Synthesis, Characterization of Some Metallic Complexes with new Azo–Schiff Base Ligand Derived from (Para-Aminobenzylamine) and Aa Study of the Anticancer Activity (MCF-7) of the new Ligand and its Complex with Zn (II)

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Abstract
The New Heterocyclic ligand 1-(4-chlorophenyl)-N-(4-((4,5-diphenyl-1H-imidazol-2-yl) diazenyl) benzyl) methanimine (4Cl-DIBM) was prepared from the condensation of para-aminobenzylamine with 4,5-diphenyl imidazole, followed by the condensation of the resulting compound with 4- chloro benzaldehyde. Different analytical and characterization techniques including (Mass,^{1}HNMR, FT-IR and UV-Vis. spectroscopy and C.H.N elemental analysis) in the investigation of Newly prepared ligand. A series of Novel solid metal complexes of this ligand with Co (II), Ni (II), Cu (II), Zn ((II), Cd (II), and Hg (II) were prepared and all complexes were characterization by techniques above, excluding the Mass and the 1H-NMR spectroscopy of some prepared solid metal complexes and the use of flame atomic absorption spectroscopy to determine the percentages of metal ions in the prepared complexes also studied the magnetic susceptibility and molar conductivity of the metal complexes dissolved in DMSO at 1 × 10^{-3} M concentration laboratory temperature. The results of this studies showed that the coordination sites for the new Azo-Schiff base ligand with Co (II), Ni (II), Cu (II), Zn ((II), Cd (II) and Hg(II) were to be through nitrogen of the imidazole ring, and the nitrogen of azo group,. The Electronic spectral and magnetic measurement data predict octahedral structure of the complexes.All complexes showed that Non-electrolytes properties. In the final stage of the study, include biological and toxicological tests for the ligand and its complex with Zn (II) on human cells for breast cancer (MCF-7) In Vitro Cytotoxicity and other normal cells. The complex of Zn (II) in this study comparing with the ligand was highly selective in killing cancer cells and it was very safe with normal not infected cells, so that it does not target healthy cells. As a result, the complex is a new drug treatment to treat breast cancer (MCF-7) with selectivity and very high effectiveness.

Keywords: Azo–Schiff base ligand, 4,5-diphenyl imidazole, para-aminobenzylamine, Metal complexes.

1. Introduction

Schiff bases and their metal complexes have received tremendous attention in the past few years, not only because of their spectroscopic properties and their applications, but also because of their physical properties, as well as the stereochemical, electrochemical (1,2) and biological (3) properties, and these properties are due to their containing the imine (C=N) group resulting from Reaction of the condensation of primary amines with aldehydes or ketones and the release of a water molecule (4,5). And the azo compounds contain (–N=N–) bridge in their composition, as these compounds are used as synthetic dyes and have applications in different fields such as pharmaceuticals, cosmetics and corrosion inhibitors (6,7).

Azo - Schiff bases compounds are a new class of chemical compounds that are receiving increasing interest in scientific research (8), compared to azo compounds and Schiff bases as they contain two active groups (-N=C-) and (-N=N-) (9), and azo - Schiff bases are considered of importance due to their electronic properties, structural flexibility and selectivity towards metal ions (10,11). Azo-schiff compounds can coordinate in many ways. They can be coordinated via azo-azomethine nitrogen (12), or by azo-nitrogen, and finally can coordinate via nitrogen atoms (azo-azomethine) (13). At the present time, Azo - Schiff bases compounds are showing remarkable applications in all areas of life (14), including industrial, biological and analytical fields, because they contain the two groups mentioned above (15), as they have been used in the industrial field as antioxidants (16), and to prevent corrosion (17) As well as for the treatment of nuclear waste (18) and in the manufacture of plastics, leather, and textiles (19). Due to the emergence of cancer tumors that are highly resistant to the effectiveness of traditional chemotherapies, it has become interesting to discover different therapeutic approaches including the development of new active drugs against resistant cancers (20). Azo - Schiff bases complexes have proven their worth as antifungals, anticancer (21), antibacterial (22) and

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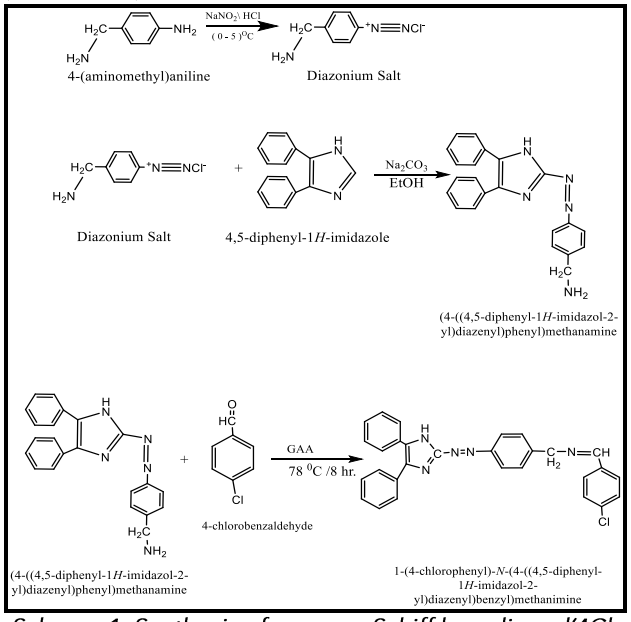
herbicides (23).
MCF-7 is a breast cancer cell line isolated in 1970 from a 69-year-old Caucasian woman (24). MCF-7 is the acronym of Michigan Cancer Foundation-7, referring to the institute in Detroit where the cell line was established in 1973 by Herbert Soule and coworkers (25). The Michigan Cancer Foundation is now known as the Barbara Ann Karmanos Cancer Institute (26).
The human breast cancer cell line MCF-7, which contains estrogen receptors, provides another experimental system to study hormone-regulated genes. Specific proteins are induced in response to estrogen (27).
Researchers everywhere in the world are working to find healthy ways to avoid, notice, and treat breast cancer and advance the value of the life of patients.

