Article

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Preparation and Characterization of Mixed Ligands Derived from Chalcone -Azo and 1,10-Phenanthroline with Some of Transition Metal Ions and Study of their Biological Activity of Some Complexes

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Abstract:

This paper reported synthesis a new ligand derived from (chalcone- azo) (DIDMN) which identified by available analytical and spectroscopic means such as (^{1}H-NMR, UV-Visible, FTIR, Mass spectrum and C.H.N), and mixed with (1,10-phen.), The mixture coordinated with (Co, Ni, Cu, Cd, Hg and Pd)(II) in mole ratio (1:1:1) (L:M:L), All complexes have been characterized by (UV-Visible, FTIR, Mass spectrum, ^{1}H-NMR and C.H.N, Magnetic Susceptibility and Molar conductivity), An octahedral geometry was suggested for all complexes, Except Pd(II) complex was square planner, The studying of biological activity appeared the inhibition ability of Pd(II) complex against breast cancer cells type (MCF-7) and also the antibacterial activity of (Hg, Cd) (II) complexes was studied on two types of pathogenic bacteria

Keywords: Anticancer activity ,Bacteria, complexes, Imidazole azo , mixed ligands

Introduction:

Recently, many researchers have been focused on preparation derived chalcones and studying its coordination with variety of metal ions [1,2] this is because these an important organic compounds have two noticeable functional groups (C=C) and (C=O) [3]. In addition, chalcones unsaturated compound that dissolve in organic solvent but not in water, chalcone complexes have been inserted in a wide range of aspects for example, industry , agriculture, pollution[4] and in medicine as anti -cancer, anti-microbial and anti- oxidant [5,6],antitumor[7],anti-HIV [8],Nowadays, the chemistry of derived imidazole attracted number of organic scientists to synthesis deferent types of these compounds and investigated them in medical chemistry to treat multiple diseases such as inflammation, diabetes , Alzheimers disease, anti-cancer and anti bacteria [9-15].Additionally, in the last century there are a significant preparation of derived the mixture of imidazole and 1,10- Phenanthroline[16,17], both compounds coordinated with metal ions through two nitrogen atoms to form a stable five- membered positions with metals which led to low oxidation state with high stability of complexes[18]. The main objective of this research is to prepare and characterize new (chalcone - imidazole) compound as a primary ligand which mixed with 1,10-phen. As a secondary ligand , this mixed coordinated with six divalent ions and characterized by deferent equipment .The second aim is evaluated the biological activity as anti-cancer and anti-bacterial of some complexes to investigate the ability of using them as new medicine.

Experimental and Instruments part :

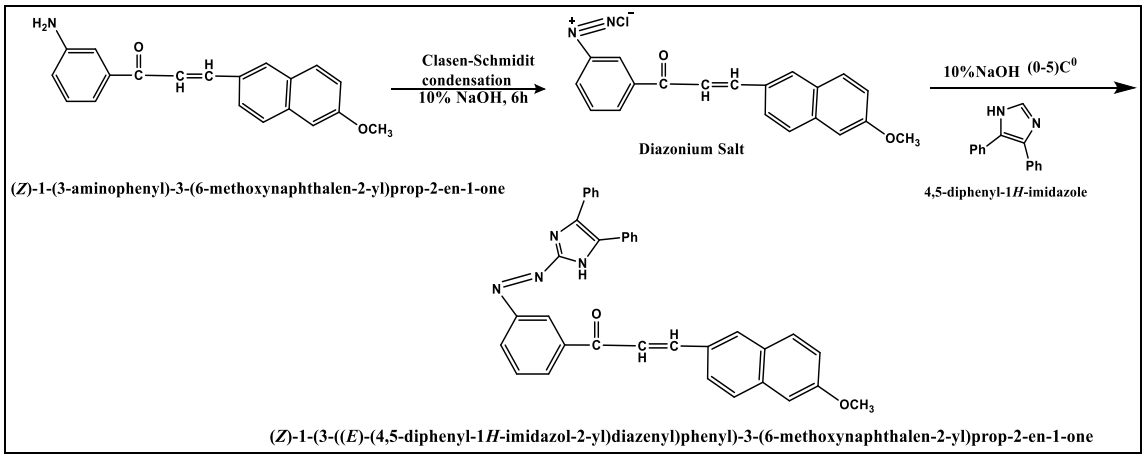
All the chemical and solvents are available purity, the chemicals and solvents were purchased from well - known international factories such as Fluka , Sigma Aldrich and BDH. In addition, the measurements have done by Varity

apparatus as Melting points Measured using (Stuart Melting Point /Germany (SPM10), (C.H.N. and M.) was found by Elemental Analyses apparatus: Flash EA/1112-thermofinniganco and Shimadzu AA-66300Atomic (Abs./F. E. S.), Molar Conductivity Measured thorough by 470WTW Apparatus at laboratory temperature in (DMSO) ($1x10^{-3}$)M., The electronic spectra were measured by Spectrometer Shimadzu (UV-Vis.)1700 at (200-1100)nm. , Magnetic Susceptibility measurements were performed using a Automatic Magnetic Susceptibility Balance Sherwood, College of Science/ University of Al-Mustansiriyah, , Mass Spectra were measured by device type (Shimadzu Technology(5975C) at (70eV)and MSD energy at temperature(50-350)C^0) , The (^{1}HNMR) Spectra were recorded by a Brucker Gmph(400MHZ) spectrophotometer (DMSO-d6 as a solvent)

Synthesis of Chalcones-Imidazole Ligand (DIDMN):

