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Design, synthesis and antimicrobial evaluation of hexadentate hydroxypyridinones with high iron(III) affinity

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Running title: hexadentate hydroxypyridinones as antimicrobials

Abstract: A range of hexadentate 3-hydroxypyridin-4-ones (HPOs) with high affinity for iron(III) have been synthesized. The log stability constants of two HPO-iron complexes ($\log K_1$) were determined to be over 34, and pFe values of the two HPOs were determined to be over 31. Antimicrobial assay indicated that they are able to markedly inhibit the growth of both Gram-positive and Gram-negative bacteria. Compounds **14a** and **14e** were found to exhibit the strongest inhibitory activity against *Staphyloccocus aureus, Bacillus subtilis, Pseudomonas aeruginosa* and *Escherichia coli,* with MIC values of 8, 8, 16 and 8 μg/mL, respectively. These results indicate that hexadentate 3-hydroxypyridin-4-ones have potential application as antimicrobial agents, especially in the treatment of wound infection.

Keywords: hydroxypyridinone; hexadentate; iron affinity; antimicrobial activity; iron chelator

Introduction

Emergence of antibiotic-resistant bacteria and fungi is a growing global problem.[1,2] There is an urgent need for the development of novel types of antimicrobial agents with a wide antimicrobial spectrum. In order to grow and thrive, bacteria need iron as a cofactor for many metabolic enzymes. Indeed, most microorganisms have developed efficient methods of absorbing iron from the environment, many involving the secretion of siderophores, which possess a high affinity and selectivity for iron(III).[3] For instance, *Staphylococcus aureus* has been demonstrated to use two hydroxycarboxylate-type siderophores, staphyloferrin A and staphyloferrin B, via the transporters Hts and Sir, respectively, to access the transferrin iron pool.[4,5]

Pseudomonas aeruginosa produces two extensively characterized siderophores, pyochelin and pyoverdin.[6] In principle, the introduction of high affinity iron selective chelating agents can limit the iron absorption of microorganism by competing with siderophores for binding iron.[7] However, the structure of antimicrobial chelators should differ appreciably from those of siderophores, otherwise the iron-chelator complex will be able to supply iron to the microorganism via the iron-siderophore transporter. In our previous work, hexadentate 3-hydroxypyridin-4-ones have been demonstrated to inhibit the growth of both Gram-positive and Gram-negative bacteria.[8-10] However, some chelators with high iron-binding constants, for instance, ethylenediamine-N, N'-bis(2-hydroxyphenyl) acetic acid (EDHPA, logK 34), N,N'-bis(2-hydroxybenzyl) ethylenediamine-N,N'-diacetic acid (HEBD, logK 40), and desferrioxamine (logK 30.4)[11] have been reported to only possess a weak ability to inhibit Gram-positive and Gram-negative bacterial growth,[12] indicating that iron affinity of the chelator is not the only factor which affects the antimicrobial potential of an iron chelator. In this study, we explore the influence of hydrophobicity on the antimicrobial efficacy of hexadentate 3-hydroxypyridin-4-ones.

Methods and Materials

Chemistry

All chemicals were of AR grade and used without any further purification. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 500 spectrometer with TMS as an internal standard. Electrospray ionization (ESI) mass spectra were obtained by infusing samples into an LCQ Deca XP ion trap instrument. High resolution mass spectra (HRMS) were determined on Waters QTOF micro.

*General procedure for preparation of **3***

A mixture of **2** (10g, 46.3mmol), amine RNH_2 (51 mmol), sodium hydroxide (4g, 100mmol) in methanol/water (40mL/40mL) was heated to reflux gently. The

reaction was monitored by TLC. After completion of the reaction (about 2h), the reactant was concentrated under reduced pressure to about half volume. Extracted with dichloromethane (3×60mL), the combined organic layers were washed with brine twice, dried over anhydrous sodium sulfate. After removal of the solvent, the crude product **3** was obtained as a brown oil.

3-(Benzyloxy)-1,2-dimethylpyridin-4(1H)-one (3a)

Yield: 88%. ^{1}HNMR (400MHz, $CDCl_3$) δ 2.06 (s, 3H, CH_3), 3.44 (s, 3H, CH_3), 5.13 (s, 2H, CH_2), 6.28 (d, *J* = 8.0Hz, 1H, Pyridinone C5-H), 7.18 (d, *J* = 8.0Hz, 1H, Pyridinone C6-H), 7.23-7.39 (m, 5H, Ar). ESI-MS: *m/z* 230 ($[M+H]^+$).

3-(Benzyloxy)-1-ethyl-2-methylpyridin-4(1H)-one (**3b)**

Yield: 86%. ^{1}H NMR (400MHz, $CDCl_3$) δ 1.28 (t, *J* = 7.6Hz, 3H, CH_3), 2.08 (s, 3H, CH_3), 3.80 (d, *J*=7.6Hz, 2H, CH_2), 5.18 (s, 2H, CH_2), 6.44 (d, *J*=7.6Hz, Pyridinone C5-H), 7.20 (d, *J*=7.6Hz, Pyridinone C6-H), 7.26-7.38 (m, 5H, Ar). ESI-MS: *m/z* 244 ($[M+H]^+$).

3-(Benzyloxy)-1-butyl-2-methylpyridin-4(1H)-one (3c)

Yield: 87%. ^{1}H NMR (400MHz, $CDCl_3$) δ 0.89 (t, *J* = 7.6Hz, 3H, CH_3), 1.24-1.30 (m, 2H, CH_2), 1.57-1.62(m, 2H, CH_2), 2.07 (s, 3H, CH_3), 3.71 (t, *J* = 7.6Hz, 2H, CH_2), 5.21 (s, 2H, CH_2), 6.41 (d, *J* = 7.6Hz, 1H, Pyridinone C5-H), 7.16 (d, *J* = 7.6Hz, 1H, Pyridinone C6-H), 7.25-7.39 (m, 5H, Ar). ESI-MS: *m/z* 272 ($[M+H]^+$).

3-(Benzyloxy)-1-hexyl-2-methylpyridin-4(1H)-one (3d)

Yield: 90%. ^{1}H NMR (400MHz, $CDCl_3$) δ 0.90 (t, *J* = 7.6Hz, 3H, CH_3), 1.21-1.30 (m, 6H, CH_2), 1.58-1.62 (m, 2H, CH_2), 2.07 (s, 3H, CH_3), 3.75 (t, *J* = 7.6Hz, 2H, CH_2), 5.23 (s, 2H, CH_2), 6.43(d, *J* = 7.6Hz, 1H, Pyridinone C5-H), 7.17(d, *J* = 7.6Hz, 1H, Pyridinone C6-H), 7.27-7.40 (m, 5H, Ar). ESI-MS: *m/z* 300 ($[M+H]^+$).

3-(Benzyloxy)-1-(2-hydroxyethyl)-2-methylpyridin-4(1H)-one (3e')

Yield: 85%. ^{1}H NMR (400MHz, $CDCl_3$) δ2.11 (s, 3H, CH_3), 3.84 (m, 4H, CH_2), 4.95 (s, 2H, CH_2), 6.15 (d, *J* = 6.0Hz, 1H, Pyridinone C5-H), 7.29-7.35 (m, 5H, Ar), 7.38(d, *J*= 6.0Hz, 1H, Pyridinone C6-H). ESI-MS: *m/z* 260 ($[M+H]^+$).

*Synthetic procedure for 3-(benzyloxy)-1-(2-(benzyloxy)ethyl) -2-methylpyridin-4(1H)-one (**3e**)*

To a solution of **3e'** (8g, 30.88mmol) in dry THF (80mL) was added sodium hydride (1.12g, 46.6 mmol) and benzyl chloride (4.0g, 32 mmol). The mixture was refluxed for 3h. Water was added dropwise cautiously to quench the reaction. The reactant was concentrated and then was dissolved in dichloromethane, washed with brine twice, dried over anhydrous sodium sulfate. After removal of the solvent, the crude product **3e** was obtained as a brown solid. Yield: 92 %. ^{1}H NMR (400MHz, $CDCl_3$) δ 2.06 (s, 3H, CH_3), 3.56 (t, *J* = 4.0 Hz, 2H, CH_2), 3.92 (t, *J* = 4.0 Hz, 2H, CH_2), 4.40 (s, 2H, CH_2), 5.16 (s, 2H, CH_2), 6.38 (d, *J* = 6.0 Hz, 1H, Pyridinone C5-H), 7.14 (d, *J* = 6.0 Hz, 1H, Pyridinone C6-H), 7.22-7.38 (m, 10H, Ph). ESI-MS: *m/z* 350 ($[M+H]^+$).

