Connor Mark (Orcid ID: 0000-0003-2538-2001)

Cannabichromene is a cannabinoid CB2 receptor agonist

Michael Udoh, Marina Santiago, Steven Devenish[a], Iain S. McGregor[a], and Mark Connor.

Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Macquarie University, [a]Lambert Initiative for Cannabinoid Therapeutics, Brain and Mind Centre, The University of Sydney; Sydney, Australia.

Correspondence: Mark Connor, Department of Biomedical Sciences, Macquarie University, Sydney, Australia. E-mail: mark.connor@mq.edu.au

Running Title: Cannabichromene is a CB2 receptor agonist.

What is already known:

- The phytocannabinoid cannabichromene (CBC) has anti-nociceptive and anti-inflammatory effects *in vitro* and *in vivo*
- How CBC exerts these effects is largely unknown

What this study adds:

- This study shows that CBC is a selective CB2 receptor agonist
- CBC has a higher *in vitro* efficacy than tetrahydrocannabinol, and activates CB2 receptor regulatory pathways

Clinical Significance:

- *Cannabis* contains a CB2-selective compound that could reduce inflammation without producing intoxication

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/bph.14815

BACKGROUND

Cannabichromene (CBC) is one of the most abundant phytocannabinoids in *Cannabis spp*. It has modest anti-nociceptive and anti-inflammatory effects and potentiates some effects of Δ^9- tetrahydrocannabinol (THC) *in vivo*. How CBC exerts these effects is poorly defined and there is little information about its efficacy at cannabinoid receptors. We sought to determine the functional activity of CBC at CB1 and CB2 receptors.

EXPERIMENTAL APPROACH

AtT20 cells stably expressing HA-tagged human CB1 and CB2 receptors were used. Assays of cellular membrane potential and loss of cell surface receptors were performed.

KEY RESULTS

CBC activated CB2 but not CB1 receptors to produce a hyperpolarization of AtT20 cells. This activation was inhibited by a CB2 antagonist AM630, and sensitive to pertussis toxin. Application of CBC reduced activation of CB2 receptors (but not CB1 receptors) by subsequent co-application of CP55,940, an efficacious CB1 and CB2 agonist. Continuous CBC application induced loss of cell surface CB2 receptors and desensitisation of the CB2-induced hyperpolarization.

CONCLUSIONS AND IMPLICATIONS

CBC is a selective CB2 receptor agonist displaying higher efficacy than THC in hyperpolarising AtT20 cells. CBC can also recruit CB2 receptor regulatory mechanisms. CBC may contribute to the potential therapeutic effectiveness of some cannabis preparations, potentially through CB2-mediated modulation of inflammation.

Keywords Cannabichromene, phytocannabinoid, cannabinoids, desensitisation, surface receptor internalisation.

Abbreviations

2-AG, 2-arachidonoyl-glycerol; AEA, anandamide; AtT20-CB1, Mouse pituitary tumour cells stably transfected with HA human CB1 cells; AtT20-CB2, Mouse pituitary tumour cells stably transfected with HA human CB2 cells; CB1, Cannabinoid receptor type 1, CB2, Cannabinoid receptor type 2; CBC, cannabichromene; CBD, Cannabidiol; CBN, Cannabinol; ECS, Endocannabinoid system; GRK, G-protein coupled receptor kinase; HA, Haemagglutinin, PTX, Pertussis toxin; THC, Tetrahydrocannabinol; THCV, Tetrahydrocannabivarin.

Introduction

Cannabichromene (CBC) is one of over 100 phytochemicals, (collectively referred to as phytocannabinoids) that are found in *Cannabis spp* (ElSohly & Gul, 2014). CBC was identified in 1966 and is one of the most abundant phytocannabinoids alongside Δ^9-tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabinol (CBN) (Izzo et al, 2009; Turner, Elshohly & Boeren, 1980). Evaluation of seized cannabis plants in USA, UK and Australia showed CBC concentrations ranging between 0.05 - 0.3%w/w (Mehmedic et al., 2010; Potter, Clark, & Brown, 2008; Swift et al., 2013). CBC, THC and CBD are directly synthesized from cannabigerolic acid, and share a common 3-pentylphenol ring (Fig.1) (Flores-Sanchez & Verpoorte, 2008). The therapeutic potential of CBC has been evident in several preclinical studies: for example, CBC decreased carrageenan-induced and lipopolysaccharide (LPS)- induced inflammation in rats and mice, respectively (Turner & Elsohly 1981; DeLong et al. 2010); modestly inhibited thermal nociception and potentiated THC anti-nociception in mice (Davis & Hatoum 1983; DeLong et al. 2010). While this may be mediated in part through changes in THC distribution in the mice (DeLong et al. 2010), the pharmacological basis for the *in vivo* actions of CBC remains unclear.

The endocannabinoid system (ECS) comprises the cannabinoid receptors (CB1 and CB2), endogenous agonists (anandamide (AEA) and 2-arachidonoyl-glycerol (2-AG)), putative endocannabinoid transporters, enzymes involved in the synthesis and metabolism of endocannabinoids (Iannotti et al, 2016). Cannabinoid receptors are differentially distributed in the body. CB1, the most abundant GPCR in the mammalian brain (Marsicano & Lutz, 1999), is predominantly expressed in the central nervous system; while CB2 is expressed abundantly in cells of the immune system and organs

such as the spleen. These distributions imply that activation of these receptors will induce different physiological responses. For example, THC causes a distinctive intoxication via stimulation of the CB1 receptors, while stimulation of CB2 receptors does not appear to contribute to the psychoactive effects of cannabis (Deng et al., 2015).

Phytocannabinoids target individual components of the ECS and act on a range of other receptors and ion channels. For example, THC activates CB1 and CB2 receptors, but also modulates GPR55, 5HT3A receptors and peroxisome proliferator-activated receptor gamma (PPARγ) (Bayewitch et al. 1996; Pertwee 1999; Barann et al. 2002; O'Sullivan et al. 2005; Ryberg et al. 2007; Lauckner et al. 2008). CBD is reported to increase anandamide levels by inhibiting the enzyme FAAH; act as a negative allosteric modulator of CB1 receptors; antagonise GPR55 receptors; activate TRPV2 receptors, and modulate T-type calcium channels (Ross et al., 2008; De Petrocellis et al., 2011; Laprairie et al., 2015). The less prevalent phytocannabinoids such as tetrahydrocannabivarin (THCV), is a low efficacy CB2 agonist, and high potency TRPV1 and TRPA1 agonist (De Petrocellis et al., 2011), while cannabinol (CBN) appears to be an agonist of CB1, CB2 receptors and TRPA1 channels (Bolognini et al., 2010; De Petrocellis et al., 2011; Rhee et al., 1997).

CBC has been reported to be a low affinity CB1/CB2 ligand in binding assays conducted on human receptors expressed in insect cells (Rosenthaler et al., 2014), and it also activates rat TRPA1 channels (De Petrocellis et al., 2011). However, receptor binding does not provide information about ligand efficacy, and whether CBC has efficacy at either receptor remains unresolved. In this study, we sought to characterise the action of CBC at human CB1 and CB2 receptors. To do this, we used an in vitro assay of inwardly rectifying potassium channel activation in intact AtT-20 cells, that we have used extensively to characterize the activity of cannabinoids at CB1 and CB2 receptors

(Banister et al. 2016; Redmond et al. 2016; Soethoudt et al. 2017; Longworth et al. 2017). Using this assay, we find that CBC is an agonist at CB2 but not CB1 receptors.