2. Materials and Methods

All chemicals were obtained from Merck, BDH and Sigma - Aldrich and used without further purification. Melting points were determined using model 9300 of ligand and its complexes. ^{1}HNMR spectra were recorded as solution in DMSO d6 as solvent using (varian 500MHZ Spectrophotometer) and Mass Spectra were recorded on Shimadzu Agilent Technologies 5975C. The UV-Visible spectra were recorded on Shimadzu spectrophotometer double band model 1700. Magnetic susceptibility measurements were carried out on a balance magnetic MSB-MKI using faraday method. The diamagnetic corrections were made by Pascal's constants. IR spectra were recorded on Shimadzu FTIR 8400 spectrometer using KBr pellet in the wavelength range 4000-400 cm 1. C.H.N Elemental analyses were performed by means of EURO 2012EA 300 C.H.N Elemental analysis. and for toxicological Studies: Autoclave Arnold Sons, USA. Biohazard safety cabinet class II BGenex, USA. Cell culture incubator, Memmert, Germany. Centrifuge Hermle, Germany. Cooling centrifuge,Beckman Model J2-21, USA. Deep freezer (-800C) Marubeni, Japan. Distillatory Ogawa seiki, Japan. Drying and sterilizing oven, Hermle, Germany. ELISA reader, Organon Teknika, Beelchum. IncubatorMemmert, Germany. Inverted microscope, Leica, Germany. Microtiter plate, multiwall plate and 96 wells Lab-TeK and Nunc, USA. Millipore filter 0.22 µm, Sartorius, Germany. pH-meter, LKB, Sweden. Sterile 25, 75 cm2 tissue culture flasks, Nunc, Denmark. Vacuum pump, Leitz, Germany. Water bath, Memmert, Germany.

Synthesis of the new Azo- Schiff base ligand(4Cl-DIBM)

The new azo-Schiff base ligand(4Cl-DIBM) was Synthesized by coupling reaction of diazonium salt with appropriate amount of (imidazole derivative) as coupling component in alkaline solution. A diazonium solution is prepared by dissolving (0.01 mol, 1.222gm) of Para aminobenzylamine in (30ml) distilled water with (8ml) of concentrated HCl acid with continuous shaking. To this mixture a solution of (0.01 mol, 0.7 gm) of sodium nitrate in 5ml of distilled water was added drop wise to the Diazonium solution with shaking and stirring to complete the process of Azotization at(0-5)^{0}C ,and left it to stand for (30min) .This diazonium solution was added drop by drop to (0.01 mol,2.202gm) of 4,5-di phenyl imidazole dissolved in(50ml)of absolute ethanol and (50ml) solution of (40% Na_2CO_3) at(0-5)^{0}C. A drop by a drop was observed to change the color to orange-red, indicating that the process of coupling between the two solutions and the formation of the azo compound then neutralized by adding drops of dilute HCl to the acidic function PH ~ 7.5. After that, the mixture was allowed to stand overnight and then the solution was filtered off, washed with distilled water, and recrystallized twice from hot ethanol and then dried in oven at 40^{0}C for 1 hours (28).
The second step included the preparation of the new Schiff base azo-ligand (4 Cl-DIBM), as it dissolved (0.01 mol, 1.400 gm) of Para chloro benzaldehyde in (10 ml) of absolute ethanol alcohol, stirring for (2 min), then adding (2-3) drops of glacial acetic acid and then left for (5 min) at laboratory temperature, After that, a solution prepared by dissolving (0.01 mol, 4.759 gm) of azo dye is added in (10 ml) of absolute ethanol alcohol, and the solution was raised for (8 hr.) at a temperature (78°C) where the azo - Schiff base ligand was obtained. the reaction was followed up by TLC technology using (0.5 ml methanol: 4.5 ml benzene), then the product was cooled, dried, collected and then recrystallized using hot absolute ethanol (29). The physical properties of it have been listed in Table 1. Scheme 1 shows the steps for preparing the new Azo - Schiff's base ligand (4 Cl-DIBM).



