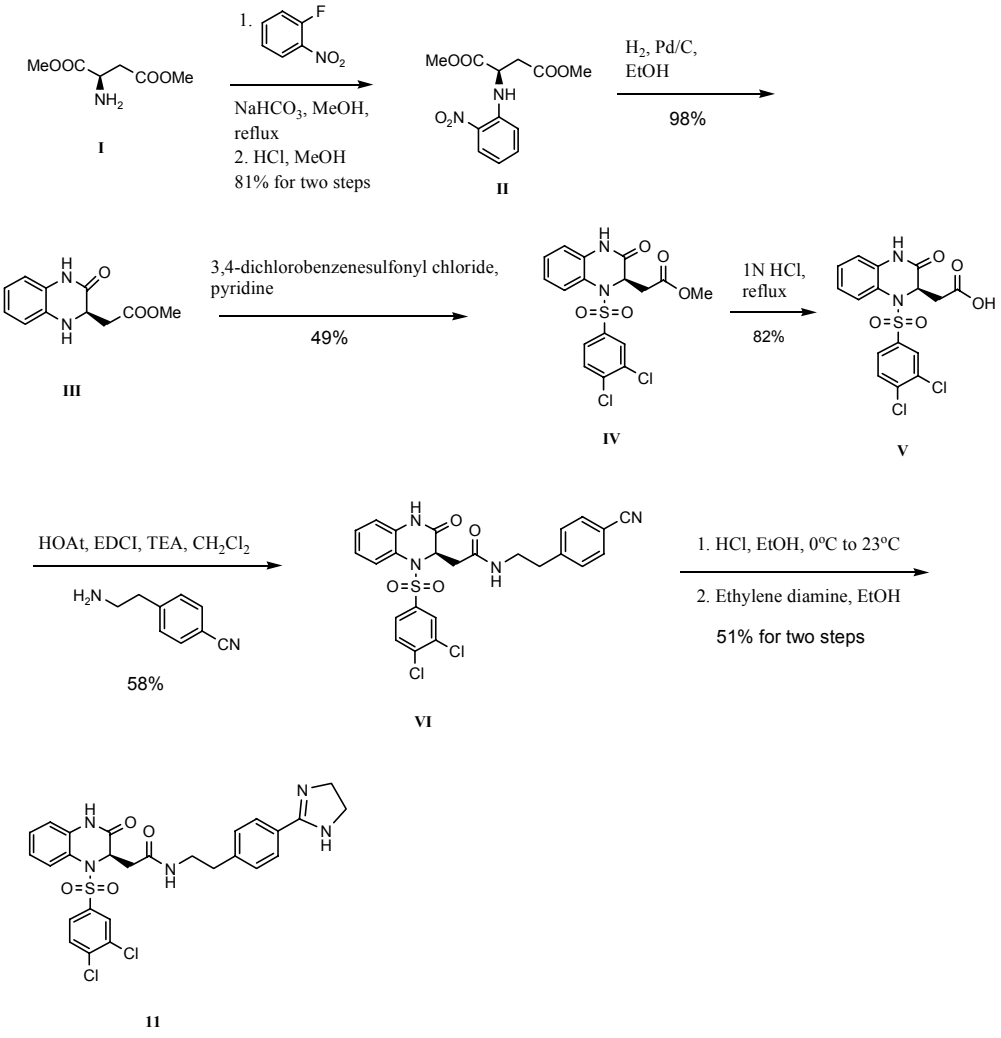
Supporting Information

Chemistry

General procedures. ^{1}H and ^{13}C (300 MHz, 400MHz, or 600 MHz) NMR spectra were recorded on a Varian VXR 300, Unity Inova 400, or Unity Inova 600 spectrometer. The chemical shifts are reported in δ (ppm) using the δ 0.00 signal of Me_4Si as an internal standard. LC/MS data were obtained on a Waters 2690 Separations Module and Micromass ZMD. High Resolution MS (HRMS) data were obtained on a Bruker 3T or 7T FTICR MS with either electrospray ionization or APCI. HPLC spectra were recorded on a Hewlett-Packard 1100 with a Vydac C-18 column or Atlantis dC_{18} column with a 5% - 95% CH_3CN/H_2O gradient at 215 nm. Chiral HPLC spectra were recorded on a Hewlett-Packard 1100 with a ChiralPak AD column utilizing 40% hexanes (containing 0.1% diethylamine) and 60% EtOH as eluant at 230 nm.

