Supplementary Material for "Discovery of a 4-Azetidinyl-1-thiazoyl-cyclohexane CCR2 Antagonist as a Development Candidate"

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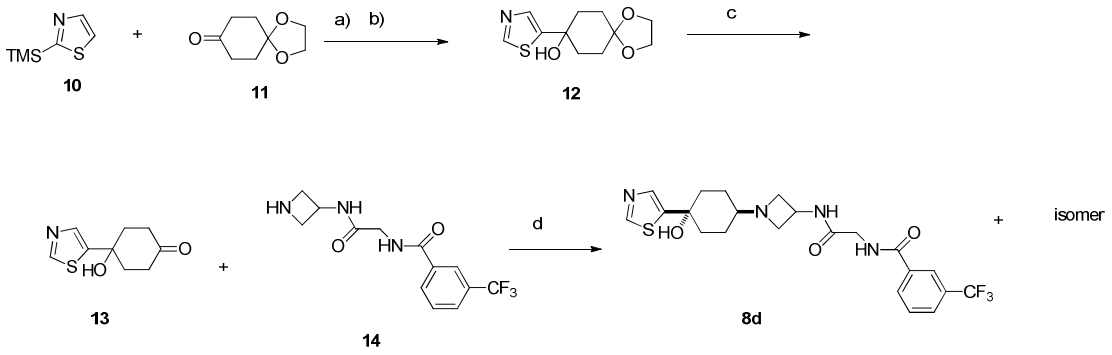
1. General Procedures

^{1}H NMR spectra were determined with a Bruker Biospin International AG-400 spectrometer at 400 MHz. Chemical shifts (δ) are reported in parts per million (ppm) relative to residual chloroform (7.26 ppm), TMS (0 ppm), or CD_3OD (4.87 ppm) as an internal reference with

coupling constants (J) reported in hertz (Hz). The peak shapes are denoted as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Electrospray (ES) mass spectra were recorded in positive or negative mode on a Micromass Platform spectrometer. The purity of all compounds described here was determined by analytical LC/MS using a Shimadzu LCMS-2110EV with analytical LC using Shiseido Capcell Pak C18 MGS3 column (3.0X50 mm, 3.5 μ), 0.05% TFA in water as mobile phase A and 0.05% TFA in acetonitrile as mobile phase B at 1 mL/min flow, gradient from 10% to 100% B in 1.7 minutes, 100% B for 1.5 minutes, 100 to 10% B in 0.13 minutes, monitored by UV absorption at 215 nm suing PDA and ELSD. The purity of all compounds was found to be > 95%. Thin-layer chromatography (TLC) was performed on Merck PLC prescored plates $60F_{254}$. Unless otherwise noted, reagents were obtained from commercial sources and were used without further purification.

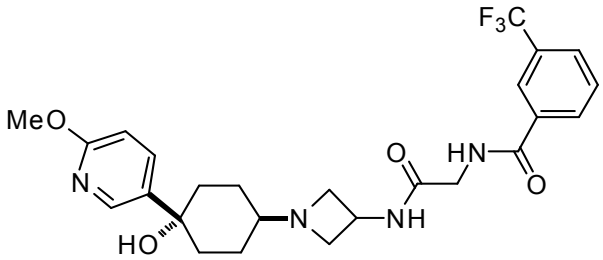
2. Synthetic procedures and data



Scheme 1. Reagents and conditions: (a) n-BuLi, -78°C. (b) TBAF, 2 steps, 55%. (C) HCl, acetone, room temperature, 90%. (d) $NaBH(OAc)_3$, TEA, DCM, room temperature, 35% along with its isomer, 40%.

All target compounds were synthesized in accordance with an analogous synthetic method described in Scheme 1.

N-({1-[4-Hydroxy-4-(6-methoxy-pyridin-3-yl)-cyclohexyl]-azetidin-3-ylcarbamoyl}-methyl)-3-trifluoromethyl-benzamide (**2f**)



8-(6-Methoxy-pyridin-3-yl)-1,4-dioxa-spiro[4.5]decan-8-ol

A solution of 5-bromo-2-methoxy-pyridine (Aldrich, 5.0 g, 26.6 mmol) in THF or ether (30 mL) at –78 °C was treated with *n*-BuLi (2.5 M in hexanes, 12 mL, 30 mmol) dropped slowly over 10 min. The reaction was stirred for an additional 20 min. at –78 °C. A solution of 1,4-dioxa-spiro[4.5]decan-8-one (Aldrich, 4.37 g, 28 mmol) in THF (10 mL) was slowly dropped into the reaction. After addition, the reaction was stirred for an additional 2 hours at –78 °C. The reaction was then quenched with water and warmed to room temperature. The solvent was removed *in vacuo* and the residue was partitioned between ethyl acetate and water. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give a yellow solid, which was then purified by silica gel column on a CombiFlash system using hexanes and ethyl acetate (from 10% ethyl acetate to 100% ethyl acetate) to afford the title compound as a yellow solid (4.25 g, yield, 60%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (s, 1H), 7.75 (d, J = 7.5 Hz, 1H), 6.72 (d, J = 7.5 Hz, 1H), 3.95 (m, 4H), 2.35 (s, br, 1H), 2.10 (m, 1H), 1.85 (m, 2H), 1.65 (m, 2H).

4-Hydroxy-4-(6-methoxy-pyridin-3-yl)-cyclohexanone

8-(6-Methoxy-pyridin-3-yl)-1,4-dioxa-spiro[4.5]decan-8-ol (4.00 g, 15.7 mmol) as prepared in the previous step was treated with 1N HCl (~ 16 mL) in acetone (20 mL) at room temperature for 4 hours. The reaction was neutralized with saturated $NaHCO_3$ solution and the solvent was removed. The residue was partitioned between ethyl acetate and water. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give a yellow solid, which was then purified by silica gel column on a CombiFlash system using hexanes and ethyl acetate (from 10% ethyl acetate to 100% ethyl acetate) to afford the title compound as a pale yellow solid (2.95 g, yield, 85%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.30 (s, 1H), 7.75 (d, J = 7.0 Hz, 1H), 6.75 (d, J = 6.5 Hz, 1H), 3.90 (s, 4H), 2.91 (m, 2H), 2.35 (d, J = 6.8 Hz, 2H), 2.22 (m, 4H).

N-(Azetidin-3-ylcarbamoylmethyl)-3-trifluoromethyl-benzamide free base, HCl and TFA salt

Refer the literature for the detail preparation.

Zhang, X.; Hufnagel, H.; Markotan, T.; Lanter, J.; Cai , C.; Hou, C.; Singer, M.; Opas, E.; McKenney, S.; Crysler, C.; Johnson, D.; Sui, Z. Overcoming hERG activity in the discovery of a series of 4-azetidinyl-1-arylcyclohexanes as CCR2 antagonists. *Bioorg. Med. Chem. Lett.* 2011, **21**, 5577-5582.

N-({1-[4-Hydroxy-4-(6-methoxy-pyridin-3-yl)-cyclohexyl]-azetidin-3-ylcarbamoyl}-methyl)-3-trifluoromethyl-benzamide (**2f**)

4-Hydroxy-4-(6-methoxy-pyridin-3-yl)-cyclohexanone (300 mg, 1.36 mmol) and N-(azetidin-3-ylcarbamoylmethyl)-3-trifluoromethyl-benzamide HCl salt (460 mg, 1.36 mmol) in DCM (5 mL) were treated with TEA (1 mL, 7.12 mmol) for 10 min followed by $NaBH(OAc)_3$ (860 mg, 4.07 mmol) for another 4 hours at room temperature. The reaction was quenched with saturated sodium bicarbonate. The organic layer was separated and the aqueous layer was extracted 3 times with chloroform and IPA "cocktail" (~ 3:1, v/v). The combined organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product, which was then purified by a CombiFlash system using ethyl acetate and 7N NH_3 in MeOH as eluent (from pure ethyl acetate to 5% 7N NH_3 in MeOH in ethyl acetate) to afford two title compounds as white solids: a less polar isomer (213 mg, yield, 31%), and a more polar isomer (250 mg, yield, 36%).

2f: less polar isomer from silica gel column

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (s, 1H), 8.11 (s, 1H), 8.05 (d, J = 5.8 Hz, 1H), 7.78 (d, J = 6.5 Hz, 1H), 7.70 (d, J = 6.5 Hz, 1H), 7.57 (t, J = 6.8 Hz, 1H), 6.70 (d, J = 6.5 Hz, 1H), 4.51 (m,1H), 4.20 (d, J = 3.2 Hz, 2H), 3.88 (s, 3H), 3.65 (t, J = 6.0 Hz, 2H), 2.92 (t, J = 5.8 Hz, 2H), 2.20 (m, 2H), 1.85 (m, 2H), 1.56 (m, 2H), 1.45 (m, 2H).

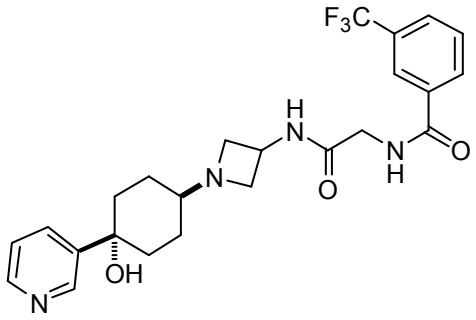
isomer: more polar isomer from silica gel column

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (s, 1H), 8.10 (s, 1H), 8.05 (d, J = 6.5 Hz, 1H), 7.80 (d, J = 6.4 Hz, 1H), 7.71 (d, J = 6.5 Hz, 1H), 7.62(d, J = 6.5 Hz, 1H), 7.60 (t, J = 6.8 Hz, 1H), 7.37 (d, J = 6.2 Hz, 1H), 6.70 (d, J = 6.8 Hz, 1H), 4.55 (m, 1H), 4.20 (d, J = 3.5 hz, 2H), 3.65 (t, J = 6.5 Hz, 2H), 3.08 (t, J = 6.5 Hz, 2H), 1.85~1.50 (m, 8H).

The following compounds were analogously synthesized from reductive amination of the corresponding cyclohexanones, as prepared in a similar synthesis of 4-hydroxy-4-(6-methoxy-

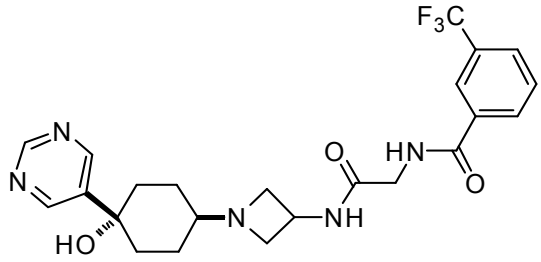
pyridin-3-yl)-cyclohexanone in the above synthesis and N-(azetidin-3-ylcarbamoylmethyl)-3-trifluoromethyl-benzamide using reductive amination described for the synthesis of **2f**.

N-{[1-(4-Hydroxy-4-pyridin-3-yl-cyclohexyl)-azetidin-3-ylcarbamoyl]-methyl}-3-trifluoromethyl-benzamide (**2a**)



Yield, 27%. The isomer was not characterized. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.65 - 8.94 (m, 1H), 8.33 - 8.58 (m, 1H), 8.12 (s, 1H), 8.01 (d, J = 6.3 Hz, 1H), 7.70 - 7.91 (m, 2H), 7.58 (t, J = 7.7 Hz, 1H), 7.25 (br. s., 1H), 4.54 (t, J = 6.1 Hz, 2H), 4.16 (br s, 1H), 3.51 - 3.72 (m, 2H), 3.00 - 3.14 (m, 2H), 2.92 (t, J = 6.6 Hz, 1H), 2.06 - 2.32 (m, 2H), 1.79 - 1.98 (m, 2H), 1.51 (br. s., 2H), 1.25 (s, 2H).