This ligand was synthesized in two steps , the first step involved synthesis of chalcone derivative by solving (0.01mole, 1.35g) from (3-amino acetophenone) in 25ml Abs. (EtOH) and (10)% NaOH, with stirr and cool at (30)min. in the middle of an ice bath. Then, (1.86g, 0.01mole) of (6- Methoxy-2- naphthaldehyde) which solved with 40ml Abs. ethanol and added to the first solution with stirring about 6hrs [19] . TLC. Paper indicates the end of the reaction time by using (Ethanol :Benzene) (3:2)ml , Rf= 0.81 , It forms a thickness yellow solution was observe, which preserved until the next day , After that ,It was acidified with (HCl) in the presence of ice cubes, Formation of a light yellow precipitate was observed, It was nominate , wash with distilled water, and crystallized with boiling acetone, In the second step ,Dissolve the chalcone derivative(0.01 mole, 3.03 g) The formed in the first step in (30 ml) from acetone and (18)% HCl dil. at temp. (0-5)C^0 and (0.74g, 0.01 mole) $NaNO_2$ in 20 ml D.W. [20], Stirred the mixture for 30 min. ,Then add diazonium salt to (2.2g , 0.01 mole) from the (4,5di phenyl imidazole) and soluble in (10% NaOH, 200ml ethanol Absolute). in very cold basic alcoholic solution to precipitate yellow of derivative chalcone-

imidazole ligand, Then it is acidified at (6.5-7) PH filtered ,washed with D.W and re-crystallize with heated acetone.



Scheme [1] : prepare the ligand (DIDMN)

Synthesis of mixed ligands (Chalcone- azo with Phenanthroline) complexes:

The mixed ligand complexes were synthesized by soluble to ion salt of (Co, Ni, Cu, Cd and Hg) (II) in aqueous solution, while ion salt of Pd(II) solved in (CH_3CN) and all metal ions solution added to acetone solution of mixed ligands (DIDMN) and (1,10-phen.) in mole ratio (LM:L)(1:1:1) , it stirred and heated about 30min. After that, the colorful precipitations of complexes of the mixed ligands were dried and re-crystallize with heated acetone.

Table [1]: Analytical and Physical Properties of Mixed Ligand (DIDMN) & (1,10-phen.) & their Mixed Ligand Complexes.

COMPOUND	*(MOLECULAR WEIGHT} g/mole*	*COLORS*	*{MELTING POINT}C^0*	*%YIELD*	*{FOUND} CALC.%*			
					%CARBON	*%HYDROGEN*	*%NITROGEN*	*%M*
$C_{35}H_{26}N_4O_2$ (DIDMN)	534.6	Yellow	187-190	86	78.56 (78.40)	4.86 (5.15)	10.47 (10.12)	---------
[Co(DIDMN)(1,10-phen.)Cl_2]	844.63	Red	192-196	80	66.77 (66.82)	4.02 (4.06)	9.94 (9.89)	6.32 (5.98)
[Ni (DIDMN)(1,10-phen.)Cl_2]	844.39	Red	204-207	79	66.79 (66.52)	4.02 (3.89)	9.94 (9.82)	6.30 (5.87)
[Cu(DIDMN)(1,10-phen.)Cl_2]	849.24	Red	212-216	78	66.41 (66.37)	4.00 (3.96)	9.89 (9.75)	6.78 (6.64)
[Hg(DIDMN)(1,10-phen.)Cl_2]	986.29	Dark yellow	296-300	75	57.18 (57.09)	3.44 (3.11)	8.51 (8.43)	----------
[Cd (DIDMN)(1,10-phen.)Cl_2]	898.11	Dark yellow	282-285	77	62.79 (62.81)	3.78 (3.98)	9.35 (9.14)	12.51 (12.43)
[Pd (DIDMN)(1,10-phen.)]Cl_2	892.12	Dark yellow	274-277	84	62.79 (62.81)	3.78 (3.98)	9.35 (9.14)	12.51 (12.43)

Bio Activity Part:

Cytotoxic activity of [Pd(DIDMN)(1,10-Phen.)]Cl_2:

A different concentrations of [Pd(DIDMN)(1,10-Phen.)]Cl_2 were taken to study the toxicity of the above- mentioned complex on infected breast cells and normal breast cells and compare the inhibitory activity between them (6.25-200)µg/ml, was considered, this search was conducted by using (M.T.T) [3-(4,5-DimethylThiazol-2-yl)-2,5- DiPhenylTetrazoliumBromide](SigmaAldrich)assay., The results of the measurements showed that the cell's ability to retain of ((Mcf7)) reached (76.46)% after the transaction with Pd(II)complex at the conc. (50)ppm., While the cell's ability to retain of ((HDF)) It arrived to(87.61)% for the same conc. mentioned above, In addition to the maximum inhibition percentage of the ((Mcf7)) after the transaction Pd(II) complex is (23.54)% at the conc.(50) ppm., While the maximum inhibition percentage of ((HDF)) is (12.39)% for the same conc. above, Finally the results of this study showed that

presented that ,(50)ppm. [Pd(DIDMN)(1,10-Phen.)]Cl_2 is an excellent concentration to kill more than half of damaged cells and has less effect on normal cells , Which is recommended to used Pd(II) complex as a new therapeutic agent for breast cancer type ((Mcf7)).

