

Synthesis and characterization of novel oxime derivatives and its application in oncology

Sara M Younes^{1*}, Shaima M Fahim²

¹ Chemical Engineering Department, Borg El Arab Higher Institute Engineering and Technology, Alexandria, Egypt

² Badr Higher Institute of Technology of Applied Healthy Science, Egypt

Abstract

The synthesize chelates of oxime as important target structures and to assess their biological activities. the development of new phagocytes with diverse biological activities is our interest and importance for oxymhydrazide chelates, the synthesis and characterization of new metal chelate from Cu (II) from (2,3-). Dihydroxy-N1, N4-bis (2 - (E) - (3- (hydroxyimino) -4-oxopentan-2-ylidene) amino (phenyl) succinamide) ligand. The structure of the ligand and its mineral chelate were confirmed using elemental and spectroscopy analysis, magnetic moment, conductivity and thermal analysis (DTA and TGA). Initial and spectral data show that chelate has a deformed octahedral structure. ESR spectra of Cu (II) chelates show the covalent bond character of a $2d_{x^2-y^2}$ ground state ESR in a deformed octahedral structure. Data on antiproliferative activity in the ligament and some of its mineral complexe show that chelators exhibit a high to moderate inhibitory effect (IC50) against human HepG2 liver cancer cells compared to the standard drug (Sorafenib (Nexavar).

Keywords: oxime, metal chelates, elements analysis, spectra analysis, magnetic moments, ESR, antitumor activity

Introduction

Oximes represents an important class of compounds due to its wide applications in industry, medicine and the detection and identification of various metal ions [1-3]. One of the most interesting aspects of oxide bonds is their ability: stabilizing various oxidation states of minerals, to develop inorganic biological models, to design selective receptors for metal ions [4-6]. In addition to the traditional applications of oxym derivatives as analytical and organic reactors, these compounds have recently attracted a lot of interest as drug candidates with a wide range of biological activity [13, 14], Anti-microbial [7], Anti-cancer [8], fungicides [9], bactericides [10], Anti-oxidant [11], Anti-doting for nerve, Anti-malarial 6, and anti-cancer treatment through histone deacetylase inhibition [12]. Thus, the increasing artificial interest in N-OH compounds holds great promise for developing new therapeutic drugs in various fields of medicine. Oxygen and chemistry is a vital research area for inorganic and inorganic chemistry. The role of oxim and its mineral complexes, especially cobaloxime, in biological systems makes it a branch interested in coordination and organic mineral chemistry [12]. Important publications on the development of coordination chemistry are associated with the preparation and characterization of oxim and its mineral complexes. These include the study of new synthetic methods and coordination patterns of types of oxide at complexity [13-17]. It is well known that oxime and oximato types can bind metal in different coordination modes [18-20]. In view of interest and importance of oxime-hydrazide chelates, we reported here synthesis and characterization of new metal chelates of Mn(II), Ni(II) Cu(II) Zn(II), Cd(II) and Pb(II) of (2,3-dihydroxy-N1,N4-bis(2-((E)-(3-(hydroxyimino)-4-oxopentan-2-ylidene)amino)phenyl)succinamide) ligand also, antitumor activity of the ligand and some of its chelates against HepG-2 have been studied.

Experimental

Instrumentation and measurement

The ligand and its mineral chelate were analyzed for C, H, N, and Cl at the Micro Analytical Center, Cairo University, Egypt. Standard analytical methods were used to determine the mineral ion content [21-22]. ¹H-NMR spectra were obtained on BRUKER 400 MHz spectrometer. Chemical shifts (ppm) are reported relative to TMS. FT-IR spectra of the ligand and its metal chelates were measured using KBr discs by a Jasco FT/IR 300E Fourier transform infrared spectrophotometer covering the range 400-4000 cm⁻¹. Electronic spectra in the 200-900 nm regions were recorded on a Perkin-Elmer 550 spectrophotometer. The thermal analysis (DTA and TG) was carried out on a Shimadzu DT-30 thermal analyzer from room temperature to 800 °C at a heating rate of 10 °C/min. Magnetic susceptibilities were measured at 25°C by the Gouy method using mercuric tetrathiocyanatocobaltate(II) as the magnetic susceptibility standard.

