Inorganica Chimica Acta 439 (2016) 8–17

Contents lists available at ScienceDirect

Inorganica Chimica Acta

journal homepage: www.elsevier.com/locate/ica

Visible light-induced cytotoxicity of a dinuclear iron(III) complex of curcumin with low-micromolar IC_{50} value in cancer cells

Tukki Sarkar [a], Ray J. Butcher [b,*], Samya Banerjee [c,*], Sanjoy Mukherjee [c], Akhtar Hussain [a,*]

[a] Department of Chemistry, Handique Girls' College, Guwahati 781001, Assam, India
[b] Department of Chemistry, Howard University, Washington, DC 20059, USA
[c] Department of Inorganic and Physical Chemistry, Indian Institute of Science, Bangalore 560 012, Karnataka, India

ARTICLE INFO

Article history:
Received 5 July 2015
Received in revised form 21 September 2015
Accepted 24 September 2015
Available online 9 October 2015

Dedicated to Professor Animesh Chakravorty on the occasion of his 80th birthday.

Keywords:
Iron complex
Photodynamic therapy
Crystal structure
Photocytotoxicity
Apoptosis
Curcumin

ABSTRACT

Photoactive metal complexes have emerged as potential candidates in the photodynamic therapy (PDT) of cancer. We present here the synthesis, characterization and visible light-triggered anticancer activity of two novel mixed-ligand oxo-bridged iron(III) complexes, viz., $[\{Fe(L)(acac)\}_2(\mu\text{-}O)](ClO_4)_2$ (**1**) and $[\{Fe(L)(cur)\}_2(\mu\text{-}O)](ClO_4)_2$ (**2**) where L is bis-(2-pyridylmethyl)-benzylamine, acac is acetylacetonate and cur is the monoanion of curcumin (bis(4-hydroxy-3-methoxyphenyl)-1,6-diene-3,5-dione). The crystal structure of complex **1** (as PF_6^- salt, **1a**) shows distorted octahedral geometry of each iron(III) centre formed by the FeN_3O_3 core. The 1:2 electrolytic complexes are stable in solution and retain their oxo-bridged identity in aqueous medium. Complex **2** has a strong absorption band in the visible region and shows promising photocytotoxicity in HeLa and MCF-7 cancer cells in visible light giving respective IC_{50} values of 3.1 ± 0.4 μM and 4.9 ± 0.5 μM while remains non-toxic in the dark (IC_{50} > 50 μM). The control complex **1** is inactive both in the light and dark. Complex **2** accumulates in cytoplasm of HeLa and MCF-7 cells as evidenced from fluorescence microscopy and triggers apoptotic cell death via light-assisted generation of reactive oxygen species (ROS). Taken together, complex **2** with its promising photocytotoxicity but negligible dark toxicity in cancer cells has significant photochemotherapeutic potential for applications in PDT.

1. Introduction

Photodynamic therapy (PDT) is a clinically approved and attractive modality for the treatment of cancer which involves selective uptake of a photosensitizer (PS) drug by neoplastic tissue followed by illumination with light specifically absorbed by the PS [1–3]. This process triggers photochemical reactions leading to the generation of cytotoxic reactive oxygen species (ROS), mainly singlet oxygen, thereby resulting in tumour regression [1,2]. PDT is a convenient substitute to the conventional cancer therapies because of its low systemic toxicity, localized drug action to photoexposed regions, and negligible disturbance to the tissues [4]. Photofrin® is currently the FDA approved clinical PDT drug. However, the drawbacks such as prolonged skin sensitivity and hepatotoxicity associated this drug have provided the chemists and pharmacologists the impetus to look for its substitute [5]. Thus photoactivatable transition metal complexes have emerged as potential alternatives to Photofrin® in the treatment of cancer [6–10]. Recent studies on photoactivatable metal complexes have focused on the use of bioessential 3*d* metal ions mainly because of their versatile coordination geometry and varied spectral and redox properties [8,9]. Furthermore, the redox active 3*d* meal complexes could offer photoredox pathways as an alternate mechanism of action [8,9].

