

Preparation and Characterization of Some Complexes with New (Azo-Schiff base) Ligand and Study of Complex as Anticancer

Layla Ali Mohammed¹, Muna Abass Hadi¹, Noor Alhuda Basim²

^{1,2}Assist. Prof., Dep. of Chemistry, College of Education for Girls, University of Kufa, Iraq, ²Lecturer, Dep. of Medical laboratory Techniques, Collage of Medical Technology, University of Islamic, Iraq

Abstract

A new series of transition metal complexes of Co(II), Ni(II), Cu(II), Zn(II), Cd(II), Hg(II), Au(III) and Pt(II) have been prepared and characterized using a new azo-Schiff base ligand derived from Schiff base (N¹Z, N²Z)-N¹, N²-bis(4-amino-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-ylidene)benzene-1,2-diamine with 5-methyl-imidazole. The structures of the new ligand azo-Schiff base and their transition metal complexes are characterized using several techniques, including analysis (C.H.N), molar conductance, magnetic measurements, electronic spectral, IR spectral studies, ¹³C-NMR, ¹H-NMR and mass spectra. The data show that these complexes have composition of [MLCl₂] where M=Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Hg(II), [ML]Cl₃ where M=Au(III) and [ML]Cl₂ where M=Pt(II). The electronic spectral, and magnetic susceptibility data of the complexes suggest octahedral geometry of all complexes, except the Au(III) and Pt(II) complex suggest a square planar geometry. The IR results demonstrate that the coordination sites are the azomethine nitrogen and azo nitrogen atoms of the ligand. The ligand behaves in a natural tetradentate manner. The biological screening effect of the Pt(II) complex is tested against human breast cancer cell line MCF-7 and normal cells (cell line WRL68). The results show the highest inhibitory effect for the complex.

Key word: transition metal, Schiff base ligand, imidazole

Introduction

Imidazole derivatives have gained great importance as they display a wide range of pharmacological properties⁽¹⁾ in addition to their excellent donor abilities and chelating agent, Schiff base and azo-Schiff bases are important for the preparation because this class of compounds possess active (π -acidic) for this reason a number of these compounds were prepared⁽²⁾. Imidazole derivatives are of high interest among researchers and are used in different fields because of their biological applications including antifungal, antioxidant⁽³⁾, antiprotozoal, antihypertensive⁽⁴⁾, antibacterial, and anticancer⁽⁵⁾. Azo dyes are highly colored and have been used for dyeing industry, electrochromism, nonlinear optical elements, printing system⁽⁶⁾, and pigments for a long time⁽⁷⁾. On the other hand, azo compounds represent (60-70%) from all azo dyes⁽⁸⁾, while Schiff bases have played an important role as chelating ligands in the development

of coordination chemistry and optical materials⁽⁹⁾, in addition to complexes showing biomedical applications like antimicrobial, anticancer⁽¹⁰⁾ and antioxidant activities⁽³⁾. Azo-Schiff base ligands and their complexes having multifunctional groups play an important role due to their complexing, catalytic properties⁽¹¹⁾. Recently, this paper reports the synthesis, characterization and biological activity study of Pt(II) complex as anticancer of the new azo-Schiff base ligand (3z)-N-(2-(((3z)-1,5-dimethyl-4-((4-methyl-1H-imidazol-2-yl)diazanyl)-2-phenyl-1,2-dihydro-3H-pyrazol-3-ylidene)amino) and its metal complexes with selected metal ions.

Measurement: The electrothermal melting point model 9300 was used to measure the melting point of the ligand and its complexes. Elemental analyses were carried out by means of a microanalytical unit of 1180 (C.H.N) elemental analyzer. Electronic spectra were recorded on Shimadzu spectrophotometer double beam model 1700 UV-Vis spectrophotometer. FTIR spectra were recorded in KBr disc on FTIR Shimadzu spectrophotometer model 8400 in wave number (4000-400) cm⁻¹. ¹H-NMR & ¹³C-NMR spectra in (ppm) unit were operating in

DMSO-*d*₆ as solvent using (Bruker-Ultra Shield 3000 MHz Switzerland). And Mass Spectra were recorded on AB Sciex-3200 QTRAP LC/MS/MS, (Mass range-*m/z*(5-2000)-quad mode and(50-1700)linear ion trap mode). Magnetic susceptibility were carried out on a balance magnetic(MSB-MKI)using faraday method. The diamagnetic corrections were made by Pascal's constants .

