Supporting Information

Gertsch *et al.* 10.1073/pnas.0803601105

SI Materials and Methods

Drugs and Antibodies. The cannabinoid receptor ligands AM630, AM251, WIN55,212–2, JWH133 (CB_2 K_i = 3.4 nM vs. CB_1 K_i = 677 nM), 2-arachidonoylglycerol (2-AG), and CP55,940 were obtained from Tocris Cookson. The CB_2 receptor selective antagonist SR 144528 (CB_2 K_i = 0.6 nM vs. CB_1 K_i = 437 nM) was obtained as a gift from Sanofi-Synthélabo Recherche. Fluo 3-AM, Pluronic F-127, and the monoclonal anti-rabbit FITC antibody were purchased from Sigma. The CB_2 rabbit polyclonal antibody (ab3561) was obtained from Abcam and was tested for differential binding to immune cells. JNK1/2 antibody phospho and non-phospho pair (ab5254) and Erk1/2 antibodies (ab50011 and ab17942) were obtained from Abcam. The radioligand [^{3}H]-CP55,940 was obtained from Perkin–Elmer, Switzerland. LPS (*Escherichia coli*, serotype 055:B5), was obtained from Fluka. Forskolin was purchased from Alexis Biochemicals. Cannabinol was obtained from Lipomed. The *Cannabis sativa* (low THC industrial hemp) flower essential oils were either purchased or provided as a gift from Profumo (oil No 1), Essentialaura (oil No 2), Olison (oil No. 3), Belladonna Magickal Arts (oil No 4), and Norfolk Essential Oils (oil No 5). (*E*)-β-caryophyllene was purified by flash column chromatography on silica gel 60/0.015–0.04 mm (Macherey-Nagel), using *n*-hexan : ethylacetate (50:1) from essential oil mixtures. The isolated compound was identified by NMR experiments and compared with a commercial reference. The purity was checked by TLC, GC and ^{1}H NMR. (*E*)-β-caryophyllene (98%), (*Z*)-β-caryophyllene (98%), α-humulene (98%), and β-caryophyllene oxide (99%), myrcene (95%), (*R*)-(+)-limonene (99%), linalool (97%), and α-pinene (mixture of isomers, 97%) were purchased from Fluka.

Cell Cultures. Human promyelocytic leukemia CB_2-expressing (positive) HL60 cells (obtained from the American Type Culture Collection; catalog no. CCL-240) were grown in Iscove's modified Dulbecco's medium with 4 mM L-glutamine and 1.5 g/liter sodium bicarbonate (ATCC) supplemented with 20% FBS, 1 g/ml fungizone (amphotericin B), 100 units/ml penicillin, and 100 g/ml streptomycin. The CB_2 receptor negative HL60 clone (lacking surface expression) was obtained from V. Dirsch (University of Vienna, Vienna, Austria) and characterized in our laboratory. It was grown in RPMI medium 1640 (Invitrogen) supplemented with 10% FBS, 1 g/ml fungizone (amphotericin B), 100 units/ml penicillin, 100 g/ml streptomycin, and 2 mM L-glutamine (all from Invitrogen). The human CB_2-expressing CHO-K1 cells were grown in the same medium as the CB_2-negative HL60 cells but supplemented with 400 μg/ml G418 (10131–027; Invitrogen). All cells were grown in a humidified incubator at 37°C and 5% CO_2.

Human Peripheral Whole Blood Cultures. Ten milliliters of peripheral whole blood was obtained from healthy volunteers in the early afternoon by authorized medical staff. The blood was collected into heparinized tubes (BD Vacutainer Systems) and gently shaken for 1 min. Two-hundred-microliter portions were then immediately aliquoted into a 96-well plate under sterile conditions. Each experiment was carried out in triplicate. Test compounds and vehicle controls were added. After 45 min of incubation in a humidified incubator at 37°C and 5% CO_2, stimulation of cells was initiated by addition of either 313 ng/ml LPS to the blood culture under gentle stirring. Volumes of stimulatory mixtures were set to 2 μl. Again, vehicle controls (ethanol, dimethyl sulfoxide, or H_2O) of the same dilutions were included. The plate was then incubated at 37°C and 5% CO_2 for 18 h. After incubation the plates were centrifuged at room temperature for 5 min at 450 rpm in an MSE Mistral 3000i centrifuge to facilitate plasma collection. For each assay at least three experiments were performed in triplicate with blood from at least three different donors (total of at least nine measurements).

FACS Analysis of CB_2 Expression. HL60 or CB_2-transfected CHO-K1 cells (10^6) were washed in PBS (Invitrogen) supplemented with 0.1% NaN_3 and 2% FBS and incubated (1:100) with the rabbit polyclonal CB_2-specific antibody (3561) for 45 min on ice in the dark. After two washing steps, the cells were incubated (1:32) with a monoclonal anti-rabbit fluorescein isothiocyanate-labeled antibody for 45 min on ice in the dark. The cells were washed twice and resuspended in 500 μl of PBS with 0.1% NaN_3 and 1% *p*-formaldehyde before analysis on a FACScan cytometer (BD Biosciences). Measurements were carried out with the CellQuest software, and relative expressions were compared with secondary antibody controls.

