Discovery of PIPE-359, a Brain-Penetrant, Selective M_1 Receptor Antagonist with Robust Efficacy in Murine MOG-EAE

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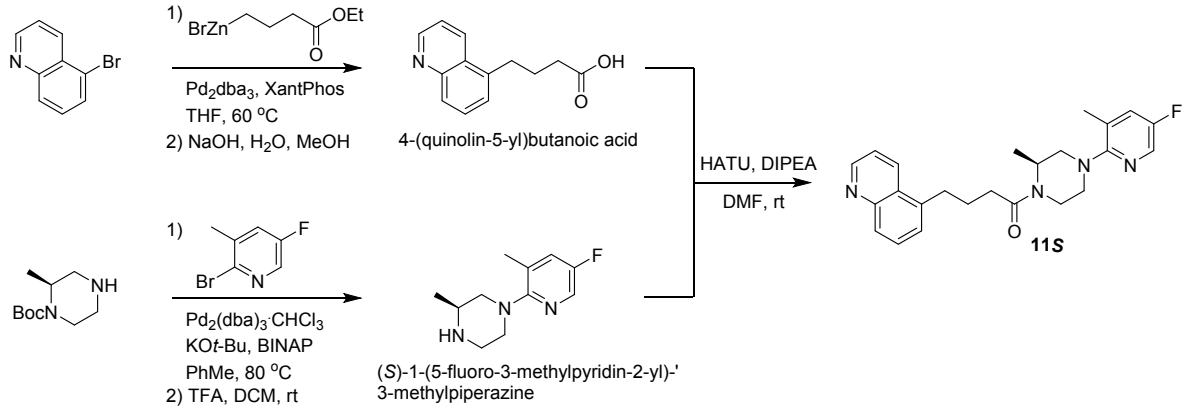
Experimental Procedures: Chemistry

Representative procedure for synthesis of 4-(quinolin-5-yl)butanoic amides (1-17). Synthesis of (*S*)-1-(4-(5-fluoro-3-methylpyridin-2-yl)-2-methylpiperazin-1-yl)-4-(quinolin-5-yl)butan-1-one (11*S*).



Synthesis of 4-(quinolin-5-yl)butanoic acid.

Step 1: To a solution of 5-bromoquinoline (3.50 g, 16.8 mmol) in THF (50 mL) at rt under N_2 was added (4-ethoxy-4-oxobutyl)zinc(II) bromide (101 mL of 0.5 M solution in THF, 50.5 mmol). $Pd_2(dba)_3$ (350 mg, 0.38 mmol) and Xantphos (427 mg, 0.74 mmol) was added and the mixture was stirred at 60 °C for 12 h. The mixture was quenched by adding sat. aqueous $NaHCO_3$, and filtered, and concentrated. The concentrate was partitioned between water and DCM. The layers were separated and the organic layer was concentrated and purified by silica gel chromatography (petroleum ether/ethyl acetate) which gave ethyl 4-(quinolin-5-yl)butanoate (2.70 g, 10.9 mmol, 64% yield) as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.92 (dd, *J* = 5.6, 1.9 Hz, 1H), 8.47 (d, *J* = 8.5 Hz, 1H), 8.02 (d, *J* = 8.5 Hz, 1H), 7.64 (dd, *J* = 8.5, 7.0 Hz, 1H), 7.45 (dd, *J* = 8.5, 4.5 Hz, 1H), 7.39 (d, *J* = 7.0 Hz, 1H), 4.15 (q, *J* = 7.0 Hz, 2H), 3.11 (m, 2H), 2.42 (t , *J* = 7.5 Hz, 2H), 2.42 (t , *J* = 7.5 Hz, 2H), 2.05 (pent, *J* = 7.5 Hz, 2H), 1.27 (t , *J* = 7.0 Hz, 3H). LRMS (ESI$^+$) calcd for $C_{15}H_{17}NO_2$ $[M+H]^+$: 244.1; found: *m/z* = 244.2.

Step 2: Ethyl 4-(quinolin-5-yl)butanoate (2.00 g, 8.22 mmol) was dissolved in MeOH (20 mL) and aqueous NaOH (20.6 mL of a 2 M solution, 41.2 mmol) was added. The resulting mixture was stirred at rt for 4 h. The mixture was concentrated to remove MeOH and adjusted to pH ~7 by adding aqueous 2 M HCl. A precipitate was formed and the solid filtered which gave 4-(quinolin-5-yl)butanoic acid (1.40 g, 6.31 mmol, 76%) as a white solid. LRMS (ESI^+) calcd for $C_{13}H_{13}NO_2$ $[M+H]^+$: 216.1; found: *m/z* = 216.1.

Synthesis of (*S*)-1-(5-fluoro-3-methylpyridin-2-yl)-'3-methylpiperazine.

Step 1. A mixture of *tert*-butyl (2*S*)-2-methylpiperazine-1-carboxylate (1.00 g, 4.90 mmol), 2-bromo-5-fluoro-3-methylpyridine (1.14 g, 5.99 mmol, 1.20 equiv), BINAP (622 mg, 1.00 mmol), *t*-BuOK (1.68 g, 15.0 mmol), $Pd_2(dba)_3{\cdot}CHCl_3$ (1.03 g, 1.00 mmol), and toluene (25 mL) was stirred under N_2 at 80 °C for 2 h. The resulting mixture was cooled to rt and concentrated in vacuo. The residue was purified by silica gel chromatography (petroleum ether/ethyl acetate) which gave *tert*-butyl (2*S*)-4-(5-fluoro-3-methylpyridin-2-yl)-2-methylpiperazine-1-carboxylate (1.00 g, 3.23 mmol, 66% yield) as a yellow oil. LRMS (ESI^+) calcd for $C_{16}H_{24}FN_3O_2$ $[M+H]^+$: 310.2; found: *m/z* = 310.2.

Step 2: A solution of *tert*-butyl (2*S*)-4-(5-fluoro-3-methylpyridin-2-yl)-2-methylpiperazine-1-carboxylate(1.00 g, 3.23 mmol) and TFA (5 mL) in DCM (10 mL) was stirred for 45 min at rt. The resulting mixture was concentrated in vacuo. The mixture was made basic by the addition of 7N methanolic ammonia solution. The resulting mixture was concentrated in vacuo. The residue was purified by reverse phase HPLC to afford (3*S*)-1-(5-fluoro-3-methylpyridin-2-yl)-3-methylpiperazine (500 mg, 2.39 mmol, 74% yield) as yellow oil. ^1H NMR (400 MHz, *d*6-DMSO): δ 8.07 (d, *J* = 2.4 Hz, 1H), 7.51 (dd, *J* = 7.2, 2.4 Hz, 1H), 3.15 (app d, *J* = 9.6 Hz, 2H), 2.93 (m, 1H), 2.89 (m, 1H), 2.88 (td, *J* = 9.2, 2.0 Hz, 1H), 2.67 (td, *J* = 9.2, 2.0 Hz, 1H), 2.39 (app t, *J* = 9.2 Hz, 1H), 2.25 (s, 3H), 1.02 (d, *J* = 5.2 Hz, 3H). LRMS (ESI^+) calcd for $C_{11}H_{16}FN_3$ $[M+H]^+$: 210.1; found: *m/z* = 210.1.

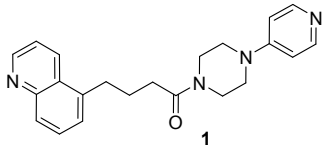
Coupling of 4-(quinolin-5-yl)butanoic acid and (*S*)-1-(5-fluoro-3-methylpyridin-2-yl)-'3-methylpiperazine. Synthesis of **11*S***.

A mixture of (3*S*)-1-(5-fluoro-3-methylpyridin-2-yl)-3-methylpiperazine (35 mg, 0.16 mmol), 4-(quinolin-5-yl)butanoic acid (34 mg, 0.16 mmol), HATU (74 mg, 0.19 mmol), DIPEA

(84 mg, 0.65 mmol) in DMF (1.5 mL) was stirred at rt overnight. The mixture was diluted with EtOAc and washed with sat. aqueous NH_4Cl and brine. The organics were dried over MgSO4, filtered, and concentrated. Purification by silica gel chromatography (50% EtOAc in hexanes gradient to 100% EtOAc) gave 1-[(2*S*)-4-(5-fluoro-3-methylpyridin-2-yl)-2-methylpiperazin-1-yl]-4-(quinolin-5-yl)butan-1-one (**11*S***, 35 mg, 0.086 mmol, 53% yield) as a white solid. ^{1}H NMR (300 MHz, CD_3OD), mixture of rotational isomers: δ 8.86 (dd, *J* = 4.3, 1.6 Hz, 1H), 8.74 (d, *J* = 8.5 Hz, 1H), 8.00 (d, *J* = 3.0 Hz, 1H), 7.93 (d, *J* = 8.5 Hz, 1H), 7.72 (dd, *J* = 8.5, 7.0 Hz, 1H), 7.60 (dd, *J* = 8.6, 4.3 Hz, 1H), 7.53 (d, *J* = 7.1 Hz, 1H), 7.41 (dd, *J* = 9.0, 3.0 Hz, 1H), 4.82 (br s, 0.5H), 4.48 (d, *J* = 13.3 Hz, 0.5H), 4.23 (br s, 0.5H), 3.82 (d, *J* = 13.3 Hz, 0.5H), 3.56 (m, 0.5H), 3.35 -3.14 (m, 4.5H), 2.91–2.41 (m, 4H), 2.37 (s, 3H), 2.11-2.99 (m, 2H), 1.42-1.32 (m, 3H). ^{13}C APT NMR (100 MHz, *d*6-DMSO), mixture of rotational isomers: δ up (*C*, *C*H_2); 170.4, 157.9, 156.9, 154.9, 148.2, 139.2, 126.5, 54.4, 54.3, 50.0, 36.2, 32.1, 31.4, 31.3, 26.5; down (*C*H, C*H*$_3$); 150.0, 132.4, 131.7, 131.5, 129.1, 127.4, 126.6, 126.5, 126.3, 121.1, 48.2, 44.2, 17.5, 16.2, 15.2. LC purity (UV diode array): 100.0%. LRMS (ESI$^+$) calcd for $C_{24}H_{27}FN_4O$ [M+H]$^+$: 407.2; found: *m/z* = 407.2. $[\alpha]^{20}_D$ +36 (c 0.24, $CHCl_3$). Anal. Calcd for $C_{24}H_{27}FN_4O$: C, 70.91; H, 6.70; N, 13.78; Found: C, 70.57; H, 6.31; N, 13.56.

The following 4-(quinolin-5-yl)butanoic amide analogs (**1**-**17**) were prepared from 4-(quinolin-5-yl)butanoic acid and readily available monopyridinyl cyclodiamines in analogous fashion as described for representative example **11*S***:

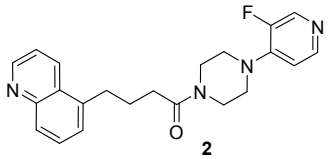
1-(4-(Pyridin-4-yl)piperazin-1-yl)-4-(quinolin-5-yl)butan-1-one (1).



1

^{1}H NMR (400 MHz, CD_3OD): δ 8.85 (d, *J* = 4.0 Hz, 1H), 8.74 (d, *J* = 8.4 Hz, 1H), 8.16 (d, *J* = 6.0 Hz, 2H), 7.92 (d, *J* = 8.4 Hz, 1H), 7.72 (app t, *J* = 7.2 Hz, 1H), 7.59 (dd, *J* = 8.8, 4.4 Hz, 1H), 7.52 (d, *J* = 6.8 Hz, 1H), 6.88 (d, *J* = 6.4 Hz, 2H), 3.77 (m, 2H), 3.70 (m, 2H), 3.47 (m, 4H), 3.21 (t, *J* = 7.6 Hz, 2H), 2.58 (t, *J* = 7.2 Hz, 2H), 2.07 (pent, *J* = 7.6 Hz, 2H). LC purity (UV 220 nm): 99.3%. LRMS (ESI$^+$) calcd for $C_{22}H_{24}N_4O$ [M+H]$^+$: 361.2; found: *m/z* = 361.1.

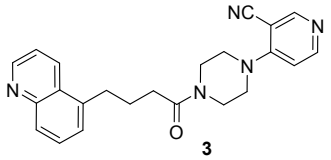
1-(4-(3-Fluoropyridin-4-yl)piperazin-1-yl)-4-(quinolin-5-yl)butan-1-one (2).



2

^{1}H NMR (400 MHz, *d*6-DMSO): δ 8.90 (d, *J* = 4.0 Hz, 1H), 8.65 (d, *J* = 8.4 Hz, 1H), 8.29 (d, *J* = 5.2 Hz, 1H), 8.17 (d, *J* = 5.6 Hz, 1H), 7.88 (d, *J* = 8.4 Hz, 1H), 7.68 (app t, *J* = 7.2 Hz, 1H), 7.59 (dd, *J* = 8.8, 4.0 Hz, 1H), 7.46 (d, *J* = 7.2 Hz, 1H), 6.98 (dd, *J* = 8.0, 5.6 Hz, 1H), 3.63 (m, 2H), 3.60 (m, 2H), 3.25 (m, 4H), 3.08 (t, *J* = 8.0 Hz, 2H), 2.48 (t, *J* = 8.0 Hz, 2H), 2.07 (pent, *J* = 8.0 Hz, 2H). LC purity (UV 254 nm): 97.7%. LRMS (ESI$^+$) calcd for $C_{22}H_{23}FN_4O$ $[M+H]^+$: 379.2; found: *m/z* = 379.1.

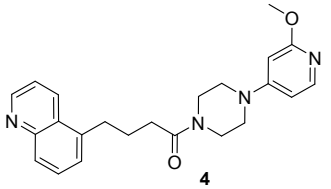
4-(4-(4-(Quinolin-5-yl)butanoyl)piperazin-1-yl)nicotinonitrile (3).



3

^{1}H NMR (400 MHz, *d*6-DMSO): δ 8.88 (d, *J* = 4.0 Hz, 1H), 8.64 (m, 2H), 8.41 (m, 1H), 7.86 (d, *J* = 8.4 Hz, 1H), 7.66 (app t, *J* = 8.4 Hz, 1H), 7.56 (dd, *J* = 8.4, 4.0 Hz, 1H), 7.44 (d, *J* = 7.2 Hz, 1H), 7.01 (d, *J* = 6.0 Hz, 1H), 3.63-3.50 (m, 8H), 3.05 (app t, *J* = 8.0 Hz, 2H), 2.45 (m, 2H), 1.88 (pent, *J* = 8.0 Hz, 2H). LC purity (UV 220 nm): 97.9%. LRMS (ESI$^+$) calcd for $C_{23}H_{23}N_5O$ $[M+H]^+$: 386.2; found: *m/z* = 386.1.

1-(4-(2-Methoxypyridin-4-yl)piperazin-1-yl)-4-(quinolin-5-yl)butan-1-one (4).

