# Supporting Information

## Design, Synthesis and Blood-Brain Barrier Transport Study of Pyrilamine Derivatives as Histone Deacetylase Inhibitors

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## 1. Figures, tables and schemes

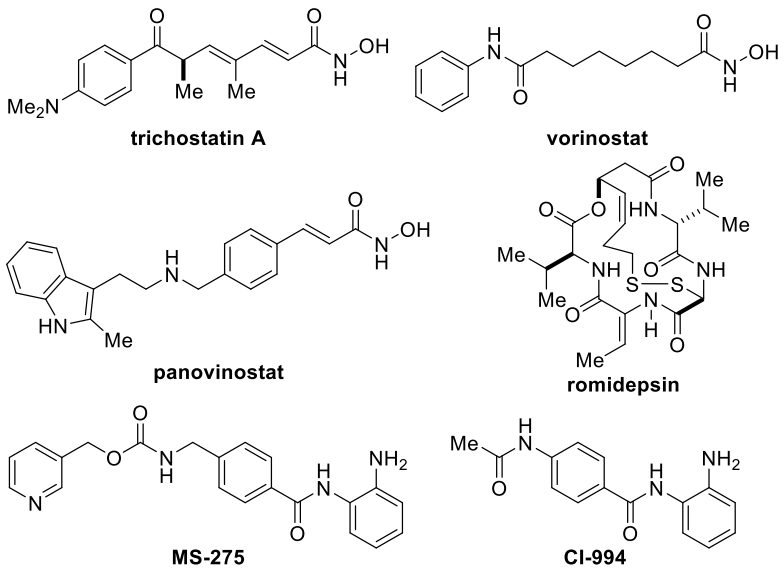



**Figure S1.** Structures of HDAC inhibitors.

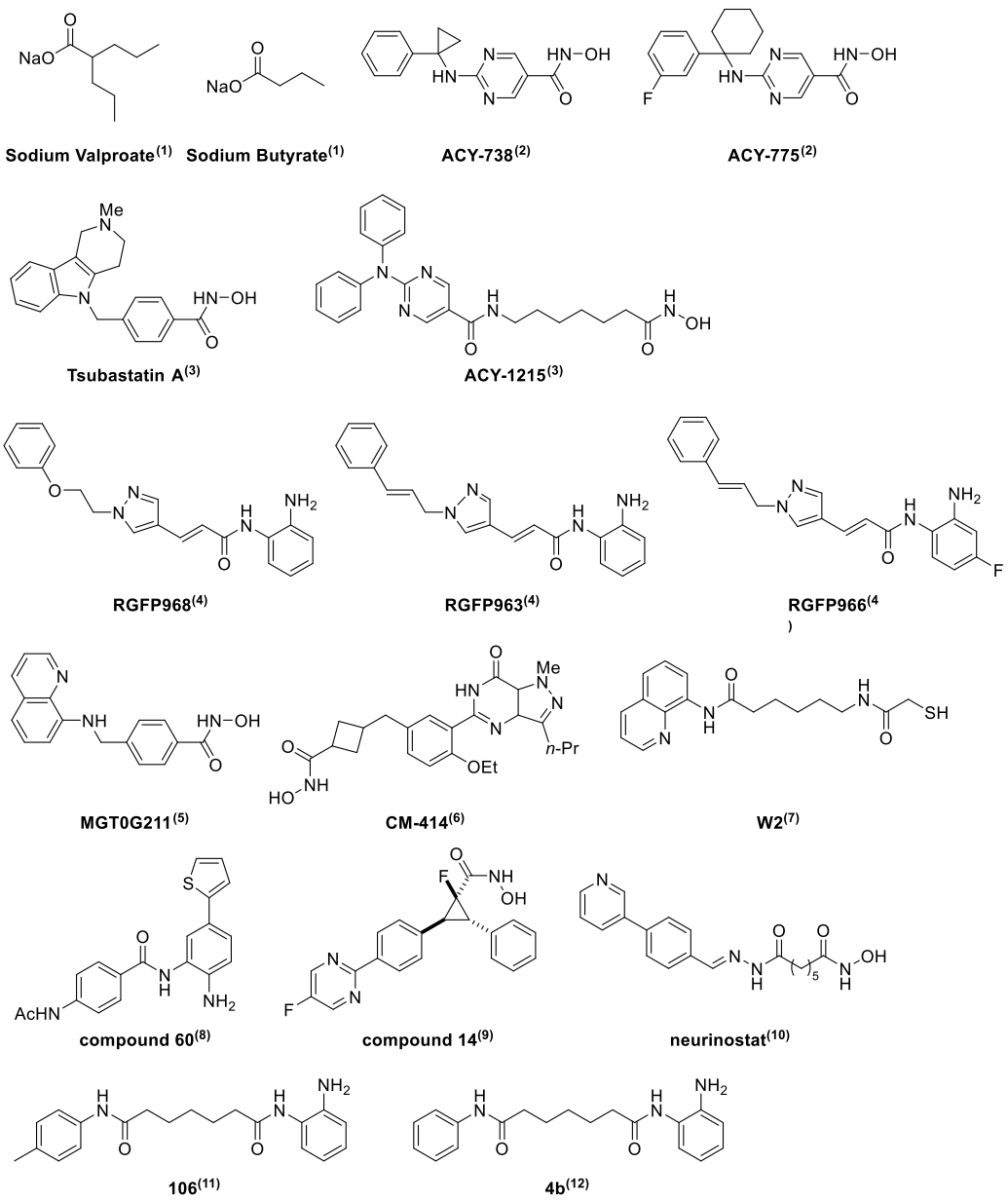



**Figure S2.** Examples of centrally-acting or CNS-penetrant HDAC inhibitors.

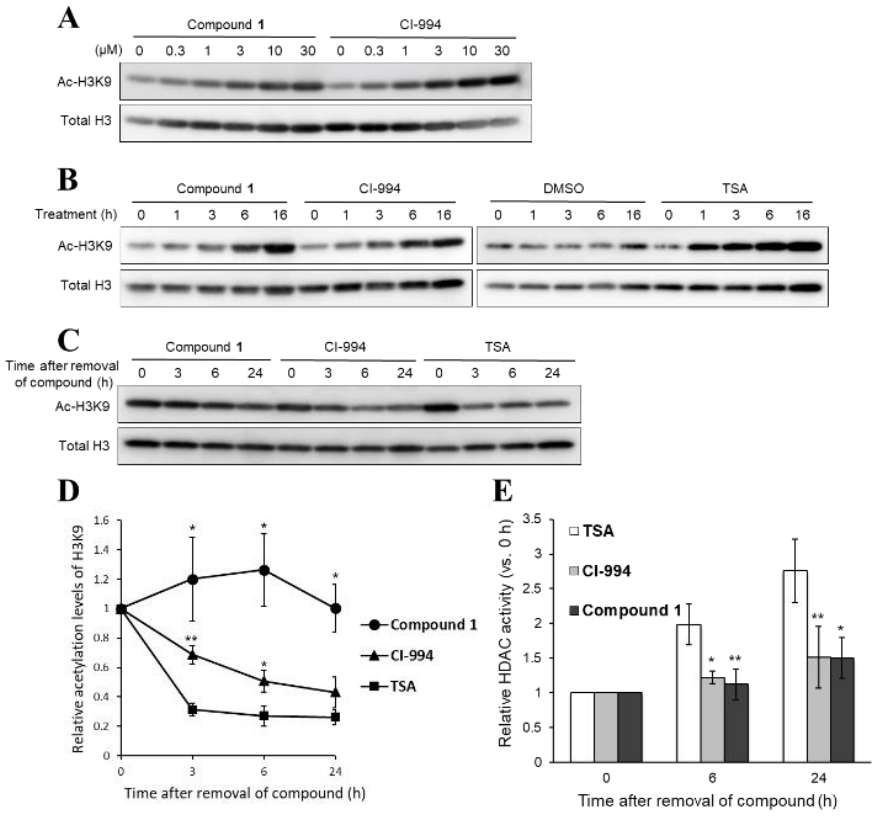



**Figure S3.** Effects of compound **1** and CI-994 on histone acetylation in HeLaS3 cells. (A) HeLaS3 cells were treated with the indicated concentrations of compound **1** or CI-994 for 16 h (A) or with 10 μM compound **1**, 10 μM CI-994, 0.2% DMSO or 1 μM TSA for the indicated time periods (B). The lysates were immunoblotted with the indicated antibodies. (C) Lasting inhibition of HDACs activities by compound **1** in cells. HeLaS3 cells were treated with 10 μM compound **1**, 10 μM CI-994 or 1 μM TSA for 16 h and then washed with DMEM for 3 times at 0 h to eliminate compounds. Cells were collected at indicated time periods and the lysates were immunoblotted with the indicated antibodies. (D) The band intensity was quantified using Image J software. Data are the means ± S.D. of three independent experiments. Student's t-test was applied for statistical analyses. *$p < 0.05$, **$p < 0.01$. Pictures show representative examples. (E) Lasting inhibition of HDAC1 activity by compound **1** *in vitro*. Flag-tagged HDAC1 proteins are incubated with 100 μM compound **1**, 100 μM CI-994 or 1 μM TSA for 1 h and then compounds were washout at 0 h. HDAC activity was measured at indicated time periods using the fluorogenic assay. Data are the means ± S.D. of three independent experiments. Student's t-test was applied for statistical analyses. *$p < 0.05$, **$p < 0.01$.

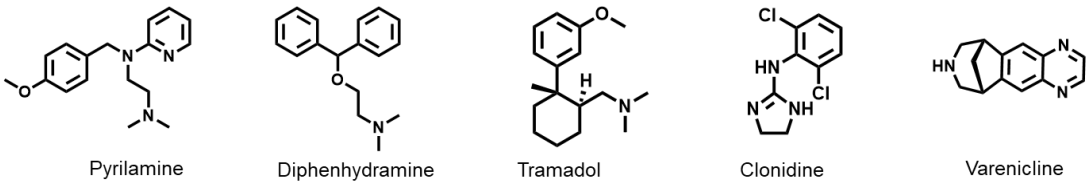



**Figure S4.** Structures of substrates of PYSOCA.

**Table S1.** Inhibitory activity of compound **1** against HDAC1–11.

| | HDAC inhibition at 30 μM (%) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | HDAC1 | HDAC2 | HDAC3 | HDAC4 | HDAC5 | HDAC6 | HDAC7 | HDAC8 | HDAC9 | HDAC10 | HDAC11 |
| **1** | 91 | 75 | 44 | 2 | 3 | 7 | 4 | 1 | 2 | 40 | 3 |
| SAHA[a] | 95 | 87 | 94 | NT | NT | 99 | NT | NT | NT | 97 | NT |
| TSA[b] | NT | NT | NT | 71 | 65 | NT | 85 | 93 | 71 | NT | 54 |

NT: Not tested.

The % inhibition values at 30 μM were determined in a fluorescence assay measuring acetylation of a synthetic substrate.

[a] Inhibitory activity was evaluated at 1 μM.

[b] Inhibitory activity was evaluated at 10 μM.

**Table S2.** $IC_{50}$ values of compound **1** against HDAC1, 2, 3 and 10.

| | HDAC $IC_{50}$ (μM) | | | |
|---|---|---|---|---|
| entry | HDAC1 | HDAC2 | HDAC3 | HDAC10 |
| **1** | 1.5 ± 0.026 | 5.5 ± 0.026 | 12.0 ± 0.38 | 15.5 ± 0.57 |
| SAHA | 0.04 ± 0.021 | 0.084 ± 0.016 | NT | NT |
| TSA | NT | NT | 0.0027 ± 0.000066 | 0.0027 ± 0.000062 |

NT: Not tested.

