Supporting Information

Discovery of Arylsulfonamide $Na_v1.7$ Inhibitors: IVIVC, MPO Methods, and Optimization of Selectivity Profile

Anthony J. Roecker,* Mark E. Layton, Joseph E. Pero, Michael J. Kelly III, Thomas J. Greshock, Richard L. Kraus, Yuxing Li, Rebecca Klein, Michelle Clements, Christopher Daley, Aneta Jovanovska, Jeanine E. Ballard, Deping Wang, Fuqiang Zhao, Andrew P. J. Brunskill, Xuanjia Peng, Xiu Wang, Haiyan Sun, Andrea K. Houghton, Christopher S. Burgey

CONTENTS

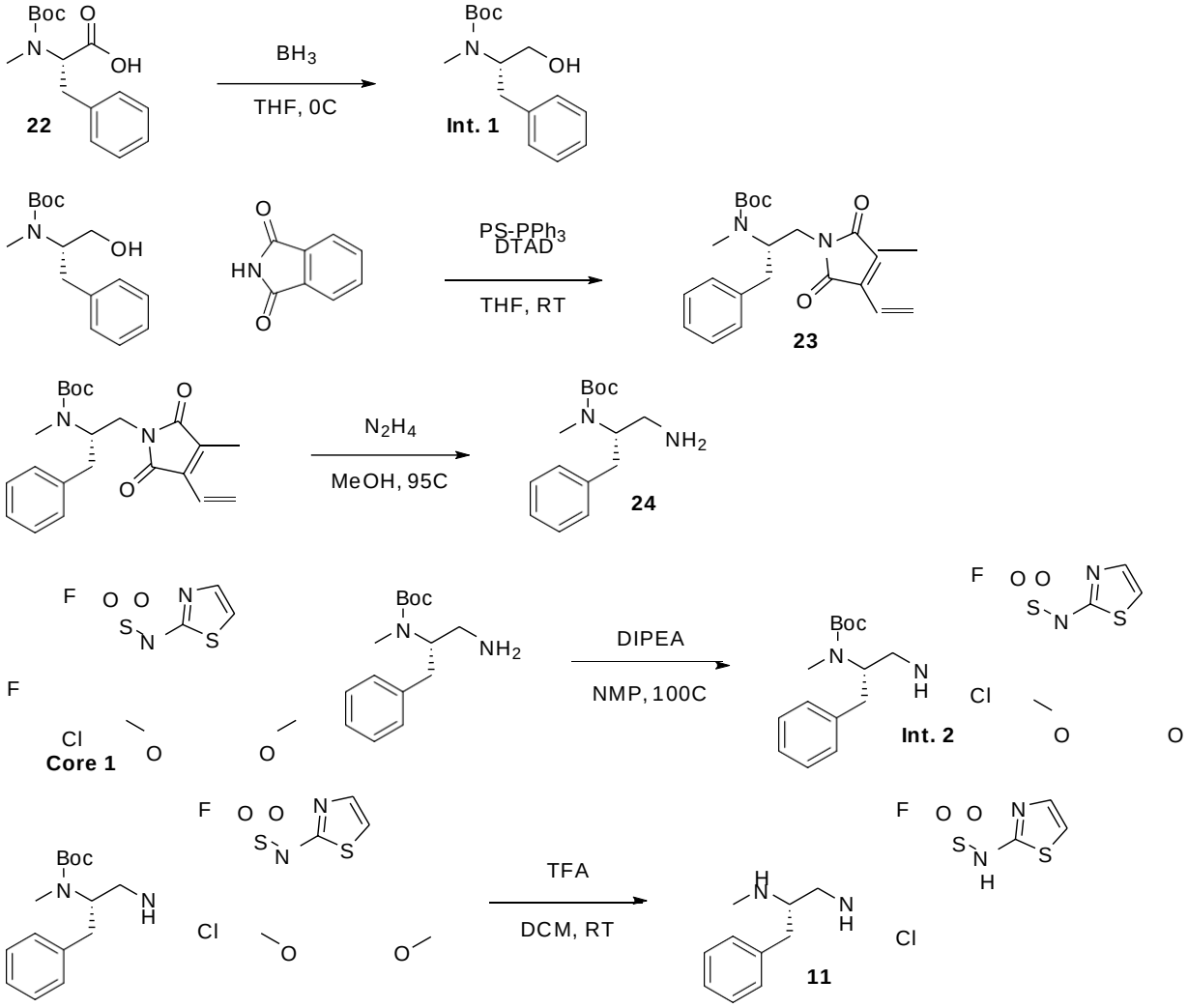
EXPERIMENTAL SECTION

1. Chemistry Experimental Procedures

Chemical Methods. All commercially available chemicals and solvents were utilized without further purification, and reactions were performed under an atmosphere of nitrogen or air unless otherwise specified. All normal phase flash chromatographic separations were performed by standard methods. The identity of the compounds prepared were confirmed by a variety of techniques. In all cases the compounds were analyzed by LC/MS or HPLC. Where utilized, unless otherwise noted, Prep HPLC was carried out on a Gilson 281 equipped with a Phenomenex Synergi C18, 100mm X 21.2 mm X 5 micron column. Conditions included a flow rate of 25 mL/min., eluted with a 0-40% acetonitrile/water eluent comprising 0.1% v/v TFA. LC/MS determinations used either an Agilent YMC J'Sphere H-80 (3 x 50 mm) 5µm column using mobile phase containing A: 0.1% Trifluoroacetic acid in water and B: acetonitrile with a gradient from 95:5 (A:B) to 0:100 (A:B) over 3.6 min and 0:100 (A:B) for 0.4 min at a flow rate of 1.4 mL/min, UV detection at 254 and 220 nm and Agilent 1100 quadrupole mass spectrometer or an Agilent TC-C18 (2.1 x 50 mm) 5µm column using mobile phase containing A: 0.0375% Trifluoroacetic acid in water and B: 0.01875% Trifluoroacetic acid in acetonitrile with a gradient from 90:10 (A:B) for 0.4 min to 90:10 to 0:100 (A:B) over 3 min and 10:90 (A:B) for 0.6 min at a flow rate of 0.8 mL/min, UV detection at 254 and 220 nm and Agilent 6110 quadrupole mass spectrometer. For some compounds, the identity of the compound was verified by proton NMR.

Proton NMR was were acquired using a Varian Unity-Inova 400 MHz NMR spectrometer equipped with a either a Varian 400 ATB PFG 5mm, Nalorac DBG 400-5 or a Nalorac IDG 400-5 probe in accordance with standard analytical techniques, unless specified otherwise, and results of spectral analysis are reported.

