DOI: http://dx.medra.org/10.17374/CI.2020.102.1.54

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NEW M-CHELATING AGENTS WANTED!

The 3-hydroxy-4-pyridinones are considered promising candidates as metals (M) chelating agents. The results of a work of synthesis of a new ligand belonging to this class of compounds, speciation studies in the presence of M^{2+} and M^{3+} and bioassays in mice treated with ^{67}Ga-citrate are reported.

Over the last decades, the increase of pollution and the onset of diseases, led the scientific community to a great interest towards the study of the impact that some substances, such as metals, organic and inorganic compounds, could have on humans and environment and the legislators to approve more specific laws to stem these issues **[1]**. To better understand their mechanism of action, it is necessary to know not only their analytical concentration, but also their "*speciation*". This term indicates the distribution of the different physical and chemical forms in which a component is present in a system and, since they may have various behavior towards humans and environment, the speciation study becomes essential to have information on their bioavailability, toxicity and environmental impact **[2]**. In the light of these considerations, the researchers have been working for the development of new chelating agents, which could be capable to remove metal cations from human body, without involving significant drawbacks such as toxicity, high costs, oral activity and undesired side effects. These potential chelators should also have a balanced lipo-hydrophilic character with high affinity towards biological membranes, as well as a higher thermodynamic stability than the commercial chelators **[3]**. The 3-hydroxy-4-pyridinones (3,4-HPs) can be considered good candidates matching these criteria. They are derivative of deferiprone and are featured by a *N*-heterocyclic aromatoid ring and ketone and a -OH groups in *ortho* position, which confer them a strong affinity towards M^{2+} and M^{3+} **[4-6]**. This class of compounds can be extrafunctionalized with the purpose of improving their bioavailability, affinity towards the biological sites and/or chelating efficiency. For these reasons, a new bis-3-hydroxy-4-pyridinone ligand (Fig. 1), containing two 3,4-HP binding units condensed to a nitrilotriacetic acid (NTA) core was synthesized with a 4 steps mechanism, starting from a natural compound like maltol, with the protection of -OH with a benzyl group, followed by a Michael addition of 1,3-diaminopropane. Then, the condensation of two equivalents of the obtained intermediate with NTA, in the presence of the coupling agent *n*-propylphosphonic anhydride (T3P) **[7-9]**, occurs, with the formation of new amidic bonds. The synthetic procedure ends with a standard catalytic hydrogenolysis to remove the benzyl protecting groups **[10]**.

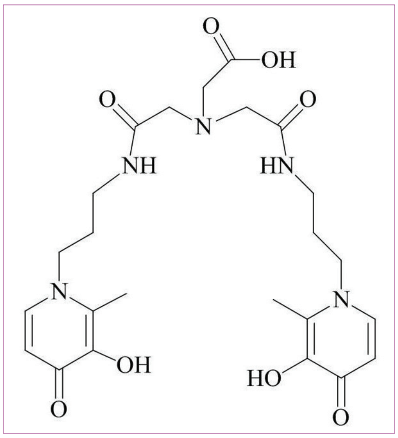

Fig. 1 - Structure of $NTA(PrHP)_2$ ligand

Furthermore, a speciation study on the ligand acid-base properties and the metal complexation in aqueous solution and in the presence of divalent (Ca^{2+}, Cu^{2+}, Zn^{2+}) and trivalent (Al^{3+}, Fe^{3+}) metal

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Anna Irto was awarded the Fernando Pulidori 2019 Prize, now in its 12th edition, on the occasion of the ISMEC 2019 Congress, held in Debrecen (H) from 11 to 14 June 2019.

$\log\beta_r^{H\,a}$ ($\log K_r^H$)^b	UV-Vis spectrophotometry T = 298.15 K	 T = 310.15 K	^{1}H NMR spectroscopy T = 298.15 K
$\log\beta_1^H$	10.84±0.03^c	10.95±0.02^c	10.85±0.002^c
$\log\beta_2^H$ ($\log K_2^H$)	20.501±0.007 (9.661)	20.99±0.03 (10.04)	20.48±0.003 (9.64)
$\log\beta_3^H$ ($\log K_3^H$)	27.65±0.14 (7.15)	27.13±0.03 (6.14)	28.07±0.014 (7.59)
$\log\beta_4^H$ ($\log K_4^H$)	32.21±0.07 (4.56)	31.25±0.01 (4.12)	32.28±0.014 (4.21)
$\log\beta_5^H$ ($\log K_5^H$)	35.72±0.09 (3.51)	34.54±0.02 (3.29)	35.79±0.010 (3.51)
$\log\beta_6^H$ ($\log K_6^H$)	38.59±0.07 (2.87)	36.95±0.04 (2.41)	38.59±0.007 (2.80)

$^a\log\beta_r^H$ equilibrium refers to eq. rH$^+$ +L^{3-} = H$_r$$L^{-(3-r)}$; $^b\log K_r^H$ equilibrium refer to eq. H$^+$ + H$_{(r-1)}$$L^{-(3-(r-1))}$ = H$_r$$L^{-(3-r)}$; ^{c}±Std. deviation.

