

Yohimbine as a Starting Point to Access Diverse Natural Product-Like Agents with Reprogrammed Activities against Cancer-Relevant GPCR Targets

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Supporting Information

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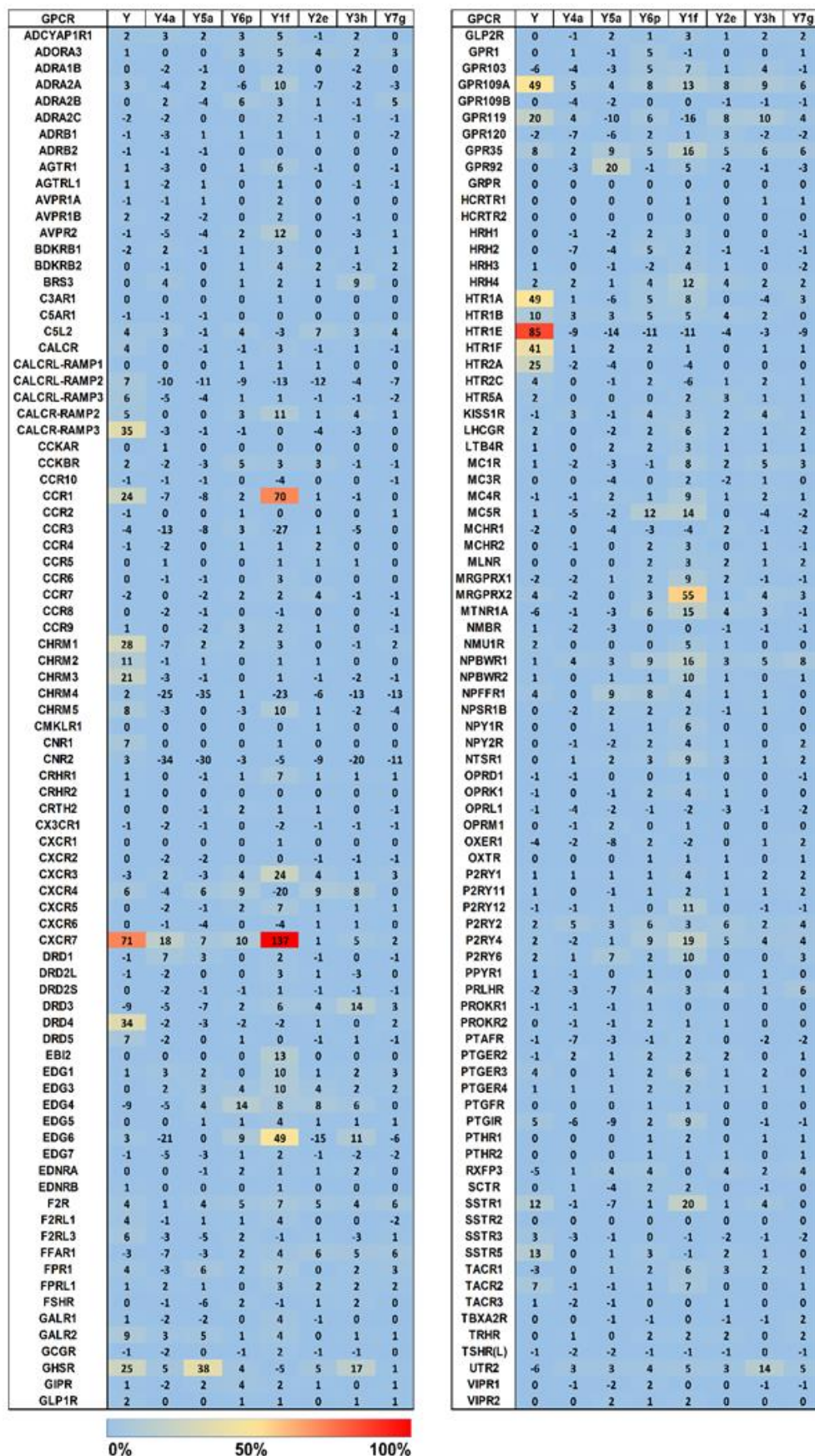
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I. General Information

Solid stocks of test compounds (~5 milligrams each) were shipped for in vitro screening against GPCR drug targets and follow up dose-response experiments at DiscoverX. Each compound was dissolved in DMSO stock solutions and tested for agonist and antagonist activity profiles against each of the 168 GPCRs in the panel.

