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

Nicholas G. Paciaroni, Verrill M. Norwood IV, Ranjala Ratnayake, Hendrik Luesch*, Robert W. Huigens III*

University of Florida, Department of Medicinal Chemistry, Center for Natural Product Drug Discovery and Development (CNPD3), College of Pharmacy, United States

*Corresponding authors email: rhuigens@cop.ufl.edu, luesch@cop.ufl.edu, luesch@cop.ufl.edu, luesch@cop.ufl.edu, luesch@cop.ufl.edu, luesch@cop.ufl.edu, luesch@cop.ufl.edu, luesch@cop.ufl.edu)

Supporting Information

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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 µL into white walled, 384well 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 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%. 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 µL of 5X sample was added to cells and incubated at 37 °C or room temperature for 30 minutes. Vehicle concentration was 1%. 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. 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. Microplates were read following signal generation with a PerkinElmer EnvisionTM 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. Doseresponse experiments (antagonists) were carried out in assays analogous to the initial screen against GPCRs. Dose-response curves for all IC_{50} values obtained during these studies can be found on later pages in this supporting information, including vohimbine-derived test compounds and known modulators (agonists and antagonists).

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

100/110	Y	Y4a	Y5a	Y6p	Y1f	Y2e	Y3h	Y7g		PCR	Y	Y4a	Y5a	Y6p	Y1f		Y3h	Y7g
ADCYAP1R1	2	3	2	3	5	-1	2	0		P2R	0	-1	2	1	3	1	2	2
ADORA3 ADRA1B	1	0	0	3	5	4	2	3		PR1 R103	0	1 4	-1 -3	5	-1	0	0 4	-1
ADRA2A	3	-4	2	-6	10	.7	-2	-3	1.000	APOIS	49	5	4	8	13	8	9	6
ADRA2B	0	2	4	6	3	1	1	5	1000.001	2109B	0	-4	.2	ő	0	-1	-1	-1
