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Synthesis, Characterization and Anti-Tumor Activity of Cu (II) and Co (II) Complexes of 3- (3, 4-Dihydroxy Benzene Acrylic Acid)

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Abstract: 3- (3, 4-dihydroxy benzene) acrylic acid abbreviated as EDA was synthesized and characterized. Cu (II) and Co (II) metal complexes of this ligand prepared by reaction of chloride salt with EDA in dry acetonitrile. Characterization of the ligand and its complexes was made by microanalyses, FT-IR, ^{1}H-NMR, Raman and UV–Visible spectroscopy. These new complexes showed excellent antitumor activity against two kinds of cancer cells that are AGS (Stomach) and MCF-7 (Breast) cells.

Key words: 3- (3, 4-dihydroxy benzene) acrylic acid · Chemical structure · Anti-tumor · AGS and MCF-7 cells

INTRODUCTION

Acrylic acid or hydroxycinnamic acid compounds are widely distributed in the plants. They usually exist as esters of organic acid or glycosides or are bound to protein and other cell wall polymers. Only a small number of them exist as free acids in nature [1, 2].

Phenolic compounds are secondary plant metabolites and naturally present in almost all plant materials, including food products of plant origin. These compounds are thought to be an integral part of both human and animal diets [3]. The chemical structure of phenolic acids shows that they are simple phenols. Hydroxycinnamic acid is the major subgroup of phenolic compounds [4, 5]. Hydroxycinnamates are phenylpropanoid metabolites and occur widely in plants [1] and plant products [6]. Hydroxycinnamates and their derivatives are bioactive plant food ingredients. They exhibit in vitro antioxidant activity, which might have beneficial health impact in vivo [7]. The other natural ligand from plants such as alkaloids compound also can be used in synthesis of metal complex [8].

In this work, the present communication describes the synthesis of a Co(II)and Cu(II) transition metals complex bearing a natural ligand (caffeic acid) and its characterization by ^{1}H-NMR, IR, UV-Vis., Raman and elemental analysis, as well as the study of its antitumor activity for first row transition metal complexes have been studied.

MATERIALS AND METHODS

3- (3,4-dihydroxy benzene acrylic acid), cobalt chloride and copper chloride, were Merck chemicals and were used without further purification. Organic solvents were reagent grade. Electronic spectra were recorded by Camspec UV–Visible spectrophotometer model Perkin Elmer Lambda 25. The IR spectra were recorded using FT-IR Bruker Tensor 27 spectrometer. ^{1}H- NMR was recorded on a Bruker AVANCE DRX 500 spectrometer at 500 and 125MHz respectively. All the chemical shifts are quoted in ppm using the high-frequency positive convention; ^{1}H -NMR spectra were referenced to external $SiMe_4$. The percent composition of elements was obtained from the Microanalytical Laboratories, Department of Chemistry, University of tarbiyatmoallem, Tehran. The human Stomach: AGS cell line and the human Breast: MCF-7 cell line, used for treatment with the drugs, was provided. AGS and MCF-7 cells were grown at 37°C in an atmosphere containing 5% CO_2, with RPMI-1640MEDIUM HEPES Modification with L-glutamine and 25mM HEPES (SIGMA-ALDRICH CHEMIE GmbH) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Gibco), 2.7% sodium bicarbonate and 500 mg/L ampicillin.

Synthesis of the Metal Complexes; General Method: A solution of metal salt dissolved in acetonitrile added a dually to a stirred acetonitrile solution of the ligand (EDA), in the molar ratio 1:1 (metal: ligand). The reaction mixture was further stirred for 4-5h to ensure of the

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completing and precipitation of the formed complexes. Finally, the complexes dried in vacuum desiccators over anhydrous $CaCl_2$.

