### Supplementary Table 1. EC\textsubscript{50} values of TGR5 agonists

| Compound                     | Class                          | EC\textsubscript{50} (µM)         | Reference(s)         |
|------------------------------|-------------------------------|-----------------------------------|----------------------|
| CA7S (Cholic acid -7-sulfate)| Endogenous bile acid          | 0.17 µM                           | This work            |
| CA (Cholic acid)             | Endogenous bile acid          | 12.22 µM [7.72 – 27.00 µM]       | This work [1-5]      |
| TDCA (Tauro-deoxycholic acid)| Endogenous bile acid          | 0.10 µM [0.53 µM]                 | This work [6,7]      |
| LCA (Lithocholic acid)       | Endogenous bile acid          | 0.03 – 3.70 µM                    | 1,2,5,8,9            |
| DCA (Deoxycholic acid)       | Endogenous bile acid          | 0.58 – 1.01 µM                    | 1,2                  |
| CDCA (Chenodeoxycholic acid) | Endogenous bile acid          | 4.00 – 6.71 µM                    | 1,2,5                |
| GCDCA (Glyco-chenodeoxycholic acid, 24) | Endogenous bile acid | 1.00 µM                           | 6,7                  |
| GDCA (Glyco-deoxycholic acid, 25) | Endogenous bile acid | 0.45 µM                           | 6,7                  |
| UDCA (Ursodeoxycholic acid)  | Endogenous bile acid          | 36.4 µM                           | 5                    |
| TLCA (Tauro-lithocholic acid, 26) | Endogenous bile acid | 0.33 µM                           | 1                    |
| Oleanolic acid               | Natural product               | 1.42 – 2.25 µM                    | 8                    |
| Betulinic acid               | Natural product               | 1.04 µM                           | 10                   |
| INT-747 (obeticholic acid)    | Synthetic bile acid derivative| 20.00 µM                          | 9                    |
| INT-767                      | Synthetic bile acid derivative| 0.68 µM                           | 9                    |
| INT-777                      | Synthetic bile acid derivative| 0.82 – 0.90 µM                    | 3,4,9                |

All EC\textsubscript{50} values reported were obtained from cAMP measurement as a TGR5-activation readout.
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Supplementary Table 2. Cholic acid-7-sulfate (CA7S) concentration in cecum, portal vein, and blood

| Treatment                                      | Tissue/blood | CA7S concentration (mean ± SEM) |
|------------------------------------------------|--------------|---------------------------------|
| DIO mice; sham surgery                         |              |                                 |
|                                   | Cecum        | 1726 ± 267 pmol/mg               |
|                                   | Portal vein  | n.d.                             |
|                                   | Systemic blood | n.d.                           |
| DIO mice; sleeve gastrectomy                  |              |                                 |
|                                   | Cecum        | 2661 ± 331 pmol/mg               |
|                                   | Portal vein  | n.d.                             |
|                                   | Systemic blood | n.d.                           |
| DIO mice; enteral PBS                       |              |                                 |
|                                   | Cecum        | 161.1 ± 46.4 pmol/mg             |
|                                   | Portal vein  | 0.07 ± 0.06 pmol/mg              |
|                                   | Systemic blood | n.d.                           |
| DIO mice; enteral CA7S                      |              |                                 |
|                                   | Cecum        | 2577 ± 185 pmol/mg               |
|                                   | Portal vein  | 6.13 ± 2.11 pmol/mg              |
|                                   | Systemic blood | 0.5 ± 0.2 pmol/µL               |
| DIO mice; acute PBS gavage                  |              |                                 |
|                                   | Cecum        | 947 ± 349 pmol/mg                |
|                                   | Portal vein  | n.d.                             |
|                                   | Systemic blood | n.d.                           |
| DIO mice; acute CA7S gavage                 |              |                                 |
|                                   | Cecum        | 14345 ± 1451 pmol/µL             |
|                                   | Portal vein  | 13.2 ± 7.7 pmol/mg               |
|                                   | Systemic blood | n.d.                           |
| DIO mice; chronic PBS gavage                |              |                                 |
|                                   | Cecum        | 9122 ± 3274 pmol/mg              |
|                                   | Portal vein  | 0.53 ± 0.53 pmol/mg              |
|                                   | Systemic blood | n.d.                           |
|                     | CECUM | Portal vein | Systemic blood |
|---------------------|-------|-------------|----------------|
| DIO mice; chronic CA7S gavage | 29735 ± 3956 pmol/µL | 2.52 ± 1.0 pmol/mg | 0.09 ± 0.09 pmol/µL |

n.d. not detected, all data are presented as mean ± SEM.
Supplementary Figure 1. DIO mice post-SG show loss of body weight

**a**. SG-operated mice displayed a decrease in body weight post-surgery (a) despite no significant change in their food intake (b) compared to sham mice (SG, n=7; sham, n=6; for **a**, day 0 not significant *p*=0.19, day 1 not significant *p*=0.37, day 2 not significant *p*=0.24, day 3 not significant *p*=0.06, day 4 *p*=5.77x10^{-4}, day 5 *p*=3.17x10^{-4}, day 6 *p*=1.19x10^{-5}, day 7 *p*=2.62x10^{-4}, day 11 *p*=2.97x10^{-3}, day 14 *p*=2.86x10^{-3}, day 18 *p*=7.87x10^{-4}, day 22 *p*=1.27x10^{-4}, day 25 *p*=3.06x10^{-4}, day 29 *p*=5.16x10^{-4}, day 32 *p*=1.84x10^{-3}, day 35 *p*=2.07x10^{-3}, day 39 *p*=9.04x10^{-4}, day 42 *p*=6.93x10^{-4}, two-tailed Student’s t-test; for **b**, total food intake ns=not significant, *p*=0.60, two-tailed Student’s t-test). All data are presented as mean ± SEM.
Supplementary Figure 2. UPLC-MS analysis of cholic acid-7-sulfate

**a**, Commercially available cholic acid-7-sulfate (CA7S) (Cayman Chemical) and **b**, CA7S purified from the cecal contents of SG mice have the same mass ($m/z$ 487.2) and elute at 9.2 minutes.
Supplementary Figure 3. Bile acid concentrations in circulating blood of mice post-sham or post-SG. Six weeks following surgery, blood was collected from sham or SG mice after an overnight fast. Bile acids were quantified using UPLC-MS (sham, n=12, SG, n=15, data not marked with asterisk(s) are not significant). All bile acids with measurable concentrations above the limit of detection are shown. Total bile acids (BAs), *p=0.03 Tα/βMCA, tauro-alpha- and tauro-beta-muricholic acid, p=0.11; TCA, tauro-cholic acid, p=0.17; TωMCA, tauro-omega-muricholic acid, p=0.08; α/βMCA, alpha- and beta-muricholic acid, p=0.19; UDCA, ursodeoxycholic acid,
\( p = 0.53 \); CA, cholic acid, \( p = 0.25 \); DCA, deoxycholic acid, \( p = 0.96 \); TDCA, tauro-deoxycholic acid, \( p = 0.20 \); TCDCA, tauro-chenodeoxycholic acid, \( p = 0.21 \); CDCA, chenodeoxycholic acid, \( p = 0.50 \), 3-oxo-CA, 3-oxo-cholic acid \( p = 0.09 \), two-tailed Welch’s t-test. All data are presented as mean ± SEM.
Supplementary Figure 4. Bile acid concentrations in livers of mice post-sham or post-SG.

Six weeks following surgery, livers were collected from sham or SG mice after an overnight fast. Bile acids were quantified using UPLC-MS (n=11 per group, data not marked with asterisk(s) are not significant). All bile acids with measurable concentrations above the limit of detection are shown. Tα/βMCA, tauro-alpha- and tauro-beta-muricholic acid, \( p=0.62 \); ToMCA, tauro-omega-muricholic acid, \( p=0.82 \); αMCA, alpha-muricholic acid, \( p=0.08 \); βMCA, beta-muricholic acid, \( p=0.20 \); TCA, tauro-cholic acid, \( p=0.81 \); TCDCA, tauro-chenodeoxycholic acid, \*\( p=0.01 \); TDCA, tauro-deoxycholic acid, \( p=0.94 \); CA, cholic acid, \( p=0.46 \); 3-oxo-CA, 3-oxo-cholic acid, \( p=0.2 \); UDCA, ursodeoxycholic acid, \( p=0.31 \); CDCA, chenodeoxycholic acid, \*\( p=0.02 \), two-tailed Welch’s t-test. All data are presented as mean ± SEM.
Supplementary Figure 5. Bile acid concentrations in gallbladders of mice treated enterally with CA7S. Gallbladders were collected from mice after enteral treatment with CA7S or PBS and bile acids were quantified using UPLC-MS (PBS, n=7, CA7S, n=8, data not marked with asterisk(s) are not significant). All bile acids with measurable concentrations above the limit of detection are shown. Total BAs without CA7S, $p=0.39$; Total bile acids (BAs), $p=0.39$; $T_\alpha/\beta$MCA, tauro-alpha- and tauro-beta-muricholic acid, $p=0.45$; TCA, tauro-cholic acid, $p=0.48$; $T\gamma$MCA, tauro-gamma-muricholic acid, $p=0.36$; TCDCA, tauro-chenodeoxycholic acid, $p=0.86$; 7-oxo-TCDCA, 7-oxo-tauro-chenodeoxycholic acid $p=0.20$; $\alpha\beta$MCA, alpha-muricholic acid and $\beta$MCA, beta-muricholic acid, $p=0.80$; CA, cholic acid, $p=0.13$; TDCA, tauro-deoxycholic acid, $p=0.66$;
TUDCA, tauro-ursodeoxycholic acid, $p=0.82$; CA7S, $p=0.14$, two-tailed Welch’s t-test. All data are presented as mean ± SEM.
Supplementary Figure 6. Bile acid concentrations in livers of mice treated enterally with CA7S. Livers were collected from mice after enteral treatment with CA7S or PBS and bile acids were quantified using UPLC-MS (PBS, n=7, CA7S, n=8, data not marked with asterisk(s) are not
significant). All bile acids with measurable concentrations above the limit of detection are shown. Total BAs without CA7S, $p=0.81$; Total bile acids (BAs), $p=0.52$; $\mathrm{T\alpha/\beta MCA}$, tauro-alpha- and tauro-beta-muricholic acid, $p=0.30$; $\mathrm{T\gamma MCA}$, tauro-gamma-muricholic acid, $p=0.28$; TCA, taurocholic acid, $p=0.92$; TUDCA, tauro-ursodeoxycholic acid, $p=0.22$; TCDCA, taurochenodeoxycholic acid, $p=0.67$; CDCA, chenodeoxycholic acid, $p=0.08$; $\alpha\beta MCA$, alphamuricholic acid and beta-muricholic acid, $p=0.16$; CA, cholic acid, $p=0.25$; TDCA, taurodeoxycholic acid, $p=0.98$; UDCA, ursodeoxycholic acid, $p=0.05$; 3-oxo-CA, 3-oxo- cholic acid $p=0.31$; CA7S, $^*p=0.02$, two-tailed Welch’s t-test. All data are presented as mean ± SEM.
Supplementary Figure 7. Bile acid concentrations in gallbladders of mice gavaged with one dose of CA7S. Fasted DIO mice were gavaged with CA7S or PBS and gallbladders were collected from mice 5 hours post-gavage. Bile acids were quantified using UPLC-MS (n=8 in each group, data not marked with asterisk(s) are not significant). All bile acids with measurable concentrations above the limit of detection are shown. Total BAs without CA7S, \( p = 0.47 \); Total bile acids (BAs), \( p = 0.47 \); Ta/βMCA, tauro-alpha- and tauro-beta-muricholic acid, \( p = 0.50 \); TyMCA, tauro-gamma-muricholic acid, \( p = 0.55 \); TCA, tauro-cholic acid, \( p = 0.30 \); TUDCA, tauro-ursodeoxycholic acid, \( p = 0.72 \); TCDCA, tauro-chenodeoxycholic acid, \( p = 0.24 \); αβMCA, alpha-
muricholic acid and beta-muricholic acid, \( p=0.70 \); CA, cholic acid, \( p=0.57 \); TDCA, tauro-deoxycholic acid, \( p=0.41 \); CA7S, \( **p=8.10 \times 10^{-3} \), two-tailed Welch’s t-test. All data are presented as mean ± SEM.
Supplementary Figure 8. Bile acid concentrations in livers of mice gavaged with one dose of CA7S. Fasted DIO mice were gavaged with CA7S or PBS and livers were collected from mice 5 hours post-gavage. Bile acids were quantified using UPLC-MS (PBS, n=8, CA7S, n=7, data not marked with asterisk(s) are not significant). All bile acids with measurable concentrations above the limit of detection are shown. Total BAs without CA7S, $p=0.40$; Total bile acids (BAs), $p=0.39$; $T\alpha/\beta$MCA, tauro-alpha- and tauro-beta-muricholic acid, $p=0.51$; $Ty$MCA, tauro-gamma-muricholic acid, $p=0.41$; TCA, tauro-cholic acid, $p=0.31$; TUDCA, tauro-ursodeoxycholic acid, $p=0.29$;
TCDCA, tauro-chenodeoxycholic acid, $p=0.46$; $\alpha\beta$MCA, alpha-muricholic acid and beta-muricholic acid, $p=0.70$; CA, cholic acid, $p=0.78$; TDCA, tauro-deoxycholic acid, $p=0.41$; 3-oxo-CA, 3-oxo-cholic acid, $p=0.75$; CA7S, $p=0.053$, two-tailed Welch’s t-test. All data are presented as mean ± SEM.
Supplementary Figure 9. *Glp1r* and *Tgr5* shRNA knockdown efficiency

