Design, Synthesis, and Evaluation of Novel 2-Substituted 3-Hydroxypyridin-4-ones: Structure−Activity Investigation of Metalloenzyme Inhibition by Iron Chelators[§]

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A range of novel 3-hydroxypyridin-4-ones with different R_2 substitutents has been synthesized for the investigation of the structure−activity relationship between the chemical nature of the ligand and the inhibitory activity of the iron-containing metalloenzyme 5-lipoxygenase. Results indicate that the molecular dimensions, together with the lipophilicity, have a critical impact on the ability of this class of chelator to inhibit 5-lipoxygenase. Hydrophilic ligands with a bulky R_2 substitutent tend to be weak inhibitors; thus 1,6-dimethyl-2-(4′-*N*-*n*-propylsuccinamido)methyl-3-hydroxypyridin-4(1*H*)-one (**22b**) which has the largest R_2 substitutent only caused 2% inhibition of the enzyme activity after 30 min incubation at 110 μM IBE (iron-binding equivalents), as compared with deferiprone which caused 40% inhibition of the enzyme activity, under the same conditions.

Transfusion dependent patients such as those suffering from β-thalassaemia develop a potentially fatal secondary haemosiderosis, and consequently, a selective iron chelator must be used to relieve such iron overload.[1] Naturally occurring siderophores are good models for such molecules but, due to their physicochemical properties, are not well absorbed from the mammalian intestine.[2] Desferrioxamine-B (DFO), the most widely used iron chelator in haematology over the past 30 years, has a major disadvantage of being orally inactive.[3] Consequently, the successful design of an orally active, nontoxic, selective iron chelator has been a much sought after goal for medicinal chemists for the past 25 years.

3-Hydroxypyridin-4-ones (HPOs) are currently one of the main candidates for the development of orally active iron chelators.[4] Indeed, the 1,2-dimethyl derivative (deferiprone, CP20) **1a** (Table 1) is the only orally active iron chelator currently available for clinical use (marketed by Apotex Inc., Toronto, Canada, as Ferriprox). Although such small bidentate ligands possess a clear advantage over hexadentate ligands with respect to oral bioavailability, they also have enhanced access to the active sites of metalloenzymes and thus tend to be more potent inhibitors than hexadentate siderophores.[4,5] In general, iron(III) chelating agents do not inhibit heme-containing enzymes or iron-sulfur cluster proteins; they do, however, interfere with enzymes containing mono-iron and bi-iron centers coordinated to oxygen ligands, including lipoxygenases and aromatic amino acid hydroxylases.[6,7] It has been previously shown that the non-heme iron-containing 5-lipoxygenase (5-LO), in which the iron center is dominated by oxygen and imidazole ligands, is rapidly inhibited by bidentate hydroxypyridinone chelators such as deferiprone, but significantly more slowly by the larger hexadentate iron chelator DFO.[8] A preliminary structure−activity investigation on a range of 3-hydroxypyridin-4-ones (Table 1) indicated that the lipophilicity of the molecule has a critical impact in controlling the ability of this class of chelator to inhibit the enzyme and that hydrophilic ligands tend to be weak inhibitors (Figure 1).[9] In these preliminary studies, however, variation of the substitution was only examined on the 1-position of the pyridinone ring (R_1) (Table 1), and it was not possible to differentiate the size and shape effects from those of hydrophobicity. As a result of previous investigations with the plant enzyme tyrosinase, it has been proposed that the bulk of the 2-substituent reduces the ability of hydroxypyridinone chelators to cause inhibition.[10] Should such a phenomenon be observed with 5-lipoxygenase, increasing the length of the R_2 substituent would be predicted to diminish enzyme inhibition. In the present study, a range of novel 3-hydroxypyridin-4-ones with different R_2 substituents has been synthesized in order to facilitate a more detailed structure/activity investigation with 5-lipoxygenase.

Results

Chemistry. The 2-alkyl-substituted 3-hydroxypyridin-4-ones **5a**−**d**, were conveniently prepared using the established methodologies outlined in Scheme 1.[11] The synthetic route employed for the 2-methoxymethyl analogue **12** is summarized in Scheme 2. Allomaltol **7** was readily prepared from commercially available kojic acid **6** in a two-step reaction by following an established procedure.[12] The α-position to the ring hydroxyl was then functionalized in an analogous fashion to that of the aldol condensation. The 3-hydroxyl group was then protected using a benzyl group. Methylation of the

[§] Abbreviations: DFO, desferrioxamine-B; HPO, 3-hydroxypyridin-4-one; 5-LO, 5-lipoxygenase; IBE, iron-binding equivalent; $D_{7.4}$, distribution coefficient at pH 7.4; MOPS, 4-morpholinepropane sulfonic acid.

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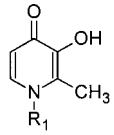
Table 1. Percentage Inhibition of 5-lipoxygenase by a Range of 3-hydroxypyridin-4-ones with Different R_1 Substituents



compound	R_1	MW	logP	% inhibition	biological activity[a]
1a (deferiprone)	CH_3	139	−0.77	39.4	−0.19
1b	CH_2CH_3	153	−0.31	58.4	0.15
1c	$CH_2CH_2CH_2CH_3$	181	0.70	80.0	0.60
1d	$CH_2CH_2CH_2CH_2CH_3$	195	1.24	90.0	0.95
1e	$CH_2CH_2CH_2CH_2CH_2CH_3$	209	1.90	93.2	1.14
1f	$CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_3$	237	2.88	98.7	1.86

[a] Calculated biological activity using the logit transformation.

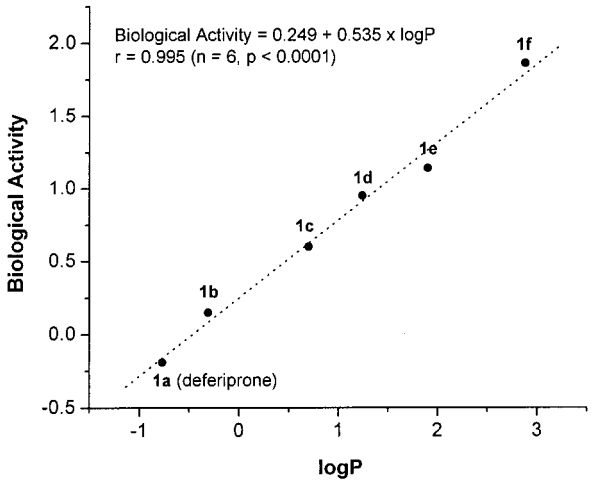
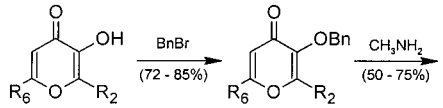


Figure 1. Relationship between lipophilicity and the inhibition of 5-lipoxygenase by HPO chelators with different R_1 substituents.

Scheme 1

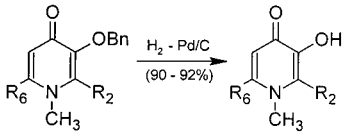


2a: R_2 = H, R_6 = H — **3a - 3d**

2b: R_2 = CH_2CH_3, R_6 = H

2c: R_2 = $CH_2CH_2CH_3$, R_6 = CH_3

2d: R_2 = $CH_2CH_2CH_2CH_3$, R_6 = CH_3

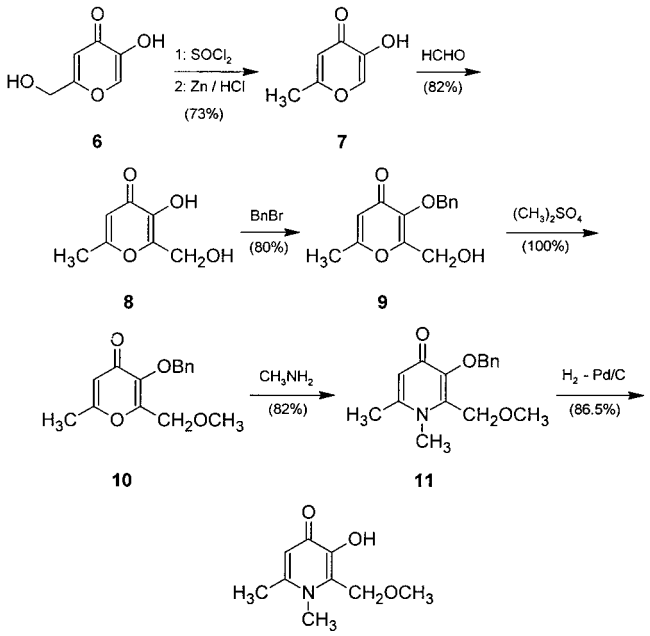


4a - 4d **5a - 5d**

2-hydroxymethyl group was achieved using dimethyl sulfate in a two-phase system of dichloromethane and 50% aqueous sodium hydroxide in the presence of a catalytic amount of tetrabutylammonium hydrogen sulfate (TBAHS). The desired product **12** was then prepared by the reaction of the protected pyranone **10** with methylamine, followed by hydrogenation to remove the benzyl group.