Experimental Procedures for the Preparation of Compound **11** in Table 1



To a solution of dimethyl D-aspartate, **I** (18g, 111.7 mmol) in MeOH (500 mL) was added 2-fluoronitrobenzene (17.33g, 122.86 mmol) and $NaHCO_3$ (9.38g, 111.7 mmol). The reaction mixture was refluxed under N_2 for approximately 2 days. The solvent was removed under reduced pressure and the residue was azeotropically dried with benzene (2 x 100 mL). Crude material was then redissolved in 200 mL of MeOH (200 mL), cooled to 0°C and the pH of the reaction mixture was adjusted to 4 with HCl(g). The reaction mixture was stirred overnight at room temperature and concentrated under reduced pressure. The residue was taken up in EtOAc and washed with a saturated $NaHCO_3$/10% Na_2CO_3 solution (9:1) (2 x 500 mL) and brine (1 x 300 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated to give dimethyl (2R)-2-(2-nitrophenylamino)butanedioate, **II** (25.54g, 81%). ^{1}H NMR ($CDCl_3$) δ 8.51 (d, *J* = 7.81 Hz, 1H), 8.21 (dd, *J* = 8.54, 1.46 Hz, 1H), 7.47 (td, *J* = 7.81, 0.90 Hz, 1H), 6.86 (d, *J* = 8.54, 1H), 6.75 (td, *J* = 7.81, 1.22 Hz, 1H), 4.70 (dd, *J* = 14.04, 5.98 Hz, 1H), 3.80 (s, 3H), 3.75 (s, 3H), 2.99 (d, *J* = 5.86, 2H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 171.30, 170.50, 143.72, 136.48, 133.36, 127.30, 116.93, 113.89, 53.22, 52.53, 52.49, 37.20. HRMS (ES) calcd. for $C_{12}H_{14}N_2O_6$ $(M + H)^+$: 283.0925, found: 283.0920.

To a solution of **II** (954 mg, 3.38 mmol) in EtOH (200 mL) was added 10% Pd/C (36 mg, 3.38mmol) and the suspension was placed on the Parr Hydrogenator (approximately 55 psi) for two days. The reaction mixture was filtered through a pad of celite and concentrated *in vacuo* to give methyl {(2R)-3-oxo-1,2,3,4-tetrahydroquinoxalin-2-yl}acetate, **III** (727 mg, 98 %) which was used without further purification. ^{1}H NMR ($CDCl_3$) δ 8.41 (s, 1H), 6.91 (td, *J* = 7.54, 1.53 Hz, 1H), 6.70-6.79 (m, 3H), 4.35 (dd, *J* = 10.51, 2.65 Hz, 1H), 3.75 (s, 3H), 3.15 (dd, *J* = 17.37, 2.65 Hz, 1H), 2.75 (dd, *J* = 17.27, 10.51 Hz, 1H). ^{13}C NMR (400 MHz, CD_3OD) δ 173.02, 169.32, 134.96, 126.93, 124.84, 120.16, 116.33, 115.30, 54.38, 52.45, 37.30. HRMS (ES) calcd. for $C_{11}H_{12}N_2O_3$ $(M + H)^+$: 221.0921, found: 221.0925.