3-(Benzyloxy)-1-(2-methoxyethyl)-2-methylpyridin-4(1H)-one (3f)

Yield: 84%. 1H NMR (400MHz, $CDCl_3$) 2.09 (s, 3H, CH_3), 3.16 (s, 3H, CH_3), 3.48 (t, *J*=4.0Hz, 2H, CH_2), 3.80 (t, *J* = 4.0Hz, 2H, CH_2), 5.15 (s, 2H, CH_2), 6.35 (d, *J* =7.2Hz, 1H, Pyridinone C5-H), 7.22-7.34 (m, 5H, Ar),7.37(d, *J*=7.2Hz, 1H, Pyridinone C6-H). ESI-MS: *m/z* 274 ($[M+H]^+$).

*General procedure for preparation of **4***

A mixture of **3** (10mmol), SeO_2 (30mmol) in acetic acid/acetic anhydride (25mL/25mL) was heated at 90-100^{o}C for 6-8h. After removal of the solvent, the residue was purified by silica gel column chromatography using ethyl acetate/methanol (50:1～20:1) as an eluent to provide aldehyde **4** as a brown oil.

3-(Benzyloxy)-1-methyl-4-oxo-1,4-dihydropyridine-2-carbaldehyde (4a)

Yield: 77%. ^{1}H NMR ($CDCl_3$, 400MHz) δ 3.78 (s, 3H, CH_3), 5.50 (s, 2H, CH_2), 7.30-7.36 (m, 5H, Ph), 6.49 (d, *J* = 7.6 Hz, 1H, C5-H in pyridinone), 7.16 (d, *J* =7.6 Hz, 1H, C6-H in pyridinone), 10.05 (s, 1H, CHO). ESI-MS: *m/z* 244 ($[M+H]^+$) .

3-(Benzyloxy)-1-ethyl-4-oxo-1,4-dihydropyridine-2-carbaldehyde (4b)

Yield: 79%. ^{1}H NMR ($CDCl_3$, 400MHz) δ 1.26 (t, *J*=7.2Hz, 3H, CH_3), 4.19 (q, *J*=7.2Hz, 2H, CH_2), 5.50 (s, 2H, CH_2), 6.54 (d, *J*=7.6Hz, 1H, C5-H in pyridinone), 7.20 (d, *J*=7.6Hz, 1H, C6-H in pyridinone), 7.30-7.35 (m, 5H, Ph), 10.03 (s, 1H, CHO). ESI-MS: *m/z* 258 ($[M+H]^+$) .

3-(Benzyloxy)-1-butyl-4-oxo-1,4-dihydropyridine-2-carbaldehyde (4c)

Yield: 78%. ^{1}H NMR ($CDCl_3$, 400MHz) δ 0.88 (t, *J*=7.2Hz, 3H, CH_3), 1.33 (m, 2H, CH_2), 1.77 (m, 2H, CH_2), 4.12 (t, *J*=7.2Hz, 2H, CH_2), 5.48 (s, 2H, CH_2), 6.50 (d, *J*=7.6Hz, 1H, C5-H in pyridinone), 7.20 (d, *J*=7.6Hz, 1H, C6-H in pyridinone), 7.29-7.34 (m, 5H, Ar), 10.01 (s, 1H, CHO). ESI-MS: *m/z* 286 ($[M+H]^+$).

3-(Benzyloxy)-1-hexyl-4-oxo-1,4-dihydropyridine-2-carbaldehyde (4d)

Yield: 79%. ^{1}H NMR ($CDCl_3$, 400MHz) δ 0.89 (t, *J*=7.2Hz, 3H, CH_3), 1.33 (m, 6H, CH_2), 1.77 (m, 2H, CH_2), 4.13 (t, *J*=7.2Hz, 2H, CH_2), 5.49 (s, 2H, CH_2), 6.51 (d, *J*=7.6Hz, 1H, C5-H in pyridinone), 7.19 (d, *J*=7.6Hz,1H, C6-H in pyridinone), 7.30 -7.34 (m, 5H, Ph), 10.01 (s, 1H, CHO). ESI-MS: *m/z* 314 ($[M+H]^+$).

3-(Benzyloxy)-1-(2-(benzyloxy)ethyl)-4-oxo-1,4-dihydropyridine-2-carbaldehyde (4e)

Yield: 71%. ^{1}H NMR ($CDCl_3$, 400MHz) δ 3.55 (t, *J*=4.4Hz, 2H, CH_2), 4.34 (t, *J*=4.4Hz, CH_2), 4.37 (s, 2H, CH_2), 5.48 (s, 2H, CH_2), 6.51 (d, *J*=7.2Hz, 1H, C5-H in pyridinone), 7.14 (d, *J*=7.2Hz, 1H, C6-H in pyridinone), 7.25-7.33 (m, 10H, Ph), 9.95 (s, 1H, CHO). ESI-MS: *m/z* 364 ($[M+H]^+$).

3-(Benzyloxy)-1-(2-methoxyethyl)-4-oxo-1,4-dihydropyridine-2-carbaldehyde (4f)

Yield: 80%. ^{1}H NMR ($CDCl_3$, 400MHz) δ 3.23 (s, 3H, CH_3), 3.47 (t, *J*=4.4Hz, 2H, CH_2), 4.31 (t, *J*=4.4Hz, 2H, CH_2), 5.50 (s, 2H, CH_2), 6.49 (d, *J*=7.6Hz, 1H, C5-H in pyridinone), 7.25 (d, *J*=7.6Hz, 1H, C6-H in pyridinone), 7.31-7.35 (m, 5H, Ph), 10.00 (s, 1H, CHO). ESI-MS: *m/z* 288 ($[M+H]^+$).

*General procedure for preparation of **5***

To a solution of **4** （10mmol） in acetone/water (20mL/20mL) was added sodium chlorite (12 mmol) and sulfamic acid (15 mmol). The mixture was stirred at room temperature overnight. After filtration and washed with water and dried in a desiccator, compounds **5** were obtained as white powders.

3-(Benzyloxy)-1-methyl-4-oxo-1,4-dihydropyridine-2-carboxylic acid (5a)

Yield: 65%. ^{1}H NMR (DMSO, 400MHz) δ 3.60 (s, 3H, CH_3), 5.03 (s, 2H, CH_2), 6.31 (d, *J*=7.2Hz, 1H, C5-H in pyridinone), 7.24-7.36 (m, 5H, Ar), 7.65 (d, *J*=7.2Hz, 1H, C6-H in pyridinone). ESI-MS: *m/z* 260 ($[M+H]^+$).

3-(Benzyloxy)-1-ethyl-4-oxo-1,4-dihydropyridine-2-carboxylic acid (5b)

Yield: 62%. ^{1}H NMR (DMSO, 400MHz) δ 1.30 (t, *J*=7.6Hz, 3H, CH_3), 4.31 (q, *J*=7.6Hz, 2H, CH_2), 5.35 (s, 2H, CH_2), 6.59 (d, *J*=7.2Hz, 1H, C5-H in pyridinone), 7.28 (d, *J*=7.2Hz, 1H, C6-H in pyridinone), 7.22-7.35 (m, 5H, Ph). ESI-MS: *m/z* 274 ($[M+H]^+$).

3-(Benzyloxy)-1-butyl-4-oxo-1,4-dihydropyridine-2-carboxylic acid (5c) Yield: 55%. ^{1}H NMR (DMSO, 400MHz) δ 0.86 (t, *J*=7.6Hz, 3H, CH_3), 1.42 (m, 2H, CH_2), 1.79

(m, 2H, CH_2), 4.25 (t, *J*=7.6Hz, 2H, CH_2), 5.45 (s, 2H, CH_2), 6.55 (d, *J*=7.2Hz, 1H, C5-H in pyridinone), 7.20 (d, *J*=7.2Hz, 1H, C6-H in pyridinone), 7.25 -7.33 (m, 5H, Ph). ESI-MS: *m/z* 302 ($[M+H]^+$).