Synthesis of ligand

The ligand, (H₆L) was prepared by

1. Adding of 5 drops of conc (30 g, 0.19 mol) tartaric acid in 25 cm³ of ethanol in presence of H₂SO₄, O-Phenylene diamine (10g, 0.09 mol) dissolved in 10cm³ of ethanol solution.
2. The mixture was refluxed with stirring for 2 hours and then left to cool at room temperature filtered off the formed hydrazide and left it to dry at room temperature.
3. The ethanolic solution of hydrazide (10g, 0.03 mol) was added to (hydroxyimino) pentane-2, 4-dione oxime (7.8g, 0.06 mol) dissolved in 30 cm³ ethanol.
4. The mixture was stirred and refluxed for three hours at 80 °C, then left to cool to room temperature.

The solid product was filtered off then dried under vacuum over anhydrous CaCl₂.

Synthesis of metal complexes

Preparation of metal chelates (A₁)

Synthesis of chelates (A₁) using (1L: 2M) were carried out by refluxing a hot ethanolic solution of the ligand (2g, 0.003 mol) with a hot ethanolic solution of the metal salts of (1.4g, 0.006 mol) of Cu (OAc)₂·H₂O, chelate (A₁). The reaction mixtures were refluxed with stirring for 1–2 hrs range, depending on the nature of the metal ion and the anion. The precipitates so formed were filtrated off, washed with ethanol, and dried in a desiccator over CaCl₂.

Antitumor evaluation

Antiproliferative activity within the compound compound was measured according to the sulfo-rhodamine B stain (SRB) assay using published methods [12]. Cells were coated in a 96 multi-Well plate (104 cells / well) for 24 h prior to treatment with complexes to allow cell binding to the plate wall. Different concentrations of the complexes in DMSO (3.9, 7.8, 15.6, 31.25, 62.5, 125, 250 and 500 µg) were added to the triple monolayer cell. The monolayer cells were incubated with the complexes for 48 h at 37 °C under

5% CO₂ atmosphere. After 48 h, cells were fixed, washed, and stained with Sulfo-Rhodamine-B stain. The excess stain was washed with acetic acid and the attached stain was recovered with Tris EDTA solution (10 M Tris HCl + 1 M M disodium EDTA, PH 7.5-8). Color intensity was measured by an ELISA reader. The relationship between the residual fraction and drug concentration is plotted to obtain a survival curve for each tumor cell line after the specific compound.

Results and Discussion

The metal complex is colored, crystalline solid, non-hygroscopic, and air stable solid at room temperature without decomposition for a long time. The complex is insoluble in water, ethanol, methanol, benzene, toluene, acetonitrile and chloroform, but appreciably soluble in both dimethylformamide (DMF) and dimethylsulfoxide (DMSO). The analytical and physical data (table 1) and spectral data. The elemental analyses indicated that, all chelates were found to be 1L: 2M molar ratio.

Table 1: Analytical and Physical Data of the ligand [H₆L] (1) and it's Metal Chelate A₁

No	Ligand /complex	Color	FW	M.P	Yield (%)	Anal. /Found (Calc.) (%)					Molar conductance
						C	H	N	M	Cl	
1	[H ₆ L]C ₂₆ H ₂₈ N ₆ O ₈	Reddish brown	552	>300	70	56.04 (56.52)	5.00 (5.11)	15.20 (15.21)	---	---	
A ₁	[(H ₆ L)(Cu) ₂ (OAc) ₄ (H ₂ O) ₂].2H ₃ OC ₃₄ H ₄₈ N ₆ O ₂₀ Cu ₂	Brown	987	>300	75	41.30 (41.34)	4.96 (9.40)	8.56 (8.51)	12.80 (12.87)		4.85

