Expedited Articles

The Desferrithiocin Pharmacophore

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The (*S*)-desferrithiocin (DFT) skeleton is shown to be a useful pharmacophore on which to design orally effective iron chelators. While the study clearly indicates that formal reduction of the desazadesmethyldesferrithiocin thiazoline to a thiazolidine (**6**), expansion of the desmethyldesferrithiocin thiazoline to a thiazine (**7**), or substitution of the thiazoline sulfur of desazadesmethyldesferrithiocin by an oxygen (**8** and **9**) lead to a substantial loss of activity, conversion of (*S*)-desmethyldesferrithiocin (**1**) to an *N*-methylhydroxamate (**4**) or to the hexacoordinate dihydroxamate ligand (**5**) results in active compounds. This investigation thus demonstrates which structural components of the siderophore are required for iron clearance after oral administration and suggests the use of the desferrithiocin platform as a vector for other chelators.

Introduction

While many organisms are auxotrophic for Fe(III), because of the insolubility of the hydroxide ($K_{sp} = 1 \times 10^{-38}$)[1] formed under physiological conditions, nature has developed rather sophisticated iron storage and transport systems. Microorganisms utilize low molecular weight ligands, siderophores, while eukaryotes tend to utilize proteins to transport iron, e.g. transferrin, and store iron, e.g. ferritin.[2]

Iron metabolism in primates 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. There are a number of scenarios which can account for "iron overload", e.g. high-iron diet, acute iron ingestion, or malabsorption of the metal. In each of these situations the patient can be treated by phlebotomy.[10] However, there are iron-overload syndromes secondary to chronic transfusion therapy, e.g. aplastic anemia and thalassemia,[11] in which phlebotomy is not an option. The patient cannot be bled, as the origin of the excess iron is the transfused red blood cells; thus the only alternative is chelation therapy. "In patients with a high transfusion regimen, erythropoeisis is suppressed and iron absorption may be near normal, but each unit of transfused RBC's contains 200 to 250 mg of iron. Most patients with thalassemia major require 200 to 300 mL/kg/year of blood, an amount equivalent to 0.25 to 0.40 mg Fe/kg/day."[12] Thus, a chelator must be able to remove a minimum of between 0.25 and 0.40 mg of Fe/kg/day.

Although considerable effort has been invested in the development of new therapeutics for managing thalassemia, the subcutaneous (sc) infusion of desferrioxamine B (DFO, Figure 1), a hexacoordinate hydroxamate iron chelator produced by *Streptomyces pilosus*,[13] is still the protocol of choice. Although the drug's efficacy and long-term tolerability are well-documented, it suffers from a number of shortcomings associated with low efficiency and marginal oral activity.

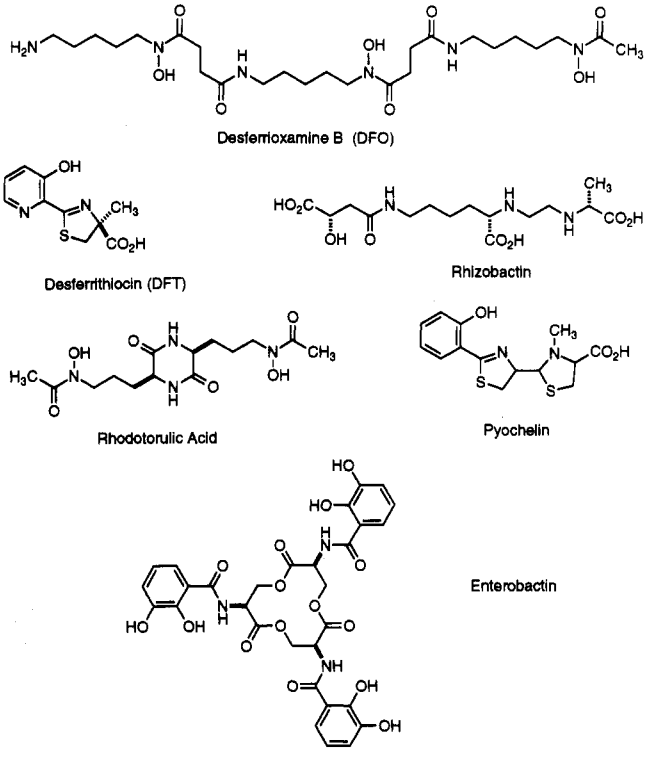

Figure 1. Microbial iron chelators, siderophores.

Desferrioxamine forms a 1:1 hexacoordinate octahedral complex *in situ* with Fe(III) with a formation constant[14] of 3×10^{30} M^{-1}. If one assumes that the same stoichiometry applies as when DFO is administered to an animal, 10% or less of the theoretical iron excretion is observed.[15] This situation is further complicated by the fact that DFO is not orally active and, when administered subcutaneously, has a very short half-life in the body and must therefore be given by continuous infusion over long periods of time. For these reasons, patient compliance is a serious problem,[16] a situation which could be solved with an orally active drug.

