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Identification of a novel hormone sensitive lipase inhibitor with a reduced potential of reactive metabolites formation

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ABSTRACT

Hormone sensitive lipase (HSL) has emerged as an attractive target for the treatment of dyslipidemia. We previously reported compound **1** as a potent and orally active HSL inhibitor. Although an attractive profile was demonstrated, subsequent studies revealed that compound **1** has a bioactivation liability. The oxygen-carbon linker in compound **1** was identified as being potentially responsible for reactive metabolite formation. By exchanging of this susceptible fragment was feasible, and a benzanilide derivative **6b** with a decreased bioactivation liability was obtained. Further modification of the novel benzanilide scaffold resulted in the identification of compound **24b**. Compound **24b** exhibited potent HSL inhibitory activity (IC_{50} = 2 nM) with a significantly reduced bioactivation potential. Oral administration of compound **24b** exhibited an antilipolytic effect on rats at 3 mg/kg.

1. Introduction

Hormone sensitive lipase (HSL) is an intracellular neutral lipase that catalyzes hydrolysis of broad substrates such as tri-, di-, and monoacylglycerol (TG, DG and MG), cholesterylester, retinyl ester and numerous water soluble ester substrates.[1] HSL contains an α/β-hydrolase fold[2] and a catalytic triad of serine, aspartic acid, and histidine,[3] which are essential for catalysis.[4] The highest expression of HSL is observed in adipose tissues (ATs) where it catalyzes lipolysis of stored triglyceride (TG); one TG molecule is sequentially broken down into one glycerol and three free fatty acids (FFAs). The cellular concentration of FFAs is tightly controlled by the balance between lipolysis of TG and esterification of FFAs by several hormones depending on energy demand.[5] In the fasted state, HSL is activated in response to catecholamines and FFAs are subsequently released into circulation as an energy source for most tissues.[6]

FFAs have a pathophysiological role in dyslipidemia as well as a physiological role in energy production. Elevation of plasma FFA level is associated with obesity and insulin resistance that induce enlargement of AT mass and attenuation of insulin-mediated AT lipolysis metabolism, which could cause dysregulation of lipolysis. Furthermore, the increased FFA flux could impair lipid profiles by enhancing hepatic production of very-low-density lipoprotein (VLDL).[7] HSL inhibition could be a promising therapeutic approach for dyslipidemia, however, the reports of HSL inhibitors have been limited.[8–17]

We previously reported that compound **1** exerted HSL inhibitory activity with an IC_{50} value of 7 nM and an in vivo antilipolytic effect at 3 mg/kg in rats.[18] Despite potent in vitro and in vivo activities, subsequent studies revealed that compound **1** has a potential to form reactive metabolites. Reactive metabolite formation could cause organ toxicity and carcinogenesis, since these metabolites covalently bind to biological macromolecules such as protein and DNA.[19] Therefore, minimizing the potential to form reactive metabolites could be required.[20]

The propensity of forming reactive metabolites was generally assessed by incubation of compounds with glutathione (GSH) in the presence of liver microsomes.[21] In the reactive metabolite trapping assay, a GSH-derived adduct of compound **1** was detected (Fig. 1).[22] Its structural elucidation using LC-MS/MS analysis provided that the site of GSH conjugation was tentatively assigned to the benzyl carbon. On the basis of the results, the proposed

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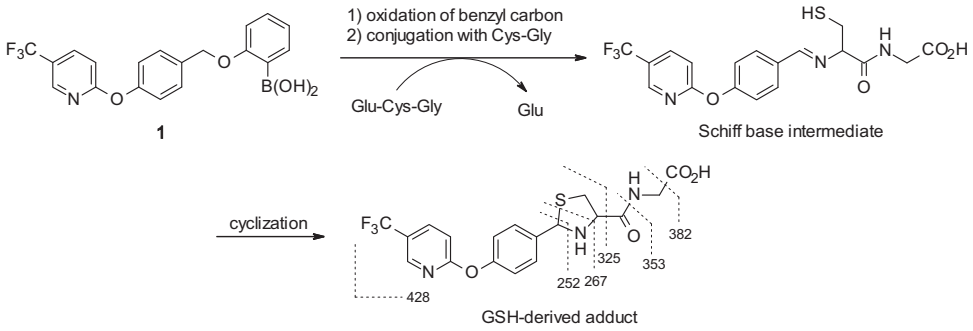


Fig. 1. Proposed mechanism of the GSH-derived adduct formation of **1**.

mechanism of the GSH-derived adduct formation involves initial oxidation of the benzyl carbon by cytochrome P450, followed by conjugation with a primary amino group of a cystein moiety that is released from GSH. The resulting Schiff base intermediate ring-contracts to a thiazolidine leading to the GSH-derived adduct.[23,24] We focused on exchanging from the susceptible fragment, namely, the oxygen-carbon linker, to an alternative fragment to circumvent the reactive metabolite liability while maintaining HSL inhibitory activity.

Herein, we report the identification of novel benzanilide derivatives that showed potent in vitro and in vivo activities with a decreased potential for forming reactive metabolites.

2. Chemistry

Scheme 1 depicts the synthesis of derivatives **3**, **6a–c** and **8**. Acylation of **2** furnished **3**. Condensation of corresponding acids (**4**[25] or **7**) with anilines (**5a** or **5b**) yielded compound **6a**, **6c** and **8**. Deprotection of pinacol group in **6a** led to boronic acid derivative **6b**.

Scheme 2 describes the synthesis of derivatives **11**, **13a–b**, **16a–b**, **19a–b** and **22a–c**. Condensation of 2-chloro-5-(trifluoromethyl) pyridine **9** with corresponding phenols followed by hydrolysis gave intermediates **10b**, **12a** and **12b**, which were converted to **11**, **13a** and **13b** in a two-step sequence. The intermediates **15a** and **15b** were synthesized from 2-cyano-5-fluoropyridine **14** by condensation and a following two-step hydrolysis. Compounds **16a** and **16b** were obtained by acylation of **5a** with the intermediates **15a** and **15b**. Compounds **19a** and **19b** were synthesized by hydrolysis of **17**[26] and following condensation. Compounds **22a–c** were prepared in a similar manner to that of compound **11**.

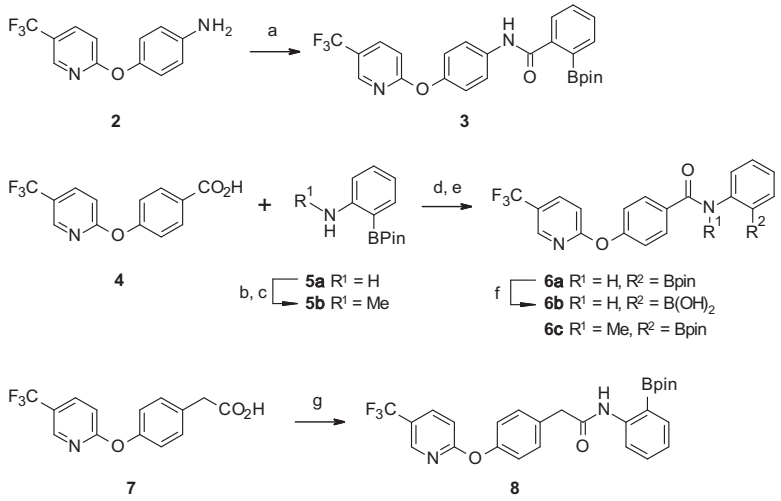
Scheme 3 illustrates the synthesis of compounds **24a–f**. Condensation of carboxylic acid **17** with corresponding anilines led to compounds **23a–f**, followed by a two-step deprotection of the pinacol group via trifluoroborate intermediates[27] afforded compounds **24a–f**.

3. Results and discussion

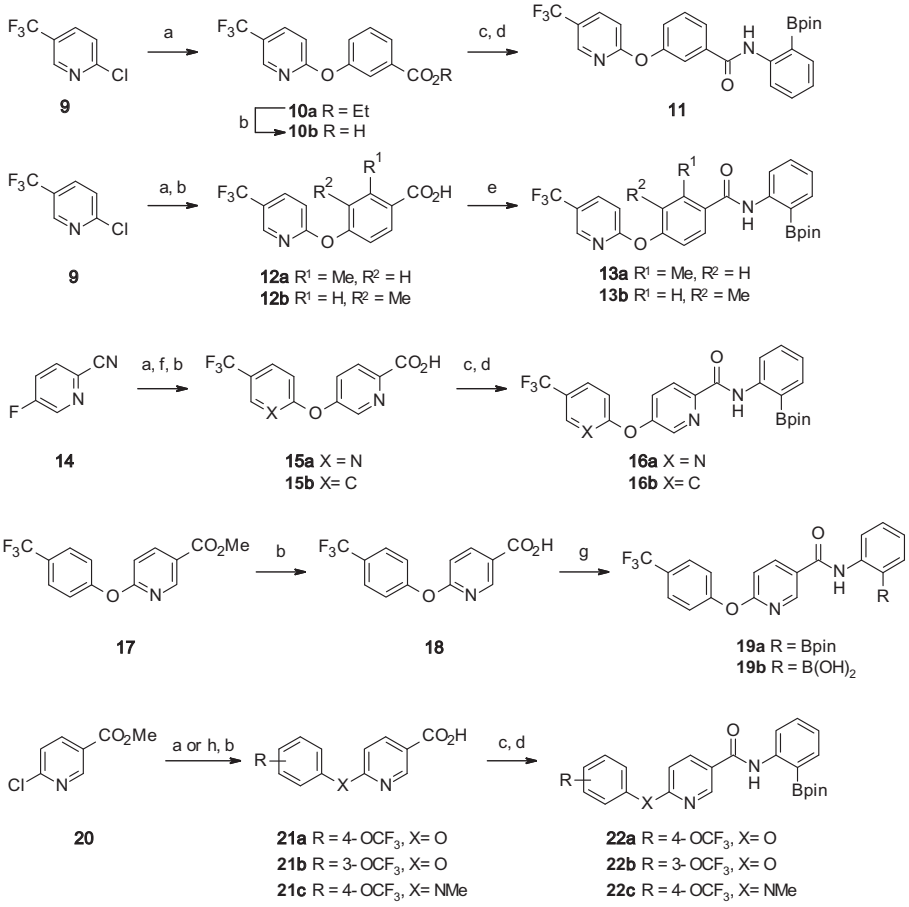
The inhibitory activity of the synthesized compounds against HSL was measured by a colorimetric assay using human HSL fractions and *p*-nitrophenyl butyrate (PNPB) as a substrate.[8]