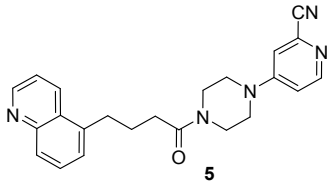


4

^{1}H NMR (400 MHz, *d*6-DMSO): δ 8.87 (dd, *J* = 4.2, 1.5 Hz, 1H), 8.62 (d, *J* = 8.2 Hz, 1H), 7.86 (d, *J* = 8.6 Hz, 1H), 7.80 (d, *J* = 6.0 Hz, 1H), 7.65 (dd, *J* = 8.5, 7.2Hz, 1H), 7.54 (dd, *J* = 8.5, 4.1 Hz, 1H), 7.44 (d, *J* = 6.8 Hz, 1H), 6.54 (dd, *J* = 6.2, 2.2 Hz, 1H), 6.10 (d, *J* = 2.0 Hz, 1H), 3.75 (s, 3H), 3.58-3.52 (m, 4H), 3.30-3.25 (m, 4H), 3.05 (app t, *J* = 8.0 Hz, 2H), 2.46 (t, *J* = 7.6 Hz, 2H), 1.87

(pent, *J* = 7.6 Hz, 2H). LC purity (UV 254 nm): 99.5%. LRMS (ESI$^+$) calcd for $C_{23}H_{26}N_4O_2$ $[M+H]^+$: 391.2; found: *m/z* = 391.1.

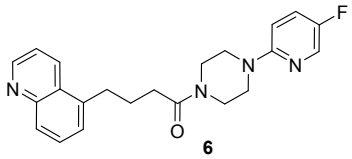
4-(4-(4-(Quinolin-5-yl)butanoyl)piperazin-1-yl)picolinonitrile (5).



5

^{1}H NMR (400 MHz, *d*6-DMSO): δ = 8.90 (dd, *J* = 4.4, 1.6 Hz, 1H), 8.64 (d, *J* = 8.2 Hz, 1H), 8.26 (d, *J* = 6.0 Hz, 1H), 7.87 (d, *J* = 8.2 Hz, 1H), 7.68 (app t, *J* = 6.8 Hz, 1H), 7.56 (dd, *J* = 8.4, 4.1 Hz, 1H), 7.49 (d, *J* = 2.8 Hz, 1H), 7.46 (d, *J* = 7.2 Hz, 1H), 7.06 (dd, *J* = 6.0, 3.2 Hz, 1H), 3.60 (m, 4H), 3.47 (m, 4H), 3.10 (app t, *J* = 8.0 Hz, 2H), 2.49 (t, *J* = 8.0 Hz, 2H), 1.90 (pent, *J* = 7.6 Hz, 2H). LC purity (UV 254 nm): 95.1%. LRMS (ESI$^+$) calcd for $C_{23}H_{23}N_5O$ $[M+H]^+$: 386.2; found: *m/z* = 386.1.

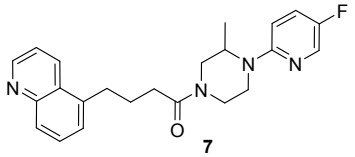
1-(4-(5-Fluoropyridin-2-yl)piperazin-1-yl)-4-(quinolin-5-yl)butan-1-one (6).



6

^{1}H NMR (400 MHz, CD_3OD): δ 8.82 (d, *J* = 3.2 Hz, 1H), 8.72 (d, *J* = 8.8 Hz, 1H), 8.06 (d, *J* = 2.8 Hz, 1H), 7.90 (d, *J* = 8.8 Hz, 1H), 7.69 (app t, *J* = 6.8 Hz, 1H), 7.57 (dd, *J* = 8.0, 4.4 Hz, 1H), 7.50 (d, *J* = 6.8 Hz, 1H), 7.40 (m, 1H), 6.83 (dd, *J* = 8.8, 3.2 Hz, 1H), 3.70 (m, 2H), 3.61 (m, 2H), 3.47(m, 4H), 3.18 (app t, *J* = 8.0 Hz, 2H), 2.55 (t, *J* = 8.0 Hz, 2H), 2.03 (pent, *J* = 7.6 Hz, 2H). LC purity (UV 220 nm): 99.2%. LRMS (ESI$^+$) calcd for $C_{22}H_{23}FN_4O$ $[M+H]^+$: 379.2; found: *m/z* = 379.1.

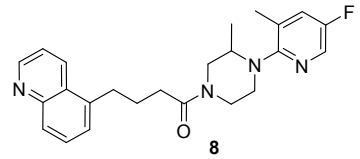
***rac*-1-(4-(5-Fluoropyridin-2-yl)-3-methylpiperazin-1-yl)-4-(quinolin-5-yl)butan-1-one (7).**



7

^{1}H NMR (400 MHz, *d*6-DMSO): δ 8.90 (dd, *J* = 4.0, 1.6 Hz, 1H), 8.61 (d, *J* = 8.8 Hz, 1H), 8.08 (d, *J* = 3.6 Hz, 1H), 7.88 (d, *J* = 8.0 Hz, 1H), 7.66 (app t, *J* = 7.6 Hz, 1H), 7.55 (dd, *J* = 8.4, 4.8 Hz, 1H), 7.48-7.42 (m, 2H), 6.79 (dd, *J* = 9.2, 3.6 Hz, 1H), 4.48-4.06 (broad m, 2H), 4.00-3.60 (broad m, 2H), 3.45-2.75 (broad m, 3H), 3.13 (app t, *J* = 8.0 Hz, 2H), 2.47 (m, 2H), 1.98 (pent, *J* = 7.6 Hz, 2H), 1.02 (d, *J* = 6.8 Hz, 3H). LC purity (UV 220 nm): 100.0%. LRMS (ESI$^+$) calcd for $C_{23}H_{25}FN_4O$ $[M+H]^+$: 393.2; found: *m/z* = 393.1.

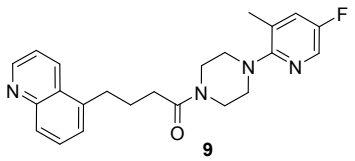
rac-1-(4-(5-Fluoro-3-methylpyridin-2-yl)-3-methylpiperazin-1-yl)-4-(quinolin-5-yl)butan-1-one (8).



8

^{1}H NMR (400 MHz, *d*6-DMSO): δ 8.90 (dd, *J* = 4.0, 1.6 Hz, 1H), 8.61 (d, *J* = 8.8 Hz, 1H), 8.14 (d, *J* = 2.8 Hz, 1H), 7.89 (d, *J* = 8.0 Hz, 1H), 7.68 (dd, *J* = 8.8, 7.2 Hz, 1H), 7.57 (dd, *J* = 8.4, 4.8 Hz, 1H), 7.54 (m, 1H), 7.48 (d, *J* = 7.2 Hz, 1H), 3.92-3.55 (m, 2H), 3.52-3.37 (m, 2H), 3.23 (m, 2H), 3.15 (app t, *J* = 8.0 Hz, 2H), 2.77 (m, 1H), 2.49 (t, *J* = 8.0 Hz, 2H), 2.30 (s, 3H), 1.95 (pent, *J* = 7.6 Hz, 2H), 0.85 (d, *J* = 6.4 Hz, 3H). LC purity (UV 220 nm): 94.0%. LRMS (ESI$^+$) calcd for $C_{24}H_{27}FN_4O$ $[M+H]^+$: 407.2; found: *m/z* = 407.2.

1-(4-(5-Fluoro-3-methylpyridin-2-yl)piperazin-1-yl)-4-(quinolin-5-yl)butan-1-one (9).

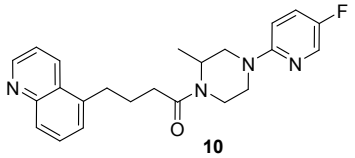


9

^{1}H NMR (400 MHz, CD_3OD): δ 8.85 (d, *J* = 3.6 Hz, 1H), 8.73 (d, *J* = 8.4 Hz, 1H), 7.99 (d, *J* = 2.4 Hz, 1H), 7.91 (d, *J* = 8.0 Hz, 1H), 7.71 (t, *J* = 6.8 Hz, 1H), 7.60 (dd, *J* = 8.4, 4.4 Hz, 1H), 7.52 (d, *J*

= 6.8 Hz, 1H), 7.41 (dd, *J* = 8.4, 3.6 Hz, 1H), 3.76 (m, 2H), 3.65 (m, 2H), 3.19 (t, *J* = 8.0 Hz, 2H), 3.11-3.05 (m, 4H), 2.57 (t, *J* = 7.2 Hz, 2H), 2.34 (s, 3H), 2.09 (pent, *J* = 7.2 Hz, 2H). LC purity (UV 220 nm): 99.9%. LRMS (ESI^+) calcd for $C_{23}H_{25}FN_4O$ $[M+H]^+$: 393.2; found: *m/z* = 393.1.

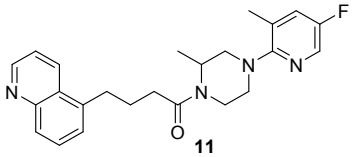
***rac*-1-(4-(5-Fluoropyridin-2-yl)-2-methylpiperazin-1-yl)-4-(quinolin-5-yl)butan-1-one (10).**



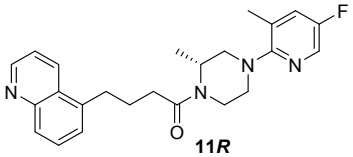
10

^1H NMR (400 MHz, *d*6-DMSO): δ 8.89 (dd, *J* = 3.6, 1.2 Hz, 1H), 8.59 (d, *J* = 8.8 Hz, 1H), 8.06 (d, *J* = 8.8 Hz, 1H), 7.88 (d, *J* = 8.0 Hz, 1H), 7.66 (app t, *J* = 8.4 Hz, 1H), 7.53 (dd, *J* = 8.8, 4.4 Hz, 1H), 7.52-7.45 (m, 2H), 6.82 (dd, *J* = 9.2, 3.2 Hz, 1H), 4.44 (broad m, 1H), 4.10-3.95 (m, 3H) 3.25 (broad m, 1H), 3.11 (m, 2H), 2.45 (m, 3H), 1.97 (pent, *J* = 7.2 Hz, 2H), 1.14 (d, *J* = 6.4 Hz, 3H). LC purity (UV 220 nm): 99.1%. LRMS (ESI^+) calcd for $C_{23}H_{25}FN_4O$ $[M+H]^+$: 393.2; found: *m/z* = 393.1.

***rac*-1-(4-(5-Fluoro-3-methylpyridin-2-yl)-2-methylpiperazin-1-yl)-4-(quinolin-5-yl)butan-1-one (11) and (*R*)-1-(4-(5-fluoro-3-methylpyridin-2-yl)-2-methylpiperazin-1-yl)-4-(quinolin-5-yl)butan-1-one (11*R*).**



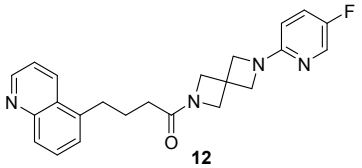
11



11*R*

The ^1H NMR and LCMS spectral data for compound **11** [LC purity (UV 220 nm): 99.4%] and compound **11*R*** [LC purity (UV 254 nm): 97.4%] were identical to compound **11*S*** shown in the representative procedure.

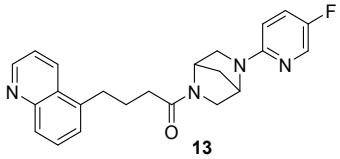
1-(6-(5-Fluoropyridin-2-yl)-2,6-diazaspiro[3.3]heptan-2-yl)-4-(quinolin-5-yl)butan-1-one (12).



12

^{1}H NMR (400 MHz, CD_3OD): δ 8.86 (d, *J* = 3.6 Hz, 1H), 8.70 (d, *J* = 8.8 Hz, 1H), 7.93 (m, 2H), 7.73 (app t, *J* = 7.2 Hz, 1H), 7.61 (dd, *J* = 8.8, 4.4 Hz, 1H), 7.52 (d, *J* = 7.2 Hz, 1H), 7.46 (dt, *J* = 7.2, 3.6 Hz, 1H), 6.46 (dd, *J* = 9.2, 4.0 Hz, 1H), 4.31 (s, 2H), 4.16 (s, 2H), 4.11 (m, 4H), 3.18 (app t, *J* = 8.0 Hz, 2H), 2.25 (t, *J* = 8.0 Hz, 2H), 2.03 (pent, *J* = 7.6 Hz, 2H). LC purity (UV 220 nm): 95.3%. LRMS (ESI$^+$) calcd for $C_{23}H_{23}FN_4O$ [M+H]$^+$: 391.2; found: *m/z* = 391.1.

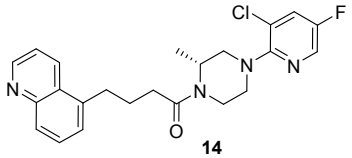
rac-1-(5-(5-Fluoropyridin-2-yl)-2,5-diazabicyclo[2.2.1]heptan-2-yl)-4-(quinolin-5-yl)butan-1-one (13).



13

^{1}H NMR (400 MHz, *d*6-DMSO), mixture of rotational isomers: δ 8.88 (m, 1H), 8.59 (m, 1H), 8.06 (app t, *J* = 2.8 Hz, 1H), 7.87 (m, 1H), 7.75-7.35 (m, 4H), 6.57 (m, 1H), 4.82 (s, 1H), 4.74 (s, 0.5H), 4.67 (s, 0.5H), 3.51 (t, *J* = 7.2 Hz, 1H), 3.35-3.15 (m, 2H), 3.07 (t, *J* = 3.2 Hz, 1H), 3.01 (t, *J* = 3.2 Hz, 1H), 2.45 (m, 2H), 2.20 (t, *J* = 6.8 Hz, 1H), 2.05-1.75 (m, 4H). LC purity (UV 220 nm): 94.9%. LRMS (ESI$^+$) calcd for $C_{23}H_{23}FN_4O$ [M+H]$^+$: 391.2; found: *m/z* = 391.1.

(*R*)-1-(4-(3-Chloro-5-fluoropyridin-2-yl)-2-methylpiperazin-1-yl)-4-(quinolin-5-yl)butan-1-one (14).

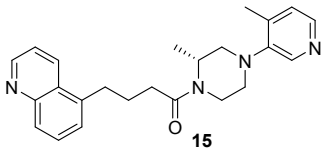


14

^{1}H NMR (300 MHz, *d*6-DMSO), mixture of rotational isomers: δ 8.91 (d, *J* = 3.5 Hz, 1H), 8.66 (d, *J* = 8.5 Hz, 1H), 8.31 (d, *J* = 2.7 Hz, 1H), 8.03 (dd, *J* = 8.1, 2.7 Hz, 1H), 7.89 (d, *J* = 8.5 Hz, 1H), 7.70 (app t, *J* = 8.4 Hz, 1H), 7.57 (dd, *J* = 8.6, 4.1 Hz, 1H), 7.47 (d, *J* = 7.1 Hz, 1H), 4.75 (br s, 0.5H), 4.39 (m, 0.5H), 4.22 (br s, 0.5H), 3.80 (m, 0.5H), 3.52 (m, 2H), 3.30 (m, 1H), 3.09 (t, *J* =

7.9 Hz, 2H), 2.80 (m, 2H), 2.44 (m, 2H), 1.89 (m, 2H), 1.26 (m, 3H). LC purity (UV 254 nm): 99.0%. LRMS (ESI$^+$) calcd for $C_{23}H_{24}ClFN_4O$ [M+H]$^+$: 427.2; found: *m/z* = 426.9.