3-(Benzyloxy)-1-hexyl-4-oxo-1,4-dihydropyridine-2-carboxylic acid (5d)

Yield: 54%. ^{1}HNMR (DMSO, 400MHz) δ 0.84 (t, *J*=6.8Hz, 3H, CH_3), 1.20-1.26 (m, 6H, CH_2), 1.66-1.72 (m, 2H, CH_2), 3.88 (t, *J*=7.6Hz, 2H, CH_2), 5.04 (s, 2H, CH_2), 6.36 (d, *J*=7.2Hz, 1H, C5-H in pyridinone), 7.27-7.42 (m, 5H, Ph), 7.72 (d, *J*=7.2Hz, 1H, C6-H in pyridinone). ESI- MS: *m/z* 330 ($[M+H]^+$).

3-(Benzyloxy)-1-(2-(benzyloxy)ethyl)-4-oxo-1,4-dihydropyridine-2-carboxylic acid (5e)

Yield: 58%, ^{1}H NMR (DMSO, 400MHz) δ 3.69 (t, *J*=4.4Hz, 2H, CH_2), 4.12 (s, 2H, CH_2) , 4.46 (t, *J*=4.4Hz, CH_2), 5.06 (s, 2H, CH_2), 6.33 (d, *J*=7.2Hz, 1H, C5-H in pyridinone), 7.22-7.41(m, 10H, Ph), 7.69 (d, *J*=7.2Hz, 1H, C6-H in pyridinone). ESI-MS: *m/z* 380 ($[M+H]^+$).

3-(Benzyloxy)-1-(2-methoxyethyl)-4-oxo-1,4-dihydropyridine-2-carboxylic acid (5f)

Yield: 60%, ^{1}H NMR (DMSO, 400MHz) δ 3.22 (s, 3H, CH_3), 3.59 (t, *J*=5.2Hz, 2H, CH_2), 4.07 (t, *J*=5.2Hz, 2H, CH_2), 5.04 (s, 2H, CH_2), 6.34 (d, *J*=7.6Hz, 1H, C5-H in pyridinone), 7.28-7.41 (m, 5H, Ph, 7.61 (d, *J*=7.6Hz, 1H, C6-H in pyridinone). ESI-MS: *m/z* 304 ($[M+H]^+$).

*Synthetic procedure for **11a***

Amine **10** was synthesized from nitromethane in four steps.[13] To a solution of **10** (1.2g, 2.4 mmol) in CH_2Cl_2 (20 mL) cooled with an ice-bath was added triethyl amine

(0.29 g, 2.88 mmol). Butyryl chloride (0.31 g, 2.88mmol) was then added dropwise. The reaction mixture was stirred at 0°C for 3h, and then at room temperature for additional 3h. The solution was washed with 5% $NaHCO_3$ (2×20 mL) and brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography （cyclohexane : ethyl acetate, 1:1） to provide **11a** as a white solid. Yield: 85%. ^{1}H NMR ($CDCl_3$, 400MHz) δ 0.93 (t, *J*=7.2Hz, 3H, CH_3), 1.30-1.38 (m, 2H, CH_2), 1.46 (m, 27H, CH_3), 1.47-1.53 (m, 6H, CH_2), 1.58-1.66 (m, 6H, CH_2), 2.08 (t, *J*=7.2Hz, 2H, CH_2), 3.08 (q, *J*=7.6Hz, 6H, CH_2), 4.74 (s, 3H, NH), 5.21 (s, 1H, NH). ESI-MS: *m/z* 573 ($[M+H]^+$).

*Synthetic procedure for **11b***

A mixture of N-carbobenzyloxy-L-leucine (0.265 g, 1 mmol), N-hydroxysuccinimide (NHS) (0.134 g, 1.2 mmol) and N, N'-dicyclohexyl carbodiimide (DCC, 0.247 g, 1.2mmol) in DMF/CH_2Cl_2 (3mL/3mL) was stirred at room temperature overnight. Compound **10** (0.502 g, 1mmol) was added to the reaction mixture and continued to stir overnight.， After removal of the solvent, the residue was dissolved in CH_2Cl_2, washed with saturated $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (cyclohexane:EtOAc, 1:1) to provide **11b** as a white solid. Yield: 88%. ^{1}H NMR (DMSO, 400MHz) δ 0.87-0.96 (m, 6H, CH_3), 1.47 (m, 27H, CH_3), 1.49-1.58 (m, 7H, $3CH_2$ and buried CH), 1.63 (m, 6H, CH_2), 1.68 (m, 2H, CH_2), 3.13 (q, *J*=7.6Hz, 6H, CH_2), 3.98 (m, 1H, CH), 5.02 (s, 2H, CH_2), 7.26 -7.35 (m, 5H, Ph). ESI-MS: *m/z* 751 ($[M+H]^+$).

*General procedure for preparation of **12.***

A solution of **11** (1.5 g) in 96% formic acid (10 mL) was stirred at room temperature for 24h. After removal of formic acid, toluene was added and then removed to get

rid of residential formic acid. The crude product **12** was obtained as a pale yellow oil in quantitative yield.

12a: Yield: 96%, ^{1}H NMR (DMSO, 400MHz) δ 0.89 (s, *J*=7.6Hz, 3H, CH_3), 1.50 (m, 2H, CH_2), 1.52 -1.64 (m, 12H, CH_2), 2.06 (t, *J*=7.6Hz, 2H, CH_2), 2.74 (t, *J*=8.0Hz, 6H, CH_2). ESI-MS: *m/z* 273 ($[M+H]^+$).

12b: Yield: 97%. ^{1}H NMR (DMSO, 400MHz) δ 0.85- 0.93 (m, 6H, CH_3), 1.45-1.65 (m, 13H, CH_2 and buried CH), 1.69 (m, 2H, CH_2), 3.10 (t, *J*=8.0Hz, 6H, CH_2), 5.01 (s, 2H, CH_2), 7.24 -7.33 (m, 5H, Ph). ESI-MS: *m/z* 451 ($[M+H]^+$).

*General procedure for preparation of **13***

A mixture of **5** (5 mmol), NHS (0.67 g, 6 mmol) and DCC (1.236g, 6 mmol) in dry DMF (20 mL) was stirred at room temperature overnight. **12** (1 mmol) was then added to the reaction mixture, followed by the addition of triethylamine (3.6 mmol). The resulting mixture was stirred at room temperature for 2 days. After removal of the solvent, the residue was purified by column chromatography using MeOH/EtOAc (1:8 to 1:3) as an eluent to give the desired product **13** as pale yellow solids.

13a. Yield: 62%. ^{1}H NMR (DMSO-d_6, 400MHz) δ 0.82 (t, *J*=7.2Hz, 3H, CH_3), 1.32 (m, 2H, CH_2), 1.35-1.49 (m, 6H, CH_2), 1.51-1.61 (m, 6H, CH_2), 1.98 (t, *J*=7.2Hz, 2H, CH_2), 3.12 (q, *J*=6.4Hz, 6H, CH_2), 3.51 (s, 9H, CH_3), 5.03 (s, 6H, CH_2), 6.19 (d, *J*=7.2Hz, 3H, C5-H in pyridinone), 6.96 (br, 1H, NH), 7.23-7.34 (m, 15H, Ph), 7.57 (d, *J*=7.2Hz, 3H, C6-H in pyridinone), 8.80 (t, *J*=5.6Hz, 3H, NH); ^{13}C NMR (100Hz, DMSO) δ 173.17, 172.25, 161.17, 144.76, 140.54, 140.20, 138.19, 128.60, 128.29, 128.18, 117.48, 73.17, 57.51, 49.10, 40.94, 38.58, 32.68, 23.24, 19.36, 14.17. ESI-MS: *m/z* 996

([M+H]$^+$); HRMS: calcd. for $C_{56}H_{66}N_7O_{10}$ ([M+H]$^+$) 996.4871, found 996.4848; calcd. for $C_{56}H_{65}N_7O_{10}Na$ ([M+Na]$^+$) 1018.4691, found 1018.4675.