Radioligand Displacement Assays on CB_1 and CB_2 Receptors. For the CB_1 receptor, binding experiments were performed in the presence of 0.39 nM the radioligand [^{3}H]CP-55,940 at 30°C in siliconized glass vials together with 7.16 μg of membrane recombinantly overexpressing CB_1 (RBHCB1M; PerkinElmer Life Sciences), which was resuspended in 0.2 ml (final volume) of binding buffer (50 mM Tris·HCl, 2.5 mM EGTA, 5 mM $MgCl_2$, 0.5 mg/ml fatty acid free BSA, pH 7.4). CB_1 receptor concentration (B_{max}) was 2.5 pmol/mg protein. Test compounds were present at varying concentrations, and the nonspecific binding of the radioligand was determined in the presence of 10 μM CP-55,940. After 90 min of incubation, the suspension was rapidly filtered through 0.05% polyethyleneimine presoaked GF/C glass fiber filters on a 96-well cell harvester and washed nine times with 0.5 ml of ice-cold washing buffer (50 mM Tris·HCl, 2.5 mM EGTA, 5 mM $MgCl_2$, 2% BSA, pH 7.4). Radioactivity on filters was measured with a Beckman LS 6500 scintillation counter in 3 ml of Ultima Gold scintillation liquid. Data collected from three independent experiments performed in triplicate were normalized between 100 and 0% specific binding for [^{3}H]CP-55,940. These data were fitted in a sigmoidal curve and graphically linearized by projecting Hill plots, which for both cases allowed the calculation of IC_{50} values. Derived from the dissociation constant (K_D) of [^{3}H]CP-55,940 (0.18 nM for CB_1 and 0.39 nM for CB_2) (see below) and the concentration-dependent displacement (IC_{50} value), inhibition constants (K_i) of competitor compounds were calculated by using the Cheng–Prusoff equation [$K_i = IC_{50}/(1 + L/K_D)$] (40). For CB_2 receptor binding studies, 3.8 μg of membrane recombinantly overexpressing CB_2 (RBXCB2M; PerkinElmer Life Sciences) was resuspended in 0.6 ml of binding buffer (see above) together with 0.11 nM the radioligand [^{3}H]CP-55,940. The CB_2 receptor radioligand binding assay was conducted in the same manner as for CB_1. CB_2 receptor concentration (B_{max}) was 4.7 pmol/mg protein. Notably, 1 mg/ml of fatty acid free BSA (BSA) increased the K_i value for *E*-BCP to ≈1400 nM (data not shown), suggesting that BSA content critically influences *E*-BCP receptor binding. This is important because differing BSA concentrations typically used in receptor-binding studies to reduce nonspecific binding may have an impact on the K_i value, depending on the incubation

times and (*E*)-BCP serum albumin interaction kinetics. B_{max} and K_D values of [^{3}H]CP-55,940 were determined by PerkinElmer, Life and Analytical Sciences, Boston, USA and are in the range of values reported in ref. 10.

Molecular Modeling. The dynamic simulations and molecular mechanics (MD/MM) calculations were carried out on each of three compounds to explore their potential conformations, using a protocol reported in ref. 1. The initial structure was first relaxed by performing a 100-step minimization with the maximum derivative being set to 0.1 kcal·mol^{-1}·Å^{-1}. Molecular dynamics simulations at a high temperature of 2000 K were then carried out to efficiently cross the energy barriers and sample local minima. Three hundred snapshots were collected at a rate of 1 ps/snapshot for postprocessing analysis. The 300 collected snapshots were further minimized with the steepest descent and followed by the conjugate gradient methods until the maximum derivative was <0.001 kcal·mol^{-1}·Å^{-1}. The determined conformers are then subject to Gaussian calculation (using HF/6–31G* basis set).

The BCP ligand docking and CB_2 protein-ligand complex studies were performed with Tripos molecular modeling packages Sybyl7.3.3 and our docking protocol published in ref. 2. First, a three-dimensional structure of the BCP molecules, including (*E*)-BCP, (*Z*)-BCP and α-humulene were built by the Sketch module in Sybyl7.3.3 and optimized by using the Tripos force field. The initial docking position of (*E*)-BCP molecules was established inside the hypothetic binding pocket that has been defined on the basis of the MOLCAD-generated solvent-accessible cavity model of the CB_2 receptor (3, 4). It was shown that the binding pocket has amphipathic characteristics, with a hydrophilic center framed by polar residues near the extracellular site and a hydrophobic center surrounded by aromatic residues in the core of the CB_2 receptor with critical residues like Tyr-190 and Phe-197 adjacent to the predicted binding pocket (5, 6). We used this binding pocket as starting point to do docking simulation of BCP binding to the CB_2 receptor.

The receptor-ligand binding geometry was optimized by using a flexible docking method with the Tripos FlexiDock program. In this docking simulation, a CB_2 binding pocket was first defined to cover all residues within 4 Å of the ligand in the initial CB_2-BCP complexes. During flexible docking by the FlexiDock module, all of the single bonds of residue side chains inside the defined CB_2 receptor binding pocket were regarded as rotatable or flexible bonds, and the ligand was allowed to rotate on all single bonds (the methyl groups in BCPs) and move flexibly within the tentative binding pocket. The atomic charges were recalculated by using the Kollman all-atom approach for the protein and the Gasteiger–Huckel approach for the ligand. No H-bonding site was marked for suitable atoms, because there is no O or N atom in BCPs. The binding interaction energy was calculated to include van der Waals, electrostatic, and torsional energy terms defined in the Tripos force field. The structure optimization was performed for 20000-generations, using a Genetic Algorithm, and the 20 best-scoring ligand-protein complexes were kept for further analyses. The Flexidock simulation indicated that the obtained 20 best scoring BCP-CB2 complex models have very similar 3D structures with little different energies, for example, (*E*)-BCP-CB_2 binding energies from −407.78 to −407.75 kcal/mol. Therefore, only one best BCP-CB_2 complex was chosen to do MD/MM simulation.

