SUPPORTING INFORMATION

A chemical probe identification platform for orphan GPCRs using focused compound screening: GPR39 as a case example

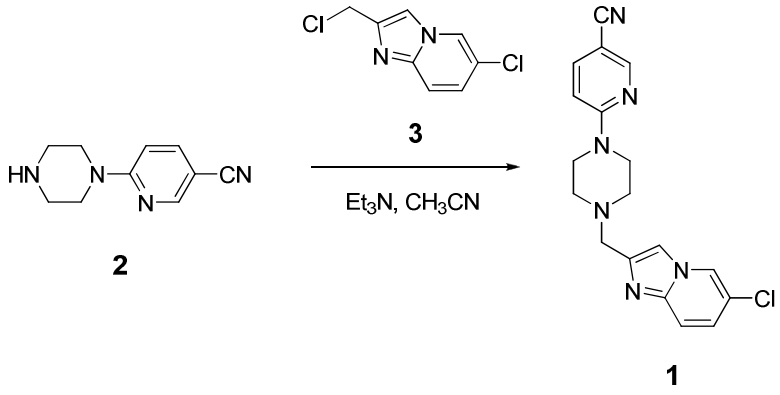
Markus Boehm,[#] David Hepworth,[#] Paula M. Loria,[//] Lisa D. Norquay,[⊥] Kevin J. Filipski,[#] Janice E. Chin,[⊥] Kimberly O. Cameron,[#] Martin Brenner,[⊥] Peter Bonnette, [⊥] Shawn Cabral,[#] Edward Conn,[#] David C. Ebner,[#] Denise Gautreau,[⊥] John Hadcock, [⊥] Esther C. Y. Lee,[#] Alan M. Mathiowetz,[#] Michelle Morin,[⊥] Lucy Rogers,[//] Aaron Smith,[#] Maria VanVolkenburg,[⊥] and Philip A. Carpino[#,*]

[⊥]Departments of Cardiovascular, Metabolic and Endocrine Diseases and [#]Cardiovascular, Metabolic and Endocrine Diseases Medicinal Chemistry, Pfizer Worldwide Research and Development, 620 Memorial Drive, Cambridge, MA 02139

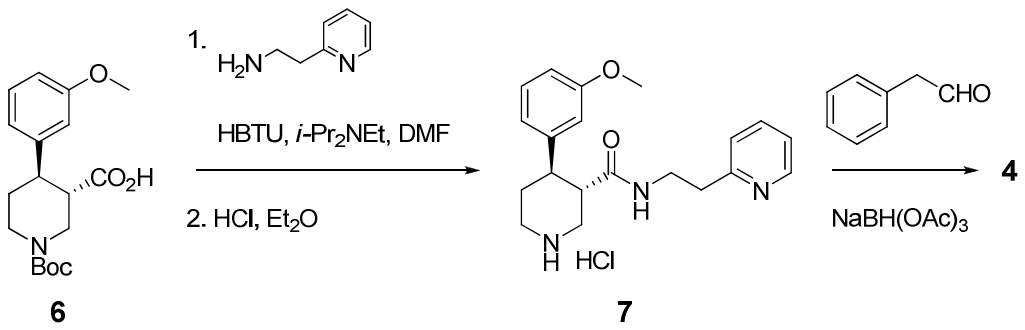
[//]Department of Pharmacokinetics, Dynamics and Metabolism – New Chemical Entities, Primary Pharmacology Group, Pfizer Worldwide Research and Development, Eastern Point Road, Groton, CT 06340

Synthetic Schemes:

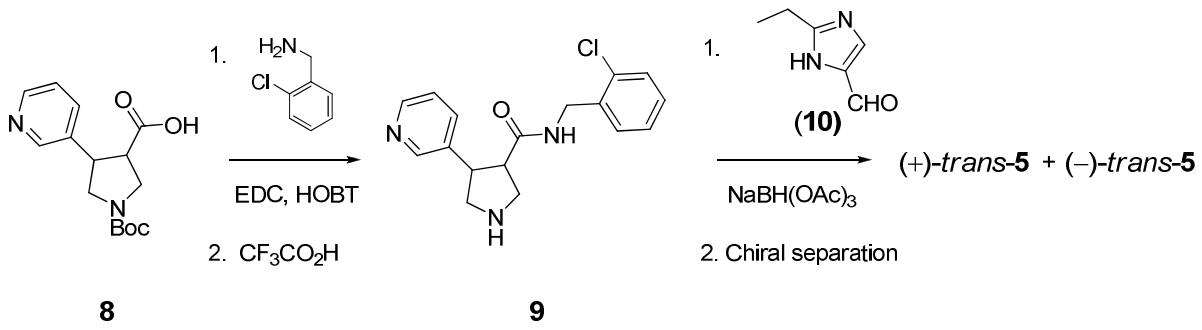
Scheme S1. Synthetic route compound **1**.



