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Characterization and antioxidant activity of newly synthesized N1-(2-chlorophenyl)-N2-phenyl-1,2-hydrazinedicarbothioamide and its Cu(II), Zn(II), ZrO(II), Hg(II) and UO_2(II) metal complexes

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Abstract

Thiourea derivatives are well known class of organic compounds having diverse application in the field of medicine, agriculture, coordination, and analytical chemistry.

This study reports novel N^1-(2-chlorophenyl)-N^2-phenyl-1,2-hydrazinedicarbothioamide and its Mn (II), Co(II), Ni(II), Cu(II), metal complexes. These synthesized compounds were characterized by H^1NMR, UV-Vis, IR spectroscopy and elemental (CHN/S) analysis. Moreover, antioxidant activity was screened for synthesized compounds. All the compounds showed significant antioxidant potential with IC_{50} values ranges from 23.81± 1.57μM - 58.32± 1.23μM. It was revealed that synthesized compounds displayed better antioxidant activity with IC_{50} values between 127.10-271.41 μM to256.42 ± 0.34 μM. Among these metal complexes, Hg complex (IC_{50} = 23.81 ± 1.57) showed highest antioxidant activity, while Zn complex (IC_{50}= 24.53 ± 1.12), Cu complex (28.10 ± 1.05) and Co complex (IC_{50}= 29.76 ± 1.21), The metal complexes UO_2(II), ZrO(II) showed moderate anti-oxidant activity. The antioxidant activity pattern of these compounds is Hg(II) > Zn(II) > Cu(II) > UO_2(II) > ZrO(II). These metal complexes showed remarkable antioxidant activity which may find their importance in avoiding or reducing the development of both heart disease and some forms of cancer.

Keywords: Thiourea derivatives, antioxidant activity, metal complexes, hydrazine, 4-phenyl-3-thiosemicarbazide, 1, 1-diphenyl-2-picrylhydrazil.

Introduction

One of the most important organic compound urea was synthesized in laboratory that played pivotal role in an organic synthesis. After that, structurally related a compound thiourea had been discovered which played a vital role in agriculture for the augmentation of crops. Thiourea is important sulphur and nitrogen-containing compounds that have drawn massive attention in drug research for last two decades (Cragg and Newman, 2005). Thiourea derivatives showed variety of biological applications such as herbicidal (Sahu and Solanki, 1991), inhibition of nitric oxide (Yon and Stoilkova, 2004), anti-viral properties (Kim, et al., 2007) and analgesic properties (Rojas, et al., 2002). Oxidation offers vital role to various organism for production of energy and to carry out various biological process. Oxidation produce free radicals and they cause various diseases like liver disease, cancer disease, cardiovascular disease, atherosclerosis, aging, ulcerative colitis, alzeihmers, Parkinsons, mild cognitive impairment. In order to reduce the free radicals damage to the human body, appropriate intake of dietary antioxidants is necessary (Wang and Luo, 2007, Qi, et al., 2005, Gu, et al., 2007).

Importance evidence shows that foods containing antioxidants and antioxidants nutrients play key role in preventing from various lethal diseases. Currently synthetic oxidants which are employed for industrial processing cause liver damage and carcinogenesis. So it is necessary to grow and utilize productive and natural antioxidant that can save human body from free radicals and also help in preventing from chronic diseases. Antioxidants show special characteristics such as they have ability to trap free radicals. The free radicals with highly reactive nature present in biological system like oxygen containing species. The reactivity of these radicals may oxidize DNA, proteins, nucleic acid and lipids and having ability to initiate degenerative disease. The group of antioxidants including flavonoids scavenges free radicals such as peroxide, phenolic acids, polyphenols, lipid peroxyl or hydroperoxide, Moreover; they inhibit lead to chronic diseases. Antioxidants are considered as health protecting agents and prevent risk for chronic diseases such as cancer and coronary heart disease. Antioxidants which are naturally present including fruits, grains, and vegetables while dietary antioxidants are produced from plants like vitamin C, vitamin E, carotenes, phenolic acids, and these are related to different classes of compounds possess different physical and chemical properties (Sravani and Paarakh, 2012).

Antioxidants enzymes consist of *glutathione peroxide*, *superoxide dismutase*, and *glutathione reductase*, these assist in catalyzing free radical reaction. Nutrient derived antioxidants are carotenoids, tocopherols and tocotrienols (vitamin E), ascorbic acid (vitamin C), and other antioxidants having low molecular weight like lipoic acid and glutathione, while metal binding proteins comprises of *lactoferrin*, *ferritin*, *albumin* and *ceruplasmin*, that keep apart from iron and copper ions and having ability to catalyze oxidation reactions (Duthie, et al., 1994).

Antioxidant activity of various substance can be assess by using (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) (DPPH) stable radical. It has ability to accept electron or hydrogen radical and reduce in occurrence of antioxidant molecule, producing colorless solution. The use of the (DPPH) radical play an important role in evaluating antioxidants activity of various substances by using spectrophotometer. This method may be useful because it can assess various products at a time. DPPH reacts with antioxidant compound which donates hydrogen and it is reduced. The color became change from deep violet to light yellow and determined by UV-VIS spectrophotometer (Kirtikar, et al., 2006, Warrier, et al., 1994, Harborne, 1998, Ghosh, 1998, Wagner and Bladet, 1996, Handa and Vasisht, 2006). Current research work has revealed that N^1-(2-chlorophenyl)-N^2-phenyl-1, 2-hydrazinedicarbothioamide and its metal complexes $Cu(L)_2$, $Zn(L)_2$, $ZrO(L)_2$,$Hg(L)_2$, and $UO_2(L)_2$ were evaluated for their antioxidant potential and displayed an outstanding antioxidant activity.

