ELSEVIER

Coordination Chemistry Reviews 232 (2002) 151–171

COORDINATION CHEMISTRY REVIEWS

www.elsevier.com/locate/ccr

Design of iron chelators with therapeutic application

Zu D. Liu, Robert C. Hider *

Department of Pharmacy, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NN, UK

Received 11 December 2001; accepted 10 February 2002

Contents

* Corresponding author. Tel.: +44-20-7848-4882; fax: +44-20-7848-4800.
E-mail address: robert.hider@kcl.ac.uk (R.C. Hider).

PII: S0010-8545(02)00050-4

Abstract

Iron overload is a serious clinical condition which can be largely prevented by the use of iron-specific chelating agents. Desferrioxamine-B, the most widely used iron chelator in haematology over the past 30 years, has a major disadvantage of being orally inactive. Consequently, the successful design of an orally active, non-toxic, selective iron chelator has become a much sought after goal. In order to identify an ideal iron chelator for clinical use, a range of specifications must be considered such as metal selectivity and affinity, kinetic stability of the complex, bioavailability and toxicity. A wide range of chelator types bind iron(III) and of these, hexa-, tri-, and bidentate are capable of providing iron(III) with the favoured octahedral environment. In this review, the comparative properties of such ligands are discussed, examples being selected from hydroxamates, aminocarboxylates, hydroxypyridinones, orthosubstituted phenols and triazoles.

Keywords: Iron chelators; Hydroxamates; Desferrioxamine-B; Hydroxypyridinones; Deferiprone

1. Introduction

Iron is a critically important metal for a wide variety of cellular events, indeed no life form is possible without the presence of this element. Iron holds this central position by virtue of its facile redox chemistry and its high affinity for oxygen. It possesses incompletely filled d-orbitals and can exist in various valencies, the most common oxidation states in aqueous media being Fe(II) and Fe(III). The redox potential between these two states is such that oxidation processes centred on iron can be coupled to a wide range of metabolic reactions.

Although iron is essential for the proper functioning of all living cells, it is toxic when present in excess. In the presence of molecular oxygen, 'loosely-bound' iron is able to redox cycle between the two most stable oxidation states thereby generating oxygen-derived free radicals such as the hydroxyl radical [1]. Hydroxyl radicals are highly reactive and capable of interacting with most types of biological molecules including sugars, lipids, proteins and nucleic acids, resulting in peroxidative tissue damage. Indeed free radicals play a significant role in lipid peroxidation, protein oxidation and nucleic acid degradation [2]. The production of such highly reactive species is undesirable and thus a number of protective strategies are adopted by cells to prevent their formation. One of the most important strategies is the tight control of iron storage, transport and distribution. In fact iron metabolism in man is highly conservative with the majority of iron being recycled within the body. Since man lacks a physiological mechanism for eliminating iron, iron homeostasis is achieved by the regulation of iron absorption. In the normal individual, iron levels are under extremely tight control and there is little opportunity for iron-catalysed free radical generating reactions to occur. However, there are situations when the iron status can change, either locally as in ischaemic tissue, or systematically as with genetic haemochromatosis or transfusion-induced iron overload. In such circumstances, the elevated levels of iron ultimately lead to free radical-mediated tissue/organ damage and eventual death [3]. Although excess iron can be removed by venesection when adequate erythropoietic reserve exists, e.g. haemochromatosis, iron chelation is the only effective way to relieve iron overload in transfusion-dependent patients such as those suffering from β-thalassaemia. Desferrioxamine-B (DFO) (**1**), the most widely used iron chelator in haematology over the past 30 years, has a major disadvantage of being orally inactive [4]. Consequently there is an urgent need for an orally active iron chelating agent [5].

In order to identify an ideal iron chelator for clinical use, careful design consideration is essential; a range of specifications must be considered such as metal selectivity and affinity, kinetic stability of the complex, bioavailability and toxicity.

2. Design features of iron chelators

2.1. Metal selectivity and affinity

In designing iron chelators for clinical application, the properties for metal selectivity and resultant ligand–metal complex stability are paramount. The selection of an appropriate ligand for metal complexation can be rationalised by classification of metals and ligands according to the concept of 'hard' and 'soft' acids and bases [6]. In theory, chelating agents can be designed for either the iron(II) or iron(III) oxidation state. High-spin iron(III) is a spherically symmetrical tripositive cation of radius 0.65 Å, and as such is classified as a hard Lewis acid by virtue of its high charge density. It forms most stable bonds with 'hard' ligands such as the charged hydroxamate oxygen atoms of DFO (**1**). In contrast the iron(II) cation, which has a lower charge density, prefers chelators containing 'soft' donor atoms, exemplified by the nitrogen-containing ligands such as 2,2′-bipyridyl and 1,10-phenanthroline. Ligands that prefer iron(II) retain an appreciable affinity for other biologically important bivalent metals such as copper(II) and zinc(II) ions (Table 1), and thus the design of a non-toxic iron(II)-selective ligands is extremely difficult and indeed may not be possible. In contrast iron(III)-selective ligands, typically oxyanions and notably hy-

Table 1
Metal affinity constants for selected ligands

Ligand	Log cumulative stability constant					
	Fe(III)	Al(III)	Ga(III)	Cu(II)	Zn(II)	Fe(II)
DFO (**1**)	30.6	25.0	27.6	14.1	11.1	7.2
2,2′-Bipyridyl (**2**)	16.3		7.7	16.9	13.2	17.2
1,10-Phenanthroline (**3**)	14.1		9.2	21.4	17.5	21.0
N,N-Dimethyl-2,3-dihydroxybenzamide (DMB) (**6**)	40.2			24.9	13.5	17.5
Acetohydroxamic acid (**7**)	28.3	21.5		7.9	9.6	8.5
Deferiprone (**11**)	37.2	35.8	32.6	21.7	13.5	12.1
EDTA (**12**)	25.1	16.5	21.0	18.8	16.5	14.3
DTPA (**13**)	28.0	18.6	25.5	21.6	18.4	16.5

Data sourced from Martell and Smith (1974–1989) [7].

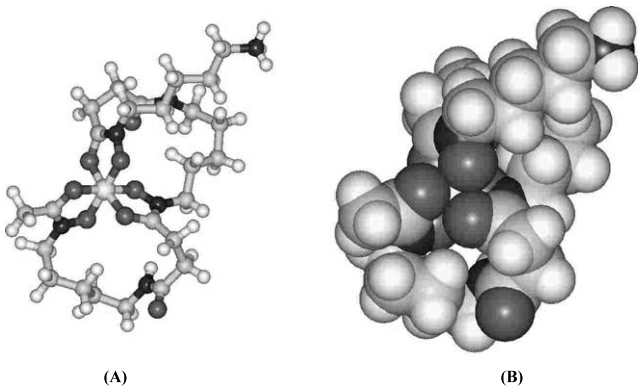
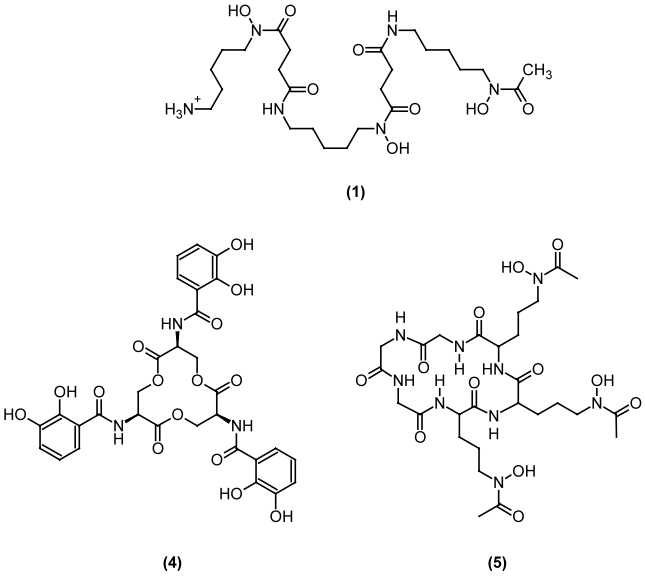


Fig. 1. Energy-minimised structure of ferrioxamine B (Λ-C-*trans*,-*trans* conformation). (A) Ball-stick model; (B) Space-filling model.

droxamates and catecholates, are generally more selective for tribasic metal cations over dibasic cations (Table 1). Most tribasic cations, for instance aluminium(III) and gallium(III), are not essential for living cells and thus in practice iron(III) provides the best target for 'iron chelator' design under biological conditions. An additional advantage of high-affinity iron(III) chelators is that, under aerobic conditions, they will chelate iron(II) cations and rapidly autoxidise them to the corresponding iron(III) species [8]. Thus, high-affinity iron(III)-selective ligands bind both iron(III) and iron(II) under most physiological conditions.

Siderophores, compounds possessing a high affinity for iron(III) and produced by microorganisms for scavenging iron from the environment, utilise the above principle [9]. Typically they are hexadentate in design and utilise catechol or hydroxamate as ligands, for instance, enterobactin (**4**), deferriferrichrome (**5**) and DFO (**1**). The stereochemistry of these molecules is such that the coordination sphere of iron(III) is completely occupied by the oxygen-containing ligands, for example, with ferrioxamine B (Fig. 1). The selectivity of these molecules for iron(III) over iron(II) is enormous leading to extremely low redox potentials; for example, enterobactin −750 mV and DFO −468 mV [10].



2.2. Ligand selection

2.2.1. Catechols

Catechol moieties possess a high affinity for iron(III). This extremely strong interaction with tripositive metal cations results from the high electron density of both oxygen atoms. However, this high charge density is also associated with the high affinity for protons (pK_a values, 12.1 and 8.4). Thus the binding of cations by catechol has marked pH sensitivity [11]. The complexes forming at pH 7.0 each bear a net charge and consequently are unlikely to permeate membranes by simple diffusion [12]. Indeed such iron complexes will tend to be trapped in intracellular compartments. For simple bidentate catechols, the 2:1 complex is the dominant form in the pH range 5.5–7.5 (Fig. 2A). With such complexes, the iron atom is not completely protected from the solvent and consequently is able to interact with hydrogen peroxide or oxygen, possibly resulting in the generation of hydroxyl radicals. An additional problem with

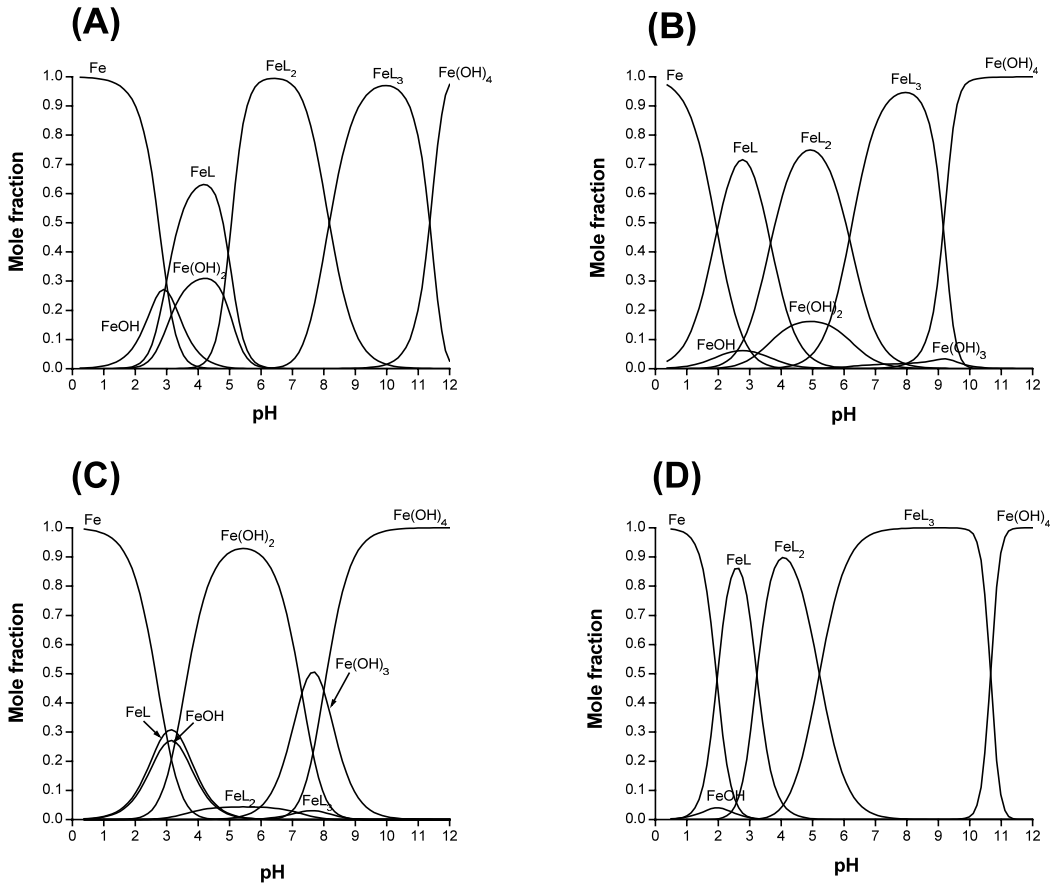


Fig. 2. Speciation plot of iron(III) in the presence of (A) *N*,*N*-dimethyl-2,3-dihydroxybenzamide (**6**), $[Fe^{3+}]_{total} = 1 \times 10^{-6}$ M; [Ligand] $= 1 \times 10^{-5}$ M; (B) acetohydroxamic acid (**7**), $[Fe^{3+}]_{total} = 1 \times 10^{-6}$ M; [Ligand] $= 1 \times 10^{-4}$ M; (C) acetohydroxamic acid (**7**), $[Fe^{3+}]_{total} = 1 \times 10^{-6}$ M; [Ligand] $= 1 \times 10^{-5}$ M; (D) 1,2-dimethyl-3-hydroxypyridin-4-one (**11**), $[Fe^{3+}]_{total} = 1 \times 10^{-6}$ M; [Ligand] $= 1 \times 10^{-5}$ M.

catechol-based ligands is their susceptibility towards oxidation [11].