Although a substantial number of synthetic iron chelators have been studied in recent years as potential orally active therapeutics, e.g. pyridoxyl isonicotinoyl hydrazone

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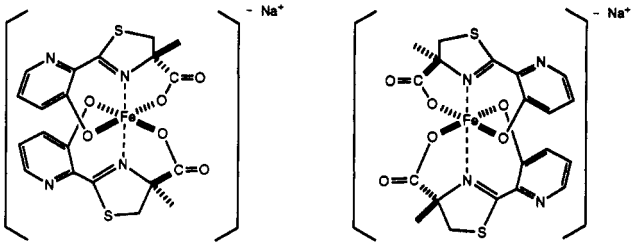


Figure 2. Proposed structure of ferrithiocin (FT) diastereomeric 2:1 complexes with Fe(III).

(PIH),[17] hydroxypyridones,[18,19] and bis(*o*-hydroxybenzyl)-ethylenediaminediacetic acid (HBED) analogues,[20] none has yet proven to be completely satisfactory. Interestingly, the siderophores have remained relatively untouched in this search. Their evaluation as iron-clearing agents has not at all paralleled the rate of their isolation and structural elucidation. In fact, until recently, beyond DFO, only two of some 100 siderophores identified have been studied in animal models: enterobactin[21] and rhodotorulic acid[22] (Figure 1). While the former was only marginally effective at clearing iron, the latter compound was reasonably active. Unfortunately both of these cyclic siderophores exhibited unacceptable toxicity, and neither possessed any oral activity. They were abandoned, as there were any number of synthetic chelators with equally unsatisfactory properties to choose from. The siderophores thus became *persona non grata* as potential clinical deferration agents.

While most siderophores fall primarily into two structural classes, hydroxamates or catecholamides,[23,24] there are a number of compounds which do not belong to either family, e.g. pyochelin,[25] rhizobactin,[26] and 2-(3′-hydroxypyrid-2′-yl)-4-methylthiazoline-4(*S*)-carboxylic acid (desferrithiocin, DFT) (Figure 1). DFT, isolated from *Streptomyces antibioticus*,[27] was shown to form a stable 2:1 complex with iron, ($K_f = 4 \times 10^{29}$ M^{-1}).[28] Furthermore, studies in rodents focused on the reduction of liver ferritin by DFT,[29] and a preliminary investigation in primates[30] suggested that it was indeed an orally active iron chelator. A more comprehensive investigation in our laboratory carried out in a bile duct cannulated rat model[31] as well as in a Cebus monkey model[15,32] supported these findings and identified the magnitude of the drug's effectiveness. DFT was shown to be more efficient when given orally than DFO given sc. It was far superior to any of the numerous hydroxypyridones (including CP20 and CP94) and PIH analogues we have investigated.[15,33] Unfortunately, animals exposed to the drug chronically presented with nephrotoxicity.[32] Nevertheless, the chelator's remarkable gastrointestinal (GI) absorption and iron-clearing efficiencies underscore the idea that the DFT skeleton represents an excellent pharmacophore on which to predicate the design of an orally effective iron chelator. However it is first necessary to identify the minimal structural components of DFT required for iron clearance in animals when the drug is given orally.

Design Concepts

On the basis of X-ray studies of the Cr(III)-DFT complex,[34] the chelator likely forms a pair of diastereomeric 2:1 complexes with Fe(III) (Figure 2). The three ligating centers in both diastereomers are the aromatic hydroxyl, the thiazoline nitrogen, and the carboxyl group. Therefore, the elements of functional significance in DFT iron binding can, in principle, be represented schematically as in Figure

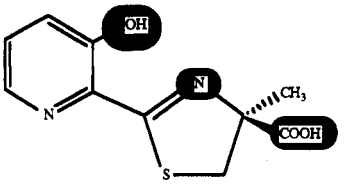


Figure 3. Chelating centers of (*S*)-desferrithiocin (DFT).

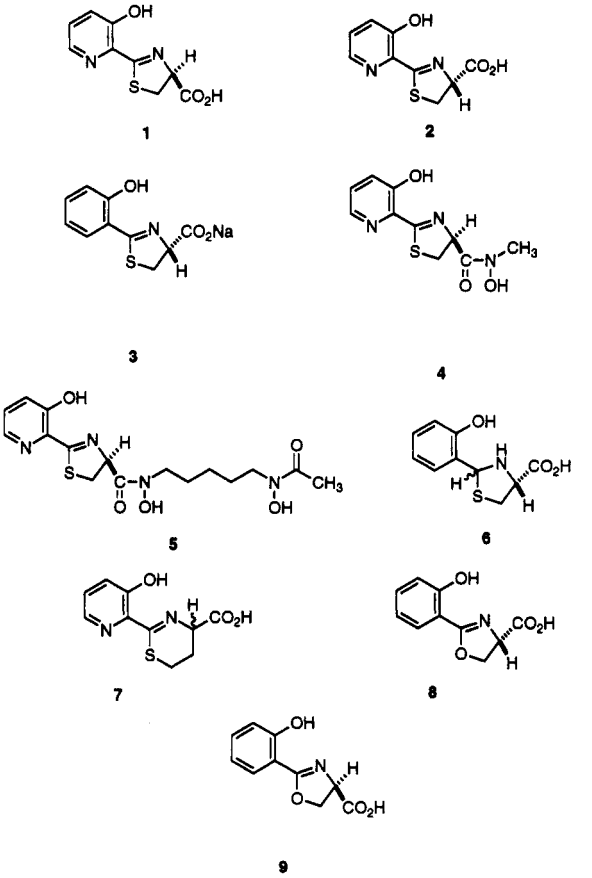
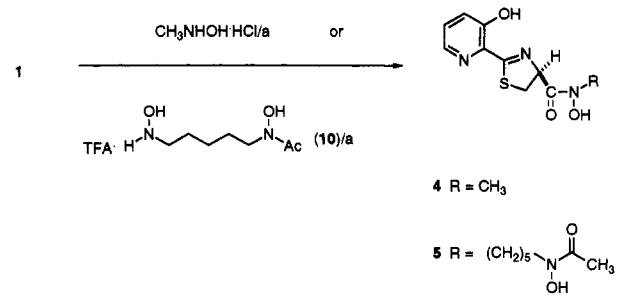


