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Hydroxypyridinone complexes with aluminium. In vitro/vivo studies and perspectives

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Contents

Abstract

Hydroxypyridinones are a class of dioxo ligands under active development, as efficient Al (and Fe) chelators for potential medical uses. A wide range of those compounds has been designed aimed at improving their physico-chemical and pharmacokinetic properties. In this paper, we make a review on the literature results related to the design, chemistry, metal binding interaction, lipo-hydrophilic character and some biological assays of bidentate and hexadentate hydroxypyridinones. Among the different types of hydroxypyridinones, the 3-hydroxy-4-pyridinones deserved special attention because they are good orally active aluminium-chelators, and they seem to be the main candidates for replacement of desferrioxamine. The interaction of the bidentate hydroxypyridinones with Al as well as the in vivo studies have been more systematically reported than those of the hexadentate derivatives. The development of the hexadentate hydroxypyridinones is quite recent but, at physiological conditions, they have higher affinity for these M^{3+} ions than the bidentate derivatives. Despite only studies on hexadentate hydroxypyridinone–iron interactions are known and described herein, special attention was deserved to these results because of the in vivo/vitro similarity between the physico-chemical properties of these ions. The high Al affinity and favourable lipo-hydrophilic balance of the hydroxypyridinones suggest that their use as Al scavengers should be highly considered in future prospects.

Keywords: Aluminium complexes; Hydroxypyridinones; Al–hydroxypyridinonates; Chelators

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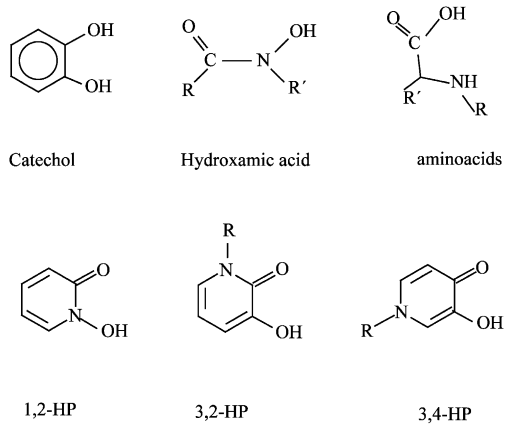
1. Introduction

Aluminium has extremely low bioavailability (despite its abundance) at normal environmental conditions. However, nowadays, it became a threat to human health, mainly due to two types of contamination: (i) environmental level, attributed to acidic rains which release Al from soils and introduce it in the food chain; (ii) therapeutic level, namely from dialysed solutions and pharmaceuticals, such as Al-containing drugs used as anti-acids. So, although Al is a nonessential element, it can accumulate in people and become of concern due to its toxicity [1]. Aluminium has been associated with a number of human diseases in relation to neurological dysfunctions (namely Alzheimer's disease [2,3], dialysis encephalopathy [4], microcytic anaemia [5] and amyotrophic lateral sclerosis [6]) as well as to bone disorders (namely vitamin-D-resistant osteomalacia [4] and osteoporosis [7]).

In fact, when Al reaches the blood circulation and is not excreted by urine, it accumulates in tissues where it becomes strongly bound. Transferrin (Tf) seems to be the main Al binding protein in plasma [8]. Since it is only ca. 30% saturated with iron in normal serum, it has got still a substantial binding capacity for chelating other trivalent metal ions, including Al. Accordingly, to treat Al accumulation and toxicity, an effective Al chelator should be able to mobilize Al from Tf. This means that the Al-chelator complexes should have greater stability constants than that with Tf ($K_{AlL} \sim 10^{13.5}$) [9].

Desferrioxamine-B (DFO-B), a trishydroxamate type siderophore, traditionally used as an iron chelator, has also been applied in the treatment of many cases of Al accumulation, over the last 20 years [10]. This was possible due to the similarity between Al and Fe (in charge, ionic radius and protein binding). However, the general use of DFO has been restricted because it is not orally active (must be administrated parenterally), it is very expensive and it has toxic side effects. For these reasons, there has been a considerable interest on identifying and testing orally active and easily produced chelators, for the in vivo Al binding in replacement of DFO [11–13]. A logical approach for the search of these compounds has been based on their analogy with siderophores, which mostly include one or more of the following bidentate binding moieties: catecholate, hydroxamate and aminocarboxylate ligands (Scheme 1). However, several disadvantages have been associated with that type of ligands for medical use, namely the instability of catechols/hydroxamates in intestine/stomach medium as well as the poor specificity of aminocarboxylates for Fe or Al [14,15].



Scheme 1.

In order to obtain a good chelator, several factors have to be taken into account besides the thermodynamic stability of the Al complex, namely the water solubility, hydrolytic stability and lipophilicity. A considerable number of other Al-sequestering ligands, including hydroxypyridinones, have recently been tested and proposed as promising orally effective Fe and Al removal chelators [16,17]. Despite the similarity between the structure of the hydroxypyridinones, and other dioxo bidentate ligands (e.g. hydroxamic acids and catechols), the hydroxypyridinones are stable at any physiological pH.

Hydroxypyridinones have attracted considerable attention for more than about two decades. Firstly, associated with the biological activity of the naturally occurring mimosine and some derivatives, as well as with their interaction with a series of transition metal ions [18–20]; lately, related to the high affinity of several types of hydroxypyridinones for the trivalent metal ions (e.g. Fe^{3+} and Al^{3+}) [13–21]. The great interest of the hydroxypyridinones as potential iron(III) chelating agents started from the preliminary investigations by Kontghiorghes [16,22] and Hider and coworkers [23–25]. They studied the promising properties of some 3-hydroxy-4-pyridinones, as potential clinical chelating agents for the replacement of DFO. The interaction of hydroxypyridinones with Al^{3+} has been the object of numerous studies by Orvig and coworkers [17,26,27], Martell and coworker [28], Yokel et al. [12] and Di Marco et al. [29]. Since the bidentate 3-hydroxy-4-pyridinones (3,4-HP) are the more encouraging alternatives to DFO, Yokel and coworkers [30,31] and Florence et al. [32] have, also recently reported biological assays on their efficacy as Al-mobilizers from animal models of Al intoxication. The interaction of hydroxypyridinones with indium and gallium has also been studied, but at a smaller extent and in relation with potential use in radiodiagnosis.

In this paper, the progress on the development of hydroxypyridinones, in the perspective of their potential use as Al chelators, is reported. Besides the description of the chemistry and the most important synthetic

pathways for a series of hydroxypyridinones, the investigations made on the Al complexes and some of the important physical and biochemical properties of ligands/complexes are also described. Special emphasis is given to the Al complexation, namely the presentation and discussion of the stability constants of the complexes, the relationship between the structure of the ligands and the Al binding affinity, as well as the in vivo permeability. Comparison between different Al chelators will be made on the basis of the calculated pM distribution curves as a function of pH. The assessment of the in vivo Al-chelation potential of 3,4-HP is discussed on the basis of reported results about the lipo-hydrophilic character of the chelators and the corresponding Al-complexes, as well as the in vivo assays about Al-mobilization.

2. Chemistry and design

The hydroxypyridinones are heterocycles with a hydroxyl group *ortho* to a ketone group, having common features both with catechols and hydroxamic acids. Due to the presence of negatively charged oxygen donors, the hydroxypyridinones are a class of hard [33] bidentate ligands, which can bind hard metal ions by two oxygen atoms yielding five-membered ring chelates. Three main types of compounds have been included under the general designation of hydroxypyridinones: 1-hydroxy-2-pyridinones (1,2-HP), 2-hydroxy-3-pyridinones (2,3-HP) and 3-hydroxy-4-pyridinones (3,4-HP) (Scheme 1).

1,2-HP can be considered as cyclic hydroxamic acids. As it can be seen in Fig. 1, they can exist in tautomeric equilibrium, while both the other types have only one tautomer. The hydroxypyridinones, in their neutral form, have one zwitterionic aromatic mesomer which makes a high charge density on the carbonyl and consequently higher binding interaction with the metal ions are found for hydroxypyridinonates than hydroxamates (Fig. 1).

As discussed below, 1,2-HP are in general more acidic

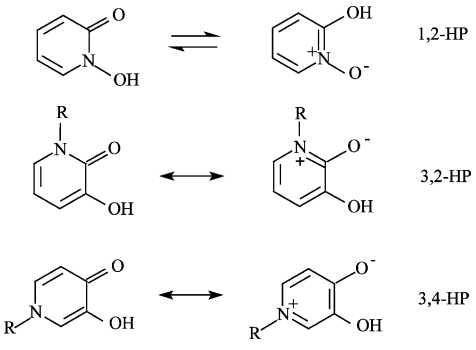


Fig. 1. Mesomeric forms of hydroxypyridinones and their abbreviations.

($pK_a < 7$) than the 3-hydroxy-2-pyridinones (3,2-HP) and the 3,4-HP. Since the 1,2-HP are in the anionic form at physiological pH, they are quite hydrophilic and have expected limitations in terms of biological applications. In contrast, the other two types are uncharged at the physiological pH and bind Al^{3+} forming also neutral 3:1 complexes in that pH ranges. 3,4-HP and 3,2-HP both have demonstrated to remove iron from iron-overload animals, although 3,4-HP were proved to be the most effective ones [24,25]. Indeed, 1,2-dimethyl-3-hydroxy-4-pyridinone (Deferiprone) is currently used in clinical trials. A series of 1-substituted-2-alkyl-3-hydroxy-4-pyridinones (see below) have been synthesised and studied as iron and Al chelators.

Although some bidentate hydroxypyridinones can meet the thermodynamic requirements to be used as drugs, the principle that to the polydentate ligands should correspond increased formation constants due to enthropic effects led to the development of several hexadentate hydroxypyridinones. A brief analysis of the chemistry of the most promising bidentate ligands (3,4-HP) as well as the design and structure of some hexadentate hydroxypyridinone are examined below.