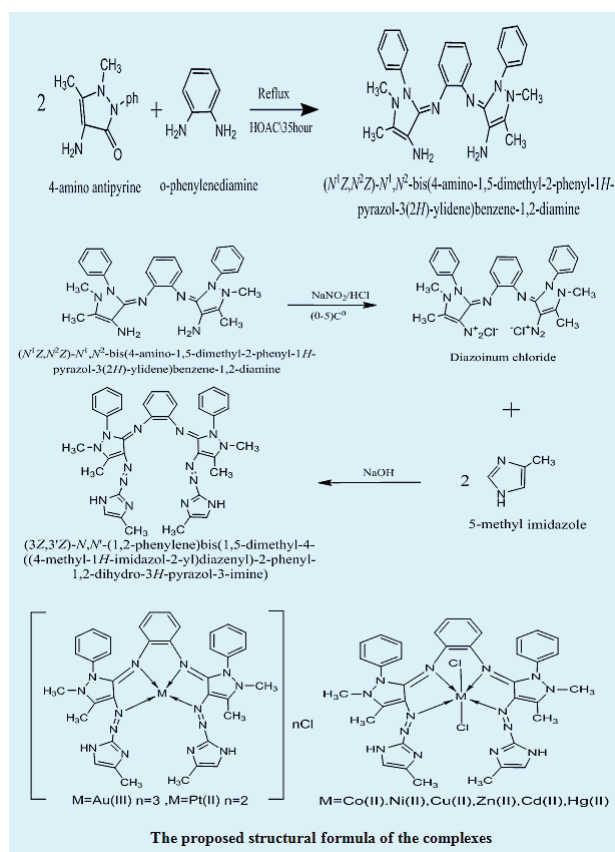
Preparation of ligands:

Preparation of the Schiff base⁽¹²⁾:

(4.06 gm ,0.02 mol) from 4-amino antipyrine dissolved in ethanol (50ml) and then mixed with (1.08g,0.01mol)(*o*-phenylene diamine) dissolved in ethanol. Three drops from glacial acetic acid were added and the mixture was refluxed with stirring for 35hrs. Schiff base ligand was isolated after the volume of the mixture was reduced to half by evaporation and precipitated product was collected by filtered off and dried over anhydrous CaCl₂. Yield:88%;mp:(125-127) C⁰.

Preparation of the new azo-Schiff base ligand:

The new azo-schiff base ligand is prepared by coupling reaction of diazonium salt with appropriate amount of (5-methyl—imidazole) as coupling component in alkaline solution. Adiazonium solution is prepared by dissolving (4.78g, 0.01 mole) of (N¹Z,N²Z)-N¹,N²-bis(4-amino-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-ylidene)benzene-1,2-diamine in 4ml of concentrated hydrochloric acid and (25ml) distilled water. To this mixture a solution of (1.4g, 0.02` mole) of sodium nitrate in 15ml of distilled water was added drop wise at (0-5)°C ,and left to stand (30min). This diazonium solution was added drop wise to 5-methyl--imidazole (1.64g,0.02mole) dissolved in (30ml) of ethanol and (50ml) of (2N) sodium hydroxide at (0-5)°C. The mixture was allowed to stand overnight. The precipitate was filtered off, washed with distilled water, and recrystallized twice from hot ethanol and then dried in oven at 40°C for 10 hours. m.p(224-226)°C,yield(60%),scheme 1.



Scheme-1:preparation of Schiff base,(azo-Schiff base) ligands and Complexes.

Preparation of metal complexes:

The metal complexes were prepared by mixing of 30ml ethanol solution of (CoCl₂.6H₂O, NiCl₂.6H₂O, CuCl₂.2H₂O, ZnCl₂, CdCl₂.2H₂O, Hg Cl₂.2H₂O,PtCl₂ ,NaAuCl₄. H₂O) with 30ml ethanol solution of (azo-Schiff base) ligand in (1:1) (metal: ligand) ratio.the resulting mixture was refluxed for 2h.The product was isolated after reduced of volume by evaporation .It was filtered off ,washed with ethanol and dried under vacuum.The complexes obtained are listed in table 2.

