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Synthesis and Cytotoxic Activity of 6-Hydroxy-4-Methyl-5,7-(Bis-Phenylazo) Coumarin with Divalent Transition Metal Ions

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Abstract

Background/Objectives: One of the severe disease intimidating human health and continues to be a main health problem worldwide is cancer. Hence, discovering new compounds with powerful anticancer activity is of extreme important. The main objective of this paper is the synthesize 6-hydroxy-4-methyl-5,7-(bis-phenylazo) coumarin and its complexes and evaluate the cytotoxic activity of the ligand and complexes against breast cancer cells. **Methods/Statistical Analysis**: A novel compounds of 6-hydroxy-4-methyl-5,7-(bis-phenylazo) coumarin have been synthesized by 6-hydroxy-4-methylcoumarin. The complexation of nickel (II), copper (II) and cobalt (II) using this ligand gave salt type complexes with the formula of $M(C_{22}H_{15}O_3N_4)$ the compounds were characterized by microelemental analysis, infra-red, nuclear magnetic resonance spectroscopic techniques and molar conductivity. Cytotoxic activity for the ligand and the complexes were evaluated against breast cancer cells by using MTT assay and the absorbance at 570 nm was measured by ELISA reader. **Findings**: The CHN analysis of the compounds are in good agreement with the calculated values and the spectroscopic analysis of the complexes indicated that the ligand coordinated to the metal centres as polydentate ligand in bis-azocoumarin and form a distorted octahedral arrangement around nickel (II), copper (II) and cobalt (II) centres. The molar conductivity of the divalent metal ions complexes was small which directly supports the fact that all of the investigated complexes are non ionic. The overall results of cells MCF7 breast cancer revealed of cell proliferation was much more highly inhibited by the ligand and complexes Cu, Co and Ni with cell viability 5.21%, 17.36%, 46.20% and 74.43%, respectively at a concentration of 30 mg/ml compared to untreated control cells and IC50 values of the ligands and complexes of Cu, Co and Ni were 1.87, 1.87, 30 and >30 g/ml, respectively. **Improvements:** Lastly, some suggestions were presented to use these compounds in vivo should be assessed to obtain worthy anticancer drugs.

Keywords: Bis-Azocoumarin, Breast Cancer MCF7, Complexes of Nickel, Copper and Cobalt, MTT Assay

1. Introduction

Coumarin is categorised as a member of the family of compounds benzopyrene, all of which consist of a benzene ring attached to a ring pyrone[1]. Coumarins include a very large class of compounds found in the kingdom[2]. Coumarin (2H-1-benzopyran-2-one), the least complex compound in great class phenolic substances are the parent molecule of coumarin derivatives. Substances are found naturally and their components include fused benzene and -pyrone rings[3]. In simple coumarins, the pharmacological and biochemical properties as well as therapeutic uses are based on the pattern of substitution[4]. In the known family of coumarin derivatives, dimeric coumarins (also called bis coumarins) occupy an interesting position. Often they are biologically active and many of them have been tested in view of therapeutic use as an antimicrobial, cardiovascular, blood thinners and antiproliferative agents[5]. Synthesized, characterized and determined cytostatic and cytotoxic nature of 8-nitro-7-hydroxycoumarin using both human (including K-562 and HL-60) and animal cell lines are grown in

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vitro[6]. The transition metals complexes of hydroxyl coumarin derivatives are a subject of increasing interest in bioinorganic and coordination chemistry. However, little is known about the complexing ability of transition metals with bis-coumarin.

A study of the literature reveals that no work has been done on the reactions of transition metal with 6-hydroxy-4-methyl-5,7-(bis-phenylazo) coumarin. Therefore, it is considered worthwhile to study complex formation and the aim of this study was to determine whether new compounds are active as cytotoxic agents. We have observed that most transition metals have activity data and literature demonstrate that acytotoxic coumarins also have these properties. Therefore, our synthesis of ligand and transition metal complexes are considered with cytotoxic detection.