ADRA2C	-2	-2	0	0	2	-1	-1	-1		R119	20	4	-10	6	-16	8	10	4
ADRB1	-1	-3	1	1	1	1	0	-2	GP	R120	-2	-7	-6	2	1	3	-2	-2
ADRB2	-1	-1	-1	0	0	0	0	0	GP	R35	8	2	9	5	16	5	6	6
AGTR1	1	-3	0	1	6	-1	0	-1		R92	0	-3	20	-1	5	-2	-1	-3
AGTRL1	1	-2	1	0	1	0	-1	-1	- 223	RPR	0	0	0	0	0	0	0	0
AVPR1A	-1	-1	1	0	2	0	0	0		RTR1	0	0	0	0	1	0	1	1
AVPR1B	2	-2	-2	0	2	0	-1	0		RTR2	0	0	0	0	0	0	0	0
AVPR2 BDKRB1	-1 -2	-5	-4	2	12 3	0	-3 1	1		RH1 RH2	0	-1 -7	-2	2	3	0	0 -1	-1 -1
BDKRB2	0	-1	0	1	4	2	-1	2		RH3	1	0	-1	-2	4	1	0	-2
BRS3	0	4	0	1	2	1	9	0		RH4	2	2	1	4	12	4	2	2
C3AR1	0	0	0	0	1	0	0	0	HT	R1A	49	1	-6	5	8	0	-4	3
C5AR1	-1	-1	-1	0	0	0	0	0	HT	R1B	10	3	3	5	5	4	2	0
C5L2	4	3	-1	4	-3	7	3	4		R1E	85	-9	-14	-11	-11	-4	-3	-9
CALCR	4	0	-1	-1	3	-1	1	-1	10000	R1F	41	1	2	2	1	0	1	1
CALCRL-RAMP1	0	0	0	1	1	1	0	0	10000	R2A	25	-2	-4	0	-4	0	0	0
CALCRL-RAMP2	7	-10	-11	-9	-13	-12	-4	-7	10000	R2C	4	0	-1	2	-6	1	2	1
CALCRL-RAMP3 CALCR-RAMP2	6	-5 0	-4	1	1 11	-1	-1 4	-2	100000	R5A SS1R	2	0	0	0	2	3	1 4	1
CALCR-RAMP3	35	.3	-1	-1	0	-4	.3	ō		CGR	2	0	-2	2	6	2	1	2
CCKAR	0	1	0	0	0	0	0	0		B4R	1	ō	2	2	3	1	1	1
CCKBR	2	2	-3	5	3	3	-1	-1	1.175.55	C1R	1	-2	-3	-1	8	2	5	3
CCR10	-1	-1	-1	0	-4	0	0	-1	M	C3R	0	0	-4	0	2	-2	1	0
CCR1	24	-7	-8	2	70	1	-1	0	M	C4R	-1	-1	2	1	9	1	2	1
CCR2	-1	0	0	1	0	0	0	1		C5R	1	-5	-2	12	14	0	-4	-2
CCR3	-4	-13	-8	3	-27	1	-5	0		HR1	-2	0	-4	-3	-4	2	-1	-2
CCR4	-1	-2	0	1	1	2	0	0		HR2	0	-1	0	2	3	0	1	-1
CCR5 CCR6	0	1	0	0	1 3	1	1	0	100000	LNR SPRX1	0	0	0	2	3	2	1	2
CCR7	-2	0	-2	2	2	4	-1	-1		PRX2	4	.2	0	3	55	1	4	3
CCR8	0	-2	-1	ô	-1	ō	0	-1		NRIA	-6	1	-3	6	15	4	3	-1
CCR9	1	0	-2	3	2	1	0	-1	1000 000	ABR	1	-2	-3	0	0	-1	-1	-1
CHRM1	28	-7	2	2	3	0	-1	2	NM	UIR	2	0	0	0	5	1	0	0
CHRM2	11	-1	1	0	1	1	0	0		3WR1	1	4	3	9	16	3	5	8
CHRM3	21	-3	-1	0	1	-1	-2	-1		3WR2	1	0	1	1	10	1	0	1
CHRM4	2	-25	-35	1	-23	-6	-13	-13	1.0710	FFR1	4	0	9	8	4	1	1	0
CHRM5 CMKLR1	8	-3	0	-3	10	1	-2	-4		SR1B Y1R	0	-2	2	2	2	-1	1	0