The R_2 substitutent of hydroxypyridinones can be further modified using the methodology illustrated in Scheme 3. The direct conversion of the benzylated 2-hydroxmethyl pyranone **9** to the corresponding *N*-methyl pyridinone analogue **13** was unsuccessful, marked

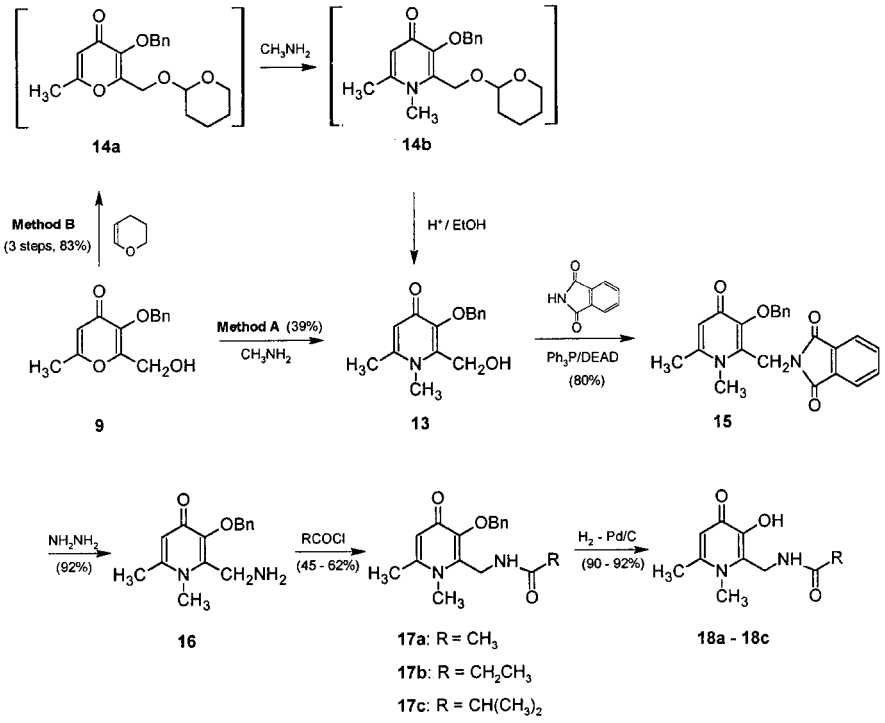
Scheme 2



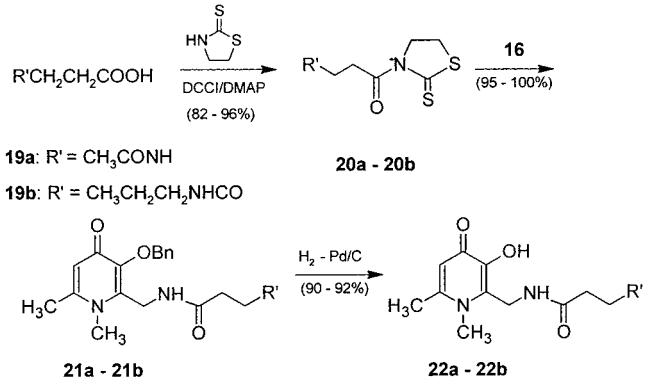
6 **7** **8** **9** **10** **11** **12**

decomposition resulting in poor yields (method A, yield <40%). Consequently, the 2-hydroxyl function of **9** was protected using 3,4-dihydro-2*H*-pyran before reacting with methylamine (method B). The pyran protecting group was then selectively removed under mild acid conditions without deprotection of the benzyl group, resulting the desired protected 2-hydroxymethyl pyridinone **13** in a much improved yield (method B, overall yield >80%). The conversion of 1,6-dimethyl-2-hydroxymethyl-3-benzyloxypyridin-4(1*H*)-one **13** to the corresponding phthalimido intermediate, **15**, was accomplished using phthalimide, triphenylphosphine, and diethyl azodicarboxylate (DEAD) in good yield. The reaction is believed to proceed through the formation of a quaternary phosphonium salt by addition of triphenylphosphine to DEAD, which reacts in situ with the pyridinone **13** to form an alkoxyphosphonium salt.[13] The phosphonium moiety was then displaced by the phthalimido anion to furnish the desired phthalimido derivative **15**, which was subsequently reacted with hydrazine to afford the amine derivative **16**. Acylation of the 2-aminomethyl pyridinone **16** with acid chlorides in the presence of catalytic bases such as pyridine afforded the protected 2-(*N*-acylaminomethyl) pyridinones **17a**−**c** in moderate yields (45−60%).

Scheme 3



Scheme 4



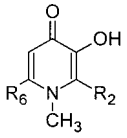
Two 3-hydroxypyridin-4-ones **22a**,**b** with further extended R_2 substitutents were also synthesized (Scheme 4). *N*-Acetyl-β-alanine **19a** and *N*-*n*-propyl succinamic acid **19b** were activated by coupling with 2-mercaptothiazoline using dicyclohexylcarbodiimide (DCCI) and 4-(dimethylamino)pyridine (DMAP) to afford the corresponding active amides **20a** and **20b** in good yields. The desired products **22a**,**b** were obtained by coupling the active amides **20a**,**b** with the protected 2-aminomethyl pyridinone **16** (~100% yield), followed by hydrogenation to remove the protecting group.

Partition Coefficients. Since the degree of ionization of the two functional groups on the pyridinone ring is relatively small at pH 7.4, the neutral species of the compounds predominates (>98%).[14,15] Hence the distribution coefficients of the compounds at pH 7.4 are expected to be almost identical to their partition coefficients. The logP values of this series of compounds cover the range −2 to +1, the most hydrophobic compound being the 2-butyl analogue **5d** (Table 2). The introduction of an oxygen atom in the R_2 substitutent reduces the lipophilicity of the molecules, thus the 2-methoxymethyl compound (logP = −0.41) is less hydrophobic than the 2-ethyl analogue (logP = −0.21). The lipophilicity of the molecules is further decreased by the introduction of an amido function, thus the diamide analogue **22a** is the most hydrophilic compound in the group (logP = −1.70).

Inhibition of 5-Lipoxygenase. Lipoxygenases are non-heme iron-containing enzymes that catalyze the site-specific oxygenation of polyunsaturated fatty acids to produce hydroperoxides. 5-Lipoxygenase (5-LO) is the principal lipoxygenase in the human neutrophil, and it catalyses the first two steps in the conversion of its substrate arachidonic acid to leukotriene A_4 which is the precursor of the potent inflammatory mediator leukotriene B_4.[16] The inhibition of the enzyme was evaluated using soybean lipoxygenase spectrophotometric assay by monitoring the reduction on the peroxidation product linoleic hydroperoxide.[8,17] Hydroxypyridinones with different R_2 substituents show variable inhibitory properties toward 5-LO, ranging from **5a** which caused nearly 60% inhibition of the enzyme activity after 30 min incubation at 110 μM IBE to **22b** which caused only 2% inhibition of the enzyme activity under the same conditions (Table 2). Deferiprone **1a** is a relatively potent inhibitor, causing 40% inhibition of the enzyme activity after 30 min incubation.