To obtain more consistent BCP-CB_2 interaction modes, further MD/MM calculations were carried out on the FlexiDock-simulated lowest-energy BCP-CB_2 complexes. In these calculations, the Tripos force field was applied to optimize the intermediate ligand-bound CB_2 receptor models with a 8 Å cutoff distance for the nonbonded interactions, and a distance-dependent dielectric function ($\varepsilon = 5r$) was used to simulate the transmembrane environment around the receptor. The detailed MD/MM protocol was described in previous publications (3, 4) Briefly: (*i*) The minimization was initially run for 500 iterations of steepest descents, followed by a conjugate gradients optimization until the maximum derivative of energy became <0.1 kcal·mol^{-1}·Å^{-1}. (*ii*) MD simulations were then performed at a constant temperature of 1000 K with a time step of 1 fs for a total of 300 ps. Initially, atomic constraints were applied to retain the backbone atoms in the seven transmembrane (7-TM) helical domains. (*iii*) 300 representative (E)-BCP-CB_2 receptor complexes were retrieved from the molecular dynamic simulations. Each of these complexes was further minimized with 500 iterations of steepest descent without the constraints defined in the MD simulation and subsequently minimized by using a conjugate gradient method until the maximum derivative of the total energy was <0.1 kcal·mol^{-1}·Å^{-1}. The obtained 300 MD/MM simulated BCP-CB2 complex models were filtered to remove the complexes with high conformational strains of CB_2 residues side chains or BCP. Finally, among the remaining MD/MM simulated conformers, the lowest-energy 3D structural model of the BCP-bound CB_2 receptor was chosen to define the binding site.

Surflex-dock was developed by Jain (7) and uses an empirical scoring function (8) and a ligand fragmentation engine (9) to dock ligands into a protein's binding site. The scoring function includes hydrophobic, polar, repulsive, entropic, and solvation terms. The Hydrophobic term is a weighted sum over all atom pairs, evolving at least one nonpolar atom, of functions capturing the positive atomic contacts and the atomic interpenetration. The polar term is a sum over all pairs of complementary polar atoms of a function capturing the effects of H-bonds and salt bridges. The repulsive term is a sum over all polar of polar atoms that are of the same sign to capture unfavorable polar contacts. The entropic term is modeling the loss of translational and rotational entropy of the ligand once it is docked by taking into account the number of rotatable ligand bonds and the ligand's molecular weight. The solvation is a function that captures the difference between the potential and actual numbers of H-bond equivalents. By considering BCP isomers as high hydrophobic ligands, the residues I110, V113, I198, F117, M265, F281, V267, and W258 with a radius of 3 Å around the hydrophobic part of the MOLCAD-generated CB_2 channel pocket was chosen to generate a protomol from the CB_2 receptor for an idealized ligand binding site. The starting placement of the ligand was set up in the protomol area during the surflex-docking procedure. Other parameters were set as defaults for Surflex-Dock, such as the starting conformations per molecule of 0, the angstroms to expand search grid of 6, the max conformations per fragment of 20, the max number of rotatable bonds per molecule of 100, and the maximum number of poses per ligand of 10. During the docking procedure, the Surflex–Dock score function positions and orients the probes to optimize ligand interactions with the CB_2 atoms.

Measurement of $[Ca^{2+}]_i$. HL60 CB2-positive cells were washed once, and cells (10^7 cells per ml) were incubated at 37°C for 20 min in Hanks's balanced salt solution containing fluo3/AM in a final concentration of 4 μM and 0.15 mg/ml Pluronic F-127. The cells were then diluted 1:5 in Hanks's balanced salt solution containing 1% FBS and incubated for 40 min at 37°C. Afterward, the cells were washed three times and resuspended in 500 μl of Ca^{2+}-free Hepes-buffered saline, containing 137 mM NaCl, 5 mM KCl, 1 mM Na_2HPO_4,5 mM glucose, 0.5 mM $MgCl_2$, 0.1 mM EGTA, 1 g/liter BSA, 10 mM Hepes, pH 7.4. Before each measurement, the cells were incubated for 7 min in a 37°C water bath. In some experiments the cells were pretreated for 4 min with SR144528 (1 μM). The cells were subsequently stimulated with 2-AG and (*E*)-BCP and vehicle controls and analyzed with the FL1 channel on a FACScan flow cytometer equipped with a 488-nm argon laser (BD Biosciences). Because the solvent (ethanol) showed a weak effect on $[Ca^{2+}]_i$ in vehicle controls, this solvent effect was subtracted from each value.

