Desazadesmethyldesferrithiocin Analogues as Orally Effective Iron Chelators

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Further structure−activity studies of desferrithiocin analogues are carried out. (*S*)-Desazadesmethyldesferrithiocin, 2-(2-hydroxyphenyl)-Δ^2-thiazoline-4(*S*)-carboxylic acid, serves as the principal framework in the current paper. Desazadesmethyldesferrithiocin can be structurally altered with facility, and data are already available on its iron-clearing properties and toxicity parameters. Four different kinds of structural modifications of this framework are undertaken: introduction of hydroxy, carboxy, or methoxy groups on the aromatic ring; alteration of the thiazoline ring; increasing the distance between the ligand donor atoms; and benz-fusion of the aromatic rings. The structural modifications described are shown to have a tremendous impact on both the iron clearance and toxicity profiles of the desazadesmethyldesferrithiocin molecule. All of the compounds are assessed in a bile-duct-cannulated rodent model to determine iron clearance efficiency. Ligands which demonstrate an efficiency of greater than 2% are carried forward to the iron-overloaded primate for iron-clearing measurements. Ligands with efficiencies greater than 3% in the primate are then evaluated in a formal toxicity study in rodents. On the basis of the results of the present work, 2-(2,4-dihydroxyphenyl)-Δ^2-thiazoline-4(*S*)-carboxylic acid is a promising candidate for clinical evaluation.

Introduction

While essentially all prokaryotes and eukaryotes have a strict requirement for iron, it is difficult for living systems to access and manage that metal, even though it represents 5% of the earth's crust. The low solubility of the predominant form of the metal, Fe(III) hydroxide ($K_{sp} = 1 \times 10^{-38}$),[1] in the biosphere has necessitated the development of rather sophisticated iron storage and transport systems in nature. Microorganisms utilize low-molecular-weight ligands, siderophores, whereas higher eukaryotes tend to utilize proteins to transport iron, e.g., transferrin, and store iron, e.g., ferritin.[2]

In primates, iron metabolism is characterized by a highly efficient recycling process[3−6] with no specific mechanism for eliminating this transition metal. Because it cannot be effectively cleared, the introduction of "excess iron"[7−9] into this closed metabolic loop leads to chronic overload and ultimately to peroxidative tissue damage. For example, patients with β-thalassemia, a hereditary anemia, require continued transfusions which increase their body iron by 200−250 mg/unit of blood. Unless these individuals receive chelation therapy, they frequently die in their third decade from iron overload. The hydroxamate desferrioxamine B (DFO), a bacterial siderophore that is commercially produced by large-scale fermentation of a strain of *Streptomyces pilosus*,[10] exhibits a high selectivity for iron and is the drug of choice for the treatment of transfusional iron overload. Unfortunately, treatment with DFO is cumbersome, inefficient, expensive, and unpleasant. Because DFO is poorly absorbed from the gastrointestinal tract and rapidly eliminated from the circulation, prolonged parenteral infusion is needed.[11−13] Effective therapy usually requires subcutaneous (sc) or intravenous (iv) administration by a portable infusion pump for 9−12 h daily. Patient compliance with such a demanding regimen becomes problematic. Thus, considerable interest has developed in the search for an iron chelator that does not require parenteral administration.

Because of the success, albeit mixed, of DFO, siderophores have served as models in the development of orally active iron chelators.[14−16] Desferrithiocin,[17−19] a metabolite of *Streptomyces antibioticus*, forms a 2:1 complex with iron(III) using the thiazoline nitrogen, the phenol oxygen, and a carboxylate oxygen[18,19] as donor sites.

While desferrithiocin [2-(3-hydroxypyrid-2-yl)-4-methyl-Δ^2-thiazoline-4(*S*)-carboxylic acid, **1**] is a very efficient iron chelator when given orally,[20,21] it presents with nephrotoxicity when administered chronically to rodents.[22] Structure−activity studies have made it possible to construct desferrithiocin analogues which are still orally effective yet have substantially reduced toxicity compared to the parent ligand.[20,21,23] However, an even broader therapeutic window would be desirable.

Thus, the goal of the current study is to assemble an orally effective iron-clearing agent with a more acceptable level of toxicity. In this paper, we focus mainly on the structure−activity relationship (SAR) with (*S*)-desazadesmethyldesferrithiocin [2-(2-hydroxyphenyl)-Δ^2-thiazoline-4(*S*)-carboxylic acid, **3**]. Previous investigations in our laboratories revealed that the (*R*)-enantiomer, sodium salt, of this molecule was a very efficient orally active chelator, but severe gastrointestinal side effects were found.[22] The animals treated with this compound at 384 μmol/kg orally (po) were dead by day +6. At necropsy, these animals displayed GI tracts which were hemorrhagic; the stomachs were grossly distended with gas and fluid; and obvious musculature was noted only at the espohageal and duodenal junc-

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tures. Although the observed glucosuria and proteinuria might have been associated with renal failure, the animals' kidneys did not display any obvious macroscopic lesions. The conditions of the GI tracts in these animals were suggestive of glucosuria secondary to acute pancreatitis and not due to renal failure. Several of the animals also had ocular and/or nasal discharges indicative of a systemic infection, which was further supported by the presence of white blood cells in the animals' urine samples and a "left shift" in peripheral blood smears. Because of the good iron-clearing properties and easily observable toxicity of this ligand, **3** seemed like an excellent model on which to base an SAR focused on toxicity issues.

Derivatives of **3** are more accessible than those of (*S*)-desmethyldesferrithiocin [2-(3-hydroxypyrid-2-yl)-Δ^2-thiazoline-4(*S*)-carboxylic acid, **2**], principally because of the absence of the 3-hydroxypicolinic acid fragment of the latter. Thus, alterations in the parent molecule which significantly reduce toxicity are likely to be more easily carried out.

Design and Synthesis

Earlier structure−activity studies on the desferrithiocin pharmacophore revealed that the three ligating centers—the aromatic hydroxyl, the thiazoline nitrogen, and the carboxyl group—are critical to the compounds' iron-clearing capabilities. Modification of some of the nonchelating fragments, such as removal of the thiazoline methyl or the aromatic nitrogen, had little impact on the deferration properties of the resulting molecules. However, either replacement of the sulfur with oxygen or expansion of the five-membered thiazoline to a six-membered Δ^2-thiazine resulted in compounds with little iron-clearing activity.[20,21] Analysis of benz-fused desazadesmethyldesferrithiocins [i.e., the naphthyl analogues 2-(2-hydroxynaphth-1-yl)-Δ^2-thiazoline-4(*R*)-carboxylic acid, 2-(2-hydroxynaphth-1-yl)-Δ^2-thiazoline-4(*S*)-carboxylic acid, 2-(3-hydroxynaphth-2-yl)-Δ^2-thiazoline-4(*R*)-carboxylic acid, and 2-(3-hydroxynaphth-2-yl)-Δ^2-thiazoline-4(*S*)-carboxylic acid] demonstrated that although the former two enantiomeric naphthyl desferrithiocins were not effective iron-clearing agents after oral administration to the rats, their positional isomers, the latter compounds, were.[23] The ineffectiveness of the former enantiomeric pair may be due to steric hindrance from the unsubstituted aromatic ring. None of the benz-fused desferrithiocins investigated were effective iron chelators in the primates, even on sc administration. While the SAR for an orally effective desferrithiocin analogue was useful, an understanding of how to minimize the toxicity of the analogues is still unclear.

Since the thiazoline nitrogen is a vinylogous extension of the aromatic ring, it seemed likely that introduction of substituents, either electron-donating or -withdrawing groups, in the 4- or 6-position of the aromatic ring would affect the donor properties of the thiazoline nitrogen. Substituents introduced at the 3- or 5-position would have inductive effects on the nitrogen but would be less profound in altering the nitrogen donor capacity. It was already clear that **3** binds to and clears iron from both rodents and primates. However, its toxicity is not acceptable and could well have its origin in how it binds iron, i.e., the metal's availability to catalyze redox processes such as Fenton chemistry. Therefore, alterations in the parent ligand which change the donor capacity of the thiazoline nitrogen are interesting due to the fact that they can affect the metal binding.

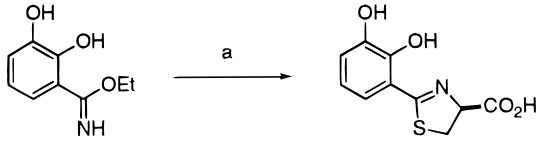
The impact of introducing electron-donating or -withdrawing groups in the aromatic ring of **3** on the iron clearance and toxicity was evaluated initially by introducing a second hydroxyl group, first at position 3 [4,5-dihydro-2-(2,3-dihydroxyphenyl)thiazole-4(*S*)-carboxylic acid, **4**] and then at position 4 [4,5-dihydro-2-(2,4-dihydroxyphenyl)thiazole-4(*S*)-carboxylic acid, **5**]. The biological results of these alterations compelled us to introduce a methoxy in the 3-position [4,5-dihydro-2-(2-hydroxy-3-methoxyphenyl)thiazole-4(*S*)-carboxylic acid, **6**] and a carboxy in the 4-position [2-(4-carboxy-2-hydroxyphenyl)-4,5-dihydrothiazole-4(*S*)-carboxylic acid, **7**].

Earlier studies demonstrated the importance of the thiazoline sulfur atom to the activity of the desferrithiocins. Substitution of an oxygen for the sulfur atom in **3** resulted in an oxazoline that was not effective at clearing iron in either rodents or primates, regardless of the mode of administration.[21] What was particularly interesting was that the oxazoline analogue was absorbed orally. To further confirm the importance of the thiazoline sulfur atom in **3**, the thiazoline was converted to a thiazole [2-(3-hydroxypyridin-2-yl)thiazole-4-carboxylic acid, **8**]. Replacing the thiazoline sulfur with either a nitrogen to yield 4,5-dihydro-2-(2-hydroxyphenyl)-1*H*-imidazole-4-carboxylic acid (**9**) or a methylene to produce 5′-methyldesazadesmethyldesthiadesferrithiocin [3,4-dihydro-5-(2-hydroxy-5-methylphenyl)-2*H*-pyrrole-2-carboxylic acid, **10**] was also performed.

Previous evaluations, while focused largely on altering the ring framework and changing the nature of the donor atoms, gave little attention to assessing the importance of the distance between the ligating centers. The intention, in this instance, is not to explore the effects of entropy but to determine how changing the distance between the centers affects oral absorption and iron clearance in animals. Therefore, changes in the distance between centers should be minor, lest large entropy effects ensue and negatively impact iron binding. Extension of the distance between the ligating centers involved either separating the thiazoline nitrogen−carboxyl bidentate fragment from the phenolic donor or separating the phenol hydroxyl−thiazoline nitrogen bidentate fragment from the carboxyl donor. The former elongation derived from insertion of a methylene bridge between the aromatic and thiazoline rings [4,5-dihydro-2-(2-hydroxyphenylmethyl)thiazole-4(*S*)-carboxylic acid, **11**], the latter one from insertion of one [4,5-dihydro-2-(2-hydroxyphenyl)thiazole-4(*S*)-acetic acid, **12**] or two [4,5-dihydro-2-(2-hydroxyphenyl)-thiazole-4(*S*)-propanoic acid, **13**] methylenes between the thiazoline ring and carboxy terminus.

Prior work with the naphthyldesferrithiocins revealed that 2-(3-hydroxynaphth-2-yl)-Δ^2-thiazoline-4(*R*)-carboxylic acid and 2-(3-hydroxynaphth-2-yl)-Δ^2-thiazoline-4(*S*)-carboxylic acid were not effective in primates, although these compounds did promote iron excretion in rodents.[23] What was interesting, and probably not unexpected, about the naphthyl compounds was the fact

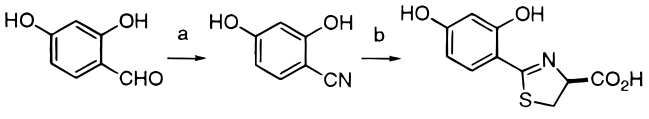
Scheme 1. Synthesis of 4,5-Dihydro-2-(2,3-dihydroxyphenyl)thiazole-4(*S*)-carboxylic Acid (**4**)[a]



4

[a] Reagents: (a) D-cysteine/*t*-BuOH/phosphate buffer/60 °C/16 h.

