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Amine-free melanin-concentrating hormone receptor 1 antagonists: Novel 1-(1*H*-benzimidazol-6-yl)pyridin-2(1*H*)-one derivatives and design to avoid CYP3A4 time-dependent inhibition

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ABSTRACT

Melanin-concentrating hormone (MCH) is an attractive target for antiobesity agents, and numerous drug discovery programs are dedicated to finding small-molecule MCH receptor 1 (MCHR1) antagonists. We recently reported novel pyridine-2(1*H*)-ones as aliphatic amine-free MCHR1 antagonists that structurally featured an imidazo[1,2-*a*]pyridine-based bicyclic motif. To investigate imidazopyridine variants with lower basicity and less potential to inhibit cytochrome P450 3A4 (CYP3A4), we designed pyridine-2(1*H*)-ones bearing various less basic bicyclic motifs. Among these, a lead compound **6a** bearing a 1*H*-benzimidazole motif showed comparable binding affinity to MCHR1 to the corresponding imidazopyridine derivative **1**. Optimization of **6a** afforded a series of potent thiophene derivatives (**6q–u**); however, most of these were found to cause time-dependent inhibition (TDI) of CYP3A4. As bioactivation of thiophenes to form sulfoxide or epoxide species was considered to be a major cause of CYP3A4 TDI, we introduced electron withdrawing groups on the thiophene and found that a CF_3 group on the ring or a Cl adjacent to the sulfur atom helped prevent CYP3A4 TDI. Consequently, 4-[(5-chlorothiophen-2-yl)methoxy]-1-(2-cyclopropyl-1-methyl-1*H*-benzimidazol-6-yl)pyridin-2(1*H*)-one (**6s**) was identified as a potent MCHR1 antagonist without the risk of CYP3A4 TDI, which exhibited a promising safety profile including low CYP3A4 inhibition and exerted significant antiobesity effects in diet-induced obese F344 rats.

1. Introduction

Obesity mainly results from an imbalance between energy intake and expenditure, leading to the storage of excess energy in the form of body fat. This fat releases numerous inflammatory adipokines and contributes to the development of various disorders such as diabetes, hypertension, dyslipidemia, depression, coronary artery disease, and cancer.[1,2] The rate of obesity is markedly increasing worldwide, resulting in a serious public health issue.[3,4] The FDA recently approved several new antiobesity drugs such as lorcaserin, combined phentermine and topiramate, combined bupropion and naltrexone, and liraglutide; however, these drugs have still not fully met doctors' and patients' needs for antiobesity treatment due to concerns regarding their efficacy, safety, and cost.[2–5]

Melanin-concentrating hormone (MCH) is a cyclic 19-amino-acid peptide, which is expressed predominantly in the lateral hypothalamic area and zona incerta, and MCH-producing neurons

Abbreviations: MCH, melanin-concentrating hormone; MCHR1, melanin-concentrating hormone receptor 1; CYP3A4, cytochrome P450 3A4; TDI, time-dependent inhibition; DIO, diet-induced obesity; hERG, human ether-a-go-go related gene; DMEDA, *N,N′*-dimethylethylenediamine; HATU, (1-[bis(dimethylamino) methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate); DIPEA, *N,N*-diisopropylethylamine; TFAA, trifluoroacetic anhydride; ADDP, 1,1′-(azodicarbonyl)dipiperidine; MW, microwave; CHO, Chinese Hamster Ovary; TM, transmembrane; GSH, glutathione; HLM, human liver microsomes.

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project throughout the brain.[6,7] Among the two subtypes of the MCH receptor that have been identified,[8–12] MCH receptor 1 (MCHR1) is widely distributed in the brain, including the hypothalamus, thalamus, olfactory cortex, amygdala, striatum, and hippocampus, in all vertebrates,[13,14] whereas MCH receptor 2 is expressed only in higher mammals[15–20] with its physiological function poorly understood.

Many studies have demonstrated that the MCH/MCHR1 pathway plays a key role in the regulation of feeding behavior and energy expenditure. MCH mRNA and peptide levels were upregulated in dietary obese rats[21] and genetically obese rodents, including *ob*/*ob* mice,[22] *db*/*db* mice,[23] $A^{y/a}$ (agouti) mice,[24] and Zucker (*fa*/*fa*) rats.[25] Chronic intracerebroventricular infusion of MCH induced hyperphagia, body weight gain, and hyperinsulinemia, particularly under high-fat diet conditions.[26–28] Transgenic mice that overexpressed MCH in the lateral hypothalamus were more susceptible to obesity and insulin resistance when fed a high-fat diet.[29] In contrast, mice that lacked MCH or MCHR1 were lean with increased metabolic rates and resistance to diet-induced obesity (DIO).[30–33] In human studies, increased levels of MCH expression were observed in the hypothalamus in obese subjects compared with lean subjects.[34] Two loss-of-function MCHR1 mutants (R210H and P377S) were identified in markedly underweight subjects, indicating the possibility that the lean phenotype could be linked to deficient MCHR1 signaling.[35]

In the course of our efforts to discover MCHR1 antagonists as novel antiobesity agents with higher therapeutic window, we recently reported novel pyridine-2(1*H*)-ones as amine-free MCHR1 antagonists that structurally featured an imidazo[1,2-*a*]pyridine-based bicyclic motif.[36] Compound **1** does not have an aliphatic amine motif, unlike most existing MCHR1 antagonists,[37] and the imidazopyridine ring itself is considered to possess intrinsic binding affinity through the putative interaction of its nitrogen atom with Asp123 and/or Tyr272 of MCHR1 (Fig. 1). Profiling of this new lead compound revealed that **1** had low potential for inhibition of the human ether-a-go-go related gene (hERG) or induction of phospholipidosis and showed potent MCHR1 antagonistic activity as well as a significant body-weight-lowering effect in DIO F344 rats after two weeks of oral administration.

Although we successfully discovered the aliphatic-amine-free MCHR1 antagonist **1**, it showed a tendency to inhibit cytochrome P450 3A4 (CYP3A4) at a concentration of 10 μM, which was considered to be due to the coordinating basic nitrogen atom on the imidazopyridine ring. Therefore, we continued a further medicinal chemistry campaign to investigate imidazopyridine variants with lower basicity, and various bicyclic motifs with hydrogen-bonding acceptors were introduced instead. Herein, we report the design of novel pyridine-2(1*H*)-ones bearing a low-basicity 1*H*-benzimidazole ring as a new bicyclic motif and subsequent optimization studies that led to the discovery of a preclinical candidate **6s** with excellent affinity for MCHR1 as well as a potent body weight-lowering effect in DIO rats based on its MCHR1 antagonistic activity. In the course of this study, compounds with thiophene as a terminal aryl ring showed time-dependent inhibition (TDI) of CYP3A4. We also discuss bioactivation mechanisms that cause CYP3A4 TDI, which were analyzed by the glutathione adduct formation study, providing possible solutions to alleviate thiophene-induced CYP3A4 TDI.

2. Chemistry

Scheme 1 illustrates a general route to synthesize 4-alkoxypyridone derivatives **5** and **6**, which involves copper coupling reactions of pyridine-2(1*H*)-ones **4a–c** with corresponding bicyclic bromides **7a–c** or **8a–h**. S_NAr reactions of various benzyl alcohols with 4-chloropyridine-*N*-oxide **2** gave intermediates **3a–c**, which were subsequently treated with acetic anhydride to yield 1-unsubstituted pyridones **4a–c**. Copper coupling reactions of **4a–c** with bicyclic bromides **7a–c** or **8a–h** afforded the target compounds. For these coupling reactions, we selected *N*,*N*′-dimethylethylenediamine (DMEDA) or *trans*-*N*,*N*′-dimethylcyclohexane-1,2-diamine as a ligand and used stoichiometric amounts of CuI owing to the low reactivity of **4a–c**.

Scheme 2 shows an alternative method to obtain 4-alkoxypyridone derivatives **6** for the screening of the 4 position on the pyridine-2(1*H*)-one ring using 4-hydroxypyridones **9a** and **9b** or 4-bromopyridone **10** as key intermediates. The intermediates **9a** and **9b** were prepared from compounds **6l** or **6y** by hydrogenative debenzylation, and subsequent bromination of **9a** using $POBr_3$ gave compound **10**. Alkylation of the intermediates **9a** and **9b** under basic or Mitsunobu conditions as well as S_NAr reactions of the intermediate **10** afforded the target compounds.

The substituents on the 2 position of the benzimidazole ring can be efficiently modified using the route described in Scheme 3. An S_NAr reaction of **11** with methylamine gave the *p*-nitrobromobenzene derivative **12a**, which was subjected to a copper coupling reaction followed by reduction of the nitro moiety by iron to give the diamine intermediate **14**. Amidation of **14** with 1 equivalent of carboxylic acids using HATU gave the corresponding amide intermediates, which were subsequently cyclized in acetic acid to afford the target compounds.

Schemes 4 and 5 illustrate the synthesis of azaimidazopyridines **7a–c**. Imidazopyridazine **7a** and imidazopyrimidine **7c** were synthesized by a one-pot alkylation–cyclization reaction using the corresponding anilines **15a** and **15b**, and 2-bromo-1-cyclopropylpropan-1-one. In contrast, imidazopyrazine **7b** was synthesized in a stepwise manner, as illustrated in Scheme 5. Aniline **16** was protected by a tosyl group and alkylated with 2-bromo-1-cyclopropylpropan-1-one to give the intermediate **18**, which was treated with trifluoroacetic anhydride (TFAA) to give the target compound **7b**.

Scheme 6 depicts the synthesis of 6-bromobenzimidazoles **8a–h**. *p*-Nitrobromobenzene derivatives **12a–c** were obtained as described in Scheme 3**.** Reduction of the nitro moiety of **12a–c** gave diamine intermediates, which were subsequently heated with the corresponding carboxylic acids in $POCl_3$ or subjected to the conditions described in Scheme 3 to afford the target compounds (paths A and B). Alternatively, the *p*-nitrobromobenzene derivative **12a** was subjected to amidation prior to reduction of the nitro moiety (path C), wherein the resulting amides **20a** and **20b** were subsequently heated with zinc in acetic acid to afford the target compounds**.**

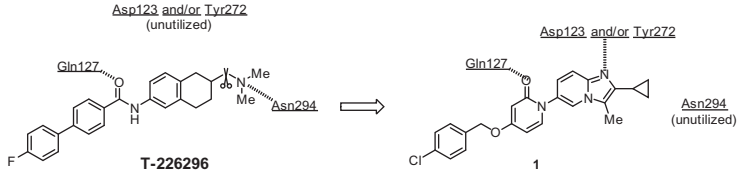
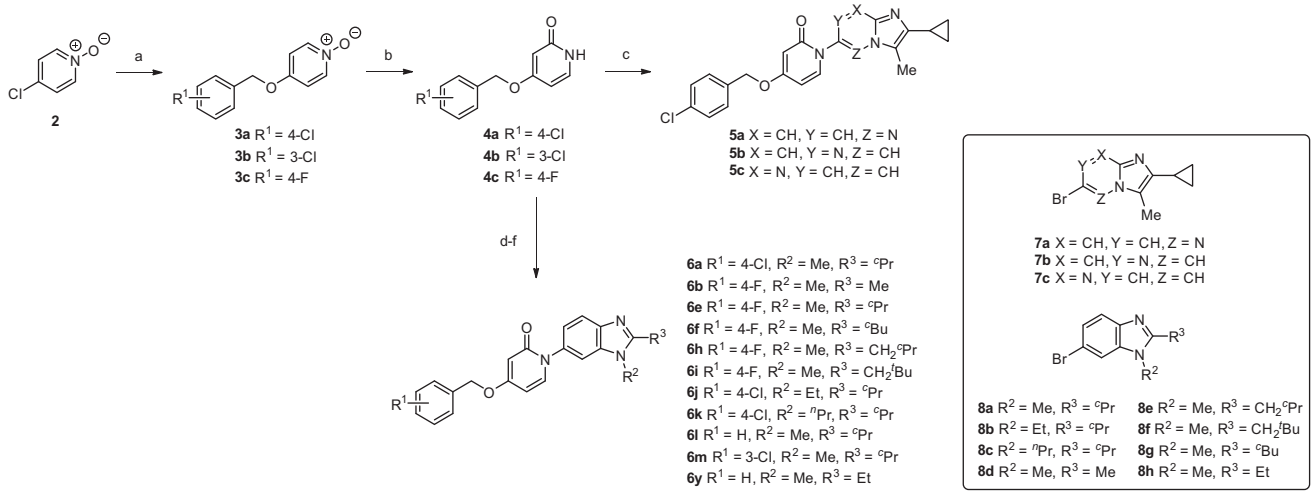
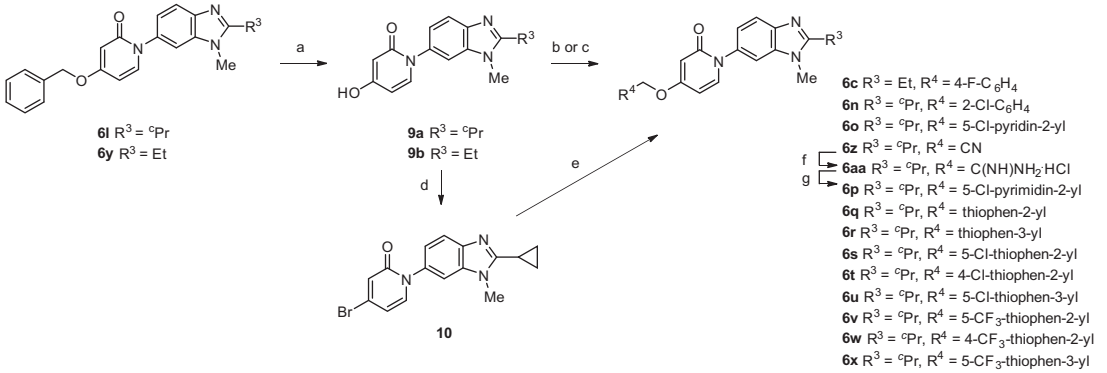


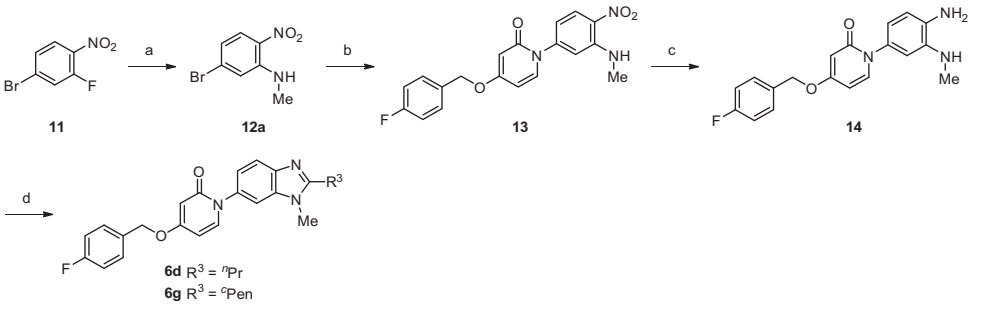
Figure 1. Chemical structures of T-226296 and the previously reported amine-free MCHR1 antagonist **1**. Dotted lines depict putative interactions with MCHR1.



Scheme 1. Synthesis of 4-alkoxypyridone derivatives **5a–c** and **6a**, **6b**, **6e**, **6f, 6h–m**, and **6y**. *Reagents and conditions:* (a) corresponding benzyl alcohol, NaH, THF, rt, 5 h, 6–66%; (b) Ac_2O, 140 °C, 2 h, 49–58%; (c) **7a–c**, CuI, DMEDA, K_2CO_3, DMSO, 150 °C, 1 h, MW irradiation, 1–42%; (d) **8**, CuI, *trans-N,N′*-dimethylcyclohexane-1,2-diamine, K_2CO_3, dioxane, 110 °C, 16 h, 18–87% (for **6a**, **6f**, **6l**, and **6m**); (e) **8**, CuI, DMEDA, K_2CO_3, DMSO, 120 °C, 1 h, MW irradiation, 11–48% (for **6h**, **6j**, and **6k**); (f) **8**, CuI, DMEDA, K_2CO_3, DMSO, 150 °C, 2 h, 22–87% (for **6b**, **6e**, **6f**, **6i**, **6l**, **6m**, and **6y**).



