Bioorganic & Medicinal Chemistry 17 (2009) 6106–6122

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Discovery of pyridone-containing imidazolines as potent and selective inhibitors of neuropeptide Y Y5 receptor

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ARTICLE INFO

Article history:
Received 20 April 2009
Revised 27 May 2009
Accepted 28 May 2009
Available online 2 June 2009

Keywords:
Neuropeptide Y Y5 receptor
Anti-obesity
Y5 antagonist
Imidazoline class

ABSTRACT

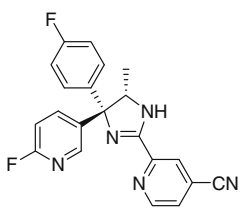
A series of 2-pyridone-containing imidazoline derivatives was synthesized and evaluated as neuropeptide Y Y5 receptor antagonists. Optimization of the 2-pyridone structure on the 2-position of the imidazoline ring led to identification of 1-(difluoromethyl)-5-[(4*S*,5*S*)-4-(4-fluorophenyl)-4-(6-fluoropyridin-3-yl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]pyridin-2(1*H*)-one (**7m**). Compound **7m** displayed statistically significant inhibition of food intake in an agonist-induced food intake model in SD rats and no adverse cardiovascular effects in anesthetized dogs. In addition, markedly higher brain penetrability and a lower plasma Occ90 value were observed in P-gp-deficient *mdr1a* (−/−) mice compared to *mdr1a* (+/+) mice after oral administration of **7m**.

1. Introduction

Neuropeptide Y (NPY) is a highly conserved 36 amino acid peptide neurotransmitter that was discovered in 1982 as a member of the pancreatic polypeptide family, along with peptide YY, a pancreatic polypeptide.[1] NPY is widely distributed in both the central and the peripheral nervous systems and has various physiological functions. The concentration of NPY and its mRNA level in the hypothalamus are markedly increased during food deprivation and in some genetic models of obesity in rodents.[2–6] Chronic central infusion of NPY in rodents results in a syndrome similar to that seen in some genetic obesity models, which is characterized by hyperphagia, insulin resistance, hyperinsulinemia, and reduced thermogenic activity in brown adipose tissue.[7] Furthermore, NPY-deficient ob/ob mice are less obese and have reduced food intake compared with ob/ob mice.[8] Thus, NPY is thought to play a major role in the physiological control of energy homeostasis and food intake.

Five distinct NPY receptor subtypes (Y1, Y2, Y4, Y5, and mouse Y6) have been cloned to date,[9] and pharmacological data suggest that the NPY Y5 receptor (Y5) is involved in feeding regulation and energy expenditure. Administration of Y5 antagonists suppresses Y5 agonist-induced food intake and diet-induced body weight gain,[10,11] and mice lacking Y5 show a reduced response to exogenously administered Y5 agonists.[12] In addition, chronic intracerebroventricular administration of the Y5-specific agonist D-Trp^{34}NPY produces obesity in rodents.[13] These results suggest that antagonism of Y5 may have considerable therapeutic benefits in treating obesity. Thus, Y5 antagonists have been studied by many pharmaceutical companies as potential anti-obesity drugs.[14] Recently, it was reported that administration of a potent and highly selective Y5 antagonist, MK-557, resulted in modest weight loss in obese human subjects.[15]

We previously reported the identification of a potent imidazoline class of Y5 antagonists **1** (Fig. 1),[16] which significantly reduced body weight of established diet-induced obesity (DIO) mice both at 3 and 10 mg/kg oral doses. However, intravenous administration of 5 mg/kg of **1** produced significant cardiotonic effects and moderate



1
hY5 *Ki*: 1.3 nM

Figure 1. Structure and Y5 binding activity of compound **1**.

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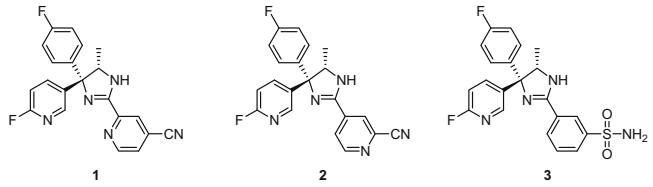
doi:10.1016/j.bmc.2009.05.069

Table 1
Effects of imidazolines on cardiovascular function in anesthetized rats[a]



Compd	LV dp/dt_{max}[b] (%)	MAP[b] (%)	Plasma level (μM)
1	18	21	6.3
2	15	−6	4.3
3[c]	20	7	4.9
4	30	19	5.1
5a	5	−15	5.3
6	−3	−19	2.6

[a] The values represent the mean for $n = 2-3$ at 3 min after 5 mg/kg iv injection of test compounds.
[b] LV dp/dt_{max} and MAP values are shown as the percent change from baseline.
[c] hY5 *Ki* = 1.7 ± 0.2 nM.

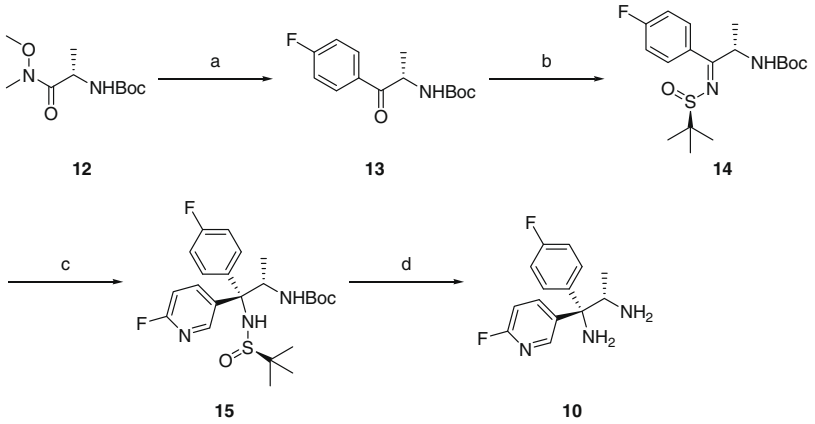
QTc prolongation in anesthetized dogs, even though compound **1** displayed excellent selectivity with respect to a panel of 167 diverse, unrelated binding sites ($IC_{50} > 1$ μM for all the binding sites tested). Therefore, we screened imidazolines with various functional groups substituted on the 2-position of the imidazoline ring using an anesthetized rat cardiovascular assay to identify imidazoline analogs with a safe cardiovascular profile (Table 1). Intravenous administration of **1** (5 mg/kg) to rats resulted in an increase in left ventricular cardiac contractility (LV dp/dt_{max}) and mean arterial pressure (MAP) at a plasma concentration of 6.3 μM, similar to those observed in anesthetized dogs.[16] 2-Cyanopyridine **2** and benzenesulfonamide **3** did not show a significant change in MAP, while increases in LV dp/dt_{max} similar to **1** were observed. Pyrazine **4** produced comparable hemodynamic effects at the same plasma drug levels to **1**. Pyridone **5a** produced a nonsignificant change in LV dp/dt_{max} and a slight decline in MAP at plasma levels of 5.3 μM. The related compound **6** with a bis(4-fluorophenyl) group had a comparable cardiovascular profile to **5a**, suggesting that the pyridone structure might prevent cardiovascular toxicities in this class. Therefore, we focused on optimization of a pyridone on the 2-position of the imidazoline ring. Synthesis, structure–activity relationships (SAR) of this series of Y5 antagonists, and identification of a novel Y5 antagonist **7m** with favorable cardiovascular safety profiles are described.

2. Chemistry

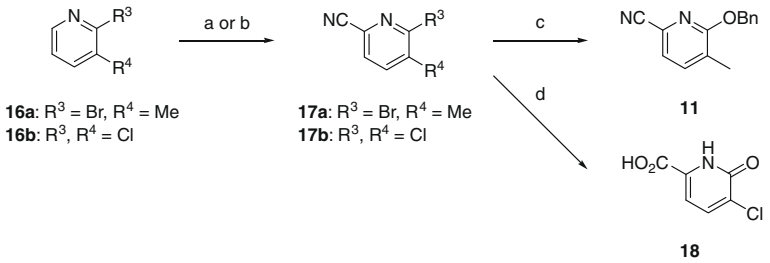
The synthetic route for intermediates, which are not commercially available, is described in Schemes 1–7. Diastereoselective synthesis of the diamine intermediate **10** was performed using (*R*)-*tert*-butyl sulfinamide, as illustrated in Scheme 1.[17] Weinreb amide **12** was converted to ketone **13**, which was thermally condensed with (*R*)-*tert*-butylsulfinamide in the presence of titanium tetraethoxide to afford ketimine **14**. Ketimine **14** was reacted with (6-fluoropyridin-3-yl)lithium in the presence of trimethylaluminum to give adduct **15** as a single diastereomer. Deprotection of the Boc and *tert*-butylsulfinyl groups was achieved under acidic conditions to furnish the key compound **10**. The stereochemistry of quaternary chiral center in **10** was determined through ^{1}H NMR comparison between diamine **10** and the authentic sample.[16]

Intermediates **11** and **18** were prepared from a 2,3-disubstituted pyridine (Scheme 2). Pyridines **16a** and **16b** were converted to the corresponding cyanopyridines **17a** and **17b** by reaction conditions a or b.[18,19] The bromine atom of **17a** was displaced with benzyl alcohol to give benzyl ether **11**. Compound **17b** was converted to the desired carboxylic acid **18** by hydrolysis with 10 N sodium hydroxide. Synthesis of compound **21** is shown in Scheme 3. 2-Cyano-5-fluoropyridine (**19**) was oxidized with urea hydrogen peroxide addition compound (UHP) and trifluoroacetic anhydride (TFAA) to give the corresponding N-oxide, which was treated with acetic anhydride to afford acetoxy compound **20**.[18,20] The nitrile and acetoxy groups of compound **20** were hydrolyzed under acidic conditions to furnish pyridone carboxylic acid **21**. Compounds **24a–c** were prepared from pyridones **22a** and **22b** as shown in Scheme 4. N-Alkylation of pyridones **22a** and **22b** followed by hydrolysis of the ester afforded carboxylic acids **24a–c**.[21] The known *N*-difluoromethyl pyridone **23d**[22] was converted to the corresponding carboxylic acid **24d** in the same manner.

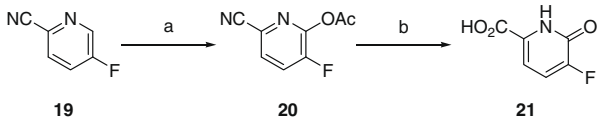
Preparation of substituted pyridones **27a**, **27b**, **30**, **31**, **34a**, and **34b** is described in Scheme 5. Bromination of 3-substituted pyridones **25a** and **25b** afforded the 5-bromo compounds **26a** and **26b**. Compound **26b** and commercially available reagent **26c** were reacted with *n*-BuLi followed by addition of CO_2 gas to give carboxylic acids **27a** and **27b**. 3-Trifluoromethylpyridone **26a** was treated



Scheme 1. Reagents and conditions: (a) 4-fluorophenylmagnesium bromide, THF, 0 °C to rt; (b) (*R*)-2-methylpropane-2-sulfinamide, $Ti(OEt)_4$, toluene, 70 °C; (c) (i) 5-bromo-2-fluoropyridine, *n*-BuLi, Et_2O, −78 °C, (ii) $AlMe_3$, **14**, −78 °C; (d) (i) TFA, rt, (ii) 8 N HCl, 1,4-dioxane, 0 °C to rt.



Scheme 2. Reagents and conditions: (a) (i) *m*CPBA, $CHCl_3$, rt, (ii) CH_3OTf, CH_2Cl_2, 0 °C to rt, then NaCN, 0 °C to rt; (b) (i) UHP, TFAA, CH_2Cl_2, 0 °C to rt; (ii) $(CH_3)_2SO_4$, 90 °C, then NaCN, H_2O, 0 °C; (c) NaH, BnOH, THF, reflux; (d) 10 N KOH, reflux.

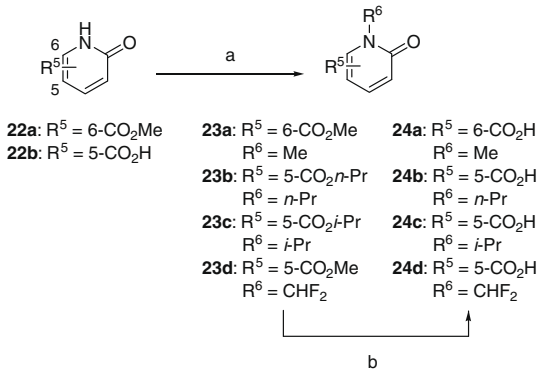


Scheme 3. Reagents and conditions: (a) (i) UHP, TFAA, CH_2Cl_2, 0 °C to rt; (ii) Ac_2O, reflux; (b) 6 N HCl, reflux.

with phenylphosphonic dichloride followed by coupling with *p*-methoxybenzyl alcohol to produce benzyl ether **28a**. The known compound **26d** was alkylated with benzyl bromide in the presence of silver carbonate to give benzyl ether **28b**. Carbonylation of **28a** and **28b** was achieved in the presence of a catalytic amount of palladium diacetate and 1,1′-bis(diphenylphosphino)ferrocene to give the corresponding *n*-propyl esters **29a** and **29b**. Removal of the *p*-methoxybenzyl group of **29a** followed by hydrolysis of the ester afforded 2-pyridone carboxylic acid **30**. Hydrolysis of **29b** furnished pyridine carboxylic acid **31**. Alkylation of 3-methylpyridone **26c** with ethyl bromide[21] or sodium chlorodifluoroacetate followed by carbonylation of the resulting N-substituted 3-methylpyridones afforded the *n*-propyl esters **33a** and **33b**, which were hydrolyzed under basic conditions, affording carboxylic acids **34a** and **34b**.

3-Fluoropyridone **37** and *N*-alkoxypyridones **39a** and **39b** were prepared according to Scheme 6. Pyridine methyl esters **35a** and **35b** were converted to the corresponding N-oxides **36a** and **36b** using UHP and TFAA.[18] Compound **36a** was heated with acetic anhydride followed by hydrolysis with 2 N sodium hydroxide to give the desired carboxylic acid **37**. Compound **36b** was reacted with TFAA to provide *N*-hydroxy-2-pyridone **38**, which was alkylated with methyl iodide or ethyl bromide followed by hydrolysis to produce carboxylic acids **39a** and **39b**.

The synthesis of 2-fluoropyridine carboxylic acid **42** is illustrated in Scheme 7. The methoxy group of compound **40**[23] was removed using sodium iodide and trimethylsilyl chloride to afford 2-fluoro-6-pyridone **41**. Alkylation of **41** with benzyl bromide followed by hydrolysis of the ester resulted in carboxylic acid **42**.



Scheme 4. Reagents and conditions: (a) R^6X, CsF, DMF, rt; (b) 1 N NaOH, MeOH.

The synthetic pathway for the desired imidazoline derivatives reported here is shown in Schemes 8 and 9. The imidazoline rings of **3′**, **5c–g**, **7a–b**, **7c′–d′**, **7e–q**, **8**, and **9′** were prepared by coupling (1*S*,2*S*)-1-(4-fluorophenyl)-1-(6-fluoropyridin-3-yl)propane-1,2-diamine (**10**) with aryl carboxylic acids, followed by thermal cyclization of the resulting amides (Scheme 8).[24] Removal of the Boc group of **3′** afforded the desired compound **3**. The benzyl group of **7c′** and **7d′** was removed under acidic conditions to yield **7c** and **7d**. Demethylation of **9′** was performed by treatment with sodium iodide and trimethylsilyl chloride to produce the imidazoline analog **9**. Imidazoline **5b** was prepared by coupling of the diamine **10** with an aryl imidate derived from **11**,[25] followed by cleavage of the benzyl group of the resulting compound **5b′** (Scheme 9).