CNR1	7	0	0	0	0	1	0	0		Y2R	0	0	1	1	6	0	0	2
CNR2	3	-34	-30	-3	.5	.9	-20	-11		SR1	0	1	2	3	9	3	1	2
CRHR1	1	0	-1	1	7	1	1	1		RD1	-1	-1	0	0	1	0	0	-1
CRHR2	1	0	0	0	0	0	0	0	OP	RK1	-1	0	-1	2	4	1	0	0
CRTH2	0	0	-1	2	1	1	0	-1	OP	RL1	-1	-4	-2	-1	-2	-3	-1	-2
CX3CR1	-1	-2	-1	0	-2	-1	-1	-1	100000	RM1	0	-1	2	0	1	0	0	0
CXCR1	0	0	0	0	1	0	0	0		ER1	-4	-2	-8	2	-2	0	1	2
CXCR2	0	-2	-2	0	0	-1	-1	-1		XTR	0	0	0	1	1	1	0	1
CXCR3 CXCR4	-3	2	-3	4	-20	4	1	3		RY1 RY11	1	1	1	1	4 2	1	2	2
CXCR5	0	.2	-1	2	7	1	1	1		RY12	-1	-1	1	ō	11	0	-1	-1
CXCR6	0	-1	-4	ō	-4	î	1	0		RY2	2	5	3	6	3	6	2	4
CXCR7	71	18	7	10	137	1	5	2		RY4	2	-2	1	9	19	5	4	4
DRD1	-1	7	3	0	2	-1	0	-1	P2	RY6	2	1	7	2	10	0	0	3
DRD2L	-1	-2	0	0	3	1	-3	0	PP	YR1	1	-1	0	1	0	0	1	0
DRD2S	0	-2	-1	-1	1	-1	-1	-1		LHR	-2	-3	-7	4	3	4	1	6
DRD3	-9	-5	-7	2	6	4	14	3		OKR1	-1	-1	-1	1	0	0	0	0
DRD4	34	-2	-3	-2	-2	1	0	2		OKR2	0	-1	-1	2	1	1	0	0
DRD5 EBI2	7	-2	0	1	0	-1	1	-1		GER2	-1	.7	-3	-1	2	0	-2	-2
EBI2 EDG1	0	0	0	0	13 10	0	0	0		GER3	-1	2	1	2	2 6	2	0	1
EDG1 EDG3	0	2	3	4	10	4	2	2		GER4	1	1	1	2	2	1	1	1
EDG4	.g	-5	4	14	8	8	6	0		GFR	ō	0	0	1	1	ō	0	0
EDG5	0	0	1	1	4	1	1	1		GIR	5	-6	-9	2	9	0	-1	-1
EDG6	3	-21	0	9	49	-15	11	-6	PT	'HR1	0	0	0	1	2	0	1	1
EDG7	-1	-5	-3	1	2	-1	-2	-2		HR2	0	0	0	1	1	1	0	1
EDNRA	0	0	-1	2	1	1	2	0		FP3	-5	1	4	4	0	4	2	4
EDNRB	1	0	0	0	1	0	0	0		CTR	0	1	-4	2	2	0	-1	0
F2R	4	1	4	5	7	5	4	6		TR1	12	-1	-7	1	20	1	4	0
F2RL1 F2RL3	4	-1	1	1	4	0	0	-2		TR2 TR3	0	0	0	0	0	0	0	0
FFAR1	6 -3	-3	-5	2	-1 4	1 6	-3 5	1 6		TR5	3 13	-3	-1 1	3	-1 -1	-2 2	-1 1	-2
FPR1	4	-3	6	2	7	0	2	3		CR1	-3	õ	1	2	6	3	2	1
FPRL1	1	2	1	ő	3	2	2	2		CR2	7	-1	-1	1	7	0	0	1
FSHR	ō	-1	-6	2	-1	1	2	ō		CR3	1	-2	-1	0	0	1	0	ō
GALR1	1	-2	-2	0	4	-1	0	0		XA2R	0	0	-1	-1	0	-1	-1	2
GALR2	9	3	5	1	4	0	1	1		RHR	0	1	0	2	2	2	0	2
GCGR	-1	-2	0	-1	2	-1	-1	0		HR(L)	-1	-2	-2	-1	-1	-1	0	-1
GHSR	25	5	38	4	-5	5	17	1		TR2	-6	3	3	4	5	3	14	5
	1	-2	2	4	2	1	0	1		PR1 PR2	0	-1	-2	2	0	0	-1	-1
GIPR GLP1R	2	0	0	1	1	0	1	1				0		1	2	0	0	0