N-{[1-(4-Hydroxy-4-pyrimidin-5-yl-cyclohexyl)-azetidin-3-ylcarbamoyl]-methyl}-3-trifluoromethyl-benzamide (**2b**)



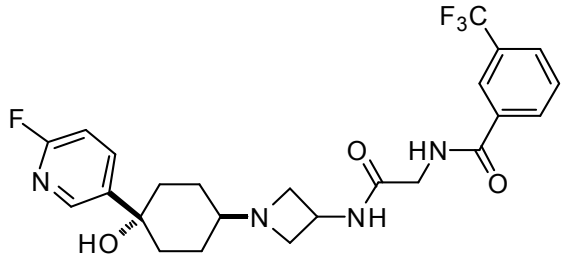
2b: less polar isomer from silica gel column (yield, 25%)

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.10 (s, 1H), 8.90 (s, 2H), 8.15 (s, 1H), 8.00 (d, J = 7.0 Hz, 1H), 7.75 (d, J = 6.6 Hz, 1H), 7.62 (t, J = 6.6 Hz, 1H), 4.52 (m, 1H), 4.15 (d, J = 3.4 Hz, 2H), 3.60 (t, J = 6.2 Hz, 2H), 2.96 (t, J = 6.5 Hz, 2H), 2.40 (s, br, 1H), 2.19 (m 2H), 1.85 (m, 2H), 1.55 (m, 4H).

isomer: more polar isomer from silica gel column (yield, 30%)

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.11 (s, 1H), 8.88 (s, 2H), 8.16 (s, 1H), 8.05 (d, J = 6.1 Hz, 1H), 7.82 (d, J = 6.2 Hz, 1H), 7.75 (m, 1H), 7.60 (m, 1H), 4.58 (m, 1H), 4.20 (d, J = 3.2 Hz, 2H), 3.65 (t, J = 6.0 Hz, 2H), 3.12 (t, J = 6.0 Hz, 2H), 2.20 (m, 2H), 1.90~1.69 (m, 4H), 1.55 (m, 2H).

N-({1-[4-(6-Fluoro-pyridin-3-yl)-4-hydroxy-cyclohexyl]-azetidin-3-ylcarbamoyl}-methyl)-3-trifluoromethyl-benzamide (**2c**)



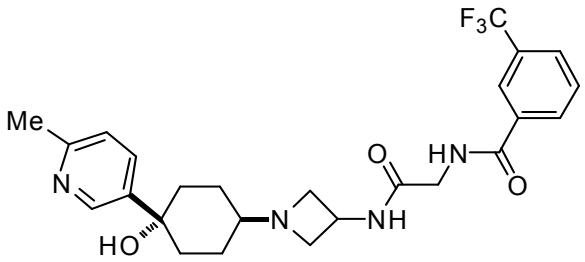
2c: less polar isomer from silica gel column (yield, 36%)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (s, 1H), 8.15 (s, 1H), 8.05 (d, J = 6.4 Hz, 1H), 7.96 (t, J = 6.8 Hz, 1H), 7.74 (d, J = 6.5 Hz, 1H), 7.55 (t, J = 7.2 Hz, 1H), 7.50 (m, 1H), 6.90 (d, J = 6.7 Hz, 1H), 4.53 (m, 1H), 4.15 (d, J = 3.5 Hz, 2H), 3.60 (t, J = 6.7 Hz, 2H), 2.88 (t, J = 6.8 Hz, 2H), 2.20 (m, 2H), 1.85 (m, 2H), 1.65 (m, 4H).

isomer: more polar isomer from silica gel column (yield, 41%)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (s, 1H), 8.10 (d, J = 6.0 Hz, 1H), 8.01 (d, J = 6.2 Hz, 1H), 7.95 (m, 1H), 7.72 (d, J = 6.2 Hz, 1H), 7.50 (t, J = 6.5 Hz, 1H), 6.85 (d, J = 6.6 Hz, 1H), 4.50 (m, 1H), 4.15 (s, br, 2H), 3.70 (t, J = 6.5 Hz, 2H), 3.10 (t, J = 5.5 Hz, 2H), 1.90~1.50 (m, 8H).

N-({1-[4-Hydroxy-4-(6-methyl-pyridin-3-yl)-cyclohexyl]-azetidin-3-ylcarbamoyl}-methyl)-3-trifluoromethyl-benzamide (**2e**)



2e: less polar isomer from silica gel column (yield, 38%)

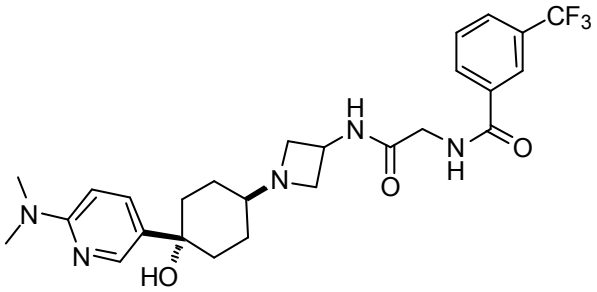
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 (s, 1H), 8.15 (s, 1H), 8.02 (d, J = 7.0 Hz, 1H), 7.70 (m, 2H), 7.68 (m, 1H), 7.55 (d, J = 6.5 Hz, 1H), 7.70 (s, 1H), 7.10 (d, J = 6.8 Hz, 1H), 4.52 (m, 1H), 4.10

(d, J = 6.0 Hz, 2H), 3.62 (t, J = 7.5 Hz, 2H), 2.98 (t, J = 7.8 Hz, 2H), 2.50 (s, 3H), 2.25 (m, 2H), 1.85 (m, 2H), 1.55 (m, 4H).

isomer: more polar isomer from silica gel column (yield, 31%)

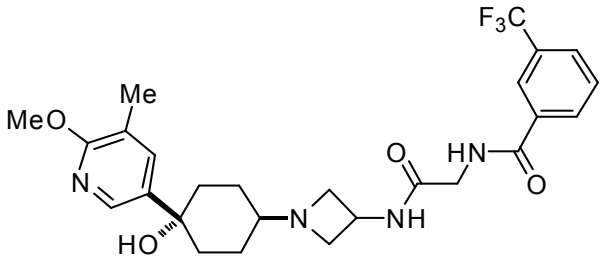
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.60 (s, 1H), 8.18 (s, 1H), 8.10 (d, J = 6.5 Hz, 1H), 7.80 (d, J = 6.8 Hz, 1H), 7.76 (d, J = 6.5 Hz, 1H), 7.60 (t, J = 7.2 Hz, 1H), 7.45 (m, 1H), 7.12 (d, J = 6.8 Hz, 1H), 4.62 (m , 1H), 4.17 (d, J = 5.8 Hz, 2H), 3.38 (br, s, 2H), 2.75 (m, 4H), 1.85 (m , 2H), 1.70 (m, 2H).

N-({1-[4-(6-Dimethylamino-pyridin-3-yl)-4-hydroxy-cyclohexyl]-azetidin-3-ylcarbamoyl}-methyl)-3-trifluoromethyl-benzamide (**2g**)



Yield, 35%. The isomer was not characterized. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (d, J = 2.3 Hz, 1H), 8.07 (s, 1H), 7.97 (d, J = 7.8 Hz, 1H), 7.70 (d, J = 8.6 Hz, 1H), 7.59 (dd, J = 8.8, 2.5 Hz, 1H), 7.46 - 7.54 (m, 2H), 4.46 - 4.57 (m, 1H), 4.09 (d, J = 5.1 Hz, 2H), 3.61 - 3.71 (m, 2H), 3.21 (br. s., 6H), 2.44 (br s., 1H), 2.11 - 2.23 (m, 2H), 1.75 - 1.86 (m, 2H), 1.65 (d, J = 6.3 Hz, 4H), 1.52 (d, J = 14.9 Hz, 2H).

N-({1-[4-Hydroxy-4-(6-methoxy-5-methyl-pyridin-3-yl)-cyclohexyl]-azetidin-3-ylcarbamoyl}-methyl)-3-trifluoromethyl-benzamide (**2h**)



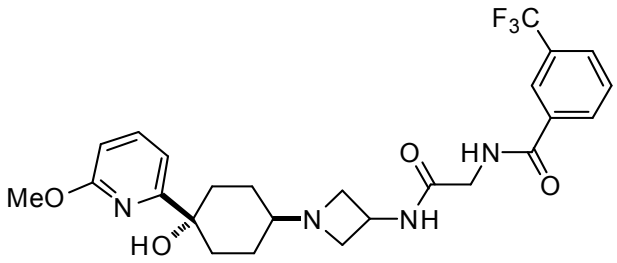
2h: less polar isomer from silica gel column (yield, 30%)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (s, 1H), 8.02 (d, J = 6.5 Hz, 1H), 7.80 (d, J = 6.6 Hz, 1H), 7.55 (t, J = 6.6 Hz, 1H), 7.60 (s, 1H), 6.72 (d, J = 6.4 Hz, 1H), 4.50 (m, 1H), 4.18 (d, J = 4.3 Hz, 2H), 3.60 (t, J = 6.8 Hz, 2H), 2.90 (t, J = 6.5 Hz, 2H), 2.20 (m, 2H), 1.85 (m, 2H), 1.62 (m, 2H), 1.43 (m, 2H).

isomer: more polar isomer from silica gel column (yield, 35%)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (s, 1H), 8.05 (d, J = 6.6 Hz, 1H), 7.78 (d, J = 6.5 Hz, 1H), 7.58 (t, J = 6.7 Hz, 1H), 7.55 (s, 1H), 6.95 (d, J = 6.6 Hz, 1H), 4.55 (m, 1H), 4.20 (d, J = 3.5 Hz, 2H), 3.66 (t, J = 7.5 Hz, 2H), 3.10 (t, J = 7.5 Hz, 2H), 1.90 ~ 1.68 (m, 6H), 1.60 (m, 2H).

N-({1-[4-Hydroxy-4-(6-methoxy-pyridin-2-yl)-cyclohexyl]-azetidin-3-ylcarbamoyl}-methyl)-3-trifluoromethyl-benzamide (**2i**)



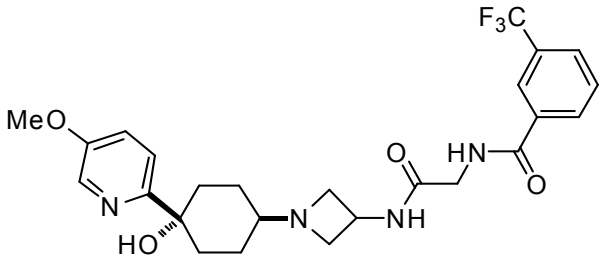
2i: less polar isomer from silica gel column (yield, 45%)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (s, 1H), 8.02 9d, J = 5.0 Hz, 1H), 7.75 (d, J = 6.0 Hz, 1H), 7.55 (m, 1H), 7.41 (m, 1H), 7.02 (d, J = 6.6 Hz, 1H), 6.62 (d, J = 6.8 Hz, 1H), 4.54 (m, 1H), 4.18 (d, J = 3.5 Hz, 2H), 3.62 (t, J = 6.8 Hz, 2H), 2.95 (t, J = 6.5 Hz, 2H), 2.10 (m, 2H), 1.90 (m, 2H), 1.52 (m, 2H), 1.45 (M, 2H).

isomer: more polar isomer from silica gel column (yield, 32%)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (s, 1H), 8.08 (d, J = 5.0 Hz, 1H), 7.85 (t, J = 4.5 Hz, 1H), 7.80 (d, J = 6.5 Hz, 1H), 7.60 (m, 2H), 6.90 (d, J = 6.6 Hz, 1H), 6.62 (d, J = 6.8 Hz, 1H), 4.52 (m, 1H), 4.20 (d, J = 5.8 Hz, 2H), 3.65 (t, J = 6.4 Hz,1H), 3.05 (t, J = 6.6 Hz, 2H), 1.85~ 1.48 (m, 8H).

