SUPPORTING INFORMATION

Discovery and *In Vitro* Characterization of SPL028: Deuterated *N,N*-Dimethyltryptamine

Marie Layzell*,[1] Peter Rands,[1] Meghan Good,[1] Zelah Joel,[1] Rick Cousins,[2] Tiffanie Benway,[1] Ellen James,[1] Carol Routledge[1]

[1]Small Pharma., 50 Featherstone Street, London, EC1Y 8RT

[2]Cinnabar Consulting Ltd., 43 Pedley Lane, Clifton, Beds SG17 5QT, UK

TABLE OF CONTENTS

Throughout the testing process, no unexpected or unusually high safety hazards were encountered. Continue to adhere to standard safety protocols for a safe working environment.

S1. Chemistry, Experimental Information

General Synthetic Methods. Reagents and solvents were obtained from commercial suppliers and used without further purification. Organic solutions were concentrated under reduced pressure on a rotary evaporator using a water bath. All new compounds gave satisfactory ^{1}H NMR, HPLC and LC/MS results. All final compounds have an HPLC purity of ≥95%. HPLC analyses were performed using the following conditions on Agilent 1100/1200 series liquid chromatograph or equivalent:

Analytical HPLC Method A

Column: Triart Phenyl 3.0 µm, 4.6 × 150 mm. Mobile phase: A = Deionised H_2O:TFA (100:0.05); B = Acetonitrile:TFA (100:0.05). Gradient: T = 0, 95% solvent A, 5% solvent B; T = 13 min, 62% solvent A, 38% solvent B; T = 26 min, 5% solvent A, 95% solvent B; T = 30.5 min, 5% solvent A, 95% solvent B; T = 31 min, 95% solvent A, 5% solvent B; stop time, 31 min. Flow = 1.0 mL/min. UV 220 nm.

Analytical HPLC Method B

Column: Acquity BEH Phenyl 4.6 x 30 mm; 1.7 µm particle size. Mobile phase: A = Deionised H_2O:TFA (100:0.03); B = Acetonitrile:TFA (100:0.03). Gradient: T = 0, 95% solvent A, 5% solvent B; T = 5 min, 95% solvent A, 5% solvent B; T = 15 min, 5% solvent A, 95% solvent B; T = 16 min, 5% solvent A, 95% solvent B; T = 16.5 min, 95% solvent A, 5% solvent B; stop time, 17 min. Flow = 2.0 mL/min. UV 220 nm.

Analytical HPLC-MS

HPLC: Column: Triart Phenyl 3.0 μm, 4.6 × 150 mm. Mobile phase: A = Deionised H_2O:TFA (100:0.05); B = Acetonitrile:TFA (100:0.05). Gradient: T = 0, 95% solvent A, 5% solvent B; T = 13 min, 62% solvent A, 38% solvent B; T = 26 min, 5% solvent A, 95% solvent B; T = 30.5 min, 5% solvent A, 95% solvent B; T = 31 min, 95% solvent A, 5% solvent B; stop time, 31 min. Flow = 1.0 mL/min. UV 220 nm.

Mass Spectrometry: System: Agilent 6100 series Quadrupole LC-MS or equivalent. Drying gas flow: 12.0 L/min. Drying gas temp: 350°C. Nebulizer pressure: 35 psig. Fragmentor: 110V. Gain: 1.0.

^{1}H Nuclear magnetic resonance (NMR) spectra were recorded on a Jeol AS400 spectrometer (399.78 MHz), except for **9i** which was recorded on a Bruker Avance Neo spectrometer (400.13 MHz). All spectra were recorded at room temperature. Chemical shifts are reported in ppm relative to deuterated solvent as an internal standard (δ_H DMSO-d_6 2.50 ppm, δ_H chloroform-d 7.26 ppm,) with the following convention for describing multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, m = multiplet, br = broad signal, dd = doublet of doublets, etc.).

Indole-3-(*N*,*N*-dimethyl)-acetamide (6). To a 5 L vessel under nitrogen was charged indole-3-acetic acid **(4)** (1 equiv, 1.467 mol), 1-hydroxybenzotriazole (~20% wet) (1.2 eq, 1.760mol) and dichloromethane (DCM) (2313mL) to give a milky white suspension. N-Ethyl-N′-(3-dimethylaminopropyl)carbodiimide hydrochloride (1.2 eq, 1.760 mol) was then charged portion-wise over 5 min at 16–22°C. The reaction mixture was stirred for 2 hours at ambient temperature before 2M dimethylamine in THF (1.5 eq, 2.200mol) was charged dropwise over 20 minutes at 20–30°C. The resultant solution was stirred at ambient temperature for 1 hour where HPLC Method A indicated 1.1% indole-3-acetic

acid **(4)** remained and 98.1% of **Indole-3-(*N,N*-dimethyl)-acetamide (6)**. The reaction mixture was then charged with 10% K_2CO_3 (1285 mL) and stirred for 5 min. The layers were separated, and the upper aqueous layer extracted with DCM (643 mL x 2). The organic extracts were combined and washed with saturated brine (643 mL). The organic extracts were then dried over $MgSO_4$, filtered and concentrated in vacuo at 45°C. This provided 303.1 g of crude **Indole-3-(*N,N*-dimethyl)-acetamide (6)** as an off-white sticky solid. The crude material was then subjected to a slurry in tert-butyl methyl ether (TBME) (2570 mL) at 50°C for 2 hours before being cooled to ambient temperature, filtered and washed with TBME (514mL x 2). The filter cake was then dried in vacuo at 50°C to afford **Indole-3-(*N,N*-dimethyl)-acetamide (6)** as an off-white solid; yield 90% (266.2 g, 2.81 mol). ^{1}H NMR (399.78 MHz, $[D_1]CHCl_3$): δ = 8.82 (br s, 1H), 7.64 (d, *J = 8 Hz*, 1H), 7.31 (d, *J = 8.4 Hz*, 1H), 7.18 (t, *J = 7.2 Hz*, 1H), 7.12 (d, *J = 7.2 Hz*, 1H), 6.96 (s, 1H), 3.83 (s, 2H), 2.99-3.00 (2 x s, 6H). HPLC method A: retention time = 13.97 min; peak area, 98.5%.

3-(3-Methylbutyl)-1H-indole or *N,N*-Dimethyltryptamine (1). To a 5L vessel under nitrogen was charged **indole-3-(*N,N*-dimethyl)-acetamide (6)** (272.5 g, 1.347 mol) and THF (1363 mL) to give an off-white suspension. 2 M $LiAlH_4$ in THF (505.3 mL, 1.213 mol) was then charged dropwise over 35 minutes at 20–56°C to give an amber solution. The solution was heated to 60°C for 2 hours where HPLC Method A indicated **indole-3-(*N,N*-dimethyl)-acetamide (6)** not detected, product 92.5%, impurity 1 2.6%, and impurity 2 1.9%. The complete reaction mixture was cooled to ambient temperature and then charged to a solution of 25% Rochelle's salts (aq) (2725 mL) dropwise over 30 minutes at 20–30°C. The resultant milky white suspension was allowed to stir at 20-25°C for 1 hour after which the layers were separated and the upper organic layer washed with saturated brine (681 mL). The organic layer was then dried over $MgSO_4$, filtered and concentrated in vacuo at 45°C.

The resultant crude oil was subjected to an azeotrope from EtOH (545 mL x 2). This afforded the title product as an oil: yield 92% (234.6 g, 1.24 mol). ^{1}H NMR (399.78 MHz, [D_1]$CHCl_3$): δ = 8.17 (br s, 1H), 7.63 (d, *J = 8.0 Hz*, 1H), 7.36 (d, *J = 8.0 Hz*, 1H), 7.20 (t, *J = 7.6 Hz*, 1H), 7.13 (d, *J = 7.2 Hz*, 1H), 7.02 (s, 1H), 2.97 (t, *J = 8.4 Hz*, 2H), 2.67 (t, *J = 8.4 Hz*, 2H), 2.37 (s, 6H). HPLC method A: retention time = 10.66 min; peak area, 95.0%.

3-(3-Methylbutyl)-1H-indole fumarate or *N,N*-Dimethyltryptamine fumarate (7). To a 5 L flange flask under nitrogen was charged fumaric acid (152.7 g 1.315 mol) and ***N,N*-Dimethyltryptamine (1)** (248.2 g, 1.315 mol) as a solution in EtOH (2928 mL). The mixture was heated to 75°C to give a dark brown solution. The solution was polish filtered into a preheated (80°C) 5 L jacketed vessel. The solution was then cooled to 70°C and seeded with Pattern A (0.1 wt%), the seed was allowed to mature for 30 min before cooling to 0°C at a rate of 5°C/hour. After stirring for an additional 4 hours at 0°C, the batch was filtered and washed with cold ethanol (496 mL x 2) and then dried at 50°C overnight. This afforded the title product as a crystalline beige-coloured solid; yield 78% (312.4 g, 1.02 mol). ^{1}H NMR (399.78 MHz, [D_6]DMSO): δ = 10.93 (br s, 1H), 7.58 (d, *J = 8.0 Hz*, 1H), 7.35 (d, *J = 8.0 Hz*, 1H), 7.19 (s, 1H), 7.08 (t, *J = 8.0 Hz*, 1H), 6.99 (t, *J = 7.6 Hz*, 1H), 6.54 (s, 2H), 3.02 (m, 4H), 2.63 (s, 6H). HPLC method A: retention time = 10.43 min; peak area, 100.0%

General Procedure for the Synthesis of Compounds 9i-9vi: To a 250 mL 3-neck flask under nitrogen was charged appropriate ratio of solid $LiAlH_4$ (1.013 g, 26.7 mmol), $LiAlD_4$ (1.120 g, 26.7 mmol) and THF (100 mL). The resultant suspension was stirred for 30 minutes before **6** (1 eq, 29.7 mmol) was charged portion-wise over 15 minutes at 20–40°C. The reaction mixture was then heated to reflux (66°C) for 2 hours where HPLC indicated no starting material **6** remained. The mixture was cooled to 0°C and quenched with 25% Rochelle's salts (aq) (120 mL) over 30

min at <30°C. The resultant milky suspension was stirred for 1 hour and then allowed to separate. The lower aqueous layer was removed and the upper organic layer washed with saturated brine (30 mL). The organics were then dried over $MgSO_4$, filtered and concentrated in vacuo. The crude was then taken up in EtOH (52 mL) and charged with fumaric acid (2.66 g, 22.917 mmol) before heating to 75°C. The resultant solution was allowed to cool to ambient temperature overnight before further cooling to 0–5°C for 1 hour. The solids were isolated by filtration and washed with cold EtOH (6.5 mL x 2). The filter cake was dried at 50°C overnight to provide target compounds **9i-vi**. ^{1}H NMR spectra, LCMS and HPLC chromatograms for compounds **9i-vi** in Figures S-S.

2-(1*H*-indol-3-yl)-*N,N*-dimethylethan-1-amine-1,1-d_2 fumarate (9i): $LiAlH_4$:$LiAlD_4$ ratio (0:1). Solid; yield 65% (5.3 g, 17.3 mmol); ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 12.96 (br s, 1H), 10.97 (br s, 1H), 7.56 (br d, J = 7.9 Hz, 1H), 7.34 (dt, J = 8.1, 1.0, 1.0 Hz, 1H), 7.18 (br d, J = 2.4 Hz, 1H), 7.07 (ddd, J = 8.2, 7.0, 1.2 Hz, 1H), 6.98 (ddd, J = 8.0, 7.0, 1.1 Hz, 1H), 6.53 (s, 2H), 2.95 (s, 2H), 2.55 (s, 6H). HPLC method A: retention time = 10.58 min; peak area = 99.3%. HPLC-MS analysis: retention time = 10.638 min, m/z = 191.1, peak area = 96.6%; retention time = 10.655 min, m/z = 190.1, peak area = 2.7%; retention time = 10.673 min, m/z = 189.1, peak area = 0.7%.

2-(1*H*-indol-3-yl)-*N,N*-dimethylethan-1-amine-1,1-d_2 fumarate (9ii): $LiAlH_4$:$LiAlD_4$ ratio (1:1). Solid; yield 63% (5.7 g, 18.6 mmol); ^{1}H NMR (399.78 MHz, [D_6]DMSO): δ = 10.92 (s, 1H), 7.57 (d, J = 7.3 Hz, 1H), 7.34 (d, J = 8.0 Hz, 1H), 7.19 (d, J = 2.5 Hz, 1H), 7.07 (td, J = 8.0, 0.9 Hz, 1H), 6.99 (td, J = 8.0, 0.9 Hz, 1H), 6.53 (s, 2H) 3.02 (m, 3H), 2.66 (m, 6H). HPLC method A: retention time = 10.60 min; peak area = 99.9%. HPLC-MS analysis: retention time = 10.650 min, m/z = 191.1, peak area = 21.7%; retention time = 10.660 min, m/z = 190.1, peak area = 48.3%; retention time = 10.678 min, m/z = 189.1, peak area = 30.0%.

2-(1*H*-indol-3-yl)-*N,N*-dimethylethan-1-amine-1,1-d_2 fumarate (9iii): $LiAlH_4$:$LiAlD_4$ ratio (1:2). Solid; yield 52% (4.2 g, 13.7 mmol); ^{1}H NMR (399.78 MHz, [D_6]DMSO): δ = 10.89 (br s, 2H), 7.56 (d, J = 7.8Hz, 1H), 7.34 (d, J = 8.2Hz, 1H), 7.18 (d, J = 2.3 Hz, 1H), 7.06 (td, J = 8.0, 0.9 Hz, 1H), 6.99 (td, J = 8.0, 0.9 Hz, 1H), 6.54 (s, 2H) 3.02 (m, 3H), 2.66 (m, 6H). HPLC method A: retention time = 10.58 min; peak area = 99.9%. HPLC-MS analysis: retention time = 10.650 min; m/z = 191.1, peak area = 36.8%; retention time = 10.660 min, m/z = 190.1, peak area = 46.8%; retention time = 10.678 min, m/z = 189.1, peak area = 16.5%.

2-(1*H*-indol-3-yl)-*N,N*-dimethylethan-1-amine-1,1-d_2 fumarate (9iv): $LiAlH_4$:$LiAlD_4$ ratio (1:3). Solid; yield 68% (5.4 g, 17.6 mmol); ^{1}H NMR (399.78 MHz, [D_6]DMSO): δ = 10.91 (br s, 1H), 7.56 (d, J = 7.8Hz, 1H), 7.34 (d, J = 8.2Hz, 1H), 7.19 (d, J = 2.3 Hz, 1H), 7.06 (td, J = 8.0, 0.9 Hz, 1H), 6.99 (td, J = 8.0, 0.9 Hz, 1H), 6.54 (s, 2H) 3.02 (s, 2.7H), 2.66 (s, 6H). HPLC method A: retention time = 10.59 min; peak area = 99.8%. HPLC-MS analysis: retention time = 10.637 min; m/z = 191.1; peak area = 49.2%: retention time = 10.649 min; m/z = 190.1; peak area = 41.5%: retention time = 10.670 min; m/z = 189.1; peak area = 9.3%.

