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Metallo – Bioactive Pyridopyrazolopyrimidine Complexes as a new Class for Anticancer Therapy

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ABSTRACT

The synthesis of bioactive pyridopyrazolopyrimidine ligands denoted I, II and III were described. The chelation abilities of the ligand towards Co(II), Cu(II) salts have been studied and give the following formula: $[Cu(H_2L^1)(HL^1)(CH_3COO)(H_2O)].3H_2O$, **(Ib)**, $[Cu(HL^1)_2(H_2O)_2].1.5H_2O$, **(Ic)**, $[Co(H_2L^1)_2Cl(OH)].H_2O$, **(Id)**, $[Cu(H_2L^2)(OH)(H_2O)].Cl.0.5H_2O$, **(IIa),** $[Cu(HL^3)Cl(H_2O)_2].H_2O$, **(IIIa)**. These complexes of these ligands have much potential as anticancer agents. Chelates behave as a neutral bidentate ligand bonded to the metal ions through either the nitrogen atom of pyrazole ring and hydroxyl oxygen atom attached to pyrimidine ring or the nitrogen atom of amide group of pyrimidine ring and carbonyl oxygen atom of amide group of pyrimidine ring in protonated or deprotonated form. These structures have been synthesized and characterized by elemental analyses, (spectral method UV–Vis., IR), magnetic susceptibility, conductance and thermogravimetric analysis (TGA) were performed. Molar conductance in DMF arrangement demonstrates that, all complexes are non-electrolytes except the complex (**IIa**) is electrolyte. All these parameters described above, confirmed the proposed structures of these metal complexes**.** The safety and tolerance assessment of these complexes were done on normal peripheral blood leucocytes isolated from eight healthy non-smoker volunteers. Besides, the anticancer properties were evaluated on acute myeloid leukemia (AML) blasts of eight patients that were recently diagnosed for AML and received no chemotherapeutics until the samples were taken. The tested complexes should be

investigated in depth towards other cancer cell lines to assess their possible integration as chemotherapeutics.

Keywords: Complexes, Synthesis, pyridopyrazolopyrimidines, Cytotoxicity.

1. INTRODUCTION

Pyrazolopyrimidines are found to possess a wide important pharmacophore and adjective structure in medicinal chemistry due to their biological importance. Functionalized pyrazolopyrimidines are known to exhibit several pharmacological activities such as CNS depressant[1], antihypertensive[2], adenosine receptors[3], tuberculostatic[4], antibacterial and antifungal[5]. Some of the pyrazolopyrimidine derivatives are known to inhibit enzymes such as xanthine oxidase[6,7]. The pyrazolo[1,5-a]pyrimidines frame work, are attractive compounds for drug discovery since many of them have been shown to exhibit excellent biological activities[8]. In addition, the pyrimidines and pyrazoles have received much attention over their years because of their interesting pharmacological properties[9,10]. Pyrazolo[3,4-*b*]pryridines have received considerable attention as a result of their biological activity. It has been shown that many of pyrazolopyridines especially pyrazolo[3,4-*b*]pryridines have antibacterial[11] and antiviral effects[12]. Some of the derivatives act as anti-metabolites and those are effective in the control of cancer[13]. Pyrazolopyridines were found to be among many systems which affects on the central nervous systems. Various pyrazolo[3,4-*b*]pryridines have been found to exhibit pharmacological properties. Some of the derivatives of pyrazolo[3,4-*b*]pryridines have been tested for anti-inflammatory[14] action, while others have been demonstrated to be good anxiolytic[15]. To enhance the activity of pyrazolopyridines and pyrazolopyrimidines, several approaches to construct another ring over those ring systems described in the literature are available on preparation of pyridopyrazolopyrimidines which left much scope for further study. Moreover, pyridopyrazolopyrimidines revealed antiproliferative activity and are used as potent kinase inhibitors[16-19]. This study aims to reprepare and characterize pyridopyrazolopyrimidin derivatives as ligands, then prepare and characterize the anticancer synthetic pyridopyrazolopyrimidines complexes and study their biological activities as anticancer agents for leukemia treatment. Among the entire diseases, cancer ranks high as a major killer worldwide.