Method

Cytotoxicity Assays

To determine the cytotoxic effect,the MTT cell viability assay was conducted on 96-well plates.Cell lines were seeded at (1×10⁴cells/well).After(24hrs).or a confluent monolayer was achieved, cells were treated with tested compound.Cell viability was measured after 72 hrs of treatment by removing the medium, adding 28μL of 2 mg/mL solution of(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) reagent MTT (and incubating the cells for 1.5h at(37°C).After removing the MTT solution,the crystals remaining in

the wells were solubilized by the addition of (130 μ L) of DMSO(Dimethyl Sulphoxide) followed by(37 $^{\circ}$ C) incubation for (15min) with shaking^(13,14).The absorbency was determined on a micro plate reader at 492 nm (test wavelength);the assay was performed in triplicate.The inhibition rate of cell growth (the percentage of cytotoxicity)was calculated as the following equation: Percent Inhibition=Rate without inhibitor-rate with inhibitor/rate without inhibitor x 100 ,[(X-Y)/X] \times 100

Results and Discussion

All our complexes are Freely soluble in DMF,DMSO,Methanol and Ethanol.Also They are stable in air.The metal complexes were characterized by elemental analysis,molar conductivities,magnetic susceptibility,IR,UV-Vis,Mass and ¹³C, ¹H,MNR spectra.The analytical data of the complexes are in

agreement with the experimental data.The value reveal that the metal to ligand ratio is(1:1) and are presented in table.2.The magnetic susceptibility of the chelate complexes at room temperature were consistent with octahedral geometry,except the Pt(II) and Au(III) complex suggest a square planar geometry around the central metal ion. Most of chelate complexes prepared in this work showed lower conductivity values of the complexes.This proves that complexes have non-electrolytic nature.Except the Pt(II) and Au(III) complex which showed higher conductivity values support the electrolytic nature of the metal complexes.

Micro analysis:

The elemental analysis data of 1:1 [M:L] ratio complexes showed that the theoretical values are in a good agreement with the found data ,as listed in table(1). The purity of azo-schiff base ligand were tested by TLC technique and C.H.N analysis.

Table(1):physical properties and analysis of Compounds

compound	Mwt	Color	m.p (C $^{\circ}$)	Yield %	Found (Calc.)%			
					%C	%H	%N	%M
L3	664	Deep red	226- 224	68	(65.06) 64.89	(5.42) 5.31	(29.51) 29.12	—
[CoL3Cl2]	793.93	Violet	>310	72	(54.41) 54.02	(4.53) 4.31	(24.68) 23.99	(7.42) 7.13
[NiL3Cl2]	793.7	Violet	>310	71	(54.42) 53.91	(4.53) 4.49	(24.69) 23. 86	(7.39) 7.06
[CuL3Cl2]	798.54	Violet	>310	80	(54.09) 53.61	(4.50) 7.28	(24.54) 24.11	(7.95) 7.27
[AuL3]Cl3	967.46	Violet	215-216	75	(44.65) 44.31	(3.72) 3.22	(20.25) 20.11	(20.35) 20.01
[PtL3]Cl2	930.09	Violet		79	(46.44) 46.09	(3.87) 3.54	(21.07) 20.96	(20.97) 20.45
[ZnL3Cl2]	800.38	Violet	270	75	(53.97) 53.20	(4.49) 4.11	(24.48) 23.97	(8.16) 7.88
[CdL3Cl2]	847.41	Violet	201- 202	62	(50.97) 50.52	(4.24) 3.94	23.12 22.89	13.26 12.79
[HgL3Cl2]	935.59	Violet	>300	69	(46.17) 54.83	(3.84) 3.41	(20.94) 20.35	—

Infrared Spectra studies of the ligand and complexes:

The important infrared spectral bands for the synthesized ligand and its chelate complexes are given in table.2. The ligand contains four potential donor sites: (1,2) the azomethine nitrogen, and (3,4) the azo group nitrogen. The IR spectrum of the ligand shows characteristic bands at (1639 and 1454) cm^{-1} due to the (C=N) and (N=N) functional groups respectively⁽¹⁵⁾. The (C=N) and (N=N) bands in the free ligand shift to (1627-1614) cm^{-1} and (1436-1409) cm^{-1} , respectively for the complexes. The reduction in bond order, upon complexation, can be attributed to delocalization of metal electron density (t_{2g}) to the π -system of the ligand. These shifts confirm the coordination of the ligand via the

nitrogen of azo methine and the azo groups to metal ions⁽¹⁶⁾. The absorption band in free ligand observed at 3423 cm^{-1} attributed to the $\nu(\text{NH})$ group⁽¹⁷⁾. This band remains unchanged in the spectra of their complexes. The absorption band in ligand azo-schiff observed at 1666 cm^{-1} attributed to the $\nu(\text{C}=\text{N})$ of the N_3 imidazole nitrogen. This band remains unchanged in the spectra of their complexes⁽¹⁸⁾. This suggests that the $\nu(\text{C}=\text{N})$ group is not taking part in coordination. New bands are attributed to $\nu(\text{M}-\text{N})$ vibrations appearance in all complexes at (447-412) cm^{-1} respectively⁽¹⁹⁾. The stretching wave number due to (N-N) in the coordinated compound was slightly affected from (1047) cm^{-1} which indicates the unsharing of this linkage of pyrazolone ring in coordination with metal ions. Representative example for their spectra is given in Fig1.

Table.2: Characteristic IR absorption bands of the ligand and its complexes in cm^{-1}

Compound	(N-H)	$\nu(\text{C}=\text{N})$ imidazole	$\nu(\text{N}=\text{N})$	$\nu(\text{C}=\text{N})$ Schiff	$\nu(\text{N}-\text{N})$	$\nu(\text{M}-\text{N})$
Ligand	3423	1666	1454	1639	1047	-----
[Cu L Cl ₂]	3421	1660	1419	1625	1047	412
[Co L Cl ₂]	3419	1666	1421	1624	1045	445
[Ni L Cl ₂]	3412	1666	1436	1625	1045	445
[PtL]C l ₂	3421	1664	1411	1631	1045	441
[Zn L Cl ₂]	3414	1665	1413	1622	1045	443
[Cd L Cl ₂]	3419	1663	1413	1625	1045	428
[Hg L Cl ₂]	3423	1665	1409	1627	1047	412
[Au L] Cl ₃	3423	1663	1412	1614	1047	447

Mass spectra:

The mass spectra of synthesized ligand and its Cu(II) complex are recorded at room temperature. The obtained peaks confirm the proposed formulae for the compounds. The mass spectrum of Ligand shows the molecular ion peak at m/z 664.3 (11.9%) compound ($\text{C}_{36}\text{H}_{36}\text{N}_{14}$) confirm the proposed formulae for compound. The mass spectrum of the Cu(II) complex exhibits the molecular

ion peak at m/z 798.2 (0.9%) to the molecular formula ($\text{Cu}(\text{C}_{36}\text{H}_{36}\text{N}_{14}\text{Cl}_2)$) consistent with the molecular weight of the Cu(II) complex.

¹HNMR spectra:

The ¹HNMR spectrum of the ligand shows the following signals: phenyl multiples at (7.3-7.5) ppm, =C-CH₃ at 2.3 ppm, N-CH₃ 3.3 ppm, CH₃ imidazole ring at

2.0 NH at 12.5 ppm⁽¹⁷⁾. There is no appreciable change in all other signals in this complexes.

¹³CNMR spectra:

The spectra of the azo-Schiff base ligand were measured at room temperature in [D6]DMSO. The ¹³C NMR spectrum of the ligand displayed characteristic signals at 8, 13.2, 39.3, 154.2 and 136.2 ppm due to (C-CH₃ imidazole)(>C-CH₃), (>N-CH₃), (>C-N=N-) antipyrine ring and (>C=N imidazole ring) of the azo-Schiff base ligand, respectively^(23,24). The peak at $\delta = 164.9$ ppm was due to azomethine carbon of the ligand⁽²³⁾. Moreover, the spectrum of the ligand showed peaks in the region of (120.1, 124.2, 127.6, 128.6, 129.01 and 144.2, 146.0 ppm) due to aromatic carbon atoms.

