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Chapter 7

Development of a Cannabinoid-based Photoaffinity Probe to Determine the Δ8/9-Tetrahydrocannabinol Protein Interaction Landscape in Neuroblastoma Cells
7.1 Introduction

Preparations of the plant Cannabis sativa have been used throughout history in various cultures as medicinal concoctions or therapeutics, as well as for recreational or religious purposes. In 1930, the isolation of cannabinol (CBN) and cannabidiol (CBD) as the first active substituents was achieved, which was followed by the discovery of Δ9-tetrahydrocannabinol (THC) in 1964. THC is the psychoactive constituent of marijuana and exists in two isomers: namely Δ9-THC and Δ8-THC, of which the latter is the most thermodynamically stable isomer. THC treatment has been associated with therapeutic effects, such as analgesia, relaxation and fatigue, appetite stimulation, antiemesis, and reduction of nausea. THC is used by patients suffering from multiple sclerosis (MS), cancer or AIDS. In addition, preclinical data of THC indicate beneficial effects in several animal models of Alzheimer’s, Parkinson’s, and Huntington’s disease. However, THC is also associated with many undesirable side effects, including induction of psychoactivity, anxiety, memory loss, cardiac arrhythmias and addiction.

Both Δ9-THC and Δ8-THC have similar affinity to the cannabinoid receptor type 1 (CB1R) and type 2 (CB2R). The CB1R is the most abundant G protein-coupled receptor (GPCR) in the mammalian brain, whereas the CB2R is predominantly present in peripheral tissues and cells of the immune system. Most of the physiological effects of THC are mediated via the CB1R and CB2R as demonstrated by the use of specific CB receptor antagonists or genetically modified mice that lack the CB receptors. It is, however, hypothesized that THC may have other non-CB receptor targets. A study, using CB1R and CB2R knockout mice, showed similar analgesia upon THC administration as compared to the equivalent wild-type mice in the tail-flick test. This effect was not observed in the hotplate test, which requires spinal processing of nociceptive information. These observations suggest the existence of another protein target in the brain. Previously, orphan GPCRs GPR55 and GPR18 and peroxisome proliferator-activated receptor gamma (PPARγ) were identified to bind to THC, but it is unclear whether these targets are responsible for some of the physiological effects of THC. Therefore, a more complete view of the protein interaction of THC in neuronal cells is desirable.

Photoaffinity-based protein profiling (pA/BPP) has been previously used to map the protein interaction landscape of small molecules. Photoaffinity probes employ a light-responsive element to covalently crosslink the compound with its target protein upon irradiation. To circumvent the problems associated with large reporter groups, photoaffinity probes with a bioorthogonal ligation handle (e.g. alkyne), to introduce a fluorescent or affinity tag (e.g. biotin) after crosslinking to a protein, have emerged as powerful tools to visualize small molecule-protein interactions in living systems (see Chapter 2). In Chapter 6, two-step pA/BPP was used to capture and visualize the CB2R on human cells. Here, it was envisioned that two-step pA/BPP could be used to map the THC interaction landscape in neuroblastoma cells.
To this end, photoaffinity probe 1 (Figure 1), a Δ⁸-THC analog carrying a diazirine as the photoreactive moiety and a terminal alkyne as the ligation handle, was developed. Probe 1 was synthesized in 14 steps and was found to have high affinity for both CBRs. The protein interaction landscape of THC was mapped in Neuro2A cells (a fast-growing neuroblastoma cell line with several neuronal properties), in which four putative novel targets of THC were identified.

![Figure 1. Design of photoaffinity probe 1. The photo-reactive diazirine is highlighted in red, the alkyne ligation handle in blue.](image)

### 7.2 Results and Discussion

#### 7.2.1 Synthesis of photoaffinity probe 1

To identify the best position in THC to introduce the photoreactive group and the ligation tag, an analysis of previously reported structure-activity relationship data of THC analogs was conducted. This led to the design of probe 1, which contains a diazirine and ligation handle on the alkyl side chain of THC. An advantage of this design is the direct coupling of the bifunctional side chain as “minimalist linker”.

