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Evaluation of 4-hydroxy-6-methyl-3-pyridinecarboxylic acid and 2,6-dimethyl-4-hydroxy-3-pyridinecarboxylic acid as chelating agents for iron and aluminium

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ABSTRACT

4-Hydroxy-6-methyl-3-pyridinecarboxylic acid (DQ6) and the new compound 2,6-dimethyl-4-hydroxy-3-pyridinecarboxylic acid (DQ726) were evaluated for possible application for iron (Fe) and aluminium (Al) chelation therapy. Metal/ligand solution chemistry, cytotoxicity, octanol/water partitioning ($D_{o/w}$), and chelation efficiency were studied. The solution chemistry of the two ligands with Fe(III) and Al(III) was investigated in aqueous 0.6 m (Na)Cl at 25 °C by means of potentiometric titrations, UV–Vis spectrophotometry, and ^{1}H NMR spectroscopy. DQ6 exhibited a high coordination efficiency towards Al(III). Fe(III)/DQ6, Al(III)/DQ726, and Fe(III)/DQ726 complexes were less stable. These results were confirmed by chelation efficiency measurements performed in an octanol/aqueous solution. Accordingly, the effects of the substitution at various ring positions of 4-hydroxy-3-pyridinecarboxylic acid were rationalised. Partitioning experiments at pH 7.4 showed both DQ6 and DQ726, and their Fe(III) and Al(III) complexes, to be hydrophilic. The toxicity of DQ6 and of DQ726 was investigated with human cancer cell lines and normal human primary cells: no cytotoxic effects were observed up to 0.1 mM, following a 3 days exposure. According to our results, DQ6 has the favourable properties to be a chelating agent for Al.

1. Introduction

Hydroxypyridinecarboxylic acids (HPs, Table 1) are new potential chelating agents for iron (Fe) and aluminium (Al) because they display a number of favourable properties. They form strong complexes with both Fe(III) and Al(III) [1–6], and have a very low affinity towards Zn(II) [7,8], which suggests the absence of essential metal decorporation *in vivo* [9]. They have a low molecular weight, which is a prerequisite for oral activity [10]. Toxic side effects induced by redox activity are unlikely for both the free ligands and the Fe(III)/ligand complexes [3,6]. The HPs investigated so far (Table 1) display negligible toxic effects ($IC_{50} > 0.1$ mM) to cancer cell lines and primary human cells, following a 3 days exposure [3,6]. DT0 is non-toxic towards animals, and it was proposed as an aspirin-like drug [11,12]. An analogue, 4-pyridoxic acid (3-hydroxy-5-hydroxymethyl-2-methyl-4-pyridinecarboxylic acid), the main metabolite of vitamin B6, is also non-toxic [13].

The simplest HPs, the unsubstituted DT0 and DQ0, have a distinct disadvantage: although their affinity towards Fe(III) and Al(III) is very high, it is still much lower than that of chelators available presently, such as deferiprone (L1). This affinity was significantly increased by methyl substitutions at the pyridinic ring. DT1 and DT2 showed a higher coordination strength than that of DT0. The complexation strength of DQ1 towards Fe(III) was slightly higher than that of DQ0; that towards Al(III) did not change. The 2-methyl substitution of DQ0 (which gives DQ2) decreased significantly the coordination strength towards Fe(III) and Al(III). The complexes of DQ716 with both Fe(III) and Al(III) are much more stable than those of the other HPs examined so far [6], so that this compound was proposed as a chelating agent for Fe and Al.

More than one HP having the proper chemical requirements would be needed, in order to perform a pharmacological screening (*e.g.* evaluation of chelation efficiency and toxicity *in vivo*) and eventually individuate at least one compound to be used as drug. Moreover, the effects of the methyl substitution at different ring positions on the metal stability of the complexes are still not clear. The metal–ligand solution chemistry of other derivatives should be

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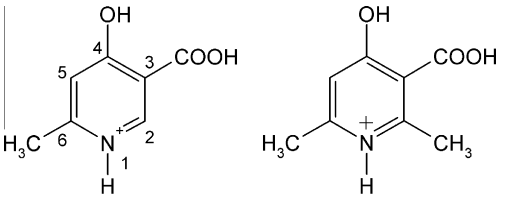
Table 1
Hydroxypyridinecarboxylic acids (HPs) examined so far.

