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ORIGINAL RESEARCH PAPER

Antitumor Activity of Asymmetric Schiff-Base Complexes of Cobalt(II) and Nickel(II)

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ABSTRACT

A new bidentate Schiff-base ligand derived from 4-nitro-o-phenylenediamine and 3-phenylpropenal and its complexes with Co(II) and Ni(II) metal ions have been synthesized. Various methods like thermogravimetric analysis (TGA), nuclear magnetic resonance (1H- and 13C-NMR), molar conductance, furrier transform infrared (FT-IR) and Ultraviolet–Visible (UV-Vis) spectroscopies are applied for characterization of Schiff-base ligand and its complexes. Based elemental analysis data the MLX2 [M=Co(II) and Ni(II); X=Cl, Br and L=N,N'–bis((E)-3–phenylallylidene)-4-nitro-1,2-phenylenediamine] formula is supposed for these metal complexes. Low molar conductivities in DMF confirm their non-electrolytic nature of them, just in the case of NiLBr2 the slight electrolyte character might be observed. Other evidences that confirmed the formation of Schiff-base complexes are changes in the location and shape of the peaks in UV-Vis and FT-IR spectra of complexes with respect to free ligand and distorted-tetrahedral structure is suggested for metal complexes. Anticancer activities of these complexes against A549 cancer cell line demonstrated these complexes are excellent candidates in the biological systems.

Keywords: *Anticancer, A549, bidentate Schiff-base, Biological*

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INTRODUCTION

Schiff-bases contain the imine group derived from the reaction of carbonyl compounds with aliphatic and aromatic amines represent a series of compounds containing N, S and O ligand donor atoms that has been widely studied [1-4]. In coordination chemistry, a wide variety of Schiff-base ligands have played a vital role, because of their ability to form stable complexes with most transition metal ions [5-7]. Schiff-base ligands and their complexes possess variety applications in the biological, analytical, and industrial areas [1, 8-10]. The study of biological properties of various Schiff-base complexes has shown these compounds have a broad spectrum of biological activities such as antibacterial, antiviral, anti-HIV, anticancer, antifungal, and anti-inflammatory [11-21]. With respect to the biological activity and desirable physicochemical, stereochemical, electrochemical, structural and catalytic properties of Schiff-base metal complexes, their values have attracted significant attention and is also relevant for their application as tools for the analysis of pharmacological constituents [1,22,23]. Furthermore, use of these complexes, specially salen and salophen to catalyze in the oxidation of various organic compounds has received much

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attention [24,25].

In this work, the synthesis and characterization of new Cobalt(II) and nickel(II) complexes of a bidentate Schiff-base ligand entitled as N,N'–bis((E)-3–phenylallylidene)-4-nitro-1,2-phenylenediamine (BPANPD) is reported. The prepared samples have been characterized by various methods. Moreover, anticancer activity of synthesized Schiff-base and its complexes against A549 cancer cell line was investigated.

EXPERIMENTAL

Materials and methods

3-phenylpropenal (*trans*-cinnamaldehyde), 4-nitro-*o*-phenylenediamine, Cobalt(II) and Nickel(II) salts, solvents, giemsa's azure eosin methylene blue solution (Giemsa) and other chemicals were purchased from Aldrich and Merck companies and were used without purification. 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) was purchased from Sigma company. A549 cancer cell line has been purchased from Iran Pasteur Institute, Tehran, Iran. ^{1}H- and ^{13}C-NMR spectra were obtained on a BRUKER 250 MHz spectrometer using $CDCl_3$ as solvent and TMS as an internal standard. FT-IR spectra of all compounds were recorded by using a Perkin Elmer spectrum version 10.01.00 FT-IR spectrometer at 4000–400 cm^{-1} by KBr tablets method. Thermogravimetric analyses (TGA) were carried out with a PL-1500 TGA apparatus under an N_2 atmosphere. Elemental analyses were performed on a Vario EL III CHNS system. UV–Vis spectra in the range of 200-800 nm were recorded using a Perkin Elmer Lambda 45 model spectrophotometer. Molar conductivities of the ligand and their complexes were determined at room temperature using Metrohm-712 conductometer. Anticancer measurements performed by using spectrophotometric plate reader Expert 96, Asys Hitch, Ec Austria. Inverted fluorescent microscope (Olympus, Japan) was used for morphology studies.

