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Synthesis, characterization and study anticancer activity of new Azo –Chalcone with mix Ligand of some divalent metal chelate complexes

Shownm Hasan Baper and Mithaq Saeed Mohammd

Department of Chemistry, Faculty of Education for Girls, University of Kufa, Iraq

Abstract

In this paper azo-chalcone compound was synthesized by coupling paramino-acetophenone with parabromobenzaldehyde to prepared chalcone , and preparing a mixture of azo-chalcone from reacted diazonium salt of Chalcone with Paracetamol new five complexes of divalent ions (Cu, Zn, Ni, Co, Cd) were prepared by mixture azo-chalcone with 8-hydroxy quinoline, The results show that the mole ratio (metal - ligand) is (1:1:1) for all chelates . The ligand characterized by ^{1}HNMR, mass spectrum and its complexes were characterized by microanalysis of the elements, UV-Vis, FTIR, molar conduction and magnetic moment azo- chalcone coordinated via (N) atom of azo group and (O) atom of OH group of Paracetamol with metal ions. Based on the obtained results, an octahedral geometry were proposed for all chelate complexes. The effect of the biological examination of Ni (II) complex was tested antitumor Breast cancer cells and compared with healthy cells to show the possibility of using these compounds in a therapeutic manner.

Keywords: *Metal complexes, azo-chalcone, geometric shape, breast cancer*

Introduction

In recent years, researchers have noticed the manufacture of many azo-chalcone derivatives (1), which may be the reason for the important role of these compounds in many aspects.

Azo compounds are important linking organic chemistry with other fields such as coordination chemistry, where they are used as rust inhibitors and anti-corrosion, such as coating metal surfaces and spoons (2) They also cause environmental risks when present in marine environments because they are highly resistant and toxic (3) It is one of the most used dyes to color materials such as dyeing textile fibers and in biomedical studies (4).It is characterized by its strong colors and its ability to resist Light, water, different atmospheric conditions, and solvents (5)) And the reason why these aromatic dyes possess the color intensity is due to the lack of localization of the

aromatic (π) electrons, or so-called resonance (6). These electrons have the ability to absorb light in the visible region between (400-750) nm. (7)

Chalcone is an aromatic ketone that forms the basic nucleus of a large number of important biological compounds that are all known as chalcones, and they are unsaturated carbonyl compounds in the two positions (α and β) (8) They are intermediates in the preparation of flavones that are widely found in plants and have obvious biological activity (9) chalcones are synthesized in room temperature, small amount of solvents, and high yield (10) in addition chalcones have been studied for a wide range of activities as anti-inflammatory (11).) Antifungal (12) Antioxidant (13) Antimalarial, Antitumor (14)

The azo-chalcone compounds have been used in many fields such as medicine, the pharmaceutical industry and dyes (15).

Paracetamol is used as an antipyretic and pain reliever without showing an effect against inflammation and thrombosis, and its action against fever is through its effect on the body temperature control centers under the hypothalamus is the same as what happens with aspirin in reducing body temperature it is non-toxic to the gastric mucosa (16) and that taking Paracetamol in therapeutic doses gives benign results, except that excessive doses of it cause hepatotoxicity (17)

Hydroxyquinoline is a white semi-alkaline crystalline powder sensitive to light, poorly soluble in water (18), originated in plants family (Euphorbiaceae), Asteraceae (19) .It is the most interesting to be explored due to its multifunctional properties such biological activities (20) anti-cancer, anti-oxidant , anti-inflammatory (21) antifungal (22) and antibacterial (23). And its therapeutic ability for some neurodegenerative diseases such as Alzheimer's and Parkinson's disease (24)

it is the only one of the seven mono-hydroxyquinoline isomers capable of forming complexes with divalent metal ions from During chelation ,the mineral imbalance is the main cause of many diseases, so 8HQ is a powerful chelating agent that restores mineral balance and is useful in the treatment of metal-related diseases (25) .

Experimental:

Materials and measurements

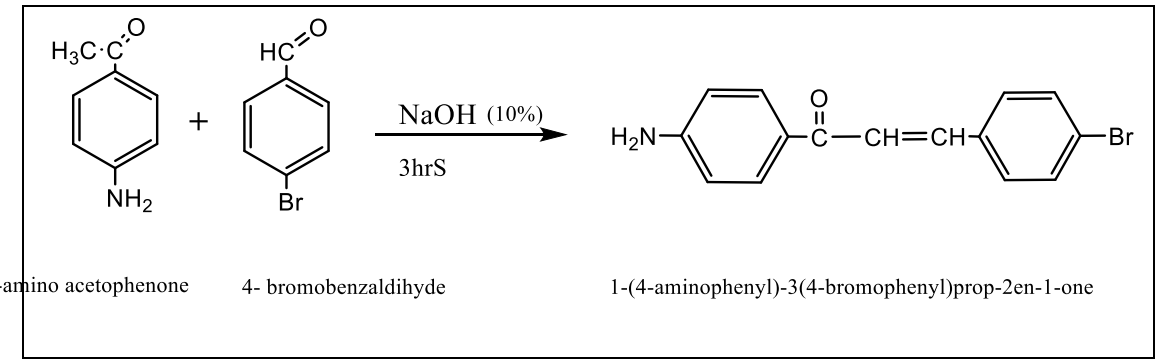
In this search all chemicals and solvents were high purity, and supplied from different companies such as Sigma Aldrich, BDH and Fluka. In addition, many equipment has been used. FTIR Spectra measured by Shimadzu FTIR 8400 in the range of (4000-400) cm^{-1}. (UV-Vis) Spectrophotometer was studied by Shimadzu UV-Vis.1700 double beam (200-1100) nm. Elemental Analysis (C.H.N.) and Metal ratio were recorded by Elemental Analyses system Instrument: Flash EA/1112-thermofinniganco and Shimadzu AA-66300Atomic Absorption/Flame Emission Spectrophotometer. Molar conductivity measurements were taken at room temperature in DMSO ($1x10^{-3}$) M by 470 WTW apparatus. Magnetic Susceptibility were recorded by Faraday method by using Sherwood scientific Balance apparatus. Mass Spectrum was carried by Shimadzu Agilent Technology (HP) Mass selective Detector (50-230)0c at (70EV). ^{1}HNMR Spectrophotometer was determined in