Scheme-1: Synthesis of new azo-Schiff base ligand(4Cl-DIBM)

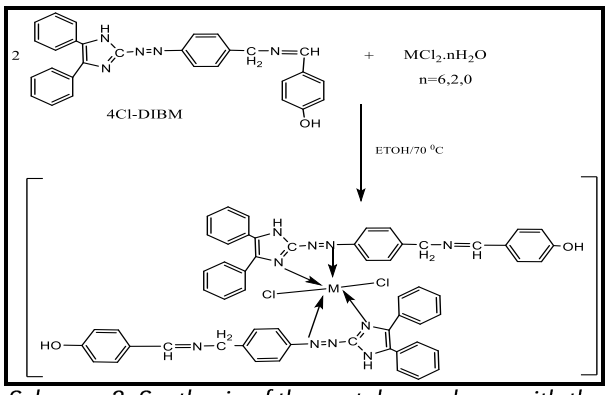
Synthesis of metal complexes

The metal complexes were synthesized by mixing of (0.0002mol) in 10ml absolute ethanol solution of each of (CoCl2.6H2O, NiCl2.6H2O, CuCl2.2H2O, ZnCl2, CdCl2and Hg Cl2) with 10ml absolute ethanol solution of (0.2g, 0.0004mol) of new azo-Schiff base ligand in (1:2) (metal: ligand) ratio. The resulting mixture was refluxed for 1h. The products of complexes were isolated after reduced of volume by evaporation. They

were filtered off and dried under vacuum the physical properties of the complexes under study are listed in Table 1. Scheme 2 illustrates the steps of preparing the metal complexes with the ligand (4Cl-DIBM).

Table (1) shows the physical properties of the new Azo Schiff base ligand and its complexes.

No	Chemical formula	Color	M.Wt g/Mole	M.P°C	Yield%	R_f
1	$C_{29}H_{22}N_5Cl$	Reddish Orange	475.970	76-78	90	0.54
2	$[Co(C_{29}H_{22}N_5Cl)_2Cl_2]$	Dark Red	1081.780	282-285 Dec	85	0.58
3	$[Ni(C_{29}H_{22}N_5Cl)_2Cl_2]$	Reddish Brawn	1081.540	235-237 Dec	88	0.67
4	$[Cu(C_{29}H_{22}N_5Cl)_2Cl_2]$	Dark Olive	1086.393	218-220	84	0.57
5	$[Zn(C_{29}H_{22}N_5Cl)_2Cl_2]$	Reddish Brawn	1088.237	242-246	89	0.69
6	$[Cd(C_{29}H_{22}N_5Cl)_2Cl_2]$	Red	1135.258	188-190 Dec	85	0.58
7	$[Hg(C_{29}H_{22}N_5Cl)_2Cl_2]$	Dark Red	1223.437	140-144 Dec	88	0.70



Scheme- 2: Synthesis of the metal complexes with the ligand (4Cl-DIBM)

Cell Cytotoxicity (In Vitro Cytotoxicity) and Viability Assays

1.cell line. The cell line of human breast cancer cells (MCF-7), which was obtained from (Pastor Institute - Iran), was used in this study, and cancer cells were sustained and developed, and tests were performed on them at the University of Tehran.

2. Development of breast cancer cell line (MCF 7-cells)

The method of (Freshney) was followed to grow the cells of the breast cancer cell line (MCF-7) as follows. The cells of the cancer cell line (MCF-7) were thawed using a water bath at a temperature of (37°C), Then the cells of the cancer cell line (MCF-7) were transferred to a container. A culture of animal cells with a diameter of (25 cm^2) contains the culture medium (RBMI-1640) and (10%) cow calf blood serum. Then, it was incubated in an incubator with a percentage of carbon dioxide (5%) at a temperature of (37°C) for 24 hours, and after 24 hours of incubation, and when it was confirmed that there was growth in the cell culture and that it was free of contamination, the cells were examined using an inverted microscope. microscope to ensure its viability, free from contamination, and its growth to the required number (approximately 500-800) thousand Cell/ml. The cells were transferred to the growth cabin and the used culture medium was disposed of by washing the cells with Physiological Slain Solution (PBS), and then a sufficient amount of trypsin was added to the cells and incubated for (30-60 sec) at a temperature of (37 °C) and it was monitored until it transformed from a mono-cell layer to a single cell, then the enzyme was stopped by adding a new growth medium containing bovine calf serum. Then the cells were collected in centrifuge tubes and placed in a centrifuge at 2000 min /r for 10 min at room temperature, to precipitate the cells and get rid of the trypsin and the used culture medium. The filtrate was disposed of, and the cells were suspended in a fresh culture medium containing 10% bovine calf serum. The number of cells was examined by taking a certain volume of the cell suspension, adding to it the same volume of Trepan Blue dye to determine the number of cells and their vitality using Hem cytometer slide and according to the equation (30).

$$C = N \times 10^4 \times F \text{ /ml}$$

Whereas: -

C = the number of cells in one ml of solution N = the number of cells in the slide

F = dilution factor 10^4 = slice dimensions

The viability of the cells in the sample was calculated using a Hemacytometer chip.

Live cell viability ratio = (number of living cells/ number of dead cells) X100 %.

The cell suspension was distributed into new containers and then incubated in a 5% CO2 incubator at (37°C) for 24 hours.

3.MTT staining test for breast cancer cell viability (MCF-7)

In this test, the cytotoxic effect of ligand (4Cl-DIBM) and zinc complex $[Zn(4Cl\text{-}DIBM)_2Cl_2]$ on breast cancer cells (MCF-7) were determined for the purpose of demonstrating their efficacy It can be used as a cancer drug.

The cancer line cells were prepared by following the above steps (31), then the cell suspension was placed in a plate with flat-bottom holes and incubated in (5%) CO2 incubator at (37°C) for 24 hr, then (200 µl) was added. From the cell suspension in each hole, followed by the addition of the prepared concentrations of complex and its ligand [Zn(4Cl-DIBM)2Cl2] and 4Cl-DIBM at (6.25, 12.5, 25, 50, 100 µg/ml) concentration to the holes., and by (3) holes for each concentration. The plate was incubated for 24 hours at a temperature of (37°C), Then 10ml of MTT solution was added to each hole at a concentration of 0.5 mg/ml. The plate was incubated for an additional 4hr at (37°C) then (100

µl) of solute solution was added Dimethyl sulfoxide to each hole to dissolve the Formazan Crystals. The absorbance of the sample was read at a wavelength of 570 nm using an ELASIS device.

