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Improvement of the antihypertensive capacity of candesartan and trityl candesartan by their SOD mimetic copper(II) complexes

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ABSTRACT

Two new complexes $[Cu(Cand)(H_2O)_4]$ **[1]** and $[Cu_2(TCand)_4(H_2O)_2]\cdot 4H_2O$ **[2]** (Cand = candesartan; TCand = trityl candesartan) have been synthesized and thoroughly characterized. The FTIR, Raman, EPR and diffuse reflectance spectra of the solid compounds show a dimeric complex for **[2]** with carboxylate bridging of the type found in copper(II) acetate. Both elemental analysis and thermal measurements allow the determination of the total stoichiometries of both complexes. The stability measurements show that the compounds are stable in ethanolic solutions at least for 1 h, while the preservation of the overall stochiometry for both species in solution has been determined by spectrophotometric titrations. By metal complexation the absence of antioxidant behavior of both sartans has been improved. Complexes **[1]** and **[2]** are strong superoxidedismutase mimetic compounds and complex **[2]** also behaves as a peroxyl radical scavenger. Furthermore, this higher antioxidant activity works in parallel with the improvement of the expansive activity over the angiotensin II-induced contracted human mesangial cells. These new complexes exhibit even higher efficiency as drugs in comparison with the free non-complexed medication with increased antioxidant ability expressing higher capacity to block the angiotensin II contractile effect. This study provides a new insight into the development of copper(II) complexes as potential drugs.

1. Introduction

Treatment of hypertension involves several different classes of drugs. Sartans are synthetic substances that have the renin–angiotensin system (RAS) as the target of their mechanism of action. They inhibit the angiotensin converting enzyme (ACE) which is mainly responsible for the conversion of angiotensin I into angiotensin II (that induces a direct vasoconstrictor action causing hypertension) [1].

Candesartan belongs to the class of AT2 receptor antagonists (AT2 blockers) and is used to treat high blood pressure but it is poorly absorbed when administered orally. Therefore, the prodrug candesartan cilexetil was developed. The prodrug undergoes rapid and complete ester hydrolysis in the intestinal wall and converted to candesartan, its active metabolite, during absorption from the gastrointestinal tract [2,3].

New therapeutical anticancer strategies proposing administration of candesartan were developed since the knowledge that angiotensin II acts as an angiogenic factor and the hypothesis that it is involved in regulation of tumor angiogenesis in cancer. Candesartan was found able for targeting tumor angiogenesis by inhibition of AT1R (angiotensin I receptor) in different kinds of cancer [4–9]. Candesartan can also attenuate induced oxidative stress and NAD(P)H oxidase activity [10]. However, it has been suggested that its antioxidant effect is independent of AT1R blockade [11].

In general, most of the sartans are composed of an appropriately substituted heterocyclic nucleus coupled to an acidic group (carboxylic or tetrazole) bearing biphenyl system through a methylene linker. In particular, candesartan has the benzimidazole group substituted with carboxyl function at the 7-position [2].

Modifications of the structure of candesartan were performed in order to improve the biological effect of the sartan. It has been demonstrated that methylation of the tetrazole ring with the concomitant loss of the acidic group produced a reduction of the potency of the drug in 1000 units of magnitude. From these data it was concluded that an ionic interaction to the receptor is more probable than H bonding interactions [12]. In order to test these observations we have also included trityl candesartan (HTCand) to perform comparisons with the behavior of candesartan (H_2Cand) (see Fig. 1).

Other structural modifications of sartans including the formation of novel molecules between losartan and compounds with antioxidant activities that produced both antihypertensive and cytoprotective effects, [13] and the so-called NO-sartans that merge both antihypertensive

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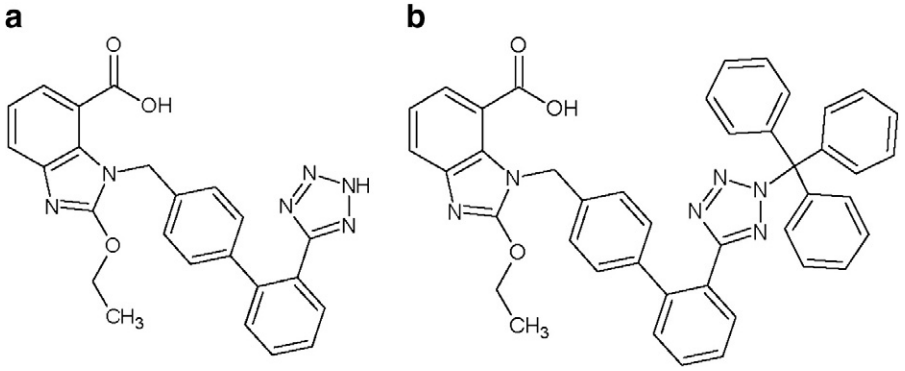

Fig. 1. a) candesartan (H_2Cand); b) trityl candesartan (HTCand).

properties using nitric oxide-releasing drugs and AT1 antagonists have been reported. These pharmacodynamic hybrids improved the action of the "native" sartans [14,15]. Taking into account that these modifications strengthen the action of the drugs, we have, therefore, undertaken the design of another class of hybrids by synthesizing coordination compounds with the biometal copper(II) and both candesartan and trityl candesartan. Metal complexes have been widely applied in clinics for centuries and the great successes achieved with platinum-based antitumor agents have promoted the development of metal-based drugs. Particularly, copper is an essential element involved in several biological functions. All tissues of the body need it for normal metabolic functions. It is able to reduce inflammations, strengthen connective tissue, restore hair color and the oxidative energy metabolism as well as fight parasites and cancer. It has been observed that the serum level of copper is often elevated in animals and humans with cancer with an associated decrease in the concentration of the antioxidant copper-dependent enzymes [16]. Besides, the role of copper in maintaining cardiovascular health has been well established. Copper is essential both for its role in antioxidant enzymes, like Cu–Zn superoxidedismutase and ceruloplasmin, as well as its role in lysyl oxidase, essential for the strength and integrity of the heart and blood vessels. Copper deficiency has produced many of the same abnormalities present in cardiovascular disease [17].

