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Synthesis and physicochemical assessment of novel 2-substituted 3-hydroxypyridin-4-ones, novel iron chelators

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Abstract

Novel 3-hydroxypyridin-4-one containing tridentate ligands were synthesised and their physicochemical properties characterised, including ionisation constants and stoichiometric titration with Fe(III). There is an urgent demand for orally active iron chelators with potential for the treatment of thalassaemia. In principle, tridentate ligands are likely to be more kinetically stable than bidentate molecules, but to date no satisfactory molecules have been identified. Fe(III) stability constants were assessed by competition with the hexadentate ligand EDTA. In all cases no evidence was found for a tridentate mode of iron chelation; instead the ligands behaved as bidentate hydroxypyridinones. As a consequence they provide no advantage over the more simple alkyl hydroxypyridinones.

Introduction

Transfusional iron overload is currently treated by the orally inactive agent, desferrioxamine B (**1**; Figure 1) (Modell et al 1982; Pippard et al 1982), administered by either subcutaneous or intravenous infusion over 8–12 h daily up to 5–7 days per week throughout the patient's life (Pippard et al 1978). Consequently patient compliance with this therapy is often limited (Hershko et al 1998).

3-Hydroxypyridin-4-ones (**2**) have emerged as promising clinical candidates for development as orally active replacements for desferrioxamine B since they possess many of the desirable molecular features deemed advantageous for effective iron chelation in-vivo (Porter et al 1994; Rai et al 1999; Liu et al 1999, 2000). The tridentate ligand desferrithiocin (**3**) (Bergeron et al 1993) is also orally active. Thus, in principle, both bidentate and tridentate ligands can be considered suitable candidates for development as clinically useful iron chelators (Hider & Hall 1991). Bidentate 3-hydroxypyridin-4-one chelating agents suffer from a number of thermodynamic disadvantages (Hider & Hall 1991) in comparison with typical hexadentate ligands such as desferrioxamine B. The iron-hexadentate complex has first-order dependence on ligand concentration, whereas the tris-bidentate complex has third-order dependence and possesses a higher kinetic stability. A further disadvantage for prototypical bidentate compounds (e.g. 1,2-dimethyl-3-hydroxy-4(1*H*)-pyridinone, **2a**; Deferiprone) is that under certain conditions they can form partially dissociated complexes at low concentration and such partially dissociated

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iron complexes can, theoretically, generate hydroxyl radicals (Halliwell & Gutteridge 1984; Hider et al 1996). The thermodynamic stability of tridentate ligands is intermediate between that of hexadentate and bidentate ligands and the tendency to form partially dissociated complexes is markedly reduced when compared with bidentate ligands having comparable affinity for iron-(III) (Hider & Hall 1991). Tridentate ligands fall into two major classes: the Y class where X is either oxygen, sulphur or nitrogen (NH) and the W class where X is either oxygen, sulphur or nitrogen (NH) but Z is limited to nitrogen (N) (Figure 1).

The fundamental difference between these two classes is that with the W class, Z acts as both ligating group and part of the linking chain. This renders it possible to use smaller chelating rings and therefore reduce the adverse changes in entropy associated with complexation (Martell et al 1987). However for optimal selectivity for iron(III) the three ligating groups should be anionic oxygen, which excludes the W class. Thus although desferrithiocin forms a stable 2:1 complex with iron(III) (Peter 1985), it also possesses appreciable affinity for iron(II) and is therefore susceptible to redox cycling under biological conditions (Baker et al 1992). Furthermore the nitrogen ligand endows the molecule with an appreciable affinity for other divalent cations, such as zinc(II) and copper(II) (Peter 1985). Desferrithiocin analogues have been investigated for their clinical potential, but to date suitable candidates have not been identified for the replacement of desferrioxamine (Bergeron et al 1991, 1999a, 1999b). The work presented in this communication is devoted to the design and evaluation of Y-class tridentate-type hydroxypyridinones. Two types have been investigated, namely a phenolate series and a carboxylate series. Thus both classes present three hard oxygen atoms as potential coordination sites for iron(III). The construction of each class involves the derivatization of substituents placed at position 2 on the 3-hydroxypyridin-4-one ring system. With the correct stereochemistry, this modification could, in principle, reduce some of the disadvantages associated with current bidentate hydroxypyridinones.

Materials and Methods

Chemistry

Chemicals were obtained from Aldrich Chemical Co. (Gillingham, UK). Melting points were determined using an Electrothermal IA 9100 Digital Melting Point Apparatus (Southend, UK) 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). Elemental analyses were performed by Micro analytical laboratories, Department of Chemistry, The University of Manchester (Manchester M13 9PL, UK).

*2-Methyl-5-hydroxy-4(1*H*)-pyranone(**6**)*

Kojic acid (100 g, 704 mmol) was dissolved in a mixture of 200 mL of thionyl chloride and 100–150 mL of petroleum ether. The mixture was then stirred for 1 h, filtered, washed with 20 mL of petroleum ether and recrystallised from water to afford 2-chloromethyl-5-hydroxy-4(1*H*)-pyranone (84.8 g, 75%) as slightly yellowish needles. m.p. 166–167°C; ^{1}H NMR (DMSO-d_6): δ = 4.65 (s, 2H, CH_2), 6.60 (s, 1H, H_3), 8.10 (s, 1H, H_6), 9.25 (s, broad, 1H, OH) ppm.; MS (EI): m/z = 160 (M^+); Anal. Calcd for $C_6H_5ClO_3$: C, 44.9; H, 3.1; Cl, 22.1%. Found: C, 44.7; H, 3.0; Cl, 22.0%.

2-Chloromethyl-5-hydroxy-4(1*H*)-pyranone 5 (30 g, 187 mmol) was added to 100 mL of distilled water and heated to 50°C with stirring. Zinc dust (24.43 g, 374 mmol) was then added. The reaction was followed by the drop-wise addition of conc. HCl (56.1 mL, 662 mmol) over a period of 20 min while the temperature of the solution was kept between 70–80°C. The reaction mixture was stirred for a further period of 3 h at 70–80°C and filtered while hot. The filtrate was extracted with 3 × 200 mL of dichloromethane. The combined organic phase was dried (Na_2SO_4) and concentrated in-vacuo to yield the crude product **6** (18.11 g) as a pale yellow solid.

Recrystallisation of the crude product from propan-2-ol afforded the pyranone **6** (15.35 g, 65%) as colourless plates. m.p. 149–150°C; ^{1}H NMR (DMSO-d_6): δ = 2.25 (s, 3H, CH_3), 6.25 (s, 1H, H_3), 8.00 (s, 1H, H_6), 8.95 (s, broad, 1H, OH) ppm.; MS (EI): m/z = 126 ($M^+\cdot$); Anal. Calcd for $C_6H_6O_3$: C, 57.1; H, 4.8%. Found: C, 57.2; H, 4.7%.

*2-Hydroxymethyl-3-hydroxy-6-methyl-4(1*H*)-pyranone (**7**)*

The pyranone **6** (10 g, 79.3 mmol) was added to an aqueous solution (100 mL) of sodium hydroxide (3.49 g, 87.2 mmol). The reaction was followed by the addition of a 37 w/v% aq. formaldehyde solution (7.1 mL, 87.2 mmol) drop-wise over a period of 10 min. The solution was left to stir overnight, acidified to pH 2 using conc. HCl and finally cooled to 5–10°C in an ice-bath for 1 h. The reaction mixture was filtered and the crystalline solid was re-crystallised from absolute ethanol to afford the pyranone **7** (8.05 g, 65%) as colourless

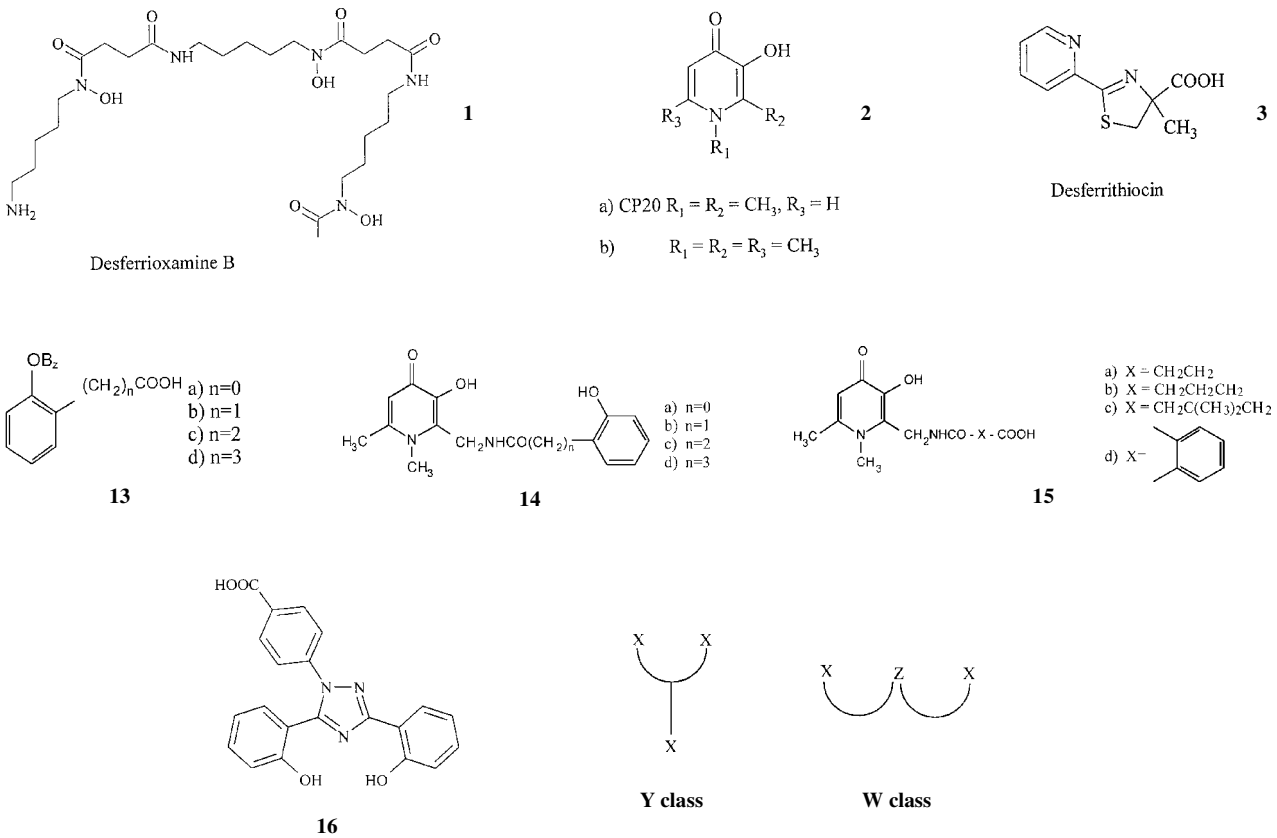


Figure 1 Structures of iron (III) chelators.

needles. m.p. 161–163°C; ^{1}H NMR (DMSO-d_6): δ = 2.30 (s, 3H, CH_3), 4.50 (s, 2H, CH_2OH), 5.30 (s, broad, 1H, CH_2OH), 6.25 (s, 1H, H_5), 8.80 (s, broad, 1H, OH); MS (EI): m/z = 156 (M^+); Anal. Calcd for $C_7H_8O_4$: C, 53.9; H, 5.2%. Found: C, 53.8; H, 5.0%.

