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DESIGN, SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIP STUDY OF PYRILAMINE DERIVATIVES AS HISTONE DEACETYLASE INHIBITORS

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Abstract – For the study on the structure-activity relationship of pyrilamine-based histone deacetylase inhibitor **1**, we focused on the structures of its benzyl and dimethylamino groups. Of the synthesized novel pyrilamine derivatives **2**–**7**, compound **2** enhanced potency against hERG inhibition, as well as decreased molecular weight and topological polar surface area.

Histone deacetylases (HDACs) are responsible for catalyzing the post translational hydrolysis of acetyl groups present on lysine residues of nuclear histones and play a role in repressing gene expression.[1] The zinc-dependent isoforms are categorized into four classes as follows: class I (HDAC1, 2, 3, 8), class IIa (HDAC4, 5, 7, 9), class IIb (HDAC6, 10) and class IV (HDAC11).[2] Since Yoshida and co-workers reported that trichostatin A (TSA, Figure 1) is a potent HDAC inhibitor,[3] many HDAC inhibitors were discovered as potent compounds for the treatment of cancer and central nervous system (CNS) diseases.[4–6] Recently, we identified a blood-brain-barrier (BBB) permeable class I HDAC inhibitor **1** containing a pyrilamine moiety as a shuttle targeting pyrilamine-sensitive proton-coupled organic cation antiporter for drug delivery to the CNS (Figure 1).[7] Compound **1** showed higher BBB permeability than CI-994 (Figure 1), a centrally active HDAC inhibitor, in rats. However, for further optimization of pyrilamine-based HDAC inhibitors, the structure-activity relationship (SAR) information about benzyl and dimethylamino groups seems necessary. Here we describe SAR study of pyrilamine-based HDAC inhibitors, which ended up with identification of compound **2** (Figure 1).

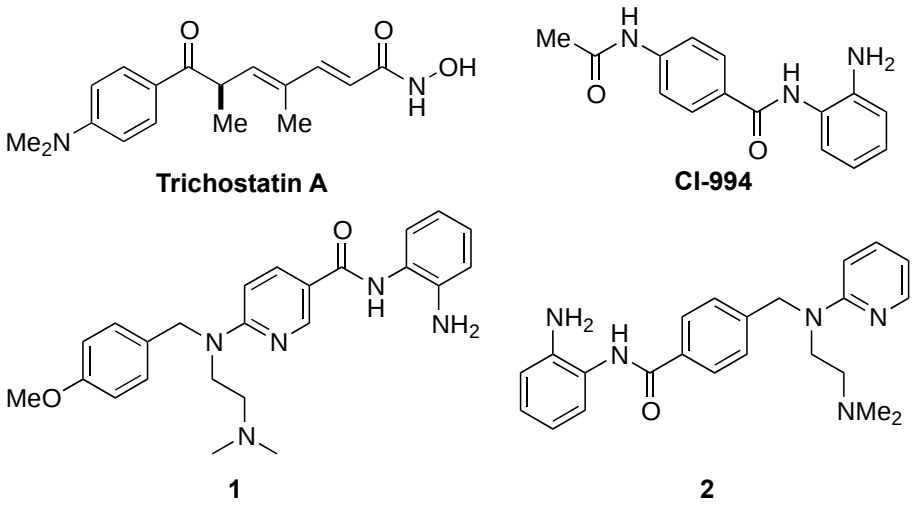


Figure 1. Chemical structures of HDAC inhibitors

Our lead optimization program of BBB permeable HDAC inhibitor **1** focused on its methoxy and dimethylamino groups based on the following reasons: (i) Demethylation of methoxy group and oxidation of benzene ring of 4-methoxybenzyl group are main pathways in metabolism of pyrilamine (Figure 2).[8] It could be that removing the methoxy group and decreasing electron density of the benzene ring of benzyl moiety are effective strategies to decrease the risk of quick metabolism of compound **1** (Figure 3). (ii) Compound **1** includes another risk to inhibit human Ether-a-go-go Related Gene (hERG) channel because lipophilic and basic compounds are typical hERG inhibitors in general. To mitigate this risk, we adopted the strategy to replace dimethylamino group by pyrrolidinyl group because bulkiness of substituents on the nitrogen atom could disturb binding to hERG channel (Figure 3).

