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Evaluation of Antitumor Activity of Copper Complexes of Coumarin in Breast and Lung Cancer

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Abstract: Considerable interest has been aroused for copper complexes of coumarins due to their wide variety of biological activity. Three copper complexes of coumarin; A1, A2 and A3 were synthesized and evaluated for antitumor activity by using MTT assay. These complexes show antitumor activity against breast cancer MCF7 with IC_{50} values of 1.87, 1.87 and 30µg/ml respectively and exhibit antitumor activity against lung cancer A549 with IC_{50} values of 7.5, 15 and 1.87µg/ml respectively.

Key words: Coumarin · Copper complexes · Antitumor

INTRODUCTION

Cancer has long been one of the serious disease threatening human health and continues to be a major health problem worldwide. Therefore, discovering new compounds with potent anticancer activity is of utmost important. Coumarins are very effective and efficient biological elements which have spasmolytic, antioxidant, anticoagulant, antibacterial, antiviral and antifungal characteristics. Extensive information regarding the antineoplastic function of coumarin derivatives is also available. They also play a role at various levels of cancer development since some of them have cytostatic characteristics, while others have cytotoxic effects on the organism [1]

Coumarin is very commonly administered as a remedial agent and has been adopted in medicine, in order to cure various lymphede-mas and malignancies. 7-hydroxy- and 4-hydroxycoumarins are elements that materialize naturally with various biological processes, for instance, the anti tumoral process. The antitumor processes of coumarin and its acknowledged metabolite 7-hydroxy-coumarin have been examined in various human tumor cell lines, according to a study by Weber *et al*. [2].

Metalo proteins, which are the hub of enzymatic process, display an important role in biological arrangements. They discover the structure of active locations and play their part as biological redox assisters. Various cases proved that transition metal complexes of coumarin exhibit increased in biological activities as compared to their ligands, as described by Kostova & Momekov [3]. As a result of their half-filled orbitals, transition metals display different oxidation states and a diversity of coordination structures and ligand spheres. A huge variety of coordination compounds and the workings of cytotoxic actions have been carried out in reference to the advancement of the advanced antitumor agents.

Recently, various researches have shown the binding of complexes coumarin derivatives with metals which have the characteristics of biological activity. According to Kostova & Stefanova [4], the research on binding characteristics of coumarin derivatives to various metal ions is an extremely essential area of study in order to completely appreciate the determinants influencing their biological activities. It has also been discovered that the attachments of a metal to a coumarin derivative not only sustains its biological activity but, in fact, boosts it.

In the present work, we tried to synthesize copper complexes from 6-hydroxy-4methyl Coumarin derivative and all complexes obtained were evaluated for antitumor activity against cancer cells MCF7 and A549 cell lines.

MATERIALS AND METHODS

Synthesis of 6-hydroxy-4-methyl Coumarin: 6-Hydroxy-4-methyl coumarin was synthesized by adding equal molar

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quantities of hydroquinone and ethyl acetoacetate in the presence of concentrated H_2SO_4 [5]. 43 mL concentrated H_2SO_4 was placed in 100 mL beaker surrounded by salt ice bath. When the temperature falls below 10°C, 20 g of hydroquinone in 25 mL of ethyl acetoacetate was dropped wisely with constant stirring The temperature was kept below 10°C by means of an ice salt bath during the addition (5 hours). The reaction mixture was kept at room temperature for about 18 hours, then poured with vigorous stirring into a mixture of crushed ice and water. The precipitate was collected by suction filtration and washed with water. The solid was dissolved in 300 mL of 5% NaOH solution, filtered and dilute (1:10). An amount of 110 ml of H_2SO_4 was added with vigorous stirring until the solution was acidic to litmus. The crude of 6-hydroxy-4-methylcoumarin was collected by filtration at the pump, washed with cold water, dried at 100°C and recrystallized from 25% ethanol.

Synthesis of 6-hydroxy-4-methyl-5-(Phenylazo) Coumarin: A well-stirred solution of aniline 0.01mole in 40 ml ethanol and 20 ml of 2M HCl was cooled in an ice salt bath and diazotized with aqueous sodium nitrite solution (20 ml, 0.01mol). The cooled (0-5°C) diazonium solution was added slowly to a well-stirred solution of (0.01mole) 6-hydroxy-4-methylcoumarin in (100 ml) ethanol containing sodium hydroxide (10 g). The reaction mixture was stirred for one hour at room temperature and then acidified with diluted HCl (100 ml, 2.5M) to neutralize the reaction mixture and precipitate the azo coumarin derivative. The product was recrystallized from ethanol to give the azo coumarin derivative 6-hydroxy -4- methyl- 5-(phenyl azo) coumarin. Colour: pale brown; (yield 60.00%), (m.p. 215-217).

