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Research Article

Anti-Cancer Screening of Some Transition Metal Ion Complexes with Coumarin Derivatives

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Abstract In this study, an equal amount of -4-methyl-5,7-(bis p-chlorophenyl azo) coumarin (ligand 6-hydroxy) and copper, cobalt, and nickel (metals) were reacted to synthesize the respective metal ion complexes. The synthesized metal complexes were characterized using various approaches such as CHN elemental analysis, ^{1}H-NMR, mass spectral data, and FTIR. The outcome of the spectroscopic analysis showed the coordination of the ligand to the metal ion that is in the complexes through hydroxyl group; the data also showed the complexes to exhibit octahedral geometry. Further studies on the solid complexes were performed by molar conductance where all the synthesized metal complexes exhibited nonconductive properties in chloroform. An MTT-based cytotoxicity screening of the complexes against lung and breast cancer cells showed Co(II), Cu(II), and Ni(II) complexes to exhibit cytotoxic activity against the studied cells at low concentrations.

Keywords bis-coumarins complexes; anti-cancer screening

1. Introduction

The metal ions-binding capacity of coumarins presents an extra means of manipulating their pharmacological response. Transition metals complexes have gained much research interest, especially 3d transition metal ions which are significantly important in coordination and bioinorganic chemistry. They can form chelates with several cations; this chelates formation is indicated by a color change at a given pH. The formation of the chelates is characterized by the high stability of the product due to the formation of 6-membered rings. Monohydroxy compounds that contain coumarin have shown great importance owing to their increased chelates-formation tendency when azo groups are introduced to such hydroxyl coumarins; the presence of these hydroxyl substituents also improves the potency of the coumarin rings in the compounds [1].

The biological activity of some 3d transition metal ions in biological systems has been reported; these ions are often referred to as metaloproteins because they are sites for the activity of most enzymes and also determine the structure of most active sites. The partially filled d orbitals of transition metals confer them the ability to exhibit several oxidation states. Their mechanism of their cytotoxic activity has been investigated in a bid to develop new antitumor agents. Some coumarin metal complexes have in some cases shown higher biological activities compared to their ligands [2,3,4,5].

Nowadays, several studies have reported the biological activities of coumarin complexes with metals. Metal ions' binding to coumarins has been reported to enhance the biological activity of such complexes. To understand the factors responsible for the biological activity of coumarin derivatives, it is necessary to investigate their binding properties to different metal ions [6,7,8,9,10]. Here, we report new experimental studies on the biological activity of complexes with coumarin derivatives.

2. Experimental

All reagents used to synthesize the complexes were commercially sourced and of the highest level of purity.

2.1. Synthesis of coumarin derivatives

A solution of p-chloro aniline was prepared and cooled in ice prior to diazotization with 20 mL of 0.01 M aqueous solution of sodium nitrite. Then, the diazonium solution (0–5 °C) was gradually introduced into 0.01 M 6-hydroxy-4-methylcoumarin solution prepared in a solution containing ethanol and sodium hydroxide (100 mL). A schematic representation of the reaction process is presented in Figure 1.

2.2. Synthesis of solid metal complexes

The process of the solid chelates synthesis involved the mixing of a hot alcoholic 0.001 M saturated metal ion solution with the pre-determined amount of each ligand

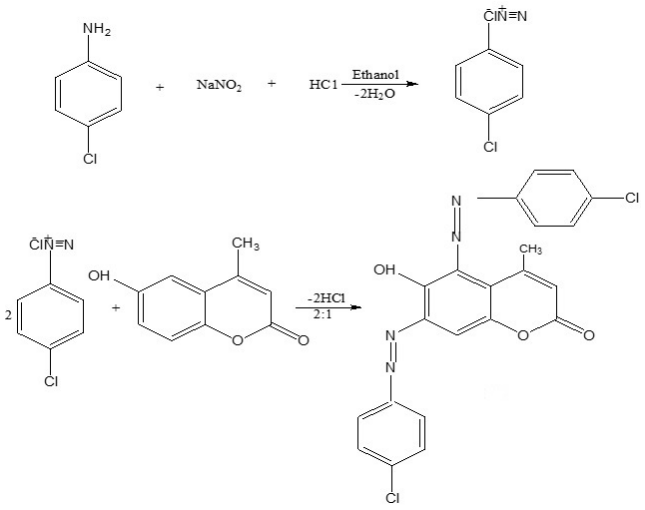

Figure 1: The reactions during the formation of 6-hydroxy-4-methyl-5,7-(bis p-chlorophenyl azo) coumarin.