To a solution of **III** (727 mg, 3.3 mmol) in pyridine (5 mL), at room temperature, was added 3,4-dichlorobenzenesulfonyl chloride (1.03 mL, 6.6 mmol). The resulting solution was stirred at room temperature overnight. Pyridine was removed *in vacuo* and the crude material was purified by flash chromatography on silica gel (25-50% EtOAc:hexanes gradient) to give methyl {(2R)-1-[(3,4-dichlorophenyl)sulfonyl]-3-oxo-1,2,3,4-tetrahydroquinoxalin-2-yl}acetate, **IV** (698 mg, 49%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (t, *J* = 8.87 Hz, 2H), 7.43 – 7.47 (m, 2H), 7.26 – 7.32 (m, 2H overlapping with $CDCl_3$), 7.20 – 7.23 (m, 1H), 6.72 (dd, *J* = 7.91, 1.32 Hz, 1H), 3.72 (s, 3H), 2.66 (dd, *J* = 15.08, 4.75 Hz, 1H), 2.45 – 2.53 (m, 1H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 168.81, 166.35, 138.75, 136.65, 133.92, 131.58, 131.25, 129.24, 129.16, 129.08, 126.25, 124.82, 121.52, 116.05, 56.42, 52.62, 35.92. HRMS (ES) calcd. for $C_{17}H_{14}Cl_2N_2O_5S$ $(M + H)^+$: 429.0073, found: 429.0054.

A solution of **IV** (698 mg, 1.63 mmol) in 1M HCl (20 mL) was refluxed overnight. The reaction mixture was concentrated under reduced pressure and dried under high vacuum to give 561 mg (82%) of {(2R)-1-[(3,4-dichlorophenyl)sulfonyl]-3-oxo-1,2,3,4-tetrahydroquinoxalin-2-yl}acetic acid, **V** which was taken on to the next step without purification. ^{1}H NMR (400 MHz, DMSO) δ 12.68 (s, 1H), 10.52 (s, 1H), 7.81

(d, *J* = 8.51 Hz, 1H), 7.51 (d, *J* = 7.68 Hz, 1H). 7.32-7.38 (m, 2H), 7.25 (dd, *J* = 8.36, 2.05 Hz, 1H), 7.17 (t, *J* = 7.67 Hz, 1H), 6.83 (d, *J* = 7.87 Hz, 1H), 4.89 (dd, *J* = 10.29, 4.25 Hz, 1H), 2.54 (dd, *J* = 15.27, 4.3 Hz, 1H), 2.17 (dd, *J* = 15.22, 10.38 Hz, 1H). ^{13}C NMR (400 MHz, DMSO) δ 170.37, 165.73, 137.94, 136.55, 133.63, 132.97, 132.39, 129.79, 128.90, 128.77, 127.33, 123.98, 121.04, 116.89, 56.79, 35.83. HRMS (ES) calcd. for $C_{16}H_{12}Cl_2N_2O_5S$ $(M + H)^+$: 415.9917, found: 415.9915.

To a solution of **V** (1.3g, 3.13 mmol) in CH_2Cl_2 (10 mL) at room temperature was added Et_3N (1.31 mL, 9.39 mmol) followed by 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (1.2g, 6.26 mmol), 1-hydroxy-7-azabenzotriazole (852 mg, 6.26 mmol), and 4-(2-aminoethyl)benzonitrile (915mg, 6.26 mmol). After stirring overnight at room temperature, the reaction mixture was diluted with CH_2Cl_2 (40 mL) and water (50 mL) and then extracted with CH_2Cl_2 (3 x 50 mL). The combined organic extracts were dried over sodium sulfate, filtered, and concentrated under reduced pressure. The crude residue was subjected to silica gel chromatography (5% MeOH in CH_2Cl_2) to give 985mg (58%) of N-[2-(4-cyanophenyl)ethyl]-2-{(2R)-1-[(3,4-dichlorophenyl)sulfonyl]-3-oxo-1,2,3,4-tetrahydroquinoxalin-2-yl}acetamide, **VI**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (d, *J* = 8.05 Hz, 2H), 7.57 (d, *J* = 8.05, 1H), 7.46 (d, *J* = 8.32 Hz, 1 H), 7.44 (d, *J* = 2.19 Hz, 1H), 7.36 (d, *J* = 8.22 Hz, 2H), 7.27-7.31 (m, 2H), 7.18 (t, *J* = 7.73 Hz, 1H), 6.72 (d, *J* = 7.95 Hz, 1H), 5.95 (bt, 1H), 5.11 (dd, *J* = 9.97, 4.39 Hz, 1H), 3.56 (dd, *J* = 13.71, 6.22 Hz, 2H), 2.94 (t, *J* = 7.04 Hz, 2H), 2.53 (dd, *J* = 15.36, 4.48, 1H), 2.34 (dd, *J* = 15.50, 9.92 Hz, 1H); LCMS (ES) *m/z* 543.1.