Synthesis of 6-hydroxy-4-methyl-5,7-(Bis Phenylazo) Coumarin: A well-stirred solution of 0.02 mole of aniline in 40 ml ethanol and 20 ml of 2 M HCl was cooled in an ice salt bath and diazotized with aqueous sodium nitrite solution (20 ml, 0.01 mol). The cooled (0-5°C) diazonium solution was added slowly to a well-stirred solution of 0.01mole of 6-hydroxy-4-methylcoumarin in 100 ml of ethanol containing 10 g of sodium hydroxide. The reaction mixture was stirred for one hour at room temperature and then acidified with diluted HCl (100 ml, 2.5M) to neutralize the reaction mixture and precipitate the azocoumarin derivative. The product was recrystallized from ethanol to give the azocoumarin derivative.

Synthesis of 6-hydroxy-4-methyl-5,7-(Bis-p-chlorophenylazo) Coumarin: A well-stirred solution of p-chloro aniline (0.02 mole in 40 ml ethanol) and 20 ml of 2M Hcl was cooled in an ice salt bath and diazotized with aqueous sodium nitrite solution (20 ml, 0.02 mol). The cooled (0-5°C) diazonium solution was added slowly to a well-stirred solution of 0.01 mole of 6-hydroxy-4-methylcoumarin in 100 ml of ethanol containing sodium hydroxide (10 g). The reaction mixture was stirred for an hour at room temperature and then acidified with diluted HCl (100 ml, 2.5 M) to neutralize the reaction mixture and precipitate the azocoumarin derivative. The product was recrystallized from ethanol to give 6-hydroxy-4-methyl-5,7-(bis-p-chlorophenylazo)coumarin.

Synthesis of 6-hydroxy-4-methyl-5-(Phenylazo) Coumarin Copper Complex (A1): The solid chelates were synthesized by mixing a hot alcoholic saturated solution of 0.001 mole of copper ion dissolved in hot ethanol with the required amount of 6-hydroxy-4-methyl-5-(phenylazo) Coumarin under investigation sufficient to form 1:1 (M:L) complex. The pH of the solution was then maintained at a value of 6.5-7.5 by addition of dilute ammonia solution (1:10) [6]. The reaction mixture was heated in a steam bath with occasional stirring for 4 hrs and evaporated till dryness. The produced complex was then dissolved in ethanol to remove unreacted species. It was then filtered off by suction and rewashed with ethanol till a colourless filtrate was obtained, suction, filtered and then finally kept in a vacuum desiccator.

Synthesis of 6-hydroxy-4-methyl-5,7-(Bis-phenyl Azo) Coumarin Copper Complex (A2): The solid chelates were synthesized by mixing a hot alcoholic saturated solution of 0.001 mole of copper ion dissolved in hot ethanol with the required amount of 6-hydroxy-4-methyl-5,7-(bis-phenylazo) Coumarin under investigation sufficient to form 1:1 (M:L) complex. The pH of the solution was then maintained at a value of 6.5-7.5 by addition of dilute (1:10) ammonia solution [6]. The reaction mixture was heated on a steam bath with occasional stirring for 4 hrs and evaporated till dryness. The produced complex was then dissolved in ethanol to remove unreacted species. It was then filtered off by suction and rewashed with ethanol till a colorless filtrate was obtained, suction, filtered and then finally kept in a vacuum desiccator.

Synthesis of 6-hydroxy-4-methyl-5,7-(Bis-p-chlorophenylazo) Coumarin Copper Complex (A3): The solid chelates were synthesized by mixing a hot alcoholic saturated solution of 0.001 mole of copper ion dissolved in hot ethanol with the required amount of 6-hydroxy-4-methyl-5,7-(bis-p-chloro phenylazo) coumarin

under investigation sufficient to form 1:1 (M:L) complex. The pH of the solution was then maintained at a value of 6.5-7.5 by addition of dilute (1:10) ammonia solution [6]. The reaction mixture was heated in a steam bath with occasional stirring for 4 hrs and evaporated till dryness. The produced complex was then dissolved in ethanol to remove unreacted species. It was then filtered off by suction and rewashed with ethanol till a colourless filtrate was obtained, suction, filtered and then finally kept in a vacuum desiccator.

Bioassay Cytotoxicity assay against breast cancer cells and lung cancer cells were performed by MTT assay.

