Design of new Ligand Derivatives from Chalcone-Imidazole with Some Transition Metal Complexes and Study Cytotoxic Activity of Pd (II) Complex

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Abstract
A new chalcone-imidazole ligand [(2E)-1- (4-((4,5-bis(4-methoxyphenyl)-1H-imidazol-2-yl)diazenyl)phenyl)-3-(4-nitrophenyl)prop-2-en-1-one] (LNO2) was synthesized and coordinated with (Co, Ni, Cu, Zn, Hg and Pd)(II) in mole ratio (1:2)(M:L), number of spectroscopic techniques have been used to characterize this ligand such as (UV-Visible, FTIR, Mass, 1H-NMR and C.H.N.). However, the complexes characterized by (C.H.N., FTIR, UV-Visible, Electrical Molar Conductivity and Magnetic Susceptibility), the results of this search suggested an Octahedral structures of all complexes, while it was square planer of Pd (II) and the data show anticancer activity of Pd (II)complex against breast cancer cells line (MCF-7) compared with healthy cells line (MCF-10A).
Keywords: coordinated, chalcone-imidazole derivatives, breast cancer.

1. Introduction

Recently, Chalcone – Azo derivatives considered vital chemical compounds, this is because chalcone derivatives have unsaturated keto-ethylenic group (CO-CH=CH-)which gave it a plenty of applications in numerous fields [1] such as in industry to enhance of fiber and polyester [2] and in Organic Chemistry to generate new organic substances [3] and in Medical and Pharmacy act as anti-inflammatory, anti- malarial, anti-Alzheimer, anti-oxidant and anti-HIV [4-7].
In addition, the chemical composition of iso group (-N=N-) has an important effect to inhibit (COVID-19) infection [8] and it plays a major role in stability of chelate complexes [9] and it reacts as reagent to extract and determine some of metal ions in different samples [10-12], this leads to azo complexes have wide applications as catalysts, anti-microbial, colorants, corrosion inhibitors and anti-cancer [13-15]. Currently, both chalcone and azo derivatives show high inhibitory activity against breast cancer cells line (MCF-7) [16].
This study has been focused on synthesized new chalcone- azo ligand with several transition metal ions and it appeared the main role of Pd (II) complex as antitumor line (MCF-7).

2. Experimental part

Materials and Instruments

All chemicals were provided by noticeable companies such as Fluka, Sigma Aldrich and BDH. Furthermore, the measurements have done by different equipment as Melting Point Measurements by Stuart Melting Point /Germany (SPM10), Elemental Analysis (C.H.N. and M.) was obtained by Elemental Analyses system Instrument: Flash EA/1112-thermofinniganco and Shimadzu AA-66300Atomic Absorption/Flame Emission Spectrophotometer. Molar Conductivity Measurements recorded by 470WTW Apparatus at room temperature in DMSO (1x10-3)M, FTIR Spectra characterized by Shimadzu FTIR 8400 at (400-4000)cm-1, UV-Visible Spectrophotometer considered by Shimadzu UV-Vis.1700 double beam at (200-1100)cm-1, Magnetic Susceptibility studied by VSM instrument :LBKFB model-Meghnatis Daghigh Kavir Company, Iran., Mass Spectra determined by Shimadzu Agilent Technology (HP)Mass Selective Detector (70EV.) at (50-230)0c, 1H-NMR Spectrophotometer applied in Bruker GmbH500MHZ in DMSO-d6 solvent .