Structure−Activity Investigation. The molecular width, the extended R_2 length, and ClogP value of the R_2 chain have been calculated for QSAR investigation (Table 2). The ACD ChemSketch program, version 4.01, was used to calculate the molecular width and the extended R_2 chain length.[18] Structures were 3D-optimized, and the widths of the molecules between R_2 and R_6 substituents along with the lengths for the R_2 chain were measured from the optimized structures. The ClogP values of the equivalent isolated R_2 chain were calculated using the CLOGP program.[19] The enzyme percentage inhibition at a fixed dose was transformed into a general biological response using the

Table 2. Physicochemical Properties and 5-Lipoxygenase Inhibitory Activity of 2-Substituted 3-Hydroxypyridin-4-ones



ligands	R_2	R_6	MW	$D_{7.4}$	logP	ClogP (R_2)	R_2 length (Å)	molecular width (Å)	% inhibition	biological activity
5a	H	H	125	0.25 ± 0.04	−0.60	0.20	1.10	4.32	59.4	0.16
1a	CH_3	H	139	0.17 ± 0.01	−0.77	0.88	2.15	5.55	39.4	−0.19
5b	CH_2CH_3	H	153	0.62 ± 0.01	−0.21	1.53	3.48	6.79	48.5	−0.03
5c	$CH_2CH_2CH_3$	CH_3	181	2.50 ± 0.10	0.40	2.05	4.70	9.25	30.0	−0.37
5d	$CH_2CH_2CH_2CH_3$	CH_3	195	8.05 ± 1.70	0.90	2.58	5.97	10.50	29.6	−0.38
12	CH_2OCH_3	CH_3	183	0.39 ± 0.07	−0.41	−0.42	4.44	8.95	14.8	−0.76
18a	$CH_2NHCOCH_3$	CH_3	210	0.15 ± 0.01	−0.82	−1.31	5.83	10.36	18.5	−0.64
18b	$CH_2NHCOCH_2CH_3$	CH_3	224	0.19 ± 0.02	−0.72	−0.78	7.12	11.67	13.5	−0.81
18c	$CH_2NHCOCH(CH_3)_2$	CH_3	238	0.45 ± 0.01	−0.35	−0.47	7.10	11.68	21.0	−0.58
22a	$CH_2NHCO(CH_2)_2NHCOCH_3$	CH_3	281	0.02 ± 0.004	−1.70	−1.67	10.88	15.43	3.8	−1.40
22b	$CH_2NHCO(CH_2)_2CONHCH_2CH_2CH_3$	CH_3	309	0.20 ± 0.005	−0.70	−0.61	13.50	18.08	2.1	−1.67

Table 3. Single Regression Analysis of the Influence of Individual Molecular Descriptors on 5-Lipoxygenase Inhibitory Activity

	molecular width	R_2 length	MW	ClogP (R_2)	logP
r	−0.949	−0.947	−0.946	0.611	0.462
r^2	0.901	0.897	0.894	0.373	0.213
r^2 (adj)	0.890	0.886	0.883	0.304	12.6
p	<0.001	<0.001	<0.001	0.046	0.153
A^a	0.713	0.258	1.246	−0.650	−0.436
B^a	−0.129	−0.144	−0.009	0.240	0.376

[a] Biological activity (Y) = $A + B \cdot$ (molecular descriptors).

logistic model.[20] Thus the biological activity adopted for the QSAR studies is expressed as[21]

$$\mathrm{BA} = \left(\frac{\mathrm{MW}}{d}\right) \log\left(\frac{P}{100 - P}\right)$$

where BA is the biological activity, P the enzyme percentage inhibition at a fixed dose, d the dose, and MW the molecular weight of the compound. Since the enzyme experiments were conducted at equimolar concentrations, the doses were equivalent and the biological response can then be simplified as

$$\mathrm{BA} = \log\left(\frac{P}{100 - P}\right)$$

The logit transformation can present more comprehensive information on the inhibitory potency of the molecules than a simple response for a fixed dose[21] and therefore will provide a more reliable statistical prediction.[21,22] Such logit transformation has been widely used in enzymology QSAR studies.[23,24]

Statistical calculations were performed with the Minitab program, version 13.[25] The influence of individual descriptors (Table 2) was first assessed through simple regression analysis between the corresponding descriptors and biological activity (BA). Goodness of fit of models were determined from the r^2 values (Table 3) and ranked according to their goodness of fit, along with the fitted parameters of the equation $Y = A + BX$. The most influential parameters were found to be the molecular width and the extended R_2 length, with r^2 values of 0.901 and 0.897, respectively (Figure 2). Since the main structure modification in this series of compounds is the R_2 substitutent, the molecular width is

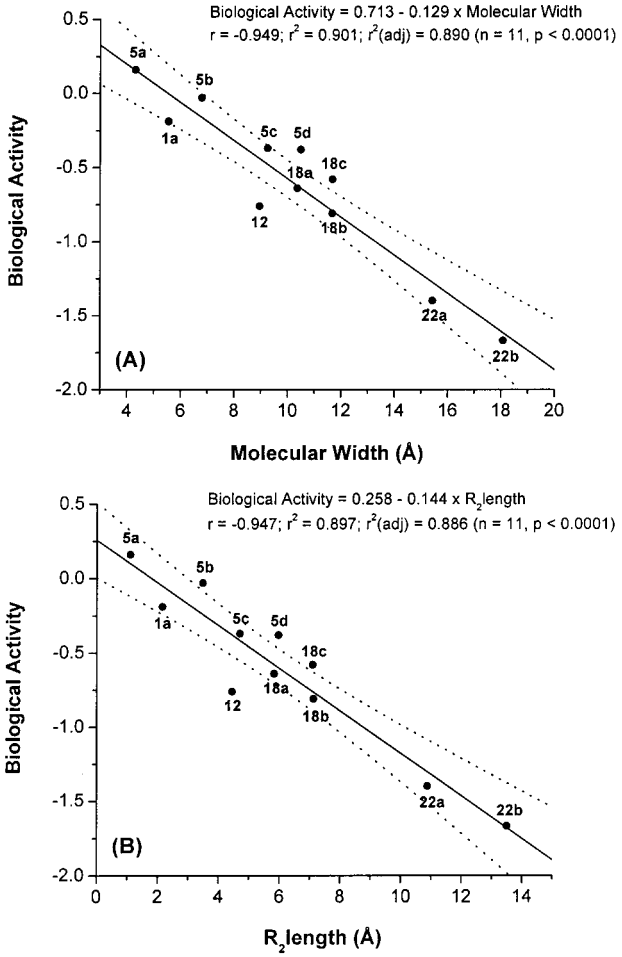


Figure 2. Correlation between (A) the molecular width and (B) the extended R_2 length with the inhibition of 5-lipoxygenase by HPO chelators with different R_2 substituents. (Dotted lines indicate 95% confidence bands.)

highly correlated with the extended R_2 length (r^2 = 0.989, p < 0.001).

Although the influence of the two lipophilicity predictors (the measured logP and the calculated ClogP for the R_2 substitutent) were found to be insignificant in the single regression analysis (Table 3), they were influential in the multiple regression analysis. The best conservative prediction model was found to be the two-

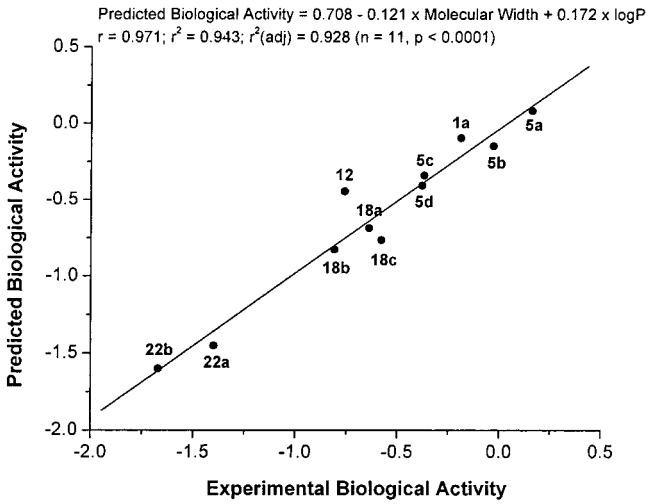


Figure 3. Two-parameter prediction model of 5-lipoxygenase inhibition by HPO chelators with different R_2 substituents using the molecular width and the measured logP value.

Table 4. Iron Mobilization Efficacy Comparison of **22a**, **22b**, and **1a** in the ^{59}Fe-Ferritin Loaded Rat Model[a]

chelator	iron mobilization (%)	efficacy (%)
control	3.87 ± 1.0	
1a	13.4 ± 5.2	9.5
22a	9.7 ± 2.4	5.9
22b	23.3 ± 3.7	19.4

[a] All chelators (450 μmol/kg) were given orally, and control rats were administered with an equivalent volume of water. Values are expressed as means ± SD (n = 5).

parameter model using the molecular width and the measured logP value (Figure 3), which provides an excellent prediction for this series of compounds (r^2 = 0.943).

Iron Mobilization Efficacy in Rats. In vivo iron scavenging abilities of **22a** and **22b** were compared with that of deferiprone **1a** in a non-iron-overloaded rat model originally developed by Pippard et al.[26] ^{59}Fe-ferritin is used to label the liver iron pool, and this is followed by a challenge with a test chelator at a time when the iron released by lysosomal degradation of ferritin is maximally available.[26,27] Since the liver is the major iron storage organ under iron-overloaded conditions, this method comes close to being an ideal animal model to assess oral bioavailability and to compare the ability of chelators to remove iron from liver.[27] All chelators were administered orally, and the dose of chelator was 450 μmol/kg. Compound **22a** was found to be a poor scavenger of iron under in vivo conditions, with an associated efficacy of 8.9% (Table 4). In contrast, the more lipophilic analogue **22b** was found to be more effective than **1a**.