*(S)-5-chloro-2-fluoro-4-((2-(methylamino)-3-phenylpropyl)amino)-N-(thiazol-2-yl)benzenesulfonamide 2,2,2-trifluoroacetate (**11**)*



(S)-tert-butyl (1-hydroxy-3-phenylpropan-2-yl)(methyl)carbamate (Intermediate 1)

To a flask containing BOC-N-ME-PHE-OH (1 g, 3.58 mmol) was added anhydrous THF (10 mL), then cooled to 0°C (ice water bath) while stirring under an atmosphere of nitrogen. Then added 1M BORANE TETRAHYDROFURAN COMPLEX (12 mL, 12.00 mmol) while stirring under nitrogen at 0°C. The reaction mixture was then permitted to stir at 0C for ~5 minutes, then

warmed to room temperature. Followed by LC/MS. After 15 minutes at room temperature the reaction mixture was cooled back to 0°C (ice water bath) then uncapped & quenched by slow addition of a saturated solution of ammonium chloride. Then diluted with water & warmed to room temperature, stirred for 15 minutes then the reaction mixture was suspended in EtOAc, the organic layer was separated. Then the organic layer was washed with saturated sodium bicarbonate (2x), followed by water, and finally brine. The organic layer was dried over sodium sulfate, filtered & concentrated to yield (S)-tert-butyl (1-hydroxy-3-phenylpropan-2-yl)(methyl)carbamate (924.3 mg, 3.48 mmol, 97% yield) which was used as is without further purification.

LRMS $C_{15}H_{23}NO_3$ [M+H]+ calculated = 266.2, observed = 266.2.

*(S)-tert-butyl (1-(1,3-dioxoisoindolin-2-yl)-3-phenylpropan-2-yl)(methyl)carbamate (**23**)*

To a flask containing (S)-tert-butyl (1-hydroxy-3-phenylpropan-2-yl)(methyl)carbamate (640 mg, 2.412 mmol), PS resin bound triphenylphosphine (1.32 g, 3.96 mmol, 3 mmol/g), & PHTHALIMIDE (468 mg, 3.18 mmol), was added anhydrous THF (5 mL), then added DTAD (760 mg, 3.30 mmol). The reaction mixture was then capped & stirred at room temperature. Followed by LC/MS. After 1 hour the reaction mixture was diluted with DCM, then filtered (to remove the resin), the filtrate was then concentrated. The resulting residue was then suspended in EtOAc, washed with saturated sodium bicarbonate, followed by water, and finally brine. The organic layer was dried over sodium sulfate, filtered & concentrated. Purification by silica gel chromatography (0-50% EtOAc/Hex; 80g ISCO). The desired fractions were concentrated, and the resulting residue was suspended in MeCN/DMSO & repurified by reverse phase chromatography (2 injections) (10-90% MeCN/H2O; 0.1% TFA in AQ; 20 min gradient; Waters 30x150 mm Sunfire 5 micron C18 column; Flow = 40 mL/min). The desired fractions were partially concentrated (to remove MeCN), then suspended in EtOAc, then washed with saturated sodium bicarbonate, followed by water, and finally brine. The organic layer was dried over sodium sulfate, filtered & concentrated to yield (S)-tert-butyl (1-(1,3-dioxoisoindolin-2-yl)-3-phenylpropan-2-yl)(methyl)carbamate (741 mg, 1.879 mmol, 78% yield).

HRMS $C_{23}H_{26}N_2O_4$ [M+H]+ calculated = 395.1965, observed = 395.1960.

*(S)-tert-butyl (1-amino-3-phenylpropan-2-yl)(methyl)carbamate (**24**)*

To a flask containing (S)-tert-butyl (1-(1,3-dioxoisoindolin-2-yl)-3-phenylpropan-2-yl)(methyl)carbamate (740 mg, 1.876 mmol) was added MeOH (10 ml) then added HYDRAZINE HYDRATE (1 ml, 20.56 mmol). The reaction mixture was then heated at 95°C in the hood open to the atmosphere with reflux condenser attached. Followed by LC/MS. After 2 hours the reaction mixture (suspension) was cooled to room temperature, then diluted with water (became clear / solubilized), then partially concentrated. The resulting residue was then suspended in EtOAc, washed with saturated sodium bicarbonate, then water, and finally brine. The organic layer was dried over sodium sulfate, then filtered & concentrated to yield (S)-tert-butyl (1-amino-3-phenylpropan-2-yl)(methyl)carbamate (480.1 mg, 1.816 mmol, 97% yield) which was used without further purification.

LRMS $C_{15}H_{24}N_2O_2$ [M+H]+ calculated = 265.2, observed = 265.4.

HRMS $C_{15}H_{24}N_2O_2$ [M+H]+ calculated = 265.1911, observed = 265.1908.

*(S)-tert-butyl (1-((2-chloro-4-(N-(2,4-dimethoxybenzyl)-N-(thiazol-2-yl)sulfamoyl)-5-fluorophenyl)amino)-3-phenylpropan-2-yl)(methyl)carbamate (**Intermediate 2**)*

To a microwave vial containing (S)-tert-butyl (1-amino-3-phenylpropan-2-yl)(methyl)carbamate (480 mg, 1.816 mmol) in NMP (10 mL) was added 5-chloro-N-(2,4-dimethoxybenzyl)-2,4-difluoro-N-(thiazol-2-yl)benzenesulfonamide (**Core 1**) (882 mg, 1.914 mmol) followed by DIPEA (0.8 mL, 4.58 mmol). The reaction mixture was then capped (not under N2) & heated at 100°C for 20 minutes under microwave irradiation. Followed by LC/MS. Then the reaction mixture was diluted with MeCN/DMSO & a few drops of water, then was purified (without workup) by reverse phase chromatography (2 injections) (25-100% MeCN/H2O; 0.1% TFA in AQ; 20 min gradient; Waters 30x150 mm Sunfire 5 micron C18 column; Flow = 40 mL/min). The desired fractions were concentrated then dissolved in MeOH/DCM & concentrated to yield (S)-tert-butyl (1-((2-chloro-4-(N-(2,4-dimethoxybenzyl)-N-(thiazol-2-yl)sulfamoyl)-5-fluorophenyl)amino)-3-phenylpropan-2-yl)(methyl)carbamate (986 mg, 1.398 mmol, 77% yield), which was used as is for the next step (significant deprotected product observed after concentration).

LRMS $C_{33}H_{38}ClFN_4O_6S_2$ [M+H]+ calculated = 705.2, observed = 705.3

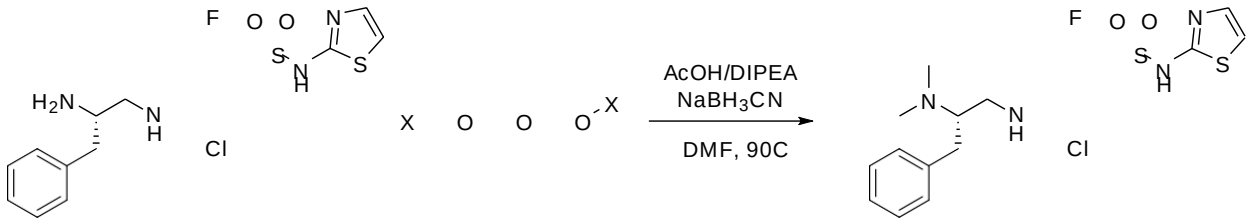
*(S)-5-chloro-2-fluoro-4-((2-(methylamino)-3-phenylpropyl)amino)-N-(thiazol-2-yl)benzenesulfonamide 2,2,2-trifluoroacetate (**11**)*