2.2.2. Hydroxamates

The hydroxamate moiety possesses a lower affinity for iron than catechol. However, it has the advantage of forming neutral *tris*-complexes with iron(III) which are in principle able to permeate membranes by non-facilitated diffusion [12]. The selectivity of hydroxamates, like catechols, favours tribasic cations over dibasic cations (Table 1). Due to the relatively low protonation constant ($pK_a \sim 9$), hydrogen ion interference at physiological pH is less pronounced than for that of catechol ligands, consequently the 3:1 complex predominates at pH 7.0 when sufficient ligand is present (Fig. 2B). However, the affinity of a simple bidentate hydroxamate ligand for iron is insufficient to solubilise iron(III) at pH 7.4 at clinically achievable concentrations (Fig. 2C), thus only tetradentate and hexadentate hydroxamates are likely to be effective iron(III) scavengers under such conditions. Many hydroxamates are metabolically labile and are only poorly absorbed via the oral route [13,14]. Numerous approaches have been explored to address this problem, however, to date no suitable hydroxamate derivative has been identified which possesses a comparable activity to that of subcutaneous DFO [15,16].

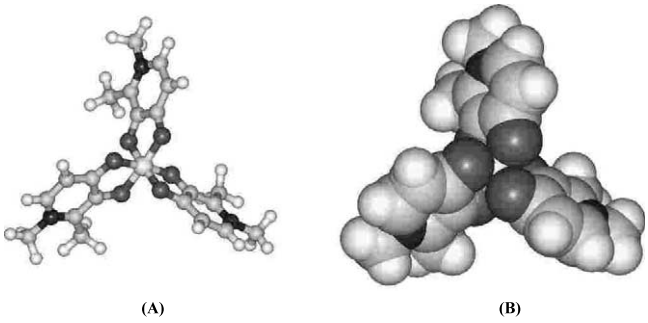
2.2.3. Hydroxypyridinones

Hydroxypyridinones (HPOs) combine the characteristics of both hydroxamate and catechol groups, forming 5-membered chelate rings in which the metal is coordinated by two vicinal oxygen atoms. The hydroxypyridinones are monoprotic acids at pH 7.0 and thus form neutral *tris*-iron(III) complexes. The affinity of such compounds for iron(III) reflects the pK_a values of the chelating oxygen atoms, the higher the affinity for iron(III), the higher the pK_a value (Table 2). There are three classes of metal chelating HPO ligands, namely 1-hydroxypyridin-2-one (**9**), 3-hydroxypyridin-2-one (**10**) and 3-hydroxypyridin-4-one (**11**). Of these, the pyridin-4-ones possess the highest affinity for iron(III) (Table 2) and are selective for tribasic metal cations over dibasic cations (Table 1). The surprisingly high pK_a value of the carbonyl function of 3-hydroxypyridin-4-one results from extensive delocalisation of the lone pair associated with the ring nitrogen atom. 3-Hydroxypyridin-4-ones form neutral 3:1 complexes with iron(III) (Fig. 3) [17],

Table 2
pK_a values and affinity constants of dioxobidentate ligands for iron(III)

Ligand	Structure	pKa_1	pKa_2	$Log\beta_3$	pFe^{3+} [1]
N,N-Dimethyl-2,3-dihydroxybenzamide (DMB) **(6)**		8.4	12.1	40.2	15
Acetohydroxamic acid **(7)**		-	9.4	28.3	13
2-Methyl-3-hydroxy-pyran-4-one (maltol) **(8)**		-	8.7	28.5	15
1-Hydroxypyridin-2-one **(9)**		-	5.8	27	16
1-Methyl-3-hydroxy-pyridin-2-one **(10)**		0.2	8.6	32	16
1,2-Dimethyl-3-hydroxy-pyridin-4-one (deferiprone) **(11)**		3.6	9.9	37.2	19

[1] $pFe^{3+} = -\log[Fe^{3+}]$ when $[Fe^{3+}]_{total}=10^{-6}$ M and $[ligand]_{total}=10^{-5}$ M at pH 7.4.



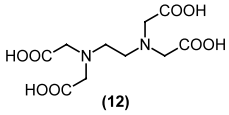
(A) (B)

Fig. 3. Energy-minimised structure of the 3:1 iron(III) complex of 1,2-dimethyl-3-hydroxypyridin-4-one (**11**). (A) Ball-stick model; (B) Space-filling model.

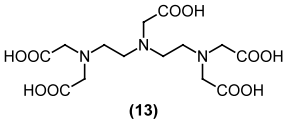
which are stable over a wide range of pH values (Fig. 2D). Although catechol derivatives possess higher β_3 values than that of 3-hydroxypyridin-4-one for iron(III), the corresponding pFe^{3+} values are lower (Table 2). This difference is due to the relatively higher affinity of catechol for protons. Indeed of all dioxygen ligand classes investigated, 3-hydroxypyridin-4-ones possess the greatest affinity for iron(III) over the physiological pH range, as indicated by their respective pFe^{3+} values (Table 2).

2.2.4. Aminocarboxylates

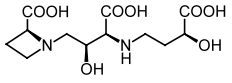
Aminocarboxylate ligands are excellent iron(III) chelating agents. Several polycarboxylate ligands such as ethylenediaminetetraacetic acid (EDTA) (**12**) and diethylenetriaminepentaacetic acid (DTPA) (**13**), have been widely investigated for their iron chelating abilities. However, the selectivity of these molecules for iron(III) is relatively poor (Table 1). This lack of selectivity leads to zinc depletion in patients receiving aminocarboxylate-based ligands such as DTPA [18]. Indeed cereals, for instance wheat, synthesise such molecules in order to scavenge both iron and zinc from the soil [19,20]. The secretion of these so-called phytosiderophores (**14**) is activated when plants are grown in soils containing low levels of either zinc or iron.



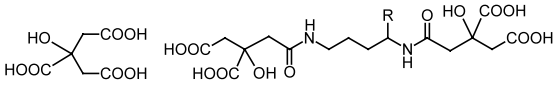
(12)



(13)



(14)



(15) (16) R = H (17) R = COOH

2.2.5. Hydroxycarboxylates

Hydroxycarboxylate ligands are strong chelating agents, which are more selective for iron(III) than the corresponding aminocarboxylates, due to all the coordinating atoms being oxygen. The interaction between iron(III) and citrate (**15**) has been well characterised [21], but by virtue of its tridentate nature, a large number of complexes have been identified [22] including iron/citrate polymers [23,24]. In contrast hexadentate hydroxycarboxylate ligands, for instance, staphloferrin (**16**) [25] and rhizoferrin (**17**) [26] have simple iron(III) complex chemistries dominated by the formation of 1:1 complexes.

2.3. Thermodynamic stability of iron(III) complexes

The coordination requirements of high spin iron(III) are best satisfied by six donor atoms ligating in an octahedral fashion to the metal centre. A factor of great importance in the stability of metal complex is the number of chelate rings formed in resultant ligand–metal complex. The number of chelating rings can be

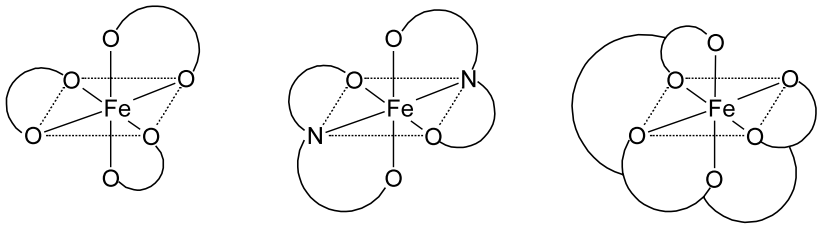
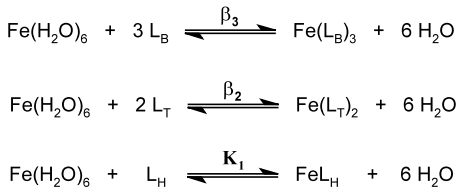


Fig. 4. Schematic representation of chelate ring formation in metal–ligand complexes.



L_B, bidentate; L_T, tridentate; L_H, hexadentate ligand

Scheme 1. The complex formation of iron(III) with bidentate, tridentate; and hexadentate ligands (charges omitted for clarity).

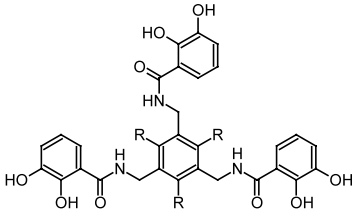
increased by increasing the number of donor atoms attached to the ligand (Fig. 4). In order to maximise the thermodynamic stability of the iron(III) complex it is necessary, therefore, to incorporate all six donors into a single molecular structure thereby creating a hexadentate ligand. This increase in thermodynamic chelate stability is largely associated with entropic changes that occur on going from free ligand and solvated free metal to ligand–metal complex (Scheme 1). Significantly the majority of natural siderophores are hexadentate ligands. The overall stability constant trends for bidentate and hexadentate ligands are typified by the bidentate ligand DMB (**6**) and the hexadentate congener MECAM (**18**), a differential of 3 log units in stability is observed (Table 3). Similarly, comparison of the bidentate ligand acetohydroxamic acid (**7**) with the linear hexadentate ligand, DFO (**1**) a positive increment of 2.3 log units is observed (Table 3).

Table 3
A comparison of the pFe^{3+} values and iron(III) stability constants for bidentate and hexadentate ligands

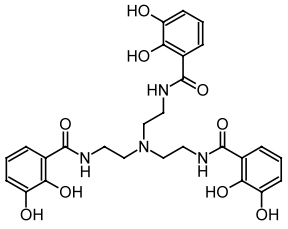
Ligand	pFe^{3+} [a]	Log stability constant
N,N-Dimethyl-2,3-dihydroxybenzamide (DMB) (**6**)	15	40.2
MECAM (**18**)	28	43
Acetohydroxamic acid (**7**)	13	28.3
DFO (**1**)	26	30.6

[a] $pFe^{3+} = -\log[Fe^{3+}]$ when $[Fe^{3+}]_{total} = 10^{-6}$ M and $[ligand]_{total} = 10^{-5}$ M at pH 7.4.

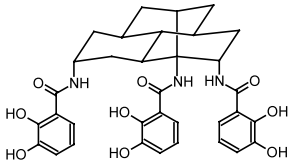
Although MECAM binds iron(III) three orders of magnitude more tightly than its bidentate analogue DMB, other hexadentate catechols for instance enterobactin bind iron(III) even more tightly (Table 4). Indeed the smaller the conformational space of the free ligand, the higher the stability of the complex; as the difference between the flexibility of the ligand and its corresponding iron complex decreases, so does the entropy difference. An extreme example would be the situation where no conformational change between free ligand and iron(III) complex occurs. Thus enterobactin (**4**), $(Et)_3$MECAM (**19**) [27] and (**21**) [28] can be considered to possess a degree of preorganisation in contrast to MECAM (**18**) and TRENCAM (**20**), which do not [29].



(**18**) R = H
(**19**) R = C_2H_5



(**20**)



(**21**)

Under biological conditions, a comparison standard which is generally more useful than the stability

Table 4
Iron(III) stability constants for a range of hexadentate catechol ligands

Ligand	Structure conformation	Log stability constant
Enterobactin (**4**)	Chiral monocyclic ring	49
(**21**)	Rigid tricyclic tripod	49
$(Et)_3$MECAM (**19**)	Planar ring with preorganised side chains	47
TRENCAM (**20**)	N-tripod	44
MECAM (**18**)	Planar ring	43

constant is the pFe^{3+} value [30]. pFe^{3+} can be defined as the negative logarithm of the concentration of the free iron(III) in solution. Typically pFe^{3+} values are calculated for total [ligand] = 10^{-5} M, total [iron] = 10^{-6} M at pH 7.4. The comparison of ligands under these conditions is useful, as the pFe^{3+} value, unlike the stability constants log K or log β_3, takes into account the effects of ligand protonation and denticity as well as differences in metal–ligand stoichiometries. The comparison of the pFe^{3+} values for hexadentate and bidentate ligands (Table 3) reveals that hexadentate ligands are far superior to their bidentate counterparts under typical in vivo conditions. Indeed when the influence of pH is monitored, the overall shift in pFe^{3+} values is even clearer (Fig. 5A). The pFe^{3+} versus pH plot provides a useful method of comparing the ability of chelators to bind iron(III) at different pH values. Thus for bidentate ligands 1-hydroxypyridin-2-one is relatively effective at acid pH values, whereas catechols dominate in the alkaline pH range (Fig. 5B). For a ligand to dominate iron(III) chelation in aqueous media it must produce a pFe^{3+} curve above that of hydroxide anion. The larger the difference, the stronger the chelator and the larger the pFe^{3+} value.

The formation of a complex will also be dependent on both free metal and free ligand concentration and as such will be sensitive to concentration changes. The degree of dissociation for a *tris*-bidentate ligand–metal complex is dependent on [ligand]^3 whilst the hexadentate ligand–metal complex dissociation is only dependent on [ligand]^1 (Scheme 2). Hence the dilution sensitivity to complex dissociation for ligands follows the order hexadentate < tridentate < bidentate. It is for this reason that the majority of natural siderophores are hexadentate compounds and can therefore scavenge iron(III) efficiently at low metal concentrations [9].

3. Critical features for clinical application

3.1. Lipophilicity and molecular weight

In order for a chelating agent to exert its pharmacological effect, a drug must be able to reach the target sites at sufficient concentration. Hence the key property for an orally active iron chelator is its ability to be efficiently absorbed from the gastrointestinal tract and to cross biological membranes thereby gaining access to the desired target sites such as the liver. There are three major factors which influence the ability of a compound to freely permeate a lipid membrane, namely, lipophilicity, ionisation state and molecular size.