2-(1*H*-indol-3-yl)-*N,N*-dimethylethan-1-amine-1,1-d_2 fumarate (9v): $LiAlH_4$:$LiAlD_4$ ratio (0:1). Solid; yield 52% (4.2 g, 13.7 mmol); ^{1}H NMR (399.78 MHz, [D_6]DMSO): δ = 10.92 (br s, 1H), 7.59 (d, J = 7.8Hz, 1H), 7.35 (d, J = 8.2Hz, 1H), 7.20 (d, J = 2.3 Hz, 1H), 7.08 (td, J = 8.0, 0.9 Hz, 1H), 6.99 (td, J = 8.0, 0.9 Hz, 1H), 6.54 (s, 2H) 3.02 (s, 3.6H), 2.66 (s, 6H). HPLC method A: retention time = 10.59 min; peak area = 99.8%. HPLC-MS analysis: retention time = 10.644 min; m/z = 191.1; peak area = 11.2%: retention time = 10.651 min; m/z = 190.1; peak area = 41.3%: retention time = 10.664 min; m/z = 189.1; peak area = 47.5%.

2-(1*H*-indol-3-yl)-*N,N*-dimethylethan-1-amine-1,1-d_2 fumarate (9vi): $LiAlH_4$:$LiAlD_4$ ratio (0:1). Solid; yield 62% (5.0 g, 16.3 mmol); ^{1}H NMR (399.78 MHz, [D_6]DMSO): δ = 10.94 (br s, 1H), 7.59 (d, *J* = 7.8Hz, 1H), 7.35 (d, *J* = 8.2Hz, 1H), 7.20 (d, *J* = 2.3 Hz, 1H), 7.08 (td, *J* = 8.0, 0.9 Hz, 1H), 6.99 (td, *J* = 8.0, 0.9 Hz, 1H), 6.54 (s, 2H) 3.02 (m, 3.5H), 2.66 (s, 6H). HPLC method A: retention time = 10.58 min; peak area = 99.4%. HPLC-MS analysis: retention time = 10.633 min, m/z = 191.1, peak area = 7.4%; retention time = 10.640 min, m/z = 190.1, peak area = 35.3%; retention time = 10.654 min, m/z = 189.1, peak area = 57.4%.

2-(1H-Indol-3-yl)-*N,N*-bis(methyl-d_3)acetamide (10): EDC.HCl (15.7 g, 81.90 mmol) was added to 3-indoleacetic acid **4** (12.0 g, 68.50 mmol) and HOBt.H_2O (1.16 g, 75.75 mmol) in DCM (108 mL) at room temperature. The reaction was stirred for 1 hour after which DIPEA (35.6 mL, 205.75 mmol) and D_6-dimethylamine HCl (9.0 g, 102.76 mmol) were added (temperature maintained below 30°C). The reaction was stirred for 1 hour at room temperature after which analysis by HPLC indicated 65.6% of **10** with 28.9% of **4** remaining. DIPEA (11.9 mL, 68.78 mmol) was added and the reaction was stirred for 1 hour at room temperature. HPLC indicated no change in conversion. Aqueous potassium carbonate (6.0 g in 54 mL water) was added and the phases were separated. The aqueous phase was extracted with DCM (2 x 30 mL). The combined organics were washed with brine (2 x 30 mL) then aqueous citric acid (20 w/w%, 50 mL), dried over $MgSO_4$ and filtered. The filtrate was stripped and the resulting solids were slurried in TBME (120 mL) and isolated by filtration. Purification by flash column chromatography yielded the title compound**.** Solid; yield 58% (8.34 g, 40.4 mmol).^{1}H NMR (399.78 MHz, [D_1]$CHCl_3$): δ = 8.53 (br s, 1H), 7.65 (d, *J = 8.0 Hz*, 1H), 7.34 (d*, J = 8.0 Hz*, 1H), 7.19 (dt, *J = 1.2, 7.6 Hz*, 1H), 7.13 (dt, *J = 1.2, 8.0 Hz* 1H), 7.02 (d, *J = 2.4 Hz*, 1H), 3.83 (s, 2H). HPLC method B: retention time = 7.04 min; peak area =98.4%.

2-(1H-indol-3-yl)-*N,N*-bis(methyl-d3)ethan-1-amine (11): $LiAlH_4$ (1 M in THF, 17.3 mL, 17.28 mmol) was added to a suspension of **9** (4.0 g, 19.20 mmol) in THF (10 mL) at <30°C. The resulting reaction was heated to 60–65°C and stirred for 2 hours. HPLC analysis indicated complete consumption of **9**. The reaction was cooled to room temperature and quenched into aqueous Rochelle's salts (10 g in 30 mL water) at <30°C. After stirring for 1 hour, the phases were separated. The aqueous was extracted with THF (20 mL). The combined organics were washed with brine (20 mL), dried over $MgSO_4$, filtered and stripped (azeotroped with ethanol, 20 mL) to afford **10**. ^{1}H NMR confirmed the identity of the title compound **11** and indicated 8.5% ethanol was present (no THF). Oil; yield 97% (3.63 g, 18.7 mmol). ^{1}H NMR (399.79 MHz, $[D_1]CHCl_3$): δ = 8.08 (br s, 1H), 7.63 (d, *J = 8.0 Hz*, 1H), 7.36 (d, *J = 8.0 Hz*, 1H), 7.20 (dt, *J = 1.2, 8.0 Hz*, 1H), 7.13 (t, *J = 7.6 Hz*, 1H), 7.03 (d, *J = 1.6 Hz*, 1H), 2.97 (t, *J = 8.4 Hz*, 2H), 2.66 (t, *J = 8.0 Hz*, 2H). HPLC method B: retention time = 2.55 min; peak area = 99.86%.

2-(1H-indol-3-yl)-*N,N*-bis(methyl-d3)ethan-1-amine fumarate (12): 11 (3.6 g active, 18.53 mmol) was dissolved in ethanol (43 mL) at room temperature. Fumaric acid (2.15 g, 18.53 mmol) was added and the solution was heated to 75°C (solids crystallized during heating and did not re-dissolve). The resulting suspension was cooled to 0–5°C and stirred for 1 hour. The solids were isolated by filtration, washed with ethanol (2 x 7 mL) and pulled dry. Further drying in a vacuum oven at 50°C yielded the desired title compound **12**. Solid; yield 87% (4.98 g, 16.6 mmol). ^{1}H NMR (400 MHz, $[D_6]$DMSO): δ = 10.93 (br s, 1H), 7.58 (d, *J = 7.6 Hz*, 1H), 7.35 (d, *J = 8..4 Hz*, 1H), 7.19 (d, *J = 1.6 Hz*, 1H), 7.08 (dt, *J = 1.2, 8.0 Hz*, 1H), 6.99 (dt, *J = 1.2, 8.0 Hz*, 1H), 6.53 (s, 2H), 3.03 (m, 4H). HPLC method A: retention time = 10.29 min; peak area = 99.9%. HPLC-MS analysis: retention time = 10.307 min, m/z = 195.2, peak area = 98.9%; retention time = 10.316 min, m/z = 194.2, peak area = 1.0%; retention time = 10.322 min, m/z = 193.2, peak area = <0.01%.

2-(1H-Indol-3-yl)-*N*,*N*-bis(methyl-d_3)ethan-1-amine-1,1-d_2 (13): $LiAlD_4$ (1 M in THF, 17.3 mL, 17.28 mmol) was added to a suspension of **10** (4.0 g, 19.20 mmol) in THF (10 mL) at <30°C. The resulting reaction was heated to 60–65°C and stirred for 2 hours. HPLC analysis indicated complete consumption of **9**. The reaction was cooled to room temperature and quenched into aqueous Rochelle's salts (10 g in 30 mL water) at <30°C. After stirring for 1 hour, the phases were separated. The aqueous was extracted with THF (20 mL). The combined organics were washed with brine (20 mL), dried over $MgSO_4$, filtered and stripped (azeotroped with ethanol, 20 mL) to give the title compound **13**. Amber oil (4.01 g). ^{1}H NMR confirmed the identity of the product and indicated 8.6% ethanol was present (no THF). ^{1}H NMR (399.78 MHz, [D_1]$CHCl_3$): δ = 8.12 (br s, 1H), 7.63 (d, *J = 8.0 Hz*, 1H), 7.36 (d, *J = 8.0 Hz*, 1H), 7.20 (dt, *J = 1.2, 8.0 Hz*, 1H), 7.13 (dt, *J = 1.2, 8.0 Hz*, 1H), 7.02 (d, *J = 1.6 Hz*, 1H), 2.95 (s, 2H). HPLC method B: retention time = 2.55 min; peak area = 91.4%.

2-(1H-Indol-3-yl)-*N*,*N*-bis(methyl-d_3)ethan-1-amine-1,1-d_2 fumarate (14): 13 (3.6 g active, 18.53 mmol) was dissolved in ethanol (43 mL) at room temperature. Fumaric acid (2.15 g, 18.53 mmol) was added and the solution was heated to 75°C (solids crystallized during heating and did not re-dissolve). The resulting suspension was cooled to 0–5°C and stirred for 1 hour. The solids were isolated by filtration, washed with ethanol (2 x 7 mL) and pulled dry. Further drying in a vacuum oven at 50°C yielded the title compound **14**. Solid: 81%, (4.62 g, 14.8 mmol). ^{1}H NMR (399.78 MHz, [D_6]DMSO): *δ* =^{1}H NMR (400 MHz, [D_6]DMSO): δ = 10.93 (br s, 1H), 7.58 (d, *J = 7.6 Hz*, 1H), 7.35 (d, *J = 8.0 Hz*, 1H), 7.19 (d, *J = 2.4 Hz*, 1H), 7.08 (dt, *J = 1.2, 8.0 Hz*, 1H), 6.99 (dt, *J = 1.2, 8.0 Hz*, 1H), 6.53 (s, 2H), 3.00 (s, 2H). HPLC method A: retention time = 10.29 min; peak area = 99.9%. HPLC-MS analysis: retention time = 10.302 min, m/z = 197.2, peak area = 96.6%; retention time = 10.317 min, m/z = 196.2, peak area = 3.3%; retention time = 10.342 min, m/z = 195.2, peak area = 0.1%.

S2. *In Vitro* Studies, Experimental Information

In vitro studies investigating metabolic stability and physicochemical properties were performed by Sygnature Discovery Ltd (Nottingham, UK). Receptor affinities were determined at Eurofins Panlabs Discovery Services, Taiwan, Ltd. (Taipei, Taiwan).

In vitro intrinsic clearance in whole hepatocytes and mitochondrial fractions of hepatocytes were used to screen all 8 deuterated DMT molecules. Human hepatocytes from 10 donors (male and female) were thawed in a water bath at 37°C and decanted into hepatocyte buffer solution (26.2 mM $NaHCO_3$, 9 mM HEPES, 2.2 mM D-fructose, Dulbecco's Modified Eagle Medium, in MilliQ purified water), which was centrifuged, the supernatant removed and resuspended in hepatocyte buffer solution at the final assay concentration (nominal 0.5 million cells/mL). **7** and deuterated DMT compounds, together with positive control stocks (diltiazem [CYP3A4 metabolism] and diclofenac [CYP2C9, CYP3A4 and glucuronidation metabolism]), were prepared at 10 mM in DMSO and diluted to 100 x the assay concentration in 9:1 acetonitrile:DMSO.

Hepatocytes were added to pre-warmed incubation tubes (37°C) together with compound stock solution (at 3.1 μM freebase; 5 μM fumarate), 0.9% (v/v) acetonitrile and 0.1% (v/v) DMSO to achieve a final count of 0.362 million viable cells/mL, shaken orbitally though the experiment. Aliquots were taken from the incubation tubes at 4, 8, 15, 30, 45, and 60 min and quenched 1:4 with ice-cold acidified methanol or acetonitrile-containing internal standard, before protein precipitation at –12 °C for a minimum of 12 hours before centrifugation at 4°C. Supernatants were transferred to 96-well plates for ultra-high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis.

MS peak areas were used to generate ln (MS response) vs time plots. Subsequent intrinsic clearance (μL/min/million cells) and half-life ($t_{1/2,}$ min) values were calculated using the following equations:

$$Half\ Life = \frac{\ln(2)}{k}$$

$$Intrinsic\ clearance = \frac{-1000k}{cell\ density(\frac{millions}{mL})}$$

$$k = slope\ of \ln(MS\ peak\ response\ or\ area) vs.\ plot$$

Human liver mitochondrial fractions (XenoTech, Sekisui, mixed gender, 5-donor pool) were assayed using a similar methodology to the human hepatocyte experiments, with a final substrate concentration of 0.6 μM (1 μM fumarate) and liver mitochondrial concentration of 0.5 mg/mL.

Statistical analysis was performed using IBM SPSS Statistics v28.0. A simple linear regression was run to assess the effect of deuteration on half-life and clearance. Half-life and clearance in **9i** and **7** were compared using an independent two-sample t-test. Statistical significance was assumed at the 5% level.

Blood/plasma ratio was determined using fresh human blood and plasma samples from a donor panel aliquoted and pre-incubated at 37 °C for 5 min. **9i** (0.6 μM freebase; 1 μM fumarate), **7** (0.6 μM freebase; 1 μM fumarate), diclofenac (positive control, 5 μM) and verapamil (positive control, 5 μM) were prepared at 2 mM in DMSO, while chloroquine (positive control, 5 μM) was prepared at 2 mM in water, before all were further diluted to 100 x the final assay concentration in methanol.

Stocks of **9i, 7** and controls were separately spiked 1:100 into the blood and plasma samples, which were incubated at 37 °C for 30 min, with shaking for 15 sec at 15 and 30 min. After 30 min plasma samples were quenched 1:3 into ice-cold methanol-containing internal standard; blood samples were centrifuged, an aliquot of the plasma was taken and supernatant quenched 1:3 into ice-cold methanol-containing internal standard, before protein precipitation between the matrix-matched plasma samples at –12 °C for a minimum of 12 hours before centrifugation at 4 °C. Supernatants were transferred to 96-well plates for LC-MS/MS analysis.

Plasma protein binding was assayed using thawed pooled donor plasma, which was centrifuged, and the resulting supernatant decanted into a fresh vessel, with pH adjusted to 7.4 using lactic acid or NaOH. Stocks of **9i** and **7** were prepared at 10 mM in DMSO and further diluted to 100 x the assay concentration, which were then spiked 1:100 into the plasma samples to achieve a final concentration of 3.2 μM (5 μM fumarate), except for **7** and **9i** which were prepared at 1 μM (1.6 μM fumarate) due to a technical error.

For each sample, 200 μL of the spiked plasma was added to the red compartment of a Rapid Equilibrium Dialysis (RED) plate (Thermo Fisher Scientific, UK) with 350 μL phosphate-buffered saline (PBS) added to the buffer compartment before sealing the RED plate with a breathable

lid and shaking on an orbital shaker in a 37 °C incubator at 5% CO_2 for 4 hours. A sample of the plasma/drug was taken and mixed with an equal volume of PBS, and a sample was taken from the buffer side and mixed with an equal volume of blank plasma before quenching 1:3 with ice-cold acetonitrile-containing internal standard. The matrix-matched quenched samples were mixed before protein precipitation at –12 °C for a minimum of 12 hours before centrifugation at 4 °C.

Supernatants were transferred to 96-well plates for LC-MS/MS analysis; values were derived for fraction of unbound and bound drug, together with recovery, following the preparation of matrix-matched standard curve and quality control samples to allow for quantification of the concentration in each sample.

