SUPPORTING INFORMATION

A novel mitragynine analog with low efficacy mu-opioid receptor agonism displays antinociception with attenuated adverse effects

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Table S1. Functional studies at KOR using cAMP inhibition & Tango-arrestin assays.

Functional data at KOR using cAMP inhibition & Tango-arrestin assays[a]				
Compd.	cAMP inhibition		β-arrestin2 recruitment	
	EC_{50} nM (pEC_{50} ± SEM)	E_{max}% ± SEM	EC_{50} nM (pEC_{50} ± SEM)	E_{max}% ± SEM
8	10.71 (7.97 ± 0.11)	86.37 ± 2.66	n.d.	< 20
9	52.92 (7.28± 0.12)	83.46 ± 3.31	n.d.	< 20
10	63.27 (7.20 ± 0.09)	93.61 ± 3.39	n.d.	< 20
4	709.1 (6.15 ± 0.11)	91.7 ± 5.02	n.d.	< 20
5	26.73 (7.57 ± 0.09	97.75 ± 2.57	n.d.	< 20
6 (SC13)	213.2 (6.67 ±0.08)	92.29 ± 2.82	n.d.	< 20
13	4974 (5.30 ± 0.19)	61.1 ± 4.6	n.d.	< 20
14	98.09 (7.01 ± 0.10)	101.08 ± 3.89	n.d.	< 20
15	72.90 (7.14 ± 0.12)	99.28 ± 3.98	n.d.	< 20
16	2484 (5.60 ±0.21)	49.33 ± 4.53	n.d.	24.83 ± 3.84
17	7822 (5.11 ± 0.13)	96.2 ± 1.2	n.d.	< 20
18	86.32 (7.06 ±0.08)	89.70 ± 2.36	n.d.	< 20
21	48.38 (7.31 ± 0.10)	82.86 ± 3.28	n.d.	< 20
20	42.69 (7.37 ± 0.09)	78.76 ± 2.28	n.d.	< 20
22	1289 (5.89 ± 0.18)	61.88 ± 7.07	n.d.	< 20
24	127.4 (6.89 ± 0.09)	72.30 ± 2.58	n.d.	< 20
23	4184 (5.38 ± 0.21)	56.39 ± 3.00	n.d.	< 20
25	22.67 (7.64 ± 0.097)	65.30 ± 2.11	n.d.	< 20
Mitragynine	68.76 (7.16 ± 0.21)	57.01 ± 3.93	n.d.	< 20
7OH	25.41 (7.59 ± 0.05)	90.84 ± 1.43	2911 (5.54 ± 0.43)	36.79 ± 13.15
U50488H	0.005 (10.29 ± 0.06)	100	7.85 (8.10 ± 0.10)	100

[a]The functional data of each assay using human kappa opioid receptor (hKOR) was normalized to E_{max} of corresponding standard U50488H. Results were analyzed using a three-parameter logistic equation in GraphPad Prism and the data are presented as mean EC_{50} (pEC_{50} ± SEM) with E_{max}% ± SEM for assays run in triplicate; nd; results could not be determined because of efficacy of β-arrestin2 recruitment was less than 20% and/or shallow slopes.

Table S2. Functional studies at DOR using cAMP inhibition & Tango-arrestin assays.

Functional data at DOR using cAMP inhibition & Tango-arrestin assays[a]				
Compd.	cAMP inhibition		β-arrestin2 recruitment	
	EC_{50} nM (pEC_{50} ± SEM)	E_{max}% ± SEM	EC_{50} nM (pEC_{50} ± SEM)	E_{max}% ± SEM
8	571.9 (6.24 ± 0.07)	115.37 ± 5.21	308.1 (6.51 ± 0.21)	53.82 ± 4.43
9	871.1 (6.06 ± 0.08)	85.53 ± 5.37	243.8 (6.61 ± 0.34)	46.88 ± 5.69
10	2640 (5.58 ± 0.07)	114.00 ± 20.9	503.8 (6.3 ± 0.26)	50.55 ± 5.7
4	85.89 (7.07 ± 0.10)	111.79 ± 5.97	260.8 (6.58 ± 0.06)	293.93 ± 7.65
5	152.6 (6.82 ± 0.10)	98.09 ± 5.45	1120 (5.95 ± 0.10)	211.34 ± 12.57
6 (SC13)	97.6 (7.01 ± 0.10)	103.15 ± 5.27	660 (6.18 ± 0.10)	191.03 ± 10.56
13	n.d	79 ± 1	n.d	< 20
14	1650 (5.78 ± 0.10)	99.15 ± 7.74	n.d	< 20
15	2179 (5.66 ± 0.12)	116.22 ± 10.3	982.2 (6.01 ± 0.2)	65.04 ± 7.32
16	176.4 (6.75 ± 0.08)	106.25 ± 3.21	160.0 (6.8 ± 0.08)	129.52 ± 4.86
17	4279 (5.37 ± 0.09)	105.87 ± 3.04	n.d	<20
18	159.2 (6.80 ± 0.09)	100.49 ± 4.21	461.7 (6.34 ± 0.1)	168.77 ± 8.85
21	2817 (5.55 ± 0.19)	70.42 ± 3.57	n.d.	<20
20	n.d.	39.03 ± 3.17	n.d.	<20
22	2349 (5.63 ± 0.13)	78.35 ± 4.37	n.d.	<20
24	4082 (5.39 ± 0.27)	86.7 ±21.92	n.d	53.14 ± 16.41
23	n.d.	50 ±4.8	2681 (5.57 ± 0.17)	81 ± 13
25	144.6 (6.84 ± 0.10)	74.92 ± 2.73	729.8 (6.14 ±0.19)	70.01 ± 8.49
Mitragynine	1179 (5.93 ± 0.11)	112.43 ± 7.75	559.3 (6.25 ± 0.25)	46.83 ± 6.11
7OH	97.83 (7.01 ± 0.08)	91.60 ± 3.72	732.4 (6.13 ± 0.06)	191.39 ± 6.47
DPDPE	2.11 (8.68 ± 0.07)	100	6.91 (8.16 ± 0.10)	100

[a]The functional data of each assay using human delta opioid receptor (hDOR) was normalized to E_{max} of corresponding standards DPDPE. Results were analyzed using a three-parameter logistic equation in GraphPad Prism and the data are presented as mean EC_{50} (pEC_{50} ± SEM) with E_{max}% ± SEM for assays run in triplicate; nd; results could not be determined because of efficacy of β-arrestin2 recruitment was less than 20% and/or shallow slopes.

