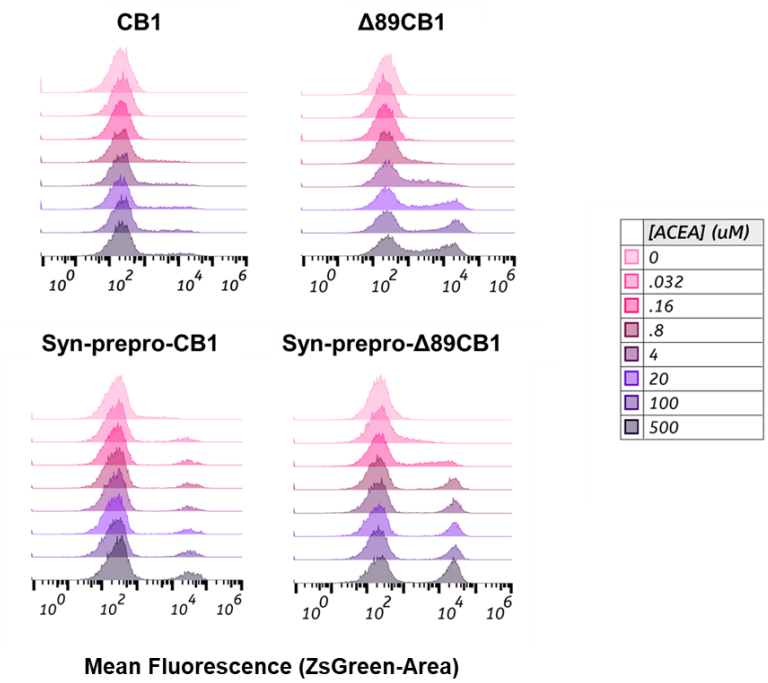
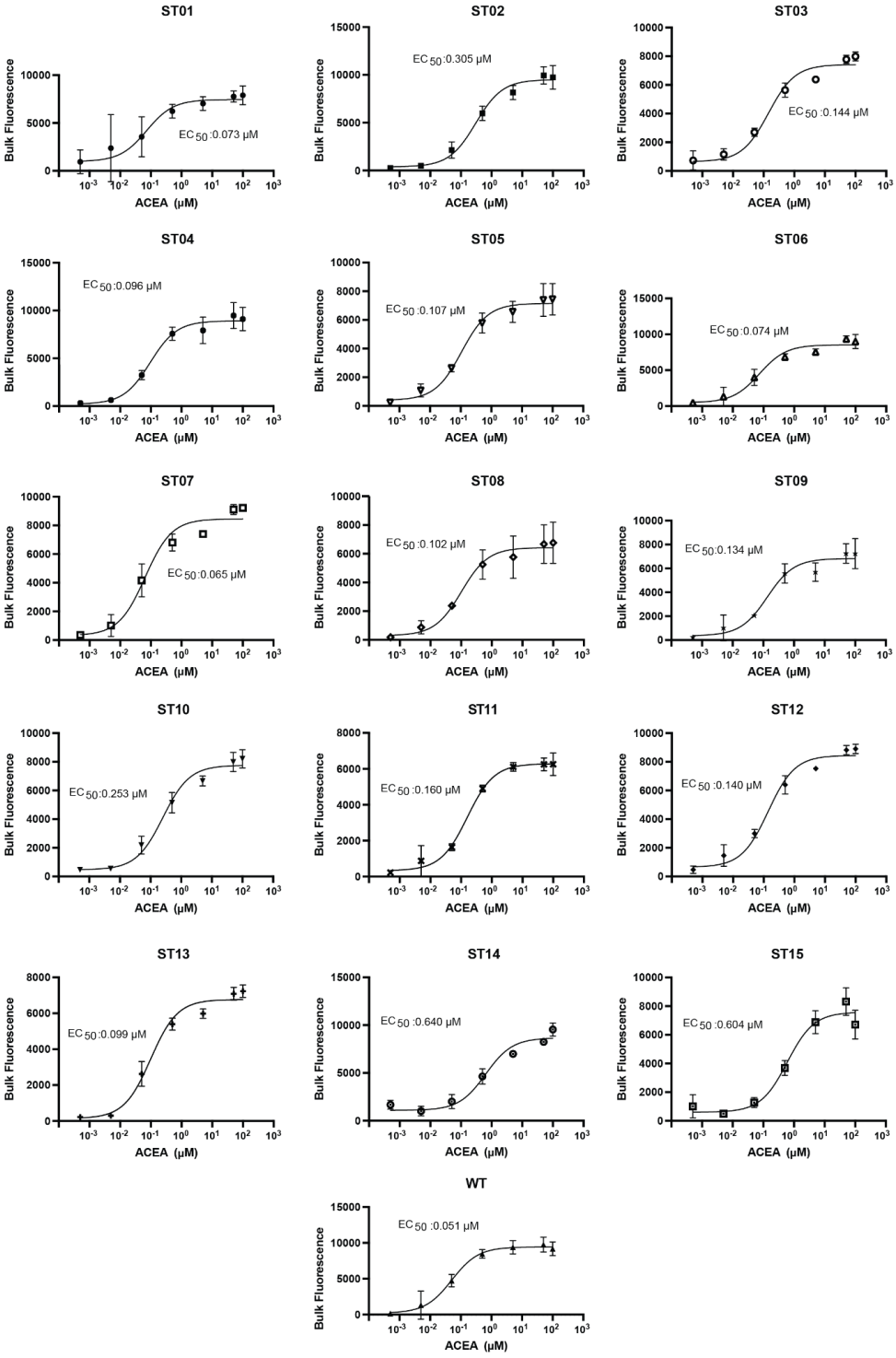
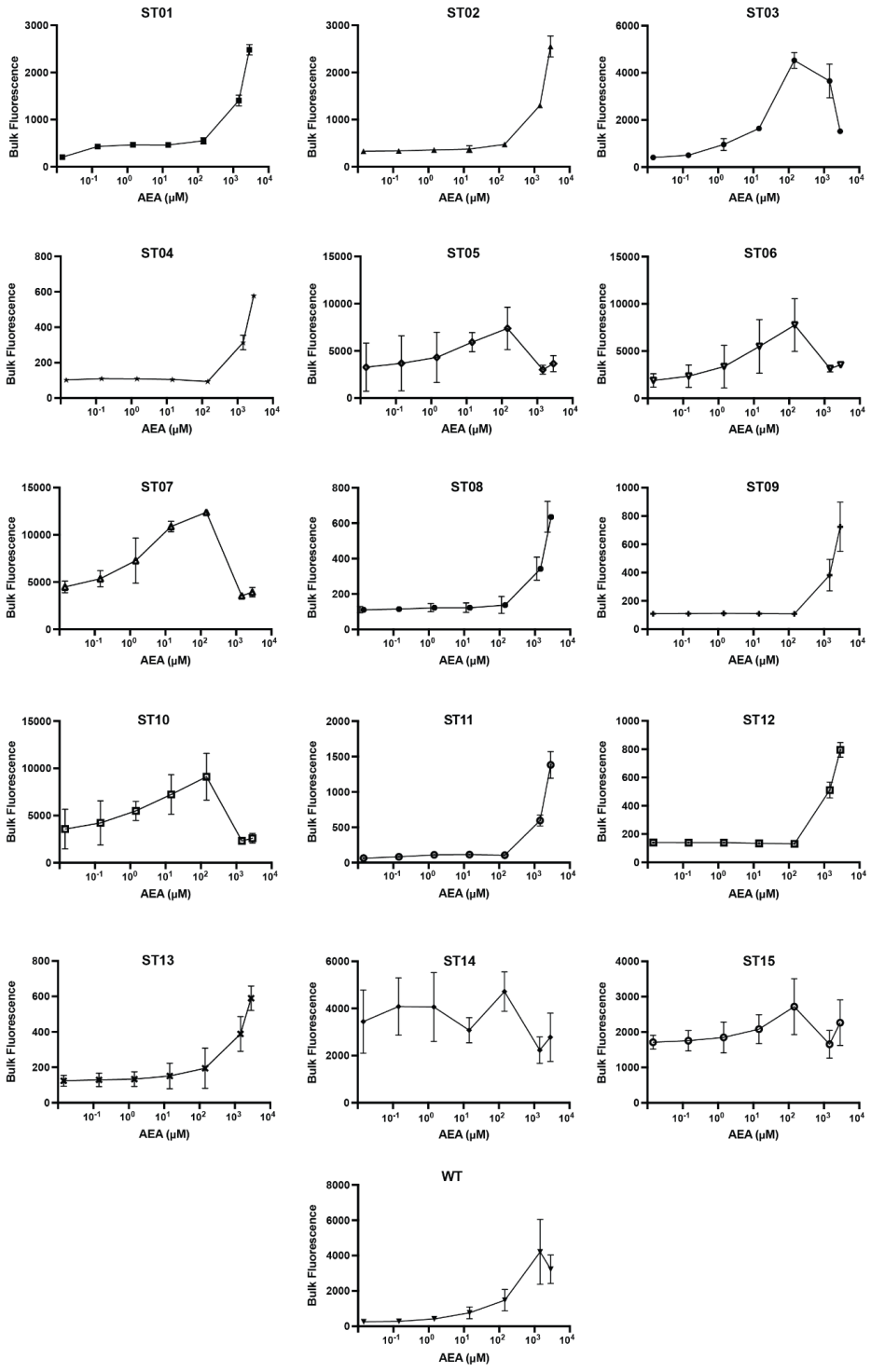


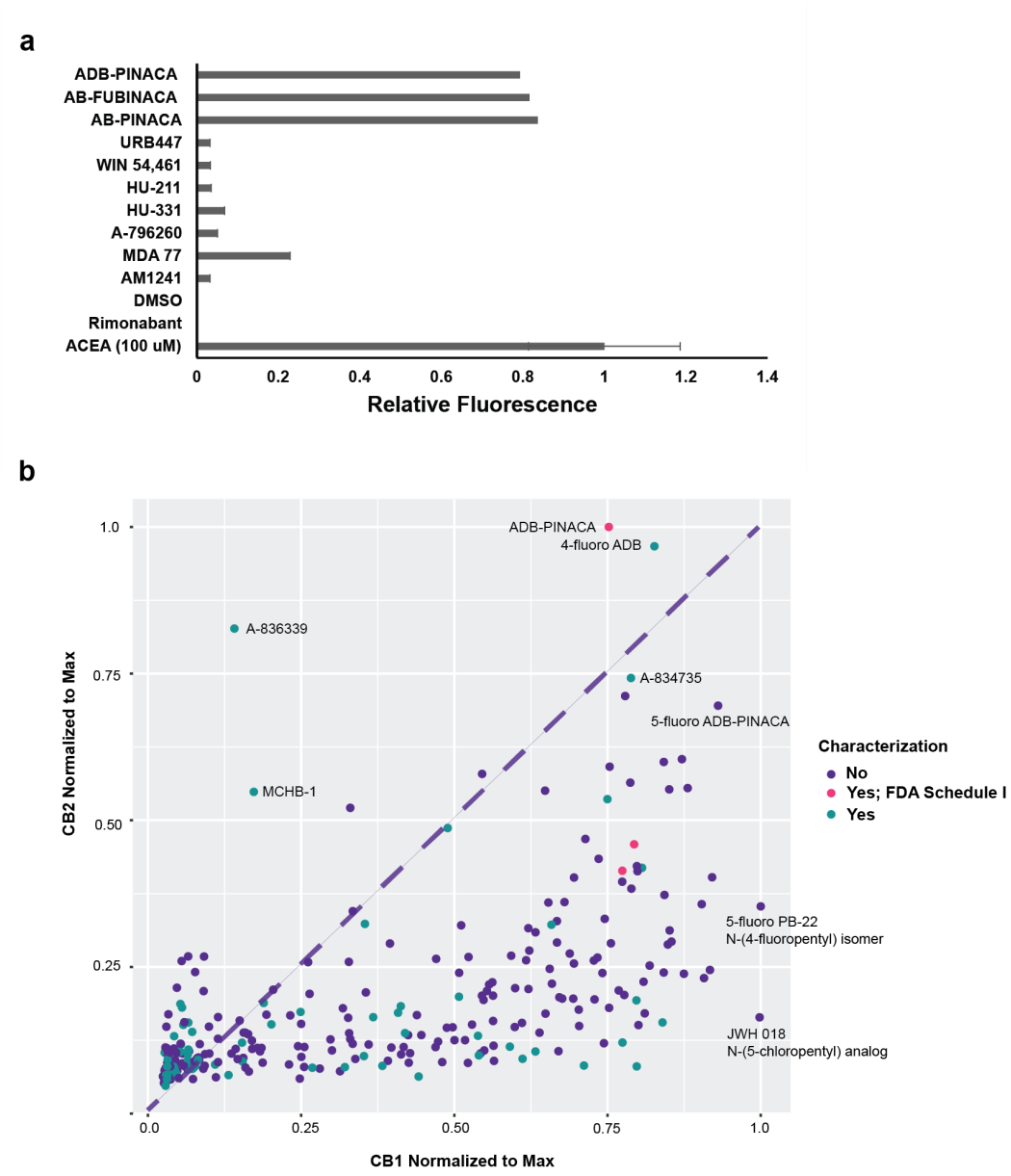
Supplementary Figure 1. Fluorescence population shifts with CB1R variants. Dose response of ACEA with four versions of the CB1 receptor: with and without the 89-residue amino terminal truncation, and with and without the synthetic pre-pro signal. Fluorescence was measured via cytometry, in which a population of singlets were binned for fluorescent response and total population ZsGreen signal (area) is shown.