3. Results and Discussion

All our complexes are Freely soluble in DMF,DMSO,Methanol and Ethanol .Also They are stable in air .The ligand and its metal complexes were characterized by elemental analysis Table (2) ,molar conductivities, magnetic susceptibility, IR,UV-Vis,(Mass and ^{1}H,MNR spectrum for the ligand only) .The analytical data of the complexes are in agreement with the experimental data .The value reveal that the metal to ligand ratio was(1:2) (M:L) and were presented in table.2.The magnetic susceptibility of the chelate complexes at room temperature were consistent with octahedral geometry, So as the around the central metal ions. All of chelate complexes prepared in this work showed lower conductivity values. This proves that complexes have non- electrolytic nature.

Table (2) shows the element Analysis the new azo Schiff base ligand(4Cl-DIBM) and its complexes.

No	Formula	M.Wt	Found) Calc. %(			
			C%	H%	N%	M%
1	(4Cl-DIBM) =C29H22CL N5	475.98	73.179 (73.547)	4.6681 (5.121)	14.7138 (15.152)	----------
2	[Co(4Cl-DIBM)2Cl2]	1081.78	64.957 65.442)(	4.0995 4.471)(	12.9478 (13.249)	5.4477 (5.801)
3	[Ni(4Cl-DIBM)2Cl2]	1081.54	64.41 (64.871)	4.1 4.475)(	12.95 (13.369)	5.4268
4	[Cu(4Cl-DIBM)2Cl2]	1086.393	64.1223 64.519)(	4.0820 4.398)(	12.8928 13.451)(	5.8492 (5.977)
5	[Zn (4Cl-DIBM)2Cl2]	1088.237	64.0136 (64.522)	4.0751 (4.529)	12.8710 (13.291)	6.0088 (6.230)
6	[Cd(4Cl-DIBM)2Cl2]	1135.258	61.3623 (61.834)	3.9063 (4.351)	12.3379 12.821)(	9.9018 (10.056)
7	[Hg(4Cl-DIBM)2Cl2]	1223.437	56.9396 (57.422)	3.6248 4.015)(	11.4486 (11.891)	16.3956-

Mass spectrum

The mass spectra of the new azo Schiff base ligand(4Cl-DIBM) was recorded at room temperature . The obtained peaks confirm the proposed formulae for the compound .The mass spectrum of Ligand show the molecular ion peak at m/z+ 475.98 compound (C29H22N5Cl)confirm the proposed formulae for compound. This small abundance (3%) was due to the large molecular weight, high bombardment energy, and the large number of heterogeneous atoms in its chemical structure, which confirm the validity of the proposed formula for the compound. Figure 1 and Scheme 3 showed the mass spectrum of ligand and the proposed mass fractionation pathway for it (32,33).

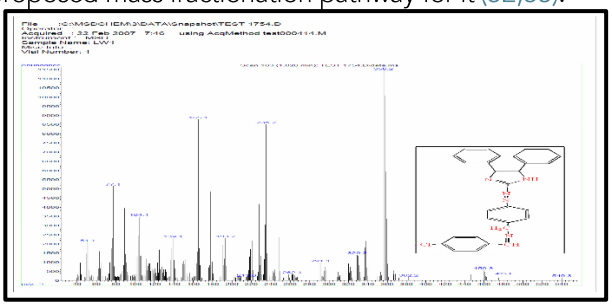
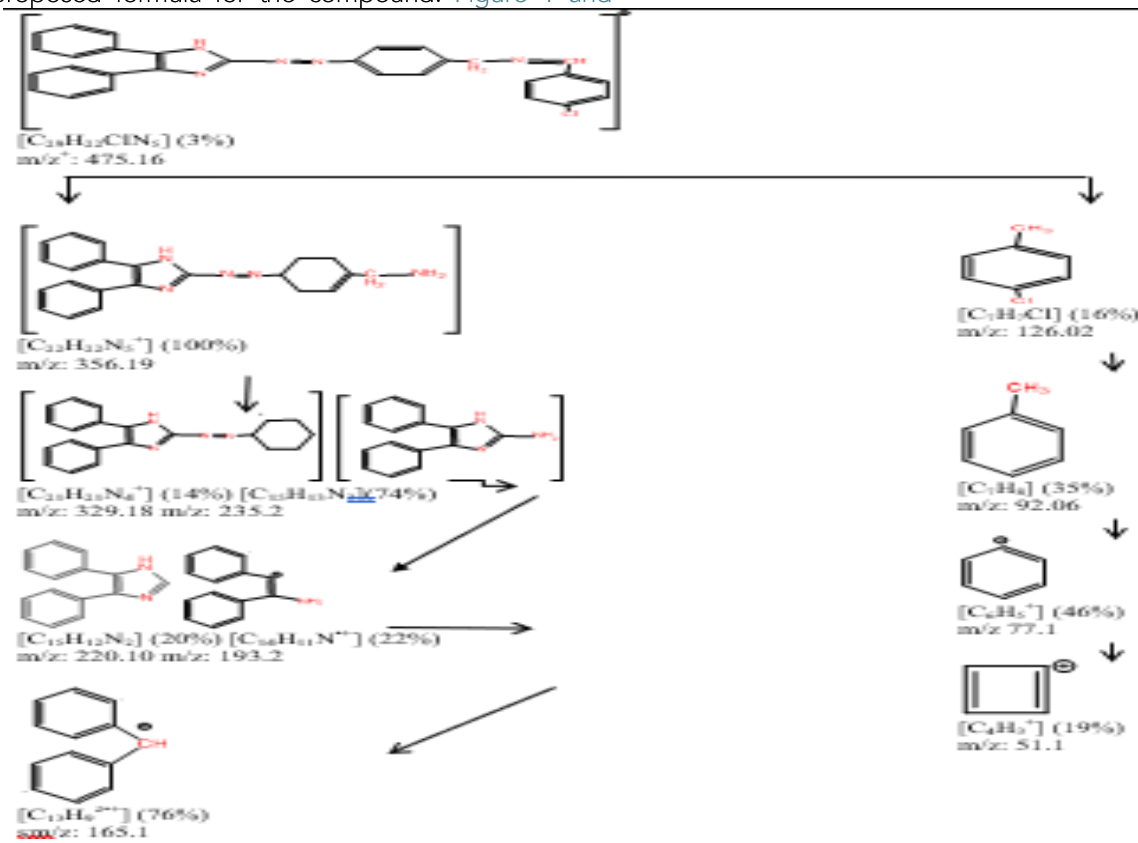