The $IC_{50}$ values were determined in a fluorescence assay measuring acetylation of a synthetic substrate.

**Table S3.** Effect of various inhibitors on the uptake of compound **1** and CI-994 by hCMEC/D3 cells.

| Inhibitor | Compound **1** | CI-994 |
|---|---|---|
| | Percentage of control | |
| Control | 100 ± 10 | 100 ± 6 |
| TEA | 84.7 ± 13.5 | 116 ± 8 |
| $MPP^+$ | 90.1 ± 13.2 | 125 ± 6 |
| L-Carnitine | 81.71 ± 5.08 | 111 ± 12 |
| Choline | 97.6 ± 0.95 | 156 ± 15 |
| Pyrilamine | 9.21 ± 3.57* | 120 ± 23 |
| Diphenhydramine | 7.88 ± 1.02* | 104 ± 5 |
| Memantine | 10.4 ± 2.2* | 80.2 ± 6.0 |
| Tramadol | 31.5 ± 5.5* | 126 ± 20 |
| Clonidine | 16.9 ± 2.1* | 113 ± 6 |
| Varenicline | 26.5 ± 3.3* | 97.6 ± 12.0 |
| Control (1% DMSO) | 100 ± 3 | 100 ± 17 |
| Decynium-22 (1% DMSO) | 117 ± 25 | 75.4 ± 9.8 |

Uptake of compound **1** by hCMEC/D3 cells was measured at 37 °C for 2 min in the absence (control) or presence of the indicated inhibitors at the concentration of 1 mM, except for decynium-22 (100 μM). Each value represents the mean ± SEM (n = 3–7). *$p$ < 0.01, significantly different from control.

**Table S4.** The $IC_{50}$ values of butyrate, valproate and CI-994 to HDAC1–3.

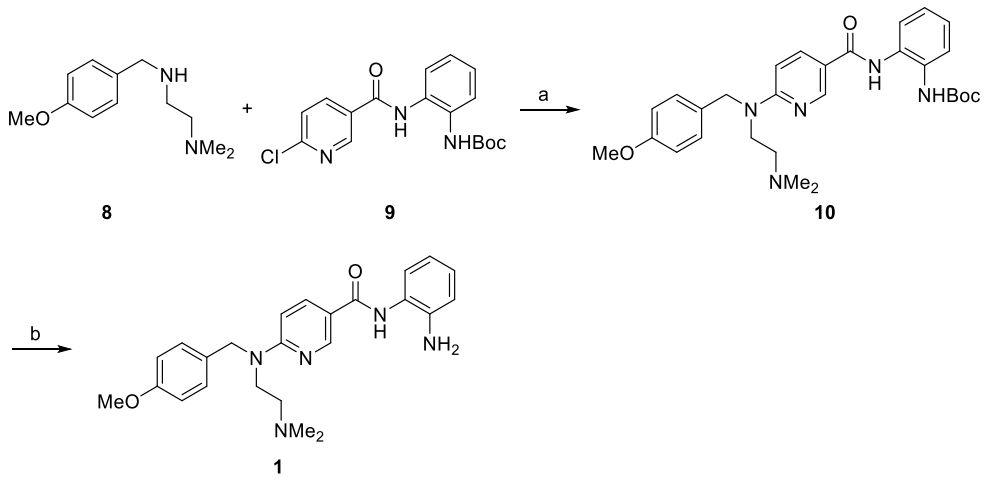
| | $IC_{50}$ (μM) | | |
|---|---|---|---|
| compound | HDAC1 | HDAC2 | HDAC3 |
| Sodium Butyrate[1] | 8.3 | 7 | 4.8 |
| Sodium Valproate[1] | 35.5 | 59.3 | 218.5 |
| CI-994[13] | 0.9 | 0.9 | 1.2 |

**Table S5.** The physicochemical properties of compounds **1**–**7**.

| compound | MW | AlogP[14,a] | logD[a] | tPSA[a] | PKa[a] |
|---|---|---|---|---|---|
| **1** | 419.5 | 3.124 | 1.902 | 83.72 | 8.86 |
| **2** | 454.0 | 3.789 | 2.567 | 83.72 | 8.86 |
| **3** | 498.4 | 3.873 | 2.651 | 83.72 | 8.86 |
| **4** | 545.4 | 3.702 | 2.481 | 83.72 | 8.86 |
| **5** | 501.6 | 4.596 | 3.374 | 111.96 | 8.86 |
| **6** | 485.6 | 4.038 | 2.816 | 96.86 | 8.86 |
| **7** | 495.6 | 4.643 | 3.421 | 83.72 | 8.86 |
| **pyrilamine** | 285.4 | 3.086 | 1.87 | 28.6 | 8.85 |

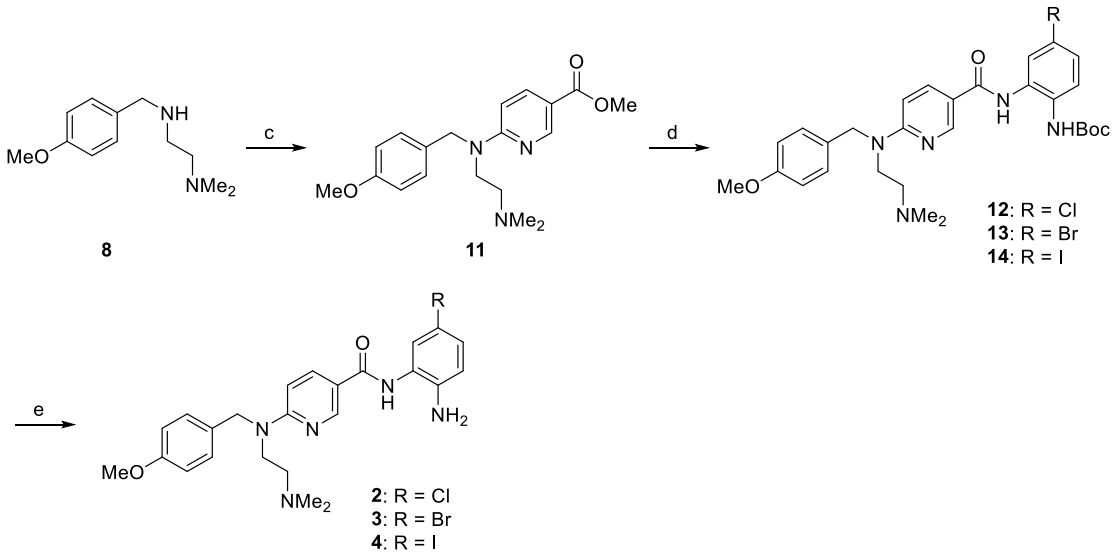
[a] Calculated by Pipeline Pilot.

**Scheme S1.** Synthetic procedures of compound **1**.



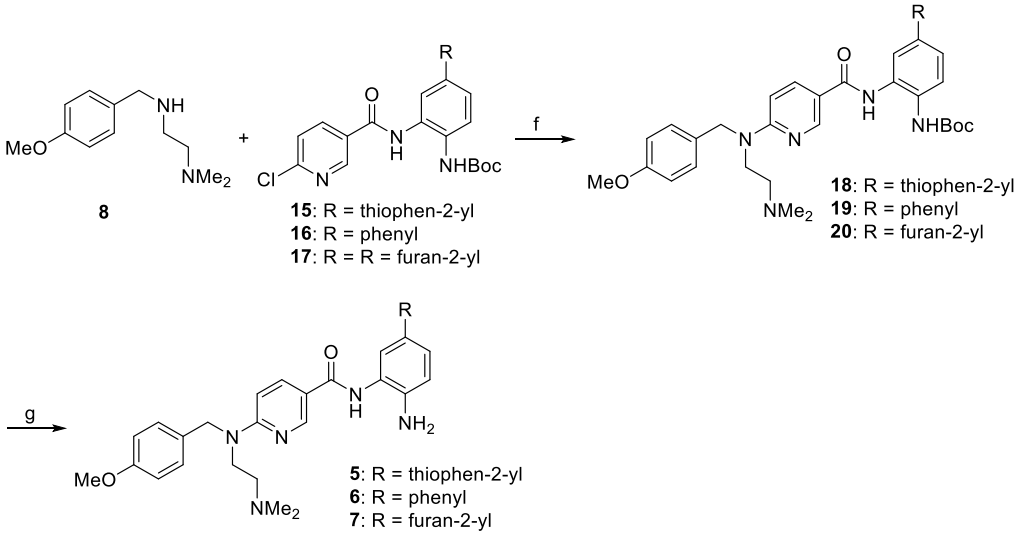

Regents and conditions: (a) pyridine, DMSO, 110 ºC, 4.5 h, microwave, 61%. (b) TFA, 0.5 h, 90%.

**Scheme S2.** Synthetic procedures of compounds **2**–**4**.



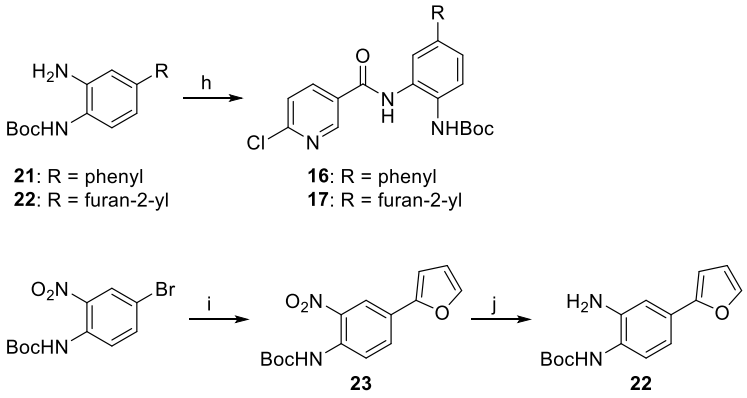

Regents and conditions: (c) methyl 6-chloronicotinate, EtN(*i*-Pr)$_2$, DMSO, 110 ºC, microwave, 23% (d) (i) NaOH, MeOH, 100 ºC, 0.5 h, microwave, then 4N HCl/EtOAc. (ii) *t*-butyl (2-amino-4-chlorophenyl)carbamate, *t*-butyl (2-amino-4-bromophenyl) carbamate, or *t*-butyl (2-amino-4-iodophenyl)carbamate, HATU, DMAP, $Et_3N$, DMF, 4 h, rt. **12**: 26%, **13**: 23%, **14**: 26%. (e) TFA, 0.5 h, **2**: 45%, **3**: 59%, **4**: 30%.

**Scheme S3.** Synthetic procedures of compounds **5**–**7**.




Regents and conditions: (f) **15**, **16** or **17**, $Et_3N$, DMSO, 110 ºC, 5 h, microwave, **18**: 39%, **19**: 26%, **20**: 39%. (g) TFA, 0.5 h, **5**: 81%, **6**: 33%, **7**: 20%.

**Scheme S4.** Synthetic procedures of compounds **16** and **17**.




Regents and conditions: (h) 6-chloronicotinoyl chloride, $Et_3N$, THF, 1 h rt, **16**: 76%, **17**: 84% (i) thiophen-2-yl boronic acid, $K_2CO_3$, $Pd(PPh_3)_4$, $H_2O$/DME (1/1), 130 ºC, 1 h, microwave, 63%. (j) $H_2$, Pd/C, MeOH, rt, 79%

## 2. Experimental Section

## HDAC inhibitory activity assay

HDAC-inhibitory activity assay of CI-994 and compounds **1**–**7**

Enzymatic activities of HDACs including HDAC1 and HDAC6 were performed by a fluorogenic assay as described previously[15]. Briefly, HDAC proteins purified from 293T cells or HeLa cells were incubated with a fluorescent peptide (2 mM Ac-KGLGK(Ac)-MCA) in 20 μL of HDAC assay buffer (20 mM Tris–HCl (pH 8.0), 150 mM NaCl, and 10% glycerol) at 37 °C for 30 min. The reaction was stopped by the addition of 20 μL of trypsin (20 mg/mL) and incubated at 37 °C for 15 min. The released aminomethylcoumarin (AMC) was measured using a fluorescence plate reader (Molecular Devices, San Jose, CA).

