Supplemental Information

Identification and optimization of small molecule agonists of the human relaxin hormone receptor RXFP1

Jingbo Xiao[1,†], Zaohua Huang[2,†], Catherine Z. Chen[1], Irina U. Agoulnik[3], Noel Southall[1], Xin Hu[1], Raisa E. Jones[1], Marc Ferrer[1], Wei Zheng[1], Alexander I. Agoulnik[2,], and Juan J. Marugan[1,*]*

[1]NIH Chemical Genomics Center, Division of Pre-Clinical Innovation, National Center for Advancing Translational Sciences, National Institutes of Health, 9800 Medical Center Drive, Rockville, Maryland 20850, USA.

[2]Department of Human and Molecular Genetics, [3]Department of Cellular Biology and Pharmacology, Herbert Wertheim College of Medicine, Florida International University, 11200 SW 8th Street, Miami, Florida 33199, USA.

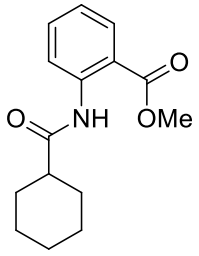
† These authors contributed equally to this project.

* To whom correspondence should be addressed: e-mail: maruganj@mail.nih.gov (J.J.M.); aagoulni@fiu.edu (A.I.A.).

Methods

General Methods for Chemistry.

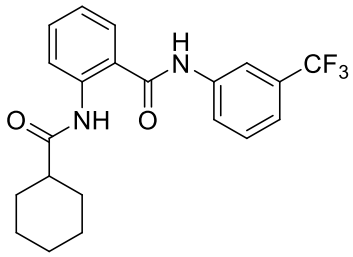
All air or moisture sensitive reactions were performed under positive pressure of nitrogen with oven-dried glassware. Anhydrous solvents such as dichloromethane, *N*,*N*-dimethylformamide (DMF), acetonitrile, methanol and triethylamine were purchased from Sigma-Aldrich (St. Louis, MO). Preparative purification was performed on a Waters semi-preparative HPLC system (Waters Corp., Milford, MA). The column used was a Phenomenex Luna C_{18} (5 micron, 30 x 75 mm; Phenomenex, Inc., Torrance, CA) at a flow rate of 45.0 mL/min. The mobile phase consisted of acetonitrile and water (each containing 0.1% trifluoroacetic acid). A gradient of 10% to 50% acetonitrile over 8 minutes was used during the purification. Fraction collection was triggered by UV detection at 220 nM. Analytical analysis was performed on an Agilent LC/MS (Agilent Technologies, Santa Clara, CA). Method 1: A 7-minute gradient of 4% to 100% acetonitrile (containing 0.025% trifluoroacetic acid) in water (containing 0.05% trifluoroacetic acid) was used with an 8-minute run time at a flow rate of 1.0 mL/min. Method 2: A 3-minute gradient of 4% to 100% acetonitrile (containing 0.025% trifluoroacetic acid) in water (containing 0.05% trifluoroacetic acid) was used with a 4.5-minute run time at a flow rate of 1.0 mL/min. A Phenomenex Luna C_{18} column (3 micron, 3 x 75 mm) was used at a temperature of 50 °C. Purity determination was performed using an Agilent diode array detector for both Method 1 and Method 2. Mass determination was performed using an Agilent 6130 mass spectrometer with electrospray ionization in the positive mode. ^{1}H NMR spectra were recorded on Varian 400 MHz spectrometers (Agilent Technologies, Santa Clara, CA). Chemical shifts are reported in ppm with undeuterated solvent (DMSO-d_6 at 2.49 ppm) as internal standard for DMSO-d_6 solutions. All of the analogs tested in the biological assays have a purity of greater than 95% based on both analytical methods. High resolution mass spectrometry was recorded on Agilent 6210 Time-of-Flight (TOF) LC/MS system. Confirmation of molecular formula was accomplished using electrospray ionization in the positive mode with the Agilent Masshunter software (Version B.02).



Methyl 2-(cyclohexanecarboxamido)benzoate. A solution of methyl 2-aminobenzoate (3.00 g, 19.9 mmol) in dichloromethane (100 mL) and triethylamine (8.30 mL, 59.5 mmol) was treated at 0 °C with cyclohexanecarbonyl chloride (2.70 mL, 19.9 mmol). The reaction was stirred at 0 °C for 2 h and room temperature for additional 2 h. The reaction mixture was concentrated and purified via silica gel chromatography using a gradient of 0-40% of EtOAc in hexanes to give 5.00 g (96%) of the title product as a white solid.

LC-MS Retention Time: t_1 (Method 1) = 6.453 min.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.65 (s, 1 H), 8.31 (dd, *J*=8.5, 1.1 Hz, 1 H), 7.90 (dd, *J*=7.9, 1.7 Hz, 1 H), 7.57 (ddd, *J*=8.6, 7.2, 1.7 Hz, 1 H), 6.87 - 7.29 (m, 1 H), 3.83 (s, 3 H), 2.31 (tt, *J*=11.3, 3.5 Hz, 1 H), 1.88 (dd, *J*=12.9, 2.5 Hz, 2 H), 1.73 (ddd, *J*=12.4, 3.3, 3.1 Hz, 2 H), 1.55 - 1.67 (m, 1 H), 1.08 - 1.47 (m, 5 H).



2-(Cyclohexanecarboxamido)-*N*-(3-(trifluoromethyl)phenyl)benzamide (1). A solution of methyl 2-(cyclohexanecarboxamido)benzoate (150 mg, 0.574 mmol) in toluene (6.00 mL) was treated at room temperature with 3-(trifluoromethyl)aniline (0.143 mL, 1.15 mmol) followed by trimethylaluminum (0.574 mL, 2.0 M in hexanes, 1.15 mmol). The reaction mixture was stirred at 100 °C overnight. After cooling, the reaction mixture was quenched with 100 µL of water and concentrated *in vacuo*. The crude residue was purified via silica gel chromatography using a gradient of 0-60% of EtOAc in hexanes to give 158 mg (71%) of the title compound as a white solid.

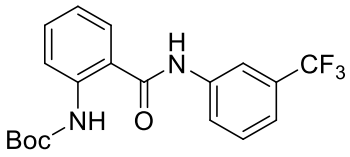
LC-MS Retention Time: t_1 (Method 1) = 6.910 min.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.62 (s, 1 H), 10.30 (s, 1 H), 8.13 (s, 1 H), 8.09 (dd, *J*=8.2, 0.8 Hz, 1 H), 7.96 (d, *J*=8.2 Hz, 1 H), 7.75 (dd, *J*=7.8, 1.6 Hz, 1 H), 7.59 (t, *J*=8.0 Hz, 1 H), 7.51 (td, *J*=7.8, 1.6 Hz, 1 H), 7.45 (dd, *J*=7.8, 0.8 Hz, 1 H), 7.22 (td, *J*=7.6, 1.2 Hz, 1 H), 2.28 (tt, *J*=11.2, 3.5 Hz, 1 H), 1.80 (dd, *J*=12.7, 2.2 Hz, 2 H), 1.68 (ddd, *J*=12.3, 3.1, 2.9 Hz, 2 H), 1.52 - 1.63 (m, 1 H), 1.04 - 1.42 (m, 5 H).

^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 173.94, 167.20, 139.67, 137.76, 131.75, 129.83, 129.30 (q, *J* = 32.3 Hz), 128.74, 124.57 (s), 124.12 (q, *J* = 273 Hz), 124.07 (s), 123.15 (s), 122.01 (s), 120.11 (q, *J* = 3.7 Hz), 116.67 (q, *J* = 4.0 Hz), 45.02, 28.99, 25.36, 25.04.

^{19}F NMR (376 MHz, DMSO-d_6) δ ppm -61.30 (s, 3 F).

HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{21}H_{22}F_3N_2O_2$, 391.1628; found 391.1632.



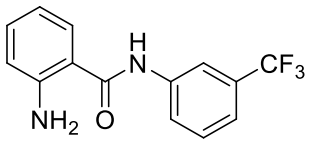
***tert*-Butyl 2-(3-(trifluoromethyl)phenylcarbamoyl)phenylcarbamate.** A solution of 2-(*tert*-butoxycarbonylamino)benzoic acid (3.00 g, 12.6 mmol) and 3-(trifluoromethyl)aniline (2.36 mL, 19.0 mmol) in dichloromethane (75.0 mL) was treated at room temperature with DMAP (1.55 g, 12.6 mmol) and EDC (4.85 g, 25.3 mmol) and stirred at room temperature for 24 h. The reaction mixture was concentrated and purified via silica gel chromatography using a gradient of 0-100% of dichloromethane in hexanes followed by 10% of EtOAc in dichloromethane to give 2.20 g (46%) of the title product as a white solid.

LC-MS Retention Time: t_1 (Method 1) = 7.042 min.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.64 (s, 1 H), 9.75 (s, 1 H), 8.14 (s, 1 H), 7.98 (d, *J*=7.6 Hz, 1 H), 7.94 (d, *J*=9.2 Hz, 1 H), 7.77 (dd, *J*=7.9, 1.5 Hz, 1 H), 7.58 (t, *J*=8.0 Hz, 1 H), 7.51 (ddd, *J*=8.5, 7.2, 1.5 Hz, 1 H), 7.45 (d, *J*=7.8 Hz, 1 H), 7.16 (td, *J*=7.6, 1.1 Hz, 1 H), 1.41 (s, 9 H).

^{19}F NMR (376 MHz, DMSO-d_6) δ ppm -61.20 (s, 3 F).

HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{19}H_{20}F_3N_2O_3$, 381.1421; found 381.1426.

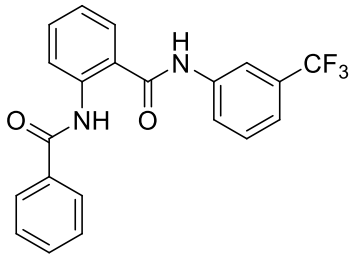


2-Amino-*N*-(3-(trifluoromethyl)phenyl)benzamide. A solution of *tert*-butyl 2-(3-(trifluoromethyl)phenylcarbamoyl)phenylcarbamate (2.11 g, 5.55 mmol) in dichloromethane (15.0 mL) was treated at 0 °C with TFA (5.34 mL, 69.3 mmol). The reaction mixture was stirred at 0 °C for 1 h and room temperature for additional 2 h. The reaction mixture was concentrated and re-dissolved in dichloromethane and washed with saturated Na_2CO_3 aqueous solution. The organic layer was separated, dried and concentrated to give 1.45 g (99%) of the title compound as a white solid.

LC-MS Retention Time: t_1 (Method 1) = 5.590 min.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.24 (s, 1 H), 8.18 (t, *J*=2.2 Hz, 1 H), 7.95 (ddd, *J*=8.4, 1.2, 1.0 Hz, 1 H), 7.63 (dd, *J*=8.0, 1.6 Hz, 1 H), 7.54 (t, *J*=8.3 Hz, 1 H), 7.34 - 7.43 (m, 1 H), 7.19 (ddd, *J*=8.4, 7.0, 1.6 Hz, 1 H), 6.74 (dd, *J*=8.3, 1.3 Hz, 1 H), 6.57 (ddd, *J*=8.1, 7.0, 1.2 Hz, 1 H), 6.34 (br. s., 2 H).

^{19}F NMR (376 MHz, DMSO-d_6) δ ppm -61.18 (s, 3 F).



2-Benzamido-*N*-(3-(trifluoromethyl)phenyl)benzamide (3). A solution of 2-amino-*N*-(3-(trifluoromethyl)phenyl)benzamide (50.0 mg, 0.178 mmol) in dichloromethane (2.00 mL) and TEA (0.075 mL, 0.535 mmol) treated at room temperature with benzoyl chloride (0.041 mL, 0.357 mmol). The reaction was stirred at room temperature for 2 h. The mixture was concentrated, re-dissolved in DMSO, filtered and purified via C_{18} reverse phase HPLC to give 35.0 mg (51%) of the title compound as a white solid.

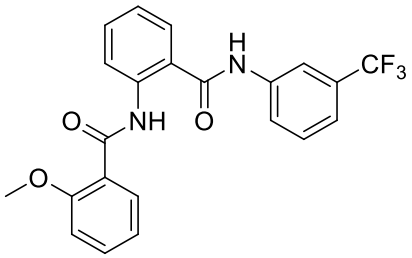
LC-MS Retention Time: t_1 (Method 1) = 6.644 min.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.37 (s, 1 H), 10.78 (s, 1 H), 8.35 (d, *J*=8.2 Hz, 1 H), 8.11 (s, 1 H), 8.02 (d, *J*=8.2 Hz, 1 H), 7.84 - 7.96 (m, 3 H), 7.51 - 7.70 (m, 5 H), 7.48 (d, *J*=8.2 Hz, 1 H), 7.32 (t, *J*=7.4 Hz, 1 H).

^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 167.56, 164.80, 139.48, 138.22, 134.47, 132.26, 131.98, 129.89, 129.33 (q, *J* = 32.3 Hz), 129.02, 128.79, 127.11, 124.40, 124.08 (q, *J* = 274 Hz), 123.79, 123.54, 121.93, 120.35 (q, *J* = 3.7 Hz), 116.96 (q, *J* = 4.0 Hz).

^{19}F NMR (376 MHz, DMSO-d_6) δ ppm -61.28 (s, 3 F).

HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{21}H_{16}F_3N_2O_2$, 385.1158; found 385.1163.



2-Methoxy-*N*-(2-((3-(trifluoromethyl)phenyl)carbamoyl)phenyl)benzamide (4). A solution of 2-amino-*N*-(3-(trifluoromethyl)phenyl)benzamide (50.0 mg, 0.178 mmol) in dichloromethane (2.00 mL) and TEA (0.075 mL, 0.535 mmol) was treated at room temperature with 2-methoxybenzoyl chloride (0.036 mL, 0.268 mmol). The reaction was stirred at room temperature overnight. The mixture was concentrated, re-dissolved in DMSO, filtered and purified via C_{18} reverse phase HPLC to give 40.0 mg (54%) of the title compound as a white solid.

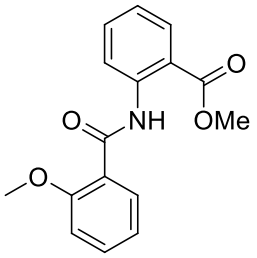
LC-MS Retention Time: t_1 (Method 1) = 6.734 min.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.57 (s, 1 H), 10.86 (s, 1 H), 8.59 (d, *J*=8.2 Hz, 1 H), 8.35 (t, *J*=2.2 Hz, 1 H), 8.04 (dd, *J*=7.8, 2.0 Hz, 1 H), 8.01 (d, *J*=9.0 Hz, 1 H), 7.81 (dd, *J*=7.8, 1.6 Hz, 1 H), 7.53 - 7.67 (m, 3 H), 7.49 (d, *J*=7.8 Hz, 1 H), 7.28 (td, *J*=7.6, 1.2 Hz, 1 H), 7.22 (d, *J*=8.2 Hz, 1 H), 7.07 - 7.15 (m, 1 H), 3.99 (s, 3 H).

^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 167.33, 163.00, 157.15, 139.67, 137.64, 133.67, 131.78, 131.50, 129.97, 129.40 (q, *J* = 32.3 Hz), 128.55, 125.46, 124.41, 123.70, 123.11, 122.20 (q, *J* = 242 Hz), 121.97, 120.84, 120.28 (q, *J* = 4.0 Hz), 116.25 (q, *J* = 4.0 Hz), 112.25, 55.58.

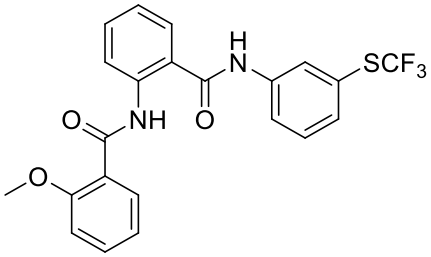
^{19}F NMR (376 MHz, DMSO-d_6) δ ppm -61.41 (s, 3 F).

HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{22}H_{18}F_3N_2O_3$, 415.1264; found 415.1266.



Methyl 2-(2-methoxybenzamido)benzoate. A solution of 2-aminobenzoate (2.57 mL, 19.9 mmol) in dichloromethane (50.0 mL) and TEA (8.30 mL, 59.5 mmol) was treated at 0 °C with 2-methoxybenzoyl chloride (2.67 mL, 19.9 mmol). The reaction mixture was stirred at 0 °C for 2 h and at room temperature for 2 h. The reaction mixture was concentrated and purified via silica gel chromatography using a gradient of 0-50% of EtOAc in hexanes to give 5.50 g (97%) of the title compound as a white solid.

LC-MS Retention Time: t_2 (Method 2) = 3.761 min; *m/z* $(M+H)^+$ 286.0.



2-Methoxy-*N*-(2-((3-((trifluoromethyl)thio)phenyl)carbamoyl)phenyl)benzamide (5). A solution of methyl 2-(2-methoxybenzamido)benzoate (200 mg, 0.701 mmol) in toluene (1.50 mL) was treated at room temperature with 3-(trifluoromethylthio)aniline (271 mg, 1.40 mmol) followed by trimethylaluminum (0.700 mL, 2.0 M in hexanes, 1.40 mmol). The reaction mixture was stirred at 100 °C overnight. After cooling, the reaction mixture was quenched with 100 µL of water and concentrated *in vacuo*. The crude residue was purified via silica gel chromatography using a gradient of 0-60% of EtOAc in hexanes to give 188 mg (60%) of the title compound as a white solid.

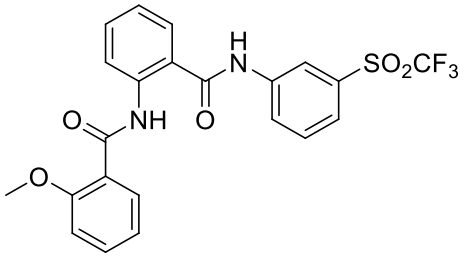
LC-MS Retention Time: t_1 (Method 1) = 7.128 min.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.55 (s, 1 H), 10.81 (s, 1 H), 8.58 (d, *J*=8.2 Hz, 1 H), 8.33 (t, *J*=2.2 Hz, 1 H), 8.03 (dd, *J*=7.8, 2.0 Hz, 1 H), 7.93 (ddd, *J*=8.2, 2.3, 1.2 Hz, 1 H), 7.80 (dd, *J*=7.8, 1.6 Hz, 1 H), 7.52 - 7.63 (m, 3 H), 7.42 - 7.51 (m, 1 H), 7.27 (td, *J*=7.6, 1.2 Hz, 1 H), 7.17 - 7.24 (m, 1 H), 7.06 - 7.16 (m, 1 H), 3.98 (s, 3 H).