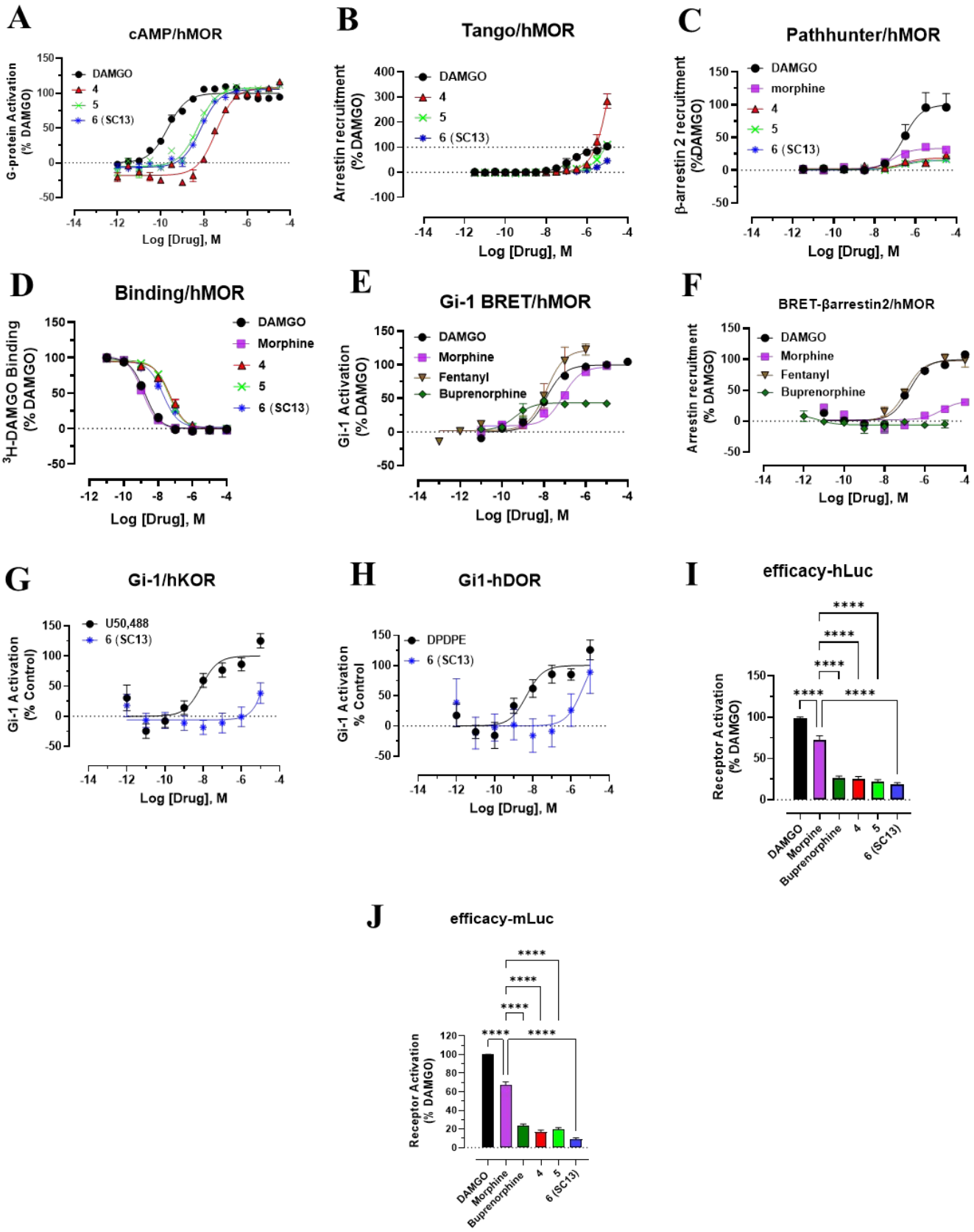

A
cAMP/hMOR
G-protein Activation (% DAMGO)
DAMGO
4
5
6 (SC13)
Log [Drug], M
B
Tango/hMOR
Arrestin recruitment (% DAMGO)
DAMGO
4
5
6 (SC13)
Log [Drug], M
C
Pathhunter/hMOR
β-arrestin 2 recruitment (%DAMGO)
DAMGO
morphine
4
5
6 (SC13)
Log [Drug], M
D
Binding/hMOR
3H-DAMGO Binding (% DAMGO)
DAMGO
Morphine
4
5
6 (SC13)
Log [Drug], M
E
Gi-1 BRET/hMOR
Gi-1 Activation (% DAMGO)
DAMGO
Morphine
Fentanyl
Buprenorphine
Log [Drug], M
F
BRET-βarrestin2/hMOR
Arrestin recruitment (% DAMGO)
DAMGO
Morphine
Fentanyl
Buprenorphine
Log [Drug], M
G
Gi-1/hKOR
Gi-1 Activation (% Control)
U50,488
6 (SC13)
Log [Drug], M
H
Gi1-hDOR
Gi-1 Activation % Control
DPDPE
6 (SC13)
Log [Drug], M
I
efficacy-hLuc
Receptor Activation (% DAMGO)
****
DAMGO
Morphine
Buprenorphine
4
5
6 (SC13)
J
efficacy-mLuc
Receptor Activation (% DAMGO)
****
DAMGO
Morphine
Buprenorphine
4
5
6 (SC13)

Figure S1. Characterization of **4**, **5** and **6** (**SC13**) in cAMP, Tango arrestin, PathHunter arrestin, binding, Nb33 recruitment at h/mMOR and Gi-signaling at hKOR and hDOR.

A) 4, **5** and **6** (**SC13**) are full agonists at hMOR in cAMP inhibition (N=3) compared to DAMGO. See table **1** (main paper) for values **B) 4**, **5** and **6** (**SC13**) showed robust arrestin recruitment (E_{max}>100% for **4**, **5** & 45% for **6** (**SC13**) with poor potency (EC_{50}>10μM) in Tango assays at MOR. See table **1** (main paper) for values. **C) 4**, **5** and **6** (**SC13**) in PathHunter-arrestin recruitment assays (n=3) show less βarrestin-2 recruitment compared to DAMGO. **β-arrestin2: 4** EC_{50} nM (pEC_{50} ± SEM) = n.d., E_{max}% ± SEM = <20%, **β-arrestin2: 5** EC_{50} nM (pEC_{50} ± SEM) = n.d., E_{max}% ± SEM = <20%, **β-arrestin2: 6** (**SC13**) EC_{50} nM (pEC_{50} ± SEM) = n.d., E_{max}% ± SEM = <20%, **β-arrestin2: morphine** EC_{50} nM (pEC_{50} ± SEM) = 80.08 (7.09 ± 0.17) nM, E_{max}% ± SEM = 33.08 ± 1.84, **β-arrestin2: DAMGO** EC_{50} nM (pEC_{50} ± SEM) = 281.57 (6.55 ± 0.2) nM. . **D)** In competitive radioligand binding assays in MOR-CHO using ^{3}H-DAMGO as radioligand, **4**, **5** and **6** (**SC 13**) labelled MOR with high to reasonable affinity. **6** (**SC13)** K_i (pK_i ± SEM) = 6.05 (8.22 ± 0.08), **5** K_i (pK_i ± SEM) = 12.33 (7.91 ± 0.03), **4** K_i (pK_i ± SEM) = 15.42 (7.81 ± 0.06), **morphine** K_i (pK_i ± SEM) = 0.37 (9.42 ± 0.04), **DAMGO** K_i (pK_i ± SEM) = 0.49 (9.31 ± 0.03). **E)** Gi-1 activation in BRET assays of controls. Fentanyl had higher efficacy over DAMGO. Efficacy of morphine was 94% and buprenorphine showed 44% efficacy. See table **S3** for values. **F)** β-arrestin2 recruitment in BRET assays of controls. Fentanyl showed robust arrestin recruitment with efficacy of 94%, morphine showed 31% efficacy while buprenorphine showed no recruitment. See table **S3** for values. **G)** No measurable Gi-1 potency was observed for **6** (**SC13**) at hKOR. **U50488** EC_{50} nM (pEC_{50} ± SEM) = 8.07 (8.09 ± 0.27) nM. **6** (**SC13**) EC_{50} nM (pEC_{50} ± SEM) = n.d., E_{max}% ± SEM = 38 ± 17. **H)** No measurable Gi-1 potency was observed for **6** (**SC 13**) at hDOR. **6** (**SC13**) EC_{50} nM (pEC_{50} ± SEM) = n.d; E_{max}% ± SEM = 88 ± 35 at hDOR. **I)** Efficacy of **4**, **5** and **6** (**SC13**) compounds, buprenorphine and morphine at the human opioid receptors in BRET-based Nb33 recruitment assaysare shown as a percentage of receptor activation relative to the full agonist, **DAMGO**. **4**, **5** and **6** (**SC13**) had significantly lower efficacy than DAMGO ($p<0.0001$) and morphine ($p<0.0001$) and similar efficacy to buprenorphine. Statistical significance was determined using one-way ANOVA followed by Dunnett's multiple comparison test, $F(5,68)=239.172.5$, $p<0.0001$. **J)** Efficacy of **4**, **5** and **6** (**SC13**) compounds, buprenorphine and morphine at the mouse opioid receptors in BRET-based Nb33 recruitment assays are shown as a percentage of receptor activation relative to the full agonist, **DAMGO**. **4**, **5** and **6** (**SC13**) had significantly lower efficacy than DAMGO ($p<0.0001$), morphine ($p<0.0001$), and similar efficacy to buprenorphine. Statistical significance was determined using one-way ANOVA followed by Dunnett's multiple comparison test, $F(5,64)=572.5$, $p<0.0001$.

Table S3. Functional binding data of the compounds at opioid receptors (hMOR/mMOR).

