Supporting Information

General information

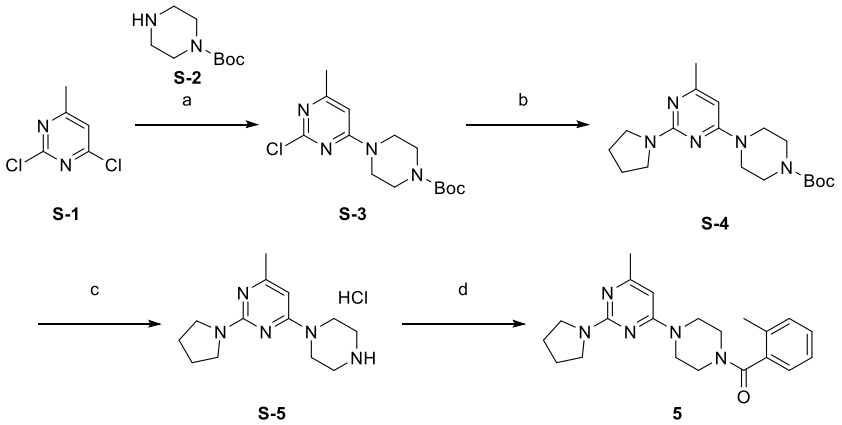
^{1}H NMR spectrum was recorded at 25 °C on a Bruker AVIII (operating at 600 MHz) or Varian Mercury 400 spectrometer (operating at 400 MHz). Chemical shifts were calculated in ppm (δ) from the residual chloroform signal at (δ_H) 7.26 ppm or internal standard, tetramethylsilane, at (δH) 0.00 ppm. The following abbreviations have been used: br = broad signal, s = singlet, d = doublet, dd = double doublet, t = triplet, q = quartet, m = multiplet, or any combination thereof. 13C NMR spectrum was recorded on a Bruker AVIII (operating at 150 MHz). Chemical shifts were calculated in ppm (δ) from the residual chloroform signal at (δ_C) 77.0 ppm. High-resolution mass spectra (HR-MS) was recorded on Thermo Fisher Scientific Orbitrap Exploris240 using electrospray ionisation (ESI) in positive mode.

The purity of the biological tested compounds was determined by an analytical LC–MS method and was found to be greater than or equal to 95% for all compounds. LC–MS analyses were performed using Waters SQD mass spectrometer linked to a Waters Acquity UPLC system with a PDA UV detector and ELSD detector. The spectrometer has an electrospray ionization (ESI) source operating in positive and negative ion mode.

Column chromatography was carried out using Biotage Isolera 4 with KP-Sil or KP-NH columns. Chemicals and solvents were used as received from vendors without further purifications.

Experimental section

Compound **5**



Step a)

To a stirred solution of 2,4-dichloro-6-methylpyrimidine **S-1** (1g, 6.135 mmol) in dry tetrahydrofuran (15 ml) was added N,N-diisopropylethylamine (3.96 g, 30.674 mmol) followed by *t*-butyl piperazine-1-carboxylate **S-2**(1.257 g, 6.748 mmol). The reaction mixture was stirred for 18 hrs at room temperature, then THF was evaporated under vacuum and the resulting crude solid was partitioned between EtOAc and water. The organic layer was separated, dried over magnesium sulfate, filtered, and then

concentrated by evaporation. The crude residue was purified by column chromatography using a KP-Sil pre-packed column with MeOH in DCM (0-5%) to afford compound **S-3** (1.81 g, 94% yield).

Step b)

A mixture of compound **S-3** (330 mg, 1.055 mmol) and pyrrolidine (881 μL, 10.55 mmol) in DMA (1.5 mL) was stirred at 120 °C for 3 hrs. Being cooled to room temperature, the reaction mixture was partitioned between EtOAc and water. The organic layer was separated, washed by water and saturated brine, dried over magnesium sulfate and then concentrated by evaporation. The crude residue was purified by column chromatography using a KP-NH pre-packed column with EtOAc in cyclohexane (0-30%) to afford compound **S-4** (327 mg, 89% yield).

^{1}H NMR (600 MHz, CHLOROFORM-d): δ ppm= 1.46-1.51 (m, 9 H), 1.87-1.96 (m, 4 H), 2.21-2.27 (m, 3 H), 3.48 (brs, 4 H), 3.50-3.62 (m, 8 H), 5.62-5.78 (m, 1 H)

Step c)

To a suspension of compound **S-4** (330 mg, 0.95 mmol) in 1,4-dioxane (1 mL) was added 4N HCl in 1,4-dioxane (1.187 mL, 4.749 mmol). Being stirred at room temperature for 3 hrs, the reaction mixture was treated with additional 4N HCl in dioxane (1 mL) and stirred for another 1 hr, and then treated with additional 4N HCl in dioxane (1 mL) and stirred for 30 min. The reaction mixture was concentrated in vacuo, then azeotroped with toluene to afford S-**5** (276.3 mg), which was used for next step without further purifications.