Materials and Methods

Compounds

CBC was synthesized according to the method of Lee & Wang (2005). Olivetol (1.80 g, 10 mmol) and citral (1.83 g, 12 mmol) were dissolved with stirring in toluene (100 mL), followed by the addition of ethylenediamine (267 uL, 240 mg, 4 mmol) and acetic acid (458 uL, 480 mg, 8 mmol). The mixture was refluxed for 6 h and concentrated under vacuum. The residue was dissolved in dichloromethane (DCM), washed with water and brine, filtered through a plug of silica, and concentrated. Column chromatography was performed multiple times, as separate runs utilising hexane with DCM (gradient from 5:1 to 1:1) and hexane with ethyl acetate or acetone (preferably acetone; gradient from 67:1 to 50:1) were necessary to remove impurities. Cannabichromene was afforded as a pale orange oil (1.20 g, 3.8 mmol, 38%), which darkened upon exposure to air and light. 1HNMR (CDCl3, 400 MHz) δ 6.66 (1H, d, J = 9.9 Hz), 6.29 (1H, s), 6.14 (1H, s), 5.51 (1H, d, J = 9.9 Hz), 5.37 (1H, s), 5.12 (1H, t, J = 6.9 Hz), 2.44 (2H, t, J = 7.67), 2.22-2.07 (2H, m), 1.81-1.66 (2H, m), 1.69 (3H, s), 1.60 (3H, s), 1.59-1.52 (2H, m), 1.41 (3H, s), 1.37-1.25 (4H, m), 0.90 (3H, t, J = 6.6 Hz); 13CNMR (CDCl3, 100 MHz) δ 153.9, 151.1, 144.8, 131.6, 127.2, 124.2, 116.9, 109.1, 107.9, 107.1, 78.3, 41.0, 35.9, 31.5, 30.6, 26.2, 25.7, 22.7, 22.5, 17.6, 14.0. structure and purity of cannabichromene (>95%) was confirmed using 1H and 13C NMR and LCMS. All physical and spectral properties were consistent with those previously reported (Lee & Wang, 2005).

THC was obtained from THCPharm (Frankfurt, Germany) while CP55,940, SR141716 and AM630 were purchased from Cayman Chemical (Michigan, USA). All drugs were

prepared as stock solutions in DMSO and diluted using a 0.01% bovine serum albumin (BSA, Sigma, Castle Hill, Australia) in HEPES-buffered low potassium Hanks Balanced Salt Solution (HBSS). HBSS comprises (mM) NaCl 145, HEPES 22, Na_2HPO_4 0.338, $NaHCO_3$ 4.17, KH_2PO_4 0.441, $MgSO_4$ 0.407, $MgCl_2$ 0.493, Glucose 5.56, $CaCl_2$ 1.26; (pH7.4, Osmolarity 315±15mosmol). Final DMSO concentration was 0.1%.

Cell Culture

Mouse pituitary tumour AtT20 FlpIn cells stably transfected with HA-tagged human CB1 (AtT20-CB1) and human CB2 (AtT20-CB2) receptors (Alexander et al. 2017; Banister, et al. 2016) were used. AtT20 FlpIn cells were made in our lab from AtT20 cells obtained from the American Type Culture Collection (ATCC, RRID:CVCL_4109), using the Flp In System from Thermo Fisher Scientific (#K601001). Tissue culture media and reagents were from Thermo Fisher Scientific, (Massachusetts, USA) or Sigma-Aldrich (Castle Hill, Australia). Tissue culture wares were sourced from Corning (Corning, NY, USA) or Becton Dickinson (North Ryde, Australia). Cells were cultured in T75 flasks using Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal bovine serum (FBS) and 1% penicillin-streptomycin (100U/ml) and incubated in a humidified atmosphere with 5% CO_2 at 37˚C. Zeocin (100 µg/ml, Invivogen, California, USA), and hygromycin (80u µg/ml, Invivogen) were used to select wild-type and transfected AtT20 cells, respectively. Cells were passaged at 80% confluency and used for assays at above 90% confluency, for up to 15 passages. For experiments, AtT20 cells were resuspended in Leibovitz's L-15 media containing 1% FBS, 1% P/S and 15mM glucose. 90 µL of the resuspended cells were plated in a black-walled, 96-well microplate (Corning, NY, USA) and incubated overnight in humidified air at 37˚C. For experiments involving pertussis toxin treatment (PTX, Hello Bio, Bristol, UK), 200 ng/ml PTX was added to the L-15 cell

suspension

Membrane Potential Assay

In this assay, a reduction in fluorescence is indicative of cellular hyperpolarisation. Changes in the membrane potential of cells were measured in duplicate, using a FLIPR Membrane Potential Assay kit (blue #R8034), and a FlexStation 3 Microplate Reader (both from Molecular Devices, Sunnyvale, CA). The dye was diluted to 50% of the manufacturers recommended concentration using HBSS. Dye (90 µL) was loaded into each well of the plate and incubated for 1 h at 37˚C prior to testing. The FlexStation 3 recorded fluorescence at 2 sec intervals ($\lambda_{excitation}$= 530, $\lambda_{emission}$= 565), and drugs were added after an initial 2 mins of baseline reading. The volume of each drug addition was 20 µL, and when two drug additions were made, each drug concentration was adjusted to accommodate the change in final volume. The cellular response to the drug is presented as a percentage change in fluorescence from baseline after subtraction of the change produced by vehicle addition. The change in fluorescence was then normalized to the change in fluorescence due to 1 µM CP55,940 (a high efficacy, non- selective CB1 and CB2 receptor agonist) (Banister et al. 2016) to allow more ready comparison across experiments. CP55,940 (1 µM) standard stimuli were obtained in independent experiments in one well of each column of each plate. Concentration-response curves were fitted to a 4-parameter sigmoidal dose-response curve in Graph Pad Prism (Version 6 GraphPad Software Inc, CA, USA; RRID:SCR_002798) to derive pEC_{50} and E_{max}.

Receptor Internalisation Assay

Changes in cell surface CB2 receptors were determined in at least 5 independent experiments, each performed in triplicate, using whole cell enzyme-linked immunosorbent assay (ELISA). Cells in L-15 media were seeded at 80,000 cells per well in a Poly-D-

lysine (Sigma, Castle Hill, Australia) coated, black walled, clear bottom 96-well plate, and incubated for 18 h at 37˚C in humidified air. After incubation, cells were treated with the drug of interest. Reported drug concentrations are final concentrations. For one drug treatment, the volume of cells in L-15 and compounds were mixed in a 1:1 ratio. For two drug treatments, the volume of cells in L-15, drug A and drug B were added in ratio 9:9:2. Following drug treatment, receptor trafficking was inhibited by placing cells on ice. Cells were then fixed with 4% paraformaldehyde (PFA) for 15 mins. Fixed cells were washed three times with 100 µL Phosphate Buffered Solution (PBS) and blocked with 1% BSA in PBS for 1 h at room temperature. Alexa Fluor® 488 anti-HA Epitope Tag Antibody (Biolegend, UK; RRID: AB_2565072), diluted to 1:250 with blocking solution, was incubated with the cells at 4˚C for 18 h. Cells were then washed three times with 100 µL PBS followed by the addition of 50 µL PBS for the quantification of fluorescence intensity using PHERAstar plate reader (BMG Labtech, Germany). Loss of cell surface receptor was calculated as the percentage decrease in fluorescence intensity after the subtraction of background fluorescence (the fluorescence of wild-type AtT20 cells incubated with the Anti-HA antibody, as above). The background fluorescence was 50 ± 3% of total fluorescence in CB2 expressing cells.

Data Analysis

Data analysis for the immunohistochemistry was blinded. For the membrane potential assay experiments, blinding is impractical, but very effort was made to vary the location within the plate and the order in which drugs were added to 96 well plates to minimize the potential confounds of evaporation or unequal exposure time to the MPA dye. All statistical analyses were conducted with GraphPad Prism, in line with the recommendations on experimental design and analysis in pharmacology (Curtis et al., 2015). Data are reported as Mean ± SEM. The equation used to fit a 4-parameter

sigmoidal dose-response is Y= Bottom + (Top-Bottom)/(1+10^((LogEC50-X)*HillSlope)). Two-tailed, unpaired *t*-tests were used to compare two data points and one-way ANOVA for more than two data points with Tukey post-hoc analysis. *P*-values < 0.05 were considered statistically significant and indicated with * graphically. Unless otherwise stated, five independent replicates were performed for each experiment.

Nomenclature of Targets and Ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org the common portal of data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding *et al.,* 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/2018 (Alexander et al., 2017).

Results

CBC evokes cellular hyperpolarisation via CB2 but not CB1 receptors

CP55,940, a non-selective CB1/CB2 receptor agonist, produced a concentration-dependent decrease in fluorescence in CB1 cells, with a pEC_{50} of 7.8 ± 0.1 (Fig. 2A). THC also evoked membrane hyperpolarisation but with a lower efficacy and potency (pEC50 of 6.6 ± 0.2; E_{max} of 53 ± 3% of CP55,940) (Fig 2A). CBC did not hyperpolarize CB1 cells, inducing a negligible change in fluorescence of 2 ± 1% at 30 µM (Fig. 2A-B).