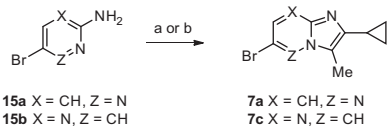
Scheme 2. Synthesis of 4-alkoxypyridone derivatives **6c**, **6n–x**, **6z**, and **6aa**. *Reagents and conditions:* (a) H_2 (1 atm), 10% Pd–C, MeOH, 3 h, 86–99%; (b) corresponding alkyl halide, K_2CO_3, DMF, 14–76% (for **6c**, **6o**, and **6z**); (c) corresponding benzyl alcohol, ADDP, PBu_3, THF, 60 °C, 3 h, 9–42% (for **6n** and **6q–v**); (d) $POBr_3$, DMF, 50 °C, 9 h, 49%; (e) corresponding benzyl alcohol, NaH, DMA, 120 °C, 10 min, 26–51% (for **6w** and **6x**); (f) NaOMe, MeOH, rt, 4 h, then NH_4Cl, rt, overnight, quant.; (g) 2-chloro-1,3-bis(dimethylamino)trimethinium hexafluorophosphate, NaOMe, MeOH, rt, 1 h, 51%.



Scheme 3. Synthesis of 4-alkoxypyridone derivatives **6d** and **6g**. *Reagents and conditions:* (a) $MeNH_2$ (40% MeOH solution), EtOH, rt, 1 h, 94%; (b) **4c**, CuI, DMEDA, K_2CO_3, DMSO, 120 °C, 1 h, MW irradiation, 44%; (c) Fe, $CaCl_2$, EtOH, water, 70 °C, 3 h, 94%; (d) corresponding carboxylic acids, HATU, DIPEA, DMF, 1 h, then AcOH, 90 °C, 1 h, 18–65%.

3. Results and discussion

The binding affinities of the compounds prepared herein to human and rat MCHR1 (hMCHR1 and rMCHR1, respectively) were evaluated in a binding assay using radiolabeled [^{125}I]MCH (4–19) and membrane fractions prepared from Chinese hamster ovary (CHO) cell lines that stably expressed MCHR1. To investigate alternative imidazopyridine motifs with lower basicity, various bicyclic motifs with lower pK_a values bearing a nitrogen atom, as in the 1 position in the imidazopyridine ring of compound **1**, were prepared (Table 1). First, we systematically introduced a nitrogen atom into the six-membered ring and prepared a series of azaimidazopyridines **5a–c**. Thus, the imidazopyrazine derivative **5b** showed an almost two-fold decrease in binding affinity relative



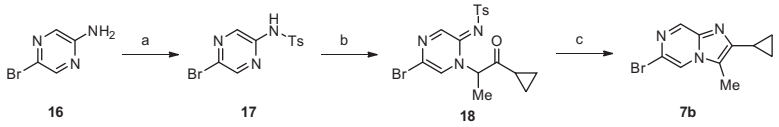
Scheme 4. Synthesis of azaimidazopyridines **7a** and **7c**. *Reagents and conditions:* (a) 2-bromo-1-cyclopropylpropan-1-one, $NaHCO_3$, DMA, 80 °C, 16 h, 76% (for **7a**); (b) 2-bromo-1-cyclopropylpropan-1-one, DMF, 100 °C, 24 h, 13% (for **7c**).

to **1** (**5b**: IC_{50} = 44 nM) and approximately 10- and 50-fold declines in potency were observed in the imidazopyridazine derivative **5a** and the imidazopyrimidine derivative **5c**, respectively (**5a**: IC_{50} = 380 nM, **5c**: IC_{50} > 1000 nM). These results indicated that the introduction of a nitrogen atom into the six-membered ring of the imidazopyridine system was basically not an appropriate approach, owing to the decrease in potency. Encouraged by the fact that the potency did not correlate with the pK_a value, we then designed compound **6a** with a low-basicity 1*H*-benzimidazole ring, which was regarded as a substructure with less risk of general safety concerns.[39,40] Compound **6a** showed as potent a binding affinity as compound **1** (**6a**: IC_{50} = 35 nM) and its pK_a value was 5.71, indicating the compound **6a** was considered as a novel low-basicity lead compound for further optimization.

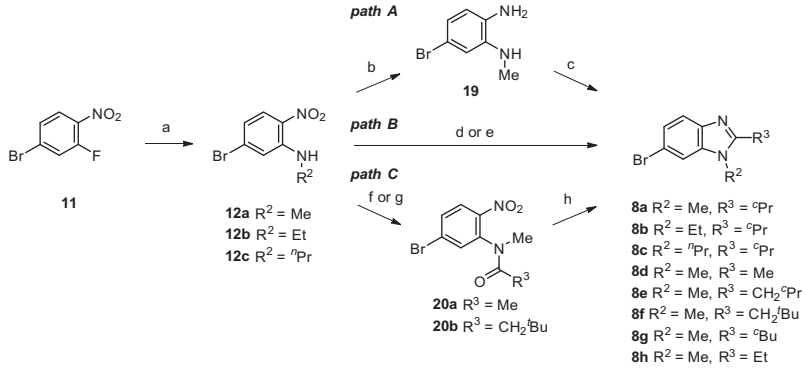
The optimization study was initiated with the 2 position on the benzimidazole ring (Table 2). During this study, 4-fluorophenyl was selected as the terminal aryl ring, which provided identical potency to the 4-chlorophenyl analog. In a previous study on imidazopyridine derivatives,[36] the introduction of a polar substituent at this position was found to be unfavorable for potency. This trend was also true with the current benzimidazole derivatives, and the introduction of a polar substituent such as an alkoxy group, alcohol, ketone, or amide decreased the affinity (data not shown). Therefore, we examined various aliphatic substituents to optimize lipophilic interaction. Investigation of the size of the substituent (**6b–d**) revealed that the potency was moderate with the methyl derivative **6b** (hMCHR1: IC_{50} = 77 nM) and increased with the ethyl derivative **6c** and "propyl derivative **6d** almost two-fold. When a cycloalkyl group was introduced at the 2 position (**6e**, **6f**, and **6g**), the cyclopropyl derivative **6e** showed a slight increase in potency (**6e** vs. **6c**). The introduction of other cyclic substituents at the 2 position, however, decreased the affinity as the ring size increased (**6e** > **6f** > **6g**). In contrast, the introduction of a cyclopropylmethyl group at the 2 position caused an almost three-fold decline in binding affinity (**6h** vs. **6d**), whereas a neopentyl group was found to lead to a deterioration in potency (**6i**). These findings indicated that the 2 position on the benzimidazole ring was directed toward a narrow lipophilic space, wherein sterically less hindering substituents with a length of two or three carbon atoms were preferred. The structure–activity relationship (SAR) study that has been discussed so far (see Table 2) revealed that a cyclopropyl group was optimal for a substituent at the 2 position.

We subsequently briefly investigated the SAR at the 1 position on the benzimidazole ring (**6j** and **6k** in Table 2). The ethyl derivative **6j** showed a slight decrease in binding affinity to rMCHR1 and further elongation of the chain resulted in a significant decline in potency, as indicated by compound **6k**. These results indicated that the 1 position possessed limited capacity to accommodate a substituent and a methyl group was optimal.

We next examined the influence of the terminal benzene ring on potency (Table 3). The unsubstituted derivative **6l** showed good binding affinities (hMCHR1: IC_{50} = 45 nM). The introduction of fluorine and chlorine atoms at the 4 position enhanced potency (**6e** and **6a**), and the 4-chloro derivative **6a** showed a two-fold increase in affinity for rMCHR1 relative to the unsubstituted derivative **6l**. In contrast, the 3-chloro and 2-chloro derivatives (**6m** and **6n**) showed significant decreases in binding affinity. Replacement of the terminal benzene ring with polar heteroaromatic rings (**6o** and **6p**) caused a decline in potency in a lipophilicity-dependent manner (**6o**: *C*log*P* = 3.56, **6p**: *C*log*P* = 2.56).[38] These findings are consistent with the results of our previous studies on dihydronaphthalenes[41] and quinolines,[42,43] wherein the left-hand side of

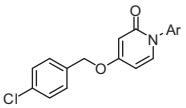


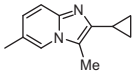
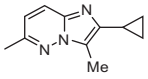
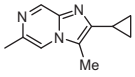
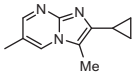
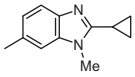
Scheme 5. Synthesis of azaimidazopyridine **7b**. *Reagents and conditions:* (a) TsCl, pyridine, rt, overnight, 51%; (b) 2-bromo-1-cyclopropylpropan-1-one, NaH, DMF, rt, overnight, $NaHCO_3$, 46%; (c) TFAA, THF, 0 °C, then 60 °C, 3 h, 80%.



Scheme 6. Synthesis of benzimidazoles **8a–h**. *Reagents and conditions:* (a) corresponding amine, EtOH, rt, 1 h, 76–94%; (b) Zn, NH_4Cl, MeOH, water, rt, 1 h, 93%; (c) cyclobutanecarboxylic acid, $POCl_3$, 120 °C, 4 h, 45% (for **8g**); (d) Zn, NH_4Cl, MeOH, water, rt, 1 h, then corresponding carboxylic acid, $POCl_3$, 120 °C, 3 h, 45–72% (for **8a**, **8c**, and **8g** in two steps); (e) Zn, AcOH, rt, 30 min, then corresponding carboxylic acid, HATU, DIPEA, DMF, rt, 1 h, then AcOH, 80 °C, 1 h, 37–96% (for **8b**, **8e**, and **8h** in three steps); (f) acetyl chloride, toluene, 90 °C, 15 h, 97% (for **20a**); (g) 3,3-dimethylbutanoyl chloride, NaH, DMF, 70 °C, overnight, 50% (for **20b**); (h) Zn, AcOH, 90 °C, 4 h, 65–78% (for **8d** and **8f**).

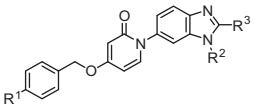
Table 1
In vitro binding affinities and pK_a values of compounds **1**, **5a–c**, and **6c**



Compound	Ar	IC_{50} (nM)[a] hMCHR1[b]	pK_a[c]
1		26	7.85
5a		380	6.35
5b		44	5.35
5c		>1000	6.35
6a		35	5.71

[a] IC_{50} values were calculated using an experiment performed in duplicate with a three-fold standard deviation.
[b] Binding affinity for human MCHR1.
[c] pK_a values were calculated using ACD Labs ver. 12.0.[38]

Table 2
In vitro binding affinities of compounds **6b–k**



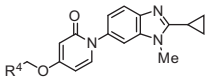
Compound	R^1	R^2	R^3	IC_{50} (nM)[a]	
				hMCHR1[b]	rMCHR1[c]
6b	F	Me	Me	77	65
6c	F	Me	Et	48	38
6d	F	Me	^{n}Pr	34	39
6e	F	Me	^{c}Pr	40	28
6f	F	Me	Cyclobutyl	240	210
6g	F	Me	Cyclopentyl	600	460
6h	F	Me	$CH_2{}^c$Pr	90	140
6i	F	Me	$CH_2{}^t$Bu	>1000	>1000
6j	Cl	Et	^{c}Pr	37	34
6k	Cl	^{n}Pr	^{c}Pr	85	110

[a] IC_{50} values were calculated using an experiment performed in duplicate with a three-fold standard deviation.
[b] Binding affinity for human MCHR1.
[c] Binding affinity for rat MCHR1.

the molecule was expected to be surrounded by a hydrophobic environment formed by Phe213, Ala216, and Phe217 on TM5 and Tyr273 on TM6,[41] which suggests that the terminal benzene ring is in a narrow hydrophobic pocket of the receptor and only the 4 position can accommodate a substituent.

In a previous report, we found that thiophene as the terminal aryl ring generally afforded better binding affinities than benzene or other heteroaromatic rings,[36] which prompted us to introduce thiophene rings into the current chemotype to increase potency (Table 4). The thiophen-2-yl derivative **6q** showed two- and three-fold weaker potencies compared with the corresponding benzene analog **6l** for hMCHR1 and rMCHR1, whereas the

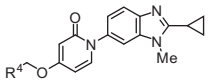
Table 3
In vitro binding affinities of compounds **6a**, **6e**, and **6l–p**



Compound	R^4	IC_{50} (nM)[a]	
		hMCHR1[b]	rMCHR1[c]
6l		45	43
6e		40	28
6a		35	21
6m		100	72
6n		160	140
6o		73	47
6p		>1000	900

[a] IC_{50} values were calculated using an experiment performed in duplicate with a three-fold standard deviation.
[b] Binding affinity for human MCHR1.
[c] Binding affinity for rat MCHR1.

Table 4
In vitro binding affinities and CYP3A4 TDI risk of compounds **6q–u**



Compound	R^4	IC_{50} (nM)[a]		CYP3A4TDI[d] (% remaining)
		hMCHR1[b]	rMCHR1[c]	
6q		76	120	NT[e]
6r		28	29	74
6s		19	11	83
6t		17	18	25
6u		14	9.3	44

[a] IC_{50} values were calculated using an experiment performed in duplicate with a three-fold standard deviation.
[b] Binding affinity for human MCHR1.
[c] Binding affinity for rat MCHR1.
[d] CYP3A4 time-dependent inhibition assay ($n = 2$). The remaining activity of CYP3A4 after pre-incubation with a test compound was determined.
[e] Not tested.

thiophen-3-yl derivative **6r** possessed better potencies relative to **6l**. The introduction of a chlorine atom onto these isomeric thiophenes revealed that both thiophen-2-yl derivatives (**6s** and **6t**) and a thiophen-3-yl derivative (**6u**) showed successful increases in potency and their binding affinities were superior to those of the lead compound **6a**. Among these, the 5-chlorothiophen-3-yl derivative **6u** showed the best potency for both species (rMCHR1: $IC_{50} = 9.3$ nM). Thus, the in vitro potency could be successfully

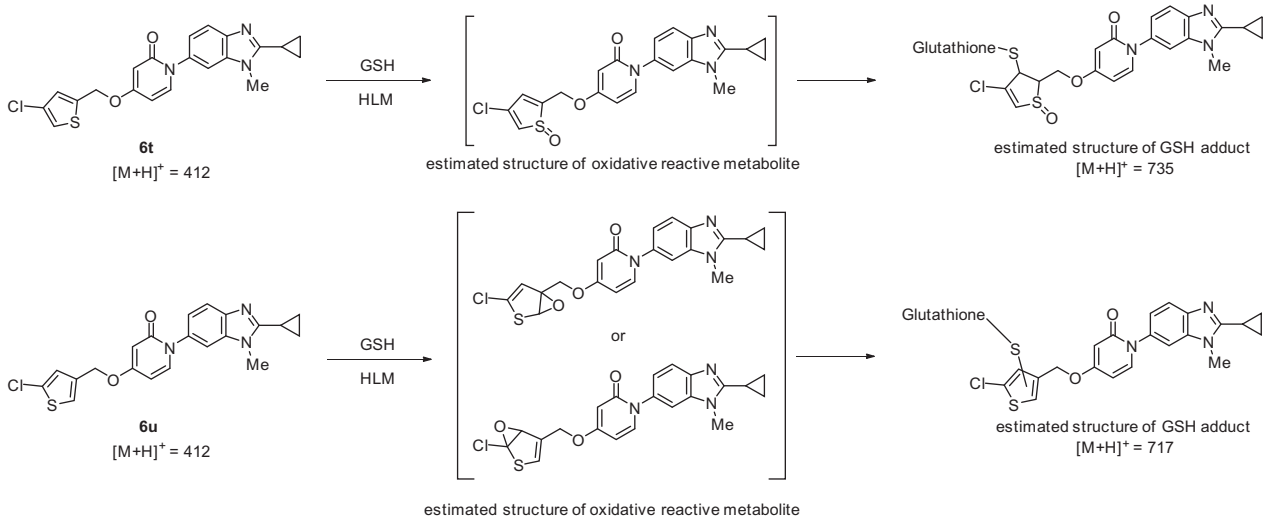
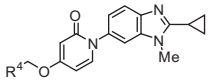


Figure 2. Glutathione adduct formation by incubation of test compounds **6t** and **6u** with GSH and human liver microsomes (HLM). The test compounds (30 μM) were incubated with HLM (1.0 mg/mL) in the presence of GSH (1 mM) at 37 °C for 60 min. The structures of the GSH adducts were estimated by LC/MS/MS analysis.

Table 5
In vitro binding affinities and CYP3A4 TDI risk of compounds **6v–x**



Compound	R^4	IC_{50} (nM)[a]		CYP3A4TDI[d] (% remaining)
		hMCHR1[b]	rMCHR1[c]	
6v		16	13	97
6w		34	17	87
6x		41	29	96

[a] IC_{50} values were calculated using an experiment performed in duplicate with a three-fold standard deviation.
[b] Binding affinity for human MCHR1.
[c] Binding affinity for rat MCHR1.
[d] CYP3A4 time-dependent inhibition assay ($n = 2$). The remaining activity of CYP3A4 after pre-incubation with a test compound was determined.

increased by the replacement of the 4-chlorophenyl moiety in **6a** with various chloro-substituted thiophenes.

