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Aliskiren Copper(II) Complex. Synthesis and Antioxidant Activity

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Abstract. Aliskiren (Alk) is a highly selective competitive inhibitor of renin used for the treatment of hypertension and related cardiovascular diseases. With the aim of improving its biological properties by the strategy of the induction of favorable conformational changes, we designed a metal-based drug. In this work we report the synthesis of a solid copper(II) complex with aliskiren, **CuAlk**, and its characterization in solution and solid-state. Moreover, and based on the association of hypertension with elevated levels of reactive oxygen species (ROS), the antioxidant properties of **CuAlk** were studied measuring the activities against 2,2-diphenyl-1-picrilhidrazil (DPPH•), hydroxyl (OH•) and peroxyl (ROO•) radicals, in addition to its superoxide dismutase (SOD) simil activity. Both the ligand and the complex were able to scavenge hydroxyl radicals, but upon complexation, the SOD mimetic activity of the ligand is enhanced.
Keywords: N-ligands, copper, antioxidants, EPR, superoxide dismutase.

Resumen. El Aliskiren (Alk) es un inhibidor por competencia altamente selectivo de la renina que se utiliza en el tratamiento de la hipertensión y enfermedades cardiovasculares relacionadas. Se diseñó un metalofármaco con la idea de mejorar las propiedades biológicas mediante la inducción de cambios conformacionales favorables. En este trabajo se reporta la síntesis de un complejo sólido de cobre(II) con aliskiren, **CuAlk**, junto con su caracterización en fase sólida y en solución. Además, de acuerdo con la relación que existe entre la hipertensión y los niveles elevados de especies reactivas de oxígeno (ROS), se estudian las propiedades antioxidantes del **CuAlk** frente a los radicales 2,2-difenil-1-picrilhidracilo (DPPH•), hidroxilo (OH•) y peroxilo (ROO•), además de la actividad superóxido dismutasa símil (SOD). Tanto el ligando, como el complejo fueron capaces de secuestrar radicales hidroxilos, pero, luego de la complejación, se mejoró la actividad SOD símil del ligando.
Palabras clave: N-ligandos, cobre, antioxidantes, EPR, superóxido dismutasa.

Introduction

Aliskiren (Alk) was the first reported compound in a novel class of renin inhibitors with the potential for treatment of hypertension and related cardiovascular diseases [1]. Being a highly selective competitive inhibitor of renin, it is able to block effectively the conversion of angiotensinogen to angiotensin I, as well as the later production of angiotensin II (Ang II). When Ang II levels are inhibited, the feedback loop is suppressed,

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resulting in further reduction in plasma renin concentrations [2]. The drug is commercially available as aliskiren hemifumarate (Fig. 1).

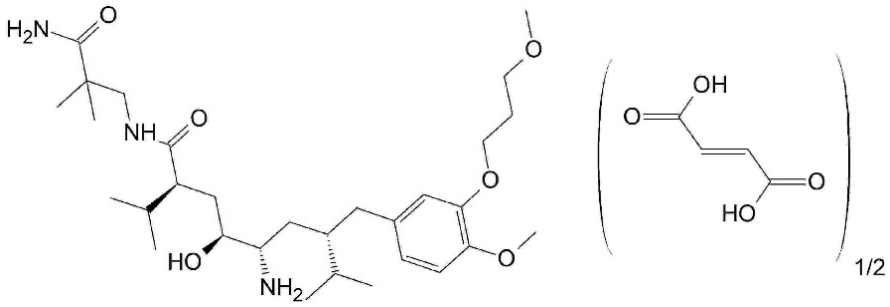

Fig. 1. Aliskiren hemifumarate (commercial drug).

In addition, it has been shown that Alk has a protective role against cisplatin induced nephrotoxicity in rats [3] and this could be associated to its antioxidant activity. It has been reported that Alk can decrease the Reactive Oxygen Species (ROS) produced by the Renin Angiotensin Aldosterone System (RAAS) at doses independent of decreasing blood pressure [4].