The aim of the current work was to generate organometallic compounds for therapeutic use, including N^1-(2-chlorophenyl)-N^2-phenyl-1, 2-hydrazinedicarbothioamide and its metal complexes $Cu(L)_2$, $Zn(L)_2$, $ZrO(L)_2$,$Hg(L)_2$, and UO2$(L)_2$. These organometallic compounds demonstrated exceptional DPPH radical scavenging action, and their use in applied medical chemistry may be significant. Characterization of synthesized metal complexes was carried out *via* different techniques such as UV/VIS spectroscopy, FTIR Spectroscopy, Elemental microanalysis (CHN/S) while thermal stability of metal complexes was evaluated through TG/DTA. These metal complexes showed remarkable antioxidant activity which may find their importance in avoiding or reducing the development of both heart disease and some forms of cancer.

Materials and Methods

Reagents

Various reagent grade chemicals were used during research study such as potassium chloride (KCl), Bovine serum albumin (Research Organics Cleveland, USA), potassium dihydrogen phosphate (KH_2PO_4), sodium dihydrogen phosphate (NaH_2PO_4), disodium hydrogen phosphate(Na_2HPO_4), sodium chloride(NaCl), sodium hydroxide (NaOH),4-phenyl-3-thiosemicarbazide, 2-chloro phenylisothiocyanate metal salts, $CuCl_2.2H_2O$, $Zn(CH_3COO)_2.2H_2O$, $ZrOCl_2.8H_2O$, $HgCl_2$, $UO_2(NO_3)_2.6H_2O$), ethanol, methanol, dimethyl sulfoxide (DMSO), dimethylformamide (DMF), were got fromSigma–Aldrich, USA. Sodium phosphate buffer (pH 7.4). Through mixing NaCl (137 mM), Na_2HPO_4 (8.1 mM), KCl(2.68 mM), and KH_2PO_4 (1.47 mM)Phosphate buffer saline (PBS) was prepared and NaOH (0.25 mM) was used for pH 10 adjustment. The solution of BSA (10 ppm) and glucose anhydrous (50 ppm) were prepared in sodium phosphate buffer while all standard test solutions were carried out by using DMSO (1mM/mL).

Instrumentation

Elemental analysis was carried out for the estimation of carbon, hydrogen, nitrogen and sulphur *via* Elemental Analyzer CHNS/O Perkin Elmer 2400 Series II.FTIR spectral data of the synthesized compounds was measured by using thermo scientific iS10 IR spectrometer. Perkin Elmer Lambda 35 UV-Vis spectrophotometer was used for evaluating electronic spectra. Structure of synthesized ligand was evaluated by ^{1}H-NMR spectrum by means of Tetra Methyl Saline as an internal standard in d_6-DMSO on a BRUKER 300 MHz spectrometer. Finnigan MAT-

311A Germany instrument for Electron impact mass spectra (EIMS). Melting point of synthesized ligand and its metal complexes was measured by Gallenkemp melting point apparatus. Electrical conductivity was estimated via Scientific Orian 5 Star conductivity meter.

Synthesis Of Ligand N1-(2-Chlorophenyl)-N2-Phenyl 1,2 Hydrazine dicarbothioamide

N^1-(2-Chlorophenyl)-N^2-Phenyl 1,2 Hydrazine dicarbothioamide was synthesized by the reaction of 0.005 moles (1gm) of 4-phenyl-3-thiosemicarbazide with the equimolar amount 0.005 moles (0.7ml) of 2-chloro phenylisothiocynate in 25 ml methanol in round bottom flask and stirred for 3 hours. After completion of reaction, off white colored precipitates was obtained by evaporating solvent, which was further washed with water and recrystallized with ethanol in order to, obtained pure precipitate of ligand **Figure 1A**. Different techniques such as UV/Vis, FTIR, H^1NMR, and Mass spectroscopic were used for characterization of ligand.

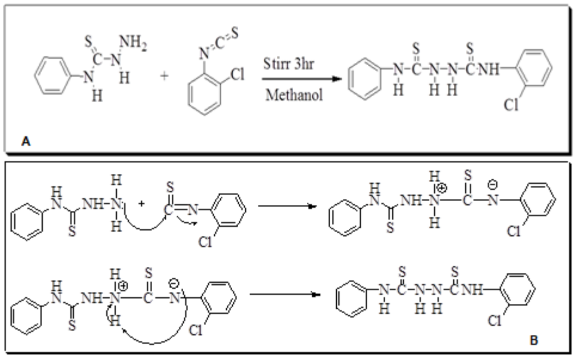

Figure 1. A. Synthetic Methodology for Ligand (L) N1-(2-chlorophenyl)-N2-phenyl-1, 2 hydrazine di Carbothioamide. **B.** Mechanism of N1-(2-chlorophenyl)-N2-phenyl-1,2-hydrazinedicarbothioamide.

Synthesis of Cu (II), Zn (II), ZrO (II), Hg (II), and UO2 (II) Metal Complexes

Ethanolic solution of N^1-(2-chlorophenyl)-N^2-phenyl-1, 2-hydrazinedicarbothioamide was mixed with different metals salts ($CuCl_2.2H_2O$, $Zn(CH_3COO)_2.2H_2O$, $ZrOCl_2.8H_2O$, $HgCl_2$, $UO_2(NO_3)_2.6H_2O$) in 2:1 ligand metal ratio. Reaction mixture was carried out for reflexing for 5 hour; and different colored precipitates were obtained by evaporating solvent and washed with dist. water. Synthesized complexes were characterized via different techniques such as CHN/S, IR, and Uv-Vis.