We have previously described the effects of two analog copper(II) complexes with losartan and valsartan and determined that upon complexation the antioxidant activities (tested for losartan–copper compound) and the inhibitory effects on the tumoral cell line proliferation were improved [18,19]. In the present communication we describe the preparation and structural characterization of two new copper(II) complexes with candesartan and trityl candesartan and determine their antioxidant behavior and the ability of AT2 to reduce planar cell surface area in cells pretreated with these new compounds in comparison with free copper(II) ion and the free ligands.

2. Materials and methods

2.1. Reagents and instrumentation

All chemicals were of analytical grade and used without further purification. Copper(II) chloride dihydrate was purchased from Riedel de Häen, pure commercial samples of candesartan and trityl candesartan (Hangzhou Garden Trading Co., Ltd (China)) were used as supplied. FTIR spectra of powdered samples (as pressed KBr pellets) were measured with a Bruker IFS 66 FTIR-spectrophotometer from 4000 to 400 cm^{-1}. A total of 60 scans were accumulated. Spectral resolution was ± 4 cm^{-1}. FT-Raman spectra were measured using the FRA 106 Raman accessory. A continuous-wave Nd:YAG laser working at 1064 nm was employed for Raman excitation. A germanium detector operating at liquid nitrogen temperature was used. Raman scattering radiation was collected with a standard spectral resolution of ± 4 cm^{-1}. Electronic absorption spectra were recorded on a Hewlett-Packard 8453 diode-array spectrophotometer, using 1 cm quartz cells. Diffuse reflectance spectra were registered with a Shimadzu UV-300 instrument, using MgO as an internal standard. Elemental analyses (EA) for carbon, hydrogen and nitrogen were performed using a Carlo Erba EA 1108 analyzer. Thermogravimetric analysis (TG) and differential thermal analysis (DTA) were performed with Shimadzu systems (models TG-50 and DTA-50, respectively), working in an oxygen flow of 50 mL/min and at a heating rate of 10 °C/min. Sample quantities ranged between 10 and 20 mg. Al_2O_3 was used as a differential thermal analysis standard. A Bruker ESP300 spectrometer operating at the X-band and equipped with standard Oxford Instruments low-temperature devices (ESR900/ITC4) was used to record the EPR spectrum of the complex at room temperature in the solid state. A computer simulation of the EPR spectra was performed using the program SimFonia [20].

2.2. Preparative

[Cu(Cand)(H_2O)$_4$] (CuCand) **[1]**: A solution of $CuCl_2 \cdot 2H_2O$ in ethanol (1 mmol, 5 mL) was added under continuous stirring to an ethanolic solution of candesartan (1 mmol, 10 mL). The solution was allowed to stir at 60 °C and an aqueous solution of 1 M NaOH was added up to pH 7–8. When the final volume of the solution was reduced by 80% a green precipitate was generated after water (5 mL) addition. The resulting solid product was filtered off, washed several times with water and dried in an oven at 60 °C. Anal. Calc.%: C, 50.2; H, 4.5; N, 14.6. Exp.%: C, 49.9; H, 4.4; N, 14.2. Thermogravimetric analysis confirmed the presence of two labile water molecules (Exp. loss: 6.4%. Calc. loss: 6.3%; endothermic peak, DTA, T<100 °C) and two additional water molecules were lost at higher temperature probably due to the stronger covalent bonds generated by the Jahn–Teller effect (Exp. loss: 6.5%. Calc. loss: 6.3%; endothermic peak, DTA, 100 °C<T<200 °C). At 800 °C the weight loss (86.1%, calc.; 86.0%, exp.) represents the formation of CuO that was characterized by FTIR spectroscopy. UV–visible spectrum (ethanol): 670 nm ($\varepsilon = 100.2$ M^{-1} cm^{-1}). Diffuse reflectance spectrum: 440 nm, 770 nm.

[Cu_2(TCand)$_4$(H_2O)$_2$]$\cdot 4H_2O$ (CuTCand) **[2]**: To an ethanolic solution of trityl candesartan (1 mmol) heated at 70 °C an ethanolic $CuCl_2 \cdot 2H_2O$ solution (0.5 mmol) was added under constant stirring. The pH value of the mixture was raised to 7 by addition of an aqueous 1 M NaOH solution. Then, the mixture was concentrated by evaporation until a final volume of 5 mL was attained. The addition of water (10 mL) gave a green solid that was filtered off, washed with water and dried in an oven at 60 °C. Anal. Calc.%: C, 69.7; H, 4.9; N, 11.3 Exp%: C, 70.0; H, 4.8; N, 11.2. By thermogravimetric analysis (TGA) it was determined that the four crystallization water molecules are lost at 80 °C with an endothermic DTA signal (weight loss, 2.4%)