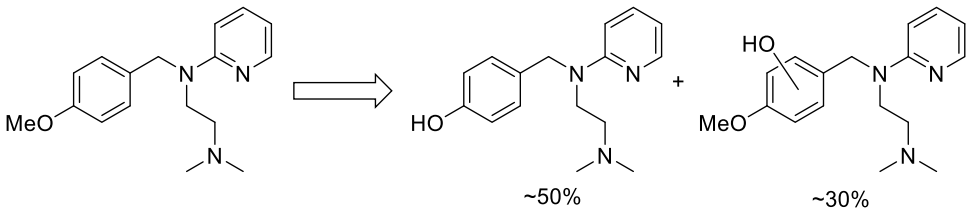


Figure 2. The pathway of metabolism of pyrilamine in human

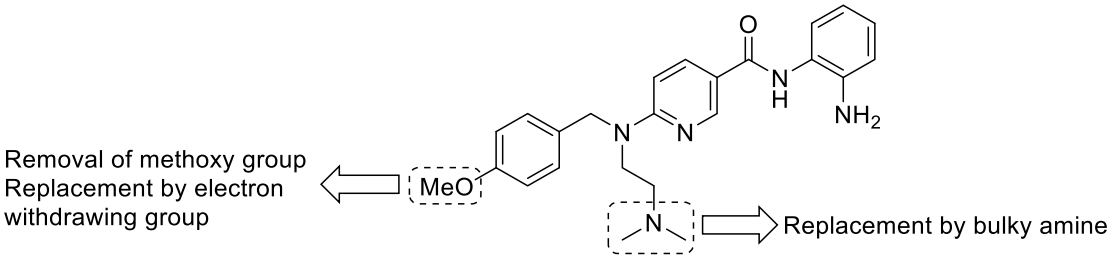
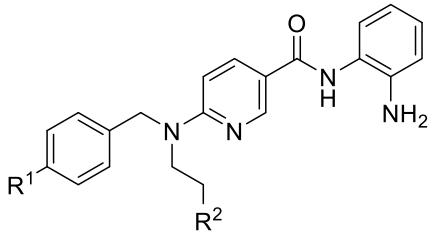


Figure 3. Our strategy to optimize compound **1**

First, we changed the methoxy group of compound **1** to hydrogen or fluorine atom to decrease electron density of benzene ring. The results are summarized in Table 1. Removal of methoxy group maintained HDAC1 inhibitory activity and decreased hERG inhibition, molecular weight and topological polar surface area (tPSA, entry 2). In addition, replacement of the methoxy group by fluorine increased hERG inhibition without potentiating HDAC1 inhibitory activity (entry 3). These results suggest that decrease of lipophilicity is appropriate strategy to decrease hERG inhibition. Second, we synthesized compounds **5**−**7** to decrease hERG inhibition by introducing pyrrolidine as bulkier amine unit. Replacement of the dimethylamino group of compound **1** by the pyrrolidin-1-yl group (**5**) not only increased hERG inhibition, but also decreased HDAC1 inhibitory activity (entry 4). In addition, compounds **6** and **7** did not significantly decrease hERG inhibitory activities compared to compounds **3** and **4** (entries 5 and 6). These results suggest that bulkiness around dimethylamino group does not link to the potency of hERG inhibition. We hypothesized that changing *N*-(2-aminophenyl)benzamide moiety, zinc binding group, from pyridine ring to benzene ring is effective to decrease electron density of benzene ring of benzyl group without increasing molecular weight, tPSA of compound **3** because carbonyl group is known as an electron withdrawing group (Figure 4). Thus, we designed compound **2**. The values of molecular weight (389.5) and tPSA (74.0 $Å^2$) of compound **2** are more suitable than those of compound **1** as CNS drugs. Fortunately, compound **2** showed increased HDAC inhibitory activity with improved hERG inhibition (entry 7).

Table 1. SAR study of pyrilamine derivatives **1**−**7**



3: R^1 = H, R^2 = NMe_2
4: R^1 = F, R^2 = NMe_2
5: R^1 = OMe, R^2 = pyrrolidin-1-yl
6: R^1 = H, R^2 = pyrrolidin-1-yl
7: R^1 = F, R^2 = pyrrolidin-1-yl

Entry	Compound	HDAC1 IC_{50} (μM)	hERG inhibition (%)[a]	Mw	tPSA (Å^2)[b]
1	**1**	4.8 ± 0.4	94	419.5	83.2
2	**3**	5.3 ± 0.3	67	389.5	74.0
3	**4**	6.3 ± 1.0	100	407.5	74.0
4	**5**	13.7 ± 0.3	97	445.6	83.2
5	**6**	5.2 ± 0.4	82	415.5	74.0
6	**7**	4.8 ± 0.9	89	433.5	74.0
7	**2**	2.2 ± 0.3	49	389.5	74.0

[a] The inhibitory activity of each compounds were evaluated at 10 μM.
[b] Calculated by ChemDraw Professional 15.

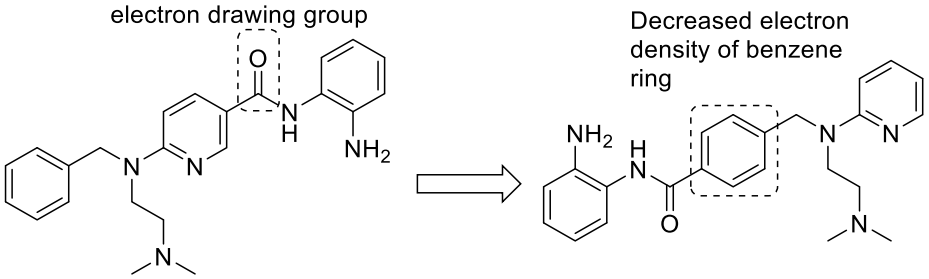


Figure 4. Drug design of compound **2**

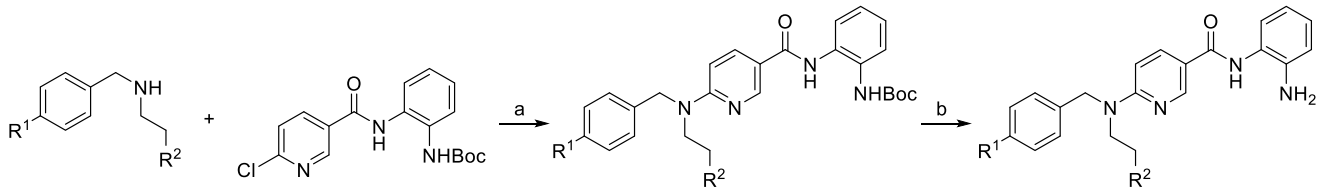
Table 2. Detailed biological activities of compounds **1** and **2**

