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Design and Synthesis of Fluorinated Iron Chelators for Metabolic Study and Brain Uptake

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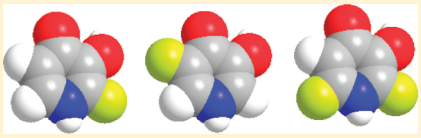
Supporting Information

ABSTRACT: A range of fluorinated 3-hydroxypyridin-4-ones has been synthesized where fluorine or fluorinated substituent was attached at 2- or 5- position of the pyridine ring in order to improve chemical and biological properties of 3-hydroxypyridin-4-ones. The synthetic route is different from conventional counterparts where a functional group is introduced to a preformed 3-hydroxypyridin-4-one ring. Herein, we introduce a novel method which starts with a fluorine containing precursor and the two hydroxyl groups at 3- and 4- positions of the pyridine ring are introduced at a later stage. The pK_a values of the free ligands and the affinity constants of their iron complexes demonstrate that the presence of fluorine dramatically alters the values. The distribution coefficient values of the free ligands and corresponding iron(III) complexes between 1-octanol and MOPS buffer (pH 7.4) are also influenced. Glucuronidation and oxidation studies of selected fluorinated 3-hydroxypyridin-4-ones demonstrate that some such fluorinated compounds have clear advantage over deferiprone in that they are metabolized more slowly. Blood–brain barrier permeability studies indicated that although lipophilicity influences the permeability it is not the only factor. Two of the selected seven fluorinated 3-hydroxypyridin-4-ones have improved brain distribution when compared with deferiprone.

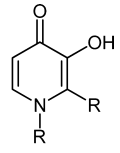


INTRODUCTION

The presence of one or more fluorine atoms in drug candidates impacts a variety of properties on such molecules, including enhanced metabolic stability, bioavailability, selective reactivity, receptor binding interactions, and changes in physical properties.[1−4] Generally, the unique characterization of fluorinated molecules is attributed to the fluorine atom possessing a high electronegativity, relatively small size, increased thermal and oxidative stability, and altered molecular lipophilicity. The participation of fluorine in medicinal chemistry and drug design cannot be underestimated; in 2007, 115 fluorinated drug candidates were investigated in phase II studies and 44 in phase III clinical trials.[5]

There is an increasing interest in the application of iron-selective chelators for the treatment and possible prevention of the onset of a range of neurodegenerative disorders, including Alzheimer's disease and Parkinson's disease.[6] Indeed one such chelator, deferiprone (DFP), a 3-hydroxypyridin-4-one (HPO), is currently being used to treat Friedreich's ataxia (Chart 1).[7] Although deferiprone is able to cross the blood–brain barrier, it is not particularly efficient at doing so.[8] In addition, deferiprone is rapidly metabolized in the liver[9] and thus the dose of deferiprone required to maintain a thalassaemia patient in negative iron balance is relatively high, typically in the region of 75 to 100 mg·kg^{-1}·day^{-1}.[10] Another HPO analogue, 1,2-diethyl-3-hydroxypyridin-4-one (CP94), has also been investigated.[11−13] Although a metabolic study in the rat demonstrated that CP94 retains the ability to chelate iron,[14] a parallel study in man demonstrated that CP94 is rapidly converted to the 3-*O*-glucuronide conjugate.[12] In an attempt to improve the distribution properties of HPOs, we have investigated the influence of introducing fluorine atoms into the aromatic nucleus. In the present study, we have synthesized a series of monofluoro, difluoro, trifluoro, and trifluoromethyl substituted HPOs. The physicochemical properties of this series of compounds have been characterized, together with their metabolic stability and rates of brain uptake.

Chart 1. Structure of DFP and CP94



DFP: R=Me
CP94: R=Et

RESULTS AND DISCUSSION

Chemistry. There are two possible approaches to the introduction of fluorine into HPOs: one is to introduce fluorine or a fluorinated moiety into the preformed HPO matrix and the other is to start with a fluorine-containing precursor and introduce chelating functional groups. In this paper, we report three fluorinated HPOs synthesized by the former approach and 23 by the latter.

The synthetic route employed by the former approach is summarized in Scheme 1. 3,4-Bis(benzyloxy)pyridine-2-carbaldehyde **2** and 3-(benzyloxy)-2-(hydroxymethyl)-1,6-dimethyl-

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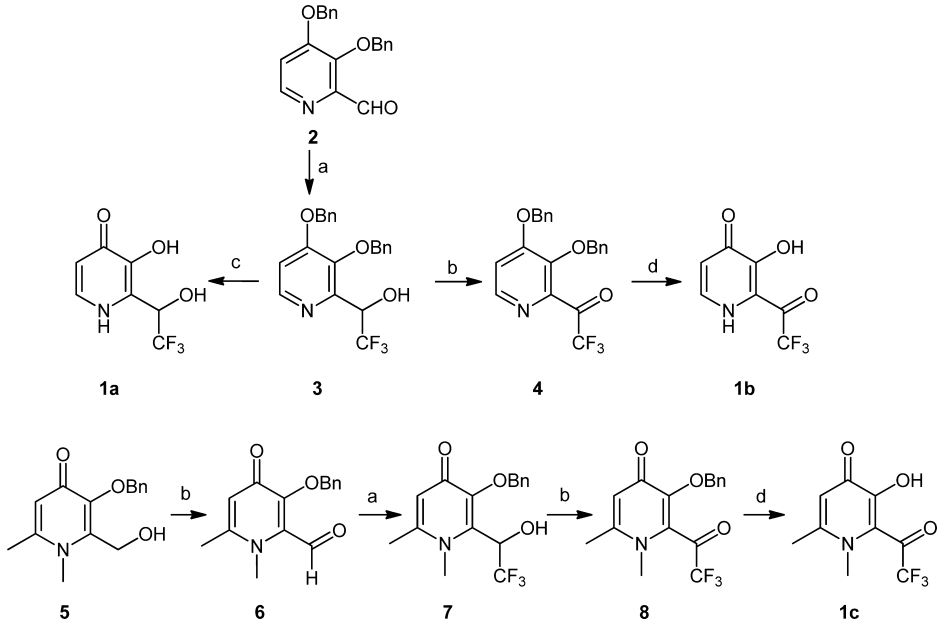
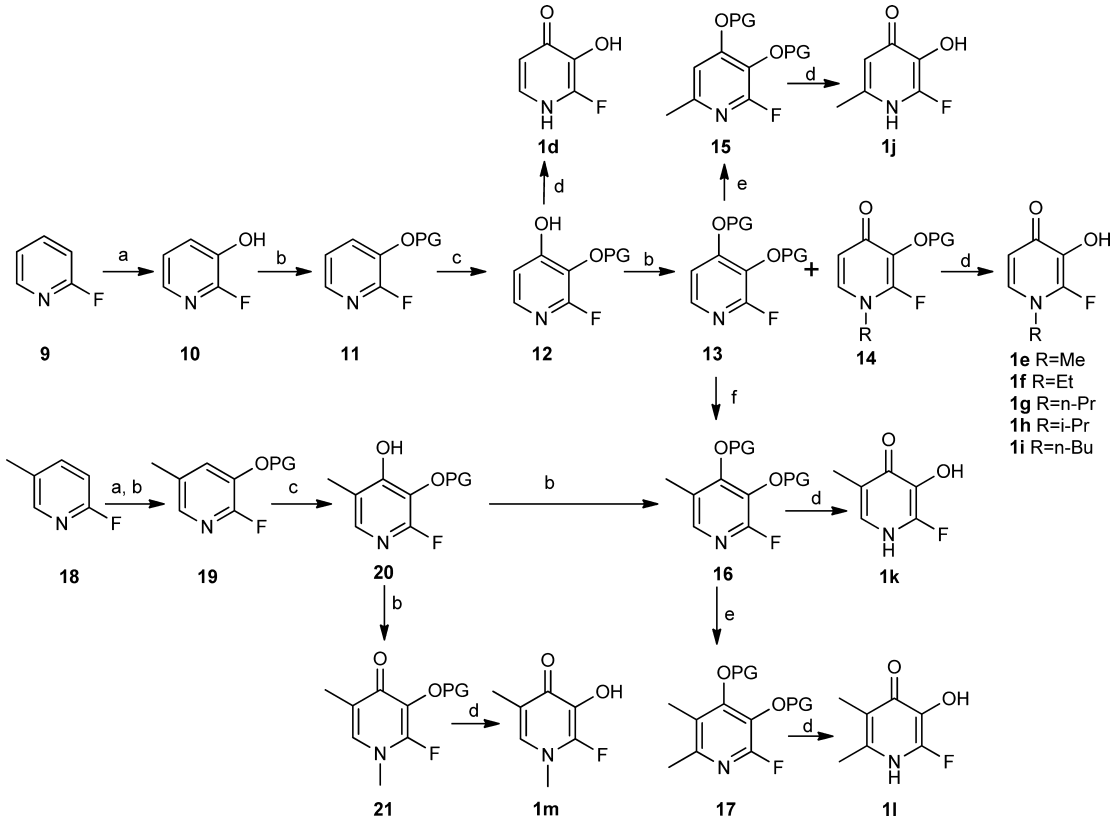
Scheme 1. Synthesis of 2-Trifluoromethyl Substitution of 3-Hydroxypyridin-4-ones[a]



[a](a) Me_3SiCF_3/TBAF; (b) DMSO/TEA/Py-SO_3; (c) Pd/C, H_2; (d) BCl_3.