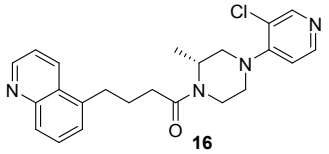
(*R*)-1-(2-Methyl-4-(4-methylpyridin-3-yl)piperazin-1-yl)-4-(quinolin-5-yl)butan-1-one (15).



15

^{1}H NMR (400 MHz, *d*6-DMSO), mixture of rotational isomers: δ 8.90 (dd, *J* = 4.2, 1.6 Hz, 1H), 8.66 (d, *J* = 8.5 Hz, 1H), 8.21-8.16 (m, 2H), 7.89 (d, *J* = 8.4 Hz, 1H), 7.70 (dd, *J* = 8.4, 7.0 Hz, 1H), 7.58 (dd, *J* = 8.6, 4.2 Hz, 1H), 7.47 (m, 1H), 7.21 (d, *J* = 4.8 Hz, 1H), 4.70 (br s, 0.5H), 4.39 (d, *J* = 12.0 Hz, 0.5H), 4.19 (br s, 0.5H), 3.67 (m, 0.5H), 3.42 (m, 0.5H), 3.15-3.00 (m, 4.5H), 2.89-2.60 (m, 3H), 2.60-2.35 (m, 2H), 2.32 (s, 3H), 1.90 (br s, 2H), 1.38-1.24 (m, 3H). LC purity (UV 220 nm): 99.0%. LRMS (ESI$^+$) calcd for $C_{24}H_{28}N_4O$ [M+H]$^+$: 389.2; found: *m/z* = 389.2.

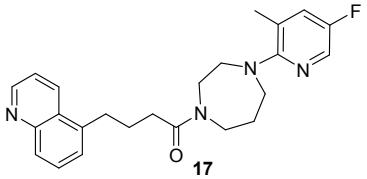
(*R*)-1-(4-(4-Chloropyridin-3-yl)-2-methylpiperazin-1-yl)-4-(quinolin-5-yl)butan-1-one (16).



16

^{1}H NMR (300 MHz, CD_3OD), mixture of rotational isomers: δ 8.86 (dd, *J* = 2.8, 1.2 Hz, 1H), 8.70 (app t, *J* = 8.4 Hz, 1H), 8.38 (s, 1H), 8.30 (d, *J* = 5.4 Hz, 1H), 7.92 (d, *J* = 8.7 Hz, 1H), 7.72 (m, 1H), 7.62 (m, *J* = 8.7, 4.5 Hz, 1H), 7.47 (d, *J* = 6.9 Hz, 1H), 7.07 (d, *J* = 5.7 Hz, 1H), 4.87 (br s, 0.5H), 4.50 (d, *J* = 12.0 Hz, 0.5H), 4.25 (br s, 0.5H), 3.88 (d, *J* = 12.0 Hz, 0.5H), 3.52 (m, 2.5H), 3.20 (m, 2.5H), 2.98-2.80 (m, 2H), 2.79-2.41 (m, 2H), 2.05 (m, 2H), 1.44 (d, *J* = 6.6 Hz, 1.5H), 1.39 (d, *J* = 6.6 Hz, 1.5H). LC purity (UV 220 nm): 99.2%. LRMS (ESI$^+$) calcd for $C_{23}H_{25}ClN_4O$ [M+H]$^+$: 429.2; found: *m/z* = 409.1.

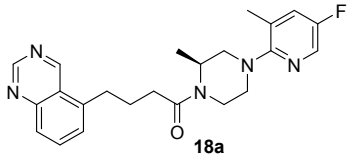
1-(4-(5-Fluoro-3-methylpyridin-2-yl)-1,4-diazepan-1-yl)-4-(quinolin-5-yl)butan-1-one (17).



17

^{1}H NMR (400 MHz, CD_3OD), mixture of rotational isomers: δ 8.83 (app t, *J* = 1.2 Hz, 1H), 8.70 (m, 1H), 7.89 (m, 2H), 7.67 (dd, *J* = 8.4, 6.8 Hz, 1H), 7.56 (m, 1H), 7.48 (m, 1H), 7.38 (m, 1H), 3.80 (app t, *J* = 5.2 Hz, 1H), 3.70-3.60 (m, 3H), 3.49 (m, 1H), 3.39 (m, 1H), 3.30 (m, 2H), 3.15 (m, 2H), 2.53 (t, *J* = 7.2 Hz, 1H), 2.49 (t, *J* = 7.2 Hz, 1H), 2.27 (s, 1.5H), 2.23 (s, 1.5H), 2.10-1.89 (m, 4H). LC purity (UV 254 nm): 99.0%. LRMS (ESI$^+$) calcd for $C_{24}H_{27}FN_4O$ $[M+Na]^+$: 429.2; found: *m/z* = 428.9.

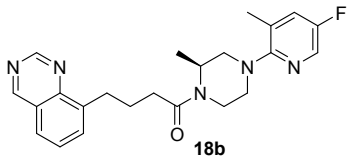
(*S*)-1-(4-(5-Fluoro-3-methylpyridin-2-yl)-2-methylpiperazin-1-yl)-4-(quinazolin-5-yl)butan-1-one (18a).



18a

Prepared as described for the synthesis of compound **11*S*** in the representative procedure starting from 5-bromoquinazoline. ^{1}H NMR (300 MHz, CD_3OD), mixture of rotational isomers: δ 9.90 (s, 1H), 9.24 (s, 1H), 8.02-7.93 (m, 3H), 7.65 (d, *J* = 7.2 Hz, 1H), 7.40 (dd, *J* = 8.4, 2.1 Hz, 1H), 4.83 (br s, 0.5H), 4.46 (d, *J* = 15.9 Hz, 0.5H), 4.26 (br s, 0.5H), 3.84 (d, *J* = 10.8 Hz, 0.5H), 3.58 (m, 0.5H), 3.22 (m, XH), 2.98-2.50 (m, 4.5H), 2.37 (s, 3H), 2.08 (m, 2H), 1.44 (d, *J* = 6.9 Hz, 1.5H), 1.37 (d, *J* = 7.2 Hz, 1.5H). LC purity (UV 220 nm): 98.6%. LRMS (ESI$^+$) calcd for $C_{23}H_{26}FN_5O$ $[M+H]^+$: 408.2; found: *m/z* = 408.3.

(*S*)-1-(4-(5-Fluoro-3-methylpyridin-2-yl)-2-methylpiperazin-1-yl)-4-(quinazolin-8-yl)butan-1-one (18b).

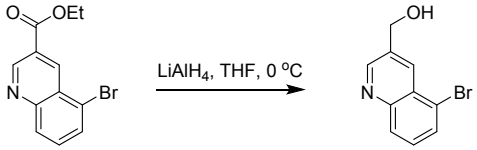


18b

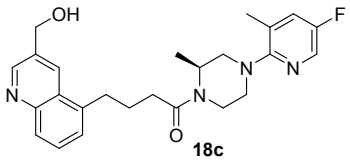
Prepared as described for the synthesis of compound **11*S*** in the representative procedure starting from 8-bromoquinazoline. ^{1}H NMR (400 MHz, CD_3OD), mixture of rotational isomers: δ 9.48 (s, 1H), 9.26 (s, 1H), 8.00-7.90 (m, 3H), 7.70 (app t, *J* = 7.6 Hz, 1H), 7.39 (dd, *J* = 8.8, 2.4 Hz, 1H), 4.80 (br s, 0.6H), 4.43 (d, *J* = 12.8 Hz, 0.4H), 4.21 (br s, 0.4H), 3.80 (d, *J* = 12.8 Hz, 0.6H), 3.57 (m, 0.6H), 3.37-3.12 (m, 4.4H), 2.95-2.41 (m, 4H), 2.38 (s, 3H), 2.08 (m, 2H), 1.43 (d, *J* = 6.4 Hz, 1.2H), 1.36 (d, *J* = 6.8 Hz, 1.8H). LC purity (UV 254 nm): 99.8%. LRMS (ESI^+) calcd for $C_{23}H_{26}FN_5O$ $[M+H]^+$: 408.2; found: *m/z* = 408.1.

(*S*)-1-(4-(5-Fluoro-3-methylpyridin-2-yl)-2-methylpiperazin-1-yl)-4-(3-(hydroxymethyl)quinolin-5-yl)butan-1-one (18c).

Synthesis of (5-bromoquinolin-3-yl)methanol.



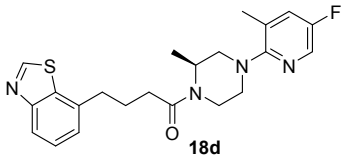
Ethyl 5-bromoquinoline-3-carboxylate (500 mg, 1.79 mmol) in THF (10 mL) was added dropwise to a stirring solution of $LiAlH_4$ (102 mg, 2.68 mmol) in THF (10 mL) at 0 °C under N_2. The mixture was stirred for 1 h at 0 °C and quenched by the addition of sodium hydroxide (0.1 mL of 15% aqueous solution). The mixture was diluted with EtOAc and the mixture was dried over anhydrous sodium sulfate. The solids were filtered, and the filtrate was concentrated in vacuo. The residue was purified by silica gel chromatography (petroleum ether/ethyl acetate) which gave (5-bromoquinolin-3-yl)methanol (267 mg, 1.12 mmol, 63% yield) as a white solid. The title compound was prepared as described for the synthesis of compound **11*S*** in the representative procedure starting from (5-bromoquinolin-3-yl)methanol.



^{1}H NMR (300 MHz, CD_3OD), mixture of rotational isomers: δ 8.85 (s, 1H), 8.61 (s, 1H), 7.98 (d, *J* = 3.0 Hz, 1H), 7.90 (d, *J* = 8.4 Hz, 1H), 7.67 (app t, *J* = 7.2 Hz, 1H), 7.50 (d, *J* = 6.9 Hz, 1H), 7.40 (dd, *J* = 8.4, 2.1 Hz, 1H), 4.88 (s, 2H), 4.80 (br s, 0.5H), 4.43 (d, *J* = 13.8 Hz, 0.5H), 4.21 (br s,

0.5H), 3.80 (d, *J* = 12.8 Hz, 0.5H), 3.57 (m, 0.5H), 3.37-3.12 (m, 4.5H), 2.95-2.48 (m, 4H), 2.35 (s, 3H), 2.04 (m, 2H), 1.46-1.32 (m, 3H). LC purity (UV diode array): 100.0%. LRMS (ESI^+) calcd for $C_{25}H_{29}FN_4O_2$ $[M+H]^+$: 437.2; found: *m/z* = 437.1.

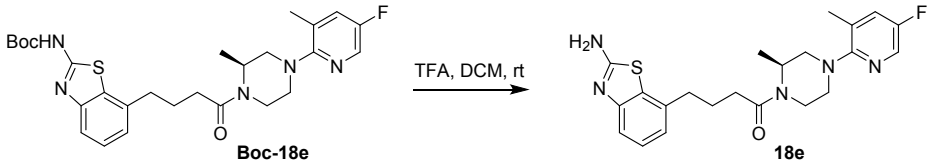
(*S*)-4-(Benzo[*d*]thiazol-7-yl)-1-(4-(5-fluoro-3-methylpyridin-2-yl)-2-methylpiperazin-1-yl)butan-1-one (18d).



18d

Prepared as described for the synthesis of compound **11*S*** in the representative procedure starting from 7-bromobenzo[*d*]thiazole. ^1H NMR (400 MHz, CD_3OD), mixture of rotational isomers: δ 9.24 (s, 1H), 7.95 (m, 2H), 7.52 (t, *J* = 7.6 Hz, 1H), 7.38 (m, 2H), 4.79 (br s, 0.5H), 4.44 (d, *J* = 13.2 Hz, 0.5H), 4.14 (br s, 0.5H), 3.75 (d, *J* = 13.2 Hz, 0.5H), 3.51 (m, 0.5H), 3.39-3.11 (m, 2.5H), 3.01 (m, 2H), 2.90-2.41 (m, 4H), 2.35 (s, 3H), 2.11 (m, 2H), 1.46 (m, 3H). LC purity (UV 254 nm): 98.4%. LRMS (ESI^+) calcd for $C_{22}H_{25}FN_4OS$ $[M+H]^+$: 413.2; found: *m/z* = 412.9.

(*S*)-4-(2-Aminobenzo[d]thiazol-7-yl)-1-(4-(5-fluoro-3-methylpyridin-2-yl)-2-methylpiperazin-1-yl)butan-1-one (18e).



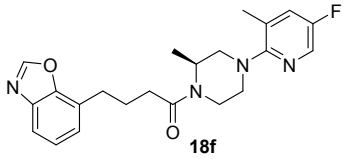
Boc-18e **18e**

Tert-butyl (*S*)-(7-(4-(4-(5-fluoro-3-methylpyridin-2-yl)-2-methylpiperazin-1-yl)-4-oxobutyl)benzo[d]thiazol-2-yl)carbamate (**Boc-18e**) was prepared as described for the synthesis of compound **11*S*** in the representative procedure starting from *tert*-butyl (7-bromobenzo[d]thiazol-2-yl)carbamate.

To a solution of *Tert*-butyl N-(7-[4-[(2S)-4-(5-fluoro-3-methylpyridin-2-yl)-2-methylpiperazin-1-yl]-4-oxobutyl]-1,3-benzothiazol-2-yl)carbamate (**Boc-18e**, 14 mg, 0.038 mmol) in DCM (2 mL) was added TFA (0.7 mL) at 0 °C. The mixture was slowly warmed to rt and stirred for 1 h. The mixture was concentrated and made basic by the addition of 7N

methanolic ammonia solution. The resulting mixture was concentrated and the residue was purified by reverse phase HPLC which gave 4-(2-amino-1,3-benzothiazol-7-yl)-1-[(2S)-4-(5-fluoro-3-methylpyridin-2-yl)-2-methylpiperazin-1-yl]butan-1-one (**18e**, 6.2 mg, 0.015 mmol, 38% yield) as a white solid after lyophilization. ^{1}H NMR (300 MHz, CD_3OD), mixture of rotational isomers: δ 7.97 (s, 1H), 7.41 (d, *J* = 2.7 Hz, 1H), 7.22 (m, 2H), 6.93 (m, 1H), 4.80 (br s, 0.5H), 4.42 (m, 0.5H), 4.21 (br s, 0.5H), 3.83 (m, 0.5H), 3.57 (m, 0.5H), 3.33-3.10 (m, 4.5H), 2.93-2.31 (m, 4H), 2.31 (s, 3H), 2.03 (m, 2H), 1.38 (m, 3H). LC purity (UV 254 nm): 99.6%. LRMS (ESI$^+$) calcd for $C_{22}H_{26}FN_5OS$ $[M+H]^+$: 428.2; found: *m/z* = 428.1.