^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 167.23, 162.98, 157.15, 140.08, 137.59, 133.68, 131.75, 131.50, 131.32, 130.33, 129.67 (q, *J* = 310 Hz), 128.60, 126.96, 124.47, 123.25 (q, *J* = 2.36 Hz), 123.11, 122.80, 121.95, 121.27, 120.85, 112.25, 55.61.

^{19}F NMR (376 MHz, DMSO-d_6) δ ppm -41.92 (s, 3 F).

HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{22}H_{18}F_3N_2O_3S$, 447.0985; found 447.0984.



2-Methoxy-*N*-(2-((3-((trifluoromethyl)sulfonyl)phenyl)carbamoyl)phenyl)benzamide (6). A solution of 2-methoxy-*N*-(2-(3-(trifluoromethylthio)phenylcarbamoyl)phenyl)benzamide (100 mg, 0.224 mmol) in dichloromethane (3.00 mL) was treated at room temperature with MCPBA (193 mg, 1.12 mmol). The reaction mixture was stirred at room temperature overnight and quenched with saturated $Na_2S_2O_3$ solution. The organic layer was separated, dried and concentrated. The crude residue was re-dissolved in DMSO, filtered and purified via C_{18} reverse phase HPLC to give 70.0 mg (65%) of the title compound as a white solid.

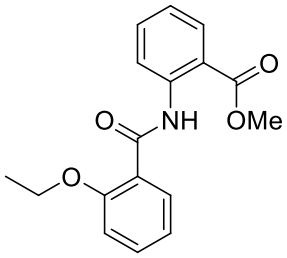
LC-MS Retention Time: t_1 (Method 1) = 6.810 min.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.59 (s, 1 H), 11.11 (s, 1 H), 8.81 - 8.87 (m, 1 H), 8.59 (dd, *J*=8.6, 0.8 Hz, 1 H), 8.25 (dt, *J*=7.1, 2.3 Hz, 1 H), 8.04 (dd, *J*=7.8, 2.0 Hz, 1 H), 7.81 - 7.93 (m, 3 H), 7.52 - 7.67 (m, 2 H), 7.29 (td, *J*=7.6, 1.2 Hz, 1 H), 7.23 (dd, *J*=8.6, 0.8 Hz, 1 H), 7.11 (td, *J*=7.5, 1.0 Hz, 1 H), 4.01 (s, 3 H).

^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 167.60, 163.03, 157.18, 140.77, 137.78, 133.73, 132.08, 131.53, 131.39, 129.94 (q, *J* = 1.0 Hz), 128.72, 128.45, 125.61, 123.93, 123.13, 122.08, 121.22, 120.86, 120.78, 119.43 (q, *J* = 328 Hz), 112.27, 55.61.

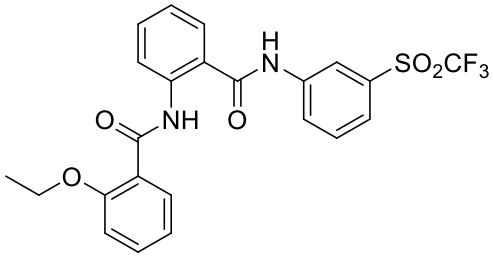
^{19}F NMR (376 MHz, DMSO-d_6) δ ppm -78.39 (s, 3 F).

HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{22}H_{18}F_3N_2O_5S$, 479.0883; found 479.0886.



Methyl 2-(2-ethoxybenzamido)benzoate. A solution of ethyl methyl 2-aminobenzoate (1.29 mL, 9.92 mmol) in dichloromethane (25.0 mL) and triethylamine (4.15 mL, 29.8 mmol) was treated at 0 °C with 2-ethoxybenzoyl chloride (1.83 g, 9.92 mmol). The reaction mixture was stirred at 0 °C for 2 h and at room temperature for 2 h. The reaction mixture was concentrated and purified via silica gel chromatography using a gradient of 0-50% of EtOAc in hexanes to give 2.70 g (91%) of the title product as a white solid which was used directly in the next reaction without further purification.

LC-MS Retention Time: t_2 (Method 2) = 3.908 min; *m/z* $(M+H)^+$ 300.1.



2-Ethoxy-*N*-(2-((3-((trifluoromethyl)sulfonyl)phenyl)carbamoyl)phenyl)benzamide (7). A solution of methyl 2-(2-ethoxybenzamido)benzoate (104 mg, 0.347 mmol) in toluene (1.50 mL) was treated at room temperature with 3-(trifluoromethylsulfonyl)aniline (117 mg, 0.521 mmol) followed by trimethylaluminum (0.521 mL, 2.0 M in hexanes, 1.04 mmol). The reaction mixture was stirred at 100 °C overnight. After cooling, the reaction mixture was quenched with 100 µL of water and concentrated *in vacuo*. The crude residue was purified via silica gel chromatography using a gradient of 0-80% of EtOAc in hexanes to give 131 mg (77%) of the title compound as a white solid.

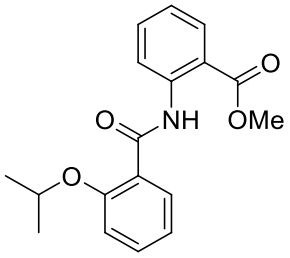
LC-MS Retention Time: t_1 (Method 1) = 6.963 min.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.35 (s, 1 H), 11.10 (s, 1 H), 8.76 (t, *J*=2.2 Hz, 1 H), 8.53 (d, *J*=8.2 Hz, 1 H), 8.23 (dt, *J*=7.1, 2.3 Hz, 1 H), 7.96 (dd, *J*=7.8, 2.0 Hz, 1 H), 7.80 - 7.92 (m, 3 H), 7.57 - 7.65 (m, 1 H), 7.53 (ddd, *J*=8.6, 7.0, 2.0 Hz, 1 H), 7.30 (td, *J*=7.6, 1.2 Hz, 1 H), 7.22 (d, *J*=8.2 Hz, 1 H), 7.04 - 7.10 (m, 1 H), 4.31 (q, *J*=6.8 Hz, 2 H), 1.31 (t, *J*=6.8 Hz, 3 H).

^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 167.44, 163.39, 156.00, 140.75, 137.73, 133.44, 132.10, 131.48, 131.34, 129.93, 128.81, 128.49, 125.58, 123.86, 123.21, 122.27, 122.08, 120.81, 120.67, 119.41 (q, *J* = 328 Hz), 113.00, 64.09, 13.80.

^{19}F NMR (376 MHz, DMSO-d_6) δ ppm -78.40 (s, 3 F).

HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{23}H_{20}F_3N_2O_5S$, 493.1040; found 493.1044.

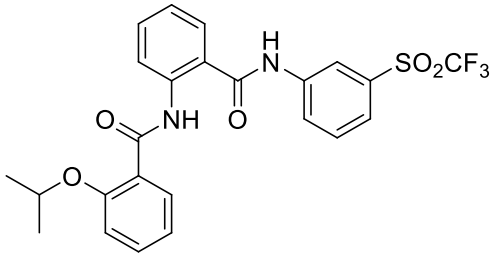


Methyl 2-(2-isopropoxybenzamido)benzoate. A solution of methyl 2-aminobenzoate (0.245 mL, 1.89 mmol) in dichloromethane (8.00 mL) and triethylamine (0.53 mL, 3.78 mmol) was treated at 0 °C with 2-isopropoxybenzoyl chloride (250 mg, 1.26 mmol). The reaction mixture was stirred at 0 °C for 2 hours and then at room temperature for another 2 hours. The reaction mixture was concentrated *in vacuo* and the crude residue was purified via silica gel chromatography using a gradient of 0-50% of EtOAc in hexanes to give 320 mg (81%) of the title compound as a colorless oil.

LC-MS Retention Time: t_1 (Method 1) = 6.576 min; t_2 (Method 2) = 3.825 min.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.59 (s, 1 H), 8.63 (d, *J*=8.6 Hz, 1 H), 7.98 (dd, *J*=7.8, 1.6 Hz, 1 H), 7.83 (dd, *J*=7.8, 2.0 Hz, 1 H), 7.64 (ddd, *J*=8.5, 7.1, 1.6 Hz, 1 H), 7.45 - 7.56 (m, 1 H), 7.13 - 7.27 (m, 2 H), 6.94 - 7.09 (m, 1 H), 4.82 (dq, *J*=6.3, 6.0 Hz, 1 H), 3.85 (s, 3 H), 1.35 (d, *J*=5.9 Hz, 6 H).

HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{18}H_{20}NO_4$, 314.1387; found 314.1399.



2-Isopropoxy-*N*-(2-(3-(trifluoromethylsulfonyl)phenylcarbamoyl)phenyl)benzamide (8). A solution of methyl 2-(2-isopropoxybenzamido)benzoate (110 mg, 0.351 mmol) in toluene (4.00 mL) was treated at room temperature with 3-(trifluoromethylsulfonyl)aniline (119 mg, 0.527 mmol) followed by trimethylaluminum (0.503 mL, 2.0 M in toluene, 1.06 mmol). The reaction mixture was stirred at 100 °C overnight. After cooling, the reaction mixture was quenched with 100 µL of water and concentrated *in vacuo*. The crude residue was purified via silica gel chromatography using a gradient of 0-80% of EtOAc in hexanes to give 130 mg (73%) of the title compound as a white solid.