In order to achieve efficient oral absorption, the chelator should possess appreciable lipid solubility which may facilitate the molecule to penetrate the gastrointestinal tract (partition coefficient greater than

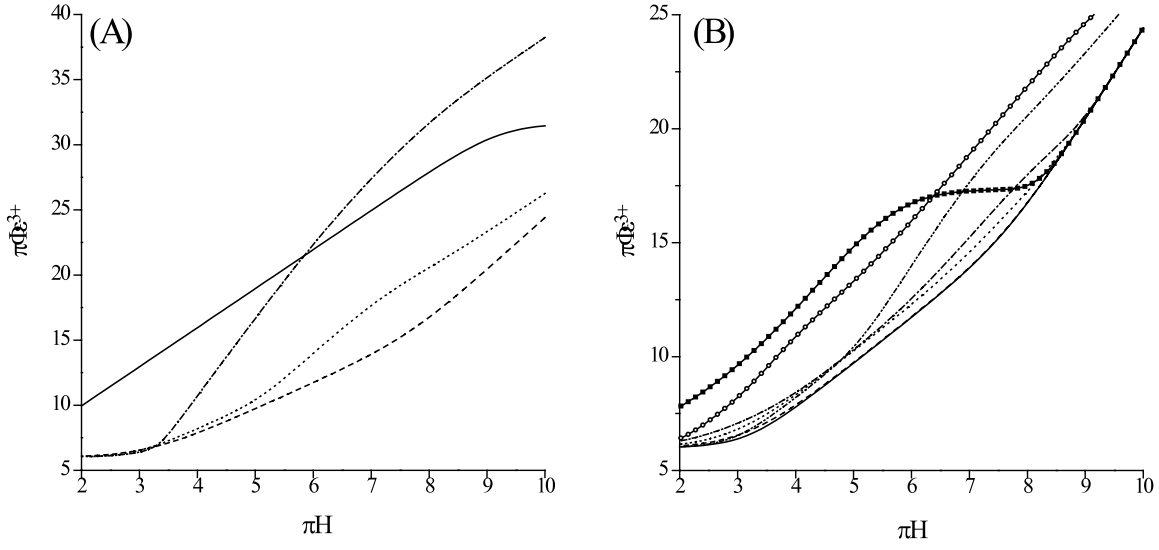
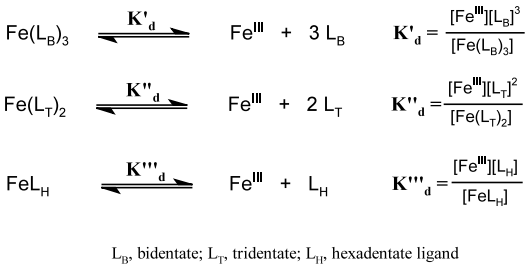


Fig. 5. Influence of pH on pFe^{3+} values of (A) DFO, MECAM and their bidentate analogues, ... *N*,*N*-dimethyl-2,3-dihydroxybenzamide (**6**); - - - - - acetohydroxamic acid (**7**); — DFO (**1**); -.-.-.-. MECAM (**18**); and (B) a range of bidentate ligands. $[Fe^{3+}]_{total} = 10^{-6}$ M; $[Ligand]_{total} = 10^{-5}$ M. — OH; -..-..- *N*,*N*-dimethyl-2,3-dihydroxybenzamide (**6**); - - - - - acetohydroxamic acid (**7**); ..... 2-methyl-3-hydroxypyran-4-one (**8**); —■— 1-hydroxypyridin-2-one (**9**); -.-.-.- 1-methyl-3-hydroxypyridin-2-one (**10**); —○— 1,2-dimethyl-3-hydroxypyridin-4-one (**11**).



L_B, bidentate; L_T, tridentate; L_H, hexadentate ligand

Scheme 2. Dissociation of bidentate, tridentate and hexadentate ligand–iron(III) complexes.

0.2) [31]. However, highly lipid-soluble chelators can also penetrate most cells and critical barriers such as the blood–brain and placental barriers, thereby enhancing possible toxic side effects. Membrane permeability can also be affected by the ionic state of the compound. Uncharged molecules penetrate cell membranes more rapidly than charged molecules [32]. It is for this reason that aminocarboxylate-containing ligands are unlikely to possess high oral activity.

Molecular size is another critical factor which influences the rate of drug absorption [33,34]. Non-facilitated diffusion is generally considered to be dominant for drugs with molecular weights < 200. The transcellular route involves diffusion into the enterocyte and thus utilises some 95% of the surface of the small intestine. In contrast, the paracellular route only utilises a small fraction of the total surface area and the corresponding flux via this route is much smaller. The 'cut-off' molecular weight for the paracellular route in the human small intestine is ca. 400 [35] and this route is unlikely to be quantitatively important for molecules with molecular weights > 200 [34]. There is no clear 'cut off' value for the transcellular route, but as judged by PEG permeability, penetration falls off rapidly with molecular weights > 500 [36,37]. Thus in order to achieve greater than 70% absorption, subsequent to oral application, the chelator molecular weight probably needs to be less than 300 [36]. This molecular-weight limit provides a considerable restriction on the choice of chelator and may effectively exclude hexadentate ligands from consideration, most siderophores, for instance DFO, have molecular weights in the range 500–900. Although EDTA has a molecular weight of only 292, it is too small to fully encompass the chelated iron, thereby facilitating the potential toxicity of the metal [31].

In contrast bidentate and tridentate ligands, by virtue of their much lower molecular weights, are predicted to possess higher absorption efficiencies. The fraction of the absorbed dose for a range of bidentate 3-hydroxypridin-4-ones has typically been found to fall between 50 and 70%, as assessed in the rabbit [38].

3.2. Iron chelator toxicity

The toxicity associated with iron chelators originates from a number of factors; including inhibition of iron-containing metalloenzymes; lack of metal selectivity, which may lead to the deficiency of other physiologically important metals such as zinc(II); redox cycling of iron-complexes between iron(II) and iron(III), thereby generating free radicals and the kinetic lability of the iron-complex leading to iron redistribution. Comparative toxicities of iron(III) chelators are summarised in Table 5.

3.2.1. Metal selectivity

An ideal iron chelator should be highly selective for iron(III) in order to minimise chelation of other biologically essential metal ions which could lead to deficiency with prolonged usage. Unfortunately, many ligands that possess a high affinity for iron(III) may also have appreciable affinities for other metals such as zinc(II), this being especially so with carboxylate- and nitrogen-containing ligands. However, this is less of a problem with the oxygen-containing bidentate catechol, hydroxamate and hydroxypyridinone ligand families which possess a strong preference for tribasic over dibasic cations (Table 1).

Although in principle, competition with copper(II) could be a problem, under most biological conditions it

Table 5
Comparative properties of bidentate, tridentate and hexadentate ligands

Bidentate ligands	Tridentate ligands	Hexadentate ligands
Possible for all coordinating atoms to be 'hard' oxygen centres which renders ligands highly selective for iron(III)	Very difficult for all coordinating atoms to be 'hard' oxygen centres which may lead to poor metal selectivity	Possible for all coordinating atoms to be 'hard' oxygen centres which renders ligands highly selective for iron(III)
Affinity for iron is concentration dependent	Affinity for iron is concentration dependent	Affinity for iron is concentration independent
Kinetically labile—iron redistribution is possible	Kinetically labile—iron redistribution is possible	Kinetically inert—iron redistribution is unlikely
Form partially coordinated 2:1 complexes	Form partially coordinated 1:1 complexes	Only form fully coordinated 1:1 complexes
Can form uncharged iron(III) complexes	All iron(III) complexes are charged	Can form uncharged iron(III) complexes
Do not form polymeric complexes	Form polymeric complexes which are likely to be trapped within cells	Do not form polymeric complexes
Penetration of BBB is dependent on lipophilicity	Penetration of BBB is dependent on lipophilicity	Generally low penetration of the BBB

is not so, as copper is extremely tightly bound to proteins and the unbound fraction is reported to be less than 10^{-20} M [39]. Copper is exchanged between proteins via specialised high affinity chaperone molecules [40].

3.2.2. Complex structure

In order to prevent free radical production, iron should be coordinated in such a manner as to avoid direct access of oxygen and hydrogen peroxide. Most hexadentate ligands such as DFO are kinetically inert and reduce hydroxyl radical production to a minimum by entirely masking the surface of the iron (Fig. 1). However, not all hexadentate ligands are of sufficient dimensions to entirely mask the surface of the bound iron, in which case the resulting complex may enhance the ability of iron to generate free radicals. This phenomenon is particularly marked at neutral or alkaline pH values when the solubility of non-complexed iron(III) is severely limited. The classic example of this type of behaviour is demonstrated by EDTA, where a seventh co-ordination site is occupied by a water molecule (Fig. 6). This water molecule is kinetically labile and is capable of rapidly exchanging with oxygen, hydrogen peroxide and many other ligands present in biological media. As a result iron(III) EDTA is toxic, readily generating hydroxyl radicals under in vivo conditions [41].

In contrast to the kinetically stable ferrioxamine complex (Fig. 1), bidentate and tridentate ligands are kinetically more labile and the iron(III) complexes tend to dissociate at low ligand concentrations. Partial dissociation of bi- and tridentate ligand iron complexes renders the iron(III) cation surface accessible to other ligands. However, the concentration dependence of 3-hydroxypyridin-4-one iron complex speciation is minimal at pH 7.4 when the ligand concentration is above 1 μM, due to the relatively high affinity of the ligand for iron(III). Thus, bidentate 3-hydroxypyridin-4-ones behave more like hexadentate ligands as the 3:1 complex is the dominant species at pH 7.4 (Fig. 2D), and the iron atom is completely coordinated (Fig. 3). This is in marked contrast to iron(III) complexes of many catechol species [12]. Even at low hydroxypyridinone concentrations there is minimal hydroxyl radical production [41,42].

3.2.3. Redox activity

Chelators that bind both iron(II) and iron(III) are capable of redox cycling, a property that has been utilised by a wide range of enzymes [43] and industrial catalysts [44]. However, this is an undesirable property for iron scavenging molecules, as redox cycling can also lead to the production of reactive oxygen radicals (Scheme 3). Significantly the high selectivity of siderophores for iron(III) over iron(II) renders redox cycling under biological conditions unlikely. Chelators with nitrogen ligands tend to possess lower redox potentials and the coordinated iron can be reduced enzymatically under biological conditions. Such complexes may redox cycle under aerobic conditions, generating oxygen radicals.

3.2.4. Enzyme inhibition

In general, iron chelators do not directly inhibit haem-containing enzymes due to the inaccessibility of porphyrin-bound iron to chelating agents. In contrast many non-haem iron-containing enzymes such as the lipoxygenase and aromatic hydroxylase families and ribonucleotide reductase are susceptible to chelator-induced inhibition [45,46]. Lipoxygenases are generally inhibited by hydrophobic chelators, therefore, the introduction of hydrophilic characteristics into a chelator tend to minimise such inhibitory potential [47]. Although this relationship holds with hydroxypyridinones, where the size of the alkyl substitution is increased in the 1-position of the pyridinone ring, in an essentially linear manner (Fig. 7A), it is less evident for compounds with large substituents in the 2-position [48]. In fact, the variation in the inhibitory properties of the HPO

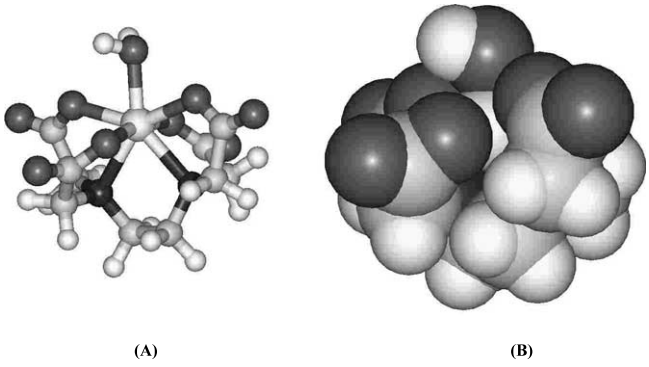
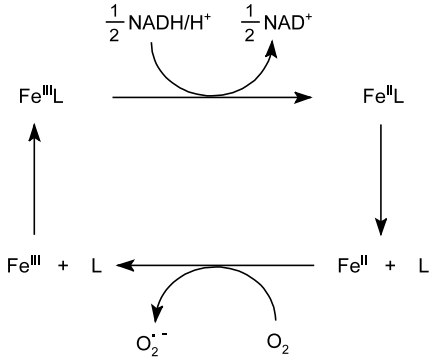


Fig. 6. Energy-minimised structure of the iron(III) complex of EDTA (**12**), the seventh co-ordination site is occupied by a water molecule, (A) Ball-stick model; (B) Space-filling model.



Scheme 3. Redox cycling of an iron complex.