To a flask containing (S)-tert-butyl (1-((2-chloro-4-(N-(2,4-dimethoxybenzyl)-N-(thiazol-2-yl)sulfamoyl)-5-fluorophenyl)amino)-3-phenylpropan-2-yl)(methyl)carbamate (986 mg, 1.398 mmol) in DCM (5 ml) was added TFA (1 ml, 12.98 mmol). The reaction mixture (became clear pink/red with TFA addition) was then stirred at room temperature open to the atmosphere. Followed by LC/MS. After 3 hours, the reaction mixture (cloudy pink) was then diluted with 4 mL DMSO, then further diluted with MeOH until the pink color disappeared. The reaction mixture was then filtered (celite) & the filtrate was partially concentrated to remove DCM & reduce volume. Then further diluted with MeOH/DMSO & purified (without workup) by reverse phase chromatography (2 injections) (5-50% MeCN/H2O; 0.1% TFA in AQ; 15 min gradient; Waters 30x150 mm Sunfire 5 micron C18 column; Flow = 40 mL/min). The desired fractions were concentrated, then re-dissolved in MeOH/DCM & concentrated to yield: (S)-5-chloro-2-fluoro-4-((2-(methylamino)-3-phenylpropyl)amino)-N-(thiazol-2-yl)benzenesulfonamide 2,2,2-trifluoroacetate (625.1 mg, 1.099 mmol, 79% yield).
LRMS $C_{19}H_{21}ClFN_4O_2S_2$ [M+H]+ calculated = 455.1, observed = 455.2.
HRMS $C_{19}H_{21}ClFN_4O_2S_2$ [M+H]+ calculated = 455.0773, observed = 455.0774.
1H NMR (400 MHz, Methanol-d4) δ 7.71 (d, J = 7.1 Hz, 1H), 7.42 – 7.29 (m, 5H), 7.11 (d, J = 4.7 Hz, 1H), 6.73 (d, J = 4.7 Hz, 1H), 6.09 (d, J = 12.3 Hz, 1H), 3.75-3.66 (m, 1H), 3.58 – 3.38 (m, 2H), 3.24 – 3.17 (m, 1H), 2.94-2.85 (m, 1H), 2.80 (s, 3H).

*(S)-4-((2-amino-3-phenylpropyl)amino)-5-chloro-2-fluoro-N-(thiazol-2-yl)benzenesulfonamide 2,2,2-trifluoroacetate (**12**)*

Synthesized in an analogous manner to 11.
LRMS $C_{18}H_{18}ClFN_4O_2S_2$ [M+H]+ calculated = 441.9, observed = 441.1.
HRMS $C_{18}H_{18}ClFN_4O_2S_2$ [M+H]+ calculated = 441.0617, observed = 441.0615.
^{1}H NMR (400 MHz, Methanol-d4) δ 7.72 (d, J = 7.1 Hz, 1H), 7.42-7.35 (m, 2H), 7.34-7.28 (m, 3H), 7.11 (d, J = 4.7 Hz, 1H), 6.73 (d, J = 4.7 Hz, 1H), 6.29 (d, J = 12.4 Hz, 1H), 3.73 – 3.64 (m, 1H), 3.47 – 3.42 (m, 2H), 3.03 – 2.97 (m, 2H).

*(S)-5-chloro-4-((2-(dimethylamino)-3-phenylpropyl)amino)-2-fluoro-N-(thiazol-2-yl)benzenesulfonamide 2,2,2-trifluoroacetate (**13**)*



To a vial containing (S)-4-((2-amino-3-phenylpropyl)amino)-5-chloro-2-fluoro-N-(thiazol-2-yl)benzenesulfonamide 2,2,2-trifluoroacetate (22 mg, 0.040 mmol) was added anhydrous DMF (0.7 ml), then PARAFORMALDEHYDE (26 mg, 0.040 mmol) followed by DIPEA (30 µl, 0.172 mmol), the reaction mixture was then stirred at room temperature for 10 minutes. Then added AcOH (45 µl, 0.786 mmol), stirred an additional 10 minutes, then added SODIUM CYANOBOROHYDRIDE (22 mg, 0.350 mmol) as a solid in 1 portion. Followed by LC/MS. The reaction was progressing but sluggish, so heated to 90°C. After 1.5 hours. the reaction mixture was diluted with MeOH/DMSO & drops of water, then filtered (syringe filter). The filtrate was then partially concentrated to reduce volume, then purified (without workup) by reverse phase chromatography (5-50% MeCN/H2O; 0.1% TFA in AQ; 15 min gradient; Waters 30x150 mm Sunfire 5 micron C18 column; Flow = 40 mL/min). The desired fractions were concentrated, then re-dissolved in MeOH/DCM & concentrated to yield (S)-5-chloro-4-((2-(dimethylamino)-3-phenylpropyl)amino)-2-fluoro-N-(thiazol-2-yl)benzenesulfonamide 2,2,2-trifluoroacetate (13.6 mg, 0.023 mmol, 59% yield).

LRMS $C_{20}H_{22}ClFN_4O_2S_2$ [M+H]+ calculated = 469.9, observed = 469.2

HRMS $C_{20}H_{22}ClFN_4O_2S_2$ [M+H]+ calculated = 469.0930, observed = 469.0929.

^{1}H NMR (400 MHz, Methanol-d4) δ 7.69 (d, J = 7.1 Hz, 1H), 7.42-7.36 (m, 4H), 7.36-7.30 (m, 1H), 7.11 (d, J = 4.7 Hz, 1H), 6.73 (d, J = 4.6 Hz, 1H), 5.92 (d, J = 12.3 Hz, 1H), 3.90 – 3.81 (m, 1H), 3.76-3.67 (m, 1H), 3.40-3.33 (m, 1H), 3.28-3.24 (m, 1H), 3.00 (s, 6H), 2.92-2.83 (m, 1H).

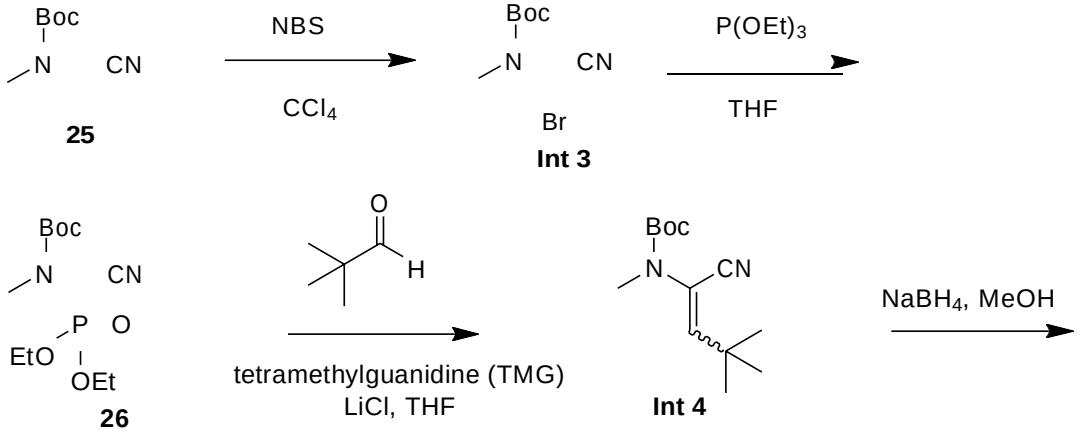
The compounds below were synthesized by analogous methods detailed above:

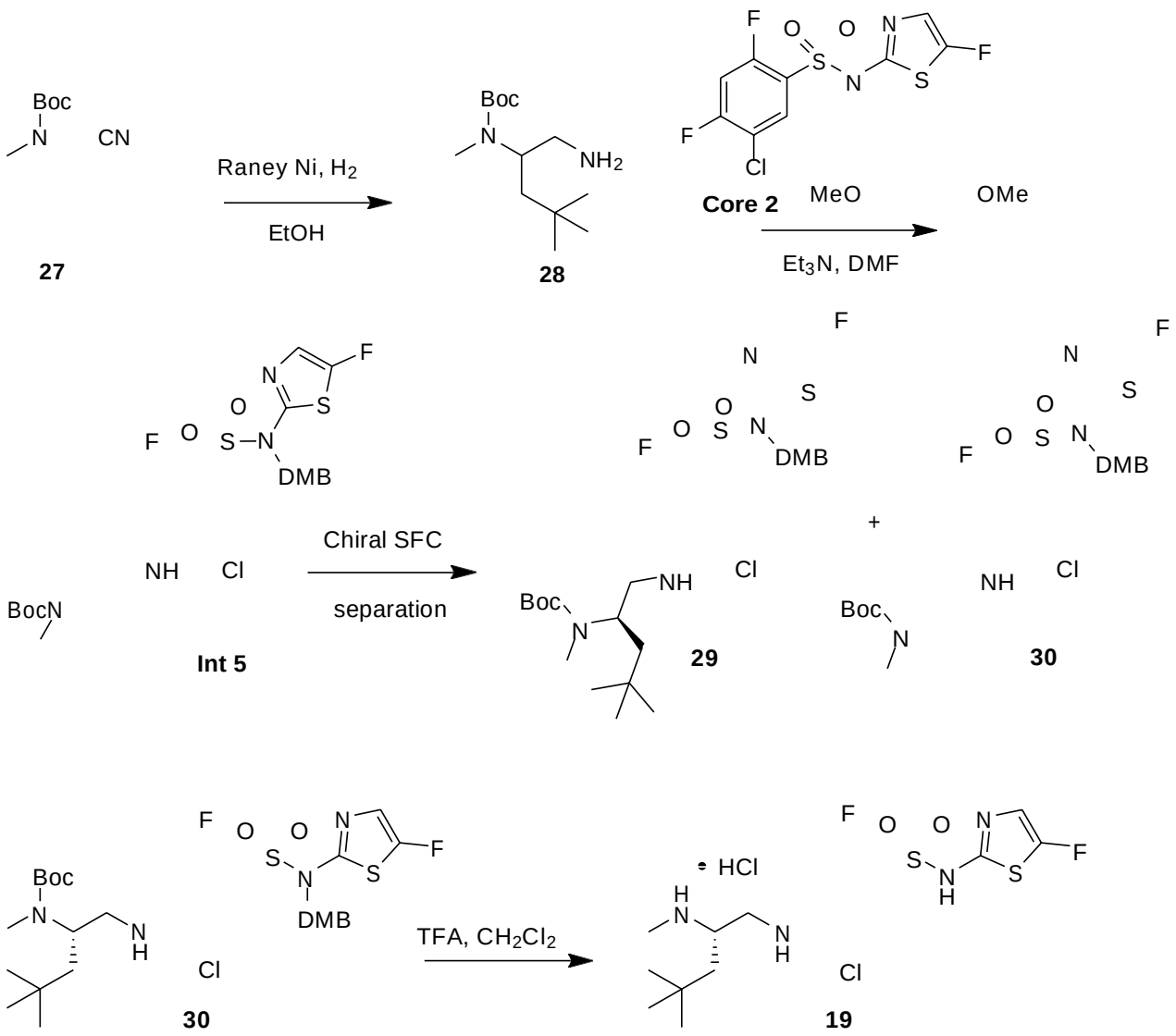
Compound number	^{1}H NMR	HRMS (M+1)

4	^{1}H NMR (400 MHz, Methanol-d4) δ 7.75 (d, J = 7.2 Hz, 1H), 7.12 (d, J = 4.8 Hz, 1H), 6.82 (d, J = 12.4 Hz, 1H), 6.74 (d, J = 4.8 Hz, 1H), 3.66 – 3.56 (m, 2H), 3.41 – 3.31 (m, 2H), 2.20 – 1.90 (m, 4H), 1.44 (s, 3H).	Calculated for $C_{15}H_{18}ClFN_4O_2S_2$: 405.0622 Observed: 405.0614
5	^{1}H NMR (400 MHz, Methanol-d4) δ 7.76 (d, J = 7.2 Hz, 1H), 7.12 (d, J = 4.8 Hz, 1H), 6.82 (d, J = 12.4 Hz, 1H), 6.74 (d, J = 4.8 Hz, 1H), 3.66 – 3.56 (m, 2H), 3.41 – 3.31 (m, 2H), 2.20 – 1.90 (m, 4H), 1.44 (s, 3H).	Calculated for $C_{15}H_{18}ClFN_4O_2S_2$: 405.0622 Observed: 405.0621
6	^{1}H NMR (500 MHz, Methanol-d4) δ 7.75 (d, J = 7.1 Hz, 1H), 7.12 (d, J = 4.7 Hz, 1H), 6.76 – 6.70 (m, 2H), 3.90-3.83 (m, 1H), 3.62-3.56 (m, 1H), 3.55-3.48 (m, 1H), 3.39-3.33 (m, 1H), 3.30-3.25 (m, 1H), 2.29-2.21 (m, 1H), 2.16-1.99 (m, 2H), 1.83-1.73 (m, 1H).	Calculated for $C_{14}H_{16}ClFN_4O_2S_2$: 391.0465 Observed: 391.0478
7	^{1}H NMR (500 MHz, Methanol-d4) δ 7.75 (d, J = 7.1 Hz, 1H), 7.12 (d, J = 4.7 Hz, 1H), 6.76 – 6.70 (m, 2H), 3.90-3.83 (m, 1H), 3.62-3.56 (m, 1H), 3.55-3.48 (m, 1H), 3.39-3.33 (m, 1H), 3.30-3.26 (m, 1H), 2.29-2.21 (m, 1H), 2.16-1.99 (m, 2H), 1.83-1.73 (m, 1H).	Calculated for $C_{14}H_{16}ClFN_4O_2S_2$: 391.0465 Observed: 391.0471
8	^{1}H NMR (400 MHz, Methanol-d4) δ 7.77 (d, J = 6.8 Hz, 1H), 7.37 – 7.31 (m, 5H), 7.13 (d, J = 4.8 Hz, 1H), 6.75 (d, J = 4.4 Hz, 1H), 6.52 (d, J = 12.4 Hz, 1H), 3.52 – 3.43 (m, 4H), 3.20 – 3.18 (m, 2H), 2.22 – 2.15 (m, 3H), 2.12 – 1.94 (m, 1H).	Calculated for $C_{21}H_{22}ClFN_4O_2S_2$: 481.0935 Observed: 481.0929
9	^{1}H NMR (400 MHz, Methanol-d4) δ 7.78 (d, J = 6.8 Hz, 1H), 7.41 – 7.32 (m, 5H), 7.14 (d, J = 4.4 Hz, 1H), 6.76 (d, J = 4.8 Hz, 1H), 6.69 (d, J = 12.8 Hz, 1H), 3.59 – 3.50 (m, 2H), 3.17 – 3.09 (m, 2H),	Calculated for $C_{20}H_{22}ClFN_4O_2S_2$: 469.0935 Observed: 469.0933

