Article

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New azo-azomethine ligand derived from Imidazole with divalent metal complexes , synthesis ,spectroscopic and biological activity studies

Zahraa Rasheed Hassani [1] , Pro. Dr.Mithaq Saeed Mohammed [2]

[1]Ph.D-Student , Department of Chemistry, College of Education for Girls, University of Kufa, Iraq.

[2]Department of Chemistry, College of Education for Girls, University of Kufa, Iraq

Abstract

The study involved the preparation of a heterocyclic aromatic Azo-Azomethine compound in a base medium . The compound was identified by partial elemental analysis, mass spectrometry, infrared spectroscopy, ultraviolet-visible spectroscopy, NMR spectroscopy and many other properties. Physical properties. In addition ,it can tained the preparation of series of octahedral coordination complexes for the ions of manganese(II), iron(II), cobalt(II), nickel(II), copper(II), zinc(II), cadmium(II) and mercury(II) a tetrahedral complex for the single positively charged silver ion and a flat square complex for the triple gold ion. The prepared new compounds proved their vital activity as antioxidant , anti-cancer compounds, and compounds with high color stability.

***Keywords** : Azo ,* Azomethene*; anticancer; antioxidant; complexes.*

1. Introduction

Geuerlly, organic compounds have two active groups in their chemical structure, the bridging Azo group (-N=N) and the azomethene group (C=N-) , which are modern compounds [1] compared to Azo compounds or Schiff bases[2] , and important in coordination chemistry[3] where they are stable complexes with transition metal ions . The nitrogen atom in both active groups has a non-acyclic electron double that imparts a lot of chemical and physical properties to their compounds .The coordination between the ligand and the metal ion is obtained by the nitrogen atom of the azomethine group and an active donor atom adjacent to the azomethine group in the Ortho position forming stable ligand rings. Or by a nitrogen atom of the bridging Azo group and an effective donor atom adjacent to the azo group that has the ability to share an electronic charge or an electronic double of the metal ion [4]. Because they

contain the Azo and azomethine groups, these compounds have different chemical and physical properties and the possibility of entering into harmony with different metal ions . These organic compounds have taken a wide place in many applications in many fields, including industrial, life and analytical, so research and studies have increased in the study and preparation of azo - azomethine compounds [5]. In the biological field, Azo-azomethine complexes have been used as anticancer agents due to their covalent bonds with nitrogen atoms present in DNA and the formation of canine rings. In the field of spectroscopy, the characteristic colors of ligands and their complexes in aqueous or organic solutions have been exploited, as spectral reagents [6]. Schiff bases are considered the raw material for the preparation of a large number of heterocyclic compounds , and their coordination complexes, as well as used in the preparation of high molecular weight polymers [7] . The number of a various studies have shown that Schiff bases have many distinctive biological activities, including antibacterial, fungal and viral[8]. In addition to its effectiveness in inhibiting various cancerous tumors and its importance in the fields of Medicine and pharmacy . It has been shown that this biological effectiveness shown on Schiff bases is due to the azomethine bond[9] .

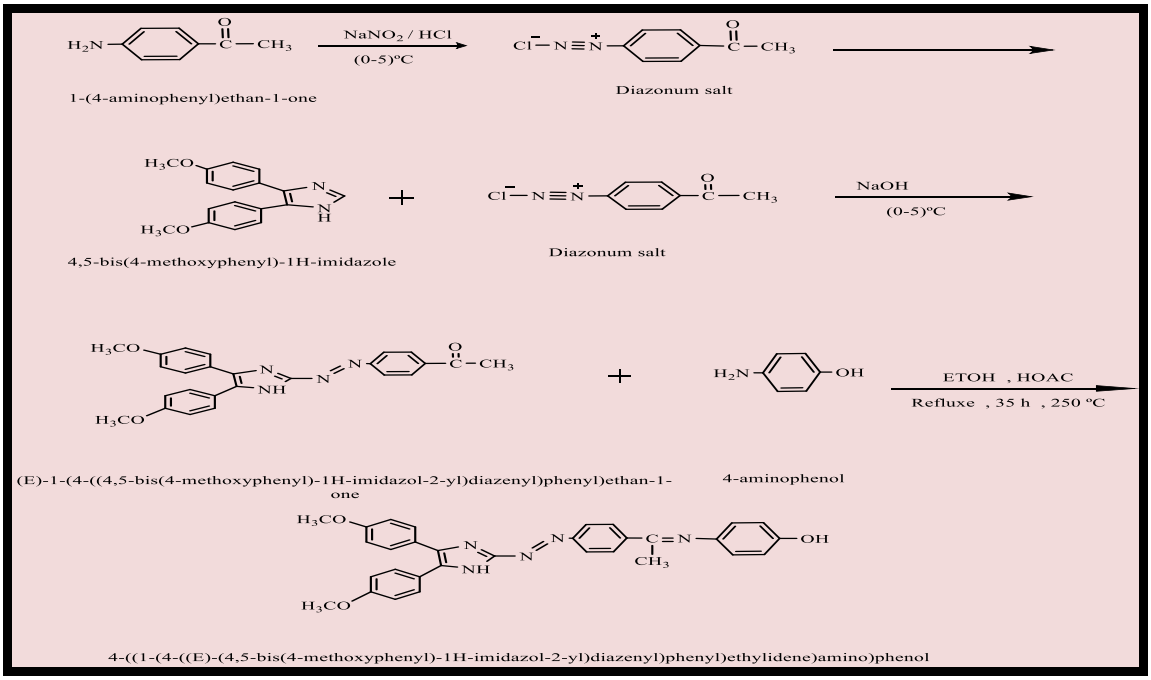
2. Experimental

2.1 Materials and Methods

All reagents and solvents used were of analytical grade from the companies BDH and FLUKA and used without further purification. Melting point temperature was using (Stuart melting point SMP10) melting point apparatus. Molar-conductance measurement was using (Digital Conductivity Series Ino.Lab.720). Magnetic Susceptibility Measurement was conducted using Balance Magnetic Susceptibility model(M.S.B Auto) . Infrared spectral analyses were recorded using (Shimadzu FTIR 8400S Spectrophotometer) in the range of 400-4000cm-1. Elemental analysis(C.H.N) was carried out using Elemental Analysis System(GMBH). For all compounds,UV-Visible Spectra were recorded on the (Shiadzu 240-UV-Vis spectrophotometer).The ^{1}H-NMR Spectra Measurement on a (BRUKER 500MHz) using DMSO as solvent .Mass Spectra Measurement were recorded on (GC-MS QP-2010) (Shimadzu Instruments , Japan).