2. EXPERIMENTAL

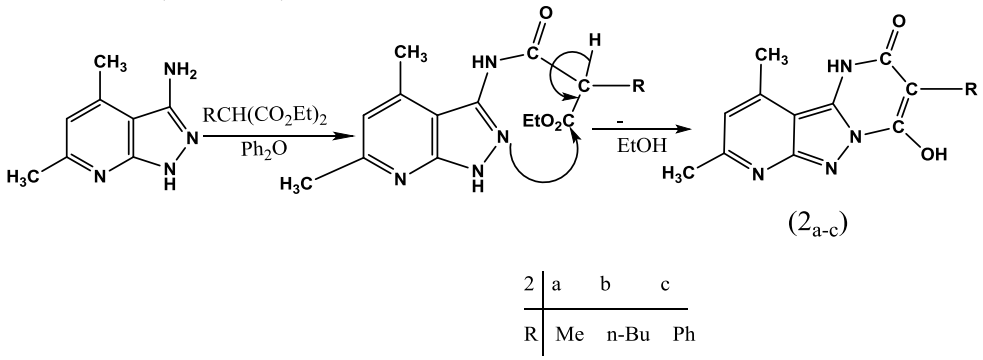
2.1. Materials and methods

All chemicals were of analytical grade (Sigma) and were used as received without further purification. Elemental analyses(C, H, and N) were carried out at the Micro analytical unit, Cairo University, Giza, Egypt. Metal content in the complexes was determined via thermogravimetric results. Infrared spectra of the free ligands and their metal complexes were performed on a Nicolet FT-IR spectrophotometer in the range 4000–400 cm^{-1}. The absorption

electronic spectra were measured in Nujol mulls using a Perkin Elmer Lambda 4B spectrophotometer. Molar conductance measurements were made in a solution of 10^{-3} M DMSO using a type CD6 N Tacussel conductivity meter. The thermal analysis (TGA&DTG) was carried out by using a Shimadzu DAT/TG-50 thermal analyzer with a heating rate of 10°C/min under air atmosphere with a flowing rate of 20 mL/min from the room temperature up to 800°C using platinum crucibles. Magnetic susceptibilities were measured at room temperature by a modified Gouy method using a Johnson Matthey magnetic susceptibility balance. The effective magnetic moments were calculated from the equation $\mu_{eff} = 2.84\,(X_M^{corrt}\,T)^{1/2}$. Melting points were measured by using Stuart melting point apparatus.

2.2 Preparation of PyridoPyrazolopyrimidines ligands (I, II and III):

PyridoPyrazolopyrimidines ligands (I, II and III) were prepared as reported.[17] Structural assignment by infrared (IR) spectroscopy showed the characteristic absorption bands at 3405-3293cm^{-1}, 3404-3276cm^{-1} and at 3430-3290 cm^{-1} due to (OH) and (NH) groups of the free ligands **(I, II and III)** respectively **.** The proposed structure for ligands **(I, II and III)**)) is shown in (Scheme 1).



Scheme 1: Preparation of PyridoPyrazolopyrimidine derivatives, where, (2_{a-c}) are the Free ligands (I, II and III) respectively.

2.3 Preparation of the metal complexes ligands (I, II and III)

All complexes were prepared in (1M:1L) by adding equimolar amounts of a hot ethanolic solution (15 mL) of appropriate metal salt to an ethanolic solution (25 mL) of ligands **(I, II and III).** The reaction mixture was stirred for one hour at 50°C and then cooled. The precipitated complexes were filtered off, washed several times with ethanol and finally dried under vacuum over anhydrous calcium chloride giving a metal-ligand mole ratio of 1:2 for (**Ib, Ic, Id)** and 1:1 for (**IIa, IIIa**).

3. CHEMISTRY

Complex (Ib),[$Cu(H_2L^1)(HL^1)(CH_3COO)(H_2O)$].$3H_2O$ Yield: 80%; m.p > 300°C; color: dark brown; molar conductivity (Λ_m): (4.90)ohm^{-1}cm^{2}mol^{-1} .Anal.Calcd.(%) for