Quantification of Cytokines in Human Blood Plasma with CBAs. Cytokine production in human peripheral whole blood was analyzed in blood plasma of whole blood cultured for 18 h at 37°C, 5% CO_2, using cytometric bead arrays (BD Biosciences) as described in ref. 40. For short, blood cultures were carried out as described above. TNF-α, IL-10, IL-6, IL-1β, and IL-8 were detected by using the human inflammation CBA kit (551811; BD Biosciences). Tests were performed according to the manufacturer's instructions. Briefly, 50 μl of supernatants were mixed with 50 μl of phycoerythrin-conjugated cytokine capture beads. For each set of experiments a standard curve was generated. Before each measurement the red and orange channels were adequately compensated, according to instructions. FL-2 was typically compensated for 40% FL-1. After 3 h of incubation, samples were rinsed, fixed with 1% paraformaldehyde, and analyzed by flow cytometry (FACScan and FACSCanto) with the CBA Analysis Software (BD Biosciences). The results were expressed as pg/ml and then analyzed for their relative expression (control versus treated sample). The lower limit of detection for each cytokine was determined as 20 pg/ml.

Determination of p38 and Erk1/2 Activation. Phsophorylation of p38 and Erk1/2 was analyzed in HL60 CB2-positive cells and $CD14^+$ peripheral blood mononuclear cells (PBMCs). PBMCs were isolated from human buffy coats by density gradient centrifugation as reported (11). Phosphoproteins were quantified by CBA Cell Signaling Flex Sets (BD Biosciences). Phosphorylated proteins from denatured cell lysate samples were measured according to manufacturer's instructions. Cells were incubated with CB_2 receptor ligands for 45 min in a humidified incubator at 37°C and 5% CO_2. Total protein was obtained from $\approx 5 \times 10^7$ PBMCs and 5×10^6 HL60 cells after treatment with BD Denature Buffer and Lysis Buffer in the presence of Triton X-100 on ice, before denaturing in a boiling water bath for 5 min. DNA was sheared in a probe sonicator and passed through a 26 gauge needle. The protein concentration was determined by using the Bio-Rad Protein Assay. Cell lysates were diluted with BD Assay Dilutent to yield samples containing 20 μg of protein, which were transferred to assay tubes containing Capture Beads. Quantification was performed on a flow cytometer (FACScan and FACSCanto) with the CBA Flex Set analysis software (BD Biosciences). The Phospho Erk1/2 Flex Set 560012 (T202/Y204) and the Phospho p38 MAPK Flex Set 560010 (T180/T182) were used. Quantification of phospho-proteins (units/ml) was based on a calibration curve, which was established for each Flex Set. Fold differences of >2-fold were regarded as meaningful.

Western Blot Analysis. Cell pellets were directly lysed in SDS sample buffer and incubated at 100°C for 10 min before loading (30 μg of total protein per lane) on a 10% SDS-polyacrylamide gel. Proteins were separated by electrophoresis, and transferred to a nitrocellulose membrane (BioRad). The membrane was blocked in 5% milk-TBST, probed with primary antibodies (all 1:500 to 1:1,000 dilutions) for non-phospho and phospho-specific Erk1/2 and JNK1/2 (from Abcam) all in 3% milk-TBST, and reacted with the horseradish peroxidase-conjugated secondary antibody (1:2000) in 3% milk-TBST. After reaction with the Western Lightning chemiluminescence reagent (NEN Life Science), the images were captured. Equal loading of the protein was confirmed by probing for non-phospho antibodies.

GC Measurements. Gas chromatography measurements were carried out with a Thermo Electron Focus GC instrument (Thermo Fisher Scientific) fitted with a BGB wax column (60 m, 0.25 mm diameter, 0.25 μm film, serial No 13651937). Ten microliters of the essential oil was diluted in 5 ml of *n*-hexane and 1 μl was injected. Nitrogen was used as the mobile phase (0.03 MPa, flow rate 10 ml/min) at a constant flow of 1.5 ml/min (split ratio 1:100). Oven temperature was increased from 65°C to 220°C at a rate of 2.5°C per min. The injector temperature was set to 220°C and the detector temperature to 250°C. Flame ionisation (FID) was used as detection method. Purified (*E*)-BCP (99%) was used as standard. Percentages of data were calculated by the area normalization method without applying FID response factor correction. Each essential oil composition was determined three times. The relative standard deviation was <5% for (*E*)-BCP.

1. Chen J-Z, *et al.* (2006) 3D-QSAR Studies of Arylpyrazole Antagonists of Cannabinoid Receptor Subtypes CB1 and CB2. A Combined NMR and CoMFA Approach. *J Med Chem* 49:625–636.
2. Chen JZ, Wang J, Xie X-Q (2007) GPCR structure-based virtual screening approach for CB2 antagonist search. *J Chem Inf Model* 47:1626–1637.
3. Abadji V, Lucas-Lenard JM, Chin C-N, Kendall DA (1999) Involvement of the carboxyl terminus of the third intracellular loop of the cannabinoid CB1 receptor in constitutive activation of GS. *J Neurochem* 72:2032–2038.
4. Xie X-Q, Chen JZ, Billings EM (2003) 3D structural model of the G-protein-coupled cannabinoid CB2 receptor. *Proteins* 53:307–319.
5. McAllister SD, *et al.* (2002) A critical role for a tyrosine residue in the cannabinoid receptors for ligand recognition. *Biochem Pharmacol* 63:2121–2136.
6. Song Z-H, Slowey C-A, Hurst DP, Reggio PH (1999) The difference between the CB1 and CB2 cannabinoid receptors at position 5.46 is crucial for the selectivity of WIN55212–2 for CB2. *Mol Pharmacol* 56:834–840.
7. Jain AN (2003) Surflex: Fully automatic flexible molecular docking using a molecular similarity-based search engine. *J Med Chem* 46:499–511.
8. Jain AN (1996) Scoring noncovalent protein-ligand interactions: A continuous differentiable function tuned to compute binding affinities. *J Comput-Aided Mol Des* 10:427–440.
9. Welch W, Ruppert J, Jain AN (1996) Hammerhead: Fast, fully automated docking of flexible ligands to protein binding sites. *Chem Biol* 3:449–462.
10. Shoemaker JL, Buckle MB, Mayeux PR, Prather PL (2005) Agonist-directed trafficking of response by endocannabinoids acting at CB2 receptors. *J Pharmacol Exp Ther* 315:828–838.
11. Raduner S, *et al.* (2006) Alkylamides from Echinacea are a new class of cannabinomimetics. Cannabinoid type 2 receptor-dependent and -independent immunomodulatory effects. *J Biol Chem* 281:14192-14206.