Step d)

Compound **S-5** (103 mg) was suspended to DCM (3 mL), followed by the addition of triethylamine (0.202 mL, 1.452 mmol) and *o*-toluoyl chloride (0.095 mL, 0.726 mmol). Being stirred at room temperature for 45 min, the reaction mixture was concentrated in vacuo and triturated with acetone to filter off triethylamine hydrochloride. The filtrate was concentrated in vacuo, then the residue was loaded to SCX-2 SPE cartridge (2g). The cartridge was washed by MeOH for 2 column volumes and then eluted with 2M ammonia solution in MeOH for 1.5 column volumes. The basic eluent fraction was concentrated in vacuo, then purified by column chromatography using KP-Sil pre-packed column with MeOH in DCM (0-3%) to afford compound **5** (63.5 mg, 48% yield).

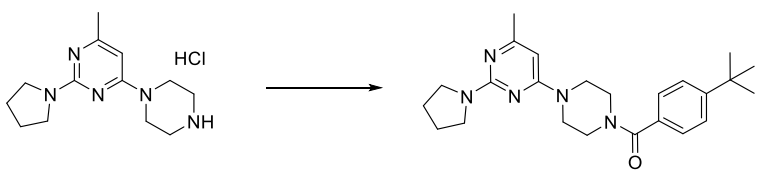
^{1}H NMR (600 MHz, CHLOROFORM-d): δ ppm= 1.87-1.95 (m, 4 H), 2.21-2.28 (m, 3 H), 2.32 (s, 3 H), 3.30 (brs, 2 H), 3.48-3.60 (m, 7 H), 3.60-3.72 (m, 2H), 3.78 - 3.99 (m, 2 H), 5.72 (s, 1 H), 7.15-7.20 (m, 1 H), 7.20-7.25 (m, 2 H)

^{13}C NMR (150 MHz, CHLOROFORM-d): δ ppm= 19.0 (s), 24.6 (s), 25.5 (s), 41.2 (s), 44.0 (s), 44.3 (s), 46.5 (s), 90.8 (s), 125.9 (s), 126.0 (s), 129.0 (s), 130.5 (s), 134.2 (s), 136.0 (s), 160.5 (s), 163.0 (s), 166.8 (s), 170.2 (s)

HR-MS m/z $[M+H]^+$: calc. for $C_{21}H_{28}N_5O^+$: 366.2288, found: 366.2281

LC-MS: RT 1.72 min, 99.6% purity

Compound **25**



To a suspension of compound **S-5** (30 mg, 0.106 mmol), 4-*t*-butyl benzoic acid (22.61 mg, 0.127 mmol) and HOBT (1.619 mg, 0.011 mmol) in DMF (1 mL) were added triethylamine (0.035 mL, 0.254 mmol) and EDC (24.32 mg, 0.127 mmol). The reaction mixture was stirred at room temperature for overnight, the diluted with saturated aqueous sodium bicarbonate and EtOAc. The organic layer was separated, washed with brine, dried over magnesium sulfate and concentrated. The crude residue was purified by column chromatography with MeOH in DCM (0-5%) and then triturated with TBME/pentane (1:3) to afford compound **25** (17.6 mg, 41% yield).

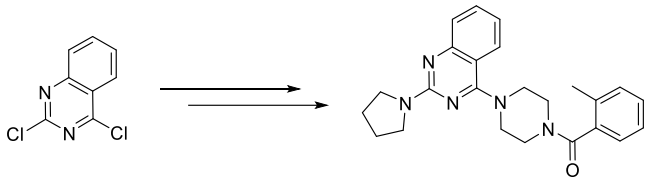
^{1}H NMR (600 MHz, CHLOROFORM-d): δ ppm= 1.33 (s, 9 H), 1.88-1.96 (m, 4 H), 2.28 (brs, 1 H), 2.25 (brs, 2 H), 3.48-3.94 (m, 1 H), 3.48-3.71 (m, 9 H), 3.82 (brs, 1 H), 5.73 (s, 1 H), 7.34-7.39 (m, 2 H), 7.41-7.46 (m, 2 H)

^{13}C NMR (150 MHz, CHLOROFORM-d): δ ppm= 24.60 (s), 24.62 (s), 25.5 (s), 31.2 (s), 34.8 (s), 44.1 (s), 46.5 (s), 90.8 (s), 125.5 (s), 127.0 (s), 132.7 (s), 153.2 (s), 160.4 (s), 163.0 (s), 166.6 (s), 170.8 (s)

HR-MS m/z $[M+H]^+$: calc. for $C_{24}H_{34}N_5O^+$: 408.2758, found: 408.2744

LC-MS: RT 2.15 min, 99.9% purity

Compound **39**

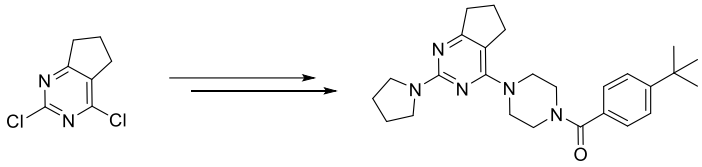


This compound was synthesized from 2,4-dichloroquinazoline in 4-step using a similar synthetic route with compound **5**.

