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Clioquinol, a Drug for Alzheimer's Disease Specifically Interfering with Brain Metal Metabolism: Structural Characterization of Its Zinc(II) and Copper(II) Complexes

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Clioquinol, a 8-hydroxyquinoline derivative, is producing very encouraging results in the treatment of Alzheimer's disease (AD). Its biological effects are most likely ascribed to complexation of specific metal ions, such as copper(II) and zinc(II), critically associated with protein aggregation and degeneration processes in the brain. We report here, for the first time, a structural characterization of the zinc(II) and copper(II) complexes of clioquinol. A ligand to metal stoichiometry of 2:1 is found in both cases, though in the presence of quite different coordination polyhedra. The present findings are discussed in the frame of modern approaches to AD treatment.

Several recent reports have provided sufficient evidence that metal ions are critically involved in the etiopathogenesis of Alzheimer's disease (AD)[1] as well as in other neurodegenerative diseases.[2,3] For instance, various research groups have shown that increases in the brain concentrations of metal ions such as zinc(II) and copper(II) greatly facilitate pathological protein aggregation processes.[2−4] Atwood and colleagues reported that zinc(II) induces aggregation in vitro of soluble β-amyloid (Aβ), at pH 7.4, and that this reaction is totally reversible with metal chelation.[5] Also, copper(II) favors Aβ aggregation when working at pH 6.8; again, the process may be reversed by removing the metal.[6] In addition, free copper(II) is known to promote potentially neurotoxic oxidation processes of biological substrates.[2]

In view of these and similar observations, Bush et al. recently proposed that the so-called "metal−protein attenuation compounds" (MPAC) approach should be seriously evaluated as an innovative strategy for the pharmacological treatment of AD.[2,7,8] This approach represents a modification of classical chelation therapy: instead of choosing strong chelating agents eventually leading to metal depletion, ligands with intermediate affinity are preferred, and these are capable of disrupting low affinity but pathologically relevant metal protein interactions, while not depleting the metal itself. Afterward, an intensive search for compounds capable of interfering selectively with pathological metal metabolism in the brain began. Among the tested ligands, clioquinol (5-chloro-7-iodo-8-hydroxyquinoline, CQ hereafter), a substance formerly used as an anti-infective agent, produced very promising effects in early clinical trials on AD patients.[8−10] The favorable pharmacological effects of CQ are probably related both to its lipophilicity and its ability to form relatively stable complexes with zinc(II) and copper(II) ions. Specifically, coordination of zinc(II) and its displacement from low affinity binding sites of the Aβ protein in the brain would result in the clearance of Aβ tangles;[2] concomitant copper(II) complexation would lead to a net decrease in neurotoxic H_2O_2 production.[2]

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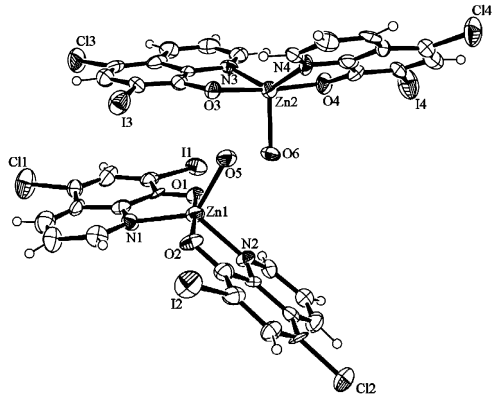

Figure 1. View of the two complex molecules in the asymmetric unit of **1** (thermal ellipsoids drawn at 50% probability). Selected bond distances (Å): Zn(1)−O(1) 2.085(13), Zn(1)−O(2) 2.060(12), Zn(1)−O(5) 2.022-(14), Zn(1)−N(1) 2.039(14), Zn(1)−N(2) 2.040(16), Zn(2)−O(3) 2.055-(12), Zn(2)−O(4) 1.994(13), Zn(2)−O(6) 2.045(14), Zn(2)−N(3) 2.078(15), Zn(2)−N(4) 2.098(16).

The coordination chemistry of CQ has been little investigated so far. However, by analogy with similar 8-hydroxyquinoline (8-HQ) ligands, it is believed that CQ, upon deprotonation of its phenolic group, forms 2:1 complexes with either zinc(II) or copper(II) ions in such a way that the resulting ML_2 species are neutral.[11] The coordination mode of CQ to zinc(II) and copper(II) is thought to match that of 8-HQ, with the pyridine nitrogen and the phenolate oxygen as metal donors of the bidentate ligand; higher stability of the copper(II) complex over the zinc(II) one is expected, in line with previous results on 8-HQ derivatives.[12] In any case, as far as we know, no detailed chemical or structural characterization has been reported for the $Zn(CQ)_2$ and $Cu(CQ)_2$ species that are believed to form in vivo following clioquinol treatment.

Prompted by the renewed, great interest in clioquinol chemistry, and by the promising results obtained in AD treatment, we have prepared and crystallized the copper(II) and zinc(II) complexes of this ligand and determined their structures by X-ray diffraction methods.

Specifically, crystals of the zinc(II) complex were prepared by mixing a THF solution of CQ with the stoichiometric amount of zinc(II) sulfate, dissolved in water (working at a 1:2 Zn/CQ ratio). Small orange needles, suitable for X-ray diffraction studies, formed within a few days, at room temperature, upon slow evaporation. Similarly, pale orange crystals of the copper complex were obtained by slow evaporation of a DMF solution of CQ after addition of the required amount of an aqueous solution of copper(II) sulfate (again at a 1:2 Cu/CQ ratio).