2. Method

2.1 Synthesis of 6-Hydroxy-4-Methyl Coumarin

6-hydroxy-4-methyl coumarin was synthesized by adding equal molar quantities of hydroquinone and ethyl acetoacetate in the presence of concentrated H_2SO_4 43 ml. In 100 ml beaker, 43 ml of concentrated H_2SO_4 was taken and the beaker was then placed in an ice bath. 20 g of hydroquinone in 25 ml of ethyl acetoacetate and H_2SO_4 was introduced drop by drop while stirring continuously upon decrease of temperature below 10°C. While addition was taking place, an ice salt bath was used to keep the temperature lower than 10°C (5 hours). At room temperature, this reaction mixture is stored for approximately 18 hours. After this, it is added into a crushed ice and water mixture with forceful stirring. Suction filtering was used to retrieve the filtrate and it was then cleaned with water. After this, 300 ml of 5% NaOH solution was used to dissolve the solid. In the solution, filtered and dilute (1:10) H_2SO_4 (110 ml) was also introduced while stirring forcefully until the nature of the solution became acidic upon litmus test. Pumps were used to retrieve the crude of 6-hydroxy-4-methyl coumarin which was cleaned with cold water and recrystallized using 25% ethanol.

2.2 Synthesis of 6-Hydroxy-4-Methyl-5,7-(Bisphenylazo) Coumarin

In an ice salt bath, fusion of 6-hydroxy-4-methyl-5,7-(bisphenylazo) coumarin a well-stirred solution of aniline (0.02 mole in 40 ml ethanol) and 20 ml of 2 M HCl was cooled down and was then diazotized with aqueous sodium nitrite solution (20 ml, 0.01 mole). After the solution of diazonium was cooled down (0-5°C), it was gradually incorporated into 0.01 mole solution of 6-hydroxy-4-methylcoumarin in 100 ml ethanol which had sodium hydroxide. Afterwards, the substance was filtered and recrystallized using pure ethanol[7]. Figure 1 describes the structural formulae of the bis-azo coumarin formed.

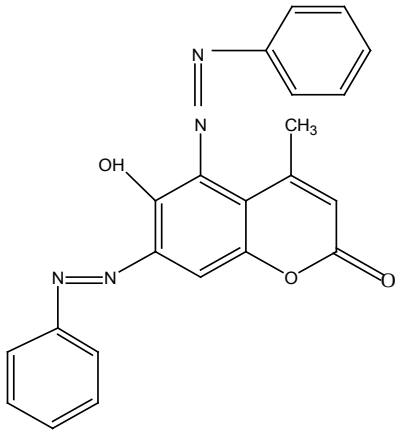

Figure 1. Structural formula of 6-hydroxy-4-methyl-5, 7-(bisphenylazo) coumarin.

2.3 Synthesis of Solid Complexes

A mixture of the a hot alcoholic saturated solution of 0.001 mole of metal ion which had been dissolved into hot ethanol along with the needed amount of the ligand being investigated which is enough for the formation of 1:1 (M:L) complexes (M = Co, Ni and Cu). The, the solution's pH was regulated at 6-7 through incorporation of dilute (1:10) ammonia solution[8]. The mixture of the reaction was then heated while stirring now and then over a steam bath for a duration of 4 hours. Afterwards, it was evaporated till it was dry. For the elimination of unreacted species, the product complexes were mixed in ethanol. After this, it was filtered using suction and washed again by ethanol. This was done until the product was a colourless filtrate, which was then suction, filtered and then stored in a vacuum desiccator. The synthesized of complexes have the following structural formula Figure 2.

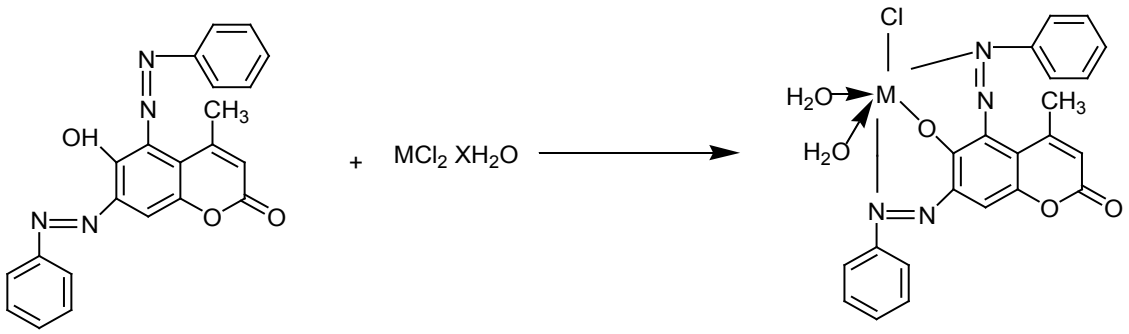

Figure 2. The proposed structure of the metal complexes (M = Co, Ni and Cu).