In terms of efficient screening, we initially synthesized and evaluated derivatives as a boronate ester since the inhibitory activity of a boronic acid was considered to be comparable to that of a corresponding ester.[18] As seen in Table 1, replacement of the oxygen-carbon linker with an amide linker led to compound **6a** with an IC_{50} value of 0.18 μM. Conversion of the boronate ester **6a** to a boronic acid **6b** resulted in a comparable HSL inhibitory activity. *N*-substituted amide **6c** or the reverse amide **3** significantly deteriorated the inhibitory activity compared with **6a**. Expanding the amide linker also resulted in a loss of the inhibitory activity (**8**). As expected, GSH-derived adduct formation in the boronic acid **6b** was not detected by the reactive metabolite trapping assay with GSH. Therefore, boronic acid **6b** was identified as a lead compound, and this benzanilide scaffold was selected for further exploration.

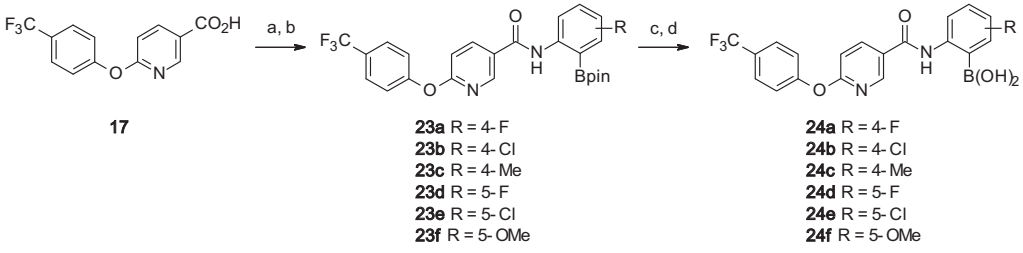
We explored the left hand moiety of the novel derivatives as boronate ester derivatives (Table 2). First, the *m*-substituted analog



Scheme 1. Reagents and conditions: (a) 2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoic acid, EDC·HCl, HOBT, DMF; (b) 1*H*-benzotriazole-1-methanol, EtOH; (c) $NaBH_4$, THF; (d) oxalyl chloride, DMF (cat.), CH_2Cl_2; (e) **5a** or **5b**, DIPEA, CH_2Cl_2; (f) $NaIO_4$, THF, H_2O, then 1 M HCl; (g) **5a**, EDC·HCl, HOBT, NMM, CH_2Cl_2.



Scheme 2. Reagents and conditions: (a) phenols, K_2CO_3, DMF; (b) 5 M NaOH, MeOH; (c) oxalyl chloride, DMF (cat.), CH_2Cl_2; (d) **5a**, DIPEA, CH_2Cl_2; (e) **5a**, EDC·HCl, HOBT, NMM, CH_2Cl_2; (f) conc. HCl, conc. H_2SO_4, MeOH; (g) **5a**, HBTU, DIPEA, DMF; (h) *N*-methyl-*p*-trifluoromethoxyaniline, AcOH.



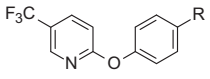
Scheme 3. Reagents and conditions: (a) oxalyl chloride, DMF (cat.), CH_2Cl_2; (b) anilines, DIPEA, CH_2Cl_2; (c) KHF_2, MeOH, H_2O; (d) TMSCl, CH_3CN, H_2O.

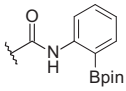
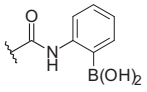
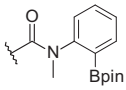
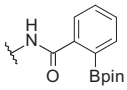
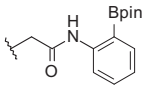
11 resulted in a loss of HSL inhibitory activity. Next, incorporation of a methyl group into the central ring on the 2-position led to a slight decrease in the inhibitory activity (**13a**), whereas that on the 3-position led to a significant loss of the inhibitory activity (**13b**). By exchanging of the central benzene ring in compound **6a** with a 2-*N*-pyridine ring led to a decrease in the inhibitory activity (**16a**). By exchanging of the left terminal pyridine ring in compound **16a** with trifluoromethyl benzene ring led to a 7-fold increase in HSL inhibitory activity with an IC_{50} value of 0.13 μM (**16b**). Furthermore, 3-*N*-pyridine **19a** showed a 10-fold increase in HSL inhibitory activity with an IC_{50} value of 0.014 μM compared to compound **16b**. Replacement of the trifluoromethyl group with a trifluoromethoxy group also showed potent inhibitory activity (**22a**), and the trifluoromethoxy group in the *meta* position maintained the inhibitory activity (**22b**). By exchanging of the ether linker in **22a** with an *N*-methyl amino linker was also tolerated (**22c**).

As a result of this investigation, the 3-*N*-pyridine was identified as the most desirable substructure in the central ring to exert potent HSL inhibitory activity. Moreover, the 3-*N*-pyridine derivative **19a**, which bears highly hydrophobic 4-trifluoromethyl benzene moiety in the left hand, exhibited the most potent HSL inhibitory activity in this series.

Encouraged by the results, we further evaluated the effects of substituents on the right hand benzene ring of compound **19a** (Table 3). Conversion of the boronate ester **19a** to a boronic acid **19b** resulted in a slight decrease in the inhibitory activity. Introduction of lipophilic substituents such as a halogen atom or a methyl group on the C-4 position in compound **19b** led to an increase in the inhibitory activity (**24a**–**24c**), and especially the 4-chlorine substituent **24b** exhibited a 23-fold increase in HSL inhibitory activity compared to **19b**. Interestingly, incorporation of a 4-chlorine group into the boronate derivative **23b** did not

Table 1
Replacement of the ether linker of compound **1**.



Compd	R	IC_{50} (μM)[a]
6a		0.18
6b		0.091
6c		8%[b]
3		8%[b]
8		12%[b]

[a] Assay results are the average of triplicates.
[b] % inhibition at 1 μM was evaluated as described in Section 5.

show a dramatic improvement in the inhibitory activity compared with the corresponding boronic acid derivative **24b**. Substitution at the C-5 position with a halogen atom resulted in a slight improvement in the inhibitory activity (**24d** and **24e**), whereas incorporation of a methoxy group did not have any effect on the IC_{50} value (**24f**). Overall, the effect of introducing substituents at the C-4 position (**24a**-**c**) was more sensitive to HSL inhibitory activity than that of C-5 (**24d**–**f**).

The reason why 4-chloro substituent **24b** exhibited potent HSL inhibitory activity might be regarded as follows:

i) An increased Lewis acidity of boronic acid

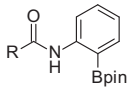
The calculated pK_a value of boronic acid in 4-chloro substituent **24b** (pK_a = 7.4) is much lower than that of the unsubstituted analog **19b** (pK_a = 8.4). The lower pK_a value increases the electrophilicity of a boron center,[28] which enhances both the formation of a dative bond between the hydroxyl group of a catalytic serine and the boron center and the stabilization of a formed boron-lipase complex.[29]

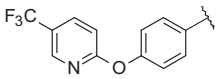
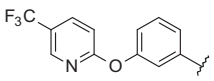
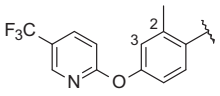
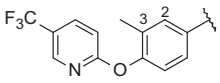
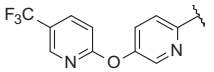
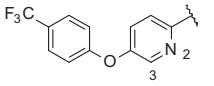
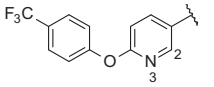
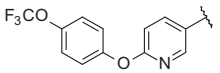
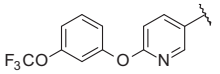
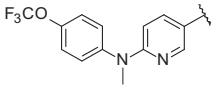
ii) Estimated the existence of binding pocket around C-4 moiety

4-Me substituent **24c** showed an adequate inhibitory activity comparable to that of a unsubstituted analog **19b** despite a nearly equivalent pK_a value of boronic acid (8.5 and 8.4, respectively). This tendency was also observed in between 4-Cl substituent **24b** and 4-F substituent **24a** (the p*K*a values of boronic acid are 7.4 and 7.3, respectively). This might imply that there was a binding pocket of the lipase around the C-4 position, which was probably hydrophobic. Thereby, compounds with substituents at the 4-position such as **24c** and **24b** resulted in enhanced HSL inhibitory activity.

The potent HSL inhibitor **24b** was obtained, and further characterization was performed (Table 4). As expected, in the reactive metabolite trapping assay, GSH-derived adducts were not detected. Compound **24b** showed high metabolic stability (81%) in rat liver microsomes, suggesting that **24b** could be devoid of the reactive intermediates formation since the metabolically labile benzyl group was replaced with the amide group that might be resistant to oxidative metabolism and further modification did

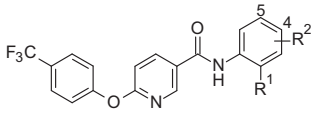
Table 2
SAR of the left hand moiety of the benzanilide derivatives.