Figure 4. Structures of desferrithiocin analogues: (*S*)-desmethyldesferrithiocin (1), (*R*)-desmethyldesferrithiocin (2), (*R*)-desazadesmethyldesferrithiocin, sodium salt (3), (*S*)-desmethyldesferrithiocin, *N*-methylhydroxamate (4), (*S*)-desmethyldesferrithiocin, *N*-[5-(acetylhydroxyamino)pentyl]hydroxamate (5), 2-(2′-hydroxyphenyl)-4(*R*)-thiazolidinecarboxylic acid (6), DL-2-(3′-hydroxypyrid-2′-yl)-4*H*-5,6-dihydro-1,3-thiazine-4-carboxylic acid (7), 2-(2′-hydroxyphenyl)-4(*S*)-oxazolinecarboxylic acid (8), 2-(2′-hydroxyphenyl)-4(*R*)-oxazolinecarboxylic acid (9).

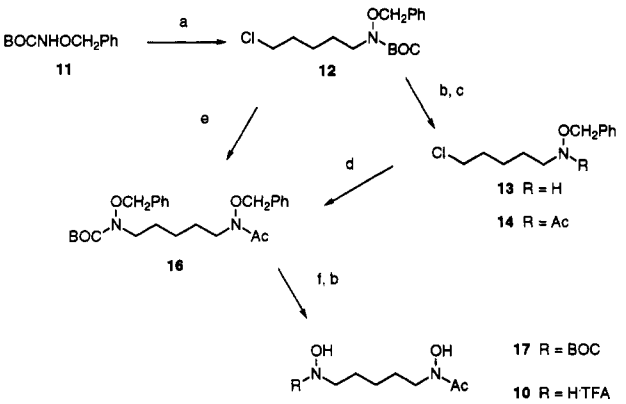
3. Any structural alterations which compromise the ability of these functional groups to coordinate with iron should significantly reduce the compound's iron-clearing properties. This is in keeping with the earlier observation that removal of the desferrithiocin hydroxyl or conversion of the carboxyl to the corresponding methyl ester resultedin inactive compounds.[31] However, modification of the nonchelating fragments such as removal of the thiazoline methyl (1 and 2) and also the aromatic nitrogen (3) (Figure 4) had little impact on the deferration properties of the resulting molecules. These observations do not necessarily mean that the chelating functionalities cannot be modified without interfering with iron clearance or for that matter that alteration of other nonchelating fragments will not impact on the molecule's iron-clearing properties. Further structure–activity studies were obviously in order. In this paper, we focus on the significance of thiazoline ring and carboxyl group modifications on the compound's activity (Figure 4). The carboxyl group modifications involved

Scheme 1.[a] Synthesis of (*S*)-Desmethyldesferrithiocin Hydroxamates 4 and 5



[a] Reagents: (a) BOP/*i*-Pr_2NEt/DMF.

Scheme 2.[a] Synthesis of *N*-[5-(Acetylhydroxyamino)-pentyl]hydroxylamine (10)



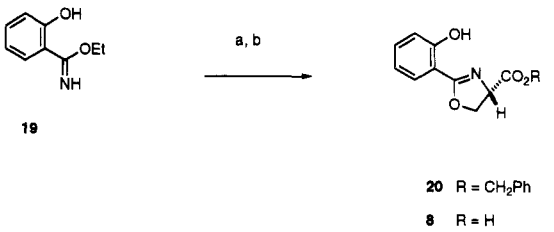
[a] Reagents: (a) $Cl(CH_2)_5Cl$ (excess)/NaH/DMF, (b) TFA/CH_2Cl_2, (c) AcCl/NaOH/CH_2Cl_2, (d) 11/NaH/DMF, (e) $AcNHOCH_2Ph$ (15)/NaH/DMF, (f) H_2/Pd–C/MeOH.

conversion of (*S*)-desmethyldesferrithiocin (1) to hydroxamates 4 and 5. The iron-clearing properties of the modified systems were then compared with that of the parent compound 1. In evaluating the significance of the thiazoline sulfur and double bond, a desazadesmethyldesferrithiocin (3) was chosen as the parent system on which to conduct the structural modifications. The sulfur of the desazadesmethyl compound was replaced with an oxygen by condensation of a 2-hydroxybenzimidate ester with (*S*)- or (*R*)-serine to generate 8 and 9. The thiazoline double-bond-reduced analogue 6 was obtained from a cyclodehydration reaction of L-cysteine with 2-hydroxybenzaldehyde. Finally the six-membered ring analogue (7) of desmethyldesferrithiocin was synthesized by a condensation of D,L-homocysteine with 3-hydroxy-2-pyridinenitrile. The resulting thiazine was compared with (*S*)-desmethyldesferrithiocin (1) as a positive control.