Curcumin (bis(4-hydroxy-3-methoxyphenyl)-1,6-diene-3,5-dione, Hcur) has been in the forefront of current research because it shows a spectrum of medicinal properties [11,12]. Curcumin is a natural *β*-diketone pigment found in the herb *Curcuma longa* L. Curcumin has received considerable attention in cancer research because it is well known for its anticancer activity against a wide range of cancers [12,13]. The anticancer activity of curcumin stems

Abbreviations: CCDC, Cambridge Crystallographic Data Centre; DCFDA, 2′,7′-dichlorofluoresceindiacetate; DMEM, Dulbecco's Modified Eagle's Medium; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; FBS, fetal bovine serum; FACS, fluorescence assisted cell sorting; FMO, frontier molecular orbital; HeLa, human cervical carcinoma; MCF-7, human breast adenocarcinoma; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PBS, phosphate buffered saline; PDT, photodynamic therapy; PI, propidium iodide; ROS, reactive oxygen species; TBAP, tetrabutylammonium perchlorate.

* Corresponding authors.
E-mail addresses: rbutcher99@yahoo.com (R.J. Butcher), samya@ipc.iisc.ernet.in (S. Banerjee), akhtariisc@gmail.com (A. Hussain).

http://dx.doi.org/10.1016/j.ica.2015.09.026
0020-1693/

from its ability to induce apoptosis in cancer cells while being harmless to the normal healthy cells [13]. Curcumin is known to interfere with the function of the transcription factor NF-κB which is a protein complex involved in the transcription of DNA [14]. However, the poor bioavailability and pharmacokinetic profile limits curcumin's applications as a clinical drug despite its negligible level of intrinsic toxicity [12–14]. While several modifications of curcumin have been studied in attempts to overcome the drawbacks, one strategy takes advantage of curcumin's metal coordinating ability via the β-diketone moiety present in its structure [15–17]. Interestingly, curcumin possesses rich photophysical properties and there are reports of curcumin showing PDT effect [18,19]. Although, there are reports on the anticancer activity of curcumin and its metal complexes, the studies on the photodynamic potential of its metal complexes are rare despite its rich photophysical and photochemical properties [20–23].

Iron is the most abundant bioessential transition metal. Therefore, its use could significantly decrease the metal-induced toxicity of the resulting complex [24]. Although, photocytotoxic iron(III) complexes are known, there is no report of its complexes with curcumin showing photocytotoxicity in visible light [24–27]. Chakravarty and coworkers have recently synthesized a mixed-ligand iron (III) complex, featuring an anthracenyl-pendant dipicolylamine base and a catecholate moiety as ligands, that shows visible and near IR light-induced cytotoxicity in human cervical carcinoma (HeLa), and human breast adenocarcinoma (MCF-7) cancer cells [24]. The interaction of curcumin with iron(III) and its consequence on the biological activity of curcumin is well documented in the literature [28–30]. Curcumin, being a hard *O,O*-donor bidentate ligand, is known to bind strongly to the iron(III) center due to its hard Lewis acidic character which is an important requisite for the stability and biological activity of the resulting complex under physiological conditions [30]. Thus, we envisaged that the combination of curcumin and iron(III) would produce a system with enhanced stability and photocytotoxicity. Recently, we have reported a series of cobalt(III) complexes of curcumin showing photocytotoxicity in HeLa cells in visible light [31]. Furthermore, the intrinsic green fluorescence of curcumin can be conveniently exploited to study the localization of the curcumin-bound complex in cancer cells [20–23].

In this work, we report for the first time the synthesis, characterization and studies on the visible light-induced anticancer activity of two novel mixed-ligand oxo-bridged iron(III) complexes of acac/curcumin and substituted dipicolylamine bases, viz., $[\{Fe(L)(acac)\}_2(\mu\text{-}O)](ClO_4)_2$ (**1**) and $[\{Fe(L)(cur)\}_2(\mu\text{-}O)](ClO_4)_2$ (**2**), where

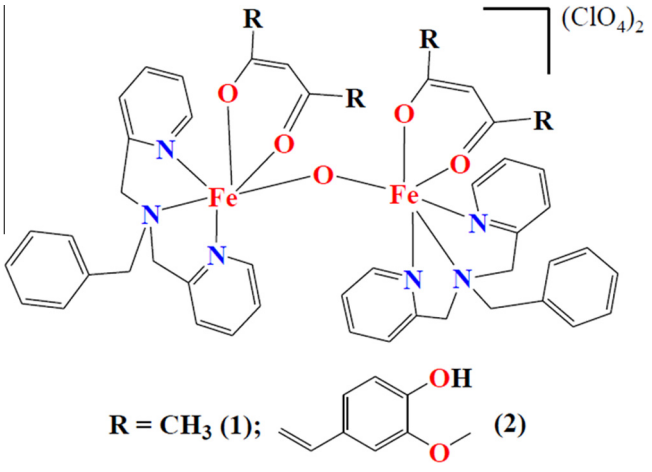

Fig. 1. The schematic representation of the complexes **1** and **2**.

