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# Evaluation of 1,2-dimethyl-3-hydroxy-4-pyridinecarboxylic acid and of other 3-hydroxy-4-pyridinecarboxylic acid derivatives for possible application in iron and aluminium chelation therapy

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ABSTRACT

Four new possible chelating agents for iron and aluminium, 1,2-dimethyl-3-hydroxy-4-pyridinecarboxylic acid (DT712), 3-hydroxy-1,2,6-trimethyl-4-pyridinecarboxylic acid, 2,6-dimethyl-3-hydroxy-4-pyridinecarboxylic acid, and 2-ethyl-3-hydroxy-1-methyl-4-pyridinecarboxylic acid, were synthesized, and their complex formation with Fe(III) and Al(III) was studied by potentiometry, UV–Vis, $^1$H NMR, and electrospray mass spectrometry (ESI-MS). Number, stoichiometry, and stability constants of metal–ligand complexes were obtained at 25 °C in aqueous (Na)Cl 0.6 m. DT712 is the most promising hydroxypyridinecarboxylic acid considered so far for iron chelation therapy, as it forms the strongest Fe(III) complexes. This compound was further investigated to better clarify its possible behaviour *in vivo* with particular respect to iron chelation therapy. UV–Vis measurements were performed to determine the kinetics by which DT712 extracts Fe(III) from transferrin. DT712 resulted to have better kinetic properties than existing iron chelators. Ternary metal/DT712/citric acid complexes were studied by ESI-MS to check the competition with a typical low molecular weight ligand in the blood. The formation of only binary Fe(III)/DT712 and Al(III)/DT712 complexes (and ternary complexes in aged solutions), suggests that DT712 effectively compete with citric acid in the metal complexation. Standard reduction potentials of Fe(III)/DT712 complexes, and the kinetic constants of complex formation, were obtained by cyclic voltammetry. Accordingly, no redox cycling is expected to occur at *in vivo* conditions, and Fe(III)/DT712 complex formation should not be kinetically limited. On the basis of the present results, DT712 is proposed as candidate for iron chelation therapy.



## 1. Introduction

The overload of iron (Fe) and aluminium (Al) produces several pathologies. Their most efficient therapeutic approach is represented by chelation therapy. The well established chelators for Fe and Al overload therapies are Desferal (DFO) and Deferiprone (L1)[1]. DFO and L1 have several drawbacks, and also the recently developed Fe chelator Deferasirox (ICL670) has controversial efficiency and side effects. Therefore, the search for alternative molecules is strongly and continuously requested [1–3].

Hydroxypyridinecarboxylic acids (HPs) are currently under investigation as possible chelating agents for Fe and Al because they display a number of favourable properties [4]. HPs form strong complexes with both Fe(III) and Al(III), and have very low affinity towards Zn(II), which suggests absence of essential metal decorporation *in vivo*. Their low molecular weight is prerequisite

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[1] CA = citric acid, DFO = Desferal, DQ5 = 4-hydroxy-5-methyl-3-pyridinecarboxylic acid, DQ6 = 4-hydroxy-6-methyl-3-pyridinecarboxylic acid, DQ715 = 1,5-dimethyl-4-hydroxy-3-pyridinecarboxylic acid, DQ716 = 1,6-dimethyl-4-hydroxy-3-pyridinecarboxylic acid, DQs = 4-hydroxy-3-pyridinecarboxylic acids, DT0 = 3-hydroxy-4-pyridinecarboxylic acid, DT1 = 3-hydroxy-1-methyl-4-pyridinecarboxylic acid, DT2 = 3-hydroxy-2-methyl-4-pyridinecarboxylic acid, DT712 = 1,2-dimethyl-3-hydroxy-4-pyridinecarboxylic acid, DT71201 = 2-ethyl-3-hydroxy-1-methyl-4-pyridinecarboxylic acid, DT726 = 2,6-dimethyl-3-hydroxy-4-pyridinecarboxylic acid, DT8126 = 3-hydroxy-1,2,6-trimethyl-4-pyridinecarboxylic acid, DTs = 3-hydroxy-4-pyridinecarboxylic acids, ESI-MS = electrospray mass spectrometry, ICL670 = Deferasirox, HP = hydroxypyridinecarboxylic acids, $k_f$ = kinetic constant for the reaction of complex formation, L1 = Deferiprone, Tf = transferrin.