Statistical analysis

The parameters were represented as the mean ± S.E. Significance of differences among means of two unpaired groups and more than two groups was determined by Student's t-test and Oneway ANOVA followed by Dunnett's test, respectively.

**Presice HDAC-inhibitory activity assay of compound 1**

$IC_{50}$ and % inhibition at 30 μM measurements were conducted by BPS Biosciences with an established fluorescence assay, using proprietary class-specific substrates and recombinant enzymes.

Assay Conditions

All of the compounds are dissolved in DMSO. The serial dilution of the compounds was first performed in 100% DMSO with the highest concentration at 3 mM. Each intermediate compound dilution (in 100% DMSO) will then get directly diluted 10x fold into assay buffer for an intermediate dilution of 10% DMSO in HDAC assay buffer and 5 μL of the dilution was added to a 50 μL reaction so that the final concentration of DMSO is 1% in all of reactions.

The enzymatic reactions for the HDAC enzymes were conducted in duplicate at 37 ºC for 30 minutes in a 50 μL mixture containing HDAC assay buffer, 5 μg BSA, an HDAC substrate, a HDAC enzyme and a test compound.

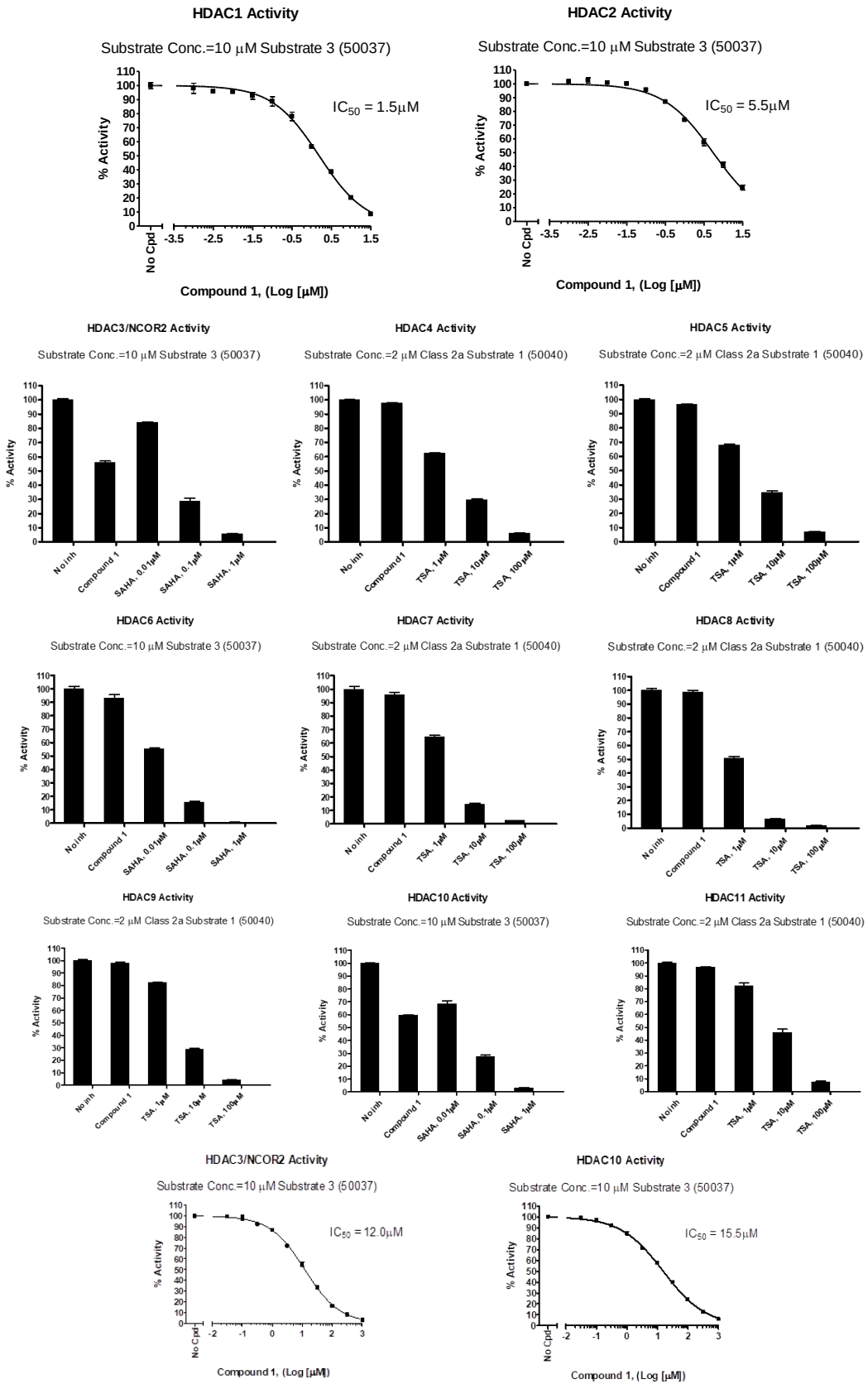
After enzymatic reactions, 50 μL of 2 × HDAC Developer was added to each well for the HDAC enzymes and the plate was incubated at room temperature for an additional 15 minutes.

Fluorescence intensity was measured at an excitation of 360 nm and an emission of 460 nm using a Tecan Infinite M1000 microplate reader.

Data Analysis

HDAC activity assays were performed in duplicates at each concentration. The fluorescent intensity data were analyzed using the computer software, Graphpad Prism. In the absence of the compound, the fluorescent intensity ($F_t$) in each data set was defined as 100% activity. In the absence of HDAC, the fluorescent intensity ($F_b$) in each data set was defined as 0% activity. The percent activity in the presence of each compound was calculated according to the following equation: %activity = $(F-F_b)/(F_t-F_b)$, where F= the fluorescent intensity in the presence of the compound.

The values of % activity versus a series of compound concentrations were then plotted using non-linear regression analysis of Sigmoidal dose-response curve generated with the equation $Y = B + (T-B)/1+10^{((LogEC50-X)\times Hill\ Slope)}$, where Y = percent activity, B = minimum percent activity, T = maximum percent activity, X = logarithm of compound and Hill Slope = slope factor or Hill coefficient. The $IC_{50}$ value was determined by the concentration causing a half-maximal percent activity.


HDAC1 Activity
Substrate Conc.=10 μM Substrate 3 (50037)
IC50 = 1.5μM
% Activity
No Cpd
Compound 1, (Log [μM])
HDAC2 Activity
Substrate Conc.=10 μM Substrate 3 (50037)
IC50 = 5.5μM
% Activity
No Cpd
Compound 1, (Log [μM])
HDAC3/NCOR2 Activity
Substrate Conc.=10 μM Substrate 3 (50037)
% Activity
No Inh
Compound 1
SAHA, 0.01μM
SAHA, 0.1μM
SAHA, 1μM
HDAC4 Activity
Substrate Conc.=2 μM Class 2a Substrate 1 (50040)
% Activity
No Inh
Compound 1
TSA, 1μM
TSA, 10μM
TSA, 100μM
HDAC5 Activity
Substrate Conc.=2 μM Class 2a Substrate 1 (50040)
% Activity
No Inh
Compound 1
TSA, 1μM
TSA, 10μM
TSA, 100μM
HDAC6 Activity
Substrate Conc.=10 μM Substrate 3 (50037)
% Activity
No Inh
Compound 1
SAHA, 0.01μM
SAHA, 0.1μM
SAHA, 1μM
HDAC7 Activity
Substrate Conc.=2 μM Class 2a Substrate 1 (50040)
% Activity
No Inh
Compound 1
TSA, 1μM
TSA, 10μM
TSA, 100μM
HDAC8 Activity
Substrate Conc.=2 μM Class 2a Substrate 1 (50040)
% Activity
No Inh
Compound 1
TSA, 1μM
TSA, 10μM
TSA, 100μM
HDAC9 Activity
Substrate Conc.=2 μM Class 2a Substrate 1 (50040)
% Activity
No Inh
Compound 1
TSA, 1μM
TSA, 10μM
TSA, 100μM
HDAC10 Activity
Substrate Conc.=10 μM Substrate 3 (50037)
% Activity
No Inh
Compound 1
SAHA, 0.01μM
SAHA, 0.1μM
SAHA, 1μM
HDAC11 Activity
Substrate Conc.=2 μM Class 2a Substrate 1 (50040)
% Activity
No Inh
Compound 1
TSA, 1μM
TSA, 10μM
TSA, 100μM
HDAC3/NCOR2 Activity
Substrate Conc.=10 μM Substrate 3 (50037)
IC50 = 12.0μM
% Activity
No Cpd
Compound 1, (Log [μM])
HDAC10 Activity
Substrate Conc.=10 μM Substrate 3 (50037)
IC50 = 15.5μM
% Activity
No Cpd
Compound 1, (Log [μM])

**Assay of effects of compound 1 and CI-994 on histone acetylation in HeLaS3 cells**

Cell culture

HeLaS3 cells were cultured in DMEM containing 10% heat inactivated fetal bovine serum and antibiotics at 37 ºC, 5% $CO_2$ in a humidified incubator.

Immunoblotting[16]

Immunoblotting was performed as described previously (Ito et al., 2015). An anti–histone H3K9Ac antibody (Millipore, Billerica, MA, USA) and an anti–histone H3 antibody (Abcam, Cambridge, UK) were used as primary antibodies.

Measurement of *in vitro* HDAC1 activity after removal of inhibitors[17]

Purified flag-tagged HDAC1 proteins and each compound were incubated in 50 μL of HDAC assay buffer (20 mM Tris-HCl [pH 8.0], 150 mM NaCl, 10% glycerol) at 4 ºC for 1 h. After addition of 20 μL of anti-flag M2 affinity gel (Sigma-Aldrich, St. Louis, MO, USA) and 100 μL of HDAC assay buffer including 0.1% Triton-X, the mixtures were rotated at 4 ºC for 30 minutes. After washing with 1 mL of HDAC assay buffer including 0.1% Triton-X for 5 times, the immune complexes were suspended in HDAC buffer and incubated at 4 ºC for 0, 6, 24 h before measurement of the HDAC1 enzymatic activity. In vitro HDAC1 activity was measured using a fluorogenic assay as described previously.