Synthesis

Synthesis of N,N'–bis((E)-3–phenylallylidene)-4-nitro-1,2-phenylenediamine (BPANPD) ligand

Preparation of ligand was done by gradually addition of 2 mmol (0.2643 g) of *trans*-cinnamaldehyde (in 10 mL ethanol) to 1 mmol (0.1531 g) of 4-nitro-*o*-phenylenediamine dissolved in ethanol (10 mL) under severe stirring for 6 h at room temperature. After cooling for 4 h, a yellow solid product was filtered, washed with ethanol (3×10 mL) and finally vacuum-dried. Purification of ligand was done by recrystallization from ethanol. The Schiff-base ligand is soluble in dichloromethane, chloroform, acetone, dimethylsulfoxide (DMSO), dimethylformamide (DMF), and slightly soluble in alcohols. Yellow color: 73% yield.

FT-IR (KBr, cm^{-1}): 3414 (w), 3324 (w), 3055 (m), 2860 (m), 1629 (s), 1583 (s), 1495 (s), 1333 (s), 1307 (s), 1150 (m) 1093 (w), 996 (w), 820 (w), 749 (m), 731 (m).

Synthesis of Co(BPANPD)X_2 complexes

To a stirring solution of 1 mmol anhydrous $CoCl_2$ in 5 mL methanol was added dropwise a solution of 0.3814 g (1 mmol) BPANPD ligand in 25 mL dichloromethane, and the solution was stirred at room temperature for 5 h. The reaction mixture was filtered, and the filtrate was evacuated for 24 h. The blue product was collected by filtration and washed with dichloromethane and dried in vacuum. Co(BPANPD)Br_2 as a deep green product was synthesis based on similar method but using anhydrous $CoBr_2$ and recrystallization from mixture of DMSO and methanol.

Synthesis of Ni(BPANPD)X_2 complexes

To a stirring solution of 1 mmol of anhydrous $NiCl_2$ in 5 mL methanol, a solution of 0.3814 g (1 mmol) BPANPD in 25 mL chloroform was added dropwise and the solution was stirred at room temperature for 3 h. The product precipitated as a green powder and collected by filtration. Recrystallization of the product was performed in the dichloromethane by ether vapor diffusion into the solution. Similar method used for synthesis of Ni(BPANPD)Br_2 by using anhydrous $NiBr_2$ and the yellow powder recrystallized from chloroform. Fig. 1 shows the structures of ligand and metal complexes.

***Co(BPANPD)Cl_2 (I)*:** Yield: 74%. FT-IR (KBr, cm^{-1}): 3443 (w), 3211 (m), 3089 (w), 2855 (w), 1637 (vs), 1524 (s), 1430 (m), 1342 (s), 1126 (m), 1038 (m), 890 (m), 829 (w), 750 (m), 506 (w).

Co(BPANPD)Br_2 (II): Yield: 68%. FT-IR (KBr, cm^{-1}): 3506 (w), 3203 (m), 3098 (w), 2853 (w), 1635 (vs), 1522 (s), 1428 (m), 1339(s), 1218 (w) 1067 (m), 961 (m),874 (w), 749 (m), 503 (w).

Ni(BPANPD)Cl_2 (III): Yield: 63%. FT-IR (KBr, cm^{-1}): 3379 (m), 3098 (w), 2971 (w), 2856 (w), 1631 (s), 1513 (s), 1450 (w), 1335 (s), 1062 (m), 966 (m),

875 (m), 746(m), 500 (w).

Ni(BPANPD)Br$_2$ (IV): Yield: 69%. FT-IR (KBr, cm^{-1}): 3445 (w), 3331 (m), 3030 (w), 2858 (w), 1631 (vs), 1526 (w), 1442 (s), 1211 (s), 1129 (m) 1029 (s), 852 (s), 801 (s), 748 (s), 492 (w).