Synthesis of Chalcone-Imidazole Ligand (LNO2)

The new ligand has been synthesized in two steps, the first step involved preparation of derivative chalcone by solving (4.05g, 0.01mole) 4- Amino acetophenone with 30ml Abs. ethanol and 10% NaOH, stirring and cooling at 30min. in ice bath. Then, (4.5g, 0.01mole) 4- Nitro Benz aldehyde solved with 50ml Abs. ethanol and added to the first solution with stirring 7hrs. [17], TLC. Paper indicated that reaction completed by using (Ethanol: Benzen)(3:2), Rf= 0.65, the dark yellow thickness solution formed which kept overnight. After that, it diluted by cold and acidified dil. HCl to precipitate derived chalcone, which filtered, dried and crystallized with hot acetone. Secondly, (2.68g, 0.01mole) derived chalcone formed at the first stage dissolved in 50ml acetone and 18% HCl dilution at (0-5)0c and (0.76g, 0.01mole) NaNO2 in 10ml D.W. [18], the mixture stirred 15 min., to presented diazonium salt which added with dropped and stirred to ice basic alcoholic solution 10%NaOH (2.8g, 0.01 mole) of 4,5-bis(4-methoxy phenyl)imidazole) in 150ml Abs. ethanol to precipitate dark red of derived chalcone-imidazole ligand. Following that, it acidified at PH = (6-7). Then, it

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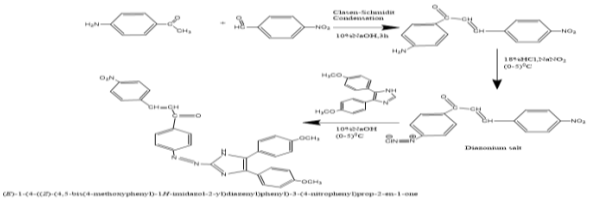
filtered, washed with D.W., Finally, it crystallized by hot acetone.



Scheme (1) Synthesized of (LNO2) Ligand

Preparation of Ligand (LNO2) complexes

The chloride salt of (Co, Ni, Cu, Zn, Hg) (II) was solved by aqueous solution. while, chloride salt of Pd (II) solved by (CH3CN) and all metal ions solution added to acetone solution of ligand (LNO2), in mole ratio (M:L)(1:2), it stirred and heated 30min. Following that, the painted precipitations of metal complexes were dried and crystallized with hot acetone.

Table (1): Some of Physical properties and analytical analysis of Ligand (LNO2) and its complexes

Compound	M.Wt g/mole	Colour	M.p.℃	Yield %	(Found) Calc.%			
					%C	%H	%N	%M
C32H25N5O5 (LNO2)	559.57	Dark red	118-121	88	68.62 (67.43)	4.46 (3.80)	12.50 (10.69)	-----
[Co(C32H25N5O5)2Cl2]	1248.98	Light purple	140-142	77	61.49 (62.35)	4.00 (3.72)	11.20 (10.55)	4.71 (3.95)
[Ni(C32H25N5O5)2(H2O)Cl]Cl	1266.74	Light purple	128-131	76	60.62 (59.50)	4.10 (3.27)	11.05 (10.92)	4.63 (3.70)
[Cu(C32H25N5O5)2Cl2]	1253.59	Dark purple	133-135	88	61.26 (60.20)	3.98 (4.00)	11.16 (10.10)	5.06 (4.92)
[Zn(C32H25N5O5)2Cl2]	1255.43	Purple	124-126	74	61.17 (60.04)	3.98 (4.47)	11.15 (10.53)	5.20 (5.11)
[Hg(C32H25N5O5)2Cl2]	1390.63	Reddish orange	136-138	77	55.22 (54.50)	3.59 (3.60)	10.06 (11.20)	-----
[Pd(C32H25N5O5)2] Cl2	1296.46	Purple	166-170	86	59.23 (58.57)	3.85 (3.95)	10.79 (9.69)	8.20 (7.85)

Cytotoxic Activity of [Pd (LNO2)$_2$] Cl$_2$

This study applied the MTT protocol [18] to compared the cytotoxic effect of Pd(II) complex on human breast cancer line (MCF-7)and healthy cells of human female line(MCF-10A) by using some of concentrations of Pd(II) complex on both breast cancer cells and healthy cells starting from (6.25µg/ml to (100 µg/ml). The result illustrated that, the cytotoxic Activity of Pd(II) complex on (MCF-7) is higher than on (MCF-10) and the best concentration of Pd(II)complex to inhibit the growth of cancerous cells is(25µg/ml) which is suggested that, Pd(II) complex could be taken as a good medicine against breast cancer line (MCF-7).

