Preparation and Characterization of New Azo-Chalcone Mixed with Imidazole Ligand Coordinated with Some Chelate Complexes and Study Antitumor Activity

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Abstract

A new series of some transition metal complexes were prepared from reaction mix ligands of new azo-chalcone with imidazole and Co, Ni, Cu, Zn and Cd (II) ions. New azo-chalcone prepared by two steps from reacted 4-bromo benzaldehyde with 4-aminoacetophenone to obtain chalcone after that reacted with paracetamol to result new azo-chalcone. The results show that the mole ratio (metal - ligand) is (1:2:2) for all the complexes under study. The ligand characterized by 1HNMR, 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 was proposed for all chelate complexes. The effect of the biological examination of Cd (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

1. Introduction

Mixed ligand complexes of transition metal ions have great interesting from the researchers who study their coordination behavior and exploiting their properties in a different field. Azo dyes are among the most important organic ligands because of their high sensitivity and selectivity to the presence of more than one coherence site in their functional group (N = N)
[1] which is the reason for their stability [2]. These dyes have a history of coloring, as they have a chromosomal force of approximately twice the chromosomal strength of anthraquinone pigments [3, 4].
Chalcones are an unsaturated ketone system and are colored compounds of flavonoids [5], a class of yellow crystalline pigments. Chalcones are found in hydroxyl form [6].
Azo dyes and chalcone dyes are important classes and are abundantly present in plants, fruits and vegetables, azo dyes and chalcone dyes are compounds that have systems linked to heterogeneous aromatic rings or degradable aliphatic groups and have appeared in many optical applications. Trans-cis isomerization [4, 7] and the two azo-chalcone formulas exhibit broad-spectrum biological and optical properties [8] and are successfully used as chemical sensors and have attracted attention due to their pharmacological activities such as Anti-inflammatories [9], antibacterial [10] antitumor [11] antioxidant anticancer and cytotoxic. These compounds are considered an important factor of beneficial chemotherapeutic agents.
Paracetamol is a chemical compound with molecular formula $C_8H_9NO_2$ [12] and it has pharmacological importance as an important analgesic and antipyretic. It has antioxidant properties as it allows to reduce the oxidation of LDL and reduce the risk to the heart and vessels.
Therefore, based on the promising biological activity of azo-chalcone in medicine and industrial fields, mix ligand complexes were prepared from of azo-chalcone with imidazole as ligand with metal ions Co, Ni, Cu, Zn and Cd (II) and their biological activities were examined

2. Experimental

Materials and measurements

In this search all chemicals and solvents were high purity, it was 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- Visible Spectrophotometer was studied by Shimadzu UV-Vis.1700 double beam (200-1100) nm. Elemental Analysis (C.H.N. and M) was 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 Spectra were carried by Shimadzu Agilent Technology (HP) Mass selective Detector (50- 230)0c at (70EV). 1H-NMR Spectrophotometer was determined in DMSO-d6 solvent in Bruker GmbH500 MHZ. Melting point measurements were recorded by Stuart Melting point (SPM10).

Preparation of the Azo-Chalcone

a- Preparation of Chalcone

Claisen-Schmidt condensation was adopted to prepare amino chalcone by dissolving (1.35gm,0.01mol) of (4-Aminoacetophenone) in (50ml) ethyl alcohol with (10%) of sodium hydroxide dissolved in distilled water while cooling the mixture in a round-bottom flask (250ml) by

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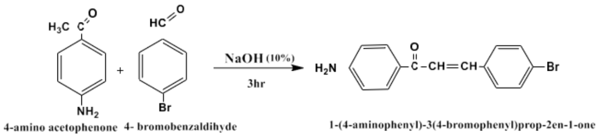
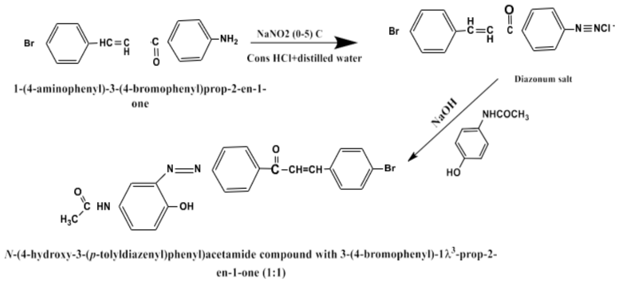
placing it in an ice bath for 30 min , followed by the gradual addition of a solution (1.86gm, 0.01mol) of 4-Bromobenzaldihyde dissolved in (25ml) of ethanol with stirring. The continuous process was carried out for 3 hours at room temperature, after which it was observed that a thick yellow solution 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 was (196-198 ºC). Scheme.1.