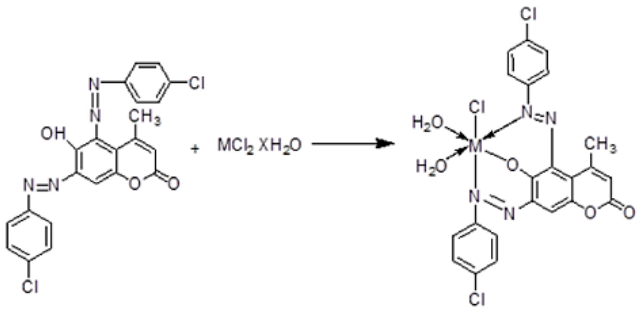

Figure 2: Schematic illustration of the reactions during the formation of coumarin complexes.

which is enough to form 1:1 molar ratio complexes. A dilute ammonia solution (1:10) was used to maintain the pH of the solution at a range of 6.5–7.5 [11]. The resulting mixture was heated with occasional stirring on a steam bath for 4 h and later to dryness. The complex resulting from the solution was then dissolved in ethanol, filtered via suction, and washed again with ethanol to get rid of the unreacted materials. The final colorless filtrate was obtained preserved in a vacuum desiccator for further use.

The schematic illustration of the chemical reaction during the formation of coumarin complexes is shown in Figure 2.

2.3. Cytotoxic activities of complexes

Most of the in vitro assays of the response of cells to external factors are based on the measurement of the viability and proliferation of such cells when exposed to such factors. One of the widely accepted methods of cell proliferation studies is the tetrazolium salts reduction method which involves the reduction of the yellow tetrazolium MTT by dehydrogenase enzymes to NADPH and NADH. The formazan formed from the dehydrogenase activity is then solubilized and spectrophotometrically quantified. This method of cell proliferation assay measures the rate of cell proliferation and the metabolic events that can result in cell death (apoptosis) or necrosis. To hasten sample handling, the number of assay steps during MTT-based cell proliferation assay has been minimized as much as possible. During MTT-based assays, the MTT reagent contributes less to the absorbance values, and for each cell type, there is a linear relationship between the number of viable cells and the color produced. This allows an accurate determination of the rate of changes in cell proliferation.

Table 1: CHN elemental analysis, physical properties, and Λm of the prepared complex.

Complexes	Co-L	Ni-L	Cu-L
MW	582.4	582.1	622
Yield %	85%	80%	90%
MP (°C)	597	631	1,010
C% (Calcd)	(45.32)	(45.35)	(42.35)
Found	45.52	45.49	43.90
H% (Calcd)	(2.40)	(2.41)	(2.35)
Found	2.31	2.90	2.62
N% (Calcd)	(9.61)	(9.62)	(9.00)
Found	8.28	7.88	8.76
M% (Calcd)	(10.11)	(10.06)	(10.81)
Found	9.50	10.99	1,074
Λm	5.6	9.3	4.5

The cytotoxicity activity of the compounds produced in this study was evaluated using MTT assay against breast cancer (MCF-7) and lung cancer (A549) cells. The cells were plated at a cell density of 2×10^5/mL in a 96-well culture plate before introducing the complexes at different concentrations of 0.468 μM/L, 0.936 μM/L, 1.875 μM/L, 3.75 μM/L, 7.5 μM/L, 15 μM/L, and 30 μM/L. The cells were allowed to incubate for 72 h at 37 °C + 5%CO_2; the control cells were left untreated. After 72 h, the cells were washed twice with PBS before adding 10 μL of 0.5 mg/mL MTT solution into the wells. The cells were further incubated for another 4 h at 37 °C + 5%CO_2 before dissolving the formed formazan crystals in 100 μL of dimethyl sulfoxide (DMSO). The absorbance of the formed purple color was determined at 570 nm in an ELISA reader.

3. Results and discussion

The synthesized complexes were characterized and found to be stable in air, soluble in $CDCl_3$ and DMSO, but insoluble in ethanol. The CHN elemental analysis shown in Table 1, the FTIR data shown in Table 2, the ^{1}H-NMR data shown in Table 3, and the mass spectral data showed a successful synthesis of the investigated complexes. From the molar

Table 2: Some of the significant IR bands exhibited by the complexes.

Complex	Band assignment					
	νOH	νC=O	νC=C	νN=N	νC−O	γOH
Co-L	3,600	1,770	1,592	1,380	1,199	850
Ni-L	3,600	1,600	1,590	1,380	1,199	800
Cu-L	3,598	1,770	1,590	1,400	1,199	850

Table 3: ^{1}H-NMR spectral data of the investigated Ni complex.