Name (IUPAC)	Acronym	References
3-Hydroxy-4-pyridinecarboxylic acid	DT0 (3H4P)	[1–3]
3-Hydroxy-1-methyl-4-pyridinecarboxylic acid	DT1 (1M3H4P)	[3–5]
3-Hydroxy-2-methyl-4-pyridinecarboxylic acid	DT2	[3,6]
4-Hydroxy-3-pyridinecarboxylic acid	DQ0 (4H3P)	[1,2,6]
4-Hydroxy-1-methyl-3-pyridinecarboxylic acid	DQ1 (1M4H3P)	[4–6]
4-Hydroxy-2-methyl-3-pyridinecarboxylic acid	DQ2	[6]
1,6-Dimethyl-4-hydroxy-3-pyridinecarboxylic acid	DQ716	[6]



DQ6 DQ726

Fig. 1. 4-Hydroxy-6-methyl-3-pyridinecarboxylic acid (DQ6), and 2,6-dimethyl-4-hydroxy-3-pyridinecarboxylic acid (DQ726) shown in their most protonated forms (H_3L^+).

studied, so that the best strategy can be developed for the identification of the strongest Fe(III) and Al(III) chelators.

This paper describes our evaluation of 4-hydroxy-6-methyl-3-pyridinecarboxylic acid (DQ6) and 2,6-dimethyl-4-hydroxy-3-pyridinecarboxylic acid (DQ726) as possible chelating agents for Fe and Al (Fig. 1). DQ6 has been synthesised and characterised previously [14], but its use as chelating agent for iron and aluminium was never explored. According to our knowledge, DQ726 is a new compound and it has never been prepared yet. Both DQ6 and DQ726 were synthesised, and their coordination properties towards Fe(III) and Al(III) were studied by means of potentiometric, UV–Vis, and (in the case of Al(III)) ^{1}H NMR measurements. Their octanol/water partitioning coefficient ($D_{o/w}$), as well as their efficiencies in chelating Fe(III) and Al(III) at physiological pH, were determined *in vitro*. Their cytotoxicity was assessed on human cancer cell lines and primary cultures of human cells.

2. Experimental

2.1. Synthesis

Melting points were determined on a Gallenkamp MFB 595 010M/B capillary melting point apparatus, and are uncorrected. Infrared (IR) spectra were measured on a Perkin–Elmer 1760 FT-IR spectrometer using potassium bromide pressed disks. Values are expressed in cm^{-1}. ^{1}H NMR spectra were recorded on Varian Gemini (200 MHz) and Bruker (300 MHz) spectrometers, using the indicated solvents. NMR data are reported as δ values (ppm) relative to tetramethylsilane as an internal standard. Elemental analyses were performed in the Microanalytical Laboratory, Department of Pharmaceutical Sciences, University of Padova, using a Perkin–Elmer elemental analyser model 240B; results fell in the range of calculated values ±0.4%. Mass spectra were obtained with a Mat 112 Varian Mat Bremen (70Ev) mass spectrometer and Applied Biosystems Mariner System 5220 LC/MS (nozzle potential 250.00). Starting materials as well as solvents were purchased from Sigma (Milan, Italy).

2.1.1. 3-(Dimethylaminomethylene)-4-oxo-6-methyl-2-pyrone (**1**)

About 10 mL (d = 0.89 mg/mL, 74 mmol) *N*,*N*-dimethylformamide dimethyl acetale were slowly added to a stirred suspension of 4-hydroxy-6-methyl-2-pyrone (5 g, 40 mmol) in 10 mL dioxane: the starting material dissolved and the solution became brown. The reaction ran at a temperature of 15 °C for 2 h, when a precipitate formed. The precipitate was collected, washed with cold dioxane and acetone, and dried *in vacuo*. Yield 60% (literature [14] 72%); mp 148–150 °C (literature [14] 152–154 °C); HRMS (ESI) calculated for $[M+H]^+$ *m/z* 179.169, found 180.174. ^{1}H NMR (DMSO-d$_2$) δ 1.10 (t, 3H, C-CH_3), 2.35 (q, 2H, C-CH_3), 3.22 (s, 3H, N-CH_3), 3.48 (s, 3H, N-CH_3), 5.55 (s, 1H, olefinic proton); 8.22 (s, 1H, olefinic proton).