RESULTS AND DISCUSSION

The reaction of Co(II) and Cu(II) salts with the ligand, EDA, results in the formation of [ML] for M=Co (II) and Cu(II). All complexes are quite stable and could be stored without any appreciable change. The EDA ligand and the $[Co(C_9H_6O_4)]Cl_2.2H_2O$ complex have 223-225°C and 195-198°C melting point respectively, but the $[Cu(C_9H_6O_4)]Cl_2.2H_2O$ complex have 169-172°C melting point. $[Co\ (C_9H_6O_4)]Cl_2.2H_2O$ complex is insoluble in common organic solvents, such as n-hexane, dichloromethane, but $[Cu\ (C_9H_6O_4)]\ Cl_2.2H_2O$ complex is insoluble in common organic solvents, such as dichloromethane. However, they are soluble in DMSO, ethanol and DMF. Their structures were characterized by elemental analysis, ^{1}H-NMR and IR. Their elemental analyses are in accord with their proposed formula.

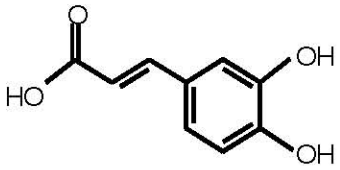
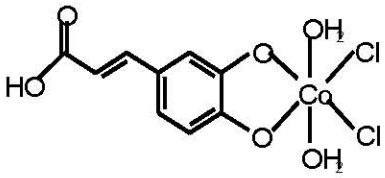


Fig. 1: Structure of the ligand, EDA.



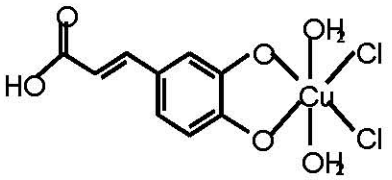
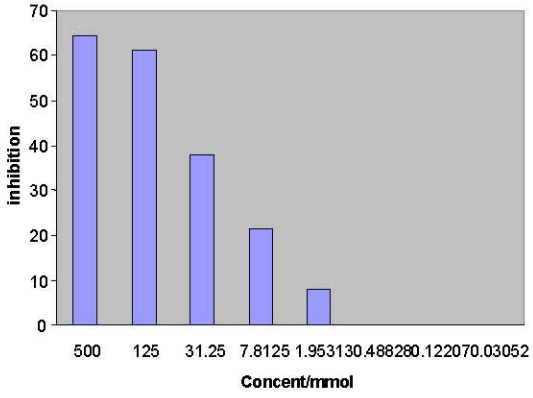
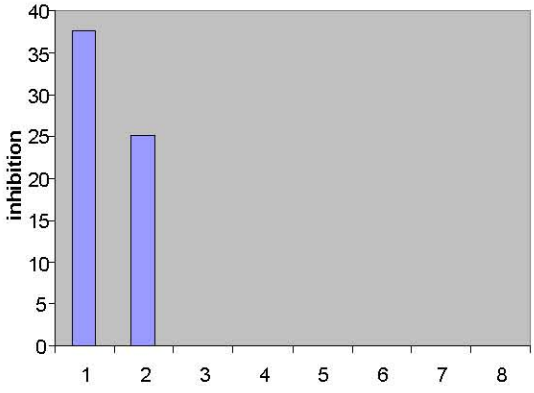


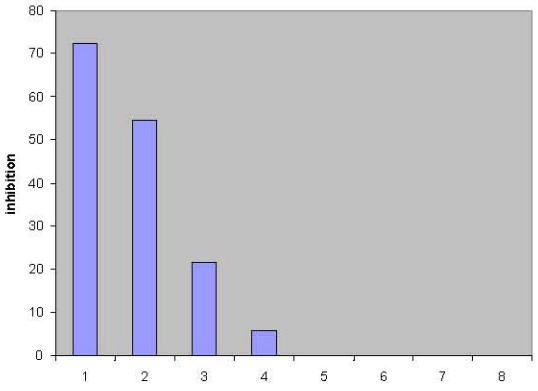
Fig. 2: Structure of Co (II) and Cu (II) complexes with ligand, EDA