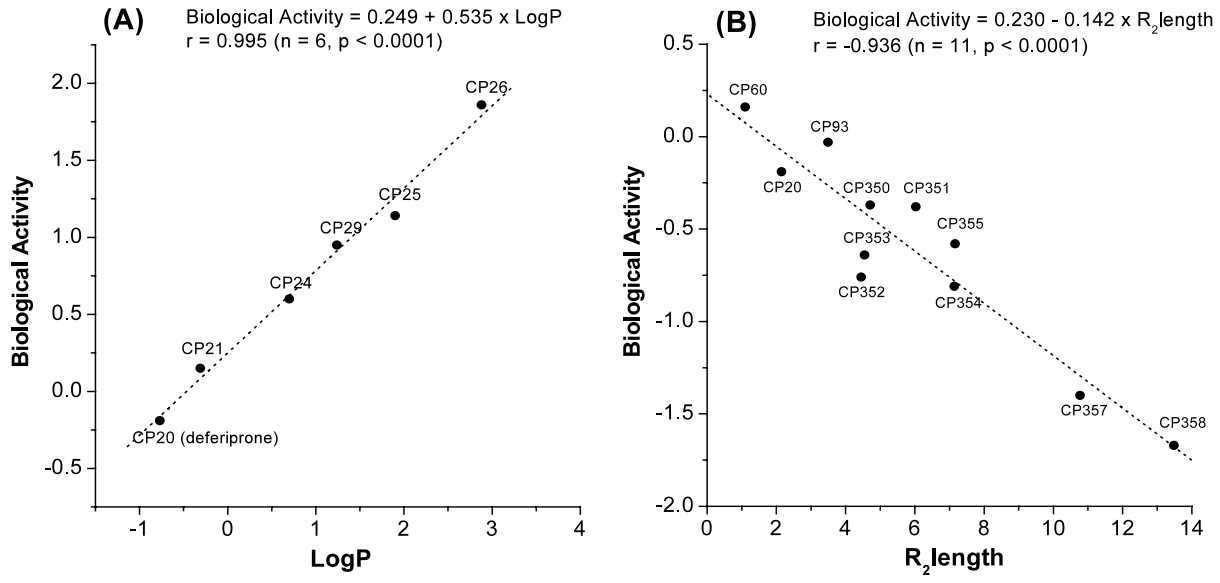


Fig. 7. Relationship between the chemical nature of the ligand and the inhibition of 5-lipoxygenase; (A) HPOs with different R_1 substituents and (B) HPOs with different R_2 substituents.

chelators possessing different R_2 substituents is more dependent on the size of the substituent than the lipophilicity of the chelator (Fig. 7B). The introduction of a hydrophilic substituent at the 2-position of hydroxypyridinones markedly reduces the inhibitory properties, presumably due to steric interference of the chelation process at the enzyme active site [48]. In contrast lipophilicity is reported to be the dominant factor in controlling the ability of HPO chelators to inhibit mammalian tyrosine hydroxylase [49], hydrophilic chelators (log $P_{\text{water/octanol}} \leq -1.0$) tending to be relatively weak inhibitors of this enzyme. Clearly by careful modification of physicochemical properties, iron chelators can be designed which exert minimal inhibitory influence on many metalloenzymes [47–50].

3.2.5. Hydrophilicity

Although bidentate and tridentate ligands possess an advantage over hexadentate ligands with respect to oral bioavailability, their enhanced ability to permeate membranes renders them potentially more toxic. Thus the penetration of the blood–brain barrier (BBB) is one of the likely side-effects associated with bidentate and tridentate ligands. The ability of a compound to penetrate the BBB is critically dependent on the partition coefficient as well as the molecular weight [51]. BBB permeability is predicted to be low for most hexadentate compounds, by virtue of their higher molecular weight. With low molecular weight molecules (M.W. < 300), penetration is largely dependent on the lipophilicity and molecules with log $P_{\text{water/octanol}}$ value less than -1.3 tend to penetrate inefficiently [52]. Thus, chelators with

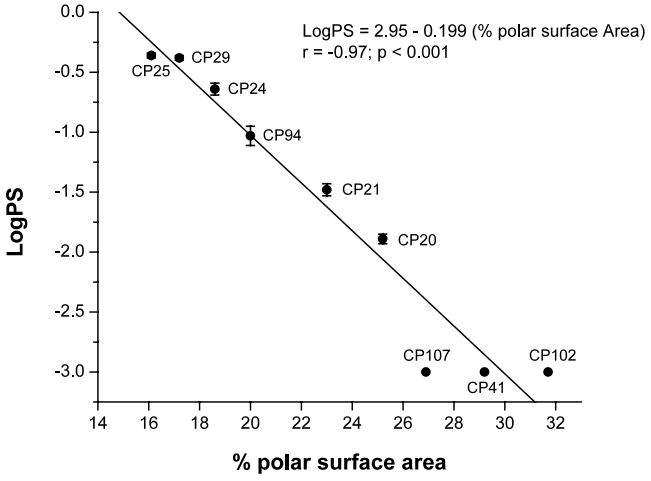


Fig. 8. Relationship between the percentage polar surface area of the HPO chelators and their blood–brain barrier permeability (log PS).

partition coefficients lower than this critical value are predicted to show poor entry into the central nervous system. Indeed brain penetration of 3-hydroxypyridin-4-ones is strongly dependent on their lipophilicity [53], a clear correction being observed between BBB permeability and the percentage polar surface area of the molecule (Fig. 8). Thus 1,ω-hydroxyalkyl hydroxypyridinones penetrate the BBB much more slowly than the simple 1-alkyl derivatives [53]. These results suggest that the biological distribution pattern of the HPOs can be significantly altered by simple modification of chemical structure without compromising their pharmacological function (selective iron chelation).

4. Iron chelators currently under investigation

4.1. Hexadentate chelators

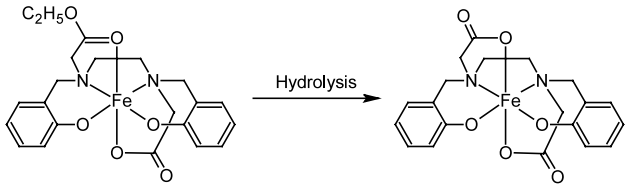
4.1.1. Desferrioxamine

Naturally occurring siderophores provide excellent models for the development of therapeutic useful iron chelators. Indeed, desferrioxamine (DFO) (**1**), a growth-promoting agent secreted by the microorganism (*Streptomyces pilosus*), is presently the therapeutic agent of choice for the clinical treatment of chronic iron overload. DFO is a *tris*-hydroxamic acid derivative and chelates ferric iron in an 1:1 molar ratio. It possesses an extremely high affinity for iron(III) and a much lower affinity for other metal ions present in biological fluids, such as zinc, calcium and magnesium. Although DFO is a large and a highly hydrophilic molecule ($\log D_{7.4} = -2$), it gains entry into the liver via a facilitated transport system. It can, therefore, interact with both hepatocellular and extracellular iron, promoting urinary and biliary iron excretion [54]. Ferrioxamine, the DFO–iron complex (Fig. 1), is kinetically inert and possesses a relatively low lipophilicity and thus is unlikely to enter cells. This property reduces the potential of iron redistribution. However, DFO is far from being an ideal therapeutic agent due to its oral inactivity and rapid renal clearance (plasma half-life of 5–10 min [55]). In order to achieve sufficient iron excretion, it has to be administered subcutaneously or intravenously for 8–12 h a day, 5–7 days a week [56]. Consequently, patient compliance with this cumbersome regimen is often poor. Moreover, although DFO has been demonstrated to be a safe drug when administered in the presence of an elevated body iron burden, intensive therapy in young patients with lower body iron stores may result in serious neurotoxicity, abnormalities of cartilage formation and other serious adverse effects [57–60].

4.1.2. Aminocarboxylates

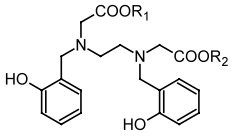
DTPA (**13**) is an aminocarboxylate hexadentate ligand and has been used in patients who develop toxic side-effects with DFO [61]. Due to its net charge at neutral pH, DTPA is largely confined to extracellular compartments in vivo and is excreted in the urine within 24 h of administration [18,62]. DTPA is not orally active and due to its relative lack of selectivity for iron(III), leads to zinc depletion [18]. Consequently, zinc supplementation is required to prevent the toxic sequelae of such depletion.

In order to enhance the selectivity of the aminocarboxylate ligands for iron(III), several analogues which contain both carboxyl and phenolic ligands have been designed [63,64]. A particularly useful compound is *N*,*N*′-bis(2-hydroxybenzyl)-ethylenediamine-*N*,*N*′-diacetic acid (HBED) (**22**) which is significantly more effective than DFO when given intramuscularly to iron



Scheme 4. The ester hydrolysis of the iron(III) complex of the mono ethyl ester derivative of HBED, me-HBED (**23**), leading to the formation of HBED (**22**).

overloaded rats [65,66]. It binds ferric iron strongly with an overall stability constant ($\log K_1$) of 40 and a pFe^{3+} value of 31, rendering this molecule a potent ligand for chelation of ferric iron in vivo. Unfortunately, HBED is not efficiently absorbed via the oral route in either primate [66,67] or man [68] because of the zwitterionic nature of the molecule. Considerable effort has been put into the design of HBED ester prodrugs [69,70]. However, most of the compounds are of little use due to the slow rate of hydrolysis of the esters, particularly in primate [67]. A compound of particular interest was found to be the mono ethyl ester, me-HBED (**23**), which possesses good oral availability [71]. The reasons for the efficacy of this compound is probably manyfold including, a disruption of intramolecular H-bonding thereby improving water solubility; an enhancement of partition constant, particularly in media of low dielectric constant; the ability of the mono ester to bind iron(III) and the activation of ester hydrolysis of the resulting iron(III) complex (Scheme 4) [72]. Unfortunately, by virtue of the presence of the two nitrogen ligands, HBED retains a relatively high affinity for zinc ($\log K_1 = 18.4$) and, therefore, would be predicted to induce undesirable side-effects due to the co-ordination of endogenous zinc. Indeed HBED is capable of removing zinc from the zinc finger protein MTF-1 [73]. Recently it has been decided not to proceed with this molecule due to associated toxicity [74].



(22) $R_1 = R_2 = H$
(23) $R_1 = C_2H_5$; $R_2 = H$

4.1.3. Catechols

Hexadentate tricatechols are iron(III) chelators per excellence, enterobactin (**4**) typifying the group. However, such molecules possess a relatively high molecular weight and therefore their oral bioavailability is poor, particularly at clinically useful doses. A further compli-

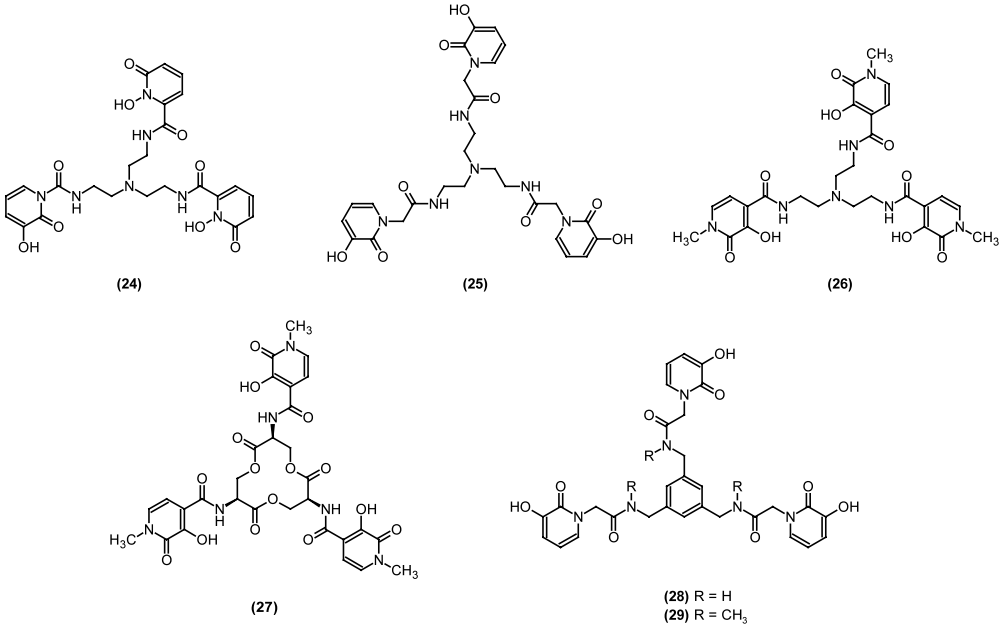
cation with such molecules is that they adopt a high net negative charge when coordinated to iron(III), which tends to minimise their rate of efflux by non-facilitated diffusion. A number of synthetic analogues have been prepared which retain the high affinity for iron(III) typical of enterobactin and yet are more stable under biological conditions, for instance, the tripodal molecule (**20**) and MECAM (**18**) [29]. Unfortunately many of these hexadentate catechols bind to the enterobactin receptor expressed by pathogenic organisms and hence will supply iron to such bacteria, an undesirable feature for clinical use.

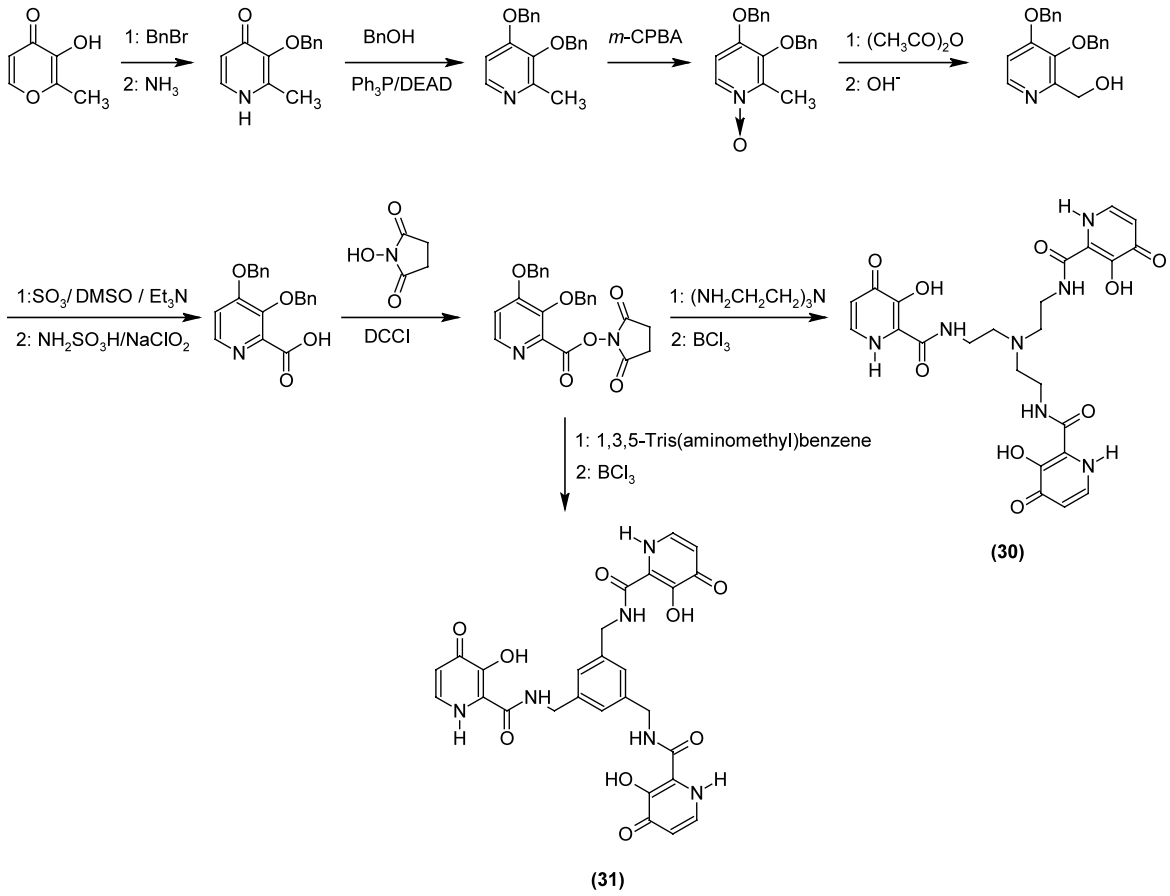
4.1.4. Hydroxypyridinones

Hexadentate siderophore analogues can be constructed by derivatising prototype bidentate hydroxypyridinones and attaching them to suitable molecular frameworks. Although enterobactin (**4**) has an extremely high stability constant for iron(III), the effectiveness of this molecule and its analogues under acid conditions is limited by their weak acid nature and the required loss of six protons on binding iron(III). In contrast hydroxypyridinones are stronger acids than catechols, and since they are monoprotic, hexadentate ligands formed from three such units only loose three protons on formation of a six-coordinated complex. Thus hexadentate HPOs compete well with hexadentate catechols at neutral pH values. Another potential advantage of these molecules is that they may not be recognised by siderophore receptors and are therefore less likely to donate iron to pathogenic organisms.