Copper(II) plays a critical role in the antioxidant defense of organisms, particularly in Cu/Zn-SOD, and in cardiovascular disease, as the heart and blood vessels are particularly susceptible to Cu deficiency [5]. Due to this, numerous copper(II) complexes with antihypertensive drugs, including β-blockers, vasodilators, ACE inhibitors, diuretics [6], and sartans, have been synthesized, some of which have improved the efficacy of the parent drug [7]. The action of these complexes is based on the ability of metal-based drugs to enhance the pharmacological effects of the original drug through various mechanisms, such as acting as a prodrug, improving targeting strategies, enhancing the lipophilic nature [8], and other mechanisms [9].

Taking this into account, we propose in this study to investigate a coordination complex containing copper(II) and Alk. In a first step, the aliskiren base (Alk base) was isolated from the hemifumarate anion. Then, a copper complex (**CuAlk**) was synthesized and characterized in both solid and solution states. This is, to our knowledge, the first metal coordination complex reported with Alk.

With the aim of studying how the *in vitro* antioxidant activity of the ligand is affected by complexation, we have performed several experiments that can be divided in three general groups: i) those based on the scavenging activity of a previously generated radical, such as 2,2-diphenyl-1-picrilhidrazil (DPPH$^{\bullet}$) or hydroxyl (OH$^{\bullet}$) radicals; ii) assays based in the mimetic activity of an antioxidant enzyme, like superoxidedismutase (SOD), in which the capacity of a compound to favor the dismutation of the generated superoxide radical is studied; iii) The third group involves the competitive methods, which use a reference compound as a probe, like the present method for the scavenge of the peroxyl (ROO$^{\bullet}$) radical, that follows the decay in the absorbance of pyranine (probe) by UV-vis spectrometry. To sum up, herein we report the synthesis and characterization of a copper(II) complex with Alk and the measurement of its antioxidant activity against different radicals.

Experimental

General section

All chemicals were used without further purification. Copper(II) nitrate hexahydrate was purchased from Biopack, and pure commercial samples of aliskiren hemifumarate (Jinlan Pharm-Drugs Technology Co., Ltd (China)). Elemental analysis for carbon, nitrogen and hydrogen were performed using a Carlo Erba EA 1108 analyzer. FTIR spectra of powdered samples (as pressed KBr pellets) were measured with an Equinox 55 FTIR-spectrophotometer from 4000 to 400 cm^{-1}. Thermogravimetric analysis (TGA) and differential thermal

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analysis (DTA) were performed on a Shimadzu system (model TG-50 and DTA-50 respectively) working in an oxygen flow (50 mL min^{-1}) at a heating rate of 10 ^oC min^{-1}. Sample quantities ranged from 5 to 10 mg. Al_2O_3 was used as a differential thermal analysis standard. UV-vis in solution and diffuse reflectance spectra were collected from 185 to 900 nm with a spectrophotometer Shimadzu UV-vis 2600, equipped with halogen and deuterium lamps. For diffuse reflectance measurements MgO was used as an internal standard. A X-band EPR spectrometer (Bruker Elexsys E500T-A) was used to record the electron paramagnetic resonance (EPR) spectra of the complexes at room temperature and 120 K (with liquid air) in solid state and at room temperature in ethanolic solution. A computer simulation of the EPR spectra was performed using the program Easyspin 5.2.28 in Matlab 2014. NMR experiments were carried out using NMR tubes adapted with J-Young valves. NMR spectra were recorded on a Bruker 400 MHz spectrometer. NMR chemical shifts are reported in ppm with d_6-DMSO as internal reference. MS experiments were performed on an LTQ Orbitrap FTMS instrument (LTQ Orbitrap Elite FTMS, Thermo Scientific, Bremen, Germany) operated in positive mode, coupled with a robotic chip-based nano-ESI source (TriVersa Nanomate, Advion Biosciences, Ithaca, NY, U.S.A.). A standard data acquisition and instrument control system were utilized (Thermo Scientific), while the ion source was controlled by Chipsoft 8.3.1 software (Advion BioScience). Samples were loaded onto a 96-well plate (Eppendorf, Hamburg, Germany) with an injection volume of 5 µL. The experimental conditions for the ionization voltage were +1.4 kV, and the gas pressure was set at 0.30 psi. The temperature of the ion transfer capillary was 120 °C. FTMS spectra were obtained in the 80-1000 m/z range in the reduced profile mode, with a resolution set to 120,000. In all spectra, one microscan was acquired with a maximum injection time value of 1000 ms.