	IC_{50} (μM)			
compound	HDAC1	HDAC4	HDAC6	hERG IC_{50} (μM)
1	4.8 ± 0.4	53.2 ± 7.2	> 100	3.7 ± 0.8
2	2.2 ± 0.3	12.1 ± 0.5	> 100	10.9 ± 0.8
TSA	0.025 ± 0.0013	0.031 ± 0.0022	0.030 ± 0.0034	NA

NA: Not available.

Finally, we evaluated isozyme selectivity of HDACs and IC_{50} values of hERG inhibition of compounds **1** and **2** (Table 2). Both compounds **1** and **2** showed higher isozyme-selectivity against HDAC1 over HDAC4 and HDAC6 than non-selective HDAC inhibitor TSA. In addition, the balance of IC_{50} values between hERG and HDAC1 suggests that the risk of QT prolongation of compound **2** is less than that of compound **1** (Table 2).

The compounds **3**−**7** were synthesized following Scheme 1. Substitution reactions of compounds **8a**, **8b**, **8c**, **8d** and **8e** with compound **9** afforded compounds **10a**−**10e**. Deprotection of Boc groups of compounds **10a**−**10e** using TFA produced target compounds **3**−**7**.



8a: R^1 = H, R^2 = NMe_2
8b: R^1 = F, R^2 = NMe_2
8c: R^1 = OMe, R^2 = pyroridin-1-yl
8d: R^1 = H, R^2 = pyroridin-1-yl
8e: R^1 = F, R^2 = pyroridin-1-yl

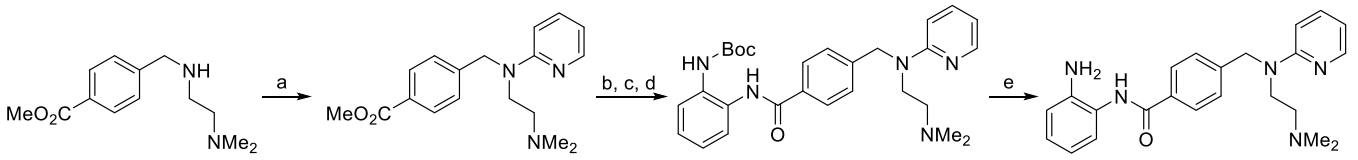
9

10a: R^1 = H, R^2 = NMe_2
10b: R^1 = F, R^2 = NMe_2
10c: R^1 = OMe, R^2 = pyroridin-1-yl
10d: R^1 = H, R^2 = pyroridin-1-yl
10e: R^1 = F, R^2 = pyroridin-1-yl

3: R^1 = H, R^2 = NMe_2
4: R^1 = F, R^2 = NMe_2
5: R^1 = OMe, R^2 = pyroridin-1-yl
6: R^1 = H, R^2 = pyroridin-1-yl
7: R^1 = F, R^2 = pyroridin-1-yl

Scheme 1. Regents and conditions: (a) pyridine, DMSO, 110 °C, 4 h, 9−15%: (b) TFA, rt, 2 h, 14−44%

The synthetic route of compound **2** is shown in Scheme 2. Substitution reaction of 2-iodopyridine with compound **11** using CuI afforded compound **12** in 56% yield. Hydrolysis of the methyl ester in **12** followed by protonation and condensation with *N*-Boc-1,2-phenylenediamine using HATU provided compound **13**. We obtained compound **2** through deprotection of compound **13** using TFA.



11 **12** **13** **2**

Scheme 2. Regents and conditions: (a) 2-iodopyridine, 2-acetylcyclohexane, CuI, Cs_2CO_3, DMF, 150 °C, 1 h, 56%: (b) NaOH, MeOH, 100 °C, 0.5 h: (c) 4 M HCl/EtOAc, (d) *N*-Boc-1,2-phenylenediamine, HATU, TEA, DMAP, DMF, rt, 24 h, 50%: (e) TFA, rt, 2 h, 54%

In summary, we successfully removed methoxy group and exchanged zinc binding group from pyridine ring to benzene ring of compound **1** without decreasing HDAC1 inhibitory activity. In addition, compound **2**, having lower molecular weight and tPSA than **1**, was improved against hERG inhibition. These results suggest that compound **2** can be a potent candidate for the treatment of CNS diseases.

EXPERIMENTAL

Melting points were recorded on Yanaco MP-500D and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on JEOL-EX-400 (400 MHz) spectrometer in the stated solvents using tetramethylsilane or residual nondeuterated solvent peak as an internal standard. Chemical shifts (δ) are expressed in parts per million. High resolution MS spectra were recorded on Thermo Fisher Scientific Q Exactive orbitrap LC-MS/MS. Reactions were followed by TLC on silica gel 60 F_{254} (E. Merck) or NH_2-silicagel 60 F_{254} (Wako) using precoated TLC plates. Synthesis using microwave was conducted with Biotage intiator^{+}. Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. All solvents were of the commercially available grade. Reactions requiring anhydrous conditions were performed under argon atmosphere.