Mechanism for the Synthesis Of N1-(2-Chlorophenyl)- N2-Phenyl-1, 2-Hydrazine Dicarbothio Amide

4-phenyl-3-thiosemicarbazide acts as nucleophile and lone pair of 4-phenyl-3-thiosemicarbazide attack on electrophilic center of ortho chloro phenylisothiocyanate and form unstable intermediate. In next step deprotonation takes place and proton loss from intermediate and form final stable product N^1-(2-chlorophenyl)-N^2-phenyl-1,2-hydrazinedicarbothioamide **Figure 1B.**

DPPH Radical Scavenging Protocol

1,1-diphenyl-2-picrylhydrazil (DPPH) inhibition involved a mixture of 95 μL of DPPH (Sigma, 300 µM) and 5 µL samples (1mM in DMSO) in ethanol solution. Solution was set incubation for 30 min at 37°C, temperature. Absorbance was checked at the wavelength of 515 nm using Perkin Elmer Lambda 35 UV-Vis spectrophotometer. All ranges of sample were measured in triplicate procedure. In order to calculate percent radical scavenging activity,

% inhibition = (1−Absorbance of analyte/Absorbance of the control group) ×100.While 50% of DPPH scavenge, radicals shown by IC_{50}values. Where DMSO was used as a control purpose and *tert*-butyl-4-hydroxyanisole was taken as standard.

Results & Discussion

Ligand (L) was synthesized by stirring the ethanolic solution of 0.2 moles of 4-phenyl-3-thiosemicarbazide with equimolar amount of 2-chloro phenylisothiocynate for 3 hours. After due time, solvent was evaporated and off white colored precipitate was obtained, which was further washed with water and recrystallized with ethanol in order to obtained pure precipitate of ligand. The ligand was characterized by UV/Vis, FTIR, H^1NMR, and Mass spectrometry.

Physical Parameters of N1-(2-Chlorophenyl)-N2-Phenyl-1, 2-Hydrazine dicarbothio Amide and Its Metal Complexes

The synthesized compound ligand (L) was appeared off white in color and found to be non-hygroscopic and stable in air. Melting point of ligand was obtained 185°C. Ligand was completely soluble in methanol, and ethanol. Molar conductance of ligand was measured in 1 x 10^{-3}M methanol solution and it was found 2.08 μs/cm, low conductance showed non-electrolytic nature as shown in **Table 1**. All the synthesized metal complexes were appeared in different colors, and found stable in air, non-hygroscopic in nature and their percentage yield found up-to 48-94% with sharp melting points. The melting points of $Cu(L)_2$, $Zn(L)_2$, $ZrO(L)_2$, $Hg(L)_2$, $UO_2(L)_2$complexes were found to be 185°C, 285°C, 245°C, 160°C, 300°C respectively. All metal $Cu(L)_2$, $Zn(L)_2$, $ZrO(L)_2$, $Hg(L)_2$, $UO_2(L)_2$ were insoluble in dichloromethane (DCM), methanol, water, ethanol, while completely soluble in dimethyl sulfoxide (DMSO) and dimethyl form amide (DMF). Molar conductance of synthesized complexes was evaluated in 0.001M DMF and measured in range of 2.08 to 57.6µs/cm at 22°C. The lower value of molar conductance showed the non-electrolytic behavior of

Table 1. Physical Parameters of Ligand (L) and Metal Complexes

Compounds	Color	UV Spectra λ max (nm)	M.P °C	Yield %	Molar Conductance μs/cm	Solubility	
						DMF & DMSO	Other Solvent*
Ligand	Off white	327, 255	185	90	2.08	Methanol DMSO	Is
$Cu(L)_2$	Greenish	340, 270	185 dec.	63	11.34	S	Is
$Zn(L)_2$	White	355, 250	285	79	8.56	S	Is
$ZrO(L)_2$	Off white	390, 350	245	40	3.44	S	Is
$Hg(L)_2$	Blackish	340,300	160	88	57.6	S	Is
$UO(L)_2$	Blackish brown	320, 270	300	64	10.6	S	Is

S= soluble, Is = insoluble, dec = decompose * = DCM, MeOH, EtOH, H_2O

Table 2. Elemental (CHN/S) Microanalyses and antioxidant activity of Ligand and Metal Complexes

Compound	Empirical Formula	M.W.	M:L ratio	Elemental analysis Found% (Calculated %)				Antioxidant activity
				C	H	N	S	
Ligand	$C_{14}N_4S_2H_{13}Cl$	336.5	____	49.92 (49.92)	3.89 (3.86)	16.63 (16.64)	19.04 (19.08)	33.41± 1.32
$Cu(L)_2$	$C_{28}N_8S_4H_{26}Cl_2Cu$	736.5	1:2	45.43 (45.62)	3.59 (3.53)	15.20 (15.20)	17.40 (17.51)	28.10± 1.05
$Zn(L)_2$	$C_{28}N_8S_4H_{26}Cl_2Zn$	738	1:2	45.50 (45.52)	3.55 (3.52)	15.16 (15.17)	17.35 (17.34)	24.53± 1.12
$ZrO(L)_2$	$C_{28}N_8S_4H_{26}Cl_2ZrO$	780	1:2	43.06 (43.07)	3.36 (3.33)	14.35 (14.35)	16.42 (16.41)	58.32± 1.23
$Hg(L)_2$	$C_{28}N_8S_4H_{26}Cl_2Hg$	873	1:2	38.46 (38.48)	3.00 (2.97)	12.82 (12.82)	14.67 (14.66)	23.81± 1.57
$UO_2(L)_2$	$C_{28}N_8S_4H_{26}Cl_2UO_2$	943	1:2	35.36 (35.36)	3.00 (2.97)	11.87 (11.87)	14.67 (14.66)	52.14± 1.45
Standard *tert*-butyl-4-hydroxyanisole	-	-	-	-	-	-	-	44.7± 1.21

metal complexes **Table 1**.