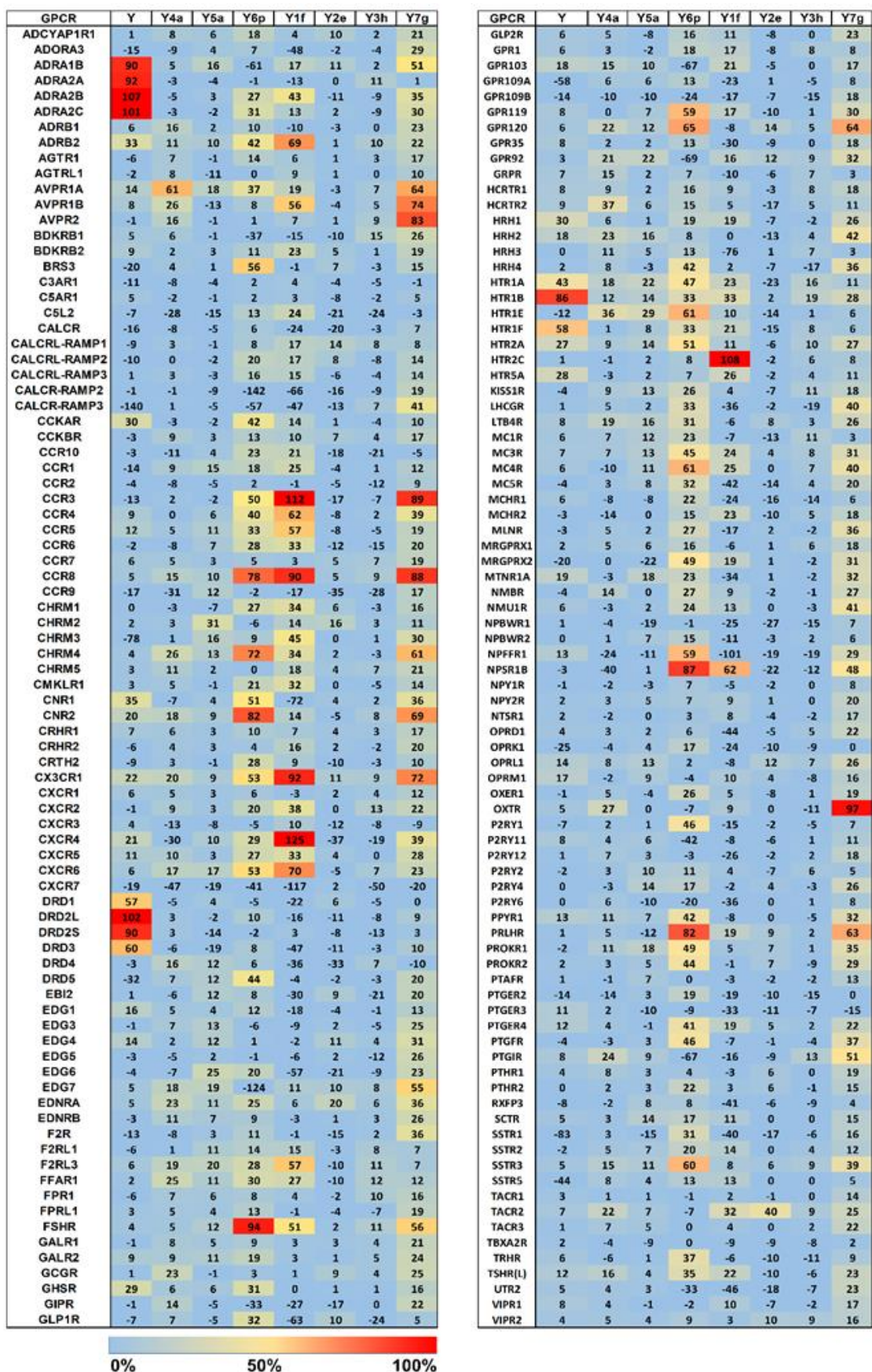
PathHunter β -Arrestin Assays were performed as follows: PathHunter cell lines were expanded from freezer stocks according to standard procedures. Cells were seeded in a total volume of 20 μ L into white walled, 384-well microplates and incubated at 37 °C prior to testing. For agonist determination, cells were incubated with sample to induce response. Intermediate dilution of sample stocks was performed to generate 5X sample in assay buffer. 5 μ L 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%. For antagonist determination, cells were pre-incubated with antagonist followed by agonist challenge at the EC₈₀ concentration. Intermediate dilution of sample stocks was performed to generate 5X sample in assay buffer. 5 μ L of 5X sample was added to cells and incubated at 37 °C or room temperature for 30 minutes. Vehicle concentration was 1%. 5 μ L 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. Assay signal was generated through a single addition of 12.5 or 15 μ L (50% v/v) of PathHunter Detection reagent cocktail, followed by a one hour incubation at room temperature. Microplates were read following signal generation with a PerkinElmer Envision™ instrument for chemiluminescent signal detection. Compound activity was analyzed using CBIS data analysis suite (ChemInnovation, CA). For agonist mode assays, percentage activity was calculated using the following formula: % Activity = 100% x (mean RLU of test sample - mean RLU of vehicle control) / (mean MAX control ligand - mean RLU of vehicle control). For antagonist mode assays, percentage inhibition was calculated using the following formula: % Inhibition = 100% x (1 - (mean RLU of test sample - mean RLU of vehicle control) / (mean RLU of EC80 control - mean RLU of vehicle control)). Note: This protocol is from DiscoverX. Dose-response experiments (antagonists) were carried out in assays analogous to the initial screen against GPCRs. Dose-response curves for all IC₅₀ values obtained during these studies can be found on later pages in this supporting information, including yohimbine-derived test compounds and known modulators (agonists and antagonists).