	2.77 (s, 3H), 1.49 (s, 3H).	
10	^{1}H NMR (400 MHz, Methanol-d4) δ 7.78 (d, J = 6.8 Hz, 1H), 7.41 – 7.32 (m, 5H), 7.14 (d, J = 4.4 Hz, 1H), 6.76 (d, J = 4.8 Hz, 1H), 6.69 (d, J = 12.4 Hz, 1H), 3.59 – 3.50 (m, 2H), 3.17 – 3.09 (m, 2H), 2.77 (s, 3H), 1.49 (s, 3H).	Calculated for $C_{20}H_{22}ClFN_4O_2S_2$: 469.0935 Observed: 469.0941
14	^{1}H NMR (400 MHz, Methanol-d4) δ 7.65 (d, J = 7.2 Hz, 1H), 7.37 – 7.30 (m, 5H), 6.98 (s, 1H), 6.06 (d, J = 12.8 Hz, 1H), 3.75 – 3.65 (m, 1H), 3.54 – 3.50 (m, 1H), 3.45 – 3.40 (m, 1H), 3.24 – 3.19 (m, 1H), 2.93 – 2.89 (m, 1H), 2.79 (s, 3H).	Calculated for $C_{19}H_{19}ClF_2N_4O_2S_2$: 473.0684 Observed: 473.0675
15	^{1}H NMR (500 MHz, DMSO-d6) δ 8.61-8.44 (m, 1H), 8.31 (s, 1H), 7.55 (d, J = 7.2 Hz, 1H), 7.38 – 7.26 (m, 5H), 6.55-6.49 (m, 1H), 6.08 (d, J = 12.7 Hz, 1H), 3.29 – 3.22 (m, 1H), 3.15-3.06 (m, 1H), 2.84-2.75 (m, 1H), 2.68-2.63 (m, 3H). *2 aliphatic protons obscured by solvent peak; compound made via parallel library chemistry.	Calculated for $C_{18}H_{19}ClFN_5O_2S_2$: 456.0731 Observed: 456.0729

*(S)-5-chloro-4-((4,4-dimethyl-2-(methylamino)pentyl)amino)-2-fluoro-N-(5-fluorothiazol-2-yl)benzenesulfonamide (**19**)*





*Preparation of tert-butyl (bromo(cyano)methyl)(methyl)carbamate (**Intermediate 3**)*

To a solution of **25** (1 g, 5.88 mmol) in CCl_4 (15 mL) was added NBS (1.150 g, 6.46 mmol). The mixture was refluxed at 80 °C for 2 h. Then the reaction was complete detected by TLC (PE: EA = 5:1). Then the reaction was cooled down to 0 °C and stirred for 0.5 h, then filtered and concentrated in vacuo to give **Intermediate 3** (1.4 g, 5.62 mmol, 96% yield) as a yellow oil which was used in next step that without further purification.

*Preparation of tert-butyl (cyano(diethoxyphosphoryl)methyl)(methyl)carbamate (**26**)*

To a solution of **Intermediate 3** (1 g, 4.01 mmol) in THF (15 mL) was added triethyl phosphite

(0.734 g, 4.42 mmol). The mixture was stirred at 75 °C under N_2 for 16 h. Then the reaction was complete detected by TLC (PE: EA = 2:1). Then the reaction was filtered and concentrated in vacuo. The residue was purified by silica gel chromatography (SiO_2, PE: EA = 5:1) to give **26** (1.2 g, 3.92 mmol, 98% yield) as a colorless oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.53-4.34 (m, 1H), 4.26 (d, *J* = 7.5 Hz, 4H), 3.08 (s, 3H), 1.48 (s, 9H), 1.41-1.32 (m, 6H)

*Preparation of tert-butyl (1-cyano-3,3-dimethylbut-1-en-1-yl)(methyl)carbamate (**Intermediate 4**)*

To a mixture of **26** (50 g, 163 mmol), LiCl (3.46 g, 82 mmol) and TMG (37.6 g, 326 mmol) in THF (500 mL) was added pivalaldehyde (28.1 g, 326 mmol) in THF (100 mL) at -78 °C. The mixture was stirred for 2 h at -78 °C and then at 30 °C for 9 h. The reaction was complete detected by TLC (PE: EA = 3:1). The reaction mixture was quenched with water (100 mL), extracted with EtOAc (200 mL*3), washed with brine (100 mL), dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by column chromatography on silica gel (SiO_2, PE: EA = 15:1) to give the product **Intermediate 4** (25 g, 92 mmol, 56.5 % yield) as a yellow oil.

^{1}H NMR (400MHz, $CDCl_3$) δ 6.19 (s, 1H), 3.00 (s, 3H), 1.44 (s, 9H), 1.23 (s, 9H).

*Preparation of tert-butyl (1-cyano-3,3-dimethylbutyl)(methyl)carbamate (**27**)*

To a solution of **Intermediate 4** (25 g, 105 mmol) in MeOH (300 ml) was added $NaBH_4$ (15.87 g, 420 mmol). Then the mixture was stirred for 2 h. The reaction was detected by LCMS. The starting material was disappeared. The mixture was concentrated in vacuo and dissolved in 20 ml DCM. The mixture was filtered and concentrated to give **27** (20g, 66.6 mmol, 63.5 % yield) as a yellow oil without the further purification. LRMS m/z (M+H): 241.1 found, 241.1 required for $C_{13}H_{24}N_2O_2$.

*Preparation of tert-butyl (1-amino-4,4-dimethylpentan-2-yl)(methyl)carbamate (**28**)*

To a solution of **27** (10 g, 41.6 mmol)) in EtOH (200 ml) was added Raney Ni (2.442 g, 41.6 mmol). Then the mixture was stirred under H_2 at 25 °C for 2 h. The reaction was detected by LCMS. The starting material was disappeared. The mixture was filtered and concentrated in

vacuo to give **28** (8 g, 26.2 mmol, 62.9 % yield) as a yellow oil. LRMS m/z (M+H): 245.2 found, 245.1 required for $C_{13}H_{28}N_2O_2$.

Preparation of tert-butyl (1-((2-chloro-4-(N-(2,4-dimethoxybenzyl)-N-(5-fluorothiazol-2-yl)sulfamoyl)-5-fluorophenyl)amino)-4,4-dimethylpentan-2-yl)(methyl)carbamate **(Intermediate 5)**

To a mixture of **28** (7.96 g, 32.6 mmol) and **Core 2** (13 g, 27.1 mmol) in DMF (300 ml) was added TEA (8.24 g, 81 mmol). Then the mixture was stirred at 25 °C for 16 h. The reaction was detected by TLC. TLC showed the starting material was disappeared. The mixture was quenched by H_2O (100 mL), and extracted by EtOAc (200mL*2). The organic layer was dried over Na_2SO_4, filtered and concentrated by vacuo to give a residue which was purified by column chromatography on silica gel (PE: EA = 5:1) to give the product **Intermediate 5** (17.5 g, 22.40 mmol, 83 % yield) as a white solid.