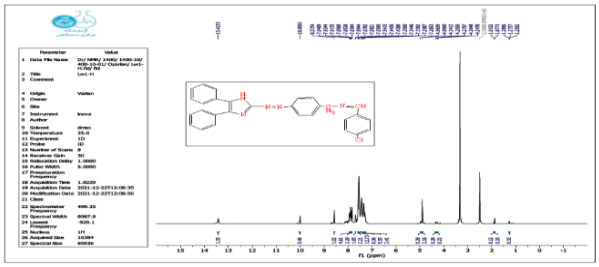
Fig1: Mass spectrum of the new azo Schiff base ligand .



Scheme -3 Mass fractionation pathways of the Ligand(4Cl-DIBM .)

^{1}H-NMR Spectra

The spectrum of newly synthesized ligand(4Cl-DIBM) gave a satisfactory data, and the molecular structure was assigned on the basic of 1H - NMR chemical shift by using DMSO-d6 as a solvent with TMS as an internal reference. The 1H-NMR spectrum of the ligand showed clear signals involved singlet at δ (2.5) (ppm) belong to the protons of solvent (DMSO) and multiples signals at δ (6.95-8) ppm which were assigned to aromatic protons of phenyl ring of Imidazole and benzilidenimin. Singlet at δ (4.8) ppm belong to the proton of methyl (CH2). Singlet at δ (8.7) ppm belong to the proton of (–CH=N), Singlet at δ (13.3) ppm belong to the proton of –C-NH imidazole ring (28), as shown in Fig. (2).



Fig(2): 1H-NMR spectrum of the ligand (4Cl-DIBM)

Infrared Spectra studies of the ligand and its complexes

The IR spectra of the complexes are compared with that of the free ligand to determine the changes that might have taken place during the Complexation (34,35), all data are listed in table (3).

Table (3) IR spectra frequencies for the new azo Schiff base ligand and its metal complexes in cm^{-1}

Compound Formula	υ(CH) Aro	υ(CH) Alpha.	υ (C=C)	υ(C=N) Imda.	υ(C=N) Shiff	υ(N=N)	C-Cl	υ(M-N)1	υ(M-N)2
(4Cl-DIBM)= $C_{29}H_{22}CL\ N_5$	3049	2850	1693	1645	1591	1444	694	-----	-----
$[Co(4Cl\text{-}DIBM)_2Cl_2]$	3057	2929	1693	1629	1598	1448	698	536	505
$[Ni(4Cl\text{-}DIBM)_2Cl_2]$	3055	2931	1693	1629	1598	1450	698	447	447
$[Cu\ (4Cl\text{-}DIBM)_2Cl_2]$	3055	2924	1691	1668	1598	1446	696	540	493
$[Zn\ (4Cl\text{-}DIBM)_2Cl_2]$	3057	2924	1691	1631	1597	1440	698	538	497
$[Cd(4Cl\text{-}DIBM)_2Cl_2]$	3057	2926	1689	1635	1598	1438	698	538	507
$[Hg(4Cl\text{-}DIBM)_2Cl_2]$	3057	2868	1689	1635	1597	1438	696	536	509

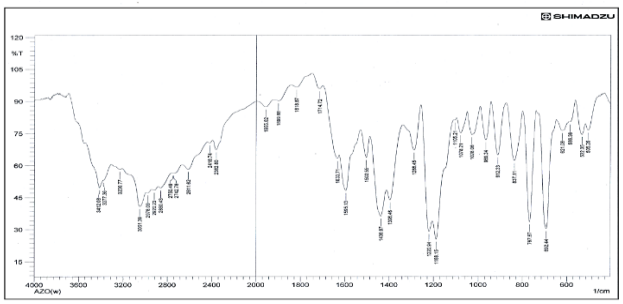


Fig (3): IR-spectra of the Azo compound

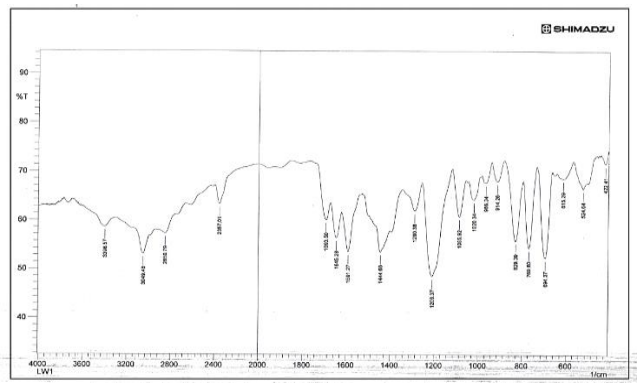


Fig (4): IR-spectra of the ligand (4Cl-DIBM)