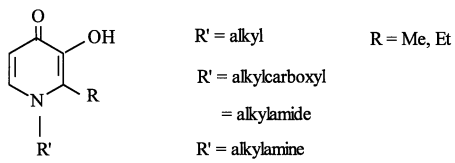
2.1. Bidentate hydroxypyridinones

Some bidentate hydroxypyridinones are commercially accessible, like 1-hydroxy-2-pyridinone, 3-hydroxy-2-pyridinone and Deferiprone (from Aldrich Chemical Co.) and mimosine (from Sigma Chemical Co.). However, a large number of derivatives have been prepared with introduction of various substituents in different positions, aimed at improving their physical and biochemical characteristics, and their in vivo permeability. A summary of the main synthetic pathways used in the preparation of 3,4-HP derivatives is schematically described below.

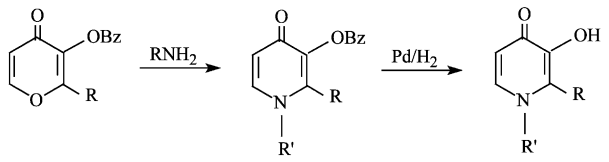
The 1-substituted-2-alkyl-3-hydroxy-4-pyridinones (Scheme 2) can be obtained from the reaction between the corresponding hydroxypyrone (e.g. the commercially available maltol, 2-methyl-3-hydroxy-4-pyrone, or ethyl maltol) and the substituted primary amine.

Harris et al. [18] and Kontoghiorghes [22] used this synthetic approach and it mostly involves an initial Michael reaction between the *O*-heterocycle and the nucleophilic amine, followed by ring-opening and ring-closure to afford the *N*-substituted *N*-heterocycle

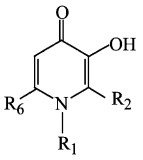
1-Substituted-2-alkyl-3-hydroxy-4-pyridinones



Scheme 2.



Scheme 3.



R_2 = CHROH

R_1, R_6 = H, CH_2, C_2H_5

Scheme 4.

[34,35]. This one-step reaction works well for the 1-alkyl and 1-alkylamino derivatives. However, for 1-alkylcarboxyl derivatives, another methodology is suitable (Scheme 3), which involves the previous protection of the pyrone-hydroxyl group with a benzyl group and its final removal by catalytic hydrogenolysis. This has been the method used by Hider and coworkers [36], Orvig and coworkers [37] and Santos et al. [38] for a series of 1,2-disubstituted-3,4-hydroxypyridinones.

More recently a series of 2-(1′-hydroxyalkyl) derivatives of 3,4-HP (Scheme 4) has also been developed by Hider et al. [39], as high efficient iron chelators and they have claimed potential for oral administration.

In this case, both the 3-hydroxyl and the 2-(1′-hydroxylalkyl) groups of the corresponding pyrone need to be protected before the usual reaction with the NH_2-R_1, leading to the *N*-substituted 3,4-HP. Therefore, a different methodology involving a one-step reaction with benzaldehyde dimethyl acetal was used, according to Scheme 5.

The two following steps are identical to those presented in Scheme 3, namely the reaction with a primary amine and subsequent hydrogenation to afford the final product. The synthesis of 2,6-substituted-3-hydroxypyridinones from the corresponding 2-alkyl pyridinone analogue has been recently proposed [40]. It involves an oxidation of the 2-alkyl-3-hydroxy-4-pyridinones, to give the substituted pyridine-3,4-dione. This can then have a Michael addition with a nucleophile to give the 6-substituted-2-alkyl-3-hydroxy-4-pyridinone.

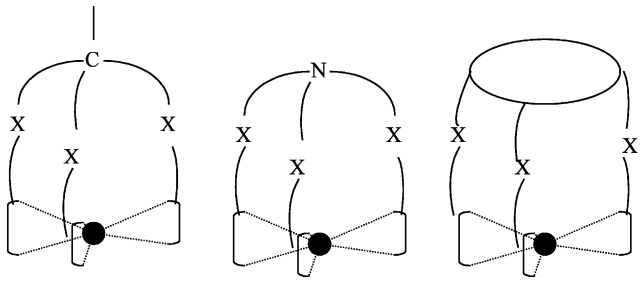


Fig. 2. Scheme of ligand frameworks and metal interaction of the hexadentate hydroxypyridinones. *X* is the point of attachment between the anchor skeleton and the binding 'arms'.

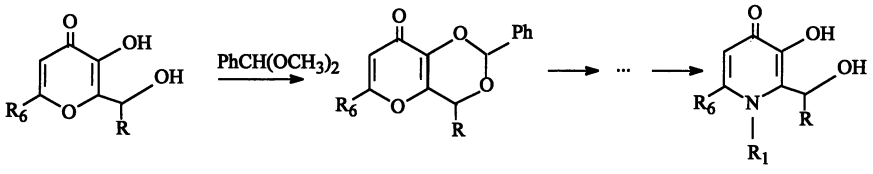
2.2. Hexadentate hydroxypyridinones

The development of hexadentate ligands to complex trivalent cations has been the object of world-wide search for orally active specific iron or Al chelators in order to replace the DFO-B. These new ligands are expected to have higher affinity for those metal ions than the bidentate ones due to enthropic contributions. Taking into account the above referred promising properties of some hydroxypyridinones, a considerable number of hexadentate ligands containing three bidentate hydroxypyridinones [41–47], as well as other bidentate binding units [48] (such as aminophenols [49,50] and catechols [51]), has been reported. They are built by an appropriate arrangement of the binding units on a framework. They are mostly tripodal ligands with a threefold symmetry, whereas the framework is a cyclic or an acyclic three-branched molecule.

Fig. 2 shows three schematic examples of ligand frameworks that have been used for several hexadentate hydroxypyridinones reported in the literature.

The points of attachment (*X*) are mainly amide linkage groups (resulting from the coupling between carboxylic acids and amine groups of each of the molecular fragments), although others have also been used. The most common bidentate hydroxypyridinone binding moieties (□) have been of type 3,2-HP and some of the corresponding hexadentate derivatives have been reported as potential therapeutic chelating drugs [25,52]. Only one of those reported tris-hydroxypyridi-

1,6-Substituted-2-hydroxyalkyl-3-hydroxy-4-pyridinones



Scheme 5.

nones is of the type 1,2-HP [41]. Concerning the multidentate derivatives with 3,4-HP, as far as it is known, there is only a very recent work on complexation studies with ligands containing two and three of these chelating moieties [53,54].

The preparation of these hexadentate derivatives involves a considerable number of steps and strategies, mostly related with protection and deprotection of the hydroxyl groups of the hydroxypyridonate ring for further reactions as well as the activation of carboxylic groups for attachment to amine groups. However, a detailed description of all the synthetic procedures is out of the aim of this paper.

The first synthetic hexadentate hydroxypyridinones were prepared by Raymond and coworkers [41] and have 1,2-HP as binding units. However, besides their effectiveness in the in vivo chelation of actinides, they proved to be quite toxic [43] and the clinical interest decreased. Since 1990, the strategy for the development of hexadentate hydroxypyridinones has been based on using the 3,2-HP binding moieties. The first hexadentate 3,2-HP derivative was prepared by the Hider and coworkers [42] and then by the Raymond and coworkers [43,44]. They both made the attachment of three 3,2-HP carboxylic derivatives to a three-branched acyclic backbone, the triaminotriethylamine (TREN). This structure was expected to give to the ligand a certain degree of preorganization. The chains were linked via formation of amide bonds (coupling between the carboxyl and the terminal amine groups). However, each research group used different 3,2-HP derivatives. Raymond et al. used the 4-carboxyl-*N*-methyl-3-hydroxy-2-pyridinone whereas Hider et al. used a longer-chain binding unit, the 1-carboxymethyl-3-hydroxy-2-pyridinone, affording the hexadentate derivatives TREN(Me-3,2-HP)$_3$ and TREN(3,2-HP)$_3$, respectively (Fig. 3). Within the same framework, other *N*-alkyl (instead of *N*-methyl) substituents have been recently used [47], to form 4-carboxyl-3-hydroxy-2-pyridinone bidentate units. Aimed at improving the preorganization of the ligand towards the metal binding, each of those research groups also used cyclic backbones. Accordingly, 1,3,5-tris(aminomethyl)benzene (ME), 1,3,5-tris(methylaminomethyl)benzene (MEC) and the enterobactin skeleton (ENTER) were used to obtain three new hexadentate hydroxypyridinones: ME(3,2-HP)$_3$, MEC(3,2-HP)$_3$ [46], ME(Me-3,2-HP)$_3$ and ENTER(Me-3,2-HP)$_3$ [43,44] (Fig. 3). Recently, Sun et al. [45] developed a new hexadentate ligand, TRIS(3,2-HP)$_3$ (Fig. 3). It also has three 3,2-HP units attached to an acyclic three-branched backbone, but with a claimed higher flexibility then the TREN(3,2-HP)$_3$ derivative. That improvement was attributed to the higher flexibility of both the molecular fragments: the anchor unit, with an apical carbon instead of a nitrogen atom; the binding arm units, with longer chain spacers as well as ether linkages instead of the usual amide ones.