***tert*-Butyl {2-[(6-{benzyl[2-(dimethylamino)ethyl]amino}pyridine-3-carbonyl)amino]phenyl}-carbamate (10a)**

A mixture of compound **8a** (0.20 g, 1.2 mmol), compound **9** (0.40 g, 1.2 mmol), pyridine (0.18 mL, 2.3 mmol), and DMSO (0.5 mL) was heated for 4 h at 110 °C in a microwave oven. Then, 10% aq. K_2CO_3 was added, and the mixture was extracted with EtOAc (2 × 30 mL). The combined organic extract was washed with brine (2 × 30 mL), dried over Na_2SO_4, and evaporated. Amino-silica gel column chromatography (hexane/EtOAc = 90/10 to 50/50) of the residue gave compound **10a** (0.070 g, 12%) as a white solid.

mp 160.5−161 °C (crystallized from hexane/EtOH)

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.51 (s, 9H), 2.28 (s, 6H), 2.53 (t, *J* = 7.1 Hz, 2H), 3.73 (t, *J* = 7.1 Hz, 2H), 4.84 (s, 2H), 6.47 (d, *J* = 9.0 Hz, 1H), 6.91 (s, 1H), 7.13−7.23 (m, 4H), 7.27−7.34 (m, 4H), 7.71 (dd, *J* = 1.4, 7.8 Hz, 1H), 7.95 (dd, *J* = 2.4, 9.0 Hz, 1H), 8.78 (d, *J* = 2.4 Hz, 1H), 8.83 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 28.3, 45.8, 46.9, 52.1, 56.7, 81.3, 104.9, 117.8, 124.4, 125.73, 125.77, 125.85, 126.9, 127.2, 128.7, 130.1, 130.9, 136.7, 137.8, 148.5, 154.5, 159.7, 164.6.

HRMS (ESI) *m/z* calcd for: $C_{28}H_{36}N_5O_3$ $(M+H)^{+}$: 490.2818, found: 490.2818.

***tert*-Butyl {2-[(6-{[2-(dimethylamino)ethyl][(4-fluorophenyl)methyl]amino}pyridine-3-carbonyl)-amino]phenyl}carbamate (10b)**

A mixture of compound **8b** (0.23 g, 1.2 mmol), compound **9** (0.40 g, 1.2 mmol), pyridine (0.18 mL, 2.3 mmol), and DMSO (0.5 mL) was heated for 4 h at 110 °C in a microwave oven. Then, 10% aq. K_2CO_3 was added, and the mixture was extracted with EtOAc (2 × 30 mL). The combined organic extract was washed with brine (2 × 30 mL), dried over Na_2SO_4, and evaporated. Amino-silica gel column chromatography (hexane/EtOAc = 90/10 to 50/50) of the residue gave compound **10b** (0.085 g, 15%) as a white solid.

mp 171.5−172 °C (crystallized from hexane/EtOH)

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.51 (s, 9H), 2.28 (s, 6H), 2.50 (t, *J* = 7.1 Hz, 2H), 3.69 (t, *J* = 7.1 Hz, 2H), 4.81 (s, 2H), 6.47 (d, *J* = 8.8 Hz, 1H), 7.00 (t, *J* = 8.6 Hz, 2H), 7.12−7.23 (m, 5H), 7.26−7.32 (m, 1H), 7.67−7.74 (m, 1H), 7.97 (dd, *J* = 2.0, 8.8 Hz, 1H), 8.77 (d, *J* = 2.0 Hz, 1H), 8.94 (brs, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 28.3, 45.8, 46.8, 51.4, 56.6, 81.3, 104.8, 115.5 (J_{CF} = 21.4 Hz), 117.9, 124.4, 125.77, 125.82, 125.89, 128.6 (J_{CF} = 8.2 Hz), 130.0, 130.8, 133.6, 136.7, 148.6, 154.6, 159.6, 162.0 (J_{CF} = 244 Hz), 164.6.

HRMS (ESI) *m/z* calcd for: $C_{28}H_{35}FN_5O_3$ $(M+H)^+$: 508.2724, found: 508.2699.

***tert*-Butyl {2-[(6-{[(4-methoxyphenyl)methyl][2-(pyrrolidin-1-yl)ethyl]amino}pyridine-3-carbonyl)-amino] phenyl}carbamate (10c)**

A mixture of compound **8c** (0.27 g, 1.2 mmol), compound **9** (0.40 g, 1.2 mmol), pyridine (0.18 mL, 2.3 mmol), and DMSO (0.5 mL) was heated for 4 h at 110 °C in a microwave oven. Then, 10% aq. K_2CO_3 was added, and the mixture was extracted with EtOAc (2 × 30 mL). The combined organic extract was washed with brine (2 × 30 mL), dried over Na_2SO_4, and evaporated. Amino-silica gel column chromatography (hexane/EtOAc = 90/10 to 50/50) of the residue gave compound **10c** (0.096 g, 15%) as a colorless amorphous powder.