**Uptake study in hCMEC/D3 cells**

hCMEC/D3 cells were cultured at 37 °C in EBM-2 medium (Lonza, Basel, Switzerland) as described previously. The cells (25-35 passages) cultured on rat collagen I-coated 24-well plates (BD Biosciences, Franklin Lakes, NJ) were washed with transport buffer (122 mM NaCl, 3.0 mM KCl, 25 mM $NaHCO_3$, 1.2 mM $MgSO_4$, 1.4 mM $CaCl_2$, 10 mM D-glucose, 10 mM HEPES, pH 7.4) and preincubated with the buffer for 20 min at 37 °C or 4 °C. Compound **1** or CI-994 dissolved in the transport buffer was applied to the cells to initiate uptake. After incubation for a designated time, the cells were washed three times with ice-cold buffer, and collected with a scraper in 200 μL of solution containing naloxone as an internal standard. Cellular protein content was measured with a Micro BCA protein assay kit (Thermo Fisher Scientific, Waltham, MA). Uptake of compounds is presented as the cell-to-medium ratio (μL/mg protein) calculated by dividing the uptake amount by the concentration in the incubation buffer. The kinetic parameters for uptake of compound **1** by hCMEC/D3 cells were obtained

from Eqs. 1 and 2 concurrently by using the nonlinear least-squares regression analysis program MULTI. In the uptake of compound compound **1** by hCMEC/D3 cells, the contribution of the non-saturable component was negligible compared to that of the saturable component.

$$V = (V_{max} \times S) / (K_m + S) \quad (1)$$
$$V = (V_{max} \times S) / (K_m \times (1 + I/K_i) + S) \quad (2)$$

where V, S, $V_{max}$, $K_m$, I, and $K_i$ are the uptake rate, drug concentration in the medium, the maximum uptake rate, the Michaelis constant, the inhibitor concentration, and the inhibitory constant, respectively.

To investigated the effect of metabolic energy depletion, the cells were preincubated with glucose-free buffer (122 mM NaCl, 3.0 mM KCl, 25 mM $NaHCO_3$, 1.2 mM $MgSO_4$, 1.4 mM $CaCl_2$, 10 mM 3-*O*-methylglucose, 10 mM HEPES, pH 7.4) containing 0.1% $NaN_3$ for 20 min. The intracellular pH was adjusted by pretreatment and by acute treatment with 30 mM $NH_4Cl$ to produce intracellular acidification and alkalization, respectively.

**In situ brain perfusion study**

Wistar rats (male) were anesthetized by intraperitoneal injection of pentobarbital, and the right carotid artery was catheterized with SP10 polyethylene tubing (Natsume Seisakusyo, Tokyo, Japan) filled with sodium heparin (100 IU/mL). Krebs-Henseleit buffer (118 mM NaCl, 4.7 mM KCl, 25 mM $NaHCO_3$, 1.2 mM $KH_2PO_4$, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 10 mM D-glucose, pH 7.4) containing 20 μM **1** or CI-994 was perfused through the catheter at the rate of 4.9 mL/min with an infusion pump (YSP-101; YMC, Kyoto, Japan). Since there was the time lag until the external carotid artery was filled with the perfusate, 5.0 s was routinely subtracted from the gross perfusion time in each experiment. At the end of the uptake period, each rat was decapitated, and the right cerebral hemisphere was collected. Brain samples were weighed, homogenized, and stored at –20 °C until determination of compounds. *In vivo* BBB permeability of compounds was represented as the permeability-surface area product ($PS_{BBB}$) calculated from the following equation.

$$PS_{BBB}\ (\mu L/min/g\ brain) = -F_{pf} \times \ln(1-K_{in}/F_{pf})$$

where $F_{pf}$ is the flow rate of the perfusate (7.54 mL/min/g of brain)[18], and $K_{in}$ is a

transfer constant for unidirectional uptake corrected for the remaining compounds in intravascular space. $K_{in}$ was calculated by fitting the following equation.

$$q_{br}/C_{pf} = K_{in} \times T + V_0$$

where $q_{br}$ is the amount of compounds in the brain, $C_{pf}$ is the concentration of compounds in the perfusate, T is the uptake time (min), and $V_0$ is the intravascular volume (0.011 mL/g brain)[19].

**LC-MS/MS analysis**

Cells were deproteinized with acetonitrile followed by filtration using a membrane filter (0.2 μm pore size). Brain samples were subjected to solid-phase extraction using an Oasis MCX cartridge (Waters, Milford, MA). Compound **1** and CI-994 were determined by means of an LC-MS/MS system composed of an Accela HPLC system and TSQ Quantum Ultra mass spectrometer (Thermo Fisher Scientific, Waltham, MA) with an electrospray ionization interface in the positive ion mode. The multiple reaction monitors were set at 420.0 to 375.1 m/z, 270.1 to 162.0 m/z, and 328.1 to 310.2 m/z for compound **1**, CI-994, and naloxone (internal standard, 30 ng/mL), respectively. Chromatographic separation was performed on a Synergi Hydro-RP column (2.0 × 50 mm, 2.5 μm, Phenomenex, Torrance, CA) at a flow rate of 0.4 mL/min and a column temperature of 40 °C. The gradient program was composed of solvent A (ammonium acetate buffer (10 mM, pH 4.0)) and solvent B (methanol) as follows: 0% B for 0-0.5 min, 0-80% B for 0.5-2 min, 80% B for 2-4.5 min, and 0% B for 4.5-6.5 min. Instrument control and data collection were carried out by Xcalibur version 2.1.0 software.

**Statistical analysis**

$V_{max}$, $K_m$, and $K_i$ values are presented as the mean ± S.D. The other values are presented as the mean ± S.E. Significance of differences between means of two unpaired groups and among more than two groups was determined by Student's t-test and one-way ANOVA followed by Dunnett's test, respectively.

## 3. Synthetic procedures

**General.**

All reagents and solvents were purchased from commercial suppliers and used without further purification. Monitoring of reactions was carried out using Merck 60 $F_{254}$ silica gel and Wako $NH_2$ silica gel 60 $F_{254}$ glass-supported TLC plates, and bands were visualized under UV light (254 nm). Nuclear magnetic resonance (NMR) spectra were recorded on a JEOL-EX-400 spectrometer at room temperature, operating at 400 MHz for $^1$H NMR and 100 MHz for $^{13}$C NMR. Chemical shifts were referenced to tetramethylsilane (TMS) in $CDCl_3$ and the residual solvent peak in DMSO-$d_6$ for $^1$H NMR. For $^{13}$C NMR, chemical shifts were referenced to the residual solvent peak in $CDCl_3$ or DMSO-$d_6$. Splitting patterns are designed as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; brs, broad singlet; dd, double doublet; dt, double triplet; and dq, double quartet. ESI mass spectra were measured on a Shimadzu LCMS-IT-TOF and Thermo Fischer Science LTQ Orbitrap Discovery MS equipment. Microwave irradiation for synthesis was conducted using Biotage Initiator$^+$. Purification by flash column chromatography was conducted using a Biotage Isolera. The following abbreviations are used for reagents and solvents: TFA (trifluoroacetic acid), EtOAc (ethyl acetate), AcOH (acetic acid), DMSO (dimethyl sulfoxide), THF (tetrahydrofurane), DME (dimethoxyethane), DMF (*N,N*-dimethylformamide).

***N*$^1$-(4-methoxybenzyl)-*N*$^2$,*N*$^2$-dimethylethane-1,2-diamine (8)**

A mixture of *N',N'*-dimethyl ethane-1,2-diamine (3.9 mL, 36 mmol), 4-methoxybenzaldehyde, (3.6 mL, 30 mmol), $NaBH(OAc)_3$ (9.54 g, 45 mmol), AcOH (2.6 mL, 45 mmol), and $CH_2Cl_2$ (90 mL) was stirred for 4 h at rt. Water (100 mL) was added and $CH_2Cl_2$ was evaporated. After the evaporation, the mixture above was added 10 % $K_2CO_3$ aq. (80 mL) and extracted with EtOAc (2 × 100 mL). Combined organic extracts were washed with brine (2×100 mL), and then dried over $Na_2SO_4$. Concentration followed by amino-silica gel flash column chromatography (hexane/EtOAc = 80/20 to 0/100) gave **2** (3.77 g, 60%) as a colorless oil.

$^1$H-NMR ($CD_3OD$, 400 MHz) δ: 2.20 (s, 6H), 2.45 (t, *J* = 7.1 Hz, 2H), 2.66 (t, *J* = 7.1 Hz, 2H), 3.68 (s, 2H), 3.78 (s, 3H), 6.84 (d, *J* = 2.0, 8.6 Hz, 2H), 7.24 (d, *J* = 2.0, 8.6 Hz, 2H).

HRMS (ESI) *m/z* calcd for: $C_{12}H_{21}N_2O$ $(M+H)^+$: 209.1649, found: 209.1645.

***t*-Butyl (2-(6-chloronicotinamido)phenyl)carbamate (9)**[20]

A mixture of *t*-butyl (2-aminophenyl)carbamate (2.0 g, 9.7 mmol), 6-chloronicotinoyl chloride (2.42 g, 14 mmol), $Et_3N$ (4.0 mL, 29 mmol) and $CH_2Cl_2$ (130 mL) was stirred for 18 h at rt. satd $NaHCO_3$ aq. (100 mL) was added and extracted with EtOAc (2×120 mL). Combined organic extracts were washed with brine (2 × 100 mL), and then dried over $Na_2SO_4$. Concentration followed by silica gel flash column chromatography (hexane/EtOAc = 80/20 to 0/100) gave **3** (2.98 g, 89%) as a white solid.

$^1$H-NMR (DMSO-$d_6$, 400 MHz) δ: 1.50 (s, 9H), 7.12 (t, $J$ = 7.8 Hz, 1H), 7.21 (t, $J$ = 7.8 Hz, 1H), 7.47 (d, $J$ = 7.8 Hz, 1H), 7.61 (d, $J$ = 7.8 Hz, 1H), 7.71 (d, $J$ = 8.4 Hz, 1H), 8.33 (d, $J$ = 8.4 Hz, 1H), 8.68 (s, 1H), 8.95 (s, 1H), 10.0 (s, 1H).

HRMS (ESI) *m/z* calcd for: $C_{17}H_{18}ClN_3O_3$ $(M\text{-}Boc+H)^+$ : 248.0585, found:248.0587.

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

A mixture of **8** (0.83 g, 4.0 mmol), **9** (0.69 g, 2.0 mmol), pyridine (0.24 mL, 3.0 mmol), and DMSO (1.0 mL) was heated for 4.5 h at 110 °C in a microwave oven. Then, 10% $K_2CO_3$ aq. 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 flash column chromatography (hexane/EtOAc = 80/20 to 0/100) of the residue gave **10** (0.51 g, 61%) as a yellow solid.

$^1$H-NMR ($CDCl_3$, 400 MHz) δ: 1.51 (s, 9H), 2.28 (s, 6H), 2.51 (t, $J$ = 7.2 Hz, 2H), 3.71 (t, $J$ = 7.2 Hz, 2H), 3.79 (s, 3H), 4.77 (s, 2H), 6.49 (d, $J$ = 9.0 Hz, 1H), 6.85 (m, 3H), 7.1-7.25 (m, 4H), 7.30 (m, 1H), 7.71 (d, $J$ = 7.6 Hz, 1H), 7.96 (dd, $J$ = 2.4, 9.0 Hz, 1H), 8.77 (d, $J$ = 2.4 Hz, 2H).

$^{13}$C-NMR ($CDCl_3$, 100 MHz) δ: 28.3, 45.8, 46.7, 51.5, 55.3, 56.6, 81.3, 104.9, 114.1, 117.7, 124.4, 125.8, 125.9, 128.2, 129.8, 130.1, 131.0, 136.6, 148.5, 154.6, 158.9, 159.8, 164.6.

