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Synthesis, spectral, antitumor , antioxidant and antimicrobial studies on Cu(II) , Ni(II) and Co(II) complexes of 4-[(1*H*-Benzoimidazol-2-ylimino)-methyl]-benzene-1,3-diol

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Abstract

A new Schiff base of 2-aminobenzimidazole with 2,4-dihydroybezaldehyde (H_3L), and its Cu(II), Ni(II) and Co(II) complexes have been synthesized and characterized by elemental analyses, molar conductance, thermal analysis (TGA), inductive poupled plasma (ICP), magnetic moment measurements, IR, EI-mass, UV-Vis. and ESR spectral studies. On the basis of spectral studies and analytical data, it is evident that the Schiff base acts as dibasic tridentate ligand coordinating via deprotonated OH, NH and azomethine nitrogen atom. The results showed that Co(II) and Ni(II) complexes have tetrahedral structure while Cu(II) complexes has octahedral geometry. The kinetic and thermodynamic parameters of the thermal decomposition stages have been evaluated. The studied complexes were tested for their *in vitro* antimicrobial activities against some bacterial strains. The anticancer activity of the ligand and its metal complexes is evaluated against human *liver carcinoma* (HEPG2) cell. These compounds exhibited a moderate and weak activity against the tested HEPG2 cell lines with IC_{50} of 9.08, 18.2 and 19.7 µg/ml for ligand, Cu(II) and Ni(II) complexes, respectively. In vitro antioxidant activity of the newly synthesized compounds has also been evaluated.

Keywords

Benzimidazole. Schiff bases complexes, Antimicrobial, Antitumor, Antioxidant

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1. Introduction

Transition metal complexes are of interest in the development of metal based anticancer agents [1-3]. The clinical success of platinum-based drugs as anticancer agents is severely affected by the serious side effects, toxicity and acquired drug resistance [4-6]. These drawbacks drive inorganic chemists to develop innovative strategies for the preparation of more effective, less toxic, target specific and preferably non- covalently bound anticancer drugs.

Imidazoles are common of large number of natural products and pharmacologically active molecules. As biologically active compounds, benzimidazoles and their derivatives exhibited many biological applications such as antitumor [7], anticancer [8], antimicrobial [9], antifungal [10], and anti-inflammatory activities [11]. Imidazole, benzimidazole and their derivatives are part of an important class of N-heterocycles which can be excellent organic ligands to generate various complexes upon ligation [12- 21]. Schiff bases are used extensively as ligands in coordination chemistry. In view of their interesting structural properties and wide ranging uses, studies of the Schiff base complexes have attracted the attention of many investigators [22-27]. Metal complexes of Schiff base compounds are a class of important compounds in medicinal and pharmaceutical field. They have wide applications in some biological aspects [26, 28-33]

The present work aims to synthesize a new Schiff base ligand namely, 4-[(1*H*-Benzoimidazol-2-ylimino)-methyl]-benzene-1,3-diol and its complexes with Co(II), Ni(II) and Cu(II) ions. The complexes have been characterized by several tools of analyses such as elemental analyses, magnetic moment measurements, , ICP , molar conductance, ESR, IR, UV–Vis spectra. In vitro antioxidant and antimicrobial activity of the free ligand and its metal complexes have been assessed. Also, the antitumor activity of the synthesized compounds has been studied.

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2. Experimental

2.1. Materials

All compounds used in the present study were of pure grade available from BDH, Aldrich or Sigma. The solvents used for the spectral studies were spectroscopic grade from Aldrich.

2.2. Synthesis of Schiff-base ligand (H_3L)

The Schiff base ligand **(Fig. 1)**, was prepared by the condensation of 1 mmol of 2-aminobenzimidazole with 1 mmol of 2,4-dihydroybezaldehyde in methanol. A 1:1 mixture of them was refluxed for 24 h. The reaction mixture was then poured onto ice water. The product which separated immediately was filtered off, washed several times with cold water and finally dried in a vacuum desiccator over anhydrous calcium chloride.

2.3. Synthesis of complexes

To a solution of ligand (0.253g, 1 mmol) in 30 ml ethanol, an ethanolic (20 ml) solution of the hydrated metal(II) chloride(0.170 g $CuCl_2.2H_2O$, 0.237g $NiCl_2.6H_2O$ and 0.237g $CoCl_2.6H_2O$, 1 mmol) was added slowly with constant stirring. The reaction mixture was heated to reflux for 4 hr. The colored precipitate appeared after adding 3 drops of triethylamine as buffering agent. The formed solid product was removed by filtration, washed several times with ethanol and dried over anhydrous $CaCl_2$. The purity of the formed compounds was monitored by TLC.

2.4. Instrumentation

The elemental microanalyses of the solid compounds were performed at the micro analytical center, Cairo University using Perkin-Elmer 2400 CHN Elemental analyzer. Metal content was estimated using inductive coupled plasma (ICP) Perkin Elmer/Optima 7000 DV at central laboratory, Tanta University. Molar conductivities in DMF (10^{-3} M) at room temperature (25°C) were measured using conductance bridge of the type 523 conductivity bridge. The Infrared spectra were recorded using a JASCO FT/IR-4100 spectrophotometer within the range 4000-400 cm^{-1} as KBr discs. Standard electron impact mass spectra (EI) were recorded using a Finnigan MAT

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8222 Spectrometer at 70 eV at micro analytical unit of Cairo University. The electronic absorption spectra were recorded using a Shimadzu UV-Vis 240 spectrophotometer. The room temperature magnetic susceptibility of the solid samples was measured using magnetic susceptibility balance (Johnson Mtthey) 436 Devon Park Drive employing the Gouy's method. The thermogravimetric analysis (TGA) of the solid samples was performed within the temperature range 25-800 $^{\circ}$C using the Shimadzu TG-50 thermogravimetric analyzer with heating rate (10 $^{\circ}$C/min.) under nitrogen atmosphere. The X-band electron spin resonance (ESR) spectra of powder samples were recorded using Joel JES-FE2XG spectrometer model equipped with an E101 micro wave bridge at room temperature. The magnetic field was calibrated with diphenylpicrylhydrazyl (DPPH).

