SUPPORTING MATERIAL FOR:

Discovery and Characterization of a Series of 3-Amino-1-Hydroxy-3,4-Dihydroquinolin-2(1*H*)-one Kynurenine Aminotransferase II Inhibitors for the Treatment of Schizophrenia

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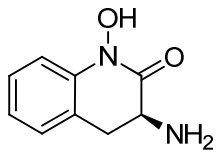
Supporting Information

General Experimental Details.

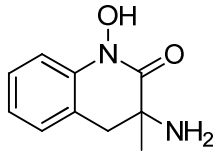
Experiments were generally carried out under inert atmosphere (nitrogen or argon), particularly in cases where oxygen- or moisture-sensitive reagents or intermediates were employed. Commercial solvents and reagents were generally used without further purification, including anhydrous solvents where appropriate (generally Sure-Seal™ products from the Aldrich Chemical Company, Milwaukee, Wisconsin). Mass spectrometry data is reported from either liquid chromatography-mass spectrometry (LCMS), atmospheric pressure chemical ionization (APCI), or gas chromatography-mass spectrometry (GCMS). Chemical shifts for nuclear magnetic resonance (NMR) data are expressed in parts per million (ppm, δ) referenced to residual peaks from the deuterated solvents employed.

General Methods for the Preparation and Analytical Data for Compounds[1,2] Illustrated in Tables 1 and 2:



7

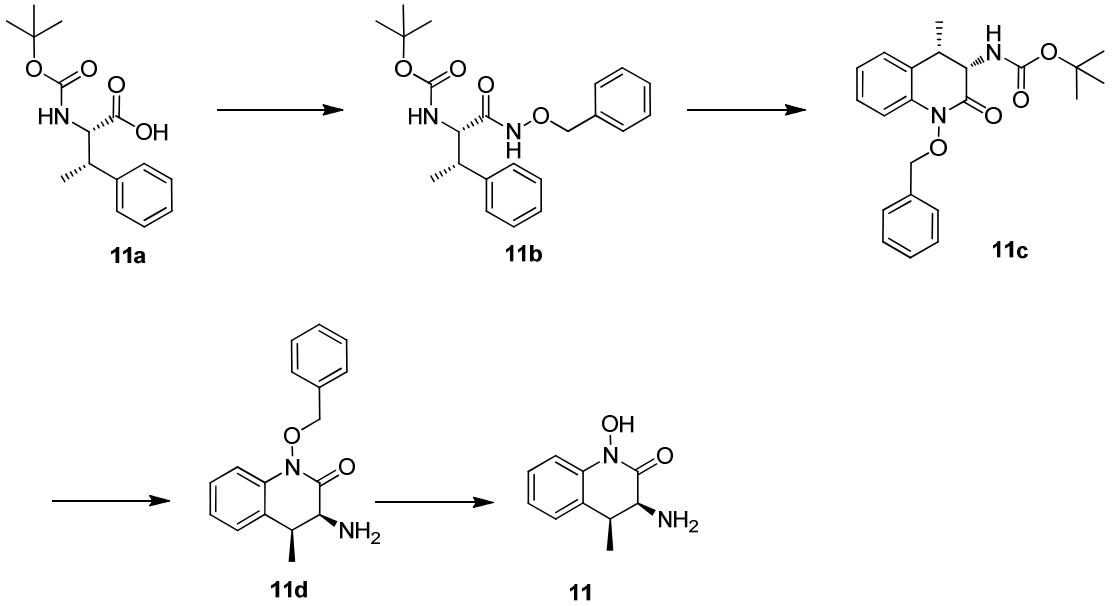
(3*S*)-3-amino-1-hydroxy-3,4-dihydroquinolin-2(1*H*)-one (7) (METHOD A) A mixture of L-2-Nitrophenylalanine (30 g, 124 mmol) and 5% Pt/C (1 g) in pyridine (1 L) was stirred at 10 ºC under H2 (30 psi) for 50 hours. After the mixture was filtered, the solid was washed with MeOH to remove pyridine and it was used for the next step directly. To a suspension this solid in MeOH (300 mL) was added in HCl/MeOH (10 mL) and the mixture was stirred at room temperature for 30 minutes. After the mixture was filtered, the filtrate was concentrated to give crude product which was washed with EtOH to give **7** (21 g, 70% for 2 steps) as a white solid. APCI *m/z* 179.1 (M+1). ^{1}H NMR (400 MHz, CD_3OD) δ 2.88 (dd, *J*=14, 15 Hz, 1H), 3.09 (dd, *J*=15.3, 6.2 Hz, 1H), 3.67 (dd, *J*=13.6, 6.1 Hz, 1H,) 7.06 (ddd, *J*=7.2, 7.2, 1.7 Hz, 1H), 7.23 (br d, *J*=7.5 Hz, 1H), 7.27-7.34 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) □ 163.0, 140.0, 129.6, 129.1, 125.6, 120.9, 114.7, 50.2, 30.3. Anal. Calcd for $C_9H_{10}N_2O_2 \cdot HCl$: C, 50.36; H, 5.17; N, 13.05. Found: C, 50.36; H, 5.21; N, 12.96. $[\alpha]^{20}_D$ = -46.9 (c = 0.55, MeOH).



10

(3-Amino-1-hydroxy-3-methyl-3,4-dihydroquinolin-2(1*H*)-one (10). Prepared in analogy to compound **7** using MeOH as solvent for the hydrogenation reaction (METHOD B): APCI *m/z* 193.0 (M+1). ^{1}H NMR (400 MHz, CD_3OD) δ 1.40 (s, 3H), 3.10 (d, *J* = 16.0 Hz, 1H), 3.35 (d, *J* = 16.0 Hz, 1H), 7.15 (m, 1H), 7.31 (br d, *J* = 8.0 Hz), 7.38 (m, 2H).

(3*S*,4*S*)-3-amino-1-hydroxy-4-methyl-3,4-dihydroquinolin-2(1*H*)-one, hydrochloride salt (**11**) **(Method E)**



11a **11b** **11c** **11d** **11**

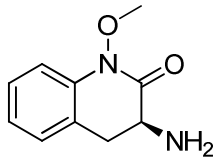
***tert*-Butyl (2*S*,3*S*)-1-(benzyloxyamino)-1-oxo-3-phenylbutan-2-ylcarbamate (11b)** (2*S*,3*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-phenylbutanoic acid (**11a**) (1.0 g, 3.6 mmol) and *O*-benzyl hydroxylamine (0.69 mg, 4.3 mmol) were combined in DCM (25 mL), and NEt_3 (5 mL, 29 mmol) and benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP, 1.58 g, 3.6 mmol) were added. The reaction was stirred for 48 hours at RT, whereupon the solvent was removed under reduced pressure. The residue was diluted with EtOAc, washed with water (3 x 20 mL), washed with saturated aqueous sodium chloride solution, dried over magnesium sulfate, filtered and concentrated *in vacuo*. The residue was purified via silica gel chromatography (Eluant: 30% EtOAc in hexane). The residue was crystallized by trituration of the oil with Et_2O to give the product as a white solid (1.30 g,

94%). LCMS *m/z* 385.0 (M+1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.28 (br d, *J*=6.8 Hz, 3H), 1.34 (s, 9H), 3.36 (m, 1H), 4.12 (dd, *J*=8.2, 8.2 Hz, 1H), 4.87 (s, 2H), 4.95 (br d, *J*=8.4 Hz, 1H), 5.40 (br s, 1H), 7.20-7.38 (m, 10H).

***tert*-Butyl (3*S*,4*S*)-1-(benzyloxy)-4-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-3-ylcarbamate (11c):** *tert*-Butyl (*2S*,3*S*)-1-(benzyloxyamino)-1-oxo-3-phenylbutan-2-ylcarbamate (**11b**) (0.50 g, 1.3 mmol) was dissolved in DCM (10 mL), in an ice-cooled flask. Phenyliodine(III) bis(trifluoroacetate) (PIFA, 0.84 g, 1.9 mmol) was added in one portion and the reaction was stirred at 0 ℃ to RT overnight. The reaction mixture was diluted with DCM (20 mL) and washed with a saturated aqueous $NaHCO_3$ solution, then with saturated aqueous sodium chloride solution, dried over magnesium sulfate, filtered and concentrated *in vacuo.* The resulting yellow oil was purified by silica gel chromatography (Eluant: 30% EtOAc in hexane) to give the product as a yellow oil still containing impurities (0.50 g). APCI *m/z* 283.3 [(M-BOC)+1].

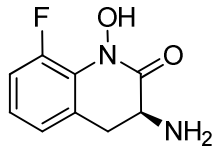
(3*S*,4*S*)-3-Amino-1-(benzyloxy)-4-methyl-3,4-dihydroquinolin-2(1*H*)-one (11d) *tert*-Butyl (3*S*,4*S*)-1-(benzyloxy)-4-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-3-ylcarbamate (**11c**) from the previous step (0.50 g, <1.3 mmol) was dissolved in THF (10 mL), and BF_3•Et_2O (0.235 mL, 1.87 mmol) was added drop-wise at RT. The reaction was refluxed for three hours. The solvent was removed *in vacuo*, and the residue was dissolved in EtOAc. The solution was basified using a 10% aqueous NaOH solution, and the organic layer was washed with water, washed with saturated aqueous sodium chloride solution, dried over magnesium sulfate, filtered and concentrated *in vacuo*. The residue was purified by silica gel chromatography (Eluant: 30% EtOAc in hexane) to give the product, still containing impurities (100 mg). LCMS *m/z* 283.1 (M+1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.14 (d, *J*=7.2 Hz, 3H), 3.12 (qd, *J*=7.1, 5.5 Hz, 1H), 3.81 (d, *J*=5.4 Hz, 1H), 5.01 (d, *J*=9.2 Hz, 1H), 5.17 (d, *J*=9.2 Hz, 1H), 7.09 (m, 1H), 7.20-7.43 (m, 6H), 7.53-7.56 (m, 2H).