Scheme S2. Synthetic route to racemic **4**



Scheme S3. Synthetic route to enantiomers (+)-*trans*-**5** and (–)-*trans*-**5**.



General Methods:
Unless otherwise stated, all reactants, reagents and solvents were obtained from commercial sources and used without further purification. Data for ^{1}H NMR spectra are reported relative to residual solvent signals (for $CDCl_3$, δ = 7.27 ppm; for DMSO-d_6, δ = 2.50 ppm; for CD_3OD, δ = 3.31 ppm) as follows: chemical shift (δ ppm), multiplicity, coupling constant (Hz) and integration. The multiplicities are denoted as follows: s, singlet; d, doublet; t, triplet; q, quartet; spt, septet; m, multiplet; br s, broad singlet; br d, broad doublet. Data for ^{13}C NMR spectra are reported in terms of chemical shift (δ ppm) relative to residual solvent signals (for $CDCl_3$, δ = 77.0 ppm; for DMSO-d_6, δ = 39.5 ppm; for CD_3OD, δ = 49.0 ppm). Flash chromatography was carried out on either a Biotage SP purification system or a Combiflash Companion from Teledyne Isco; Biotage SNAP, KPsil or Redisep Rf silica columns were used. Except where otherwise noted, all reactions were run under an inert atmosphere of nitrogen gas using anhydrous solvents at room temperature (~23 °C). The terms "concentrated" and "evaporated" refer to the removal of solvent at reduced pressure on a rotary evaporator with a water bath temperature not exceeding 60 °C.

6-(4-((6-Chloro*H*-imidazo[1,2-a]pyridin-2-yl)methyl)piperazin-1-yl)nicotinonitrile (1):
A mixture of 6-(piperazin-1-yl)nicotinonitrile hydrochloride (**3**, 500 mg, 2.20 mmol), 6-chloro-2-(chloromethyl)imidazo[1,2-a]pyridine (**2**, 470 mg, 2.30 mmol), and triethylamine (0.1 mL) in acetonitrile (10 mL) was stirred at 78 ^{o}C for 4 h. The mixture was cooled and partitioned between dichloromethane (40 mL) and aqueous sodium bicarbonate (40 mL). The organic layer was separated, dried over sodium sulfate, filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography, eluting with a gradient from 10% to 50% ethyl acetate in petroleum ether to give the title compound **1** (320 mg, 41%) as a white solid.
HRMS (ES^+): Calc'd for $C_{18}H_{17}ClN_6$ (M+H): 353.1276 found: 353.1276.
^{1}H NMR (400 MHz, CD_3OD) δ = 9.15 (d, *J*=1.2 Hz, 1H), 8.59 (s, 1H), 8.52 (d, *J*=2.0 Hz, 1H), 8.13 (dd, *J*=9.6, 1.6 Hz, 1H), 8.06 (d, *J*=9.4 Hz, 1H), 7.91 (dd, *J*=9.4, 2.3 Hz, 1H), 7.09 (d, *J*=9.4 Hz, 1H), 4.84 (s, 2H), 4.15 (br. s, 4H), 3.58 (br. s, 4H).
^{13}C NMR (100 MHz, CD_3OD) δ 158.3, 151.4, 140.7, 139.6, 135.6, 127.4, 125.9, 125.6, 118.5, 117.2, 113.1, 107.3, 98.2, 51.1, 49.1, 41.6.