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.51 (s, 9H), 1.75−1.82 (m, 4H), 2.51−2.58 (m, 4H), 2.68 (t, *J* = 7.3 Hz, 2H), 3.72 (t, *J* = 7.3 Hz, 2H), 3.80 (s, 3H), 4.78 (s, 2H), 6.49 (d, *J* = 9.0 Hz, 1H), 6.82−6.89 (m, 2H), 7.03−7.19 (m, 5H), 7.29 (dd, *J* = 1.6, 8.5 Hz, 1H), 7.66 (d, *J* =7.6 Hz, 1H), 7.95 (dd, *J* = 2.4, 9.0 Hz, 1H), 8.78 (d, *J* = 2.4 Hz, 1H), 8.90 (brs, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 23.4, 28.3, 47.6, 51.3, 53.1, 54.4, 55.2, 81.1, 104.8, 113.9, 117.5, 124.4, 125.66, 125.71, 128.2, 129.8, 130.2, 130.7, 136.6, 148.6, 154.5, 158.7, 159.6, 164.7.

HRMS (ESI) *m/z* calcd for: $C_{31}H_{40}N_5O_4$ $(M+H)^+$: 546.3080, found: 546.3072.

***tert*-Butyl {2-[(6-{benzyl[2-(pyrrolidin-1-yl)ethyl]amino}pyridine-3-carbonyl)amino]phenyl}-carbamate (10d)**

A mixture of compound **8d** (0.24 g, 1.2 mmol), compound **9** (0.40 g, 1.2 mmol), pyridine (0.18 mL, 2.3 mmol), and DMSO (0.5 mL) was heated for 4 h at 110 °C in a microwave oven. Then, 10% aq. K_2CO_3 was added, and the mixture was extracted with EtOAc (2 × 30 mL). The combined organic extract was washed with brine (2 × 30 mL), dried over Na_2SO_4, and evaporated. Amino-silica gel column chromatography (hexane/EtOAc = 90/10 to 50/50) of the residue gave compound **10d** (0.066 g, 11%) as a colorless amorphous powder.

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.50 (s, 9H), 1.80−1.83 (m, 4H), 2.55 (brs, 4H), 2.70 (t, *J* = 7.8 Hz, 2H), 3.70 (t, *J* = 7.8 Hz, 2H), 4.84 (s, 2H), 6.51 (d, *J* = 9.0 Hz, 1H), 6.87−6.91 (m, 1H), 6.99 (t, *J* = 8.8 Hz, 2H), 7.11−7.23 (m, 5H), 7.28 (dd, *J* = 1.7, 7.8 Hz, 1H), 7.71 (dd, *J* = 1.4, 7.9 Hz, 1H), 7.97 (dd, *J* = 2.4, 8.9 Hz, 1H), 8.77 (d, *J* = 2.4 Hz, 1H), 8.86 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 23.5, 28.3, 47.8, 52.1, 53.2,

54.4, 81.3, 104.9, 117.8, 124.4, 125.73, 125.77, 125.82, 127.0, 127.2, 128.6, 130.1, 130.9, 136.7, 137.9, 148.6, 154.5, 159.7, 164.6.

HRMS (ESI) *m/z* calcd for: $C_{30}H_{38}N_5O_3$ $(M+H)^+$: 516.2975, found: 516.2950.

***tert*-Butyl{2-[(6-{[(4-fluorophenyl)methyl][2-(pyrrolidin-1-yl)ethyl]amino}pyridine-3-carbonyl)-amino]phenyl}carbamate (10e)**

A mixture of compound **8e** (0.26 g, 1.2 mmol), compound **9** (0.40 g, 1.2 mmol), pyridine (0.18 mL, 2.3 mmol), and DMSO (0.5 mL) was heated for 4 h at 110 °C in a microwave oven. Then, 10% aq. K_2CO_3 was added, and the mixture was extracted with EtOAc (2 × 30 mL). The combined organic extract was washed with brine (2 × 30 mL), dried over Na_2SO_4, and evaporated. Amino-silica gel column chromatography (hexane/EtOAc = 90/10 to 0/100) of the residue gave compound **10e** (0.055 g, 9%) as a white solid.

mp 112.5−113 °C (crystallized from hexane/EtOH)

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.50 (s, 9H), 1.77−1.84 (m, 4H), 2.52−2.58 (m, 4H), 2.70 (t, *J* = 7.8 Hz, 2H), 3.71 (t, *J* = 7.8 Hz, 2H), 4.83 (brs, 2H), 6.50 (d, *J* = 9.0 Hz, 1H), 6.87 (s, 1H), 7.00 (t, *J* = 8.5 Hz, 2H), 7.11–7.24 (m, 4H), 7.29 (dd, *J* = 1.8, 7.8 Hz, 1H), 7.71 (dd, *J* = 1.8, 7.8 Hz, 1H), 7.97 (dd, *J* = 2.4, 9.0 Hz, 1H), 8.77 (d, *J* = 2.4 Hz, 1H), 8.86 (brs, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 23.5, 28.3, 47.8, 51.3, 53.1, 54.4, 81.3, 104.8, 115.4 (J_{CF} = 21.4 Hz), 117.9, 124.4, 125.73, 125.76, 125.9, 128.7 (J_{CF} = 8.2 Hz), 130.0, 130.9, 133.7, 136.7, 148.5, 154.5, 159.5, 162.0 (J_{CF} = 244 Hz), 164.5.