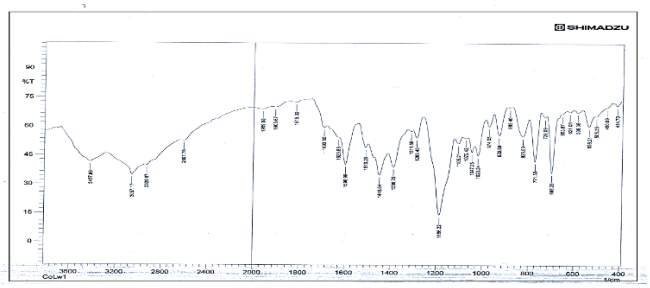


Fig (5): IR-spectra of Co (II) complex

Magnetic susceptibility

The results of the magnetic susceptibility measurements are listed in the table (4) where the magnetic moment value of the magnetic moment of Co(II) ,Ni (II) and Cu(II) Complexes reach (5.03, 2.95 ,1.86) B.M respectively, which indicates the presence of the paramagnetic characteristic (36). As for the complexes of Zn (II), Cd (II) and Hg(II) they have shown Di magnetic properties due to Electron cover saturation (nd) in the electrons(37).

Measurement of molar conductivity

From the results obtained, the molar electrical conductivity measurements for solutions of Chelate complexes of ions under study with the new ligand and with concentration of (1×10-3) molar per complex at the laboratory temperature and using DMSO as solvent, were ranged from (10.00-16.36) S. cm2.mol-1and listed in table (4), We find the lack of ionic properties of all these complexes. These results are identical to what was stated in the literature for metallic complexes devoid of ionic properties (38).

Table (4) molar conductivity and Magnetic susceptibility values for the complexes

compounds	μ_{eff} (B.M)	$\Lambda_M(S.cm^2 \cdot mol^{-1})$
$[Co(4Cl\text{-}DIBM)_2Cl_2]$	13.90	5.03
$[Ni(4Cl\text{-}DIBM)_2Cl_2]$	11.40	2.95
$[Cu(4Cl\text{-}DIBM)_2Cl_2]$	15.66	1.86
$[Zn(4Cl\text{-}DIBM)_2Cl_2]$	10.00	Dia
$[Cd(4Cl\text{-}DIBM)_2Cl_2]$	16.36	Dia
$[Hg(4Cl\text{-}DIBM)_2Cl_2]$	15.57	Dia

Electronic spectra

The electronic absorption spectra are very useful in the estimation of effects equipped thru other approaches of structural exploration.

The spectrum of the new ligand (4Cl-DIBM) in solvent (DMSO) showed two absorption peaks, one at (280 nm, 35714.28 cm-1) due to the electron transition of the type (π → π*) while the second peak was attributed at (430 nm, 23255.8 cm-1) to the electron transition (n → π*) due to the ligand having double bonds with atoms having unshared electron pairs.

The spectrum of the ligand was compared with that of the cobalt (II) complex, which showed an absorption peak at (292 nm, 34246.57 cm 1) due to intra-ligand charge transfer (IL. CT) and an absorption peak at (475 nm, 21052.63 cm 1) has been attributed to the electron transition ν3 = 4T1g → 4T1g (P) This fact is consistent with the literature on the appearance of this band in octahedral cobalt (II) complexes (39).

The UV-visible spectrum of nickel (II) complex solution recorded an absorption peak at (295 nm, 33898.30 cm-1) due to internal charge transfer in the ligand (IL. CT), while the absorption peak was detected at (480 nm, 20833.33 cm 1). to the electron transition ν3=3A2g(F)→3T1g (P) and this is consistent with what was mentioned in the literature regarding octahedral nickel (II) complexes.

While the UV-visible spectrum of copper (II) complex solution showed an absorption peak at (286 nm, 34965.03 cm-1) that was attributed to the internal charge transfer in the ligand (IL. CT) and a broad absorption peak at (580 nm, 19685.03 cm 1) due to the to the electron transition ((2Eg→2T2g), and this is consistent with what was mentioned in the literature (40).

As for electronic spectra of the zinc (II), Cadmium (II) and mercury (II) complexes with new ligand (4Cl-DIBM), they does not possess type (d-d) electronic transmissions because of the fullness of the five (d) orbitals. As new peaks appeared in the metal ion complexes that were not visible in the ligand spectrum, this indicates the consistency of the metal ion with the new ligand due to the charge transfer (C.T) (41). the spectrum of the free ligand is red-shifted in complexes due to ligand to metal charge transfer (LMCT) transition, suggesting an octahedral geometry around metal (II) in the complexes as showed in Fig.(6) ,(7)and (8) .

Table (5) shows the electronic spectra of ligand and its metal complexes in ethanol solvent.

