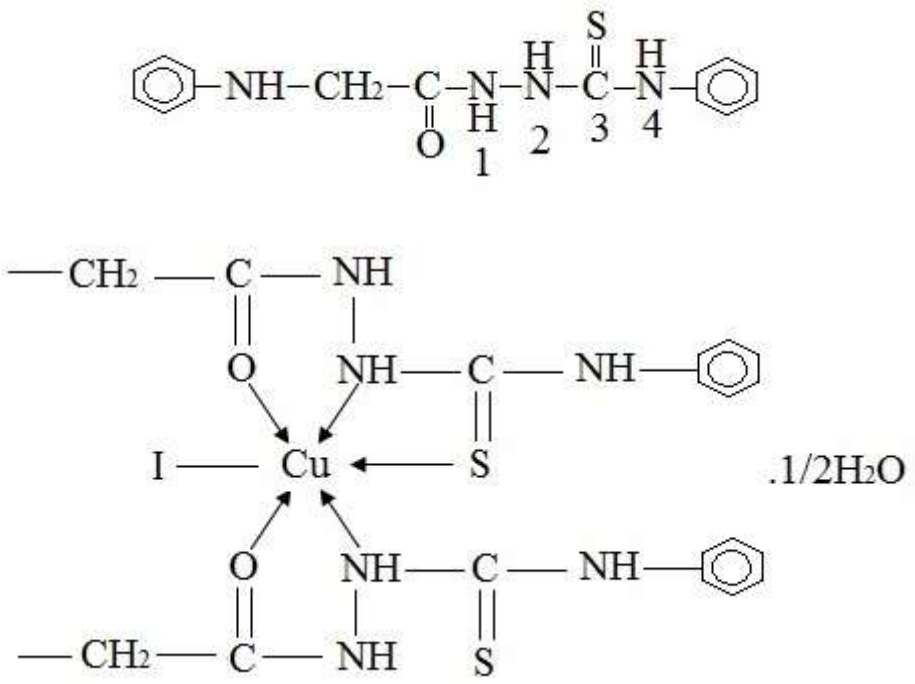
Antibacterial tests

The in vitro antibacterial activity studies were carried out as described by (El-Boraey *et al*., 2016) with some modification, the inhibitory effect of both the synthesized ligand and its complex was tested on the pathogenic gram-positive organism *Streptococcus pyogenes* and the gram-negative bacterium *Escherichia coli*. Biological effect of the ligand and its complex was carried out on Brain Heart Infusion (BHI) was used to grow *S. pyogenes* cells and Nutrient Broth (NB) medium was used to grow *E. coli* cells. Compounds under investigation were dissolved in DMSO which has no inhibition activity on both microbes. Two different concentrations (1 µg/ml and 5 µg/ml) were prepared. Bacterial strains were prepared by activating them on the proper broth media with shaking. The bacteria were then cultured for 24hrs at 37^o C in an incubator. With this subcultures, fresh broth media were inoculated by one mille of the standard bacterial culture.

For growth studies, *S. pyogenes* and *E. coli* cultures were inoculated and grown aerobically on BHI broth medium and NB medium respectively. Growth was calculated turbidometrically at 650 nm using conventional spectrophotometer, in which turbidity produced is measured by taking absorbance and compared with turbidity produced by control. After growing bacterial cultures on media that contain the ligand, absorption measurements of complex and control were measured by spectrophotometer after 24hrs and 48hrs of incubation to determine the number of viable cells count per milliliter of sample and were used to calculate the inhibition percentage.

RESULTS AND DISCUSSION

The ligand of 2-(anilinoacetyl) – *N* - phenylhydrazine – 1 - carbothioamide (H_2L) was confirmed by elemental analysis as shown in table (1), infrared as shown in table (2) and ^{1}H NMR spectroscopy. The reaction of the ligand H_2L with CuI produce complex of the general formulae $[Cu(H_2L)_2I.\ 1/2H_2O]$. These air stable complexes are non-hygroscopic, partially soluble in most organic solvents, but freely soluble in DMF and DMSO. Values of molar conductivities were measured in DMF (10^{-3}M) solution. Table (1) shows that, the complex is non-electrolyte, indicating that coordination of the anion to the ligand. The solid complex is colored, insoluble in water, methanol and ethanol but soluble in DMF at 10^{-3}M (Al-Shaheen and Al-Mula 2014).



Scheme 1. Chemical structure of ligand (H_2L) and copper(I) complex

^{1}H NMR Spectra

The ^{1}H NMR spectrum of the ligand (H_2L) in DMSO –d^6 revealed a chemical shift (δ/ppm) at 9.045 ppm and at 9.733 ppm attributed to N(4)H and N(1)H. The peaks of N(1)H and N(2)H appeared as signals at 8.9 and 8.3 ppm .The singlet peak appeared at 3.331 ppm due to CH_2 group and the multiple peak at 7.4 ppm due to aromatic protons of phenyl group **(El-Saied et al., 2017).**

Table 1. Analytical and physical data for the ligand (H_2L, $C_{15}H_{15}N_4OS$) and Cu(I) complex

No.	Compound	Color	Found (Cal. %)					
			C	H	N	I	Cu	Λ_M
	H_2L	Buff	60 (60.3)	503 (5.4)	18.6 (18.5)	-	-	-
1	$Cu(H_2L)_2I.1/2H_2O$	Green	45.5(47.0)	4.1(3.6)	7.8(10.7)	16(15.8)	8.0(8.4)	28

Where, Λ_M = molar conductivity ohm^{-1} cm^2 mol^{-1} in 10^{-3}M in DMF solution.

