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Research Article

Synthesis, Characterization, and Anticancer Activity Studies of New N-(5-phenyl-1,3,4- oxadizole-2-yl) propane hydrazide and its Transition Metal Complexes

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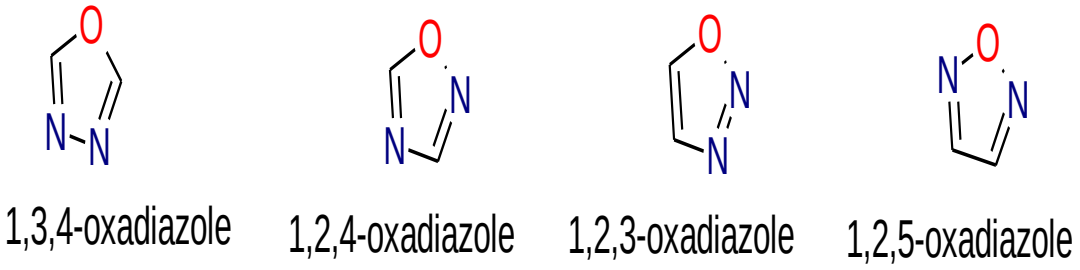
ABSTRACT
A new ligand of N-(5-phenyl-1,3,4- oxadizole-2-yl) propane hydrazide and its Cu(II), Co(II), and Ni(II) complexes were synthesized. These ligand and its complexes have been characterized by ^{1}HNMR,mass,and Fourier transform infrared (FTIR)spectra, as well as magnetic susceptibility, elemental analysis[C, H, N] and conductance measurements. The program of Hyperchem 7.51 has been used up for theoretical accounts using PM method to study the electrostatic potential that provided good information about the complexity site.Depending to the results obtained we can suggested square planer geometrics for Co(II) and Ni(II) complex, while tetrahedral geometry for Cu(II) complex. In otherwise the ligand and its complexes screened for their anticancer activity. This research showed excellent results in comparison with Ciprofloxacin as standard drug

Keywords: ligand, complexes, characterization, HyperChem, electrostatic potential, anticancer

INTRODUCTION
Oxdiazoles and their derivative compounds can regard as simple five membered heterocyclic have one oxygen and two nitrogen atoms. The Oxadiazoles subsist in different isomeric forms such as 1,2,5-oxadiazoles , 1,2,4-oxadiazoles , 1,2,3-oxadiazoles and 1,3,4-oxadiazoles [1,2].The isomer 1,3,4-oxadiazoles reverts to the diazoketone tautomer (unstable) [3] .



compounds containing 1,3,4-oxadiazole moiety play an important application in the field of biological activities as antibacterial [4,5], antifungal [6], anti-inflammatory[7], anti-cancer[8], anticonvulsant[9], antiviral[10], anti-HIV[11], anti-diabetic[12], anti-tubercular[13], and lipid peroxidation inhibitor[14]. Other application of1,3,4-oxadiazole as insecticidal[15], antioxidant[16], corrosion inhibitor[17], fluorescent and colorimetric chemical sensors[18], dyes[19], polymers material[20], and light emitting diodes[21].

EXPERIMENTAL
Materials
Methyl benzoate (Sigma-Aldrich, 99%) and other materials (BDH, ≈99%). All chemicals used as received without further purification.

Synthesis of the ligand
Synthesisof Benzohydrazide (A)
A mixture of methyl benzoate (15.2mL 0.1mol) and hydrazine hydrate (10mL, 0.2mol) in ethanol absolute (100 ml) were refluxed for 4 hours, the mixture was evaporated to half volume, cooled, filtered and washed with ethanol absolute[22] ,the solid (A) was lighting white, and its melting point was (118-122°C), with yield 95.32%.

Synthesis of 5-phenyl-1,3,4-oxadiazole-2-thiol (B)

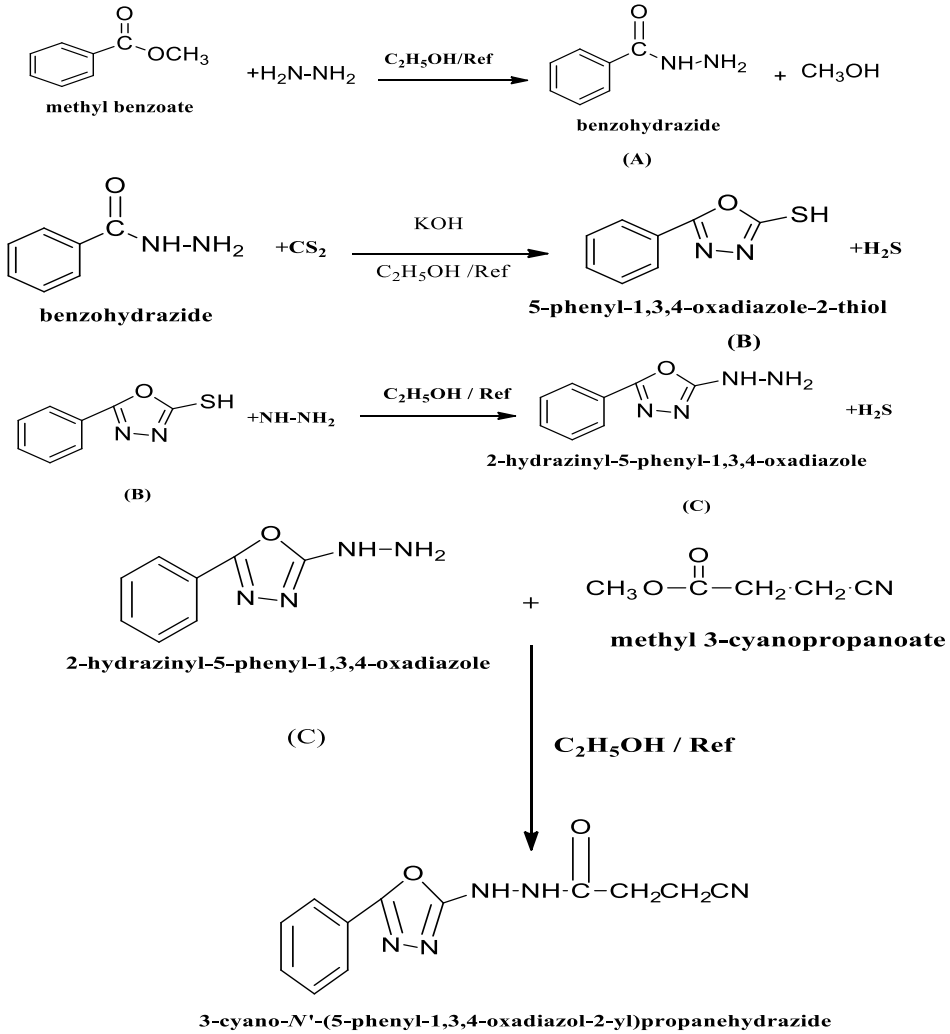
The Benzohydrazide (A) (13g, 0.1mol) , (5.6g, 0.1mol) of Potassium Hydroxide putted in ice path andcarbon disulfide (7.6mL,0.1mol) were added drop wise, then the mixture were refluxed in ethanol absolute (100ml) for continuous 22 hours until the H_2S gas emission was stopped. The solvent was evaporated and acidified with HCl (10%) then the precipitated was filtered and the result solid was recrystallized from ethanol absolute[23].The solid (B) was white, with melting point (218-221˚C), and its yield was90.12%.