HRMS (ESI) *m/z* calcd for: $C_{29}H_{38}N_5O_4$ $(M+H)^+$: 520.2919, found: 520.2921

***N*-(2-Aminophenyl)-6-((2-(dimethylamino)ethyl)(4-methoxybenzyl)amino) nicotinamide (1)**

A mixture of **10** (0.18 g, 0.35 mmol) and TFA (1.0 mL) was stirred for 0.5 h at rt, and then poured into 10% $K_2CO_3$ aq. (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 (1.0 mL) and hexane (10 mL) was added. The resulting solid was collected by filtration to

give **1** (0.093 g, 90%) as a yellow solid.
$^{1}$H-NMR ($CDCl_3$, 400 MHz) δ: 2.28 (s, 6H), 2.52 (t, *J* = 7.3 Hz, 2H), 3.71 (t, *J* = 7.3 Hz, 2H), 3.79 (s, 3H), 3.88 (brs, 2H), 4.78 (s, 2H), 6.51 (d, *J* = 9.0 Hz, 1H), 6.8-6.9 (m, 4H), 7.08 (m, 1H), 7.15 (d, *J* = 8.7 Hz, 2H), 7.30 (m, 1H), 7.64 (brs, 1H), 7.93 (dd, *J* = 2.4, 9.0 Hz, 1H), 8.73 (d, *J* = 2.4 Hz, 1H).
$^{13}$C-NMR ($CDCl_3$, 100 MHz) δ: 45.6, 46.7, 51.5, 55.3, 56.6, 105.1, 114.1, 117.6, 118.3, 119.7, 124.8, 125.2, 127.0, 128.1, 129.6, 136.8, 140.7, 148.1, 158.8, 159.7, 164.6.
HRMS (ESI) *m/z* calcd for: $C_{24}H_{30}N_5O_2$ $(M+H)^+$: 420.2394, found: 420.2401

**HCl salt of compound 1**
4N HCl in EtOAc was added to compound **1** (56 mg) and the resulting solid was collected by filtration to give the HCl salt of compound **1** (68 mg, 91%) as a white crystal.

**Methyl 6-{[2-(dimethylamino)ethyl][(4-methoxyphenyl)methyl]amino}pyridine -3-carboxylate (11)**
A mixture of **8** (2.0 g, 9.6 mmol), methyl 6-chloronicotinate (1.7 g, 9.6 mmol), *N*,*N*-diisopropylethylamine (2.5 mL, 14 mmol) and DMSO (1.0 mL) was stirred for 4 h at 110 ºC using microwave and then the mixture was added 10% citric acid aq. (40 mL). The solution was extracted with EtOAc (2 × 30 mL) and the water layer was alkalized with $K_2CO_3$. This alkalized solution was extracted with EtOAc (2 × 30 mL). Combined organic extracts were washed with brine (2 × 30 mL), and then dried over $Na_2SO_4$. Concentration followed by amino silica gel column chromatography (hexane/EtOAc = from 7/3 to 1/1) gave **11** (0.75 g, 23%) as a colorless oil.
$^{1}$H-NMR ($CDCl_3$, 400 MHz) δ: 2.26 (s, 6H), 2.49 (t, *J* = 7.5 Hz, 2H), 3.66 (t, *J* = 7.5 Hz, 2H), 3.80 (s, 3H), 3.86 (s, 3H), 4.77 (s, 2H), 6.44 (d, *J* = 9.0 Hz, 1H), 6.84 (d, *J* = 8.4 Hz, 2H), 7.14 (d, *J* = 8.4 Hz, 2H), 7.94 (dd, *J* = 2.4, 9.0 Hz, 1H), 8.81 (d, *J* = 2.4 Hz, 1H). $^{13}$C-NMR ($CDCl_3$, 100 MHz) δ: 45.9, 46.7, 51.4, 51.6, 55.3, 56.6, 104.6, 114.0, 114.1, 128.2, 129.7, 138.3, 151.3, 158.8, 160.2, 166.7.
HRMS (ESI) *m/z* calcd for: $C_{19}H_{26}N_3O_3$ $(M+H)^+$: 344.1969, found: 344.1971.

***t*-Butyl {4-chloro-2-[(6-{[2-(dimethylamino)ethyl][(4-methoxyphenyl)methyl] amino}pyridine-3-carbonyl)amino]phenyl}carbamate (12)**
A mixture of **11** (0.22 g, 0.64 mmol), NaOH (0.23 g, 5.8 mmol) and MeOH (3 mL) was heated for 0.5 h at 100 ºC in a microwave oven and then the mixture was evaporated to remove MeOH. The remaining solid was added 4N HCl/EtOAc (3 mL) and resulting

solid was filtered and washed with EtOH. Evaporating the filtrate gave white solid and a mixture of this and *t*-butyl (2-amino-4-chlorophenyl)carbamate (0.22 g, 0.90 mmol), HATU (0.51 g, 1.4 mmol), $Et_3N$ (0.25 mL, 1.8 mmol), DMAP (0.032 g, 0.27 mmol) and DMF (3.0 mL) was stirred for 8 h at rt. The mixture was added 10% $K_2CO_3$ aq. and extracted with EtOAc (2 × 30 mL). Combined organic extracts were washed with brine (2 × 30 mL), and then dried over $Na_2SO_4$. Concentration followed by amino silica gel column chromatography (hexane/EtOAc = from 10/0 to 0/10) gave **12** (0.091 g, 26 %) as a white solid.

$^1$H-NMR ($CDCl_3$, 400 MHz) δ: 1.51 (s, 9H), 2.26 (s, 6H), 2.50 (t, *J* = 7.3 Hz, 2H), 3.71 (t, *J* = 7.3 Hz, 2H), 3.80 (s, 3H), 4.77 (s, 2H), 6.48 (d, *J* = 9.0 Hz, 1H), 6.80 (s, 1H), 6.85 (d, *J* = 8.6 Hz, 2H), 7.10 (dd, *J* = 2.4, 8.6 Hz, 1H), 7.15 (d, J = 8.6 Hz, 2H), 7.20 (d, *J* = 8.6 Hz, 1H), 7.81 (d, *J* = 2.4 Hz, 1H), 7.93 (dd, *J* = 2.4, 9.0 Hz, 1H), 8.76 (d, *J* = 2.4 Hz, 1H), 8.86 (s, 1H). $^{13}$C-NMR ($CDCl_3$, 100 MHz) δ: 28.2, 45.8, 46.6, 51.4, 55.3, 56.5, 81.7, 104.9, 114.0, 117.1, 125.3, 125.4, 125.5, 128.1, 128.3, 131.0, 136.6, 148.6, 154.4, 158.8, 159.7, 164.5.

HRMS (ESI) *m/z* calcd for: $C_{29}H_{37}ClN_5O_4$ $(M+H)^+$:554.2529, found:554.2549

**_t_-Butyl {4-bromo-2-[(6-{[2-(dimethylamino)ethyl][(4-methoxyphenyl)methyl]amino}pyridine-3-carbonyl)amino]phenyl}carbamate (13)**

A mixture of **11** (0.088 g, 0.26 mmol), NaOH (0.093 g, 2.3 mmol) and MeOH (0.5 mL) was heated for 0.5 h at 100 ºC in a microwave oven and then the mixture was evaporated to remove MeOH. The remaining solid was added 4N HCl/EtOAc (3 mL) and resulting solid was filtered and washed with EtOH. Evaporating the filtrate gave white solid and a mixture of this and *t*-butyl (2-amino-4-bromophenyl)carbamate (0.16 g, 0.55 mmol), HATU (0.32 g, 0.83 mmol), $Et_3N$ (0.23 mL, 1.7 mmol), DMAP (0.038 g, 0.33 mmol) and DMF (3.8 mL) was stirred for 20 h at rt. The mixture was added 10 % $K_2CO_3$ aq. and extracted with EtOAc (2 × 30 mL). Combined organic extracts were washed with brine (2 × 30 mL), and then dried over $Na_2SO_4$. Concentration followed by amino silica gel column chromatography (hexane/EtOAc = from 10/0 to 0/10) gave **13** (0.036 g, 23%) as a yellow white solid.

$^1$H-NMR ($CDCl_3$, 400 MHz) δ: 1.51 (s, 9H), 2.28 (s, 6H), 2.51 (t, *J* = 7.1 Hz, 2H), 3.79 (t, *J* = 7.1 Hz, 2H), 3.79 (s, 3H), 4.77 (s, 2H), 6.49 (d, *J* = 9.0 Hz, 1H), 6.85 (d, *J* = 8.6 Hz, 2H), 6.91 (brs, 1H), 7.13-7.15 (m, 3H), 7.18 (d, *J* = 8.6 Hz, 3H), 7.23 (dd, J = 2.4, 8.6 Hz, 1H), 7.90 (s, 1H), 7.93 (dd, *J* = 2.4, 9.0 Hz, 1H), 8.76 (d, *J* = 2.4 Hz, 1H), 8.88 (s, 1H). $^{13}$C-NMR ($CDCl_3$, 100 MHz) δ: 28.3, 45.8, 46.7, 51.5, 55.3, 56.6, 81.6, 105.0,

114.1, 117.2, 118.4, 125.7, 128.2, 128.5, 129.2, 129.7, 132.2, 136.6, 148.6, 154.4, 158.9, 159.8, 164.7.
HRMS (ESI) *m/z* calcd for: $C_{29}H_{37}BrN_5O_4$ $(M+H)^+$: 598.2023, found:598.2035.

**_t_-Butyl {2-[(6-{[2-(dimethylamino)ethyl][(4-methoxyphenyl)methyl]amino} pyridine-3-carbonyl)amino]-4-iodophenyl}carbamate (14)**

A mixture of **11** (0.084 g, 0.24 mmol), NaOH (0.090 g, 2.2 mmol) and MeOH (1.0 mL) was heated for 0.5 h at 100 ºC in a microwave oven and then the mixture was evaporated to remove MeOH. The remaining solid was added 4N HCl/EtOAc (3 mL) and resulting solid was filtered and washed with EtOH. Evaporating the filtrate gave white solid and a mixture of this and *t*-butyl (2-amino-4-iodophenyl)carbamate (0.15 g, 0.45 mmol), HATU (0.26 g, 0.67 mmol), $Et_3N$ (0.19 mL, 1.4 mmol), DMAP (0.016 g, 0.14 mmol) and DMF (5.0 mL) was stirred for 20 h at rt. The mixture was added 10 % $K_2CO_3$ aq. and extracted with EtOAc (2 × 30 mL). Combined organic extracts were washed with brine (2 × 30 mL), and then dried over $Na_2SO_4$. Concentration followed by amino silica gel column chromatography (hexane/EtOAc = from 10/0 to 0/10) gave **14** (0.041 g, 26%) as a colorless solid.
$^1$H-NMR ($CDCl_3$, 400 MHz) δ: 1.51 (s, 9H), 2.26 (s, 6H), 2.50 (t, *J* = 7.1 Hz, 2H), 3.71 (t, *J* = 7.1 Hz, 2H), 3.78 (s, 3H), 4.77 (s, 2H), 6.49 (d, *J* = 9.0 Hz, 1H), 6.84 (d, *J* = 8.6 Hz, 2H), 6.90 (brs, 1H), 7.04 (d, *J* = 8.6 Hz, 1H), 7.15 (d, *J* = 8.6 Hz, 2H), 7.42 (dd, *J* = 2.4, 8.6 Hz, 1H), 7.93 (dd, *J* = 2.4, 9.0 Hz, 1H), 8.03 (d, *J* = 2.4 Hz, 1H), 8.76 (d, *J* = 2.4 Hz, 1H), 8.80 (s, 1H). $^{13}$C-NMR ($CDCl_3$, 100 MHz) δ: 28.3, 45.8, 46.7, 51.5, 55.3, 56.6, 81.6, 88.9, 105.0, 114.1, 117.1, 125.8, 128.2, 129.7, 130.2, 132.0, 134.2, 134.6, 136.6, 148.7, 154.3, 158.9, 159.8, 164.7.
HRMS (ESI) *m/z* calcd for: $C_{29}H_{37}IN_5O_4$ $(M+H)^+$: 646.1885, found: 646.1878.