Scheme 2. Synthesis of 2-Fluoro Substituted 3-Hydroxypyridin-4-one Derivatives[a]



[a](a) (i) LDA in THF at −75 °C for 0.5 h, (ii) $B(OMe)_3$ at −75 °C for 2 h, (iii) CH_3CO_3H at 0 °C for 1 h; (b) K_2CO_3, RI in acetone refluxed overnight; (c) (i) LTMP in THF at −75 °C for 1 h, (ii) $B(OMe)_3$ at −75 °C for 2 h, (iii) CH_3CO_3H at 0 °C for 1 h; (d) BBr_3 in DCM at 0 °C overnight; (e) (i) LDMEA in THF at −75 °C for 20 h, (ii) MeI; (f) (i) LTMP in THF at −75 °C for 20 h, (ii) MeI (PG = protected group).

pyridin-4-one **5** were readily prepared from commercially available maltol and kojic acid respectively by following a previous reported procedure.[15,16] Selective oxidation of the alcohol **5** to its corresponding aldehyde **6** proceeded effectively by using the sulfur trioxide pyridine complex (SO_3·Py) in combination with dimethyl sulfoxide as the oxidizing reagent. The trifluoromethylation reaction was carried out by reacting the aldehyde with $TMSCF_3$ in the presence of a catalytic

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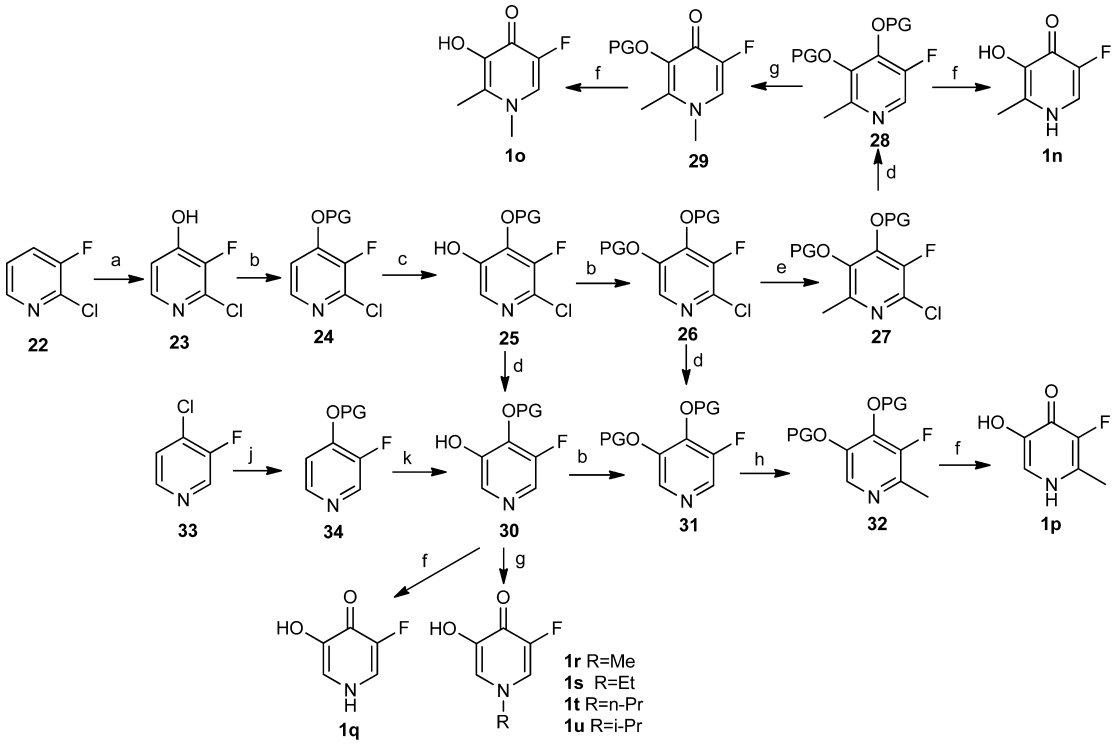
Scheme 3. Synthesis of 5-Fluoro Substituted 3-Hydroxypyridin-4-one Derivatives[a]



[a](a) (i) LDA in THF at −75 °C for 2 h, (ii) $B(OMe)_3$ at −75 °C for 2 h, (iii) CH_3CO_3H at 0 °C for 1 h; (b) K_2CO_3, RI in acetone refluxed overnight; (c) (i) LTMP in THF at −75 °C for 20 h, (ii) $B(OMe)_3$ at −75 °C for 2 h, (iii) CH_3CO_3H at 0 °C for 1 h; (d) $Pd(OH)_2/H_2/Et_3N$; (e) (i) LTMP in THF at −75 °C for 24 h, (ii) MeI; (f) BBr_3 in DCM at 0 °C overnight; (g) RI in acetone, reflux overnight; (h) (i) LDA in THF at −75 °C for 20 h, (ii) MeI; (j) NaOMe, reflux overnight; (k) LDA in THF at −75 °C for 20 h, (ii) $B(OMe)_3$ at −75 °C for 2 h, (iii) CH_3CO_3H at 0 °C for 1 h (PG = protected group).

amount of tetrabutylammonium fluoride, followed by desilylation. The resulting alcohol was either hydrogenated to afford deprotected product **1a** or further oxidized to aldehydes **4** and **8**, respectively. As hydrogenation would reduce the ketone to its corresponding alcohol, boron trichloride was selected to remove the benzyl protecting group to form **1b** and **1c**, respectively.

We also investigated the ability of several fluoronitrogen agents such as Selectfluor to introduce fluorine directly into the heterocyclic ring, but unfortunately all such attempts failed. Therefore, the second approach which used commercial available fluorine-containing building blocks as starting materials was adopted.

The general methodology adopted for the synthesis of 2-fluoro substituted HPOs **1d−m** started from 2-fluoropyridine or 2-fluoro-5-methylpyridine is summarized in Scheme 2. 2-Fluoro-3-hydroxypyridine **10** was obtained by treating 2-fluoropyridine **9** with lithium diisopropylamide (LDA), followed by successive additions of trimethylborate and peracetic acid. Lithiation occurs selectively at C3 of 2-fluoropyridine due to a strong inductive effect of the fluorine atom.[17,18] Before a second lithiation at C4 by using lithium 2,2,6,6-tetramethylpiperidine (LTMP), the 3-hydroxy group of the compound **10** required protection. Reacting **10** with methyl iodide in the presence of potassium carbonate affords a quantitative yield of 2-fluoro-3-methoxypyridine **11**. A similar methodology was adopted to introduce a second hydroxyl group at C4 of compound **11**. We attempted to protect the 4-hydroxy group of **12** also using K_2CO_3/RI reagents. However, at this stage, a mixture of two alkylated products **13** and **14** were isolated, where the alkyl group was attached either on the oxygen of the 4-hydroxy or the nitrogen of the pyridine ring. The formation of **13** and **14** can be explained by the tautomerism of compound **12** between 4-hydroxypyridine and pyridine-4-one tautomers. By increasing the size of the alkyl group, the ratio of **13**:**14** can be dramatically increased. For example, a ratio of 4:5 for methyl group is enhanced to 30:1 for the isopropyl group. The 4-alkoxypyridine isomer **13** can be selectively methylated at either C5 or C6 to afford **16** and **15**, respectively, depending on the choice of lithiation reagents. LTMP favors the deprotonation of the pyridine derivative at C5, while the unimetal superbase (USB: 1:1 ratio of butyllithium and lithium 2-(dimethylamino)ethoxide)[17,19] prefers at C6. In contrast to the pyridine isomer **13**, the pyridinone **14** cannot be methylated at either of the apparently vacant positions.

Compound **16** can also be produced from 2-fluoro-5-methylpyridine **18**, where only two alkoxy groups at both C3 and C4 are involved, in a similar way to that mentioned above. However, analogue **15** cannot be produced from the similar route, as 2-fluoro-6-methylpyridine is prone to be lithiated at the 6-methyl group in preference to C3. Compound **16** was again methylated by USB, followed by trapping with methyl iodide to afford the dimethyl substituted compound **17**. Again, when compound **20** was refluxed with K_2CO_3/MeI in acetone, an equimolecular mixture of **16** and **21** was obtained. A conventional method to introduce an alkyl group on the N1 of pyridine ring using alkyl iodide or dimethyl sulfate to form 1-alkylpyridinium failed with 2-fluoropyridine derivatives, most likely due to the strong influence of fluorine at C2. The alkyl

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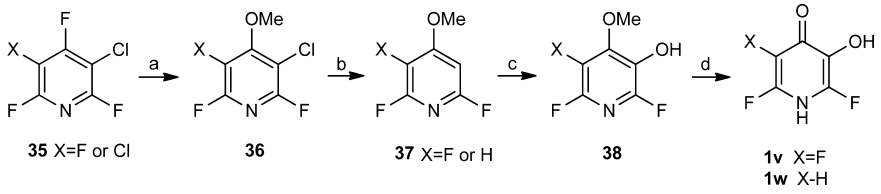
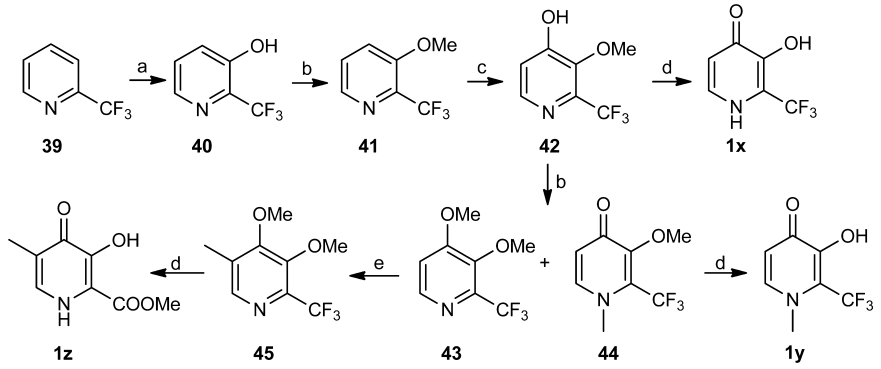
Scheme 4. Synthesis of Multifluoro Substituted 3-Hydroxypyridin-4-ones[a]



[a](a) NaOMe; (b) Pd/C, $HCOONH_4$; (c) (i) LDA in THF at −75 °C for 0.5 h, (ii) $B(OMe)_3$ at −75 °C for 2 h, (iii) CH_3CO_3H at 0 °C for 1 h; (d) BBr_3, overnight.

