

Supplementary Information for

Large library docking for cannabinoid-1 receptor agonists with reduced side effects

Tia A. Tummino^{1,2,†}, Christos Iliopoulos-Tsoutsouvas^{3,†}, Joao M. Braz^{4,†}, Evan S. O'Brien⁵, Reed M. Stein^{1,2}, Veronica Craik⁴, Ngan K. Tran³, Suthakar Ganapathy³, Fangyu Liu¹, Yuki Shiimura^{5,6}, Fei Tong³, Thanh C. Ho³, Dmytro S. Radchenko⁷, Yurii S. Moroz^{7,8,9}, Sian Rodriguez Rosado⁴, Karnika Bhardwaj⁴, Jorge Benitez⁴, Yongfeng Liu¹⁰, Herthana Kandasamy¹¹, Claire Normand¹¹, Meriem Semache¹¹, Laurent Sabbagh¹¹, Isabella Glenn¹, John J. Irwin¹, Kaavya Krishna Kumar^{5,*}, Alexandros Makriyannis^{3,12*}, Allan I. Basbaum^{4,*}, & Brian K. Shoichet^{1,*,#}

†Contributed equally.

#Lead Contact

*Correspondence: kaavyak@stanford.edu, a.makriyannis@northeastern.edu, allan.basbaum@ucsf.edu, bshoichet@gmail.com

This file includes:

Supplementary Tables 1 to 8

Additional supplementary materials:

Synthetic procedures, chemical characterization and spectral data is supplied as

Supplemental_Information_QC.pdf

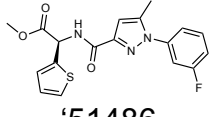
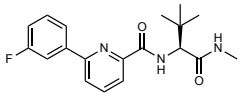
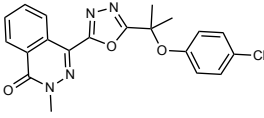
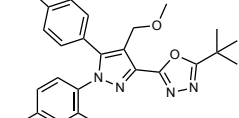
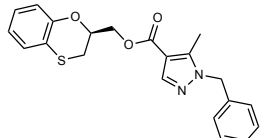
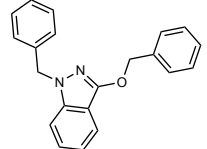
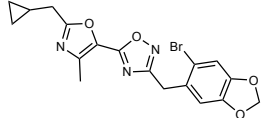
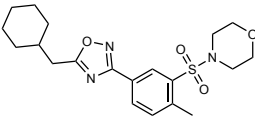
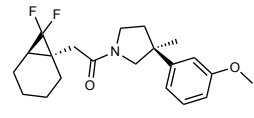
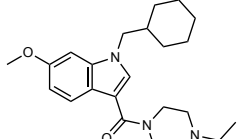
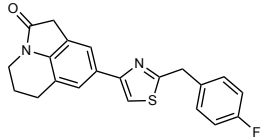
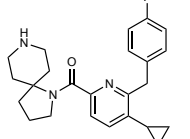
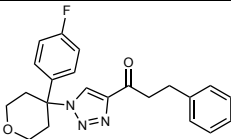
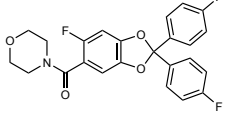
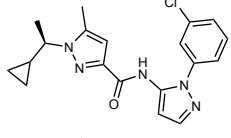

Supplementary_Table_9.xlsx

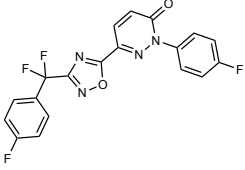
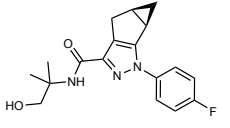
Model_final.pdb

Map_final.mrc

PDB_Validation_report.pdf

Supplementary Table 1. Binding affinities for hits identified in initial CB1 docking screen.

Compound	Global rank	rCB1 affinity ^a K _i [95% CI] nM pK _i [95% CI]	Tc ^b	Nearest ChEMBL ligand ^c
 '51486	117390	731 [552 – 969] 6.14 [6.01 – 6.26]	0.30	 CHEMBL4110127
 '0450	6582	691 [459 – 1033] 6.16 [5.99 – 6.34]	0.36	 CHEMBL519214
 '7800	12210	1007 [615 – 1654] 6.0 [5.78 – 6.21]	0.28	 CHEMBL3116279
 '7019	20488	4039 [3027 – 5379] 5.39 [5.27 – 5.52]	0.24	 CHEMBL472680
 '7218	29322	52.2% [24.79]	0.31	 CHEMBL3347301
 '1038	47606	53.6% [2.91]	0.28	 CHEMBL3890211
 '7337	24720	57.0% [3.04]	0.29	 CHEMBL259699
 '7902	139929	57.1% [0.02]	0.31	 CHEMBL3915046