(3*S*,4*S*)-3-Amino-1-hydroxy-4-methyl-3,4-dihydroquinolin-2(1*H*)-one, hydrochloride salt (11) (3*S*,4*S*)-3-Amino-1-(benzyloxy)-4-methyl-3,4-dihydroquinolin-2(1*H*)-one (**11d**) from the previous reaction (100 mg, <0.35 mmol) was dissolved in EtOH (4 mL) and 1-methyl-1,4-cyclohexadiene (1 mL), and treated with $Pd(OH)_2$ (10 mg, 035 mol). The reaction was refluxed for 1 h, then filtered through a Celite pad, which was subsequently washed with EtOAc. Concentration of the filtrate *in vacuo* provided a solid, which was purified by silica gel chromatography (Eluant: 30% MeOH in EtOAc) to afford the free base of the product. R_f = 0.3 (20% MeOH in EtOAc). A 1N solution of HCl in Et_2O was used to make the hydrochloride salt (15 mg, 6% over three steps). LCMS *m/z* 193.1 (M+1). ^{1}H NMR (400 MHz, CD_3OD) δ 1.13 (d, *J*=7.0 Hz, 3H), 3.18 (m, 1H), 4.01 (d, *J*=5.5 Hz, 1H), 7.10 (ddd, *J*=7.2, 7.2, 1.2 Hz, 1H), 7.22-7.37 (m, 3H).



9

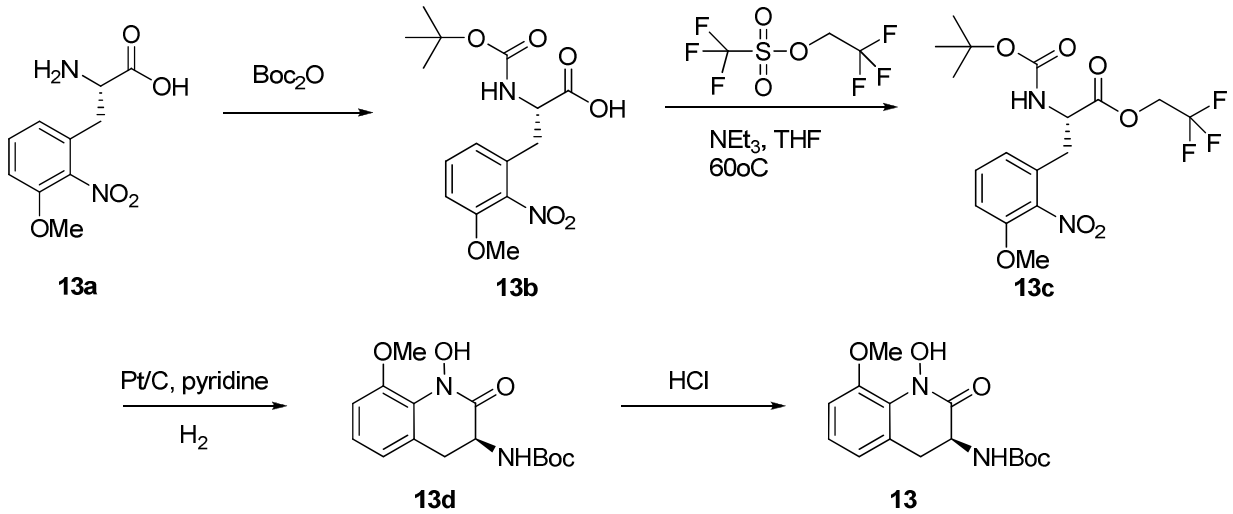
(3*S*)-3-amino-1-methoxy-3,4-dihydroquinolin-2(1*H*)-one (9) (Method E). Prepared from *N*-Boc-L-phenylalanine in analogy to compound **11**; APCI *m/z* 193.1 (M+1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.58 (dd, *J* = 16.0, 8.0 Hz, 1H), 3.70 (m, 1H), 3.79 (s, 3H), 4.66 (dd, *J* = 16.0, 8.0 Hz, 1H), 7.05 (dd, *J* = 8.0, 8.0 Hz, 1H), 7.14 (d, *J* = 8.0 Hz, 1 H), 7.24-7.29 (m, 2H), 9.01 (br s, 2H).

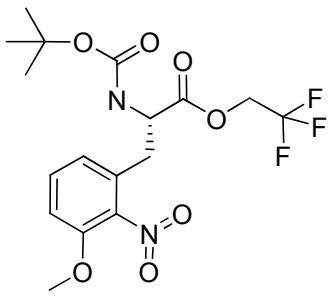


12

(3*S*)-3-amino-8-fluoro-1-hydroxy-3,4-dihydroquinolin-2(1*H*)-one (12). Prepared in analogy to compound **24**; LCMS m/z 196.9 (M+1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.09 (br s, 2H), 2.78 (dd, *J*=15, 14 Hz, 1H), 2.96 (dd, *J*=15.4, 5.8 Hz, 1H), 3.56 (dd, *J*=13.4, 5.6 Hz, 1H), 7.02-7.14 (m, 3H), 10.42 (br s, 1H).

General Experimental for trifluoroethylester formation and cyclization (METHOD D) (13):



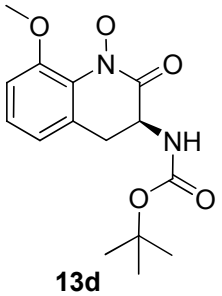


13c

2,2,2-Trifluoroethyl *N*-(*tert*-butoxycarbonyl)-3-methoxy-2-nitro-L-phenylalaninate (13c) To a solution of 1-(bromomethyl)-3-methoxy-2-nitrobenzene (494 mg, 2.01 mmol, 1 eq) in CH_2Cl_2 (10 mL) cooled to -30 ^oC was added *tert*-butyl *N*-(diphenylmethylidene)glycinate (890 mg, 3.01 mmol, 1.5 eq) and (-)-(O)-(9)-allyl-N-(9-anthracenylmethyl) cinchodinium bromide (128 mg, 0.201 mmol, 0.1 eq). CsOH (439 mg, 2.61 mmol, 1.3 eq) was then added and the mixture was allowed to stir for 18 h. The reaction mixture was washed with water, dried (Na_2SO_4), filtered and concentrated. The crude mixture was purified by flash chromatography on silica gel eluting with CH_2Cl_2 (isocratic) to give *tert*-butyl *N*-(diphenylmethylidene)-3-methoxy-2-nitro-L-phenylalaninate (870 mg, 94%). ^1H NMR (400 MHz, $CDCl_3$) δ 7.63 - 7.58 (m, 2H), 7.41 - 7.18 (m, 7H), 6.86 (d, *J* = 8.2 Hz, 2H), 6.71 (d, *J* = 7.0 Hz, 1H), 4.21 (dd, *J* = 4.7, 8.8 Hz, 1H), 3.85 (s, 3H), 3.34 - 2.95 (m, 2H), 1.42 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.3, 170.2, 150.9, 139.5, 136.2, 131.7, 130.5, 130.4, 130.3, 129.1, 128.7, 128.4, 128.2, 127.8, 123.9, 110.7, 81.8, 77.6, 76.9, 66.3, 56.6, 34.7, 28.2. HRMS (*m/z*): $[M+H]^+$ calcd for $C_{27}H_{29}N_2O_5$, 461.20701; found, 461.2069. $[\alpha]^{20}_D$ -128.8 (c = 0.182, MeOH).

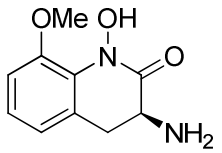
To a solution of *tert*-butyl *N*-(diphenylmethylidene)-3-methoxy-2-nitro-L-phenylalaninate (396 mg, 0.86 mmol, 1 eq) in CH_2Cl_2 (20 mL) was added TFA (3 mL). The reaction was allowed to stir at rt for 18 h. The reaction mixture was concentrated to remove TFA. The residue was partitioned between 4 M HCl and Et_2O. The aqueous layer was washed several times with Et_2O. The aqueous layer was then concentrated to give 3-methoxy-2-nitro-L-phenylalanine (**13a**) as the HCl salt. The amino acid was dissolved in THF (10 mL) and 1 M NaOH (10 mL). Boc_2O (375 mg, 1.72 mmol, 2 eq) was added and the mixture was allowed to stir at rt overnight. The reaction mixture was neutralized using aq. NH_4Cl solution and extracted with EtOAc. The organic layers were dried ($MgSO_4$), filtered and concentrated to give *N*-(*tert*-butoxycarbonyl)-3-methoxy-2-nitro-L-phenylalanine (**13b**, 240 mg), which was used in the next step without purification. To a solution of *N*-(*tert*-butoxycarbonyl)-3-methoxy-2-nitro-L-phenylalanine **13b** (100 mg, 0.294 mmol, 1 eq) in THF (10 mL) was added NEt_3 (0.102 mL, 0.735

mmol, 2.5 eq) and trifluoroethyl trifluoromethanesulfonate (89 mg, 0.382 mmol, 1.3 eq). The reaction was heated at 60 °C overnight. The reaction mixture was partitioned between EtOAc and water. The organic layer was washed with water, dried (MgSO4), filtered and concentrated. The crude product mixture was purified on a 12 g silica column eluting with 0-100% EtOAc/heptane to give 2,2,2-trifluoroethyl *N*-(*tert*-butoxycarbonyl)-3-methoxy-2-nitro-L-phenylalaninate **(13c)** as a white solid (84 mg, 67% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (t, *J* = 8.1 Hz, 1H), 7.00 - 6.87 (m, 2H), 5.17 (d, *J* = 7.6 Hz, 1H), 4.67 - 4.57 (m, 1H), 4.57 - 4.43 (m, 2H), 3.88 (s, 3H), 3.16 - 3.06 (m, 1H), 2.95 (dd, *J* = 8.5, 14.3 Hz, 1H), 1.40 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.1, 155.2, 151.2, 142.3, 131.4, 129.6, 122.3, 122.8, 111.7, 80.7, 61.6, 56.6, 54.2, 33.3, 28.4. Anal. Calcd for $C_{17}H_{21}F3N_2O_7$: C, 48.34; H, 5.01; N, 6.63. Found: C, 48.55; H, 5.00; N, 6.54. $[\alpha]^{20}_D$ = -6.6 (c = 0.11, MeOH).