Scheme 5. Synthesis of Trifluoromethylated 3-Hydroxypyridin-4-ones[a]



[a](a) (i) LTMP/16 h, (ii) $B(OMe)_3$/2 h, (iii) CH_3CO_3H/1 h; (b) CH_3I/K_2CO_3; (c) same as (a) except 3 h with LTMP; (d) BBr_3; (e) LTMP/MeI.

protecting group was then readily removed using BBr_3 to form the corresponding iron chelating products **1d−m**.

The synthesis of 5-fluoro substituted HPOs **1n−u** is outlined in Scheme 3. Although 3-fluoropyridine is a commercially available chemical and its lithiation by LDA occurs at C4 rather than C2, further lithiation readily lead to attacks at C2, as the proton at this position is more acidic than those at the 5- and 6-positions due to the fluorine at C3. Therefore, 2-chloro-3-fluoropyridine **22** was selected for the starting material, where the C2 was chlorine blocked. In similar fashion to that of 2-fluoropyridine, the hydroxyl group was subsequently introduced at C4 and C5 to form intermediate **25**. Methylation occurs at C6 when the 5-hydroxy group of **25** was protected to afford **27**. The blocking chlorine atom of compounds **25**, **26**, and **27** was removed by hydrogenation to produce **30**, **31**, and **28**, respectively. Compound **30** can be also obtained from commercially available 4-chloro-3-fluoropyridine **33** in a two-step manner via intermediate **34**. Compound **31** can be obtained from **25** by either protection of 5-hydroxy group followed by reduction to remove the chlorine atom or a reverse procedure via **30**. Because of the presence of a fluorine at C3, C2 of compound **31** prefer to be deprotonated over C6, followed by quenching by methyl iodide to obtain **32**. In contrast to the 2-fluoropyridine analogues, which failed to produce 1-alkylpyridinium salts by reacting with alkyl iodide, when 3-fluoropyridine derivatives **28** and **30** were treated with alkyl iodide, a clear new spot at the bottom of TLC plate was detected. The resulting product was identified as the 1-alkylpyridin-4-one derivatives **29** and **1r−u**, respectively. A plausible mechanism for this unexpected product is explained by the formation of the unstable 1-alkylpyridinium salt intermediate.[20] 4- and 5-Alkyl protecting groups of **28−30** and **32** were removed by BBr_3 to afford the chelators **1n−q**, respectively.

The syntheses of tri- and difluoro HPOs **1v** and **1w** started with the pentahalo substituted pyridine **35** (Scheme 4). Treatment of the commercially available 3-chloro-2,4,5,6-tetrafluoropyridine or 3,5-dichloro-2,4,6-trifluoropyridine **35** with 1 equiv of sodium methoxide yielded **36** in a good yield. Treatment of **36** with 10% Pd/C in the presence of ammonium formate at 50 °C for 10 h gave compound **37** with a high yield, followed by subsequent lithiation, electrophilic substitution, and oxidation as outlined above, introduced a hydroxyl group to afford compound **38**. The 4-methyl protecting group was removed to produce **1v** and **1w**, respectively.

In similar fashion to that of 2-fluoro HPOs, the synthesis of 2-trifluoromethyl HPOs was initiated from 2-trifluoromethylpyridine **39** (Scheme 5). Because of the lower electron withdrawing influence of the trifluoromethyl group compared to fluorine at C2, deprotonation at C3 requires a stronger base, such as LTMP (pK_a 37.3), rather than LDA (pK_a 35.7), as a lithiation reagent. After two hydroxyl groups were introduced at C3 and C4 in a similar procedure as reported for that of 2-fluoropyridine, compound **42** was treated with methyl iodide in the presence of K_2CO_3 to afford two methylated products **43** and **44** in a ratio of 3:2. Intermediate **43** was then methylated at C5 using LTMP/MeI reagents to obtain **45** in a high yield. Deprotection of compounds **42** and **44** with BBr_3 gave the expected products **1x** and **1y**, respectively. However, when **45** was treated with BBr_3 in same way, no peak was found in ^{19}F NMR spectrum. Combined with the NMR, MS, and elemental analysis data, the product was deduced to be compound **1z**. This result can be explained by the fact that the trifluoromethyl was substituted to tribromomethyl with BBr_3 and then undergoes hydrolysis with methanol to form the ester (**1z**).

Determination of Acid Dissociation Constants. pK_a values of all the F-HPOs **1a−z** are presented in Table 1. Similar to nonfluorinated analogues, all 1-alkyl substituted F-HPOs contain two pK_as, assigned to dissociable 3-hydroxy

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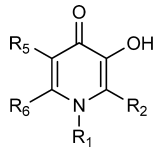
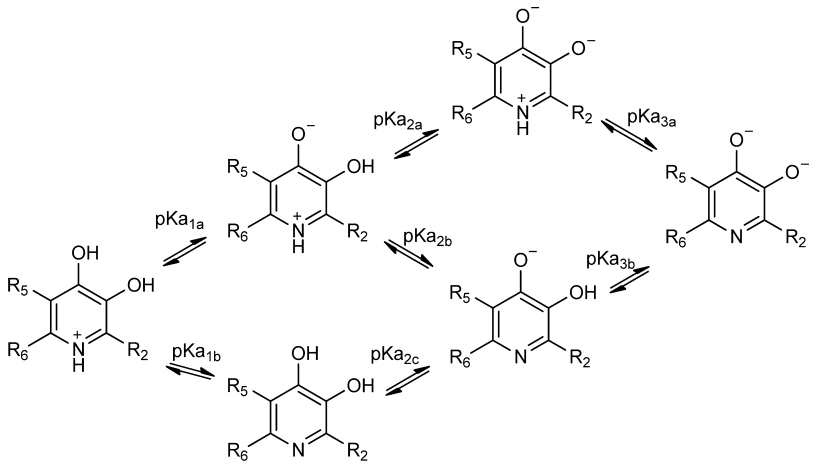
Table 1. pK_as and Iron Affinities of Fluorinated 3-Hydroxypyridin-4-ones



compd	R_1	R_2	R_5	R_6	pK_{a1}	pK_{a2}	pK_{a3}	log β_3	pFe^{3+}
DFP	Me	Me	H	H	3.68	9.77		36.4	20.6
CP94	Et	Et	H	H	3.81	9.93		36.8	20.5
1a	H	$CH(OH)CF_3$	H	H	2.6	8.2		33.0	20.8
1b	H	$COCF_3$	H	H	2.8	6.4	9.7	28.6	19.0
1c	Me	$COCF_3$	H	Me	3.85	9.15		35.5	18.7
1d	H	F	H	H	0.9	6.5	10.7	37–39[a]	18–20[a]
1e	Me	F	H	H	2.4	7.7		30.3	19.4
1f	Et	F	H	H	2.46	7.72		30.5	19.6
1g	*n*-Pr	F	H	H	2.49	7.89		30.7	19.5
1h	*i*-Pr	F	H	H	2.51	7.88		31.3	20.0
1i	*n*-Bu	F	H	H	2.61	7.79		31.2	20.1
1j	H	F	H	Me	1.15	6.7	11.08	37–39[a]	17–19[a]
1k	H	F	Me	H	1.42	6.66	11.10	37–39[a]	16.5–18.5[a]
1l	H	F	Me	Me	2.01	6.98	11.56	37–39[a]	16.5–18[a]
1m	Me	F	Me	H	2.5	8.35		33.0	18.0
1n	H	Me	F	H	2.2	8.93	12.05	33.0	18.9
1o	Me	Me	F	H	2.24	9.08		33.0	18.5
1p	H	H	F	Me	1.94	8.68	12.04	32.0	18.6
1q	H	H	F	H	1.6	8.2	11.7	29.9	17.8
1r	Me	H	F	H	1.76	8.18		30.9	18.9
1s	Et	H	F	H	1.78	8.25		31.9	19.7
1t	*n*-Pr	H	F	H	1.49	8.12		30.6	18.7
1u	*i*-Pr	H	F	H	1.72	8.12		29.7	17.9
1v	H	F	F	F	<0.8	4.4	9.9	35.9	18.8
1w	H	F	H	F	<0.8	5.7	10.6	38.6	19.8
1x	H	CF_3	H	H	1.54	6.5	10.4	38.5–39.5[a]	20–21[a]
1y	Me	CF_3	H	H	<0.8	7.4		26.5	16.2
1z	H	COOMe	Me	H	3.84	8.98		33.5	19.5

[a]Range of values quoted due to the multiple protonation of the iron complexes.