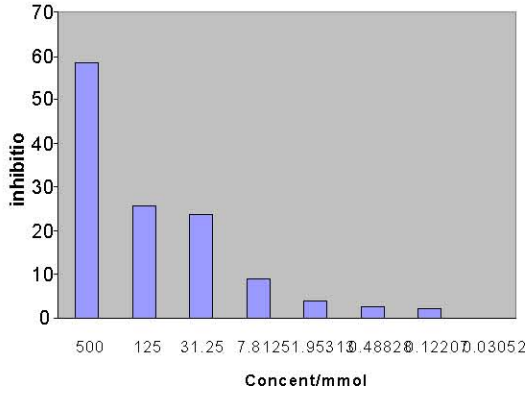
Caffeic acid +Cu (AGS)



Caffeic acid +Cu (MCF-7)



Caffeic acid +Co (AGS)



Caffeic acid +Co (MCF-7)

Fig. 3: Dose-dependent growth inhibition of AGS and MCF7 cell lines by total methanol extract after 48 h Viability was quantitated by MTT assay

Table 1: Effects of complexes on cell lines

Cell lines	EDACC Co (II)	EDACC Cu (II)	EDA
AGS	125-500	31/25-500	ND
MCF-7	500	500	ND

ND: IC_{50} and IC_{70} not determined. Growth inhibition not responsive at concentration tested

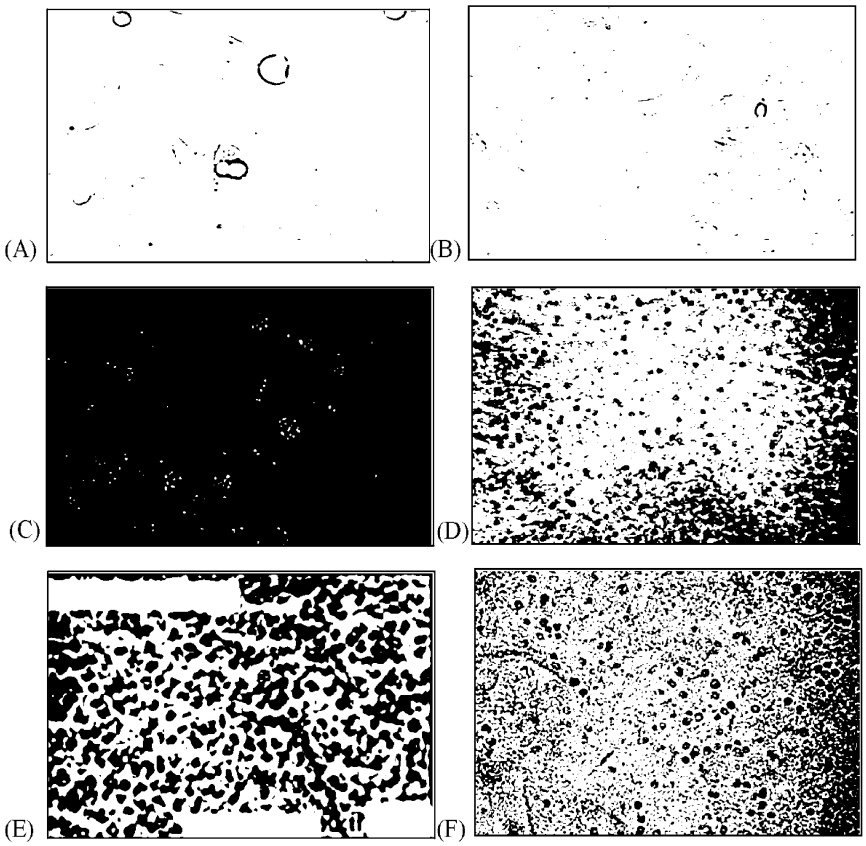


Fig. 4: Normal cell line AGS and MCF-7 (A, B). Cell lines after treatment with EDACC, C: AGS (Caffeic acid + Cu) D: MCF-7 (Caffeic acid + Cu). E: AGS (Caffeic acid + Co), F: MCF-7 (Caffeic acid + Co)

The spectral data of the complexes have good relationship with the literature data.