***N*-(2-Amino-5-chlorophenyl)-6-{[2-(dimethylamino)ethyl][(4-methoxyphenyl)methyl]amino}pyridine-3-carboxamide (2)**

A mixture of **12** (0.082 g, 0.15 mmol) and TFA (1.0 mL) was stirred for 0.5 h at rt, and then poured into 10% $K_2CO_3$ aq. (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 (1.0 mL) and hexane (10 mL) was added. The resulting solid was collected by filtration to give **2** (0.030 g, 45%) as a white solid.
$^1$H-NMR ($CDCl_3$, 400 MHz) δ: 2.28 (s, 6H), 2.51 (t, *J* = 7.1 Hz, 2H), 3.71 (t, *J* = 7.3 Hz, 2H), 3.80 (s, 3H), 4.78 (s, 2H), 6.50 (d, *J* = 9.0 Hz, 1H), 6.75 (d, *J* = 8.6 Hz, 1H), 6.85

(d, $J$ = 8.6 Hz, 2H), 7.01 (dd, $J$ = 2.4, 8.6 Hz, 1H), 7.15 (d, $J$ = 8.6 Hz, 2H), 7.41 (d, $J$ = 2.4 Hz, 1H), 7.71 (brs, 1H), 7.91 (dd, $J$ = 2.4, 9.0 Hz, 1H), 8.71 (d, $J$ = 2.4 Hz, 1H).
$^{13}$C-NMR ($CDCl_3$, 100 MHz) δ: 45.9, 46.8, 51.5, 55.3, 56.6, 105.2, 114.1, 117.1, 119.3, 124.3, 124.7, 126.1, 126.5, 128.1, 129.6, 136.8, 138.8, 148.2, 158.8, 159.8, 164.5.
HRMS (ESI) *m/z* calcd for: $C_{24}H_{29}ClN_5O_2$ $(M+H)^+$: 454.2004, found: 454.1992.

**_N_-(2-Amino-5-bromophenyl)-6-{[2-(dimethylamino)ethyl][(4-methoxyphenyl)methyl]amino}pyridine-3-carboxamide (3)**

A mixture of **13** (0.056 g, 0.093 mmol) and TFA (1.0 mL) was stirred for 2 h at rt, and then poured into 10 % $K_2CO_3$ aq. (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 (1.0 mL) and hexane (10 mL) was added. The resulting solid was collected by filtration to give **3** (0.027 g, 59%) as a white solid.
$^1$H-NMR ($CDCl_3$, 400 MHz) δ: 2.31 (s, 6H), 2.55 (t, $J$ = 7.1 Hz, 2H), 3.72 (t, $J$ = 7.1 Hz, 2H), 3.79 (s, 3H), 3.97 (brs, 2H), 4.76 (s, 2H), 6.51 (d, $J$ = 9.0 Hz, 1H), 6.70 (d, $J$ = 8.3 Hz, 2H), 6.85 (d, $J$ = 8.8 Hz, 4H), 7.14-7.18 (m, 3H), 7.51 (d, $J$ = 1.9 Hz, 1H), 7.74 (s, 1H), 7.90 (dd, $J$ = 2.4, 9.0 Hz, 1H), 8.70 (d, $J$ = 2.4 Hz, 1H). $^{13}$C-NMR ($CDCl_3$, 100 MHz) δ: 45.7, 46.6, 51.6, 55.3, 56.6, 105.3, 111.1, 114.1, 117.3, 119.6, 126.3, 127.6, 127.7, 128.1, 129.5, 136.8, 139.6, 148.2, 158.9, 159.8, 164.5.
HRMS (ESI) *m/z* calcd for: $C_{24}H_{29}BrN_5O_2$ $(M+H)^+$: 498.1499, found: 498.1504.

**_N_-(2-Amino-5-iodophenyl)-6-{[2-(dimethylamino)ethyl][(4-methoxyphenyl)methyl]amino}pyridine-3-carboxamide (4)**

A mixture of **14** (0.040 g, 0.062 mmol) and TFA (1.0 mL) was stirred for 2 h at rt, and then poured into 10% $K_2CO_3$ aq. (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 (1.0 mL) and hexane (10 mL) was added. The resulting solid was collected by filtration to give **4** (0.010 g, 30%) as a white solid.
$^1$H-NMR ($CDCl_3$, 400 MHz) δ: 2.30 (s, 6H), 2.57 (t, $J$ = 7.3 Hz, 2H), 3.73 (t, $J$ = 7.3 Hz, 2H), 3.79 (s, 3H), 3.90 (s, 2H), 4.77 (s, 2H), 6.51 (d, $J$ = 9.0 Hz, 1H), 6.59 (d, $J$ = 8.5 Hz, 1H), 6.85 (d, $J$ = 8.3 Hz, 2H), 7.14 (d, $J$ = 8.5 Hz, 2H), 7.33 (dd, $J$ = 2.4, 8.6 Hz, 1H), 7.63 (s, 2H), 7.90 (dd, $J$ = 2.4, 9.0 Hz, 1H), 8.71 (d, $J$ = 2.4 Hz, 1H). $^{13}$C-NMR ($CDCl_3$, 100 MHz) δ: 45.6, 46.5, 51.6, 55.3, 56.5, 80.1, 105.3, 114.1, 117.2, 120.0, 126.3, 128.1, 129.4, 133.5, 135.5, 136.8, 140.6, 148.2, 158.9, 159.8, 164.5.

HRMS (ESI) *m/z* calcd for: $C_{24}H_{29}IN_5O_2$ $(M+H)^+$: 546.1360, found: 546.1377.

**_t_-Butyl (4-(furan-2-yl)-2-nitrophenyl)carbamate (23)**

A mixture of *t*-butyl (4-bromo-2-nitrophenyl)carbamate (0.20 g, 0.63 mmol), furan-2-ylboronic acid (0.085 g, 0.76 mmol), $K_2CO_3$ (0.26 g, 1.9 mmol ), $Pd(PPh_3)_4$ (0.072 g, 0.063 mmol) and $H_2O$/DME (1/1, 3.0 mL) was heated for 1 h at 130 ºC using microwave. 10% $K_2CO_3$ aq. (20 mL) was added and extracted with EtOAc (2 × 20 mL). Combined organic extracts were washed with brine (2 × 20 mL), and then dried over $Na_2SO_4$. Concentration followed by silica gel flash column chromatography (hexane/EtOAc = from hexane only to 9/1) gave **23** (0.12 g, 63%) as a yellow solid.

$^1$H-NMR (DMSO-$d_6$, 400 MHz) δ: 1.50 (s, 9H), 6.64 (m, 1H), 7.11 (d, $J$ = 3.4 Hz, 1H), 7.71 (d, $J$ = 8.8 Hz, 1H), 7.80 (s, 1H), 7.96 (m, 1H), 8.19 (m, 1H), 9.65 (s, 1H).

LC/MS (ESI) *m/z*: 304 $[M+H]^+$.

**_t_-Butyl [2-amino-4-(furan-2-yl)phenyl]carbamate (22)**

To a solution of **23** (0.60 g, 2.0 mmol) in MeOH (70 mL) was added Pd/C (0.10 g). The solution above was filtered by celite to remove Pd/C. Concentration followed by amino silica gel flash column chromatography (hexane/EtOAc = from 95/5 to 60:40) gave **22** (0.43g, 79%) as a white solid.

$^1$H-NMR ($CDCl_3$, 400 MHz) δ: 1.50 (s, 9H), 3.77 (brs, 2H), 6.22 (brs, 1H), 6.43 (m, 1H), 6.55 (d, $J$ = 3.2 Hz, 1H), 7.10 (m, 2H), 7.32 (d, $J$ = 8.3 Hz, 1H), 7.41 (s, 1H).

HRMS (ESI) *m/z* calcd for: $C_{10}H_{11}N_2O$ $(M\text{-Boc}+H)^+$: 175.0866, found: 175.0867.

**_t_-Butyl {2-[(6-chloropyridine-3-carbonyl)amino]-4-(furan-2-yl)phenyl}carbamate (17)**

A mixture of **22** (0.30 g, 1.1 mmol), 6-chloronicotinoyl chloride (0.21 g, 0.12 mmol), triethylamine (0.61 mL, 4.4 mmol), and THF (3.0 mL) was stirred for 8 h at rt. Water was added and extracted with EtOAc (2 × 30 mL). Combined organic extracts were washed with brine (2 × 30 mL), and then dried over $Na_2SO_4$. Concentration gave a white solid. The white solid was suspended in EtOAc (1 mL) and then hexane (10 mL) was added. Resulting solid was filtered to give **17** (0.38 g, 84%) as a white pale.

$^1$H-NMR ($CDCl_3$, 400 MHz) δ: 1.50 (s, 9H), 6.43 (m, 1H), 6.55 (m, 1H), 7.00 (brs, 1H), 7.16 (d, $J$ = 8.3 Hz, 1H), 7.37-7.41 (m, 3H), 7.98 (s, 1H), 8.20 (dd, $J$ = 2.2, 8.3 Hz, 1H), 8.99 (m, 1H), 9.75 (s, 1H). $^{13}$C-NMR ($CDCl_3$, 100 MHz) δ: 28.2, 81.9, 105.5, 111.7, 121.0, 121.6, 124.1, 124.7, 128.6, 128.8 128.8 130.2, 137.8, 142.2, 149.0, 152.6, 154.6, 154.9, 162.9.

HRMS (ESI) *m/z* calcd for: $C_{21}H_{21}ClN_3O_4$ $(M+H)^+$: 414.1215, found: 414.1215

***t*-Butyl {3-[(6-chloropyridine-3-carbonyl)amino][1,1'-biphenyl]-4-yl}carbamate (16)**

A mixture of **21** (0.30 g, 1.1 mmol), 6-chloronicotinoyl chloride (0.21 g, 0.12 mmol), $Et_3N$ (0.59 mL, 4.2 mmol), and THF (3.0 mL) was stirred for 8 h at rt. Water was added and extracted with EtOAc (2 × 30 mL). Combined organic extracts were washed with brine (2 × 30 mL), and then dried over $Na_2SO_4$. Concentration gave a white solid. The white solid was suspended in EtOAc (1 mL) and then hexane (10 mL) was added. Resulting solid was filtered to give **16** (0.34 g, 76%) as a white pale.

$^1$H-NMR ($CDCl_3$, 400 MHz) δ: 1.50 (s, 9H), 6.82 (s, 1H), 7.21 (d, *J* = 8.8 Hz, 1H), 7.34 (m, 1H), 7.41 (m, 5H), 7.56 (m, 2H), 8.04 (m, 1H), 8.23 (dd, *J* = 2.4 Hz, 8.3 Hz, 2H), 8.99 (d, *J* = 2.4 Hz, 1H), 9.76 (brs, 1H). $^{13}$C-NMR ($CDCl_3$, 100 MHz) δ: 28.3, 82.2, 124.2, 124.5, 124.6, 124.8, 127.0, 127.6, 128.7, 128.8, 130.5, 137.9, 139.3, 140.0, 148.8, 154.6, 155.0, 162.7.

HRMS (ESI) *m/z* calcd for: $C_{23}H_{23}ClN_3O_3$ $(M+H)^+$: 424.1422, found: 424.1423.

***t*-Butyl {2-[(6-{[2-(dimethylamino)ethyl][(4-methoxyphenyl)methyl]amino} pyridine-3-carbonyl)amino]-4-(thiophen-2-yl)phenyl}carbamate (18)**

A mixture of **8** (0.12 g, 0.56 mmol), **15**[22] (0.20 g, 0.43 mmol), pyridine (0.11 mL, 1.4 mmol), and DMSO (1.0 mL) was heated for 5 h at 110 ºC in a microwave oven. 10% $K_2CO_3$ aq. was added and extracted with EtOAc (2 × 30 mL). Combined organic extracts were washed with brine (2 × 30 mL), and then dried over $Na_2SO_4$. Concentration followed by amino-silica gel flash column chromatography (hexane/EtOAc = from 8/2 to EtOAc only) gave **18** (0.10 g, 39%) as a white solid.

