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RESEARCH ARTICLE

Synthesis, Characterization and Biological Study of the Ligand 2-methyl-5-[(2*E*)-2-(4-Nitrobenzylidene) Hydrazinyl]- 1, 3, 4-Oxadiazole with some of its Transition Metal Complexes

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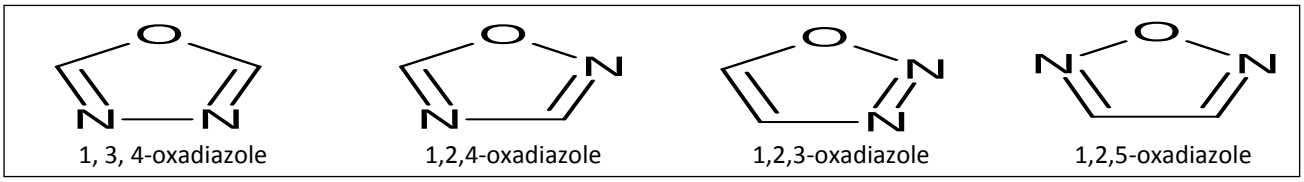
Abstract

A new series of transition metal ions (Cr (III), Co (III), Cu (II) and Ni (II) complexes with the ligand 2-methyl-5-[(2E)-2-(4-nitrobenzylidene) hydrazinyl]-1, 3, 4-oxadiazole were synthesized. A ligand was synthesized by reaction hydrazine hydrate with ethyl acetate in ethanol as solvent , the product reaction with potassium hydroxide and carbon disulphide to give 5-methyl-1,3,4-oxadiazole-2-thaiol, the product was reacted with hydrazine hydrate again to produce 2-hydrazinyl-5-methyl-1,3,4-oxadiazol. The resultant was mixed with parnitro-benzldehyde to produce the ligand (L2). The newly synthesized compounds were characterized by Fourier transform infrared (FTIR) Spectroscopy, Nuclear Magnetic Resonance Spectroscopy (1H NMR), elemental analyses (C, H, N), Mass Spectra data. The program Hyperchem 7.51 have been used for theoretical calculation using PM3 method to study the electrostatic potential, electron density, heat of complexation. It has been shown through all the above diagnostic measures that ligand is formed with the (Cu, Co and Ni) ions tetrahedral complexes and with the Cr formed octahedral complex. The biological study of ligand and its complexes showed that all prepared compounds have antibacterial against three types of bacteria and there was high percentage of hemolysis.

Keyword: *1, 3, 4-Oxadiazole derivative, Antibacterial, Hyperchem, Hemolysis.*

Introduction



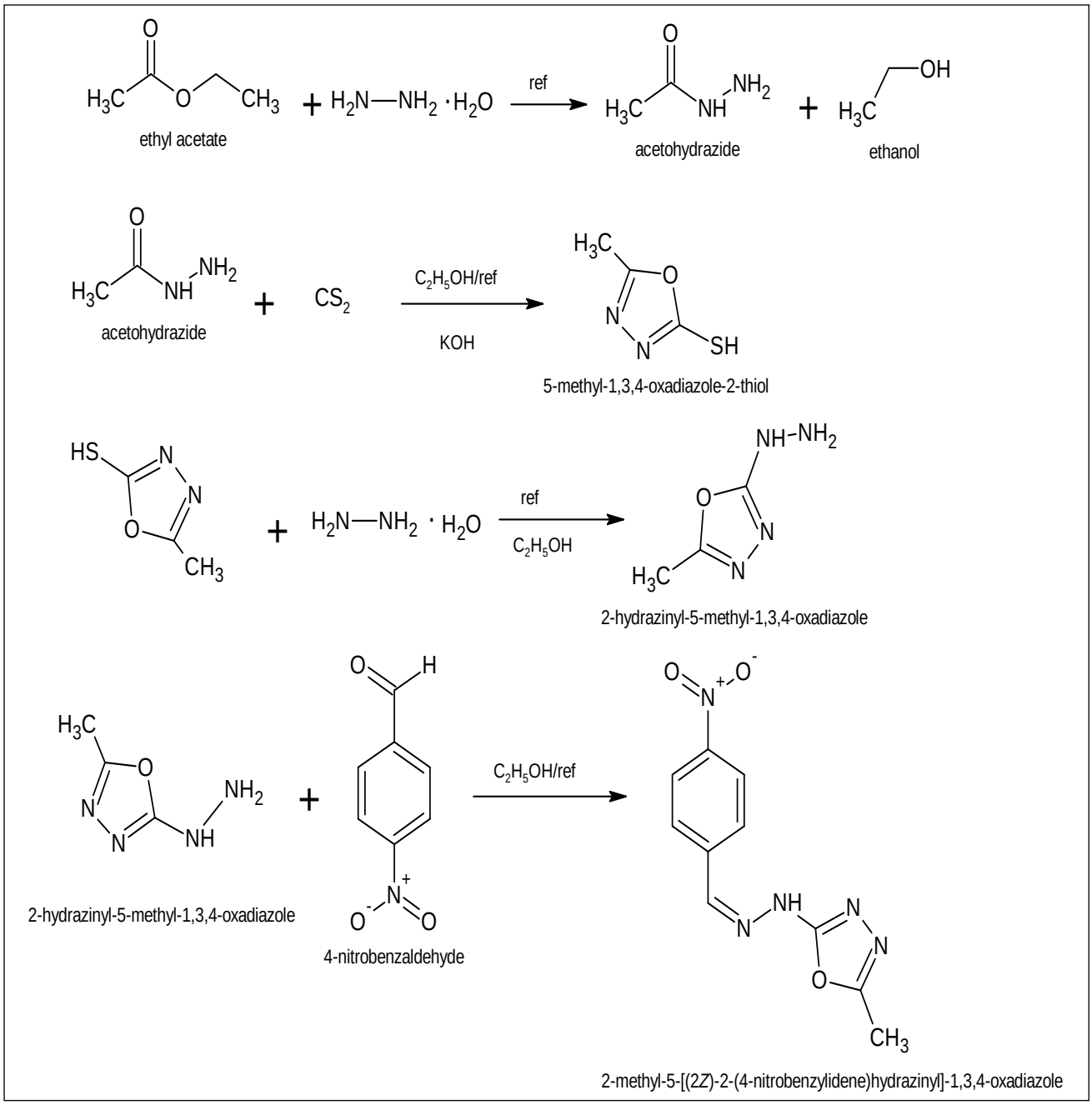
A Cyclic organic compound containing all atoms in ring formation is named a carboxylic compound. If at least one atom other than carbon forms a part of the ring system designated as a hetro cyclic compound. The most common hetro atoms are sulfur, nitrogen and oxygen [1]. 1,3,4-Oxadiazole is a heterocyclic compound containing an oxygen atom and two nitrogen atoms in a five membered ring.It is derived from furan by substitution of two methine groups (=CH) with two pyridine type nitrogen's (-N=) [2, 3]. At room temperature it is liquid in nature and having boiling point at 150°C. 1, 3, 4-oxadiazole have good thermal stability [4]. There are three other known isomers for 1,3,4 oxadiazole, namely; 1,2,4-oxadiazole 1,2,3-oxadiazole and 1,2,5-oxadiazole.

However; 1,3,4-oxadiazole and 1,2,4-oxadiazole are better known, and more widely studied by researchers [5]. The 1,3,4-oxadiazole-derivatives are the most stable among the oxadiazole isomers and attracted considerable attention and became an important construction motif for the development of new drugs.Compounds containing 1,3,4-oxadiazole cores have a broad biological activity spectrum including antibacterial [6], antifungal [7], analgesic [8], anti-inflammatory [9], antiviral [10] anticancer [11], antihypertensive [12] and anticonvulsant [13]. The ability of 1, 3, 4-oxadiazoles to undergo various chemical reactions has made them important for molecule planning because of their privileged structure, which has enormous biological potential [14].