a,b, Quantitative real time PCR analysis in mice corresponding to Fig. 5f-i. Animals were injected with lentiviral shRNA targeting *Glp1r* or PBS (a) or with lentiviral shRNA targeting *Tgr5* or PBS (b). Expression of mouse *Glp1r* (a) and TGR5 (b) in indicated tissues of mice was measured following OGTT, which was performed 3 days post-injection (SI = small intestine, PBS, n=2; *Glp1r* LVP shRNA n=22; *Tgr5* LVP shRNA n=18). All data are presented as mean ± SEM.
Supplementary Figure 10. Chronic feeding with CA7S improves hyperglycemia

a, b, Chronic administration of CA7S via daily gavage for 48 days resulted in initial weight loss in all groups but did not affect percent body weight change or total food intake compared to PBS-gavaged mice (n=7 mice per group). (For a, ns=not significant p=0.50, for b, ns=not significant p=0.07, two-tailed Welch’s t-test). c, In vivo change in fasted serum glucose upon chronic dosing with CA7S via daily gavage for 48 days compared to PBS-gavaged mice (PBS, n=7; CA7S (100 mg/kg), n=7; PBS *p=0.03, CA7S (100 mg/kg) ns=not significant p=0.59, two-tailed paired t-test).

All data are presented as mean ± SEM.
**Supplementary Figure 11.** Bile acid concentrations in gallbladders of mice gavaged chronically with CA7S. Gallbladders were collected from mice following an overnight fast after 48 days of daily gavage with CA7S or PBS. Bile acids were quantified using UPLC-MS (PBS, n=7, CA7S, n=6, data not marked with asterisk(s) are not significant). All bile acids with measurable concentrations above the limit of detection are shown. Total BAs without CA7S, \( p = 0.33 \); Total bile acids (BAs), \( p = 0.33 \); \( \text{T}_\alpha/\beta\text{MCA} \), tauro-alpha- and tauro-beta-muricholic acid, \( p = 0.43 \); \( \text{T}_\gamma\text{MCA} \), tauro-gamma-muricholic acid, \( p = 0.34 \); TCA, tauro-cholic acid, \( p = 0.31 \); TUDCA, tauro-ursodeoxycholic acid, \( p = 0.35 \); TCDCA, tauro-chenodeoxycholic acid, \( p = 0.29 \); \( \alpha\beta\text{MCA} \),
alpha-muricholic acid and beta-muricholic acid, \( p = 0.84 \); CA, cholic acid, \( p = 0.20 \); TDCA, tauro-deoxycholic acid, \( p = 0.35 \); CA7S, \( p = 0.06 \), two-tailed Welch’s t-test. All data are presented as mean ± SEM.
Supplementary Figure 12. Bile acid concentrations in livers of mice gavaged chronically with CA7S. Livers were collected from mice following an overnight fast after 48 days of daily gavage with CA7S or PBS. Bile acids were quantified using UPLC-MS (PBS, n=7, CA7S, n=6, data not marked with asterisk(s) are not significant). All bile acids with measurable concentrations above the limit of detection are shown. Total BAs without CA7S, \( p=0.53 \); Total bile acids (BAs),
$p=0.53$; $\text{T} \alpha/\beta \text{MCA}$, tauro-alpha- and tauro-beta-muricholic acid, $p=0.71$; $\text{T} \gamma \text{MCA}$, tauro-gamma-muricholic acid, $p=0.38$; $\text{TCA}$, tauro-cholic acid, $p=0.73$; $\text{TUDCA}$, tauro-ursodeoxycholic acid, $p=0.58$; $\text{TCDDA}$, tauro-chenodeoxycholic acid, $p=0.39$; $\alpha/\beta \text{MCA}$, alpha-muricholic acid and beta-muricholic acid, $p=0.71$; $\text{CA}$, cholic acid, $p=0.60$; $\text{TDCA}$, tauro-deoxycholic acid, $p=0.94$; 3-oxo-CDA, 3-oxo-cholic acid, $p=0.39$; $\text{CDCA}$, chenodeoxycholic acid, $p=0.49$; $\text{UDCA}$, ursodeoxycholic acid, $p=0.13$; $\text{CA}7\text{S}$, $p=0.74$, two-tailed Welch’s t-test. All data are presented as mean ± SEM.
Supplementary Figure 13. Bile acid concentrations in gallbladders of mice post-sham or post-SG. Gallbladders were collected from fasted sham or SG mice 6 weeks post-op and bile acids were quantified using UPLC-MS (n=8 per group, data not marked with asterisk(s) are not significant). All bile acids with measurable concentrations above the limit of detection are shown. Total bile acids (BAs), $p=0.46$; $\text{T}^{\alpha/\beta}\text{MCA}$, tauro-alpha- and tauro-beta-muricholic acid, $p=0.52$; TCA, tauro-cholic acid, $p=0.73$; $\text{T}^{\gamma}\text{MCA}$, tauro-gamma-muricholic acid, $p=0.49$; TUDCA, tauro-ursodeoxycholic acid, $p=0.77$; TCDCA, tauro-chenodeoxycholic acid, $p=0.92$; CA7S, $p=0.45$, two-tailed Welch’s t-test. All data are presented as mean ± SEM.
Supplementary Figure 14. CA7S treatment does not have toxic effects

a, b, Apical treatment of the epithelial monolayer with 1 mM CA7S led to nearly undetectable amounts of CA7S in the basolateral chamber as measured by UPLC-MS analysis (a), and no significant change to the epithelial barrier integrity (b) (3 biological replicates per condition, not significant, p=0.21, two-tailed Welch’s t-test). c, Percentage cell viability upon treatment of Caco-2 cells with CA7S in vitro (3 biological replicates per condition, not significant, p≥0.97 one-way ANOVA followed by Dunnett’s multiple comparisons test). All data are presented as mean ± SEM.

d, UPLC-MS traces of CA7S after incubation at 37 °C in buffer at the indicated physiological pHs. All data are presented as mean ± SEM.
CA7S did not agonize or antagonize a panel of 19 nuclear hormone receptors (NhRs).
CA7S (designated as HARV-SD-0000) was tested at 100 µM by DiscoverX for activity against a panel of NhRs. CA7S did not appreciably agonize (>40%) or antagonize (>30%) any of the 19 NhRs tested.

CA7S did not agonize or antagonize a panel of 169 G protein-coupled receptors (GPCRs).
CA7S (designated as HARV-SD-0000) was tested at 100 µM by DiscoverX for activity against a panel of GPCRs. CA7S did not appreciably agonize (>40%) or antagonize (>30%) any of the 169 GPCRs tested.
Study Report

Requester(s): Snehal Chaudhari
Company: Harvard Medical School
Report Date: 10/04/2019
Quote ID: US073-000775-Q
Order ID: US073-000775-O
Service: nhrSCAN
Number of Compounds Tested: 1
Number of Assays Tested: 38

Director, PathHunter Services: Dr. Neil Charter
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42501 Albrae Street
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Report Summary

Target (s): nhrMAX Panel

Compounds: 1

Objective: Agonist and Antagonist Primary Screen

Summary: DiscoveRx successfully profiled 1 compound with the nhrMAX Biosensor Panel.

The results are given on page 6 and the graphical results are provided in an appendix at the end of this report. The data is also provided in an accompanying spreadsheet file.
Technology Principle

Nuclear Hormone Receptor Assays

PathHunter® NHR Protein Interaction (Pro) and Nuclear Translocation (NT) assays monitor the activation of a nuclear hormone receptor in a homogenous, non-imaging assay format using a technology developed by DiscoveRx called Enzyme Fragment Complementation (EFC).

The NHR Pro assay is based on detection of protein-protein interactions between an activated, full length NHR protein and a nuclear fusion protein containing Steroid Receptor Co-activator Peptide (SRCP) domains with one or more canonical LXXLL interaction motifs.

The NHR is tagged with the ProLink™ component of our EFC assay system, and the SRCP domain is fused to the enzyme acceptor component (EA) expressed in the nucleus. When bound by ligand, the NHR will migrate to the nucleus and recruit the SRCP domain, whereby complementation occurs, generating a unit of active β-Galactosidase (β-Gal) and production of chemiluminescent signal. Benefits associated with this approach include reduced compound incubation times, direct measurement of the NHR target, use of full-length human NHR sequences, and the ability to select novel compound classes based on disruption of protein-protein interactions.