2.5. Antibacterial Studies

Antimicrobial activity of the tested samples was determined using a modified Kirby-Bauer disc diffusion method [34] at the micro-analytical unit of Cairo University. Briefly, 100 µl of the test bacteria was grown in 10 ml of fresh media until they reached 10^6 cells/ml for bacteria [35]. 100 µl of microbial suspension was spread onto agar plates corresponding to the broth in which they were maintained. Isolated colonies of each organism that might be playing a pathogenic role should be selected from primary agar plates and tested for susceptibility by disc diffusion method **[36].** From the available many media, NCCLS recommends Mueller-Hinton agar since it results in good batch-to-batch reproducibility. Disc diffusion method for filamentous fungi was tested by using approved standard method (M38-A) developed by the NCCLS **[37]** for evaluating the susceptibilities of filamentous fungi to antifungal agents. Disc diffusion method for yeasts developed by using approved standard method (M44-P) by the NCCLS **[38].** Plates inoculated with Gram positive bacteria as *Staphylococcus aureus NCTC 6356* and Gram negative bacteria as *Escherichia coli NRRL-B-3704*. They were incubated at 35-37 °C for 24-48 hours and, then the diameters of the inhibition zones were measured in millimeters **[39].** Standard discs of tetracycline (antibacterial agent) and amphotericin B (antifungal agent) served as positive controls for antimicrobial activity but filter discs impregnated with 10 µl of solvent (distilled water, DMSO) were used as a negative control. The agar used is Meuller-Hinton agar that is rigorously tested for composition and pH. Further the depth of the agar in the plate is a factor to be considered in the disc diffusion method.

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This method is well documented and standard zones of inhibition have been determined for susceptible and resistant values. Blank paper disks (Schleicher & Schuell, Spain) with a diameter of 8.0 mm were impregnated 10 µl of tested concentration of the stock solutions. When a filter paper disc impregnated with a tested chemical is placed on agar the chemical will diffuse from the disc into the agar. This diffusion will place the chemical in the agar only around the disc. The solubility of the chemical and its molecular size will determine the size of the area of chemical infiltration around the disc. If an organism is placed on the agar it will not grow in the area around the disc if it is susceptible to the chemical. The area of no growth around the disc is known as a "Zone of inhibition" or "Clear zone". For the disc diffusion, the zone diameters were measured with slipping calipers of the National Committee for Clinical Laboratory Standards [32, 33]. Agar-based methods such as Etest and disk diffusion can be good alternatives because they are simpler and faster than broth-based methods [40, 41].

2.6. Antioxidant studies

The antioxidant activity of ligand and its complexes as well as the standard ascorbic acid was assessed on the basis of the radical scavenging effect of the stable DPPH (1,1-diphenyl-2-picryl hydrazyl) free radical. The radical scavenging effects of antioxidants on DPPH are due to their hydrogen donating ability which causes an absorbance drop at 517 nm. In the DPPH radical scavenging, antioxidants react with the DPPH radical, which is a stable free radical and exists naturally in deep violet color, to turn into a yellow colored (diphenyl--picryl hydrazine). The degree of discoloration indicates the radical scavenging potential of the antioxidant [42]. 1µg of the studied samples and ascorbic acid (reference compound) was dissolved in 1 ml of DMSO. 250 µl of each solution were added to 1 ml DPPH/DMSO solution (6 mg/50 ml) and the total volume was adjusted to 3 ml with DMSO. After overtaxing, the mixture was incubated for 30 min at room temperature. Absorbance was measured at 517 nm. Absorbance of blank sample containing the same amount of DMSO and DPPH solution was prepared and measured as well. The experiment was carried out in triplicate. The scavenging potential was compared with a solvent control (0% radical scavenging) and ascorbic acid.

2.7. Measurement of Potential cytotoxicity

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The human cancer cell line used for in vitro screening experiment is *liver Carcinoma* (HEPG2) cell lines. It was obtained frozen in liquid nitrogen (-180 °C) from the American Type Culture Collection. The tumor cell lines were maintained in the National Cancer Institute, Cairo, Egypt, by serial sub-culturing. Potential cytotoxicity of the compounds was tested using Skehan et al. method **[43].**

Cells were plated in 96-multiwell plate (104 cells/well) for 24 hrs before treatment with the compound to allow attachment of cell to the wall of the plate. Different concentration of the compounds under test (0, 5, l0, 12, 25 and 50 µg/ml) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 hrs at 37 °C and in atmosphere of 5% CO_2. After 48 hrs, Cells were fixed, washed and stained with Sulfo-Rhodamine-B stain. Excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fraction and drug cone is plotted to get the survival curve of each tumor cell line after the specified compound. An Elisa reader (TECAN SUNRISE), Potential cytotoxicity of the compounds was measured in (Pharmacology Unit, Cancer Biology Department, National Cancer Institute, Cairo University). Doxorubicin was used as standard cytotoxins

3. Results and Discussion

All the metal chelates are colored and stable towards air and moisture. The analytical results of the complexes are consistent with the proposed molecular formulae and confirm the formation of 1:1 and 2:1 (M:L) complexes **(Table 1).** The observed molar conductance values of all complexes in 10^{-3} molar DMF solution reveals their non-electrolytic nature [44]

3.1. ^{1}H-NMR Spectru of the Ligand

^{1}H-NMR spectrum of the Schiff base H_3L was recorded in d^6-DMSO. The ^{1}H-NMR spectrum of the ligand exhibited a multiple at 6.39-7.64 ppm which assigned to aromatic protons. The azomethine proton (-CH=N-) appeared as a singlet at 8.13ppm. The ligand spectrum showed a peak at 9.90 ppm, belonging to the phenolic OH proton. The most downfield signals observed as a singlet is due to NH proton of imidizole moiety and appeared at 12.52 ppm **[45].**

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3.2. EI-mass spectra

The constitutions and purities of the prepared ligand (H_3L) and its metal complexes are confirmed using ESI mass spectrometry. The mass spectra of H_3L, $[CuHL(H_2O)_3]$, $[Ni_2HLCl_2.EtOH]$ and [CoHL.EtOH] showed peaks at m/z 253, 368.25, 485.20, and 355.20, respectively, corresponding to the molecular weight of the parent ion $[H_3L^+]$ and $[ML^+]$.The results of both elemental analyses and MS of the prepared complexes are in satisfactory agreement with each other's which confirm the proposed molecular formula (Table 1).

3.3. Infrared spectral studies

The IR spectra of the Schiff base and its metal complexes along with their tentative assignments are reported in Table (2) and shown in Fig(2).