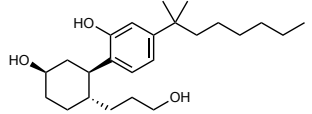
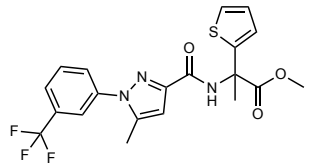
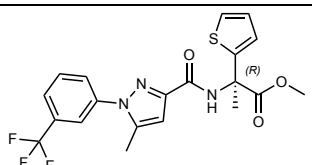
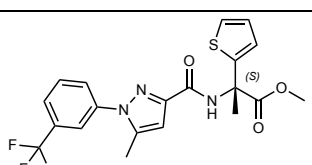
 <p>'2443</p>	21964	51.1% [4.87]	0.23	 <p>CHEMBL3354970</p>
--	-------	--------------	------	--

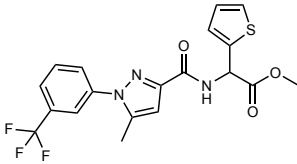
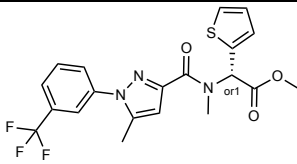
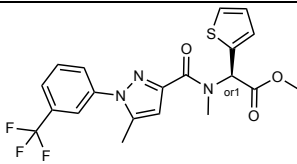
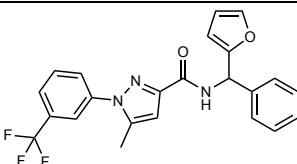
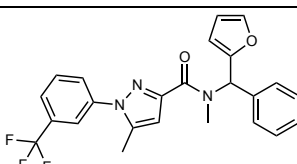
^aBinding affinity to rCB1 represented as K_i [95% CI] and pK_i [95% CI] from three independent experiments in triplicate when measured. Otherwise, % radioligand displacement [S.D] from three replicates in a single-point competition experiment at 10 μM

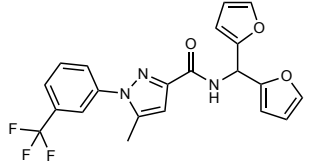
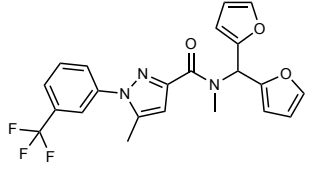
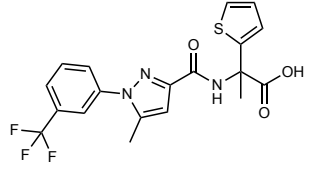
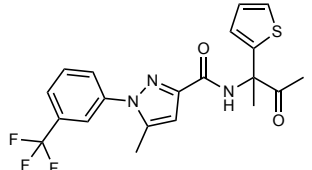
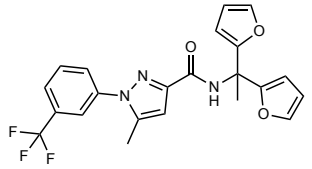
^bTanimoto coefficient based on ECFP4 fingerprints

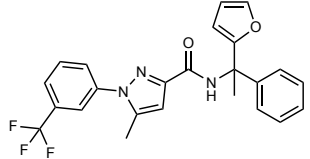
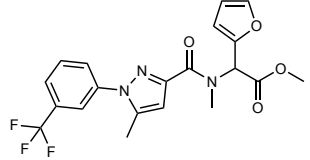
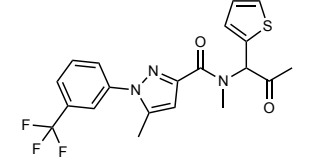
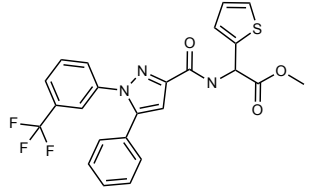
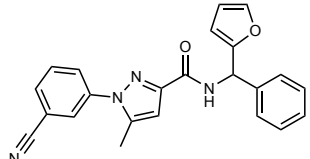
^cCorresponding ChEMBL ligand with the most similar fingerprint

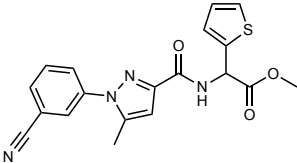
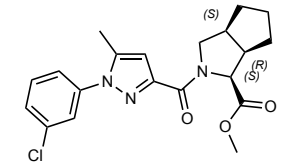
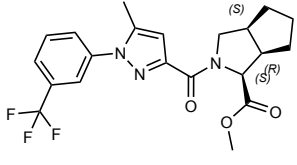
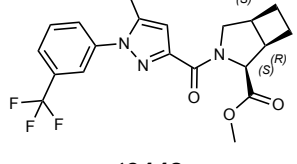
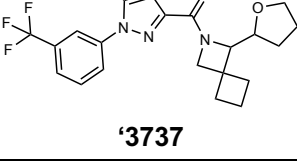
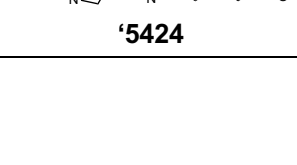
Supplementary Table 2. Binding affinities and functional activities for active analogs at CB1.