Compounds	ʌmax (nm)	Absorption bands(cm 1)	Transitions	∈ $(\frac{M}{Cm})$	Geometry	Hybridization
(4Cl-DIBM) = $C_{29}H_{22}CL\ N_5$	430 280	35714.28 23255.81	π →π* n →π*	8230 7850	----	----
$[Co(4Cl\text{-}DIBM)_2Cl_2]$	475 292	34246.57 21052.63	IL. CT = $^4T_1g \rightarrow {}^4T_1g_{(P)}$ ν3	6570 5750	Octahedral	Sp^3d^2
$[Ni(4Cl\text{-}DIBM)_2Cl_2]$	295480	33898.30 20833.33	IL. CT ν3 = $^3A_2g_{(F)} \rightarrow {}^3T_1g_{(P)}$	15340 12110	Octahedral	Sp^3d^2
$[Cu(4Cl\text{-}DIBM)_2Cl_2]$	580 286	34965.03 19685.03	IL. CT $^2E_g \rightarrow {}^2T_{2g}$	2353014150	Octahedral	Sp^3d^2
$[Zn(4Cl\text{-}DIBM)_2\ Cl_2]$	475	21881.83	ML.CT	9380	Octahedral	Sp^3d^2
$[Cd(4Cl\text{-}DIBM)_2\ Cl_2]$	262 451	38167.93 22172.94	IL. CT ML. CT	26780 7410	Octahedral	Sp^3d^2
$[Hg(4Cl\text{-}DIBM)_2Cl_2]$	280 456	35714.28 21929.82	IL. CT ML. CT	23980 5960	Octahedral	Sp^3d^2

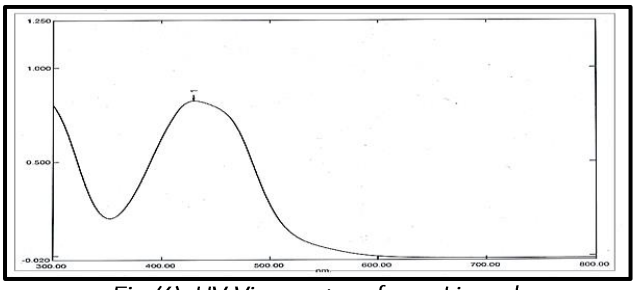


Fig (6): UV-Vis spectra of new Ligand

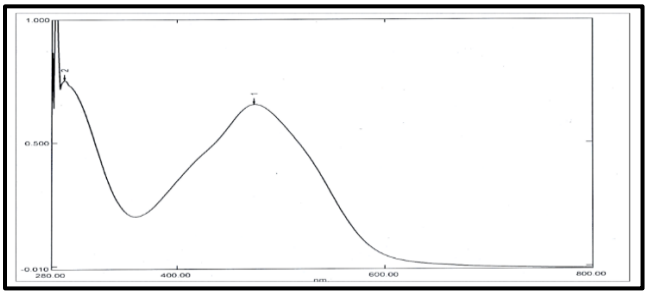


Fig (7): UV-Vis spectra of Co (II)complex

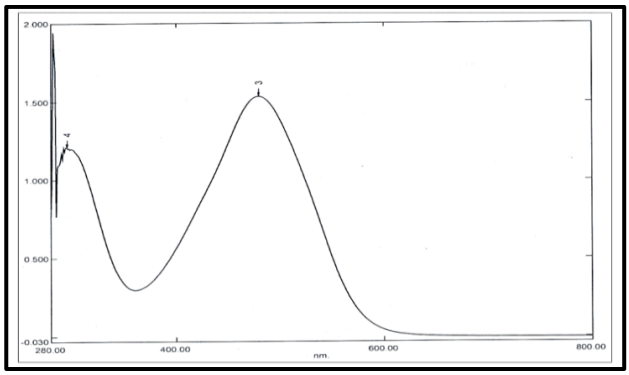


Fig (8): UV-Vis spectra of Ni (II) complex

Proposed Structural

From the results reached it is possible to propose the octahedral structure of all metal complexes with new Azo Schiff base ligand(4Cl-DIBM). The Proposed Structural of metallic complexes can be illustrated in the Fig (9) .

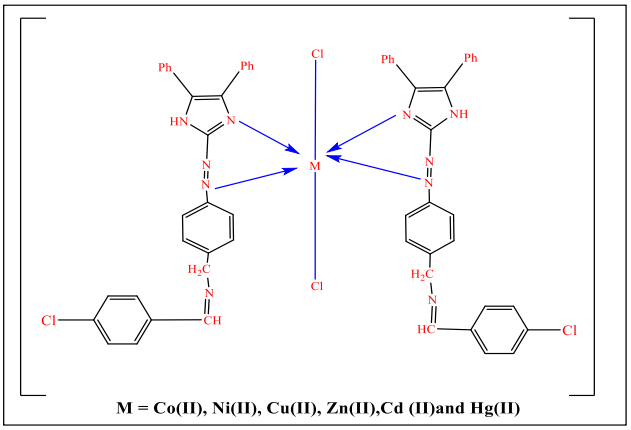


Fig.(9): Proposed Structural of the metallic complexes

Anticancer Screening (In Vitro Cytotoxicity)