Elemental CHN/S Micro-Analysis of Ligand and Metal Complexes

The Elemental CHN/S micro-analysis data showed good agreement with proposed formulae and verified the structure of it. Elemental CHN/S analysis results showed close agreement with calculated values. The formula of synthesized ligand was also confirmed by elemental analysis as shown in **Table 2**. The Elemental CHN/S micro-analysis data showed good agreement with the suggested formulae for N^1-(2-chlorophenyl)-N^2-phenyl-1,2-hydrazinedicarbothioamide Ligand(L) and also proposed composition of all synthesized metal complexes. Elemental CHN/S analysis results approved that calculated values are closed with observed values. The formula of the synthesized ligand and metal complexes was confirmed by CHN/S elemental analysis with 1:2 metal ligand ratios as shown in **Table 2.**

Spectral Analysis

The characterization of synthesized ligand (L) and its metal complexes was carried out via different spectroscopic techniques such as UV/VIS spectroscopy, FTIR Spectroscopy, H^1NMR and Elemental microanalysis (CHN/S).

Electronic Spectra

The absorption band of ligand (L) was carried out in methanol and bands were appeared at 327 nm and 255 nm respectively. These bands were appeared due to $\pi \rightarrow \pi^*$ intra ligand transitions. The UV-Vis spectra **Figure 2a-f** of all the synthesized metal complexes were taken in DMF and displayed absorption bands to longer wave length than ligand **Table 1**. The bathochromic shift was supporting evidence for complex formation. The absorption bands were appeared at 270, 340 for $Cu(L)_2$, 250, 355 for $Zn(L)_2$ 350, 390 for $ZrO(L)_2$ 300, 340 for $Hg(L)_2$ 270, 320 for $UO_2(L)_2$ as shown in **Table 1.** The λ_{max} value of metal complexes increase in comparison to ligand was an indication of complex formation. The bathochromic shift might be occurred due to ligand to metal charge transfer LMCT or π to π^* transition.

IR Spectral Data

FTIR spectral data of ligand (L) has presented in **Table 3** FTIR spectrum **Figure 3a** of the ligand exhibited strong absorption at 3226, 1537, 1129, and 685cm^{-1}, these peaks are assigned for N-H, -C=C-, -C=S, -C-Cl stretching vibrations respectively. The FTIR functionalities of synthesized metal complexes are presented in **Table 3**. Polydentate nature of ligand was observed by its structure illustration, so it may be suggested that IR measurements are highly

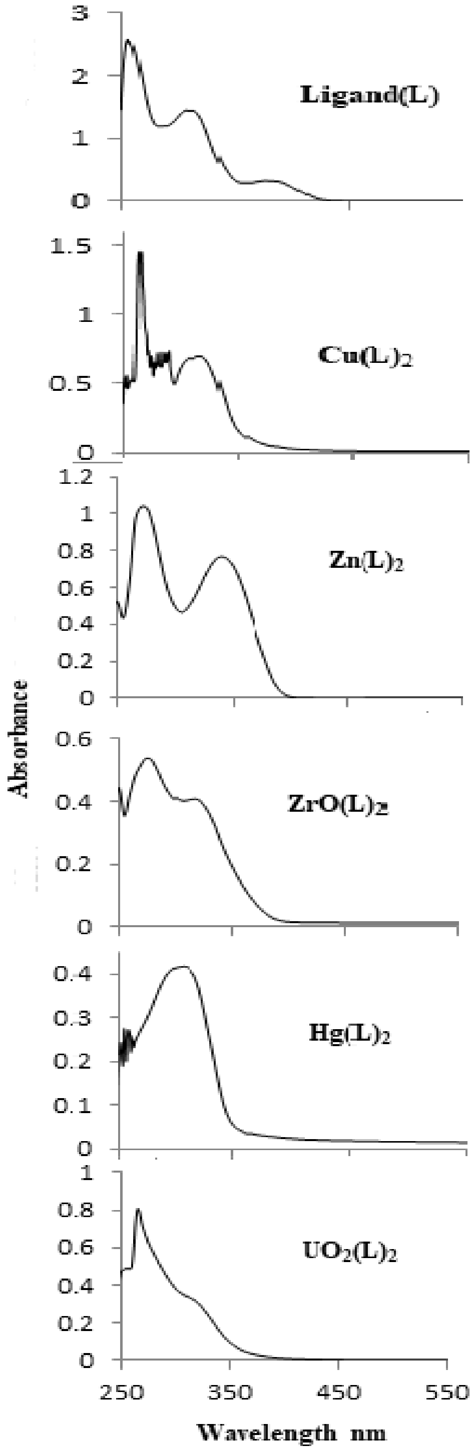

Figure 2. Electronic spectra of different compounds.