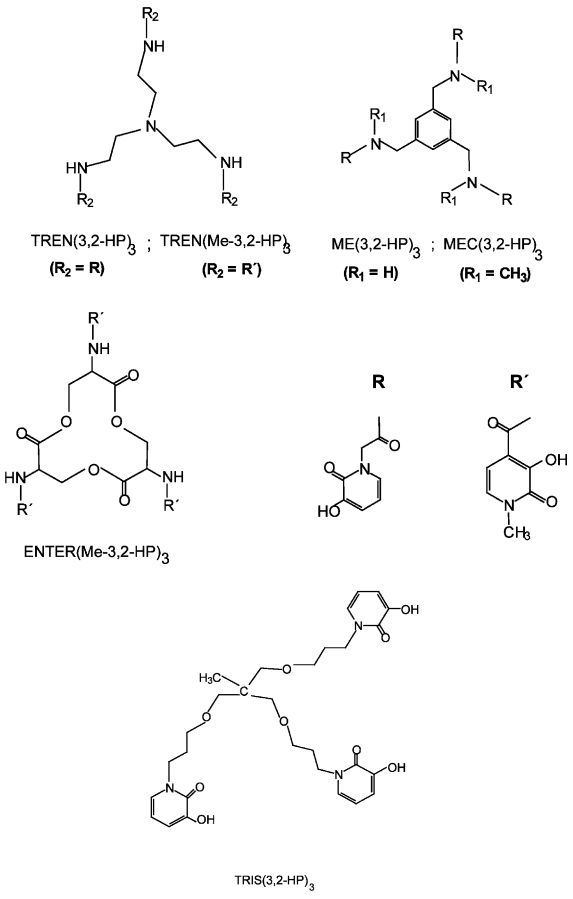
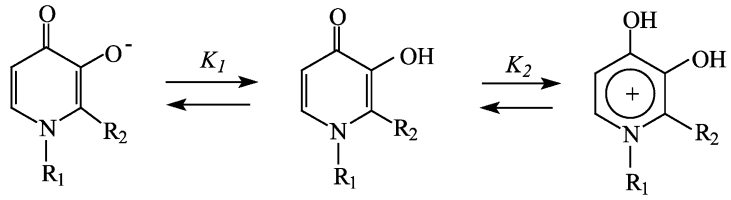


Fig. 3. Molecular structure of hexadentate hydroxypyridinones.

3. Acid–base properties

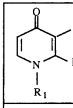
The study of the proton affinity of hydroxypyridinones is crucial in order to evaluate the assessment of these ligands as metal chelating agents, as well as to understand their behaviour in terms of lipo-hydrophilic balance and biodistribution. The hydroxypyridinones, in their neutral form, have a zwitterionic aromatic character, which makes high charge density on the carbonyl oxygen atoms. This is due to the charge transfer from the nitrogen lone pair to carbonyl oxygen, which is promoted by the restoration of the aromatic resonance of the ring. The protonation equilibria for the 3,4-hydroxypyridinones are shown in Scheme 6, where K_1 and K_2 are the equilibrium constants for the protonation of the 3-hydroxy and 4-hydroxy (pyridinium) groups, respectively. The corresponding log K_i are presented in Table 1.

Among the hydroxypyridinones (1,2-HP; 3,2-HP and 3,4-HP), there is a trend on the stepwise protonation constants according to the order 3,4-HP > 3,2-HP > 1,2-HP (Table 2). This can be rationalized in terms of the



Scheme 6.

Table 1
Stepwise protonation constants (log K_i) of a set of 1,2-substituted 3-hydroxy-4-pyridinone as well as global formation constants (log β_i) of the Al(III) and Fe(III) complexes and corresponding log β_{eff}; pM * values at pH 7.4

	R_2	R_1	Acid-Base			Fe(III) complexation				Al(III) complexation				
			Log K_1	Log K_2	Log K_3	Log β_1	Log β_2	Log β_3	pFe	Log β_1	Log β_2	Log β_3	Log β_{eff}	pAl
1	H	H [a]	9.01	3.34	-	14.2	25.8	35.1	20.8	-	-	-	-	-
			9.6 [b]	3.6 [b]				36.9 [b]						
2	Me	H	9.76 [b]	3.70 [b]	-	15.4 [b]	27.43 [b]	37.2 [b]	20.7					
			9.80 [c]	3.65 [c]	-					11.87 [c]	22.54 [c]	32.05 [c]	24.9 [c]	15.4
3	Me	Me	9.64 [b]	3.56 [b]	-	14.92 [b]	27.15 [b]	37.2 [b]	21.0					
			9.86 [c]	3.70 [c]	-					11.91 [c]	22.83 [c]	32.25 [c]	24.9 [c]	15.4
			9.77 [d]	3.68 [d]		15.1 [d]	26.6 [d]	35.88 [d]	19.3	12.20 [d]	23.25 [d]	32.62 [d]	25.51	16.0
4	Me	Et	9.88 [b]	3.65 [b]	-	15.33 [b]	27.83 [b]	37.7 [b]	20.8					
			9.81 [c]	3.64 [c]	-					11.75 [c]	22.52 [c]	32.17 [c]	24.9 [c]	15.5
5	Et	Et [f]	9.93	3.81	-	15.20	26.86	36.80	19.7	-	-	-	-	-
6	Me	$(CH_2)_5CH_3$ [c]	9.92	3.59	-	-	-	-	-	11.51	22.49	31.71	24.2	14.7
7	Me	C_6H_6 [e]	9.40	3.06	-	-	-	-	-	11.36	21.78	30.74	24.7	15.3
8	H	$CH_2CH(NH_2)CO_2H$ [g]	8.76	2.48	7.14	-	-	34.74	-	-	-	29.19	-	-
9	Me	$(CH_2)_2CO_2H$	9.83 [h]	3.34 [h]	4.13 [h]	18.03 [i]	30.17 [i]	37.97 [i]	21.5 [i]	13.04 [j]	24.06 [j]	32.81 [j]	-	16.1
10	Me	$(CH_2)_3NH_2$	9.09 [h]	3.20 [h]	10.07 [h]	-	-	34.89 [i,j]	17.4 [i]	14.97 [i,k]	39.12 [i,k]	54.26 [i,k]	-	13.2
11	Me	$(CH_2)_3CO_2H$ [k]	9.91	3.53	4.38	16.30	27.38	35.63	20.1	13.03	22.97	31.27	-	14.4

[a]Ref. [20]; [b]Ref. [36]; [c]Ref. [26]; [d]Ref. [28]; [e]Ref. [27]; [f]Ref. [38]; [g]Ref. [20]; [h]Ref. [32]; [i]Ref. [56]; [j][57]; [k]values are referred to log β_{MHiLi}.
* $C_L/C_M = 10$, $C_M = 1$ μM.

increased stability of the protonated form of the ligand, as the positive charge of the ring nitrogen is further away from the positive proton.

Since the 1,2-HP are very acidic (log $K_1 = 5.5–6$) [21,55], they are anionic at physiological pH, thus being quite hydrophilic and with limitations for biological applications. Conversely, 3-hydroxy-2-pyridinones (Table 2) [21,29] and the 3,4-HP (Table 1) are more basic (log $K_1 = 8.6–9.0$ and 9.5–9.9, respectively), and so neutral at physiological pH.

The effect of ring substituents is clearly shown by a detailed analysis of Table 1, for the 3,4-HP, and of Table 2, for the different types of hydroxypyridinones. There is an increase on the corresponding log K_1 values, according to the order ethyl > methyl > hydrogen (see for example the *N*-substitution of 3,4-HP, Table 1) [36]. This is due to the electron-donation effect of the alkyl groups. However, that effect is smaller than ca. 1 log unit and so it does not affect the predominance of the neutral species in a wide pH range, including the physiological pH. On the other hand, for the *N*-aryl substituted 3,4-HP, the log K_is are lower than the corresponding values for the *N*-alkyl derivatives (cf. 9.40 and 3.03 for 1-phenyl-2-methyl-3-hydroxy-4-pyridinone [27]; 9.56 and 3.56 for the 1,2-dimethyl-3-hydroxy-4-pyridinone [36], Table 1). This difference is consistent with the electron-withdrawing and the -donation effects of phenyl and alkyl groups, respectively. Identical effects were observed with the introduction of alkylamine and alkylcarboxyl groups [38,56,57]. Remarkable are the attempts made on improving the physico-chemical properties of the hydroxypyridinones by introducing other substituents, like the hydroxyl or hydroxyalkyl groups. In fact, the introduction of the 4-hydroxyl group in 1,2-HP produces a considerable increase (ca. 1) on the log K_1 corresponding to the *N*-hydroxyl proton of 1,2-HP (Table 2), with subsequent expected improvements in the chelating properties [22,28].

Concerning the hexadentate ligands, the log K_1 values of the 3-phenolic groups of 3,2-HP reflect, to some extent, the type of spacer. However, comparison be-

Table 2
Stepwise protonation constants (log K_i) of a set of different types of hydroxypyridinones as well as global formation constants (log β_i) of the Al(III) and Fe(III) complexes and corresponding log β_{eff}; pM * values at pH 7.4

Chelator	Acid–base		Fe(III) complexation				Al(III) complexation				
	Log K_1	Log K_2	Log β_1	Log β_2	Log β_3	Log β_{eff}	Log β_1	Log β_2	Log β_3	Log β_{eff}	pAl
3-Hydroxy-4-pyridinone	9.6 [a]	3.6 [a]			36.9 [a]	25.82	–	–	–	–	–
(**1**)	9.01 [b]	3.34 [b]	14.2 [b]	25.8 [b]	35.1 [b]						
3-Hydroxy-2-pyridinone	8.66 [b]	(0.1)	11.7 [b]	21.5 [b]	32.3 [a]	25.82	8.59 [c]	16.34 [c]	23.11 [c]	19.79	12.3
(**2**)	8.59 [c]				29.6 [b]						
1,4-Dihydroxy-2-pyridinone	8.28 [d]	6.14 [d]	–	21.3 [d]	28.18 [d]	26.9	–	21.20 [d]	25.16 [d]	22.52	15.1
(**3**)	8.4 [e]	6.7 [e]			29.9 [e]						
1-Methyl-3-hydroxy-2-pyridinone	8.89 [d]	–	11.8 [d]	21.6 [d]	29.99 [d]	25.52	9.41 [d]	17.79 [d]	25.10 [d]	20.53	12.2
(**4**)	8.8 [e]	3.0 [e]									
1,2-Dimethyl-3-hydroxy-4-pyridinone	9.77 [d]	3.68 [d]	15.1 [d]	26.6 [d]	35.88 [d]	27.4	12.20 [d]	23.25 [d]	32.62 [d]	25.51	16.0
(**5**)	9.7 [e]	3.3 [e]			34.5 [e]						
1-Hydroxy-2-pyridinone	5.86 [f]	(1.2)	10.6	20.1	27.2	–	8.16	15.54	21.59	–	12.4
(**6**)	5.78 [f]	(−0.9)	9.0	16.6	26.9						

[a]Ref. [36]; [b]Ref. [21]; [c]Ref. [29]; [d]Ref. [28]; [e]Ref. [22]; [f]Ref. [55].
* $C_L/C_M = 10$, $C_M = 1$ μM.

tween them is rendered difficult, because some of them are quite lipophilic, thus being determined by quite different methods and experimental conditions. Also in some cases, the intrinsic value [58] or just the average value is reported for pK_as of those groups.