II. Supplementary Figure 1. Agonistic Activity Heatmap of Yohimbine Analogues against 168 GPCRs.



Rows correspond to GPCR drug targets. Columns correspond to percent activation of compounds screened in agonist mode at 20 μ M against each GPCR.

III. Supplementary Figure 2. Antagonistic Activity Heatmap of Yohimbine Analogues against 168 GPCRs.



Rows correspond to GPCR drug targets. Columns correspond to percent inhibition of compounds screened in antagonist mode at 20 μ M against each GPCR.

IV. Supplementary Figure 3. Y7g (NP-8-25) Dose-Dependent Inhibition against Select GPCRs.

Compound Name	Project ID	Assay Name	Assay Format	Assay Target	Result Type	RC50 (uM)	Hill	Curve Bottom	Curve Top	Max Response	Result Graph
NP-8-25	UFLD10-01-d-00001-000-00	Arrestin	Antagonist	AVPR2	IC50	0.4593884	2.4376	10.336	100.27	101.04	<p>NP-8-25 AVPR2 Max = 100.3 Slope = 2.438 Min = 10.34 IC50 = 0.4594 R2 = 0.9862</p>
NP-8-25	UFLD10-01-d-00001-000-00	Arrestin	Antagonist	CCR3	IC50	5.481103	1.3171	-3.3154	100	96.651	<p>NP-8-25 CCR3 Max = 100 Slope = 1.317 Min = -3.315 IC50 = 5.481 R2 = 0.9674</p>
NP-8-25	UFLD10-01-d-00001-000-00	Arrestin	Antagonist	CCR8	IC50	6.036859	1.7916	-8.2358	80	82.175	<p>NP-8-25 CCR8 Max = 80 Slope = 1.792 Min = -8.236 IC50 = 6.037 R2 = 0.9542</p>
NP-8-25	UFLD10-01-d-00001-000-00	Arrestin	Antagonist	OXTR	IC50	1.164683	1.819	-4.1245	100	96.486	<p>NP-8-25 OXTR Max = 100 Slope = 1.819 Min = -4.125 IC50 = 1.165 R2 = 0.9934</p>