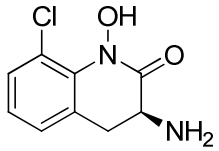
13d

***tert*-Butyl [(3*S*)-1-hydroxy-8-methoxy-2-oxo-1,2,3,4-tetrahydroquinolin-3-yl]carbamate (13d)** To a solution of 2,2,2-trifluoroethyl *N*-(*tert*-butoxycarbonyl)-3-methoxy-2-nitro-L-phenylalaninate **(13c)** (0.8 g, 1.89 mmol) in pyridine (60 mL) was added 5% Pt/C catalyst (0.4 g). The reaction was shaken on a Parr shaker at 30 psi of H_2 for 3 h. The reaction mixture was filtered through Celite with ethyl acetate washing and concentrated. The crude residue was purified by flash chromatography on silica gel eluting with 30% ethyl acetate in petroleum ether to afford the title compound (300 mg, 51% yield). ^{1}H NMR (400 MHz, CD_3OD) δ 7.16 - 7.09 (m, 1H), 7.00 (d, *J* = 8.2 Hz, 1H), 6.93 (d, *J* = 8.0 Hz, 1H), 6.84 (d, *J* = 7.4 Hz, 1H), 4.37 - 4.24 (m, 1H), 3.88 (s, 3H), 3.03 - 2.89 (m, *J* = 9.4 Hz, 2H), 1.46 (s, 9H). ^{13}C NMR (100 MHz, CD_3OD) δ 167.6, 150.3, 128.3, 127.3, 126.1, 119.3, 112.1, 79.2, 55.3, 50.6, 32.1, 27.1.



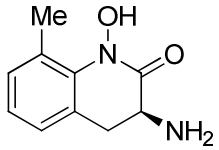
13

(3*S*)-3-Amino-1-hydroxy-8-methoxy-3,4-dihydroquinolin-2(1*H*)-one (13) To a solution of *tert*-butyl [(3*S*)-1-hydroxy-8-methoxy-2-oxo-1,2,3,4-tetrahydroquinolin-3-yl]carbamate **(13d)** (0.3 g, 0.97 mmol) in anhydrous Et_2O (4 mL) was added 2 M HCl in Et_2O. The reaction mixture was maintained at rt with stirring for 2h, then concentrated in vacuo to obtain a solid. The solid was washed with diethyl ether under Ar atmosphere and dried under vacuum at 45 ºC for 4 h to afford the title compound as a solid (80 mg, 40%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.38 (br s, 1H), 8.61 (br s, 3H), 7.20 - 7.11 (m, 1H), 7.10 - 7.01 (m, 1H), 6.92 (d, *J* = 7.7 Hz, 1H), 4.40 - 4.22 (m, 1H), 3.81 (s, 3H), 3.16 - 2.99 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.8, 150.0, 128.3, 126.2, 125.3, 119.7, 113.0, 56.2, 48.5, 29.7. HRMS (*m/z*): $[M+H]^+$ calcd for $C_{10}H_{13}N_2O_3$, 209.0920; found, 209.0918.



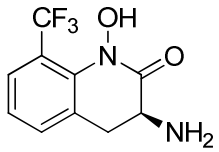
14

(3*S*)-3-Amino-8-chloro-1-hydroxy-3,4-dihydroquinolin-2(1*H*)-one hydrochloride (14) Prepared in analogy to compound **13 (Method D)**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.00 (br s, 1H), 7.42 (d, *J* = 7.9 Hz, 1H), 7.33 (d, *J* = 7.4 Hz, 1H), 7.22 - 7.04 (m, 1H), 4.40 (dd, *J* = 7.0, 13.0 Hz, 1H), 3.34 - 3.05 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.0, 136.4, 130.9, 126.9, 126.6, 126.1, 122.6, 48.1, 29.6. Anal. Calcd for $C_9H_{10}Cl_2N_2O_2$: C, 43.40; H, 4.05; N, 11.25. Found: C, 43.10; H, 3.81; N, 11.06. $[\alpha]^{20}_D$ - 6.23 (c = 0.39, MeOH).



15

(3*S*)-3-Amino-1-hydroxy-8-methyl-3,4-dihydroquinolin-2(1*H*)-one (15) Prepared in analogy to compound **13 (Method D)**:^{1}H NMR (400 MHz, CD_3OD) δ 7.20 - 7.09 (m, 2H), 7.09 - 7.01 (m, 1H), 4.30 - 4.07 (m, 1H), 3.19 - 3.07 (m, 2H), 2.47 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ 164.3, 137.5, 132.2, 128.6, 125.6, 125.5, 123.5, 49.4, 30.6, 19.8. HRMS (*m/z*): $[M+H]^+$ calcd for $C_{10}H_{13}N_2O_2$, 193.0971; found, 193.0971. $[\alpha]^{20}_D$ = -9.25 (c = 0.4, MeOH).

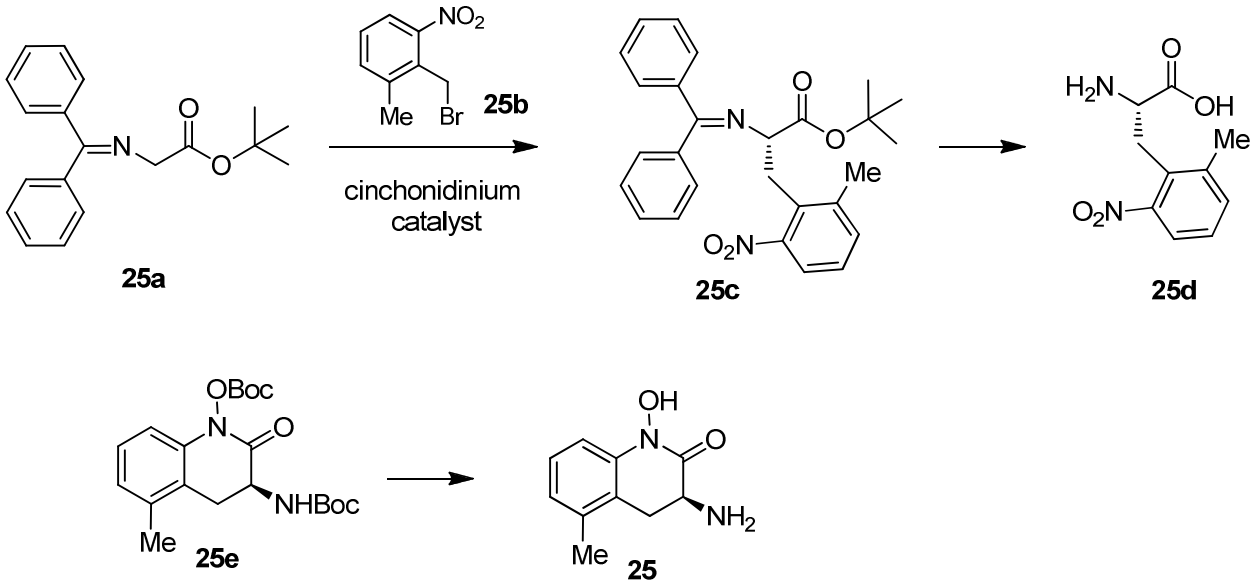


16

(3*S*)-3-Amino-1-hydroxy-8-(trifluoromethyl)-3,4-dihydroquinolin-2(1*H*)-one (16)

Prepared in analogy to compound **13 (Method D)**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.12 - 10.93 (m, 1H), 8.74 - 8.59 (m, 2H), 7.76 - 7.71 (m, 1H), 7.69 - 7.64 (m, 1H), 7.39 - 7.25 (m, 1H), 4.57 - 4.41 (m, 1H), 3.27 - 3.05 (m, 2H). ^{13}C NMR (100 MHz, CD_3OD) δ 162.9, 137.0, 131.7, 127.6, 125.0, 124.7, 124.4, 122.0, 48.4, 29.7. HRMS (*m/z*): $[M+H]^+$ calcd for $C_{10}H_{10}F_3N_2O_2$, 247.0687; found, 247.0689.

General Experimental for Asymmetric Alkylation/$SnCl_2$ cyclization (METHOD C) (25):

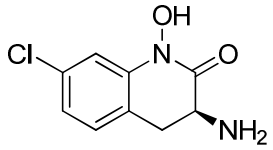


***tert*-Butyl N-(diphenylmethylidene)-2-methyl-6-nitro-L-phenylalaninate (25c)** To a solution of 2-(bromomethyl)-1-methyl-3-nitrobenzene **(25b)** (1.02 g, 4.41 mmol, 1 eq) in CH_2Cl_2 (10 mL) cooled to -30 °C was added *tert*-butyl *N*-(diphenylmethylidene)glycinate (**25a**) (1.43 g, 4.86 mmol, 1.1 eq) and (-)-(O)-(9)-allyl-*N*-(9-anthracenylmethyl) cinchodinium bromide (281 mg, 0.44 mmol, 0.1 eq). $CsOH\text{-}H_2O$ (1.10 g, 6.62 mmol, 1.5 eq) was added and the mixture was allowed to stir for 18 h. The reaction mixture was washed with water, dried (Na_2SO_4), filtered and concentrated. The crude mixture was purified by flash chromatography on silica gel, eluting with CH_2Cl_2 (isocratic) to give tert-butyl N-(diphenylmethylidene)-2-methyl-6-nitro-L-phenylalaninate **(25c)** (1.41 g, 72% yield, 91% ee).

