SUPPLEMENTAL INFORMATION

Table S1. ***Related to Table 1*****. Affinity and selectivity of SR compounds from radioligand binding assays.** To determine selectivity at the opioid receptors, specific competition binding was assessed with ~1 nM ^{3}H-DAMGO, ^{3}H-diprenorphine and ^{3}H-U69,593 in CHO-hMOR, -hDOR and -hKOR membranes, respectively. *See also:* Table 1 for structures, Figure S1 for synthesis of the compounds and Figure S2 for KOR and NOP activity counter-screens.

Agonist	Binding affinities (K_i, nM)			K_i ratios	
	hMOR	hDOR	hKOR	hDOR/ hMOR	hKOR/ hMOR
DAMGO	2.1±0.34	>10,000	989±116	> 6421	475
Morphine	6.4±0.72	112±28	52±1.0	17	8
Fentanyl	2.8±0.73	2459±850	260±55	874	92
Sufentanil	0.24±0.07	283±76	11±3.3	1166	44
SR-11501	3.0±0.85	>10,000	13±1.1	>4794	4
SR-14968	0.29±0.11	>10,000	31±4.3	>55,999	108
SR-14969	0.86±0.33	>10,000	33±2.3	>48,993	38
SR-15098	14±3.8	>10,000	146±24	>3254	10
SR-15099	11±2.6	>10,000	110±45	>2395	10
SR-17018	11±2.4	>10,000	68±21	>3730	6

Table S2. ***Related to Table 1 and Figures 1 and S3*****. MOR agonist potency and efficacy measures in cell-based assays and in brainstem from mice.** Potencies (EC_{50}) and efficacies (E_{MAX}) of the SR compounds and several clinically used opioids. Inhibition of forskolin-stimulated cAMP was determined in CHO-hMOR cells, ^{35}S-GTPγS binding was determined in membranes from CHO-mMOR cells or from mouse brainstems and βarrestin2 recruitment was assessed with the imaging based transfluor assay with U2OS-βarrestin2-GFP-mMOR cells. Data are presented as mean ± S.E.M. of 3 or more assays run in duplicate or triplicate. E_{MAX} values were calculated relative to DAMGO. *See also:* Table 1 for potencies and efficacies in the GTPγS binding and βarrestin2-translocation assays at the human MOR and Figures 1B and S3 for the corresponding concentration response curves.

Agonist	Human MOR		Mouse MOR					
	cAMP (CHO)		GTPγS (CHO)		GTPγS (brain)		βarr2 (imaging)	
	EC_{50} nM	E_{MAX} %	EC_{50} nM	E_{MAX} %	EC_{50} nM	E_{MAX} %	EC_{50} nM	E_{MAX} %
DAMGO	5.2±0.6	100	34±2.1	100	400±33	100	170±13	100
Sufentanil	0.03±0.01	100±1	2.7±0.3	82±1	4.8±1.7	32±3	1.0±0.2	85±7
Fentanyl	0.54±0.11	98±2	87±11	82±1	170±64	33±3	31±4.8	93±4
SR-11501	7.9±0.81	98±1	133±12	73±2	396±68	38±3	140±23	81±4
Morphine	26±3.9	97±1	81±7.4	82±1	159±19	41±1	425±51	37±4
SR-14969	14±2.7	98±2	40±2.6	94±1	159±30	93±10	891±72	89±5
SR-14968	7.2±0.75	100±2	11±1.2	96±1	26±1.8	91±3	628±207	91±3
SR-15098	110±13	103±1	230±35	70±1	219±24	41±3	>10,000	17±5[a]
SR-15099	75±15	101±2	212±22	70±1	180±27	34±3	>10,000	12±6[a]
SR-17018	76±11	105±3	193±29	72±1	288±60	37±4	>10,000	11±6[a]

[a]percent of maximum stimulation at the 10 μM concentration is presented rather than E_{MAX}.

Table S3. *Related to Figure 2.* Percent free compound concentrations in mouse plasma. Plasma protein binding was performed to determine the % free for each compound by equilibrium dialysis. C57BL/6J mice were treated with 6 mg/kg, i.p. as indicated (1 mg/kg for fentanyl) and the total plasma concentration was determined by LC/MS 1 hour after treatment (15 minutes for fentanyl, due to its shorter half-life). The estimated free concentration was then calculated. Mean ± S.E.M. (n = 3-9). *See also:* Figure 2 for the concentration of compounds in plasma over time.

Agonist	% Free	Plasma Concentration (nM)	
		Total	Estimated Free
Fentanyl (1)	11	46±6.6	5
SR-11501 (6)	4.7	427±104	20
Morphine (6)	72	575±71	414
SR-14969 (6)	5.5	2206±499	121
SR-14968 (6)	3.2	2080±156	67
SR-15098 (6)	5.6	4191±87	235
SR-15099 (6)	8.9	2274±374	202
SR-17018 (6)	4.6	1704±558	78

Table S4. ***Related to Table 3 and Figures 3 and S4.*** **Number of animals used.** The number of C57BL/6J and MOR-KO animals used (*n*) for both the antinociception and respiration assays. Two animals were removed from the analysis of the respiratory assays for SR-11501 (48 mg/kg) due to mortality and are not counted here. *See also:* Table 3 for ED_{50} values, Figures 3 and S4 for in vivo responses.

Agonist	Antinociception		Respiration	
	C57BL/6J	MOR-KO	C57BL/6J	MOR-KO
Fentanyl	6-7	4	6-8	7
SR-11501	5-6	5	4-8	7
Morphine	7-14	5	6-15	9
SR-14969	5	5	4-6	7
SR-14968	5-7	5	4-6	7
SR-15098	8-9	5	10-13	--
SR-15099	5-17	5	10-13	--
SR-17018	5-6	6	8	--

Table S5. ***Related to Figure 4 and Tables 2 and 3.*** **Correlation of bias factors derived from different** ***in vitro*** **assays to therapeutic windows calculated from different combinations of** ***in vivo*** **responses.** Bias factors representing G protein signaling over βarrestin2 recruitment calculated from the indicated assays were plotted against therapeutic windows calculated for different animal tests (ED_{50} respiration/ ED_{50} antinociception). The R^2 values from the linear regression analysis are provided. Abbreviations: CHO cells (*CHO*), mouse brainstem (*brain*), human MOR (*hMOR*), mouse MOR (*mMOR*), hot plate (*HP*), tail flick (*TF*), % arterial oxygen saturation (O_2), breath rate (*BR*). *See also:* Figure 4 for graphical bias correlation of first entry; Table 2 for bias factors and Table 3 for therapeutic windows.

Bias Factor from:			Therapeutic Window from:			
MOR species	G protein assay	βarrestin2 assay	O_2/HP	O_2/TF	BR/HP	BR/TF
hMOR	CHO-GTPγS	EFC	0.9589	0.8639	0.8001	0.7969
hMOR	CHO-cAMP	EFC	0.9525	0.9454	0.5209	0.6619
mMOR	CHO-GTPγS	Imaging	0.8805	0.6802	0.7150	0.6800
mMOR	Brain-GTPγS	Imaging	0.8277	0.6413	0.8537	0.8088