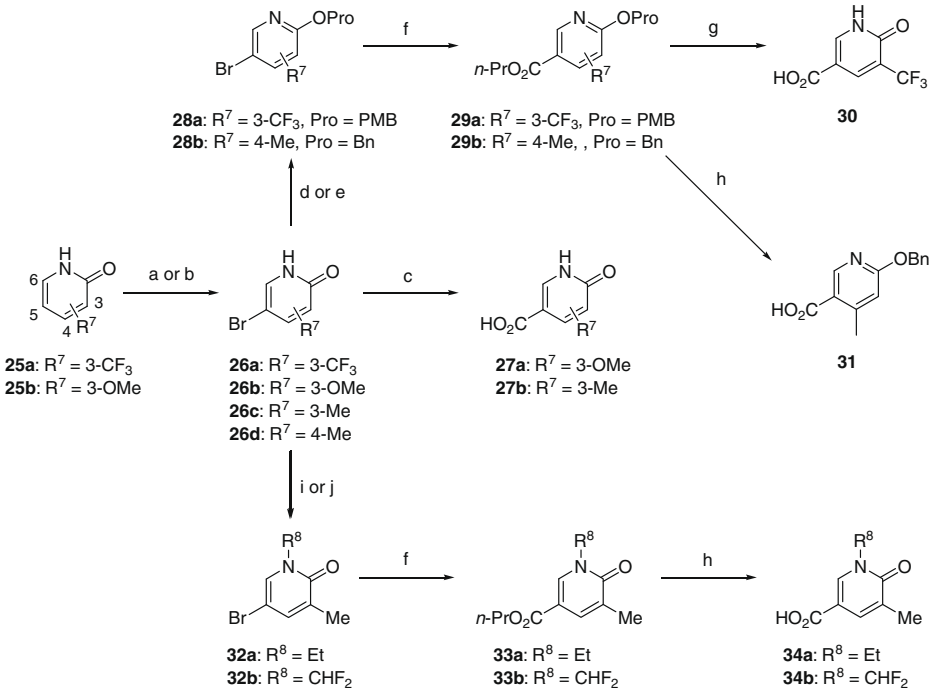
3. Results and discussion

3.1. SAR of pyridone-containing imidazolines

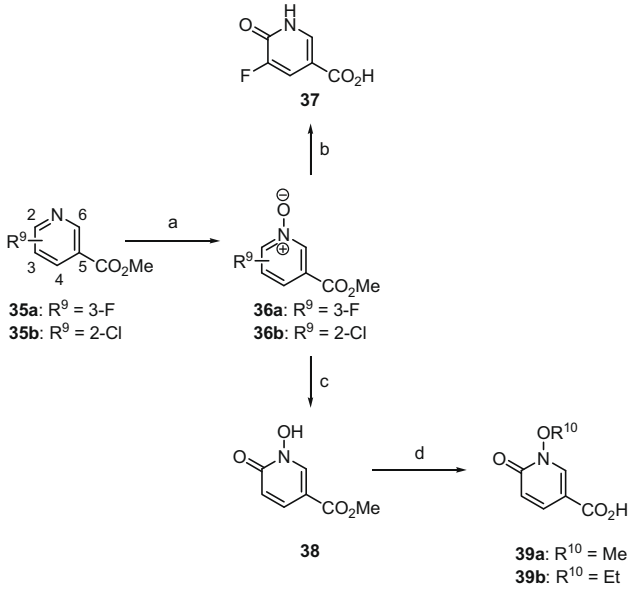
The series of imidazoline compounds was tested in a [^{125}I]PYY binding assay using LMtk$^-$ cell membranes expressing human recombinant Y5 receptors.[26] Rat hepatic clearance was examined by the in vitro serum incubation method to predict in vivo clearance in the rat liver, as previously reported by our laboratory.[27] The substitutions on the 4- and 5-positions of imidazoline, (4*S*)-4-(4-fluorophenyl)-4-(6-fluoropyridin-3-yl), and (5*S*)-methyl groups as in **5a** were selected due to their favorable effects on Y5 potency, metabolic stability, and human Ether-à-go-go related gene (hERG) affinity, as described in our previous report.[16]

We initially prepared pyridone regioisomers **7a**, **8**, and **9** (Table 2). Compound **7a** with 5-pyridin-2(1*H*)-one (5-pyridone) exhibited a slightly reduced Y5 activity compared to the 6-pyridin-2(1*H*)-one (6-pyridone) **5a**. Replacement of the 6-pyridone with a 3-pyridin-2(1*H*)-one (3-pyridone) or 4-pyridin-2(1*H*)-one (4-pyridone) as in **8** and **9** was deleterious to Y5 potency. We therefore focused on investigation of SAR for substitution effects on the 5- and 6-pyridone rings. Note that metabolic stability of the 5-pyridone **7a** was considerably better than that of 6-pyridone **5a** in rat hepatocytes.

The effects of substitutions on the 6-pyridone ring of **5a** are shown in Table 3. 3-Methyl substitution as in **5b** resulted in a higher hepatic clearance than **5a**, while retaining Y5 potency. Y5 affinities for 4- and 5-methyl derivatives **5c** and **5d** were more than fivefold less potent compared to **5a**. Based on these observations, additional 3-substituted pyridone derivatives were prepared and evaluated. 3-Fluoro substitution led to a significant improvement in hepatic clearance, although the derivative exhibited slightly decreased Y5 activity. The 3-chloro compound **5f** showed a similar in vitro profile to **5e**. The *N*-methyl derivative **5g** showed a significant loss of Y5 potency.



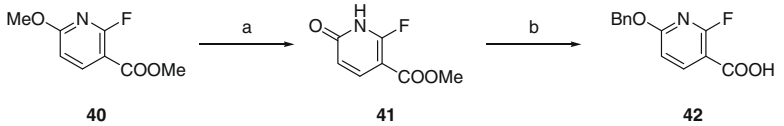
Scheme 5. Reagents and conditions: (a) NaOAc, Br_2, AcOH, 80 °C; (b) Br_2, CH_2Cl_2, rt; (c) *n*-BuLi, THF, −78 °C, then CO_2 gas; (d) (i) $PhPOCl_2$, 140 °C, (ii) *p*-methoxybenzyl alcohol, NaH, THF, 80 °C; (e) Ag_2CO_3, BnBr, benzene, rt; (f) $Pd(OAc)_2$, dppf, Et_3N, *n*-PrOH, DMF, CO gas, 100 °C; (g) (i) TFA, rt, (ii) 4 N NaOH, 1,4-dioxane, MeOH, rt; (h) 3 N NaOH, MeOH, 0 °C–rt; (i) EtBr, CsF, DMF, rt; (j) $CClF_2CO_2Na$, NaH, LiBr, DMF, 145 °C.



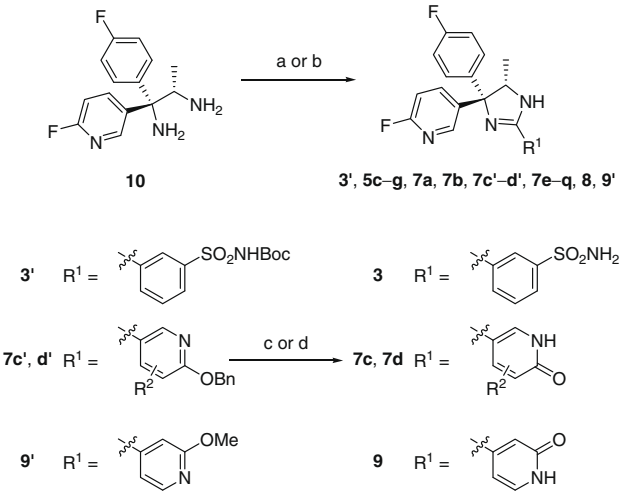
Scheme 6. Reagents and conditions: (a) UHP, TFAA, CH_2Cl_2 or CH_3CN, 0 °C to rt; (b) (i) Ac_2O, 140 °C, (ii) 2 N NaOH, rt; (c) TFAA, CH_3CN, rt; (d) (i) $R^{10}X$, K_2CO_3, DMF, rt, (ii) 1 N NaOH, MeOH, rt.

Next, the effects of substitutions on the 5-pyridone ring of **7a** were investigated as shown in Table 4. 3-Methyl substitution as in **7b** led to slight improvement in Y5 potency, relative to the parent compound **7a**. However, 4- or 6-substitution, as in **7c** and **7d**, resulted in significant loss of Y5 potency. Therefore, we focused on the 3-substituted 5-pyridone derivatives. The 3-fluoro compound **7e** and the parent **7a** were equipotent, and the 3-chloro, 3-trifluoromethyl, and 3-methoxy derivatives **7f**–**h** exhibited improved Y5 activities. The rat hepatic clearance of **7e**–**h** was comparable to the unsubstituted pyridone **7a**. Next, we investigated the effects of N-substitution on the 5-pyridone of **7a**. N-Methylation as in **7i** led to a slight decrease in Y5 potency, whereas the ethyl derivative **7j** was 2.5-fold more potent than **7a**. However, the predicted metabolic turnover of **7j** was much higher than that of the parent **7a**. The N-substituted pyridones **7k** and **7l** also showed a comparable profile to **7j** in terms of Y5 affinity and predicted rat hepatic clearance, suggesting that *N*-substituents of pyridones of **7j**–**l** were metabolically soft spots. Based on these results, introduction of a difluoromethyl group on the nitrogen of the pyridone ring of **7a** was attempted. The difluoromethyl compound **7m** showed improved Y5 inhibitory activity and an acceptable hepatic clearance, compared to **7a**. The *N*-methoxy analog **7n** was found to have a comparable profile to **7m**. Extension of the alkyl group as in **7o** decreased Y5 potency and increased hepatic clearance. The 1,3-disubstituted 5-pyridones **7p** and **7q** exhibited reduced Y5 potency compared to the 3-methyl compound **7b**.

Among the 5- and 6-pyridone derivatives described in Tables 3 and 4, the potent (hY5 $K_i \leqslant 4.0$ nM) and metabolically stable (predicted $CL_h \leqslant 30$ mL/min/kg) imidazolines **7f**, **7g**, **7m**, and **7n**



Scheme 7. Reagents and conditions: (a) NaI, TMSCl, CH_3CN, rt; (b) (i) BnBr, CsF, DMF, rt; (ii) 1 N NaOH, MeOH, 0 °C to rt.

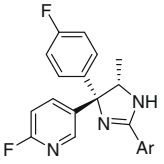


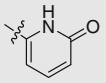
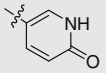
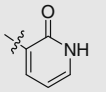
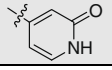
Scheme 8. Reagents and conditions: (a) (i) R^1CO_2H, DMI, Et_3N, $CHCl_3$, 0 °C, (ii) neat, 150 °C; (b) (i) R^1CO_2H, WSC·HCl, pyridine, $CHCl_3$, rt, (ii) $Yb(OTf)_3$ or $Sc(OTf)_3$, toluene, 100–150 °C; (c) TFA, rt or 50 °C; (d) NaI, TMSCl, CH_3CN, rt, then **9′**, rt.

were tested for P-glycoprotein (P-gp) susceptibility, which was assessed using human *MDR1*- and *mdr1a*-transfected porcine renal epithelial (LLC-PK1) cell monolayers and obtained transcellular transport ratios.[28] The transcellular transport ratios of the tested compounds (B-to-A/A-to-B ratio) are summarized in Table 5. In this P-gp transport assay, a compound with a B-to-A/A-to-B ratio above 3 is considered to be a P-gp substrate. All the tested compounds were mouse P-gp substrates (B-to-A/A-to-B ratio = 4–12), and the N-unsubstituted 5-pyridones **7f** and **7g** were also found to be human P-gp substrates. In contrast, the N-substituted compounds **7m** and **7n** were found not to be human P-gp substrates. Given that the N-unsubstituted 5-pyridone derivative **7a** is a substrate for human P-gp (B-to-A/A-to-B ratio = 8.5), the NH group on the 5-pyridone ring may increase susceptibility to human P-gp.

The antagonistic activity of the negligible human P-gp substrates **7m** and **7n** was determined by measuring the ability of each compound to inhibit NPY-induced $[Ca^{2+}]_i$ increase in LMtk$^-$ cells expressing the recombinant human Y5 receptor (Table 6).[29] In this functional assay, both compounds showed potent antagonistic activities. Compounds **7m** and **7n** were also evaluated for hERG K^+ channel binding activity using the [^{35}S]*N*-[(4*R*)-1′-[(2*R*)-6-cyano-1,2,3,4-tetrahydro-2-naphthalenyl]-3,4-dihydro-4-hydroxyspiro[2*H*-1-benzopyran-2,4′-piperidin]-6-yl]methanesulfonamide competitive binding assay to assess QTc prolongation liability (Table 6).[30] These compounds were found to have negligible hERG inhibitory activities. Compound **7m** was chosen for further studies on the basis of its potent functional activity. This compound displayed excellent selectivity over other NPY receptors (hY1, hY2, and hY4 binding: >10 μM)[31] and over 168 diverse unrelated binding sites (IC_{50} > 1.0 μM for all binding sites tested).

Table 2
SAR of pyridone analogues[a]



Compd	Ar	hY5 binding[b] *Ki* (nM)	CL_H[c] (mL/min/kg)
5a		1.4 ± 0.2	31
7a		7.4 ± 0.5	12
8		600	[d]
9		42 ± 4	[d]

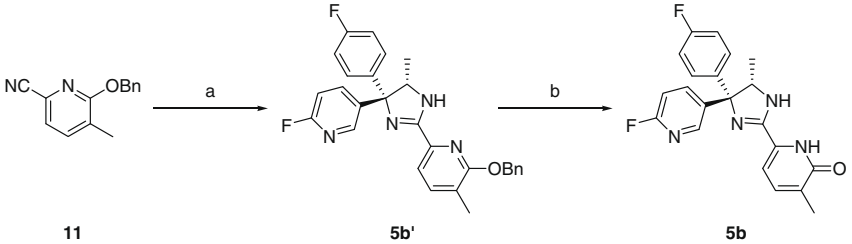
[a] The values represent the mean ± SE for $n = 3$ or the mean for $n = 2$.
[b] [^{125}I]PYY binding assay in LMtk$^-$ cells expressing human recombinant Y5 receptors.
[c] CL_H was determined by the serum incubation method using isolated rat hepatocytes.
[d] Not determined.

3.2. Pharmacokinetic, in vivo efficacy, and cardiovascular safety studies with 7m

The pharmacokinetic parameters of **7m** were evaluated in rats and rhesus monkeys (Table 7).[32] Compound **7m** displayed excellent profiles in both species. The brain penetrability of imidazoline **7m** was assessed in Sprague-Dawley (SD) rats. Compound **7m** had a brain-to-plasma ratio of 0.42 (brain = 0.91 nmol/g, plasma = 2.20 μM) 2 h after 10 mg/kg oral administration. This low ratio is presumably due to the effect of P-gp efflux.

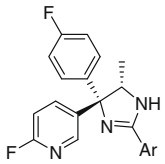
Having demonstrated the excellent potency, selectivity, and suitable pharmacokinetic profiles of **7m**, this compound was tested in an agonist-induced acute food intake model with SD rats (Fig. 2).[33] The compound was orally administered 2 h before animals were treated with either the Y5 selective agonist D-Trp^{34}NPY or artificial CSF, and cumulative food intake was measured for the following 2 h. Compound **7m** showed statistically significant and dose-dependent inhibition of the Y5 selective agonist-induced food intake in this feeding model.

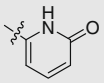
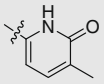
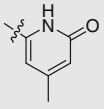
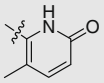
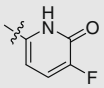
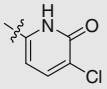
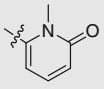
The potential cardiovascular effects of **7m** were evaluated in anesthetized dogs (Table 8). QTc interval, mean arterial pressure (MAP), left ventricular systolic pressure (LVSP), cardiac output (CO), heart rate (HR), and cardiac contractility (LV dp/dt_{max}) were assessed after cumulative intravenous infusion of 1, 2, and



Scheme 9. Reagents and conditions: (a) (i) NaOMe, MeOH, rt, then MsOH, (ii) **10**, MeOH, rt; (b) TFA, rt or 50 °C.

Table 3
SAR of 6-pyridone derivatives[a]



Compd	Ar	hY5 binding[b] *Ki* (nM)	CL_H[c] (mL/min/kg)
5a		1.4 ± 0.2	31
5b		2.6 ± 0.3	55
5c		8.6 ± 0.7	[d]
5d		28 ± 5	[d]
5e		6.9 ± 0.9	19
5f		8.6 ± 1.8	19
5g		350 ± 29	[d]

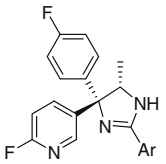
[a] The values represent the mean ± SE for $n = 3$ or the mean for $n = 2$.
[b] [^{125}I]PYY binding assay in LMtk$^-$ cells expressing human recombinant Y5 receptors.
[c] CL_H was determined by the serum incubation method using isolated rat hepatocytes.
[d] Not determined.