In CB2 cells, CP55,940 produced a concentration-dependent decrease in fluorescence (pEC50 of 7.1 ± 0.1) (Fig. 2C). The maximum effect of THC (10 µM) was 22 ± 3% of CP55,940 (Fig. 2C). In contrast to AtT20-CB1 cells, application of CBC to AtT20-CB2 cells resulted in a significant hyperpolarisation, reaching a maximum of 52 ± 4% of the maximum effect of CP55,940 at the highest concentration of CBC tested (30 µM, Fig. 2C-D). 30 µM CBC produced a negligible change in fluorescence when applied to wild-type AtT20 cells (2

± 0.4%).

CBC-induced hyperpolarisation is blocked by AM630 and is pertussis sensitive

Pre-treatment of AtT20-CB2 cells with AM630 (3 μM, 5 mins), a CB2 receptor selective antagonist (Ignatowska-Jankowska, Jankowski, & Swiergiel, 2011), inhibited the 10 μM CBC response by 93 ± 6% compared to vehicle pre-treated cells (Fig. 3A-B). AM630 similarly inhibited the responses to CP55,940 (300 nM, Fig. 3B). Overnight incubation of CB2 cells with 200 ng/ml pertussis toxin strongly reduced responses to 10 μM CBC (8 ± 4% of CP 55,940) and 1 μM CP55,940 (11 ± 4%).

CBC inhibits CP55,940 responses at CB2 receptors, but not CB1 receptors

The effect of CBC on responses to CP55,940 and THC was investigated by pre-incubating cells with CBC (10 μM, 5 mins). In CB1 cells, CBC did not significantly affect the subsequent response to either CP55,940 (100 nM) or THC (10 μM) (Fig. 4A-B). In CB2 cells, the CP55,940 (300 nM) response was significantly inhibited by prior application of CBC (10 μM, 5 mins, 44 ± 5%) (Fig. 4C & 4D) or CP55,940 (100 nM, 5 mins, 23 ± 5%) (Suppl Fig 1A, B) respectively. Simultaneous application of CP55,940 (300 nM) and CBC (10 μM) produced a similar change fluorescence to application of CP55,940 (300 nM) alone (112 ± 5 %, Suppl Fig 1C, D).

CBC treatment causes cell surface loss of CB2 receptors

CB2 receptors undergo agonist-induced loss of surface receptors following prolonged stimulation (Grimsey et al, 2011). We found that 1 μM CP55,940 internalized CB2 surface receptors to 59 ± 3% of basal surface level (BSL) after 30 mins treatment. CBC also internalized CB2 surface receptors (10 μM, 77 ± 5%; 30 μM, 71 ± 3%). When CB2

cells were pre-treated with AM630 (3 μM, 5 mins), 10 μM CBC did not produce significant loss of surface CB2 receptors after 30 mins treatment (105 ± 4% of BSL) (Fig. 5A). The amount of cell surface receptors did not change when the cells were exposed to AM630 (3 μM), followed by vehicle for 30 minutes (97 ± 8% of BSL). To assess the possible role of G protein receptor kinases in CBC-mediated receptor internalisation, we pre-treated cells with Compound 101 (10 μM), a GRK2/3 kinase inhibitor (Lowe et al., 2015), for 1 h and then challenged them with CBC. There was no significant change in 10 μM CBC internalisation of CB2 surface receptors following Compound 101 pre-treatment (Fig. 5B).

Effect of CBC on CB2 signalling desensitisation

In the membrane potential assay, prolonged stimulation of AtT20 cells expressing cannabinoid receptors results in the slow reversal of cellular hyperpolarisation (Cawston et al., 2013). Continuous stimulation of CB2 receptors for 30 mins by 1 μM CP55,940 or 10 μM CBC resulted in a reversal of the cellular hyperpolarization by 88 ± 3% (Fig 6A) and 73 ± 6%, (Fig 6B-C), respectively. The desensitisation did not change significantly when cells were pre-treated with Compound 101 (10 μM, 60mins) (Fig 6A-C).

Discussion

In this study, we have discovered that CBC is a phytocannabinoid with selective CB2 receptor agonist actions. We have also provided evidence that it signals through the Gi/o type G-proteins, induces CB2 receptor internalisation and signalling desensitisation that is independent of GRK2/3 kinases.

CBC produced a dose-dependent cell activation indicated by cellular hyperpolarisation in CB2 cells but with no analogous hyperpolarisation in CB1 cells. This is consistent with -

a previous finding that CBC apparently does not stimulate [^{35}S]GTPγS binding via CB1 expressed in CHO cells (Romano et al., 2013) or inhibit AC activity in N18 cells natively expressing mouse CB1 receptors (Howlett, 1986). Although no cannabinoid-antagonist dependent effects have been elucidated in other assays, CBC has been reported to weakly inhibit cellular AEA uptake and the 2-AG hydrolysing enzyme monoacylglycerol lipase (MAGL), both of which may conceivably lead to an indirect activation of cannabinoid receptors through increase in extracellular endocannabinoids (Ligresti, 2006; De Petrocellis et al., 2011). However, the rapid onset of cellular hyperpolarisation in CB2 cells upon addition of CBC suggests a direct receptor activation. Our findings are also consistent with previous studies which concluded that CBC does not significantly affect the CB1 receptor mediated psychoactive effects of cannabis *in-vivo* (DeLong et al., 2010; Ilan et al., 2005).

Cannabinoid receptors mediate downstream signalling predominantly through the $G_{i/o}$ protein family (Mallipeddi, et al, 2017), but CB_1 can couple G_s-proteins when there is no functional $G_{i/o}$-coupling (Bonhaus et al., 1998; Glass & Felder, 1997), and affect G_q in some environments (Lauckner, Hille, & Mackie, 2005). The loss of CBC signalling upon PTX treatment confirms $G_{i/o}$-protein coupling in the hyperpolarization assay, consistent with previous findings with these cells (Banister et al., 2016).

CBC-induced hyperpolarisation in CB2 cells was absent in wild type AtT20 cells and blocked by the selective CB2 receptor antagonist AM630. This blockade is likely due to competitive binding at the CB2 receptor site, supporting the hypothesis that CBC effects are mediated through the CB2 receptor orthosteric site. It is noteworthy that SR144,528, a CB2 antagonist, does not block the anti- inflammatory effects of CBC either *in vitro* (inhibition of nitrite formation in peritoneal macrophages) or *in vivo* (LPS-induced paw oedema) assays (DeLong et al., 2010; Romano et al., 2013). The receptor mechanisms

underlying these anti-inflammatory effects are not yet fully defined.

THC is a low efficacy agonist in many assays of CB1 and CB2 receptor function (Bayewitch et al., 1996; Soethoudt et al., 2017). Therefore, we investigated whether CBC could be acting as an antagonist at the CB1 receptor, since it had been previously reported to bind at the CB1 receptors, albeit with lower affinity than CB2 receptors (Rosenthaler et al., 2014). Using sub-maximal concentrations of a high efficacy agonist (300 nM CP55,940), and maximum concentration of a lower efficacy agonist (10 μM THC), we found that CBC did not alter the onset, and extent, of cellular hyperpolarisation in CB1 receptor expressing cells. These suggest that CBC does not significantly interact with the CB1 receptor site. However, CBC significantly reduced the extent of CP55,940-induced hyperpolarization in CB2 cells after 5 mins treatment. This is likely due to receptor desensitisation, as CBC (10 μM) and CP55940 (300 nM) added at the same time produced a similar effect to CP55940 (300 nM) alone. We showed that lower concentration of CP55940 (100 nM) also produced a modest degree of desensitisation to subsequent addition of a higher concentration (300 nM) of same drug.