The IR spectrum of the free ligand showed two bands at 3443 and 3299 cm^{-1} which assigned to phenolic OH and NH groups, respectively. All complexes showed a broad absorption band within the range 3443-3384 cm^{-1} due to $\nu_{(OH)}$ of water or ethanol molecules attached to metal(II) ion. The disappearance $\nu_{(N-H)}$ band of imidazole ring for all complexes indicated the participation of the deprotonated N-H in chelation. The band appearing at 1604 cm^{-1} is due to $\upsilon_{C=N}$ of azomethine group of the free ligand. This band was shifted to lower frequency (13-27 cm^{-1}) on complex formation. This shift is due to coordination of azomethine nitrogen to the central metal ion. The band δ_{OH} appeared at(1327 cm^{-1} for the free ligand H_3L) was shifted to 1311 , 1308 and 1309 cm^{-1} for complexes 1, 2 and 3, respectively. The above observations were supported by the appearance of a new band at 569-555 and 461-498 cm^{-1}, which were tentatively assigned to the ν(M–O) and ν(M–N) modes, respectively **[46].**

3.4. Thermogravimetric analysis of the complexes

The thermal behavior of the solid metal complexes was studied using TG technique. The stages of decomposition, temperature range, weight loss percentages as well as the decomposition products are given in Table 3. The thermal decomposition of complexes 1 and 3 takes place in two steps. The first decomposition step appears within the temperature range 29-177 and 29-120°C with mass loss of 14.63 and 12.35% which corresponded to elimination of coordinated water and ethanol molecules for complexes 1 and 3,respectively. The second step within the

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temperature range 177-512 and 120-519 °C with mass loss of 19.80 and 16.21% represents the degradation of the organic ligand leaving CuO + C and CoO as the final product. The thermal decomposition of complex 2 takes place in three steps. The first decomposition step appears within the temperature range 30-162 °C with mass loss of 9.45 % corresponding to the elimination of coordinated ethanol molecule. The second step appears within the temperature range 162-328 °C with mass loss of 14.89 % due to the loss of chloride ions The third step appears within the temperature range 328-460°C with mass loss of 23.72% due to the degradation of the organic ligand leaving NiO as the final product.

The thermodynamic parameters of the different decomposition processes of the complexes namely, enthalpy (ΔH^*), entropy (ΔS^*) and free energy of the decomposition (ΔG^*) as well as the activation energy (E^*), order (n) and pre-exponential factor (A), were evaluated graphically by using Coats–Redfern **[47]**.

The calculated values of n, E^*, A, ΔS^*, ΔH^* and ΔG^* for the decomposition steps are given in **Table (4)**.

From the results listed in **Table (4),** the following features can be deduced:
Kinetics of the thermal decomposition stages of all complexes under study obeys, in most cases, the first order kinetics. The values of ΔG* increased for the subsequently decomposition stages due to increasing the values of (TΔS*) from one stage to another due to the structural rigidity of the remaining complex after the expulsion of one and more ligand as competed with the precedent complex, which require more energy. The positive values of ΔG* suggested that all the decomposition steps are non-spontaneous process. The positive ΔH* values indicate that the decomposition processes are endothermic. The negative values of the activation entropies ΔS^* indicate a more ordered activated complex than the reactants and/or the reactions are slower than normal [**48**].

3.5. Electronic absorption and magnetic moment measurements

The electronic absorption spectra of the complexes **1**-**3** were studied in Nujol mull. The spectrum of Cu(II) complex **1** displayed a band at 22026 cm^{-1} assigned to $^2Eg \rightarrow {}^2T_{2g}$ transition assuming octahedral geometry around the central Cu(II) ion. The electronic absorption spectrum of Ni(II) complex showed two bands at 16286 and

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22522 cm^{-1} assignable to $^3T_1(F) \rightarrow {}^3A_2$, $^3T_1(F) \rightarrow {}^3T_1(p)$, respectively,assuming the tetrahedral geometry around the Ni(II) ion**.** The electronic absorption spectrum of Co(II) complex displayed two bands at 15527 and 21929 cm^{-1} which is assigned to $^4A_2 \rightarrow {}^4T_1(F)$ ν_2 and $^4A_2 \rightarrow {}^4T_1(P)$ ν_3 transition, respectively, due to tetrahedral arrangement.

The ligand field parameter B (interelectronic repulsion of the d electrons in complex), β (The Nephelauxetic effect) and 10Dq are calculated according to the equation reported for the tetrahedral Co(II) complex **[49]**

$\mu_{eff} = 3.87(1- 4\lambda/10Dq)$, $\lambda = -178 Cm^{-1}$

$B = [4(\nu_3-15Dq)^2 – 10Dq^2] / [60((\nu_3-15Dq) – 180Dq]$

$\nu_1 = 10Dq$

$\beta = B(complex) / B(free\ ion)$

where *B* (free ion) for Co(II) is 963cm^{-1}. The 10Dq, B and β values are 7060 cm^{-1}, 695.66 cm^{-1} and 0.62, respectively. These results show that the interelectronic repulsion of the d-electrons in a complex is less than in the free ion. The value of B in a complex is 72% of the free-ion value. The β value is related directly to covalence. The reduction of B by complex formation is caused by delocalization of the d-electron cloud on the ligand, which is in turn caused by the formation of covalent bond. The data show the Co(II) complex has covalent character [50].

The magnetic moment data of the solid complexes at room temperature showed that all complexes are paramagnetic. The magnetic moment value of Cu(II) complex (1.90 BM) is higher than the theoretical spin only value of Cu(II) complex refers to spin – orbital coupling. The magnetic moment value of Ni(II) complex (2.65 BM) is lower than spin only value of Ni(II) complex may be due dimmerization and antiferromagnetic interaction**[51].** The magnetic moment value of Co(II) complex (4.26 BM) is higher than the theoretical spin only value of Co(II) complex referring to orbital contribution for Co(II) complex**[51].** These values indicate octahedral structure for complex 1 and tetrahedral structure for complexes **2** and **3**.

3.6. ESR spectra

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The X-band ESR spectra of the powdered Cu(II) Schiff base complex was recorded at room temperature (25°C). The profile of the ESR spectrum of Cu(II) complex is consistent with octahedral geometry around Cu(II) center which agree with the electronic spectral data. The ESR spectrum of Cu(II) complex exhibited two g-values. The calculated values of g_{II} and $g\perp$ of Cu(II) complex showed the order $g_{II} > g\perp > 2.0023$ which is consistent with d_{x2-y2} ground state **[52]**. The g-value of Cu(II) complex has a positive contribution from the value of free electron (2.0023) due to the measurable covalent character in the bonding between the Schiff base ligand and Cu(II) ion. The value $g_{II} < 2.3$ indicated that the Cu(II)-Schiff base complex has the covalent character **[53, 54]**

The geometric parameter G, which measure the exchange interaction between the metal center in a polycrystalline compounds is calculated by using the expression:

$$G = (g_{II} - 2.0023)/ (g\perp - 2.0023)$$

The calculated G value was found greater than 4 (**Table** 5) which indicates no interaction between the adjacent Cu(II) centers **[54,55]**