^{1}H NMR (500 MHz, $CDCl_3$) δ 1.47 (s, 9H) 2.38 (s, 3H) 3.54 (dd, *J*=13.9, 3.4 Hz, 1H) 3.67 - 3.72 (m, 1H) 4.28 (dd, *J*=10.5, 3.4 Hz, 1H) 7.20 - 7.40 (m, 12H) 7.48 - 7.52 (m, 1H) 7.55 - 7.62 (m, 5H) 7.81 - 7.84 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 197.0, 170.9, 170.6, 151.7, 140.9, 139.1, 135.9, 134.5, 132.7,130.5, 130.2, 129.0, 128.2, 127.6, 127.0, 122.5, 81.6, 66.2, 31.3, 28.3. Anal. Calcd for $C_{27}H_{28}N_2O_4$: C, 72.95; H, 6.35; N, 6.30. Found: C, 72.94; H, 6.51; N, 6.70.

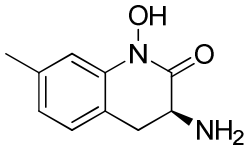
2-Methyl-6-nitro-L-phenylalanine (25d) To a solution of tert-butyl *N*-(diphenylmethylidene)-2-methyl-6-nitro-L-phenylalaninate **(25c)** (190 mg, 0.43 mmol, 1 eq) in CH_2Cl_2 (5 mL) was added TFA (5 mL). The reaction was allowed to stir at rt for 18 h. The reaction mixture was concentrated to remove TFA. The residue was partitioned between 4 M HCl and Et_2O. The aqueous layer was washed several times with Et_2O. The aqueous layer was then concentrated to give 2-methyl-6-nitro-L-phenylalanine **(25d)** (95 mg, 99% yield) as the HCl salt. ^{1}H NMR (500 MHz, CD_3OD) δ 2.53 (s, 3H) 3.43 (dd, *J*=14.2, 7.1 Hz, 1H) 3.62 (dd, *J*=14.3, 8.9 Hz, 1H) 4.26 (t, *J*=8.0 Hz, 1H) 7.44 (t, *J*=7.9 Hz, 1H) 7.60 (d, *J*=7.6 Hz, 1H) 7.82 (d, *J*=8.0 Hz, 1H) ^{13}C NMR (100 MHz, CD_3OD) δ 169.8, 151.2, 140.7, 135.5, 128.3, 122.9, 52.2, 29.6, 19.1, LC-MS- m/e = 224 (M+1). Anal. Calcd for $C_{10}H_{13}ClN_2O_4$: C, 46.08; H, 5.03; Cl, 13.60; N, 10.75. Found; C, 45.89; H, 4.92; Cl, 13.92; N, 10.58. $[\alpha]^{20}_D$ = - 62.6 (c = 0.11, MeOH).

(3*S*)-3-Amino-1-hydroxy-5-methyl-3,4-dihydroquinolin-2(1*H*)-one (25) To a solution of 2-methyl-6-nitro-L-phenylalanine **25d** (450 mg, 1.73 mmol, 1 eq) in THF:MeOH (50 mL:50 mL) at 0 °C was added $SnCl_2$ (1.64 g, 8.63 mmol, 5 eq) and NaOAc.3H_2O (2.35 g, 17.3 mmol, 10 eq). The mixture was allowed to stir, gradually warming to rt over 5 h. NEt_3 (2.4 mL, 17.3 mmol, 10 eq) and Boc_2O (1.13 g, 5.13 mmol, 3 eq) were then added and the mixture was allowed to stir overnight at rt. The mixture was concentrated and the residue was taken up in EtOAc and H_2O. The organic layer was washed several times with water, dried ($MgSO_4$), filtered and concentrated. The crude product was purified by flash chromatography on silica gel, eluting with 0-100% EtOAc/heptane (containing 1% NEt_3) to give di-Boc-protected product **25e** (660 mg, 89% yield). To a solution of Product **25e** (600 mg, 1.53 mmol, 1 eq) in Et_2O (10 mL) was added 2 M HCl/ Et_2O (10 mL). The reaction mixture was stirred overnight at rt. The solid precipitate was filtered off and washed with Et_2O to give (3*S*)-3-amino-1-hydroxy-5-methyl-3,4-dihydroquinolin-2(1*H*)-one **(25)** (280 mg, 80% yield). ^{1}H NMR (500 MHz, CD_3OD) δ 2.36 (s, 3H) 2.89 (t, *J*=14.2 Hz, 1H) 3.40 (dd, *J*=15.0, 4.3 Hz, 1H) 4.14 (br s, 1H) 7.00 - 7.04 (m, 1H) 7.21 - 7.29 (m, 2H). ^{13}C NMR (500 MHz, CD_3OD) δ 139.1, 136.1, 127.8, 126.2, 118.8, 111.6, 99.9, 49.0, 27.0, 18.2. HRMS (*m/z*): $[M+H]^+$ calcd for $C_{10}H_{12}N_2O_2$, 193.0971; found, 193.0970. $[\alpha]^{20}_D$ = -18.3 (c = 0.11, MeOH)



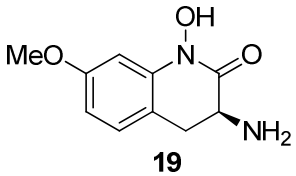
17

(3*S*)-3-Amino-7-chloro-1-hydroxy-3,4-dihydroquinolin-2(1*H*)-one (17) Prepared in analogy to **25**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.02 (br s, 1H), 8.73 (br s, 2H), 7.32 (d, *J* = 7.8 Hz, 1H), 7.20 (s, 1H), 7.10 (d, *J* = 7.8 Hz, 1H), 4.36 (dd, *J* = 5.7, 14.4 Hz, 1H), 3.20 (dd, *J* = 6.2, 15.2 Hz, 1H), 3.11 - 2.97 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.5, 141.0, 133.2, 130.4, 123.8, 119.6, 113.4, 48.5, 28.9. HRMS (*m/z*): $[M+H]^+$ calcd for $C_9H_{10}ClN_2O_2$, 213.0425; found, 213.0423.



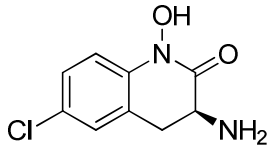
18

(3*S*)-3-Amino-1-hydroxy-7-methyl-3,4-dihydroquinolin-2(1*H*)-one hydrochloride (18) Prepared in analogy to **25**:^{1}H NMR (400 MHz, CD_3OD) δ 7.23 (s, 1H), 7.18 (d, *J* = 7.6 Hz, 1H), 6.96 (dd, *J* = 0.7, 7.5 Hz, 1H), 4.30 (dd, *J* = 6.6, 14.6 Hz, 1H), 3.25 (dd, *J* = 6.5, 14.7 Hz, 1H), 3.12 (t, *J* = 14.7 Hz, 1H), 2.36 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ 163.2, 140.2, 140.0, 129.2, 126.4, 118.0, 115.5, 50.5, 30.2, 21.6. HRMS (*m/z*): $[M+H]^+$ calcd for $C_{10}H_{12}N_2O_2$, 193.0977; found, 193.0980. $[\alpha]^{20}_D$ -29.1 (*c* 0.45, MeOH).



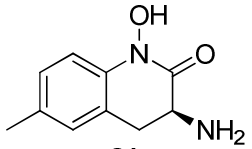
19

(3*S*)-3-Amino-1-hydroxy-7-methoxy-3,4-dihydroquinolin-2(1*H*)-one (19) Prepared in analogy to **25**: ^{1}H NMR (400 MHz, CD_3OD) δ 7.20 (d, *J* = 8.2 Hz, 1H), 6.95 (d, *J* = 2.5 Hz, 1H), 6.69 (dd, *J* = 2.5, 8.4 Hz, 1H), 4.29 (dd, *J* = 6.4, 14.6 Hz, 1H), 3.81 (s, 3H), 3.25 - 3.16 (m, 1H), 3.13 - 3.00 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.0, 159.5, 140.3, 129.2, 112.0, 108.8, 100.0, 55.6, 48.6, 28.2. HRMS (*m/z*): $[M+H]^+$ calcd for $C_{10}H_{13}N_2O_3$, 209.0920; found, 209.0915. $[\alpha]^{20}_D$ -36.79 (c = 0.41, MeOH).



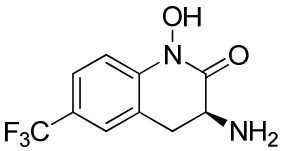
20

(3*S*)-3-Amino-6-chloro-1-hydroxy-3,4-dihydroquinolin-2(1*H*)-one (20) Prepared in analogy to **25**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.95 (s, 1H), 8.69 (br s, 3H), 7.43 (d, J = 2.0 Hz, 1H), 7.38 - 7.32 (m, 1H), 7.22 (d, J = 8.6 Hz, 1H), 4.36 (dd, J = 6.6, 14.4 Hz, 1H), 3.25 - 3.00 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.2, 137.7, 128.5, 128.1, 122.8, 115.5, 48.5, 29.1.Anal. Calcd for $C_9H_{10}Cl_2N_2O_2$: C, 43.40; H, 4.05; N, 11.25. Found C, 43.46; H, 3.60; N, 11.03. HRMS (*m/z*): $[M+H]^+$ calcd for $C_9H_{10}ClN_2O_2$, 213.0425; found, 213.0420.



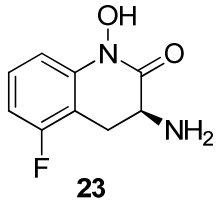
21

(3*S*)-3-Amino-1-hydroxy-6-methyl-3,4-dihydroquinolin-2(1*H*)-one hydrochloride (21)
Prepared in analogy to **25**: ^{1}H NMR (400 MHz, CD_3OD) δ 7.27 (d, J = 8.4 Hz, 1H), 7.19 (d, J = 8.4 Hz, 1H), 7.13 (s, 1H), 4.30 (dd, J = 6.6, 14.4 Hz, 1H), 3.23 (dd, J = 6.6, 14.8 Hz, 1H), 3.14 (t, J = 14.6 Hz, 1H), 2.32 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ 162.9, 137.8, 135.8, 130.2, 129.9, 120.9, 115.0, 50.4, 30.6, 20.9. HRMS (*m/z*): $[M+H]^+$ calcd for $C_{10}H_{12}N_2O_2$, 193.0977; found, 193.0970. $[\alpha]^{20}_D$ -55.7 (*c* 0.28, MeOH).