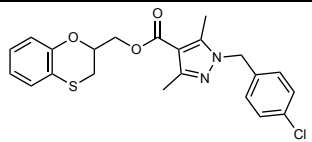
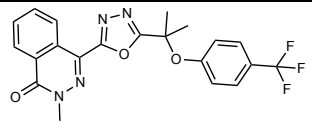
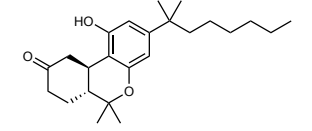
Compound	rCB1 binding K _i [95% CI] (nM) pK _i [95% CI] E _{max} [SEM] Significance ^a	hCB1 Lance Ultra cAMP EC ₅₀ [95% CI] (nM) pEC ₅₀ [95% CI] E _{max} [95% CI] Significance ^a	hCB1 Cerep cAMP EC ₅₀ [95% CI] (nM) pEC ₅₀ [95% CI] E _{max} [95% CI]	hCB1 Glosensor cAMP EC ₅₀ [95% CI] (nM) pEC ₅₀ [95% CI] E _{max} [95% CI]	hCB1 Tango β-arrestin recruitment EC ₅₀ [95% CI] (nM) pEC ₅₀ [95% CI] E _{max} [95% CI]	hCB1 DiscoverX β-arrestin recruitment EC ₅₀ [95% CI] (nM) pEC ₅₀ [95% CI] E _{max} [95% CI]
 CP-55,940	2.9 [2.05 – 4.2] 8.5 [8.4 – 8.7] 98% [3.5]	6.2 [4.7 – 8.0] 8.2 [8.1 – 8.3] 85% [86 – 85]	0.026	0.028 [0.02 – 0.04] 10.6 [10.5 – 10.7] 96% [93 – 99]	8.9 [7.5 – 10.6] 8.1 [8.0 – 8.1] 100% [96 – 104]	4.0 [3.2 – 4.9] 8.4 [8.3 – 8.5] 108% [99 – 109]
 '4042	1.86 [1.37 – 2.52] 8.7 [8.6 – 8.9] 99% [3.0] ns	3.3 [1.9 – 5.6] 8.5 [8.3 – 8.7] 78% [78 – 79] ns	0.008 [0.006 – 0.01] 11.1 [11.0 – 11.2] 96% [102 – 107]	0.039 [2.9 – 5.4] 10.4 [10.3 – 10.5] 91% [87 – 94]	10.7 [8.7 – 13.3] 8.0 [7.9 – 8.1] 102% [98 – 105]	2.3 [2.5 – 4.8] 8.7 [8.3 – 9.6] 71% [60 – 65]
 '1350	0.95 [0.74 – 1.24] 9.02 [8.9 – 9.1] 106% [2.9] *	1.6 [0.7 – 3.6] 8.8 [8.4 – 9.2] 78% [77 – 80] **	--	--	--	--
 '8690	90.2 [56.7 – 143] 7.1 [6.9 – 7.3] 100% [4.0] ****	473 [109 – 1822] 6.3 [5.8 – 7.0] 53% [45 – 65] ****	--	--	--	--

 <p>'60154</p>	44.3 [33.9 – 58.0] 7.4 [7.2 – 7.5] 110% [2.9]	351 [93] 6.5 [7.0] 67% [59 – 89]	--	25.2 [16 – 40] 7.6 [7.4 – 7.8] 82% [74 – 189]	819 [718 – 934] 6.1 [6.0 – 6.1] 39% [37 – 40]	--
 <p>'1066</p>	1719 [736 – 4048] 5.8 [5.4 – 6.1] 97% [8.0]					
 <p>'6000</p>	1455 [943 – 2249] 5.8 [5.7 – 6.0] 96% [4.0]					
 <p>'1081</p>	116 [76.3 – 178] 6.9 [6.8 – 7.1] 96% [4.0]	--	--	--	--	--
 <p>'1082</p>	850 [488 – 1491] 6.1 [5.8 – 6.3] 94% [5.7]	--	--	--	--	--

 <p>'1090</p>	<p>90.8 [42.7 – 192] 7.0 [6.7 – 7.4] 99% [7.13]</p>	<p>--</p>	<p>--</p>	<p>--</p>	<p>--</p>	<p>--</p>
 <p>'4388</p>	<p>1360 [998 – 1857] 5.9 [5.7 – 6.0] 113% [4.4]</p>	<p>--</p>	<p>--</p>	<p>--</p>	<p>--</p>	<p>--</p>
 <p>'4051</p>	<p>5328 [3774– 7507] 5.3 [5.1 – 5.4] 103% [3.8]</p>	<p>--</p>	<p>--</p>	<p>--</p>	<p>--</p>	<p>--</p>
 <p>'4156</p>	<p>13.5 [6.3 – 30.1] 7.9 [7.5 – 8.2] 91% [7.5]</p>	<p>--</p>	<p>--</p>	<p>--</p>	<p>--</p>	<p>--</p>
 <p>'4936</p>	<p>7.5 [3.9 – 14.3] 8.1 [7.9 – 8.4] 88% [6.3]</p>	<p>--</p>	<p>--</p>	<p>--</p>	<p>--</p>	<p>--</p>