Synthetic Methods

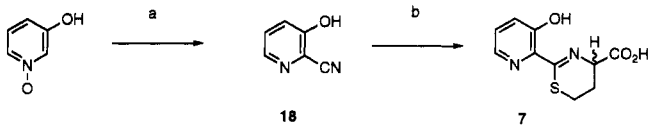
The key step in the synthesis of the hydroxamates involved N-acylation of either *N*-methylhydroxylamine or *N*-[5-(acetylhydroxyamino)pentyl]hydroxylamine (10) with (*S*)-desmethyldesferrithiocin (1) employing benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) as a condensing agent[35] (Scheme 1). However, synthesis of the hydroxylamine hydroxamate required for accessing dihydroxamate 5 (Figure 4) was somewhat more complicated (Scheme 2). *N*-(*tert*-Butoxycarbonyl)-*O*-benzylhydroxylamine (11)[36] was first alkylated with excess 1,5-dichloropentane (NaH/DMF) to produce the monohalide 12. The *tert*-butoxycarbonyl (BOC) protecting group was next removed with trifluoroacetic acid (TFA)/CH_2Cl_2, providing *N*-(5-chloropentyl)-*O*-benzylhydroxylamine (13). This stable amino halide can be acylated with a variety of activated esters; thus a series of desmethyldesferrithiocin dihydroxamate analogues could readily be generated. Exposure of 13 to acetyl chloride under Schotten–Baumann conditions led to *N*-acetyl-*N*-(5-chloropentyl)-*O*-benzylhydroxylamine (14), which was reacted with the anion of *N*-BOC-*O*-benzylhydroxylamine (11) in DMF to produce fully protected hydroxylamine hydroxamate 16. A less versatile but more direct route to synthon 16 involved N-alkylation of *N*-acetyl-*O*-benzylhydroxylamine (15)[37] with chloride 12. Hydrogenation of 16 to di-*N*-hydroxy compound 17 followed by cleavage of the BOC protecting group furnished hydroxylamine hydroxamate 10. Hydrogenolysis of the benzyl groups of 16 before removal of the BOC protecting moiety ensured that overreduction of the benzyloxyamine to a primary amine did not occur. Coupling of hydroxylamine 10 with (*S*)-desmethyldesferrithiocin (1) using BOP completed the synthesis of chelator 5 (Scheme 1). It is noteworthy that when acylations of *N*-methylhydroxylamine or hydroxylamine 10 with acid 1 were carried out with *N*-hydroxysuccinimide and 1,3-dicyclohexylcarbodiimide, the resulting products were almost completely racemized as indicated by rotation values that approached zero relative to those obtained with the BOP-promoted condensations.

Scheme 3.[a] Synthesis of Oxazoline Analogue 8



[a] Reagents: (a) L-serine benzyl ester·HCl/CH_3OH, (b) H_2/Pd–C/CH_3OH.

Scheme 4.[a] Synthesis of DL-2-(3′-Hydroxypyrid-2′-yl)-4*H*-5,6-dihydro-1,3-thiazine-4-carboxylic acid (7)



[a] Reagents: (a) TMS-CN/NEt_3/CH_3CN. (b) DL-homocysteine/phosphate buffer (pH 5.95)/CH_3OH.

Enantiomeric oxazolines 8 and 9, oxygen analogues of the desazadesmethyldesferrithiocin system 3 (Figure 4) were prepared by condensation of the benzyl ester of L- or D-serine,[38] respectively, with ethyl 2-hydroxybenzimidate (19)[39] followed by hydrogenolysis of benzyl ester 20 or 21, respectively, under mild conditions (1 atm, 10% Pd/C, CH_3OH) (Scheme 3). As will be described below, neither enantiomer was active either orally or when given subcutaneously. Because of this we next elected to explore both a thiazolidine and a thiazine analogue. Condensation of 2-hydroxybenzaldehyde with L-cysteine produced diastereomeric thiazolidine 6,[40] the formal reduction product of the thiazoline double bond of the desazadesmethyl chelator 3 (Figure 4). Condensation of 3-hydroxy-2-cyanopyridine (18),[41] obtained from 3-hydroxypyridine *N*-oxide, with DL-homocysteine afforded racemic dihydro-1,3-thiazine 7 (Scheme 4) a six membered analogue of enantiomers 1 and 2 (Figure 4). If either of these mixtures

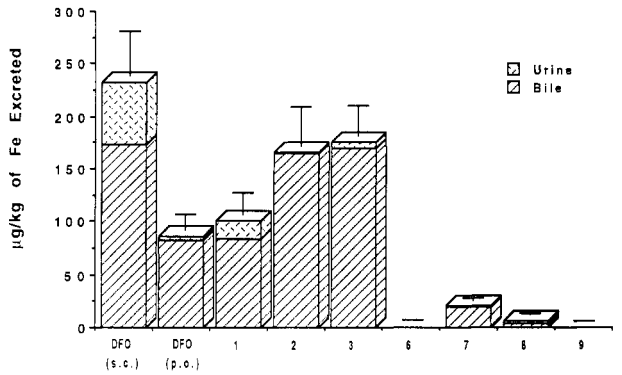


Figure 5. Response of the bile duct cannulated rats to DFO administered sc and po at 150 μmol/kg, analogues **1**, **2**, **3**, **6**, and **7** given orally at 150 μmol/kg and analogues **8** and **9** dosed at 300 μmol/kg po.