Synthesis of 2-hydrazinyl-5-phenyl-1,3,4-oxdiazole(C)

A compound (B) (6.5g, 0.05mol) and hydrazine hydrate (7.5ml،0.057mol) in ethanol absolute as solvent (50 ml) were refluxed for 22 hours until there is no emitted H_2S gas. A white precipitate appeared in round bottom[24]. The white precipitate was filtered and recrystallized from ethanol absolute.The product (C) was whitish brown. It have melting point(226˚C), with yield 74.8%.

Synthesis of3-Cyno-N-(5-phenyl-1,3,4 -oxadizole-2-yl) propane hydrazide(L)

A mixture containing (0.22mole, 2.48g) of ethyl cyanoacetate was prepared with (0.022mole, g4) of the compound (C) prepared in the first step in (30 ml) of absolute ethanol, and the mixture was refluxed for 4 h. Follow the reaction with TLC[25] [26 technique, then solution was concentrated to half volume, and let the solution cooledand filtered.The precipitate was recrystallized with absolute ethanol to give dark brown color crystals of the product, which have melting point (224_226 ° C), and the yield (70.5%) as shown in the scheme below.



Scheme 1: Synthesis reaction of the ligand

Preparation of complexes

The complexes were synthesized by mix (0.2g, 0.00077mol) from ligand with the same moles of salts ($CoCl_2.6H_2O$,$CuCl_2.2H_2O$, and $NiCl_2.6H_2O$) both alone in (100ml) ethanol absolute and refluxed for 2 hrs. (Monitored by TLC).Then the

precipitate was filtered and wash several times with ethanol or aqueous ethanol to removed unreacted salts or ligand,then precipitated complexes was dried[27] .

Analysis and physical measurements

Physical properties and elemental microanalysis CHN shown in table 1.

Table 1: Physical properties and elemental microanalysis data of the ligand and its complexes

No.	Formula	Color	C%	H%	N%	Λ $Scm^2 mol^{-1}$	M.p °C	μeff B.M
1	$C_{12}H_{11}N_5O_2$	Light brown	56.03%Exp. 56%Cal.	4.31%Exp. 3.85%Cal.	27.22%Exp. 27.95%Cal.	___	226	___
2	$[Co(L_3)_2]$	grey	___	___	___	16	199	1.9
3	$[Ni(L_3)Cl_2]$	Olive green	___	___	___	14	173	0.7
4	$[Cu(L_3)Cl_2]$	Black	___	___	___	15	223	2.2

Anticancer activity

Maintenance of cell cultures

HeLa cells maintained in RPMI-1640 supplemented with 10% fetal bovine serum, 100 units/mL penicillin, and 100 μg/mL streptomycin. All cells passaged using Trypsin-EDTA reseeded at 80% confluence twice a week, and incubated at 37 °C[28].

Cytotoxicity Assays(MTT assay)

To determine the cytotoxic effect, the MTT cell viability assay conducted on 96-well plates. Cell lines were seeded at 1 × 10^4cells/well. After 24 h or a confluent monolayer was achieved, cells were treated with tested compounds at(ligand and complex Ni(II) different concentration. Cell viability measured after 72 h of treated cells by removing the medium, adding 28 μL of 2 mg/mL solution of MTT and incubating the cells for 2.5 h at 37 °C. After removing the MTT solution, the crystals remaining in the wells were solubilized by the addition of 130 μL of DMSO (Dimethyl Sulphoxide) followed by 37 °C incubation for 15 min with shaking [29]. The absorbency was determined on a micro-plate reader at 492 nm (test wavelength); the assay performed in triplicate. The inhibition rate of cell growth (the percentage of cytotoxicity) calculated as the following equation:

$$\text{Cytotoxicity} = \text{A-B/A} * 100$$

Where A and B are the optical density of control and the optical density of test

For visualize the shape of cells under inverted microscope, 200 μL of cell suspensions seeded in 96-well micro-titration plates at density 1x10^4 cells mL^{-1} and incubated for 24 h at 37°C. Then the medium removed and added ligand and complex Ni(II) after 24hr , the plates were stained with 50 μL with Crystal violet and incubated at 37°C for 15 min, the stain was washed gently with tap water until the dye was removed. The cell observed under inverted microscope at 100x magnification microscope filed and photographed with digital camera [30].

