Supporting information

Design and synthesis of fluorinated iron chelators for metabolic study and brain uptake

*Yongmin Ma, Sourav Roy, Xiaole Kong, Yulin Chen, Dingyong Liu, Robert C Hider**

Institute of Pharmaceutical Science, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London, SE1 9NH, UK.

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I. General information.

Fluorinated pyridines were purchased from Fluorochem. Reagents were from Sigma-Aldrich and reagent grade quality and were used without further purification. Column chromatography purifications were performed on Merck silica gel 60 (0.04-0.063 mm). ^{1}H, ^{13}C and ^{19}F NMR spectra were recorded on a Bruker Avance 400 (400 MHz) NMR spectrometer. Chemical shifts (δ) are reported in ppm downfield from the internal standard tetramethylsilane (TMS) for ^{1}H and ^{13}C NMR. ESI mass spectra were obtained by infusing samples into an LCQ Deca XP ion trap mass instrument. HRMS were monitored on MicroMass Q-TOF instrument. The chemical purity of the final compounds was determined using the following conditions: an Hewlett-Packard 1090M HPLC system consisted of an autoinjector, an autosampler, a diode-array detector and a reversed-phase polymer HPLC column (PLRP-S 100 Å, 25×0.46 cm ID, 5 μm, Polymer Laboratories Ltd, Church Stretton, Shropshire, UK); the gradient mobile phase system included (A): 1-heptanesulfonic acid solution (5 mM, pH2.0) and (B): acetonitrile; the percentage of acetonitrile was increased from 2–40% in 20 min and maintained until 25 min; the flow-rate was 1 ml/min and the analytes were monitored at 270 nm. The purity of each compound was ≥95% in this analysis.

II. General procedure of the intermediates and compound characterization

1-(3,4-bis(Benzyloxy)pyridin-2-yl)-2,2,2-trifluoroethanol (3). A mixture of 2-formyl-3,4-dibenzyloxypyridine **2** (20 mmol) and (trifluoromethyl)trimethylsilane (24 mmol) in 20 mL of THF was cooled to 0 ^{o}C and treated with a catalytic amount of tetrabutylammonium fluoride (TBAF; 50 mg). The mixture was brought to ambient temperature and stirred for 1 h. The mixture was analysed by TLC for the completion of the reaction. The resulting compounds were then hydrolysed with aqueous HCl and extracted with ethyl acetate (50 mL × 3). The organic phase was combined, dried by anhydrous Na_2SO_4 and concentrated under reduced pressure to afford a white solid. The compound was further purified by flash column chromatography ($CHCl_3$/MeOH, 9:1). Yield 92%. ^{1}H NMR ($CDCl_3$): δ 8.37 (d, J=5.5Hz, 1H), 7.58-7.49 (m, 5H), 7.45 (s, 5H), 7.11 (d, J=5.5Hz, 1H), 5.42-5.37 (m, 1H), 5.34 (s, 2H), 5.32 (d, J=10.8Hz, 1H), 5.19 (d, J=10.8Hz, 1H). ESI-MS: 390 $(M+1)^+$.

1-(3,4-bis(Benzyloxy)pyridin-2-yl)-2,2,2-trifluoroethanone (4). To a solution of 1-(3,4-bis(Benzyloxy)pyridin-2-yl)-2,2,2-trifluoroethanol (10 mmol) in chloroform (100 mL), was added dimethyl sulfoxide (DMSO) (15 mL) and triethylamine (TEA) (8.4 mL, 6 equiv.). The reaction mixture was then cooled in an ice-bath followed by the slow addition of sulfur trioxide pyridine complex (8 g, 50 mmol, 5 equiv.). The mixture was allowed to thaw at room temperature and left to stir overnight. Water (3×) was used to wash the organic fraction, which was subsequently dried over anhydrous sodium sulfate, filtered, and concentrated in *vacuo*. The dark green residue obtained was loaded on to a silica gel column (eluant: ethyl acetate) to yield a white solid. Yield 45%. ^{1}H NMR ($CDCl_3$): δ 8.28 (t, J=5.5Hz, 1H), 7.49-7.10 (m, 10H), 6.10 (s, 1H), 5.27 (s, 2H), 5.24 (s, 2H). ESI-MS: 388 $(M+1)^+$.

An analogous reaction as described for compound 4 starting from 3-(benzyloxy)-2-(hydroxymethyl)-1,6-dimethylpyridin-4(1*H*)-one **5** gave 3-(benzyloxy)-1,4-dihydro-1,6-dimethyl-4-oxopyridine-2-carbaldehyde **6**. ^{1}H NMR ($CDCl_3$): δ 10.06 (s, 1H), 7.36-7.29 (m, 5H), 6.47 (s, 1H), 5.50 (s, 2H), 3.68 (s, 3H), 2.31 (s, 3H). ESI-MS: 258 $(M+1)^+$.

An analogous reaction as described for compound **3** starting from compound **6** gave 3-(benzyloxy)-2-(2,2,2-trifluoro-1-hydroxyethyl)-1,6-dimethylpyridin-4(1*H*)-one **7**. ^{1}H NMR ($CDCl_3$): δ 7.82 (br s, 1H), 7.35-7.32 (m, 5H), 6.38 (s, 1H), 5.88 (m, 1H), 5.38 (m, 1H), 4.22 (m, 1H), 3.47 (s, 3H), 2.01 (s, 3H). ^{19}F NMR: -73.7 (s). ESI-MS: 328 $(M+1)^+$.

An analogous reaction as described for compound **4** starting from compound **7** gave 3-(benzyloxy)-2-(2,2,2-trifluoroacetyl)-1,6-dimethylpyridin-4(1*H*)-one **8**. ^{1}H NMR ($CDCl_3$): δ 7.34-7.30 (m, 5H), 6.41 (s, 1H), 5.42 (s, 2H), 4.22 (m, 1H), 3.33 (s, 3H), 2.32 (s, 3H). ^{19}F NMR: -76.01 (s). ESI-MS: 326 $(M+1)^+$.

2-Fluoropyridin-3-ol (10). A solution of 2-fluoropyridine (10 mmol) in anhydrous THF (20ml) under N_2 was cooled to –78^{o}C in a dry ice/acetone bath. To this solution was added a solution of lithium diisopropylamide (LDA; 11 mmol) in hexane slowly. The mixture was stirred for 0.5 h at –78^{o}C. To the mixture was added trimethoxyborane (2.4 ml) and stirred for 2h, followed by an addition of peracetic acid (3.6 ml; 32% in dilute acetic acid). The mixture was allowed to warm to 0^{o}C under stirring for 1h. After the mixture was cooled to –20 ^{o}C, sodium dithionite (4 g in 10 ml water) was added slowly. The mixture was extracted with ethyl acetate and the extract was dried and concentrated. The residue was purified by chromatography, eluting with 1:5 EtOAc:hexane to give a

white solid (92%). ^{1}H NMR ($CDCl_3$): δ 9.46 (brs, 1H, OH), 7.45-7.47 (m, 1H, C-6H), 7.13-7.18 (m, 1H, C-4H), 6.85-6.88 (m, 1H, C-5H). ESI-MS: 114 $(M+1)^+$.

2-Fluoro-3-methoxypyridine (11). 2-Fluoropyridin-3-ol (10 mmol), methyl iodide (20 mmol) and potassium carbonate (20 mmol) in acetone (100 ml) was refluxed overnight. The inorganic salt was filtered and the solvent was evaporated. The residue was purified on column (eluent: EtOAc: hexane = 1:5) to afford a colorless liquid (95%). ^{1}H NMR ($CDCl_3$): δ 7.74-7.75 (m, 1H, C-6H), 7.26-7.31 (m, 1H, C-4H), 7.11-7.15 (m, 1H, C-5H), 3.91 (s, 3H, CH_3). ^{19}F NMR ($CDCl_3$): -90.15 (s). ESI-MS: 128 $(M+1)^+$.

2-Fluoro-3-methoxypyridin-4-ol (12). At –78 ^{o}C, 2,2,6,6-tetramethylpiperidine (12 mmol) was added to a solution of n-butyllithium (11 mmol; 2 M in hexane) in THF (20 ml) under N_2, after 15 min, followed by 2-fluoro-3-methoxypyridine (10 mmol). After stirring for 0.5 h, to this solution was added trimethoxyborane (2.4 ml) and stirred for 2 h, followed by an addition of peracetic acid (3.6 ml; 32% in dilute acetic acid). The mixture was allowed to warm to 0 ^{o}C under stirring for 1h. After the mixture was cooled to –20 ^{o}C, sodium dithionite (4 g in 10 ml water) was added slowly. The mixture was extracted with DCM and the extract was dried and concentrated. The residue was purified by chromatography, eluting with 1:9 MeOH:DCM to give a fluorescent white solid (87%). ^{1}H NMR (d_6-DMSO): δ 7.63 (d, J=5.4Hz, 1H, C-6H), 6.80 (d, J=5.6Hz, 1H, C-5H). 3.86 (s, 1H, CH_3). ^{19}F NMR (d_6-DMSO): -83.03 (s). ESI-MS: 144 $(M+1)^+$.

General procedure for preparation of 3,4-dihydroxyl protected 2-fluoropyridine **13** and 3-hydroxyl protected 2-fluoro-1-alkylpyridin-4(*1H*)-one **14**.

To a solution of **12** (5 mmol) in acetone (20 ml) was added potassium carbonate (10 mmol) and alkyl iodide (10 mmol). The mixture was refluxed overnight. inorganic salt was filtered and the solvent was evaporated. The residue was purified on column chromatography, eluting first with EtOAc to afford **13** and then MeOH:DCM = 1:4 to afford **14**.

2-Fluoro-3,4-dimethoxypyridine (13a) (yield: 40%); ^{1}H NMR ($CDCl_3$): δ 7.82 (dd, J=0.7, 5.6Hz, 1H, C-6H), 6.76 (d, J=5.6Hz, 1H, C-5H), 3.95 (s, 3H, 4-OCH_3), 3.93 (d, J=1.3Hz, 3H, 3-OCH_3). ESI-MS: 158 $(M+1)^+$.