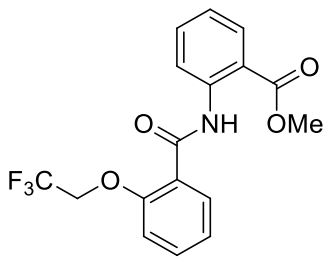
LC-MS Retention Time: t_1 (Method 1) = 6.974 min; t_2 (Method 2) = 3.881 min.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.11 (s, 1 H), 11.09 (s, 1 H), 8.75 (s, 1 H), 8.48 (d, *J*=8.2 Hz, 1 H), 8.20 (dt, *J*=7.4, 2.2 Hz, 1 H), 7.91 (dd, *J*=7.8, 1.6 Hz, 1 H), 7.77 - 7.89 (m, 3 H), 7.57 - 7.66 (m, 1 H), 7.51 (ddd, *J*=8.6, 7.0, 2.0 Hz, 1 H), 7.30 (td, *J*=7.5, 1.0 Hz, 1 H), 7.20 (d, *J*=8.6 Hz, 1 H), 7.05 (t, *J*=7.4 Hz, 1 H), 4.75 - 4.85 (m, 1 H), 1.32 (d, *J*=5.9 Hz, 6 H).

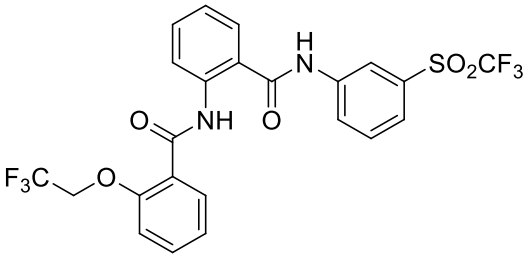
^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 167.24, 163.63, 155.29, 140.75, 137.61, 133.30, 132.06, 131.52, 131.28, 129.97 (br.), 128.89, 128.51, 125.58, 123.93, 123.29, 122.79, 122.56, 120.82, 120.44, 119.40 (q, *J* = 328 Hz), 113.98, 71.19, 21.05.

^{19}F NMR (376 MHz, DMSO-d_6) δ ppm -78.38 (s, 3 F).

HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{24}H_{22}F_3N_2O_5S$, 507.1196; found 507.1221.



Methyl 2-(2-(2,2,2-trifluoroethoxy)benzamido)benzoate. A solution of 2-(2,2,2-trifluoroethoxy)benzoic acid (250 mg, 1.14 mmol) in dichloromethane (8.00 mL) and HATU (432 mg, 1.14 mmol) was treated at room temperature with methyl 2-aminobenzoate (0.147 mL, 1.14 mmol). The reaction was stirred at room temperature overnight. The mixture was concentrated *in vacuo* and purified via silica gel chromatography using a gradient of 0-80% of EtOAc in hexanes to give 281 mg (70%) of the title compound.



2-(2,2,2-Trifluoroethoxy)-*N*-(2-((3 ((trifluoromethyl)sulfonyl)phenyl)carbamoyl)phenyl)benzamide (9). A solution of methyl 2-(2-(2,2,2-trifluoroethoxy)benzamido)benzoate (150 mg, 0.425 mmol) in toluene (4.00 mL) was treated at room temperature with 3-(trifluoromethylsulfonyl)aniline (143 mg, 0.637 mmol) followed by trimethylaluminum (0.637 mL, 2.0 M in toluene, 1.06 mmol). The reaction mixture was stirred at 100 °C overnight. After cooling, the reaction mixture was quenched with 100 µL of water and concentrated *in vacuo*. The crude residue was purified via silica gel chromatography using a gradient of 0-80% of EtOAc in hexanes to give 130 mg (73%) of the title compound as a white solid.

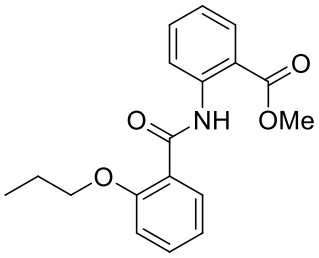
LC-MS Retention Time: t_1 (Method 1) = 6.804 min.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.10 (s, 1 H), 11.05 (s, 1 H), 8.64 (s, 1 H), 8.44 (d, *J*=8.2 Hz, 1 H), 8.27 (dt, *J*=7.1, 2.3 Hz, 1 H), 7.79 - 7.92 (m, 4 H), 7.60 - 7.69 (m, 1 H), 7.52 - 7.60 (m, 1 H), 7.37 (d, *J*=8.2 Hz, 1 H), 7.31 (t, *J*=7.6 Hz, 1 H), 7.19 (t, *J*=7.4 Hz, 1 H), 4.94 (q, *J*=9.0 Hz, 2 H).

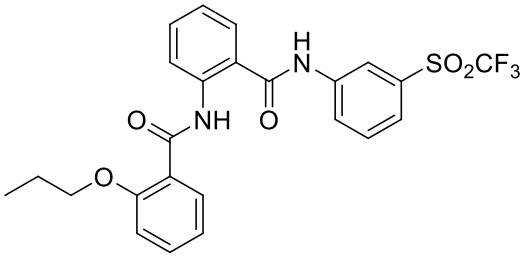
^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 167.46, 163.36, 154.24, 140.76, 137.68, 132.87, 132.27, 131.25, 130.85, 129.87, 128.99, 128.60, 125.45, 124.28, 123.83, 123.63 (q, *J* = 280 Hz), 123.49, 122.52, 121.97, 121.05, 119.42 (q, *J* = 329 Hz), 114.03, 65.00 (q, *J* = 35.0 Hz).

^{19}F NMR (376 MHz, DMSO-d_6) δ ppm -72.46 (t, *J*=9.4 Hz, 3 F), -78.48 (s, 3 F).

HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{23}H_{17}F_6N_2O_5S$, 547.0757; found 547.0762.



Methyl 2-(2-propoxybenzamido)benzoate. A solution of 2-propoxybenzoic acid (250 mg, 1.39 mmol) in dichloromethane (8.00 mL) and HATU (528 mg, 1.39 mmol) was treated at room temperature with methyl 2-aminobenzoate (0.180 mL, 1.39 mmol). The reaction was stirred at room temperature overnight. The mixture was concentrated *in vacuo* and purified via silica gel chromatography using a gradient of 0-100% of EtOAc in hexanes to give 400 mg (92%) of the title compound as a colorless oil.



2-Propoxy-*N*-(2-((3-((trifluoromethyl)sulfonyl)phenyl)carbamoyl)phenyl)benzamide (10). A solution of methyl 2-(2-propoxybenzamido)benzoate (150 mg, 0.479 mmol) in toluene (3.00 mL) was treated at room temperature with 3-(trifluoromethylsulfonyl)aniline (162 mg, 0.718 mmol) followed by trimethylaluminum (0.718 mL, 2.0 M in toluene, 1.44 mmol). The reaction mixture was stirred at 110 °C overnight. After cooling, the reaction mixture was quenched with 100 µL of water and concentrated *in vacuo*. The crude residue was purified via silica gel chromatography using a gradient of 0-80% of EtOAc in hexanes to give 84.0 mg (35%) of the title compound as a white solid.

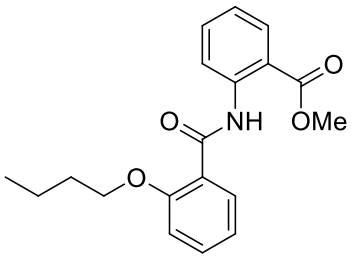
LC-MS Retention Time: t_1 (Method 1) = 7.147 min.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.26 (s, 1 H), 11.10 (s, 1 H), 8.72 (s, 1 H), 8.54 (d, *J*=8.6 Hz, 1 H), 8.24 (dt, *J*=7.1, 2.3 Hz, 1 H), 7.94 (dd, *J*=7.8, 2.0 Hz, 1 H), 7.78 - 7.91 (m, 3 H), 7.56 - 7.67 (m, 1 H), 7.52 (ddd, *J*=8.5, 7.1, 2.0 Hz, 1 H), 7.25 - 7.33 (m, 1 H), 7.20 (d, *J*=8.2 Hz, 1 H), 7.07 (t, *J*=7.4 Hz, 1 H), 4.16 (t, *J*=7.0 Hz, 2 H), 1.71 (sxt, *J*=7.2 Hz, 2 H), 0.77 (t, *J*=7.4 Hz, 3 H).

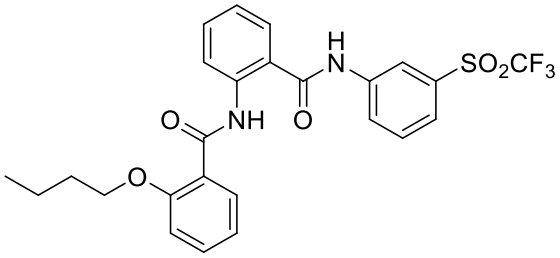
^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 167.42, 163.49, 156.14, 140.78, 137.75, 133.37, 132.14, 131.39, 131.33, 129.95 (br.), 128.90, 128.48, 125.54, 123.80, 123.21, 122.25, 122.15, 120.88, 120.68, 119.43 (q, J = 328 Hz), 113.09, 69.82, 21.24, 9.74.

^{19}F NMR (376 MHz, DMSO-d_6) δ ppm -78.43 (s, 3 F).

HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{24}H_{22}F_3N_2O_5S$, 507.1196; found 507.1219.



Methyl 2-(2-butoxybenzamido)benzoate. A solution of 2-butoxybenzoic acid (300 mg, 1.545 mmol) in dichloromethane (8.00 mL) and HATU (587 mg, 1.55 mmol) was treated at room temperature with methyl 2-aminobenzoate (0.300 mL, 2.32 mmol). The reaction was stirred at room temperature overnight. The mixture was concentrated *in vacuo* and purified via silica gel chromatography using a gradient of 0-100% of EtOAc in hexanes to give 400 mg (79%) of the title compound as a colorless oil.