DMSO-d6 solvent in Bruker GmbH500 MHZ. Melting point measurements were recorded by Stuart Melting point (SPM10)

Preparation of the chalcone –azo ligand

A- Preparation of derivative chalcone

Claisen-Schmidt condensation was adopted (26) to prepare amino Chalcone by dissolving (2.7gm,0.04mol) of (4-Aminoacetophenone) in (25ml) ethyl alcohol and (0.025mol ،1gm) of sodium hydroxide dissolved in (50ml) distilled water And (25ml) ethanol while cooling the mixture in a round-bottom flask (250ml) by placing it in an ice bath (30 min), followed by the gradual addition of a solution (3.7gm, 0.04mol) of 4-Bromobenzaldihyde dissolved in (50ml) of ethanol with stirring. The continuous process was carried out for three hours at room temperature, after which it was observed that a thick yellow solution formed, it was kept in the refrigerator for the next day, then the contents of the flask were poured into a beaker with a capacity of 1000ml containing ice grits and acidification was carried out with dilute hydrochloric acid, a dark yellow precipitate was observed. It was filtered and washed several times with cold distilled water, dried and recrystallized with hot acetone, and the melting point at (196-198 ºC) Scheme (1) shows of the reaction

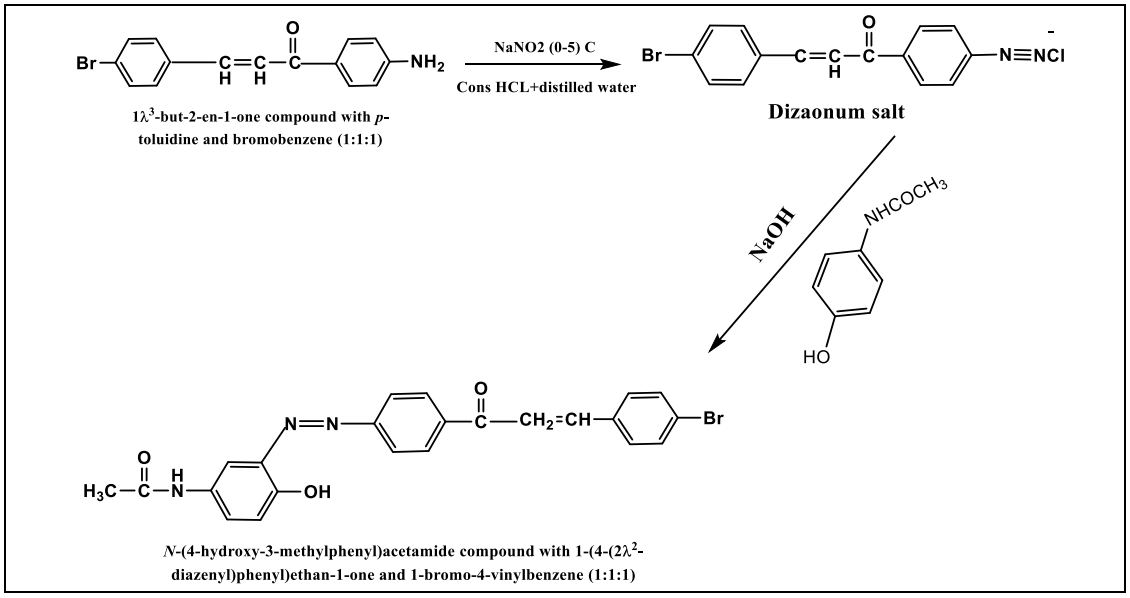


Scheme (1) Preparation of chalcone

B-Preparation of chalcone-azo ligand

Two step to preparate of azo-chalcone ligand included dissolving (3.02g,0.01mol) from derivative chalcone (in 100ml) of acetone with 18% HCl solution in (0-5 ˚C) and added (0.7g, 0.01mole) NaNO2 dissolved in 10 ml distal water drop by drop with continuous stirring and maintaining the temperature within the mentioned range for a period of(15 minutes) to ensure the formation of diazonium salt which added with dropping and stirring to ice basic solution of Paracetamol (2.8gm,0.01mol) dissolved in 100 ml ethanol and(15ml) sodium hydroxide solution (10%) at (0- 5˚C) Continuous stirring where red precipitate was observed, left for the next day to complete reaction after that, it acidified by dil. HCl at PH = (6.5-7) then, it filtered, washed with cold distilled water to remove sodium chloride salt and were

crystallized with hot acetone, then drieds, and calculated the percentage of the product .Scheme.2.