Intermediate 5 (17.5 g, 24.88 mmol) was resolved under SFC condition below:

Column: Chiralpak AD-3 150×4.6 mm I.D.,

3 um Mobile phase:iso-propanol (0.05% DEA) in CO_2 from 5% to 40%;

Flow rate: 2.4 mL/min; Wavelength: 220nm

Enantiomer **30** (8 g, 10.24 mmol, 41.1 % yield; peak 1: Rt = 4.99 min, 99% ee) as a colorless oil and Enantiomer **29** (8 g, 10.24 mmol, 41.1 % yield; peak 2: Rt = 5.42 min, 99% ee) as a colorless oil were obtained.

Preparation of (S)-5-chloro-4-((4,4-dimethyl-2-(methylamino)pentyl)amino)-2-fluoro-N-(5-fluorothiazol-2-yl)benzenesulfonamide (**19**)

To a solution of enantiomer **30** (5 g, 7.11 mmol) in 80 ml DCM was added TFA (10 ml). The mixture was stirred at 25 °C under N_2 for 1 h. The reaction was detected by LCMS. The starting material was disappeared. The mixture was concentrated and dissolved in 100 mL DMF and filtered. The filtrate was purified by prep-HPLC (HCl) to give **19** (2 g, 4.33 mmol, 60.9 % yield) as a white solid. LRMS m/z (M+H): 453.1 found, 453.0 required for $C_{17}H_{23}ClF_2N_4O_2S_2$.

Preparative HPLC on a fitted with a Phenomenex Synergi Max-RP 250*80 10u using water and acetonitrile as the eluents.

Mobile phase A: 0.05% HCl water. Mobile phase B: acetonitrile.

Gradient: 19-39 % B, 0-10.0 min; 100% B, 10.1-12.0 min; 10% B, 12.1-15 min.

FlowRate: 120 mL/min.

^{1}H NMR (400MHz, CD_3OD) δ 7.74 (d, *J* = 6.2 Hz, 1H), 7.00 (s, 1H), 6.70 (d, *J* = 12.6 Hz, 1H), 3.60-3.43 (m, 3H), 2.73 (s, 3H), 1.64 (s, 2H), 1.02 (s, 9H); **^{13}C NMR** (126 MHz, Methanol-d4) δ 161.77, 159.32, 150.51, 148.78, 129.20, 117.73, 113.42, 105.75, 99.02, 55.06, 45.60, 41.67, 29.81, 29.56, 28.32. **LRMS** *m/z* (M+H) 453.1 found, 453.0 required for $C_{17}H_{23}ClF_2N_4O_2S_2$; **HRMS** *m/z* (M+H) 453.0986 found, 453.0997 calculated for $C_{17}H_{23}ClF_2N_4O_2S_2$.

The compounds below were synthesized by analogous methods detailed above:

Compound number	^{1}H NMR	HRMS (M+1)
16	^{1}H NMR (400 MHz, Methanol-d4) δ 7.74 (d, J = 6.8 Hz, 1H), 7.00 (s, 1H), 6.63 (d, J = 12.4 Hz, 1H), 3.55 – 3.48 (m, 2H), 3.31 – 3.25 (m, 1H), 2.72 (s, 3H), 2.55 – 2.46 (m, 1H), 2.23 – 2.15 (m, 2H), 2.00 – 1.72 (m, 6H).	Calculated for $C_{17}H_{21}ClF_2N_4O_2S_2$: 451.0841 Observed: 451.0833
17	^{1}H NMR (400 MHz, Methanol-d4) δ 7.75 (d, J = 7.2 Hz, 1H), 7.00 (s, 1H), 6.71 (d, J = 12.4 Hz, 1H), 3.70 – 3.50 (m, 3H), 2.75 (s, 3H), 1.69 – 1.66 (m, 2H), 0.95 – 0.85 (m, 1H), 0.65 – 0.63 (m, 2H), 0.25 – 0.23 (m, 2H).	Calculated for $C_{16}H_{19}ClF_2N_4O_2S_2$: 437.0684 Observed: 437.0677
18	^{1}H NMR (400 MHz, Methanol-d4) δ 7.75 (d, J = 6.8 Hz, 1H), 7.00 (s, 1H), 6.69 (d, J = 12.4 Hz, 1H), 3.70 – 3.67 (m, 2H), 3.55 – 3.48 (m, 1H), 2.74 (s, 3H), 2.00 – 1.95 (m, 1H), 1.48 – 1.42 (m, 1H), 1.15 (s, 3H), 0.50 – 0.44 (m, 4H).	Calculated for $C_{17}H_{21}ClF_2N_4O_2S_2$: 451.0841 Observed: 451.0838
20	^{1}H NMR (400 MHz, Methanol-d4) δ 7.59 (d, J =	Calculated for

	7.2 Hz, 1H), 6.84 (s, 1H), 6.49 (d, J = 12.4 Hz, 1H), 3.48 – 3.41 (m, 1H), 3.30 – 3.28 (m, 2H), 2.52 (s, 3H), 1.01 – 0.97 (m, 1H), 0.84 – 0.78 (m, 1H), 0.00 (s, 9H).	$C_{16}H_{23}ClF_2N_4O_2S_2Si$: 469.0766 Observed: 469.0761
21	^{1}H NMR (400 MHz, Methanol-d4) δ 7.73 (d, J = 7.2 Hz, 1H), 6.98 (s, 1H), 6.65 (d, J = 12.8 Hz, 1H), 3.55 – 3.41 (m, 3H), 2.68 (s, 3H), 2.50 (s, 1H, 1.95 – 1.78 (m, 8H).	Calculated for $C_{18}H_{21}ClF_2N_4O_2S_2$: 463.0841 Observed: 463.0840

2. In Vitro Pharmacology Procedures

Cell Harvest and Preparation

$Na_v1.x$ channels were studied in stably transfected HEK293 cell lines expressing the alpha subunit for each Na_v. For each $Na_v1.x$ sub-type, cells were dissociated from culture flasks by removing growth media, adding 4mL of TrpLE select, and incubating at room temperature (RT) for approximately 1 minute until cells were observed to be detached from the flask bottom via visual inspection under the microscope. A calcium and magnesium free phosphate-buffered saline (PBS) containing 10% fetal bovine serum (FBS) (6 mL) was added to block TrpLE activity. The cell suspension was transferred to a 15 mL falcon tube and cells were centrifuged for 1 min at 1000 rpm. After centrifugation, the supernatant was removed via aspiration and the cell pellet was resuspended in 2 mL of the 10% FBS-PBS solution + 2 mL of 40 mM Na-N-methyl D-glucamine (Na-NMDG) external solution and allowed to recover on the benchtop before recording. Cells were kept at RT in the 10% FBS – 40 mM Na-NMDG solution for the duration of the recordings (up to 6 hours).