Table [2]: Inhibiting Activity of pd (II) Complex Towards Breast Cancer Cells line ((Mcf7)) and healthy cells ((HDF)) at 24h.,(37)C^0

CONC. (ppm.)	Percentage Rate (%) for Each Cell Line			
	{Mcf7}		{HDF}	
	Infected breast cells		Healthy breast cells	
	Cell Retention Capacity	Cell Inhibition.	Cell Retention Capacity	Cell Inhibition.
6.25	98.37	1.63	97.38	2.62
12.5	88.07	11.93	92.68	7.32
25	84.71	15.29	91.23	8.77
50	76.46	23.54	87.61	12.39
100	69.20	30.8	79.39	20.61
200	63.45	36.55	63.47	36.53

Anti- bacterial activity of [Cd(DIDMN)(1,10-Phen.)Cl_2]and [Hg(DIDMN)(1,10-Phen.) Cl_2]:

The antibacterial activity of cadmium and mercury complexes was carried out on two types of pathogenic bacteria ,Gram-positive bacteria ((*S. aureus*)) &((*Enterococcus faecium))*and Gram- negative bacteria *((E. Coli.))& ((Proteus mirabilis))* ,A serial dilution was prepared by mixing (1ml)of each complex with 1ml of DMSO (V/V) .,This process was carried out using the agar well diffusion methods. After the preparation of Mueller Hinton Agar medium , The chosen pathogens were swabbed along the solidified agar face, followed by making(6) holes in agar plates with a (5mm) diameter and then fill each hole with(100µl) of each fresh bacterial isolates at increasing conc. of (6.75,12.5, 25,50 and 100) µg/ml., Then leave it to dry for ten minutes at (37) C^0 for (24)hrs. ,The biological

activity was measuring by calculating mean inhibition zone for each bacterial isolated.

Table [3]: Biological activity for(Cd (II) &Hg (II)) complexes on (E .coli),(P. mirabilis)

Gram Negative Bacteria(G-)	Complexes	Mean Inhibition zone* (mm) of a concentration:				
		100%	50%	25%	12.5%	6.75%
E. Coli.	[Cd (DIDMN)(1,10-Phen.)Cl_2]	13	15	15	----	----
	[Hg(DIDMN)(1,10-Phen.) Cl_2]	16	16	20	16	16
Proteus mirabilis	[Cd (DIDMN)(1,10-Phen.)Cl_2]	16	20	16	–	–
	[Hg(DIDMN)(1,10-Phen.) Cl_2]	19	16	16	15	15

Table [4]: Biological activity of Cd (II) , Hg (II) complexes on (E. faecium),(S. aureus)

Gram Positive Bacteria (G+)	Complexes	Mean Inhibition zone* (mm) of a concentration:				
		100%	50%	25%	12.5%	6.75%
Enterococcus faecium	[Cd (DIDMN)(1,10-Phen.)Cl_2]	15	17	15	__	__
	[Hg(DIDMN)(1,10-Phen.) Cl_2]	18	18	19	19	20
Staphylococcus aureus	[Cd (DIDMN)(1,10-Phen.)Cl_2]	18	18	18	18	19
	[Hg(DIDMN)(1,10-Phen.) Cl_2]	18	18	18	19	20

Result and Discussion:

(^{1}H-NMR) Spectrum of (DIDMN)Ligand:

The main signals in the ^{1}H-NMR Spectrum of Ligand were characterized as the following: The multiple signals were seen in the range δ(7.5-8.5) ppm which were due to the aromatic protons[21], while two significant signals at δ(6.9)ppm and δ (7.4)ppm attributed to protons of derived chalcone (CH=CH-CO)[22] , In addition the signal at δ (13.6) ppm it is due to the protons of the imidazole derivative (N-H) [23], and significant signal at δ (3.5) ppm the methoxy group (O-CH_3) [24].

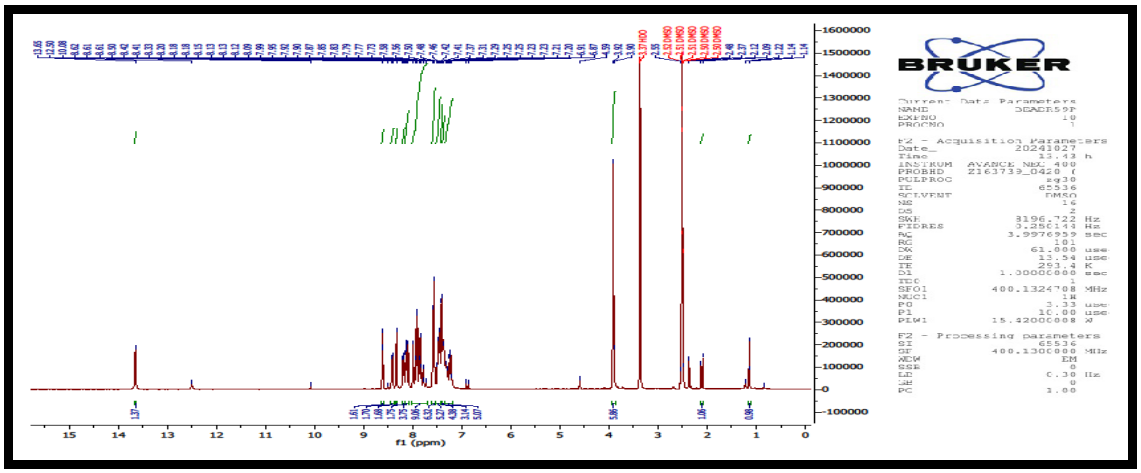
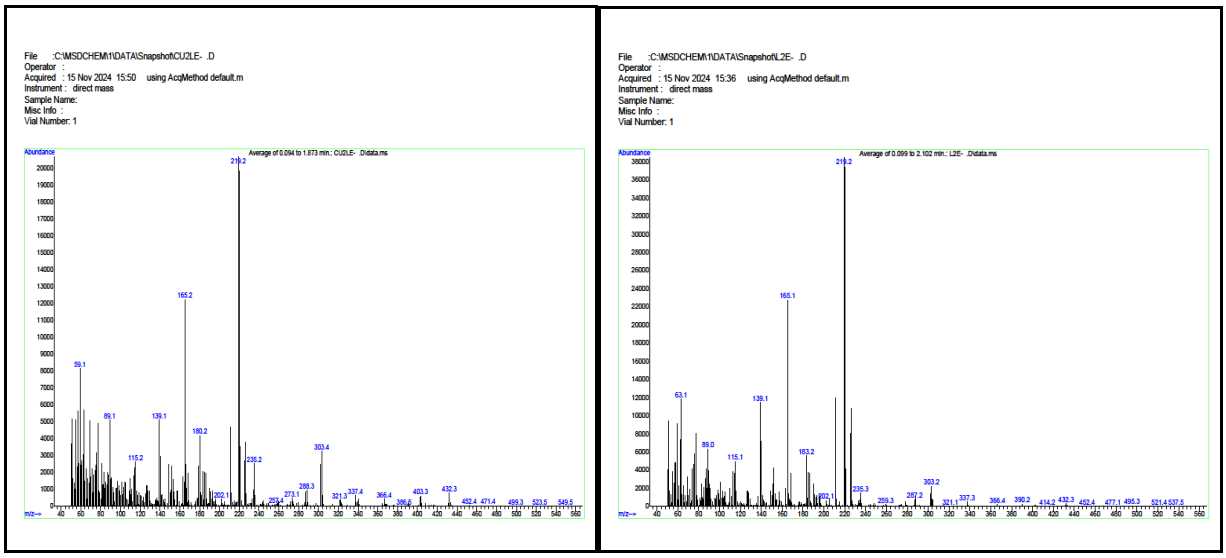