Stimulation of both CB1 and CB2 receptors have been implicated in antinociception (Bisogno et al., 2009; Guindon, Desroches, & Beaulieu, 2007; Kinsey et al., 2010; La Rana et al., 2006; Lichtman et al., 2004). CB1 receptors are involved in the attenuation of synaptic transmission of nociception in the brain and primary afferent neurons, while CB2 contributes to antinociception by inhibiting the release of proinflammatory factors around nociceptive neuron terminals (Manzanares, Julian, & Carrascosa, 2006). Since THC analgesia is at least partly mediated through CB1 receptors (Mao et al., 2000) and CBC is a ligand for CB2 receptors, it is possible that the potentiation of THC analgesia by CBC, in addition to pharmacokinetic interaction (Davis & Hatoum, 1983; DeLong et al., 2010) may be a result of CBC stimulation of CB2-mediated inhibition of the release

of pro- inflammatory factors. Apart from CB2-related anti-inflammatory activities, CBC may act directly or indirectly on proteins such as TRPA1 or adenosine A1 receptors (De Petrocellis et al., 2008; Maione et al., 2011; Shinjyo & Di Marzo, 2013).

Upon sustained exposure to agonists, CB2 receptors undergo receptor internalisation, resulting in signalling desensitisation (Bouaboula, Dussossoy, & Casellas, 1999; Shoemaker et al, 2005). Our results show that CBC caused both loss of surface receptors and signalling desensitisation of CB2 receptors. However, the loss of cell surface receptors was less than that observed with CP55,940. This may be due to lower efficacy of CBC in comparison to CP55,940, which is among the most efficacious cannabinoids for internalisation (Atwood et al., 2012). CBC-induced loss of surface CB2 receptors was antagonised by AM630, an effect that further underlines the agonist effect of CBC is CB2 receptor-mediated. AM630 is an inverse agonist at CB2 receptors (Ross et al, 1999), and has previously been reported to increase (Grimsey et al., 2011), or have no effect (Atwood et al., 2012), on CB2 surface receptor levels. Under our experimental conditions, AM630 did not have any appreciable effect on the cell surface receptors.

In the canonical view, GPCR signal desensitisation is usually mediated by GPCR kinase (GRK)-mediated phosphorylation of GPCRs with phosphorylated receptors interacting with arrestins to prevent further downstream signalling (Gainetdinov et al., 2004). Information about the mechanisms of desensitisation of CB2 receptor signalling is sparse, and the GRK involved in CBC-induced CB2 surface internalisation and desensitisation have not been identified. Our results suggest that GRK2/3 kinases are likely not involved in these processes, consistent with previous findings suggesting that GRK2/3 were not likely involved in CB2 internalisation (Bouaboula et al., 1999).

Beta-caryophyllene, which is a terpenoid found in relative abundance within cannabis and food plants, is a naturally occurring CB2-selective agonist (Gertsch et al., 2008). It

has both *in vitro* and *in vivo* CB2-mediated anti-inflammatory activities. Here, we have shown that CBC, a phytocannabinoid, is also CB2-selective agonist. This selectivity implies that CBC and/or its derivatives may be further investigated as a potential therapeutic agent that influences the non- psychotropic CB2 receptor pathways of the ECS. Understanding its mechanism of anti-inflammatory activity *in-vitro* and *in-vivo,* as well as activity at other targets, would be valuable in developing its therapeutic potential. Notably, the combination of CBC with THC produces enhanced anti- nociception and anti-inflammatory responses *in vivo* (Davis & Hatoum, 1983; DeLong et al., 2010). This may reflect pharmacokinetic interactions with THC, but also the pharmacodynamic effects of CBC itself on inflammatory processes. Future research might further investigate CBC in combination with THC and cannabidiol to formulate an optimal analgesic cannabis-based medicine, with minor psychotropic effects and potentiated analgesia.

Acknowledgements

M.U is a recipient of the Macquarie University Postgraduate Research Scholarships for International Students. We thank Dr Samuel Banister for his helpful comments on this study.

Author contributions

M.U, M.S and M.C designed and analysed experiments. S.D synthesized CBC, M.U conducted all other experiments. M.U, S.D, M.S, I.M and M.C prepared the manuscript. All authors have seen the final paper.

Conflict of interest

The authors declare they have no conflicts of interest associated with this work.

Declaration of Transparency and Scientific Rigour

This Declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical research as stated in the *BJP* guidelines for Design & Analysis, and Immunoblotting and Immunochemistry, and as recommended by funding agencies, publishers and other organisations engaged with supporting research.

References

Alexander, S. P., Christopoulos, A., Davenport, A. P., Kelly, E., Marrion, N. V, Peters, J. A., … & Davies J.A.; CGTP Collaborators. (2017). THE CONCISE GUIDE TO PHARMACOLOGY 2017/18: G

protein-coupled receptors. *British Journal of Pharmacology*, *174*, S17–S129. https://doi.org/10.1111/bph.13878

Atwood, B. K., Wager-Miller, J., Haskins, C., Straiker, A., & Mackie, K. (2012). Functional Selectivity in CB2 Cannabinoid Receptor Signaling and Regulation: Implications for the Therapeutic Potential of CB2 Ligands. *Molecular Pharmacology*, *81(2)*, 250–263. https://doi.org/10.1124/mol.111.074013

Banister, S. D., Longworth, M., Kevin, R., Sachdev, S., Santiago, M., Stuart, J., …& Kassiou, M. (2016). Pharmacology of Valinate and tert-Leucinate Synthetic Cannabinoids 5F-AMBICA, 5F-AMB, 5F-ADB, AMB-FUBINACA, MDMB-FUBINACA, MDMB-CHMICA, and Their Analogues. *ACS Chemical Neuroscience*, *7*(9), 1241–1254. https://doi.org/10.1021/acschemneuro.6b00137

Barann, M., Molderings, G., Brüss, M., Bönisch, H., Urban, B. W., & Göthert, M. (2002). Direct inhibition by cannabinoids of human 5-HT3A receptors: probable involvement of an allosteric modulatory site. *British Journal of Pharmacology*, *137*(5), 589–596. https://doi.org/10.1038/sj.bjp.0704829

Bayewitch, M., Rhee, M. H., Avidor-Reiss, T., Breuer, A., Mechoulam, R., & Vogel, Z. (1996). (-)-Delta9-tetrahydrocannabinol antagonizes the peripheral cannabinoid receptor-mediated

inhibition of adenylyl cyclase. *The Journal of Biological Chemistry*, *271*(17), 9902–9905. https://doi.org/10.1074/jbc.271.17.9902

Bisogno, T., Ortar, G., Petrosino, S., Morera, E., Palazzo, E., Nalli, M., … & Di Marzo V.;Endocannabinoid Research Group. (2009). Development of a potent inhibitor of 2-arachidonoylglycerol hydrolysis with antinociceptive activity in vivo. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, *1791*(1), 53–60. https://doi.org/10.1016/j.bbalip.2008.10.007

Bolognini, D., Costa, B., Maione, S., Comelli, F., Marini, P., Di Marzo, V., … & Pertwee, R. G. (2010). The plant cannabinoid Delta9-tetrahydrocannabivarin can decrease signs of inflammation and inflammatory pain in mice. *British Journal of Pharmacology*, *160*(3), 677–687. https://doi.org/10.1111/j.1476-5381.2010.00756.x

Bonhaus, D. W., Chang, L. K., Kwan, J., & Martin, G. R. (1998). Dual Activation and Inhibition of Adenylyl Cyclase by Cannabinoid Receptor Agonists: Evidence for Agonist-Specific Trafficking of Intracellular Responses. *The Journal of Pharmacology and Experimental Therapeutics*, *287*, 884–888.

Bouaboula, M., Dussossoy, D., & Casellas, P. (1999). Regulation of peripheral cannabinoid receptor CB2 phosphorylation by the inverse agonist SR 144528. Implications for receptor biological responses. *The Journal of Biological Chemistry*, *274*(29), 20397–20405. https://doi.org/10.1074/JBC.274.29.20397

Cawston, E. E., Redmond, W. J., Breen, C. M., Grimsey, N. L., Connor, M., & Glass, M.