It is usual to determine the covalent bonding parameters for the Cu(II) ion in various ligand field environments. Molecular orbital coefficient, α^2 (a measure of the covalency in the in-plane σ-bonding between a copper 3d orbital and the ligand orbitals), β^2 (covalent in-plane π –bonding) and γ^2 (out-of-plane π –bonding) were calculated by using the following equations**[56-58].**

$$\alpha^2 = - (A_{\parallel}/0.036) + (g_{\parallel} - 2.0023) + 3/7(g\perp - 2.0023) + 0.04$$

$$\beta^2 = (g_{\parallel} - 2.0023)\, E / 8\lambda\alpha^2$$

$$\gamma^2 = (g\perp - 2.0023)\, E / 2\lambda\alpha^2$$

The α^2 value = 0.5, it indicates a complete covalent bonding, while the value of α^2 = 1.0 suggests complete ionic bonding. The observed value of α^2 (0.59) indicates that the Cu(II) complex has covalent character. For the Cu(II) complex, the high value of β^2 compared to α^2 indicated that the in-plane π –bonding is less covalent than the in-plane σ-bonding

The ESR parameters are used to evaluate the orbital reduction factors K by using the expression **[59]** $K = (K_{\parallel}^2 + 2\, K\perp^2)/ 3$, where $K_{\parallel}$ and $K\perp$ are the parallel and perpendicular components of the orbital reduction factor. The low value of K than

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unity was indicative of its covalent nature which in agreement with the conclusion obtained from the value of g_{II}. Hathaway **[60]** has pointed out that for pure σ-bonding, $K_{\|} \approx K_{\perp} = 0.77$ and for in-plane π-bonding $K_{\|} < K_{\perp}$, while for out-of plane π-bonding $K_{\|} > K_{\perp}$. For the studied Cu(II) complex, the observed order $K_{\|}(0.51) > K_{\perp}(0.31)$ implies a greater contribution from out-of-plane π-bonding than for in-plane π-bonding in metal–ligand π-bonding[60].

Based on the analytical, spectral data and magnetic moment data, the complexes were formulated in Fig 3.

3.7. Antibacterial Results

The synthesized ligand and its complexes were screened for their antibacterial activity against E. coli (Gram-negative bacteria) and S. aureus (Gram-positive bacteria). The standard drugs, tetracycline and amphotericin B, were also tested for their antibacterial activities at the same concentration and conditions of the test compounds (Table 6).

From the value for gram's positive bacteria (S. aureus) one can arrange the complexes as Cu(II) > Co(II) > Ni(II) > Ligand. On the other hand, the inhibition values against gram's negative bacteria (E. coli) can arranged the compound as Co(II) > Ligand > Cu(II) > Ni(II). The antimicrobial activity of the ligand and its metal complexes could not reach the effectiveness of the standard antimicrobial drugs such as tetracycline or amphotericin B.

3.8. Antioxidant results

Hydroxyl radical is highly reactive oxygen centered radical formed from the reactions of various hydroperoxides with transition metal ions. Among all the free radicals, hydroxyl radical is by far the most potent and therefore the most dangerous oxygen metabolite and hence the elimination of this radical is one of the major aims of antioxidant administration**[61].** Hydroxyl radical is known to be capable of abstracting hydrogen atoms from membrane lipids and brings about peroxide reaction of lipids. The antioxidant activity of free ligand , complexes and the standard

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ascorbic acid were assessed on the basis of the radical scavenging effect of the stable DPPH (1,1-diphenyl-2-picryl hydrazyl) free radical. The results were shown in **Fig. S1.**

By comparing the antioxidant activity of the ligand (IC_{50} value is 77.85 µg/ml) with that of the metal complexes (IC_{50} values of Cu(II), Ni(II) and Co(II) complexes are 83.73, 99.86 and 57.77 µg/ml, respectively) it become clear that: Co(II) complex possesses higher scavenging activity towards $^{\cdot}OH$ than the parent ligand. It exhibited excellent scavenging activity against the DPPH radical. The values were found to be close to the values of standard ascorbic acid (IC_{50} value is 67.44 µg/ml). Cu(II) and Ni(II) complexes possesses higher scavenging activity towards $^{\cdot}OH$ than the free ligand. For Co(II) complex, the observed lower IC50 value in antioxidant assays demonstrated that Co(II) complex has the potential as drugs to eliminate the radicals. The antioxidant activity was decreased in the order Co(II) > free ligand >Cu(II) > Ni(II).

3.9. Antitumor results

The anticancer activity of the free ligand and its Cu(II) and Ni(II) complexes was determined in vitro against human cancer cell line *liver Carcinoma* (HEPG2). The values of IC_{50}, compared with the standard drug doxorubicin are collected in table 7. Fig.S2 represents the cytotoxicity of ligand , complexes and doxorubicin (Dox) drug against the *liver Carcinoma* (HEPG2) cell line, using different concentrations of compounds or Dox. Untreated cells were used as a control. From these figures it is clear that Ni(II)complex inhibited the growth of the tested cells than Cu(II) complex. This indicated that the type of ion may be the reason for the different anticancer activity **[62].**

The SB ligand and its Ni (II) and Cu (II) complexes showed an inhibition of cell viability and gave the IC_{50} values of 9.03, 18.20 and 19.7 µg/ml against HEPG2, respectively, compared with IC_{50} value of (4.73 µg/ml) for the standard cytotoxin drug doxorubicin. According to Shier **[63]** the compounds exhibiting IC_{50} activity within the range of 10-25 µg/ml are considered weak anticancer drugs, while those of IC_{50} activity between 5 and 10 µg/ml are moderate and compounds of activity below 5.00 µg/ml are considered strong agents. Accordingly, the studied complexes showed

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a weak antitumor activity, but the free ligand showed a moderate activity against the HEPG2.

4. Conclusion

A new Schiff base of 2-aminobenzimidazole with 2,4-dihydroybezaldehyde (H_3L), and its Cu(II), Ni(II) and Co(II) complexes were synthesized and characterized by elemental analysis, spectral studies (mass spectra, IR, ESR, UV-Vis) and magnetic data. The electronic and ESR spectral studies suggested tetrahedral geometry for Ni(II) and Co(II) complexes. The Cu(II) complex have octahedral geometry. The kinetic and thermodynamic parameters of the thermal decomposition stages have been evaluated using Coats-Redfern methed. The antimicrobial activity of the ligand and its metal complexes could not reach the effectiveness of the standard antimicrobial druges such as tetracycline or amphotericin B. The antioxidant activity complex exhibited excellent scavenging activity against the DPPH radical. The values were found to be close to the value of standard ascorbic acid. The antioxidant activity decreases in the order Co(II) > free ligand >Cu(II) > Ni(II). In vitro antitumor activity of these complexes showed a weak antitumor activity, but the free ligand showed a moderate activity against the HEPG2.

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References

[1] H.T. Chifotides, K.R. Dunbar, Acc. Chem. Res. 38 (2005) 146-156.