***Trans*-4-(3-methoxyphenyl)-*N*-(2-(pyridin-2-yl)ethyl)piperidine-3-carboxamide hydrochloride (7):**

Step 1: Diisopropylethylamine (1.54 g, 11.9 mmol) was added at room temperature to a solution of *trans*-1-(*tert*-butoxycarbonyl)-4-(3-methoxyphenyl)piperidine-3-carboxylic acid[1] (**6**, 2.0 g, 6.0 mmol), *O*-(benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate (2.21 g, 5.96 mmol) in dry *N*,*N*-dimethylformamide (50 mL). After the solution was stirred for 10 min, 2-(pyridin-2-yl)ethanamine (727 mg, 5.96 mmol) was added dropwise at room temperature. The solution was stirred for 20 h, diluted with ethyl acetate, and washed with water. The organic layer was concentrated under reduced pressure to afford a residue which was purified by column chromatography (ethyl acetate/heptanes) to obtain 1.6 g of racemic-*trans*-*tert*-butyl 4-(3-methoxyphenyl)-3-((2-(pyridin-2-yl)ethyl)carbamoyl) piperidine-1-carboxylate (61% yield).
LCMS (ES^+): Calc'd for $C_{25}H_{33}N_3O_4$ (M+H): 440.3 found: 440.3.
Step 2: A round bottomed flask was charged with the product from step 1 (1.6 g, 3.64 mmol) and diethyl ether (30 mL), then was cooled to 0 ^oC. Saturated hydrogen chloride in diethyl ether (20 mL) was added at 0 °C. The reaction mixture was stirred for 2 h at room temperature. The excess hydrogen chloride in diethyl ether was distilled off. Additional diethyl ether was added and distilled off to afford 1.0 g (81% yield) of **7** which was taken on to the next step without further purification.
LCMS (ES+): Calc'd for $C_{20}H_{25}N_3O_2$ (M+H): 340.2 found: 340.3.

***Trans*-4-(3-methoxyphenyl)-1-phenethyl-*N*-(2-(pyridin-2-yl)ethyl)piperidine-3-carboxamide (4):**
A round bottomed flask was charged with *trans*-4-(3-methoxyphenyl)-*N*-(2-(pyridin-2-yl)ethyl)piperidine-3-carboxamide hydrochloride (**7**, 400 mg, 1.17 mmol), dry 1,2-dichloroethane (12 mL), and diisopropylethylamine (0.5 mL) and was stirred for 30 minutes at room temperature. 2-Phenylacetaldehyde (141 mg, 1.17 mmol) and sodium triacetoxyborohydride (750 mg, 3.53 mmol) were added and the reaction mixture was stirred for 20 h and diluted with ethyl acetate. The organic layers was separated, washed with water, and concentrated under reduced pressure to afford a residue that was purified via prep TLC, eluting with 3% methanol in dichloromethane, to provide 78 mg of **4** (15% yield).
HRMS (ES^+): Calc'd for $C_{28}H_{33}N_3O_2$ M+H): 444.2646 found: 444.2660.
^1H NMR: ^1H NMR (600 MHz, CD_3OD) δ 8.38 (d, *J*=5.3 Hz, 1H) 7.64 (td, *J*=7.6, 1.8 Hz, 1H) 7.28 (t, *J*=7.6 Hz, 2H) 7.23 (d, *J*=8.2 Hz, 2H) 7.19 (q, *J*=7.7 Hz, 3H) 6.87 (d, *J*=7.6 Hz, 1H) 6.81 (d, *J*=7.6 Hz, 1H) 6.79 - 6.72 (m, 2H) 3.75 (s, 3H) 3.38 (dt, *J*=13.5, 6.7 Hz, 1H) 3.24 - 3.15 (m, 2H) 3.11 (bd, *J*=9.4 Hz, 1H) 2.88 - 2.82 (m, 2H) 2.81 (m, 2H) 2.75 - 2.64 (m, 4H) 2.57 (dt, *J*=13.5, 6.7 Hz, 1H) 2.37 (t, *J*=11.2 Hz, 1H) 2.32 (td, *J*=11.4, 3.5 Hz, 1H) 1.82 - 1.92 (m, 2H).
^{13}C NMR (151 MHz, CD_3OD) δ 173.3, 159.8, 158.7, 148.3, 144.7, 139.5, 137.1, 129.1, 128.3, 128.1, 125.9, 123.6, 121.6, 119.5, 113.0, 111.7, 59.9, 56.1, 54.2, 53.2, 49.6, 44.5, 38.5, 36.7, 32.5, 32.1.

***Trans*-*N*-(2-chlorobenzyl)-4-(pyridin-3-yl)pyrrolidine-3-carboxamide (9):**
Step 1: A round bottomed flask was charged with racemic-*trans*-1-(*tert*-butoxycarbonyl)-4-(pyridin-3-yl)pyrrolidine-3-carboxylic acid (**8**, 300 mg, 1.03 mmol), 2-chlorobenzyl amine (218 mg, 1.54 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (237 mg, 1.24 mmol), 1-hydroxy-1,2,3-benzotriazole (158 mg, 1.16 mmol), *N*,*N*-dimethylformamide (1 mL), and dichloromethane (5 mL), then stirred at room temperature for 15 h. The reaction was diluted with dichloromethane and 1M $NaHSO_4$ was added. The layers were separated and the organic layer was washed with 1M $NaHSO_4$, saturated sodium bicarbonate solution, brine and then dried over sodium sulfate. The mixture was filtered and