3. Result and Discussion

FTIR Spectra of (LNO2) Ligand and its complexes

The spectrum of Free ligand (LNO2) show strong band at 1658cm-1 referred to (C=O) group [19], this band did not suffer from significant changes in spectra of complexes, while the bands of (N=N), (N-H) and (C=N) group illustrated noticeable changed in the spectra of complexes compared with Free ligand because of the coordination with the ligand .Additionally, there are new bands of (M-N) for all complexes, while (M-O) for Ni(II) complex, which overlapped with (N-H) imidazole [20] .

Table (2): FT-IR spectral data (cm^{-1}) of ligand (LNO2) and its complexes.

compound	υ (N-H)	C=N) (υ	N=N) (υ	υ(C=O) Chalcone	O-H)υ(Water	M-N) (υ	M-O)υ(
LNO2	3495	1598	1454	1658	.....	.....	.....
[Co(LNO2)$_2$Cl$_2$]	3475	1519	1413	1662	.....	476	.....
[Ni(LNO2)$_2$(H$_2$O)Cl]Cl	3417*	1517	1436	1662	3417*	480	542
[Cu(LNO2)$_2$Cl$_2$]	3415	1517	1413	1604	.....	476	.....
[Zn(LNO2)$_2$Cl$_2$]	3477	1519	1415	1662	.....	478	.....
[Hg(LNO2)$_2$Cl$_2$]	3437	1518	1402	1600	.....	478	.....
[Pd(LNO2)$_2$]Cl$_2$	3419	1519	1413	1664	.....	476	.....
(O-H)water. *υ(N-H) imidazole overlapped with υ							

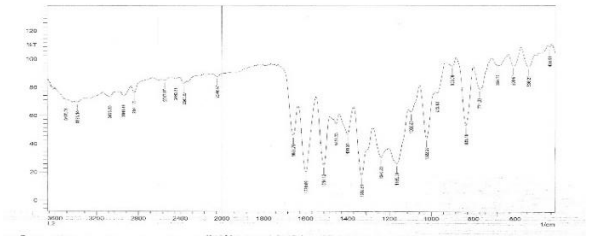


Fig. (1) FTIR Spectra of ligand (LNO2)

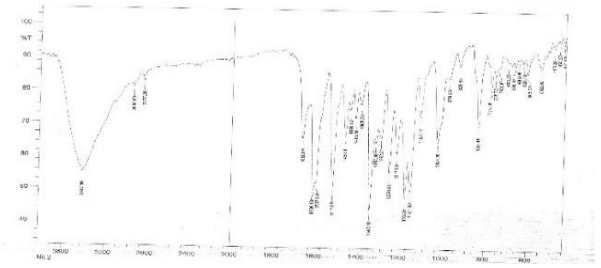


Fig. (2) FTIR Spectra of [Ni(LNO2)$_2$(H$_2$O)Cl]Cl

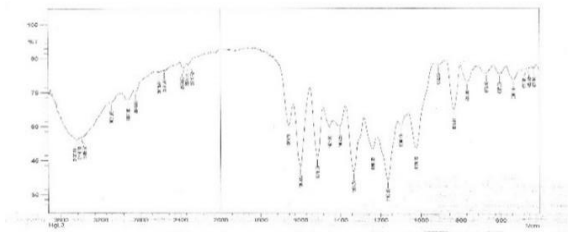


Fig. (3) FTIR Spectra of [Hg(LNO2)2)Cl2]

Electronic Spectra of (LNO2) Ligand and its complexes

All electronic absorption spectra of (LNO2) Ligand and its complexes were determined in the range (200-1100) nm at room temperature by dissolving DMSO solution (1x10-4) M, the electronic spectral data of complexes appeared redshift, this could be attribute to the coordination of ligand via donating atoms with vacant orbitals of the metal ions [17,21], the results were observed in the table (3).