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

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

GPCR	Y	Y4a	Y5a	Y6p	Y1f	Y2e	Y3h	¥7g	GPCR	
ADCYAP1R1	1	8	6	18	4	10	2	21	GLP2R	
ADORA3	-15	-9	4	7	-48	-2	-4	29	GPR1	
ADRA1B	90	5	16	-61	17	11	2	51	GPR103	
ADRA2A ADRA2B	92	-3	4	-1	-13	0	11	1	GPR109A	-
ADRA2B ADRA2C	107	-5	3	27	43	-11	-9 -9	35	GPR109B	
ADRA2C		-3	-2	31	13	2	1000	30	GPR119	
ADRB1	6	16	2	10	-10	-3	0	23	GPR120	
AGTR1	33	11	10	42	69	1	10	22	GPR35	
AGTRL1	-6	7	-1	14	6	1	3	17	GPR92	
AVPR1A	-2	8	-11	0	9	1	0	10	GRPR	
	14	61	18	37	19	-3	7	64	HCRTR1	
AVPR1B	8	26	-13	8	56	-4	5	74	HCRTR2	
AVPR2	-1	16	-1	1	7	1	9	83	HRH1	
BDKRB1	5	6	-1	-37	-15	-10	15	26	HRH2	
BDKRB2	9	2	3	11	23	5	1	19	HRH3	
BR\$3	-20	4	1	56	-1	7	-3	15	HRH4	
C3AR1	-11	-8	-4	2	4	-4	-5	-1	HTR1A	-
C5AR1	5	-2	-1	2	3	-8	-2	5	HTR18	
C5L2	-7	-28	-15	13	24	-21	-24	-3	HTR1E	
CALCR	-16	-8	-5	6	-24	-20	-3	7	HTR1F	
CALCRL-RAMP1	-9	3	-1	8	17	14	8	8	HTR2A	
CALCRL-RAMP2	-10	0	-2	20	17	8	-8	14	HTR2C	
CALCRL-RAMP3	1	3	-3	16	15	-6	-4	14	HTR5A	1
CALCR-RAMP2	-1	-1	-9	-142	-66	-16	-9	19	KISS1R	
CALCR-RAMP3	-140	1	-5	-57	-47	-13	7	41	LHCGR	
CCKAR	30	-3	-2	42	14	1	-4	10	LTB4R	
CCKBR	-3	9	3	13	10	7	4	17	MC1R	
CCR10	-3	-11	4	23	21	-18	-21	-5	MC3R	
CCR1	-14	9	15	18	25	-4	1	12	MC4R	
CCR2	-4	-8	-5	2	-1	-5	-12	9	MCSR	
CCR3	-13	2	-2	50	112	-17	-7	89	MCHR1	
CCR4	9	0	6	40	62	-8	2	39	MCHR2	
CCR5	12	5	11	33	57	-8	-5	19	MLNR	
CCR6	-2	-8	7	28	33	-12	-15	20	MRGPRX1	
CCR7	6	5	3	5	3	5	7	19	MRGPRX2	
CCR8	5	15	10	78	90	5	9	88	MTNR1A	-
CCR9	-17	-31	12	-2	-17	-35	-28	17	NMBR	
CHRM1	0	-3	-7	27	34	6	-3	16	NMU1R	
CHRM2	2	3	31	-6	14	16	3	11	NPBWR1	
CHRM3	-78	1	16	9	45	0	1	30	NPBWR2	
CHRM4	4	26	13	72	34	2	-3	61	NPFFR1	