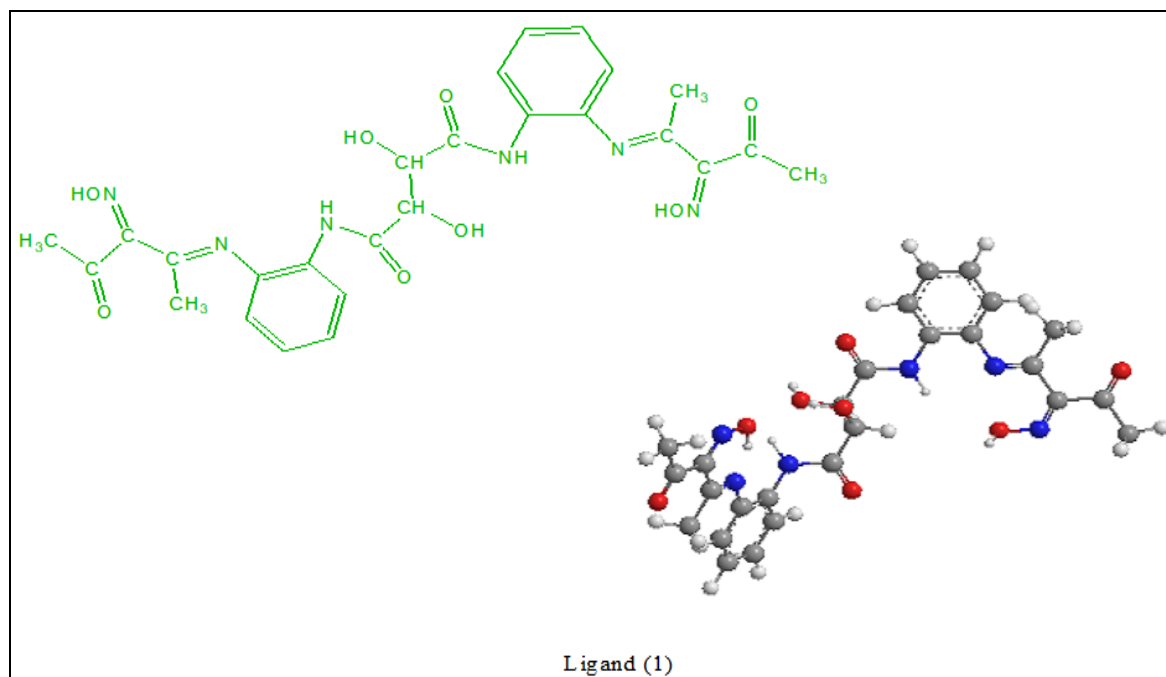


Fig 1: Proposed structures of the ligand

Conductance measurements

Measurements of molar connections of mineral chelates in DMSO solvent were measured at a concentration of 10⁻³. The low amounts of molar conductivity Ω⁻¹cm²mol⁻¹ (listed in Table 1) indicate that all chelate has a non-electrolytic nature [23].

These values agree well with the analytical data dedicated to the participation of groups of anions in metal coordination [24].

Mass spectra

Mass spectrometry to confirm the molecular ion peaks of the H₆L Schiff base and investigate fragment types. The recorded mass spectrum of the H₆L ligand revealed a molecular ion peak strongly confirming the proposed formula. Showed a molecular ion peak at m / z 552 amu, confirming the weight of its formula (FW 552) and the purity of the prepared ligand. The prominent mass fragmentation peaks observed at m/z = 43, 79, 107, 150,

285, 341, 414, 492 and 552 amu corresponding to C_4H_7 , C_6H_7 , C_8H_{11} , $C_9H_{12}NO$, $C_{15}H_{15}N_3O_3$, $C_{17}H_{17}N_4O_4$, $C_{20}H_{24}N_5O_5$, $C_{24}H_{24}N_6O_6$ and $C_{26}H_{28}N_6O_8$ moieties respectively supported the suggested structure of the ligand (Table 2).

Table 2: Mass spectrum of the ligand [H_6L] (1)

m/z	Rel. Int.	Fragment
43	24.29	C_4H_7
79	68.51	C_6H_7
107	85.55	C_8H_{11}
150	26.84	$C_9H_{12}NO$
285	38.08	$C_{15}H_{15}N_3O_3$
341	39.30	$C_{17}H_{17}N_4O_4$
414	34.22	$C_{20}H_{24}N_5O_5$
492	29.83	$C_{24}H_{24}N_6O_6$
552	125.43	$C_{26}H_{28}N_6O_8$

Mass spectra of chelate (6) (A1)

The mass spectrum of the [H_6L] $Cu(OAc)_4 \cdot 2H_2O$ chelate (A1) showed the molecular ion peak at m/z 987 amu, confirming its formula weight (F.W. 987). The mass fragmentation patterns observed at m/z = 83, 183, 313, 368, 551, 602, 705, 891 and 987 amu correspond to C_6H_{11} , $C_{10}H_{17}NO_2$, $C_{15}H_{25}N_2O_5$, $C_{17}H_{24}N_2O_7$, $C_{28}H_{29}N_3O_9$, $C_{29}H_{20}N_3O_{12}$, $C_{30}H_{33}N_4O_{14}Cu$, $C_{32}H_{40}N_6O_{16}Cu_2$ and $C_{34}H_{40}CuN_6O_{16}Cu_2$ and moieties, respectively, supported the suggested structure of the complex [Table 2].