2.2 Preparation of Azo-Azo methine ligand

The new Azo-Azo methine ligand prepared using the traditional method (diazonium salt) with appropriate amount of (amidazole derivative) in alkaline-solution [10]. By dissolve (1.3 gm,0.01 mol) of 4-amino acetophenone in(50ml)distilled water with (3ml) of concentrated HCl acid with continuous shaking .To this mixture a solution of (0.7gm, 0.01mol) of sodeum nitrat in(10ml) to complate the Azotization process, at (0-5)ºC for (30min) .This solution was added to (2.8gm ,0.01mol) of the iamidazole derivative dissolved in (100 ml) of ethanol and (50 ml) of (0.1M) sodium hydroxide at (0-5)ºC.The mixture has staraed to night after that it washed with water, recrystallized and dried in oven at 44ºC for 2 hours. In absolut alcohole (50ml) dissolved (2.1gm ,0.01mol) from azo-azo methine dye and mixed with (0.545gm) para-aminophenol dissolve in absolut alcohol . From glacial acetic acid added three drop and for(35hrs) stirring the mixture in refluxed after the volume of the mixture was reduced to half by eveporation and precipiteted product was recrystellized and dried[11] .



Schme (1):synthesis of Azo-Azo methine ligand

2.3Metal complex synthesis

The new azo-azo methine ligand in an acetone soluteon [0.49 gm,0.0005M] is added to each of the water solution of the salt for select metals [Mn(II) ,Fe(II),Co (II) ,Ni(II) ,Cu(II) ,Zn(II) ,Cd(II) , Hg(II) ,and Ag(I)] (0.0005M) (0.031,0.084 , 0.059, 0.059, 0.042, 0.034, 0.045, 0.067 and 0.437) gm respctively in term of mole ratio [1:2] [M:L] and (0.099gm)from Au (III)and the mole ratio [1: 1][M:L] . The combinetion then was reafluxed for three hours, and the results were analyzed using a TLC tachnique. The colored pracipitate is filtared and rins with ethenol many times then allowed to dry[12]

2.4 Anti-oxidant Activity

solutaon of DPPH with concantration 0.1 μg/ mL prepared used ethanol as solvant. From the solution above, 3 ml was withdrewn and add to 1ml of azo-azo methine solution in ethanol at many concantrations . The mixtur was keept in darkroom. After 30min, the absorbence at 517 nm ageinst blank sample lacking scavanger was measeured. The absorbence of the clear result solution was measured at 517 nm using ethanol as blank. Ascorbic acid (A.A) was used as positive controls[13] . The inhibition percentage calculated by:

% Inhibition= [(A Control - A Sample) /A Control] × 100

A Control: absorbence of DPPH and A Sampl: absorbance of DPPH in sample.

2.5Anti-cancer effectiveness

The cytotoxic activity of **[Au ($C_{31}H_{27}N_5O_3$) Cl_2] Cl** ageinst liver cancer cell line (HEPG2) and normal cell line (HFF). MTT-assay was used the ligand after incubation for 24 at 37 ºC with concentrations of (0.5, 1,5, 10, 50 and 100) μg/ mL. The extant of the toxice impact calculats by comparing the percentage of inhibition to the conterol [14].

3.Results and Discussion

The azo-azo methine was red powder, All complaxes are fine powder, varry in color depend of metal , Stable , and solubel in most organic solvents as methenol, ethanol, acetone, di-methylformamide and di-methyl sulfoxied giving stable solutions at room temperature, but in-soluble in water[15].

3.1 Element investigation

Table-1 has shown all the calculeted-valuaes is good agreement with data ,which used to test the purity of ligand .It suggeest that the metale, and the ligand are cooredinated in the ratio [2:1] or [1:1] (M:L)[16]. The analytical and physical data of the ligand and its chelate complexes are presented in Table (1).

Table (1): Some physicael and analytical data of the ligcnd and its complexes

Compound	M.wt. g/mole	M.p. °C	Yield%	Color	M:L	Found (Caled) % C	H	N	M
$C_{31}H_{27}N_5O_3$	517.589	145	85	Deep Red	....	70.22 (71.7)	5.95 (5.21)	14.59 (13.51)	…… …….
[Mn($C_{31}H_{27}N_5O_3)_2Cl_2$]	1161.016	132	65	Red	1:2	63.09 (64.05)	4.74 (4.64)	11.87 (12.05)	4.65 (4.73)
[Fe ($C_{31}H_{27}N_5O_3)_2$ SO_4]	1187.082	255	75	Red	1:2	61.71 (62.64)	4.67 (4.54)	12.64 (11.78)	3.45 (4.70)
[Co ($C_{31}H_{27}N_5O_3)_2Cl_2$]	1215.068	191	78	Purple Red	1:2	60.26 (61.15)	4.89 (4.43)	12.91 (11.50)	4.78 (4.86)
[Ni ($C_{31}H_{27}N_5O_3)_2Cl_2$]	1164.788	142	76	Purple	1:2	62.61 (63.83)	3.89 (4.63)	11.91 (12.01)	4.96 (5.03)
[Cu ($C_{31}H_{27}N_5O_3)_2Cl_2$]	1169.618	155	75	Red Brown	1:2	62.64 (63.53)	3.88 (4.61)	12.85 (11.95)	5.14 (5.42)
[Zn($C_{31}H_{27}N_5O_3)_2C_4H_6O_4$]	1218.558	136	74	Red	1:2	60.35 (61.00)	4.87 (4.42)	12.83 (11.48)	5.28 (5.36)
[Cd ($C_{31}H_{27}N_5O_3)_2$ Cl_2]	1218.478	135	71	Red	1:2	60.41 (61.00)	3.73 (4.42)	11.24 (11.48)	9.74 (9.21)
[Hg ($C_{31}H_{27}N_5O_3)_2$ Cl_2]	1306.668	127	77	Red	1:2	56.17 (56.91)	4.22 (4.13)	10.55 (10.71)	15.60 (15.34)
[Ag ($C_{31}H_{27}N_5O_3)_2$] NO_3	1205.051	150	74	Light Red	1:2	60.73 (61.67)	4.77 (4.47)	11.50 (11.60)	8.81 (8.94)
[Au ($C_{31}H_{27}N_5O_3$) Cl_2] Cl	820.908	140	67	Red	1:1	44.26 (45.01)	3.87 (3.26)	8.27 (8.47)	23.43 (23.84)

