Discovery of Tetrasubstituted Imidazolines as Potent and Selective Neuropeptide Y Y5 Receptor Antagonists: Reduced Human Ether-a-go-go Related Gene Potassium Channel Binding Affinity and Potent Antiobesity Effect

Nagaaki Sato,* Makoto Ando, Shiho Ishikawa, Makoto Jitsuoka, Keita Nagai, Hirobumi Takahashi, Aya Sakuraba, Hiroyasu Tsuge, Hidefumi Kitazawa, Hisashi Iwaasa, Satoshi Mashiko, Akira Gomori, Ryuichi Moriya, Naoko Fujino, Tomoyuki Ohe, Akane Ishihara, Akio Kanatani, and Takehiro Fukami

Tsukuba Research Institute, Merck Research Laboratories, Banyu Pharmaceutical Company, Ltd., Okubo 3, Tsukuba 300-2611, Japan

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A series of novel imidazoline derivatives was synthesized and evaluated as neuropeptide Y (NPY) Y5 receptor antagonists. Optimization of previously reported imidazoline leads, **1a** and **1b**, was attempted by introduction of substituents at the 5-position on the imidazoline ring and modification of the bis(4-fluorphenyl) moiety. A number of potent derivatives without human ether-a-go-go related gene potassium channel (hERG) activity were identified. Selected compounds, including **2a**, were shown to have excellent brain and CSF permeability. Compound **2a** displayed a suitable pharmacokinetic profile for chronic in vivo studies and potently inhibited D-Trp^{34}NPY-induced acute food intake in rats. Oral administration of **2a** resulted in a potent reduction of body weight in a diet-induced obese mouse model.

Introduction

Neuropeptide Y (NPY),[a] identified in 1982, is a 36-amino acid peptide neurotransmitter. NPY is a member of the pancreatic polypeptide family, which includes peptide YY and pancreatic polypeptide.[1] NPY is widely distributed in both the central and the peripheral nervous systems and has various physiological functions. Concentrations of NPY and its mRNA in the hypothalamus are affected by feeding status, including food deprivation and refeeding.[2,3] Chronic central infusion of NPY in rodents results in a syndrome similar to that in some genetic obesity models, characterized by hyperphagia, insulin resistance, hyperinsulinemia, and reduced thermogenic activity in brown adipose tissue.[4] Furthermore, NPY-deficient *ob*/*ob* mice are less obese and have reduced food intake compared with *ob*/*ob* mice.[5] Therefore, NPY is thought to have a major role in the physiological control of energy homeostasis.

Five distinct NPY receptor subtypes (Y1, Y2, Y4, Y5, and mouse y6) have been cloned to date,[6] and pharmacological data suggest that the NPY Y5 receptor is involved in regulation of feeding and energy expenditure. Administration of Y5 antagonists suppressed Y5 agonist-induced food intake and diet-induced body weight gain,[7,8] and mice lacking the Y5 receptor showed a reduced response to exogenously administered Y5 receptor agonists.[9] In addition, chronic intracerebroventricular administration of a Y5-specific agonist, D-Trp^{34}NPY, lead to obesity.[10] These results suggest that Y5 is a key regulator involved in the development of obesity in rodents; hence, Y5 antagonists have been targeted by many pharmaceutical companies as potential antiobesity drugs.[11] There are conflicting reports regarding the antiobesity effects of Y5 antagonists in rodents.[8,11c,d] Therefore, Y5 specific antagonists, which are effective in wild-type mice but ineffective in Y5 knockout mice, have considerable utility to examine the mechanism-based efficacy of Y5 antagonists in vivo. Our laboratory has reported two structurally diverse Y5 specific antagonists, **25** and **26**.[8] Chronic administration of compound **25** suppressed body weight gain in diet-induced obese (DIO) rodents while having no effects on lean and genetically obese rodents, highlighting the pathophysiological roles of Y5 in the diet-induced obesity that may represent the significant populations of obese human subjects in the westernized countries.[8a] Recently, it was reported that administration of a potent and highly selective NPY Y5 receptor antagonist, MK-557, resulted in modest weight loss in obese human subjects.[12] Considering the well-tolerated Y5 antagonist treatment in humans and possibility of the combination therapy with Y1 antagonists, development of safe and mechanistically validated Y5 antagonists is of considerable importance.[11d]

We previously reported the discovery of a potent imidazoline class of Y5 antagonists, **1a** and **1b** (Figure 1). After oral administration, compound **1b** was shown to inhibit food intake

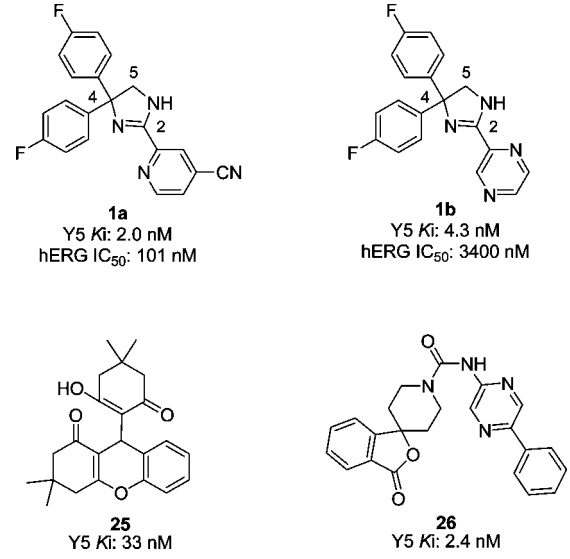

Figure 1. Structures of Y5 antagonist **1a**, **1b**, **25**, and **26**.

* To whom correspondence should be addressed. Phone: +81-29-877-2004. Fax: +81-29-877-2029. E-mail: nagaaki_sato@merck.com.

[a] Abbreviations: aCSF, artificial cerebrospinal fluid; APCI, atmospheric pressure chemical ionization; Boc, *tert*-butoxycarbonyl; CNS, central nervous system; CSF, cerebrospinal fluid; DIO, diet-induced obesity; ESI, electrospray ionization; hERG, human ether-a-go-go related gene potassium channel; NOE, nuclear Overhauser effect; NPY, neuropeptide Y; SAR, structure—activity relationship; SD rats, Sprague—Dawley rats.

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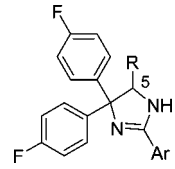
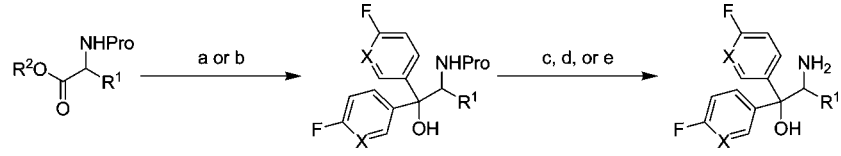
Table 1. In Vitro Profiles of 5-Substituted Derivatives **1c**−**h**[a]



compd	R	Ar	hY5R binding[b] *Ki* (nM)	hERG binding[c] IC_{50} (nM)	log$D_{7.4}$[d]	predicted CL_H[e] (mL/min/kg)
1a	H		2.0 ± 0.3	101 ± 14	4.0	12
1b	H		4.3 ± 0.2	3400 ± 170	3.5	51
1c	(*S*)-Me		1.0 ± 0.2	1730 ± 30	4.1	28
1d	(*R*)-Me		300 ± 41	ND[f]	ND[f]	ND[f]
1e	(*S*)-Et		2.6 ± 0.2	490 ± 40	ND[f]	ND[f]
1f	(*S*)-*n*-Pr		8.6 ± 0.9	490 ± 30	ND[f]	ND[f]
1g	(*R*)-CH_2OH		3.0 ± 0.1	6100±1000	3.4	11
1h[g]	(*S*)-Me		1.9 ± 0.2	>10000	3.7	57

[a] The values represent the mean ± SEM or the mean (n = 3−4). [b] [^{125}I]PYY binding assay in LMtk$^-$ cells expressing human recombinant Y5 receptors; see Experimental Section. [c] Displacement binding assay of [^{35}S]*N*-[(4*R*)-1′-[(2*R*)-6-cyano-1,2,3,4-tetrahydro-2-naphthyl]-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. [d] Octanol−water distribution coefficient at pH 7.4; see ref 24 for experimental details. [e] CL_H was determined by the serum incubation method using isolated rat hepatocytes. [f] ND = not determined. [g] Hydrochloride salt.

Scheme 1. Preparation of Aminoalcohol Intermediates **11a**−**f**[a]



5 : R^1 = (*S*)-Me, R^2 = Et, Pro = Boc
6 : R^1 = (*R*)-Me, R^2 = Me, Pro = Boc
7 : R^1 = (*S*)-Et, R^2 = Me, Pro = Boc
8 : R^1 = (*S*)-*n*-Pr, R^2 = Me, Pro = Boc
9 : R^1 = (*S*)-CH_2OMOM, R^2 = Me, Pro = Cbz

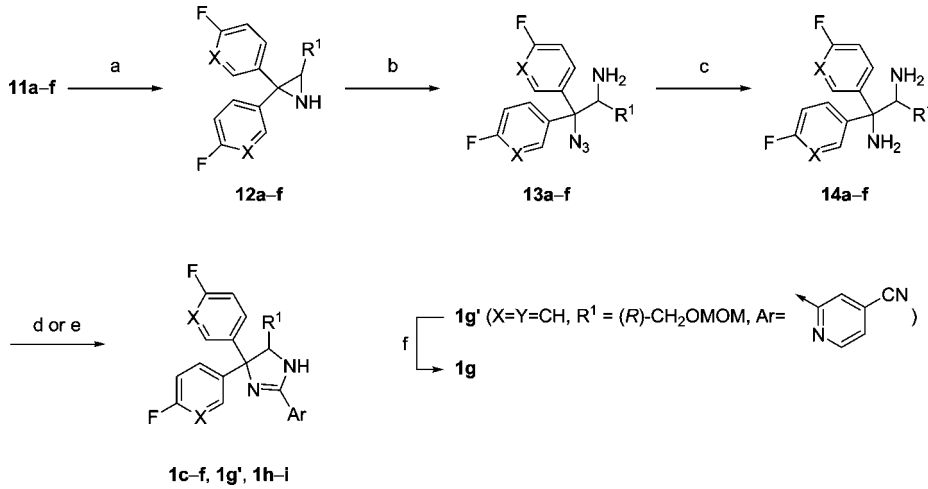
10a : X = CH, R^1 = (*S*)-Me
10b : X = CH, R^1 = (*R*)-Me
10c : X = CH, R^1 = (*S*)-Et
10d : X = CH, R^1 = (*S*)-*n*-Pr
10e : X = CH, R^1 = (*S*)-CH_2OMOM
10f : X = N, R^1 = (*S*)-Me

[a] Reagents and conditions: (a) 4-fluorophenylmagnesium bromide, THF, 0 °C to rt. (b) (i) 5-Bromo-2-fluoropyridine, *n*-BuLi, Et_2O, −78 °C; (ii) **5**, Et_2O, −78 °C to rt. (c) 4 N HCl−EtOAc, rt. (d) 10% Pd/C, H_2, MeOH, rt; (e) TFA, CH_2Cl_2, rt.

in rats induced by centrally administered Y5-preferring agonist, D-Trp^{34}NPY, where the minimum effective dose of **1b** was 30 mg/kg.[13] Further investigation of leads **1a** and **1b** was necessary to address issues such as moderate in vivo potency and potent human ether-a-go-go related gene potassium channel (hERG) inhibitory activity[14] in order to identify clinical candidates from this class (Table 1). In this report, compounds **1a** and **1b** were further optimized by modification of the 2, 4, and 5-positions of the imidazoline substituents, resulting in the identification of a potent and selective derivative, **2a**.