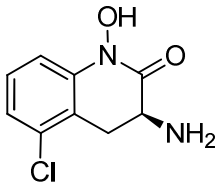
22

(3*S*)-3-Amino-1-hydroxy-6-(trifluoromethyl)-3,4-dihydroquinolin-2(1*H*)-one (22) Prepared in analogy to **25**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.15 (br s, 1H), 8.88 (br s, 3H), 7.74 (s, 1H), 7.69 (d, J = 8.6 Hz, 1H), 7.42 (d, J = 8.6 Hz, 1H), 4.44 (dd, J = 6.4, 14.6 Hz, 1H), 3.44 - 3.30 (m, 1H), 3.29 - 3.14 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.8, 143.0, 126.0, 125.9, 124.6, 124.3, 121.7, 114.2, 48.4, 29.1. Anal. Calcd for $C_{10}H_{10}F_3N_2O_2Cl$: C, 42.49; H, 3.57; N, 9.91. Found: C, 42.21; H, 3.35; N, 9.70.



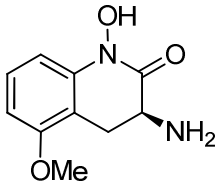
23

(3*S*)-3-Amino-5-fluoro-1-hydroxy-3,4-dihydroquinolin-2(1*H*)-one (23) Prepared in analogy to **25**: ^{1}H NMR (400 MHz, CD_3OD) δ 7.39 - 7.28 (m, 1H), 7.18 (d, *J* = 8.2 Hz, 1H), 6.89 (t, *J* = 8.7 Hz, 1H), 4.35 (dd, *J* = 6.6, 14.6 Hz, 1H), 3.53 (dd, *J* = 6.6, 15.2 Hz, 1H), 2.93 (t, *J* = 14.9 Hz, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ 161.7, 159.5, 140.4, 129.7, 111.0, 109.5, 107.4, 48.5, 22.0. HRMS (*m/z*): $[M+H]^+$ calcd for $C_9H_{10}FN_2O_2$, 197.0721; found, 197.0716.



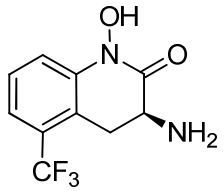
24

(3*S*)-3-amino-5-chloro-1-hydroxy-3,4-dihydroquinolin-2(1*H*)-one (24) 2-chloro-6-nitro-L-phenylalanine, hydrochloride salt was prepared in analogy to **25d** and converted to the title product in analogy to **7** with use of methanol as hydrogenation solvent (**Method B**): LCMS *m/z* 212.9 (M+1). ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 2.98 (dd, *J* = 15, 15 Hz, 1H), 3.53 (dd, *J* = 15.5, 6.7 Hz, 1H), 4.50 (dd, *J*=14.5, 6.6 Hz, 1H), 7.25 (dd, *J*=8.0, 1.1 Hz, 1H), 7.28 (dd, *J*=8.2, 1.1 Hz, 1H), 7.39 (br dd, *J*=8, 8 Hz, 1H), 8.63 (br s, 3H), 11.02 (s, 1H).



26

(3*S*)-3-Amino-1-hydroxy-5-methoxy-3,4-dihydroquinolin-2(1*H*)-one (26) 2-Methoxy-6-nitro-L-phenylalanine, hydrochloride salt was prepared in analogy to **25d** and converted to the title product in analogy to **7** with use of methanol as hydrogenation solvent **(Method B)**: LCMS *m/z* 209.0 (M+1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.72 (dd, *J*=15, 15 Hz, 1H), 3.46 (dd, *J*=15.5, 6.9 Hz, 1H), 3.83 (s, 3H), 4.34 (dd, *J*=14.4, 6.8 Hz, 1H), 6.82 (d, *J*=8.2 Hz, 1H), 6.92 (d, *J*=8.1 Hz, 1H), 7.31 (dd, *J*=8.3, 8.3 Hz, 1H), 8.66 (br s, 3H), 10.83 (br s, 1H).



27

(3*S*)-3-Amino-1-hydroxy-5-trifluoromethyl-3,4-dihydroquinolin-2(1*H*)-one (27) Prepared in analogy to **25**: LCMS *m/z* 247.4 (M+1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.13 (dd, *J* = 16, 16 Hz, 1H), 3.46 (m, 1H), 4.47 (dd, *J* = 16.0, 8.0 Hz, 1H), 7.45 (d, *J* = 4.0 Hz, 1H), 7.51-7.59 (m, 2H), 8.75 (br s, 2H), 11.10 (br s, 1H).

Protocols for Inhibition Assays Using Recombinant Kynurenine Aminotransferase II.

Human KAT II inhibition spectra assay:

Formation of kynurenic acid (KYNA) is indirectly assessed by a decrease in light absorbance at 370 nm (OD370) as the L-kynurenine (KYN) substrate is converted by the human KAT II (hKAT II) enzyme into KYNA. An inhibitor would therefore inhibit the decrease in OD370.

The protocol was performed by placing the following reagents into a Costar 384 well black plate (30 µL total assay volume/well):

- 10 µL of 3x concentrated compound;
- 10 µL of 3x concentrated substrate mix (BGG (Sigma G-5009); 3 mM L-Kynurenine in 150 mM Tris Acetate (Sigma K3750); 3 mM α-ketoglutaric acid in 150 mM Tris Acetate (Sigma K2010); and 210 µM pyridoxal 5-phosphate (PLP) in 150 mM Tris Acetate (Sigma 9255)); and
- 10 µL of 3x concentrated enzyme (90 nM enzyme in 150 mM Tris Acetate with 0.3% bovine serum).

Plates were sealed and incubated at 37 ℃ for 15-20 h before reading OD370 on a SpectraMax Plus plate reader. IC_{50} values were generated by comparing the efficacy of compounds across a concentration range to inhibit a reduction in the OD370 value relative to assay wells with DMSO added in place of concentrated compound.

KAT II kinetic assay:

Test compounds were dissolved in 100% DMSO and diluted to the required concentrations in 100% DMSO. An additional aqueous dilution was made so that the compound at 3x final concentration was 1.0% DMSO in the assay specific buffer. Compounds were tested at 11 concentrations. Final DMSO concentrations in the assay plate were equal to 0.33%.

Assay Methodology

KATII enzyme activity was followed by measuring the loss of absorbance of the L-KYN substrate at an absorbance wavelength of 370 nm. The KAT II assays were run in a 384 well format at a final volume of 30 µL using 150 mM Tris Acetate buffer (pH7.0), 1 mM L-KYN,1 mM α-ketoglutaric acid, 70 µM PLP, 0.1% BGG and either 30 nM human KATII enzyme or 5 nM rat KAT II enzyme. Compound was diluted in 100% DMSO and spotted prior to the addition of the other reagents. Enzyme

was always added last. Assay plates were sealed around the edges with tape and immediately read on a SpectraMax plate reader at an absorbance wavelength of 370 nm. The SpectraMax plate reader was set up to read every 5 min for 16 hours.

The following steps are taken to ensure consistent production of kinetic read data:

1. A 10 μL aliquot of the compound dilutions (described above in compound preparation) was added to the assay plate by hand followed by a quick spin to ensure compound was collected at bottom of well.
2. A 10 μL aliquot of substrate mix containing the L-KYN, α-ketoglutaric acid and PLP was then added to the assay plate via a Multidrop instrument.
3. Finally, a 10 μL aliquot of a 3x concentration of enzyme stock soluton was added last to initiate the reaction via a Multidrop instrument.
4. The microplate lid was placed onto the assay plate and taped to seal in humidity, and the assay plate was put into the SpectraMax reader. A quick vibration on the plate platform was done to ensure mixing, and the absorbance was read (wavelength of 370 nm) every 5 min over 16 h at room temperature.

Determination of Potencies (k_{inact}/K_I values)

The direct substrate absorbance loss assay described above was performed for the determination of potencies (k_{inact}/K_I values). The overall potency, k_{inact}/K_I values, were determined using the general approaches previously reported.[3,4] Reaction progress curves (decrease in OD 370 nm with time) were obtained in the presence of eleven concentrations of inhibitor with top dose at 1 μM and diluted by 2 fold to 1 nM. Data analysis was performed using GraphPad Prism version 5.01 for Windows, GraphPad Software, San Diego, California USA. Each progress curve was fit to a one phase exponential decay model to determine $k_{observed}$ (k_{obs}) values at each inhibitor concentration. For the human KATII enzyme, a 6 hour time window (5 minutes to 360 minutes) was used to derive the *kobs* value across all inhibitor concentrations. The inhibitor dissociation constant (K_I) and the first-order rate constant of enzyme inactivation at infinite inhibitor concentration (k_{inact}) were then obtained by fitting the *kobs vs*. [I] curves as described in the references.[3,5]

Experimental Methods for Scheme 1 (NMR studies)

All NMR spectra were collected at 298K on a Bruker 600 MHz spectrometer equipped with 5mm cryoprobe. The human KAT II protein (550uM) was solubilized in 30mM Tris buffer, pH 8.0, with 200 mM NaCl and small amount of glycerol. All spectra were collected using 30 degree read pulse with 1.5 s recycling delay, and the total acquisition time for each spectrum was 16 hours. The spectra were processed using the Topspin 2.0 software from Bruker. Prediction of ^{13}C chemical shifts was by the use of CNMR predictor of the software package ACDlab 9.0.

KAT II Protein Isolation and X-ray Crystallography Experimental Protocols

Expression and Purification:

Full length human KAT II (residues 1-425), with a C-terminal hexa-histidine tag was expressed in Sf21 insect cells. 4 L of cells were resuspended in 120 mL Y PER and 60 mL B PER, 4 protease free EDTA tablets, 30 uL Benzamase. The cell paste was left to re-suspend with gentle stirring at 4° C for 20 minutes. The cell lysate was cleared by centrifugation (15,000g, 4° C, 45 minutes). The protein supernatant was passed through a Ni NTA column equilibrated in 30 mM Tris pH 8.00, 150 mM NaCl, 1 mM TCEP and 10 mM imidazole. KAT II was removed from the column using the above buffer in 200 mM imidazole (10mL/L). The protein was then diluted in MonoQ buffer to reduce the salt concentration (30 mM Tris pH 8.00, 10 mM NaCl and 1 mM TCEP), and was removed from the column using a salt gradient which worked out to be typically 200 mM NaCl. Yield of protein was about 17.5 mg/L. Protein was concentrated to 8 mg/ml and frozen at -80° C. Yield from 4 L was 70mg.