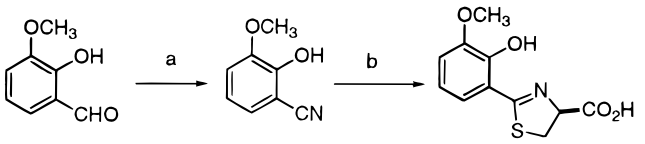
Scheme 2. Synthesis of 4,5-Dihydro-2-(2,4-dihydroxyphenyl)thiazole-4(*S*)-carboxylic Acid (**5**)[a]



16 **5**

[a] Reagents: (a) $CH_3CH_2NO_2/CH_3COONa/CH_3COOH$; (b) D-cysteine/$CH_3OH$/phosphate buffer/70 °C/54 h.

Scheme 3. Synthesis of 4,5-Dihydro-2-(2-hydroxy-3-methoxyphenyl)thiazole-4(*S*)-carboxylic Acid (**6**)[a]



17 **6**

[a] Reagents: (a) $CH_3CH_2NO_2/CH_3COONa/CH_3COOH$; (b) D-cysteine/$CH_3OH$/phosphate buffer/70 °C/17 h.

that the iron excretion was almost totally biliary. It was hoped that, because of the increased lipophilicity of these compounds, they would have a more protracted half-life and thus promote greater iron excretion than the parent ligand **3**; this did not occur. In this work, the benz-fused ligands 2-(3-hydroxyquinolin-2-yl)-Δ^2-thiazoline-4(*R*)-carboxylic acid (**14**) and 2-(3-hydroxyquinolin-2-yl)-Δ^2-thiazoline-4(*S*)-carboxylic acid (**15**) were synthesized and evaluated.

Synthetic Methods

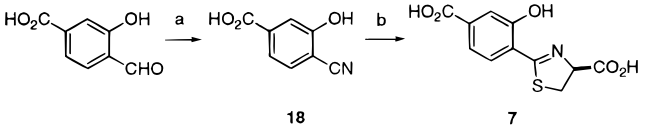
The preparation of analogue **4** was accomplished in moderate yield (30%) by reaction of ethyl 2,3-dihydroxybenzimidate[24] with D-cysteine in phosphate buffer (pH 5.95) and *tert*-butyl alcohol (Scheme 1).

Chelator **5** was synthesized in 66% yield by condensation of 2,4-dihydroxybenzonitrile (**16**) with D-cysteine in phosphate buffer and methanol (Scheme 2). Nitrile **16** was prepared from 2,4-dihydroxybenzaldehyde with nitroethane in sodium acetate and acetic acid.[25]

The key step of the synthesis of **6** in 90% yield was cyclocondensation of D-cysteine with 2-hydroxy-3-methoxybenzonitrile (**17**) in phosphate buffer (pH 5.95) and methyl alcohol (Scheme 3). Intermediate **17** was obtained from *o*-vanillin by treatment with nitroethane in sodium acetate and acetic acid.

Dicarboxylic acid **7** was synthesized by a similar reaction sequence (Scheme 4). 4-Formyl-3-hydroxybenzoic acid was converted to 4-cyano-3-hydroxybenzoic acid (**18**) using nitroethane in sodium acetate and acetic acid. Cyclization of **18** with D-cysteine in phosphate buffer (pH 5.95) and methanol completed the synthesis of chelator **7**.

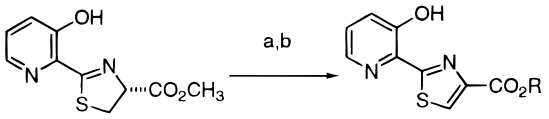
Scheme 4. Synthesis of 2-(4-Carboxy-2-hydroxyphenyl)-4,5-dihydrothiazole-4(*S*)-carboxylic Acid (**7**)[a]



18 **7**

[a] Reagents: (a) $CH_3CH_2NO_2/CH_3COONa/CH_3COOH$; (b) D-cysteine/$CH_3OH$/phosphate buffer/70 °C/23 h.

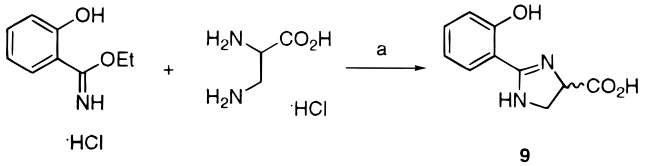
Scheme 5. Synthesis of 2-(3-Hydroxypyridin-2-yl)thiazole-4-carboxylic Acid (**8**)[a]



19 R = CH_3
8 R = H

[a] Reagents: (a) MnO_2/CH_2Cl_2; (b) CH_3OH/NaOH; 85% H_3PO_4.

Scheme 6. Synthesis of 4,5-Dihydro-2-(2-hydroxyphenyl)-1*H*-imidazole-4-carboxylic Acid (**9**)[a]



9

[a] Reagents: (a) NEt_3/CH_3OH.

The synthesis of the thiazole **8** was accomplished by dehydrogenation of the methyl ester of (*R*)-desmethyldesferrithiocin[20] with manganese dioxide[26] in CH_2Cl_2, generating **19**, which was saponified to the aromatized analogue of desferrithiocin, **8**[27] (Scheme 5).

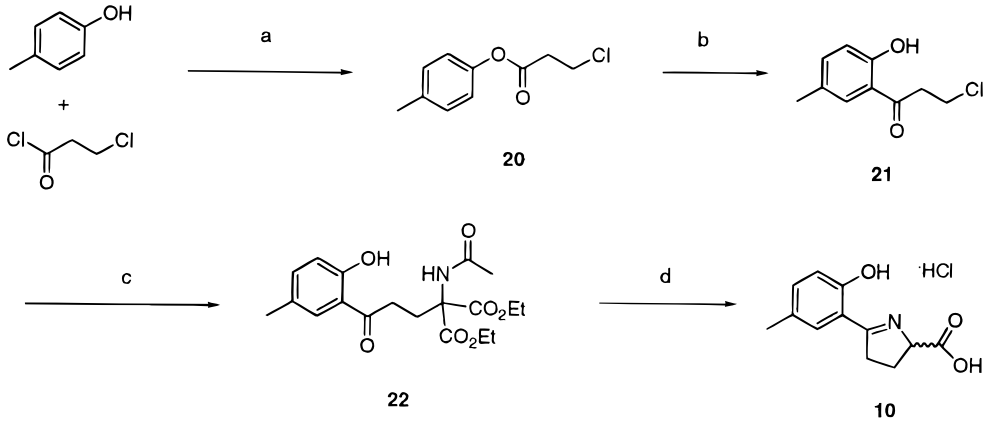
The synthesis of **9** was carried out by cyclocondensation of ethyl 2-hydroxybenzimidate[28] with 2,3-diaminopropionic acid in methanol/triethylamine in 53% yield (Scheme 6). Chelator **9** could also be depicted as the isomeric imine[29] since unsymmetrical N,N′-disubstituted amidines form a single tautomeric compound.[30]

Racemic 5′-methyldesazadesmethyldesthiadesferrithiocin (**10**) was generated by first acylating *p*-cresol with 3-chloropropionyl chloride in pyridine, resulting in **20** (Scheme 7). When **20** was heated with aluminum chloride at 90 °C for 1 h, a Fries rearrangement gave β-chloro ketone **21**. C-Alkylation of diethyl acetamidomalonate (NaOEt/EtOH) with chloride **21** produced open-chain intermediate **22**. Refluxing diester acetamide **22** with concentrated HCl for 15 h effected cyclization to **10** in high yield.[31] Through use of this efficient route, imine chelator **10** was generated and stored in multigram quantities as its hydrochloride salt.

Ligand **11** was synthesized in 54% yield by cyclocondensation of D-cysteine with 2-hydroxyphenylacetonitrile[32] in phosphate buffer (pH 5.95)/CH_3OH (Scheme 8).

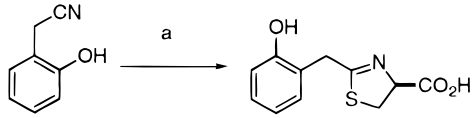
Compound **12**, the next higher homologue of **3**, was generated from 3(*S*)-amino-4-mercaptobutanoic acid (**26**)[33] and 2-cyanophenol in methanolic phosphate buffer, albeit in only 8% yield (Scheme 9). The β-amino acid **26** was prepared in four steps from *N*-t-BOC-β-*tert*-butyl-L-aspartic acid *N*-hydroxysuccinimide ester, which was first reduced to alcohol **23**[34] with $NaBH_4$ in THF.[35] After activation of **23** to **24** with *p*-toluenesulfonyl

Scheme 7. Synthesis of 3,4-Dihydro-5-(2-hydroxy-5-methylphenyl)-2*H*-pyrrole-2-carboxylic Acid (**10**)[a]



[a] Reagents: (a) pyr/CH_2Cl_2; (b) $AlCl_3$/90 °C/1 h; (c) $AcNHCH(CO_2Et)_2$/NaOEt/EtOH; (d) 12 N HCl/reflux/15 h.

Scheme 8. Synthesis of 4,5-Dihydro-2-(2-hydroxyphenylmethyl)thiazole-4(*S*)-carboxylic Acid (**11**)[a]



11

[a] Reagents: (a) D-cysteine/CH_3OH/phosphate buffer/reflux/25 h.

chloride in pyridine, a nucleophilic displacement with potassium thioacetate[36] in DMF resulted in fully protected amino acid **25**, which was unmasked to the thiol amino acid **26** in refluxing 6 N HCl.

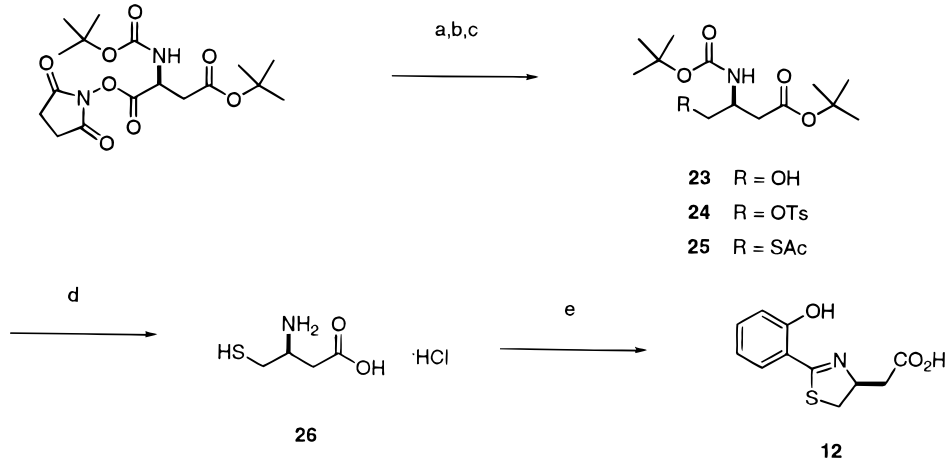
Chelator **13,** the bis-homologue of **3**, was made from cyclocondensation of 4(*S*)-amino-5-mercaptopentanoic acid (**30**)[33,36] with 2-cyanophenol in methanolic phosphate buffer in 35% yield; thus, this route to **13** is practical (Scheme 10). The γ-amino acid **30** was prepared in four steps from γ-*tert*-butyl-*N*-BOC-L-glutamate, which was activated as a mixed anhydride (ethyl chloroformate/triethylamine/THF) and selectively reduced to alcohol **27** with $NaBH_4$ in aqueous THF.[37] Activation of **27** by conversion to **28** with *p*-toluenesulfonyl chloride in pyridine and reaction of **28** with potassium thioacetate in DMF resulted in triprotected amino acid **29**, which was transformed to the thiol amino acid **30** in refluxing 6 N HCl.