[2] P.J. Bednarski, F.S. Mackay, P.J. Sadier, Anticancer Agents Med. Chem. 7 (2007) 75-93

[3] M.J. Rose, N.L. Fry, R. Marlow, L. Hinck, P.K. Mascharak, J. Am. Chem. Soc. 130 (2008) 8834-8846.

[4] M. Skander, P. Retailleau, B. Bourri, L. Schio, P. Mailliet, A. Marinetti, J. Med. Chem. 53 (2010) 2146-2154.

[5] Z Wu, Q. Liu, X. liang, X. Yang, N. Wang, X. Wang, H. Sun, Y. Lu, Z. Guo, J. Biol. Inorg. Chem. 14 (2009) 1313-1323

[6] R. Gust, W. Beckb, G. Jaouenc, H. Schenberger. Coord. Chem. Rev. 253 (2009) 2742-2759.

[7] J. Mann, A. Baron, Y. Opoku-Boahen, E. Johansson, G. Parkinson, L.R. Kelland, S. Neidle. J. Med. Chem., 44 (2001) 138-144.

[8] A. Gellis, H. Kovacic, N. Boufatah, P. Vanelle. Eur. J. Med. Chem., 43 (2008) 1858-1864.

[9] O. Guven, T. Erdogan, H. Goker, S. Yildiz. Bioorg. Med. Chem. Lett., 17 (2007) 2233-2236.

[10] H. Torres-Gomez, E. Hernandez-Nuez, I. Leon-Rivera, J. Guerrero-Alvarez, R. Cedillo-Rivera, R. Moo-Puc, R. Argotte-Ramos, M.C. Rodriguez-Gutierrez, M.J. Chan-Bacab, G. Navarrete- Vazquez. Bioorg. Med. Chem. Lett., 18 (2008) 3147-3151.

[11] S.M. Sondhi, S. Rajvanshi, M. Johar, N. Bharti, A. Azam, A.K. Singh, Eur. J. Med. Chem., 37 (2002) 835-843.

[12] S. Yurdakul, M. Kurt, J. Mol. Struct. 650 (2003) 181-190.

[13] T. Bando, H. Iida, Z.F. Tao, A. Narita, N. Fukuda, T. Yamori, H. Sugiyama,

ACCEPTED MANUSCRIPT

Chem. Biol. 10 (2003) 751-758.

[14] F. Arjmand, B. Mohani, S. Ahmad, Eur. J. Med. Chem. 40 (2005) 1103-1110.

[15] R. Sang, L. Xu, Inorg. Chem. 44 (2005) 3731-3737.

[16] N. Pashchevskaya, M. Nazarenko, S. Bolotin, A. Oflidi, V. Panyushkin, Russ J. Inorg. Chem. 9 (2010) 1510-1517.

[17] H. Wu, X. Huang, B. Liu, F. Kou , F. Jia , J. Yuan, Y. Bai J. Coord. Chem., 64 (2011) 4383-4396.

[18] Y. Li, P. Yang, Inorg. Chem. Commun. 14 (2011) 545-549.

[19] H. Wu, K. Wang, B. Liu, F. Kou, F. Jia, J. Yuan, Y. Bai, Inorg. Chim. Acta , 384 (2012) 302-308.

[20] L. Qin, L. Liu, X Du, G. Cui, Transition Met. Chem. 38 (2013) 85-91.

[21] H. Wu, J. Yuan, Y. Zhang, F. Shi, G. Pan, J. Kong, X. Fan, Inorg. Chim. Acta 404 (2013) 13-22.

[22] A. Emara. Spectrochim. Acta, A, 77 (2010) 117-125.

[23] W.Y. Bi, X.Q. Lu¨ , W.L. Chai, J.R. Song, W.Y. Wong, W.K. Wong, R.A. Jones. J. Mol. Struct., 891 (2008) 450-455.

[24] O. A. Ali Spectrochim. Acta A: 132 (2014) 52-62.

[25] A. Singh, O. Pandey, S. Sengupta, , Spectrochim. Acta A, 113 (2013) 393-399.

[26] R.M. Issa, M. Gaber , N.A. Al–Wakiel, S. K. Fathalla, Chin. J. Chem., 30 (2012) 547-556.

[27] N. El- Wakiel, Y. El-Sayed, M. Gaber, J. Mol. Struct.,1001 (2011) 1-11.

[28] D. Nasrin, M.A. Alam, M.N. Hossain, M. Nazimuddin, Chem. J. 3 (2013) 13-19

[29] M.I. Khan, A. Khan, I. Hussain, M.A. Khan, S. Gul, M. Iqbal, I. Ur-Rahman, F. Khuda, Inorg. Chem. Commun. 35 (2013) 104-109.

ACCEPTED MANUSCRIPT

[30] O.E. Sherif, N.S. Abdel-Kader, Spectrochim. Acta A, 117 (2014) 519-526.

[31] M. Hanif, Z.H. Chohan, Spectrochim. Acta A, 104 (2013) 468-476.

[32] K. Ghosh, P. Kumar, N. Tyagi, U.P. Singh, N. Goel. Inorg. Chem. Commun., 14 (2011) 489 -492.

[33] M. Gaber, N. A. El-Wakiel, H. El-Ghamry, S. K. Fathalla J. Mol. Struct. 1076 (2014) 251-261.

[34] A..Bauer, W.M. Kirby, C. Sherris, M. Turck, Am. J. Clin. Path. 45 (1966) 493-496.

[35] M.A. Pfaller, L. Burmeister, M.S. Bartlett, M.G. Rinaldi, J. Clin. Microbiol. 26 (1988) 1437-1441.

[36] National Committee for Clinical Laboratory Standards. Performance antimicrobial susceptibility of Flavobacteria. 41, (1997).

[37] National Committee for Clinical Laboratory Standards. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Conidium-Forming Filamentous Fungi: Proposed Standard M38-A. NCCLS, Wayne, PA, USA, (2002).

[38] National Committee for Clinical Laboratory Standards. Method for Anlifungal Disk Diffusion Susceptibility Testing of Yeast: Proposed Guideline M44-P. NCCLS, Wayne, PA, USA, (2003).

[39] National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A3. National Committee for Clinical Laboratory Standards, Villanova, Pa. (1993).

[40] L.D. Liebowitz, H.R. Ashbee, E.G.V. Evans, Y. Chong, N. Mallatova, M. Zaidi, D. Gibbs, Diagn. Microbiol. Infect. Dis. 4, (2001) 27-33.

[41] M.J. Matar, L. Ostrosky-Zeichner, V.L. Paetznick, J.R. Rodriguez, E. Chen, J.H. Rex, Antimicrob. Agents Chemother. 47 (2003) 1647-1651.