 <p>'5806</p>	<p>8.0 [3.0 – 21.8] 8.1 [7.7 – 8.5] 100% [10.5]</p>					
 <p>'6425</p>	<p>934 [583 – 1501] 6.0 [5.8 – 6.2] 108% [5.8]</p>	--	--	--	--	--
 <p>'6829</p>	<p>1046 [669 – 1643] 6.0 [5.8 – 6.2] 106% [5.8]</p>	--	--	--	--	--
 <p>'8079</p>	<p>18.5 [13.8 – 25.0] 7.7 [7.6 – 7.9] 104% [3.0]</p>	--	--	--	--	--
 <p>'12565</p>	<p>301 [195 – 462] 6.5 [6.3 – 6.7] 97% [4.0]</p>	--	--	--	--	--

 <p>'31604</p>	<p>801 [596 – 1,076] 6.1 [6.0 – 6.2] 100% [2.8]</p>	--	--	--	--	--
 <p>'10010</p>	<p>1,196 [952 – 1,505] 5.9 [5.8 – 6.0] 101% [2.9]</p>	--	--	N.D.	> 10,000	--
 <p>'6439</p>	<p>251 [173 – 364] 6.6 [6.4 – 6.8] 106% [3.9]</p>	--	--	N.D.	> 10,000	--
 <p>'6448</p>	<p>866 [564 – 1,317] 6.1 [5.9 – 6.3] 103% [4.1]</p>	--	--	--	--	--
 <p>'3737</p>	<p>173 [94 – 322] 6.76 [6.5 – 7.03] 112% [8.8]</p>	--	326 [168 – 1044] 6.5 [6.0 – 6.8] 99% [91 – 135]	N.D.	> 10,000	> 10,000
 <p>'5424</p>	<p>876 [683 – 1123] 6.1 [6.0 – 6.2] 104% [2.6]</p>	--	--	--	--	--

 <p>'5463</p>	<p>825 [396 – 1,755] 6.1 [5.8 – 6.4] 88% [6.6]</p>	--	--	--	--	--
 <p>'2153</p>	<p>163 [90 – 287] 6.79 [6.5 – 7.0] 82.6% [4.5]</p>	--	--	--	--	--
 <p>Nabilone</p>	<p>2.3 [1.4 – 3.8] 8.6 [8.4 – 8.9] 81% [3.7]</p>	--	--	--	--	--

N.D. = best fit values were not determined due to compound inactivity or poor data quality

-- Not tested

^aOne-way ANOVA statistical significance of individual comparisons to CP-55,940 after correction with Dunnett's test of multiple hypotheses. ns = not significant, * p<0.05, ** p<0.01, *** p<0.001, **** p<0.001

Supplementary Table 3. Cryo-EM data collection, model refinement, and validation statistics.

Data Collection	Global Refinement
Voltage (kV)	300
Magnification	96,000
Total electron dose (e ⁻ /Å ²)	56.6
Defocus range (μm)	-0.7 - -2.0
Calibrated pixel size (Å)	0.8521
Micrographs collected	8324
Data Processing	
Extracted particles	4,967,593
Particles used for final reconstruction	465,411
Final map resolution (Å, 0.143 FSC)	3.3
Map resolution range (Å)	2.6 - 4.2
Map sharpening B factor (Å ²)	175.3
Model Content	
Initial models used (PDB code)	6N4B (CB1/G _i /scFv16)
Total number of atoms	8,528
No. of protein residues	1116
No. of ligands	1
Model Validation	
CC map vs. model (%)	69.05
RMSD	
Bond lengths (Å) / Bond angles (°)	0.003 / 0.663
Ramachandran plot statistics	
Favored (%)	94.12
Allowed (%)	5.88
Outliers (%)	0.0
Rotamer outliers (%)	0.0
C-beta deviations	0.0
Clash score	7.19

Supplementary Table 4. Functional activities for select analogs versus a variety of transducers and hCB1 in the bioSens-All® platform.