RESULTS AND DISCUSSION

FT-IR spectra

The FT-IR of the synthesized ligand and its complexes carried out using KBr disc to ligand and CsI for complexes. The free ligand (L) exhibited six major bands at (3186cm^{-1}), (1689cm^{-1}), (1535cm^{-1}), (1064cm^{-1}), (1311cm^{-1}), and (1081cm^{-1})[31].Which are attributable to (υNH_2), (υO=C-N)imide, (υC=N)Oxa, (υC-O-C)sym, (υC-O-C)asy and structure movement bands respectively, as shown below(table 2).New bands were formed attributed to the coordinated (M- N), and (M-Cl) bonds and appeared at the regions (501-478cm^{-1}),and (324-277cm^{-1}) respectively. This indicates that the coordinate occurred through the(N), and (Cl) atoms.

Table 2: The Infrared spectra of L and its metal complexes (υ cm^{-1})

Assignment	wave length/cm^{-1}			
	L_2	$[Co(L_2)]_2$]	$[Ni(L_2)Cl_2]$	$[Cu(L_2)Cl_2]$
N-H	3186	3294	3294	3502 3448
C-H(Aliphatic)	2931	2931	2924	2970
C-H(Aromatic)	3109	3109	3109	3116
O=C-N	1689	1689	1689	1689
C=N(Oxadiazole)	1535	1489	1489	1473
C≡N	2240	2240	2240	2240
C-O-C	1064(sy) 1311(asy)	1234(sy) 1319(asy)	1234(sy) 1311(asy)	1234(sy) 1350(asy)
Skatel movement	1072	1072	1072	1072
M-N	———	501	478	378
M-Cl	———	324	277	277

Nuclear Magnetic Resonance

The Alecand L spectrum showed a triple signal at (2H, 3.96ppm) returned to CH_2 (1), another triple signal at (2H, 3.22ppm) returning to CH_2 (2) protons, and a broad mono signal was observed at (9.82ppm, 1H). The spectrum showed multiple signals at (7.39ppm to 8.04ppm), returning to the aromatic ring protons (5H) with the proton NH (2)[32.]

Mass spectra

The mass spectra of ligand appeared molecular ion peak at 257 m/z, which is in conformity with the molecular formula $C_{12}H_{11}N_5O_2$.Other peaks are due to the subsequent fragments like $[C_9H_9N_4O]^+$=189 m/z, $[C_8H_7N_4O]^+$=175 m/z, $[C_8H_6N_3O]^+$=160 m/z, $[C_8H_5N_2O]^+$=145 m/z, $[C_8H_9N_2]^+$=133 m/z, $[C_7H_7N]^+$=104 m/z, $[C_6H_5]^+$=77 m/z, and $[C_5H_5]^+$=64 m/z.

The mass spectrum characteristic of the Co(II) was characterized by the appearance of a molecular partial ion peak at 571 m/z, and anther peak appears at 314m/z indicating the loss of one ligand, confirming our conclusion of the molecular formula of the complex.

The mass spectral of the Ni(II) complexes showed molecular ion peaks at 386 m/z corresponding to $[Ni(L)Cl_2]^{\cdot+}$ stoichiometry . This complex shows another a fragmentation peaks at 351 m/z, 315 m/z due to loss one and two chlorine atom respectively. The mass spectral of the Cu(II) complexes showed molecular ion peaks at 391 m/z corresponding to $[Cu(L)Cl_2]^{\cdot+}$ stoichiometry . This complex shows another a fragmentation peaks at 356 m/z, 320 m/z due to loss one and two chlorine atom respectively.

Magnetic sensibility

The magnetic momentum for each metal complexes listed in table 1. These magnetic measurements give an idea about the electronic state of the transition metal ion of the complexes.The observed magnetic momentum value was 1.9 BM for Co(II) complex with six paired electrons, This value confirms that cobalt(II) square planer geometry .0.7 BM for Ni(II) suggesting square planer geometry [33] .the value of magnetic momentum or Cu is 2.2 confirm tetrahedral geometry

Anticancer Profiles

Cancer cell line of ovaries was exhibited for concentrations ranging from (6.25,100μg / ml) to both the (L) and the complex [Ni (L) CI2] for 24hr and 37C°. The toxicological effect evaluated by the embarrassment of the percentage of growth inhibition rate.

The study showed that there is a significant effect of these compounds when used on ovarian cancer cells called line(SKOV-3 cells).

Table (3-25)[34] shows the effect of ligand (L) on the growth of cells of ovarian cancer, where the lowest rate of cell growth was found at the lowest concentration 6.25μg/ml and the highest inhibition rate at concentration100 μg / ml.

As for the effect of the complex[Ni (L) Cl_2]on the growth of cells of ovarian cancer, where the lowest inhibition of cell growth was found at the lowest concentration6.25μg/ml and the highest inhibition rate at the concentration100 μg/ ml.

We also note that ligand has less toxic activity against the cancer cells of the ovarian cancer cell line (SKOV-3 cells) than the effectiveness of nickel complex (II) as shown in Table 3-25

The results also showed that the type and concentration of the compound used are two important factors in determining the rate of cell inhibition, as it was found that the increase in the concentration of both the ligand and its complex of nickel (II) increases the rate of inhibition of cell growth of cancerous lines,

Table 3: Effect of Ni-II and L-Complex (L) on Cancer Cells of the Ovary Cancer Line

Inhibition rate (s. error ± mean)	Con. Used	Compound
1.80±13.73	6.25	L
1.89±18.37	12.5	
1.48±24.66	25	
1.80±30.26	50	
2.96±40.88	100	
1.45±17.33	6.25	[Ni(L) Cl_2]
2.03±26.33	12.5	
3.53±48.33	25	
1.766±6.66	50	
3.22±77.00	100	