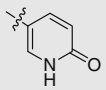
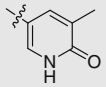
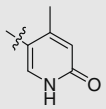
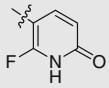
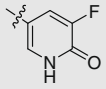
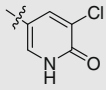
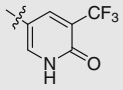
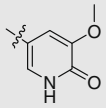
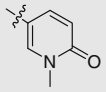
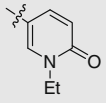
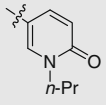
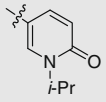
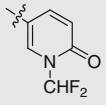
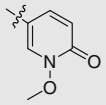
5 mg/kg/10 min of **7m**, which produced peak plasma concentrations of 7.7, 14.3, and 26.9 μM at the end of the each dosing period, respectively. In these cardiovascular studies, no significant treatment-related adverse cardiovascular effects were observed.

3.3. Receptor occupancy of 7m in P-gp-deficient *mdr1a* (−/−) and wild type *mdr1a* (+/+) CF-1 mice

Finally, brain NPY Y5 receptor occupancy was evaluated in rodents using an ex vivo receptor occupancy method to estimate plasma levels resulting in a high degree of receptor occupancy. Our previous studies indicated that high and sustained Y5 receptor occupancy is required for efficacy in both rodents with DIO and humans.[11,15] Since **7m** is a significant substrate for rodent P-gp as shown in Table 5, brain penetration by **7m** in rodents is limited by P-gp mediated efflux, leading to limited receptor occupancy and therefore limited efficacy in rodents. However, **7m** is a weak or negligible human P-gp substrate, and therefore, we speculated that **7m** shows higher brain penetrability and better receptor occupancy in humans than in rodents. To demonstrate the potential of **7m**, brain and plasma concentrations and receptor occupancy of **7m** were studied in P-gp-deficient *mdr1a* (−/−) and wild type *mdr1a* (+/+) CF-1 mice (Table 9). After oral administration of 10 mg/kg of **7m**, the brain-to-plasma ratio was 2.05 in *mdr1a*

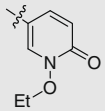
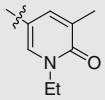
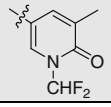
Table 4
SAR of 5-pyridone derivatives[a]



Compd	Ar	hY5 binding[b] *Ki* (nM)	CL_H[c] (mL/min/kg)
7a		7.4 ± 0.5	12
7b		4.3 ± 0.8	24
7c[d]		630 ± 83	[e]
7d		>1000	[e]
7e		11 ± 2	9
7f		3.4 ± 0.5	19
7g		3.8 ± 1.0	13
7h		5.0 ± 0.7	14
7i		13 ± 2	[e]
7j		2.9 ± 0.1	50
7k		4.7 ± 1.2	57
7l		3.6 ± 0.2	58
7m		2.8 ± 0.6	23
7n		2.8 ± 0.1	25

(*continued on next page*)

Table 4 (*continued*)

Compd	Ar	hY5 binding[b] *Ki* (nM)	CL_H[c] (mL/min/kg)
7o		7.3 ± 0.5	51
7p		6.6 ± 0.4	55
7q		8.5	[e]

[a] The values represent the mean ± SE for $n = 3$ or the mean for $n = 2$.
[b] [^{125}I]PYY binding assay in LMtk$^-$ cells expressing human recombinant Y5 receptors.
[c] CL_H was determined by the serum incubation method using isolated rat hepatocytes.
[d] Trifluoroacetate.
[e] Not determined.

Table 5
P-gp susceptibility of compounds **7f**, **7g**, **7m**, and **7n**[a]

Compd	P-gp susceptibility transcellular transport ratio (B-to-A)/(A-to-B)	
	Human *MDR1*	Mouse *mdr1a*
7f	9.1	12
7g	12	5.2
7m	1.6	7.0
7n	1.9	4.0

[a] The values represent the mean for $n = 3$. Transcellular transport ratios ((B-to-A)/(A-to-B)) were obtained from *MDR1*- and mouse *mdr1a*-transfected LLC-PK1 cell monolayers.

(−/−) CF-1 mice, which is fourfold higher than that seen in *mdr1a* (+/+) CF-1 mice. Encouraged by these results, ex vivo receptor occupancy studies were carried out in *mdr1a* (−/−) and *mdr1a* (+/+) CF-1 mice to obtain plasma level-receptor occupancy relationships (Fig. 3A). As expected, a remarkable leftward shift of the titration curve for *mdr1a* (−/−) mice was observed in the plasma level-receptor occupancy relationship. The plasma level required to achieve 90% receptor occupancy (Occ90) is 33 nM in *mdr1a* (−/−) mice, whereas the Occ90 value (170 nM) is higher in *mdr1a* (+/+) mice (Table 9). High and sustained receptor occupancy was observed after oral administration of 1 and 3 mg/kg **7m** in *mdr1a* (−/−) mice (Fig. 3B). These results suggest that **7m** may provide a high degree of brain receptor occupancy at a very low plasma concentration in humans.

Table 6
Functional Y5 antagonistic activity and hERG affinity of **7m** and **7n**[a]

Compd	$[Ca^{2+}]_i$ response IC_{50}[b] (nM)	hERG IC_{50}[c] (nM)
7m	8.7 ± 0.1	>10
7n	15.7 ± 4.0	>10

[a] The values represent the mean ± SE for $n = 3$.
[b] Antagonistic activities (human recombinant Y5 receptor/Gqi5 in CHO cells) at 100 nM NPY stimulation.
[c] Displacement binding assay of [^{35}S]*N*-[(4*R*)-1′-[(2*R*)-6-cyano-1,2,3,4-tetrahydro-2-naphthalenyl]-3,4-dihydro-4-hydroxyspiro[2*H*-1-benzopyran-2,4′-piperidin]-6-yl]methanesulfonamide in membranes derived from HEK293 cells stably transfected with the hERG gene expressing the I_{Kr} channel protein.

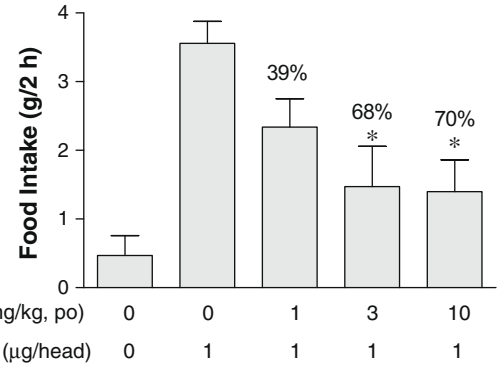


Figure 2. Effect of **7m** on food intake induced by the NPY selective agonist, D-Trp^{34}NPY. Compound **7m** was orally administered 2 h before 3rd ventricle injection of D-Trp^{34}NPY (1 μg/head). The values represent the mean ± SEM ($n = 7–10$). * $P < 0.05$ (compared with only the D-Trp^{34}NPY treated group).

Table 7
Pharmacokinetic parameters of **7m**[a]

Species	IV CL_P (mL/min/kg)	V_{dss} (L/kg)	PO $AUC_{0-\infty}$ (μM h)	C_{max} (μM)	*F* (%)
Rat[b]	23	2.6	4.8	1.1	92
Monkey[c]	3.5	3.8	11	0.64	92

[a] The values represent the mean for $n = 3$.
[b] Oral dose = 3 mg/kg. Intravenous dose = 1 mg/kg.
[c] Oral dose = 1 mg/kg. Intravenous dose = 0.3 mg/kg.

4. Conclusion

In summary, a series of 2-pyridone-containing imidazoline derivatives was synthesized and evaluated as NPY Y5 antagonists. Compound **7m** was found to have potent Y5 antagonistic activity and negligible susceptibility to human P-gp. In addition, imidazoline **7m** showed statistically significant inhibition of food intake in the agonist-induced food intake model and no adverse cardiovascular effects in anesthetized dogs. Because **7m** is a significant substrate for rodent P-gp, ex vivo receptor occupancy studies were conducted in *mdr1a* (−/−) and *mdr1a* (+/+) mice to obtain plasma-receptor occupancy relationships. Compound **7m** displayed markedly higher brain penetrability and a lower plasma Occ90 value in *mdr1a* (−/−) than in *mdr1a* (+/+) mice, suggesting that **7m** could potentially show higher brain penetrability and receptor occupancy in humans than in rodents. Based on the profiles described in this report, compound **7m** was selected as a clinical development candidate for the treatment of obesity and CNS-related dysfunctions. Progress in the development of this compound will be reported elsewhere.

5. Experimental

5.1. Materials and methods

Unless otherwise noted, all solvents, chemicals, and reagents were obtained commercially and used without purification. Silica gel column chromatography was carried out on a Wakogel® C-300 (Wako, mesh 45–75 μm) or with prepacked silica gel columns (KP-Sil™ silica) from Biotage under the indicated conditions. Preparative HPLC purification was carried out on a YMC-Pack *Pro* C18 (YMC, 50 mm × 30 mm id S-5 μm), eluting with a gradient of 0.1% CF_3CO_2H–CH_3CN/0.1% aqueous CF_3CO_2H = 10/90 to 50/50 over 8 min at a flow rate of 40 mL/min. Preparative thin-layer chromatography (TLC) was performed on a TLC Silica Gel 60 F (Merck

Table 8
Cardiovascular effects of **7m** in anesthetized dogs[a]

IV dose[b] (mg/kg)	Plasma conc[c] (μM)	QTc[d] (%)	MAP[d] (%)	LVSP[d] (%)	CO[d] (%)	HR[d] (%)	LV dp/dt_{max}[d] (%)
1	7.7	+2	+2	+3	+4	+3	−2
2	14.3	−2	−3	−1	+7	−2	−10
5	26.9	−4	−5	−4	+5	−2	−16

[a] The values represent the mean for $n = 3$.
[b] Doses infused over 10 min were 1, 2, and 5 mg/kg, yielding rising cumulative doses of 1, 3, and 8 mg/kg.
[c] Plasma concentrations were measured at the end of the dosing period.
[d] The parameters were measured at 0, 10, and 20 min during the cardiovascular study. Each value is described as a maximum percentage change from baseline; see Section 5 for details.

KGaA). The ^{1}H NMR spectra were obtained at 400 MHz on a MERCURY-400 (Varian), 400 MHz on a JMN-AL400 (JEOL), 200 MHz on a Gemini 200 (Varian), or 300 MHz on a Gemini 300 (Varian) spectrometer, with chemical shift (δ, ppm) reported relative to TMS as an internal standard. Mass spectra were recorded with electron-spray ionization (ESI) or atmospheric pressure chemical ionization (APCI) on a Waters micromass ZQ, micromass Quattro II or micromass Q-Tof-2 instrument. High resolution mass spectra (HRMS) were recorded with electron-spray ionization on a micromass Q-Tof-2 instrument. The purity of target compounds was determined by HPLC with the two different eluting methods. Following HPLC systems were used for purity assessment; Method A: Hewlett–Packard agilent 1100 series with a SPELCO Ascentis (4.6 mm × 150 mm id S-2.7 μm), eluting with a gradient of 0.1% H_3PO_4/CH_3CN = 95/5 to 10/90 over 7 min followed by 10/90 isocratic over 1 min at a flow rate of 1.5 mL/min, 40 °C of column temperature, detection with UV 210 nm; Method B: Hewlett–Packard agilent 1100 series with a SPELCO Ascentis (4.6 mm × 150 mm id S-2.7 μm), eluting with a gradient of 10 mM potassium phosphate (pH 6.6)/CH_3CN = 95/5 to 20/80 over 7 min followed by 20/80 isocratic over 1 min at a flow rate of 1.5 mL/min, 40 °C of column temperature, detection with UV 210 nm. Optical rotations of compounds **7f**, **7g**, **7m**, and **7n** were recorded on a JASCO P-1020 polarimeter.

5.2. Chemistry

5.2.1. *tert*-Butyl ({3-[(4*S*,5*S*)-4-(4-fluorophenyl)-4-(6-fluoropyridin-3-yl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]phenyl}sulfonyl)carbamate (3′)

To a stirred solution of 3-(chlorosulfonyl)benzoic acid (10.0 g, 45.3 mmol) in chloroform (100 mL) was added *tert*-butylamine (16.3 mL, 320 mmol) at 0 °C, and the mixture was stirred at 0 °C for 1 h. The reaction mixture was poured into 300 mL of 10% aqueous sodium hydrogen sulfate, and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, concentrated, and vacuum-dried to give 3-[(*tert*-butoxycarbonyl)sulfamoyl]benzoic acid (12.0 g). To a stirred solution of the crude benzoic acid (733 mg), (1*S*,2*S*)-1-(4-fluorophenyl)-1-(6-fluoropyridin-3-yl)propane-1,2-diamine (**10**) (500 mg, 1.90 mmol), and triethylamine (795 μL, 5.70 mmol) in chloroform (8.0 mL) was added a 25 wt. % solution of 2-chloro-1,3-dimethylimidazolinium chloride in dichloromethane (1.06 mL) at 0 °C, and the mixture was stirred at 0 °C for 20 min. The resultant mixture was partitioned between ethyl acetate and saturated aqueous sodium hydrogen carbonate, and the layers were separated. The aqueous layer was extracted with ethyl acetate, and the combined organic layers were washed with brine, dried over sodium sulfate, and concentrated. The residue was purified by silica gel flash chromatography with 33% ethyl acetate in hexanes to give *N*-[(1*S*,2*S*)-1-amino-1-(4-fluorophenyl)-1-(6-fluoropyridin-3-yl)propan-2-yl]-3-sulfamoylbenzamide as a colorless foam (893 mg). The neat amide was heated to 150 °C for 3 days. The resulting reaction mixture was purified by silica gel flash column chromatography with 50% ethyl acetate in hexanes to give **3′** (741 mg, 73% yield over 3 steps) as a pale-yellow foam. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.87 (3H, d, J = 6.5 Hz), 1.24 (9H, s), 4.65 (1H, q, J = 6.5 Hz), 4.78 (1H, s), 5.27 (1H, s), 6.87 (1H, dd, J = 3.0, 8.5 Hz), 7.00 (2H, t, J = 8.7 Hz), 7.15–7.30 (2H, m), 7.56 (1H, t, J = 7.5 Hz), 7.91 (1H, dt, J = 2.3, 8.2 Hz), 7.98 (1H, d, J = 7.7 Hz), 8.14 (1H, d, J = 7.7 Hz), 8.30 (1H, s), 8.39 (1H, s); MS (ESI): m/z 429 $[M-Boc+H]^+$.

5.2.2. 3-[(4*S*,5*S*)-4-(4-Fluorophenyl)-4-(6-fluoropyridin-3-yl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]benzenesulfonamide (3)

Compound **3′** (198 mg, 0.409 mmol) was dissolved in TFA (5.0 mL), and the mixture was stirred at room temperature for 20 h. After being concentrated, the residue was partitioned between ethyl acetate and saturated aqueous sodium hydrogen carbonate, and the layers were separated. The aqueous layer was extracted with ethyl acetate, and the combined organic layers were washed with brine, dried over sodium sulfate, and concentrated. The residue was purified by silica gel flash column chromatography with 67% ethyl acetate in hexanes to give **3** (154 mg, 88% yield) as a colorless foam. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.84 (3H, d, J = 6.6 Hz), 4.60–4.72 (1H, m), 5.60 (2H, brs), 6.82 (1H, dd, J = 3.0, 8.6 Hz), 6.98 (2H, t, J = 8.6 Hz), 7.15–7.30 (2H, m), 7.51 (1H, t, J = 7.8 Hz), 7.77–7.87 (1H, m), 7.93 (1H, d, J = 7.7 Hz), 8.06 (1H, d, J = 7.7 Hz), 8.25–8.38 (2H, m); HRMS (ES^+) calcd for $C_{21}H_{19}F_2N_4O_2S$ $[M+H]^+$ m/z 429.1197, found m/z 429.1186; HPLC purity: (A) 99.2% (t_R = 3.9 min), (B) 97.3% (t_R = 5.9 min).

5.2.3. 2-(Benzyloxy)-6-[(4*S*,5*S*)-4-(4-fluorophenyl)-4-(6-fluoropyridin-3-yl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]-3-methylpyridine (5b′)

To a stirred solution of 6-(benzyloxy)-5-methylpyridine-2-carbonitrile (**11**) (52 mg, 0.23 mmol) in methanol (5.0 mL) was added

Table 9
Brain penetration and plasma Occ90 values of **7m** in P-gp-deficient *mdr1a* (−/−) and wild type *mdr1a* (+/+) CF-1 mice[a]

	Plasma[b] (μM)	Brain[b] (nmol/g)	Brain/plasma[c]	Plasma Occ90[d] (nM)
mdr1a (+/+)	8.36	4.00	0.48	170
mdr1a (−/−)	7.01	14.8	2.05	33

[a] The values represent the mean for $n = 3$.
[b] The brain and plasma concentrations were obtained 2 h after oral administration of **7m** (10 mg/kg) in mice.
[c] The ratios were obtained from the mean values.
[d] Plasma Occ90 was determined by ex vivo receptor occupancy; see Section 5 for details.