Scheme (2) Preparation of azo- chalcone ligand

Preparation of Mix Ligand Complexes

The chelate complexes of the ligand mixture prepared by the same steps were followed to dissolve known weights of the metal salts whose solid complexes were desired, which are all of the complexes of cobalt (II), nickel (II), copper (II), zinc (II) and cadmium (II). Solutions salts were combined with a (0.464mol , 0.001 g) of organic ligand (azo-chalcone) and (0.001 mol, 0.145 g) of ligand (8- hydroxy quinoline) in a molar ratio (1: 1: 1) (M: L: L) and the solutions of the complexes were heated. For 20 min, complex deposits were obtained after getting rid of the acetone. hot acetone, the precipitate were collected ,dried and melting point were recorded .Table (2) show some physical properties of (L1) and (L2) and their complexes

Cytotoxic Activity of Ni (II)complex

A different concentrations of [Ni(L_1)(L_2)] was used to inhibit cell growth of breast cancer cells (MCF-7) (6.25-100) µg/ml as well as healthy cells (MCF-10A) number of the viable cells of (MCF-7) after treatment with Ni(II) complex is 31.51% at the concentration 50 µ g/ml, while the number of viable cells of (MCF-10A) at the same concentration is 92.94% .The highest inhibition ratio of the (MCF-7) after addition of Ni(II) complex is 68.49 % at the concentration 50 µ g/ml, compared with the highest inhibition ratio of (MCF-10A) is 7.06% at the same concentration .The results presented that , 50 µ g/ml [Ni(L_1)(L_2)] is an excellent concentration to kill more than half of damaged cells and has less effect on healthy cells , which is indicated that Ni(II) complex could be used as an important therapy breast cancer (27) .

Table (1): Effect of [Ni(L_1) (L_2)] on breast cellular cancerous cell (MCF-7) Viability compared with healthy cells (MCF-10A) at the same concentration using 24 hours MTT test at 37 C^0

Con. (µg. mL^{-1})	Mean Percentage (%) for each cell line			
	MCF-7		MCF-10A	
	Cancerous line cells of MCF-7		Normal line cells of MCF-10A	
	Cell Viability	Cell Inhibition	Cell Viability	Cell Inhibition
6.25	90.43	9.57	96.80	3.2
12.5	80.61	19.39	96.77	3.23
25	61.73	38.27	95.44	4.56
50	31.51	68.49	92.94	7.06
100	23.60	76.4	63.83	36.17

Results and discussion:

The ligand and its chelate complexes were isolated in their pure solid form preparation for proving their chemical formula by many spectral and analytical means, including the infrared, ultraviolet - visible spectrum. The mass spectrum and the nuclear magnetic resonance spectrum of the proton were recorded to ligand, as well as the ratio of carbon, hydrogen, and nitrogen elements in their complexes, molar conductivity, and magnetic moments. The solid complexes are stable at room temperature and soluble in acetone, DMF and DMSO but insoluble in water. The elemental analyses and metal contents data were surmised in table (2) for ligand and complexes are in a good agreement with the suggested

Table (2): Some of physical properties of (L1) and (L2) and their complexes

NO	Formula	M: L	Color	m.p.C Ċ	Yield %	C Found (cal)%	H Found (cal)%	N Found (cal)%	M Found (cal)%
1	$HL_1=C_{23}H_{18}N_3O_3Br$	-	Red	137-140	96	59.5 (59.42)	3.89 (3.34)	9.05 (4.98)	___
3	$[Co(C_{23}H_{17}N_3O_3Br)(C_9H_6NO)(H_2O)_2]$	1:1:1:2	Dark brown	300 Dec	82	54.75 (54.32)	3.86 (3.24)	7.98 (7.43)	8.39 (8.12)
4	$[Ni(C_{23}H_{17}N_3O_3Br)(C_9H_6NO)(H_2O)_{2]}$	1:1:1:2	Dark red	270-272	78	54.77 (54.49)	3.86 (3.38)	7.98 (7.47)	8.36 (8.06)
5	$[Cu(C_{23}H_{17}N_3O_3Br)(C_9H_6NO)(H_2O)_2]$	1:1:1:2	Dark red	202-206	76	54.39 (54.09)	3.83 (3.51)	7.92 (7.44)	8.99 (8.45)
6	$[Zn(C_{23}H_{17}N_3O_3Br)C_9H_6NO)(H_2O)_2]$	1:1:1:2	Red	100-103	85	54.25 (54.02)	3.82 (3.45)	7.90 (7.52)	9.22 (9.04)
7	$[Cd(C_{23}H_{17}N_3O_3Br)(C_9H_6NO)(H_2O)_2]$	1:1:1:2	Dark red	257-259	90	50.87 (50.48)	3.58 (3.22)	7.41 (7.16)	14.88 (14.43)

Mass Spectrum of azo-chalcone ligand

The mass spectrum of the azo-Chalcone ligand (HL) was recorded and it showed many fragments of mass fragmentation with relative abundance, including the peak of fragmentation(464) (M / ž) attributed to a mother molecule (azo - Chalcone) ligand was prepared for the study of this research and is a statement of the correctness of its molecular structure. The fragment at (m/z=421 and 393) corresponding to ($C_{21}H_{16}N_3O_2Br$) and ($C_{21}H_{17}NO$ Br), fragment at (m/z*=365 and 341) which due to ($C_{20}H_{16}NO$ Br) and ($C_{18}H_{15}NO$ Br) Fig.5 and scheme.3, showed the mass spectrum and fragmentation pattern of Azo-Chalcone ligand.