Scheme 6. Proton Dissociation Equilibria of the 1-Nonsubstituted F-HPOs (Either R_2 or R_5 = F or F-Containing Group) (For Clarity, the Resonance Forms Are Not Presented)



(pK_{a2}) and 4-oxo group (pK_{a1}).[21] However, both pK_as are lower when compared to those of nonfluorinated HPOs, for example, compare **1e** with DFP. This is due to the strong electron withdrawing property of fluorine (Table 1). 1-Unsubstituted F-HPOs possess an additional pK_a which is due to dissociation of 1-NH. This phenomenon does not occur on nonfluorinated analogues (**1z**) as the pK_a value falls outside the detectable range (pH 1–12).[22] The ionization equilibrium of this type of F-HPOs is shown in Scheme 6. According to our observations on the absorption spectra shifts with increased pH

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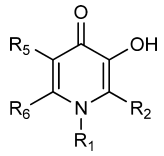
Table 2. Comparison of Distribution Coefficient of Free Ligand and Iron Complex



compd	R_1	R_2	R_5	R_6	log $D_{7.4}$ (n = 7) (free ligand)	F_n	log P (free ligand)	log $D_{7.4}$ (n = 4)[a] (Fe^{3+} complex)
DFP	Me	Me	H	H	−0.77 ± 0.02	0.995	−0.77	−2.60 ± 0.05
CP94	Et	Et	H	H	0.23 ± 0.01	0.997	0.25	−0.62 ± 0.02
1a	H	$CH(OH)CF_3$	H	H	0.08 ± 0.01	0.863	0.14	−0.34 ± 0.01
1b	H	$COCF_3$	H	H	−0.97 ± 0.02	0.999	−0.96	−1.08 ± 0.04
1c	Me	$COCF_3$	H	Me	−1.30 ± 0.02	0.982	−1.27	0.86 ± 0.04
1d	H	F	H	H	−0.21 ± 0.01	0.112	0.74	−3.00 ± 0.19
1e	Me	F	H	H	−1.15 ± 0.02	0.666	−0.97	−3.75 ± 0.24
1f	Et	F	H	H	−0.75 ± 0.01	0.676	−0.58	−2.08 ± 0.03
1g	*n*-Pr	F	H	H	−0.21 ± 0.05	0.755	−0.08	−0.54 ± 0.05
1h	*i*-Pr	F	H	H	−0.54 ± 0.02	0.74	−0.41	−1.03 ± 0.06
1i	*n*-Bu	F	H	H	0.23 ± 0.02	0.71	0.38	0.99 ± 0.04
1j	H	F	H	Me	0.25 ± 0.01	0.166	1.03	−1.91 ± 0.02
1k	H	F	Me	H	0.50 ± 0.02	0.154	1.32	−1.88 ± 0.03
1l	H	F	Me	Me	1.07 ± 0.04	0.275	1.64	−0.96 ± 0.00
1m	Me	F	Me	H	−0.69 ± 0.01	0.899	−0.65	−2.27 ± 0.06
1n	H	Me	F	H	−0.003 ± 0	0.971	−0.01	−1.34 ± 0.09
1o	Me	Me	F	H	−0.81 ± 0.01	0.98	−0.80	−2.80 ± 0.04
1p	H	H	F	Me	−0.73 ± 0.01	0.95	−0.71	−1.62 ± 0.06
1q	H	H	F	H	−0.92 ± 0.02	0.863	−0.85	−2.67 ± 0.11
1r	Me	H	F	H	−1.39 ± 0.01	0.858	−1.32	−3.00 ± 0.06
1s	Et	H	F	H	−0.95 ± 0.01	0.876	−0.89	−1.92 ± 0.01
1t	n-Pr	H	F	H	−0.63 ± 0.03	0.839	−0.46	−0.61 ± 0.04
1u	i-Pr	H	F	H	−1.00 ± 0.02	0.832	−0.92	
1v	H	F	F	F	−1.31 ± 0.01	0.001	1.70	−1.93 ± 0.17
1w	H	F	H	F	−0.36 ± 0.03	0.02	1.35	−3.17 ± 0.51
1x	H	CF_3	H	H	−0.08 ± 0.02	0.112	0.87	−1.28 ± 0.01
1y	Me	CF_3	H	H	−0.75 ± 0.01	0.5	−0.45	−0.54 ± 0.03
1z	H	COOMe	Me	H	−0.32 ± 0.02	0.974	−0.31	0.41 ± 0.07

[a]Distribution coefficients were measured using a 10:1 molar ratio of ligand to iron to ensure that the 3:1 neutral complexes formed.

over the range of 1−12 and computer modeling predictions,[23] **1b** and 5-F-HPOs (**1n**, **1p**, **1q**) have the same sequence of pK_a values, that is, the lowest pK_a value (<3) is assigned to the protonation of the 4-oxo group, followed by 3-hydroxy group in the range of 6−9 and the pK_a value above 9.5 is assigned to the dissociation of 1-NH (route pK_{a1a}−pK_{a2a}−pK_{a3a} in Scheme 6). However, when fluorine or trifluoromethyl is introduced at the 2-position of 1-unsubstituted HPOs (**1d**, **1j**−**1l**, **1x**), fluorine or trifluoromethyl group dramatically decreases the pK_a value of 1-NH to the range 6.5−7. Correspondingly, the pK_a of 3-hydroxy group is increased due to the influence of 4-O anion. The ionization of this group adopts the route pK_{a1a}−pK_{a2b}−pK_{a3b} (Scheme 6). This result is consistent with the previous report that introduction of 2-F on the pyridine decreases the pK_a value of 1-NH from 5.17 to −0.44[24] and also with our computer modeling predictions.[23] Furthermore, when two fluorines were introduced at the *ortho*- position of 1-NH (**1v** and **1w**), the pK_a value of 1-NH further drops to <1 and the assignment of the ionization route follows 1-NH to 4-oxo to 3-hydroxy (route pK_{a1b}−pK_{a2c}−pK_{a3b} in Scheme 6).

In comparison with the 1-nonsubstituted analogue **1d**, an introduction of methyl group at N1 (**1e**) dramatically influences the pK_a values of the 4-oxo and 3-hydroxy groups. However, this phenomenon does not occur when fluorine is at C5 position (**1n** and **1o**). On increasing the R_1 chain length (**1e**−**1i**), the pK_{a1} was increased slightly from 2.4 to 2.61 but the pK_{a2} value was unchanged. Again, this trend does not hold when fluorine is at C5 (**1r**−**1t**). A shift of the methyl group from C6 to C5 (**1j** and **1k**), is associated with a slight increase of the pK_{a1} value from 1.15 to 1.42 due to the electron donation of methyl group at the *ortho*- position. Compared to **1q**, an introduction of methyl group at C6 (**1p**) increases both pK_{a1} and pK_{a2}. A further increase was observed when the methyl group was introduced at C2 (**1n**). In comparison with 2-fluoro-1-alkyl substituted HPOs, the 5-fluoro analogues have lower pK_{a1} values but higher pK_{a2} values (**1e** vs **1r**; **1f** vs **1s**, **1g** vs **1t**). This phenomenon can be explained by the fluorine at the *ortho*- position having a stronger influence on the pK_a value than that at the *meta*- position. Similarly, by shifting the fluorine from C5 to C2 in the 1-nonsubstituted F-HPOs (**1q** vs **1d** and **1p** vs **1j**), the pK_a value of 1-NH is markedly decreased due to the shift of fluorine from *meta*- to *ortho*- position. Surprisingly, the pK_{a1} value, which is assigned to the 4-oxo group, is also decreased (Table 1).

When two or more fluorine atoms are introduced on the pyridinone ring, the pK_a value of 1-NH function is further decreased to <1 (**1d** to **1w** or **1v**). In contrast, the pK_a value of the 4-oxo function is increased whereas the pK_a value of the 3-hydroxy function remains in a similar range. Replacement of F- with CF_3- at position 2 generates similar pK_a values (**1d** to **1x**)

following the same sequence. This is confirmed by the finding that two similar pK_a values were determined when the 3-hydroxy of **1x** is protected. The values are 1.4 and 6.1, assigned to 4-oxo and 1-NH, respectively. Similar to the pair of **1d** and **1e**, an introduction of methyl on N1 dramatically decreases the pK_a value of 3-hydroxy (**1y** vs **1x**).

Determination of Iron(III) Affinity Constants. Stability constants of all F-HPOs ligands were evaluated using an automated spectrophotometric titration system. The value log β_3 was obtained by sum of the logarithms of three stepwise equilibrium constants K_1, K_2, and K_3 and are presented in Table 1. The values were found to be related to their pK_a values of both 3-hydroxy and 4-oxo moieties. For *N*-alkyl F-HPOs, due to their lower pK_a values compared with those of DFP and CP94, the iron stability constants (log β_3) fall correspondingly. This trend also holds for **1b** and 1-nonsubstituted 5-F-HPOs (**1n**, **1p**, and **1q**) where both chelating related pK_a values (pK_{a1} and pK_{a2}) are less than that of DFP and CP94. The lowest log β_3 value was found to be 26.5 for **1y**, where the pK_a values of 3-hydroxy and 4-oxo groups are at 7.4 and <0.8, respectively. However, log β_3 values are dramatically increased when pK_a values of the 3-hydroxy group (pK_{a3}) rise to above 10. 1-Nonsubstituted 2-F-HPOs (**1d**, **1j**−**1l**) and multifluoro substituted HPOs (**1v**−**1x**) fell into this group. For example, although the pK_a value of the 4-oxo group for **1j** is only 1.15, much lower than that of DFP, the increase of pK_a of the 3-hydroxy for **1j** (11.08 to 9.77 for DFP) leads to a larger log β_3 value than its counterpart (Table 1). The largest log β_3 value in this series is 38.6 for **1w**, where two fluorines were attached at C2 and C6, respectively, and pK_a values of the 3-hydroxy and 4-oxo groups are 10.6 and 5.7, respectively. However, it is difficult to determine the log β_3 values of **1d**, **1j**−**1l**, and **1x** precisely due to their 1-NH pK_a values being close to 7. This results in a complication associated with the protonation of the iron complexes, thereby introducing more species in the stoichiometric model. Thus only ranges of the log β_3 and pFe values are provided (Table 1).