3.2 ^{1}HNMR Spectra of Azo-Azo methine ligand

In Fig(1) , ^{1}HNMR-spectrum of this ligand was recorded by di-methyl sulfoxied . At δ 2.5 ppm, signal of solvent . Protons of benzene and phenyl rings were seen in(δ 6.4 –8.4 ppm) . OCH3-imidezole ring at 3.8 ppm . The signal at δ 12.5 ppm refer to NH-imidazole , at δ 9.2 ppm owaing to (OH) [17] .

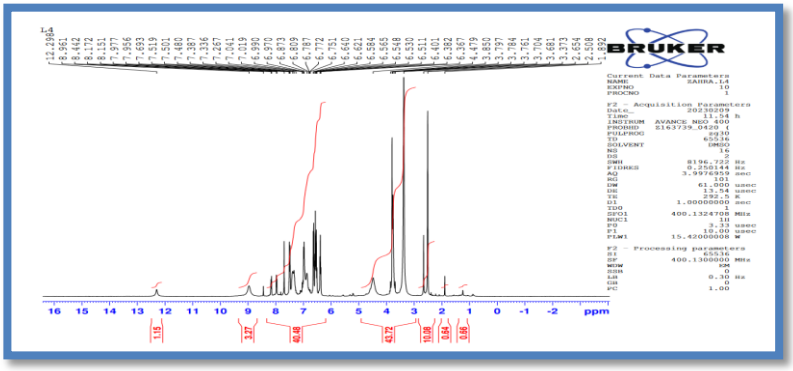


Fig.(1) The ^{1}HNMR spectrum of the ligand

3.3 Mass specatrum of Azo-Azo methine ligand

The mass spectrum of azo-azo methine ligand was displayed a well defained molcular ion paek at(m/z 109.2) which equivelent to molecular mass.The friagment at (m/z 238.3) corresponding to the schiff molecule after losing imidazole. The friagment at (m/z 280 and 93) due to 4,5-Bis-(*p*-.Methoxy phenyl) imidazole and phanol molecular respectivaly[18]. The sucessive fragmantation peaks are shown in Fig.(2).

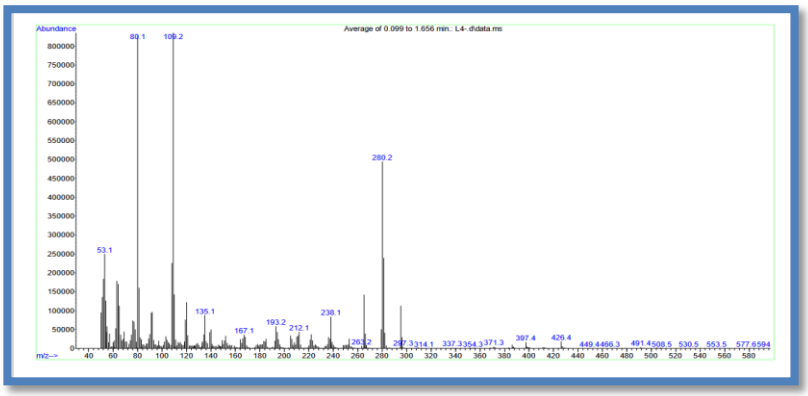
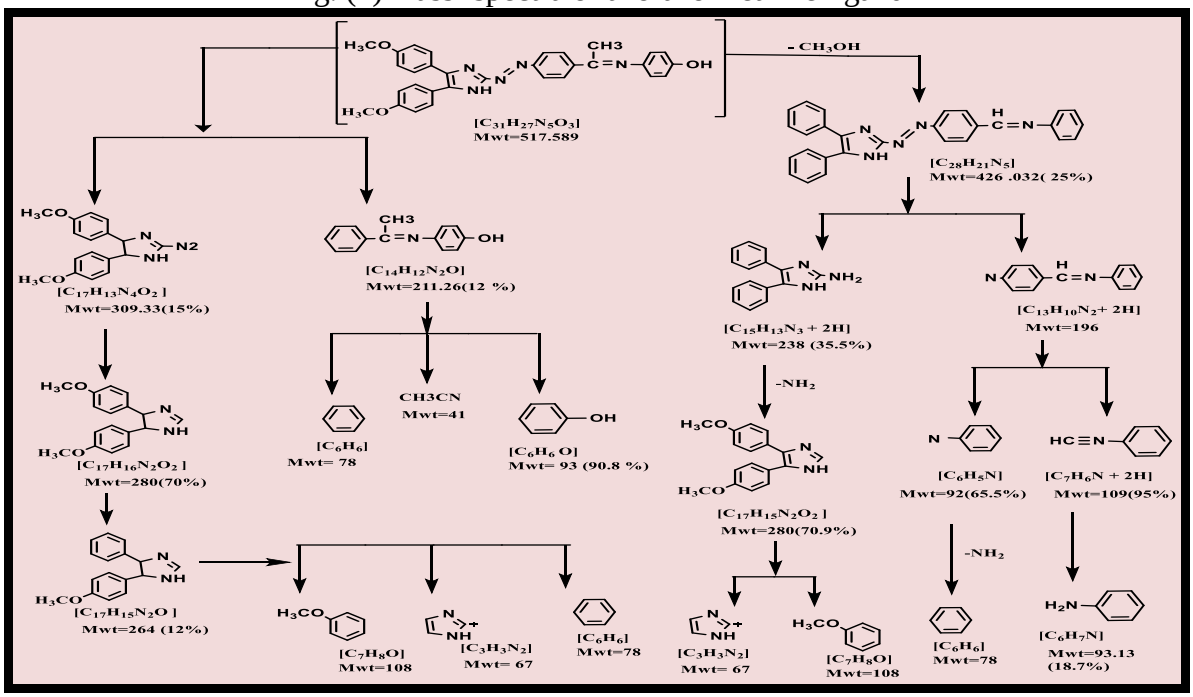