2-Fluoro-3-methoxy-1-methylpyridin-4(*1H*)-one (14a) (yield: 53%); ^{1}H NMR ($CDCl_3$): δ 7.03 (t, J=7.3Hz, 1H, C-6H), 6.36 (d, J=7.6Hz, 1H, C-5H), 3.94 (s, 3H, 3-OCH_3), 3.63 (d, J=3.0Hz, 3H, 1-CH_3). ESI-MS: 158 $(M+1)^+$.

4-Ethoxy-2-fluoro-3-methoxypyridine (13b) (yield: 65%); ^{1}H NMR ($CDCl_3$): δ 7.78 (dd, J=0.5, 5.7Hz, 1H, C-6H), 6.73 (d, J=5.7Hz, 1H, C-5H), 4.16 (q, J=7.0Hz, 2H, CH_2), 3.92 (d, J=1.0Hz, 3H, 3-OCH_3), 1.49 (t, J=7.0Hz, 3H, CH_3). ESI-MS: 172 $(M+1)^+$.

1-Ethyl-2-fluoro-3-methoxypyridin-4(*1H*)-one (14b) (yield: 28%); ^{1}H NMR ($CDCl_3$): δ 7.59 (t, J=7.6Hz, 1H, C-6H), 6.17 (d, J=7.7Hz, 1H, C-5H), 4.00 (dq, J=4.9, 7.2Hz, 2H, CH_2), 3.76 (s, 3H, 3-OCH_3), 1.32 (t, J=7.2Hz, 3H, CH_3). ESI-MS: 172 $(M+1)^+$.

3-Ethoxy-2-fluoro-1-propylpyridin-4(*1H*)-one (14c) (yield: 13%); ^{1}H NMR ($CDCl_3$): δ 7.00 (t, J=7.2Hz, 1H, C-6H), 6.37 (d, J=7.6Hz, 1H, C-5H), 4.22 (q, J=7.0Hz, 2H, CH_2), 3.85 (m, 2H, CH_2), 1.81 (m, 2H, CH_2), 1.34 (t, J=7.0Hz, 3H, CH_3), 0.98 (t, J=7.4Hz, 3H, CH_3). ^{19}F NMR: -117.6 (s).ESI-MS: 200 $(M+1)^+$.

3-Ethoxy-2-fluoro-1-isopropylpyridin-4(*1H*)-one (14d) (yield: 3%); ^{1}H NMR ($CDCl_3$): δ 7.13 (dd, J=6.7, 7.8Hz, 1H, C-6H), 6.41 (d, J=7.8Hz, 1H, C-5H), 4.56 (m, 1H,

CH), 4.22 (q, J=7.1Hz, 2H, CH_2), 1.49 (d, J=6.8Hz, 6H, $2CH_3$), 1.35 (t, J=7.1Hz, 3H, CH_3). ^{19}F NMR: -118.73 (s).ESI-MS: 200 $(M+1)^+$.

1-Butyl-2-fluoro-3-ethoxypyridin-4(*1H*)-one (14e) (yield: 45%, using LiOH instead of K_2CO_3 as the base)); ^1H NMR ($CDCl_3$): δ 7.03 (t, J=7.4Hz, 1H, C-6H), 6.37 (d, J=7.6Hz, 1H, C-5H), 4.22 (q, J=7.0Hz, 2H, CH_2), 3.88 (dt, J=2.2 7.2Hz, 2H, CH_2), 1.79-1.71 (m, 2H, CH_2), 1.41-1.26 (m, 5H, CH_2 and CH_3), 0.97 (t, J=7.4Hz, 3H, CH_3). ^{19}F NMR: -116.25 (s).ESI-MS: 214 $(M+1)^+$.

2-Fluoro-4-ethoxy-3-methoxy-6-methylpyridine (15). At –78 ^oC, N,N-Dimethylethanolamine (10 mmol) was added to a solution of n-butyllithium (20 mmol; 2.5 M in hexane) in THF (20 ml), after 5 min, followed by 2-fluoro-4-ethoxy-3-methoxypyridine (5 mmol). After stirring at this temperature for 20 h, to this solution was added methyl iodide (20 mmol) and stirred for 2 h, the temperature was allowed to thaw to room temperature for another 2 h. The mixture was extracted with ethyl acetate and the extract was dried and concentrated. The residue was purified by chromatography, eluting with 1:9 EtOAc:hexane to give a colorless liquid (66%). ^1H NMR ($CDCl_3$): δ 6.57 (s, 1H, C-5H), 4.14 (q, J=7.0Hz, 2H, CH_2), 3.87 (d, J=1.0Hz, 3H, 3-OCH_3), 2.40 (s, 3H, 6-CH_3), 1.48 (t, J=7.0Hz, 3H, CH_3). ^{19}F NMR ($CDCl_3$): -86.16 (s). ESI-MS: 186 $(M+1)^+$.

3,4-Dihydroxyl protected 2-Fluoro-5-methylpyridine (16).

Route one: At –78^oC, 2,2,6,6-tetramethylpiperidine (12 mmol) was added to a solution of n-butyllithium (11 mmol; 2.5 M in hexane) in THF (20 ml), after 15 min, followed by 2-fluoro-4-ethoxy-3-methoxypyridine (10 mmol). After 20 h, to this solution was added methyl iodide (20 mmol) and stirred for 2h, the temperature was allowed to thaw to room temperature for another 2h. The mixture was extracted with ethyl acetate and the extract was dried and concentrated. The residue was purified by chromatography, eluting with 1:9 EtOAc:hexane to give a colorless liquid 2-fluoro-4-ethoxy-3-methoxy-5-methylpyridine (**16a**) (81%). ^1H NMR ($CDCl_3$): δ 7.62 (s, 1H, C-6H), 4.33 (q, J=7.1Hz, 2H, CH_2), 3.89 (d, J=1.5Hz, 3H, 3-OCH_3), 2.15 (dd, J=0.9, 1.2Hz, 3H, 5-CH_3), 1.39 (t, J=7.0Hz, 3H, CH_3). ^{19}F NMR ($CDCl_3$): -85.64 (s). ESI-MS: 186 $(M+1)^+$.

Route two: 2-Fluoro-4-hydroxy-3-methoxy-5-methylpyridine **20** (10 mmol), methyl iodide (20 mmol) and potassium carbonate (20 mmol) in acetone (100 ml) was refluxed overnight. The inorganic salt was filtered and the solvent was evaporated. The residue was purified on column eluting first with EtOAc:hexane=1:3 to afford a colorless liquid 3,4-dimethoxy-2-fluoro-5-methylpyridine (**16b**) (40%). ^1H NMR ($CDCl_3$): δ 7.61 (s, 1H, C-6H), 4.07 (s, 3H, 4-OCH_3), 3.90 (d, J=1.5Hz, 3H, 3-OCH_3), 2.14 (t, J=1.0Hz, 3H, 5-CH_3). ^{19}F NMR ($CDCl_3$): -85.42 (s). ESI-MS: 172 $(M+1)^+$. Then eluting with MeOH:DCM=1:4 to afford a pale yellow liquid 2-fluoro-3-methoxy-1,5-dimethylpyridin-4(*1H*)-one (**21**) (43%). ^1H NMR ($CDCl_3$): δ 7.02 (dd, J=0.8, 6.8Hz, 1H, C-6H), 3.70 (s, 3H, 3-OCH_3), 3.51 (d, J=3.0Hz, 3H, 1-CH_3), 1.86 (d, J=0.9Hz, 3H, 5-CH_3). ^{19}F NMR ($CDCl_3$): -111.11 (s). ESI-MS: 172 $(M+1)^+$.

5,6-Dimethyl-2-fluoro-4-ethoxy-3-methoxypyridine (17). At –78 ^oC, N,N-Dimethylethanolamine (10 mmol) was added to a solution of n-butyllithium (20 mmol; 2.5 M in hexane) in THF (20 ml), after 5 min, followed by 2-fluoro-4-ethoxy-3-methoxy-5-methylpyridine **16** (5 mmol). After 16 h, to this solution was added methyl iodide (20 mmol) and stirred for 2 h, the temperature was allowed to thaw to room temperature for another 2 h. The mixture was extracted with ethyl acetate and the extract was dried and concentrated. The residue was purified by chromatography, eluting with 1:9

EtOAc:hexane to give a colorless liquid (73%). ^{1}H NMR ($CDCl_3$): δ 4.26 (q, J=7.1Hz, 2H, CH_2), 3.87 (d, J=1.3Hz, 3H, 3-OCH_3), 2.35 (s, 3H, 6-CH_3), 2.11 (s, 3H, 5-CH_3), 1.39 (t, J=7.1Hz, 3H, CH_3). ^{19}F NMR ($CDCl_3$): -87.00 (s). ESI-MS: 200 $(M+1)^+$.

2-Fluoro-3-methoxy-5-methylpyridine (19). A solution of 2-fluoro-5-methylpyridine (**18**, 2 mmol) in anhydrous THF (10ml) was cooled to –78^{o}C. To this solution was added a solution of lithium diisopropylamide (LDA; 2.2 mmol) in hexane slowly at same temperature. After 1h at –78^{o}C, to the mixture was added trimethoxyborane (0.48ml) and stirred for 2h, followed by an addition of peracetic acid (0.72 ml; 32% in dilute acetic acid). The mixture was allowed to warm to 0^{o}C under stirring for 1h. After the mixture was cooled to –20 ^{o}C, sodium dithionite (0.8g in 2ml water) was added dropwise. The mixture was extracted with ethyl acetate and the extract was dried and concentrated. The residue was purified by chromatography, eluting with 1:1 EtOAc:hexane to give the expected product as a white crystal (69%). ^{1}H NMR ($CDCl_3$): δ 7.53 (dd, J=1.9, 2.6Hz, 1H, C-6H), 7.22-7.19 (m, 1H, C-4H), 6.25 (brs, 1H, OH), 2.29 (d, J=0.5Hz, 3H, 5-CH_3). ^{19}F NMR ($CDCl_3$): -97.13 (s). ESI-MS: 128 $(M+1)^+$. This compound (10 mmol), methyl iodide (20 mmol) and potassium carbonate (20 mmol) in acetone (100 ml) was then refluxed overnight. The inorganic salt was filtered and the solvent was evaporated. The residue was purified on column (EtOAc:hexane =1:3) to afford a colorless liquid (95%). ^{1}H NMR ($CDCl_3$): δ 7.53 (d, J=0.5Hz, 1H, C-6H), 7.09 (dd, J=1.8, 9.8Hz, 1H, C-4H), 3.89 (s, 3H, 3-OCH_3), 2.32 (s, 3H, 5-CH_3). ^{19}F NMR ($CDCl_3$): -90.84 (s). ESI-MS: 142 $(M+1)^+$.