(2013). Real-time characterization of cannabinoid receptor 1 (CB1) allosteric modulators reveals novel mechanism of action. *British Journal of Pharmacology*, *170*(4), 893–907. https://doi.org/10.1111/bph.12329

Curtis, M. J., Bond, R. A., Spina, D., Ahluwalia, A., Alexander, S. P. A., Giembycz, M. A., … & McGrath, J. C. (2015). Experimental design and analysis and their reporting: new guidance for publication in BJP. *British Journal of Pharmacology*, *172*(14), 3461–3471. https://doi.org/10.1111/bph.12856

Davis, W. M., & Hatoum, N. S. (1983). Neurobehavioral actions of cannabichromene and interactions with Δ9-tetrahydrocannabinol. *General Pharmacology*, *14*(2), 247–252. https://doi.org/10.1016/0306-3623(83)90004-6

De Petrocellis, L., Ligresti, A., Moriello, A. S., Allarà, M., Bisogno, T., Petrosino, S., …& Di Marzo, V. (2011). Effects of cannabinoids and cannabinoid-enriched *Cannabis* extracts on TRP channels and endocannabinoid metabolic enzymes. *British Journal of Pharmacology*, *163*(7), 1479–1494. https://doi.org/10.1111/j.1476-5381.2010.01166.x

De Petrocellis, L., Vellani, V., Schiano-Moriello, A., Marini, P., Magherini, P. C., Orlando, P., & Di Marzo, V. (2008). Plant-Derived Cannabinoids Modulate the Activity of Transient Receptor Potential Channels of Ankyrin Type-1 and Melastatin Type-8. *Journal of Pharmacology and Experimental Therapeutics*, *325*(3), 1007–1015. https://doi.org/10.1124/jpet.107.134809

DeLong, G. T., Wolf, C. E., Poklis, A., & Lichtman, A. H. (2010). Pharmacological evaluation

of the natural constituent of Cannabis sativa, cannabichromene and its modulation by Δ9-tetrahydrocannabinol. *Drug and Alcohol Dependence*, *112*(1–2), 126–133. https://doi.org/10.1016/J.DRUGALCDEP.2010.05.019

Deng, L., Guindon, J., Cornett, B. L., Makriyannis, A., Mackie, K., & Hohmann, A. G. (2015). Chronic cannabinoid receptor 2 activation reverses paclitaxel neuropathy without tolerance or cannabinoid receptor 1-dependent withdrawal. *Biological Psychiatry*, *77*(5), 475–487. https://doi.org/10.1016/j.biopsych.2014.04.009

Elsohly M and Gul W (2014) Constituents of cannabis sativa, in: Handbook of Cannabis (Pertwee RG ed) pp. 3–22, Oxford University Press, Oxford, UK. ISBN 978-0-19-966268-5 https://doi.org/10.1093/acprof:oso/9780199662685.003.0001

Flores-Sanchez, I. J., & Verpoorte, R. (2008). Secondary metabolism in cannabis. *Phytochemistry Reviews*, *7*(3), 615–639. https://doi.org/10.1007/s11101-008-9094-4

Gainetdinov, R. R., Premont, R. T., Bohn, L. M., Lefkowitz, R. J., & Caron, M. G. (2004). Desensitization of G protein–coupled receptors and neuronal functions. *Annual Review of Neuroscience*, *27*, 107–144. https://doi.org/10.1146/annurev.neuro.27.070203.144206

Gertsch, J., Leonti, M., Raduner, S., Racz, I., Chen, J.-Z., Xie, X.-Q., … & Zimmer, A. (2008). Beta-caryophyllene is a dietary cannabinoid. *Proceedings of the National Academy of Sciences*, *105*(26), 9099–9104. https://doi.org/10.1073/pnas.0803601105

Glass, M., & Felder, C. C. (1997). Concurrent stimulation of cannabinoid CB1 and dopamine

D2 receptors augments cAMP accumulation in striatal neurons: evidence for a Gs linkage to the CB1 receptor. *The Journal of Neuroscience*, *17*(14), 5327–5333. https://doi.org/10.1523/JNEUROSCI.17-14-05327.1997

Grimsey, N. L., Goodfellow, C. E., Dragunow, M., & Glass, M. (2011). Cannabinoid receptor 2 undergoes Rab5-mediated internalization and recycles via a Rab11-dependent pathway. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, *1813*(8), 1554–1560. https://doi.org/10.1016/J.BBAMCR.2011.05.010

Guindon, J., Desroches, J., & Beaulieu, P. (2007). The antinociceptive effects of intraplantar injections of 2-arachidonoyl glycerol are mediated by cannabinoid CB $_2$ receptors. *British Journal of Pharmacology*, *150*(6), 693–701. https://doi.org/10.1038/sj.bjp.0706990

Harding SD, Sharman JL, Faccenda E, Southan C, Pawson AJ, Ireland S et al. (2018). The IUPHAR/BPS Guide to PHARMACOLOGY in 2018: updates and expansion to encompass the new guide to IMMUNOPHARMACOLOGY. *Nucleic Acids Research, 46*, D1091-D1106. https://doi.org/10.1093/nar/gkx1121

Howlett, A.C (1987). Cannabinoid inhibition of adenylate cyclase: Relative activity of constituents and metabolites of Marihuana. *Neuropharmacology*, *26*(5), 507-512. https://doi.org/10.1016/0028-3908(87)90035-9

Iannotti, F. A., Di Marzo, V., & Petrosino, S. (2016). Endocannabinoids and endocannabinoid-related mediators: Targets, metabolism and role in neurological disorders. *Progress in Lipid Research*, *62*, 107–128. https://doi.org/10.1016/J.PLIPRES.2016.02.002

Ignatowska-Jankowska, B., Jankowski, M. M., & Swiergiel, A. H. (2011). Cannabidiol decreases body weight gain in rats: Involvement of CB2 receptors. *Neuroscience Letters*, *490*(1), 82–84. https://doi.org/10.1016/J.NEULET.2010.12.031

Ilan, A. B., Gevins, A., Coleman, M., ElSohly, M. A., & de Wit, H. (2005). Neurophysiological and subjective profile of marijuana with varying concentrations of cannabinoids. *Behavioural Pharmacology*, *16*(5–6), 487–496. https://doi.org/10.1097/00008877-200509000-00023

Izzo, A. A., Borrelli, F., Capasso, R., Di Marzo, V., & Mechoulam, R. (2009). Non-psychotropic plant cannabinoids: new therapeutic opportunities from an ancient herb. *Trends in Pharmacological Sciences*, *30*(10), 515–527. https://doi.org/10.1016/J.TIPS.2009.07.006

Kinsey, S. G., Long, J. Z., Cravatt, B. F., & Lichtman, A. H. (2010). Fatty acid amide hydrolase and monoacylglycerol lipase inhibitors produce anti-allodynic effects in mice through distinct cannabinoid receptor mechanisms. *The Journal of Pain: 11*(12), 1420–1428. https://doi.org/10.1016/j.jpain.2010.04.001

La Rana, G., Russo, R., Campolongo, P., Bortolato, M., Mangieri, R. A., Cuomo, V., … & Calignano, A. (2006). Modulation of Neuropathic and Inflammatory Pain by the Endocannabinoid Transport Inhibitor AM404 [N-(4-Hydroxyphenyl)-eicosa-5,8,11,14-tetraenamide]. *Journal of Pharmacology and Experimental Therapeutics*, *317*(3), 1365–1371. https://doi.org/10.1124/jpet.105.100792

Laprairie, R. B., Bagher, A. M., Kelly, M. E. M., & Denovan-Wright, E. M. (2015).

Cannabidiol is a negative allosteric modulator of the cannabinoid CB1 receptor. *British Journal of Pharmacology*, *172*(20), 4790–4805. https://doi.org/10.1111/bph.13250

Lauckner, J. E., Hille, B., & Mackie, K. (2005). The cannabinoid agonist WIN55,212-2 increases intracellular calcium via CB 1 receptor coupling to Gq/11 G proteins. *Proceedings of the National Academy of Sciences of the United States of America*, 102(52):19144-19149. https://doi.org/10.1073/pnas.0509588102

Lauckner, J. E., Jensen, J. B., Chen, H.-Y., Lu, H.-C., Hille, B., & Mackie, K. (2008). GPR55 is a cannabinoid receptor that increases intracellular calcium and inhibits M current. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(7), 2699–2704. https://doi.org/10.1073/pnas.0711278105

Lee, Y.-R. & Wang, X. (2005). Concise Synthesis of Biologically Interesting (±)-Cannabichromene, (±)-Cannabichromenic Acid, and (±)-Daurichromenic Acid. *Bulletin of the Korean Chemical Society.* 26. 1933-1936. https://doi.org/10.5012/bkcs.2005.26.12.1933.