Complex	Chemical shift (δ) ppm	Assignment
Ni-L	7.73	Aromatic C−H protons
	6.60	Pyrone ring C−H
	2.49	CH_3 pyrone ring

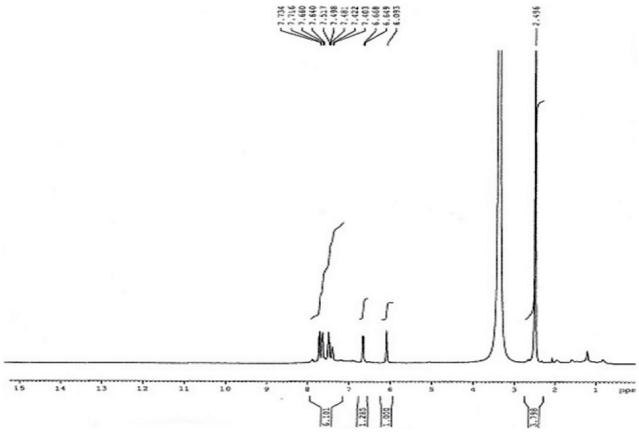

Figure 3: ^{1}H-NMR spectrum of complex (Ni-L).

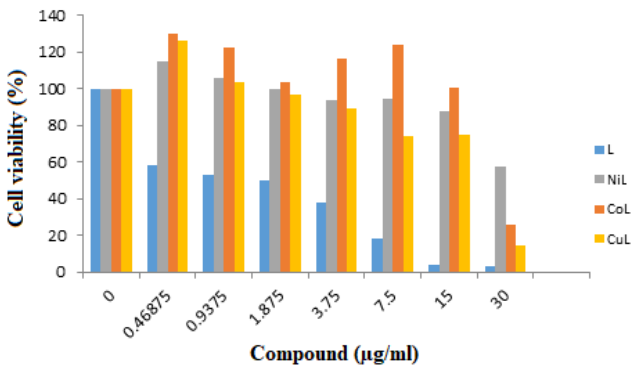

Figure 4: Effect of ligand L and complexes on cell viability of breast cancer cells MCF-7.

conductance studies, all the complexes were observed to be neutral and nonconductive. The FTIR spectra showed a shift in the observed band for the vibration of the N=N bond in the complexes compared to the free ligands to a lower wavelength, indicating the site of the chelation process. The upward shift in the OH band (peaked at 3,339 cm^{-1} and 3,600 cm^{-1} in the free ligands and complexes, resp.) indicates coordination via the hydroxyl group. The observed band at the wavelength range of 3,600 cm^{-1} indicates the vibration of the OH group of the water of hydration and water of coordination. The bands assigned to the carboxyl group are the most important bands in the free ligands group; these bands shifted to a lower wave number (in the range 1,199 cm^{-1}) due to complexing with C−O. In the ^{1}H-NMR report, the obtained data showed the absence of

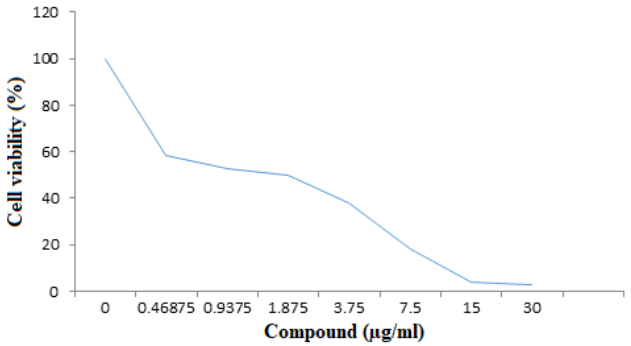

Figure 5: Effect of ligand L and complexes on cell viability of lung cancer cells A549.

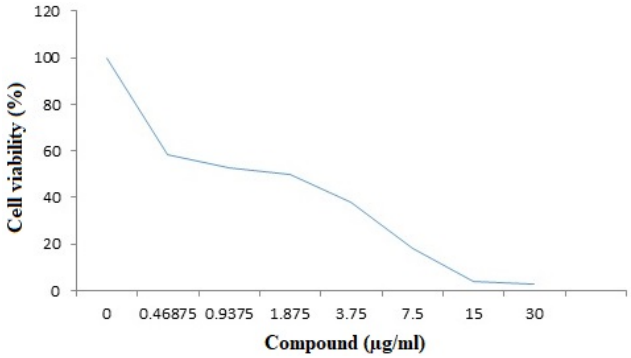

Figure 6: Effect of ligand L on cell viability of breast cancer cells MCF-7.

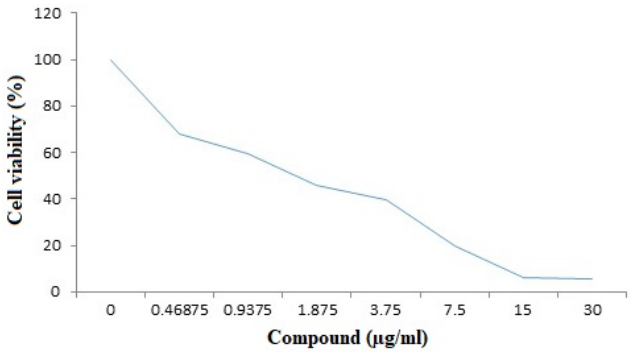

Figure 7: Effect of Cu-L complex on cell viability of lung cancer cells A549.

