Synthesis, Spectroscopic Characterization and in Vitro Cytotoxicity Assay of Mropholine Mannich Base Derivatives of Benzimidazole with Some Heavy Metals

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Abstract

Mannich bases derivatives of Benzimidazole were prepared from condensation reaction of 2-mercaptobenzimidazole with formaldeyhed once with mropholine and another with pyrazinamide to prepare L_1 and L_2 respectively, coordinated with three metal ions of Pd(II), Pt(IV) and Au(III). The structures of these compounds were confirmed by metal and elemental analyses, UV-Vis and FT-IR spectroscopy, magnetic susceptibility, conductivity measurement at room temperature, ^{1}HNMR and ^{13}CNMR. Experimental results showed that the ligands (L_1&L_2) coordinated as bi-dentate and tridentate with metal ions respectively. Cytotoxicity of Mannich bases and their metal complexes were examined against mice cell line RAW 264.7 using MTT method .Each cell line was injected by following doses (400,200,100 and 50) µg/ml of prepared compounds by using mice cell as a negative control and cis-platin as a positive control. The ligands and Pd(II), Pt(IV), Au(III) complexes showed good activity at various concentrations especially Pd(II) complexes of both complexes. [DOI: 10.22401/JNUS.21.3.06]

Keywords: Mannich Bases, Benzimidazole, 2-mercaptobenzamidazole, cytotoxicity, complexes.

Introduction

Benzimidazole is a heterocyclic secondary amino Benzo derivative of imidazole. [1a, b]. The imino group (-NH) present in benzimidazole shows both acidic and basic characteristics i.e. it is strongly acidic and weakly basic, therefore Benzimidazole is known to possesses both of these characteristics [1b]. Benzimidazole derivatives were successfully used to develop as Anti-inflammatory, Antioxidant, Antineoplastic, Anthelmintic and Antiviral [1b,2a]. Benzimidazole and their derivatives have multiple applications in coordination chemistry such as (bioinorganic chemistry, photophysics and photochemistry) [2b]. From condensation reaction three components of Benzimidazole is very significant for the synthesis of different useful and active compounds. Mannich base is product from condensation three compounds of active hydrogen containing compound, HCHO and secondary amine [3a]. In recent years, the metal complexes of Mannich bases have been studied over a wide range due to the sensitivity and selectivity of the ligands towards different metal ions [3a]. The organic chelating compounds consisting amide moiety as a functional group have strong ability to form metal complexes and show a variety of biological activities such as, antifungal, anti HIV activity, antibacterial, Tuberculosis activity, antiviral, antiulcer and antihypertensive [3b,4]. In the immediate work, a new Mannich base obtained from the reaction of morpholine, formaldehyde and 2-mercaptobenzimdazole and their metal ion complexes with Palladium, Platinum and gold were synthesized and characterized using different physicochemical techniques. The proposed structure of the synthesized Mannich bases is given in Scheme1. The cytotoxic activities of theses ligands and their metal ion complexes were studied *in vitro* against mice cell line.

Material and Methods

In this paper the chemicals were used of highest purity. Micro elemental data were measured by Euro EA 3000 elemental analyzer. Metal contents were carried out by using shimadzu atomic absorption 680 Flam spectrophotometer. Conductance data were obtained in 10^{-3}M in DMF solution of the complexes using WTW conductometer at 25°C. Infrared spectra were measurement using Shimadzu and perkin Elmer FT-Infrared spectrophotometer using CsI pellets.