*1,6-Dimethyl-2-hydroxymethyl-3-benzyloxy-4(1H)-pyridinone (**9**)*

Aqueous sodium hydroxide (4.23 g, 105.7 mmol) (5 mL) was added to 100 mL of methanol containing the pyranone **7** (15 g, 96.1 mmol) and heated to reflux. Benzyl bromide (12.6 mL, 105.7 mmol) was added dropwise over a period of 30 min and the mixture was refluxed for a further 6 h. The reaction mixture was concentrated in-vacuo. The residue was then taken up into 200 mL of dichloromethane and filtered. The organic phase was washed with 5% aq. w/v sodium hydroxide solution (2×100 mL) and water (100 mL), dried (Na_2SO_4), and concentrated in-vacuo to yield the crude product, 2-hydroxymethyl-3-benzyloxy-6-methyl-4(1*H*)-pyranone, as a yellow crystalline solid. Further purification by column chromatography on silica gel (eluant: EtOAc) afforded pure compound **8** (17.76 g, 75%) as a white crystalline solid. m.p. 114–116°C; ^{1}H NMR (DMSO-d_6): δ = 2.25 (s, 3H, CH_3), 4.35 (d, 2H, CH_2OH, J = 6 Hz), 5.10 (s, 2H, CH_2Ph), 5.50 (t, 1H, OH, J = 6 Hz), 6.30 (s, 1H, H_5), 7.40 (m, 5H, Aromatic); MS (EI): m/z = 246 (M^+); Anal. Calcd for $C_{14}H_{14}O_4$: C, 68.3; H, 5.7%. Found: C, 68.3%; H, 5.6%.

2-Hydroxymethyl-3-benzyloxy-6-methyl-4-(1*H*)-pyranone **8** (3.00 g, 12.2 mmol) was dissolved in a mixture of 10 mL of tetrahydrofuran and 10 mL of 40% w/w aqueous methylamine in a sealed thick-walled glass tube. The reaction mixture was stirred at 70°C overnight and then concentrated in-vacuo. The crude product was purified by column chromatography on silica gel (eluant: EtOH). The crystalline solid was re-crystallised from 2-propanol to afford the pyridinone **9** (1.58 g, 50%) as colourless needles. m.p. 181–183°C; ^{1}H NMR (DMSO-d_6/CD_3OD): δ = 2.35 (s, 3H, CH_3), 3.70 (s, 3H, NCH_3), 4.60 (s, 2H, CH_2OH), 5.05 (s, 2H, CH_2Ph), 6.35 (s, 1H, H_5), 7.40 (m, 5H, Aromatic); MS (EI): m/z = 259 ($M^{+\cdot}$); Anal. Calcd for $C_{15}H_{17}NO_3$: C, 69.5; H, 6.6; N, 5.4%. Found: C, 69.2; H, 6.4; N, 5.2%.

*1,6-Dimethyl-2-aminomethyl-3-benzyloxy-4(1H)-pyridinone (**12**)*
The pyridinone **9** (10 g, 38.6 mmol) was added to 200 mL of dry distilled THF containing triphenyl phosphine (12.14 g, 46.3 mmol), and phthalimide (6.81 g, 46.3 mmol) and cooled to 0°C in an ice-bath. Diethyl azodicarboxylate (7.3 mL, 46.3 mmol) was added drop-wise by syringe over a period of 30 min at 0°C. The reaction mixture was then allowed to warm slowly to room temperature and stirred overnight. The precipitate was isolated by filtration, washed with 10 mL THF, and dried under high vacuum to afford 1,6-dimethyl-2-phthalimidomethyl-3-benzyloxy-4(1*H*)-pyridinone **10** (11.93 g, 80%) as a white amorphous powder. m.p. 256°C (dec.); ^{1}H NMR ($CDCl_3/CD_3OD$): δ = 2.35 (s, 3H, CH_3), 3.65 (s, 3H, NCH_3), 4.85 (s, 2H, CH_2N), 5.30 (s, 2H, CH_2Ph), 6.45 (s, 1H, H_5), 7.40 (m, 5H, Aromatic), 7.80 (m, 4H, phthalimide aromatic); MS (EI): m/z = 388 (M^+); Anal. Calcd for $C_{23}H_{20}N_2O_4$: C, 71.1; H, 5.2; N, 7.2%. Found: C, 70.8; H, 5.1; N, 7.0%.

1,6-Dimethyl-2-phthalimidomethyl-3-benzyloxy-4-(1*H*)-pyridinone **10** (6.52 g, 16.8 mmol) was then dissolved in 60 mL of ethanol containing 10 mL of 5.5% w/v aq. hydrazine and refluxed for 3 h. The reaction mixture was chilled to 0°C, acidified to pH 1 with conc. HCl and filtered. The filtrate was then concentrated in-vacuo and the residue was recrystallised from ethanol–diethyl ether to afford the dihydrochloride salt of the pyridinone **11** (5 g, 90%) which was then dissolved in 50 mL of water and the resulting solution adjusted to pH 12 using 10 M NaOH. The solution was extracted with 3 × 100 mL of dichloromethane. The organic phase was dried (Na_2SO_4) and the solvent removed under reduced pressure to afford the pyridinone **12** as the free base (3.90 g, 100% recovery) isolated as a white solid. m.p. 143–144°C; ^{1}H NMR ($CDCl_3$): δ = 0.95 (s, broad, 2H, NH_2), 2.20 (s, 3H, CH_3), 3.55 (s, 3H, NCH_3), 3.70 (s, 2H, CH_2N), 5.25 (s, 2H, CH_2Ph), 6.25 (s, 1H, H_5), 7.35 (m, 5H, aromatic); MS (EI): m/z = 258 ($M^+\cdot$); Anal. Calcd for $C_{15}H_{18}N_2O_2$: C, 69.7; H, 7.0; N, 10.9%. Found: C, 70.0; H, 7.1; N, 10.6%.

Synthesis of chemical intermediates
2-Benzyloxybenzyl bromide Distilled water (20 mL) containing sodium hydroxide (6.78 g, 0.17 mol) was added to 150 mL of methanol containing 2-hydroxybenzyl alcohol (19.09 g, 0.154 mol) drop-wise over a period of 5 min. Benzyl bromide (28.26 g, 0.17 mol) was then added over a period of 20 min, and the solution was stirred under a nitrogen atmosphere overnight. The solution was concentrated in-vacuo (400 mL of dichloromethane was added). The organic phase was washed with 2 × 300 mL of 5% w/v NaOH solution, dried (Na_2SO_4), filtered and concentrated in-vacuo. Further purification by column chromatography (eluant: 20% EtOAc–petroleum ether) on silica gel gave 2-benzyloxybenzyl alcohol (29.5 g, 90%) as an oil. ^{1}H–NMR ($CDCl_3$): δ = 2.35 (t, 1H, OH, J = 6 Hz), 4.65 (d, 2H, CH_2OH, J = 6 Hz), 5.0 (s, 2H, CH_2Ph), 6.8–7.3 (m, 4H, aromatic), 7.3 (s, 5H, Ph) ppm; MS (EI): m/z = 214 (M^+). Anal. Calcd for $C_{14}H_{14}O_2$: C, 78.5; H, 6.6%. Found: C, 78.1; H, 6.8%.

2-Benzyloxybenzyl alcohol (1.0 g, 4.67 mmol) and triphenylphosphine (1.63 g, 6.21 mmol) were then dissolved in 15 mL of dry THF and treated drop-wise with 6 mL acetonitrile containing carbon tetrabromide (2.01 g, 6.07 mmol) such that the temperature did not rise above the ambient temperature. The mixture was stirred at room temperature overnight (~ 18 h) and concentrated in-vacuo. The residue was chromatographed (eluant: 20% petroleum ether–EtOAc) on silica gel to give 2-benzyloxybenzyl bromide (1.16 g, 90%) as an oil. ^{1}H NMR ($CDCl_3$): δ = 4.5 (s, 2H, CH_2), 5.05 (s, 2H, CH_2Ph), 6.8–7.2 (m, 4H, aromatic), 7.35 (s, 5H, Ph) ppm; MS (EI): m/z = 276/278 [$M^+/(M^+\cdot+2)$]. Anal. Calcd for $C_{14}H_{13}BrO$: C, 60.7; H, 4.7; Br, 28.8%. Found: C, 60.9; H, 5.0; Br, 28.6%.

1-Bromo-2-(2-benzyloxyphenyl)ethane The general method for preparation was the same as for 2-benzyloxybenzyl bromide. Further purification of the benzyloxyphenethyl alcohol by column chromatography (eluant: 20% EtOAc–petroleum ether) on silica gel gave 2-benzyloxyphenethyl alcohol (90%) as an oil. ^{1}H NMR ($CDCl_3$): δ = 2.05 (s, broad, 1H, OH), 2.80 (t, 2H, CH_2), 3.7 (t, 2H, CH_2OH), 4.9 (s, 2H, CH_2Ph), 6.7–7.2 (m, 9H, aromatic) ppm. MS (EI): m/z = 228 (M^+). Anal. Calcd for $C_{15}H_{16}O_2$: C, 78.9; H, 7.1%. Found: C, 78.9; H, 7.3%.

The residue resulting from the reaction of 2-benzyloxy-phenethyl alcohol, triphenylphosphine and carbon tetrabromide was chromatographed (eluant: petroleum ether–EtOAc 19:1) on silica gel to give 1-bromo-2-(2-benzyloxyphenyl) ethane (9.31 g, 90%) as an oil. ^{1}H NMR ($CDCl_3$): δ = 3.2 (t, 2H, CH_2), 3.6 (t, 2H, CH_2Br), 5.2 (s, 2H, CH_2Ph), 6.8–7.3 (m, 4H, aromatic), 7.3 (s, 5H, Ph) ppm. MS (EI): m/z = 290/292 [$M^+/(M^+\cdot+2)$]. Anal. Calcd for $C_{15}H_{15}BrO$: C, 61.9; H, 5.2; Br, 27.4%. Found: C, 61.7; H, 5.3; Br, 27.0%.

*5.1.5.3 3-(2-Benzyloxyphenyl)-propanoic acid (**13c**)*
Distilled water (5 mL) containing NaOH (0.44 g, 11 mmol) was added to 50 mL of methanol containing methyl-3-(2-hydroxyphenyl)-propionate (1.80 g,

10 mmol) over a period of 5 min. Then benzyl bromide (1.88 g, 11 mmol) was added to the above solution over a period of 5 min and the mixture was stirred overnight. The reaction mixture was concentrated in-vacuo, dissolved in 100 mL of dichloromethane and filtered. The filtrate was washed with 3×50 mL of 5% NaOH solution, dried (Na_2SO_4), filtered and concentrated in-vacuo. The residue was chromatographed (eluant: 20% EtOAc–petroleum ether) on silica gel to yield methyl-3-(2-benzyloxyphenyl)-propionate (1.76 g, 65%) as an oil. ^{1}H NMR ($CDCl_3$): δ = 2.65 (t, 2H, CH_2COOMe), 3.0 (t, 2H, CH_2CH_2COOMe), 3.65 (s, 3H, CH_3), 5.1 (s, 2H, CH_2Ph), 6.8–7.3 (m, 4H, aromatic), 7.4 (s, 5H, Ph) ppm. MS (CI): m/z = 288 ($M^+\cdot$ +18); Anal. Calcd for $C_{17}H_{18}O_3$: C, 75.5; H, 6.7%. Found: C, 75.8; H; 6.9%.

THF (10 mL) containing methyl-3-(2-benzyloxyphenyl)-propionate (1.08 g, 4 mmol) was then added to 10 mL of distilled water containing KOH (2.24 g, 40 mmol), and refluxed for 4 h. The volume of solution was reduced to less than 10 mL in-vacuo, then 20–30 mL of distilled water was added. The residue was extracted with 3×30 mL of dichloromethane. The aqueous phase was acidified to pH 1 with conc. HCl, extracted with 3×30 mL of dichloromethane and dried (Na_2SO_4). Following filtration the solvent was removed in-vacuo to afford 3-(2-benzyloxyphenyl)-propanoic acid (0.92 g, 90%) as an oil. ^{1}H NMR ($CDCl_3$): δ = 2.75 (t, 2H, CH_2COOH), 2.95 (t, 2H, CH_2CH_2COOH), 5.1 (s, 2H, CH_2Ph), 7–7.5 (m, 4H, aromatic), 7.4 (s, 5H, Ph), 8.15 (s, broad, 1H, COOH) ppm. MS (CI): m/z = 274 ($M^+\cdot$ +18); Anal. Calcd for $C_{16}H_{16}O_3$: C, 75.0; H, 6.3%. Found: C, 74.6; H, 6.3%.