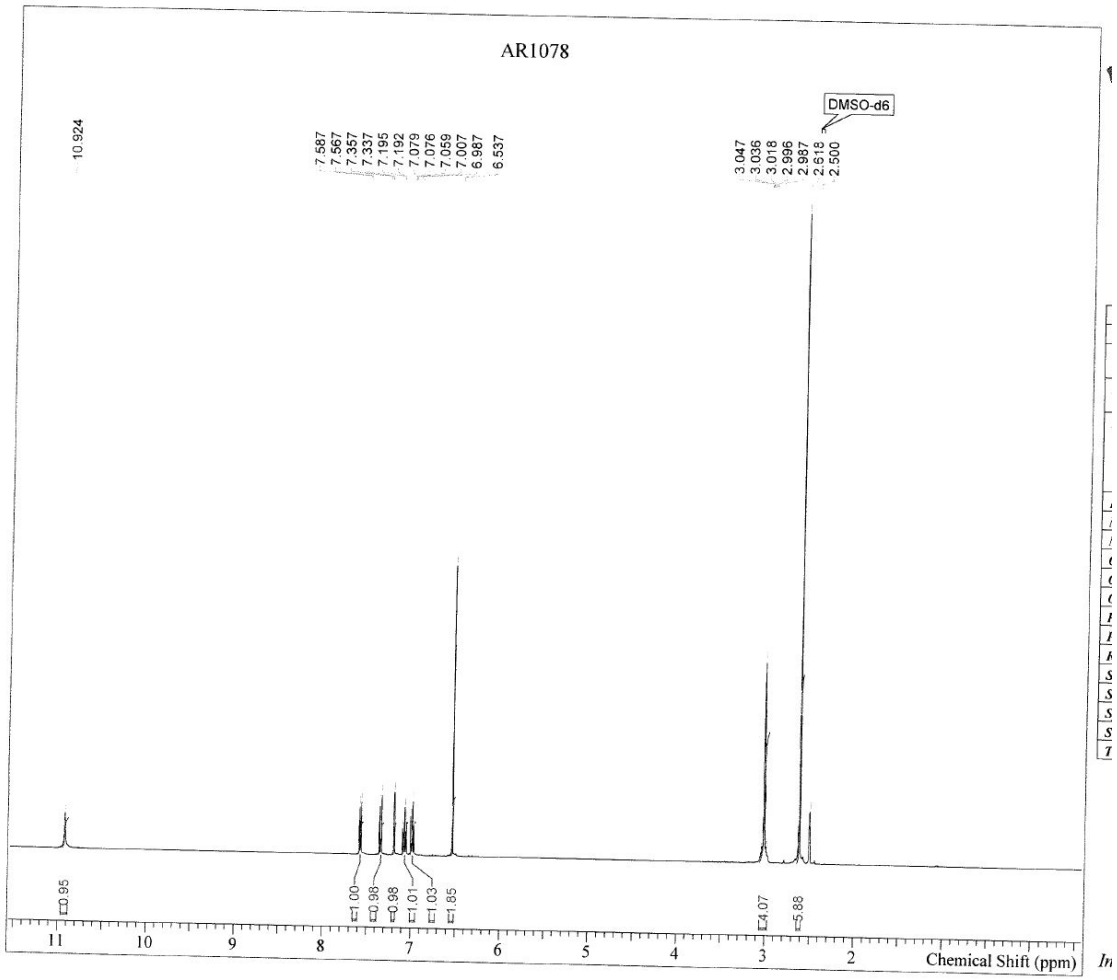
The distribution coefficient log $D_{7.4}$ measures the partition of substances in octanol and aqueous solutions in a solution at pH 7.4, i.e., approximating that of blood. A 10 mM stock of **9i** and **7** diluted in DMSO were prepared together with 10 mM control markers (ketoconazole, propranolol and verapamil). These were added to incubation tubes together with octanol before shaking for 5 min; buffer was added, and the tubes were shaken for a further 90 min at room temperature. The tubes were then centrifuged at room temperature and samples taken from both octanol and buffer fractions, which were diluted to give similar responses on LC-MS/MS before being transferred to 96-well plates with methanol-containing internal standard prior to LC-MS/MS analysis.

Competitive binding of **9i** and **7** against specific radiolabelled probes was assessed at a fixed concentration of 6 μM (10 μM fumarate) using a standard panel of receptors, transporters, ion channels and enzymes, including those of particular interest (details in Tables S2 and S3). The

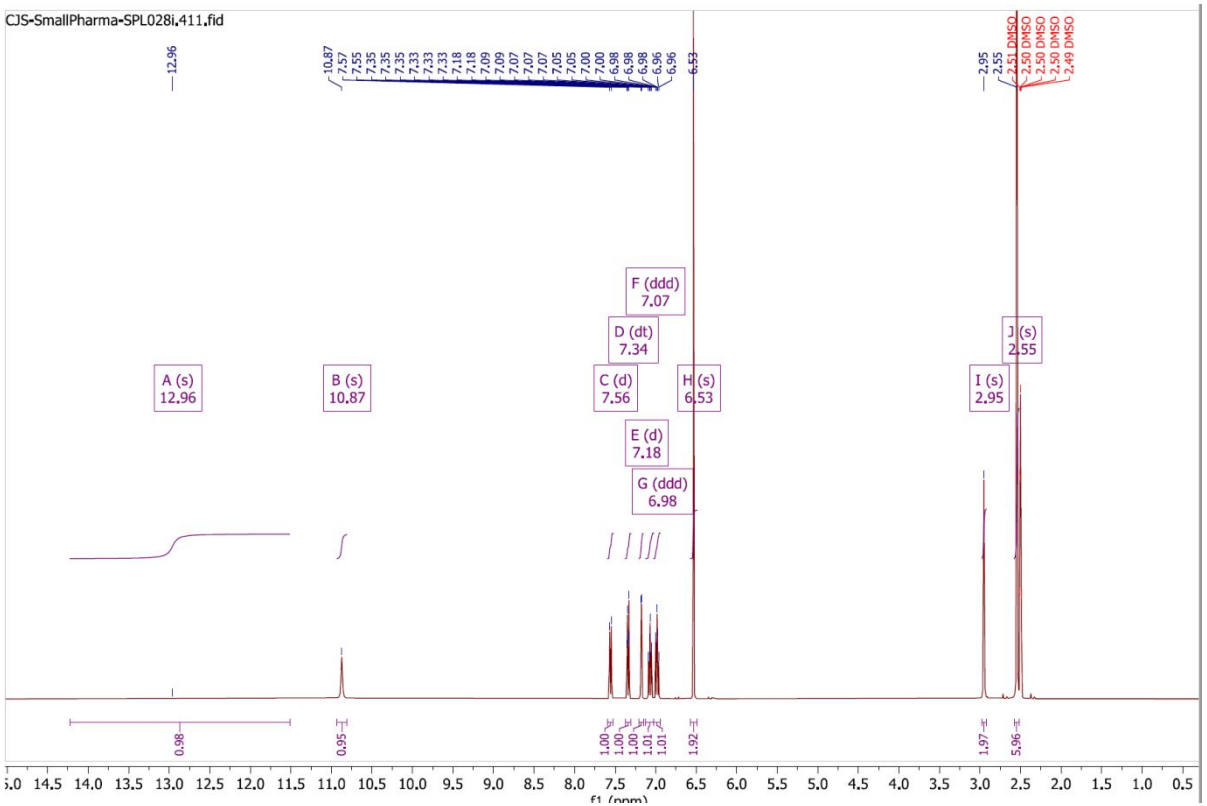
receptor/ion channel screening panel consisted of 92 receptors, including the Safety Screen 87 Panel, 5-HT_7, 5-HT_6, 5-HT_{5A}, α_{2C} and σ_1 receptors. The 5-HT_{2D}, $mGluR_2$ and imidazoline I_2 receptor were not tested as they were not available at the time of assay. *In vitro* inhibition of several enzymes by **9i** and **7** was assessed, including MAO-A and MAO-B, sodium/potassium ATPase, acetylcholinesterase, cyclo-oxygenase (COX-1 and COX-2), angiotensin converting enzyme (ACE), cathepsin G, phosphodiesterases (3A and 4D2), serine/threonine protein kinase PKCα, insulin receptor protein kinase and lymphocyte-specific protein tyrosine kinase (Supplementary Table 2). Effects of **9i** and **7** on enzyme activity were measured using spectrophotometric quantitation.

IC_{50}/K_i values were subsequently determined for **9i** and **7** only where radioligand binding was inhibited by >50% at a concentration of 10 μM. Freebase concentrations of **9i** and **7** used for IC_{50}/K_i determination were 0.003, 0.01, 0.03, 0.1, 0.3, 1, 3, and 10 μM. Mean values were calculated from two separate assays. IC_{50} and Hill coefficient (nH) values were determined by a non-linear, least squares regression analysis using MathIQ™ (ID Business Solutions Ltd., UK). Ki values were calculated by applying the Cheng and Prusoff equation[1] of the observed IC_{50} for the test compound, the assay radioligand concentration and historical K_D values of the ligand (obtained experimentally at Eurofins Panlabs, Inc.). nH defines the slope of the competitive binding curve where values significantly different than 1.0, may suggest that the binding displacement does not follow the laws of mass action with a single binding site.

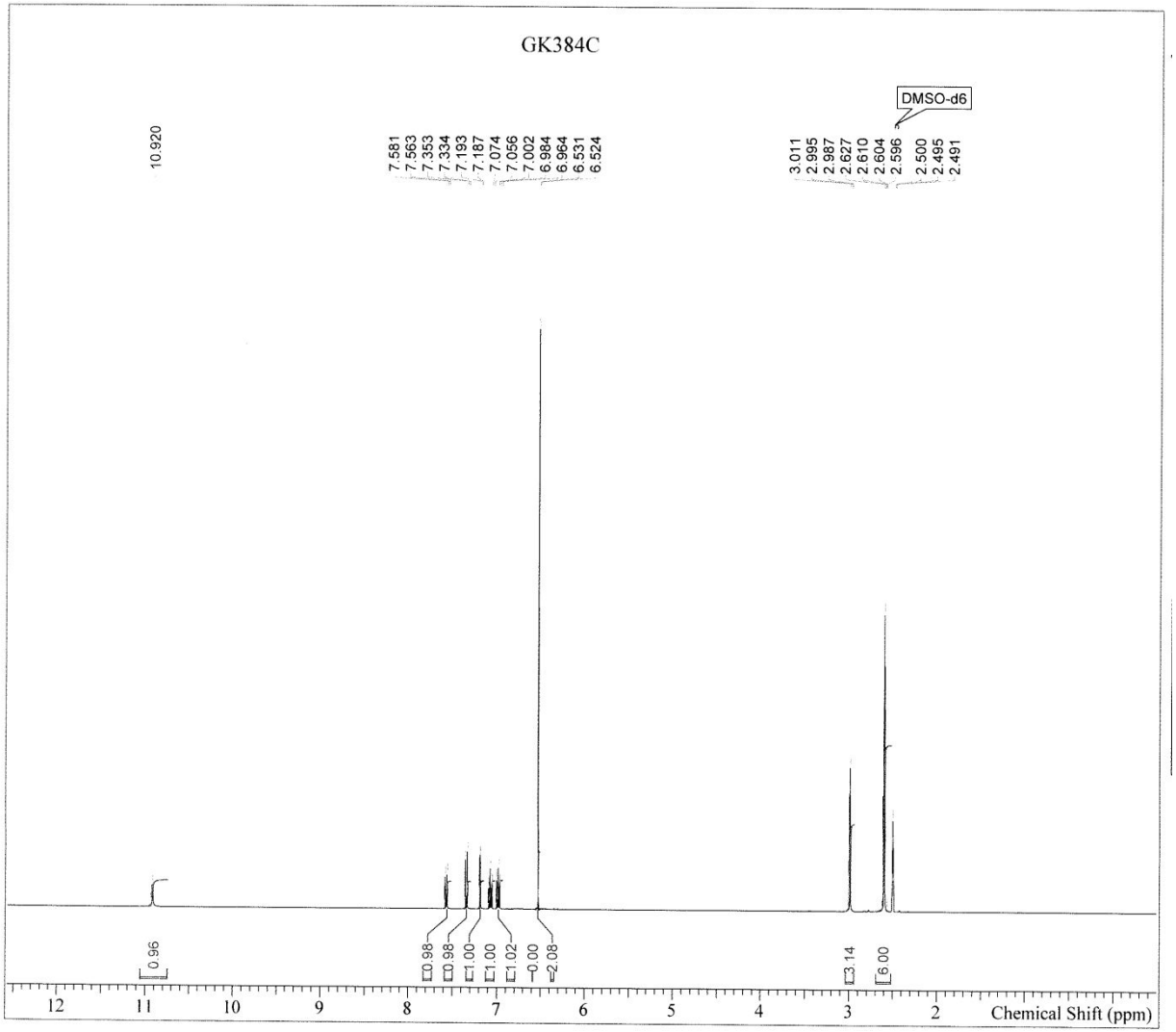
S3. [1]H-NMR data for test compounds 7, 9i-9vi, 12 and 14.