Evolution of anticancer activity of MLX$_2$ Schiff-base complexes

At first, A549 cancer cells were cultured in a flask including DMEM/F12 culture medium containing 10% of the fetal bovine serum (FBS) and the flask was moved to the incubator at 37 °C in a humidified atmosphere with 5% CO_2. The flasks were examined daily with inverted microscope and cell division, density, and cell morphology was controlled. The culture medium was altered every 3 days and the cell was separated by trypsin/EDTA. Then for anticancer experiments, A549 cells were plated into 96-well plates at a density 1×10^4 per well and later left for 24 h to gain exponential growth. The cells were treated with Schiff-base ligand and its complexes with the concentration of 1, 0.1 and 0.01 mg mL^{-1} and maintained at 37 °C with CO_2 for 24 and 72 h. The sample without treatment was served as a control group. To carry out this experiment, MTT solution containing 5 mg mL^{-1} MTT powder was made in the dark. After that, the solution was combined with DMEM/F12 culture medium in a ratio of 1:9 (9 parts of culture medium and 1 part of MTT solution), and in each well, 100 µL of the above mentioned solution was added and the plate was incubated for 4 h. Then, the wells were empty again and in each well, 100 µL of dimethylsulfoxide was added to dissolve the sediment and incubated for 30 min. Afterward, the contents of each well once slowly sampled via the sampler, and in the end, by using ELISA and a wavelength of 570 nm, the absorption of each well was studied. Finally, Giemsa stain was applied for the staining of treatment and control groups. The fixation of cells was performed by methanol and followed by the use of 4% Giemsa stain for 15 min. Later these cells were washed with water and studied under an inverted microscope (Olympus, Japan).

RESULTS AND DISCUSSION

Characterization of BPANPD Schiff-base ligand and its metal complexes

The reaction of MX_2 (M=Co, Ni; X=Cl, Br) with BPANPD in a 1:1 molar ratio gives complexes that stoichiometry's are confirmed by elemental analysis. Elemental analyses and other physical properties of the ligand and its complexes are summarized in Table 1. These compounds are stable under ordinary laboratory condition. Low molar conductance values in the range of 3.59–55.74 S cm^2 mol^{-1} from 10^{-3} M solutions of the complexes in DMF (Table 1), showed they are non-electrolytes [26,27]. The Λ_M of $NiLBr_2$ (55.74 S cm^2 mol^{-1}) near to 1:1 electrolyte type (65-90 S cm^2 mol^{-1}), and might be behave as slight electrolyte [28].

The ^{1}H- and ^{13}C-NMR chemical shifts of Schiff-base ligand in $CDCl_3$ are shown in Fig. 2. The ^{1}H-NMR spectrum of the BPANPD shows peaks at 8.4 (bs, 2H) for azomethine, 7.07-8.00 (m, 13 H) for aromatic rings and 5.01 and 6.70 (d, 4H) ppm for olefinic hydrogen. Peak at 161.20 for azomethine and at 148.64, 145.30, 138.70, 135.49, 135.32, 129.96, 128.98, 128.04, 127.67, 124.17, 113.07 and 112.87 ppm for aromatic and olefinic carbons are appeared in ^{13}C-NMR spectrum of this ligand. Due

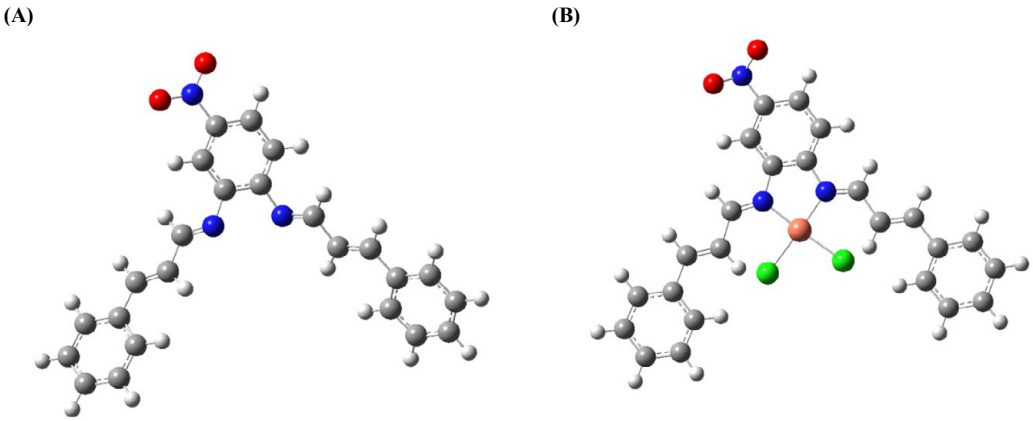

Fig. 1. Schematic view of the Schiff-base ligand (A) and metal complexes (B) [Metal (orange), Halogen (green), Oxygen (red), Nitrogen (blue), Carbon (gray) and Hydrogen (white)].

