

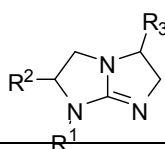
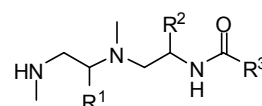
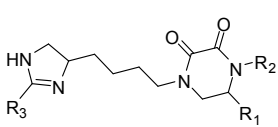
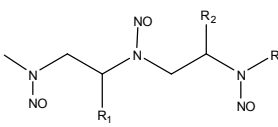
SUPPORTING INFORMATION

Direct High Throughput *In Vivo* Screening: Identification of Individual, Novel Antinociceptive Compounds from a Library of 734,821 Pyrrolidine bis-Piperazines

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Supplemental Table 1. 10 small-molecule libraries samples contained in the Scaffold Ranking Library. For the Scaffold Ranking Library each row indicates one sample. By way of example sample #8 (Library 1954) contains a total of 738,192 compounds in the single sample of the Scaffold Ranking Library. Also shown on this table are the number of samples in each of the specific Positional Scanning Libraries (Library 1954 has 120 samples), the number of compounds in each of these samples (1954 has either 17,576 or 28,392 depending on the specific sample), a generic name for the Library (1954 is Pyrrolidine Bis-piperazine) and a structure depicting the common scaffold shared by all the compounds in a particular Library as well as the sites of diversity.

Library	Samples	Compounds/mix	Total	Name	Structure
531	141	2,009 -2,499	102,459	Bicyclic guanidine	
914	150	2,500	125,000	N-acyl triamine	
1275	116	1,258 – 1,665	56,610	Dihydroimidazolyl-butyl-diketopiperazine	
1433	74	361 - 684	12,996	Nitrosamine	

1481	135	1,872 – 2,304	89,856	Poly-phenylurea	
1952	120	17,576 - 28,392	738,192	Pyrrolidine pentamine	
1953	120	17,576 - 28,392	738,192	Pyrrolidine Bis-diketopiperazine	
1954	120	17,576 - 28,392	738,192	Pyrrolidine Bis-piperazine	
1989	188	103,823	4,879,681	Cyclic tetrapeptide (thiazole)	
2048	83	510-1156	17,340	Cyclic guanidine linked sulfonamide	

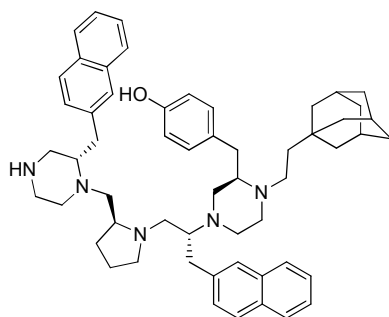
Supplemental Table 2. Values for the inhibition of Mu-, Delta-, and Kappa-Opioid receptor-selective radioligand binding to membrane protein by samples from TPI-2213-series (at 500 nM each).

<i>% Radioligand Binding ± SD</i>			
500 nM 2213:	$[^3\text{H}]\text{DAMGO}$ (MOR)	$[^3\text{H}]\text{DPDPE}$ (DOR)	$[^3\text{H}]\text{U69,593}$ (KOR)
TPI 2213-1	111.34 ± 6.06	93.30	104.10 ± 7.66
TPI 2213-2	113.27 ± 6.78	98.87	99.35 ± 11.4
TPI 2213-3	108.61 ± 4.67	84.28	92.55 ± 11.1
TPI 2213-4	108.42 ± 3.77	97.32	95.67 ± 9.08
TPI 2213-5	106.85 ± 6.13	90.99	97.52 ± 9.24
TPI 2213-6	103.46 ± 11.3	91.08	89.12 ± 7.34
TPI 2213-7	103.30 ± 7.70	92.15	90.50 ± 5.34
TPI 2213-8	103.10 ± 3.60	87.44	93.06 ± 11.8
TPI 2213-9	110.52 ± 5.93	90.38	106.27 ± 11.2
TPI 2213-10	106.82 ± 5.22	90.85	100.98 ± 5.53
TPI 2213-11	113.92 ± 14.1	86.22	100.70 ± 3.33
TPI 2213-12	111.75 ± 5.90	94.42	98.66 ± 5.78
TPI 2213-13	106.28 ± 8.95	85.62	90.65 ± 7.32
TPI 2213-14	103.50 ± 7.93	90.65	92.79 ± 10.2
TPI 2213-15	103.21 ± 8.18	95.20	94.37 ± 6.42
TPI 2213-16	95.90 ± 7.14	84.76	91.54 ± 9.00
TPI 2213-17	109.34 ± 6.25	104.82	117.24 ± 14.4
TPI 2213-18	107.07 ± 4.54	99.98	109.00 ± 10.5
TPI 2213-19	108.19 ± 8.06	91.78	89.54 ± 5.16
TPI 2213-20	105.28 ± 11.1	87.99	110.47 ± 2.67
TPI 2213-21	107.93 ± 3.51	96.11	103.28 ± 5.60
TPI 2213-22	105.25 ± 6.16	88.65	93.32 ± 5.31
TPI 2213-23	97.85 ± 6.39	94.03	100.94 ± 6.69
TPI 2213-24	101.03 ± 9.67	89.97	79.15 ± 5.39
TPI 2213-25	109.23 ± 6.97	90.29	113.79 ± 7.82
TPI 2213-26	103.83 ± 9.57	91.66	105.65 ± 15.8
TPI 2213-27	107.01 ± 10.6	91.66	113.75 ± 6.79
TPI 2213-28	97.65 ± 5.81	91.97	106.52 ± 8.23
TPI 2213-29	110.32 ± 8.69	92.15	116.64 ± 4.17
TPI 2213-30	100.53 ± 4.20	91.06	105.69 ± 5.42
TPI 2213-31	104.81 ± 9.30	88.89	93.80 ± 5.58
TPI 2213-32	98.44 ± 7.86	87.70	94.05 ± 7.44
TPI 2213-33	107.66 ± 7.75	102.18	116.27 ± 10.4
TPI 2213-34	102.76 ± 8.82	92.00	108.70 ± 7.63
TPI 2213-35	109.83 ± 8.82	91.46	106.29 ± 8.06
TPI 2213-36	102.48 ± 8.67	92.99	111.04 ± 8.68
TPI 2213-37	95.25 ± 7.33	88.49	93.36 ± 9.08
TPI 2213-38	96.60 ± 5.65	88.67	97.84 ± 7.00
TPI 2213-39	101.21 ± 8.14	90.47	98.24 ± 6.23
TPI 2213-40	97.85 ± 7.32	83.53	103.62 ± 4.87
TPI 2213-41	105.45 ± 6.43	94.51	93.57 ± 8.41
TPI 2213-42	106.81 ± 7.64	91.38	90.42 ± 5.28