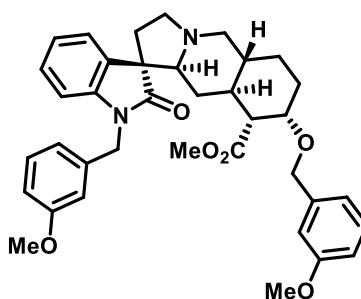
Positive-control comparators evaluated alongside Y7g (NP-8-25).

Compound Name	Project ID	Assay Name	Assay Format	Assay Target	Result Type	RCS0 (uM)	Hill	Curve Bottom	Curve Top	Max Response	Result Graph
Vasopressin	UFL010-01-d-00001-000-00	Arrestin	Agonist	AVPR2	EC50	9.556304E-05	2.3922	3.7904	100	85.911	<p>Vasopressin AVPR2</p> <p>Max = 100 Slope = 2.392 Min = 3.79 EC50 = 9.556e-5 R2 = 0.92</p>
Tolvaptan	UFL010-01-d-00001-000-00	Arrestin	Antagonist	AVPR2	IC50	7.338717E-06	1.8815	0	104.12	105.76	<p>Tolvaptan AVPR2</p> <p>Max = 104.1 Slope = 1.881 Min = 0 IC50 = 7.339e-6 R2 = 0.9898</p>
CCL13	UFL010-01-d-00001-000-00	Arrestin	Agonist	CCR3	EC50	0.03832658	1.2184	1.1819	100	100	<p>CCL13 CCR3</p> <p>Max = 100 Slope = 1.218 Min = 1.182 EC50 = 0.03833 R2 = 0.9921</p>
CCL1	UFL010-01-d-00001-000-00	Arrestin	Agonist	CCR8	EC50	0.09674311	0.99411	-0.72743	100	91.176	<p>CCL1 CCR8</p> <p>Max = 100 Slope = 0.9941 Min = -0.7274 EC50 = 0.09674 R2 = 0.9923</p>
Oxytocin	UFL010-01-d-00001-000-00	Arrestin	Agonist	OXTR	EC50	0.006488585	0.97039	0.96685	100	100	<p>Oxytocin OXTR</p> <p>Max = 100 Slope = 0.9704 Min = 0.9668 EC50 = 0.006489 R2 = 0.9865</p>

Continued. Positive-control comparators evaluated alongside Y7g (NP-8-25).

Compound Name	Project ID	Assay Name	Assay Format	Assay Target	Result Type	RC50 (uM)	Hill	Curve Bottom	Curve Top	Max Response	Result Graph
L-368,899	UFL010-01-d-00001-000-00	Arrestin	Antagonist	OXTR	IC50	0.004615725	0.93537	0	99.978	100.14	<p>L-368,899 OXTR</p> <p>Max = 99.98 Slope = 0.9354 Min = 0 IC50 = 0.004616 R2 = 0.995</p>

Summary of investigations with Y7g (NP-8-25).