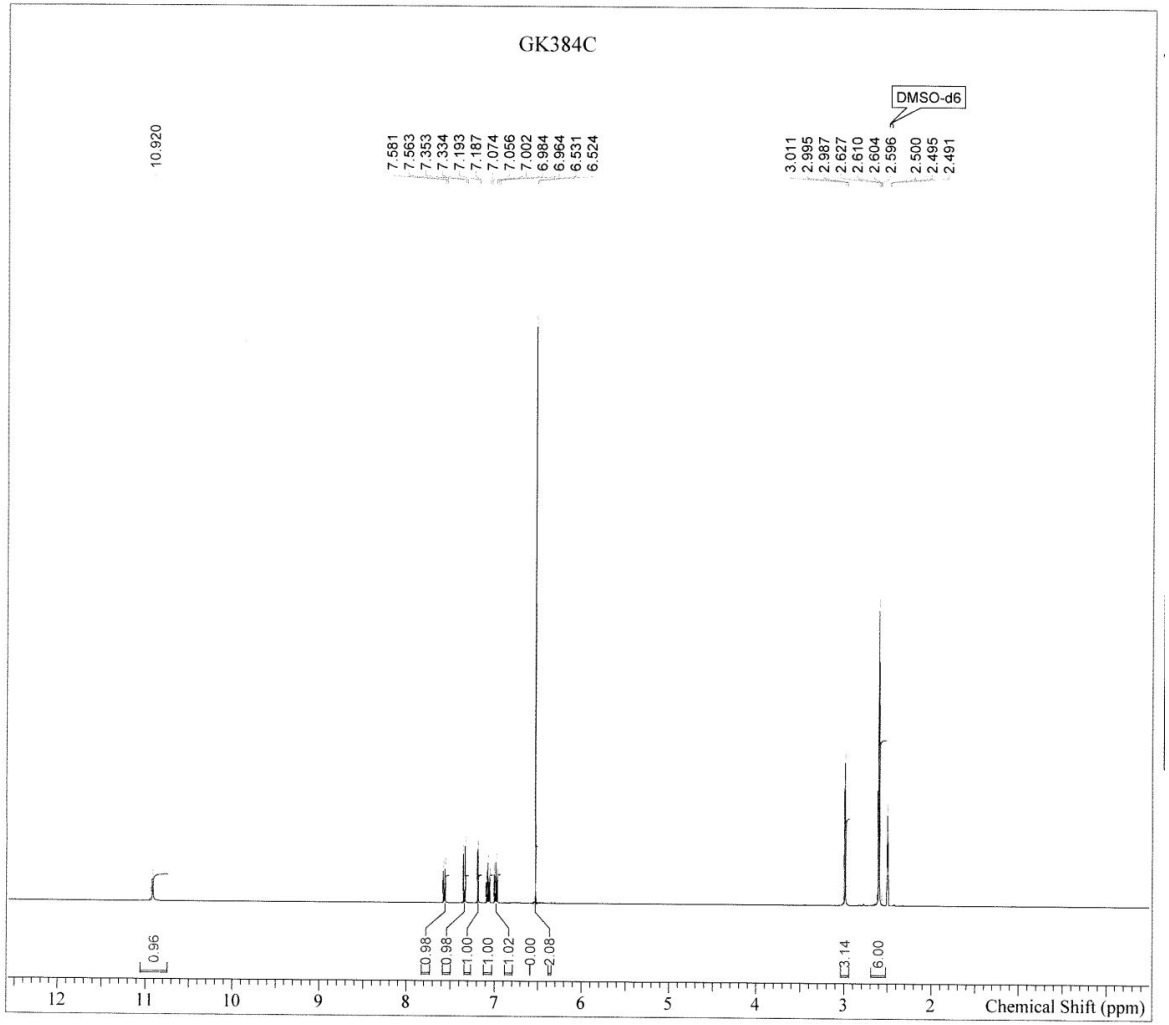
Compound example **7**



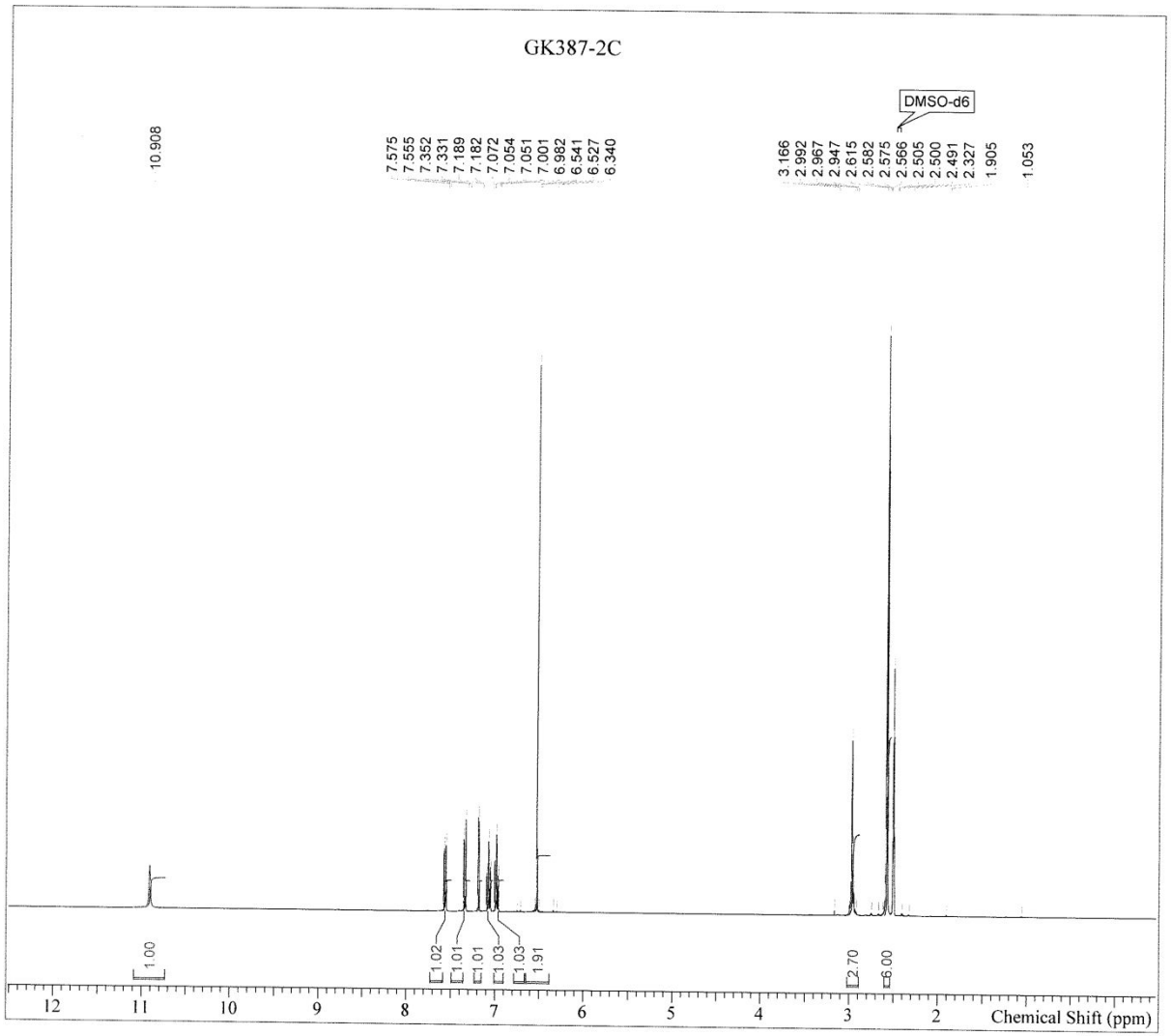
Compound example **9i**



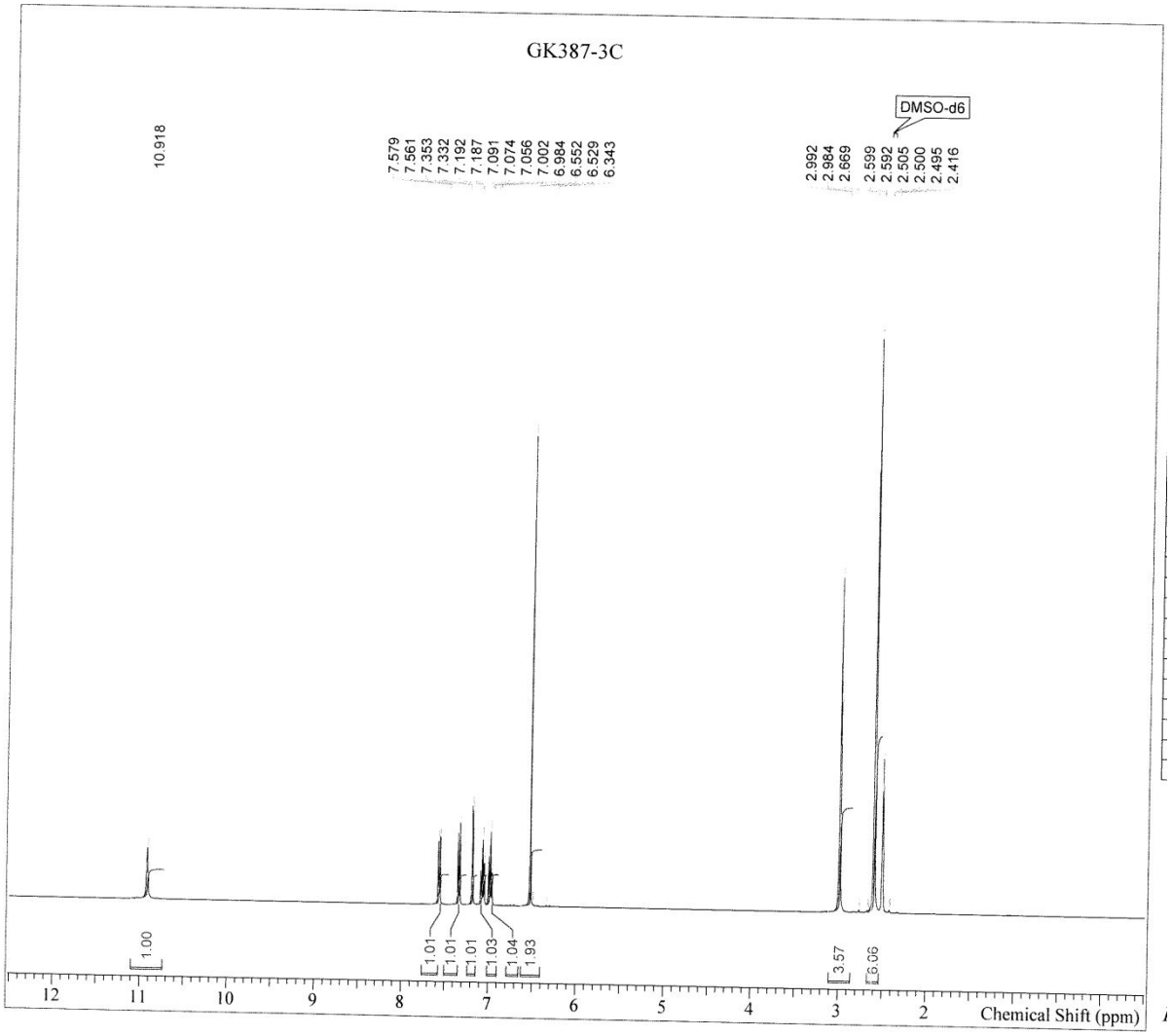
Compound example **9ii**



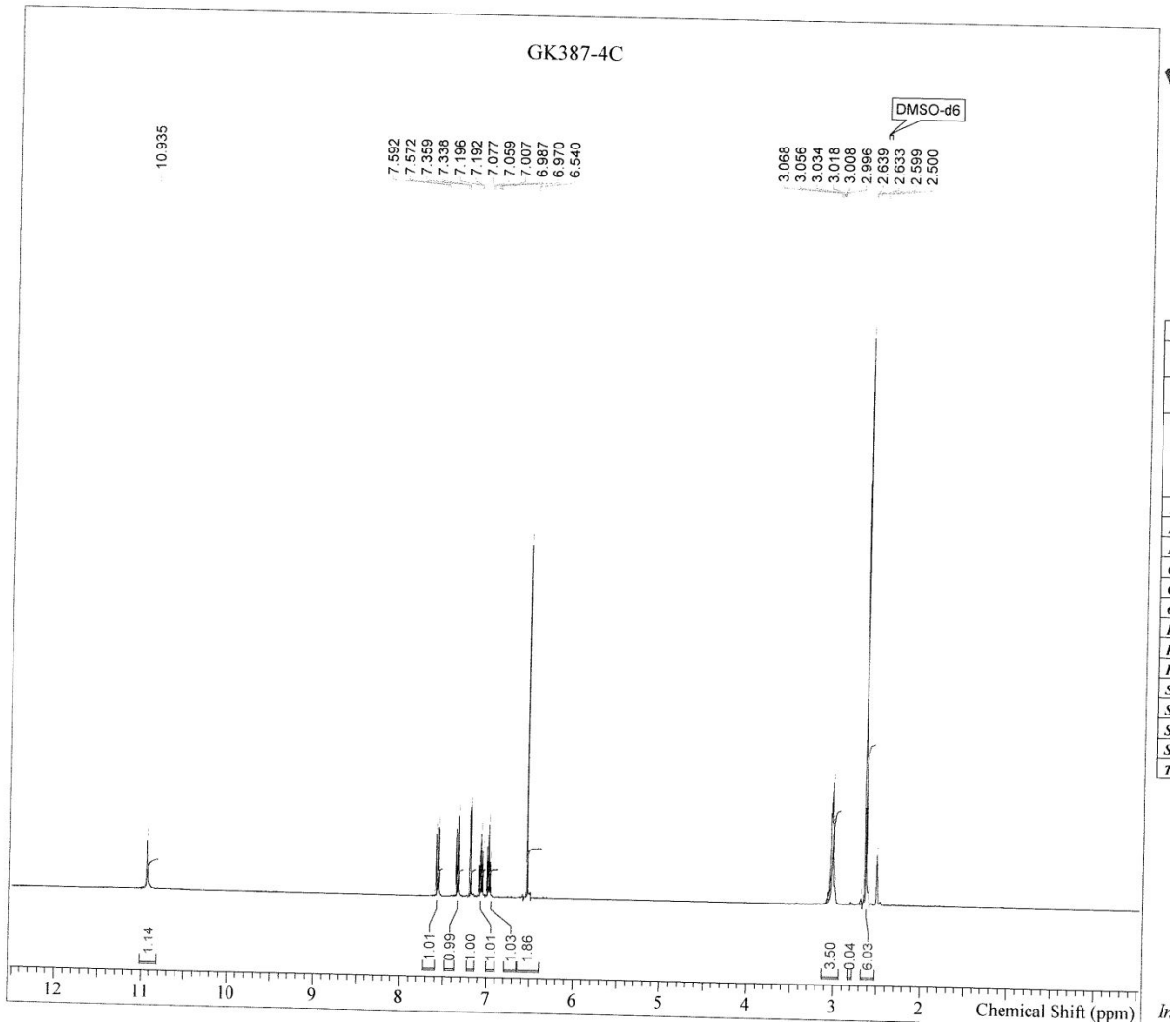
Compound example **9iii**



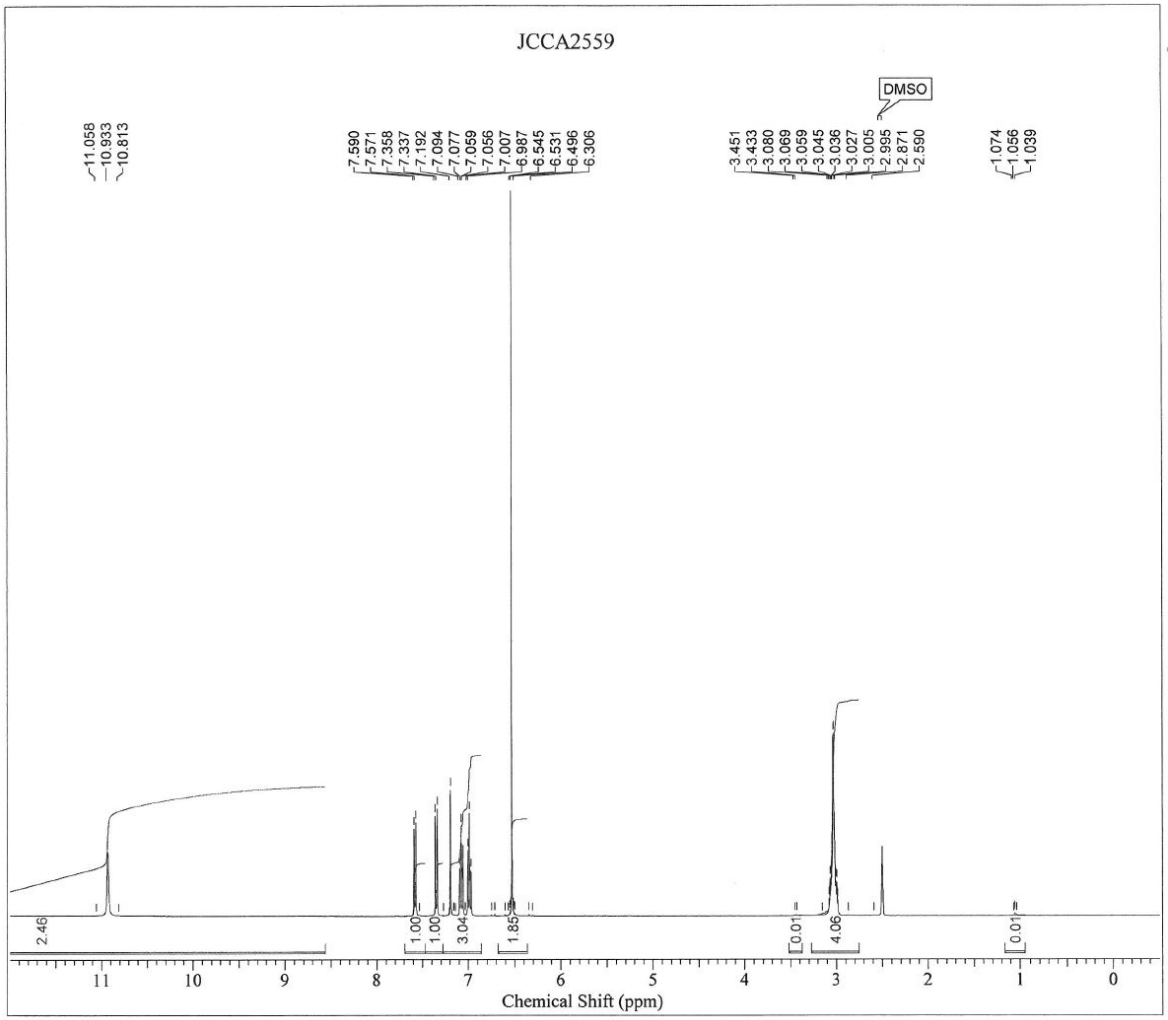
Compound example **9iv**



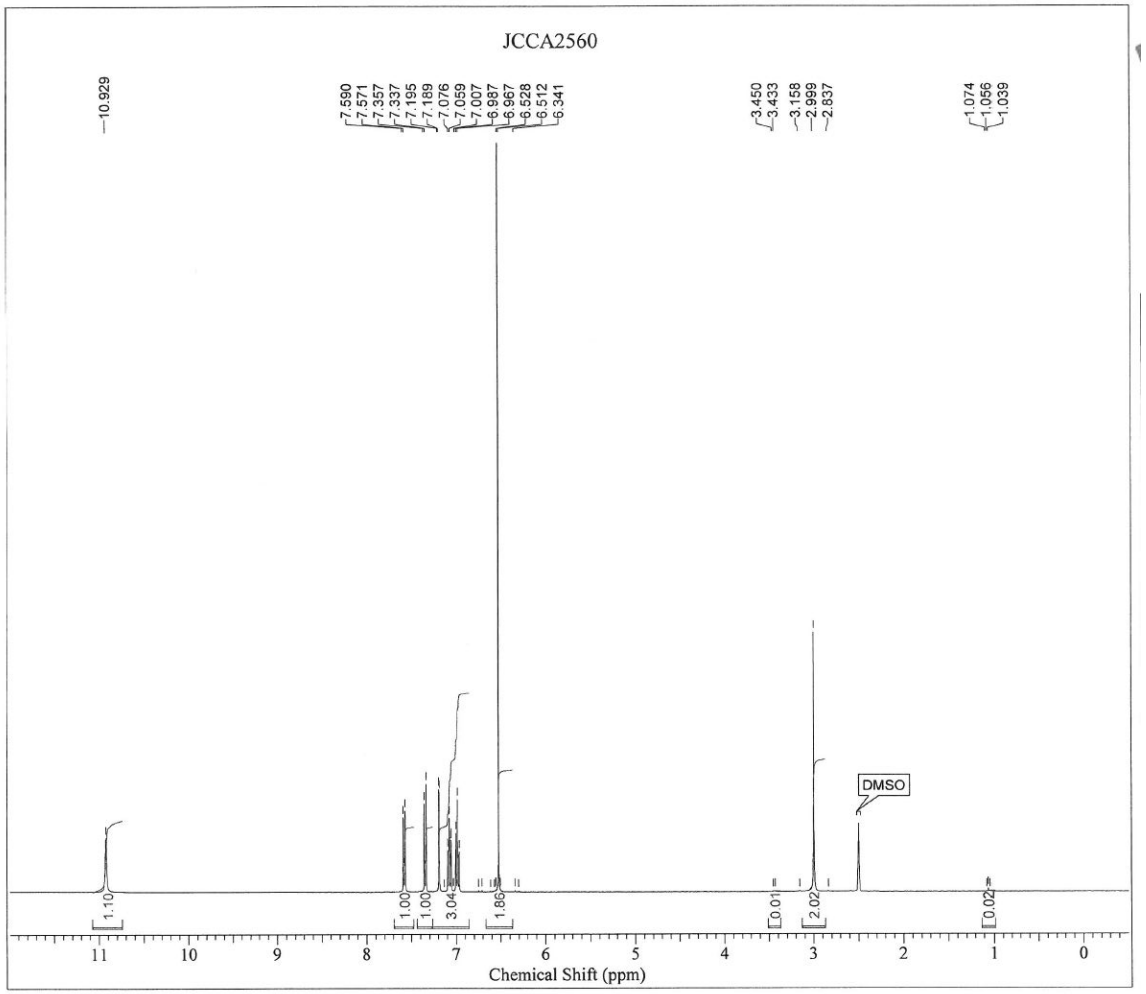
Compound example **9v**



Compound example **9vi**

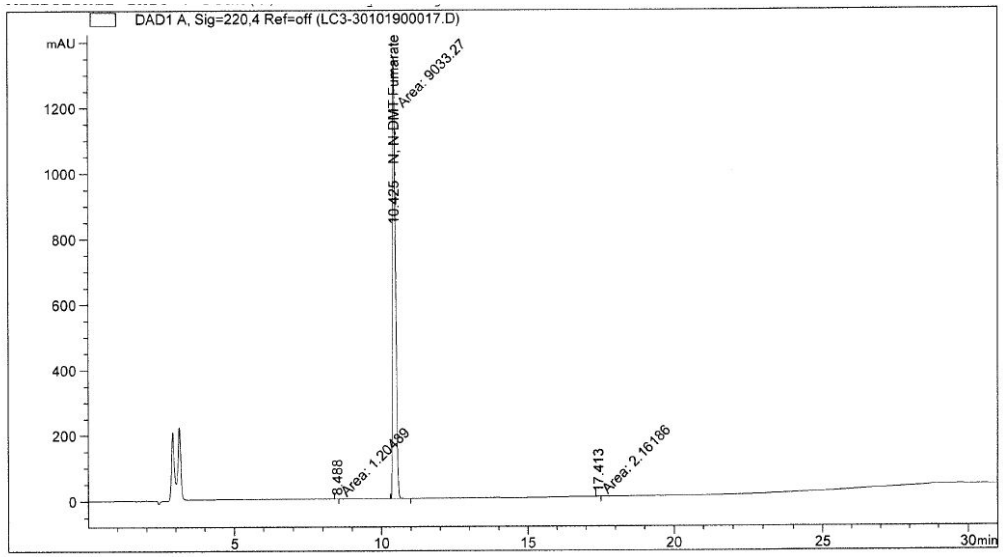


Compound example **12**



Compound example **14**

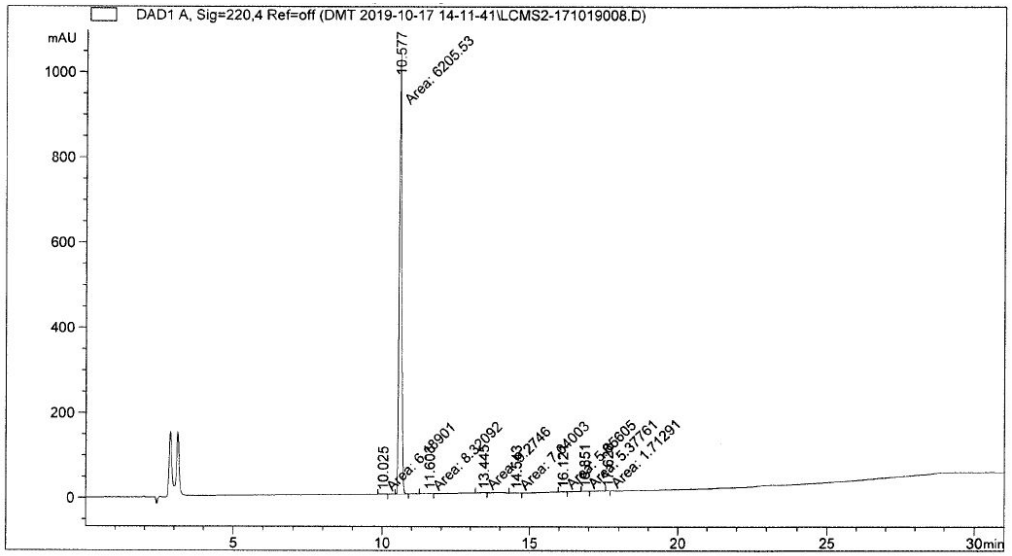
S4. HPLC data for test compounds 7, 9i-9vi, 12 and 14.



Signal 1: DAD1 A, Sig=220,4 Ref=off

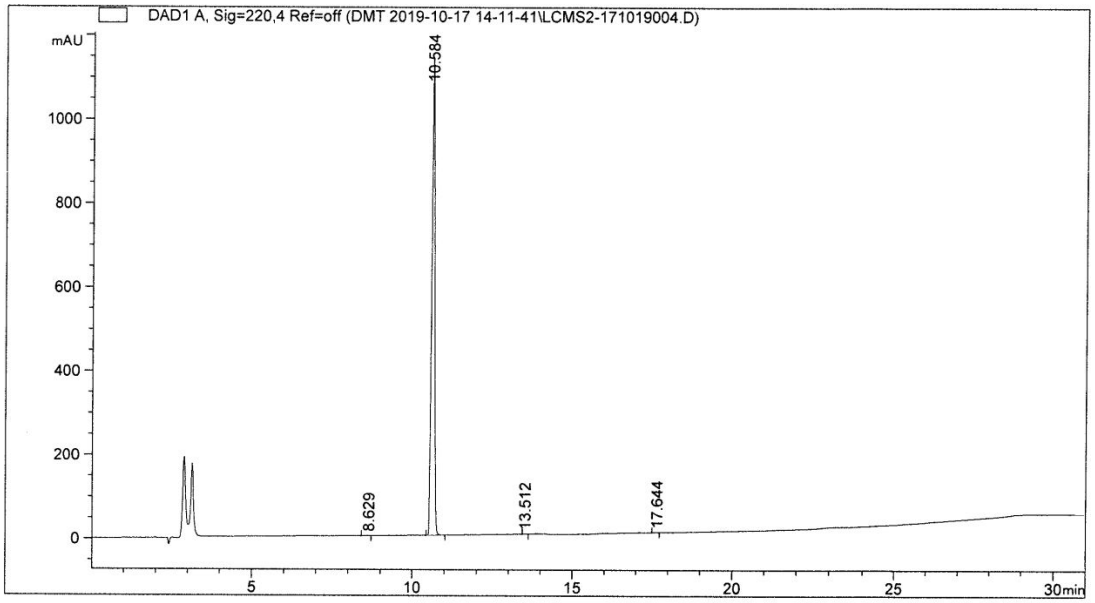
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.741	MM	0.0742	3.88420	8.72299e-1	0.0482
2	10.291	MM	0.1138	8049.75830	1178.90552	99.8572
3	11.460	MM	0.0933	1.67731	2.99644e-1	0.0208
4	12.309	MM	0.2037	5.94807	4.86751e-1	0.0738