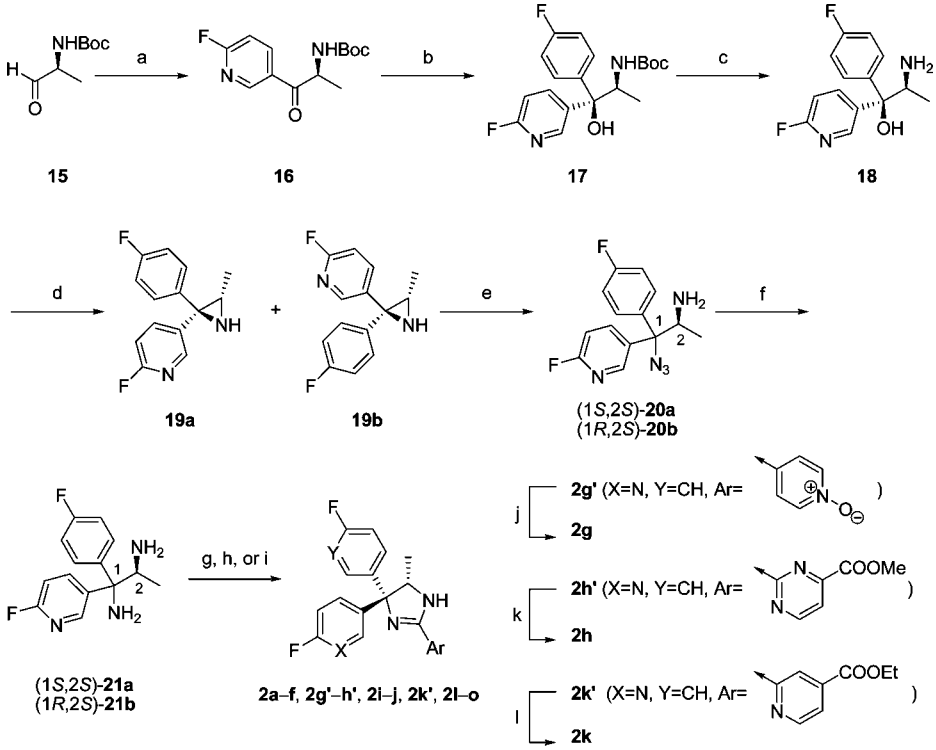
Chemistry. The synthetic route for the derivatives reported herein is illustrated in Schemes 1−4. The aminoalcohol intermediates **11a**−**f** having bis(4-fluorophenyl) or bis(6-fluoro-3-pyridyl) groups were prepared from esters **5**,[15a] **6**, **7**,[15b] **8**,[15c] and **9**[15d] (Scheme 1). Esters **5**−**8** were converted to **10a**−**d** by coupling with 4-fluorophenylmagnesium bromide, followed by

Scheme 2. Preparation of Compounds **1c**−**i**[a]



[a] Reagents and conditions: (a) PPh_3, Br_2, Et_3N, toluene, 0 °C. (b) NaN_3, NH_4Cl, DMF, H_2O, 110 °C. (c) 10% Pd/C, H_2, MeOH, rt. (d) Cat. $Yb(OTf)_3$ or $Sc(OTf)_3$, ArCN, toluene, 100−110 °C. (e) Methyl arylcarboximidoate, MeOH, rt. (f) 6 N HCl, THF, rt.

Scheme 3. Preparation of Compounds **2a**−**o**[a]



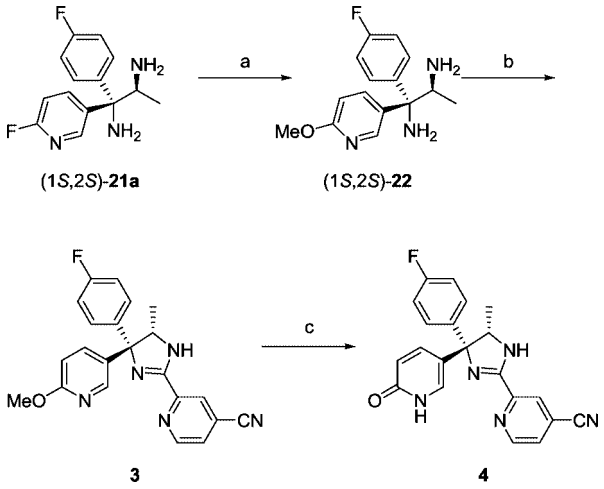
[a] Reagents and conditions: (a) (i) 5-Bromo-2-fluoropyridine, *n*-BuLi, Et_2O, −78 °C; (ii) **15**, Et_2O, −78 °C to rt; (iii) DMSO, oxalyl chloride, CH_2Cl_2, −78 °C, then DIEA, −78 to 0 °C. (b) 4-Fluorophenylmagnesium bromide, THF, 0 °C. (c) TFA, CH_2Cl_2, rt. (d) PPh_3, CCl_3CCl_3, Et_3N, CH_3CN, 50 °C—rt. (e) NaN_3, NH_4Cl, DMF, H_2O, 70 °C. (f) 10% Pd/C, H_2, MeOH, rt. (g) Cat. $Yb(OTf)_3$ or $Sc(OTf)_3$, ArCN, toluene, 100−110 °C. (h) Methyl arylcarboximidoate, MeOH, rt. (i) (i) ArCOOH, WSC·HCl, pyridine, rt; (ii) $Yb(OTf)_3$ or $Sc(OTf)_3$, toluene, 130 °C. (j) TMSCN, Et_3N, CH_3CN, 80 °C. (k) (i) NH_3, MeOH, −40 °C to rt; (ii) TFAA, Et_3N, THF, −78 °C to rt. (l) NH_3, MeOH, −40 °C to rt.

deprotection of the *tert*-butoxycarbonyl (Boc) group under acidic conditions to afford the corresponding aminoalcohol **11a**−**d**. Ester **9** was converted to the corresponding *N*-protected aminoalcohol **10e** in the same manner, which was hydrogenated to give aminoalcohol **11e**. Compound **11f** was prepared from ethyl ester **5**. Ester **5** was coupled with (2-fluoropyridine-5-yl)lithium, followed by deprotection of the Boc group to furnish the desired intermediate **11f**.

Synthesis of the imidazoline derivatives **1c**−**i** from aminoalcohols **11a**−**f** is shown in Scheme 2. Treatment of the aminoalcohols **11a**−**f** with triphenylphosphine and bromine provided the corresponding aziridines **12a**−**f**.[16] The aziridine ring was opened using sodium azide to give **13a**−**f**,[17] which were reduced by hydrogenation to give diamines **14a**−**f**.[18] Formation of the imidazoline rings of **1c**−**f**, **1g′**, and **1h**−**i** was accomplished using reaction conditions d[19] or e,[20] as depicted in Scheme 2. The methoxymethyl group of **1g′** was cleaved under acidic conditions to give the desired imidazoline **1g**.

Synthesis of compounds **2a**−**o** is illustrated in Scheme 3. Ketone **16** was prepared by the coupling of known aldehyde **15** with (2-fluoropyridin-5-yl)lithium, followed by oxidation of the resultant alcohol. Coupling of **16** with 4-fluorophenylmag-

Scheme 4. Preparation of Compounds **3** and **4**[a]



[a] Reagents and conditions: (a) 10% HCl—MeOH, rt. (b) Pyridine-2,4-dicarbonitrile, cat. $Yb(OTf)_3$, toluene, 150 °C. (c) TMSCl, NaI, CH_3CN, rt.

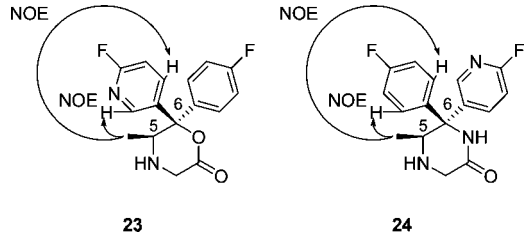


Figure 2. Determination of the absolute configurations of compounds **23** and **24** by NOE.

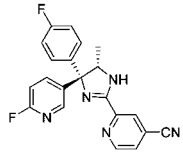
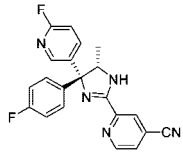
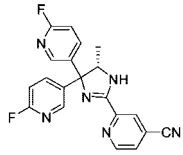
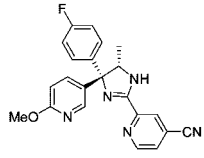
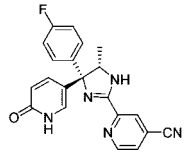
nesium bromide was followed by removal of the Boc group to give aminoalcohol **18** as a single isomer. Aminoalcohol **18** was converted to diamines **21a** and **21b** using the same reaction sequence as described for the preparation of **14a**—**f** from **11a**—**f**. Aziridine formation of **18** was effected by treatment with a preheated mixture of triphenylphosphine and hexachloroethane to afford aziridines **19a** and **19b** in 39% and 22% yields, respectively. Aziridines **19a** and **19b** were then reacted with sodium azide followed by hydrogenation to give **21a** and **21b**, which were converted to the imidazoline derivatives **2a**—**f**, **2g′**—**h′**, **2i**—**j**, **2k′**, and **2l**—**o** by reaction conditions g, h, or i (Scheme 3). Compound **2g′** was converted to the pyridine derivative **2g** by treatment with trimethylsilyl cyanide. The ester group of **2h′** was converted to the corresponding amide, which was subsequently dehydrated by trifluoroacetic anhydride in the presence of triethylamine to give cyanide **2h**. Compound **2k′** was reacted with ammonia to give amide **2k**. Compounds **3** and **4** were prepared from the diamine **21a** as shown in Scheme 4. The 2-fluoro group of **21a** was displaced by a methoxy group by treatment with a methanolic solution of hydrogen chloride to give **22**, which was coupled with pyridine-2,4-dicarbonitrile to give the imidazoline **3**. Deprotection of the methoxy group of **3** afforded **4**.

Stereochemical Assignments of the Intermediates (18 and 21a). The stereochemistry of the 1-position of **18** and **21a** was supported by nuclear Overhauser effect (NOE) studies of the corresponding morpholinone and piperazinone derivatives **23** and **24**, as shown in Figure 2. Conversion to the cyclic derivatives **23** and **24** was achieved without epimerization of the chiral centers.[21] The absolute configuration of the 5-methyl group of **23** and **24** is *S*, which were derived from starting L-alanine. For compound **23**, a characteristic NOE was observed between the *ortho*-protons of the 6-fluoro-3-pyridyl group and the methyl proton at the 5-position. For compound **24**, a characteristic NOE was observed between the *ortho*-protons of the 4-fluorophenyl group and the methyl proton at the 5-position. On the basis of these results, the absolute configurations of **23** and **24** were determined to be 5*S*,6*R* and 5*S*,6*S*, respectively.