Fig. (2) mass- spectra of azo-azo methine ligand



Schem (2): Suggested mass fragmentation pathways for Ligand

3.4 Infrared Spectra of Azo-Azo methine ligand

The IR spactra of azo-azo methine ligand and its complexes recorded in 4000-400 cm^{-1} .The IR spectral data refer to that ligand beheves as a nautral bidantate chilating agent, and the coordination fram the N-azo atom nearest to a phenyl ring, and N3-imidazole ring atom to give five-mambered chelat ring.The Figures (3) - (13) show the spectra azo-azo methine ligand and its complexes [19].

Table (2): Chrcterestic FT-IR frequancies (cm^{-1}) of the azo-azo methine ligand and its complexes

Compound	ν (O─H)	υ(N=H) imidazole	υ(C=N) schiff	ν (C═N) imidazole	ν (N═N) azo	ν (M─N)
$C_{31}H_{27}N_5O_3$	3469	3379 s	1674 m	1606 s	1465 m	.....

[Mn($C_{31}H_{27}N_5O_3)_2Cl_2$]	3412 s	3178 m	1674 s	1600 m	1456 w	438
[Fe ($C_{31}H_{27}N_5O_3)_2$ SO_4]	3414 br	3234 br	1678 m	1606 m	1458 w	435
[Co ($C_{31}H_{27}N_5O_3)_2Cl_2$]	3412 s	3200 w	1674 w	1593 s	1463 m	425
[Ni ($C_{31}H_{27}N_5O_3)_2Cl_2$]	3417 br	3250 w	1670 w	1610 m	1456 m	422
[Cu ($C_{31}H_{27}N_5O_3)_2Cl_2$]	3417 br	3325 m	1668 m	1597 s	1460 m	435
[Zn($C_{31}H_{27}N_5O_3)_2C_4H_6O_4$]	3419 br	3381 s	1670 w	1606 s	1462 s	435
[Cd ($C_{31}H_{27}N_5O_3)_2$ Cl_2]	3475 s	3304 s	1674 s	1602 s	1460 s	453
[Hg ($C_{31}H_{27}N_5O_3)_2$ Cl_2]	3421 br	3285 w	1670 m	1602 s	1460 m	435
[Ag ($C_{31}H_{27}N_5O_3)_2$] NO_3	3415 br	3250 w	1674 s	1606 s	1458 w	418
[Au ($C_{31}H_{27}N_5O_3$) Cl_2] Cl	3414 br	3236 w	1674 w	1614 s	1465 w	420

S= strong, m = medium, w = weak , br = broad

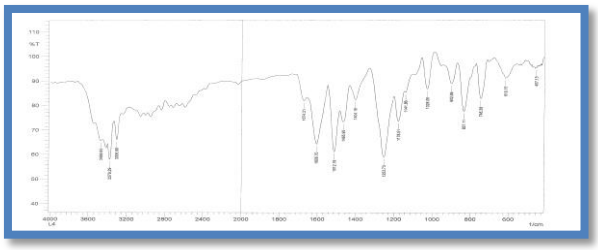


Fig. 3: Theazo-azo methine ligand IR spectra

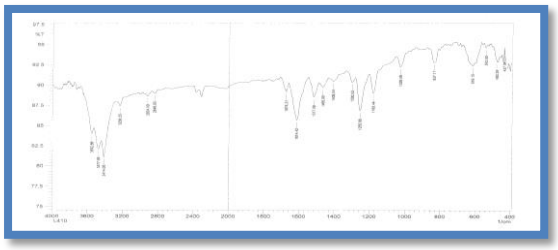


Fig. 4: IR Spec.of [Au (L) Cl_2]Cl

Fig. 5: IR Spe. of [Mn $(L)_2$ Cl_2]

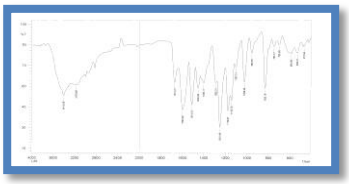


Fig.6: IR Spe. of [Fe $(L)_2$ SO_4

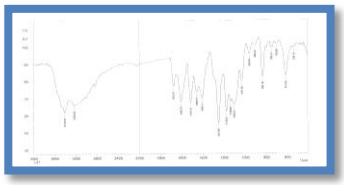


Fig7 :IR Spe. of [Co $(L)_2$ Cl_2]

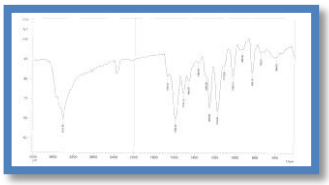


Fig.8 : IR Spe. of [Ni $(L)_2$ Cl_2]

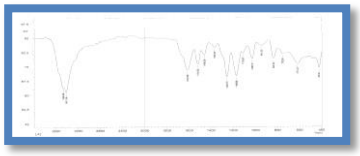


Fig. 9: IR Spe. of [Cu $(L)_2$ Cl_2]