Analysis of [Co ($C_9H_6O_4$)] $Cl_2.2H_2O$ (EDACC): Dark Blue crystals; yield 86%. ^{1}H-NMR (™ ppm DMSO, 500MHz): 5. 97-7.21 [5H, aroma]; 11.91 [1H, s, acid); 8.98-9, 36[2H, s, alkene]. IR absorptions (cm^{-1} KBr): 1620 (C=C), 972 (=C-H), 1352 (C-O), 574 (Co-O) and 456 (Co-Cl). UV- Vis (MϵXN): λ max 320 nm (∑ 610), λ 240nm (∑ 1353). Raman shift (cm^{-1}): 500 (Co-O), 325 (Co-Cl), 975(C-H), 1618(C=C), 1189(C-O) (Fig. 2. Left).

Analysis of [Cu ($C_9H_6O_4$)] $Cl_2.2H_2O$ (EDACC): Orange crystals; yield 95%. IR absorptions (cm^{-1} KBr): 1609 (C=C), 897 (C=H), 1281(C=O), 573(Cu-O), 455(Cu-Cl). UV- Vis (MeCN): λmax 240 nm (∑ 1210), λ 280-330 nm (∑ 560) (Fig. 2. Right).

***In vitro* Activities:** EDA, [Co ($C_9H_6O_4$)]$Cl_2.2H_2O$ and [Cu ($C_9H_6O_4$)]$Cl_2.2H_2O$ three compounds were assayed for cytotoxicity in vitro against AGS (human Stomach) cells and MCF-7(human Breast) cells. The two cell lines were provided by the Pasteur Institute Laboratory of natural and biomimetic in Iran. The procedure for cytotoxicity studies was similar to that reported earlier [9]. Briefly, in order to calculate the concentration of each drug that produces a 72% inhibition of cell growth (AGS), [10]. After incubation periods 72 hrs for all cell lines, the cell concentrations were determined both in control and in drug-treated cultures. Cytotoxicity of total acetonitryl extract of caffeic acid and its different fractions were examined on cell lines. The percentage of cell viability was determined by MTT assay. All experiments were done in six times.

Cytotoxicity of Various Complexes of Caffeic Acid: The results showed that caffeic acid had cytotoxic effect on AGS and MCF7 Cell lines at 72 hrs incubation time. The inhibitory concentration of 50% (IC_{50}) of (caffeic acid+ Cu) on AGS was 31 mmol and (caffeic acid+ Co) on AGS was 125 mmol and (Caffeic acid+ Co) on MCF7 cell line was >500 mmol. The inhibitory concentration of 70% (IC_{70}) of (caffeic acid+ Cu) on AGS was 500 mmol and (caffeic acid+ Co) on AGS was 500 mmol. Also the result showed this extract decreased cell viability in a centration-dependent manner and the toxicity started at a concentration as little as 500 mmol (Fig. 3).

Among the complexes, Caffeic acid + Co was found to be more effective than the others. Tables 1 show inhibitory effects in terms of IC50 values against one normal Chang tow cancer cell lines (AGS stomach, MCF-7 breast) by EDACC (Acrylic acid + Co(II) and Cu(II) salts) extracts.

CONCLUSION

It is clear from the above discussion that Co (II) and Cu (II) complexes offer a new outlook for chemotherapy. The results of antitumor activity show that the metal complexes exhibit antitumor properties and it is important to note that they show enhanced inhibitory activity compared to the parent ligand. The mechanism by which these complexes act as antitumor agents is apoptosis. It has also been proposed that concentration plays a vital role in increasing the degree of inhabitation [11].

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