The Infrared Spectra of The Ligand and Copper(I) Complex

Fundamental IR spectral bands for the ligand and copper complex are given in table (2). The IR spectrum of the free ligand is characterized mainly by the strong bands at 3339 cm^{-1}, 3291 cm^{-1}, 3250 cm^{-1}, 1674 cm^{-1} and 744 cm^{-1} are attributed to the stretching frequencies of υ(N4-H), υ(N2- H), υ(N1-H), υ(C=O) and υ(C=S) wagging vibrations, respectively. This supports the nature of the ligand H_2L as bidentate one and coordination take place via(C=O) and (N2-H). The bonding mode of the ligand to Cu ion has been judged by a careful comparison of the infrared spectra of the complex with that of the free ligand. In general, the infrared spectra of the Cu(I) complex shows significant changes compared to the spectrum of the free ligand. The IR spectra of complex show strong band at 3430,

3304 , 3183 , 1599 and 754 cm^{-1} which attributed to the stretching frequencies of υ(N4-H), υ(N2-H), υ(N1-H), υ(C=O) and υ(C=S) wagging vibrations, respectively. The new bands appeared at 605 and 508 cm^{-1} assigned to υ(Cu-O) and υ(Cu-N) respectively, (Meena and Jain 2014).

Table 2. Infrared spectral bands (cm^{-1}) for ligand (H_2L) and Cu(I) complex

No.	Compound	ν(N4-H)/ ν(OH)	ν(N2-H)	ν(N1-H)	ν(C=O)	ν(C=S)	ν(Cu-O)	ν(CU-N)
	H_2L	3339	3291	3250	1674	744	-	-
1	$Cu(H_2L)_2I.1/2H_2O$	3430	3304	3183	1599	754	508	410

The electronic spectra of the ligand and copper(I) complex

The electronic spectra of ligand and Cu(I) complex were recorded in DMF solution (10^{-3}M). In UV spectra of ligand shows λ_{max} at 272 nm with a shoulder band. It indicates that in DMF solution the ligand exists in thiol form. While in Cu(I) ions have the d^{10} configuration and therefore their complexes should not exhibit any d-d transition. The copper complex of this ion was found to be diamagnetic[47] and octahedral geometry **(Majeed and Alabdeen 2012).**

Thermal Studies (TG/DTG)

The thermal properties of ligand and its copper complexes were investigated by thermogravimetric analysis (TG/DTG),under nitrogen atmosphere from room temperature in the range 25-800°C as shown in table (3) **(Donia et al., 2003).**

Ligand

The TG curve of the ligand shows that the ligand is thermally stable up to 127°C after this point melting point at 128°C. Also the TG curve shows decomposition step in the temperature range 128.5–799 °C, with total mass loss of 100% (found 97.9%).

Copper(I) Complex

The TG curves of Cu(I) complex show mass loss in the temperature range 29.3 - 38.5°C (Calc./Found % 1.1/ (1.0)), associated with DTG peak at 43°C is assigned to release of half molecule of water of crystallization.

Table 3. Thermal data of ligand and copper(I) complex.

No.	Compound	TG/°C	Mass loss% Cal. (F.)	Reaction	Leaving species
	H_2L	128 128.5-799	- 100(97.9)	- c	Melting decomposition Decompostion
1	$Cu(H_2L)_2I.\ 1/2H_2O$	29.3 - 138.5 138.5 -248.5 248.5 -511 511-799.7	1.1(1.0) 28.0(28.1) 37.5(37.7) 37.5(37.7)	D c c f	$-1/2H_2O$ - 0.75L -L -2CuO

Where, d =Dehydration, c= decomposition and f =Final product percent

Antibacterial Activity:

The antibacterial studies of the prepared compounds screened against both gram-positive and gram-negative bacteria proved that these compounds exhibit remarkable antibacterial activity and can be used in the future as therapeutic drugs for pathogenic bacterial diseases in table (4) showed antibacterial activity against the tested microbes. Generally, it was found that the antibacterial activity of both the synthetic

ligand and Cu(I) complex was proportionally increased with increased concentration. The tested compounds are found to have remarkable biological activity. For 1 µg/ml concentration of both synthetic ligand and Cu(I) complex, the antibacterial activity of the tested compounds was found to follow the order : Cu(I) complex < ligand in case of *E. coli* as shown in figure (1). On the other hand, a higher antibacterial activity was recorded when using the ligand with *S. pyogenes* as shown in figure (2) with the same concentration. (El-Boraey *et al*., 2016).