Lichtman, A. H., Shelton, C. C., Advani, T., & Cravatt, B. F. (2004). Mice lacking fatty acid amide hydrolase exhibit a cannabinoid receptor-mediated phenotypic hypoalgesia. *Pain*, *109*(3), 319–327. https://doi.org/10.1016/j.pain.2004.01.022

Ligresti, A., Moriello AS., Starowicz K., Matias I., Pisanti S., De Petrocellis L., …& Di Marzo V. (2006). Antitumor Activity of Plant Cannabinoids with Emphasis on the Effect of Cannabidiol on Human Breast Carcinoma. *Journal of Pharmacology and Experimental Therapeutics*, *318*(3), 1375–1387. https://doi.org/10.1124/jpet.106.105247

Longworth, M., Banister, S. D., Boyd, R., Kevin, R. C., Connor, M., McGregor, I. S., & Kassiou, M. (2017). Pharmacology of Cumyl-Carboxamide Synthetic Cannabinoid New Psychoactive Substances (NPS) CUMYL-BICA, CUMYL-PICA, CUMYL-5F-PICA, CUMYL-5F-PINACA, and Their Analogues. *ACS Chemical Neuroscience*, *8*(10), 2159–2167. https://doi.org/10.1021/acschemneuro.7b00267

Lowe, J. D., Sanderson, H. S., Cooke, A. E., Ostovar, M., Tsisanova, E., Withey, S. L., … & Bailey, C. P. (2015). Role of G Protein-Coupled Receptor Kinases 2 and 3 in μ-Opioid Receptor Desensitization and Internalization. *Molecular Pharmacology*, *88*(2), 347–356. https://doi.org/10.1124/mol.115.098293

Maione, S., Piscitelli, F., Gatta, L., Vita, D., De Petrocellis, L., Palazzo, E., …& Di Marzo, V. (2011). Non-psychoactive cannabinoids modulate the descending pathway of antinociception in anaesthetized rats through several mechanisms of action. *British Journal of Pharmacology*, *162*(3), 584–596. https://doi.org/10.1111/j.1476-5381.2010.01063.x

Mallipeddi, S., Janero, D. R., Zvonok, N., & Makriyannis, A. (2017). Functional selectivity at G-protein coupled receptors: Advancing cannabinoid receptors as drug targets. *Biochemical Pharmacology*, *128*, 1–11. https://doi.org/10.1016/j.bcp.2016.11.014

Manzanares, J., Julian, M., & Carrascosa, A. (2006). Role of the cannabinoid system in pain control and therapeutic implications for the management of acute and chronic pain episodes. *Current Neuropharmacology*, *4*(3), 239–257. https://doi.org/10.2174/157015906778019527

Mao, J., Price, D. D. ., Lu, J., Keniston, L., & Mayer, D.J . (2000). Two distinctive

antinociceptive systems in rats with pathological pain. *Neuroscience Letters*, *280*(1), 13–16. https://doi.org/10.1016/S0304-3940(99)00998-2

Marsicano, G., & Lutz, B. (1999). Expression of the cannabinoid receptor CB1 in distinct neuronal subpopulations in the adult mouse forebrain. *European Journal of Neuroscience*, *11*(12), 4213–4225. https://doi.org/10.1046/j.1460-9568.1999.00847.x

Mehmedic, Z., Chandra, S., Slade, D., Denham, H., Foster, S., Patel, A. S., … & ElSohly, M. A. (2010). Potency trends of Δ9-THC and other cannabinoids in confiscated cannabis preparations from 1993 to 2008. *Journal of Forensic Sciences*, *55*(5), 1209–1217. https://doi.org/10.1111/j.1556-4029.2010.01441.x

O'Sullivan, S. E., Tarling, E. J., Bennett, A. J., Kendall, D. A., & Randall, M. D. (2005). Novel time-dependent vascular actions of Delta9-tetrahydrocannabinol mediated by peroxisome proliferator-activated receptor gamma. *Biochemical and Biophysical Research Communications*, *337*(3), 824–831. https://doi.org/10.1016/j.bbrc.2005.09.121

Pertwee, R. G. (1999). Pharmacology of cannabinoid receptor ligands. *Current Medicinal Chemistry*, *6*(8), 635–664.

Potter, D. J., Clark, P., & Brown, M. B. (2008). Potency of Δ^9 –THC and Other Cannabinoids in Cannabis in England in 2005: Implications for Psychoactivity and Pharmacology. *Journal of Forensic Sciences*, *53*(1), 90–94. https://doi.org/10.1111/j.1556-4029.2007.00603.x

Redmond, W. J., Cawston, E. E., Grimsey, N. L., Stuart, J., Edington, A. R., Glass, M., &

Connor, M. (2016). Identification of N-arachidonoyl dopamine as a highly biased ligand at cannabinoid CB1 receptors. *British Journal of Pharmacology*, *173*(1), 115–127. https://doi.org/10.1111/bph.13341

Rhee, M. H., Vogel, Z., Barg, J., Bayewitch, M., Levy, R., Hanus, L., … & Mechoulam, R. (1997). Cannabinol derivatives: binding to cannabinoid receptors and inhibition of adenylylcyclase. *Journal of Medicinal Chemistry*, *40*(20), 3228–3233. https://doi.org/10.1021/jm970126f

Romano, B., Borrelli, F., Fasolino, I., Capasso, R., Piscitelli, F., Cascio, M., … & Izzo, A. (2013). The cannabinoid TRPA1 agonist cannabichromene inhibits nitric oxide production in macrophages and ameliorates murine colitis. *British Journal of Pharmacology*, *169*(1), 213–229. https://doi.org/10.1111/bph.12120

Rosenthaler, S., Pöhn, B., Kolmanz, C., Huu, C. N., Krewenka, C., Huber, A., … & Moldzio, R. (2014). Differences in receptor binding affinity of several phytocannabinoids do not explain their effects on neural cell cultures. *Neurotoxicology and Teratology*, *46*, 49–56. https://doi.org/10.1016/j.ntt.2014.09.003

Ross, R. A., Brockie, H. C., Stevenson, L. A., Murphy, V. L., Templeton, F., Makriyannis, A., & Pertwee, R. G. (1999). Agonist-inverse agonist characterization at CB1 and CB2 cannabinoid receptors of L759633, L759656, and AM630. *British Journal of Pharmacology*, *126*(3), 665– 672. https://doi.org/10.1038/sj.bjp.0702351

Ryberg, E., Larsson, N., Sjögren, S., Hjorth, S., Hermansson, N.-O., Leonova, J., … &

Greasley,

P. J. (2007). The orphan receptor GPR55 is a novel cannabinoid receptor. *British Journal of Pharmacology*, *152(7)*, 1092–1101. https://doi.org/10.1038/sj.bjp.0707460

Shinjyo, N., & Di Marzo, V. (2013). The effect of cannabichromene on adult neural stem/progenitor cells. *Neurochemistry International*, *63*(5), 432–437. https://doi.org/10.1016/j.neuint.2013.08.002

Shoemaker, J. L., Joseph, B. K., Ruckle, M. B., Mayeux, P. R., & Prather, P. L. (2005). The Endocannabinoid Noladin Ether Acts as a Full Agonist at Human CB2 Cannabinoid Receptors. *The Journal of Pharmacology and Experimental Therapeutics*, *314(2)*, 868–875. https://doi.org/10.1124/jpet.105.085282

Soethoudt, M., Grether, U., Fingerle, J., Grim, T. W., Fezza, F., de Petrocellis, L., …& van der Stelt, M. (2017). Cannabinoid CB2 receptor ligand profiling reveals biased signalling and off-target activity. *Nature Communications*, *8*, 13958. https://doi.org/10.1038/ncomms13958

Swift, W., Wong, A., Li, K. M., Arnold, J. C., & McGregor, I. S. (2013). Analysis of cannabis seizures in NSW, Australia: cannabis potency and cannabinoid profile. *PloS One*, *8*(7), e70052. https://doi.org/10.1371/journal.pone.0070052

Sylantyev, S., Jensen, T. P., Ross, R. A., & Rusakov, D. A. (2013). Cannabinoid- and lysophosphatidylinositol-sensitive receptor GPR55 boosts neurotransmitter release at central synapses. *Proceedings of the National Academy of Sciences*, *110*(13), 5193–5198. https://doi.org/10.1073/pnas.1211204110