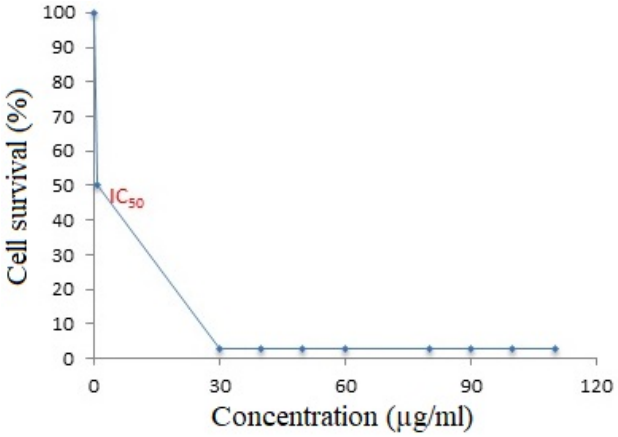

Figure 8: IC_{50} value of L on MCF-7 cells.

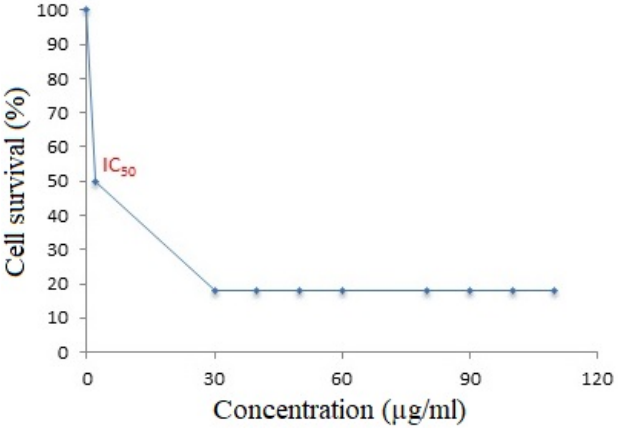

Figure 9: IC_{50} value of CuL on A549 cells.

OH (which appeared at 12.54 ppm in the free ligands) in the Ni complex, thereby confirming the deprotonation of OH. It also confirmed that the ligand coordinated to the metals via the hydroxyl group.

In the spectra of the complexes, the pyrone ring exhibited the signals observed at 6.60 ppm while those observed at 7.73–7.36 ppm were assigned to the protons of the aromatic ring as shown in Figure 3.

3.1. Cytotoxic activity of ligand and complexes

At 30 μg/mL, the ligand exhibited cytotoxic activity than the complexes (Cu, Co, and Ni) with values of 2.81% and 65.44% for MCF-7 and A549, respectively. Some coumarins which contain halogen groups are highly cytotoxic [12]. Thus, the presence of p-chloro substituents plays important roles in the antibreast cancer activity. Against the lung cancer cells A549, ligand L showed weak cytotoxicity; therefore, p-chloro substituent is selectively active against MCF-7. Moreover, the cell viability of the three complexes of Cu, Co, and Ni against MCF-7 was 14.57%, 26.09%, and 57.54%, respectively. According to the obtained results, the

Table 4: IC_{50} values of ligand and complexes in MCF-7 and A549 cell lines.

Compounds	MCF-7	A549
L	1 μg/mL	$>$ 30 μg/mL
CuL	30 μg/mL	1.875 μg/mL
NiL	$>$ 30 μg/mL	$>$ 30 μg/mL
CoL	30 μg/mL	$>$ 30 μg/mL

Cu complex was the most cytotoxic agent against MCF-7 cells compared to the tested Co and Ni complexes.

Cu complexes exhibited a high cytotoxicity against MCF-7 and A549 while the other complexes showed moderate to weak activities against both cell lines. Furthermore, the IC_{50} results signified that the ligand is the most active against MCF-7 (at the concentration of 1 μg/mL) while Cu complex is the most active against A549 (at the concentration of 1.875 μg/mL).

4. Conclusions

The synthesized coumarin-metal complexes in this study showed the formation of their structure through the binding of the nitrogen atoms of the bis azo group in the ligands with the OH group of the aromatic ring in the coumarins. The synthesized complexes were characterized by ^{1}H-NMR. The complex formation resulted in the disappearance of the OH group in the free ligands. The antiproliferative activity of the synthesized coumarin-metal ion complexes was determined and the results showed a significant activity of the complexes against the studied cell lines.

Conflict of interest The authors declare that they have no conflict of interest.

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