Results and Discussion

The compounds were tested using the [^{125}I]PYY binding assay in membranes isolated from LMtk$^-$ cells transfected with cloned human Y5 receptors. Selected compounds were 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-naphthyl]-3,4-dihydro-4-hydroxyspiro[2*H*-1-benzopyran-2,4′-piperidin]-6-yl]methanesulfonamide competitive binding assay to assess QTc prolongation liability.[22] Rat hepatic clearance was assessed by the in vitro serum incubation method previously reported by our laboratory.[23] The 5-substituent of the imidazoline ring was investigated initially (Table 1). Compound **1c**, having a 5*S*-methyl group, was twice as potent as the parent **1a** and showed reduced hERG activity. In contrast, the 5*R* isomer **1d** showed a marked loss of potency. Therefore, the 5*S*-methyl group of **1c** was further substituted as in the derivatives **1e**, **1f**, and **1g**. The ethyl and *n*-propyl derivatives (**1e** and **1f**) were not as potent as **1c**, and their hERG activities were not improved. The hydroxymethyl derivative **1g** displayed ca. a 3-fold decrease in both hY5 and hERG activities compared to **1c**. A 5*S*-methyl group was introduced into pyrazine lead **1b** to afford **1h**, resulting in loss of the hERG activity while its hY5 activity was retained. Unfortunately, compound **1h** displayed relatively large predicted rat hepatic clearance (predicted $CL_H = 57$ mL/min/kg). When compounds **1c**, **1g**, and **1h** were compared, hERG activity was found to decrease as lipophilicity decreased. The literature also suggests that a decrease in lipophilicity is effective for reducing hERG activity.[25] Hence, modification of the lipophilic 4,4-bis(4-fluorophenyl) group was attempted (Table 2). Compounds **2a** and **2b**, containing a 6-fluoropyridin-3-yl group,[26] displayed excellent hY5 binding and negligible hERG binding activities. The log $D_{7.4}$ values of **2a** and **2b** were found to be significantly lower than that of **1c**. It should be noted that the metabolic stabilities of epimers **2a** and **2b** were strikingly different based on their predicted rat hepatocyte clearance. Clearly, the nitrogen atom of the fluoropyridine is recognized by metabolic enzymes. The bis(fluoropyridine) derivative **1i** showed a further decrease in log $D_{7.4}$ and negligible hERG activity. The methoxypyridine derivative **3** exhibited potent hY5 activity; however, moderate hERG activity and poor rat hepatic clearance were observed. The pyridone derivative **4** was devoid of hY5 potency. Modification of the 4- and 5-substituents of lead compounds **1a** and **1b** led to the identification of compound **2a**, which has both improved hY5 and hERG activities and good predicted rat hepatic clearance. At this point, we decided to revisit the structure—activity relationship (SAR) for the 2-substituent, using compound **2a** as a template (Table 3). The 2-subsituents were selected on the basis of our previous SAR study.[13] The cyanophenyl derivative **2c** exhibited high affinity for hY5 but moderate hERG affinity, probably due to its lipophilicity. In contrast, the methanesulfonylphenyl derivative **2d** had a slightly reduced hY5 activity relative to **2a** while its hERG activity was negligible. When the cyanopyridine isomers **2e**, **2f**, and **2g** were compared, **2g** was found to have a binding profile comparable to the parent **2a**, while **2e** and **2f** showed decreased activities. In addition, **2g** displayed good rat hepatic clearance. The

Table 2. In Vitro Profiles of Imidazoline Derivatives **1i, 2a**, **2b**, **3**, and **4**[a]

compd		hY5R binding[b] *Ki* (nM)	hERG binding[c] IC_{50} (nM)	log$D_{7.4}$[d]	predicted CL_H[e] (mL/min/kg)
2a		1.3 ± 0.2	>10000	3.2	18
2b		1.6 ± 0.2	>10000	2.9	56
1i		3.1 ± 0.5	>10000	2.2	52
3[f]		1.1 ± 0.1	6300 ± 330	3.5	44
4		145 ± 23	ND[g]	ND[g]	ND[g]

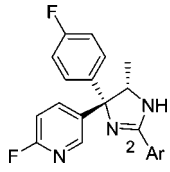
[a] The values represent the mean ± SEM or the mean ($n = 3-4$). [b] [^{125}I]PYY binding assay in LMtk$^-$ cells expressing human recombinant Y5 receptors. [c] Displacement binding assay of [^{35}S]*N*-[(4*R*)-1′-[(2*R*)-6-cyano-1,2,3,4-tetrahydro-2-naphthyl]-3,4-dihydro-4-hydroxyspiro[2*H*-1-benzopyran-2,4′-piperidin]-6-yl]methanesulfonamide in membranes derived from HEK293 cells stably transfected with the hERG gene expressing the I_{Kr} channel protein. [d] Octanol−water distribution coefficient at pH 7.4; see ref 24 for experimental details. [e] CL_H was determined by the serum incubation method using isolated rat hepatocytes. [f] Hydrochloride salt. [g] ND = not determined.

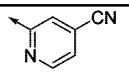
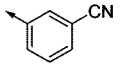
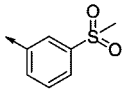
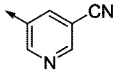
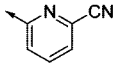
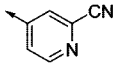
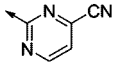
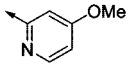
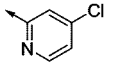
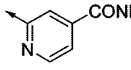
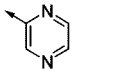
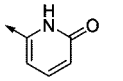
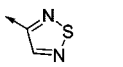
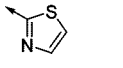
cyanopyrimidine derivative **2h** showed decreased activity. The 4-substituted pyridin-2-yl derivatives **2i**, **2j**, and **2k** were prepared and evaluated. The methoxy and chloro derivatives (**2i** and **2j**) were relatively lipophilic (log $D_{7.4}$ values >3.5) and showed moderate hERG binding activities. The aminocarbonyl derivative **2k** showed decreased hY5 activity. The pyrazine and pyridone derivatives (**2l** and **2m**) displayed suitable hY5 and hERG activities. Regarding five-membered heterocycles, the thiadiazole derivative **2n** exhibited a binding profile comparable to its pyrazine bioisoster analogue **2l**. The thiazole derivative **2o** exhibited decreased activity. Thus, this 2-substituent SAR study revealed that a variety of aryl groups are tolerated in terms of hY5 activity, and that a log $D_{7.4}$ value below 3.3 seems to be necessary to eliminate hERG activity.

Compounds **1g**, **2a**, **2c**, **2e**, **2g**, and **2m** were evaluated for brain and CSF permeability in Sprague−Dawley (SD) rats (Table 4). The data for previously reported compounds **1a**, **1b**, and **1j** are included in Table 3 for comparison.[13] With the exception of **2m**, all the newly reported test compounds showed good brain permeability based on brain concentrations and brain-to-plasma ratios. The relatively low brain permeability of **2m** is probably attributed to its mouse P-gp susceptibility (the mouse P-gp transcellular transport ratio (B-to-A/A-to-B ratio) for **2m** is 15.2).[27] In addition to the brain concentration, free brain concentration is a critical factor to take into consideration for efficacy of central nervous system (CNS) targeting agents. The free brain concentration is estimated from the CSF concentration for moderate to high-permeability compounds.[28] The CSF levels of the 4-(6-fluoropyridin-3-yl)imidazoline derivatives **2a**, **2c**, **2e**, and **2g** are remarkably high due to their high brain levels and improved CSF-to-brain ratios. It should be noted that an excellent correlation was observed between the log $D_{7.4}$ values and CSF-to-brain ratios (Figure 3). This correlation is generally valid for a series of structurally close analogues that have similar pK_a values (data not shown), probably because basicity is an important parameter that significantly influences brain tissue binding.

Highly brain and CSF permeable **2a** was chosen for further studies based on its high Y5 affinity ($K_i = 1.3$ nM), reduced hERG binding (IC_{50} >10 μM), and good metabolic stability in rat hepatocytes ($CL_H = 18$ mL/min/kg). Antagonistic activity of **2a** was assessed by measuring the antagonist inhibition of NPY-induced $[Ca^{2+}]_i$ increase in LMtk$^-$ cells expressing recombinant human Y5 receptor. In this functional assay, **2a** showed potent antagonistic activity, with an IC_{50} value of 2.0 ± 0.3 nM. Compound **2a** also displayed excellent selectivity

Table 3. In Vitro Profiles of Imidazoline Derivatives **2c**−**o**[a]



compd	Ar	hY5R binding[b] *Ki* (nM)	hERG binding[c] IC_{50} (nM)	log$D_{7.4}$[d]	predicted CL_H[e] (mL/min/kg)
2a		1.3 ± 0.2	>10000	3.2	18
2c		0.8 ± 0.1	4330 ± 940	3.6	9.0
2d		2.5 ± 0.6	>10000	2.9	6.0
2e		3.2 ± 0.3	>10000	2.9	12
2f		5.9 ± 0.7	6930 ± 1970	3.3	13
2g		1.0 ± 0.1	>10000	3.2	13
2h		3.6 ± 0.3	>10000	2.2	30
2i		2.5 ± 0.4	6330 ± 380	3.6	58
2j[g]		1.8 ± 0.3	7800 ± 910	3.8	58
2k		5.1 ± 0.7	>10000	2.4	7
2l		2.2 ± 0.1	>10000	2.5	42
2m		1.4 ± 0.2	>10000	2.4	31
2n		2.0 ± 0.3	>10000	3.1	58
2o		4.1 ± 0.4	>10000	3.0	ND[f]

[a] The values represent the mean ± SEM or the mean (n = 3−4). [b] [^{125}I]PYY binding assay in LMtk$^-$ cells expressing human recombinant Y5 receptors. [c] Displacement binding assay of [^{35}S]*N*-[(4*R*)-1′-[(2*R*)-6-cyano-1,2,3,4-tetrahydro-2-naphthyl]-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. [d] Octanol−water distribution coefficient at pH 7.4; see ref 24 for experimental details. [e] CL_H was determined by the serum incubation method using isolated rat hepatocytes. [f] ND = not determined. [g] Hydrochloride salt.

with respect to other NPY receptor subtypes (hY1, hY2, and hY4 binding: >10 μM)[29] and to a panel of 167 diverse, unrelated binding sites (IC_{50} >1 μM for all the binding sites tested). Compound **2a** was shown to have good pharmacokinetic profiles both in rats and monkeys (Table 5). Note that the plasma clearance value (CL_P = 20 mL/min/kg) is in good agreement with the predicted in vitro rat hepatic clearance (CL_H = 18.0 mL/min/kg).

Compound **2a** was tested in an agonist-induced acute food intake model (Figure 4). The compound was orally administered 2 h prior to icv dosing of either theY5 selective agonist D-Trp^{34}NPY or artificial CSF, then cumulated food intake was measured for 2 h. Compound **2a** showed potent dose-dependent inhibition of food intake, with 93% inhibition achieved at 1 mg/kg.