Discussion

It has been previously established that direct access and chelation of the iron center is the major mechanism for lipoxygenase inhibition by small bidentate HPO chelators.[8] Indeed, using crystallographic studies, Boyington and co-workers have demonstrated the existence of two internal channels. Of the two channels, the one which provides the access route for oxygen is likely to accommodate small chelators, the other channel which binds the lipid substrate being too narrow (3.5 Å).[28] This oxygen channel is lined by hydrophobic amino acids, and therefore the ingress of lipophilic chelators will be favored, while that of hydrophilic molecules will be retarded. Thus hydrophilic chelators are predicted to be less inhibitory to 5-lipoxygenase than hydrophobic analogues. Although this relationship holds well for hydroxypyridinones, where the size of the alkyl substitutent is increased in the 1-position of the pyridinone ring (Figure 1), it is less evident for compounds with large substituents in the 2-position. In fact, the variation in the inhibitory properties of the HPO chelators possessing different R_2 substituents is more dependent on the size of the substituent than the lipophilicity of the chelator (Table 3). Although no simple correlation exists between the lipophilicity of these chelators and their ability to inhibit 5-LO, a correlation was observed between the molecular dimensions and inhibitory properties—inhibition decreasing with increasing the width of the molecule and in particular the R_2 substituents (Figure 2). Thus **5a**, which only has a hydrogen at the 2-position (molecular width = 4.32 Å; R_2 length = 1.10 Å), causes the maximum inhibition observed with this series, whereas **22b** which has a relatively rigid large substituent (molecular width = 18.08 Å; R_2 length = 13.50 Å) was found to cause the minimum inhibition (Table 2). Clearly, the strong dependence of the inhibition of 5-LO activity on the size of the 2-substituent is readily explained by restricted access of the chelator to the enzyme iron-binding site. Significantly the oxygen channel of lipoxygenase is conical in shape and 18 Å in length, tapering from 11 Å diameter at the protein surface to 2.5 Å at the iron-binding site, with most of this channel being 8 Å in diameter.[28] Thus the introduction of bulky substituents at the 2-position of the pyridinone ring will decrease the accessibility of the chelator to the iron center and therefore reduce its inhibitory ability. Multiple regression analysis using a two-parameter model further indicates that the inhibition of 5-LO activity by HPO chelators depends on the molecular dimensions as well as its lipophilicity (Figure 3). Ligands which have lengthy rigid R_2 substituents coupled with an overall hydrophilic nature of the molecule tend to be weak inhibitors. The inhibitory property of **22b** approaches that of DFO, which does not inhibit the enzyme activity even after 24 h incubation at a concentration of 110 μM IBE.

In vivo iron mobilization efficacy of the two compounds which possess the least inhibitory activity, namely **22a** and **22b**, have been compared with deferiprone **1a** in the ^{59}Fe-ferritin labeled rat model. Compound **22a** was found to be less effective than **1a** (Table 4). This may be attributed to the relatively low logP value associated with this molecule (Table 2). Such low lipophilicity may lead to poor oral absorption and liver extraction. Consequently, inefficient iron mobilization is expected. In contrast, the more hydrophobic analogue **22b** was found to be twice as effective than **1a**. This molecule therefore has a clear advantage over **1a** of being a powerful iron scavenger as well as a weaker inhibitor of 5-lipoxygenase and probably other related metalloenzymes.

In summary, 5-lipoxygenase was found to be more susceptible toward inhibition by small hydrophobic HPO chelators. In contrast, hydrophilic molecules with bulky

R_2 substituents are relatively weak inhibitors. This finding will facilitate the design of nontoxic orally active iron chelators.

Experimental Section

Chemistry. Ethyl maltol (**2b**) was purchased from Pfizer Ltd. (Widnes, U.K.). Kojic acid **(6)** was purchased from Fluka. All other chemicals were obtained from Aldrich. Melting points were determined using an electrothermal IA 9100 digital melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded using a Perkin-Elmer (60 MHz) NMR spectrometer. Chemical shifts (δ) are reported in ppm downfield from the internal standard tetramethylsilane (TMS). Mass spectra (FAB) analyses were carried out by the Mass Spectrometry Facility, Department of Pharmaceutical and Biological Chemistry, The School of Pharmacy, 29/39 Brunswick Square, London WC 1AX, U.K. (in 3-nitrobenzyl alcohol matrix). Elemental analyses were performed by Micro Analytical Laboratories, Department of Chemistry, The University of Manchester, Manchester M13 9PL, U.K.

3-Hydroxypyran-4(1*H*)-one (pyromeconic acid) (2a), **2-propyl-3-hydroxypyran-4(1*H*)-one (2c)**, and **2-butyl-3-hydroxypyran-4(1*H*)-one (2d)** were synthesized as described by Ellis et al.[12]

3-Benzyloxypyran-4(1*H*)-one (3a): To a solution of pyromeconic acid (**2a**) (5.6 g, 50 mmol, 1 equiv) in methanol (50 mL) was added sodium hydroxide (2.2 g, 55 mmol, 1.1 equiv) dissolved in water (5 mL), and the mixture was heated to reflux. Benzyl bromide (9.4 g, 0.55mol, 1.1 equiv) was added dropwise over 30 min, and the resulting mixture was refluxed for 6 h. After removal of solvent by rotary evaporation, the residue was mixed with water (50 mL) and extracted with dichloromethane (3 × 50 mL). The combined extracts were washed with 5% aqueous sodium hydroxide (50 mL) followed by water (2 × 50 mL). The organic fraction was then dried over anhydrous sodium sulfate, filtered, and rotary evaporated to give the crude product. Further purification by column chromatography on silica gel (eluant: ethyl acetate) furnished a yellow crystalline solid (8.2 g, 80%): mp 82−84 °C (lit. value[12] 84−85 °C); ^{1}H NMR ($CDCl_3$) δ 5.10 (s, 2H, **CH_2**Ph), 6.50 (d, 1H, **5-H**(pyranone)), 7.55 (s, 5H, **Ar**), 8.25 (d, 1H, **6-H**(pyranone)), 8.35 (s, 1H, **2-H**(pyranone)).

Analogous syntheses starting with **2b**−**d** gave compounds **3b**−**d**.

2-Ethyl-3-benzyloxypyran-4(1*H*)-one (3b) (85%): mp 33−34 °C (lit. value[11] 33−34 °C); ^{1}H NMR ($CDCl_3$) δ 1.0 (t, 3H, 2-CH_2**CH_3**), 2.55 (q, 2H, 2-**CH_2**CH_3), 5.13 (s, 2H, **CH_2**Ph), 6.3 (d, 1H, **5-H**(pyranone)), 7.35 (s, 5H, **Ar**), 7.6 (d, 1H, **6-H**(pyranone)).

2-Propyl-3-benzyloxy-6-methyl-pyran-4(1*H*)-one (3c) (72%): mp 65−68 °C; ^{1}H NMR ($CDCl_3$) δ 0.85 (t, 3H, 2-CH_2-CH_2**CH_3**), 1.25−1.50 (m, 2H, 2-CH_2**CH_2**CH_3) 2.15 (s, 3H, **6-CH_3**), 2.40 (t, 2H, 2-**CH_2**CH_2CH_3), 5.10 (s, 2H, **CH_2**Ph), 6.05 (s, 1H, **5-H** (pyranone)), 7.30 (s, 5H, **Ar**); MS (FAB) *m*/*z*, 258 [M^+]. Anal. ($C_{16}H_{18}O_3$) C, H.

2-Butyl-3-benzyloxy-6-methyl-pyran-4(1*H*)-one (3d) (82%): mp 52−54 °C; ^{1}H NMR ($CDCl_3$) δ 0.80 (t, 3H, 2-CH_2-CH_2CH_2**CH_3**), 1.05−1.45 (m, 4H, 2-CH_2**CH_2CH_2**CH_3) 2.10 (s, 3H, **6-CH_3**), 2.35 (t, 2H, 2-**CH_2**$CH_2CH_2CH_3$), 5.00 (s, 2H, **CH_2**-Ph), 6.00 (s, 1H, **5-H** (pyranone)), 7.20 (s, 5H, **Ar**); MS (FAB) *m*/*z*, 272 [M^+]. Anal. ($C_{17}H_{20}O_3$) C, H.

