Supplemental Table 1. MOR species specificity profile for TRV130 and reference ligands; functional potency and efficacy.

	hMOR							
compound	cAMP				β-arrestin2			
	pEC_{50}	**SD**	**EC_{50} (nM)**	**Efficacy (%)**	**pEC_{50}**	**SD**	**EC_{50} (nM)**	**Efficacy (%)**
TRV130	8.1	0.04	7.9	83	7.4	0.22	40	14
fentanyl	8.2	0.05	6.3	112	6.6	0.03	251	478
morphine	7.3	0.03	50	100	6.3	0.10	501	99
hydromorphone	7.8	0.03	16	100	6.9	0.11	126	89
buprenorphine	8.7	0.04	2.0	52			N.Q.	
oxymorphone	7.8	0.02	16	98	6.8	0.06	159	84
DAMGO	7.8	0.03	16	111	6.1	0.02	794	884

	mMOR							
compound	cAMP				β-arrestin2			
	pEC_{50}	**SD**	**EC_{50} (nM)**	**Efficacy (%)**	**pEC_{50}**	**SD**	**EC_{50} (nM)**	**Efficacy (%)**
TRV130	9.4	0.06	0.4	104	7.9	0.06	13	74
fentanyl	9.2	0.05	0.6	101	7.2	0.07	63	164
morphine	7.6	0.04	25	100	6.0	0.06	1000	100
hydromorphone	8.2	0.03	6.3	102	6.7	0.06	200	106
buprenorphine	8.7	0.04	2.0	83			N.Q.	
oxymorphone	8.2	0.03	6.3	99	6.5	0.06	316	101
DAMGO	8.3	0.04	5.0	103	6.2	0.02	631	235

	rMOR							
compound	cAMP				β-arrestin			
	pEC_{50}	**SD**	**EC_{50} (nM)**	**Efficacy (%)**	**pEC_{50}**	**SD**	**EC_{50} (nM)**	**Efficacy (%)**
TRV130	8.4	0.03	4.0	90			N.Q.	
fentanyl			N.D.				N.D.	
morphine	8.0	0.04	10	100	6.4	0.12	398	100
hydromorphone	8.5	0.06	3.2	95	7.1	0.28	79	80
buprenorphine	8.4	0.05	4.0	79			N.Q.	
oxymorphone	8.5	0.03	3.2	95	6.8	0.13	159	74
DAMGO	8.6	0.03	2.5	95	6.0	0.03	1000	1118

Potencies and efficacies were determined at the hMOR, mMOR and rMOR species orthologues
Efficacy values are calculated as % maximal response to morphine
(n >10 independent experiments performed in duplicate)
N.Q. is not quantifiable N.D. is not determined

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Supplemental Table 2. Quantification of Bias signaling.

	hMOR						
	cAMP			β-arrestin2			
compound	pEC_{50}	Efficacy	RAi G	pEC_{50}	Efficacy	RAi β	Bias Ratio
TRV130	8.1	83	5.24	7.4	14	1.77	3.0
fentanyl	8.2	112	8.93	6.6	478	9.61	0.9
morphine	7.3	100	1.00	6.3	99	1.00	1.0

Efficacy values are calculated as % maximal response to morphine

(n >10 independent experiments performed in duplicate)

human mu-opioid receptor (hMOR)

Intrinsic relative activity (RAi) and Bias Ratio values are calculated as indicated in the methods

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Supplemental Table 3. Kinetic parameters of TRV130 and reference opioid ligands binding to the human MOR in HEK293 cell membranes.

Ligand	k_{on}	k_{off} ($t_{½}$)	k_{off} / k_{on}	*Competition binding,* K_i
	$\times 10^{8}\ M^{-1}\ min^{-1}$	min^{-1}	*nM*	*nM*
TRV130	15 ± 2.8	0.37 ± 0.1 (2 min)	0.25 ± 0.04	6
fentanyl	0.45 ± 0.3	0.22 ± 0.04 (3 min)	13 ± 10	5.7
morphine	1.7 ± 1.3	0.54 ± 0.15 (1.2 min)	6.8 ± 4.5	6.3
naloxone	24 ± 16	0.47 ± 0.18 (1.4 min)	0.3 ± 0.1	7.6
[^{3}H]diperenorphine	2.7 ± 0.3	0.09 (7.3 min)	0.3 ± 0.03	0.4*

*Affinity (K_d) constant derived from independent direct radioligand saturation studies

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Supplemental Table 4. TRV130 is selective for the MOR. From greater than 120 targets including GPCRs, ion channels, transporters and enzyme targets evaluated, TRV130 only showed weak interaction at the receptors listed.

Receptor	*pIC_{50}*	*pK_i*	*K_i (μM)*	*nH*
human α_{2C}	*5.0*	*5.5*	*3.0*	*1.2*
human D_{2S}	*4.8*	*5.2*	*6.7*	*0.7*
human D_3	*4.8*	*5.5*	*3.4*	*0.9*
human 5-HT_{1A}	*5.7*	*5.9*	*1.2*	*1*
rat σ 1 and 2	*5.9*	*6.0*	*1.1*	*0.5*

Agonist radioligands used for; hD_{2S}, h5-HT_{1A} and σ receptor assays-[^{3}H]-7-OH-DPAT, [^{3}H]-8-OH-DPAT and [^{3}H]-DTG, respectively

Antagonist radioligands used for; $h\alpha_{2c}$ and hD_3 receptor assays -[^{3}H]RX 821002 and [^{3}H]methyl-spiperone, respectivley

K_i values determined using Cheng and Prusoff correction, *nH* equals the Hill slope

Supplemental Table 5. ED_{50} values (95% confidence intervals and percent maximal responses; mg/kg, s.c.) of TRV130 and morphine for antinociceptive responses and therapeutic index (side effect) assays.

	TRV130		Morphine	
Assay	ED_{50} mg/kg (95% CI)	Max	ED_{50} mg/kg (95% CI)	Max
mouse Hot Plate	0.88 (0.6-1.4)	100%	4.9 (3.3-6.7)	85%
rat Hot Plate	0.32 (0.1-1.0)	100%	3.2 (1-10)	100%
rat Tail Flick	0.22 (0.2-0.3)	100%	1.0 (0.7-1.5)	100%
mouse Glass Bead	1.7 (0.8-3.6)	53%	4.3 (1.1-12)	100%
mouse Fecal Boli	2.4 (0.6-10)	53%	2.2 (0.8-6.0)	100%
mouse Charcoal GIT	0.33 (0.2-0.6)	100%	0.65 (0.45-0.9)	100%
rat Respiratory Depression	2.1 (2-2.2)	67%	6.2 (4.7-8.2)	80%

CI, confidence intervals. GIT, gastrointestinal transit

Max, maximal response achieved in the assay

ED_{50} values were determined from the sigmoidal log dose response curves best fit by nonlinear regression analysis using GraphPad Prism