Experimental

Preparation Method of Ligand (L2)

Ethyl acetate (0.1 mol, 8.8 g) and hydrazine hydrate (0.1 mol, 5 g) were mixed gently and heated under reflux for 4 hrs. With 100 ml of absolute ethanol [15].The crude product was filtered and washed with ethanol to give the desired product (C). A mixture of C (0.1mol, 7g) and (0.1 mol, 5.7g) of potassium hydroxide with 100 ml from ethanol was heated until potassium hydroxide dissolved. Then the mixture was cooled at 0 C°, then (0.1 mol, 6.5ml) from carbon disulphide was added. The mixture was heated under reflux for 24 hrs. The resultant mixture was concentrated, and carefully acidified with hydrochloric acid HCl (10%) to give yellow precipitate. Then the crude product was filtered and the crystal was recrystallized from ethanol absolute [16] to give the product (D) as yellow solid, (yield 74%). A mixture D (0.1mol, 11.6 g) (and hydrazine hydrate (0.1 mol, 5g) dissolved in (50 ml) of ethanol and reflux for (19 hrs.). The crude product was concentrated and then cooled [17] Solid product was filtered and recrystallized from ethanol to give the product (E) as yellow solid, yield (80%).Synthesis L2 was started with (0.01 mol, 1.51 g) para naitro-benzldehyd, which was mixed with 15 ml ethanol. After this (0.02 mol, 2.24 g) of compound E was added to this alcoholic solution. Mixture was allowed to reflux for 5 hours. Product was filtered, and recrystallized from ethanol, yield 88% as the Scheme (1).



Scheme 1: steps to prepared 1, 3, 4-oxadiazole derivative (L2)

Preparation Method of Complexes

All complexes were prepared by refluxing the respective hydrated metal chloride (0.001mol) in 15 ml ethanol with 50 ml of an ethanolic solution of ligand (0.001 mol) for 3 hrs. The separated solids were filtered and washed with ethanol and cold water to remove unreacted salts or ligand, then precipitated complexes dried in air [18].

Table 1: Molecular formula and some physical properties of prepared complexes

NO.	Formula	M.Wt	Color	M.P.^{o}C	Yield%
1	L2	247	Light yellow	214-216	88.5
2	[Cr(L_2) Cl_3H_2O]	423	Orange	187-188	75.4
3	[Co(L_2) Cl_2]	376	Olive	193-195	76.3
4	[Cu(L_2) Cl_2]	381.5	Brown	204-205	83.1
5	[Ni(L_2) Cl_2]	376.6	Light green	175-177	88.4

Biological Study

Antibacterial Activity

Antibacerial activity of the prepared ligand and its complexes was studied, three types of bacteria were used, *Staphylococcus aureus*, *Streptococcus pyogenes* and *Enterobacter aerogenes*.

These species have been selected because of their importance in the medical field as they cause multiple types of diseases. The method which used to calculate the inhibitory effect of chemical compounds was Disc Diffusion method [19, 20].

- Prepare Muller Hinton agar plates according to the manufacture instructions.
- Make refreshment for bacterial isolates by incubated in nutrient broth at 37 ^{o}C overnight.
- Different concentrations of the ligand and its complexes were prepared using diluted DMSO as solvent.
- Filter paper discs were prepared and soaked in the solutions of the ligand and its complexes overnight.
- A lawn of bacteria was made by sterile swab on the Muller Hinton agar plates then soaked filter paper discs put on the plates with use two types of control, negative control (filter paper disc soaked in diluted DMSO) and positive control (antibiotic disc). The plates incubated at 37 ^{o}C for 24 hrs.
- Inhibition zone on the plated measured in mm unit.

Hemolysis Assay

The Hemolysis assay was done as described by Henkelman.S. Et al [21].5mL of blood was collected from healthy volunteers (exclusion criteria include the volunteers not have bleeding disorder) in the tubes containing 5.4 mg of EDTA to prevent coagulation and centrifuged at 1000 rpm for 10 min at 4C. Plasma was removed carefully and the white buffy layer was completely removed by aspiration with a pipette with utmost care. The erythrocytes were then washed for additional three times with 1X PBS, pH 7.4 for 5 min. Washed erythrocytes were stored at 4oC and used within 6 h for the hemolysis assay.

50 uL of 10 dilution (100 uL Erythrocytes suspension: 900 uL 1XPBS) of erythrocytes suspension was mixed with 100 uL of test samples (20-80ng/mL), 100 uL of 1XPBS was used as negative control and 100 uL of 1% SDS as positive controls. Reaction mixture was incubated at 370C water bath for 60 min. The volume of reaction mixture was made upto 1 mL by adding 850 uL of 1XPB. Finally it was centrifuged at 300rpm for 3min and the resulting hemoglobin in supernatant was measured at 540 nm by spectrophotometer to determine the concentration of hemoglobin. The percentage hemolysis was calculated as follows

% Hemolysis inhibition= 100-[Sample/ Control] x 100.

Experimental Section

Infrared absorption spectra were recorded on FTIR spectrophotometer on a model (Shimadzu FT-IR Spectrometer) in the range (200-4000) cm^{-1} using KBr and CsI disks. ^{1}H-NMR spectra were recorded at 500 MHZ utilize a model Bruker DRX, TMS was use as standard, DMSO-d^{6} used as solvent. Mass spectra (MS) were registered in the range (0-800) m/e on 5973 network mass selective detector. Elemental C, H, N and S analysis were carried out on analyzer 2400 Perkin Elmer.

Melting points were recorded in open capillary tubes using an electro thermal melting point/SMP31 device.

Result and Discussion

Elemental Analysis

The purity of 2-methyl-5-[(2E)-2-(4-nitrobenzylidene)hydrazinyl]-1,3,4-oxadiazol and its complexes were checked by TLC .Elemental analysis (C, H, N) tabulated in Table (2).The theoretical values were in a good accordance with the experimental values.

Table 2: Elemental analysis (C, H, N)

Compound	Elemental analysis theoretical l			Elemental analysis Experimental l		
	H%	C%	N%	H%	C%	N%
L_2	3.6	48.58	28.34	4.2	49.1	27.4

FTIR Spectra of L2 and its Complexes

The structure of the prepared ligand (L2) and its metal complexes were confirmed by infrared spectroscopy. The characteristic bands are shown in the Table (3).The infrared spectrum of the ligand (L2) show band at (v 3465) cm-1 indicating the absorption for v (NH) [22]. The ligand also shows the presence six major bands at (3070, 2954, 1680, 1581, 1103 and 1030) cm^{-1}which are assigned to (v CH (aromatic), CH (Aliphatic), CH=N (aso), C=N (endocyclic) (C-O) and structure movement band respectively. New bands were found attributed to coordinate (v M-N and M-Cl) these appear at region (509-586) and (239-285) cm^{-1} respectively [23, 24], and (M-O) at (401) cm^{-1} this indicate that the coordination occur through N and O atoms only. Moreover the bands stretching showed shifted to higher and lower value, this indicates the coordination with metal ion as show the Table (3) and The Figures (1-5).

Table 3: FT-IR absorption bands of the prepared L2 and their complexes

NO.	Compoud	υNH cm^{-1}	CH-aliphtic	υCH-aromatic cm^{-1}	υC=N endo cm^{-1}	υCH=N cm^{-1}	υC-Ocm^{-1}	Structural movement cm^{-1}	υM-O cm^{-1}	υM-N cm^{-1}	υM-Cl cm^{-1}
1	**L2**	**3465**	**2954**	**3070**	**1581**	**1680**	**1103)**	**1030**		**-------**	**--------**
2	**[Cr(L2)Cl_3H_2O]**	**3209**	**2971**	**3109**	**1527**	**1604**	**1103**	**1033**	**401**	**586**	**270**
3	**[Co(L2)Cl_2]**	**3400**	**2931**	**3109**	**1589**	**1697**	**1113**	**1030**		**529**	**285**
4	**[Cu(L2)Cl_2]**	**3348**	**2924**	**3070**	**1589**	**1690**	**1103**	**1025**		**555**	**250**
5	**[Ni(L2)Cl_2]**	**3340**	**2931**	**3062**	**1597**	**1689**	**1103**	**1025**		**509**	**239**