Further evaluation of these thiophene derivatives, however, revealed that compounds **6r**, **6t**, and **6u** caused CYP3A4 TDI. Because CYP3A4 is the most abundant CYP isoform and is responsible for the oxidative metabolism of a wide variety of clinical drugs, CYP3A4 TDI may preclude the drug development from the perspective of drug–drug interactions. Given the fact that benzene and pyridine derivatives (**6e** and **6o**) did not show potential for CYP3A4 TDI, we hypothesized that the thiophene substructure was responsible for the risk of CYP3A4 TDI. This hypothesis was also supported by the reported recognition that thiophenes were regarded as toxicophores in some cases owing to the potential risk of oxidative bioactivation leading to the formation of electrophilic species, which are linked to toxicity caused by conjugation with biomolecules and a mechanism-based inactivation of CYP3A4, resulting in clinical drug–drug interactions.[44,45] To investigate this assumption, compounds **6t** and **6u** were tested in glutathione (GSH) trapping experiments. When **6t** and **6u** were incubated in human liver microsomes with GSH followed by analysis using liquid chromatography coupled with mass spectrometry (LC/MS), accurate masses corresponding to "parent mass + GSH + O" and "parent mass + GSH − H_2" were observed with **6t** and **6u**, respectively (Fig. 2). Furthermore, from the product ion spectra of the GSH adducts it appeared that the GS moiety was substituted on the thiophene ring. These findings suggested that the formation of a GSH adduct of **6t** would be caused by the generation of a sulfoxide by microsomal oxidation followed by 1,4-addition of GSH to yield a GSH adduct at the β position to the sulfur atom. In contrast, **6u** would be oxidized to an epoxide, followed by nucleophilic attack of GSH and subsequent loss of water to generate the GSH adduct. These mechanistic analyses of the formation of GSH adducts of **6t** and **6u** suggested that CYP3A4 TDI that was observed with thiophene derivatives was triggered by oxidation of the

Table 6
Pharmacokinetic parameters of **6s** and **6v** in rats.[a]

Compound	F[b] (%)	iv (0.1 mg/kg)		po (1 mg/kg)		
		CL_{total}[c] (mL h^{-1} kg^{-1})	V_{ss}[d] (mL kg^{-1})	C_{max}[e] (ng mL^{-1})	T_{max}[f] (h)	AUC_{0-8h}[g] (ng h mL^{-1})
6s	23	450	920	164.7	1.0	514.9
6v	57	285	979	297.8	2.7	2015.6

[a] $n = 3$; SD rats (male, eight weeks old).
[b] Bioavailability.
[c] Total clearance.
[d] Volume of distribution at steady state.
[e] Maximal plasma concentration.
[f] Time of maximal concentration.
[g] Area under the plasma concentration–time curve (0–8 h).

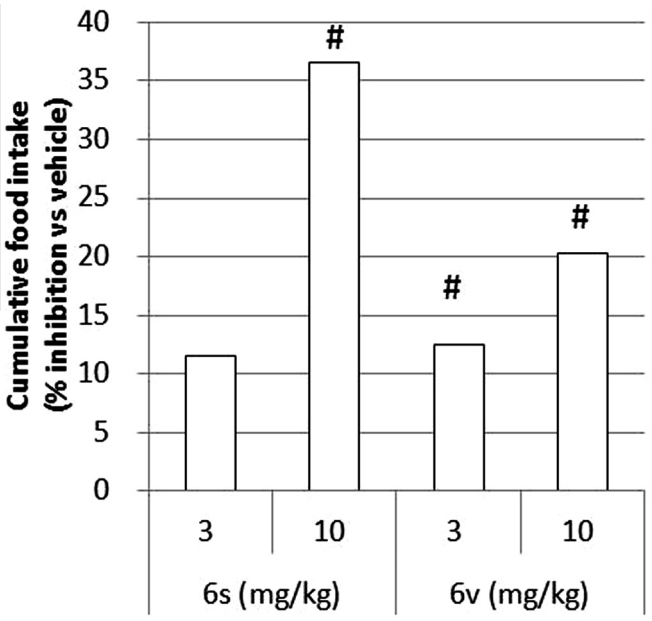


Figure 3. Results for **6s** and **6v** in a two-day food intake study in DIO F344 rats. Inhibition of cumulative food intake over two days in DIO F344 rats. The compounds were administered once daily, and food intake from the initial administration to two days later was measured. The cumulative food intake inhibition rate was calculated by dividing the average food intake of each treatment group by that of the vehicle group ($n = 6$ for each group). (#) $p < 0.025$ vs. the vehicle group (Williams test).

thiophenes to form reactive species such as electrophilic sulfoxides or epoxides. Therefore, we assumed that the risk of CYP3A4 TDI could be reduced by the following two strategies: (1) preventing the oxidation of thiophenes with increased steric influence around the sulfur atom and (2) decreasing the electron density of thiophenes. Indeed, compound **6s**, which had substituents on both carbons in positions α to the sulfur atom, did not show a risk of TDI, which would indicate the effectiveness of the strategy of masking the sulfur atom to prevent sulfoxide formation.

We then investigated the second strategy and examined the influence of decreasing the electron density of the thiophene ring on the risk of CYP3A4 TDI by introducing the strongly electron-withdrawing trifluoromethyl group and prepared a series of trifluoromethyl-substituted thiophene derivatives (Table 5). The 5-trifluoromethylthiophen-2-yl derivative **6v** showed comparable potency to the corresponding chloro analog **6s** for both species; however, the 4-trifluoromethylthiophen-2-yl derivative **6w** showed a slight decrease in potency for hMCHR1 relative to **6t**. The 5-trifluoromethylthiophen-3-yl derivative **6x** showed a three-fold reduction in affinity compared with the chloro analog **6u**. Evaluation of CYP3A4 TDI of these thiophene derivatives showed that not only compound **6v**, which had both α carbons substituted, but also compounds **6w** and **6x**, which had one α carbon unsubstituted, gave negative results. Considering the fact that the corresponding chloro-substituted analogs **6t** and **6u** were CYP3A4 TDI-positive, this clearly indicated that decreasing the electron density of the thiophene ring by replacement with a trifluoromethyl group was effective in reducing the risk of CYP3A4 TDI and that trifluoromethyl-substituted thiophenes are considered to be less susceptible to bioactivation and safer alternatives to chloro-substituted thiophenes.

Among the compounds discussed so far, we selected **6s** and **6v**, which showed potent in vitro binding affinities for both MCHR1s and did not possess the potential for CYP3A4 TDI. Secondary functional cell-based assays of the inhibition of MCH-stimulated Ca^{2+} mobilization in CHO cells confirmed that these two compounds functioned as MCHR1 antagonists (**6s**: $IC_{50} = 24$ nM, **6v**: $IC_{50} = 18$ nM). Pharmacokinetic screening of **6s** and **6v** resulted in reasonable oral availability and plasma exposure (Table 6).

To evaluate their pharmacological effects, compounds **6s** and **6v** (3 and 10 mg/kg) were orally administered to DIO F344 rats fed a high-fat diet *ad libitum*. The results of a two-day in vivo study are shown in Figure 3. Compound **6s** significantly and dose-dependently suppressed food intake in DIO rats by 11.6% and 36.5% at 3 and 10 mg/kg, respectively. Compound **6v** showed an almost equal anorectic effect at 3 mg/kg; however, its efficacy at 10 mg/kg was a slightly weaker than that of **6s** (20.3%). It was assumed that the low solubility of **6v** (0.25 μg/mL at pH 6.8) relative to **6s** (2.3 μg/mL at pH 6.8) caused a non-linear pharmacokinetic profile and caused lower plasma exposure at a dose of 10 mg/kg of **6v**. Given these results, we selected **6s** for further pharmacological evaluation.

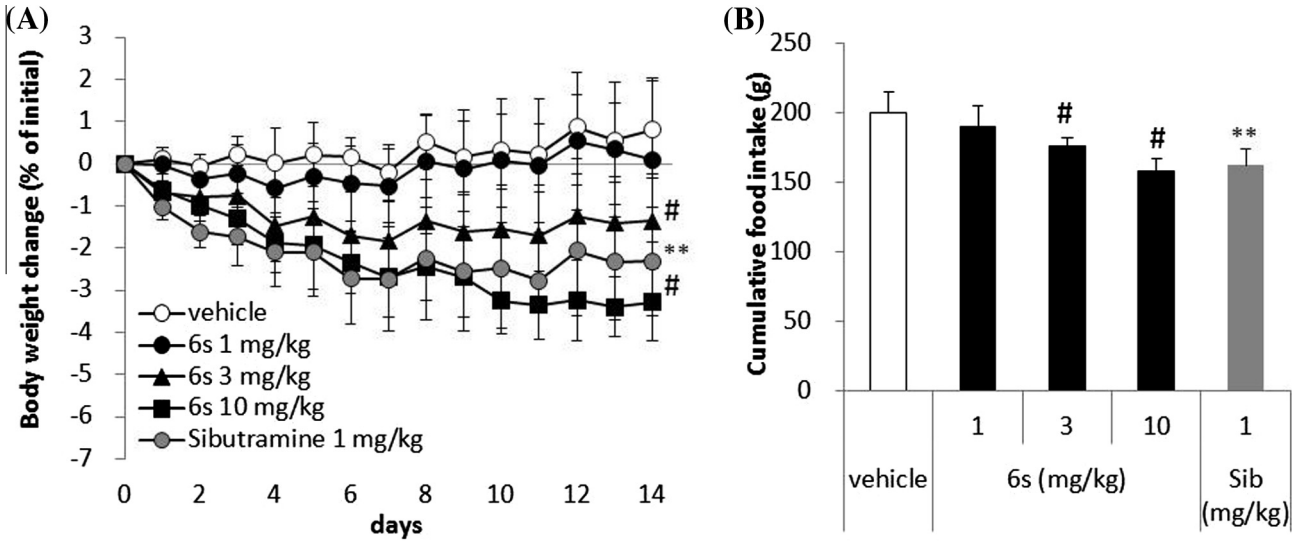


Figure 4. Results for **6s** in a repeated-dose study in DIO F344 rats. (A) Body weight change from initial value during two weeks of dosing. (B) Cumulative food intake for two weeks of dosing. The compounds were administered once daily for two weeks and the body weight and food intake were measured before drug administration. Each data point represents mean ± SD ($n = 5$ or 6 for each group). (#) $p < 0.025$ vs. the vehicle group (Williams test), (**) $p < 0.01$ vs. the vehicle group (Student's *t*-test).

The effect of **6s** on body weight reduction was assessed using a two-week repeated dosing study on DIO rats (Fig. 4). A significant change in body weight was observed from 3 mg/kg, and once daily oral administration of 3 and 10 mg/kg of **6s** resulted in body weight reductions of 2.2% and 4.1%, respectively, relative to the vehicle group. The cumulative food intakes of these two cohorts were significantly reduced by 11.6% and 20.9%, respectively. It was noteworthy that 10 mg/kg of **6s** showed a more potent antiobesity effect relative to 1 mg/kg of sibutramine (4.1% vs 3.1%), whereas the anorectic effects in these two cohorts were almost the same (20.9% vs 18.7%). The involvement of MCHR1 antagonists in energy expenditure has been reported,[46] which would make an additional contribution to the antiobesity effect of **6s**, resulting in greater loss of body weight relative to sibutramine. The trough plasma concentration at 10 mg/kg was 0.79 μM, which was equivalent to an unbound drug concentration of 15.8 nM (unbound fraction of **6s** in DIO rat plasma was 0.02). These results indicated that a unbound concentration of more than 1.44 times the IC_{50} was maintained in this cohort throughout the study, which was considered to be the necessary plasma level to afford a body weight reduction of 4.7%. Compound **6s** was confirmed to show acceptable brain exposure (brain/plasma ratio = 0.5), which suggested that **6s** was blood–brain barrier-permeable.

To clarify that the anorectic property was linked to MCHR1 antagonism, the effect of **6s** in MCHR1-deficient and wild-type mice was examined. Compound **6s** exerted no body-weight-lowering effect in MCHR1-deficient mice, whereas it significantly reduced body weight in wild-type mice in a dose-dependent manner (Fig. 5). Compound **6s** also exhibited a trend toward decreasing cumulative food intake in a dose-dependent manner in MCHR1-deficient mice. These results indicated that the anorectic effect of **6s** and the resulting weight loss were based on its MCHR1 antagonistic activity.

The findings discussed so far indicated that we have successfully identified a low-basicity MCHR1 antagonist, **6s**. Compound **6s** possessed a much lower potential for reversible CYP3A4 inhibition, which prevented previously reported imidazopyridine derivatives from proceeding for development, and did not display potential for phospholipidosis in an in vitro assay. Furthermore, compound **6s** did not inhibit hERG channel in a patch clamp test (IC_{50} > 10 μM), providing better safety profile in terms of drug-induced cardiovascular risk. Therefore, compound **6s** could be a promising antiobesity agent that exerted a body-weight-lowering effect based on its MCHR1 antagonistic activity.

4. Conclusion

To investigate imidazopyridine variants with lower basicity and potential for CYP3A4 inhibition, we designed pyridine-2(1*H*)-ones bearing various less basic bicyclic motifs. Among these, a lead compound **6a** bearing a 1*H*-benzimidazole motif showed comparable binding affinity for MCHR1 to the corresponding imidazopyridine derivative **1**. During optimization studies, a series of potent thiophene derivatives were shown to induce CYP3A4 TDI via bioactivation of the thiophene ring. We found that blockage of the α carbon atoms of thiophenes as well as the introduction of the bulky and strongly electron-withdrawing trifluoromethyl group were effective in preventing this bioactivation mechanism, which led to the identification of **6s** and **6v** as potent MCHR1 antagonists without the risk of CYP3A4 TDI. Note that the trifluoromethyl group possessed a beneficial effect in preventing CYP3A4 TDI of thiophenes even though one α carbon atom was left unsubstituted, as in **6w** and **6x**, which indicated that trifluoromethyl-substituted thiophenes could be used in drug design as safer building blocks without the risk bioactivation. In summary, the preclinical candidate 4-[(5-chlorothiophen-2-yl)methoxy]-1-(2-cyclopropyl-1-methyl-1*H*-benzimidazol-6-yl)pyridin-2(1*H*)-one (**6s**), which exhibited a significant body-weight-lowering effect in DIO rats on oral administration at 5 and 10 mg/kg, was expected to be a new antiobesity agent based on its MCHR1 antagonistic activity.

5. Experimental

5.1. Chemistry

Melting points were determined on a Yanaco melting point apparatus Mp-500D and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AVANCE III (300 MHz) or a Bruker Advance III plus (400 MHz) spectrometer. Chemical shifts are given in parts per million (ppm) downfield from tetramethylsilane (δ) as the internal standard in deuterated solvent, and coupling constants

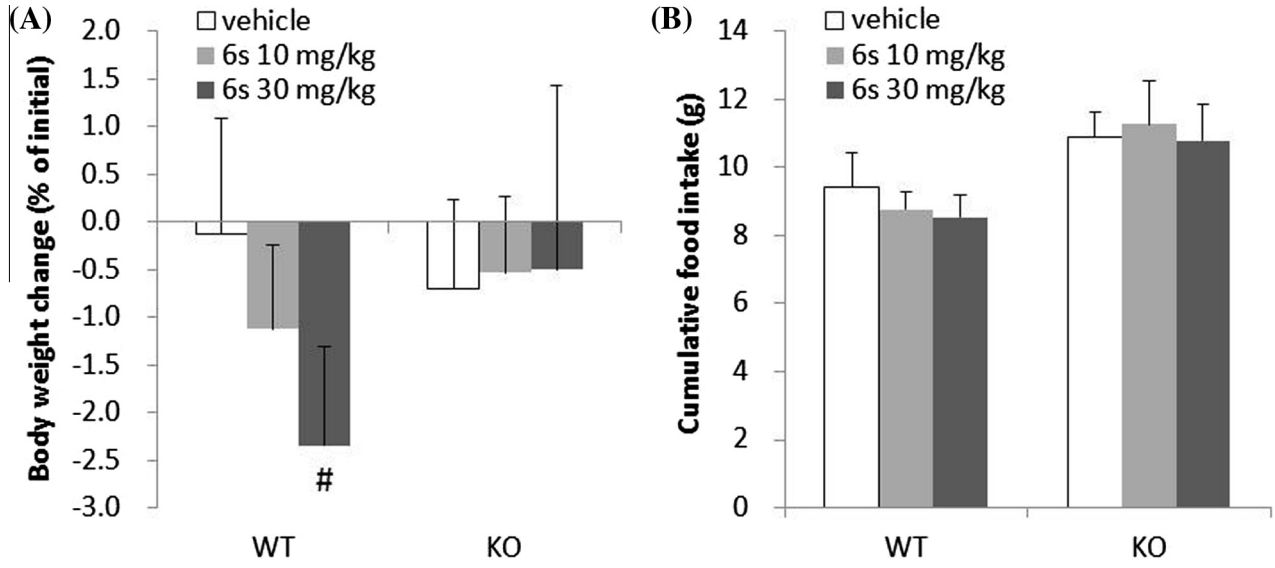


Figure 5. Results for **6s** in a three-day study in MCHR1-deficient (KO) and wild-type (WT) mice. The mice were fed a high-fat diet. The body weight change (A) and cumulative food intake (B) were measured for three days. Each data point represents mean + SD (n = 6 for each group). (#) p < 0.025 vs. the vehicle group (Williams test).