The synthesis of probe 1 commenced with reduction of commercially available 3,5-dihydroxybenzoic acid 2 to corresponding benzyl alcohol 3 in near-quantitative yield, using dimethyl sulfide complex of borane, along with co-reagent trimethoxyborate (Scheme 1). Benzyl alcohol 3 was oxidized to aldehyde 4 using a stoichiometric amount of Jones reagent, which prevented overoxidation to the benzoid acid. Protection of the aldehyde was performed under Lewis acidic conditions, which resulted in 1,3-dithiolane 5 in excellent yield. Electrophilic aromatic substitution of resorcinol derivative 5 under acidic conditions with the commercially available chiral monoterpenic (S)-cis-verbenol yielded bicyclic intermediate 6 in moderate yield. The tricyclic intermediate 7 was obtained in moderate yield by ring-closing rearrangement of bicyclic dithiolane 6, due to the generation of side products. Δ⁸-THC was synthesized in two steps from olivetol and (S)-cis-verbenol using the same procedures (Scheme 2, Experimental section), in a similar yield, and comparable to literature. Intermediate 7 was deprotected by Ag(I) salts, using a AgNO₃/wet EtOH system. Overoxidation of the resulting aldehyde to the equivalent benzoic acid was prevented using a modified workup, comprised of additional washing steps with 10 wt. % Na₂SO₃ (aq.), on top of the sole filtration step described in the literature. The resulting aldehyde was not isolated but subjected directly to phenol protection with TBS ether, to yield aldehyde 8 in excellent yield over two steps.
Reduction of 8 to benzyl alcohol 9 with LiBH₄ proceeded with near-quantitative yield, and a subsequent Appel reaction afforded benzyl bromide 10 in excellent yield. Benzyl mercaptan 11 was obtained by substitution of the bromide by thiourea, followed by cleavage of the amidine moiety from the sulfur atom with NaOH (aq).

The synthesis of minimalist linker 17 started with the functionalization of commercially available ethyl acetoacetate 12 to propargyl ketoester 13 via generation of the dienolate under strongly basic conditions, followed by regiospecific electrophilic attack by propargyl bromide. Ketoester 13 was then protected with ethylene glycol to the corresponding ketal, with azeotropic removal of water under acidic conditions, followed by direct reduction of the ester group with LiAlH₄, afforded corresponding alcohol 14 with excellent yield over two steps.

Scheme 1. Synthesis of probe 1. Reagents and conditions: a) BH₃Me₂S, B(OMe)₃, THF, rt, 16 hr, 99%; b) CrO₃, H₂SO₄, Acetone, 0°C, 10 min, 86%; c) 1,2-ethanediol, BF₃·Et₂O, THF, rt, 16 hr, 99%; d) (S)-cis-verbenol, CSA, CHCl₃, rt, 2 hr, 60%; e) BF₃·Et₂O, DCM, 0°C-rt, 1.5 hr, 62%; f) AgNO₃, EtOH/H₂O (10:1), rt, 18 h; g) TBSCI, imidazole, DCM, rt, 3 hr, 87% (yield over 2 steps); h) LiBH₄, THF, rt, 30 min, 99%; i) CBr₄, PPh₃, DCM, rt, 1 hr, 98%; j) Thiourea, ETOH, 40°C, 1 hr; k) 1 M NaOH (aq.), ETOH, rt, 1 hr, 80% (2 steps); l) LDA, THF, -40°C, 30 min; then propargyl bromide, 0°C, 1 hr, 76%; m) ethylene glycol, TsOH, PhMe, reflux in Dean-Stark apparatus, 3 h; n) LiAlH₄, THF, 0°C, 1 hr, 92% (2 steps); o) TsOH, 19:1 acetone/H₂O, 50°C, 2 hr, 98%; p) NH₃ (l), reflux, 5 h; then NH₂SO₃H in MeOH, rt, 16 h; q) Li₃, Et₃N, DCM, 0°C, 83% (2 steps); r) Li₃, PPh₃, imidazole DCM, rt, 1 hr, 90%; s) K₂CO₃, 2:1 THF/DMF, 30°C, 22 h; t) TBAF, THF, 0°C, 15 min, 84% (2 steps).
Deprotection of ketal 14 afforded ketone 15 in a near-quantitative yield, which was next functionalized by refluxing in liquid NH₃, followed by addition of hydroxylamine-O-sulfonic acid. The resulting crude diaziridine was subsequently oxidized to diazirine 16 using molecular iodine in mild basic conditions and was obtained in high yield over two steps. 16 then underwent a modified Appel reaction to generate minimalist linker 17 as alkyl iodide, in excellent yield.

Finally, minimalist linker 17 was coupled overnight at 30°C to resorcinol mercaptan 11 using K₂CO₃ in a 2:1 THF/DMF solvent system and the crude sulfide underwent rapid TBS ether deprotection in the presence of TBAF, affording target probe 1 in high yield over two steps. Overall, probe 1 was synthesized from commercially available 3,5-dihydroxybenzoic acid 2 in 14 steps, with a total yield of 18%.

7.2.2 CBR binding affinity of probe 1
To test the affinity of probe 1 on both the CB₁R and CB₂R, a [³H]CP55940 displacement assay was used (Figure 2). Probe 1 bound to the CB₁R and CB₂R with a pKᵢ value of 8.5 ± 0.1 and 8.0 ± 0.4, respectively, which is similar as previously reported for Δ⁹-THC and Δ⁸-THC.¹⁴,¹⁵

![Figure 2. CBR binding affinity of probe 1. Binding affinity of probe 1 was measured on membrane fractions of (A) CB₁R- or (B) CB₂R-overexpressing CHO cells, using previously described [³H]CP55940 displacement assays (Chapter 3).](image)