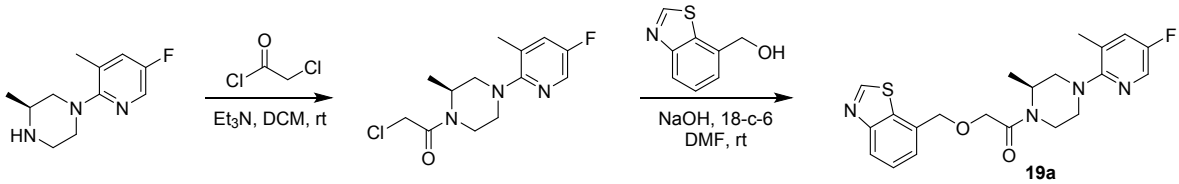
(*S*)-4-(Benzo[d]oxazol-7-yl)-1-(4-(5-fluoro-3-methylpyridin-2-yl)-2-methylpiperazin-1-yl)butan-1-one (18f).



18f

Prepared as described for the synthesis of compound **11*S*** in the representative procedure starting from 7-bromobenzo[d]oxazole. ^{1}H NMR (300 MHz, CD_3OD), mixture of rotational isomers: δ 8.50 (s, 1H), 7.99 (s, 1H), 7.59 (m, 1H), 7.42-7.15 (m, 3H), 4.80 (br s, 0.5H), 4.44 (d, *J* = 10.8 Hz, 0.5H), 4.20 (br s, 0.5H), 3.77 (m, 0.5H), 3.55 (m, 0.5H), 3.44-2.70 (m, 6.5H), 2.60-2.25 (m, 5H), 2.11 (m, 2H), 1.51-1.22 (m, 3H). LC purity (UV 254 nm): 98.5%. LRMS (ESI$^+$) calcd for $C_{22}H_{25}FN_4O_2$ $[M+H]^+$: 397.2; found: *m/z* = 397.1.

(*S*)-2-(Benzo[*d*]thiazol-7-ylmethoxy)-1-(4-(5-fluoro-3-methylpyridin-2-yl)-2-methylpiperazin-1-yl)ethan-1-one (19a).



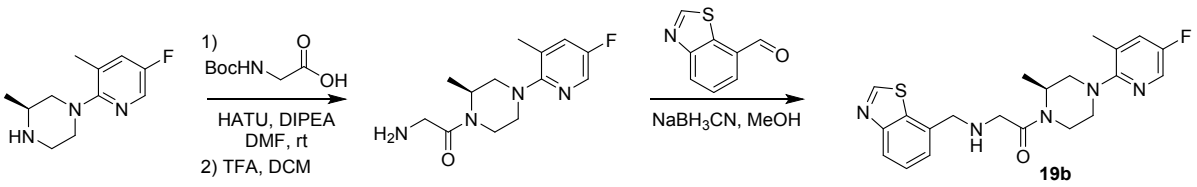
19a

Step 1. To a solution of (3*S*)-1-(5-fluoro-3-methylpyridin-2-yl)-3-methylpiperazine (180 mg, 0.86 mmol), and Et_3N (261 mg, 2.58 mmol), in DCM (4 mL) at rt was added 2-chloroacetyl chloride (146 mg, 1.29 mmol). The mixture was stirred for 1 h, diluted with DCM and washed with water. The organics were dried over Na_2SO_4, filtered, and concentrated which gave 2-

chloro-1-[(2*S*)-4-(5-fluoro-3-methylpyridin-2-yl)-2-methylpiperazin-1-yl] ethan-1-one (160 mg, 0.56 mmol, 65% yield) as a brown oil that was used directly in the next reaction. LRMS (ESI$^+$) calcd for $C_{13}H_{17}ClFN_3O$ $[M+H]^+$: 286.1; found: *m/z* = 286.1.

Step 2. A mixture of 2-chloro-1-[(2*S*)-4-(5-fluoro-3-methylpyridin-2-yl)-2-methylpiperazin-1-yl]ethan-1-one (70 mg, 0.24 mmol), benzo[*d*]thiazol-7-ylmethanol (49 mg, 0.29 mmol), NaOH (29 mg, 0.73 mmol), 18-crown-6 (32 mg, 0.12 mmol) in DMF (2 mL) was stirred at rt for 1 h. The resulting solution was stirred for 1 hr at 25 ^oC. The mixture was stirred for 1 h, diluted with DCM and washed with water. The organics were dried over Na_2SO_4, filtered, and concentrated. Purification by reverse phase HPLC gave (*S*)-2-(benzo[*d*]thiazol-7-ylmethoxy)-1-(4-(5-fluoro-3-methylpyridin-2-yl)-2-methylpiperazin-1-yl)ethan-1-one (**19a**, 36 mg, 0.087 mmol, 72% yield) as a white solid after lyophilization. ^1H NMR (300 MHz, CD_3OD), mixture of rotational isomers: δ 9.27 (s, 1H), 8.04 (d, *J* = 7.5 Hz, 1H), 7.96 (d, *J* = 3.0 Hz, 1H), 7.57 (app t, *J* = 7.7 Hz, 1H), 7.48 (d, *J* = 7.5 Hz, 1H), 7.39 (dd, *J* = 9.0, 2.7 Hz, 1H), 4.93 (s, 2H), 4.76 (br s, 0.5H), 4.47-4.12 (m, 2.5H), 3.77 (d, *J* = 12.7 Hz, 0.5H), 3.57 (m, 0.5H), 3.33-3.10 (m, 3H), 2.96-2.70 (m, 2H), 2.35 (s, 3H), 1.39 (m, 3H). LC purity (UV 254 nm): 99.6%. LRMS (ESI$^+$) calcd for $C_{21}H_{23}FN_4O_2S$ $[M+H]^+$: 415.2; found: *m/z* = 414.8.

(*S*)-2-((Benzo[d]thiazol-7-ylmethyl)amino)-1-(4-(5-fluoro-3-methylpyridin-2-yl)-2-methylpiperazin-1-yl)ethan-1-one (19b).

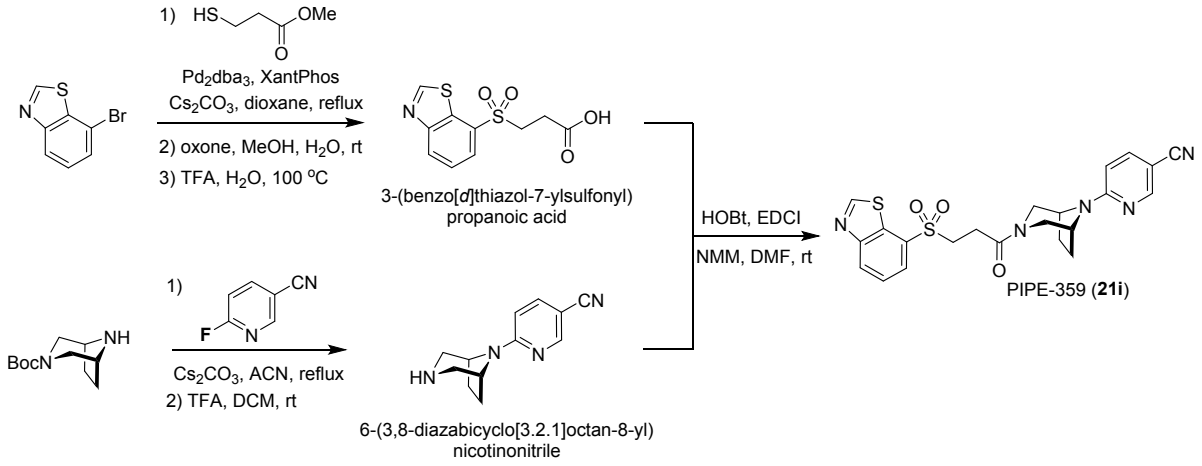


Step 1. A mixture of (3*S*)-1-(5-fluoro-3-methylpyridin-2-yl)-3-methylpiperazine (150 mg, 0.72 mmol), 2-[[(*tert*-butoxy)carbonyl]amino]acetic acid (188 mg, 1.08 mmol), HATU (409 mg, 1.08 mmol), DIPEA (278 mg, 2.15 mmol) in DMF (3 mL) was stirred at rt for 1 h. The mixture was stirred for 1 h, diluted with DCM and washed with water. The organics were dried over Na_2SO_4, filtered, and concentrated. The material was used directly in the next reaction.

Step 2. The crude residue from above was dissolved in DCM (4.0 mL) and TFA (2 mL) was added. The mixture was stirred at rt for 1 h and concentrated in vacuo. Purification by

reverse phase HPLC gave (*S*)-2-amino-1-(4-(5-fluoro-3-methylpyridin-2-yl)-2-methylpiperazin-1-yl)ethan-1-one (90 mg, 0. 34 mmol, 47% yield over two steps) as a yellow oil after concentration. LRMS (ESI$^+$) calcd for $C_{13}H_{19}FN_4O$ [M+H]$^+$: 267.2; found: *m/z* = 267.1. Step 3. A mixture of 2-amino-1-[(2*S*)-4-(5-fluoro-3-methylpyridin-2-yl)-2-methylpiperazin-1-yl]ethan-1-one (35 mg, 0.13 mmol) and 1,3-benzothiazole-7-carbaldehyde (32 mg, 0.20 mmol) in MeOH was stirred at rt for 1 h at which time $NaBH_3CN$ (17 mg, 0.26 mmol) was added. The resulting solution was stirred for an additional 2 h. The mixture was filtered and the filtrates were concentrated in vacuo. The concentrate was purified by reverse phase HPLC which gave 2-[[(1,3-benzothiazol-7-yl)methyl]amino]-1-[(2*S*)-4-(5-fluoro-3-methylpyridin-2-yl)-2-methylpiperazin-1-yl]ethan-1-one (**19b**, 17 mg, 0.041 mmol, 32% yield) as a white solid after lyophilization. ^{1}H NMR (300 MHz, CD_3OD), mixture of rotational isomers: δ 9.22 (s, 1H), 8.01 (m, 2H), 7.55 (app t, *J* = 7.5 Hz, 1H), 7.47 (d, *J* = 7.2 Hz, 1H), 7.39 (dd, *J* = 8.7, 2.7 Hz, 1H), 4.77 (br s, 0.5H), 4.40 (m, 0.5H), 4.13 (s, 2H), 4.11 (br s, 0.5H), 3.70-3.49 (m, 3.5H), 3.33-3.10 (m, 3H), 2.90-2.69 (m, 2H), 2.34 (s, 3H), 1.36 (m, 3H). LC purity (UV 254 nm): 99.1%. LRMS (ESI$^+$) calcd for $C_{21}H_{24}FN_5OS$ [M+H]$^+$: 414.2; found: *m/z* = 413.8.

Representative procedure for synthesis of 3-(benzo[*d*]thiazol-7-ylsulfonyl)propanamides (19c, 20a-h, 21c-l) and (*S*)-4-(benzo[*d*]thiazol-7-ylsulfonyl)-1-(4-(5-fluoro-3-methylpyridin-2-yl)-2-methylpiperazin-1-yl)butan-1-one (19d). Synthesis of 6-(3-(3-(benzo[*d*]thiazol-7-ylsulfonyl)propanoyl)-3,8-diazabicyclo[3.2.1]octan-8-yl)nicotinonitrile (PIPE-359, 21i).



Synthesis of 3-(benzo[*d*]thiazol-7-ylsulfonyl)propanoic acid.

Step 1. A mixture of 7-bromobenzo[*d*]thiazole (5.00 g, 23.4 mmol), methyl 3-sulfanylpropanoate (5.63 g, 46.8 mmol), $Pd_2(dba)_3$ (2.15 g, 2.35 mmol), Xantphos (2.72 g, 4.70 mmol), and Cs_2CO_3 (15.30 g, 47.0 mmol), in dioxane (50 mL) was heated to reflux (110 °C oil bath temperature) under N_2 at for 3 h at which time the mixture was cooled, filtered, and concentrated in vacuo. The concentrate was dissolved in EtOAc and washed with sat. aqueous NH_4Cl (3×). The organics were dried over Na_2SO_4, filtered, and concentrated. Purification by silica gel chromatography (petroleum ether/ethyl acetate) gave methyl 3-(benzo[*d*]thiazol-7-ylthio)propanoate (5.50 g, 21.7 mmol, 93% yield) as a yellow solid.

Step 2. Methyl 3-(benzo[*d*]thiazol-7-ylthio)propanoate (5.50 g, 21.7 mmol) was dissolved in MeOH (60 mL). Oxone® (potassium monopersulfate triple salt) (26.7 g, 43.4 mmol) was added portion-wise at rt over 10 min. The mixture was stirred overnight and partitioned between water and DCM. The layers were separated and the aqueous phase was back-extracted with DCM (2×). The organics were washed with with 3 x 100 mL of 10% aqueous sodium bisulfite (3×). The organics were dried over Na_2SO_4, filtered, and concentrated. Purification by silica gel chromatography (petroleum ether/ethyl acetate) gave methyl 3-(benzo[*d*]thiazol-7-ylsulfonyl)propanoate (5.30 g, 18.6 mmol, 86% yield) as a yellow solid.

Step 3: A mixture of methyl 3-(benzo[*d*]thiazol-7-ylsulfonyl)propanoate (5.30 g, 18.6 mmol), TFA (15 mL) and H_2O (15 mL) was heated to 100 °C and stirred for 2.5 h. The resulting mixture was concentrated in vacuo to give 3-(benzo[*d*]thiazol-7-ylsulfonyl)propanoic acid as a yellow solid which contained minor impurities and was used directly in the coupling reactions without further purification. ^{1}H NMR (400 MHz, *d*6-DMSO): δ 12.40 (br s, 1H), 9.59 (s, 1H), 8.48 (dd, *J* = 6.8, 0.8 Hz, 1H), 8.05 (d, *J* = 6.4 Hz, 1H), 7.84 (app t, *J* = 6.4 Hz, 1H), 3.64 (t, *J* = 6.0 Hz, 2H), 2.56 (t, *J* = 6.0 Hz, 2H). LRMS (ESI$^+$) calcd for $C_{10}H_9NO_4S_2$ $[M+H]^+$: 272.0; found: *m/z* = 271.9.

Synthesis of 6-(3,8-diazabicyclo[3.2.1]octan-8-yl)nicotinonitrile.

Step 1. A mixture of 6-fluoronicotinonitrile (5.10 g, 24.0 mmol), *tert*-butyl 3,8-diaza-bicyclo[3.2.1]octane-3-carboxylate (2.93 g, 24.0 mmol), and Cs_2CO_3 (23.5 g, 72.3 mmol) in ACN (50 mL) was heated to reflux and stirred for 16 h. The mixture was cooled to rt and filtered. The filtrates were concentrated and partitioned between sat. aqueous NH_4Cl and

EtOAc. The layers were separated and the organics were washed with additional sat. aqueous NH_4Cl (2x), concentrated, dried over Na2SO4, filtered, and concentrated. Purification by silica gel chromatography (petroleum ether/ethyl acetate) gave *tert*-butyl 8-(5-cyanopyridin-2-yl)-3,8-diaza-bicyclo[3.2.1]octane-3-carboxylate (6.50 g, 20.7 mmol, 86% yield) as a light yellow solid. LRMS (ESI^+) calcd for $C_{17}H_{22}N_4O_2$ $[M+H]^+$: 315.2; found: *m/z* = 315.1.