13b. Yield: 65%. ^{1}H NMR (CD_3OD, 400MHz) δ 0.92 (t, *J*=7.2Hz, 3H, CH_3), 1.41 (t, *J*=7.2Hz, 15H, $3CH_3$ and $3CH_2$), 1.55-1.60 (m, 8H, CH_2), 2.08 (t, *J*=7.6Hz, 2H, CH_2), 3.21 (t, *J*=7.2Hz, 6H, CH_2), 3.98 (q, *J*=7.2Hz, 6H, CH_2), 5.11 (s, 6H, CH_2), 6.51 (d, *J*=7.2Hz, 3H, C5-H in pyridinone), 7.29-7.43 (m, 15H, Ph), 7.74 (d, *J*=7.2Hz, 3H, C6-H in pyridinone); ^{13}C NMR (100Hz, DMSO) δ173.11, 172.22, 161.16, 144.64, 139.92, 139.16, 138.27, 128.59, 128.21, 128.11, 118.01, 73.17, 57.50, 54.41, 49.07, 38.60, 32.84, 23.21, 19.41, 16.96, 14.19. ESI-MS: *m/z* 1038 ([M+H]$^+$); HRMS: calcd. for $C_{59}H_{72}N_7O_{10}$ ([M+H]$^+$) 1038.5341, found 1038.5332; calcd. for $C_{59}H_{71}N_7O_{10}Na$ ([M+Na]$^+$) 1060.5160, found 1060.5148.

13c. Yield: 70%. ^{1}H NMR ($CDCl_3$, 400MHz) δ 0.81-0.89 (m, 12H, CH_3), 1.25 -1.42 (m, 14H, CH_2), 1.46-1.70 (m, 12H, CH_2), 1.99 (t, *J*=7.6Hz, 2H, CH_2), 3.12 (t, *J*=4.0Hz, 6H, CH_2), 3.79 (t, *J*=8.0Hz, 6H, CH_2), 5.06 (s, 6H, CH_2), 6.25 (d, *J*=7.2Hz, 3H, C5-H in pyridinone), 7.28-7.39 (m, 15H, Ph), 7.68 (d, *J*=7.2Hz, 3H, C6-H in pyridinone); ^{13}C NMR (100Hz, DMSO) δ173.11, 172.26, 161.06, 144.68, 139.94, 139.73, 138.22, 128.57, 128.21, 128.11, 117.64, 73.11, 57.54, 53.62, 49.13, 38.61, 33.10, 32.76, 23.26, 19.55, 19.38, 14.17, 13.92. ESI-MS: *m/z* 1122 ([M+H]$^+$); HRMS: calcd. for $C_{65}H_{84}N_7O_{10}$ ([M+H]$^+$) 1122.6280, found 1122.6267; calcd. for $C_{65}H_{83}N_7O_{10}Na$ ([M+Na]$^+$) 1144.6099, found 1144.6107.

13d. Yield: 76%. ^{1}H NMR ($CDCl_3$, 400MHz) δ 0.89 (m, 12H, CH_3), 1.08 (m, 6H, CH_2), 1.29 (m, 24H, CH_2), 1.53 (m, 2H, CH_2), 1.77 (m, 6H, CH_2), 1.92 (t, *J*=7.6Hz, 2H, CH_2), 3.10 (t, *J*=7.6Hz, 6H, CH_2), 3.75-3.79 (m, 6H, CH_2), 4.98 (s, 6H, CH_2), 6.21 (d, *J*=7.2Hz, 3H, C5-H in pyridinone), 7.15 (d, *J*=7.2Hz, 3H, C6-H in pyridinone), 7.25-7.37 (m, 15H,

Ph); ^{13}C NMR (100Hz, DMSO) δ173.11, 172.21, 161.07, 144.69, 139.91, 139.73, 138.25, 128.55, 128.20, 128.07, 117.63, 73.08, 57.44, 53.90, 49.08, 38.65, 32.76, 31.21, 31.02, 25.92, 23.28, 22.38, 19.38, 14.30, 14.17. ESI-MS: *m/z* 1206 ($[M+H]^+$); HRMS: calcd. for $C_{71}H_{95}N_7O_{10}Na$ ($[M+Na]^+$) 1228.7038, found 1228.7004.

13e. Yield: 68%, ^{1}H NMR (CD_3OD, 400MHz) δ 0.88 (t, *J*=7.2Hz, 3H, CH_3), 1.34 (m, 6H, CH_2), 1.52 (m, 8H, CH_2), 2.04 (t, *J*=7.2Hz, 2H, CH_2), 3.13 (t, *J*=7.6Hz, 6H, CH_2), 3.72 (t, *J*=4.8Hz, CH_2), 4.11 (t, *J*=4.8Hz, 6H, CH_2), 4.45 (s, 6H, CH_2), 5.09 (s, 6H, CH_2), 6.45 (d, *J*=7.6Hz, 3H, C5-H in pyridinone), 7.25-7.38 (m, 30H, Ph), 7.68 (d, *J*=7.6Hz, 3H, C6-H in pyridinone); ^{13}C NMR (100Hz, DMSO) δ173.36, 172.27, 161.10, 144.75, 140.82, 139.77, 138.50, 138.21, 128.75, 128.58, 128.22, 128.12, 127.95, 127.74, 117.08, 73.17, 72.51, 69.10, 53.54, 49.10, 32.83, 29.53, 26.96, 23.25, 19.40, 14.18. ESI-MS: *m/z* 1356 ($[M+H]^+$); HRMS: calcd. for $C_{80}H_{89}N_7O_{13}Na$ ($[M+Na]^+$) 1378.6416, found 1378.6425.

13f. Yield: 71%, ^{1}H NMR (CD_3OD, 400MHz) δ 0.92 (t, *J*=7.6Hz, 3H, CH_3), 1.39 (m, 6H, CH_2), 1.57 (m, 8H, CH_2), 2.09 (t, *J*=7.6Hz, 2H, CH_2), 3.19 (t, *J*=7.2Hz, 6H, CH_2), 3.31 (s, 9H, CH_3), 3.65 (t, *J*=4.8Hz, CH_2), 4.10 (t, *J*=4.8Hz, 6H, CH_2), 5.12 (s, 6H, CH_2), 6.47 (d, *J*=7.2Hz, 3H, C5-H in pyridinone), 7.24-7.37 (m, 15H, Ph), 7.69 (d, *J*=7.2Hz, 3H, C6-H in pyridinone); ^{13}C NMR (100Hz, DMSO) δ173.27, 172.20, 161.05, 144.70, 140.67, 139.74, 138.21, 128.58, 128.22, 128.12, 117.12, 73.10, 71.16, 58.68, 57.44, 53.22, 49.08, 38.59, 32.80, 23.22, 19.37, 14.18. ESI-MS: *m/z* 1128 ($[M+H]^+$); HRMS: calcd. for $C_{62}H_{78}N_7O_{13}$ ($[M+H]^+$) 1128.5658, found 1128.5674; calcd. for $C_{62}H_{77}N_7O_{13}Na$ ($[M+Na]^+$) 1150.5477, found 1150.5496.

13g. Yield: 74%, ^{1}H NMR (CD_3OD, 400MHz) δ 0.90 (d, *J*=6.8Hz, 6H, CH_3), 1.35-1.41 (m, 16H, $3CH_3$, $3CH_2$ and CH), 1.50-1.57 (m, 8H, CH_2), 3.18 (t, *J*=7.6Hz, 6H, CH_2), 3.97 (q, *J*=7.2Hz, 6H, CH_2), 4.07-4.12 (m, 1H, CH), 5.01 (s, 2H, CH_2), 5.11 (s, 6H, CH_2), 6.50

(d, *J*=7.2Hz, 3H, C5-H in pyridinone), 7.27-7.43 (m, 20H, Ph), 7.72 (d, *J*=7.2Hz, 3H, C6-H in pyridinone). ESI-MS: *m/z* 1215 ($[M+H]^+$); HRMS: calcd. for $C_{69}H_{83}N_8O_{12}$ ($[M+H]^+$) 1215.6130, found 1215.6086; calcd. for $C_{69}H_{82}N_8O_{12}Na$ ($[M+Na]^+$) 1237.5950, found 1237.5925.