7.2.3 Two-step photoaffinity labeling of CB₁R and CB₂R
The ability of probe 1 to label CBRs in membranes of CB₂R- or CB₁R-overexpressing CHO cells was tested using a two-step photoaffinity labeling assay for gel-based imaging as previously described in Chapter 6. Probe 1 at a concentration of 2 μM, which is more than sufficient to fully occupy the binding site of the receptors, did not label either one of the CBRs (Figure 3). Of note, positive control LEI121, a CB₂R-selective photoaffinity probe developed in Chapter 6, did show profound labeling of CB₂R. This may indicate that the diazirine of probe 1 is not properly aligned with the amino acid residues in the binding site of CB₁R and CB₂R to form a covalent bond with the protein.
Figure 3. Gel-based analysis of two-step photo-affinity labeling efficiency of probe 1 and LEI121. Probe 1 was not able to covalently label the CBRs in membranes from (A) CB2R- or (B) CB1R-overexpressing CHO cells, whereas LEI121 (Chapter 5) specifically labeled CB2R (A).

7.2.4 Chemoproteomic profiling of THC protein targets using probe 1

Next, the ability of probe 1 as a chemical tool to identify protein targets of THC was evaluated. Live Neuro2A cells (a fast-growing neuroblastoma cell line with neuronal properties and therefore a suitable test-system) were incubated with probe 1 (10 μM). Vehicle-treated and non-irradiated cells were used as control. Ligation with biotin-N₃ for affinity enrichment on avidin agarose beads enabled identification of nearly 800 proteins by mass spectrometry-based proteomics (Figure 4A), based on two independent experiments. Nearly 200 proteins were more than two-fold enriched by probe 1 compared to the untreated control, of which ~50 proteins were also found in the “CRAPome” database (Contaminant Repository for Affinity Purification). The CRAPome database constitutes a list of frequently identified proteins (e.g. ribosomal proteins or histones) in photoaffinity labeling experiments regardless of the type of probe. These CRAPome proteins can, therefore, be considered as false positives, suggesting that nearly 150 unique probe targets were identified. Gene ontology analysis revealed that protein targets of probe 1 are mostly located in the endoplasmic reticulum (ER), mitochondria and membranes or in the cytoplasm (Figure 4B). The proteins are mostly associated with energy metabolism and protein transport (Figure 4C). Probe targets that were more than five-fold enriched are shown in Table S1. To assess which of the probe targets also interact with THC, competition experiments with probe 1 (1 μM) and Δ⁸-THC (10 μM) or Δ⁹-THC (10 μM) were performed. This resulted in one putative protein target of Δ⁸-THC (Cox4i1) and three for Δ⁹-THC (Reep5, Mtch2, Gnb1) (Figure 4A,D (red dots)) for which the labeling of the protein by probe 1 was reduced by 40-70% (Figure 4E, Table S2). It should be noted that putative protein target Reep5 was enriched only 1.5-fold by probe 1, but is listed because it had the largest reduction after THC-pretreatment (69 ± 6%).
Cox4i1 is involved in energy metabolism, whereas Reep5, Mtc2, Gnb1 are associated with protein modification and transport, energy metabolism, apoptosis and DNA maintenance, or signal transduction, respectively (Table 1). Interestingly, these four putative protein targets are associated with various neurological diseases as reported in the KEGG and OMIM database (Table 2).37, 38

Figure 4. Proteomic analysis of proteins targeted by probe 1. A) Representative plot showing the level of enrichment by probe 1 after UV-irradiation. B-C) Pie charts showing gene ontology analysis of the cellular location (B) and cellular function (C) of identified probe targets (N=2, n=2). D) Volcano plots showing the fold change in abundance of probe targets after pretreatment with either Δ⁸-THC and Δ⁹-THC (ratio THC pretreated samples over non-competition samples). Proteins of which the abundance were >40% lowered by THC (p-value <0.05) are shown in red. Statistics performed was an unpaired student’s t-test. E) Inhibition of putative protein targets by Δ⁸-THC and Δ⁹-THC (Mean ± SEM, N=3, n=3).
Table 1. THC protein targets as identified by competitive proteomics. Data from this table is shown in Figure 4. Inhibition data is the mean ± SEM (N=3, n=3). Gene ontology data is derived from the Uniprot database, combined with the DAVID Bioinformatics Database.

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Protein name</th>
<th>Inhibition ± SEM</th>
<th>Mitochondrion</th>
<th>Membrane</th>
<th>Extracellular</th>
<th>Cytoplasmic</th>
<th>Synaptic vesicle</th>
<th>Endoplasmic reticulum</th>
<th>Nuclear</th>
<th>RNA maintenance</th>
<th>DNA maintenance</th>
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<tbody>
<tr>
<td>Δ²-THC</td>
<td>Reep5</td>
<td>69 ± 6</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
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<td>x</td>
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<tr>
<td></td>
<td>Mitch2</td>
<td>56 ± 7</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
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<tr>
<td></td>
<td>Gnb1</td>
<td>42 ± 8</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
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<td></td>
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<tr>
<td>Δ⁴-THC</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Cox4i1</td>
<td>46 ± 9</td>
<td>x</td>
<td>x</td>
<td>x</td>
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<td></td>
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<td></td>
<td>x</td>
</tr>
</tbody>
</table>

Table 2. Putative THC protein targets with hits in the KEGG and/or OMIM database. Inhibition data is the mean ± SEM (N=3, n=3). Putative protein targets were analyzed using the KEGG and OMIM database and were enriched ~2x or more after UV-irradiation.