Y7g (NP-8-25)

Compound Name	Project ID	Assay Name	Assay Format	Assay Target	Result Type	RC50 (uM)	Hill	Curve Bottom	Curve Top	Max Response
Vasopressin	UFL010-01-d-00001-000-00	Arrestin	Agonist	AVPR2	EC50	9.556304E-05	2.3922	3.7904	100	85.911
Tolvaptan	UFL010-01-d-00001-000-00	Arrestin	Antagonist	AVPR2	IC50	7.338717E-06	1.8815	0	104.12	105.76
CCL13	UFL010-01-d-00001-000-00	Arrestin	Agonist	CCR3	EC50	0.03832658	1.2184	1.1819	100	100
CCL1	UFL010-01-d-00001-000-00	Arrestin	Agonist	CCR8	EC50	0.09674311	0.99411	-0.72743	100	91.176
Oxytocin	UFL010-01-d-00001-000-00	Arrestin	Agonist	OXTR	EC50	0.006488585	0.97039	0.96685	100	100
L-368,899	UFL010-01-d-00001-000-00	Arrestin	Antagonist	OXTR	IC50	0.004615725	0.93537	0	99.978	100.14
NP-8-25	UFL010-01-d-00001-000-00	Arrestin	Antagonist	AVPR2	IC50	0.4593884	2.4376	10.336	100.27	101.04
NP-8-25	UFL010-01-d-00001-000-00	Arrestin	Antagonist	CCR3	IC50	5.481103	1.3171	-3.3154	100	96.651
NP-8-25	UFL010-01-d-00001-000-00	Arrestin	Antagonist	CCR8	IC50	6.036859	1.7916	-8.2358	80	82.175
NP-8-25	UFL010-01-d-00001-000-00	Arrestin	Antagonist	OXTR	IC50	1.164683	1.819	-4.1245	100	96.486

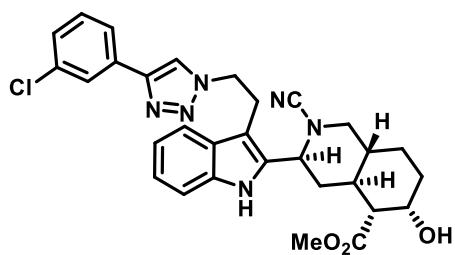
V. Supplementary Figure 4. Y1f (NP-7-53) Dose-Dependent Inhibition against Select GPCRs.

Compound Name	Project ID	Assay Name	Assay Format	Assay Target	Result Type	RC50 (uM)	Hill	Curve Bottom	Curve Top	Max Response	Result Graph
NP-7-53	UFL008-01-d-00001-000-00	Arrestin	Antagonist	CCR3	IC50	8.290315	4.3604	-2.3235	102.68	102.35	<p>NP-7-53 CCR3 Max = 102.7 Slope = 4.36 Min = -2.324 IC50 = 8.29 R2 = 0.975</p>
NP-7-53	UFL008-01-d-00001-000-00	Arrestin	Antagonist	CCR8	IC50	7.291603	2.5391	3.1561	87.175	85.051	<p>NP-7-53 CCR8 Max = 87.18 Slope = 2.539 Min = 3.156 IC50 = 7.292 R2 = 0.9694</p>
NP-7-53	UFL008-01-d-00001-000-00	Arrestin	Antagonist	CX3CR1	IC50	8.468666	2.3088	-0.26463	103.73	102.15	<p>NP-7-53 CX3CR1 Max = 103.7 Slope = 2.309 Min = -0.2646 IC50 = 8.469 R2 = 0.9878</p>
NP-7-53	UFL008-01-d-00001-000-00	Arrestin	Antagonist	CXCR4	IC50	7.936554	3.5291	10.203	105	100.4	<p>NP-7-53 CXCR4 Max = 105 Slope = 3.529 Min = 10.2 IC50 = 7.937 R2 = 0.8533</p>
NP-7-53	UFL008-01-d-00001-000-00	Arrestin	Antagonist	HTR2C	IC50	0.1756965	1.1212	0	106.22	110.1	<p>NP-7-53 HTR2C Max = 106.2 Slope = 1.121 Min = 0 IC50 = 0.1757 R2 = 0.9791</p>

Positive-control comparators evaluated alongside Y1f (NP-7-53).