13h. Yield: 72%, ^{1}H NMR ($CDCl_3$, 400MHz) δ 0.89-0.97 (m, 15H, CH_3), 1.30 -1.39 (m, 13H, 6CH_2 and CH), 1.50-1.56 (m, 8H, CH_2), 1.75-1.83 (m, 6H, CH_2), 3.17 (t, *J*=8.0Hz, 6H, CH_2), 3.93 (t, *J*=8.0Hz, 6H, CH_2), 4.07-4.12 (m, 1H, CH), 5.01 (s, 2H, CH_2), 5.12 (s, 6H, CH_2), 6.50 (d, *J*=4.0Hz, 3H, C5-H in pyridinone), 7.28-7.43 (m, 20H, Ph), 7.72 (d, *J*=4.0Hz, 3H, C6-H in pyridinone). ESI-MS: *m/z* 1299 ($[M+H]^+$); HRMS: calcd. for $C_{75}H_{95}N_8O_{12}$ ($[M+H]^+$) 1299.7069, found 1299.7067; calcd. for $C_{75}H_{94}N_8O_{12}Na$ ($[M+Na]^+$) 1321.6889, found 1321.6903.

13i. Yield: 71%, ^{1}H NMR(CD_3OD, 400MHz) δ 0.91 (d, *J*=6.8Hz, 6H, CH_3), 1.32 -1.41 (m, 6H, CH_2), 1.51-1.59 (m, 7H, 3CH_2 and CH), 1.63-1.74 (m, 2H, CH_2), 3.18 (t, *J*=4.0Hz, 6H, CH_2), 3.29 (s, 9H, CH_3), 3.65 (t, *J*=4.0Hz, 6H, CH_2), 4.03 (m, 1H, CH), 4.09 (t, *J*=4.0Hz, 6H, CH_2), 5.02 (s, 2H, CH_2), 5.12 (s, 6H, CH_2), 6.45 (d, *J*=4.0Hz, 3H, C5-H in pyridinone), 7.28-7.42 (m, 20H, Ar), 7.66 (d, *J*=4.0Hz, 3H, C6-H in pyridinone). ESI-MS: *m/z* 1305 ($[M+H]^+$); HRMS: calcd. for $C_{72}H_{89}N_8O_{15}$ ($[M+H]^+$) 1305.6447, found 1305.6412; calcd. for $C_{72}H_{88}N_8O_{15}Na$ ($[M+Na]^+$) 1327.6267, found 1327.6260.

*General procedure for preparation of **14***

To a suspension of **13 (13a-i)** (1mmol) and concentrated hydrochloric acid (1mL) in MeOH (30 mL) was added 5% Pd/C (0.2 g). Hydrogenation was carried out at 30 psi H_2 for 8h. After filtration to remove the catalyst, the filtrate was concentrated to dryness. The residue was purified by crystallization from methanol/acetone. Hydrochlorides of hexadentate ligand **14** were obtained as white solids.

14a. Yield: 95%, ^{1}H NMR (CD_3OD, 500MHz) δ 0.97 (t, *J*=5.0Hz, 3H, CH_3), 1.67 (m, 8H, CH_2), 1.88 (m, 6H, CH_2), 2.25 (t, *J*=5.0Hz, 2H, CH_2), 3.47 (t, *J*=7.5Hz, 6H, CH_2), 4.10 (s, 9H, CH_3), 7.27 (d, *J*=5.0Hz, 3H, C5-H in pyridinone), 8.25(d, *J*=5.0Hz, 3H, C6-H in pyridinone). ^{13}CNMR (100Hz, DMSO) δ 171.3, 160.6, 158.2, 141.9, 137.1, 136.1, 111.2, 57.0, 56.2, 43.2, 39.7, 37.4, 22.2, 18.8, 13.2. ESI-MS: *m/z* 726 ($[M+H]^+$); HRMS: calcd. for $C_{35}H_{48}N_7O_{10}$ ($[M+H]^+$), 726.3462; found, 726.3496.

14b. Yield: 94%, ^{1}H NMR (CD_3OD, 500MHz) δ 0.98 (t, *J*=7.5Hz, 3H, CH_3), 1.57 (t, *J*=7.0Hz, 9H, CH_3), 1.67 (m, 8H, CH_2), 1.88 (m, 6H, CH_2), 2.22 (t, *J*=7.5Hz, 2H, CH_2), 3.47 (t, *J*=7.5Hz, 6H, CH_2), 4.41 (q, *J*=7.0Hz, 6H, CH_2), 7.29 (d, *J*=6.5Hz, 3H, C5-H in pyridinone), 8.33 (d, *J*=6.5Hz, 3H,C6-H in pyridinone). ^{13}C NMR (100Hz, DMSO) δ 171.9, 161.3, 158.5, 142.7, 138.1, 136.7, 111.8, 57.1, 56.3, 40.1, 39.3, 37.9, 22.5, 18.8, 13.7, 13.3. ESI-MS: *m/z* 768 ($[M+H]^+$); HRMS: calcd. for $C_{38}H_{54}N_7O_{10}$ ($[M + H]^+$), 768.3931; found, 768.3944.

14c. Yield: 96%, ^{1}H NMR (CD_3OD, 400MHz) δ 0.83-0.88 (m, 12H, CH_3), 1.24 -1.49 (m, 14H, CH_2), 1.46-1.77 (m, 12H, CH_2), 2.04 (t, *J*=7.6Hz, 2H, CH_2), 3.21-3.27 (m, 6H, CH_2), 4.20 (t, *J*=6.4Hz, 6H, CH_2), 7.42 (d, *J*=8.0Hz, 3H, C5-H in pyridinone), 8.31 (d, *J*=8.0Hz, 3H, C6-H in pyridinone). ^{13}C NMR (100Hz, DMSO) δ 171.8, 161.4, 158.6, 142.8, 138.2, 136.8, 111.9, 57.0, 56.2, 40.0, 39.0, 37.9, 37.5, 32.3, 22.6, 18.8, 13.7, 13.3. ESI-MS: *m/z* 852 ($[M+H]^+$); HRMS: calcd. for $C_{44}H_{66}N_7O_{10}$ ($[M+H]^+$), 852.4871; found, 852.4882.

14d. Yield: 96%, ^{1}H NMR (CD_3OD, 400MHz) δ 0.90-0.99 (m, 12H, CH_3), 1.25 -1.40 (m, 20H, CH_2), 1.64 (m, 6H, CH_2), 1.90 (m, 12H, CH_2), 2.20 (t, *J*=7.6Hz, 2H, CH_2), 3.44 (t, *J*=6.4Hz, 6H, CH_2), 4.33 (t, *J*=6.4Hz, 6H, CH_2), 7.26 (d, *J*=6.4Hz, 3H, C5-H in pyridinone), 8.29 (d, *J*=6.4Hz, 3H, C6-H in pyridinone). ^{13}C NMR (DMSO, 100Hz) δ

171.9, 161.3, 158.5, 142.7, 138.1, 136.7, 111.8, 57.0, 56.2, 40.1, 39.3, 37.9, 32.5, 30.4, 30.3, 25.1, 22.6, 21.7, 18.8, 13.6. ESI-MS: *m/z* 936 ($[M+H]^+$); HRMS: calcd. for $C_{50}H_{78}N_7O_{10}$ ($[M+H]^+$), 936.5809; found, 936.5818.

14e. Yield: 93%, ^{1}H NMR (CD_3OD, 400MHz) δ 0.98 (t, *J*=7.2Hz, 3H, CH_3), 1.66 (m, 8H,CH_2), 1.87 (m, 6H, CH_2), 2.28 (t, *J*=7.2Hz, 2H, CH_2), 3.45 (t, *J*=7.2Hz, 6H, CH_2), 3.93 (t, *J*=4.8Hz, 6H, CH_2), 4.55 (t, *J*=4.8Hz, 6H, CH_2), 7.27 (d, *J*=6.8Hz, 3H, C5-H in pyridinone), 8.24 (d, *J*=6.8Hz, 3H, C6-H in pyridinone). ^{13}C NMR (100Hz, DMSO) δ 171.0, 161.3, 158.3, 142.7, 138.9, 136.5, 111.2, 68.7, 59.4, 56.4, 40.1, 37.4, 25.1, 22.2, 18.8, 13.3. ESI-MS: *m/z* 816 ($[M+H]^+$)); HRMS: calcd. for $C_{38}H_{54}N_7O_{13}$ ($[M + H]^+$), 816.3779; found, 816.3798.