RESULTS AND DISCUSSION

Three newly synthesized copper complexes (A1, A2 and A3) were characterized using elemental analysis (C, H, N), spectroscopic techniqes (FTIR, UV visible, MS) and conductivity measurements. Analytical data and some physical properties of the compounds are listed in Table 1.1.

All the complexes are coloured solids, soluble in DMSO and chloroform. The elemental analysis results show good agreement between the experimental and theoretical values based on the suggested formula in each case. The sharp melting points of all complexes indicate that they are fairly pure compounds. The molar conductance shows that A1, A2 and A3 complexes are non-electrolytes in nature with the values of 13.3, 14.1 and 4.5 ohm^{-1} cm^2 $mole^{-1}$ respectively.

On complexation (Figure 1.1), there are clear differences between the infrared spectra of the free ligands and their complexes. In the infrared spectra of the complexes (Table 1.2), the band is observed within the range of 1410-1450 cm^{-1} assigned to $^{i}_{N=N}$ in the free ligands are shifted to the lower wave number on complex formation, indicating that it is centre of chelation. Coordination via the hydroxyl group is indicated by increased in the frequency of the hydroxyl band, $^{v}_{O-H}$. In the free ligands. it is found at 3339-3200 cm^{-1} and increased to 3697-3598 cm^{-1} in the complexes. Meanwhile, $^{v}_{C=}$o band is observed in the range of 1605-1590 cm^{-1} in free ligands and increased to 1650-1770 cm^{-1} due to complexation. The most important bands in the free ligands are those assigned to the carboxyl group observed at 1248 cm^{-1}. Due to the complexation, these bands are shifted to 1199 cm^{-1}.

The presence of water molecules within the coordination sphere of complexes are supported by the presence of a band at 814-850 cm^{-1} due to $\gamma(OH_2)$. The spectra of metal complexes must exhibit bands in the ranges of 514-580 cm^{-1} and 428-468 cm^{-1} which may assigned to (M◎N) and (M-O) stretching frequencies, respectively [7]. In other words, these bands are possibly due to the formation of coordination and covalent bonds between the donor atoms N and O and the central metal ion.

The electronic absorption spectra of the investigated coumarin derivatives without copper metal exhibit two bands at 270 nm and 330 nm. The first band may be assigned to the π-π* transition within the phenyl moiety and the second band may be ascribed to the n-π* transitions within the -N=N- followed by intramolecular charge (C.T.) or intra-ligand transitions within the ligands. However, in the presence of copper metal, the electronic absorption spectra of the complexes exhibit two absorption bands at 270 nm and 299 nm. This small change in the n-π* transition upon complexation could be attributed to the electron delocalization on the ligands upon coordination with metal ions. A shoulder at 364 nm may be attributed to charge transfer $^2A_{2g} \rightarrow {}^2T_{1g}$ transitions and an octahedral configuration is suggested around the central metal ion [8-10].

Evaluation of cytotoxicity activity against breast and cancer cells using MTT assay are listed in Table 1.3. Complex A1 shows antitumor activity against breast cancer cells MCF7 with an IC_{50} value of 1.87µg/ml and antitumor activity against lung cancer cells A549 with an IC_{50} value of 7.5µg/ml. Thus, it can be concluded that the structure of complex A1 is more selective towards antitumor activity against breast cancer cells MCF7 (Figures 1.2 & 1.5). On the other hand, complex A2 exhibits stronger antitumor activity with an IC_{50} value of 1.87µg/ml in breast cancer cells MCF7 than lung cancer cells A549 which shows moderate activity with an IC_{50} value of 15µg/ml (Figures 1.3 & 1.6). Meanwhile, complex A3 shows antitumor activity against lung cancer cells A549 with an IC_{50} value of 1.87µg/ml and weak activity against breast cancer cells MCF7, with an IC_{50} value of 30µg/ml (Figures 1.4 & 1.7). Hence, A3 complex is more selectively active on the lung cancer cells A549. It is noteworthy that, the complexes share similar structures of coumarin but have different substitutions on some positions. It is suggested that, the linear skeleton of coumarin and copper metals are favourable for antitumor activity [11]. On the other hand, the electron accepting and donating properties of the molecules are important factors for estimating the antitumor activity of complexes [12].