Into a solution of **VI** (985 mg, 1.81 mmol) in EtOH (100 mL) at 0°C was bubbled HCl(g) for ~10 minutes. The reaction mixture was then capped and allowed to slowly warm to room temperature. After overnight stirring, the reaction mixture was concentrated *in vacuo*. The residue was dissolved in EtOH and to this solution was added ethylene diamine (0.13 mL, 2.00 mmol). After overnight stirring, the solvent was removed *in vacuo*. The residue was partitioned between CH_2Cl_2 and water. The aqueous layer was extracted with CH_2Cl_2 (2 x 100 mL). The combined organic phases were washed with brine (1 x 200 mL), dried over sodium sulfate, filtered, and concentrated. The reaction product was subjected to silica gel chromatography eluting first with 20% MeOH in CH_2Cl_2, then with 20% MeOH in CH_2Cl_2 containing 1% NH_4OH to yield 541 mg of title compound, **11** (51% overall yield for two steps). ^{1}H NMR (400 MHz, CD_3OD) δ 7.79 (d, *J* = 34.07 Hz, 2H), 7.63 (d, *J* = 8.41 Hz, 1H), 7.51 (dd, *J* = 7.96, 1.28 Hz, 1H), 7.44 (d, *J* = 8.23 Hz, 2H), 7.41 (d, *J* = 2.10 Hz, 1H), 7.30-7.34 (m, 2H), 7.16 (td, *J* = 7.68, 1.37 Hz, 1H), 6.81 (dd, *J* = 8.13, 1.46 Hz, 1H), 5.13 (q, *J* = 4.94 Hz, 1H), 3.94 (s, 4H), 3.47-3.54 (m, 1H), 3.37-3.41 (m, 1H), 2.87-1.92 (m, 2H), 2.40 (dd, *J* = 14.36, 4.67 Hz, 1H), 2.25 (dd, *J* = 14.45, 4.30 Hz, 1H); ^{13}C NMR (400 MHz, CD_3OD) δ 170.03, 168.09, 167.79, 148.69, 139.48, 137.89, 134.49, 134.26, 132.58, 131.22, 130.26, 130.02, 129.90, 129.61, 127.77, 124.89, 122.63, 121.68, 117.42, 58.14, 45.97, 41.25, 37.79, 36.55. Purity: 96.36% by HPLC. 63.52% ee by chiral HPLC. HRMS (ES) calcd. for $C_{27}H_{25}Cl_2N_5O_4S$ $(M + H)^+$: 586.1073, found: 586.1039.

Compound 3:

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (d, *J* = 8.06 Hz, 1H), 7.44 (d, *J* = 7.87 Hz, 1H), 7.29-7.26 (1 H overlapping with $CDCl_3$), 7.06 (dd, *J* = 13.10, 7.79 Hz, 2H), 6.94 (t, *J* = 7.79 Hz, 1H), 6.88 – 6.82 (m, 2H), 6.84 (s, 2H), 4.94 (dd, *J* = 10.35, 4.13 Hz, 1H), 3.49 (s, 3H), 2.69 (dd, *J* = 14.47, 4.03 Hz, 1H) 2.47 (td, *J* = 14.65, 4.21 Hz, 1H), 2.44 (s, 6H), 2.25 (s, 3H). ^{13}C NMR (600 MHz, $CDCl_3$, obtained from HMQC and gHMBC studies) δ 167.1, 165.1, 147.7, 143.8, 141.1, 132.7, 132.3, 130.2, 128.8, 128.4, 127.4, 124.0, 123.9, 122.1, 120.9, 119.9, 116.0, 109.8, 55.6, 55.5, 38.4, 23.0, 21.1. Purity: 97.10% by HPLC. 75.01% ee by chiral HPLC. HRMS (ES) calcd. for $C_{26}H_{27}N_3O_5S$ $(M + H)^+$: 494.1744, found: 494.1752.