$C_{32}H_{46}N_8O_{10}Cu$,FW= (766.318): C, 50.15; H, 6.05; N, 14.63, Cu, 8.29; Found(%) C, 50.19; H, 4.73; N, 15.22, Cu, 8.61. IR (cm^{-1}),3422(m) υ(OH) phenolic υ(OH) of H_2O[20], the bands characteristic to υ(NH) and υ(C=O) were absent[21], 1461(m) υ **(N=C−O),** 1382(w) υ(C-O) amide[22], 1272(sh) Phenolic υ(C−OH), 1027(m) 1027(m) υ(N−N) of pyrazole[23], 599w υ(M.O), 531(vw) υ(M←O), 457(vw) υ(M←N)[24-26]**,** 1598(s) and 1421(m)cm^{-1} due to $\nu_{assy.}(OAc^-)$ and $\nu_{sym..}(OAc^-)$ respectively. The separation $\Delta\nu$=177cm^{-1} suggests the unidantate coordination of (OAc^-) ion in complex (Ib)[27]. UV-Vis: (192-250, 296−308), (515), (792) due to ($\pi - \pi^*$)[32], $^2A_{1g}(D) \longrightarrow {}^2B_{1g}(D)$, (d−d transition of an octahedral geometry)[33] respectively , 2.04(BM)[34] and mass loss Calcd.(%): (2.35), (4.70), (2.35), (30.46), (41.92), (18.22); Found (%): (2.35), (4.68), (2.35), (30.55), (41.16), (18.91) at **TG** range (^0C): (27−127), (127−282), (282−394), (394−491), (491−899), (At 899) due to loss of hydrated (H_2O), lattice ($2H_2O$), coordinated (H_2O), partial ligand pyrolysis, complete ligand pyrolysis and (CuO + C residue) respectively.

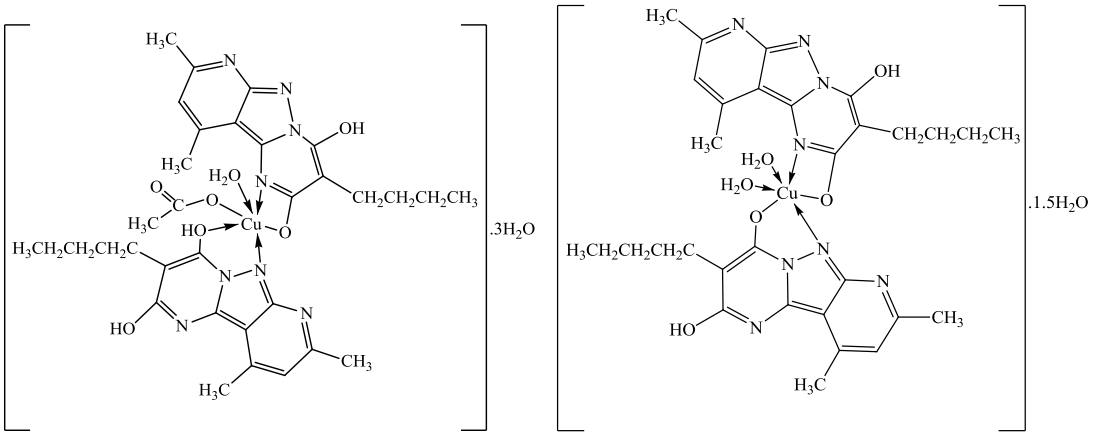
Complex (Ic), $[Cu(HL^1)_2(H_2O)_2].1.5H_2O$ Yield: 84%; m.p > 300°C; color: pale green; molar conductivity ($\mathbf{\Lambda}_m$): (11.60) $ohm^{-1}cm^2mol^{-1}$. Anal.Calcd.(%) for $C_{30}H_{41}N_8O_{7.5}Cu$,FW= (697.240): C, 51.68; H, 5.93; N, 16.07, Cu, 9.11; Found(%) C, 52.68; H, 4.96; N, 17.03, Cu, 9.03. IR (cm^{-1}), 3442(m) υ(OH) phenolic υ(OH) of H_2O[20], the bands characteristic to υ(NH) and υ(C=O) were absent[21], 1428(sh) υ**(N=C−O) ,** 1395(m) υ(C-O) amide[22], 1219(m) Phenolic υ(C−OH), 1027(m) υ(N−N) of pyrazole[23], 601w υ(M−O), 445(vw) υ(M←N) [24-26]. UV-Vis: (192−250), (344−350), (448−515), (706) due to ($\pi - \pi^*$)[32], (^{2}eg(D) $\longrightarrow {}^2B_{1g}$**(D)**), ($n - \pi^*$), (d−d transition of an octahedral geometry)[33] respectively; 1.91(**BM**)[34] and mass loss Calcd.(%): (3.87), (2.58), (40.00), (36.11), (17.44) ; Found (%): (3.52), (2.70), (39.60), (36.90), (17.28) at **TG** rang(^0C): (28−100), (100−209), (209−453), (453−669), (669−899) due to loss of hydrated 1.5(H_2O), coordinated (H_2O), [[coordinated (H_2O) & partial ligand pyrolysis]], complete ligand pyrolysis and (CuO + C residue) respectively.

