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Searching for new aluminium chelating agents: A family of hydroxypyrone ligands

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ABSTRACT

Attention is devoted to the role of chelating agents in the treatment of aluminium related diseases. In fact, in spite of the efforts that have drastically reduced the occurrence of aluminium dialysis diseases, they so far constitute a cause of great medical concern. The use of chelating agents for iron and aluminium in different clinical applications has found increasing attention in the last thirty years. With the aim of designing new chelators, we synthesized a series of kojic acid derivatives containing two kojic units joined by different linkers. A huge advantage of these molecules is that they are cheap and easy to produce. Previous works on complex formation equilibria of a first group of these ligands with iron and aluminium highlighted extremely good pMe values and gave evidence of the ability to scavenge iron from inside cells. On these bases a second set of bis-kojic ligands, whose linkers between the kojic chelating moieties are differentiated both in terms of type and size, has been designed, synthesized and characterized. The aluminium^{III} complex formation equilibria studied by potentiometry, electrospray ionization mass spectroscopy (ESI-MS), quantum-mechanical calculations and ^{1}H NMR spectroscopy are here described and discussed, and the structural characterization of one of these new ligands is presented. The in vivo studies show that these new bis-kojic derivatives induce faster clearance from main organs as compared with the monomeric analog.

1. Introduction

Overviews on the pathological effects of aluminium overload in humans, and on its role in neurodegenerative diseases have been recently presented [1,2]. Aluminium was regarded as a non-toxic metal ion till the seventies of the last century, and its products have a number of applications, in medicine, in food processing, in water treatment, etc. The awareness that neurological and bone diseases in patients under dialysis treatment were related with aluminium toxicity encouraged the research on the management of aluminium intoxication. The reduction of all parenteral and oral aluminium exposures contributed to decrease aluminium dependent diseases in the last 20 years [3,4]. The aluminium chelation was recommended when patients did not clinically improve when aluminium exposure ceased [5]. Deferoxamine was the first aluminium chelator introduced in clinical practice that reduces not only bone aluminium deposit but also aluminium burden in the brain [6–12]. The acute neurological complications, which may be developed during Deferoxamine therapy for aluminium bone diseases, limited this treatment only to those patients with serum aluminium levels higher than 200 μg/L, or with aluminium bone concentration ten times greater than normal values [5,13,14]. Different aluminium chelators have been then introduced [15].

The aluminium chelation therapy has been founded on that in use for iron. Actually, massive research efforts due to the worldwide diffusion of iron overload diseases have lead to significant improvements in iron chelation. Evidence has been given for the utility, in aluminium dependent pathologies, of the knowledge acquired on iron chelating agents [16,17].

With the aim of designing new ligands that form high stability complexes, which satisfy the chemical and biological requirements for

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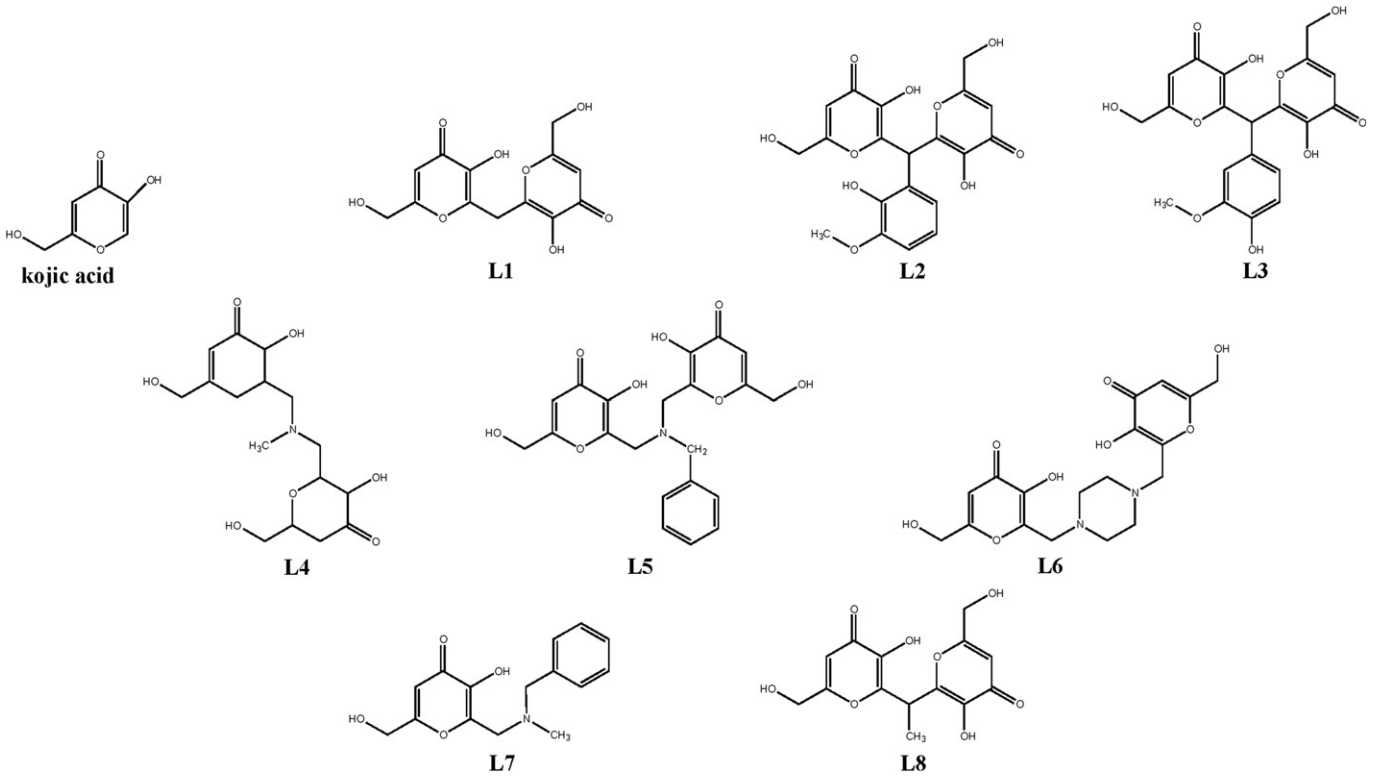