The excellent in vivo efficacy of **2a** in the acute food intake model prompted us to evaluate **2a** for chronic efficacy. The chronic antiobesity effect of **2a** was evaluated in established DIO mice fed a moderately high-fat diet (Figure 5). Compound **2a** was orally administered once daily for 4 days, then twice daily for an additional 15 days. Compound **2a** significantly reduced the body weight of the established DIO mice at doses of 3 and 10 mg/kg (Figure 5a), with apparent maximum efficacy at 3 mg/kg. Body weight reduction was not observed in DIO Y5 receptor deficient mice following 3 mg/kg twice daily dosing of **2a** for 15 days, clearly demonstrating the Y5-specific efficacy of compound **2a** in DIO mice (Figure 5b). Plasma exposure on the final day was monitored (Figure 6). The plasma exposure was dose-proportional in the DIO wild-type mice (3 mg/kg vs 10 mg/kg), and the plasma levels after 3 mg/kg dosing were comparable in the DIO wild-type and Y5 deficient mice.

Conclusion

Previously reported imidazoline leads **1a** and **1b** were optimized to remove hERG activity and potentiate in vivo efficacy. Introduction of substituents at the 5-position of the imidazoline ring and modification of the lipophilic 4,4-bis(4-fluorophenyl) group led to the identification of the potent and selective Y5 receptor antagonist **2a**, which showed negligible hERG activity and good pharmacokinetic profile and brain penetration in rats. Compound **2a** potently suppressed Y5 selective agonist D-Trp^{34}NPY-induced food intake in SD rats. The Y5 specific chronic antiobesity effect of **2a** was demonstrated by body weight reduction in established DIO mice.

The potential cardiovascular effects of compound **2a** were evaluated in anesthetized and ventilated dogs.[31] At 2 mg/kg iv dosing (C_{max} = 12 μM), no adverse treatment-related cardiovascular effects were observed. However, at 5 mg/kg iv dosing (C_{max} = 25 μM), **2a** produced significant changes in mean arterial pressure (+28%), left ventricular systolic pressure (+29%), cardiac contractility (+50%), cardiac output (+46%), femoral blood flow (−32%), and a moderate prolongation of the QTc interval (+7%).

Further characterization of compound **2a** is ongoing to assess its potential as a clinical development candidate. Efforts to mitigate the cardiovascular liability by further structural modification are also ongoing.

Experimental Section

Chemistry. General Procedures. Unless otherwise noted, all solvents, chemicals, and reagents were obtained commercially and used without purification. The ^{1}H NMR spectra were obtained at 400 MHz on a MERCURY-400 (Varian) or a JMN-AL400 (JEOL) spectrometer, with chemical shift (δ, ppm) reported relative to TMS as an internal standard. Mass spectra were recorded with electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) on a Waters micromass ZQ, micromass Quattro II or micromass Q-Tof-2 instrument. Flash chromatography was carried out with prepacked silica gel columns (KP-Sil silica) from Biotage.

Table 4. Brain and CSF Permeability of Selected Compounds[a]

compd	plasma[b] (μM)	brain level[b] (nmol/g)	CSF level[b] (μM)	brain-to-plasma ratio	CSF-to-brain ratio	log $D_{7.4}$
1a[c]	1.3	6.4	0.025	4.9	0.004	4.0
1b[c]	1.2	5.9	0.064	4.9	0.011	3.5
1g	1.1	2.3	0.057	2.1	0.025	3.4
1j[c]	1.4	8.1	0.027	5.8	0.003	4.3
2a	2.6	7.0	0.21	2.7	0.029	3.2
2c	2.6	6.1	0.13	2.3	0.022	3.6
2e	4.4	5.3	0.46	1.2	0.087	2.9
2g	3.7	3.2	0.22	0.9	0.069	3.2
2m	3.7	0.36	0.050	0.1	0.139	2.4

[a] The plasma, brain, and CSF concentrations were determined 2 h after oral administration of 10 mg/kg of test compounds. [b] The values represent the mean ($n = 2-3$). [c] The synthesis and brain penetration data of, **1a**, **1b**, and 3-[4,4-bis(4-fluorophenyl)-4,5-dihydro-1*H*-imidazol-2-yl]benzonitrile (**1j**) were reported previously. See ref 13 for details.

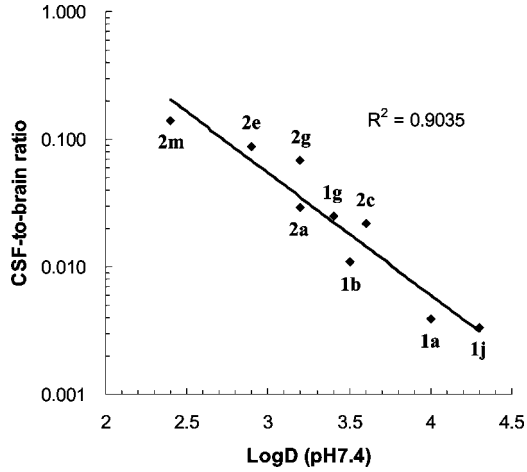


Figure 3. Relationship between log $D_{7.4}$ values and CSF-to-brain ratios of compounds in Table 4.

Table 5. Phamacokinetic Parameters of **2a**[a]

species	iv CL_P (mL/min/kg)	V_{dss} (L/kg)	PO $AUC_{0-\infty}$ (μM·h)	C_{max} (μM)	F (%)
rat[b]	20	6.2	1.8	0.50	77
monkey[c]	8.0	4.4	4.6	0.59	76

[a] The values represent the mean ($n = 3$). [b] 1 mg/kg po and 1 mg/kg iv in $n = 3$ animals/dose. [c] 1 mg/kg po and 0.3 mg/kg iv in $n = 3$ animals/dose.

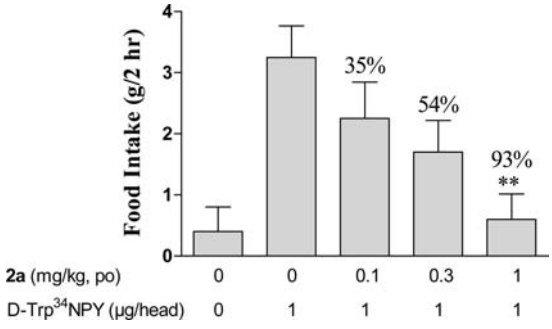


Figure 4. Effect of **2a** on food intake induced by D-Trp[34]NPY. Compound **2a** was orally administered 2 h before third ventricle injection of D-Trp[34]NPY (1 μg/head). The values represent the mean ± SEM ($n = 5-9$). ** $P < 0.01$ (compared with only D-Trp[34]NPY treated group).

Preparative thin-layer chromatography (TLC) was performed on a TLC Silica Gel 60 F (Merck KGaA). Preparative HPLC purification was carried out on a YMC-Pack *Pro* C18 (YMC, 50 mm × 30 mm id), eluting with a gradient of CH_3CN/0.1% aq CF_3CO_2H = 10/90 to 50/50 over 8 min at a flow rate of 40 mL/min. Purity of the target compounds was determined by HPLC with the two different eluting methods as follows. Analytical HPLC was performed on a SPELCO Ascentis Express (150 mm × 4.6 mm id), eluting with a gradient of (A) 0.1% H_3PO_4/CH_3CN = 95/5 to 10/90 over 7 min followed by 10/90 isocratic over 1 min and (B) 10 mM potassium phosphate buffer (pH 6.6)/CH_3CN = 95/5 to 20/80 over 7 min followed by 20/80 isocratic over 1 min (detection at 210 nm). All tested compounds had a purity of >95%. High resolution mass spectra were recorded with electron-spray ionization (ESI) on a micromass Q-Tof-2 instrument.

2-[(5*S*)-4,4-Bis(4-fluorophenyl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]-4-pyridinecarbonitrile (1c). To a solution of **14a** (200 mg, 0.76 mmol) and 2,4-pyridinedicarbonitrile (108 mg, 0.84 mmol) in toluene (5.0 mL) was added ytterbium tris(trifluoromethanesulfonate) (47 mg, 0.076 mmol), and the mixture was heated to 100 °C for 15 h in a sealed tube. 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 preparative HPLC with 10−50% 0.1% CF_3CO_2H-CH_3CN in 0.1% aq CF_3CO_2H to give **1c** (49.6 mg, 17% yield) as a colorless solid; HPLC purity: 98.8%. ^{1}H NMR (400 MHz, CD_3OD): δ 0.89 (3H, d, $J = 6.4$ Hz), 4.83−4.90 (1H, m), 7.00−7.10 (4H, m), 7.20−7.25 (2H, m), 7.50−7.55 (2H, m), 7.82 (1H, dd, $J = 1.2$, 4.8 Hz), 8.37−8.43 (1H, m), 8.87−8.90 (1H, m). HRMS (ES^+) calcd for $C_{22}H_{17}N_4F_2$ $[M + H]^+$ m/z 375.1421, found m/z 375.1418. $[\alpha]^{25}_D$: −341° (*c* 1.00, CH_3OH).

2-[(5*R*)-4,4-Bis(4-fluorophenyl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]-4-pyridinecarbonitrile (1d). Compound **1d** was prepared from **14b** using the procedure described for **1c** as a pale-pink solid (22% yield); HPLC purity: 98.5%. ^{1}H NMR (400 MHz, CD_3OD): δ 0.88 (3H, d, $J = 6.8$ Hz), 4.80−4.90 (1H, m), 6.98−7.10 (4H, m), 7.18−7.26 (2H, m), 7.48−7.54 (2H, m),7.78−7.83 (1H, m), 8.40 (1H, s), 8.86 (1H, d, $J = 4.8$ Hz); HRMS (ES^+) calcd for $C_{22}H_{17}N_4F_2$ $[M + H]^+$ m/z 375.1421, found m/z 375.1413. $[\alpha]^{25}_D$: +317° (*c* 0.60, CH_3OH).

2-[(5*S*)-5-Ethyl-4,4-bis(4-fluorophenyl)-4,5-dihydro-1*H*-imidazol-2-yl]-4-pyridinecarbonitrile (1e). Compound **1e** was prepared from **14c** using the procedure described for **1c** as a pale-yellow solid (28% yield); HPLC purity: 97.7%. ^{1}H NMR (400 MHz, CD_3OD): δ 0.90−1.10 (4H, m), 1.16−1.28 (1H, m), 4.50−4.65 (1H, m), 6.97−7.10 (4H, m), 7.15−7.23 (2H, m), 7.47−7.55 (2H, m), 7.83 (1H, dd, $J = 1.2$, 4.8 Hz), 8.42 (1H, s), 8.85−8.90 (1H, m). HRMS (ES^+) calcd for $C_{23}H_{19}N_4F_2$ $[M + H]^+$ m/z 389.1578, found m/z 389.1571. $[\alpha]^{25}_D$: −255° (*c* 1.00, CH_3OH).