By using the pK_a and log β_3 values, the pFe^{3+} value, which is defined as the negative logarithm of concentration of free iron(III) at pH 7.4 under conditions $\text{ligand}_{[\text{total}]} = 10^{-5}$ M and $\text{iron}_{[\text{total}]} = 10^{-6}$ M, was calculated. pFe^{3+} values of 1-alkyl 2-F-HPOs fall in the same range as that of DFP and CP94, with slightly higher values when the R_1 chain length was increased (**1e**−**1i**). However, a dramatic drop of pFe^{3+} value occurs with the 1-unsubstituted counterparts (**1d**, **1j**−**1l**), although the stability constant of **1j**−**1l** are higher than that of DFP. In contrast, there is no marked difference of pFe^{3+} values between 1-alkyl and 1-unsubstituted 5-F-HPOs, with both groups having marginally smaller values than that of DFP.

Determination of Distribution Coefficients. The distribution coefficients ($D_{7.4}$) of the free ligands and their iron(III) complexes between an aqueous phase buffered at pH 7.4 and octanol are presented in Table 2. By replacing hydrogen with fluorine, both distribution coefficients of free ligands and iron complexes fall in a similar range (compare **1m** and **1o** with DFP), with fluorine located at the second position possessing slightly more lipophilic character. In general, the increase in the alkyl chain length at N1 results in an increase in the $D_{7.4}$ values of both the ligands and the iron(III) complexes (**1e**−**1i** and **1r**−**1t**). Similar to our previous report,[25] 1-unsubstituted F-HPOs have much higher distribution coefficients compared to their 1-methyl substituted analogues (**1d** to **1e**; **1n** to **1o**; **1x** to **1y**). This trend also holds for the corresponding iron(III) complexes except for the pair **1x** and **1y**, where the log $D_{7.4}$ value of **1x** is 0.67 higher than that of **1y** in contrast to the value of its iron(III) complex, which is 0.74 lower than the counterpart. In comparison with 3-F analogues, 2-F-HPOs are slightly more hydrophobic. For example, the log $D_{7.4}$ value of **1d** is −0.21 whereas that of **1q** is −0.92. The same phenomenon is found by comparing **1e** with **1r**, **1f** with **1s**, and **1g** with **1t**. Among these compounds, **1l** and **1r** possess the highest and the lowest $D_{7.4}$ values, respectively, but do not form the most hydrophobic and hydrophilic iron(III) complexes, respectively. On the other hand, compounds containing similar D_{ligand} values do not necessarily mean that they have similar D_{complex} values (compare **1f** to **1y**). This observation is different from our previous report that a linear relationship exists between D_{ligand} and D_{complex}.[25] In most cases, iron(III) complexes are more hydrophilic than their corresponding free ligands. However, **1c**, which is in the hydrophilic class, possesses relatively hydrophobic property when chelating with iron.

Glucuronidation and Oxidation. One of the main problems for HPOs is that they undergo rapid metabolism in the liver and are glucuronidated at the 3-hydroxyl group, resulting in the formation of the nonchelating 3-*O*-glucuronide metabolite.[9] To understand the potential influence of fluorine on the metabolic stability of HPO, several F-HPO chelators were selected for incubation with guinea pig hepatic microsomes. Three were selected which contained a nonalkylated nitrogen atom (**1b**, **1d**, and **1x**) and three possessed an *N*-alkylated atom (**1e**, **1f**, and **1m**). The ratio of glucuronidated HPOs after 1 h incubation to the total added iron chelators was expressed as a percentage conversion. The glucuronidation rates of those F-HPO chelators and deferiprone are presented in Figure 1. **1d** and **1x**, where the N atom on the ring is

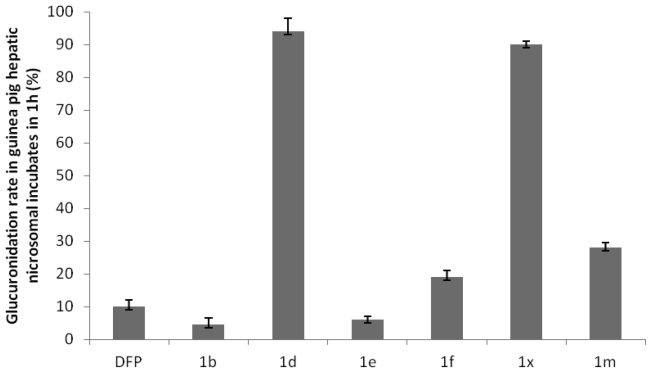


Figure 1. Comparison of the glucuronidation rates of a series of F-HPO chelators with deferiprone incubated for 1 h in guinea pig hepatic microsomes (mean ± SD) (n = 4 or 5).

unsubstituted, were almost completely metabolized after 1 h. In contrast, **1f** and **1m**, which have similar log *P* values, were only moderately metabolized under the same conditions, but more rapid than that of deferiprone. Interestingly, substitution of the ethyl group with methyl at N1 (compare **1e** to **1f**) or removing the methyl group at C5 (**1m** to **1e**) leads to increased metabolic stability (Figure 1). Substitution of hydrogen by a methyl group on the heterocyclic nitrogen (compare **1e** to **1d**) dramatically influences metabolic stability. To our surprise, compound **1b**, which has no substitution on N1, was demonstrated to possess a lower glucuronidation rate than deferiprone. This phenomenon may be attributed to the

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influence of 2-trifluoroacetyl group, where the presence of a strong intramolecular H-bond between the C=O at position 2 and 3-hydroxy group prevents the rapid metabolism of 3-hydroxyl group.

The two compounds with the lowest rates of glucuronidation (**1b** and **1e**) together with deferiprone were further investigated for phase 1 metabolism. Table 3 shows the relative resistance of

Table 3. Comparison of the Cytochrome P-450 Involved Oxidative Metabolism Rates of Deferiprone, 1b and 1e Incubated 1 h in Guinea Pig Hepatic Microsomes (Mean ± SD) (n = 5)

substrate	percentage of metabolite (%)
DFP	19.8 ± 1.5
1b	0.6 ± 2.4
1e	3.0 ± 2.0

1b and **1e** to microsomal oxidation as compared to deferiprone. In similar fashion to the glucuronidation result, both F-HPOs were found to possess lower rates of oxidative metabolism than that of deferiprone, indicating potential for improved maintenance of the drug level at a given dose. From this preliminary investigation, it was decided that with the exception of **1b**, *N*-alkylated hydroxypyridinones should be selected for BBB permeability measurements.

Blood–Brain Barrier (BBB) Permeability. Hydroxypyridinones for this study were selected on their basis: their affinity for iron(III) (pFe > 19.0), log *P* value (0 to −1), and with the exception of **1b**, the existence of an *N*-alkyl group. The range of log *P* values was chosen to minimize liver first pass extraction. By adopting these limits, the fluorinated HPOs were reduced to **1b**, **1e**–**1h**, and **1s**–**1u**. As **1e**–**1h** is closely related, we further selected **1f** and **1g** from this subgroup. The brain uptake values at steady-state for these selected F-HPOs and deferiprone are presented in Figure 2. Two F-HPOs (**1g** and **1t**) demonstrated

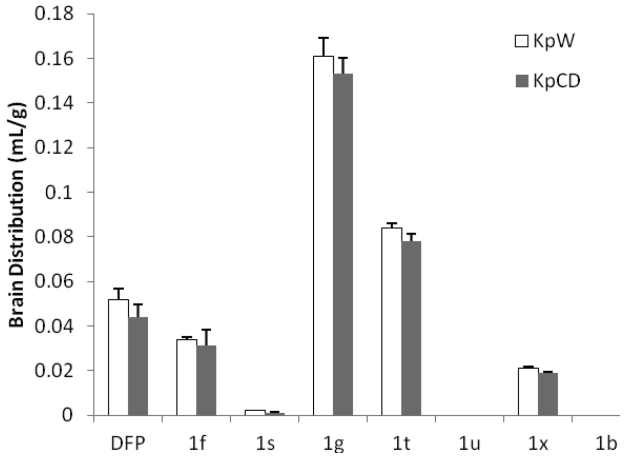

Figure 2. K_pW and K_pCD values of the selected F-HPOs compared to deferiprone. Values are mean ± SEM where n = 6–12.