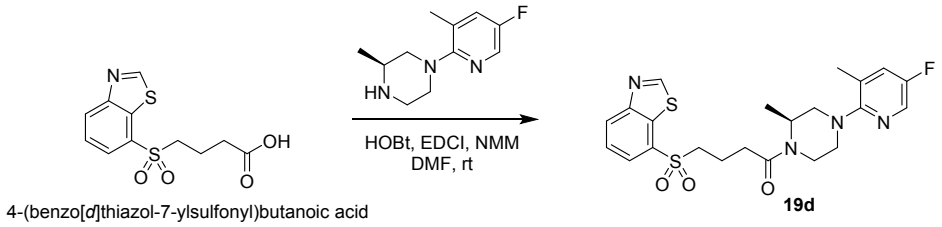
Step 2. A solution of *tert*-butyl 8-(5-cyanopyridin-2-yl)-3,8-diaza-bicyclo[3.2.1]-octane-3-carboxylate (6.50 g, 20.7 mmol) in DCM (100 mL) containing TFA (25 mL) was stirred for 1.5 h at rt. The mixture was concentrated and made basic by the addition of 7N methanolic ammonia solution. The resulting mixture was concentrated and purified by reverse phase HPLC which gave 6-(3,8-diaza-bicyclo[3.2.1]octan-8-yl)nicotinonitrile (4.47 g, 21.0 mmol, 100% yield) as a yellow semi-solid. ^1H NMR (400 MHz, *d*6-DMSO): δ 8.43 (d, *J* = 2.0 Hz, 1H), 7.77 (dd, *J* = 7.2, 2.0 Hz, 1H), 7.76 (d, *J* = 7.2 Hz, 1H), 4.50 (br s, 2H), 2.76 (d, *J* = 8.8 Hz, 2H), 2.55 (d, *J* = 8.8 Hz, 2H), 1.95 (m, 2H), 1.87 (m, 2H). LRMS (ESI^+) calcd for $C_{12}H_{14}N_4$ $[M+H]^+$: 215.1; found: *m/z* = 215.1.

Coupling of 3-(benzo[*d*]thiazol-7-ylsulfonyl)propanoic acid and 6-(3,8-diazabicyclo[3.2.1]octan-8-yl)nicotinonitrile. Synthesis of Synthesis of PIPE-359 (**21i**).

A mixture of 6-(3,8-diaza-bicyclo[3.2.1]octan-8-yl)nicotinonitrile (3.00 g, 14.00 mmol), 3-(1,3-benzothiazole-7-sulfonyl)propanoic acid (3.79 g, 14.00 mmol), HOBt (2.84 g, 21.00 mmol), EDCI (4.03 g, 21.0 mmol) and NMM (4.25 g, 42.0 mmol) in DMF (40 mL) was stirred at rt for 1.5 h. The mixture was diluted with EtOAc and the organics were washed with sat. aqueous NH_4Cl solution (3×). The mixture was dried over anhydrous sodium sulfate, filtered, and concentrated. Purification by reverse phase HPLC gave 6-(3-(3-(benzo[*d*]thiazol-7-ylsulfonyl)propanoyl)-3,8-diazabicyclo[3.2.1]octan-8-yl)nicotinonitrile [PIPE-359 (**21i**), 2.80 g, 5.87 mmol, 42% yield] as a white solid. ^1H NMR (400 MHz, *d*6-CD_3OD): δ 9.39 (s, 1H), 8.41 (dd, *J* = 2.4, 0.8 Hz, 1H), 8.38 (dd, *J* = 8.0, 1.2 Hz, 1H), 8.07 (dd, *J* = 7.2 Hz, 1H), 7.78 (app t, *J* = 8.0 Hz, 1H), 7.74 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.81 (d, *J* = 8.8 Hz, 1H), 4.72 (br s, 1H), 4.64 (br s, 1H), 3.99 (d, *J* = 13.2 Hz, 1H), 3.71-3.6- (3H), 3.35 (m, 1H), 2.90 (dt, *J* = 17.2, 6.8 Hz, 1H), 2.78-2.70 (m, 2H), 2.01 (m, 2H), 1.81 (m, 1H), 1.64 (m, 1H). ^{13}C APT NMR (100 MHz, *d*6-DMSO), mixture of rotational isomers: δ up (*C*, *C*H_2); 168.9, 156.9, 154.6, 132.6, 131.9, 118.6,

95.5, 50.6, 48.7, 46.0, 26.3, 26.2; down (*C*H, C*H*$_3$); 159.2, 153.1, 140.0, 128.6, 126.9, 126.8, 108.4, 52.7, 52.6. LC purity (UV diode array): 100.0%. LRMS (ESI$^+$) calcd for $C_{22}H_{21}N_5O_3S_2$ [M+H]$^+$: 468.1; found: *m/z* = 468.1. Anal. Calcd for $C_{22}H_{21}N_5O_3S_2$: C, 56.51; H, 4.53; N, 14.98; Found: C, 56.49; H, 4.49; N, 14.89.

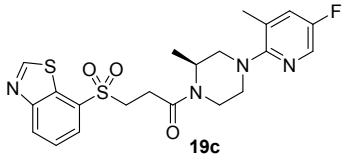
(*S*)-4-(Benzo[*d*]thiazol-7-ylsulfonyl)-1-(4-(5-fluoro-3-methylpyridin-2-yl)-2-methylpiperazin-1-yl)butan-1-one (19d).



4-(Benzo[*d*]thiazol-7-ylsulfonyl)butanoic acid was prepared as described for the synthesis of 3-(benzo[*d*]thiazol-7-ylsulfonyl)propanoic acid in the representative procedure for the synthesis of PIPE-359 (**21i**). A mixture of (*S*)-1-(5-fluoro-3-methylpyridin-2-yl)-3-methylpiperazine (50 mg, 0.24 mmol), 4-(benzo[*d*]thiazol-7-ylsulfonyl)butanoic acid (75 mg, 0.26 mmol), EDCI (45 mg, 0.29 mmol), HOBt (39 mg, 0.29 mmol) and NMM (73 mg, 0.72 mmol) in DMF (2 mL) was stirred at rt overnight. The mixture was filtered and purified by reverse phase HPLC which gave **(***S*)-4-(benzo[*d*]thiazol-7-ylsulfonyl)-1-(4-(5-fluoro-3-methylpyridin-2-yl)-2-methylpiperazin-1-yl)butan-1-one (**19d**, 25 mg, 0.052 mmol, 22% yield) after lyophilization. ^{1}H NMR (400 MHz, *d*6-DMSO), mixture of rotational isomers: δ 9.58 (s, 1H), 8.46 (d, *J* = 8.0 Hz, 1H), 8.09-8.06 (m, 2H), 7.84 (t, *J* = 7.8 Hz, 1H), 7.52 (m, 1H), 4.53 (br s, 0.5H), 4.20 (d, *J* = 11.6 Hz, 0.5H), 4.00 (br s, 0.5H), 3.58 (m, 0.5H), 3.46 (m, 2H), 3.31-3.20 (m, 1.5H), 3.13 (d, *J* = 13.0 Hz, 1H), 2.91 (m, 0.5H), 2.70-2.30 (m, 4H), 2.27 (s, 3H), 1.74 (m, 2H), 1.27 (d, *J* = 4.8 Hz, 1.5H), 1.15 (d, *J* = 6.0 Hz, 1.5H). LC purity (UV 254 nm): 95.8%. LRMS (ESI$^+$) calcd for $C_{22}H_{25}FN_4O_3S_2$ [M+H]$^+$: 477.1; found: *m/z* = 477.6.

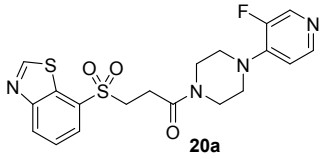
The following 3-(benzo[*d*]thiazol-7-ylsulfonyl)propanamide analogs (**19c**, **20a-h** from table 5) were prepared from 3-(benzo[*d*]thiazol-7-ylsulfonyl)propanoic acid and readily available monopyridinyl piperidines using coupling conditions as described as described for the synthesis of PIPE-359 (**21i**) in the representative procedure:

(*S*)-3-(Benzo[*d*]thiazol-7-ylsulfonyl)-1-(4-(5-fluoro-3-methylpyridin-2-yl)-2-methylpiperazin-1-yl)propan-1-one (19c).



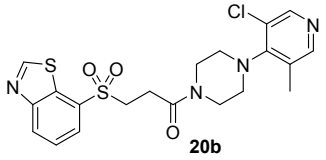
^{1}H NMR (400 MHz, *d*6-DMSO), mixture of rotational isomers: δ 9.59 (s, 1H), 8.46 (d, *J* = 8.0 Hz, 1H), 8.10-8.05 (m, 2H), 7.83 (t, *J* = 7.6 Hz, 1H), 7.52 (dd, *J* = 8.8, 2.4 Hz, 1H), 4.38 (br s, 0.5H), 4.08 (m, 1H), 3.73-3.65 (m, 2.5H), 3.24-3.10 (m, 2H), 3.00-2.52 (m, 5H), 2.29 (s, 3H), 1.29 (d, *J* = 6.4 Hz, 1.5H), 1.09 (d, *J* = 6.8 Hz, 1.5H). LC purity (UV 254 nm): 96.5%. LRMS (ESI$^+$) calcd for $C_{21}H_{23}FN_4O_3S_2$ $[M+H]^+$: 463.1; found: *m/z* = 463.3.

3-(Benzo[*d*]thiazol-7-ylsulfonyl)-1-(4-(3-fluoropyridin-4-yl)piperazin-1-yl)propan-1-one (20a).



^{1}H NMR (400 MHz, *d*6-DMSO): δ 9.57 (s, 1H), 8.45 (d, *J* = 8.0 Hz, 1H), 8.27 (d, *J* = 6.0 Hz, 1H), 8.14 (d, *J* = 5.6 Hz, 1H), 8.05 (d, *J* = 7.3 Hz, 1H), 7.81 (app t, *J* = 8.0 Hz, 1H), 6.94 (dd, *J* = 8.4, 6.0 Hz, 1H), 3.66 (t, *J* = 7.2 Hz, 2H), 3.48 (m, 2H), 3.40 (m, 2H), 3.22 (m, 2H), 3.13 (m, 2H), 2.75 (t, *J* = 7.2 Hz, 2H). LC purity (UV 254 nm): 95.5%. LRMS (ESI$^+$) calcd for $C_{19}H_{19}FN_4O_3S_2$ $[M+H]^+$: 435.1; found: *m/z* = 435.0.

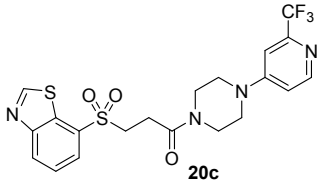
3-(Benzo[*d*]thiazol-7-ylsulfonyl)-1-(4-(3-chloro-5-methylpyridin-4-yl)piperazin-1-yl)propan-1-one (20b).



^{1}H NMR (400 MHz, *d*6-DMSO): δ 9.59 (s, 1H), 8.48 (dd, *J* = 8.4, 1.2 Hz, 1H), 8.33 (s, 1H), 8.25 (s, 1H), 8.08 (d, *J* = 7.2 Hz, 1H), 7.84 (app t, *J* = 7.6 Hz, 1H), 3.70 (t, *J* = 7.1 Hz, 2H), 3.46-3.42

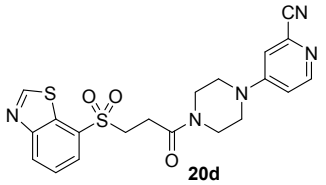
(m, 4H), 3.16 (m, 2H), 3.02 (m, 2H), 2.78 (t, *J* = 7.1 Hz, 2H), 2.27 (s, 3H). LC purity (UV 254 nm): 98.8%. LRMS (ESI$^+$) calcd for $C_{20}H_{21}ClN_4O_3S_2$ [M+H]$^+$: 465.1; found: *m/z* = 464.9.

3-(Benzo[*d*]thiazol-7-ylsulfonyl)-1-(4-(2-(trifluoromethyl)pyridin-4-yl)piperazin-1-yl)propan-1-one (20c).



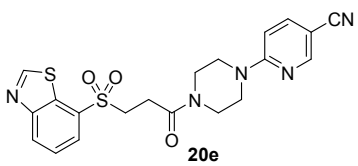
^{1}H NMR (400 MHz, CD_3OD): δ 9.42 (s, 1H), 8.42 (d, *J* = 8.4 Hz, 1H), 8.26 (d, *J* = 6.0 Hz, 1H), 8.11 (d, *J* = 7.6 Hz, 1H), 7.83 (app t, *J* = 7.6 Hz, 1H), 7.22 (d, *J* = 2.6 Hz, 1H), 7.00 (dd, *J* = 6.0, 2.4 Hz, 1H), 3.71 (t, *J* = 7.2 Hz, 2H), 3.69-3.43 (m, 8H), 2.93 (t, *J* = 7.2 Hz, 2H). LC purity (UV 254 nm): 98.6%. LRMS (ESI$^+$) calcd for $C_{20}H_{19}F_3N_4O_3S_2$ [M+H]$^+$: 485.1; found: *m/z* = 485.1.

4-(4-(3-(Benzo[*d*]thiazol-7-ylsulfonyl)propanoyl)piperazin-1-yl)picolinonitrile (20d).



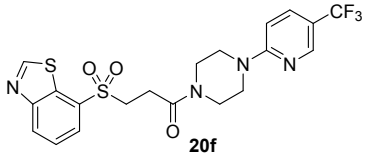
^{1}H NMR (400 MHz, *d*6-DMSO): δ 9.60 (s, 1H), 8.48 (dd, *J* = 8.4, 0.8 Hz, 1H), 8.25 (d, *J* = 6.0 Hz, 1H), 8.08 (dd, *J* = 7.6 Hz, 1H), 7.83 (app t, *J* = 8.0 Hz, 1H), 7.50 (d, *J* = 2.8 Hz, 1H), 7.3 (dd, *J* = 6.4, 2.8 Hz, 1H), 3.67 (t, *J* = 7.2 Hz, 2H), 3.53-3.32 (m, 8H), 2.77 (t, *J* = 7.2 Hz, 2H). LC purity (UV 254 nm): 96.4%. LRMS (ESI$^+$) calcd for $C_{20}H_{19}N_5O_3S_2$ [M+H]$^+$: 442.1; found: *m/z* = 442.0.

6-(4-(3-(Benzo[*d*]thiazol-7-ylsulfonyl)propanoyl)piperazin-1-yl)nicotinonitrile (20e).