The syntheses of quinoline desmethyldesferrithiocins 4,5-dihydro-2-(3-hydroxyquinolin-2-yl)thiazole-4(*R*)-carboxylic acid (**14**) and 4,5-dihydro-2-(3-hydroxyquinolin-2-yl)thiazole-4(*S*)-carboxylic acid (**15**) were achieved in overall yields of 40% and 32%, respectively (Scheme 11). The key step was accomplished by the cyclocondensation of L- or D-cysteine, respectively, with 3-hydroxy-2-cyanoquinoline (**33**) in phosphate buffer (pH 5.95) and methanol. 3-Aminoquinoline was converted to 3-hydroxyquinoline (**31**) upon heating with aqueous sodium bisulfite for 3 days. N-Oxidation of **31** with the urea/H_2O_2 adduct (UHP)−phthalic anhydride system furnished *N*-oxide **32**, which was transformed to cyano derivative **33** by heating with trimethylsilyl cyanide (3−4 equiv),[38] generated in situ from potassium cyanide and chlorotrimethylsilane, in triethylamine and DMF.

Biological Evaluations

The iron-clearing properties of the compounds were tested both in a non-iron-overloaded, bile-duct-cannulated rat model and in an iron-overloaded *Cebus apella* monkey model. The non-iron-overloaded, bile-duct-cannulated rat model[20,22,39] represents a very useful and rapid initial screen of potential iron chelators. The procedures employed in this model allow us to measure the total amount of iron cleared, the rate at which various chelators induce iron clearance in the bile and

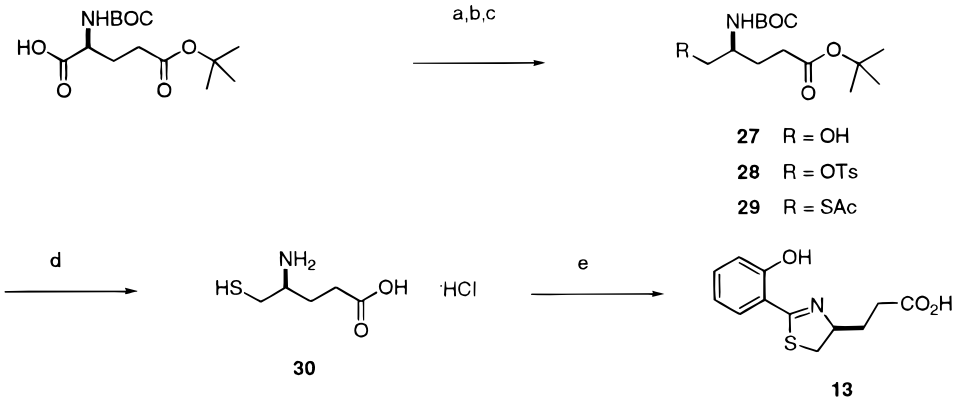
Scheme 9. Synthesis of 4,5-Dihydro-2-(2-hydroxyphenyl)thiazole-4(*S*)-acetic Acid (**12**)[a]



26 **12**

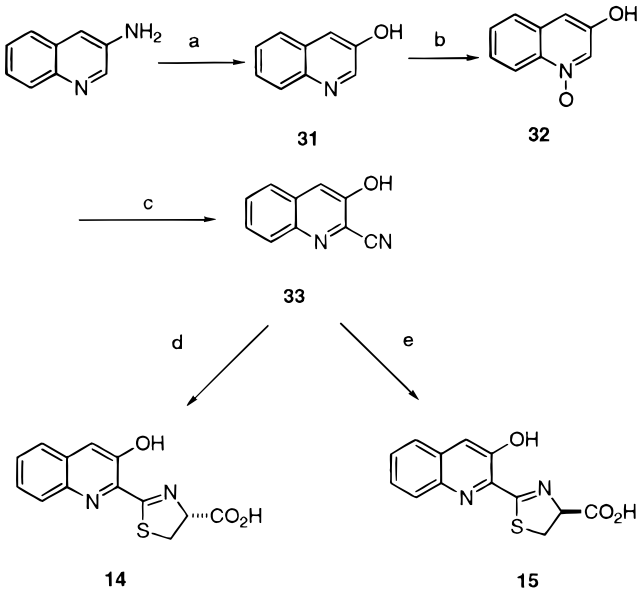
[a] Reagents: (a) $NaBH_4$/THF; (b) TsCl/pyr; (c) CH_3COSK/DMF; (d) 6 N HCl/130 °C; (e) 2-cyanophenol/phosphate buffer/MeOH/50 °C/1 day.

Scheme 10. Synthesis of 4,5-Dihydro-2-(2-hydroxyphenyl)thiazole-4(*S*)-propanoic Acid (**13**)[a]



[a] Reagents: (a) $ClCO_2Et/NEt_3$/THF, $NaBH_4$ (aq); (b) TsCl/pyr; (c) CH_3COSK/DMF; (d) 6 N HCl/130 °C; (e) 2-cyanophenol/phosphate buffer/MeOH/45 °C/2.5 days.

Scheme 11. Synthesis of 4,5-Dihydro-2-(3-hydroxyquinolin-2-yl)thiazole-4(*R*)-carboxylic Acid (**14**) and 4,5-Dihydro-2-(3-hydroxyquinolin-2-yl)thiazole-4(*S*)-carboxylic Acid (**15**)[a]



[a] Reagents: (a) $NaHSO_3$/NaOH; (b) UHP/phthalic anhydride/CH_2Cl_2; (c) TMSCl/KCN (3−4 equiv each)/NEt_3/DMF; (d) L-cysteine/phosphate buffer (pH 5.95)/CH_3OH/60 °C/1 day; (e) D-cysteine/phosphate buffer (pH 5.95)/CH_3OH/60 °C/1 day.

urine, and the relative biliary versus urinary iron excretion. Whereas the interruption of enterohepatic circulation caused by cannulation of the bile duct may lead to an overestimation of fecal iron excretion, if the ligand of interest does not induce iron clearance in the bile or the urine, then additional investigations are unnecessary. This measurement is a good "first-line" assessment of a chelator's efficacy, and many chelators have appeared outstanding in rodents. Nevertheless, these ligands have failed at the clinical level. Because of the many similarities of the iron-overloaded *C. apella* monkey to humans,[40,41] it serves as an intermediate screen for evaluating iron chelators before human studies.

The biological results will be separated into two sections, rodent data and primate data, because of the large number of compounds evaluated. Within each section, the compounds will be grouped according to the kinds of structural alterations in the desferrithiocin framework they represent: addition of electron-donating or -withdrawing groups to the aromatic ring, replacement of the thiazoline sulfur by nitrogen or a methylene, separation of donor sites, or benz-fusion.

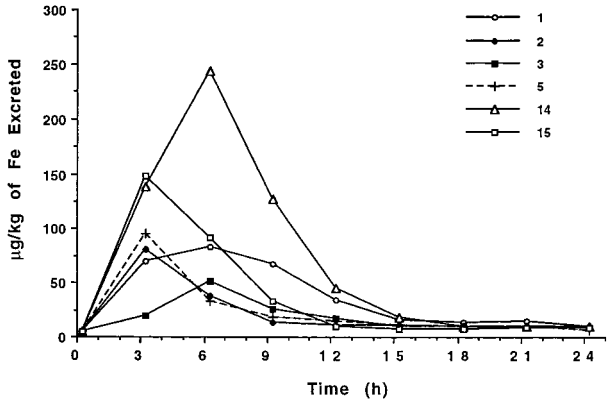


Figure 1. Chelator-induced iron excretion in the bile of the bile-duct-cannulated rats over time to ligands **1**, **2**, **3**, **5**, **14**, and **15** administered po at 150 μmol/kg.

Chelator-Induced Iron Clearance in Rodents. When assessing the effects of structural alterations of the desferrithiocin skeleton (Table 1), three earlier compounds should also be considered for comparative purposes: **1**, **2**, and **3**. In Table 1, the iron-clearing efficiencies of the compounds in rodents are shown, along with the relative fractions excreted in the bile and in the urine. Where relevant, biliary iron clearance vs time curves are provided (Figure 1).

In the first family of compounds, the aromatic ring of desazadesmethyldesferrithiocin was substituted with electron-donating and -withdrawing groups. Three different functional groups were employed: hydroxyl, methoxyl, and carboxyl. The additional substituents were introduced at the 3- or 4-position of the aromatic ring of desazadesmethyldesferrithiocin. The relationship of these substituents regarding the electronics of the thiazoline nitrogen is an interesting one. Introduction of a hydroxyl at the 3-position is electron-withdrawing by induction at the thiazoline nitrogen; the same is true when a methoxyl is put at this position. A comparison of the 3-hydroxylated ligand (**4**) with the 3-methoxylated analogue (**6**) attests to possible confounding effects by the potential auxiliary coordination site in **4**. In both cases, the groups are electron-withdrawing; however, the methoxyl group cannot serve as a ligand donor.

Table 1. Summary of Evaluations of the Desferrithiocins

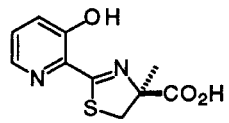
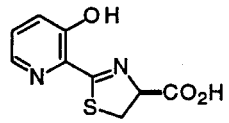
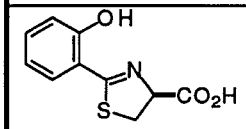
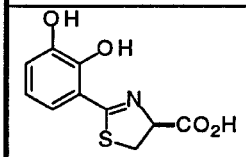
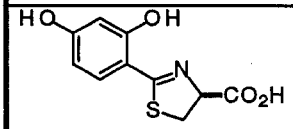
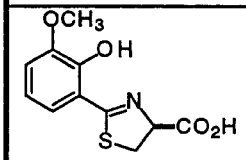
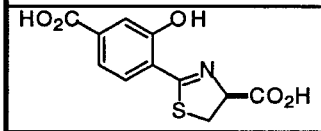
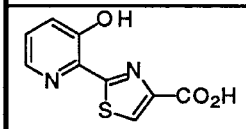
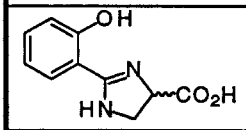
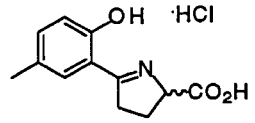
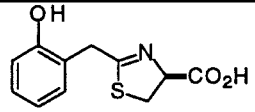
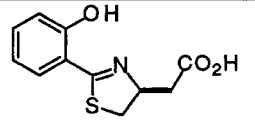
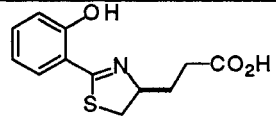
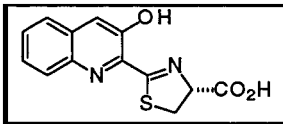
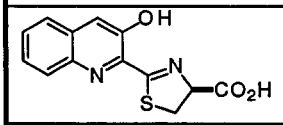
compound structure	number	efficiency % (rat)[a]	efficiency % (monkey)[b]
	**1**	5.5 ± 3.2 [93 bile, 7 urine]	150 μmol/kg 16.1 ± 8.5 (po) [78 stool, 22 urine]
	**2**	2.4 ± 0.6 (po) [82 bile, 18 urine]	150 μmol/kg 4.8 ± 2.7 (po) [48 stool, 52 urine] 300 μmol/kg 8.0 ± 2.5 (po) [42 stool, 58 urine]
	**3**	1.4 ± 0.6 (po) [100 bile, 0 urine]	300 μmol/kg 12.4 ± 7.6 (po) [90 stool, 10 urine]
	**4**	≤ 0.5 (po) ≤ 0.5 (sc)	N.D.[c]
	**5**	2.4 ± 0.9 (po) [100 bile, 0 urine]	150 μmol/kg 4.2 ± 1.4 (po) [70 stool, 30 urine]
	**6**	0.9 ± 0.3 (po) [100 bile, 0 urine] 0.5 ± 0.9 (sc) [96 bile, 4 urine]	N.D.
	**7**	≤ 0.5 (po) ≤ 0.5 (sc)	N.D.
	**8**	≤ 0.5 (po)	N.D.
	**9**	≤ 0.5 (po) ≤ 0.5 (sc)	N.D.
	**10**	≤ 0.5 (po) ≤ 0.5 (sc)	N.D.
	**11**	≤ 0.5 (po) ≤ 0.5 (sc)	N.D.
	**12**	≤ 0.5 (po)	N.D.
	**13**	≤ 0.5 (po)	N.D.