Table 1.1: Analytical and physical data of copper complexes

Compound Colour	Yield (%)	Mp (°C)	% C	% H	% N
A1 Brown	80	899	44.44 (45.30)	3.93 (3.14)	6.48 (7.90)
A2 Dark yellow	85	966	50.96 (50.50)	3.08 (3.16)	10.81 (9.20)
A3 Dark blue	90	1010	42.35 (43.90)	2.35 (2.62)	9.00 (8.76)

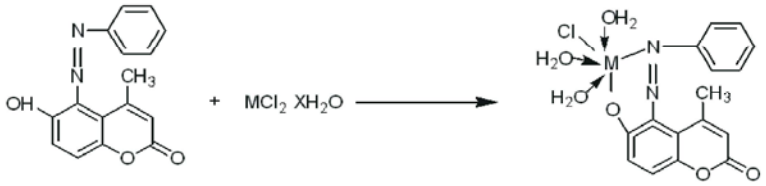
* The calculated values are in brackets

Table 1.2: Major FTIR absorption bands (cm^{-1}) of copper complexes

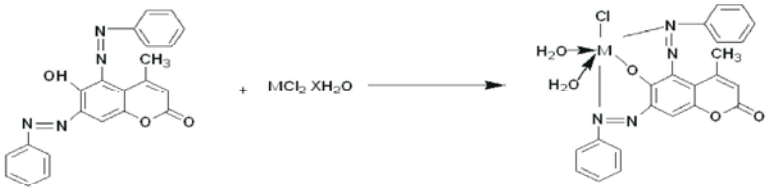
Compound	ν_{O-H}	$\nu_{N=N}$	$\nu_{c=o}$	$\gamma_{(O-H)2}$
A1	3598	1382	1679	814
A2	3697	1380	1650	800
A3	3598	1400	1770	850

Table 1.3: IC50 values of complexes in MCF7 and A549 cell lines

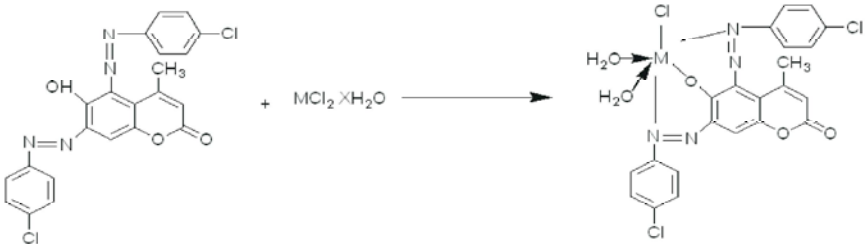
Compounds	MCF7 (µg/ml)	A549 (µg/ml)
A1	1.87	7.5
A2	1.87	15
A3	30	1.87



A1



A2



A3

Fig. 1.1: Schematic diagram illustrates the reactions occurred during the preparation of complexes (M=Cu)

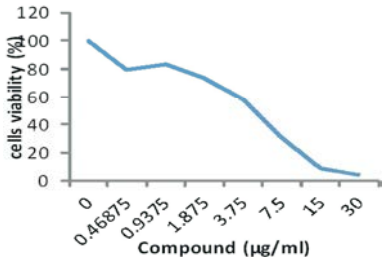


Fig. 1.2: Effect of A1 complex on cell viability of lung cancer cells A549

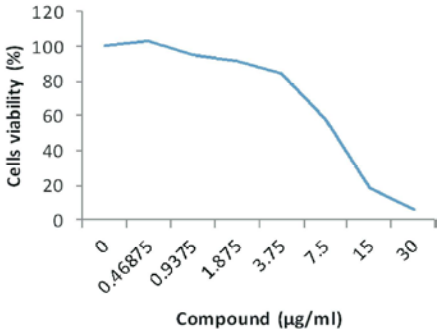


Fig. 1.3: Effect of A2 complex on cell viability of lung cancer cells A549

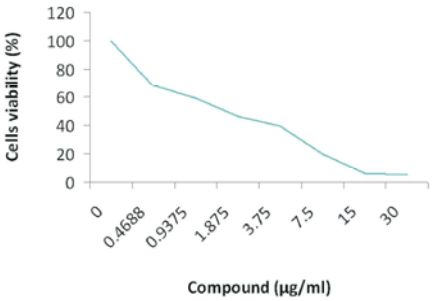


Fig. 1.4: Effect of A3 complex on cell viability of lung cancer cells A549

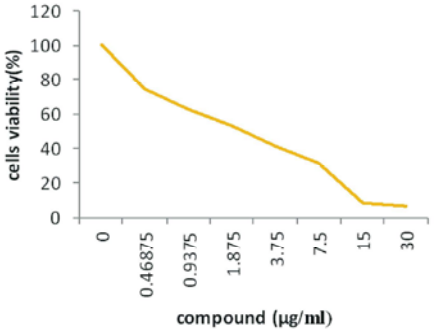


Fig. 1.5: Effect of complex A1 on cell viability of breast cancer cells MCF7

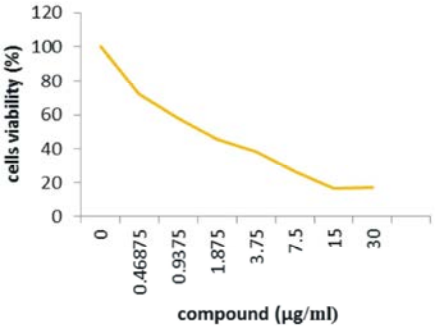


Fig. 1.6: No text of specified style in document.: Effect of A2 complex on cell viability of breast cancer cells MCF7