Compound 4

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.24 (dd, *J* = 8.09, 1.37 Hz, 1H), 7.42 (d, *J* = 7.63 Hz, 1H), 7.27 (dd, overlapped with $CDCl_3$, 1H), 7.07 (td, *J* = 7.94, 1.53 Hz, 2H), 6.94 (t, *J* = 7.33 Hz, 1H), 6.88 (td, *J* = 8.09, 1.22 Hz, 2H), 6.84 (s, 2H), 4.95 (dd, *J* = 10.38, 4.27 Hz, 1H), 3.87 (s, 3H), 2.69 (dd, *J* = 14.35, 4.28 Hz, 1H), 2.47 (td, overlapped with singlet at 2.44, 1H), 2.44 (s, 6H), 2.21 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 167.56, 165.24, 147.76, 143.85, 141.11, 132.70, 132.33, 130.26, 128.69, 128.39, 127.33, 123.98, 123.87, 122.07, 120.94, 119.98, 119.91, 116.25, 116.17, 109.81, 55.56, 55.46, 38.40, 23.03, 21.05. Purity: 96.48% by HPLC. 88.91% ee by chiral HPLC. HRMS (ES) calcd. for $C_{26}H_{27}N_3O_5S$ $(M + H)^+$: 494.1744, found: 494.1739.

Compound 5

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.26 (dd, *J* = 7.93, 1.53 Hz, 1H), 7.96 (bs, 1H), 7.69 (d, *J* = 7.94 Hz, 1H), 7.43 – 7.51 (m, 2H), 7.28 – 7.35 (m, 2H), 7.18 (td, *J* = 8.54, 1.52 Hz, 1H), 7.06 (dd, *J* = 7.63, 1.53 Hz, 1H), 6.94 (t, *J* = 7.78 Hz, 1H), 6.89 (dd, *J* = 8.24, 1.22 Hz, 1H), 6.79 (d, *J* = 7.93 Hz, 1H), 5.31 (dd, *J* = 9.77, 4.58 Hz, 1H), 3.91 (s, 3H), 2.73 (dd, *J* = 14.80, 4.42 Hz, 1H), 2.56 (dt, *J* = 14.80, 4.88 Hz, 1H). ^{13}C NMR (600 MHz, CD_3OD, obtained from HMQC and gHMBC studies) δ 168.5, 168.3, 151.7, 139.4, 138.1, 134.5, 134.4, 132.5, 130.2, 130.1, 130.0, 127.9, 126.6, 124.9, 123.8, 122.7, 121.4, 117.4, 111.9, 58.2, 56.2, 38.5. Purity: 97.37% by HPLC. 84.15% ee by chiral HPLC. HRMS (ES) calcd. for $C_{23}H_{19}Cl_2N_3O_5S$ $(M + H)^+$: 520.0495, found: 520.0488.

Compound 6

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.28 (d, *J* = 8.05 Hz, 1H), 8.00 (bs, 1H), 7.70 (d, *J* = 7.81 Hz, 1H), 7.47 (d, *J* = 8.05 Hz, 1H), 7.33 – 7.44 (m, 3H), 7.22 (t, *J* = 7.69 Hz, 1H), 7.06 (t, *J* = 7.81 Hz, 1H), 6.88 – 6.97 (m, 3H), 5.29 (dd, *J* = 10.37, 3.78 Hz, 1H), 3.93 (s, 3H), 2.83 (s, 3H), 2.68 (dd, *J* = 15.01, 3.54 Hz, 1H), 2.44 (t, *J* = 12.94 Hz, 1H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 165.04, 164.98, 147.93, 138.42, 136.03, 135.16, 133.52, 130.85, 129.43, 129.42, 128.95, 127.21, 126.40, 124.29, 124.11, 121.06, 120.22, 115.30, 109.92, 56.79, 55.63, 37.98, 28.71. Purity: 98.70% by HPLC. 59.98% ee by chiral HPLC. HRMS (ES) calcd. for $C_{24}H_{21}Cl_2N_3O_5S$ $(M + H)^+$: 534.0652, found: 534.0664.