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for oral activity [5]. Toxic side effects induced by redox activity are unlikely for both free ligands and Fe(III)/ligand complexes. HPs investigated so far display negligible toxic effects ($IC_{50}$ > 0.1 mM) to cancer cell lines and primary human cells, following a 3 days exposure.

Among the possible HP isomers, 4-hydroxy-3-pyridinecarboxylic acids (DQs) substituted at the pyridinic ring have been mostly considered up to now [4,6]. Although their affinity towards Fe(III) and Al(III) is very high, it is lower than that of chelators available presently, such as L1. This is the only disadvantage highlighted up to now for all DQs, and therefore the major efforts have been directed to the synthesis of derivatives displaying higher metal affinity, and to the rationalization of their metal complexation affinity in relation to their ring substitution. To this connection, the effect of the electrondonating methyl substitution on the Fe(III) and Al(III) complex stability was experimentally determined [6]. The 1-, 5- and 6-methyl substitutions enhance the complex stability for both metal ions, *i.e.* the coordinating oxygens increase more their Lewis than their Brønsted basicity. The 5-methyl substitution is slightly more efficient than the 6-methyl one because the former is closer to the coordinating oxygens than the latter. The 1-methyl substitution has a very strong effect on the Fe(III) complex stability, and a much lower one on the Al(III) complex stability. On the other hand, the 2-methyl substitution should be avoided because the ortho effect caused on the carboxylic group inhibits the complex formation. Finally, the methyl effects appear to be addictive. Among the DQs studied up to now, 1,5-dimethyl-4-hydroxy-3-pyridinecarboxylic acid (DQ715) [6] and 1,6-dimethyl-4-hydroxy-3-pyridinecarboxylic acid (DQ716) [7] are the most promising derivatives for Fe chelation therapy, whereas 4-hydroxy-5-methyl-3-pyridinecarboxylic acid (DQ5) [6] and 4-hydroxy-6-methyl-3-pyridinecarboxylic acid (DQ6) [7] are the most promising DQs for Al chelation therapy.

The 3-hydroxy-4-pyridinecarboxylic acids (DTs) isomers are now considered, where the hydroxy and carboxylic groups are exchanged with respect to the DQs. Three simple DTs have been studied previously: 3-hydroxy-4-pyridinecarboxylic acid (DT0) [8,9], 3-hydroxy-1-methyl-4-pyridinecarboxylic acid (DT1) [10,11], and 3-hydroxy-2-methyl-4-pyridinecarboxylic acid (DT2) [12]. The DTs expected to display the highest metal affinity can now be selected on the basis of the methylation results obtained for the DQs, assuming the same effects of the different substituent positions. It follows that 5-methyl derivatives should be avoided, and that 1-, 2-, and 6-methylated DTs should display the highest metal ion affinity, with the 1-derivatives forming stronger complexes with Fe(III) than with Al(III). Also, results on DQs suggest that polymethylation should lead to stronger complexes than monomethylation.

The dimethylated derivatives 1,2-dimethyl-3-hydroxy-4-pyridinecarboxylic acid (DT712) and 2,6-dimethyl-3-hydroxy-4-pyridinecarboxylic acid (DT726) have been considered in this paper. Measurements for Fe(III) only were performed also on the ethyl analogue of DT712, 2-ethyl-3-hydroxy-1-methyl-4-pyridinecarboxylic acid (DT71201). A trimethylated DT was considered as well, 3-hydroxy-1,2,6-trimethyl-4-pyridinecarboxylic acid (DT8126). The compounds studied in this work are shown in Fig. 1.

The synthesis of DT712 was recently performed in our laboratories, and it is described [13], whereas that of DT8126, DT726 and DT71201 is new. The Fe(III) and Al(III) coordination properties of these DTs were studied by potentiometric, UV–Vis, and (in the case of Al(III)) $^1$H NMR measurements. The ability of DT712 and of DQ715 to extract Fe(III) from transferrin was studied by UV–Vis, in order to determine the kinetic constant of the metal extraction. Binary metal/DT712 and ternary metal/DT712/citric acid solutions were studied by electrospray mass spectrometry to evaluate the Fe(III) and Al(III) competition between the chelator and a typical low molecular weight ligand in the blood. Fe(III)/DT712 and Fe(III)/DT8126 complexes were studied by cyclic voltammetry to determine the standard reduction potentials of the complexes and the kinetics of complex formation.