14f. Yield: 96%, ^{1}H NMR (CD_3OD, 500MHz) δ 0.98 (t, *J*=7.5Hz, 3H, CH_3), 1.66 (m, 8H, CH_2), 1.86 (m, 6H, CH_2), 2.23 (t, *J*=7.5Hz, 2H, CH_2), 3.42 (s, 9H, CH_3), 3.46 (t, *J*=7.5Hz, 6H, CH_2), 3.79 (t, *J*=5.0Hz, 6H, CH_2), 4.55 (t, *J*=5.0Hz, 6H, CH_2), 7.27 (d, *J*=7.0Hz, 3H, C5-H in pyridinone), 8.23 (d, *J*=7.0Hz, 3H, C6-H in pyridinone). ^{13}C NMR (100Hz, DMSO) δ 171.0, 161.3, 158.3, 142.7, 138.9, 136.5, 111.2, 69.9, 58.8, 57.8, 56.2, 40.0, 39.6, 25.1, 22.6, 18.8, 13.6. ESI-MS: *m/z* 858 ($[M+H]^+$); HRMS: calcd. for $C_{41}H_{60}N_7O_{13}$ ($[M+H]^+$), 858.4248; found, 858.4272.

14g. Yield: 94%, ^{1}H NMR (CD_3OD, 400MHz) δ 0.86 (d, *J*=6.4Hz, 6H, CH_3), 1.38 (t, *J*=7.2Hz, 9H, CH_3), 1.45-1.54 (m, 7H, $3CH_2$ and CH), 1.57-1.78 (m, 8H, CH_2), 3.24 (m, 6H, CH_2), 3.84 (m, 1H, CH), 4.20 (t, *J*=7.2Hz, 6H, CH_2), 7.41 (d, *J*=7.2Hz, 3H, C5-H in pyridinone), 8.32 (d, *J*=7.2Hz, 3H, C6-H in pyridinone). ^{13}C NMR (100Hz, DMSO) δ 167.9, 161.1, 158.5, 142.3, 137.4, 136.6, 112.1, 58.2, 56.1, 51.8, 40.4, 40.0, 32.2, 32.1, 23.7, 22.4, 16.4, 13.2. ESI-MS: *m/z* 811 ($[M+H]^+$); HRMS: calcd. for $C_{40}H_{59}N_8O_{10}$ ($[M+H]^+$), 811.4353; found, 811.4329.

14h. Yield: 95%, ^{1}H NMR ($CDCl_3$, 400MHz) δ 0.85-0.91 (m, 15H, CH_3), 1.22 -1.29 (m, 6H, CH_2), 1.42-1.81 (m, 21H, $10CH_2$ and CH), 3.24-3.28 (m, 6H, CH_2), 3.82-3.87 (m, 1H, CH), 4.20 (t, *J*=7.2Hz, 6H, CH_2), 7.41 (d, *J*=6.8Hz, 3H, C5-H in pyridinone), 8.28-8.30 (m, 3H, C6-H in pyridinone). ^{13}C NMR (100Hz, DMSO) δ 168.41, 161.5, 158.8, 142.8, 138.0, 136.6, 111.9, 58.1, 56.2, 51.1, 40.5, 40.1, 32.4, 32.1, 23.6, 22.6, 22.3, 22.0, 18.9, 13.3. ESI-MS: *m/z* 895 ($[M+H]^+$); HRMS: calcd. for $C_{46}H_{71}N_8O_{10}$ ($[M+H]^+$), 895.5292; found, 895.5310.

14i. Yield: 94%, ^{1}H NMR (CD_3OD, 400MHz) δ 0.89 (t, *J*=6.4Hz, 6H, CH_3), 1.39 -1.55 (m, 7H, CH_2 and CH), 1.57-1.63 (m, 8H, CH_2), 3.14-3.35 (m, 6H, CH_2), 3.22 (s, 9H, CH_3), 3.67 (t, *J*=4.8Hz, 6H, CH_2), 3.81-3.87 (m, 1H, CH), 4.35 (t, *J*=4.8Hz, 6H, CH_2), 7.31 (d, *J*=6.8Hz, 3H, C5-H in pyridinone), 8.09 (d, *J*=6.8Hz, 3H, C6-H in pyridinone). ^{13}C NMR (100Hz, DMSO) δ 168.4, 161.5, 158.8, 142.8, 138.0, 136.6, 111.9, 70.0, 59.8, 58.0, 56.2, 51.1, 40.5, 40.1, 32.4, 32.5, 22.7, 18.8, 13.3. ESI-MS: *m/z* 901 ($[M+H]^+$); HRMS: calcd. for $C_{43}H_{65}N_8O_{13}$ ($[M+H]^+$), 901.4670; found, 901.4643.

Physico-chemical properties of hexadentate hydroxypyridinones

pKa determination

The titration system used in this determination comprised an autoburette (Metrohm Dosimat 765 l mL syringe) and a HP 8453 UV-visible spectrophotometer. 0.1M KCl electrolyte solution was used to maintain the ionic strength. The temperature of the test solutions was maintained in a thermostatic cuvette holder at 25±0.1°C using a Cary 1 controller. An argon atmosphere was applied to the entire titration equipment. The initial sample concentration was approximately 7×10^{-5}M. pKa values were analyzed from these data by pHab.[14]

Determination of iron(III) affinity

The automatic titration system used in this study comprised of an autoburette (Metrohm Dosimat 765 liter ml syringe) and Mettler Toledo MP230 pH meter with Metrohm pH electrode (6.0133.100) and a reference electrode (6.0733.100). 0.1 M KCl electrolyte solution was used to maintain the ionic strength. The temperature of the test solutions was maintained in a thermostatic jacketed titration vessel at 25 ^{o}C ± 0.1 ^{o}C by using a Techne TE-8J temperature controller. The solution under investigation was stirred vigorously during the experiment. A Gilson Mini-plus#3 pump with speed capability (20 mL/min) was used to circulate the test solution through a Hellem quartz flow cuvette. For stability constant determinations, a 50 mm path length cuvette was used, and for pKa determinations, a cuvette path length of 10 mm was used. The flow cuvette was mounted on an HP 8453 UV-visible spectrophotometer.[15,16] All instruments were interfaced to a computer and controlled by a Visual Basic program. Automatic titration and spectral scans adopted the following strategy: the pH of a solution was increased by 0.1 pH unit by the addition of KOH from the autoburette; when pH readings varied by <0.001 pH unit over a 3 s period, an incubation period was activated. For pKa determinations, a period of 1 min was adopted; for stability constant determinations, a period of 5 min was adopted. At the end of the equilibrium period, the spectrum of the solution was then recorded. The cycle was repeated automatically until the defined end point pH value was achieved. All the titration data were analyzed with the pHab program.[14,16] The species plot was calculated with the HYSS program.[17] Analytical grade reagent materials were used in the preparation of all solutions.

Antimicrobial assay

Bacterial strains

P. aeruginosa, *S. aureus*, *B. subtilis* and *E. coli* were purchased from China General Microbiological Culture Collection (CGMCC). The media used in this study were

nutrient agar medium (NA) and tryptone soybean broth (TSB), which were purchased from Beijing Land Bridge Technology Co. Ltd. These four bacterial strains were inoculated in a tube containing an inclined plane of NA and cultured at 37 ^{o}C for 24 h. This gel was then used to inoculate into 5 mL of TSB and incubated at 37 ^{o}C for 24 h before transfer 50 μL into another tube of fresh TSB. This transfer was incubated at 37 °C to an optical density of approximately 5.0×10^{5} colony-forming units (CFU/ml).

Determination of Minimum Inhibitory Concentration (MIC)

All assays were cultured at 37 ^{o}C for 24 h in 10×100-mm tubes. The incubation medium was Trypticase Soy Broth (TSB). All tubes contained 80 μL of antimicrobial agent with different concentrations up to 2048 μL /mL, 20μL of bacterial inoculum with $1\text{-}2\times10^{5}$ cells/mL , with a total volume of 100μL. After incubation at 37℃ for 2 h, 900 μL of sterilized TSB was added to each tube to reach a final volume of 1 mL , and cultured at 37℃ for 24 h. Minimum inhibitory concentrations (MIC) were determined by visual inspection of the turbidity of broth in tubes.[18] All assays were carried out in triplicate.