CHRM5	3	11	2	0	18	4	7	21	NPSR18	
CMKLR1	3	5	-1	21	32	0	-5	14	NPY1R	
CNR1	35	-7	4	51	-72	4	2	36	NPY2R	
CNR2	20	18	9	82	14	-5	8	69	NTSR1	
CRHR1	7	6	3	10	7	4	3	17	OPRD1	
CRHR2	-6	4	3	4	16	2	-2	20	OPRK1	
CRTH2	-9	3	-1	28	9	-10	-3	10	OPRL1	
CX3CR1	22	20	9	53	02	11	9	72	OPRM1	
CXCR1	6	5	3	6	-3	2	4	12	OXER1	
CXCR2	-1	9	3	20	38	0	13	22	OXTR	
CXCR3								And Address of Concession	P2RY1	
	4	-13	-8	-5	10	-12	-8	-9	1.0000000000000000000000000000000000000	
CXCR4	21	-30	10	29		-37	-19	39	P2RY11	
CXCR5 CXCR6	11	10	3	27	33	4	0	28	P2RY12	
1000000000	6	17	17	53	70	-5	7	23	P2RY2	
CXCR7	-19	-47	-19	-41	-117	2	-50	-20	P2RY4	
DRD1	57	-5	4	-5	-22	6	-5	0	P2RY6	
DRD2L	102	3	-2	10	-16	-11	-8	9	PPYR1	
DRD2S	90	3	-14	-2	3	-8	-13	3	PRLHR	
DRD3	60	-6	-19	8	-47	-11	-3	10	PROKR1	
DRD4	-3	16	12	6	-36	-33	7	-10	PROKR2	
DRD5	-32	7	12	44	-4	-2	-3	20	PTAFR	
EBI2	1	-6	12	8	-30	9	-21	20	PTGER2	1.5
EDG1	16	5	4	12	-18	-4	-1	13	PTGER3	
EDG3	-1	7	13	-6	-9	2	-5	25	PTGER4	
EDG4	14	2	12	1	-2	11	4	31	PTGFR	
EDG5	-3	-5	2	-1	-6	2	-12	26	PTGIR	
EDG6	-4	-7	25	20	-57	-21	-9	23	PTHR1	
EDG7	5	18	19	-124	11	10	8	55	PTHR2	
EDNRA	5	23	11	25	6	20	6	36	RXFP3	
EDNRB	-3	11	7	9	-3	1	3	26	SCTR	
F2R	-13	-8	3	11	-1	-15	2	36	SSTR1	10
F2RL1	-6	1	11	14	15	-3	8	7	SSTR2	
F2RL3	6	19	20	28	57	-10	11	7	SSTR3	
FFAR1	2	25	11	30	27	-10	12	12	SSTRS	100
FPR1	-6	7	6	8	4	-2	10	16	TACR1	
FPRL1	3	5	4	13	4	-4	-7	19	TACR2	
FSHR	4	s	12	94	51	2	11	56	TACR3	
GALR1	-1	8	5	9	3	3	4	21	TBXA2R	
GALR2	9	9	11	19	3	1	5	24	TRHR	
GCGR	1	23	-1	3	1	9	4	24	TSHR(L)	
GHSR								Conception of the local distance of the loca	100 B	
	29	6 14	6 -5	31 -33	0 -27	1	1	16	VIPR1	
				- 33	-61	- 4/	0	22	VIPE1	6 C
GIPR GLP1R	-1 -7	7	-5	32	-63	10	-24	5	VIPR2	