Table 1 (Continued)

compound structure	number	efficiency % (rat)[a]	efficiency % (monkey)[b]
	**14**	12.3 ± 3.2 (po) [100 bile, 0 urine]	75 μmol/kg ≤ 0.5 (po)
	**15**	5.9 ± 3.2 (po) [90 bile, 10 urine]	150 μmol/kg 3.5 ± 1.8 (po) [68 stool, 32 urine]

[a] In the rats, doses were 150 μmol/kg via the route indicated. The net iron excretion was calculated by subtracting the iron excretion of control animals from the iron excretion of treated animals. Efficiency of chelation is defined as (net iron excretion)/(total iron-binding capacity of chelator) administered, expressed as a percent. Directly beneath the efficiency calculation is the percentage breakdown of iron excretion in the bile and urine, respectively. [b] In the monkeys, the doses and routes were as shown in the table. The efficiency of each compound was calculated by averaging the iron output for 4 days before the administration of the drug, subtracting these numbers from the 2-day iron clearance after the administration of the drug, and then dividing by the theoretical output; the result is expressed as a percent. Directly beneath the efficiency calculation is the percentage breakdown of iron excretion in the stool and urine, respectively. [c] N.D., not determined.

When the hydroxyl group is in the 4-position (**5**), it donates electrons to the nitrogen, while a carboxyl group at this carbon (**7**) withdraws electrons from the nitrogen. Within this family of compounds (**4**−**7**), only one, 4′-hydroxydesazadesmethyldesferrithiocin (**5**), cleared iron from the rats. The efficiency was 2.4 ± 0.9% when administered po, and 100% of this excretion was biliary. Interestingly, only when the position 4 substituent was electron-donating to the nitrogen as in **5** was the compound effective; compound **7**, which contains an electron-withdrawing carboxyl at this position, was ineffective (Table 1).

Modifications of the thiazoline ring included oxidation of desmethyldesferrithiocin (**2**) to the thiazole (**8**), replacement of the sulfur of **3** with a nitrogen to yield an imidazoline (**9**), and introduction of a methylene group in place of the sulfur (**10**). In the case of **10** the 5′-methyl compound was prepared in place of the unsubstituted desazadesmethyldesthiadesferrithiocin analogue simply because of synthetic facility. The latter two compounds have an asymmetric center that is racemized; this could confound the biological findings in that it would be uncertain whether one enantiomer in the mixture was the greater contributor to the iron clearance observed. In fact, one might argue that the presence of an enantiomer might result in antagonistic activity. However, it was decided that had notable activity been seen in the racemates of these compounds, the pure enantiomers would have been synthesized and evaluated. Of the three thiazoline ring-modified systems (**8**−**10**) described, none performed well (Table 1).

Three ligands in which the donor atoms were separated were constructed: one in which a methylene group was placed between the aromatic and thiazoline rings (**11**), one in which a methylene group was placed between the thiazoline ring and the carboxyl group (**12**), and one in which two methylene groups were inserted between the thiazoline ring and the carboxyl group (**13**). When compared in the rodent with unmodified desazadesmethyldesferrithiocin (**3**), all three of these did poorly with efficiencies of ≤0.5% when given orally. Subcutaneous administration, which improves the iron clearance of certain ligands,[42] did not affect the performance of **11** (Table 1). Because of these results, these compounds were not carried forward into the primates or toxicity studies.

The benz-fused ligands evaluated were active. (*R*)-Quinoline **14** was exceptionally effective in rodents with an efficiency of 12.3 ± 3.2% (Table 1). Essentially all of the iron was excreted in the bile. However, (*S*)-enantiomer **15** was considerably less so; the efficiency was 5.9 ± 3.2% with 90% of the iron excreted in the bile and 10% in the urine. The principal interest in these systems is related to the potential of increased lipophilicity, which may provide a more protracted increase in iron excretion relative to desmethyldesferrithiocin. An examination of the quinoline desferrithiocin analogue-induced iron excretion in the bile (Figure 1) reveals that the iron clearance time for (*R*)-enantiomer **14**, 12 h, was longer than that of the (*S*)-enantiomer **15** or the parent compound **3** (9 h). Interestingly, the point of peak iron excretion for **14**, 6 h, is the same as that for **3** and **1**, yet the peak for both **15** and the less lipophilic **5** is earlier, at 3 h, similar to that of **2** (Figure 1).

Chelator-Induced Iron Clearance in Primates. The decision to move a compound forward into evaluation in the primate model is based solely on its performance in the initial screen in rodents. Of the four aromatic ring-modified analogues evaluated (**4**−**7**), only **5** was considered for primate studies. It performed well in the primate model; the efficiency was 4.2 ± 1.4% when given po at a dose of 150 μmol/kg (Table 1). Iron clearance was largely in the stool, 70% vs 30% in the urine. This is comparable to the efficiency of **2**, 4.8 ± 2.7%, when given at the same dose. However, when **2** and **3** were given at 300 μmol/kg, **2**, at 8.0 ± 2.5% with 42% of the iron in the stool, was not as efficient as the parent **3**, at 12.4 ± 7.6% with 90% of the iron in the stool. This suggests that **5** may not be as efficient as **3**; unfortunately, **3** is unacceptably toxic.

Because none of the thiazoline-modified or "donor-separated" ligands were effective in the rodents, they were not assessed in the primates. Benz-fused systems **14** and **15** were both evaluated in the primates. (*R*)-Enantiomer **14** performed poorly, whereas (*S*)-enantiomer **15**, the less effective enantiomer in the rodents, did clear iron from the primates. At a dose of 150 μmol/kg po, the efficiency of this ligand was 3.5 ± 1.8%; 68% of the iron excreted was found in the stool and 32% in the urine.

Toxicity. If the ligands were shown to be effective in the primates, a 10-day toxicity trial was initiated in

Table 2. Chronic Toxicity Evaluations of Effective Desferrithiocin Analogues

compd no.	dose/day[a] (μmol/kg) × no. of days	no. of deaths	comments
1	384 × 10	5/5	All animals dead by day 5: severe nephrotoxicity.[22]
2	384 × 10	0/5	All histopathologies normal; well-tolerated for the 10-day test period.[22]
	250 × 30	0/5	All histopathologies normal; well-tolerated for the 30-day test period.
3	384 × 10	5/5	All animals dead by day 6: severe GI toxicity.
5	384 × 10	0/5	All histopathologies normal; well-tolerated for the 10-day test period.
	250 × 30	0/5	All histopathologies normal; well-tolerated for the 30-day test period.

[a] The compounds were given to rats po at the doses and test periods shown.

rodents. The drugs were administered orally to normal rats by gavage once a day for 10 days, or until the animals expired. The data for **1** and **2**, while historical,[22] are included in Table 2 for the reader's convenience. Desferrithiocin (**1**), the natural product, was a very effective iron chelator when given orally to rats or monkeys. However, the compound also presented with significant side effects, particularly nephrotoxicity, when administered chronically to rats. At a dose of 384 μmol/kg/day, 5 out of 5 animals were dead within 10 days (Table 2). Abstraction of the methyl group from the thiazoline ring of **1** to yield **2** resulted in a compound that was far less toxic. When administered po at a dose of 384 μmol/kg/day for 10 days (3840 μmol/kg total dose), **2** was well-tolerated, and no deaths were observed. Extensive histological evaluation revealed no drug-related abnormalities.

However, removal of both the aromatic nitrogen and the thiazoline methyl group to generate **3** resulted in a ligand that, while very effective at clearing iron, was also highly toxic. In contrast to the nephrotoxicity of desferrithiocin (**1**), the primary toxicity was gastrointestinal in nature. At a daily dose of 384 μmol/kg, due to the rapidly deteriorating condition of the animals, the experiment was stopped after the fifth dose. All of the animals were dead by day 6. At necropsy, the stomachs of all of the animals were hemorrhagic and grossly distended with gas and fluid; the stomach walls were paper thin. The intestines were also hemorrhagic, and pressure necrosis of the spleen, from the grossly distended stomach, was noted in two of the animals. As with the corresponding (*R*)-enantiomer,[22] this compound is a severe gastrointestinal irritant.

Interestingly, when an electron-donating hydroxyl group is added to position 4 of the aromatic ring of **3** as in **5**, the gastrointestinal toxicity problem found in **3** was absent; no deaths occurred when this compound was given at 384 μmol/kg/day for 10 days (3840 μmol/kg total dose) (Table 2). Histopathological analysis of tissues (see Experimental Section for a list) revealed no drug-related abnormalities when compared to Cremophor/water-treated controls.

Since analogues **2** and **5** appeared to be virtually nontoxic in the 10-day toxicity study, these ligands were further evaluated under an expanded dosing protocol. In the longer term study, the rats (n = 5/group) were given 30 daily doses of analogues **2** and **5** over a 32-day period. The compounds were administered orally by gavage as a suspension in water at 250 μmol/kg/dose, for a total dose of 7500 μmol/kg. A necropsy was performed 1−2 days after the last dose of the drug had been given, and extensive tissues (see Experimental Section) were examined by an outside pathologist.

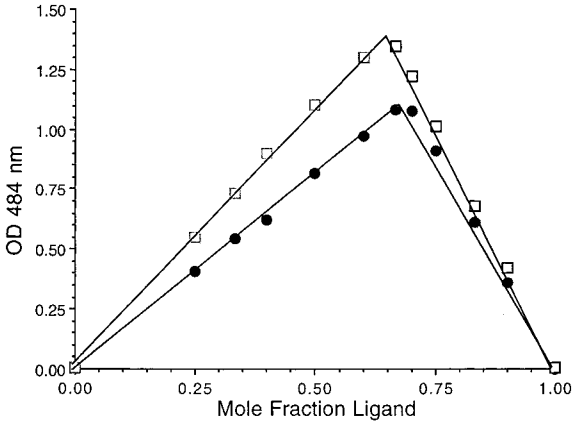


Figure 2. Job's plots of **3** and **5**. The plots are superimposed on each other to show the similarity between the two graphs. Solutions containing different ligand: Fe(III) ratios were prepared so that [ligand] + [Fe(III)] = 0.5 mM for **5** and 1.0 mM for **3**. Compound **3** is indicated by the open squares, and analogue **5** is shown by the filled circles. The data points were fitted to the mole fractions (1) from 0 to 0.667 and (2) from 0.667 to 1.000; r^2 = 0.993−1.000. Theoretical mole fraction maximum for a 2:1 ligand−Fe complex is 0.667. The observed maximum for **3** is 0.68; the observed maximum for **5** is 0.655.

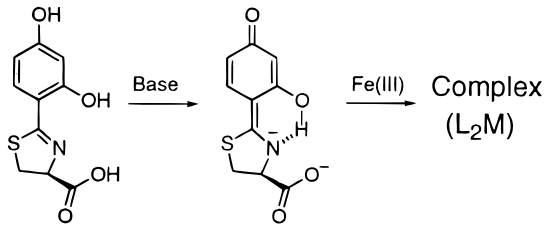


Figure 3. Proposed quinone configuration of ligand **5**. Addition of base may result in a quinone structure such that the electrons are redistributed to the thiazoline nitrogen and carboxylate oxygen; the ligand-to-metal complex is 2:1 (L_2M).

Histopathological analysis revealed no difference between the tissues from control vs analogue-treated animals.