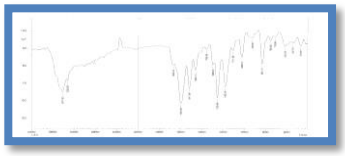


Fig. 10: IR Spe. of [Zn $(L)_2$ $C_4H_6O_4$]

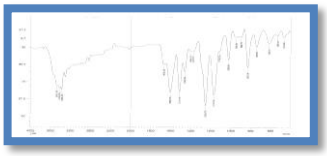


Fig.11: IR Spe.of [Cd $(L)_2$ Cl_2]

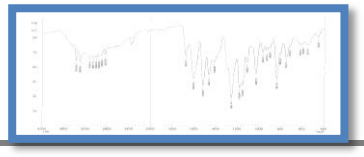


Fig.12: IR Spe. of [Hg $(L)_2$ Cl_2]

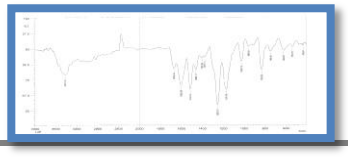
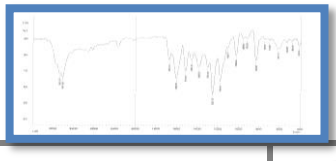


Fig.13: IR Spe. of [Ag $(L)_2$]NO_3]



3.5 Electronic Transfers

At 25ºC temperature, the spectra of elecetronic absorpetion of all the compounds were logged by exhausting solution of ethanol in(200-1100 nm) [20]. Molar conductance data of the complexes were measured in the solvent DMSO and the complexes were found to be non-electrolytic ,except silver complex and gold.Conductivity value of the chelate [21] Conductivity of complexes are lie in the range (8.23 - 45.72) $S.cm^2.mol^{-1}$ nature The data of the azo-azo methine ligand and its complexes are suimmerized in Table (3), The Figures (14) - (24) show the spectra of azo-azo methine ligand and its complexes.

Table 3. Electronic data , Magnetic measurements, Geometry, Hybridization and Conductivity

Compound	Absorption Bands(n.m)	Transition	Conductivity S. cm^2 .mol^{-1}	μeff (B.M)	Geometry	Hybridization
$C_{31}H_{27}N_5O_3$	284 439	π→ π* n→ π*	…….	…….	.....	.....
[Mn($C_{31}H_{27}N_5O_3)_2Cl_2$]	237 294 481	π→ π* ILCT MLCT	9.13	3.42	Octahedral	SP^3d^2
[Fe ($C_{31}H_{27}N_5O_3)_2$ SO_4]	295 478	π→ π* MLCT	13.42	4.37	Octahedral	SP^3d^2
[Co ($C_{31}H_{27}N_5O_3)_2Cl_2$]	276 418 541	π→ π* ILCT MLCT	9.47	2.19	Octahedral	SP^3d^2
[Ni ($C_{31}H_{27}N_5O_3)_2Cl_2$]	274 438 513	π→ π* ILCT MLCT	6.23	3.14	Octahedral	SP^3d^2
[Cu ($C_{31}H_{27}N_5O_3)_2Cl_2$]	246 417	π→ π* MLCT	12.72	2.78	Octahedral	SP^3d^2
[Zn($C_{31}H_{27}N_5O_3)_2C_4H_6O_4$]	286 447	π→ π* MLCT	15.17	Dia	Octahedral	SP^3d^2
[Cd ($C_{31}H_{27}N_5O_3)_2$ Cl_2]	285 448	π→ π* MLCT	8.15	Dia	Octahedral	SP^3d^2
[Hg ($C_{31}H_{27}N_5O_3)_2$ Cl_2]	288 485	π→ π* MLCT	16.22	Dia	Octahedral	SP^3d^2
[Ag ($C_{31}H_{27}N_5O_3)_2$] NO_3	276 445	π→ π* MLCT	61.72	Dia	Tetrahedral	SP^3
[Au ($C_{31}H_{27}N_5O_3$) Cl_2] Cl	271 448	π→ π* MLCT	38.23	Dia	Square planar	dSP^2

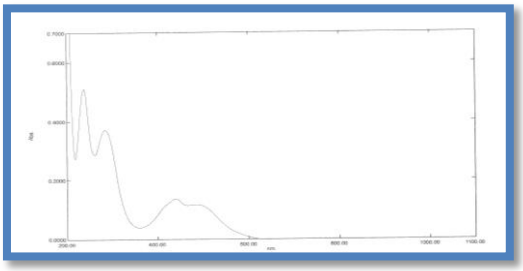


Figure 14: UV-Vis. of the azo- schiff ligand

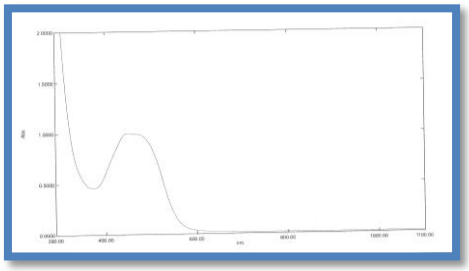


Figure15: UV-Vis of [Au (L) Cl_2]Clcomplex

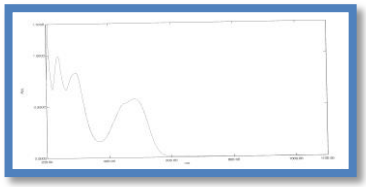


Figure16: UV-Vis of [Mn $(L)_2Cl_2$]

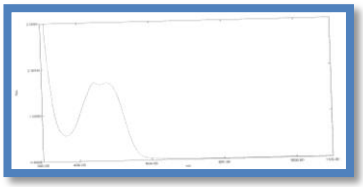


Figure17: UV-Vis of [Fe $(L)_2SO_4$]

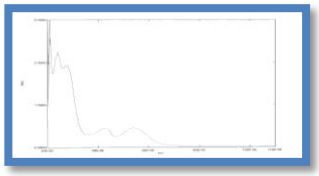


Figure 18: UV-Vis of [Co $(L)_2$ Cl_2]