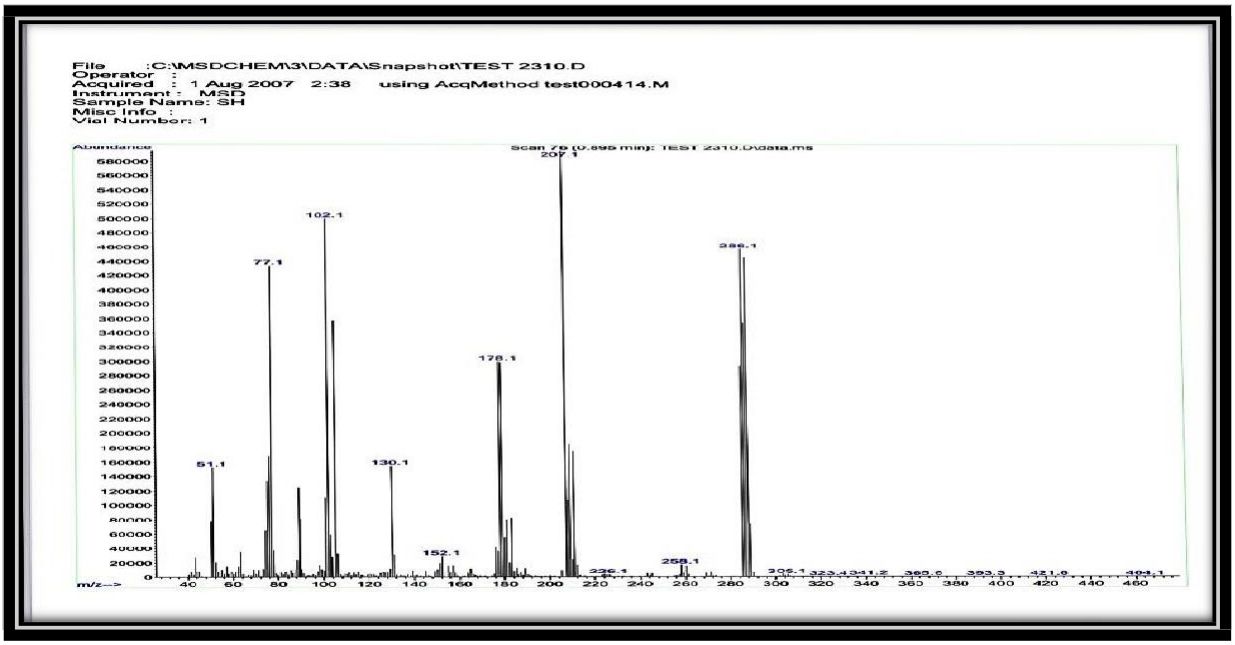
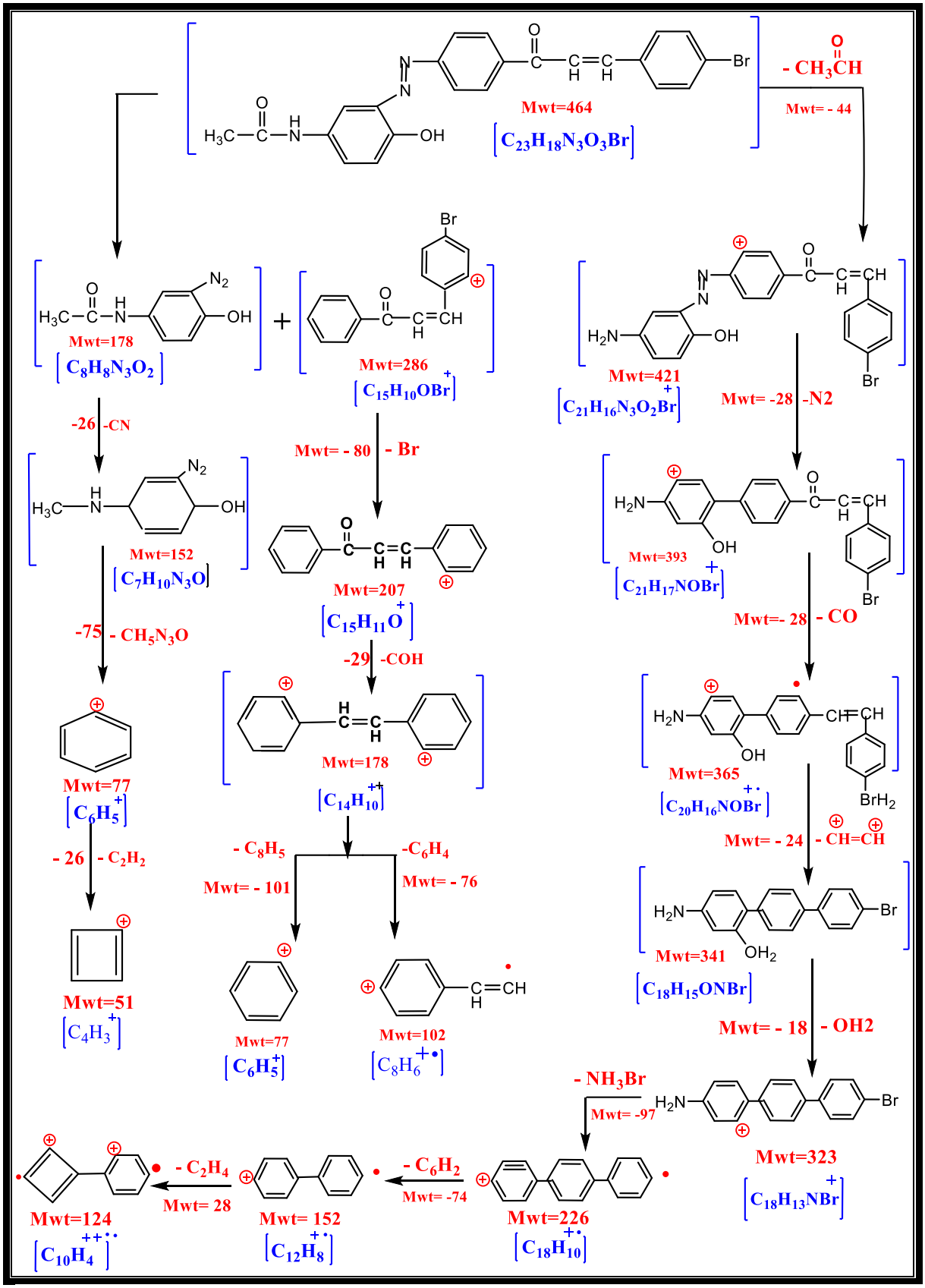