<table>
<thead>
<tr>
<th>Gene name</th>
<th>KEGG pathway</th>
<th>OMIM database</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ²-THC</td>
<td>Reep5</td>
<td>Ras signaling pathway, Chemokine signaling pathway, PI3K-Akt signaling pathway, Circadian entrainment, Retinoic acid endocannabinoid signaling, Glutamatergic synapse, Cholinergic synapse, Serotoninergic synapse, GABAergic synapse, Dopaminergic synapse, Phototransduction, Morphine addiction, Alcoholism, Pathways in cancer</td>
</tr>
<tr>
<td></td>
<td>Gnb1</td>
<td></td>
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<tr>
<td>Δ⁴-THC</td>
<td>Cox4i1</td>
<td>Oxidative phosphorylation, Metabolic pathways, Cardiac muscle contraction, Non-alcoholic fatty liver disease (NAFLD), Alzheimer's disease, Parkinson's disease, Huntington's disease</td>
</tr>
</tbody>
</table>

7.3 Conclusions

The aim of this chapter was to identify unknown protein targets of THC using photo-affinity labeling and chemical proteomics. To this end, Δ⁸-THC-derived probe 1 was synthesized in 14 steps with a total yield of 18%. Probe 1 had nanomolar affinity for both CBRs, but was not able to covalently label the CBRs in an established gel-based photo-affinity labeling assay. Different positioning of the photoreactive group in the probe, e.g. on the tricyclic core of the scaffold, might allow the covalent capturing of CBRs. Photoaffinity labeling of the proteome of live Neuro2A cells resulted in the identification of ~150 target proteins. Competition studies with THC significantly reduced enrichment of four proteins by probe 1, which suggests that THC has a limited interaction profile in Neuro2A cells. Reep5, Mitch2 and Gnb1 were identified as putative protein targets of Δ⁸-THC, whereas Cox4i1 was targeted by Δ⁴-THC. These targets are mostly involved in protein handling, energy metabolism, apoptosis or DNA maintenance, which may suggest that long-term exposure of THC may affect a variety of (epigenetic) functions of brain cells. Of note, the affinity and functional activity of THC on these four proteins needs to be further validated in orthogonal experiments using recombinant expression systems.

Taken together, the identification of the putative protein hits described is a first step towards a better understanding of the protein interaction profile of THC, which could ultimately lead to the development of novel therapeutics based on THC.
7.4 Experimental Section

7.4.1 Chemistry

7.4.1.1 General remarks

All reactions were performed using air- or flame-dried glassware. Solvents were purchased from Sigma-Aldrich, dry solvents were analytically dried by storing them for 24 hr on activated molecular sieves. Use of dry solvents is mentioned explicitly. Reagents were purchased from Sigma-Aldrich, Acros Organics, and Merck and used without further purification. All moisture sensitive reactions performed under an Ar atmosphere are mentioned explicitly. 1H and 13C NMR spectra were recorded on a Bruker AV 400 MHz spectrometer at 400 and 100 MHz, respectively, using CDCl 3 or CD 3 OD as solvent, unless stated otherwise. Chemical shift values are reported in ppm with TMS or solvent resonance as the internal standard (CDCl 3 /TMS, δ 0.00 for 1H (TMS), δ 77.16 for 13C (CDCl 3 ); CD 3 OD, δ 3.31 for 1H, δ 49.00 for 13C). Data are reported as follows: chemical shifts (δ) in ppm, multiplicity (s = singlet, d = doublet, dd = doublet of doublet, ddd = doublet of doublet of doublet, dt = doublet of triplet, t = triplet, td = triplet of doublet, qt = quartet, bs = broad singlet, m = multiplet), coupling constants (Hz), and integration. High resolution mass spectra were recorded on a Thermo Scientific LTQ Orbitrap XL. Liquid Chromatography was performed on a Finnigan Surveyor LC/MS system, equipped with a C18 column. TLC analysis was performed on Merck silica gel 60/Kieselguhr F254, 0.25 mm TLC plates. Compounds were visualized by UV irradiation or with a KMnO 4 stain (K 2 CO 3 (40 g), KMnO 4 (6 g), and H 2 O (600 mL)). Molecules shown are drawn using the Chemdraw Professional 16.0.