Figure [1]: (^{1}HNMR) Spectrum of (DIDMN)Ligand

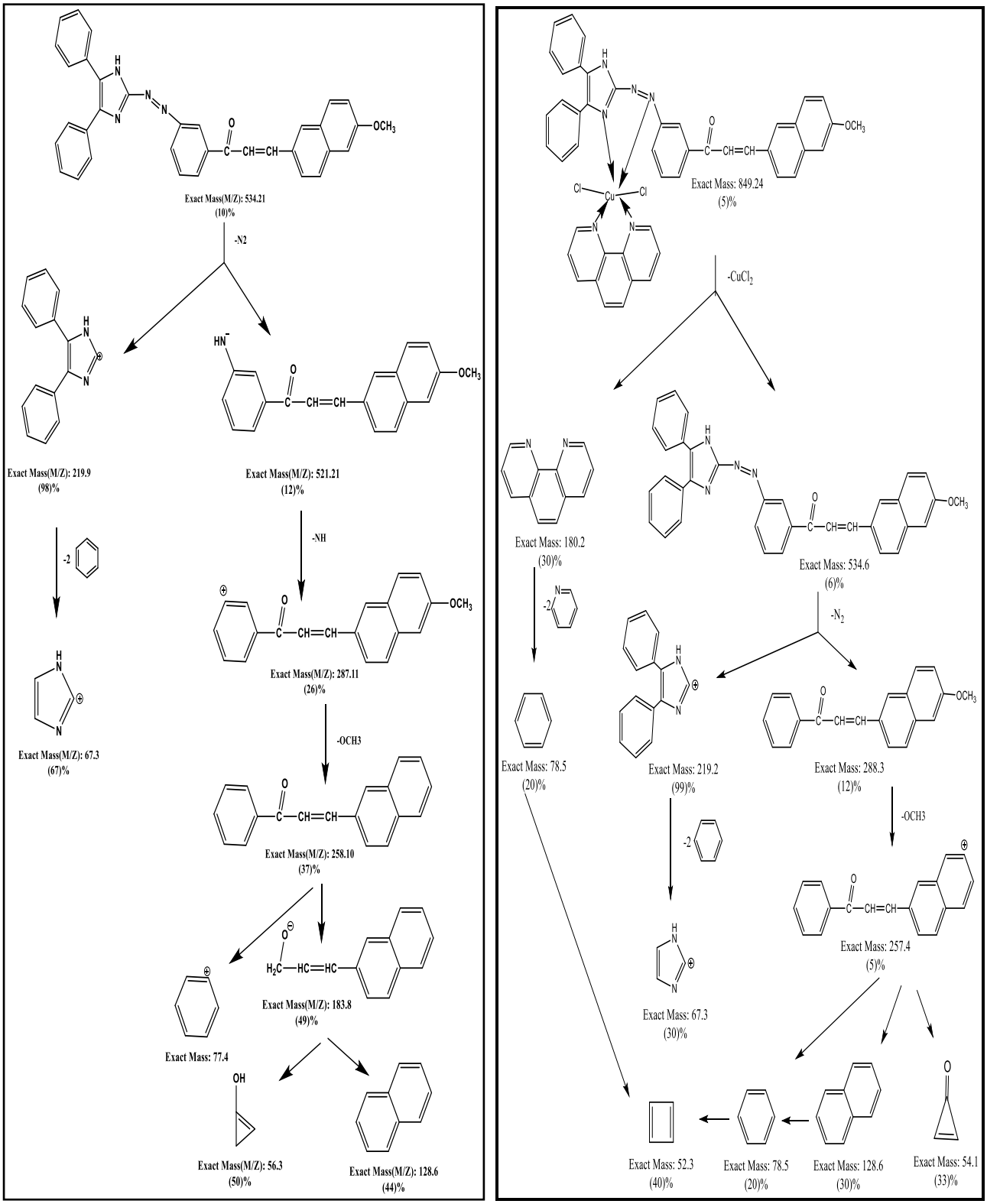
Mass Spectra of (DIDMN)Ligand and [Cu(DIDMN)1,10-Phen.)Cl_2]complex:

The Mass fragmentation pathways of both ligand (DIDMN) and Cu(II) complex shows loss of (N2) molecule for azo group at (m/z* = 521.2) and showed the peak of (1,10-phen.) at (m/z* = 180.2) , also the molecular peak at (m/z* = 219.2) of derived imidazole . The figure (2) and scheme (2).The explain for results of the proposed mass fragmentation pathways for both (DIDMN) and Cu (II) complex.