Crystallization:

Crystals were grown by vapour diffusion as hanging drops. Protein at 10 mg/ml was incubated at room temperature for 2 h with compound (1 mM) and pyridoxal phosphate (2 mM). 2μl drops, containing equal volumes of protein solution and reservoir solution (200 mM NaCl, 0.1M NaCitrate pH 5.6, 24% PEG4K), were equilibrated against 1mL of reservoir solution. Plate-like crystals appeared after a few days.

Structure determination and refinement:

Diffraction data were collected from a single crystal flash frozen in a stream of dry nitrogen at 100 K, using 25% glycerol as a cryoprotectant. Data to 3.2 Å resolution were collected on a Rigaku Saturn CCD detector, using a Rigaku FR-E generator with focusing optics as the x-ray source. The crystals are orthorhombic (space group $P2_12_12_1$), with unit cell dimensions of a=65.20, b=116.60 c=129.27 Å. The structure was solved by molecular replacement, using the previously reported structure of human KAT II as a starting model.[5] The structure was refined to a final R/R_{free} of 20.0/27.5%. The final refined model contains two protein molecules, two PLP-inhibitor complexes, 2 chloride ions and one water molecule. The entire polypeptide chain of human KAT II is modeled, except for residues 17-33 in molecule A, and residues 18-28 in molecule B, which were too disordered to be modeled with confidence at this resolution. Complete data collection and refinement statistics are given in Table S1.

Table S1. Data Collection and Refinement Statistics

Data Collection		
Space Group		$P2_12_12_1$
Unit cell: *a,b,c*, Å		65.20, 116.60, 129.27
Resolution (Å)		3.2
Total measurements		92666 (8827)*
Unique reflections		16095 (1605)
R_{sym} (%)		10.5 (31.0)
<I>/<SigI>		25.84 (7.77)
Completeness		99.4 (99.9)
Redundancy		5.76 (5.5)
*Numbers in parentheses reflect the values in the highest resolution shell (3.2-3.31 Å).		
Model Refinement		
Maximum resolution (Å)		3.2
R_{work}[a] (%)		20.0
R_{free} (%)		27.5
Mean B value (Å^2)		56.6
Rms deviations from ideal geometry		
Bonds (Å)	0.010	
Angles (°)		1.33
Peptide Omega Torsion Angles (°)		2.89
Number of TLS Groups		2
Water molecules		1
Ions		2 Chloride

[a]$R_{work} = \Sigma||F_{obs}| - |F_{calc}|/\Sigma|F_{obs}|$. R_{free} is equivalent to R_{work}, except that it is calculated for a randomly chosen 5% of reflections omitted from the refinement process.

Plasma Protein and Nonspecific Binding of PF-04859989

Plasma and brain free fraction for PF-04859989 was determined through the use of a 96-well equilibrium dialysis apparatus.[6] Spectra-Por 2 membranes with molecular cutoff of 12 to 14 kDa used for the dialysis were obtained from Spectrum Laboratories Inc. (Rancho Dominguez, CA). The membranes were conditioned in deionized water for 20 min followed by 30% ethanol for 20 min. Following a thorough rinse with deionized water, the membranes were subsequently stored in 0.10 M sodium phosphate buffer (pH 7.4) until needed. Previously frozen rat plasma and brains (Pel-Freez Biologicals, Rogers, AR) were thawed the day of the experiment. Brain samples were diluted with 2 volumes of 0.10 M sodium phosphate buffer and homogenized via a Kinematica Polytron homogenizer (Kinematica, Inc., Bohemia, NY). Plasma and brain homogenate (1/3 total dilution) were pH adjusted to 7.4 and then spiked with the test drug (1 µM) and 150 µL aliquots were loaded into the 96-well equilibrium dialysis plate and dialyzed versus 150 µL of 0.10 M sodium phosphate buffer. The apparatus was covered with a CO_2 permeable membrane and equilibrium was achieved following a 6 h incubation in a cell culture incubator (37° Celsius, 5% CO_2). After reaching equilibrium, 100 µL aliquots of each matrix were removed from the 96-well equilibrium dialysis apparatus and added to equal amount of blank matrix in 1.2 mL polypropylene tubes to employ a mixed matrix analysis technique. Mixed matrix analysis allows for direct comparison of analytical data when all samples are composed of an identical matrix, thus buffer samples are added to control plasma or control brain homogenate and plasma or brain homogenate samples are added to an equal volume of control buffer. The samples were analyzed identically to the *in vivo* samples (via LC-MS/MS) with the output of analyte area/internal standard ratios in place of concentration data. For plasma, the unbound fraction was determined as the ratio of analyte/internal standard area counts determined in buffer and plasma. As described by Kalvass and Maurer[7] the unbound fraction in total (undiluted) brain was calculated via the below equation, where D and fu,measured represent the fold dilution of brain tissue and the free fraction determined as the ratio of area counts in buffer versus diluted brain tissue, respectively.

$$Total\ (undiluted)\ fu = \frac{1/\mathrm{D}}{\left(\left(\frac{1}{\mathrm{fu,measured}}\right) - 1\right) + 1/\mathrm{D}}$$

Animals

Male Sprague-Dawley rats weighing 200 to 250 g were obtained from Charles River Laboratories International Inc. (Wilmington, MA). Upon arrival, the rats were maintained for at least 2 days on a 12-h

light/dark cycle in a temperature- and humidity-controlled environment with free access to food and water. The rats were housed in clear polycarbonate boxes containing sawdust. The study was conducted in accordance with approved Pfizer Animal Care and Use Procedures.

Dose Administration and Sample Collection

Rats were administered a single subcutaneous dose at 10 mg/kg (n = 3) of PF-04859989. Dosing solution was prepared as a solution in sterile water and administered in a 2 mL/kg volume in the mid-back region. Rats were euthanized in a CO_2 chamber at 0.25 h postdose. CSF was collected via puncture of the cisterna magna using a 23 gauge needle attached to polyethylene tubing and a syringe. Whole blood was collected by cardiac puncture into Vacutainer tubes containing heparin and stored on ice until centrifuged for the preparation of plasma. Whole brains were collected by decapitation, rinsed with phosphate-buffered saline, and weighed. CSF and whole brains were immediately frozen on dry ice upon collection.

Sample Analysis of PF-04859989

All samples were quantified via HPLC-MS/MS. Whole brain samples were thawed, diluted 1:4 (w/v) with water, and homogenized using a Kinematica Polytron homogenizer (Kinematica, Inc., Bohemia, NY). Standards and controls were prepared in a similar manner using a brain homogenate prepared from untreated animals. Plasma, csf, and brain homogenate samples were prepared for analysis by deproteination with acetonitrile containing a structurally similar internal standard. A 50 µL of sample was precipitated with 100 µl of acetonitrile, vortexed, and centrifuged. Following centrifugation, the supernatant was transferred to a clean 96-well injection block and evaporated under a flow of nitrogen at 37º Celsius and reconstituted in 75 µl of 50/50 methanol/water. 10 µl was subsequently injected on the LC-MS/MS for analysis.

LC-MS/MS analysis was carried out for using a high-performance liquid chromatography system consisting of a Shimadzu LC-20 binary pump (Shimadzu Scientific Instruments, Columbia, MD) with CTC PAL autosampler (Leap Technologies, Carrboro, NC) interfaced to Micromass Quattro Ultima mass spectrometer (Waters, Milford, MA). The mass spectrometer was fitted with an electrospray ionization Z-spray interface that was operated in a positive ion mode. Multiple reaction monitoring was performed with the transition m/z 178.9 to 116.1 for PF-04859989.

PF-04859989 and the internal standard were separated on a Phenomenex Synergi Hydro-RP column (30 x 2.0 mm, 2.5 µm) by gradient elution. Mobile phase A consisted of 20 mM ammonium acetate with

0.1% formic acid and 0.1% isopropanol and mobile phase B consisted of acetonitrile. During the first half-minute of the chromatographic run, the mobile phase solvents were held at a constant ratio 100:0 (A:B) that was followed by a 1.0 minute linear gradient to a mobile phase solvent ratio of 10:90 (A:B). The intermediate condition was held for 0.5 minute followed by an immediate return to the starting conditions. The starting conditions were then maintained for a one-minute period of re-equilibration. A flow rate of 0.3 ml/min was used. All raw data was processed using MassLynx Software ver. 4.0 (Waters, Milford, MA). The lower limit of quantification (LLOQ) for PF-04859989 was 1.0 ng/ml for plasma, csf, and brain homogenate (conversion to 5 ng/g) samples. The upper limit of quantification (ULOQ) was 1000 ng/mL. Pharmacokinetic parameters were determined by non-compartmental analysis with use of linear trapezoidal method for AUC calculation using Watson Bioanalytical LIMS (v7.2.0.03, Thermo Electron Corp., Philadelphia, PA).

Experimental Methods for Figure 6.

Animal care and handling. All animals used in these studies were housed two per cage, in environmentally controlled animal quarters (light/dark-4:00 am/4:00 pm) with free access to food and water for not less than 7 days prior to testing except as noted in method. All procedures were in accordance with the Pfizer Laboratory Animal Care Program and were approved by the Institutional Animal Care and use Committee of Pfizer Global Research and Development, Groton CT.