concentrated *in vacuo* to provide *trans*-1-(*tert*-butoxycarbonyl)-4-(pyridin-3-yl)pyrrolidine-3-carboxylic acid (220 mg, 52% yield) that was taken on to the next step without further purification.
LCMS (AP^+): Calc'd for $C_{22}H_{26}ClN_3O_3$ (M+H): 416.2 found: 416.1.
Step 2: A round bottomed flask was charged with the product from step 1 (220 mg, 0.529 mmol), 5 mL dichloromethane, and 0.8 mL TFA (10 mmol), then was stirred at room temperature for 15 h. The reaction was concentrated *in vacuo* and the residue dissolved in methanol. A spatula tip of potassium carbonate was added and the mixture was stirred for 5 minutes, filtered through celite, and concentrated *in vacuo* to provide 500 mg of a crude product **9** that was taken onto the next step without further purification.
LCMS (ES^+): Calc'd for $C_{17}H_{18}ClN_3O$ (M+H): 316.1 found: 316.0.

_N_-(2-Chlorobenzyl)-1-((2-ethyl-1_H_-imidazol-4-yl)methyl)-4-(pyridin-3-yl)pyrrolidine-3-carboxamide [(+)-*trans*-5 and (–)-*trans*-5]:
A round bottomed flask was charged with racemic-*trans*-*N*-(2-chlorobenzyl)-4-(pyridin-3-yl)pyrrolidine-3-carboxamide (**9**, 167 mg, 0.529 mmol), 2-ethyl-1H-imidazole-5-carbaldehyde (**10**, 79 mg, 0.64 mmol), acetic acid (0.03 mL, 0.53 mmol), 1,2-dichloroethane (5 mL), and methanol (3 mL). Sodium triacetoxyborohydride (157 mg, 0.741 mmol) was added and the reaction was stirred at room temperature for 15 h. The reaction was quenched with the addition of 1M HCl. The aqueous layer was washed twice with methyl *tert*-butyl ether, basified with 2M NaOH, and extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash column chromatography (5% to 10% MeOH in dichloromethane with 0.5% ammonium hydroxide) was then used to provide 110 mg of the racemic title compound.
HRMS (ES^+): Calc'd for $C_{23}H_{26}ClN_5O$ (M+H): 424.1899 found**:** 424.1911.
^{1}H NMR (400 MHz, CD_3OD) δ ppm 8.47 (d, *J*=2.0 Hz, 1H) 8.41 (dd, *J*=5.1, 1.6 Hz, 1H) 7.84 (dt, *J*=8.2, 2.0 Hz, 1H) 7.34 - 7.43 (m, 2H) 7.20 - 7.28 (m, 2H) 7.12 - 7.19 (m, 1H) 6.85 (s, 1H) 4.49, 4.36 (AB q, *J*=15.6 Hz, 2H), 3.62 - 3.76 (m, 3H) 3.17 (dt, *J*=12.7, 8.7 Hz, 2H) 3.07 (q, *J*=7.8 Hz, 1H) 2.92 (dd, *J*=9.0, 7.4 Hz, 1H) 2.86 (dd, *J*=9.6, 7.2 Hz, 1H) 2.71 (q, *J*=7.4 Hz, 2H) 1.29 (t, *J*=7.6 Hz, 3 H).
^{13}C NMR (151 MHz, CD_3OD) δ 173.8, 149.8, 148.1, 147.1, 139.5, 135.9, 135.4, 133.0, 129.1, 128.9, 128.5, 126.7, 124.0, 60.2, 57.2, 52.8, 50.4, 45.2, 40.8, 21.0, 11.8.
This material was then subjected to chiral SFC (Column: Chiralpak AD-H 250×21.2 mm, 5μm with supercritical carbon dioxide (80)/methanol (20) and ammonium hydroxide (0.2); detection at 210 nm, 80.0 mL/min, 120 Bar backpressure) to provide the separated enantiomers.
Peak 1, (+)-*trans*-**5**, 34 mg obtained: $[\alpha]_D^{20}$ = + 42.8 (c = 1.0, MeOH).
Peak 2, (–)-*trans*-**5**, 37 mg obtained: $[\alpha]_D^{20}$ = – 37.7 (c = 1.0, MeOH).