^{1}H NMR (400 MHz, *d*6-DMSO): δ 9.58 (s, 1H), 8.50 (d, *J* = 1.6 Hz, 1H), 8.46 (dd, *J* = 6.4, 0.8 Hz, 1H), 8.07 (d, *J* = 6.0 Hz, 1H), 7.87 (dd, *J* = 7.2, 1.6 Hz, 1H), 7.83 (app t, *J* = 6.0 Hz, 1H), 6.90 (d, *J* = 7.2 Hz, 1H), 3.67 (m, 4H), 3.56 (m, 2H), 3.47 (m, 2H), 3.38 (m, 2H), 2.77 (t, *J* = 6.0 Hz, 2H). ^{13}C APT NMR (100 MHz, *d*6-DMSO): δ up (*C*, *C*H_2); 167.0, 158.9, 132.6, 132.2, 118.6, 95.4, 50.6, 43.9, 43.5, 43.4, 40.7, 25.9; down (*C*H, C*H*$_3$); 159.3, 152.4, 140.0, 128.7, 126.9, 106.5. LC purity (UV 254 nm): 98.8%. HRMS (ESI$^+$) calcd for $C_{20}H_{19}N_5O_3S_2$ [M+H]$^+$: 442.1008; found: 442.1007.

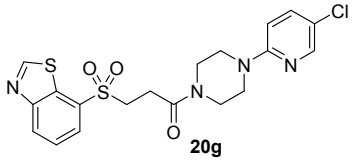
3-(Benzo[*d*]thiazol-7-ylsulfonyl)-1-(4-(5-(trifluoromethyl)pyridin-2-yl)piperazin-1-yl)propan-1-one (20f).



20f

^{1}H NMR (300 MHz, CD_3OD): δ 9.42 (s, 1H), 8.44 (dd, *J* = 8.4, 1.2 Hz, 1H), 8.38 (m, 1H), 8.12 (d, *J* = 7.5 Hz, 1H), 7.84 (app t, *J* = 8.1 Hz, 1H), 7.76 (dd, *J* = 9.0, 2.4 Hz, 1H), 6.88 (d, *J* = 9.0 Hz, 1H), 3.77-3.69 (m, 4H), 3.66-3.51 (m, 6H), 2.92 (t, *J* = 7.2 Hz, 2H). LC purity (UV 220 nm): 100.0%. LC purity (UV 254 nm): 99.6%. LRMS (ESI$^+$) calcd for $C_{20}H_{19}F_3N_4O_3S_2$ [M+H]$^+$: 485.1; found: *m/z* = 485.1.

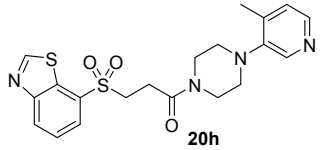
3-(Benzo[*d*]thiazol-7-ylsulfonyl)-1-(4-(5-chloropyridin-2-yl)piperazin-1-yl)propan-1-one (20g).



20g

^{1}H NMR (400 MHz, *d*6-DMSO): δ 9.58 (s, 1H), 8.46 (dd, *J* = 8.0, 0.8 Hz, 1H), 8.12-8.06 (m, 2H), 7.84 (app t, *J* = 8.0 Hz, 1H), 7.62 (dd, *J* = 9.2, 2.8 Hz, 1H), 6.86 (d, *J* = 9.2 Hz, 1H), 3.69 (t, *J* = 6.8 Hz, 2H), 3.70-3.30 (m, 8H), 3.3 (m, 4) 2.77 (t, *J* = 6.8 Hz, 2H). LC purity (UV 220 nm): 100.0%. LC purity (UV 254 nm): 98.1%. LRMS (ESI$^+$) calcd for $C_{19}H_{19}ClN_4O_3S_2$ [M+H]$^+$: 451.1; found: *m/z* = 451.0.

3-(Benzo[d]thiazol-7-ylsulfonyl)-1-(4-(4-methylpyridin-3-yl)piperazin-1-yl)propan-1-one (20h).

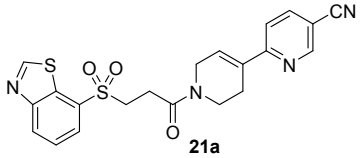


20h

^{1}H NMR (400 MHz, *d*6-DMSO): δ 9.59 (s, 1H), 8.46 (dd, *J* = 6.4, 0.8 Hz, 1H), 8.19 (s, 1H), 8.15 (d, *J* = 3.6 Hz, 1H), 8.08 (d, *J* = 6.0 Hz, 1H), 7.84 (app t, *J* = 6.0 Hz, 1H), 7.18 (d, *J* = 3.6 Hz, 1H), 3.69 (t, *J* = 5.6 Hz, 2H), 3.47 (m, 2H), 3.40 (m, 2H), 2.86 (m, 2H), 2.77 (m, 4H), 2.26 (s, 3H). LC purity (UV diode array): 100.0%. LRMS (ESI$^+$) calcd for $C_{20}H_{22}N_4O_3S_2$ [M+H]$^+$: 431.1; found: *m/z* = 431.0.

The following 3-(benzo[*d*]thiazol-7-ylsulfonyl)propanamide analogs (**21a-h, 21j-l**) from table 7 were prepared from 3-(benzo[*d*]thiazol-7-ylsulfonyl)propanoic acid and readily available 6-(cyclodiamino)nicotinonitriles (for **21e-h** and **21j-l**), 1',2',3',6'-tetrahydro-[2,4'-bipyridine]-5-carbonitrile (for **21a**), or 6-(piperidin-4-yl)nicotinonitrile (for **21b**) using coupling conditions as described for the synthesis of PIPE-359 (**21i**) in the representative procedure:

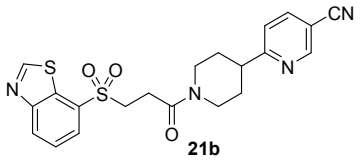
1'-(3-(Benzo[*d*]thiazol-7-ylsulfonyl)propanoyl)-1',2',3',6'-tetrahydro-[2,4'-bipyridine]-5-carbonitrile (21a).



21a

^{1}H NMR (300 MHz, CD_3OD), mixture of rotational isomers: δ 9.38 (s, 1H), 8.56 (d, *J* = 1.5 Hz, 1H), 8.40 (dd, *J* = 8.1, 0.9 Hz, 1H), 8.13-8.08 (m, 2H), 7.82 (m, 1H), 7.71 (app t, *J* = 7.2 Hz, 1H), 6.83 (m, 1H), 4.22 (m, 0.8H), 4.08 (m, 1.2H), 3.73 (t, *J* = 6.9 Hz, 2H), 3.70-3.60 (m, 2H), 2.97 (m, 2H), 2.71 (m, 1.2H), 2.53 (m, 0.8H). LC purity (UV 254 nm): 97.9%. LRMS (ESI$^+$) calcd for $C_{21}H_{18}N_4O_3S_2$ [M+H]$^+$: 439.1; found: *m/z* = 439.1.

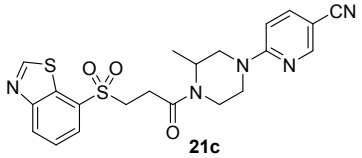
6-(1-(3-(Benzo[*d*]thiazol-7-ylsulfonyl)propanoyl)piperidin-4-yl)nicotinonitrile (21b).



21b

^{1}H NMR (300 MHz, *d*6-DMSO): δ 9.58 (s, 1H), 8.95 (d, *J* = 1.5 Hz, 1H), 8.45 (dd, *J* = 8.1, 0.9 Hz, 1H), 8.24 (dd, *J* = 8.4, 2.4 Hz, 1H), 8.07 (d, *J* = 7.2 Hz, 1H), 7.83 (app t, *J* = 8.1 Hz, 1H), 7.51 (d, *J* = 7.8 Hz, 1H), 4.27 (d, *J* = 12.7 Hz, 1H), 3.84 (d, *J* = 13.2 Hz, 1H), 3.68 (t, *J* = 6.6 Hz, 2H), 3.00 (m, 2H), 2.77 (m, 2H), 2.52 (m, 1H), 1.83 (m, 2H), 1.41 (m, 1H), 1.37 (m, 1H). LC purity (UV 254 nm): 100.0%. LRMS (ESI$^+$) calcd for $C_{21}H_{20}N_4O_3S_2$ [M+H]$^+$: 441.1; found: *m/z* = 441.1.

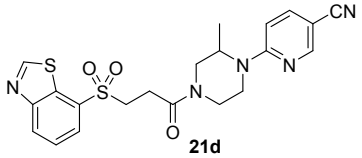
rac-6-(4-(3-(Benzo[d]thiazol-7-ylsulfonyl)propanoyl)-3-methylpiperazin-1-yl)nicotinonitrile (21c).



21c

^{1}H NMR (400 MHz, *d*6-DMSO), mixture of rotational isomers: δ 9.58 (s, 1H), 8.47 (d, *J* = 2.0 Hz, 1H), 8.45 (d, *J* = 6.8 Hz, 1H), 8.06 (d, *J* = 6.0 Hz, 1H), 7.86 (dd, *J* = 7.6, 2.0 Hz, 1H), 7.81 (m, 1H), 6.92 (d, *J* = 7.6 Hz, 0.4H), 6.88 (d, *J* = 7.6 Hz, 0.6H), 4.31-4.00 (m, 4H), 3.68 (m, 3H), 3.35-3.10 (m, 2H), 2.98-2.63 (m, 2H), 1.06 (d, *J* = 5.2 Hz, 1.2H), 0.86 (d, *J* = 5.2 Hz, 1.8H). LC purity (UV diode array): 90.0%. LRMS (ESI$^+$) calcd for $C_{21}H_{21}N_5O_3S_2$ [M+H]$^+$: 456.1; found: *m/z* = 456.0.

rac-6-(4-(3-(Benzo[*d*]thiazol-7-ylsulfonyl)propanoyl)-2-methylpiperazin-1-yl)nicotinonitrile (21d).

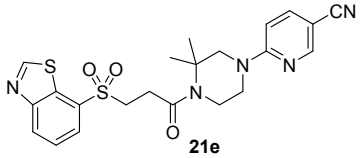


21d

^{1}H NMR (400 MHz, *d*6-DMSO), mixture of rotational isomers: δ 9.58 (s, 1H), 8.50 (app t, *J* = 1.7 Hz, 1H), 8.46 (d, *J* = 6.8 Hz, 1H), 8.07 (app t, *J* = 6.4 Hz, 1H), 7.86 (dt, *J* = 7.2, 0.9 Hz, 1H), 7.82 (m, 1H), 6.85 (d, *J* = 7.2 Hz, 1H), 4.55 (m, 1H), 4.11 (app t, *J* = 12.8 Hz, 1H), 3.99 (m, 1H), 3.80 (d, *J* = 10.0 Hz, 0.5H), 3.72-3.62 (m, 2.5H), 3.38-3.08 (m, 3H), 2.90 (m, 0.5H), 2.80-2.70 (m,

1.5H), 1.03 (d, *J* = 5.2 Hz, 1.5H), 0.95 (d, *J* = 5.2 Hz, 1.5H). LC purity (UV diode array): 100.0%. LRMS (ESI$^+$) calcd for $C_{21}H_{21}N_5O_3S_2$ [M+H]$^+$: 456.1; found: *m/z* = 456.0.

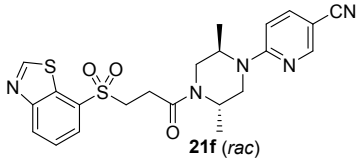
6-(4-(3-(Benzo[*d*]thiazol-7-ylsulfonyl)propanoyl)-3,3-dimethylpiperazin-1-yl)nicotinonitrile (21e).



21e

^{1}H NMR (400 MHz, *d*6-DMSO): δ 9.56 (s, 1H), 8.48 (s, 1H), 8.46 (d, *J* = 7.6 Hz, 1H), 8.07 (d, *J* = 7.6 Hz, 1H), 7.88 (dd, *J* = 7.2, 2.0 Hz, 1H), 7.81 (app t, *J* = 8.0 Hz, 1H) 6.71 (br s, 1H), 3.78 (m, 2H), 3.72 (m, 2H), 3.65 (t, *J* = 6.8 Hz, 2H), 3.48 (m, 2H), 2.78 (t, *J* = 6.8 Hz, 2H), 1.13 (s, 6H). LC purity (UV 254 nm): 99.2%. LRMS (ESI$^+$) calcd for $C_{22}H_{23}N_5O_3S_2$ [M+H]$^+$: 470.1; found: *m/z* = 470.1.

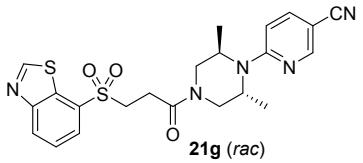
***rac*-(*trans*)-6-(4-(3-(Benzo[*d*]thiazol-7-ylsulfonyl)propanoyl)-2,5-dimethylpiperazin-1-yl)nicotinonitrile (21f).**



21f (*rac*)

^{1}H NMR (400 MHz, CD_3OD), mixture of rotational isomers: δ 9.44 (s, 1H), 8.44 (s, 1H), 8.43 (d, *J* = 8.0 Hz, 1H), 8.11 (dd, *J* = 7.2, 4.4 Hz, 1H), 7.84 (app t, *J* = 8.0Hz, 1H), 7.76 (dd, *J* = 8.8, 2.0 Hz, 1H), 6.86 (dd, *J* = 8.8, 5.2 Hz, 1H), 4.80-4.60 (m, 1H), 4.31-4.10 (m, 1H), 3.72 (m, 3H), 3.61-3.51 (m, 2H), 3.13 (m, 1H), 2.85 (m, 2H), 1.25 (d, *J* = 6.8 Hz, 1.5H), 1.15 (d, *J* = 6.8 Hz, 1.5H), 1.06 (m, 3H). LC purity (UV 254 nm): 98.5%. LRMS (ESI$^+$) calcd for $C_{22}H_{23}N_5O_3S_2$ [M+H]$^+$: 470.1; found: *m/z* = 470.1.

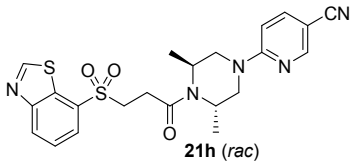
***rac*-(*trans*)-6-(4-(3-(Benzo[*d*]thiazol-7-ylsulfonyl)propanoyl)-2,6-dimethylpiperazin-1-yl)nicotinonitrile (21g).**



21g (*rac*)

^{1}H NMR (300 MHz, CD_3OD): δ 9.41 (s, 1H), 8.42 (m, 2H), 8.10 (d, *J* = 7.2 Hz, 1H), 7.82 (app t, *J* = 8.1 Hz, 1H), 7.76 (dd, *J* = 9.3, 2.4 Hz, 1H), 6.78 (d, *J* = 9.3 Hz, 1H), 4.65 (m, 1H), 4.65 (m, 1H), 4.55 (m, 1H), 4.25 (d, *J* = 13.5 Hz, 1H), 3.85 (d, *J* = 13.8 Hz, 1H), 3.71 (m, 2H), 2.98-2.80 (m, 3H), 1.17 (d, *J* = 6.9 Hz, 3H), 1.08 (d, *J* = 6.9 Hz, 3H). LC purity (UV 254 nm): 99.2%. LRMS (ESI$^+$) calcd for $C_{22}H_{23}N_5O_3S_2$ $[M+H]^+$: 470.1; found: *m/z* = 470.4.

