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Photocytotoxicity of copper(II) complexes of curcumin and N-ferrocenylmethyl-L-amino acids

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ABSTRACT

Copper(II) complexes [Cu(Fc-aa)(cur)] (**1**–**3**) of curcumin (Hcur) and N-ferrocenylmethyl-L-amino acids (Fc-aa), viz., ferrocenylmethyl-L-tyrosine (Fc-TyrH), ferrocenylmethyl-L-tryptophan (Fc-TrpH) and ferrocenylmethyl-L-methionine (Fc-MetH), were prepared and characterized. The DNA photocleavage activity, photocytotoxicity and cellular localization in HeLa and MCF-7 cancer cells of these complexes were studied. Acetylacetonate (acac) complexes [Cu(Fc-aa)(acac)] (**4**–**6**) were prepared and used as controls. The chemical nuclease inactive complexes showed efficient pUC19 DNA cleavage activity in visible light. Complexes **1**–**3** showed high photocytotoxicity with low dark toxicity thus giving remarkable photodynamic effect. FACScan analysis showed apoptosis of the cancer cells. Fluorescence microscopic studies revealed primarily cytosolic localization of the complexes.

1. Introduction

Curcumin which is the major yellow pigment extracted from the rhizome of turmeric plant (*Curcuma Longa* Linn) is well known for its medicinal values for a variety of ailments [1–4]. Its various pharmacologic effects like anti-arthritic, anti-inflammatory, antioxidant, anti-proliferative and anti-angiogenic properties have been extensively investigated. In addition, curcuminoids are used for the treatment of HIV, multiple sclerosis, Alzheimer disease (AD), microbial and parasitic infections [5–8]. Curcumin and its analogs have been studied as anti-tumor agents in various cancer cell lines and solid tumors [9–12]. *In vitro* studies have revealed that the drug induces mitochondria-mediated pathway of apoptosis involving caspases and Bcl-2 family members [12]. Curcumin with all its medicinal values, however, suffers from several limitations like bioavailability, instability in a biological medium, susceptibility to oxidation, etc [2,13,14]. This fluorescent pigment which absorbs at 420 and 450 nm and emits in green rapidly degrades in a cellular medium [3]. This predicament could be circumvented by coordination of the α,β-unsaturated 1,3-diketone moiety of curcumin (1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) to various metal ions (Fig. 1) [1]. While metals are known to form complexes with 1,3-diketones like acetylacetone (Hacac), very few complexes of curcumin are evaluated for their anti-proliferative properties [15–20]. Recently, ternary complexes of zinc are studied for their chemotherapeutic activities [20]. Although curcumin has very rich photophysical and photochemical properties due to the presence of an unsaturated β-diketone moiety which induces keto–enol tautomerism, this compound has not been utilized to design photoactivatable chemotherapeutic (PACT) agents which could act at low concentrations by selectively photo-exposing the cancer cells thus leaving the unexposed normal cells unaffected [21]. The metal-based curcumin complexes as a new generation PACT agents could be developed as potential substitutes of the macrocyclic organic dyes like porphyrins and phthalocyanines that

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Abbreviations: ct-DNA, calf thymus DNA; DMEM, Dulbecco's Modified Eagle's medium; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; DNA, deoxyribonucleic acid; EB, ethidium bromide; EDTA, ethylenediaminetetraacetate; FBS, fetal bovine serum; Fc, ferrocenyl; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; GSH, glutathione; NC, nicked circular; PBS, phosphate buffered saline; PDT, photodynamic therapy; PI, propidium iodide; SC, supercoiled; SCE, saturated calomel electrode; SOD, superoxide dismutase; TAE, tris-acetate-EDTA; TBAP, tetrabutylammonium perchlorate; TEMP, 2,2,6,6-tetramethyl-4-piperidone; Tris, tris(hydroxymethyl)aminomethane.