(*J*) are in hertz (Hz). Data are reported as follows: chemical shift, integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, m = multiplet, dd = doublet of doublets, td = triplet of doublets, and br s = broad signal), and coupling constants. Reagents and solvents were obtained from commercial sources and used without further purification. Reaction progress was determined by thin layer chromatography (TLC) analysis on Merck Kieselgel 60 F254 plates or Fuji Silysia NH plates. Chromatographic purification was performed on silica gel columns [(Merck Kieselgel 60, 70–230 mesh size or 230–400 mesh size, Merck) or (Chromatorex NH-DM 1020, 100–200 mesh size)] or on Purif-Pack (SI or NH, particle size: 60 μm, Fuji Silysia Chemical, Ltd.). LC−MS analysis was performed on a Shimadzu liquid chromatography–mass spectrometer system, operating in APCI (+ or −) or ESI (+ or −) ionization mode. Analytes were eluted using a linear gradient of 0.05% TFA containing water/acetonitrile or 5 mM ammonium acetate containing water/acetonitrile mobile phase and detected at 220 nm. Analytical HPLC was performed with Corona Charged Aerosol Detector (CAD) or photo diode array detector. The column was a Capcell Pak C18AQ (50 mm × 3.0 mm I.D., Shiseido, Japan) or L-column 2 ODS (30 mm × 2.0 mm I.D., CERI, Japan) with a temperature of 50 °C and a flow rate of 0.5 mL/min. Mobile phase A and B under a neutral condition were a mixture of 50 mmol/L ammonium acetate, water and acetonitrile (1:8:1, v/v/v) and a mixture of 50 mmol/L ammonium acetate and acetonitrile (1:9, v/v), respectively. The ratio of mobile phase B was increased linearly from 5% to 95% over 3 min, 95% over the next 1 min. Mobile phase A and B under an acidic condition were a mixture of 0.2% formic acid in 10 mmol/L ammonium formate and 0.2% formic acid in acetonitrile, respectively. The ratio of mobile phase B was increased linearly from 14% to 86% over 3 min, 86% over the next 1 min. The purities of compounds submitted for biological evaluation were >95% as determined by elemental analyses within ±0.4% of the calculated values or analytical HPLC. Yields are not optimized.

5.1.1. 4-[(4-Chlorobenzyl)oxy]pyridine 1-oxide (3a)

A solution of (4-chloropheny)methanol (49.5 g, 347 mmol) in THF (200 mL) was added dropwise to a suspension of NaH (60% oil dispersion, 16.7 g, 419 mmol) in THF (200 mL) at 0 °C. After the mixture was stirred at 0 °C for 30 min, **2** (45.0 g, 347 mmol) was added portionwise to the reaction mixture. After completion of the addition, the mixture was stirred at rt for 5 h. The mixture was quenched with water (400 mL) at 0 °C and extracted with EtOAc/THF (1:1) four times. The organic layers were combined, passed through NH-silica gel pad (EtOAc/MeOH) and concentrated. The filtrate was concentrated, and the residual solid was washed with IPE and dried to give the title compound (54.3 g, 66%) as a brown solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.17 (2H, s), 7.08 (2H, d, J = 6.9 Hz), 7.48 (4H, s), 8.10 (2H, d, J = 7.0 Hz). MS (ESI/APCI) m/z 236.0 $[M+H]^+$.

5.1.2. 4-[(3-Chlorobenzyl)oxy]pyridine 1-oxide (3b)

The title compound was prepared in 45% yield using (3-chlorophenyl)methanol in an analogous manner to **3a**. Brown solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.18 (2H, s), 7.08 (2H, dd, J = 5.6, 1.8 Hz), 7.42–7.43 (3H, m), 7.53 (1H, br s), 8.10 (2H, dd, J = 5.6, 1.8 Hz). MS (ESI/APCI) m/z 236.0 $[M+H]^+$.

5.1.3. 4-[(4-Fluorobenzyl)oxy]pyridine 1-oxide (3c)

The title compound was prepared in 6% yield using (4-fluorophenyl)methanol in an analogous manner to **3a**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 5.15 (2H, s), 7.04–7.13 (2H, m), 7.19–7.30 (2H, m), 7.46–7.57 (2H, m), 8.07–8.14 (2H, m). MS (ESI/APCI) m/z 220.1 $[M+H]^+$.

5.1.4. 4-[(4-Chlorobenzyl)oxy]pyridine-2(1*H*)-one (4a)

A mixture of **3a** (54.3 g, 230 mmol) and acetic anhydride (540 mL, 5.71 mol) was stirred at 140 °C for 2 h. After concentration of the mixture, the residue was dissolved in MeOH (300 mL). Water (450 mL) was added to the mixture, followed by stirring at rt for 1 h. The resulting precipitate was collected by filtration, washed with IPA, and dried to give the title compound (29.3 g, 54%) as a gray solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.99 (2H, s), 5.93 (1H, d, J = 2.3 Hz), 6.03 (1H, dd, J = 7.4, 2.5 Hz), 7.23 (1H, d, J = 7.2 Hz), 7.29–7.44 (4H, m). ^{13}C NMR (75 MHz, DMSO-d_6) δ 68.4, 98.0, 99.1, 128.5, 129.7, 132.7, 135.0, 135.5, 163.9, 167.2. MS (ESI/APCI) m/z 236.0 $[M+H]^+$.

5.1.5. 4-[(3-Chlorobenzyl)oxy]pyridine-2(1*H*)-one (4b)

The title compound was prepared in 49% yield using **3b** in an analogous manner to **4a**. Off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.07 (2H, s), 5.76 (1H, d, J = 2.4 Hz), 5.92 (1H, dd, J = 7.2 2.4 Hz), 7.25 (1H, d, J = 7.3 Hz), 7.34–7.45 (3H, m), 7.50 (1H, br s), 11.10 (1H, br s). MS (ESI/APCI) m/z = 236.0 $[M+H]^+$.

5.1.6. 4-[(4-Fluorobenzyl)oxy]pyridine-2(1*H*)-one (4c)

The title compound was prepared in 58% yield using **3c** in an analogous manner to **4a**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 5.04 (2H, s), 5.78 (1H, d, J = 2.3 Hz), 5.90 (1H, dd, J = 7.2, 2.7 Hz), 7.17–7.28 (3H, m), 7.43–7.53 (2H, m), 11.10 (1H, br s). ^{13}C NMR (75 MHz, DMSO-d_6) δ 68.5, 97.9, 99.1, 115.3 (d, J = 21 Hz), 130.2 (d, J = 8.3 Hz), 132.2 (d, J = 3 Hz), 135.4, 161.9 (d, J = 242.3 Hz), 164.0, 167.3. MS (ESI/APCI) m/z = 220.1 $[M+H]^+$.

5.1.7. 4-[(4-Chlorobenzyl)oxy]-1-(2-cyclopropyl-3-methylimidazo[1,2-*b*]pyridazin-6-yl)pyridin-2(1*H*)-one (5a)

The title compound was prepared in 28% yield using **4a** and **7a** in an analogous manner to **5c**. Pale yellow crystals; mp 234–236 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.87–1.08 (4H, m), 2.17 (1H, br s), 2.52 (3H, br s), 5.19 (2H, s), 6.04 (1H, s), 6.23 (1H, d, J = 7.8 Hz), 7.30 (1H, d, J = 9.3 Hz), 7.50 (4H, s), 7.82 (1H, d, J = 7.8 Hz), 8.02 (1H, d, J = 9.4 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ 7.6, 8.29, 8.31, 69.0, 97.7, 101.1, 115.6, 120.9, 124.0, 128.6, 129.8, 132.8, 134.7, 136.5, 137.8, 146.2, 148.1, 162.5, 167.5. MS (ESI/APCI) m/z = 407.3 $[M+H]^+$. Anal. Calcd for $C_{22}H_{19}ClN_4O_2$: C, 64.94; H, 4.71; N, 13.77. Found: C, 64.86; H, 4.63; N, 13.70.

5.1.8. 4-[(4-Chlorobenzyl)oxy]-1-(2-cyclopropyl-3-methylimidazo[1,2-*a*]pyrazin-6-yl)pyridin-2(1*H*)-one (5b)

The title compound was prepared in 42% yield using **4a** and **7b** in an analogous manner to **5c**. Pale yellow crystals; mp 221–222 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.92–1.04 (4H, m), 2.14–2.24 (1H, m), 2.54 (3H, s), 5.18 (2H, s), 6.01 (1H, d, J = 2.4 Hz), 6.17 (1H, dd, J = 7.7, 2.6 Hz), 7.50 (4H, s), 7.78 (1H, d, J = 7.7 Hz), 8.68 (1H, s), 8.83 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 7.5, 8.1, 8.7, 68.8, 97.7, 100.4, 115.1, 119.4, 128.5, 129.7, 132.8, 134.8, 135.8, 138.0, 138.3, 138.9, 149.2, 162.5, 166.9. MS (ESI/APCI) m/z = 407.4 $[M+H]^+$. Purity 97.8% (HPLC).

5.1.9. 4-[(4-Chlorobenzyl)oxy]-1-(2-cyclopropyl-3-methylimidazo[1,2-*a*]pyrimidin-6-yl)pyridin-2(1*H*)-one (5c)

A mixture of **4a** (83 mg, 0.35 mmol), **7c** (89 mg, 0.35 mmol), DMEDA (0.075 mL, 0.71 mmol), CuI (66.6 mg, 0.35 mmol), K_2CO_3 (146 mg, 1.06 mmol), and DMSO (3 mL) was heated at 150 °C for 1 h under microwave irradiation. The mixture was poured into 28% NH_3 solution at rt and extracted with EtOAc. The organic layer was separated, washed with 0.1 N NaOH solution and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (NH silica gel, hexane/EtOAc = 50/50) to give the title compound (1.1 mg, 0.77%) as white crystals. ^{1}H

NMR (400 MHz, DMSO-d_6) δ 0.95 (4H, d, J = 2.0 Hz), 2.06–2.17 (1H, m), 2.45 (3H, s), 5.18 (2H, s), 6.01–6.06 (1H, m), 6.16–6.24 (1H, m), 7.50 (4H, s), 7.68–7.75 (1H, m), 8.34–8.41 (1H, m), 8.87–8.94 (1H, m). MS (ESI/APCI) m/z = 407.4 $[M+H]^+$. Purity 99.2% (HPLC).

5.1.10. 4-[(4-Chlorobenzyl)oxy]-1-(2-cyclopropyl-1-methyl-1*H*-benzimidazol-6-yl)pyridin-2(1*H*)-one (6a)

To a stirred degassed mixture of **8a** (502 mg, 2.0 mmol), **4a** (470 mg, 2.0 mmol), and K_2CO_3 (552 mg, 4.0 mmol) in dioxane (15 mL) were added CuI (76 mg, 0.4 mmol) and *trans-N,N′*-dimethyl-cyclohexane-1,2-diamine (56 mg, 0.4 mmol). The reaction vessel was sealed and heated at 110 °C for 16 h. The reaction mixture was cooled to rt and concentrated. The resulting residue was diluted with DCM (250 mL), washed with brine (100 mL), dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography (silica gel, DCM/MeOH = 97/3 to 96/4) to give the title compound (150 mg, 18%) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 1.14–1.20 (4H, m), 2.24 (1H, m), 3.90 (3H, s), 5.16 (2H, s), 6.09 (1H, d, J = 2.6 Hz), 6.27 (1H, dd, J = 7.6, 2.7 Hz), 7.15 (1H, dd, J = 8.5, 2.0 Hz), 7.41–7.50 (5H, m), 7.69 (2H, t, J = 8.3 Hz). MS (ESI/APCI) m/z = 406.0 $[M+H]^+$. Anal. Calcd for $C_{23}H_{20}ClN_3O_2$: C, 68.06; H, 4.97; N, 10.35. Found: C, 67.96; H, 5.01; N, 10.30.

5.1.11. 1-(1,2-Dimethyl-1*H*-benzimidazol-6-yl)-4-[(4-fluorobenzyl)oxy]pyridin-2(1*H*)-one (6b)

The title compound was prepared in 25% yield using **4c** and **8d** in an analogous manner to **6e**. White solid; mp 256–258 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.55 (3H, s), 3.73 (3H, s), 5.14 (2H, s), 5.99 (1H, s), 6.09 (1H, d, J = 6.8 Hz), 7.06 (1H, d, J = 8.5 Hz), 7.26 (2H, t, J = 8.7 Hz), 7.45–7.64 (5H, m). ^{13}C NMR (101 MHz, DMSO-d_6) δ 13.4, 29.7, 68.8, 97.8, 99.8, 108.6, 115.4 (d, J = 22.2 Hz), 117.8, 120.2, 130.2 (d, J = 8.1 Hz), 132.1 (d, J = 3.0 Hz), 134.6, 135.6, 139.7, 141.5, 141.5, 153.6, 161.9 (d, J = 245.4 Hz), 162.7, 166.6. MS (ESI/APCI) m/z = 364.3 $[M+H]^+$. Anal. Calcd for $C_{21}H_{18}FN_3O_2$: C, 69.41; H, 4.99; N, 11.56. Found: C, 69.29; H, 5.04; N, 11.45.

5.1.12. 1-(2-Ethyl-1-methyl-1*H*-benzimidazol-6-yl)-4-[(4-fluorobenzyl)oxy]pyridin-2(1*H*)-one (6c)

A suspension of **9b** (5.00 g, 18.6 mmol), 1-(chloromethyl)-4-fluorobenzene (5.37 g, 37.1 mmol), K_2CO_3 (7.70 g, 55.7 mmol), and DMF (50 mL) was stirred at rt for 19 h. The resulting precipitate was collected by filtration, and the solid was washed with IPE and water successively to give a crude product (3.89 g). Other two bathes using 35 g and 50 g of **9b** gave 33.2 g and 50.0 g of crude product, respectively. Three lots were combined and recrystallized from MeOH–water to give the title compound (71.4 g, 57%, three bathes) as a white solid; mp 228–229 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.46 (3H, t, J = 7.6 Hz), 2.93 (2H, q, J = 7.5 Hz), 3.73 (3H, s), 5.02 (2H, s), 6.01–6.11 (2H, m), 7.05–7.16 (3H, m), 7.24–7.45 (6H, m), 7.77 (1H, d, J = 8.5 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ 11.3, 20.1, 29.5, 68.9, 97.8, 99.8, 108.8, 115.4 (d, J = 21.0 Hz), 118.1, 120.3, 130.3 (d, J = 8.3 Hz), 132.2 (d, J = 3.0 Hz), 134.7, 135.7, 139.7, 141.5, 157.8, 162.0 (d, J = 243.0 Hz), 162.8, 166.7. MS (ESI/APCI) m/z = 378.3 $[M+H]^+$. Anal. Calcd for $C_{22}H_{20}FN_3O_2$: C, 70.01; H, 5.34; N, 11.13. Found: C, 69.90; H, 5.26; N, 11.12.

5.1.13. 4-[(4-Fluorobenzyl)oxy]-1-(1-methyl-2-propyl-1*H*-benzimidazol-6-yl)pyridin-2(1*H*)-one (6d)

The mixture of **14** (90 mg, 0.27 mmol), HATU (106 mg, 0.28 mmol), ^{n}butyric acid (0.024 mL, 0.27 mmol), DIPEA (0.136 mL, 0.80 mmol), and DMF (2 mL) was stirred at ambient temperature for 1 h. The mixture was quenched with water and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was dissolved with AcOH (2.0 mL) and stirred at 90 °C for 1 h. After evaporating, the residue was purified by column chromatography (NH silica gel, hexane/EtOAc = 90/10 to 0/100). The residual solid was recrystallized from EtOAc–MeOH to give the title compound (48.7 mg, 47%) as an off-white solid; mp 217–219 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.00 (3H, t, J = 7.4 Hz), 1.74–1.88 (2H, m), 2.86 (2H, t, J = 7.6 Hz), 3.74 (3H, s), 5.13 (2H, s), 5.98 (1H, s), 6.09 (1H, dd, J = 7.6, 3.0 Hz), 7.06 (1H, dd, J = 8.5, 2.1 Hz), 7.21–7.31 (2H, m), 7.50–7.56 (3H, m), 7.56–7.61 (2H, m). ^{13}C NMR (101 MHz, DMSO-d_6) δ 13.7, 20.1, 28.4, 29.6, 68.8, 97.8, 99.8, 108.7, 115.3 (d, J = 21.2 Hz), 118.0, 120.3, 130.2 (d, J = 8.1 Hz), 132.1 (d, J = 3.0 Hz), 134.6, 135.6, 135.6, 139.7, 141.5, 156.7, 161.9 (d, J = 245.4 Hz), 162.7, 162.8, 166.6. MS (ESI/APCI) m/z = 392.2 $[M+H]^+$. Anal. Calcd for $C_{23}H_{22}FN_3O_2 \cdot 0.1H_2O$: C, 70.25; H, 5.69; N, 10.69. Found: C, 70.28; H, 5.57; N, 10.71.