Compd	R	IC_{50} (μM)[a]
6a		0.18
11		33%[b]
13a		0.38
13b		30%[b]
16a		0.92
16b		0.13
19a		0.014
22a		0.024
22b		0.021
22c		0.040

[a] Assay results are the average of triplicates.
[b] % inhibition at 1 μM was evaluated as described in Section 5.

Table 3
SAR of the right hand moiety of the benzanilide derivatives.



Compd	R^1	R^2	IC_{50} (μM)[a]	pK_a[b]
19a	Bpin[d]	H	0.014	NC[c]
19b	$B(OH)_2$	H	0.046	8.4
24a	$B(OH)_2$	4-F	0.013	7.3
24b	$B(OH)_2$	4-Cl	0.002	7.4
24c	$B(OH)_2$	4-Me	0.020	8.5
23b	Bpin[d]	4-Cl	0.008	NC[c]
24d	$B(OH)_2$	5-F	0.016	8.5
24e	$B(OH)_2$	5-Cl	0.024	8.2
24f	$B(OH)_2$	5-OMe	0.059	8.8

[a] Assay results are the average of triplicates.
[b] Calculated by ACD/Percepta (version 2014).
[c] Not calculated.
[d] Bpin: pinacolboronate.

Table 4
Pharmaceutical profiles of compound **24b**.

Reactive metabolite formation[a]	ND[b]
Metabolic stability[c]	81%
C_{max}[d] (μg/mL)	3.35
AUC[d] (μg·h/mL)	19.65

[a] Reactive metabolite assessment was conducted using isotope labeled glutathione as a trapping agent in NADPH-supplemented human liver microsomes.
[b] Not detected.
[c] % remaining after 30 min in rat liver microsomes (0.5 mg/mL).
[d] 3 mg/2 mL/kg of compound solution in propylene glycol/Tween80 = 4:1 (v/v) solution.

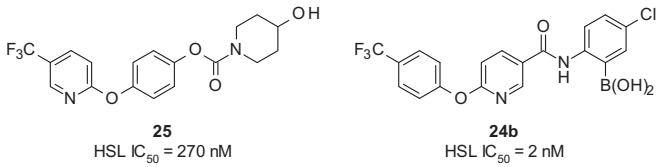


Fig. 2. Structures of **25** and **24b**.

not increase metabolically vulnerable sites that led to bioactivation. Owing to this metabolic stability, compound **24b** showed good oral exposure (C_{max} = 3.35 μg/mL and AUC = 19.65 μg·h/mL at 3 mg/kg p.o.) in rats.

After these promising profiles were identified, we examined an antilipolytic effect of compound **24b** in rats. To evaluate in vivo potency, a head-to-head comparison was performed by measuring the plasma glycerol level as a lipolytic end point.[30] For the comparison, compound **25** (Fig. 2), which was identified by Ebdrup et al. as a potent orally active HSL inhibitor and exerted the antilipolytic effect at a dose of 10 mg/kg p.o. due to its good PK profiles,[14] was selected.

As shown in Fig. 3, oral administration of compound **24b** relatively lowered plasma glycerol level. The reduced plasma glycerol AUC value of compound **24b** at 3 mg/kg p.o. was higher than that of **25** at 10 mg/kg p.o., with the value of 17%, due to its potent HSL inhibitory activity (IC_{50} = 2 nM) compared with compound **25** (IC_{50} = 270 nM).[18] This indicates that compound **24b** is an orally active HSL inhibitor that has the potential for acting as an antilipolytic agent.

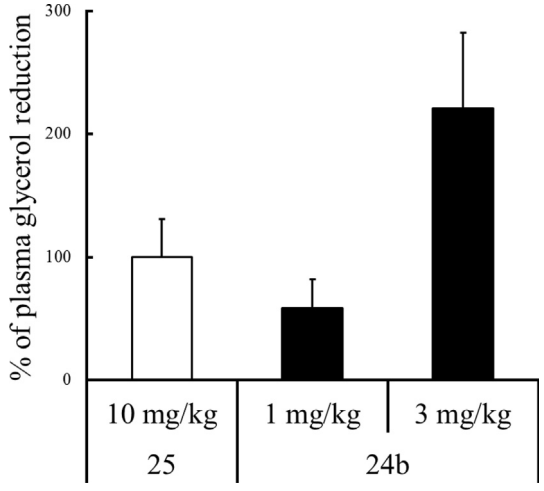


Fig. 3. The acute effect of HSL inhibition in rats treated with **25** at 10 mg/kg (white square) or **24b** at 1 and 3 mg/kg (black square). Data are reported as a percentage of plasma glycerol reduction of the area under the curve (AUC) (0–7 h), setting at 100% the reduction measured in compound **25**. Data are mean ± SEM n = 3–4.

4. Conclusion

Starting from compound **1**, by exchanging from the oxygen-carbon linker to the amide linker resulted in a promising lead compound **6b** with the moderate HSL inhibitory activity and a reduced bioactivation liability. Further optimization of the left hand and the right hand moiety of **6b** led to the identification of compound **24b** with an IC_{50} value of 2 nM and a significantly reduced reactive metabolite liability. Reflecting its in vitro efficacy, the oral administration of compound **24b** at 3 mg/kg in rats led to a decrease in plasma glycerol level. Further optimization of the derivatives is currently underway, which will clarify the biological importance of the HSL inhibition.

5. Experimental

5.1. Chemistry

Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. NMR spectra were recorded on a Varian Mercury 400 or 500 spectrometer with tetramethylsilane as an internal reference. Mass spectra were recorded on an Agilent Technologies Agilent 1100 series LC/MS. TLC analysis was performed on 60F354 plates (Merck). Flash column chromatography was performed on a Shoko scientific SI series on a Shoko Scientific Purif-α2. Purities of assayed compounds were in all cases greater than 90%, as determined by NMR analysis and LC/MS. The following abbreviations are used: EDC·HCl, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; HOBT, 1-hydroxybenzotriazole monohydrate; DMF, *N,N*-dimethylformamide; AcOEt, ethyl acetate; EtOH, ethanol; THF, tetrahydrofuran; DIPEA, *N,N*-diisopropylethylamine; MeOH, methanol; DMSO, dimethylsulfoxide; DMA, *N,N*-dimethylacetamide; EDTA, ethylenediaminetetraacetic acid; DTT, dithiothreitol; PNPB, *p*-nitrophenyl butyrate.

5.1.1. 2-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)-N-(4-{[5-(trifluoromethyl)pyridin-2-yl]oxy}phenyl)benzamide (**3**)

A mixture of **2** (0.15 g, 0.60 mmol), 2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoic acid (0.15 g, 0.60 mmol), EDC·HCl (0.13 g, 0.66 mmol), HOBT (0.10 g, 0.66 mmol) and DMF (6.0 mL) was stirred at room temperature overnight. The reaction mixture was diluted with water and extracted with AcOEt. The organic layer was washed with H_2O and brine, dried over Na_2SO_4 and evaporated. The resulting residue was purified by silica gel chromatography (hexane-AcOEt). The obtained solid was triturated in hexane/AcOEt and filtered to give **3** (0.011 g, 4%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ: 8.46 (1H, s), 8.31 (1H, s), 7.90 (1H, dd, J = 8.6, 2.3 Hz), 7.74–7.70 (4H, m), 7.51–7.48 (2H, m), 7.16 (2H, d, J = 9.0 Hz), 7.01 (1H, d, J = 9.0 Hz), 1.38 (12H, s). MS (ESI$^+$) m/z: 485 $(M+H)^+$. HRMS (ESI$^+$) m/z: 485.1855 $(M+H)^+$ (calcd for $C_{25}H_{25}BF_3N_2O_4$: 485.1854).

5.1.2. N-Methyl-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) aniline (**5b**)

Step A: To a mixture of **5a** (0.44 g, 2.0 mmol) and EtOH (9.0 mL) was added 1*H*-benzotriazole-1-methanol (0.30 g, 2.0 mmol). After stirring at room temperature overnight, the reaction mixture was concentrated in vacuo and the obtained residue was triturated with hexane. The resulting precipitate was collected by filtration to give *N*-(1*H*-Benzotriazol-1-ylmethyl)-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (0.59 g, 84%) as a brown solid. ^{1}H NMR ($CDCl_3$) δ: 8.03 (1H, d, J = 8.2 Hz), 7.67 (1H, d, J = 8.2 Hz), 7.62 (1H, dd, J = 7.4, 1.6 Hz), 7.44–7.40 (1H, m), 7.33–7.28 (2H, m), 7.09 (1H, t, J = 7.2 Hz), 7.02 (1H, d, J = 8.2 Hz), 6.73 (1H, td,

J = 7.4, 0.8 Hz), 6.16 (2H, d, *J* = 7.4 Hz), 1.37 (12H, s). MS (ESI^-) *m/z*: 349 (M−H). Step B: To a solution of *N*-(1*H*-Benzotriazol-1-ylmethyl)-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (0.37 g, 1.1 mmol) in THF (10 mL) was added sodium tetrahydroborate (0.040 g, 1.1 mmol). The mixture was stirred at 76 °C for 1 h. After cooling to room temperature, the reaction mixture was diluted with water and extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was purified by silica gel chromatography (hexane-AcOEt) to give **5b** (0.19 g, 75%) as a colorless solid. ^1H NMR ($CDCl_3$) δ: 7.63 (1H, dd, *J* = 7.2, 1.8 Hz), 7.35–7.30 (1H, m), 6.62 (1H, td, *J* = 7.2, 1.0 Hz), 6.54 (1H, d, *J* = 8.2 Hz), 2.85 (3H, s), 1.33 (12H, s). MS (FAB) *m/z*: 256 $(M+Na)^+$.