4. Aluminium complexation

4.1. Bidentate hydroxypyridinones

The metal binding efficiency is one of the most important criteria for choosing a chelator for clinical use. The hydroxypyridinones are expected to form very stable complexes with Al due to high partial negative charge of the chelating oxygen atoms and to the 'acidity' of the phenolic group.

The strong interaction of hydroxypyridinones with the Group 13 metal ions, but particularly with Fe^{3+} and Al^{3+}, was first outlined about 30 years ago in a study with mimosine, a naturally occurring aminoacid coupled to a 3,4-HP [1-(α-aminopropanocarboxyl)-3-hydroxy-4-pyridinone] (log $\beta_{FeL_3} = 34.74$; log $\beta_{AlL_3} = 29.18$) [20]. Later, Raymond and coworkers [21] studied the interaction with iron of various types of hydroxypyridinones (1,2-HP, 2,3-HP and 3,4-HP). 1,2-HP proved to be good chelators in acidic medium, due to the high acidity of the ligands, whereas 3,4-HP were more effective in the neutral range of pH (pH 6–9). This seemed to contrast with the catechol, which has high affinity for the iron (log $\beta_{FeL_3} = 44.9$), but only in basic conditions, because the log K_a values are very high (13.3, 9.22) [59].

Since then, much effort has been devoted to study the effect of different substituents in the metal binding efficacy, mostly with the iron(III) (Hider [36,37], Kontoghiorghes [21] and Martell and coworker [28]). Different groups (Orvig [26,37], Martell [28], Di Marco [29] and Santos [56,57]) also studied the effect of substituents on the binding affinity with other metal ions of Group 13, including the Al^{3+}, for the most promising chelators. That evaluation was based on the stability constants of the corresponding metal complexes, which are mostly calculated by potentiometric and spectrophotometric titration techniques. In some cases, the Al speciation and validation of the proposed models are also aided by ^{27}Al-NMR spectroscopy [26].

Regarding the interaction of the bidentate hydroxypyridinones with Al as well as the iron, for comparison purposes, some literature results are summarized in Tables 1 and 2. They are mostly reported as global formation constants, log β_3, ($\beta_3 = [ML_3]/[M][L]^3$). However, comparison between the metal binding affinity of different ligands has to take into account their different proton concentration dependency. Accordingly, pM values (pM = −log [M] for $C_L/C_M = 10$ and $C_L = 10^{-5}$ M) or effective overall formation constants [log β_{eff} = log $\beta_3 - 3 \times$ (log K_1 − pH), K_1 being the highest protonation constant [60]] at physiological pH have been used.

Among the different types of hydroxypyridinones, the 3,4-HP have been the most systematically studied in their interaction with Al, namely the 2-methyl derivative with a range of *N*-substituents (Table 1). Comparison between the log β_{eff}, calculated for the corresponding Al complexes (AlL_3) at the physiological pH, indicates that the introduction of a *N*-alkyl function produces just a very small increase on the overall constants (log $\beta_3 \simeq$ 32.05 vs. 32.25) but no significant effect on the conditional constants (log $\beta_{eff} = 24.9$). A small decrease on β_{eff} was found for the complex with 2-methyl-1-hexyl-3-hydroxy-4-pyridinone (log $\beta_{eff} = 24.2$). The *N*-phenyl substitution produces also only a small decrease on that conditional constant (log $\beta_{eff} = 24.9$ vs. 24.7). Thus,

the electron-withdrawing and -donating effects of the *N*-alkyl and *N*-aryl substituents, respectively, produce only minor differences on the overall β_3 values. This is due to small changes in the pK_as and those differences become even neglected at the level of the conditional constants at the physiological pH. Noteworthy is the effect of introducing charged groups which produces remarkable increase on the binding affinity (pAl) as the charge is negative (e.g. *N*-alkylcarboxylate, **9**) or positive (e.g. *N*-alkylamonia, **10**). Although these substituents maintain practically constant the thermodynamic properties, for an eventual competition with Tf for the Al (log β_{eff} = 20.0) [9], some of them produce quite considerable changes on the physicochemical properties, such as the lipo-hydrophilicity balance, as indicated by the different distribution coefficients (see below).

A comparative analysis of the Al affinity of different types of bidentate hydroxypyridinones can be made on the basis of the log β_{eff} or the pM values presented in Table 2. Although being limited to the physiological pH, they give a clear indication that among the hydroxypyridinone presented in this table, the 1,2-dimethyl-3-hydroxy-4-pyridinone is the strongest Al chelator (ca. pAl 16) [26,28]. This is due to the electron-donation effect of the two alkyl groups and also to the fact that the 3,4-HP have larger distances between the inherent positive charges of the metal complexes (metal cation and pyridinium nitrogen).

Noteworthy is the extra-stabilization achieved with the introduction of a hydroxyl group at position 4 of 1,2-HP, to give the 1,4-dihydroxy-2-pyridinone. Its affinity for the Al (pAl 15.1) is much higher than that of the parent compound 1,2-HP (pAl 12.4) and it seems to be even higher than that of tranferrin (pAl 14.5) [9].

For a more detailed comparison of the Al affinity among the bidentate hydroxypyridinones (see Tables 1 and 2), the corresponding pAl versus pH plots have been calculated and are shown in Figs. 4 and 5. It should be

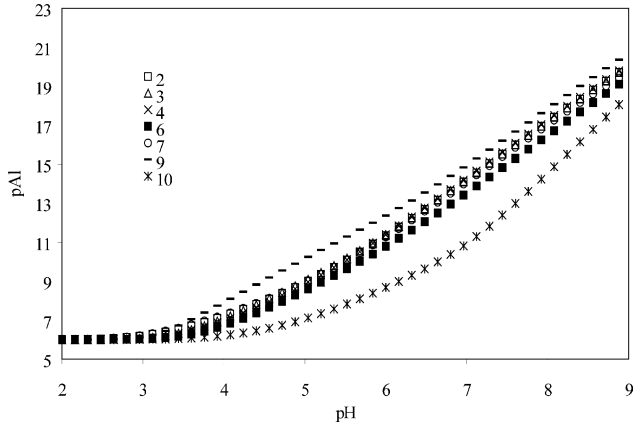


Fig. 4. Aluminium complexation strength, for various bidentate 3,4-HP presented in Table 1, reported as pAl versus pH (pAl = $-\log[Al^{3+}]$ with $C_L/C_{Al} = 10$ and $C_{Al} = 10^{-6}$ M).

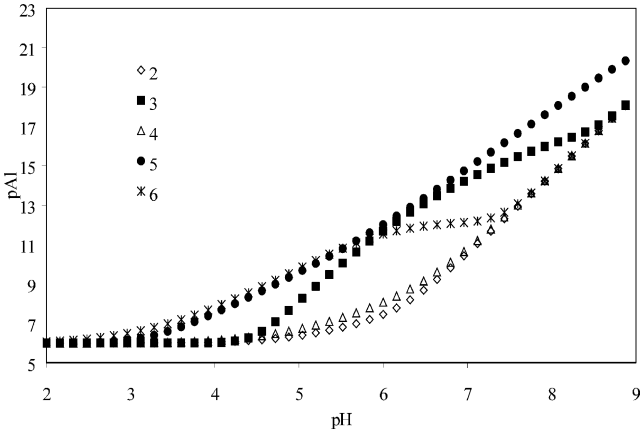


Fig. 5. Aluminium complexation strength for the various types of hydroxypyridinones presented in Table 2, reported as pAl versus pH (pAl = $-\log[Al^{3+}]$ with $C_L/C_{Al} = 10$ and $C_{Al} = 10^{-6}$ M).

mentioned that these pAl values were calculated based on the equilibrium constants reported in the literature, neglecting effects of different ionic strengths.

Fig. 4 shows that the 3,4-HP derivatives present a steady variation of pAl with pH and that the graphical results are a confirmation of the effect of the *N*-substitution on the affinity order, suggested by the pAl values at physiological pH (Table 1). Thus, the effect of the alkyl substituents is rather small, as compared with those of the polar (charged) substituents which, by Coulombic effects, can interact with the positive charge of the metal cation in the complex. The 3,4-HP with *N*-alkylcarboxylate or *N*-alkylammonium groups (**9**, **10** in Fig. 4) are illustrative examples of those cases.