Totals :				8061.26788	1180.56421	

Compound example **7**



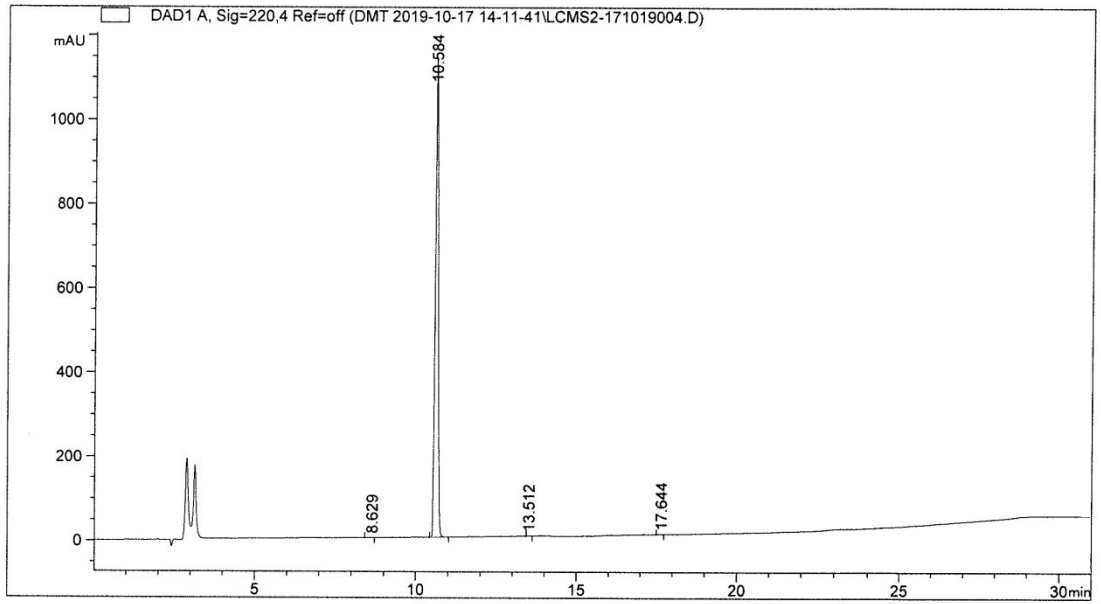

```
=====================================================================
                    Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=220,4 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  10.025 MM    0.1932    6.18901 5.34007e-1   0.0990
   2  10.577 MM    0.0997 6205.53418 1037.36975  99.3028
   3  11.603 MM    0.3375    8.32092 4.10872e-1   0.1332
```

Compound example **9i** (SPL028i GSK387-5C)



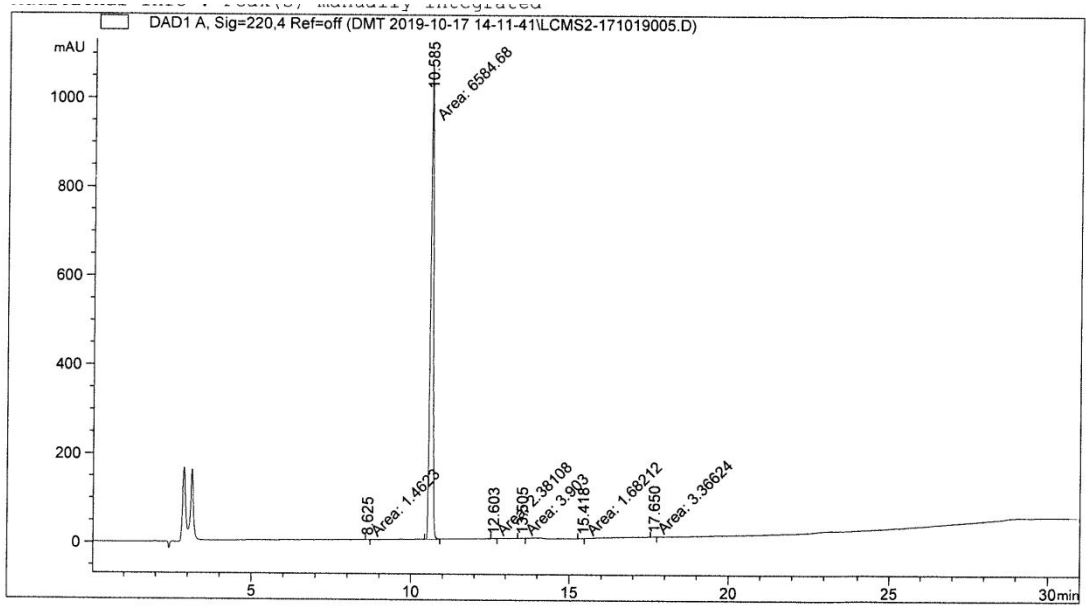

```
=====================================================================
                        Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=220,4 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   8.629 MM    0.1301    2.96942 3.80264e-1   0.0406
   2  10.584 MM    0.1066 7295.20459 1140.14697  99.8607
   3  13.512 MM    0.1110    2.72276 4.08686e-1   0.0373
```