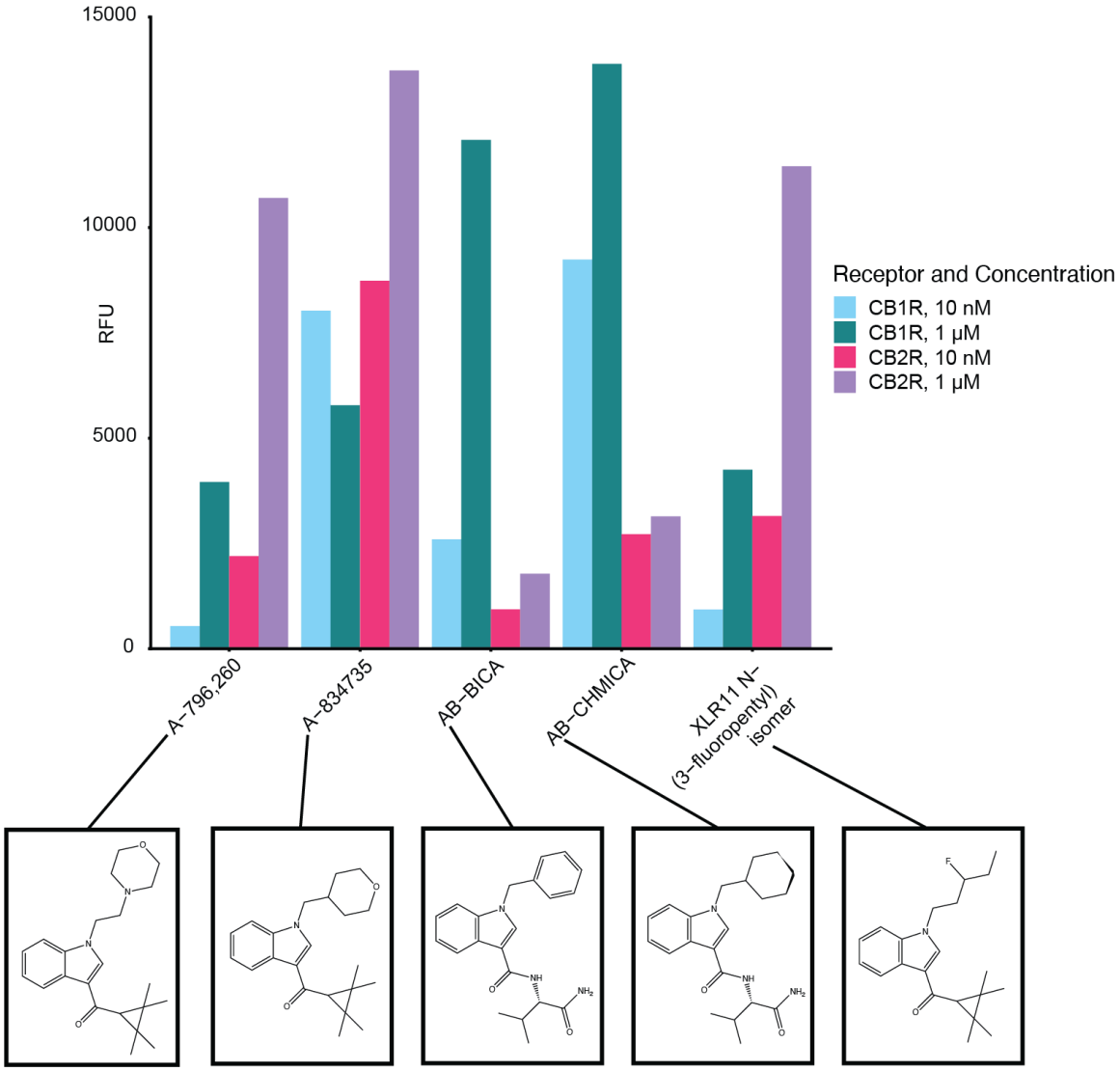
Supplementary Figure 2. CB1R dose responses with sterol-modified strains. For all experiments, *n*=3. Nonlinear regression was performed with a 4-parameter logistic equation.



