## Molecular Pharmacology

Probing the CB1 cannabinoid receptor binding pocket with AM6538, a high-affinity irreversible antagonist.

Robert B. Laprairie ${ }^{1}$, Kiran Vemuri ${ }^{2}$, Edward L Stahl ${ }^{1}$, Anisha Korde ${ }^{2}$, Jo-Hao Ho ${ }^{1}$, Travis W. Grim ${ }^{1}$, Tian Hua ${ }^{3}$, Yiran $\mathrm{Wu}^{3}$, Raymond C. Stevens ${ }^{4}$, Zhi-Jie Liu ${ }^{3}$, Alexandros Makriyannis ${ }^{2}$, Laura M. Bohn ${ }^{1 *}$
${ }^{1}$ Departments of Molecular Medicine and Neuroscience, The Scripps Research Institute, Jupiter, FL 33458, USA; ${ }^{2}$ Center for Drug Discovery and Departments of Chemistry and Chemical Biology and Pharmaceutical Sciences, Northeastern University, Boston, MA 02115, USA; ${ }^{3}$ iHuman Institute, ShanghaiTech University, Shanghai 201210, China; ${ }^{4}$ Departments of Biological Sciences and Chemistry, Bridge Institute, Michelson Center for Convergent Bioscience, University of Southern California, Los Angeles, California, 90089.

## Supplemental Data



Figure S1. Modulation of $\mathrm{hCB}_{1}$-depedent signaling. (A) 3xHA-hCB ${ }_{1} \mathrm{CHO}$ cells were treated with forskolin and vehicle or $0.03 \mathrm{nM}-10 \mu \mathrm{M} \mathrm{CP55,940}, \mathrm{THC} ,\mathrm{or} \mathrm{JWH-018} \mathrm{for} 30 \mathrm{~min}$ and inhibition of forskolin-stimulated cAMP accumulation was quantified. Data were normalized by setting vehicle treatment as $0 \%$ and CP55,940 $E_{\max }$ as $100 \%$ within assays performed on the same plate, mean with SD plotted. Measures derived from 3 parameter non-linear regression (Prism 6.0) for each individual assay were averaged (with $95 \%$ confidence intervals): CP55,940 ( $\mathrm{n}=11$ ): $\mathrm{EC}_{50} 4.8(1.1-8.5) \mathrm{nM}, E_{\max } 100 \%$; JWH-018 (n=5): $\mathrm{EC}_{50} 1.4(0.04-3.2) \mathrm{nM}, E_{\max } 91(80-101) \%$; THC (n=6): $\mathrm{EC}_{50} 51(6.7-110) \mathrm{nM}, E_{\max } 54(42-67) \%$. (B) hCB ${ }_{1}$ CHO PathHunter (DiscoveRx) cells were treated with vehicle or agonist for 90 min and $\beta$ arrestin2 recruitment was quantified following substrate incubation. Data were normalized by setting vehicle treatment as $0 \%$ and CP55,940 $E_{\max }$ as $100 \%$ within assays performed on the same plate. Data are presented as the mean with SD.. Measures derived from 3 parameter non-linear regression (Prism 6.0) for each individual assay were averaged (with $95 \%$ confidence interval) to provide: CP55,940 ( $\mathrm{n}=12$ ): $\mathrm{EC}_{50} 18$ (11-26) nM, $E_{\max } 100 \%$; JWH-018(n=5): $\mathrm{EC}_{50} 1.4(0.71-2.1) \mathrm{nM} ; E_{\max } 90(79-101) \%$, (n=7): EC $5070(8.9-$ $150) \mathrm{nM}, E_{\max } 48(43-54) \%$. (C) $\Delta \Delta \mathrm{LogR}$ values estimated using the operational model and displayed as mean with $95 \%$ confidence interval from the data in A and B.
Data related to figure 3: (D) 3xHA-hCB ${ }_{1}$ CHO cells were treated with vehicle ( $1 \%$ DMSO in PBS) or $1 \mu \mathrm{M}$ antagonist for 6 h with 5 washes, followed by forskolin stimulation ( $20 \mu \mathrm{M}, 30 \mathrm{~min}$ ). The 6 hour antagonist treatment did not affect cAMP accumulation compared to vehicle without antagonist. Data are mean with SD; $\mathrm{n}=$ 6 (basal and vehicle), 3 (SR141716A, AM6538, AM4112, AM6542).


Figure S2. Inhibition of forskolin-stimulated cAMP accumulation: antagonist competition at of $\mathrm{hCB}_{1}$ by SR141716A, AM6538, AM4112, and AM6542. 3xHA-hCB ${ }_{1}$ CHO cells were co-treated with $20 \mu \mathrm{M}$ forskolin and vehicle or $0.03 \mathrm{nM}-10 \mu \mathrm{M}$ CP55,940, THC, or JHW-018 $\pm$ SR141716A, AM6538, AM4112, or AM6542 for 30 min and inhibition of forskolin-stimulated cAMP accumulation was quantified. Figures A, B, E, and F were published in the supplemental information from Hua et al., 2016 and are shown here for comparison. Concentrationresponse data were globally fit to a competitive nonlinear regression model (eq. 1) in Prism 6.0. Data were normalized by setting the vehicle treatment as $0 \%$ and maximum stimulation obtained with CP55,940 as $100 \%$; mean with SD; $\mathrm{n}=5$ (THC with AM4112, THC with AM6542), $\mathrm{n}=3$ for all other treatments; experiments performed in duplicate. $\mathrm{pA}_{2}$ parameters are presented in table 1. $\mathrm{pA}_{2}$ values for inhibition of forskolin-stimulated cAMP accumulation with THC excluded 1 and $10 \mu \mathrm{M}$ AM4112 and AM6542 in global nonlinear regression analysis.


Figure S3. $\beta$ arrestin2 recruitment: antagonist competition at of $\mathrm{hCB}_{1}$ by SR141716A, AM6538, AM4112, and AM6542. $\mathrm{hCB}_{1}$ CHO DiscoveRx cells were treated with $0.03 \mathrm{nM}-10 \mu \mathrm{M}$ CP55,940, THC, or JHW-018 $\pm$ SR141716A, AM6538, AM4112, or AM6542 for 90 min and ßarrestin2 recruitment was quantified. Concentrationresponse data were globally fit to a competitive nonlinear regression model (eq. 1) in Prism 6.0. Figures A, B, E, and F were published in the supplemental information from Hua et al., 2016 and are shown here for comparison. Data were normalized to the vehicle ( $0 \%$ ) and maximum stimulation obtained with CP55,940 ( $100 \%$ ); mean with SD; $\mathrm{n}=4$ (JWH-018 with AM6542); $\mathrm{n}=3$ for all other treatments; experiments performed in duplicate. $\mathrm{pA}_{2}$ parameters are presented in table 1.

Hua T, Vemuri K, Pu M, Qu L, Han GW, Wu Y, Zhao S, Shui W, Li S, Korde A, Laprairie RB, Stahl E L, Ho J-H, Zvonok N, Zhou H, Kufareva I, Wu B, Zhao Q, Hanson MA, Bohn LM, Makriyannis A, Stevens RC, and Liu Z-J (2016) Crystal structure of the human cannabinoid receptor CB1. Cell 167: 750-762.