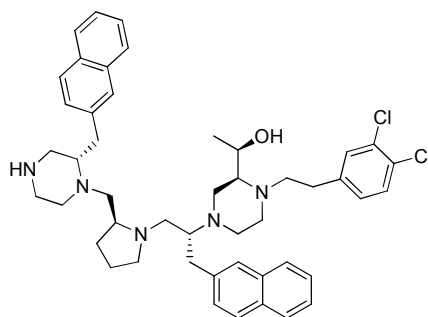
TPI 2213-43	104.91 ± 10.5	97.82	86.73 ± 7.34
TPI 2213-44	106.43 ± 5.02	90.91	89.27 ± 7.49
TPI 2213-45	103.03 ± 6.80	84.18	86.91 ± 10.9
TPI 2213-46	98.24 ± 5.74	78.22	88.88 ± 7.43
TPI 2213-47	97.24 ± 3.20	83.80	91.14 ± 14.5
TPI 2213-48	93.16 ± 6.67	90.63	85.79 ± 12.1
TPI 2213-49	107.09 ± 7.04	91.97	105.58 ± 10.9
TPI 2213-50	104.48 ± 11.6	88.41	93.05 ± 7.65
TPI 2213-51	106.24 ± 4.40	89.65	90.88 ± 4.67
TPI 2213-52	103.21 ± 5.78	81.30	88.37 ± 5.94
TPI 2213-53	104.06 ± 8.28	85.80	90.60 ± 5.38
TPI 2213-54	102.29 ± 7.59	87.34	97.28 ± 6.93

Membrane protein was incubated with a single TPI-2213 compound (500 nM) in the presence of 0.87 nM [³H]DAMGO, 2 nM [³H]DPDPE, or 2 nM [³H]U69,593 in 50 mM Tris-HCl, pH 7.5 at 25°C as described in “Experimental Procedures.” Data are listed as the mean % Radioligand binding ± SD from two experiments performed in duplicate for MOR and KOR, and one experiment performed in duplicate (DOR) without SD values.

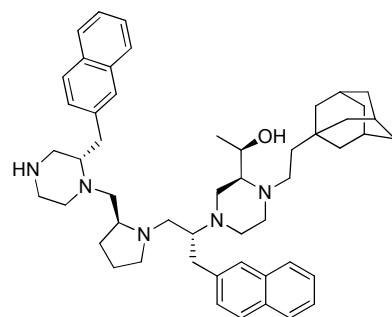
Supplemental Figure 1. Chemical Structures of Individual Compounds.



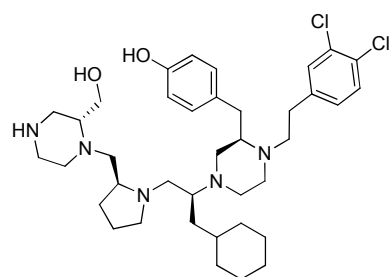
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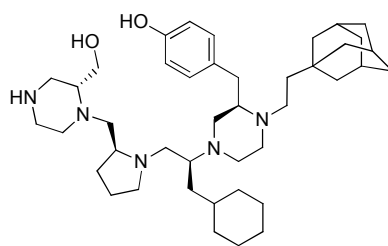
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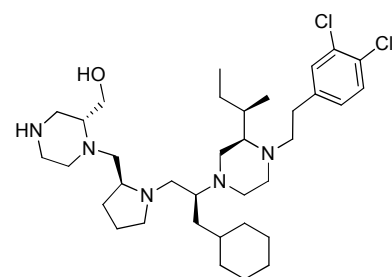
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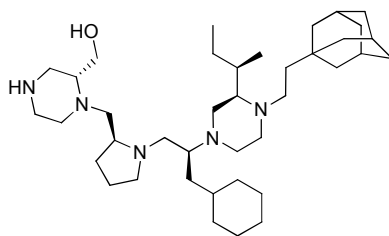
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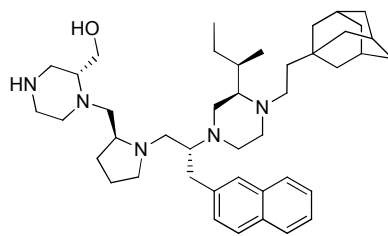
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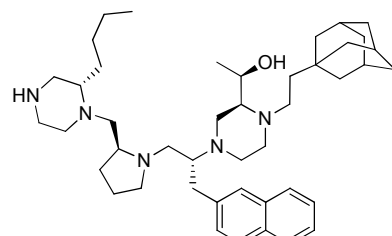
2213-23



2213-24



2213-32



2213-54