Several hexadentate ligands based on 1-hydroxypyridin-2-one and the 3-hydroxypyridin-2-one moiety have been investigated, for example (**24**) [75], (**25**) [76], (**26**) [77], (**27**) [78], (**28**) and (**29**) [79]. Although the pFe^{3+} values of the hexadentate ligands were significantly higher (ca. 7 and 8 log units) than those of the corresponding bidentate ligands, a clear decrease in the formation constants of up to 2 log units was observed with the hexadentate ligands when compared with the bidentate analogues, indicating the lack of ligand predisposition for metal binding [75–79]. In order to provide the correct geometry for metal binding, it is important to attach the molecular scaffold to the *ortho* position to the chelating oxygens [80]. Furthermore, the introduction of an amide group adjacent to the coordinating phenolates further contributes to the stability of the resulting iron-complex via a hydrogen-bond effect [80]. Recently, a novel synthetic route has been developed for constructing hexadentate ligands from the 3-hydroxypyridin-4-one unit (Scheme 5). This method leads to hexadentate 3-hydroxypyridin-4-ones (**30**) and (**31**) which possess the appropriate geometry for iron chelation [81]. Preliminary physicochemical characterisation indicates that such hexadentate ligands are extremely potent iron chelators, particularly over the pH range of 2–9, with an associated pM value of 30.5 for (**30**) at pH 7.4.

Hexadentate pyridinone molecules are likely to be less toxic than their bidentate analogues because of a more restricted biodistribution. However, by virtue of their higher molecular weight, such molecules, like siderophores, possess relatively low oral bioavailability. The





Scheme 5. Synthesis of novel hexadentate 3-hydroxypyridin-4-one ligands.

fraction of the oral dose absorbed for the hexadentate (**29**) for instance was found to be only 3.5% in mice [77].

4.2. Tridentate chelators

Unlike hexadentate and bidentate molecules, it is not possible to synthesise simple tridentate ligands which only possess oxygen anion coordination sites [82], the central ligand typically being nitrogen (Fig. 4). This has the adverse effect of rendering the iron(III) complex more easily reduced and therefore more susceptible towards redox cycling. Furthermore, the presence of two nitrogens in the coordination sphere reduces the selectivity of iron(III) over zinc(II) [12]. An additional problem with tridentate ligands is that, without exception, they form charged iron(III) complexes, an undesirable feature for efficient iron extraction from intracellular sites.

4.2.1. PIH analogues

Pyridoxal isonicotinoyl hydrazone (PIH) (**32**), together with a wide range of analogues have been subjected to extensive evaluation as iron(III) chelators [83–85]. Many have been demonstrated to be orally active in rodents. The efficiency of in vivo iron removal

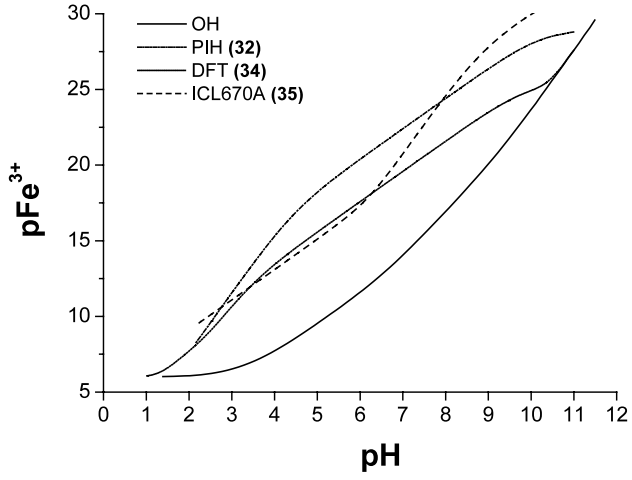


Fig. 9. Influence of pH on pFe^{3+} values of pyridoxal isonicotinoyl hydrazone (**32**), desferrithiocin (**34**) and the triazole ICL670A (**35**); $[Fe^{3+}]_{total} = 10^{-6}$ M; $[Ligand]_{total} = 10^{-5}$ M.

increases with lipophilicity of both the ligand and the iron complex [86], but as more lipophilic chelators are likely to be associated with enhanced toxicity, log $P_{water/octanol}$ values close to unity are preferred [86]. Many of the PIH analogues are uncharged at neutral pH values and therefore gain ready access to cells, indeed

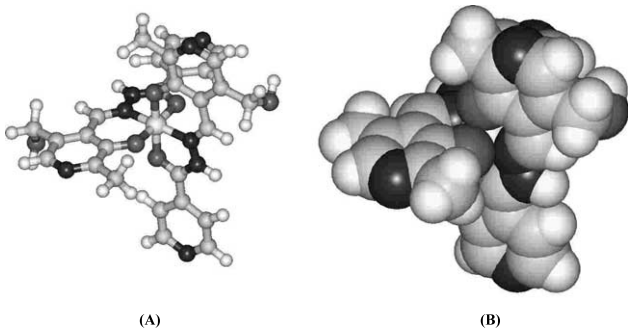


Fig. 10. Energy-minimised structure of the 2:1 iron(III) complex of pyridoxal isonicotinoyl hydrazone (**32**); (A) Ball-stick model; (B) Space-filling model.

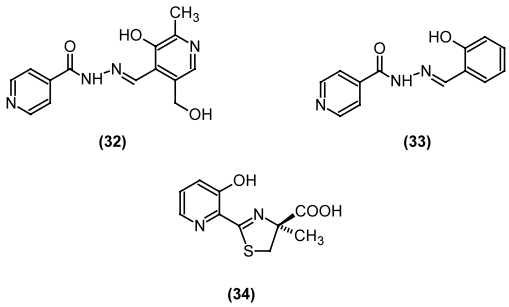
salicylaldehyde isonicotinoyl hydrazone (SIH) (**33**) gains entry to a range of cell types more rapidly than most other chelators, including the smaller hydroxypyridinones [87]. The binding of iron(III) by PIH and related ligands is complicated because of the existence of a number of dissociable protons both in the free and coordinated states. Never-the-less the 2:1 complex is the favoured species over the range pH 4–8 and the affinity for iron(III) compares well with other tridentate ligands (Fig. 9).

PIH was selected for human balance studies because the two components of the condensed molecule, isoniazid and pyridoxal have been used safely to treat tuberculosis [88]. No significant toxicity was observed at a dose of 30 mg kg^{-1} per 24 for 6 days, but iron excretion at this dose was insufficient to produce negative iron balance [88]. This dose is much lower than those used in animals (typically > 100 mg kg^{-1}) and ideally the study should be repeated at a higher dose [89].

The two nitrogen atoms in the coordinating sphere of the complex (Fig. 10) [90] endow PIH analogues with the ability to bind iron(II) with appreciable affinity [91]. Such complexes are likely to readily redox cycle.

4.2.2. Desferrithiocins

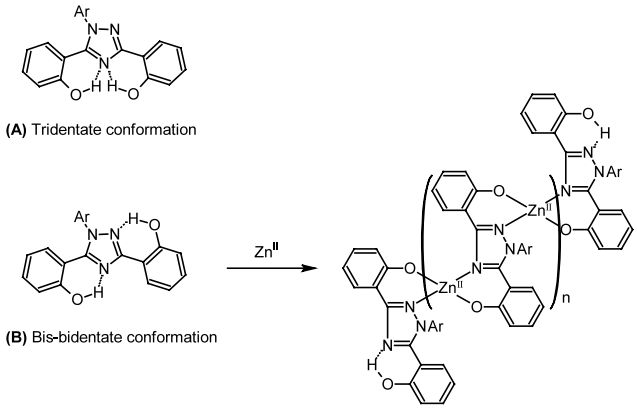
Desferrithiocin (DFT) (**34**) is a siderophore isolated from *Streptomyces antibioticus*. It forms a 2:1 complex with iron(III) at neutral pH using a phenolate oxygen, a carboxylate oxygen and a nitrogen atom as ligands [92]. It possesses a high affinity for ferric iron (log $\beta_2 = 29.6$), however, by virtue of the presence of the nitrogen and carboxylate ligands, it also binds zinc tightly (log $\beta_2 =$ 15.3) [93]. Long term studies of DFT in normal rodents and dogs at low doses have shown toxic side effects, such as reduced body weight and neurotoxicity [94]. A range of synthetic analogues of DFT have been prepared [95], however, to date no suitable candidates have been identified for the replacement of DFO, mainly because of the adverse toxicity of the iron complexes of this class of molecule [96].



4.2.3. Triazoles

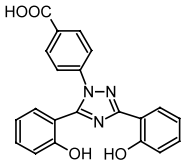
Recently, triazoles have been investigated as ligands by Novartis [97]. These molecules chelate iron(III) with two phenolate oxygens and one triazolyl nitrogen. The lead compound ICL670A (**35**) possesses a pFe^{3+} value of 22.5 and is extremely hydrophobic, with a log $P_{water/octanol}$ value of 3.8 and a log $D_{7.4}$ value of 1.0 [98]. As a result it can penetrate membranes easily and possesses good oral availability. Indeed, when orally administered to hypertransfused rats, ICL670A promotes the excretion of chelatable iron from hepatocellular iron stores four to five times more effectively than DFO [99]. By virtue of a high proportion of the molecule binding to albumin, it is largely retained in the extracellular space and therefore is remarkably nontoxic, despite its strong lipophilic character. It has been demonstrated to be a highly effective iron scavenging molecule in a range of species, including the marmoset. The vast majority of ICL670A-induced iron excretion is via the bile and faeces, only a relatively small fraction is excreted via the urine. ICL670A has successfully completed phase I trials and is currently in phase II trials.

ICL670A (**35**) forms a 2:1 iron complex (**36**) which possesses a net charge of 3- and a molecular weight over 800 [100]. Should such a complex form intracellularly, it is likely that, as with tricatechols, the iron will remain

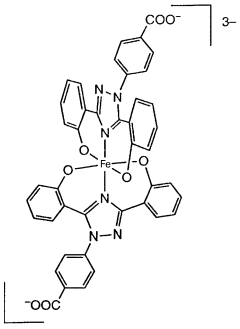


Scheme 6. Two possible conformations of the triazole derivatives: (A) the tridentate conformation and (B) the bis-bidentate conformation. The bis-bidentate structure has a strong tendency to form polymeric complexes.

trapped within the cell. Furthermore, the triazoles can exist in two conformations: one a tridentate structure and the alternate a *bis*-bidentate structure with a strong tendency to form polymeric complexes (Scheme 6) [101]. The latter conformation favours zinc(II) since 50% of the coordinating ligands are nitrogen.



(35)



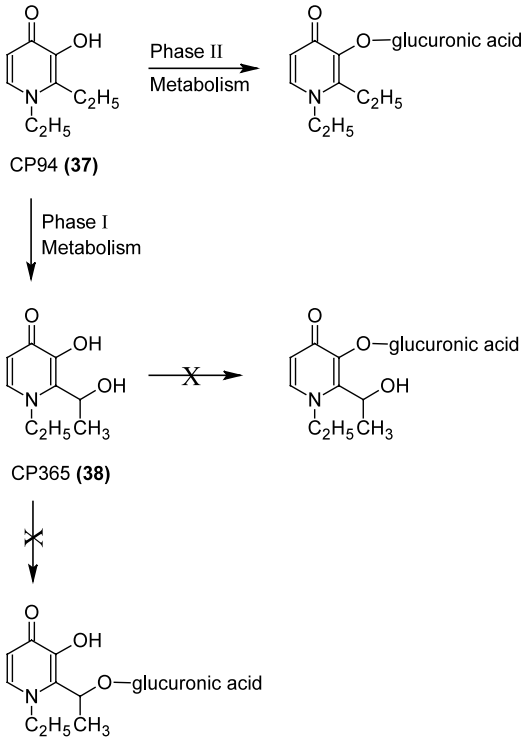
(36)

4.3. Bidentate chelators

On the basis of selectivity and affinity, particularly considering the pFe^{3+} value, 3-hydroxypyridin-4-one is the optimal bidentate ligand for the chelation of iron(III) over the pH range of 6.0–9.0 (Fig. 5B).