Compound 7

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.61 (dd, *J* = 5.86, 1.71 Hz, 1H), 7.93 (d, *J* = 9.03 Hz, 1H), 7.71 (d, *J* = 7.33 Hz, 1H), 7.68 (tt, *J* = 7.57, 1.71 Hz, 1H), 7.32 (dd, *J* = 12.70, 1.96 Hz, 1H), 7.29 (dd, *J* = 13.42, 1.47 Hz, 1H), 7.19 (td, *J* = 7.82, 1.22 Hz, 1H), 6.78 (d, *J* = 6.83 Hz, 1H), 6.61 (d, *J* = 9.04 Hz, 1H), 5.30 (dd, *J* = 9.65, 4.52 Hz, 1H), 3.96 (s, 3H), 3.88 (s, 3H), 3.85 (s, 3H), 2.72 (dd, *J* = 14.90, 4.64 Hz, 1H), 2.53 (td, *J* = 14.65, 4.89 Hz, 1H). ^{13}CNMR (400 MHz, $CDCl_3$) δ 167.20, 165.21, 150.39, 143.17, 141.73, 138.73,

136.54, 133.84, 132.06, 131.22, 129.38, 128.79, 126.45, 124.88, 124.69, 121.56, 116.62, 115.62, 107.10, 61.51, 61.15, 56.65, 56.36, 38.63, 38.54. Purity: >99% by HPLC. HRMS (ES) calcd. for $C_{25}H_{23}Cl_2N_3O_7S$ $(M + H)^+$: 580.0707, found: 580.0700.

Compound 8

^{1}H NMR (400 MHz, CD_3OD) δ 7.63 (d, *J* = 8.41 Hz, 1H), 7.61 (dd, *J* = 8.04, 1.28 Hz, 1H), 7.49 (d, *J* = 8.51 Hz, 2H), 7.45 (d, *J* = 2.10, 1H), 7.35 (m, 2H), 7.29 (d, *J* = 8.42 Hz, 2H), 7.19 (td, *J* = 7.77, 1.33 Hz, 1H), 6.86 (dd, *J* = 8.05, 1.28 Hz, 1H), 5.26 (q, *J* = 4.97 Hz, 1H), 3.18 (t, *J* = 7.54 Hz, 2H), 2.93 (t, *J* = 7.54 Hz, 2H), 2.66 (dd, *J* = 14.40, 4.90 Hz, 1H) 2.49 (td, *J* = 14.35, 4.29 Hz, 1H). ^{13}C NMR (600 MHz, CD_3OD, obtained from HMQC and gHMBC studies) δ 168.4, 168.1, 139.4, 138.6, 138.0, 134.5, 134.2, 133.9, 132.6, 130.20, 130.19, 130.0, 129.8, 127.8, 125.0, 122.7, 122.2, 117.4, 58.1, 42.0, 38.7, 34.1. Purity: 98.80% by HPLC. 81.82% ee by chiral HPLC. HRMS (ES) calcd. for $C_{24}H_{22}Cl_2N_4O_4S$ $(M + H)^+$: 533.0812, found: 533.0777.

Compound 9

^{1}H NMR (400 MHz, CD_3OD) δ 7.60 (dd, *J* = 8.28, 1.42 Hz, 2H). 7.42 (t, *J* = 1.97 Hz, 1.H), 7.41 (dd, *J* = 8.68, 1.73 Hz, 2H), 7.31 (m, 2H), 7.17 (m, 3H), 6.83 (dt, *J* = 8.04, 1.28 1H), 5.23 (q, *J* = 5.00 Hz, 1H), 2.90 (t, *J* = 7.77, 2H), 2.68 (t, *J* = 7.68 Hz, 2H), 2.62 (dd, *J* = 14.35, 4.84 Hz, 1H), 2.45 (td, *J* = 10.24, 4.11 Hz, 1H), 1.94 (m, 2H). ^{13}C NMR (400 MHz, CD_3OD) δ 168.26, 168.21, 139.46, 138.03, 137.91, 137.86, 134.52, 134.30, 132.59, 130.25, 130.08, 129.87, 129.74, 127.84, 124.99, 122.79, 121.92, 117.46, 58.18, 40.28, 38.74, 32.95, 30.40. Purity: 100% by HPLC. 68.64% ee by chiral HPLC. HRMS (ES) calcd. for $C_{25}H_{24}Cl_2N_4O_4S$ $(M + H)^+$: 547.0968, found: 547.0934.