Table 1. Physical data and elemental analysis of the ligand and its complexes

Compound	MW (g mol^{-1})	M. P. (°C)	Λ_M (S cm^2 mol^{-1})	Found (Calculated) (%)		
				C	H	N
BPANPD	381.43	175	3.59	75.67 (75.57)	5.14 (5.03)	10.97 (11.02)
I	511.25	>300 [d]	25.64	56.33 (56.38)	3.81 (3.75)	8.29 (8.22)
II	600.15	>300 [d]	37.48	48.25 (48.05)	3.23 (3.19)	7.09 (7.00)
III	511.01	265	33.59	56.48 (56.41)	3.55 (3.75)	8.03 (8.22)
IV	599.91	287 [d]	55.74	48.08 (48.05)	2.98 (3.75)	7.23 (7.01)

d: decomposition temperature

I: Co(BPANPD)Cl_2, II:Co(BPANPD)Br_2, III: Ni(BPANPD)Cl_2, IV: Ni(BPANPD)Br_2

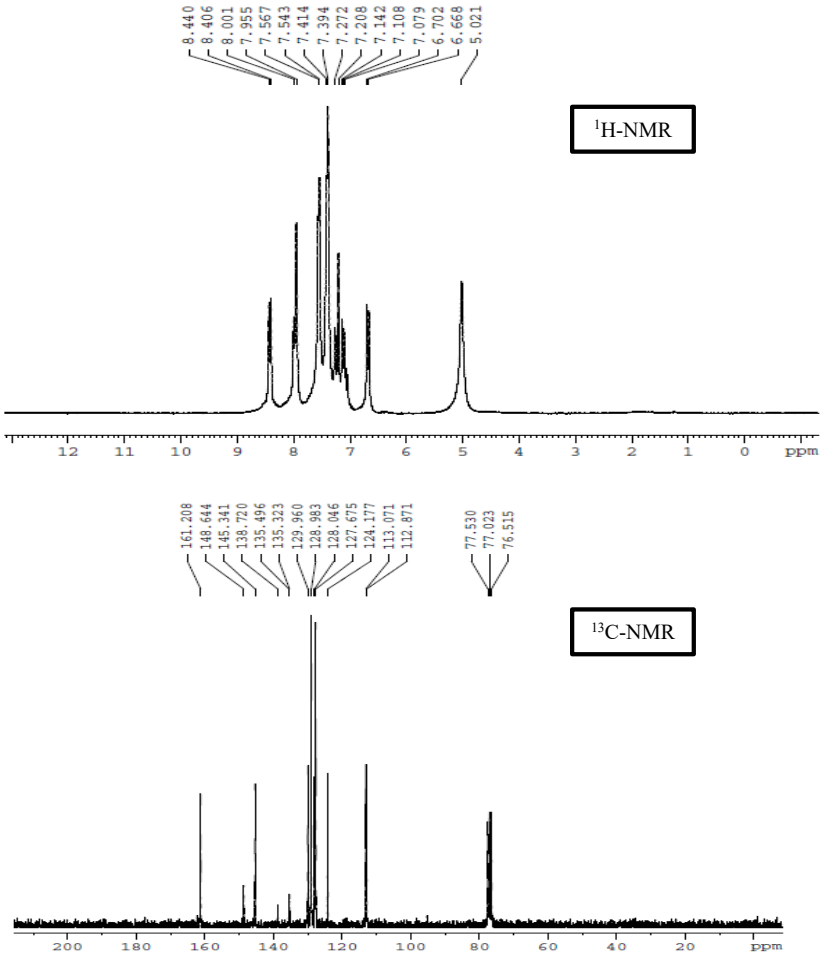

Fig. 2. ^{1}H-NMR and ^{13}C-NMR of the BPANPD Schiff-base ligand in $CDCl_3$.

to paramagnetic properties of cobalt (II) and nickel (II) complexes recording of NMR spectra was not possible.

To clarify the mode of bonding and the effect of the coordination metal ion on the band frequency of the ligand, the FT-IR spectra of the free ligand and their complexes were recorded and compared each other (Fig. S1). Data are presented in Table 2. In FT-IR spectrum of the ligand, the absence of functional group absorption frequencies of the initially materials and appearance of a strong absorption peak at 1629 cm^{-1} can be considered as evidences for formation of -C=N bond in the free ligand [29]. In the metal complexes, the stretching band of the C=N is shifted near 2–8 cm^{-1} to higher frequencies, at 1631–1637 cm^{-1}. Observation of these changes verifies that the azomethine nitrogen atoms are coordinated to the metal ions. After