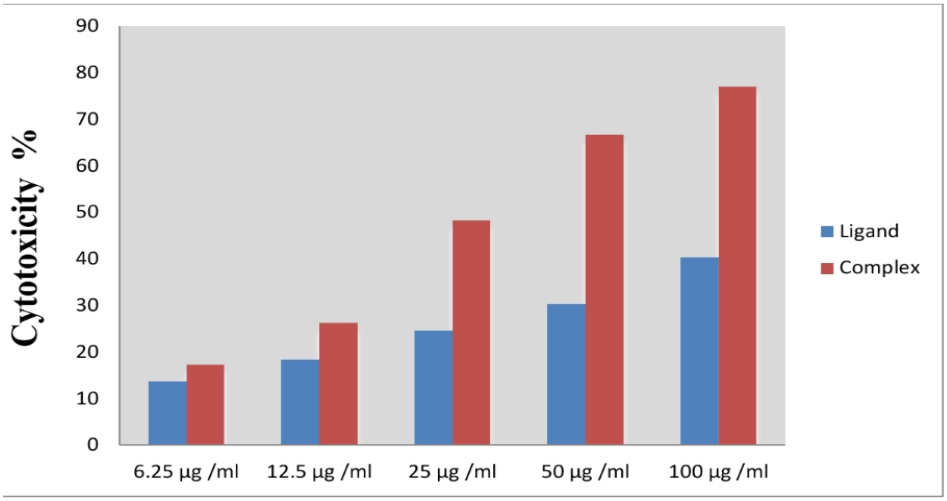
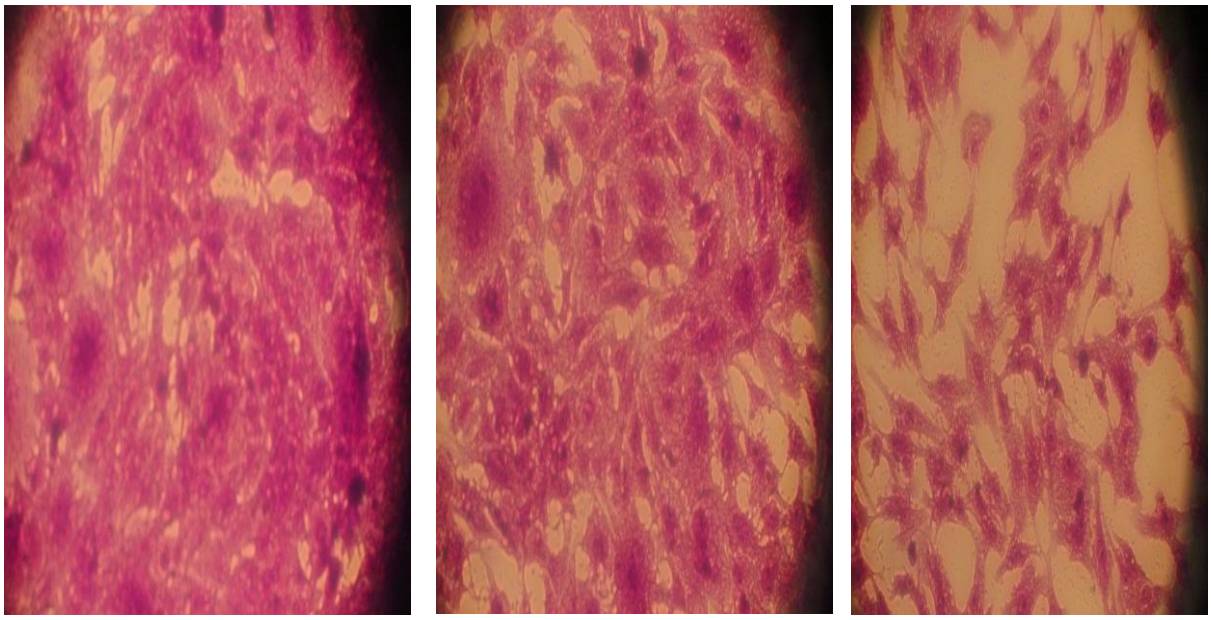


Fig.1: Cytotoxicity (L) form and complex of Ni (II) on SKOV-3 cells



1 2

Fig.2: Microscopic images of the effect of cytotoxicity: (1) Control untreated cells, (2)Picture is due to the effect of the L, and (3) Picture is due to the complex effect [Ni (L) Cl2]

The results indicated the presence of toxic effects on the growth of cancer cell lines of ligand concentrations. This inhibition of ligandis due to its oxidizole ring, which is effective in inhibiting the growth of cancer cells.

CONCLUSION

The 1,3,4-oxdiazole derivative acts as a bi-dentate ligand. The spectroscopic dataexhibit the involvement of two NH_2 groups in coordination to the central transition metal ion. Various techniques have been used such as(FTIR, ^{1}H.NMR and Mass) spectraas well as Molar conductance and magnetic susceptibility to characterization oftransition metal complexes .a square plainer geometry for Ni(II) and tetrahedral geometry for Cu(II) complex is proposed.