Compound 10

^{1}H NMR (300 MHz, CD_3OD), δ 7.81 (s, 4H), 7.63 (d, *J* = 8.55 Hz, 1H), 7.60 (dd, *J* = 7.08, 1.47 Hz, 1H), 7.44 (d, *J* = 1.96 Hz, 1H), 7.33 (m, 2H), 7.18 (td, *J* = 7.81, 1.47 Hz, 1H), 6.85 (dd, *J* = 8.06, 1.61 Hz, 1H), 5.26 (q, *J* = 4.80 Hz, 1H), 4.08 (s, 4H), 2.72 (dd, *J* = 14.65, 4.88 Hz, 1H), 2.53 (td, *J* = 14.65, 4.64 Hz, 1H). ^{13}C NMR (400 Mhz, DMSO) δ 167.51, 166.02, 164.76, 144.84, 144.71, 137.93, 136.72, 133.69, 132.99, 132.43, 130.48, 129.69, 128.95, 128.81, 127.36, 128.97, 121.23, 119.46, 119.38, 117.12, 116.87, 56.54, 44.90, 38.08. Purity: 93.40% by HPLC. HRMS (ES) calcd. for $C_{25}H_{21}Cl_2N_5O_4S$ $(M + H)^+$: 558.0764, found: 558.0746.

Pharmacology

Receptor Binding Assays.

Radioligand binding assays were performed using membranes from CHO cells that stably express the human or rat bradykinin B_1 receptors or CHO cells that express the human bradykinin B_2 receptor. For all receptor types, cells were harvested from culture flasks in PBS/1mM EDTA and centrifuged at 1000xg for 10 minutes. The cell pellets were homogenized with a polytron in ice-cold 20 mM HEPES, pH 7.4 and 1mM EDTA (lysis buffer) and centrifuged at 50,000xg for 20 minutes. The membrane pellets were re-homogenized in lysis buffer, centrifuged again at 50,000xg and the final pellets are resuspended at 5 mg protein/mL in assay buffer (20 mM HEPES, pH 7.4, 120 mM NaCl, 5 mM KCl) supplemented with 1% BSA and frozen at -80 °C.

On the day of assay, membranes were centrifuged at 14,000xg for 5 minutes and resuspended to the desired protein concentration in assay buffer containing 100 nM enaliprilat, 140 μg/mL bacitracin and 0.1% BSA. [^{3}H]-des-Arg^{10}, Leu^9-kallidin was the radioligand used for the human bradykinin B_1 receptors; [^{3}H]-des-Arg^{10}-kallidin was used for the rat bradykinin B_1 receptors; and [^{3}H]-bradykinin was used to label the human bradykinin B_2 receptor.

For all assays, compounds were diluted from DMSO stock solutions with 4 μL added to assay tubes for a final DMSO concentration of 2%. This was followed by the addition of 100 μL of the radioligand and 100 μL of the membrane suspension. Nonspecific binding for the bradykinin B_1 receptor binding assays was determined using 1 μM des-Arg^{10}-kallidin, while nonspecific binding for the bradykinin B_2 receptor was determined with 1 μM bradykinin. Tubes were incubated at room temperature (22°C) for 60 minutes followed by filtration using a Tomtec 96-well harvesting system. Radioactivity retained by the filter was counted using a Wallac beta-plate scintillation counter.

CHO Human and Rat Bradykinin B_1 FLIPR Protocol.

