Journal of Molecular Structure 1155 (2018) 765–775

Contents lists available at ScienceDirect

Journal of Molecular Structure

journal homepage: http://www.elsevier.com/locate/molstruc

Hydrothermal preparation and physicochemical studies of new copper nano-complexes for antitumor application

M. Saif [a, *], Hoda F. El-Shafiy [a], Mahmoud M. Mashaly [a], Mohamed F. Eid [a], A.I. Nabeel [b], R. Fouad [a]

[a] Department of Chemistry, Faculty of Education, Ain Shams University, Roxy 11711, Cairo, Egypt
[b] Biochemistry Laboratory, Faculty of Education, Ain Shams University, Roxy, Cairo, Egypt

ARTICLE INFO

Article history:
Received 2 February 2017
Received in revised form
9 November 2017
Accepted 10 November 2017
Available online 17 November 2017

Keywords:
Hydrothermal
Surfactant
Nano-complex
Optimization
Cytotoxicity
Toxicity

ABSTRACT

Two novel nano-complexes $[(Cu)_2(L)(NO_3)_2(OH_2)]$ (CuH) and $[Cu(HL)(OH_2)_2(NO_3)]$ (CuCTH)were synthesized by hydrothermal method at 200 °C for 48 h in absence and presence of surfactant (CTAB), respectively. Introducing surfactant (CTAB) leads to changing stoichiometric metal/ligand ratio from binuclear (CuH) to mononuclear (CuCTH) nano-complexes. CuH shows irregular nano-flake shape while CuCTH have separately uniform nano-spherical morphology. Thermal analysis revealed that CuCTH is thermally stable in comparison with CuH Nano-complex. CuCTH absorption peak shifted to shorter wavelength (blue shift) and sharpness of the peak also decreased in presence of CTAB. The role of CTAB in the crystal growth is discussed. CuH and CuCTH nano-complexes were tested for their *in vitro* cytotoxicity against Ehrlich Ascites Carcinoma cell line (E.A.C.). Both nano-complexes effectively inhibited E.A.C. growth with IC_{50}value of 37 and 25 μM for CuH and CuCTH, respectively. The high antitumor activity of CuCTH was attributed to several factors such as spherical morphology, smaller size, chemical structure, and geometry. The LD_{50} for high cytotoxic CuCTH nano-complex on mice was found to be 100 mg/kg with strong abscess in abdomen side effect. To overcome this side effect, different molar ratio of CuCTH and previously prepared ZnNano-complexes were tested for their *in vitro*cytotoxicity and in *vivo* toxicity. Obtained results show that the 2:8 M ratio between CuCTH and Zn nano-complexes gives very low toxicity without any side effects. Also, geometric optimization and conformational analysis were performed using semi-empirical PM_3 method. Energy gap (ΔE), dipole moment, and structure activity relationship were performed and discussed.

1. Introduction

Cancer has become one of the most serious, global health threats in recent years; millions of people die of cancer every year. Nowadays, cancer is mostly treated by conventional approaches like chemotherapy, surgical resection and radiotherapy. However, these methods are highly aggressive, non-specific, and often accompanied by significant side effects, because they also show conspicuous toxicity to normal cells and tissues. Having lower toxicity as well as higher efficiency and stability than those of conventional dosage forms, nano-particles allow sustained and controlled delivery of anticancer agents, and also can be used to deliver drugs by altering signal transduction or modulating tumor microenvironment [1]. Physicochemical properties of nano-particles, including particle size, morphology, and surface chemistry have been identified as strongly affecting drug delivery and cellular toxicity [2]. Till now, many methods, such as arc discharge, laserablation, wet-chemistry, and microwave-assisted have developed Nano and microcrystals with various morphologies. Hydrothermal synthetic techniques can be used to form novel nano-structures and compounds from relatively insoluble and unreactive precursors. Reactants are combined in varying molar ratios, fill volumes, pressures, and temperatures. They produce metastable compounds that are often not accessible by higher temperature synthesis [3]. Hydrothermal method, moreover, seems to be a more promising way for the synthesis of nano-crystalline materials due to its low cost, high efficiency, and its potential for large-scale production [4]. Furthermore, the crystalline size, distribution, and the morphology control are easily manufactured by addition of

* Corresponding author.
E-mail address: mona_saif1@yahoo.com (M. Saif).

https://doi.org/10.1016/j.molstruc.2017.11.043
0022-2860/

counter ion, capping molecule, change of the reactant concentration, hydrothermal temperature, and processing time [5–8].