Compound		hCB1 G ₁	hCB1 G _{oB}	hCB1 G _z
CP-55,940	EC ₅₀ [95% CI] (nM)	0.46 [0.4 – 0.5]	0.63 [0.6 – 0.7]	0.28 [0.18 – 0.5]
	pEC ₅₀ [95% CI]	9.3 [9.3 – 9.4]	9.2 [9.2 – 9.3]	9.6 [9.4 – 9.8]
	E _{max} [95% CI]	100 [98 – 102]	100 [99 – 101]	102 [102 – 104]
'51486	EC ₅₀ [95% CI] (nM)	849 [745– 947]	711 [579– 873]	1118 [363– 3447]
	pEC ₅₀ [95% CI]	6.1 [6.0 – 6.1]	6.2 [6.1 – 6.2]	6.0 [5.5 – 6.4]
	E _{max} [95% CI]	73 [70 – 75]	74 [70 – 75]	130 [996 – 163]
'60154	EC ₅₀ [95% CI] (nM)	2.5 [1.9 – 3.4]	18.4 [15.8– 21.5]	17.5 [7.5 – 41]
	pEC ₅₀ [95% CI]	7.6 [7.5 – 7.7]	7.7 [7.7 – 7.8]	7.8 [7.4 – 8.1]
	E _{max} [95% CI]	92 [90 – 94]	100 [98 – 100]	121 [103 – 140]
'1081	EC ₅₀ [95% CI] (nM)	150 [116 – 197]	91.7 [55 – 153]	225 [73 – 694]
	pEC ₅₀ [95% CI]	6.8 [6.7 – 6.9]	7.0 [6.8 – 7.3]	6.7 [6.2 – 7.1]
	E _{max} [95% CI]	49 [48 – 51]	45 [43 – 47]	72 [55 – 89]
'1082	EC ₅₀ [95% CI] (nM)	°N.D.	N.D.	N.D.
	pEC ₅₀ [95% CI]			
	E _{max} [95% CI]			
'1087	EC ₅₀ [95% CI] (nM)	N.D.	N.D.	N.D.
	pEC ₅₀ [95% CI]			
	E _{max} [95% CI]			
'1090	EC ₅₀ [95% CI] (nM)	35.6 [28.5 – 45]	37.3 [30 – 47]	126 [45 – 354]
	pEC ₅₀ [95% CI]	7.5 [7.3 – 7.5]	7.4 [7.3 – 7.5]	6.9 [6.5 – 7.4]
	E _{max} [95% CI]	81 [80 – 82]	90 [88 – 92]	115 [92 – 137]
'4388	EC ₅₀ [95% CI] (nM)	N.D.	N.D.	N.D.
	pEC ₅₀ [95% CI]			
	E _{max} [95% CI]			
'6829	EC ₅₀ [95% CI] (nM)	4056 [2417 – 9228]	1011 [706 – 1449]	1347 [720 – 2522]
	pEC ₅₀ [95% CI]	5.4 [5.0 – 5.6]	6.0 [5.8 – 6.2]	5.9 [6.0 – 6.2]
	E _{max} [95% CI]	68 [57 – 77]	49 [45 – 53]	70 [57 – 83]
'4051	EC ₅₀ [95% CI] (nM)	6523 [5770 – 7511]	7988 [6709 – 9511]	2431 [1069 – 5531]
	pEC ₅₀ [95% CI]	5.2 [5.1 – 5.2]	5.1 [5.0 – 5.2]	5.6 [5.3 – 6.0]
	E _{max} [95% CI]	108 [104 – 113]	115 [106 – 121]	114 [86 – 141]
'12565	EC ₅₀ [95% CI] (nM)	104 [79 – 138]	44.8 [27 – 73]	54.6 [22.1 – 135]
	pEC ₅₀ [95% CI]	7.0 [6.9 – 7.1]	7.4 [7.1 – 7.6]	7.3 [6.9 – 7.7]
	E _{max} [95% CI]	28 [27 – 28]	25 [24 – 26]	51 [41 – 61]
'10010	EC ₅₀ [95% CI] (nM)	814 [717 – 932]	801 [582– 1102]	1396 [506 – 3853]
	pEC ₅₀ [95% CI]	6.1 [6.0 – 6.1]	6.1 [6.0 – 6.2]	5.9 [5.4 – 6.3]
	E _{max} [95% CI]	74 [70 – 74]	71 [67 – 75]	144 [113 – 175]
'6439	EC ₅₀ [95% CI] (nM)	65.5 [56.8 – 755]	60.9 [52.9 – 70]	339 [96 – 1195]
	pEC ₅₀ [95% CI]	7.2 [7.1 – 7.2]	7.2 [7.2 – 7.3]	6.5 [5.9 – 7.0]
	E _{max} [95% CI]	95 [93 – 96]	100 [99 – 101]	162 [125 – 199]
'6448	EC ₅₀ [95% CI] (nM)	345 [321 – 372]	310 [267 – 360]	728 [381 – 1393]
	pEC ₅₀ [95% CI]	6.5 [6.4 – 6.5]	6.5 [6.4 – 6.6]	6.1 [5.9 – 6.4]
	E _{max} [95% CI]	87 [86 – 88]	90 [87 – 91]	151 [130 – 172]
'3737a	EC ₅₀ [95% CI] (nM)	2804 [2436 – 3285]	6729 [2678 – 16910]	2470 [998 – 6108]
	pEC ₅₀ [95% CI]	5.6 [5.5 – 5.6]	5.2 [4.8 – 5.6]	5.6 [5.2 – 6.1]
	E _{max} [95% CI]	98 [94 – 102]	118 [94 – 143]	140 [9109 – 172]
'3737b	EC ₅₀ [95% CI] (nM)	3026 [2486 – 3822]	19780 [4343 – 90080]	30840 [1133 – 839000]
	pEC ₅₀ [95% CI]	5.5 [5.4 – 5.6]	4.7 [4.1 – 5.4]	4.5 [3.1– 6.0]
	E _{max} [95% CI]	89 [84 – 94]	152 [94 – 208]	231 [34 – 428]
'3737c	EC ₅₀ [95% CI] (nM)	N.D.	N.D.	N.D.
	pEC ₅₀ [95% CI]			
	E _{max} [95% CI]			
'3737d	EC ₅₀ [95% CI] (nM)	N.D.	N.D.	N.D.
	pEC ₅₀ [95% CI]			
	E _{max} [95% CI]			