2-Fluoro-4-hydroxy-3-methoxy-5-methylpyridine (20). At –78^{o}C, 2,2,6,6-tetramethylpiperidine (12 mmol) was added to a solution of n-butyllithium (11 mmol; 2 M in hexane) in THF (20 ml), after 15 min, followed by 2-fluoro-3-methoxy-5-methylpyridine **19** (10 mmol). After 2 h, to this solution was added trimethoxyborane (2.4 ml) and stirred for 2h, followed by an addition of peracetic acid (3.6 ml; 32% in dilute acetic acid). The mixture was allowed to warm to 0^{o}C for 1h. After the mixture was cooled to –20 ^{o}C, sodium dithionite (4 g in 10 ml water) was added slowly. The mixture was extracted with ethyl acetate and the extract was dried and concentrated. The residue was purified by chromatography, eluting with 1:3 EtOAc:hexane to give a white solid (78%). ^{1}H NMR ($CDCl_3$): δ 7.58 (s, 1H, C-6H), 6.67 (brs, 1H, OH), 3.99 (d, J=2.2Hz, 3H, 3-OCH_3), 2.18 (t, J=1.0Hz, 3H, 5-CH_3). ^{19}F NMR ($CDCl_3$): -86.22 (s). ESI-MS: 158 $(M+1)^+$.

2-Chloro-3-fluoro-4-hydroxypyridine (23). A solution of 2-chloro-3-fluoropyridine (2 mmol) in anhydrous THF (10 ml) was cooled to –78 ^{o}C. To this solution was added a solution of lithium diisopropylamide (LDA; 2.2 mmol) in hexane slowly at same temperature. After 2 h at –78^{o}C, to the mixture was added trimethoxyborane (0.48 ml) and stirred for 2 h, followed by an addition of peracetic acid (0.72 ml; 32% in dilute acetic acid). The mixture was allowed to warm to 0 ^{o}C under stirring for 1h. After the mixture was cooled to –20^{o}C, sodium dithionite (0.8 g in 2 ml water) was added dropwise. The mixture was extracted with ethyl acetate and the extract was dried and concentrated. The residue was purified by chromatography, eluting with 1:19 MeOH:DCM to give the expected product as a white solid (80%). ^{1}H NMR (d_6-DMSO): δ 11.86 (brs, 1H, OH), 7.89 (d, J=5.3Hz, 1H, C-6H), 6.95 (t, J=5.8Hz, 1H, C-5H). ^{19}F NMR (d_6-DMSO): -141.29 (s). ESI-MS: 148 $(M+1)^+$.

General procedure for the preparation of **4-hydroxy protected 2-chloro-3-fluoro-pyridine** (**24**). 2-Chloro-3-fluoro-4-hydroxypyridine **23** (10 mmol), alkyl iodide (20 mmol) and potassium carbonate (20 mmol) in acetone (100 ml) was refluxed overnight. The inorganic salt was filtered and the solvent was evaporated. The residue was purified on column (eluent: ethyl acetate: hexane = 1:5) to afford a colorless liquid.

2-Chloro-3-fluoro-4-methoxypyridine (24a) (yield: 52%). ^{1}H NMR ($CDCl_3$): δ 8.08 (dd, J=1.0, 5.8Hz, 1H, C-6H), 6.88 (t, J=5.7Hz, 1H, C-5H), 3.97 (s, 3H, CH_3). ^{19}F NMR ($CDCl_3$): -143.49 (s). ESI-MS: 162 $(M+1)^+$.

2-Chloro-4-ethoxy-3-fluoropyridine (24b) (yield: 58%). ^{1}H NMR ($CDCl_3$): δ 8.05 (dd, J=1.0, 5.5Hz, 1H, C-6H), 6.85 (t, J=5.6Hz, 1H, C-5H), 4.19 (q, J=7.0Hz, 2H, CH_2), 1.50 (t, J=7.0Hz, 3H, CH_3). ^{19}F NMR ($CDCl_3$): -143.04 (s). ESI-MS: 176 $(M+1)^+$.

2-Chloro-3-fluoro-4-propoxypyridine (24c) (yield: 81%). ^{1}H NMR ($CDCl_3$): δ 8.04 (dd, J=1.0, 5.6Hz, 1H, C-6H), 6.85 (t, J=5.6Hz, 1H, C-5H), 4.07 (t, J=6.5Hz, 2H, CH_2), 1.93-1.84 (m, 2H), 1.07 (t, J=7.4Hz, 3H, CH_3). ^{19}F NMR ($CDCl_3$): -143.07 (s). ESI-MS: 190 $(M+1)^+$.

2-Chloro-3-fluoro-4-isopropoxypyridine (24d) (yield: 86%). ^{1}H NMR ($CDCl_3$): δ 8.03 (dd, J=1.0, 5.6Hz, 1H, C-6H), 6.84 (t, J=5.7Hz, 1H, C-5H), 4.69 (m, 1H, CH), 1.42 (d, J=6.1Hz, 6H, $2CH_3$). ^{19}F NMR ($CDCl_3$): -142.38 (s). ESI-MS: 190 $(M+1)^+$.

General procedure for the preparation of **4-hydroxy protected 2-chloro-3-fluoro-5-hydroxypyridine (25)**

At –78 ^{o}C, 2,2,6,6-tetramethylpiperidine (12 mmol) was added to a solution of n-butyllithium (11 mmol; 2 M in hexane) in THF (20 ml), after 15 min, followed by 24 (10 mmol). After 20 h, to this solution was added trimethoxyborane (2.4 ml) and stirred for 2 h, followed by an addition of peracetic acid (3.6 ml; 32% in dilute acetic acid). The mixture was allowed to warm to 0 ^{o}C under stirring for 1 h. After the mixture was cooled to –20 ^{o}C, sodium dithionite (4 g in 10 ml water) was added slowly. The mixture was extracted with ethyl acetate and the extract was dried and concentrated. The residue was purified by chromatography, eluting with 1:1 EtOAc:hexane to give a white solid.

2-Chloro-3-fluoro-5-hydroxy-4-methoxypyridine (25a) (96%). ^{1}H NMR ($CDCl_3$): δ 7.88 (d, J=0.8Hz, 1H, C-6H), 5.88 (brs, 1H, OH), 4.22 (d, J=4.0Hz, 3H, CH_3). ^{19}F NMR ($CDCl_3$): -139.00 (s). ESI-MS: 178 $(M+1)^+$.

2-Chloro-4-ethoxy-3-fluoro-5-hydroxypyridine (25b) (91%). ^{1}H NMR ($CDCl_3$): δ 7.88 (d, J=0.7Hz, 1H, C-6H), 6.0 (brs, 1H, OH), 4.51 (dq, J=2.5, 7.1Hz, 2H, CH_2), 1.46 (dt, J=0.7, 7.1Hz, 3H, CH_3). ^{19}F NMR ($CDCl_3$): -138.26 (s). ESI-MS: 192 $(M+1)^+$.

2-Chloro-3-fluoro-5-hydroxy-4-propoxypyridine (25c) (90%). ^{1}H NMR ($CDCl_3$): δ 7.88 (s, 1H, C-6H), 5.90 (brs, 1H, OH), 4.39 (dt, J=2.6, 6.7Hz, 2H, CH_2), 1.83 (m, 2H, CH_2), 1.04 (t, J=7.4Hz, 3H, CH_3). ^{19}F NMR ($CDCl_3$): -133.63 (s). ESI-MS: 206 $(M+1)^+$.

2-Chloro-3-fluoro-5-hydroxy-4-isopropoxypyridine (25d) (88%). ^{1}H NMR ($CDCl_3$): δ 7.89 (d, J=0.7Hz, 1H, C-6H), 5.93 (brs, 1H, OH), 4.95 (m, 1H, CH), 1.40 (dd, J=0.9, 6.1Hz, 6H, $2CH_3$). ^{19}F NMR ($CDCl_3$): -136.99 (s). ESI-MS: 206 $(M+1)^+$.

General procedure for the preparation of **4,5-dihydroxyl protected 2-chloro-3-fluoropyridine (26)**

25 (10 mmol), methyl iodide (20 mmol) and potassium carbonate (20 mmol) in acetone (100 ml) was refluxed overnight. The inorganic salt was filtered and the solvent was evaporated. The residue was purified on column (eluent: EtOAc: hexane = 1:3) to afford a white solid.

2-Chloro-4,5-dimethoxy-3-fluoropyridine (26a) (yield: 97%). ^{1}H NMR ($CDCl_3$): δ 7.80 (d, J=0.9Hz, 1H, C-6H), 4.14 (s, 3H, 4-OCH_3), 3.95 (s, 3H, 5-OCH_3). ^{19}F NMR ($CDCl_3$): -138.34 (s). ESI-MS: 192 $(M+1)^+$.

2-Chloro-4-ethoxy-3-fluoro-5-methoxypyridine (26b) (yield: 90%). ^{1}H NMR ($CDCl_3$): 7.80 (d, J=0.9Hz, 1H, C-6H), 4.40 (dq, J=1.8, 7.1Hz, 2H, CH_2), 3.94 (s, 3H, OCH_3), 1.42 (dt, J=0.6, 7.1Hz, 3H, CH_3). ^{19}F NMR ($CDCl_3$): -137.55 (s). ESI-MS: 206 $(M+1)^+$.

