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RESEARCH ARTICLE

Cytotoxic Activities of Newly Synthesized Co(II), Ni(II) and Cu(II) Complexes with Hexadentate Hydrazonic Ligands

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Abstract: In this paper, we report the synthesis, characterization and antitumor studies of Co(II), Ni(II) and Cu(II) complexes with two potentially hexadentate ligands. Antitumor activity of the prepared ligands and its complexes against human breast cancer cell line MCF-7 was determined. The level of total protein, nucleic acids (RNA and DNA) and nitric oxide (NO) in MCF-7 treated cells was estimated. Antitumor activities of the complexes are accompanied with over-production of free and superoxide radicals allowed reactive oxygen species-mediated tumor cell death. The results show significant antitumor activity comparable to cisplatin

Keywords: MCF-7, Nucleic acids, Antitumor activities, Transition metal complexes, Hexadentate hydrazonic ligands, Cytotoxicity

Introduction

There is currently considerable interest in the coordination chemistry of polydentate ligands containing oxygen and nitrogen as donor atoms. Although acyclic Schiff bases have not been extensively studied but they found to have a flexible cavity size which is considered as an advantage. Some studies[1-4] regarding pentadentate bis acylhydrazones of 2,6-diacetylpyridine suggests that 2,9-diformylphenanthroline has been widely used as a metal-binding reagent in most aspects of coordination chemistry while some of its derivatives are used as hexadentate ligands[5-6].

Divalent transition metal complexes of the hexadentate ligands were reported classically in many studies[7-12]. Use of metal complexes in biological (antibacterial, antimicrobial) and medicinal chemistry (anticancer) has been growing rapidly in recent years[13-29].

In this paper, we report the synthesis and characterization of complexes of (2,9-diformyl-phenanthroline)-bis(benzoyl) hydrazone and (2,9-diformylphenanthroline)bis(2-pyridyl) hydrazone with Co(II), Ni(II) and Cu(II) and use the prepared complexes as a new class of coordination chemistry-based antitumor agents.

Experimental

All reagents were obtained from Sigma-Aldrich. $CoCl_26H_2O$, $NiCl_26H_2O$ and $CuCl_22H_2O$ used were all of AR grade. Yields refer to analytically pure compounds and were not optimized. ^{1}H NMR was recorded on Perkin Elmer 283B and 300 MHz Varian XL-300 instruments in DMSO-d_6 as solvent. IR spectra were recorded on a Perkin Elmer (Spectrum 1000) Fourier-transform infrared (FT-IR) spectrometer, using KBr pellets. Elemental analyses were determined at the College of Science, King Saud University, Riyadh and the results are in agreement with calculated values. Molar conductance Λ_m, in Ω^{-1} cm^2 mol^{-1} units, at 25 °C of freshly prepared (0.001 mol dm^{-3}) metal chelates in ethanol was determined using a YSI-32 model conductometer. The magnetic susceptibility was measured at room temperature by the Guoy method. 2, 9-Diformyl-1,10-phenanthroline (DFF) and H_2L^1 were made according to the literature methods[3,21].

Synthesis of ligand H_2L^2

A solution of 2-pyridylhydrazine (0.23 g, 2.1 mmol) in methanol (20 mL) was refluxed with solution of 2,9-diformylphenanthroline (0.25 g, 1.05 mmol) in methanol (75 mL). After few minutes, a brown precipitate appeared. The solution was refluxed for additional 2 h. After cooling, the precipitate was filtered off and washed with methanol[4]. Yield: 64%, m p = 211-213 °C ^{1}H NMR (300 MHz, [d_6] DMSO, 25 °C): δ = 6.86 (t, $J_{H,H}$ = 7Hz, 2H, H_7), 7.43 (d, $J_{H,H}$ = 8 Hz, 2H, H_5), 7.72 (d, $J_{H,H}$ = 7 Hz, 2H, H_6), 7.95 (s, 2H, H_1), 8.19 (d, $J_{H,H}$ = 5 Hz, 2H, H_8), 8.35 (d, $J_{H,H}$ = 8 Hz, 2H, H_3), 8.45 (m, 4H, H_2 + H_4), 10.41 (s, 2H, N-H, exchange with D_2O) IR (cm^{-1}): υNH = 3268, υC=N = 1573 MS-CI (pos ions): *m/z* 420 [MH$^+$, 100], Anal calc for $C_{28}H_{20}N_6O_23H_2O$: C, 63.90, H, 4.93, N, 15.95 Found: C, 64.04, H, 469, N, 15.62%.