PatchXpress Electrophysiology Assay

Whole-cell sodium currents were recorded from HEK293 cells stably expressing mouse $Na_v1.7\alpha$ channels using the PatchXpress 7000A automated patch clamp platform (Molecular Devices, LLC; Sunnyvale, CA). External solution containing 150 mM NaCl, 120 mM NMDG, 1mM KCl, 0.5 mM $MgCl_2$, 5 mM HEPES, 2.7 mM $CaCl_2$, pH to 7.3 with NaOH and internal solution recording containing 30 mM CsCl, 5 mM HEPES, 10 mM EGTA, 120 mM CsF, 5 mM NaF, 2

mM $MgCl_2$, pH to 7.3 with CsOH were used for recordings. An inactivation curve was established for each cell to determine the voltage at which 50% of the channels reside in the inactivated state ($V_{0.5\ inact}$). For compound testing cells were held at a potential 20 mV negative to $V_{0.5inact}$. Cells were exposed to test compounds for 5 minutes before measuring current inhibition using the following pulse protocols. From the set holding potential, an 8 second pre-pulse to -115 mV (hyperpolarized protocol) or 7 mV positive to $V_{0.5\ inact}$ (depolarized protocol) was given prior to a 20 ms test pulse to -20 mV. The protocol was applied to cells every 10 seconds in the absence, presence of compound and after washout. At least three compound concentrations were tested for IC_{50} determinations. Raw data from PatchXpress experiments were analyzed using the DataXpress 2 software (Molecular Devices). IC_{50} values were calculated using a 4 parameter logistic function (Hill equation).

Qube Electrophysiology Assay

Whole-cell sodium currents were recorded from HEK cells stably expressing human $Na_v1.7$ α or human Nav1.6 α channels using the Qube automated patch clamp platform (Sophion Bioscience Inc.). A total of 10 concentrations of compound were tested by 10-point titrations using the Echo acoustic liquid handling instrument and glass coated 384 well plates. 384 whole-cell Nav current recordings per Qchip were performed on the Qube. Before compound addition, cells in each Qchip well were monitored for stability for at least 5 minutes. Cells were then incubated with a single concentration of compound. For each concentration, compound was applied 3 times to each well to achieve equilibrium. Washout was performed to measure recovery of the sodium current. The following external and internal solutions were used: External solution: 150 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, 12 mM Dextrose, pH 7.3 with NaOH; Internal solution: 30 mM CsCl, 5 mM HEPES, 10 mM EGTA, 120 mM CsF, 5 mM NaF, 2 mM $MgCl_2$, pH=7.3 with CsOH. *Human Nav1.7 current recordings.* Cells were held at -75 mV. A train of 6 consecutive test pulses to -10 mV was applied at a frequency of 0.1 Hz in the absence and presence of compound. Each test pulse was preceded by an 8 second prepulse to -115 mV. Cells in each Qchip well were exposed to a single compound concentration for 5 minutes at -75 mV before inhibition was measured. *Human Nav1.6 current recordings.* The holding potential was set at -65 mV. A train of 6 consecutive test pulses to -10 mV was applied at a frequency of 0.1 Hz in the absence and presence of compound. Each test pulse was preceded

by an 8 second prepulse to -115 mV. Cells in each Qchip well were exposed to a single compound concentration for 5 minutes at -65 mV before inhibition was measured. Raw data from Qube experiments were analyzed using the Sophion Biosince Inc. software. IC_{50} values were calculated using a 4 parameter logistic function (Hill equation).

Manual Patch Clamp Electrophysiology Assay

Recordings were obtained in manual patch clamp in the whole cell configuration. Data was recorded using a Multiclamp 700A amplifier (Molecular Devices) controlled by pClamp 9 on a Windows XP computer. Data was digitized using a Digidata 1330. The pipette manipulator was controlled by a Sutter MP-285 (Sutter Instruments). A Nikon TE-2000 was used to visualize the cells. Cells were deposited in a small diamond-shaped bath (Warner Instruments, part number RC-24) filled with external solution. Flow of compound into the bath was controlled by a 8 port manifold (Warner Instruments, part number MP-8) fed by gravity. The bath level was controlled via vacuum suction of the bath using the house vacuum.

Signals were low-pass filtered at 5 kHz and the sampling interval was not below 200 ms. Pipette resistance was between 1.1 and 1.5 MΩ. Once a stable whole cell recording was established, a protocol was run to measure the total amount of $Na_v1.x$ current in the cell ("NaV17 -120 to 0 mV 0.1 Hz" protocol). The holding potential was set to -120 mV and there was a 20ms voltage step to 0mV, then back to -120 mV. This repeated at a 0.1 Hz frequency. 5 - 10 stable sweeps were collected before proceeding. Cells were discarded if more than 15 nA of current was present in this protocol.

Recording quality and cell health were determined by two protocols: "I-V" and “Steady-State Inactivation”. Cells were discarded if the cell appeared to be escaping the voltage clamp of the amplifier during the I-V protocol. For each cell, the V_{mid} (Voltage at which 50% of $Na_v1.x$ channels are inactivated) for the cell was calculated by opening the data file in Clampfit and plotting the peak current during the 0 mV step vs. the pre-pulse voltage, then fitting the curve with a Boltzmann equation. Cells were discarded if their Vmid fell outside a pre-defined range (-90 mV to -55 mV) or if the slope of the fitted curve was greater than 7.

Once a stable recording was obtained, compound potency was examined in a protocol that started from a hyperpolarized potential (HP) of $V_{1/2}$ – 20 mV for 50 ms, stepped to -115 mV for

8 s, stepped to -130 mV for 100 ms, then stepped to 0 mV for 20 ms, and then returned to $V_{1/2}$-20 mV for 50 ms. Sweeps were collected at an interval of 0.1 Hz. Sweeps were collected in vehicle control until steady state was reached, then drug was washed into the bath for 5 minutes at the HP. After 5 minutes, the protocol was repeated, and three sweeps were collected.

Recording Solutions

The 150 mM NaCl external solution contained: 150 mM NaCl, 1 mM KCl, 0.5 mM $MgCl_2$, 5 mM HEPES, 2 mM $CaCl_2$. This solution was made by Hyclone (NA 788, Lot# RRG146895 and Part# RR14915.01, manufactured 7/2014) and stored at 4°C until the day of first use. Thereafter it was stored at RT. This solution was used for human $Na_v1.7$, human $Na_v1.6$, mouse $Na_v1.7$, and rhesus $Na_v1.7$ recordings.

The 40mM NaCl external solution contained: 40 mM NaCl, 120 mM NMDG, 1 mM KCl, 0.5 mM $MgCl_2$, 5 mM HEPES, 2 mM $CaCl_2$. This solution was made by Hyclone (NA 832, Part# RR14916.01, Lot# RRC146768, manufactured 4/2014) and stored at 4°C until the day of first use. Thereafter it was stored at RT. This solution was used for human $Na_v1.7$ recordings when the currents recorded in the 150mM NaCl external exceeded 10nA in peak magnitude.

The internal solution contained: 30 mM CsCl, 5 mM HEPES, 10 mM EGTA, 120 mM CsF, 5 mM NaF, 2 mM $MgCl_2$, pH = 7.3 with CsOH, 320 mOsm. This solution was made by MRL Lab Services (Part # NC739, lot # NF13169, manufactured 7/2013) and stored at 4°C until use. The internal solution was brought to RT before being used for experiments.

3. Multiparameter Optimization (MPO) and Physicochemical Property Calculation Methods

Polar surface area (PSA) was calculated as specified in Clark, D.E. *J. Pharm. Sci.* **1999**, *88*, 807-814. cLogD was calculated via an internal MSD method cited in Cheminfromatics Developments: History, Reviews, and Current Research – Chapter 5: Cheminformatics in Drug Design (2004, IOS Press), p. 111-127. This method corrects cLogD from ACDLabs (https://www.acdlabs.com/products/percepta/) by subtracting cLogP from ACDLabs and adding cLogP from Biobyte software (http://www.biobyte.com/). The MPO data was analyzed using

TIBCO Spotfire 7.11 with lead discovery – Business Intelligence Analytics Software & Data Visualization.