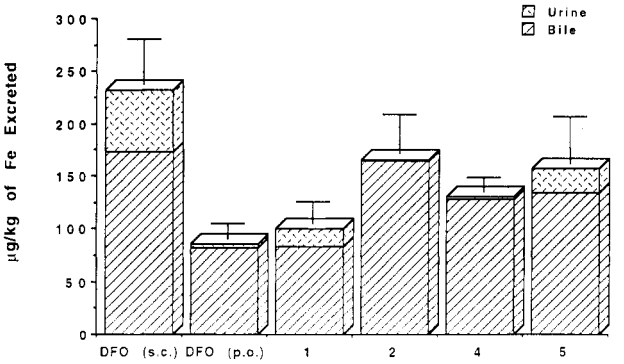


Figure 6. Response of the bile duct cannulated rats to DFO administered sc and po at 150 μmol/kg, and analogues **1**, **2**, **4**, and **5** given orally at 150 μmol/kg.

had shown significant activity, synthesis and testing of its pure stereoisomers would have been pursued.

Iron-Clearance Studies

Rodent Model. The non-iron-overloaded bile duct cannulated rat represents a very useful and rapid initial screen of new iron chelators.[31-33] The procedures employed in this study allow us to measure both the rate at which various chelators induce iron clearance in the bile and urine and the total iron cleared. While there is a problem of interruption of enterohepatic circulation, which can 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.

Thiazoline-Ring-Modified Pharmacophore. All of the chelators in Figure 4 were administered orally (po) in 40% Cremophor RH-40/water. The Cremophor vehicle was utilized to assure that all of the chelators were equally soluble. DFO was administered po and sc at 150 μmol/kg, and (*S*)- and (*R*)-desmethyldesferrithiocin (**1** and **2**) and (*R*)-desazadesmethyldesferrithiocin, sodium salt (**3**), were given po at 150 μmol/kg in the same vehicle. DFO cleared 232 ± 42 μg/kg of iron when administered sc with an efficiency of 2.8 ± 0.7% and 86 ± 14 μg/kg of iron when given po with an efficiency of 1.0 ± 0.5%. The sc route is clearly more effective ($p < 0.001$). Desmethyldesferrithiocin in the (*S*)-configuration (**1**), given at the same dose, induced 101 ± 20 μg/kg of iron (Figure 5) with an efficiency of 2.4 ± 0.6%. The (*R*)-enantiomer (**2**) at the same dose was slightly more effective, generating 166 ± 137 μg/kg of iron with an efficiency of 3.9 ± 1.8% ($p > 0.12$). (*R*)-Desazadesmethyldesferrithiocin, sodium salt (**3**), behaved very similarly to (*R*)-desmethyldesferrithiocin (**2**) ($p > 0.85$). At 150 μmol/kg of **3**, 176 ± 28 μg/kg of iron was excreted with an efficiency of 4.2 ± 1.6%.

Substitution of the desazadesmethyldesferrithiocin sulfur with an oxygen to form either the corresponding (*S*)- or (*R*)-desazadesmethyldesferrithiocin oxazoline analogues (**8** or **9**) resulted in compounds that were essentially inactive at doses as high as 300 μmol/kg po. Even when the oxazoline analogues were administered sc at a dose of 300 μmol/kg, there was no iron clearance response. The same scenario was realized on expansion of the desmethyldesferrithiocin ring to a dihydro-1,3-thiazine system (**7**), or when the desazadesmethyldesferrithiocin double bond was formally reduced to the corresponding secondary amine, thiazolidine (**6**).

On the basis of simple coordination-chemistry considerations, the poor iron-clearing properties observed on expanding the thiazoline ring or replacing sulfur with an oxygen make little sense. Furthermore, although it is true that conversion of the thiazoline to a thiazolidine might be expected to result in a ligand with different iron-binding properties than the parent desazadesmethyldesferrithiocin, its complete inactivity is difficult to rationalize.

Carboxylate-Modified Pharmacophore. Conversion of (*S*)-desmethyldesferrithiocin (**1**) to the corresponding *N*-methylhydroxamate (**4**) resulted in a compound which was active. At 150 μmol/kg, the drug induced 131 ± 13 μg/kg of iron (Figure 6) with an efficiency of 3.1 ± 0.4%. It is important to point out that Job's plots revealed that this compound was forming a 2:1 complex with Fe(III). Thus its efficiency is between those of desmethyldesferrithiocins **1** and **2**. This stoichiometry is probably related to the fact that the distance between the hydroxamate and the aromatic oxygen and thiazoline nitrogen is not sufficient to utilize all of the coordinating capacity of the hydroxamate. Normally, we would have expected **4** to form a 3:2 complex with Fe(III). In addition, the corresponding (*S*)-desmethyldesferrithiocin dihydroxamate hexacoordinate ligand (**5**) was also tested. When the ligand was administered po at a dose of 150 μmol/kg, its iron clearance was 156 ± 45 μg/kg. The stoichiometry of the Fe(III)–**5** chelate, although largely 3:2 from the Job's plots, is more complicated and thus the efficiency of the ligand is difficult to calculate. However, based on the fraction of 3:2 complex, its efficiency is similar to that of (*R*)-desmethyldesferrithiocin (**2**) (Figure 6).