Ca^{2+} flux assay:
CHO-k1 cells stably expressing recombinant hGPR39 (CHO-FlpIn hGPR39) were cultured in growth media (DMEM:F-12, 10% heat inactivated FBS, 1x L-glutamine, 1x pen/strep, 500 μg/ml hygromycin) until 70-80% confluency. Cells were harvested with cell dissociation solution, plated at 15,000 cells/well in 50 μL growth media, and incubated for 24 hours at 37 °C (95% O_2, 5% CO_2). After 24 h, media was removed from cell plates and replaced with 25 μL Fluo-4 No Wash Calcium Dye (Invitrogen, #F36205). Cell plates were incubated at 37 °C (95% O_2, 5% CO_2) for 40 minutes, followed by a 20 minute equilibration at room temperature. Compound half log serial dilutions were prepared at 100x final assay

concentration (FAC) in 100% DMSO and a 3x FAC compound plate was prepared by diluting a 1 μL/100% DMSO spot 1:33 in assay buffer (HBSS, 20 mM HEPES) containing either buffer or $ZnSO_4$ (120 μM or 300 μM). After 1 h incubation, cell and compound plates were positioned in the FLIPR Tetra (Molecular Devices). A 10 second baseline was measured followed by 12.5 μL compound addition and the read continued for a further 90 seconds, at one second intervals. Dose response curves were generated using the FLIPR Tetra Max-Min Stat file.

cAMP assay:

CHO-k1 cells stably expressing recombinant hGPR39 (CHO-FlpIn hGPR39) were cultured at 37 °C (95% O_2, 5% CO_2) in growth media (DMEM: F-12, 10% heat inactivated FBS, 1x L-glutamine, 1x pen/strep, 500 μg/mL hygromycin) until 70-80% confluency. Cells were harvested with cell dissociation solution and re-suspended in assay buffer (HBSS, 20 mM Hepes, 125 μM IBMX). 2.5 μL of 160 μM $ZnSO_4$ (40 μM FAC, prepared in assay buffer) was added to assay plates containing 100 nL of compound in DMSO, followed by 7.5 μL of the cell solution (2000 cells/well). Cells, compound, and $ZnSO_4$ were incubated for 30 minutes at room temperature. cAMP levels were determined using the Dynamic Range cAMP detection kit (Cisbio). Briefly, 5 μL/well of the cAMP-d2 reagent was added, followed by 5 μL/well of the cAMP-cryptate reagent. Detection reagents were incubated for 60 minutes at room temperature, after which plates were read using the Perkin Elmer Envision (Channel 1: 340 nm_{ex} and 665 nm_{em}, Channel 2: 340 nm_{ex} and 615 nm_{em}). Raw counts were interpolated using a cAMP standard curve to quantify the nM amount of cAMP generated by the cells in each well.

Knockdown of GPR39 in HT29 cells:

HT29 human intestinal epithelial cells (ATCC HTB-38) were cultured in McCoy's 5A media (Invitrogen #16600) with 10% FBS. Cells were trypsinized to release and 1 x 10^6 cells were re-suspended in 100 μL of room temperature Nucleofector Solution according the kit instructions (Lonza # VCA-1001). GPR39 Silencer Select siRNA (4 μL, Ambion #s6072, GGCACTATTTCATTAGTTT) or scrambled control siRNA (Ambion #4390843) was added and cells were electroporated on a Nucleofector 2B instrument (Lonza) utilizing the W-017 program optimized for HT29 cells. Cells were allowed to recover by addition of 80 μL of 37°C RPMI 1640 for 15 minutes. Growth media (4 mL) was added and cells were plated in replicate plates for RNA isolation and FLIPR assay. A non-transfected control cell plate was also seeded. At 24-, 48-, and 72-h post-transfection, cells were analyzed by qRT-PCR for GPR39 gene expression levels. Cyclophilin B expression levels were used as the internal control. Briefly, cDNA was prepped using the High Capacity cDNA Reverse Transcription Kit (ABI #4374967) and RT-PCR was performed using the ABI Step One Real Time PCR System and custom primer probe sets [GPR39 forward (5' CCA GGA GGC ACA CCA TCA TC 3'), GPR39 reverse (5' CCA GCA TAC GGC CAA TGT C 3'), and GPR39 MGB probe (5' CTG AGG CTG ATT GTT G 3'), cyclophilin B forward (5' CCC ACC GTG TTC TTC GAC AT 3'), cyclophilin B reverse (5'TTT CTG CTG TCT TTG GGA CCT T 3') and cyclophilin MGB probe (ABI #4316034; 5' CCT TTG AGC TGT TTG CA 3')]. mRNA knockdown was 81% at 24 hr, 69% at 48 hr, and 48% at 72 hr. Cells from the 72 h time point were assayed for activity in the presence of 40 μM $ZnSO_4$ by FLIPR as described.

