Journal of Inorganic Biochemistry 186 (2018) 116–129

Contents lists available at ScienceDirect

Journal of Inorganic Biochemistry

journal homepage: www.elsevier.com/locate/jinorgbio

Bifunctional 3-hydroxy-4-pyridinones as effective aluminium chelators: synthesis, solution equilibrium studies and *in vivo* evaluation

Anna Irto[a], Paola Cardiano[a], Karam Chand[b], Rosalia Maria Cigala[a], Francesco Crea[a], Concetta De Stefano[a], Lurdes Gano[c], Silvio Sammartano[a,*], Maria Amélia Santos[b,*]

[a] Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche e Ambientali, Università di Messina, Viale F. Stagno d'Alcontres, 31, 98166 Messina, Italy
[b] Centro de Química Estrutural, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovísco Pais 1, 1049-001 Lisboa, Portugal
[c] Centro de Ciências e Tecnologias Nucleares (C2TN), Instituto Superior Técnico, Universidade de Lisboa, Estrada Nacional 10, 2695-066 Bobadela LRS, Portugal

ARTICLE INFO

Keywords:
3-Hydroxy-4-pyridinones
Speciation
Protonation
Al^{3+} complexation
Sequestering ability
In vivo chelation

ABSTRACT

This paper reports the results on the study of a set of synthesized bifunctional 3-hydroxy-4-pyridinones chelators as potential aluminium sequestering agents. They were *N*-functionalized with alkyl-amino, -carboxylic and –(amino-carboxylic) groups, envisaging the improvement of the Al^{3+} sequestering capacity, in comparison with the marketed chelating drug deferiprone. The main focus of this work was given to the assessment of their binding ability towards Al^{3+}, which was studied by potentiometric and UV–Vis spectrophotometric measurements carried out at $T = 298.15$ K. The speciation models were characterized by $Al_pL_qH_r^{(3p+r-qz)}$ species with different stoichiometry. Depending on ligand side-chain structures and on their thermodynamic properties, different trends of stability was found. Furthermore, the sequestering ability of the ligands towards Al^{3+} was investigated by the calculation of $pL_{0.5}$ values at different experimental conditions. These results clearly indicate that the presence of amino-carboxylic groups in the ligands increases the sequestering ability towards Al^{3+}. The *in silico* evaluation of pharmacokinetic descriptors indicated no violation to the Lipinski's rule and drug-likeness properties. Furthermore, the *in vivo* bioassays on a model of metal-overload mice showed for three investigated ligands a higher metal-sequestering capacity than for the chelating drug deferiprone, thus suggesting their potential interest as Al-chelating drug candidates.

1. Introduction

The treatment of diseases related to the accumulation of hard metal cations in the human body is based on the chelating therapy. This approach consists in the administration of chelators to patients, to induce the sequestration of the metal ions (*e.g.* Fe^{3+}, Al^{3+}, etc.) and their systemic excretion [1–4]. Intake of metals in the human body can occur through the diet, the environment or other external sources; once absorbed, they reach the blood and human organs, competing with other essential metals for vital functions. For a long time the Deferoxamine (DFO or Desferal®), was used as chelator in the treatment of iron overload [5]. It is a microbial trishydroxamic acid [6] able to form with Fe^{3+}, as hexadentate ligand, a metal–ligand complex, having high stability from the thermodynamic point of view ($\log K_{[FeL]^{2+}} = 30.6$ at $I = 0.10$ M in $KCl_{(aq)}$ and $T = 298.15$ K) [7,8]. On the other hand, it is featured by various side effects, such as hydrophilic character, oral inactivity, toxicity, being also very expensive [9]. To overcome these drawbacks, the employment of a new class of chelating agents has been developed, *i.e.* the family of 3-hydroxy-4-pyridinones (3,4-HPs), derivatives of 1,2-dimethyl-3-hydroxy-4-pyridinone, commercially known as deferiprone (DFP) or Ferriprox®, and approved as oral drug for the treatment of patients affected by iron overload. These molecules are a class of bidentate compounds, characterized by an aromatoid *N*-heterocycle with hydroxyl and ketone groups in ortho position. Unlike DFO, 3,4-HPs can be effective in all biological conditions, do not cause undesired effects and are also economically affordable [10–13]. These products can be synthesized from maltol, a natural origin compound [14].