2-[(5*S*)-4,4-Bis(4-fluorophenyl)-5-propyl-4,5-dihydro-1*H*-imidazol-2-yl]-4-pyridinecarbonitrile (1f). Compound **1f** was prepared from **14d** using the procedure described for **1c** as a colorless amorphous (39% yield); HPLC purity: 98.2%. ^{1}H NMR (400 MHz, CD_3OD): δ 0.83 (3H, t, $J = 7.2$ Hz), 0.95−1.20 (2H, m), 1.30−1.60 (2H, m), 4.60−4.70 (1H, m), 6.98−7.12 (4H, m), 7.14−7.22 (2H, m), 7.47−7.56 (2H, m), 7.83 (1H, dd, $J = 1.6$, 4.8 Hz), 8.42 (1H,

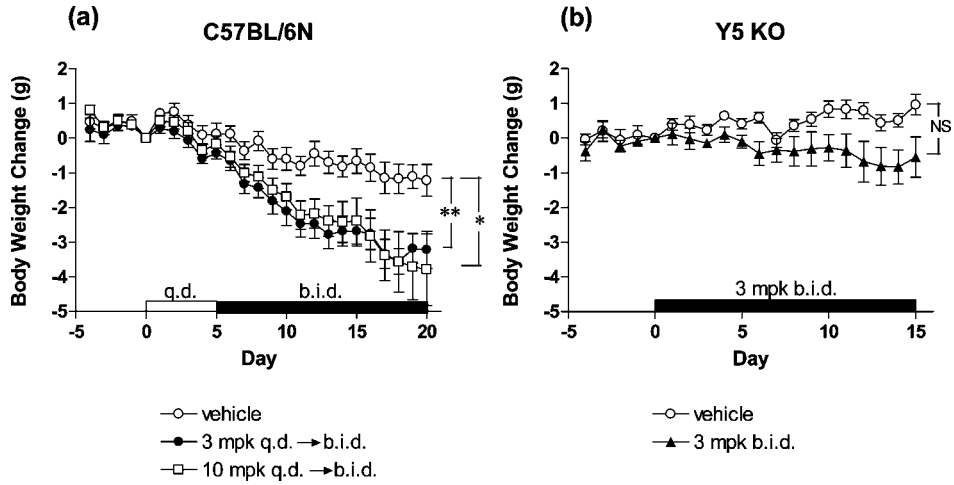


Figure 5. Effect of compound **2a** on body weight of established DIO mice (a) and established DIO Y5 receptor deficient mice (b) fed a moderately high-fat diet. The values represent the mean ± SEM ($n = 7-10$). * $P < 0.05$, ** $P < 0.01$ (compared with vehicle).

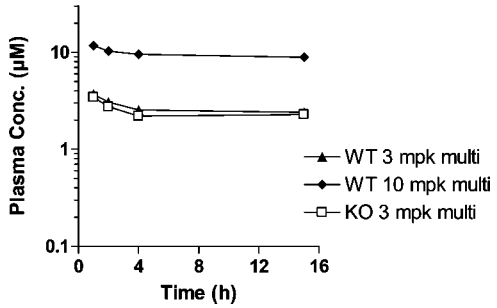


Figure 6. Plasma drug levels after the final oral dosing of compound **2a** in chronic in vivo efficacy studies in MHF diet-fed C57BL/6N mice and MHF diet-fed Y5 receptor deficient mice. See Experimental Section for details.

s), 8.84−8.90 (1H, m). HRMS (ES^+) calcd for $C_{24}H_{21}N_4F_2$ [M + H]$^+$ *m*/*z* 403.1734, found *m*/*z* 403.1729. $[\alpha]^{25}_D$: −262° (*c* 0.56, CH_3OH).

2-[(5*R*)-4,4-Bis(4-fluorophenyl)-5-(hydroxymethyl)-4,5-dihydro-1*H*-imidazol-2-yl]-4-pyridinecarbonitrile (1g). To a solution of **14e** (1.50 g, 4.65 mmol) and 2,4-pyridinedicarbonitrile (601 mg, 4.65 mmol) in toluene (30 mL) was added ytterbium tris(trifluoromethanesulfonate) (289 mg, 0.465 mmol), and the mixture was heated to reflux for 2 days in a sealed tube. 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, concentrated, and vacuum-dried. The crude material was dissolved in THF (20 mL) and treated with 6 N hydrochloric acid (10 mL), and the mixture was stirred for 1 day. After being neutralized with solid sodium hydrogen carbonate, the resulting mixture was partitioned between ethyl acetate and saturated aqueous sodium hydrogen carbonate. The layers were separated, and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and concentrated. The residue was purified by flash chromatography with 50% ethyl acetate in hexanes to give **1g** (385 mg, 21% yield over 2 steps) as a pale-yellow foam; HPLC purity: 96.7%. ^{1}H NMR (300 MHz, $CDCl_3$): tautomers δ 3.08−3.20 (0.6H, m), 3.30 (1.4H, dd, J = 4.5, 10.8 Hz), 4.69 (0.7H, dd, J = 4.5, 8.9 Hz), 4.68 (0.3H, dd, J = 4.3, 8.2 Hz), 6.59 (0.7H, s), 6.66 (0.3H, s), 6.93−7.09 (4H, m), 7.18−7.30 (2H, m), 7.30−7.40 (0.6H, m), 7.45−7.55 (1.4H, m), 7.55−7.63 (1H, m), 8.45 (0.3H, s), 8.57 (0.7H, s), 8.73−8.80 (1H, m). HRMS (ES^+) calcd for $C_{22}H_{17}N_4OF_2$ [M + H]$^+$ *m*/*z* 391.1370, found *m*/*z* 391.1367. $[\alpha]^{25}_D$: −313° (*c* 1.00, $CHCl_3$).

2-[(5*S*)-4,4-Bis(4-fluorophenyl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]pyrazine Hydrochloride (1h Hydrochloride). To a stirred solution of **14a** (50.0 mg, 0.191 mmol) in methanol (1.0 mL) was added methyl pyrazine-2-carboximidoate methanesulfonate[30] (44.0 mg, 0.191 mmol) at room temperature, and the mixture was stirred at room temperature for 2 days under a nitrogen atmosphere. The reaction 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 flush chromatography with 33% ethyl acetate in hexanes to give **1h**. A hydrochloride salt of **1h** was prepared by treatment with 4 N hydrogen chloride in dioxane as a colorless foam (49.0 mg, 73% yield); HPLC purity: 99.4%. ^{1}H NMR (300 MHz, $CDCl_3$): tautomers δ 0.90 (2.1H, d, J = 6.4 Hz), 0.99 (0.9H, d, J = 6.9 Hz), 4.70 (0.7H, q, J = 6.4 Hz), 5.03 (0.3H, q, J = 6.9 Hz), 5.97−6.00 (0.7H, m), 6.43−6.45 (0.3H, m), 6.95−7.08 (4H, m), 7.25−7.37 (2.8H, m), 7.51−7.56 (1.2H, m), 8.53−8.55 (1H, m), 8.66 (1H, d, J = 2.5 Hz), 9.42 (0.3 H, d, J = 1.7 Hz), 9.54 (0.7H, d, J = 1.5 Hz). HRMS (ES^+) calcd for $C_{20}H_{17}N_4F_2$ [M + H]$^+$ *m*/*z* 351.1421, found *m*/*z* 351.1412. $[\alpha]^{25}_D$: −289° (*c* 0.21, CH_3OH).

2-[(5*S*)-4,4-Bis(6-fluoro-3-pyridyl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]-4-pyridinecarbonitrile (1i). Compound **1i** was prepared from **14f** using the procedure described for **1c** as a pale-yellow solid (46% yield); HPLC purity: 99.6%. ^{1}H NMR (300 MHz, $CDCl_3$) tautomers δ 0.93 (2.7H, d, J = 6.4 Hz), 1.01 (0.3H, d, J = 6.8 Hz), 4.77 (0.9H, d, J = 6.4 Hz), 5.00−5.10 (0.1H, m), 6.25 (0.9H, brs), 6.60 (0.1H, brs), 6.89−6.92 (2H, m), 7.62 (1H, dd, J = 1.3, 5.0 Hz), 7.69−7.76 (1H, m), 7.89−7.95 (1H, m), 8.14−8.15 (1H, m), 8.40−8.43 (1H, m), 8.53 (1H, s), 8.77 (1H, d, J = 5.0 Hz). HRMS (ES^+) calcd for $C_{20}H_{15}N_6F_2$ [M + H]$^+$ *m*/*z* 377.1326, found *m*/*z* 377.1327. $[\alpha]^{25}_D$: −333° (*c* 0.50, CH_3OH).

2-[(4*S*,5*S*)-4-(4-Fluorophenyl)-4-(6-fluoro-3-pyridyl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]-4-pyridinecarbonitrile (2a). Compound **2a** was prepared from **21a** using the procedure described for **1c** as a colorless amorphous (69% yield); HPLC purity: 98.1%. ^{1}H NMR (300 MHz, $CDCl_3$): tautomers δ 0.87 (2.4H, d, J = 6.7 Hz), 0.98 (0.6H, d, J = 7.1 Hz), 4.73 (0.8H, q, J = 6.7 Hz), 4.98 (0.2H, d, J = 7.1 Hz), 6.15 (0.8H, s), 6.52 (0.2H, s), 6.89 (0.8H, dd, J = 2.8, 8.5 Hz), 6.85−6.95 (0.2H, m), 6.95−7.10 (2H, m), 7.15−7.30 (2H, m), 7.61 (1H, d, J = 4.4 Hz), 7.72−7.82 (0.2H, m), 7.90 (0.8H, dt, J = 2.8, 8.5 Hz), 8.20−8.30 (0.2H, m), 8.39 (0.8H, d, J = 3.5 Hz), 8.42−8.47 (0.2H, m), 8.54 (0.8H, d, J = 1.6 Hz), 8.70−8.80 (1H, m). HRMS (ES^+) calcd for $C_{21}H_{16}N_5F_2$ [M + H]$^+$ *m*/*z* 376.1374, found *m*/*z* 376.1369. $[\alpha]^{25}_D$: −417° (*c* 1.00, $CHCl_3$).

2-[(4*R*,5*S*)-4-(4-Fluorophenyl)-4-(6-fluoro-3-pyridyl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]-4-pyridinecarbonitrile (2b). Compound **2b** was prepared from **21b** using the procedure described for **1c** as a yellow solid (55% yield); HPLC purity: 95.5%. ^{1}H NMR (300 MHz, $CDCl_3$): tautomers δ 0.96 (2.4H, d, J = 6.5 Hz), 1.01 (0.6H, d, J = 6.9 Hz), 4.76 (0.8H, q, J = 6.5 Hz), 5.07 (0.2H, q, J = 6.9 Hz), 6.14 (0.8H, brs), 6.54 (0.2H, brs), 6.84−6.91 (1H, m), 7.00−7.10 (2H, m), 7.30−7.37 (0.4H, m), 7.48−7.56 (1.6H, m), 7.60−7.70 (2H, m), 8.08 (0.8H, d, J = 2.6 Hz), 8.15 (0.2H, d, J = 2.6 Hz), 8.45 (0.2H, s), 8.57 (0.8H, s), 8.74−8.78 (1H, m). HRMS (ES$^+$) calcd for $C_{21}H_{16}N_5F_2$ [M + H]$^+$ *m/z* 376.1374, found *m/z* 376.1378. $[\alpha]^{25}_D$: −291° (*c* 0.80, CH_3OH).