Scheme (1) The preparation of Chalcone

b- Preparation of Azo-Chalcone

Two step to preparation of azo-chalcone included dissolved (3.02g,0.01mol) from chalcone (in 100ml) of acetone with 18% HCl solution in (0-5 C) and add (0.76g, 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- 5C) 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 returned It was crystallized with hot acetone, then dried, and calculated the percentage of the product .Scheme.2.



Scheme (2) Preparation of azo- chalcone

Preparation of Mix Ligand Complexes

The chelate complexes of mix ligand were prepared by dissolved metal chloride salts of cobalt (II), nickel (II), copper (II), zinc (II) and cadmium (II)in distal water then added to acetone solutions of (0.0005 g, 0.464mol) of organic ligand (azo-chalcone) and (0.068g, 0.0005 mol) of (Imidazole) in a molar ratio (1: 2:2) (M: L: L) and the solutions of the complexes were heated for 20 min. Its crystals were recrystallized from hot acetone, the precipitate was collected and recorded the melting point of the solid recrystallization.

Table (1): Some of physical properties of (L1) and (L2) and their complexes

NO	Formula	M: L	Color	m.p. C̊	Yield %	C%	H%	N%	M% Calc. (Found)
1	HL1=(C23H18N3O3Br)2	____	Red	137-140	96	59.5 (59.42)	3.89 (3.34)	9.05 (4.98)	____
3	[Co(C23H17N3O3Br)2	1:2:2	Dark-brown	134-137	77	55.7	3.76	12.49	5.25
	(C3H3N2)2]					(55.49)	(3.21)	(12.22)	)5.12)
4	[Ni(C23H17N3O3Br)2	1:2:2	Dark brown	88-90	85	55.72	3.76	12.49	5.23
	(C3H3N2)2]					)55.41)	(3.36)	(12.10)	(5.01)
5	[Cu(C23H17N3O3Br)2	1:2:2	Reddish brown	161-164	80	55.48	3.74	12.44	5.64
	(C3H3N2)2]					)52.24)	(3.25)	(12.32)	(5.32)
6	[Zn(C23H17N3O3Br)2	1:2:2	Red	96-100	78	55.3	3.74	12.42	5.79
	(C3H3N2)2]					(55.11)	(3.35)	(11.19)	(5.58)
7	[Cd(C23H17N3O3Br)2	1:2:2	Orange	250-252	82	53.17	3.59	11.92	9.57
	(C3H3N2)2]					(52.89)	(3.14)	(11.64)	(9.18)

Cytotoxic Activity of Cd (II)complex

A different concentrations of [Cd(L1)(L2)] 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 Cd(II) complex is 33.42% at the concentration 50 µ g/ml, while the number of viable cells of (MCF-10A) at the same concentration is 72.22%.The highest inhibition ratio of the (MCF-7) after addition of Cd(II) complex is 66.58 % at the concentration 50 µ g/ml, compared with the highest inhibition ratio of (MCF-10A) is 27.78% at the same concentration .The results presented that , 50 µ g/ml [Cd(L1)(L2)] is an excellent concentration to kill more than half of damaged cells and has less effect on healthy cells , which is indicated that Cd(II) complex could be used as an important therapy breast cancer

Table (2): Effect of [Cd(L1) (L2)] 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 C0

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	70.79	29.21	95.97	4.03
12.5	62.88	37.12	95.88	4.12
25	53.32	46.68	94.12	5.88
50	33.42	66.58	72.22	27.78
100	20.54	79.46	51.04	48.96

3. Results and Discussion

The ligands and its complexes were isolated in their pure solid form preparation for proving their chemical formula by many spectral and analytical means, including the infrared, ultraviolet and visible spectra. The mass spectra and the nuclear magnetic resonance spectra of the proton were recorded, 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 (1) for ligands and complexes are in a good agreement with the suggested formula.

FTIR Spectra

The IR spectra is illustrated noticeable information about functional groups of azo- chalcone, free ligand and mixed ligands complexes. The FT-IR spectrum of the azo-chalcone was given an absorption beam at the 3433cm^{-1} site belonging to the (OH) group of paracetamol overlapping with the (NH) group [13] and the appearance of an absorption band at 1662cm^{-1} due to the carbonyl (C=O) group [14] and an absorption band at 1597cm^{-1} due to (C=N) group in imidazole ring ,absorption band at the site 1489cm^{-1} for the (N=N) azo group, 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 (27) **.**The spectra of chelate complexes appeared some changes in functional groups compared with free ligand because of coordination with metal ions. In addition, new bands illustrated which attributed to (M-N) and (M- O) respectively.