Fig. 5 illustrates the pH dependence on the Al affinity of the different types of hydroxypyridinones. The 1,2-HP (**6**) is a good Al chelator, but only in the acidic pH range (pH < 6), due to the high acidity of the phenolic proton (see Table 2). Introduction of an extra 4-hydroxyl group **3** produces a shift of the pH range of higher binding efficacy to higher values, mainly due to the increase of the log K_as. Thus, in the neutral range of pH, pAl of **3** is very similar to that of 3,4-HP (**5**). The 3,2-HP have their highest affinity for Al^{3+} in neutral pH ranges, but they always present lower affinity than the 3,4-HP, independently of the substituents in question. As stated above, the highest efficacy of the 3,4-HP, when compared with the other two types of hydroxypyridinones, is mostly attributed to electrostatic repulsions between the positive charge of the pyridinium nitrogen and that of the metal ion, which decreases with increasing distance between these two positive centres. A stabilizing effect is also accomplished with the introduction of electron-donor substituents, which makes the 1,2-dialkyl-3-hydroxy-4-pyridinone(s) the best chelators (Table 2 or Fig. 5), in the neutral pH range.

In terms of the effect of other substituents on the physico-chemical properties of hydroxypyridinones, many other studies have been performed, but they

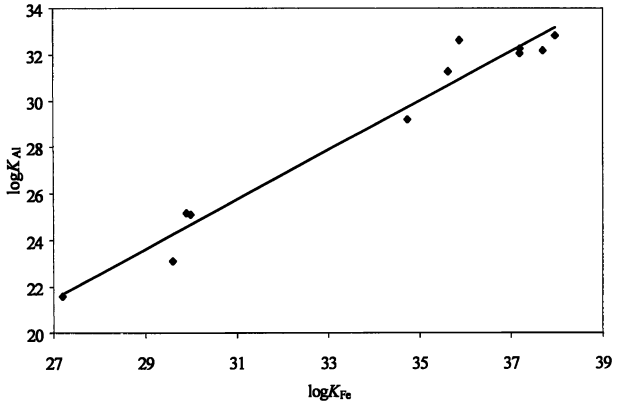


Fig. 6. Relationship for the complexation of Fe^{3+} and Al^{3+} with the set of bidentate hydroxypyridinones, reported in Tables 1 and 2. Each point consists of the Fe^{3+} global stability constant (log β_{ML3}) of a given ligand as the x coordinate and the Al^{3+} global stability constant of the same ligand as the y coordinate.

have been confined to the iron(III) interaction. Considerable improvements were obtained on the pFe values (two orders of magnitude higher than Deferiprone) by the introduction of 1-hydroxyalkyl groups at the 2-position together with 6-methyl group [36]. A large number of 2-alkyl-3,4-HP derivatives, containing various *N*-substituents (alkyl, alkylhydroxyl, alkylcarboxyl, alkylester and alkylamines) have also been recently developed [61–63], although their study was restricted to biological assays, as potential iron chelators. Kontoghiorghes [22] also evaluated the effect of some substituents (e.g. 4-hydroxy, 4-metoxy; 4-(2′-metoxyethoxy); 4-nitro; 5-nitro) on the effectiveness of 1,2-HP derivatives as iron chelators. However, among all these 1,2-HP derivatives, the 1,4-dihydroxy derivatives proved to be the most effective on the release of iron from Tf, because of favourable thermodynamic properties of the metal complex and of ligand/complex solubility at the physiological pH. So, its affinity towards Al was then later studied by Martell and coworker [28].

Despite the great importance of hydroxypyridinones as Al chelators, their interaction with Fe^{3+} has been studied at much higher extent than with Al^{3+}. This led the author of this article to make a comparative analysis of the global stability constants reported for a set of complexes of bidentate hydroxypyridinones with both these metal ions. The plot the stability constants of the iron complexes of each ligand (log β_{FeL_3}) presented in Tables 1 and 2, (as the x coordinates) and the stability constants of the corresponding Al^{3+} complexes(as the y coordinate), shows an approximate linear correlation for the affinity of these ligands towards these metal ions (Fig. 6). The existence of such a relationship is important because the strength of the Al interaction with the most promising hydroxypyridinones can be broadly extrapolated, in cases for which the chelating properties (values of stability constants) are only known for the iron.

As a summary, it may be stated that among the bidentate hydroxypyridinones studied, the 1,2-dialkyl-3-hydroxy-4-pyridinones and the 1-alkylcarboxyl-2-methyl-3-hydroxy-4-pyridinone are the most effective Al chelators at physiological conditions and, based on this feature, it can be predicted that they can be used as therapeutic agents against Al overload.

4.2. Hexadentate hydroxypyridinones

The comparison between the effectiveness of the ligands, based on Figs. 4 and 5, was made on the assumption of normal diluted conditions (10^{-5} M for C_L and 10^{-6} M for C_M) and it holds for ligands which are all bidentate (monochelating compounds) and form the same type of complexes. However, the comparison with ligands having higher denticity, like the tris-hydroxypyridinones or even Tf, has to be made with care because of the dilution effect outlined by Martell and coworker [64]. In fact, at a given concentration of a M^{3+} metal ion, the concentration of the mono–hexadentate complex (ML) is first-order-dependent on the ligand concentration while the concentration of a tris–bidentate complex (ML_3') is third-order-dependent. So, for more diluted conditions, the competition of a monochelating compound for a three-charged metal ion usually decreases, as compared to that of a trischelating agent. Therefore, under the diluted conditions that prevail in physiological systems, the hexadentate hydroxypyridinones, either because of their indifference to that dilution effect or due to enthropic contributions (displacement of coordinated water molecules by ligands), are expected to have higher affinity for M^{3+} than the corresponding bidentate hydroxypyridinones.

Although a considerable number of hexadentate hydroxypyridinones has been developed world-wide by several research groups (see above), they have been mostly aimed at the replacement of the DFO in iron chelating therapy. Therefore, based on the similarity between the physico-chemical properties of Fe^{3+} and Al^{3+} (as illustrated in Fig. 6 for the bidentate hydroxypyridinones), we have decided to include herein a summary of the iron chelating properties (Table 3) and the distribution coefficients of a series of hexadentate hydroxypyridinones shown in Fig. 3.

Concerning the tris-hydroxypyridinones with acyclic framework, TREN(3,2-HP)$_3$ [42] presents a pFe value (25.8) much higher than the corresponding value for the bidentate *N*-ethyl derivative (18.3), although the calculated log K_{ML} (28.8) is lower than log β_{ML_3} (32.3) obtained for the corresponding bidentate analogue. This was attributed to the lack of ligand preorganization, although the existence of a hydrogen bond network between the amide hydrogen and the carbonyl oxygen

Table 3
Distribution coefficients (*n*-octanol/water, pH 7.4), pK_a values, global stability constants (log β_{FeL_3}/log β_{FeL}) and pFe values for some hexadentate hydroxypyridinones as well as for one related bidentate ligand.

Ligand	D_{ligand}	pK_a	log β_{FeL_3}/log β_{FeL}	pFe
1-Et-3,2-HP [a] (**1**)	1.57	8.99	32.3	18.3
TREN(3,2-HP)$_3$ [a] (**2**)	0.025	9.249; 8.686; 8.132 (pK_a int = 8.60) [b]; 5.993	28.8 [b]	25.8
ME(3,2-HP)$_3$ [c](**3**)	0.018 [a]	9.43; 8.83; 8.20 (pK_a int = 8.68)	28.20 [a]	24.8 [c]
ME(Me-3,2-HP)$_3$ [c](**4**)	0.030 [a]	9.49; 8.98; 8.27 (pK_a int = 8.74)	28.70	25.1
TRIS(3,2-HP)$_3$ [d](**5**)	–	9.50 [e]; 1.92; 0.6	37.6	32.6
TREN(Me-3,2-HP)$_3$ (**6**)	–	6.95; 5.80; 4.96 [f]; 8.20	26.7 [g]	26.7 [g]
ENTER(Me-3,2-HP)$_3$ [g](**7**)	–	6.1 [e]	26.7	27.4

[a]Ref. [42]; [b]pK_a intrinsic according to Ref. [58]; [c]Ref. [41]; [d]Ref. [45]; [e]average value; [f]Ref. [43]; [g]Ref. [44].

atoms has been considered as a possible reason for the weakening of that metal binding interaction [65]. The TREN(Me-3,2-HP)$_3$ derivative [43], having the same spacer backbone, but slightly different 3,2-HP binding units, presents higher affinity for the iron (pFe 26.7) than DFO (pFe 26.3) [59]. The TRIS(3,2-HP)$_3$ derivative, with an apical carbon instead of a nitrogen atom, presented the highest pFe value (32.6) [45]. It seems there is a gain in stability of this complex, relative to the TREN derivatives, attributed to the claimed higher flexibility of this ligand. However, comparison between these results is questionable because, for this last compound, the measurements were performed in non-aqueous media and the log K_a has an unexpectedly high value (average 9.5).

Regarding the hexadentate hydroxypyridinones with cyclic backbones, both the ligands with 1,3,5-tris(aminomethyl)benzene frameworks, ME(3,2-HP)$_3$ and MEC(3,2-HP)$_3$ [46], show a decrease in the metal binding affinity (pFe 24.8 and 25.1, respectively), as compared with the corresponding TREN analogue. Besides the expected change in the lipophilicity, the *N*-methyl substitution on the framework, made no differentiation between the metal affinities of both of these cyclic derivatives, thus indicating that only negligible

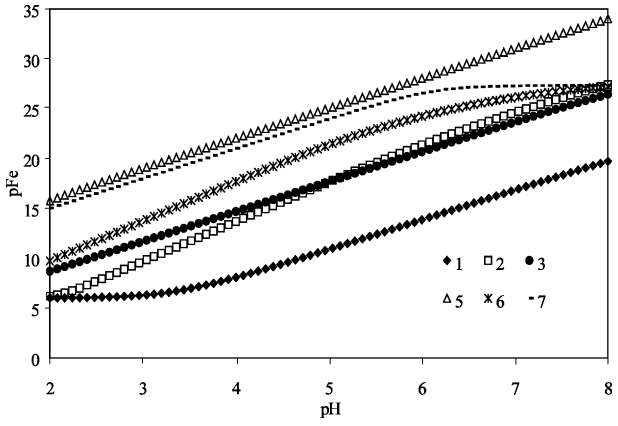


Fig. 7. Iron complexation strength, for some hexadentate hydroxypyridinones presented in Table 3, reported as pFe versus pH (pFe = $-\log[Fe^{3+}]$ with $C_L/C_{Fe} = 10$ and $C_{Fe} = 10^{-6}$ M).

effects could be due to eventual intramolecular H-bond network. The tris-hydroxypyridinone with the enterobactin framework (macrocyclic triserine skeleton), ENTER(Me-3,2-HP)$_3$ [44], presented also a higher affinity for iron (pFe 27.4) than DFO. However, comparisons involving these cyclic backbone derivatives should be made with precaution because, due to poor water solubility, their equilibrium studies were performed in ethanol/water mixed solutions.