Receptors	Compounds	G protein activation (BRET) assay		Arrestin recruitment (BRET) assay		Figure
		EC_{50} nM (pEC_{50} ± SEM)	E_{max}% ± SEM	EC_{50} nM (pEC_{50} ± SEM)	E_{max}% ± SEM	
hMOR	**4**	1250 (5.90 ± 0.14)	60.50 ± 5.15	nd	nd	**2A-B**
	5	252.79 (6.6 ± 0.15)	61.76 ± 4.05	nd	nd	**2A-B**
	6 (SC13)	145.13 (6.84 ± 0.08)	69.65 ± 2.61	nd	nd	**2A-B**
	DAMGO	17.35 (7.76 ± 0.09)	100	161.33 (6.79 ± 0.06)	100	**2A-B**
Receptors	**Controls**	**G protein activation (BRET) assay**		**Arrestin recruitment (BRET) assay**		**Figure**
		EC_{50} nM (pEC_{50} ± SEM)	Emax% ± SEM	EC_{50} nM (pEC_{50} ± SEM)	Emax% ± SEM	
hMOR	**Buprenorphine**	0.42 (9.38 ± 0.24)	43.63 ± 2.36	nd	nd	**S1E-F**
	Morphine	81.34 (7.09 ± 0.12)	97.21 ± 4.36	6774.6 (5.17 ± 0.64)	32.59 ± 10.55	**S1E-F**
	Fentanyl	13.17 (7.88 ± 0.18)	122.45 ± 9.11	118.64 (6.93 ± 0.13)	97.93 ± 4.17	**S1E-F**
	DAMGO	12.58 (7.9 ± 0.07)	100	175 (6.76 ± 0.08)	100	**S1E-F**
Receptors	**Compounds**	**Nb33 recruitment assay (hMOR)**		**Nb33 recruitment assay (mMOR)**		**Figure**
		EC_{50} nM (pEC_{50} ± SEM)	Emax% ± SEM	EC_{50} nM (pEC_{50} ± SEM)	Emax% ± SEM	
	DAMGO	265.45 (6.58 ± 0.05)	100 ± 2.17	154.25 (6.81 ± 0.04)	100 ± 1.50	**2C-D**
	Buprenorphine	3.25 (8.49 ± 0.31)	23.83 ± 1.89	3.65 (8.44 ± 0.34)	20.55 ± 1.62	**2C-D**
	Morphine	1600 (5.78 ± 0.17)	71.86 ± 4.79	584.83 (6.23 ± 0.09)	69.62 ± 2.44	**2C-D**
	6 (SC13)	730.53 (6.14 ± 0.21)	21.13 ± 2.23	12.27 (7.91 ± 0.40)	7.69 ± 1.1	**2C-D**

The functional data of each assay using human/mouse mu opioid receptor (hMOR/mMOR) were normalized to E_{max} of corresponding standards. Results were analyzed using a three-parameter logistic equation in GraphPad Prism and the data are presented as mean EC_{50} (pEC_{50} ± SEM) with E_{max}% ± SEM for assays run in triplicate.

Table S4. Potency and efficacy table for TRUPATH assay

Potency table (Figure 2G):

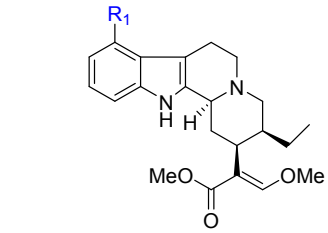
Compounds	Gi1 EC_{50} nM (pEC_{50} ± SEM)	Gi2 EC_{50} nM (pEC_{50} ± SEM)	Gi3 EC_{50} nM (pEC_{50} ± SEM)	Goa EC_{50} nM (pEC_{50} ± SEM)	Gob EC_{50} nM (pEC_{50} ± SEM)	Gz EC_{50} nM (pEC_{50} ± SEM)	βarr1 EC_{50} nM (pEC_{50} ± SEM)	βarr2 EC_{50} nM (pEC_{50} ± SEM)
DAMGO	17.39 (7.76 ± 0.08	8.53 (8.07 ± 0.05	40.04 (7.4 ± 0.06)	6.52 (8.19 ± 0.08)	4.38 (8.36 ± 0.07)	1.63 (8.79 ± 0.04)	321.08 (6.49 ± 0.04)	161.07 (6.79 ± 0.05)
4	1000 (5.90 ± 0.13)	319.95 (6.49 ± 0.21)	790.84 (6.10 ± 0.29	247.62 (6.61 ± 0.21)	184.07 (6.73 ± 0.16)	83.78 (7.08 ± 0.1)	nd	nd
5	255.75 (6.59 ± 0.14)	148.26 (6.83 ± 0.22)	1500 (5.83 ± 0.59)	64.83 (7.19 ± 0.14)	100.91 (7 ± 0.13)	37.96 (7.42 ± 0.10)	nd	nd
6 (SC13)	145.27 (6.84 ± 0.07)	121.81 (6.91 ± 0.21)	255.34 (6.59 ± 0.22)	52.94 (7.28 ± 0.14)	62.17 (7.21 ± 0.08)	14.53 (7.84 ± 0.09)	nd	nd
Buprenorphine	0.37 (9.43 ± 0.18)	0.13 (9.89 ± 0.22)	0.17 (9.77 ± 0.23)	0.17 (9.77 ± 0.1)	0.55 (9.26 ± 0.18)	0.2 (9.7 ± 0.09)	nd	nd
Fentanyl	12.35 (7.91 ± 0.16)	1.15 (8.94 ± 0.17)	31.1 (7.51 ± 0.17)	3.78 (8.42 ± 0.12)	4.08 (8.39 ± 0.08)	1.39 (8.86 ± 0.16)	300.74 (6.52 ± 0.13)	114.29 (6.94 ± 0.11)
Morphine	51.66 (7.29 ± 0.13	18 (7.74 ± 0.21)	88.27 (7.05 ± 0.16)	11.2 (7.95 ± 0.15)	15.16 (7.82 ± 0.1)	8.22 (8.08 ± 0.18)	nd	4930 (5.31 ± 00.58)

Efficacy table (Figure 2H):

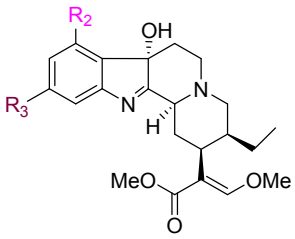
Compounds	Gi1 E_{max}% ± SEM	Gi2 E_{max}% ± SEM	Gi3 E_{max}% ± SEM	Goa E_{max}% ± SEM	Gob E_{max}% ± SEM	Gz E_{max}% ± SEM	βarr1 E_{max}% ± SEM	βarr2 E_{max}% ± SEM
DAMGO	100	100	100	100	100	100	100	100
4	60.50 ± 5.01	70.21 ± 7.05	42.77 ± 7.19	64.87 ± 6.28	63.87 ± 4.8	79.47 ± 3.42	nd	nd
5	61.81 ± 3.94	74.75 ± 7.38	32.74 ± 12.54	65.42 ± 3.92	71.24 ± 4.09	81.4 ± 3.16	nd	nd
6 (SC13)	69.66 ± 2.32	81.45 ± 7.62	59.78 ± 6.13	75.37 ± 4.26	73.84 ± 2.58	85.66 ± 2.93	nd	nd
Buprenorphine	43.56 ± 2.27	59.88 ± 3.69	40.02 ± 2.63	65.49 ± 1.92	65.45 ± 3.58	80.62 ± 2.14	nd	nd
Fentanyl	122.08 ± 8.82	94.47 ± 5.92	111.58 ± 8.99	100.36 ± 4.47	98.40 ± 2.91	93.92 ± 5.69	83.24 ± 3.96	97.81 ± 4.04
Morphine	94.89 ± 4.91	88.54 ± 7.04	86.34 ± 6.08	88.37 ± 4.83	94.38 ± 3.38	87.7 ± 5.52	nd	31.68 ± 9.51

Pharmacological parameters from **Figure 2G** and **2H** for all responding transducers. Potency [EC_{50} nM (pEC_{50} ± SEM)] and efficacy (E_{max}% ± SEM) are reported as estimates from simultaneous curve fitting of all biological replicates and include standard error. nd: E_{Max}<10%.

Table S5. Compounds and data used to build the statistical models.