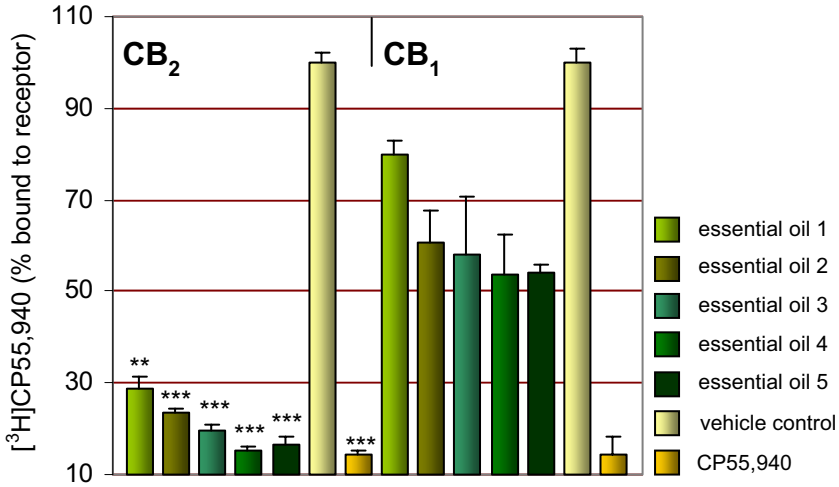

Fig. S1. Displacement of [^{3}H]CP55,940 from HEK293 cells expressing hCB$_2$ or hCB$_1$ receptors by different commercial *Cannabis sativa* essential oils (see *Materials and Methods*). Five micrograms per milliliter of each essential oil was dissolved in dimethyl sulfoxide and tested for [^{3}H]CP55,940 displacement. CP55,940 (5 μM) was used as positive control. Essential oils showing a displacement >50% were considered meaningful. Data show mean values of three independent experiments ± SEM (**, $P < 0.01$; ***, $P < 0.001$ from ANOVA identifying hits that displace [^{3}H]CP55,940 by >50%).