Synthesis of metal complexes

Synthesis of metal complexes with H_2L^1

The Co(II), Ni(II) and Cu(II) complexes were prepared by refluxing the respective hydrated metal chloride (0.2 mmol) in warm ethanol (60 mL) with H_2L^1 (0.2 mmol), the solution turns from brown to yellow. After refluxing for 4 h, the solution was concentrated to 20 mL and when a mixture of diethyl ether (20 mL) and ammonia (15 mL) was added to it, a yellow powder precipitated, filtered off, washed with ether and dried.

Synthesis of metal complexes with H_2L^2

The ligand (02 mmol) was dissolved in warm THF (60 mL) after the addition of the metal salts (0.2 mmol), the solution turned from brown to yellow and the complexes precipitated. The yellow-brown powder filtered off, washed with ether and dried.

Characterization of the metal complexes

$[Co(H_2L^1)]Cl_22H_2O$, (**1**): Yield: 72%, mp: > 300 °C, ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 7.57-7.66 (m, 6H, H_6 + H_7), 7.97 (d, $J_{H,H}$ = 8 Hz, 4H, H_5), 8.09 (s, 2H, H_1), 8.38 (d, $J_{H,H}$ = 8 Hz, 2H, H_3), 8.60 (d, $J_{H,H}$ = 8 Hz, 2H, H_2), 8.84 (s, 2H, H_4), 12.23 (s, 2 H, N-H, exchange with D_2O) IR (KBr, ν, cm^{-1}): 3420 (OH), 3176 (NH), 1631 (CO), 1555 (C=N), 453(Co-O), 393 (Co-N) Anal calcd for $C_{28}H_{20}N_6O_2Cl_2Co2H_2O$: C, 52.68, H, 3.79, N, 13.17 Found: C, 52.68, H, 3.74, N, 13.20% Λ_M (Ω^{-1} cm^2 mol^{-1}): 7.46 μ_{eff} : 3.29 BM.

$[Ni(H_2L^1)]Cl_22H_2O$, (**2**): Yield: 69%, mp: > 300 °C ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): δ = 7.55-7.68 (m, 6H, H_6 + H_7), 7.98 (d, $J_{H,H}$ = 7 Hz, 4H, H_5), 8.08 (s, 2H, H_1), 8.39 (d, $J_{H,H}$ = 8 Hz, 2H, H_3), 8.59 (d, $J_{H,H}$ = 8 Hz, 2H, H_2), 8.84 (s, 2H, H_4), 10.17 (s, 2 H, N-H, exchange with D_2O) IR (KBr, ν, cm^{-1}): 3419(OH), 3168 (NH), 1620(CO), 1549 (C=N), 461(Ni-O), 384(Ni-N), Anal calc for $C_{28}H_{20}N_6O_2Cl_2Ni2H_2O$: C, 52.63, H, 3.79, N, 13.15 Found: C, 52.49, H, 3.72, N, 13.26 % Λ_M (Ω^{-1} cm^2 mol^{-1}): 81.8 μ_{eff} : 2.95 BM.

$[Cu(H_2L^1)]Cl_2H_2O$, (**3**): Yield: 71%, mp: > 300 °C, ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): δ = 7.57-7.65 (m, 6H, H_6 + H_7), 7.97 (d, $J_{H,H}$ = 8 Hz, 4H, H_5), 8.28 (m, 4H, H_1 + H_3), 8.58 (d, $J_{H,H}$ = 8 Hz, 2H, H_2), 9.01 (s, 2H, H_4), 9.71 (s, 2 H, N-H, exchange with D_2O), IR (KBr, ν, cm^{-1}): 3420(OH), 3198(NH), 1634(CO), 1545 (C=N), 448(Cu-O), 369 (Cu-N), Anal calc for $C_{28}H_{20}N_6O_2Cl_2CuH_2O$: C, 53.73, H, 3.54, N, 13.43, Found: C, 53.49, H, 3.55, N, 13.6 %, Λ_M (Ω^{-1} cm^2 mol^{-1}): 70.5, μ_{eff} :1.93 BM.