N-({1-[4-Hydroxy-4-(5-methoxy-pyridin-2-yl)-cyclohexyl]-azetidin-3-ylcarbamoyl}-methyl)-3-trifluoromethyl-benzamide (**2j**)



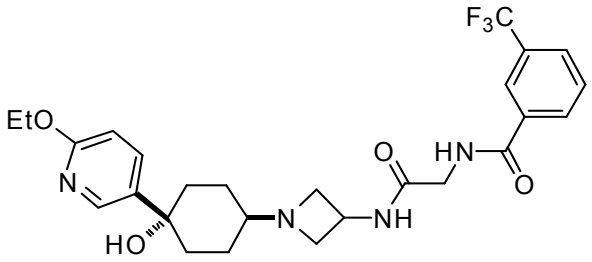
2j: less polar isomer from silica gel column (yield, 28%)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (s, 1H), 8.14 (s, 1H), 8.10 (s, 1H), 8.02 (d, J = 6.5 Hz, 1H), 7.72 (d, J = 7.1 Hz, 1H), 7.52 (t, J = 6.8 Hz, 1H), 7.40 (s, 1H), 4.50 (m, 1H), 4.18 (d, J = 3.0 Hz, 2H), 3.55 (t, J = 6.0 Hz, 2H), 2.85 (t, J = 6.0 Hz, 2H), 2.31 (s, br, 1H), 2.15 (t, J = 9.0 Hz, 2H), 1.85 (m, 2H), 1.45 (m, 4H).

isomer: more polar isomer from silica gel column (yield, 32%)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (s, 1H), 8.23 (d, J = 6.8 Hz, 2H), 8.05 (d, J = 6.7 Hz, 1H), 7.78 (m, 2H), 7.55 (m, 2H), 7.44 (s, 1H), 4.55 (m, 1H), 4.15 (d, J = 3.0 Hz, 2H), 3.65 (t, J = 7.0 Hz, 2H0, 3.10 (d, J = 5.8 Hz, 2H), 2.15 (s, br, 1H), 1.95~1.55 (m, 8H).

N-({1-[4-(6-Ethoxy-pyridin-3-yl)-4-hydroxy-cyclohexyl]-azetidin-3-ylcarbamoyl}-methyl)-3-trifluoromethyl-benzamide (**2k**)



2k: less polar isomer from silica gel column (yield, 37%)

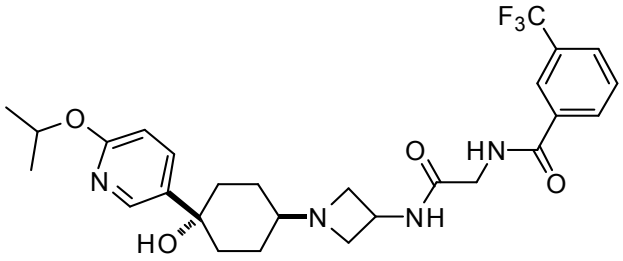
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (s, 1H), 8.15 (s, 1H), 8.08 (m, 2H), 7.80 (m, 2H), 7.62 (m, 2H), 6.75 (d, J = 6.5 Hz, 1H), 4.53 (m, 1H), 4.35 (q, J = 7.8 Hz, 2H), 4.22 (d, J = 6.1 Hz, 2H), 3.75 (t, J = 6.5 Hz, 2H), 2.95 (t, J = 6.5 Hz, 2H), 2.40 (m, 2H), 2.26 (m, 2H), 1.92 (m, 2H), 1.65 (m, 2H), 1.48 (t, J = 7.5 Hz, 3H).

isomer: less polar isomer from silica gel column (yield, 39%)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (s, 1H), 8.15 9s, 1H), 8.04 (d, J = 6.2 Hz, 1H), 7.85 (t, J = 5.5 Hz, 1H), 7.72 (d, J = 6.7 Hz, 1H), 7.70 (t, J = 6.4 Hz, 1H), 6.68 (d, J = 7.0 Hz, 1H), 6.10 (s,

br, 1H), 5.72 (s, br, 1H), 4.51 (m,1H), 4.30 (q, J = 7.8 Hz, 2H), 4.18 (d, J = 3.5 Hz, 2H), 3.62 (t, J = 6.9 Hz, 2H), 3.10 (t, J = 6.5 Hz, 2H), 1.85 (m, 2H), 1.70 (m, 4H), 1.55 (m, 2H), 1.38 (t, J = 8.0 Hz, 3H).

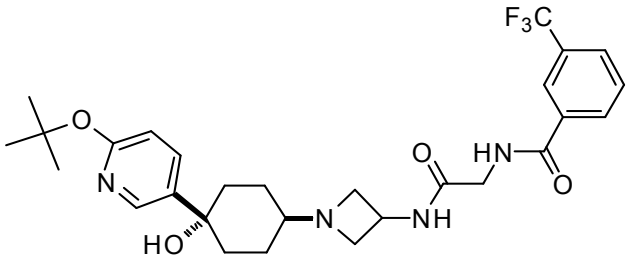
N-({1-[4-Hydroxy-4-(6-isopropoxy-pyridin-3-yl)-cyclohexyl]-azetidin-3-ylcarbamoyl}-methyl)-3-trifluoromethyl-benzamide (**2m**)



NaH (95%, 51 mg, 2 mmol) was added into IPA (4 mL) at 0°C slowly until bubble disappeared. To this solution was added N-({1-[4-(6-fluoro-pyridin-3-yl)-4-hydroxy-cyclohexyl]-azetidin-3-ylcarbamoyl}-methyl)-3-trifluoromethyl-benzamide (**2c**, 200 mg, 0.40 mmol) in DMF (1 mL). After addition, the reaction was heated for another 2 hours at 80°C. The reaction solution was quenched with MeOH and partitioned between DCM and water. The organic layer was separated and the aqueous layer was extracted 3 times with chloroform and IPA "cocktail" (~ 3:1, v/v). The combined organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product, which was then purified by a CombiFlash system using ethyl acetate and 7N NH_3 in MeOH as eluent (from pure ethyl acetate to 5% 7N NH_3 in MeOH in ethyl acetate) to afford the title compound as a white solid (110 mg, yield, 51%) .

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (s, 1H), 8.15 (s, 1H), 8.05 (d, J = 6.8 Hz, 1H), 7.75 (d, J = 6.8 Hz, 1H), 7.70 (d, J = 6.8 Hz, 1H), 7.52 (t, J = 6.5 Hz, 1H), 6.68 (d, J = 6.0 Hz, 1H), 5.20 (m, 1H), 4.62 (m, 1H), 4.15 (d, J = 4.0 Hz, 2H), 3.60 (t, J = 7.0 Hz, 2H), 3.05 (s., br, 2H), 2.30 (s, 1H), 2.20 (m, 2H), 2.05 (m, 2H), 1.65 (m, 2H), 1.52 (m, 2H), 1.31 (d, J = 6.0 Hz, 6H).

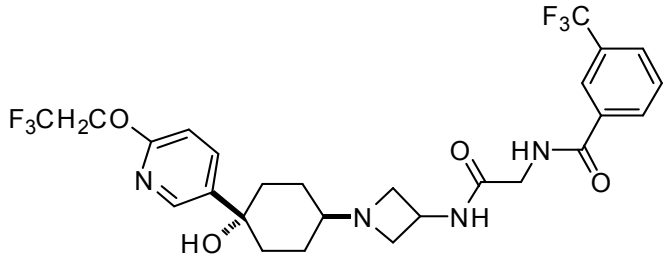
N-({1-[4-(6-tert-Butoxy-pyridin-3-yl)-4-hydroxy-cyclohexyl]-azetidin-3-ylcarbamoyl}-methyl)-3-trifluoromethyl-benzamide (**2n**)



The compound was prepared as a white solid from **2c** and t-BuOK using the procedure described for the synthesis of **2m**.

Yield, 25%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (s, 1H), 8.10 (s, 1H), 8.00 (d, J = 6.5 Hz, 1H), 7.80 (d, J = 6.5 Hz, 1H), 7.61 (d, J = 6.6 Hz, 1H), 7.52 (t, J = 5.5 Hz, 1H), 7.35 (m, 1H), 6.88 (m, 1H), 6.60 (d, J = 6.8 Hz, 1H), 4.52 (m, 1H), 4.15 (d, J = 4.0 Hz, 2H), 3.65 (t, J = 7.0 Hz, 2H), 2.95 (t, J = 7.0 Hz, 2H), 2.30 (s, 1H), 2.21 (m, 1H), 1.80 (m, 6H), 1.52 (s, 9H), 1.35 (m, 2H).

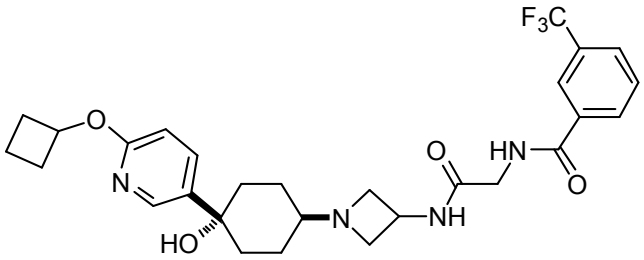
N-[(1-{4-Hydroxy-4-[6-(2,2,2-trifluoro-ethoxy)-pyridin-3-yl]-cyclohexyl}-azetidin-3-ylcarbamoyl)-methyl]-3-trifluoromethyl-benzamide (**2l**)



The compound was prepared as a white solid from **2c** and CF_3CH_2OH (Sigma) using the procedure described for the synthesis of **2m**.

Yield, 30%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (s, 1H), 8.15 (s, 1H), 8.05 (d, J = 6.4 Hz, 1H), 7.78 (d, J = 6.6 Hz, 1H), 7.70 (t, J = 6.6 Hz, 1H), 7.65 (m, 1H), 7.55 (t, J = 5.8 Hz, 2H), 6.70 (d, J = 6.8 Hz, 1H), 4.75 (q, J = 8.5 Hz, 2H), 4.55 (m, 1H), 4.15 (d, J = 4.0 Hz, 2H), 3.62 (t, J = 6.0 Hz, 2H), 2.95 (t, J = 7.0 Hz, 2H), 2.30 (s, 1H), 1.85 (m, 4H), 1.55 (m, 2H), 1.45 (m, 2H).

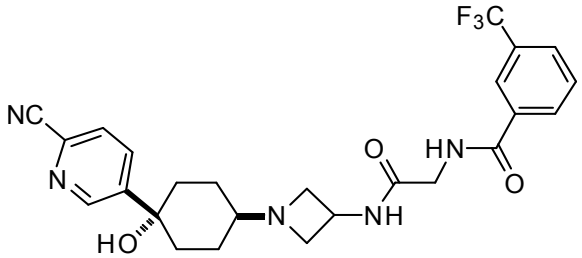
N-({1-[4-(6-Cyclobutoxy-pyridin-3-yl)-4-hydroxy-cyclohexyl]-azetidin-3-ylcarbamoyl}-methyl)-3-trifluoromethyl-benzamide (**2o**)



The compound was prepared as a white solid from **2c** and cyclobutanol (Aldrich) using the procedure for the synthesis **2m**.

Yield, 27%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (s, 1H), 8.10 (s, 1H), 8.01 (d, J = 6.5 Hz, 1H), 7.75 (d, J = 6.6 Hz, 1H), 7.70 (d, J = 6.8 Hz, 1H), 7.55 (t, J = 6.6 Hz, 1H), 7.25 (m, 1H), 6.65 (d, J = 6.0 Hz, 1H), 5.10 (m, 1H), 4.50 (m, 1H), 4.10 (d, J = 4.0 Hz, 2H), 3.60 (t, J = 7.0 Hz, 2H), 2.90 (t, J = 7.0 Hz, 2H), 2.40 (m, 1H), 2.37 (m, 4H), 2.15 (m, 2H), 2.10 (m, 2H), 1.83 (m, 2H), 1.80 ~ 1.45 (4H).

