SUPPLEMENTAL INFORMATION

Table S1. Transduction coefficients and relative activity for GAT100, Org27569, and PSNCBAM-1.

Cell model	Orthosteric ligand	Allosteric ligand	β-arrestin1		pPLCβ3		pERK1/2		cAMP	
			LogR	ΔLogR	LogR	ΔLogR	LogR	ΔLogR	LogR	ΔLogR
HEK293A	2-AG	GAT100	7.9 ± 0.2	0.0 ± 0.2	7.2 ± 0.1	0.0 ± 0.1	7.1 ± 0.1	0.0 ± 0.1	6.8 ± 0.1	0.0 ± 0.1
		Org27569	4.0 ± 0.4	-3.9 ± 0.4	4.3 ± 0.9	-2.9 ± 0.9	4.3 ± 0.1	2.2 ± 0.1	3.5 ± 0.8	-3.3 ± 0.8
		PSNCBAM-1	4.9 ± 0.4	-3.0 ± 0.4	5.5 ± 0.7	-1.7 ± 0.7	4.8 ± 0.2	2.7 ± 0.2	4.0 ± 0.5	-2.8 ± 0.5
	AEA	GAT100	7.3 ± 0.2	-0.6 ± 0.2	7.8 ± 0.8	0.6 ± 0.8	7.1 ± 0.1	0.0 ± 0.1	6.3 ± 0.1	-0.5 ± 0.1
		Org27569	6.1 ± 0.4	-1.8 ± 0.4	7.0 ± 0.6	-0.2 ± 0.6	4.1 ± 0.8	-3.0 ± 0.8	6.2 ± 0.6	-0.6 ± 0.6
		PSNCBAM-1	6.2 ± 0.2	-1.7 ± 0.8	7.2 ± 0.2	0.0 ± 0.2	6.6 ± 0.2	-0.5 ± 0.2	4.4 ± 0.7	-2.4 ± 0.7
	CP55,940	GAT100	7.6 ± 0.1	-0.3 ± 0.1	7.1 ± 0.1	-0.1 ± 0.1	7.0 ± 0.1	-1.0 ± 0.1	6.4 ± 0.1	-0.4 ± 0.1
		Org27569	7.7 ± 0.5	-0.2 ± 0.5	7.3 ± 0.1	0.1 ± 0.1	6.3 ± 0.4	-0.8 ± 0.4	6.6 ± 0.6	-0.2 ± 0.6
		PSNCBAM-1	6.7 ± 0.7	-1.2 ± 0.7	7.5 ± 0.5	0.3 ± 0.5	6.6 ± 0.6	-0.5 ± 0.6	6.7 ± 0.6	-0.1 ± 0.6
Neuro2a	2-AG	GAT100	6.9 ± 0.3	0.0 ± 0.3	7.3 ± 0.1	0.0 ± 0.1	7.1 ± 0.1	0.0 ± 0.1	-	-
		Org27569	6.8 ± 0.6	-0.1 ± 0.6	7.4 ± 0.2	0.1 ± 0.2	6.5 ± 0.2	-0.6 ± 0.2	-	-
		PSNCBAM-1	6.9 ± 0.9	0.0 ± 0.9	6.3 ± 0.5	-1.0 ± 0.5	6.6 ± 0.4	-0.5 ± 0.4	-	-
	AEA	GAT100	7.9 ± 0.2	1.0 ± 0.2	7.1 ± 0.1	-0.2 ± 0.1	7.2 ± 0.1	0.1 ± 0.1	-	-
		Org27569	6.8 ± 0.4	-0.1 ± 0.4	6.6 ± 0.2	-0.7 ± 0.2	6.8 ± 0.2	-0.3 ± 0.2	-	-
		PSNCBAM-1	6.7 ± 0.3	-0.2 ± 0.3	6.4 ± 0.7	-0.9 ± 0.7	7.0 ± 0.4	-1.0 ± 0.4	-	-
	CP55,940	GAT100	8.0 ± 0.1	1.1 ± 0.1	7.9 ± 0.2	0.6 ± 0.2	7.2 ± 0.1	0.1 ± 0.1	-	-
		Org27569	7.2 ± 0.8	0.3 ± 0.8	7.0 ± 0.4	-0.3 ± 0.4	6.9 ± 0.9	-0.2 ± 0.9	-	-
		PSNCBAM-1	8.0 ± 0.2	1.1 ± 0.2	8.9 ± 0.2	1.6 ± 0.2	8.3 ± 0.9	1.2 ± 0.9	-	-

Table S1 Continued. Transduction coefficients and relative activity for GAT100, Org27569, and PSNCBAM-1.