MG: R_1 = OMe
8: R_1 = furan-3-yl
9: R_1 = Ph
10: R_1 = Me



7OH: R_2 = OMe, R_3 = H
4: R_2 = Ph, R_3 = H
6 (SC13): R_2 = furan-3-yl, R_3 = H
5: R_2 = Me, R_3 = H
11-F: R_2 = OMe, R_3 = F

Drug	G-protein (E_{max} ± SEM, %DAMGO)
MG	<10
7OH	31.15 ± 4.93
8	<10
9	<10
10	<10
4	60.50 ± 5.15
5	61.76 ± 4.05
6 (SC13)	69.65 ± 2.61
11-F	<10
Morphine	97.21 ± 4.36
Buprenorphine	43.63 ± 2.36

Table S6. Average Structural Interaction Fingerprints (SIFt) probability for each ligand simulated.

Residue	MG	7OH	8	9	10	4	5	6 (SC13)	11-F	bpr	morphine
G efficacy	10%	31%	10%	10%	10%	61%	62%	70%	10%	43%	97%
Y(1.39)_Apolar	-	34%	-	-	-	44%	-	41%	68%	-	-
Y(1.39)_Aro_E2F	-	12%	-	-	-	-	-	15%	30%	-	-
L/M(2.57)_Apolar	-	-	-	-	-	-	-	-	32%	-	-
Q(2.60)_Apolar	100%	100%	100%	100%	99%	100%	93%	100%	97%	83%	-
Q(2.60)_Hbond_1Wat	-	-	-	-	-	-	-	-	14%	-	-
N/V(2.63)_Apolar	57%	71%	87%	84%	72%	84%	69%	79%	54%	26%	-
Y(2.64)_Apolar	20%	61%	15%	37%	-	38%	-	24%	55%	67%	29%
Y(2.64)_Aro_E2F	-	18%	-	24%	-	11%	-	-	14%	-	-
W(23.50)_Apolar	93%	43%	100%	93%	75%	63%	46%	53%	25%	-	-
I/L(3.29)_Apolar	99%	64%	100%	100%	100%	78%	90%	89%	37%	62%	-
D(3.32)_Apolar	100%	100%	100%	100%	100%	100%	98%	100%	99%	98%	100%
D(3.32)_Elec_ProN	99%	76%	97%	95%	100%	87%	73%	90%	54%	96%	98%
D(3.32)_Hbond_1Wat	-	27%	-	-	-	12%	23%	-	56%	24%	-
D(3.32)_Hbond_ProA	99%	65%	96%	90%	99%	70%	73%	82%	35%	58%	86%
Y(3.33)_Apolar	100%	98%	100%	100%	100%	100%	99%	100%	98%	100%	100%
Y(3.33)_Aro_E2F	-	-	-	-	-	-	-	-	-	20%	-
Y(3.33)_Hbond_1Wat	27%	25%	-	-	-	29%	25%	30%	21%	69%	56%
Y(3.33)_Hbond_2Wat	49%	55%	65%	58%	58%	60%	57%	62%	41%	32%	44%
M(3.36)_Apolar	98%	98%	99%	100%	98%	99%	99%	97%	95%	92%	99%
F(3.37)_Apolar	-	12%	-	-	-	-	-	26%	-	-	-
C(45.50)_Apolar	85%	47%	97%	97%	62%	81%	76%	77%	24%	23%	-
C(45.50)_Hbond_1Wat	20%	34%	-	-	-	-	25%	12%	14%	-	-
C(45.50)_Hbond_2Wat	-	28%	-	-	-	13%	18%	23%	23%	24%	-
T/S(45.51)_Apolar	-	-	-	-	-	-	17%	-	-	-	-
L(45.52)_Hbond_2Wat	-	13%	-	-	-	-	19%	-	-	17%	-
E(5.35)_Hbond_2Wat	-	-	-	-	-	-	-	-	-	20%	15%
K(5.39)_Hbond_2Wat	33%	15%	27%	25%	34%	14%	11%	11%	11%	11%	22%
V(5.42)_Apolar	56%	72%	46%	59%	52%	78%	78%	83%	77%	99%	88%
A(5.46)_Apolar	-	-	-	-	-	-	-	14%	10%	-	-
W(6.48)_Apolar	80%	97%	90%	87%	76%	99%	90%	100%	92%	85%	41%
I(6.51)_Apolar	97%	99%	97%	98%	98%	99%	100%	93%	97%	98%	95%
H(6.52)_Apolar	99%	96%	100%	99%	95%	99%	92%	95%	91%	95%	91%
H(6.52)_Aro_E2F	-	-	-	-	-	-	-	-	-	84%	77%
V/I(6.55)_Apolar	70%	38%	62%	48%	55%	55%	66%	47%	35%	89%	55%
W/Y(7.35)_Apolar	-	14%	-	-	-	11%	39%	16%	-	99%	73%
W/Y(7.35)_Hbond_1Wat	-	30%	-	-	-	27%	23%	21%	47%	-	-
W/Y(7.35)_Hbond_2Wat	18%	28%	26%	33%	27%	32%	27%	38%	23%	-	27%
W/Y(7.35)_Hbond_ProD	-	-	-	-	-	-	-	-	-	-	-
H/Y(7.36)_Apolar	-	57%	-	10%	-	86%	28%	82%	75%	-	-
H/Y(7.36)_Aro_E2F	-	36%	-	-	-	56%	13%	49%	44%	-	-
H/Y(7.36)_Hbond_2Wat	-	12%	-	-	-	10%	-	-	14%	-	-
I(7.39)_Apolar	69%	99%	92%	96%	84%	99%	98%	99%	100%	92%	98%
G(7.42)_Apolar	47%	94%	75%	79%	61%	92%	64%	91%	96%	95%	-
Y(7.43)_Apolar	58%	96%	83%	100%	74%	98%	98%	99%	95%	100%	86%
Y(7.43)_Hbond_1Wat	-	10%	-	-	-	-	-	-	27%	22%	-
Q(2.60)_Hbond_2Wat	18%	-	29%	23%	31%	-	-	-	-	-	-
S(2.61)_Apolar	-	-	-	-	-	20%	-	31%	31%	-	-
V(3.28)_Apolar	89%	-	99%	98%	76%	22%	17%	37%	-	-	-
T/S(45.51)_Hbond_2Wat	-	14%	-	-	-	-	18%	-	-	24%	-
Y(1.39)_Aro_F2F	-	-	-	-	-	-	-	-	13%	-	-
H(6.52)_Hbond_1Wat	-	-	-	-	-	-	-	-	10%	-	-
Y(1.39)_Hbond_2Wat	12%	-	20%	15%	21%	-	-	-	-	-	-
Q(2.60)_Hbond_ProD	22%	-	-	-	-	-	-	-	-	-	-
K(5.39)_Apolar	21%	-	-	-	-	-	-	-	-	35%	-
F(5.43)_Apolar	13%	-	-	-	-	-	-	-	-	-	-
H(6.52)_Hbond_2Wat	21%	11%	21%	17%	28%	-	-	-	-	-	-
N/V(2.63)_Hbond_2Wat	-	13%	-	-	-	-	-	-	-	-	-
W(23.50)_Aro_E2F	-	-	31%	18%	19%	34%	-	31%	-	-	-
W(23.50)_Aro_F2F	-	-	70%	69%	-	12%	-	13%	-	-	-
C(3.25)_Apolar	-	-	37%	15%	-	-	-	-	-	-	-
L/M(5.38)_Hbond_2Wat	-	-	11%	-	-	-	-	-	-	-	-
I/L(3.29)_Hbond_1Wat	-	-	-	-	-	-	17%	-	-	-	-
L(45.52)_Apolar	-	-	-	-	-	-	18%	-	-	-	-
F(2.59)_Apolar	-	-	-	40%	-	-	-	-	-	-	-
Y(2.64)_Hbond_2Wat	-	-	-	10%	-	-	-	-	-	-	10%
W/Y(7.35)_Aro_E2F	-	-	-	-	-	-	-	-	-	-	-

Y(2.64)_Aro_F2F	-	-	-	-	-	-	-	-	-	-	-
Y(2.64)_Hbond_1Wat	-	-	-	-	-	-	-	-	-	-	13%

Table S7. Interactions in the top statistical models that are predicted to either enhance (negative coefficients) or reduce (positive coefficients) ligand-induced MOR activation and consequent G protein signaling.