3-[(4*S*,5*S*)-4-(4-Fluorophenyl)-4-(6-fluoro-3-pyridyl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]benzonitrile (2c). To a stirred solution of the **21a** (100 mg, 0.380 mmol) and 3-cyanobenzoic acid (84.0 mg, 0.571 mmol) in chloroform (2.0 mL) and pyridine (2.0 mL) was added 1-(3-diemthylaminopropyl)-3-ethylcarbodiimide hydrochloride (109 mg, 0.570 mmol) at 0 °C, and the mixture was allowed to warm to room temperature and stirred for 18 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, concentrated, and vacuum-dried. The crude material was dissolved in toluene (2.0 mL) and treated with ytterbium tris(trifluoromethanesulfonate) (24.0 mg, 0.0380 mmol), and the mixture was heated to 130 °C for 15 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 flash chromatography with 50% ethyl acetate to give **2c** (140 mg, 98% yield over 2 steps) as a colorless foam; HPLC purity: 95.3%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.89 (3H, d, J = 6.5 Hz), 4.66−4.74 (1H, m), 5.10 (1H, brs), 6.85−6.90 (1H, m), 7.02 (2H, t, J = 8.4 Hz), 7.20−7.30 (2H, m), 7.57 (1H, t, J = 7.9 Hz), 7.74−7.79 (1H, m), 7.85−7.95 (1H, m), 8.07−8.12 (1H, m), 8.19 (1H, s), 8.37−8.42 (1H, m). HRMS (ES$^+$) calcd for $C_{22}H_{17}N_4F_2$ [M + H]$^+$ *m/z* 375.1421, found *m/z* 375.1419. $[\alpha]^{25}_D$: −296° (*c* 1.00, $CHCl_3$).

2-Fluoro-5-{(4*S*,5*S*)-4-(4-fluorophenyl)-5-methyl-2-[3-(methylsulfonyl)phenyl]-4,5-dihydro-1*H*-imidazol-4-yl}pyridine (2d). Compound **2d** was prepared from **21a** and 3-(methylsulfonyl)benzoic acid using the procedure described for **2c** as a pale-yellow amorphous (24% yield over 2 steps); HPLC purity: 97.5%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.90 (3H, d, J = 6.3 Hz), 3.10 (3H, s), 4.70−4.80 (1H, m), 5.25 (1H, brs), 6.87 (1H, dd, J = 2.8, 8.3 Hz), 7.02 (2H, t, J = 8.6 Hz), 7.20−7.30 (2H, m), 7.66 (1H, t, J = 7.8 Hz), 8.89 (1H, dt, J = 2.8, 8.3 Hz), 8.07 (1H, dd, J = 1.2, 7.8 Hz), 8.23 (1H, d, J = 7.8 Hz), 8.37 (2H, s). HRMS (ES$^+$) calcd for $C_{22}H_{20}N_3O_2F_2S$ [M + H]$^+$ *m/z* 428.1244, found *m/z* 428.1238. $[\alpha]^{25}_D$: −263° (*c* 0.90, $CHCl_3$).

5-[(4*S*,5*S*)-4-(4-Fluorophenyl)-4-(6-fluoro-3-pyridyl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]-3-pyridinecarbonitrile (2e). Compound **2e** was prepared from **21a** and 5-cyanonicotinic acid[30] using the procedure described for **2c** as a colorless foam (17% yield over 2 steps); HPLC purity: 98.6%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (3H, d, J = 6.3 Hz), 4.75 (1H, q, J = 6.3 Hz), 5.38 (1H, s), 6.89 (1H, dd, J = 2.9, 8.6 Hz), 7.03 (2H, t, J = 8.6 Hz), 7.20−7.30 (2H, m), 7.85−7.95 (1H, m), 8.35−8.45 (1H, m), 8.54 (1H, s), 8.96 (1H, d, J = 2.0 Hz), 9.23 (1H, d, J = 2.0 Hz). HRMS (ES$^+$) calcd for $C_{21}H_{16}N_5F_2$ [M + H]$^+$ *m/z* 376.1374, found *m/z* 376.1370. $[\alpha]^{25}_D$: −245° (*c* 0.56, CH_3OH).

6-[(4*S*,5*S*)-4-(4-Fluorophenyl)-4-(6-fluoro-3-pyridyl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]-2-pyridinecarbonitrile (2f). Compound **2f** was prepared from **21a** and pyridine-2,6-dicarbonitrile using the procedure described for **1c** as a colorless oil (13% yield); HPLC purity: 97.3%. ^{1}H NMR (300 MHz, $CDCl_3$): tautomers δ 0.88 (2.4H, d, J = 6.5 Hz), 0.95−1.00 (0.6H, m), 4.72 (0.8H, q, J = 6.5 Hz), 4.95−5.05 (0.2H, m), 6.17 (1H, s), 6.82−6.92 (1H, m), 6.98−7.10 (2H, m), 7.20−7.30 (2H, m), 7.79 (1H, d, J = 6.6 Hz), 7.85−8.00 (2H, m), 8.39 (1H, d, J = 1.6 Hz), 8.51 (1H, d, J = 7.3 Hz). HRMS (ES$^+$) calcd for $C_{21}H_{16}N_5F_2$ [M + H]$^+$ *m/z* 376.1374, found *m/z* 376.1380. $[\alpha]^{25}_D$: −335° (c 0.20, CH_3OH).

4-[(4*S*,5*S*)-4-(4-Fluorophenyl)-4-(6-fluoro-3-pyridyl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]pyridine 1-oxide (2g′). To a stirred solution of 4-pyridinecarbonitrile 1-oxide (100 mg, 0.83 mmol) in methanol (4.0 mL) was added sodium methoxide (13.5 mg, 0.25 mmol) at room temperature, and the mixture was stirred at room temperature for 16 h before being neutralized with methanesulfonic acid (70 μL, 1.1 mmol). To the mixture was added **21a** (158 mg, 0.60 mmol) in methanol (2.0 mL) at room temperature, and the mixture was stirred at room temperature for an additional 16 h and 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, filtered, and concentrated. The residue was purified by flush chromatography with 10% methanol in chloroform to give **2g′** (216 mg, 77% yield) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.89 (3H, d, J = 7.5 Hz), 4.69 (1H, q, J = 7.5 Hz), 5.10−5.25 (1H, m), 6.87 (1H, dd, J = 3.0, 7.5 Hz), 7.15−7.30 (2H, m), 7.75 (2H, d, J = 6.8 Hz), 7.80−7.92 (1H, m), 8.20 (2H, J = 6.8 Hz), 8.37 (1H, s).MS (ESI): *m/z* 367 [M + H]$^+$.

4-[(4*S*,5*S*)-4-(4-Fluorophenyl)-4-(6-fluoro-3-pyridyl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]-2-pyridinecarbonitrile (2g). To a stirred solution of **2g′** (172 mg, 0.47 mmol) and triethylamine (332 μL, 2.4 mmol) in acetonitrile (5.0 mL) was added trimethylsilanecarbonitrile (301 μL, 2.4 mmol) at room temperature, and the mixture was heated to 90 °C for 17 h in a sealed tube. The reaction mixture was concentrated, and the resulting residue was purified by flash chromatography with 50% ethyl acetate in hexanes to give **2g** (108 mg, 61% yield) as a colorless foam; HPLC purity: 99.1%. ^{1}H NMR (300 MHz, $CDCl_3$): tautomers δ 0.89 (2.7H, d, J = 6.5 Hz), 0.96 (0.3H, d, J = 6.7 Hz), 4.74 (0.9H, q, J = 6.5 Hz), 4.90−5.00 (0.1H, m), 5.12 (0.9H, s), 5.40 (0.1H, s), 6.88 (1H, dd, J = 3.0, 8.6 Hz), 7.00−7.10 (2H, m), 7.18−7.30 (2H, m), 7.80−7.95 (2H, m), 8.12 (0.1H, s), 8.18 (0.9H, d, J = 1.0 Hz), 8.20−8.25 (0.1H, s), 8.38 (0.9H, d, J = 2.1 Hz), 8.82 (1H, d, J = 5.1 Hz). HRMS (ES$^+$) calcd for $C_{21}H_{16}N_5F_2$ [M + H]$^+$ *m/z* 376.1374, found *m/z* 376.1369. $[\alpha]^{25}_D$: −322° (*c* 0.68, CH_3OH).

Ethyl 2-[(4*S*,5*S*)-4-(4-Fluorophenyl)-4-(6-fluoro-3-pyridyl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]-4-pyrimidinecarboxylate (2h′). Compound **2h′** was prepared from **21a**, ethyl 2-cyanopyrimidine-4-carboxylate,[30] and scandium tris(trifluoromethanesulfonate) in place of ytterbium tris(trifluoromethanesulfonate) using the procedure described for **1c** as a brown amorphous (85% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.90 (3H, d, J = 6.3 Hz), 1.46 (3H, t, J = 7.2 Hz), 4.51 (2H, q, J = 7.2 Hz), 4.80 (1H, q, J = 6.3 Hz), 6.80−6.90 (1H, m), 6.95−7.10 (3H, m), 7.20−7.40 (2H, m), 7.90−8.00 (1H, m), 8.03 (1H, d, J = 4.9 Hz), 8.43 (1H, brs), 9.14 (1H, d, J = 4.9 Hz). MS (ESI): *m/z* 424 [M + H]$^+$.

2-[(4*S*,5*S*)-4-(4-Fluorophenyl)-4-(6-fluoro-3-pyridyl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]-4-pyrimidinecarbonitrile (2h). Compound **2h′** (405 mg, 0.96 mmol) was dissolved in methanol (10 mL) and cooled to −78 °C. Ammonia gas was bubbled into the solution for 20 min, and the mixture was allowed to warm to room temperature and stirred for 22 h. The resulting mixture was concentrated, and the residue was dissolved in ethyl acetate and filtered. The filtrate was concentrated, and the residue was purified by flash chromatography with 50% ethyl acetate in chloroform and then 10% methanol in chloroform to give 2-[(4*S*,5*S*)-4-(4-fluorophenyl)-4-(6-fluoro-3-pyridyl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]-4-pyrimidinecarboxamide (351 mg) as a colorless amorphous. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.90 (3H, d, J = 6.3 Hz), 4.70−4.85 (1H, m), 5.80−5.90 (1H, m), 6.24 (1H, brs), 6.80−6.95 (1H, m), 6.98−7.10 (3H, m), 7.20−7.35 (2H, m), 7.90−8.00 (1H, m), 8.21 (1H, d, J = 4.9 Hz), 8.42 (1H, brs), 9.08 (1H, d, J = 4.9 Hz). MS (ESI): *m/z* 395 [M + H]$^+$. To a stirred solution of the amide (337 mg, 0.85 mmol) and triethylamine (1.20 mL, 8.62 mmol) in THF (1.7 mL) was added trifluoroacetic anhydride (360 μL, 2.55 mmol) at −78 °C, and the mixture was allowed to warm to room

temperature and stirred for 19 h. The reaction mixture 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 magnesium sulfate, and concentrated. The residue was purified by flash chromatography with 50% ethyl acetate in chloroform to give **2h** (253 mg, 62% yield over 2 steps) as a brown amorphous; HPLC purity: 97.1%. ^{1}H NMR (300 MHz, CD_3OD): δ 0.86 (3H, d, J = 6.6 Hz), 4.80−4.93 (1H, m), 6.95−7.13 (3H, m), 7.26−7.35 (2H, m), 8.00−8.10 (2H, m), 8.35−8.40 (1H, m), 9.18 (1H, d, J = 4.9 Hz). HRMS (ES^+) calcd for $C_{20}H_{15}N_6F_2$ $[M + H]^+$ m/z 377.1326, found m/z 377.1326. $[\alpha]^{25}_D$: −351° (c 1.00, CH_3OH).