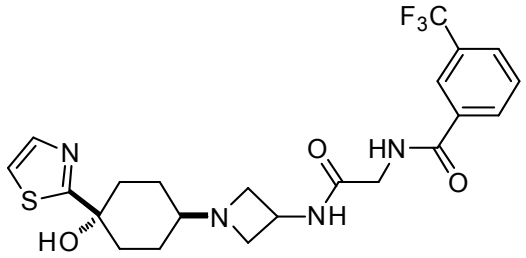
N-({1-[4-(6-Cyano-pyridin-3-yl)-4-hydroxy-cyclohexyl]-azetidin-3-ylcarbamoyl}-methyl)-3-trifluoromethyl-benzamide (**2d**)



N-({1-[4-(6-fluoro-pyridin-3-yl)-4-hydroxy-cyclohexyl]-azetidin-3-ylcarbamoyl}-methyl)-3-trifluoromethyl-benzamide **6a** (70 mg, 0.14 mmol), KCN (Aldrich, 46 mg, 0,70 mmol) and 18-crown-6 (Aldrich, 190 mg, 0.70 mmol) in DMF (1 mL) were heated to 150 °C in a sealed tube overnight. The reaction solution was loaded on a silica gel column with a CombiFlash system using ethyl acetate and 7N NH_3 in MeOH as eluent (from pure ethyl acetate to 5% 7N NH_3 in MeOH in ethyl acetate) to afford the title compound as a white solid (34 mg, yield, 48%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (s, 1H), 8.15 (s, 1H), 8.01 (d, J = 6.0 Hz, 1H), 7.80 (d, J = 6.5 Hz, 1H), 7.61 (t, J = 6.8 Hz, 1H), 7.35 (s, br, 1H), 7.88 (d, J = 6.2 Hz, 1H), 4.52 (m, 1H), 4.18 (d, J = 3.0 Hz, 2H), 3.62 (t, J = 7.0 Hz, 2H), 2.90 (t, J = 6.8 Hz, 2H), 2.32 (s, 1H), 2.20 (m, 2H), 1.80 (4H), 1.55 (m, 2H).

N-{[1-(4-Hydroxy-4-thiazol-2-yl-cyclohexyl)-azetidin-3-ylcarbamoyl]-methyl}-3-trifluoromethyl-benzamide (**8a**)



8-Thiazol-2-yl-1,4-dioxa-spiro[4.5]decan-8-ol

A solution of *n*-BuLi (2.5 M in hexanes, 26 mL, 65 mmol) was dropped slowly into a solution of thiazole (Aldrich, 5.0 g, 59 mmol) in THF (50 mL) at –78 °C over 10 min. The reaction was stirred for additional 20 min. at –78 °C. A solution of 1,4-dioxa-spiro[4.5]decan-8-one (9.36 g, 60 mmol) in THF (20 mL) was slowly dropped into the reaction. After addition, the reaction was stirred for additional 2 hours at –78 °C. The reaction was then quenched with water solution and warmed to room temperature. The solvent was removed *in vacuo* and the residue was partitioned between ethyl acetate and water. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give yellow solid, which was then purified by silica gel column on a CombiFlash system using hexanes and ethyl acetate (from 10% ethyl acetate to 100% ethyl acetate) to afford the title compound as a white solid (10.1 g, 71%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (s, 1H), 7.28 (s, 1H), 4.01 (m, 4H), 3.30 (s, 1H), 2.35 (m, 2H), 2.23 (m, 2H), 2.05 (m, 2H), 1.85 (m, 2H).

4-Hydroxy-4-thiazol-2-yl-cyclohexanone

The compound was prepared as a white solid from de-protection of 8-thiazol-2-yl-1,4-dioxa-spiro[4.5]decan-8-ol, as prepared using the procedure described in the synthesis of **2f**.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (s, 1H), 7.32 (s, 1H), 2.93 (m, 2H), 2.40 (m, 4H), 2.31 (m, 2H).

N-{[1-(4-Hydroxy-4-thiazol-2-yl-cyclohexyl)-azetidin-3-ylcarbamoyl]-methyl}-3-trifluoromethyl-benzamide (**8a**)

The compound was prepared as a white solid from reductive amination of 4-hydroxy-4-thiazol-2-yl-cyclohexanone and N-(azetidin-3-ylcarbamoylmethyl)-3-trifluoromethyl-benzamide using the procedure described in the synthesis of **2f**.

8a: less polar isomer from silica gel column (yield, 39%)

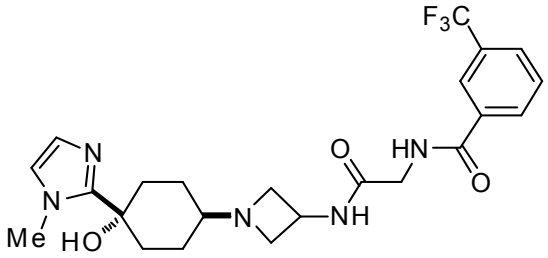
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (s, 1H), 8.02 (d, J = 6.0 Hz, 1H), 7.75 (d, J = 6.5 Hz, 1H), 7.70 (m, 1H), 7.65 (s, 1H), 7.55 (t, J = 6.8 Hz, 1H), 7.43 (d, J = 6.0 Hz, 1H), 7.22 (d, J = 6.2 Hz, 1H), 4.48 (m, 1H), 4.18 (d, J = 6.7 Hz, 2H), 3.51 (t, J = 7.0 Hz, 2H), 2.98 (t, J = 7.0 Hz, 2H), 2.30 (m, 2H), 1.81 (m, 2H), 1.68 (m, 2H), 1.48 (m, 2H).

isomer: more polar isomer from silica gel column (yield, 35%)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (s, 1H), 8.02 (d, J = 7.0 Hz, 1H), 7.75 (d, J = 7.0 Hz, 1H), 7.70 (s, 1H), 7.55 (t, J = 6.5 Hz, 1H), 7.32 (d, 4.5 Hz, 1H), 7.01 (d, J = 4.0 Hz, 1H), 4.55 (m, 1H), 4.15 (d, J = 7.8 Hz, 2H), 3.65 9t, J = 7.5 Hz, 2H), 3.08 (t, J = 7.5 Hz, 2H), 2.05 (m, 2H), 1.80 (m, 4H), 1.70 (m, 2H).

The following compounds were analogously synthesized from reductive amination of the corresponding cyclohexanones, as prepared in a similar synthesis of 4-hydroxy-4-(6-methoxy-pyridin-3-yl)-cyclohexanone, and N-(azetidin-3-ylcarbamoylmethyl)-3-trifluoromethyl-benzamide using the procedure described for the synthesis of **2f**.

N-({1-[4-Hydroxy-4-(1-methyl-1H-imidazol-2-yl)-cyclohexyl]-azetidin-3-ylcarbamoyl}-methyl)-3-trifluoromethyl-benzamide (**4**)



4: less polar isomer from silica gel column (yield, 40%)

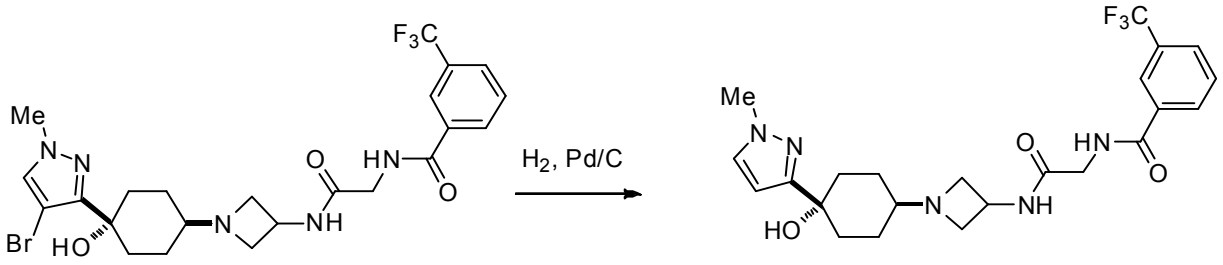
^{1}H NMR (400 MHz, *d*$_4$-MeOH) δ 8.15 (s, 1H), 8.06 (d, J = 6.5 Hz, 1H), 7.80 (d, J = 6.4 Hz, 1H), 7.65 (t, J = 6.5 Hz, 1H), 6.93 (d, J = 4.5 Hz, 1H), 6.75 (d, J = 4.0 Hz, 1H), 4.34 (m, 1H), 3.98 (s, 2H), 3.79 (s, 3H), 3.65 (t, J = 6.0 Hz, 2H), 3.04 (t, J = 6.0 Hz, 2H), 2.35 (m, 1H), 2.30 (m, 2H), 1.85 (m, 2H), 1.65 (m, 2H), 1.30 (m, 2H).

isomer: more polar isomer from silica gel column (yield, 38%)

^{1}H NMR (400 MHz, *d*$_4$-MeOH) δ 8.14 (s, 1H), 8.06 (d, J = 6.0 Hz, 1H), 7.78 (d, J = 6.2 Hz, 1H), 7.58 (t, J = 6.5 Hz, 1H), 6.88 (s, 1H), 6.72 (s, 1H), 4.41 (m, 1H), 3.98 (s, 2H), 3.90 (t, J = 6.0 Hz,

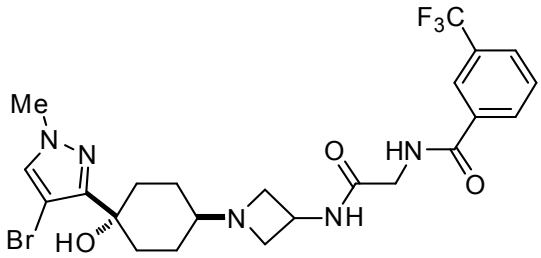
2H), 3.80 (s, 3H), 3.12 (s, 3H), 3.36 (t, J = 6.0 Hz, 2H), 2.55 (m, 1H), 2.16 (m, 2H), 1.85 (m, 2H), 1.35 (m, 2H), 1.70 (m, 2H), 1.55 (m, 2H).

N-({1-[4-Hydroxy-4-(1-methyl-1H-pyrazol-3-yl)-cyclohexyl]-azetidin-3-ylcarbamoyl}-methyl)-3-trifluoromethyl-benzamide (**5**)



5

N-({1-[4-(4-Bromo-1-methyl-1H-pyrazol-3-yl)-4-hydroxy-cyclohexyl]-azetidin-3-ylcarbamoyl}-methyl)-3-trifluoromethyl-benzamide



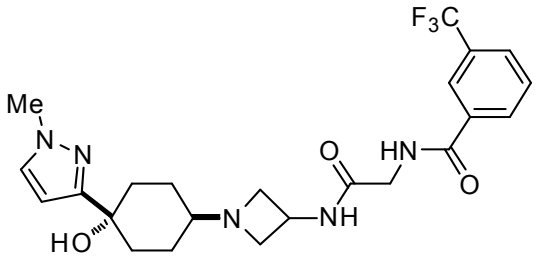
The compound: less polar isomer from silica gel column (yield, 30%)

^{1}H NMR (400 MHz, *d*$_4$-MeOH) δ 8.25 (s, 1H), 8.16 (d, J = 6.5 Hz, 1H), 7.90 (d, J = 6.4 Hz, 1H), 7.68 (t, J = 6.5 Hz, 1H), 7.32 (s, 1H), 4.42 (m, 1H), 4.11 (s, 3H), 4.05 (s, 2H), 3.68 (t, J = 6.0 Hz, 2H), 3.05 (t, J = 6.0 Hz, 2H), 2.55 (m, 2H), 2.40 (m, 1H), 1.96 (m, 2H), 1.70 (m, 2H), 1.55 (m, 2H).

isomer: more polar isomer from silica gel column (yield, 27%)

^{1}H NMR (400 MHz, *d*$_4$-MeOH) δ 8.24 (s, 1H), 8.20 (d, J = 6.6 Hz, 1H), 7.88 (d, J = 6.4 Hz, 1H), 7.70 (t, J = 6.8 Hz, 1H), 7.35 (s, 1H), 4.50 (m, 1H), 4.15 (s, 3H), 4.06 (s, 2H), 3.75 (t, J = 6.8 Hz, 2H), 3.12 (t, J = 6.8 Hz, 2H), 2.35 (m, 3H), 1.95 (m, 2H), 1.75 (m, 2H), 1.60 (m, 2H).