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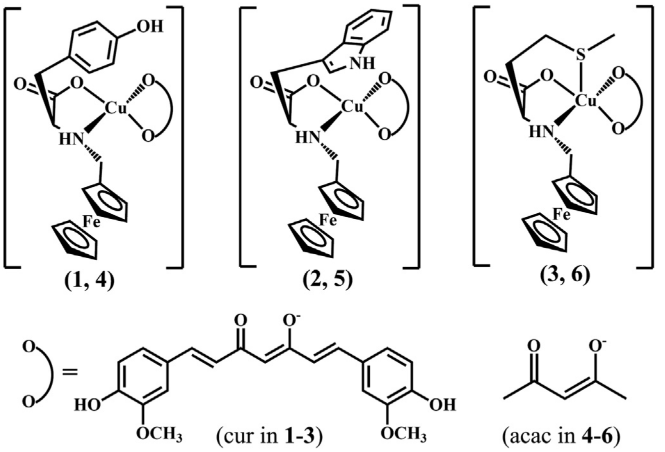

Fig. 1. Schematic drawings of curcumin (Hcur), complexes **1**–**3** (having curcumin ligand) and **4**–**6** (having acac ligand).

are used in photodynamic therapy (PDT) of cancer [22]. The curcumin metal complexes with their better stability and solubility in a biological medium could overcome the problem associated with its low bioavailability and instability in a human body.

We have chosen both curcumin and N-ferrocenylmethyl-L-amino acids to design suitable copper(II)-based PACT agents considering the biological importance of ferrocene conjugates like ferrocifen and ferroquine showing anti-cancer and anti-malarial activity [23,24]. Attachment of a ferrocene moiety to the breast cancer drug tamoxifen significantly enhanced the overall activity of the drug. Metal-arene half-sandwich complexes are also extensively studied for their antitumor properties, particularly as anti-metastasis agents [25–27]. We have reported Cu(II) and V(IV) complexes having pendant ferrocenyl moiety showing visible light induced DNA cleavage activity and photocytotoxicity in visible light [28–31]. Although these complexes show good photocytotoxicity, the PDT effect is, however, low due to significant dark toxicity arising from the redox activation of the metal by cellular thiols. To reduce the dark cellular toxicity and to enhance the PDT effect, we have now designed new copper(II) complexes of curcumin and reduced Schiff base ligands of bio-essential L-amino acids conjugated to a ferrocenyl moiety. The presence of curcumin and the ferrocenyl moiety in the ternary structure leads to remarkable PDT effect of the complexes.

Herein, we report the synthesis and anticancer activity of three curcumin (Hcur) copper(II) complexes [Cu(Fc-aa)(cur)] (**1**–**3**) having N-ferrocenylmethyl-L-amino acid ligands (Fc-aa), viz., Fc-TyrH, Fc-TrpH or Fc-MetH using L-tyrosine, L-tryptophan or L-methionine (Fig. 1). In addition, three acetylacetonato (acac) complexes [Cu(Fc-aa)(acac)] (**4**–**6**) having the N-ferrocenylmethyl-L-amino acid ligands (Fc-aa) are prepared and used as controls. The ternary complexes have the natural pigment curcumin, biochemically essential amino acids and the ferrocenyl moiety. Curcumin dye upon binding to Cu(II) ion is expected to be stable in the biological pH. The dye being a photosensitizer could be useful for photo-activated chemotherapeutic study [3]. Its emission property could be used for cellular imaging by fluorescence microscopy. The ferrocenyl moiety with its redox active and lipophilic nature is expected to enhance the cellular uptake of the designed complexes. The complexes were tested for their toxicity in HeLa and MCF-7 cancer cell lines in dark and visible light and the results are compared with those obtained for free curcumin. Significant results of this work include remarkable PDT effect of the curcumin complexes showing high photocytotoxicity and low dark toxicity. Interestingly, fluorescence microscopic studies show desirable cytosolic localization of the complexes **1**–**3** for their PDT action [22,32,33].