Compound example **9ii** (SPL028ii GK384C)



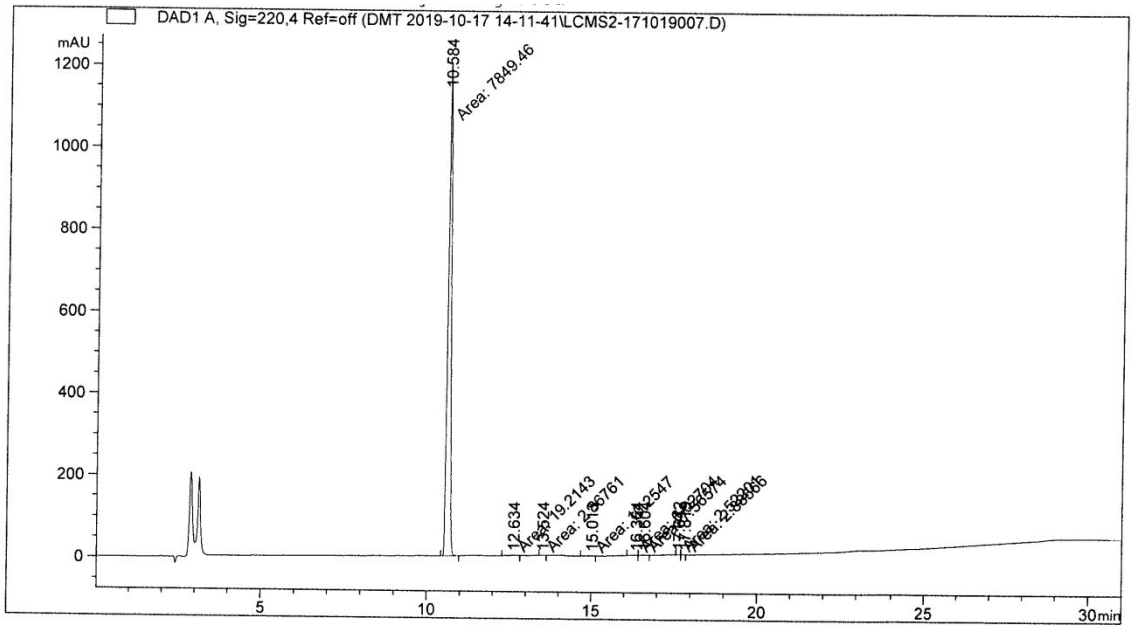

```
=====================================================================
                        Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=220,4 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   8.629 MM    0.1301    2.96942 3.80264e-1   0.0406
   2  10.584 MM    0.1066 7295.20459 1140.14697  99.8607
   3  13.512 MM    0.1110    2.72276 4.08686e-1   0.0373
```

Compound example **9iii** (SPL028iii GK387-1C)



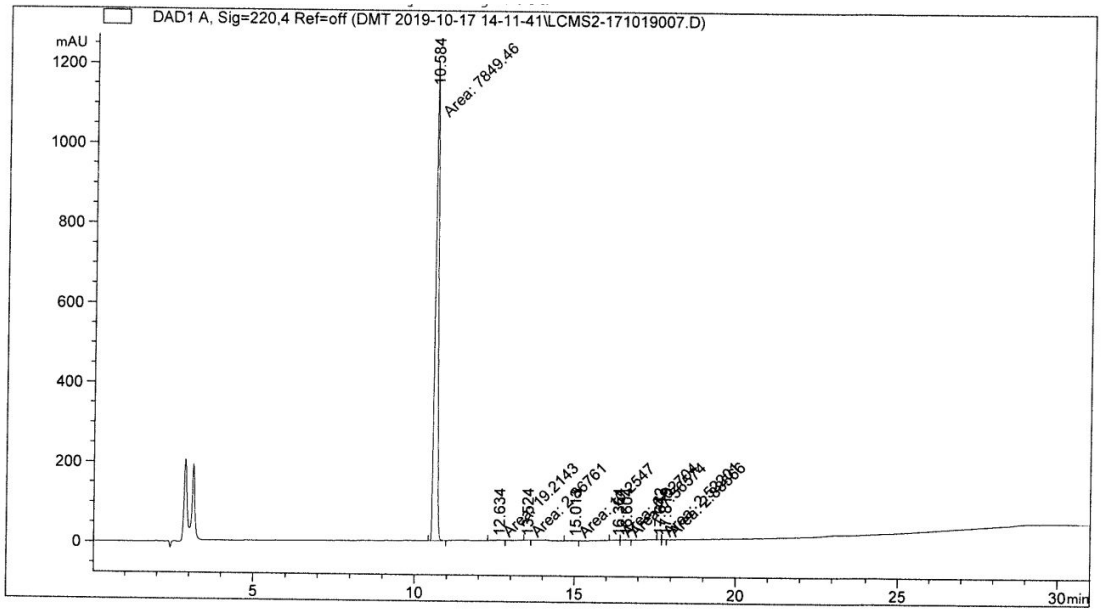

```
=====================================================================
                    Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=220,4 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   8.625 MM    0.0761    1.46230 3.20251e-1   0.0222
   2  10.585 MM    0.1025 6584.67969 1070.60669  99.8061
   3  12.603 MM    0.1202    2.38108 3.30208e-1   0.0361
```

Compound example **9iv** (SPL028iv GK387-2C)




```
=====================================================================
                        Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=220,4 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  10.584 MM    0.1084 7849.46289 1206.84558  99.3502
   2  12.634 MM    0.2874   19.21431    1.11412   0.2432
   3  13.524 MM    0.0899    2.86761 5.31860e-1   0.0363
```

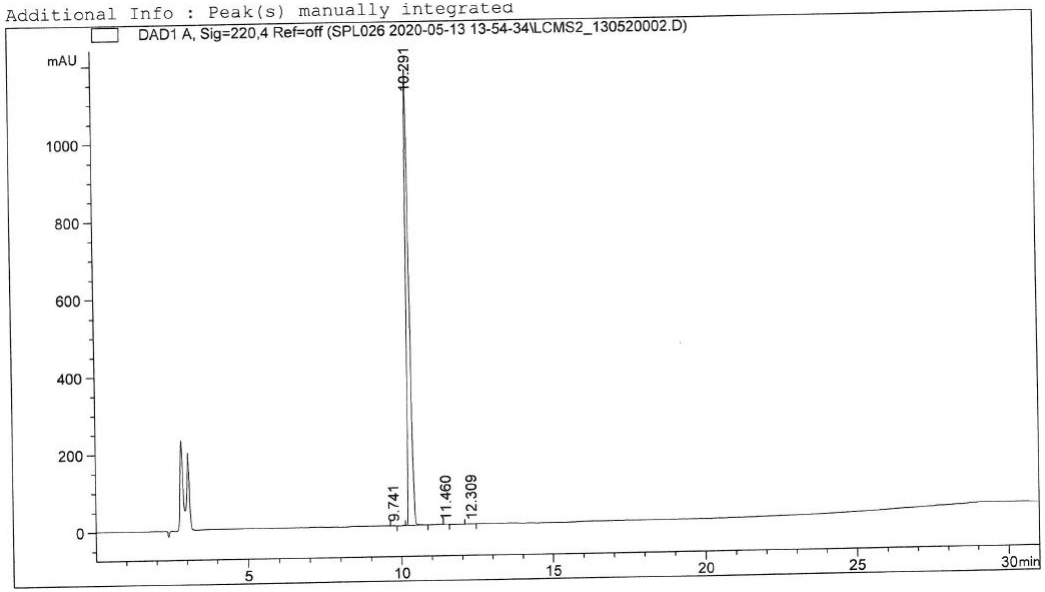
Compound example **9v** (SPL028v GK387-3C)




```
=====================================================================
                        Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=220,4 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  10.584 MM    0.1084 7849.46289 1206.84558  99.3502
   2  12.634 MM    0.2874   19.21431    1.11412   0.2432
   3  13.524 MM    0.0899    2.86761 5.31860e-1   0.0363
```

Compound example **9vi** (SPL028vi GK387-4C)

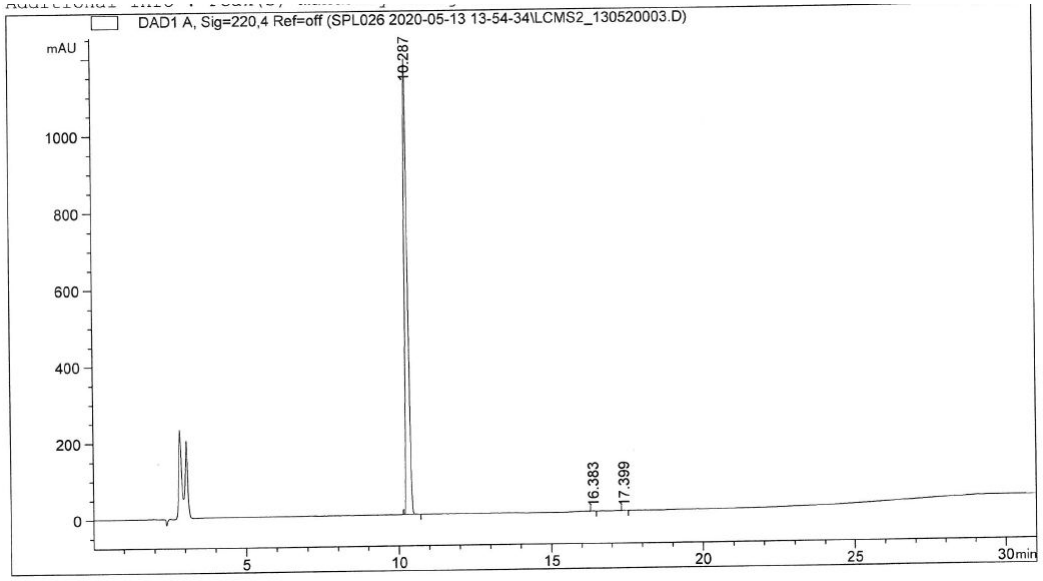
Additional Info : Peak(s) manually integrated



Signal 1: DAD1 A, Sig=220,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.741	MM	0.0742	3.88420	8.72299e-1	0.0482
2	10.291	MM	0.1138	8049.75830	1178.90552	99.8572
3	11.460	MM	0.0933	1.67731	2.99644e-1	0.0208
4	12.309	MM	0.2037	5.94807	4.86751e-1	0.0738
Totals :				8061.26788	1180.56421	

Compound example **12** (JCCA2559)



Signal 1: DAD1 A, Sig=220,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.741	MM	0.0742	3.88420	8.72299e-1	0.0482
2	10.291	MM	0.1138	8049.75830	1178.90552	99.8572
3	11.460	MM	0.0933	1.67731	2.99644e-1	0.0208
4	12.309	MM	0.2037	5.94807	4.86751e-1	0.0738
Totals :				8061.26788	1180.56421	

Compound example **14** JCCA2560

S5. Table S1: Mean In Vitro Percent Inhibition with 10 µM 9i

	9i
Adenosine A_1 (human)	-3
Adenosine A_{2A} (human)	8
Adrenergic α_{1A} (human)	85
Adrenergic α_{1B} (human)	87
Adrenergic α_{1D} (human)	63
Adrenergic α_{2A} (human)	69
Adrenergic α_{2B} (human)	80
Adrenergic α_{2C} (human)	58
Adrenergic β_1 (human)	8
Adrenergic β_2 (human)	-8
Androgen (testosterone)	7
Angiotensin AT_1 (human)	8
Bradykinin B_2 (human)	-1
Cannabinoid CB_1 (human)	12

Cannabinoid CB_2 (human)	-14
Chemokine CCR_1 (human)	-13
Chemokine $CXCR_2$ (human)	-6
Cholecystokinin CCK_1 (human)	-8
Cholecystokinin CCK_2 (human)	-5
D_1 (human)	8
D_{2L} (human)	39
D_{2S} (human)	41
Endothelin ET_A (human)	27
Estrogen Erα (human)	7
$GABA_A$ (chloride channel, TBOB) (rat)	0
$GABA_A$ (flunitrazepam, central) (rat)	-4
$GABA_A$ (Ro-15-1788, hippocampus) (rat)	3
$GABA_{B1A}$ (human)	-10
Glucocorticoid (human)	1
Glutamate, AMPA (rat)	-4

Glutamate, kainate (rat)	8
Glutamate, NMDA (agonism) (rat)	-3
Glutamate, NMDA (glycine) (rat)	-10
Glutamate, NMDA (phencyclidine) (rat)	5
Glutamate, NMDA (polyamine)	7
Glycine, strychnine-sensitive (rat)	-4
Histamine H_1 (human)	91
Histamine H_2 (human)	7
Leukotriene, $CysLT_1$ (human)	-3
Melanocortin MC_1 (human)	-1
Melanocortin MC_4 (human)	-2
Muscarinic M_1 (human)	3
Muscarinic M_2 (human)	4
Muscarinic M_3 (human)	15
Muscarinic M_4 (human)	0
Neuropeptide Y, Y_1 (human)	5

Nicotinic, $nACh_{\alpha 1}$ (human)	-1
Nicotinic, $nACh_{\alpha 3\beta 4}$ (human)	64
Opioid, δ_1 (human)	-11
Opioid, κ (human)	-1
Opioid, μ (human)	5
Platelet activating factor (human)	5
PPARγ (human)	-3
Progesterone, PR_B (human)	-21
Serotonin 5-HT_{1A} (human)	98
Serotonin 5-HT_{1B} (human)	76
Serotonin 5-HT_{2A} (human)	94
Serotonin 5-HT_{2B} (human)	96
Serotonin 5-HT_{2C} (human)	93
Serotonin 5-HT_3 (human)	6
Serotonin 5-HT_{5A} (human)	70
Serotonin 5-HT_6 (human)	86

Serotonin 5-HT_7 (human)	96
Sigma, σ_1 (human)	45
Tachykinin, NK_1	18
Vasopressin, $V1_A$	-5
Adenosine (guinea pig)	6
Dopamine (human)	22
GABA (rat)	0
Norepinephrine (human)	49
Serotonin	25
Calcium, L-type (benzothiazepine) (rat)	29
Calcium, L-type (dihydropyridine) (rat)	-19
Calcium, L-type (phenylalkylamine) (rat)	47
Calcium, N-type (rat)	-5
Potassium channel, K_{ATP} (hamster)	15
Potassium channel, hERG (human)	22
Sodium channel, site 2 (rat)	13

Sodium/potassium ATPase (pig)	4
Acetylcholinesterase (human)	39
Angiotensin converting enzyme (human)	5
Cyclooxygenase, COX-1 (human)	14
Cyclooxygenase, COX-2 (human)	15
Monoamine oxidase A (human)	84
Monoamine oxidase B (human)	6
Peptidase, Cathepsin G (human)	1
Phosphodiesterase, PDE3A (human)	-6
Phosphodiesterase, PDE4D2 (human)	-2
Serine/threonine protein kinase, PCKα (human)	-2
Insulin receptor protein tyrosine kinase (human)	36
Lymphocyte-specific protein tyrosine kinase (human)	9

Biological Methods

All radioligands and reagents used in the in vitro studies were supplied by Perkin Elmer and cell lines or tissue by Perkin Elmer, EPDST, Charles River, and Eurofins Discovery Services.