2-Butoxy-*N*-(2-((3-((trifluoromethyl)sulfonyl)phenyl)carbamoyl)phenyl)benzamide (11). A solution of methyl 2-(2-butoxybenzamido)benzoate (150 mg, 0.458 mmol) in toluene (3.00 mL) was treated at room temperature with 3-(trifluoromethylsulfonyl)aniline (155 mg, 0.687 mmol) followed by trimethylaluminum (0.688 mL, 2.0 M in toluene, 1.38 mmol). The reaction mixture was stirred at 110 °C overnight. After cooling, the reaction mixture was quenched with 100 µL of water and concentrated *in vacuo*. The crude residue was purified via silica gel chromatography using a gradient of 0-80% of EtOAc in hexanes to give 72.0 mg (30%) of the title compound as a white solid.

LC-MS Retention Time: t_1 (Method 1) = 7.325 min; t_2 (Method 2) = 3.971 min.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.27 (s, 1 H), 11.10 (s, 1 H), 8.71 (s, 1 H), 8.55 (d, *J*=8.2 Hz, 1 H), 8.26 (dt, *J*=7.3, 2.0 Hz, 1 H), 7.93 (dd, *J*=7.8, 2.0 Hz, 1 H), 7.81 - 7.91 (m, 3 H), 7.58 - 7.68 (m, 1 H), 7.47 - 7.57 (m, 1 H), 7.29 (t, *J*=7.4 Hz, 1 H), 7.22 (d, *J*=8.2 Hz, 1 H), 7.08 (t, *J*=7.4 Hz, 1 H), 4.19 (t, *J*=6.8 Hz, 2 H), 1.57 - 1.73 (m, 2 H), 1.23 (sxt, *J*=7.5 Hz, 2 H), 0.70 (t, *J*=7.4 Hz, 3 H).

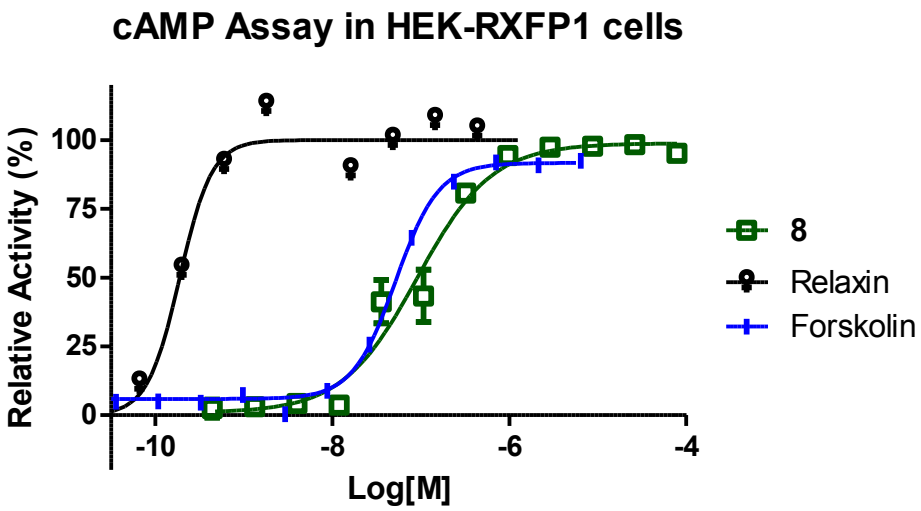
^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 167.41, 163.52, 156.13, 140.81, 137.81, 133.37, 132.20, 131.36, 131.34, 129.96 (br.), 128.94, 128.41, 125.54, 123.59, 123.18, 122.30, 122.06, 120.85, 120.69, 113.09, 68.19, 29.93, 18.34, 13.37.

^{19}F NMR (376 MHz, DMSO-d_6) δ ppm -78.42 (s, 3 F).

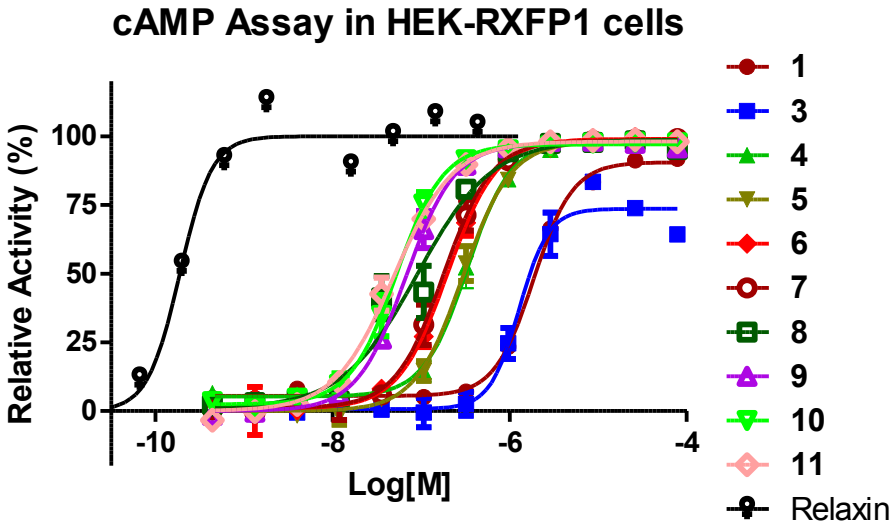
HRMS (ESI) *m/z* $(M+H)^+$ calcd. for $C_{25}H_{24}F_3N_2O_5S$, 521.1353; found 521.1359.

Supplemental Figure S1 a-f. Concentration-response curves from selected assays for tested compounds. a. cAMP assay in HEK-RXFP1 cells for relaxin, forskolin and compound **8**; **b.** cAMP assay in HEK-RXFP1 cells for compounds **1** and **3-11**; **c.** cAMP assay in THP1 cells; **d.** cAMP assay in HEK-RXFP2 cells; **e.** cAMP assay in HEK-V1b cells; and **f.** ATP cytotoxicity in HEK-RXFP1 cells. Compounds were run in triplicate, with 12-point titrations over the presented concentration range. Responses are plotted as the mean values ± s.e.m for relative activity, normalized to positive control (100%) and vehicle control (0%), versus log molar concentration.

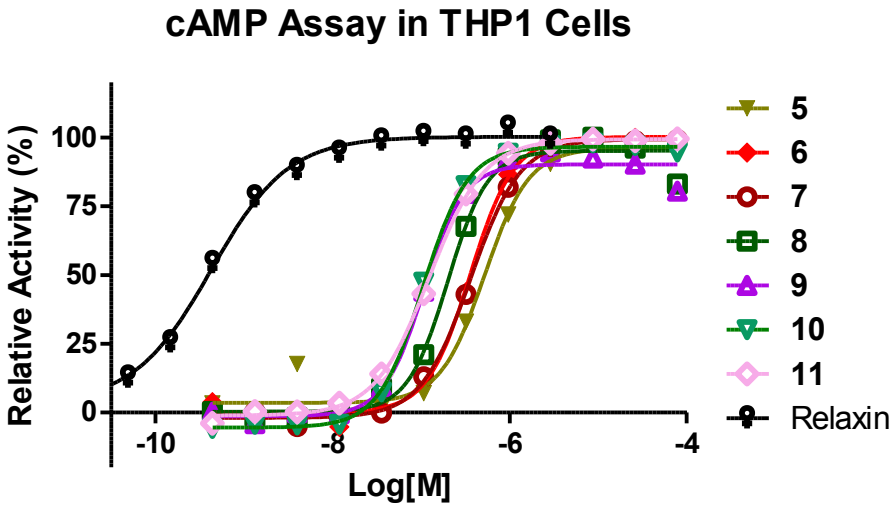
a.



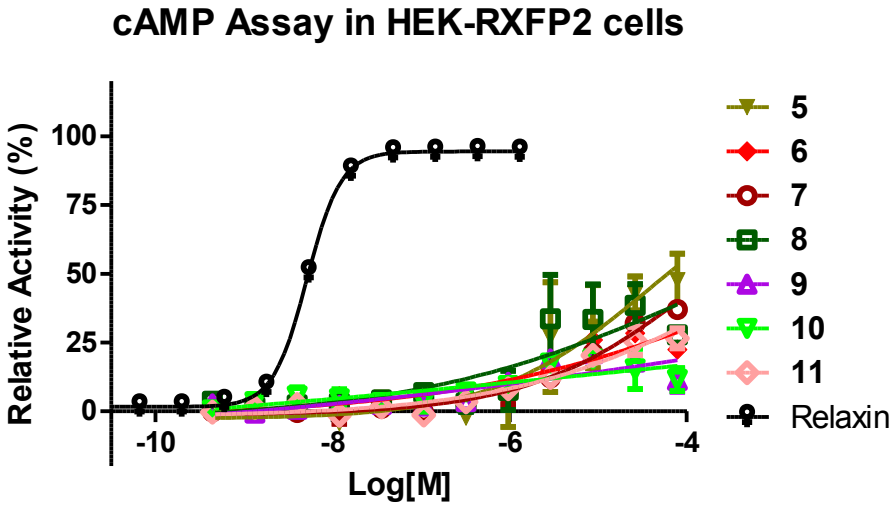
b.