The 3,4-HP herein studied are extra-functionalized (*see* Fig. 1), envisaging the improvement of their affinity towards biological sites and drug-likeness properties. Although some of these compounds (*L1*: 4-(3-hydroxy-2-methyl-4-oxopyridin-1(4H)-yl)butanoic acid; *L5*: 1-(3-aminopropyl)-3-hydroxy-2-methylpyridin-4(1H)-one; *L4*: (*S*)-2-amino-5-(3-hydroxy-2-methyl-4-oxopyridin-1(4H)-yl)pentanoic acid) have already been studied by some of the authors [15–17], the fact that the ligand with the amino-carboxylic side chain (*L4*) presented a good *in vivo*

* Corresponding authors.
E-mail addresses: ssammartano@unime.it (S. Sammartano), masantos@ist.utl.pt (M.A. Santos).

https://doi.org/10.1016/j.jinorgbio.2018.05.017
Received 14 February 2018; Received in revised form 25 May 2018; Accepted 26 May 2018
Available online 30 May 2018
0162-0134/

Abbreviations table

DFB	Deferoxamine or Desferal®
DFP	1,2-dimethyl-3-hydroxy-4-pyridinone, deferiprone or Ferriprox®
L1	4-(3-hydroxy-2-methyl-4-oxopyridin-1(4H)-yl)butanoic acid
L2	(*S*)-2-amino-4-((2-(3-hydroxy-2-methyl-4-oxopyridin-1(4H)-yl)ethyl)amino)-4-oxobutanoic acid
L3	(*S*)-2-amino-4-((3-(3-hydroxy-2-methyl-4-oxopyridin-1(4H)-yl)propyl)amino)-4-oxobutanoic acid;
L4	(*S*)-2-amino-5-(3-hydroxy-2-methyl-4-oxopyridin-1(4H)-yl)pentanoic acid
L5	1-(3-aminopropyl)-3-hydroxy-2-methylpyridin-4(1H)-one
EthylL	2-(3-hydroxy-2-ethyl-4-oxopyridin-1(4H)-yl)acetic acid
UV–Vis spectrophotometry	Ultraviolet-Visible spectrophotometry
NMR	Nuclear Magnetic Resonance
ESI-MS	Electrospray Ionization Mass Spectroscopy
MS-LC Ion Trap	Mass-Liquid Chromatography Ion Trap
BnCl	benzyl chloride
MeOH	methanol
EtOH	ethanol
10% Pd/C	palladium on activated carbon, 10% (w/w)
TLC	Tin Layer Chromatography
DCM	dichloromethane
DMF	*N*-dimethylformamide
DMSO	dimethyl sulfoxide
ACN	acetonitrile
Et_2O	diethyl ether
TMS	tetramethylsilane

performance in terms of metal clearance, led us to develop other two new analogues (*L2:* (*S*)-2-amino-4-((2-(3-hydroxy-2-methyl-4-oxopyridin-1(4H)-yl)ethyl)amino)-4-oxobutanoic acid; *L3:* (*S*)-2-amino-4-((3-(3-hydroxy-2-methyl-4-oxopyridin-1(4H)-yl)propyl)amino)-4-oxobutanoic acid) containing the same terminal group but with an extra amide bond. So the set of five bifunctional ligands containing as side groups amino-carboxylic, as well as amino, carboxylic groups (for comparison purposes) were prepared and evaluated for their Al^{3+}-sequestering capacity. Their preparation involved standard reactions of maltol with bifunctional amines, while in some cases further coupling reactions with a cyclic amino-acid anhydride with the formation of new amidic bonds [5,11–13]. The synthetic procedures used in the preparation of a set of the bifunctional 3-hydroxy-4-pyridinones are schematically described in Scheme 1. The acid-base properties were investigated by Ultraviolet-Visible (UV–Vis) spectrophotometry, spectrofluorimetry, while the binding ability towards Al^{3+} was studied by potentiometry and UV–Vis spectrophotometry. With the purpose of confirming the speciation models proposed on the basis of the cited investigations, the protonation behavior of *L2* as well as its interaction with Al^{3+} was further studied by ^{1}H Nuclear Magnetic Resonance (NMR) spectrometry. The measurements were carried out at $I = 0.15$ M