Aliskiren free base

0.25 g of the aliskiren hemifumarate (0.4 mmol) were dissolved in 15 mL H_2O and 1.5 mL NH_4OH were added, obtaining a cloudy suspension. The free base ($C_{30}H_{53}N_3O_6$) was obtained by two subsequent extractions with ethyl acetate (7.0 mL each). The organic phase was washed with 5 mL of distilled water and then all the volatiles were removed using a rotavapor, obtaining a colorless gel. A colorless solid can be collected by cooling the gel formed in the previous step. (0.167 g, 67 % yield). The absence of the fumarate bands [10] at 1565 cm^{-1} ($\nu_{as\ COO-}$) and 1635 cm^{-1} ($\nu_{s\ COO-}$) in the FTIR spectrum confirms its separation of the aliskiren base (Fig. S1). UV (EtOH) λ_{max} (log ε) 227 (3.998) nm (shoulder); 280 (3.468) nm; IR (KBr) ν_{max} 3408, 3355, 2958, 2931, 2873, 1663, 1650, 1608, 1591, 1515, 1469, 1443, 1425, 1386, 1369, 1260 1236, 1191, 1161, 1138, 1122, 1052, 1028, 807, 767, 607, 552, 435 cm^{-1}; ^{1}H NMR ($(CD_3)_2SO$, 400 MHz) δ 6.84 (1H, t), 6.54 (1H, s), 6.09 (2H, d), 6.0 (1H, s), 5.95 (1H, d), 2.96 (4H, s), 2.74 (3H, t), 2.50 (3H, s), 1.81 (3H, s), 1.70 (1H, m), 1.62 (2H, m), 1.40 (1H, t), 1.20 (2H, t), 0.90 (4H, m), 0.56 (1H, t), 0.43 (2H, m), 0.32 (6H, s), 0.10 (6H, t), 0.03 (6H, t); ^{13}C NMR ($(CD_3)_2SO$, 100 MHz) δ 166.60, 151.3, 113.8, 111.7, 71.4, 68.4, 65.1, 57.7, 55.3, 49.4, 46.2, 42.4, 33.3, 32.9, 30.1, 28.6, 23.1, 20.5, 19.8, 19.4, 16.9.

$[Cu(Alk)_2(H_2O)_2](NO_3)_2$ (CuAlk)