Electronic Spectra:

Electronic spectra provide the most detailed information about the electronic structure. The UV-Vis spectrum of the (azo-schiff base) ligand exhibits two charge transfer (CT) bands at (310 nm)(32258 cm⁻¹) and (370 nm)(27027 cm⁻¹) attributed to ($\pi-\pi^*$) and ($n-\pi^*$) transition within the (azo-schiff base) ligand. In the

spectrum of the complexes, the CT band at (310 nm) remains as such, in agreement with the $\pi-\pi^*$ transition of the (azo-schiff base) ligand. The band observed at (370 nm) in the spectrum of the free ligand is red-shifted to (529-546 nm) in complexes due to ligand to metal charge transfer (LMCT) transition⁽²⁵⁾, suggesting an octahedral geometry around metal(II) in the complexes⁽²⁶⁾. The electronic transitions, magnetic properties and conductivity values of the ligand and its complexes these are listed in table.3.

Magnetic measurements:

The Co(II) complex has a magnetic moment of 5.01 B.M, which is in agreement with the reported value for octahedral Co(II) complexes⁽²⁷⁾. The Ni(II) complex shows a magnetic moment value of (2.9) within the range of (2.9, 3.3) B.M⁽²⁸⁾ suggesting an octahedral environment. The Cu(II) complex shows a magnetic moment value of 1.71 B.M, and consistent with a distorted octahedral geometry⁽²⁹⁾. The Zn(II), Cd(II), Hg(II), Au(III), Pt(II) are diamagnetic and according to the empirical formulae of complexes, an octahedral geometry is proposed⁽³⁰⁾ except the Au(III) and Pt(II) complex suggest a square planar geometry⁽³¹⁾.

Table.3: Electronic spectra, conductivity and magnetic moment of complexes

Comp.	Absorption Bands(nm)	Absorption Bands(cm ⁻¹)	Transition	μ_{eff} (B.M)	Conductivity S.mol ⁻¹ . cm ²	Geometry	Hybridization
Ligand	310 370	32285 27027	$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$	-----	-----	-----	-----
(Pt L)Cl ₂	543	18416	M \rightarrow L, CT	Dia	75.03	square-planar	dsp ²
(Co LCl ₂)	540	18518	M \rightarrow L, CT	5.01	6.14	Octahedral distorted	sp ³ d ²
[Ni LCl ₂)	539	18552	M \rightarrow L, CT	2.9	7.16	Octahedral (regular)	sp ³ d ²
[Cu LCl ₂)	546	18315	M \rightarrow L, CT	1.71	9.22	Octahedral distorted	sp ³ d ²
(Au L)Cl ₃	535	18691	M \rightarrow L, CT	Dia	166.17	square-planar	dsp ²

Cont... Table.3:Electronic spectra, conductivity and magnetic moment of complexes

(Zn LCl ₂)	530	18867	M→L,CT	Dia	10.23	Octahedral (regular)	sp ³ d ²
(Cd LCl ₂)	529	118903	M→L,CT	Dia	8.01	Octahedral (regular)	sp ³ d ²
(Hg LCl ₂)	532	18796	M→L,CT	Dia	6.33	Octahedral (regular)	sp ³ d ²

Anticancer activity:

Evaluation of newly synthesized complex in cancer therapy are studied. The antitumor activities of the synthesized Pt(II) complex was tested against breast cancer cell line MCF-7 and normal cells (cell line WRL68). The results showed that the highest inhibitory effect was reported for complex give IC₅₀ value (93) and (1193), respectively. The cell cytotoxic effect of tested Pt(II) complex was calculated. The optical density was measured with the micro plate reader to determine the number of viable cells and the Inhibition Rate was calculated as

$$\text{Percent Inhibition} = \frac{\text{Rate without inhibitor} - \text{rate with inhibitor}}{\text{rate without inhibitor}} \times 100, [(X - Y) / X] \times 100$$

The (50%) inhibitory concentration (IC₅₀), the concentration required to cause toxic effects in (50%) of intact cells, was estimated from graphic plots of the dose response curve for each concentration. using Graph pad Prism 6. The anticancer activity of the synthesized Pt(II) complex was determined against an human breast cancer cell line MCF-7 and normal cells (cell line WRL68) using different concentrations was evaluated. The cytotoxicity of Pt(II) complex on normal cells (WRL68) were studied using MTT assay. The results indicate effect of toxicity was very low on normal cells (WRL68). The platinum complex exhibit good results and expressed as IC₅₀ value (μg/ml). The results are shown in fig.(1).