Table 2. FT-IR and UV-Vis spectral data of BPANPD ligand and its complexes

Compound	υ(C-H) imine	υ(C=N)	υ(NO_2)	υ(M-N)	λ (nm), ε(M^{-1} cm^{-1})
BPANPD	2860(m)	1629(s)	1583(w), 1333(vs)	----	298 (5659), 372(7815)
I	2855(w)	1637(vs)	1524(s), 1342(vs)	506(w)	300 (6321), 359 (6196)
II	2853(w)	1635(vs)	1522(s), 1339(vs)	503(w)	321 (7456), 334 (7538), 363 (8136)
III	2856(w)	1631(vs)	1513(s), 1335(vs)	500(w)	314 (5148), 333 (5018), 361 (5465)
IV	2858(w)	1631(s)	1526(s), 1337(s)	492(w)	317(4564), 359(4671)

s= strong, vs=very strong w= weak, m= medium.
I: $Co(BPANPD)Cl_2$, II:$Co(BPANPD)Br_2$, III: $Ni(BPANPD)Cl_2$, IV: $Ni(BPANPD)Br_2$

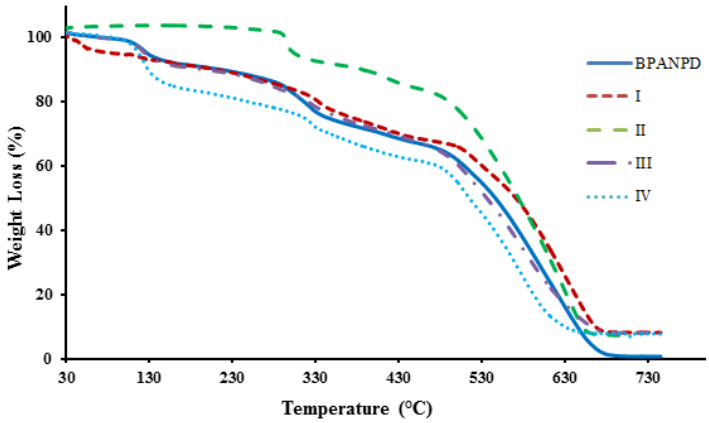

Fig. 3. TGA isotherms of Schiff-base ligand and its complexes [I: $Co(BPANPD)Cl_2$, II: $Co(BPANPD)Br_2$, III: $Ni(BPANPD)Cl_2$, IV: $Ni(BPANPD)Br_2$].

complexation the peak at 2860 cm^{-1} assigned to iminic C-H shifted smoothly to lower frequency. The very strong bands for the ligand at 1583 and 1333 cm^{-1} can be attributed to the asymmetric (ν_{asym}) and symmetric stretching (ν_{sym}) of NO_2 groups.

The absorption spectra of the ligand and its complexes have been obtained at concentration of 10^{-5} M in DMF solvent in the 200–800 nm region (Fig. S2). The spectral data are given in the latter column of Table 2. The spectrum of ligand exhibit two absorption bands in the regions 298, 372 nm assigned to the π-π* transitions of the phenylene rings and the iminic systems, respectively [30,31]. As seen in Table 2, the absorption bands of all complexes are different from the spectrum of the free ligand. The first band was shifted to higher wavelengths by 2–23 nm (red shifted) while the second one shifted to lower wavelengths 9–13 nm. These changes in electronic spectra are due to coordination of the ligand to the metal ions [32]. According to spectral and physical evidences and comparing with previously reports, distorted-tetrahedral structure is suggested for the complexes [33-35]. TGA method was used to determine the thermal properties and stability of ligand and its complexes within a temperature range from room temperature to 800 °C (Fig. 3). Obtained thermograms show that ligand undergoing complete decomposition while complexes generated metal oxide as residual. From TGA plots and based on start decomposition temperature at 100 °C could be suggested that there is no water molecules presence in their structures [36].

Anticancer activity

The in vitro cytotoxic activities of the ligand and its complexes were examined on A549 cancer cell lines. Cell proliferation was estimated by MTT assay. According to Fig. 4, the results showed that ligand and its complexes inhibited cellular proliferation of A549 cells in a dose-dependent manner. Comparing to each other the cytotoxic activity of $NiLBr_2$ complex was more. As the most effective compound, the cell viability of A549 in response to 0.01, 0.1, and 1 mg mL^{-1} of $NiLBr_2$ was 88, 23 and 9.5% at 24 h respectively. The IC_{50} for ligand, $CoLBr_2$ and $NiLBr_2$ was verified to be approximate 0.06, 0.5 and 0.04 mg mL^{-1} respectively, which decreased A549 cells' growth up to 50%, in