'7019	EC ₅₀ [95% CI] (nM) pEC ₅₀ [95% CI] E _{max} [95% CI]	N.D.	N.D.	b ₋₋
'5424	EC ₅₀ [95% CI] (nM) pEC ₅₀ [95% CI] E _{max} [95% CI]	N.D.	N.D.	--
'7800	EC ₅₀ [95% CI] (nM) pEC ₅₀ [95% CI] E _{max} [95% CI]	6793 [983] 5.2 [6.0] 34 [24]	597 [398 – 1017] 6.2 [5.9 – 6.4] 21 [20 – 23]	--
'5463	EC ₅₀ [95% CI] (nM) pEC ₅₀ [95% CI] E _{max} [95% CI]	4941 [1763 – 26860000] 5.3 [3.4 – 5.8] 31 [24 – 88]	7472 [1125] 5.1 [6.0] 32 [22]	--
'0450	EC ₅₀ [95% CI] (nM) pEC ₅₀ [95% CI] E _{max} [95% CI]	56310 [2177] 4.3 [5.7] 49 [26]	2509 [680 – 11020000] 5.6 [3.0 – 6.2] 36 [30 – 117]	--
'2153	EC ₅₀ [95% CI] (nM) pEC ₅₀ [95% CI] E _{max} [95% CI]	1011 [371 – 6576] 6.0 [5.2 – 6.4] 89 [78 – 118]	2061 [620 – 25390] 5.7 4.6 – 6.2] 109 [91 – 162]	--

^aN.D. = best fit values were not able to be determined due to compound inactivity or poor data quality

^b-- Not tested

Supplementary Table 5. Detailed functional activities for select analogs and controls versus a variety of transducers and hCB1 in the bioSens-All® platform.

Compound		hCB1 G _{i1}	hCB1 G _{i2}	hCB1 G _{oB}	hCB1 G _z	hCB1 G _{i3}	hCB1 G _{i5}	hCB1 Barr2 + GRK2
CP-55,940	EC ₅₀ [95% CI] (nM)	0.46 [0.4 – 0.5]	0.55 [0.4 – 0.7]	0.63 [0.6 – 0.7]	0.28 [0.18 – 0.5]	2.4 [1.65 – 3.4]	0.26 [0.22 – 2.9]	3.1 [1.97 – 4.8]
	pEC ₅₀ [95% CI]	9.34 [9.3 – 9.4]	9.26 [9.1 – 9.4]	9.20 [9.2 – 9.3]	9.55 [9.4 – 9.8]	8.62 [8.5 – 8.8]	9.59 [9.5 – 9.7]	8.51 [8.3 – 8.7]
	E _{max} [95% CI]	100 [98 – 102]	100 [96 – 103]	100 [98 – 101]	101 [96 – 107]	100 [95 – 105]	100 [98 – 102]	89 [82 – 98]
'4042	EC ₅₀ [95% CI] (nM)	0.48 [0.4 – 0.6]	0.56 [0.4 – 0.9]	0.64 [0.5 – 0.8]	0.43 [0.3 – 0.6]	2.1 [0.5 – 9.6]	0.37 [0.28 – 0.49]	3.6 [2.1 – 6.8]
	pEC ₅₀ [95% CI]	9.32 [9.2 – 9.4]	9.25 [9.0 – 9.5]	9.20 [9.1 – 9.3]	9.37 [9.3 – 9.4]	8.69 [8.0 – 9.3]	9.43 [9.3 – 9.6]	8.44 [8.2 – 8.7]
	E _{max} [95% CI]	102 [100 – 104]	101 [95 – 106]	103 [99 – 106]	97 [93 – 102]	64 [54 – 84]	105 [102 – 108]	72 [71 – 74]
'1350	EC ₅₀ [95% CI] (nM)	0.23 [0.18 – 0.3]	0.28 [0.19 – 0.4]	0.29 [0.24 – 0.34]	0.35 [0.22 – 0.53]	0.83 [0.23 – 2.7]	0.22 [0.18 – 0.27]	2.2 [1.1 – 4.4]
	pEC ₅₀ [95% CI]	9.63 [9.5 – 9.7]	9.54 [9.4 – 9.7]	9.54 [9.5 – 9.6]	9.46 [9.3 – 9.7]	9.08 [8.6 – 9.6]	9.66 [9.6 – 9.8]	8.66 [8.4 – 9.0]
	E _{max} [95% CI]	92 [90 – 95]	95 [90 – 99]	98 [94 – 98]	94 [88 – 100]	56 [48 – 67]	99 [96 – 101]	63 [60 – 72]
'8690	EC ₅₀ [95% CI] (nM)	19.1 [14.4 – 25.8]		18 [14 – 23]	33 [15 – 117]			
	pEC ₅₀ [95% CI]	7.72 [7.6 – 7.8]	^b --	7.8 [7.7 – 7.9]	7.5 [6.9 – 7.8]	--	--	^a N.D.
	E _{max} [95% CI]	82 [77 – 85]		83 [80 – 87]	98 [86 – 121]			
2-AG	EC ₅₀ [95% CI] (nM)	224 [147 – 353]	62 [42 – 918]	93 [65 – 135]	493 [15]	4470 [1618 – 398200]	32 [25 – 40]	1025 [774 – 1446]
	pEC ₅₀ [95% CI]	6.65 [6.5 – 6.8]	7.2 [7.0 – 7.4]	7.0 [6.9 – 7.2]	6.3 [7.8]	5.4 [5.1 – 5.8]	7.5 [7.4 – 7.6]	6.0 [5.8 – 6.1]
	E _{max} [95% CI]	122 [113 – 133]	112 [106 – 119]	114 [108 – 121]	205 [112]	183 [133 – 607]	112 [108 – 116]	200 [184 – 220]

^aN.D. = best fit values were not able to be determined due to compound inactivity or poor data quality

^b-- Not tested

Supplementary Table 6. Relative efficacy calculations for '4042 and '1350 versus CP-55,940.