*4-(2-Benzyloxyphenyl)-n-butanoic acid (**13d**)* Sodium hydride (1.28 g of 60% w/w dispersion in oil, 31.95 mmol) was added to 60 mL of dry THF containing diethyl malonate (9.57 g, 60 mmol) at room temperature. The reaction was followed by the addition of 10 mL of dry THF containing 1-bromo-2-(2-benzyloxyphenyl)ethane (8.86 g, 30.43 mmol) drop-wise over a period of 10 min and stirred overnight at room temperature. The residue was concentrated and chromatographed (eluant: 10% EtOAc–petroleum ether) on silica gel to give a mixture of diethyl [2-(2-benzyloxyphen)-ethyl]-malonate and diethyl malonate (10.96 g) as an oil. THF (75 mL) containing the resulting crude residue (10.96 g) was added to 100 mL of distilled water containing KOH (33.66 g, 600 mmol) over a period of 10 min. The mixture was refluxed for 48 h and concentrated in-vacuo. Next, 100 mL of distilled water was added to the obtained crude residue and the pH of the solution was adjusted to 1–3 by conc. HCl. Then the mixture was refluxed for 4 h, extracted with 4×200 mL of dichloromethane, dried (Na_2SO_4) and concentrated in-vacuo. The obtained crude residue was chromatographed (eluant: 50% EtOAc–petroleum ether) on silica gel to yield 4-(2-benzyloxyphenyl)-n-butanoic acid (1.24 g, 25%). m.p. 98–99°C; ^{1}H-NMR ($CDCl_3$): δ = 1.95 (m, 2H, CH_2CH_2COOH), 2.35 (t, 2H, CH_2COOH), 2.75 (t, 2H, CH_2), 5.05 (s, 2H, CH_2Ph), 6.8–7.4 (m, 4H, aromatic), 7.4 (s, 5H, Ph), 9.6 (s, broad, 1H, COOH) ppm. MS (EI): m/z = 270 (M^+); Anal. Calcd for $C_{17}H_{18}O_3$: C, 75.5; H, 6.7%. Found: C, 75.5; H, 6.7%.

N-[(2-benzyloxyphenyl)carboxy] succinimide derivatives (general method)
N-Hydroxy succinimide (22 mmol) and 4-dimethylaminopyridine (2 mmol) were added to 100 mL of dichloromethane containing ω-(2-benzyloxyphenyl)-n-alkanoic acid (20 mmol). After 5 min, dicyclohexyldicarbodiimide (22 mmol) was added to the solution which was stirred overnight under a nitrogen atmosphere. After filtration the filtrate was washed with 2×200 mL NaOH (0.2 M), concentrated in-vacuo and chromatographed on silica gel.

*N-[(2-benzyloxyphenyl)carboxy]succinimide (**14a**)*
The eluant for column chromatography was 40% EtOAc–petroleum ether. Recrystallisation of the crude compound from EtOAc gave the product **14a** (yield 70%) as white crystals. m.p. 118–119°C; ^{1}H NMR ($CDCl_3$): δ = 2.75 (s, 4H, CH_2), 5.05 (s, 2H, CH_2Ph), 6.8–7.3 (m, 8H, aromatic), 7.8 (m, 1H, aromatic-H *ortho* to carbonyl) ppm. MS (EI) m/z = 325 (M^+); Anal. Calcd for $C_{18}H_{15}NO_5$: C, 66.5; H, 4.6; N, 4.3%. Found: C, 66.6; H, 4.7; N, 4.3%.

*N-[(2-benzyloxybenzyl)carboxy]succinimide (**14b**)*
The eluant for column chromatography was 90% EtOAc–petroleum ether. Recrystallisation of the crude compound from EtOAc gave the product **14b** (yield 70%) as a white crystalline solid. m.p. 136.5–138.5°C; ^{1}H NMR ($CDCl_3$): δ = 2.7 (s, 4H, CH_2), 3.9 (s, 2H, CH_2CO), 5.05 (s, 2H, CH_2Ph), 6.8–7.3 (m, 4H, aromatic-H), 7.3 (s, 5H, Ph) ppm. MS (EI): m/z = 339 (M^+); Anal. Calcd for $C_{19}H_{17}NO_5$: C, 67.3; H, 5.1; N, 4.1%. Found: C, 67.3; H, 5.2; N, 4.2%.

*N-[2-(2-benzyloxyphenyl)-ethylcarboxy] succinimide (**14c**)* The eluant for column chromatography was 40% EtOAc–petroleum ether. Recrystallisation of the crude compound from EtOAc gave the product **14c** (yield 94%) as a white crystalline solid. m.p. 130–131°C; ^{1}H NMR ($CDCl_3$): δ = 2.75 (s, 4H, CH_2CH_2), 3.0

(t, 2H, CH_2CO), 3.0 (t, 2H, CH_2CH_2CO), 5.05 (s, 2H, CH_2Ph), 6.8–7.3 (m, 4H, aromatic), 7.35 (s, 5H, Ph) ppm. MS (EI): m/z = 353 (M^+); Anal. Calcd for $C_{20}H_{19}NO_5$: C, 68.0; H, 5.4; N, 4.0%. Found: C, 68.0; H, 5.3; N, 3.9%.

N-*[3-(2-benzyloxyphenyl)-prop-1-yl]carboxy]succinimide (**14d**)* The eluant for column chromatography was 40% EtOAc–petroleum ether. Recrystallisation of the crude compound from EtOAc gave the product **14d** (yield 70%) as a wax. ^{1}H NMR ($CDCl_3$): δ = 2.1 (m, 2H, CH_2CH_2CO), 2.6 (t, 2H, CH_2CO), 2.6 (t, 2H, $CH_2CH_2CH_2CO$), 2.8 (s, 4H, CH_2CH_2), 5.1 (s, 2H, CH_2Ph), 6.8–7.4 (m, 4H, aromatic), 7.4 (s, 5H, Ph) ppm. MS (EI): m/z = 367 (M^+); Anal. Calcd for $C_{21}H_{21}NO_5$: C, 68.7; H, 5.8; N, 3.8%. Found: C, 68.4; H, 5.8; N, 3.7%.

N-*(Benzylsuccinyl)-2-mercaptothiazoline.* A mixture of succinic anhydride (8.2 g, 82 mmol), benzyl alcohol (10.6 g, 98 mmol) and 4-dimethylaminopyridine (530 mg) in 200 mL of THF was refluxed under nitrogen gas for 12 h, acidified by conc. HCl and concentrated in-vacuo. The residue was taken up into EtOAc (400 mL) and washed with 2×200 mL HCl (1 M) and 2×125 mL NaOH (1.25 M). The basic layer was acidified to pH 4–5 with a 1 M HCl solution, extracted with 4×300 mL EtOAc, dried (Na_2SO_4), filtered and concentrated in-vacuo. The residue was recrystallised from EtOAc–petroleum ether to yield benzyl succinate (14.3 g, 84%) as white crystals. m.p. 56–57°C; ^{1}H NMR (DMSO-d_6): δ = 2.35 (t, 2H, CH_2), 2.45 (t, 2H, CH_2), 5.0 (s, 2H, CH_2Ph), 7.25 (s, 5H, Ph) ppm. MS (EI): m/z = 208 (M^+); Anal. Calcd for $C_{11}H_{12}O_4$: C, 63.5; H, 5.8%. Found: C, 63.1; H, 5.8%.

1,3-Dicyclohexylcarbodiimide (2.13 g, 10.3 mmol) was added to 150 mL of dichloromethane containing benzylsuccinate (2.0 g, 9.61 mmol), 4-dimethylaminopyridine (100 mg), 2-mercaptothiazoline (1.51 g, 9.61 mmol). The mixture was stirred overnight under nitrogen gas and filtered. The filtrate was washed with 3×150 mL NaOH (0.1 M), dried (Na_2SO_4) and chromatographed (eluant: 40% EtOAc–petroleum ether) on silica gel to yield *N*-(benzylsuccinyl)-2-mercaptothiazoline (2.43 g, 82%) as an oil. ^{1}H NMR ($CDCl_3$): δ = 2.7 (t, 2H, CH_2COOBn), 3.2 (t, 2H, CH_2S), 3.5 (t, 2H, CH_2CON), 4.5 (t, 2H, CH_2N), 5.1 (s, 2H, CH_2Ph), 7.2 (s, 5H, Ph) ppm. MS (EI): m/z = 309 (M^+); Anal. Calcd for $C_{14}H_{15}NO_3S_2$: C, 54.4; H, 4.9; N, 4.5; S, 20.7%. Found: C, 54.6; H, 5.2; N, 4.7; S, 20.9%.

N-*(Benzyl glutaryl)-2-mercaptothiazoline* The preparation of this compound was undertaken as for the corresponding succinyl analogue above. Benzyl-glutarate was separated (7.36 g, 40%) as a pale yellow oil. ^{1}H NMR (DMSO-d_6): δ = 2.05 (m, 2H, CH_2), 2.45 (s, 4H, $CH_2CH_2CH_2$), 5.10 (s, 2H, CH_2Ph), 7.35 (s, 5H, Ph) ppm. MS (EI): m/z = 222 (M^+); Anal. Calcd for $C_{12}H_{14}O_4$: C, 64.9; H, 6.4%. Found: C, 64.7; H, 6.5%.

1,3-Dicyclohexylcarbodiimide (1.1 g, 5.31 mmol) was then added to 150 mL of dichloromethane containing benzylglutarate (1.1 g, 4.96 mmol), 4-dimethylaminopyridine (100 mg) and 2-mercaptothiazoline (0.59 g, 4.96 mmol). The mixture was stirred overnight under nitrogen gas and filtered. The filtrate was washed with 3×150 mL NaOH (0.1 M), dried (Na_2SO_4) and chromatographed (eluant: 40% EtOAc–petroleum ether) on silica gel to yield *N*-(benzyl glutaryl)-2-mercaptothiazoline (1.34 g, 84%) as an oil. ^{1}H NMR ($CDCl_3$): δ = 1.6 (m, 2H, CH_2), 2.55 (s, 2H, CH_2COOBn), 3.2 (t, 2H, CH_2S), 3.4 (s, 2H, CH_2CON), 4.5 (t, 2H, CH_2N), 5.1 (s, 2H, CH_2Ph), 7.2 (s, 5H, Ph) ppm. MS (EI): m/z = 323 (M^+); Anal. Calcd for $C_{15}H_{17}NO_3S_2$: C, 55.7; H, 5.3; N, 4.3; S, 19.8%. Found: C, 55.4; H, 5.6; N, 4.4; S, 19.7%.

N-*(Benzyl 3,3-dimethylglutaryl)-2-mercaptothiazoline* The preparation of this compound was the same as for the succinyl derivative. The residue was chromatographed (eluant: 40% EtOAc–petroleum ether) on silica gel to yield benzyl-3,3-dimethylglutarate (18.6 g, 75%) as an oil. ^{1}H NMR (DMSO-d_6): δ = 1.05 (s, 6H, CH_3), 2.25 (s, 2H, CH_2), 2.4 (s, 2H, CH_2), 5.0 (s, 2H, CH_2Ph), 7.3 (s, 5H, Ph) ppm. MS (EI): m/z = 250 (M^+); Anal. Calcd for $C_{14}H_{18}O_4$: C, 67.2; H, 7.2%. Found: C, 67.2; H, 7.0%.