			Morphine		4, 5 & 6 (SC13)		Buprenorphine	
Residue	Interaction	Avg. Coeff	Prob.	Effect	Prob.	Effect.	Prob.	Effect
Interactions reducing G protein signaling								
Y(1.39)	Apolar	7.46	—		28%	⇊	—	
V(2.63)	Apolar	2.81	—		77%	⇊	26%	↓
C(45.50)	Apolar	2.43	—		78%	⇊	23%	↓
L(3.29)	Apolar	2.30	—		86%	⇊	62%	⇊
W(23.50)	Apolar	1.99	—		54%	⇊	—	
Interactions enhancing G protein signaling								
H(6.52)	Aro_E2F	-2.32	77%	⇈	—		84%	⇈
Y(7.36)	Apolar	-5.76	—		65%	⇈	—	
Y(7.36)	Aro_E2F	-8.65	—		39%	⇈	—	

Table S8. Selected models for the prediction of the negative log of the efficacy -log(E_{Max}) as a function of interaction probabilities. The R^2 on the full training set and the LOO-RMSE are reported for each model, as well as the values of the coefficient estimates and their standard errors.

Model ID	R^2	LOO-RMSE	Interaction	Coefficient	std. Error
1	0.936	0.312	(intercept)	0.176	0.244
			Y(1.39)_Apolar	7.633	1.083
			N/V(2.63)_Apolar	2.954	0.41
			H/Y(7.36)_Apolar	-6.353	0.756
2	0.93	0.494	(intercept)	0.513	0.218
			H/Y(7.36)_Aro_E2F	-7.572	1.151
			Y(1.39)_Apolar	6.749	1.068
			W(23.50)_Apolar	1.973	0.317
3	0.918	0.531	(intercept)	0.335	0.279
			Y(1.39)_Apolar	9.097	1.466
			C(45.50)_Apolar	2.428	0.424
			H/Y(7.36)_Apolar	-6.558	0.99
4	0.916	0.366	(intercept)	0.19	0.279
			H/Y(7.36)_Aro_E2F	-9.397	1.349
			Y(1.39)_Apolar	7.077	1.217
			N/V(2.63)_Apolar	2.659	0.463
5	0.907	0.386	(intercept)	-0.06	0.361
			Y(1.39)_Apolar	8.523	1.468
			I/L(3.29)_Apolar	2.394	0.441
			H/Y(7.36)_Apolar	-5.918	0.955
6	0.904	0.484	(intercept)	-0.068	0.348
			H/Y(7.36)_Aro_E2F	-8.988	1.395
			Y(1.39)_Apolar	8.093	1.337
			I/L(3.29)_Apolar	2.211	0.408
7	0.901	0.624	(intercept)	0.583	0.274
			Y(1.39)_Apolar	6.629	1.363
			W(23.50)_Apolar	2.014	0.38
			H/Y(7.36)_Apolar	-4.657	0.915
8	0.899	0.403	(intercept)	2.327	0.195
			Y(1.39)_Apolar	5.867	1.255
			H(6.52)_Aro_E2F	-2.321	0.41
			H/Y(7.36)_Apolar	-5.324	0.872

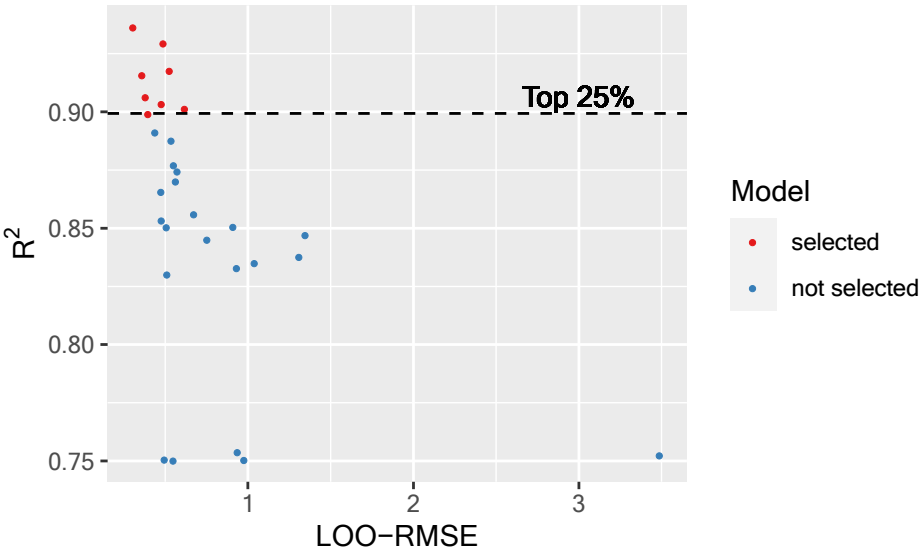


Figure S2. Full training set R^2 validation and leave-one-out (LOO) cross-validation root mean square error (RMSE) for models with R^2>0.75. Models with R^2 in the top quartile (red points) were selected as best performing models on experimental data.

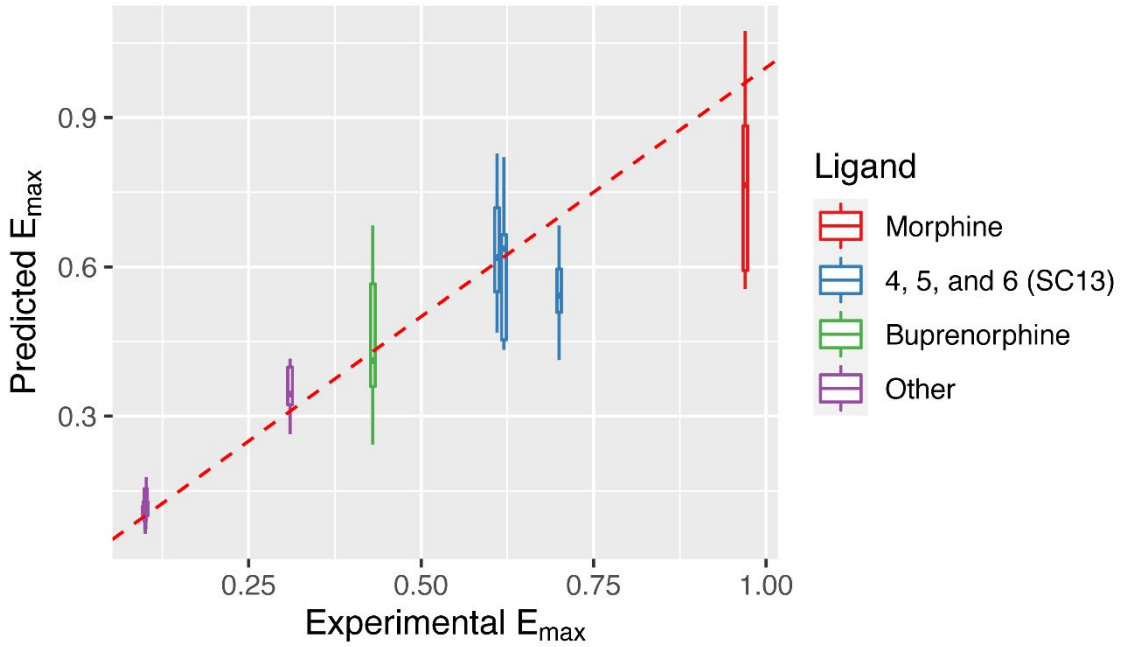
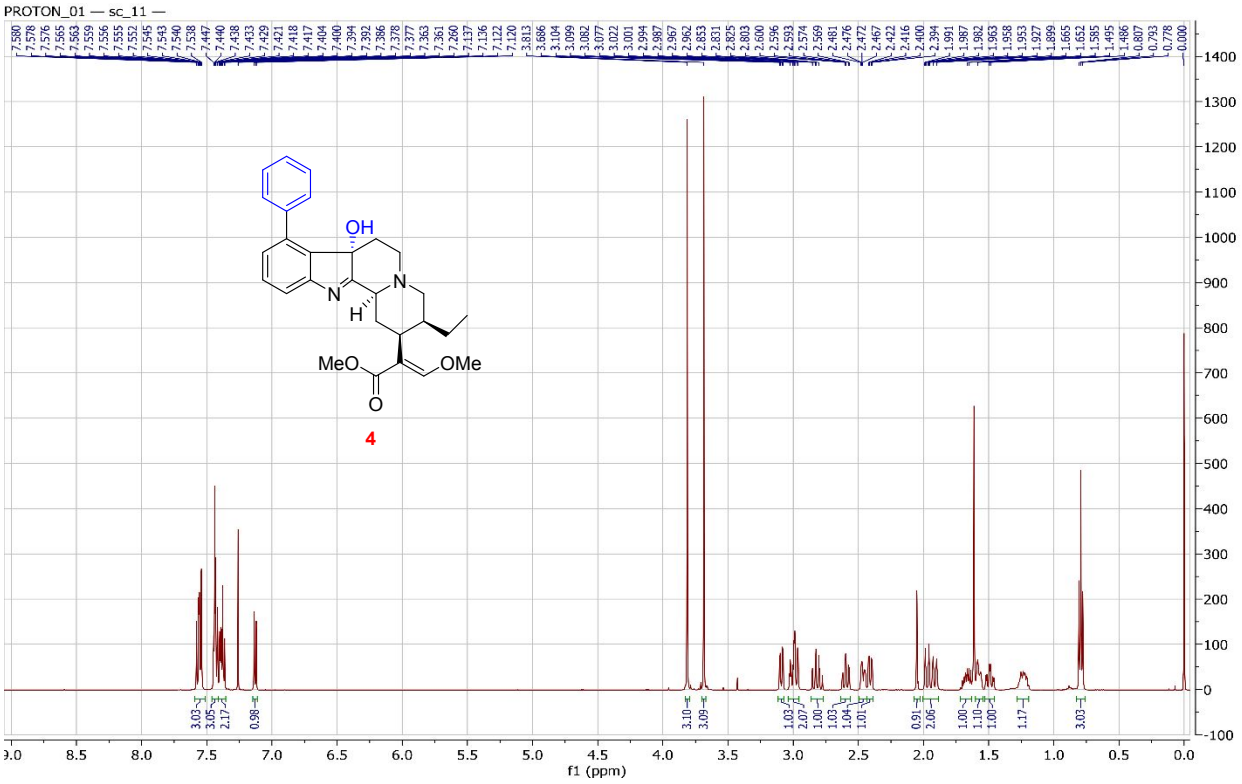