Complex (Id), $[Co(H_2L^1)_2Cl(OH)].H_2O$ Yield: 81%; m.p > 300°C; color: light green; molar conductivity ($\mathbf{\Lambda}_m$): (19.00)$ohm^{-1}cm^2mol^{-1}$.Anal.Calcd.(%) for $C_{30}H_{39}N_8O_6CoCl$,FW= (702.070): C, 51.27; H, 5.60; N, 15.96; Co, 8.39; Found(%) C, 52.70; H, 5.46; N, 16.16, Cu, 8.49. IR (cm^{-1}), 3419(m) υ(OH) of H_2O/EtOH[20], 3205(m) υ(NH) amide , 1635(sh) υ(C=O) amide[28-30], 1239(sh) υ(C−OH), 980(w) υ(N−N) of pyrazole as ligand(I), 532(vvw) υ(M←O), 454(vw) υ(M←N)[24-26]. UV-Vis: (436−486), (574−670) due to ($^4T_{1g}(F) \longrightarrow {}^4T_{2g}(p)$), $^4T_{1g} \longrightarrow {}^4A_{2g}$(octahedral geometry) respectively; 4.17(**BM**) and mass loss Calcd.(%): (2.63), (7.47), (79.23), (10.67); Found (%): (2.73), (7.27), (79.20), (10.80) at **TG**A range (^0C): (23−69), (69−228), (228−479, (479−899) due to loss of hydrated (H_2O), [(OH) + 0.5Cl_2 gas], complete ligand pyrolysis and (CoO) respectively.

Complex (IIa), $[Cu(H_2L^2)(OH)(H_2O)].Cl.0.5H_2O$**:**Yield:68%; m.p > 300°C; color: yellowish brown; molar conductivity ($\mathbf{\Lambda}_m$): (60.40)$ohm^{-1}cm^2mol^{-1}$. Anal.Calcd.(%) for $C_{12}H_{16}N_4O_{4.5}CuCl$,FW= (387.288): C, 37.21; H, 4.16; N, 14.47, Cu, 16.41;Found (%) C, 37.90; H, 3.71; N, 14.88; Cu, 15.87. IR (cm^{-1}), 3430(m,br) υ(OH) of H_2O/EtOH[20], 3290(vw) υ(NH) amide , 1640(sh) υ(C=O) amide[28-30], 1232(s) υ(C−OH) as ligand(II), 983(vw)