higher K_p values than deferiprone. **1f** and **1x** are also able to permeate the BBB but with less efficiency than deferiprone. Three other F-HPOs, **1s**, **1u**, and **1b**, were found to possess poor BBB permeability. 2-F-HPOs have slightly more lipophilic character when compared to the 5-F analogues, and this may account for the corresponding higher K_p values (**1f** to **1s** and **1g** to **1t**). Additional methylene groups on the F-HPO also improve brain uptake, probably due to changes in lipophilicity (**1g** to **1f** and **1t** to **1s**). However, a poor permeability was observed for **1u**, an isomer of **1t**. This phenomenon may result from its increased hydrophilicity (log *P* value of −0.92 for **1u** compared to −0.46 for **1t**). The bulky nature of the isopropyl group may be another factor which influences the permeability. **1x** is more lipophilic than **1g**. However, its K_p value is lower than that of **1g**, which indicates that the lipophilicity is not the only determining factor for brain distribution. Although **1b** was demonstrated to be metabolically more stable than DFP, it failed to cross BBB. An objective of this study was to identify correlations between the brain distribution and log *P* values of F-HPOs. A clear correlation between the K_pCD values of the F-HPOs and their log *P* values were observed, when the data for **1x** is excluded from the study (Figure 3). **1x** is the only member

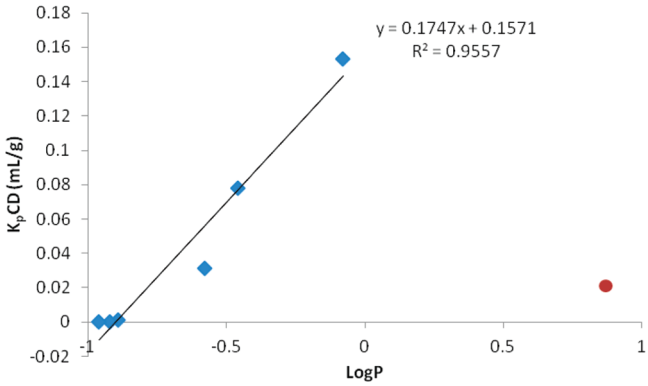

Figure 3. Correlation of *K*pCD values against log *P* of F-HPOs (**1b**, **1f**, **1g**, **1s**, **1t**, **1u**, and **1x**). *y*-Errors are excluded for simplicity. The trendline is drawn excluding the brown circle on the right.

of the group possessing a trifluoromethyl function and despite the relatively high log *P* value of the free ligand, its ability to permeate the BBB was extremely low.

CONCLUSION

The synthetic route of fluorinated HPOs adopted in this study is different from the conventional method for the HPO synthesis and offers a guideline for the synthesis of other functionalized 3-hydroxypyridin-4-ones where the functional groups are difficult to introduce onto the preformed 3-hydroxypyridin-4-one structure. This synthetic approach may be helpful to organic chemists working in the heterocyclic fluorine chemistry field. The physicochemical properties of F-HPOs demonstrate that the introduction of fluorine to 3-hydroxypyridin-4-ones markedly influences the pK_a, log β_3 values, and the distribution coefficients of both the free ligands and the iron complexes, when compared with nonfluorinated analogues. Selected F-HPOs were found to possess a range of metabolic stabilities, some being more stable than the clinically used deferiprone. Two of the F-HPOs, namely **1g** and **1t**, were found to possess superior brain uptake than deferiprone.

EXPERIMENTAL SECTION

General Method. 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

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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. Purity (≥95%) was determined via HPLC analysis.

3-Hydroxy-2-(2,2,2-trifluoro-1-hydroxyethyl)pyridin-4(1*H*)-one (1a). To a solution of 1-(3,4-bis(benzyloxy)pyridin-2-yl)-2,2,2-trifluoroethanol **3** (1 g) in methanol (15 mL) was added a catalytic amount of Pd/C (5%, 0.1 g) and 1 mL of concentrated HCl. The reaction was hydrogenated at room temperature and 2 atm for 3 h. Then the catalyst was filtered off through Celite, and the residue was concentrated under reduced pressure to afford the title compound as an off-white solid. Recrystallization from EtOH/acetone gave a white solid. Yield 88%. ^{1}H NMR (DMSO-d_6): δ 8.16 (d, J = 6.5 Hz, 1H), 7.48 (d, J = 6.5 Hz, 1H), 5.77 (q, J = 6.6 Hz, 1H), 4.30 (br s, 3H). ^{19}F NMR: −72.24 (d, J = 6.6 Hz, 3F). ^{13}C NMR: 63.58 (q, J = 32 Hz, CHOH), 112.06 (CH-5), 123.90 (q, J = 282 Hz, CF_3), 132.99 (C-2), 135.80 (CH-6), 143.38 (C-3), 161.63 (CO-4). HRMS: calcd for $C_7H_7NO_3F_3$ $(M + 1)^+$, 210.0378; found, 210.0382.

General Procedure for the Preparation of 1b−q and 1v−1z. Methyl or ethyl protecting 3,4-dihydroxypyridine or 3-hydroxy-1-alkylpyridin-4-one was dissolved into CH_2Cl_2 (20 mL) and flushed with nitrogen at −5 °C. Boron trichloride or boron tribromide (1 M in CH_2Cl_2, 8 mL) was slowly added, and the reaction mixture was stirred at room temperature for 20 h. The excess BCl_3/BBr_3 was eliminated at the end of the reaction by the addition of methanol (10 mL) and left to stir for another half an hour. After removal of the solvents under reduced pressure, the residues were purified by recrystallization to afford white solids.

*3-Hydroxy-2-(2,2,2-trifluoroacetyl)pyridin-4(1H)-one Hydrochloride (**1b**).* Yield: 70%. ^{1}H NMR (DMSO-d_6): δ 8.17 (d, J = 6.4 Hz, 1H), 7.46 (d, J = 6.4 Hz, 1H), 4.08 (br s, 2H). ^{19}F NMR: −78.54 (s, 3F). ^{13}C NMR: 113.37 (CH-5), 121.70 (q, J = 155 Hz, CF_3), 131.37 (C-2), 134.53 (CH-6), 145.45 (C-3), 163.31 (CO-4), 168.68 ($COCF_3$). HRMS: calcd for $C_7H_5NO_3F_3$ $(M + 1)^+$, 208.0222; found, 208.0219.

*2-(2,2,2-Trifluoroacetyl)-3-hydroxy-1,6-dimethylpyridin-4(1H)-one Hydrochloride (**1c**).* Yield: 65%. ^{1}H NMR (DMSO-d_6) δ 8.73 (brs, 1H, OH), 7.28 (s, 1H, C-5H), 4.18 (s, 3H, NCH_3), 2.62 (CH_3). ^{19}F NMR: −81.77 (d, J = 7.4 Hz). ^{13}C NMR: 21.13 (s, CH_3), 41.48 (s, J = 20 Hz, NCH_3), 113.58 (s, CH-5), 122.20 (d, J = 306 Hz, CF_3), 134.54 (s, C-2), 145.17 (s, C-3), 150.91 (s, C-6), 160.93 (s, C-4), 214.00 (s, $COCF_3$). HRMS: calcd for $C_9H_9NO_3F_3$ $(M + 1)^+$, 236.0535; found, 236.0539.

*2-Fluoro-3-hydroxypyridin-4(1H)-one Hydrobromide (**1d**).* Yield: 60%. ^{1}H NMR (CD_3OD): δ 7.41 (d, J = 5.5 Hz, 1H, C-6H), 6.72 (d, J = 5.5 Hz, 1H, C-5H)), 4.94 (brs, 2H, OH and NH). ^{19}F NMR (CD_3OD): −85.48 (s). ^{13}C NMR (CD_3OD): 111.67 (d, J = 3 Hz, C-5), 129.35 (d, J = 29 Hz, C-3), 137.17 (d, J = 16 Hz, C-6), 155.75 (d, J = 228 Hz, C-2), 157.58 (d, J = 9 Hz, C-4). HRMS: calcd for $C_5H_5NO_2F$ $(M + 1)^+$, 130.0304; found, 130.0314.

*2-Fluoro-3-hydroxy-1-methylpyridin-4(1H)-one Hydrobromide (**1e**).* Yield: 66%. ^{1}H NMR (CD_3OD): δ 8.06 (dd, J = 5.1, 7.1 Hz, 1H, C-6H), 7.13 (dd, J = 0.8 7.1 Hz, 1H, C-5H), 4.96 (brs, 1H, OH), 4.09 (d, J = 3.8 Hz, 3H, CH_3). ^{19}F NMR (CD_3OD): −102.49 (s). ^{13}C NMR (CD_3OD): 40.70 (d, J = 6 Hz, CH_3), 111.39 (s, C-5), 133.03 (d, J = 12 Hz, C-3), 136.10 (d, J = 5 Hz, C-6), 152.32 (d, J = 265 Hz, C-2), 165.05 (d, J = 10 Hz, C-4). HRMS: calcd for $C_6H_7NO_2F$ $(M + 1)^+$, 144.0461; found, 144.0460.

*1-Ethyl-2-fluoro-3-hydroxypyridin-4(1H)-one Hydrobromide (**1f**).* Yield: 59%. ^{1}H NMR (DMSO-d_6): δ 8.17 (dd, J = 5.3, 7.1 Hz, 1H, C-6H), 7.11 (d, J = 7.1 Hz, 1H, C-5H), 4.37 (dq, J = 2.5, 7.2 Hz, 2H, CH_2), 1.41 (t, J = 7.2 Hz, 3H, CH_3). ^{19}F NMR (DMSO-d_6): −106.84 (s). ^{13}C NMR (DMSO-d_6): 14.81 (s, CH_3), 48.69 (d, J = 4 Hz, CH_2), 110.50 (s, C-5), 131.15 (d, J = 12 Hz, C-3), 133.60 (d, J = 5 Hz, C-6), 149.98 (d, J = 264 Hz, C-2), 163.89 (d, J = 11 Hz, C-4). HRMS: calcd for $C_7H_9NO_2F$ $(M + 1)^+$, 158.0617; found, 158.0622.