The Conductivity and Magnetic Susceptibility measurements

The molar conductivity shows an ionic property of (Ni, Pd) (II)complexes and non-ionic properties of (Co, Cu, Zn, Hg) (II)complexes, these measurements recorded at room temperature and (1x10-3) M DMSO solution. The Magnetic moment indicated that, Para magnetic properties with high spin of (Co, Ni, Cu) (II)complexes [22]. While Dia magnetic properties with low spin of (Zn, Hg, Pd) (II)complexes [23,24]. All results have been noticed in the table (3).

Table (3): Electronic data (nm, cm 1), Magnetic measurements, Geometry, Hybridization and Conductivity.

Compounds	λ_{max} (nm)	Absorption bands(cm^{-1})	Transitions	μ_{eff} (B.M)	Geometry	Hybridization	Conductivity $S.mol^{-1}.cm^2$
LNO2	266 323 502	37593 30959 19920	π →π* π →π* C.T.	----	----	----	----
$[Co(LNO2)_2Cl_2]$	508 396 320	19685 25252 31250	$^4T_1g_{(F)}$ → $^4T_2g_{(P)}$ M.L.C.T *I.LC.T.*	4.70	Octahedral	Sp^3d^2	15.8
$[Ni(LNO2)_2(H_2O)Cl]Cl$	529 324 250	18903 30864 40000	$^3A_2g_{(F)}$ →$^3T_2g_{(F)}$ *I.LC.T.* *I.LC.T.*	2.61	Octahedral	Sp^3d^2	54
$[Cu(LNO2)_2Cl_2]$	553 410 321	18083 24390 31152	M.L.C.T *I.LC.T.* *I.LC.T.*	1.74	Octahedral	Sp^3d^2	17.8
$[Zn(LNO2)_2Cl_2]$	507	19723	M.L.C.T.	Dia	Octahedral	Sp^3d^2	7.4
$[Hg(LNO2)_2Cl_2]$	505	19801	M.L.C.T	Dia	Octahedral	Sp^3d^2	3.7
$[Pd(LNO2)_2]Cl_2$	577	17331	^1A_1g→^1B_1g	Dia	Square planer	dsp^2	73

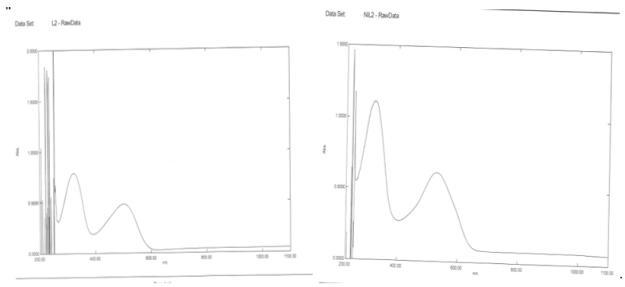


Fig. (4): UV-Vis. spectra of (LNO2) ligand and Ni(II) complex

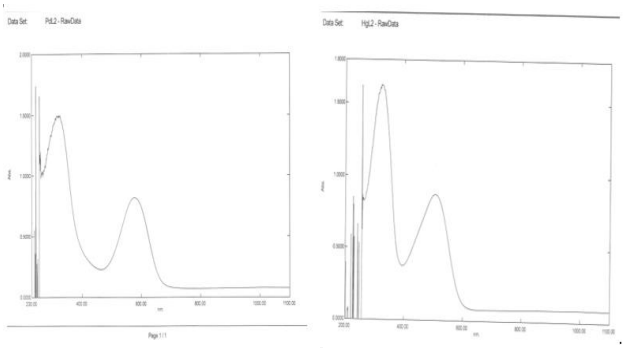


Fig. (5): UV-Vis. spectra of Pd(II)complex and Hg(II) complex

^{1}H-NMR Spectrum of (LNO2) Ligand

1H-NMR Spectrum of (LNO2) Ligand was characterized in DMSO-d6. Two significant signals at (6.9) ppm and (7.2) ppm mentioned to protons of derived chalcone (CH=CH-CO) [25]. The multiple signals were seen in the range (7.7-8.9) ppm attributed to aromatic protons. In addition, sharp singlet signal at (3.5) ppm to (OCH3) group in aromatic ring [21] and weak singlet signal presented at (13.8) ppm referred to proton of derived imidazole (N-H) [26].