(A) (B)

Figure [2]: (A) Mass Spectrum of (DIDMN)Ligand & (B) Mass Spectrum of Cu(II).



(A) **(B)**

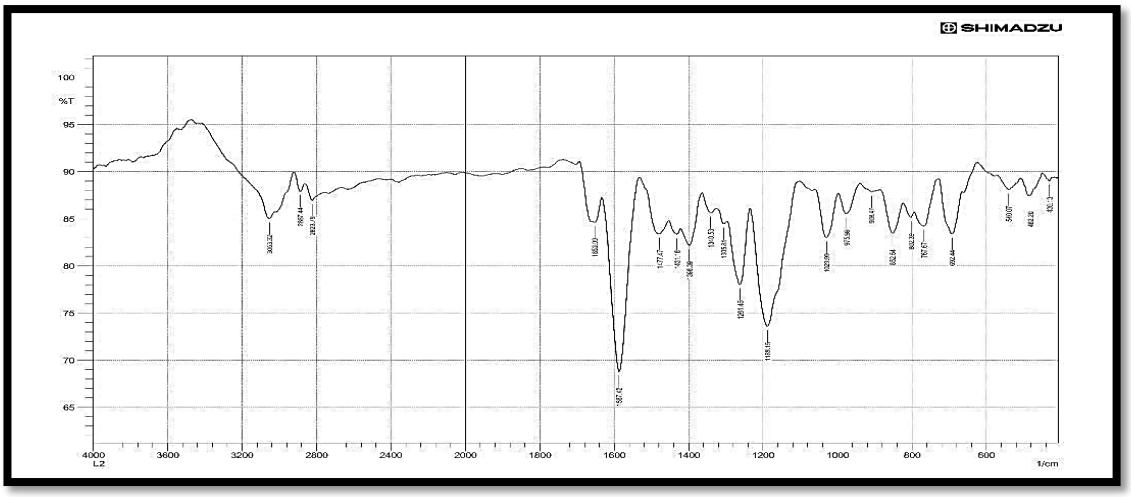
Scheme [2]: (A) Mass fragmentation paths of (DIDMN)Ligand & (B) Mass fragmentation paths of Cu(II)complex

(Infrared Spectra) of (DIDMN) Ligand and it is Mixed Ligands Complexes:

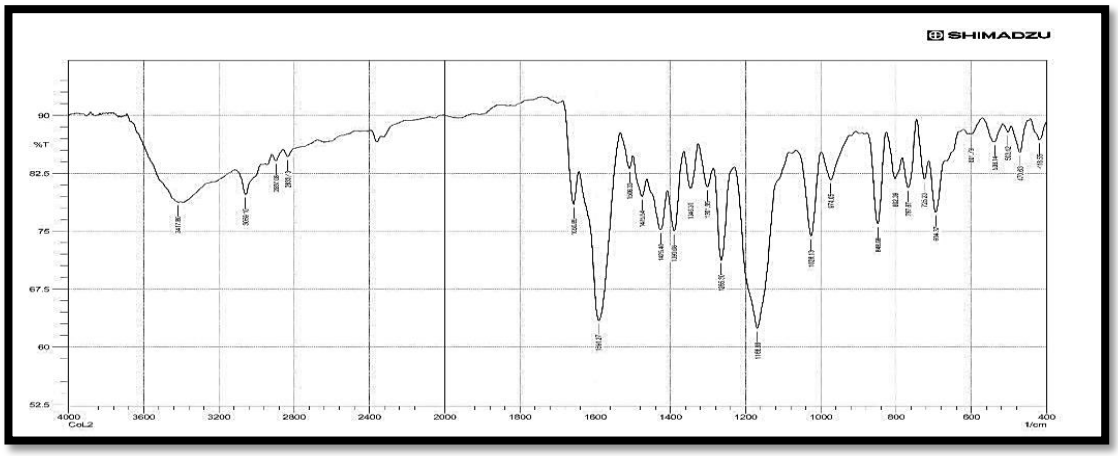
The data of infra-red spectrophotometer were showed a noticeable shifting of υ(C=N) of primary ligand (DIDMN) and secondary ligand (1,10-phen.) as a result of coordination through their nitrogen atom [25,26] . In addition, the frequency of υ(N=N) of primary ligand were shifted because of participated with metal ions through one of nitrogen atom [27], while υ(C=O) of derived chalcone did not share with coordination [28]. The appearance of new peaks indicates the bonds υ(M-N) present in the complexes in the IR spectrum [29].