Fig (1) Mass Spectrum of the Ligand (Azo- Chalcone)



Scheme (3): Suggested mass fragmentation pathways for ligand (Azo-Chalcone)

^{1}H NMR Spectra

The results of the ^{1}HNMR spectrum of azo-chalcone at experimental temperature was recorded in DMSO-d6 with TMS as a reference. The spectrum showed signal at (6.9ppm)due to the α protons, the signal at (7.4 ppm) belonged to the β protons due to the effect of electronic succession of the carbonyl group in the beta site(28) ,the signal between (7.6 -8.2 ppm) referred to the phenyl ring (29) and a signal appeared at (2.06ppm) representing the CH_3 aliphatic group of Paracetamol , also a signal showed at (9.98 ppm) due to the OH group and a signal appeared at (10.67 ppm) representing the NH group .

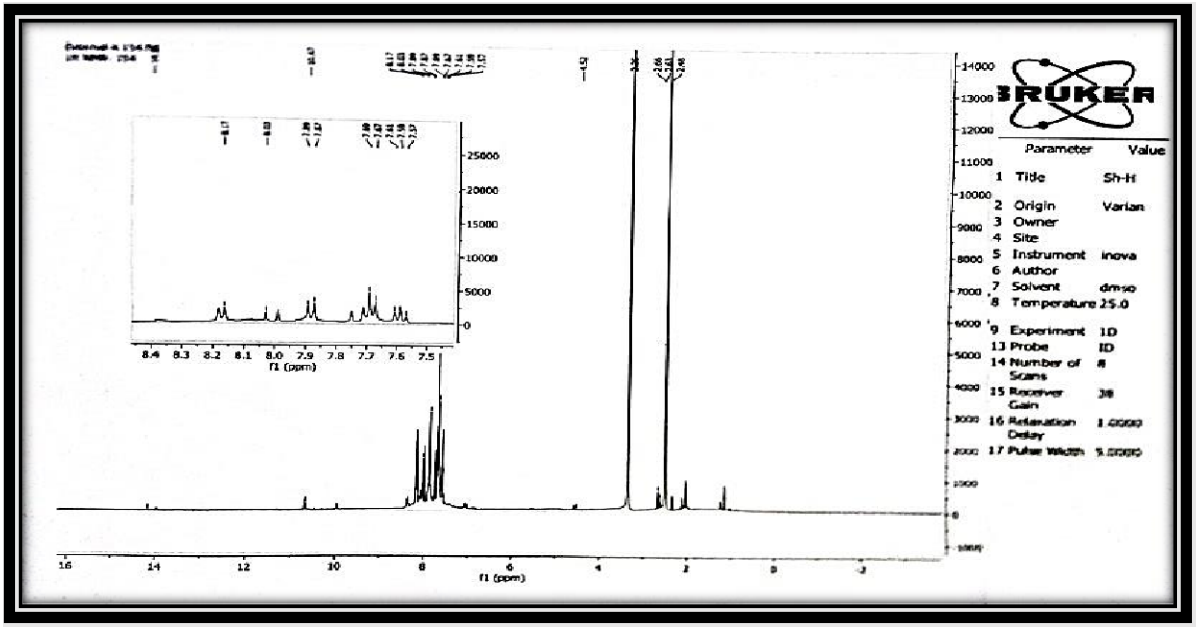


Fig (2) Spectrum of the ^{1}H-NMR proton of the ligand (Azo- Chalcone)

FT-IR Spectra

The FTIR spectra is illustrated noticeable information about functional groups of azo-chalcone, free ligand and mixed ligands complexes. The FTIR spectrum of the azo-chalcone was given an absorption band at the 3433cm^{-1} belonging to the (OH) group of paracetamol overlapping with the (NH) group(30) and the appearance of an absorption band at 1662cm^{-1} due to the carbonyl (C=O) group in Paracetamol (31),absorption band at the site 1489cm^{-1} for the (N=N) azo group (32) , absorption bands at the 2927cm^{-1} and 3057cm^{-1} referred to the aliphatic and aromatic C-H group and an absorption band at the 1020cm^{-1} attributed to the (C-Br) group(31) .The spectra of chelate complexes appeared and an absorption band at 1602cm^{-1} due to (C=N) group in 8-Oxin (33)

changes in functional groups compared with free ligand because of coordination with metal ions. In addition, new bands at (600-400 cm^{-1}) which attributed to (M-N) and (M- O) respectively. Fig(3) and fig(4).