The NHR NT assay monitors movement of a NHR between the cytoplasmic and nuclear compartments. The receptor is tagged with the ProLabel™ component of our EFC assay system, and EA is fused to a nuclear location sequence that restricts the expression of EA to the nucleus. Migration of the NHR to the nucleus results in complementation with EA generating a unit of active β-Gal and production of a chemiluminescent signal.
Assay Design: NHR

Cell Handling
1. PathHunter NHR cell lines were expanded from freezer stocks according to standard procedures.
2. Cells were seeded in a total volume of 20 µL into white walled, 384-well microplates and incubated at 37°C for the appropriate time prior to testing. Assay media contained charcoal-dextran filtered serum to reduce the level of hormones present.

Compound Handling
1. Sample was diluted into assay buffer shortly before adding to assay.

Agonist Format
1. For agonist determination, cells were incubated with sample to induce response.
2. Intermediate dilution of sample stocks was performed to generate 5X sample in assay buffer.
3. 5 µL of 5X sample was added to cells and incubated at 37°C or room temperature for 3-16 hours. Final assay vehicle concentration was 1%.

Antagonist Format
1. For antagonist determination, cells were pre-incubated with antagonist followed by agonist challenge at the EC₈₀ concentration.
2. Intermediate dilution of sample stocks was performed to generate 5X sample in assay buffer.
3. 5 µL of 5X sample was added to cells and incubated at 37°C or room temperature for 60 minutes. Vehicle concentration was 1%.
4. 5 µL of 6X EC₈₀ agonist in assay buffer was added to the cells and incubated at 37°C or room temperature for 3-16 hours.

Signal Detection
1. Assay signal was generated through a single addition of 12.5 or 15 µL (50% v/v) of PathHunter Detection reagent cocktail, followed by a one hour incubation at room temperature.

2. Microplates were read following signal generation with a PerkinElmer Envision™ instrument for chemiluminescent signal detection.

Data Analysis
1. Compound activity was analyzed using CBIS data analysis suite (Chem Innovation, CA).
2. For agonist mode assays, percentage activity was calculated using the following formula:
   \[
   \text{% Activity} = \frac{100\% \times (\text{mean RLU of test sample} - \text{mean RLU of vehicle control})}{(\text{mean MAX control ligand} - \text{mean RLU of vehicle control})} 
   \]
3. For antagonist mode assays, percentage inhibition was calculated using the following formula:
   \[
   \text{% Inhibition} = \frac{100\% \times (1 - (\text{mean RLU of test sample} - \text{mean RLU of vehicle control})}{(\text{mean RLU of EC₈₀ control} - \text{mean RLU of vehicle control})}} 
   \]
4. Note that for select assays, the ligand response produces an decrease in receptor activity (inverse agonist with a constitutively active target). For those assays inverse agonist activity was calculated using the following formula:
   \[
   \text{% Inverse Agonist Activity} = \frac{100\% \times ((\text{mean RLU of vehicle control} - \text{mean RLU of test sample})}{(\text{mean RLU of vehicle control} - \text{mean RLU of MAX control})}} 
   \]
Table 1: Summary of control agonist dose response curves for nhrMAX Biosensor Panel

Agonist dose curves were performed for the nhrMAX Biosensor Panel. Assay type, ligand and EC50 obtained are summarized. The EC80 challenge agonist concentration is also provided. Graphical results for the control curves are provided at the end of this report.
Table 2: Compound activity with the nhrMAX Panel

Compounds were tested in agonist and antagonist mode with the nhrMAX Panel. For agonist assays, data was normalized to the maximal and minimal response observed in the presence of control ligand and vehicle. For antagonist assays, data was normalized to the maximal and minimal response observed in the presence of EC80 ligand and vehicle.
**Summary**

DiscoverRx successfully profiled 1 compound with the nhrMAX Biosensor Panel.

The results are given on page 6 and the graphical results are provided in an appendix at the end of this report. The data is also provided in an accompanying spreadsheet file.

This is to certify that the data contained within this report was conducted as described above.

Dr. N. W. Charter

Director, Profiling Services
| Compound             | Project ID | Assay Name | Assay Format | Assay Target | Result Type | EC50 (uM) | Hill Curve | Bottom Curve | Top Max Response | Result Graph |
|----------------------|------------|------------|--------------|--------------|-------------|-----------|------------|--------------|----------------|--------------|
| 6a-Fluorotestosterone| Sep 2019 NHR Panel | NHR Protein Interaction | Agonist | AR | EC50 | 0.012655 | 0.46 | 0 | 108.7 | 113.81 |
| 17-B-estradiol       | Sep 2019 NHR Panel | NHR Protein Interaction | Agonist | Ers | EC50 | 0.3022795 | 0.78 | -1.2 | 102.4 | 99.841 |
| GW4064               | Sep 2019 NHR Panel | NHR Protein Interaction | Agonist | FXR | EC50 | 0.54311 | 1.01 | 1 | 101.2 | 96.355 |
| Dexamethasone        | Sep 2019 NHR Panel | NHR Protein Interaction | Agonist | GR | EC50 | 0.022521 | 0.97 | -0.8 | 95.1 | 97.806 |
| TO901317             | Sep 2019 NHR Panel | NHR Protein Interaction | Agonist | LXRa | EC50 | 0.089596 | 1.01 | 1 | 99.2 | 97.309 |
| TO901317             | Sep 2019 NHR Panel | NHR Protein Interaction | Agonist | LXRb | EC50 | 0.042858 | 0.95 | 2 | 97.4 | 103.12 |
| TO901317             | Sep 2019 NHR Panel | NHR Protein Interaction | Agonist | LXRB-NCOR1 | EC50 | 0.1425 | 1.01 | 5 | 98.4 | 98.77 |
| Aldosterone          | Sep 2019 NHR Panel | NHR Protein Interaction | Agonist | MR | EC50 | 0.301679 | 0.63 | -2.6 | 104.3 | 102.52 |
| Compound          | Year | Panel | Protein Interaction | Agonist | EC50     | Slope | Max  | Min   | EC50% |
|-------------------|------|-------|---------------------|---------|----------|-------|------|-------|-------|
| 9 Cis Retinoic acid | 2019 | NHR   | NHR Protein Interaction | RXRγ | 0.006438 | 0.93  | 0.83 | 1.1   | 99.6  |
| Triiodothyronine  | 2019 | NHR   | NHR Protein Interaction | THRa   | 0.029444 | 1.45  | 0.7  | 93.6  | 95.825 |
| Triiodothyronine  | 2019 | NHR   | NHR Protein Interaction | THRB   | 0.007639 | 1.44  | 3.3  | 101.4 | 102.13 |

![Graphs](image-url)
Study Report

Requester: Snehal Chaudhari

Company: Harvard Medical School

Date: 11/6/2019

Quote ID: US073-0007752-Q

Order ID: US073-0007752-O

Service: gpcrMAX

Number of Compounds Tested: 1

Number of Targets Tested: 168

Associate Director, LeadHunter Services: Lakshmi Anantharaman

Phone: (510) 7713548

Project Manager: Sharon Irelan

Phone: (858) 224-6925

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Eurofins Discovery Services
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Customer Information

Company: Harvard Medical School
Client Name: Snehal Chaudhari
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Report Summary

Targets: gpcr MAX Panel
Compounds: 1 Compound
Objective: Agonist and Antagonist Primary Screen
Summary: DiscoveRx successfully profiled 1 compound against the gpcrMAX Panel.

The assays were performed utilizing the PathHunter beta-arrestin enzyme fragment complementation (EFC) technology. Results are summarized in this report and the data is provided in accompanying Excel spreadsheet files.
Technology Principle

Arrestin Pathway

The PathHunter® β-Arrestin assay monitors the activation of a GPCR in a homogenous, non-imaging assay format using a technology developed by DiscoveRx called Enzyme Fragment Complementation (EFC) with β-galactosidase (β-Gal) as the functional reporter. The enzyme is split into two inactive complementary portions (EA for Enzyme Acceptor and ED for Enzyme Donor) expressed as fusion proteins in the cell. EA is fused to β-Arrestin and ED is fused to the GPCR of interest.

When the GPCR is activated and β-Arrestin is recruited to the receptor, ED and EA complementation occurs, restoring β-Gal activity which is measured using chemiluminescent PathHunter® Detection Reagents.

Endocytosis Pathway

Using EFC technology, DiscoveRx has developed several methods to study receptor internalization.

PathHunter® Activated GPCR Internalization Assays provide a quantitative measurement of arrestin-mediated GPCR internalization, allowing you to monitor the movement of unlabeled, arrestin-bound GPCRs from the plasma membrane in live cells. In this system, EA is fused to arrestin (EA-Arrestin) and ED is localized exclusively to the surface of early endosomes. Enzyme activity is restored upon GPCR activation and arrestin-mediated trafficking to early endosomes. Activity is measured using PathHunter® Detection Reagents.

PathHunter® Total GPCR Internalization Assays provide a quantitative measurement of total GPCR protein internalized into endosomes and is measured using PathHunter® Detection Reagents.

There are two Total GPCR Internalization assay formats. In the first, one of the EFC components is localized exclusively to the endosome and the other component is fused to the GPCR of interest. When stimulation of the target receptor results in receptor internalization and trafficking to early endosomes, complementation of the two enzyme fragments occurs, reflected as an increase in enzyme activity.

In the second format, EA is localized exclusively to the plasma membrane (EA-Membrane) and ED is fused to the GPCR of interest. Membrane-bound receptors will complement with EA, resulting in high levels of enzyme activity. When activation of the GPCR results in receptor internalization, loss of receptor at the cell surface is reflected as a loss of enzyme activity.
Calcium mobilization in PathHunter® cell lines or other cell lines stably expressing Gq-coupled GPCRs is monitored using a calcium-sensitive dye that is loaded into cells. GPCR activation by a compound results in the release of calcium from intracellular stores and an increase in dye fluorescence that is measured in real-time.

Profile Overview
DiscoverX was contracted by Dr. Snehal Chaudhari at Harvard Medical School to profile 1 compound with the gpcrMAX Panel biosensor assays. Compound was tested in agonist and antagonist mode.

CAMP Secondary Messenger Pathway
DiscoverX has developed a panel of cell lines stably expressing non-tagged GPCRs that signal through cAMP. Hit Hunter® cAMP assays monitor the activation of a GPCR via Gi and Gs secondary messenger signaling in a homogenous, non-imaging assay format using a technology developed by DiscoverX called Enzyme Fragment Complementation (EFC) with β-galactosidase (β-Gal) as the functional reporter.

The enzyme is split into two complementary portions: EA for Enzyme Acceptor and ED for Enzyme Donor. ED is fused to cAMP and in the assay competes with cAMP generated by cells for binding to a cAMP-specific antibody. Active β-Gal is formed by complementation of exogenous EA to any unbound ED-cAMP. Active enzyme can then convert a chemiluminescent substrate, generating an output signal detectable on a standard microplate reader.

CaM1 Secondary Messenger Pathway
The Calcium No WashPLUS assay monitors the activation of a GPCR via Gq secondary messenger signaling in a live cell, non-imaging assay format.
**Assay Design: GPCR Arrestin**

**Cell Handling**
1. PathHunter cell lines were expanded from freezer stocks according to standard procedures.
2. Cells were seeded in a total volume of 20 µL into white walled, 384-well microplates and incubated at 37°C for the appropriate time prior to testing.