S6: Table S2. In Vitro Receptor, Transporter and Ion Channel Binding Assays

	Source	**B_{max} (pmole/mg protein)**	**Incubation**	**Radioligand**	**Specific binding (K_D)***	**Non-specific ligand**
Receptors						
Adenosine A_1	Human recombinant CHO-K1 cells	2.70	90 min at 25 °C; 20 mM HEPES (pH 7.4), 10 mM magnesium chloride, 100 mM sodium chloride	1.0 nM [^{3}H]-DPCPX	85% (1.40 nM)	100 µM R(-)-PIA
Adenosine A_{2A}	Human recombinant HEK-293 cells	7.0	90 min at 25 °C; 50 mM TRIS-HCl (pH 7.4), 10 mM	0.05 µM [^{3}H]-CGS-21680	85% (0.064 µM)	50.0 µM NECA

			magnesium chloride, 1 mM EDTA, 2 IU/mL adenosine deaminase			
Adrenergic α_{1A}	Human recombinant Chem-1 cells	8.70	60 min at 25 °C; 50 mM HEPES (pH 7.4), 5 mM magnesium chloride, 1 mM calcium chloride, 0.2% BSA	0.2 nM [^{3}H]-prazosin	90% (0.57 nM)	10.0 μM phentolamine
Adrenergic α_{1B}	Human recombinant Chem-1 cells	6.20	60 min at 25 °C; 50 mM HEPES (pH 7.4), 10 mM magnesium chloride, 1 mM EDTA	0.2 nM [^{3}H]-prazosin	90% (0.13 nM)	10.0 μM phentolamine

Adrenergic α_{1D}	Human recombinant HEK-293 cells	0.17	60 min at 25 °C; 50 mM TRIS-HCl (pH 7.4)	0.6 nM [^{3}H]-prazosin	80% (0.58 nM)	10.0 μM phentolamine
Adrenergic α_{2A}	Human recombinant CHO-K1 cells	16.0	60 min at 25 °C; 50 mM TRIS-HCl (pH 7.4), 1 mM EDTA	1.50 nM [^{3}H]-rauwolscine	95% (1.50 nM)	10.0 μM WB-1401
Adrenergic α_{2B}	Human recombinant CHO-K1 cells	12.0	60 min at 25 °C; 50 mM TRIS-HCl (pH 7.4), 1 mM EDTA, 12.5 mM magnesium chloride, 0.2% BSA	2.50 nM [^{3}H]-rauwolscine	90% (2.10 nM)	10 μM prazosin
Adrenergic α_{2C}	Human recombinant CHO-K1 cells	12.0	60 min at 25 °C; 50 mM TRIS-HCl (pH 7.4), 1 mM EDTA	0.50 nM [^{3}H]-rauwolscine	95% (0.4 nM)	10 μM WB-401

Adrenergic β_1	Human recombinant CHO-K1 cells	0.071	120 min at 25 °C; 50 mM TRIS-HCl (pH 7.4), 1.4 mM ascorbic acid, 5 mM EDTA, 120 mM magnesium chloride, 1.5 mM calcium chloride, 0.001% BSA	0.03 nM [$_{125}$I]-cyanopindolol	95% (0.041 nM)	100 μM S(-)-propanolol
Adrenergic β_2	Human recombinant CHO cells	0.44	60 min at 25 °C; 50 mM TRIS-HCl (pH 7.4), 0.5 mM EDTA, 5 mM magnesium chloride, 120 mM NaCl	0.2 nM [^{3}H]-CGP-12177	95% (0.44 nM)	10.0 μM ICI-118551

Androgen (testosterone)	Human LNCaP clone FGC cells	0.25	20 h at 4 °C; 25 mM HEPES (pH 7.4), 10% glycerol, 1 mM EDTA, 10 mM molybdate	0.5 nM [3H]-methyltrienolone	75% (0.71 nM)	1.0 μM testosterone
Angiotensin AT_1	Human recombinant CHO-K1 cells	16.0	180 min at 37 °C; 50 mM TRIS-HCl (pH 7.4), 5 mM magnesium chloride, 0.1% BSA, 1 mM EDTA	20.0 pM [^{125}I]-(Sar^1, Ile^8)-angiotensin II	90% (0.056 nM)	
Bradykinin B_2	Human recombinant Chem-1 cells	9.40	60 min at 25 °C; 50 mM HEPES (pH 7.4), 0.2% BSA, 1 mM calcium chloride, 5 mM magnesium	0.5 nM [^{3}H]-bradykinin	90% (0.85 nM)	5.0 μM bradykinin

			chloride, 0.05 mM bacitracin			
Cannabinoid CB_1	Human recombinant Chem-1 cells	15.0	60 min at 37 °C; 50 mM HEPES (pH 7.4), 0.2% BSA, 1 mM calcium chloride, 5 mM magnesium chloride	2.0 nM [^{3}H]-SR141716A	78% (18.0 nM)	10.0 μM CP-55,940
Cannabinoid CB_2	Human recombinant CHO-K1 cells	1.70	90 min at 37 °C; 20 mM HEPES (pH 7.0), 0.5% BSA	2.40 nM [^{3}H]-WIN-55,212-2	80% (4.90 nM)	10 uM R(+)-WIN-55,212-2
Chemokine CCR_1	Human recombinant Chem-2 cells	1.10	180 min at 25 °C; 50 mM HEPES (pH 7.4), 0.2% BSA, 1 mM calcium chloride, 5	0.1 nM [^{125}I]-MIP-1α	72% (0.46 nM)	0.1 μM MCP-3

			mM magnesium chloride			
Chemokine $CXCR_2$	Human recombinant CHO-K1 cells	0.28	60 min at 25 °C; 25 mM HEPES (pH 7.4), 0.2% BSA, 2 mM calcium chloride, 1 mM magnesium chloride	15.0 pM [^{125}I]-IL-8 (80%)	80% (12.0 pM)	10.0 nM IL-8
Cholecystokinin CCK_1	Human recombinant 1321-N1 cells	2.30	180 min at 25 °C; 25 mM HEPES (pH 7.4), 0.1% BSA, 1 mM calcium chloride, 5 mM magnesium chloride	0.11 nM [^{125}I]-CCK-8	85% (0.59 nM)	1.0 μM L-364,718

Cholecystokinin CCK_2	Human recombinant Chem-1 cells	9.40	180 min at 25 °C; 50 mM HEPES (pH 7.4), 0.1% BSA, 1 mM calcium chloride, 5 mM magnesium chloride	0.05 nM [^{125}I]-CCK-8	90% (0.069 nM)	1.0 μM sincalide
Dopamine D_1	Human recombinant CHO cells	0.63	120 min at 37 °C; 50 mM TRIS-HCl (pH 7.4), 1.4 mM ascorbic acid, 0.001% BSA, 150 mM sodium chloride	1.40 nM [^{3}H]-SCH-23390	90% (1.40 nM)	10.0 μM (+)-butaclamol
Dopamine D_{2L}	Human recombinant CHO cells	0.48	1.0% DMSO 120 min at 25 °C; 50 mM TRIS-HCl (pH 7.4), 1.4 mM ascorbic	0.16 nM [^{3}H]-spiperone	85% (0.08 Nm)	10.0 μM haloperidol

			acid, 0.001% BSA, 150 mM sodium chloride			
Dopamine D_{2S}	Human recombinant CHO cells	1.60	120 min at 25 °C; 50 mM TRIS-HCl (pH 7.4), 1.4 mM ascorbic acid, 0.001% BSA, 150 mM sodium chloride	0.16 nM [^{3}H]-spiperone	90% (0.09 nM)	10.0 μM haloperidol
Endothelin ET_A	Human recombinant CHO-S cells	0.35	120 min at 37 °C; 50 mM TRIS-HCl (pH 7.4), 0.1% BSA, 0.5 mM calcium chloride	0.03 nM [^{125}I]-endothelin-1	90% (0.048 nM)	0.1 μM endothelin-1
Estrogen ERα	Human recombinant insect Sf9 cells	1400	120 min at 25 °C; 10 mM TRIS-HCl (pH	0.5 nM [^{3}H]-estradiol	85% (0.2 nM)	1.0 μM diethylstilbestrol

			7.4), 0.1% BSA, 10% glycerol, 1 mM DTT			
$GABA_A$ (chloride channel, TBOB)	Wistar rat brain (minus cerebellum)	2.10	120 min at 25 °C; 50 mM potassium phosphate (pH 7.4), 200 mM sodium chloride	3.0 nM [^{3}H]-TBOB	78% (0.026 µM)	200 µM picrotoxin
$GABA_A$ (flunitrazepam, central)	Wistar rat brain (minus cerebellum)	1.20	60 min at 25 °C; 50 mM phosphate buffer (pH 7.4)	1.0 nM [^{3}H]-flunitrazepam	91% (4.40 nM)	10.0 µM diazepam
$GABA_A$ (Ro-15-1788, hippocampus)	Wistar rat hippocampus	1.20	120 min at 4 °C; 50 mM TRIS-citrate (pH 7.4)	1.0 nM [^{3}H]-Ro-15-1788	96% (1.20 nM)	10.0 µM diazepam
$GABA_{B1A}$	Human recombinant CHO-K1 cells	48.0	180 min at 25 °C; 50 mM TRIS-HCl (pH	4.0 nM [^{3}H]-CGP-54626	90% (3.30 nM)	30.0 mM GABA

			7.4), 2.5 mM calcium chloride, 0.1% BSA			
Glucocorticoid	Human recombinant insect cells	1.0	24 h min at 4 °C; 1.5 mM potassium phosphate, 8 mM sodium phosphate (pH 7.4), 137 mM sodium chloride, 2.7 mM potassium chloride, 0.2% BSA	5.0 nM [^{3}H]-dexamethasone	97% (4.60 nM)	10.0 μM dexamethasone
Glutamate, AMPA	Wistar rat cerebral cortex	17.0	90 min at 4 °C; 50 mM TRIS-HCl (pH 7.4), 200 mM potassium thiocyanate	5.0 nM [^{3}H]-AMPA	90% (0.99 μM)	1.0 mM L-glutamic acid

Glutamate, kainate	Wistar rat cerebral cortex	0.21	120 min at 4 °C; 50 mM TRIS-HCl (pH 7.4)	6.0 nM [^{3}H]-kainic acid	80% (5.40 nM)	1.0 mM L-glutamic acid
Glutamate mGluR$_5$	Human recombinant CHO-K1 cells	0.68	120 min at 25 °C; 25 mM HEPES (pH 7.4), 2.5 mM calcium chloride, 1 mM magnesium chloride	0.03 μM [^{3}H]-quisqualic acid	85% (0.026 μM)	1.0 mM L-glutamic acid
Glutamate, NMDA (agonism)	Wistar rat cerebral cortex	2.30	20 min at 4 °C; 50 mM TRIS-HCl (pH 7.4)	2.0 nM [^{3}H]-CGP-39653	70% (19.0 nM)	1.0 mM L-glutamic acid
Glutamate, NMDA (glycine)	Wistar rat cerebral cortex	3.70	30 min at 4 °C; 50 mM HEPES (pH 7.7)	0.33 nM [^{3}H]-MDL-105,519	85% (6.0 nM)	10.0 μM MDL-105,519
Glutamate, NMDA (phencyclidine)	Wistar rat cerebral cortex	0.78	45 min at 25 °C; 10 mM TRIS-HCl (pH 7.4)	4.0 nM [^{3}H]-TCP	94% (8.40 nM)	1.0 μM MK-801

Glutamate, NMDA (polyamine)	Wistar rat cerebral cortex	1.10	120 min at 4 °C; 50 mM TRIS-HCl (pH 7.4)	2.0 nM [^{3}H]-ifenprodil	80% (0.026 μM)	10.0 μM ifenprodil
Glycine, strychnine-sensitive	Wistar rat spinal cord	0.35	10 min at 4 °C; 50 mM potassium phosphate (pH 7.4), 200 mM sodium chloride	10.0 nM [^{3}H]-strychnine	77% (13.0 nM)	1.0 mM glycine
Histamine H_1	Human recombinant CHO-K1 cells	6.70	180 min at 25 °C; 50 mM TRIS-HCl (pH 7.4), 5 mM magnesium chloride	1.20 nM [^{3}H]-pyrilamine	94% (1.10 nM)	1.0 μM pyrilamine
Histamine H_2	Human recombinant CHO-K1 cells	6.90	120 min at 25 °C; 50 mM phosphate buffer (pH 7.4), 0.2% gelatin	0.1 nM [^{125}I]-aminopotentidine	90% (0.45 nM)	3.0 μM tiotidine

Leukotriene $CysLT_1$	Human recombinant CHO-K1 cells	3.0	30 min at 25 °C; 50 mM TRIS-HCl (pH 7.4), 5 mM calcium chloride, 5 mM magnesium chloride, 100 μg/ml bacitracin, 1 mM benzamidine, 0.1 mM PMSF	0.3 nM [^{3}H]-leukotriene D4	93% (0.21 nM)	0.3 μM leukotriene D4
Melanocortin MC_1	Human recombinant CHO-K1 cells	0.53	120 min at 37 °C; 25 mM HEPES-KOH (pH 7.0), 100 mM sodium chloride, 1 mM 1,10-phenanthroline, 1.5 mM calcium chloride, 1 mM magnesium	0.04 nM [^{125}I]-NDP-α-MSH	90% (0.037 nM)	1.0 μM NDP-α-MSH

			sulfate, 1 complete protease inhibitor tablet 100/ml			
Melanocortin MC_4	Human recombinant HEK-293 cells	3.90	120 min at 37 °C; 25 mM HEPES-KOH (pH 7.0), 100 mM sodium chloride, 1 mM 1,10-phenanthroline, 1.5 mM calcium chloride, 1 mM magnesium sulfate, 1 complete protease inhibitor tablet 100/ml	20.0 pM [^{125}I]-NDP-α-MSH	90% (0.5 nM)	3.0 μM NDP-α-MSH

Muscarinic M_1	Human recombinant CHO-K1 cells	2.0	120 min at 25 °C; 50 mM TRIS-HCl (pH 7.4), 10 mM magnesium chloride, 1 mM EDTA	0.8 nM [^{3}H]-N-methylscopolamine	95% (0.26 nM)	1.0 μM atropine
Muscarinic M_2	Human recombinant CHO-K1 cells	5.10	120 min at 25 °C; 50 mM TRIS-HCl (pH 7.4), 10 mM magnesium chloride, 1 mM EDTA	0.8 nM [^{3}H]-N-methylscopolamine	95% (0.58 nM)	1.0 μM atropine
Muscarinic M_3	Human recombinant CHO-K1 cells	5.40	120 min at 25 °C; 50 mM TRIS-HCl (pH 7.4), 10 mM magnesium chloride, 1 mM EDTA	0.8 nM [^{3}H]-N-methylscopolamine	95% (0.75 nM)	1.0 μM atropine

Muscarinic M_4	Human recombinant CHO-K1 cells	1.60	120 min at 25 °C; 50 mM TRIS-HCl (pH 7.4), 10 mM magnesium chloride, 1 mM EDTA	0.8 nM [^{3}H]-N-methylscopolamine	95% (0.22 nM)	1.0 μM atropine
Neuropeptide Y Y_1	Human SK-N-MC cells	0.58	60 min at 37 °C; 25 mM HEPES (pH 7.4), 1 mM magnesium chloride, 2.5 mM calcium chloride, 0.1% BSA, 0.01% bacitracin	15.0 pM [^{125}I]-peptide YY	80% (0.24 nM)	1.0 μM neuropeptide Y (human, rat)
Nicotinic $nAChR_{\alpha 1}$	Human RD cells	1.0	120 min at 25 °C; 147 mM sodium chloride (pH 7.4), 4 mM potassium chloride,	0.6 nM [^{125}I]-α-bungarotoxin	85% (1.10 nM)	1.0 μM α-bungarotoxin