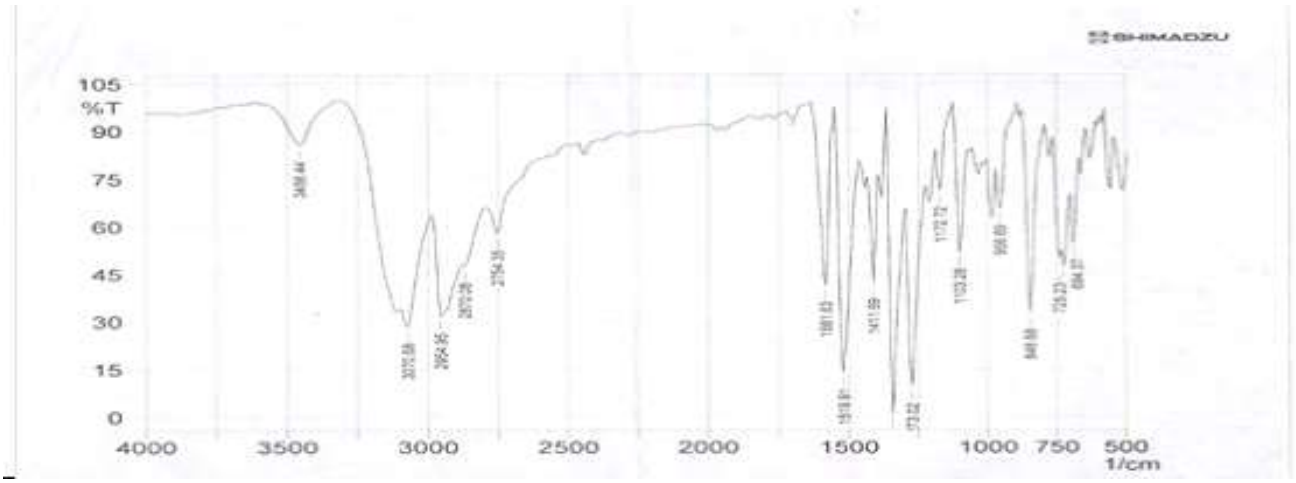


Figure 1: FTIR Spectrum of (L2)

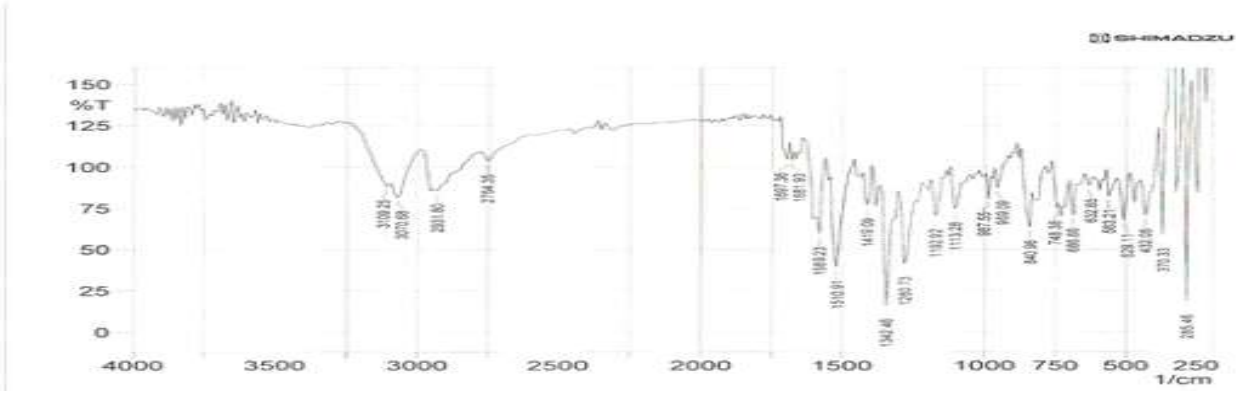


Figure 2: FTIR Spectrum of [Co (L2) Cl_2]

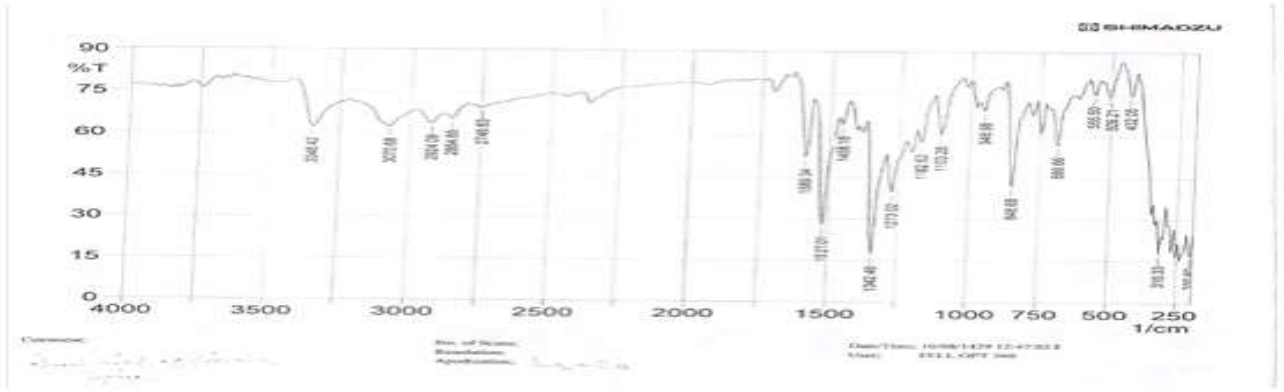


Figure 3: FTIR Spectrum of [Cu (L2) Cl2]

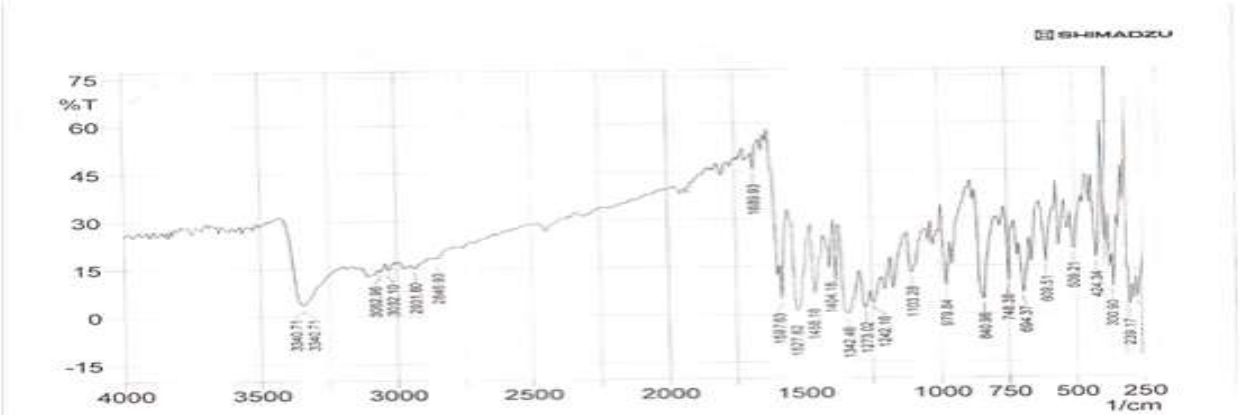


Figure 4: FTIR Spectrum of [Ni (L2) Cl2]

Figure 5: FTIR Spectrum of [Cr (L2) Cl_3H_2O]

Nuclear Magnetic Resonance Spectra Study of L2 (^{1}H-NMR)

1H NMR spectrum of ligand shows the signals (ppm) at 2.5(s, 3H, CH_3), 8.2-8.4 (4H, Ar-H), 10.4 (s, 1H, N=CH) [25], 11.9 (s, 1H, NH) [26], Figure (6).