4. Representative Procedure for Mouse Formalin Paw Test

C57BL/6 male mice (n = 8/group, 25 – 30g) were acclimated to the observation chamber for 15 min prior to start of experiments. The animals received an oral gavage (5 ml/kg) of 30, 100, or 300 mg/kg of compound **19** or vehicle (30% Captisol in water) 15 min before intraplantar formalin injection. 30 µL of 5% formalin was injected into their left hind paw and formalin-evoked spontaneous nociceptive behaviors characterized by licking/biting or shaking of the injected paw were continuously recorded by video recording (in seconds) for up to 120 min following injection. The total time spent exhibiting formalin-evoked nociceptive behaviors was reported from 0-5 min (1st phase) and 20-35 min (2nd phase). At the end of experiments (1.5 hours post compound administration), samples were collected by intracardiac puncture for PK analyses.

5. X-ray Crystallography Data

S1. Crystal Data and Structure Refinement for Compound 19 (CCDC 2045967)

A single crystal grown from methanol by solvent evaporation was selected for single crystal X-ray data analysis. The crystal was a small block with dimensions of 0.10 mm x 0.08 mm x 0.06 mm. Data collection was performed on a Bruker Apex II system at 100K. The unit cell was determined to be monoclinic in the chiral space group $P2_1$. The structure contained two cations of protonated **19** and two chloride anions in the in the crystallographic asymmetric unit.

The tautomeric state of the molecule is as shown in Figure S1 , with a proton located on the nitrogen of the fluorothiazole ring rather than on the sulfonamide nitrogen. Absolute configuration was established by anomalous-dispersion effects in diffraction measurements on the crystal and confirmed that the stereochemistry was as shown.

Crystallographic data is summarized in Table S1. Figure S1 shows a thermal ellipsoid representation of Compound **19** with thermal ellipsoids set at the 50% probability level. Coordinates, refinement details and structure factors have been deposited with the Cambridge Crystallographic Data Centre (CCDC 2045967).

Figure S1: Thermal ellipsoid representation of Compound **19** with thermal ellipsoids set at the 50% probability level.

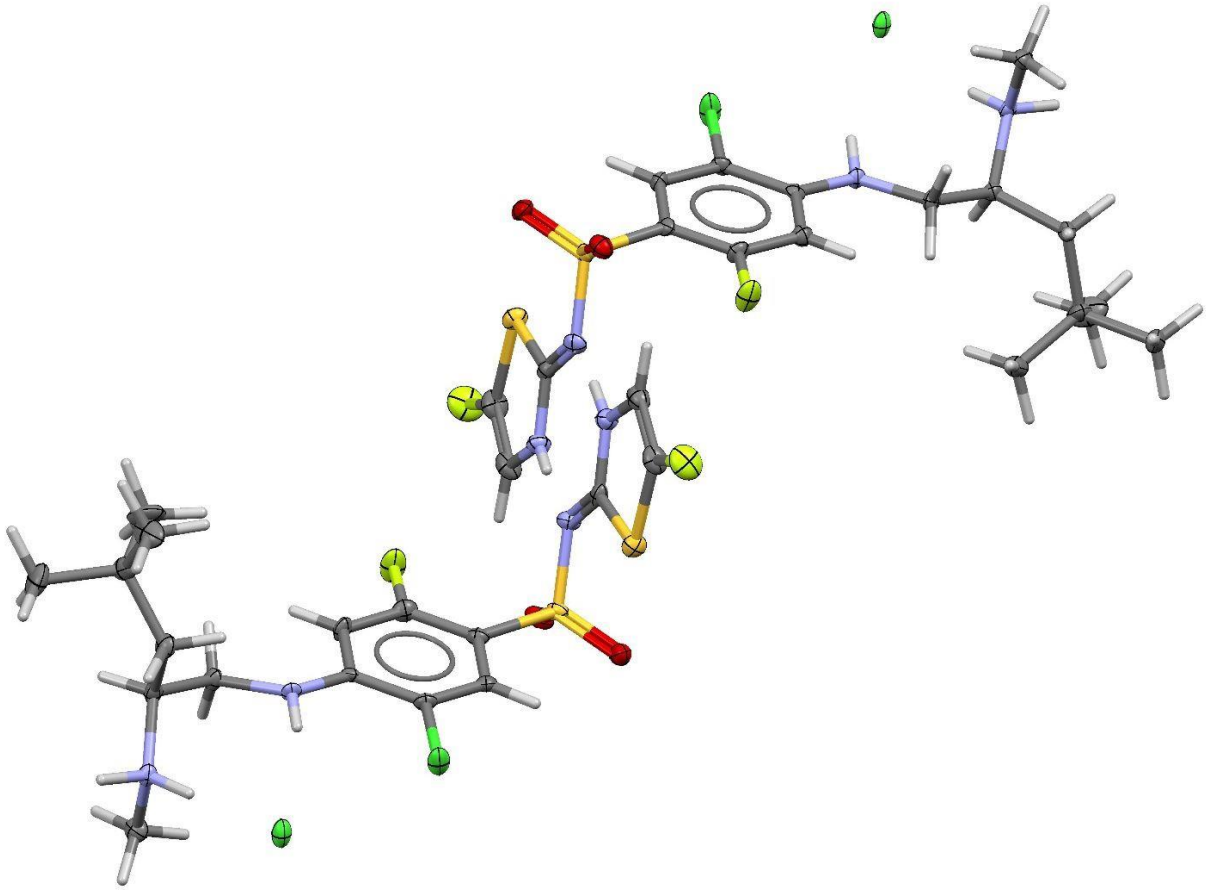


Table S1. Crystal Data and Structure Refinement for Compound 19 [CCDC 2045967]

Identification code	mdl064	
Empirical formula	$(C17\ H24\ Cl\ F2\ N4\ O2\ S2)^{+}\ .\ Cl^{-}$	
Formula weight	489.42	
Temperature	100(2) K	
Wavelength	1.54178 Å	
Crystal system	Monoclinic	
Space group	$P2_1$	
Unit cell dimensions	a = 11.5999(2) Å	α= 90°
	b = 7.29640(10) Å	β= 101.8597(9)°
	c = 26.9054(5) Å	γ = 90°
Volume	2228.60(6) $Å^3$	
Z	4	
Density (calculated)	1.459 g/cm^3	
Absorption coefficient	4.713 mm^{-1}	
F(000)	1016	
Crystal size	0.100 x 0.080 x 0.060 mm^3	
Theta range for data collection	1.678 to 68.246°	
Index ranges	-13<=h<=13, -8<=k<=8, -30<=l<=32	
Reflections collected	24085	
Independent reflections	8100 [R(int) = 0.0537]	
Completeness to theta = 68.246°	99.8 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.754 and 0.588	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	8100 / 1 / 555	
Goodness-of-fit on F^2	1.027	
Final R indices [I>2sigma(I)]	R1 = 0.0438, wR2 = 0.0983	
R indices (all data)	R1 = 0.0490, wR2 = 0.1007	
Absolute structure parameter	-0.008(13)	
Largest diff. peak and hole	0.356 and -0.281 $e.Å^{-3}$	