$[Co(H_2L^2)]Cl_2$, (**4**): Yield: 65%, mp: > 300 °C, ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): δ = 6.90 (t, $J_{H,H}$ = 6 Hz, 2H, H_7), 7.45 (d, $J_{H,H}$ = 8 Hz, 2H, H_5), 7.79 (t, $J_{H,H}$ = 8 Hz, 2H, H_6), 7.98 (s, 2H, H_1), 8.20 (d, $J_{H,H}$ = 6 Hz, 2H, H_8), 8.39 (d, $J_{H,H}$ = 8 Hz, 2H, H_3), 8.46-8.50 (s + d, 4H, H_4 + H_2), 10.33 (s, 2H, N-H, exchange with D_2O), IR (KBr, ν, cm^{-1}): 3410 (OH), 3259 (NH), 1551 (C=N), 388 (Co-N), Anal calc for $C_{24}H_{18}N_8Cl_2Co$: C, 52.57, H, 3.31, N, 20.44, Found: C, 52.68, H, 3.23, N, 20.36%, Λ_M (Ω^{-1} cm^2 mol^{-1}): 83.5, μ_{eff} : 3.22 BM.

$[Ni(H_2L^2)]Cl_2$, (**5**): Yield: 78%, mp: > 300 °C, ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): δ = 6.95 (t, $J_{H,H}$ = 6 Hz, 2H, H_7), 7.52 (d, $J_{H,H}$ = 8 Hz, 2H, H_5), 7.81 (t, $J_{H,H}$ = 8 Hz, 2H, H_6), 7.98 (s, 2H, H_1), 8.18 (d, $J_{H,H}$ = 6 Hz, 2H, H_8), 8.31 (d, 2H, $J_{H,H}$ = 8 Hz, H_3), 8.46-8.55 (s + d, 4H, H_4 + H_2), 10.38 (s, 2H, N-H, exchange with D_2O), IR (KBr, ν, cm^{-1}): 3446 (OH), 3233 (NH), 1544 (C=N), 374(Ni-N), Anal calc for $C_{24}H_{18}N_8Cl_2Ni$: C, 52.60, H, 3.31, N, 20.45, Found: C, 52.84, H, 3.51, N, 20.15%, Λ_M (Ω^{-1} cm^2 mol^{-1}): 88.2, μ_{eff} : 3.06 BM.

$[Cu(H_2L^2)]Cl_2$, (**6**): Yield: 76%, mp: > 300 °C, ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): δ = 6.88 (t, $J_{H,H}$ = 6 Hz, 2H, H_7), 7.44 (d, $J_{H,H}$ = 8 Hz, 2H, H_5), 7.77 (t, $J_{H,H}$ = 8 Hz, 2H, H_6), 7.99 (s, 2H, H_1), 8.20 (d, $J_{H,H}$ = 6 Hz, 2H, H_8), 8.29 (d, 2H, $J_{H,H}$ = 8 Hz, H_3), 8.37-8.50 (s + d, 4H, H_4 + H_2), 10.29 (s, 2H, N-H, exchange with D_2O), IR (KBr, ν, cm^{-1}): 3425(OH), 3273(NH), 1547 (C=N), 376 (Cu-N), Anal calc for $C_{24}H_{18}N_8Cl_2Cu$: C, 52.14, H, 3.28, N, 20.27, Found: C, 51.95, H, 3.43, N, 20.36%, Λ_M (Ω^{-1} cm^2 mol^{-1}):75.7, μ_{eff} : 1.91 BM.

Antitumor studies

Cell culture

The human breast cancer cell line MCF-7 cells were cultured in Dulbecco's modified Eagle's medium (Gibco, Carlsbad, CA, USA) supplemented with 10% fetal calf serum (Gibco) and 1% penicillin-streptomycin antibiotics (Cellgro, Manassas, VA, USA) at 37 °C in humidified atmosphere containing 5% CO_2. Cells at concentration of $0.50x10^6$ were grown in a 25 cm^3 flask in 5 cm^3 complete culture medium.

Estimation of *in vitro* tumor cell growth inhibition was assessed by incubating $0.65x10^5$ MCF-7 cells in 1 cm^3 phosphate buffer saline with varying concentrations of metal(II) complexes and cisplatin (as a control drug) at 37 °C for 24 h in CO_2 atmosphere. Cells were cultured for 48 h to ensure total attachment. Afterwards, the tested compounds were added to the cells. Cell survival was evaluated at the end of the incubation period by MTT colorimetric assay in all cellular experiments, results were compared with untreated cells.