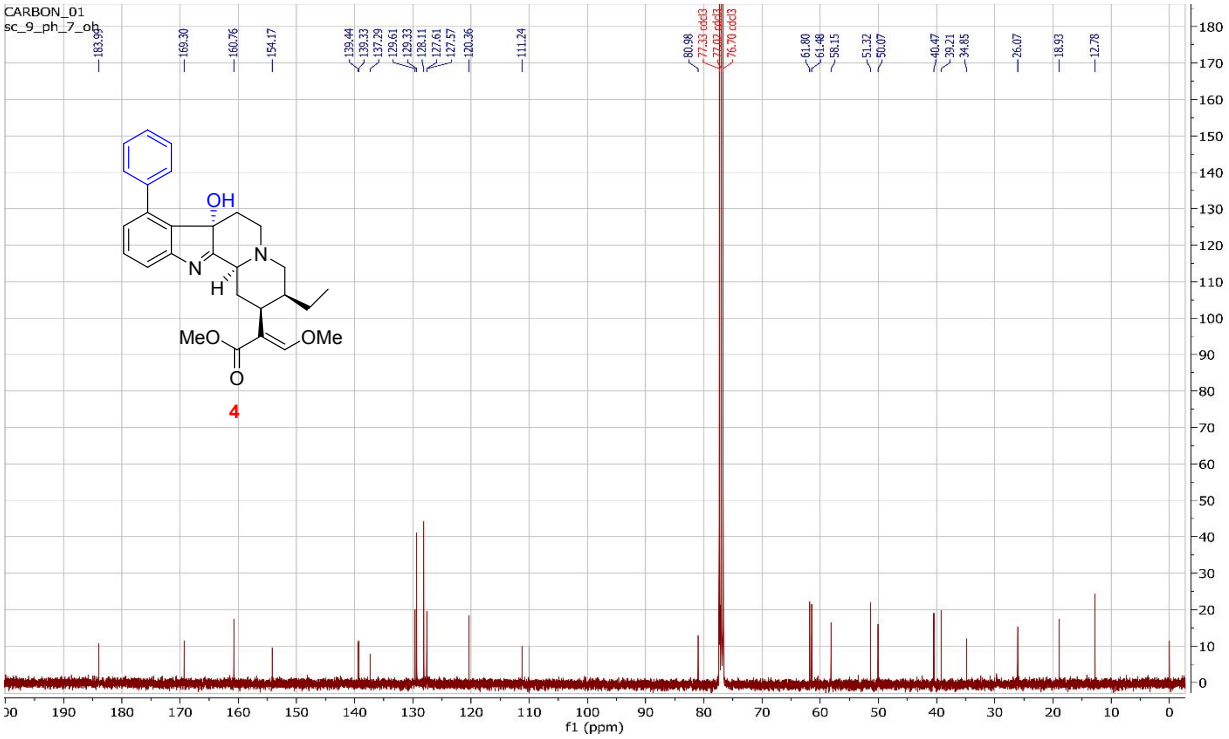
Figure S3. Values of the negative logarithm of the G protein efficacy E_{max} predicted from the selected top 25% models, compared to the experimental values for **morphine** (red), the **4**, **5** and **6** (**SC13**) ligands (blue), **buprenorphine** (green), and the remaining 6 ligands in the training set (purple).

^{1}H and ^{13}C NMR spectra of **4**, **5** and **6** (**SC13**)

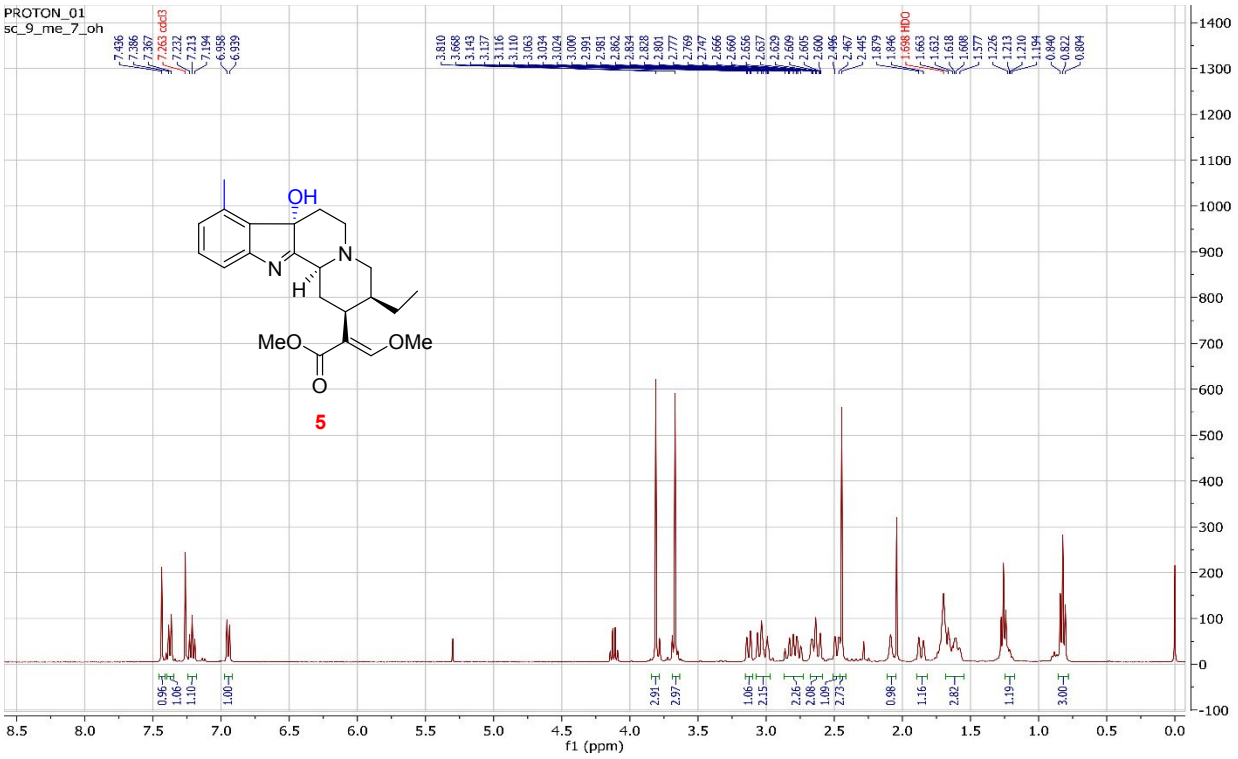
^{1}H NMR of 4 (500 MHz, $CDCl_3$)