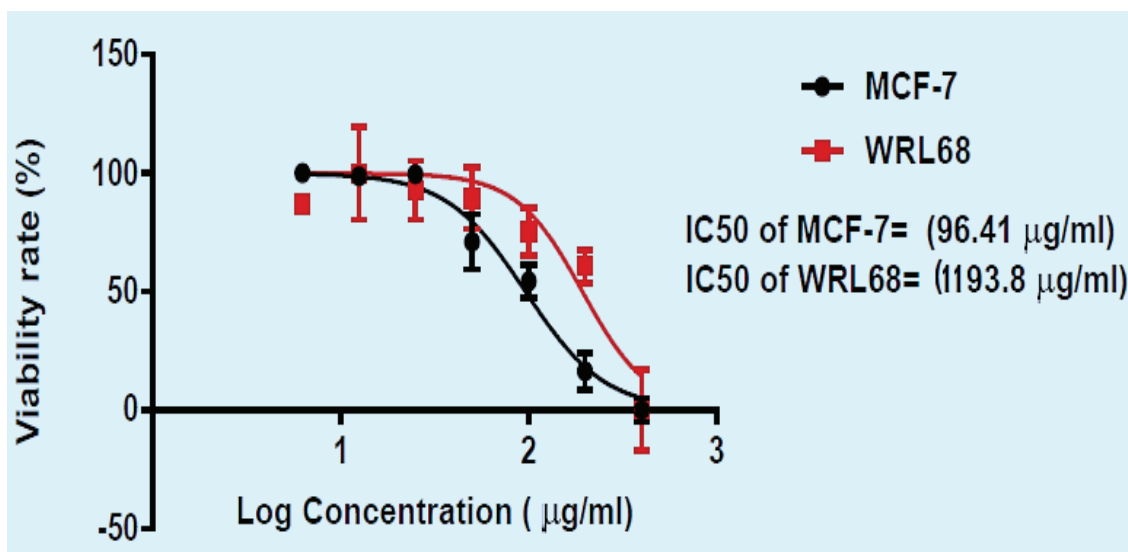


Fig.1: Curve with plotting of IC₅₀ of :a human breast cancer cell line MCF-7 & (b) WRL68

Treating tumors or tumor metastases

Killing cancer cells because of DNA binding cannot account for a selective mechanism of cell recognition and of discriminating toxicity. Provided that tumor cells differ from their healthy counterparts for the activated genes rather than for DNA content (which is almost identical in terms of nucleotide content, e.g.: the number of guanines and their sequence, in any cell of the body including the tumor mass), the question remains open why, besides the high sensitivity of testicular tumors that can be cured, other tumors towards which platinum drugs are applied in a number of combination chemotherapeutic regimens show [32].

Conductivity measurement:

Molar conductance (Λ_m) measurements of the metal complexes table(3) carried out using DMSO as solvent at the concentration of $10^{-3}M$ in room temperature. All chelate complexes prepared in this work showed conductivity values ranged between (6.14-10.23) $s.mol^{-1}.cm^2$ that non-electrolyte and no conductive species⁽¹⁵⁾ While the molar conductance of Au(III) and Pt(II) complex are (75.03-166.17) $s.mol^{-1}.cm^2$ indicating the electrolytic nature (1:3) and (1:2) electrolyte of this complexes furthermore the chloride ions are located outside the coordination sphere⁽¹⁶⁾.

Conclusions

In this paper we have explored the synthesis and coordination chemistry of some monomeric complexes obtained from the reaction of the tetra dentate ligand with some metal ions as shown in Figure.(2). The mode of bonding and overall structure of the complexes were determined through physico-chemical and spectroscopic methods. Complex formation study via molar ratio has been investigated and results were consistent

to those found in the solid complexes with a ratio of (M:L) as (1:1). The biological screening effect of the Pt(II) complex are tested against human breast cancer cell line MCF-7 and WRL68. The results show the highest inhibitory effect for complex.

Conflict of Interest: There is no any Conflict of Interest

Ethical Clearance: Ethics committee refer that there is no plagiarism and there is no mistakes or wrong results in work.