Tab. 1 - Protonation constants^{a,b} of NTA(PrHP)$_2$ determined by UV-Vis spectrophotometry and ^{1}H NMR spectroscopy at I = 0.15 mol L^{-1} in NaCl$_{(aq)}$ and T = 298.15 and 310.15 K

cations, was performed. The analytical techniques used for these studies were UV-Vis spectrophotometry, spectrofluorimetry, potentiometry and ^{1}H NMR spectroscopy. NaCl was selected as ionic medium (ionic strength I = 0.15 mol L^{-1}), since it is the main inorganic component of most of natural **[11, 12]** and biological fluids, namely blood plasma (I ~ 0.16 mol L^{-1}) **[13]**. The elaboration of experimental data using suitable computer programs **[14-16]** allowed the determination of equilibrium constants and speciation models, considered the best possible ones on the base of some criteria, already reported in the literature **[17]**. Concerning the ligand acid-base properties, the structure of NTA(PrHP)$_2$ is featured by six different protonable sites: two hydroxyl groups of the *N*-aromatoid rings, an amino and a carboxylic groups on the NTA side of the molecule, two pyridinone nitrogen atoms (proton supplied by excess of inorganic acid) **[18]**. For all the possible protonable groups the protonation constants were successfully determined with a good accordance among the different analytical techniques, as reported in Tab. 1 **[3]**. The effect of temperature on the acid-base properties of NTA(PrHP)$_2$ can be better estimated by the analysis of the distribution diagram in Fig. 2, built at I = 0.15 mol L^{-1} in NaCl$_{(aq)}$ at T = 298.15 K and T = 310.15 K (physiological conditions). The percentages of all the species exceed the 35% and their formation at higher temperature is shifted towards more acidic pHs than at T = 298.15 K; at physiological pH values the main species is, in both cases, H$_2$$L^-$. The investigation on the binding ability of the ligand towards Ca^{2+}, Cu^{2+}, Zn^{2+}, Al^{3+} and Fe^{3+} led to the determination of speciation models featured by M$_p$$L_qH_r^{(np-3q+r)}$ species with different stoichiometry, including protonated, simple metal-ligand, hydrolytic mixed and polynuclear species, as reported in Tab. 2, taking into account the different charges and structures of the ligands and the various hydrolytic behaviours of the metal cations **[3]**. As an example, the stability constants refined for the ML$^{(n-3)}$ species

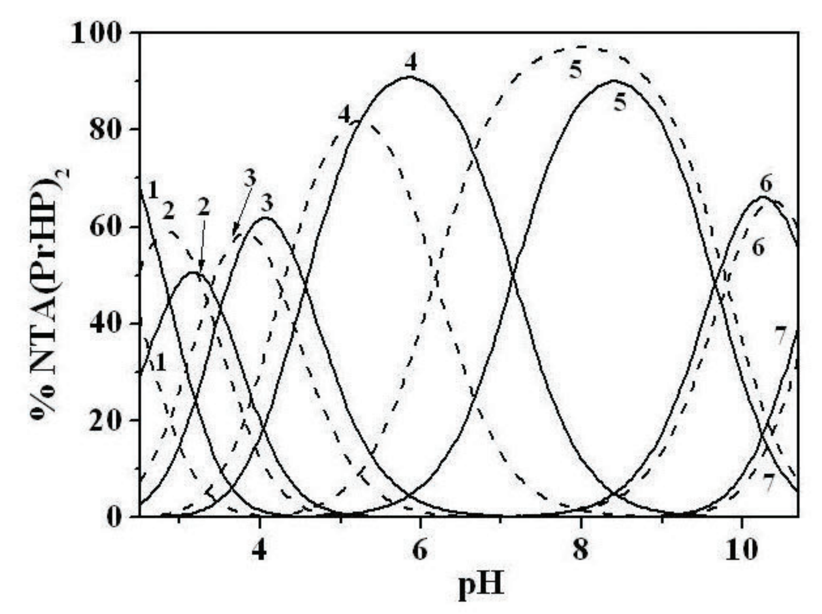

Fig. 2 - Distribution diagram of NTA(PrHP)$_2$ at I = 0.15 mol L^{-1} in NaCl$_{(aq)}$ and T = 298.15 K (solid line) and T = 310.15 K (dashed line). c_L = 1.0·10^{-5} mol L^{-1}. Species: 1. H$_6$$L^{3+}$; 2. H$_5$$L^{2+}$; 3. H$_4$$L^+$; 4. H$_3$$L^0_{(aq)}$; 5. H$_2$$L^-$; 6. H$L^{2-}$; 7. L^{3-}