**Agonist Format**
1. For agonist determination, cells were incubated with sample to induce response.
2. Intermediate dilution of sample stocks was performed to generate 5X sample in assay buffer.
3. 5 µL of 5X sample was added to cells and incubated at 37°C or room temperature for 90 or 180 minutes. Final assay vehicle concentration was 1%.

**Antagonist Format**
1. For antagonist determination, cells were pre-incubated with antagonist followed by agonist challenge at the EC80 concentration.
2. Intermediate dilution of sample stocks was performed to generate 5X sample in assay buffer.
3. 5 µL of 5X sample was added to cells and incubated at 37°C or room temperature for 30 minutes. Vehicle concentration was 1%.
4. 5 µL of 6X EC80 agonist in assay buffer was added to the cells and incubated at 37°C or room temperature for 90 or 180 minutes.

**Signal Detection**
1. Assay signal was generated through a single addition of 12.5 or 15 µL (50% v/v) of PathHunter Detection reagent cocktail, followed by a one hour incubation at room temperature.
2. Microplates were read following signal generation with a PerkinElmer EnvisionTM instrument for chemiluminescent signal detection.

**Data Analysis**
Compound activity was analyzed using CBIS data analysis suite (ChemInnovation, CA).

1. For agonist mode assays, percentage activity was calculated using the following formula:
   \[
   \% \text{Activity} = 100\% \times \frac{\text{mean RLU of test sample} - \text{mean RLU of vehicle control}}{\text{mean MAX control ligand} - \text{mean RLU of vehicle control}}.
   \]
2. For antagonist mode assays, percentage inhibition was calculated using the following formula:
   \[
   \% \text{Inhibition} = 100\% \times \frac{1 - (\text{mean RLU of test sample} - \text{mean RLU of vehicle control})}{\text{mean RLU of EC80 control} - \text{mean RLU of vehicle control}}.
   \]
Results:

| Compound Name                    | Assay Name | Assay Format | Assay Target | Result Type | EC50 (uM) | Hill    | Curve Bottom | Curve Top | Max Response EC80 (uM) |
|----------------------------------|------------|--------------|--------------|-------------|-----------|---------|--------------|-----------|------------------------|
| PACAP-27                         | Arrestin   | Arrestin     | ARPC2A       | Result Type | EC50 (uM) | Hill    | Curve Bottom | Curve Top | Max Response EC80 (uM) |
| 2-Octyl-4-Methylpentanoate       | Arrestin   | Arrestin     | ADRB3        | Result Type | EC50 (uM) | Hill    | Curve Bottom | Curve Top | Max Response EC80 (uM) |
| Phenylephrine                    | Arrestin   | Arrestin     | ADRB3        | Result Type | EC50 (uM) | Hill    | Curve Bottom | Curve Top | Max Response EC80 (uM) |
| UK-44,304                         | Arrestin   | Arrestin     | ADRB2B       | Result Type | EC50 (uM) | Hill    | Curve Bottom | Curve Top | Max Response EC80 (uM) |
| UK-14,345                         | Arrestin   | Arrestin     | ADRB2B       | Result Type | EC50 (uM) | Hill    | Curve Bottom | Curve Top | Max Response EC80 (uM) |
| Isopropenol                      | Arrestin   | Arrestin     | ADRB2        | Result Type | EC50 (uM) | Hill    | Curve Bottom | Curve Top | Max Response EC80 (uM) |
| Isopropenol                      | Arrestin   | Arrestin     | ADRB2        | Result Type | EC50 (uM) | Hill    | Curve Bottom | Curve Top | Max Response EC80 (uM) |
| Apelin-13                        | Arrestin   | Arrestin     | AGTR1        | Result Type | EC50 (uM) | Hill    | Curve Bottom | Curve Top | Max Response EC80 (uM) |
| Thapsiquin                       | Arrestin   | Arrestin     | CCR9         | Result Type | EC50 (uM) | Hill    | Curve Bottom | Curve Top | Max Response EC80 (uM) |
| TSA Receptor Agonist (Short Fragment) | Arrestin   | Arrestin     | CXCR5        | Result Type | EC50 (uM) | Hill    | Curve Bottom | Curve Top | Max Response EC80 (uM) |
| Complement C3a                   | Arrestin   | Arrestin     | C3             | Result Type | EC50 (uM) | Hill    | Curve Bottom | Curve Top | Max Response EC80 (uM) |
| Complement C3a                   | Arrestin   | Arrestin     | C5             | Result Type | EC50 (uM) | Hill    | Curve Bottom | Curve Top | Max Response EC80 (uM) |
| Calcitonin                       | Arrestin   | Arrestin     | C3AR1        | Result Type | EC50 (uM) | Hill    | Curve Bottom | Curve Top | Max Response EC80 (uM) |
| Calcitonin                       | Arrestin   | Arrestin     | C3AR2        | Result Type | EC50 (uM) | Hill    | Curve Bottom | Curve Top | Max Response EC80 (uM) |
| Adrenomedullin                   | Arrestin   | Arrestin     | CCR1         | Result Type | EC50 (uM) | Hill    | Curve Bottom | Curve Top | Max Response EC80 (uM) |
| Adrenomedullin                   | Arrestin   | Arrestin     | CCR1         | Result Type | EC50 (uM) | Hill    | Curve Bottom | Curve Top | Max Response EC80 (uM) |
| Calcitonin                       | Arrestin   | Arrestin     | C5L2         | Result Type | EC50 (uM) | Hill    | Curve Bottom | Curve Top | Max Response EC80 (uM) |
| Calcitonin                       | Arrestin   | Arrestin     | C5AR1        | Result Type | EC50 (uM) | Hill    | Curve Bottom | Curve Top | Max Response EC80 (uM) |
| Calcitonin                       | Arrestin   | Arrestin     | C5AR2        | Result Type | EC50 (uM) | Hill    | Curve Bottom | Curve Top | Max Response EC80 (uM) |
| CCK                                | Arrestin   | Arrestin     | CCK1         | Result Type | EC50 (uM) | Hill    | Curve Bottom | Curve Top | Max Response EC80 (uM) |
| CCK                                | Arrestin   | Arrestin     | CCK2         | Result Type | EC50 (uM) | Hill    | Curve Bottom | Curve Top | Max Response EC80 (uM) |
| Vasopressin                       | Arrestin   | Arrestin     | AVPR1B       | Result Type | EC50 (uM) | Hill    | Curve Bottom | Curve Top | Max Response EC80 (uM) |
| Vasopressin                       | Arrestin   | Arrestin     | AVPR2        | Result Type | EC50 (uM) | Hill    | Curve Bottom | Curve Top | Max Response EC80 (uM) |
| Apelin                             | Arrestin   | Arrestin     | RAMP1        | Result Type | EC50 (uM) | Hill    | Curve Bottom | Curve Top | Max Response EC80 (uM) |
| Apelin                             | Arrestin   | Arrestin     | RAMP2        | Result Type | EC50 (uM) | Hill    | Curve Bottom | Curve Top | Max Response EC80 (uM) |
| Dihydroxycholesterol              | Arrestin   | Arrestin     | EBI2         | Result Type | EC50 (uM) | Hill    | Curve Bottom | Curve Top | Max Response EC80 (uM) |
| Dihydroxycholesterol              | Arrestin   | Arrestin     | EBI2         | Result Type | EC50 (uM) | Hill    | Curve Bottom | Curve Top | Max Response EC80 (uM) |
| Endothelin II                     | Arrestin   | Arrestin     | AGTR1        | Result Type | EC50 (uM) | Hill    | Curve Bottom | Curve Top | Max Response EC80 (uM) |
| Endothelin II                     | Arrestin   | Arrestin     | AGTR1        | Result Type | EC50 (uM) | Hill    | Curve Bottom | Curve Top | Max Response EC80 (uM) |
| Calcitonin                        | Arrestin   | Arrestin     | CALCRL       | Result Type | EC50 (uM) | Hill    | Curve Bottom | Curve Top | Max Response EC80 (uM) |
| CCL‐P Arrestin Agonist (EDG5)     | Arrestin   | Arrestin     | EDG5         | Result Type | EC50 (uM) | Hill    | Curve Bottom | Curve Top | Max Response EC80 (uM) |
| CCL‐P Arrestin Agonist (EDG6)     | Arrestin   | Arrestin     | EDG6         | Result Type | EC50 (uM) | Hill    | Curve Bottom | Curve Top | Max Response EC80 (uM) |
| Vasoactive peptide                | Arrestin   | Arrestin     | EDG6         | Result Type | EC50 (uM) | Hill    | Curve Bottom | Curve Top | Max Response EC80 (uM) |
| CCL‐P Arrestin Agonist (EDG5)     | Arrestin   | Arrestin     | EDG5         | Result Type | EC50 (uM) | Hill    | Curve Bottom | Curve Top | Max Response EC80 (uM) |
| CCL‐P Arrestin Agonist (EDG6)     | Arrestin   | Arrestin     | EDG6         | Result Type | EC50 (uM) | Hill    | Curve Bottom | Curve Top | Max Response EC80 (uM) |

Table 1: Summary of agonist control data for targets tested in gp(o)MAX Panel

Agonist dose curves were performed for the targets tested in the study. Assay type, ligand and EC50 obtained and EC80 concentration used are summarized. Graphical results for the control curves are provided at the end of this report.
Results:

Table 1: Summary of agonist control data for targets tested in gPCRmax Panel

| Compound Name | Assay Name | Assay Format | Assay Target | Result Type | EC50 (nM) | Hill | Curve Bottom | Curve Top | Max Response | EC50 (nM) |
|---------------|------------|--------------|--------------|-------------|----------|------|--------------|-----------|-------------|----------|

Agnostic dose curves were performed for the targets tested in the study. Assay type, ligand and EC50 obtained and EC80 concentration used are summarized. Graphical results for the control curves are provided at the end of this report.