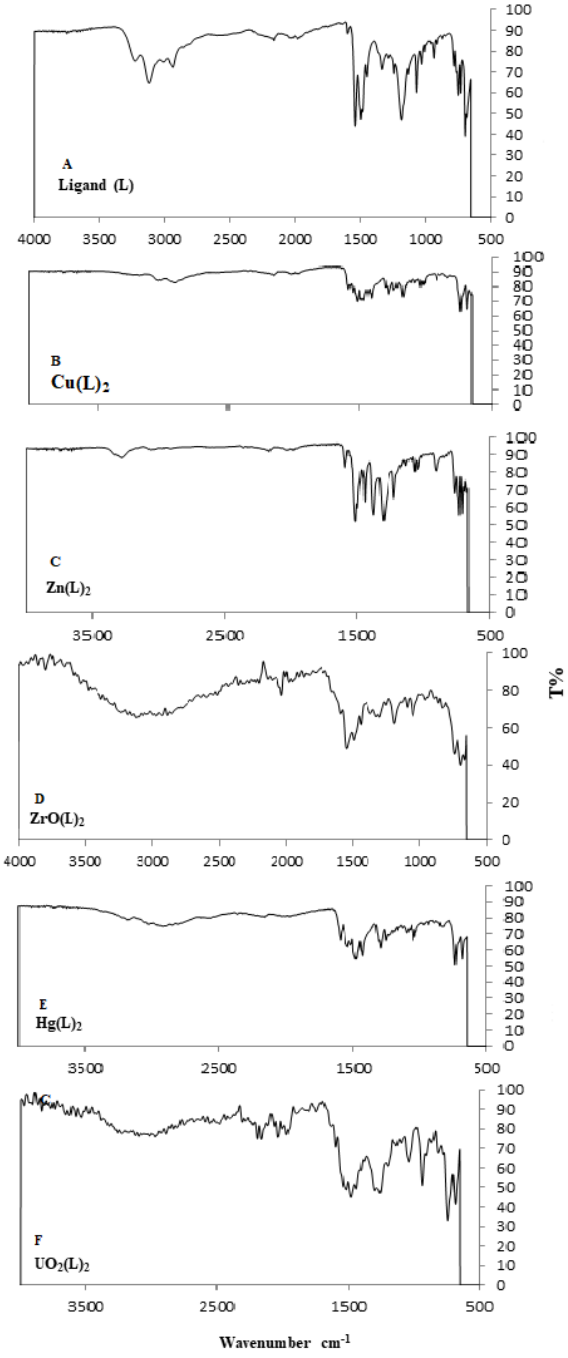

Figure 3. A-F. FTIR spectral analysis of different compounds

representing with respect to the complexation behavior with various metal ions. The peaks **Figure 3a-f** which were observed in the metal complexes and did not observe in ligand that confirms the presence of donor groups in ligand, where the characteristic FTIR absorption bands in the region of 3183- 3273cm^{-1}assigned for N-H stretching and 1096-1133cm^{-1}assigned for the (C=S) which confirms the presence of a thioamide group.

Table 3. FTIR Values for Ligand and Metal Complexes

Compound	**(-NH)** **ν cm^{-1}**	**(-C=C-)** **ν cm^{-1}**	**(-C=S)** **ν cm^{-1}**	**(-C-Cl)** **ν cm^{-1}**
Ligand(L)	3226	1537	1129	685
$Cu(L)_2$	3282	1520	1099	685
$Zn(L)_2$	3273	1510	1053	689
$ZrO(L)_2$	3126	1548	1096	692
$Hg(L)_2$	3183	1540	1053	688
$UO_2(L)_2$	3230	1519	1133	685

(H^1NMR) Spectral Data of Ligand

The H^1NMR spectrum of ligand (L) was taken in DMSO-d_6. The synthesized ligand contained two aromatic ring systems denoted by A and B. The H^1NMR spectrum of the ligand showed doublet for one proton at 7.02ppm for H-6 of ring A, while multiple appeared for three protons at 7.52 ppm for H-3,4, and 5 of ring A, a doublet assigned for one proton at 7.37ppm for H-2 of ring A. Moreover, doublet was observed for one proton at 7.84ppm for H-6' of ring B, multiple was appeared for two protons at 8.03ppm for H-4' and 5' of ring B. Finally a doublet for one proton at 8.05ppm assigned for H- 3' of ring B. **Figure 4a-b.**

Mass Spectrum of N1-(2-chlorophenyl)-N2-phenyl-1,2 Hydrazine dicarbothioamide

The mass spectrum of synthesized ligand N^1-(2-chlorophenyl)-N^2-phenyl-1,2-hydrazinedicarbothioamide showed molecular ion peak at *m/z* 336 (7%), while base peak observed at *m/e*=210 (100%)**.** The other *m/e* peaks at 165, 135, 126, 91, 89, and 59 was observed for relative fragmented ions **Figure - 5a-b.** The reported molecular ion peaks in the mass spectrum of synthesized ligand endorsed the proposed formula mass of the ligand.

The Proposed Structural Interpretation of Metal Complexes

On the basis of above spectroscopic techniques such as FTIR, UV-VIS, elemental analysis, and thermal analysis results, the suggested structure of the metal complexes are displayed**.** It is determined that *N* and *S* atoms of ligand are coordinated to metals in 2:1 ratio in such way that octahedral structure is assigned metal complexes.