Turner, C. E., Elsohly, M. A., & Boeren, E. G. (1980). Constituests of Cannabis sativa L. XVII. A review of the natural constituents. *Journal of Natural Products, 43*(2), 169–234. https://doi.org/10.1021/np50008a001

Turner, C. E., & Elsohly, M. A. (1981). Biological activity of cannabichromene, its homologs and isomers. *Journal of Clinical Pharmacology*, *21*(S1), 283S–291S. https://doi.org/10.1002/j.1552-4604.1981.tb02606.x

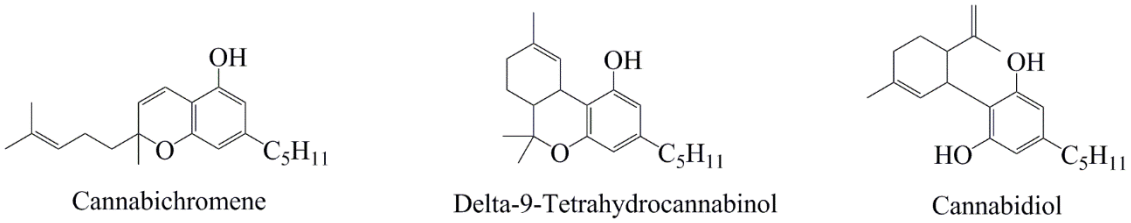

Figure 1: Chemical structures of some phytocannabinoids

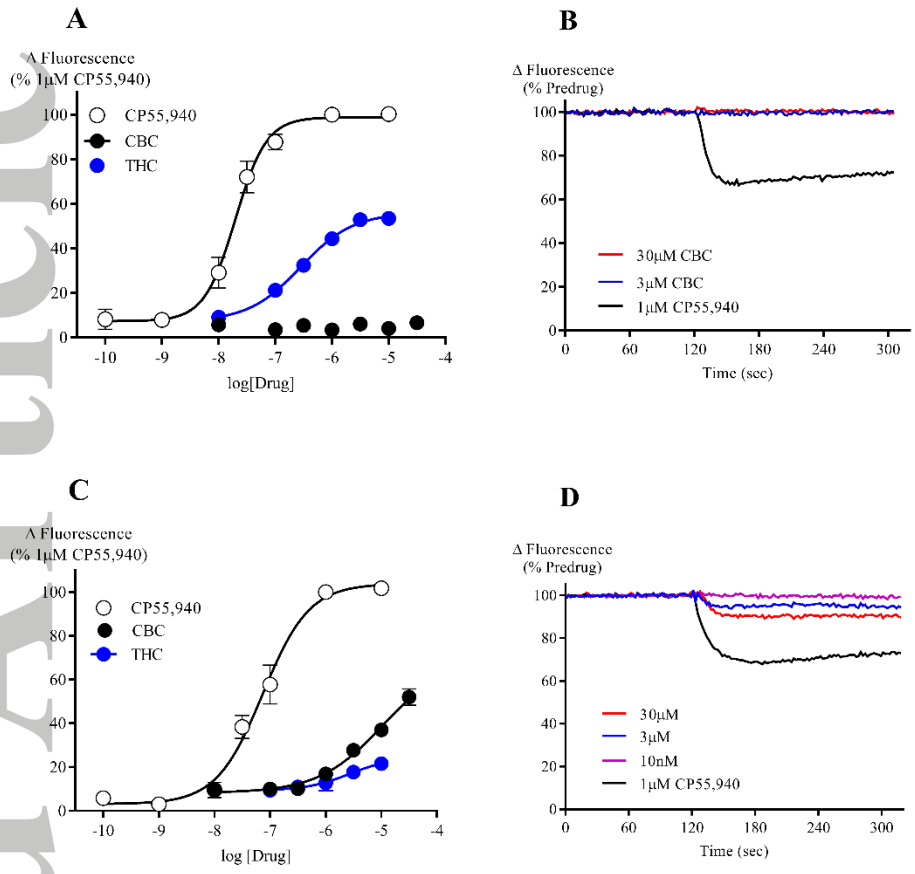

Figure 2. CBC activates CB2 but not CB1 receptors. (A) Concentration-response curves of CP55,940, THC and CBC in AtT20 CB1 cells (n=5). Results are expressed as mean ± SEM after normalization to 1 µM CP55,940 hyperpolarisation. (B) Representative traces of changes in fluorescence, due to CBC and 1 µM CP55,940-induced hyperpolarisation in AtT20 CB1 cells. Drugs were added after 120sec of baseline reading and read over 300sec. (C) Concentration- response curves of CP55,940, THC and CBC in AtT20 CB2 cells (n=5). Results are expressed as mean ± SEM after normalization to 1 µM CP55,940 hyperpolarisation. (D) Representative traces of changes in fluorescence, due to CBC and 1 µM CP55,940-induced hyperpolarisation in AtT20 CB2 cells. Drugs were added after 120sec of baseline reading and read over 300sec.

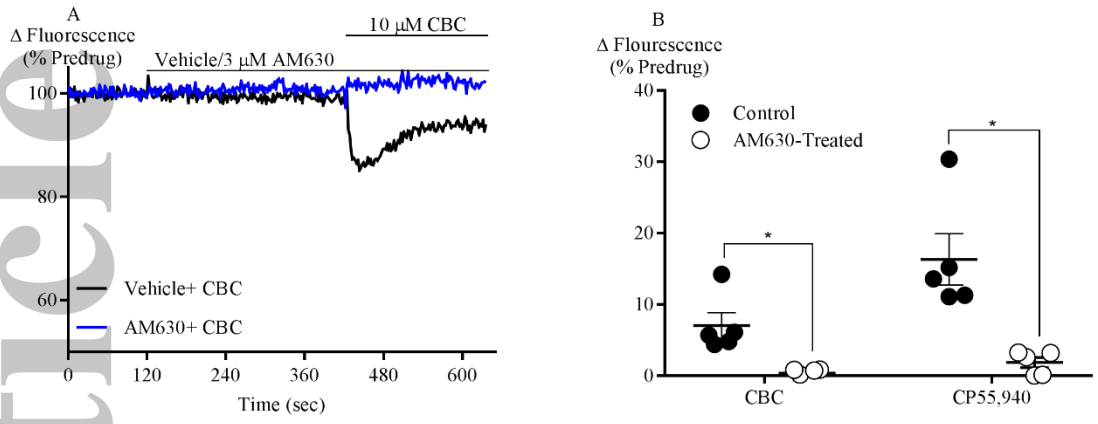

Figure 3. CBC activation of CB2 receptors is blocked by AM630. (A) A representative trace of change in fluorescence of AtT20-CB2 cells after 5 mins pre-treatment with vehicle and 3 μM AM630, followed by the addition of 10 μM CBC. (B) Responses to CBC (10 μM) and CP55,940 (300 nM) in AtT20-CB2 cells with or without pre-incubation of AM630 (3 μM) for 5 mins. Results are expressed as mean ± SEM (*n*=5).