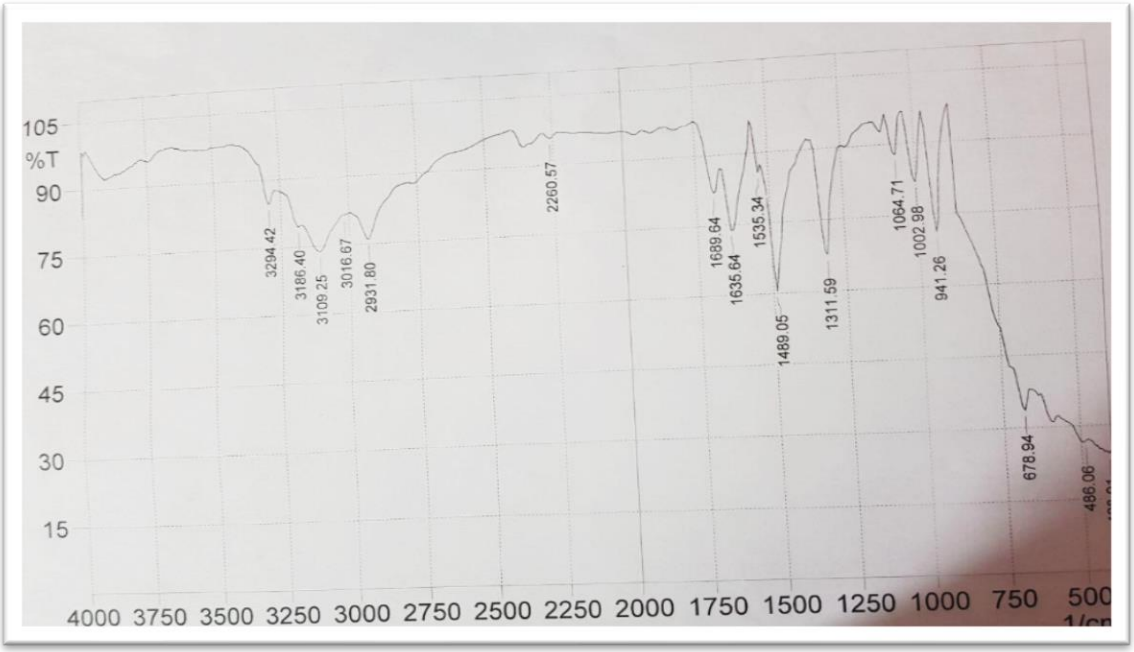


Fig.3: FT.IR spectrum of the lignd

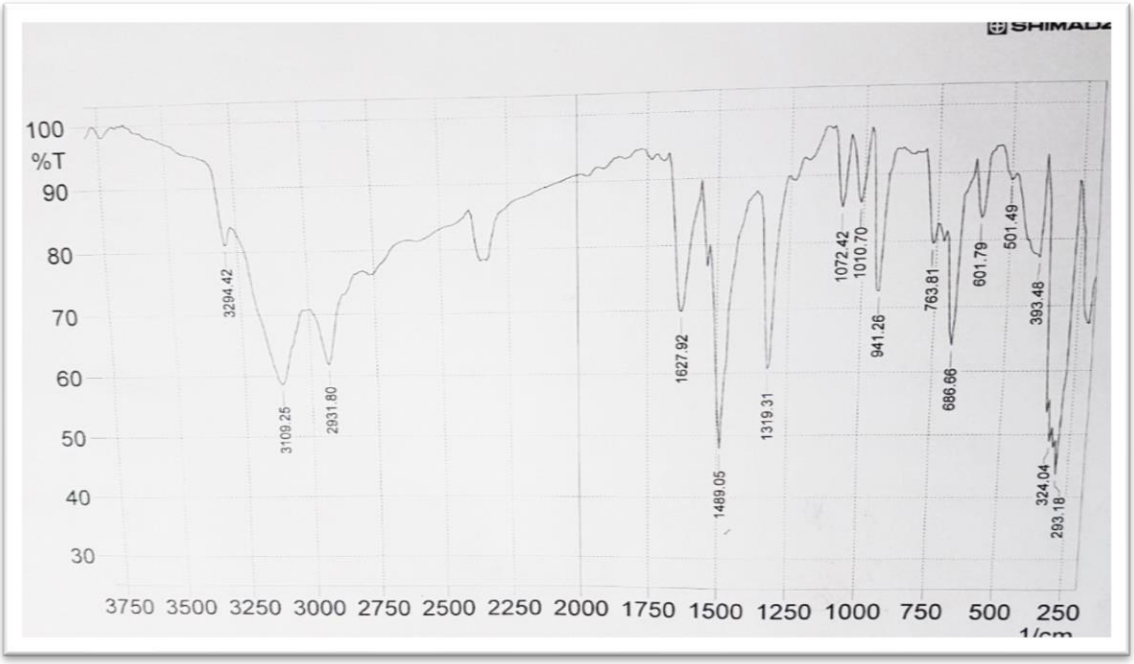


Fig.4: FT.IR spectrum of $[Co(L)_2]$

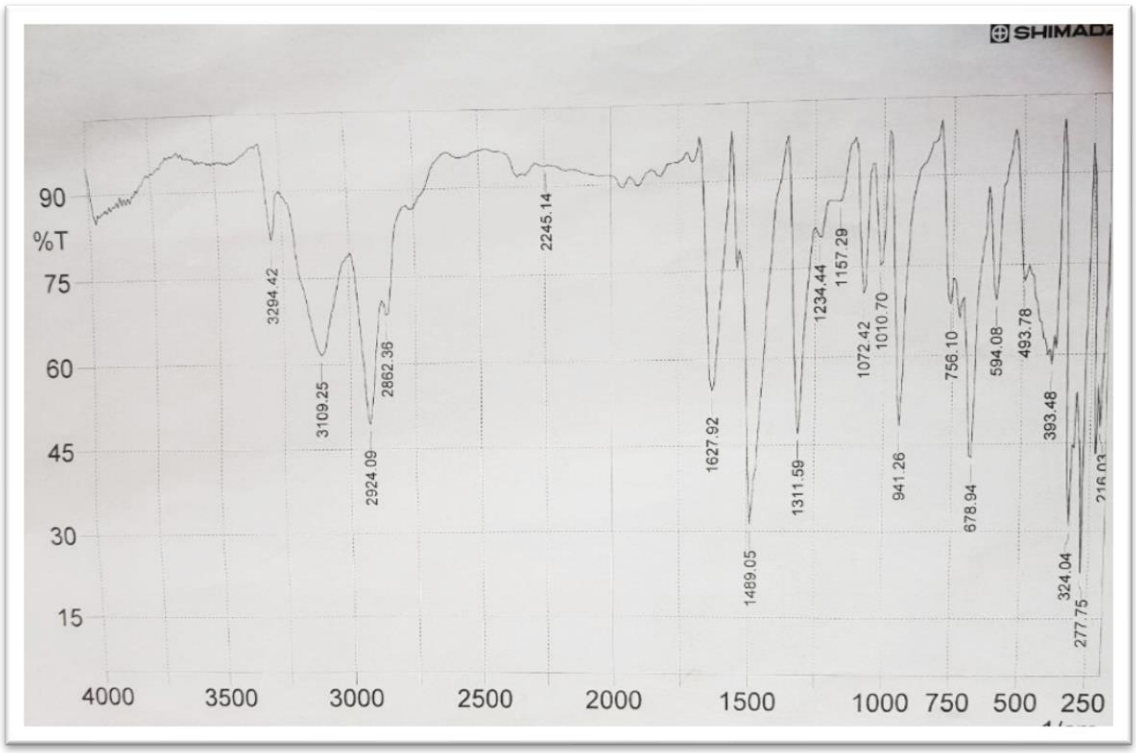


Fig.5: FT.IR sectrum of [Ni(L)Cl_2]