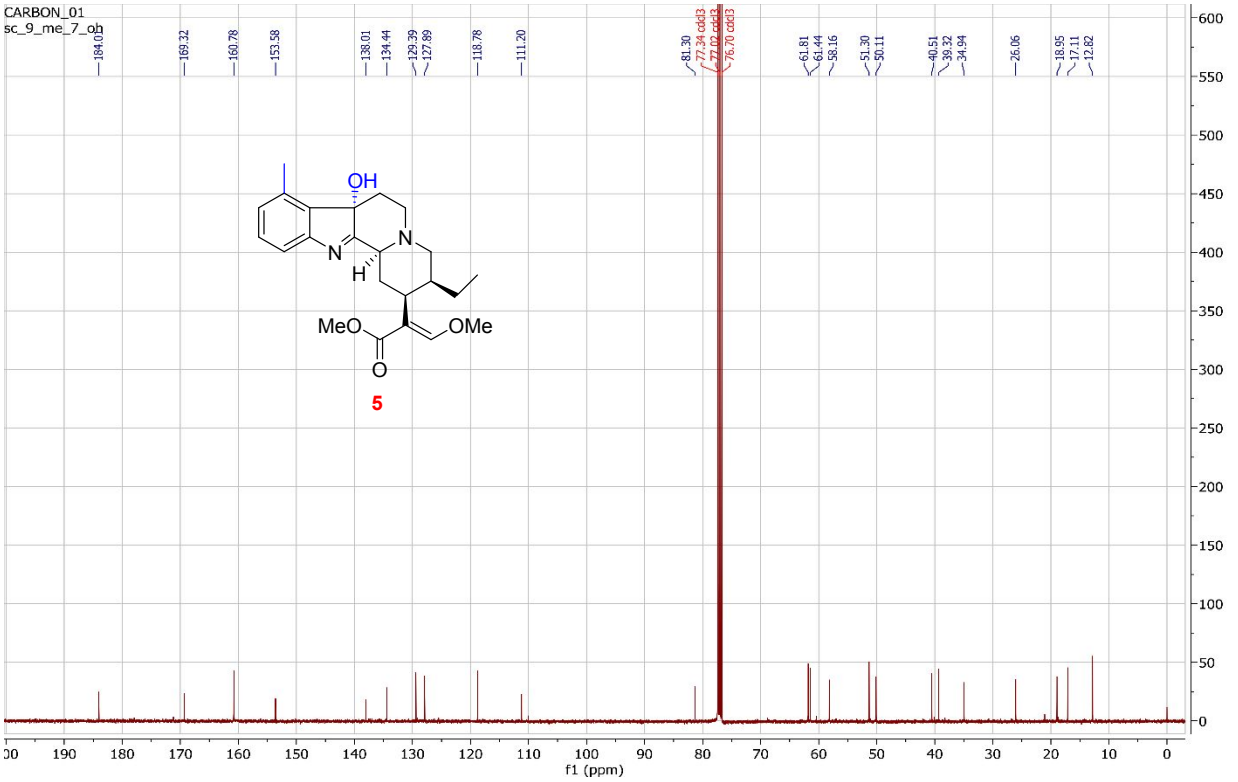
^{13}C NMR of 4 (100 MHz, $CDCl_3$)



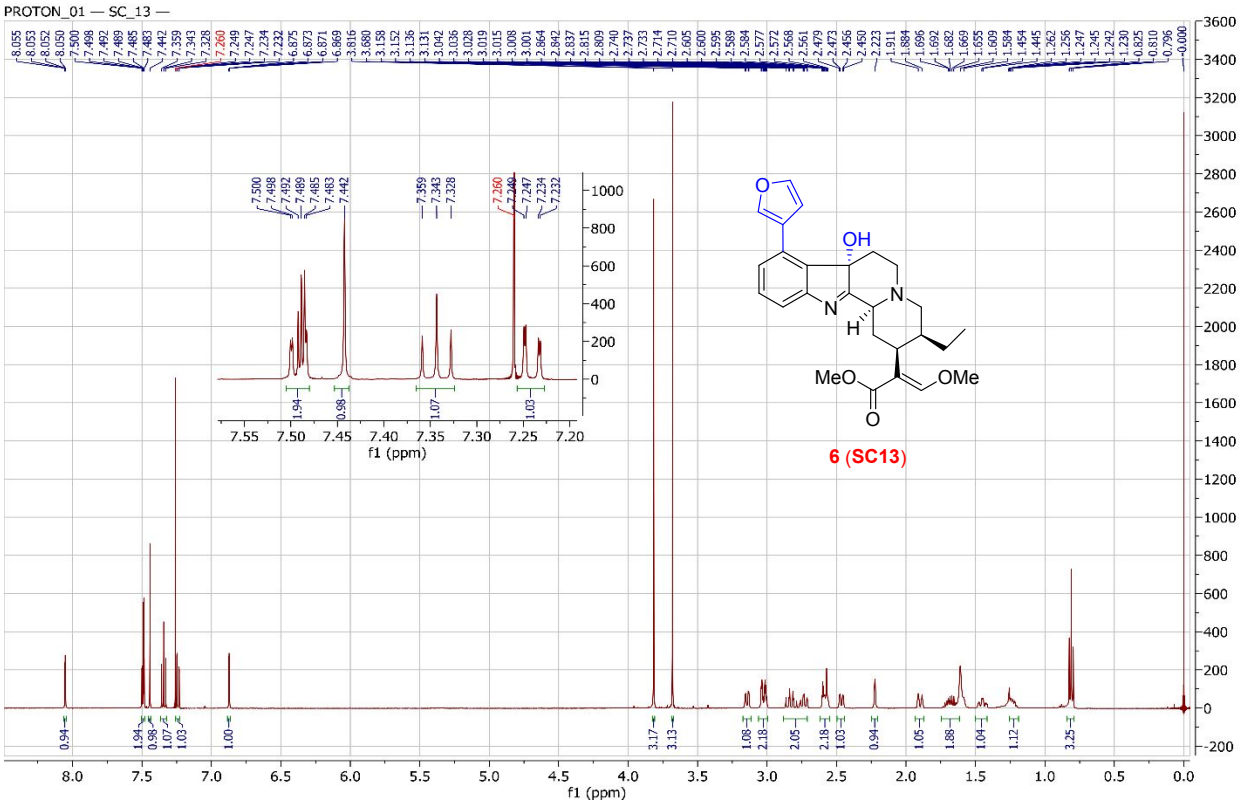
^{1}H NMR of 5 (400 MHz, $CDCl_3$)