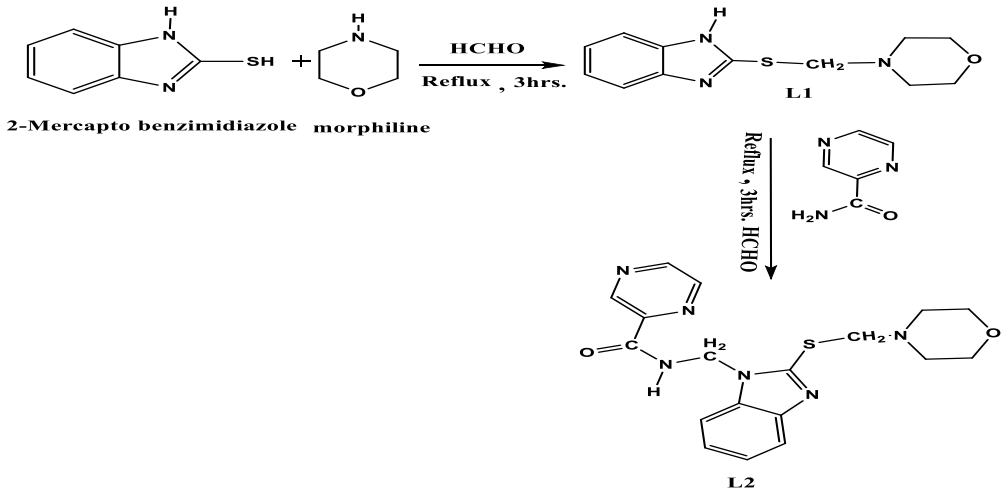
Absorbance in uv-Visible region was recorded in ethanol solution using uv-Vis.1800 PC Shimadzu Spectrophotometer. The ^{1}H,^{13}C NMR of the compounds were recorded on a Fourier transform Varian spectrometer operating Bruker at 400 MHZ employing DMSO d_6 solvent and TMS as internal reference. Magnetic susceptibility measurements of the complexes were made by using Balance of Johnson Mattey catalytic system division at 25°C. Melting point apparatus of Gallen kamp M.F.B-60 was used to measure the melting points of all prepared compounds. Optical density of each well in cell culture plates in cytotoxic assay was read by micro ELISA reader ASYS, Austria at a transmitting wave length on 620 nm, Plates of cell culture were incubated at 37°C in SANYO, incubator Japan.

1-Preparation of 2-(Morpholin-N-methyl) mercapto-1H-benzimidazole(L_1).

This compound was prepared according to the literature[5] and as represented in the Scheme (1).

2-Synthesis of N-((2-((Morpholinomethyl) thiol)-1H-benzomidazol-yl)methyl) pyrazine-2-carboxamide)(L_2).

The ligand (L_2) was synthesized by mannich condensation reaction between equal mole of 2-(Morpholin-N-methyl)mercapto-1H-benzimidazole and pyrazinamide (L_1). The mixture was dissolved in methanol (30 ml) in a beaker under ice-cold condition constant stirring. In the same solution, formaldehyde (0.01 mol) was added gradually and heated to reflux for (3 hrs), then kept overnight in the freezer. Color solid mass was obtained and it was washed, and recrystallized from ethanol (yield 70 %). It melts at (132-134)°C, as represented in Scheme (1).



Scheme (1): General steps of the prepared L_1 and L_2.

3-General Procedure to Prepare the New Complexes

The Mannich bases reaction occurs in ethanol with metal ion salts of $PdCl_2$, $H_2PtCl_6.6H_2O$ and $HAuCl_4.6H_2O$, 1:2 and 1:1 molar ratio for L_1 and L_2 respectively. The mixture was then refluxed for (3 hrs.); the color solid complexes were formed, and then filtered, washed with ethanol and dried in dissector.

4-Formation of Metal Complexes for (L_1& L_2) in Solution State

In solution state , Studied of the complexes formation of L_1 and L_2 with Pd(II), Pt(IV) and Au(III) by using ethanol as a solvent, in order to determined M: L ratio in the prepared complexes using molar ratio method [6]. A set of solutions were prepared having a various volume of ligands and constant concentration of the metal ions (0.25-3). From the

relationship between the absorption of the absorbed light and the mole ratio of M:L at λmax the M:L ratio was determined.

5-Cytotoxic assays

Cytotoxicity effect of both ligands and to metal ion complexes on RAW 264.7 (mice carcinoma) was done in a sterile area using a laminar air flow cabinet biosafety, RAW 264.7 cell line was used in this study were equipped from Biotechnology Center/Al-Nahrain University. The cells were grown in Modified Eagles Medium (MEM) with 10% fetal bovine serum, (100 U/ml) of penicillin and (100 µg) of streptomycin/ml in a humidified incubator with (5% CO_2) at (37°C). The manufactured products were subjected to a screening system for evaluation of their cytotoxicity activity on mention cell line in comparison to the known anticancer drug (cis-Pt). Cell survival was further assessed by (3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT)) dye reduction assay which is based on the capability of viable cells to metabolize a yellow tetrazolium salt to violet formazan product that can be detected spectrophotometrically. Exponentially growing cells (RAW 264.7) were plated in triplicate in 96-well sterilized microtiter-plates at a density of (1×10^5 cells/well). After 24 hrs, cells were treated with decreasing doses of the compounds under investigation and incubated in (5% CO_2) atmosphere with high humidity. After 48 hrs of compounds exposure, the cells were incubated with MTT (0.5 mg/1ml) distilled water for another 4 hrs at 37°C. The blue MTT formazan precipitate was then solubilized in detergent (50%) final concentration of N, N-dimethylformamide and 10% of sodium dodecyl sulphate then incubated for an additional 2 hrs. Absorbance was measured at 620 nm on a multi-well ELISA plate reader [7].