Metal Complex Stoichiometry. In an attempt to determine whether there was anything unusual about the nature of the Fe(III) complex of **3** versus the Fe(III) complex of **5**, we ran Job's plots on both of these ligands. Not surprisingly, like **1** and **2**, both these desaza chelators formed 2:1 complexes with Fe(III) (Figure 2). Our principal concern was related to the 4-hydroxyl of analogue **5** and its role in metal complex formation. It is possible that the 4-hydroxyl could be in a quinone configuration on complexation (Figure 3). How this configuration might affect the metal chelation is unclear. We are currently investigating both the thermodynamics of formation and the structure of the complex.

Partition Studies. In a search for the origin of the differences in toxicity between the natural product **1** and

Table 3. Octanol−Water Distribution Coefficients[a]

compd no.	oct−H_2O K_{app}	log *P*
1	0.017	−1.77
2	0.013	−1.87
3[b]	0.12	−0.93
5	0.063	−1.2

[a] Data are expressed as distribution coefficients uncorrected for partial ionization of the acids and are all measured in TRIS buffer, pH 7.4. The measurements were done using a "shake flask" direct measurement. [b] The (*R*)-enantiomer of **3**, sodium salt, was tested.

analogues **3** and **5**, we ran a simple partition study. The measurement was conducted in an octanol−water−TRIS buffer mixture. While there are small differences in log *P* (Table 3), it would be difficult to attribute the tremendous differences in toxicity between these ligands on the small differences in partition values.

Conclusion

It is now clear that there are very significant boundary conditions on the kinds of structural alterations one can make on the desferrithiocin framework which are compatible with maintaining its iron-clearing properties. It is equally certain that the toxicity profiles of the desferrithiocins are just as sensitive to structural changes.

At the level of the bile-duct-cannulated rodent model, desazadesmethyldesferrithiocin (**3**) was less effective than either desferrithiocin (**1**) or desmethyldesferrithiocin (**2**) and, in fact, more toxic than **2**. However, ligand **3** has the singular advantage in that, owing to the absence of a picolinic acid fragment, **3** and its analogues are easy to assemble. Thus, structure−activity studies focused on understanding and ameliorating toxicity are easier to carry out.

Again in the rodent, taking (*S*)-desazadesmethyldesferrithiocin (**3**) as the parent compound, introduction of a hydroxyl or methoxyl in the 3-position of the aromatic ring (**4** and **6**, respectively) or a carboxyl in the 4-position of the aromatic ring (**7**), all inductively electron-withdrawing groups, substantially reduces the compound's iron-clearing capabilities. Introduction of a hydroxyl in the 4-position, an electron-donating group, results in a ligand (**5**) which is comparable in effectiveness in rodents to **3** and is far less toxic than **3**. In the primates, **5** does not perform as well as **3**, but the toxicity of **3** renders this a moot point.

Modifications of the thiazoline ring of **2** or **3** obliterate the iron-clearing properties as observed in the rodents. Either oxidation of the thiazoline ring to a thiazole (**8**) or replacement of the sulfur atom by nitrogen (**9**) or a methylene group (**10**) results in ineffective compounds. Any attempt to separate the chelating sites in **3** by inserting methylenes also abolishes the iron-clearing abilities as exemplified by analogues **11**, **12**, and **13**. Finally, benz-fusion results in a pair of quinoline ligands: the (*R*)-enantiomer **14** performs exceptionally well in rodents but poorly in primates; the (*S*)-enantiomer **15** clears iron from both rodents and primates.

From our perspective, the most interesting finding in the present study is related to the profound effect of introducing a hydroxyl group at the 4-position of the aromatic ring of desazadesmethyldesferrithiocin on the compound's toxicity. We were unable to demonstrate any significant relationship in the differences in partition values and changes in toxicity between the hydroxylated and parent molecule. Although the stoichiometry of the iron complexes for both ligands **3** and **5** is identical, we have not yet explored the role of the 4′-hydroxyl on either the stability of the complex or how it affects the ability of the complex to participate in Fenton chemistry. The 4′-hydroxyl could form a quinone-like structure (Figure 3), in which the charge on the nitrogen is enhanced for donation to Fe(III). These issues are currently under consideration.

Experimental Section

Chelators **1**, **2**, and **3** were prepared by methodology developed in these laboratories.[20] *N*-t-BOC-*β*-*tert*-butyl-L-aspartic acid *N*-hydroxysuccinimide ester and *N*-t-BOC-L-glutamic acid *γ*-*tert*-butyl ester were obtained from Sigma Chemical Co. (St. Louis, MO). All other reagents were purchased from Aldrich Chemical Co. (Milwaukee, WI) and were used without further purification. Distilled solvents and glassware that had been presoaked in 3 N HCl for 15 min were employed for reactions involving chelators. Fisher Optima grade solvents were routinely used, and DMF was distilled. Organic extracts were dried with sodium sulfate. Phosphate buffer was made up to 0.1 M at a pH of 5.95.[43] Silica gel 32−63 (40 *μ*m "flash") from Selecto, Inc. (Kennesaw, GA), silica gel 60 (70−230 mesh) from EM Science (Darmstadt, Germany), or Lipophilic Sephadex LH-20 from Sigma Chemical Co. was used for column chromatography. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Proton NMR spectra were run at 90 or 300 MHz in deuterated organic solvents ($CDCl_3$ not indicated) or in D_2O with chemical shifts in parts per million downfield from tetramethylsilane or 3-(trimethylsilyl)propionic-*2,2,3,3-d*$_4$ acid, sodium salt, respectively. Coupling constants (*J*) are in Hertz. Mass spectra were carried out on a Fennigan 4516 instrument. Optical rotations were run at 589 nm (sodium D line) with *c* as g of compound/100 mL of solution. Elemental analyses were performed by Atlantic Microlabs (Norcross, GA). Cremophor RH-40 was obtained from BASF (Parsippany, NJ). Sprague−Dawley rats were purchased from Charles River (Wilmington, MA). Nalgene metabolic cages, rat jackets, and fluid swivels were purchased from Harvard Bioscience (South Natick, MA). Intramedic polyethylene tubing (PE 50) was obtained from Fisher Scientific (Pittsburgh, PA). *C. apella* monkeys were obtained from World Wide Primates (Miami, FL). Ultrapure salts were obtained from Johnson Matthey Electronics (Royston, U.K.). Imferon, an iron dextran solution, was obtained from Fisons (Bedford, MA).

4,5-Dihydro-2-(2,3-dihydroxyphenyl)thiazole-4(*S*)-carboxylic Acid (4). $NaHCO_3$ (0.71 g, 8.45 mmol) was added in portions to a solution of D-cysteine hydrochloride monohydrate (1.47 g, 8.40 mmol) in water (20 mL). Phosphate buffer (40 mL), *tert*-butyl alcohol (60 mL), and ethyl 2,3-dihydroxybenzimidate[24] (1.21 g, 6.65 mmol) were added, and the mixture was stirred at 60 °C under Ar for 16 h. Volatile components were removed under reduced pressure, and saturated $NaHCO_3$ (100 mL) was added. The aqueous layer was washed with EtOAc (3 × 50 mL), acidified with 1 N HCl to pH 3, and extracted with EtOAc (3 × 50 mL). The combined organic extracts were concentrated in vacuo. Purification of the residue on a Sephadex LH-20 column, eluting with 4% EtOH/toluene, yielded 474 mg (30%) of **4** as a yellow solid, mp 88 °C: $[\alpha]^{22}_D$ +38.7° (*c* 1.00, CH_3OH); NMR (CD_3OD) *δ* 3.67 (m, 2 H), 5.41 (t, 1 H, *J* = 9), 6.75 (t, 1 H, *J* = 8), 6.95 (m, 2 H). Anal. ($C_{10}H_9NO_4S$) C, H, N.

4,5-Dihydro-2-(2,4-dihydroxyphenyl)thiazole-4(*S*)-carboxylic Acid (5). D-Cysteine hydrochloride monohydrate (6.80 g, 38.7 mmol) was added to a solution of **16** (3.50 g, 25.9 mmol) in a mixture of degassed CH_3OH (105 mL) and phosphate buffer (70 mL). Sodium bicarbonate (3.25 g, 38.7 mmol) was carefully added, and the mixture was stirred at 70 °C under Ar for 54 h. Volatile components were removed under reduced

pressure, and the solution was acidified with 1 N HCl to pH 2. The resulting brown precipitate was filtered, and the solid was washed with H_2O (40 mL) and EtOH (20 mL). The crude product was dissolved in saturated $NaHCO_3$ (700 mL), and the aqueous solution was washed with EtOAc (2 × 200 mL). The aqueous layer was filtered through a fine frit and acidified with 1 N HCl to pH 2. The precipitated product was collected. The latter aqueous layer was extracted with EtOAc (4 × 400 mL), and the extracts were concentrated in vacuo. The residue was combined with the precipitated product and dried under high vacuum at 40 °C for 12 h to give 4.08 g (66%) of **5** as a yellow solid, mp 266−268 °C dec (lit. mp of (*R*)-enantiomer 261−262 °C[44]): $[\alpha]^{23}_D$ +27.0° (*c* 1.02, DMF); NMR (DMSO-d_6) δ 3.61 (m, 2 H), 5.38 (dd, 1 H, J = 7.2, 9.4), 6.31 (d, 1 H, J = 2.3), 6.38 (dd, 1 H, J = 2.3, 8.6), 7.25 (d, 1 H, J = 8.6), 10.25 (br s, 1 H), 12.60 (br s, 1 H), 13.15 (br s, 1 H). Anal. ($C_{10}H_9NO_4S$) C, H, N.

4,5-Dihydro-2-(2-hydroxy-3-methoxyphenyl)thiazole-4(*S*)-carboxylic Acid (6). D-Cysteine (2.78 g, 22.9 mmol) was added to a solution of **17** (2.28 g, 15.3 mmol) in a mixture of degassed CH_3OH (30 mL) and phosphate buffer (20 mL). The mixture was stirred at 70 °C under Ar for 17 h. Volatile components were removed under reduced pressure; the suspension was acidified with 1 N HCl to pH 2.5 and extracted with EtOAc (3 × 200 mL). Solvent was removed by rotary evaporation. Purification of the residue on a Sephadex LH-20 column, eluting with 4% EtOH/toluene, yielded 3.50 g (90%) of **6** as a yellow solid, mp 65 °C: $[\alpha]^{22}_D$ +31.8° (*c* 1.15, CH_3OH); NMR (CD_3OD) δ 3.69 (d, 2 H, J = 9), 5.42 (t, 1 H, J = 9), 6.87 (t, 1 H, J = 8), 7.09 (m, 2 H). Anal. ($C_{11}H_{11}NO_4S$) C, H, N.

2-(4-Carboxy-2-hydroxyphenyl)-4,5-dihydrothiazole-4(*S*)-carboxylic Acid (7). D-Cysteine (780 mg, 6.44 mmol) was added to a solution of **18** (700 mg, 4.29 mmol) in a mixture of degassed CH_3OH (30 mL) and phosphate buffer (20 mL). The pH was adjusted to 6 with 1 N NaOH, and the mixture was stirred at 70 °C under Ar for 23 h. Volatile components were removed under reduced pressure, and the suspension was acidified with 1 N HCl to pH 2. The aqueous layer was extracted with EtOAc (4 × 100 mL). The combined organic extracts were evaporated in vacuo. Purification of the residue on a Sephadex LH-20 column, eluting with 6% EtOH/toluene, gave 604 mg (53%) of **7** as a yellow solid, mp 240 °C dec: $[\alpha]^{22}_D$ +19.6° (*c* 1.02, CH_3OH); NMR (DMSO-d_6) δ 3.67 (dd, 1 H, J = 7.6, 11.4), 3.76 (dd, 1 H, J = 9.7, 11.4), 5.52 (dd, 1 H, J = 7.6, 9.7), 7.49 (m, 2 H), 7.60 (d, 1 H, J = 8.1), 12.60 (br s, 1 H), 13.30 (br s, 2 H). Anal. ($C_{11}H_9NO_5S$) C, H, N.