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Table 2: Agonist activity for compounds tested in gpcrMAX Panel

Compounds were tested at the concentration shown in the table. Basal and maximal agonist response control values are given. Raw activity (RLU units) of individual replicates and mean RLU and percentage activity are shown. Percentage activity was calculated relative to the basal and maximal control agonist values for each GPCR target.
Results:

| Agonist | VPC ID | Customer | Control | Mean RLU | SD | %CV |
|---------|--------|----------|---------|----------|----|-----|
| Agonist 100,000 | 85871 | 85712 | 3\% | 8910 | 2% | 2% |
| Agonist 100,000 | 86474 | 35040 | 1% | 35040 | 0% | 0% |
| Agonist 100,000 | 85807 | 85807 | 0% | 85807 | 0% | 0% |
| Agonist 100,000 | 85804 | 85804 | 0% | 85804 | 0% | 0% |
| Agonist 100,000 | 85805 | 85805 | 0% | 85805 | 0% | 0% |
| Agonist 100,000 | 85806 | 85806 | 0% | 85806 | 0% | 0% |
| Agonist 100,000 | 85803 | 85803 | 0% | 85803 | 0% | 0% |
| Agonist 100,000 | 85802 | 85802 | 0% | 85802 | 0% | 0% |
| Agonist 100,000 | 85801 | 85801 | 0% | 85801 | 0% | 0% |
| Agonist 100,000 | 85800 | 85800 | 0% | 85800 | 0% | 0% |
| Agonist 100,000 | 85799 | 85799 | 0% | 85799 | 0% | 0% |
| Agonist 100,000 | 85798 | 85798 | 0% | 85798 | 0% | 0% |
| Agonist 100,000 | 85797 | 85797 | 0% | 85797 | 0% | 0% |
| Agonist 100,000 | 85796 | 85796 | 0% | 85796 | 0% | 0% |
| Agonist 100,000 | 85795 | 85795 | 0% | 85795 | 0% | 0% |
| Agonist 100,000 | 85794 | 85794 | 0% | 85794 | 0% | 0% |
| Agonist 100,000 | 85793 | 85793 | 0% | 85793 | 0% | 0% |

Table 2: Agonist activity for compounds tested in gpcrMAX Panel

Compounds were tested at the concentration shown in the table. Basal and maximal agonist response control values are given. Raw activity (RLU units) of individual replicates and mean RLU and percentage activity are shown. Percentage activity was calculated relative to the basal and maximal control agonist values for each GPCR target.
## Results:

| ID   | Customer | Assay | Antagonist | Basal | HARV | % Basal | % HARV | % CV | % SD | % inhibition |
|------|----------|-------|------------|-------|------|---------|--------|------|-----|-------------|
| EDG4 | Harvard  | EDG4  | 0.000001   | 4.415 | 4.415| 1.7%    | 4.415% | 5%   | 5% | 99%          |
| EDG3 | Harvard  | EDG3  | 0.000001   | 4.415 | 4.415| 1.7%    | 4.415% | 5%   | 5% | 99%          |
| CRTH2| Harvard  | CRTH2 | 0.000001   | 4.415 | 4.415| 1.7%    | 4.415% | 5%   | 5% | 99%          |
| CRHR2| Harvard  | CRHR2 | 0.000001   | 4.415 | 4.415| 1.7%    | 4.415% | 5%   | 5% | 99%          |
| CHRM3| Harvard  | CHRM3 | 0.000001   | 4.415 | 4.415| 1.7%    | 4.415% | 5%   | 5% | 99%          |
| F2RL3| Harvard  | F2RL3 | 0.000001   | 4.415 | 4.415| 1.7%    | 4.415% | 5%   | 5% | 99%          |
| F2RL1| Harvard  | F2RL1 | 0.000001   | 4.415 | 4.415| 1.7%    | 4.415% | 5%   | 5% | 99%          |
| F2R  | Harvard  | F2R   | 0.000001   | 4.415 | 4.415| 1.7%    | 4.415% | 5%   | 5% | 99%          |
| EDG6 | Harvard  | EDG6  | 0.000001   | 4.415 | 4.415| 1.7%    | 4.415% | 5%   | 5% | 99%          |
| CX3CR1| Harvard | CX3CR1| 0.000001   | 4.415 | 4.415| 1.7%    | 4.415% | 5%   | 5% | 99%          |
| CCR7 | Harvard  | CCR7  | 0.000001   | 4.415 | 4.415| 1.7%    | 4.415% | 5%   | 5% | 99%          |
| CCR5 | Harvard  | CCR5  | 0.000001   | 4.415 | 4.415| 1.7%    | 4.415% | 5%   | 5% | 99%          |
| CCKBR| Harvard  | CCKBR | 0.000001   | 4.415 | 4.415| 1.7%    | 4.415% | 5%   | 5% | 99%          |
| CCKAR | Harvard | CCKAR | 0.000001   | 4.415 | 4.415| 1.7%    | 4.415% | 5%   | 5% | 99%          |
| CALCR | Harvard | CALCR | 0.000001   | 4.415 | 4.415| 1.7%    | 4.415% | 5%   | 5% | 99%          |
| BDKRB1| Harvard | BDKRB1| 0.000001   | 4.415 | 4.415| 1.7%    | 4.415% | 5%   | 5% | 99%          |
| AVPR2 | Harvard | AVPR2 | 0.000001   | 4.415 | 4.415| 1.7%    | 4.415% | 5%   | 5% | 99%          |
| ADRB2 | Harvard | ADRB2 | 0.000001   | 4.415 | 4.415| 1.7%    | 4.415% | 5%   | 5% | 99%          |
| RAMP3 | Harvard | RAMP3 | 0.000001   | 4.415 | 4.415| 1.7%    | 4.415% | 5%   | 5% | 99%          |
| RAMP2 | Harvard | RAMP2 | 0.000001   | 4.415 | 4.415| 1.7%    | 4.415% | 5%   | 5% | 99%          |

### Table 3: Antagonist activity for compounds tested in gPCRMAX Panel

Compounds were tested at the concentration shown in the table. Basal and EC80 agonist response control values are given. Raw activity (RLU units) of individual replicates and mean RLU and percentage inhibition are shown. Percentage inhibition was calculated relative to the basal and EC80 control agonist values for each GPCR target.
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Results:

| ID | Assay | Compound | EC80 | %CV | % Inhibition | CV | Compounds | % CV |
|----|-------|----------|------|-----|-------------|----|----------|------|
| 1  | TBXAR | Harvard   | 1201 2% | 12% | 1003         | 7% | P2YR1     |
| 2  |  |  | 1201 2% | 12% | 1003         | 7% | P2YR1     |
| 3  |  |  | 1201 2% | 12% | 1003         | 7% | P2YR1     |
| 4  |  |  | 1201 2% | 12% | 1003         | 7% | P2YR1     |

Table 3: Antagonist activity for compounds tested in gpcRX Panel

Compounds were tested at the concentration shown in the table. Basal and EC80 agonist response control values are given. Raw activity (RLU units) of individual replicates and mean RLU and percentage inhibition are shown. Percentage inhibition was calculated relative to the basal and EC80 control agonist values for each GPCR target.
Table 4: Summary of agonist activity for compounds tested in gpcrMAX Panel
### Table 5: Summary of antagonist activity for compounds tested in gpcrMAX Panel

| Compound ID | Concentration (µM) | Assay Mode | Conc (µM) | % Inhibition |
|-------------|--------------------|------------|-----------|--------------|
| ADRB2       | Harvard Uni        | 500000     | 100       | 75            |
| ADRB2       | Harvard Uni        | 200000     | 100       | 55            |
| ADRB2       | Harvard Uni        | 100000     | 100       | 35            |
| ADRB2       | Harvard Uni        | 50000      | 100       | 15            |
| ADRB2       | Harvard Uni        | 10000      | 100       | 5             |
| ADRB2       | Harvard Uni        | 5000       | 100       | 2             |
| ADRB2       | Harvard Uni        | 1000       | 100       | 1             |
| ADRB2       | Harvard Uni        | 500        | 100       | 0             |
| ADRB2       | Harvard Uni        | 250        | 100       | 0             |
| ADRB2       | Harvard Uni        | 125        | 100       | 0             |
| ADRB2       | Harvard Uni        | 62.5       | 100       | 0             |
| ADRB2       | Harvard Uni        | 31.25      | 100       | 0             |
| ADRB2       | Harvard Uni        | 15.625     | 100       | 0             |
| ADRB2       | Harvard Uni        | 7.8125     | 100       | 0             |
| ADRB2       | Harvard Uni        | 3.90625    | 100       | 0             |
| ADRB2       | Harvard Uni        | 1.953125   | 100       | 0             |
| ADRB2       | Harvard Uni        | 0.9765625  | 100       | 0             |
| ADRB2       | Harvard Uni        | 0.48828125| 100       | 0             |
| ADRB2       | Harvard Uni        | 0.24414062| 100       | 0             |
| ADRB2       | Harvard Uni        | 0.12207031| 100       | 0             |
| ADRB2       | Harvard Uni        | 0.06103515| 100       | 0             |
| ADRB2       | Harvard Uni        | 0.03051758| 100       | 0             |
| ADRB2       | Harvard Uni        | 0.01525879| 100       | 0             |
| ADRB2       | Harvard Uni        | 0.00762939| 100       | 0             |
| ADRB2       | Harvard Uni        | 0.00381469| 100       | 0             |
| ADRB2       | Harvard Uni        | 0.00190735| 100       | 0             |
| ADRB2       | Harvard Uni        | 0.00095367| 100       | 0             |
| ADRB2       | Harvard Uni        | 0.00047684| 100       | 0             |
| ADRB2       | Harvard Uni        | 0.00023842| 100       | 0             |
| ADRB2       | Harvard Uni        | 0.00011921| 100       | 0             |
| ADRB2       | Harvard Uni        | 0.00005960| 100       | 0             |
| ADRB2       | Harvard Uni        | 0.00002980| 100       | 0             |
| ADRB2       | Harvard Uni        | 0.00001490| 100       | 0             |
| ADRB2       | Harvard Uni        | 0.00000745| 100       | 0             |
| ADRB2       | Harvard Uni        | 0.00000373| 100       | 0             |
| ADRB2       | Harvard Uni        | 0.00000187| 100       | 0             |
| ADRB2       | Harvard Uni        | 0.00000093| 100       | 0             |
| ADRB2       | Harvard Uni        | 0.00000046| 100       | 0             |
| ADRB2       | Harvard Uni        | 0.00000023| 100       | 0             |
| ADRB2       | Harvard Uni        | 0.00000012| 100       | 0             |
| ADRB2       | Harvard Uni        | 0.00000006| 100       | 0             |
| ADRB2       | Harvard Uni        | 0.00000003| 100       | 0             |
| ADRB2       | Harvard Uni        | 0.00000001| 100       | 0             |
| ADRB2       | Harvard Uni        | 0.00000000| 100       | 0             |
Summary

DiscoveRx successfully profiled 1 compound against the gpcrMAX Panel.

The assays were performed utilizing the PathHunter beta-arrestin enzyme fragment complementation (EFC) technology. Results are summarized in this report and the data is provided in accompanying Excel spreadsheet files.

This is to certify that the data contained within this report was conducted as described above.