7.4.1.2 Synthetic procedures to photoaffinity probe 1 (scheme 1)

3,5-Dihydroxybenzal alcohol (3): A flame-dried 500 mL round bottom flask was charged with a magnetic stirring bar, purged with Ar, and borane-dimethylsulfide complex (18.8 mL, 100 mmol, 3 eq), along with trimethoxy borate (35.6 mL, 313.2 mmol, 4.7 eq) and dry THF (30 mL) were added at room temperature. The flask was purged with Ar again and 3,5-dihydroxybenzoic acid 2 (10.28 g, 66.6 mmol, 1 eq) in dry THF (50 mL) was added dropwise over 20 min at room temperature, throughout which rigorous hydrogen gas evolution occurred. The reaction was allowed to stir for 18h at room temperature. Upon completion MeOH (100 mL) was added dropwise, throughout which minor hydrogen gas and heat evolution occurred. The solution was filtered through celite, and the filtrate concentrated, and then subsequently coevaporated four times with MeOH (100 mL each), to give 3,5-dihydroxybenzyl alcohol 2 (9.31 g, 66.3 mmol, 99%) as white/gray amorphous crystals. R: 0.5 (50% EtOAc/pentane). 1H NMR (400 MHz, MeOD) δ 6.32 (d, J = 2.2 Hz, 2H), 6.18 (t, J = 2.2 Hz, 1H), 4.47 (s, 2H).

3,5-Dihydroxybenzaldehyde (4): A 500 mL round bottom flask was charged with a magnetic stirring bar, and benzyl alcohol 3 (7.82 g, 55 mmol, 1 eq) and acetone (340 mL) was added. The solution was cooled to 0°C using an ice bath, upon which freshly made 0.9 M Jones reagent (58.5 mL, 52.5 mmol, 1.05 eq) was added dropwise over 10 min. The reaction was stirred for an additional 10 min at 0°C, upon which iPrOH was added (5 mL) and the reaction stirred an additional 5 min, until all yellow color had disappeared, indicating full reduction of residual CrO 3. The reaction was diluted with Et 2O (1.5 L), and transferred to a separating funnel. The organic layer was washed with a 1:1 (v/v) solution of sat. NaHCO 3/brine (150 mL) and then washed successively with brine (8 x 150 mL). The organic layer was dried over MgSO 4 and concentrated, to give 3,5-dihydroxybenzaldehyde 4 (6.53 g, 47.3 mmol, 86%) as light brown amorphous crystals. R: 0.4 (40% EtOAc/pentane). 1H NMR (400 MHz, MeOD) δ 9.77 (s, 1H), 6.79 (d, J = 2.2 Hz, 2H), 6.55 (t, J = 2.2 Hz, 1H).

2-(3,5-Dihydroxyphenyl)-1,3-dithiolane (5): A 500 mL round bottom flask was charged with a magnetic stirring bar, and aldehyde 4 (2.9 g, 21 mmol, 1 eq), and purged with Ar. Dry THF (15 mL) was added, and shortly after dry DCM (180 mL) and 1,2-ethanediithiol (2.65 mL, 31.51 mmol, 1.5 eq) were added. BF 3 ·Et 2 O (0.95 mL, 6.93 mmol, 0.33 eq) was added dropwise, upon which the reaction was allowed to stir for 16h at room temperature. The reaction was quenched with sat. NaHCO 3 (200 mL), and transferred to a separating funnel.
The pH of the aqueous layer was adjusted to pH 7 with 1 M HCl aq. solution, and the subsequently extracted with DCM (2 x 200 mL), and with EtOAc (200 mL). The combined organic layers were dried over MgSO₄, and concentrated. The resulting brown syrup was dissolved in tBuOMe (20 mL), cooled in an ice bath, and ice-cold hexane (200 mL) was added. The slurry was filtered and the solids washed generically with ice-cold hexane (100 mL), to give 5 (4.56 g, 21 mmol, 99%) as off-white flaky crystals. Rr: 0.5 (40% EtOAc/pentane). ¹H NMR (400 MHz, MeOD) δ 6.47 (d, J = 2.1 Hz, 2H), 6.15 (t, J = 2.1 Hz, 1H), 5.50 (s, 1H), 3.49 – 3.42 (m, 2H), 3.33 – 3.27 (m, 2H).