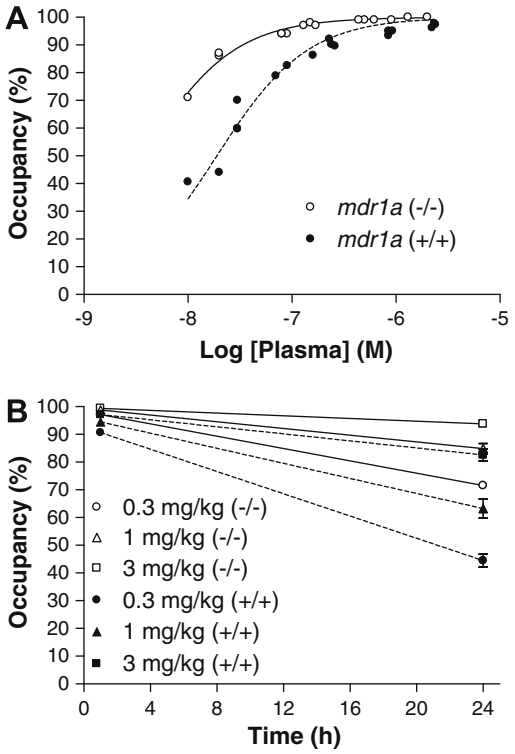


Figure 3. Brain Y5 receptor occupancy and drug exposure levels after oral administration of **7m**. (A): Relationship between plasma concentrations of **7m** and Y5 receptor occupancy in P-gp-deficient *mdr1a* (−/−) and wild type *mdr1a* (+/+) CF-1 mice. Receptor occupancy and exposure were determined 1 or 24 h following oral administration of vehicle or compound **7m** (0.3, 1.0, and 3.0 mg/kg). See Section 5 for details. (B) Receptor occupancy of **7m** at 1 or 24 h after oral administration (0.3, 1.0, and 3.0 mg/kg) in P-gp-deficient *mdr1a* (−/−) and wild type *mdr1a* (+/+) CF-1 mice.

a 25 wt. % solution of sodium methoxide in methanol (9.0 μL, 0.038 mmol) at room temperature, and the mixture was heated to 50 °C overnight. After methanesulfonic acid (17 μL, 0.27 mmol) was added, the mixture was treated with **10** (50 mg, 0.19 mmol), and the resulting mixture was heated to 80 °C overnight. The reaction mixture was partitioned between chloroform and saturated aqueous sodium hydrogen carbonate, and the layers were separated. The aqueous layer was extracted with chloroform, and the combined organic layers were dried over sodium sulfate and concentrated. The residue was purified by silica gel flash column chromatography with 20% ethyl acetate in hexanes to give **5b′** (75 mg, 84% yield over 2 steps) as a colorless oil. ^{1}H NMR (300 MHz, CD_3OD): δ 0.83 (3H, d, J = 6.2 Hz), 2.24 (3H, s), 4.75 (1H, q, J = 6.2 Hz), 5.48 (2H, s), 6.95–7.10 (3H, m), 7.20–7.38 (5H, m), 7.40–7.50 (2H, m), 7.55 (1H, d, J = 6.2 Hz), 7.66 (1H, d, J = 6.2 Hz), 8.02 (1H, dt, J = 2.5, 7.5 Hz), 8.32 (1H, d, J = 2.5 Hz); MS (ESI): m/z 471 $[M+H]^+$.

5.2.4. 6-[(4*S*,5*S*)-4-(4-Fluorophenyl)-4-(6-fluoropyridin-3-yl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]-3-methylpyridin-2(1*H*)-one (5b)

Compound **5b′** (75 mg, 0.16 mmol) was dissolved in TFA (10 mL), and the mixture was stirred at room temperature for 2 days. After being concentrated, the residue was partitioned between chloroform and saturated aqueous sodium hydrogen carbonate, and the layers were separated. The aqueous layer was extracted with chloroform, and the combined organic layers were dried over sodium sulfate and concentrated. The residue was purified by preparative TLC with 10% methanol in chloroform to give **5b** (37 mg, 61% yield) as a pale-brown solid. ^{1}H NMR (300 MHz, CD_3OD): δ 0.83 (3H, d, J = 6.6 Hz), 2.14 (3H, s), 4.79 (1H, q, J = 6.6 Hz), 6.80–6.88 (1H, m), 6.98–7.15 (3H, m), 7.24–7.36 (2H, m), 7.45–7.52 (1H, m), 7.95–8.06 (1H, m), 8.28–8.35 (1H, m); HRMS (ES^+) calcd for $C_{21}H_{19}F_2N_4O$ $[M+H]^+$ m/z 381.1527, found m/z 381.1519; HPLC purity: (A) 98.4% (t_R = 4.1 min), (B) 98.2% (t_R = 5.7 min).

5.2.5. 6-[(4*S*,5*S*)-4-(4-Fluorophenyl)-4-(6-fluoropyridin-3-yl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]-4-methylpyridin-2(1*H*)-one (5c)

To a stirred solution of **10** (150 mg, 0.57 mmol) and 4-methyl-6-oxo-1,6-dihydropyridine-2-carboxylic acid (105 mg, 0.68 mmol) in chloroform (5.0 mL) and pyridine (5.0 mL) was added 1-(3-diemthylaminopropyl)-3-ethylcarbodiimide hydrochloride (164 mg, 0.86 mmol) at 0 °C, and the mixture was allowed to warm to room temperature and stirred for 72 h. After being concentrated, the residue was partitioned between ethyl acetate and saturated aqueous sodium hydrogen carbonate, and the layers were separated. The aqueous layer was extracted with ethyl acetate, and the combined organic layers were washed with brine, dried over sodium sulfate, and concentrated. The residue was purified by silica gel flash column chromatography with 0–10% methanol in chloroform to give *N*-[(1*S*,2*S*)-1-amino-1-(4-fluorophenyl)-1-(6-fluoropyridin-3-yl)propan-2-yl]-4-methyl-6-oxo-1,6-dihydropyridine-2-carboxamide (145 mg) as a pale-red amorphous. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.15 (3H, d, J = 6.3 Hz), 2.34 (3H, s), 5.30–5.45 (1H, m), 6.48–6.52 (1H, m), 6.76 (1H, dd, J = 3.2, 8.5 Hz), 6.98–7.08 (3H, m), 7.49–7.52 (2H, m), 7.80–7.90 (2H, m), 8.40 (1H, d, J = 2.4 Hz); MS (ESI): m/z 399 $[M+H]^+$. The amide was suspended in toluene (5.0 mL) and treated with ytterbium tris(trifluoromethanesulfonate) (46 mg, 0.073 mmol), and the mixture was heated to 150 °C for 6 h in a sealed tube. The reaction mixture was partitioned between chloroform and saturated aqueous sodium hydrogen carbonate, and the layers were separated. The aqueous layer was extracted with chloroform, and the combined organic layers were dried over sodium sulfate and concentrated. The residue was purified by preparative TLC with 10% methanol in chloroform to give **5c** (54 mg, 25% yield over 2 steps) as a colorless foam. ^{1}H NMR (400 MHz, CD_3OD): δ 0.83 (3H, d, J = 6.3 Hz), 2.28 (3H, d, J = 1.0 Hz), 4.82 (1H, q, J = 6.3 Hz), 6.49–6.51 (1H, m), 6.80–6.84 (1H, m), 7.01–7.09 (3H, m), 7.25–7.35 (2H, m), 7.99–8.04 (1H, m), 8.31 (1H, d, J = 2.4 Hz); HRMS (ES^+) calcd for $C_{21}H_{19}F_2N_4O$ $[M+H]^+$ m/z 381.1527, found m/z 381.1519; HPLC purity: (A) 99.6% (t_R = 4.3 min), (B) 99.2% (t_R = 5.5 min).

5.2.6. 6-[(4*S*,5*S*)-4-(4-Fluorophenyl)-4-(6-fluoropyridin-3-yl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]-5-methylpyridin-2(1*H*)-one (5d)

Compound **5d** was prepared from **10** and 3-methyl-6-oxo-1,6-dihydropyridine-2-carboxylic acid using the procedure described for **5c** as a colorless solid (28% yield over 2 steps). ^{1}H NMR (400 MHz, CD_3OD): δ 0.86 (3H, d, J = 6.0 Hz), 2.16 (3H, s), 4.80–4.90 (1H, m), 6.58 (1H, d, J = 9.2 Hz), 7.03 (1H, dd, J = 2.8 Hz, 8.8 Hz), 7.07 (2H, t, J = 8.8 Hz), 7.30–7.40 (2H, m), 7.48 (1H, d, J = 9.2 Hz), 7.88 (1H, s), 7.90–8.00 (1H, m), 8.31 (1H, d, J = 2.0 Hz); HRMS (ES^+) calcd for $C_{21}H_{19}F_2N_4O$ $[M+H]^+$ m/z 381.1527, found m/z 381.1524; HPLC purity: (A) 99.7% (t_R = 3.8 min), (B) 98.4% (t_R = 5.5 min).

5.2.7. 3-Fluoro-6-[(4*S*,5*S*)-4-(4-fluorophenyl)-4-(6-fluoropyridin-3-yl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]pyridin-2(1*H*)-one (5e)

Compound **5e** was prepared from **10** and 5-fluoro-6-oxo-1,6-dihydropyridine-2-carboxylic acid (**21**) using the procedure described for **5c** as a colorless solid (6% yield over 2 steps). ^{1}H NMR (400 MHz, CD_3OD): δ 0.60–1.00 (3H, m), 4.90–5.10 (1H, m), 6.80–7.40 (7H,m), 7.88 (1H, s), 8.00–8.40 (1H, m); HRMS (ES^+) calcd

for $C_{20}H_{16}F_3N_4O$ [M+H]$^+$ *m/z* 385.1276, found *m/z* 385.1270; HPLC purity: (A) 98.7% (t_R = 4.0 min), (B) 98.0% (t_R = 5.1 min).

5.2.8. 3-Chloro-6-[(4*S*,5*S*)-4-(4-fluorophenyl)-4-(6-fluoropyridin-3-yl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]pyridin-2(1*H*)-one (5f)

Compound **5f** was prepared from **10** and 5-chloro-6-oxo-1,6-dihydropyridine-2-carboxylic acid (**18**) using the procedure described for **5c** as a colorless solid (18% yield over 2 steps). ^{1}H NMR (300 MHz, CD_3OD): δ 0.89 (3H, d, *J* = 6.6 Hz), 4.93 (1H, q, *J* = 6.6 Hz), 6.96 (1H, d, *J* = 7.6 Hz), 7.00–7.15 (3H, m), 7.20–7.35 (2H, m), 7.76 (1H, d, *J* = 7.6 Hz), 8.04 (1H, dt, *J* = 2.6, 8.5 Hz), 8.34 (1H, d, *J* = 2.6 Hz); HRMS (ES$^+$) calcd for $C_{20}H_{16}ClF_2N_4O$ [M+H]$^+$ *m/z* 401.0981, found *m/z* 401.0982; HPLC purity: (A) 99.2% (t_R = 4.1 min), (B) 98.8% (t_R = 5.6 min).

5.2.9. 6-[(4*S*,5*S*)-4-(4-Fluorophenyl)-4-(6-fluoropyridin-3-yl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]-1-methylpyridin-2(1*H*)-one (5g)

Compound **5g** was prepared from **10** and 1-methyl-6-oxo-1,6-dihydropyridine-2-carboxylic acid (**24a**) using the procedure described for **5c** as a colorless solid (5% yield over 2 steps). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.87 (3H, d, *J* = 6.5 Hz), 2.68 (3H, s), 4.70 (1H, q, *J* = 6.5 Hz), 5.19 (1H, s), 6.37 (1H, dd, *J* = 1.2 Hz, 6.7 Hz), 6.60–6.65 (1H, m), 6.85–6.95 (1H,m), 7.00–7.10 (2H, m), 7.20–7.40 (3H, m), 7.75–7.85 (1H, m), 8.41 (1H, s); HRMS (ES$^+$) calcd for $C_{21}H_{19}F_2N_4O$ [M+H]$^+$ *m/z* 381.1527, found *m/z* 381.1529; HPLC purity: (A) 99.9% (t_R = 3.6 min), (B) 99.8% (t_R = 5.3 min).

5.2.10. 5-[(4*S*,5*S*)-4-(4-Fluorophenyl)-4-(6-fluoropyridin-3-yl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]pyridin-2(1*H*)-one (7a)

Compound **7a** was prepared from **10** and 6-oxo-1,6-dihydropyridine-3-carboxylic acid using the procedure described for **5c** as a colorless solid (26% yield over 2 steps). ^{1}H NMR (300 MHz, CD_3OD): δ 0.83 (3H, d, *J* = 6.5 Hz), 4.74 (1H, q, *J* = 6.5 Hz), 6.56 (1H, d, *J* = 10.3 Hz), 7.00–7.10 (3H, m), 7.20–7.30 (2H, m), 7.89 (1H, s), 7.95–8.10 (2H, m), 8.27 (1H, d, *J* = 2.7 Hz); HRMS (ES$^+$) calcd for $C_{20}H_{17}F_2N_4O$ [M+H]$^+$ *m/z* 367.1370, found *m/z* 367.1360; HPLC purity: (A) 99.9% (t_R = 3.6 min), (B) 99.7% (t_R = 5.0 min).

5.2.11. 5-[(4*S*,5*S*)-4-(4-Fluorophenyl)-4-(6-fluoropyridin-3-yl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]-3-methylpyridin-2(1*H*)-one (7b)

Compound **7b** was prepared from **10** and 5-methyl-6-oxo-1,6-dihydropyridine-3-carboxylic acid (**27b**) using the procedure described for **5c** as a colorless solid (49% yield over 2 steps). ^{1}H NMR (400 MHz, CD_3OD): δ 0.83 (3H, d, *J* = 6.8 Hz), 2.14 (3H, s), 4.72 (1H, q, *J* = 6.8 Hz), 7.03 (1H, dd, *J* = 2.4, 8.4 Hz), 7.05–7.09 (2H, m), 7.23–7.27 (2H, m), 7.89 (1H, s), 7.90–7.91 (1H, m), 7.95–8.00 (1H, m), 8.26 (1H, s); HRMS (ES$^+$) calcd for $C_{21}H_{19}F_2N_4O$ [M+H]$^+$ *m/z* 381.1527, found *m/z* 381.1528; HPLC purity: (A) 99.9% (t_R = 3.7 min), (B) 99.7% (t_R = 5.3 min).

5.2.12. 2-(Benzyloxy)-5-[(4*S*,5*S*)-4-(4-fluorophenyl)-4-(6-fluoropyridin-3-yl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]-4-methylpyridine (7c′)

Compound **7c′** was prepared from **10**, 6-(benzyloxy)-4-methylpyridine-3-carboxylic acid (**31**), and scandium tris(trifluoromeths anesulfonate) in place of ytterbium tris(trifluoromethanesulfonate) using the procedure described for **5c** as a pale-brown oil (26% yield over 2 steps). ^{1}H NMR (400 MHz, CD_3OD): δ 0.84 (3H, d, *J* = 6.4 Hz), 2.35 (3H, s), 4.77 (1H, q, *J* = 6.4 Hz), 5.35 (2H, s), 6.73 (1H, s), 7.00–7.10 (3H, m), 7.20–7.40 (7H, m), 7.96 (1H, dt, *J* = 2.4, 8.8 Hz), 8.17 (1H, s), 8.30 (1H, d, *J* = 3.2 Hz); MS (ESI): *m/z* 471 [M+H]$^+$.

5.2.13. 5-[(4*S*,5*S*)-4-(4-Fluorophenyl)-4-(6-fluoropyridin-3-yl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]-4-methylpyridin-2(1*H*)-one trifluoroacetate (7c trifluoroacetate)

Compound **7c′** (49 mg, 0.10 mmol) was dissolved in TFA (5.0 mL), and the mixture was stirred at room temperature overnight. After being concentrated, the residue was purified by preparative HPLC with 10–50% 0.1% CF_3CO_2H/CH_3CN in 0.1% aqueous CF_3CO_2H, and the combined fractions were concentrated and vacuum-dried to give a trifluoroacetate salt of **7c** (27 mg, 55% yield) as a pale-brown solid. ^{1}H NMR (400 MHz, CD_3OD): δ 1.06 (3H, d, *J* = 6.4 Hz), 2.32 (3H, d, *J* = 1.2 Hz), 5.34 (1H, q, *J* = 6.4 Hz), 6.48 (1H, s), 7.10–7.40 (5H, m), 8.00–8.10 (1H, m), 8.06 (1H, s), 8.35 (1H, d, *J* = 2.8 Hz); HRMS (ES$^+$) calcd for $C_{21}H_{19}F_2N_4O$ [M+H]$^+$ *m/z* 381.1527, found *m/z* 381.1526; HPLC purity: (A) 99.0% (t_R = 3.7 min), (B) 98.9% (t_R = 5.1 min).