Results and Discussion

Chemistry

Hexdentate ligands can be formed by conjugating three bidentate ligands onto a suitable tripodel backbone. However, in order for the ligand to adopt the correct geometry for iron(III) binding, it is essential that the backbone be connected to the ring at the ortho position relative to the oxygen anion,[19] namely, connecting to position-2 or 5 at pyridinone ring. In this work, we firstly designed and synthesized a range of benzyl protected bidentate hydroxypyridinones (HPO) containing free carboxyl group at position-2 starting from maltol (Scheme 1). The benzylation of the starting material, maltol (**1**) was achieved by the treatment with benzyl chloride to

obtain **2**. Treatment of **2** with primary amine under basic conditions with reflux provided HPO derivatives **3** in 84-90% yield, which underwent the selective oxidation of the methyl group at position-2 with selenium dioxide in acetyl anhydride to generate aldehydes **4**. Further oxidation of **4** in the presence of sodium hypochlorite and sulfanic acid provided the carboxylic acids **5** in good yield. The tripodal molecules (**12**) containing three free amino groups were synthesized starting from nitromethane (**6**) (Scheme 2). In the presence of Triton B, nitromethane underwent a Michael addition with acrylonitrile to produce **7** in good yield. Reduction of **7** with B_2H_6 generated **8**, in which three amino groups were then protected with Boc to give **9**. The nitro group in **9** was reduced by hydrogenation in the presence of Raney nickel to produce **10**.[13] Reaction of **10** with butyryl chloride gave **11a**; coupling of **10** with N-Cbz-L-leucine in the presence of O-(6-chloro-1-hydrocibenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HCTU) provided **11b**. Treatment of **11a** and **11b** with formic acid generated triamines **12a** and **12b**, respectively. Activation of 12 to an active ester was achieved in the presence of N-hydroxysuccinimide (NHS) and (DCC). Hexadentate hydroxypyridinones **14** were synthesised by the coupling of the active ester with triamines followed by the removal of the benzyl protecting groups under hydrogenation (Scheme 3).

Physico-chemical characterisation

pKa values

The pH dependent UV spectra of **14c** (Figure 1) was recorded between 250 and 400 nm over the pH range 1.728 - 11.754 for the free ligand. The speciation spectra demonstrate a clear shift in λ_{max} from 290 to 320 nm, which reflects the pH dependence of the ligand ionization equilibrium. The ligand **14** can be considered as a trimer of the corresponding bidentate ligand and it therefore possesses two sets of intrinsic pKa values. Using the spectrophotometric titration method, the p*K*a values

of **14c** obtained from nonlinear least-squares regression analysis were found to be 1.99, 2.29, 2.42, 7.46, 8.37 and 8.95. Of the six p*K*a values, the three lower values correspond to the 4-oxo functions and the higher three correspond to the 3-hydroxyl functions. The pKa values of chelator **14h** were determined to be 1.92, 2.72, 3.95, 7.43, 8.40, 9.28 and 10.79 using the same method as that for **14c.** The value 10.79 is assigned to the amino group of leucine residue.

Iron(III) affinity

The stability constant of an iron-ligand complex is one of the key parameters related to the chelation efficacy of a ligand. The UV spectra of **14c** in the presence of iron at different pH values are shown in Figure 2. The stability constant of the iron(III) complexes ($\log K_1$) of **14c** and **14h** were determined to be 34.9 and 34.6, respectively.

The pFe^{3+} value, defined as the negative logarithm of concentration of the free iron(III) in solution (when $[Fe^{3+}]_{Total} = 10^{-6}$M; $[Ligand]_{Total} = 10^{-5}$M; pH = 7.4), is a more suitable parameter for comparison than the stability constant, since it takes into account the effect of ligand basicity, denticity and the degree of protonation. The pFe^{3+} values of **14c and 14h**, calculated by using the measured stability constant $\log K_1$ and pKa values, are extremely high, being 31.9 and 31.3 respectively. For comparative purposes, the pFe^{3+} value for most widely clinically used iron chelator desferioxamine is 26.6.

Antimicrobial evaluation

The antimicrobial activity of these hexadentate hydroxypyridinones was evaluated using minimum inhibition concentration assay. A clinically used bidentate hydroxypyridinone (Deferiprone) and a commercially available chelator, ethylenediamine tetraacetic acid (EDTA), were adopted as comparators. It was found

that all the iron chelators exhibited marked inhibition against tested Gram-positive bacteria (*S. aureus* and *B. subtilis*) and two Gram-negative bacteria (*E. coli* and *P. aeruginosa*) (Table 1). Deferiprone was found to possess the weakest inhibition against all of the four tested bacterial strains, which can be attributed to its relatively lower iron affinity (pFe = 20.5). In the case of *S. aureus*, **14a**, **14b**, **14c**, **14e**, **14f** and **14h** were found to exhibit strong inhibitory activity, with a MIC of 8 μg/mL, which is lower than EDTA (MIC 32 μg/mL), whereas **14g** has a MIC of 512 μg/mL. Against *B. subtilis*, most of the chelators (**14a**, **14b**, **14c**, **14e**, **14f** and **14h**) showed stronger inhibitory activity than EDTA, with a MIC of 8 μg/mL, while **14g** and **14i** showed relatively low inhibitory effect. In the case of *P. aeruginosa*, almost all the chelators exhibited stronger inhibition (MIC 16-32 μg/mL) than EDTA (MIC 128μg/mL), with the exception of **14i**, which has a similar effect to EDTA. In the case of *E. coli*, again almost all the chelators were found to possess stronger inhibition (MIC 8-32 μg/mL) than EDTA (MIC 64 μg/mL), with the exception of **14i**, which has a similar effect to EDTA.

Most of the chelators investigated in this study were found to possess stronger antimicrobial activity than that of EDTA. This is presumably due to these chelators possessing a higher affinity for iron(III) than EDTA (logK = 28.6).[20] Based on the similar affinity constants for iron(III) of compounds **14c** and **14h**, it is most probable that the affinity for iron(III) remains largely unchanged for the entire group (**14a-14i**) and thus any variation of antibacterial influence is unlikely to be related to differences in iron affinity. The calculated partition coefficients (ClogP) of the chelators,[21] are also presented in Table 1. Overall, **14i** and **14g** showed relatively weaker inhibitory effect than the other hexadentate hydroxypyridinones, indicating that the presence of a charged amino group has a detrimental influence on antibacterial effect – possibly by adversely influencing the penetration of compounds into the surface of bacteria. Significantly compound **14h** is not so severely influenced by the presence of the amino function and this compound is

markedly more hydrophobic than both **14i** and **14g**. On consideration of the ClogP values of the noncharged chelators (**14a-14f**), those with values falling in the range -4.38 to +1.74 all have similar efficacies. Whereas the most hydrophobic chelator **14d** with a ClogP value of +4.77 has a low efficacy against most of the organisms. Thus in summary it would appear that the non-charged state of chelator is important for activity as is a reasonable water solubility (negative ClogP value). It is proposed that these chelators inhibit the growth of Gram-positive bacteria by scavenging iron in the immediate environment on the surface of the bacteria and for Gram negative bacteria by entering and scavenging iron in the periplasmic space.

Conclusion

The inhibition of bacterial iron uptake represents a promising alternative approach for the design of new antimicrobials, the hexadentate 3-hydroxypyridin-4-ones were found to possess strong inhibitory activity against the growth of both Gram-positive and Gram-negative bacteria. Overall, the more hydrophilic **14a** and **14e** showed optimal bacterial growth inhibitory effect. These hexadentate chelators have potential as antimicrobial agents, particularly in the treatment of external infections.

Acknowledgments

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Disclosure

All authors declare that they have no conflict of interest.