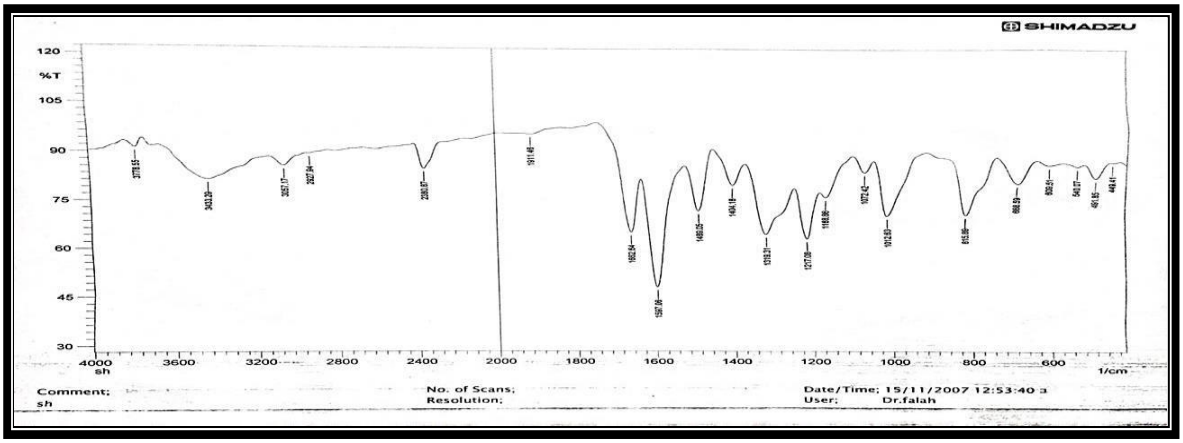


Fig. (3) FT-IR spectrum of (azo-chalcone)

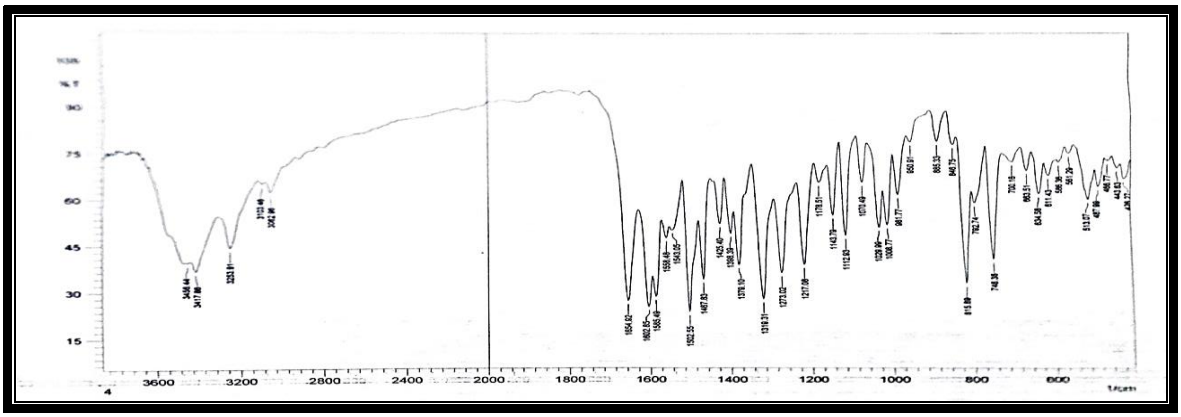


Fig (4) FT-IR spectrum of [Cu($C_{23}H_{17}N_3O_3Br$) (C_9H_6NO) (H_2O)$_2$]

Table (3): Some of physical properties of (L_1), (L_2), and their complexes

Compound	ν(O─H) Paracetamol & $_{H2O}$	ν (C=O)	υ(C=C) Chalcone	υ(C=N) 8-OXIN	υ (N=N)	υ (M –N)	υ (M -O)
$HL_1=C_{23}H_{18}N_3O_3Br$	3433	1653	1598	1597	1489	___	___
$[Co(C_{23}H_{17}N_3O_3Br)(C_9H_6NO)(H_2O)_2]$	3388	1656	1579	1604	1465	487	447
$[Ni(C_{23}H_{17}N_3O_3Br)(C_9H_6NO)(H_2O)_{2]}$	3404	1656	1581	1604	1469	468	445
$[Cu(C_{23}H_{17}N_3O_3Br)(C_9H_6NO)(H_2O)_2]$	3456	1654	1585	1602	1467	466	443
$[Zn(C_{23}H_{17}N_3O_3B)(C_9H_6NO)(H_2O)_2]$	3455	1658	1562	1604	1467	487	449
$[Cd(C_{23}H_{17}N_3O_3Br)(C_9H_6NO)(H_2O)_2]$	3415	1658	1581	1602	1462	493	447

Electronic spectra and Magnetic susceptibility

UV-Visible Spectroscopy, one of the significant methods in which electronic spectral data were recorded and compared with free ligand. In this study the measurement was solvated in DMSO solvent at room temperature, where the spectrum of the ligand (azo-chalcone) appeared shows two main bands, the first one at (262) nm for the ($\pi \rightarrow \pi^*$) and band at (320) nm for the electronic transition ($n \rightarrow \pi^*$) which attributed to the azo group (N=N) , this band agonized from a red shift to longer wavelengths depending on coordination with chelate metal ions(34). The table (4) includes the electronic transition of Co (II), Ni (II), Cu (II), Zn (II) and Cd (II) complexes with charge transfer transition .The value of the magnetic moment gives information about predictable coordination of the metal ion and it suggests the geometric shape of complex's .The magnetic moment value of Co(II)complex suggested an octahedral geometry with high spin state. In addition, the high spin state of Ni (II) complex and Cu (II) complex's which seems to be an octahedral geometry.

However, metal complexes of Zn (II), Cd (II), were diamagnetic because of Zn (II), Cd (II)have d^{10} with an octahedral geometry (35).