5.2.14. 6-(Benzyloxy)-2-fluoro-3-[(4*S*,5*S*)-4-(4-fluorophenyl)-4-(6-fluoropyridin-3-yl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]pyridine (7d′)

Compound **7d′** was prepared from **10**, 6-(benzyloxy)-2-fluoropyridine-3-carboxylic acid (**42**), and scandium tris(trifluoromeths anesulfonate) in place of ytterbium tris(trifluoromethanesulfonate) using the procedure described for **5c** as a colorless oil (51% yield over 2 steps). ^{1}H NMR (400 MHz, CD_3OD): δ 0.83 (3H, d, *J* = 6.4 Hz), 4.76 (1H, q, *J* = 6.4 Hz), 5.37 (2H, s), 6.82 (1H, dd, *J* = 1.0, 8.3 Hz), 7.00–7.10 (3H, m), 7.25–7.40 (5H, m), 7.40–7.45 (2H, m), 7.95–8.05 (1H, m), 8.24 (1H, t, *J* = 9.0 Hz), 8.31 (1H, d, *J* = 2.9 Hz); MS (ESI): *m/z* 475 [M+H]$^+$.

5.2.15. 6-Fluoro-5-[(4*S*,5*S*)-4-(4-fluorophenyl)-4-(6-fluoropyridin-3-yl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]pyridin-2(1*H*)-one (7d)

Compound **7d′** (84 mg, 0.18 mmol) was dissolved in TFA (10 mL), and the mixture was heated to 50 °C for 3 days. After being concentrated, the residue was purified by preparative HPLC with 10–50% 0.1% CF_3CO_2H/CH_3CN in 0.1% aqueous CF_3CO_2H, to give **7d** (2.3 mg, 3% yield) as a pale-brown solid. ^{1}H NMR (400 MHz, CD_3OD): δ 1.00 (3H, d, *J* = 6.4 Hz), 5.08 (1H, q, *J* = 6.4 Hz), 6.60–6.63 (1H, m), 7.05–7.15 (4H, m), 7.80–8.05 (3H, m), 8.30–8.40 (1H, m); HRMS (ES$^+$) calcd for $C_{20}H_{16}F_3N_4O$ [M+H]$^+$ *m/z* 385.1276, found *m/z* 385.1271; HPLC purity: (A) 97.0% (t_R = 4.0 min), (B) 96.6% (t_R = 4.5 min).

5.2.16. 3-Fluoro-5-[(4*S*,5*S*)-4-(4-fluorophenyl)-4-(6-fluoropyridin-3-yl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]pyridin-2(1*H*)-one (7e)

Compound **7e** was prepared from **10** and 5-fluoro-6-oxo-1,6-dihydropyridine-3-carboxylic acid (**37**) using the procedure described for **5c** as a colorless amorphous (36% yield over 2 steps). ^{1}H NMR (300 MHz, CD_3OD): δ 0.84 (3H, d, *J* = 6.3 Hz), 4.70–4.90 (1H, m), 7.00–7.15 (3H, m), 7.20–7.30 (2H, m), 7.82 (1H, dd, *J* = 2.3, 11.2 Hz), 7.90–8.05 (2H, m), 8.28 (1H, d, *J* = 2.3 Hz); HRMS (ES$^+$) calcd for $C_{20}H_{16}F_3N_4O$ [M+H]$^+$ *m/z* 385.1276, found *m/z* 385.1278; HPLC purity: (A) 95.3% (t_R = 3.7 min), (B) 95.3% (t_R = 5.1 min).

5.2.17. 3-Chloro-5-[(4*S*,5*S*)-4-(4-fluorophenyl)-4-(6-fluoropyridin-3-yl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]pyridin-2(1*H*)-one (7f)

Compound **7f** was prepared from **10** and 5-chloro-6-oxo-1,6-dihydropyridine-3-carboxylic acid using the procedure described for **5c** as a colorless solid (29% yield over 2 steps). ^{1}H NMR (300 MHz, CD_3OD): δ 0.84 (3H, d, *J* = 6.6 Hz), 4.80 (1H, q, *J* = 6.6 Hz), 7.00–7.15 (3H, m), 7.20–7.30 (2H, m), 7.89 (1H, s), 7.95–8.05 (1H, m), 8.04 (1H, d, *J* = 2.4 Hz), 8.21 (1H, d, *J* = 2.4 Hz), 8.25–8.30 (1H, m); HRMS (ES$^+$) calcd for $C_{20}H_{16}ClF_2N_4O$ [M+H]$^+$

m/z 401.0981, found *m/z* 401.0983; HPLC purity: (A) 98.7% (t_R = 3.9 min), (B) 98.8% (t_R = 5.4 min); $[\alpha]_D^{25}$ −239 ° (*c* 1.0, CH_3OH).

5.2.18. 5-[(4*S*,5*S*)-4-(4-Fluorophenyl)-4-(6-fluoropyridin-3-yl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]-3-(trifluoromethyl)-pyridin-2(1*H*)-one (7g)

Compound **7g** was prepared from **10** and 6-oxo-5-(trifluoromethyl)-1,6-dihydropyridine-3-carboxylic acid (**30**) using the procedure described for **5c** as a pale-yellow solid (25% yield over 2 steps). ^{1}H NMR (300 MHz, CD_3OD): δ 0.85 (3H, d, *J* = 6.5 Hz), 4.73–4.90 (1H, m), 7.02–7.14 (3H, m), 7.21–7.30 (2H, m), 7.99 (1H, ddd, *J* = 2.7, 7.6, 8.6 Hz), 8.28 (1H, d, *J* = 2.5 Hz), 8.32 (1H, d, *J* = 2.6 Hz), 8.39 (1H, d, *J* = 1.9 Hz); HRMS (ES^+) calcd for $C_{21}H_{16}F_5N_4O$ $[M+H]^+$ *m/z* 435.1244, found *m/z* 435.1250; HPLC purity: (A) 97.9% (t_R = 4.2 min), (B) 97.2% (t_R = 5.9 min); $[\alpha]_D^{25}$ −255 ° (*c* 0.45, CH_3OH).

5.2.19. 5-[(4*S*,5*S*)-4-(4-Fluorophenyl)-4-(6-fluoropyridin-3-yl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]-3-methoxypyridin-2(1*H*)-one (7h)

Compound **7h** was prepared from **10** and 5-methoxy-6-oxo-1,6-dihydropyridine-3-carboxylic acid (**27a**) using the procedure described for **5c** as a colorless solid (25% yield over 2 steps). ^{1}H NMR (400 MHz, CD_3OD): δ 0.85 (3H, d, *J* = 6.2 Hz), 3.88 (3H, s), 4.74 (1H, q, *J* = 6.2 Hz), 7.02–7.10 (3H, m), 7.24–7.28 (2H, m), 7.43 (1H, d, *J* = 2.0 Hz), 7.64 (1H, d, *J* = 2.0 Hz), 7.96–8.01 (1H, m), 8.27 (1H, d, *J* = 2.0 Hz); HRMS (ES^+) calcd for $C_{21}H_{19}F_2N_4O_2$ $[M+H]^+$ *m/z* 397.1476, found *m/z* 397.1472; HPLC purity: (A) 95.4% (t_R = 3.7 min), (B) 97.1% (t_R = 5.1 min).

5.2.20. 5-[(4*S*,5*S*)-4-(4-Fluorophenyl)-4-(6-fluoropyridin-3-yl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]-1-methylpyridin-2(1*H*)-one (7i)

Compound **7i** was prepared from **10** and 1-methyl-6-oxo-1,6-dihydropyridine-3-carboxylic acid using the procedure described for **5c** as a colorless amorphous (40% yield over 2 steps). ^{1}H NMR (300 MHz, CD_3OD): δ 0.83 (3H, d, *J* = 6.5 Hz), 3.60 (3H, s), 4.74 (1H, q, *J* = 6.5 Hz), 6.57 (1H, d, *J* = 9.6 Hz), 7.00–7.15 (3H, m), 7.20–7.30 (2H, m), 7.89 (1H, s), 7.90–8.05 (2H, m), 8.26 (2H, dd, *J* = 2.4, 8.0 Hz); HRMS (ES^+) calcd for $C_{21}H_{19}F_2N_4O$ $[M+H]^+$ *m/z* 381.1527, found *m/z* 381.1526; HPLC purity: (A) 98.0% (t_R = 3.7 min), (B) 97.8% (t_R = 5.3 min).

5.2.21. 1-Ethyl-5-[(4*S*,5*S*)-4-(4-fluorophenyl)-4-(6-fluoropyridin-3-yl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]pyridin-2(1*H*)-one (7j)

Compound **7j** was prepared from **10** and 1-ethyl-6-oxo-1,6-dihydropyridine-3-carboxylic acid using the procedure described for **5c** as a colorless solid (38% yield over 2 steps). ^{1}H NMR (300 MHz, CD_3OD): δ 0.83 (3H, d, *J* = 6.5 Hz), 1.37 (3H, t, *J* = 7.2 Hz), 4.07 (2H, q, *J* = 7.2 Hz), 4.75 (1H, q, *J* = 6.5 Hz), 6.57 (1H, d, *J* = 9.6 Hz), 7.00–7.15 (3H, m), 7.20–7.30 (2H, m), 7.89 (1H, s), 7.90–8.00 (2H, m), 8.20–8.30 (2H, m); HRMS (ES^+) calcd for $C_{22}H_{21}F_2N_4O$ $[M+H]^+$ *m/z* 395.1683, found *m/z* 395.1675; HPLC purity: (A) 99.5% (t_R = 3.9 min), (B) 98.7% (t_R = 5.7 min).

5.2.22. 5-[(4*S*,5*S*)-4-(4-Fluorophenyl)-4-(6-fluoropyridin-3-yl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]-1-propylpyridin-2(1*H*)-one (7k)

Compound **7k** was prepared from **10** and 6-oxo-1-propyl-1,6-dihydropyridine-3-carboxylic acid (**24b**) using the procedure described for **5c** as a colorless solid (47% yield over 2 steps). ^{1}H NMR (400 MHz, CD_3OD): δ 0.84 (3H, d, *J* = 5.6 Hz), 0.97 (3H, t, *J* = 7.6 Hz), 1.80 (2H, sextet, *J* = 7.6 Hz), 3.98 (2H, t, *J* = 7.6 Hz), 4.70–4.80 (1H, m), 6.56 (1H, d, *J* = 9.6 Hz), 7.00–7.10 (3H, m), 7.20–7.30 (2H, m), 7.90–8.00 (2H, m), 8.20–8.30 (2H, m); HRMS (ES^+) calcd for $C_{23}H_{23}F_2N_4O$ $[M+H]^+$ *m/z* 409.1840, found *m/z* 409.1839; HPLC purity: (A) 98.9% (t_R = 4.1 min), (B) 98.3% (t_R = 6.0 min).

5.2.23. 5-[(4*S*,5*S*)-4-(4-Fluorophenyl)-4-(6-fluoropyridin-3-yl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]-1-(propan-2-yl)pyridin-2(1*H*)-one (7l)

Compound **7l** was prepared from **10** and 6-oxo-1-(propan-2-yl)-1,6-dihydropyridine-3-carboxylic acid (**24c**) using the procedure described for **5c** as a colorless solid (38% yield over 2 steps). ^{1}H NMR (400 MHz, CD_3OD): δ 0.84 (3H, d, *J* = 6.4 Hz), 1.42 (3H, d, *J* = 4.4 Hz), 1.44 (3H, d, *J* = 4.4 Hz), 4.75 (1H, q, *J* = 6.4 Hz), 5.18 (1H, qui, *J* = 4.4 Hz), 6.56 (1H, d, *J* = 9.2 Hz), 7.00–7.10 (3H, m), 7.20–7.30 (2H, m), 7.90–8.00 (2H, m), 8.27 (2H, d, *J* = 2.4 Hz); HRMS (ES^+) calcd for $C_{23}H_{23}F_2N_4O$ $[M+H]^+$ *m/z* 409.1840, found *m/z* 409.1850; HPLC purity: (A) 99.4% (t_R = 4.1 min), (B) 98.0% (t_R = 6.0 min).

5.2.24. 1-(Difluoromethyl)-5-[(4*S*,5*S*)-4-(4-fluorophenyl)-4-(6-fluoropyridin-3-yl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]pyridin-2(1*H*)-one (7m)

Compound **7m** was prepared from **10** and 1-(difluoromethyl)-6-oxo-1,6-dihydropyridine-3-carboxylic acid (**24d**) using the procedure described for **5c** as a colorless amorphous (23% yield over 2 steps). ^{1}H NMR (300 MHz, CD_3OD): δ 0.83 (1H, d, *J* = 6.5 Hz), 4.76 (1H, q, *J* = 6.5 Hz), 6.62 (1H, d, *J* = 9.9 Hz), 7.00–7.15 (3H, m), 7.20–7.30 (2H, m), 7.78 (1H, t, *J* = 59.9 Hz), 7.90–8.10 (2H, m), 8.25–8.35 (2H, m); HRMS (ES^+) calcd for $C_{21}H_{17}F_4N_4O$ $[M+H]^+$ *m/z* 417.1338, found *m/z* 417.1336; HPLC purity: (A) 99.1% (t_R = 4.1 min), (B) 97.6% (t_R = 6.4 min); $[\alpha]_D^{25}$ −300 ° (*c* 0.63, CH_3OH).

5.2.25. 5-[(4*S*,5*S*)-4-(4-Fluorophenyl)-4-(6-fluoropyridin-3-yl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]-1-methoxypyridin-2(1*H*)-one (7n)

Compound **7n** was prepared from **10** and 1-methoxy-6-oxo-1,6-dihydropyridine-3-carboxylic acid (**39a**) using the procedure described for **5c** as a colorless solid (9% yield over 2 steps). ^{1}H NMR (400 MHz, CD_3OD): δ 0.83 (3H, d, *J* = 6.4 Hz), 4.08 (3H, s), 4.75 (1H, q, *J* = 6.4 Hz), 6.69 (1H, d, *J* = 9.6 Hz), 7.02 (1H, dd, *J* = 2.4, 8.4 Hz), 7.04–7.08 (2H, m), 7.24–7.27 (2H, m), 7.95–7.99 (2H, m), 8.26 (1H, d, *J* = 2.8 Hz), 8.47 (1H, d, *J* = 2.4 Hz); HRMS (ES^+) calcd for $C_{21}H_{19}F_2N_4O_2$ $[M+H]^+$ *m/z* 397.1476, found *m/z* 397.1469; HPLC purity: (A) 98.4% (t_R = 3.7 min), (B) 96.1% (t_R = 5.5 min); $[\alpha]_D^{25}$ −308 ° (*c* 0.75, CH_3OH).

5.2.26. 1-Ethoxy-5-[(4*S*,5*S*)-4-(4-fluorophenyl)-4-(6-fluoropyridin-3-yl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]pyridin-2(1*H*)-one (7o)

Compound **7o** was prepared from **10** and 1-ethoxy-6-oxo-1,6-dihydropyridine-3-carboxylic acid (**39b**) using the procedure described for **5c** as a colorless solid (22% yield over 2 steps). ^{1}H NMR (400 MHz, CD_3OD): δ 0.80–0.90 (3H, m), 1.40 (3H, t, *J* = 7.0 Hz), 4.33 (2H, d, *J* = 7.0 Hz), 4.80–4.90 (1H, m), 6.69 (1H, d, *J* = 9.6 Hz), 7.00–7.10 (3H, m), 7.20–7.30 (2H, m), 7.94–8.02 (2H, m), 8.25–8.30 (1H, m), 8.46 (1H, d, *J* = 2.4 Hz); HRMS (ES^+) calcd for $C_{22}H_{21}F_2N_4O_2$ $[M+H]^+$ *m/z* 411.1633, found *m/z* 411.1621; HPLC purity: (A) 99.8% (t_R = 3.9 min), (B) 99.6% (t_R = 5.8 min).