^{1}H NMR (400 MHz, CHLOROFORM-d): δ ppm= 1.84-2.06 (m, 4 H), 2.35 (s, 3 H), 3.38-3.49 (m, 2 H), 3.50-3.60 (m, 2 H), 3.60-3.68 (m, 4 H), 3.74 (t, *J*=5.1 Hz, 2 H), 3.95-4.12 (m, 2 H), 7.03 (ddd, *J*=8.2, 6.4, 1.8 Hz, 1 H), 7.15-7.37 (m, 5 H), 7.44-7.57 (m, 2 H), 7.62-7.70 (m, 1 H)

MS-ESI m/z $[M+H]^+$: calc. 402; found 402

Compound **41**



This compound was synthesized from 2,4-dichloro-6,7-dihydro-5H-cyclopenta[d]pyrimidine in 4-step using a similar synthetic route with compound **5**.

^{1}H NMR (400 MHz, CHLOROFORM-d): δ ppm= 1.25-1.40 (m, 9 H), 1.92 (brt, *J*=6.42 Hz, 4 H), 1.95-2.03 (m, 2 H), 2.76 (br t, *J*=7.61 Hz, 2 H), 2.84 (t, *J*=7.06 Hz, 2 H), 3.52 (brs, 5 H), 3.58-3.92 (m, 4 H), 7.36 (d, *J*=8.25 Hz, 2 H), 7.43 (d, *J*=8.25 Hz, 2 H)

MS-ESI m/z $[M+H]^+$: calc. 434; found 434

LC-MS: RT 2.25 min, 97.9% purity

5-Day Chaperone Assay with 4-MUG

Human fibroblasts were seeded at 5000 cell per well into a 96 well plate Falcon plate and cultured for 24hrs. Medium was removed from the cells and compounds resuspended in medium were transferred to the cells. Following 5 day compound treatment at 37C and 5% CO2, cells were washed twice with PBS, followed by lysing of the cells in AB + 0.2% Triton + 1 X Protease Inhibitor Cocktail (Thermo Scientific 78430). 4-Methylumbelliferyl β-D-glucopyranoside (4-MUG; Merck) resuspended in Assay Buffer (0.01% Tween-20 and 10mM sodium taurocholate hydrate in citrate/phosphate (pH4.5)) was added to all wells to a FRC of 200uM and incubated at room temperature for 18hrs. Enzymatic reaction was stopped via addition of Stop Solution (0.5 M NaOH and 0.5 M glycine at pH 10.0) and fluorescence (ex340nm/em480nm) was measured on a Pherastar FS (BMG). Fluorescence measurements were normalised to the maximal response of the known GCase PC Isofagomine (IFG) (100%). The concentration of each compound producing 50% of its maximal response (Emax) was calculated as an AC_{50}.

Lysosomal GCase Immunocytchemistry (ICC) assay

Human fibroblasts were seeded at 5000 cell per well into a 96 well plate Falcon plate and cultured for 24hrs. Medium was removed from the cells and compounds resuspended in medium were transferred to the cells. Following 5-day compound treatment at 37C and 5% CO2, cells were washed twice with TBS containing 0.1% tween 20 (TBS-Tween hereafter). Cells were fixed in 4%PFA (Santa Cruz SC-281692) for 30 min at room temperature, fixed in methanol for 30 min at room temperature, permeabilised using 0.3% Triton-X-100 in TBS-Tween for 60 minutes at room temperature and blocked with 1X Blockace (Biorad) for 1 hour at room temperature. Anti-GCase and -Cathepsin-D antibodies (diluted in 0.2% Blockace in TBS-Tween) were added overnight at 4ºC, and following cell washing, secondary Alexa Fluor conjugated antibodies and Hoescht (5ug/mL) in TBS-Tween were added for 30 min at room temperature. Cells were then washed (3x TBS-Tween) prior to image acquisition and image analysis using a Cellomics VTi (ThermoFisher Scientific) high content imaging system. GCase levels was measured as GCase fluorescence intensity colocalised with Cathepsin-D fluorescence. Measurements were then normalised to the maximal response of the known GCase PC Isofagomine (IFG) (100%). The concentration of each compound producing 50% of its maximal response was calculated as an EC_{50}.