*2-Fluoro-3-hydroxy-1-propylpyridin-4(1H)-one Hydrobromide (**1g**).* Yield: 70%. ^{1}H NMR (DMSO-d_6): δ 8.14 (dd, J = 5.3, 7.1 Hz, 1H, C-6H), 7.09 (d, J = 7.1 Hz, 1H, C-5H), 4.30 (t, J = 7.1 Hz, 2H, CH_2), 1.85−1.78 (m, 2H, CH_2), 0.89 (t, J = 7.3 Hz, 3H, CH_3). ^{19}F NMR: −107.45 (s). ^{13}C NMR: 10.31 (s, CH_3), 22.54 (s, CH_2), 54.31 (d, J = 2 Hz, CH_2), 110.47 (s, CH-5), 131.19 (d, J = 11 Hz, C-3), 134.02 (d, J = 4 Hz, CH-6), 149.88 (d, J = 264 Hz, C-2), 164.42 (d, J = 10 Hz, C-4). HRMS: calcd for $C_8H_{11}NO_2F$ $(M + 1)^+$, 172.0774; found, 172.0777.

*2-Fluoro-3-hydroxy-1-isopropylpyridin-4(1H)-one Hydrobromide (**1h**).* Yield: 60%. ^{1}H NMR (DMSO-d_6): δ 8.25 (dd, J = 5.1, 7.2 Hz, 1H, C-6H), 7.13 (d, J = 7.2 Hz, 1H, C-5H), 5.13−4.93 (m, 1H, CH), 4.70 (brs, OH), 1.53 (d, J = 6.7 Hz, 6H, $2CH_3$). ^{19}F NMR: −105.59. ^{13}C NMR: 21.32 (s, CH_3), 55.77 (d, J = 5 Hz, CH), 110.64 (s, C-5H), 131.09 (d, J = 13 Hz, C-3), 143.76 (d, J = 22 Hz, CH-6), 149.90 (d, J = 272 Hz, C-2), 163.01 (d, J = 11 Hz, C-4). HRMS: calcd for $C_8H_{11}NO_2F$ $(M + 1)^+$, 172.0774; found, 172.0774.

*1-Butyl-2-fluoro-3-hydroxypyridin-4(1H)-one Hydrobromide (**1i**).* Yield: 64%. ^{1}H NMR (DMSO-d_6): δ 8.18 (d, J = 5.4, 7.0 Hz, 1H, C-6H), 7.12 (d, J = 7.1 Hz, 1H, C-5H), 4.34 (t, J = 7.1 Hz, 2H, CH_2), 1.80−1.72 (m, 2H, CH_2), 1.35−1.26 (m, 2H, CH_2), 0.91 (t, J = 7.4 Hz, 3H, CH_3). ^{19}F NMR: −107.11 (s). ^{13}C NMR: 13.30 (s, CH_3), 18.79 (s, CH_2), 31.06 (s, CH_2), 52.81 (d, J = 3 Hz, CH_2), 110.46 (s, CH-5), 131.20 (d, J = 11 Hz, C-3), 134.01 (d, J = 4 Hz, CH-6), 149.91 (d, J = 264 Hz, C-2), 164.25 (d, J = 10 Hz, C-4). HRMS: calcd for $C_9H_{13}NO_2F$ $(M + 1)^+$, 186.0930; found, 186.0925.

*2-Fluoro-3-hydroxy-6-methyl-pyridin-4(1H)-one Hydrobromide (**1j**).* Yield: 78%. ^{1}H NMR (DMSO-d_6): δ 6.61 (s, 1H, C-5H), 5.99 (brs, OH and NH), 2.21 (s, 3H, CH_3). ^{19}F NMR (DMSO-d_6): −90.71 (s). ^{13}C NMR (DMSO-d_6): 22.23 (s, CH_3), 109.50 (d, J = 3 Hz, C-5), 125.04 (d, J = 29 Hz, C-3), 144.52 (d, J = 13 Hz, C-6), 152.92 (d, J = 227 Hz, C-2), 156.26 (d, J = 8 Hz, C-4). HRMS: calcd for $C_6H_7NO_2F$ $(M + 1)^+$, 144.0461; found, 144.0463.

*2-Fluoro-3-hydroxy-5-methyl-pyridin-4(1H)-one Hydrobromide (**1k**).* Yield: 74%. ^{1}H NMR (DMSO-d_6): δ 7.59 (brs, OH and NH), 7.36 (s, 1H, C-6H), 2.07 (s, 3H, CH_3). ^{19}F NMR (DMSO-d_6): −91.60 (s). ^{13}C NMR (DMSO-d_6): 12.53 (s, CH_3), 119.84 (d, J = 3 Hz, C-5), 126.56 (d, J = 29 Hz, C-3), 135.54 (d, J = 16 Hz, C-6), 152.69 (d, J = 224 Hz, C-2), 154.12 (C-4). HRMS: calcd for $C_6H_7NO_2F$ $(M + 1)^+$, 144.0461; found, 144.0478.

*2-Fluoro-3-hydroxy-5,6-dimethyl-pyridin-4(1H)-one Hydrobromide (**1l**).* Yield: 75%. ^{1}H NMR (DMSO-d_6): δ 6.23 (brs, OH and NH), 2.23 (s, 3H, 6-CH_3), 2.02 (s, 3H, 5-CH_3). ^{19}F NMR (DMSO-d_6): −94.63 (s). ^{13}C NMR (DMSO-d_6): 11.03 (s, 5-CH_3), 20.43 (s, 6-CH_3), 117.10 (d, J = 4 Hz, C-5), 124.59 (d, J = 29 Hz, C-3), 142.63 (d, J = 13 Hz, C-6), 150.60 (d, J = 227 Hz, C-2), 155.10 (d, J = 8 Hz, C-4). HRMS: calcd for $C_7H_9NO_2F$ $(M + 1)^+$, 158.0617; found, 158.0609.

*2-Fluoro-3-hydroxy-1,5-dimethyl-pyridin-4(1H)-one Hydrobromide (**1m**).* Yield: 79%. ^{1}H NMR (DMSO-d_6): δ 8.19 (d, J = 5.4 Hz, 1H, C-6H), 6.15 (brs, 1H, OH), 3.95 (d, J = 3.7 Hz, 3H, 1-CH_3), 2.14 (s, 3H, 5-CH_3). ^{19}F NMR (DMSO-d_6): −105.50 (s). ^{13}C NMR (DMSO-d_6): 12.60 (s, 5-CH_3), 39.48 (d, J = 5 Hz, 1-CH_3), 120.41 (s, C-5), 129.51 (d, J = 13 Hz, C-3), 133.68 (d, J = 5 Hz, C-6), 149.57 (d, J = 263 Hz, C-2), 162.73 (d, J = 9 Hz, C-4). HRMS: calcd for $C_7H_9NO_2F$ $(M + 1)^+$, 158.0617; found, 158.0627.

*5-Fluoro-3-hydroxy-2-methylpyridin-4(1H)-one Hydrobromide (**1n**).* Yield: 69%. ^{1}H NMR (DMSO-d_6): δ 8.55 (d, J = 5.0 Hz, 1H, C-6H), 4.54 (brs, OH and NH), 2.54 (s, 3H, CH_3). ^{19}F NMR (DMSO-d_6): −148.82 (s). ^{13}C NMR (DMSO-d_6): 14.04 (s, CH_3), 121.88 (d, J = 32 Hz, C-6), 137.31 (s, C-2), 144.11 (d, J = 7 Hz, C-3), 148.73 (d, J = 238 Hz, C-5), 150.48 (d, J = 12 Hz, C-4). HRMS: calcd for $C_6H_7NO_2F$ $(M + 1)^+$, 144.0461; found, 144.0468.

*5-Fluoro-3-hydroxy-1,2-dimethyl-pyridin-4(1H)-one Hydrobromide (**1o**).* Yield: 62%. ^{1}H NMR (DMSO-d_6): δ 8.72 (d, J = 6.1 Hz, 1H, C-6H), 4.89 (brs, OH), 3.99 (s, 3H, 1-CH_3), 2.47 (s, 3H, 2-CH_3). ^{19}F NMR (DMSO-d_6): −149.61 (d, J = 6.1 Hz). ^{13}C NMR (DMSO-d_6): 12.70 (s, 2-CH_3), 43.91 (s, 1-CH_3), 127.24 (d, J = 35 Hz, C-6), 139.28 (s, C-2), 144.58 (d, J = 7 Hz, C-3), 147.60 (d, J = 237 Hz, C-5), 149.80 (d, J = 13 Hz, C-4). HRMS: calcd for $C_7H_9NO_2F$ $(M + 1)^+$, 158.0617; found, 158.0617.

*3-Fluoro-5-hydroxy-2-methylpyridin-4(1H)-one Hydrobromide (**1p**).* Yield: 72%. ^{1}H NMR (DMSO-d_6): δ 8.04 (d, J = 0.5 Hz, 1H, C-6H), 4.30 (brs, OH and NH), 2.64 (d, J = 2.8 Hz, 3H, CH_3). ^{19}F NMR (DMSO-d_6): −146.32 (s). ^{13}C NMR (DMSO-d_6): 12.67 (s,

CH_3), 123.02 (s, C-6), 134.77 (d, J = 27 Hz, C-2), 145.25 (d, J = 7 Hz, C-5), 147.32 (d, J = 238 Hz, C-3), 150.60 (d, J = 11 Hz, C-4). HRMS: calcd for $C_6H_7NO_2F$ $(M + 1)^+$, 144.0461; found, 144.0468.