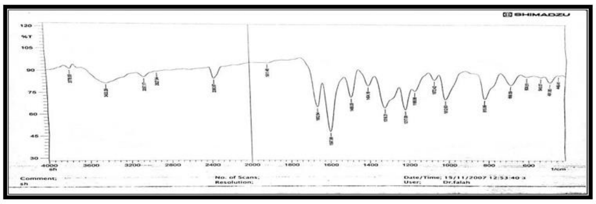


Fig (1) FT-IR spectrum of (azo-chalcone)

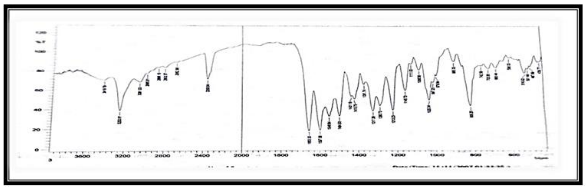


Fig (2) FT-IR spectrum of [Ni(C23H17N3O3Br)2 (C3H3N2)2] complex

Table (3): Some of physical properties of (L1), (L2) and their complexes							
Compound	**ν(O—H) paracetamol**	**ν (C=O)**	**υ(C=C) Chalcone**	**υ(C=N) Imidazole**	**υ (N=N)**	**υ (M –N)**	**υ (M -O)**
HL1=C23H18N3O3Br	3433	1662	______	1597	1489	____	____
[Co(C23H17N3O3Br)2 (C3H3N2)2]	_____	1660	1540	1597	1490	584	489
[Ni(C23H17N3O3Br)2 (C3H3N2)2]	_____	1653	1548	1597	1494	516	439
[Cu(C23H17N3O3Br)2 (C3H3N2)2]	_____	1658	1587	1604	1485	536	441
[Zn (C23H17N3O3Br)2 (C3H3N2)2]	_____	1658	1583	1604	1487	528	447
[Cd(C23H17N3O3Br)2 (C3H3N2)2]	___	1658	1548	1598	1487	497	432

Electronic spectra

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 (π →π*) the second band appeared at (320) nm for the electronic transition (n→π*) 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 [15]. 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 ions and it suggested the geometric shape of complexes. 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), Cu (II) of Zn (II)and Cd (II**)** complexes which seem to be an octahedral geometry. Zn (II) and Cd (II) diamagnetic moments for nd^{10} and the electronic spectra of their complexes don't show any d-d transitions.

Table (4): Electronic spectra, Conductivity and Magnetic Moment of Ligand and its Complexes				
Compound	**Absorption bands (nm)**	**Transition**	**Conductivity S. cm2.mol-1 in (DMSO)**	**Jeff (B.M)**
HL1=C23H18N3O3Br	262-320	π →π*-n→π*	_	___
[Co(C23H17N3O3Br)2 (C3H3N2)2]	409-564	π →π* MLCT	3.5	4.2
[Ni(C23H17N3O3Br)2 (C3H3N2)2]	329-741-533	π →π* MLCT ILCT	4.6	3.01
[Cu(C23H17N3O3Br)2 (C3H3N2)2]	352-524	π →π*-ML CT	8.7	1.78

$[Cd(C_{23}H_{17}N_3O_3Br)_2(C_3H_3N_2)_2]$	350-660-440	π →π* ML CT ILCT	1.8	Día
z				

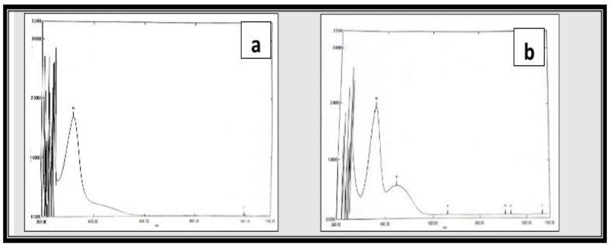


Fig (3) UV-Vis spectrum of(a) Azo chalcone& (b) of $[Cd(C_{23}H_{17}N_3O_3Br)_2(C_3H_3N_2)_2]$ complex

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, the signal between (7.6 -8.2 ppm) referred to the phenyl ring and a signal appeared at (2.06ppm) representing the CH3 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.