An ethanolic solution of $Cu(NO_3)_2$ (2 mmol, 2mL) was added dropwise to an ethanolic aliskiren base solution (1 mmol, 5 mL). This excess of copper(II) was added to favour the formation of the complex. Afterwards, the pH was adjusted to 12 with 1 M NaOH, leading to the precipitation of the excess of Cu(II) as a light blue solid ($Cu(OH)_2$, confirmed by FTIR spectroscopy) and the formation of a purple solution. After removing the solid by filtration, the purple solution was acidified with HNO_3 (0.1 mol/L) until pH 7 were the colour changes to blue. Then, the solution was slowly evaporated until 1 mL and afterwards, 3 mL of water were added, after an oily solid was obtained. The blue solid was separated from the solution and dried in oven at 60 °C (0.87 g, 70 % yield). Amorphous powder: UV-vis (EtOH) λ_{max} (log ε) 644 (1.504) nm; Diffuse reflectance λ_{max} 646 nm (Fig. S2), IR (KBr) ν_{max} 3431, 3335, 2959, 2832, 2873, 1665, 1658, 1650, 1608, 1591, 1515, 1468, 1443, 1425, 1384, 1260, 1235, 1191, 1161, 1138, 1121, 1052, 1029, 807, 768, 631 cm^{-1}; ^{1}H NMR ($(CD_3)_2SO$, 400 MHz) δ 6.80, 6.60, 3.90, 3.72, 3.44, 3.24, 2.50, 1.90, 1.02, 0.80 (very broad peaks); ^{13}C NMR ($(CD_3)_2SO$, 100 MHz) δ 147.0, 146.4, 111.2, 67.8, 64.7, 57.1, 54.8, 28.3; FTMS *m/z* (rel. int.) 1165.7 [M^+, $CuAlk_2^+$](26.1), 1164.7 [M H-1$^+$] (37.5), 1166.7 [MH^+] (25.0), 1842.0 (2.3), 1841 (1.7), 1718 (4.9), 1717.1 (5.4), 1716.1 (5.0), 1286 (1.0), 1188.7 (2.2), 676.2 (1.3) (Fig. S3 and S4); Anal. C 54.2 %, H 8.4%, N 8.3 %, calc. for $CuC_{60}H_{110}N_8O_{20}$ (Mw 1325.5 g/mol), C 54.3 %, H 8.2 %, N 8.4 %; Thermogravimetric analysis (TGA) range 50-100 °C (Calc. loss: 2.1 %, Exp. loss: 1.7 %, two labile water molecules per copper atom) with a broad endothermic peak, around 100 °C, DTA, At 800 °C the

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weight loss (94.0 %, calc.; 94.1 %, exp.) represents the formation of CuO that was characterized by FTIR spectroscopy. No suitable crystals for X-ray structural determinations were obtained.

Spectrophotometric titration

Prior to determining the stoichiometry of the complex (CuAlk) in solution, it was necessary to remove fumarate anion from Alk hemifumarate. To accomplish this, 0.1218 g of Alk hemifumarate (0.1 mmol fumarate, 0.2 mmol Alk) were dissolved in 10 ml of 96 % ethanol. Next, 0.0242 g of $Cu(NO_3)_2.3H_2O$ (0.1 mmol) were added to the solution. The resulting mixture formed a light blue solid (copper fumarate by FTIR), which was then filtered and washed three times with 96 % ethanol. The remaining ethanolic solution and the washed solution (Alk base) were transferred quantitatively to a graduated flask and made up to 25 mL (8 mM). Subsequently, the molar ratio method was applied by adding varying quantities of ethanolic solution of $Cu(NO_3)_2.3H_2O$ (0.01 M) in a ligand-to-metal ratio ranging from 10 to 0.7. pH values were adjusted to 7.5 using 0.1 M NaOH solution in 96 % ethanol (Fig. S5).

Antioxidant properties

In these experiments the compounds were dissolved in the minimum quantity of ethanol (EtOH) to avoid their precipitation and were then added to the aqueous buffer and substrate solutions. The basal measurements were performed using the same proportion of EtOH. The final concentration of EtOH in each test achieved a maximum of 2 % in the final reaction mixtures. Due to the required conditions for each study, the maximum concentration tested for each compound can differ among experiments.

Scavenging of the hydroxyl radical (OH•)

Hydroxyl radicals were generated by the ascorbate–iron–H_2O_2 system. The reaction mixture was prepared by the addition of all the reagents up to the following concentrations: 3.75 mM 2-deoxyribose, 2.0 mM H_2O_2, 0.1 mM Fe^{+3}-EDTA and the test compound in 20 mM KH_2PO_4–KOH buffer (pH 7.4). The reaction was initiated by the addition of 0.1 mM of sodium ascorbate, followed by incubation (heating and shaking at 37 °C for 30 min). The extent of deoxyribose degradation was measured by the thiobarbituric acid method [11,12]. The solutions of $FeCl_3$, ascorbate, and H_2O_2 were prepared in distilled water immediately before use.