5.2.27. 1-Ethyl-5-[(4*S*,5*S*)-4-(4-fluorophenyl)-4-(6-fluoropyridin-3-yl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]-3-methylpyridin-2(1*H*)-one (7p)

Compound **7p** was prepared from **10** and 1-ethyl-5-methyl-6-oxo-1,6-dihydropyridine-3-carboxylic acid (**34a**) using the procedure described for **5c** as a colorless solid (23% yield over 2 steps). ^{1}H NMR (400 MHz, CD_3OD): δ 0.84 (3H, d, *J* = 6.8 Hz), 1.37 (3H, t, *J* = 7.2 Hz), 2.16 (3H, s), 4.08 (2H, q, *J* = 6.8 Hz), 4.74 (1H, q, *J* = 6.8 Hz), 7.03 (1H, dd, *J* = 2.4, 8.0 Hz), 7.05–7.09 (2H, m),

7.24–7.28 (2H, m), 7.84 (1H, dd, J = 1.2, 2.4 Hz), 7.95–8.01 (1H, m), 8.12 (1H, dd, J = 0.8, 2.4 Hz), 8.27 (1H, d, J = 2.8 Hz); HRMS (ES^+) calcd for $C_{23}H_{23}F_2N_4O$ $[M+H]^+$ m/z 409.1840, found m/z 409.1835; HPLC purity: (A) 98.3% (t_R = 4.1 min), (B) 97.2% (t_R = 6.1 min).

5.2.28. 1-(Difluoromethyl)-5-[(4*S*,5*S*)-4-(4-fluorophenyl)-4-(6-fluoropyridin-3-yl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]-3-methylpyridin-2(1*H*)-one (7q)

Compound **7q** was prepared from **10** and 1-(difluoromethyl)-5-methyl-6-oxo-1,6-dihydropyridine-3-carboxylic acid (**34b**) using the procedure described for **5c** as a yellow solid (17% yield over 2 steps). ^1H NMR (400 MHz, CD_3OD): δ 0.81–0.88 (3H, m), 2.16 (3H, s), 4.72–4.78 (1H, brs), 7.10–7.09 (3H, m), 7.23–7.28 (2H, m), 7.80 (1H, t, J = 60 Hz), 7.92–8.20 (2H, m), 8.16–8.19 (1H, br s), 8.25–8.30 (1H, brs); HRMS (ES^+) calcd for $C_{22}H_{19}F_4N_4O$ $[M+H]^+$ m/z 431.1495, found m/z 431.1485; HPLC purity: (A) 99.6% (t_R = 4.3 min), (B) 99.1% (t_R = 6.9 min).

5.2.29. 3-[(4*S*,5*S*)-4-(4-Fluorophenyl)-4-(6-fluoropyridin-3-yl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]pyridin-2(1*H*)-one (8)

Compound **8** was prepared from **10** and 2-oxo-1,2-dihydropyridine-3-carboxylic acid using the procedure described for **5c** as a pale-yellow solid (11% yield over 2 steps). ^1H NMR (300 MHz, CD_3OD): δ 1.03 (3H, d, J = 6.6 Hz), 5.24 (1H, q, J = 6.6 Hz), 6.65 (1H, dd, J = 6.4, 7.4 Hz), 7.10–7.40 (5H, m), 7.89 (1H, s), 7.96 (1H, dd, J = 2.1, 6.3 Hz), 8.05–8.15 (1H, m), 8.38 (1H, d, J = 2.8 Hz), 8.45 (1H, dd, J = 2.1, 7.4 Hz); HRMS (ES^+) calcd for $C_{20}H_{17}F_2N_4O$ $[M+H]^+$ m/z 367.1370, found m/z 367.1361; HPLC purity: (A) 95.1% (t_R = 3.8 min), (B) 95.8% (t_R = 5.1 min).

5.2.30. 4-[(4*S*,5*S*)-4-(4-Fluorophenyl)-4-(6-fluoropyridin-3-yl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]-2-methoxypyridine (9′)

Compound **9′** was prepared from **10** and 2-methoxypyridine-4-carboxylic acid using the procedure described for **5c** as a colorless amorphous (46% yield over 2 steps). ^1H NMR (300 MHz, CD_3OD): δ 0.80–0.90 (3H, m), 3.92 (3H, s), 4.70–4.90 (1H, m), 7.00–7.40 (7H, m), 7.95–8.05 (1H, m), 8.20–8.40 (2H, m); MS (ESI): m/z 381 $[M+H]^+$.

5.2.31. 4-[(4*S*,5*S*)-4-(4-Fluorophenyl)-4-(6-fluoropyridin-3-yl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]pyridin-2(1*H*)-one (9)

To a stirred solution of sodium iodide (352 mg, 2.35 mmol) in acetonitrile (10 mL) was added trimethylsilyl chloride (298 μL, 2.35 mmol) at room temperature, and the mixture was stirred at room temperature for 15 min. To the resulting mixture was added a solution of **9′** (180 mg, 0.47 mmol) in acetonitrile (10 mL) at room temperature, and the mixture was stirred at room temperature for 3 days. After being concentrated, the residue was partitioned between chloroform and saturated aqueous sodium hydrogen carbonate, and the layers were separated. The aqueous layer was extracted with chloroform, and the combined organic layers were dried over sodium sulfate and concentrated. The residue was purified by preparative TLC with 10% methanol in chloroform to give **9** (153 mg, 89% yield) as a colorless solid. ^1H NMR (300 MHz, CD_3OD): δ 0.83 (3H, d, J = 6.3 Hz), 4.70–4.90 (1H, m), 6.83 (1H, dd, J = 1.3, 6.9 Hz), 6.92 (1H, s), 7.00–7.10 (3H, m), 7.20–7.30 (2H, m), 7.49 (1H, d, J = 6.7 Hz), 7.84 (1H, s), 7.90–8.10 (1H, m), 8.28 (1H, s); HRMS (ES^+) calcd for $C_{20}H_{17}F_2N_4O$ $[M+H]^+$ m/z 367.1370, found m/z 367.1373; HPLC purity: (A) 99.8% (t_R = 3.6 min), (B) 99.5% (t_R = 5.1 min).

5.2.32. (1*S*,2*S*)-1-(4-Fluorophenyl)-1-(6-fluoropyridin-3-yl)-propane-1,2-diamine (10)

Compound **15** (5.37 g, 11.5 mmol) was dissolved in TFA (11 mL) at 0 °C, and the mixture was allowed to warm to room temperature and stirred for 1 h. After being concentrated, the residue was dissolved in dioxane (13 mL), and the mixture was cooled to 0 °C. 8 N HCl (13 mL) was added slowly to the mixture at 0 °C, and the resulting mixture was allowed to warm up to room temperature and stirred for 2 h. After being cooled to 0 °C, the mixture was basified with 8 N NaOH (20 mL). The resultant mixture was saturated with solid NaCl and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, concentrated, and vacuum-dried to give **10** (3.02 g, 100%) as a yellow oil. ^1H NMR (400 MHz, $CDCl_3$): δ 1.00 (3H, d, J = 6.2 Hz), 4.04 (1H, q, J = 6.2 Hz), 6.85 (1H, dd, J = 3.4, 8.8 Hz), 7.00–7.05 (2H, m), 7.30–7.40 (2H, m), 7.90–8.00 (1H, m), 8.39 (1H, d, J = 2.0 Hz); MS (ESI): m/z 264 $[M+H]^+$.

5.2.33. 6-(Benzyloxy)-5-methylpyridine-2-carbonitrile (11)

To a stirred solution of **17a** (1.31 g, 6.7 mmol) and benzyl alcohol (763 μL, 7.4 mmol) in THF (50 mL) was added sodium hydride (60% dispersion in oil, 320 mg, 8.0 mmol), and the mixture was heated to reflux for 1 h. After being cooled to room temperature, the mixture was partitioned between ethyl acetate and saturated aqueous sodium hydrogen carbonate, and the layers were separated. The aqueous layer was extracted with ethyl acetate, and the combined organic layers were washed with brine, dried over sodium sulfate, and concentrated. The residue was purified by silica gel flash column chromatography with 5% ethyl acetate in hexanes to give **11** (1.05 g, 70% yield) as a pale-pink oil. ^1H NMR (400 MHz, $CDCl_3$): δ 2.28 (3H, s), 5.40 (2H, s) 7.20–7.30 (1H, m), 7.30–7.40 (3H, m), 7.40–7.50 (3H, m); MS (ESI): m/z 225 $[M+H]^+$.

5.2.34. *tert*-Butyl [(2*S*)-1-(4-fluorophenyl)-1-oxopropan-2-yl]carbamate (13)

To a stirred solution of *N*′-(*tert*-butoxycarbonyl)-*N*-methoxy-*N*-methyl-L-alaninamide (**12**) (20.0 g, 86.1 mmol) in THF (300 mL) was added a 2 M THF solution of 4-fluorophenylmagnesium bromide (100 mL, 200 mmol) at 0 °C, and the mixture was allowed to warm up to room temperature and stirred overnight. After being cooled to 0 °C, saturated aqueous ammonium chloride was added to the mixture. The resulting mixture was extracted twice with ethyl acetate, and the combined organic layers were washed with brine, dried over sodium sulfate, concentrated, and vacuum-dried to give **13** (23.1 g, 100% yield) as a pale-brown solid. ^1H NMR (400 MHz, $CDCl_3$): δ 0.95 (3H, t, J = 7.6 Hz), 1.30 (9H, s), 5.24 (1H, qui, J = 7.6 Hz), 5.20–5.40 (1H, m), 7.16 (2H, t, J = 8.0 Hz), 7.95–8.05 (2H, m); MS (ESI): m/z 268 $[M+H]^+$.

5.2.35. *tert*-Butyl [(1*E*,2*S*)-1-{[(*R*)-*tert*-butylsulfinyl]imino}-1-(4-fluorophenyl)propan-2-yl]carbamate (14)

To a stirred solution of **13** (64.8 g, 243 mmol) and (*R*)-2-methylpropane-2-sulfinamide (35.4 g, 292 mmol) in toluene (130 mL) was added titanium tetraethanolate (127 mL, 607 mL), and the mixture was heated to 70 °C for 17 h under a nitrogen atmosphere. After being cooled to room temperature, brine (240 mL) and ethyl acetate (480 mL) was added to the mixture, and the resulting mixture was stirred at room temperature for 20 min. The resultant suspension was filtered though Celite pad, and the filtrate was washed with water and brine, dried over sodium sulfate, and concentrated. The residue was purified by silica gel flash column chromatography with 10–15% ethyl acetate in hexanes to give **14** (54.3 g, 60% yield) as a pale-yellow solid. ^1H NMR (300 MHz, $CDCl_3$): δ 1.32 (9H, s), 1.44 (9H, s), 1.51 (3H, d, J = 7.2 Hz), 5.00–5.40 (1H, br s), 7.11 (2H, t, J = 8.0 Hz), 7.20–8.00 (2H, m); MS (ESI): m/z 371 $[M+H]^+$.

5.2.36. *tert*-Butyl [(1*S*,2*S*)-1-{[(*R*)-*tert*-butylsulfinyl]amino}-1-(4-fluorophenyl)-1-(6-fluoropyridin-3-yl)propan-2-yl]carbamate (15)

To a stirred solution of 5-bromo-2-fluoropyridine (7.87 g, 44.7 mmol) in diethyl ether (200 mL) was added slowly a 2.66 M solution of *n*-BuLi in hexanes (18.5 mL, 49.2 mmol) at −78 °C,

and the mixture was stirred at −78 °C for 30 min. A solution of **14** (5.52 g, 14.9 mmol) and trimethylaluminum (1 M hexane solution, 19.4 mL, 19.4 mmol) in diethyl ether (100 mL), which was stirred at 0 °C for 15 min in advance, was added to the resultant orange suspension at −78 °C. The resulting mixture was stirred at −78 °C for 30 min before adding saturated aqueous sodium sulfate and solid magnesium sulfate. After being warmed to room temperature, the suspension was filtered through Celite pad, and the filtrate was concentrated. The residue was triturated with diisopropyl ether, and the resulting precipitate was collected by filtration. The resultant solid was vacuum-dried to give **15** (5.37 g, 77% yield) as a pale-brown solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.99 (3H, d, J = 6.7 Hz), 1.44 (9H, s), 1.48 (9H, s), 4.37 (1H, s), 5.03 (1H, m), 6.81 (1H, brs), 6.87 (1H, dd, J = 3.2, 8.8 Hz), 7.03 (2H, m), 7.29 (2H, m), 7.97 (1H, m), 8.22 (1H, d, J = 2.6 Hz); MS (ESI): m/z 468 $[M+H]^+$.

5.2.37. 6-Bromo-5-methylpyridine-2-carbonitrile (17a)

To a stirred solution of 2-bromo-3-methylpyridine (**16a**) (2.94 g, 17.1 mmol) in chloroform (30 mL) was added *m*CPBA (4.43 g, 25.7 mmol) at room temperature, and the mixture was stirred at room temperature overnight. After being cooled to 0 °C, saturated aqueous sodium hydrogen sulfite was added to the mixture. The resulting mixture was extracted twice with chloroform, and the combined organic layers were washed with saturated aqueous sodium hydrogen carbonate, dried over sodium sulfate, concentrated, and vacuum-dried to give 2-bromo-3-methylpyridine 1-oxide (2.43 g). To a stirred solution of the crude in dichloromethane (30 mL) was added methyl trifluoromethanesulfonate (1.61 mL, 14.1 mmol) at 0 °C, and the mixture was allowed to warm up to room temperature and stirred for 2 h. After being concentrated, the residue was dried in vacuo. The resulting crude was dissolved in DMF (30 mL) and treated with NaCN (946 mg, 19.3 mmol) at 0 °C, and the mixture was allowed to warm to room temperature and stirred for 19 h. The mixture was partitioned between ethyl acetate and saturated aqueous sodium hydrogen carbonate, and the layers were separated. The aqueous layer was extracted with ethyl acetate, and the combined organic layers were washed with brine, dried over sodium sulfate, and concentrated. The residue was purified by silica gel flash column chromatography with 10% ethyl acetate in hexanes to give **17a** (1.31 g, 52% yield over 3 steps) as a pale-yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.49 (3H, s), 7.56 (1H, d, J = 8.0 Hz), 7.63 (1H, d, J = 8.0 Hz); MS (ESI): m/z 197 $[M+H]^+$.

5.2.38. 5,6-Dichloropyridine-2-carbonitrile (17b)

To a stirred solution of 2,3-dichloropyridine (**16b**) (10.0 g, 67.6 mmol) and urea hydrogen peroxide addition compound (13.3 g, 142 mmol) in dichloromethane (100 mL) was added dropwise TFAA (19.1 mL, 135 mmol) at 0 °C, and the mixture was allowed to warm to room temperature and stirred for 3 h. After being cooled to 0 °C, saturated aqueous sodium hydrogen sulfite was added to the mixture. The resulting mixture was poured into 0.5 N HCl, and the layers were separated. The aqueous layer was extracted twice with chloroform, and the combined organic layers were stirred with saturated sodium hydrogen carbonate for 5 min. The layers were separated, and the organic layer was dried over sodium sulfate, concentrated, and vacuum-dried to give 2,3-dichloropyridine 1-oxide (11.0 g) as a colorless solid. The crude pyridine 1-oxide was added to a stirred dimethyl sulfate (6.67 mL, 70.4 mmol) in small portions, and the mixture was heated to 90 °C for 30 min. After being cooled to room temperature, the mixture was diluted with water (50 mL). To the stirred aqueous solution was added NaCN (3.94 g, 80.5 mmol) at 0 °C, and the mixture was stirred at 0 °C for 1 h under a nitrogen atmosphere. The resulting precipitate was collected by filtration and washed with water and vacuum-dried. The solid was triturated with 5% methanol in diisopropyl ether, and the precipitate was collected and dried in vacuo to give **17b** (4.32 g, 37% yield over 2 steps) as a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.61 (1H, d, J = 10.6 Hz), 7.94 (1H, d, J = 10.6 Hz); MS (ESI): m/z 173 $[M+H]^+$.

5.2.39. 5-Chloro-6-oxo-1,6-dihydropyridine-2-carboxylic acid (18)

Compound **17b** (500 mg, 2.48 mmol) in 10 N KOH (10 mL) was heated to reflux for 2 h. The mixture was cooled to 0 °C and acidified to pH 2–3 with concentrated HCl. The resulting precipitate was collected, washed with water, and vacuum-dried. The resultant crude was dissolved in water (30 mL) and few drops of 28% aqueous ammonia solution, and the mixture was acidified to pH 2–3 with concentrated HCl. The precipitate was collected, washed with water, and dried in vacuo to give **18** (290 mg, 52% yield) as pale-yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.60–6.70 (1H, m),7.70–7.80 (1H, m); MS (ESI): m/z 172 $[M-H]^-$.