$\log\beta_{pqr}$ ^{a} $(\log K_{pqr})^{b}$	Ca^{2+}	Cu^{2+}	Zn^{2+}	Al^{3+}	Fe^{3+}
$\log\beta_{113}$ $(\log K_{113})$	38.76±0.03^{i} (11.11)	38.86±0.01^{i} (11.21)	37.93±0.03^{i} (10.28)	-	-
$\log\beta_{112}$ $(\log K_{112})$	35.50±0.06 (15.00)	35.77±0.03 (15.27)	34.96±0.01 (14.46)	-	37.67±0.09^{i} (17.17)
$\log\beta_{111}$ $(\log K_{111})$	29.508±0.007 (18.668)	31.73±0.02 (20.89)	29.72±0.03 (18.88)	30.72±0.02^{i} (19.88)	34.06±0.08 (23.22)
$\log\beta_{110}$	19.70±0.02	21.08±0.01	19.85±0.07	26.810±0.008	27.25±0.05
$\log\beta_{11-1}$ $(\log K_{11-1})^{c}$	-	-	-	-	17.34±0.05 (3.64)
$\log\beta_{210}$ $(\log K_{210})^{d}$	32.49±0.01 (5.68)	31.95±0.04 (5.76)	31.98±0.03 (5.63)	32.49±0.01 (5.68)	42.01±0.18 (14.76)
$\log\beta_{22-2}$ $(\log K_{22-2})^{e}$	43.25±0.06 (10.76)	42.82±0.19 (10.87)	-	43.25±0.06 (10.76)	-
$\log\beta_{230}$ $(\log K_{230})^{f}$	-	-		-	69.17±0.03 (14.67)
$\log\beta_{231}$ $(\log K_{231})^{g}$	-	-	-	-	81.058±0.008 (15.72)
$\log\beta_{233}$ $(\log K_{233})^{h}$	90.88±0.03 (9.61)	90.53±0.06 (9.26)	-	90.88±0.03 (9.61)	-

$^{a}\log\beta_{pqr}$ refer to equilibrium: $pM^{n+} + qL^{3-} + rH^{+} = M_pL_qH_r^{(np-3q+r)}$; $^{b}\log K_{pqr}$ refer to equilibrium: $pM^{n+} + qH_rL^{-(3-r)} = M_pL_qH_r^{(np-3q+r)}$; $^{c}\log K_{11-1}$ refers to equilibrium: $Fe^{3+} + L^{-} + H_2O = FeL(OH)^{-} + H^{+}$; $^{d}\log K_{210}$ refers to equilibrium: $M^{3+} + ML^{0}_{(aq)} = M_2L^{3+}$; $^{e}\log K_{22-2}$ refers to equilibrium: $Al_2L^{3+} + L^{3-} + 2H_2O = Al_2L_2(OH)_2^{2-} + 2H^{+}$; $^{f}\log K_{230}$ refers to equilibrium: $2FeL^{0}_{(aq)} + L^{3-} = Fe_2L_3^{3-}$; $^{g}\log K_{231}$ refers to equilibrium: $2FeL^{0}_{(aq)} + HL^{2-} = Fe_2L_3H^{2-}$; $^{h}\log K_{233}$ refers to equilibrium: $2AlL^{0}_{(aq)} + H_3L^{0}_{(aq)} = Al_2L_3H^{0}_{3\,(aq)}$; ^{i}±Std. Deviation

Tab. 2 - Experimental$^{a,\,b\text{-}h}$ stability constants of M^{n+}/NTA(PrHP)$_2$ species determined by UV-Vis spectrophotometric titrations at I = 0.15 mol L^{-1} in $NaCl_{(aq)}$ and T = 298.15 K

follow the trend: $Fe^{3+}>Al^{3+}>Cu^{2+}>Zn^{2+}>Ca^{2+}$. ^{1}H NMR and computational studies (DFT-optimization, B3LYP/6-31G(d)) [19] allowed to suggest, for $ZnLH_3^{2+}$ species, an interaction of the metal cation with the carbonyl oxygen atoms from the two amide groups and the carboxylic acid from the NTA moiety. Concerning the Al^{3+}/NTA(PrHP)$_2$ interactions, as observable in Fig. 3, computational studies indicated for the $Al_2L_3H_3{}^{0}{}_{(aq)}$ complex an approximately D_3-symmetric structure, with the three ligand molecules arranged in a C-shaped conformation that allows each pyridinone moiety to interact with a different Al^{3+} cation (Fig. 3 a)). On the other hand, the $Al_2L_2(OH)_2{}^{2-}$ complex (Fig. 3 b)) is featured by two hydroxide ions placed in a bridging position between the metals. The sequestering ability of the ligand towards the metal cations was investigated by the determination, at different experimental pHs, of the $pL_{0.5}$ [20], an