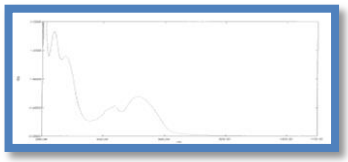


Figure19: UV-Vis of [Ni $(L)_2$ Cl_2]

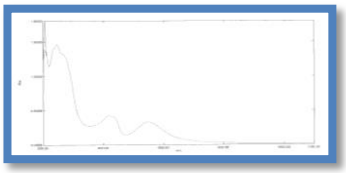


Figure20: UV-Vis of [Cu $(L)_2$ Cl_2]

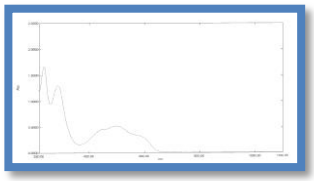


Figure21:UV-Vis of $[Zn(L)_2C_4H_6O_4]$

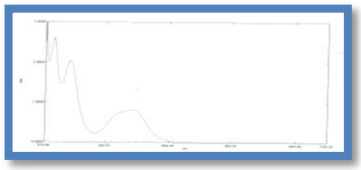


Figure22: UV-Vis of [Cd $(L)_2$ Cl_2]

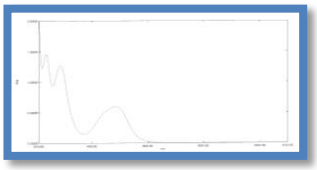


Figure23: UV-Vis of [Hg $(L)_2$ Cl_2]

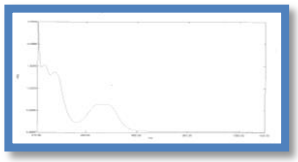


Figure24: UV-Vis of [Ag $(L)_2]NO_3$

3.6 Anticancer effectiveness

The liver is the largest organ in the body, the liver performs several functions, most notably the production of bile, which has an important role in the digestive process. And the metabolism of carbohydrates, proteins and fats. As well as detoxification of the blood. It has an important role in the production of blood coagulation factors. Liver cancer is a tumor that arises in liver cells and develops due to mutations in their genetic material, which leads to abnormal cell growth, resulting in changes in the genetic material of cells, which turns them from normal cells to cancerous, and several factors play a role in increasing the risk of disease, including infection with viral hepatitis "B" or "C", cirrhosis, diabetes, obesity and alcohol abuse , As well as exposure to the so-called "aflatoxins", which are toxins produced by mold that grows in crops when stored poorly, such as peanuts and corn, and these toxins eventually pass to the foods we eat, fatty changes in the liver and some genetic liver diseases such as Wilson's disease[22].Our study is evaluating the anti-cancer activities of [Au($C_{31}H_{27}N_5O_3)Cl_2$] Cl against liver cancer cell lines (HEPG2 cells) .The anti-tumor actevity and growth inhibitary actevity were spcified by IC50 [23]. IC50 is the conecntration of compound that decrease cell growth by 50% in the same conditions. The results showed IC50 revealing that the compound is much more efficient against HEPG2 cell lines .

Table (6). Biological effectiveness of **[Au ($C_{31}H_{27}N_5O_3$) Cl_2] Cl** on cellular cells of liver cancer HEPG2 Compare it to the normal HFF cellular line for the same concentration and use the test MTT for 72 hours and tem. 37 °C.

Con. ($\mu g.mL^{-1}$)	Parcentage (%) for each cell line			
	Cancarous line cells of HEPG2		Normel line cells of HFF	
	Cell Viab.	Cell Inhib.	Cell Viab.	Cell Inhib.
0.5	97.91	2.09	104.92	4.92
1	92.83	7.17	96.62	3.38
5	89.99	10.01	87.89	12.11
10	82.87	17.13	78.4	21.6
50	67.25	32.75	77.19	22.81
100	58.02	41.98	72.35	27.65

Fig (26) Anticancer activity data of **[Au ($C_{31}H_{27}N_5O_3$) Cl_2] Cl** cells against unhealthy and healthy Cells.