5.1.3. N-[2-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]-4-{[5-(trifluoromethyl)pyridin-2-yl]oxy}benzamide (**6a**)

Step A: To a solution of **4** (1.1 g, 4.0 mmol) in CH_2Cl_2 (24 mL) was added oxalyl chloride (1.0 mL, 12 mmol) followed by DMF (4 drops) at room temperature. The reaction mixture was stirred at room temperature for 1 h and concentrated in vacuo to give crude 4-{[5-(trifluoromethyl)pyridin-2-yl]oxy}benzoyl chloride, which was used directly in the next step. Step B: Crude 4-{[5-(trifluoromethyl)pyridin-2-yl]oxy}benzoyl chloride was dissolved in CH_2Cl_2 (24 mL) at 0 °C. To the solution was added DIPEA (0.75 mL, 4.4 mmol) and **5a** (0.88 g, 4.4 mmol). After stirring at room temperature for 1 h, the mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was purified by silica gel chromatography (hexane-AcOEt) to give **6a** (0.56 g, 29%) as a colorless solid. ^1H NMR ($CDCl_3$) δ: 10.28 (1H, s), 8.75 (1H, d, *J* = 8.2 Hz), 8.47–8.46 (1H, m), 8.13 (2H, d, *J* = 8.6 Hz), 7.96 (1H, dd, *J* = 8.8, 2.5 Hz), 7.83 (1H, dd, *J* = 7.4, 1.6 Hz), 7.54 (1H, td, *J* = 7.8, 1.4 Hz), 7.28 (2H, d, *J* = 9.0 Hz), 7.11 (2H, dd, *J* = 15.4, 8.0 Hz), 1.41 (12H, s). MS (ESI^+) *m/z*: 485 $(M+H)^+$. HRMS (ESI^-) *m/z*: 483.1712 $(M-H)^-$ (calcd for $C_{25}H_{23}BF_3N_2O_4$: 483.1708).

5.1.4. {2-[(4-{[5-(Trifluoromethyl)pyridin-2-yl]oxy}benzoyl)amino]phenyl}boronic acid (**6b**)

To a solution of **6a** (0.46 g, 0.95 mmol), THF (24 mL) and H_2O (6.0 mL) was added sodium periodate (0.61 g, 2.7 mmol). After stirring at room temperature for 1 h, sodium periodate (0.61 g, 2.7 mmol) was additionally added, and the reaction mixture was stirred at room temperature for 1 h. 1 M HCl aq. (1.2 mL) was added, and the mixture was stirred overnight. The reaction mixture was diluted with water and extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was purified by silica gel chromatography (AcOEt-MeOH). The obtained solid was triturated in hexane/AcOEt and filtered to give **6b** (0.15 g, 39%) as a colorless solid. ^1H NMR (CD_3OD) δ: 8.45 (1H, s), 8.24 (2H, d, *J* = 9.0 Hz), 8.14 (1H, dd, *J* = 8.6, 2.3 Hz), 7.49 (1H, d, *J* = 7.0 Hz), 7.42 (2H, d, *J* = 9.0 Hz), 7.35–7.24 (4H, m). MS (ESI^-) *m/z*: 401 $(M-H)^-$. HRMS (ESI^-) *m/z*: 401.0931 $(M-H)^-$ (calcd for $C_{19}H_{13}BF_3N_2O_4$: 401.0926).

5.1.5. N-Methyl-N-[2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]-4-{[5-(trifluoromethyl)pyridin-2-yl]oxy}benzamide (**6c**)

Compound **6c** was prepared in a similar manner described for **6a**. Yield: 30%. ^1H NMR ($CDCl_3$) δ: 8.40 (1H, s), 7.84 (1H, d, *J* = 9.0 Hz), 7.74 (1H, d, *J* = 7.4 Hz), 7.37 (3H, d, *J* = 8.6 Hz), 7.26–7.19 (1H, m), 7.07 (1H, d, *J* = 7.8 Hz), 6.88–6.86 (3H, m), 3.43 (3H, s), 1.33 (12H, br s). MS (ESI^+) *m/z*: 499 $(M+H)^+$. HRMS (ESI^+) *m/z*: 499.2010 $(M+H)^+$ (calcd for $C_{26}H_{27}BF_3N_2O_4$: 499.2010).

5.1.6. N-[2-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]-2-(4-{[5-(trifluoromethyl)pyridin-2-yl]oxy}phenyl)acetamide (**8**)

A mixture of **7** (0.59 g, 2.0 mmol), **5a** (0.66 g, 2.6 mmol), EDC·HCl (0.50 g, 2.6 mmol), HOBT (0.50 g, 2.6 mmol), 4-methylmorpholine (0.33 mL, 3.0 mmol) and CH_2Cl_2 (12 mL) was stirred at room temperature overnight. The reaction mixture was diluted with water and extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was purified by silica gel chromatography (hexane-AcOEt). The obtained solid was triturated in hexane/AcOEt and filtered to give **8** (0.50 g, 50%) as a colorless solid. ^1H NMR ($CDCl_3$) δ: 9.41 (1H, s), 8.48 (1H, d, *J* = 8.2 Hz), 8.42 (1H, s), 7.89 (1H, dd, *J* = 8.4, 2.9 Hz), 7.76 (1H, dd, *J* = 7.4, 1.6 Hz), 7.47–7.43 (3H, m), 7.14 (2H, d, *J* = 8.6 Hz), 7.07 (1H, td, *J* = 7.3, 0.9 Hz), 7.01 (1H, d, *J* = 8.6 Hz), 3.72 (2H, s), 1.34 (12H, s). MS (ESI^+) *m/z*: 499 $(M+H)^+$. HRMS (ESI^+) *m/z*: 499.2011 $(M+H)^+$ (calcd for $C_{26}H_{27}BF_3N_2O_4$: 499.2010).

5.1.7. Ethyl 3-{[5-(trifluoromethyl)pyridin-2-yl]oxy}benzoate (**10a**)

A mixture of 2-chloro-5-(trifluoromethyl)pyridine (5.45 g, 30 mmol), ethyl 3-hydroxybenzoate (5.5 g, 33 mmol), K_2CO_3 (4.98 g, 36 mmol) and DMF (20 mL) was stirred at 100 °C for 5 h. After cooling to room temperature, the reaction mixture was diluted with water and extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was purified by silica gel chromatography (hexane-AcOEt) to give **10a** (7.42 g, 79%) as a colorless oil. ^1H NMR ($CDCl_3$) δ: 8.43 (1H, s), 7.94 (2H, tt, *J* = 8.8, 2.4 Hz), 7.82 (1H, t, *J* = 2.0 Hz), 7.51 (1H, t, *J* = 7.8 Hz), 7.36 (1H, dq, *J* = 8.0, 1.2 Hz), 7.06 (1H, d, *J* = 9.0 Hz), 4.38 (2H, q, *J* = 7.0 Hz), 1.39 (3H, t, *J* = 7.0 Hz). MS (ESI^+) *m/z*: 312 $(M+H)^+$.

5.1.8. 3-{[5-(Trifluoromethyl)pyridin-2-yl]oxy}benzoic acid (**10b**)

A mixture of **10a** (0.15 g, 0.48 mmol), 5 M NaOH aq. (0.19 mL, 9.5 mmol) and MeOH (4.8 mL) was stirred at 60 °C for 20 min. After cooling to room temperature, the reaction mixture was concentrated in vacuo and the obtained residue was diluted with 1 M HCl aq. The resulting precipitate was collected by filtration to give **10b** (0.11 g, 78%) as a colorless solid. ^1H NMR (DMSO-D_6) δ: 8.59–8.59 (1H, m), 8.27 (1H, dd, *J* = 8.4, 2.9 Hz), 7.84 (1H, dt, *J* = 7.7, 1.3 Hz), 7.68 (1H, dd, *J* = 2.3, 1.6 Hz), 7.55 (1H, t, *J* = 7.8 Hz), 7.43 (1H, dq, *J* = 8.1, 1.2 Hz), 7.31 (1H, d, *J* = 8.6 Hz). MS (ESI^+) *m/z*: 284 $(M+H)^+$.

5.1.9. N-[2-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]-3-{[5-(trifluoromethyl)pyridin-2-yl]oxy}benzamide (**11**)

Compound **11** was prepared in a similar manner described for **6a**. Yield: 13%. ^1H NMR ($CDCl_3$) δ: 10.28 (1H, s), 8.74 (1H, d, *J* = 8.6 Hz), 8.46 (1H, s), 7.95 (2H, dq, *J* = 10.5, 2.9 Hz), 7.86–7.82 (2H, m), 7.57–7.52 (2H, m), 7.37–7.35 (1H, m), 7.12 (1H, td, *J* = 7.4, 0.8 Hz), 7.08 (1H, d, *J* = 8.6 Hz), 1.34 (12H, s). MS (ESI^+) *m/z*: 485 $(M+H)^+$.

5.1.10. 2-Methyl-4-{[5-(trifluoromethyl)pyridin-2-yl]oxy}benzoic acid (**12a**)

Step A: methyl 2-methyl-4-{[5-(trifluoromethyl)pyridin-2-yl]oxy}benzoate was prepared in a similar manner described for **10a**. Yield: 67%. ^1H NMR ($CDCl_3$) δ: 8.45 (1H, d, *J* = 1.0 Hz), 8.02 (1H, d, *J* = 7.8 Hz), 7.93 (1H, dd, *J* = 8.8, 2.4 Hz), 7.06–7.01 (3H, m), 3.90 (3H, s), 2.63 (3H, s). MS (ESI^+) *m/z*: 312 $(M+H)^+$. Step B: Compound **12a** was prepared in a similar manner described for **10b**. Yield: 74%. ^1H NMR ($CDCl_3$) δ: 8.47 (1H, s), 8.16 (1H, d, *J* = 9.0 Hz), 7.95 (1H, dd, *J* = 8.6, 2.7 Hz), 7.09–7.06 (3H, m), 2.68 (3H, s). MS (ESI^+) *m/z*: 298 $(M+H)^+$.