Thermogravimetric Analysis

Thermogravimetric analysis deals with properties of substances in which mass of sample is changed as temperature change. Thermal stability and decay array of the N^1-(2-chlorophenyl)-N^2-phenyl-1, 2-hydrazine dicarbothioamide. Ligand (L) and its Metal complexes were recognized by recording derivative of thermo gram (DTG) thermo gravimetric analysis (TGA), and differential thermal analysis (DTA) curves.

A. TGA, DTG and DTA of N1-(2-chlorophenyl)-N2-phenyl-1,2- Hydrazine dicarbothioamide Ligand (L)

The TGA of N^1-(2-chlorophenyl)-N^2-phenyl-1,2-hydrazinedicarbothioamide Ligand (L) showed weight loss in two stages. In first stage thermal decomposition of TGA curve was seen between 130-200ºC with weight loss 33.9%, second stage was observed between 200-325ºCwith weight loss 41.6%.While the DTG thermo gram peaks were observed at 130ºC and 170ºC respectively as shown in **Table 4**. DTA thermo gram showed one endothermic peak at 166.1% **Table 4** which indicated some physical phenomenon such as melting point of ligand while there was no exothermic peak was detected.

B. TGA, DTG and DTA of Cu (L)2 Complex

Thermal decomposition of Cu $(L)_2$ Complex was observed in three steps via TGA. First step was detected at 80-170ºC with weight loss of 54.9%, second step was seen at175-210ºC with weight loss 11.2%, while third step was observed at 270-350ºC with weight loss of 21%. On the contrary, DTG thermogram peaks appeared at 100ºC and 300ºC as shown in **Table 4**. One endothermic peak of DTA was observed at 92.05ºC **Table 4.** Which showed some physical phenomenon such as melting point of ligand, although no any exothermic peak was detected.

C. TGA, DTG and DTA of Zn (L)2 Complex

Thermal decomposition of Zn $(L)_2$ complex was appeared in two steps through TGA. First step was detected at 100ºC-200ºC with weight loss of 5.2%, while second thermal decomposition was seen at 275ºC-350ºC with weight loss of 25.7%. In DTG thermo gram peaks were observed at 280ºC and 370ºC respectively which shows maximum weight loss of Zn complex **Table 4.** One endothermic peak of DTA was observed at 414ºC **Table 4** which indicates some physical phenomenon such as melting point of ligand, although there no any exothermic peak was observed.

D. TGA, DTG and DTA of ZrO (L)2 Complex

Thermal decomposition of $ZrO(L)_2$ Complex was seen in three steps. First step was seen at 100ºC-160ºC with weight loss of 51.01%, second step was detected between 160ºC-270ºC with weight loss of 20.4%.

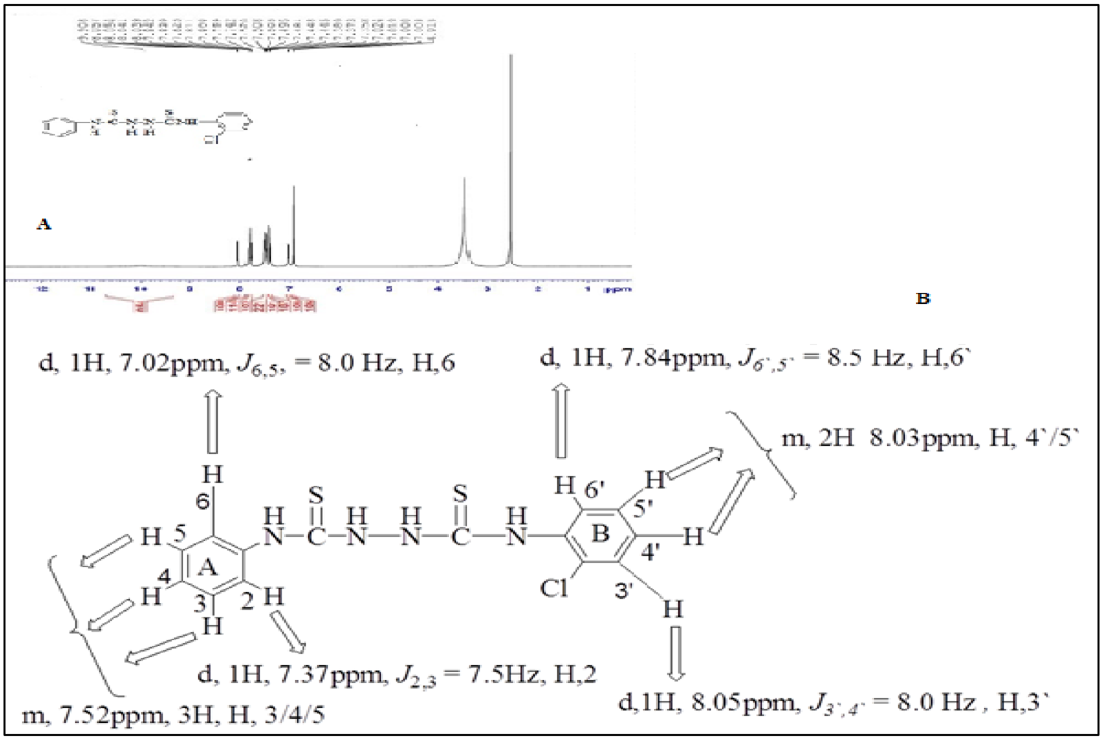

Figure 4. H1NMRSpectrum (A) and description (B) of Ligand (L).