5.1.14. 1-(2-Cyclopropyl-1-methyl-1*H*-benzimidazol-6-yl)-4-[(4-fluorobenzyl)oxy]pyridin-2(1*H*)-one (6e)

To a solution of **4c** (2.44 g, 11.2 mmol), **8a** (2.8 g, 11.15 mmol), K_2CO_3 (4.62 g, 33.5 mmol), and DMEDA (1.20 mL, 11.15 mmol) in DMSO (56 mL) was added CuI (2.12 g, 11.2 mmol), and the mixture was stirred at 150 °C under Ar atmosphere for 2 h. After cooling to 0 °C, 28% NH_3 solution (56.0 mL) was added, and the mixture was allowed to warm to rt for 2 h. The precipitate was collected by filtration, washed with water and IPE, dissolved in THF (500 mL), and filtered through a short NH silica-gel column (EtOAc). The filtrate was concentrated and the residue was purified by column chromatography (NH silica gel, hexane/EtOAc = 90/10 to 0/100), followed by recrystallized from EtOH–water to give the title compound (1.60 g, 37%) as an off-white solid; mp 221–223 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.99–1.15 (4H, m), 2.20–2.33 (1H, m), 3.85 (3H, s), 5.13 (2H, s), 5.98 (1H, d, J = 2.6 Hz), 6.09 (1H, dd, J = 7.7, 2.8 Hz), 7.00–7.09 (1H, m), 7.21–7.32 (2H, m), 7.43–7.67 (5H, m). ^{13}C NMR (101 MHz, DMSO-d_6) δ 7.0, 8.3, 29.6, 68.8, 97.8, 99.8, 108.6, 115.3 (d, J = 22.2 Hz), 117.6, 120.5, 130.2 (d, J = 9.1 Hz), 132.1 (d, J = 3.0 Hz), 134.5, 135.7, 139.6, 141.0, 158.2, 161.9 (d, J = 245.4 Hz), 162.7, 166.6. MS (ESI/APCI) m/z = 390.2 $[M+H]^+$. Anal. Calcd for $C_{23}H_{20}FN_3O_2 \cdot 0.1H_2O$: C, 70.61; H, 5.20; N, 10.74. Found: C, 70.53; H, 5.19; N, 10.69.

5.1.15. 1-(2-Cyclobutyl-1-methyl-1*H*-benzimidazol-6-yl)-4-[(4-fluorobenzyl)oxy]pyridin-2(1*H*)-one (6f)

The title compound was prepared in 22% yield using **4c** and **8g** in an analogous manner to **6a**. White solid; mp 246–249 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.91–1.94 (1H, m), 2.05–2.12 (1H, m), 2.39–2.46 (4H, m), 3.66 (3H, s), 3.89 (1H, m), 5.13 (2H, s), 5.98 (1H, d, J = 2.8 Hz), 6.08 (1H, dd, J = 7.6, 2.8 Hz), 7.07 (1H, dd, J = 8.4, 2.0 Hz), 7.26 (2H, t, J = 8.8 Hz), 7.51–7.55 (3H, m), 7.57–7.63 (2H, m). ^{13}C NMR (101 MHz, DMSO-d_6) δ 18.1, 26.4, 29.4, 31.5, 68.8, 97.8, 99.8, 108.7, 115.3 (d, J = 21.2 Hz), 118.2, 120.3, 130.2 (d, J = 9.1 Hz), 32.1 (d, J = 3.0 Hz), 134.7, 135.9, 139.6, 141.4, 159.2, 161.9 (d, J = 245.4 Hz), 162.7, 166.6. MS (ESI/APCI) m/z = 404.0 $[M+H]^+$. Anal. Calcd for $C_{24}H_{22}FN_3O_2 \cdot 0.11H_2O$: C, 71.10; H, 5.52; N, 10.36. Found: C, 71.14; H, 5.42; N, 10.32.

5.1.16. 1-(2-Cyclopentyl-1-methyl-1*H*-benzimidazol-6-yl)-4-[(4-fluorobenzyl)oxy]pyridin-2(1*H*)-one (6g)

The title compound was prepared in 22% yield using cyclopentanecarboxylic acid in an analogous manner to **6d**. White solid; mp 262–263 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.59–1.85 (4H, m), 1.88–1.99 (2H, m), 2.07 (2H, br s), 3.45 (1H, t, J = 7.7 Hz), 3.76 (3H, s), 5.13 (2H, s), 5.99 (1H, d, J = 2.6 Hz), 6.09 (1H, dd, J = 7.5, 2.6 Hz), 7.06 (1H, dd, J = 8.5, 2.1 Hz), 7.26 (2H, t, J = 8.9 Hz), 7.46–7.64 (5H, m). ^{13}C NMR (101 MHz, DMSO-d_6) δ 25.3, 29.6, 31.1, 36.2, 68.8, 97.8, 99.8, 108.7, 115.3 (d, J = 21.2 Hz), 118.1, 120.2,

130.2 (d, J = 8.1 Hz), 132.1 (d, J = 3.0 Hz), 134.6, 135.9, 139.7, 141.3, 160.3, 161.9 (d, J = 245.4 Hz), 162.7, 166.6. MS (ESI/APCI) m/z = 418.1 $[M+H]^+$. Anal. Calcd for $C_{25}H_{24}FN_3O_2 \cdot 0.14H_2O$: C, 71.49; H, 5.83; N, 10.00. Found: C, 71.48; H, 5.67; N, 10.04.

5.1.17. 1-[2-(Cyclopropylmethyl)-1-methyl-1*H*-benzimidazol-6-yl]-4-[(4-fluorobenzyl)oxy]pyridin-2(1*H*)-one (6h)

The title compound was prepared in 37% yield using **4c** and **8e** in an analogous manner to **6j**. Pale yellow solid; mp 206–214 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.27–0.37 (2H, m), 0.57–0.68 (2H, m), 1.12–1.25 (1H, m), 2.87 (2H, d, J = 6.4 Hz), 3.75 (3H, s), 5.02 (2H, s), 5.96–6.13 (2H, m), 7.04–7.16 (3H, m), 7.31 (1H, d, J = 7.2 Hz), 7.35–7.45 (3H, m), 7.79 (1H, d, J = 8.3 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ 4.5, 8.9, 29.8, 31.1, 68.8, 97.8, 99.8, 108.8, 115.3 (d, J = 21.2 Hz), 118.1, 120.3, 130.2 (d, J = 8.1 Hz), 132.1 (d, J = 3.0 Hz), 134.7, 135.6, 139.7, 141.6, 156.4, 161.9 (d, J = 245.4 Hz), 162.7, 166.6. MS (ESI/APCI) m/z = 404.2 $[M+H]^+$. Anal. Calcd for $C_{24}H_{22}FN_3O_2$: C, 71.45; H, 5.50; N, 10.42. Found: C, 70.88; H, 5.57; N, 10.13.

5.1.18. 1-[2-(2,2-Dimethylpropyl)-1-methyl-1*H*-benzimidazol-6-yl]-4-[(4-fluorobenzyl)oxy]pyridin-2(1*H*)-one (6i)

The title compound was prepared in 34% yield using **4c** and **8f** in an analogous manner to **6e**. White solid; mp 238–239 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.04 (9H, s), 2.81 (2H, s), 3.77 (3H, s), 5.14 (2H, s), 5.99 (1H, d, J = 2.6 Hz), 6.09 (1H, dd, J = 7.6, 2.6 Hz), 7.08 (1H, dd, J = 8.5, 2.1 Hz), 7.20–7.32 (2H, m), 7.48–7.56 (3H, m), 7.61 (2H, dd, J = 7.9, 5.3 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ 29.4, 30.4, 32.4, 39.0, 68.8, 97.8, 99.8, 109.0, 115.3 (d, J = 21.2 Hz), 118.1, 120.4, 130.2 (d, J = 8.1 Hz), 132.1 (d, J = 3.0 Hz), 134.6, 135.3, 139.7, 141.6, 155.0, 161.9 (d, J = 245.4 Hz), 162.8, 166.7. MS (ESI/APCI) m/z = 420.2 $[M+H]^+$. Anal. Calcd for $C_{25}H_{26}FN_3O_2$: C, 71.58; H, 6.25; N, 10.02. Found: C, 71.46; H, 61.7; N, 9.97.

5.1.19. 4-[(4-Chlorobenzyl)oxy]-1-(2-cyclopropyl-1-ethyl-1*H*-benzimidazol-6-yl)pyridin-2(1*H*)-one (6j)

A mixture of **4a** (100 mg, 0.42 mmol), **8b** (124 mg, 0.47 mmol), CuI (81 mg, 0.42 mmol), DMEDA (0.048 mL, 0.42 mmol), K_2CO_3 (147 mg, 1.06 mmol), and DMSO (2.5 mL) was heated 120 °C for 1 h under microwave irradiation. The mixture was quenched with 28% ammonia solution at rt and extracted with EtOAc. The organic layer was separated, washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (NH silica gel, hexane/EtOAc = 75/25 to 0/100). The solid was crystallized from IPA–hexane to give the title compound (85 mg, 48%) as a light pink solid; mp 203–204 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.08–1.19 (2H, m), 1.20–1.31 (2H, m), 1.46 (3H, t, J = 7.4 Hz), 1.93–2.05 (1H, m), 4.29 (2H, q, J = 7.2 Hz), 5.02 (2H, s), 6.01–6.08 (2H, m), 7.10 (1H, dd, J = 8.7, 1.9 Hz), 7.28–7.42 (6H, m), 7.70 (1H, d, J = 8.3 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ 7.0, 7.1, 8.4, 15.0, 37.7, 68.7, 97.9, 99.8, 108.4, 117.8, 120.4, 128.5, 129.7, 132.7, 134.5, 134.8, 135.0, 139.8, 141.5, 157.6, 162.8, 166.6. MS (ESI/APCI) m/z = 420.1 $[M+H]^+$. Anal. Calcd for $C_{24}H_{22}ClN_3O_2$: C, 68.65; H, 5.28; N, 10.01. Found: C, 68.53; H, 5.29; N, 9.73.

5.1.20. 4-[(4-Chlorobenzyl)oxy]-1-(2-cyclopropyl-1-propyl-1*H*-benzimidazol-6-yl)pyridin-2(1*H*)-one (6k)

The title compound was prepared in 11% yield using **4a** and **8c** in an analogous manner to **6j**. Off-white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.00 (3H, t, J = 7.5 Hz), 1.08–1.20 (2H, m), 1.23–1.33 (2H, m), 1.85–1.96 (2H, m), 1.96–2.04 (1H, m), 4.20 (2H, t, J = 7.3 Hz), 5.02 (2H, s), 6.00–6.13 (2H, m), 7.01–7.16 (3H, m), 7.28–7.36 (2H, m), 7.37–7.45 (2H, m), 7.70 (1H, d, J = 8.3 Hz). MS (ESI/APCI) m/z = 434.2 $[M+H]^+$.

5.1.21. 4-(Benzyloxy)-1-(2-cyclopropyl-1-methyl-1*H*-benzimidazol-6-yl)pyridin-2(1*H*)-one (6l)

The title compound was prepared in 87% yield using 4-(benzyloxy)pyridin-2(1*H*)-one and **8a** in an analogous manner to **6a**. White solid; mp 210–211 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.95–1.19 (4H, m), 2.18–2.34 (1H, m), 3.85 (3H, s), 5.15 (2H, s), 5.98 (1H, d, J = 3.0 Hz), 6.10 (1H, dd, J = 7.6, 2.6 Hz), 6.96–7.11 (1H, m), 7.29–7.66 (8H, m). ^{13}C NMR (101 MHz, DMSO-d_6) δ 7.1, 8.3, 29.5, 69.6, 97.8, 99.8, 108.5, 117.7, 120.3, 127.8, 128.1, 128.5, 134.5, 135.8, 135.9, 139.6, 141.4, 158.3, 162.8, 166.7. MS (ESI/APCI) m/z = 372.0 $[M+H]^+$. Anal. Calcd for $C_{23}H_{21}N_3O_2$: C, 74.37; H, 5.70; N, 11.31. Found: C, 74.19; H, 5.76; N, 11.16.

5.1.22. 4-[(3-Chlorobenzyl)oxy]-1-(2-cyclopropyl-1-methyl-1*H*-benzimidazol-6-yl)pyridin-2(1*H*)-one (6m)

The title compound was prepared in 29% yield using **4b** and **8a** in an analogous manner to **6a**. White solid; mp 220–222 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.02–1.11 (4H, m), 2.25–2.29 (1H, m), 3.85 (3H, s), 5.18 (2H, s), 5.97 (1H, d, J = 2.4 Hz), 6.12 (1H, dd, J = 7.5, 2.5 Hz), 7.04 (1H, dd, J = 8.5, 1.6 Hz), 7.43–7.48 (3H, m), 7.49–7.55 (3H, m), 7.59 (1H, d, J = 7.6 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ 7.1, 8.4, 29.6, 68.6, 97.9, 99.8, 108.6, 117.8, 120.4, 126.4, 127.5, 128.1, 130.5, 133.1, 134.4, 135.8, 138.5, 139.8, 141.4, 158.3, 162.8, 166.5. MS (ESI/APCI) m/z = 405.8 $[M+H]^+$. Anal. Calcd for $C_{23}H_{20}ClN_3O_2$: C, 68.06; H, 4.97; N, 10.35. Found: C, 67.94; H, 4.91; N, 10.31.

5.1.23. 4-[(2-Chlorobenzyl)oxy]-1-(2-cyclopropyl-1-methyl-1*H*-benzimidazol-6-yl)pyridin-2(1*H*)-one (6n)

To a suspension of **9a** (100 mg, 0.36 mmol) and (2-chlorophenyl)methanol (101 mg, 0.71 mmol) in THF (2 mL) were added tributylphosphine (0.266 ml, 1.07 mmol) and ADDP (269 mg, 1.07 mmol) at 60 °C, and the mixture was stirred at the same temperature for 3 h. After solvent was removed by evaporation, the residue was purified by column chromatography (silica gel, hexane/EtOAc = 90/10 to 0/100, then EtOAc/MeOH = 100/0 to 85/15), followed by column chromatography (NH silica gel, hexane/EtOAc = 90/10 to 0/100, then EtOAc/MeOH = 100/0 to 85/15). The residual solid was recrystallized by EtOH–hexane to give the title compound (60.0 mg, 42%) as an off-white solid; mp 199–201 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.01–1.13 (4H, m), 2.23–2.31 (1H, m), 3.85 (3H, s), 5.20 (2H, s), 6.02 (1H, d, J = 2.6 Hz), 6.10 (1H, dd, J = 7.5, 2.5 Hz), 7.06 (1H, dd, J = 8.6, 1.6 Hz), 7.40–7.48 (2H, m), 7.50–7.57 (3H, m), 7.59 (1H, d, J = 7.5 Hz), 7.61–7.66 (1H, m). ^{13}C NMR (75 MHz, DMSO-d_6) δ 7.1, 8.4, 29.6, 67.3, 97.7, 99.6, 108.6, 117.8, 120.4, 127.5, 129.5, 130.4, 130.7, 133.0, 133.1, 134.4, 135.8, 139.8, 141.4, 158.3, 162.8, 166.7. MS (ESI/APCI) m/z = 406.1 $[M+H]^+$. Anal. Calcd for $C_{23}H_{20}ClN_3O_2$: C, 68.06; H, 4.97; N, 10.35. Found: C, 68.11; H, 4.88; N, 10.31.

5.1.24. 4-[(5-Chloropyridin-2-yl)methoxy]-1-(2-cyclopropyl-1-methyl-1*H*-benzimidazol-6-yl)-pyridin-2(1*H*)-one (6o)

The title compound was prepared in 14% yield using **9a** and (5-chloropyridin-2-yl)methanol in an analogous manner to **6c**. White solid; mp 234–236 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.04–1.09 (4H, m), 2.26–2.32 (1H, m), 3.84 (3H, s), 5.23 (2H, s), 5.96 (1H, s), 6.12 (1H, dd, J = 7.7, 2.6 Hz), 7.04 (1H, m), 7.50–7.52 (2H, m), 7.59–7.61 (2H, m), 8.02–8.04 (1H, m), 8.67 (1H, s). ^{13}C NMR (75 MHz, DMSO-d_6) δ 7.1, 8.4, 29.6, 69.8, 98.0, 99.7, 108.5, 117.8, 120.4, 123.5, 130.5, 130.5, 134.4, 135.8, 136.9, 139.9, 141.4, 147.8, 154.1, 158.3, 162.7, 166.5. MS (ESI/APCI) m/z = 407.4 $[M+H]^+$. Anal. Calcd for $C_{22}H_{19}ClN_4O_2$: C, 64.94; H, 4.71; N, 13.77. Found: C, 64.77; H, 4.83; N, 13.50.