1,3-Dicyclohexylcarbodiimide (4.42 g, 21.4 mmol) was added to 150 mL of dichloromethane containing benzyl-3,3-dimethylglutarate (5.01 g, 20 mmol), 4-dimethylaminopyridine (400 mg), 2-mercaptothiazoline (2.39 g, 20 mmol). The mixture was stirred overnight under a nitrogen blanket and filtered. The filtrate was washed with 3×400 mL of NaOH (0.1 M), dried (Na_2SO_4), and chromatographed (eluant: 20% EtOAc–petroleum ether) on silica gel to yield *N*-(benzyl 3,3-dimethylglutaryl)-2-mercaptothiazoline (4.87 g, 70%) as an oil. ^{1}H NMR ($CDCl_3$): δ = 1.1 (s, 6H, CH_3), 2.55 (s, 2H, CH_2COOBn), 3.1 (t, 2H, CH_2S), 3.35 (s, 2H, CH_2CON), 4.4 (t, 2H, CH_2N), 5.0 (s, 2H, CH_2Ph), 7.3 (s, 5H, Ph) ppm. MS (EI): m/z = 351 (M^+); Anal. Calcd for $C_{17}H_{21}NO_3S_2$: C, 58.1; H, 6.0; N, 4.0; S, 18.2%. Found: C, 58.5; H, 6.2; N, 4.3; S, 17.9%.

N-*(Benzyl phthalyl)-2-mercaptothiazoline* The preparation of this compound was the same as for the succinyl derivative. Benzyl phthalate (15.37 g, 60%) was then separated as an oil. IR (KCl): broad 3500–3000, 1720, 1457, 1279, 743 cm^{-1}; ^1H NMR (DMSO-d_6): δ = 5.0 (s, 2H, CH_2), 7.0–7.3 (m, 9H, aromatic), 8.95 (s, broad, 1H, COOH) ppm. MS (FAB): m/z = 256 (M^+).

1,3-Dicyclohexylcarbodiimide (4.42 g, 21.4 mmol, 1.07 equiv.) was added to 150 mL of dichloromethane containing benzylphthalate (5.13 g, 20 mmol), 4-dimethylaminopyridine (400 mg) and 2-mercaptothiazoline (2.39 g, 20 mmol). The mixture was stirred overnight under nitrogen and filtered. The filtrate was washed with 3×400 mL of NaOH (0.1 M), dried (Na_2SO_4) and chromatographed (eluant: 30% EtOAc–petroleum ether) on silica gel to yield *N*-(benzyl phthalyl)-2-mercaptothiazoline (5.65 g, 79%) as an oil. IR (oil): 3020, 2990, 1710, 1680, 1450, 1370, 1310, 1270, 1230, 1140, 750, 740 cm^{-1}; ^1H NMR ($CDCl_3$): δ = 3.15 (t, 2H, CH_2S), 4.5 (t, 2H, CH_2N), 5.3 (s, 2H, CH_2Ph), 7.3–7.5 (m, 8H, aromatic) ppm. MS (FAB): m/z = 357 (M^+); Anal. Calcd for $C_{18}H_{15}NO_3S_2$: C, 60.5; H, 4.2; N, 4.0; S, 17.9%. Found: C, 60.3; H, 4.4; N, 4.1; S, 18.1%.

N-*(2,3-Dimethoxybenzyl)phthalamide* Dichloromethane (10 mL) containing 2,3-dimethoxybenzylamine (0.84 g, 5 mmol) was added to 15 mL of dichloromethane containing phthalic anhydride (0.74 g, 5 mmol). The mixture was stirred overnight, filtered and washed with 10 mL dichloromethane to give the compound (1.42 g, 90%) as a white powder after drying. m.p. 170–172°C; ^1H NMR (DMSO-d_6): δ = 3.7 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 4.4 (d, 2H, $HNCH_2$, J = 6 Hz), 6.8–7.8 (m, 7H, aromatic), 8.6 (t, 1H, NH, J = 6 Hz) ppm. MS (FAB): m/z = 316 (M^+); Anal. Calcd for $C_{17}H_{17}NO_5$: C, 64.8; H, 5.4; N, 4.4%. Found: C, 64.5; H, 5.4; N, 4.5%.

2-Benzyloxybenzyl chloride Dichloromethane (50 mL) containing the 2-benzyloxybenzyl alcohol (40 mmol) was added to 12.6 mL of thionyl chloride in dichloromethane (50 mL) over a period of 10 min under nitrogen, refluxed for 18 h and concentrated in-vacuo. Column chromatography (eluant: 80% petroleum ether–chloroform) of the residue on silica gel gave the product (5.54 g, 60%) as an oil. ^1H NMR ($CDCl_3$): δ = 4.6 (s, 2H, CH_2), 5.0 (s, 2H, CH_2Ph), 6.8–7.3 (m, 4H, aromatic), 7.3 (s, 5H, Ph) ppm; MS (EI): m/z = 232/234 [$M^+/(M^+\cdot + 2)$]. Anal. Calcd for $C_{14}H_{13}ClO$: C, 72.3; H, 5.6; Cl 15.2%. Found: C, 71.9; H, 5.9, Cl, 15.6%.

1-Chloro-2-(2-benzyloxyphenyl)ethane Dichloromethane (50 mL) containing 2-benzyloxyphenethyl alcohol (5.37 g, 23.5 mmol) was added to a mixture of 5.60 g of thionyl chloride in 30 mL of dichloromethane over a period of 10 min under nitrogen, refluxed for 18 h and concentrated in-vacuo. Column chromatography (eluant: chloroform) of the residue on silica gel afforded the product (3.48 g, 60%) as an oil. ^1H NMR ($CDCl_3$): δ = 3.1 (t, 2H, CH_2CH_2Cl), 3.7 (t, 2H, CH_2Cl), 5.0 (s, 2H, CH_2Ph), 6.7–7.3 (m, 4H, aromatic), 7.3 (s, 5H, Ph) ppm. MS (EI): m/z = 246/248 [$M^+/(M^+\cdot + 2)$]. Anal. Calcd for $C_{15}H_{15}ClO$: C, 73.0; H, 6.1; Cl, 14.4%. Found: C, 73.1; H, 6.2; Cl, 14.0%.

*Phenolate-type 3-hydroxypyridin-4-one (**14**) (general method)*

Dichloromethane (30 mL) containing 1,6-dimethyl-2-amino-methyl-3-benzyloxy-4(1*H*)-pyridinone **12** (5.73 mmol) was added to 30 mL of dichloromethane containing *N*-((2-benzyloxyphenyl)carboxy) succinimide derivative (5.21 mmol) and stirred overnight. The solution was then washed first with 2×60 mL NaOH (0.2 M) and then with 2×60 mL HCl (0.1 M), dried (Na_2SO_4) and concentrated in-vacuo. The residue was finally chromatographed on silica gel.

1,6-Dimethyl-2-(2-benzyloxybenzamido)methyl-3-benzyloxy-4(1H)-pyridinone The eluant for column chromatography was EtOH; yield 90%; m.p. 73–75°C; ^1H NMR ($CDCl_3$): δ = 2.46 (s, 3H, CH_3), 3.81 (s, 3H, NCH_3), 4.71 (d, 2H, $HNCH_2$, J = 6 Hz), 5.11 (s, 2H, CH_2Ph), 5.19 (s, 2H, CH_2Ph), 7.03–7.49 (m, 13H, aromatic), 7.82 (s, 1H, H_5), 8.10 (m, 1H, aromatic-H *ortho* to carbonyl), 8.10 (t, 1H, NH, J = 6 Hz) ppm. MS (FAB): m/z = 469 (M^+); Anal. Calcd for $C_{29}H_{28}N_2O_4 \cdot \frac{5}{2}H_2O$: C, 67.8; N, 5.5%. Found: C, 67.7; N, 5.5%.

1,6-Dimethyl-2-[(2-benzyloxyphenyl)-acetamido]methyl-3-benzyloxy-4(1H) pyridinone The eluant for column chromatography was first EtOAc then EtOH; yield 90%; m.p. 87–89°C; ^1H NMR ($CDCl_3$): δ = 2.27 (s, 3H, CH_3), 3.59 (s, 2H, CH_2CONH), 3.66 (s, 3H, NCH_3), 4.48 (d, 2H, $HNCH_2$, J = 5.4 Hz), 4.99 (s, 2H, CH_2Ph), 5.14 (s, 2H, CH_2Ph), 6.68–7.42 (m, 16H, aromatic, H_5 and NH) ppm. MS (FAB): m/z = 483 (M^+); Anal. Calcd for $C_{30}H_{30}N_2O_4 \cdot 3H_2O$: C, 67.2; N, 5.2%. Found: C, 67.6; N, 5.3%.

1,6-Dimethyl-2-[3-(2-benzyloxyphenyl)-propanamido]-methyl-3-benzyloxy-4(1H)-pyridinone The eluant for

column chromatography was 90% EtOAc–MeOH; yield 90% as a white crystalline solid; m.p. 83–85°C; ^{1}H NMR ($CDCl_3$): δ = 2.15 (s, 3H, CH_3), 2.41 (t, 2H, CH_2CONH), 2.95 (t, 2H, CH_2CH_2CONH), 3.15 (s, 3H, NCH_3), 4.27 (d, 2H, $HNCH_2$, J = 5.6 Hz), 5.05 (s, 2H, CH_2Ph), 5.16 (s, 2H, CH_2Ph), 5.62 (t, 1H, NH, J = 5.6 Hz), 6.25 (s, 1H, H_5), 6.82–7.41 (m, 14H, aromatic) ppm. MS (EI): m/z = 497 (M^+); Anal. Calcd for $C_{31}H_{32}N_2O_4 \cdot H_2O$: C, 72.4; H, 6.7; N, 5.4%. Found: C, 72.6; H, 6.4; N, 5.6%.

*1,6 - Dimethyl-2-[4-(2-benzyloxyphenyl)-n-butanamido]-methyl-3-benzyloxy-4(1*H*)-pyridinone* The eluant for column chromatography was 90% EtOAc–MeOH. Recrystallisation of the crude compound from EtOAc gave 1,6-dimethyl-2-[4-(2-benzyloxyphenyl)-n-butanamido]methyl-3-benzyloxy-4(1*H*)-pyridinone (yield 70%) as a wax. ^{1}H NMR ($CDCl_3$): δ = 1.95 (m, 2H, CH_2CH_2CONH), 1.95 (t, 2H, CH_2-CO), 2.2 (s, 3H, CH_3), 2.65 (t, 2H, CH_2), 3.4 (s, 3H, NCH_3), 4.3 (d, 2H, $HNCH_2$, J = 6 Hz), 5.05 (s, 2H, CH_2Ph), 5.2 (s, 2H, CH_2Ph), 5.4 (t, 1H, HN, J = 6 Hz), 6.35 (s, 1H, H_5), 6.8–7.4 (m, 14H, aromatic) ppm. MS (FAB): m/z = 511 (M^+); Anal. Calcd for $C_{32}H_{34}N_2O_4 \cdot \frac{1}{2}2H_2O$: C, 74.0; H, 6.8; N, 5.4%. Found: C, 73.9; H, 6.6; N, 5.4%.

*Phenolate-type 3-hydroxy-4(1*H*)-pyridinone (**14**) (general method)*

Nitrogen gas was passed over a solution of 30–40 mL of methanol containing compound **19** (2.4 mmol) for a period of 3–5 min. The reaction was then followed by the addition of palladium 5% on activated carbon (5–10% w/w of compound **19**). Nitrogen gas was again passed over the solution for a period of 3–5 min. Finally, hydrogen gas was passed over the solution overnight. The mixture was warmed, filtered, concentrated in-vacuo and recrystallized from methanol and diethyl ether.