Scavenging of the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH•)

A methanolic solution of DPPH• was added to the compound solutions (final concentration of each compound 50, 100, 250 and 500 μM) dissolved in 0.1 M tris(hydroxymethyl)aminomethane-HCl buffer (pH 7.1) at 25 °C. The absorbance at 517 nm was measured after 60 min of the initiation of the reaction in the dark and compared with the absorbance of the control (Fig. S6).

Inhibition of the peroxyl radical (ROO•)

The decrease in pyranine (probe) intensity at 454 nm was followed by UV-vis spectrophotometry. Peroxyl radicals were generated by the thermal decomposition of AAPH (2,2-azobis(2-amidinopropane) dihydrochloride) [13], which can generate free radicals at a steady rate for extended periods of time (half-life of 175 h). Reaction mixtures of 3.0 mL contained 50 mM of AAPH, 0.05 mM of pyranine and different concentrations in the range 0.1-0.5 mM of the compounds to be studied. The absorbance spectra were measured with a thermostated cell at 37 °C. The delay of pyranine consumption (lag phase) was calculated as the time before the consumption of pyranine started (Fig. S7).

Superoxide dismutase assay

The superoxide dismutase (SOD) activity was examined indirectly using the nitroblue tetrazolium (NBT) assay. The ability of the compounds to inhibit the reduction of NBT by the superoxide anion generated by the phenazine methosulfate (PMS) and reduced nicotinamide adenine dinucleotide (NADH) system was determined. The system contained 0.5 mL of the sample, 0.5 mL of 1.40 mM NADH, and 0.5 mL of 300 μM NBT, in 0.1 M KH_2PO_4-NaOH buffer (pH 7.4). After incubation at 25 °C for 15 min, the reaction was started by the addition of 0.5 mL of 120 μM PMS [14]. Then, the reaction mixture was incubated for 5 min, and the absorbance of the band at 560 nm was measured. Each experiment was performed in triplicate and at least three

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independent experiments were performed in each case. The SOD activity is expressed as the concentration of the tested compounds that produces the 50 % inhibition of the NBT reduction by superoxide produced in the system (IC_{50} values) and was evaluated from the absorbance decrease at 560 nm in comparison with the blank (the reaction mixture without the addition of the different compounds) (Fig. S8).

Results and discussion

Characterization of the structure of the complex

CuAlk was characterized by FTMS, FTIR, EPR, NMR and diffuse reflectance spectroscopies. The room temperature EPR spectra (Fig. 2) of solid-state showed an elongated octahedral geometry, $g_{//} > g_{\perp}$, $d_{x^2-y^2}$ ground state. The g values of $g_{//} = 2.221$; $g_{\perp} = 2.052$ and $A_{//} = 196 \times 10^{-4}$ cm^{-1}; $A_{\perp} = 23 \times 10^{-4}$ cm^{-1} are in agreement with a coordination environment of two nitrogen and two oxygen atoms in the equatorial plane, CuN_2O_2 [15-17]. Values of $G = (g_{//} - 2) / (g_{\perp} - 2) = 4.25$ (higher than 4) show that no significant magnetic exchange is present. Furthermore, low-temperature EPR measurement at 120 K has been performed but no super hyperfine coupling constant for nitrogen has been observed (Fig. S9). In addition, the diffuse reflectance spectrum (Fig. S2) showed a very broad band at 646 nm, consistent with a tetragonal distortion (Jahn-Teller effect) where more than one d-d transition is allowed [17,18], as was also suggested by EPR spectroscopy.