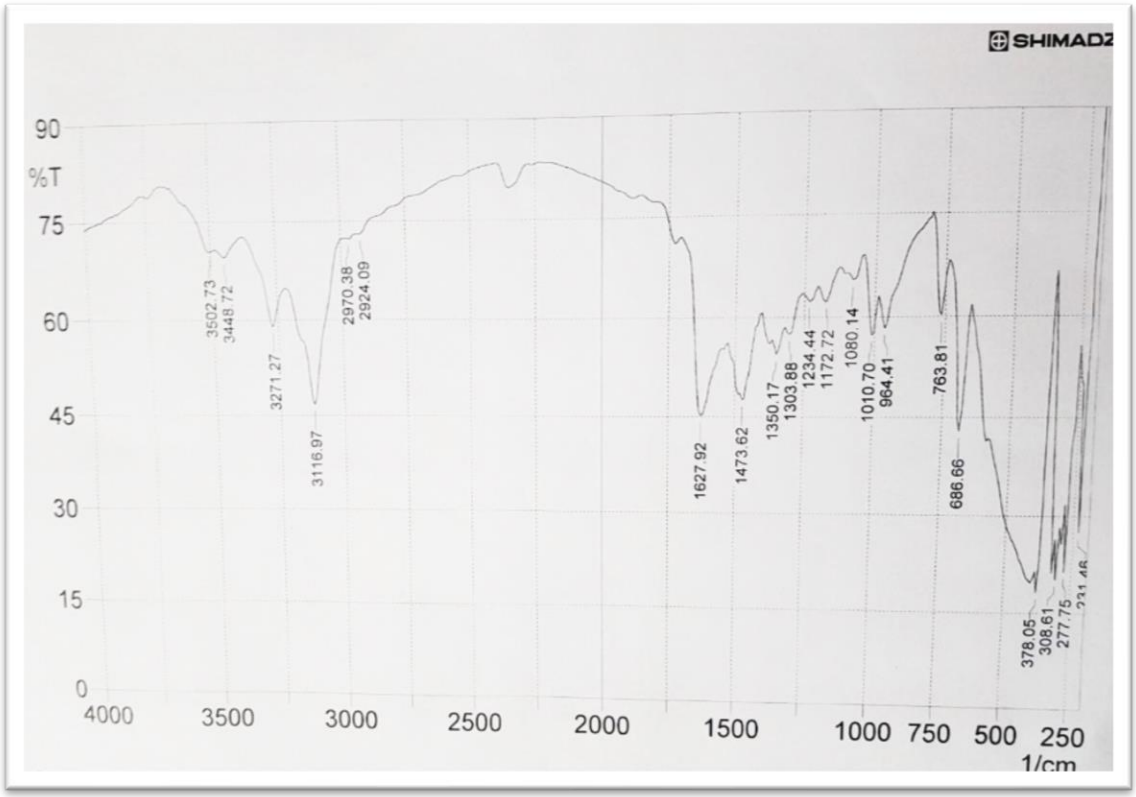


Fig.6: FT.IR sectrum of [Cu(L)Cl2 /

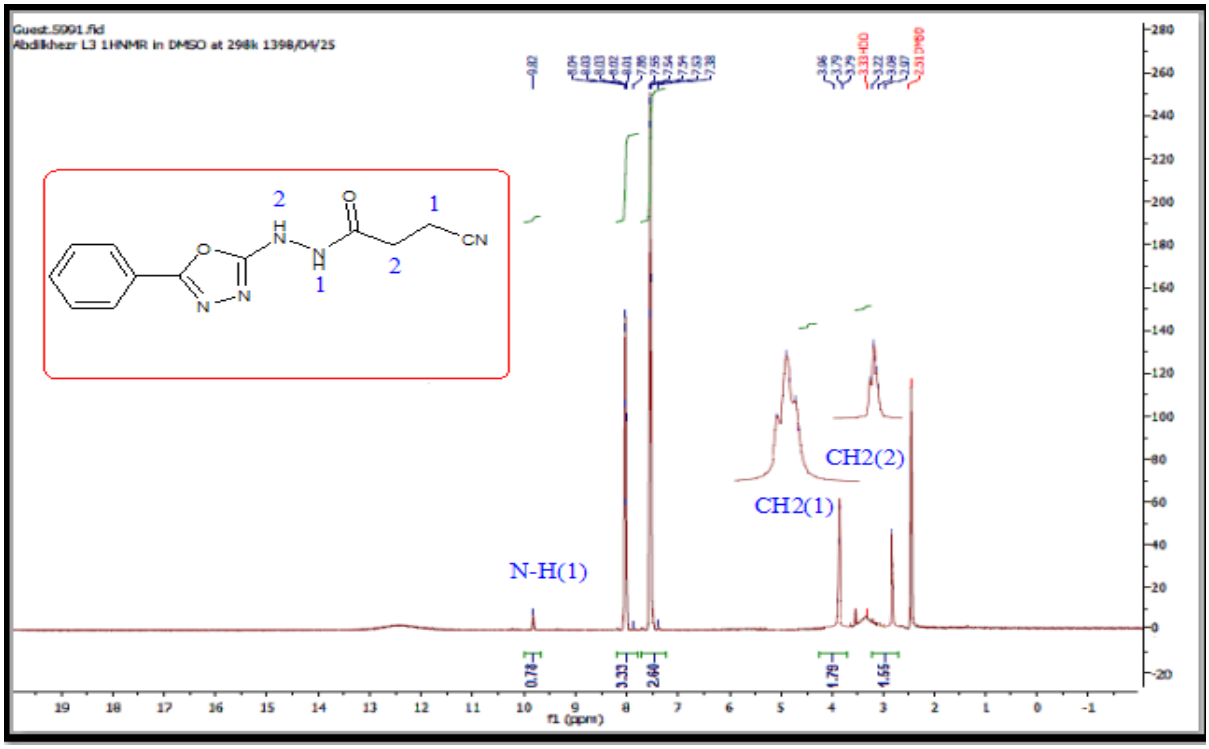


Fig.7: 1 H-NMR spectrum of the lignd