*1,6 - Dimethyl-2-(2-hydroxybenzamido)methyl-3-hydroxy-4(1*H*)-pyridinone (**14a**)* Yield 35%; m.p. 221–223°C; IR (KCl): 3244, 2568, 1545, 1489, 1450, 1372, 1252, 753 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ = 2.57 (s, 3H, CH_3), 3.95 (s, 3H, NCH_3), 4.85 (d, 2H, $HNCH_2$, J = 5 Hz), 6.88–6.98 (m, 2H, aromatic), 7.24 (s, 1H, H_5), 7.39 (m, 1H, aromatic-H *para* to carbonyl), 7.9 (m, 1H, aromatic-H *ortho* to carbonyl), 9.28 (t, 1H, NH, J = 5 Hz), 12.08 (s, broad, 1H, OH) ppm. MS (accurate mass, high resolution, FAB): Actual. m/z = 289.1188 (M^+). Measured. m/z = 289.1198 (M^+); Anal. Calcd for $C_{15}H_{16}N_2O_4 \cdot 2H_2O$: C, 55.5; H, 6.2; N, 8.6%. Found: C, 55.6; H, 5.7; N, 8.5%.

*1,6-Dimethyl-2-[(2-hydroxyphenyl)-acetamido]methyl-3-hydroxy-4(1*H*)-pyridinone (**14b**)* Yield 20%; m.p. 182–183°C; IR (KCl): 3206, 1670, 1628, 1540, 1460, 1360, 760 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ = 2.5 (d, 3H, CH_3, J = 1.6 Hz), 3.43 (s, 2H, CH_2CONH), 3.84 (s, 3H, NCH_3), 4.61 (d, 2H, $HNCH_2$, J = 5 Hz), 6.70–7.07 (m, 4H, aromatic), 7.16 (s, 1H, H_5), 8.72 (t, 1H, NH, J = 5 Hz), 9.6 (s, broad, 1H, OH) ppm. MS (accurate mass, high resolution, FAB): Actual. m/z = 303.1345 (M^+). Measured. m/z = 303.1344 (M^+); Anal. Calcd for $C_{16}H_{18}N_2O_4 \cdot 2H_2O$: C, 55.8; H, 6.5; N, 8.3%. Found: C, 55.7; H, 6.0; N, 8.1%.

*1,6-Dimethyl-2-[3-(2-hydroxyphenyl)-propanamido]-methyl-3-hydroxy-4(1*H*)-pyridinone (**14c**)* Yield 50%; m.p. 248–250°C; IR (KCl): 3284, 1636, 1569, 1507, 1450, 1243, 770 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ = 2.27 (s, 3H, CH_3), 2.38 (t, 2H, CH_2CH_2CONH), 2.75 (t, 2H, CH_2CH_2CONH), 3.37 (s, 3H, NCH_3), 4.42 (d, 2H, $HNCH_2$, J = 5 Hz), 6.12 (s, 1H, H_5), 6.65–7.03 (m, 4H, aromatic), 8.20 (t, 1H, NH, J = 5 Hz) ppm. MS (accurate mass, high resolution, FAB): Actual. m/z = 317.1501 (M^+). Measured. m/z = 317.1509 (M^+); Anal. Calcd for $C_{17}H_{20}N_2O_4 \cdot \frac{1}{3}CH_3OH$: C, 63.7; H, 6.6; N, 8.6%. Found: C, 63.9; H, 6.5; N, 8.4%.

*1,6-Dimethyl-2-[4-(2-hydroxyphenyl)-n-butanamido]-methyl-3-hydroxy-4(1*H*)-pyridinone (**14d**)* Yield 70%; m.p 242–244°C. IR (KCl): 3194, 1647, 1580, 1510, 1450, 1398, 1309, 1263, 750 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ = 1.7 (m, 2H, CH_2CH_2CONH), 2.15 (t, 2H, CH_2CONH), 2.25 (s, 3H, CH_3), 2.5 (t, 2H, CH_2), 3.45 (s, 3H, NCH_3), 4.45 (d, 2H, $HNCH_2$, J = 6 Hz), 6.15 (s, 1H, H_5), 8.15 (t, 1H, HN, J = 6 Hz), 6.7–7.05 (m, 4H, aromatic), 8.15 (t, 1H, HN, J = 6 Hz) ppm. MS (accurate mass, high resolution, FAB): Actual. m/z = 331.1658 (M^+). Measured. m/z = 331.1664 (M^+); Anal. Calcd for $C_{18}H_{22}N_2O_4 \cdot \frac{1}{4}CH_3OH$: C, 64.8; H, 6.9; N, 8.3%. Found: C, 64.6; H, 6.8; N, 8.2%.

*Carboxylate-type of 3-hydroxypyridin-4-one (**15**)*

*1,6-Dimethyl-2-(benzylsuccinamido)methyl-3-benzyloxy-4(1*H*)-pyridinone* The general method was the same as that for compound **14**. The compound (yield 99%) was separated as an oil. ^{1}H NMR ($CDCl_3$): δ = 2.2

(s, 3H, CH_3), 2.30 (s, 2H, CH_2CONH), 2.45 (t, 2H, CH_2COOBn), 3.41 (s, 3H, CH_3N), 4.40 (d, 2H, CH_2N), 5.1 (s, 2H, CH_2Ph), 5.2 (s, 2H, CH_2Ph), 6.09 (s, 1H, H_5), 7.35 (s, 10H, Ph), 8.19 (t, 1H, NH) ppm. MS (FAB): m/z = 449 (M^+); Anal. Calcd for $C_{26}H_{28}N_2O_5$: C, 69.6; H, 6.3; N, 6.3%. Found: C, 69.5; H, 6.6; N, 6.2%.

1,6-Dimethyl-2-(benzylglutaramido)methyl-3-benzyloxy-4(1H)-pyridinone The general method was the same as that for compound **14**. The compound (yield 98%) was separated as an oil. ^{1}H NMR ($CDCl_3$): δ = 1.8 (s, 2H, CH_2), 2.15 (m, 4H, $CH_2CH_2CH_2$), 2.3 (s, 3H, CH_3), 3.45 (s, 3H, CH_3N), 4.4 (d, 2H, CH_2N), 5.15 (s, 2H, CH_2Ph), 5.25 (s, 2H, CH_2Ph), 6.1 (s, 1H, H_5), 7.4 (s, 10H, Ph), 8.1 (t, 1H, NH) ppm. MS (FAB): m/z = 463 (M^+); Anal. Calcd for $C_{27}H_{30}N_2O_5$: C, 70.1; H, 6.5; N, 6.1%. Found: C, 69.8; H, 6.5; N, 6.1%.

1,6-Dimethyl-2-(benzyl-3,3-dimethylglutaramido)methyl-3-benzyloxy-4(1H)-pyridinone The general method was the same as that for compound **14**. The residue was chromatographed (eluant: 80% EtOAc–MeOH) on silica gel. The compound (yield 74%) was separated as an oil. ^{1}H NMR ($CDCl_3$): δ = 1.05 (s, 6H, $C(CH_3)_2$), 2.15 (s, 2H, CH_2CONH), 2.15 (d, 3H, CH_3, J = 0.4 Hz), 2.4 (s, 2H, CH_2COOBn), 3.45 (s, 3H, CH_3N), 4.35 (d, 2H, CH_2N, J = 6 Hz), 5.1 (s, 2H, CH_2Ph), 5.2 (s, 2H, CH_2Ph), 6.25 (q, 1H, H_5, J = 0.4 Hz), 6.6 (t, 1H, NH, J = 6 Hz), 7.35 (s, 10H, Ph) ppm. MS (FAB): m/z = 491 (M^+); Anal. Calcd for $C_{29}H_{34}N_2O_5 \cdot \frac{3}{2}H_2O$: C, 67.3; N, 5.4%. Found: C, 67.3; N, 5.5% (H analysis ~8% low).

*1,6-Dimethyl-2-succinamidomethyl-3-hydroxy-4(1H)-pyridinone (**15a**)* The general method was the same as that for compound **14**. The residue was recrystallized from methanol–diethyl ether. The compound (yield 70%) was separated as a white crystalline solid. m.p. 223–224°C; IR (KCl): broad 3500–3000, 2960, 1713 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ = 2.24 (s, 3H, CH_3), 2.30 (t, 2H, CH_2COOH), 2.45 (t, 2H, CH_2CH_2COOH), 3.41 (s, 3H, CH_3N), 4.40 (d, 2H, CH_2N), 6.09 (s, 1H, H_5), 6.90 (s, broad, 1H, OH), 8.20 (t, 1H, NH) ppm. MS (FAB): m/z = 269 (M^+); Anal. Calcd for $C_{12}H_{16}N_2O_5$: C, 53.7; H, 6.0; N, 10.4%. Found: C, 53.6; H, 5.8; N, 10.3%.

*1,6-Dimethyl-2-glutaramidomethyl-3-hydroxy-4(1H)-pyridinone (**15b**)* The general method was the same as that for compound **14**. The residue was recrystallized from methanol–diethyl ether. The compound (yield 70%) was separated as white crystals. m.p. 228–229°C; IR (KCl): broad 3500–3000, 2960, 1715 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ = 1.68 (m, 2H, CH_2), 2.15 (m, 4H, $CH_2CH_2CH_2$), 2.25 (s, 3H, CH_3), 3.42 (s, 3H, CH_3N), 4.37 (d, 2H, CH_2N), 6.09 (s, 1H, H_5), 7.3 (s, broad, 1H, OH), 8.13 (t, 1H, NH) ppm. MS (FAB): m/z = 283 (M^+); Anal. Calcd for $C_{13}H_{18}N_2O_5$: C, 55.3; H, 6.4; N, 9.9%. Found: C, 55.4; H, 6.5; N, 9.8%.

*1,6-Dimethyl-2-(3,3-dimethylglutaramido)methyl-3-hydroxy-4(1H)-pyridinone (**15c**)* The general method was the same as that for compound **14**. The residue was recrystallized from methanol–diethyl ether. The compound (yield 55%) was separated as white crystals. m.p. 200–202°C; IR (KCl): broad 3500–3000, 2960, 1713, 1506, 1249, 711 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ = 1.0 (s, 6H, $C(CH_3)_2$), 2.2 (s, 2H, CH_2), 2.3 (s, 2H, CH_2), 2.3 (s, 3H, CH_3), 3.5 (s, 3H, CH_3N), 4.45 (d, 2H, CH_2N, J = 6 Hz), 6.25 (s, 1H, H_5), 7.2 (s, broad, 1H, OH), 8.2 (t, 1H, NH, J = 6 Hz) ppm. MS (accurate mass, high resolution, FAB): Actual. m/z = 311.1607 (M^+). Measured m/z = 311.1623 (M^+); Anal. Calcd for $C_{15}H_{22}N_2O_5 \cdot \frac{3}{5}H_2O$: C, 56.1; H, 7.3; N, 8.7%. Found: C, 56.5; H, 7.1; N, 8.9%.

*1,6-Dimethyl-2-phthalamidomethyl-3-hydroxy-4(1H)-pyridinone hydrochloride (**15d**)* Dichloromethane (10 mL) containing 1,6-dimethyl-2-aminomethyl-3-benzyloxy-4(1*H*)-pyridinone (**12**) (1 g, 3.88 mmol) was added to 15 mL of dichloromethane containing phthalic anhydride (0.58 g, 3.88 mmol). The mixture was stirred overnight, filtered and washed with 10 mL of dichloromethane to give 1,6-dimethyl-2-phthalamidomethyl-3-benzyloxy-4(1*H*)-pyridinone (1.32 g, 84%) as white crystals. m.p. 186–188°C; ^{1}H NMR (DMSO-d_6): δ = 2.3 (s, 3H, CH_3), 3.55 (s, 3H, NCH_3), 4.55 (d, 2H, $HNCH_2$, J = 6 Hz), 5.1 (s, 2H, CH_2Ph), 6.2 (s, 1H, H_5), 7.2–7.8 (m, 9H, aromatic), 8.55 (t, 1H, NH, J = 6 Hz) ppm. MS (FAB): m/z = 407 (M^+); Anal. Calcd for $C_{23}H_{22}N_2O_5$: C, 68.0; H, 5.4; N, 6.9%. Found: C, 68.1; H, 5.0; N, 6.7%.