2-[(4*S*,5*S*)-4-(4-Fluorophenyl)-4-(6-fluoro-3-pyridyl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]-4-(methyloxy)pyridine (2i). Compound **2i** was prepared from **21a** and 4-(methyloxy)-2-pyridinecarbonitrile[30] using the procedure described for **1c** as a colorless foam (62% yield); HPLC purity: 95.5%. ^{1}H NMR (300 MHz, $CDCl_3$): tautomers δ 0.87 (3H, d, J = 6.3 Hz), 3.92 (3H, s), 4.60−4.75 (0.8H, m), 4.90−5.00 (0.2H, m), 5.55−5.70 (0.2H, m), 6.22 (0.8H, brs), 6.83−7.08 (4H, m), 7.20−7.30 (2H, m), 7.75−7.85 (1H, m), 7.90−8.00 (1H, m), 8.35−8.45 (2H, m). HRMS (ES^+) calcd for $C_{21}H_{19}N_4OF_2$ $[M + H]^+$ m/z 381.1527, found m/z 381.1523. $[\alpha]^{25}_D$: −334° (c 0.52, CH_3OH).

4-Chloro-2-[(4*S*,5*S*)-4-(4-fluorophenyl)-4-(6-fluoro-3-pyridyl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]pyridine Hydrochloride (2j Hydrochloride). Compound **2j** was prepared from **21a** and 4-chloro-2-pyridinecarboxylic acid using the procedure described for **2c**. A hydrochloride salt of **2j** was prepared by treatment with 4 N hydrogen chloride in dioxane as a colorless foam (39% yield over 2 steps); HPLC purity: 97.9%. ^{1}H NMR (300 MHz, CD_3OD): δ 1.09 (3H, d, J = 6.7 Hz), 5.42 (1H, q, J = 6.7 Hz), 7.18−7.35 (5H, m), 7.85−7.90 (1H, m), 8.12 (1H, ddd, J = 2.9, 8.8, 8.8 Hz), 8.31 (1H, dd, J = 0.7, 2.0 Hz), 8.40−8.45 (1H, m), 8.81 (1H, d, J = 5.3 Hz). HRMS (ES^+) calcd for $C_{20}H_{16}N_4F_2Cl$ $[M + H]^+$ m/z 385.1032, found m/z 385.1029. $[\alpha]^{25}_D$: −161° (c 1.01, CH_3OH).

Methyl 2-[(4*S*,5*S*)-4-(4-fluorophenyl)-4-(6-fluoro-3-pyridyl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]-4-pyridinecarboxylate (2k′). Compound **2k′** was prepared from **21a** and 4-methoxypyridine-2-carbonitrile[31] using the procedure described for **1c** as a yellow oil (87% yield). ^{1}H NMR (300 MHz, $CDCl_3$): tautomers δ 0.88 (2.4H, d, J = 6.3 Hz), 0.90−1.05 (0.6H, m), 3.99 (3H, s), 4.70 (0.8H, q, J = 6.3 Hz), 4.92−5.05 (0.2H, m), 6.19 (0.8H, brs), 6.60 (0.2H, brs), 6.82−6.95 (1H, m), 6.95−7.10 (2H, m), 7.20−7.32 (2H, m), 7.90−8.00 (2H, m), 8.38 (0.2H, s), 8.41 (0.8H, s), 8.73 (1H, d, J = 5.0 Hz), 8.79 (0.8H, s), 8.81 (0.2H, s). MS (ESI): m/z 409 $[M + H]^+$.

2-[(4*S*,5*S*)-4-(4-Fluorophenyl)-4-(6-fluoro-3-pyridyl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]-4-pyridinecarboxamide (2k). Compound **2k′** (339 mg, 0.83 mmol) was dissolved in methanol (5.0 mL) and cooled to −40 °C. Ammonia gas was bubbled into the solution for 15 min, and the mixture was allowed to warm to room temperature and stirred for 3 days. The resulting mixture was concentrated, and the residue was dissolved in ethyl acetate and filtered. The filtrate was concentrated, and the residue was purified by flash chromatography with 20% methanol in chloroform to give **2k** (265 mg, 81% yield) as a colorless solid; HPLC purity: 99.3%. ^{1}H NMR (400 MHz, $CDCl_3$): tautomers δ 0.88 (2.6H, d, J = 6.4 Hz), 0.95−1.05 (0.4H, m), 4.65−4.75 (0.8H, m), 4.90−5.05 (0.2H, m), 5.99 (1H, brs), 6.27 (0.8H, s), 6.50−6.80 (1.2H, m), 6.88 (1H, brd, J = 7.6 Hz), 7.02 (2H, t, J = 8.8 Hz), 7.20−7.30 (2H, m), 7.85−8.00 (2H, m), 8.30−8.40 (1H, m), 8.40−8.60 (1H, m), 8.73 (1H, d, J = 4.8 Hz). HRMS (ES^+) calcd for $C_{21}H_{18}N_5OF_2$ $[M + H]^+$ m/z 394.1479, found m/z 394.1476. $[\alpha]^{25}_D$: −287° (c 0.32, CH_3OH).

2-[(4*S*,5*S*)-4-(4-Fluorophenyl)-4-(6-fluoro-3-pyridyl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]pyrazine (2l). Compound **2l** was prepared from **21a** using the procedure described for **1h** as a pale-yellow oil (94% yield); HPLC purity: 97.9%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (3H, d, J = 6.0 Hz), 4.60−4.80 (1H, m), 6.11 (1H, s), 6.88 (1H, dd, J = 2.6, 8.6 Hz), 7.02 (2H, t, J = 8.6 Hz), 7.20−7.35 (2H, m), 7.90−8.00 (1H, m), 8.41 (1H, s), 8.50−8.60 (1H, m), 8.80 (1H, d, J = 2.6 Hz), 9.51 (1H, s). HRMS (ES^+) calcd for $C_{19}H_{16}N_5F_2$ $[M + H]^+$ m/z 352.1374, found m/z 352.1371. $[\alpha]^{25}_D$: −335° (c 0.53, CH_3OH).

6-[(4*S*,5*S*)-4-(4-Fluorophenyl)-4-(6-fluoro-3-pyridyl)-5-methyl-4,5-dihydro-1*H*-imidazol-2-yl]-2(1*H*)-pyridinone (2m). Compound **2m** was prepared from **21a** and 6-*oxo*-1,6-dihydropyridine-2-carboxylic acid using the procedure described for **2c** as a colorless amorphous (58% yield over 2 steps); HPLC purity: 97.6%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.87 (3H, d, J = 6.5 Hz), 4.70−4.80 (1H, m), 5.65−5.85 (1H, m), 6.65−6.80 (2H, m), 6.86−6.92 (1H, m), 6.98−7.06 (2H, m), 7.15−7.24 (2H, m), 7.45−7.52 (1H, m), 7.80−7.88 (1H, m), 8.33 (1H, s). HRMS (ES^+) calcd for $C_{20}H_{17}N_4OF_2$ $[M + H]^+$ m/z 367.1370, found m/z 367.1374. $[\alpha]^{25}_D$: −259° (c 0.70, CH_3OH).

2-Fluoro-5-[(4*S*,5*S*)-4-(4-fluorophenyl)-5-methyl-2-(1,2,5-thiadiazol-3-yl)-4,5-dihydro-1*H*-imidazol-4-yl]pyridine (2n). Compound **2n** was prepared from **21a** and 1,2,5-thiadiazole-3-carboxylic acid using the procedure described for **2c** as a colorless oil (54% yield over 2 steps); HPLC purity: 99.5%. ^{1}H NMR (300 MHz, $CDCl_3$): tautomers δ 0.88 (2.4H, d, J = 6.5 Hz), 0.98 (0.6H, d, J = 6.9 Hz), 4.70 (0.8H, q, J = 6.5 Hz), 4.90−5.00 (0.2H, m), 5.72 (0.8H, s), 6.08 (0.2H, s), 6.85−6.95 (1H, m), 7.00−7.10 (2H, m), 7.20−7.30 (2H, m), 7.73−7.82 (0.2H, m), 7.90 (0.8H, dt, J = 2.6, 7.6 Hz), 8.25−8.30 (0.2H, m), 8.39 (0.8H, d, J = 1.7 Hz), 9.14 (0.2H, s), 9.20 (0.8H, s). HRMS (ES^+) calcd for $C_{17}H_{14}N_5F_2S$ $[M + H]^+$ m/z 358.0938, found m/z 358.0937. $[\alpha]^{25}_D$: −369° (c 1.00, CH_3OH).

2-Fluoro-5-[(4*S*,5*S*)-4-(4-fluorophenyl)-5-methyl-2-(1,3-thiazol-2-yl)-4,5-dihydro-1*H*-imidazol-4-yl]pyridine (2o). Compound **2o** was prepared from **21a** and 1,3-thiazole-2-carboxylic acid using the procedure described for **2c** as a pale-yellow amorphous (9% yield over 2 steps); HPLC purity: 97.7%. ^{1}H NMR (300 MHz, $CDCl_3$): tautomers δ 0.88 (2.4H, d, J = 6.6 Hz), 0.97 (0.6H, d, J = 6.7 Hz), 4.69 (0.8H, q, J = 6.6 Hz), 4.88−4.98 (0.2H, m), 5.98 (0.8H, brs), 6.40 (0.2H, brs), 6.85−6.95 (1H, m), 7.01 (2H, t, J = 8.7 Hz), 7.20−7.30 (2H, m), 7.51 (1H, d, J = 3.1 Hz), 7.70−8.00 (2H, m), 8.27 (0.2H, brs), 8.40 (0.8H, brs). HRMS (ES^+) calcd for $C_{18}H_{15}N_4F_2S$ $[M + H]^+$ m/z 357.0985, found m/z 357.0984. $[\alpha]^{25}_D$: −339° (c 0.57, CH_3OH).

2-{(4*S*,5*S*)-4-(4-Fluorophenyl)-5-methyl-4-[6-(methyloxy)-3-pyridyl]-4,5-dihydro-1*H*-imidazol-2-yl}-4-pyridinecarbonitrile Hydrochloride (3 Hydrochloride). Compound **3** was prepared from **22** using the procedure described for **1c**. A hydrochloride salt of **3** was prepared by treatment with 4 N hydrogen chloride in dioxane as a pale-yellow solid (76% yield over 2 steps); HPLC purity: 97.3%. ^{1}H NMR (300 MHz, CD_3OD): δ 0.84 (3H, d, J = 6.3 Hz), 3.87 (3H, s), 4.79−4.88 (1H, m), 6.75 (1H, d, J = 8.9 Hz), 7.00−7.10 (2H, m), 7.22−7.32 (2H, m), 7.74 (1H, dd, J = 2.6, 8.9 Hz), 7.80 (1H, dd, J = 1.4, 5.0 Hz), 8.25 (1H, d, J = 2.6 Hz), 8.40 (1H, s), 8.85 (1H, d, J = 5.0 Hz). HRMS (ES^+) calcd for $C_{22}H_{19}N_5OF$ $[M + H]^+$ m/z 388.1574, found m/z 388.1566. $[\alpha]^{25}_D$: −255° (c 0.97, CH_3OH).