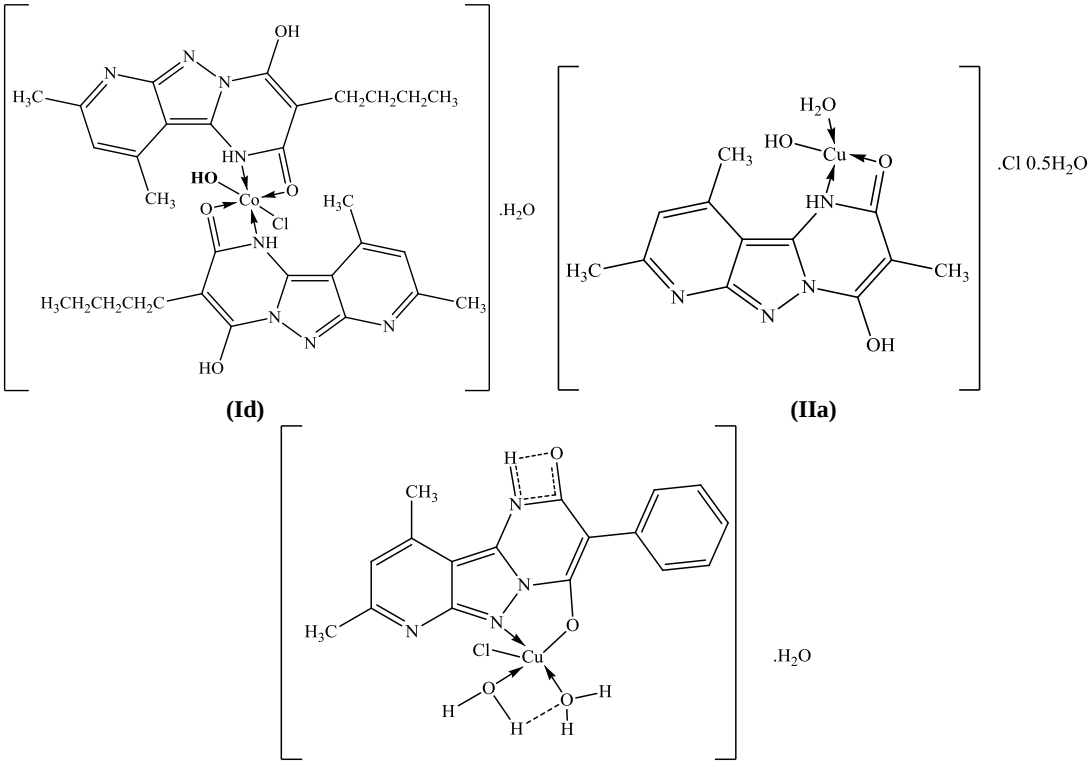
υ(N−N) of pyrazole as ligand(II), 503(w) υ(M←O), 444(vvw) υ(M←N)[24-26]. UV-Vis: (336), (452), (510-580) due to ($\pi - \pi^*$), ($n - \pi^*$)[32], (d−d transition of square planer)[35] respectively; 1.85 **(BM)** and mass loss Calcd.(%): (2.32), (17.68), (60.54), (19.46); Found (%): (2.25), (18.36), (60.52), (18.87) at **TG** range(^{0}C): (29−135), (135−336), (336−649), (649−899) due to loss of hydrated 0.5(H_2O), [coordinated 2(H_2O) + 0.5Cl_2 gas], complete ligand pyrolysis and (Cu + C residue) respectively.

Complex (IIIa), [$Cu(HL^3)Cl(H_2O)_2$].H_2O**:** Yield: 65%; m.p > 300^{o}C; color: light brown; molar conductivity (Λ_m): (35.20)ohm^{-1}cm^{2}mol^{-1}.Anal.Calcd.(%) for $C_{17}H_{19}N_4O_5CuCl$, FW= (458.362): C, 44.54; H, 4.18; N, 12.23, Cu, 13.86; Found(%) C, 44.05; H, 3.07p; N, 12.29 ; Cu,14.17. IR (cm^{-1}), 3506(sh) due to inter or intramolecular hydrogen bonding between (H_2O) molecules, 3429(m) υ (OH) phenolic υ (OH) of H_2O[20], 3190(sh) υ(NH) amide [Upon comlexation, the band of (NH) exhibits downshift by about 100cm^{-1} in complex (**IIIa**) may be due to [[hydrogen bonding between (NH) amide proton and (C=O) amide oxygen]], 1648(sh) υ(C=O) amide as its ligand (III)[31], 1272(w) υ(C−OH), 1023(w) υ(N−N) of pyrazole[23], 603(w) υ (M−O), 427(vw) υ(M←N) [24-26]. UV-Vis: (420-515), (660-680) due to ($\pi - \pi^*$, $n - \pi^*$)[32], (d-d transition of square pyramidal)[36] respectively; 1.78(**BM**) and mass loss Calcd.(%): (3.93), (7.86), (7.73), (61.80), (18.67); Found (%): (3.64), (7.20), (8.18), (61.91), (19.07) at **TG**A range (^{0}C): (25−173), (173−260), (260−283),(283−490), (490−899) due to loss of hydrated (H_2O), coordinated 2(H_2O) , (Partial dissociation of ligand due to loss of 0.5 mole of Cl_2 gas], complete ligand pyrolysis, (CuO + C residue) respectively. The elemental analysis, IR, lectronic spectra, and magnetic moment measurements, as well as thermal analyses are compatible with the suggested structure (Fig. 2).