Table [5]: (FT-IR) spectral data (cm^{-1}) of ligand (DPIAB) and Complexes

Compound	υ (N-H)	υ (C=N)	υ (N=N)	υ(C=O) Chalcone	υ(M-N) Imidazol	υ(M-N) 1,10phen.
(DIDMN)=L_2	3415	1587	1438	1647	_____	_____
[Co(DIDMN)(1,10phen.)]Cl_2	3417	1591	1425	1656	470	418
[Ni(DIDMN)(1,10phen.)Cl_2]	3377	1589	1427	1656	472	420
[Cu(DIDMN)(1,10phen.)Cl_2]	3414	1585	1402	1647	484	422
[Hg(DIDMN)(1,10phen.)Cl_2]	3414	1585	1417	1649	540	480
[Cd(DIDMN)(1,10phen.)Cl_2]	3415	1585	1427	1645	472	408
[Pd(DIDMN)(1,10phen.)]Cl_2	3414	1589	1415	1651	491	439



Figure[3]: FTIR Spectrum of ligand (DIDMN)



Figure[4]: FTIR Spectrum of Co (II) complex

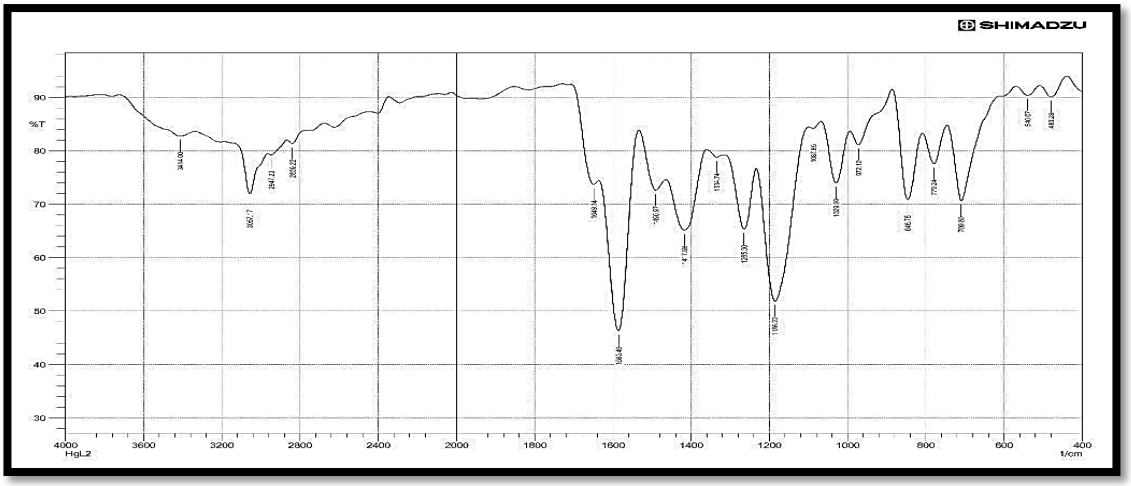


Figure [5]: FTIR Spectrum of Hg (II) complex

Electronic Spectra of (DIDMN) Ligand and mixed ligands complexes:

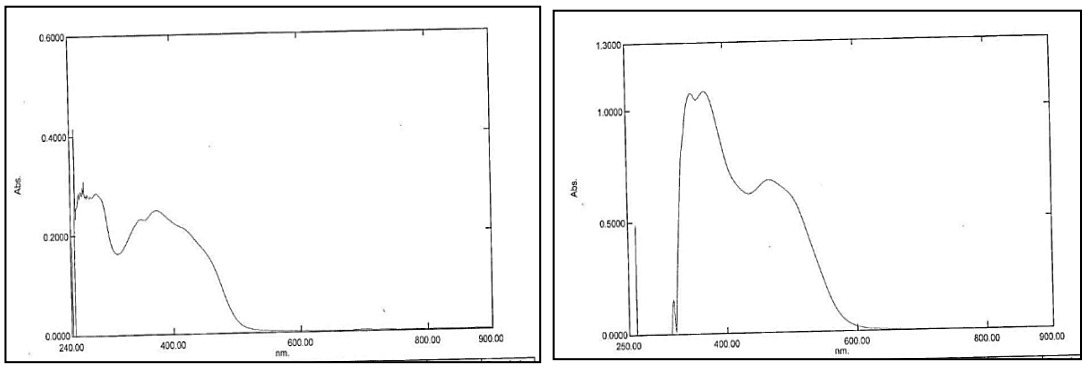
The primary ligand (DIDMN) and mixed ligands complexes were dissolved by acetone solution at (10^{-4})M, the electronic absorption spectral results of complexes illustrated red shift this is because the matching of mixed ligands via donating atoms with vacant orbital's of the metal ions [30]. The results were noticed in the table (6).

Analytical Measurements (Molar Conductivity and Magnetic Susceptibility):

Molar conductance measurements of all metal complexes were checked at the laboratory temperature for (10^{-3}**)** M of (DMSO) solution, The results presented non-ionic properties of all complexes except Pd(II) complex which was an ionic properties. The Magnetic Susceptibility indicated that, Para magnetic properties with high spin of (Co, Ni, Cu) (II) complexes. However, Dia magnetic properties with low spin of (Cd, Hg, Pd) (II)complexes. All results have been established in the table(6). The results of mentioned measurements were suggested an Octahedral shapes for all prepared complexes but a square planer shape of Pd (II) complex, as structured in figure (8).