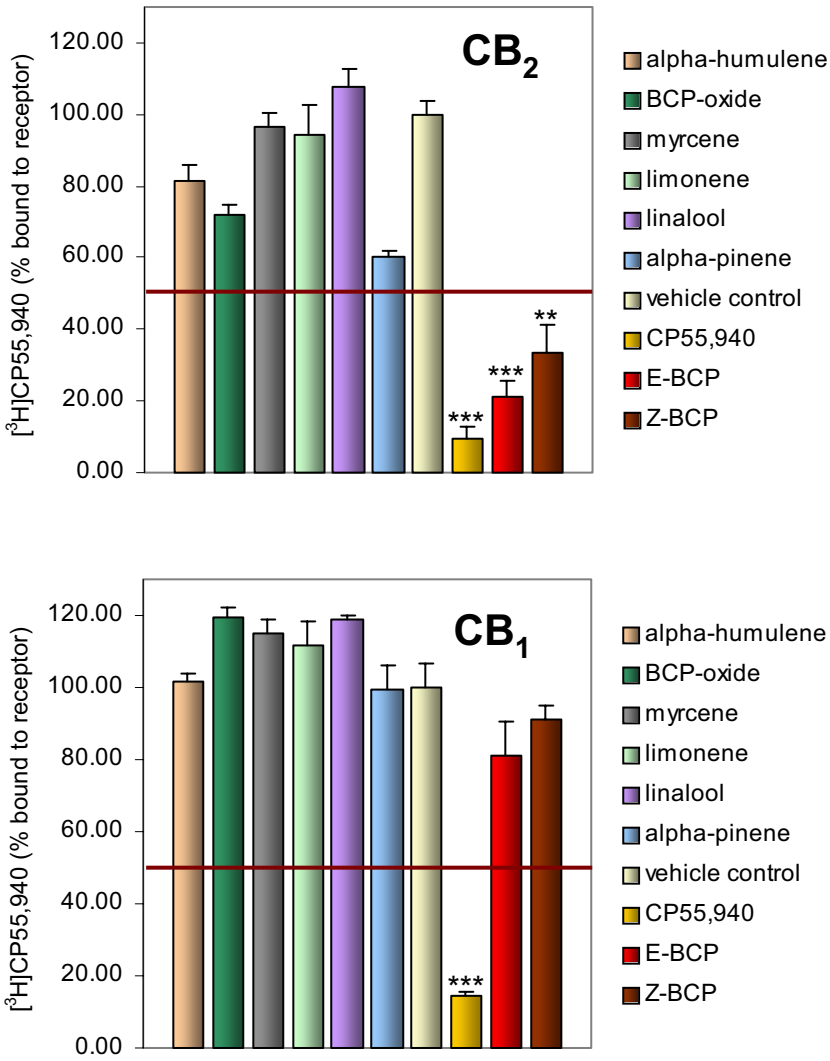

Fig. S2. Screening of the major chemical constituents of *Cannabis* essential oil on displacement of [^{3}H]CP55,940 from HEK293 cells expressing *h*CB_2 and *h*CB_1 receptors. Ten micromolar concentrations of each compound dissolved in dimethyl sulfoxide was assayed for competitive CB_2 and CB_1 receptor binding. Significant displacement (>50%) of [^{3}H]CP55,940 from *h*CB_2 receptors was obtained with (*E*)-BCP, (*Z*)-BCP and the CP55,940 positive control. Data show mean values of three experiments ± SEM (**, $P < 0.01$; ***, $P < 0.001$ from ANOVA identifying hits that displace [^{3}H]CP55,940 by >50%).

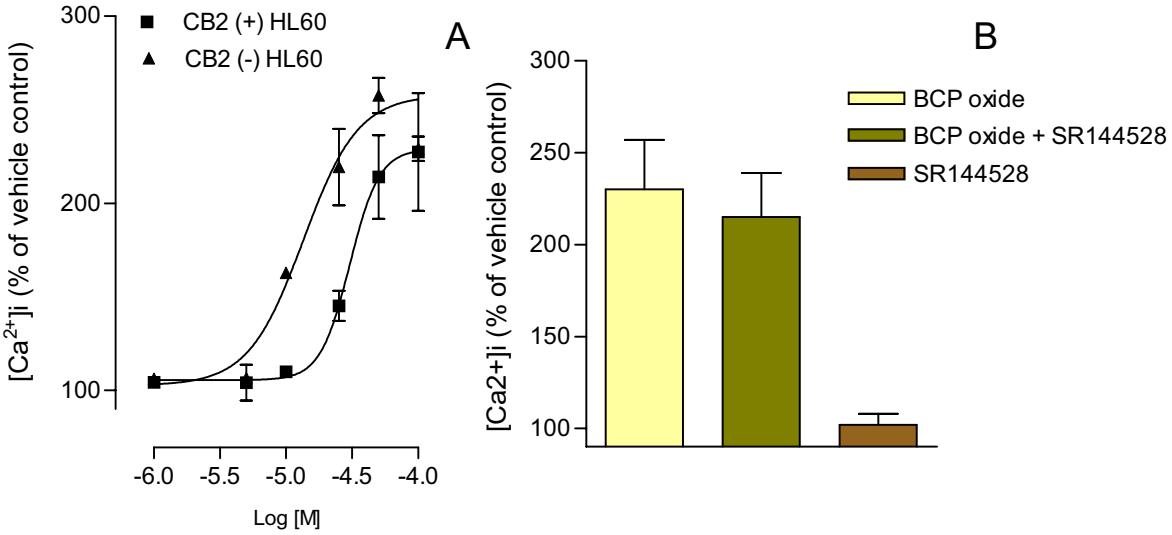

Fig. S3. BCP oxide induces intracellular calcium $[Ca^{2+}]_i$ by CB_2 receptor-independent mechanisms (*A*) BCP oxide triggers $[Ca^{2+}]_i$ transients in HL60 cells expressing the CB_2 receptor but also HL60 cells lacking CB_2 receptor surface expression. Data show the values of three independent experiments ± SEM. (*B*) The CB_2 receptor-selective antagonist SR144528 (1 μM) cannot inhibit BCP oxide induced $[Ca^{2+}]_i$ in CB_2 expressing HL60 cells. Data show the values of three independent experiments ± SEM.