The results of the present study clearly indicate that formal reduction of the desazadesmethyldesferrithiocin thiazoline to a thiazolidine (**6**), expansion of the desmethyldesferrithiocin thiazoline to a thiazine (**7**), or substitution of the thiazoline sulfur of desazadesmethyldesferrithiocin by an oxygen (**8** and **9**) lead to a substantial loss of activity of the desferrithiocin pharmacophore. However, conversion of (*S*)-desmethyldesferrithiocin (**1**) to an *N*-methylhydroxamate (**4**) or to the hexacoordinate dihydroxamate ligand (**5**) results in active compounds. These studies in conjunction with our previous work[31] define a number of critical structural components of a molecule containing the desferrithiocin pharmacophore required for oral activity. Of the nonchelating functionalities, the aromatic nitrogen and the thiazoline methyl of DFT are not necessary while the sulfur atom is required. Of the chelating functionalities, the aromatic hydroxyl and thiazoline nitrogen are critical for

activity, while the carboxyl group can be altered. However, the carboxyl changes are restricted, that is, conversion to simple esters is problematic while the desmethyldesferrithiocin hydroxamates are active. Previous studies in our laboratories have shown that small hydroxamates are not absorbed across the GI tract.[42] If in fact the desmethyldesferrithiocin hydroxamates are absorbed across the GI tract intact, this suggests that the desferrithiocin pharmacophore may serve as a vector for other kinds of chelators. We are currently preparing radiolabeled hydroxamates to study GI transport.

Experimental Section

L-Serine benzyl ester hydrochloride was obtained from Sigma Chemical Co. All other reagents were purchased from Aldrich Chemical Co. and were used without further purification. Fisher Optima-grade solvents were routinely used, and DMF was distilled. Organic extracts were dried with sodium sulfate. Glassware that was presoaked in 3 N HCl for 15 min, and distilled solvents were employed for reactions involving chelators. Silica gel 60 (70–230 mesh) obtained 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 recorded at 90 or 300 MHz in $CDCl_3$ unless otherwise indicated with chemical shifts given in parts per million downfield from an internal tetramethylsilane standard. Coupling constants (J) are in hertz. Mass spectra were carried out on a Fennigan 4516 instrument. Optical rotations were run in CH_3OH at 589 nm (sodium D line) at 25 °C with c as grams of compound per 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.

Atomic Absorption Iron Determinations. Bile and urine samples were analyzed on a Perkin-Elmer 5100 PC atomic absorption spectrophotometer fitted with a model AS-51 autosampler using a quartz sampling probe as previously described.[15]

Bile Duct Cannulation. Male Sprague-Dawley rats averaging 400 g were housed in Nalgene plastic metabolic cages and were given free access to water. The animals were anesthetized using sodium pentobarbital (50 mg/kg), given ip. The bile duct was cannulated using 22-gauge PE 50 tubing, which was inserted approximately 2 cm into the duct and tied firmly in place.

A skin tunneling needle was inserted from the shoulder area around to the abdominal incision. The cannula was threaded through the needle until it emerged from the shoulder opening. The cannula was then passed from the animal to the swivel inside a metal torque-transmitting tether, which was attached to a rodent jacket. The cannula was directed from the animal to a Gilson micro fraction collector by a fluid swivel mounted above the metabolic cage. This system allowed the animal to move freely in the cage while continuous bile samples were being collected. Bile samples were collected in plastic disposable tubes at 3-h intervals for 24 h. Urine samples were collected in plastic disposable tubes for 24 h.

Preparation of Drugs. Drug solutions were prepared in 60% water, 40% Cremophor RH-40.

(*S*)-Desmethyldesferrithiocin, *N*-Methylhydroxamate (4). BOP (442.3 mg, 1.0 mmol) was added to a solution of **1** (224.2 mg, 1.0 mmol) and *N*-methylhydroxylamine hydrochloride (83.52 mg, 1.0 mmol) in DMF (8 mL) at 0 °C. A solution of diisopropylethylamine (DIEA, 129.2 mg, 1.0 mmol) in DMF (2 mL) was added dropwise to the above solution at 0 °C. The mixture was stirred at 0 °C for 15 min and at room temperature overnight. Solvent was removed under high vacuum, and the residue was treated with EtOAc (30 mL). The organic phase was washed with 10-mL portions of saturated $NaHCO_3$, saturated NaCl, 10% citric acid, and saturated NaCl, and solvent was removed by rotary evaporation. Purification of the residue on a Sephadex LH-20 column, eluting with 3% EtOH/toluene, produced 120 mg (47%) of **4** as a yellow solid: $[\alpha]_D$ −41.3° (c 2.34); NMR ($CDCl_3/d_6$-DMSO) δ 3.27 (s, 3 H), 3.53 (dd, 2 H, J = 9, 6), 5.70 (t, 1 H, J = 9), 7.30 (d, 2 H, J = 3), 8.10 (t, 1 H, J = 3). Anal. ($C_{10}H_{11}N_3O_3S$) C, H, N.