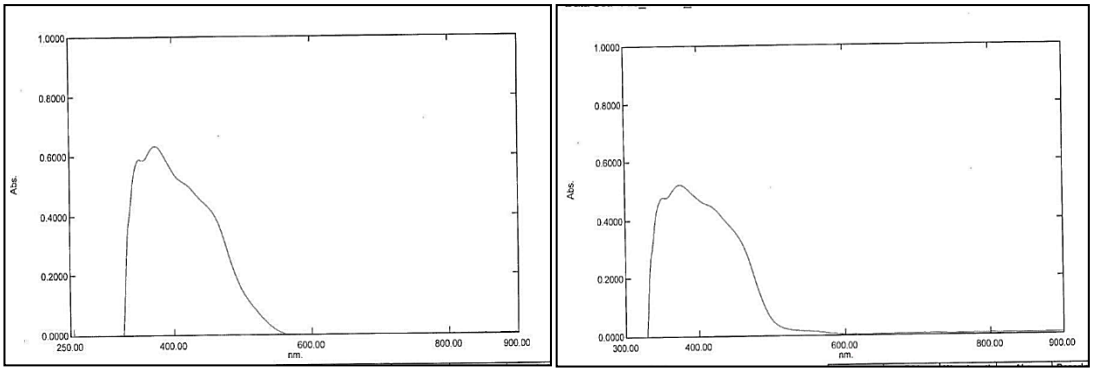
Table[6]: Magnetic Susceptibility results & Electronic transition value (nm,cm^{-1}), Conductivity, Geometry , and Hybridization

compound	λ_{max} (nm)	Abs. bands(cm^{-1})	Transitions	μ_{eff} (B.M)	Geometry	Hybridization	Conductivity $S.mol^{-1}.cm^2$
$C_{35}H_{26}N_4O_2$ (DIDMN)	282 352 377	35460 28409 26525	$\pi \rightarrow \pi^*$ $\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$	----	----	----	----
[Co((DIDMN)(1,10-phen.)Cl_2]	488	20491	*I.LC.T.*	3.83	Octahedral	Sp^3d^2	6.8
[Ni((DIDMN)(1,10-phen.)Cl_2]	494	20242	*I.LC.T.*	2.65	Octahedral	Sp^3d^2	13.2
[Cu((DIDMN)(1,10-phen.)Cl_2]	468	21367	*I.LC.T.*	1.73	Octahedral	Sp^3d^2	3.2
[Hg((DIDMN)(1,10phen.)Cl_2]	380	26315	*M.L.C.T.*	Dia.	Octahedral	Sp^3d^2	15.5
[Cd((DIDMN)(1,10phen.)Cl_2]	387	25839	*M.L.C.T*	Dia.	Octahedral	Sp^3d^2	13
[Pd((DIDMN)(1,10phen.)]Cl_2	385	25974	*M.L.C.T*	Dia.	Square planer	dsp^2	79.8



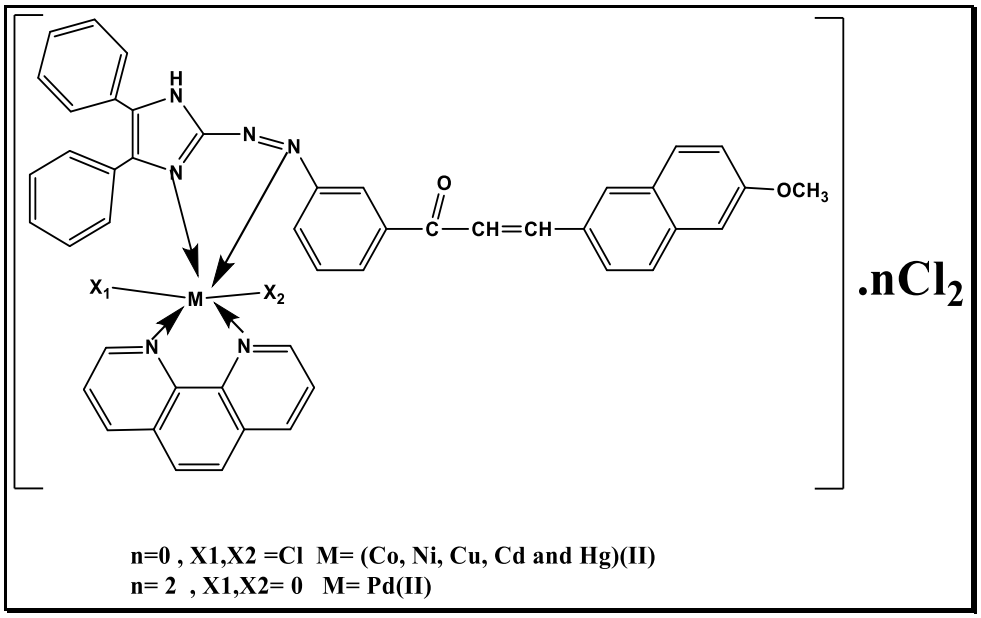
(A) **(B)**

Figure [6] : (A) UV-Vis. spectrum of (DIDMN) ligand &(B) UV-Vis. Spectrum of Copper complex



(C) **(D)**

Figure[7]: (C) UV-Vis. spectrum of Mercury complex &(D)UV-Vis. Spectrum of Palladium complex