CHO cells engineered to stably express the human or rat bradykinin B_1 receptor were seeded at a density of 25,000 cells per well in a 96-well plate in 200 μL cell culture media (Iscove's modified DMEM containing 1 mg/mL G418 and 10% heat inactivated fetal calf serum). After overnight incubation at 37 °C, the cell plates were washed twice with Hank's buffered salt solution and the cells were incubated for 60 minutes at 37 °C with Hank's solution containing 4 μM of FLUO-3 acetoxymethyl ester and 1 mM probenecid. The cells were then washed four times with dye-free salt solution containing probenecid and then 100 μL of salt solution with 1 mM probenecid was added to each well. des-Arg^{10}-kallidin-induced elevation of cytosolic calcium was determined using a Fluorescence Imaging Plate Reader (FLIPR, Molecular Devices Corp., Sunnyvale, CA). All assays were conducted at 37 °C. Antagonist was added to the appropriate wells in a volume of 50 μL of Hank's solution two minutes prior to the addition of 3 nM of des-Arg^{10}-kallidin in a 50 μL volume. Changes in cellular fluorescence due to increased cytosolic calcium ion concentrations in response to agonist were determined using an excitation wavelength of 488 nm and a 510-570 nm bandwidth emission filter. Curve fitting and IC_{50} calculations were performed using GraphPad Prism software. At least eight concentrations of antagonist were used to generate each inhibition curve.

Rabbit Inflammatory Hyperalgesia Assay Protocol:

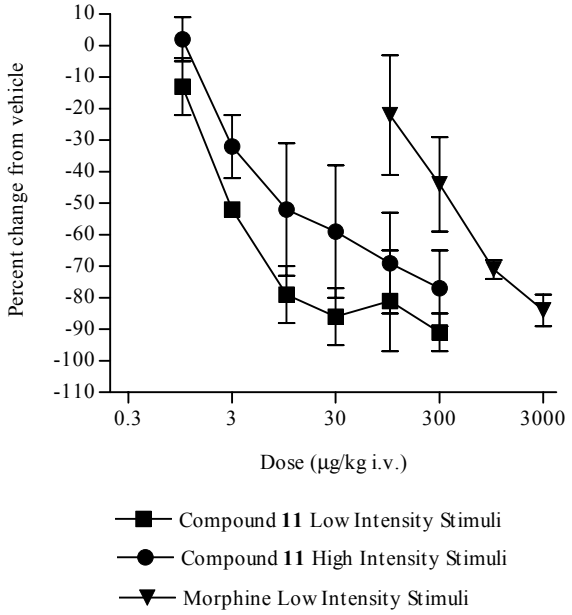
Male New Zealand Rabbits (2.5-3.2kg) were anesthetized with an i.v. injection of Saffan. Cannulae were placed into the left carotid artery to monitor blood pressure, the femoral vein for drug administration, and the trachea to allow artificial respiration. The animal's core body temperature was maintained via a thermostatically controlled heating blanket. Rabbits were spinalized at C2 and decerebrated at the level of the colliculli. Complete Freund's adjuvant (0.5ml of 1mg/ml mycobacterium tuberculosis) was injected

intra-plantar into the paw and inflammation allowed to develop for a period of at least 1 hour.

A bipolar electrode was placed into the semitendinous/femoris muscle to record muscle single motor unit activity and the appropriate receptive field was determined by mechanical stimulation of the foot. A pinch stimulator was used to apply force to the receptive field to elicit motor unit firing. Two intensities were used; a low intensity which was the least force sufficient to produce a measurable response (threshold) and a higher intensity (5 x threshold) that produced a more substantial response. Single motor unit recordings were made and consequently care was taken to ensure that with high intensity stimuli no other motor unit recordings were sampled.

Following three cycles of consistent and stable responses to low and high intensity stimuli, vehicle was given intravenously (1ml/kg) and the effects on motor unit firing rate were measured. Thereafter, a cumulative dose-response curve was constructed for compound **11** (1-300μg/kg).

Rabbit Inflammatory Hyperalgesia Assay Results:



Effect of BK B_1 receptor antagonist compound **11** and morphine, in a rabbit inflammatory hyperalgesia assay.