$^1$H-NMR ($CDCl_3$, 400 MHz) δ: 1.50 (s, 9H), 2.28 (s, 6H), 2.51 (t, *J* = 7.1 Hz, 2H), 3.70 (t, *J* = 7.1 Hz, 2H), 3.83 (s, 3H), 4.73 (s, 2H), 6.47 (d, *J* = 9.0 Hz, 1H), 6.85 (d, *J* = 8.5 Hz, 2H), 7.02 (m, 2H), 7.15 (d, *J* = 8.5 Hz, 2H), 7.23 (m, 2H), 7.35 (m, 2H), 7.92 (s, 1H), 7.97 (dd, *J* = 2.4 Hz, 9.0 Hz, 1H), 8.80 (d, *J* = 2.4 Hz, 1H), 8.86 (brs, 1H). $^{13}$C-NMR ($CDCl_3$, 100 MHz) δ: 28.3, 45.8, 46.7, 51.5, 55.3, 56.6, 81.4, 104.9, 114.1, 117.6, 124.3, 124.4, 124.7, 128.2, 128.7, 129.4, 129.8, 131.0, 136.7, 138.9, 140.1, 148.6, 153.1, 154.4, 158.9, 159.8, 164.8.

28.3, 45.8, 46.7, 51.6, 55.3, 56.6, 81.3, 104.9, 114.1, 117.5, 123.4, 124.8, 128.0, 128.2, 129.6, 129.8, 131.0, 132.1, 136.7, 143.3, 148.7, 154.4, 158.9, 159.8, 164.9.

HRMS (ESI) *m/z* calcd for: $C_{33}H_{39}N_5NaO_4S$ $(M+Na)^+$: 624.2615 found: 624.2632.

***t*-Butyl {3-[(6-{[2-(dimethylamino)ethyl][(4-methoxyphenyl)methyl]amino} pyridine-3-carbonyl)amino][1,1'-biphenyl]-4-yl}carbamate (19)**

A mixture of **8** (0.34 g, 1.7 mmol), **16** (0.6 g, 1.4 mmol), pyridine (0.34 mL, 4.4 mmol), and DMSO (3.0 mL) was heated for 5 h at 110 ºC in a microwave oven. 10% $K_2CO_3$ aq. was added and extracted with EtOAc (2 × 30 mL). Combined organic extracts were washed with brine (2 × 30 mL), and then dried over $Na_2SO_4$. Concentration followed by amino-silica gel flash column chromatography (hexane/EtOAc = from 8/2 to EtOAc only) gave **19** (0.22 g, 26%) as a yellow solid.

$^1$H-NMR ($CDCl_3$, 400 MHz) δ: 1.50 (s, 9H), 2.28 (s, 6H), 2.50 (t, *J* = 7.3 Hz, 2H), 3.70 (t, *J* = 7.3 Hz, 2H), 3.79 (s, 3H), 4.77 (s, 2H), 6.48 (d, *J* = 9.0 Hz, 1H), 6.85 (d, *J* = 8.6 Hz, 2H), 6.93 (brs, 1H), 7.16 (d, *J* = 8.6 Hz, 2H), 7.3-7.4 (m, 5H), 7.56 (d, *J* = 8.8 Hz, 2H), 7.94 (m, 1H), 7.97 (dd, *J* = 2.4, 9.0 Hz, 1H), 8.80 (d, *J* = 2.4 Hz, 1H), 8.86 (brs, 1H). $^{13}$C-NMR ($CDCl_3$, 100 MHz) δ: 28.3, 45.8, 46.7, 51.5, 55.3, 56.6, 81.4, 104.9, 114.1, 117.6, 124.3, 124.4, 124.7, 127.1, 127.3, 128.2, 128.7, 129.4, 129.8, 131.0, 136.7, 138.9, 140.1, 148.6, 154.5, 158.9, 159.8, 164.8.

HRMS (ESI) *m/z* calcd for: $C_{35}H_{42}N_5O_4$ $(M+H)^+$: 596.3232, found: 596.3258.

***t*-Butyl {2-[(6-{[2-(dimethylamino)ethyl][(4-methoxyphenyl)methyl]amino} pyridine-3-carbonyl)amino]-4-(furan-2-yl)phenyl}carbamate (20)**

A mixture of **8** (0.20 g, 0.48 mmol), **17** (0.12 g, 0.58 mmol), pyridine (0.12 mL, 1.5 mmol ), and DMSO (1.0 mL) was heated for 5 h at 110 ºC in a microwave oven. 10 % $K_2CO_3$ aq. was added and extracted with EtOAc (2 × 30 mL). Combined organic extracts were washed with brine (2 × 30 mL), and then dried over $Na_2SO_4$. Concentration followed by amino-silica gel flash column chromatography (hexane/EtOAc = from 8/2 to EtOAc only) gave **20** (0.11 g, 39%) as a yellow solid.

$^1$H-NMR ($CDCl_3$, 400 MHz) δ: 1.50 (s, 9H), 2.29 (s, 6H), 2.51 (t, *J* = 7.1 Hz, 2H), 3.71 (t, *J* = 7.1 Hz, 2H), 3.80 (s, 3H), 4.78 (s, 2H), 6.43 (m, 1H), 6.48 (d, *J* = 9.0 Hz, 1H), 6.61 (d, *J* = 3.4 Hz, 1H), 6.85 (d, *J* = 8.8 Hz, 2H), 6.92 (brs, 1H), 7.16 (d, *J* = 8.8 Hz, 2H), 7.31 (d, *J* = 9.3 Hz, 1H), 7.4-7.5 (m, 2H), 7.97 (dd, *J* = 2.4, 9.0 Hz, 1H), 8.00 (m, 1H), 8.80 (d, *J* = 2.4 Hz, 1H). $^{13}$C-NMR ($CDCl_3$, 100 MHz) δ: 28.3, 45.8, 46.6, 51.5, 55.3, 56.6, 81.3, 105.0, 105.2, 111.7, 114.1, 117.6, 120.9, 121.2, 124.7, 128.2, 128.6, 129.3, 129.7, 131.0, 136.6, 142.0, 148.6, 153.1, 154.4, 158.9, 159.8, 164.8.

HRMS (ESI) *m/z* calcd for: $C_{33}H_{40}N_5O_5$ $(M+H)^+$: 586.3024, found: 586.3046.

***N*-[2-Amino-5-(thiophen-2-yl)phenyl]-6-{[2-(dimethylamino)ethyl][(4-methoxyphenyl)methyl]amino}pyridine-3-carboxamide (5)**

A mixture of **18** (0.10 g, 0.16 mmol) and TFA (6.0 mL) was stirred for 0.5 h at rt. The mixture above was poured into 10% $K_2CO_3$ aq. (30 mL) and extracted with EtOAc (2 × 20 mL). Combined organic extracts were washed with brine (2 × 30 mL), and then dried over $Na_2SO_4$. Concentration gave a colorless oil. The oil was suspended in EtOAc (1.0 mL) and hexane (10 mL) was added. Resulting solid was filtered to give **5** (0.065 g, 81%) as a white solid.

$^1$H-NMR ($CDCl_3$, 400 MHz) δ: 2.28 (s, 6H), 2.51 (t, *J* = 7.1 Hz, 2H), 3.70 (t, *J* = 7.1 Hz, 2H), 3.79 (s, 3H), 3.97 (brs, 2H), 4.78 (s, 2H), 6.50 (d, *J* = 9.2 Hz, 1H), 6.82-6.87 (m, 3H), 7.01-7.03 (m, 1H), 7.15-7.19 (m, 4H), 7.33 (dd, *J* = 2.4, 8.3 Hz, 1H), 7.53 (d, *J* = 2.4 Hz, 1H), 7.70 (brs, 1H), 7.93 (d, *J* = 2.4, 9.0 Hz, 1H), 8.74 (d, *J* = 2.4 Hz, 1H).

$^{13}$C-NMR ($CDCl_3$, 100 MHz) δ: 45.9, 46.8, 51.6, 55.3, 56.6, 105.2, 114.1, 117.4, 118.6, 121.9, 122.9, 123.6, 124.8, 124.9, 126.4, 127.9, 128.2, 129.7, 136.8, 140.4, 144.2, 148.2, 158.9, 159.8, 164.8.

HRMS (ESI) *m/z* calcd for: $C_{28}H_{32}N_5O_2S$ $(M+H)^+$: 502.2271, found: 502.2276.

***N*-(4-Amino[1,1'-biphenyl]-3-yl)-6-{[2-(dimethylamino)ethyl][(4-methoxyphenyl)methyl]amino}pyridine-3-carboxamide (6)**

A mixture of **19** (0.22 g, 0.41 mmol) and TFA (3.0 mL) was stirred for 0.5 h at rt. The mixture above was poured into 10% $K_2CO_3$ aq. (30 mL) and extracted with EtOAc (2 × 20 mL). Combined organic extracts were washed with brine (2 × 30 mL), and then dried over $Na_2SO_4$. Concentration gave a colorless oil. The oil was suspended in EtOAc (1.0 mL) and hexane (10 mL) was added. Resulting solid was filtered to give **7** (0.068 g, 33%) as a white solid.

$^1$H-NMR ($CDCl_3$, 400 MHz) δ: 2.34 (s, 6H), 2.53 (t, *J* = 7.3 Hz, 2H), 3.73 (t, *J* = 7.3 Hz, 2H), 3.80 (s, 3H), 4.00 (brs, 2H), 4.77 (s, 2H), 6.50 (d, *J* = 9.0 Hz, 1H), 6.84 (m, 2H), 6.87 (d, *J* = 8.3 Hz, 1H), 7.15 (d, *J* = 8.8 Hz, 2H), 7.28 (m, 1H), 7.32 (dd, *J* = 2.2, 8.3 Hz, 1H), 7.39 (m, 2H), 7.51 (m, 1H), 7.53 (m, 2H), 7.77 (brs, 1H), 7.93 (dd, *J* = 2.4 Hz, 9.0 Hz, 1H), 8.75 (d, *J* = 2.4 Hz, 1H). $^{13}$C-NMR ($CDCl_3$, 100 MHz) δ: 45.8, 46.7, 51.6, 55.3, 56.6, 105.2, 114.1, 117.5, 118.6, 123.9, 125.0, 125.7, 126.6, 128.1, 128.7, 129.6, 132.9, 136.8, 140.2, 140.5, 148.2, 158.9, 159.8, 164.7.

HRMS (ESI) *m/z* calcd for: $C_{30}H_{34}N_5O_2$ $(M+H)^+$: 496.2707, found: 496.2722.