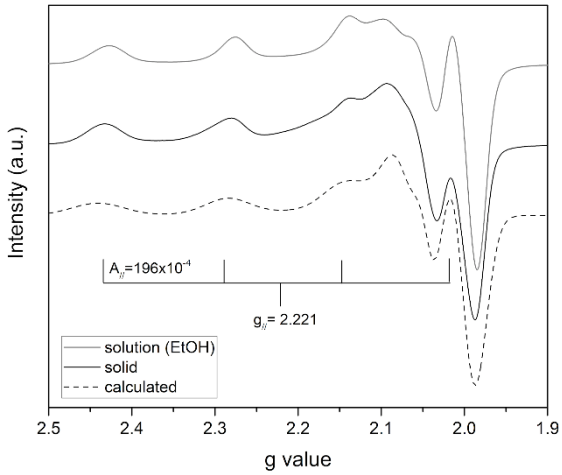

Fig. 2. Experimental (solid lines) and calculated (dotted lines) room temperature Band-X EPR of **CuAlk**. Solid complex in black and ethanolic solution (0.025M) in gray. Calculated values of $g_{//}$ and $A_{//}$ are also shown in the Figure.

Alk molecule (Fig. 1) contains a primary aliphatic amine, a hydroxyl group, and primary and secondary amide groups. Although there have been reports of copper complexes with deprotonated amide groups at neutral pH [19,20], Alk also possesses an amine and hydroxyl groups with higher basicity (the amine group in the free base exhibits a pKa value of 9.49 [1]). Consequently, we propose that the interaction between Alk and Cu(II) occurs through its aliphatic amine and hydroxyl groups. Therefore, only small changes are expected in FTIR spectrum of Alk, according to a previous report by Aydoğmuş et al. [21], since several absorption bands for stretching of O-H and N-H bonds are in the same region, and only a few of them are modified by metal complexation. However, clearer changes have been observed in the EPR and NMR spectra. The FTIR spectral changes are shown in Fig. S1 and listed in Table 1. In the 3500-3000 cm^{-1} region, only a change in the pattern of the broad band is observed due to the modification of the stretching frequencies of O-H and N-H (amine) involved in the coordination and the presence of coordinated water molecules in the metal complex. In the 1700-1600 cm^{-1} region, no changes in amide band [22] have been observed. The sharp band in 1384 cm^{-1} in

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CuAlk is assigned to an ionic nitrate (non-coordinated) stretching mode [23]. The decrease of intensity of the band at 1138 cm^{-1}, assigned to the deformation N-H and rocking NH_2 modes [24], and the increase of intensity of the band due to the C-N stretching plus NH_2 torsion in 1050 cm^{-1}, present as a small shoulder next to the strong band in 1095 cm^{-1} are the main changes observed due to coordination of Alk to Cu(II).

Table 1. Tentative assignment of the main bands observed in FTIR spectra. Values are expressed in cm^{-1}.

Assignment	Alk base	CuAlk
ν N-H (amine and amide) + ν O-H (Alk) + ν O-H (water)	3355 (br,s) 3408 (br)	3335 (br, s) 3431 (br)
ν C=O (amide) + δ (N-H)	1663 (vs) 1650 (sh) 1608 (sh) 1591 (sh)	1665 (vs) 1658 (s) 1650 (sh) 1608 (sh) 1591 (sh)
ν N-O (nitrate)	-	1384 (vs)
δ (N-H) + γ NH_2	1138 (m)	1138 (m/w)
ν C-N aliphatic amine + τ NH_2	1052 (sh, w)	1052 (m)

Abbreviations: vs: very strong, s: strong, br: broad, sh: shoulder, m: medium, w: weak.

The measured ^{1}H NMR and ^{13}C NMR spectra of the free ligand, Alk, are in agreement with the reported values (Fig. S10 and S11) [25]. On the other hand, the ^{1}H NMR spectrum of a solution of **CuAlk** in d_6-DMSO showed broad resonances, as expected for the coordination to a paramagnetic Cu(II) center. The ^{13}C NMR spectrum suggests the coordination to the Cu(II) center, which may result in a reduced number of resonances and a broader range of chemical shifts.

Even though it is difficult to establish differences between the vibrational modes of the three N-containing groups, the tentative assignment presented here is supported by the higher basicity of a primary amine in comparison with primary and secondary amides [21], and the formation of a 5-member ring with Cu(II), and the EPR, NMR and FTMS spectral information. Based on these findings, we propose that **CuAlk** has a structure in which a Cu(II) center is tetragonally coordinated by two nitrogen and two oxygen atoms from the ligand in the equatorial plane (Fig. 3).