[42] A. Ardestani, R.Yazdanparast, Food Chem., 104(2007) 21-29.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

[43] P. Skehan, R. Storeng, et al, J. Natl Cancer Inst, 82 (1990) 1107-1112.

[44] W.J. Greay, Coord. Chem. Rev. 7 (1971) 81-122.

[45] H.T. Chifotides, K.R. Dunbar, N. Katsaros, G. Pneumatikakis, J. Inorg. Biochem. 55 (1994) 203-216.

[46] H. N. Aliyu, A. S. Mohammed, Glo. Adv. Res. J. Microbiol, 1(5), (2012) 67-71.

[47] A. W. Coats, J. P. Redfern, Nature, 201 (1964) 68-69.

[48] N. Madhu, P. Radhakrishnan, W. Linert, J. Therm. Anal. Calorim. 84 (2006) 607-611.

[49] O. M.I. Adly, Spectrochim. Acta A, 79 (2011) 1295-1303.

[50] D. Nichlis, Complexes and First-Row Transition Elements, Chapter 5 (1974) 92.

[51] M. M.Al-Neaimi, M.M. Al-khuder. Spectrochim Acta A, 105 (2013) 365-373.

[52] D. Kivelson, R.Neiman, J. Chem. Phys.35 (1961) 149-155.

[53] B.J. Hathaway, D.E. Billing, Coord. Chem. Rev. 5 (1970) 143-207.

[54] M. Gaber, Y.S.El-Sayed, K.Y.El-Baradie, R.M.Fahmy, Spectrochim. Acta A,107 (2013) 359-370.

[55] B.J.Hathaway, Struct. Bond 57 (1984) 55-118.

[56] N.Raman, A.Kulandaisamy Transition Met. Chem., 29(2004) 129-135.

[57] C.J.Carrano,C. Nunn, R.Quan,J. Bonadies,V. Pecoraro, Inorg. Chem.29 (1990) 944-951.

[58] M.S.Masoud, A. Soayed, A.E. Ali, Spectrochim.Acta A , 60 (2004) 1907-1915.

[59] M. Gaber, Y. El-Sayed, K. .El-Baradie, R. Fahmy, J. Mol. Struct. 1032 (2013) 185-194.

[60] B.J. Hathaway, Struct. Bond 14 (1973) 49-67.

[61] C.T.Ballhausen, An Introduction to Ligand Field, Mc Graw Hill, NewYork 1962, A.P.Lerer, Inorganic Electronic Spectroscopy Elsevier, Amsterdam 1984.

[62] B.N.Meyer, N.R.Ferrigni, J.E.Putnam, L.B.Jacobsen, D,E,Nichols, J.L.Mclauhglin, Planta Med. 45(1982) 31-34.

[63] Z.H.Chohan, A.Scozzafava, C.T.Supuran, J. Enzyme, Inhib. Med. Chem. 17 (2002) 261-266.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

[64] Z.H. Chohan, H. Pervez, M.K. Khan, A. Rauf, G. Maharvi, C.T. Supuran, J. Enzyme, Inhib. Med. Chem. 19 (2004) 85-90.

[65] N. Udilova, A. Kozlov,W. Breberschulte, K. Frei, K. Ehrenberger, N. Nhol, Biochem. Pharm.65 (2003) 59-65.

[66] X-Riera, V. Moreno, C. Ciudad, V. Noe, M. Font-Bardra, X. Solans, Bioinorg. Chem. Appl. 2007 (2007)

[67] W.T. Shier, Mammalian Cell Culture on $5 a Day: A Lab Manual of Low Cost Methods (University of the Philippines, Los Banos, 1991.

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Table 1. Physical characteristics, analytical and molar conductance data of the complexes.

No	compound	Color (Λ_m)[a]	Yield %	% Elemental analysis (found)				Mol. wt
				C	H	N	M	
Lig	H_3L $C_{14}H_{11}N_3O_2$	Dark yellow	85	66.40 (66.31)	4.38 (4.80)	16.60 (16.15)	--	253 (253)*
1	$[CuHL(H_2O)_3]$ $C_{14}H_{15}CuN_3O_5$	Brown 0.19	82	45.59 (45.04)	4.07 (4.02)	11.39 (11.09)	17.23 (17.31)	368.50 (368.25)*
2	$[Ni_2HLCl_2.EtOH]$ $C_{16}H_{15}Ni_2Cl_2N_3O_3$	Reddish brown 0.90	80	39.57 (40.09)	3.11 (3.03)	8.65 (8.27)	24.47 (24.17)	485.20 (485.20)*
3	[CoHL.EtOH] $C_{16}H_{15}CoN_3O_3$	Olive 0.50	82	53.94 (53.38)	4.20 (4.08)	11.82 (11.34)	16.49 (16.40)	355.90 (355.20)*

All the synthesized complexes decompose without melting above 300 °C,

[a]Λ_m = Molar conductance (Ω^{-1} cm^2 mol^{-1}).

*molecular weight from mass spectrum

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Table 2. IR Spectral data (cm^{-1}) of the ligand and its Cu(II) , Ni(II) and Co(II) complexes

Compounds	ν_{OH}	ν_{NH}	$\nu_{C=N}$ *Schiff*	$\nu_{OH.}$	ν_{M-O}	ν_{M-N}
H_3L	3443	3299	1604	1327	------	-----
$[CuHL(H_2O)_3]$	3443	--	1577	1311	555	461
$[Ni_2HLCl_2.EtOH]$	3409	--	1591	1308	564	491
[CoHL.EtOH]	3384	--	1584	1309	569	498

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Table 3. Thermogravimetric analysis data (TGA) of the metal complexes

No	*Temp. range (°C)*	*Mass loss %*		**Assignment**
		Found	Calc.	
1	29-200	10.98	11.42	Loss of coordinated water molecules
	200-370	23.18	22.53	Loss of coordinated chloride ions
	370-727	77.55	77.94	Dissociation of the organic ligand with formation of CuO + C as final products
2	87-167	18.61	18.00	Loss of coordinated ethanol molecule
	167-267	13.80	13.87	Loss of coordinated chloride ions
	267-690	87.86	88.47	Dissociation of the organic ligand with formation of CoO as final product
3	29-120	12.35	12.95	Loss of coordinated ethanol molecule
	120-519	83.09	83.46	Dissociation of the organic ligand with formation of CoO as final product

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Table 4. The activation parameters (Δ H*, ΔS*, ΔG*) for decomposition of Cu(II), Ni(II) and Co(II) complexes