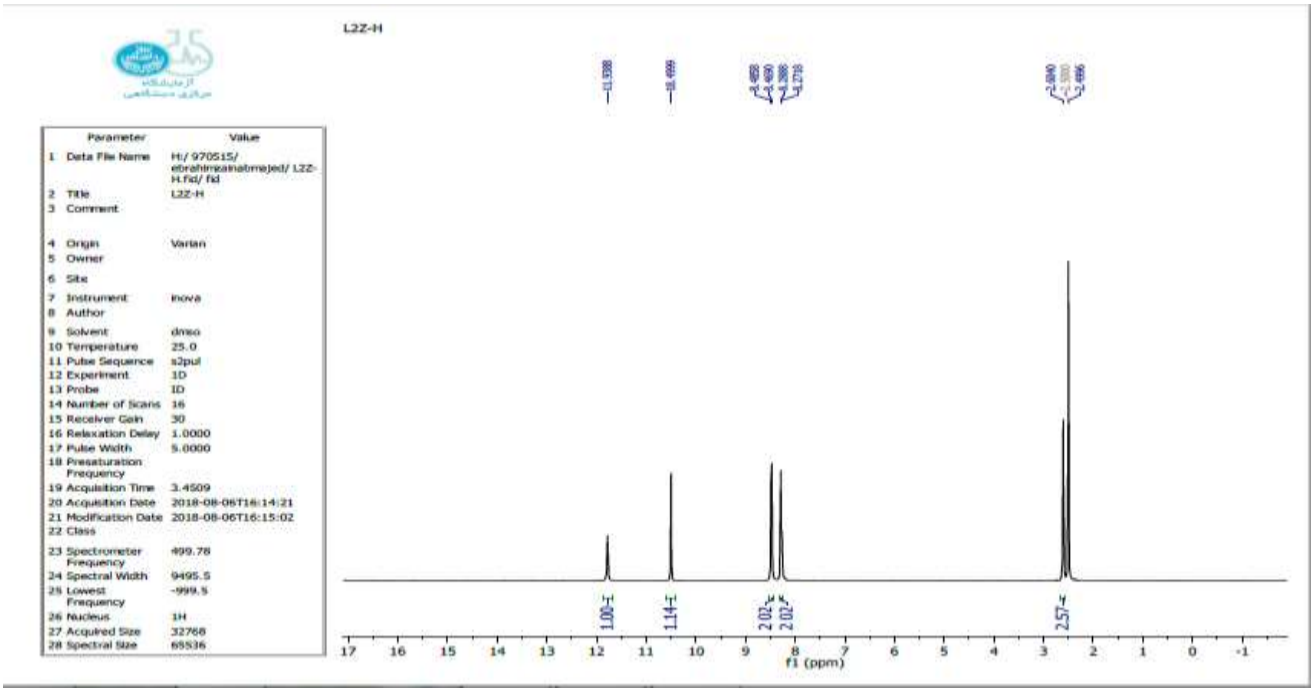


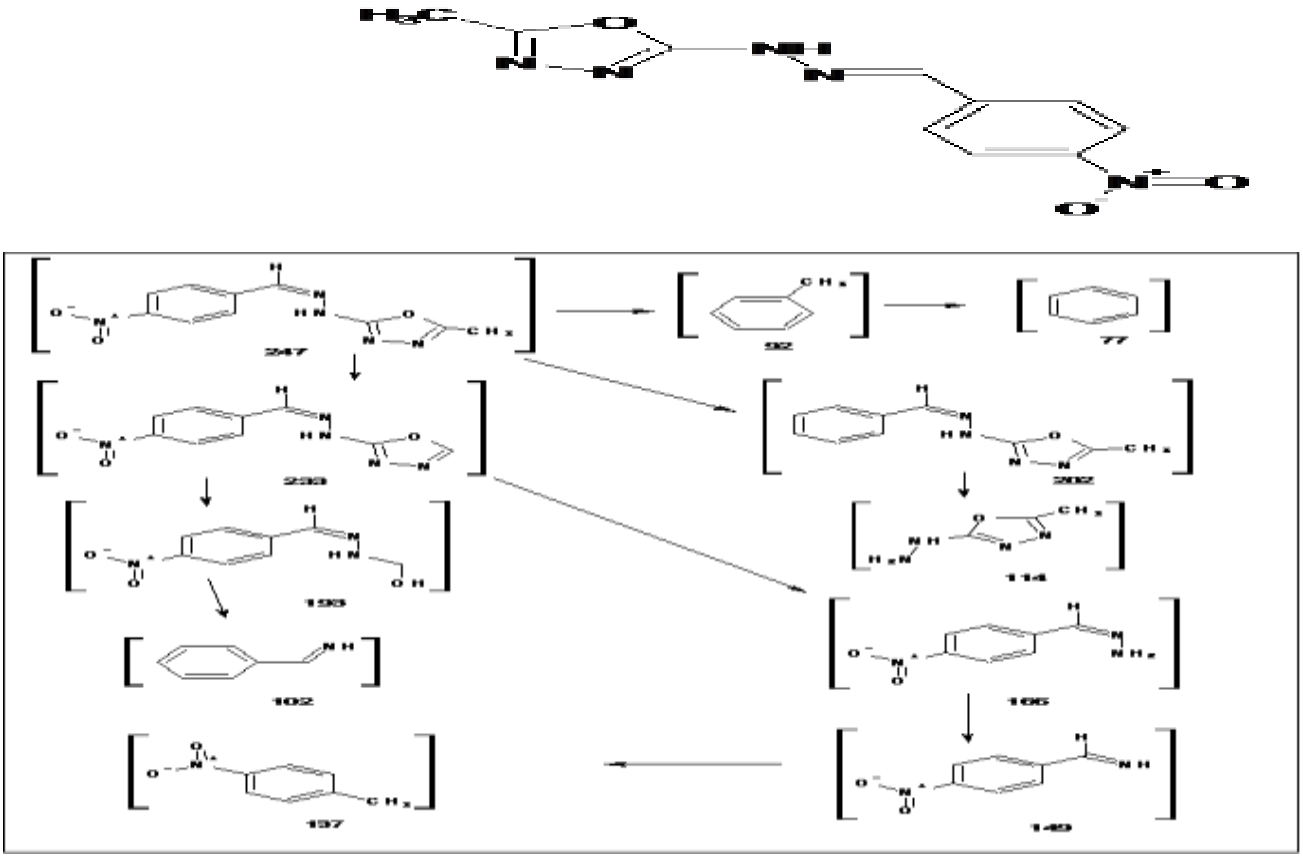
Figure 6: ^{1}HNMR Spectrum of L2

Mass Spectrum of L2

Mass spectrum of ligand shows the molecular ion peak at 247 m/z, base peak at 115m/z and other peaks list in Table (4) which confirms the structural formula of L2As in the following Scheme (2) Figure (7).

Table 4: Proposed nuts for ligand (L2)

Molecular	m/z	Molecular	m/z
$[C_{10}H_9N_5O_3]^{+\cdot}$	247	$[C_7H_7NO_2]^{+\cdot}$	137
$[C_9H_6N_5O_3]^{+\cdot}$	233	$[C_3H_6N_4O]^{+\cdot}$	115
$[C_{10}H_9N_4O]^{+\cdot}$	205	$[C_7H_6N]^{+\cdot}$	102
$[C_8H_7N_3O_3]^{+\cdot}$	193	$[C_7H_6]^{+\cdot}$	89
$[C_7H_6N_3O_2]^{+\cdot}$	165	$[C_6H_6]^{+\cdot}$	77
$[C_7H_5N_2O_2]^{+\cdot}$	149		



Scheme 2: Proposed fragments of L2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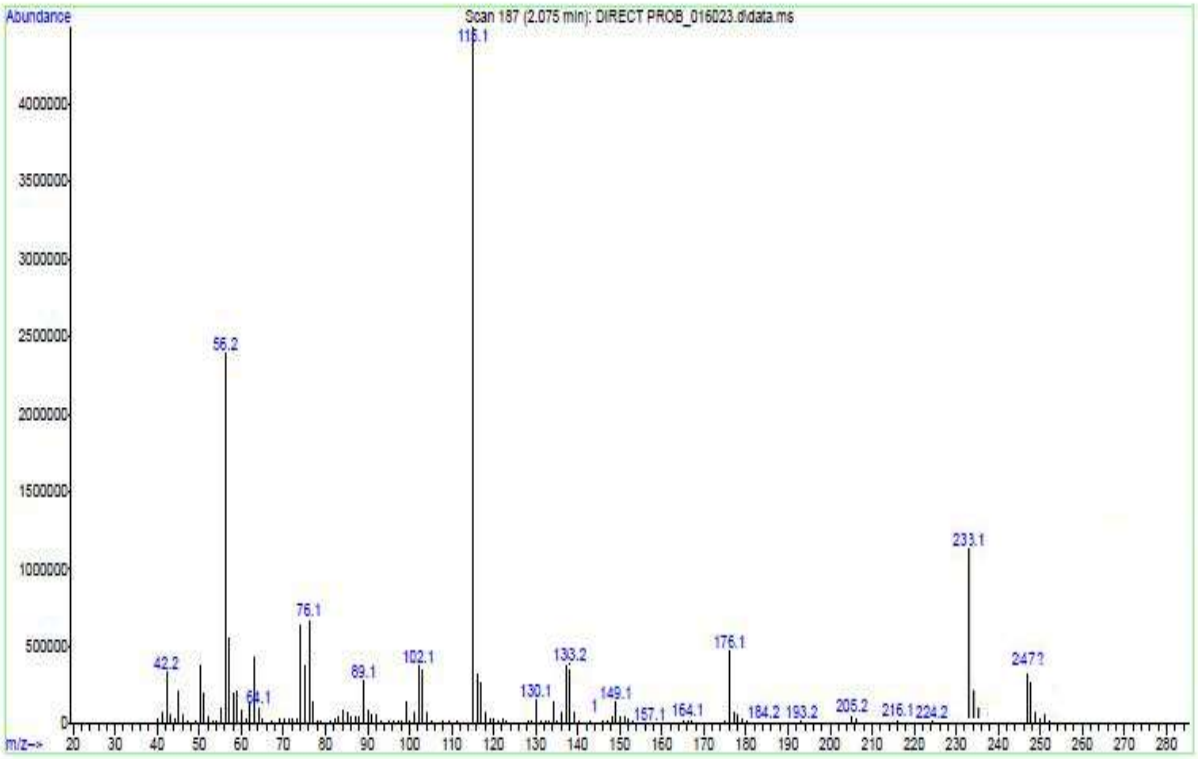