Cell-free GCase inhibitory assay with 4-MUG

Recombinant GCase (Cerezyme, Genzyme) resuspended in Assay Buffer ("AB"; 0.01% Tween-20 and 10mM sodium taurocholate hydrate in citrate/phosphate (pH4.5)) was added to all wells to a final reaction concentration (FRC) of 15nM and incubated with test compound for 10 minutes at room temperature. Subsequently, 4-Methylumbelliferyl β-D-glucopyranoside (4-MUG; Merck) resuspended in AB was added to all wells to a FRC of 200uM and incubated for 30 minutes at room temperature. Enzymatic reaction was stopped via addition of a Stop Solution (0.5 M NaOH and 0.5 M glycine at pH 10.0) and fluorescence (ex340nm/em480nm) was measured on a Pherastar FS (BMG). Fluorescence measurements were normalised to the maximal response of the known GCase inhibitor Isofagomine

(IFG) (100%). The concentration of each compound producing 50% of its maximal response was calculated as an IC_{50}.

Lysosome live cell GCase activity assay with PFB-FDGlu

Human fibroblasts were seeded at 10,000 cells per well into 96-well Ultra CellCarrier tissue culture plates (Perkin Elmer) and cultured for 24hrs. Growth medium was removed and replaced with test compounds resuspended in Assay Medium (DMEM with high glucose and Glutamax, 1% heat-inactivated FBS, and sodium pyruvate at 1mM) and incubated for 30 minutes at 37°C, 5% CO_2. 5-(pentafluorobenzoylamino) fluorescein Di-β-D-glucopyranoside (PFB-FDGlu; Eisai in-house; FRC 400uM) and Hoechst dye (Merck; FRC 2ug/mL) diluted in Assay Medium was added to compound-treated cells and incubated for 2hrs at 37°C, 5% CO_2. Plates were washed twice with FluoroBrite (ThermoFisher) before imaging on a Cellomics VTi (ThermoFisher Scientific) high content imaging system. Cellular fluorescence measurements were normalised to the maximal response of the known GCase inhibitor Isofagomine (IFG) (100%). The concentration of each compound producing 50% of its maximal response was calculated as an IC_{50}.

Solubility

Solubilities of the test compounds in PBS (Dulbecco's phosphate buffered saline, pH7.0 to 7.3) were determined using a high-throughput HPLC method. A 10 mM solution of test compound in DMSO was prepared and diluted to give a 1:100 DMSO solution of test medium in the microwell of the filter plate. After gyratory shaking of the filter plate for 15 min at room temperature, the solution was filtered. An aliquot of this filtered solution was analysed by HPLC-UV method.

Liver microsomal stability

Test compound (0.1 μM) was incubated with human liver microsomes (0.2 mg/mL) in the presence or absence of NADPH-generating system for 15 min at 37 °C. After deproteination by the addition of an equal volume of acetonitrile/methanol (7:3) mixture containing 0.1 μM propranolol as the internal standard to the reaction mixture, the test compound in the sample was measured by LC–MS/MS. The residual ratio of the compound in the presence of NADPH relative to that in the absence of NADPH was evaluated.

MDR1 transport assay

LLC-PK1 and MDR1 gene-transfected LLC-PK1 (LLC-MDR1) cells were individually seeded at a density of 6.0×10^5 cells/cm^2 onto porous membrane filters of 24-well cell culture insert plates. Cells were cultured in 5% CO_2/95% air humidified atmosphere at 37 degree for 5 to 6 days to prepare cell monolayer and were used for the transport studies. For transcellular transport experiments, each cell monolayer was pre-incubated at 37 °C for 2 h in Hanks' balanced salt solution containing 10 mM HEPES (HBSS buffer). Transcellular transport experiments were initiated by adding the HBSS buffer containing test compounds (1 μM) to the apical or basal side of the cell culture inserts. After

incubation at 37 ℃ for 2 h, a portion of HBSS buffer was collected from the receiver side, and permeated amount of the test compound was analyzed by LC-MS/MS. The apparent permeability coefficient (Papp) of the test compounds were estimated using Eq. 1, where Q, *t*, C_0, and *A* represent permeated amount of test compounds, incubation time, initial concentration of test compounds, and membrane area, respectively.

$$Papp = Q/t/C_0/A \quad (1)$$

Flux ratios (FRs) across the cell monolayer were defined by Eq. 2, where $P_{app,\ b\ to\ a}$ and $P_{app,\ a\ to\ b}$ represent the Papp in the basal-to-apical direction and the apical-to-basal direction, respectively, and the corrected FR was defined by Eq. 3.

$$FR = P_{app;\ b\ to\ a}/P_{app,\ a\ to\ b} \quad (2)$$

$$\text{Corrected FR (CFR)} = (\text{FR in LLC-MDR1 cells})/(\text{FR in LLC-PK1 cells}) \quad (3)$$