HRMS (ESI) *m/z* calcd for: $C_{30}H_{37}FN_5O_3$ $(M+H)^+$: 534.2880, found: 534.2855.

***N*-(2-Aminophenyl)-6-{benzyl[2-(dimethylamino)ethyl]amino}pyridine-3-carboxamide (3)**

A mixture of compound **10a** (0.063 g, 0.13 mmol) and TFA (5 mL) was stirred for 2 h at rt, and then poured into 10% aq. K_2CO_3 (30 mL). The mixture was extracted with EtOAc (2 × 20 mL). The combined organic extract was washed with brine (2 × 30 mL), dried over Na_2SO_4, and evaporated to give a colorless oil. Amino-silica gel column chromatography (hexane/EtOAc = 90/10 to 0/100) of the residue gave compound **3** (0.022 g, 44%) as a colorless amorphous powder.

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.28 (s, 6H), 2.53 (t, *J* = 7.1 Hz, 2H), 3.73 (t, *J* = 7.1 Hz, 2H), 3.86 (s, 2H), 4.84 (s, 2H), 6.50 (d, *J* = 9.0 Hz, 1H), 6.80–6.86 (m, 2H), 7.07 (t, *J* = 7.6 Hz, 1H), 7.21 (d, *J* = 7.3 Hz, 2H), 7.28–7.34 (m, 4H), 7.69 (s, 1H), 7.92 (dd, *J* = 2.0, 9.0 Hz, 1H), 8.73 (d, *J* = 2.0 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 45.8, 46.9, 52.2, 56.7, 105.2, 117.7, 118.3, 119.8, 124.8, 125.2, 126.8, 127.0, 127.2, 128.7, 136.9, 137.8, 140.7, 148.1, 159.8, 164.6.

HRMS (ESI) *m/z* calcd for: $C_{23}H_{28}N_5O$ $(M+H)^+$: 390.2294, found: 390.2288.

***N*-(2-Aminophenyl)-6-{[2-(dimethylamino)ethyl][(4-fluorophenyl)methyl]amino}pyridine-3-carboxamide (4)**

A mixture of compound **10b** (0.073 g, 0.14 mmol) and TFA (5 mL) was stirred for 2 h at rt, and then poured into 10% aq. K_2CO_3 (30 mL). The mixture was extracted with EtOAc (2 × 20 mL). The combined organic extract was washed with brine (2 × 30 mL), dried over Na_2SO_4, and evaporated to give a colorless oil. Amino-silica gel column chromatography (hexane/EtOAc = 90/10 to 0/100) of the residue gave compound **4** (0.014 g, 24%) as a colorless amorphous powder.

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.28 (s, 6H), 2.51 (t, J = 7.1 Hz, 2H), 3.69 (t, J = 7.1 Hz, 2H), 3.88 (brs, 2H) 4.82 (s, 2H), 6.50 (t, J = 9.0 Hz, 1H), 6.81–6.87 (m, 2H), 7.00 (t, J = 8.8 Hz, 2H), 7.08 (dt, J = 1.2, 7.6 Hz, 1H), 7.20 (dd, J = 5.4, 8.2 Hz, 2H) 7.30 (d, J = 7.6 Hz, 1H), 7.67 (s, 1H), 7.94 (dd, J = 2.4, 9.0 Hz, 1H), 8.72 (d, J = 2.4 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 45.8, 46.9, 51.4, 56.6, 105.1, 115.5 (J_{CF} = 21.4 Hz), 117.9, 118.3, 119.8, 124.8, 125.2, 127.0, 128.5 (J_{CF} = 7.6 Hz), 133.6, 137.0, 140.8, 148.2, 159.6, 162.1 (J_{CF} = 244 Hz), 164.6.

HRMS (ESI) *m/z* calcd for: $C_{23}H_{27}FN_5O$ $(M+H)^+$: 408.2200, found: 408.2194.

***N*-(2-Aminophenyl)-6-{[(4-methoxyphenyl)methyl][2-(pyrrolidin-1-yl)ethyl]amino}pyridine-3-carboxamide (5)**

A mixture of compound **10c** (0.075 g, 0.14 mmol) and TFA (5 mL) was stirred for 2 h at rt, and then poured into 10% aq. K_2CO_3 (30 mL). The mixture was extracted with EtOAc (2 × 20 mL). The combined organic extract was washed with brine (2 × 30 mL), dried over Na_2SO_4, and evaporated to give a colorless oil. Amino-silica gel column chromatography (hexane/EtOAc = 90/10 to 0/100) of the residue gave compound **5** (0.016 g, 26%) as a colorless amorphous powder.

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.76–1.82 (m, 4H), 2.53–2.59 (m, 4H), 2.70 (t, J = 7.3 Hz, 2H), 3.72 (t, J = 7.3 Hz, 2H), 3.79 (s, 3H), 3.87 (brs, 2H), 4.79 (s, 2H), 6.53 (d, J = 9.0 Hz, 1H), 6.84–6.86 (m, 4H), 7.05–7.10 (m, 1H), 7.15 (d, J = 8.6 Hz, 2H), 7.28–7.33 (m, 1H), 7.65 (s, 1H), 7.93 (dd, J = 2.2, 9.0 Hz, 1H), 8.72 (d, J = 2.2 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 23.5, 47.8, 51.5, 53.2, 54.4, 55.3, 105.2, 114.1,117.5, 118.4, 119.8, 124.8, 125.2, 127.0, 128.2, 129.8, 136.8, 140.7, 148.1, 158.9, 159.7, 164.6.