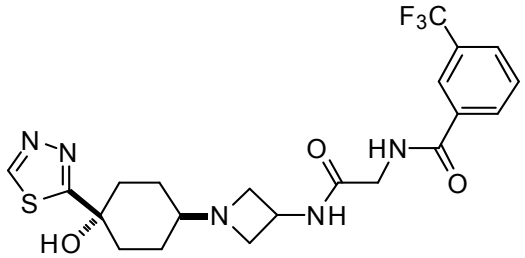
N-({1-[4-Hydroxy-4-(1-methyl-1H-pyrazol-3-yl)-cyclohexyl]-azetidin-3-ylcarbamoyl}-methyl)-3-trifluoromethyl-benzamide (**5**)



N-({1-[4-(4-Bromo-1-methyl-1H-pyrazol-3-yl)-4-hydroxy-cyclohexyl]-azetidin-3-ylcarbamoyl}-methyl)-3-trifluoromethyl-benzamide from the previous step (100 mg, 0.18 mmol) in MeOH (25 mL) was passed through a 10% Pd on C cartridge in a H-Cube under full hydrogen mode (flow rate 1 mL/min). The resulting solution was concentrated to give the title compound as a yellow solid (57 mg, yield, 66%).

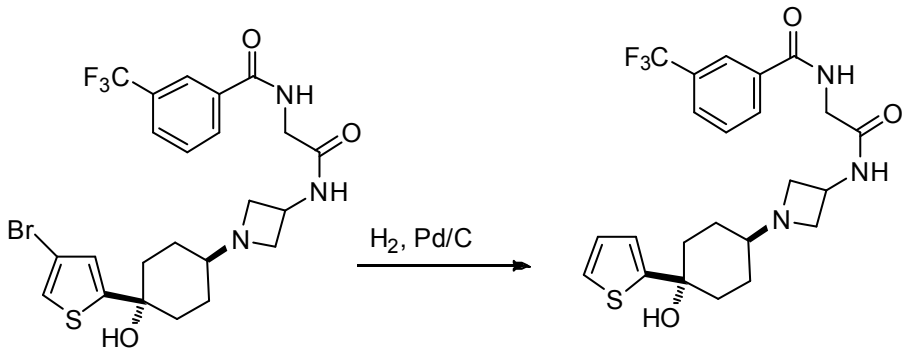
^{1}H NMR (400 MHz, d_4-MeOH) δ 8.21 (s, 1H), 8.20 (d, J = 6.5 Hz, 1H), 7.90 (d, J = 6.5 Hz, 1H), 7.72 (t, J = 6.5 Hz, 1H), 7.32 (s, 1H), 6.28 (s, 1H), 4.51 (m, 1H), 4.15 (s, 2H), 4.08 (s, 3H), 3.95 (t, J = 6.0 Hz, 2H), 3.52 (t, J = 6.0 Hz, 2H), 2.85 (m, 1H), 2.26 (m, 2H), 2.02 (m, 2H), 1.82 (m, 2H), 1.45 (m, 2H).

N-{[1-(4-Hydroxy-4-[1,3,4]thiadiazol-2-yl-cyclohexyl)-azetidin-3-ylcarbamoyl]-methyl}-3-trifluoromethyl-benzamide (**6**)



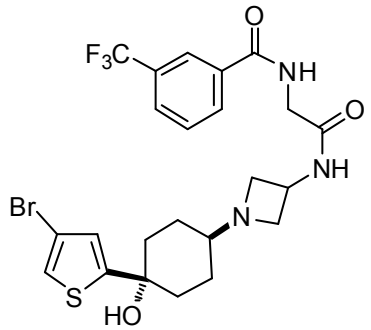
Yield, 25%. The isomer was not characterized. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.10 (s, 1H), 8.10 (s, 1H), 7.71 (d, J = 5.8 Hz, 1H), 7.30 (d, J = 6.5 Hz, 1H), 7.25 (t, J = 5.6 Hz, 1H), 7.18 (s, 1H), 6.65 (m, 1H), 4.50 (m, 1H), 4.18 (d, J = 4.2 Hz, 2H), 3.60 (t, J = 7.0 Hz, 2H), 3.10 (d, J = 6.5 Hz, 2H), 2.45 (m, 1H), 2.35 (m, 1H), 2.20 (m, 2H), 1.95 (m, 4H), 1.60 (m, 2H).

N-{[1-(4-Hydroxy-4-thiophen-2-yl-cyclohexyl)-azetidin-3-ylcarbamoyl]-methyl}-3-trifluoromethyl-benzamide (**7**)



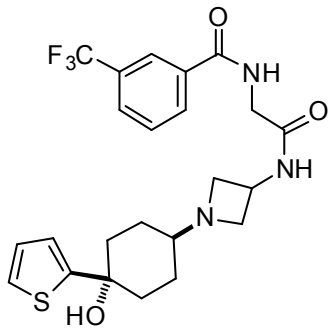
7

N-({1-[4-(4-Bromo-thiophen-2-yl)-4-hydroxy-cyclohexyl]-azetidin-3-ylcarbamoyl}-methyl)-3-trifluoromethyl-benzamide



Yield, 42%. The isomer was not characterized. ^{1}H NMR (400 MHz, d_4-MeOH) δ 8.12 (s, 1H), 8.04 (d, J = 7.8 Hz, 1H), 7.76 (d, J = 7.8 Hz, 1H), 7.59 (t, J = 7.8 Hz, 1H), 7.15 (d, J = 5.3 Hz, 1H), 6.82 (d, J = 5.3 Hz, 1H), 4.34 (t, J = 6.8 Hz, 1H), 3.50 - 3.58 (m, 4H), 2.82 (t, J = 7.5 Hz, 2H), 2.46 - 2.60 (m, 2H), 2.22 (t, J = 3.4 Hz, 1H), 1.66 - 1.80 (m, 2H), 1.49 (d, J = 13.6 Hz, 2H), 1.36 (dd, J = 13.9, 4.5 Hz, 2H).

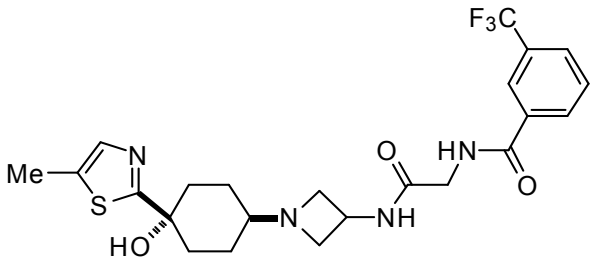
N-{[1-(4-Hydroxy-4-thiophen-2-yl-cyclohexyl)-azetidin-3-ylcarbamoyl]-methyl}-3-trifluoromethyl-benzamide (**7**)



The compound was prepared as a white solid from reductive de-bromination (hydrogenation) of N-({1-[4-(4-bromo-thiophen-2-yl)-4-hydroxy-cyclohexyl]-azetidin-3-ylcarbamoyl}-methyl)-3-trifluoromethyl-benzamide using the procedure described in the synthesis of **5**.

^{1}H NMR (400 MHz, *d*$_4$-MeOH) δ 8.12 (s, 1H), 8.06 (d, J = 7.8 Hz, 1H), 7.77 (d, J = 7.8 Hz, 1H), 7.60 (t, J = 7.8 Hz, 1H), 7.22 (d, J = 5.1 Hz, 1H), 6.96 - 7.07 (m, 1H), 6.82 - 6.90 (m, 1H), 4.43 - 4.57 (m, 1H), 4.14 (t, J = 8.3 Hz, 2H), 3.96 (br s, 2H), 3.21 (d, J = 1.8 Hz, 2H), 3.14 (br. s., 1H), 2.17 - 2.32 (m, 2H), 1.89 - 2.00 (m, 2H), 1.74 (t, J = 13.4 Hz, 2H), 1.38 (d, J = 9.3 Hz, 2H).

N-({1-[4-Hydroxy-4-(5-methyl-thiazol-2-yl)-cyclohexyl]-azetidin-3-ylcarbamoyl}-methyl)-3-trifluoromethyl-benzamide (**8b**)



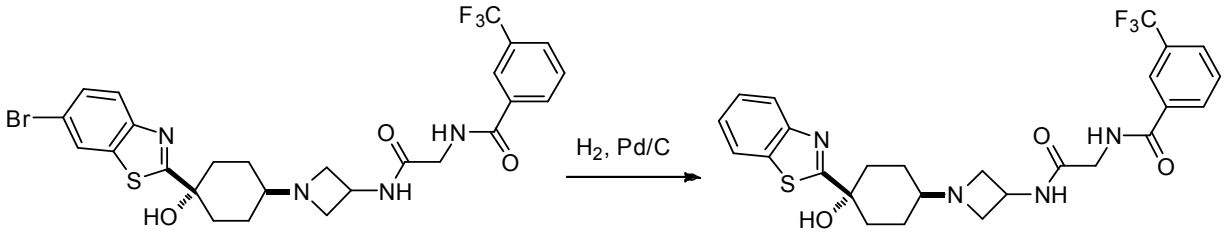
8b: less polar isomer from silica gel column (yield, 35%)

^{1}H NMR (400 MHz, *d*$_4$-MeOH) δ 8.21 (s, 1H), 8.15 (d, J = 6.5 Hz, 1H), 7.82 (d, J = 6.0 Hz, 1H), 7.65 (t, J = 7.2 Hz, 1H), 4.40 (m, 1H), 3.55 (t, J = 7.5 Hz, 2H), 3.30 (s, 3H), 2.98 (t, J = 7.2 Hz, 2H), 2.30 (m, 2H), 1.80 (m, 2H), 1.65 (m, 2H), 1.42 (m, 2H).

isomer: more polar isomer from silica gel column (yield, 41%)

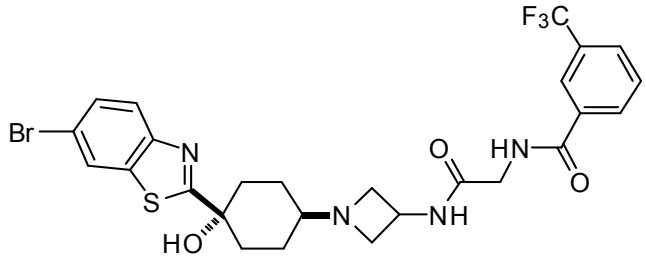
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (s, 1H), 8.06 (d, J = 6.0 Hz, 1H), 7.78 (d, J = 6.4 Hz, 1H), 7.62 (t, J = 6.5 Hz, 1H), 7.32 (s, 1H), 4.62 (m, 1H), 4.18 (d, J = 4.5 Hz, 2H), 3.89 (t, J = 6.8 Hz, 2H), 3.50 (s, br, 2H), 2.52 (s, br, 1H), 2.10 ~ 1.89 (m, 3H), 1.82 ~ 1.63 (m, 3H).

N-{[1-(4-Benzothiazol-2-yl-4-hydroxy-cyclohexyl)-azetidin-3-ylcarbamoyl]-methyl}-3-trifluoromethyl-benzamide (**8c**)



8c

N-({1-[4-(6-Bromo-benzothiazol-2-yl)-4-hydroxy-cyclohexyl]-azetidin-3-ylcarbamoyl}-methyl)-3-trifluoromethyl-benzamide



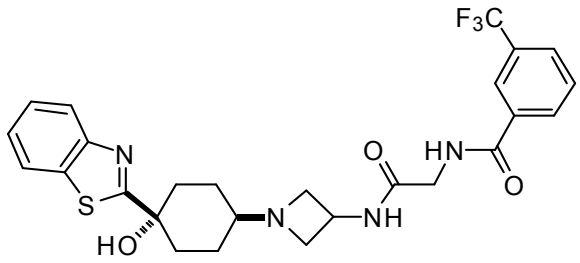
The compound : less polar isomer from silica gel column (yield, 33%)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (s, 1H), 8.02, 8.00 (s, 1H), 7.80 (d, J = 6.5 Hz, 1H), 7.77 (d, J = 6.2 Hz, 1H), 7.61 (m, 1H), 7.57 (m, 2H), 4.55 (m, 1H), 4.20 (s, 2H), 3.60 (t, J = 6.5 Hz, 2H), 3.10 (t, J = 6.4 Hz, 2H), 2.38 (m, 3H), 1.85 (m, 2H), 1.74 (m, 2H), 1.60 (m, 2H).

isomer: more polar isomer from silica gel column (yield, 25%)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (s, 1H), 8.02 (m, 2H), 7.80 (m, 2H), 7.65 (d, J = 6.5 Hz, 1H), 7.60 (m, 1H), 7.45 (m, 1H), 4.58 (m, 1H), 4.20 (s, 2H), 3.65 (t, J = 6.5 Hz, 2H), 3.02 (t, J = 6.6 Hz, 2H), 2.23 (m, 1H), 2.10 (m, 4H), 1.80 (m, 2H), 1.65 (m, 2H).