Copper is the most studied metal among all transition metal ions. It exhibits considerable biochemical action either binding to albumins and other proteins or binding to ligands forming complexes that interact with bio-molecules such as nucleic acids. The main interest in copper complexes arises from their potential use as antiviral, antimicrobial, anti-inflammatory, antitumor, and as enzyme inhibitors [9].

So we focus on the hydrothermal synthesis and physicochemical properties of copper nano-complexes in absence and presence ofcapping agent (CTAB) for antitumor applications. Our previous work [10] shows that Zn nano-complex is characterized with moderate antitumor activity and very low toxicity. So, the cytotoxicity and toxicity of a mixture between CuCTH with previously prepared Zn(II) complex [10] were investigated.

2. Experimental

2.1. Materials

The starting materials used in these studies are analytical grade. They include methyl salicylate (Chemical Laboratory), hydrazine hydrate (Panreac Quimica). Copper nitrate, phosphorous oxychloride, o-hydroxyacetophenone, and cetryltrimethyl ammonium bromide (CTAB)from BDH. Analar or Merck chemicals were also used. All solvents used are of spectroscopic grade. Tumor cells from mice (Ehrlich Acites Carcinoma (E.A.C.)) were obtained from National Cancer Institute. Zn(II) Nano-complex was prepared according to our previous published paper [10].

2.2. Characterization

Optical properties were recorded on a 'JASCO' model V-550 UV–Vis spectrophotometer (Japan). FT-IR spectra (4000-400 cm^{-1}) were carried out using 'FT-IR Nicolet IS10' spectrometer (USA). The phase and crystallographic structure of the product were identified by X-ray diffraction The XRD; PHILIPS diffractometer with CuKα1 radiation (k = 1.54056 Å). An accelerating voltage of 40 kV and an emission current of 30 mA were used. Morphology and particle size of the product were investigated by the transmission electron microscope (TEM, JEM-2100 (JEOL)) operated at 200 kV accelerating voltage. Magnetic susceptibilities of complexes were measured by 'Gouy' method at room temperature using 'shelwood' magnetic susceptibility scientific balance, Cambridge Science Park, (England). Effective magnetic moments were calculated using the relation $\mu_{eff} = 2.828\ (\chi_m.T)^{1/2}$B.M., where χ_m is the molar susceptibility corrected using Pascal's constant for diamagnetism of all atoms in compounds. TGA-DTG measurements were carried out on a 'Shimadzu' thermogravimetric analyzer using 'TA-50 WSI' program (Japan) with temperature range from 30 to 800 °C at nitrogen atmosphere and the heating rate is 10°/min. Microanalyses of carbon, hydrogen, and nitrogen were carried out at the Ministry of Defense, Chemical War Department. Melting or decomposition points were carried out on a melting point apparatus (stuart), England. Molar conductivities of 10^{-3} M solutions of solid complexes in DMF were measured on 'Corning' conductivity meter 14831 model 441NY. Visible light optical microscope was used. An attempt to gain better insight on the molecular structures of the ligand and their Nano-complexes (CuH and CuCTH), geometric optimization, and conformational analysis has been performed using semi-empirical 'PM3' method [11] force field as implemented in 'HyperChem 7.52' program [12].

2.3. Synthesis of the organic ligand, H_2L

The ligand (11E)-2-hydroxy-N/-((4-oxo-4H-chromen-3-yl) methylene)benzohydrazide) was prepared according to literature method [7] (Scheme 1). (Yield: 93%. m. p~249 C°. Anal. Calcd. for $C_{17}H_{12}N_2O_4$: C, 66.23; H, 3.92; N, 9.08%. Found: C, 66.11; H, 3.88; N, 9.00%. IR (cm^{-1}), 3247 [ν(N–H)], 1659 [$\nu(C{=}O)_{\gamma\text{pyrone}}$] and 1570 [ν(C=N)]. UV/Vis (DMF) λ_{max}/nm: 255, 307 and 440.