1-Methyl-3-hydroxypyridin-4(1*H*)-one Hydrochloride (5a). To a solution of **3a** (2.0 g, 10 mmol) in ethanol (15 mL) was added 15 mL of 40% aqueous methylamine, and the reaction mixture was sealed in a thick-walled glass tube. After being stirred at 70 °C for 12 h, the resulting solution was reduced in volume to 10 mL by rotary evaporation prior to addition of water (40 mL). The mixture was then adjusted to pH 1 with concentrated hydrochloric acid and washed with diethyl ether (2 × 50 mL). Subsequent adjustment of the aqueous fraction to pH 9 with 10 N sodium hydroxide solution was followed by extraction into dichloromethane (4 × 50 mL), the combined organic layers then being dried over anhydrous sodium sulfate, filtered, and rotary evaporated to give a brown oil. Purification by column chromatography on silica gel (eluant: methanol:chloroform, 1:9 v/v) gave the protected pyridinone (**3a**) as a yellow oil, which was subsequently dissolved in ethanol (25 mL)/water (5 mL) and adjusted to pH 1 with hydrochloric acid prior to hydrogenolysis for 2 h in the presence of 5% Pd/C catalyst (0.40 g). Filtration followed by rotary evaporation gave a light pink solid, which was further purified by recrystallization from methanol/diethyl ether to yield a white crystalline solid (1.16 g, 72%): mp 122−123 °C; ^{1}H NMR (DMSO-d_6) δ 4.1 (s, 3H, **N-CH_3**), 7.3 (d, 1H, **5-H**(pyridinone)), 8.15 (d, 1H, **6-H**(pyridinone)), 8.25 (d, 1H, **2-H** (pyridinone)); MS (FAB) *m*/*z*, 126 [$(M - Cl)^+$]. Anal. ($C_6H_8NO_2Cl$) C, H, N.

Analogous syntheses starting with **3b**−**d** gave compounds **5b**−**d**.

1-Methyl-2-ethyl-3-hydroxypyridin-4(1*H*)-one hydrochloride (5b) (76%): mp 185−190 °C; ^{1}H NMR (DMSO-d_6) δ 1.20 (t, 3H, 2-CH_2**CH_3**), 3.00 (q, 1H, 2-**CH_2**CH_3), 3.95 (s, 3H, **N-CH_3**), 7.35 (d, 1H, **5-H**(pyridinone)), 8.2 (d, 1H, **6-H** (pyridinone)), 7.8−8.6 (br., **OH**); MS (FAB) *m*/*z*, 154 [$(M - Cl)^+$]. Anal. ($C_8H_{12}NO_2Cl$) C, H, N.

1,6-Dimethyl-2-propyl-3-hydroxypyridin-4(1*H*)-one hydrochloride (5c) (63%): mp 210−213 °C; ^{1}H NMR (DMSO-d_6) δ 1.10 (t, 3H, 2-CH_2CH_2**CH_3**), 1.50−1.85 (m, 2H, 2-CH_2**CH_2**-CH_3) 2.65 (s, 3H, **6-CH_3**), 3.05 (t, 2H, 2-**CH_2**CH_2CH_3), 3.95 (s, 3H, **N-CH_3**), 7.05 (s, 1H, **5-H**(pyridinone)); MS (FAB) *m*/*z*, 182 [$(M - Cl)^+$]. Anal. ($C_{10}H_{16}NO_2Cl$) C, H, N.

1,6-Dimethyl-2-butyl-3-hydroxypyridin-4(1*H*)-one hydrochloride (5d) (51%): mp 193−196 °C; ^{1}H NMR (DMSO-d_6) δ 1.05 (t, 3H, 2-$CH_2CH_2CH_2$**CH_3**), 1.35−1.80 (m, 4H, 2-CH_2**CH_2CH_2**CH_3) 2.65 (s, 3H, **6-CH_3**), 3.00 (t, 2H, 2-**CH_2**-$CH_2CH_2CH_3$), 4.00 (s, 3H, **N-CH_3**), 6.95 (s, 1H, **5-H**(pyridinone)); MS (FAB) *m*/*z*, 196 [$(M - Cl)^+$]. Anal. ($C_{11}H_{18}NO_2Cl$) C, H, N.

2-Methyl-5-hydroxypyran-4(1*H*)-one (allomaltol) (7) was synthesized from kojic acid (**6**) in two steps using the established procedure.[12]

2-Hydroxymethyl-3-hydroxy-6-methyl-pyran-4(1*H*)-one (8). Allomaltol **7** (12.6 g, 100 mmol) was added to an aqueous solution of sodium hydroxide (4.4 g, 110 mmol) in distilled water (100 mL) and stirred at room temperature for 5 min. Formaldehyde solution (35%, 9 mL) was added dropwise over 10 min, and the solution was stirred for 12 h. Acidification to pH 1 using concentrated hydrochloric acid and cooling to 3−5 °C for 12 h gave a crystalline deposit (12.8 g, 82%): mp 159−161 °C (lit. value[12] 157−158 °C); ^{1}H NMR (DMSO-d_6) δ 2.3 (s, 3H, **6-CH_3**), 4.5 (s, 2H, 2-**CH_2**OH), 4.6−5.7 (br., 1H, 2-CH_2**OH**), 6.25 (s, 1H, **5-H**), 8.7−9.2 (br., 1H, **3-OH**).

2-Hydroxymethyl-3-benzyloxy-6-methyl-pyran-4(1*H*)-one (9). Sodium hydroxide (4.84 g, 121 mmol, 1.1 equiv) dissolved in 10 mL of distilled water was added to a solution of **8** (17.2 g, 110 mmol, 1 equiv) in methanol (100 mL), and the reaction mixture was heated to reflux. Benzyl bromide (20.7 g, 121 mmol, 1 equiv) was added dropwise over 30 min and the resulting mixture then refluxed for 12 h. The reaction mixture was concentrated in vacuo, the residue taken up into dichloromethane (300 mL), and the inorganic salts filtered off. The dichloromethane layer was washed with 5% sodium hydroxide solution (2 × 100 mL) and water (100 mL), dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo to yield the crude product as a yellow crystalline solid. Recrystallization from dichloromethane/petroleum ether 40/60 afforded a white crystalline solid (21.6 g, 80%): mp 115−116 °C; ^{1}H NMR ($CDCl_3$) δ 2.2 (s, 3H, **6-CH_3**), 2.6 (br., s, 1H, 2-CH_2**OH**), 4.3 (br., s, 2H, 2-**CH_2**OH), 5.2 (s, 2H, **CH_2**Ph), 6.15 (s, 1H, **5-H**(pyranone)), 7.4 (s, 5H, **Ar**); MS (FAB) *m*/*z*, 246 [M^+]. Anal. ($C_{14}H_{14}O_4$) C, H.

2-Methoxymethyl-3-benzyloxy-6-methyl-pyran-4(1*H*)-one (10). To a solution of **9** (2.46 g, 10 mmol, 1 equiv) in 30 mL of dichloromethane were added 30 mg of tetrabutylammonium hydrogen sulfate and 50% (w/w) aqueous sodium hydroxide (from 1.2 g of sodium hydroxide and 1.2 g of water), and the mixture was stirred vigorously for 30 min. The reaction mixture was then cooled in an ice−water bath, and

1.9 g (15 mmol, 1.5 equiv) of dimethyl sulfate was added dropwise over a period of 1 h. The mixture was stirred vigorously at room temperature for 3 h until TLC analysis (eluant: ethyl acetate) revealed that the reaction was completed. A total of 2 mL of concentrated aqueous ammonia was added and stirred for a further 30 min at room temperature to decompose excess dimethyl sulfate. The reaction mixture was poured into water, and 50 mL of dichloromethane was added. The dichloromethane layer was washed with water, dried (Na_2SO_4), and concentrated in vacuo to yield the crude product as an orange oil (2.6 g, 100%) which solidified on cooling. Recrystallization from dichloromethane/petroleum ether 40/60 afforded a white crystalline solid (2.35 g, 90%): mp 33−35 °C; ^{1}H NMR ($CDCl_3$) δ 2.25 (s, 3H, **6-CH$_3$**), 3.26 (s, 3H, O**CH$_3$**), 4.2 (s, 2H, 2-**CH$_2$**OCH$_3$), 5.18 (s, 2H, **CH$_2$**Ph), 6.16 (s, 1H, **5-H**(pyranone)), 7.35 (s, 5H, **Ar**); MS (FAB) *m*/*z*, 260 [M$^+$]. Anal. ($C_{15}H_{16}O_4$) C, H.