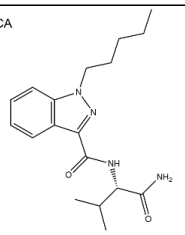
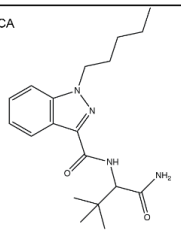
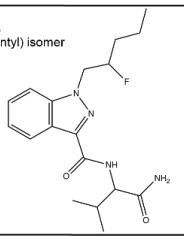
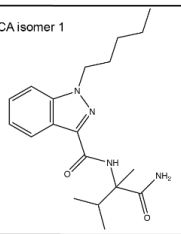
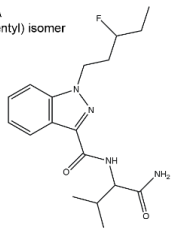
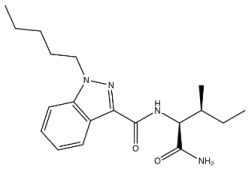
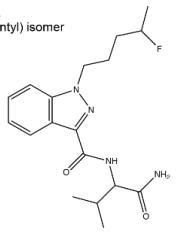
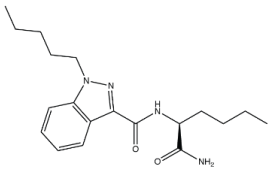
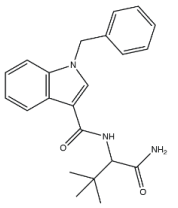
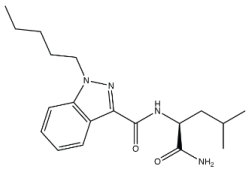
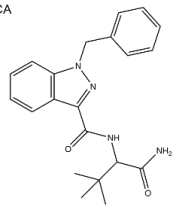
Supplementary Figure 3. CB2R doses responses with sterol-modified strains. For all experiments, *n*=3; data points for consecutive doses are connected with lines as a guide to the eye.



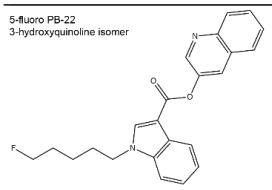
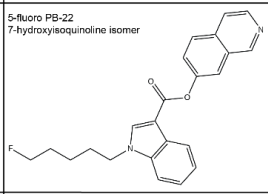
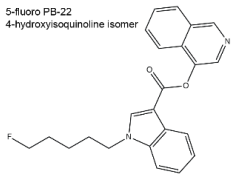
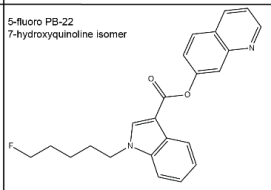
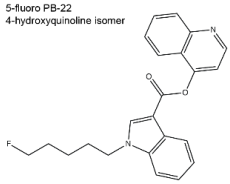
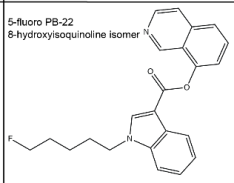
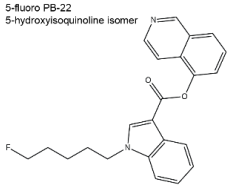
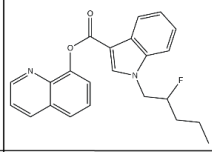
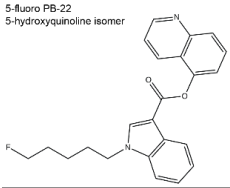
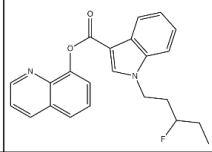
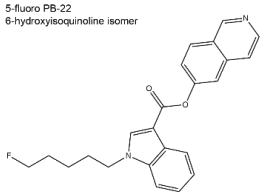
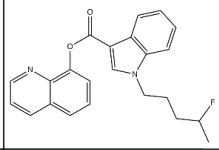
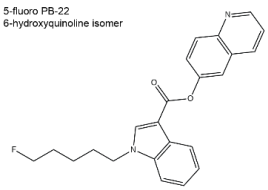
Supplementary Figure 4. Relative signaling of synthetic cannabinoid compounds at cannabinoid receptors. (A) Signaling with control compounds at CB1R. Known agonists ADB-PINACA, AB-FUBINACA, and AB-PINACA show strong signaling at 10 nM compared to a positive control (100 µM ACEA). All other compounds (10 nM) are not predicted to activate CB1R. DMSO vehicle was also tested. (B) Synthetic cannabinoid compounds screened against CB1R and CB2R at 10 nM, normalized to the maximum signal for each receptor at this concentration. Large distances from the right and left of the plotted line connote rough bias towards CB1R and CB2R, respectively. Compounds are labeled based on if they, to our knowledge, have been characterized biochemically against one or both cannabinoid receptors.