(Ib) **(Ic)**

Continued Figure 2



(Id) **(IIa)**

(IIIa)

Figure 2. Structural representation of the complexes

4. ANTICANCER ACTIVITY

Materials and methods

Peripheral blood leucocytes' isolation and incubation:

The safety assessment of our chemicals administration was done on other eight healthy non-smoker volunteers leukocytes. Moreover, the anticancer properties were evaluated on peripheral venous blood samples of eight male acute myeloid leukemia (AML) patients that were recently diagnosed for AML and did not receive any chemotherapeutics until the samples were taken. About one milliliters of peripheral venous blood samples were collected using sterile syringes and then transferred to sterile K_2-EDTA containing tubes (KIMICO vacutainer). All samples were transported to the laboratory within three to four hours. The study plan was approved by Menoufia University ethical committee. Peripheral blood leukocytes were isolated by incubation with four volumes of erythrocyte lysing buffer (0.015M NH_4C1, 1mM $NaHCO_3$, 0.l mM EDTA). Then, they were centrifuged for 10 minutes at 1500 rpm using cooling centrifuge (Sigma 3K 30, Germany). These steps were repeated

until a white pellet appeared[37]. Isolated human normal leucocytes and myeloid leukemia cells were preceded for short-term culture in serum free RPMI-1640 medium supplemented with 1% (100U/ml penicillin and 100 µg/ml streptomycin) for three hours of tested extracts incubation at 37ºC and humidified 5% CO_2 atmosphere[38].

Assessment of cytotoxicity in NPBL and AML cells

After the various treatments incubation for 3h, determination of cytotoxicity was done by the charged cationic trypan blue dye (0.4%, Lonza) uptake method following incubation for 5 minutes at 37 °C for control and treated groups of both normal and AML cells.

5. RESULTS

Cytotoxicity in NPBL and AML cells

After the various treatments and incubations for 1h, determination of cytotoxicity was done by the charged cationic trypan blue dye uptake method. Results revealed significant cytotoxicity towards AML cells while cytotoxicity towards NPBL were not observed (Figure 3 and 4).

Cytotoxicity of (1,5,10) µg/ml for complexes (Ib, Ic, Id, IIa and IIIa) on normal blood cells after direct exposure for one hour of incubation:

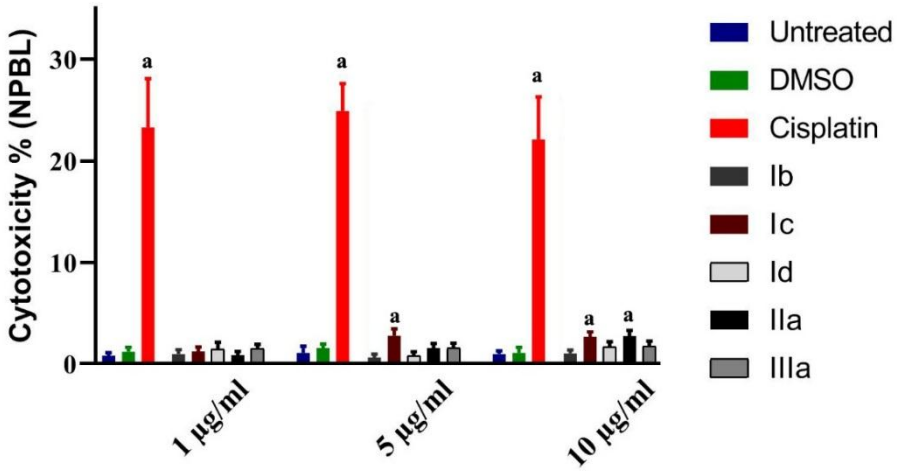


Figure (3) Cytotoxicity of complexes (Ib,Ic,Id, IIa and IIIa) on NPBL after direct exposure for 1h of incubation using trypan blue staining method. Data represent the average of three independent experiments, (n=3). Bars: standard deviation and a: significant with respect to the control (*P*= 0.05). Cisplatin (3 µg/mL).