2-(3-Hydroxypyridin-2-yl)thiazole-4-carboxylic Acid (8). Compound **19** (1.00 g, 4.23 mmol) was stirred with 1 N NaOH (60 mL) and CH_3OH (80 mL) for 5 h at room temperature. After the bulk of the CH_3OH was removed by rotary evaporation, the concentrate was cooled to 0 °C and acidified to a pH of 2 with 85% H_3PO_4. The precipitate was filtered, washed with water (2×), and recrystallized with aqueous EtOH to give 0.80 g (85%) of **8** as a yellow solid: NMR (DMSO-d_6) δ 6.1 (br s, 2 OH), 7.35−7.60 (m, 2 H), 8.11−8.26 (m, 1 H), 8.50 (s, 1 H). Anal. ($C_9H_6N_2O_3S$) C, H, N.

4,5-Dihydro-2-(2-hydroxyphenyl)-1*H*-imidazole-4-carboxylic Acid (9).[29] To an ice-cooled solution of D,L-2,3-diaminopropionic acid monohydrochloride (141 mg, 1.0 mmol) and triethylamine (111 mg, 1.1 mmol) in CH_3OH (8 mL) was added a solution of ethyl 2-hydroxybenzimidate hydrochloride[28] (200 mg, 0.99 mmol) in CH_3OH (4 mL). The reaction mixture was refluxed for 5 h, and the solvent was removed in vacuo. Silica gel flash chromatography (CH_3OH:CH_2Cl_2 = 3:7) gave 108 mg (53%) of **9** as a colorless solid, mp 162 °C: NMR (DMSO-d_6) δ 3.86 (dd, 1 H, J = 7, 12), 3.40 (m, 1 H), 4.50 (dd, 1 H, J = 7, 11), 6.86 (m, 2 H), 7.35 (m, 1 H), 7.69 (m, 1 H); $C_{10}H_{10}N_2O_3$ MS (EI) 206 (M^+, 61%), 161 ($M^+ - CO_2H$, 100%).

3,4-Dihydro-5-(2-hydroxy-5-methylphenyl)-2*H*-pyrrole-2-carboxylic Acid Hydrochloride (10). Concentrated HCl (140 mL) was added to **22** (18.08 g, 48 mmol), and the reaction mixture was heated at reflux under a nitrogen balloon with periodic venting for 15 h. Solvent was removed under high vacuum, the residue was dissolved in H_2O (120 mL), and evaporation was repeated. Distilled H_2O (120 mL) was added to the solid, and solvent was removed by lyophilization to give 12.96 g (quantitative) of **10** as a green solid: NMR (D_2O) δ 2.30 (s, 3 H), 2.33−2.46 (m, 1 H), 2.67−2.82 (m, 1 H), 3.59−3.68 (m, 2 H), 5.10 (dd, 1 H, J = 10, 6), 7.04 (d, 1 H, J = 8), 7.52−7.58 (m, 1 H), 7.59−7.62 (m, 1 H). Anal. ($C_{12}H_{14}ClNO_3$) C, H, N.

4,5-Dihydro-2-(2-hydroxyphenylmethyl)thiazole-4(*S*)-carboxylic Acid (11). $NaHCO_3$ (1.90 g, 22.6 mmol) was added in portions to a solution of D-cysteine hydrochloride monohydrate (3.95 g, 22.5 mmol) in water (20 mL). Phosphate buffer (40 mL), degassed CH_3OH (60 mL), and 2-hydroxyphenylacetonitrile[32] (2.00 g, 15.0 mmol) were added, and the mixture was refluxed under Ar for 25 h. Volatile components were removed under reduced pressure, and saturated $NaHCO_3$ (100 mL) was added. The aqueous layer was washed with EtOAc (3 × 50 mL), acidified with 1 N HCl to pH 3, and extracted with EtOAc (3 × 50 mL). The combined organic extracts were evaporated in vacuo. Purification of the residue on a Sephadex LH-20 column, eluting with 15% EtOH in toluene, afforded 1.91 g (54%) of **11** as a colorless solid, mp 129 °C: $[\alpha]^{22}_D$ −13.7° (*c* 1.00, CH_3OH); NMR (CD_3OD) δ 2.88 (dd, 1 H, J = 5.7, 13.8), 2.96 (dd, 1 H, J = 4.8, 13.8), 3.54 (d, 1 H, J = 14.7), 3.65 (d, 1 H, J = 14.7), 4.63 (m, 1 H), 6.80 (m, 2 H), 7.12 (m, 2 H); HRMS calcd for $C_{11}H_{11}NO_3S$ (M^+) 237.0460, found 237.0476.

4,5-Dihydro-2-(2-hydroxyphenyl)thiazole-4(*S*)-acetic Acid (12). Degassed 6 N HCl (100 mL) was added to **25** (3.18 g, 9.53 mmol), and the reaction mixture was heated at reflux under a nitrogen balloon with periodic venting for 1 day. Solvent was removed under high vacuum, degassed H_2O (50 mL) was added, and evaporation was repeated to give 1.55 g (95%) of **26**,[33] which was used directly. Degassed phosphate buffer (55 mL) and distilled CH_3OH (86 mL) were added to **26** (1.54 g, 9.0 mmol) and 2-cyanophenol (1.04 g, 8.77 mmol). The pH was raised to 7 with 50% (w/w) NaOH (40 drops), and the reaction mixture was heated at 50 °C for 1 day under N_2. After the bulk of solvents were removed by rotary evaporation, 0.5 M citric acid (100 mL) was added, followed by extraction with EtOAc (5 × 20 mL). The organic extracts were washed with H_2O (25 mL) and brine (25 mL), and solvent was taken off in vacuo. Purification of the concentrate on a Sephadex LH-20 column, eluting with 3% EtOH/toluene, produced 0.17 g (8%) of **12** as a yellow solid, mp 157−158.5 °C: $[\alpha]^{21}_D$ −23.7° (*c* 1.34, CH_3OH); NMR δ 2.76 (dd, 1 H, J = 16, 8), 2.98 (dd, 1 H, J = 16, 6), 3.16 (dd, 1 H, J = 11, 7), 3.61 (dd, 1 H, J = 11, 8), 4.2 (br s, 2 H), 5.05−5.16 (m, 1 H), 6.85−7.03 (m, 2 H), 7.33−7.45 (m, 2 H). Anal. ($C_{11}H_{11}NO_3S$) C, H, N.

4,5-Dihydro-2-(2-hydroxyphenyl)thiazole-4(*S*)-propanoic Acid (13). Degassed phosphate buffer (45 mL) and distilled CH_3OH (50 mL) were added to **30** (1.58 g, 8.5 mmol) and 2-cyanophenol (0.953 g, 8.00 mmol). The pH was raised to 6 with 50% (w/w) NaOH (44 drops), and the reaction mixture was heated at 45 °C for 2.5 days under N_2. After the bulk of solvents were removed by rotary evaporation, the concentrate was acidified to a pH of 2 with aqueous citric acid and extracted with EtOAc (6 × 50 mL). The organic extracts were washed with H_2O (2×) and brine, and solvent was taken off in vacuo. Purification of the concentrate on a Sephadex LH-20 column, eluting with 2% EtOH/toluene, produced 0.701 g (35%) of **13** as a light-green solid, mp 108−108.5 °C: $[\alpha]^{21}_D$ −70.6° (*c* 0.622, $CHCl_3$); NMR δ 2.11 (q, 2 H, J = 8), 2.64 (t, 2 H, J = 7), 3.04 (dd, 1 H, J = 11, 9), 3.49 (dd, 1 H, J = 11, 9), 4.70−4.82 (m, 1 H), 6.84−7.01 (m, 2 H), 7.31−7.43 (m, 2 H). Anal. ($C_{12}H_{13}NO_3S$) C, H, N.

4,5-Dihydro-2-(3-hydroxyquinolin-2-yl)thiazole-4(*R*)-carboxylic Acid (14). Phosphate buffer (7 mL) was added to **33** (0.34 g, 2.00 mmol) and L-cysteine (0.73 g, 6.0 mmol) in distilled CH_3OH (20 mL). The reaction mixture was heated at 60 °C for 1 day, and the volatiles were removed in vacuo. The residue was taken up in $KHCO_3$ (30 mL), and the solid was filtered. The filtrate was extracted with Et_2O (3 × 20 mL), and the aqueous portion was acidified to a pH of 3 with 1 N HCl. Solid was filtered and dissolved in H_2O (10 mL), which

was then extracted with Et_2O (3 × 50 mL). The organic extracts were washed with H_2O (20 mL), and the volume of solvent was lowered to ca. 20 mL under reduced pressure. The concentrate was combined with hexane (20 mL); filtration gave 0.5 g (91%) of **14** as a solid, mp 170 °C: NMR (acetone-d_6) δ 3.69−3.82 (m, 2 H), 5.71 (dd, 1 H, J = 10, 8), 7.57−7.65 (m, 2 H), 7.78 (s, 1 H), 7.83−7.89 (m, 1 H), 7.96−8.03 (m, 1 H). Anal. ($C_{13}H_{10}N_2O_3S$) C, H, N.

4,5-Dihydro-2-(3-hydroxyquinolin-2-yl)thiazole-4(*S*)-carboxylic Acid (15). D-Cysteine (0.73 g, 6.0 mmol) and **33** (0.34 g, 2.00 mmol) were reacted by the method of **14** to furnish 0.4 g (73%) of **15** as a solid, mp 170 °C: $[\alpha]^{22}_D$ −16.9° (*c* 0.90, DMF); NMR (acetone-d_6) δ 3.70−3.84 (m, 2 H), 5.72 (dd, 1 H, J = 10, 8), 7.58−7.66 (m, 2 H), 7.80 (s, 1 H), 7.85−7.92 (m, 1 H), 7.97−8.04 (m, 1 H). Anal. ($C_{13}H_{10}N_2O_3S$) C, H, N.

2,4-Dihydroxybenzonitrile (16).[45] A mixture of 2,4-dihydroxybenzaldehyde (5.00 g, 36.2 mmol), sodium acetate (5.94 g, 72.4 mmol), nitroethane (5.44 g, 72.4 mmol), and glacial acetic acid (10 mL) was refluxed for 6 h. After cooling, the mixture was poured onto ice (100 g) and extracted with EtOAc (4 × 50 mL). The combined organic layers were washed with saturated $NaHCO_3$ until the pH of the aqueous layer remained at 8, and the solvent was removed in vacuo. Silica gel flash chromatography (cyclohexane:EtOAc = 1:1) afforded 2.87 g (59%) of **16** as a pale-yellow solid: NMR (DMSO-d_6) δ 6.33 (d, 1 H, J = 8.6), 6.43 (s, 1 H), 7.37 (d, 1 H, J = 8.6), 10.35 (s, 1 H), 10.78 (s, 1 H); IR (KBr) 2200 cm^{-1}.

2-Hydroxy-3-methoxybenzonitrile (17).[46] A mixture of *o*-vanillin (4.93 g, 32.4 mmol), sodium acetate (5.32 g, 64.9 mmol), nitroethane (4.87 g, 64.9 mmol), and glacial acetic acid (10 mL) was refluxed for 6 h. After cooling, the reaction mixture was poured onto ice (100 g) and extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with saturated $NaHCO_3$ until the pH of the aqueous layer remained at 8, and the solvent was removed in vacuo. Silica gel flash chromatography (CH_2Cl_2) gave 2.60 g (54%) of **17** as a pale-yellow oil: NMR (DMSO-d_6) δ 6.90 (t, 1 H, J = 8), 7.15 (dd, 1 H, J = 8, 1.4), 7.25 (dd, 1 H, J = 8, 1.4).

4-Cyano-3-hydroxybenzoic Acid (18). A mixture of 4-formyl-3-hydroxybenzoic acid[47] (2.00 g, 12.0 mmol), sodium acetate (1.97 g, 24.0 mmol), nitroethane (1.80 g, 24.0 mmol), and glacial acetic acid (4.4 mL) was refluxed for 3.5 h. After cooling, the mixture was poured into H_2O (300 mL) and extracted with EtOAc (4 × 100 mL). The organic phase was removed by rotary evaporation. Silica gel flash chromatography (90/10/5 $CHCl_3$/acetone/HOAc) gave 1.42 g (72%) of **18** as a pale-yellow solid: NMR (DMSO-d_6) δ 7.44 (dd, 1 H, J = 1.3, 8.1), 7.56 (d, 1 H, J = 1.3), 7.74 (d, 1 H, J = 8.1), 11.50 (br s, 1 H), 13.20 (br s, 1 H). Anal. ($C_8H_5NO_3$) C, H, N.