*5.1.11. 3-Methyl-4-{[5-(trifluoromethyl)pyridin-2-yl]oxy}benzoic acid (**12b**)*

Step A: Methyl 3-methyl-4-{[5-(trifluoromethyl)pyridin-2-yl]oxy}benzoate was prepared in a similar manner described for **10a**. Yield: 80%. ^{1}H NMR ($CDCl_3$) δ: 8.41 (1H, s), 8.01 (1H, d, J = 1.2 Hz), 7.95–7.93 (2H, m), 7.12 (1H, d, J = 8.6 Hz), 7.06 (1H, d, J = 8.6 Hz), 3.92 (3H, s), 2.22 (3H, s). MS (ESI$^+$) m/z: 312 (M+H)$^+$. Step B: Compound **12b** was prepared in a similar manner described for **10b**. Yield: 77%. ^{1}H NMR ($CDCl_3$) δ: 8.42 (1H, s), 8.07 (1H, s), 8.02 (1H, dd, J = 8.6, 2.0 Hz), 7.95 (1H, dd, J = 8.6, 2.3 Hz), 7.15 (1H, d, J = 8.2 Hz), 7.09 (1H, d, J = 8.6 Hz), 2.24 (3H, s). MS (ESI$^+$) m/z: 298 (M+H)$^+$.

*5.1.12. 2-Methyl-N-[2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) phenyl]-4-{[5-(trifluoromethyl)pyridin-2-yl]oxy}benzamide (**13a**)*

Compound **13a** was prepared in a similar manner described for **8**. Yield: 12%. ^{1}H NMR ($CDCl_3$) δ: 9.84 (1H, s), 8.67 (1H, d, J = 8.2 Hz), 8.44 (1H, s), 7.91 (1H, dd, J = 9.0, 2.3 Hz), 7.79 (1H, dd, J = 7.4, 1.6 Hz), 7.70 (1H, d, J = 8.6 Hz), 7.51 (1H, td, J = 7.8, 1.8 Hz), 7.12–7.02 (4H, m), 2.59 (3H, s), 1.32 (12H, s). MS (ESI$^+$) m/z: 499 (M+H)$^+$. HRMS (ESI$^+$) m/z: 499.2010 (M+H)$^+$ (calcd for $C_{26}H_{27}BF_3N_2O_4$: 499.2010).

*5.1.13. 3-Methyl-N-[2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) phenyl]-4-{[5-(trifluoromethyl)pyridin-2-yl]oxy}benzamide (**13b**)*

Compound **13b** was prepared in a similar manner described for **8**. Yield: 17%. ^{1}H NMR ($CDCl_3$) δ: 10.22 (1H, s), 8.76 (1H, d, J = 8.3 Hz), 8.44 (1H, s), 7.99 (1H, d, J = 2.0 Hz), 7.95 (2H, td, J = 5.7, 2.8 Hz), 7.83 (1H, dd, J = 7.6, 1.7 Hz), 7.53 (1H, td, J = 7.8, 1.8 Hz), 7.18 (1H, d, J = 8.3 Hz), 7.12 (1H, td, J = 7.3, 1.0 Hz), 7.07 (1H, d, J = 8.8 Hz), 2.28 (3H, s), 1.41 (12H, s). MS (ESI$^+$) m/z: 499 (M+H)$^+$. HRMS (ESI$^+$) m/z: 499.2015 (M+H)$^+$ (calcd for $C_{26}H_{27}BF_3N_2O_4$: 499.2010).

*5.1.14. 5-{[5-(Trifluoromethyl)pyridin-2-yl]oxy}pyridine-2-carboxylic acid (**15a**)*

Step A: A mixture of 5-(trifluoromethyl)pyridin-2-ol (1.0 g, 8.2 mmol), 2-cyano-5-fluoropyridine (1.5 g, 9.0 mmol), K_2CO_3 (1.4 g, 9.8 mmol) and DMA (3.0 mL) was stirred at 100 °C for 3 h. After cooling to room temperature, the reaction mixture was diluted with water and extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was purified by silica gel chromatography (hexane-AcOEt) to give 5-{[5-(trifluoromethyl)pyridin-2-yl]oxy}pyridine-2-carbonitrile (1.9 g, 86%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ: 8.80 (1H, d, J = 2.0 Hz), 8.02 (1H, dd, J = 8.4, 2.5 Hz), 7.89 (1H, d, J = 9.0 Hz), 7.72 (1H, s), 7.59 (1H, dd, J = 9.8, 2.7 Hz), 6.78 (1H, d, J = 9.8 Hz). MS (ESI$^+$) m/z: 266 (M+H)$^+$. Step B: A mixture of 5-{[5-(trifluoromethyl)pyridin-2-yl]oxy}pyridine-2-carbonitrile (1.9 g, 7.0 mmol), conc. hydrochloric acid (6.0 mL), conc. sulfuric acid (3.0 mL) and MeOH (20 mL) was stirred at 85 °C for 4 h. After cooling to room temperature, the reaction mixture was concentrated in vacuo. The residue was diluted with 5 M NaOH aq. and extracted with AcOEt. The resulting residue was purified by silica gel chromatography (hexane-AcOEt) to give methyl 5-{[5-(trifluoromethyl)pyridin-2-yl]oxy}pyridine-2-carboxylate (1.1 g, 3.8 mmol) as a pale yellow solid. ^{1}H NMR ($CDCl_3$) δ: 8.79 (1H, d, J = 2.9 Hz), 8.31 (1H, d, J = 8.3 Hz), 7.99 (1H, dd, J = 8.3, 2.4 Hz), 7.73 (1H, s), 7.57 (1H, dd, J = 9.5, 2.7 Hz), 6.77 (1H, d, J = 9.8 Hz), 4.06 (3H, s). MS (ESI$^+$) m/z: 299 (M+H)$^+$. Step C: Compound **15a** was prepared in a similar manner described for **10b**. Yield: 91%. ^{1}H NMR ($CDCl_3$) δ: 8.81 (1H, d, J = 2.0 Hz), 8.43 (1H, d, J = 8.2 Hz), 8.09 (1H, dd, J = 8.4, 2.5 Hz), 7.78 (1H, s), 7.61 (1H, dd, J = 9.6, 2.5 Hz), 6.82 (1H, d, J = 9.8 Hz). MS (ESI$^+$) m/z: 285 (M+H)$^+$.

*5.1.15. 5-[4-(Trifluoromethyl)phenoxy]pyridine-2-carboxylic acid (**15b**)*

Step A: A mixture of 4-(trifluoromethyl)phenol (2.9 g, 18 mmol), 2-cyano-5-fluoropyridine (2.0 g, 9.0 mmol), K_2CO_3 (2.7 g, 20 mmol) and DMA (20 mL) was stirred at 100 °C for 6 h. After cooling to room temperature, the reaction mixture was diluted with water and extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was purified by silica gel chromatography (hexane-AcOEt) to give 5-[4-(trifluoromethyl)phenoxy]pyridine-2-carbonitrile (4.0 g, 92%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ: 8.50 (1H, d, J = 2.4 Hz), 7.71–7.69 (3H, m), 7.35 (1H, dd, J = 8.8, 2.9 Hz), 7.19 (2H, d, J = 8.8 Hz). MS (ESI$^+$) m/z: 264 (M+H)$^+$. Step B: A mixture of 5-[4-(trifluoromethyl)phenoxy]pyridine-2-carbonitrile (1.0 g, 3.8 mmol), conc. hydrochloric acid (3.0 mL), conc. sulfuric acid (1.5 mL) and MeOH (10 mL) was stirred at 85 °C for 3 h. After cooling to room temperature, the reaction mixture was concentrated in vacuo. The residue was diluted with 5 M NaOH aq. and extracted with AcOEt. The resulting residue was purified by silica gel chromatography (hexane-AcOEt) to give methyl 5-[4-(trifluoromethyl)phenoxy]pyridine-2-carboxylate (0.74 g, 2.5 mmol) as a colorless solid. Yield: 65%. ^{1}H NMR ($CDCl_3$) δ: 8.54 (1H, d, J = 2.4 Hz), 8.16 (1H, d, J = 8.8 Hz), 7.68 (2H, d, J = 8.3 Hz), 7.39 (1H, dd, J = 8.5, 2.7 Hz), 7.16 (2H, d, J = 8.3 Hz), 4.02 (3H, s). MS (ESI$^+$) m/z: 298 (M+H)$^+$. Step C: Compound **15b** was prepared in a similar manner described for **10b**. Yield: 82%. ^{1}H NMR ($CDCl_3$) δ: 8.39 (1H, d, J = 2.7 Hz), 8.24 (1H, d, J = 8.6 Hz), 7.72 (2H, d, J = 8.6 Hz), 7.48 (1H, dd, J = 8.6, 2.7 Hz), 7.20 (2H, d, J = 9.0 Hz). MS (ESI$^+$) m/z: 284 (M+H)$^+$.

*5.1.16. N-[2-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]-5-{[5-(trifluoromethyl)pyridin-2-yl]oxy}pyridine-2-carboxamide (**16a**)*

Compound **16a** was prepared in a similar manner described for **6a**. Yield: 32%. ^{1}H NMR ($CDCl_3$) δ: 11.83 (1H, s), 8.82 (1H, d, J = 8.3 Hz), 8.75 (1H, d, J = 2.4 Hz), 8.52 (1H, d, J = 8.3 Hz), 7.99 (1H, dd, J = 8.3, 2.4 Hz), 7.86 (1H, dd, J = 7.3, 1.5 Hz), 7.80 (1H, d, J = 1.0 Hz), 7.61–7.57 (2H, m), 7.18 (1H, td, J = 7.3, 1.0 Hz), 6.82 (1H, d, J = 9.8 Hz), 1.46 (12H, s). MS (ESI$^+$) m/z: 486 (M+H)$^+$. HRMS (ESI$^+$) m/z: 486.1804 (M+H)$^+$ (calcd for $C_{24}H_{24}BF_3N_3O_4$: 486.1806).