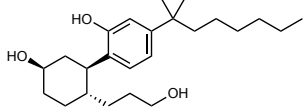
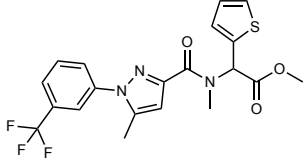
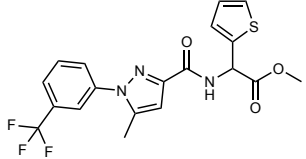
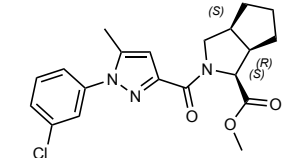
Target	Sensor	Compound	Mean log (E _{max} /EC ₅₀)	SEM log (E _{max} /EC ₅₀)	Mean Δlog (E _{max} /EC ₅₀)	SEM Δlog (E _{max} /EC ₅₀)	t-test to CP-55,940 ^a	RE ^b
hCB1	G _{i1}	CP-55,940	9.34	0.07	0.00	0.09		1.00
		'4042	9.33	0.14	-0.01	0.15	ns	0.97
		'1350	9.59	0.06	0.30	0.09	****	1.98
	G _{i2}	CP-55,940	9.24	0.13	0.00	0.18		1.00
		'4042	9.25	0.03	0.01	0.13	ns	1.01
		'1350	9.52	0.03	0.28	0.13	**	1.89
	G _{oB}	CP-55,940	9.19	0.07	0.00	0.10		1.00
		'4042	9.21	0.09	0.02	0.11	ns	1.04
		'1350	9.09	0.44	0.35	0.44	****	2.24
	G _z	CP-55,940	9.53	0.23	0.00	0.33		1.00
		'4042	9.32	0.19	-0.21	0.30	ns	0.62
		'1350	9.42	0.22	-0.11	0.32	ns	0.77
	G ₁₃	CP-55,940	8.63	0.23	0.00	0.32		1.00
		'4042	8.59	0.25	-0.04	0.33	ns	0.91
		'1350	8.85	0.05	0.22	0.23	*	1.64
	G ₁₅	CP-55,940	9.59	0.02	0.00	0.02		1.00
		'4042	9.46	0.12	-0.13	0.12	ns	0.74
		'1350	9.65	0.06	0.06	0.06	ns	1.14
β _{arr2+} GRK2	CP-55,940	8.28	0.25	0.00	0.35		1.00	
	'4042	8.19	0.03	-0.09	0.03	*	0.81	
	'1350	8.34	0.08	0.06	0.08	ns	1.16	
hCB2	G _{i1}	CP-55,940	8.86	0.12	0.00	0.17		1.00
		'4042	8.49	0.03	-0.20	0.13	ns	0.63
		'1350	8.32	0.10	-0.54	0.16	*	0.29
	G _{i2}	CP-55,940	8.97	0.00	0.00	0.00		1.00
		'4042	8.74	0.00	-0.23	0.00	c--	0.59
		'1350	8.57	0.00	-0.40	0.00	--	0.40
	G _{oB}	CP-55,940	8.76	0.09	0.00	0.13		1.00
		'4042	8.43	0.19	-0.33	0.21	n.s.	0.47
		'1350	8.36	0.01	-0.40	0.09	***	0.40
	G _z	CP-55,940	8.90	0.39	0.00	0.55		1.00
		'4042	8.48	0.51	-0.42	0.64	n.s.	0.38
		'1350	8.24	0.23	-0.65	0.45	n.s.	0.22
	β _{arr2+} GRK2	CP-55,940	7.83	0.13	0.00	0.18		1.00
		'4042	7.74	0.00	-0.09	0.13	***	0.82
		'1350	7.79	0.09	-0.04	0.16	n.s.	0.92

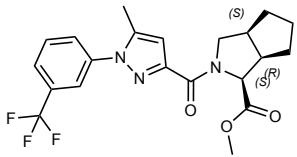
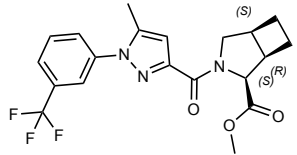
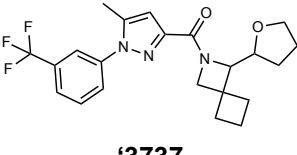
^aStatistical significance of all comparisons of compound activities (Mean Δlog (E_{max}/EC₅₀)) to CP-55,940 control by unpaired t-test. ns = not significant, * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001

^bRE, relative efficacy = 10^{Δlog (E_{max}/EC₅₀)}

^c--, not determined

Supplementary Table 7. Binding affinities and functional activities for select active analogs at CB2.