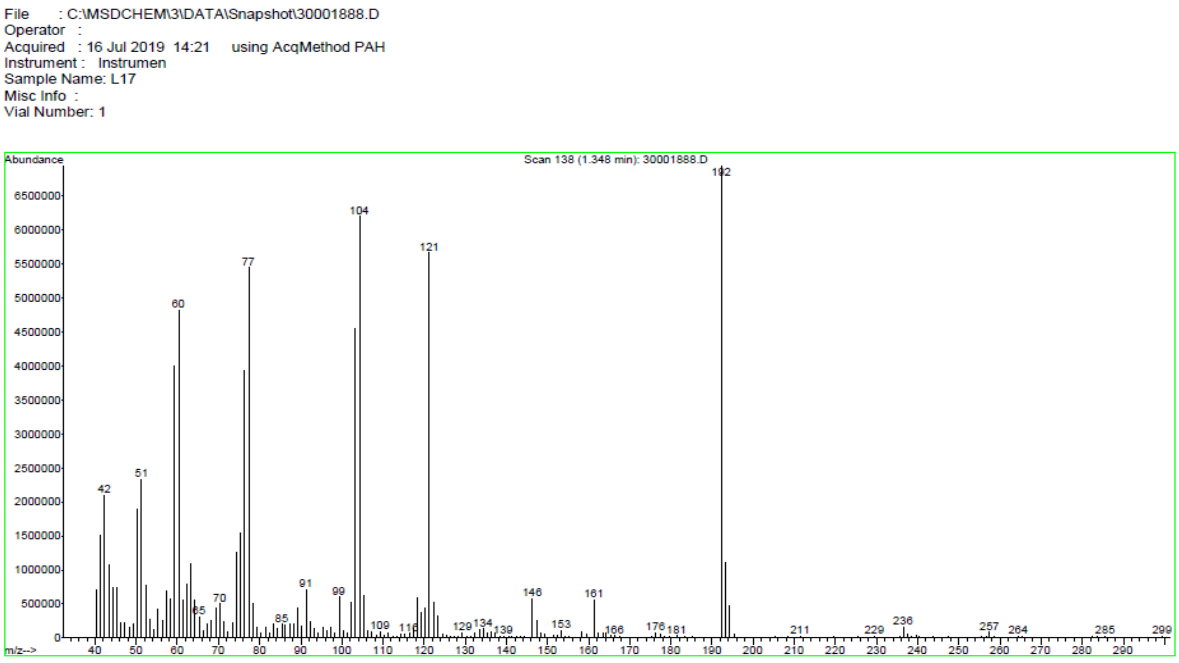


Fig.8: Mass spectrum of the lignd

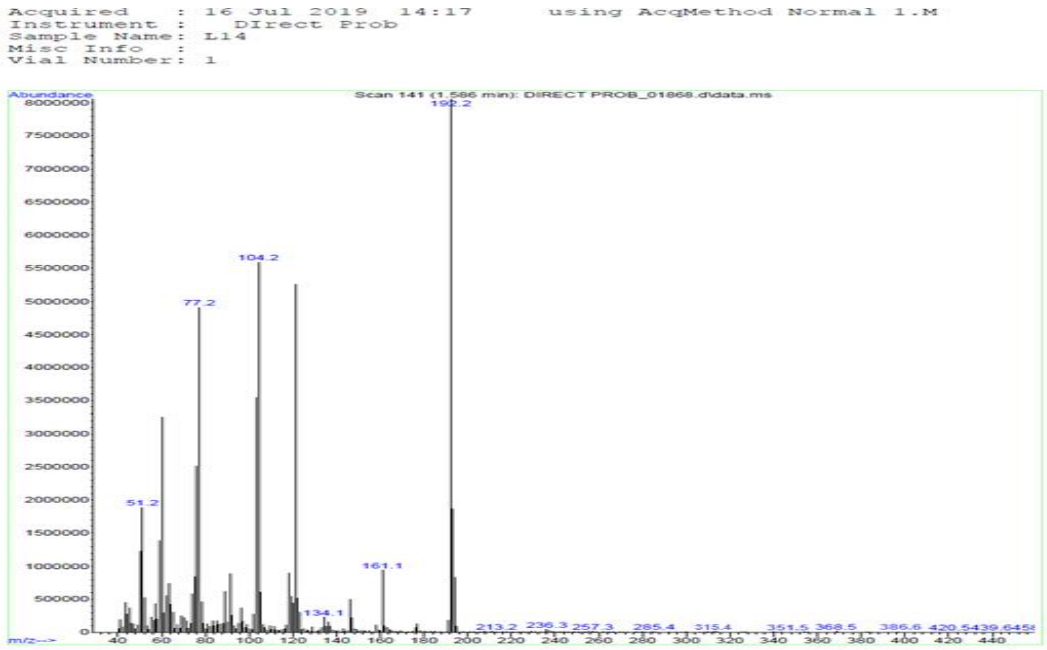


Fig.9: Mass sectrum of [Ni(L)Cl2]