*5.1.17. N-[2-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]-5-[4-(trifluoromethyl)phenoxy]pyridine-2-carboxamide (**16b**)*

Compound **16b** was prepared in a similar manner described for **6a**. Yield: 11%. ^{1}H NMR ($CDCl_3$) δ: 11.71 (1H, s), 8.75 (1H, d, J = 7.8 Hz), 8.44 (1H, d, J = 2.4 Hz), 8.32 (1H, d, J = 8.8 Hz), 7.82 (1H, dd, J = 7.3, 1.5 Hz), 7.68 (2H, d, J = 8.8 Hz), 7.54–7.50 (1H, m), 7.46 (1H, dd, J = 8.5, 2.7 Hz), 7.17–7.11 (3H, m), 1.42 (12H, s). MS (ESI$^+$) m/z: 485 (M+H)$^+$. HRMS (ESI$^+$) m/z: 485.1855 (M+H)$^+$ (calcd for $C_{25}H_{25}BF_3N_2O_4$: 485.1854).

*5.1.18. 6-[4-(Trifluoromethyl)phenoxy]pyridine-3-carboxylic acid (**18**)*

Compound **18** was prepared in a similar manner described for **10b**. Yield: 80%. ^{1}H NMR ($CDCl_3$) δ: 8.90 (1H, d, J = 2.0 Hz), 8.39 (1H, dd, J = 8.6, 2.7 Hz), 7.72 (2H, d, J = 8.6 Hz), 7.31 (2H, d, J = 8.6 Hz), 7.08 (1H, dd, J = 8.6, 0.8 Hz). MS (ESI$^+$) m/z: 284 (M+H)$^+$.

*5.1.19. N-[2-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]-6-[4-(trifluoromethyl)phenoxy]pyridine-3-carboxamide (19a) and {2-[({6-[4-(trifluoromethyl)phenoxy]pyridin-3-yl}carbonyl)amino] phenyl}boronic acid (**19b**)*

A mixture of **18** (0.68 g, 2.4 mmol), **5a** (0.44 g, 2.0 mmol), DIPEA (1.4 mL, 6 mmol), 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (0.91 g, 2.4 mmol), and DMF (12 mL) was stirred at room temperature overnight. The reaction mixture was diluted with water and extracted with AcOEt. The organic layer was washed with H_2O and brine, dried over Na_2SO_4

and evaporated. The resulting residue was purified by silica gel chromatography (AcOEt-MeOH) to give **19a** (0.094 g, 10%) as a colorless solid and **19b** (0.18 g, 23%) as a colorless solid.

19a: ^{1}H NMR ($CDCl_3$) δ: 10.23 (1H, s), 8.84 (1H, d, J = 2.3 Hz), 8.67 (1H, d, J = 8.2 Hz), 8.36 (1H, dd, J = 8.6, 2.7 Hz), 7.83 (1H, dd, J = 7.4, 1.6 Hz), 7.70 (2H, d, J = 8.6 Hz), 7.53 (1H, td, J = 7.8, 1.4 Hz), 7.30 (2H, d, J = 8.2 Hz), 7.14 (1H, td, J = 7.4, 0.8 Hz), 7.06 (1H, d, J = 8.6 Hz), 1.41 (12H, s). MS (ESI^+) m/z: 485 $(M+H)^+$. HRMS (ESI^+) m/z: 485.1848 $(M+H)^+$ (calcd for $C_{25}H_{25}BF_3N_2O_4$: 485.1854).

19b: ^{1}H NMR (CD_3OD) δ: 8.92 (1H, d, J = 2.3 Hz), 8.58 (1H, dd, J = 8.8, 2.5 Hz), 7.77 (2H, d, J = 9.0 Hz), 7.51 (1H, t, J = 3.9 Hz), 7.39 (2H, d, J = 8.6 Hz), 7.31–7.28 (4H, m). MS (ESI^-) m/z: 401 $(M-H)^-$. HRMS (ESI^-) m/z: 401.0913 $(M-H)^-$ (calcd for $C_{19}H_{13}BF_3N_2O_4$: 401.0926).

5.1.20. 6-[4-(Trifluoromethoxy)phenoxy]pyridine-3-carboxylic acid (**21a**)

Step A: Methyl 6-[4-(trifluoromethoxy)phenoxy]pyridine-3-carboxylate was prepared in a similar manner described for **10a**. Yield: 71%. ^{1}H NMR ($CDCl_3$) δ: 8.81 (1H, dd, J = 2.3, 0.8 Hz), 8.31 (1H, dd, J = 8.6, 2.3 Hz), 7.29–7.26 (2H, m), 7.21–7.17 (2H, m), 6.98 (1H, dd, J = 8.6, 0.8 Hz), 3.93 (3H, s). MS (ESI^+) m/z: 314 $(M+H)^+$. Step B: Compound **21a** was prepared in a similar manner described for **10b**. Yield: 83%. ^{1}H NMR ($CDCl_3$) δ: 8.88 (1H, dd, J = 2.3, 0.8 Hz), 8.35 (1H, dd, J = 8.6, 2.3 Hz), 7.30–7.27 (2H, m), 7.22–7.19 (2H, m), 7.02 (1H, dd, J = 8.6, 0.8 Hz). MS (ESI^+) m/z: 300 $(M+H)^+$.

5.1.21. 6-[3-(Trifluoromethoxy)phenoxy]pyridine-3-carboxylic acid (**21b**)

Step A: Methyl 6-[3-(trifluoromethoxy)phenoxy]pyridine-3-carboxylate was prepared in a similar manner described for **10a**. Yield: 78%. ^{1}H NMR ($CDCl_3$) δ: 8.82 (1H, dd, J = 2.3, 0.8 Hz), 8.32 (1H, dd, J = 8.6, 2.3 Hz), 7.44 (1H, t, J = 8.2 Hz), 7.12–7.09 (3H, m), 6.99 (1H, d, J = 8.6 Hz), 3.93 (3H, s). MS (ESI^+) m/z: 314 $(M+H)^+$. Step B: Compound **21b** was prepared in a similar manner described for **10b**. Yield: 76%. ^{1}H NMR ($CDCl_3$) δ: 8.90 (1H, d, J = 2.3 Hz), 8.37 (1H, dd, J = 8.6, 2.3 Hz), 7.46 (1H, t, J = 8.4 Hz), 7.14 (2H, dd, J = 8.4, 2.2 Hz), 7.09–7.08 (1H, m), 7.03 (1H, dd, J = 8.6, 0.8 Hz). MS (ESI^+) m/z: 300 $(M+H)^+$.

5.1.22. 6-{Methyl[4-(trifluoromethoxy)phenyl]amino}pyridine-3-carboxylic acid (**21c**)

Step A: A mixture of methyl 6-chloronicotinate (0.34 g, 2.0 mmol), *N*-methyl-*p*-trifluoromethoxyaniline (0.38 g, 2.0 mmol) and acetic acid (3.0 mL) was heated at 180 °C with microwave-assistance for 30 min with stirring. After cooling to room temperature, the mixture was diluted with 1 M NaOH aq. and extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was purified by silica gel chromatography (hexane-AcOEt) to give methyl 6-{methyl[4-(trifluoromethoxy)phenyl]amino}pyridine-3-carboxylate (0.066 g, 10%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ: 8.88 (1H, d, J = 2.3 Hz), 7.91 (1H, dd, J = 9.0, 2.3 Hz), 7.31 (4H, s), 6.44 (1H, dd, J = 9.0, 0.8 Hz), 3.88 (3H, s), 3.53 (3H, s). MS (ESI^+) m/z: 327 $(M+H)^+$. Step B: Compound **21c** was prepared in a similar manner described for **10b**. Yield: 58%. ^{1}H NMR ($CDCl_3$) δ: 8.94 (1H, d, J = 2.3 Hz), 7.93 (1H, dd, J = 9.0, 2.3 Hz), 7.31–7.30 (4H, m), 6.44 (1H, dd, J = 9.0, 0.8 Hz), 3.54 (3H, s). MS (ESI^+) m/z: 313 $(M+H)^+$.

5.1.23. N-[2-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]-6-[4-(trifluoromethoxy)phenoxy]pyridine-3-carboxamide (**22a**)

Compound **22a** was prepared in a similar manner described for **6a**. Yield: 74%. ^{1}H NMR ($CDCl_3$) δ: 10.22 (1H, s), 8.84 (1H, d, J = 2.7 Hz), 8.67 (1H, d, J = 8.6 Hz), 8.34 (1H, dd, J = 8.6, 2.7 Hz), 7.83 (1H, dd, J = 7.4, 1.2 Hz), 7.53 (1H, td, J = 7.9, 1.6 Hz), 7.29–7.27 (2H, m), 7.21 (2H, d, J = 9.0 Hz), 7.13 (1H, td, J = 7.4, 0.8 Hz), 7.01 (1H, dd, J = 8.6, 0.8 Hz), 1.41 (12H, s). MS (ESI^+) m/z: 501 $(M+H)^+$. HRMS (ESI^+) m/z: 501.1807 $(M+H)^+$ (calcd for $C_{25}H_{25}BF_3N_2O_5$: 501.1803).