***N*-[2-Amino-5-(furan-2-yl)phenyl]-6-{[2-(dimethylamino)ethyl][(4-methoxyphenyl) methyl]amino}pyridine-3-carboxamide (7)**

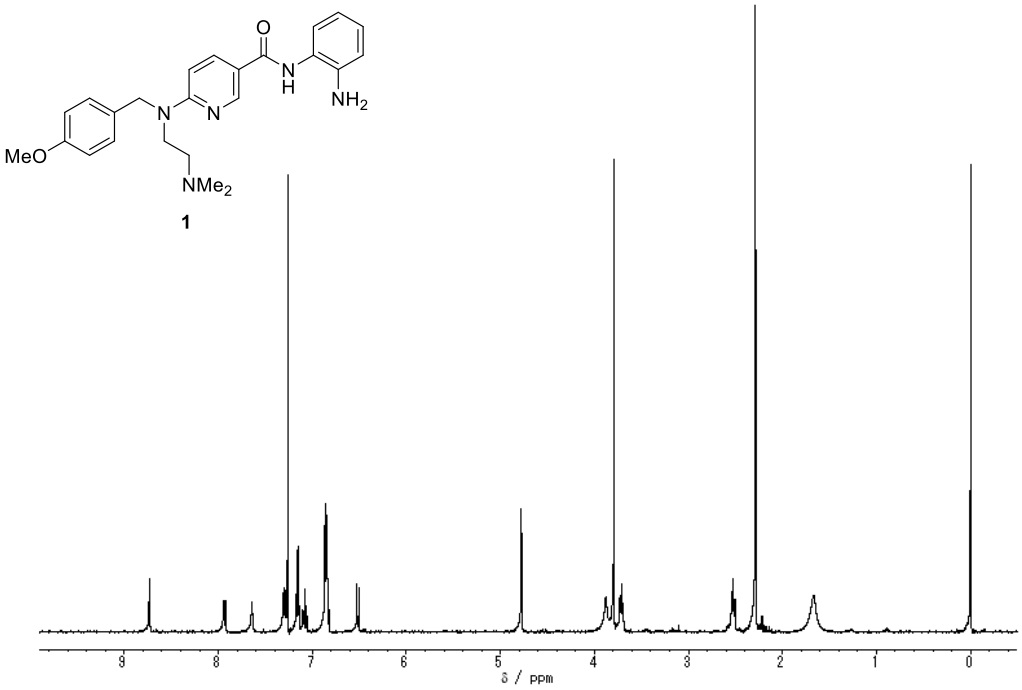
A mixture of **20** (0.060 g, 0.10 mmol) and TFA (3.0 mL) was stirred for 0.5 h at rt. The mixture above was poured into 10% $K_2CO_3$ aq. (30 mL) and extracted with EtOAc (2 × 20 mL). Combined organic extracts were washed with brine (2 × 30 mL), and then dried over $Na_2SO_4$. Concentration gave a colorless oil. The oil was suspended in EtOAc (1.0 mL) and hexane (10 mL) was added. Resulting solid was filtered to give **6** (0.010 g, 20%) as a white solid.

$^1$H-NMR ($CDCl_3$, 400MHz) δ: 2.27 (s, 6H), 2.51 (t, *J* = 7.1 Hz, 2H), 3.70 (t, *J* = 7.1 Hz, 2H), 3.79 (s, 3H), 3.97 (brs, 2H), 4.78 (s, 2H), 6.41 (dd, *J* = 2.0, 3.4 Hz, 1H), 6.47 (d, *J* = 3.4 Hz, 1H), 6.50 (d, *J* = 9.0 Hz, 1H), 6.82 (d, *J* = 8.6 Hz, 1H), 6.85 (d, *J* = 8.6 Hz, 2H), 7.15 (d, *J* = 8.6 Hz, 2H), 7.38 (m, 2H), 7.60 (d, *J* = 2.0 Hz, 1H), 7.70 (brs, 1H), 7.92 (dd, *J* = 2.4, 9.0 Hz, 1H), 8.74 (d, *J* = 2.4 Hz, 1H). $^{13}$C-NMR ($CDCl_3$, 400 MHz) δ: 45.9, 46.8, 51.5, 55.3, 56.6, 103.2, 105.2, 111.5, 114.1, 117.4, 118.5, 120.9, 122.8, 123.2, 124.8, 128.1, 129.7, 136.8, 140.3, 141.2, 148.2, 153.9, 158.9, 159.8, 164.7.

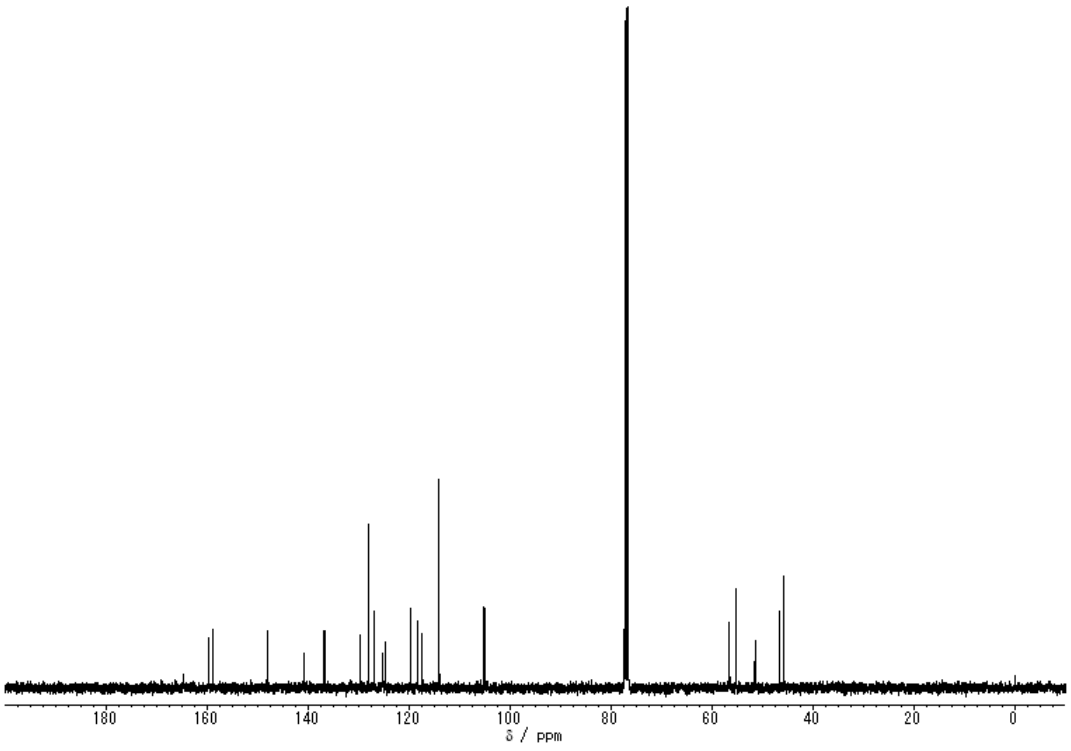
HRMS (ESI) *m/z* calcd for: $C_{28}H_{32}N_5O_3$ $(M+H)^+$: 486.2500, found: 486.2519

## 3. NMR spectra of compounds

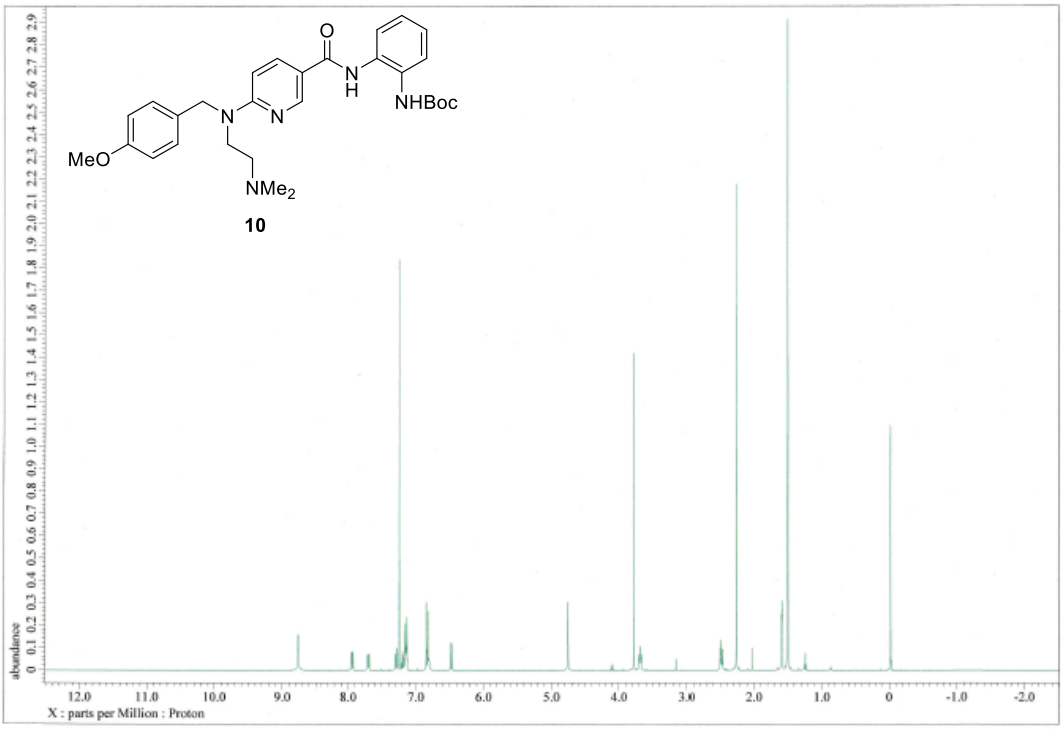
$^{1}$H-NMR spectrum of compound **1** ($CDCl_3$, 400 MHz)



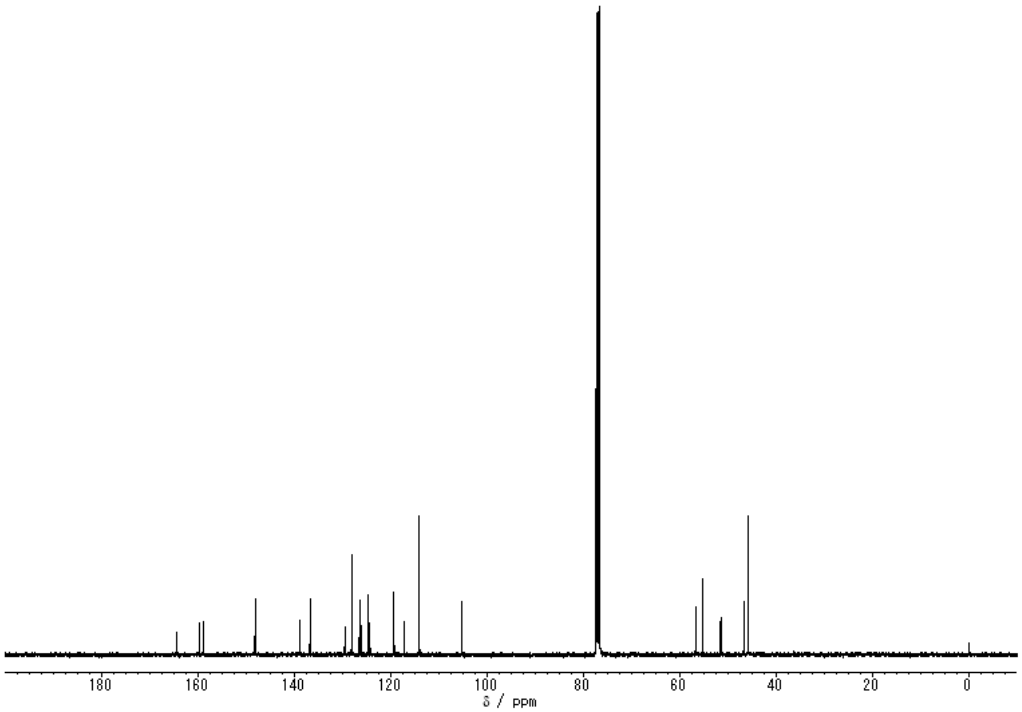

$^{13}$C-NMR spectrum of compound **1** ($CDCl_3$, 100 MHz)



$^{1}$H-NMR spectrum of compound **10** ($CDCl_3$, 400 MHz)




$^{13}$C-NMR spectrum of compound **10** ($CDCl_3$, 100 MHz)



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