For further comparison between the metal affinities of these ligands at different pH, Fig. 7 shows their pFe values plotted as a function of the pH, using the model conditions ($C_L/C_M = 10$, $C_L = 10^{-5}$ M).

All the hexadentate ligands present higher affinity for the iron than the bidentate analogue, practically irrespective of the pH range. Among this set of ligands, tris(2,3-HP)$_3$ and ENTER(Me-3,2-HP)$_3$ have the highest metal affinities, which are identical in the neutral or in the acid range. However, for pH > 6, the ENTER and the TREN derivatives show no further influence of the pH on the metal binding because the complex is practically deprotonated. The TREN derivatives **2** and **6** also present extra losses on the binding efficacy in acidic pH range due to the protonation of the anchor *N*-amine group. The four orders of magnitude higher value of the pFe of the TREN derivative **6** as compared to that of **2**, in the neutral or acid pH range, may be attributed to the electron-donating effect of the *N*-methyl group.

Therefore, these ligands can be considered as good chelating agents for iron and the same should be valid for Al. In fact, Al^{3+} has a smaller size than the Fe^{3+} (ionic radius 0.50 and 0.64, respectively) and it may also be more easily accommodated in the tripodal cavity, namely in the less flexible ones. So, it is admissible that the drop on the stability constant for the Al complexes relative to the corresponding Fe complexes (observed for the bidentate ligands), may be even lower for a hexadentate than a bidentate hydroxypyridinone. Thus, as the hexadentate hydroxypyridinones can compete for Fe with Tf, the same could hold for Al.

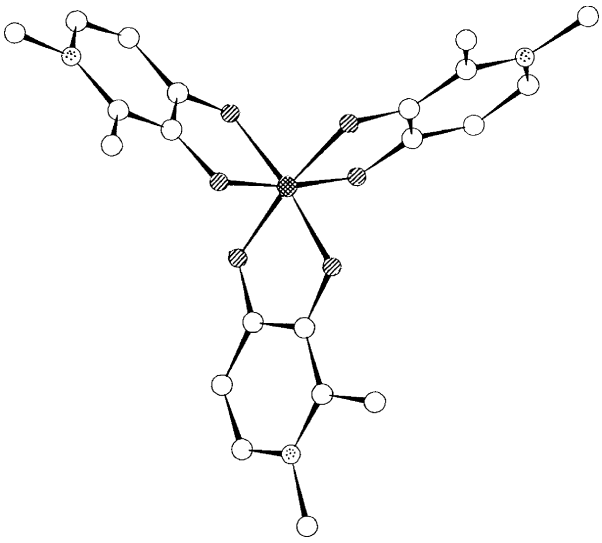


Fig. 8. CERIUS^2 molecular model of the complex [Al(1,2-dimethyl-3-hydroxy-4-pyridinone)$_3$], from the corresponding X-ray structure (Ref. [37]), showing the *fac* symmetry of the metal-containing portion of the compound. The metal ion is double hatched, the oxygen atoms are hatched and the nitrogen atoms are dotted; hydrogen atoms are omitted for clarity.

4.3. Crystal structure

The crystal structures of the Al complexes have been reported for *N*-alkyl- and *N*-aryl-3-hydroxy-4-pyridinones as well as for the 3-hydroxy-1-methyl-2-pyridinone. The [Al(1,2-dimethyl-3-hydroxy-4-pyridinone)$_3$·12H$_2$O] complex [37] crystallises as *facial* (*fac*) isomers (all the hydroxo oxygen atoms bound in *trans* position to the carbonyl oxygen atoms) with a threefold symmetry, as shown in the corresponding CERIUS^2 [66] molecular model presented in Fig. 8. The *fac* configuration may be favoured due to the existence of some stabilization when all strong donor atoms are coordinated in *trans* position to the weaker donor atoms. Two different metal–oxygen distances were found, as expected, with shorter Al–O distances for the 3-hydroxy oxygens compared to those of the 4-carbonyl oxygens. Noteworthy is the extensive hydrogen-bonding network, which involves interaction between the complex molecules, via the formation of head-to-tail dimers with pairs of C=O···H–O–C hydrogen bonds linking the dimers. On the other hand, all six *O*-chelating atoms are hydrogen bonded with two water molecules, thus forming dodecahydrate complexes. The hydrogen bonding between the ligand and water molecules is arranged in a cyclic way. Their similarity to water channels led this type of complexes to be called as exoclathrate [37]. It was admitted that hydrogen bonding between this complex and water might have contributed to the *fac* geometry adopted in the solid state.

The crystal structure reported for the Al complexes with *N*-aryl-3-hydroxy-4-pyridinone [27] also showed the existence of *fac* isomers and the exoclathrate lattice with water molecules, as found for the *N*-alkyl derivatives [37]. That exoclathrate structure was not observed in the complex with the *N*-ethyl and *N*-*p*-tolyl-2-methyl-3-hydroxy-4-pyridinone, probably due to steric reasons. The complex [Al(3-hydroxy-1-methyl-2-pyridinone)$_3$] also presented a threefold symmetry, crystallizing in space group *R*3*c* (rhombohedral crystal system), but with racemic characteristics [29].

Concerning the hexadentate 3-hydroxy-2-pyridinones, as stated above, there is no studies reported on their Al complexes. However, similarly to the X-ray structures reported for the iron complexes with TREN(3,2-HP)$_3$ [67] and ENTER(3,2-HP)$_3$ [44], the Al complexes with any of those supported tris-chelating ligands, are expected to have an approximate C_3 point symmetry, but with deviations from the octahedral geometry of the coordination to the central metal atom, due to steric constrains imposed by the frameworks.

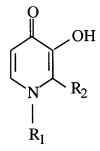
5. Distribution coefficients

The importance of the hydroxypyridinones in chelating therapy (mostly decorporation of Fe^{3+} and Al^{3+}, but also incorporation of other M^{3+} metal ions such as Ga^{3+} and In^{3+}) has determined that much of the effort on designing these ligands has been channelled in the introduction of suitable substituents. These studies aimed at improving the metal affinity of the sequestering agents, at physiological conditions, as well as the drug absorption by the target organs and further excretion. So, according to the organ to selectively deliver the drug, its design must take into account the expected lipo-hydrophilic balance for both the chelator and the metal complex, in order to conjugate the maximization of the scavenger efficacy and the minimization of the potential toxicity.

The lipo-hydrophilic character of hydroxypyridinones has been usually evaluated through the octanol/water distribution (partition) coefficient (*D* or log *D*), which is basically a concentration ratio of a species in octanol phase and in aqueous phase (pH 7.4), by established methods [68–70]. Spectrophotometric techniques ('shakeflask' or continuous flow methods) are mostly used with measurements based on the benzenoid band (π–π*), which is at ca. 280–290 nm for the ligands, but becomes batocromically shifted (ca. 300 nm) upon complexation. The aqueous and octanol phases are presaturated with respect to each other, before use. However, for water insoluble compounds a different technique has also been used, based on the evaluation of the solubility and solvation in alcohol/water mixture [71].

A systematic study is known about the effect of a wide range of *N*-substituents on the distribution coefficients of 3,4-HP, in relation with their ability as orally active

Table 4
Distribution coefficient (D) (log D) in n-octanol/water of some 3,4-HP and corresponding Al complexes as well as their in vivo efficiency as Al chelators



R_1	R_2	Chelator D	Chelator Log D	Al complex D	Al complex log D	Efficiency (%) [d]
$-CH_2CH_3$ [a]	Ethyl	2.1	0.32 0.23 [c]	2.1	0.32	3.5
$-CH_3$ [a]	Me	0.094	-1.03 -0.77 [c]	0.0009	-3.05	2.8
$-CH_3$ [a]	Ethyl	0.28	-0.55 -0.21 [c]	0.0070	-2.15	4.5
$-CH_2CH_3$ [a]	Me	0.16	-0.80 -0.31 [c]	0.0084	-2.08	5.0
$-(CH_2)_2CH_3$ [a]	Me	0.78	-0.11 0.18 [c]	0.22	-0.66	--
$-(CH_2)_3CH_3$ [a]	Me	1.5	0.18 0.70 [c]	17	1.23	6.5
$-(CH_2)_2OCH_3$ [a]	Me	0.24	-0.62 -0.41 [c]	0.0013	-2.89	11.7
$-(CH_2)_3OCH_2CH_3$ [a]	Me	0.68	-0.17 0.12 [c]	0.22	-0.66	3.5
$-(CH)_2OH$ [d]	Me	all in aqueous		all in aqueous		3.3
-Ph [b]	Me	13.2	1.12	126	2.10	--
-Ph-p-CH_3 [b]	Me	52.5	1.72	446	2.65	--
-Ph-p-OCH_3 [b]	Me	18.6	1.27	288	2.46	--
-Ph-p-NO_2 [b]	Me	5.8	0.76	29	1.46	--

[a]Ref. [12]; [b]Ref. [27]; [c]Ref. [72]; [d]Ref. [30].

iron chelators with clinical potential [36,37,72]. However, much less is known for the Al hydroxypyridinonate complexes.