5-(1,3-Dithiolan-2-yl)-2-((1R,2S,5S)-4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-yl)benzene-1,3-diol (6): A 500 mL round bottom flask was charged with a magnetic stirring bar, dithiolane 5 (2.2 g, 10.3 mmol, 1 eq), and purged with Ar. Dry CHCl₃ (90 mL) was added, along with anhydrous camphorsulfonic acid (0.26 g, 1.03 mmol, 0.1 eq), and the flask purged with Ar again. (S)-cis-Verbenol (1.73 g, 11.35 mmol, 1.1 eq, 50% ee) in dry CHCl₃ (10 mL) was added dropwise, and the reaction allowed to stir at room temperature for 3 h. Upon completion the reaction was quenched with an aqueous solution of 1:4 (v/v) sat. NaHCO₃ /brine (450 mL), and was transferred to a separating funnel. The pH of the aqueous layer was adjusted to pH 7 with 1 M HCl aq. solution, and subsequently extracted with CHCl₃ (2 x 120 mL) and with EtOAc (120 mL). The combined organic layer was dried over MgSO₄, and concentrated. After concentration, the crude residue (~4 g) was purified by flash column chromatography (150 g silica), eluting with 10% EtOAc/pentane (8 CV) to give 6 (2.17 g, 6.24 mmol, 60%) as a viscous yellow oil, which forms a foamy amorphous white solid under reduced pressure at room temperature. Rr: 0.65 (20% EtOAc/pentane). ¹H NMR (400 MHz, CDCl₃) δ 5.68 (d, J = 1.3 Hz, 1H), 5.48 (s, 1H), 3.92 (dd, J = 5.0, 2.5 Hz, 1H), 3.46 – 3.43 (m, 2H), 3.33 – 3.30 (m, 2H), 2.33 – 2.23 (m, 2H), 2.19 – 2.16 (m, 1H), 1.85 (s, 3H), 1.50 – 1.46 (m, 1H), 1.32 (s, 3H), 0.95 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 153.2, 140.5, 116.3, 115.0, 55.8, 48.0, 47.1, 40.9, 40.2, 38.1, 28.0, 26.1, 23.9, 20.6. LC-MS (ESI+) m/z: calculated for C₂₀H₂₅O₂S₂ [M + H]⁺: 349.13, found 349.07.

(6aR,10aR)-3-(1,3-Dithiolan-2-yl)-6,6,9-trimethyl-6a,7,10a-tetrahydro-6H-benzo[c]chromene-1-ol (7): A flame-dried 1000 mL round bottom flask was charged with a magnetic stirring bar, bicyclic resorcinol derivative 6 (5.75 g, 16.51 mmol, 1 eq) was added. The flask was purged with Ar, and dry DCM (375 mL) was added, and the solution cooled to 0°C. BF₃·Et₂O (4.2 mL, 33 mmol, 2 eq) was added dropwise over 5 min, upon which the reaction was allowed to warm to room temperature, and was stirred for 1.5 h. Upon completion the reaction was quenched with an aqueous solution of 1:4 (v/v) sat. NaHCO₃ /brine (450 mL), and was transferred to a separating funnel. The pH of the aqueous layer was adjusted to pH 7 with 1 M HCl aq. solution, and subsequently extracted with CHCl₃ (2 x 450 mL). The combined organic layer was dried over MgSO₄, and concentrated. After concentration, the crude residue (~6 g) was purified by flash column chromatography (225 g silica), eluting first with 6% EtOAc/pentane (6 CV), then 8% EtOAc/pentane (8 CV) to give 7 (3.39 g, 10.3 mmol, 62%) as a viscous dark yellow oil, which forms a foamy amorphous yellow solid under reduced pressure at room temperature. Rr: 0.55 (1% TFA/DCM). ¹H NMR (400 MHz, CDCl₃) δ 6.53 (s, 2H), 6.48 (d, J = 1.1 Hz, 1H), 5.48 (s, 1H), 5.41 (d, J = 3.8 Hz, 1H), 5.26 (s, 1H), 3.49 – 3.37 (m, 2H), 3.35 – 3.25 (m, 2H), 3.19 (dd, J = 16.1, 3.5 Hz, 1H), 2.69 (td, J = 10.8, 4.6 Hz, 1H), 2.13 (dd, J = 11.0, 3.5 Hz, 1H), 1.81 (m, 3H), 1.69 (s, 3H), 1.37 (s, 3H), 1.09 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 155.3, 155.0, 140.2, 134.8, 119.4, 113.2, 109.9, 106.7, 77.13, 55.8, 44.8, 40.2, 40.1, 35.8, 31.80, 28.0, 27.6, 23.6, 18.7. LC-MS (ESI+) m/z: calculated for C₂₀H₂₅O₂S₂ [M + H]⁺: 349.13, found 349.07.

