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Combined chelation of bi-functional bis-hydroxypiridinone and mono-hydroxypiridinone: Synthesis, solution and *in vivo* evaluation

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ABSTRACT

3-Hydroxy-4-pyridinones (3,4-HP) are well known iron-chelators with applications in medicinal chemistry, mainly associated with their high affinity towards trivalent *hard* metal ions (e.g. M^{3+}, M = Fe, Al, Ga) and use as decorporating agents in situations of metal accumulation. The polydenticity and the extra-functionality of 3,4-HP derivatives have been explored, aimed at improving the chelating efficacy and the selectivity of the interaction with specific biological receptors. However, the ideal conjugation of both features in one molecular unity usually leads to high molecular weight compounds which can have crossing-membrane limitations.

Herein, a different approach is used combining a arylpiperazine-containing bis-hydroxypyridone (H_2L^1) with a biomimetic mono-hydroxypyridinone, ornithine-derivative (HL^2), to assess the potential coadjuvating effect that could result from the administration of both compounds for the decorporation of *hard* metal ions. This work reports the results of solution and *in vivo* studies on their chelating efficacy either as a simple binary or a ternary system (H_2L^1:HL^2:M^{3+}), using potentiometric and spectrophotometric methods. The solution complexation studies with Fe(III) indicate that the solubility of the complexes is considerably increased in the ternary system, an important feature for the metal complex excretion, upon the metal sequestration. The results of the *in vivo* studies with ^{67}Ga-injected mice show differences on the biodistribution profiles of the radiotracer, upon the administration of each chelating agent, that are mainly ascribed to the differences of their extra-functional groups and lipo/hydrophilic character. However, administration of both chelating agents leads to a more steady metal mobilization, which may be attributed to an improved access to different cellular compartments.

1. Introduction

Metal ions as iron and aluminium, when in excess or accumulated in tissues, can cause organ dysfunctions and be responsible for serious pathologies or neuropathologies. Hemochromatosis and β-thalassemia (requiring frequent blood transfusions) are examples of iron overload diseases [1,2]. Aluminium accumulation can cause lesions in the central nervous system tissues and be responsible for numerous human neurological disorders [3].

Desferrioxamine (DFO) is a hexadentate tris-hydroxamic siderophore which, for decades, has been used for the excretion of body iron excess. Due to drawbacks associated with DFO, other iron-chelating drugs have been discovered, including 1,2-dimethyl-3-hydroxy-4-pyridinone (commercially available as deferriprone, DFP), an orally active iron-chelating drug also currently used in β-thalassemic patients [4,5]. The combined chelation therapy with DFO and DFP is also being used in special situations of regularly transfused iron-overload patients [6–9], improving the metal mobilization, either in an additive or a synergistic manner, probably through iron "shuttling" from DFP to DFO [10]. The Al-decorporation has also been tested with this and other chelating combinations [11], such as ascorbate (AS) and DFO and/or Feralex-G, (a glycosyl-hydroxypyridinone derivative) [12].

Since the discover of DFP, 3-hydroxy-4-pyridinones (3,4-HP) have been quite explored due to their high affinity towards trivalent *hard* metal ions (M = Fe, Al, Ga). In fact, besides the above referred interest for aluminum and iron chelation, the complexation with gallium is also of interest because gallium radionuclides can be used as imaging diagnosis tools, namely ^{68}Ga in positron emission tomography (PET) [13,14]. In order to obtain better chelating agents for clinical application, several aspects of the 3,4-HP derivatives

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have been explored, namely their polydenticity [15–18] and extrafunctionality [19–21], to improve the chelating efficacy and the selectivity of the biological interaction, respectively. The potential use of 3,4-HPs with complementary properties (e.g. denticity and molecular size) for combined chelation has also been recently analysed [22].

We have developed a new bis-hydroxypyridone extrafunctionalized with an arylpiperazine group, which is known to be recognized by serotonin and sigma receptors, thus providing targeting properties for brain and tumour-imaging agents [23–26]. In this ligand IDAPipPr(3,4-HP)$_2$ (H_2L^1), two 3,4-HP chelating moieties are appended to an iminodiacetic acid (IDA) scaffold (see Scheme 1), which has *N*-attached a 1,4-dissubstituted arylpiperazine. However, the saturation of all the M^{3+} six coordination sites by a bis-hydroxypyridinonate ligand can lead to the formation of a dinuclear complex, with expected higher problems on crossing biological membranes than a mononuclear complex. Also, the water solubility is often much lower for a polynuclear than a mononuclear species. Therefore, to overpass these potential limitations of H_2L^1 bioavailability and to take profit of eventual coadjuvating effects, the chelating properties of a combination of this bis-hydroxypyridinone ligand with a biomimetic mono-hydroxypyridinone (ornithine-derivative) were also explored.