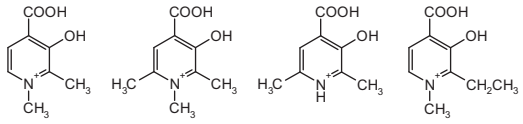


**Fig. 1.** 1,2-Dimethyl-3-hydroxy-4-pyridinecarboxylic acid (DT712), 3-hydroxy-1,2,6-trimethyl-4-pyridinecarboxylic acid (DT8126), 2,6-dimethyl-3-hydroxy-4-pyridinecarboxylic acid (DT726), and 2-ethyl-3-hydroxy-1-methyl-4-pyridinecarboxylic acid (DT71201). All ligands are shown in their most protonated form.

## 2. Experimental

### 2.1. Synthesis of 1,2-dimethyl (DT712), 1,2,6-trimethyl (DT8126), 2,6-dimethyl (DT726) and 2-ethyl-1-methyl (DT71201) HP derivatives

Melting points were determined on a Gallenkamp MFB 595 010M/B capillary melting point apparatus, and are uncorrected. $^1$H NMR spectra were recorded on a Bruker 400 MHz spectrometer, with the solvents indicated. Chemical shifts are reported in $\delta$ (ppm) values downfield from tetramethylsilane which is taken as internal reference. Coupling constants are given in Hertz. In the case of multiplets, chemical shifts were measured at the approximate center. Integrals were satisfactorily in line with those expected on the basis of compound structure. Elemental analyses were performed in the Microanalytical Laboratory, Department of Pharmaceutical Sciences, University of Padova, on a Perkin-Elmer C, H, N elemental analyzer model 240B, and analyses indicated by the symbols of the elements were within ±0.4% of the theoretical values. Mass spectra were obtained on a Mat 112 Varian Mat Bremen (70 eV) mass spectrometer and Applied Biosystems Mariner System 5220 LC/Ms (nozzle potential 250.00). Chemical reactions were monitored by analytical thin-layer chromatography (TLC) on Merck silica gel 60 F-254 glass plates. Solutions were concentrated on a rotary evaporator under reduced pressure. Starting materials were purchased from Aldrich Chimica and Acros, solvents from Carlo Erba, Fluka and Lab-Scan.

The synthetic procedure leading to DT726 (**5a**), DT8126 (**6a**), and DT71201 (**6b**) is reviewed in Scheme 1 and it is described in the following subsections. DT712 (**5b**) has been synthesized as described [13].

#### 2.1.1. Synthesis of D,L-alanine ethyl ester hydrochloride (*2a*)

A solution of commercial D,L-alanine (5.00 g, 56.12 mmol) in 150 mL absolute ethanol and 5 mL HCl 37% was refluxed for 5 h. The solvent was then evaporated and the uncoloured oily residue was dried under vacuum; Yield 92%; $^1$H NMR ($CDCl_3$): $\delta$ 1.05 (t, 3H, $J$ = 7.07 Hz, –OCH$_2$C*H*$_3$), 1.45 (d, 3H, $J$ = 7.25 Hz, –CHC*H*$_3$), 3.48 (q, 2H, $J$ = 7.07 Hz, –OC*H*$_2$CH$_3$), 4.02 ppm (q, 1H, $J$ = 7.25 Hz, –C*H*CH$_3$); HRMS (ESI, 140 eV): $m/z$ [M+H$^+$] calcd for $C_5H_{12}NO_2$: 118.0868 found: 118.0794.

#### 2.1.2. Synthesis of ethyl 2-acetamidopropanoate (*3a*)

In a 250 mL round bottom flask, to 5 g (32.54 mmol) of ethylalanine hydrochloride **2a** were added 25 mL of triethylorthoacetate, and the reaction mixture was refluxed for 2 h (oil bath). In the meantime, the colourless solution became yellow–brown. At the end, the solution was concentrated in a rotary evaporator giving a light yellow oily product. Yield 80–90%; $^1$H NMR ($CDCl_3$): $\delta$