2-Chloro-4-ethoxy-3-fluoro-5-methoxy-6-methylpyridine (27). At –78^{o}C, 2,2,6,6-tetramethylpiperidine (12 mmol) was added to a solution of n-butyllithium (11 mmol; 2 M in hexane) in THF (20 ml), after 15 min, followed by 2-Chloro-4-ethoxy-3-fluoro-5-methoxypyridine **26b** (10 mmol). After 24 h, to this solution was added methyl iodide (20 mmol) and stirred for 2h, the temperature was allowed to thaw to room temperature for another 2 h. The mixture was extracted with ethyl acetate and the extract was dried and concentrated. The residue was purified by chromatography, eluting with 1:9 EtOAc:hexane to give a colorless liquid (81%). ^{1}H NMR ($CDCl_3$): δ 4.39 (dq, J=2.0, 7.1Hz, 2H, CH_2), 3.85 (s, 3H, OCH_3), 2.40 (d, J=1.2Hz, 3H, 6-CH_3), 1.43 (dt, J=0.6, 7.1Hz, 3H, CH_3). ^{19}F NMR ($CDCl_3$): -139.08 (s). ESI-MS: 220 $(M+1)^+$.

4-Ethoxy-3-fluoro-5-methoxy-6-methylpyridine (28). To a solution of 2-chloro-4-ethoxy-3-fluoro-5-methoxy-6-methylpyridine **27** (5 mmol) in ethyl acetate (20mL), catalytic 20% $Pd(OH)_2/C$ (0.5 g) and triethylamine (10 mmol) were added. The mixture was hydrogenated at room temperature and 3 atms for 24 h. Then the catalyst was filtered off through celite, and the clear solution, taken to dryness, afforded the title compound. Column chromatography eluting with 1:3 EtOAc:hexane yields a colorless liquid (81%). ^{1}H NMR ($CDCl_3$): δ 8.08 (d, J=2.5Hz, 1H, C-2H), 4.36 (dq, J=1.9, 7.0Hz, 2H, CH_2), 3.86 (s, 3H, OCH_3), 2.43 (d, J=1.2Hz, 3H, 6-CH_3), 1.43 (dt, J=0.5, 7.0Hz, 3H, CH_3). ^{19}F NMR ($CDCl_3$): -148.94 (s). ESI-MS: 186 $(M+1)^+$.

3-Fluoro-5-methoxy-1,6-dimethylpyridin-4(*1H*)-one (29). A solution of 4-ethoxy-3-fluoro-5-methoxy-6-methylpyridine (5 mmol) in acetone (30 mL) was added methyl iodide (50 mmol) and the mixture was heated at 60 ^{o}C for 20 h. The solvent was then evaporated and the residue was purified by column chromatography eluting with 1:9 MeOH:DCM to afford a white solid (78%). ^{1}H NMR ($CDCl_3$): δ 7.36 (d, J=6.4Hz, 1H, C-2H), 3.92 (s, 3H, OCH_3), 3.63 (s, 3H, 1-CH_3), 2.36 (s, 3H, 6-CH_3). ^{19}F NMR ($CDCl_3$): -153.68 (s). ESI-MS: 172 $(M+1)^+$.

4-Hydroxyl protected 3-fluoro-5-hydroxypyridine (30)

Route 1: A solution of 3-fluoro-4-methoxypyridine **34** (2 mmol) in THF (10ml) was cooled to –78 ^{o}C. To this solution was added a solution of lithium diisopropylamide (LDA; 4.2 mmol) in hexane slowly at same temperature. After 20h at –78^{o}C, to the mixture was added trimethoxyborane (0.48ml) and stirred for 2h, followed by an addition of peracetic acid (0.72 ml; 32% in dilute acetic acid). The mixture was allowed to warm to 0^{o}C under stirring for 1h, and then cooled to –20 ^{o}C, sodium dithionite (0.8g in 2ml water) was added slowly. The mixture was extracted with DCM and the extract was dried and concentrated. The residue was purified by chromatography, eluting with 1:8 MeOH:DCM to give the expected product 5-fluoro-4-methoxypyridin-3-ol (**30a**) as a white solid (52%). ^{1}H NMR (d_6-DMSO): δ 8.50 (brs, 1H, OH), 7.96 (s, 1H, C-6H), 7.95 (s, 1H, C-2H), 3.93 (s, 3H, OCH_3). ^{19}F NMR (d_6-DMSO): -148.83 (s). ESI-MS: 144 $(M+1)^+$.

Route 2: To a solution of compound **25b-d** (5 mmol) in ethyl acetate (20mL), catalytic 20% $Pd(OH)_2/C$ (0.5 g) and triethylamine (10 mmol) were added. The mixture was hydrogenated at room temperature and 3 atms for 20 h. Then the catalyst was filtered off through celite, and the clear solution, taken to dryness, afforded the title compound. Column chromatography eluting with EtOAc yields white solid 4-ethoxy-3-fluoro-5-hydroxypyridine (**30b**) (85%). ^{1}H NMR ($CDCl_3$): δ 8.08 (s, 1H, C-6H), 8.01 (d, J=3.0Hz, 1H, C-2H), 4.46 (dq, J=2.1, 7.0Hz, 2H, CH_2), 1.43 (dt, J=0.6, 7.0Hz, 3H, CH_3). ^{19}F NMR ($CDCl_3$): -147.43 (s). ESI-MS: 158 $(M+1)^+$; 3-fluoro-5-hydroxy-4-propoxypyridine (**30c**) ^{1}H NMR ($CDCl_3$): δ 8.09 (s, 1H, C-6H), 8.02 (d, J=3.0Hz, 1H, C-2H), 6.65 (brs, 1H, OH), 4.35 (dt, J=2.3, 6.6Hz, 2H, CH_2), 1.86-1.77 (m, 2H, CH_2), 1.03 (t, J=7.4Hz, 3H, CH_3). ^{19}F NMR ($CDCl_3$): -147.46 (s). ESI-MS: 172 $(M+1)^+$; 3-fluoro-5-hydroxy-4-isopropylpyridine (**30d**) ^{1}H NMR ($CDCl_3$): δ 8.10 (s, 1H, C-6H), 8.02 (d, J=2.9Hz, 1H, C-2H), 6.90 (brs, 1H, OH), 4.94-4.87 (m, 1H, CH), 1.38 (dd, J=0.8, 6.1Hz, 6H, $2CH_3$). ^{19}F NMR ($CDCl_3$): -146.40 (s). ESI-MS: 172 $(M+1)^+$.

4-Ethoxy-3-fluoro-5-methoxypyridine (31)

Route 1: **30b** (10 mmol), methyl iodide (20 mmol) and potassium carbonate (20 mmol) in acetone (100 ml) was refluxed overnight. The inorganic salt was filtered and the solvent was evaporated. The residue was purified on column (eluent: ethyl acetate: hexane = 1:3) to afford a colorless liquid (89%). ^{1}H NMR ($CDCl_3$): δ 8.13 (d, J=2.3Hz, 1H, C-2H), 8.06 (s, 1H, C-6H), 4.37 (dq, J=1.6, 7.0Hz, 2H, CH_2), 3.95 (s, 3H, OCH_3), 1.41 (dt, J=0.6, 7.1Hz, 3H, CH_3). ^{19}F NMR ($CDCl_3$): -147.41 (s, 1F). ESI-MS: 172 $(M+1)^+$.

Route 2: To a solution of **26b** (5 mmol) in ethyl acetate (20 mL), catalytic 20% $Pd(OH)_2/C$ (0.5 g) and triethylamine (10 mmol) were added. The mixture was hydrogenated at room temperature and 3 atms for 24 h. Then the catalyst was filtered off through celite, and the clear solution, taken to dryness, afforded the title compound. Column chromatography eluting with 1:3 EtOAc:hexane yields a colorless liquid (85%).

4-Ethoxy-3-fluoro-5-methoxy-2-methylpyridine (32). A solution of **31** (2 mmol) in anhydrous THF (10ml) was cooled to –78^{o}C. To this solution was added a solution of lithium diisopropylamide (LDA; 2.2 mmol) in hexane slowly at same temperature. After 20 h, to this solution was added methyl iodide (20 mmol) and stirred for 2h, the temperature was allowed to thaw to room temperature for another 2h. The mixture was extracted with ethyl acetate and the extract was dried and concentrated. The residue was purified by chromatography, eluting with EtOAc to give a colorless liquid (62%). ^{1}H NMR ($CDCl_3$): δ 7.93 (s, 1H, C-6H), 4.33 (dq, J=1.4, 7.0Hz, 2H, CH_2), 3.92 (s, 3H, OCH_3), 2.44 (d, J=3.5Hz, 3H, 2-CH_3), 1.40 (t, J=7.0Hz, 3H, CH_3). ^{19}F NMR ($CDCl_3$): -143.79 (s). ESI-MS: 186 $(M+1)^+$.

3-Fluoro-4-methoxypyridine (34). To a solution of 4-chloro-3-fluoropyridine (**33**, 10 mmol) in anhydrous THF and MeOH (15 ml : 15 ml) was added sodium methoxide (25% in MeOH, 10.5 mmol) dropwise. The mixture was refluxed overnight until the reaction completed (monitored by TLC). The mixture was poured into 100ml water and extracted with DCM (100 ml × 3). The organic extract was dried over Na_2SO_4 and concentrated. The residue was purified by chromatography (eluent: EtOAc), to give a colorless liquid (55%). ^{1}H NMR ($CDCl_3$): δ 8.34 (d, J=3.3Hz, 1H, C-2H), 8.29 (d, J=5.5Hz, 1H, C-6H), 6.89-6.92 (q, J=5.5, 7.2Hz, 1H, C-5H), 3.95 (s, 3H, CH_3). ^{19}F NMR ($CDCl_3$): -156.45 (s). ESI-MS: 128 $(M+1)^+$.