Results and Discussion

Some of physical properties of the prepared compounds can be shown in Table (1), all the complexes prepared have a different color, some of them are partly soluble in most organic solvents, but other of them soluble in DMF. The results of elemental analysis (C H N and S) and molecular formulae are shown in Table (1). At the maximum absorption, absorbance As and Am of the solution were measured at wavelength maximum. The stability constant K and molar absorptivity ε_{max} have been calculated. The data of complexes formation can be listed in Table (2).

Table (1)

Shows the physical characterization, elemental analysis and metal content for all compounds.

Comp.	*m.p^{O}C*	*Color*	*Yield %*	*Elemental analysis Calc. (Found)*				*Metal % Found (Calc.)*	*µs.cm^{-1}*
				C	*H*	*N*	*S*		
$C_{12}H_{15}N_3OS(L_1)$ 249.33	199-200	off white	83	57.75 (56.12)	6.01 (6.679)	16.84 (17.22)	12.83 (12.98)	------	-----
$[PdL_2]Cl_2.C_2H_5OH$ 722. 08	184-186	Brown	88	43.20 (43.76)	4.98 (5.06)	11.63 (12.00)	8.86 (8.90)	14.73 (15.56)	72
$[PtL_2Cl_2]Cl_2. H_2O$ 853.66	210-212	Brown	92	33.73 (34.17)	3.74 (3.71)	9.83 10.33)(	7.50 (8.11)	22.84 (22.92)	154
$[AuL_2Cl_2]Cl.0.5\ C_2H_5OH$ 825.06	130-132	Dark brown	77	36.36 (36.99)	3.99 (4.58)	10.18 (10.48)	7.75 (8.03)	23.86 (24.11)	77
$C_{18}H_{20}N_6O_2S(L_2)$ 384.46	132-134	off white	70	56.18 (55.81)	5.20 (5.15)	21.84 (21.06)	8.32 (8.20)	-----	----
$[PdLCl]Cl.C_2H_5OH$ 607.88	148-150	Dark brown	84	39.48 (39.43)	4.27 (3.72)	13.81 (14.47)	5.26 (5.78)	17.52 (16.69)	63
$[PtLCl_3]Cl.H_2O$ 739.46	212d	Reddish orange	88	29.21 (29.92)	2.97 (3.61)	11.35 (12.04)	4.32 (5.12)	26.37 (25.67)	66
$[AuLCl_3]. C_2H_5OH$ 733.86	140-142	Brownish Yellow	72	32.70 (33.15)	3.54 (3.98)	11.44 (11.00)	4.36 (5.21)	26.83 (27.00)	33

d= decomposition degree

Table (2)
Stability constant of all prepared complexes at (25°C).

Comp.	A_S	A_m	α	ε_{max} *L.mol^{-1} cm^{-1}*	K L.mol^{-1} *(*): L^2.mole^{-2}*	$\lambda_{max\ (nm)}$
PdL_1	1.129	1.285	0.121	12850	$*5.9 \times 10^4$	440
PtL_1	0.333	0.400	0.167	4000	$*2.97 \times 10^4$	408
AuL_1	1.200	1.299	0.076	12990	$*1.59 \times 10^5$	390
PdL_2	0.221	0.321	0.311	3210	5.7×10^6	408
PtL_2	0.258	0.321	0.196	3210	2.6×10^7	384
AuL_2	0.993	1.111	0.106	11110	1.86×10^9	366

K=Stability constant
A_s: Average of triplicate absorption of the solution containing a stoichometric volume of ligand and metal ion.
A_m: Average of triplicate absorption of solution containing the same amount of metal and fivefold excess of ligand