Nitrogen gas was passed over a solution of 20 mL of methanol, 40 mL dimethylformamide and 5 mL of conc. HCl containing 1,6-dimethyl-2-phthalamidomethyl-3-benzyloxy-4(1*H*)-pyridinone (1.15 g, 2.83 mmol) for a period of 3–5 min. The reaction was followed by the addition of palladium 5% w/w on activated carbon. Then nitrogen gas was again passed over the solution for a period of 3–5 min. Finally, hydrogen gas was passed over the solution overnight. The mixture was

warmed, filtered, concentrated in-vacuo, and recrystallized from methanol–diethyl ether to give compound **15d** (0.45 g, 45%). m.p. 240–241°C; IR (KCl): broad 3500–2500, 2665, 1766, 1710, 1616, 1546, 1468, 1319, 1131, 727 cm^{-1}; ^1H NMR (DMSO-d_6): δ = 2.57 (s, 3H, CH_3), 3.92 (s, 3H, NCH_3), 5.14 (s, 2H, CH_2), 7.23 (s, 1H, H_5), 7.87 (s, 4H, aromatic) ppm. MS (FAB): m/z = 317 ($M^{+\cdot}+1$); Anal. Calcd for $C_{16}H_{17}ClN_2O_5$: C, 54.5; H, 4.9; N, 7.9; Cl, 10.1%. Found: C, 54.3; H, 5.0; N, 8.0; Cl, 10.5%.

General procedures for physicochemical characterisation

The system used in this study comprises a UV–visible spectrophotometer (Perkin-Elmer Lambda 5 with thermostatted cell holders in size of 1-, 10- or 50-mm quartz flow cells), autoburette (Metrohm Dosimat 665 (1-mL syringe) and peristaltic pump (Watson-Marlow 101U/R M2) all interfaced to a computer. Solution temperature was maintained at 25±0.1°C (thermostatted jacketed titration vessel). KCl 0.1 M electrolyte solution was used throughout. Titration data was analysed using NONLINW1 (Taylor et al 1988) (non-linear least-squares regression analysis).

Ferric chloride 17.906 mM in 1% HCl (atomic absorption standard, Aldrich) was used as the iron stock solution. 4-Morpholinepropanesulfonic acid (MOPS, pH 7.4) 50 and 100 mM (BDH, Analar grade) and 18 MΩ water (Millipore) were used in the preparation of all solutions. EDTA was purchased from BDH (Analar grade) (EDTA-trisodium salt).

pK_a Determination

The electrodes were calibrated by titrating a volumetric standard strong acid, 150 μL HCl (0.2 M), with KOH (0.2 M), under an argon gas atmosphere. Following electrode calibration, 300 μL HCl (0.2 M) was added and the solution alkalimetrically titrated. For **14** and **15d** ($2–4\times10^{-5}$ M (gravimetric)), the spectrophotometric method was employed. However, for **15a–c** (4.4×10^{-4} M (gravimetric)) pK_a determination was carried out using simultaneous spectrophotometric and potentiometric titrimetry. The NONLINW program uses the Gauss–Newton–Marquart equations to refine pK_a values. These parameters are refined to optimise overlap with the experimental titration data. The best fitted curve obtained from the potentiometric plot of pH vs 0.2 M KOH or the spectrophotometric plot of absorbance at a specific wavelength vs pH, is used to validate the optimised parameters. For potentiometric titrations, the refined parameters are initial acid concentration, pK_w, electrode zero concentration of carbon dioxide and the pK_a values. For spectrophotometric titrations the refined parameters are extinction coefficient of protonated and deprotonated species, pK_a values and electrode zero. As the Gauss–Newton–Marquart algortihm is based on the non-linear least-squares method, using the pK_a value observed from the experimental curve avoids any possible uncertainty associated with inverting the matrix.

pH-Dependent UV spectrophotometric titration of iron(III)–ligand complexes

Electrode calibration and base-line correction for UV spectrophotometry were carried out using 25 mL of 85% methanol–0.1 M KCl under an argon atmosphere. The solution was re-acidified using HCl (0.2 M) and the pH of the solution was adjusted to 1.5–2. Then iron(III) (final concentration, 1 μM) and the phenolate-type 3-hydroxypyridin-4(1*H*)-one **14** (final concentrations of 2 and 3 μM) were added to the above solution. To provide a control solution, the 85% alcoholic (methanol)–0.1 M KCl solution was also used for titration of the iron(III)-**2** complex. The method used for the carboxylate type **15** was similar to that used for the phenolate type **14**. However due to the greater aqueous solubility of **15**, a 0.1 M KCl solution was employed.

A set of ligand–iron(III) complex samples were prepared with the ligand:iron(III) ratio in the range of 1:1 to 3.8:1. The final concentration of iron(III) was 4×10^{-5} M. The equilibrium absorbance (λ = 450 nm) was plotted against the ligand:iron(III) ratio.

*Spectrophotometric iron(III)–**15c** stability constants determination*

With a bidentate ligand there are 3 mononuclear complexes possible as defined by equations 1–5, the overall cumulative constant β_3 being the value which is generally used to compare ligand affinities. With a tridentate ligand there are only 2 mononuclear complexes as defined by equations 1, 2 and 4. The values of β_1, β_2 and, significantly, β_3 were determined for iron(III)–**15c**.

Iron(III) (final concentration, 1.1×10^{-4} M) and the carboxylate-type 3-hydroxypyridin-4(1*H*)-one **15c** (final concentration 1.1×10^{-3} M) were added to a 0.1 M KCl solution (pH 1.5–2) and alkalimetrically titrated. In similar fashion to the pK_a determination, the extinction, the extinction coefficients of the three iron(III)–ligand species (equations 1, 2 and 3) and the electrode zero were inserted in the *STABOPT* programme for optimisation (Hider et al 2000). The three values β_1, β_2

and β_3 were estimated from curve-fitting analyses using the Gauss–Newton–Marquart algorithm.

$$[M]+[L]\overset{K_1}{\rightleftharpoons}[ML]; \qquad K_1 = \frac{[ML]}{[M][L]} = \beta_1 \tag{1}$$

$$[ML]+[L]\overset{K_2}{\rightleftharpoons}[ML_2]; \qquad K_2 = \frac{[ML_2]}{[ML][L]} \tag{2}$$

$$[ML_2]+[L]\overset{K_3}{\rightleftharpoons}[ML_3]; \qquad K_3 = \frac{[ML_3]}{[ML_2][L]} \tag{3}$$

$$[M]+2[L]\overset{\beta_2}{\rightleftharpoons}[ML_2]; \qquad \beta_2 = K_1K_2 \tag{4}$$

$$[M]+3[L]\overset{\beta_3}{\rightleftharpoons}[ML_3]; \qquad \beta_3 = K_1K_2K_3 \tag{5}$$

Spectrophotometric competition studies between EDTA and iron(III)–ligand complexes

To a solution containing a final concentration of 2.6×10^{-5} M iron(III) and 2.6×10^{-4} M EDTA, was added a set volume of **15c** (3.9384 mM) in 3-(*N*-morpholino) propanesulfonic acid (MOPS) buffer (0.1 M, pH 7.4). The absorbance of the solution was monitored at λ = 456 nm to measure the Z value for each addition of the titrant (equation 6).

$$Z = (A - A_E)/(A_L - A_E) \tag{6}$$

where A is the absorbance of the competing system when both EDTA and the ligand are present in solution, A_E is the absorbance of the species [FeEDTA] in the absence of the ligand and A_L is the absorbance of the species [FeL_n] in the absence of EDTA. Data which possess a Z value between 0.2–0.8 were used to calculate the conditional stability constant (equation 7).

$$\begin{aligned} K^* &= (1-Z)([L]_T - nZ[Fe]_T)/Z([E]_T - (1-Z)[Fe]_T) \\ &= K_E/K_L \end{aligned} \tag{7}$$

where $[Fe]_T$, $[L]_T$, and $[E]_T$ are constant during the assay; n (iron(III) binding stoichiometry) is equal to one and three for a hexadentate ligand and a bidentate ligand, respectively. Having measured the overall conditional stability constant for the ligand, K_L, it is possible to calculate the overall stability constant, β, for the ligand by using equation 8.

$$K = \beta(\alpha)^n \tag{8}$$

where α is the fraction of the fully ionised species such as [$EDTA^{4-}$] and n is the iron(III) binding stoichiometry; α for EDTA can be calculated from the pK_a values of EDTA (2.00, 2.67, 6.16 and 10.26) as a function of pH. Consequently, the conditional stability constant for EDTA at pH 7.4, K_E, can be derived from the literature value for the absolute stability constant of EDTA ($\log\beta_E$ = 25.1) (Martell & Smith 1974–1989).

Results

Chemistry

To explore ways of further improving the Fe(III)-chelation characteristics of the 3-hydroxy-4(1*H*)-pyridinone unit, a range of 2-substituted derivatives were prepared and evaluated. The synthesis of both classes of 2-substituted 3-hydroxypyridin-4-one, **14** and **15**, involved the condensation of suitably derivatised carboxylic acids with the key amine intermediate 1,6-dimethyl-2-aminomethyl-3-benzyloxypyridin-4-one **12**.

*Preparation of 1,6-dimethyl-2-aminomethyl-3-benzyloxypyridin-4-one (**12**)*

A useful eight-step synthesis of this intermediate has been developed from kojic acid (**4**) (Tilbrook 1995) (Figure 2). Allomaltol (**6**) was obtained by chlorination of the hydroxymethyl group of kojic acid (**4**) and subsequent reduction to the methyl group using zinc/HCl. Selective hydroxymethylation at position 2 of allomaltol (**6**) was achieved with formaldehyde under basic conditions which affords alcohol **7**. Subsequent benzylation of the phenolic hydroxyl group and subsequent treatment with methylamine provided 1,6-dimethyl-2-hydroxymethyl-3-benzyloxy-pyridin-4-one (**9**). This intermediate was converted to amine **12** via the phthalamido intermediate **10** under Mitsunobu conditions (Mitsunobu et al 1972).

Preparation of hydroxypyridin-4-one phenolate derivatives

For the syntheses of **13c** and **13d**, the corresponding W hydroxy 2 alkyl phenol was suitably protected (phenolic hydroxyl group blocked as a benzyl ether) and converted to the corresponding bromide (Fieser & Fieser 1979; Mitsunobu 1981). Conversion of the bromide to the corresponding malonate and subsequent hydrolysis provided the required carboxylic acid intermediate **13**. Intermediate **13c** was also prepared directly by benzylation and subsequent hydrolysis of methyl-3-(2-hydroxy-phenyl)propionate. This latter method was preferred for the preparation of **13c**. The general preparative route for the hydroxypyridin-4-one phenolate derivatives involves the formation and isolation of suitably protected phenol derivatives, namely the *N*-hydroxysuccinimide esters. Reaction between key amine