2.4 Cytotoxic Activities of Ligand and Complexes by using (MTT) Assay

Study of the cytotoxic activity was by using MTT assay, which is used to evaluate these compounds on viability cancer breast MCF7 Cells. Absorption of the samples was measured by an ELISA reader at 570 nm. Survival fraction was calculated as percentage of the untreated control.

3. Results and Discussion

3.1 Infrared Spectral

Data and band assignments of the investigated ligand are shown in Table 1 which describes the pattern of band allocation in the ligand under study. Through IR spectra and the frequency bands data, we observed that a broad band is present at 3399 cm^{-1}, resembling the stretching vibratory motion of the OH group of the ligand being studied. –C=C bands were observed to be present at 1500 cm^{-1} through analysis of the IR spectra of the ligand being studied. The presence of bands in the area of 3000 cm^{-1} is a result of the stretching vibration of Ar-H. Whereas the ones present in the area of 1198 cm^{-1} are a result of the stretching vibration of aliphatic C-H. At 896 cm^{-1}, we find the -CH of the aromatic rings. The location and substituent type are determinants for the amount and shape of the bands. The IR spectra of this ligand show presence of the bands at 1250 cm^{-1}. This has been allocated to the C-N stretching vibration whereas the stretching vibration of N = N is the determined one for the bands present at 1448 cm^{-1}[9]. While at 1665 cm^{-1}, the bands are allotted to the C = O group stretching vibration. In the solid complexes, the infrared spectral illustrates remarkable variations. These provide a rational conception regarding their structure. In Table 2, it was observed through the infrared spectra of the complexes, that at 1448 cm^{-1}, the bands present have been allocated to the N = N, which is located in the free ligand. This is transported to the number of lower wave in complex formation in the range of 1380-1384 cm^{-1}. This shows that this region is an area of complexation. The bands present in complexes within the range of 3585-3598 cm^{-1} are allocated to the OH coordination and hydration water. In the range 1670-1683 cm^{-1}, the C = O is the assigned group of the bands seen there. While C = C is the assigned group of bands in the 1592-1655 cm^{-1} and C–O is the group for the bands present in the 1199 cm^{-1} range. As a result of complexation, these are transferred to the lower wave number. In the 800 cm^{-1} range, bands are observed due to the spectra of metal complexes and can be allocated to OH. Another way to describe this is that the presence of these bands is a probable result of the coordination and covalent bonds presence among donor atoms (N and O) and the central metal ion.

Table 1. IR Frequencies and band assignments of the investigated ligand

Band Assignment	L (cm^{-1})
$\acute{\imath}_{OH}$	3399
$\acute{\imath}_{C\text{-}H\ aromatic}$	3000
$\acute{\imath}_{C=O}$	1600
$\acute{\imath}_{C=C}$	1500
$\acute{\imath}_{N=N}$	1448
$\acute{\imath}_{C\text{-}N}$	1250
$\acute{\imath}_{C\text{-}H}$	1198
$\tilde{a}_{C\text{-}H}$	896

Table 2. Frequencies and band assignment of metal complexes (cm^{-1})

Complex	$\acute{\imath}_{OH}$	$\acute{\imath}_{C=O}$	$\acute{\imath}_{C=C}$	$\acute{\imath}_{N=N}$	$\acute{\imath}_{C\text{-}O}$	ãoH
Co-L (1:1)	3598	1650	1600	1382	1199	800
Ni-L(1:1)	3598	1655	1592	1384	1199	800
Cu-L (1:1)	3597	1650	1592	1380	1199	800

3.2 ^{1}H NMR Spectra

In DMSO, ^{1}H NMR spectra of the observed ligand (L) and Ni (II) complex was considered as solvent. Table 3 summarizes the values of chemical shift in various proton kinds in the ligand (L) and Ni complex observed. In DMSO, the ^{1}H NMR spectra of the ligand (L) observed also executes a sharp signal at 12.44 ppm, which is allocated to the OH group protons. At 2.1 ppm, the aliphatic protons of the methyl groups in the pyrone ring. At 7.73-7.39 ppm, the signals observed were allotted to the aromatic ring protons whereas at 7.73-7.39 ppm, the signals found are allocated to the pyrone ring protons[10]. The signals at the Ni-L complex were seen to vanish at 12.44 ppm in the free ligand. This describes the role in complexation deprotonation and involvement of the OH group. In the free ligand, every other observed signal describes up field shift as a result of complexation Figures 3 and 4.