N-{[1-(4-Benzothiazol-2-yl-4-hydroxy-cyclohexyl)-azetidin-3-ylcarbamoyl]-methyl}-3-trifluoromethyl-benzamide (**8c**)



The compound was prepared as a white solid from reductive de-bromination (hydrogenation) of N-({1-[4-(6-bromo-benzothiazol-2-yl)-4-hydroxy-cyclohexyl]-azetidin-3-ylcarbamoyl}-methyl)-3-trifluoromethyl-benzamide from the previous step using the procedure described in the synthesis of **5**.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (s, 1H), 8.05 (d, J = 6.6 Hz, 1H), 7.93 (d, J = 6.4 Hz, 1H), 7.89 (d, J = 6.0 Hz, 1H), 7.80 (d, J = 6.7 Hz, 1H), 7.55 (t, J = 6.2 Hz, 1H), 7.48 (t, J = 6.4 Hz, 1H), 7.37 (t, J = 6.1 Hz, 1H), 4.50 (m, 1H), 4.19 (d, J = 3.2 Hz, 2H), 3.64 (t, J = 6.8 Hz, 2H), 3.10 (s, 1H), 3.02 (t, J = 6.7 Hz, 2H), 2.40 (m, 1H), 2.25 (m, 2H), 1.85 (m, 2H), 1.72 (m, 2H), 1.60 (m, 2H).

N-{[1-(4-Hydroxy-4-thiazol-5-yl-cyclohexyl)-azetidin-3-ylcarbamoyl]-methyl}-3-trifluoromethyl-benzamide (**8d**)

8-Thiazol-5-yl-1,4-dioxa-spiro[4.5]decan-8-ol

A solution of 2-trimethylsilanyl-thiazole (Fluka, 5.0 g, 31.8 mmol) in THF (20 mL) at –78 °C was treated with n-BuLi (2.5 M in hexanes, 15.3 mL, 38.2 mmol) dropped slowly over 10 min. The reaction was stirred for an additional 20 min. at –78 °C. A solution of 1,4-dioxa-spiro[4.5]decan-8-one (Aldrich, 6.0 g, 38 mmol) in THF (15 mL) was slowly dropped into the reaction. After addition, the reaction was stirred for an additional 2 hours at –78 °C. The reaction was then quenched with water and warmed to room temperature. The solvent was removed in vacuo and the residue was partitioned between ethyl acetate and water. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give a yellow solid. To this solid in THF (20 mL) was added TBAF (1.0 N in THF, 30 mL) at room temperature. The reaction was stirred for 1 hour. The solvent was removed and the residue was partitioned between ethyl acetate and water. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered, concentrated and then purified by silica gel column on a CombiFlash system using hexanes and ethyl acetate (from 10% ethyl acetate to 100% ethyl acetate) to afford the title compound as a white solid (4.22 g, yield, 55%).

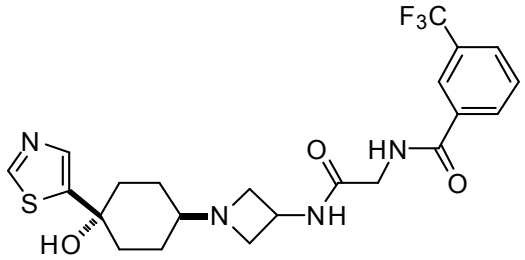
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.65 (s, 1H), 7.72 (s, 1H), 4.01 (m, 4H), 2.20 (m, 2H), 2.08 (m, 4H), 1.69 (m, 2H).

4-Hydroxy-4-thiazol-5-yl-cyclohexanone

8-Thiazol-5-yl-1,4-dioxa-spiro[4.5]decan-8-ol (4.35 g, 18.0 mmol) as prepared in the previous step was treated with 1N HCl (~ 10 mL) in acetone (20 mL) at room temperature for 4 hours. The reaction was neutralized with saturated $NaHCO_3$ solution and the solvent was removed. The residue was partitioned between ethyl acetate and water. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give a yellow solid, which was then purified by silica gel column on a CombiFlash system using hexanes and ethyl acetate (from 10% ethyl acetate to 100% ethyl acetate) to afford the title compound as a pale yellow solid (3.2 g, yield, 90%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (s, 1H), 7.73 (s, 1H), 2.96 (m, 2H), 2.35 (m, 4H), 2.23 (m, 2H).

N-{[1-(4-Hydroxy-4-thiazol-5-yl-cyclohexyl)-azetidin-3-ylcarbamoyl]-methyl}-3-trifluoromethyl-benzamide (**8d**)



4-Hydroxy-4-thiazol-5-yl-cyclohexanone, as prepared in the previous step, (2.0g , 10.1 mmol) and N-(azetidin-3-ylcarbamoylmethyl)-3-trifluoromethyl-benzamide, HCl salt (5.1 g, 15.0 mmol) as prepared in Step D of Example 1, in DCM (20 mL) was treated with TEA (2.8 mL, 20 mmol) for 10 min followed by $NaBH(OAc)_3$ (5.28 g, 25 mmol) for another 4 hours at room temperature. The reaction was quenched with saturated sodium bicarbonate. The organic layer was separated and the aqueous layer was extracted 3 times with chloroform and IPA "cocktail" (~ 3:1, v/v). The combined organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product, which was then purified by a CombiFlash system using ethyl acetate and 7N NH_3 in MeOH as eluent (from pure ethyl acetate to 5% 7N NH_3 in MeOH in ethyl acetate) to afford two title compounds as white solids: a less polar isomer and a more polar isomer.

8d: less polar isomer from silica gel column (1.71 g, yield, 35%)

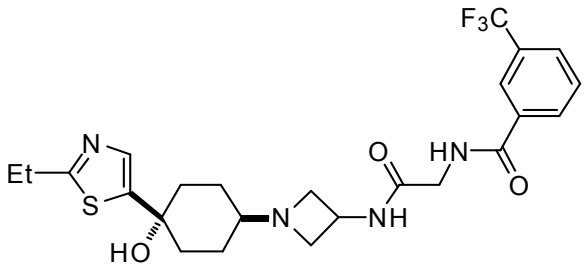
^{1}H NMR (400 MHz, d_4-MeOH) δ 8.90 (s, 1H), 8.25 (s, 1H), 8.20 (d, J = 6.3 Hz, 1H), 7.88 (d, J = 6.5 Hz, 1H), 7.85 (s, 1H), 7.69 (t, J = 6.5 Hz, 1H), 4.45 (m, 1H), 4.05 (s, 2H), 3.68 (t, J = 7.0 Hz,

2H), 3.02 (t, J = 7.0 Hz, 2H), 2.35 (s, br, 1H), 2.25 (m, 2H), 1.90 (m, 2H), 1.82 (m, 2H), 1.38 (m, 2H).

isomer: more polar isomer from silica gel column (1.95 g, yield, 40%)

^{1}H NMR (400 MHz, d_4-MeOH) δ 8.88 (s, 1H), 8.20 (s, 1H), 8.16 (d, J = 6.5 Hz, 1H), 7.87 (d, J = 6.5 Hz, 1H), 7.74 (s, 1H), 7.70 (t, J = 6.5 Hz, 1H), 4.46 (m, 1H), 4.05 (s, 2H), 4.68 (t, J = 7.0 Hz, 2H), 3.70 (t, J = 7.2 Hz, 2H), 3.07 (t, J = 7.0 Hz, 2H), 2.20 (m, 1H), 2.05 (m, 2H), 1.85 (t, J = 7.8 Hz, 2H), 1.74 (m, 2H), 1.65 (m, 2H).

N-({1-[4-(2-Ethyl-thiazol-5-yl)-4-hydroxy-cyclohexyl]-azetidin-3-ylcarbamoyl}-methyl)-3-trifluoromethyl-benzamide (**8e**)



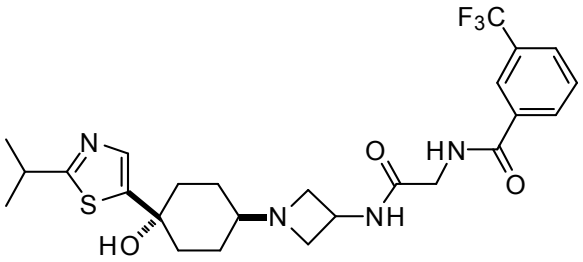
8e: less polar isomer from silica gel column (yield, 31%)

^{1}H NMR (400 MHz, d_4-MeOH) δ 8.16 (s, 1H), 8.08 (d, J = 6.5 Hz, 1H), 7.80 (d, J = 6.6 Hz, 1H), 7.64 (t, J = 6.4 Hz, 1H), 7.49 (s, 1H), 4.38 (m, 1H), 3.98 (s, 2H), 3.58 (d, J = 6.0 Hz, 2H), 2.95 (m, 4H), 2.30 (m, 1H), 2.15 (m, 2H), 1.80 (m, 2H), 1.65 (m, 2H), 1.32 (t, J = 7.5 Hz, 3H), 1.28 (m, 2H).

isomer: more polar isomer from silica gel column (yield, 36%)

^{1}H NMR (400 MHz, d_4-MeOH) δ 8.15 (s, 1H), 8.10 (d, J = 6.8 Hz, 1H), 7.82 (d, J = 6.4 Hz, 1H), 7.65 (t, J = 6.5 Hz, 1H), 7.38 (s, 1H), 4.45 (m, 1H), 4.02 (s, 2H), 3.74 (t, J = 6.0 Hz, 2H), 3.15 (t, J = 7.0 Hz, 2H), 2.92 (q, J = 7.2 Hz, 2H), 2.30 (m, 1H), 2.06 (m, 2H), 1.79 (m, 2H), 1.65 (m, 2H), 1.52 (m, 2H).

N-({1-[4-Hydroxy-4-(2-isopropyl-thiazol-5-yl)-cyclohexyl]-azetidin-3-ylcarbamoyl}-methyl)-3-trifluoromethyl-benzamide (**8f**)



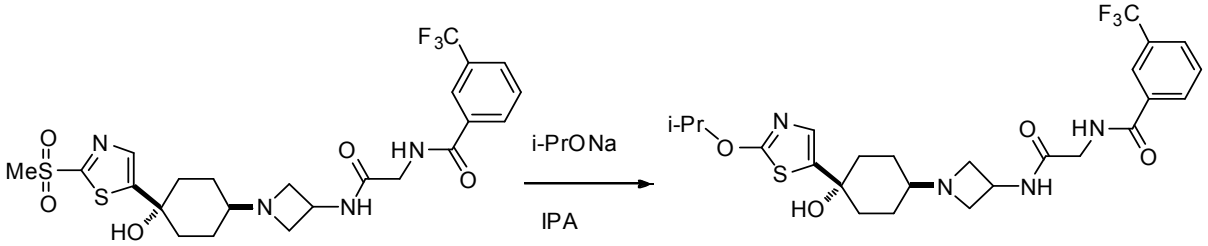
8f: less polar isomer from silica gel column (yield, 42%)

^{1}H NMR (400 MHz, d_4-MeOH) δ 8.25 (s, 1H), 8.12 (d, J = 6.5 Hz, 1H), 7.85 (d, J = 6.6 Hz, 1H), 7.70 (t, J = 6.4 Hz, 1H), 7.50 (s, 1H), 4.42 (m, 1H), 4.05 (s, 2H), 3.65 (d, J = 6.0 Hz, 2H), 3.30 (m, 1H), 3.01 (t, J = 6.0 Hz, 2H), 2.30 (m, 2H), 2.25 (m, 2H), 1.85 (m, 2H), 1.70 (m, 2H), 1.35 (d, J = 7.5 Hz, 6H).

isomer: more polar isomer from silica gel column (yield, 40%)

^{1}H NMR (400 MHz, d_4-MeOH) δ 8.25 (s, 1H), 8.15 (d, J = 6.5 Hz, 1H), 7.90 (d, J = 6.4 Hz, 1H), 7.75 (t, J = 6.5 Hz, 1H), 7.48 (s, 1H), 4.55 (m, 1H), 4.12 (t, J = 6.0 Hz, 2H), 4.05 (s, 2H), 3.72 (t, J = 6.0 Hz, 2H), 2.85 (m, 1H), 2.06 (m, 2H), 1.85 (m, 4H), 1.65 (m, 2H).