1H -NMR Spectra

The 1H -NMR spectrum of the ligand (1) was recorded in DMSO- d_6 . The spectrum showed two broad peaks at 10[s, 1H] and 92[s, 1H] assigned for protons of (OH) oxime group. The two peaks were weakened in the spectra of the Cu (II) chelate (A1) may be formed of hydrogen bonding of oxime group. Protons signals of acetyl and methyl groups appeared at 3.80 [3H] and 3.50 [3H] ppm respectively, these signals are disappeared upon adding D_2O . However, the aromatic signal protons appear in the 6.32-7.42 ppm region. The proton of NH group appears at 8.01 ppm [25].

Electronic spectra and magnetic moments.

The electron spectra and magnetic moments of the bonding show bands at 280, 330 nm can be mapped to the $\pi \rightarrow \pi^*$ transition which is almost unchanged upon mixing, however, the second range can be mapped to $n \rightarrow \pi^*$ and the charge transfer shifts of the azomethene and carbonyl groups [26]. These bands were shifted to lower energy upon chelate formation, indicating participation of these groups in coordination with the metal ions. The electronic spectra of copper(II) chelate (A1) were nearly identical and showing bands in the 270-265, 305-302, 312-310, 465-450, 580-570

and 615-605 nm ranges.

The first three bands are assigned to intraligand transitions, however the other bands are assigned $2B_{1g} \rightarrow {}^2A_{1g}$ $v_1(dx^2-y^2 \rightarrow dz^2)$, ${}^2B \rightarrow {}^2B_{2g}$, $v_2(dx^2-y^2 \rightarrow dxy)$, and ${}^2B_{1g} \rightarrow {}^2E_g$, $v_3(dx^2-y^2 \rightarrow dxy, dyz)$ transitions, respectively. These transitions indicated that the copper (II) ion has a tetragonally distorted octahedral geometry. This could be due to the Jahn-Teller effect that operates on the d9 electronic ground state of six coordinate system, elongating one trans pair of coordinate bonds and shortening the remaining four ones [27]. The magnetic moments for copper (II) chelates at room temperature were in the 1.61-1.71 range BM, supporting that the chelates have octahedral geometry [28]. [29], [30-31], [32], (Table 3) are due to intra ligand transitions within the ligand and show diamagnetic property.

Table 3: The electronic absorption spectral bands (nm) and magnetic moments (B.M.) for the ligand [H_6L] (1), and its chelate.

No.	λ_{max} (nm)	μ_{eff} in B.M.
(1)	280 nm (log ϵ = 3.98), 330 nm (log ϵ = 4.25)	-
(6)	265, 305, 310, 465, 580, 610	1.67

Electron Spin Resonance (ESR)

To obtain further information about the stereochemistry and the nature of the metal ligand bonding ligand, ESR spectra are carried out. ESR spectra of solid copper (II) chelate (A1) (Table 4) showed that the chelates exhibited anisotropic signals with g values $g_{||} = 2.21$ and 2.18 , $g_{\perp} = 2.07$ and 2.06 respectively. These values are characteristic for a species d9 configuration with an axial symmetry type of $d(x^2-y^2)$ ground state. The values of $g_{||}$ and g_{\perp} are closer to 2.00 and $g_{||} > g_{\perp} > g_e$ (2.0023) indicating that the chelates possessed a tetragonal distortion copper (II) geometry corresponding to an elongation along the four fold symmetry z-axis [33-35]. Also, the value of $g_{||}/A_{||}$ may be considered as a diagnostic of the stereochemistry. It has been suggested, that this quotient may be used as an empirical index of geometry. The range reported for square-planar chelates are 105-135 cm^{-1} and for tetrahedrally distorted chelates 150-250 cm^{-1} . The $g_{||}/A_{||}$ values for the complex under consideration lie just in the range which expected for tetragonal distorted octahedral copper (II) chelates [36]. In addition, the exchange coupling interaction between copper (II) ions is explained by Hathaway expression which stated that $G = (g_{||} - 2)/(g_{\perp} - 2)$. If the value of G is greater than four, the exchange interaction is negligible whereas when the value of G is less than four a considerable interaction is present in solid complexes. The G values of the all copper (II) chelates are 3.0 and 3.7 (Table 4). The orbital populations for chelates (A1) are 94.5%, indicating a $d(x^2-y^2)$ ground state.