3-Chloro-2,5,6-trifluoro-4-methoxypyridine (36a) and **3,5-dichloro-2,6-difluoro-4-methoxypyridine (36b)**. To a solution of 3-chloro-2,4,5,6-tetrafluoropyridine or 3,5-dichloro-2,4,6-trifluoropyridine (60 mmol) in anhydrous THF and MeOH (50 ml : 50 ml) was added sodium methoxide (25% in MeOH, 60 mmol) dropwise at ice-bath. The mixture was stirred at room temperature for 20 h. After removing the solvent by reduced pressure, the residue was dissolved in 100 ml water and extracted with ethyl acetate (100 ml × 3). The organic extract was dried over Na_2SO_4 and concentrated. The residue was purified by chromatography (eluent: ethyl acetate:hexane = 1:15), to give a colorless liquid 3-Chloro-2,5,6-trifluoro-4-methoxypyridine (87%), ESI-MS at 198 $(M+1)^+$; or 3,5-dichloro-2,6-difluoro-4-methoxypyridine (82%), ESI-MS at 214 $(M+1)^+$.

2,3,6-Trifluoro-4-methoxypyridine (37a) and **2,6-difluoro-4-methoxypyridine (37b)**. Compound **36** (15 mmol), ammonium formate (30 mmol) and 10% palladium on charcoal (0.5 g) in methanol (60 ml) was heated at 50^{o}C for 10h. The reaction mixture was filtered and the solvents evaporated. The residue was purified by chromatography (eluent: ethyl acetate:hexane = 1:15) to give a white crystal. 2,3,6-Trifluoro-4-methoxypyridine (**37a**) (90%). ^{1}H NMR ($CDCl_3$): δ 6.40-6.42 (m, 1H), 4.00 (s, 3H). ^{19}F NMR: -75.45 (dd, J=14.6, 20.4Hz, 1F), -95.90 (dd, J=15.0, 19.0Hz, 1F), -174.96 (t, J=21.0Hz, 1F). ESI-MS: 164 $(M+1)^+$. 2,6-Difluoro-4-methoxypyridine (**37b**) (92%). ^{1}H NMR ($CDCl_3$): δ 6.31 (s, 2H), 3.90 (s, 3H). ^{19}F NMR: -73.81 (s, 2F). ESI-MS: 146 $(M+1)^+$.

2,5,6-Trifluoro-4-methoxypyridin-3-ol (38a) and **2,6-difluoro-4-methoxypyridin-3-ol (38b)**. A solution of compound **37** (2 mmol) in THF (10ml) was cooled to –78 ^{o}C. To this solution was added a solution of lithium diisopropylamide (LDA; 2.2 mmol) in hexane slowly at same temperature. After 0.5 h at –78 ^{o}C, to the mixture was added trimethoxyborane (0.48 ml) and stirred for 2 h, followed by an addition of peracetic acid (0.72 ml; 32% in dilute acetic acid). The mixture was allowed to warm to 0 ^{o}C under stirring for 1h, then cooled to –20 ^{o}C, sodium dithionite (0.8 g in 2 ml water) was added dropwise. The mixture was extracted with ethyl acetate and the extract was dried and concentrated. The residue was purified by chromatography, eluting with 1:5 ethyl acetate:hexane to give the expected product as a white solid. 2,5,6-Trifluoro-4-methoxypyridin-3-ol (**38a**) (82%). ^{1}H NMR ($CDCl_3$): δ 3.98 (s, 3H). ^{19}F NMR: -93.08 (dd, J=16.5, 24.7Hz, 1F), -98.16 (dd, J=16.6, 22.4Hz, 1F), -154.44 (t, J=23.2Hz, 1F). HRMS: Calcd for $C_6H_6NO_2F_3$ $(M+1)^+$, 180.0272; Found, 180.0295. 2,6-difluoro-4-methoxypyridin-3-ol (**38b**) (78%). ^{1}H NMR ($CDCl_3$): δ 6.37 (d, J=1.0Hz, 1H), 5.15 (br s, 1H), 4.00 (s, 3H). ^{19}F NMR: -76.41 (d, J=15.3Hz, 1F), -92.95 (d, J=15.3Hz, 1F). ESI-MS: 162 $(M+1)^+$.

2-**(Trifluoromethyl)pyridin-3-ol (40).** At –78 ^{o}C, 2,2,6,6-tetramethylpiperidine (12 mmol) was added to a solution of n-butyllithium (11 mmol; 2 M in hexane) in THF (20 ml), after 15 min, followed by 2-trifluoromethylpyridine (10 mmol). After 16 h, to this solution was added trimethoxyborane (2.4 ml) and stirred for 2 h, followed by an addition of peracetic acid (3.6 ml; 32% in dilute acetic acid). The mixture was allowed to warm to 0 ^{o}C under stirring for 1h, then cooled to –20 ^{o}C, sodium dithionite (4 g in 10 ml water) was added dropwise. The mixture was extracted with DCM and the extract was dried and concentrated. The residue was purified by chromatography, eluting with 1:9 MeOH:DCM to give a fluorescent white solid (72%). ^{1}H NMR (d_6-DMSO): δ 11.1 (br s, 1H), 8.19 (dd,

J=1.2, 4.3Hz, 1H), 7.58 (dd, J=4.3, 8.4Hz, 1H), 7.52 (dd, J=0.7, 8.3Hz, 1H). ESI-MS: 164 $(M+1)^+$.

2-(Trifluoromethyl)-3-methoxypyridine (41). 2-(Trifluoromethyl)pyridin-3-ol (10 mmol), methyl iodide (20 mmol) and potassium carbonate (20 mmol) in acetone (100 ml) was refluxed for 6 h. The inorganic salt was filtered and the solvent was evaporated. The residue was purified on column (eluent: ethyl acetate: hexane = 1:1) to afford a white solid (94%). ^{1}H NMR ($CDCl_3$): δ 8.27 (dd, J=0.9, 4.5Hz, 1H), 7.47 (dd, J=4.5, 8.5Hz, 1H), 7.39 (d, J=8.4Hz, 1H), 3.95 (s, 3H). ESI-MS: 178 $(M+1)^+$.

2-(Trifluoromethyl)-3-methoxypyrid-4-ol (42). At –78 ^{o}C, 2,2,6,6-tetramethylpiperidine (12 mmol) was added to a solution of n-butyllithium (11 mmol; 2 M in hexane) in THF (20 ml), after 15 min, followed by 2-(trifluoromethyl)-3-methoxypyridine (10 mmol). After 3 h, to this solution was added trimethoxyborane (2.4 ml) and stirred for 2h, followed by an addition of peracetic acid (3.6 ml; 32% in dilute acetic acid). The mixture was allowed to warm to 0 ^{o}C under stirring for 1h, then cooled to –20 ^{o}C, sodium dithionite (4 g in 10 ml water) was added dropwise. The mixture was extracted with DCM and the extract was dried and concentrated. The residue was purified by chromatography, eluting with 1:1 ethyl acetate:hexane to give a white solid (92%). ^{1}H NMR (d_6-DMSO): δ 11.81 (br s, 1H), 8.30 (d, J=5.2Hz, 1H), 7.32 (d, J=5.2Hz, 1H), 4.02 (s, 3H). ^{19}F NMR: -60.23 (s, 3F). ESI-MS: 194 $(M+1)^+$.

2-(Trifluoromethyl)-3,4-dimethoxypyridine (43) and **3-Methoxy-1-methyl-2-trifluoromethyl-1H-pyridin-4-one (44)**. 2-(Trifluoromethyl)-3-methoxypyrid-4-ol **42** (10 mmol), methyl iodide (20 mmol) and potassium carbonate (20 mmol) in acetone (100 ml) was refluxed overnight. The inorganic salt was filtered and the solvent was evaporated. The residue was purified on column, eluting with MeOH:DCM = 1:19 to afford **43** and **44**. 2-(trifluoromethyl)-3,4-dimethoxypyridine (**43**) (yield: 52%). ^{1}H NMR ($CDCl_3$): δ 8.31 (d, J=5.4Hz, 1H, C-6H), 7.02 (d, J=5.4Hz, 1H, C-5H), 3.97 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3). ^{19}F NMR ($CDCl_3$): -66.52 (s). ESI-MS: 208 $(M+1)^+$. 3-Methoxy-1-methyl-2-trifluoromethyl-1H-pyridin-4-one (**44**) (yield: 37%). ^{1}H NMR ($CDCl_3$): δ 7.26 (d, J=7.6Hz, 1H), 6.47 (d, J=7.6Hz, 1H), 3.97 (s, 3H), 3.77 (d, J=2.2Hz, 3H). ^{19}F NMR: -58.72 (s, 3F).

2-(Trifluoromethyl)-3,4-dimethoxy-5-methylpyridine (45). At –78^{o}C, 2,2,6,6-tetramethylpiperidine (12 mmol) was added to a solution of n-butyllithium (11 mmol; 2.5 M in hexane) in THF (20 ml), after 15 min, followed by 2-(trifluoromethyl)-3,4-dimethoxypyridine (10 mmol). After 20 h, to this solution was added methyl iodide (20 mmol) and stirred for 2 h, the temperature was allowed to thaw to room temperature for another 1 h. The mixture was extracted with ethyl acetate and the extract was dried and concentrated. The residue was purified by chromatography, eluting with 1:3 EtOAc:hexane to give white crystal (yield: 75%). ^{1}H NMR ($CDCl_3$): 8.19 (s, 1H, C-6H), 4.00 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 2.28 (s, 3H, CH_3). ^{19}F NMR ($CDCl_3$): -66.37 (s). ESI-MS: 222 $(M+1)^+$.

III. Physicochemical property determination

pKa and Iron stability constants

The automatic titration system used in this study comprised 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 ℃ ± 0.1 ℃ 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 the 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. 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.[1] The species plot was calculated with the HYSS program.[2] Analytical grade reagent materials were used in the preparation of all solutions.