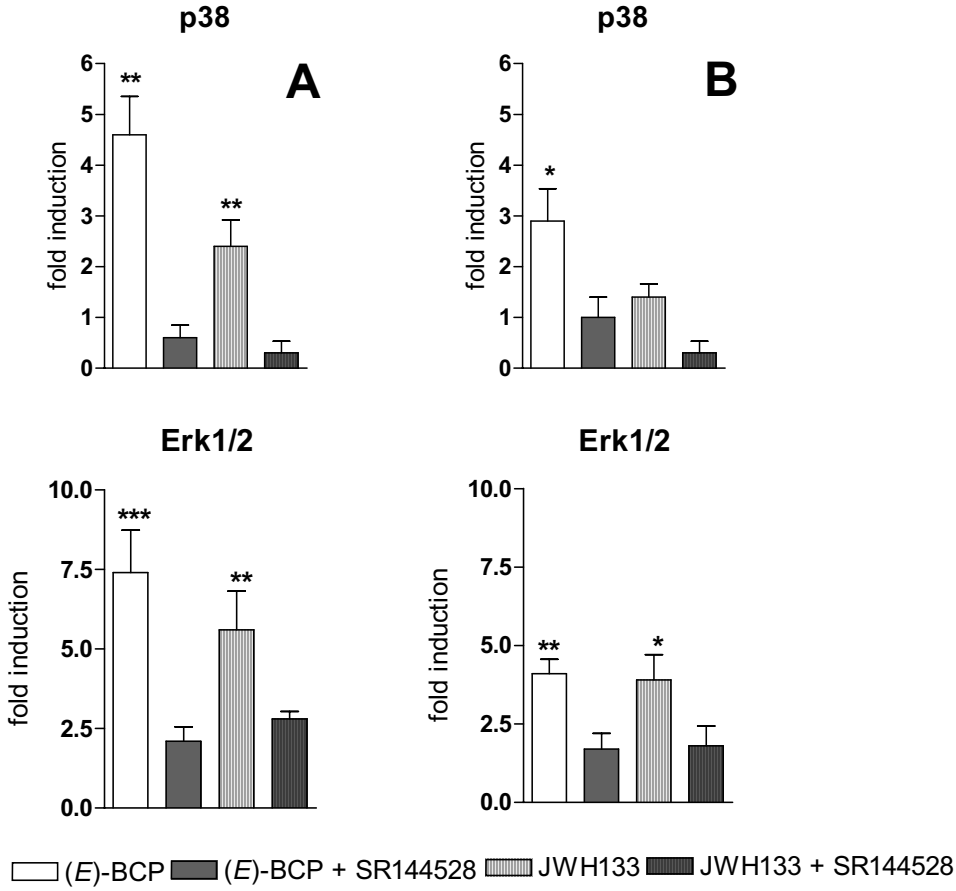

Fig. S4. (*E*)-BCP (1 μM) triggered activation of MAP kinases p38 and Erk1/2. (*A*) p38 and Erk1/2 induction (relative amount of phosphorylated protein after 30 min incubation) in CB_2 receptor positive HL60 cells (10^6 cells per ml) compared with the induction triggered by the selective CB_2 receptor agonist JWH133 (1 μM). The induction was efficiently blocked by prior incubation of cells with SR144528 (1 μM for 1 h). (*B*) Same experiments carried out with primary human $CD14^+$ monocytes (2×10^6 cells per ml). Shown is the fold-induction relative to vehicle control after 30 min as mean values of three independent experiments $\pm$ SEM (*, $P < 0.05$; **, $P < 0.0$

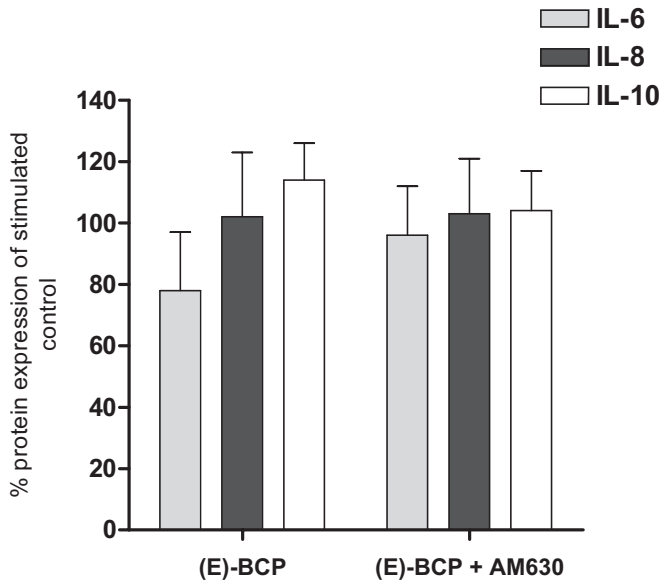

Fig. S5. Modulatory effects of *E*-BCP *in vitro* (followed from Fig. 4A) (*A*) 500 nM (*E*)-BCP does not significantly inhibit LPS-stimulated IL-6, -8, and -10 protein expression in human peripheral whole blood. Data show values from four independent experiments ± SEM from CBA FACS experiments.