1,6-Dimethyl-2-methoxymethyl-3-benzyloxypyridin-4(1*H*)-one Hydrochloride (11). To a solution of **10** (3.12 g, 12 mmol, 1 equiv) in ethanol (10 mL)/water (10 mL) was added 2.8 g (36 mmol, 3 equiv) of 40% aqueous methylamine, followed by 2 N sodium hydroxide solution until pH 13 was obtained. The reaction mixture was sealed in a thick-walled glass tube and stirred at 70 °C for 12 h. After adjustment to pH 1 with concentrated hydrochloric acid, the solvent was removed by rotary evaporation prior to addition of water (50 mL) and washing with diethyl ether (3 × 50 mL). Subsequent adjustment of the aqueous fraction to pH 7 with 10 N sodium hydroxide solution was followed by extraction into dichloromethane (4 × 50 mL), the combined organic layers then being dried over anhydrous sodium sulfate and filtered and the solvent removed in vacuo. The residue was redissolved in 30 mL methanol and adjusted to pH 1 with concentrated hydrochloric acid. The solution was reconcentrated in vacuo to yield the crude product. Recrystallization from methanol/diethyl ether gave a white crystalline solid (3.05 g, 82%): mp 125−128 °C; ^{1}H NMR (DMSO-d_6) δ 2.6 (s, 3H, **6-CH$_3$**), 3.26 (s, 3H, O**CH$_3$**), 3.86 (s, 3H, **N-CH$_3$**), 4.6 (s, 2H, 2-**CH$_2$**OCH$_3$), 5.04 (s, 2H, **CH$_2$**Ph), 5.5−6.5 (br., 1H, **OH**), 7.2−7.8 (m, 6H, **Ar** and **5-H**(pyridinone)); MS (FAB) *m*/*z*, 274 [(M − Cl)$^+$].

1,6-Dimethyl-2-methoxymethyl-3-hydroxypyridin-4(1*H*)-one Hydrochloride (12). A solution of **11** (1.55 g, 5 mmol) in methanol (40 mL)/water (10 mL) was subjected to hydrogenolysis in the presence of 5% Pd/C catalyst (0.3 g) for 3 h. Following filtration, the filtrate was concentrated in vacuo and the crude material recrystallized from methanol/diethyl ether to give a white solid (0.95 g, 86.5%): mp 156−159 °C; ^{1}H NMR (DMSO-d_6) δ 2.53 (s, 3H, **6-CH$_3$**), 3.28 (s, 3H, O**CH$_3$**), 3.83 (s, 3H, **N-CH$_3$**), 4.68 (s, 2H, 2-**CH$_2$**OCH$_3$), 7.25 (s, 1H, **5-H**(pyridinone)), 6.0−8.5 (br., 2H, **OH**); MS (FAB) *m*/*z*, 184 [(M − Cl)$^+$]. Anal. ($C_9H_{14}NO_3Cl$) C, H, N.

1,6-Dimethyl-2-hydroxymethyl-3-benzyloxypyridin-4(1*H*)-one (13): Method A. To a solution of **9** (2.46 g, 10 mmol) in ethanol (10 mL) was added 10 mL of 40% aqueous methylamine. The reaction mixture was sealed in a thick-walled glass tube and stirred at 70 °C for 12 h. After removal of the solvent, the residue was purified by column chromatography on silica gel (eluant: ethanol) followed by recrystallization from propan-2-ol to afford the titled compound as a white crystalline solid (1.02 g, 39%).

Method B: To a solution of **9** (2.46 g, 10 mmol, 1 equiv) in dichloromethane (30 mL) was added 3,4-dihydro-2*H*-pyran (1.7 g, 20 mmol, 2 equiv) followed by *p*-toluenesulfonic acid monhydrate (30 mg, cat.). After being stirred at room temperature for 3 h, the reaction mixture was washed with 5% aqueous sodium carbonate (20 mL) followed by water (2 × 20 mL). The organic fraction was then dried over anhydrous sodium sulfate, filtered, and rotary evaporated to yield a light yellow oil, which was then dissolved in ethanol (10 mL)/40% aqueous methylamine (10 mL). The reaction mixture was sealed in a thick-walled glass tube and stirred at 70 °C for 12 h. After removal of the solvent by rotary evaporation, the residue was redissolved in ethanol (20 mL) and 2 N hydrochloric acid (5 mL) and refluxed for 4 h. The solvent was removed by rotary evaporation prior to addition of water (50 mL) and washing with diethyl ether (2 × 20 mL). Subsequent adjustment of the aqueous fraction to pH 9 with 10 N sodium hydroxide solution was followed by extraction into dichloromethane (4 × 50 mL), the combined organic layers then being dried over anhydrous sodium sulfate, filtered, and rotary evaporated to give a light brown solid. Recrystallization from methanol/diethyl ether afforded the pure product (2.15 g, 83%) as a white crystalline solid: mp 181−183 °C; ^{1}H NMR (DMSO-d_6) δ 2.35 (s, 3H, **6-CH$_3$**), 3.75 (s, 3H, **N-CH$_3$**), 4.6 (s, 2H, 2-**CH$_2$**-OH), 5.05 (s, 2H, **CH$_2$**Ph), 6.35 (s, 1H, **5-H** (pyridinone)), 7.4 (s, 5H, **Ar**); MS (FAB) *m*/*z*, 259 [M$^+$]. Anal. ($C_{15}H_{17}NO_3$) C, H, N.

1,6-Dimethyl-2-phthalimidomethyl-3-benzyloxypyridin-4(1*H*)-one (15). To a solution of triphenyl phosphine (12.2 g, 46 mmol, 1.2 equiv) and phthalimide (6.8 g, 46 mmol, 1.2 equiv) in dry THF (200 mL) was added 10 g of **13** (38 mmol, 1 equiv), and the mixture was cooled to 0 °C in an ice bath. Diethyl azodicarboxylate (7.3 mL, 46 mmol, 1.2 equiv) was added dropwise with stirring over 30 min, after which the reaction mixture was allowed to warm slowly to room temperature and then stirred overnight. The resulting precipitate was isolated by filtration, washed with THF (l0 mL), and dried under high vacuum to yield a white amorphous powder (11.9 g, 80%): mp 250 °C (dec); ^{1}H NMR (DMSO-d_6) δ 2.25 (s, 3H, **6-CH$_3$**), 3.55 (s, 3H, **N-CH$_3$**), 4.75 (s, 2H, **CH$_2$**N), 5.15 (s, 2H, **CH$_2$**Ph), 6.30 (s, 1H, **5-H**(pyridinone)), 7.15 (s, 5H, **benzyl Ar**), 7.65 (m, 4H, **phthalimide Ar**); MS (FAB) *m*/*z*, 388 [M$^+$]. Anal. ($C_{23}H_{20}N_2O_4$) C, H, N.

1,6-Dimethyl-2-aminomethyl-3-benzyloxypyridin-4(1*H*)-one (16). To a solution of **15** (6.0 g. 15 mmol) in ethanol (50 mL) was added 10 mL of 5.5% aqueous hydrazine. After being refluxed for 3 h, the reaction mixture was chilled to 0 °C, acidified to pH 1 with concentrated hydrochloric acid, and filtered. The filtrate was concentrated in vacuo, and the residue was redissolved in distilled water (50 mL), adjusted to pH 12 with l0 N sodium hydroxide, and extracted with dichloromethane (3 × l00 mL). The combined organic extracts were dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure to yield an orange oil. Purification by column chromatography on silica gel (eluant: methanol:chloroform, 20:80 v/v) furnished a white solid (3.56 g, 92%): mp 143−144 °C; ^{1}H NMR ($CDCl_3$) δ 2.30 (s, 3H, **6-CH$_3$**), 3.65 (s, 3H, **N-CH$_3$**), 3.80 (s, 2H, **CH$_2$N**), 5.35 (s, 2H, **CH$_2$**Ph), 6.35 (s, 1H, **5-H**(pyridinone)), 7.45 (m, 5H, **Ar**); MS (FAB) *m*/*z*, 258 [M$^+$]. Anal. ($C_{15}H_{18}N_2O_2$) C, H, N.