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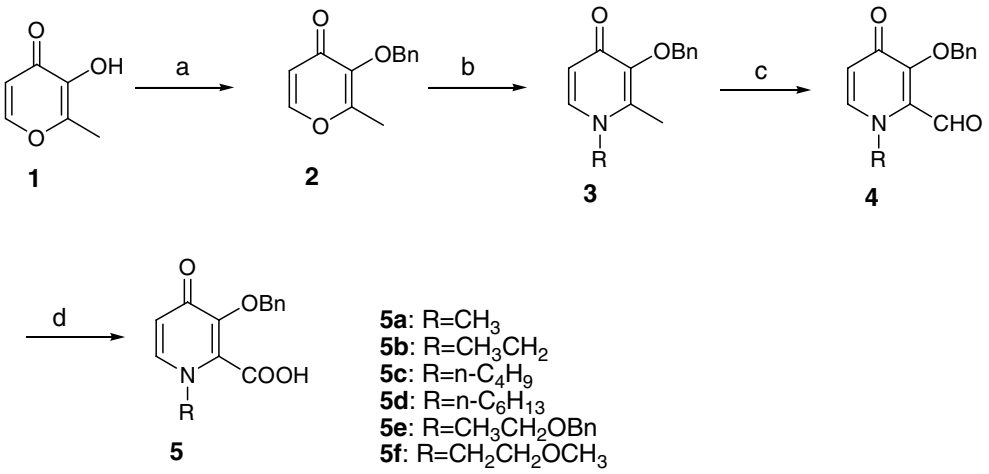
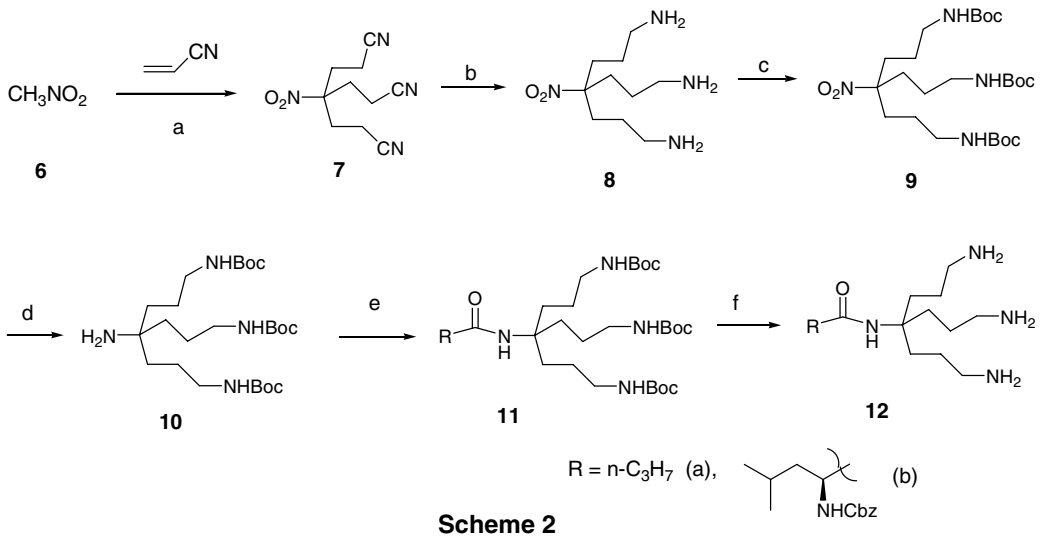
Scheme 1



Scheme 1

Reagents and conditions：(a) BnCl, NaOH, CH_3OH, reflux, 7-8h; (b) RNH_2, NaOH, CH_3OH/H_2O, reflux, 2.5h; (c) SeO_2, $CH_3COOH/(CH_3CO)_2O$, reflux; (d) NH_2SO_3H,$NaClO_2$, CH_3COCH_3/H_2O

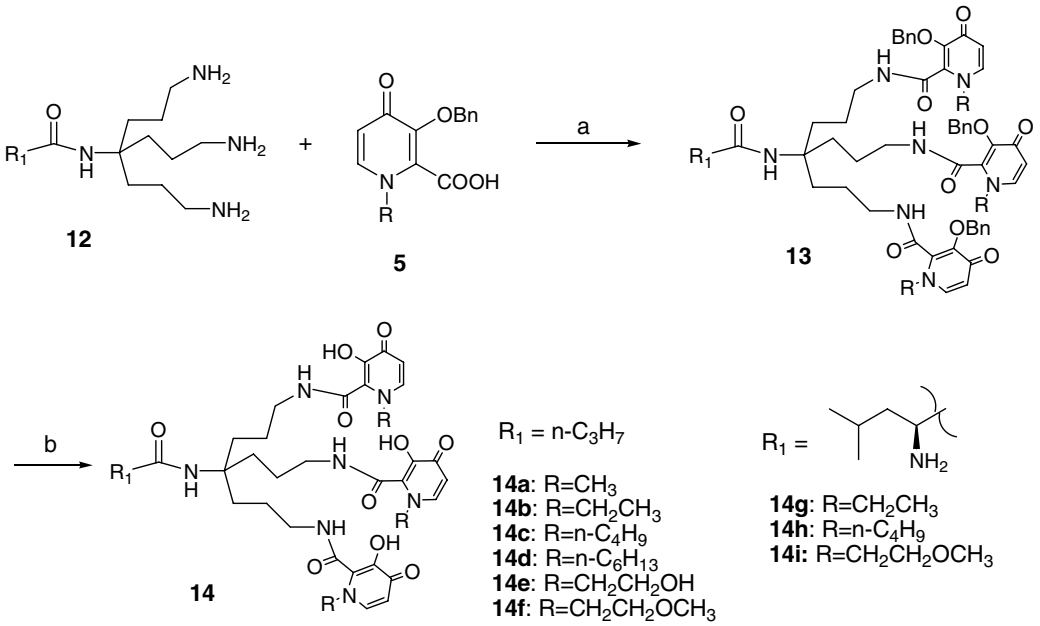
Scheme 2



Scheme 2

Reagents and conditions：(a) Triton B, 30 ^{o}C, overnight.(b) BH_3-THF, 24h; (c) $(Boc)_2O$, CH_3OH; (d) H_2, Raney-Ni, EtOH, 5d; (e) A. for **11a**: n-C_3H_7COCl, Et_3N,DCM; B. for 11b: Cbz-L-Leu, NHS, DCC, Et_3N, DMF. (f) HCOOH, rt, 24h.

Scheme 3



Scheme 3

Reagents and conditions ： (a) NHS,DCC,DMF,4d. (b) CH_3OH, Pd/C, 8h.

Figure Legends

Figure 1. Titration of **14c.** [**14c**] = 67.8 µM, pH was changed from 1.728 to 11.754 by the addition of KOH in 20.290 mL of 0.1M KCl at 25 ^{o}C.

Figure 2. Titration of **14c** with iron(III). [**14c**]= 23.3 µM, [Fe^{3+}]= 22.5 µM with the addition of KOH, start in 20.058 ml of 0.1M KCl at 25^{o}C, pH from 10.584 to pH 12.980.

Figure 1

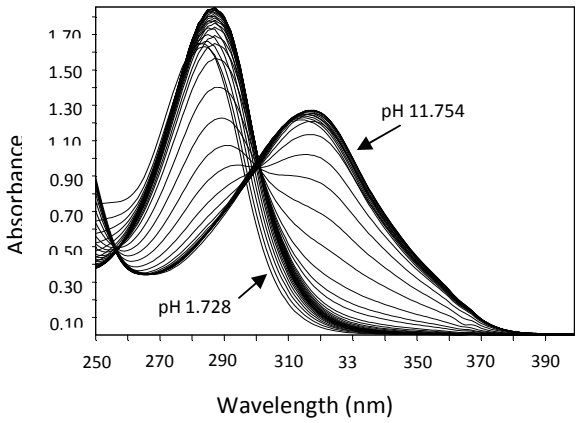


Figure 2

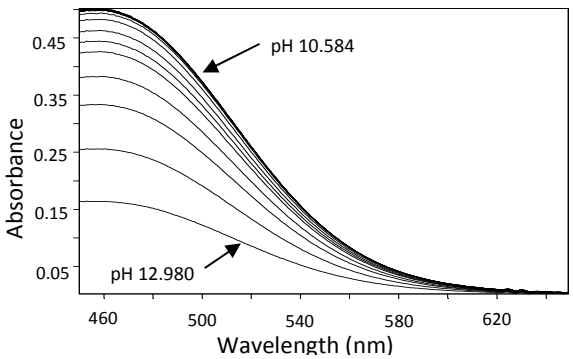


Table 1. Antimicrobial activity of chelators (MIC, μg/mL)

Compounds	*S. aureus*	*B. subtilis*	*P. aeruginosa*	*E. coli*	ClogP
Deferiprone	512	512	512	512	-0.60
EDTA	32	64	128	64	-5.19
14a	8	8	16	8	-2.57
14b	8	8	32	8	-1.44
14c	8	8	32	8	1.74
14d	32	64	32	32	4.77
14e	8	8	16	8	-4.38
14f	8	8	32	16	-2.62
14g	512	256	32	32	-4.87
14h	8	8	32	8	0.82
14i	64	512	128	64	-3.54