*3-Fluoro-5-hydroxypyridin-4(1H)-one Hydrobromide (**1q**).* Yield: 65%. ^{1}H NMR (DMSO-d_6): δ 8.64 (dd, J = 1.0, 4.9 Hz, 1H, C-2H), 8.12 (s, 1H, C-6H). ^{19}F NMR (DMSO-d_6): −146.96 (s). ^{13}C NMR (DMSO-d_6): 123.91 (d, J = 33 Hz, C-2), 124.66 (s, C-6), 146.93 (d, J = 8 Hz, C-5), 149.50 (d, J = 240 Hz, C-3), 151.58 (d, J = 12 Hz, C-4). HRMS: calcd for $C_5H_5NO_2F$ $(M + 1)^+$, 130.0304; found, 130.0301.

*2,3,6-Trifluoro-5-hydroxypyridin-4(1H)-one Hydrochloride (**1v**).* Yyield: 72%. ^{19}F NMR (DMSO-d_6): δ −93.30 (dd, J = 18.8, 22.6 Hz, 1F), −100.84 (dd, J = 18.8, 26.3 Hz, 1F), −166.00 (dd, J = 22.6, 26.3 Hz, 1F). ^{13}C NMR: 127.06 (d, J = 31 Hz, C-3), 135.07 (dd, J = 18, 241 Hz, C-5), 140.40 (dd, J = 14, 224 Hz, C-2), 144.28 (dd, J = 15, 230 Hz, C-6), 147.78 (s, C-4). HRMS: calcd for $C_5H_3NO_2F_3$ $(M + 1)^+$, 166.0116; found, 166.0139.

*2,6-Difluoro-3-hydroxypyridin-4(1H)-one Hydrobromide (**1w**).* Yield: 67%. ^{1}H NMR (DMSO-d_6): δ 10.81 (br s, 1H), 8.93 (br s, 1H), 6.31 (d, J = 0.5 Hz, 1H). ^{19}F NMR: −79.37 (d, J = 15.7 Hz, 1F), −90.59 (d, J = 15.7 Hz, 1F). ^{13}C NMR: 96.13 (dd, J = 4.5, 40.5 Hz, CH-5), 127.60 (dd, J = 6.5, 27.5 Hz, C-3), 152.80 (dd, J = 19, 232 Hz, C-2), 155.39 (dd, J = 18, 231 Hz, C-6), 161.74 (dd, J = 8, 12 Hz, CO-4). HRMS: calcd for $C_5H_4NO_2F_2$ $(M + 1)^+$, 148.0210; found, 148.0236.

*2-(Trifluoromethyl)-3-hydroxypyridin-4(1H)-one Hydrochloride (**1x**).* Yield: 76%. ^{1}H NMR (DMSO-d_6): δ 8.52 (br s), 7.99 (d, J = 5.6 Hz, 1H), 7.29 (d, J = 5.6 Hz, 1H). ^{19}F NMR: −63.49 (s, 3F). ^{13}C NMR: 113.72 (s, CH-5), 121.58 (q, J = 273 Hz, CF_3), 129.34 (q, J = 33 Hz, C-2), 138.83 (s, CH-6), 143.55 (s, C-3), 157.63 (s, CO-4). HRMS: calcd for $C_6H_5NO_2F_3$ $(M + 1)^+$, 180.0274; found, 180.0275.

*3-Hydroxy-1-methyl-2-trifluoromethyl-1H-pyridin-4-one Hydrobromide (**1y**).* Yield: 52%. ^{1}H NMR (DMSO-d_6): δ 8.84 (d, J = 6.8 Hz, 1H), 7.68 (d, J = 6.7 Hz, 1H), 4.24 (d, J = 1.1 Hz, 3H). ^{19}F NMR: −57.37 (s, 3F). ^{13}C NMR: 45.77 (d, J = 4 Hz, CH_3), 108.09 (s, CH-5), 119.35 (q, J = 37 Hz, C-2), 119.76 (q, J = 274 Hz, CF_3), 146.98 (s, CH-6), 150.09 (s, C-3), 164.85 (s, CO-4). HRMS: calcd for $C_7H_7NO_2F_3$ $(M + 1)^+$, 194.0459; found, 194.0443.

*Methyl 3-Hydroxy-5-methyl-4-oxo-1,4-dihydropyridine-2-carboxylate Hydrobromide (**1z**).* Yield: 78%. ^{1}H NMR (DMSO-d_6): δ 8.15 (s, 1H), 5.65 (brs), 4.07 (s, 3H), 2.28 (s, 3H). ^{13}C NMR: 13.42 (CH_3), 53.70 (OCH_3), 121.36 (C-5), 124.12 (C-2), 136.87 (C-6), 146.79 (C-3), 162.32 (CO-4), 164.06 (2-COO). HRMS: calcd for $C_8H_{10}NO_4$ $(M + 1)^+$, 184.0610; found, 184.0606.

General Procedure for Preparation of 1r−u. A solution of **30** (5 mmol) in acetone (30 mL) was added alkyl iodide (10 mmol), and the mixture was heated at 60 °C overnight. The solvent was then evaporated and the residue was recrystallized to afford white solids.

*3-Fluoro-5-hydroxy-1-methylpyridin-4(1H)-one (**1r**).* Yield: 86%. ^{1}H NMR (DMSO-d_6): δ 8.68 (d, J = 5.2 Hz, 1H, C-2H), 8.08 (s, 1H, C-6H), 5.20 (brs, 1H, OH), 4.04 (s, 3H, CH_3). ^{19}F NMR (DMSO-d_6): −147.39 (s). ^{13}C NMR (DMSO-d_6): 45.95 (s, CH_3), 127.81 (d, J = 35 Hz, C-2), 128.53 (s, C-6), 146.91 (d, J = 8 Hz, C-5), 148.96 (d, J = 238 Hz, C-3), 150.94 (d, J = 12 Hz, C-4). HRMS: calcd for $C_6H_7NO_2F$ $(M + 1)^+$, 144.0461; found, 144.0465.

*1-Ethyl-3-fluoro-5-hydroxypyridin-4(1H)-one (**1s**).* Yield: 82%. ^{1}H NMR (DMSO-d_6): δ 8.04 (d, J = 6.0 Hz, 1H, C-2H), 7.57 (s, 1H, C-6H), 4.62 (brs, OH), 3.93 (q, J = 7.1 Hz, 2H, CH_2), 1.32 (t, J = 7.1 Hz, 3H, CH_3). ^{19}F NMR (DMSO-d_6): −156.02 (d, J = 6.0 Hz). ^{13}C NMR (DMSO-d_6): 15.98 (s, CH_3), 51.51 (s, CH_2), 121.69 (s, C-6), 124.36 (d, J = 35 Hz, C-2), 149.35 (d, J = 15 Hz, C-5), 150.63 (d, J = 233 Hz, C-3), 160.66 (d, J = 13 Hz, C-4). HRMS: calcd for $C_7H_9NO_2F$ $(M + 1)^+$, 158.0617; found, 158.0609.

*3-Fluoro-5-hydroxy-1-propylpyridin-4(1H)-one (**1t**).* Yield: 70%; refluxed for 3 days. ^{1}H NMR (DMSO-d_6): δ 8.03 (dd, J = 2.1, 7.1 Hz, 1H, C-2H), 7.53 (d, J = 2.0 Hz, 1H, C-6H), 3.85 (t, J = 7.1 Hz, 2H, CH_2), 1.77−1.68 (m, 2H, CH_2), 0.81 (t, J = 7.4 Hz, 3H, CH_3). ^{19}F NMR: −156.36 (d, J = 7.4 Hz). ^{13}C NMR: 10.29 (s, CH_3), 23.46 (s, CH_2), 57.62 (s, CH_2), 121.95 (s, CH-6), 124.73 (d, J = 35 Hz, CH-2), 149.23 (d, J = 13 Hz, C-5), 150.51 (d, J = 233 Hz, C-3), 160.75 (d, J = 13 Hz, C-4). HRMS: calcd for $C_8H_{11}NO_2F$ $(M + 1)^+$, 172.0774; found, 172.0769.

*3-Fluoro-5-hydroxy-1-isopropylpyridin-4(1H)-one (**1u**).* Yield: 65%; refluxed in 2-iodopropane only. ^{1}H NMR (DMSO-d_6): δ 8.45 (dd, J = 2.0, 6.7 Hz, 1H, C-2H), 7.91 (d, J = 1.9 Hz, C-6H), 4.53−4.46 (m, 1H, CH), 1.44 (d, J = 6.7 Hz, 6H, $2CH_3$). ^{19}F NMR: −150.74 (s). ^{13}C NMR: 22.06 (s, CH_3), 60.13 (s, CH), 123.01 (s, C-6), 123.65 (d, J = 34 Hz, C-2), 148.24 (d, J = 10 Hz, C-5), 150.11 (d, J = 237 Hz, C-3), 155.79 (d, J = 13 Hz, C-4). HRMS: calcd for $C_8H_{11}NO_2F$ $(M + 1)^+$, 172.0774; found, 172.0768.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Details of the synthesis, physicochemical property, metabolism and blood−brain barrier studies, the relationship between log D_{ligand} and log $D_{iron\ complex}$ of the fluorinated HPOs. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

DFP, deferiprone; HPO, 3-hydroxypyridin-4-one; CP94, 1,2-diethyl-3-hydroxypyridin-4-one; LDA, lithium diisopropylamide; LTMP, lithium 2,2,6,6-tetramethylpiperidine; USB, unimetal superbase; BBB, blood−brain barrier

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