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Curcumin derivatives as metal-chelating agents with potential multifunctional activity for pharmaceutical applications

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ABSTRACT

Curcuminoids represent new perspectives for the development of novel therapeutics for Alzheimer's disease (AD), one probable mechanism of action is related to their metal complexing ability. In this work we examined the metal complexing ability of substituted curcuminoids to propose new chelating molecules with biological properties comparable with curcumin but with improved stability as new potential AD therapeutic agents. The K2T derivatives originate from the insertion of a $-CH_2COOC(CH_3)_3$ group on the central atom of the diketonic moiety of curcumin. They retain the diketo-ketoenol tautomerism which is solvent dependent. In aqueous solution the prevalent form is the diketo one but the addition of metal ion (Ga^{3+}, Cu^{2+}) causes the dissociation of the enolic proton creating chelate complexes and shifting the tautomeric equilibrium towards the keto–enol form. The formation of metal complexes is followed by both NMR and UV–vis spectroscopy. The density functional theory (DFT) calculations on K2T21 complexes with Ga^{3+} and Cu^{2+} are performed and compared with those on curcumin complexes. $[Ga(K2T21)_2(H_2O)_2]^+$ was found more stable than curcumin one. Good agreement is detected between calculated and experimental ^1H and ^{13}C NMR data. The calculated O – H bond dissociation energy (BDE) and the O – H proton dissociation enthalpy (PDE), allowed to predict the radical scavenging ability of the metal ion complexed with K2T21, while the calculated electronic affinity (EA) and ionization potential (IP) represent yardsticks of antioxidant properties. Eventually theoretical calculations suggest that the proton-transfer-associated superoxide-scavenging activity is enhanced after binding metal ions, and that Ga^{3+} complexes display possible superoxide dismutase (SOD)-like activity.

1. Introduction

Curcumin is a homodimer of feruloyl-methane containing a 3-methoxy and 4-hydroxy phenyl group, a heptadiene with two Michael acceptors, and an α,β-diketone (Scheme 1). The importance given to this phytocompound, extracted from *Curcuma longa* L. (turmeric), to related curcuminoids and their conjugates, is attested by the ever-increasing in vitro and in vivo tests reported in literature. In the past few decades, numerous studies on curcumin have demonstrated its multiple pharmacological activities and promising application as a novel drug in various diseases [1]. Curcumin is well studied due to its putative cancer prevention and anti-cancer activities which are mediated influencing multiple signaling pathways [2,3]. Recently, curcumin has been considered as a key molecule for the development of novel therapeutics for Alzheimer's disease (AD) [4]. In fact several studies revealed that curcumin is able to destabilize the preformation of β-amyloid (Aβ) fibrils in the central nervous system. Based on the assay results of mitochondrial metabolic markers, curcumin was found to protect human neuroblastoma SH-SY5Y cells against Aβ-induced damage of mitochondrial energy metabolism [5].

The utility of curcumin is however limited by its poor water-solubility, fast degradation and relatively low in vivo bioavailability. The recent quest for a 'supercurcumin' has led to different proposed formulations with various oils and with inhibitors of metabolism (e.g., piperine), liposomal and polymeric nanoparticles encapsulations [6] and conjugation of curcumin prodrugs [7]. PEGylation has been an instrumental avenue to increase water-solubility, stability and bioactivity of curcumin [8,9]. An alternative way is to functionalize curcumin in order to improve its bioavailability without compromising its beneficial properties. In this way we have recently synthesized some new curcumin derivatives with greater chemical stability; among these the *tert*-butyl ester derivatives (Scheme 2) have shown cytotoxicity against different tumorogenic cell lines, with IC_{50} values similar or in many cases lower than curcumin itself [10]. The presence, in these compounds, of a substituent on the central C atom of the β-diketo moiety of curcumin does not significantly alter the ability of the molecules to undergo a keto–enol tautomerism which is a fundamental feature of curcumin.

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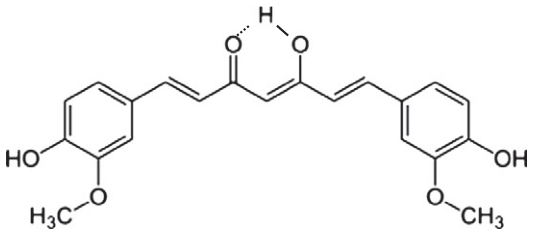


Scheme 1. Curcumin.

Another interesting domain of investigation is curcumin metal-chelation capacity, bearing probable correlation with its cytoprotective potency. Iron complexes of curcumin seem to have high potential in the treatment of cancer [11], while gallium complexes have remarkable antiviral effects on HSV-1 in cell culture [12].

^{1}H NMR data state that the dissociated keto–enol moiety of the ligand is involved in metal chelation and the formed complexes have high stability at physiological pH [13,14]. Various computational and analytical reports were published on curcumin interaction with Cu^{2+} ion, too [15–17]. The interaction of curcumin with Cu^{+} and Cu^{2+} ions may be involved in the observed anticancer properties [18,19] while the interactions of Cu^{2+} with Aβ are generally accepted to be a crucial process in the development of neurotoxicity in AD [20,21]. In fact drugs for the treatment of this disease seem to be correlated with metal complexation, acting as superoxide scavengers. Thus the skill of curcumin to chelate metal ions such as iron and copper could be a useful feature in developing new treatment for AD. In addition Zn-curcumin was found to increase superoxide dismutase (SOD) activity in chronic gastric ulcers in rats [22].