5.2.40. 6-Cyano-3-fluoropyridin-2-yl acetate (20)

To a stirred solution of 5-fluoropyridine-2-carbonitrile (**19**) (421 mg, 3.45 mmol) and urea hydrogen peroxide addition compound (649 mg, 6.90 mmol) in dichloromethane (40 mL) was added dropwise TFAA (1.02 mL, 7.25 mmol) at 0 °C, and the mixture was allowed to warm to room temperature and stirred for 3 h. Saturated aqueous sodium hydrogen sulfite was added to the mixture, and the layers were separated. The aqueous layer was extracted twice with chloroform, and the combined organic layers were dried over sodium sulfate, concentrated, and vacuum-dried to give 5-fluoropyridine-2-carbonitrile 1-oxide (272 mg) as a yellow solid. The crude was treated with acetic anhydride (10 mL), and the mixture was heated to reflux for 16 h. After being concentrated, the residual oil was purified by silica gel flash column chromatography with 10% ethyl acetate in hexanes to give **20** (290 mg, 81% yield over 2 steps) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.41 (3H, s), 7.42–7.56 (2H, m); MS (ESI): m/z 139 $[M-Ac+H]^+$.

5.2.41. 5-Fluoro-6-oxo-1,6-dihydropyridine-2-carboxylic acid (21)

Compound **20** (227 mg, 1.26 mmol) was treated with 6 N HCl (20 mL), and the mixture was heated to reflux for 16 h. After being cooled to room temperature, the resulting precipitate was collected, washed with water, and vacuum-dried to give **21** (151 mg, 76% yield) as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.81 (1H, dd, J = 3.2, 9.2 Hz), 7.66 (1H, t, J = 9.2 Hz); MS (ESI): m/z 158 $[M+H]^+$.

5.2.42. Methyl 1-methyl-6-oxo-1,6-dihydropyridine-2-carboxylate (23a)

To a stirred solution of methyl 6-oxo-1,6-dihydropyridine-2-carboxylate (**22a**) (200 mg, 1.30 mmol) and methyl iodide (243 μL 3.90 mmol) in DMF (5.0 mL) was added CsF (592 mg, 3.90 mmol) at room temperature, and the mixture was stirred at room temperature for 2 days. The mixture was partitioned between ethyl acetate and saturated aqueous sodium hydrogen carbonate, and the layers were separated. The organic layer was washed with water, dried over sodium sulfate, and concentrated. The residue was purified by silica gel flash column chromatography with 5% methyl alcohol in chloroform to give **23a** (103 mg, 48% yield) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.69 (3H, s), 3.93 (3H, s), 6.70–6.80 (2H, m), 7.33 (1H, dd, J = 6.8, 8.8 Hz); MS (ESI): m/z 168 $[M+H]^+$.

5.2.43. Propyl 6-oxo-1-propyl-1,6-dihydropyridine-3-carboxylate (23b)

To a stirred solution of 6-oxo-1,6-dihydropyridine-3-carboxylic acid (**22b**) (2.00 g, 14.4 mmol) and *n*-propyl bromide (5.23 mL, 57.6 mmol) in DMF (50 mL) was added CsF (8.75 g, 57.6 mmol) at room temperature, and the mixture was stirred at room temperature for 17 h. The mixture was partitioned between ethyl acetate and water, and the layers were separated. The organic layer was washed with brine, dried over sodium sulfate, and concentrated. The residue was purified by silica gel flash column chromatography with 10% ethyl acetate in hexanes to give **23b** (240 mg, 7% yield) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.90–1.10 (6H, m), 1.70–1.90 (4H, m), 3.95 (2H, t, J = 6.7 Hz), 4.23 (2H, t, J = 6.7 Hz), 6.55 (1H, d, J = 9.2 Hz), 7.81 (1H, dd, J = 2.8, 9.2 Hz), 8.15 (1H, d, J = 2.8 Hz); MS (ESI): m/z 224 $[M+H]^+$.

5.2.44. Propan-2-yl 6-oxo-1-(propan-2-yl)-1,6-dihydropyridine-3-carboxylate (23c)

Compound **23c** was prepared from 6-oxo-1,6-dihydropyridine-3-carboxylic acid (**22b**) and *i*-propyl bromide using the procedure described for **23b** as a colorless oil (0.7% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ1.35 (6H, d, J = 4.6 Hz), 1.42 (6H, d, J = 4.4 Hz), 5.10–5.30 (2H, m), 6.52 (1H, d, J = 9.2 Hz), 7.80 (1H, dd, J = 1.6, 9.2 Hz), 8.21 (1H, d, J = 1.6 Hz); MS (ESI): m/z 224 $[M+H]^+$.

5.2.45. 1-Methyl-6-oxo-1,6-dihydropyridine-2-carboxylic acid (24a)

To a stirred solution of **23a** (98 mg, 0.59 mmol) in methyl alcohol (3.0 mL) was added 1 N NaOH (1.18 mL, 1.18 mmol) at 0 °C, and the mixture was allowed to warm to room temperature and stirred for 30 min. The mixture was partitioned between ethyl acetate and 0.5 N HCl, and the layers were separated. The aqueous layer was extracted with ethyl acetate, and the combined organic layers were washed with brine, dried over sodium sulfate, concentrated, and vacuum-dried to give **24a** (18 mg, 20% yield) as a colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.49 (3H, s), 6.55–6.60 (1H, m), 6.65–6.75 (1H, m), 7.43 (1H, dd, J = 6.8, 9.4 Hz); MS (ESI): m/z 154 $[M+H]^+$.

5.2.46. 6-Oxo-1-propyl-1,6-dihydropyridine-3-carboxylic acid (24b)

Compound **24b** was prepared from **23b** using the procedure described for **24a** as a colorless solid (62% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.43 (3H, t, J = 7.5 Hz), 1.82 (2H, sextet, J = 7.5 Hz), 3.95 (2H, t, J = 7.5 Hz), 6.19 (1H, d, J = 9.5 Hz), 7.87 (1H, dd, J = 1.6, 9.6 Hz), 8.21 (1H, d, J = 1.6 Hz), 12.40 (1H, s); MS (ESI): m/z 182 $[M+H]^+$.

5.2.47. 6-Oxo-1-(propan-2-yl)-1,6-dihydropyridine-3-carboxylic acid (24c)

Compound **24c** was prepared from **23c** using the procedure described for **24a** as a colorless solid (81% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.66 (6H, d, J = 4.4 Hz), 4.49 (1H, septet, J = 4.4 Hz), 6.19 (1H, d, J = 9.5 Hz), 7.87 (1H, dd, J = 1.6, 9.5 Hz), 8.14 (1H, d, J = 1.6 Hz), 12.43 (1H, brs); MS (ESI): m/z 182 $[M+H]^+$.

5.2.48. 1-(Difluoromethyl)-6-oxo-1,6-dihydropyridine-3-carboxylic acid (24d)

Compound **24d** was prepared from **23d** using the procedure described for **24a** as a colorless solid (73% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.56 (1H, d, J = 9.6 Hz), 7.82 (1H, t, J = 58.8 Hz), 7.86 (1H, dd, J = 1.6, 9.6 Hz), 8.24 (1H, d, J = 1.6 Hz); MS (ESI): m/z 190 $[M+H]^+$.

5.2.49. 5-Bromo-3-(trifluoromethyl)pyridin-2(1*H*)-one (26a)

To a stirred solution of 3-(trifluoromethyl)pyridin-2(1*H*)-one (**25a**) (2.61 g, 16.0 mmol) and sodium acetate (1.48 g, 18.0 mmol) in acetic acid (30 mL) was added bromine (900 μL, 18.0 mmol) at room temperature, and the mixture was heated to 80 °C for 14 h. After being concentrated, the residue was partitioned between ethyl acetate and saturated aqueous sodium hydrogen carbonate, and the layers were separated. The aqueous layer was extracted with ethyl acetate, and the combined organic layers were dried over magnesium sulfate and concentrated. The residue was triturated with hexanes, collected by filtration, and vacuum-dried to give **26a** (3.52 g, 91% yield) as a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.73 (1H, d, J = 2.6 Hz), 7.90 (1H, dd, J = 0.7, 2.6 Hz); MS (ESI): m/z 242 $[M+H]^+$.

5.2.50. 5-Bromo-3-methoxypyridin-2(1*H*)-one (26b)

To a stirred solution of 3-methoxypyridin-2(1*H*)-one (**25b**) (1.00 g, 8.00 mmol) in dichloromethane (10 mL) was added dropwise bromine (453 μL, 8.79 mmol) at room temperature, and the mixture was stirred at room temperature for 3.5 h. After being cooled to 0 °C, saturated aqueous sodium hydrogen carbonate was added to the mixture. The resulting mixture was extracted with ethyl acetate, and the organic layer was washed with brine, dried over sodium sulfate, and concentrated. The residue was purified by silica gel flash column chromatography with 33% ethyl acetate in hexanes to give **26b** (600 mg, 37% yield) as a pale-brown solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.86 (3H, s), 6.79 (1H, d, J = 2.0 Hz), 7.11 (1H, d, J = 2.0 Hz); MS (ESI): m/z 204 $[M+H]^+$.

5.2.51. 5-Methoxy-6-oxo-1,6-dihydropyridine-3-carboxylic acid (27a)

To a stirred solution of **26b** (422 mg, 2.07 mmol) in THF (15 mL) was added a 1.59 M solution of *n*-BuLi in hexanes (2.74 mL, 4.35 mmol) at −78 °C, and the mixture was stirred at −78 °C for 30 min. After CO_2 gas was bubbled into the reaction mixture at −78 °C for 1 h, the resulting mixture was stirred at −78 °C for 1 h and warmed up to room temperature. After being concentrated, the residue was dissolved in water and acidified with 4 N HCl. The mixture was extracted five times with ethyl acetate, and the combined organic layers were washed with brine, dried over sodium sulfate, concentrated, and vacuum-dried to give **27a** (131 mg, 37% yield) as a brown solid. ^{1}H NMR (400 MHz, CD_3OD): δ 3.85 (3H, s), 7.28 (1H, d, J = 2.0 Hz), 7.76 (1H, d, J = 2.0 Hz); MS (ESI): m/z 170 $[M+H]^+$.

5.2.52. 5-Methyl-6-oxo-1,6-dihydropyridine-3-carboxylic acid (27b)

Compound **27b** was prepared from **26c** using the procedure described for **27a** as a pale-pink solid (61% yield). ^{1}H NMR (200 MHz, CD_3OD): δ 2.11 (3H, s), 7.80–7.90 (1H, m), 7.95–8.05 (1H, m); MS (ESI): m/z 152 $[M-H]^-$.

5.2.53. 5-Bromo-2-[(4-methoxybenzyl)oxy]-3-(trifluoromethyl)-pyridine (28a)

Compound **26a** (1.98 g, 8.17 mmol) in phenylphosphonic dichloride (4.0 mL) was heated to 140 °C for 4 h. After being cooled to room temperature, the mixture was poured into ice water, and extracted three times with chloroform. The combined organic layers were washed with saturated aqueous sodium hydrogen carbonate and brine, dried over sodium sulfate, concentrated, and vacuum-dried to give 5-bromo-2-chloro-3-(trifluoromethyl)pyridine (1.96 g) as a brown oil. To a stirred solution of the crude and 4-methoxybenzyl alcohol (940 μL, 7.54 mmol) in THF (15 mL) was added sodium hydride (60% dispersion in oil, 350 mg, 8.75 mmol) at room temperature, and the mixture was heated to 80 °C for 1 h. After being cooled to room temperature, the mixture was quenched with water. The mixture was extracted three times with ethyl acetate, and the combined organic layers were washed with brine, dried over magnesium sulfate, and

concentrated. The residue was purified by silica gel flash column chromatography with 4% ethyl acetate in hexanes to give **28a** (2.18 g, 80% yield over 2 steps) as a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.81 (3H, s), 5.42 (2H, s), 6.86–6.94 (2H, m), 7.34–7.40 (2H, m), 7.95 (1H, d, J = 2.4 Hz), 8.35 (1H, d, J = 2.4 Hz); MS (ESI): not detected.

5.2.54. 2-(Benzyloxy)-5-bromo-4-methylpyridine (28b)

To a stirred solution of **26d** (1.00 g, 5.30 mmol) and benzyl bromide (1.90 mL, 15.9 mmol) in benzene (20 mL) was added silver carbonate (1.50 g, 5.30 mmol) at room temperature, and the mixture was stirred at room temperature for 4 days under light shielding conditions. After being filtered through a pad of Celite, the filtrate was concentrated. The residue was purified by silica gel flash column chromatography with 5% ethyl acetate in hexanes to give **28b** (1.40 g, 93% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.34 (3H, s), 5.33 (2H, s), 6.71 (1H, s), 7.30–7.45 (5H, m), 8.18 (1H, s); MS (ESI): m/z 278 $[M+H]^+$.

5.2.55. Propyl 6-[(4-methoxybenzyl)oxy]-5-(trifluoromethyl)-pyridine-3-carboxylate (29a)

To a stirred solution of **28a** (1.01 g, 2.78 mmol), 1,1′-bis(diphenylphosphino)ferrocene (308 mg, 0.555 mmol), and triethylamine (0.77 mL, 5.53 mmol) in DMF (3.7 mL) and n-propyl alcohol (7.4 mL) was added palladium diacetate (62.9 mg, 0.280 mmol), and the mixture was heated to 100 °C under an atmospheric pressure of carbon monoxide for 13 h. After being cooled to room temperature, the black solution was filtered through a pad of SiO_2 and washed with ethyl acetate. The filtrate was washed with water and brine, dried over magnesium sulfate, and concentrated. The residue was purified by silica gel flash column chromatography with 6% ethyl acetate in hexanes to give **29a** (919 mg, 89% yield) as a pale-yellow oil. ^{1}H NMR^{1}H NMR (300 MHz, $CDCl_3$): δ 1.02 (3H, t, J = 6.7 Hz), 1.78 (2H, sextet, J = 6.7 Hz), 3.81 (3H, s), 4.30 (2H, t, J = 6.7 Hz), 5.53 (2H, s), 6.90 (2H, d, J = 8.5 Hz), 7.39 (2H, d, J = 8.5 Hz), 8.44 (1H, d, J = 2.0 Hz), 8.96 (1H, d, J = 2.0 Hz); MS (ESI): m/z 370 $[M+H]^+$.

5.2.56. Propyl 6-(benzyloxy)-4-methylpyridine-3-carboxylate (29b)

Compound **29b** was prepared from **28b** using the procedure described for **29a** as a pale-yellow solid (89% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.03 (3H, t, J = 7.3 Hz), 1.74–1.81 (2H, m), 2.57 (3H, s), 4.25 (2H, t, J = 6.6 Hz), 5.42 (2H, s), 6.65 (1H, s), 7.26–7.40 (3H, m), 7.41–7.43 (2H, m), 8.79 (1H, s); MS (ESI): m/z 286 $[M+H]^+$.

5.2.57. 6-Oxo-5-(trifluoromethyl)-1,6-dihydropyridine-3-carboxylic acid (30)

Compound **29a** (492 mg, 1.33 mmol) was dissolved in TFA (1.3 mL), and the mixture was stirred at room temperature for 1 h. After being concentrated, the residue was partitioned between ethyl acetate and saturated aqueous sodium hydrogen carbonate, and the layers were separated. The aqueous layer was extracted with ethyl acetate, and the combined organic layers were dried over magnesium sulfate and concentrated to give propyl 6-oxo-5-(trifluoromethyl)-1,6-dihydropyridine-3-carboxylate as a yellow amorphous. The crude product was dissolved in methyl alcohol (5.0 mL) and treated with 4 N NaOH (0.67 mL), and the mixture was stirred at room temperature for 4 days. After being concentrated, the residue was diluted with ethyl acetate and water. The layers were separated, and the organic layer was washed with water. The combined aqueous layers were acidified with concentrated HCl, and the resulting precipitate was collected and dried in vacuo to give **30** (115 mg, 42% yield over 2 steps) as a colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.15 (1H, s), 8.20–8.25 (1H, m), 12.80–12.90 (1H, m), 13.10–13.25 (1H, m); MS (ESI): m/z 206 $[M-H]^-$.

5.2.58. 6-(Benzyloxy)-4-methylpyridine-3-carboxylic acid (31)

Compound **31** was prepared from **29b** using the procedure described for **24a** as a pale-brown solid. (81% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.50 (3H, s), 5.39 (2H, s), 6.82 (1H, s), 7.31–7.44 (5H, m), 8.64 (1H, s), 12.87 (1H, s); MS (ESI): m/z 244 $[M+H]^+$.