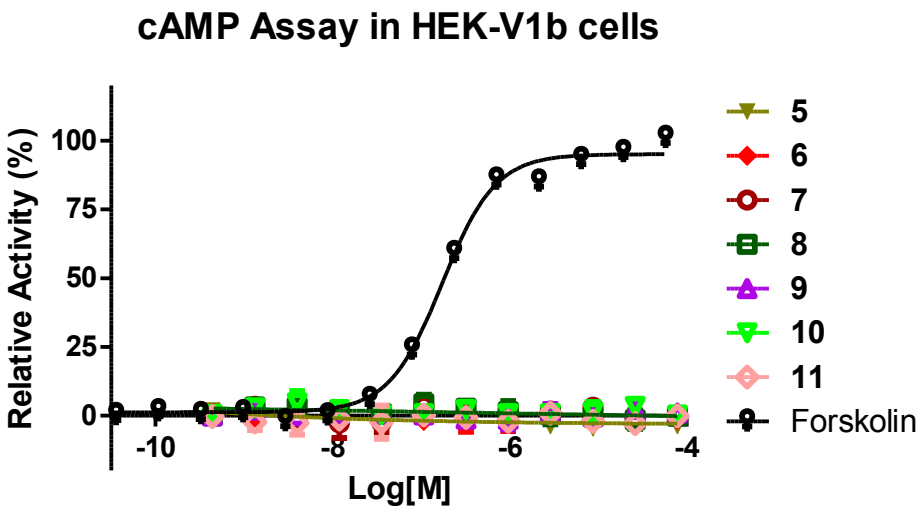
c.


cAMP Assay in THP1 Cells
Relative Activity (%)
100
75
50
25
0
-10
-8
-6
-4
Log[M]
5
6
7
8
9
10
11
Relaxin

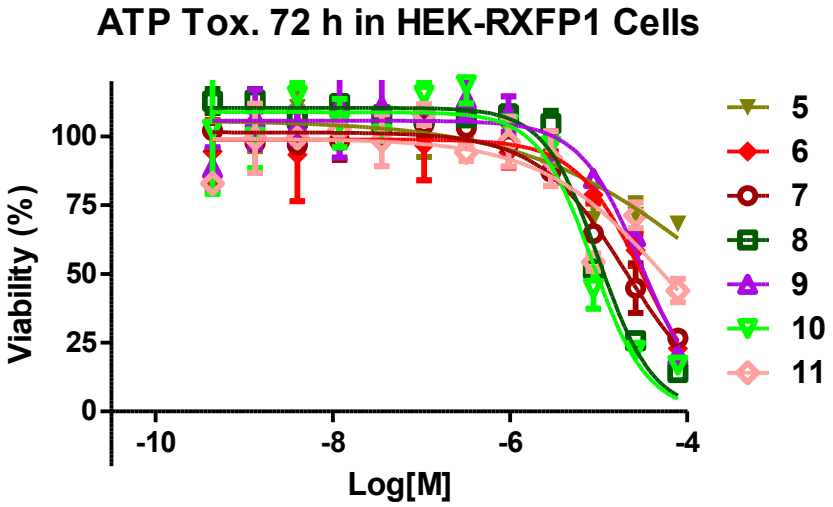
d.


cAMP Assay in HEK-RXFP2 cells
Relative Activity (%)
100
75
50
25
0
-10
-8
-6
-4
Log[M]
5
6
7
8
9
10
11
Relaxin

e.


cAMP Assay in HEK-V1b cells
Relative Activity (%)
100
75
50
25
0
-10
-8
-6
-4
Log[M]
5
6
7
8
9
10
11
Forskolin

f.


ATP Tox. 72 h in HEK-RXFP1 Cells
Viability (%)
100
75
50
25
0
-10
-8
-6
-4
Log[M]
5
6
7
8
9
10
11

Supplemental Table S1 a-e. Metabolic stability of compound in mouse liver microsomes (MLM) with and without NADPH. The study was performed by Pharmaron via fee-for-service type of contract (www.pharmaron.com). Make a master solution containing microsomes (0.5 mg/mL), phosphate buffer (5 mM), ultra-pure H_2O and $MgCl_2$ solution (5 mM) and then add 5 μL of 200 μM test compounds or control solution (verapamil). The final concentration of test compounds or verapamil in the reaction system was 2 μM. Pre-warm the mixture at 37°C for 5 min. The reaction was started with the addition of 50 μL of 10 mM NADPH solution at the final concentration of 1 mM and carried out at 37°C. 50 μL of ultra-pure H_2O was used instead of NADPH solution in the negative control. Aliquots of 50 μL were taken from the reaction solution at 0, 15, 30, 45 and 60 min. The reaction was stopped by the addition of 4 volumes of cold methanol at the designated time points. Samples were centrifuged at 16,000 g for 10 minutes to precipitate protein. Aliquot of 100 μL of the supernatant was used for LC/MS/MS analysis. All experiments were performed in duplicate.

a.

Incubation T	Compou	
	W	With
0		
15		
30		
45		
60		

b.

Incubation T	Compou	
	W	With
0		
15		
30		
45		
60		

c.

Incubation T	Compou	
	W	With
0		
15		
30		
45		
60		

d.

Incubation T	Compound **10**	
	With N	**Without N**
0	100.	100.0
15	92.3	98.29
30	82.8	98.29
45	76.7	97.14
60	74.4	93.71

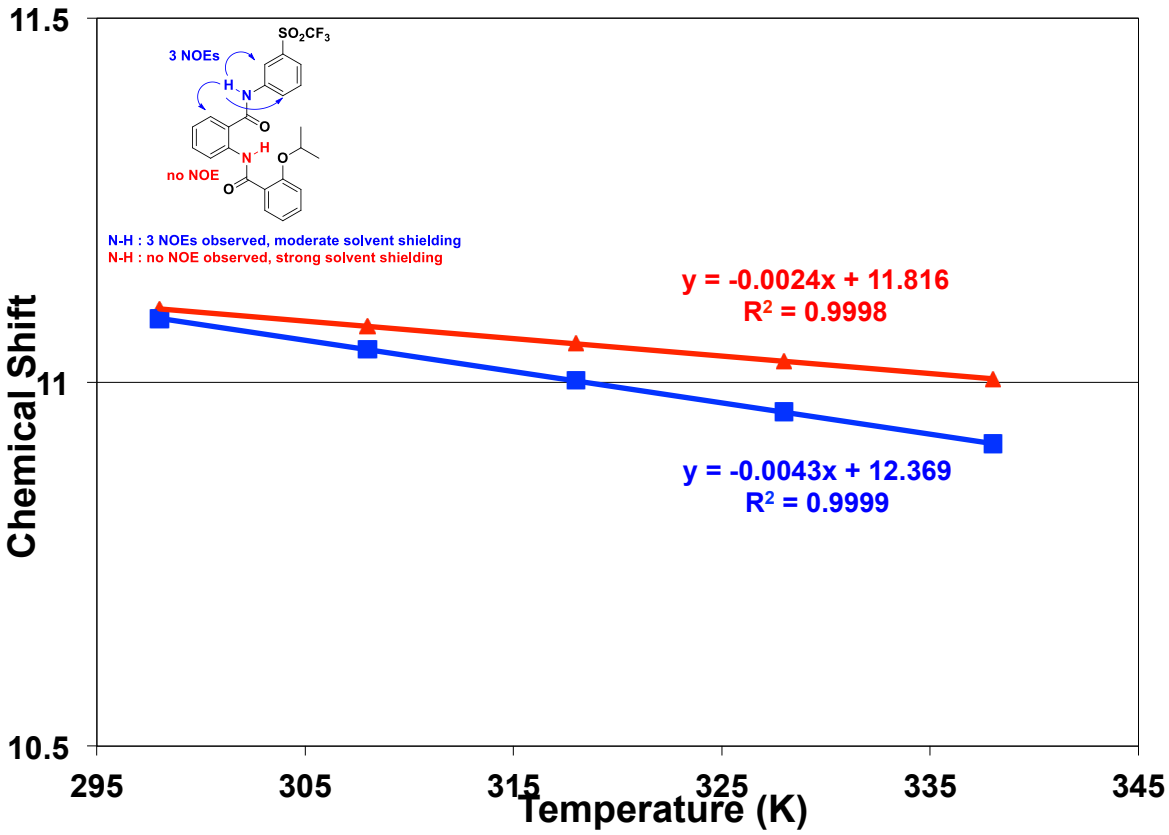
e.

Incubation T	Compou	
	W	With
0		
15		
30		
45		
60		

Supplemental Table S2. The stability results of compound 8 and control compound propantheline in human and mouse plasma. The study was performed by Pharmaron via fee-for-service type of contract (www.pharmaron.com). The stock solution of test compound was prepared in DMSO at the concentration of 100 μM and then human or mouse plasma was spiked to reach a final concentration of 1 μM. The final percent volume of organic solvents was 1%. Add 500 μL of spiked plasma sample into the centrifuge tube, and incubate at 37°C at approximately 60 rpm on an orbital shaker. Aliquots of 100 μL were taken from the reaction solution at 0, 60 and 120 minutes. The reaction was stopped by the addition of 4 volumes of cold methanol at the designated time points. Samples were centrifuged at 20,000 g for 15 minutes to precipitate protein. An aliquot of 150 μL of the supernatant was used for LC/MS/MS analysis. All experiments were performed in duplicate. The LC system comprised a Shimadzu liquid chromatograph separation system equipped with degasser DGU-20A3, solvent delivery unit LC-20AD, system controller CBM-20A, column oven CTO-10ASVP and CTC Analytics HTC PAL System. Mass spectrometric analysis was performed using an API 4000 instrument from AB Inc (Canada) with an ESI interface. The data acquisition and control system were created using Analyst 1.5 software from ABI Inc.