Cell model	Orthosteric ligand	Allosteric ligand	β-arrestin1		pPLCβ3		pERK1/2		cAMP	
			Log$R$	ΔLog$R$	Log$R$	ΔLog$R$	Log$R$	ΔLog$R$	Log$R$	ΔLog$R$
ST$Hdh^{Q7/Q7}$	2-AG	GAT100	8.0 ± 1.0	0.0 ± 1.0	7.3 ± 0.1	0.0 ± 0.1	7.2 ± 0.1	0.0 ± 0.1	-	-
		Org27569	7.9 ± 0.6	-0.1 ± 0.6	8.5 ± 0.7	0.5 ± 0.7	7.4 ± 0.6	0.2 ± 0.6	-	-
		PSNCBAM-1	7.8 ± 0.2	-0.2 ± 0.2	7.2 ± 0.2	-0.8 ± 0.2	7.0 ± 0.3	-0.2 ± 0.3	-	-
	AEA	GAT100	8.0 ± 0.7	0.0 ± 0.7	7.1 ± 0.1	-0.9 ± 0.1	7.1 ± 0.1	-0.1 ± 0.1	-	-
		Org27569	7.2 ± 0.2	-0.8 ± 1.1	7.9 ± 0.6	-0.1 ± 0.6	6.3 ± 0.9	-0.9 ± 0.9	-	-
		PSNCBAM-1	7.8 ± 0.6	-0.2 ± 0.6	7.3 ± 0.5	-0.7 ± 0.5	6.8 ± 0.2	-0.4 ± 0.2	-	-
	CP55,940	GAT100	7.9 ± 0.1	-0.1 ± 0.1	7.2 ± 0.1	-0.8 ± 0.1	7.3 ± 0.1	1.0 ± 0.1	-	-
		Org27569	7.3 ± 0.3	-0.7 ± 0.3	7.4 ± 0.6	-0.6 ± 0.6	7.0 ± 0.3	-0.2 ± 0.3	-	-
		PSNCBAM-1	7.7 ± 0.7	-0.3 ± 0.7	7.3 ± 0.2	-0.7 ± 0.2	6.8 ± 0.3	-0.4 ± 0.3	-	-

Log$R$ and ΔLog$R$ determined using operational model analysis as described in Materials and Methods (eq. 1 - 3). Calculations are from data presented in figures 3 (β-arrestin1), 5 (pPLCβ3), 7 (pERK1/2), and 9 (cAMP) in cells treated with 500 nM orthosteric agonist + 1 nM - 10 µM allosteric modulator. ΔLog$R$ for GAT100 compared with Org27569 and PSNCBAM-1. Data are mean ± SEM from at least four independent experiments. N = 4.

Table S2. Ligand/Receptor Interaction Energies for GAT100

Residues	Electrostatic (kcal/mol)	Van der Waals (kcal/mol)	Total Energy (kcal/mol)
CP55,940	0.26	-2.34	-2.08
Q1.32	0.05	-0.32	-0.27
D(184)	-0.80	-2.06	-2.86
S(185)	0.00	-0.11	-0.11
F3.25	-0.23	-1.94	-2.17
L3.29	-0.04	-0.21	-0.25
F(268)	-0.40	-5.03	**-5.44**
P(269)	-1.11	-3.77	**-4.88**
H(270)	0.14	-0.25	-0.11
I(271)	0.03	-0.20	-0.18
P6.50	0.26	-0.33	-0.07
L6.51	0.65	-3.26	-2.61
L6.52	-0.08	-0.18	-0.26
I6.54	-0.39	-4.22	**-4.61**
M6.55	0.68	-1.68	-0.99
D6.58	-2.40	-2.49	**-4.89**
M(371)	0.01	-0.56	-0.55
K(373)	0.66	-0.22	0.44
K(376)	0.70	-1.23	-0.52
V7.34	-0.47	-1.22	-1.69
F7.35	-0.55	-6.53	**-7.09**
	-3.1	**-38.1**	**-41.2**