1. Effect of ligand (4CI-DIBM) on the growth of breast cancer cell lines (MCF-7) and Healthy cells (MCF-10A)

The results showed in Table (6) the effect of the ligand (4CI-DIBM) on the growth of breast cancer cell line (MCF-7) as well as healthy cells (MCF-10A). The highest inhibition of the ligand (4CI-DIBM)) for the cancerous cell line (MCF-7) (82.04%) at the concentration (100 µg/ml), while the lowest percentage of ligand inhibition (4CI-DIBM) was for the healthy cell line (MCF-10A) cells (16.54%) at the concentration (50 µg). \ml). The results also showed that the best percentage of ligand inhibition (4CI-DIBM) for breast cancer cell line (MCF-7) and cell line (MCF-10A) at concentration (100 µg/ml). On both types of cancerous and healthy line cells, there is the so-called half-inhibitory concentration (IC50), which is the concentration needed to kill nearly half of the cells (1,2). It was (IC50) = (12.19%), and we note that this concentration is low compared to the healthy line cells (MCF-10A), where the half-inhibited concentration reached (IC50) = (231.80%). This important result indicates the possibility of using the ligand (4CI -DIBM) as a new treatment against this type of cancer (MCF-7) (42,43). Figure (10) shows the half-inhibitory concentration of cancerous cells (MCF-7) and healthy cells (MCF-10A).

Table (6) Effect of the ligand (4CI-DIBM) on(MCF-7) and their comparison with (MCF-10A) for the same concentration using the MTT test for 24 hours at a temperature of 37^{0}C.

Con. (µg. mL^{-1})	Mean Percentage (%) for each cell line			
	Cancerous line cells of (MCF-7)		Normal line cells of (MCF-10A)	
	Cell Viability	Cell Inhibition	Cell Viability	Cell Inhibition
6.25	59.64	40.36	76.34	23.66
12.5	48.62	51.38	74.55	25.45
25	41.02	58.98	78.91	21.09
50	29.42	70.58	83.46	16.54
100	17.96	82.04	80.99	19.01

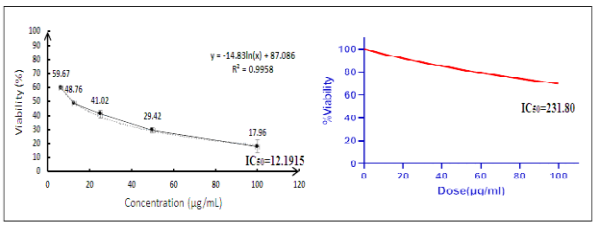


Fig. (10) the half-inhibitory concentration of the ligand (4CI-DIBM) for (MCF-7) and (MCF-10A) respectivly.

2. Effect of [Zn (C29H 22N5Cl)2 Cl2] on growth of breast cancer cell (MCF-7) and Healthy cells (MCF-10A)

The effect of the complex [Zn (C29H 22N5Cl)2 Cl2] on the growth of breast cancer cell line (MCF-7) cells and on the growth of healthy cells (MCF-10A) was studied. The highest percentage of inhibition of complex was (86.19%) at concentration (100 µg/ml), while the lowest percentage of inhibition of the complex of healthy cell line cells was (14.89%)) at concentration (50 µg/ml). The results also showed that the best percentage of inhibition of the complex [Zn(C29H 22N5Cl)2 Cl2] for the breast cancer cell line (MCF-7) and the cell line of healthy cells (MCF-10A) at the concentration (50 µg/ml).

Where it was observed that the half-inhibitory concentration of the complex [Zn(C29H 22N5Cl)2 Cl2] in the cells of the breast cancer cell line (MCF-7) equals (9.4684 µg/ml), which is very low compared to the half-inhibitory concentration of the cells of the healthy line, which is equal to (230.6 µg/ml). ml). Where it is an excellent result because we need a very high concentration to kill half of the healthy cells, and this result indicates the possibility of using the complex [Zn (C29H 22N5Cl)2 Cl2] as a new treatment against this type of cancer. Figure (11) shows the half inhibitory concentration of cancer cells (MCF-7) and healthy cells (MCF-10A).

Table (7) Effect of the complex [Zn($C_{29}H_{22}N_5Cl)_2$ Cl_2] on(MCF-7) and its comparison with (MCF-10A) for the same concentration using the MTT test for 24 hours at a temperature of 37^{0}C.

Con. (µg. mL^{-1})	Mean Percentage (%) for each cell line			
	Cancerous line cells of (MCF-7)		Normal line cells of (MCF-10A)	
	Cell Viability	Cell Inhibition	Cell Viability	Cell Inhibition
6.25	66.99	33.01	80.70	19.30
12.5	37.71	62.26	73.72	26.28
25	25.96	74.31	83.57	16.43
50	16.30	83.70	85.11	14.89
100	13.81	86.19	77.41	22.59

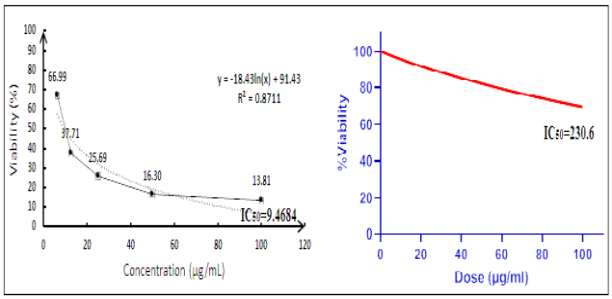


Fig. (10) the half-inhibitory concentration of the complex [Zn (C29H 22N5Cl)2 Cl2] for (MCF-7) and (MCF-10A) respectivly.

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