In vitro cytotoxicity assay

The effect of complexes on growth of MCF-7 cells was estimated by MTT colorimetric assay[30]. This method is based on the selective ability of living cells to reduce the yellow tetrazolium soluble salt of MTT (3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl tetrazolium bromide) to a purple-blue insoluble formazan product by mitochondrial enzymes. The amount of formazan produced is directly proportional to the number of living, not dead cells, present during MTT exposure. The crystals of formazan were dissolved in DMSO and the optical density was measured spectrophotometrically (Microplates reader, Asys Hitech,

Austria). Cells ($0.65x10^5$ cells/well) were plated separately in a sterile flat-bottomed 96-well microplate (BD Falcon) and treated with 30 mm^3 of different concentration of complexes and cisplatin (5, 10, 25, or 50 $\mu g/cm^3$) for 24 h at 37 °C in a humidified 5% CO_2 atmosphere. Then, incubation media were removed and 40 mm^3 MTT solution/well was added and incubated for an additional 6 h MTT crystals were solubilized by adding 200 mm^3. DMSO/well and the plate were shaken gently for 15 min at room temperature. The results were determined photometrically using a microplate enzyme-linked immunosorbent assay.

ELISA reader and absorbance at 570 nm data are expressed as percentage relative viability compared with untreated cells calculated using the following equation:

(Absorbance of treated cells) / (Absorbance of control cells) x 100

The cytotoxic concentration was expressed by half-maximal inhibitory concentration IC_{50}. The IC_{50} calculations were performed using Microsoft Excel and Microcal Origin software for PC. After that, the cells in the culture medium were treated with 20 mm^3 of 1/10 of the IC_{50} values of each complex, with the standard reference drug cisplatin or DMSO (as control) for comparison, then incubated for 24 h at 37 °C in a humidified 5% CO_2 atmosphere. The MCF-7 cells were harvested and homogenizer until complete cell disruption.

Biochemical assays

Antioxidants status assay

Enzyme activities and the level of both reduced glutathione (GSH) and lipid peroxidation (LP) were expressed in cell lysates as a function of total cellular protein[31] activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) were determined as described in literature[32-34]. Levels of reduced glutathione (GSH), nitric oxide (NO) and hydrogen peroxide (H_2O_2) were determined using the methods of Montgomery and Dymock[35], Ellman[36] and Wolf[37].

Estimation of nucleic acids and protein

Total protein and nucleic acids (DNA and RNA) were precipitated and measured in cell homogenates. Total cellular protein was assayed by the method of Lowry *et al.*[32]. Total DNA was extracted and assayed according to the method described by Zhou *et al.*[38]. Total RNA was extracted and assayed using Ribolyser (Hybaid, Heidelberg/Germany).

Statistical analysis

The results are reported as mean ± standard error (SE) for at least six experiments statistical differences were analyzed using one-way analysis of variance (ANOVA) test followed by *t*-test, wherein differences were considered significant at $p < 005$.

Results and Discussion

Synthesis and characterization of the ligands and complexes

The hydrazonic ligand H_2L^1 (Figure 1a) was synthesized, following previously described method[3]. H_2L^2 (Figure 1b) was analogously prepared by condensation of 2,9-diformyl-phenanthroline and 2-pyridylhydrazine in both cases, the spectroscopic characterization does not show any remarkable aspects. In solution, only one set of signals is present in their ^1H NMR spectrum. Accordingly, the ligands show C_{2v} point group symmetry.

The complexes of H_2L^1 (Figure 2a) were obtained by adding the metal chlorides to a refluxing solution of the ligand in ethanol. The complexes were isolated as air stable solids after partial removal of the solvent. No deprotonation of H_2L^1 (in the IR spectra, υ(N-H)

3200 cm^{-1}, in the ^1H NMR spectra, δ(N-H) in the 10-12 ppm range). On the other hand, the reaction between the metal chlorides and a refluxing solution of H_2L^2 in THF leads to the corresponding complexes (Figure 2b).