L is bis-(2-pyridylmethyl)-benzylamine, acac is acetylacetonate and cur is the monoanion of curcumin (bis(4-hydroxy-3-methoxyphenyl)-1,6-diene-3,5-dione) (Fig. 1). The strongly chelating *N,N,N*-donor tridentate dipicolylamine base (L) was chosen for the stability of the resulting complexes. The structurally analogous complex **1**, having the photo-inactive acetylacetonate (acac) instead of photoactive curcumin, was synthesized, structurally characterized by single crystal diffraction methods, and studied as a control to compare the data and understand the role of curcumin (Fig. 1). Significant results of this work include: (i) the remarkable photocytotoxicity of complex **2** in visible light with negligible dark toxicity, (ii) the apoptotic mode of cell death due to the photo-assisted generation of intracellular ROS, and (iii) the cytosolic localization of complex **2** thereby making the cytosolic organelles as the potential targets.

2. Experimental

2.1. Materials

Unless otherwise specified, all the reagents and chemicals used in this work were obtained from commercial sources such as Sigma–Aldrich, U.S.A.; E-Merck; Alfa Aesar, UK; and Rankem, India, and used without further purification. Solvents were purified and distilled prior to their use by standard techniques [32]. Curcumin (95% curcuminoid content, *ca* 80% curcumin) was purchased from Sigma–Aldrich, U.S.A. and purified into three individual components by following a literature method [33]. Tris-(hydroxymethyl)aminomethane–HCl (Tris–HCl) buffer solution (pH = 7.2) was prepared using deionized and sonicated double distilled water. DMEM, propidium iodide, Hoechst 33342, MTT and 2′,7′-dichlorofluorescein diacetate (DCFDA) were procured from Sigma–Aldrich (U.S.A) and used as received. The *N,N,N*-donor dipicolylamine base bis-(2-pyridylmethyl)-benzylamine (L) was prepared by following a literature method [34].

2.2. General methods

The elemental analyses were done using a Thermo Finnigan Flash EA 1112 CHNS analyzer. The infrared (IR) spectra were recorded on a Bruker ATR FT-IR spectrometer. Electronic absorption spectra were recorded on a Perkin Elmer Lambda 25 spectrophotometer. Molar conductivity measurements were performed using a calibrated digital conductivity meter (Labtronics, India). Room temperature electrochemical measurements were carried out on a Biologic SP-50 Potentiostat/Galvanostat (Biologic Instruments, France) with a three electrode setup consisting of a platinum working electrode, platinum wire auxiliary electrode, and calomel reference electrode (SCE) at a scan rate of 100 mV s^{-1}. The electrochemical measurements were done using solutions of the metal complexes prepared in HPLC grade dimethylformamide (DMF). TBAP (0.1 M) was used as a supporting electrolyte in DMF. The ESI-MS measurements were carried out using Agilent Technologies 6538 UHD Accurate-mass Q-TOF LC/MS mass spectrometer. Room temperature magnetic susceptibilities of the DMSO-d_6 solutions of the Fe(III) complexes containing 1% TMS (v/v) as the internal reference were obtained by a solution NMR method with a Bruker AMX-400 NMR spectrometer [35]. The magnetic moments were calculated by the Evans method using the equation: $\mu_{eff} = 0.0618\ (\Delta f T/fM)$, where Δf is the observed shift in frequency of the TMS signal, T is the temperature (K), f is the operating frequency (MHz) of the NMR spectrometer, and M is the molarity of the complex in the solution [36]. Fluorescence quantum yields were determined using a PerkinElmer LS 55 fluorescence spectrometer using coumarin-153 laser dye as a reference