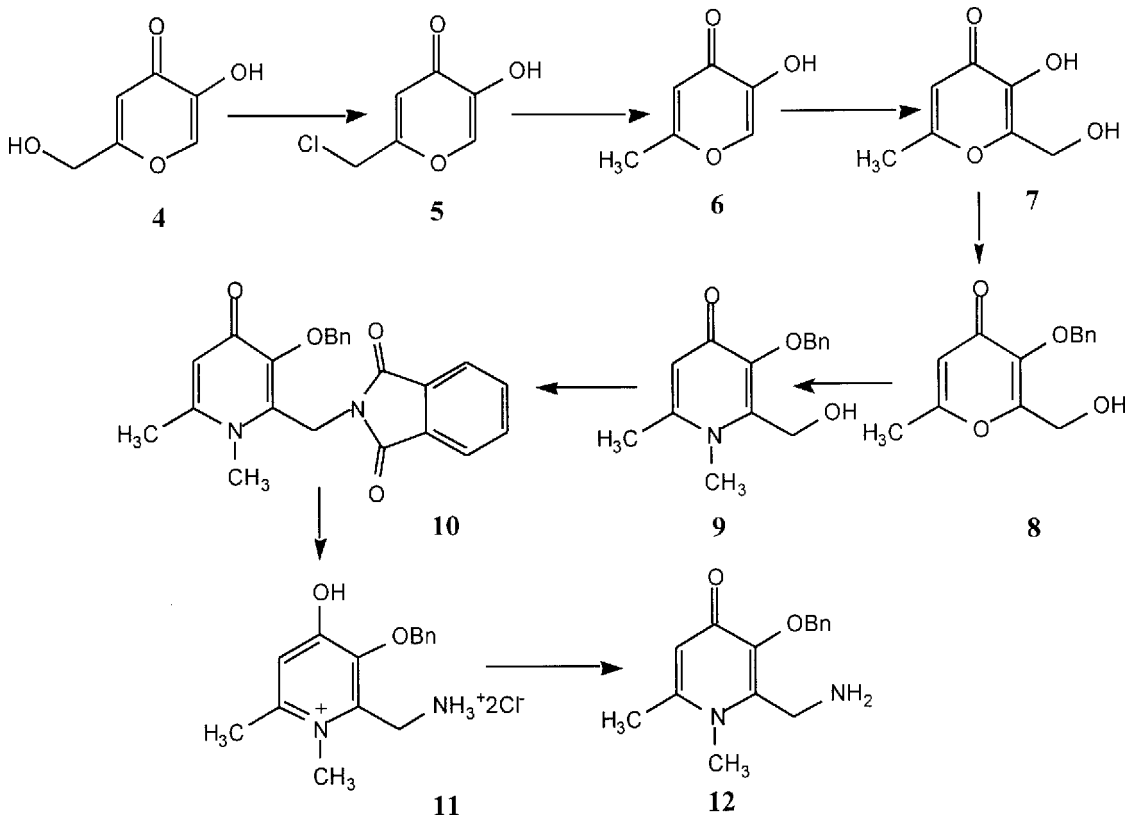


Figure 2 Preparation of amine intermediate **12**.

12 and the activated carboxylate derivatives followed by catalytic hydrogenation provided the desired potentially tridentate ligands **14**.

Preparation of hydroxypyridin-4-one carboxylate derivatives

The synthesis of the required congeneric series of hydroxypyridin-4-ones possessing a terminal carboxyl group appended to position 2 involves the formation and ammonolysis of the corresponding intermediate. Subsequent hydrogenation permitted the isolation of the desired potentially tridentate ligands **15a–c**. Interestingly this method failed to provide access to derivative **15d**. However, ammonolysis of phthalic anhydride with amine **12** yielded the corresponding conjugate. Deprotection using catalytic hydrogenation subsequently gave **15d**.

Physicochemical properties

To fully characterise the ability of this series of molecules to chelate iron, the pK_a values and iron(III) dissociation constants were determined.

Table 1 pK_a values for potential tridentate compounds type **14** and **15**.

Ligand	pK_a values	
	Spectrophotometric data	Potentiometric data
14a	3.38, 7.45, 9.69	
14b	3.39, 9.19, 10.02	
14c	3.16, 8.98, 10.35	
14d	3.09, 9.32, 10.53	
15a	3.28, 9.55	3.30, 4.41, 9.53
15b	3.25, 9.53	3.35, 4.42, 9.57
15c	3.23, 9.56	3.36, 4.37, 9.55
15d	3.40, 4.06, 9.69	

Standard deviation (s.d.) for the pK_a determination of the compounds **14** and **15** were in the range of 0.01–0.04 units

pK_a Determination

The pK_a values of the 3-hydroxypyridin-4(1*H*)-ones **14** and **15**, determined using the potentiometric or spectrophotometric methods, were found to be in close agreement (Table 1). Spectrophotometric titration of the

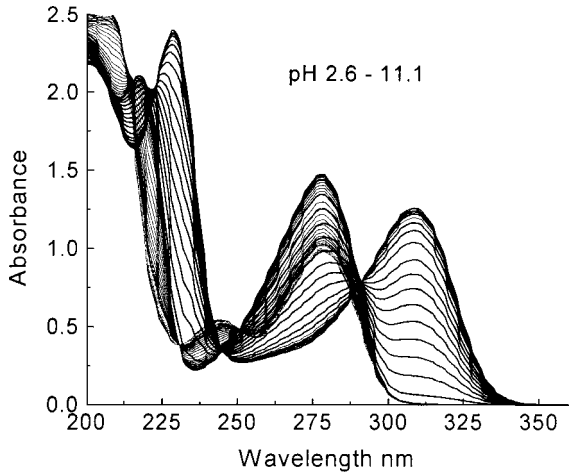


Figure 3 UV spectra of the carboxylate-type 3-hydroxy-4(1*H*)-pyridinone **15c**. The spectra were recorded between 200 and 360 nm over the pH range 2.6–11.1. [**15c**] = 4.4×10^{-4} M in 25.75 mL of 0.1 M KCl solution.

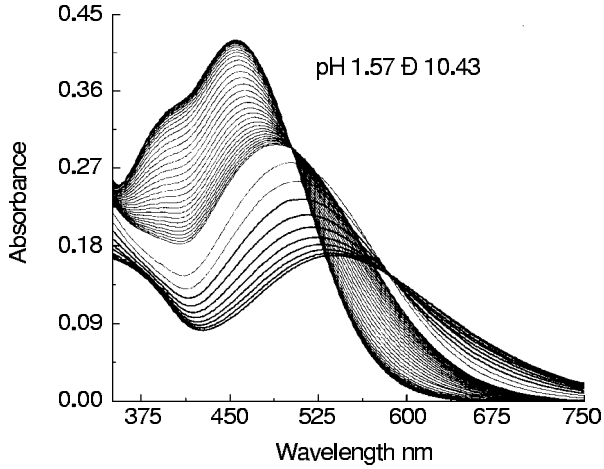


Figure 4 Visible spectra for iron–**15d** complexes. [Fe^{3+}] = 8.74×10^{-5} M; [**15d**] = 4.37×10^{-4} M; 0.1 M KCl solution containing 50% methanol. The calibration curve of electrolyte containing 50% methanol was obtained with an electrode zero of 10.94 mV. A mixed solvent was used for this study due to the relative insolubility of iron–**15d** complexes.

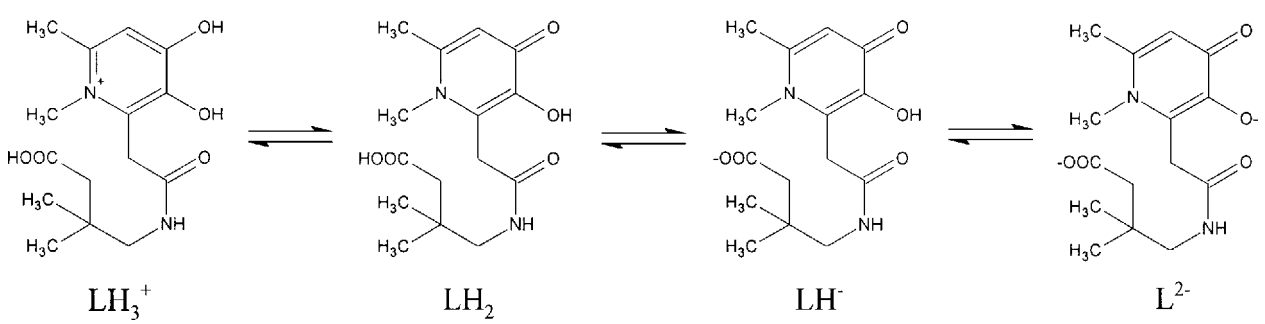
hydroxypyridinone **14** provided a series of closely related values with the single exception of the phenolic function of **14a** which had a pK_a value of 7.45 as compared with the range 10.02–10.53 for the other member of series. This lower pK_a value is probably due to hydrogen-bond formation between the NH of the amide and the oxyanion of the phenolate moiety **14a**. The phenolic pK_a values of ligands **14b–d** resemble that of *o*-methylphenol (10.28) (Perrin et al 1981).

The speciation spectra of **15c** (Figure 3) indicate that **15c** exists predominantly in the form LH_3^+ at pH 2 and as the pH rises, the fraction of this species declines and the LH_2, LH^- and L^{2-} species appear in sequence. This leads to a shift in λ_{max} from $\sim$276 nm to the longer wavelength of $\sim$308 nm, which can be assigned to the deprotonation of the phenolic OH groups. The speciation plots corresponding to **14** and **15** indicate that, at pH 7.4, the LH^- species (equation 9) dominates for the carboxylate-type 3-hydroxypyridin-4-ones **15** and LH_2 dominates for the phenolic type. The ionised fraction of the phenolic –OH of both types of pyridinone derivative is negligible at pH 7.4.

pH-Dependent titration of iron(III)–ligand complexes

To investigate whether the 2-substituted-3-hydroxypyridin-4(1*H*)-one derivatives **14** and **15** behave as either tridentate or bidentate ligands, the iron(III)-ligand complex speciation was investigated over the pH range of 2–11.

The iron(III)–ligand complex speciation spectra of the carboxylate-type ligand **15d** are shown in Figure 4. The phenolate-type 3-hydroxypyridin-4(1*H*)-one **14**–iron(III) complexes possess very low aqueous solubility at neutral pH. Consequently, 50% aqueous methanol



Equation 9

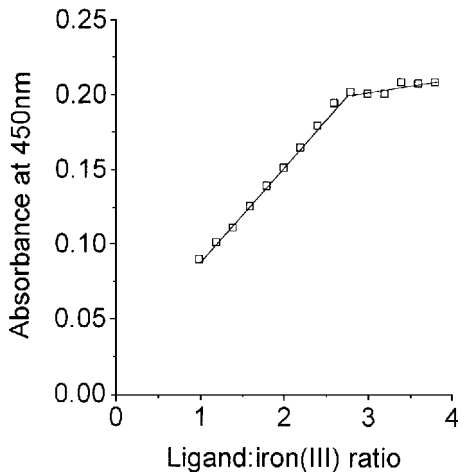


Figure 5 Stoichiometric titration curve for **15c**–iron(III). $[Fe^{3+}] = 4 \times 10^{-5}$ M, in 0.1 M MOPS buffer pH 7.4. The data of the absorbance ($\lambda_{450\ nm}$) of the iron–**15c** complex plotted against the molar ratio were fitted using linear regression.

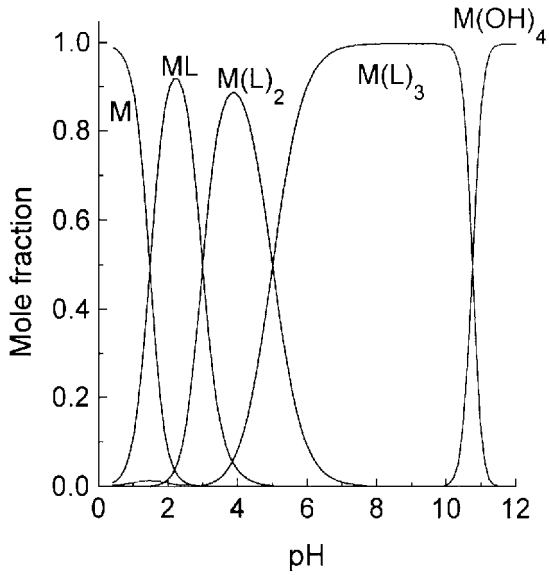


Figure 6 Speciation plot of the gem-dimethylcarboxylate **15c**. $[Fe^{3+}] = 10^{-6}$ M; [L] = 10^{-5} M.

was employed for these titrations. The series of spectra were very similar to those previously reported for bidentate hydroxypyridinones (Motekaitis & Martell 1991). Thus for **15d** the isosbestic point (~ 590 nm) delineates the transition from the FeL to FeL_2 and as the pH continues to rise from 4.5 to 7.0, the species FeL_2 disappears and the concentration of FeL_3 undergoes a corresponding increase. The isosbestic point (~ 500 nm) records the transition from FeL_2 to FeL_3. The stoichiometric titration curve for **15c** (Figure 5) demonstrates the existence of a 3:1 ligand–metal binding ratio. Similar results were obtained for the bidentate ligands **2a** and **2b**.