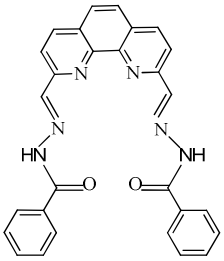

Figure 1a. (2,9-Diformylphenanthroline) bis(benzoyl) hydrazone (Ligand 1 - H_2L^1)

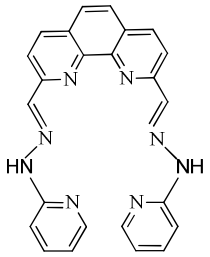

Figure 1b. (2,9-Diformylphenanthroline)-bis(2-pyridyl) hydrazone (Ligand 2 - H_2L^2)

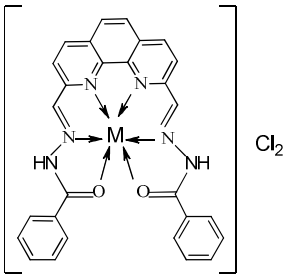

Figure 2a. Metal complexes of H_2L^1

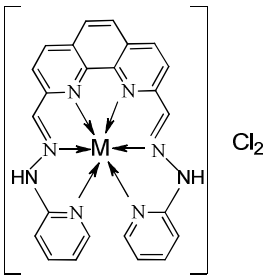

Figure 2b. Metal complexes of H_2L^2

Upon coordination, the (C=O) absorption frequencies shifts to 1620-1634 cm^{-1} (1682-1653 cm^{-1} in the free ligand) and the (C=N) absorption frequencies shifts to 1555-1544 cm^{-1} (1573-1569 cm^{-1} in the free ligand) in addition, (M-O) absorption bands appear at 461-448 cm^{-1} for H_2L^1 complexes, whilst (M-N) absorption bands appear at 393-369 cm^{-1} for all complexes. In conclusion, the ligands behave as hexadentate ligands and the chloride ions assures the neutrality of the system.

The observed molar conductance of 0.001 mol dm^{-3} metal chelates in ethanol represents a 1:2 (cation:anion) electrolytes. Therefore, elemental analysis, spectroscopic data and molar conductance led us to conclude that the two ligands are neutral and hexadentate hydrazonic ligands.

Magnetic moments

The magnetic moments of the four Ni(II) and Cu(II) complexes within the reported ranges of the spin only value 2.83 and 1.73 BM corresponding to two and one unpaired electrons. The observed lower moments of the two Co(II) complexes may be seen anomalous, but normal magnetic moments of six-coordinate Co(II) complex should fall near the extremes of the range from *ca* 1.80 to 5.20 BM One survey of cobalt(II) chemistry[39] shows that there are moments falling through-out this entire range.

Electronic spectra of the metal complexes

Cobalt(II) complexes show two broad bands at 578 and 424 nm which may tentatively be assigned to $^4T_{1g}(F) \rightarrow {}^4T_{2g}$ and $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$, respectively. Nickel(II) complexes show three spin allowed bands at 852, 621 and 398 nm assignable to $^3A_{2g} \rightarrow {}^3T_{2g}$, $^3A_{2g} \rightarrow {}^3T_{1g}$ and

$^3A_{2g} \rightarrow {}^3T_{1g}$[40]. Copper(II) complexes, only shows one band at 396 nm. The electronic spectra of the complexes in solution along with the magnetic moments are agreed well with the pseudo-octahedral geometry around the metal ions for all complexes.

Antitumor activity

In the present study, human breast cancer cell line MCF-7 was treated with the two ligands and their complexes at different concentrations. Growth arrest and apoptosis on the cell density was measured by the 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide (MTT) assay. The doses of the tested compounds were selected based on the preliminary studies. The tumor cell line showed normal growth in our culture system DMSO did not seem to have any noticeable effect on cellular growth. The screening results are summarized in Table 1. It is evident that all of the tested complexes showed anticancer activity, with IC_{50} values ranging from 4.50 to 10.50 µg cm^3. It is clear that comparison of the cytotoxicity of the tested complexes against the MCF-7 cell line has shown that the growth inhibitory potency follows the order Ni > Cu > Co. The results indicate a strong correlation between cisplatin (IC_{50}: 4 µg/cm^3) and Ni complex (IC_{50}: 4.50 µg cm^3). The $[Ni(H_2L^2)]Cl_2$ complex was the best compound, exerting a significant cytotoxic effect on MCF-7 cells compared with cisplatin. The median growth inhibitory concentration (IC_{50}) after 24 h was 10.50 µg/cm^3 for $[Co(H_2L^1)]Cl_2 2H_2O$ (**1**), 5.50 µg/cm^3 for $[Ni(H_2L^1)]Cl_2 2H_2O$ (**2**), 6.50 µg cm^3 for $[Cu(H_2L^1)]Cl_2H_2O$ (**3**), 8.50 µg cm^3 for $[Co(H_2L^2)]Cl_2$ (**4**), 4.50 µg cm^3 for $[Ni(H_2L^2)]Cl_2$ (**5**) and 6.00 µg cm^3 for $[Cu(H_2L^2)]Cl_2$ (**6**).