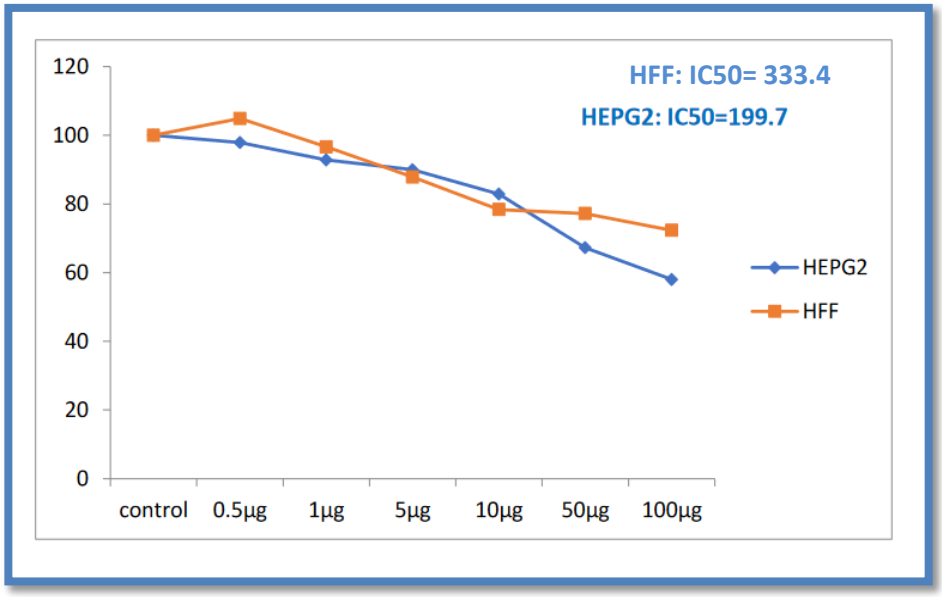
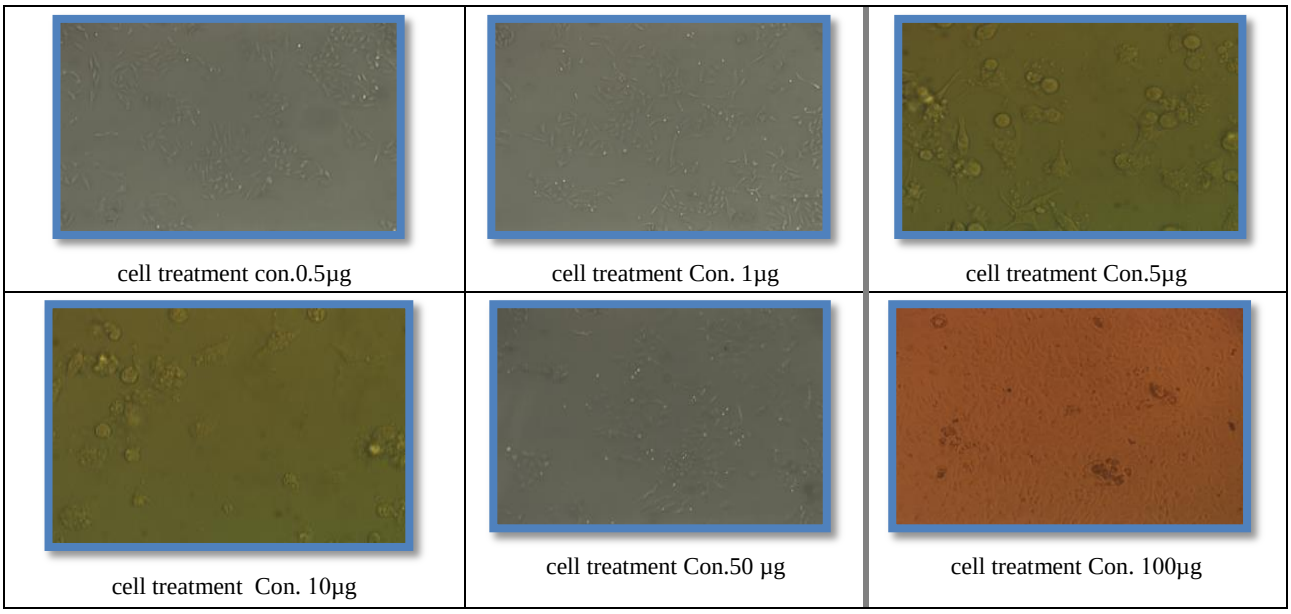


Fig (27) Cancer cells treating By[$Au(C_{31}H_{27}N_5O_3)Cl_2$]Cl with different concentrations after adding (MTT)

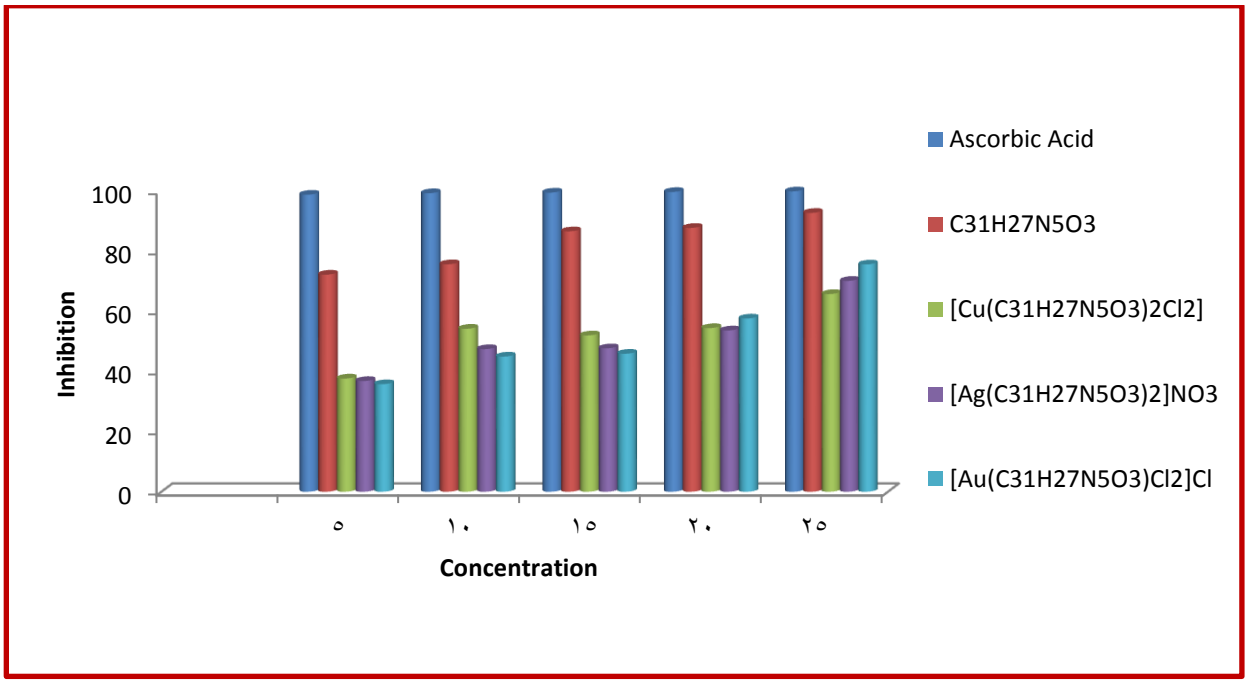


3.7Antioxidant Activity

Oxygen is an essential and important element in the production of energy by the oxidation of food, however, the reduction of this element is not complete, even under natural conditions. And from some oxygen-derived free radicals, such as nitric oxide •[NO], superoxide superoxide • - [O2], hydroxide •[OH], and peroxide •[RO2] are carcinogenic and affect disorders in the human body by oxidizing nucleic acids, proteins, lipids, or DNA, as intermediate groups of naturally active chemicals often arise from trophic transformation processes called free radicals, and these antigens protect the cell from damage caused by radicals this is done by removing the active substances of oxygen from the body through the defense systems of antioxidants, thereby protecting the body from the occurrence of oxidative stress. Antioxidants have several mechanisms, namely, the breaking of radical chain reactions, the absorption of ultraviolet and visible radiation, the inhibition of free radicals, the arrest of electron transfer and the removal of heavy metals by chelation [24]. The antioxidant capacity of compounds and the knowledge of the oxidative mechanism are evaluated in the laboratory by the DPPH and ABTS test method and other modern methods[25]. Decreasing DPPH-solution absorbence indicated of incraesing DPPH-radical scaivenging actievity . The DPPH percintage inhibition of the azo-azo methine ligand and its complexes shown in the table 7.