2.1.2. 4-Hydroxy-6-methylpyridin-3-carboxyl acid (**2**)

About 1 g (5.58 mmol) of pyrone derivative **1** was suspended in 30% aqueous ammonia (20 mL) and 1 mL $NH(CH_3)_2$. After stirring for 30 min at room temperature, the solution was evaporated under reduced pressure to about 1/3 of its volume and the remaining solution cooled (ice-bath) and acidified to pH 3 with HCl 1 M. The formed precipitate was collected and dried yielding a solid product which was re-crystallized from water to give pure product. Yield 64% (literature [14] 49%); mp 264–266 °C (literature [14] 267–268 °C); HRMS (ESI) calculated for $[M+H]^+$ *m/z* 153.125, found 154.258. ^{1}H NMR (D_2O + NaOD) δ 2.15 (s, 3H, C-CH_3), 7.22 (s, 1H), 8.52 (s, 1H). *Anal.* Calc. for $C_8NO_3H_9$: C, 57.48; H, 5.43; N, 8.38. Found: C, 51.30; H, 5.60; N, 7.40%.

2.1.3. Ethyl 4-hydroxy-2,6-dimethylpyridin-3-carboxylate (**3**)

About 5 g (35 mmol) of 2,2,6-trimethyl-4(1*H*)-1,3-dioxin-4-one and 2.5 g (19 mmol) of ethyl-(*Z*)-3-aminobut-2-enoate (ethyl-3-crotonate) were heated in a flask at 120–130 °C for 1 h, until no more water formed (Dean–Stark apparatus). On cooling, diethyl ether (20 mL) was added to the reaction mixture and a precipitate formed, which was collected and washed with a small amount of diethyl ether and dried to give solid product; yield 45% (literature [15] 40%); mp 162 °C (literature [15] 168 °C); HRMS (ESI) calculated for $[M+H]^+$ *m/z* 167.160, found 168.178. ^{1}H NMR (D_2O + NaOH) δ 1.30 (t, 3H, C-CH_3), 2.55 (s, 6H, CH_3), 4.29 (q, 2H, C-CH_2), 6.81 (s, 1H).

2.1.4. 4-Hydroxy-2,6-dimethylpyridin-3-carboxyl acid (**4**)

About 1 g (5.98 mmol) of pyridin-carboxylate derivative **3** was suspended in NaOH 0.5 M (20 mL) and refluxed for 4 h. Then, the resulting solution was cooled, acidified with aqueous HCl 2 M to pH 6 and extracted with chloroform to remove the unreacted ester. Further acidification of aqueous solution to pH 3 and cooling (ice-bath) gave a white solid which was re-crystallized from water to yield pure crystalline product. Yield 90%; mp = 320–325 °C (decomp.); HRMS (ESI) calculated for $[M+H]^+$ *m/z* 167.160, found 168.178; ^{1}H NMR (DMSO-d$_6$) δ 2.33 (s, 3H, CH_3), 2.71 (s, 3H, CH_3), 6.66 (s, 1H, H-5), 12.82 (s, 1H, N*H*), 16.15 (s, 1H, COO*H*). *Anal.* Calc. for $C_8NO_3H_9$: C, 57.33; H, 5.42; N, 8.36. Found: C, 54.40; H, 5.05; N, 7.80%.

2.2. Thermodynamic study

All potentiometric measurements were performed using a Radiometer ABU93 tri-burette apparatus. UV–Vis and ^{1}H NMR spectra were recorded using a Perkin–Elmer Lambda 20 spectrophotometer and a Bruker DRX-400 spectrometer operating at 400.13 MHz, respectively. All analyte concentrations were expressed in the molality scale (mol/kg of water). For potentiometric and UV–Vis