5.2.59. 5-Bromo-1-ethyl-3-methylpyridin-2(1*H*)-one (32a)

Compound **32a** was prepared from **26c** and ethyl bromide using the procedure described for **23a** as a colorless solid (69% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.38 (3H, t, J = 7.8 Hz), 2.15 (3H, s), 3.96 (2H, q, J = 7.8 Hz), 7.20–7.25 (1H, m), 7.25–7.30 (1H, m); MS (ESI): m/z 216 $[M+H]^+$.

5.2.60. Propyl 1-ethyl-5-methyl-6-oxo-1,6-dihydropyridine-3-carboxylate (33a)

Compound **33a** was prepared from **32a** using the procedure described for **29a** as a brown oil (90% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.01 (3H, t, J = 7.6 Hz), 1.39 (3H, t, J = 6.8 Hz), 1.75 (2H, sextet, J = 6.8 Hz), 2.16 (3H, s), 4.03 (2H, q, J = 7.6 Hz), 4.21 (2H, t, J = 6.8 Hz), 7.69 (1H, dd, J = 1.2, 2.4 Hz), 8.04 (1H, d, J = 2.4 Hz); MS (ESI): m/z 224 $[M+H]^+$.

5.2.61. Propyl 1-(difluoromethyl)-5-methyl-6-oxo-1,6-dihydropyridine-3-carboxylate (33b)

To a stirred solution of **26c** (2.30 g, 12.2 mmol) and lithium bromide (2.12 g, 24.4 mmol) in DMF (10 mL) was sodium hydride (60% dispersion in oil, 540 mg, 13.5 mmol) at 0 °C, and the mixture was allowed to warm to room temperature and stirred for 15 min. To the resultant mixture was added sodium chloro(difluoro)acetate (3.72 g, 24.2 mmol), and the mixture was heated to 140 °C for 30 min. After being concentrated, the residue was partitioned between ethyl acetate and saturated aqueous ammonium chloride, and the layers were separated. The aqueous layer was extracted with ethyl acetate, and the combined organic layers were washed with brine, dried over sodium sulfate, and concentrated. The residue was purified by silica gel flash column chromatography with 0–10% ethyl acetate in hexanes to give **32b** (870 mg) as a pale-yellow oil. Compound **33b** was prepared from the crude **32b** using the procedure described for **29a** as a brown oil (18% yield over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.03 (3H, t, J = 7.2 Hz), 1.77 (2H, sextet, J = 7.2 Hz), 2.17 (3H, s), 4.25 (2H, t, J = 7.2 Hz), 7.70 (1H, t, J = 63.2 Hz), 7.70–7.75 (1H, m), 8.15–8.20 (1H, m); MS (ESI): m/z 246 $[M+H]^+$.

5.2.62. 1-Ethyl-5-methyl-6-oxo-1,6-dihydropyridine-3-carboxylic acid (34a)

Compound **34a** was prepared from **33a** using the procedure described for **24a** as a colorless solid (97% yield). ^{1}H NMR (400 MHz, CD_3OD): δ 1.34 (3H, t, J = 7.2 Hz), 2.12 (3H, s), 4.07 (2H, q, J = 7.2 Hz), 7.79 (1H, d, J = 2.8 Hz), 8.29 (1H, d, J = 2.8 Hz); MS (ESI): m/z 182 $[M+H]^+$.

5.2.63. 1-(Difluoromethyl)-5-methyl-6-oxo-1,6-dihydropyridine-3-carboxylic acid (34b)

Compound **34b** was prepared from **33b** using the procedure described for **24a** as a colorless solid (72% yield). ^{1}H NMR (400 MHz, CD_3OD): δ 2.14 (3H, s), 7.77 (1H, t, J = 60.0 Hz), 7.83–7.84 (1H, m), 8.25 (1H, d, J = 2.0 Hz); MS (ESI): m/z 204 $[M+H]^+$.

5.2.64. Methyl 5-fluoropyridine-3-carboxylate 1-oxide (36a)

To a stirred solution of methyl 5-fluoropyridine-3-carboxylate (**35a**) (880 mg, 5.7 mmol) and urea hydrogen peroxide addition

compound (1.1 g, 12 mmol) in dichloromethane (14 mL) was added dropwise TFAA (1.6 mL, 11 mmol) at 0 °C, and the mixture was allowed to warm to room temperature and stirred for 17 h. After being cooled to 0 °C, saturated aqueous sodium hydrogen sulfite was added to the mixture. The layers were separated, and the aqueous layer was extracted three times with chloroform. The combined organic layers were washed with saturated aqueous sodium hydrogen carbonate, dried over sodium sulfate, concentrated, and vacuum-dried to give **36a** (740 mg, 76% yield) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.98 (3H, s), 7.60–7.65 (1H, m), 8.20–8.30 (1H, m), 8.60 (1H, t, J = 2.0 Hz); MS (ESI): *m/z* 172 $[M+H]^+$.

5.2.65. Methyl 6-chloropyridine-3-carboxylate 1-oxide (36b)

To a stirred solution of methyl 6-chloropyridine-3-carboxylate (**35b**) (9.80 mg, 49.1 mmol) and urea hydrogen peroxide addition compound (9.70 g, 103 mmol) in acetonitrile (100 mL) was added dropwise TFAA (13.9 mL, 98.2 mmol) at 0 °C, and the mixture was allowed to warm to room temperature and stirred for 3 h. After being concentrated, the residue was partitioned between ethyl acetate and saturated aqueous sodium hydrogen sulfite, and the layers were separated. The aqueous layer was extracted with ethyl acetate, and the combined organic layers were dried over sodium sulfate and concentrated. The residue was purified by silica gel flash column chromatography with 0–80% ethyl acetate in hexanes to give **36b** (9.45 g, 89% yield) as a colorless solid. ^{1}H NMR (400 MHz, CD_3OD): δ 3.96 (3H, s), 7.86 (1H, d, J = 8.6 Hz), 7.95 (1H, dd, J = 1.8, 8.6 Hz), 8.88 (1H, d, J = 1.8 Hz); MS (ESI): *m/z* 188 $[M+H]^+$.

5.2.66. 5-Fluoro-6-oxo-1,6-dihydropyridine-3-carboxylic acid (37)

Compound **36a** (334 mg, 1.95 mmol) in acetic anhydride (15 mL) was heated to 140 °C for 5 h. The mixture was concentrated, and the residual oil was heated to 50 °C for 15 min and concentrated. The residual brown solid was suspended in dichloromethane and filtered. The resulting solid was dried in vacuo to give methyl 5-fluoro-6-oxo-1,6-dihydropyridine-3-carboxylate (360 mg) as a brown solid. The crude ester was dissolved in 2 N NaOH (2.5 mL) and stirred at room temperature for 17 h. Concentrated HCl was added to adjust pH to ca. 3, and the resulting precipitate was collected, washed with water and diethyl ether, and dried in vacuo to give **37** (270 mg, 88% over 2 steps) as a pale-brown solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.62 (1H, dd, J = 2.3, 11.2 Hz), 7.86 (1H, s), 12.60 (1H, brs), 12.99 (1H, brs); MS (ESI): *m/z* 158 $[M+H]^+$.

5.2.67. Methyl 1-hydroxy-6-oxo-1,6-dihydropyridine-3-carboxylate (38)

To a stirred solution of **36b** (2.60 g, 12.1 mmol) in acetonitrile (10 mL) was added TFAA (20 mL) at room temperature, and the mixture was stirred at room temperature for 1 h. After being concentrated, solid sodium hydrogen carbonate and methyl alcohol was added to the residue, and the resulting suspension was filtered. The filtrate was concentrated, and the residue was purified by silica gel flash column chromatography with 0–5% methyl alcohol in chloroform to give **38** (1.34 g, 65% yield) as a pale-yellow solid. ^{1}H NMR (400 MHz, CD_3OD): δ 3.83 (3H, s), 6.52 (1H, d, J = 9.2 Hz), 7.80 (1H, dd, J = 2.4, 9.2 Hz), 8.51 (1H, d, J = 2.4 Hz); MS (ESI): *m/z* 170 $[M+H]^+$.

5.2.68. 1-Methoxy-6-oxo-1,6-dihydropyridine-3-carboxylic acid (39a)

To a stirred solution of **38** (420 mg, 2.48 mmol) and iodomethane (465 μL, 7.44 mmol) in DMF (10 mL) was added potassium carbonate (1.03 g, 7.44 mmol) at room temperature, and the mixture was stirred at room temperature overnight. After being concentrated, the residue was partitioned between ethyl acetate and water, and the layers were separated. The aqueous layer was extracted with ethyl acetate, and the combined organic layers were washed with brine, dried over sodium sulfate, and concentrated. The residue was purified by silica gel flash column chromatography with 0–75% ethyl acetate in hexanes to give methyl 1-methoxy-6-oxo-1,6-dihydropyridine-3-carboxylate (418 mg) as a pale-yellow solid. The resultant ester was dissolved in methanol (5 mL) and treated with 1 N NaOH (4.48 mL, 4.48 mmol), and the mixture was stirred at room temperature for 6 h. After being concentrated, 8 N HCl was added to the residue. The resulting precipitate was collected by filtration, washed with water, and dried in vacuo to give **39a** (254 mg, 62% yield over 2 steps) as a colorless solid. ^{1}H NMR (400 MHz, CD_3OD): δ 4.07 (3H, s), 6.63 (1H, d, J = 9.6 Hz), 7.94 (1H, dd, J = 2.4, 9.6 Hz), 8.58 (1H, d, J = 2.4 Hz); MS (ESI): *m/z* 170 $[M+H]^+$.

5.2.69. 1-Ethoxy-6-oxo-1,6-dihydropyridine-3-carboxylic acid (39b)

Compound **39b** was prepared from **38** and ethyl bromide using the procedure described for **39a** as a colorless solid (30% yield over 2 steps). ^{1}H NMR (400 MHz, CD_3OD): δ 1.38 (3H, t, J = 7.2 Hz), 4.31 (2H, t, J = 7.2 Hz), 6.63 (1H, d, J = 9.6 Hz), 7.93 (1H, dd, J = 2.2, 9.6 Hz), 8.55 (1H, d, J = 2.2 Hz); MS (ESI): *m/z* 184 $[M+H]^+$.

5.2.70. Methyl 2-fluoro-6-oxo-1,6-dihydropyridine-3-carboxylate (41)

To a stirred solution of sodium iodide (8.50 g, 56.5 mmol) in acetonitrile (60 mL) was added trimethylsilyl chloride (7.17 mL, 56.5 mmol) at 0 °C, and the mixture was allowed to warm to room temperature and stirred for 15 min. To the resulting mixture was added **40** (2.09 g, 11.3 mmol) at room temperature, and the mixture was stirred at room temperature for 17 h. After being concentrated, water and saturated aqueous sodium hydrogen sulfite were added to the residue. The resulting precipitate was collected by filtration and vacuum-dried to give **41** (1.33 g, 69% yield) as a pale-yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.93 (3H, s), 6.71 (1H, dd, J = 1.0, 8.4 Hz), 8.34 (1H, dd, J = 8.4, 9.5 Hz), 10.84 (1H, brs); MS (ESI): *m/z* 172 $[M+H]^+$.

5.2.71. 6-(Benzyloxy)-2-fluoropyridine-3-carboxylic acid (42)

Methyl 6-(benzyloxy)-2-fluoropyridine-3-carboxylate was prepared from **41** and benzyl bromide using the procedure described for **23a** as a colorless oil. Subsequently, compound **42** was prepared from the resultant benzyl ether using the procedure described for **24a** as a colorless solid (55% yield over 2 steps). ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.36 (2H, s), 6.90 (1H, dd, J = 1.2, 8.5 Hz), 7.34–7.41 (3H, m), 7.43–7.50 (2H, m), 8.28 (1H, dd, J = 8.5, 10.0 Hz), 13.23 (1H, s); MS (ESI): *m/z* 248 $[M+H]^+$.

5.3. Biology

5.3.1. Anesthetized rats cardiovascular assay

Male Sprague-Dawley rats were anesthetized with the long-acting barbiturate, inactin (100 mg/kg). Catheters were inserted into the femoral artery and vein for measurement of arterial pressure and compound administration, respectively. Additional catheter was placed in the left ventricle through the carotid artery for measurement of left ventricular pressure (LVP). The index of cardiac contractility, left ventricular dp/dt_{max} (LV dp/dt_{max}) is derived from LVP. Rats were randomly chosen for each treatment group. Following a control period, vehicle (60% propylene glycol/distilled water; 1 mL/kg) was administered. Forty-five minutes later, compound was administered. A small volume blood sample was collected at 3 and 30 min after administration for drug level determination.

5.3.2. Anesthetized dog cardiovascular assay

Male beagle dogs (n = 2–3/group) were anesthetized by 5% isoflurane vapor mixed with oxygen (2 L/min) and N_2O (3 L/min). Anesthesia was maintained by 1.5–2.0% isoflurane vapor through an endotracheal tube with an artificial ventilator (SAFER100, Aika, Tokyo). Ventilatory volume and/or rate were adjusted to maintain the level of PCO_2 within the normal range. Briefly, polyethylene catheters were inserted into the left femoral artery and vein for measurement of aortic blood pressure and infusion of compound **7m**, respectively. A 7-F Swan-Gantz catheter was advanced into the pulmonary artery through the left jugular vein for measurement of cardiac output via thermodilution utilizing a cardiac output computer (MTC-6210, Nihon Kohden, Tokyo, Japan). Central venous pressure and pulmonary arterial pressure were measured through the distal and proximal ports of the catheter, respectively. A microtip catheter (Miller, Model MPC-500, 5F) was advanced into the left ventricle via the left carotid artery for measurement of left ventricular pressure. A standard limb lead II electrocardiogram (ECG) was recorded via subcutaneous limb leads. The QT and PR intervals were determined using commercial software (SBP-4/8, Softron, Tokyo, Japan). The rate-corrected QT interval (QTc) was calculated using a Bazzett's formula: $QTc = QT/(RR^{1/2})$. Cardiac contractility (LV dp/dt_{max}) was obtained by differentiating the LVP signal with an electronic differentiator (EQ-601G, Nihon Kohden, Tokyo, Japan). Following the completion of the surgical protocol, animals were allowed to stabilize for at least 1 h and baseline data were collected. Compounds were infused over 10 min at 1, 2 and 5 mg/kg, yielding rising cumulative doses of 1, 3 and 8 mg/kg, respectively. The parameters were measured at 0, 10 and 20 min during the cardiovascular study. A blood sample was collected immediately after the end of dosing.

5.3.3. Brain Y5 receptor occupancy study

Male *mdr1a* (+/+) and *mdr1a* (-/-) CF-1 mice (Charles River, Tokyo, Japan) were administered vehicle or the Y5 antagonist (0.3, 1 and 3 mg /kg) by oral gavage. One and 24 h after drug administration animals were killed by collecting whole blood from the heart under isoflurane anesthesia. Whole brains rapidly removed and were frozen in dry ice-cold isopentane and stored at −80 °C until use. Serial coronal sections, 20 μm in thickness, of the anterior striatal (0.2∼1.0 mm anterior to Bregma) regions were cut by cryostat microtome and stored at −80 °C. Because we have confirmed that equivalent Y5 receptor occupancy was observed in both striatal and posterior hypothalamic regions after oral administration of the Y5 antagonist in the previous studies (data not shown), striatal sections were used for the Y5 receptor occupancy assay. Striatal sections were incubated with 100 pM of concentration of another Y5 antagonist with ^{35}S label (compound A; Banyu Pharmaceutical) in 50 mM Tris HCl buffer for 30 min at room temperature. Compound A is a spiroindoline class Y5 antagonist, binds to Y5 receptor with high affinity (IC_{50} for human Y5 = 0.99 nM), and is highly selective for Y5 because the binding was displaceable by Y5-selective compounds and was completely abolished in the Y5 receptor KO mouse brain (data not shown). Nonspecific binding was evaluated by using an adjacent brain slice by the addition of a 10,000-fold excess of nonlabeled Y5 antagonist. Following incubation, the striatal sections were washed, air-dried and exposed to BAS 5000 imaging plates (Fuji, Kanagawa, Japan), and autoradiographic images were quantified. Ex vivo receptor labeling by ^{35}S compound A in drug-treated animals was calculated and expressed as follows: RO (%) = 100 × [1 − (receptor labeling of drug-treated animals/receptor labeling of vehicle-treated animals)]. Compound **7m** (0.3, 1 and 3 mg /kg) was orally administered to three animals per strain, and blood samples were collected at 1 and 24 h. Plasma levels of the Y5 antagonist after oral dosing were measured by HPLC.

Acknowledgement

We thank Mr. Hirokazu Ohsawa for the measurement of HRMS.

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