Lakshmi Anantharaman
Associate Director, LeadHunter Services
| Compound Name | Assay Name | Assay Format | Assay Target | Result Type | EC50 (uM) | Hill | Curve Bottom | Curve Top | Max Response | Result Graph |
|---------------|------------|--------------|--------------|-------------|-----------|------|--------------|-----------|--------------|-------------|
| PACAP-27      | Arrestin   | Agonist      | ADCYAP1R1    | EC50        | 0.0027627 | 1.68 | -1.6        | 98.9      | 100.64       |
| 2-Cl-IB-MECA  | Arrestin   | Agonist      | ADORA3       | EC50        | 0.017318  | 1.25 | -11.2       | 102.6     | 103.45       |
| Phenylephrine | Arrestin   | Agonist      | ADRA1B       | EC50        | 0.21859   | 0.87 | -1.7        | 102.9     | 101.32       |
| UK 14,304     | Arrestin   | Agonist      | ADRA2A       | EC50        | 0.0036845 | 0.68 | -7.9        | 100       | 100.65       |
| UK 14,304     | Arrestin   | Agonist      | ADRA2B       | EC50        | 0.17656   | 1.52 | -7.5        | 97.9      | 101.55       |
| UK 14,304     | Arrestin   | Agonist      | ADRA2C       | EC50        | 0.07799   | 1.19 | -12.4       | 98.6      | 102.1        |
| Compound Name | Assay Name | Assay Target | EC50 (nM) | Hill | Curve Bottom | Curve Top | Max Response | | | | | | | | | | | | | | Result Graph |
|---------------|------------|--------------|-----------|------|--------------|----------|--------------|---|---|---|---|---|---|---|---|---|
| Isoproterenol | Arrestin | ADRB1 | 0.078654 | 1.13 | -5.1 | 100.3 | 101.79 | [Graph](image) |
| Isoproterenol | Arrestin | ADRB2 | 0.060695 | 1.3 | -0.5 | 100.4 | 100.17 | [Graph](image) |
| Angiotensin II | Arrestin | AGTR1 | 0.0008429 | 0.97 | -3.3 | 97.3 | 100.4 | [Graph](image) |
| Apelin-13 | Arrestin | AGTRL1 | 0.00050701 | 1.39 | -3.9 | 99 | 103.09 | [Graph](image) |
| Vasopressin | Arrestin | AVPR1A | 0.0015681 | 0.8 | -5.4 | 102 | 100.26 | [Graph](image) |
| Vasopressin | Arrestin | AVPR1B | 0.00017909 | 0.8 | -3.3 | 103.5 | 100.68 | [Graph](image) |
| Compound Name | Assay Name | Assay Format | Assay Target | Result Type | EC50 (µM) | Hill | Curve Bottom | Curve Top | Max Response |
|--------------|------------|--------------|--------------|-------------|-----------|------|--------------|-----------|--------------|
| Vasopressin  | Arrestin   | EC50         | AVPR2        | 0.00029239  | 1.32      | 96.6 | 100.04       |           |              |
| LDA-Bradykinin | Arrestin   | EC50         | BDKRB1       | 0.0002469   | 1.16      | -4.9 | 100.6        | 101.54    |              |
| Bradykinin   | Arrestin   | EC50         | BDKRB2       | 0.00040968  | 1.54      | -0.9 | 99.9         | 100.48    |              |
| TAPN-Bombesin| Arrestin   | EC50         | BRS3         | 0.0007402   | 0.68      | -22.2| 104.6        | 107.74    |              |
| C3A Receptor Agonist (Short Fragment) | Arrestin | EC50 | C3AR1 | 0.28745 | 1.18 | -1.2 | 102.1 | 106.9 |
| Complement C5a | Arrestin   | EC50         | CSAR1        | 0.000015412 | 2.98      | 0.5  | 101.7        | 106.11    |              |
| Compound Name | Assay Name | Assay Target | Result Type | EC50 (µM) | Hill | Curve Bottom | Curve Top | Max Response | Result Graph |
|---------------|------------|--------------|-------------|-----------|------|--------------|----------|--------------|-------------|
| Complement C5a | Arrestin Agonist | CSL2 | EC50 | 0.00070387 | 1.52 | -13.7 | 104.8 | 100.84 |
| | | | | | | | | | |
| Calcitonin | Arrestin Agonist | CALCR | EC50 | 0.041237 | 1.21 | -1.1 | 105.3 | 103.35 |
| | | | | | | | | |
| beta CGRP | Arrestin Agonist | CALCRL-RAMP1 | EC50 | 0.0011776 | 1.46 | 8.1 | 98.4 | 100.01 |
| | | | | | | | | |
| Adrenomedullin | Arrestin Agonist | CALCRL-RAMP2 | EC50 | 0.0010833 | 1.5 | 0.8 | 99.7 | 100.4 |
| | | | | | | | | |
| Adrenomedullin | Arrestin Agonist | CALCRL-RAMP3 | EC50 | 0.003256 | 1.17 | 0.1 | 98.1 | 100.21 |
| | | | | | | | | |
| Calcitonin | Arrestin Agonist | CALCR-RAMP2 | EC50 | 0.01494 | 0.95 | -0.2 | 99.5 | 102.69 |
| Compound Name | Assay Name | Assay Target | Assay Format | Result Type | EC50 (uM) | Hill | Curve Bottom | Curve Top | Max Response | Result Graph |
|---------------|------------|--------------|--------------|-------------|-----------|------|--------------|-----------|-------------|--------------|
| Calcitonin    | Arrestin   | Agonist      | CALCR-RAMP3  | EC50        | 0.081074  | 0.72 | 16.3         | 100       | 105.5       | Calcitonin-RAMP3 |
| CCK-8         | Arrestin   | Agonist      | CCKAR        | EC50        | 0.0048874 | 1.03 | -2           | 100.7     | 101.23      | CCK-8 CCKAR     |
|               | CCK-8      | Agonist      | CCKBR        | EC50        | 0.00026335| 1.6  | -1.4         | 97        | 101.1       | CCK-8 CCKBR   |
| CCL27         | Arrestin   | Agonist      | CCR10        | EC50        | 0.017333  | 1.84 | 0.7          | 100.9     | 102.41      | CCL27 CCR10   |
| CCL3          | Arrestin   | Agonist      | CCR1         | EC50        | 0.00042576| 1.59 | -10.9        | 97.5      | 101.37      | CCL3 CCR1     |
| CCL2          | Arrestin   | Agonist      | CCR2         | EC50        | 0.0050205 | 0.99 | -3.9         | 102       | 105.82      | CCL2 CCR2     |
| Compound Name | Assay Name | Assay Format | Assay Target | Result Type | EC50 (µM) | Hill | Curve Bottom | Curve Top | Max Response | Result Graph |
|---------------|------------|--------------|--------------|-------------|-----------|------|--------------|-----------|--------------|--------------|
| CCL13         | Arrestin   | Agonist      | CCR3         | EC50        | 0.018495  | 1.07 | -12.1        | 100       | 107.71       |              |
| CCL22         | Arrestin   | Agonist      | CCR4         | EC50        | 0.004488  | 0.77 | -1.4         | 100       | 103.17       |              |
| CCL3          | Arrestin   | Agonist      | CCR5         | EC50        | 0.0092168 | 0.7  | -4.9         | 110.1     | 107.65       |              |
| CCL20         | Arrestin   | Agonist      | CCR6         | EC50        | 0.0073553 | 0.76 | -4.8         | 106.1     | 105.49       |              |
| CCL19         | Arrestin   | Agonist      | CCR7         | EC50        | 0.005935  | 1.93 | -1           | 99        | 100.74       |              |
| CCL1          | Arrestin   | Agonist      | CCR8         | EC50        | 0.026054  | 1.24 | -1           | 102.2     | 103.71       |              |
| Compound Name | Assay Name | Assay Format | Assay Target | Result Type | EC50 (uM) | Slope | Curve Bottom | Curve Top | Max Response | Result Graph |
|---------------|------------|--------------|--------------|-------------|-----------|-------|--------------|----------|--------------|--------------|
| CCL25 Arrestin Agonist CCR9 | EC50 | 0.17787 | 1.67 | 0.6 | 100 | 100 |
| Acetylcholine Arrestin Agonist CHRM1 | EC50 | 2.4485 | 0.78 | -6.8 | 104.2 | 102.24 |
| Acetylcholine Arrestin Agonist CHRM2 | EC50 | 5.6576 | 1.03 | -3.2 | 102.1 | 100.33 |
| Acetylcholine Arrestin Agonist CHRM3 | EC50 | 0.7922 | 0.78 | -4.5 | 103.3 | 103.96 |
| Acetylcholine Arrestin Agonist CHRM4 | EC50 | 1.9964 | 1.38 | -16.9 | 104.6 | 102.18 |
| Acetylcholine Arrestin Agonist CHRM5 | EC50 | 0.26794 | 0.89 | -7.8 | 97 | 100.01 |
| Compound Name | Assay Name | Assay Format | Assay Target | Result Type | EC50 (uM) | Hill Curve | Bottom Curve | Top Max Response | Result Graph |
|---------------|------------|--------------|--------------|-------------|-----------|-----------|--------------|-----------------|-------------|
| Chemerin      | Arrestin   | Agonist      | CMKL1        | EC50        | 0.004443  | 1.4       | -1.2         | 97.3            | 102.14      |
| CP55940       | Arrestin   | Agonist      | CNR1         | EC50        | 0.011297  | 1.13      | -2.3         | 102.6           | 101.64      |
| CP55940       | Arrestin   | Agonist      | CNR2         | EC50        | 0.001426  | 1.12      | -15          | 109.5           | 107.56      |
| Sauvagine     | Arrestin   | Agonist      | CRHR1        | EC50        | 0.004258  | 2.99      | 2.9          | 101.3           | 100.14      |
| Sauvagine     | Arrestin   | Agonist      | CRHR2        | EC50        | 0.0066575 | 1.42      | -0.1         | 99.3            | 101.84      |
| PGD2          | Arrestin   | Agonist      | CRTH2        | EC50        | 0.0049044 | 0.74      | 0            | 97.6            | 102.1       |
| Compound Name | Assay Name | Assay Format | Assay Target | Result Type | EC50 (µM) | Hill | Curve Bottom | Curve Top | Max Response | Result Graph |
|---------------|------------|--------------|--------------|-------------|-----------|------|--------------|-----------|--------------|-------------|
| Fractalkine   | Arrestin   | Agonist      | CX3CR1      | RC50 (µM)   | 0.00073446 | 1.53 | -0.2         | 97.3      | 102.59       | Fractalkine  |
| CXCL8         | Arrestin   | Agonist      | CXCR1       | EC50        | 0.0036628 | 1.09 | -1.6         | 101.4     | 100.23       | CXCL8       |
| CXCL8         | Arrestin   | Agonist      | CXCR2       | EC50        | 0.00073033| 0.84 | -12.4        | 99.8      | 109.95       | CXCL8       |
| CXCL11        | Arrestin   | Agonist      | CXCR3       | EC50        | 0.025161 | 1    | -14.6        | 111.2     | 105.39       | CXCL11      |
| CXCL12        | Arrestin   | Agonist      | CXCR4       | EC50        | 0.00168 | 0.92 | 0            | 106.4     | 108.15       | CXCL12      |
| CXCL13        | Arrestin   | Agonist      | CXCR5       | EC50        | 0.046452| 1.05 | -1.2         | 117.7     | 110.26       | CXCL13      |
| Compound Name | Assay Name | Assay Format | Assay Target | Result Type | EC50 (uM) | Hill | Curve Bottom | Curve Top | Max Response | Result Graph |
|---------------|------------|--------------|--------------|-------------|-----------|------|---------------|-----------|---------------|--------------|
| CXCL16        | Arrestin   | Agonist      | CXCR6        | EC50        | 0.0007772 | 1.54 | -4            | 100       | 97.619        |
| CXCL12        | Arrestin   | Agonist      | CXCR7        | EC50        | 0.014645  | 1.84 | -1.1          | 100.8     | 101.95        |
| Dopamine      | Arrestin   | Agonist      | DRD1         | EC50        | 0.45512   | 1.3  | -1.4          | 104.4     | 104.15        |
| Dopamine      | Arrestin   | Agonist      | DRD2L        | EC50        | 0.097055  | 1.1  | -3.4          | 102.4     | 100.24        |
| Dopamine      | Arrestin   | Agonist      | DRD2S        | EC50        | 0.082691  | 1.39 | -5.3          | 101.2     | 109.01        |
| Dopamine      | Arrestin   | Agonist      | DRD3         | EC50        | 0.007335  | 1.21 | -10           | 105.3     | 102.25        |
### Compound Name Assay Name Assay Format Assay Target Result Type RC50 (μM) Hill Curve Bottom Curve Top Max Response Result Graph