Scheme 1. Chemical structures and acronyms of studied ligands.

an effective chelating agent such as selectivity, lipophilicity and bioavailability, our group has synthesized some derivatives of kojic acid, and studied their complex formation equilibria with Fe^{III} and Al^{III}, as well as those with the parent ligand kojic acid (Scheme 1).

In previous works, the formation of MeL, MeL_2, and MeL_3 complexes of Al^{III} and Fe^{III} with kojic acid was remarked, and of diverse protonated species of Me_2L_2 and MeL_2 complexes with L1 [18], and with the related compounds in which vanillin and *o*-vanillin (L2 and L3) substituents were inserted on the linker [19]. The found pFe values (23.1 for L1, 18.9 for L2 and 22.2 for L3), lower than that for desferal (26.6) and comparable with that of deferiprone (20.7), and the fact that these ligands are easily and cheap to produce were very encouraging. We have recently synthesized a new set of bis-kojic ligands in which different linkers connecting the two kojic coordinating moieties have been designed for improving the interaction between the kojic units and the metal ions.

In this paper we will report the study on the complex formation equilibria of ligands L4–L8 as well as the structure characterization of L4 by X-ray diffraction. The in vivo efficacy of the ligands L4, L5, L7 and L8 as potential sequestering agents was also studied and reported herein, namely for the Ga-67 mobilization in mice previously injected with the radiotracer ^{67}Ga-citrate, as an animal model of Al-overload.

2. Experimental

2.1. Reagents

All the products, NaOH, KOH, and $AlCl_3$ purchased from Aldrich, HCl from Fluka, KCl from Carlo Erba (Milan, Italy), were used without further purification. An already described method was used for 0.1 M carbonate free KOH solution [20]. Ligand solutions were acidified with stoichiometric equivalents of HCl. Al^{III} solution was prepared by dissolving the required amount of $AlCl_3$ in pure double distilled water to which a stoichiometric amount of HCl was previously added to prevent hydrolysis. This solution was standardized by EDTA titration.

2.2. Synthesis

The synthesis of the ligands in Scheme 1 has been previously reported [21].

2.2.1. Synthesis of the L4 crystal

L4 (15 mg) was dissolved in distilled water (3 mL), aided by drop wise addition of HCl 0.01 M. Afterwards, isopropanol (3 mL) was added and the solution was left stirring for 30 min and then filtered into a crystallization device to remove possible impurities. The solution was placed into an acetone chamber diffusion, where acetone acts as antisolvent in crystallization process. After three weeks, parallelepiped colorless crystals appeared suitable for X-ray diffraction (XRD). It is also possible to obtain single crystals of L4 without acetone diffusion, leaving the solution to stand at room temperature. However, the quality of the crystals is lower, hence good quality data could not be obtained.

2.3. Potentiometric measurements

Potentiometric measurements of the complex formation equilibria were carried out under the same conditions described in a previous publication [18]. The operating ligand concentrations ranged from 3×10^{-4} to 3×10^{-3} M according to the examined ligand. The studies of complex formation were carried using constant ligand concentration, and 1:1, 1:2, and 1:3 metal/ligand molar ratios. To take into account the low complex formation rate with Al(III), a suitable procedure was used: the titrations started 1 h after the mixing of the reagents, long delay times between two subsequent additions were used (2–7 min) and the achievement of the equilibrium was checked using a drift parameter