(6aR,10aR)-1-(((tert-Butyldimethylsilyl)oxy)-6,6,9-trimethyl-6a,7,10a-tetrahydro-6H-enzo[c]chromene-3-carbaldehyde (8): A 100 mL round bottom flask was charged with a magnetic stirring bar, tricyclic dithiolane 7 (482 mg, 1.38 mmol, 1 eq), and EtOH (40 mL). AgNO₃ (756 g, 4.43 mmol, 3.2 eq) was added, followed by millipore H₂O (4 mL), and the flask was sealed with a septum and allowed to stir at room temperature for 18 hr, upon which the reaction was diluted with EtOAc (75 mL), and filtered through celite, washing solids with additional EtOAc (50 mL). The combined filtrate was transferred to a separating funnel and washed with an aqueous solution of 1:1 (v/v) 10% Na₂SO₃/Brine (2 x 50 mL), then with H₂O (50 mL), and brine (50 mL).
The organic layer was dried over MgSO₄ and concentrated. The crude aldehyde was subsequently dissolved in dry DMF (4 mL) and transferred to a 10 mL round bottom flask, and purged with Ar. tert-Butylidimethylsilyl chloride (243 mg, 1.6 mmol, 1.25 eq) was added, followed by imidazole (217 mg, 3.2 mmol, 2.5 eq). The reaction was purged again with Ar and stirred for 3 h at room temperature. Upon completion the reaction was quenched with 0.2 M HCl (25 mL), EtOAc (25 mL) was added, and transferred to a separating funnel. The layers were separated, and the aqueous layer was extracted again with EtOAc (25 mL). The combined organic layer was washed with H₂O (20 mL), and brine (40 mL), and subsequently dried over MgSO₄ and concentrated. After concentration, the crude residue (~600 mg) was purified by flash column chromatography (20 g silica), eluting with 10% CHCl₃/pentane, to give aldehyde 8 (464 mg, 1.20 mmol, 87% over two steps) as a clear, viscous oil. Rf: 0.1 (10% CHCl₃/pentane). ¹H NMR (400 MHz, CDCl₃) δ 9.81 (s, 1H), 6.96 (d, J = 0.7 Hz, 1H), 6.87 (s, 1H), 5.43 (d, J = 2.8 Hz, 1H), 3.24 (dd, J = 16.5, 3.3 Hz, 1H), 2.66 (td, J = 10.8, 4.3 Hz, 1H), 2.24 – 2.05 (m, 1H), 1.81 (t, J = 10.9 Hz, 3H), 1.69 (s, 3H), 1.40 (s, 3H), 1.08 (s, 3H), 1.01 (s, 3H), 0.32 (s, 3H), 0.18 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 191.8, 156.0, 155.7, 135.9, 134.7, 124.9, 124.7, 119.5, 114.2, 110.1, 77.3, 45.2, 37.4, 35.7, 33.0, 30.5, 29.8, 28.1, 27.4, 26.0, 25.9, 23.4, 18.4, -3.5, -4.3.

((6aR,10aR)-1-((tert-Butyldimethylsilyl)oxy)-6,6,9-trimethyl-6a,7,10a-tetrahydro-6H-benzo[c]chromen-3-yl) methanol (9): A flame-fried 10 mL round bottom flask was charged with a magnetic stirring bar, aldehyde 8 (193 mg, 0.5 mol, 1 eq), and the flask was purged with Ar. Dry THF was added (2 mL) and the flask cooled to 0°C in an ice water bath. 2 M LiBH₄ in THF (0.375 mL, 0.75 mmol, 1.5 eq) was added dropwise, upon which the reaction was allowed to warm to room temperature, and was stirred for 30 min. Upon completion the reaction was quenched with H₂O (50 mL), and transferred to a separating funnel. The aqueous layer was extracted with EtO₂ (3 x 40 mL), and the combined organic layer dried over MgSO₄ and concentrated. After concentration, the residue (~200 mg) was filtered through a short pad of silica (5 g), eluting with CHCl₃ to give primary alcohol 9 (192 mg, 495 μmol, 99%) as a turbid, colorless syrup. Rf: 0.4 (CHCl₃). ¹H NMR (400 MHz, CDCl₃) δ 6.46 (d, J = 1.2 Hz, 1H), 6.39 (d, J = 1.2 Hz, 1H), 5.41 (d, J = 3.6 Hz, 1H), 4.53 (s, 2H), 3.30 – 3.14 (m, 1H), 2.59 (td, J = 10.8, 4.2 Hz, 1H), 2.26 – 2.02 (m, 1H), 1.91 – 1.57 (m, 4H), 1.68 (s, 3H), 1.37 (s, 3H), 1.07 (s, 3H), 1.00 (s, 3H), 0.27 (s, 3H), 0.15 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 155.5, 155.1, 140.4, 135.0, 119.4, 116.7, 109.9, 109.4, 76.9, 65.3, 45.4, 36.1, 32.5, 28.2, 27.5, 26.1, 23.5, 18.4, -3.4, -4.2.

((6aR,10aR)-3-(Bromomethyl)-6,6,9-trimethyl-6a,7,10a-tetrahydro-6H-benzo[c]chromen-1-yl)oxy) (tert-butyl)dimethylsilylane (10): A 10 mL round bottom flask was charged with a magnetic stirring bar, 9 (192 mg, 495 μmol, 1 eq), DCM (2.5 mL) and Cb₃ (172 mg, 519 μmol, 1.05 eq). The flask was cooled to 0°C in an ice water bath, and PPh₃ (136 mg, 0.519 mmol, 1.05 eq) was added. The reaction was purged with Ar, and allowed to come to room temperature, and stirred for 1 h. Upon completion the reaction was concentrated under reduced pressure, and hexane (1 mL) was added. The resulting slurry was purified by flash column chromatography (15 g silica), eluting first with pentane (6 CV), then with 25% Et₂O/pentane (6 CV) to give 10 (218 mg, 483 μmol, 98%) as a clear, viscous oil. Rf: 0.35 (pentane). ¹H NMR (400 MHz, CDCl₃) δ 6.48 (d, J = 1.6 Hz, 1H), 6.40 (d, J = 1.5 Hz, 1H), 5.41 (d, J = 2.5 Hz, 1H), 4.41 – 4.27 (m, 2H), 3.22 (dd, J = 16.6, 3.6 Hz, 1H), 2.58 (td, J = 10.8, 4.2 Hz, 1H), 2.20 – 2.05 (m, 1H), 1.84 – 1.72 (m, 3H), 1.63 (s, 3H), 1.37 (s, 3H), 1.04 (s, 3H), 1.00 (s, 9H), 0.28 (s, 3H), 0.15 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 155.2, 155.0, 136.7, 135.0, 119.4, 117.8, 112.1, 111.6, 76.9, 45.3, 36.0, 33.9, 32.5, 28.2, 27.5, 26.1, 23.5, 18.5, -3.4, -4.2.