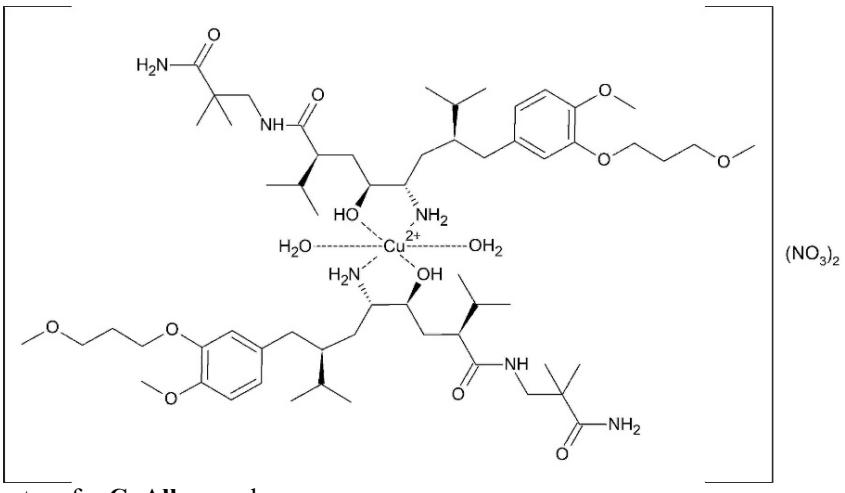

Fig. 3. Proposed structure for **CuAlk** complex.

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The stability of **CuAlk** in ethanolic solution was determined using UV-vis spectroscopy. The observed UV-vis transition with a maximum at λ=644 nm and an ε= 31.9 M^{-1} cm^{-1} corresponds to d-d transitions, and therefore metal-centered, no band for Alk base has been observed in this region. No changes were observed up to 2 h, and the spectral patterns observed were similar to those of the blue solid obtained by diffuse reflectance spectroscopy (Fig. S2). Hence, we propose that the structure of the solid was retained upon dissolution and the complex remained stable at least during 2 h. Furthermore, a spectrophotometric titration of Alk:Cu (Fig. S5) was conducted using various ligand-to-metal molar ratios in ethanolic solutions ranging from L:M 10 to 0.7 at pH 7.5. The results indicated a final stoichiometry of 2:1 (L:M), which is consistent with the structural determination of the solid complex. The recorded FTMS spectrum in EtOH solution shows a complex with two ligands per Cu center as the most abundant species in the solution, indicating also, that the stoichiometry of the complex is retained in solution. The spectrum also shows the presence of different fragments, such as the free ligand and other Cu:L complexes with lower intensities (Fig. S3 and S4).

Antioxidant activities

The antioxidant properties of the free ligand and **CuAlk** were studied by different techniques: the scavenging power of the $DPPH^{\bullet}$, the scavenging of $OH^{\bullet}$ radical, the inhibition of the production of peroxyl radical ($ROO^{\bullet}$, lag phase) and the ability of mimic the SOD activity according to previously reported methods [26].

Neither Alk or **CuAlk** have shown significant scavenging activities on $DPPH^{\bullet}$ or on $ROO^{\bullet}$ radicals at concentrations up to 500 μM (Figs. S6, S7). Even though Cu(II) did not show any scavenging activity on $OH^{\bullet}$ radical in the range of concentrations studied, Alk and **CuAlk** exhibited a significant scavenging activity of this radical (Fig. 4). The statistical analysis of the data shows no difference between them, with an inhibition close to 40 % at the maximum concentration tested (100 μM).