LogP and $logD_{7.4}$ of 3-hydroxypyridin-4-ones

The relative concentrations of a compound allowed to partition between an aqueous (aq) and octanol (oct) layer are measured to determine these values (equation 1, 2). A physiologically relevant value, logD is commonly measured at pH 7.4 ($logD_{7.4}$). LogP is calculated from pKa scheme at a pH where the solute is in a non-ionised state.

$$\mathbf{LogP = Log ([solute]_{oct} /[un\text{-}ionised\ solute]_{aq})} \qquad \mathbf{Eq.1}$$

$$\mathbf{LogD = Log ([solute]_{oct} /([un\text{-}ionised\ solute]_{aq} + [ionised\ solute]_{aq}))} \qquad \mathbf{Eq.2}$$

$$\mathbf{LogD_{(pH)} = LogP - log(1+10^{(pH\text{-}pKa)})} \qquad \mathbf{Eq.3}$$

LogP values of the 3-hydroxypyridin-4-ones were calculated substituting the values of $logD_{7.4}$ and the basic pK_a (higher value) of each chelator in equation 3.

A traditional shake flask method using 1-octanol and MOPS buffer at pH 7.4 was used to measure the distribution coefficient ($logD_{7.4}$) of all compounds and their iron(III) complex. Both the phases (MOPS buffer and 1-octanol) were pre-saturated with each other for 24 hr before use, a 2 mL sample of the HPO solution in MOPS was shaken vigorously with 1, 2, 3 and 4 ml of the pre-saturated 1-octanol in a 15mL screw top plastic tube (BD Biosciences, Oxford, UK) for 2 minutes using a vortex genie (Scientific industries, NY, USA). The tubes were then shaken vigorously on a Grant Incubator OLS 200 mechanical shaking water bath (Grant, Cambridgeshire, UK) for 2 hours at 25 ± 1°C and speed of 100 shakes/min. Both layers were then separated by centrifugation at 700 g for 5 minutes on a Spectrafuge 16M centrifuge (Pegasus Scientific Inc, MD, USA). An aliquot of the aqueous phase was transferred to a 1.5 mL semi-micro UV cuvette suitable for 220-900 nm spectral range (Sigma-Aldrich, UK) and its absorbance was measured at the peak absorbance recorded earlier. For every HPO all values were recorded in

triplicate and used to calculate the $D_{7.4}$ values from the ratio of the decrease in absorbance of the aqueous phase using the following formula:

$$\mathbf{D_{7.4} = (A_0 - A_1)\ x\ Vw\ /\ A_1\ x\ Vo}$$

Where A_0 = initial absorbance of the aqueous phase; A_1 = absorbance at equilibrium of aqueous phase after the addition of octanol; Vw = volume of the aqueous layer used in partitioning; and Vo = total volume of 1-octanol layer used in partitioning.

Vw and Vo were determined by the difference in weight before and after addition of the 2 mL aqueous phase to the 1, 2, 3 and 4 mL of 1-octanol prior to vortexing of the two phases.

Table 1S Iron affinities of fluorinated 3-hydroxypyridin-4-ones

Compounds	Affinity constants for Fe(III)				pFe^{3+}
	$\log K_1$	$\log K_2$	$\log K_3$	$\log \beta_3$	(pH 7.4)
DFP	14.6	12.2	9.7	36.4	20.6
CP94	15.2	11.8	9.8	36.8	20.5
1a	13.0	11.0	9.0	33.0	20.8
1b	12.1	9.4	7.2	28.6	19.0
1c	14.3	11.8	9.4	35.5	18.7
1e	11.9	10.2	8.2	30.3	19.4
1f	12.0	10.3	8.2	30.5	19.6
1g	12.2	10.3	8.2	30.7	19.5
1h	12.3	10.5	8.5	31.3	20.0
1i	12.4	10.5	8.3	31.2	20.1
1m	13.1	11.1	8.8	33.0	20.5
1n	13.0	11.1	8.9	33.0	18.9
1o	13.6	11.0	8.4	33.0	18.5
1p	12.5	10.7	8.8	32	18.6
1q	11.6	10.2	8.1	29.9	17.8
1r	12.1	10.3	8.4	30.9	18.9
1s	12.7	10.6	8.6	31.9	19.7
1t	11.9	10.3	8.4	30.6	18.7
1u	11.8	10.0	7.9	29.7	17.9
1v	15.1	11.9	8.9	35.9	18.8
1w	16.5	12.8	9.3	38.7	19.8
1y	10.8	8.2	7.5	26.5	16.2
1z	14.7	11.9	6.9	33.5	19.5

IV. Glucuronidation and oxidation study

In Vitro Glucuronidation

Tris-HCl buffer (50 mM, pH7.4) was used to prepare UDPGA (20 mM) and $MgCl_2$ (50 mM) solutions. The male Dunkin-Hartley guinea pig hepatic microsome (In Vitro Technologies GmbH, Leipzig, Germany) (2 mg protein/ml) was diluted 1:1 by Tris-HCl buffer (50 mM, pH7.4), which contained triton-X100 3µl/ml. The incubates consisted of microsomes (1 mg protein), UDPGA (2 mM), $MgCl_2$ (5 mM) and a HPO compound (substrate, Figure 1) (0.2 mM). Tris-HCl buffer (50 mM, pH7.4) was used to adjust the final volume of the incubates to 1.0 ml. The incubation was carried out in a shaking water bath at 37 ^{o}C for 1h. When the incubation was completed, 0.1ml of trichloroacetic acid (25% w/v) was added to the samples to stop the reaction. Then samples were added to the internal standard solution (CP95, 2 mM, 0.1 ml) and centrifuge at 2000 rpm for 10 min. The supernatant was separated and loaded on HPLC column for analysis.

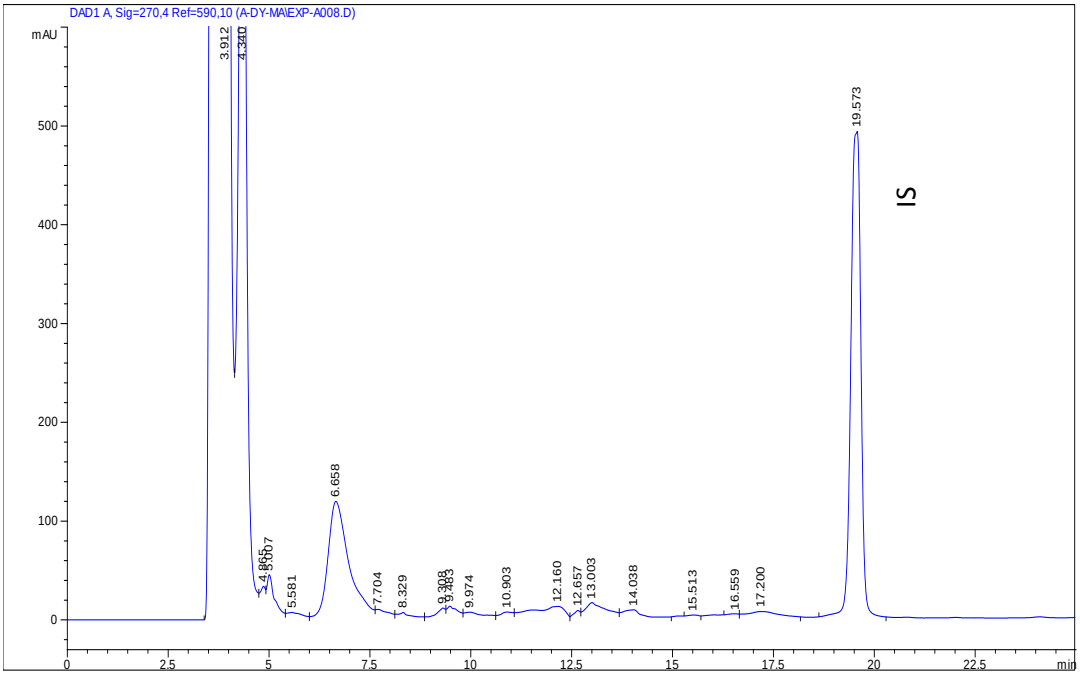
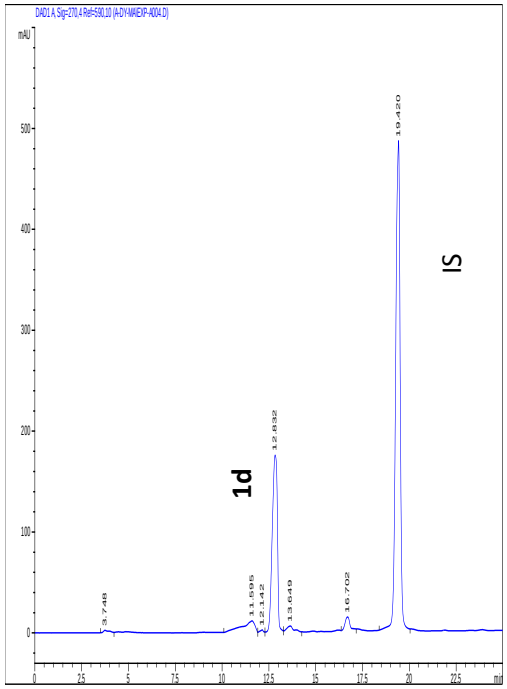
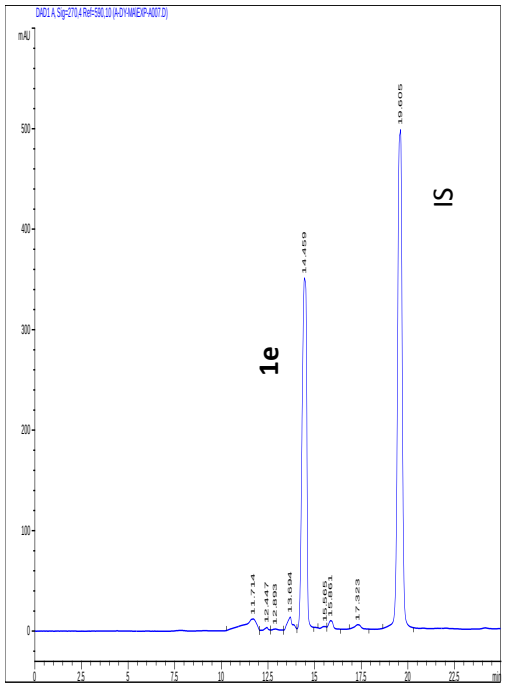


Figure 1S. The chromatogram of the blank incubate.