1-FT-IR spectra

The bands of infrared spectra of the chelating ligands and all metal ion complexes with their Transmittance bands are given in Table (3). To diagnose the coordination sites that may be involved in coordination, the infrared spectra of these complexes were compared with those the free ligands. The infrared IR bands of L_1 shown at (2931-2870), (1608) and (1357 cm^{-1}) for Mannich group[8a, b], ν C=N and ν CN respectively[8b,9a]. This band is shifted by (23-47),(27-31) and (15-17) cm^{-1} in the metal ion complexes respectively. Another sharp bands appeared at 1107 & 1111cm^{-1} for CNC of morpholine moiety[9b], this bands is shifted to higher and some other to lower frequency, indicate that these complexes coordinated through of the nitrogen atom from the morpholine ring for L_1 and L_2 and the nitrogen of imidazole to the central metal ion. In Infrared spectra of complexes for L_1, the ν,δ NH[9b], ν CS and ν CSC [10a] bands stayed at the same location of free ligands, this proves the amine and sulphur is not coordinated, as opposed to link areas for L_1,the L_2 coordinated with ν, δ NH, ν CS and ν CSC [9b,10a], the shifting of these bands stretching vibration to the lower wave number side as compared to the free ligand is indicative of participation of sulphur and NH group in coordination [9b,10a]. The presence of a broad band of water molecules and sharp bands of ethanol molecules in out of coordination sphere is determined by the appearance of band at (3456-3406) and (3421) cm^{-1} [10b], (3498, 3532) and (3510, 3521) cm^{-1} [10b] for L_1 and L_2 respectively, indicating the coordination of nitrogen and chloride[10a] to metal ion appeared medium and weak intensity bands ν(M-N) observed near at (555-597), (559-582) cm^{-1} and ν(M-Cl) observed at (322 and 333) and (322, 325 and 327) cm^{-1}. The most diagnostic FT.IR bands for the ligands and their complexes in addition to other bands are shown in Table (3).

Table (3)
IR Absorption data of ligands and their metal ion complexes.

Comp.	ν CH_2-N	ν NH	δ NH	ν C=O amide	ν CN	ν CSC	ν CS	νNCS	ν CNC morphline	ν NCN	ν C-O-C	ν C=N	ν M-N	ν M-S	others
L1	2931 2870	3147	1438	----	1357	1180	740	1068 1161	1107	-----	1215	1593		----	-----
PdL1	2954 2888	3155	1435	-----	1342	1180	740	1041 1147	1130	-----	1215	1624	570	-----	C_2H_5OH 3532 Pd-Cl 330
PtL1	2908 2823	3143	1433	-----	1342	1180	744	1037 1145	1130	-----	1222	1620	559	-----	ν H_2O 3456-3406 Pt-Cl 333
AuL1	2951 2886	3142	1444	-----	1340	1188	744	1045 1149	1130	-----	1220	1620	597	-----	C_2H_5OH 3498 Au-Cl 322
L2	2931 2866	3282	1462	1674	1357	1161	740	1049 1139	1111	1373	1215	1608 1581	----	---	----
PdL2	2966 2888	3260	1450	1675	1342	1172	760	1061 1153	1081	1387	1216	1610 1583	570	435	νC_2H_5OH 3521 Pd-Cl 327
PtL2	2981 2888	3268	1450	1671	1340	1176	763	1059 1153	1080	1387	1216	1610 1583	559	451	ν H_2O 3421 Pt-Cl 322
AuL2	2978 2872	3261	1492	1678	1341	1176	762	1061 1150	1089	1389	1219	1610 1573	582	450	νC_2H_5OH 3510 Au-Cl 325

2-Magnetic moments, electronic spectral data and conductivity measurements

The spectral data of the ligands and their metal ion complexes were carried out in ethanol solution, listed in Table (4). The ligands exhibits strong absorption bands at (33003, 3587sh, 40485, 45454, and 33112, 37453, 40485, 45045) nm attributed to n →π* and π→ π*, respectively [11a]. Diagnosis geometry of the synthesized complexes depend on the UV -vis spectrum and the complexes were shown to have a square planner or octahedral geometry. The electronic spectral for metal complexes of Pd(II), Pt(VI) and Au(III) were studies.

PdL_1 &PdL_2:The electronic spectra of Pd complexes show a broad bands at 24509, 24390, 27322 and 31250 cm^{-1} which attributed to$^1A_1g\rightarrow{}^1B_1g(\nu1)$ transition and the later one is due to $^1A_1g\rightarrow{}^1Eg(\nu2)$ transitions respectively of square planer geometry [11a,11b]. Another bands appeared at 32786, 42553 and 35714, 43478 cm^{-1} can be assigned to L→Pd CT transitions due to charge transfer transition for L_1 and L_2 respectively [12a, b]. The measured magnetic moment is zero B.M showed that the complexes to be low spin, and the conductivity in DMF indicted that the both complexes are ionic[11a].