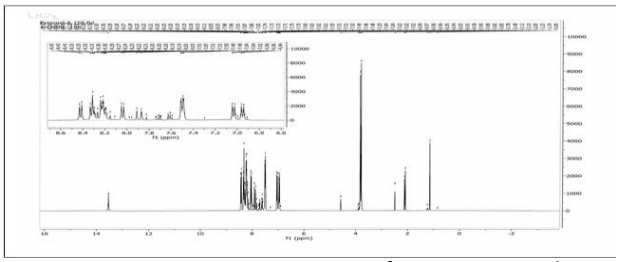


Fig. (6): 1H-NMR Spectrum of (LNO2) Ligand

Mass Spectrum of (LNO2) Ligand

Mass spectrum gives information about the

expected molecular ion peaks [27], the base peak was gotten at (m/z=559.4) equivalent molecular formula of (LNO2) Ligand, another peak at (m/z=281.1) due to 4,5-Bis – (4-methoxy phenyl) imidazole. The fig. (7) illustrated several peaks explained fragmentations of molecular ions at(m/z).

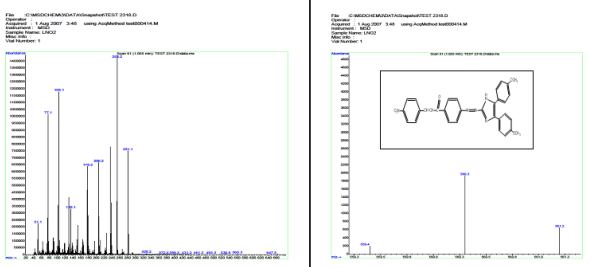
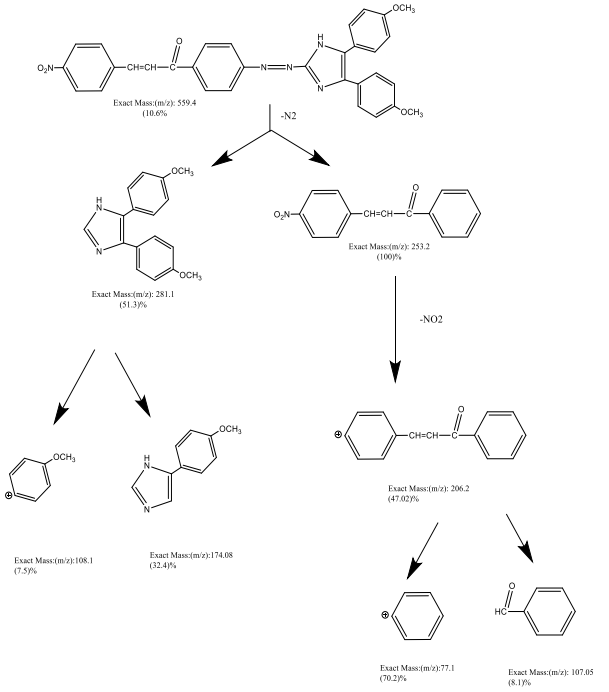
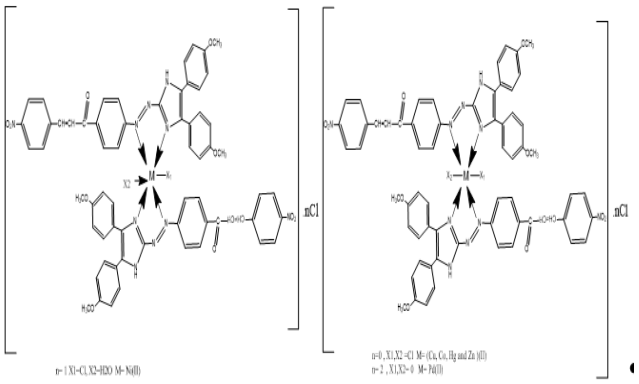