Table 4: ESR data for metal (II) complex:-

No.	G ^c	ΔE_{xy}	ΔE_{xz}	K_{\perp}^2	$K_{ }^2$	K	K^2	$g_{ }/A_{ }$	α^2	β^2	β_1^2	-2B	ad ² (%)
(A)	3.0	17241	21505	0.88	0.54	0.8	0.65	170	0.64	1.37	0.84	222	94.5%

a) $g_{iso} = (2g_{\perp} + g_{||})/3$, b) $A_{iso} = (2A_{\perp} + A_{||})/3$, c) $G = (g_{||} - 2)/(g_{\perp} - 2)$

Where λ^o is the spine orbit coupling of free copper ion (-828 cm^{-1}) and ΔE_{xy} and ΔE_{xz} are the electronic transition energies of $2B^1 \rightarrow 2B^2$ and $2B^1 \rightarrow {}^2E$ respectively. For the purpose of calculation, it was assumed that, the maximum in the band corresponds to ΔE_{xy} and ΔE_{xz} can be taken from the wave length of these bands. From the above relations,

the orbital reduction factors ($K_{||}$, K_{\perp} and K) which are a measure of covalence can be calculated. For an ionic environment, $K=1$ and for a covalent environment $K < 1$; the lower the value of K, the greater is the covalent character. The values of K for copper complexes are less than one which inductive to considerable covalent bond

character. The plane and out-of-plane π -bonding coefficients (β_1^2 and β_2^2) respectively are dependent upon to values of ΔE_{xy} and ΔE_{xz} . The copper chelates showed β_1^2 values 0.84 and 0.69 range indicating a moderate degree of covalent character in the in-plane π -bonding, while β_2^2 are in 1.37 and 1.07 ranges indicating ionic character in the out-of-plane π -bonding. Where $2B^0$ is the calculated dipolar coupling for unit occupancy of d orbital. The orbital populations for chelates (A1) are 94.5%, indicating a d (x^2-y^2) ground state.

Thermal analyses

The thermal data of the chelate (A1) corroborate the stoichiometric formula, number of water molecules, and end products. DTA-TGA curves of chelate (A1). Thermogram of chelate (A1) $[(H_6L) Cu (OAc)_4(H_2O)_2] \cdot 2H_2O$ exhibited

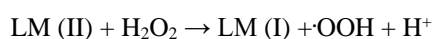
multiple decomposition steps, the first step involving breaking of H-bondings accompanied with endothermic peak at 45 °C. In the second step, two molecules of hydrated water were lost endothermically with a peak at 80 °C accompanied by 3.9% (Cal. 3.6%) weight loss. Two molecules of coordinated water were lost endothermically with a peak at 165 °C accompanied by 3.71% (Cal. 3.78%) weight loss 25.12.1% (Cal. 25.79%) weight loss accompanied by an endothermic peak at 270 °C was assigned to loss of four coordinated acetate groups (4OAc). The endothermic peak observed at 390 °C refers to the melting point of the chelate. The final step observed as exothermic peaks at 440-620 °C range with 23.30% weight loss (Cal. 23.26%), refers to complete oxidative decomposition of the chelate which ended up with the formation of CuO.

Table 5: Thermal analyses for metal (II) chelates

Compound No. Molecular formula	Temp. (°C)	DTA (peak)		TGA (Wt.loss %)		Assignments
		Endo	Exo	Calc.	Found	
Chelate (6) $[(H_2L) Cu(OAc)_4(H_2O)_2] \cdot 2H_2O$ $H_3OC_{34}H_{48}Cu_2N_6O_{20}$	45	Endo	-	-	-	Broken of H-bondings
	80	Endo	-	3.6	3.9	Loss of (2H ₂ O) hydrated water molecules
	165	Endo	-	3.78	3.71	Loss of 2(H ₂ O) coordinated water molecules
	270	Endo	-	25.79	25.12	Loss of coordinated(4 OAc) group
	390	Endo	-	-	-	Melting point
	440,480,510,520,620	-	Exo	23.26	23.30	Decomposition process with the formation of (2CuO)