Insulin secretion assay in human islets:

Cultured human islets were pre-incubated in low glucose media (2.8 mM glucose; 0.75 h) to reduce insulin release to basal levels. Following pre-incubation, compound **1** was used at both low and stimulatory glucose concentrations (11.2 mM glucose) for 1 h and GSIS was determined by quantitation of insulin release into the supernatant (Alpco EIA).[2]

Table S4. CEREP screening data for compound **1**.

Cerep catalog reference	**Assay**	**Percent inhibition of control specific binding or enzyme activity**
439	solute carrier family 6 (neurotransmitter transporter, serotonin), member 4 (SLC6A4)	42
131	5-hydroxytryptamine (serotonin) receptor 1A (HTR1A)	15
471	5-hydroxytryptamine (serotonin) receptor 2A (HTR2A)	23.5
1333	5-hydroxytryptamine (serotonin) receptor 2B (HTR2B)	24.5
808	5-hydroxytryptamine (serotonin) receptor 2C (HTR2C)	23
411	5HT3 receptor	8.5
808-4eh	5-hydroxytryptamine (serotonin) receptor 4 (HTR4)	4
144	5-hydroxytryptamine (serotonin) receptor 7 (adenylate cyclase-coupled) (HTR7)	7.5
4	adenosine A2a receptor (ADORA2A)	20.5
3441	angiotensin I converting enzyme (peptidyl-dipeptidase A) 1 (ACE)	3
363	acetylcholinesterase (Yt blood group) (ACHE)	22.5
802-1bA	adrenergic, alpha-2A-, receptor (ADRA2A)	47
1344	adrenergic, alpha-2B-, receptor (ADRA2B)	56
821-1h	angiotensin II receptor, type 1 (AGTR1)	-25
20	adrenergic, beta-1-, receptor (ADRB1)	8
802-2bh	adrenergic, beta-2-, receptor, surface (ADRB2)	-5.67
802-3h	adrenergic, beta-3-, receptor (ADRB3)	-2
36	cannabinoid receptor 1 (brain) (CNR1)	0
37	cannabinoid receptor 2 (macrophage) (CNR2)	3
39	cholecystokinin A receptor (CCKAR)	-15
41	cholecystokinin B receptor (CCKBR)	-9
1552	solute carrier family 5 (choline transporter), member 7 (SLC5A7)	25.5
727	prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase) (PTGS2)	7.5
44	D1 dopamine receptor (Non-specific)	5.33
803-3h	dopamine receptor D3 (DRD3)	11
803-Uh	solute carrier family 6 (neurotransmitter transporter, dopamine), member 3 (SLC6A3)	37.7
114	opioid receptor, delta 1 (OPRD1)	-0.5
825-1h	endothelin receptor type A (EDNRA)	-3