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)	HIII	Curve Bottom	Curve Top	Max Response	Result Graph
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 AVPR2 100 25 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
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 CCR3 100 50 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
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 CCR8 100 25 0 8 9 9 0 8 9 9 0 0.00 1 0.1 0.1 0 Max = 80 Stope = 1.792 Min = -8.238 IC50 = 6.037 R2 = 0.9542
NP-8-25	UFL010-01-d-00001- 000-00	Arrestin	Antagonist	OXTR	IC50	1.164683	1.819	-4.1245	100	96.486	NP-8-25 OXTR 100 25 0 0 0.001 0.01 0.10 1 10 Max = 100 Slope = 1.819 Min = -4.125 IC50 = 1.165 R2 = 0.9934

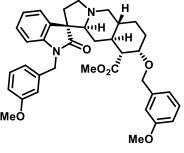
Positive-control comparators evaluated alongside Y7g (NP-8-25).

Compound Name	Project ID	Assay Name	Assay Format	Assay Target	Result Type	RC50 (uM)	HIII	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	Vasopressin AVPR2 100 75 50 25 0 1e-5 0.0001 0.01 0.01 0.10 Max = 100 Stope = 2.392 Min = 3.79 EC50 = 9.558e-5 R2 = 0.92
Tolvaptan	UFL010-01-d-00001- 000-00	Arrestin	Antagonist	AVPR2	1050	7.338717E- 05	1.8815	O	104.12	105.76	Tokvaptan AVPR2 100 75 0 1e-6 1e-5 0.0001 0.01 0.01 Max = 104.1 Stope = 1.881 Min = 0 IC50 = 7.339e-6 R2 = 0.9898
CCL13	UFL010-01-d-00001- 000-00	Arrestin	Agonist	CCR3	EC50	0.03832658	1.2184	1.1819	100	100	CCL13 CCR3 100 75 50 25 0 25 0 1e-5 0.0001 0.01 0.01 0.10 Max = 100 Store = 1.218 Min = 1.182 EC50 = 0.03833 R2 = 0.9921
CCL1	UFL010-01-d-00001- 000-00	Arrestin	Agonist	CCR8	EC50	0.09674311	0.99411	-0.72743	100	91.176	CCL1 CCR8 100 75 50 25 0 1e-5 0.0001 0.001 0.01 0.10 Max = 100 Slope = 0.9941 Max = 100 Slope = 0.9941 Min = -0.7274 EC50 = 0.09674 R2 = 0.9923
Oxytocin	UFL010-01-d-00001- 000-00	Arrestin	Agonist	OXTR	EC50	0.006488585	0.97039	0.96685	100	100	Orytocin OXTR 100 75 50 25 0 1e-6 1e-5 0.0001 0.001 0.01 Max = 100 Slope = 0.9704 Min = 0.9668 EC50 = 0.006489 R2 = 0.9865

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

Compound Name	Project ID	Assay Name	Assay Format	Assay Target	Result Type	RC50 (uM)	H	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	O	99.978	100.14	L-368.899 OXTR 100 25 0 25 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0

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



Y7g (NP-8-25)

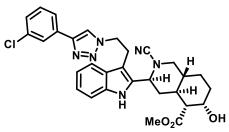
Compound Name	Project ID	Assay Name	Assay Format	Assay Target	Result Type	RC50 (uM)	Hill	Curve Bottom	Curve Top	Max Response
Vasopressin	UFL010-01-d-00001-000-00	Arrestin	Agonist	AVPR2	EC50	9.556304E-05	2.3922	3.7904	100	85.911
Tolvaptan	UFL010-01-d-00001-000-00	Arrestin	Antagonist	AVPR2	IC50	7.338717E-06	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)	HE	Curve Bottom	Curve Top	Max Response	Result Graph
NP-7-53	UFL008-01-0-00001- 000-00	Arrestin	Antagonist	CCR3	IC50	8.290315	4.3604	-2.3235	102.68	102.35	NP-7-53 CCR3 100 75 0 25 0 -2 -2 -2 -2 -2 -2 -2 -2 -2 -2 -2 -2 -2
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 CCCRR 100 75 50 25 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
NP-7-53	UFL008-01-0-00001- 000-00	Arrestin	Antagonist	CX3CR1	IC50	8.468666	2.3068	-0.26463	103.73	102.15	NP-7-53 CX3CR1 100 75 0 23 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
NP-7-53	UFL008-01-d-00001- 000-00	Arrestin	Antagonist	CXCR4	1050	7.936554	3.5291	10.203	105	100.4	NP-7-53 CXCR4 100 50 50 50 50 50 50 50 50 50 50 50 50 5
NP-7-53	UFL008-01-d-00001- 000-00	Arrestin	Antagonist	HTR2C	IC50	0.1756965	1.1212	O	106.22	110.1	NP-7-53 HTR2C 100 75 50 25 0 001 0.01 0.10 1 00 Max = 105.2 Slope = 1.121 Min = 0 IC50 = 0.1757 R2 = 0.9791