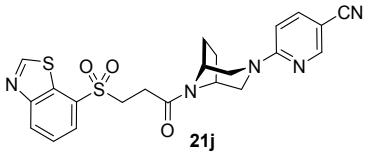
rac-(_trans_)-6-(4-(3-(Benzo[_d_]thiazol-7-ylsulfonyl)propanoyl)-3,5-dimethylpiperazin-1-yl)nicotinonitrile (21h).



21h (*rac*)

^{1}H NMR (400 MHz, *d*6-DMSO): δ 9.59 (s, 1H), 8.46 (m, 2H), 8.06 (d, *J* = 7.6 Hz, 1H), 7.89-7.81 (m, 2H), 7.01 (d, *J* = 9.2 Hz, 1H), 4.42-4.20 (m, 3H), 4.11 (m, 1H), 3.72 (m, 2H), 3.10 (m, 1H), 2.98 (m, 2H), 2.61 (m, 1H), 1.11 (d, *J* = 5.2 Hz, 3H), 0.91 (d, *J* = 5.2 Hz, 3H). LC purity (UV 254 nm): 96.3%. LRMS (ESI$^+$) calcd for $C_{22}H_{23}N_5O_3S_2$ $[M+H]^+$: 470.1; found: *m/z* = 470.3.

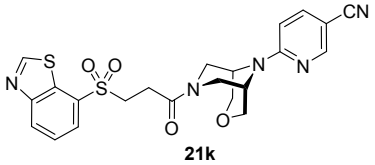
6-(8-(3-(Benzo[_d_]thiazol-7-ylsulfonyl)propanoyl)-3,8-diazabicyclo[3.2.1]octan-3-yl)nicotinonitrile (21j).



21j

^{1}H NMR (400 MHz, *d*6-DMSO): δ 9.56 (s, 1H), 8.49 (d, *J* = 2.0 Hz, 1H), 8.44 (d, *J* = 8.0 Hz, 1H), 8.06 (d, *J* = 7.6 Hz, 1H), 7.86 (dd, *J* = 9.2, 2.0 Hz, 1H), 7.86 (app t, *J* = 8.0 Hz, 1H), 6.83 (d, *J* = 9.2 Hz, 1H), 4.39 (dd, *J* = 7.2, 6.0 Hz, 1H), 4.07 (m, 2H), 3.71 (t, *J* = 7.2 Hz, 2H), 3.00 (d, *J* = 12.4 Hz, 1H), 2.85-2.71 (m, 3H), 2.61 (m, 1H), 1.90 (m, 1H), 1.71-1.45 (m, 3H). LC purity (UV 254 nm): 97.8%. LRMS (ESI$^+$) calcd for $C_{22}H_{21}N_5O_3S_2$ $[M+H]^+$: 468.1; found: *m/z* = 468.0.

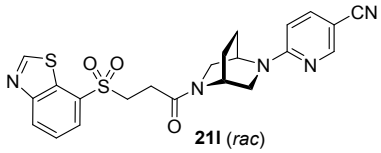
6-(7-(3-(Benzo[*d*]thiazol-7-ylsulfonyl)propanoyl)-3-oxa-7,9-diazabicyclo[3.3.1]nonan-9-yl)nicotinonitrile (21k).



21k

^{1}H NMR (400 MHz, CD_3OD): δ 9.41 (s, 1H), 8.46 (d, *J* = 2.0 Hz, 1H), 8.41 (d, *J* = 8.0 Hz, 1H), 8.10 (d, *J* = 8.0 Hz, 1H), 7.81 (m, 2H), 6.90 (d, *J* = 9.2 Hz, 1H), 4.60 (br s, 1H), 4.51 (d, *J* = 10.0 Hz, 2H), 4.05 (dd, *J* = 18.4, 13.2 Hz, 2H), 3.91 d, *J* = 11.6 Hz, 1H), 3.78 (m, 2H), 3.72-3.63 (m, 2H), 3.46 (m, 1H), 2.95-2.85 (m, 3H). LC purity (UV 254 nm): 99.0%. LRMS (ESI$^+$) calcd for $C_{22}H_{21}N_5O_4S_2$ $[M+H]^+$: 484.1; found: *m/z* = 484.1.

***rac*-6-(5-(3-(Benzo[d]thiazol-7-ylsulfonyl)propanoyl)-2,5-diazabicyclo[2.2.2]octan-2-yl)nicotinonitrile (21l).**



21l (*rac*)

^{1}H NMR (400 MHz, CD_3OD), mixture of rotational isomers: δ 9.39 (s, 0.4H), 9.36 (s, 0.6H), 8.40 (m, 2H), 8.07 (m, 1H), 7.80 (d, *J* = 8.0 Hz, 1H), 7.75 (m, 2H), 6.59 (m, 1H), 4.60 (br s, 0.6H), 4.28 (br s, 0.4H), 3.75-3.68 (m, 4H), 3.52-3.35 (m, 2H), 2.92-2.69 (m, 2H), 2.0-1.76 (m, 4H). LC purity (UV 254 nm): 99.3%. LRMS (ESI$^+$) calcd for $C_{22}H_{21}N_5O_3S_2$ $[M+H]^+$: 468.1; found: *m/z* = 468.2.

Figure S1. ^{1}H (CD_3OD) and ^{13}C APT (*d*6-DMSO) NMR data for compound 11*S*

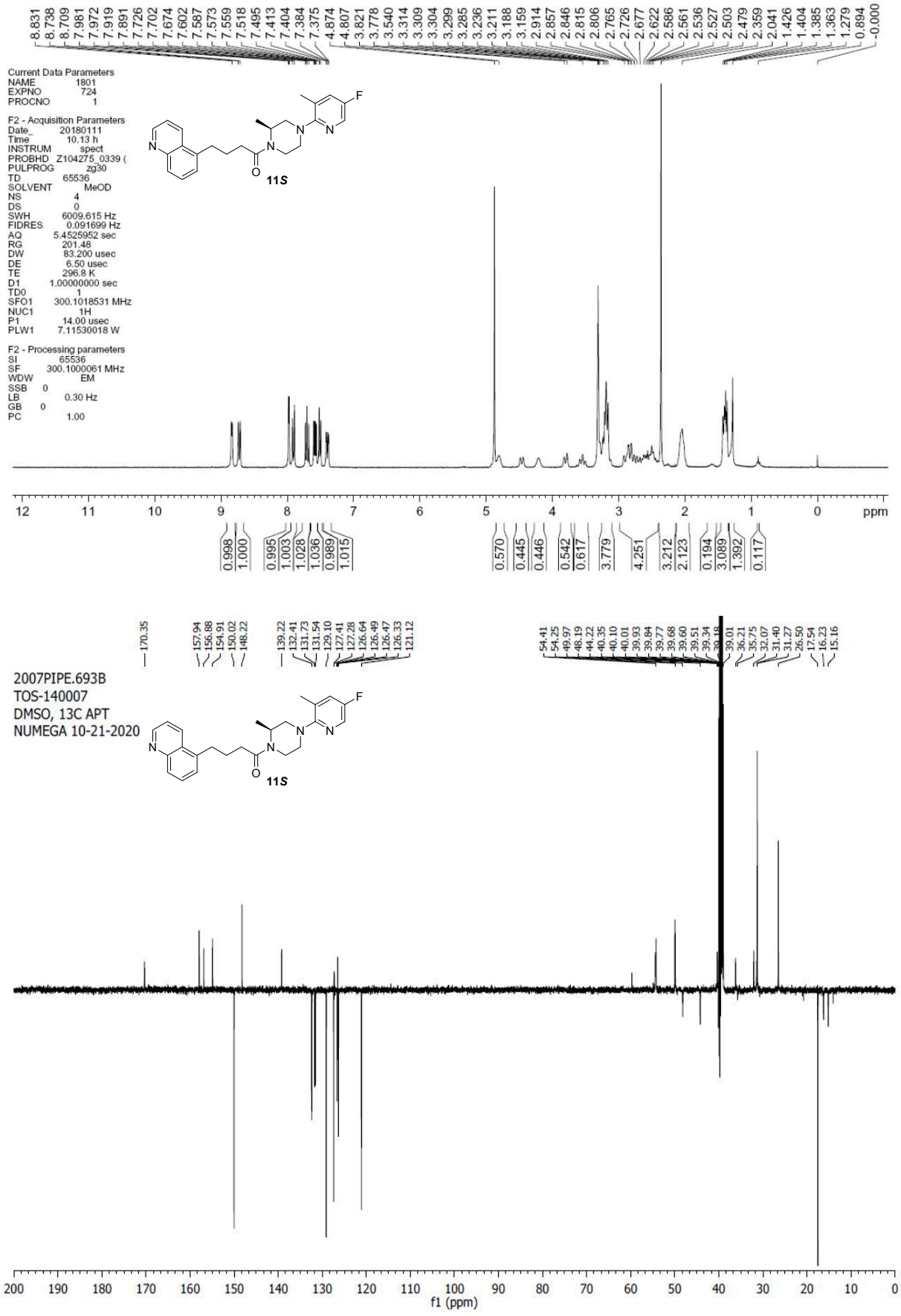
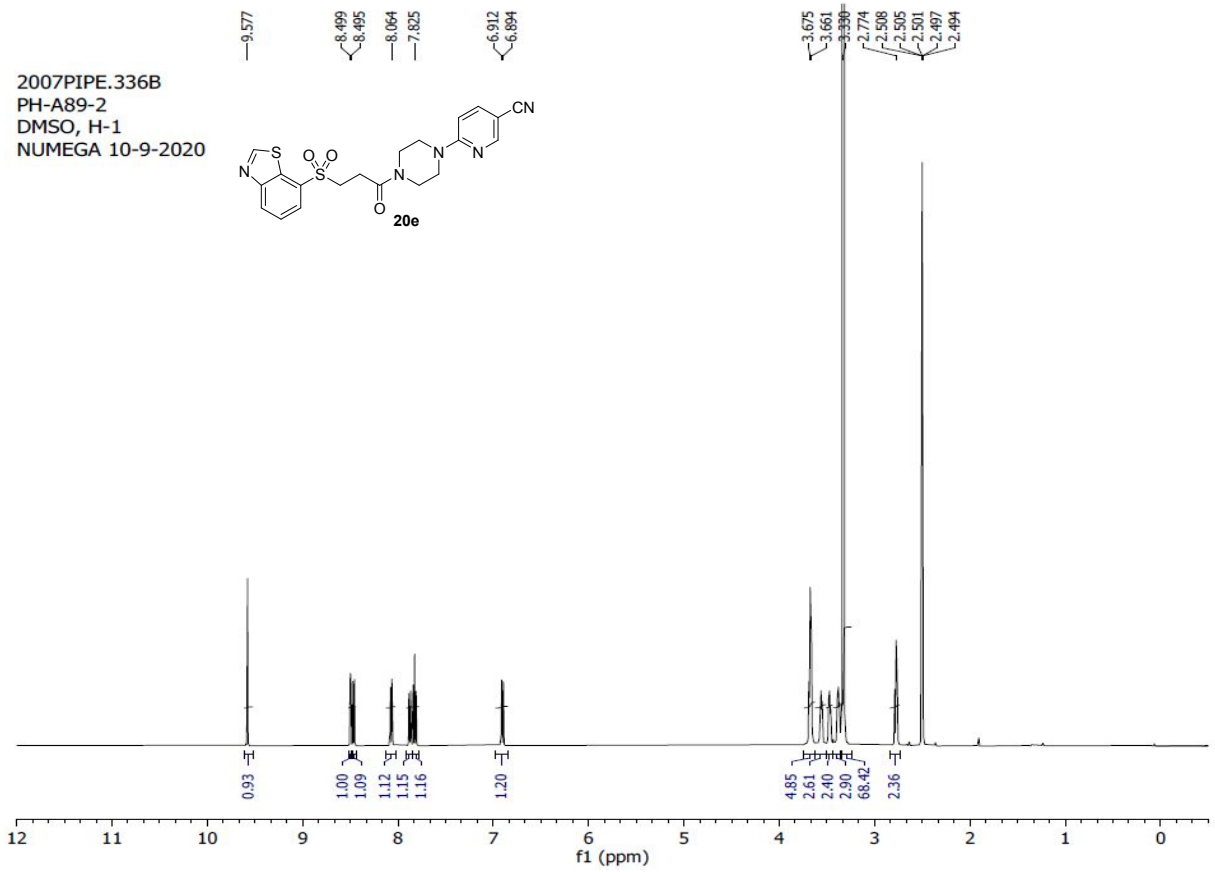


Figure S2. ^{1}H (*d*6-DMSO) and ^{13}C APT (*d*6-DMSO) NMR data for compound 20e