(*S*)-Desmethyldesferrithiocin, *N*-[5-(Acetylhydroxyamino)pentyl]hydroxamate (5). BOP (178.7 mg, 0.404 mmol) was added to a solution of **10** (86.0 mg, 0.404 mmol) and **1** (90.58 mg, 0.404 mmol) in DMF (8 mL) at 0 °C. A solution of DIEA (52.2 mg, 0.404 mmol) in DMF (2 mL) was added dropwise to the cold solution. The mixture was stirred at 0 °C for 15 min and at room temperature overnight. Solvent was removed under high vacuum, and the residue was treated with EtOAc (40 mL). Product was isolated and purified by the procedure of **4** to furnish 93 mg (60%) of **5** as an oil: $[\alpha]_D$ −16.7° (c 9.85); NMR (CD_3OD) δ 1.30–1.90 (m, 6 H), 2.06 (s, 3 H), 3.40–3.80 (m, 6 H), 5.93 (t, 1 H, J = 9), 7.33 (d, 2 H, J = 3), 8.06 (t, 1 H, J = 3); HRMS calcd $C_{16}H_{23}N_4O_5S$ 383.1389 (M + 1), $C_{16}H_{22}N_4O_5S$ (M), 382.1311, found 383.1406 (M + 1), 382.1344 (M).

***N*-(*tert*-Butoxycarbonyl)-*N*-(5-chloropentyl)-*O*-benzylhydroxylamine (12).** NaH (80%, 0.591 g, 19.7 mmol) was added in portions to a solution of **11**[36] (4.0 g, 17.91 mmol) in DMF (60 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min, and 1,5-dichloropentane (12.76 g, 89.55 mmol) in DMF (60 mL) was slowly added. The mixture was stirred at 0 °C for an additional 20 min and at 70–80 °C overnight. Solvent was removed under high vacuum, and the residue was cooled to 0 °C and quenched with H_2O (50 mL) at 0 °C. Extraction with CH_2Cl_2 (4 × 40 mL), evaporation of solvent, and purification by silica gel column chromatography, eluting with 1:29:70 EtOAc/$CHCl_3$/hexane, gave 4.32 g (74%) of **12** as an oil: NMR δ 1.47 (s, 9 H), 1.30–1.90 (m, 6 H), 3.40 (t, 2 H, J = 6), 3.47 (t, 2 H, J = 6), 4.80 (s, 2 H), 7.30 (s, 5 H). Anal. ($C_{17}H_{26}ClNO_3$) C, H, N.

***N*-(5-Chloropentyl)-*O*-benzylhydroxylamine (13).** Trifluoroacetic acid (10 mL) was added dropwise to a solution of **12** (3.00 g, 9.15 mmol) in CH_2Cl_2 (60 mL) at 0 °C. The solution was stirred at 0 °C for 20 min and at room temperature for 15 min, and volatile material was removed in vacuo. The residue was treated with saturated $NaHCO_3$ (60 mL) and extracted with CH_2Cl_2 (4 × 50 mL). Evaporation of solvent and purification on a short silica gel column, eluting with CH_2Cl_2, gave **13** (quantitative) as an oil: NMR δ 1.33–1.90 (m, 6 H), 2.87 (t, 2 H, J = 6), 3.43 (t, 2 H, J = 6), 4.30 (br s, 1 H), 4.60 (s, 2 H), 7.18 (s, 5 H). Anal. ($C_{12}H_{18}ClNO$) C, H, N.

***N*-Acetyl-*N*-(5-chloropentyl)-*O*-benzylhydroxylamine (14).** Aqueous 1 N NaOH (25 mL) was added to a solution of **13** (2.13 g, 9.35 mmol) in CH_2Cl_2 (35 mL) at 0 °C. Acetyl chloride (1.123 g, 14.02 mmol) in CH_2Cl_2 (15 mL) was added dropwise at 0 °C, and the biphasic mixture was stirred at 0 °C for 20 min and then at room temperature overnight. The layers were separated, and the aqueous portion was further extracted with CH_2Cl_2 (3 × 50 mL). The organic extracts were washed with brine (100 mL) and concentrated. Purification by silica gel column chromatography using 20% EtOAc/hexane gave 2.41 g (96%) of **14** as an oil: NMR δ 1.33–1.90 (m, 6 H), 2.07 (s, 3 H), 3.48 (t, 2 H, J = 6), 3.60 (t, 2 H, J = 6), 4.80 (s, 2 H), 7.33 (s, 5 H). Anal. ($C_{14}H_{20}ClNO_2$) C, H, N.

***N*-Acetyl-*N'*-(*tert*-butoxycarbonyl)-*N,N'*-bis(benzyloxy)-1,5-pentanediamine (16). Method A.** NaH (80%, 0.323 g, 10.7 mmol) was added in portions to a solution of **11** (2.19 g, 9.79 mmol) in DMF (45 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min, and **14** (2.4 g, 8.9 mmol) in DMF (15 mL) was slowly added following the procedure of **12**. Silica gel column chromatography using 6:2:1 hexane/EtOAc/$CHCl_3$ generated 3.85 g (95%) of **16** as an oil: NMR δ 1.23–1.80 (m, 6 H), 1.47 (s, 9 H), 2.03 (s, 3 H), 3.36 (t, 2 H, J = 6), 3.57 (t, 2 H, J = 6), 4.76 (s, 2 H), 4.78 (s, 2 H), 7.30 (s, 5 H). Anal. ($C_{26}H_{36}N_2O_5$) C, H, N.

Method B. NaH (80%, 1.16 g, 38.7 mmol) was added in portions to **15**[37] (5.9 g, 35.5 mmol) in DMF (50 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min, and a solution of **12** (10.6 g, 32.3 mmol) in DMF (30 mL) was slowly introduced, following the procedure of **12**. Silica gel column chromatography using 10% acetone/hexane gave 7.1 g (50%) of **16** as an oil.