Table 7: Antioxidant activity of the azo-azo methine ligand and its complexes

Con. (µg. mL^{-1})	Inhibition				
	Ascorbic Acid	$C_{31}H_{27}N_5O_3$	$[Cu(C_{31}H_{27}N_5O_3)_2Cl_2]$	$[Ag(C_{31}H_{27}N_5O_3)_2]NO_3$	$[Au(C_{31}H_{27}N_5O_3)Cl_2]Cl$
5	98.7	72.22	37.74	36.91	35.85
10	99.2	75.66	54.30	47.54	45.05
15	99.4	86.58	52.10	47.80	46.00
20	99.6	87.70	54.51	53.74	57.69
25	99.8	92.69	65.80	70.16	75.60

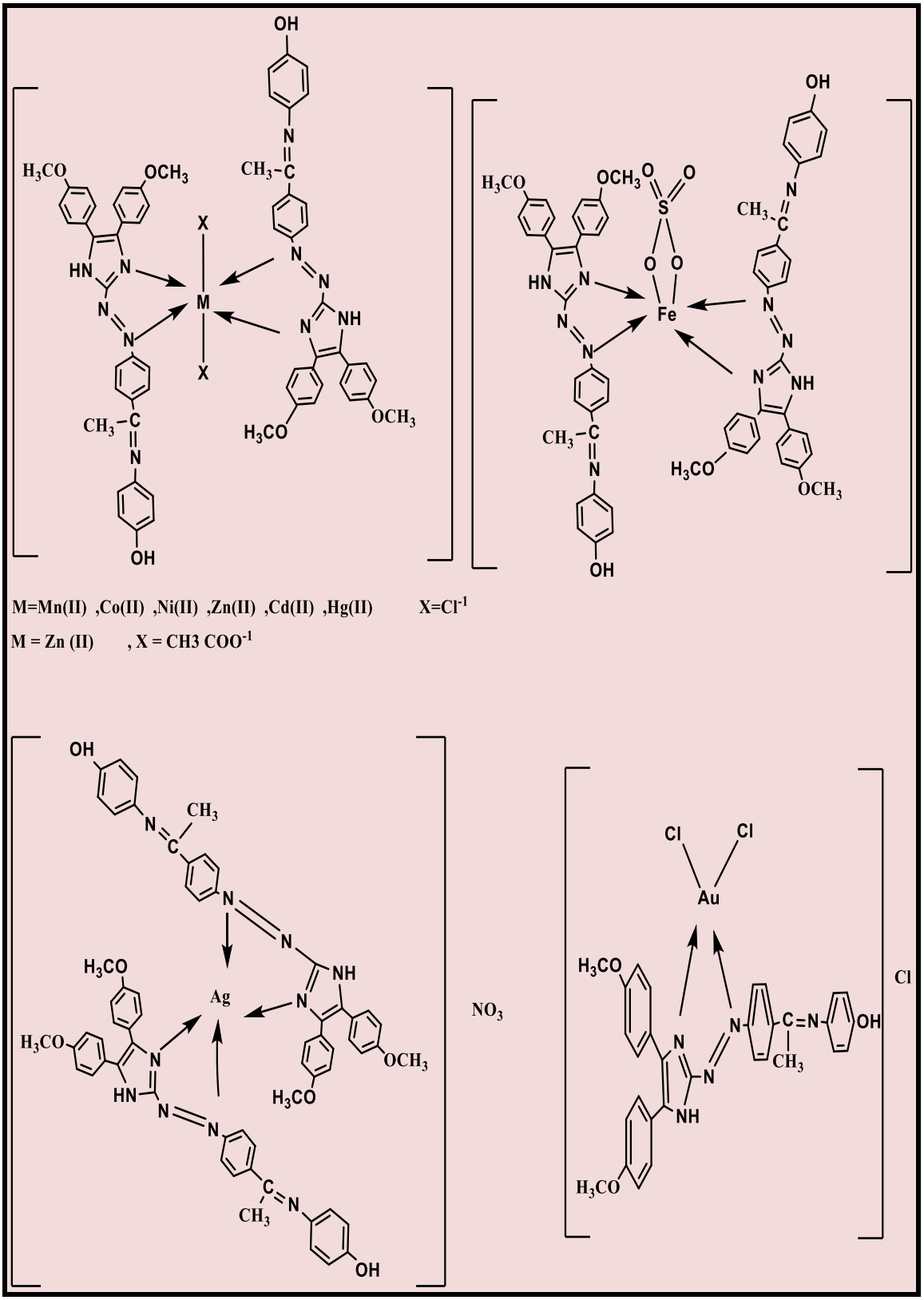


Fig(28) Scavenging activity of the azo-azo methine ligand and its complexes

4 .Conclusion

The conclusions were made based on the results of the spectral analysis of ligand and its complexes . The prepared compounds were stable and the study confirmed that the prepared azo-azo methine ligand and its complexes is bioactive as an antioxidant and the gold ion(III) complex as an anticancer.Au(III) complexes was square planar, whereas Ag(I) complex was Tetrahedral and the other complexes were octahedral.The synthasized complexes were as high stabeility. The ligand was synthaesized by the diazoetization method and the ligand acted as neutrel N, N-bidantate cheleting ligand bends to the metal ions pentegonal chilating ring.

Scheme(2) Suggested geometries of the ligand complexes



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