PtL_1&PtL_2:The Pt (IV) ion with d^6 configuration and the magnetic moment equal to zero in their complexes can be suggested an octahedral geometry of theses complexes. The octahedral coordinated of Pt(IV) ion in the position (26041, 33557) & (24390, 26666) cm^{-1} has the ground state^1A_1g and excited state should be ^1T_1g, ^1T_2g [11a,12a], in addition to a weak bands assigned to spin forbidden, singlet –triplet transition may be observed for two prepared complexes at lower energies than the spin allowed transitions[11b,12b]. The prepared brown and reddish orange of Pt(IV) complexes showed another bands at (39215, 32362 and 47393) cm^{-1} which assigned to ligand→Pt CT transition respectively[10a]. The conductivity measurement showed that both complexes to be ionic[12b].

AuL_1&AuL_2:The spectra of these complexes gave three absorption bands appeared at (27282, 36037, 25641 and 31250), (40816, 45454, 34364 and 47619) cm^{-1} for L_1 and L_2 respectively, which indicate an octahedral geometry of these complexes [11b,13a], assigned to$^3A_2g\rightarrow{}^3T_2g$, $^3A_2g\rightarrow{}^3T_1g$, $^3A_2g\rightarrow{}^3T_{1g(P)}$ and L→Au CT transition respectively [11b,12b,13b]. It is possible to calculate the values of Dq and *B′* using band position of ν_1 and ν_2. Value of ν_3 was calculated to be around 52919 and 56465 cm^{-1} for $AuL_1$and AuL_2 respectively [13b]. The ratio ν_2/ν_1 =1.32 &1.21 fits the diagram at 3.1& 3.5 Dq/B′, B' will be 874.0 and 719.5,15 for L_1& B'=13110,10792 for L_2.The value of the constant field splitting 10Dq will be 27094

and 25182 cm^{-1} for L_1 and L_2 respectively; it is approximately equal to the first transition. The values are in the rang (77 &33) $\mu s.cm^{-1}$of the complexes in DMF, this data indicate ionic and nonionic complexes for AuL_1 and AuL_2 respectively. Magnetic moments of two complexes are shown in Table (4).

Table (4)
Electronic spectra, conductance in DMF and magnetic moment for ligands and their metal ion Complexes.

Molecular formula	L_1	L_2	PdL_1	PtL_1	AuL_1	PdL_2	PtL_2	AuL_2
Absorption Bands(cm^{-1})	33003,3587sh 40485,45454	33112,37453 40485,45045	24509 27322 32786, 42553	10695 26041 33557 39215	27282 36037 $52919_{(cal.)}$ 40816,45454	22727 31250 35714,43478	10309 24390 26666 32362,47393	25641 31250 $56465_{(cal.)}$ 34364,47619
Assignments	(n→π*) (π→π*)	(n→π*) (π→π*)	$^1A_1g\rightarrow{}^1B_1g$ $^1A_1g\rightarrow{}^1Eg$ L→PdCT	$^1A_1g\rightarrow{}^3T_2g,{}^3T_1g$ $^1A_1g\rightarrow{}^1T_1g$ $^1A_2g\rightarrow{}^1T_2g$ L→Pt CT	$^3A_2g\rightarrow{}^3T_2g$ $^3A_2g\rightarrow{}^3T_1g$ $^3A_2g\rightarrow{}^3T_1g_{(P)}$ L→Au CT	$^1A_1g\rightarrow{}^1B_1g$ $^1A_1g\rightarrow{}^1Eg$ L→PdCT	$^1A_1g\rightarrow{}^3T_2g,{}^3T_1g$ $^1A_1g\rightarrow{}^1T_1g$ $^1A_1g\rightarrow{}^1T_2g$ L→Pt CT	$^3A_2g\rightarrow{}^3T_2g$ $^3A_2g\rightarrow{}^3T_1g$ $^3A_2g\rightarrow{}^3T_1g_{(P)}$ L→Au CT
μ_{eff} *B.M.*	------	------	0.00	0.00	(2.82)	0.00	0.00	(2.82)
Suggested structure	------	------	Sq.P	Oh	Oh	Sq.P	Oh	Oh