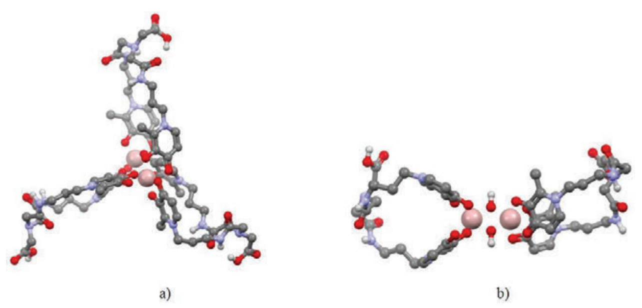

Fig. 3 - Ball-and-stick view of the DFT-optimized geometry of the $Al_2L_3H_3{}^{0}{}_{(aq)}$ (a) and $Al_2L_2(OH)_2{}^{2-}$ (b) complexes. Oxygen: red; carbon: grey; hydrogen: white, nitrogen: blue; aluminium: pink. Some hydrogens have been omitted for clarity

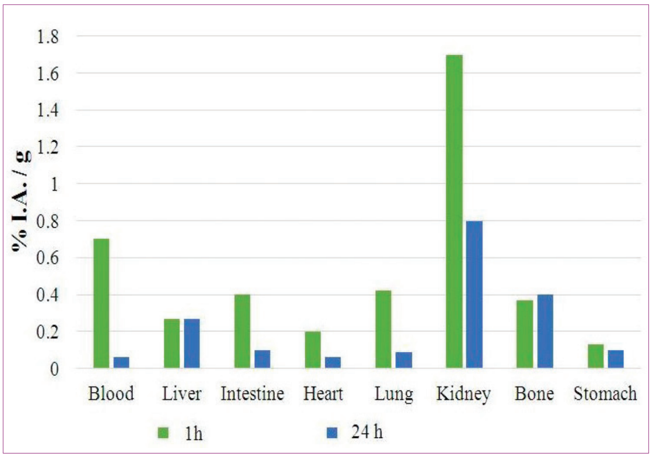

Fig. 4 - Biodistribution of ^{67}Ga after intraperitoneal injection of NTA(PrHP)$_2$, expressed as % I. A./g at 1 and 24 h post-injection in female Balb-C mice

empirical and objective parameter, which represent the total concentration of ligand required to sequester the 50% of metal cation, if present in trace (10^{-12} mol L^{-1}) in solution. It was observed that NTA(PrHP)$_2$ assume high pL$_{0.5}$ values and its sequestering ability towards the metal cations varies during the whole pH range of investigation. The determination pM **[21]** parameter at pH = 7.4 showed that the metal-ligand affinity follows the trend: $Fe^{3+}>Al^{3+}>Cu^{2+}>Zn^{2+}\geq Ca^{2+}$, indicating the NTA(PrHP)$_2$ tendency to sequester *hard* trivalent metal cations, without involving, from the thermodynamic point of view, a possible competition with M^{2+}. Biodistribution studies were carried out in Balb-C mice previously injected with ^{67}Ga-citrate to assess the ability of the new bis-3-hydroxy-4-pyridinone ligand as *in vivo* chelating agent for the mobilization of trivalent metal cations. This study (Fig. 4) indicated that the radiometal is rapidly cleared from the bloodstream mainly by the kidneys through urinary excretion; the overall rate of radioactivity excretion was also high, reaching the 84.6±4.0 and 95.0±2.5 % I.A./g (percent of injected activity per gram of organ) at 1 h and 24 h, respectively.

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Cercasi nuovi agenti chelanti di metalli!

I 3-idrossi-4-piridinoni sono considerati promettenti candidati come agenti chelanti dei metalli (M). Sono riportati i risultati di un lavoro di sintesi di un nuovo legante appartenente a questa classe di composti, studi di speciazione in presenza di M^{2+} e M^{3+} e saggi biologici su topi trattati con ^{67}Ga-citrato.