5.1.25. 4-[(5-Chloropyrimidin-2-yl)methoxy]-1-(2-cyclopropyl-1-methyl-1*H*-benzimidazol-6-yl)pyridin-2(1*H*)-one (6p)

A mixture of **6aa** (100 mg, 0.27 mmol), 2-chloro-1,3-bis (dimentylamino)trimethinium hexafluorophosphate (98 mg, 0.32 mmol), sodium methoxide (43.4 mg, 0.80 mmol), and MeOH (3 mL) was stirred at rt for 1 h. The mixture was concentrated and the residue was diluted with water. The aqueous phase was extracted with EtOAc. The combined organic layers were washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (NH silica gel, hexane/EtOAc = 90/10 to 0/100) to give the title compound (56.0 mg, 51%) as a white solid; mp 217–219 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.94–1.17 (4H, m), 2.26 (1H, br s), 3.85 (3H, s), 5.36 (2H, s), 5.87 (1H, br s), 6.13 (1H, d, J = 9.0 Hz), 7.04 (1H, d, J = 9.0 Hz), 7.51 (2H, br s), 7.59 (1H, d, J = 6.9 Hz), 9.02 (2H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 7.0, 8.3, 29.5, 69.5, 97.9, 99.6, 108.5, 117.7, 120.3, 129.8, 134.4, 135.8, 139.8, 141.4, 156.1, 158.3, 162.5, 162.6, 166.6. MS (ESI/APCI) m/z = 408.3 $[M+H]^+$.

5.1.26. 1-(2-Cyclopropyl-1-methyl-1*H*-benzimidazol-6-yl)-4-(thiophen-2-ylmethoxy)pyridin-2(1*H*)-one (6q)

The title compound was prepared in 30% yield using **9a** and thiophen-2-ylmethanol in an analogous manner to **6n**. White solid; mp 222–223 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.04–1.11 (4H, m), 2.24–2.28 (1H, m), 3.85 (3H, s), 5.34 (2H, s), 6.05–6.07 (2H, m), 7.03–7.08 (2H, m), 7.27 (1H, d, J = 2.8 Hz), 7.50–7.52 (2H, m), 7.57 (1H, d, J = 7.2 Hz), 7.61 (1H, d, J = 4.9 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ 7.1, 8.3, 29.5, 64.4, 97.8, 99.7, 108.5, 117.8, 120.4, 126.9, 127.3, 128.3, 134.4, 135.8, 137.8, 139.7, 141.4, 158.3, 162.7, 166.3. MS (ESI/APCI) m/z = 377.8 $[M+H]^+$. Purity 99.4% (HPLC).

5.1.27. 1-(2-Cyclopropyl-1-methyl-1*H*-benzimidazol-6-yl)-4-(thiophen-3-ylmethoxy)pyridin-2(1*H*)-one (6r)

The title compound was prepared in 37% yield using **9a** and thiophen-3-ylmethanol in an analogous manner to **6n**. Off-white solid; mp 223–225 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.02–1.09 (4H, m), 2.24–2.28 (1H, m), 3.85 (3H, s), 5.13 (2H, s), 5.99 (1H, d, J = 2.6 Hz), 6.06 (1H, dd, J = 7.5, 2.6 Hz), 7.04 (1H, dd, J = 8.4, 1.7 Hz), 7.19 (1H, d, J = 4.2 Hz), 7.50–7.52 (2H, m), 7.56–7.60 (2H, m), 7.64 (1H, m). ^{13}C NMR (101 MHz, DMSO-d_6) δ 7.1, 8.3, 29.5, 65.1, 97.6, 99.8, 108.5, 117.8, 120.4, 124.7, 126.8, 127.6, 134.5, 135.8, 136.7, 139.6, 141.4, 158.3, 162.8. MS (ESI/APCI) m/z = 378.2 $[M+H]^+$. Anal. Calcd for $C_{21}H_{19}N_3O_2S{\cdot}0.12H_2O$: C, 66.44; H, 5.11; N, 11.07. Found: C, 66.49; H, 5.09; N, 11.08.

5.1.28. 4-[(5-Chlorothiophen-2-yl)methoxy]-1-(2-cyclopropyl-1-methyl-1*H*-benzimidazol-6-yl)pyridin-2(1*H*)-one (6s)

The title compound was prepared in 33% yield using **9a** and (5-chlorothiophen-2-yl)methanol in an analogous manner to **6n**. White solid; mp 218–220 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.02–1.11 (4H, m), 2.23–2.28 (1H, m), 3.85 (3H, s), 5.29 (2H, s), 6.03–6.07 (2H, m), 7.02 (1H, dd, J = 8.5, 1.8 Hz), 7.08 (1H, d, J = 3.7 Hz), 7.16 (1H, d, J = 3.8 Hz), 7.51 (2H, dd, J = 5.4, 3.5 Hz), 7.57 (1H, d, J = 7.4). ^{13}C NMR (75 MHz, DMSO-d_6) δ 7.1, 8.4, 29.6, 64.4, 97.9, 99.6, 108.5, 117.8, 120.4, 126.6, 128.3, 129.1, 134.4, 135.8, 137.3, 139.8, 141.4, 158.3, 162.7, 166.1. MS (ESI/APCI) m/z = 412.3 $[M+H]^+$. Anal. Calcd for $C_{21}H_{18}ClN_3O_2S$: C, 61.23; H, 4.40; N, 10.20. Found: C, 61.34; H, 4.43; N, 10.21.

5.1.29. 4-[(4-Chlorothiophen-2-yl)methoxy]-1-(2-cyclopropyl-1-methyl-1*H*-benzimidazol-6-yl)pyridin-2(1*H*)-one (6t)

The title compound was prepared in 24% yield using **9a** and (4-chlorothiophen-2-yl)methanol in an analogous manner to **6n**. Off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.04–1.09 (4H, m), 2.26 (1H, m), 3.85 (3H, s), 5.32 (2H, s), 6.03–6.08 (2H, m), 7.04 (1H, dd, J = 8.5, 1.8 Hz), 7.28 (1H, s), 7.50–7.52 (2H, m), 7.58 (1H, d, J = 7.5 Hz), 7.64 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 7.1, 8.3, 29.5, 64.0, 97.9, 99.6, 108.5, 117.8, 120.3, 122.1, 123.0, 127.8, 134.4, 135.8, 139.4, 139.8, 141.4, 158.3, 162.7, 166.1. MS (ESI/APCI) m/z = 412.2 $[M+H]^+$. Purity > 99.9% (HPLC).

5.1.30. 4-[(5-Chlorothiophen-3-yl)methoxy]-1-(2-cyclopropyl-1-methyl-1*H*-benzimidazol-6-yl)pyridin-2(1*H*)-one (6u)

The title compound was prepared in 9% yield using **9a** and (5-chlorothiophen-3-yl)methanol in an analogous manner to **6n**. White solid; mp 236–237 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.02–1.11 (4H, m), 2.24–2.32 (1H, m), 3.85 (3H, s), 5.05 (2H, s), 5.98 (1H, d, J = 2.6 Hz), 6.06 (1H, dd, J = 7.6, 2.7 Hz), 7.03 (1H, dd, J = 8.4, 1.8 Hz), 7.20 (1H, d, J = 1.4 Hz), 7.51 (2H, dd, J = 5.4, 3.5 Hz), 7.56–7.58 (2H, m). ^{13}C NMR (101 MHz, DMSO-d_6) δ 7.1, 8.3, 29.5, 65.0, 97.7, 99.7, 108.5, 117.7, 120.3, 124.2, 127.0, 128.8, 134.4, 135.8, 136.5, 139.7, 141.4, 158.3, 162.7, 166.5. MS (ESI/APCI) m/z = 412.0 $[M+H]^+$. Anal. Calcd for $C_{21}H_{18}ClN_3O_2S$: C, 61.23; H, 4.40; N, 10.20. Found: C, 61.19; H, 4.39; N, 10.17.

5.1.31. 1-(2-Cyclopropyl-1-methyl-1*H*-benzimidazol-6-yl)-4-{[5-(trifluoromethyl)thiophen-2-yl]methoxy}pyridin-2(1*H*)-one (6v)

The title compound was prepared in 35% yield using **9a** and [5-(trifluoromethyl)thiophen-2-yl]methanol in an analogous manner to **6n**. White solid; mp 256–257 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.99–1.14 (4H, m), 2.22–2.31 (1H, m), 3.85 (3H, s), 5.45 (2H, s), 6.06 (1H, d, J = 2.5 Hz), 6.10 (1H, dd, J = 7.5, 2.7 Hz), 7.05 (1H, dd, J = 8.5, 1.8 Hz), 7.38 (1H, d, J = 2.8 Hz), 7.49–7.54 (2H, m), 7.60 (1H, d, J = 7.5 Hz), 7.69 (1H, d, J = 2.8 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ 7.0, 8.3, 29.5, 64.0, 98.0, 99.5, 108.5, 117.8, 120.3, 125.0 (q, J = 269.7 Hz), 128.1, 129.4 (q, J = 37.4 Hz), 130.0 (q, J = 4.0 Hz), 134.4, 135.8, 139.9, 141.4, 143.4 (d, J = 2.0 Hz), 158.3, 162.6, 166.0. MS (ESI/APCI) m/z = 446.1 $[M+H]^+$. Anal. Calcd for $C_{22}H_{18}F_3N_3O_2S$: C, 59.32; H, 4.07; N, 9.43. Found: C, 59.43; H, 4.10; N, 9.42.

5.1.32. 1-(2-Cyclopropyl-1-methyl-1*H*-benzimidazol-6-yl)-4-{[4-(trifluoromethyl)thiophen-2-yl]methoxy}pyridin-2(1*H*)-one (6w)

NaH (60% oil dispersion, 87 mg, 2.18 mmol) was added to a solution of [4-(trifluoromethyl)thiophen-2-yl]methanol (397 mg, 2.18 mmol) in DMA at 0 °C. After being stirred at the same temperature for 30 min, **10** (500 mg, 1.45 mmol) was added to the reaction mixture. The mixture was stirred at 120 °C for 10 min. The mixture was quenched with water at 0 °C and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (NH silica gel, hexane/EtOAc = 100/0 to 0/100), followed by preparative HPLC (L-Column 2 ODS, eluted with H_2O in acetonitrile containing 0.1% TFA). The desired fraction was neutralized with satd $NaHCO_3$ solution and extracted with EtOAc. The organic layer was separated, dried over $MgSO_4$, and concentrated in vacuo to give the title compound (330 mg, 51%) as a pale yellow solid; mp 218–219 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.95–1.14 (4H, m), 2.21–2.33 (1H, m), 3.85 (3H, s), 5.39 (2H, s), 6.05–6.10 (2H, m), 7.05 (1H, dd, J = 8.5, 1.2 Hz), 7.52 (2H, dd, J = 5.0, 3.2 Hz), 7.56–7.67 (2H, m), 8.33 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 7.0, 8.3, 29.5, 63.9, 97.9, 99.6, 108.5, 117.8, 120.3, 122.0 (q, J = 270.7 Hz), 124.57, 124.59, 129.3 (q, J = 35.4 Hz), 129.6 (q, J = 4.0 Hz), 134.4, 135.8, 139.8, 141.4, 158.3, 162.7, 166.1. MS (ESI/APCI) m/z = 446.1 $[M+H]^+$. Anal. Calcd for $C_{22}H_{18}F_3N_3O_2S{\cdot}0.25H_2O$: C, 58.72; H, 4.14; N, 9.34. Found: C, 58.89; H, 4.21; N, 9.29.

5.1.33. 1-(2-Cyclopropyl-1-methyl-1*H*-benzimidazol-6-yl)-4-{[5-(trifluoromethyl)thiophen-3-yl]methoxy}pyridin-2(1*H*)-one (6x)

The title compound was prepared in 26% yield using **10** and [5-(trifluoromethyl)thiophen-3-yl]methanol in an analogous manner to **6x**. White solid; mp 236–237 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.02–1.12 (4H, m), 2.22–2.31 (1H, m), 3.85 (3H, s), 5.16 (2H, s), 6.01 (1H, d, J = 2.5 Hz), 6.09 (1H, dd, J = 7.6, 2.4 Hz), 7.04 (1H, dd, J = 8.5, 1.3 Hz), 7.48–7.55 (2H, m), 7.59 (1H, d, J = 7.5 Hz), 7.81 (1H, s), 8.06 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 7.0, 8.3, 29.5, 64.5, 97.7, 99.7, 108.5, 117.8, 120.3, 122.4 (q, J = 270.0 Hz), 129.6, 129.8 (q, J = 37.4 Hz), 130.2 (q, J = 3.0 Hz), 134.4, 135.8, 137.2, 139.7, 141.4, 158.3, 162.7, 166.4. MS (ESI/APCI) m/z = 446.3 [M+H]$^+$. Anal. Calcd for $C_{22}H_{18}F_3N_3O_2S$: C, 59.32; H, 4.07; N, 9.43. Found: C, 59.36; H, 4.25; N, 9.34.

5.1.34. 4-(Benzyloxy)-1-(2-ethyl-1-methyl-1*H*-benzimidazol-6-yl)pyridin-2(1*H*)-one (6y)

The title compound was prepared in 47% yield using 4-(benzyloxy)pyridin-2(1*H*)-one and **8h** in an analogous manner to **6e**. White solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.46 (3H, t, J = 7.5 Hz), 2.93 (2H, q, J = 7.6 Hz), 3.73 (3H, s), 5.06 (2H, s), 6.03–6.13 (2H, m), 7.13 (1H, dd, J = 8.4, 1.9 Hz), 7.28–7.48 (7H, m), 7.77 (1H, d, J = 8.4 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ 11.3, 20.1, 29.5, 69.6, 97.8, 99.9, 108.8, 118.1, 120.3, 127.9, 128.2, 128.5, 134.7, 135.7, 135.9, 139.7, 141.5, 157.8, 162.8, 166.8. MS (ESI/APCI) m/z = 360.3 [M+H]$^+$. Anal. Calcd for $C_{22}H_{21}N_3O_2 \cdot 0.92H_2O$: C, 70.28; H, 6.12; N, 11.18. Found: C, 70.18; H, 5.72; N, 11.13.

5.1.35. {[1-(2-Cyclopropyl-1-methyl-1*H*-benzimidazol-6-yl)-2-oxo-1,2-dihydropyridin-4-yl]oxy}acetonitrile (6z)

A mixture of **9a** (1.00 g, 3.55 mmol), bromoacetonitrile (0.27 mL, 3.91 mmol), K_2CO_3 (1.47 g, 10.7 mmol), and DMF (10 mL) was stirred at 80 °C for 2 h. The mixture was poured into water and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, and concentrated. The residue was purified by column chromatography (NH silica gel, hexane/EtOAc = 97/3 to 0/100) to give the title compound (0.87 g, 76%) as a white solid; mp 184–186 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.97–1.20 (4H, m), 2.27 (1H, br s), 3.85 (3H, s), 5.25 (2H, s), 6.08 (1H, br s), 6.14 (1H, d, J = 7.65 Hz), 7.07 (1H, d, J = 8.5 Hz), 7.49–7.59 (2H, m), 7.67 (1H, d, J = 7.4 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ 7.1, 8.4, 29.5, 53.3, 98.2, 98.9, 108.6, 115.7, 117.8, 120.3, 134.2, 135.8, 140.4, 141.5, 158.4, 162.4, 164.9. MS (ESI/APCI) m/z = 321.3 [M+H]$^+$.

5.1.36. 2-{[1-(2-Cyclopropyl-1-methyl-1*H*-benzimidazol-6-yl)-2-oxo-1,2-dihydropyridin-4-yl]oxy}ethanimidamide hydrochloride (6aa)

Sodium methoxide (2.53 mg, 0.050 mmol) was added to a solution of **6z** (300 mg, 0.94 mmol) in MeOH (4 mL) and the mixture was stirred at rt for 4 h. To the solution was added ammonium chloride (52.6 mg, 0.98 mmol) and the mixture was stirred at rt overnight. The solvent was evaporated to give the title compound (368 mg, quant.) as a light brown solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.19 (4H, m), 2.27 (1H, br s), 3.86 (3H, s), 4.99 (2H, s), 5.87 (1H, s), 6.16 (1H, d, J = 5.3 Hz), 7.04 (1H, d, J = 7.8 Hz), 7.48–7.57 (2H, m), 7.68 (1H, d, J = 7.5 Hz), 9.04 (3H, br s). MS (ESI/APCI) m/z = 321.3 [M+H]$^+$.