Cytotoxicity of (10µg/ml) for complexes (Ib-Ic,Id, IIa and IIIa) on leukemia blood cells (AML) after direct exposure for one hour:

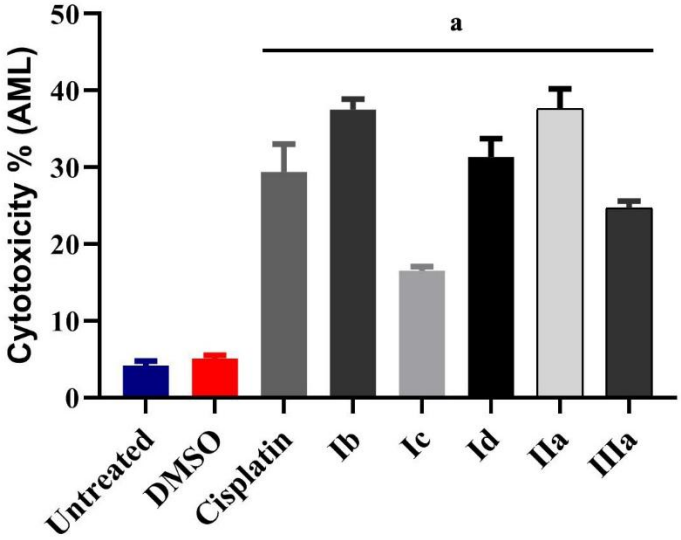


Figure (4) Cytotoxicity of complexes (Ib-Id, IIa and IIIa) on AML after direct exposure for one hour of incubation using trypan blue staining method. Data represent the average of three independent experiments, (n=3). Bars: standard deviation and a: significant with respect to the control (*P*= 0.05). Cisplatin (3 µg/mL).

6. DISCUSSION

The cytotoxicity of the copper complexes (Ib, IIa) and cobalt complex (Id) are more active than standard drug used. This can be explained as Cu (II) ion binds to DNA. It seems that, changing the anion, molar ratio and the nature of the metal ion has effect on the biological behavior, due to alter binding ability of DNA binding, so testing of different complexes is very interesting from this point of view. Chemotherapeutic activity of the complexes may be attributed to the central metal atom which was explained by Tweedy's chelation theory[39.40]. Also, the positive charge of the metal increases the acidity of coordinated ligand that bears protons, leading to stronger hydrogen bonds which enhance the biological activity[41, 42]. Moreover, Gaetke and Chow had reported that, metal has been suggested to facilitate oxidated tissue injury through a free-radical mediated pathway analogous to the Fenton reaction[42]. By applying the ESR-trapping technique, evidence for metal - mediated hydroxyl radical formation *in vivo* has been obtained. ROS are produced through a Fenton-type reaction as follows:

$$LM(II) + H_2O_2 \rightarrow LM(I) + .OOH + H^+$$
$$LM(I) + H_2O_2 \rightarrow LM(II) + .OH + OH^-$$

Where L, organic ligand

Also, metal could act as a double-edged sword by inducing DNA damage and also by inhibiting their repair[43]. The OH radicals react with DNA sugars and 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 via a similar reaction to those discussed below for the direct effects of radiation on DNA[44]. pyridopyrazolopyrimidines revealed antiproliferative activity and are used as potent kinase inhibitors[16-19]. The tested complexes were found to be less toxic than the free ligands. This indicates enhancing of antitumor activity upon coordination.

CONCLUSION

Novel bioactive pyridopyrazolopyrimidine metal complexes have been synthesized and characterized using chemical and physicochemical analysis. These structures have been synthesized and characterized by elemental analyses, (spectral method UV–Vis., IR), magnetic susceptibility, conductance and thermogravimetric analysis. TGA were performed. Molar conductance in DMF arrangement demonstrates that, all complexes are non-electrolytes except the complex (**IIa**) is electrolyte. All these parameters described above, confirmed the proposed structures of these metal complexes. These complexes of these ligands have much potential as anticancer agents. Chelates behave as a neutral bidentate ligand bonded to the metal ions through either the nitrogen atom of pyrazole ring and hydroxyl oxygen atom attached to pyrimidine ring or the nitrogen atom of amide group of pyrimidine ring and carbonyl oxygen atom of amide group of pyrimidine ring in protonated or deprotonated form.**.** The safety and tolerance assessment of these complexes were done on normal peripheral blood leucocytes isolated from eight healthy non-smoker volunteers. Besides, the anticancer properties were evaluated on acute myeloid leukemia (AML) blasts of eight patients that were recently diagnosed for AML and received no chemotherapeutics until the samples were taken. The tested complexes should be investigated in depth towards other cancer cell lines to assess their possible integration as chemotherapeutics. These compounds are promising candidates as anticancer agents because of their high cytotoxic activities.

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