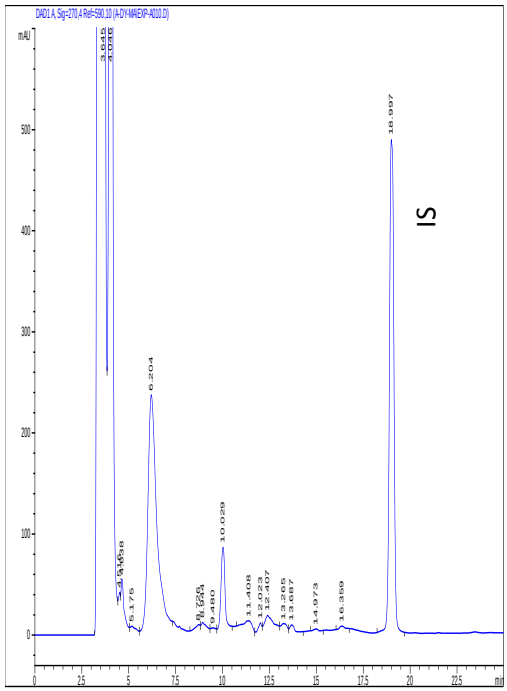
1d standard



1e standard



1d incubate



1e incubate

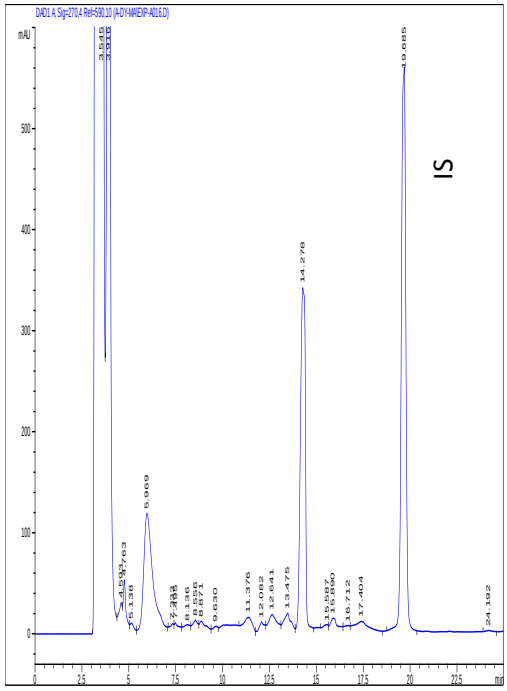
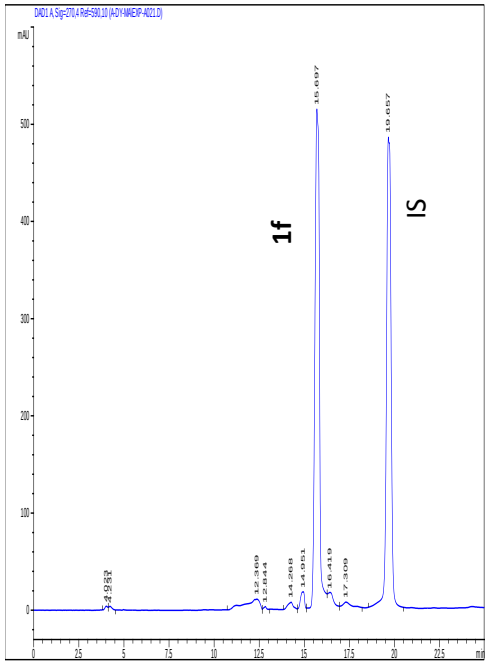
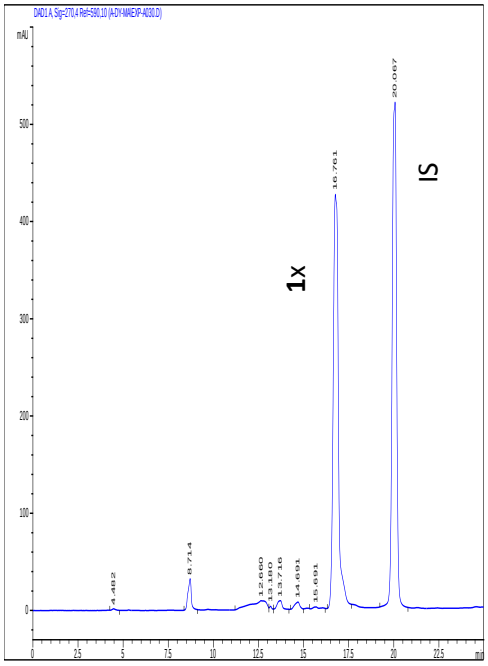


Figure 2S (A). Chromatograms of the standards and incubates of **1d** and **1e**.

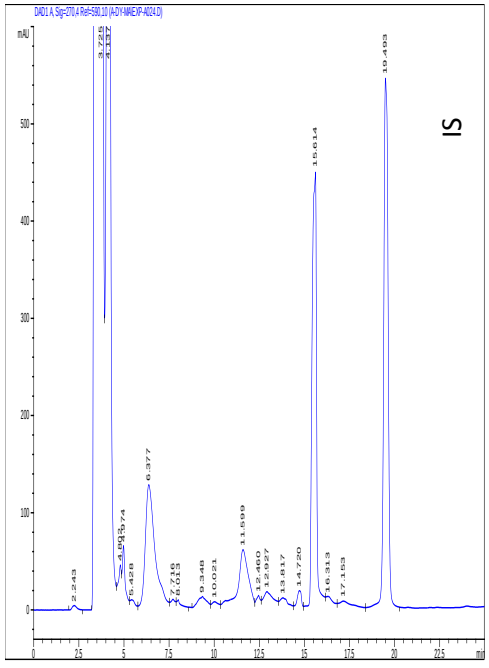
1f standard



1x standard



1f incubate



1x incubate

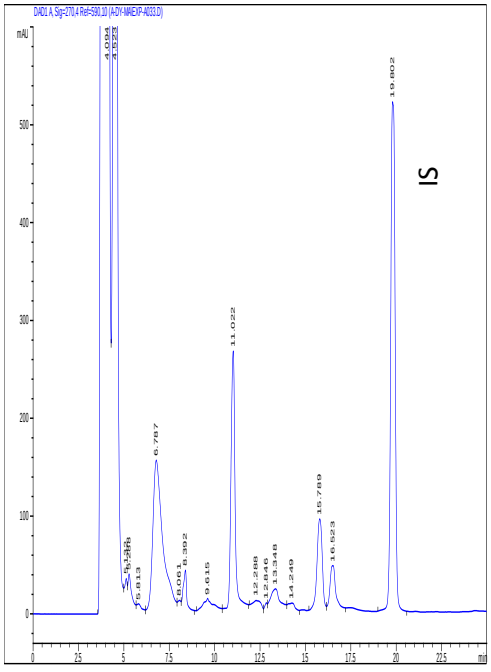
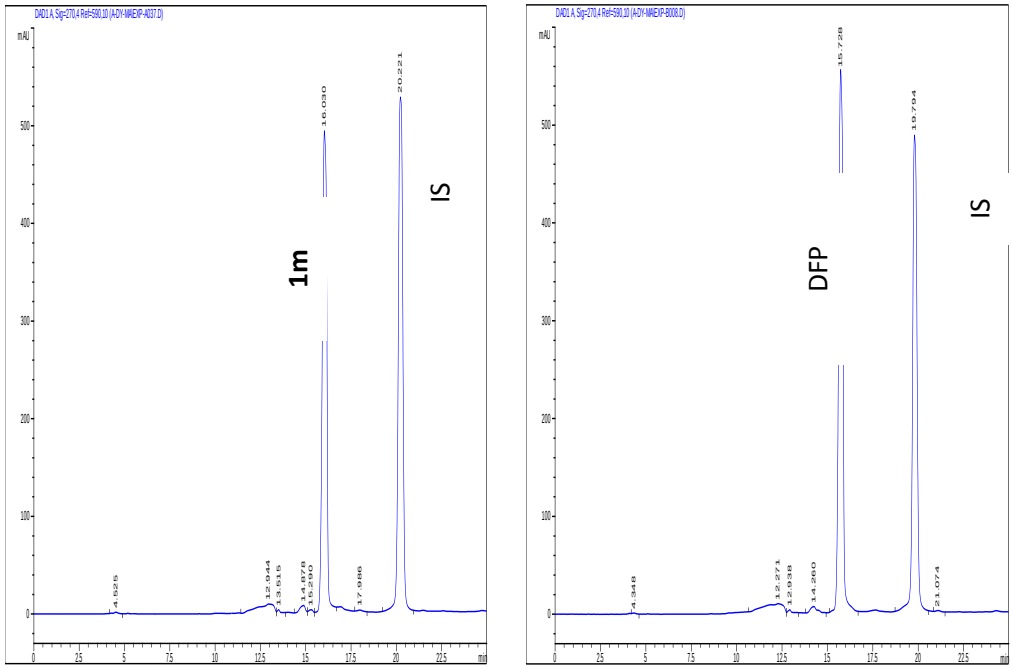


Figure 2S (B). Chromatograms of the standards and incubates of **1f** and **1x**.