5.1.37. 6-Bromo-2-cyclopropyl-3-methylimidazo[1,2-*b*]pyridazine (7a)

To a solution of **15a** (1.0 g, 5.75 mmol) in DMA (10 mL) was added 2-bromo-1-cyclopropylpropan-1-one (1.40 mL, 11.5 mmol) and $NaHCO_3$ (0.97 g, 11.5 mmol) at rt, and the mixture was stirred at 80 °C for 16 h. The mixture was poured into water and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc = 100/0 to 70/30) to give the title compound (1.10 g, 76%) as yellow crystals. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.87–1.01 (4H, m), 2.14 (1H, br s), 2.51–2.53 (3H, s), 7.28 (1H, d, J = 9.3 Hz), 7.90 (1H, d, J = 9.3 Hz). MS (ESI/APCI) m/z = 252.0 [M+H]$^+$.

5.1.38. 6-Bromo-2-cyclopropyl-3-methylimidazo[1,2-*a*]pyrazine (7b)

To a solution of **18** (1.24 g, 2.92 mmol) in THF (10 mL) was added TFAA (0.826 mL, 5.84 mmol) at 0 °C, and the mixture was heated at 60 °C for 3 h. The mixture was poured into satd $NaHCO_3$ solution at rt and extracted with EtOAc. The organic layer was separated, washed with 1 N NaOH solution and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc = 100/0 to 50/50) to give the title compound (0.59 g, 80%) as pale yellow crystals. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.81–1.07 (4H, m), 2.15 (1H, t, J = 4.8 Hz), 2.53 (3H, s), 8.61 (1H, s), 8.68 (1H, s). MS (ESI/APCI) m/z = 252.2 [M+H]$^+$.

5.1.39. 6-Bromo-2-cyclopropyl-3-methylimidazo[1,2-*a*]pyrimidine (7c)

To a solution of **15b** (500 mg, 2.87 mmol) in DMF (10 mL) was added 2-bromo-1-cyclopropylpropan-1-one (0.70 mL, 5.75 mmol) at rt, and the mixture was stirred at 100 °C for 24 h. The mixture was poured into 1 N NaOH solution and extracted with EtOAc. The organic layer was separated, washed with 1 N NaOH solution and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (NH silica gel, hexane/EtOAc = 100/0 to 50/50) to give the title compound (89 mg, 12%) as pale yellow crystals. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.84–1.02 (4H, m), 2.04–2.15 (1H, m), 2.50 (3H, br s), 8.41 (1H, s), 8.98 (1H, s).

5.1.40. 6-Bromo-2-cyclopropyl-1-methyl-1*H*-benzimidazole (8a)

A mixture of **12a** (4.20 g, 18.2 mmol), zinc (5.94 g, 90.9 mmol), NH_4Cl (9.7 g, 182 mmol), MeOH (50 mL), and water (25 mL) was stirred at rt for 3 h. After MeOH was removed by evaporation, the mixture was neutralized with satd $NaHCO_3$ solution and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. Then the residue was dissolved in $POCl_3$ (1.68 mL, 18.0 mmol) and cyclopropanecarboxylic acid (2.86 mL, 36.0 mmol) was added to the mixture at rt. The mixture was stirred at 120 °C for 3 h. After cooling to 0 °C, ice water and satd $NaHCO_3$ solution were carefully added, and the mixture was extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, concentrated to give a brown solid. This solid was dissolved in 1 N HCl solution and washed with EtOAc. The aqueous layer was basified with 4 N NaOH solution and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated to give the title compound (3.3 g, 72%) as a brown solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.95–1.14 (4H, m), 2.23 (1H, tt, J = 7.9, 5.1 Hz), 3.83 (3H, s), 7.24 (1H, dd, J = 8.5, 2.1 Hz), 7.41 (1H, d, J = 8.7 Hz), 7.75 (1H, d, J = 1.9 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ 7.0, 8.4, 29.5, 112.4, 113.4, 119.6, 123.9, 137.2, 141.1, 157.9. Anal. Calcd for $C_{11}H_{11}BrN_2$: C, 52.61; H, 4.42; N, 11.16. Found: C, 52.37; H, 4.31; N, 11.14.

5.1.41. 6-Bromo-2-cyclopropyl-1-ethyl-1*H*-benzimidazole (8b)

Zinc (8.0 g, 122 mmol) was added to a solution of **12b** (3.0 g, 12.2 mmol) in AcOH (60 mL) at rt. The mixture was stirred at ambient temperature for 30 min. The insoluble material was removed by filtration and the filtrate was concentrated in vacuo.

The mixture was neutralized with satd $NaHCO_3$ solution and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo to give an intermediate 4-bromo-N^2-ethylbenzene-1,2-diamine.

HATU (4.89 g, 12.9 mmol) was added to a solution of the intermediate 4-bromo-N^2-ethylbenzene-1,2-diamine, DIPEA (6.40 mL, 36.7 mmol), and cyclopropanecarboxylic acid (0.98 mL, 12.2 mmol) in DMF (40 mL), and the mixture was stirred at rt for 1 h. The mixture was quenched with water and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was dissolved in AcOH (40 mL) and the mixture was stirred at 80 °C for 1 h. After concentration of the mixture, the residue was neutralized with satd $NaHCO_3$ solution and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc = 100/0 to 0/100) to give the title compound (1.2 g, 37%) as a pale yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.03–1.18 (2H, m), 1.19–1.30 (2H, m), 1.46 (3H, t, J = 7.2 Hz), 1.96 (1H, tt, J = 8.2, 4.9 Hz), 4.25 (2H, q, J = 7.3 Hz), 7.29 (1H, dd, J = 8.3, 1.9 Hz), 7.43 (1H, d, J = 1.5 Hz), 7.50 (1H, d, J = 8.3 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ 7.0, 8.4, 14.9, 37.6, 112.3, 113.5, 119.7, 123.9, 136.1, 141.2, 157.3. MS (ESI/APCI) m/z = 265.1 $[M+H]^+$. Anal. Calcd for $C_{12}H_{13}BrN_2$: C, 54.36; H, 4.94; N, 10.57. Found: C, 54.29; H, 4.87; N, 10.58.

5.1.42. 6-Bromo-2-cyclopropyl-1-propyl-1*H*-benzimidazole (8c)

The title compound was prepared in 56% yield using **12c** in an analogous manner to **8a**. Pink solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.00 (4H, t, J = 7.4 Hz), 1.07–1.16 (2H, m), 1.21–1.30 (2H, m), 1.80–2.01 (3H, m), 4.13–4.21 (2H, m), 7.26–7.32 (1H, m), 7.42 (1H, d, J = 1.9 Hz), 7.50 (1H, d, J = 8.7 Hz). MS (ESI/APCI) m/z = 279.1 $[M+H]^+$.

5.1.43. 6-Bromo-1,2-dimethyl-1*H*-benzimidazole (8d)

To a solution of **20a** (30.0 g, 109 mmol) in AcOH (300 mL) was added zinc powder (35.9 g, 549 mmol) at rt. After being stirred over 90 °C for 4 h, the reaction mixture was allowed to cool to rt, and the zinc dust was removed by filtration with Celite pad and washed with EtOAc. The filtrate was concentrated and partitioned between EtOAc and satd $NaHCO_3$ solution. The resulting precipitate was removed by filtration with Celite pad, and the filtrate was extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$, and concentrated. The residue was purified by chromatography (NH silica gel, hexane/EtOAc = 100/0 to 0/100) to give the title compound (16.0 g, 65%) as a purple solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.56–2.61 (3H, m), 3.69 (3H, s), 7.32 (1H, dd, J = 8.5, 1.3 Hz), 7.43 (1H, s), 7.53 (1H, d, J = 8.4 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ 13.4, 29.8, 112.6, 113.6, 119.6, 123.8, 137.1, 141.3, 153.3. MS (ESI/APCI) m/z = 225.1 $[M+H]^+$. Anal. Calcd for $C_9H_9BrN_2$: C, 48.02; H, 4.03; N, 12.45. Found: C, 48.12; H, 3.99; N, 12.52.

5.1.44. 6-Bromo-2-(cyclopropylmethyl)-1-methyl-1*H*-benzimidazole (8e)

The title compound was prepared in 96% yield using **12a** and cyclopropylacetic acid in an analogous manner to **8b**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.31 (2H, d, J = 6.0 Hz), 0.63 (2H, dd, J = 7.9, 1.1 Hz), 1.08–1.27 (1H, m), 2.83 (2H, d, J = 6.4 Hz), 3.72 (3H, s), 7.34 (1H, d, J = 1.9 Hz), 7.45 (1H, d, J = 1.9 Hz), 7.58 (1H, d, J = 8.7 Hz). MS (ESI/APCI) m/z = 265.1 $[M+H]^+$.

5.1.45. 6-Bromo-2-(2,2-dimethylpropyl)-1-methyl-1*H*-benzimidazole (8f)

The title compound was prepared in 78% yield using **20b** in an analogous manner to **8d**. White solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.02 (9H, s), 2.77 (2H, s), 3.75 (3H, s), 7.28 (1H, dd, J = 8.7, 1.9 Hz), 7.51 (1H, d, J = 8.3 Hz), 7.76 (1H, d, J = 1.9 Hz). MS (ESI/APCI) m/z = 281.0 $[M+H]^+$.

5.1.46. 6-Bromo-2-cyclobutyl-1-methyl-1*H*-benzimidazole (8g)

To a mixture of cyclobutanecarboxylic acid (352 μL, 3.73 mmol) and **19** (500 mg, 2.48 mmol) was added $POCl_3$ (10 mL) and the mixture was heated under reflux for 4 h. The mixture was cooled to rt and poured into ice-cold satd $NaHCO_3$ solution (100 mL). The mixture was extracted with EtOAc, and the organic layer was washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The reside was purified by column chromatography (silica gel, hexane/EtOAc = 70/30) to afford the title compound (300 mg, 45%) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.89–1.95 (1H, m), 2.01–2.12 (1H, m), 2.37–2.46 (4H, m), 3.64 (3H, s), 3.80–3.88 (1H, m), 7.27 (1H, dd, J = 8.4, 1.6 Hz), 7.51 (1H, d, J = 8.4 Hz), 7.75 (1H, d, J = 1.6 Hz). MS (ESI/APCI) m/z = 266.8 $[M+H]^+$.

5.1.47. 6-Bromo-2-ethyl-1-methyl-1*H*-benzimidazole (8h)

The title compound was prepared in 57% yield using **12a** and propanoic acid in an analogous manner to **8e**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.45 (3H, t, J = 7.5 Hz), 2.89 (2H, q, J = 7.5 Hz), 3.69 (3H, s), 7.32 (1H, dd, J = 8.5, 1.8 Hz), 7.44 (1H, d, J = 1.5 Hz), 7.57 (1H, d, J = 8.5 Hz). MS (ESI/APCI) m/z = 239.0 $[M+H]^+$.

5.1.48. 1-(2-Cyclopropyl-1-methyl-1*H*-benzimidazol-6-yl)-4-hydroxypyridin-2(1*H*)-one (9a)

A mixture of **6l** (2.4 g, 6.46 mmol), 10% Pd–C (1.2 g), and MeOH (60 mL) was hydrogenated under H_2 atmosphere (1 atm) at rt for 3 h. The inorganic material was removed by filtration and the filtrate was concentrated in vacuo to give the title compound (1.57 g, 86%) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.99–1.13 (4H, m), 2.21–2.30 (1H, m), 3.85 (3H, s), 5.65 (1H, br s), 5.95 (1H, d, J = 7.4 Hz), 7.02 (1H, dd, J = 8.4, 2.0 Hz), 7.40–7.58 (3H, m), 10.78 (1H, br s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 7.1, 8.4, 29.6, 108.4, 109.0, 117.9, 120.0, 121.9, 133.8, 135.3, 135.8, 140.2, 141.7, 158.6, 160.4. MS (ESI/APCI) m/z = 282.1 $[M+H]^+$. Anal. Calcd for $C_{16}H_{15}N_3O_2 \cdot 0.11H_2O$: C, 67.84; H, 5.42; N, 14.83. Found: C, 67.80; H, 5.42; N, 14.81.

5.1.49. 1-(2-Ethyl-1-methyl-1*H*-benzimidazol-6-yl)-4-hydroxypyridin-2(1*H*)-one (9b)

The title compound was prepared in 99% yield using **6y** in an analogous manner to **9a**. Pale yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.33 (3H, t, J = 7.5 Hz), 2.90 (2H, q, J = 7.5 Hz), 3.74 (3H, s), 5.65 (1H, d, J = 2.5 Hz), 5.96 (1H, dd, J = 7.5, 2.5 Hz), 7.04 (1H, dd, J = 8.4, 2.0 Hz), 7.49–7.62 (3H, m), 10.88 (1H, br s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 11.3, 20.0, 29.5, 98.4, 100.2, 108.7, 117.9, 120.3, 135.0, 135.7, 139.9, 141.4, 157.7, 163.0, 167.2. MS (ESI/APCI) m/z = 470.2 $[M+H]^+$. Anal. Calcd for $C_{15}H_{15}N_3O_2 \cdot 1.35H_2O$: C, 61.36; H, 6.08; N, 14.31. Found: C, 61.49; H, 5.73; N, 14.39.

5.1.50. 4-Bromo-1-(2-cyclopropyl-1-methyl-1*H*-benzimidazol-6-yl)pyridin-2(1*H*)-one (10)

To a solution of **9a** (1.0 g, 3.55 mmol) in DMF (15 mL) was added phosphoryl tribromide (1.22 g, 4.27 mmol) at ambient temperature. The mixture was stirred at 50 °C for 9 h. The mixture was poured into satd $NaHCO_3$ solution and extracted with EtOAc. The

organic layer was separated, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residual solid was recrystallized from EtOH–hexane to give the title compound (600 mg, 49%) as a brown solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.99–1.16 (4H, m), 2.22–2.32 (1H, m), 3.85 (3H, s), 6.54 (1H, dd, J = 7.3, 2.0 Hz), 6.84 (1H, d, J = 2.0 Hz), 7.09 (1H, dd, J = 8.4, 1.9 Hz), 7.50–7.61 (2H, m), 7.67 (1H, d, J = 7.3 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ 7.1, 8.3, 29.5, 98.5, 99.9, 108.5, 117.7, 120.4, 134.7, 135.8, 140.0, 141.3, 158.2, 162.9, 166.8. MS (ESI/APCI) m/z = 345.0 $[M+H]^+$. Anal. Calcd for $C_{16}H_{14}BrN_3O$: C, 53.78; H, 4.36; N, 11.76. Found: C, 53.85; H, 4.10; N, 11.94.

5.1.51. 5-Bromo-*N*-methyl-2-nitroaniline (12a)

To a solution of **11** (25.0 g, 114 mmol) in EtOH (100 mL) was added methylamine (40% in MeOH, 34.8 mL, 341 mmol) at rt. The mixture was stirred at rt for 1 h and then cooled to 0 °C. The precipitate was collected by filtration, and washed with EtOH and IPE successively to give the title compound (24.8 g, 94%) as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.95 (3H, d, J = 4.9 Hz), 6.83 (1H, dd, J = 9.1, 1.9 Hz), 7.17 (1H, d, J = 1.9 Hz), 7.98 (1H, d, J = 9.1 Hz), 8.23 (1H, br s).

5.1.52. 5-Bromo-*N*-ethyl-2-nitroaniline (12b)

The title compound was prepared in 80% yield using ethylamine in an analogous manner to **12a**. Pale yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.38 (3H, t, J = 7.2 Hz), 3.33 (2H, qd, J = 7.2, 5.1 Hz), 6.75 (1H, dd, J = 9.1, 1.9 Hz), 7.01 (1H, d, J = 1.9 Hz), 7.98 (1H, br s), 8.03 (1H, d, J = 9.1 Hz).

5.1.53. 5-Bromo-2-nitro-*N*-propylaniline (12c)

The title compound was prepared in 76% yield using ^{n}propylamine in an analogous manner to **12a**. Orange solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.06 (3H, t, J = 7.5 Hz), 1.77 (2H, m), 3.25 (2H, td, J = 7.1, 5.1 Hz), 6.74 (1H, dd, J = 9.0, 1.9 Hz), 7.01 (1H, d, J = 1.9 Hz), 7.92–8.11 (2H, m).