Complex	n	step	r	E*	Δ H*	A	-ΔS*	Δ G*
Cu(II) complex	0 1	1st 2nd	0.9529 0.9100	6.8 53.45	3.58 49.62	4.15E+8 319327	0.090 0.152	38.60 119.4
Ni(II) complex	0 1 1	1st 2nd 3rd	0.9953 0.9906 0.9923	16.39 59.86 92.7	13.52 56.99 89.8	3329959 14054 506	0.111 0.175 0.203	51.55 117.3 159.6
Co(II) complex	1 1	1st 2nd	0.9952 0.9833	44.3 82.2	41.44 75.34	1797.5 3942	0.192 0.193	107.6 234.9

E*, ΔH* , ΔG* ($KJ.mol^{-1}$), ΔS* ($J.K^{-1}.mol^{-1}$), A**:** the pre-exponential factor(s^{-1})

Table 5. ESR spectral data of Cu(II) complex at 25°C

Compound	G	$K_{\parallel}$	$K_{\perp}$	K	α^2	β^2	γ^2	$g_{\parallel}$	$g_{\perp}$	g_{av}
$[CuHL(H_2O)_3]$	6.20	0.51	0.31	0.15	0.59	0.86	0.52	2.267	2.04	2.21

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Table 6. Antimicrobial activities of the investigated ligand and its complexes.

Compounds	Inhibition zone diameter(mm/ mg compound)	
	E. coli	*St.aur*
H_3L	12	9
$[CuHL(H_2O)_3]$	11	12
$[Ni_2HLCl_2.EtOH]$	10	10
[CoHL.EtOH]	17	11
Tetracycline	33	30
Ampicilin	22	18

Table 7. Antitumor data of the investigated ligand and its complexes.

compound	stander	ligand	Cu complex	Ni complex
IC_{50} ug/ml	4.73	9.08	18.2	19.7

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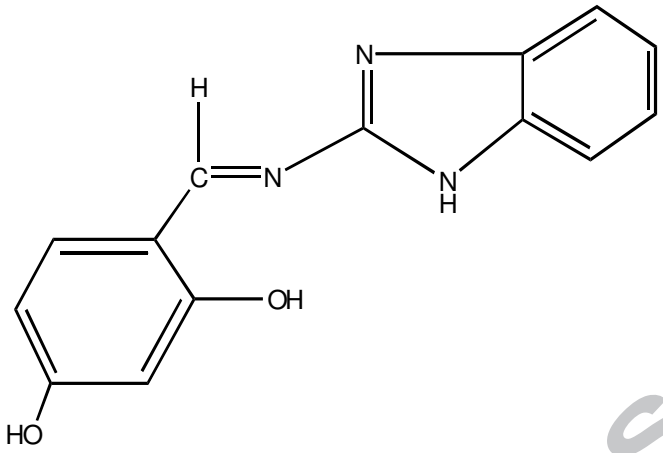

Fig. 1 The structure of Schiff base (H_3L).

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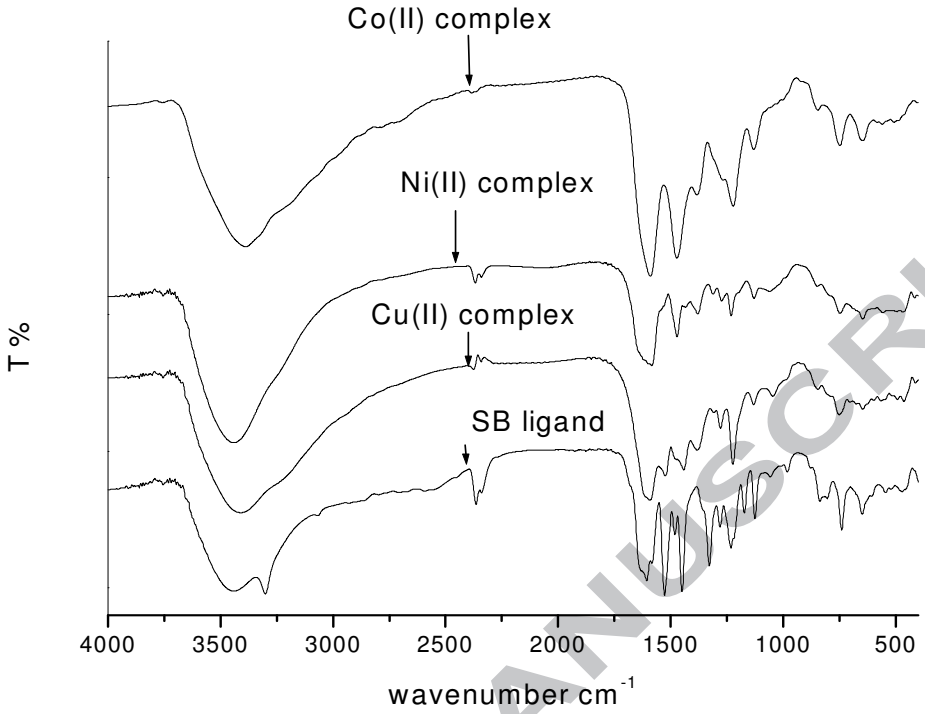

Fig.2 IR spectra of the ligand and its Cu(II) , Ni(II) and Co(II) complexes.

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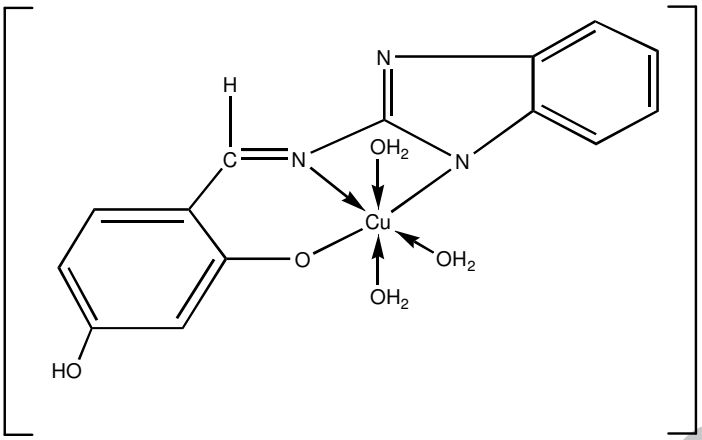
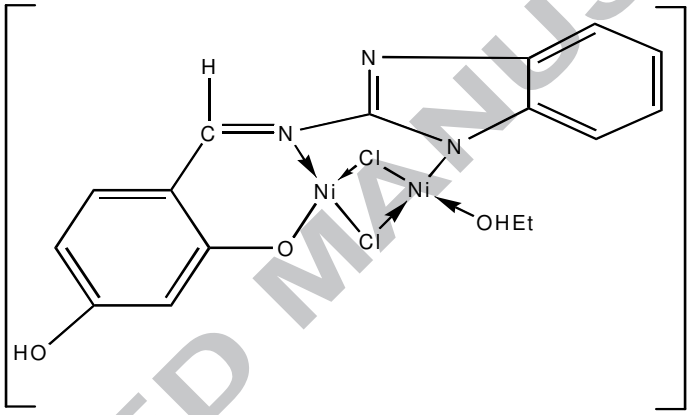
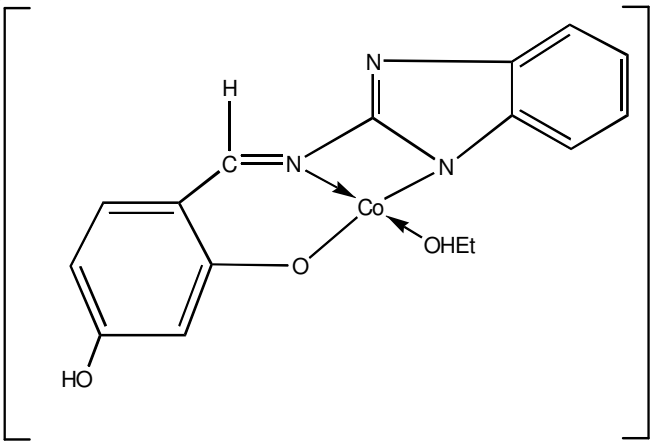