Compou	**Incul Time**	**Remaining percentage (%**	
		in human p	**in mouse p**
Propanthe		100.0	100.0
	(	31.38	26.31
	1	6.22	7.46
8		100.0	100.0
	(	103.7	100.3
	1	104.9	99.03

Supplemental Figure S2. Three dimensional conformation of compound 8 in solution state. Solution state of three dimensional conformation in DMSO-d_6 determined by NMR. Variable temperature NMRs were recorded in a temperature interval of 10 K from 298 K to 338 K at the concentration of 5 mM for compound **8** in DMSO-d_6.



Supplemental Table S3 a-g. Summary of X-ray data for compound 8

a. X-ray crystal data and structure refinement for compound 8.

Identification code	Compound **8**	
Empirical formula	C24 H21 F3 N2 O5 S	
Formula weight	506.49	
Temperature	123(2) K	
Wavelength	0.71073 Å	
Crystal system	Triclinic	
Space group	P-1	
Unit cell dimensions	a = 9.0601(5) Å	α= 94.231(3)
	b = 10.3804(6) Å	β= 92.208(3)°
	c = 13.5868(7) Å	γ = 114.264(2
Volume	1158.41(11) Å^3	
Z	2	
Density (calculated)	1.452 g/cm^3	
Absorption coefficient	0.203 mm^{-1}	
F(000)	524	
Crystal size	0.28 x 0.12 x 0.09 mm^3	
Theta range for data collection	2.16 to 25.37°	
Index ranges	-10<=h<=10, -12<=k<=12, -14<=l<=	
Reflections collected	15065	
Independent reflections	4206 [R(int) = 0.0387]	
Completeness to theta = 25.00°	99.1 %	
Absorption correction	Multi-scan	
Max. and min. transmission	0.9819 and 0.9453	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	4206 / 0 / 318	
Goodness-of-fit on F^2	1.065	
Final R indices [I>2sigma(I)]	R1 = 0.0344, wR2 = 0.0900	
R indices (all data)	R1 = 0.0413, wR2 = 0.0967	
Largest diff. peak and hole	0.358 and -0.428 e Å^{-3}	

b. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (Å^{2}x 10^3) for compound 8. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

c. Bond lengths [Å] for compound 8.

B	Bond le	B	Bond le	B	Bond le
S(	1.42	C(3)	0	C(15	1.3
S(	1.42	C(4)	1.4	C(15	0
S(	1.75	C(5)	1.4	C(16	0
S(	1.84	C(6)	1.3	C(17)·	0
F(	1.3	C(6	1.4	C(17)·	0
F(	1.3	C(7)	1.3	C(17)·	0
F(	1.3	C(8)	1.5	C(18	1.3
O(1	1.3	C(9)	1.4	C(18	0
O(1	1.46	C(9)	1.4	C(19	1.3
O(	1.2	C(10	1.3	C(19	0
O(	1.2	C(11	1.5	C(20	1.3
N(	1.3	C(11	1.5	C(20	0
N(	1.4	C(11		C(21	0
N(		C(12)·	0	C(22	1.3
N(	1.3	C(12)·	0	C(22	0
N(	1.4	C(12)·	0	C(23	1.3
N(		C(13	1.3	C(23	0
C(2	1.3	C(13	0	C(24	0
C(	1.3	C(14	1.3		
C(	1.3	C(14	0		

d. Bond angles [°] for compound 8.

Bon	Bonc	Bond	Bond	Bond	Bond
O(3)-S(1	1	C(7)-C(6)-	12	C(16)-C(15)·	
O(3)-S(1	1	C(18)-C(7)·	11	C(15)-C(16)·	12
O(2)-S(1	1	C(18)-C(7)·	12	C(15)-C(16)·	
O(3)-S(1	1	C(6)-C(7)-	11	C(10)-C(16)·	
O(2)-S(1	1	O(5)-C(8)-	12	C(11)-C(17)-I	
C(2)-S(1	1	O(5)-C(8)-	11	C(11)-C(17)-I	
C(10)-O(1	11	N(2)-C(8)-	11	H(17A)-C(17)·	
C(5)-N(1	12	C(13)-C(9)-	11	C(11)-C(17)-I	
C(5)-N(1		C(13)-C(9)·	11	H(17A)-C(17)·	
C(4)-N(1		C(10)-C(9)·	12	H(17B)-C(17)·	
C(8)-N(2	12	O(1)-C(10)-	12	C(19)-C(18)	12

C(8)-N(2		O(1)-C(10)	11	C(19)-C(18)·	
C(7)-N(2		C(16)-C(10)	11	C(7)-C(18)-	
F(2)-C(1	10	O(1)-C(11)-	10	C(20)-C(19)·	12(
F(2)-C(1	10	O(1)-C(11)-	10	C(20)-C(19)·	
F(3)-C(1	10	C(12)-C(11)·	11	C(18)-C(19)·	
F(2)-C(1	11	O(1)-C(11)-		C(21)-C(20)·	11
F(3)-C(1	10	C(12)-C(11)·		C(21)-C(20)·	
F(1)-C(1	11	C(17)-C(11)·		C(19)-C(20)·	
C(24)-C(	12	C(11)-C(12)-		C(20)-C(21)	12
C(24)-C(	11	C(11)-C(12)-		C(20)-C(21)·	
C(3)-C(2	11	H(12A)-C(12)		C(6)-C(21)-	
C(4)-C(3	11	C(11)-C(12)-		C(23)-C(22)	12(
C(4)-C(3		H(12A)-C(12)		C(23)-C(22)·	
C(2)-C(3		H(12B)-C(12)·		C(4)-C(22)-	
C(3)-C(4)	11	C(14)-C(13)	12	C(22)-C(23)·	12(
C(3)-C(4	12	C(14)-C(13)·		C(22)-C(23)·	
C(22)-C(	11	C(9)-C(13)-		C(24)-C(23)·	
O(4)-C(5	12	C(13)-C(14)·	11	C(2)-C(24)-	11
O(4)-C(5	12	C(13)-C(14)·		C(2)-C(24)-	
N(1)-C(5	11	C(15)-C(14)·		C(23)-C(24)·	
C(21)-C(	11	C(14)-C(15)·	12		
C(21)-C(	12	C(14)-C(15)·			

e. Anisotropic displacement parameters ($Å^2 x\ 10^3$) for compound 8. The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2\ a^{*2}U^{11} + ... + 2\ h\ k\ a^*\ b^*\ U^{12}]$.

f. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$ x 10^3) for compound 8.

g. Hydrogen bonds for compound 8 [Å and °].

D-H..	d(	d(	d	<(
N(1)-H(1)..			2.3	
N(2)-H(2)			2.(	
N(2)-H(2)			2.'	

Supplemental Table S4 a-b. a. Individual and mean plasma concentration-time data of compound **8** after an intraperitoneal (IP) dose of 30 mg/kg in male C57BL/6 mice. **b.** Individual and mean heart concentration-time data of compound **8** after an IP dose of 30 mg/kg in male C57BL/6 mice. (BQL = Below quantifiable limit of 1 ng/mL for compound **8** in male C57BL/6 tissues. NA = Not available. SD = Standard deviation in the mean (ng/mL) values across the three individuals. CV = Coefficient of variation for the individual sample values.)

a.

Dose (mg/kg)	Dose route	Sampling time (hr)	Concentration (ng/mL) Individual			Mean (ng/mL)	Mean (μM)	SD	CV(%)
30	IP	0	BQL	BQL	BQL	BQL	BQL	NA	NA
		0.083	2650	2660	2050	2453	4.8438	349	14.2
		0.25	2970	2880	2890	2913	5.7520	49.3	1.69
		0.5	2860	3810	3040	3237	6.3904	505	15.6
		1	5030	4890	4190	4703	9.2861	450	9.57
		2	2660	2580	3810	3017	5.9560	688	22.8
		4	1630	755	811	1065	2.1034	490	46.0
		8	210	321	225	252	0.4975	60.2	23.9
		12	123	74.6	132	110	0.2169	30.9	28.1
		24	21.5	17.9	52.5	30.6	0.0605	19.0	62.1
		48	0.914	2.90	13.0	5.60	0.0111	6.48	116

PK parameters	Unit	Estimate
T_{max}	hr	1.00
C_{max}	μM	9.29
Terminal $t_{1/2}$	hr	8.56
AUC_{last}	hr*ng/mL	15882
AUC_{INF}	hr*ng/mL	15951

b.