Methyl 2-(3-Hydroxypyridin-2-yl)thiazole-4-carboxylate (19).[27] Manganese(IV) oxide[26] (2.63 g, 30.3 mmol) was added to methyl 4,5-dihydro-2-(3-hydroxypyridin-2-yl)thiazole-4(*R*)-carboxylate[20] (0.725 g, 3.04 mmol). The reaction mixture was stirred for 1 day at room temperature and filtered through Celite, which was washed with $CHCl_3$. The filtrate was dried in vacuo to generate 0.63 g (88%) of **19** as a white solid: NMR δ 3.95 (s, 3 H), 7.13−7.42 (m, 3 H), 8.02−8.20 (m, 2 H). Anal. ($C_{10}H_8N_2O_3S$) C, H, N.

4-Methylphenyl 3-Chloropropanoate (20). 3-Chloropropionyl chloride (12.5 mL, 0.131 mol) was added over 3 min to a solution of *p*-cresol (12.90 g, 0.119 mol) in pyridine (9.5 mL, 0.12 mol) and CH_2Cl_2 (53 mL) at 0 °C. After the reaction mixture was stirred for 1 day at 0 °C to room temperature, solvent was removed by rotary evaporation. The concentrate was treated with brine (50 mL) and 0.5 M citric acid (150 mL) and extracted with EtOAc (3 × 100 mL). The organic extracts were washed with 100 mL: 1 N HCl, H_2O, cold 0.1 N NaOH, H_2O, and brine. After solvent removal, the residue was purified by silica gel flash column chromatography eluting with 7.5% EtOAc/hexane to give 12.90 g (55%) of **20**[48] as a colorless liquid: NMR δ 2.34 (s, 3 H), 3.03 (t, 2 H, J = 7), 3.86 (t, 2 H, J = 7), 6.95−7.01 (m, 2 H), 7.17 (d, 2 H, J = 8).

2-(3-Chloropropionyl)-4-methylphenol (21). $AlCl_3$ (38.9 g, 0.292 mol) was added to **20** (12.89 g, 64.9 mmol); the exothermic reaction was controlled by brief cooling in ice water. The reaction mixture was heated at 90−95 °C with stirring under a nitrogen balloon with periodic venting of the HCl for 69 min. The reaction flask was cooled to 0 °C, and cold 0.5 N HCl (300 mL) was added, slowly at first. The aqueous phase was extracted with EtOAc (250 mL, 3 × 100 mL). The organic extracts were washed with H_2O (100 mL) and brine (100 mL). After solvent removal, the solid was chromatographed on a silica gel flash column eluting with 26% CH_2Cl_2/petroleum ether yielding 10.21 g (79%) of **21** as a pale-green solid: NMR δ 2.32 (s, 3 H), 3.48 (t, 2 H, J = 7), 3.92 (t, 2 H, J = 7), 6.91 (d, 1 H, J = 8), 7.31 (dd, 1 H, J = 8, 2), 7.49 (s, 1 H), 11.84 (s, 1 H). Anal. ($C_{10}H_{11}ClO_2$) C, H. Recrystallization from cyclohexane gave **21** as colorless plates, mp 63.8−64 °C.

Diethyl (Acetylamino)[3-(2-hydroxy-5-methylphenyl)-3-oxopropyl]propanedioate (22). Diethyl acetamidomalonate (12.84 g, 59.11 mmol) was added to freshly prepared 0.29 M NaOEt (225 mL) in EtOH at 0 °C. After brief sonication, the solution was transferred to an addition funnel and added over 6 min to a suspension of **21** (10.68 g, 53.74 mmol) in EtOH (50 mL) at 0 °C. After the reaction mixture was stirred for 18 h at room temperature, solvent was removed in vacuo. Cold 0.25 N HCl (200 mL) was added, and the aqueous phase was extracted with $CHCl_3$ (200 mL, 2 × 100 mL). The organic layer was washed with H_2O (100 mL). After solvent removal, the solid was purified by silica gel flash column chromatography using 4% acetone/CH_2Cl_2 to furnish 18.10 g (89%) of **22** as a solid: NMR δ 1.26 (t, 6 H, J = 7), 2.04 (s, 3 H), 2.30 (s, 3 H), 2.78 (t, 2 H, J = 7), 2.98 (t, 2 H, J = 7), 4.17−4.34 (m, 4 H), 6.79 (s, 1 H), 6.88 (d, 1 H, J = 8), 7.25−7.30 (m, 1 H), 7.47 (s, 1 H), 11.97 (s, 1 H). Anal. ($C_{19}H_{25}NO_7$) C, H, N. Recrystallization from cyclohexane afforded **22** as colorless needles, mp 110.3−111.4 °C.

***tert*-Butyl 3(*S*)-(*tert*-Butoxycarbonylamino)-4-hydroxybutanoate (23).** Sodium borohydride (1.23 g, 32.8 mmol) was added to a solution of *N*-t-BOC-β-*tert*-butyl-L-aspartic acid *N*-hydroxysuccinimide ester (5.02 g, 13.0 mmol) in THF at 0 °C. After the reaction mixture was stirred for 4 h at room temperature, 1:1 ice water/brine was added, slowly at first, to the flask at 0 °C, followed by cautious addition of 0.5 M citric acid (100 mL). The biphasic mixture was extracted with EtOAc (4 × 100 mL). The organic extracts were washed with 5% $NaHCO_3$ (75 mL) and brine (75 mL) and concentrated. Purification by silica gel flash column chromatography using 42% EtOAc/hexane gave 3.60 g (quantitative) of **23**[34] as a colorless liquid: $[\alpha]^{23}_D$ +3.0° (*c* 1.14, $CHCl_3$); NMR δ 1.44 and 1.45 (2 s, 18 H), 2.43−2.82 (m, 3 H), 3.62−4.04 (m, 3 H), 5.22 (br s, 1 H). Anal. ($C_{13}H_{25}NO_5$) C, H, N.

***tert*-Butyl 3(*S*)-(*tert*-Butoxycarbonylamino)-4-(*p*-tolylsulfonyloxy)butanoate (24).** A solution of *p*-toluenesulfonyl chloride (3.09 g, 16.2 mmol) in pyridine (15 mL) was added by a cannula to **23** (3.56 g, 12.9 mmol) in pyridine (15 mL) at <0 °C (ice−salt bath) over 5 min. The reaction mixture stood at 4 °C for 1 day. After removal of solvent under high vacuum, H_2O (100 mL) and EtOAc (100 mL) were added, and the layers were separated. The aqueous phase was extracted further with EtOAc (2 × 50 mL). The organic extracts were washed with 75 mL: 0.5 M citric acid, H_2O, and brine. Removal of the solvent gave 5.8 g (quantitative) of **24** as a green oil, which was used directly in the next step because of its instability. A sample (0.219 g) was chromatographed on flash silica gel, eluting with 20% EtOAc/petroleum ether, to give 0.124 g of **24**: NMR δ 1.40 and 1.41 (2 s, 18 H), 2.45 (s, 3 H), 2.49 (d, 2 H, J = 6), 4.04−4.19 (m, 3 H), 5.00 (br s, 1 H), 7.35 (d, 2 H, J = 8), 7.78 (d, 2 H, J = 8).

***tert*-Butyl 4-(Acetylthio)-3(*S*)-(*tert*-butoxycarbonylamino)butanoate (25).** Potassium thioacetate (4.3 g, 38 mmol) was added to a solution of **24** (5.8 g, 12.9 mmol) in DMF (45 mL). After the reaction mixture was stirred for 20 h at room temperature, solvent was removed under high vacuum. After 50% aqueous brine (100 mL) and EtOAc (150 mL) were added, the layers were separated. The aqueous phase was extracted further with EtOAc (4 × 50 mL). The organic extracts were washed with 100 mL: saturated $NaHCO_3$, H_2O, 0.5 M citric

acid, H_2O (the layers were filtered), and brine. After solvent removal, the residue was purified by silica gel flash column chromatography using 17% EtOAc/hexane to provide 3.29 g (77%) of **25** as an oil: $[\alpha]^{25}_D$ +6.1° (*c* 1.37, $CHCl_3$); NMR δ 1.43 and 1.46 (2 s, 18 H), 2.35 (s, 3 H), 2.48 (d, 2 H, $J = 6$), 3.05−3.20 (m, 2 H), 3.97−4.13 (m, 1 H), 4.97−5.15 (m, 1 H). Anal. ($C_{15}H_{27}NO_5S$) C, H, N.

***tert*-Butyl 4(*S*)-(*tert*-Butoxycarbonylamino)-5-hydroxypentanoate (27).** Triethylamine (2.0 mL, 14 mmol) and ethyl chloroformate (1.4 mL, 15 mmol) were added successively by syringe to *N*-t-BOC-L-glutamic acid γ-*tert*-butyl ester (4.33 g, 14.3 mmol) in THF (100 mL) at <0 °C (ice−salt bath). After stirring in the cold bath for 37 min, solids were filtered through a glass frit (40−60 μm) and were washed with THF (35 mL). The filtrate was transferred to a 250-mL addition funnel and added to a solution of sodium borohydride (1.48 g, 39 mmol) in H_2O (20 mL) at 0 °C over 30 min. The reaction mixture was maintained at 0 °C for 5.5 h and then stirred for 20 h (0 °C to room temperature). After the bulk of solvents were removed by rotary evaporation, the concentrate was quenched with ice water (50 mL) and 1 N HCl (50 mL). After extraction with EtOAc (4 × 100 mL), the extracts were washed with 100 mL: 0.5 M citric acid, saturated $NaHCO_3$, H_2O, and brine and concentrated. Purification by silica gel flash column chromatography using 42% EtOAc/hexane generated 3.46 g (84%) of **27** as a colorless oil: $[\alpha]^{26}_D$ −13.5° (*c* 1.88, $CHCl_3$); NMR δ 1.45 and 1.46 (2 s, 18 H), 1.7−1.9 (m, 2 H), 2.28−2.38 (m, 2 H), 2.73 (br s, 1 H), 3.49−3.68 (m, 3 H), 4.83−4.95 (br s, 1 H). Anal. ($C_{14}H_{27}NO_5$) C, H, N.

***tert*-Butyl 4(*S*)-(*tert*-Butoxycarbonylamino)-5-(*p*-tolylsulfonyloxy)pentanoate (28).** A solution of *p*-toluenesulfonyl chloride (2.90 g, 15.2 mmol) in pyridine (10 mL) was added to a solution of **27** (3.45 g, 11.9 mmol) in pyridine (15 mL) at <0 °C (ice−salt bath) over 3 min. The reaction mixture was placed at 4 °C for >1 day. After removal of solvent under high vacuum, H_2O (100 mL) and EtOAc (100 mL) were added, and the layers were separated. The aqueous phase was extracted further with EtOAc (4 × 50 mL). The organic extracts were washed with 100 mL: 0.5 M citric acid, H_2O, and brine. Removal of the solvent gave 5.45 g (quantitative) of **28** as a solid, which was used directly in the next step. An analytical sample (0.169 g) was purified by silica gel flash column chromatography using 24% EtOAc/hexane, furnishing 0.093 g of **28** as a white solid, mp 88−91 °C: $[\alpha]^{25}_D$ −20.4° (*c* 3.45, $CHCl_3$); NMR δ 1.39 and 1.40 (2 s, 18 H), 1.78 (q, 2 H, $J = 7$), 2.21−2.29 (m, 2 H), 2.45 (s, 3 H), 3.70−3.84 (m, 1 H), 3.94−4.09 (m, 2 H), 4.67 (d, 1 H, $J = 10$), 7.32−7.38 (m, 2 H), 7.76−7.81 (m, 2 H). Anal. ($C_{21}H_{33}NO_7S$) C, H, N.