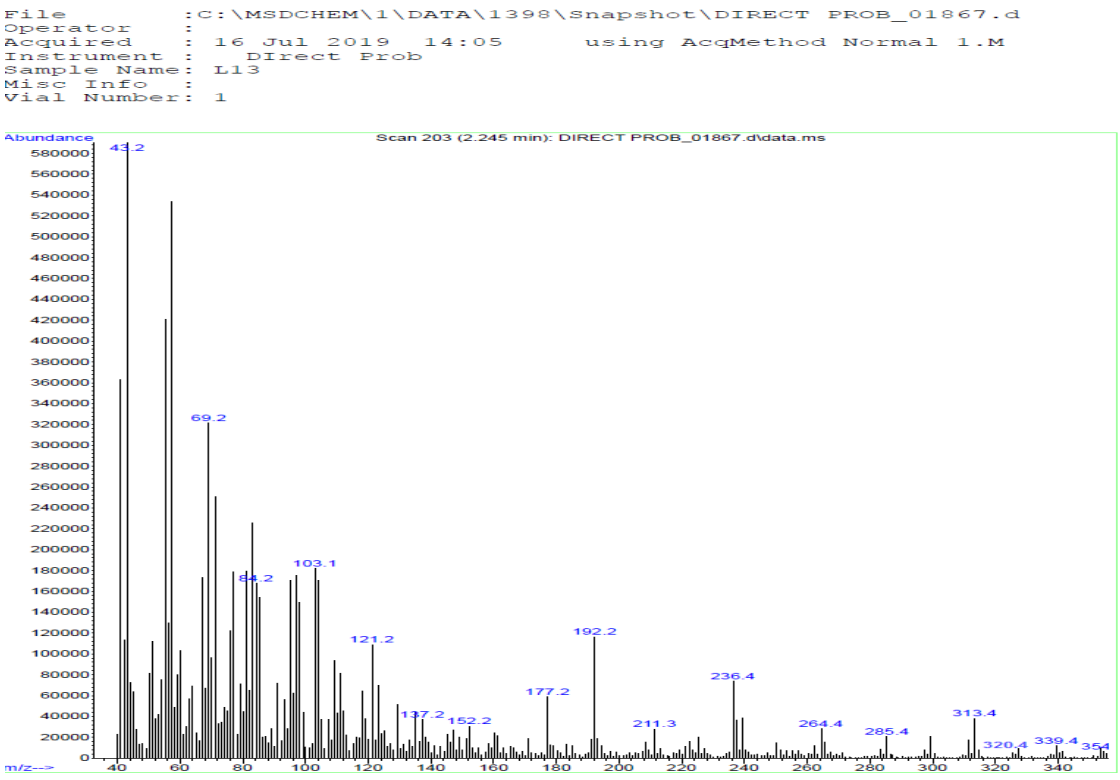


Fig.10: Mass sectrum of [Cu(L)Cl2]

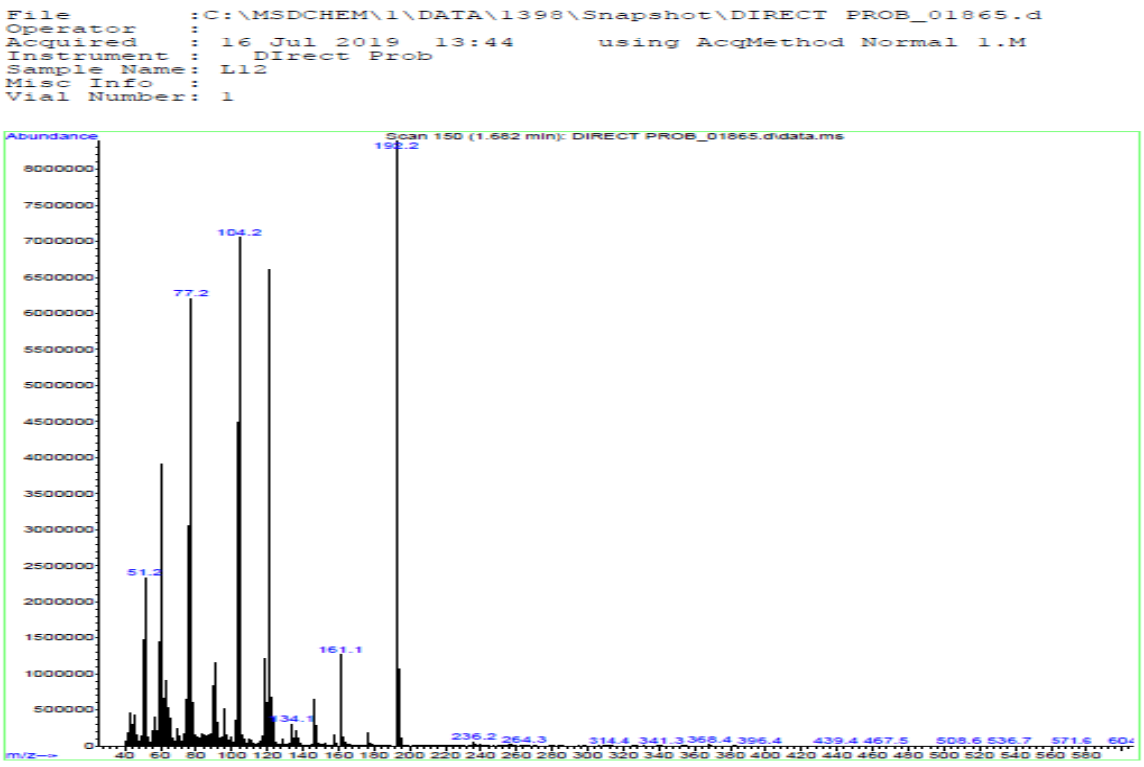


Fig.11: Mass sectrum of [Co(L)2]

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