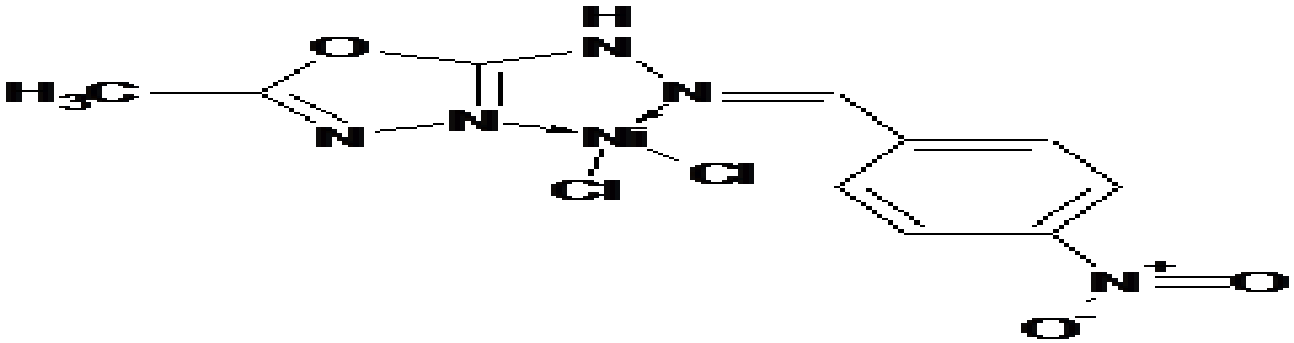
Figure 7: Mass spectra of L2

Mass Spectrum of Complex [Ni(L2)Cl$_2$]

Mass spectrum of complex appearance of the molecular ion peak at 376 m/z , the spectrum showed other peaks (Figure 8) list in Table (5).

Table 5: Proposed nuts for [Ni(L2)Cl2]$^{+\cdot}$

NO.	Molecular	m/z
1	[Ni(L2)Cl$_2$]$^{+\cdot}$	376.4
2	[Ni(L2)Cl]$^{+\cdot}$	341.3
3	[Ni(L2)]$^{+\cdot}$	305.2
4	[C$_{10}$H$_9$N$_5$O$_3$]$^{+\cdot}$	247.2

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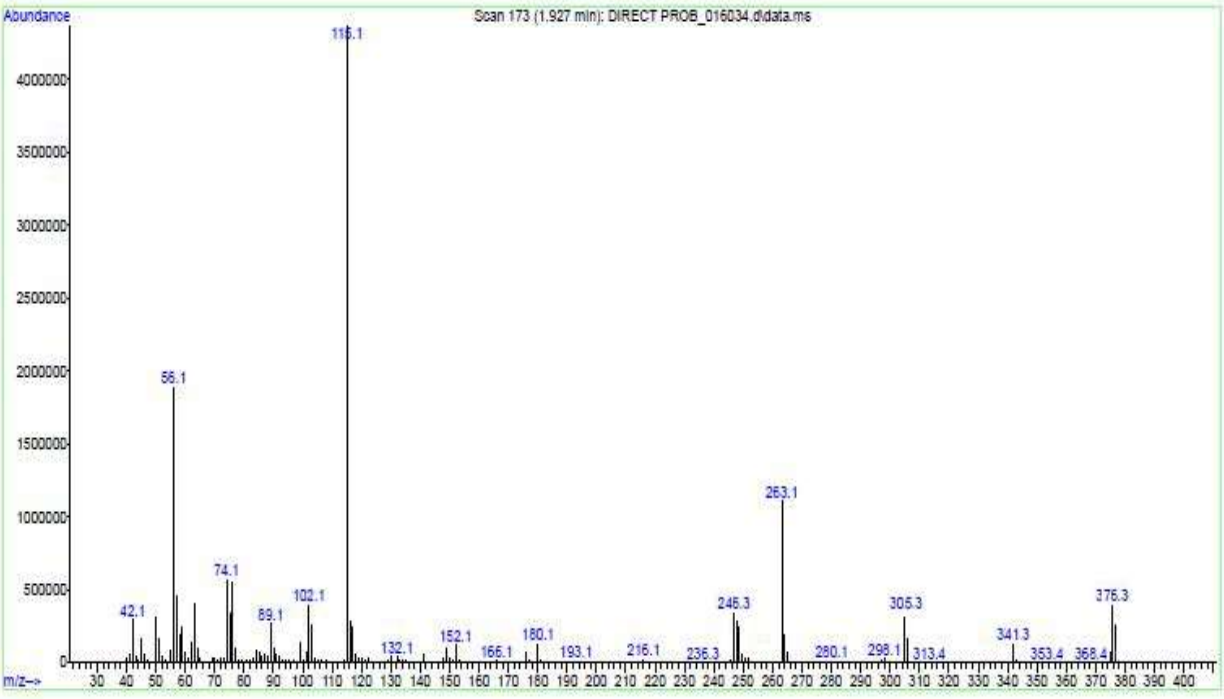


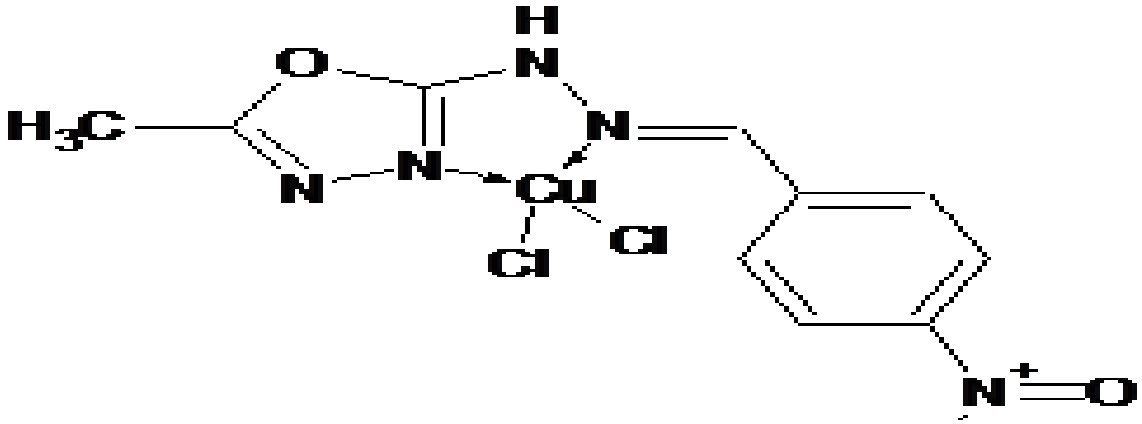
Figure 8: Mass spectra of [Ni (L2) Cl_2]

Mass Spectrum of Complex [Cu (L2) Cl_2]

Mass spectrum of complex appearance of the molecular ion peak at 381.5m/z, the spectrum showed other peaks (Figure 9) list in Table (6).