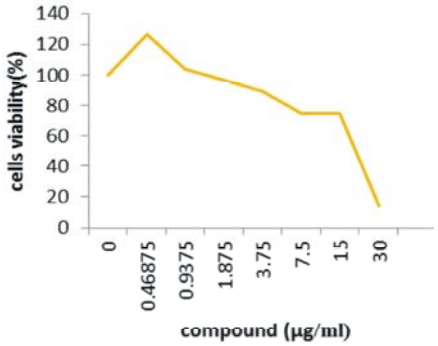


Fig. 1.7: Effect of A3 complex on cell viability of breast cancer cells MCF7

CONCLUSION

Three copper complexes from three different coumarin derivatives were successfully synthesized and evaluated for antitumor activity against cancer cells (MCF7 breast cancer cells and A549 lung cancer cells). It was found that complexes A1 and A2 exhibit strong antitumor activity against breast cancer cells MCF7, while complex A3 exhibits strong antitumor activity against lung cancer cells A549.

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REFERENCES

1. Stanchev, S., G. Momekov, F. Jensen and I. Manolov, 2008. Synthesis, computational study and cytotoxic activity of new 4-hydroxycoumarin derivatives. European Journal of Medicinal Chemistry, 43(4): 694-706.
2. Weber, U.S., B. Steffen and C.P. Siegers, 1998. Antitumor-activities of coumarin, 7-hydroxy-coumarin and its glucuronide in several human tumor cell lines. Research Communications in Molecular Pathology and Pharmacology, 99(2): 193-206.
3. Kostova, I.G. Momekov, 2006. New zirconium (IV) complexes of coumarins with Cytotoxic activity. Journal of Medicinal Chemistry, 40: 717-726.
4. Kostova, I. and T. Stefanova, 2010. Cytotoxicity of new Ho (III) and Pr (III) complexes. Journal of Rare Earths, 28: 40-46.

5. Sen, Kalyanamaysen and P. Bagchi, 1959. Studies on the ultraviolet absorption spectra of coumarons and chromones II Hydroxy derivatives. The Journal of Organic Chemistry, 24(3): 316-319.
6. Ibrahim, D.M. and S.A. Abdel-Latif, 2013. Complexation, Spectroscopic, Thermal, Magnetic And Conductimetric Studies On Some 8-(Arylazo) Coumarins With Divalent Transition Metal Ions IOSR Journal of Applied Chemistry, 5: 40-50.
7. Emara, A.A., 1999. Novel asymmetric tetradentate Schiff base ligands derived from 6-metwia-formyl4-hydroxy-2-(1H)-quinolone and their metal complexes. Synthesis and Reactivity in Inorganic and Metal-organic Chemistry, 29(1): 87-103.
8. Shin, Y.G.K., B.S. Brunschwig, C. Creutz and N. Sutin, 1996. Electroabsorption Spectroscopy of Charge-Transfer States of Transition-Metal Complexes. 2. Metal-to-Ligand and Ligand-to-Metal Charge-Transfer Excited States of Pentaammineruthenium Complexes. The Journal of Physical Chemistry, 100(20): 8157-8169.
9. Anacona, J.R., T. Martell and I. Sanchez, 2005. Metal complexes of a new ligand derived from 2, 3-quinoxalinedithiol and 2, 6-bis(bromomethyl) pyridine. Journal of the Chilean Chemical Society, 50(1): 375-378.
10. Al-Ramadane, O.M., O.A. Akrawi, A.A. Ibrahim and A.S. Al-Kazzaz, 2009. Synthesis Characterization and Theoretical Study of Some Transition Metal Complexes with Some Terpyridines. National Journal of Chemistry, 35: 512-520.
11. Promsuwan, P. and C. Yenjai, 2013. Synthesis and Cytotoxicity of Coumarin Derivatives and Nordentatin. Asian Journal of Chemistry, 25(7): 3629-3632.
12. Ishihara, M., Y. Yokote and H. Sakagami, 2006. Quantitativestructure-cytotoxicity relationship analysis of coumarin and its derivatives by semiempirical molecular orbital method. Anticancer Research, 26(4b): 2883-2886.