**Interaction energies equal to -3.0 kcal/mol or better are shown in bold

Table S3. Ligand/Receptor Interaction Energies for CP55,940**

Residues	Electrostatic (kcal/mol)	Van der Waals (kcal/mol)	Total Energy (kcal/mol)
GAT100	0.26	-2.34	-2.08
Q1.32	-2.73	-2.16	**-4.89**
L1.33	-0.14	-0.23	-0.37
I1.35	0.00	-0.55	-0.55
A1.36	1.06	-2.00	-0.93
S1.39	0.07	-2.14	-2.07
L1.30	-0.02	-1.62	-1.64
G1.43	0.09	-0.28	-0.19
I2.56	0.17	-2.18	-2.01
F2.57	0.09	-3.13	**-3.04**
S2.60	0.18	-1.59	-1.40
D2.63	1.27	-0.30	0.96
F2.64	-0.19	-3.36	**-3.55**
F3.25	-0.18	-4.53	**-4.71**
K3.28	-13.05	-0.36	**-13.40**
L3.29	-0.12	-0.27	-0.39
G3.31	-0.07	-0.17	-0.24
V3.32	-0.05	-2.25	-2.30
S3.35	-0.08	-1.01	-1.10
F3.36	0.03	-1.02	-1.00
K(373)	0.36	-0.51	-0.15
T7.33	-0.02	-0.25	-0.27
F7.35	-0.05	-1.61	-1.66
A7.36	-0.77	-2.38	**-3.15**
S7.39	-0.91	-2.20	**-3.10**
M7.40	0.00	-0.26	-0.26
C7.42	0.03	-1.90	-1.87
L7.43	0.03	-4.51	**-4.48**
L7.44	0.00	-0.17	-0.17
N7.45	0.03	-0.84	-0.81
S7.46	0.05	-1.02	-0.97
T7.47	0.00	-0.04	-0.04
	-14.6	**-47.2**	**-61.8**

**Interaction energies equal to -3.0 kcal/mol or better are shown in bold

Figure S1. The RMSD of the transmembrane residues of CB1 relative to the initial model in the equilibration run. The CB1 receptor conformation at 70ns was extracted and used for ligand docking studies.

RMSD of CB1R TM helices in equilibration run

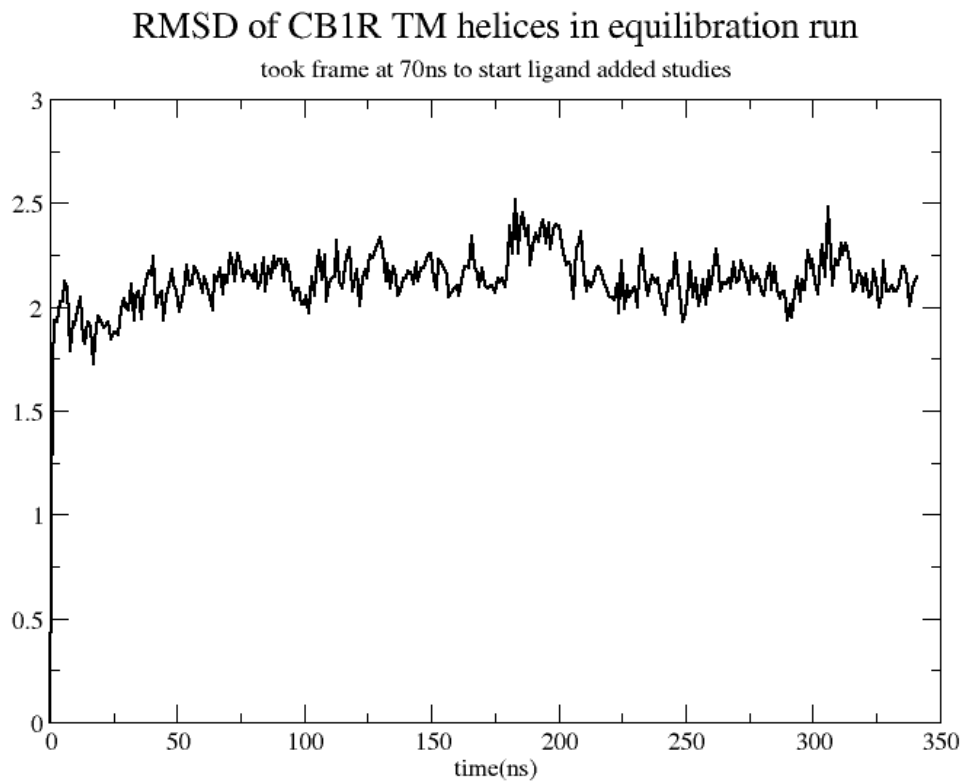took frame at 70ns to start ligand added studies

Figure S2. An overlay is presented here of the ORG27569 global minimum energy conformation (cyan) and the docked conformation of GAT100 (purple) illustrated in Figure 2.