Microdialysis in freely moving rats. Presurgerized male Sprague-Dawley rats (280 – 340 g) were ordered from Charles River (Raleigh, NC) with the guide cannula installed according to the following specifications. Microdialysis probe guide cannula (Bioanalytical Systems, Inc., West Lafayette, ID) were implanted in the PFC (bregma: AP +3.7 mm, ML -0.7 mm, DV -2.0 mm; Paxinos and Watson 1997) under isoflurane anesthesia and fixed to the skull using bone screws and dental acrylic. Animals were allowed to recover for a minimum of 24 h. Approximately 18 h before testing, guide stylets were removed, and probes (BR-4; Bioanalytical Systems, Inc.) with a polyacrylonitrile membrane (OD 340 mm, MW cut-off 30,000 daltons) and a 4 mm dialysis tip were inserted. The inlet and outlet of the probe were connected with flexible PEEK tubing (inside diameter 0.005", Upchurch Scientific) and the probes were perfused at (1.5 μl/min) with artificial CSF (147 mM NaCl, 2.7 mM KCl, 1.0 mM $MgCl_2$ hexahydrate, 1.3 mM $CaCl_2$ dihydrate) with a CMA/100 (CMA/Microdialysis, North Chelmsford, MA) microperfusion pump. On test day, microdialysate samples (30-μl) were continuously collected and automatically injected every 20 min for analysis by on-line HPLC/Fluorimetric detection.

Rats were dosed subcutaneously (SC) with vehicle (2 ml/kg) or test compound dissolved in vehicle. Vehicle consisted of 1% acetic acid in distilled water (v/v). Test compound or vehicle was administered approximately 2 hours after basal KYNA levels had stabilized and the effects on KYNA levels were monitored for at least 4 hours.

Table S2. Human and Rat KAT II Potency for Analogs of 7

Compound	R^1	R^2	R^3	R^4	hKAT II IC_{50}[a] (nM)	rKAT II IC_{50}[a] (nM)	hKAT II k_{inact}/K_i[b] ($M^{-1}s^{-1}$)	rKAT II k_{inact}/K_i[b] ($M^{-1}s^{-1}$)
7	H	H	H	H	23± (n = x)	263± (n = x)	18,500±7770 (n = 20)	573±303 (n = 16)
12	F	H	H	H	40±8 (n = 8)	631±110 (n =7)	5310±828 (n = 3)	57± 21 (n = 3)
13	OMe	H	H	H	572± 94 (n = 3)	>10,000 (n = 3)	21.3± (n =3)	--
14	Cl	H	H	H	252±55 (n = 6)	>10,000 (n = 6)	42.0±7.1 (n = 4)	--
15	Me	H	H	H	1050±79 (n = 2)	>10,000 (n = 2)	--	--
16	CF_3	H	H	H	174 ±28 (n = 4)	>6810 (n = 5)	541±300 (n = 5)	--
17	H	Cl	H	H	29±9 (n = 3)	118±14 (n = 3)	28,300±10,900 (n = 5)	1170 ±293 (n = 3)
18	H	Me	H	H	37±3 (n = 3)	368±18 (n = 3)	33,400±15,400 (n = 3)	590±14 (n = 3)
19	H	OMe	H	H	22±4 (n = 8)	137±15 (n = 8)	31,700±8500 (n = 3)	1840±783 (n = 4)
20	H	H	Cl	H	36±4 (n = 7)	258±30 (n = 7)	19,000±16,600 (n = 5)	465±336 (n = 4)
21	H	H	Me	H	30±4 (n = 3)	402±72 (n = 3)	11,900±2420 (n = 3)	167±73 (n = 3)
22	H	H	CF_3	H	29±7 (n = 9)	488±149 (n = 9)	15,300±4900 (n = 3)	102±58 (n = 3)
23	H	H	H	F	45±11 (n = 6)	2060±80 (n = 5)	6680±1740 (n = 3)	--
24	H	H	H	Cl	349±105 (n = 9)	>7970 (n = 10)	9.5±4.0 (n = 4)	--
25	H	H	H	Me	319± 75 (n = 6)	>10,000 (n = 6)	--	--
26	H	H	H	OMe	179± 32 (n = 9)	>4920 (n = 8)	1940±1010 (n = 3)	--
27	H	H	H	CF_3	>10,000 (n = 8)	>10,000 (n = 8)	--	--

a Values represent the geometrical mean ± SEM; *b* Values represent the arithmetic mean ± SD.

Table S3. CEREP Screening data for PF-04859989[8] (all assays at 10 uM PF-04859989)

Assay		CEREP Catalog Ref	% Inhibition of Control Specific Binding or Enzyme Activity
Adenosine	A3 (h) (agonist radioligand)	0006	-6
Adenosine	A1 (h) (antagonist radioligand)	0002	4
Adenosine	A2A (h) (agonist radioligand)	0004	-1
Adrenergic	alpha 2C (h) (antagonist radioligand)	0016	2
Adrenergic	beta 3 (h) (antagonist radioligand)	0227	2
Adrenergic	alpha 1 (non-selective) (antagonist radioligand)	0008	-9
Adrenergic	alpha 2A (h) (antagonist radioligand)	0013	-1
Adrenergic	alpha 2B (h) (antagonist radioligand)	1344	8
Adrenergic	beta 1 (h) (agonist radioligand)	0018	-6
Adrenergic	beta 2 (h) (agonist radioligand)	0020	-3
Androgen	AR (h) (agonist radioligand)	0933	-3
Angiotensin II	AT1 (h) (antagonist radioligand)	0024	-27
Benzodiazepine	BZD (central) (agonist radioligand)	0028	-1
Calcium channel	Ca2+ channel (L, dihydropyridine site) (antagonist radioligand)	0161	8
Calcium channel	Ca2+ channel (L, diltiazem site) (benzothiazepines) (antagonist radioligand)	0162	-11
Calcium channel	Ca2+ channel (L, verapamil site) (phenylalkylamine) (antagonist radioligand)	0163	-9
Calcium channel	Ca2+ channel (N) (antagonist radioligand)	0164	-9
Canabinoid	CB1 (h) (agonist radioligand)	0036	6
Canabinoid	CB2 (h) (agonist radioligand)	0037	-6
Chemokine	CXCR4 (h) (agonist radioligand)	1022	0
Chloride channel	Cl- channel (GABA-gated) (antagonist radioligand)	0170	-5
Cholecystokinin	CCK1 (CCKA) (h) (agonist radioligand)	0039	27
Cholecystokinin	CCK2 (CCKB) (h) (agonist radioligand)	0041	-6
Choline	choline transporter (CHT1) (h) (antagonist radioligand)	1552	-1
Dopamine	D1 (h) (antagonist radioligand)	0044	-29
Dopamine	D2S (h) (antagonist radioligand)	0046	-15
Dopamine	D3 (h) (antagonist radioligand)	0048	-14
Dopamine	dopamine transporter (h) (antagonist radioligand)	0052	0
Dopanmine	D2S (h) (agonist radioligand)	1322	6
Dopanmine	D4.4 (h) (antagonist radioligand)	0049	4
Endothelin	ETA (h) (agonist radioligand)	0054	-12
Endothelin	ETB (h) (agonist radioligand)	0056	3
GABA	GABA transporter (antagonist radioligand)	0060	11
GABA-A	GABAA (agonist radioligand)	0058	6
GABA-B	GABAB(1b) (h) (antagonist radioligand)	0885	12
Glucocorticoid	GR (h) (agonist radioligand)	0469	1

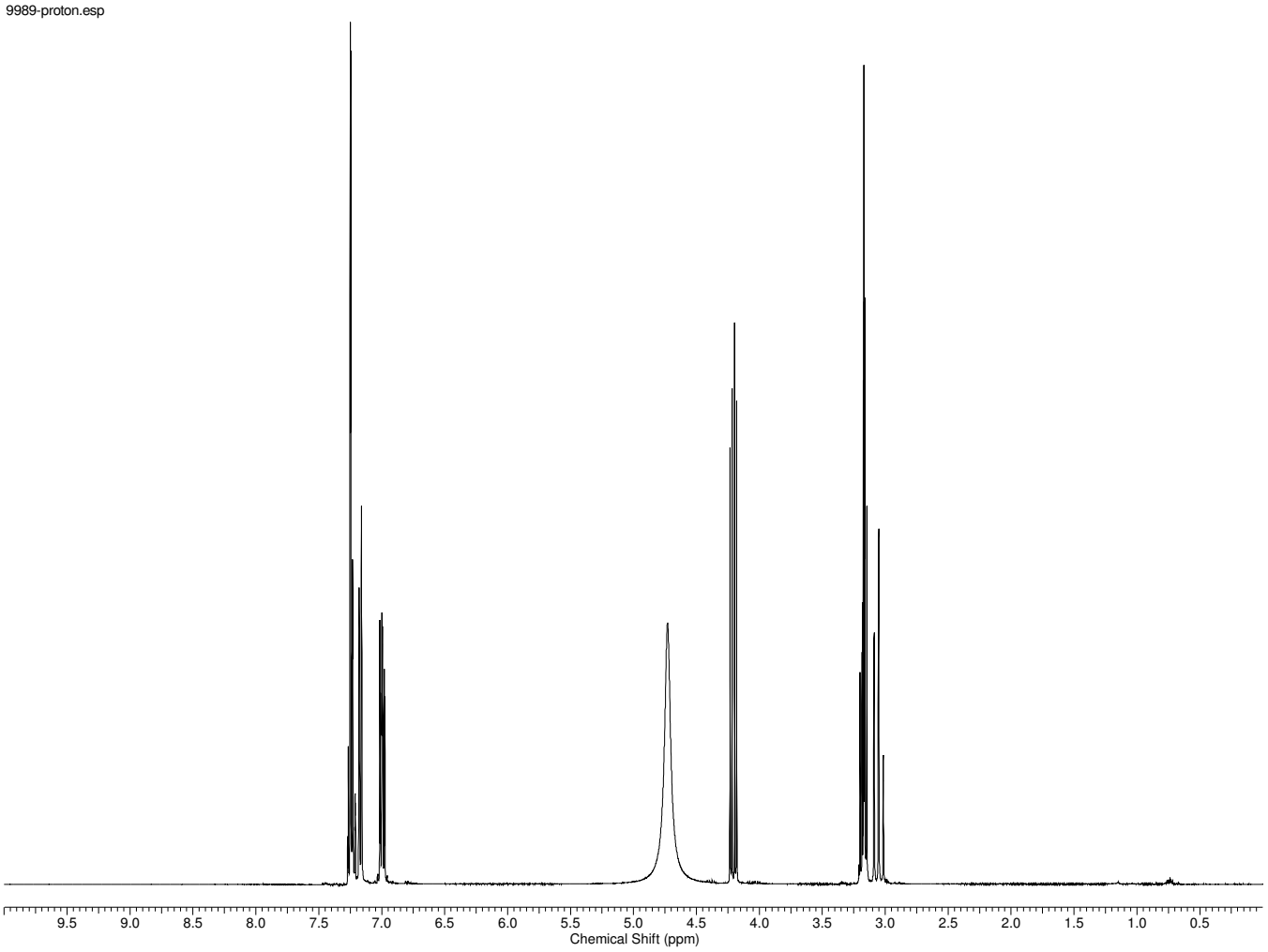
Table S2., Cont'd. CEREP Screening data for PF-04859989 (all assays at 10 uM PF-04859989)