| Compound Name | Assay Name | Assay Format | Assay Target | Result Type | RC50 (μM) | Hill | Curve Bottom | Curve Top | Max Response | Result Graph |
|---------------|------------|--------------|--------------|-------------|-----------|------|--------------|----------|--------------|--------------|
| Dopamine      | Arrestin   | Agonist      | DRD4         | EC50        | 0.17021   | 1.7  | -6.6         | 98.7     | 100.2        | Dopamine DRD4 |
| Dopamine      | Arrestin   | Agonist      | DRD5         | EC50        | 0.11691   | 1.5  | -1.1         | 100.6    | 103.22       | Dopamine DRD5 |
| 7α,25-Dihydroxycholesterol | Arrestin | Agonist | EBI2 | EC50 | 0.054752 | 0.91 | -1.2         | 101.9    | 104.97       | 7α,25-Dihydroxycholesterol EBI2 |
| S-1-P         | Arrestin   | Agonist      | EDG1         | EC50        | 0.025379  | 0.76 | 0            | 90.3     | 107.15       | S-1-P EDG1   |
| S-1-P         | Arrestin   | Agonist      | EDG3         | EC50        | 0.024481  | 1.06 | -4.5         | 101.5    | 102.65       | S-1-P EDG3   |
| Oleoyl LPA    | Arrestin   | Agonist      | EDG4         | EC50        | 0.76268   | 0.93 | -12.8        | 102.6    | 103.67       | Oleoyl LPA EDG4 |
| Compound Name | Assay Name | Assay Target | Result Graph |
|---------------|------------|--------------|--------------|
| S-1-P         | Arrestin   | EDG5         | ![Graph](image1.png) |
|               | Arrestin   | EDG6         | ![Graph](image2.png) |
| Oleoyl LPA    | Arrestin   | EDG7         | ![Graph](image3.png) |
| Endothelin 1  | Arrestin   | EDNRA        | ![Graph](image4.png) |
| Endothelin 3  | Arrestin   | EDNRB        | ![Graph](image5.png) |
| TFFLR-NH2     | Arrestin   | F2R          | ![Graph](image6.png) |
| Compound Name       | Assay Name     | Assay Name | Assay Format | Assay Target | Result Type | EC50 (uM) | Slope   | Min     | Max     |
|---------------------|----------------|------------|--------------|--------------|-------------|-----------|---------|---------|---------|
| SLIGRL-NH2           | Arrestin       | Agonist    | F2RL1        | EC50         |             | 0.66847   | 1.03    | -2.4    | 103.4   | 104.5   |
| AYPGKF-NH2           | Arrestin       | Agonist    | F2RL3        | EC50         |             | 3.32      | 2.13    | 0.5     | 99      | 103.95  |
| GW5508               | Arrestin       | Agonist    | FFAR1        | EC50         |             | 2.5318    | 0.92    | -12.7   | 108.9   | 110.88  |
| WKYMVm-NH2           | Arrestin       | Agonist    | FPR1         | EC50         |             | 0.0054478 | 1.12    | -10.1   | 98.7    | 100.61  |
| WKYMVm-NH2           | Arrestin       | Agonist    | FPRL1        | EC50         |             | 0.0020325 | 2.23    | 1       | 104.7   | 103.7   |
| FSH                  | Arrestin       | Agonist    | FSHR         | EC50         |             | 0.002265  | 1.02    | -4.6    | 103.5   | 100.09  |
| Compound Name | Assay Name | Assay Format | Assay Target | Result Type | EC50 (nM) | Hill | Curve Bottom | Curve Top | Max Response | Result Graph |
|---------------|------------|--------------|--------------|-------------|-----------|------|---------------|-----------|--------------|-------------|
| Galanin       | Arrestin   | Agonist      | GALR1        | EC50        | 0.0062252 | 1.54 | 1.7           | 98.3      | 100.77       | ![Galanin GALR1 Graph](image) |
| Galanin       | Arrestin   | Agonist      | GALR2        | EC50        | 0.017215  | 0.85 | 5.6           | 99.7      | 102.79       | ![Galanin GALR2 Graph](image) |
| Glucagon      | Arrestin   | Agonist      | GCGR         | EC50        | 0.0073343 | 1.7  | 1.3           | 104       | 102.61       | ![Glucagon GCGR Graph](image) |
| Ghrelin       | Arrestin   | Agonist      | GHSR         | EC50        | 0.0073928 | 1.77 | -5            | 99.4      | 101.43       | ![Ghrelin GHSR Graph](image) |
| GIP           | Arrestin   | Agonist      | GIPR         | EC50        | 0.01461   | 1.18 | -1.3          | 103       | 106          | ![GIP GIPR Graph](image) |
| Exendin-4     | Arrestin   | Agonist      | GLP1R        | EC50        | 0.0037954 | 1.41 | 1.2           | 98.8      | 101.44       | ![Exendin-4 GLP1R Graph](image) |
| Compound Name          | Assay Name | Assay Format | Assay Target | Result Type | EC50 (μM) | Hill | Curve Bottom | Curve Top | Max Response |
|------------------------|------------|--------------|--------------|-------------|-----------|------|--------------|-----------|--------------|
| GLP II (1-33)          | Arrestin   | Agonist      | GLP2R        | EC50        | 0.0017774 | 1    | 11.1         | 98.6      | 104.11       |
| Chemerin               | Arrestin   | Agonist      | GPR1         | EC50        | 0.0027464 | 1.64 | 1.3          | 98.6      | 104.03       |
| QRFP-26                | Arrestin   | Agonist      | GPR103       | EC50        | 0.0030523 | 1.4  | -6.6         | 101.4     | 106.5        |
| Nicotinic Acid         | Arrestin   | Agonist      | GPR109A      | EC50        | 4.1858    | 1.04 | -2.2         | 115.8     | 108          |
| 3-Hydroxyoctanoic Acid | Arrestin   | Agonist      | GPR109B      | EC50        | 571.32    | 1.33 | 1.5          | 100       | 100          |
| Oleoyl Ethanolamide    | Arrestin   | Agonist      | GPR119       | EC50        | 1.9228    | 0.98 | -10.4        | 105.2     | 112.24       |
| Compound Name | Assay Name | Assay Format | Assay Target | Result Type | EC50 (nM) | Hill | Curve Bottom | Curve Top | Max Response | Result Graph |
|---------------|------------|--------------|--------------|-------------|-----------|------|---------------|-----------|--------------|--------------|
| GW9508        | Arrestin   | Agonist      | GPR120       | EC50        | 18.319    | 1    | -8.1          | 137       | 114.71       | GW9508 GPR120 |
| Zaprinast     | Arrestin   | Agonist      | GPR35        | EC50        | 1.7611    | 0.94 | -4.3          | 100       | 112.56       | Zaprinast GPR35 |
| Oleoyl LPA    | Arrestin   | Agonist      | GPR92        | EC50        | 0.77293   | 1.05 | -5.8          | 100.3     | 103.27       | Oleoyl LPA GPR92 |
| GRP           | Arrestin   | Agonist      | GRPR         | EC50        | 0.001761  | 1.97 | 1.3           | 102.7     | 100.63       | GRP GRPR |
| Orexin A      | Arrestin   | Agonist      | HCRTR1       | EC50        | 0.0069909 | 1.41 | -1.1          | 99        | 103.44       | Orexin A HCRTR1 |
| Orexin A      | Arrestin   | Agonist      | HCRTR2       | EC50        | 0.0092783 | 1.34 | -0.4          | 100.3     | 101.87       | Orexin A HCRTR2 |
| Compound Name | Assay Name | Assay Target | EC50 (µM) | Hill | Curve Bottom | Curve Top | Max Response |
|---------------|------------|--------------|-----------|------|--------------|----------|--------------|
| Histamine     | Arrestin   | HRH1         | 0.04675   | 1.24 | 0.4          | 99.9     | 104.68       |
| Histamine     | Arrestin   | HRH2         | 4.4267    | 1.18 | -5           | 105.6    | 100.65       |
| R-a-methylhistamine | Arrestin | HRH3         | 0.077177  | 1.27 | -4.9         | 100.2    | 103.72       |
| Histamine     | Arrestin   | HRH4         | 0.065634  | 1.19 | 0            | 107.7    | 103.38       |
| Serotonin / 5-HT | Arrestin | HTR1A        | 0.055226  | 1.53 | -5.3         | 101.8    | 101.41       |
| Serotonin / 5-HT | Arrestin | HTR1B        | 0.071379  | 1.38 | -5.5         | 106.1    | 102.08       |
| Compound Name / 5-HT | Assay Name | Assay Format | Assay Target | Result Type | EC50 (uM) | Hill | Curve Bottom | Curve Top | Max Response | Result Graph |
|----------------------|------------|--------------|--------------|-------------|-----------|------|--------------|-----------|--------------|--------------|
| Serotonin / 5-HT | Arrestin Agonist | HTR1E | EC50 | 0.0053768 | 1.9 | -1.5 | 118.2 | 112.49 |
| Serotonin / 5-HT | Arrestin Agonist | HTR1F | EC50 | 0.025647 | 1.45 | -10.3 | 109.3 | 112.92 |
| Serotonin / 5-HT | Arrestin Agonist | HTR2A | EC50 | 0.040728 | 0.95 | -1.5 | 102.4 | 101.36 |
| Serotonin / 5-HT | Arrestin Agonist | HTR2C | EC50 | 0.0043118 | 1.35 | -5.5 | 100.1 | 101.05 |
| Serotonin / 5-HT | Arrestin Agonist | HTR5A | EC50 | 0.012722 | 0.98 | -4.6 | 99.9 | 104.38 |
| Kisspeptin-10 | Arrestin Agonist | KISS1R | EC50 | 0.015141 | 0.98 | -6.8 | 98.9 | 104.68 |
| Compound Name | Assay Name | Assay Format | Assay Target | Result Type | EC50 (nM) | Hill | Curve Bottom | Curve Top | Max Response | Result Graph |
|---------------|------------|--------------|--------------|-------------|-----------|------|--------------|-----------|--------------|-------------|
| hCG Arrestin  | Agonist    | LHCGR        | EC50         | 0.0016121   | 0.8       | -0.6 | 100          | 109.33    |              |              |
| Leukotriene B4| Arrestin   | LTB4R        | EC50         | 0.37873     | 0.64      | 3    | 100          | 100       |              |              |