5.1.24. N-[2-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]-6-[3-(trifluoromethoxy)phenoxy]pyridine-3-carboxamide (**22b**)

Compound **22b** was prepared in a similar manner described for **6a**. Yield: 39%. ^{1}H NMR ($CDCl_3$) δ: 10.23 (1H, s), 8.84 (1H, d, J = 2.4 Hz), 8.67 (1H, d, J = 8.3 Hz), 8.36 (1H, dd, J = 8.3, 2.4 Hz), 7.83 (1H, dd, J = 7.3, 1.5 Hz), 7.53 (1H, td, J = 7.9, 1.6 Hz), 7.45 (1H, dt, J = 10.3, 3.2 Hz), 7.14–7.12 (4H, m), 7.03 (1H, d, J = 8.8 Hz), 1.40 (12H, s). MS (ESI^+) m/z: 501 $(M+H)^+$. HRMS (ESI^+) m/z: 501.1806 $(M+H)^+$ (calcd for $C_{25}H_{25}BF_3N_2O_5$: 501.1803).

5.1.25. 6-{Methyl[4-(trifluoromethoxy)phenyl]amino}-N-[2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]pyridine-3-carboxamide (**22c**)

Compound **22c** was prepared in a similar manner described for **6a**. Yield: 32%. ^{1}H NMR ($CDCl_3$) δ: 10.11 (1H, s), 8.90 (1H, d, J = 2.3 Hz), 8.67 (1H, d, J = 8.2 Hz), 7.96 (1H, dd, J = 8.8, 2.5 Hz), 7.80 (1H, dd, J = 7.4, 1.6 Hz), 7.50 (1H, td, J = 7.9, 1.6 Hz), 7.34–7.30 (4H, m), 7.09 (1H, td, J = 7.3, 1.0 Hz), 6.52 (1H, d, J = 9.0 Hz), 3.55 (3H, s), 1.41 (12H, s). MS (ESI^+) m/z: 514 $(M+H)^+$.

5.1.26. N-[4-Fluoro-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]-6-[4-(trifluoromethyl)phenoxy]pyridine-3-carboxamide (**23a**)

Compound **23a** was prepared in a similar manner described for **6a**. Yield: 51%. ^{1}H NMR (CD_3OD) δ: 8.86 (1H, dd, J = 2.7, 0.8 Hz), 8.50 (1H, dd, J = 8.6, 2.7 Hz), 7.76 (2H, d, J = 8.6 Hz), 7.38 (2H, d, J = 9.0 Hz), 7.33–7.21 (2H, m), 7.15 (1H, dd, J = 8.6, 3.1 Hz), 7.03 (1H, td, J = 8.6, 3.1 Hz), 1.36 (12H, s). MS (ESI^+) m/z: 503 $(M+H)^+$.

5.1.27. N-[4-Chloro-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]-6-[4-(trifluoromethyl)phenoxy]pyridine-3-carboxamide (**23b**)

Compound **23b** was prepared in a similar manner described for **6a**. Yield: 62%. ^{1}H NMR (CD_3OD) δ: 8.86 (1H, t, J = 1.6 Hz), 8.50 (1H, dd, J = 8.8, 2.5 Hz), 7.77 (2H, d, J = 9.0 Hz), 7.56 (1H, d, J = 2.3 Hz), 7.40 (2H, d, J = 8.6 Hz), 7.33–7.29 (3H, m), 1.37 (12H, s). MS (ESI^+) m/z: 519 $(M+H)^+$. HRMS (ESI^-) m/z: 517.1334 $(M-H)^-$ (calcd for $C_{25}H_{22}BClF_3N_2O_4$: 517.1319).

5.1.28. N-[4-Methyl-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]-6-[4-(trifluoromethyl)phenoxy]pyridine-3-carboxamide (**23c**)

Compound **23c** was prepared in a similar manner described for **6a**. Yield: 37%. ^{1}H NMR ($CDCl_3$) δ: 10.17 (1H, s), 8.83 (1H, d, J = 2.0 Hz), 8.55 (1H, d, J = 8.2 Hz), 8.35 (1H, dd, J = 8.6, 2.3 Hz), 7.69 (2H, d, J = 8.6 Hz), 7.63 (1H, d, J = 2.0 Hz), 7.34 (1H, dd, J = 8.6, 2.0 Hz), 7.30 (2H, d, J = 8.6 Hz), 7.05 (1H, d, J = 8.6 Hz), 2.33 (3H, s), 1.40 (12H, s). MS (ESI^+) m/z: 499 $(M+H)^+$.

5.1.29. N-[5-Fluoro-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]-6-[4-(trifluoromethyl)phenoxy]pyridine-3-carboxamide (**23d**)

Compound **23d** was prepared in a similar manner described for **6a**. Yield: 61%. ^{1}H NMR ($CDCl_3$) δ: 10.33 (1H, s), 8.83 (1H, dd, J = 2.7, 0.8 Hz), 8.49 (1H, dd, J = 12.1, 2.3 Hz), 8.35 (1H, dd, J = 8.6, 2.7 Hz), 7.80 (1H, dd, J = 8.6, 7.0 Hz), 7.70 (2H, d, J = 8.2 Hz), 7.30 (2H, d, J = 9.0 Hz), 7.06 (1H, d, J = 8.6 Hz), 6.83 (1H, td, J = 8.2, 2.3 Hz), 1.40 (12H, s). HRMS (ESI^-) m/z: 501.1595 $(M-H)^-$ (calcd for $C_{25}H_{22}BF_4N_2O_4$: 501.1614).

*5.1.30. N-[5-Chloro-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) phenyl]-6-[4-(trifluoromethyl)phenoxy]pyridine-3-carboxamide (**23e**)*

Compound **23e** was prepared in a similar manner described for **6a**. Yield: 37%. ^{1}H NMR (CD_3OD) δ: 8.86 (1H, dd, J = 2.7, 0.8 Hz), 8.50 (1H, dd, J = 8.8, 2.5 Hz), 7.77 (2H, d, J = 9.0 Hz), 7.62 (1H, d, J = 7.8 Hz), 7.42–7.40 (3H, m), 7.30–7.26 (2H, m), 1.37 (12H, s). MS (ESI^+) m/z: 519 $(M+H)^+$.

*5.1.31. N-[5-Methoxy-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]-6-[4-(trifluoromethyl)phenoxy]pyridine-3-carboxamide (**23f**)*

Compound **23f** was prepared in a similar manner described for **6a**. Yield: 42%. ^{1}H NMR (CD_3OD) δ: 8.83 (1H, d, J = 2.7 Hz), 8.48 (1H, dd, J = 8.6, 2.3 Hz), 7.77 (2H, d, J = 9.0 Hz), 7.58 (1H, d, J = 8.2 Hz), 7.40 (2H, d, J = 8.6 Hz), 7.27 (1H, d, J = 9.0 Hz), 7.22 (1H, d, J = 2.3 Hz), 6.85 (1H, dd, J = 8.2, 2.3 Hz), 3.82 (3H, s), 1.37 (12H, s). MS (ESI^+) m/z: 515 $(M+H)^+$.

*5.1.32. {5-Fluoro-2-[({6-[4-(trifluoromethyl)phenoxy]pyridin-3-yl}carbonyl)amino]phenyl}boronic acid (**24a**)*

Step A: To a solution of **23a** (0.51 g, 1.0 mmol) in MeOH (50 mL) was added 5.6 M KHF_2 aq. (1.26 mL). After stirring at room temperature for 15 min, the reaction mixture was concentrated in vacuo. The residue was dissolved in hot acetone and filtered. The filtrate was concentrated in vacuo to give crude potassium {5-fluoro-2-[({6-[4-(trifluoromethyl)phenoxy]pyridin-3-yl}carbonyl)amino]-phenyl}(trifluoro)borate(1-), which was used directly in the next step. Step B: Crude potassium {5-fluoro-2-[({6-[4-(trifluoromethyl)phenoxy]pyridin-3-yl}carbonyl)amino]phenyl}(trifluoro)-borate(1-) was dissolved in CH_3CN (50 mL) and H_2O (0.11 mL) and chlorotrimethylsilane (0.77 ml, 6.1 mmol) was added. After stirring overnight, the resulting mixture was evaporated. The resulting residue was diluted with saturated $NaHCO_3$ solution and extracted with AcOEt. The organic layer was washed with H_2O (twice) and brine, dried over Na_2SO_4, filtered and evaporated. The resulting residue was purified by silica gel chromatography (hexane-AcOEt). The obtained solid was triturated in hexane/AcOEt and filtered to give **24a** (0.16 g, 38%) as a colorless solid. ^{1}H NMR (CD_3OD) δ: 8.91 (1H, s), 8.56 (1H, dd, J = 8.6, 2.0 Hz), 7.77 (2H, d, J = 9.0 Hz), 7.38 (2H, d, J = 9.0 Hz), 7.30 (1H, br s), 7.24 (1H, d, J = 8.6 Hz), 7.16 (1H, dd, J = 8.6, 2.7 Hz), 7.03–7.01 (1H, br s). MS (ESI^-) m/z: 419 $(M-H)^-$. HRMS (ESI^-) m/z: 419.0843 $(M-H)^-$ (calcd for $C_{19}H_{12}BF_4N_2O_4$: 419.0832).

*5.1.33. {5-Chloro-2-[({6-[4-(trifluoromethyl)phenoxy]pyridin-3-yl}carbonyl)amino]phenyl}boronic acid (**24b**)*

Compound **24b** was prepared in a similar manner described for **24a**. Yield: 47%. ^{1}H NMR (CD_3OD) δ: 8.91 (1H, s), 8.56 (1H, d, J = 8.6 Hz), 7.77 (2H, d, J = 8.6 Hz), 7.44 (1H, s), 7.39 (2H, d, J = 8.2 Hz), 7.29–7.26 (3H, m). MS (ESI^-) m/z: 435 $(M-H)^-$. HRMS (ESI^-) m/z: 435.0529 $(M-H)^-$ (calcd for $C_{19}H_{12}BClF_3N_2O_4$: 435.0536).