825-2h	endothelin receptor type B (EDNRB)	7
469	nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor) (NR3C1)	1
870	histamine receptor H1 (HRH1)	57.7
805-2hc	histamine receptor H2 (HRH2)	-12
805-3h	histamine receptor H3 (HRH3)	9.5
806-1h	cholinergic receptor, muscarinic 1 (CHRM1)	16.3
806-2h	cholinergic receptor, muscarinic 2 (CHRM2)	27
806-3h	cholinergic receptor, muscarinic 3 (CHRM3)	19.5
118	opioid receptor, mu 1 (OPRM1)	6
936	Nicotinic acetylcholine receptor complex	19
355	solute carrier family 6 (neurotransmitter transporter, noradrenalin), member 2 (SLC6A2)	54
826-1h	tachykinin receptor 1 (TACR1)	28
2881	mitogen-activated protein kinase 14 (MAPK14)	-3
808-6h	5-hydroxytryptamine (serotonin) receptor 6 (HTR6)	2
801-3h	adenosine A3 receptor (ADORA3)	-6
802-1bCh	adrenergic, alpha-2C-, receptor (ADRA2C)	59
724-3	caspase 3, apoptosis-related cysteine peptidase (CASP3)	3
877-4h	chemokine (C-X-C motif) receptor 4 (CXCR4)	-4
900-2	cytochrome P450, family 2, subfamily C, polypeptide 19 (CYP2C19)	15
900-4	cytochrome P450, family 2, subfamily C, polypeptide 9 (CYP2C9)	35
900-3	cytochrome P450, family 3, subfamily A, polypeptide 4 (CYP3A4)	32
9900-3r	cytochrome P450, family 3, subfamily A, polypeptide 4 (CYP3A4)	-12
803-2ha	dopamine receptor D2 (DRD2)	10
805-4	histamine receptor H4 (HRH4)	16
887-h	cysteinyl leukotriene receptor 1 (CYSLTR1)	10
806-4h	cholinergic receptor, muscarinic 4 (CHRM4)	28
889-3h	melanocortin 3 receptor (MC3R)	-4
889-4h	melanocortin 4 receptor (MC4R)	-9
Cat. 849-1hc	melanin-concentrating hormone receptor 1 (MCHR1)	5
846-h	motilin receptor (MLNR)	-6
826-2h	tachykinin receptor 2 (TACR2)	30
752-e	phosphodiesterase 5A, cGMP-specific (PDE5A)	1
816-4h	somatostatin receptor 4 (SSTR4)	11
871-h	tumor necrosis factor receptor superfamily, member 1A (TNFRSF1A)	2
831-2h	arginine vasopressin receptor 2 (AVPR2)	0
832-1h	vasoactive intestinal peptide receptor 1 (VIPR1)	-4
781-zp	zeta-chain (TCR) associated protein kinase 70kDa (ZAP70)	0
801-2bh	adenosine A2b receptor (ADORA2B)	-2
933	androgen receptor (AR)	2
821-2h	angiotensin II receptor, type 2 (AGTR2)	13
823-2h	bradykinin receptor B2 (BDKRB2)	-2
822-3h	bombesin-like receptor 3 (BRS3)	3
724-9	caspase 9, apoptosis-related cysteine peptidase (CASP9)	1
877-2h	chemokine (C-C motif) receptor 2 (CCR2)	5
726	prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and	15

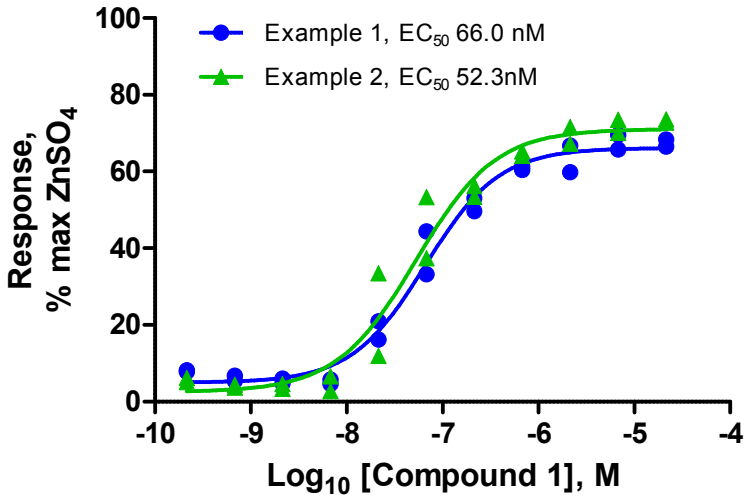
	cyclooxygenase) (PTGS1)	
900-5	cytochrome P450, family 2, subfamily B, polypeptide 6 (CYP2B6)	1
900-42	cytochrome P450, family 3, subfamily A, polypeptide 5 (CYP3A5)	18
813-ah	estrogen receptor 1 (ESR1)	-5
753-1	matrix metallopeptidase 1 (interstitial collagenase) (MMP1)	10
753-2	matrix metallopeptidase 2 (gelatinase A, 72kDa gelatinase, 72kDa type IV collagenase) (MMP2)	10
753-9	matrix metallopeptidase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase) (MMP9)	11
754-h	membrane metallo-endopeptidase (MME)	-4
888-h	opiate receptor-like 1 (OPRL1)	-5
781	v-yes-1 Yamaguchi sarcoma viral related oncogene homolog (LYN)	-1
830-h	platelet-activating factor receptor (PTAFR)	13
827-1h	neuropeptide Y receptor Y1 (NPY1R)	-2
895-5	Rat Glycine receptor (alpha1+beta or alpha3+beta)	-19
861-N	Rat N-type calcium channel (non-specific)	1
163	Rat L-type calcium channels (non-specific)	12.5
161	Rat L-type calcium channels (non-specific)	-6.5
162	Rat L-type calcium channels (non-specific)	14
28	Rat GABA A Receptor (GABA Binding Site)	-17
58	Rat GABA A Receptor (GABA Binding Site)	-15
64	Rat AMPA receptor (Glutamate receptors 1-4)	-5
8	Rat Alpha1 adrenergic receptor (Non-specific)	4
60	Rat GABA Transporter (Non-specific)	-8.5
895-2	Rat Ionotropic Kainate Receptor	-12
169	Rat Na+ (sodium) Channel (Non-specific)	12.3
807-n1	Rat Nicotinic achr neuronal (alpha-bgtx-insensitive)	6
895-3	Rat NMDA receptor (non specific)	21
855	Rat Thyroid hormone receptor (Non-specific)	4
443	monoamine oxidase A	12.5
132	5-hydroxytryptamine (serotonin) receptor 1B	7.5
809-1p	similar to nischarin, partial	-5
752-f	Bovine PDE6 (Phosphodiesterase 6; Non-specific)	-1
863-3	Rat SKCa channel (non-specific)	-9
170	Rat chloride channel (Non-specific)	10
889-1	melanocortin 1 receptor ()	4
891	opioid receptor, sigma 1	45
900-51	cytochrome P450, family 2, subfamily D, polypeptide 6 (CYP2D6)	1
819-h	glucagon receptor (GCGR)	-4
772-1s	Bos taurus xanthene dehydrogenase (XDH)	17
768-f	FYN oncogene related to SRC, FGR, YES (FYN)	-3
782	Pig Catechol O-methyltransferase (COMT)	18
900-1	cytochrome P450, family 1, subfamily A, polypeptide 2 (CYP1A2)	10
900-7	cytochrome P450, family 2, subfamily E, polypeptide 1 (CYP2E1)	0
900-3t	cytochrome P450, family 3, subfamily A, polypeptide 4 (CYP3A4)	13
900-3m	cytochrome P450, family 3, subfamily A, polypeptide 4 (CYP3A4)	7
1386	urotensin 2 receptor (UTS2R)	19.5