4.3.1. Dialkylhydroxypyridinones

The 1,2-dimethyl derivative (deferiprone, L1, CP20) (**11**) is the only orally active iron chelator currently available for clinical use (marketed by Apotex Inc. Toronto, Canada as Ferriprox™). Unfortunately, the dose required to keep a previously well chelated patient in negative iron balance with Ferriprox™ is relatively high, in the region of 75–100 mg kg^{-1} day^{-1} [102] and side effects have been observed in some patients receiving deferiprone [103,104]. One of the major reasons for the limited efficacy of deferiprone in clinical use is that it undergoes extensive metabolism in the liver. The 3-hydroxyl functionality, which is crucial for scavenging iron, is a prime target for glucuronidation. Urinary recovery studies conducted on deferiprone in both rats and man have shown that, respectively, $>44\%$ and $>85\%$ of the administered dose is recovered in the urine as the non-chelating 3-*O*-glucuronide conjugate [105]. The use of deferiprone for the treatment of iron overload remains a hotly debated subject at the present time [104,106–111], however, until a superior orally active chelator becomes available, deferiprone remains in the unique position of being the only orally active chelator available for clinical use.



Scheme 7. Metabolism of CP94 (**37**) via phase I and II metabolic pathways. The 2,1′-hydroxylated metabolite CP365 (**38**) is the major metabolite in the rat, whereas phase II glucuronidation is the major metabolic route of CP94 in man.

The 1,2-diethyl analogue CP94 (**37**) has also been widely investigated [112–115]. This chelator is more efficient at iron removal than deferiprone in several mammalian species, e.g. rat [112] and cebus monkey [115]. The presumed reason for the greater efficacy of CP94 in the rat is its unusual metabolic pathway which leads to the formation of the 2-(1′-hydroxyethyl) metabolite CP365 (**38**) (Scheme 7) [105,116]. This metabolite does not undergo further metabolism to form a glucuronide conjugate and hence retains the ability to chelate iron. Promising results obtained in rat models, led to the limited clinical evaluation of CP94 in thalassaemic patients [114]. Unfortunately, the metabolism of CP94 in man does not parallel that of the rat. The main urinary metabolite of CP94 in man is the 3-*O*-glucuronide conjugate ($>85\%$) [113]. Extensive conversion to this metabolite was found to severely limit clinical efficacy.

4.3.2. Hydroxypyridinone prodrugs

The critical dependence of chelator efficacy on metabolic behaviour has led to a concept of ligand design

which minimises conjugation reactions with glucuronic acid. Despite the limited efficacy of CP94 in man, the superior extracellular and intracellular iron mobilisation ability in the rat provided important information for chelator design. The lack of glucuronidation of the 2-(1′-hydroxyethyl) metabolite of CP94 led to the investigation of the possibility of developing structurally related compounds. Indeed, 1-hydroxyalkyl derivatives of HPOs such as CP41 (**39**) and CP102 (**40**), which are not extensively conjugated with glucuronic acid, have been identified [117,118]. Although the use of 1-hydroxyalkyl derivatives of HPO may offer a significant improvement over previously evaluated HPOs [119–121], a possible disadvantage of these compounds, especially the more hydrophilic analogues, is the reduced liver extraction.

One of the potential problems associated with orally active bidentate HPOs is that by virtue of their relatively low molecular weight and favourable distribution coefficients, they rapidly penetrate most cells and critical barriers such as the blood–brain barrier and the placental barrier. Ideally, the distribution of iron chelators developed for the treatment of general iron overload, such as β-thalassaemia major, is best limited to the extracellular space and the liver. The anticipated partition coefficient (log P) values for an ideal iron chelator are outlined in Table 6. Clearly, there is no single compound fulfilling these requirements since the optimal partition coefficient for absorption from the gastrointestinal tract is quite different from that necessary to limit access to the brain and placenta. In principle this problem can be overcome by the use of prodrugs, whereby a hydrophobic prodrug is absorbed from the gastrointestinal tract and then efficiently extracted by the liver during the 'first pass'. Once in the hepatocyte, the ester link is cleaved to a much more hydrophilic chelator (Fig. 11). This chelator can scavenge iron in the hepatocyte, but also efflux into the systemic circulation, thereby scavenging the extracellular iron pool. The hydrophilic nature of the iron-chelating metabolite restricts its ability to cross critical membrane barriers and thereby minimises toxicity problems.

Table 6
Anticipated optimal partition coefficients of an ideal iron chelator

	log P
Good absorption from the gastrointestinal tract	> -1
Efficient liver extraction	> 0
Poor entry into peripheral cells (thymus, muscle, heart, bone marrow)	< -3
Poor ability to penetrate the blood-brain and maternal/placental barriers	< -3

A wide range of ester prodrugs of 1-hydroxyalkyl HPOs has been investigated [122–124]. *In vitro* esterase studies indicate that the pivaloyl esters and the aromatic ester analogues partially fulfil the requirements for relatively efficient liver extraction [122–126]. Preliminary pharmacokinetic and absorption studies in the rat have demonstrated that the prodrugs are rapidly absorbed from the gastrointestinal tract in the intact form and subsequently undergo extensive first pass metabolism [125,126]. In many cases the ester prodrug leads to superior iron excretion via the bile than the corresponding alcohol (Table 7), indicating selective delivery to the liver.

4.3.3. 'High pFe^{3+}' hydroxypyridinones

An undesirable feature associated with bidentate hydroxypyridinone chelators is the kinetic lability of

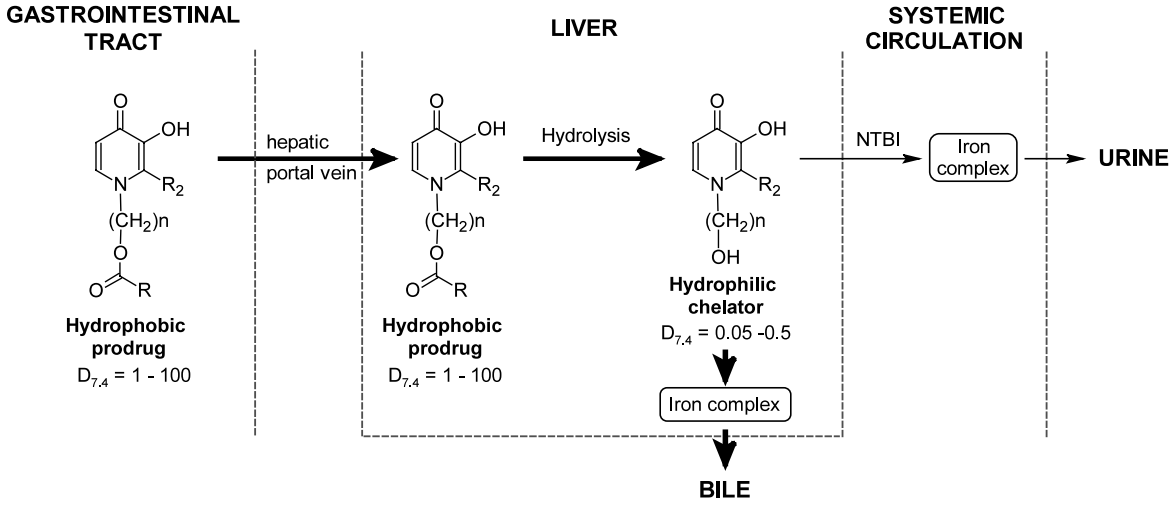
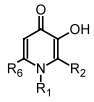


Fig. 11. Schematic representation of the use of ester prodrugs of 1-hydroxyalkyl HPOs to enhance both the absorption from the gastrointestinal tract and liver extraction from the hepatic portal vein. In the hepatocyte, the prodrug is rapidly converted to a hydrophilic chelator which will scavenge iron in the hepatocyte. This hydrophilic chelator can efflux into the systemic circulation, thereby scavenging the extracellular iron pool. Due to its hydrophilic nature, the molecule will not be expected to readily penetrate critical membrane barriers such as the blood–brain barrier.

Table 7
Physicochemical properties and iron mobilisation efficacies of selected 3-hydroxypyridin-4-ones



Ligand	R_1	R_2	R_6	log P	pFe^{3+}	Efficacy (%)
CP20 (**11**)	CH_3	CH_3	H	-0.77	19.4	9.5
CP94 (**37**)	CH_2CH_3	CH_2CH_3	H	0.25	19.7	55.8
CP102 (**39**)	CH_2CH_2OH	CH_2CH_3	H	-0.66	ND	12.9
CP41 (**40**)	$(CH_2)_3OH$	CH_3	H	-0.89	ND	26.0
CP117 (**41**)	$CH_2CH_2OOC(CH_3)_3$	CH_2CH_3	H	1.16	ND	19.1
CP165 (**42**)	$(CH_2)_3OOC(CH_3)_3$	CH_3	H	0.94	ND	35.5
CP283 (**43**)	$(CH_2)_3OOCC_6H_5$	CH_3	H	1.25	ND	32.5
CP361 (**47**)	CH_3	$CH(OH)CH_3$	CH_3	-0.60	21.5	44.5
CP365 (**38**)	CH_2CH_3	$CH(OH)CH_3$	H	-0.58	21.4	50.6
CP502 (**48**)	CH_3	$CONHCH_3$	CH_3	-1.36	21.7	54.8
CP511 (**49**)	H	$CONHCH_3$	CH_3	1.08	22.8	46.2

[a] Iron mobilisation efficacy of chelators (450μmol/kg) was measured using the ^{59}Fe-ferritin loaded rat model.

the iron(III) complexes. Ideally, the dominant species under most physiological conditions should be the fully coordinated 3:1 species. Clearly, chelators with high pFe^{3+} values are predicted not only to scavenge iron more effectively at low ligand concentrations, but also to dissociate less readily and therefore form lower concentrations of the partially coordinated complexes.

In order to further improve chelation efficacy and minimise drug-induced toxicity, considerable effort has been applied to the design of novel hydroxypyridinones with enhanced pFe^{3+} values [127,128]. Novartis synthesised a range of bidentate hydroxypyridinone ligands, which possess an aromatic substituent at the 2-position. The aromatic group is reported to stabilise the resulting iron complex and hence increase the pFe^{3+} values [127]. The lead compound (**44**) was found to be orally active [129] and highly effective at removing iron from both the iron-loaded rat and marmoset [127]. Recently, we have demonstrated that the introduction of either a 1′-hydroxyalkyl group (**45**) [130] or an amido function (**46**) [131] at the 2-position of 3-hydroxypyridin-4-ones enhances the affinity for iron(III) in the pH range 5–8. This effect results from stabilising the ionised species, due to the combined effect of intramolecular hydrogen-bonding between the 2-substitutent with the adjacent 3-hydroxyl function and electron withdrawal from the pyridinone ring. Although such an effect reduces the overall iron(III) stability constant, it also reduces the pK_a values of the chelating function. These combined changes result in an increase in the corresponding pFe^{3+} values [128,130,131]. Interestingly, the Novartis

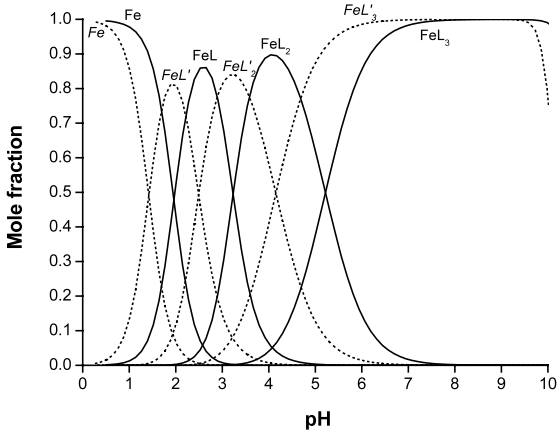
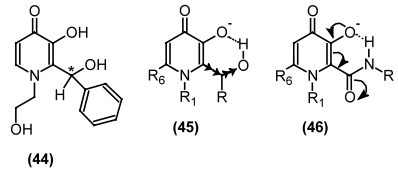


Fig. 12. Comparison of the speciation plots of iron(III) in the presence of deferiprone (**11**) and CP502 (**48**); L = deferiprone; L′ = CP502; $[Fe^{3+}]_{total} = 1 \times 10^{-6}$ M and [Ligand] = 1×10^{-5} M.

lead compound (**44**) also possesses an 1′-hydroxyl group at the 2-position and this is almost certainly responsible for the observed enhanced pFe^{3+} value.



The enhancement of pFe^{3+} values has a dramatic effect on the speciation plot of the iron(III), the *N*-methyl amido derivative CP502 (**47**) dissociating less readily, leading to lower concentrations of the L_2Fe^+ complex when compared with deferiprone (Fig. 12). These novel high pFe^{3+} HPOs show great promise in their ability to remove iron under *in vivo* conditions [128,130,131] (Table 7). Detailed dose–response studies suggest that chelators with high pFe^{3+} values scavenge iron more effectively at lower doses when compared with simple dialkyl substituted hydroxypyridinones. A small number of related compounds have been selected for pre-clinical investigation by Apotex.

5. Therapeutic applications of iron(III)-selective chelators

5.1. General iron overload

The most frequent treatment of inherited haematological diseases such as β-thalassaemia major is to increase the haemoglobin levels by blood transfusion [132]. Unfortunately, regular blood transfusion leads to elevated body iron levels due to the inability of man to excrete iron. Each unit of blood (400 ml) contains ca. 250 mg of iron and thus if received more than twice per annum, the patient will begin to accumulate excess iron. With thalassaemia major, transfusions are usually

provided at monthly intervals and therefore without chelation therapy a patient will accumulate over 2.5 g of iron per annum. A normal adult human only has a total of 4–5 g of body iron. Excess iron, which rapidly accumulates, is mainly located within the liver and the spleen. With untreated patients, death generally occurs in the second decade of life as a result of infection or heart disease [3]. Iron chelation therapy prevents the development of iron overload and as a consequence the life style of thalassaemia major patients has been dramatically improved. Over 50 000 cases with thalassaemia major occur worldwide annually, particularly in the Mediterranean and Far East Regions. Bone marrow transplantation can be considered for young patients but this is expensive and of limited availability. Consequently effective orally active iron chelators are much sought after [133]. More recently transfusion has been increasingly introduced for the treatment of sickle cell anaemia, due to its beneficial effect in the treatment of crises and in the reduction of the incidence of strokes [134,135]. Again an effective orally active iron chelator would permit regular transfusion of such patients without the worry of inducing an associated iron overloaded state. Thalassaemia and sickle cell genes are widely dispersed in tropical and subtropical regions of the world since such genes endow protection against malaria infection.