Source of Funding: Self funding.

References

- 1- Isra, W.; Hesham, B.; Htham, M Y.; Ahmed, A j. Synthesis and characterization of new imidazole azo ligand with some of transition metal ions, and their biological effect on two pathogenic bacteria of burn patients: *Int. Journal. Research in Pharm. Sci.* **2019**, *10*, 3.
- 2- Saad, MM.; Ali, KI. Preparation and Identification of new azo-schiff base ligand (NASAR) and its divalent transition metal Complexes: *J. Pharm. Sci. Res.* **2018**, *10*(9).
- 3- S. Slassi, A. F. Tailler, G. Larcher, A. Amine, and A. El-Ghayoury; Imidazol and Azo-Based Schiff Bases ligands as Highly Active Antifungal and Antioxidant Components: *Heteroatom Chemistry.* **2019**, *8*, 14.
- 4- S. Dutta; Synthesis and anthelmintic activity of some novel 2-substituted-4,5-diphenyl imidazoles: *Acta Pharmaceutica.* **2010**, *60*, 229.
- 5- G. K. Sharma, S. Kumar, and D. Pathak; Synthesis, antibacterial and anticancer activities of some novel imidazoles: *Scholars Research Library.* **2010**, *2*, 223.
- 6- Yildiz, M. Kaya, S. Dincer; Mononuclear Fe(III), Cr(III), Co(II) metal complexes based on azo-anthraquinone moieties: synthesis, characterization and antibacterial activities: *Chem. Sci. Trans.* **2013**, *2*, 547.
- 7- Noroozi, P N.; Gholsanamloo, M M. Par, H. Rashidnejad, A. Gharib, K. Nejati; Synthesis, Characterization and spectroscopic properties of new azo dyes derived from aniline derivatives based on acetylacetone and azo-metal(II) complexes and singular value decomposition (SVD) investigation: *Iranian Chemical Communication.* **2019**, *7*, 19.
- 8- Tan, G N. Synthesis, properties and Application of organic dyes and pigments (Ph. D. Thesis). Wageningen University: 2001.
- 9- Hiroki Sato, Izumi Beppu, Tomoyuki Haraguchi, Takashiro Akitsu, Rakesh Parida. Optical properties of chiral Schiff base MnII, CoII: *J. Indian Chem. Soc.* **2018**, *95*.
- 10- G. Valarmathy, R. Subbalakshmi, R. Renganathan, and R. Kokila; Synthesis of Schiff base ϵ -2-((3-Hydroxyphenyl)imino)methyl)-6-methoxyphenol Containing N and O Donors and its Metal Complexes: Spectral, Thermal,

Redox Behaviour, Fluorescence Quenching, Antimicrobial and Anticancer studies: Asian Journal of Chemistry: **2018**,30, 645.

- 11- Taha, N.I., O. N. Tapabashi, M. N. El-Subeyhi; green synthesis of new Schiff bases and bis-azo-schiff bases derived from 2,6-diaminopyridine: *Int. J. Org. Chem.*, **2018**,8,309.
- 12- Layla Ali Mohammed, Raheem Tahir Mehdi, Abid Allah Mohammed Ali, Synthesis and Biological Screening of the Gold Complex as Anticancer and Some Transition Metal Complexes with New Heterocyclic Ligand Derived from 4-Amino Antipyrine. *Nano Biomed. Eng.*, 2018,10,3.
- 13- A. Accardo, L. Alo, M. Aurilio, Receptor binding peptides for target- selective delivery of nano particles encapsulated drugs. *Int. J. Nanomedicine*, 2014, 1537-1557.
- 14- Rajsh, Jumar. Anticancer activity of eco-friendly gold nano particles against lung and liver cancer cells, *J. Genetic. Engine. erring. Biotechnology*, (2016),14:195-202.
- 15- Bergmo, A. and G. Sava, Linking the future of anticancer metal-complexes to the therapy of tumour metastases. *Chemical Society Reviews*, 2015,44(24):8818-8835.
- 16- Suresh, M. .;Prakash, V. preparation and characterization of Cr(II), Mn(II), Co, Cu(II), and Cd(II) Chelates of Schiff bases derived from Vanillin. *Inte. J. physical Sci.*, 2010,5(4):2203-2211.