Fig. (7): Mass Spectrum of (LNO2) Ligand



Scheme (2) Suggested fragmentations of (LNO2) Ligand



Scheme (3) Suggested geometries of (LNO2) complexes

Effect of [Pd (LNO2)$_2$] Cl_2 on cancerous cells line (MCF-7)

The results show that, the number of the viable cells of (MCF-7) after treatment with Pd (II)complex is 49.87% at the concentration 25µg/ml. While the number of viable cells of (MCF-10A) at the same concentration is 98.41% and the highest inhibition ratio of the (MCF-7) after addition of Pd (II)complex is 50.13%compared with the highest inhibition ratio of healthy cells (MCF-10A) is 1.59% at the same concentration, this indicates that 25µg/ml is the best concentration to kill more than half of cancer cells. However, it has less effect on healthy cells. In addition, the result demonstrated IC50= 31.88 of cancerous cells (MCF-7). While IC50= 177.9 of healthy cells (MCF-10A), that means the concentration which kills half of (MCF-7) is the lowest the concentration to kill (MCF-10A), these results approved that, Pd (II)complex could be used as an excellent breast cancer therapy.

Table (4): Cytotoxic Activity of [Pd(LNO2)$_2$]Cl_2 on Breast cancer cells line (MCF-7) and healthy cells (MCF-10A) at the same concentration using 24h. MTT test 37^{0}c.

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	93.11	6.89	97.29	2.71
12.5	63.27	36.73	97.52	2.48
25	49.87	50.13	98.41	1.59
50	40.43	59.57	78.33	21.67
100	29.59	70.41	58.28	41.72

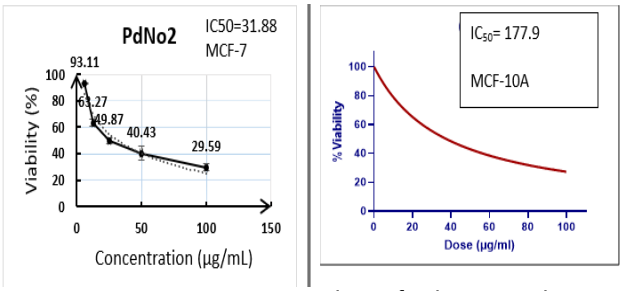


Fig. (8): Anti-cancer activity data of Pd (II) complex on (MCF-7) and (MCF-10A)

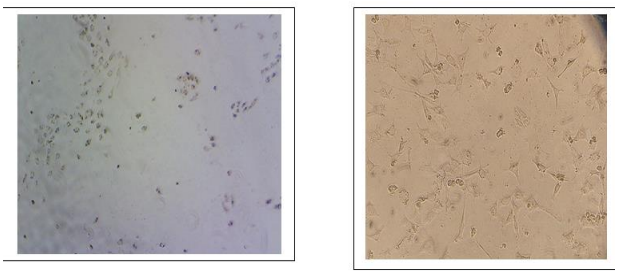


Fig. (9): Anti-cancer activity of Pd(II)complex on : (A)(MCF-7) at 25µg/ml and (B)(MCF-10A) at 25µg/ml under inverted microscope

4. Conclusion

This search described the synthesized of new derived chalcone – imidazole ligand (LNO2), which coordinated as mole ratio (M: L) (1:2) with some metal ions, this ligand and its complexes were characterized by different techniques, the results appeared an Octahedral geometry of all complexes excluding Pd (II)complex was square planner geometry. The cytotoxic result suggested that Pd (II)complex may be reserved as suitable breast anti-cancer medicine line (MCF-7).

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