Table 6: proposed nuts for [Cu (L2) Cl_2] $^{+\cdot}$

NO.	Molecular	m/z
1	$[Cu(L2)Cl_2]^{+\cdot}$	381.5
2	$[Cu(L2)Cl]^{+\cdot}$	346.4
3	$[Cu(L2)]^{+\cdot}$	310.5
4	$[C_{10}H_9N_5O_3]^{+\cdot}$	247

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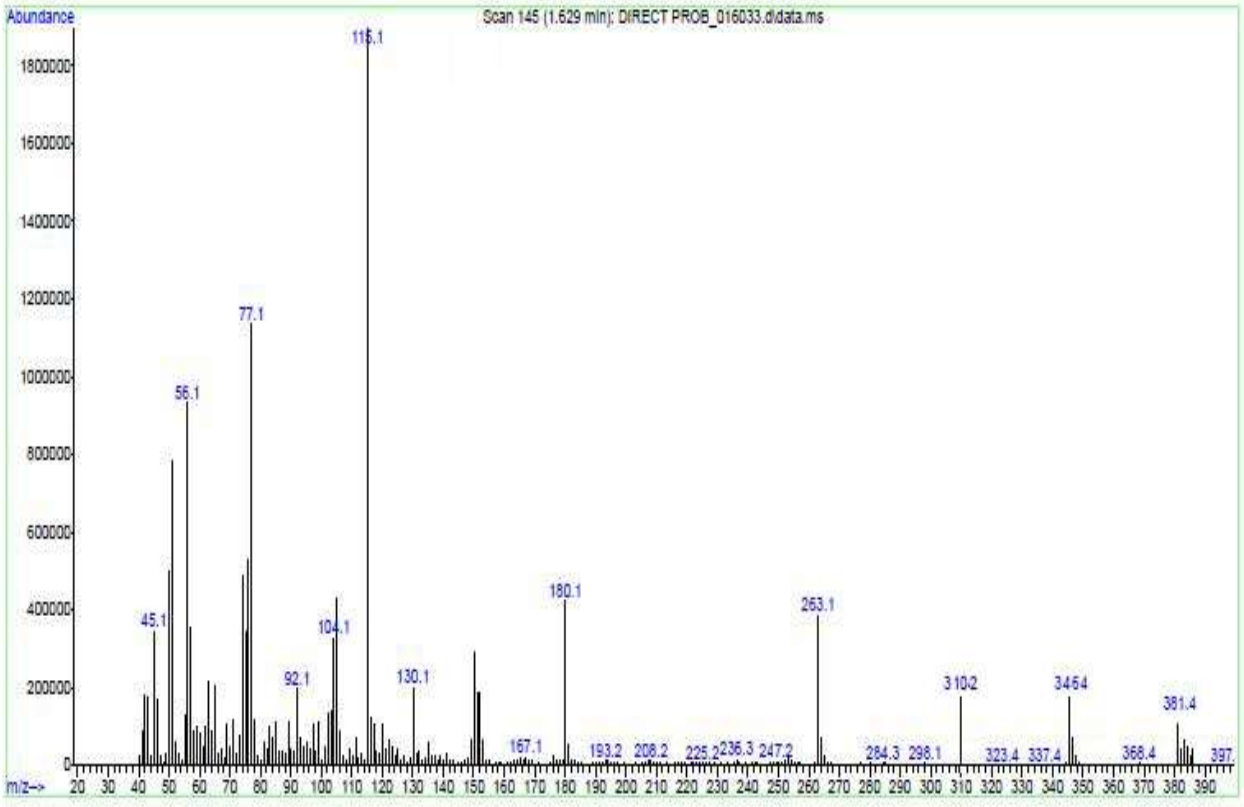


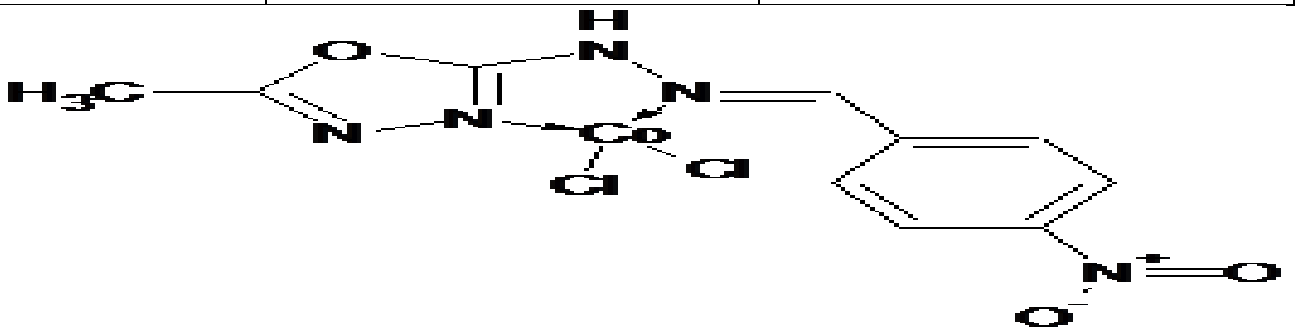
Figure 9: Mass spectra of [Cu(L2) Cl_2]

Mass Spectrum of Complex [Co(L2)Cl_2]

Mass spectrum of complex appearance of the molecular ion peak at 376.5 m/z, the spectrum showed other peaks (Figure 10) list in Table (7).

Table 7: Proposed nuts for [Co (L2) Cl_2] $^{+\cdot}$

NO.	Molecular	m/z
1	$[Co(L2)Cl_2]^{+\cdot}$	376.5
2	$[Co(L2)Cl]^{+\cdot}$	341.2
3	$[Co(L2)]^{+\cdot}$	306.2
4	$[C_{10}H_9N_5O_3]^{+\cdot}$	247.3

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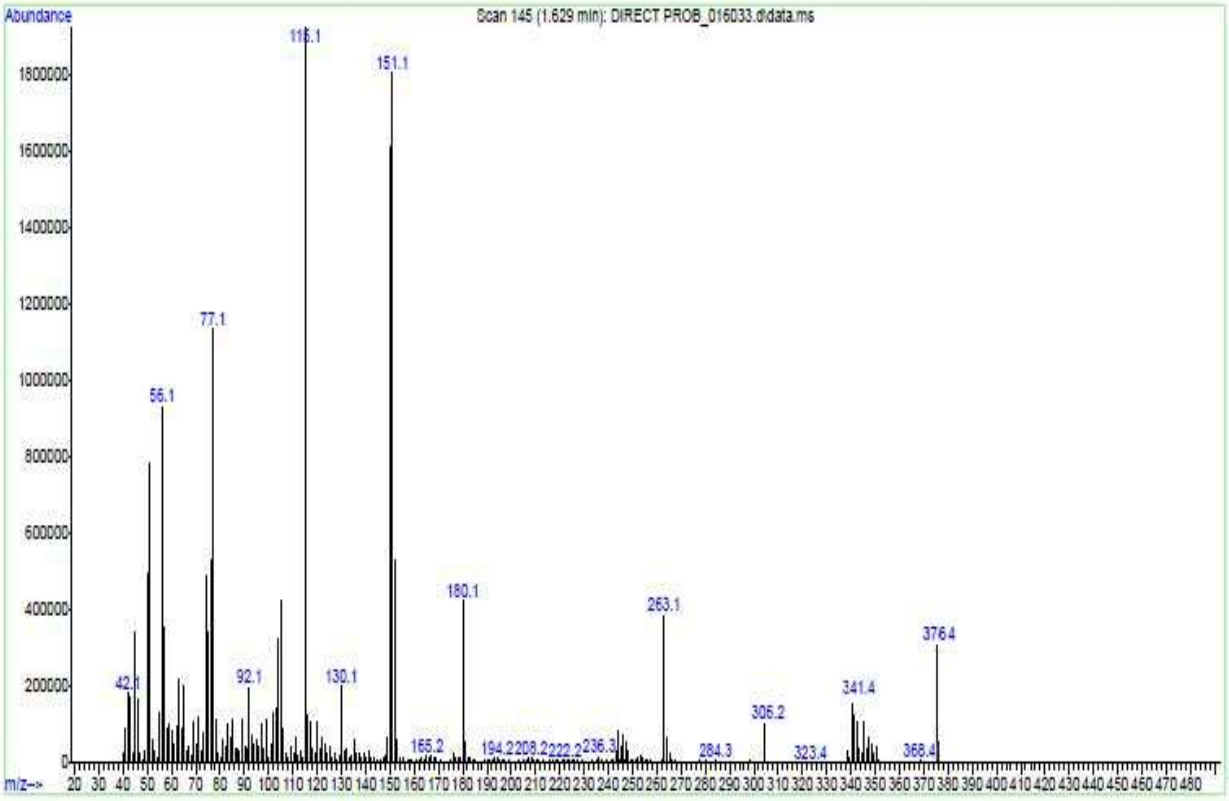


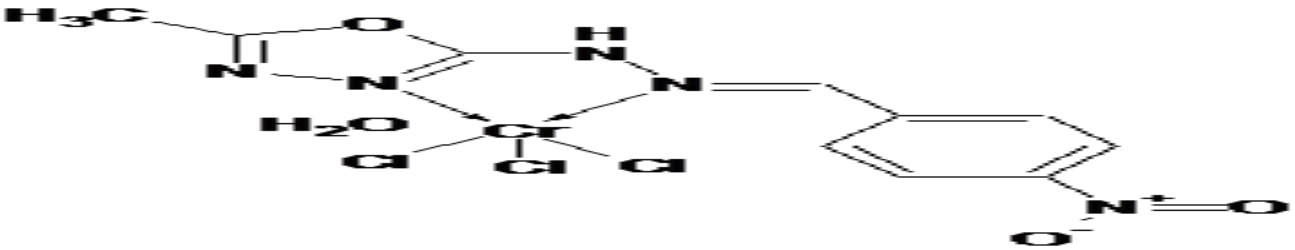
Figure 10: Mass spectra of [Co (L2) Cl_2]

Mass Spectrum of Complex [Cr (L2) Cl_3H_2O]

Mass spectrum of complex appearance of the molecular ion peak at 423.4 m/z, the spectrum showed other peaks (Figure 11) list in table.