The structure of the zinc(II) complex (**1**) consists of $[Zn(CQ)_2(H_2O)]$, THF, and water solvate molecules.[13] The asymmetric unit contains two symmetry-independent $[Zn(CQ)_2(H_2O)]$ molecules (Figure 1), where the metal ions exhibit similar coordination environments. Each zinc(II) cation is coordinated by the oxygen and nitrogen atoms of two ligand anions, and by a water oxygen, in an approximately trigonal bipyramidal environment. The phenolic oxygens occupy the axial positions of the trigonal bipyramids [O1−Zn1−O2 172.5(6)° and O3−Zn2−O4 173.3(6)°], while the two nitrogens and the water oxygen lie in the equatorial plane. Distances to the metal roughly agree with those found for other five-coordinate zinc(II) complexes formed by 8-hydroxyquinolines,[14,15] although detailed comparisons are difficult due to differences in geometries and to a pronounced scattering of distance values. The CQ ligands in the complex molecules of **1** form interplanar angles of 135.3(4)° (Zn1) and 147.2(3)° (Zn2). As it appears from Figure 1, two ligands, one from each of the symmetry-independent molecules, interact via π-stacking at ca. 3.4 Å distance, their planes forming a 6.0(4)° angle. Such interactions, extended through the operation of inversion centers, give rise to parallel pillars of $[Zn(CQ)_2(H_2O)]_2$ units growing along the [111] crystallographic direction.

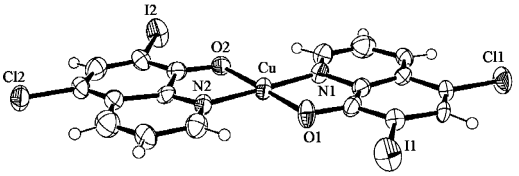

Figure 2. View of the complex molecule in the structure of **2** (thermal ellipsoids drawn at 50% probability). Selected bond distances (Å): Cu−O(1) 1.915(9), Cu−O(2) 1.922(9), Cu−N(1) 1.964(10), Cu−N(2) 1.984-(10).

In the structure of the copper derivative (**2**), the $[Cu(CQ)_2]$ molecules (one per asymmetric unit) are planar and pseudocentrosymmetric.[16] The Cu(II) ion is in a tight square planar environment (Figure 2) of two oxygen and two nitrogen atoms, forming distances to the donor atoms significantly shorter than those found for **1**. Such distances agree with those [Cu−O 1.94(1) Å, Cu−N 1.98(1) Å] found in an earlier study of an essentially four-coordinate 8-HQ copper(II) derivative,[17] whereas they are slightly shorter (by 0.04−0.07 Å) than the analogous distances in a five-coordinate[18] and a six-coordinate[19] complex. In addition, each copper(II) ion in **2** forms long-range interactions, within the 3.4−3.8 Å interval, on both sides of the molecular plane, with the carbon atoms of the aromatic rings of contiguous molecules. Such interactions give rise to stacks extending in the direction of the crystallographic *a*-axis.

While many metal complexes formed by the 8-HQ ligand, possibly substituted in a variety of ways, are known, and the structures of many of these have been determined, to

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(13) Crystal data for **1**: $C_{40}H_{30}Cl_4I_4N_4O_8Zn_2$, $P\bar{1}$, $a = 10.265(5)$ Å, $b = 11.432(5)$ Å, $c = 20.320(5)$ Å, $\alpha = 99.610(5)°$, $\beta = 95.560(5)°$, $\gamma = 92.510(5)°$, $V = 2335.6(16)$ Å^3, $Z = 2$, R1 = 0.075.
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(16) Crystal data for **2**: $C_{18}H_8Cl_2CuI_2N_2O_2$, $P2_1$, $a = 4.880(4)$ Å, $b = 10.827(5)$ Å, $c = 18.012(7)$ Å, $\beta = 91.13(7)°$, $V = 951.5(10)$ Å^3, $Z = 2$, R1 = 0.032.
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the best of our knowledge no structure of metal complexes of the present 5-chloro-7-iodo-8-quinoline ligand has been reported so far, and only nickel(II) complexes of the CQ-related 5,7-dichloro-8-quinoline have been structurally characterized.[20,21] Thus, detailed structural information on the coordinating properties of this interesting ligand is provided here for the first time.

Presently, clioquinol is behaving very well in experimental treatments of AD, both preclinical and clinical.[8,9] The high lipophilicity of the free ligand may explain its facile access to the brain while selective metal chelation may account for its effects against neurodegeneration.

We have shown that neutral ML_2 species are readily obtained when clioquinol is reacted in vitro either with zinc(II) or copper(II) ions. Owing to the relatively large affinity constants of CQ for both metal ions,[11] it is likely that such ML_2 species may form in vivo as well. However, recent reports point out that CQ treatment does not result in eventual copper and zinc depletion in the brain.[9,22] Conversely, it is hypothesized that clioquinol just produces mobilization and transfer of selected metal ions among different intracellular sites, with no significant changes in total metal concentrations.[2,8] Specifically, intracellular mobilization of zinc(II) and its removal from low affinity binding sites on the Aβ protein would be very effective in preventing and/or clearing formation of neurotoxic β-amyloid aggregates, while complexation of copper(II) might lead to a diminished production of highly neurotoxic hydrogen peroxide.[2]

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Supporting Information Available: Crystallographic data for **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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