1,6-Dimethyl-2-(*N*-acetylaminomethyl)-3-benzyloxypyridin-4(1*H*)-one Hydrochloride (17a). A solution of acetyl chloride (0.59 g. 7.5 mmol, 1.5 equiv) in dry DMF (5 mL) was added dropwise to a solution of **16** (1.29 g, 5 mmol, 1 equiv) in DMF (30 mL) containing 2 mL of pyridine (25 mmol, 5 equiv) at 0 °C under nitrogen. The reaction mixture was then stirred under nitrogen at room temperature for 12 h. Following filtration, the solvent was removed under high vacuum. The residue was dissolved in dichloromethane (200 mL), washed with 1 N hydrochloric acid (50 mL) and distilled water (2 × 50 mL), dried over anhydrous sodium sulfate, and filtered, and the solvent was then removed in vacuo. The residue was redissolved in 30 mL of methanol and adjusted to pH 1 with concentrated hydrochloric acid. The solution was reconcentrated in vacuo to yield the crude product. Recrystallization from methanol/diethyl ether afforded a white crystalline solid (0.76 g. 45%): mp 166−168 °C; ^{1}H NMR (DMSO-d_6) δ 1.90 (s, 3H, CO**CH$_3$**), 2.60 (s, 3H, **6-CH$_3$**), 3.95 (s, 3H, **N-CH$_3$**), 4.65 (br, d, 2H, **CH$_2$**NH), 5.15 (s, 2H, **CH$_2$**Ph), 7.25 (s, 1H, **5-H**(pyridinone)), 7.45 (m, 5H, **Ar**); MS (FAB) *m*/*z*, 301 [(M − Cl)$^+$].

Analogous reactions of **16** with propionyl chloride or isobutyryl chloride gave compounds **17b** and **17c**.

1,6-Dimethyl-2-(*N*-propionylaminomethyl)-3-benzyloxypyridin-4(1*H*)-one hydrochloride (17b): (50%); mp 148−152 °C; ^{1}H NMR (DMSO-d_6) δ 0.95 (t, 3H, COCH$_2$**CH$_3$**), 2.10 (q, 2H, CO**CH$_2$**CH$_3$), 2.55 (s, 3H, **6-CH$_3$**), 3.80 (s, 3H,

N-CH$_3$), 4.40 (br, 2H, **CH$_2$**NH), 5.10 (s, 2H, **CH$_2$**Ph), 7.15 (s, 1H, **5-H**(pyridinone)), 7.30 (m, 5H, **Ar**); MS (FAB) *m*/*z*, 315 [(M − Cl)$^+$].

1,6-Dimethyl-2-(*N*-isobutyrylaminomethyl)-3-benzyloxypyridin-4(1*H*)-one hydrochloride (17c): (62%); mp 174−176 °C; ^{1}H NMR (DMSO-d_6) δ 0.85 (d, 6H, COCH(**CH$_3$**)$_2$), 1.95−2.30 (m, 1H, CO**CH**(CH$_3$)$_2$), 2.50 (s, 3H, **6-CH$_3$**), 3.75 (s, 3H, **N-CH$_3$**), 4.30 (br, 2H, **CH$_2$**NH), 5.05 (s, 2H, **CH$_2$**Ph), 7.05 (s, 1H, **5-H**(pyridinone)), 7.15 (m, 5H, **Ar**); MS (FAB) *m*/*z*, 329 [(M − Cl)$^+$].

Analogous hydrogenation procedures to the preparation of **12** starting with **17a**−**c** gave compounds **18a**−**c**.

1,6-Dimethyl-2-(*N*-acetylaminomethyl)-3-hydroxypyridin-4(1*H*)-one hydrochloride (18a): mp 236−238 °C; ^{1}H NMR (DMSO-d_6) δ 1.85 (s, 3H, CO**CH$_3$**), 2.55 (s, 3H, **6-CH$_3$**), 3.90 (s, 3H, **N-CH$_3$**), 4.60 (br, 2H, **CH$_2$**NH), 7.20 (s, 1H, **5-H**(pyridinone)), 8.70 (t, 1H, **NH**CO); MS (FAB) *m*/*z*, 211 [(M − Cl)$^+$]. Anal. ($C_{10}H_{15}N_2O_3Cl$) C, H, N.

1,6-Dimethyl-2-(*N*-propionylaminomethyl)-3-hydroxypyridin-4(1*H*)-one hydrochloride (18b): mp 245−257 °C; ^{1}H NMR (DMSO-d_6) δ 0.90 (t, 3H, COCH$_2$**CH$_3$**), 2.05 (q, 2H, CO**CH$_2$**CH$_3$), 2.45 (s, 3H, **6-CH$_3$**), 3.75 (s, 3H, **N-CH$_3$**), 4.45 (br, 2H, **CH$_2$**NH), 7.05 (s, 1H, **5-H**(pyridinone)), 8.65 (t, 1H, **NH**CO); MS (FAB) *m*/*z*, 225 [(M − Cl)$^+$]. Anal. ($C_{11}H_{17}N_2O_3$-Cl) C, H, N.

1,6-Dimethyl-2-(*N*-isobutyrylaminomethyl)-3-hydroxypyridin-4(1*H*)-one hydrochloride (18c): mp 243−245 °C; ^{1}H NMR (DMSO-d_6) δ 0.85 (d, 6H, COCH(**CH$_3$**)$_2$), 2.15−2.45 (m, 1H, CO**CH**(CH$_3$)$_2$), 2.40 (s, 3H, **6-CH$_3$**), 3.70 (s, 3H, **N-CH$_3$**), 4.45 (br, 2H, **CH$_2$**NH), 6.95 (s, 1H, **5-H**(pyridinone)), 8.70 (t, 1H, **NH**CO); MS (FAB) *m*/*z*, 239 [(M − Cl)$^+$]. Anal. ($C_{12}H_{19}N_2O_3$-Cl) C, H, N.

3-(3′-Acetamidopropionyl)-1,3-thiazolidine-2-thione (20a). To a vigorously stirred solution of *N*-acetyl-β-alanine (4.59 g, 35 mmol, 1 equiv) in dichloromethane (200 mL) was added dicyclohexylcarbodiimide (DCCI) (7.93 g, 38.5 mmol, 1.1 equiv), followed by the addition of 2-mercaptothiazoline (4.58 g, 38.5 mmol, 1.1 equiv) and a catalytic amount of 4-(dimethylamino)pyridine (DMAP) (300 mg). The mixture was stirred for 24 h, the white precipitate of *N*,*N*′-dicyclohexylurea (DCU) filtered from the yellow solution, and the filtrate volume adjusted to 400 mL with dichloromethane. The dichloromethane layer was washed with 0.1 N sodium hydroxide solution (3 × 150 mL) and water (200 mL), dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo to yield the crude product as a yellow oil. Purification by column chromatography on silica gel (eluant: methanol: chloroform, 1:9 v/v) followed by recrystallization from chloroform/petroleum ether 40−60 °C afforded a yellow crystalline solid (7.80 g, 96%): mp 112−115 °C; ^{1}H NMR ($CDCl_3$) δ 2.0 (s, 3H, **CH$_3$**CO), 3.2−3.85 (m, 6H, CO**CH$_2$CH$_2$**NH and **CH$_2$**N), 4.60 (t, 2H, **CH$_2$**S), 6.0−6.80 (br., 1H, **NH**); MS (FAB) *m*/*z*, 232 [M$^+$]. Anal. ($C_8H_{12}N_2O_2S_2$) C, H, N, S.

An analogous procedure starting with *N*-*n*-propyl succinamic acid **19b** gave 3-(*N*-*n*-propylsuccinamido)-1,3-thiazolidine-2-thione (**20b**) (82%): mp 73−78 °C; ^{1}H NMR ($CDCl_3$) δ 0.95 (t, 3H, **CH$_3$**CH$_2$CH$_2$NH), 1.15−1.80 (m, 2H, CH$_3$**CH$_2$**CH$_2$NH), 2.55 (t, 2H, CH$_2$**CH$_2$**CONH), 3.0−3.85 (m, 6H, CH$_3$CH$_2$**CH$_2$**NH, **CH$_2$**CH$_2$CONH and **CH$_2$**N), 4.50 (t, 2H, **CH$_2$**S), 5.30−6.30 (br., 1H, **NH**); MS (FAB) *m*/*z*, 260 [M$^+$]. Anal. ($C_{10}H_{16}N_2O_2S_2$) C, H, N, S.