Table 8 proposed nuts for [Cr (L2) Cl_3H_2O] $^{+\cdot}$

NO.	Molecular	m/z
1	$[Cr(L2)Cl_3H_2O]^{+\cdot}$	423.3
2	$[Cr(L2)Cl_3]^{+\cdot}$	405.4
3	$[Cr(L2)Cl_2]^{+\cdot}$	369.9
4	$[Cr(L1)Cl]^{+\cdot}$	334.4
5	$[Cr(L1)]^{+\cdot}$	298.1
6	$[C_{10}H_9N_5O_3]^{+\cdot}$	247

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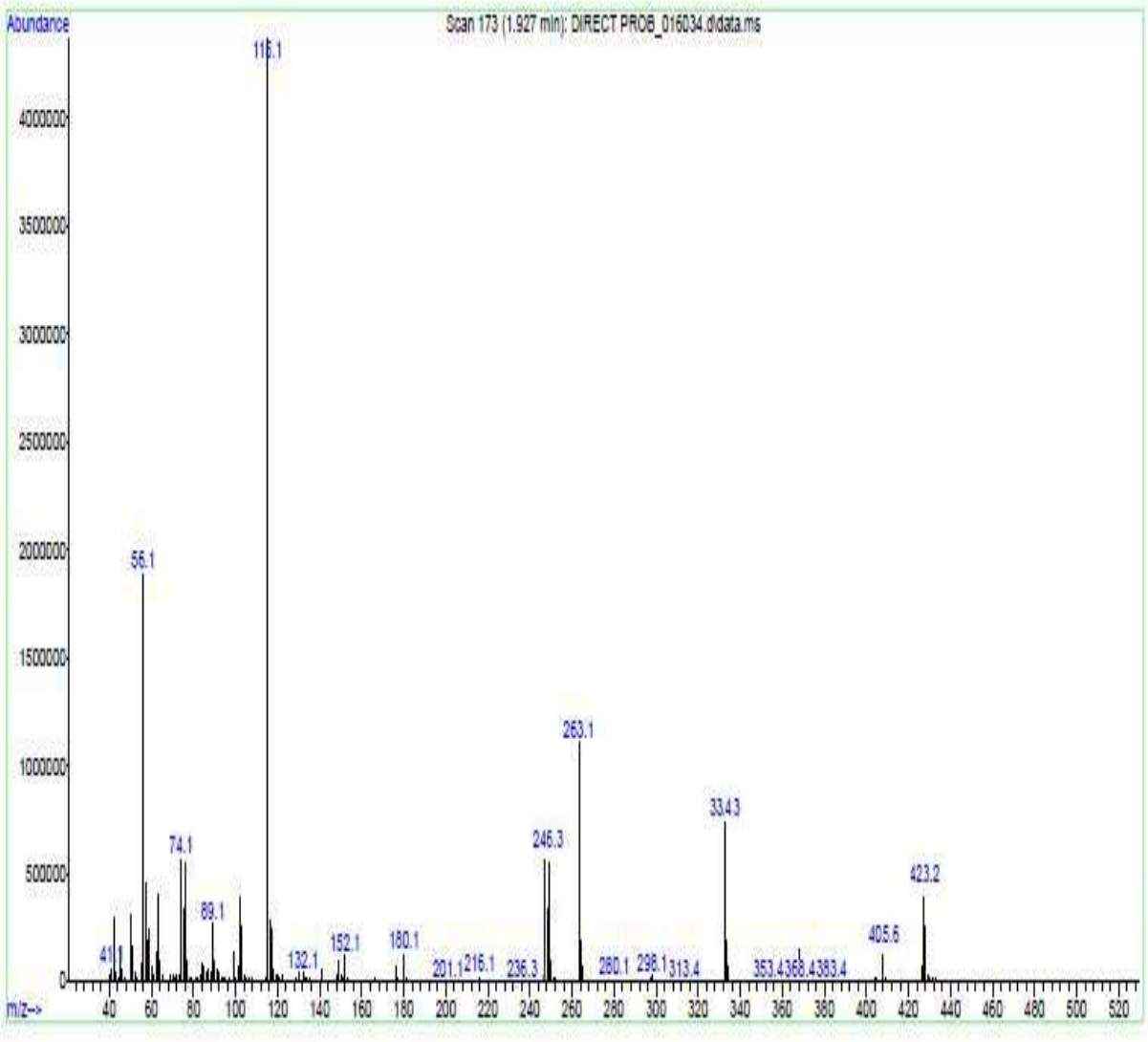

Figure 11: Mass spectra of [Cr (L2) Cl_3 H_2O]

Theoretical Study of L2

The free ligands (L1) and its complexes with transition metal ions (Cr^{+3}, Cu^{+2}, Co^{+2}, Ni^{+2}) have been studied theoretically to predict the most stable structure among others. The program Hyperchem 7.51 have been used for theoretical calculation using PM3 method to study the electrostatic potential, electron density, heat of formation, heat of complexation, binding energy, symmetry and other geometrical parameters as shown the Figure (12).