Herein we report the synthesis of the new tetradentate ligand IDAPipPr(3,4-HP)$_2$ (see Scheme 1), the aqueous solution studies and the *in vivo* assays for metal decorporation. The solution studies allowed the determination of the acid–base properties of this ligand and its chelating ability towards M(III) metal ions (M = Fe, Al, Ga), either alone or combined with the Orn(3,4-HP) chelating agent (HL^2). These equilibrium studies were performed using potentiometric and spectrophotometric (UV–Vis, ^{1}H NMR) measurements. The lipo/hydrophilic character was also evaluated based on the 1-octanol/water partition coefficient of the compounds.

The *in vivo* biodistribution studies were performed to assess the ability of these chelating systems for the removal of a radiotracer (^{67}Ga) injected in mice, as a model of iron-overload animal. The chelating ability of H_2L^1 and HL^2, when used separately and in combination, were evaluated and compared. Also, the biodistribution of the ^{67}Ga–L^1 complex in different organs was assayed. The results are discussed in comparison with others previously reported for analogous compounds.

2. Experimental

2.1. Reagents and solutions

All chemicals used were p.a. grade. When anhydrous conditions were necessary, the solvents were dried using methods described in literature [27]. The chemical reactions were followed by TLC. After synthesis and recrystallization of the ligands, the concentrations of their stock solutions were confirmed by the Gran's method [28]. The metal ion (Al, Fe, Ga) stock solutions were prepared, respectively, from $AlCl_3 \cdot 6H_2O$ (in diluted HCl solution), $FeCl_3$ (in diluted HCl solution), $Ga(ClO_4)_3$ (in diluted $HClO_4$ solution).

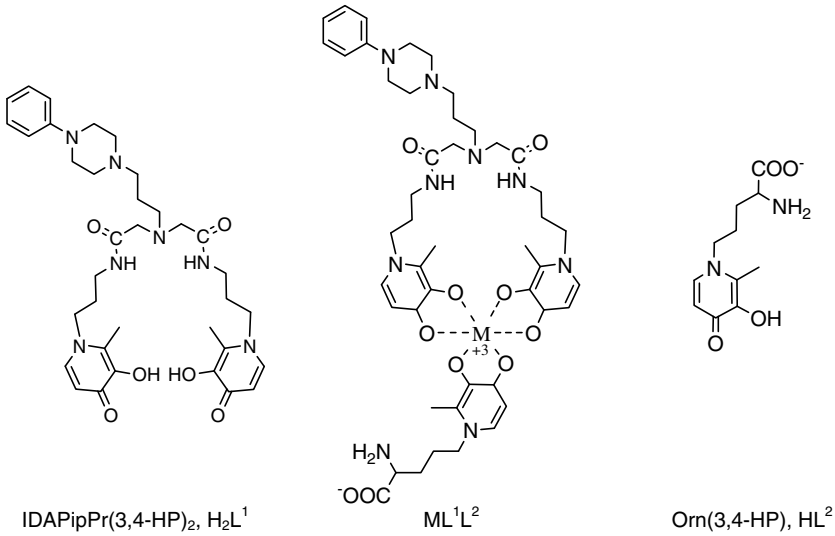
Except in the case of Ga(III), the concentrations of the metal ion stock solutions were determined gravimetrically via precipitation of quinolin-8-olates. For the measurement of the concentration of Ga(III), known amount of EDTA was added and the excess of EDTA was determined with $ZnCl_2$ stock solution. The HCl concentration of the Fe(III) and Ga(III) solutions were determined by pH-potentiometry.

2.2. Synthesis of the ligand

2.2.1. *N-(3-(4-Phenylpiperazin-1-yl)propyl)imino-bis(acetyl(1-(3′-aminopropyl)-3-benzyloxy-2-methyl-4-pyridinone))*

1-(3̀-Aminopropyl)-3-benzyloxy-2-methyl-4-pyridinone hydrochloride [17] (1.004 g, 3.24 mmol) was dissolved in dry methanol (10 mL) cooled in an ice-bath to 0 °C. KOH pellets (397 mg, 7.08 mmol) were added to the cooled solution, under nitrogen. The mixture was stirring for 30 min, the formed KCl was filtered out, the solution was evaporated and the free 1-(3′-aminopropyl)-3-benzyloxy-2-methyl-4-pyridinone was dried under vacuum.

In a different flask, *N*-(3-(4-phenylpiperazin-1-yl)propyl)iminodiacetic acid [29] (625 mg, 1.67 mmol) was dissolved in dry dimethylformamide (DMF, 55 mL). The solution was cooled down in a ice bath, and *N*-methyl-morpholine (0.400 mL, 3.64 mmol) and *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU, 1003 mg, 3.12 mmol) were added. The yellow solution obtained was stirred at 0 °C under nitrogen atmosphere for 50 min. The solution was filtered and then added to a solution of free 1-(3′-aminopropyl)-3-benzyloxy-2-methyl-4-pyridinone in dry DMF (*ca.* 6 mL) and cooled down with a NaCl–ice cooling bath.



Scheme 1.