2.4. Synthesis of copper complexes

2.4.1. Synthesis of CuH nano-complex

A mixture of free ligand (H_2L) and $Cu(NO_3)_2 \cdot 3H_2O$ in molar ratio1:1was dispersed in H_2O, sealed in 100 mL of Teflon-lined autoclave with 70% filling, and heated at 200 °C for 48h [13,14]. After the reactor was slowly cooled to room temperature, deep brown crystals were obtained. The crystals were filtered off, washed with distilled water, and dried in a desiccator at room temperature over anhydrous $CaCl_2$. (Yield: 65%. m. p.˃300C°. Anal. Calcd. for $C_{17}H_{12}N_4O_{11}Cu_2$: C, 35.48; H, 2.10; N, 9.73; Cu, 22.08%. Found: C, 35.99; H, 2.15, N, 9.89, Cu, 21.62%. IR (cm^{-1}),1622 [$\nu(C{=}O)_{\gamma\text{pyrone}}$], 1556 [ν(C=N)], 1509 [$\nu(C{=}N)_{enolic}$], 1278 and1054 [ν(coord.NO_3)], 625, 583 and 528 [ν(M-O)], 432 and 419 [ν(M–N)]. UV/Vis (DMF) λ_{max}/nm: 593. μ_{eff} (B.M.) = 2.0. Λ_M(Scm^2mol^{-1}): 18; soluble in DMF and DMSO.

2.4.2. Synthesis of CuCTH nano-complex

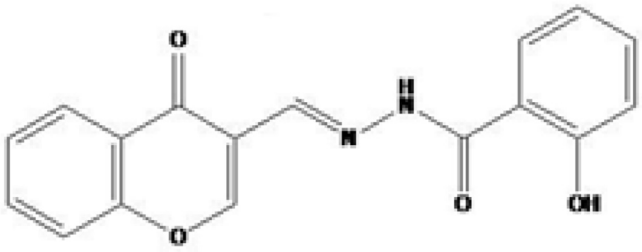
The complex CuCTH was prepared as the previous method except for adding CTAB (0.1 mmol) [.Very fine brown crystals were obtained. (Yield: 60%. m. p.˃300C°. Anal. Calcd. for $C_{17}H_{15}N_3O_9Cu$: C, 43.55; H, 3.22; N, 8.96; Cu, 13.55%. Found: C, 43.35; H, 3.15; N, 8.89; Cu, 13.34%. IR (cm^{-1}), 1622 [$\nu(C{=}O)_{\gamma\text{pyrone}}$], 1555 [ν(C=N)], 1519 [$\nu(C{=}N)_{enolic}$], 1277 and1055 [ν(coord.NO_3)], 623, 585 and 528 [ν(M–O)], 473 and 432 [ν(M-N)]. UV/Vis (DMF) λ_{max}/nm: tailing from 450 to 800 with hump at 588. μ_{eff} (B.M.) = 1.8. Λ_M(Scm^2mol^{-1}): 21; soluble in DMF and DMSO.

Scheme 2 shows the chemical structure of both, CuH and CuCTH nano-complexes [$(Cu)_2(L)(NO_3)_2(OH_2)$]and [$Cu(HL)(OH_2)_2(NO_3)$], respectively.

2.5. Cytotoxicity experiments

2.5.1. In vitro antitumor experiments

Antitumor activity of CuH and CuCTH nano-complexes as well as different molar ratio of CuCTH and Zn(II) Nano-complexes was carried out using E.A.C. according to *Mclimans* et al. (1957) [15]. A set of sterile test tubes was used, where 2.5×10^5 tumor cells per ml were suspended in phosphate buffer saline. Four tubes with three different concentrations for each compound (25, 50, 100, 200 μg/ml DMSO) were made and 2.5×10^5 tumor cells were added for each tube. Tubes were kept for 2 h at 37 °C. Samples were taken volume by volume with trypan blue on slides and were covered and



Scheme 1. The chemical structure of organic ligand (H_2L).