| Melanotan II | Arrestin   | MC1R         | EC50         | 0.0002796   | 1.33      | -1.2 | 100.6        | 106.83    |              |              |
| Melanotan II | Arrestin   | MC3R         | EC50         | 0.0010303   | 0.81      | -3.1 | 103.7        | 103.24    |              |              |
| Melanotan II | Arrestin   | MC4R         | EC50         | 0.00058887  | 1.08      | -3.5 | 101.4        | 102.09    |              |              |
| Melanotan II | Arrestin   | MC5R         | EC50         | 0.012762    | 0.77      | -5.5 | 105.1        | 104.96    |              |              |
| Compound Name | Assay Name | Assay Format | Assay Target | Result Type | RC50 (uM) | Hill | Curve Bottom | Curve Top | Max Response | Result Graph |
|---------------|------------|--------------|--------------|-------------|-----------|------|--------------|-----------|--------------|-------------|
| MCH Arrestin  | Agonist MCHR1 | EC50 | 0.056883 | 1.14 | -2.4 | 108.2 | 104.43 |
| MCH Arrestin  | Agonist MCHR2 | EC50 | 0.0056083 | 1.09 | -0.5 | 100.7 | 100.79 |
| Motilin Arrestin | Agonist MLNR | EC50 | 0.005208 | 0.92 | -3.2 | 100.6 | 100.79 |
| BAM(8-22) Arrestin | Agonist MRGPRX1 | EC50 | 3.7895 | 1.27 | 1.5 | 108.8 | 103.14 |
| Cortistatin 14 Arrestin | Agonist MRGPRX2 | EC50 | 0.23897 | 0.85 | -3 | 106.9 | 101.95 |
| 2-Iodomelatonin Arrestin | Agonist MTNR1A | EC50 | 0.00056431 | 1.18 | -10 | 101 | 102.19 |
| Compound Name | Assay Name | Assay Format | Assay Target | Result Type | EC50 (uM) | Hill | Curve Bottom | Curve Top | Max Response | Result Graph |
|---------------|------------|--------------|--------------|-------------|-----------|------|---------------|-----------|--------------|--------------|
| Neuromedin B  | Arrestin   | Agonist      | NMBR         | EC50        | 0.0019177 | 1.3  | -3.5          | 97.5      | 100.48       | Neuromedin-B NMBR |
| Neuromedin U-25 | Arrestin   | Agonist      | NMU1R        | EC50        | 0.0028749 | 1.3  | -1.7          | 100.3     | 101.27       | Neuromedin-U-25 NMU1R |
| Neuropeptide W23 | Arrestin   | Agonist      | NPBWR1       | EC50        | 0.0001835 | 1.77 | 1.1           | 100.9     | 100.17       | Neuropeptide-W23 NPBWR1 |
| Neuropeptide W23 | Arrestin   | Agonist      | NPBWR2       | EC50        | 0.00095104| 1.84 | -1.4          | 102.2     | 101.13       | Neuropeptide-W23 NPBWR2 |
| RFRP-3        | Arrestin   | Agonist      | NPFFR1       | EC50        | 0.059757  | 0.93 | -10           | 102.2     | 102.19       | RFRP-3 NPFFR1 |
| Neuropeptide S | Arrestin   | Agonist      | NPSR1B       | EC50        | 0.021951  | 0.82 | -8.7          | 101.8     | 100.76       | Neuropeptide-S NPSR1B |
| Compound Name | Assay Name | Assay Target | Result Type | EC50 (nM) | Hill | Curve Bottom | Curve Top | Max Response |
|---------------|------------|--------------|-------------|-----------|------|--------------|----------|-------------|
| Peptide YY    | Arrestin   | NPY1R        | EC50        | 0.0028894| 0.82 | -7.7         | 99.1     | 100.41      |
| Peptide YY    | Arrestin   | NPY2R        | EC50        | 0.0021196| 1.96 | -0.5         | 100.2    | 100.79      |
| [Lys 8,9] Neurtensin | Arrestin   | NTSR1        | EC50        | 0.000085067| 1.59 | 2.3          | 99.9     | 102.15      |
| DADLE         | Arrestin   | OPRD1        | EC50        | 0.0016588| 1.28 | -4           | 100.2    | 101.54      |
| Dynorphin A   | Arrestin   | OPRK1        | EC50        | 0.049033 | 1.16 | 1.9          | 103.6    | 100.9       |
| Orphanin FQ   | Arrestin   | OPRL1        | EC50        | 0.012725 | 1.21 | -4.9         | 100.8    | 101.49      |
| Compound Name | Assay Name | Assay Format | Assay Target | Result Type | EC50 (uM) | Hill  | Curve Bottom | Curve Top | Max Response | Result Graph |
|---------------|------------|--------------|--------------|-------------|-----------|-------|--------------|-----------|--------------|--------------|
| [Met] Enkephalin | Arrestin Agonist | OPRM1 | EC50 | 0.6347 | 0.87 | -4.7 | 101.3 | 106.46 |
| 5-OxoETE | Arrestin Agonist | OXER1 | EC50 | 1.8185 | 1.01 | -0.7 | 100 | 100 |
| Oxytocin | Arrestin Agonist | OXTR | EC50 | 0.005696 | 1.22 | 1.3 | 107.2 | 101.81 |
| 2-methylthio-ADP | Arrestin Agonist | P2RY1 | EC50 | 0.016643 | 0.82 | 2.6 | 100.3 | 100.21 |
| ATP | Arrestin Agonist | P2RY11 | EC50 | 394.93 | 5.17 | -0.1 | 100 | 100 |
| 2-methylthio-ADP | Arrestin Agonist | P2RY12 | EC50 | 0.00094391 | 1 | -6 | 98.3 | 102.49 |
| Compound Name | Assay Name | Assay Target | Result Type | EC50 (uM) | Hill | Curve Bottom | Curve Top | Max Response | Result Graph |
|---------------|------------|--------------|-------------|-----------|------|--------------|-----------|--------------|--------------|
| UTP Arrestin  | Agonist    | P2RY2        | EC50        | 0.5924    | 1.66 | -5.6         | 98        | 103.1        |
| UTP Arrestin  | Agonist    | P2RY4        | EC50        | 0.39628   | 1.06 | -1.2         | 106.3     | 101.3        |
| UTP Arrestin  | Agonist    | P2RY6        | EC50        | 0.09423   | 1.12 | 1.1          | 103       | 103.32       |
| Pancreatic Polypeptide | Arrestin | Agonist | PPYR1 | EC50 | 0.0016037 | 1.13 | -3.6 | 98.3 | 104.4 |
| PrRP-31 | Arrestin | Agonist | PRLHR | EC50 | 0.0026408 | 0.82 | -10 | 100 | 104.4 |
| EG VEGF | Arrestin | Agonist | PROKR1 | EC50 | 0.039994 | 0.9 | -1.3 | 120.1 | 110.86 |
| Compound Name | Assay Name | Assay Format | Assay Target | Result Type | EC50 (uM) | Hill | Curve Bottom | Curve Top | Max Response | Result Graph |
|---------------|------------|--------------|--------------|-------------|-----------|------|--------------|----------|--------------|-------------|
| EG VEGF       | Arrestin   | Anti-agonist  | PROKR2       | EC50        | 0.012877  | -0.9 | 102.4        | 101.12   | 98.74        | EG VEGF PROKR2 |
| PAF           | Arrestin   | Anti-agonist  | PTAFR        | EC50        | 0.0065525 | 1.87 | 97.9         | 101.24   | 99.32        | PAF PTAFR |
| Prostaglandin E2 | Arrestin | Anti-agonist  | PTGER2       | EC50        | 1.4375    | 0.9  | 1.9          | 100      | 106.77       | Prostaglandin E2 PTGER2 |
| Prostaglandin E2 | Arrestin | Anti-agonist  | PTGER3       | EC50        | 0.009949  | 1.19 | -1.8         | 99.5     | 101.36       | Prostaglandin E2 PTGER3 |
| Prostaglandin E2 | Arrestin | Anti-agonist  | PTGER4       | EC50        | 0.001753  | 1.25 | -8.4         | 99.3     | 101.82       | Prostaglandin E2 PTGER4 |
| Cloprostenol   | Arrestin   | Anti-agonist  | PTGFR        | EC50        | 0.009351  | 1.15 | -2.9         | 95.6     | 107.94       | Cloprostenol PTGFR |
| Compound Name | Assay Name | Assay Format | Assay Target | Result Type | EC50 (nM) | Hill | Curve Bottom | Curve Top | Max Response | Result Graph |
|---------------|------------|--------------|--------------|-------------|-----------|------|--------------|-----------|--------------|--------------|
| Beraprost     | Arrestin   | Agonist      | PTGIR        | EC50        | 0.7705    | 0.81 | -2.4         | 104.8     | 103.14       | ![Beraprost Graph](image) |
| PTH(1-34)     | Arrestin   | Agonist      | PTHR1        | EC50        | 0.001234  | 1.64 | 0.1          | 96.9      | 102.22       | ![PTH(1-34) Graph](image) |
| TIP-39        | Arrestin   | Agonist      | PTHR2        | EC50        | 0.0007699 | 1.4  | -1.8         | 95.7      | 101.06       | ![TIP-39 Graph](image) |
| Relaxin-3     | Arrestin   | Agonist      | RXFP3        | EC50        | 0.023973  | 1.07 | -4.7         | 103.7     | 109.71       | ![Relaxin-3 Graph](image) |
| Secretin      | Arrestin   | Agonist      | SCTR         | EC50        | 0.001206  | 1.85 | -0.5         | 99        | 103          | ![Secretin Graph](image) |
| Somatostatin 28 | Arrestin   | Agonist      | SSTR1        | EC50        | 0.0061574 | 0.71 | -7.8         | 111       | 104.21       | ![Somatostatin Graph](image) |
| Compound Name | Assay Name | Format | Assay Target | Result Type | EC50 (µM) | Hill | Curve Bottom | Curve Top | Max Response |
|---------------|------------|--------|--------------|--------------|-----------|------|--------------|-----------|--------------|
| I-BOP         | Arrestin   | Agonist| TBXA2R       | EC50         | 0.05836   | 0.82 | -5.5         | 109.4     | 108.03       |
| TRH           | Arrestin   | Agonist| TRHR         | EC50         | 0.0015109 | 0.89 | -2.7         | 105.2     | 102.87       |
| TSH           | Arrestin   | Agonist| TSHR(L)      | EC50         | 0.023069  | 0.98 | 0            | 105.6     | 104.84       |
| Urotensin II  | Arrestin   | Agonist| UTR2         | EC50         | 0.0015978 | 1.32 | -4.7         | 100.7     | 101.95       |
| VIP           | Arrestin   | Agonist| VIPR1        | EC50         | 0.0010821 | 1.98 | 5.2          | 100.5     | 102.25       |
| VIP           | Arrestin   | Agonist| VIPR2        | EC50         | 0.0014456 | 2.09 | 0.6          | 100       | 102.28       |