N-({1-[4-Hydroxy-4-(2-methanesulfonyl-thiazol-5-yl)-cyclohexyl]-azetidin-3-ylcarbamoyl}-methyl)-3-trifluoromethyl-benzamide (**8g**)



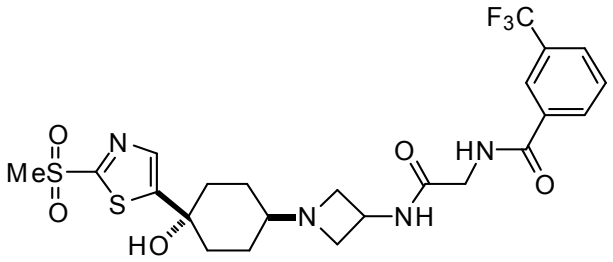
8g

4-Hydroxy-4-(2-methanesulfonyl-thiazol-5-yl)-cyclohexanone

The compound was prepared as a white solid from OXONE oxidation of 8-(2-methylsulfanyl-thiazol-5-yl)-1,4-dioxa-spiro[4.5]decan-8-ol (analogous preparation) .

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (s, 1H), 3.33 (s, 3H), 2.95 (m, 2H), 2.42 (m, 4H), 2.30 (m, 2H).

N-({1-[4-Hydroxy-4-(2-methanesulfonyl-thiazol-5-yl)-cyclohexyl]-azetidin-3-ylcarbamoyl}-methyl)-3-trifluoromethyl-benzamide



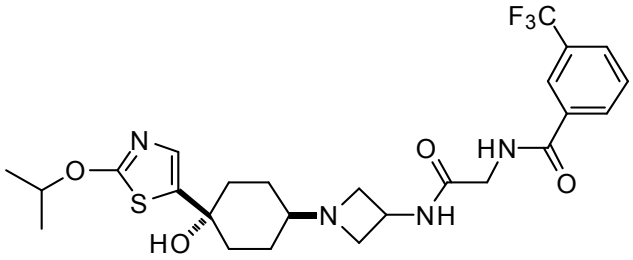
the compound: less polar isomer from silica gel column (yield, 28%)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (s, 1H), 8.05 (d, J = 6.5 Hz, 1H), 7.85 (s, 1H), 7.78 (d, J = 6.6 Hz, 1H), 7.63 (t, J = 6.6 Hz, 1H), 7.45 (t, J = 3.0 Hz,1H), 7.01 (d, J = 5.6 Hz, 1H), 4.65 (m, 1H), 4.15 (d, J = 3.5 Hz, 2H), 3.60 (t, J = 6.5 Hz, 2H), 3.35 (s, 3H), 2.98 (t, J = 6.1 Hz, 2H), 2.35 (m, 1H), 2.22 (m, 2H), 1.87 (m, 2H), 1.79 (m, 2H), 1.46 (m, 2H).

isomer: more polar isomer from silica gel column (yield, 30%)

^{1}H NMR (400 MHz, d_4-MeOH) δ 8.15 (s, 1H), 8.06 (d, J = 6.8 Hz, 1H), 7.80 (d, J = 6.5 Hz, 1H), 7.55 (t, J = 6.6 Hz, 1H), 7.50 (s, 1H), 4.45 (m, 1H), 3.98 (s, 2H), 3.75 (t, J = 6.0 Hz, 2H), 3.21 (d, J = 6.5 Hz, 2H), 2.85 (m, 1H), 2.65 (m, 1H), 2.10 (m, 2H), 2.00 (m, 2H), 1.75 (m, 2H), 1.42 (m, 2H).

N-({1-[4-Hydroxy-4-(2-isopropoxy-thiazol-5-yl)-cyclohexyl]-azetidin-3-ylcarbamoyl}-methyl)-3-trifluoromethyl-benzamide (**8g**)

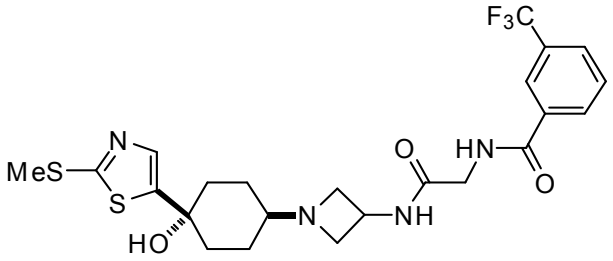


A solution of N-({1-[4-Hydroxy-4-(2-methanesulfonyl-thiazol-5-yl)-cyclohexyl]-azetidin-3-ylcarbamoyl}-methyl)-3-trifluoromethyl-benzamide (less polar isomer from previous step, 100 mg, 0.18 mmol) was treated with i-PrONa (prepared by ~ 30 mg NaH in 3 mL i-PrOH) at 80 °C in a seal tube for 4 hours. The crude solution was directly purified by a CombiFlash system using ethyl acetate and 7N NH_3 in MeOH as eluent (from pure ethyl acetate to 5% 7N NH_3 in MeOH in ethyl acetate) to afford the title compound as a white solid (62 mg, yield, 64%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (s, 1H), 8.03 (d, J = 6.5 Hz, 1H), 7.81 (d, J = 6.5 Hz, 1H), 7.58 (d, J = 6.6 Hz, 1H), 7.35 (m, 1H), 7.25 (d, J = 4.0 Hz,1H), 5.12 (m, 1H), 4.55 (m, 1H), 4.15

(d, J = 3.5 Hz, 2H), 3.60 (t, J = 6.5 Hz, 2H), 3.02 (m, J = 6.1 Hz, 2H), 2.25 (m, 1H), 2.15 (m, 2H), 2.00 (m, 2H), 1.85 (m, 4H), 1.41 (d, J = 7.2 Hz, 6H), 1.35 (m, 2H).

N-({1-[4-Hydroxy-4-(2-methylsulfanyl-thiazol-5-yl)-cyclohexyl]-azetidin-3-ylcarbamoyl}-methyl)-3-trifluoromethyl-benzamide (**8h**)



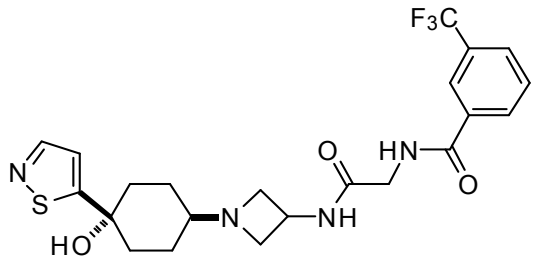
8h: less polar isomer from silica gel column (yield, 41%)

^{1}H NMR (400 MHz, d_4-MeOH) δ 8.10 (s, 1H), 8.05 (d, J = 6.5 Hz, 1H), 7.76 (d, J = 6.6 Hz, 1H), 7.60 (t, J = 6.6 Hz, 1H), 7.23 (s, 1H), 4.35 (m, 1H), 3.98 (d, J = 3.5 Hz, 2H), 3.55 (t, J = 6.5 Hz, 2H), 3.02 (t, J = 6.1 Hz, 2H), 2.90 (m, 1H), 2.70 (s, 3H), 2.02 (m, 2H), 1.75 (m, 2H), 1.48 (m, 2H), 1.28 (m, 2H).

isomer: more polar isomer from silica gel column (yield, 35%)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (s, 1H), 8.02 (d, J = 6.8 Hz, 1H), 7.80 (d, J = 6.5 Hz, 1H), 7.62 (t, J = 6.6 Hz, 1H), 7.40 (s, 1H), 4.52 (m, 1H), 4.18 (d, J = 5.0 Hz, 2H), 3.65 (t, J = 6.0 Hz, 2H), 3.11 (d, J = 6.5 Hz, 2H), 2.85 (m, 1H), 2.80 (s, 3H), 2.10 (m, 2H), 1.75 (m, 2H), 1.62 (m, 2H), 1.42 (m, 2H).

N-{[1-(4-Hydroxy-4-isothiazol-5-yl-cyclohexyl)-azetidin-3-ylcarbamoyl]-methyl}-3-trifluoromethyl-benzamide (**9**)



9: less polar isomer from silica gel column, prepared as hemi-succinate (yield, 38%),

^{1}H NMR (400 MHz, *d_4*-MeOH) δ 8.38 (s, 1H), 8.25 (s, 1H), 8.18 (d, J = 6.5 Hz, 1H), 7.90 (d, J = 6.6 Hz, 1H), 7.72 (t, J = 6.6 Hz, 1H), 7.28 (s, 1H), 4.55 (m, 1H), 4.10 (s, 2H), 4.05 (t, J = 7.5 Hz,

2H), 3.55 (t, J = 7.5 Hz, 2H), 2.90 (m, 1H), 2.55 (s, 2H), 2.22 (m, 2H), 1.97 (m, 2H), 1.79 (m, 2H), 1.58 (m, 2H).

isomer: more polar isomer from silica gel column (yield, 25%)

^{1}H NMR (400 MHz, *d*$_4$-MeOH) δ 8.11 (s, 1H), 8.05 (d, J = 6.5 Hz, 1H), 7.80 (d, J = 6.6 Hz, 1H), 7.62 (t, J = 6.6 Hz, 1H), 7.15 (s, 1H), 4.45 (m, 1H), 3.95 (s, 2H), 3.65 (t, J = 7.0 Hz, 2H), 3.01 (t, J = 6.5 Hz, 2H), 2.95 (m, 1H), 2.01 (m, 2H), 1.97 (m, 2H), 1.68 (m, 2H), 1.40 (m, 2H).

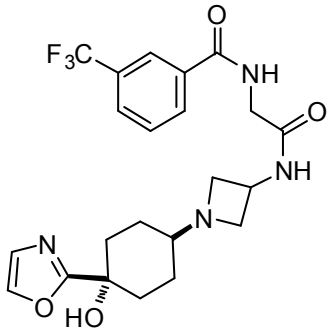
N-{[1-(4-Hydroxy-4-oxazol-2-yl-cyclohexyl)-azetidin-3-ylcarbamoyl]-methyl}-3-trifluoromethyl-benzamide (**3**)

4-Hydroxy-4-oxazol-2-yl-cyclohexanone

Borane-THF (Aldrich, 1.0 M, 30.4 mL, 30.4 mmol) and oxazole (Aldrich, 2.0 mL, 30.41 mmol) in THF (20 mL) were pre-stirred at –78 °C for 1 hour before addition of *n*-butyllithium (2.5 M, 12.1 mL, 30.4 mmol) followed by 1,4-cyclohexanedione monoethylene acetal (5.2 g, 33.45 mmol). After maintaining the reaction at –78 °C for 4 hours, 1N HCl was added to the reaction and allowed to stir at room temperature overnight followed by aqueous work up with $NaHCO_3$ and EtOAc. The organic layer was dried (Na_2SO_4) and concentrated *in vacuo* and purified using column chromatography (0-100% EtOAc/Hexanes with a few 7N NH_3 in MeOH) to afford the title compound (2.9 g, yield, 51%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (s, 1H), 7.09 (s, 1H), 2.76 - 2.98 (m, 2H), 2.19 - 2.48 (m, 4H), 1.86 - 2.15 (m, 2H).