Assay		CEREP Catalog Ref	% Inhibition of Control Specific Binding or Enzyme Activity
Glutamate	PCP (antagonist radioligand)	0124	0
Glutamate	AMPA (agonist radioligand)	0064	-11
Glutamate	kainate (agonist radioligand)	0065	1
Glutamate	NMDA (antagonist radioligand)	0066	14
Glutamate	glycine (strychnine-insensitive) (antagonist radioligand)	0068	10
Histamine	H4 (h) (agonist radioligand)	1384	-7
Histamine	H1 (h) (antagonist radioligand)	0870	-7
Histamine	H2 (h) (antagonist radioligand)	1208	-2
Histamine	H3 (h) (agonist radioligand)	1332	-7
Imidazoline	I1 (agonist radioligand)	0642	93 (Note: Ki=1.6E-06)
Leukotriene	BLT1 (LTB4) (h) (agonist radioligand)	1209	-4
Leukotriene	CysLT1 (LTD4) (h) (agonist radioligand)	0086	-3
Melanin concentrating hormone	MCH1 (h) (agonist radioligand)	1115	19
Melanocortin	MC1 (agonist radioligand)	0644	1
Melanocortin	MC3 (h) (agonist radioligand)	0447	-12
Melanocortin	MC4 (h) (agonist radioligand)	0420	-9
Melatonin	MT1 (ML1A) (h) (agonist radioligand)	1538	2
Melatonin	MT3 (ML2) (agonist radioligand)	0088	15
Monoamine oxidase-A	MAO-A (antagonist radioligand)	0443	7
Motolin	motilin (h) (agonist radioligand)	0470	6
Muscarinic	M4 (h) (antagonist radioligand)	0096	0
Muscarinic	M5 (h) (antagonist radioligand)	0097	-4
Muscarinic	M1 (h) (antagonist radioligand)	0091	-3
Muscarinic	M2 (h) (antagonist radioligand)	0093	-5
Muscarinic	M3 (h) (antagonist radioligand)	0095	-10
Neurokinin	NK1 (h) (agonist radioligand)	0100	-14
Neurokinin	NK2 (h) (agonist radioligand)	0102	-3
Neuropeptide Y	Y1 (h) (agonist radioligand)	0106	-8
Nicotinic	N neuronal alpha -BGTX-insensitive (alpha 4beta 2) (agonist radioligand)	0110	0
Nicotinic	N muscle-type (h) (antagonist radioligand)	0936	-4
Norepinepherine	norepinephrine transporter (h) (antagonist radioligand)	0355	1
Opioid	delta 2 (DOP) (h) (agonist radioligand)	0114	-9
Opioid	kappa (KOP) (agonist radioligand)	1971	-9
Opioid	mu (MOP) (h) (agonist radioligand)	0118	0
peroxisome proliferator activated receptor	PPARgamma (h) (agonist radioligand)	0641	-8
Phosphodiesterase	rolipram (antagonist radioligand)	0379	4

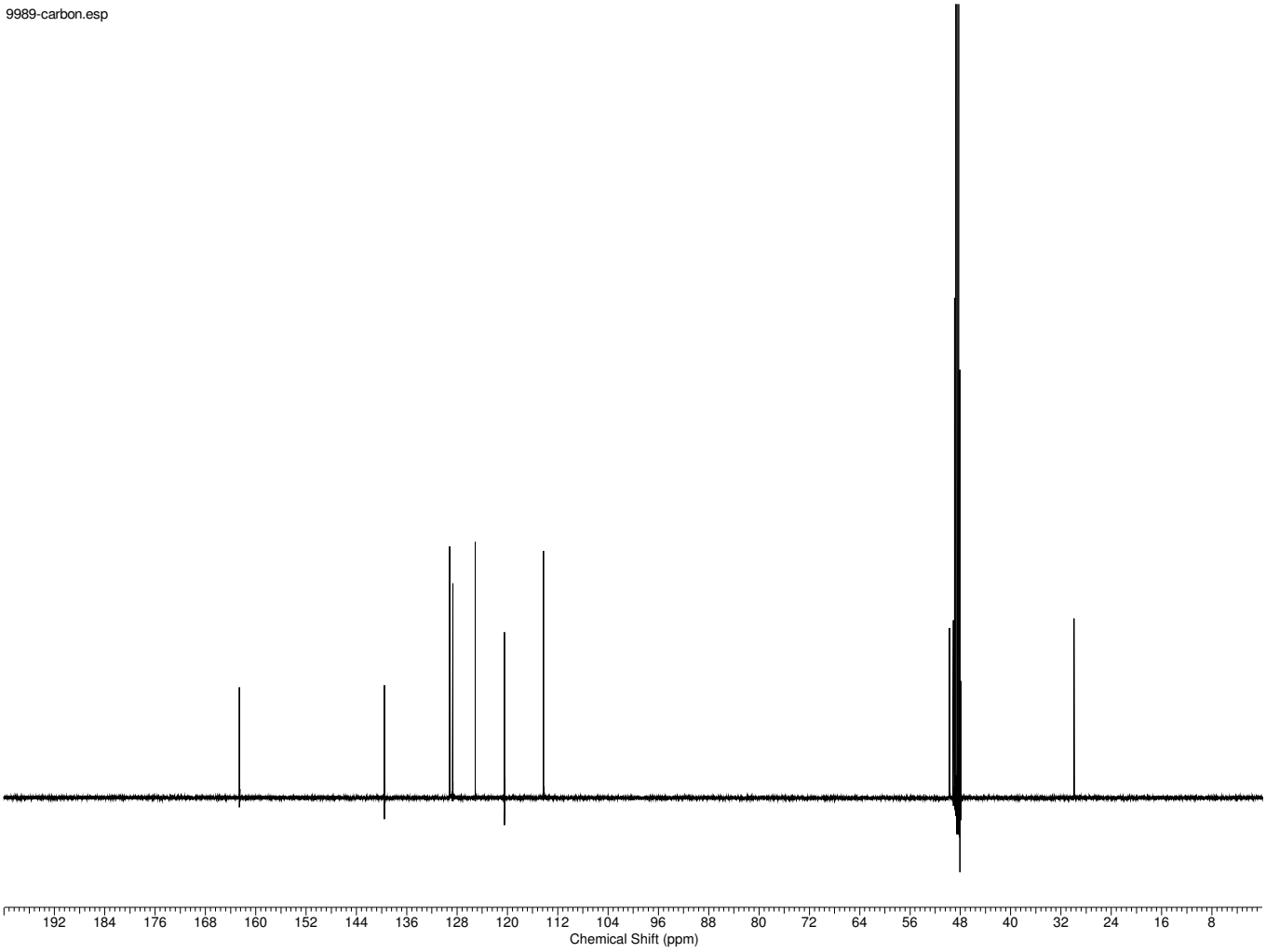
Table S2., Cont'd. CEREP Screening data for PF-04859989 (all assays at 10 uM PF-04859989)

Assay		CEREP Catalog Ref	% Inhibition of Control Specific Binding or Enzyme Activity
Phosphodiesterase	rolipram (antagonist radioligand)	0379	4
Serotonin	5-HT1D (agonist radioligand)	1974	-11
Serotonin	5-HT2A (h) (antagonist radioligand)	0135	1
Serotonin	5-HT2C (h) (antagonist radioligand)	0137	8
Serotonin	5-HT6 (h) (agonist radioligand)	0142	0
Serotonin	5-HT1A (h) (agonist radioligand)	0131	6
Serotonin	5-HT1B (antagonist radioligand)	0132	3
Serotonin	5-HT2A (h) (agonist radioligand)	0471	-3
Serotonin	5-HT2B (h) (agonist radioligand)	1333	6
Serotonin	5-HT2C (h) (agonist radioligand)	1003	-1
Serotonin	5-HT3 (h) (antagonist radioligand)	0411	5
Serotonin	5-HT4e (h) (antagonist radioligand)	0501	-8
Serotonin	5-HT7 (h) (agonist radioligand)	0144	28
Serotonin	5-HT transporter (h) (antagonist radioligand)	0439	-5
Sigma	sigma (non-selective) (agonist radioligand)	0146	-6
Small conductance calcium-activated potassium channel	SKCa channel (antagonist radioligand)	0167	1
Sodium channel	Na+ channel (site 2) (antagonist radioligand)	0169	6
Somatostatin	sst4 (h) (agonist radioligand)	0482	-13
Thyroid hormone	TR (TH) (agonist radioligand)	0156	8
TNF-alpha	TNF-alpha (h) (agonist radioligand)	0076	-22
Urotensin	UT (h) (agonist radioligand)	1386	3
Vasoactive intestinal peptide	VPAC1 (VIP1) (h) (agonist radioligand)	0157	-5
Vasopressin	V2 (h) (agonist radioligand)	0497	-5
Vasopressin	V1a (h) (agonist radioligand)	0159	-5

^{1}H NMR spectrum of PF-04859989 (7)



^{13}C NMR spectrum of PF-04859989 (7)



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