Cu(II) complex



Ni(II) complex



Co(II) complex

Fig. 3 The structures of the metal complexes

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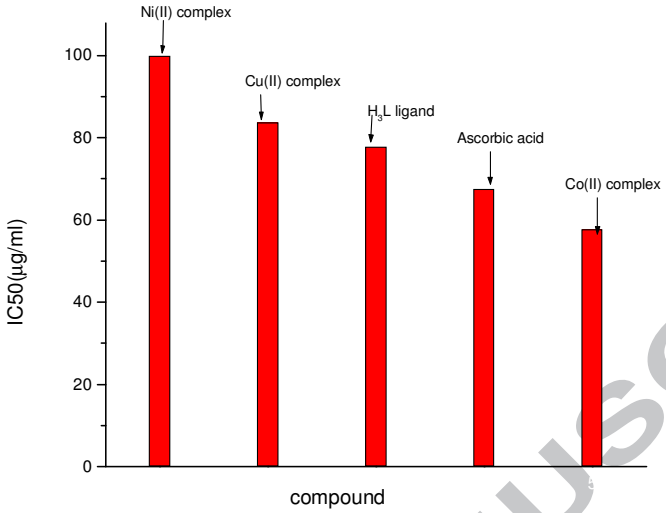

Fig.S1 Trends in the inhibition of hydroxyl radical by the H_3Lligand and its complexes.

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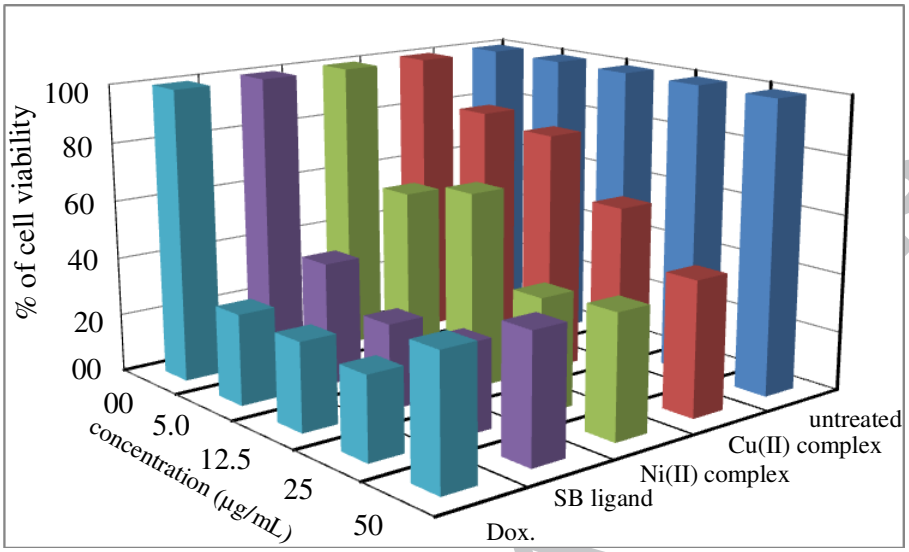

Fig. S2 In vitro cytotoxicity of H_3L ligand ,complexes and doxorubicin (Dox) drug against human *liver Carcinoma* cell line (HEPG2)

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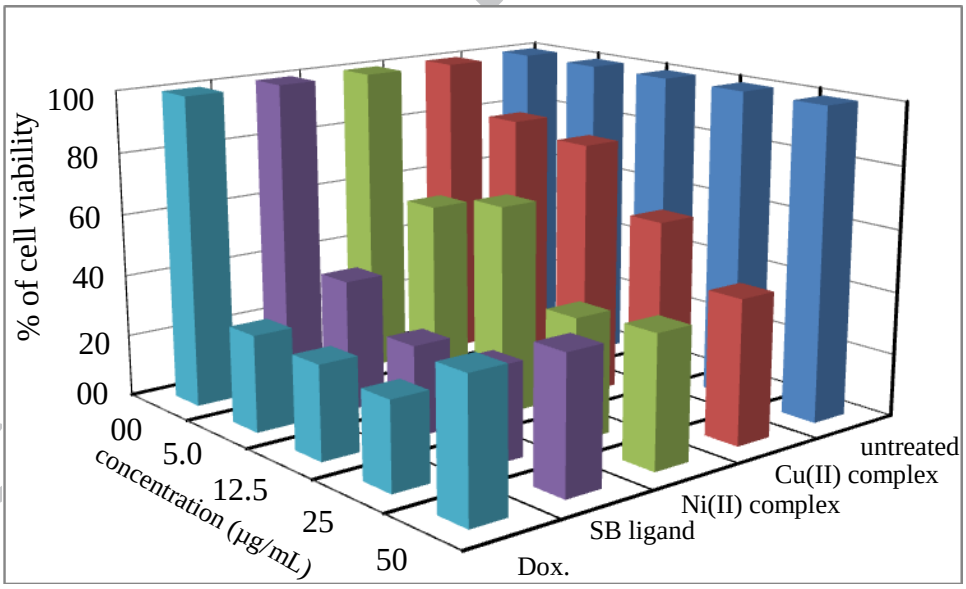

In vitro cytotoxicity of H_3L ligand ,complexes and doxorubicin (Dox) drug against human *liver Carcinoma* cell line (HEPG2)

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Schiff base complexes of 2-aminobenzimidazole ►Spectral studies ► Thermal analysis ► Antimicrobial, Antitumor, Antioxidant activities

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