Table 1. Effect of treatment with the prepared complexes on MCF-7 cells cytotoxicity (IC_{50}) and the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) as well as the levels of reduced glutathione (GSH) and hydrogen peroxide (H_2O_2)

Complexes	IC_{50} (µg/cm^3)	SOD (U/mg protein)	CAT (U/mg protein)	GSH-Px (U/mg protein)	GSH (nmol/mg protein)	H_2O_2 (nmol/mg protein)
Control (DMSO)	--	40.20±4.75	7.40±0.70	9.60±1.00	39.00±4.00	15.70±1.60
Cisplatin	4.00±0.36	130.80±15.65	2.96±0.22	4.40±0.40	21.60±2.40	47.50±5.70
H_2L^1	18.00±0.36	58.80±8.45	6.76±0.28	8.32±0.82	34.30±3.41	22.50±4.10
$[Co(H_2L^1)]Cl_2.2H_2O$	10.50±0.36	63.90±5.30	6.14±0.19	7.31±0.07	27.25±3.75	33.15±3.75
$[Ni(H_2L^1)]Cl_2.2H_2O$	5.50±0.36	93.40±10.73	3.82±0.74	5.16±0.25	24.15±3.70	44.55±4.08
$[Cu(H_2L^1)]Cl_2.H_2O$	6.50±0.36	84.50±5.34	4.67±0.42	5.82±0.40	25.40±3.40	38.45±4.25
H_2L^2	14.50±0.36	76.50±11.25	5.66±1.53	7.17±0.35	27.15±1.92	31.20±2.18
$[Co(H_2L^2)]Cl_2$	8.50±0.36	80.40±2.34	5.20±0.13	6.43±0.29	26.30±4.18	36.70±0.85
$[Ni(H_2L^2)]Cl_2$	4.50±0.36	114.74±11.31	2.63±0.27	4.64±0.51	22.45±0.40	46.90±1.68
$[Cu(H_2L^2)]Cl_2$	6.00±0.36	89.15±7.15	4.11±0.62	5.63±0.21	24.90±1.15	39.50±1.14

Data are expressed as mean ± standard error (SE) of six separate experiments. Differences were considered significant at $p < 0.05$

A differential cytotoxic effect towards tumor cells may be possible based on a reduced ability to detoxify free radicals. Free radicals and in particular superoxide radical ($O_2^{\cdot -}$) cause cellular disruption due to peroxidation of membrane lipids, several enzymes have evolved to cope with $O_2^{\cdot -}$ produced by metabolic reactions in cells in an oxygen environment. (a) Superoxide dismutase (SOD) which converts $O_2^{\cdot -}$ to H_2O_2 and O_2, (b) Catalase (CAT) which serves to reduce the H_2O_2 to H_2O, (c) Glutathione peroxidase (GSH-Px) which acts complementarily to catalase in elimination of H_2O_2 especially in tissues or compartments devoid of catalase, (d) Glutathione reductase (GSH) which catalyzes the reduction of the oxidized form of glutathione produced by glutathione peroxidase by reduced pyridine nucleotides[41].

To elucidate the mechanisms by which the prepared complexes exert their antitumor activities, we estimated the activities of the free-radical-metabolizing enzymes including superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px). The levels of the oxidative stress parameters including hydrogen peroxide (H_2O_2), nitric oxide (NO) and reduced glutathione (GSH) in MCF-7 cells treated with the prepared complexes were studied as well as the effect of these complexes on the levels of total protein and nucleic acids.