*5.1.34. {5-Methyl-2-[({6-[4-(trifluoromethyl)phenoxy]pyridin-3-yl}carbonyl)amino]phenyl}boronic acid (**24c**)*

Compound **24c** was prepared in a similar manner described for **24a**. Yield: 41%. ^{1}H NMR (CD_3OD) δ: 8.91 (1H, dd, J = 2.7, 0.8 Hz), 8.56 (1H, dd, J = 8.6, 2.7 Hz), 7.78 (2H, d, J = 8.2 Hz), 7.40 (2H, d, J = 8.2 Hz), 7.32–7.29 (2H, m), 7.21–7.18 (2H, m), 2.37 (3H, s). MS (ESI^-) m/z: 415 $(M-H)^-$. HRMS (ESI^-) m/z: 415.1100 $(M-H)^-$ (calcd for $C_{20}H_{15}BF_3N_2O_4$: 415.1082).

*5.1.35. {4-Fluoro-2-[({6-[4-(trifluoromethyl)phenoxy]pyridin-3-yl}carbonyl)amino]phenyl}boronic acid (**24d**)*

Compound **24d** was prepared in a similar manner described for **24a**. Yield: 40%. ^{1}H NMR (CD_3OD) δ: 8.93 (1H, d, J = 2.4 Hz), 8.58 (1H, dd, J = 8.8, 2.4 Hz), 7.78 (2H, d, J = 8.3 Hz), 7.50 (1H, dd, J = 8.3, 6.8 Hz), 7.40 (2H, d, J = 8.8 Hz), 7.30 (1H, d, J = 8.8 Hz), 7.06–7.05 (2H, m). MS (ESI^-) m/z: 419 $(M-H)^-$. HRMS (ESI^-) m/z: 419.0825 $(M-H)^-$ (calcd for $C_{19}H_{12}BF_4N_2O_4$: 419.0832).

*5.1.36. {4-Chloro-2-[({6-[4-(trifluoromethyl)phenoxy]pyridin-3-yl}carbonyl)amino]phenyl}boronic acid (**24e**)*

Compound **24e** was prepared in a similar manner described for **24a**. Yield: 66%. ^{1}H NMR (CD_3OD) δ: 8.91 (1H, br s), 8.57–8.55 (1H, m), 7.75 (2H, d, J = 8.2 Hz), 7.43 (1H, d, J = 7.8 Hz), 7.37 (2H, d, J = 8.2 Hz), 7.22 (1H, br s), 7.17 (1H, d, J = 8.2 Hz), 7.13 (1H, br s). MS (ESI^-) m/z: 435 $(M-H)^-$. HRMS (ESI^-) m/z: 435.0561 $(M-H)^-$ (calcd for $C_{19}H_{12}BClF_3N_2O_4$: 435.0536).

*5.1.37. {4-Methoxy-2-[({6-[4-(trifluoromethyl)phenoxy]pyridin-3-yl}carbonyl)amino]phenyl}boronic acid (**24f**)*

Compound **24f** was prepared in a similar manner described for **24a**. Yield: 25%. ^{1}H NMR (CD_3OD) δ: 8.92 (1H, d, J = 2.4 Hz), 8.57 (1H, dd, J = 8.8, 2.4 Hz), 7.78 (2H, d, J = 8.8 Hz), 7.41 (3H, d, J = 7.8 Hz), 7.30 (1H, d, J = 8.8 Hz), 6.92 (1H, dd, J = 8.1, 2.2 Hz), 6.88 (1H, d, J = 2.4 Hz), 3.83 (3H, s). MS (ESI^-) m/z: 431 $(M-H)^-$. HRMS (ESI^-) m/z: 432.1084 $(M-H)^-$ (calcd for $C_{20}H_{15}BF_3N_2O_5$: 431.1032).

*5.1.38. 4-{[5-(trifluoromethyl)pyridin-2-yl]oxy}phenyl 4-hydroxypiperidine-1-carboxylate (**25**)*

Compound **25** was prepared in the same manner previously reported by Ebdrup et al.[14] ^{1}H NMR (DMSO-D_6) δ: 8.58–8.57 (1H, m), 8.24 (1H, dd, J = 8.8, 2.5 Hz), 7.25 (1H, d, J = 9.0 Hz), 7.21–7.18 (4H, m), 3.89–3.86 (1H, m), 3.76–3.70 (2H, m), 3.31–3.28 (1H, m), 3.17–3.14 (1H, m), 1.81–1.77 (2H, m), 1.42–1.38 (2H, m). HRMS (ESI^+) m/z: 383.1207 $(M+H)^+$ (calcd for $C_{18}H_{18}F_3N_2O_4$: 383.1213).

5.2. Pharmacology

5.2.1. In vitro HSL assay

Recombinant human HSL was expressed in *Spodoptera frugiperda* Sf9 insect cells using the Bac-to-Bac® Baculovirus Expression System (Invitrogen, Inc.). The infected Sf9 cells were suspended in 3 pellet volumes with a homogenization buffer (25 mM sucrose pH 7.5, 1 mM EDTA, 1 mM DTT and protease inhibitors) to the whole volume of 30 mL. The homogenate was sonicated on an ice bath. Insoluble material was removed by centrifugation at 18,000 rpm for 20 min. The obtained 20 mL of clear supernatant was utilized for the in vitro assay. The solution of human HSL extract diluted by 6-fold (10 μL) and various concentrations of compounds in DMSO (10 μL) were added to a solution of 1.0 mM PNPB in a phosphate buffer (0.1 M NaH_2PO_4 pH 7.25, 0.15 M NaCl, and 1 mM DTT) (180 μL). The enzymatic reactions were carried out at 37 °C for 5 min. The solution absorbances were measured spectrophotometrically at a λ of 405 nm at 0 and 5 min. Each assay was carried out in triplicate. IC_{50} values were obtained by fitting data to a nonlinear curve fitting program (GraphPad Software, Inc., La Jolla CA).

5.2.2. In vivo inhibition of lipolysis

Male Wistar rats 8 weeks of age were purchased from Japan SLC, Inc. All animals were held under standard laboratory conditions (12 h light per day, lights on at 7:00 AM, 23 ± 2 °C, 55 ± 10% humidity) with food and water available ad libitum. Overnight-fasted rats received vehicle (propylene glycol/Tween80 = 4:1 (v/v)) (n = 4) or HSL inhibitor in the vehicle (1 mg/kg, 3 mg/kg and 10 mg/kg body weight p.o.; n = 3 per group). EDTA-treated blood samples were collected just prior to and after dosing at 0.5, 1, 3, 5, and 7 h from tail veins. The blood was centrifuged at 12,000 rpm for 5 min at 4 °C to obtain the plasma. The plasma samples were stored at -20 °C before analysis. Plasma glycerol levels

were determined using a Free Glycerol Determination Kit (Sigma-Aldrich). All experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee at Daiichi Sankyo Co., Ltd. The relative value was calculated as the following formula:

Plasma glycerol reduction of the $AUC_{0\text{-}7\ h}$ (%) relative to compound **25** = {[Plasma glycerol $AUC_{0\text{-}7\ h}$ value (vehicle group) − Plasma glycerol $AUC_{0\text{-}7\ h}$ value (test group)]/[Plasma glycerol $AUC_{0\text{-}7\ h}$ value (vehicle group) − Plasma glycerol $AUC_{0\text{-}7\ h}$ value (compound **25** group)]} × 100

5.2.3. Pharmacokinetic evaluation in rats

Each plasma sample was prepared as described above, followed by mixing with water, acetonitrile, and IS (internal standard) acetonitrile solution (20 μM of niflumic acid). For each standard sample, each standard solution of compound **24b** (0.02, 0.05, 0.2, 0.5, 2, 5, 20 and 50 μM) was added to blank plasma and mixed with water, acetonitrile and IS acetonitrile solution. After vortex mixing for 5 min at room temperature, the mixtures were added to a Captiva plate (Agilent Technologies) and filtrated. The filtrates were subjected to LC-MS/MS analysis using an API 4000QTRAP (Applied Biosystems/MDS SCIEX). C_{max} and $AUC_{0\text{-}7h}$ were determined by a non-compartmental model using the BioBook function of E-WorkBook Suite (ID Business Solutions Ltd.).

5.2.4. GSH trapping experiments

For the GSH trapping experiments, each test compound (500 μM) was incubated with human liver microsomes (2 mg of protein/mL) supplemented with an NADPH generating system and an equimolar mixture of GSH and stable isotope-labeled GSH ([$^{13}C_2$, ^{15}N-Gly]GSH). After 60-min incubation, the reaction was terminated with acetonitrile containing propranolol, followed by centrifugation and concentration of the supernatant. The incubation without NADP or a substrate was performed to obtain control samples. The analytical samples were subjected to LC-MS/MS analysis in a full scan MSE mode using a Q-Tof Xevo mass spectrometer (Waters). The data were analyzed by Metabolynx software (Waters). The structure elucidation of GSH adducts of compound **1** was performed by LC/MS and LC-MS/MS analysis using an LTQ Orbitrap XL (Thermo Fisher Scientific).

5.2.5. Metabolic stability

Compound **24b** (1 μM) and rat liver microsomes (0.5 mg/mL) were incubated in sodium phosphate buffer (pH 7.4) at 37 °C with an NADPH generating system. After 20-min incubation, the reaction was terminated with MeOH. After centrifuging each solution separately at 3500 rpm for 10 min at 4 °C, the remaining parent compound was determined by LC-MS/MS.

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