HRMS (ESI) *m/z* calcd for: $C_{26}H_{32}N_5O_2$ $(M+H)^+$: 446.2556, found: 446.2553.

***N*-(2-Aminophenyl)-6-{benzyl[2-(pyrrolidin-1-yl)ethyl]amino}pyridine-3-carboxamide (6)**

A mixture of compound **10d** (0.057 g, 0.11 mmol) and TFA (5 mL) was stirred for 2 h at rt, and then poured into 10% aq. K_2CO_3 (30 mL). The mixture was extracted with EtOAc (2 × 20 mL). The combined organic extract was washed with brine (2 × 30 mL), dried over Na_2SO_4, and evaporated to give a colorless oil. Amino-silica gel column chromatography (hexane/EtOAc = 90/10 to 0/100) of the residue gave compound **6** (0.010 g, 22%) as a colorless amorphous powder.

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.76–1.82 (m, 4H), 2.53–2.61 (m, 4H), 2.72 (t, *J* = 7.3 Hz, 2H), 3.76 (t, *J* = 7.3 Hz, 2H), 3.88 (brs, 2H), 4.86 (s, 2H), 6.54 (d, *J* = 9.0 Hz, 1H), 6.82–6.88 (m, 2H), 7.06–7.13 (m, 1H), 7.21–7.23 (m, 2H), 7.31–7.33 (m, 4H), 7.62 (brs, 1H), 7.94 (dd, *J* = 2.2, 9.0 Hz, 1H), 8.72 (d, *J* = 2.2 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 23.5, 47.9, 52.1, 53.2, 54.4, 105.2, 117.8, 118.4, 119.8, 124.8, 125.1, 126.9, 127.0, 127.2, 128.7, 136.9, 137.8, 140.7, 148.1, 159.8.

HRMS (ESI) *m/z* calcd for: $C_{25}H_{30}N_5O$ $(M+H)^+$: 416.2450, found: 416.2446.

***N*-(2-Aminophenyl)-6-{[(4-fluorophenyl)methyl][2-(pyrrolidin-1-yl)ethyl]amino}pyridine-3-carboxamide (7)**

A mixture of compound **10e** (0.043 g, 0.081 mmol) and TFA (5 mL) was stirred for 2 h at rt, and then poured into 10% aq. K_2CO_3 (30 mL). The mixture was extracted with EtOAc (2 × 20 mL). The combined organic extract was washed with brine (2 × 30 mL), dried over Na_2SO_4, and evaporated to give a colorless oil. The oil was suspended in EtOAc (1 mL) and hexane (10 mL) was added. The resulting solid was collected by filtration to give compound **7** (0.005 g, 14%) as a colorless amorphous powder.

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.77–1.84 (m, 4H), 2.53–2.59 (m, 4H), 2.70 (t, *J* = 7.6 Hz, 2H), 3.71 (t, *J* = 7.6 Hz, 2H), 3.88 (s, 2H), 4.83 (s, 2H), 6.52 (d, *J* = 9.0 Hz, 1H), 6.81–6.84 (m, 2H), 6.96–7.03 (m, 2H), 7.07 (dt, *J* = 1.2, 7.6 Hz, 1H), 7.15–7.24 (m, 2H), 7.28 (d, *J* = 7.9 Hz, 1H), 7.72 (brs, 1H), 7.94 (dd, *J* = 2.2, 9.0 Hz, 1H), 8.71 (d, *J* = 2.2 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 23.4, 47.8, 51.3, 53.2, 54.4, 105.0, 115.5 (J_{CF} = 20.6 Hz), 117.8, 118.3, 119.8, 124.8, 125.2, 127.0, 128.6 (J_{CF} = 8.2 Hz), 133.6, 137.0, 140.7, 148.1, 159.6, 162.1 (J_{CF} = 244 Hz), 164.5.

HRMS (ESI) *m/z* calcd for: $C_{25}H_{29}FN_5O$ $(M+H)^+$: 434.2356, found: 434.2350.

Methyl 4-({[2-(dimethylamino)ethyl](pyridin-2-yl)amino}methyl)benzoate (12)

A mixture of compound **11** (0.27 g, 1.2 mmol), 2-iodopyridine (0.13 mL, 1.3 mmol), 2-acetylcyclohexane (0.083 g, 0.64 mmol), CuI (0.078 g, 0.41 mmol), Cs_2CO_3 (0.38 g, 1.3 mmol), DMF (0.6 mL) was heated for 1 h at 150 °C in a microwave oven. Then, 10% aq. K_2CO_3 was added, and the mixture was extracted with EtOAc (2 × 30 mL). The combined organic extract was washed with brine (2 × 30 mL), dried over Na_2SO_4, and evaporated. Amino-silica gel column chromatography (hexane/EtOAc = 90/10 to 50/50) of the residue gave compound **12** (0.11 g, 56%) as a colorless oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.25 (s, 6H), 2.50 (t, *J* = 7.1 Hz, 2H), 3.64 (t, *J* = 7.1 Hz, 2H), 3.89 (s, 3H), 4.85 (s, 2H), 6.42–6.47 (m, 1H), 6.53–6.57 (m, 1H), 7.28–7.32 (m, 2H), 7.37–7.43 (m, 1H), 7.94–7.98 (m, 2H), 8.14–8.17 (m, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 45.9, 46.9, 51.8, 52.0, 56.8, 105.6, 112.1, 126.9, 128.8, 129.8, 137.3, 144.7, 148.1, 157.9, 167.0.