3.3 The Molar Conductivities

For complexes present in chloroform, we calculated the molar conductivities for the solid complexes. These were found to be present in the complexes Co, Ni and Cu 12.3, 12.3 and 14.1 ohm^{-1} cm^2 mol^{-1} respectively. Regarding the ionic complexes of the divalent metal ions, the measured values were found to be small. The presence of chloride ions in the coordination sphere can be a contributing factor in the decreased values of conductivity as compared to the relation of ions to the metal ions in the formation of complexes. Additionally, on adding $AgNO_3$, no white precipitate is produced. Through this, we can prove that the nature of the studied complexes is that of non-ionic or non-electrolytes[11].

Table 3. ^{1}H NMR The spectral data found in studied ligand as well as Ni complex

Ligands and complexes	Chemical Shift, (ppm)	Assignment
L	12.44	OH proton
	7.58	Aromatic C-H protons
	7.14	Pyrone ring C-H
	2.55	CH_3 pyrone ring
Ni-L	7.73	Aromatic C-H protons
	6.60	Pyrone ring C-H
	2.49	CH_3 pyrone ring
	3.50	H_2O of coordination

3.4 Cytotoxic Activity of Ligand and Complexes

Cytotoxic activity of ligand and complexes, upon studying

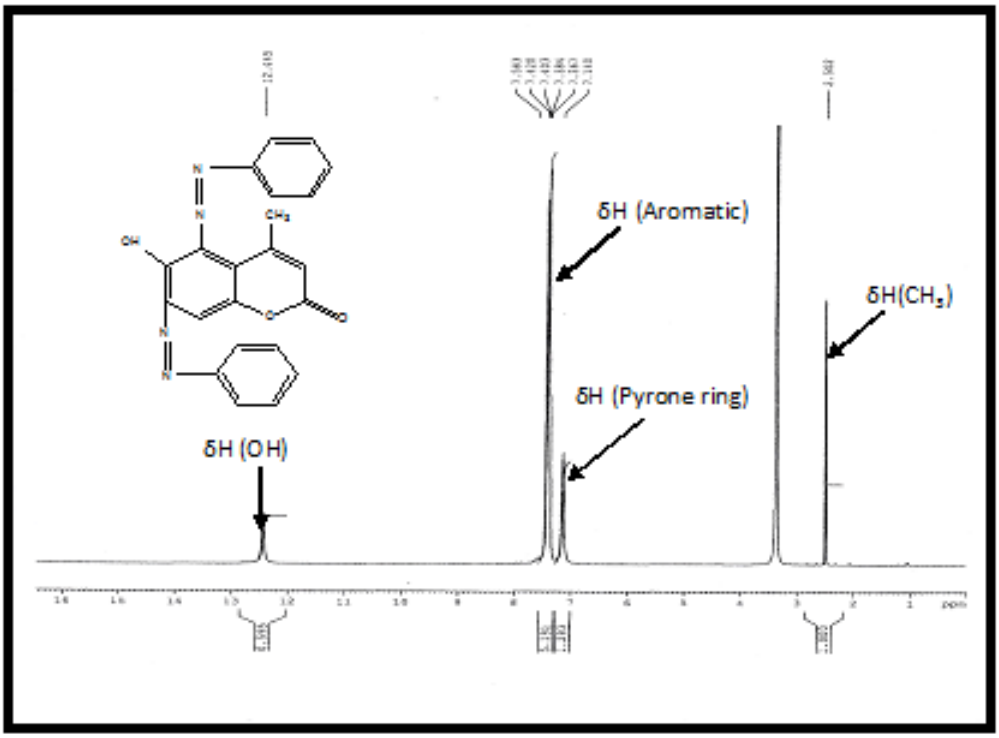

Figure 3. Proton NMR spectrum of ligand.