Cytotoxicity Activity

via a similar reaction to those discussed below for the direct effects of radiation. The cytotoxic activity of the oxime - hydrazone ligand H_6L (1) and its metal chelate (6) was evaluated against human liver HepG2 cancer cell, (HepG2 cell line) within 0.1–100 $\mu\text{g/L}$ concentration range as shown in [Figure 2]. The IC_{50} values were calculated for each compound and results are presented in [Figure 3] and Table (6). As shown, the chelates displayed significantly cytotoxic activities compared to Sorafenib (Nexavar) standard drug. Cytotoxicity activity of the complex may be attributed to the central metal atom which was explained by Tweedy's complexation theory [38]. Cytotoxicity results indicated that all tested complexes ($IC_{50} = 6\text{--}50\mu\text{M}$) and then chelate (A1) with IC_{50} value 54 μM . It was observed that all complexes are more active than the free ligand. This indicated enhancing of the antitumor activity upon coordination. The enhancement of cytotoxic activity may be assigned to that the positive charge of the metal increased the acidity of coordinated ligand that bears protons, leading to stronger hydrogen bonds which enhanced the biological activity [39]. It seems that changing the anion, coordination sites, and the nature of the metal ion has a pronounced effect on the biological behavior by altering the binding ability of DNA [40]. Gaetke and Chow had reported that metal has been suggested to facilitate oxidative tissue injury through a free radical mediated pathway analogous to the Fenton reaction [41]. By applying the ESR-trapping technique, evidence for metal - mediated hydroxyl radical formation *in vivo* has been obtained [42-47]. Reactive oxygen species are produced through a Fenton-type reaction as follows:



Where L, organic ligand. Furthermore, metal could act as a double-edged sword by inducing DNA damage and also by inhibiting their repair [48]. The OH radicals react with DNA sugars and radicals react with DNA sugars and bases, resulting in the release of free bases and strand break occurs. Bases and the most significant and well characterized of the OH reactions is hydrogen atom abstraction from the C4 on the deoxyribose unit to yield sugar radicals with subsequent β -elimination. By this mechanism strand break occurs as well as the release of the free bases. Another form of attack on the DNA bases is by solvated electrons, probably on DNA.

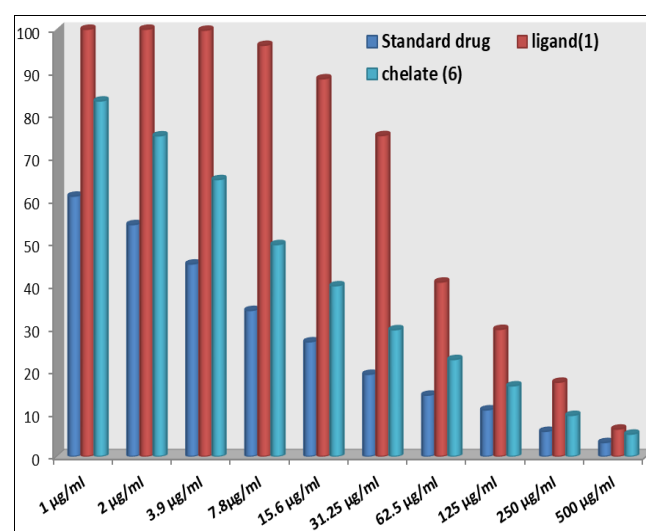


Fig 2: Mean inhibition zone of the ligand and metal chelate A1 (6) against Liver carcinoma HepG2.