***tert*-Butyl 5-(Acetylthio)-4(*S*)-(*tert*-butoxycarbonylamino)pentanoate (29).** Potassium thioacetate (3.99 g, 34.9 mmol) in DMF (20 mL) was added by cannula to a solution of **28** (5.43 g, 12.2 mmol) in DMF (15 mL) at 0 °C over 10 min. After the reaction mixture was stirred for 21 h at room temperature, solvent was removed under high vacuum. After 50% aqueous brine (100 mL) and EtOAc (150 mL) were added, the layers were separated. The aqueous phase was extracted further with EtOAc (4 × 75 mL). The organic extracts were washed with 100 mL: saturated $NaHCO_3$, H_2O (2×), and brine. After solvent removal, the residue was purified by silica gel flash column chromatography using 20% EtOAc/hexane and recolumned with 18% EtOAc/hexane to give 3.07 g (72%) of **29** as a solid, mp 50−52 °C: $[\alpha]^{26}_D$ −20.9° (*c* 1.02, $CHCl_3$): NMR δ 1.43 and 1.44 (2 s, 18 H), 1.66−1.90 (m, 2 H), 2.27−2.33 (m, 2 H), 2.35 (s, 3 H), 2.96−3.14 (m, 2 H), 4.56 (d, 2 H, $J = 9$). Anal. ($C_{16}H_{29}NO_5S$) C, H, N.

5-Mercapto-4(*S*)-aminopentanoic Acid (30). Degassed 6 N HCl (40 mL) was added to **29**, and the reaction mixture was heated at reflux under a nitrogen balloon for 1 day. Solvent was removed under high vacuum, degassed H_2O (5 mL) was added, and evaporation was repeated to give 0.125 g (quantitative) of **30**,[33,36] which was used directly in the next step: NMR (D_2O) δ 1.93−2.11 (m, 2 H), 2.44−2.60 (m, 2 H), 2.76 (dd, 1 H, $J = 14, 6$), 2.96 (dd, 1 H, $J = 14, 5$), 3.42−3.52 (m, 1 H).

3-Hydroxyquinoline (31). 3-Aminoquinoline (10.00 g, 69.4 mmol) and $NaHSO_3$ (40 g, 0.38 mol) were heated at reflux in water (100 mL) for 3 days. The reaction mixture was basified to a pH of 8 with 30% NaOH and brought to reflux. After cooling to room temperature, solid was filtered to provide 9.7 g (96%) of **31** as a solid, mp 168 °C (lit. mp > 150 °C dec[49]): NMR (DMSO-d_6) δ 7.45−7.58 (m, 3 H), 7.76−7.82 (m, 1 H), 7.86−7.96 (m, 1 H), 8.58 (m, 1 H), 10.20 (br s, 1 H). Anal. (C_9H_7NO) C, H, N.

3-Hydroxyquinoline *N*-Oxide (32). Urea hydrogen peroxide addition compound (7.87 g, 83.7 mmol), phthalic anhydride (12.3 g, 83.0 mmol), and **31** (4.84 g, 33.3 mmol) in CH_3OH (250 mL) and CH_2Cl_2 (250 mL) were refluxed for 15 h. Solvent was removed in vacuo, and the oil was diluted with $CHCl_3$ (200 mL). Solid was filtered, and the filtrate was evaporated. Purification by silica gel flash column chromatography using 75:20:5 hexane/EtOAc/EtOH and then 10% EtOH/$CHCl_3$ and recrystallization from EtOH/$CHCl_3$ furnished 3.8 g (71%) of **32** as a solid, mp 218 °C: NMR (DMSO-d_6) δ 7.23 (m, 1 H), 7.48−7.64 (m, 2 H), 7.89 (m, 1 H), 8.29 (m, 1 H), 8.38 (m, 1 H), 10.65 (s, 1 H). Anal. ($C_9H_7NO_2$) C, H, N.

2-Cyano-3-hydroxyquinoline (33). Triethylamine (2.4 mL, 18 mmol) was added to a vigorously stirred mixture of **32** (0.51 g, 3.2 mmol) and KCN (0.39 g, 6.0 mmol) in DMF (60 mL). Within 10 min chlorotrimethylsilane (1.9 mL, 15 mmol) was added, whereupon the temperature rose to 45 °C. After the reaction mixture was heated for 2 days at 100−110 °C, inorganic salts were filtered at room temperature and washed with DMF. The filtrate was evaporated under high vacuum, and the concentrate was taken up in $CHCl_3$ (50 mL). Solid precipitated and was filtered to give 0.35 g (64%) of **33** as a solid, mp 183 °C: NMR (DMSO-d_6) δ 7.62−7.71 (m, 2 H), 7.84 (s, 1 H), 7.91−8.02 (m, 2 H). Anal. ($C_{10}H_6N_2O$) C, H, N.

Cannulation of Bile Duct in Rats. The cannulation has been described previously.[20,23,42] Briefly, male Sprague−Dawley rats averaging 400 g were housed in Nalgene plastic metabolic cages during the experimental period and given free access to water. The animals were anesthetized using sodium pentobarbital (55 mg/kg) given ip. The bile duct was cannulated using 22-gauge polyethylene tubing. The cannula was inserted into the duct about 1 cm from the duodenum and tied in place. After threading through the shoulder, the cannula was passed from the rat to the swivel inside a metal torque-transmitting tether, which was attached to a rodent jacket around the animal's chest. The cannula was directed from the rat to a Gilson microfraction collector (Middleton, WI) by a fluid swivel mounted above the metabolic cage. Bile samples were collected at 3-h intervals for 24 h. Urine samples were taken every 24 h. Sample collection and handling were as previously described.[20]

Iron Loading of *C. apella* Monkeys. After intramuscular anesthesia with ketamine, an intravenous infusion was started in a leg vein. The iron dextran was added to approximately 90 mL of sterile normal saline and administered to the animals by slow infusion at a dose of 200−300 mg of iron/kg of body weight over 45−60 min. Two to three infusions, separated by between 10 and 14 days, were necessary to provide about 500 mg of iron/kg of body weight. After administration of iron dextran, the serum transferrin iron saturation rose to between 70% and 80%. The serum half-life of iron dextran in humans is 2.5−3.0 days.[50] We waited at least 20 half-lives, 60 days, before using any of the animals in experiments evaluating iron-chelating agents.

Primate Fecal and Urine Samples. Fecal and urine samples were collected at 24-h intervals. The collections began 4 days prior to the administration of the test drug and continued for an additional 5 days after the drug was given. Fecal samples were assayed for the presence of occult blood, weighed, and mixed with distilled deionized H_2O before autoclaving for 30 min. The mixture was then freeze-dried, and a known portion of the powder was mixed with low-iron HNO_3 and refluxed for 24 h. Once any particulate matter in the digested samples was removed by centrifugation, iron

concentrations were determined by flame atomic absorption spectroscopy as previously described.[23,41] Monkey urine samples were acidified and reconstituted to initial volume after sterilization, if necessary.

Drug Preparation and Administration. The iron chelators were solubilized in 40% Cremophor RH-40/water (v/v) and given po or sc to the rats at a dose of 150 μmol/kg. In the primates, the compounds were solubilized in 40% Cremophor RH-40/H_2O (v/v) and given po at a dose of 75, 150, or 300 μmol/kg as indicated in Table 1.

Calculation of Iron Chelator Efficiency. The efficiency of each chelator was calculated on the basis of a 2:1 ligand−iron complex. The efficiencies in the rodent model were calculated by subtracting the iron excretion of control animals from the iron excretion of treated animals. This number was then divided by the theoretical output; the result is expressed as a percentage. In the monkeys the numbers were generated by averaging the iron output for 4 days before the administration of the drug, subtracting these numbers from the 2-day iron clearance after the administration of the drug and then dividing by the theoretical output; the result is expressed as a percentage.

Toxicity Evaluations in Rodents. Male Sprague−Dawley rats averaging 450 g were housed in polycarbonate cages with Beta-Chips (Northeastern Products Corp., Warrensburg, NY) provided as bedding. Before the first drug administration, the rats were weighed and evaluated for their general condition. In the initial toxicological evaluations, the rats (n = 5/group) were given analogues **1**, **2**, **3**, and **5** at a dose of 384 μmol/kg once daily for 10 days following an overnight fast. The drugs were solubilized in 40% Cremophor RH-40/H_2O (v/v) and administered orally by gavage. The rats were fed 3 h after drug administration and were allowed access to food for 5 h. The amount of food and water consumed was recorded daily. In addition, the animals were weighed and evaluated for their activity level and general appearance on a daily basis. Control animals were given an equivalent volume of 40% Cremophor/H_2O (v/v) orally by gavage and were maintained on the same feeding schedule as the drug-treated animals.

A necropsy was performed whenever an animal died during the course of the study, and any gross abnormalities were noted. Animals that survived the 10-day exposure to the drug were sacrificed 24 h after the final dose and extensive tissues, including: adrenal gland, bone marrow, stomach, small intestine, large intestine, testicle, kidney, liver, lung, lymph node, pancreas, skeletal muscle, spleen, thymus, thyroid, and bladder, were sent to an outside pathologist for histological evaluation.

Analogues that were determined to be "nontoxic" in the initial 10-day study were further evaluated under an expanded dosing protocol. In the longer term study, the rats (n = 5/group) were given 30 daily doses of analogues **2** and **5** over a 32-day period. To more closely mimic potential clinical applications, no Cremophor vehicle was used; the drugs were given orally by gavage as a suspension in H_2O at a dose of 250 μmol/kg. Control rats received an equivalent amount of H_2O orally. Since animals in the initial studies lost about 1% of their body weight per day due to the restricted access to food, the animals in the long-term study were given food ad libitum. Inasmuch as rats are nocturnal and consume the majority of their food at night, the drugs were given late in the afternoon to minimize the amount of food present in the animals' stomachs. Prior to each daily drug administration, the rats were weighed and evaluated for their general condition. Food intake and water intake were carefully monitored. A necropsy was performed 1−2 days after the last dose of the drug had been given, and extensive tissues, including: adrenal gland, aorta, bone marrow, brain, eye, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, testicle, heart, kidney, liver, lung, mesenteric lymph node, pancreas, prostate, mandibular salivary gland, skeletal muscle, spleen, thymus, thyroid, trachea, and bladder, were examined by an outside pathologist.

Determination of Stoichiometry of Ligand−Fe(III) Complexes. The stoichiometry of ligand−Fe(III) complexes was determined spectrophotometrically from Job's plots for ligands **2**, **3**, and **5**. Ligand **1**, known to form a 2:1 ligand−Fe(III) complex,[19] was run as a positive control. Solutions were monitored at the visible λ_{max} (484 nm). Solutions containing different ligand:Fe(III) ratios were prepared by mixing appropriate volumes of 0.5 mM ligand in 100 mM TRIS Cl, pH 7.4, and 0.5 mM Fe(III) nitrilotriacetate (NTA) in 100 mM TRIS Cl, pH 7.4, so that [ligand] + [Fe(III)] = 0.5 mM for **5** and 1.0 mM for **3**. The 0.5 mM Fe(III)−NTA solution was prepared immediately prior to use by dilution of a 50 mM Fe(III)−NTA stock solution with TRIS buffer. The Fe(III)−NTA stock solution was prepared by mixing equal volumes of 100 mM ferric ammonium sulfate and 200 mM trisodium NTA. The iron content was verified by atomic absorption spectroscopy.

Determination of Partition Coefficients. The octanol−H_2O partition data are expressed as distribution coefficients uncorrected for partial ionization of the acids and were all measured at pH 7.4 (50 mM TRIS buffer) using UV spectrometry. The measurements were done using a "shake flask" direct measurement.[51] Three to five minutes of mixing was followed by between 1 and 24 h of settling time. The equilibrations and measurements were made at 24 °C using a Shimazu model 2501PC UV spectrometer. HPLC grade 1-octanol was obtained from Sigma-Aldrich (St. Louis, MO).

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