			2.2 mM calcium chloride			
Nicotinic $nAChR_{\alpha3\beta4}$	Human recombinant CHO-K1 cells	1.50	60 min at 25 °C; 50 mM TRIS-HCl (pH 7.4)	0.05 nM [^{3}H]-epibatidine	90% (0.13 nM)	10.0 μM epibatidine
Opioid δ_1	Human recombinant HEK-293 cells	1.40	60 min at 25 °C; 50 mM TRIS-HCl (pH 7.4), 1 mM EDTA, 10 mM magnesium chloride	1.30 nM [^{3}H]-naltrindole	95% (0.053 nM)	1.0 μM naltrindole
Opioid κ	Human recombinant HEK-293 cells	1.10	60 min at 25 °C; 50 mM TRIS-HCl (pH 7.4)	0.6 nM [^{3}H]-diprenorphine	90% (0.41 nM)	10.0 μM naloxone
Opioid μ	Human recombinant CHO-K1 cells	3.80	60 min at 25 °C; 50 mM TRIS-HCl (pH 7.4)	0.6 nM [^{3}H]-diprenorphine	90% (0.41 nM)	10.0 μM naloxone

Platelet activating factor	Human recombinant CHO-K1 cells	0.78	60 min at 25 °C; 25 mM HEPES (pH 7.4), 10 mM magnesium chloride, 0.3% BSA	1.0 nM [^{3}H]-PAF	80% (1.50 nM)	10.0 μM C16-PAF
PPARγ	Human recombinant insect cells	5400	24 h at 4 °C; 10 mM TRIS-HCl (pH 7.4), 1 mM EDTA, 10% glycerol, 1 mM DTT, 1 μg/ml benzamidine, 10 mM sodium molybdate, 0.1% non-fat dried milk	5.0 nM [^{3}H]-rosiglitazone	95% (20.0 nM)	10.0 μM rosiglitazone
Progesterone PR_B	Human T-47D cells	0.0002 fmole/cell	20 h at 4 °C; 5 mM sodium phosphate (pH 7.4), 10% glycerol, 10 mM	0.5 nM [^{3}H]-progesterone	91% (2.0 nM)	1.0 μM R-5020

			monothiolglycerol, 20 mM sodium molybdate			
Serotonin 5-HT_{1A}	Human recombinant CHO-K1 cells	1.30	60 min at 25 °C; 50 mM TRIS-HCl (pH 7.4), 0.1% ascorbic acid, 0.5 mM EDTA, 10 mM magnesium sulfate	1.50 nM [^{3}H]-8-OH-DPAT	75% (2.0 nM)	10.0 μM metergoline
Serotonin 5-HT_{1B}	Human recombinant Chem-1 cells	22.0	90 min at 37 °C; 50 mM TRIS-HCl (pH 7.4), 1 mM EDTA, 10 mM magnesium chloride	1.0 nM [^{3}H]-GR125743]	90% (3.20 nM)	10.0 μM serotonin

Serotonin 5-HT_{2A}	Human recombinant CHO-K1 cells	0.51	60 min at 25 °C; 50 mM TRIS-HCl (pH 7.4)	0.5 nM [^{3}H]-ketanserin	90% (0.2 nM)	1.0 μM mianserin
Serotonin 5-HT_{2B}	Human recombinant CHO-K1 cells	1.10	60 min at 25 °C; 50 mM TRIS-HCl (pH 7.4), 0.1% ascorbic acid, 4 mM calcium chloride	1.20 nM [^{3}H]-lysergic acid diethylamide	80% (2/10 nM)	10.0 μM serotonin
Serotonin 5-HT_{2C}	Human recombinant CHO-K1 cells	4.90	60 min at 25 °C; 50 mM TRIS-HCl (pH 7.4), 0.1% ascorbic acid, 10 μM pargyline	1.0 nM [^{3}H]-mesulergine	90% (1.10 nM)	1.0 μM mianserin
Serotonin 5-HT_3	Human recombinant HEK-293 cells	11.0	60 min at 25 °C; 50 mM TRIS-HCl (pH 7.4), 1 mM EDTA, 5	0.69 nM [^{3}H]-GR-65630	90% (0.2 nM)	10.0 μM MDL-72222

			mM magnesium chloride			
Serotonin 5-HT_{5A}	Human recombinant CHO-K1 cells	4.30	60 min at 25 °C; 50 mM TRIS-HCl (pH 7.4), 0.5 mM EDTA, 10 mM magnesium chloride	1.70 nM [^{3}H]-lysergic acid diethylamide	80% (1.80 nM)	100 µM serotonin
Serotonin 5-HT_6	Human recombinant HeLa cells	1.70	120 min at 37 °C; 50 mM TRIS-HCl (pH 7.4), 150 mM sodium chloride, 2 mM ascorbic acid, 0.001% BSA	1.50 nM [^{3}H]-lysergic acid diethylamide	90% (1.30 nM)	5.0 µM serotonin
Serotonin 5-HT_7	Human recombinant CHO-K1 cells	0.95	120 min at 25 °C; 50 mM TRIS-HCl (pH 7.4), 0.5 mM EDTA,	5.50 nM [^{3}H]-lysergic acid diethylamide	90% (7.40 nM)	10.0 µM serotonin

			10 mM magnesium chloride			
Sigma σ1	Human Jurkat cells	1.08	120 min at 37 °C; 50 mM TRIS-HCl (pH 8.0)	15.0 nM [^{3}H]-pentazocine	90% (16.0 nM)	10.0 μM haloperidol
Tachykinin NK_1	Human recombinant CHO cells	1.70	90 min at 4 °C; 20 mM HEPES (pH 7.4), 1 mM manganese chloride, 0.1% BSA	0.8 nm [^{3}H]-substance P	90% (2.10 nM)	10.0 μM L-703,606
Vasopressin V_{1A}	Human recombinant HEK-293 cells	2.80	120 min at 25 °C; 50 mM TRIS-HCl (pH 7.4), 5 mM magnesium chloride, 0.1% BSA	0.03 nM [^{125}I] Phenylacetyl-Tyr(Me)-PheGlnAsnArgPro-ArgTyr	85% (7.40 pM)	10.0 uM (Arg^{8})-vasopressin

Transporters

Adenosine	Duncan Hartley-derived Guinea pig cerebral cortex	0.24	30 min at 25 °C; 50 mM TRIS-HCl (pH 7.4)	0.5 nM [^{3}H]-nitro-benzylthioinosine	90% (0.26 nM)	5.0 μM nitro-benzyl-thioinosine
Dopamine	Human recombinant CHO-S cells	0.047	180 min at 4 °C; 50 mM TRIS-HCl (pH 7.4), 100 mM sodium chloride, 1 μM leupeptin, 10 μM PMSF	0.15 nM [^{3}H]-RTI-55	90% (0.58 nM)	10.0 μM nomifensine
GABA	Wistar rat brain (minus cerebellum)	60.0	20 min at 25 °C; 10 mM HEPES (pH 7.5), 120 mM sodium chloride, 4 mM	6.0 nM [^{3}H]-GABA	80% (0.3 μM)	10.0 μM NO-711

			calcium acetate, 10 uM isoguvacine, 10 uM S(-)-baclofen			
Norepinephrine	Human recombinant MDCK cells	2.50	180 min at 4 °C; 50 mM TRIS-HCl (pH 7.4), 100 mM sodium chloride, 1 μM leupeptin, 10 μM PMSF	0.2 nM [^{3}H]-RTI-55	75% (0.024 μM)	10.0 μM desipramine
Serotonin	Human recombinant HEK-293 cells	4.40	60 min at 25 °C; 50 mM TRIS-HCl (pH 7.4), 120 mM sodium chloride, 5 mM potassium chloride	0.4 nM [^{3}H]-paroxetine	95% (0.078 nM)	10.0 μM imipramine

Ion channels

Calcium, L-type (benzothiazepine)	Wistar rat brain (minus cerebellum)	0.21	180 min at 4 °C; 50 mM TRIS-HCl (pH 7.4), 0.1% BSA	2.0 nM [^{3}H]-diltiazem	73% (16.0 nM)	10.0 µM diltiazem
Calcium, L-type (dihydropyridine)	Wistar rat cerebral cortex	0.23	90 min at 25 °C; 50 mM TRIS-HCl (pH 7.4)	0.1 nM [^{3}H]-nitrendipine	91% (0.18 nM)	1.0 µM nitrendipine
Calcium, L-type (phenylalkylamine)	Wistar rat cerebral cortex	1.60	60 min at 25 °C; 50 mM HEPES (pH 7.4)	0.4 nM [^{3}H]-(-)-desmethoxy verapamil	80% (14.0 nM)	10 µM desmethoxy verapamil
Calcium, N-type	Wistar rat frontal brain	0.88	30 min at 4 °C; 20 mM TRIS-HCl (pH 7.4), 0.5% BSA	10.0 pM [^{125}I]-ω-conotoxin GVIA	96% (0.051 nM)	0.1 µM ω-conotoxin GVIA
Potassium, ATP	Hamster pancreatic HIT-T15 beta cells	1.0	120 min at 25 °C; 50 mM MOPS (pH 7.4), 0.1 mM calcium chloride	5.0 nM [^{3}H]-glyburide	90% (0.64 nM)	1.0 µM glyburide

Potassium, hERG	Human recombinant HEK-293 cells	6.30	60 min at 25 °C; 10 mM HEPES (pH 7.4), 0.1% BSA, 5 mM potassium chloride, 0.8 mM magnesium chloride, 130 mM sodium chloride, 1 mM EGTA, 10 mM glucose	1.50 nM [^{3}H]-astemizole	90% (6.80 nM)	10.0 μM astemizole
Sodium, site 2	Wistar rat brain (minus cerebellum)	0.7	60 min at 37 °C; 10 mM HEPES, 50 mM TRIS-HCl (pH 7.4), 130 mM choline potassium chloride, 0.8 mM magnesium chloride, 5.5 mM	5.0 nM [^{3}H]-batrachotoxinin	77% (0.052 μM)	100 μM veratridine

glucose, 40 μg/ml

LqTx

*Historical values. Vehicle for SPL026 and SPL028 was 1.0% dimethylsulfoxide was unless otherwise stated.

BSA, bovine serum albumin; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid); EGTA, ethylene glycol tetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; MOPS, 3-(N-morpholino)propanesulfonic acid; PMSF, phenylmethylsulfonyl fluoride; TRIS-HCl, tris(hydroxymethyl)aminomethane hydrochloride

S7. Table S3: *In Vitro* Enzyme Inhibition Assays

Enzyme	Tissue	Substrate	Incubation buffer	Pre-incubation	Incubation	Spectro-photometric quantitation
Sodium/potassium ATPase	Pig heart	100 μM ATP	50 mM TRIS-HCl (pH 7.0), 20 mM potassium chloride, 5 mM magnesium chloride, 100 mM sodium chloride	15 min at 37 °C	60 min at 37 °C	Pi
Acetylcholinesterase	Human recombinant HEK-293 cells	700 μM acetylthiocholine	0.1 M sodium phosphate	15 min at 25 °C	20 min at 37 °C	Thiocholine

Cyclo-oxygenase 1	Human recombinant Baculovirus infected Sf9 cells	3.0 μM arachidonic acid	200 mM TRIS-HCl (pH 8.0), 6 μM EDTA, 10 μM hematin	15 min at 25 °C	3 min at 25 °C	Resorufin
Cyclo-oxygenase 2	Human recombinant Insect Sf9 cells	3.0 μM arachidonic acid	200 mM TRIS-HCl (pH 8.0), 6 μM EDTA, 10 μM hematin	15 min at 25 °C	3 min at 25 °C	Resorufin
Monoamine oxidase A	Human recombinant Insect cells	50.0 μM kynuramine	100 mM potassium phosphate (pH 7.4)	15 min at 37 °C	60 min at 37 °C	4-hydroxy-quinoline
Monoamine oxidase B	Human recombinant Insect cells	50.0 μM kynuramine	100 mM potassium phosphate (pH 7.4)	15 min at 37 °C	60 min at 37 °C	4-hydroxy-quinoline

Angiotensin converting enzyme	Human recombinant mouse myeloma cells	25.0 μM Abz-FRK (Dnp)-P	50 mM MES (pH 6.5)	15 min at 25 °C	30 min at 25 °C	Abz-FR-OH
Cathepsin G	Human neutrophils	10.0 μM N-succinyl-ala-ala-pro-phe-AMC	50 mM sodium acetate (pH 5.5), 1 mM DTT, 2 mM EDTA	15 min at 25 °C	30 min at 25 °C	AMC
Phosphodiesterase PDE3A	Human recombinant Insect Sf9 cells	0.1 μM FAM-cAMP	10 mM TRIS-HCl (pH 7.2), 10 mM magnesium chloride, 0.05% sodium azide, 0.1% phosphate-free BSA	15 min at 25 °C	15 min at 25 °C	Fluorescein-AMP-IMAP
Phosphodiesterase PDE4D2	Human recombinant	0.1 μM FAM-cAMP	10 mM TRIS-HCl (pH 7.2), 10 mM	15 min at 25 °C	15 min at 25 °C	Fluorescein-AMP-IMAP

	Insect Sf9 cells		magnesium chloride, 0.05% sodium azide, 0.1% phosphate-free BSA			
Serine/threonine protein kinase, PKCα	Human recombinant Insect Sf9 cells	250 µg/mL histone	20 mM HEPES (pH 7.2), 8 mM magnesium chloride, 0.08 mM calcium chloride, 100 µg/mL phosphatidylserine, 20 µg/mL diacylglycerol	15 min at 25 °C	10 min at 25 °C	[^{32}P]-histone
Insulin receptor protein tyrosine kinase	Human recombinant Insect cells	200 µg/mL poly(glu:tyr)	50 mM HEPES (pH 7.4), 10 mM magnesium chloride, 1 mM DTT, 200 µM sodium orthovanadate,	15 min at 37 °C	30 min at 37 °C	[^{32}P]-poly(glu:tyr)

			2 mM manganese chloride			
Lymphocyte-specific protein tyrosine kinase	Human recombinant Insect cells	200 µg/mL poly(glu:tyr)	50 mM HEPES (pH 7.4), 20 mM magnesium chloride, 1 mM DTT, 200 µM sodium orthovanadate, 2 mM manganese chloride	15 min at 37 °C	30 min at 37 °C	[^{32}P]-poly(glu:tyr)

Vehicle for SPL026 and SPL028 was 1.0% dimethylsulfoxide was unless otherwise stated.

AMP-IMAP, adenosine monophosphate-immobilized metal ion affinity for phosphochemicals; BSA, bovine serum albumin; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid); FAM-cAMP, green fluorescent cyclic adenosine monophosphate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; MES, 2-(N-morpholino)ethanesulfonic acid; TRIS-HCl, tris(hydroxymethyl)aminomethane hydrochloride

References

1. Cheng, Y.; Prusoff, W. H. Relationship between the inhibition constant (K1) and the concentration of inhibitor which causes 50 per cent inhibition (I50) of an enzymatic reaction. *Biochem. Pharmacol*. **1973**, *22*, 3099–3108.