2-[(4*S*,5*S*)-4-(4-Fluorophenyl)-5-methyl-4-(6-*oxo*-1,6-dihydro-3-pyridyl)-4,5-dihydro-1*H*-imidazol-2-yl]-4-pyridinecarbonitrile (4). To a stirred solution of sodium iodide (90 mg, 0.60 mmol) in acetonitrile (5.0 mL) was added chloro(trimethyl)silane (76 μL, 0.60 mmol) at 0 °C, and the mixture was stirred at room temperature for 15 min. To the resultant mixture was added a free base of **3** (45 mg, 0.12 mmol) in acetonitrile (2.0 mL), and the mixture was allowed to warm up to room temperature and stirred for 2 days. The resulting mixture was concentrated, and the residue was poured into a mixture (1:1) of saturated aqueous sodium thiosulfate and saturated aqueous sodium hydrogen carbonate, which was extracted with chloroform twice. 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 **4** (14.6 mg, 33% yield) as a pale-yellow solid; HPLC purity: 98.1%. ^{1}H NMR (400 MHz, CD_3OD): δ 0.80 (3H, d, J = 6.8 Hz), 4.60−4.70 (1H, m), 6.50 (1H, d, J = 9.2 Hz), 7.10 (2H, t, J = 8.8 Hz), 7.35 (2H, dd, J = 5.4, 8.8 Hz), 7.52 (1H, d, J = 2.6 Hz), 7.62 (1H, dd, J = 2.6, 9.2 Hz), 7.84 (1H, dd, J = 1.5, 5.2 Hz), 8.41 (1H, s), 8.87 (1H, d, J = 1.5, 5.2 Hz). HRMS (ES^+) calcd for $C_{21}H_{17}N_5OF$ $[M + H]^+$ m/z 374.1417, found m/z 374.1412. $[\alpha]^{25}_D$: −333° (c 0.41, CH_3OH).

(5*S*,6*R*)-6-(4-Fluorophenyl)-6-(6-fluoro-3-pyridyl)-5-methyl-3-morpholinone (23). To a stirred solution of **18** (30 mg, 0.11 mmol) and triethylamine (40 μL, 0.28 mmol) in chloroform (3.0 mL) was added chloroacetyl chloride (11 μL, 0.14 mmol) at 0 °C, and the mixture was allowed to warm to room temperature and stirred for 2 h. The mixture was poured into saturated aqueous sodium hydrogen carbonate and extracted with chloroform twice. The combined organic layers were washed with brine, dried over magnesium sulfate, concentrated, and vacuum-dried. The crude material was dissolved in dimethylformamide (3.0 mL) and treated with sodium hydride (60% dispersion in oil, 4.0 mg, 0.28 mmol) at 0 °C, and the mixture was allowed to warm to room temperature and stirred for 1 h. The mixture was poured into water and extracted with diethyl ether. The organic layer was washed with brine, dried over magnesium sulfate, and concentrated. The residue was purified by preparative TLC with 50% ethyl acetate in hexanes to give **23** (14 mg, 40% yield) as a colorless amorphous. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.10 (3H, d, J = 6.5 Hz), 3.87 (1H, d, J = 17.2 Hz), 4.33 (1H, d, J = 17.2 Hz), 4.32−4.42 (1H, m), 6.87 (1H, dd, J = 3.0, 8.6 Hz), 7.00−7.10 (2H, m), 7.35−7.45 (2H, m), 7.60−7.70 (1H, m), 8.13 (1H, s). MS (ESI) *m*/*z* 305 $[M + H]^+$.

(5*S*,6*S*)-6-(4-Fluorophenyl)-6-(6-fluoro-3-pyridyl)-5-methyl-2-piperazinone (24). To a stirred solution of **21a** (30 mg, 0.11 mmol) and triethylamine (40 μL, 0.29 mmol) in chloroform (3.0 mL) was added chloroacetyl chloride (11 μL, 0.14 mmol) at 0 °C, and the mixture was allowed to warm to room temperature and stirred for 1 h. The reaction mixture was poured into saturated aqueous sodium hydrogen carbonate, and the layers were separated. The aqueous layer was extracted with chloroform, and the combined layers were washed with brine, dried over magnesium sulfate, and concentrated. The residue was dissolved in methanol (3.0 mL) and treated with triethylamine (32 μL, 0.23 mmol), and the mixture was heated to 85 °C for 3 days in a sealed tube. After being cooled to room temperature, the reaction mixture was concentrated. The residue was purified by flash chromatography with 10% methanol in chloroform to give **24** (13 mg, 61% yield) as a pale-brown amorphous. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.04 (3H, d, J = 6.6 Hz), 3.08 (1H, d, J = 18.3 Hz), 3.56 (1H, d, J = 18.3 Hz), 4.40−4.50 (1H, m), 6.92 (1H, dd, J = 3.0, 8.6 Hz), 7.01 (2H, t, J = 8.9 Hz), 7.15−7.30 (2H, m), 7.80−7.90 (1H, m), 8.37 (1H, d, J = 2.6 Hz). MS (ESI): *m*/*z* 304 $[M + H]^+$.

NPY Y5 Receptor Binding Assay. The assay was performed according to previously described procedures with membranes prepared as follows;[29] LMtk$^-$ cells were washed with 10 mM MOPS buffer (pH 7.4) containing 154 mM NaCl, 10 mM KCl, 0.8 mM $CaCl_2$, and 20% sucrose, homogenized and centrifuged at 1000 g for 15 min. The supernatant was centrifuged at 90000*g* for 50 min. The pellets were resuspended in 5 mM Hepes/Tris buffer (pH 7.4) and centrifuge again. The membrane fraction was resuspended by a homogenizer in the same buffer and used for the assay.

$[Ca^{2+}]_i$ Response Assay. CHO-K1 cells stably expressing humanY5 and chimeric G protein (Gqi5) were seeded into 96-well plates and incubated with a cytoplasmic calcium indicator, Fluo-4AM. After the cells were washed four times, the intracellular Ca^{2+} mobilization evoked by 100 nM of human NPY was monitored as a change in cell fluorescence intensity by FLIPR (Molecular Devices). Varying concentration of Y5 antagonists were added to the plate 5 min prior to the addition of human NPY. The antagonistic activities were calculated as IC_{50} values.

Plasma, Brain, and Cerebrospinal Fluid Concentrations in SD Rats. Test compound was suspended in 0.5% methylcellulose and orally administered to male Sprague−Dawley rats (7−10 weeks old, 200−400 g) at 10 mg/kg. At designated time after administration, blood was collected with a heparinized syringe from the abdominal aorta under isoflurane anesthesia. Then, the head skin was cut open, and a dental 30 G needle was inserted between the cervical vertebrae, and it was further inserted into the cavum subarachnoideale. After 50−100 μL cerebrospinal fluid had been collected by a 1 mL syringe through a tube connected to the needle, the brain was extracted. The blood sample was centrifuged (4 °C, 6000 rpm, 10 min) to separate plasma, and the plasma sample was deproteinized with 3-fold amount of ethanol containing an internal standard. The brain sample was homogenized with 2.0 mL of water by ultrasonification, and an aliquot of the homogenate was deproteinized with 3-fold amount of ethanol containing an internal standard. The cerebrospinal fluid was mixed with 3-fold amount of ethanol containing an internal standard. These samples were allowed to stand at −20 °C for 20 min and then centrifuged (4 °C, 12000*g*, 10 min). The supernatant was analyzed by LC/MS/MS, and the concentration of the test compound in the plasma, brain, and cerebrospinal fluid were measured by the method using a relative calibration curve.

Pharmacokinetics. Pharmacokinetic characterizations were conducted in male SD rats and male rhesus monkeys following single oral and single intravenous administration. Single doses of **2a** were administered either intravenously in a vehicle of PEG400/EtOH/H_2O = 50/10/40 (rat) or 60% propylene glycol (pH 3) (monkey) or orally by gavage in a vehicle of 0.5% methylcellurose aqueous suspension. Doses of 1 (iv) and 1 (po) mg/kg for rats and 0.3 (iv) and 1 (po) mg/kg for monkeys were used. Blood samples for the determination of drug plasma concentrations were obtained at multiple time points up to 8 h after administration. Blood samples were centrifuged to separate the plasma, and the plasma samples were deproteinized with ethanol containing an internal standard. Compound **2a** and the internal standard were detected by LC-MS/MS in a positive ionization mode using the electrospray ionization probe, and the precursor to product ion combinations were monitored in multiple reaction monitoring mode.

In Vivo Efficacy Study: Acute Feeding in SD Rats. Sprague−Dawley (SD) rats (10−15 weeks old) were anesthetized with sodium pentobarbital (50 mg/kg, ip), and a sterile 26-gauge guide cannula (Plastics One Inc., Roanoke, VA) was stereotaxically implanted into the third ventricle. The stereotaxic coordinates used were 2.2 mm posterior to the bregma and 8.0 mm from the surface of the skull, using a flat skull position. Cannula was attached to the skull with dental cement, and an antibiotic (Cefamedin α, 50 mg/kg) was subcutaneously injected. The experiments were conducted during light cycle at least 1 wk after the surgery. Test compounds were suspended in 0.5% methylcellulose in water, and D-Trp^{34}NPY was dissolved in artificial cerebrospinal fluid (aCSF). Test compounds or the vehicle were administered orally to SD rats. Two hours after the oral dosing, D-Trp^{34}NPY (1 μg/0.4 μL) or the vehicle was injected into the third ventricle. Food intake was monitored for 2 h after the D-Trp^{34}NPY injection. Data are expressed as means ± SEM. Significant differences in D-Trp^{34}NPY-induced feeding were analyzed by Dunnett's test.

In Vivo Efficacy Study: Chronic Dosing in DIO Mice. Male C57BL/6N and Y5R KO mice (18−20 weeks old) were fed moderately high-fat diet (MHF diet; Oriental Bioservice Kanto Inc.) ad libitum for about 6 months before the experiment was initiated. The MHF diet provides 52.4% energy as carbohydrate, 15.0% as protein, and 32.6% as fat (4.4 kcal/g). The MHF diet-fed C57BL/6N mice were divided into three groups matched for body weight, and each group was orally administered either vehicle (0.5% methylcellulose in water) or a test compound at doses of 3 and 10 mg/kg, qid for 5 days and bid for another 15 days. The MHF diet-fed Y5R KO mice were treated with the test compound at 3 mg/kg bid for 15 days. Administration was performed about 1 h before the start of the dark period after measurement of body weight and about 2 h after the start of the light period. At the end of the experiment, plasma drug levels were measured at 1, 2, 4, and 15 h after the final dosing. Data are expressed as means ± SEM (body weight change) or means (plasma levels). Body weight changes were analyzed by repeated-measures one-way ANOVA followed by Bonferroni test.

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Supporting Information Available: Procedures for the synthesis of intermediates, biological methods, HPLC retention times, purity, and HPLC traces for compounds **1g**, **2a**, **2c**, **2e**, **2g**, and **2m**. This material is available free of charge via the Internet at http://pubs.acs.org.

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