Compound	rCB2 binding EC ₅₀ [95% CI] (nM) pEC ₅₀ [95% CI] E _{max} [SEM]	hCB2 Cerep cAMP EC ₅₀ [95% CI] (nM) pEC ₅₀ [95% CI] E _{max} [95% CI]	hCB2 BRET2 + GoA EC ₅₀ [95% CI] (nM) pEC ₅₀ [95% CI] E _{max} [95% CI]	hCB2 Tango β-arrestin recruitment EC ₅₀ [95% CI] (nM) pEC ₅₀ [95% CI] E _{max} [95% CI]
 CP-55,940	--	0.082	13.1 [9.4 – 18.5] 7.9 [7.7 – 8.0] 99% [94 – 105]	22.9 [21.3 – 24.8] 7.6 [7.60 – 7.67] 100% [98 – 102]
 '4042	2.18 [1.7 – 2.8] 8.7 [8.6 – 8.8] 103% [8.8]	0.011 [0.002 – 0.02] 10.95 [10.7 – 11.6] 72% [77 – 84]	7.9 [1.6 – 40.6] 8.1 [7.4 – 8.8] 29% [22 – 36]	33.9 [21.7 – 61.5] 7.5 [7.2 – 7.7] 28% [26 – 32]
 '60154	b ₋₋₋	--	594 [125 – 2813] 6.2 [5.6 – 6.9] 31% [20 – 42]	3341 [2707 – 4371] 5.5 [5.4 – 5.6] 21% [19 – 23]
 10010	--	--	^a N.D.	556 [506 – 611] 6.3 [6.2 – 6.3] 18% [17 – 19]

 <p>'6439</p>	--	--	97.1 [12.6 – 749] 7.0 [6.13 – 67.9] 81% [34 – 128]	338 [282– 415] 6.5 [6.4 – 6.6] 14% [13 – 15]
 <p>'6448</p>	--	--	--	--
 <p>'3737</p>	--	4.3 [.0004 – 22.6] 8.4 [7.7 – 12.5] 45% [42 – 80%]	N.D.	1500 [1274 – 1805] 5.8 [5.7 – 5.9] 9% [8 – 9]

^aN.D. = best fit values were not determined due to compound inactivity or poor data quality

^b-- Not tested

Supplementary Table 8. Functional activities for select analogs and controls versus a variety of transducers and hCB2 in the bioSensAll platform.

Compound		hCB2 G _{i1}	hCB2 G _{i2}	hCB2 G _{oB}	hCB2 G _z	hCB2 Barr2 + GRK2
CP-55,940	EC ₅₀ [95% CI] (nM)	1.4 [1.1 – 1.7]	1.1 [0.9 – 1.3]	1.7 [1.5 – 2.1]	1.05 [0.6 – 1.9]	14.3 [11.6 – 17.9]
	pEC ₅₀ [95% CI]	8.87 [8.7 – 8.9]	8.97 [8.9 – 9.1]	8.76 [8.7 – 8.8]	8.98 [8.7 – 9.2]	7.84 [7.8 – 7.9]
	E _{max} [95% CI]	100 [97 – 103]	100 [97 – 104]	100 [98 – 103]	98 [89 – 109]	100 [95 – 104]
'4042	EC ₅₀ [95% CI] (nM)	2.7 [2.0 – 3.4]	1.3 [0.9 – 1.7]	2.5 [1.9 – 3.3]	1.36 [0.5 – 3.1]	5.5 [3.5 – 8.7]
	pEC ₅₀ [95% CI]	8.58 [8.5 – 8.7]	8.90 [8.8 – 9.0]	8.60 [8.5 – 8.7]	8.87 [8.5 – 9.3]	8.26 [8.1 – 8.5]
	E _{max} [95% CI]	82 [79 – 85]	70 [67 – 73]	61 [59 – 63]	56 [47 – 68]	33 [33 – 33]
'1350	EC ₅₀ [95% CI] (nM)	3.55 [2.98 – 4.2]	1.6 [1.4 – 1.9]	2.6 [2.15 – 3.1]	2.6 [1.6 – 3.9]	4.2 [2.8 – 6.3]
	pEC ₅₀ [95% CI]	8.45 [8.4 – 8.5]	8.79 [8.7 – 8.9]	8.58 [8.5 – 8.7]	8.58 [8.4 – 8.8]	8.38 [8.2 – 8.6]
	E _{max} [95% CI]	74 [72 – 77]	61 [59 – 63]	59 [57 – 61]	52 [46 – 60]	30 [28 – 33]
2-AG	EC ₅₀ [95% CI] (nM)	217 [186 – 256]	96 [70 – 129]	394 [349 – 447]	2123 [931 – 87070]	1854 [1110–4061]
	pEC ₅₀ [95% CI]	6.66 [6.6 – 6.7]	7.0 [6.9 – 7.2]	6.4 [6.3 – 6.5]	5.7 [4.1 – 6.0]	5.7 [5.4 – 6.0]
	E _{max} [95% CI]	105 [102 – 109]	78 [74 – 84]	101 [98 – 104]	96 [76 – 233]	118 [104 – 140]