3-^{1}HNMR and ^{13}CNMR spectra

Mode of the ligands and Pd, Pt complexes are also provided by the ^{1}H-NMR, ^{13}CNMR spectra of the Mannich bases and the diamagnetic of Pd (II) and Pt (IV) complexes, which were recorded in DMSO d_6. The ^{1}HNMR spectra of the ligands show signals of aromatic protons appeared at (7.13-7.32) & (7.17-7.67) δ respectively[14a]. The (N-H) proton chemical shift take place at (12.25,8.98) δ [11a,14a] which gave rise to singlet, doublet for L_1 and L_2 respectively. The peak at (3.90),(3.97) and (5.15) ppm are attributed to the CH_2-N groups of Mannich bases present in L_1 and L_2[14b,2a]. The signals appeared in the position of (δ 3.57,3.59 and δ 2.49,2.48) are due to -O-CH_2 and N-CH_2 proton of morpholine moiety for L_1 and L_2 respectively [14a]. Other bands appeared at δ (8.86,9.29 and 9.99) are due to pyrazine proton for L_2 ligand [15a].

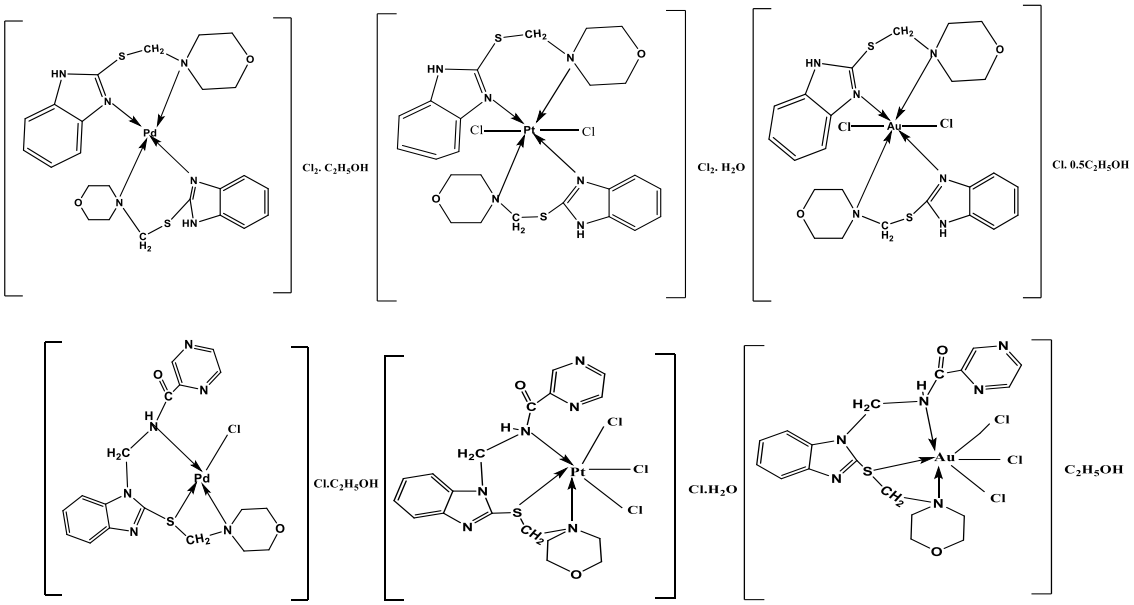
In Pd (II) and Pt(IV) complexes, the (d, N-H) proton shifted down field to 9.00 δ, evidence the coordination of NH moiety to Palladium and Platinum ion in L_2[15b]. The signal due to morpholine N-CH_2 protons is also shifted slightly downfield and appeared at 2.69 & 2.68 δ in the complexes L_1 and L_2. This is indication to the coordination of nitrogen for morpholine [14a].

In the ^{13}C-NMR spectra of the ligands $L_1$and L_2, the signals in the range from (47.96, 68.78) & (48.01, 68.99) ppm were assigned to carbon atoms of morpholine (N-CH_2,OCH_2) [14a]. Aromatic ring carbon atoms of benzimidazole were determined in the range of 114.05-138.92 and 114.08-139.58 ppm respectively [10a,14a]. Additional signals were determined at 61.0, 60.64, 61.64ppm and 147.01,151.34 ppm [14b], which are assigned to the carbon atom of CH_2-N of Mannich bases and (NCN) for benzimidazole for L_1 &L_2 respectively [16]. Other bands are shown in Table (5).