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

Compound Name	Project ID	Assay Name	Assay Format	Assay Target	Result Type	RC50 (uM)	HIII	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	CCL13 CCR3 100 75 50 25 0 1e-5 0.001 0.01 0.10 Max = 100 Slope = 1.218 Min = 1.182 EC50 = 0.03833 R2 = 0.9921
CCL1	UFL008-01-d-00001- 000-00	Arrestin	Agonist	CCR8	ECSD	0.09674311	0.99411	-0.72743	100	91.176	COL1 CCR9 100 75 50 25 0 1e-5 0.0001 0.01 0.01 0.01 Max = 100 Slope = 0.9941 Min = -0.7274 EC50 = 0.09674 R2 = 0.9923
Fractalkine	UFL008-01-d-00001- 000-00	Arrestin	Agonist	CX3CR1	EC50	0.002201558	1.0354	-1.0393	99.659	100	Fractalkine CX3CR1 100 75 50 25 0 1e-6 1e-5 0.0001 0.001 0.01 Max = 98.66 Stope = 1.035 Min = -1.039 EC50 = 0.002202 R2 = 0.9957
CXCL12	UFL008-01-d-00001- 000-00	Arrestin	Agonist	CXCR4	EC50	0.002401872	0.73368	-4.3946	100	102.31	CXCL12 CXCR4 100 75 50 25 0 1e-5 0.0001 0.001 0.01 0.10 Max = 100 Slope = 0.7337 Min = -4.395 EC50 = 0.002402 R2 = 0.9657
Serotonin / 5- HT	UFL008-01-d-00001- 000-00	Arrestin	Agonist	HTR2C	EC50	0.002948356	0.99676	5.1362	100	105.56	Serotonin / 5-HT HTR2C 100 75 50 25 0 0.0001 0.001 0.01 0.10 1 Max = 100 Slope = 0.9958 Min = 5.138 EC50 = 0.002948 R2 = 0.9675



Y1f (NP-7-53)

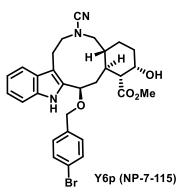
Compound Name	Project ID	Assay Name	Assay Format	Assay Target	Result Type	RC50 (uM)	Hill	Curve Bottom	Curve Top	Max Response
CCL13	UFL008-01-d-00001-000-00	Arrestin	Agonist	CCR3	EC50	0.03832658	1.2184	1.1819	100	100
CCL1	UFL008-01-d-00001-000-00	Arrestin	Agonist	CCR8	EC50	0.09674311	0.99411	-0.72743	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. 1 362	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. 1 561	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	NP-7-115 FSHR 100 25 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
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 NPSR1B 100 25 0 25 0 0 8 8 9 0 0 8 8 9 0 100 100 100 100 100 100 100 100 100
NP-7-115	UFL009-01-d-00001- 000-00	Arrestin	Antagonist	PRLHR	IC50	4.892051	3.0216	7.135	100	100.2	NP-7-115 PRLHR 100 25 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0

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	0.002949578	1.5455	8.2508	100	111.49	FSH FSHR 100 75 50 25 0 1e-6 1e-5
Neuropeptide S	UFL009-01-d-00001- 000-00	Arrestin	Agonist	NPSR1B	EC50	0.01398175	1.1544	D	97.292	103.44	Neuropeptide S NPSR1E 100 75 50 25 0 0.001 0.01 0.10 1 10 Max = 97.29 Slope = 1.154 Min = 0 EC50 = 0.01396 R2 = 0.9767
SHA 68	UFL009-01-d-00001- 000-00	Arrestin	Antagonist	NPSR1B	IC50	0.08218263	1.9069	0	109.26	112.12	SHA 68 NPSR1B 100 75 50 25 0.0001 0.001 0.01 0.10 1 Max = 109.3 Slope = 1.907 Min = 0 IC50 = 0.08218 R2 = 0.9926
PrRP-31	UFL009-01-d-00001- 000-00	Arrestin	Agonist	PRLHR	EC50	0.001670129	1.2469	-2.7102	100	101.88	PrRP-31 PRLHR 100 75 50 25 0 1e-5 0.0001 0.001 0.01 0.10 Max = 100 Slope = 1.247 Min = -2.71 EC50 = 0.00157 R2 = 0.9948



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. <mark>1</mark> 607	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