804-1b	gamma-aminobutyric acid (GABA) B receptor, 1 (GABBR1)	2
831-1ah	arginine vasopressin receptor 1A (AVPR1A)	6
124	Rat NMDA receptor (non specific)	3.5
854-gh	peroxisome proliferator-activated receptor gamma (PPARG)	0.5
885-hr	leukotriene B4 receptor 2 (LTB4R2)	66
900-46	cytochrome P450, family 2, subfamily C, polypeptide 8 (CYP2C8)	12
892-2	Hamster Melatonin-2 Target Placeholder	74
808-1dc	5-hydroxytryptamine (serotonin) receptor 1D	20
1971	opioid receptor, kappa 1	18.5
892-1h	melatonin receptor 1A (MTNR1A)	10
752-2a	phosphodiesterase 2A, cGMP-stimulated (PDE2A)	8
2705	phosphodiesterase 3B, cGMP-inhibited (PDE3B)	7
2434	phosphodiesterase 4D, cAMP-specific (phosphodiesterase E3 dunce homolog, Drosophila) (PDE4D)	1.5
3344	5-hydroxytryptamine (serotonin) receptor 2B (HTR2B)	1
1626	cholinergic receptor, muscarinic 2 (CHRM2)	19
442	adenosine A1 receptor (ADORA1)	5
3029	Combination: Nicotinic alpha-4/beta-2	3
1744	NA	-14
2885	Aurora Kinase (Non-specific)	-7
889	opioid receptor, sigma 1	77
3020	phosphodiesterase 1A, calmodulin-dependent (PDE1A)	5
3056	ABL Tyrosine Kinase (Non-specific)	-0.5
2338	ADRA1A/ADRA2A adrenergic alpha 1a and 2a	41.5

Table S5. Donor characteristics for human islet experiments.

	Age	**Sex**	**BMI**
Donor 1	47	F	27.1
Donor 2	35	M	35.4
Donor 3	60	M	27

Figure S6. Representative dose-response curves of **1** in hGPR39 Ca^{2+} assay.



References

(1) Corminboeuf, O.; Bezencon, O.; Remen, L.; Grisostomi, C.; Richard-Bildstein, S.; Bur, D.; Prade, L.; Strickner, P.; Hess, P.; Fischli, W.; Steiner, B.; Treiber, A. Piperidine-based renin inhibitors: Upper chain optimization. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6291-6296.

(2) Wijesekara, N.; Chimienti, F.; Wheeler, M. B. Zinc, a regulator of islet function and glucose homeostasis. *Diabetes, Obes. Metab.* **2009**, *11*, 202-214.