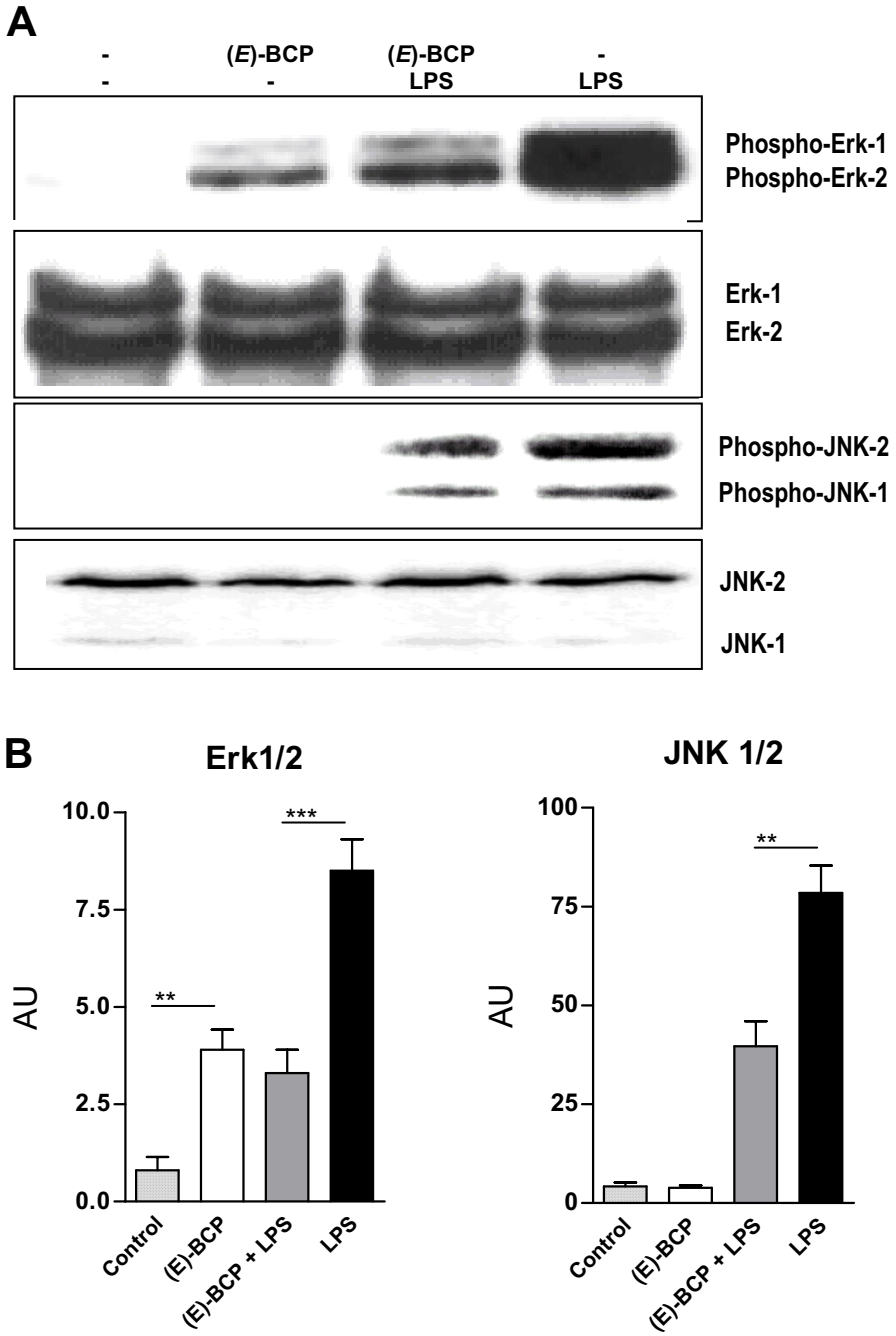

Fig. S6. (*E*)-BCP inhibits LPS-stimulated MAP kinases Erk-1/2 and JNK-1/2 in human monocytes. (*A*) LPS-stimulated (313 ng/ml) Erk-1/2 (30 min) and JNK-1/2 (2 h) phosphorylation in primary human CD14$^+$ monocytes (10^6 cells per ml) is inhibited by (*E*)-BCP (incubation of 500 nM for 1 h before stimulation) as shown by Western blot analyses. Images are representative of three experiments (*B*) Same experiment performed with quantitative CBA Flexset FACS measurements of phospho-Erk-1/2 and phospho-JNK-1/2. Mean values of three independent experiments ± SEM show induction or inhibition (AU) relative to controls (**, $P < 0.01$; ***, $P < 0.001$).

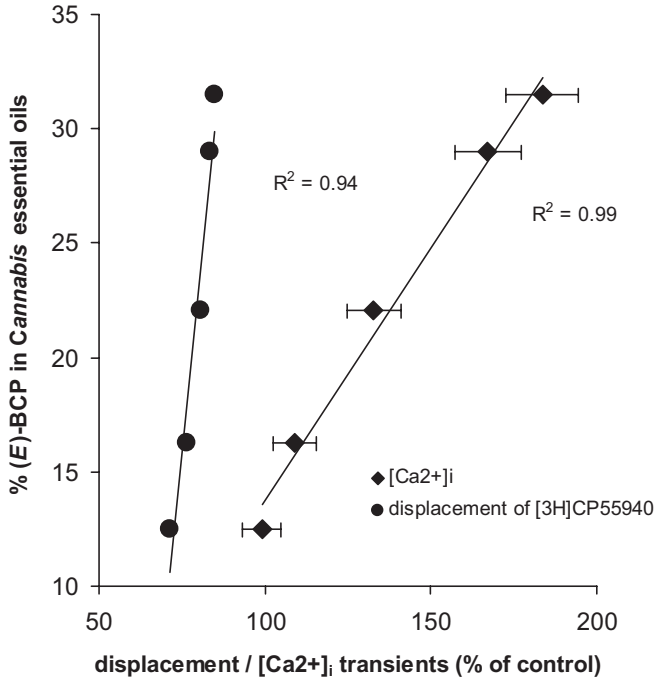

Fig. S7. Correlation between (*E*)-BCP content in *Cannabis* essential oil and CB_2 receptor interaction. 10 μg/ml of each essential oil was incubated with CB_2 receptor-transfected HEK293 cellular membranes and HL60 cells for competitive [^{3}H]CP55940 displacement and [Ca^{2+}]i transients, respectively. The (*E*)-BCP content in *Cannabis* essential oils (determined by GC) clearly correlates with CB_2 receptor binding interaction and receptor activation. Data show mean values of three independent experiments $\pm$ SEM.