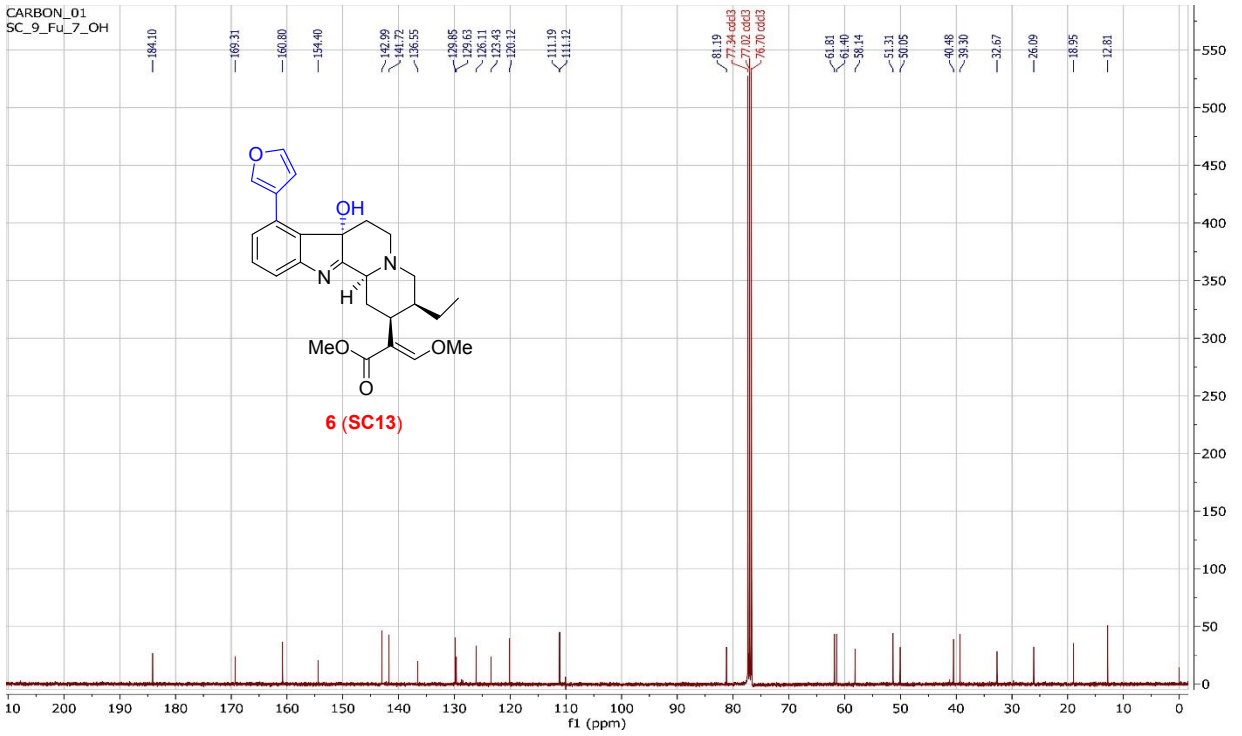
^{13}C NMR of 5 (100 MHz, $CDCl_3$)



^{1}H NMR of 6 (SC13) (500 MHz, $CDCl_3$)



^{13}C NMR of 6 (SC13) (100 MHz, $CDCl_3$)



HPLC method to determine purity

Instrument: Agilent 1200 Series HPLC

Column: Higgins Analytical CLIPEUS C18 column (5μm, 150 × 4.6 mm),

Method: Gradient elution program–(acetonitrile/water 5/95/95/5, 0.1% TFA), flow rate = 0.65 ml/min.

HPLC profile of 4

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Sample Name: SC11

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    Acq. Instrument : Instrument 1                    Location : Vial 92
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                                                     Inj Volume : 10 µl
    Acq. Method     : C:\Chem32\1\DATA\MURTHY\MURTHY100720 2020-10-07 23-15-32\MURTHY100120-1.M
    Last changed    : 10/7/2020 11:11:21 PM by Grant
    Analysis Method : C:\CHEM32\1\DATA\MURTHY\MURTHY100720 2020-10-07 23-15-32\MURTHY100120-1.M
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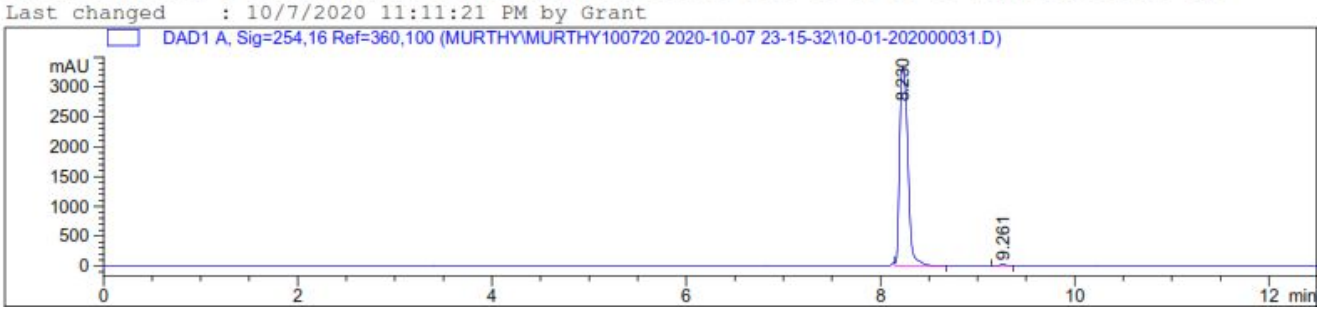


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                            Area Percent Report
    ======================================================================

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    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: DAD1 A, Sig=254,16 Ref=360,100

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [mAU*s]     [mAU]       %
    ----|-------|----|-------|----------|----------|--------|
       1   8.230 VV    0.0967 1.97031e4  3343.78052  99.3420
       2   9.261 VV    0.0632  130.51276   30.92712   0.6580

    Totals :                  1.98336e4  3374.70764

Instrument 1 10/8/2020 10:14:44 PM Grant
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HPLC profile of 5

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Sample Name: SC12

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    Acq. Instrument : Instrument 1                    Location : Vial 43
    Injection Date  : 10/17/2020 3:01:34 PM               Inj :   1
                                                    Inj Volume : 10 µl
    Acq. Method     : C:\Chem32\1\DATA\MURTHY\MURTHY101720 2020-10-17 14-46-55\MURTHY100120-1.M
    Last changed    : 10/7/2020 11:11:21 PM by Grant
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    Last changed    : 10/7/2020 11:11:21 PM by Grant
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    ======================================================================
                              Area Percent Report
    ======================================================================

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    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: DAD1 A, Sig=254,16 Ref=360,100

    Peak RetTime Type  Width     Area      Height     Area
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    ----|-------|----|-------|----------|----------|--------|
       1   7.418 VV    0.0539  118.41225   32.93866   1.7806
       2   7.606 VV    0.0590 6418.16650 1665.01318  96.5136
       3   7.930 VV    0.0619   16.27796    3.80944   0.2448
       4   8.521 VV    0.0615   97.15552   23.88614   1.4610

   Totals :                  6650.01222 1725.64742

Instrument 1 10/18/2020 10:42:24 AM Peter
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HPLC profile of 6 (SC13)

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Sample Name: SC13

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                                                   Inj Volume : 10 µl
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    Last changed    : 10/7/2020 11:11:21 PM by Grant
    Analysis Method : C:\CHEM32\1\DATA\MURTHY\MURTHY100720 2020-10-07 23-15-32\MURTHY100120-1.M
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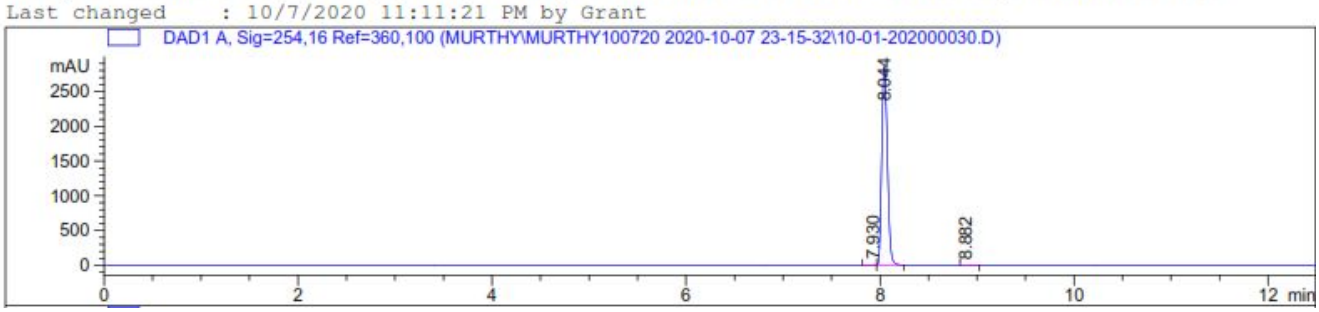


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                              Area Percent Report
    ========================================================================

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    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: DAD1 A, Sig=254,16 Ref=360,100

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [mAU*s]     [mAU]       %
    ----|-------|----|-------|----------|----------|--------|
       1   7.930 VV    0.0543   29.86893    8.64324   0.2560
       2   8.044 VV    0.0632 1.16139e4  2868.91309  99.5341
       3   8.882 VB    0.0639   24.49723    5.73100   0.2099

   Totals :                  1.16683e4  2883.28733

Instrument 1 10/8/2020 10:14:16 PM Grant
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