1,6-Dimethyl-2-(3′-acetamidopropionamido)methyl-3-benzyloxypyridin-4(1*H*)-one (21a). To a solution of **20a** (1.16 g, 5 mmol, 1 equiv) in dichloromethane (50 mL) was added **16** (1.29 g, 5 mmol, 1 equiv), and the reaction mixture was allowed to reflux for 6 h. The dichloromethane layer was washed with 0.1 N sodium hydroxide solution (3 × 30 mL) and water (30 mL) and dried over anhydrous sodium sulfate, and the solvent was removed in vacuo. The crude product was purified by column chromatography on silica gel (eluant: methanol:chloroform, 15:85 v/v) to afford a white crystalline solid (1.76 g, 95%): mp 176−178 °C; ^{1}H NMR ($CDCl_3$) δ 2.0 (s, 3H, **CH$_3$**CO), 2.30 (s, 3H, **6-CH$_3$**), 2.4 (t, 2H, CO**CH$_2$**CH$_2$-NH), 3.20−3.80 (m, 2H, COCH$_2$**CH$_2$**NH), 3.50 (s, 3H, **N-CH$_3$**), 4.40 (d, 2H, **CH$_2$**NHCO), 5.15 (s, 2H, **CH$_2$**Ph), 6.30 (s, 1H, **5-H**(pyridinone)), 6.50−7.30 (br., 2H, **NH**), 7.40 (m, 5H, **Ar**); MS (FAB) *m*/*z*, 371 [M$^+$].

An analogous procedure starting with **20b** gave **1,6-Dimethyl-2-(4′-*N*-*n*-propylsuccinamido)methyl-3-benzyloxypyridin-4(1*H*)-one (21b):** (100%): mp 124−127 °C; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, **CH$_3$**CH$_2$CH$_2$NH), 1.10−1.80 (m, 2H, CH$_3$**CH$_2$**CH$_2$NH), 2.25 (s, 3H, **6-CH$_3$**), 2.50 (s, 4H, **CH$_2$CH$_2$**-CONH), 2.85−3.40 (m, 2H, CH$_3$CH$_2$**CH$_2$**NH), 3.40 (s, 3H, **N-CH$_3$**), 4.40 (d, 2H, **CH$_2$**NHCO), 5.10 (s, 2H, **CH$_2$**Ph), 6.25 (s, 1H, **5-H**(pyridinone)), 6.50 (t, 1H, **NH**), 7.05 (t, 1H, **NH**), 7.35 (m, 5H, **Ar**); MS (FAB) *m*/*z*, 399 [M$^+$].

1,6-Dimethyl-2-(3′-acetamidopropionamido)methyl-3-hydroxypyridin-4(1*H*)-one Hydrochloride (22a). A solution of **21a** (1.39 g, 3.75 mmol) in ethanol (50 mL) was subjected to hydrogenolysis in the presence of 5% Pd/C catalyst (0.3 g) for 3 h. The catalyst was removed by filtration, and the filtrate was acidified to pH 1 with concentrated hydrochloric acid. After removal of the solvent in vacuo, the residue was purified by recrystallization from methanol/diethyl ether to give a white solid (1.07 g, 90%): mp 198−200 °C; ^{1}H NMR (DMSO-d_6) δ 1.70 (s, 3H, **CH$_3$**CO), 2.20 (t, 2H, CO**CH$_2$**CH$_2$-NH), 2.50 (s, 3H, **6-CH$_3$**), 2.90−3.50 (m, 2H, COCH$_2$**CH$_2$**NH), 3.80 (s, 3H, **N-CH$_3$**), 4.55 (d, 2H, **CH$_2$**NHCO), 7.30 (s, 1H, **5-H**(pyridinone)), 7.90 (t, 1H, **NH**), 8.80 (t, 1H, **NH**); MS (FAB) *m*/*z*, 282 [(M − Cl)$^+$]. Anal. ($C_{13}H_{20}N_3O_4Cl$) C, H, N.

An analogous procedure starting with **21b** gave **1,6-Dimethyl-2-(4′-*N*-*n*-propylsuccinamido)methyl-3-hydroxypyridin-4(1*H*)-one hydrochloride (22b):** (92%); mp 193−195 °C; ^{1}H NMR (DMSO-d_6) δ 0.79 (t, 3H, **CH$_3$**CH$_2$CH$_2$NH), 1.00−1.70 (m, 2H, CH$_3$**CH$_2$**CH$_2$NH), 2.35 (s, 4H, **CH$_2$CH$_2$**-CONH), 2.55 (s, 3H, **6-CH$_3$**), 2.70−3.20 (m, 2H, CH$_3$CH$_2$**CH$_2$**-NH), 3.90 (s, 3H, **N-CH$_3$**), 4.60 (d, 2H, **CH$_2$**NHCO), 7.4 (s, 1H, **5-H**(pyridinone)), 7.90 (t, 1H, **NH**), 8.80 (t, 1H, **NH**), 7.10−9.20 (br., **OH**); MS (FAB) *m*/*z*, 310 [(M − Cl)$^+$]. Anal. ($C_{15}H_{24}N_3O_4Cl$) C, H, N.

Determination of Distribution Coefficients. Distribution coefficients between 1-octanol and MOPS buffer (pH 7.4) were determined using an automated continuous flow technique.[11,14,15] The aqueous and octanol phases were presaturated with respect to each other before use. The aqueous phase (50 mM MOPS buffer, pH 7.4) was separated from the two phase system (1-octanol/MOPS buffer, pH 7.4) by means of a hydrophilic cellulose filter (5 μm diameter, 589/3 Blauband filter paper, Schleicher and Schuell) mounted in the gel-filtration column adjuster. A known volume (25−50 mL) of MOPS buffer (saturated with octanol) was taken in the flat base mixing chamber. After a baseline was obtained, the solution was used for reference absorbance. The ligand to be examined was dissolved in buffer (saturated with octanol) so as to give an absorbance of between 0.5 and 1.5 absorbance units at the preselected wavelength (∼280 nm). The "online spectrophotometer" permitted continuous monitoring of the equilibrium of the aqueous phase. Once a stable UV absorbance was obtained, an aliquot of octanol was added and reequilibration monitored. This cycle was repeated until a predefined total volume of added octanol was reached. The distribution coefficient ($D_{7.4}$) was calculated for each octanol addition using the following equation

$$D_{7.4} = \frac{A_0 - A_1}{A_1}\frac{V_w}{V_o}$$

where A_0 = initial absorbance of the aqueous phase, A_1 = absorbance at equilibrium of aqueous phase after the addition of octanol, V_w = volume of the aqueous (MOPS buffer), and V_o = total volume of octanol after each addition.

Biological Method. (a) Lipoxygenase Assay. Soybean lipoxygenase (sbLPO) is a 5-lipoxygenase which catalyses the peroxidation of its preferential substrate linoleic acid to linoleic hydroperoxide. The inhibition of the enzyme was monitored by the decrease in absorbance at 234 nm on conversion to the product, linoleic hydroperoxide, relative to a control. The rate of reaction in the absence and presence of various compounds

was determined using the molar absorption coefficient of linoleic hydroperoxide ($e = 23600\ M^{-1}\ cm^{-1}$). Soybean lipoxygenase and linoleic acid were purchased from Sigma, U.K. sbLPO was prepared as a 10 000 units/mL stock in 0.2 M borate buffer, pH 9.0, and linoleic acid as a 3.2 M stock in ethanol. The enzyme (final concentration 500 units/3.0 mL reaction volume) was preincubated with chelator or respective diluent at room temperature for 30 min, and the reaction was initiated by the addition of linoleic acid (667 pM in borate buffer).

(b) Iron Mobilization Efficacy Study in ^{59}Fe-Ferritin Loaded Rat. Male Wistar rats were purchased (local breed) from A. Tuck & Son (Battlesbridge, Essex SS1, U.K.) and housed in the Biological Service Unit, King's College London. The animals were maintained at a temperature between 20 and 23 °C, with food and water ad libitum. Hepatocytes of normal fasted rats (190−230 g) were labeled with ^{59}Fe by administration of ^{59}Fe-ferritin through a tail vein.[27] One hour later, each rat was administered a chelator (450 μmol/kg) orally. Control rats were administered with an equivalent volume of water. The rats were placed in individual metabolic cages and urine and feces collected. Rats were allowed access to food 1 h after oral administration of chelator. There was no restriction of water throughout the study period. The investigation was terminated 24 h after the ^{59}Fe-ferritin administration, rats were sacrificed, and the liver and gastrointestinal tract (including its content and feces) were removed for γ counting. The "iron mobilization efficacy" was calculated according to following equations:

$$\text{iron mobilization (\%)} = \frac{^{59}\text{Fe} - \text{activity}_{(\text{gut and faeces})}}{^{59}\text{Fe} - \text{activity}_{(\text{gut and feces})} + {}^{59}\text{Fe} - \text{activity}_{(\text{liver})}} \times 100\%$$

$$\text{efficacy (\%)} = \text{iron mobilization (\%)} - \text{control (\%)}$$

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