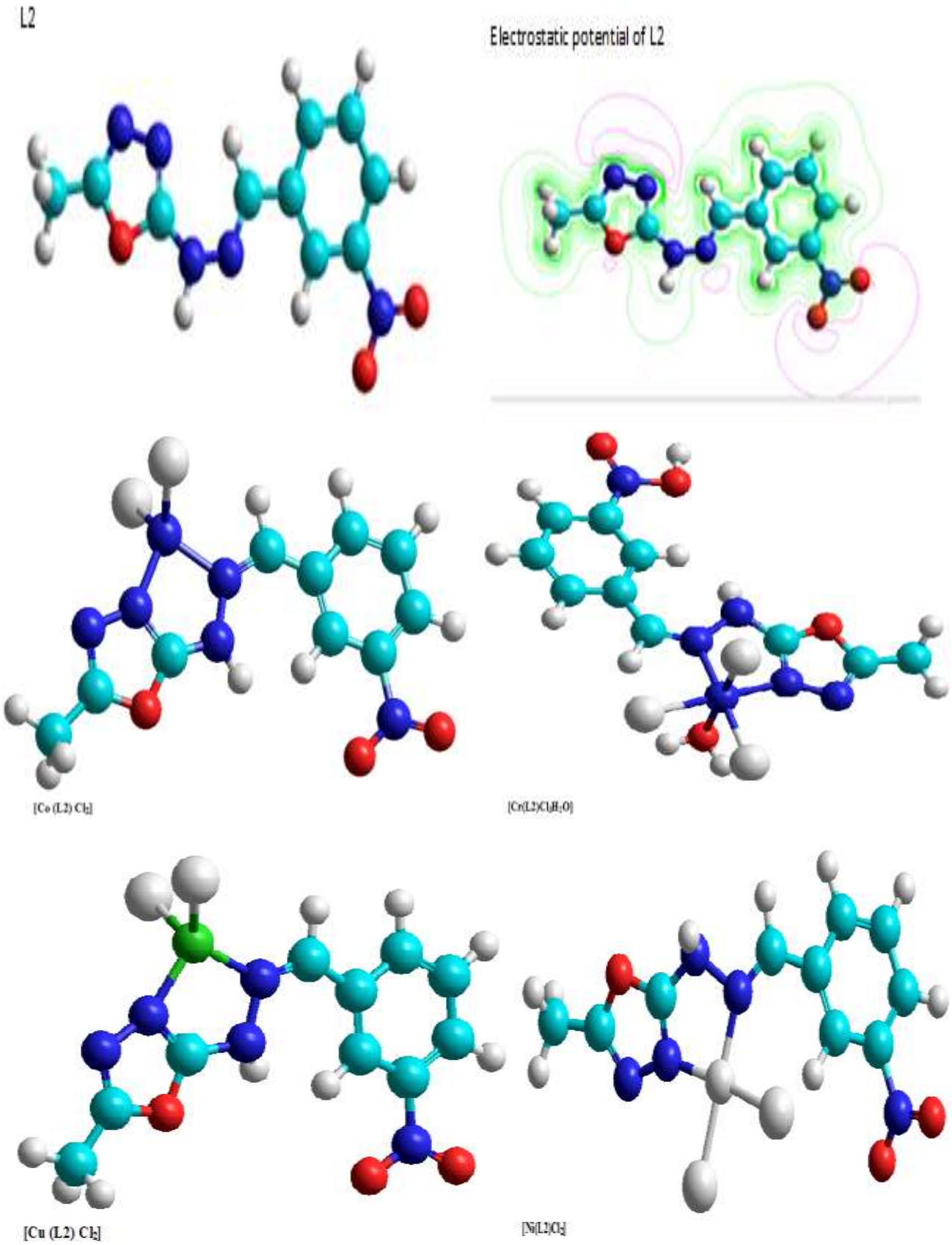

Figure 12: L1 and electrostatic potential

Biological Study

Antibacterial Effect

The L2 showed high inhibition zone (16 mm) at concentration 1.2 mg/ml against *Enterobacter aerogenes* and showed 11 mm and 8 mm against *Streptococcus pyogenes* and *Staphylococcus aureus* respectively. The complexes of the ligand also showed highest inhibition zone at concentration 1.2 mg/ml with variable effect of bacterial isolates, for

example [Ni (L2) Cl_2] and [Co (L2) Cl_2] express 12 and 17 mm inhibition zone against *Staphylococcus aureus* respectively, [Cu (L2) Cl_2] showed highest inhibition zone 12 mm against *Enterobacter aerogenes* while [Cr (L2) Cl_3 H_2O] showed same inhibition zone 11 mm against both *Enterobacter aerogenes* and *Staphylococcus aureus* Figure (13). Table (9) showed in details the effect of the ligand and its complexes with different concentrations against three species of bacteria.

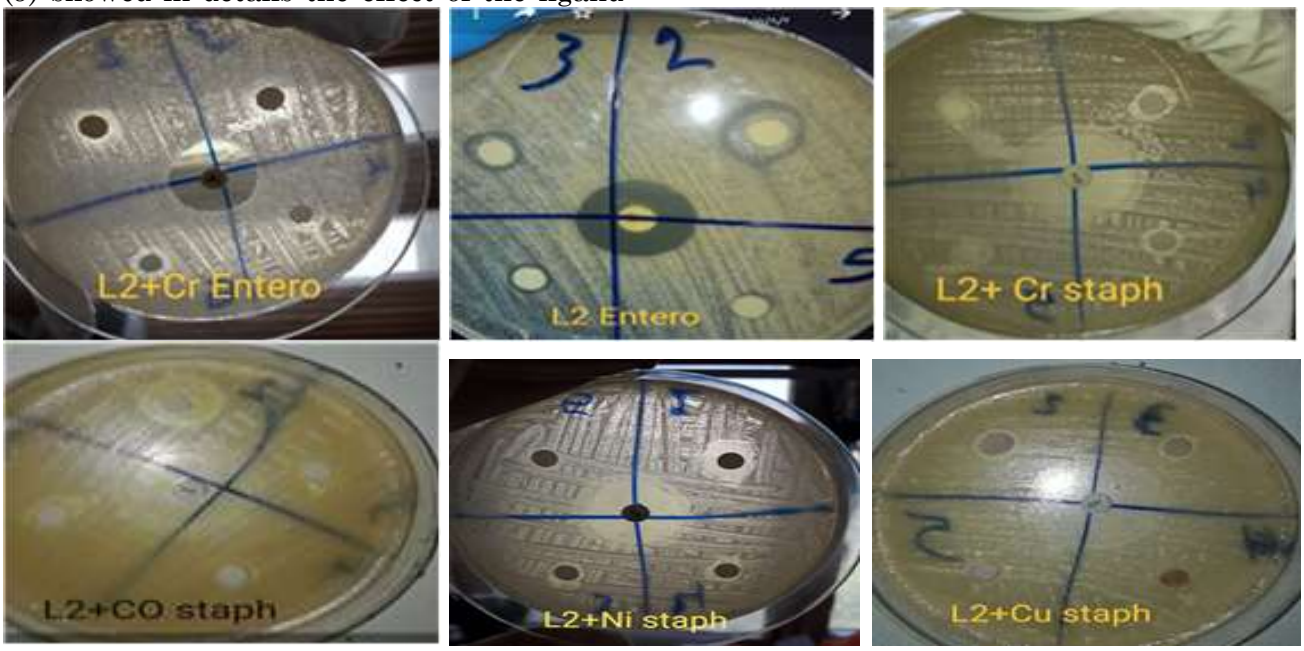

Figure 13: Effect of Ligand and its complexes against three species of bacteria

Table 9: The effect of the ligand and its complexes with different concentrations against three species of bacteria

Compound	Concentration mg/ml	Inhibition Zone (mm)		
		Streptococcus pyogenes	***Staphylococcus aureus***	***Enterobacter aerogenes***
L2	1.2	11	8	16
L2	0.62	10	8	11
L2	0.31	10	No inhibition	10
L2	0.15	10	No inhibition	10
Antibiotic		30	28	20
[Ni(L2)Cl_2]	1.2	8	12	9
[Ni(L2)Cl_2]	0.62	8	10	9
[Ni(L2)Cl_2]	0.31	8	10	9
[Ni(L2)Cl_2]	0.15	8	No inhibition	9
Antibiotic		28	25	15
[Co(L2)Cl_2]	1.2	8	17	8
[Co(L2)Cl_2]	0.62	8	11	8
[Co(L2)Cl_2]	0.31	8	9	No inhibition
[Co(L2)Cl_2]	0.15	7	8	No inhibition
Antibiotic		28	20	20
[Cu(L2)Cl_2]	1.2	10	8	12
[Cu(L2)Cl_2]	0.62	8	8	No inhibition
[Cu(L2)Cl_2]	0.31	No inhibition	8	No inhibition
[Cu(L2)Cl_2]	0.15	No inhibition	No inhibition	No inhibition
Antibiotic		27	15	18
[Cr(L2)Cl_3H_2O]	1.2	8	11	11
[Cr(L2)Cl_3H_2O]	0.62	8	8	10
[Cr(L2)Cl_3H_2O]	0.31	No inhibition	8	10

$[Cr(L2)Cl_3H_2O]$	0.15	No inhibition	7	10
Antibiotic		23	24	20

Hemolysis

The results showed there was high percentage of hemolysis ranged between 55% to 81 % regardless of concentration of the compounds (Table 10) and this indicate to the strong effect of these compounds on human RBC leading to destruction of these RBC. These results need to more studies on the molecular level to investigate the level of effect of these compounds on the cell membrane of RBC and trying to find solution in the chemical design of these compounds to treat this issue of hemolysis. On the other hand, this compound could be used as antibacterial agents especially for these bacteria that known as multidrug resistant even with the presence of hemolysis because the issue of hemolysis could be override by blood transfusion.

Table 10: hemolysis percentages of ligand (L2) and its complexes

Compound	mg/ml Concentration	Hemolysis%
L2	0.2	68.69
L2	0.1	61.73
L2	0.05	55.90
$[Ni(L2)Cl_2]$	0.2	81.57
$[Ni(L2)Cl_2]$	0.1	74.67
$[Ni(L2)Cl_2]$	0.05	72.74
$[Co(L2)Cl_2]$	0.2	68.11
$[Co(L2)Cl_2]$	0.1	58.38
$[Cu(L2)Cl_2]$	0.1	67.90
$[Cu(L2)Cl_2]$	0.05	60.45
$[Cu(L2)Cl_2]$	0.025	58.17
$[Cr(L2)Cl_3H_2O]$	0.2	63.97
$[Cr(L2)Cl_3H_2O]$	0.1	62.93
$[Cr(L2)Cl_3H_2O]$	0.025	57.97

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