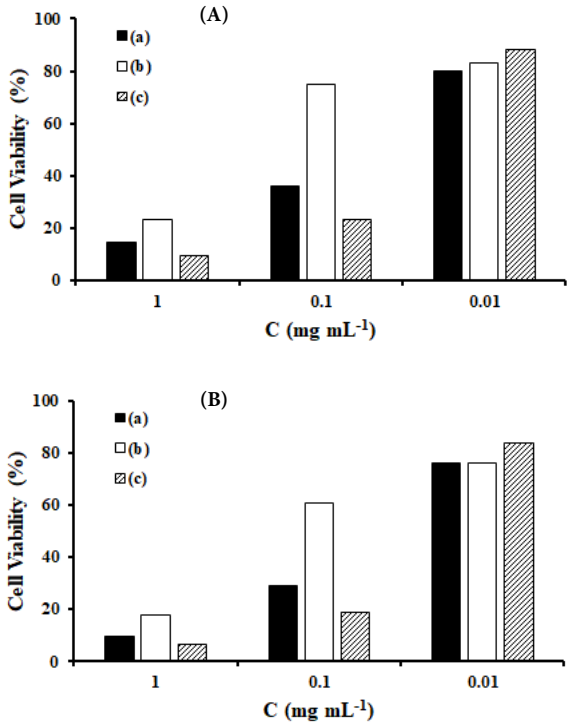

Fig. 4. Diagram of cell viability by MTT assay. Formosan absorbance expressed as a measure of cell viability from the A549 cancer cells cultured with a concentration of 0.01, 0.1 and 1 mg mL^{-1} of ligand and its complexes after 24 h (A) and 72 h (B). [Ligand (a), $CoLBr_2$ (b) and $NiLBr_2$ (c), $p<0.05$ and Values are mean (n=3)].

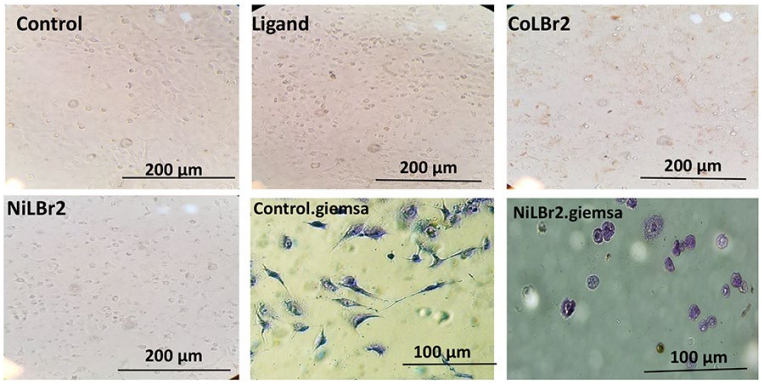

Fig. 5. Giemsa staining and morphologic observation for a survey of cytotoxicity of ligand and its complexes with the IC_{50} concentration in A549 cells after 24 h.

comparison to the untreated cells.

As proved in Fig. 5 ligand and its complexes treatment-induced alterations in cell morphology, like nuclear condensation, large vacuoles, cellar rounding, and shrinkage showed cytoskeleton disruption. The control samples presented natural morphology were homogeneously stained with the cytoplasm less than the nucleus and the nucleolus stayed obviously visible. On the other hand, if these cells exposed to ligand and its complexes, they became rounded and compared to the control cells, there was a meaningful decrease in cell length.

CONCLUSIONS

In summary, a new asymmetric bidentate Schiff-base ligand derived from condensation of 4-nitro-*o*-phenylenediamine and *trans*-cinnamaldehyde and its complexation capacity towards Co(II) and Ni(II) has been studied. The structures of the ligand and complexes were confirmed by elemental analysis,

thermal studies, ^{1}H-NMR, ^{13}C-NMR, FT-IR, UV–Vis spectra and molar conductance. The suggested structure of the complexes is distorted-tetrahedral and the Schiff base ligand act as bidentate chelating agent. Also, thermal studies of all compounds were found that all compounds are decomposed and lose more than 90 % of its weight up to 750 °C. Finally, in vitro anticancer properties of all compounds were screened against A549 cancer cell line. These results exhibited more anticancer activity of bromide complex of nickle (II) with respect to free ligand and cobalt (II) bromide.

SUPPLEMENTARY MATERIAL

consists of FT-IR and UV-Vis spectra of ligand and metal complexes.

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