1m standard

DFP standard



1m incubate

DFP incubate

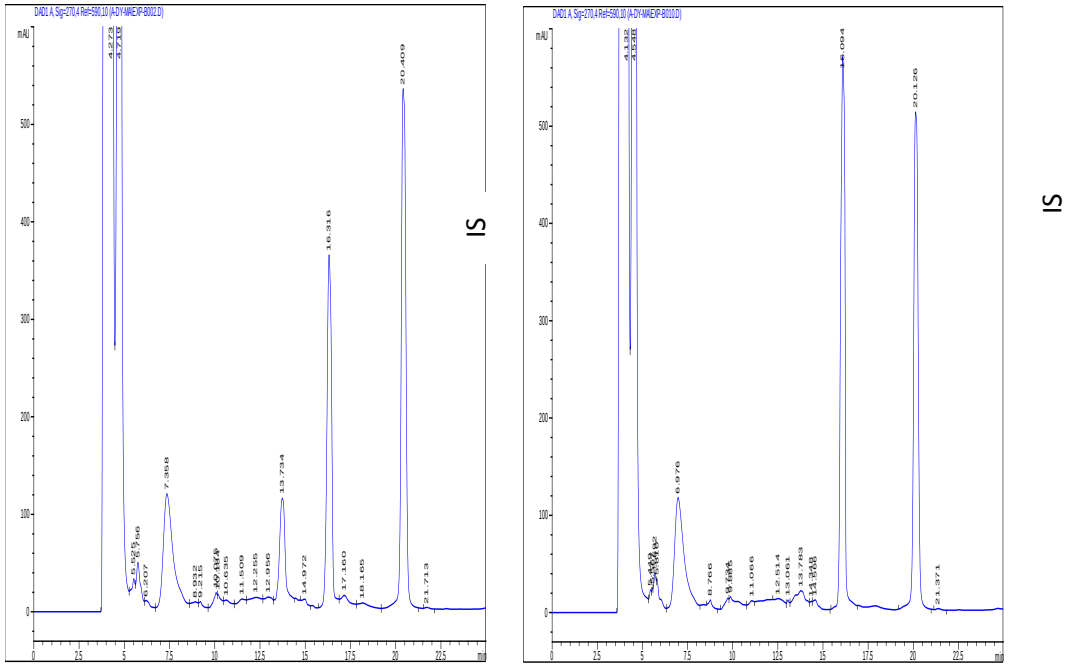
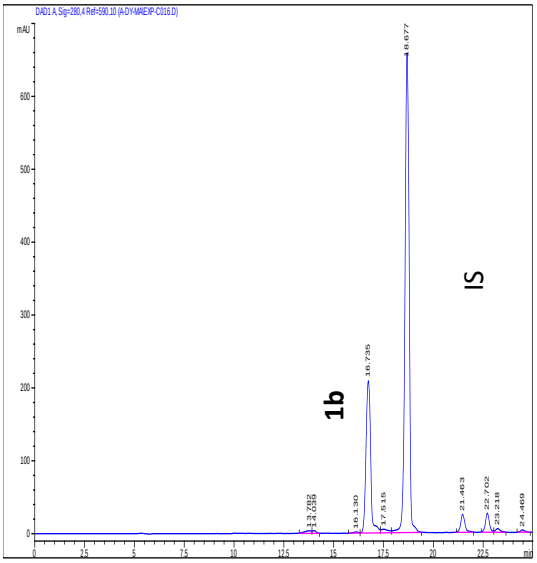


Figure 2S (C). Chromatograms of the standards and incubates of **1m** and deferiprone.

1b standard



1b incubate

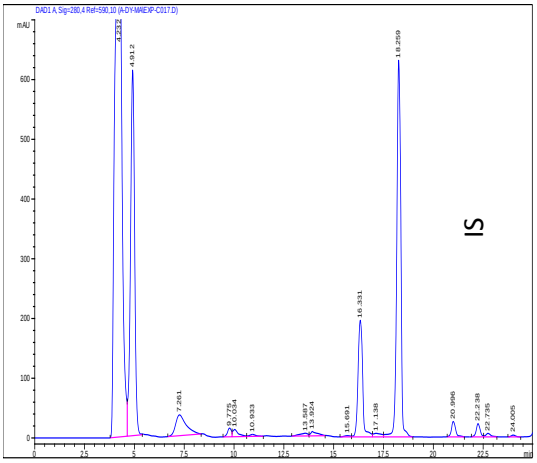


Figure 2S (D). Chromatograms of the standard and incubate of **1b**.

Oxidation

Tris-HCl buffer (50 mM, pH7.4) was used to prepare NADPH (20 mM) and $MgCl_2$ (50 mM) solutions. The guinea pig microsomal preparation (2 mg protein/ml) was diluted 1:1 by Tris-HCl buffer (50 mM, pH7.4) (without triton-X100). The incubates consisted of microsomes (1 mg protein), NADPH (2 mM), $MgCl_2$ (5 mM) and a HPO compound (substrate, Table 1) (0.2 mM). Tris-HCl buffer (50 mM, pH7.4) was used to adjust the final volume of the incubates to 1.0 ml. The incubation was carried out in a shaking water bath at 37 ^{o}C for 1h. When the incubation was completed, 0.1 ml of trichloroacetic acid (25% w/v) was added to the samples to stop the reaction. Then samples were added to the internal standard solution (CP94, 2 mM, 0.1 ml) and centrifuge at 2000 rpm for 10 min. The supernatant was separated and loaded on HPLC column[3] for analysis.

V. Blood-brain barrier permeability study

An in situ brain perfusion technique is used to determine the BBB influx efficiency of 3-hydroxypyridin-4-ones. The technique involves the surgical exposure of right and left carotid arteries in anesthetised adult male Guinea pigs. The internal carotid arteries are cannulated with fine silicon tubing while the external and common carotid arteries remain ligated to prevent backflow of the perfusate. A steady rate perfusion is carried out using a peristaltic pump with a flow rate of 6 mL/min for 15-20 min. The perfusion buffer is a solution of the iron chelator(s) in Ringer's solution (NaCl 117 mM, KCl 4.7 mM, $MgSO_4$ 0.8 mM, $NaHCO_3$ 24.8 mM, KH_2PO_4 1.2 mM, $CaCl_2$ 2.5 mM, D-glucose 10 mM, bovine serum albumin 39 g/l, Evans blue, 0.055 g/l), aerated with 95% O_2, 5% CO_2 and warmed to 37 ^oC. A BBB impermeable vascular marker (D-^{3}H-Mannitol) is also added to this perfusate to account for perfusate remaining in the vascular space at the end of the perfusion. Pressure is monitored to ~80 mm Hg throughout the perfusion. Soon after cannulation and initiation of perfusion the jugular veins were severed to allow drainage. The perfusion is terminated by decapitation at the end of the perfusion period. The brain is removed from the skull with much care so as to maintain its intact morphology. The brain is dissected to remove the cerebellum and choroid plexuses.

The two brain hemispheres once divided are weighed and individually prepared for analysis by scintillation and HPLC. Each brain hemisphere is then prepared to obtain extracts of 'whole brain' and 'capillary depleted' brain tissue. The brain is homogenised in 2x its weight of physiological buffer followed by 3x its weight of 30% dextran. Representative samples for scintillation counting are collected from this homogenate. Half of the remaining amount of brain homogenate is vortexed with trifluoroacetic acid and the other half remains untreated. Both these halves are centrifuged at 13000 rpm, 4 ^oC for 40 mins. The clear supernatant from the trifluoroacetic acid treated half of the homogenate is separated for HPLC analysis to determine the amount of chelator present in 'whole brain' extract. The supernatant brain tissue and fluid is separated from the precipitated capillaries in the other (untreated) half of the homogenate. This brain tissue and fluid is treated (similarly to the 'whole brain') with trifluoroacetic acid followed by centrifugation to obtain the 'capillary depleted brain' extract for HPLC analysis[3].

VI Relationship of logD between free ligand and iron complex

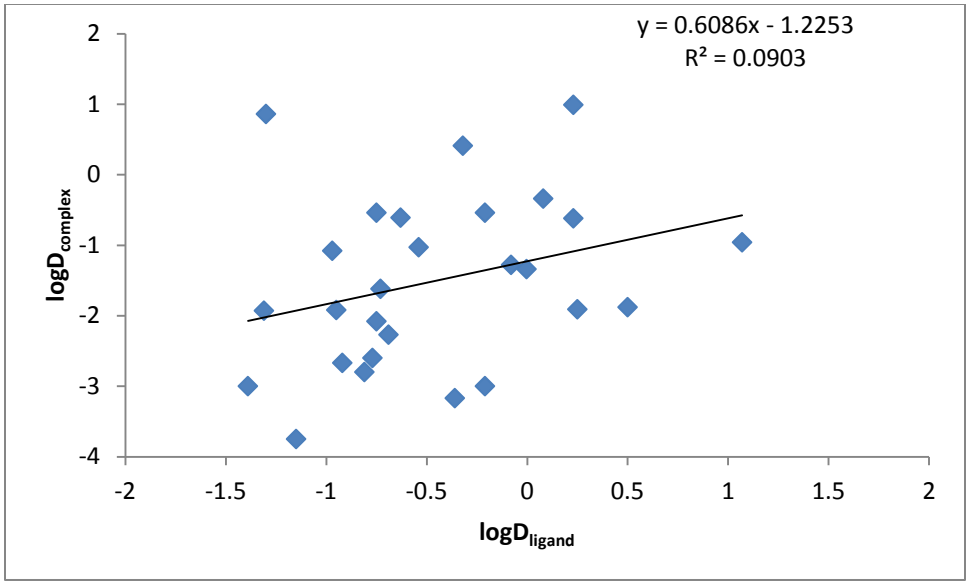


Figure 3S Relationship between the logD value of iron complex ($logD_{complex}$) and logD value of free ligand ($logD_{ligand}$). There is not good correlation between these two values.

References:

1. Gans, P.; Sabatini, A.; Vacca, A. Determination of equilibrium constants from spectrophometric data obtained from solutions of known pH: The program pHab. *Ann Chim-Rome* **1999,** 89, 45-49.
2. Gans, P.; Alderighi, L.; Ienco, A.; Peters, D.; Sabatini, A.; Vacca, A. Hyperquad simulation and speciation (HySS): a utility program for the investigation of equilibria involving soluble and partially soluble species. *Coordin Chem Rev* **1999,** 184, 311-318.
3. Liu, D. Y.; Liu, Z. D.; Lu, S. L.; Hider, R. C. Gradient ion-pair high-performance liquid chromatographic method for analysis of 3-hydroxypyridin-4-one iron chelators. *J Chromatogr B* **1999,** 730, 135-139.