Iron overload can also result from the hyperabsorption of iron from the diet. Adult man typically absorbs 1 mg of iron each day and this intake is tightly controlled. There are several inherited diseases which are associated with enhanced iron absorption, for example genetic haemochromatosis, which is common in Northern Europe and North America. Symptoms resulting from iron overload, e.g. heart failure, liver cirrhosis or diabetes typically present in the fourth or fifth decade of such patients when total body iron stores have reached levels of 20–40 g [136]. Iron absorption may also be increased secondary to anaemia such as that found in patients with thalassaemia intermedia. With these patients, the degree of anaemia is not sufficiently severe to necessitate regular blood transfusions. However, due to increased absorption of iron via gut, they develop the symptoms of iron overload typically in the fourth or fifth decade of life [137]. Thalassaemia intermedia is extremely common in South East Asia, where in some regions over 50% of the population carry the gene.

5.2. Removal of non-transferrin bound iron

Under normal conditions, serum transferrin is 20–35% saturated with iron and this is the only major form of non-heme iron in plasma. However, under conditions of iron overload, transferrin becomes saturated and an appreciable proportion of iron released by the reticuloendothelial system is therefore unable to bind to transferrin and so remains in the form of non-transferrin bound iron (NTBI). The speciation of this iron pool is unknown at the present time, but some fractions are toxic, generating hydroxyl radicals. More importantly NTBI is taken up by cells independently of the transferrin receptor. Thus highly vascular tissues, for instance endocrine tissue and heart muscle, also become iron loaded. The resulting hydroxyl radical generation in iron loaded cells leads to severe damage and eventually cell death.

Transferrin also contributes to defence against infection by depriving microorganisms of iron [138]. Thus the presence of NTBI presents a weakness, rendering the host more susceptible towards infection. For this reason it is probably sensible to remove all traces of NTBI, especially in patients with a suppressed immune system. Patients receiving chemotherapy for the treatment of cancer experience an elevation of NTBI. This is due to the toxic side effects on bone marrow, which reduces the demand of marrow cells for iron. Consequently transferrin becomes saturated and iron entering the extracellular space from the gastrointestinal tract and the reticuloendothelial system forms NTBI, rendering the host more susceptible towards infection. Removal of NTBI by chelation could become a useful therapy in such cases.

5.3. Localised iron overload

Although considerable evidence has been produced suggesting that oxygen-derived free radicals play a major role in producing tissue damage associated with both reperfusion of ischaemic tissue and local inflammation, a clear role for an iron scavenger in the reversal of such phenomena has not been proven. Desferrioxamine and hydroxypyridinones have been implicated in the treatment of both pathologies but only at relatively high chelator concentrations and not in the clinical situation. There is considerable potential for the use of iron-selective chelators, should the role of 'loosely-bound' iron be unambiguously associated with hydroxyl radical production under clinically relevant circumstances [139].

It is well established that there are elevated levels of iron in the substantia nigra of Parkinsonian brains [140]. This iron could be partially or totally responsible for the enhancement of lipid peroxidation which also occurs in this brain region in patients suffering from Parkinson's disease. Iron-specific chelators could, in principle, minimise such damage, thereby slowing down the progression of the disease. Hydroxypyridinones capable of crossing the blood–brain barrier have potential for such therapy.

5.4. Selective inhibition of iron-containing enzymes

The inhibition of ferrochelatase increases the level of its substrate protoporphyrin IX. On irradiation this tetrapyrrole generates hydroxyl radicals, thereby rendering photodynamic therapy of skin and bladder cancers a real possibility [141]. The rapidly metabolised, hydrophobic hydroxypyridinone, CP94, gains ready access to ferrochelatase and gives rise to a synergistic effect with 5-aminolevulinic acid on the selective necrosis of surface tumours. This work has recently been extended to the treatment of pancreatic and colon cancers [142].

The inhibition of ribonucleotide reductase renders it possible to synchronise cell cycling which has relevance in combination chemotherapy where optimal synergism is obtained when the cell cycle is synchronised throughout the tumour. Both desferrioxamine and hydroxypyridinones are known to facilitate cell synchronisation and may therefore have relevance to the chemotherapy of some tumour classes [143].

5.5. Antimalarial iron chelators

The antimalarial activity of desferrioxamine has been demonstrated under both *in vitro* and *in vivo* conditions [144]. Primary lesions are associated with the nucleus which is consistent with ribonucleotide reductase serving as the target. Orally active hydroxypyridinones have also been demonstrated to possess antimalarial activity under *in vitro* conditions [145]. The introduction of an iron chelator to control this parasite infection is a novel approach to chemotherapy and may find benefit against many drug-resistant strains now present in Africa and South East Asia. The design of chelators with an affinity for one of the parasite-induced transport proteins present in the erythrocyte membrane could offer a degree of target selectivity [146].

6. Conclusions

Over the past 30 years many attempts have been directed at the design of non-toxic orally active iron chelators, but only one clinically useful compound has emerged to date, deferiprone. Since 1995 a number of significant advances have been made and the authors are confident that other more efficacious orally active chelators will soon join deferiprone. Several lead compounds have been identified and are under active development for the treatment of transfusional iron overload. The successful introduction of such compounds will impact considerably on the therapeutic outcome and quality of life for the thalassemic population. There is potential for iron chelation in a wide range of clinical situations. Once a chelator has been clinically proven in thalassemic patients, such compounds will almost certainly find application for the treatment of other disease states, for instance sickle cell anaemia.

References

[1] B. Halliwell, J.M.C. Gutteridge, Free Radicals in Biology and Medicine, second ed., Clarendon Press, Oxford, 1989.
[2] R.R. Crichton, Inorganic Biochemistry of Iron Metabolism, Ellis Harwood, New York, London, 1991.
[3] G.M. Brittenham, in: R. Hoffman, E. Benz, S. Shattil, B. Furie, H. Cohen (Eds.), Hematology: Basic Principles and Practice, Churchill Livingstone, New York, 1991, p. 327.
[4] C. Hershko, A.M. Konijn, G. Link, Br. J. Haematol. 101 (1998) 399.
[5] N.C. Andrews, New Engl. J. Med. 341 (1999) 1986.
[6] R.G. Pearson, J. Am. Chem. Soc. 85 (1963) 3535.
[7] A.E. Martell, R.M. Smith, Critical Stability Constant, vol. 1–6, Plenum Press, London, 1974–1989.
[8] D.C. Harris, P. Aisen, Biochim. Biophys. Acta 329 (1973) 156.
[9] R.C. Hider, Struct. Bond 38 (1984) 25.
[10] K.N. Raymond, G. Muller, B.F. Matzanke, Top. Curr. Chem. 58 (1984) 49.
[11] R.C. Hider, A.R. Mohd-Nor, J. Silver, I.E.G. Morrison, L.V.C. Rees, J. Chem. Soc. Dalton Trans. 2 (1981) 609.
[12] R.C. Hider, A.D. Hall, Prog. Med. Chem. 28 (1991) 41.
[13] H. Keberle, Ann. New York Acad. Sci. 119 (1964) 758.
[14] J.B. Summers, B.P. Gunn, J.G. Martin, H. Mazdiyasni, A.O. Stewart, P.R. Young, A.M. Goetze, J.B. Bouska, R.D. Dyer, D.W. Brooks, G.W. Carter, J. Med. Chem. 31 (1988) 3.
[15] R.W. Grady, J.H. Graziane, H.A. Alkers, A. Cerami, J. Pharmacol. Exp. Ther. 196 (1976) 478.
[16] A. Winston, D.V.P.R. Varaprasad, J.J. Metterville, H. Rosenkrantz, J. Pharmacol. Exp. Ther. 232 (1985) 644.
[17] E.T. Clarke, A.E. Martell, J. Reibenspies, Inorg. Chim. Acta 196 (1992) 177.
[18] M.J. Pippard, M.J. Jackson, K. Hoffman, M. Petrou, C.B. Model, Scand. J. Haematol. 36 (1986) 466.
[19] Y. Sugiura, H. Tanaka, Y. Mino, T. Ishida, N. Ota, M. Inoue, K. Nomoto, H. Yoshioka, T. Takemoto, J. Am. Chem. Soc. 103 (1981) 6979.
[20] N. von Wiren, H. Khodr, R.C. Hider, Plant Physiol. 124 (2000) 1149.
[21] R.B. Martin, J. Inorg. Biochem. 28 (1986) 181.
[22] J.L. Pierre, I. Gautier-Luneau, Biometals 13 (2000) 91.
[23] T.G. Spiro, G. Bates, P. Saltman, J. Am. Chem. Soc. 89 (1967) 5559.
[24] J. Strouse, S.W. Layten, C.E. Strouse, J. Am. Chem. Soc. 99 (1977) 562.
[25] J. Meiwes, H.P. Fiedler, H. Haag, H. Zahner, S. Konetschnyrapp, G. Jung, FEMS Microbiol. Lett. 67 (1990) 201.
[26] A. Thieken, G. Winkelmann, FEMS Microbiol. Lett. 94 (1992) 37.
[27] T.D.P. Stack, Z.G. Hou, K.N. Raymond, J. Am. Chem. Soc. 115 (1993) 6466.
[28] B. Tse, Y. Kishi, J. Org. Chem. 59 (1994) 7807.
[29] S.J. Rodgers, C.W. Lee, C.Y. Ng, K.N. Raymond, Inorg. Chem. 26 (1987) 1622.
[30] R.C. Hider, Z.D. Liu, S. Piyamongkol, Transfus. Sci. 23 (2000) 201.
[31] G.S. Tilbrook, R.C. Hider, in: A. Sigel, H. Sigel (Eds.), Metal Irons in Biological Systems: Iron Transport and Storage in Microorganisms, Plants and Animals, vol. 35, Marcel Dekker, New York, 1998, p. 691.
[32] A.T. Florence, D. Attwood, Physicochemical Principles of Pharmacy, second ed., Macmillan, London, 1988.