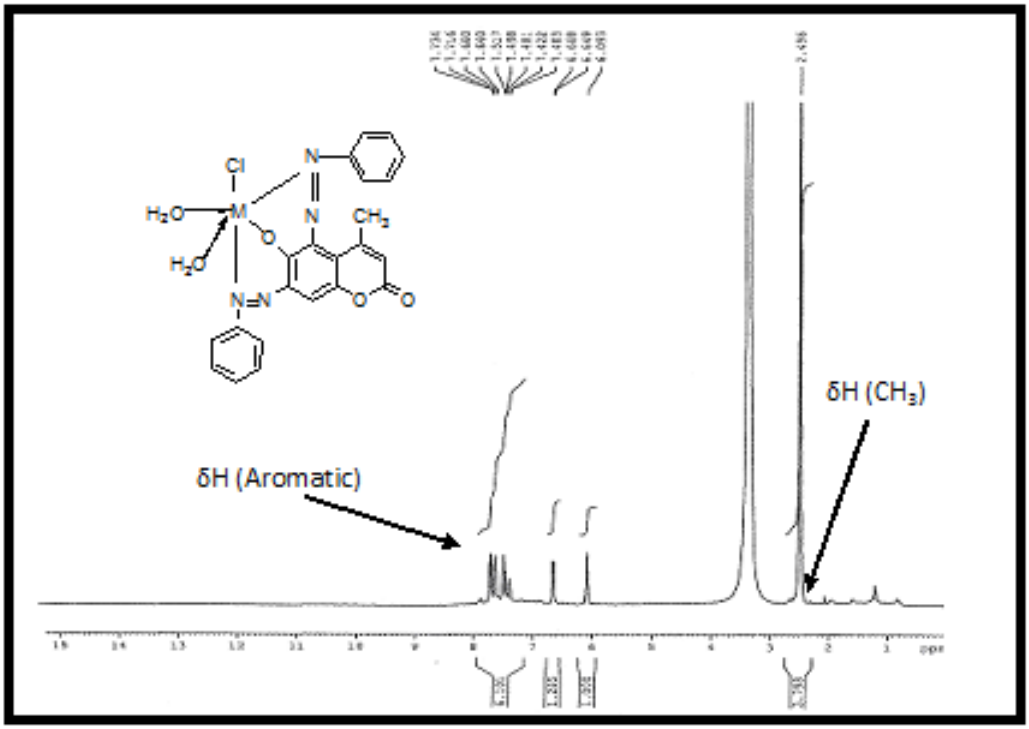

Figure 4. Proton NMR spectrum of complex.

the complete results, the ligand was observed to hinder the cell proliferation as compared to Cu, Co and Ni complexes having values of 5.21, 17.36, 46.20 and 74.43 % respectively in a 30 µg/ml concentration in contrast to the control cells which have not been treated cells (Table 4 and Figure 5).

Table 4. Effect of ligand and complexes on cell viability of cancer cells

Concentration (µg/ml)	Cell viability (%)			
	Ligand	Cu- L	Ni-L	Co-L
0.0	100± 0.81	100± 0.81	100± 0.81	100± 0.81
0.468	56.53± 0.46	72.32± 0.57	102.76± 0.88	105.97± 0.80
0.937	52.74± 0.43	58.21± 0.45	85.33± 0.73	74.79± 0.56
1.875	55.84± 0.45	45.96± 0.36	90± 0.77	85.55± 0.65
3.75	52.70± 0.43	38.61± 0.30	92.72± 0.79	61.08± 0.46
7.5	53.68± 0.44	26.65± 0.21	98.48± 0.84	72.63± 0.55
15	42.52± 0.34	18.68± 0.131	91.05± 0.78	61.39± 0.47
30	5.21± 0.04	17.36± 0.137	74.43± 0.63	46.20± 0.35

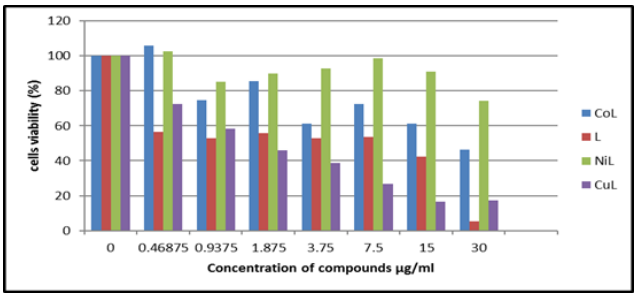

Figure 5. Effect of ligand and complexes on cell viability of cancer cells.

Table 5. IC_{50} values of ligand and complexes in MCF7 cell lines

Compounds	MCF7
L	1.87 µg/ml
Cu- L	1.87 µg/ml
Ni-L	>30 µg/ml
Co-L	30 µg/ml

4. Conclusions

The synthesized 6-hydroxy-4-methyl-5, 7-(bis-phenyl azo) coumarin complexes show that the structure of the complexes are formed through the OH group in the aromatic ring and coordination of the nitrogen atoms of

the bis azo group in ligand. The ligand and complexes with Co (II), Cu (II) and Ni (II) have biological activity against the breast cancer cells MCF7 at low concentrations.

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