N-{[1-(4-Hydroxy-4-oxazol-2-yl-cyclohexyl)-azetidin-3-ylcarbamoyl]-methyl}-3-trifluoromethyl-benzamide (**3**)



Yield, 31%. The isomer was not characterized. ^{1}H NMR (400 MHz, *d*$_4$-MeOH) δ 8.20 - 8.32 (m, 1H), 8.10 - 8.20 (m, 1H), 7.87 (s, 1H), 7.90 (s, 1H), 7.71 (t, J = 8.6 Hz, 1H), 7.07 - 7.22 (m, 1H),

4.43 (t, J = 6.9 Hz, 1H), 3.71 (d, J = 5.3 Hz, 2H), 3.54 - 3.65 (m, 2H), 3.01 (t, J = 7.7 Hz, 2H), 2.76 - 2.96 (m, 1H), 2.52 (d, J = 13.4 Hz, 2H), 2.21 - 2.33 (m, 2H), 2.07 - 2.18 (m, 2H), 1.76 - 1.92 (m, 2H).

3. In vitro biological assays

a) MCP-1 Receptor Binding Assay in THP-1 Cells:

THP-1 cells were obtained from American Type Culture Collection (Manassas, VA, USA). The THP-1 cells were grown in RPMI-1640 supplemented with 10% fetal bovine serum in a humidified 5% CO_2 atmosphere at 37°C. The cell density was maintained at 0.5·106 cells/mL. THP-1 cells were incubated with 0.5 nM ^{125}I labeled MCP-1 (Perkin-Elmer Life Sciences, Inc. Boston, MA) in the presence of varying concentrations of either unlabeled MCP-1 (R & D Systems**,** Minneapolis, MN) or test compound for 2 hours at 30^{o}C in a 96 well plate. Cells were then harvested onto a filter plate, dried, and 20 μL of Microscint 20 was added to each well. Plates were counted in a TopCount NXT, Microplate Scintillation & Luminescence Counter (Perkin-Elmer Life Sciences, Inc. Boston, MA). Blank values (buffer only) were subtracted from all values and drug treated values were compared to vehicle treated values. 1 μM cold MCP-1 was used for nonspecific binding.

b) MCP-1 Induced Chemotaxis in THP-1 Cells:

MCP-1 induced chemotaxis was run in a 24-well chemotaxis chamber. MCP-1 (0.01 μg/mL) was added to the lower chamber and 100 μL of THP-1 cells (1 x 10 ^{7} cell/mL) was added to the top chamber. Varying concentrations of test compound were added to the top and bottom chambers. Cells were allowed to chemotax for 3 hours at 37 ^{o}C and 5% CO_2. An aliquot of the cells which had migrated to the bottom chamber was taken and counted then compared to vehicle.

c) hERG [^{3}H]-astemizole binding experiment:

This assay is a 384 well in-plate vacuum filtration binding assay. Assay reagents are added into a prepared/blocked 384 well assay plate in the following order: 1) hERG Membrane diluted in Assay Buffer; 2) Test Compound; 3) ^{3}H Astemizole diluted in Assay Buffer. Assay reagents are incubated in the filter plate for 1 hour and then washed 6x with ice-cold wash buffer. Plates are allowed to dry overnight at room temperature. The following morning, plates are sealed and scintillant is added to each well. Following a 2-hour incubation with scintillant, plates are placed

on the TopCount and counted 1 minute per well. Data is calculated using raw CPM. Where applicable, IC_{50} values are calculated using raw CPM values. Curves are fitted individually from singlet 11 point dosing curves + 1% DMSO Control.

d) hERG Patch Express experiment:

Experiments were performed using HEK293 cells stably expressing the HERG potassium channel. Cells were grown at 37°C and 5% CO_2 in culture flasks in MEM Medium supplemented with 10% heat-inactivated fetal bovine serum, 1% L-Glutamine-Penicillin-Streptomycin-solution, 1% non-essential amino acids (100x), 1% sodium pyruvate (100 mM) and 0.8% Geneticin (50 mg/ml). Before use the cells were subcultured in MEM medium in the absence of 5 ml L-Glutamine-Penicillin-Streptomycin. For use in the automated patch-clamp system PatchXpress 7000A (Axon Instruments) cells were harvested to obtain cell suspension of single cells. Extracellular solution contained (mM): 150 NaCl, 4 KCl, 1 $MgCl_2$, 1.8 $CaCl_2$, 10 HEPES, 5 Glucose (pH 7.4 with NaOH). Pipette solution contained (mM): 120 KCl, 10 HEPES, 5 EGTA, 4 ATP-Mg_2, 2 $MgCl_2$, 0.5 $CaCl_2$ (pH7.2 with KOH). Patch-clamp experiments were performed in the voltage-clamp mode and whole-cell currents were recorded with an automated patch-clamp assay utilizing the PatchXpress 7000A system (Axon Instruments). Current signals were amplified and digitized by a Multiclamp amplifier, stored and analyzed by using the PatchXpress, DataXpress software and Igor 5.0 (Wavemetrics). The holding potential was -80 mV. The HERG current (K^+-selective outward current) was determined as the maximal tail current at -40 mV after a 2 second depolarization to +60 mV. Pulse cycling rate was 15 s. Before each test pulse a short pulse (0.5 s) from the holding potential to -60 mV was given to determine (linear) leak current. After establishing whole-cell configuration and a stability period, the vehicle was applied for 5 minutes followed by the test substance by increasing concentrations of 3×10^{-6} M, 10^{-5} M and 3×10^{-5} M. Each concentration of the test substance was applied twice. The effect of each concentration was determined after 5 min as an average current of 3 sequential voltage pulses. To determine the extent of block the residual current was compared with vehicle pre-treatment. Data are presented as mean values ± standard error of the mean (SEM).

4. In vivo assays

a) Guinea pig CV study

Arterial Pressure, Heart Rate and ECG in Anesthetized Guinea Pigs:

Female Hartley guinea pigs weighing between 980-1058 g were anesthetized with pentobarbital (50 mg/kg, i.p.). The trachea was cannulated to allow ventilation with room air with a respirator. The ECG was recorded using a modified chest lead with the electrodes oriented along the long axis of the heart. A carotid artery and a jugular vein were cannulated for recording arterial blood pressure and administration of compound, respectively. Signals were recorded and analyzed using a Ponemah P3 Plus Data Aquisition System. Mean, systolic and diastolic arterial pressures, heart rate and ECG intervals (PR, QRS and QT) were determined. Corrected QT (QTc) was derived using Bazett's formula. The compound was solubilized in 20% hydroxypropyl-β-cyclodextrin for dosing solutions of 0.5 mL/kg. Each dose of the compound was infused intravenously over 5 minutes. Incremental doses of 0.1, 0.2, 0.7, 2 and 7 mg/kg were administered every 20 minutes to yield cumulative doses of 0.1, 0.3, 1, 3 and 10 mg/kg. Equivalent volumes of vehicle were administered to a separate group of animals. Data for each parameter are reported as a time course and as a dose response summary based on peak percent changes or the integrated average percent change following each dose.

b) Dog CV study

Cardio-hemodynamic, cardio-electrophysiological and pulmonary/respiratory effects in artificially ventilated, anesthetized dogs:

Tthe potential cardio-hemodynamic, cardiac electrophysiological and pulmonary effects of the compound were evaluated at increasing intravenous doses of 0.16 to 5 mg/kg (total dose = 9.86 mg/kg i.v.; n = 4; 2 F, 2 M) infused over 5 min at 30-min intervals, in neuromuscular blocked, artificially ventilated anesthetized dogs (median bodyweight: 11.2 kg, age ranging from 11 to 16 months). The results were compared to vehicle dogs from experiments conducted in the same time-frame (0.032 up to 1 ml/kg i.v.; total volume = 1.97 ml/kg; n = 4; 2 F, 2 M). Total intravenous anesthesia was induced with a potent long-acting morphinomimetic, lofentanil 0.075 mg/kg i.v., an anticholinergic, scopolamine 0.015 mg/kg i.v., and a muscle relaxant, succinylcholine 1 mg/kg i.v.. Intermittent positive pressure ventilation was applied with oxygen-enriched pressurized air at 20 respirations per min. Hypnosis and analgesia were maintained with a continuous

i.v. infusion of etomidate at 1.5 mg/kg/h, and hourly slow bolus injections of fentanyl

0.025 mg/kg i.v.. The physiological condition and stability of each preparation was monitored by measurement of arterial blood gases and metabolic blood parameters.

The compound was administered as a 5-min infusion in a dose range of 0.16 to 5 mg/kg (total dose = 9.86 mg/kg i.v.) at 30-min intervals according to the following dosing scheme: 0 min: 0.16 mg/kg; 30 min: 0.32 mg/kg; 60 min: 0.63 mg/kg; 90 min: 1.25 mg/kg; 120 min: 2.5 mg/kg; 150 min: 5 mg/kg. The actual changes and percentage changes from the control baseline value for each parameter were calculated at 5, 10, 20 and 30 min after the start of the administration, both in the compound and the vehicle group. Only the percentage changes from the control baseline value at 5 min and at 30 min were reported in the tables. Plasma levels of the compound, measured as the free base, were determined at the end of each 5 min infusion period (i.e. at assumed Cmax) and 30 min after the onset of each infusion, using a qualified research LC-MS/MS method. The ECG and RV MAP signals during each 5-min infusion period and at 30 min after the onset of each infusion were inspected on-line and again off-line after the experiment for a range of parameters.

c) PK studies

Mice, rats, dogs, monkeys or other species are normally fasted overnight, then dosed intravenously (IV) at levels of 1-3 mg/kg and by oral gavage at a levels of 10-30 mg/kg with drug candidate. Food is returned at 4 hr post dose. Blood samples (0.25 – 2.0 ml depending on the species) are collected post dose into tubes containing an appropriate anticoagulant. Blood samples are centrifuged for cell removal, and 100 mL plasma is transferred to a clean vial, placed on dry ice, and subsequently stored in a -70^{o}C freezer prior to analysis. Plasma samples are normally prepared as follows. Two volumes of acetonitrile containing internal standard is added to one volume of plasma to precipitate proteins. Samples are centrifuged (3000 g for 5 min) and supernatant removed for analysis by LC-MS-MS. Calibration standards are prepared by adding appropriate volumes of stock solution directly into plasma and treated identically to collected plasma samples. Calibration standards are typically prepared in the range of 2 ng/ml to 10 ug/mL for quantitation. QC samples are prepared in parallel at high, medium and low concentrations in an identical manner and they are used to ensure the quality of the assay results. No more that 2 of the 6 QC standards are allowed to differ by more than 20% of their nominal value. LC-MS-MS analysis is performed utilizing multiple reaction monitoring for detection of

characteristic ions for each drug candidate, additional related analytes and internal standard. Detection below 2 ng/mL may require alternate sample preparation procedures and will increase analysis turnaround times. Plasma concentrations are measured as described above to determine a concentration vs time profile. The area under the plasma concentration vs time curve (AUC) is calculated using the linear trapezoidal method. Fitting of the data to obtain pharmacokinetic parameters is generally carried out using non-compartmental analysis. Subsequent fitting with compartmental models can be carried out to aid in simulation of different dosing regimens. Compartmental analysis requires a data set with well defined phases to be useful. Parameters are expressed for individual animals as well as mean $\pm$ standard deviations. The CV% is a measure of the variability of the individual parameters. The exception is the mouse where individual time points are obtained from individual animals so only a mean curve is obtained.

5. ADME profile of 8d

In inhibition assays with recombinant CYP450 enzymes, **8d** had no significant activity against any of the five major human CYP450 enzymes. **8d** was not a CYP inducer at concentrations up to 30 μM as measured in the human Puracyp PXR cell line. It was stable in human, rat, and mouse liver microsomes (> 86% remaining at 10 min). **8d** has high aqueous solubility (pH 7.4, 40 μM). It demonstrated relatively low protein binding (67-77%) in human, rat, mouse, dog and monkey plasma.