Antibacterial activity of 5 µg/ml concentration for both the free acyclic ligand and its complex followed the order ligand complex when compounds were used with both *S. pyogenes* and *E. coli* (Sönmez *et al.,* 2010). Results in Figure (1) suggested that in case of 1 µg/ml Cu(I) complex, the chelation could facilitate the ability to cross the cell membrane of *E. coli* and can be explained by Tweedy's chelation theory. Chelation complication could enhance the lipophilic nature of the central metal atom, which subsequently favors its permeation through the lipid layer of the cell membrane (Tweedy, 1964). Complex when used with both concentrations (1 µg/ml and 5 µg/ml) in case of the gram-positive *S. pyogene* bacterium. It also has been observed that some moieties such as N(2)H linkage introduced into such compounds exhibits extensive biological activity (Singh *et al*., 2013) . The antibacterial studies of the prepared compounds screened against both gram-positive and gram-negative bacteria proved that these compounds exhibit remarkable antibacterial activity and can be used in the future as therapeutic drugs for pathogenic bacterial diseases.

Table 4. Antibacterial activity of ligand and its metal complex

No.	Compound	Inhibition %			
		E. coli		*S. pyogenes*	
		1µg/ml	5µg/ml	1µg/ml	5µg/ml
	H_2L	73.41	94.56	94.23	95.76
1	$Cu(H_2L)_2I.1/2H_2O$	11.17	3.62	35.41	41.35

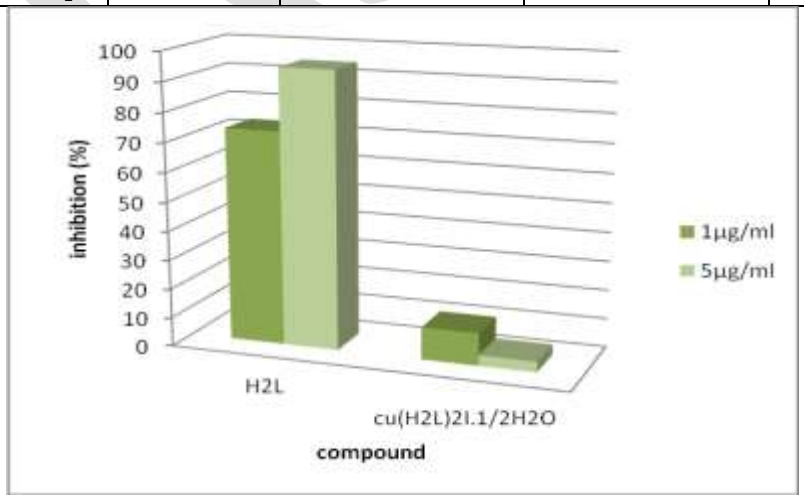


Figure 1. antibacterial activity of ligand and Cu(I) aganist *E. coli*.

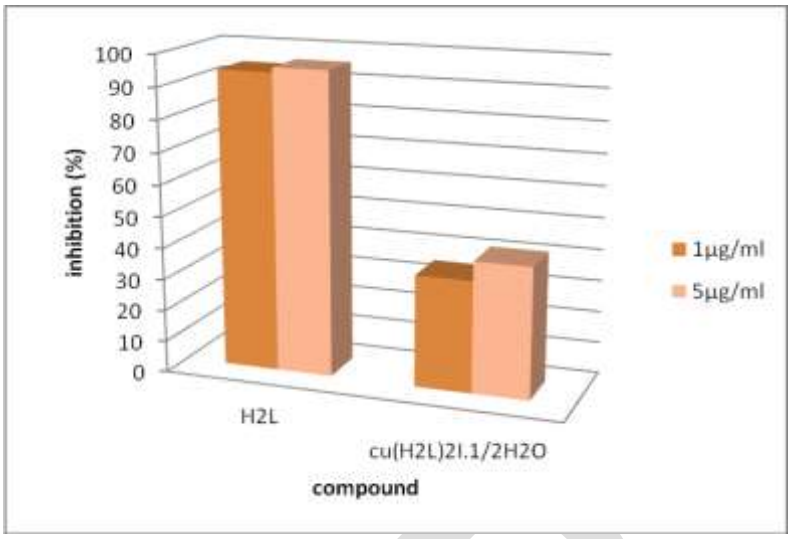


Figure 2. antibacterial activity of ligand and Cu(I) aganist *S. pyogenes*.

CONCLUSION

In this work, synthesis and characterization of ligand and its Cu (I) complex is reported. The analytical and physicochemical analysis confirmed the composition and the structure of the newly obtained compound.

1-The spectral analysis of infrared of the Cu(I) complex strong and large band.

2- The results obtained can be summarized as follows:

a- The result confirmed that the ligand behaved as neutral bidentate, coordination take place via carbonyl oxygen(C=O)and N(2)H groups. Copper Complex is more thermally stable than free ligand Copper(I) complexes is mononuclear, octahedral geometry.

b- The antibacterial activity of the Cu(I) complex screened against both gram-positive and gram-negative bacteria proved that these compounds exhibit the antibacterial studies of the prepared compounds screened against both gram-positive and gram-negative bacteria proved that these compound exhibit remarkable antibacterial activity and can be used in the future as therapeutic drugs for pathogenic bacterial diseases.

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