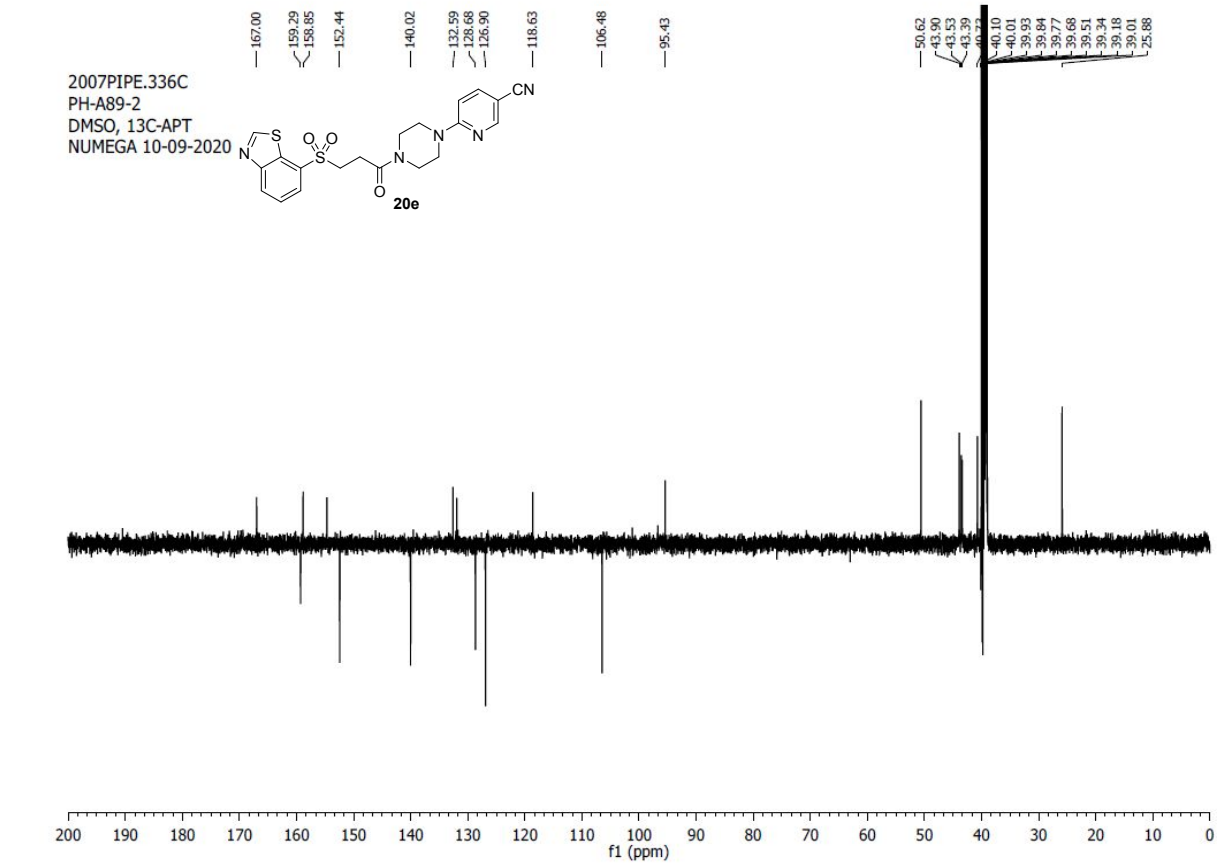
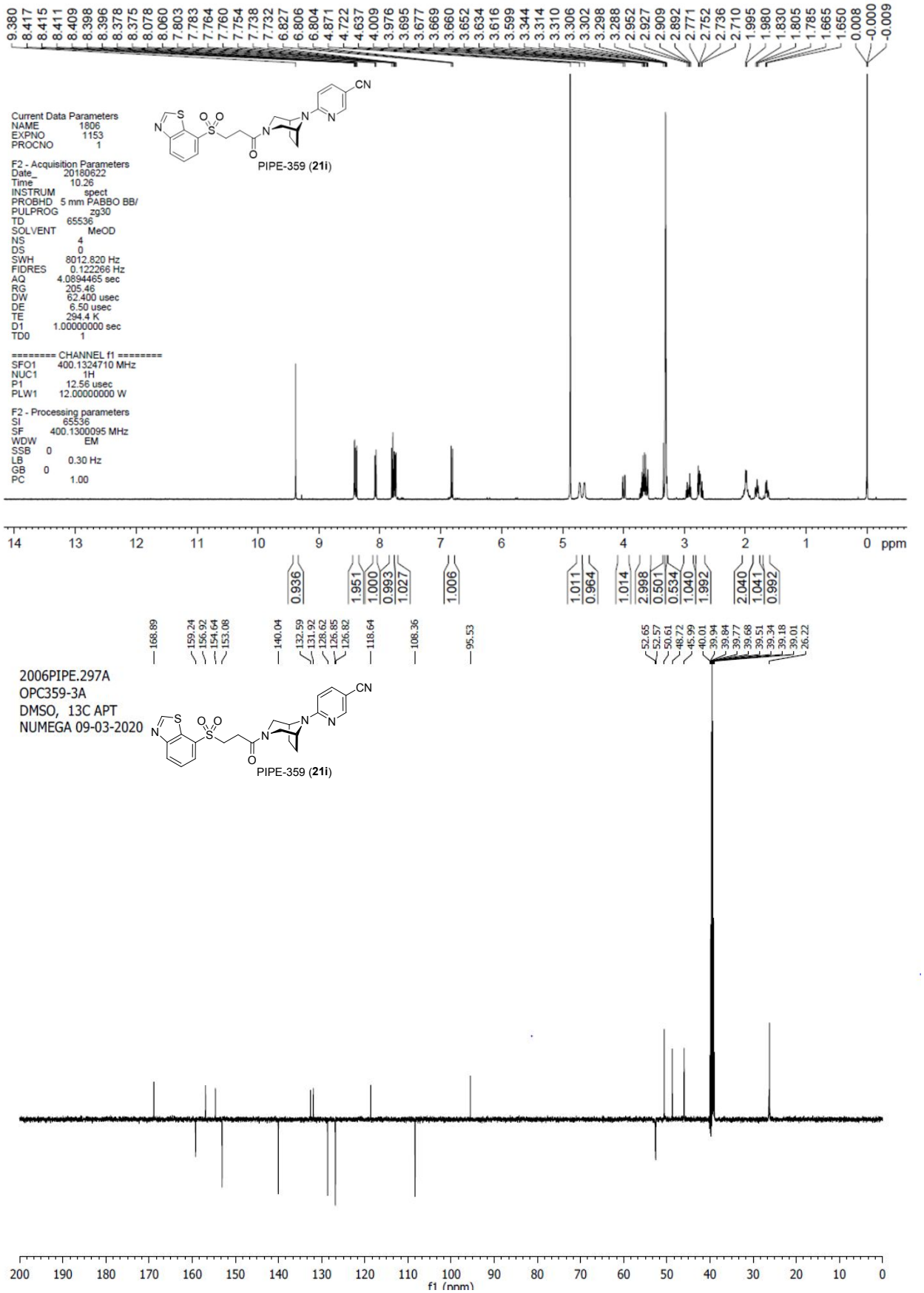


Figure S3. ^{1}H (CD_3OD) and ^{13}C APT (*d*6-DMSO) NMR data for PIPE-359 (21i)



Experimental Procedures: Biology

In vitro functional assay of muscarinic acetylcholine receptor activity (hM_nR).

CHO-K1 cells stably expressing human muscarinic receptor (hM1R, hM_2R, hM_3R or hM_4R) with aequorin (Perkin Elmer) were grown in F12 media (Gibco) containing 10% FBS (ATCC), 0.4 mg/mL geneticin (Sigma-Aldrich) and 0.25 mg/mL Zeocin (Invitrogen). Cells were grown as per the manufacturer's protocol. For compound testing, cells were grown to confluency and detached gently with Accutase (Sigma-Aldrich) followed by centrifugation for 5 min at 150 x g. Cells were then re-suspended in assay buffer (i.e. DMEM/F-12 HEPES without phenol red (Invitrogen) with 0.1% BSA (Sigma-Aldrich)) at a density of 5 x 106 cells/ml. Under sterile conditions, 5 μM coelenterazine (Invitrogen) was added to the cells, mixed, then incubated at room temperature protected from light, with gentle agitation, for 4 h. Primary compound plates were prepared in 100% DMSO in opaque 96-well plates (VWR) and serially diluted in half log increments. Secondary compound plates were prepared at 3x concentration in assay buffer. Compounds were added to white, clear bottom tissue culture treated 96-well plates (Fisher Scientific). Coelenterazine-loaded cells were then added at 5 x 105 cells/well. Compounds and cells were incubated at room temperature for 30 min in the dark. Acetylcholine, at EC_{80} concentration, was added and calcium flux measured using a FlexStation 3 (Molecular Devices). Sigmoidal dose-response curves were generated by measuring luminescence over 40 sec and calculating the area under the curve. Dose response curves and IC_{50} values were generated using Prism (GraphPad). Compounds were tested at a final concentration range of 100 pM to 10 μM in 0.1% DMSO.

Radioligand binding assay (RBA).

Radioligand binding assays were performed by Beacon Discovery (San Diego, CA) using commercially available [^{3}H]-*N*-methyl scopolamine as the radioligand and nonspecific binding was determined in the presence of unlabeled *N*-methyl scopolamine at a saturating concentration of 10 μM. Competition experiments utilized human muscarinic receptor (hM_1R, hM_2R, hM_3R or hM_4R) expressing HEK293 cell membranes (15-25 μg membrane protein/well) and radioligand at final assay concentrations of 0.4 to 0.6 nM. Experiments comprised addition of 95 μL of assay buffer (20 mM HEPES, pH 7.4, 10 mM MgCl2), 50 μL of membranes, 50 μL of radioligand stock, and 5 μL of PIPE-359 (**21i**) diluted in assay buffer to 96-well microtiter plates, which was then incubated for 1 h at room temperature. Assay incubations were terminated by rapid filtration through PerkinElmer F/C filtration plates

under reduced pressure using a 96-well Packard filtration apparatus, followed by washing three times with ice cold assay buffer. Plates were then dried at 45 °C for a minimum of 2 h. Finally, 25 µL of BetaScint™ scintillation cocktail was added to each well and the plates were counted in a Packard TopCount® scintillation counter. In each competition study, PIPE-359 (**21i**) was dosed at ten concentrations with duplicate determinations at each test concentration.

Experimental Procedures: in vitro ADMET

Microsomal stability assay.

Assays were performed by Pharmaron, Inc. (Beijing, China). Test compound was incubated in a master solution having a final concentration of 100 mM phosphate buffer and 0.5 mg/mL microsomal protein (rat, human, or mouse). Following the addition of NADPH (10 mM final), the incubation plate was pre-warmed for ten minutes in a water bath at 37 °C. The reaction was started with the addition of the control compound or test compound solutions (1 µM final). Verapamil was used as a positive control in this study.

Aliquots were taken from the reaction solution at 15 min or 0.5, 15, 30, 45 and 60 minutes for full time-course assays. The reaction was stopped by the addition of 5 volumes of cold acetonitrile with IS (100 nM alprazolam, 200 nM caffeine and 100 nM tolbutamide). Samples were centrifuged at 3,220 g for 40 minutes. Finally, an aliquot of 150 µL of the supernatant was mixed with 150 µL of ultra-pure water and then analyzed by LC-MS/MS to determine the percent remaining of the test or control compound.

All calculations were carried out using Microsoft Excel. Analyte peak areas were determined from extracted ion chromatograms. The slope value, k, was determined by linear regression of the natural logarithm of the remaining percentage of the parent drug vs. incubation time curve. In vitro half-life ($t_{1/2}$) was determined from the slope value: -(0.693/k). Conversion of the in vitro $t_{1/2}$ (min) into the in vitro intrinsic clearance (in vitro CL_{int}, in µL/min/mg protein) was done using the following equation (mean of duplicate determinations): $(0.693/t_{1/2})$*(volume of incubation (uL)/amount of protein (mg). Note: All points with < 10% left of 0.5 min. sample were excluded, but a minimum of two points were required.

hERG inhibition (manual patch clamp) assay.

Assays were performed by Pharmaron, Inc. (Beijing, China). HERG stably expressed HEK293 cell line (Cat# K1236) was purchased from Invitrogen. The cells are cultured in 85% DMEM, 10% dialyzed FBS, 0.1 mM NEAA, 25 mM HEPES, 100 U/mL Penicillin-Streptomycin, 5µg/mL

Blasticidin and 400 μg/mL Geneticin. Cells are split using TrypLE™ Express approximately 3×/week, and maintained between ~40% to ~80% confluence. Before the assay, the cells were induced with doxycycline at 1 μg/mL for 48 h. On the experiment day, the induced cells were resuspended and plated onto the coverslips at 5×10^5 cells/6 cm cell culture dish prior to use. Under a microscope (Olympus IX73), a desirable cell was located and suction was applied to form a Gigaohm seal. Brief, strong suction until the membrane patch has ruptured. A commercial patch-clamp amplifier (Axon Multiclamp 700B) was used for the whole cell recordings. The membrane potential was set to -60 mV to ensure hERG channels were closed. Spikes of capacity current were cancelled using the C_{slow} on the amplifier. Holding potential was set to -90 mV for 0.5s; and current recorded at 50 kHz filtered at 10 kHz. Leaking current is tested by depolarizing membrane potential to -80 mV for initial holding voltage of -90 mV. The hERG current was elicited by depolarizing at +30 mV for 4.8 seconds and then the voltage was taken back to -50 mV for 5.2 seconds to remove the inactivation and observe the deactivating tail current. The maximum amount of tail current size was used to determine hERG current amplitude. The current was recorded 120 seconds to assess stability. Only stable cells with recording parameters passing acceptance criteria were applied for the perfusion of working solutions. Prior to the experiment, working solution was prepared by dilution of stock solution (10 mM test compound in DMSO) in using extracellular solution (32 mM NaCl, 4 mM KCl, 3 mM $CaCl_2$, 0.5 mM $MgCl_2$, 11.1 mM glucose, and 10 mM HEPES) to reach final concentrations of 0.3, 1.0, 3, 10, and 30 μM. Vehicle control (DMSO) was applied to the cells to establish the baseline. Dofetilide was (at same concentrations as test substrate) was used as positive control. Once the hERG current was found to be stabilized for 5 minutes, working solution was applied and hERG current in the presence of test compound (at aforementioned concentrations) was recorded for approximately 5 minutes to reach steady state and then 5 sweeps were captured. In order to ensure the good performance of cultured cells and operations, the positive control, Dofetilide, with 5 doses was also used to test the same batch of cells. Percent current inhibition was calculated using the following equation: Peak current inhibition = $[1-(\text{peak tail current}_{\text{test compound}} / \text{peak tail current}_{\text{vehicle}})] \times 100$. The dose response curve of test compounds was plotted with %inhibition against the concentration of test compounds using Graphpad Prism 6.0, and the data was fit to a sigmoid dose-response curve with a variable slope.

Cytochrome P450 (CYP) inhibition assay.

Assays were performed by Pharmaron, Inc. (Beijing, China). Inhibition of cytochrome P450 enzymes 2C9, 2D6, and 3A4 by PIPE-359 (**21i**) was assessed in human liver microsomes (150-donor pooled, Bioreclamation IVT). Specific aspects of the incubation conditions for each CYP isozyme experiment are defined in Table S1. In general, microsomes and marker substrates at concentrations defined in Table S1 were mixed with phosphate buffer (100 mM final) and were kept on ice. The master solution of buffer, microsomes, and substrates was then dispensed into a 96 deep well incubation plate along with the addition of a working solution of PIPE-359 (**21i**) (10 µM final incubation concentration). The incubation plate was pre-warmed in a water bath at 37°C for 15 minutes before the reactions were initiated by the addition of NADPH solution (1 mM final) in phosphate buffer. After the addition of NADPH, the plate was incubated at 37°C for the corresponding time found in Table 1. The assay was performed in duplicate. The reactions were terminated by the addition of 1 volume of cold acetonitrile containing 3% formic acid and internal standards (200 nM Labetalol, 100 nM Alprazolam and 100 nM tolbutamide). The plate was centrifuged at 4000 rpm for 30 minutes followed by placement on ice for 20 minutes, then re-centrifuged at 4000 rpm for 30 minutes to precipitate protein. Next, 100 µL of the supernatant was transferred to a new plate and mixed with 100 µL of ultra-pure water and then analyzed by LC-MS/MS. The conversion of CYP2C9 substrate diclofenac to 4-OH-diclofenac metabolite, CYP2D6 substrate dextromethorphan to dextrorphan metabolite, and CYP3A4 substrate midazolam to was monitored by LC-MS/MS. The inhibition of each CYP enzyme in human liver microsomes was measured as the percentage decrease in the activity of marker metabolite formation compared to non-inhibited controls (= 100% activity). Ketoconazole, sulfaphenazole and quinidine were used as positive control inhibitors for CYP 3A4, 2C9 and 2D6 enzymes, respectively.

Table S1. Test System

CYP450 isozyme	Marker Substrate (conc,)	Control Inhibitor (conc.)	Protein Conc.	Incubation Time (min.)
2C9	Diclofenac (6 µM)	Sulfaphenazole (5 µM)	0.2 mg/mL	5
2D6	Dextromethorphan (2 µM)	Quinidine (0.5 µM)	0.2 mg/mL	20
3A4	Midazolam (1 µM)	Ketoconazole (0.5 µM)	0.2 mg/mL	5