***N*-Acetyl-*N'*-(*tert*-butoxycarbonyl)-*N,N'*-dihydroxy-1,5-pentanediamine (17).** A solution of **16** (1020 mg, 2.234 mmol) was dissolved in distilled CH_3OH (80 mL) and degassed with N_2.

10% Pd–C (0.2 g) was added, and the suspension further degassed by N_2. Hydrogenation (1 atm, room temperature) was carried out for 2 h. The catalyst was filtered and washed with distilled CH_3OH (60 mL). After solvent removal, the crude oil was purified by Sephadex LH-20 column chromatography using 4% EtOH/toluene to give 490 mg (79%) of **17** as an oil: NMR (CD_3OD) δ 1.20–1.83 (m, 6 H), 1.46 (s, 9 H), 2.07 (s, 3 H), 3.40–3.73 (m, 4 H). Anal. ($C_{12}H_{24}N_2O_5$) C, H, N.

***N*-Acetyl-*N*,*N*'-dihydroxy-1,5-pentanediamine Trifluoroacetate (10).** Trifluoroacetic acid (6 mL) was added dropwise to **17** (540 mg, 1.954 mmol) in CH_2Cl_2 (20 mL) at 0 °C following the procedure of **13**. The concentrate was repeatedly treated with dry benzene and dried in vacuo to provide **10** in quantitative yield as an oil: NMR (CD_3OD) δ 1.23–1.87 (m, 6 H), 2.07 (s, 9 H), 3.06–3.23 (m, 2 H), 3.60 (t, 2 H, J = 6).

2-(2'-Hydroxyphenyl)-4(*R*)-thiazolidinecarboxylic acid (6) was prepared as a white solid from 2-hydroxybenzaldehyde and L-cysteine by a known procedure, mp 167–169 °C (dec) (lit. mp 171–173 °C (dec),[40] 164–166 °C[43]).

Benzyl 2-(2'-Hydroxyphenyl)-4(*S*)-oxazolinecarboxylic Acid (20). L-Serine benzyl ester hydrochloride (1.0 g, 4.3 mmol) and **19**[39] (0.788 g, 4.8 mmol) were heated at reflux in CH_3OH (30 mL) under nitrogen for 12 h. Solvent was removed by rotary evaporation and the residue treated with EtOAc (30 mL) and H_2O (30 mL). After stirring for 30 min, the layers were separated, and the aqueous phase was further extracted with EtOAc (30 mL). Concentration of the organic extracts and purification by silica gel column chromatography using $CHCl_3$ produced 703 mg (56%) of **20** as a pale yellow solid: NMR δ 4.4–4.7 (m, 1 H), 4.85–5.10 (dd, 2 H, J = 11, 7), 5.19 (s, 2 H), 6.7–7.9 (m, 9 H), 11.55 (s, 1 H). Anal. ($C_{17}H_{15}NO_4$) C, H, N.

2-(2'-Hydroxyphenyl)-4(*S*)-oxazolinecarboxylic Acid (8). Compound **20** (1.0 g, 3.4 mmol) was dissolved in distilled CH_3OH (30 mL), and 10% Pd–C (36 mg) was introduced under N_2. The mixture was stirred under hydrogen (1 atm, room temperature) for 1 h, solids were filtered, and the filtrate was concentrated. Chromatography with Sephadex LH-20 eluting with 1% EtOH/toluene afforded 453 mg (65%) of **8** as a glass: NMR (10:1 $CDCl_3$/d_6-DMSO) δ 4.7–5.1 (m, 3 H), 6.7–7.7 (m, 6 H). Anal. ($C_{10}H_9NO_4$) C, H, N.

Benzyl 2-(2'-Hydroxyphenyl)-4(*R*)-oxazolinecarboxylic acid (21) was prepared from D-serine benzyl ester benzenesulfonate[38] and **19** by the method of **20**.

2-(2'-Hydroxyphenyl)-4(*R*)-oxazolinecarboxylic acid (9) was synthesized from **21** by the method of **8**. Anal. ($C_{10}H_9NO_4$) C, H, N.

DL-2-(3'-Hydroxypyrid-2'-yl)-4*H*-5,6-dihydro-1,3-thiazine-4-carboxylic Acid (7). DL-Homocysteine (928 mg, 6.66 mmol) was suspended in distilled CH_3OH (20 mL) and phosphate buffer (12 mL) at pH 5.95. The mixture was degassed with N_2, and **18**[41] (400 mg, 3.33 mmol) was added in portions. The mixture was stirred at 50 °C for 4 days; after 5 h, the pH was adjusted to 6. Solvent was removed by rotary evaporation, and the pH was adjusted to 2.8 by concentrated phosphoric acid at 0 °C. The precipitate was filtered, washed with ice water, and dried azeotropically with benzene and then under high vacuum to produce 546 mg (71%) of **7** as a solid: NMR δ 1.66–1.80 (m, 1 H), 2.32–2.52 (m, 1 H), 3.00–3.30 (m, 2 H), 4.58 (dd, 1 H, J = 10, 4.5), 7.34 (d, 2 H, J = 3), 8.20 (t, 1 H, J = 3), 13.10 (br s, 1 H), 14.10 (br s, 1 H). Anal. ($C_{10}H_{10}N_2O_3S$) C, H, N.

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