Concerning the assessment of hydroxypyridinones as potential Al chelators in biological systems, it is usually evaluated in vitro through the octanol/water partition (distribution) coefficients of chelators/Al-complexes [36]. Table 4 summarizes the reported results (D and log D values) for a set of 3,4-HP and the corresponding Al-complexes. Two slightly different methodologies have been used: Yokel et al. [11,12] used the method proposed by Scherrer [70] with an octanol/water system containing insoluble Al (as the borate) to simulate the mobilization of Al from biological Al-binders such as Tf. Other authors, such as Porter and Hider [36,72] and also Orvig et al. [27], used slightly different methods [69], where the presence of insoluble Al source in the octanol/water system is not required. So, comparison between distribution coefficients of different authors must be made with precaution because they may give slightly different values.

From the set of results presented in Table 4, it can be seen that all the N-aryl-3-hydroxy-4-pyridinones (aryl = phenyl, p-tolyl, p-methoxyphenyl, p-nitrophenyl) [27] presented log $D \geqslant 1$, being therefore much more lipophilic than any of the N-alkyl derivatives (log $D < 1$). The corresponding Al complexes show an increase in lipophilicity, when compared to the corresponding ligands ($\log D_{\text{complex}}/\log D_{\text{ligand}} \sim 2$), similarly to what was found later by Hider and coworkers [72], for the iron complexes of a set of lipophilic N-alkyl-3-hydroxy-4-pyridinones. To such an increase on the Al-complex lipophilicity is expected an enhancement on the lipid solubility and membrane permeability, but a decrease on the renal excretion. Therefore, some doubts

can be raised about the interest of these aryl derivatives as scavengers of Al in overload animals. However, they seem to fit important criteria that have been referred as necessary to cross the blood–brain barrier (BBB) [73], such as neutral charge, lipid solubility and molecular weight below 500. Concerning the octanol/water distribution coefficients for a series of 1,2-dialkyl-3-hydroxy-4-pyridinones, the values obtained by Yokel et al. [12] are similar to those reported by Hider and coworkers [72] for the same type of compounds.

Thus, just by changing the 1,2-substituents, it is possible to obtain good Al chelators with a wide range of lipophilicity. The Al complexes of *N*-alkyl derivatives are in general more hydrophilic than the corresponding ligands, this feature being in favour of Al clearance in biological systems. On the contrary, the *N*-aryl-3-hydroxy-4-pyridinones are quite lipophilic ($1 < D < 2$) and their Al-complexes are even more lipophilic than the corresponding ligands, which limits their use in Al clearance. Such behaviour can be attributed to the fact that the complex presents a more extended hydrophobic surface to its environment than the ligands.

The relevance given to the lipo-hydrophilic balance of ligands for potential chelation therapy led Burgess et al. [71,74,75] to evaluate the lipo-hydrophilic character of a series of poorly water-soluble 3,4-HP, but using a different technique, which is based on their solubility and solvation in alcohol/water mixtures. The solubility is evaluated by measuring the concentrations of saturated solutions obtained by shaking an excess of ligand (or complex) in a range of methanol/water mixtures (0–100% V/V, methanol percentage in the solvent mixture). The concentration data (ca. solubility = *S*) is converted in the named transfer chemical potential, $S_m\mu^o$.

$$S_m\mu^o = -RT \ln(S_{\text{in medium 2}}/S_{\text{in medium 1}})$$

So, the more lipophilic the species is, the higher is its solubility in methanol and the more negative is $S_m\mu^o$.

Burgess et al. [71,75] performed that type of solvation studies for a set of *N*-alkyl- and *N*-aryl-3-hydroxy-4-pyridinones. The calculated $S_m\mu^o$ values for 1-(*p*-hexylphenyl)-2-methyl-3-hydroxy-4-pyridinone, 1-phenyl-3-hydroxy-4-pyridinone and 1,2-dimethyl-3-hydroxy-4-pyridinone were 23.4, −7.7 and −4.0 kJ mol^{-1}, respectively, which means that they are ca. 10^4, 23 and 5, respectively, times more soluble in methanol than in water. Although this is in agreement with expectations, due to the presence of lipophilic groups, noteworthy is the fact that some of the studied compounds, namely the least lipophilic (1,2-dimethyl-3-hydroxy-4-pyridinone), presents a maximum solubility (minimum transfer chemical potential at around 80%) (see Fig. 9). This can be rationalized as a synergic solvation, i.e. it is better solvated by both the components of the mixture. The existence of such a minimum for the transfer chemical potential can give an indication of an adequate hydrolipophilicity and therefore a good combination of conditions for oral administration and crossing membrane ability.

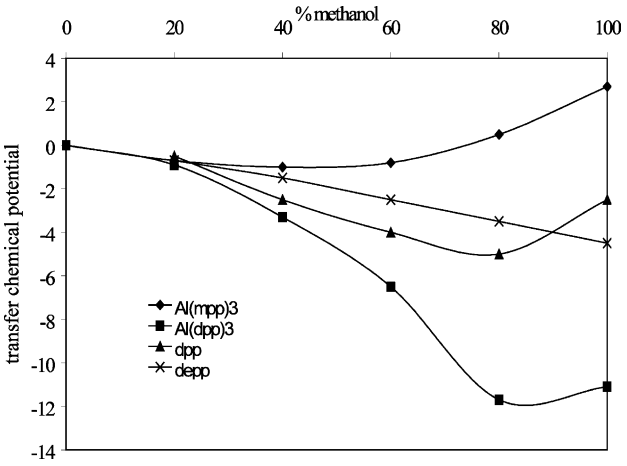


Fig. 9. Transfer chemical potentials for two alkyl-3-hydroxy-4-pyridinones and two Al complexes from water in methanol/water mixtures at 298 K, according to Refs. [71,74]. Abbreviations: mpp, [3-hydroxy-2-methyl-4-pyridinone]; dpp, [1,2-dimethyl-3-hydroxy-4-pyridinone]; depp, [1,2-diethyl-3-hydroxy-4-pyridinone].

The solubility/solvation of some Al complexes with hydroxypyridinones were also studied by the same method [71,74]. Fig. 9 shows the expected effect of changing the lipophilicity of two ligands and two Al-complexes. The Al-complex of the 1,2-dimethyl-3-hydroxy-4-pyridinone also presents a minimum for the transfer chemical potential (maximum solvation stabilization), and a higher lipophilicity than the corresponding ligand. This seems to be in some contradiction with evaluations based on the distribution coefficients (see Table 4) where the increase on the lipophilicity of the Al-complexes, as compared to the corresponding ligands was only found for the most lipophilic hydroxypyridinones. Such a mixed hydro-lipophilicity character (i.e. the existence of a transfer chemical potential minimum) is admitted to be a determinant feature for the proposed chelating therapeutical function of these chelators.

Finally, taking into account the similarity between Al and Fe, it seems worthwhile to make a brief analysis of a systematic study performed by Hider et al. [36,37] on the effect of a wide range of *N*- and *C*-substituents on the distribution coefficients (*D*) of a series 3,4-HP. To a one $-CH_2-$ unit elongation of the *N*-alkyl chain on a series of 2-alkyl-3-hydroxy-4-pyridinones corresponds an increase on *D* of ca. 2.3×10^4, while the introduction of a hydroxyl group in the alkylic chain can produce a reduction of *D* (6–27 times smaller). However, the major decrease of lipophilicity was observed with the introduction of charged groups, namely *N*-carboxyalkyl ($D < 0.001$ for $R = (CH_2)_2COOH$) and *N*-aminoalkyl groups ($D = 0.008$ for $R = (CH_2)_3NH_2$). The effect of *C*-ring substitution is similar to that of *N*-ring substitution. A special emphasis was given to the ester derivatives,

which were considered as prodrugs [37]. In fact, due to their favourable lipophilicity, they could be selectively delivered to certain organs, such as the liver (the main iron-storage organ under iron-overload conditions). Once there, they could be hydrolysed to give a more hydrophilic compound (*N*-hydroxyalkyl derivative), which could complex the metal ion and be easily extracted, thus minimizing the potential toxicity. Noteworthy is the correlation found between the effect of the *N*-substitution on the log *D* of the ligands and the corresponding iron complexes [72]. It was found a biphasic linear relationship, which means there are two slopes: a lower slope (0.49) for the hydrophilic ligands (log $D < -1$) but a higher slope (2.53) for the lipophilic ones. This is probably due to the fact that the more hydrophilic ligands and complexes have a similar aqueous solvation effect.

In summary, a considerable number of 3,4-HP, having different substituents, induces a wide range of lipo-hydrophilic characteristics (distribution coefficients) on the chelator/metal-complex. They can provide useful indication, namely in terms of the ability of the chelators to penetrate the sites of Al storage, but also on the ability of Al elimination upon complexation.

6. Biological assays

Based on the in vitro results, there is a set of 1-alkyl-2-substituted 3,4-HP which, due to the high Al-binding affinity and favourable intermediate lipophilicity ($D = 0.2–1.0$), [76] should be good candidates for Al-chelation therapy. Some recent studies have been conducted to evaluate the in vivo Al chelation efficacy of several bidentate 3,4-HP in animal models of Al accumulation and toxicity. On the other side, besides the high molecular mass of the hexadentate hydroxypyridinones, which is expected to render in difficulties for membrane crossing, their extremely high Al-affinity may overcome this problem, because only low dosage levels are need. So, both the bidentate and the hexadentate are analysed in terms of reported in vivo results and perspectives.