Compound Name	Project ID	Assay Name	Assay Format	Assay Target	Result Type	EC50 (uM)	Hill	Curve Bottom	Curve Top	Max Response	Result Graph
CCL13	UFL008-01-d-00001-000-00	Arrestin	Agonist	CCR3	EC50	0.03832658	1.2184	1.1819	100	100	
CCL1	UFL008-01-d-00001-000-00	Arrestin	Agonist	CCR8	EC50	0.09674311	0.99411	-0.72743	100	91.176	
Fractalkine	UFL008-01-d-00001-000-00	Arrestin	Agonist	CX3CR1	EC50	0.002201558	1.0354	-1.0393	99.659	100	
CXCL12	UFL008-01-d-00001-000-00	Arrestin	Agonist	CXCR4	EC50	0.002401872	0.73368	-4.3946	100	102.31	
Serotonin / 5-HT	UFL008-01-d-00001-000-00	Arrestin	Agonist	HTR2C	EC50	0.002948356	0.99676	5.1362	100	105.56	

Summary of investigations with Y1f (NP-7-53).



Y1f (NP-7-53)

Compound Name	Project ID	Assay Name	Assay Format	Assay Target	Result Type	RC50 (uM)	Hill	Curve Bottom	Curve Top	Max Response
CCL13	UFL008-01-d-00001-000-00	Arrestin	Agonist	CCR3	EC50	0.03832658	1.2184	1.1819	100	100
CCL1	UFL008-01-d-00001-000-00	Arrestin	Agonist	CCR8	EC50	0.09674311	0.99411	-0.72743	100	91.176
Fractalkine	UFL008-01-d-00001-000-00	Arrestin	Agonist	CX3CR1	EC50	0.002201558	1.0354	-1.0393	99.659	100
CXCL12	UFL008-01-d-00001-000-00	Arrestin	Agonist	CXCR4	EC50	0.002401872	0.73368	-4.3946	100	102.31
Serotonin / 5-HT	UFL008-01-d-00001-000-00	Arrestin	Agonist	HTR2C	EC50	0.002948356	0.99676	5.1362	100	105.56
NP-7-53	UFL008-01-d-00001-000-00	Arrestin	Antagonist	CCR3	IC50	8.290315	4.3604	-2.3235	102.68	102.35
NP-7-53	UFL008-01-d-00001-000-00	Arrestin	Antagonist	CCR8	IC50	7.291603	2.5391	3.1561	87.175	85.051
NP-7-53	UFL008-01-d-00001-000-00	Arrestin	Antagonist	CX3CR1	IC50	8.468666	2.3088	-0.26463	103.73	102.15
NP-7-53	UFL008-01-d-00001-000-00	Arrestin	Antagonist	CXCR4	IC50	7.936554	3.5291	10.203	105	100.4
NP-7-53	UFL008-01-d-00001-000-00	Arrestin	Antagonist	HTR2C	IC50	0.1756965	1.1212	0	106.22	110.1

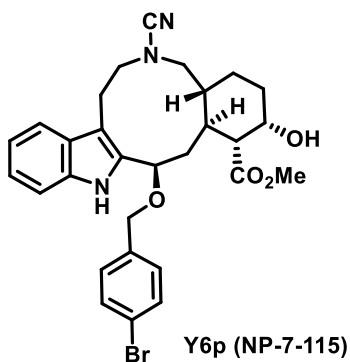
VI. Supplementary Figure 5. Y6p (NP-7-115) Dose-Dependent Inhibition against Select GPCRs.