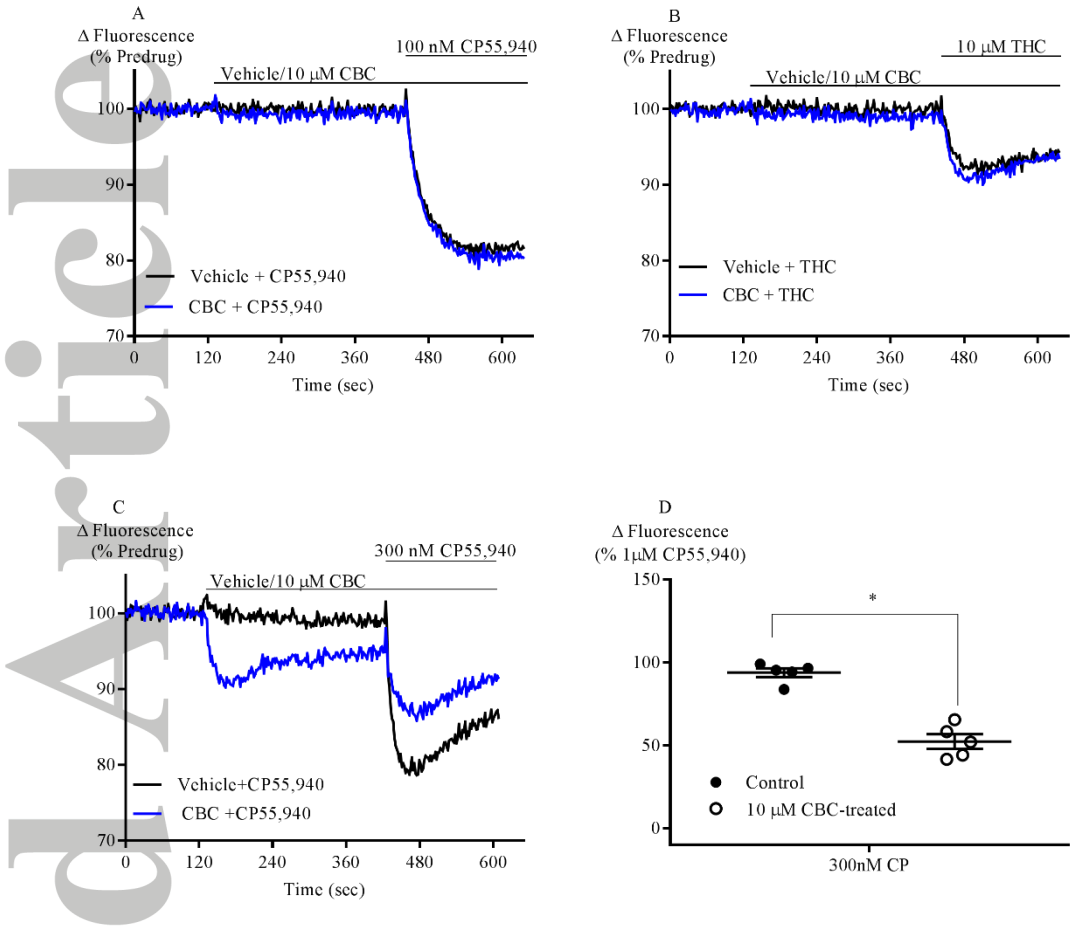

Figure 4. CBC antagonises CP55,940 and THC response in CB2 cells. Representative traces of the effect of CBC (10 μM) on (A) CP55,940 (100 nM) on fluorescence in AtT20-CB1 cells loaded with a membrane potential-sensitive dye. (B) THC (10 μM) hyperpolarisation in AtT20-CB1. (C) CP55,940 (300 nM) hyperpolarisation in AtT20-CB2 cells. After 2mins baseline reading, cells were pre-treated with vehicle or 10 μM CBC for 5 mins, followed by the addition CP55,940 (D) Summary data of the effect of 10 μM CBC on 300 nM CP55,940 in AtT20-CB2 cells. Results are expressed as mean ± SEM after normalization to 1 μM CP55,940 hyperpolarisation (n=5) *(Note: Truncated axes).*

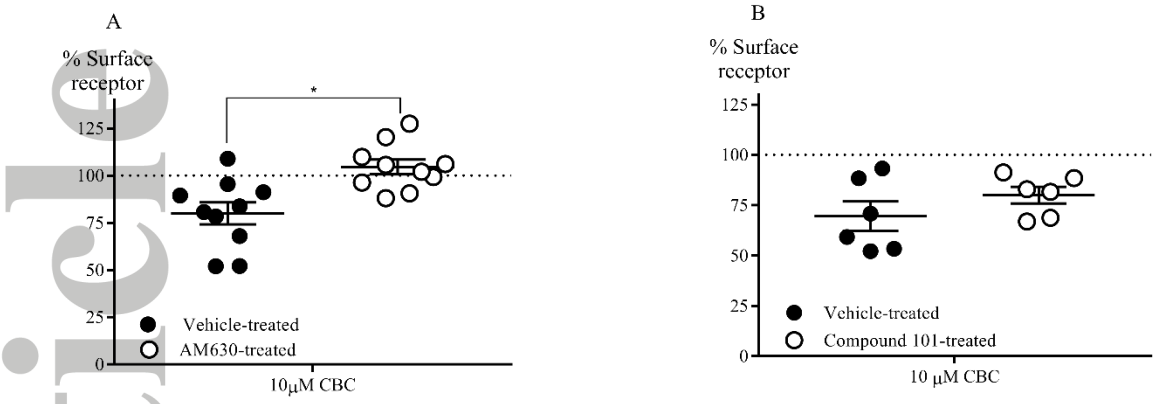

Figure 5. Effect of AM630 and compound 101 on CBC internalisation of CB2 cell surface receptors. (A) Summary data of the effect of AM630 on CBC internationalization of surface receptors. Cells were pre-treated with AM630 (3 μM, 5 mins) followed by CBC (10 μM, 30 mins) in the continuous presence of antagonist (*n=10)* (B)Summary data of the effect of compound 101 on CBC internationalization of surface receptors. Cells were pre-treated with compound 101 (10 μM, 60mins), followed by CBC (10 μM, 30 mins) in the continuous presence of the GRK2/3 inhibitor (n=5). All Results are expressed as mean percentage of the basal surface receptor level (BSL)± SEM, which is the percentage of vehicle- treated AtT20-CB2 cells, after subtraction of background signal.

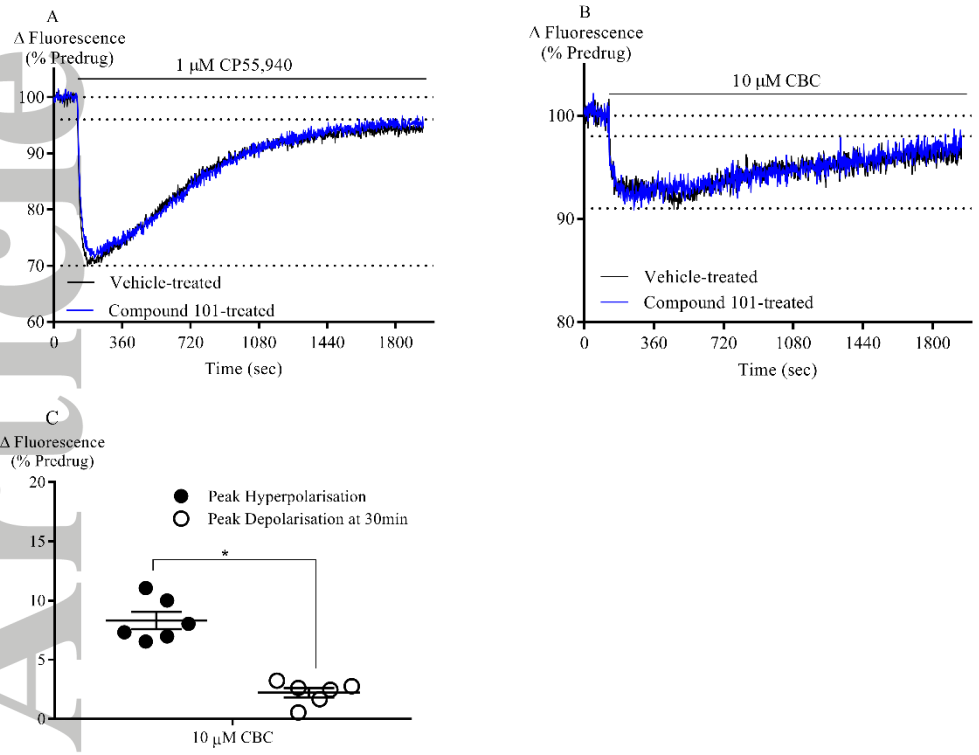

Figure 6. Desensitisation of AtT20-CB2 cells signalling. (A) A representative trace of 1 μM CP55,940 desensitisation of AtT20-CB2 cell signalling in the presence of vehicle or compound 101. (B) A representative trace of 10 μM CBC desensitisation of AtT20 CB2 cell signalling in the presence of vehicle or compound 101. Cell were pre-incubated with compound 101 (10 μM, 60 mins) before CP55,940 or CBC addition. CP55,940 or CBC were added after 2 mins of baseline reading and read for 30 mins. (C) Summary data of CBC (10 μM, 30 mins) desensitisation of AtT20-CB2 receptor signalling. Peak hyperpolarisation was determined within 5 mins of drug addition and peak depolarisation was determined at 30 mins of drug addition. All data are expressed as mean change in florescence due to cellular hyperpolarisation ± SEM, after subtraction of baseline (n=6).

(Note: truncated axes)