Table (5)
^{13}CNMR ,^{1}HNMR spectral of L_1 and L_2 and Pd(II), Pt(IV) complexes.

^{13}CNMR	^{1}HNMR
^{13}CNMR(DMSO-d_6) ppm: L_1:114.05-138.29 (4C-Ar), 47.96, 68.78 (N-CH_2,O-CH_2), 61.0 (CH_2-N), 147.01(N-C=N-),	DMSO d_6,ppm δL_1: 7.13-7.32 (m,4H,Ar), 2.49(t,4H,CH_2), 3.57(t,4H,CH_2), 3.90(S,2H,CH_2), 12.25(S,1H,NH).
^{13}CNMR(DMSO-d_6) δL2: 114.08-139.58(4C-Ar), 48.01, 68.99 (N-CH_2,O-CH_2), 60.64 61.64 (CH_2-N), 151.34(N-C=N-), 172.07(-C=O), 145.56(C-C=O pyr.), 144.94, 145.01, 146.47(C-Pyr.ring).	DMSO d_6, δ L_2: 7.17-7.67 (m,4H,Ar), 2.48 (t,4H,CH_2), 3.59 (t,4H,CH_2), 3.97(S,2H,S-CH_2), 5.15(d,2H,N-CH2), 8.89(t,1H,NH), 8.88, 9.29, 9.99(d,3H,CH-Npyr.)

Accordingly, the *proposed structure* model for these newly complexes prepared which is characterized by physical and spectroscopic analysis can be shown in following figure:



Fig(1): Suggested Structures of Prepared Complexes.

4-In vitro cytotoxicity

Cancer and some malignant diseases occur as a result of several factors, such as DNA damage, oxidative stress and chronic inflammation. These diseases can be controlled by resistant to carcinogens and or to inhibit advancement of the disease by chemotherapy [17a]. The newly synthesized products were evaluated for their *in vitro* cytotoxic activity against RAW 264.7 cell line. Our results showed that all of the newly synthesized products especially metal ion complexes exhibited a strong growth inhibition activity on the tested cell line in comparison to the reference anticancer drug i.e (cis-Pt). Benzimidazole is an important pharmacophore and a privileged structure in medicinal chemistry. Nowadays is a moiety of choice which possesses many pharmacological properties [17b], in L_1 complexes the activity of these complexes also comes from presence of morpholine moiety beside the benzimidazole group because it is considered an important building blocks in the field of medicinal chemistry especially as anticancer drugs *in vivo & in vitro* [18a]. In the L_2 the presence of pyrazine group, which possessed a wide range of biological activities being found in nature and in many drugs [18b], and due to the importance of pyrazine nucleus in drug discovery and according to continuous searches of new potent and safe anticancer agents we were attempted to test compounds contain of this moiety against cancer cell line

and good results were obtained for this group i.e in L_2 compare to L_1 complexes. From the research results, some factors are responsible for the pharmacological efficacy of these compounds (ligands and complexes) such as size of metal, polarity, charge distribution, ionic character and geometry shape. The results exhibited the higher inhibition rate of Pd(II) complexes of both ligands compare to Au(III) & Pt(IV) complexes and this may be due to square planner geometry of these complexes which is preferred to cell than the octahedral geometry. Many of the prepared Pd (II) complexes showed a special antitumor activity *in vitro* compared to the pt(IV) complexes due to the fact that these compounds are effective (high lability) in biological fluids. The mechanism of these complexes appears based on effects on DNA [19]. Octahedral Platium(IV) complexes have a capability towards ligands substitution by a dissociative mechanism versus an associative mechanism for Platinum (II), We conclude from this result, Pt(IV) compounds are relatively more inert. This is favored for oral bioavailability and lower toxicity, but it is undesirable for DNA interpolation *in vitro*. In spite of that, several Platinum (IV) complexes are showing considerable activity in initial trials, with functionality thought to depend on the *in vivo* reduction of Pt(IV) to Pt(II), producing reactive intermediates capable of interacting with DNA. (The reduction of Pt(IV) to Pt(II) compounds by biological agents is necessary to exert their antitumor activity). The reduction potential of Pt (IV) complexes depends on the type of equatorial and axial ligands [19,20]. Gold complexes have shown great efficacy as an anticancer drug, due to the fact that many Au(I) and Au (III) compounds inhibit the growth of cancer cells, The cytotoxic effects of these complexes are associated with DNA damage and mitochondrial function inhibition, and their interaction with many intracellular targets such as cysteine, nitrogen bases, glutathione reductase, thioredoxin reductase and selenocysteine [21a,b]. The gold (III) complex have octahedral geometry and high charge of gold (III) may be reduced inside the cell into low charge gold(I) and make more stable complex which may be due to increasing the inhibition rates on RAW. 264.7 cell line. The *In vitro* cytotoxicity results are summarized in Table (6) and Fig.(2).