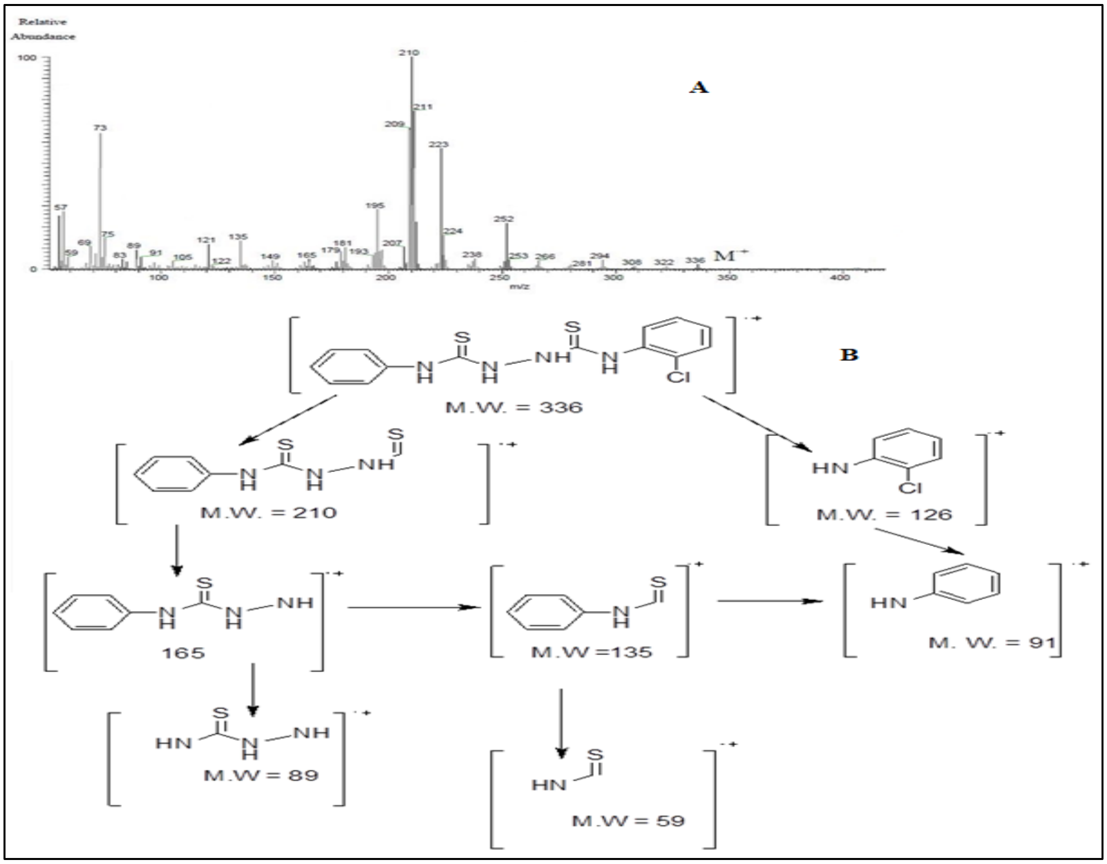

Figure 5. Mass spectrum (A) and Mass Fragmentation (B) of Ligand (L)

While third step was appeared at 270°C-360°C with weight loss of 26.8%. In DTG thermo gram peaks were observed at 110°C and 210°C respectively which shows maximum weight loss of $ZrO(L)_2$ complex as shown in **Table 4**. One endothermic peak of DTA was observed at 325.54°C **Table 4**, although no any exothermic peak was detected.

Table 4. Thermogravimetric analysis of ligand and Complexes

Compound	n	**Temperature**			**Total Weight Loss %**	**DTG T_{max} Loss °C**	**DTA Exo °C**	**DTA Endo °C**
		Stage I	**Stage II**	**Stage III**				
Ligand(L)	2	130-200°C (33.9%)	200-325°C (41.6%)	-	75.5	130 170	-	166.1
$Cu(L)_2$	3	80-170°C (54.9%)	175-210°C (11.2%)	270-350°C (21%)	87.1	100 300	—	92.05
$Zn(L)_2$	2	100-200°C (5.2%)	275-350°C (25.7%)	-	31.04	280 370	—	414
$ZrO(L)_2$	3	100-160°C (51.01%)	160-270°C (20.4%)	270-360°C (26.8%)	98.21	110 210	—	325.54
$Hg(L)_2$	3	100-150°C (5.6%)	160-250°C (8.6%)	300-350°C (15.6%)	29.93	250 310	468.7	193
$UO_2(L)_2$	3	110-190°C (40.9%)	190-270°C (11.4%)	270-370°C (17.6%)	69.9	160	112	90 200

E. TGA, DTG and DTA of Hg (L)2 Complex

Thermal decomposition of Hg $(L)_2$ Complex was observed in three steps. First step was seen at 100°C-150°C with weight loss of 5.6%, second step of thermal decomposition was observed between 160°C-250°C with weight loss of 8.6%. While third step was appeared between 300°C-350°C with weight loss 15.6%. In DTG thermograme peaks were observed at 250°C-310°C which showed maximum weight loss of Hg $(L)_2$ as shown in **Table 4**. One endothermic peak of DTA was observed at 193°C **Table 4** which indicates some physical phenomenon such as melting point of ligand, although no any exothermic peak was detected.