In summary, the curcumin derivatives here investigated are designed as potential neuroprotective agents, acting as metal-chelators with improved stability at physiological pH and increased cytotoxic activity with respect to curcumin. The aim of this work is to investigate by means of experimental and theoretical approach, the ability of these compounds (Scheme 2) to interact with Ga^{3+} and Cu^{2+} ions, to compare the results with the lead compound curcumin, and to evaluate their potential application as therapeutics.

2. Experimental section

2.1. General procedure and chemicals

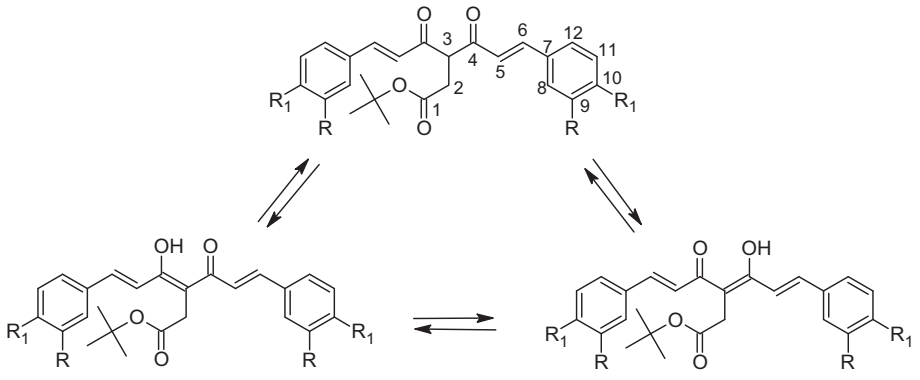
All chemicals were reagent grade and used without further purification unless otherwise specified. They were purchased from Sigma-Aldrich.

Compounds **K2T21**, **K2T23**, **K2T24**, and **K2T33** were synthesized and characterized as reported in ref. [10].

K2T31 [(3Z-5E)-*tert*-butyl-4-hydroxy-6-(4-hydroxyphenyl)-acrolyl) hexa3,5-dienoate] was here synthesized according to ref. [10]. Orange–red powder, 40% yield; **KE** 60%: ^{1}H NMR (MeOD-d_4) δ 3.60 (s, 2H; H-2), 7.11 (d, 2H; H-5, J = 15.2 Hz), 7.68 (d, 2H; H-6, J = 15.2 Hz), 6.85 (d, 4H; H-8, J = 8.1 Hz), 7.54 (d, 4H; H-9, J = 8.1 Hz), 1.45 (s, 9H; –COO(C$\underline{H}_3$)$_3$); ^{13}C NMR (MeOD-d_4) δ 171.0 (C-1), 32.7 (C-2),104.8 (C-3), 183.9 (C-4), 118.5 (C-5), 142.9 (C-6), 126.8 (C-7), 116.7 (C-8), 131.2 (C-9), 160.2 (C-10), 81.0 (–COOC(CH$_3$)$_3$), 26.8 (–C(C$\underline{H}_3$)$_3$). **DK** 40%: ^{1}H NMR (MeOD-d_4) δ 2.88 (d, 2H; H-2), 4.85 (t, 1H; H-3), 6.88 (d, 2H; H-5, J = 15.9 Hz), 7.70 (d, 2H; H-6, J = 15.9 Hz), 6.85 (d, 4H; H-8, J = 8.1 Hz), 7.54 (d, 4H; H-9, J = 8.1 Hz), 1.45 (s, 9H; –COO(C$\underline{H}_3$)$_3$); ^{13}C NMR (MeOD-d_4) δ 171.0 (C-1), 33.4 (C-2), 59.0 (C-3), 194.2 (C-4), 122.6 (C-5), 146.1 (C-6), 129.8 (C-7),116.7 (C-8), 131.2 (C-9), 160.2 (C-10), 81.0 (–COOC(CH$_3$)$_3$), 26.8 (–C(C$\underline{H}_3$)$_3$).

2.2. UV–vis spectroscopy

UV–vis spectrophotometric measurements were performed using Jasco V-570 spectrophotometer at 25.0 ± 0.1 °C in the 200–600 nm spectral range employing 1 cm quartz cells. Owing to the poor water solubility of the compounds, a methanol mother solution (5×10^{-3} M) was diluted in water in order to obtain 5×10^{-5} M solutions used for pH-metric titrations. The pH value was varied by adding small amounts of concentrated NaOH or HCl to obtain at least 25 different values in the pH range 4–9. The metal–ligand solutions were obtained by adding appropriate quantities of $GaCl_3$ or $CuCl_2$ water solutions as to obtain 1:1, 1:2, 1:3 and 1:4 M:L molar ratios ([L] = 5×10^{-5} M). A constant ionic strength of 0.1 M ($NaNO_3$) was maintained in all experiments. Each titration was performed at least three times. The overall protonation constants ($\log\beta_{LH}$) and the overall stability constants of metal complexes



R= R_1= H **K2T33**

R= OCH_3, R_1 = H **K2T23**

R = OCH_3, R_1 = OH **K2T21**

R = OCH_3, R_1 = $OCOCH_3$ **K2T24**

R = H, R_1 = OH **K2T31**

Scheme 2. General scheme of tautomeric equilibrium between keto–enol (KE) and di-keto (DK) forms of curcuminoids.