HRMS (ESI) *m/z* calcd for: $C_{18}H_{24}N_3O_2$ $(M+H)^+$: 314.1869, found: 314.1860.

***tert*-Butyl {2-[4-({[2-(dimethylamino)ethyl](pyridin-2-yl)amino}methyl)benzamido]phenyl}-carbamate (13)**

A mixture of compound **12** (0.10 g, 0.32 mmol), NaOH (0.12 g, 3.1 mmol), MeOH (1 mL) was heated for 0.5 h at 100 °C in a microwave oven. Then, 4M HCl/EtOAc (5 mL) was added and evaporated to remove MeOH and EtOAc. After that, the mixture of the residue, *N*-Boc-1,2-phenylenediamine (0.11 g, 0.53 mmol), HATU (0.25 g, 0.65 mmol), DMAP (0.0052 mg, 0.043 mmol), TEA (0.012 mL, 0.87 mmol) and DMF (2 mL) was stirred at rt for 24 h. Then, 10% aq. K_2CO_3 was added, and the mixture was extracted with EtOAc (2 × 20 mL). The combined organic extract was washed with brine (2 × 30 mL), dried over Na_2SO_4, and evaporated. Amino-silica gel column chromatography (hexane/EtOAc = 90/10 to 0/100) of the residue gave compound **13** (0.10 g, 50%) as a white solid.

mp 131.5−132 °C (crystallized from hexane/EtOAc)

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.48 (s, 9H), 2.26 (s, 6H), 2.52 (t, *J* = 7.3 Hz, 2H), 3.64 (t, *J* = 7.3 Hz, 2H), 4.85 (s, 2H), 6.45 (d, *J* = 8.8 Hz, 1H), 6.54–6.59 (m, 1H), 7.05 (brs, 1H), 7.12 (dt, *J* = 1.5, 7.8 Hz, 1H), 7.18 (dt, *J* = 1.5, 7.8 Hz, 1H), 7.22–7.26 (m, 1H), 7.30 (d, *J* = 8.1 Hz, 2H), 7.38–7.44 (m, 1H), 7.72–7.75 (m, 1H), 7.87 (d, *J* = 8.1 Hz, 2H), 8.14–8.18 (m, 1H), 9.13 (brs, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 28.2, 45.8, 47.0, 51.8, 56.7, 81.2, 105.7, 112.1, 124.4, 125.7, 125.8, 125.9, 127.0, 127.6, 130.0, 130.8, 132.9, 137.3, 143.4, 148.1, 154.6, 157.9, 165.5.

MS (ESI) *m/z* calcd for: $C_{28}H_{36}N_5O_3$ $(M+H)^+$: 490.2818, found: 490.2811.

***N*-(2-Aminophenyl)-4-({[2-(dimethylamino)ethyl](pyridin-2-yl)amino}methyl)benzamide (2)**

A mixture of compound **13** (0.090 g, 0.18 mmol) and TFA (5 mL) was stirred for 2 h at rt. Then the reaction mixture was poured into 10% aq. K_2CO_3 (30 mL) dropwise. The mixture was extracted with EtOAc (2 × 20 mL). The combined organic extract was washed with brine (2 × 30 mL), dried over Na_2SO_4, and evaporated to give a colorless oil. The oil was suspended in EtOAc (1 mL) and solidified by addition of hexane (10 mL). The resulting solid was collected by filtration to give compound **2** (0.038 g, 54%) as a colorless amorphous powder.

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.27 (s, 6H), 2.53 (t, *J* = 7.3 Hz, 2H), 3.66 (t, *J* = 7.3 Hz, 2H), 3.86 (brs, 2H), 4.86 (s, 2H), 6.46 (d, *J* = 8.6 Hz, 1H), 6.54–6.66 (m, 1H), 6.78–6.88 (m, 2H), 7.08 (dt, *J* = 1.4, 7.7 Hz, 1H), 7.30 (d, *J* = 8.5 Hz, 1H), 7.34 (d, *J* = 8.1 Hz, 2H), 7.36–7.44 (m, 1H), 7.82 (d, *J* = 8.1 Hz, 2H), 7.86 (brs, 1H), 8.14–8.18 (m, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 45.8, 47.0, 51.8, 56.8, 105.7, 112.2, 118.4, 119.8, 124.6, 125.2, 127.2, 127.3, 127.6, 132.8, 137.4, 140.7, 143.6, 148.1, 157.9, 165.6.

MS (ESI) *m/z* calcd for: $C_{23}H_{28}N_5O$ $(M+H)^+$: 390.2294, found: 390.2284.

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