Spectrophotometric determination of iron(III)–ligand stability constants

Since both the phenolate-type 3-hydroxy-4(1*H*)-pyridinone **14** and the carboxylate-type **15** apparently behave as bidentate ligands, it was decided to measure the iron(III)–ligand stability constants for one of these ligands, namely water soluble **15c**. The log absolute stability constant values of **15c** were determined as $\log\beta_1 = 14.9 \pm 0.21$, $\log\beta_2 = 27.0 \pm 0.43$ and $\log\beta_3 = 36.6 \pm 0.54$, which are close to the corresponding values of analogous bidentate 3-hydroxypyridin-4(1*H*)-one **2b** (15.1, 27.2, 37.0). A value of $\log\beta_3 = 36.4 \pm 0.61$ was also determined using competition studies between EDTA and **15c** for iron(III). This value is similar to that of the similarly substituted bidentate ligand 1,2,6-trimethyl-3-hydroxy-4(1*H*)-pyridinone, **2b** ($\log\beta_3 = 37.7 \pm 0.5$) (Tilbrook 1995). The corresponding speciation plot of iron(III) in the presence of **15c** (Figure 6) demonstrates that the ML_3 species dominates at pH 7–10.

Discussion

The preparation of eight potentially tridentate 3-hydroxy-4(1*H*)-pyridinone derivatives, **14** and **15**, are described. The design of the molecules was such as to present iron(III) with three hard oxygen ligands. Energy minimisation studies indicated that **14c**, **14d** and **15a**–**d** were all capable of coordinating iron(III) in tridentate mode. However pH-dependent UV spectrophotometric titrations of the iron(III)–ligand complexes (both types 20 and 23) failed to produce any evidence for tridentate chelation. Data for iron(III)–**15d** is presented in Figure 4 and is typical of the bidentate hydroxypyridin-4-one mode of chelation (Motekaitis & Martell 1991). Similar results were determined for the other six ligands. These findings, together with stoichiometric titration of the carboxylate-type 3-hydroxypyridin-4-one **15c** (Figure 5), confirm that the compound-type **15** behaves as a bidentate ligand under the conditions investigated. Thus, with the stoichiometric titration, the increase in absorbance with introduction of ligand, saturated at an iron : ligand ratio of 2.7. Spectrophotometric titration of the **15c**–iron(III) complex yielded three stability constant values; namely $\log K_1 = 14.9$, $\log K_2 = 12.0$ and $\log K_3 = 9.7$ with an overall absolute stability constant

of $\log\beta_3 = 36.6$. This value was found to agree with the independent value obtained from competition studies between EDTA and the ligand **15c** for iron(III), $\log\beta_3 = 36.4 \pm 0.6$.

The reason for compounds **14c–d** and **15a–d** not acting as tridentate ligands is due to the major unfavourable entropic contribution associated with the third ligating group placed at ring position 2. A large degree of conformational freedom is associated with the pendant 2-substituent in the free ligand which would be largely lost upon chelation with Fe(III). In contrast, desferrithiocin (**3**) is relatively pre-organised, consisting of a five-membered heterocyclic ring directly linked to a pyridine ring in both the free ligand and metal complex and, therefore, there is a minimal loss of entropy associated with the ligand during the formation of the iron complex. This pre-organisation is only achievable using a nitrogen atom as both a linking and ligating element. A negative oxygen atom lacks this ability and can only use one valency for connection to the organic part of the ligand and so can not serve to connect adjacent chelating functions. This point is made clear by the comparison of analogous dicatechols and *bis*-amino phenols, where very long connecting bridges are required for ligands limited to negative oxygen donors (Evers et al 1989). Recently, a novel tridentate iron(III) chelator has been reported to be orally active in a range of animal models (Heinz et al 1999). However, this triazole (**16**) also contains a nitrogen atom, and consequently is predicted to have an appreciable affinity for divalent metals (Ryabukhin et al 1987).

Conclusion

To achieve high iron(III) selectivity under biological conditions, it is essential to use only hard oxygen ligands such as catechols, hydroxamates or pyridinonates. This can be readily achieved in both the bidentate (Hider & Hall 1991) and hexadentate (Raymond et al 1984) mode, but not in the tridentate mode. Therefore, orally active iron chelators are almost certainly limited to the bidentate class. At present there is no chelator type which is superior to that of the 3-hydroxypyridin-4-one group.

References

Baker, E., Peter, W. H., Jacobs, A. (1992) Desferrithiocin is an effective iron chelator in vivo and in vitro but ferrithiocin is toxic. *Br. J. Haematol.* **81**: 424–431

Bergeron, R. J., Weigand, J., Dionis, J. B., Elgi-Kormakka, M., Frei, J., Huxley-Tencer, A., Peter, H. H. (1991) Evaluation of desferrithiocin and its synthetic analogues as orally effective iron chelators. *J. Med. Chem.* **34**: 2072–2078

Bergeron, R. J., Strieff, R. R., Creary, E. A., Daniels, R. D., King, W., Luchetta, G., Weigand, J., Moerker, T., Peter, H. H. (1993) A comparative study of the iron-clearing properties of desferrithiocin analogues with desferrioxamine B in a cebus monkey model. *Blood* **81**: 2166–2173

Bergeron, R. J., Weigand, J., Weimar, W. R., Vinson, J. R. T., Bussenius, J., Yao, G. W., McManis, J. S. (1999a) Desazadesmethyldesferrithiocin analogues as orally effective iron chelators. *J. Med. Chem.* **42**: 95–108

Bergeron, R. J., Weigand, J., McManis, J. S., McCosar, B. H., Weimar, W. R., Brittenham, G. M., Smith, R. E. (1999b) Effects of C-4 stereochemistry and C-4′ hydroxylation on the iron clearing efficiency and toxicity of desferrithiocin analogues. *J. Med. Chem.* **42**: 2432–2440

Evers, A., Hancock, R. D., Martell, A. E., Motekaitis, R. J. (1989) Metal-iron recognition in ligands with negatively charged oxygen donor groups-complexation of Fe(III), Ga(III), In(III), Al(III) and other highly charged metal-ions. *J. Inorg. Chem.* **28**: 2189–2195

Fieser, L. F., Fieser, M. (1979) *Reagents for organic synthesis.* John Wiley & Sons, Inc., New York

Halliwell, B., Gutteridge, M. C. (1984) Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochem. J.* **219**: 1–14

Heinz, U., Hegetschweiler, K., Acklin, P., Faller, B., Lattmann, R., Schnebli, H. P. (1999) 4-[3,5-Bis(2-hydroxyphenyl)-1,2,4-triazol-1-yl]benzoic acid: a novel efficient and selective iron(III) complexing agent. *Angew. Chem. Int. Ed.* **38**: 2568–2570

Hershko, C., Konijn, A. M., Link, G. (1998) Iron chelators for thalassaemia. *Br. J. Haematol.* **101**: 399–406

Hider, R. C., Hall, A. D. (1991) Clinically useful chelators of tripositive elements. *Prog. Med. Chem.* **28**: 41–173

Hider, R. C., Choudhury, R., Rai, B. L., Dehkordi, L. S., Singh, S. (1996) Design of orally active iron chelators. *Acta Haematol.* **95**: 6–12

Hider, R. C., Liu, Z. D., Khodr, H. H. (2000) Metal chelation of polyphenols. *Methods Enzymol.* **335**: 190–203

Liu, Z. D., Khodr, H. K., Liu, D. Y., Lu, S. L., Hider, R. C. (1999) Synthesis, physicochemical characterisation and biological evaluation of 2-(1′-hydroxyalky)-3-hydroxypyridin-4-ones: novel iron chelators with enhanced pFe^{3+} values. *Med. Chem.* **42**: 4814–4823

Liu, Z. D., Khodr, H. K., Lu, S. L., Hider, R. C. (2000) Design, synthesis and evaluation of N-basic substituted 3-hydroxypyridin-4-ones: orally active iron chelators with lysosomotrophic potential. *J. Pharm. Pharmacol.* **52**: 263–272

Martell, A. E., Smith, R. M. (1974–1989) *Critical stability constants.* Vols 1–6, Plenum Press, New York

Martell, A. E., Motekaitis, R. J., Murase, I., Sala, L. F., Stoldt, R., Ng, C. Y., Rosenkrantz, H., Metterville, J. J. (1987) Development of iron chelators for Cooleys anemia. *Inorg. Chim. Acta* **138**: 215–230

Mitsunobu, O. (1981) The use of diethyl azodicarboxylate and triphenylphosphine in synthesis and transformation of natural-products. *Synthesis* **13**: 1–28

Mitsunobu, O., Wada, M., Sano, T. (1972) Stereospecific and stereoselective reactions. 1. Preparation of amines from alcohols. *J. Am. Chem. Soc.* **94**: 679–680

Modell, B., Letsky, E. A., Flynn, D. M., Peto, R., Weatherall, D. J. (1982) Survival and desferrioxamine in thalassaemia major. *Br. Med. J.* **284**: 1081–1084

Motekaitis, R. J., Martell, A. E. (1991) Stabilities of the iron(III) chelates of 1,2-dimethyl-3-hydroxy-4-pyridinone and related ligands. *Inorg. Chem. Acta* **183**: 71–80

Perrin, D. D., Dempsey, B., Serjeant, E. P. (1981) Prediction of pK_a values for phenols, aromatic carboxylic acids and aromatic amines. pK_a Prediction for organic acids and bases. Chapman & Hall, London

Peter, H. (1985) Industrial aspects of iron chelators: pharmaceutical applications. In: Spike, G., Montrevil, J., Crichton, R. R., Mazurier, J. (eds) *Proteins of iron storage and transport*. Elsevier, New York

Pippard, M. J., Letsky, E. A., Callender, S. T., Weatherall, D. J. (1978) Prevention of iron loading in transfusion-dependent thalassaemia. *Lancet* ii: 1178–1180

Pippard, M. J., Callender, S. T., Finch, C. A. (1982) Ferrioxamine excretion in iron-loaded man. *Blood* **60**: 288–294

Porter, J. B., Singh, S., Hoyes, K. P., Epemolu, O., Abeysinghe, R. D., Hider, R. C. (1994) Lessons from preclinical and clinical studies with 1,2-diethyl-3-hydroxypyridin-4-one, CP94 and related compounds. *Adv. Exper. Med. Biol.* **356**: 361–370

Rai, B. L., Liu, Z. D., Liu, D. Y., Lu, S. L., Hider, R. C. (1999) Synthesis, physicochemical properties and biological evaluation of ester prodrugs of 3-hydroxypyridin-4-one: design of orally active chelators with clinical potential. *Eur. J. Med. Chem.* **34**: 475–485

Raymond, K. N., Müller, G., Matzanke, B. F. (1984) Complexation of iron by siderophores: a review of their solution and structural chemistry and biological function. *Top. Curr. Chem.* **123**: 49–102

Ryabukhin, Y. I., Shibaeva, N. V., Kuzharov, A. S., Korobkova, V. G., Khokhlov, A. V., Garnovskii, A. D. (1987) Synthesis and investigation of complex compounds of transition metals. *Koord. Khim.* **13**: 869–874

Taylor, P. D., Morrison, I. E. G., Hider, R. C. (1988) Microcomputer application of non-linear regression analysis to metal-ligand equilibria. *Talanta* **35**: 507–512

Tilbrook, G. S. (1995) *Design and synthesis of hexadentate iron(III) chelating agents.* Ph.D. Thesis, University of London