Compound Name	Project ID	Assay Name	Assay Format	Assay Target	Result Type	RC50 (uM)	Hill	Curve Bottom	Curve Top	Max Response	Result Graph
NP-7-115	UFL009-01-d-00001-000-00	Arrestin	Antagonist	FSHR	IC50	8.03491	2.2975	-3.1607	99.537	98.194	<p>NP-7-115 FSHR</p> <p>Max = 99.54 Slope = 2.297 Min = -3.161 IC50 = 8.035 R2 = 0.9484</p>
NP-7-115	UFL009-01-d-00001-000-00	Arrestin	Antagonist	NPSR1B	IC50	10.31193	2.9899	4.9977	100	98.409	<p>NP-7-115 NPSR1B</p> <p>Max = 100 Slope = 2.99 Min = 4.998 IC50 = 10.31 R2 = 0.9784</p>
NP-7-115	UFL009-01-d-00001-000-00	Arrestin	Antagonist	PRLHR	IC50	4.892051	3.0216	7.135	100	100.2	<p>NP-7-115 PRLHR</p> <p>Max = 100 Slope = 3.022 Min = 7.135 IC50 = 4.892 R2 = 0.9622</p>

Positive-control comparators evaluated alongside Y6p (NP-7-115).

Compound Name	Project ID	Assay Name	Assay Format	Assay Target	Result Type	RC50 (uM)	Hill	Curve Bottom	Curve Top	Max Response	Result Graph
FSH	UFL009-01-d-00001-000-00	Arrestin	Agonist	FSHR	EC50	0.002949678	1.5455	8.2508	100	111.49	<p>FSH FSHR</p> <p>Max = 100 Slope = 1.545 Min = 8.251 EC50 = 0.00295 R2 = 0.9773</p>
Neuropeptide S	UFL009-01-d-00001-000-00	Arrestin	Agonist	NPSR1B	EC50	0.01396175	1.1544	0	97.292	103.44	<p>Neuropeptide S NPSR1B</p> <p>Max = 97.29 Slope = 1.154 Min = 0 EC50 = 0.01396 R2 = 0.9767</p>
SHA 68	UFL009-01-d-00001-000-00	Arrestin	Antagonist	NPSR1B	IC50	0.08218263	1.9069	0	109.26	112.12	<p>SHA 68 NPSR1B</p> <p>Max = 109.3 Slope = 1.907 Min = 0 IC50 = 0.08218 R2 = 0.9926</p>
PrRP-31	UFL009-01-d-00001-000-00	Arrestin	Agonist	PRLHR	EC50	0.001670129	1.2469	-2.7102	100	101.88	<p>PrRP-31 PRLHR</p> <p>Max = 100 Slope = 1.247 Min = -2.71 EC50 = 0.00167 R2 = 0.9948</p>

Summary of investigations with **Y6p (NP-7-115)**.



Compound Name	Project ID	Assay Name	Assay Format	Assay Target	Result Type	RC50 (uM)	Hill	Curve Bottom	Curve Top	Max Response
FSH	UFL009-01-d-00001-000-00	Arrestin	Agonist	FSHR	EC50	0.002949578	1.5455	8.2508	100	111.49
Neuropeptide S	UFL009-01-d-00001-000-00	Arrestin	Agonist	NPSR1B	EC50	0.01396175	1.1544	0	97.292	103.44
SHA 68	UFL009-01-d-00001-000-00	Arrestin	Antagonist	NPSR1B	IC50	0.08218263	1.9069	0	109.26	112.12
PrRP-31	UFL009-01-d-00001-000-00	Arrestin	Agonist	PRLHR	EC50	0.001670129	1.2469	-2.7102	100	101.88
NP-7-115	UFL009-01-d-00001-000-00	Arrestin	Antagonist	FSHR	IC50	8.03491	2.2975	-3.1607	99.537	98.194
NP-7-115	UFL009-01-d-00001-000-00	Arrestin	Antagonist	NPSR1B	IC50	10.31193	2.9899	4.9977	100	98.409
NP-7-115	UFL009-01-d-00001-000-00	Arrestin	Antagonist	PRLHR	IC50	4.892051	3.0216	7.135	100	100.2