5.1.54. 4-[(4-Fluorobenzyl)oxy]-1-[3-(methylamino)-4-nitrophenyl] pyridin-2(1*H*)-one (13)

The title compound was prepared in 44% yield using **4c** and **12a** in an analogous manner to **6a**. Yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.96 (3H, d, J = 4.9 Hz), 5.13 (2H, s), 6.00 (1H, d, J = 2.3 Hz), 6.14 (1H, dd, J = 7.7, 2.8 Hz), 6.69 (1H, dd, J = 9.0, 1.9 Hz), 6.96 (1H, d, J = 1.9 Hz), 7.25 (2H, t, J = 8.9 Hz), 7.52 (2H, dd, J = 8.3, 5.7 Hz), 7.64 (1H, d, J = 7.5 Hz), 8.13 (1H, d, J = 9.0 Hz), 8.27 (1H, d, J = 4.9 Hz). MS (ESI/APCI) m/z = 370.1 $[M+H]^+$.

5.1.55. 1-[4-Amino-3-(methylamino)phenyl]-4-[(4-fluorobenzyl) oxy]pyridin-2(1*H*)-one (14)

A mixture of **13** (90 mg, 0.24 mmol), iron (54.4 mg, 0.97 mmol), calcium chloride (54.1 mg, 0.49 mmol), EtOH (1.5 mL), and water (1.5 mL) was heated at 70 °C for 3 h. The inorganic material was removed by filtration, and the filtrate was concentrated. The residue was neutralized with satd $NaHCO_3$ solution and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, and concentrated to give the title compound (78 mg, 94%) as an brown solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.85 (3H, s), 3.29–3.41 (2H, m), 4.99 (2H, s), 5.90–6.08 (2H, m), 6.52–6.65 (2H, m), 6.74 (1H, d, J = 7.9 Hz), 7.09 (2H, t, J = 8.7 Hz), 7.23 (1H, s), 7.39 (2H, dd, J = 8.7, 5.3 Hz). MS (ESI/APCI) m/z = 340.1 $[M+H]^+$.

5.1.56. *N*-(5-Bromopyrazin-2-yl)-4-methylbenzenesulfonamide (17)

To a solution of **16** (2.0 g, 11.5 mmol) in pyridine (40 mL) was added TsCl (3.29 g, 17.2 mmol) at rt, and the mixture was stirred at rt overnight. The solvent was evaporated, and the residue was poured into satd NH_4Cl solution, extracted with EtOAc, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc = 100/0 to 50/50) to give the title compound (1.92 g, 51%) as white crystals. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.36 (3H, s), 7.40 (2H, d, J = 8.2 Hz), 7.82 (2H, d, J = 8.3 Hz), 8.18 (1H, s), 8.44 (1H, s), 11.67 (1H, br s). MS (ESI/APCI) m/z = 328.0 $[M-H]^-$.

5.1.57. *N*-[(2*E*)-5-Bromo-1-(1-cyclopropyl-1-oxopropan-2-yl) pyrazin-2(1*H*)-ylidene]-4-methylbenzenesulfonamide (18)

To a solution of **17** (1.73 g, 5.27 mmol) in DMF (20 mL) was added NaH (60% oil dispersion, 0.32 g, 7.9 mmol) at 0 °C, and the mixture was stirred at rt for 30 min. To the mixture was added 2-bromo-1-cyclopropylpropan-1-one (1.87 g, 10.5 mmol) at rt and the resulting mixture was stirred overnight. The mixture was poured into water and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc = 100/0 to 50/50) to give the title compound (1.02 g, 46%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.74–1.00 (4H, m), 1.71 (3H, t, J = 7.3 Hz), 2.21–2.29 (1H, m), 2.36 (3H, s), 5.63 (1H, d, J = 7.3 Hz), 7.34 (2H, d, J = 8.0 Hz), 7.69 (2H, d, J = 8.2 Hz), 8.31 (1H, s), 8.76 (1H, s).

5.1.58. 4-Bromo-N^2-methylbenzene-1,2-diamine (19)

A solution of **12a** (350 mg, 1.51 mmol), zinc (495 mg, 7.57 mmol), and NH_4Cl (810 mg, 15.15 mmol) in MeOH (4 mL)/water (2 mL) was stirred at rt for 1 h. The insoluble material was removed by filtration and neutralized satd $NaHCO_3$ solution. The mixture was concentrated and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo to give the title compound as a brown solid (282 mg, 93%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.68 (3H, d, J = 4.9 Hz), 4.60 (2H, s), 4.87 (1H, d, J = 4.9 Hz), 6.32–6.58 (3H, m). MS (ESI/APCI) m/z = 202.09 $[M+H]^+$.

5.1.59. *N*-(5-Bromo-2-nitrophenyl)-*N*-methylacetamide (20a)

To a solution of **12a** (50.0 g, 216 mmol) in toluene (500 mL) was added acetyl chloride (30.8 mL, 432 mmol) at rt. After being stirred at 90 °C for 15 h, acetyl chloride (7.69 mL, 108 mmol) was added and the mixture was stirred at 90 °C for further 5 h. The reaction mixture was cooled to rt, poured into EtOAc, washed with satd $NaHCO_3$ solution and brine, dried over Na_2SO_4, and concentrated. The residual solid was recrystallized from EtOAc–hexane to give the title compound (57 g, 97%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.65–2.26 (3H, m), 3.00–3.52 (3H, m), 7.67–8.20 (3H, m). MS (ESI/APCI) m/z = 272.9 $[M+H]^+$.

5.1.60. *N*-(5-Bromo-2-nitrophenyl)-*N*,3,3-trimethylbutanamide (20b)

To a mixture of **12a** (300 mg, 1.30 mmol), 3,3-dimethylbutanoyl chloride (0.45 mL, 3.25 mmol), and DMF (5 mL) was added NaH (60% oil dispersion, 57.1 mg, 1.43 mmol), and the mixture was heated at 70 °C overnight. The mixture was poured into water and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, concentrated, and purified by column chromatography (silica gel, hexane/EtOAc = 100/0 to 75/25) to give the title compound (214 mg, 51%) as a solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.86–1.06 (9H, m), 1.67–2.39 (2H, m), 3.02–3.45 (3H, m), 7.68–8.13 (3H, m). MS (ESI/APCI) m/z = 329.0 $[M+H]^+$.

5.2. Determination of hMCHR1 competitive inhibitory activity of test compound using binding assay

5.2.1. Preparation of membrane fraction

Using hMCHR1-expressing CHO cell clone 57,[47] MCHR1-expressing CHO cellular membrane fractions were prepared by

the following method. In phosphate buffered saline (pH 7.4) supplemented with 5 mM EDTA (ethylenediaminetetraacetic acid) were respectively suspended human MCHR1-expressing CHO cells (1×10^8 cells) and centrifuged. Homogenate buffer (10 mL, 10 mM $NaHCO_3$, 5 mM EDTA, pH 7.5, 0.5 mM PMSF (phenylmethylsulfonyl fluoride), 20 mg/L leupeptin, 4 mg/L E-64, 1 mg/L pepstatin A) was added to the pellets of the cells and, using Polytron homogenizer, the mixture was homogenated. The supernatant obtained after centrifugation at $400\times g$ for 10 min was further centrifuged at $100{,}000\times g$ for 1 h to give precipitate of the membrane fraction. The precipitate were suspended in 2 mL of assay buffer [20 mM Tris–HCl (pH 7.5), 5 mM EDTA, 0.5 mM PMSF, 20 mg/L leupeptin, 4 mg/L E-64, 1 mg/L pepstatin A]. The membrane fractions were suspended in assay buffer to a protein concentration of 2 mg/mL, and after dispensing, preserved at −80 °C and used upon thawing each time when in use.

5.2.2. Binding assay

An MCHR1-expressing CHO cellular membrane fraction (173 μL) diluted with an assay buffer was dispensed to a 96-well polypropylene plate (3363, Corning). DMSO solution (2 μL), 33 μM cold MCH (1–19) diluted with DMSO solution (2 μL), or a test compound solution diluted with DMSO solution to various concentrations (2 μL) was added, and last, [^{125}I]-MCH(4–19) diluted with assay buffer (hereinafter, sometimes to be referred to as "hot MCH", 25 μL) was added to each well. The mixture was reacted with stirring at room temperature for 1 h, and the plate was set on FilterMate harvester (PerkinElmer). Using a polyethylenimine-treated glass filter plate (GF/C, PerkinElmer), which had been previously set, the plate was suction-filtered and washed three times with washing buffer (50 mM Tris–HCl buffer pH 7.5). The glass filter plate was dried, MicroScint 0 (PerkinElmer) was added at 25 μL/well, and the resulting radioactivity was measured by TopCount liquid scintillation counter (PerkinElmer). The binding inhibition rate of the test compound was calculated by the following formula.

$$\begin{aligned}&\text{Binding inhibition}\,(\%) = 100\\&\quad-(\text{radioactivity upon addition of test compound and hot MCH}\\&\quad-\text{radioactivity upon addition of cold MCH and hot MCH solution})\\&\quad/(\text{radioactivity upon addition of DMSO solution and hot MCH}\\&\quad-\text{radioactivity upon addition of cold MCH and hot MCH solution})\times 100.\end{aligned}$$

5.3. Measurement of MCH receptor 1 antagonistic activity of test compound using Ca^{2+} mobilization assay

Using an expression vector plasmid introduced with human MCHR1 gene for expression in animal cells, human MCHR1 gene was introduced into CHO cells (CHO dhfr$^-$) by Lipofectamine LTX (Invitrogen). The cells were cultured in selection MEMα medium [445 mL of MEMα medium without nucleic acid and added with 5 mL of penicillin–streptomycin (Invitrogen) and 50 mL of dialyzed fetal bovine serum]. Colony 24 clones grown in the selection medium, which were human MCHR1 gene-expressing CHO cell candidates, were selected. From these clones, clone no.4 which showed the highest response to the change of Ca^{2+} concentration on stimulation by the addition of 25 nM ligand MCH (4–19) was selected by Ca^{2+} mobilization assay. In the following test, this human MCHR1-expressing CHO cell (clone no.4) was used. An integrated dispensing function fluorometer (CellLux, PerkinElmer) was used for Ca^{2+} mobilization assay. The CHO cells were sown in a 96-well plate (type 3904, Corning) with a black wall and clear well bottom at a density of 20000 cells/well and cultured in an incubator for about 24 h at 5% CO_2, 37 °C. The medium was removed, and the cells were washed with phosphate buffered saline (PBS). A Ca^{2+} indicator dye reagent (DOJINDO LABORATORIES, Ca screening no-wash kit Fluo4) was added at 100 μL/well, and the dye was allowed to penetrate into the cell for 30 min in an incubator at 5% CO_2, 37 °C. The plate was set on a plate reader. First, a test compound solution diluted with an assay buffer [10 mM HEPES (pH 7.4): 1× Assay Buffer (DOJINDO LABORATORIES, attached to Ca screening no-wash kit Fluo4) containing 0.1% BSA] or DMSO solution was added at 50 μL/well, and then ligand MCH (4–19) peptide (final concentration 2 nM) diluted with assay buffer or DMSO was added at 50 μL/well, during which changes in intracellular fluorescence were measured at 2 s intervals. The antagonistic activity of the test compound was calculated by the following formula and shown as an inhibition rate (%) wherein the intracellular fluorescence activity resulting from the stimulation by the addition of ligand MCH (4–19) peptide was 100% and that of the well added with DMSO solution alone was 0%.

$$\begin{aligned}&\text{Inhibitory rate}\,(\%) = 100\\&\quad-[\text{fluorescence activity upon addition of test compound}\\&\quad\text{and MCH(4—19) peptide solution} - \text{fluorescence activity}\\&\quad\text{upon addition of DMSO solution only}]/[\text{fluorescence activity}\\&\quad\text{upon addition of DMSO solution and MCH(4—19) peptide solution}\\&\quad-\text{fluorescence activity upon addition of DMSO solution only}]\\&\quad\times 100.\end{aligned}$$

5.4. Evaluation of time-dependent inhibition (TDI) of CYP3A4 (single-point assay)

Human liver microsomes were purchased from Xenotech, LLC (Lenexa, KS). A mixture of a test compound (30 μM) and microsomes in phosphate buffer (pH 7.4) was preincubated at 37 °C in the presence of an NADPH-generating system containing $MgCl_2$, glucose-6-phosphate, β-NADP$^+$, and glucose-6-phosphate dehydrogenase. After preincubation, enzymatic activity of CYP3A4 in the incubation mixture was determined by measuring 6β-hydroxytestosterone in the reaction with testosterone by UPLC. The activity (% of control) for each preincubation time was calculated to the following: {(activity with test compound)/(activity with DMSO)} × 100. The remaining activity (% remaining) after preincubation was calculated to the following: {activity with preincubation (% of control)}/{activity without preincubation (% of control)} × 100.

5.5. GSH trapping experiment

5.5.1. Instrument

LC/MS system consisted of UPLC system (Waters, Milford, MA) and SYNAPT Q-TOF mass spectrometer (Waters) equipped with an electrospray ionization source.

5.5.2. Microsomal incubation with GSH

For the GSH trapping experiments each test compound (30 μM) was incubated with human liver microsomes (final protein concentration 1.0 mg/mL; XenoTech, LLC. Lenexa, KS) in the presence of an NADPH-regenerating system and GSH (1 mM) in phosphate buffer (pH 7.4) at 37 °C. The reaction was terminated after 60 min by the addition of an equal volume of acetonitrile. After centrifugation at 15000 rpm for 10 min, 5 μL of supernatant was injected into LC/MS system.

5.5.3. LC/MS/MS analysis

Microsomal incubation mixtures were separated on a BEH C_{18} column (1.7 μm, 2.1 × 100 mm; Waters) using solvent A (5% acetonitrile in 5 mM aqueous ammonium acetate) and solvent B (90% acetonitrile in 50 mM aqueous ammonium acetate). At a flow rate of 0.5 mL/min, the initial elution gradient was 98% solvent A

and 2% solvent B with a linear gradient to 70% solvent B over 10 min and returned to initial condition. The column was allowed to equilibrate at 2% solvent B for 5 min before the next injection. The column temperature was 40 °C and the eluents were monitored with a PDA detector. The mass spectrometry was run in positive ion mode. The source settings were 1.20 kV capillary voltage, 35 V sampling corn voltage, 120 °C source temperature, 350 °C desolvation temperature. GSH adducts were analyzed based on their product ion spectra of the protonated molecules upon CID ramped from 15 V to 40 V.

5.6. Animal experiments

All animal experiments were performed in compliance with the Guidelines for the Care and Use of Laboratory Animals of Takeda Pharmaceutical Company Ltd.

5.6.1. Pharmacokinetic analysis in rats

Test compounds were administered intravenously (iv, 0.1 mg/kg) or orally (po, 1 mg/kg, suspended in 0.5% methylcellulose aqueous solution) by cassette dosing to fed Sprague-Dawley rats. After administration, blood samples were collected and centrifuged to obtain the plasma fraction. The plasma samples were deproteinized by mixing with acetonitrile followed by centrifugation. The compound concentrations in the supernatant were measured by LC/MS/MS.

5.6.2. Evaluation of anorectic effect using male DIO-F344/Jcl rats

Male DIO-F344/Jcl rats (45 weeks old) fed with a high-fat diet (D12451: Research Diets) from 5 weeks old were used. From before the start of experiment, the rats were singly housed, given a powder high-fat diet (D12451 M: Research Diets), and habituated to oral administration with tap water. The rats were grouped based on both the food intake and the body weight of day 0. The rats were orally administered vehicle (0.5% methylcellulose solution) or compounds suspended in vehicle at 2 mL/kg 2 h before the onset of dark period for 2 days ($n = 6$ for each group). The food intake for 2 days from the initial administration was measured. The food intake inhibition rate of each compound administration group to the vehicle group was calculated.

5.6.3. Evaluation of antiobesity effect using male DIO-F344/Jcl rats

DIO-F344 rats (41 weeks old) were habituated and grouped prior to treatment as described above. The rats were orally administered vehicle (0.5% methylcellulose solution) or compounds suspended in vehicle at 2 mL/kg for 2 weeks ($n = 5$ or 6 for each group). Sibutramine was used as a positive control in this study. The compounds were administered after measurement of body weight at 1–3 h before the onset of dark period, and food intake was measured every 2 or 3 days. The change in body weight was presented as percentage from initial body weight.

5.6.4. In vivo selectivity of anorectic effect by using MCHR1-deficient mice

Male MCHR1-deficient mice and wild-type litter mate mice (27 weeks old) loaded with a high-fat diet (D12451) from 7 weeks of age were used. Before the start of the experiment, the mice were independently raised, a high-fat diet (D12451) was given, and tap water was administered for acclimation. The mice were grouped on the basis of food intake from day −4 to day −1 and body weight of day −1 as indices. Each group was orally administered vehicle (0.5% methylcellulose solution) or compounds suspended in vehicle at 10 mL/kg for 3 days ($n = 6$ for each group). Food intake for 3 days was measured.

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