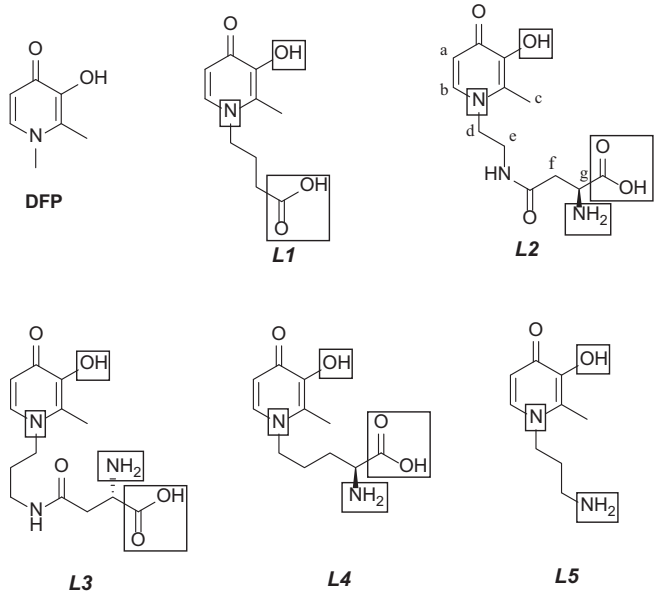

Fig. 1. Structures of deferiprone (DFP) and bifunctional 3-hydroxy-4-pyridinones under study. For *L2*, the letters stand for the ^{1}H NMR titrations peaks assignment. For each ligand the protonable groups are highlighted.

in NaCl, the main inorganic component of most of natural [18,19] and biological fluids [20]. The investigations on the acid-base properties of these 3-hydroxy-4-pyridinones (3,4-HPs) were performed at $T = 298.15$ and 310.15 K, with the aim of assessing their behavior also at physiological conditions. To complete this study, the sequestering ability of the ligands towards the Al^{3+} was investigated, by means of a sigmoidal equation and of $pL_{0.5}$ parameter, previously proposed by the research group [21]. *In vivo* assays on metal-sequestration of a ^{67}Ga treated mice model were also carried out for the 3,4-HP-amino-acid derivatives (*L2, L3, L4*) in comparison with the market drug (DFP).

2. Materials and methods

2.1. Chemicals for solution studies

All the reagents were of the highest available purity and the solutions were prepared with analytical grade water ($R = 18\,M\Omega\,cm^{-1}$) using grade A glassware. Hydrochloric acid and sodium hydroxide solutions were prepared by diluting concentrated ampoules (Riedel-deHäen) and were standardized against sodium carbonate and potassium hydrogen phthalate, respectively. NaOH solutions were preserved from atmospheric CO_2 using soda lime traps. The aluminium solutions were prepared by weighing $AlCl_3$ hexahydrate, without further purification, and standardized with EDTA standard solutions; their purity resulted always ≥98% [22]. NaCl aqueous solutions were prepared by weighing the pure salt (Fluka), previously dried in an oven at $T = 383.15$ K for at least 2 h.

2.2. General synthetic information

Analytical grade reagents were purchased from Sigma-Aldrich, Fluka and Acros and were used as supplied. The solvents, if necessary, were dried according to standard methods [23]. All the reactions were TLC (Tin Layer Chromatography) controlled and the most common used mobile phases were dichloromethane (DCM): methanol (MeOH): ammonium hydroxide (NH_4OH) solvents mixtures: S1 (DCM-MeOH 9.5:0.5), S2 (DCM-MeOH-NH_4OH 8.5:1:0.5), S3 (DCM-MeOH-NH_4OH 8:2:0.5) (for more details *see* Abbreviations table). Moreover, ferric chloride (to check the presence of phenol groups), ninhydrine (for amino groups) and Dragendorff's reagent (if quaternary nitrogen groups were present) assays were carried out. The purity of the synthesized compounds was checked by ^{1}H and ^{13}C NMR (proton and 13-carbon Nuclear Magnetic Resonance) experiments, performed with Bruker AVANCE III 300 MHz and 400 MHz spectrometers, in deuterated solvents (D_2O, Methanol-d_4, Dimethyl sulfoxide-d_6 (DMSO-d_6)). Melting point of each 3-hydroxy-4-pyridinone was measured using a Leica Galen III hot stage apparatus. The following abbreviations are used: s = singlet, d = doublet, t = triplet, m = multiplet. Electrospray