The results showed that the order of antitumor activity of the compounds was **5 > 2 > 6 > 3 > 4 > 1.** The highest activity was found for Ni complexes, which resulted in the highest SOD activity and H_2O_2 and low activities of CAT and GSH-Px as well as GSH level. These results indicate that the antitumor effect of the present complexes may be exerted at least partly by production of reactive oxygen species. As shown in Table 2, the level of total protein and nucleic acids were significantly lower than in the control, while the level of NO was significantly higher in MCF-7 cells treated with most complexes as compared to the control cells. The antitumor activities are accompanied by dose-dependent increases in SOD activities of treated cells compared with the control group. This means that ligands and their complexes can cause H_2O_2 production.

The H_2O_2 produced should be rapidly removed through the activation of CAT and GSH-Px. The present results show that activities of CAT and GSH-Px and the level of reduced GSH are lowered in groups treated with ligands and complexes (in dose-dependent manner) compared with the control group (Table 1).

Table 2. Effect of prepared complexes on the level of total protein, nucleic acids (RNA and DNA) and nitric oxide (NO) in MCF-7 treated cells

Complexes	Protein, μg 10^{-6} cells	RNA, μg 10^{-6} cells	DNA, μg 10^{-6} cells	NO (μmol/mg protein)
Control (DMSO)	110.50± 12.30	15.30±1.60	8.50±0.80	1.90±0.16
Cisplatin	33.60±3.70	3.40±0.40	2.50±0.30	4.20±0.37
H_2L^1	82.20±7.32	11.20±0.26	7.95±0.23	2.10±0.45
$[Co(H_2L^1)]Cl_2.2H_2O$	64.50±3.83	9.60±0.33	7.20±0.04	2.85±0.19
$[Ni(H_2L^1)]Cl_2.2H_2O$	42.15±4.67	6.20±0.71	4.30±0.41	3.85±0.42
$[Cu(H_2L^1)]Cl_2.H_2O$	53.85±4.23	7.65±0.35	5.70±0.37	3.25±0.16
H_2L^2	75.80±6.14	9.80±0.37	7.65±0.35	2.50±0.09
$[Co(H_2L^2)]Cl_2$	59.25±1.80	8.25±0.67	6.10±0.22	3.10±0.37
$[Ni(H_2L^2)]Cl_2$	36.50±5.31	5.10±0.54	3.75±0.82	4.15±0.30
$[Cu(H_2L^2)]Cl_2$	45.10±2.01	7.20±8.7	5.25±0.08	3.60±0.25

Data are expressed as mean ± standard error (SE) of six separate experiments. Differences were considered significant at p <0.05

The present results show that activities of CAT and GSH-Px and the levels of reduced GSH are reduced in all treated groups compared to the DMSO-treated group. Consequently, the excess H_2O_2 produced in tumor cells with the complexes cannot be removed in other words, the accumulation of H_2O_2 and other free radicals in tumor cells is partly the cause of tumor cell killing thus, the results of the present study are consistent with the hypothesis that the prepared complexes exert their antitumor effects because they produce reactive oxygen species moreover, the results show that treatment with these complexes leads to an increase in the level of NO which leads to apoptosis (programmed cell death), whereas the increase in reactive oxygen species (ROS) leads to necrosis (cell death), so, the way antitumor cell dies reflects the radical balance in the system in addition, our results show that the increase in NO levels is accompanied by depletion of the total protein and nucleic acid levels compared to the control.

In 2000, Huang *et al.*[42] observed that selective inhibition of SOD kills human cancer cells but not normal cells, suggesting that regulation of free-radical-producing agents may also have important clinical applications.

Conclusion

The present study suggests that the synthesized complexes possess significant antitumor activity comparable to the activity of the commonly used anticancer drug cisplatin. These complexes exert their antitumor activities by regulating free radical production by increasing the activity of superoxide dismutase with depletion of catalase and glutathione peroxidase activities moreover, these complexes enhanced the reduction of intracellular reduced glutathione level, hydrogen peroxide, nitric oxide and other free radicals causing tumor cell death, as monitored by reduction in the synthesis of protein and nucleic acids. Further additional cytotoxicity tests and anticancer studies *in vitro* and *in vivo* for the ligand and complexes will be carried out in the near future.

Conflicts of interest

No conflicts of interest that may affect the authors of the manuscript exist.

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