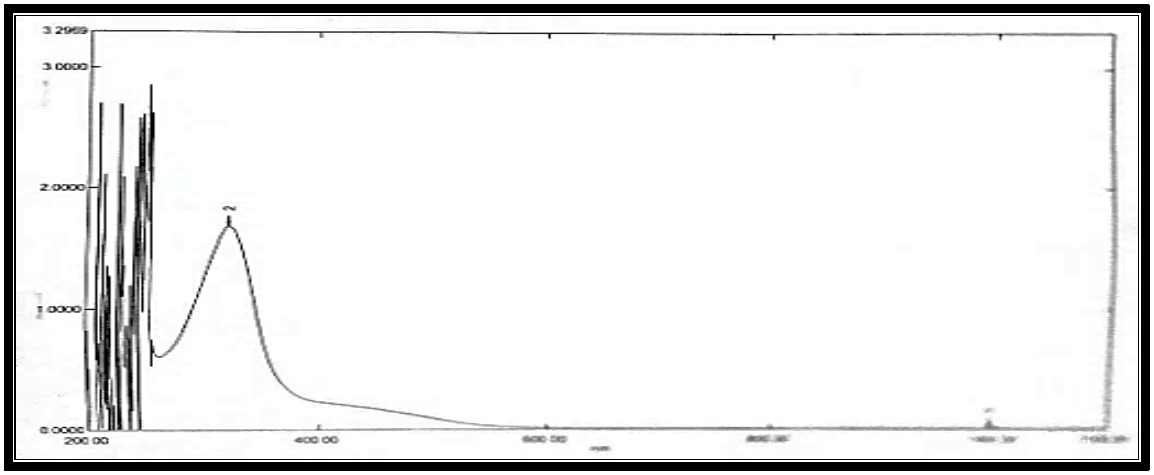


Fig. (5)) UV-Vis spectrum of (azo-chalcone)

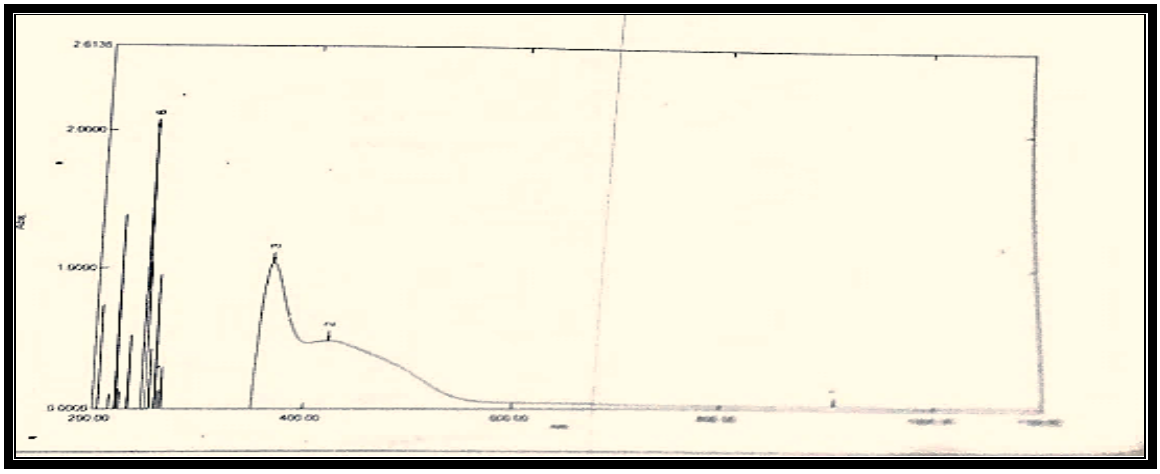


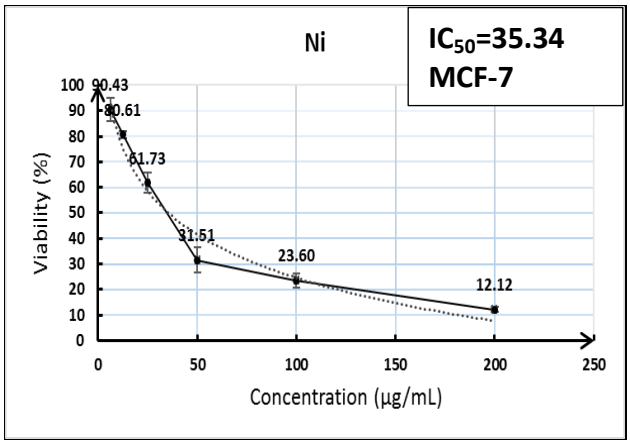
Fig. (6)) UV-Vis spectrum of [Zn($C_{23}H_{17}N_3O_3Br$) (C_9H_6NO) (H_2O)$_2$] complex

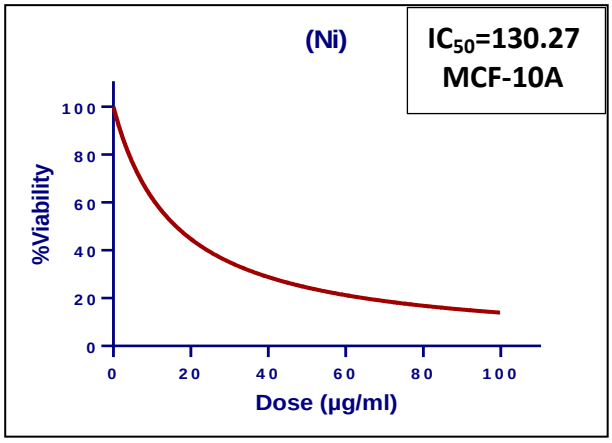
Table (4): Electronic spectra, Conductivity and Magnetic Moment of Ligands and its Complexes