Supplementary Figure 5. Structure and signaling of isolated synthetic cannabinoid compounds that show biased activity. (A) Select compounds from the synthetic cannabinoid screen at both CB1R and CB2R. For all experiments, *n*=1.

Compound	Normalized Activity at 1 μM	Compound	Normalized Activity at 1 μM
AB-PINACA 	CB1R: 0.550 CB2R: 0.533	ADB-PINACA 	CB1R: 0.590 CB2R: 0.700
AB-PINACA N-(2-fluropentyl) isomer 	CB1R: 0.632 CB2R: 0.340	ADB-PINACA isomer 1 	CB1R: 0.810 CB2R: 0.303
AB-PINACA N-(3-fluropentyl) isomer 	CB1R: 0.700 CB2R: 0.310	ADB-PINACA isomer 2 	CB1R: 0.753 CB2R: 0.568
AB-PINACA N-(4-fluropentyl) isomer 	CB1R: 0.680	ADB-PINACA isomer 3 	CB1R: 0.735 CB2R: 0.460
ADB-BICA 	CB1R: 0.842 CB2R: 0.255	ADB-PINACA isomer 4 	CB1R: 0.671 CB2R: 0.561
ADB-BINACA 	CB1R: 0.916 CB2R: 0.676		

Supplementary Table 1. Structure and activity of PINACA compounds.

5-fluoro PB-22 isomer	Normalized Activity at 1 μM	5-fluoro PB-22 isomer	Normalized Activity at 1 μM
5-fluoro PB-22 3-hydroxyquinoline isomer 	CB1R: 0.530 CB2R: 0.148	5-fluoro PB-22 7-hydroxyisoquinoline isomer 	CB1R: 0.530 CB2R: 0.339
5-fluoro PB-22 4-hydroxyisoquinoline isomer 	CB1R: 0.570 CB2R: 0.786	5-fluoro PB-22 7-hydroxyquinoline isomer 	CB1R: 0.550 CB2R: 0.199
5-fluoro PB-22 4-hydroxyquinoline isomer 	CB1R: 0.629 CB2R: 0.247	5-fluoro PB-22 8-hydroxyisoquinoline isomer 	CB1R: 0.560 CB2R: 0.771
5-fluoro PB-22 5-hydroxyisoquinoline isomer 	CB1R: 0.540 CB2R: 0.906	5-fluoro PB-22 N-(2-fluoropentyl) isomer 	CB1R: 0.531 CB2R: 0.582
5-fluoro PB-22 5-hydroxyquinoline isomer 	CB1R: 0.604 CB2R: 0.496	5-fluoro PB-22 N-(3-fluoropentyl) isomer 	CB1R: 0.530 CB2R: 0.532
5-fluoro PB-22 6-hydroxyisoquinoline isomer 	CB1R: 0.525 CB2R: 0.331	5-fluoro PB-22 N-(4-fluoropentyl) isomer 	CB1R: 0.560 CB2R: 0.636
5-fluoro PB-22 6-hydroxyquinoline isomer 	CB1R: 0.500 CB2R: 0.141		

Supplementary Table 2. Structure and activity of 5-fluoro PB-22 compounds.