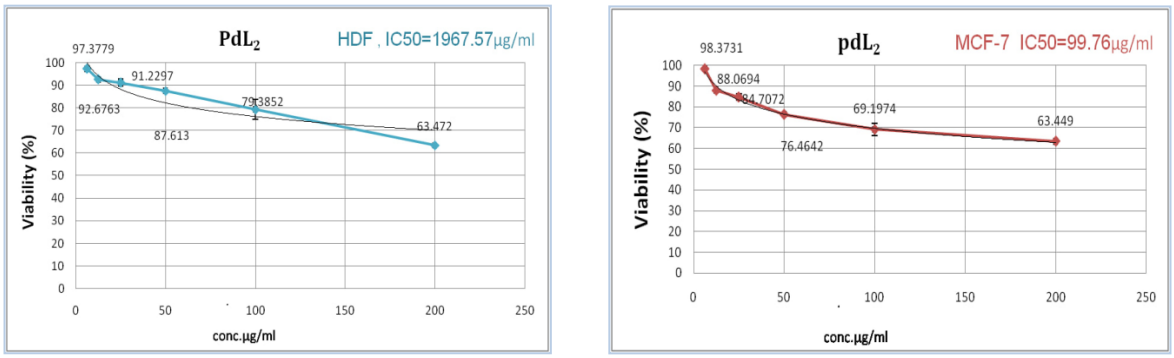


Figure[8]: suggested geometrical shape of complexes

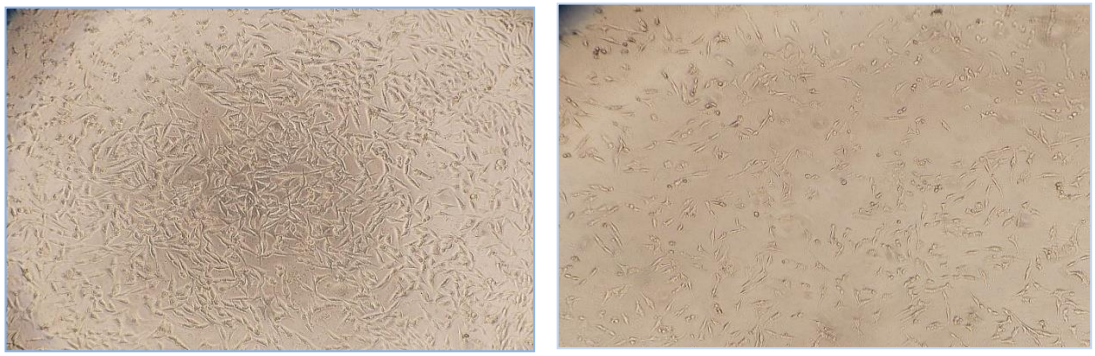
The influence inhibiting of (Pd, Cd and Hg)(II) complexes:

The results of this search evaluated the significant fatal effect of (Palladium , Cadmium and Mercury)(II) complexes as the following. The study illustrated the highest inhibition of Pd(II) complex against a human breast cancerous cells line (Mcf7) compared with uninfected cells line (HDF) which was appeared at IC_{50}= 99.76µg/ml for (Mcf7), while IC_{50}= 1967.57µg/ml for (HDF), Thus this result indicates that the palladium complex can be used as an effective drug treatment

for this type of cancer breast, In addition to , the result showed that, Hg(II) complex was the best as anti-bacteria compared with Cd(II) complex and both of them had anti-bacterial activity towards Gram Positive bacteria more than Gram Negative bacteria.



Figure[9]:Anti-cancer activity data of Pd(II) complex on (MCF7) and (HDF)



(A) **(B)**

Figure[10]: Shows a comparison of inhibitory efficacy of [Pd(L_2)(1,10-phen.)]Cl_2 on (A) :infected breast cells & (B): normal breast cells.

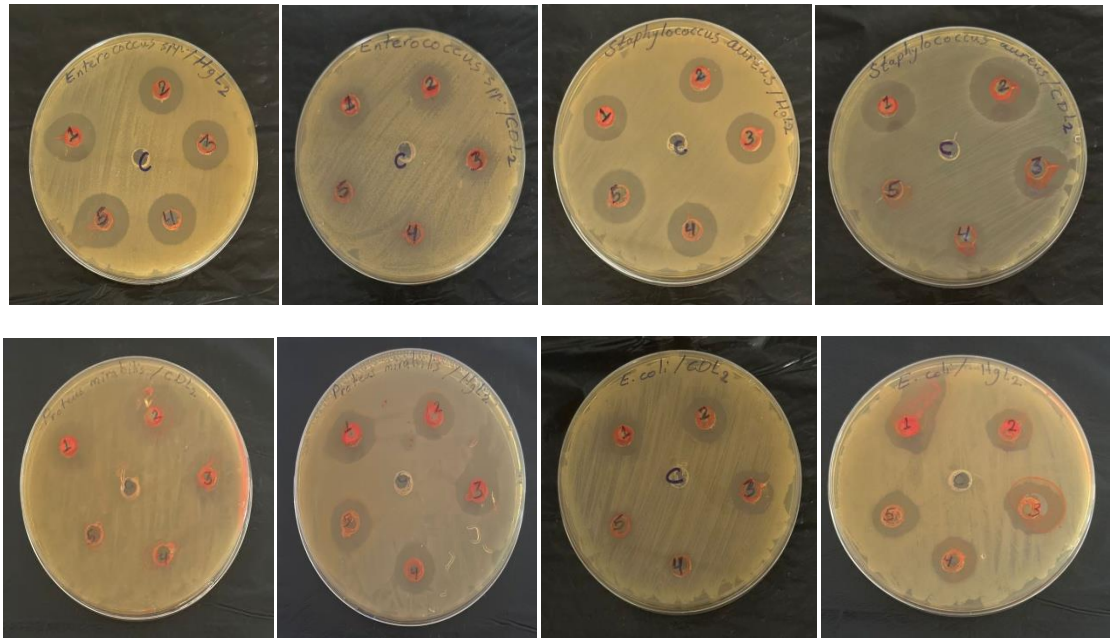


Figure [11]:Anti bacterial activity of Cd (II) and Hg(II) complexes on two pathogenic bacteria

Conclusion:

This paper described the synthesized of new derived chalcone – imidazole ligand (DIDMN) , which coordinated as a primary ligand and (1,10-phen.) as a secondary ligand with some of transition metal ions in mole ratio (L:M:L)(1:1:1), this ligand and its mixed ligands complexes were characterized by different means , the measurement results showed an Octahedral Geometric shape of all complexes except Palladium complex was Square planner geometry, The cytotoxic result suggested that, (Pd) complex it can make use of it the treatment of this type of breast cancer and other types from cancers, While both (Cd, Hg) (II) complexes had anti-bacterial activity towards some of Gram Positive and negative bacteria .

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