Table (6)
Shows the percentage inhibition rate on (RAW. 264.7).

Compounds	***Inhibition rate %***			
	400 µg/ml	***200µg/ml***	***100 µg/ml***	***50 µg/ml***
Cis-Pt	76.37	72.78	67.88	63.67
L_1	62.50	57.50	56.00	47.75
PtL_1	79.75	76.00	73.50	69.00
PdL_1	78.00	73.25	71.25	71.00
AuL_1	80.00	67.75	64.00	62.50
L_2	70.75	69.25	68.75	65.50
PtL_2	74.66	73. 58	68.75	64.30
PdL_2	78.41	76. 50	74.58	73.15
AuL_2	77.47	75.13	72.75	68.99

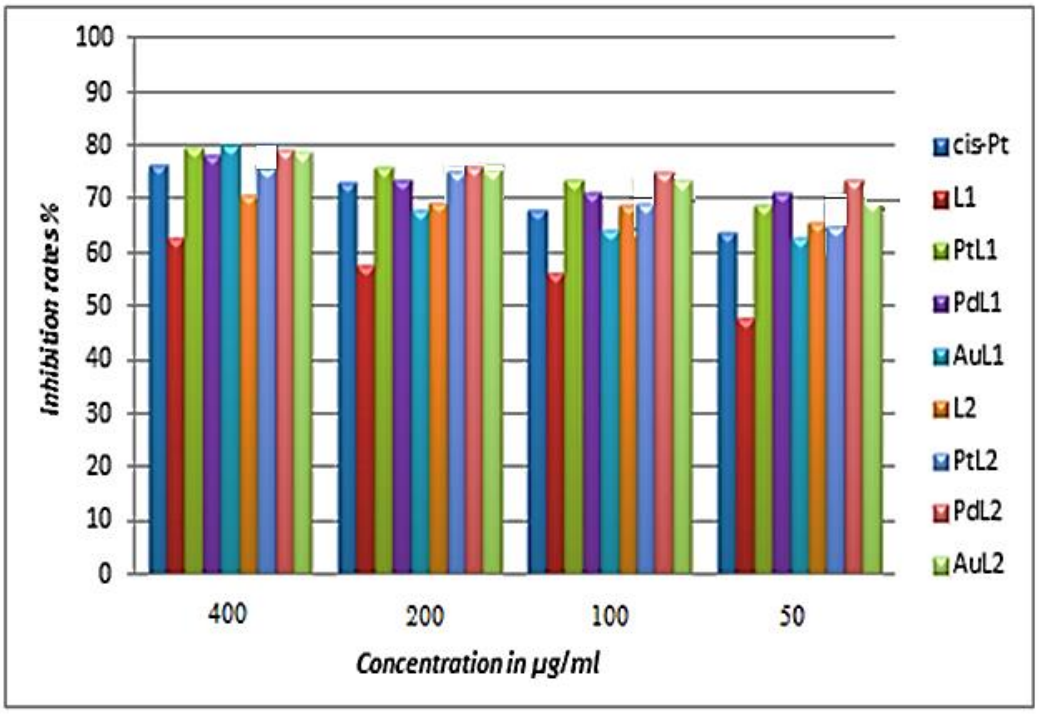


Fig.(2): Shows the percentage inhibition rate on (RAW. 264.7) after time of exposure 48 hrs.

Conclusions

The structures of the prepared compounds were suggested according to physical and spectral analysis. All data revealed that the ligands acted as bidentate and tridentate for L_1, L_2 respectively. Based on the electronic spectral data and the magnetic susceptibility measurements, the complexes exhibited square planner geometry of palladium ion and octahedral geometry of platinum and gold ions complexes. The ligands and the metal ion complexes were screened for their cytotoxic activities. The cytotoxic studies showed that the palladium ion complexes of both ligands more active than the other compounds.

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