[33] D. Holander, D. Ricketts, C.A.R. Boyd, Can. J. Gastroenterol. 2 (1988) 35A.
[34] U. Fagerholm, D. Nilsson, L. Knutson, H. Lennernas, Acta Physiol. Scand. 165 (1999) 315.
[35] S. Travis, I. Menzies, Clin. Sci. 82 (1992) 471.
[36] D.G. Maxton, I. Bjarnason, A.P. Reynolds, S.D. Catt, T.J. Peters, I.S. Menzies, Clin. Sci. 71 (1986) 71.
[37] M. Kim, J. Nutr. 126 (1996) 2172.
[38] R.A. Yokel, A.M. Fredenburg, K.A. Meurer, T.L. Skinner, Drug Metab. Dispos. 23 (1995) 1178.
[39] T.V. O'Halloran, Science 261 (1993) 715.
[40] T.D. Rae, P.J. Schmidt, R.A. Pufahl, V.C. Culotta, T.V. O'Halloran, Science 284 (1999) 805.
[41] S. Singh, H. Khodr, M.I. Taylor, R.C. Hider, Biochem. Soc. Symp. 61 (1995) 127.
[42] R.C. Hider, R. Kayyli, P. Evans, Blood 94 (1999) 406A.
[43] S.J. Lippard, J.M. Berg, Principles of Bioinorganic Chemistry, University Science Books, California, 1994.
[44] G.J.P. Britovsek, V.C. Gibson, D.F. Wass, Angew Chem. Int. Ed. 38 (1999) 428.
[45] R.C. Hider, Toxicol. Lett. 82-3 (1995) 961.
[46] R.C. Hider, S. Singh, J.B. Porter, Proc. R. Soc. Edinburgh 99B (1992) 137.
[47] R.D. Abeysinghe, P.J. Robert, C.E. Cooper, K.H. MacLean, R.C. Hider, J.B. Porter, J. Biol. Chem. 271 (1996) 7965.
[48] Z.D. Liu, R.S. Kayyali, R.C. Hider, A.E. Theobald, J. Med. Chem. 45 (2002) 631.
[49] Z.D. Liu, M. Lockwood, S. Rose, A.E. Theobald, R.C. Hider, Biochem. Pharmacol. 61 (2001) 285.
[50] C.E. Cooper, G.R. Lynagh, K.P. Hoyes, R.C. Hider, R. Cammack, J.B. Porter, J. Biol. Chem. 271 (1996) 20291.
[51] V.A. Levin, J. Med. Chem. 23 (1980) 682.
[52] W.H. Oldendorf, Proc. Soc. Exp. Biol. Med. 147 (1974) 813.
[53] M.D. Habgood, Z.D. Liu, L.S. Dehkordi, H.H. Khodr, J. Abbott, R.C. Hider, Biochem. Pharmacol. 57 (1999) 1305.
[54] C. Hershko, R.W. Grady, A. Cerami, J. Lab. Clin. Med. 92 (1978) 144.
[55] M.R. Summers, A. Jacobs, D. Tudway, P. Perera, C. Ricketts, Br. J. Haematol. 42 (1979) 547.
[56] M.J. Pippard, S.T. Callender, D.J. Weatherall, Clin. Sci. Mol. Med. 54 (1978) 99.
[57] J.B. Porter, M.C. Jawson, E.R. Huehns, C.A. East, J.W.P. Hazell, Br. J. Haematol. 73 (1989) 403.
[58] M.H. Freedman, D. Grisaru, N.F. Olivieri, I. MacLusky, P.S. Thorner, Am. J. Dis. Child 144 (1990) 565.
[59] N.F. Olivieri, J.R. Buncic, E. Chew, T. Gallant, R.V. Harrison, N. Keenan, W. Logan, D. Mitchell, G. Ricci, B. Skarf, New Engl. J. Med. 314 (1986) 869.
[60] N.F. Olivieri, J. Harris, G. Koren, S. Khattak, M.H. Freedman, D.M. Templeton, J.D. Bailey, B.J. Reilly, Am. J. Pediatr. Hematol. Oncol. 14 (1992) 48.
[61] M.J. Jackson, D.P. Brenton, B. Modell, J. Inher. Met. Dis. 6 (1983) 97.
[62] R.M. Bannerman, S.T. Callender, D.L. Williams, Br. Med. J. 2 (1962) 1573.
[63] F. L'Eplattenier, I. Murase, A.E. Martell, J. Am. Chem. Soc. 89 (1967) 837.
[64] A.E. Martell, R.J. Motekaitis, E.T. Clarke, Can. J. Chem. 64 (1986) 449.
[65] E.H. Lau, E.A. Cerny, B.J. Wright, Y.E. Rahman, J. Lab. Clin. Med. 101 (1983) 806.
[66] R.J. Bergeron, J. Wiegand, G.M. Brittenham, Blood 91 (1998) 1446.
[67] H.H. Peter, R.J. Bergeron, R.R. Streiff, J. Wiegand, in: R.J. Bergeron, G.M. Brittenham (Eds.), The Development of Iron Chelators for Clinical Use, CRC Press, London, 1993, p. 373.
[68] R.W. Grady, A.D. Salbe, M.W. Hilgartner, P.J. Giardina, Adv. Exp. Med. Biol. 356 (1994) 351.
[69] C.G. Pitt, Y. Bao, J. Thompson, M.C. Wani, H. Rosenkrantz, J. Metterville, J. Med. Chem. 29 (1986) 1231.
[70] F. Gasparini, T. Leutert, D.L. Farley, International Patent WO 95/16663 (1995).
[71] N. Lowther, B. Tomlinson, R. Fox, B. Faller, T. Sergejew, H. Donnelly, J. Pharm. Sci. 87 (1998) 1041.
[72] B. Faller, C. Spanka, T. Sergejew, V. Tschinke, J. Med. Chem. 43 (2000) 1467.
[73] R.C. Hider, D. Bittel, G.K. Andrews, Biochem. Pharmacol. 57 (1999) 1031.
[74] R. Lattmann, P. Acklin, P. Buhlmayer, A. Crowe, B. Faller, Y. Jin, D. Ledergerber, H.P. Nick, T. Sergejew, C. Spanka, A. Wong, P. Zbinden, H.P. Schnebli, 11th International Conference on Oral Chelation in the Treatment of Thalassaemia, Catania, Italy, 22–25 March 2001, p. 89.
[75] D.L. White, P.W. Durbin, N. Jeung, K.N. Raymond, J. Med. Chem. 31 (1988) 11.
[76] M. Streater, P.D. Taylor, R.C. Hider, J.B. Porter, J. Med. Chem. 33 (1990) 1749.
[77] J.D. Xu, B. Kullgren, P.W. Durbin, K.N. Raymond, J. Med. Chem. 38 (1995) 2606.
[78] M. Meyer, J.R. Telford, S.M. Cohen, D.J. White, J. Xu, K.N. Raymond, J. Am. Chem. Soc. 119 (1997) 10093.
[79] B.L. Rai, H. Khodr, R.C. Hider, Tetrahedron 55 (1999) 1129.
[80] B. O'sullivan, J.D. Xu, K.N. Raymond, in: D.G. Badman, R.J. Bergeron, G.M. Brittenham (Eds.), Iron Chelators: New Development Strategies, The Saratoga Group, Florida, 2000, p. 177.
[81] Z.D. Liu, S. Piyamongkol, R.C. Hider, BIO IRON 2001-World Congress on Iron Metabolism. Cairns, Australia, 18–23 August 2001, p. 79.
[82] M.Y. Moridani, G.S. Tilbrook, H.H. Khodr, R.C. Hider, J. Pharm. Pharmacol. 54 (2002) 349.
[83] C. Hershko, S. Avramovici-Grisaru, G. Link, L. Gelfand, S. Sarel, J. Lab. Clin. Med. 98 (1981) 99.
[84] D.K. Johnson, M.J. Pippard, T.B. Murphy, N.J. Rose, J. Pharmacol. Exp. Ther. 221 (1982) 399.
[85] M. Cikrt, P. Ponka, E. Necas, J. Neuwirt, Br. J. Haematol. 45 (1980) 275.
[86] J.T. Edward, P. Ponka, D.R. Richardson, Biometals 8 (1995) 209.
[87] G. Zanninelli, H. Glickstein, W. Breuer, P. Milgram, P. Brissot, R.C. Hider, A.M. Konijn, J. Libman, A. Shanzer, Z.I. Cabantchik, Mol. Pharmacol. 51 (1997) 842.
[88] G.M. Brittenham, Semin. Hematol. 27 (1990) 112.
[89] D.R. Richardson, P. Ponka, J. Lab. Clin. Med. 131 (1998) 306.
[90] J. Webb, L.M. Vitolo, Birth Defects Original Article Ser. 23 (1988) 63.
[91] S. Avramovicigrisaru, S. Sarel, S. Cohen, R.E. Bauminger, Israel J. Chem. 25 (1985) 288.
[92] F.N. Hahn, T.J. McMurry, A. Hugi, K.N. Raymond, J. Am. Chem. Soc. 112 (1990) 1854.
[93] G. Anderegg, M. Raber, J. Chem. Soc. Chem. Commun. (1990) 1194.
[94] L.C. Wolfe, Sem. Haematol. 27 (1990) 117.
[95] R.J. Bergeron, J. Wiegand, J.B. Dionis, M. Egli-Karmakka, J. Frei, A. Huxley-Tencer, H.H. Peter, J. Med. Chem. 34 (1991) 2072.
[96] E. Baker, W.H. Peter, A. Jacobs, Br. J. Haematol. 81 (1992) 424.
[97] R. Lattmann and P. Acklin, International Patent WO 97/49395 (1997).
[98] H.P. Nick, P. Acklin, B. Faller, Y. Jin, R. Lattmann, M.-C. Rouan, T. Sergejew, H. Thomas, H. Wiegand, H.P. Schnebli, in: D.G. Badman, R.J. Bergeron, G.M. Brittenham (Eds.), Iron Chelators: New Development Strategies, The Saratoga Group, Florida, 2000, p. 311.

[99] C. Hershko, A.M. Konijn, H.P. Nick, W. Breuer, Z.I. Cabantchik, G. Link, Blood 97 (2001) 1115.
[100] U. Heinz, K. Hegetschweiler, P. Acklin, B. Faller, R. Lattmann, H.P. Schnebli, Angew Chem. Int. Ed. 38 (1999) 2568.
[101] Y.I. Ryabukhin, N.V. Shibaeva, A.S. Kuzharov, V.G. Korobkova, A.V. Khokhlov, A.D. Garnovskii, Sov. J. Coordinat. Chem. (1988) 493.
[102] J.A.B. Balfour, R.H. Foster, Drugs 58 (1999) 553.
[103] G.M. Brittenham, Blood 80 (1992) 569.
[104] N.F. Olivieri, G.M. Brittenham, C.E. McLaren, D.M. Templeton, R.G. Cameron, R.A. McClelland, A.D. Burt, K.A. Fleming, New Engl. J. Med. 339 (1998) 417.
[105] S. Singh, O. Epemolu, P.S. Dobbin, G.S. Tilbrook, B.L. Ellis, L.A. Damani, R.C. Hider, Drug Metab. Dispos. 20 (1992) 256.
[106] D.G. Nathan, New Engl. J. Med. 332 (1995) 953.
[107] A.V. Hoffbrand, F. Al-Refaie, B. Davis, N. Siritanakatkul, B.F.A. Jackson, J. Cochrane, E. Prescott, B. Wonke, Blood 91 (1998) 295.
[108] K.V. Kowdley, M.M. Kaplan, New Engl. J. Med. 339 (1998) 468.
[109] F. Tricta, M. Spino, New Engl. J. Med. 339 (1998) 1710.
[110] A. Maggio, M. Capra, C. Ciaccio, C. Magnano, M. Rizzo, et al., Blood 94 (1999) 34B.
[111] A.R. Cohen, R. Galanello, A. Piga, R. Gamberini, V. DeSanctis, F. Tricta, Blood 94 (1999) 406A.
[112] J.B. Porter, J. Morgan, K.P. Hoyes, L.C. Burke, E.R. Huehns, R.C. Hider, Blood 76 (1990) 2389.
[113] J.B. Porter, R.D. Abeysinghe, K.P. Hoyes, C. Barra, E.R. Huehns, P.N. Brooks, M.P. Blackwell, M. Araneta, G. Britenham, S. Singh, P. Dobbin, R.C. Hider, Br. J. Haematol. 85 (1993) 159.
[114] J.B. Porter, S. Singh, P.H. Katherine, O. Epemolu, R.D. Abeysinghe, R.C. Hider, Adv. Exp. Med. Biol. 356 (1994) 361.
[115] R.J. Bergeron, R.R. Streiff, J. Wiegand, G. Luchetta, E.A. Creary, H.H. Peter, Blood 79 (1992) 1882.
[116] S.L. Lu, I. Gosriwatana, D.Y. Liu, Z.D. Liu, A.I. Mallet, R.C. Hider, Drug Metab. Dispos. 28 (2000) 873.
[117] S. Singh, R.O. Epemolu, R. Ackerman, J.B. Porter, R.C. Hider, 3rd NIH-Sponsored Symposium on The Development of Iron Chelators for Clinical Use. Gainsville, FL (1992) Abstract: 52.
[118] S. Singh, R. Choudhury, R.O. Epemolu, R.C. Hider, Eur. J. Drug Metab. Pharmacokinet. 21 (1996) 33.
[119] G. Zanninelli, R. Choudury, O. Loreal, D. Guyader, G. Lescoat, J. Arnaud, R. Verna, B. Cosson, S. Singh, R.C. Hider, P. Brissot, J. Hepatol. 27 (1997) 176.
[120] B.L. Rai, L.S. Dekhordi, H. Khodr, Y. Jin, Z. Liu, R.C. Hider, J. Med. Chem. 41 (1998) 3347.
[121] Z.D. Liu, S.L. Lu, R.C. Hider, Biochem. Pharmacol. 57 (1999) 559.
[122] B.L. Rai, Z.D. Liu, D.Y. Liu, S.L. Lu, R.C. Hider, Eur. J. Med. Chem. 34 (1999) 475.
[123] Z.D. Liu, D.Y. Liu, S.L. Lu, R.C. Hider, J. Pharm. Pharmacol. 51 (1999) 555.
[124] Z.D. Liu, D.Y. Liu, S.L. Lu, R.C. Hider, Arzneim-Forsch/Drug Res. 50 (2000) 461.
[125] R. Choudhury, R.O. Epemolu, B.L. Rai, R.C. Hider, S. Singh, Drug Metab. Dispos. 25 (1997) 332.
[126] D.Y. Liu, Z.D. Liu, S.L. Lu, R.C. Hider, Pharmacol. Toxicol. 86 (2000) 228.
[127] P. Zbinden, US Patent 5,688,815 (1997).
[128] R.C. Hider, G.S. Tilbrook, Z.D. Liu, International Patent WO 98/54138 (1998).
[129] N. Lowther, P. Fox, B. Faller, H. Nick, Y. Jin, T. Sergejew, Y. Hirschberg, R. Oberle, H. Donnelly, Pharmaceut. Res. 16 (1999) 434.
[130] Z.D. Liu, H.H. Khodr, D.Y. Liu, S.L. Lu, R.C. Hider, J. Med. Chem. 42 (1999) 4814.
[131] Z.D. Liu, S. Piyamongkol, D.Y. Liu, H.H. Khodr, S.L. Lu, R.C. Hider, Bioorgan. Med. Chem. 9 (2001) 563.
[132] D.J. Weatherall, J.B. Clegg, The Thalassaemia Syndromes, third ed., Blackwell Scientific Press, Oxford, 1981.
[133] J.B. Porter, Drug Safety 17 (1997) 407.
[134] W. Reed, E.P. Vichinsky, Annu. Rev. Med. 49 (1998) 461.
[135] W. Reed, E.P. Vichinsky, Education Program Book, International Society of Haematology, Toronto, 2000, p. 13.
[136] J.C. Barton, C.Q. Edwards, Hemochromatosis: Genetics Pathophysiology, Diagnosis and Treatment, Cambridge University Press, Cambridge, 2000.
[137] M.J. Pippard, S.T. Callender, G.T. Warner, D.J. Weatherall, Lancet II (1979) 819.
[138] J.J. Bullen, E. Griffiths, Iron and Infection: Molecular, Physiological, and Clinical Aspects, Wiley, New York, 1999.
[139] R.C. Hider, S. Singh, J.B. Porter, Proc. R. Soc. Edinburgh 99B (1992) 137.
[140] D.T. Dexter, F.R. Wells, F. Agid, Y. Agid, A.J. Lees, P. Jenner, C.D. Marsden, Lancet II (1987) 1219.
[141] A. Curnow, B.W. McIlroy, M.J. Postle-Hacon, J.B. Porter, A.J. MacRobert, S.G. Bown, Br. J. Cancer 78 (1998) 1278.
[142] A. Curnow, B.W. McIlroy, M.J. Postle-Hacon, J.B. Porter, A.J. MacRobert, S.G. Bown, Photochem. Photobiol. 69 (1999) 71.
[143] K.P. Hoyes, R.C. Hider, J.B. Porter, Cancer Res. 52 (1992) 4591.
[144] V.R. Gordeuk, P.E. Thuma, G.M. Brittenham, S. Zulu, G. Simwanza, A. Mhangu, G. Flesch, D. Parry, Blood 79 (1992) 308.
[145] C. Hershko, V.R. Gordeuk, P.E. Thuma, E.N. Theanacho, D.T. Spira, R.C. Hider, T.E.A. Peto, G.M. Brittenham, J. Inorg. Biochem. 47 (1992) 267.
[146] R.C. Hider, Z.D. Liu, J. Pharm. Pharmacol. 49 (1997) 59.