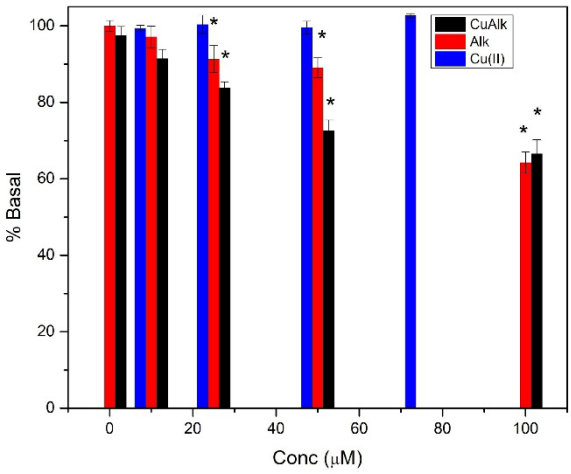

Fig. 4. Scavenging of hydroxyl radical ($OH^{\bullet}$) for **CuAlk** (black), Alk (red) and copper(II) salt (blue). * Significant values in comparison with the control ($p < 0.05$).

For **CuAlk**, the measured SOD activity (IC_{50} = 0.69 μM) (Table 2) is significantly lower than the corresponding value obtained for the metallic cation and comparable to the activity reported for the bovine enzyme (IC_{50}=0.21 μM) [26]. Alk base has not shown significant activity since at the maximum concentration tested (100 μM), an inhibition of only the 65 % of the NBT reduction was observed. In general terms, at neutral pH the value of the rate constant for the second order spontaneous dismutation of the native enzyme is about 2 x 10^7 M^{-1} s^{-1} [27]. From the obtained IC_{50} values, we have compared the activities from the calculation of the k_{McCF} (McCord-Fridovich) constant that standardizes the values and is independent of both detector concentration and nature [28]. The constant is calculated from the equation: $K_{McCF} = k_{detector}$·[detector]/IC_{50}, where k_{NBT} (pH = 7.8) = 5.94 × 10^4 mol^{-1} L s^{-1} and [detector]= NBT concentration in the mixture. Again, a SOD

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mimetic value in the order of the value of the native enzyme was obtained for the complex. Based on these results, it is possible to conclude that the complexation of Cu(II) by Alk enhanced considerably its SOD mimetic behaviour. Just a few reported complexes exhibited such an enhancement in the SOD mimetic activity [29].

Table 2. IC_{50} values and kinetic constants of **CuAlk**, Cu(II) salt and bovine erythrocyte superoxide dismutase.

	IC_{50} (µM)	**K_{McCF} ($mol^{-1}L\ s^{-1}$)**
Cu(II)	3.20[a]	1.3 x 10^6
Alk	100 [b]	ND[c]
CuAlk	0.69	6.4 x 10^6
SOD	0.21[a]	2.1 x 10^7

[a] according to [26], [b] corresponds to 65 % inhibition, [c] not determined

It was suggested that the SOD mimicking ability depends on the coordination geometry around the metal center and the ligand system used, which affects its redox potentials [29]. For example, in copper(II) complexes with peptides and amide groups, a higher SOD-like activity is observed when the complex has a specific nitrogen donor set forming the metal binding site, and the Cu(II) center is weakly coordinated to solvent molecules in the axial position [30]. In this case, the improvement of **CuAlk** in comparison to Alk, could be due to the complexation of copper(II) with N and O donor atoms, according to the previous suggestions.

Conclusions

In this work, a new copper(II) complex $[Cu(Alk)_2(H_2O)_2](NO_3)_2$, **CuAlk** was synthesized by the addition of cupric nitrate to an ethanolic solution of aliskiren. In this new compund, the renin inhibitor aliskiren is acting as a bidentate ligand. The complex is stable in solution at physiological pH value. Neither the complex, nor the ligand were able to scavenge the $DPPH^{\bullet}$ and $ROO^{\bullet}$ radicals. On the other hand, both compounds have shown activity against $OH^{\bullet}$ radical. Furthermore, the complexation of Cu(II) with aliskiren has shown an increase in the SOD mimetic activity, considerably improving the antioxidant properties of the metal center.

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