F. TGA. DTG and DTA of UO2 (L)2 Complex

Thermal decomposition of UO_2 $(L)_2$ was observed in three steps. First step of TGA was seen at 110°C-190°C with weight loss of 40.9%, second step was observed between 190-270 with weight loss, while third step was appeared at 270-370°C with weight loss 17.6%. In DTG thermo gram peak was observed at 160°C which showed maximum weight loss of UO_2 $(L)_2$ **Table 4**. Two endo thermic peaks were detected at 90°C and 200°C, while one exothermic peak was detected at 112°C **Table 4.**

Antioxidant Activity

N^1-(2-chlorophenyl)-*N*^{2}-phenyl-1,2hydrazinedi Carbothioamide Ligand (L) and their metal complexes evaluated for DPPH radical scavenging activity and results shown in **Table 4**. All the compounds showed significant antioxidant potential with IC_{50} values ranges from 23.81± 1.57μM - 58.32± 1.23μM. The ligand (IC_{50} =33.41 ± 1.32μM) as well as metal complex , $Cu(L)_2$ (IC_{50} = 28.10± 1.05μM), $Zn(L)_2$ (IC_{50} = 24.53± 1.12μM), $ZrO(L)_2$ (IC_{50} = 58.32± 1.23μM), $Hg(L)_2$ (IC_{50} = 23.81± 1.57μM), $UO_2(L)_2$ (IC_{50} =52.14± 1.45μM) showed far better activity than the standard (IC_{50} = 44.7± 1.21). Among these metal complexes, Hg complex (IC_{50} = 23.81 ± 1.57) showed highest antioxidant activity, while Zn complex (IC_{50}= 24.53 ± 1.12), Cu complex (28.10 ± 1.05) were also found to be remarkable antioxidant and they displayed better activity than standard. The metal complexes $UO_2(L)_2$, $ZrO(L)_2$ showed moderate anti-oxidant activity. These compounds were found be less active than other metal complexes as well as standard. The antioxidant activity pattern of these compounds is $Hg(L)_2 > Zn(L)_2 > Cu(L)_2 > UO_2(L)_2 > ZrO(L)_2$.

Conclusion

Ligand and its metal complexes were screened for antioxidant activity. Among these metal complexes $Hg(L)_2$ $Zn(L)_2$, $Cu(L)_2$exhibited better antioxidant potential. It indicates that these complexes have excellent potential towards antioxidant activity. Therefore these compounds may be used for the development of organometallic compounds which may find their importance in avoiding or reducing the development of both heart disease and some forms of cancer.

References

Cragg GM, Newman DJ (2005). Biodiversity: A continuing source of novel drug leads. Pure Appl Chem; 77:7-24.

Duthie, G.G., and Brown, K.M (1994) Reducing the Risk of Cardiovascular Disease, ch 2, p. 19-38, In: Functional Foods, ed. Goldberg, I. Chapman and Hall: New York.

Ghosh, M.N,(1998). Fundamentals of Experimental Pharmacology, 2nd Edn., Scientific Book Agency, Calcutta,,174-179.

Gu, C.Q.; Li, J.W.; Chao, F.; Jin, M.; Wang, X.W.; Shen, Z.Q(2007). Isolation, identification and function of a novel anti-HSV-1 protein from *Grifola frondosa*. *Antiviral Res.*, *75*, 250 257.

Handa, SS, Vasisht, K, *et.al*, (2006).Compendium of Medicinal and Aromatic Plants-Asia, II, ICS-UNIDO, AREA Science Park, Padriciano, Trieste, Italy,, 79-83.

Harborne, J.B,(1998).Phytochemical methods- A guide to modern techniques of plant analysis, 3rd Edn, Springer (India) Pvt. Ltd, New delhi,, 5-32.

Kim YJ, Ryu JH, Cheon YJ, Lim HJ, Jeon R(2007). Design and synthesis of urea and thiourea derivatives and their inhibitory activities on lipopolysaccharide-induced NO production. Bio-org Med Chem Lett, 17:3317-3321.

Kirtikar, K.R, Basu, B.D, (2006). Indian medicinal plants, International book distributors, Dehradun, 993-994.

Qi, H.M.; Zhang, Q.B.; Zhao, T.T.; Chenc, R.; Zhang, H.; Niu, X.Z.(2005). Antioxidant activity of di-valent sulfate content derivatives of polysaccharide extracted from *Ulva pertusa* (Chlorophyta) *in vitro*. *Int. J. Biol. Macromol.*, *37*, 195–199.

Rojas J, Paya M, Domínguez JN, Ferrandiz L, Bioorg K(2002). The synthesis and effect of fluorinated chalcone derivatives on nitric oxide production. Medicinal Chemistry Letters, 12: 1951-1954

Sahu MP, Solanki NS (1991). Role of sulfihydral compounds in improving dry matter portioning and grain production of maize. Journal of Agronomy Crop Science,; 167: 356-359.

Sravani, T, Paarakh, P.M, "Antioxidant activity of Hedychium spicatum Buch. Ham Rhizomes" *Indian journal of natural products & resources*, Sep.2012, 3(3), 354-358.

Wagner, H, Bladet, S, et.al,(1996)Plant Drug Analysis-A TLC Atlas, 1st Edn, Springer verlag Berlin, Heidel berg, New York, 195-214.

Wang, Z.; Luo, D (2007). Antioxidant activities of different fractions of polysaccharide purified from *Gynostemma pentaphyllum Makino. Carbohydr. Polym.* , *68*, 54–58.

Warrier, P.K, Nambier, VPK, Raman Kutty C, (1994). Indian medicinal plants- A compendium of 500 species, Orient longman Ltd, Madras, Vol-I, 95-97.

Yon ova PA, Stoilkova GM (2004). Synthesis and biological activity of urea and Thiourea derivatives from 2- aminoheterocyclic compounds. Journal of Plant Growth Regulation; 23:280-291.