2. Results and discussion

2.1. Synthesis and general aspects

The N-ferrocenylmethyl-L-amino acid ligands were prepared by condensing ferrocene carboxaldehyde with the respective amino acid and subsequently reducing the Schiff base with sodium borohydride [34]. Ternary copper(II) complexes [Cu(Fc-aa)(cur)] (**1**–**3**) having N-ferrocenylmethyl-L-amino acids (Fc-aa) were synthesized in good yield (~75%) from a reaction of the respective amino acid reduced Schiff base (Fc-Tyr, Fc-Trp and Fc-Met) with copper(II) acetate monohydrate and curcumin (Hcur) in methanol (Fc, ferrocenyl moiety) (Fig. 1). To establish the role of curcumin on the photocytotoxic effect of **1**–**3**, copper(II) complexes [Cu(Fc-aa)(acac)] (**4**–**6**) were prepared as controls by reacting respective N-ferrocenylmethyl-L-amino acids (Fc-aa) with copper(II) acetate monohydrate and acetylacetone (Hacac) (Fig. 1). The complexes were characterized from their physicochemical data (Table 1). The major mass peak observed in the ESI-MS spectra is assignable to the $[M + Na]^+$ species having ^{63}Cu. An additional minor mass peak observed with ~2 *m/z* unit higher is due to the isotopic abundance of ^{65}Cu. The IR spectra of the complexes showed a characteristic stretching band near 1620 cm^{-1} due to asymmetric COO stretch. In addition, C=O and C=C stretching bands due to curcumin and acetylacetonato ligand were observed at ~1590 and ~1500 cm^{-1}, respectively [35]. The complexes are non-electrolytic as evidenced from their molar conductance values of 16–19 S m^2 M^{-1} in DMF at 25 °C. The magnetic moment values of ~1.8 μ_B at 25 °C suggest the presence of one-electron paramagnetic 3d^9-Cu(II) in the complexes. The UV–visible spectra of **1**–**3** in DMF–Tris–HCl buffer (1/1 v/v) showed an intense curcumin-based $\pi \rightarrow \pi^*$ band at ~430 nm which masks the Fc-centered and Cu(II)-centered d–d band (Fig. 2) [29,36]. The curcumin complexes **1**–**3** showed an emission spectral band at 514 nm on excitation at 430 nm in DMF at 25 °C giving

Table 1
Selected physicochemical data for the complexes [Cu(Fc-aa)(cur)] (**1**–**3**) and [Cu(Fc-aa)(acac)] (**4**–**6**).

Complex	1	2	3	4	5	6
IR/cm^{-1} [ν (C=O, C=C)]	1594, 1501	1593, 1502	1589, 1504	1580, 1515	1575, 1517	1575, 1519
λ_{max}/nm (ε/M^{-1} cm^{-1})[a]	432 (45,780)	431 (57,170)	432 (62,010)	445 (355), 615 (110)	445 (370), 612 (100)	445 (340), 620 (110)
E_f/V (ΔE_p/mV)[b]	0.42 (100)	0.40 (110)	0.44 (80)	0.45 (125)	0.48 (115)	0.49 (110)
Λ_M[c]/S m^2 M^{-1}	17	16	19	18	18	16
μ_{eff}[d]/μ_B	1.79	1.83	1.81	1.81	1.79	1.82

[a] In DMF–Tris–HCl buffer (1:1 v/v).

[b] Fe(III)–Fe(II) couple in DMF–0.1 M TBAP, $E_f = 0.5\,(E_{pa} + E_{pc})$, $\Delta E_p = (E_{pa} - E_{pc})$, where E_{pa} and E_{pc} are the anodic and cathodic peak potentials, respectively. The potentials are vs. SCE. Scan rate = 50 mV s^{-1}.

[c] Molar conductivity in DMF.

[d] Magnetic moment at 298 K using DMSO-d_6 solution of the complexes.