6.1. Bidentate hydroxypyridinones

A quite large number of in vivo assays has been performed by Hider [61–63] for a large set of bidentate *N*-substituted 3,4-HP, aimed at screening and identifying the most promising iron chelators, in ^{59}Fe loaded rats. There are also some studies about the effects of various *N*-substituted 3,4-HP on in vivo interactions with ^{111}In [77] and ^{67}Ga [38,78], for potential radiodiagnosis purposes. Concerning in vivo studies on decorporation of aluminum with hydroxypyridinonates, some interesting results were recently reported by Yokel et al. [30,31] and Florence et al. [32]. Both these research groups used Al-loaded animals, as models of this metal accumulation and toxicity, and bidentate *N*-substituted-2-alkyl-3-hydroxy-4-pyridinones as chelating agents, in comparison with DFO, the drug currently used to treat Al intoxication.

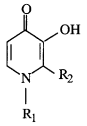
The biological assays performed by Yokel et al. [30] were conducted on Al-loaded rabbits with a set of eight 3,4-hydroxypyridinones. Their ability to solubilize Al (borate) in an octanol/water system had been previously evaluated and related with Al mobilization from tranferrin [12]. In these in vivo studies, the efficiency of the Al chelators was based on calculated urinary plus biliary Al excretion. Table 4 shows the calculated efficiencies for that set of Al-chelators together with the distribution coefficients for chelators and Al complexes. The efficiencies range from 2.8 to 11.7%, thus being higher than the corresponding value for DFO (2.1%). These results show the absence of any significant correlation between the lipophilicity of the chelators and the total Al output, although to the higher lipophilic complexes correspond higher biliary Al excretion. Thus, they further suggest that the less lipophilic and orally active chelators can be useful for Al decorporation of patients with normal renal functions, whereas the more lipophilic ones may be more indicated for patients with lack of those functions (dialysed patients).

Three of those hydroxypyridinones with a wide range of lipophylicity [1,2-dimethyl, 1,2-diethyl and the 1-(ethan-1′-ol)-2-methyl 3,4-HP derivatives with $D = 0.094$, 2.1, < 0.001, respectively] were also tested in their ability to cross the BBB in rats [31]. These results indicated that the two more lipophilic ones could easier diffuse across the BBB, although the 1,2-dimethyl derivative shows absence of toxicity.

Concerning the in vivo studies performed by Florence et al. [32], Al-loaded rats were used to study the effect of lipophilicity of two hydroxypyridinones (1,2-dialkyl-3,4-HP, alkyl = methyl, ethyl) on the mobilization of Al from specific tissues (liver and brain). It was shown that, upon intra-peritoneal administration, the DFO was more effective (74%) than any of those hydroxypyridinones, 1,2-dimethyl (14%) and 1,2-diethyl (44%) derivatives, in mobilizing liver Al, whereas the most lipophilic one (the 1,2-diethyl derivative) was the most efficient in mobilizing brain Al. This different behaviour was rationalized in terms of different facility of both ligand to cross the BBB. Furthermore, based on the relatively low efficacy of each of these chelators to mobilize the iron from brain [79], as compared with the Al, it was suggested that the administration of the more lipophilic chelator in patients with excess of Al might not interfere with the iron homeostasis.

Finally, since the hydroxypyridinones have been studied at a much smaller extent for their efficacy in Al- than in Fe-mobilization, a summary of recent in vivo results on the iron removal from mice with 3,4-HP

Table 5
Measured partition coefficients in octanol/MOPS (pH 7.4) and cyclohexane/MOPS (pH 7.4) as well as blood–brain–barrier permeabilities (log PS) of selected 1,2-substituted-3,4-HP [83]

Chelator	R_1	R_2	Log $D_{(octanol)}$	Log $D_{(cyclohexane)}$	Log PS
	CH_3	CH_3	-0.770	-2.796	-1.89
	CH_2CH_3	CH_3	-0.310	-2.155	-1.48
	$(CH_2)_4CH_3$	CH_3	0.703	-1.602	-0.64
	$(CH_2)_5CH_3$	CH_3	1.241	-1.432	-0.38
	$(CH_2)_6CH_3$	CH_3	1.898	-1.000	-0.36
	CH_2CH_3	C_2H_5	0.230	-1.854	-1.03
	$(CH_2)_4OH$	CH_3	-0.886	-2.886	<-3.00
	$(CH_2)_2OH$	C_2H_5	-0.658	-2.886	<-3.00
	$(CH_2)_4OH$	C_2H_5	-0.284	-0.284	<-3.00

containing several types of *N*-substituents (alkyl, alkylhydroxyl, alkylcarboxyl, alkylester and alkylamines) is included herein. It was demonstrated that compounds with $D = 0.5–2.0$ were in general more effective in Fe removal than those with $D < 0.2$ [26,61]. Some hydrophilic compounds (namely complexes having a net charge -3 at pH 7.4) presented higher efficacy than Deferiprone, which was attributed to easier excretion (lower $D_{complex}/D_{ligand}$) [80]. Some esters or the corresponding 1-(hydroxyalkyl)-3-hydroxy-4-pyridinones can be considered as prodrugs or drugs, presenting higher efficacy than Deferiprone, thus being also potential chelators for the treatment of Al overload patients. Whereas the more lipophilic ones were supposed to target the liver hepatocytes, some hydrophilic basic amine derivatives could target the lysosomes [62,63]. However, their efficacy seems also to be correlated with their lipophilicity.

6.2. Hexadentate hydroxypyridinones

In vitro results have shown that the hexadentate hydroxypyridinones are more effective chelators than the corresponding bidentate compounds (see above), namely as Fe mobilizators from hypatocytes [43]. In the absence of identical studies for Al, a brief summary of the Fe in vivo studies will be presented. Yokel et al. [52] tested the in vivo Fe chelation efficacy of the hexadentate TREN-(Me-3,2-HP) in comparison with the corresponding bidentate derivative (Me-3,2-HP).The results indicated that, in spite of the expected lower gastrointestinal absorption (probably due to the high molecular weight), this hexadentate hydroxypyridinone achieved useful oral bioavailability and, at lower concentrations, it is a more efficient Fe chelator than its bidentate analogue. The fourfold increase in Fe excretion in the Fe-loaded rats is mainly due to the biliary Fe excretion, which suggested the uptake of the chelator by the liver cells (hepatocytes) as happens with the 3,4-HP derivatives (see above), but without a great penetration in the general circulation (plasma).

Finally it should be mentioned that the ligands TREN(Me-3,2-HP)$_3$ and ME(Me-3,2-HP)$_3$ were also tested in their efficiency for removal of ^{238}Pu from rats [81]. They proved to be highly effective at low dosage. When injected, both of them presented higher efficacy than the currently used $CaNa_3$–DPTA. However, when orally administered, only the TREN derivative was more effective than the standard ligand.

6.3. BBB permeability of hydroxypyridinones

The interest of hydroxypyridinones in chelating therapy has been mainly addressed to the sequestering of iron in peripheral tissue, being not expected to enter the central nervous system (CNS). However, Al may be present in the CNS and, mainly, in the brain at toxic level [82]. Therefore, the drug to be used must be able to cross the BBB. Since the hydroxypyridinones are potential chelating drugs, it is of considerable interest to analyse the relationship between their structure and their BBB permeability. Hider and coworkers [83], based on previous literature data [73,84] about the dependence between BBB permeability and partition coefficients (octanol/water and cyclohexane/water), selected a set of 3,4-HP and investigated the relationship between their chemical structure and BBB permeability. For that purpose, partition coefficients of a series of 3,4-HP derivatives were measured in octanol/water and cyclohexane/water systems, as well as their BBB permeability. This liposolubility parameter was determined by infusion of each 3,4-HP into rat's brain, by cannulla methods. The permeability parameter measured, [permeability × surface area (PS)] was roughly evaluated as the ratio between the solute concentration in the brain and the solute concentration in the perfusate. Table 5 contains a set of nine 3,4-HP with various *N*-alkyl and *N*-hydroxyalkyl substituents [83].

Both sets of compounds showed an increase in the partition coefficients with the increase of the length of the alkyl chain, although the corresponding hydroxyalkyl derivatives gave always the lower values. The *N*-alkyl substituted 3,4-HP showed that to a higher lipophilicity [log $D_{octanol}$ and log $D_{cyclohexane}$) is associated a higher brain penetration (log PS). However, no increase in BBB permeability was observed with lipophilicity of the hydroxyalkyl 3,4-HP. This was attributed to the existence of hydrogen bonds between the solute and water, which have to be broken. So, for these ligands, the molecular volume seems to have a positive effect on lipid solubility and BBB permeability, while the hydrogen bond basicity/polarity has negative effects.

In summary, a single change in 3,4-HP structure (*N*-alkylation) increases the ability to cross BBB (in spite of

increasing molecular mass), while the *N*-hydroxyalkylation makes it more difficult.

7. Conclusions

This paper illustrates the advances made in the chemistry and the design of new hydroxypyridinones for Al chelation. It also shows how these features are correlated with the lipo-hydrophilic balance and the biological behaviour, taking into account their potential use in therapy of Al intoxication.

The relative affinities for the Al and the desirable lipo-hydrophilic balance of chelators–metal complexes at physiological conditions suggest that, among the different type of compounds (1,2-HP, 3,2-HP and 3,4-HP), in general, the 3,4-HP can be better therapeutic agents against Al overload than other hydroxypyridinones. These bidentate ligands are more efficient than Tf at higher concentrations. For lower concentrations, recent studies of some hexadentate hydroxypyridinones with iron suggested that they could reach useful levels of concentration in the plasma scavenging these metal ions and so, maybe they can be thought as potential Al antitoxic agents. Bidentate 3,4-HP have already been in vivo tested and some of them proved to reduce Al-toxicity in animals.

However, much multidisciplinary collaborative research efforts are encouraged for increasing our understanding of the Al in biological systems and identifying key deficits of the known Al-chelating agents, such as the hydroxypyridinones, in the perspective of improving their design and efficacy.

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