Compound	Absorption bands(nm)	Transition	Conductivity $S.cm_2.mol^{-1}$ in(DMSO)	μeff (B.M)
$HL_1=C_{23}H_{18}N_3O_3Br$	262 320	π →π* n→π*	----------	----
$[Co(C_{23}H_{17}N_3O_3Br)$ $(C_9H_6NO)(H_2O)_2]$	371 402	π →π* MLCT	16.6	3.9
$[Ni(C_{23}H_{17}N_3O_3Br)$ $(C_9H_6NO)(H_2O)_2]$	379 410	π →π* MLCT	5.2	2.9
$[Cu(C_{23}H_{17}N_3O_3Br)$ $(C_9H_6NO)(H_2O)_2]$	368 421	π →π* MLCT	18.4	1.76
$[Zn(C_{23}H_{17}N_3O_3Br)$ $(C_9H_6NO)(H_2O)_2]$	364 420	π →π* MLCT	1.6	Dia
$[Cd(C_{23}H_{17}N_3O_3Br)$ $(C_9H_6NO)(H_2O)_2]$	377 404	π →π* MLCT	14.1	Dia

Cytotoxic Activity of [Cd (L_1) (L_2)(H_2O)$_2$] on breast Cancerous cells line (MCF-7)

The result illustrates that, IC50=35.34 for breast cancer cells (MCF-7) while IC50=130.27 for healthy cells (MCF-10A) which explains that , the concentration which kills half of (MCF-7) is the lowest the concentration to kill (MCF-10A) , that is approved an importance of Ni(II) complex as new treatment for inhibition of breast cancer cells.





Fig(7) Anticancer activity data of Ni (II) complex cells against unhealthy

Anticancer activity data of Ni(II) complex cells against healthy

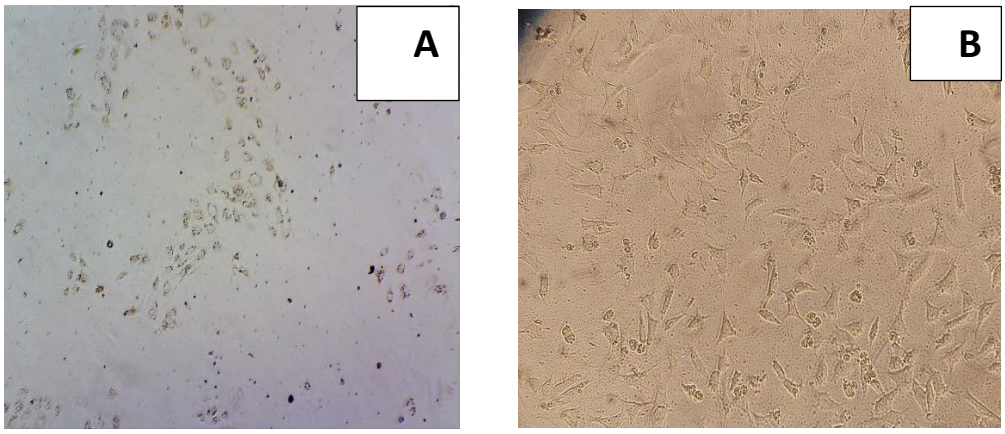
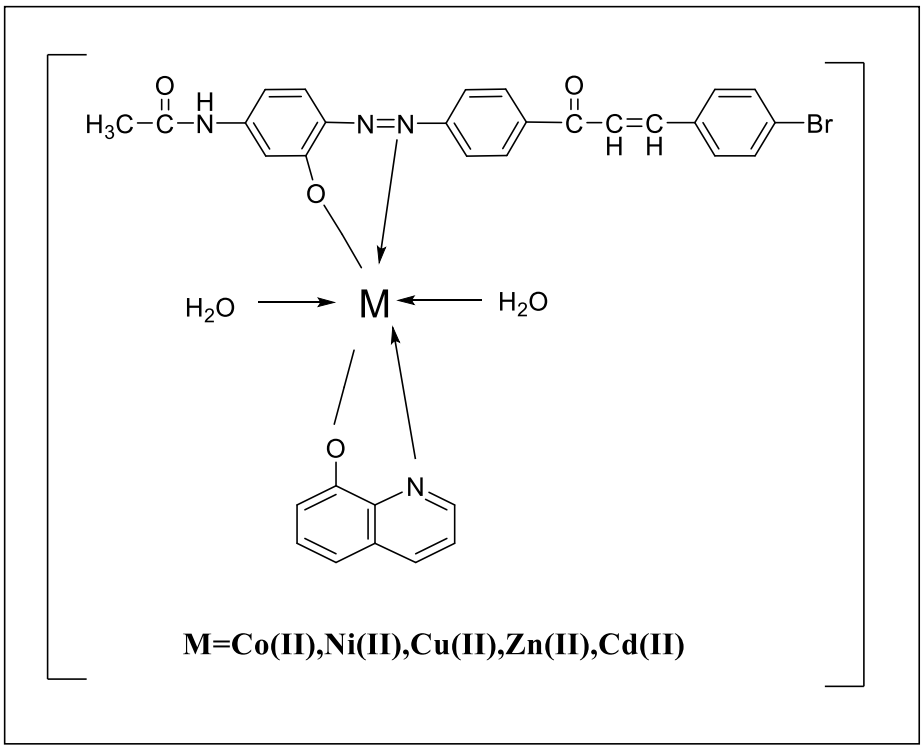


Fig (8): Anti- cancer activity of Ni (II) on: (A)(MCF-7) at 50µg /ml and (B) (MCF-10A) at the same conc. Under inverted microscope

Conclusion

This paper contains new mixed 8-oxin and Azo-Chalcone ligands and its complexes were prepared and characterized by spectral equipment. The results appeared an octahedral geometry for all complexes. In addition, Ni (II) complex has high cytotoxicity which suggested the possibility of using as new anticancer medicine.



Fig(9): Suggested geometries of the mix ligand complexes

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