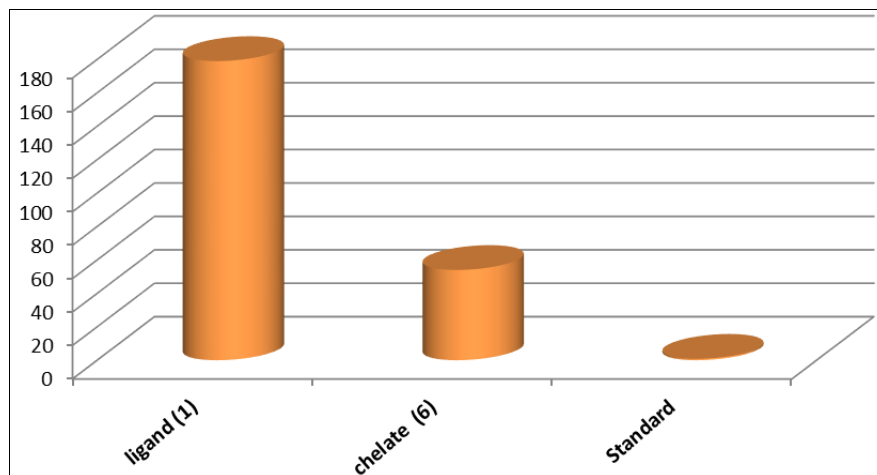
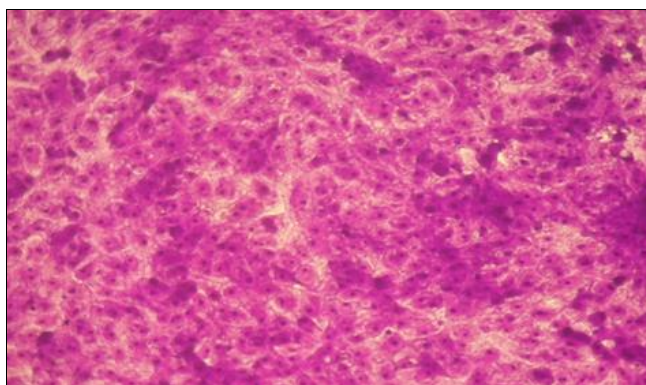


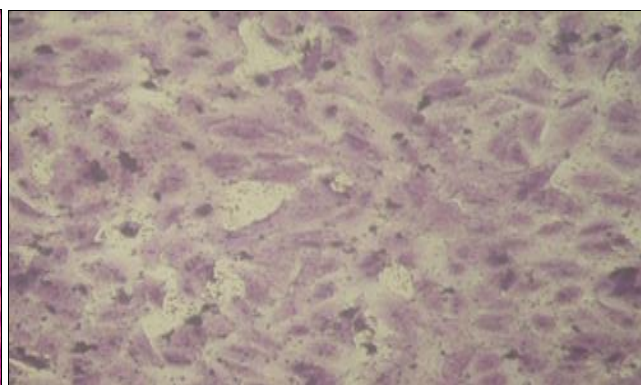
Fig 3: IC₅₀ for the ligand and some of its metal chelate.

Table 6: Cytotoxic activity (IC₅₀) of the ligand and metal chelate against human liver HEPG-2.

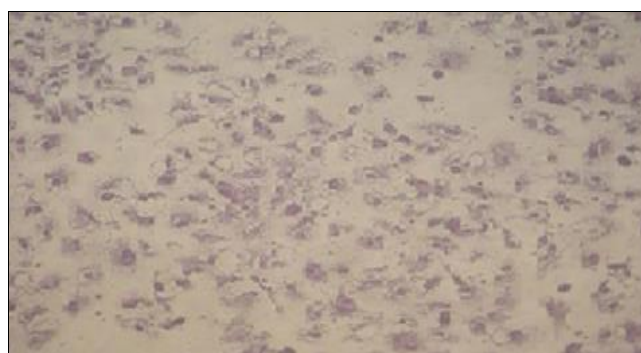
Compound No.	Compound	(IC ₅₀) HepG-2/ml)
Ligand (1)	[H ₆ L]	179
A1(6)	[(H ₆ L)(Cu) ₂ (OAc) ₄ (H ₂ O) ₂].2H ₃ O	54
Standard	Doxrubien	0.47



Control HepG2



HepG-2 cells treated with chelate (6) at 250 ug/ml



HepG-2 cells treated with chelate (6) at 3.9 ug/ml

Fig 4: the cytotoxicity of control HepG2 and tested chelate

From the (figure 4), we found that: 1) Decrease in the number of available cells. 2) Most of the remaining observed degeneration changes in the form of the irregular cell membrane opaque and not well formed chromatin regulated of swelling cytoplasm, other showed optatic change in the formed of chrunked cells and increase esinophilia cells, and picknitoic nucleus.

Conclusions

In our study, new oxime metal (II) chelates were prepared. The chelates are non-electrolytic in nature as suggested by molar conductance measurements. The ligand coordinated

to the central metal ion through amide oxygen and nitrogen atoms, one-imine nitrogen, one oxime-nitrogen atoms forming five-membered rings including the metal ions. The antitumor activities of the ligand as well as its metal chelate were assessed that, the toxicity of both ligand and metal complex were found to be concentration dependent, the cell viability decreased with increasing the concentration of chelates.

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