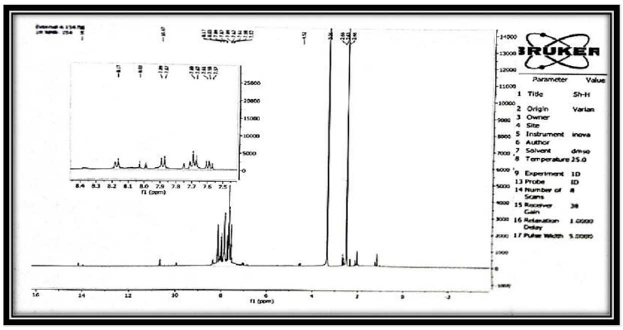


Fig (4) Spectrum of the ^{1}H-NMR proton of the ligand (Azo- Chalcone)

Mass Spectrum of Azo-Chalcone

The mass spectrum of the azo-Chalcone (HL) was recorded and it showed many fragments of mass fragmentation with relative abundance, including the peak of fragmentation (464) (M / z) 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 ($C_{21}H_{17}NOBr$), fragment at (m/z*=365 and 341) which due to ($C_{20}H_{16}NOBr$) and ($C_{18}H_{15}NOBr$) Fig.5 and scheme.3, showed the mass spectrum and fragmentation pattern of Azo-Chalcone ligand

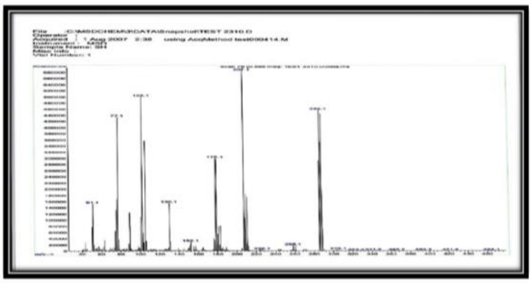
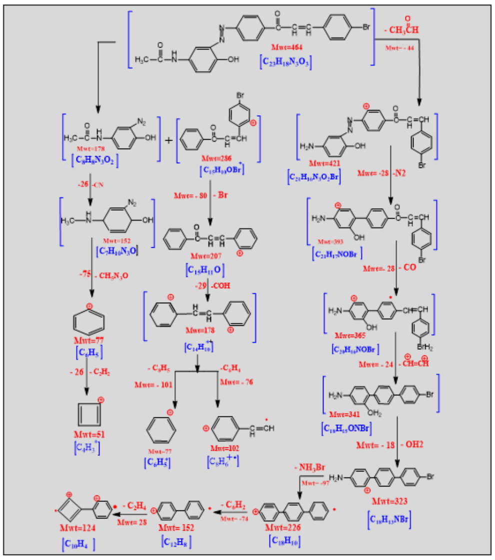


Fig (5) Mass Spectrum of the Ligand (Azo- Chalcone)



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

Cytotoxic Activity of [Cd (L1) (L2)] on breast Cancerous cells line (MCF-7)

The result illustrates that, IC_{50}= 23.24 for breast cancer cells (MCF-7) while IC_{50}=198.41 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 Cd (II) complex as new treatment for inhibition of breast cancer cells.

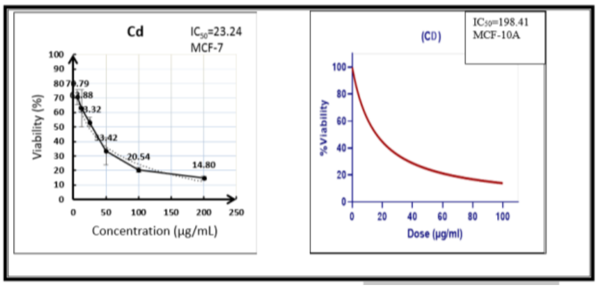


Fig (6) Anticancer activity data of Cd (II)complex cells Against unhealthy
Anticancer activity data of Cd (II)complex against healthy cells

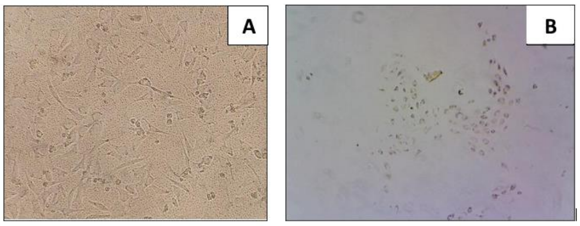


Fig (7): Anti- cancer activity of Cd (II) on: (A)(MCF-7) at 50µg /ml and (B) (MCF-10A) at the same conc. Under inverted microscope

Conclusion

This paper contains new mixed Imidazole 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, Cd (II) complex has high cytotoxicity which suggested the possibility of using as new anticancer medicine.

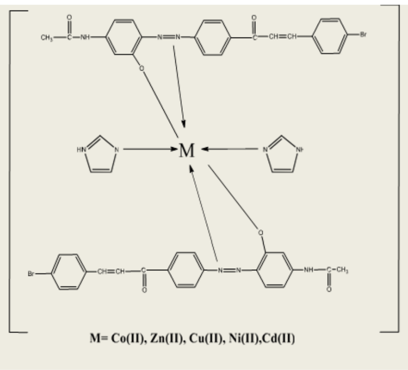


Fig (8) Suggested geometries of the mix ligand complexes

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