Dose (gr/kg)	Dose route	Sampling time (hr)	Concentration (ng/g) Individual			Mean (ng/g)	Mean (μmol/kg)	SD	CV(%)
30	IP	0	BQL	BQL	BQL	BQL	BQL	NA	NA
		0.083	9670	9210	12500	10460	20.6519	1782	17.0
		0.25	9920	12600	14200	12240	24.1663	2163	17.7
		0.5	13100	11700	10900	11900	23.4950	1114	9.36
		1	14000	14700	14800	14500	28.6284	436	3.01
		2	11400	9370	9560	10110	19.9609	1121	11.1
		4	5880	3250	3840	4323	8.5359	1380	31.9
		8	1070	1410	931	1137	2.2449	246	21.7
		12	631	440	787	619	1.2228	174	28.1
		24	107	73.9	332	171	0.3376	140	82.1
		48	4.58	10.1	49.1	21.3	0.0420	24.3	114

PK parameters	Unit	Estimate
T_{max}	hr	1.00
C_{max}	μmol/kg	28.6
Terminal $t_{1/2}$	hr	7.48
$AUC_{heart}(AUC_{last})$	hr*ng/mL	60167
AUC_{INF}	hr*ng/mL	60397
AUC_{heart}/AUC_{plasma}	%	379

Supplemental Table S5 a-e. The determination of oral bioavailability of compound 8 in mice. a. Individual and mean plasma concentration-time data of compound **8** after an oral gavage (PO) dose of 30 mg/kg in male C57BL/6 mice. **b.** Individual and mean heart concentration-time data of compound **8** after a PO dose of 30 mg/kg in male C57BL/6 mice. **c.** Individual and mean plasma concentration-time data of compound **8** after an intravenous (IV) dose of 3 mg/kg in male C57BL/6 mice. **d.** Individual and mean heart concentration-time data of compound **8** after an IV dose of 3 mg/kg in male C57BL/6 mice. (BLOQ = below limit of quantification of 1 ng/mL for compound **8** in male C57BL/6 tissues). **e.** Calculation of oral bioavailability (F%) of compound **8** in mice. BLOQ = Below quantifiable limit of 1 ng/mL for compound **8** in male C57BL/6 tissues. NA = Not available. SD = Standard deviation in the mean values across the three individuals.

a.

Plasma concentration of compound **8** after 30 mg/kg oral administration							
Time	Individual			Mean	SD	Mean	SD
hr	ng/mL			ng/mL	ng/mL	uM	uM
0.17	292	116	126	178	98.85	0.351	0.195
0.5	318	374	227	306.33	74.19	0.605	0.146
1	327	224	265	272	51.86	0.537	0.102
1.5	247	185	160	197.33	44.79	0.39	0.088
2	385	103	184	224	145.19	0.442	0.287
3	90.3	149	122	120.43	29.38	0.238	0.058
5	28.3	26.2	91.7	48.73	37.23	0.096	0.073
7	6.87	7.54	18.8	11.07	6.7	0.022	0.013
24	2.13	2.64	3.1	2.62	0.49	0.005	0.001

b.

Heart concentration of compound **8** after 30 mg/kg oral administration							
Time	Individual			Mean	SD	Mean	SD
hr	ng/g			ng/g	ng/g	umol/Kg	umol/Kg
0.17	490	545	372	469	88.39	0.926	0.175
0.5	546	1270	354	723.33	483.06	1.428	0.954
1	541	716	325	527.33	195.86	1.041	0.387
1.5	749	1040	1290	1026.33	270.76	2.026	0.535
2	847	246	451	514.67	305.52	1.016	0.603
3	429	614	469	504	97.34	0.995	0.192
5	57.7	98.6	233	129.77	91.71	0.256	0.181
7	25.5	23.4	53.2	34.03	16.63	0.067	0.033
24	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ

c.

Plasma concentration of compound **8** after 3 mg/kg IV administration							
Time	Individual			Mean	SD	Mean	SD
hr	ng/mL			ng/mL	ng/mL	uM	uM
0.08	467	273	401	380.33	98.64	0.751	0.195
0.25	337	174	151	220.67	101.4	0.436	0.2
0.5	216	104	14.8	111.6	100.82	0.22	0.199
1	171	115	8.87	98.29	82.35	0.194	0.163
2	149	83	11.6	81.2	68.72	0.16	0.136
3	17.1	85.9	61.7	54.9	34.9	0.108	0.069
5	11.6	16.2	42.6	23.47	16.73	0.046	0.033
7	37.8	11.3	16.1	21.73	14.12	0.043	0.028
24	NS	6.12	2.75	4.44	2.38	0.009	0.005

d.

Heart concentration of compound **8** after 3 mg/kg IV administration							
Time	Individual			Mean	SD	Mean	SD
hr	ng/g			ng/g	ng/g	umol/Kg	umol/Kg
0.08	2220	1060	2980	2086.67	966.92	4.12	1.909
0.25	1800	1000	367	1055.67	718.12	2.084	1.418
0.5	428	2590	22.2	1013.4	1380.37	2.001	2.725
1	1040	820	259	706.33	402.72	1.395	0.795
2	951	810	71.6	610.87	472.31	1.206	0.933
3	61.7	661	189	303.9	315.74	0.6	0.623
5	23.4	40.5	21.3	28.4	10.53	0.056	0.021
7	351	40.7	207	199.57	155.28	0.394	0.307
24	94.3	30.6	40.9	55.27	34.19	0.109	0.068

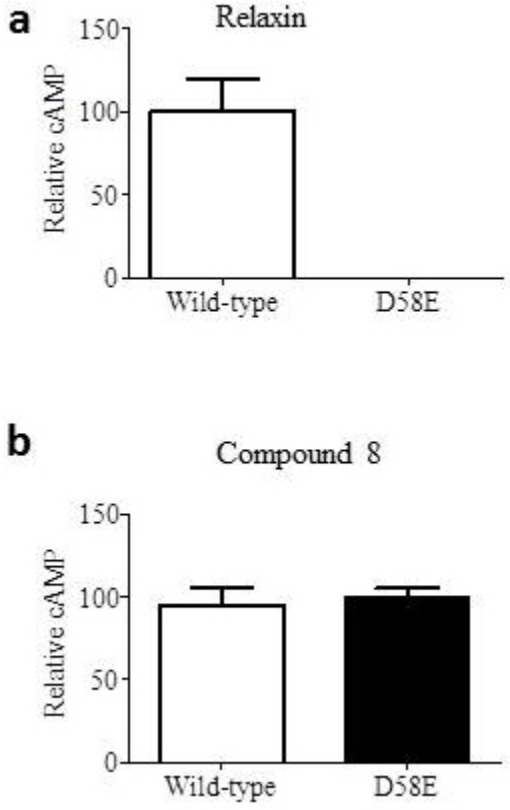
e.

Parameter	Units	IV (1	PO (	IV (	PO
AUClast	hr*ng/mL	1	9	5	2
AUCINF_c	hr*ng/mL	1	1	5	2
Cl_obs	mL/min/kg	6		8	
MRTlast	hr			5	
Vss_obs	L/kg	2		4	
t1/2	hr			6	
C0	ng/mL	4		2	
Tmax	hr				
Cmax	ng/mL		1		1
F	%				

a This short half-life reflects a below limit of quantification (BLOQ) of compound **8** at 24 h time point in heart tissues for the given dose, which the model takes as zero concentration and very likely underestimates the elimination half-life of the compound in this tissue.

AUClast = the integral of the concentration-time curve using only the observed values. AUCINF_obs = the integral of the concentration-time curve, predicting concentrations at additional timepoints using the elimination half-life. Cl_obs = observed clearance. MRTlast = mean residence time based on the last observation. Vss_obs = volume of distribution. t1/2 = elimination half-life. C0 = concentration immediately after dosing. Tmax = time of maximum concentration observed. Cmax = maximum concentration observed. F = oral bioavailability.

Supplemental Figure S3 a-b. Activation of LDLa domain mutant $D_{58}E$ RXFP1 receptor with compound 8. a. HEK293T cells transiently transfected with wild-type and mutant human RXFP1 receptor stimulated with relaxin at 90 ng/ml (150 nM). The mutant receptor did not respond to relaxin stimulation. Data were normalized as 100% cAMP response in cells transfected with wild-type receptor. **b.** Stimulation with compound **8** at 66nM induced the same cAMP response in cells transfected with the wild-type and $D_{58}E$ RXFP. Data were normalized as 100% cAMP response in cells transfected with wild-type RXFP1. Transfection efficiency and the surface expression of the wild-type and mutant receptor were similar (25). The bars represent the mean values ± s.e.m.



Supplemental Table S6. Evaluation of the affinity of compound 8 for the agonist site of the human relaxin RXFP1 receptor in transfected HEK-293 cells determined in a radioligand binding assay. The assay was performed at Cerep with catalog ref. 2058 (www.cerep.com) via fee-for-service type of contract. Cell membrane homogenates (120 µg protein) are incubated for 60 min at 22°C with 0.02 nM [^{125}I]relaxin 2 in the absence or presence of the test compound in a buffer containing 50 mM Tris-HCl (pH 7.4), 2 mM EDTA, 0.02% bacitracin and 0.5% BSA. Nonspecific binding is determined in the presence of 1 µM relaxin 2. Following incubation, the samples are filtered rapidly under vacuum through glass fiber filters (GF/B, Packard) presoaked with 0.5% PEI and rinsed several times with an ice-cold buffer containing 50 mM Tris-HCl and 500 mM NaCl using a 96-sample cell harvester (Unifilter, Packard). The filters are dried then counted for radioactivity in a scintillation counter (Topcount, Packard) using a scintillation cocktail (Microscint 0, Packard). The results are expressed as a percent inhibition of the control radioligand specific binding. The standard reference compound is relaxin 2, which is tested in each experiment at several concentrations to obtain a competition curve from which its IC_{50} is calculated.

Co	Conce	% Inh Contro Bi	% Spe			Ref Cor	IC5	Ki R
	1					H2		3.1
	5					H2		3.1
	2					H2		3.1
	1					H2		3.1
	6					H2		3.1
	3					H2		3.1

Supplemental Table S7. Calculated chemical properties by ChemOffice12.

Compo	Molecular We	cL (partition	Number of don	Number of accep
	390.3	5.(	5	2
	429.4	3.1	5	3
	384.3	5.(	5	2
	414.3	5.1	6	2
	446.4	5.4	6	2
	478.4	4.9	8	2
	492.4	5.4	8	2
	506.4	5.1	8	2
	546.4	5.1	1	2
1	506.4	5.9	8	2
1	520.5	6.3	8	2