((6aR,10aR)-1-((tert-Butyldimethylsilyl)oxy)-6,6,9-trimethyl-6a,7,10a-tetrahydro-6H-benzo[c]chromen-3-yl)methanethiol (11): A 10 mL round bottom flask was charged with a magnetic stirring bar, bromide 10 (31 mg, 68 μmol, 1 eq), and EtOH (1.3 mL). Thiourea (10 mg, 134 μmol, 2 eq) was added, the reaction heated to 40°C in a warm water bath, and stirred for 1 h. Upon completion, the reaction was cooled, dry N₂ gas was bubbled through the reaction for 5 min, and subsequently 1 M NaOH (0.2 mL) was added, and the reaction was stirred another 1 h. Upon completion the reaction was quenched with 0.1 M HCl (10 mL), and transferred to a separating funnel. The aqueous layer was extracted with EtO₂ (20 mL), and the organic layer was washed with sat. NaHCO₃ (10 mL), H₂O (10 mL), brine (10 mL), dried with MgSO₄, and concentrated.
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After concentration, the crude residue (~25 mg) was purified by flash column chromatography (2 g silica, eluting first with pentane (4 CV), then 5% CHCl₃/pentane (8 CV), to give 11 (22 mg, 54 μmol, 80% over two steps) as a turbid, viscous oil. Rf: 0.5 (5% CHCl₃/pentane). H NMR (400 MHz, CDCl₃) δ 6.41 (d, J = 1.7 Hz, 1H), 6.35 (d, J = 1.7 Hz, 1H), 5.41 (d, J = 3.8 Hz, 1H), 3.66 – 3.53 (m, 2H), 3.22 (dd, J = 16.6, 4.3 Hz, 1H), 2.57 (td, J = 10.9, 4.3 Hz, 1H), 2.23 – 2.07 (m, 1H), 1.92 – 1.68 (m, 3H), 1.68 (s, 3H), 1.36 (s, 3H), 1.07 (s, 3H), 1.00 (s, 9H), 0.27 (s, 3H), 0.15 (s, 3H). C NMR (100 MHz, CDCl₃) δ 155.02, 140.3, 135.0, 119.4, 116.2, 111.2, 110.5, 76.9, 45.4, 36.1, 32.4, 28.9, 28.2, 27.6, 26.1, 23.5, 18.5, -3.4, -4.2.

Ethyl 3-oxohept-6-ynoate (13): A flame-dried Schlenk tube was charged with a stirring bar and purged multiple times with Ar. Dry THF (30 mL) and then freshly distilled disopropylamine (9.71 mL, 69.28 mmol) were added, and the solution cooled to -78°C. 1.6 M nBuLi in hexanes (39.38 mL, 63 mmol) was added dropwise, and stirred for 15 min. The generated LDA solution (0.8 M by titration, 73 mL, 2.12 eq) was transferred via cannula to a flame-dried 250 mL round bottom flask. The flask was cooled to -40°C, upon which ethyl acetoacetate 12 (3.47 mL, 27.5 mmol, 1 eq) in dry THF (25 mL) was added dropwise. The reaction was stirred for 30 min, upon which propargyl bromide (80% in toluene, 3 mL, 28 mmol, 1.01 eq) was added dropwise, and the reaction was allowed to warm to 0°C and stirred for 1 h. Upon completion the reaction was quenched with 0.5 M HCl (200 mL), and transferred to a separating funnel. The aqueous layer was extracted with EtO (2 x 200 mL). The combined organic layers were washed with brine (100 mL), dried over MgSO₄, and concentrated. After concentration, the crude residue (~25 mg) was purified by flash column chromatography (2 g silica), eluting with pentane (4 CV), then 50% CHCl₃/pentane (9 CV) to give 13 (3.49 g, 20.8 mmol, 76%) as a clear oil. Rf: 0.4 (50% CHCl₃/hexane). H NMR (400 MHz, CDCl₃) δ 4.00 (m, 4H), 3.75 (t, J = 6.2 Hz, 2H), 2.73 (s, 1H), 2.42 (m, 2H), 1.97 (t, J = 2.7 Hz, 1H), 1.29 (t, J = 7.1 Hz, 3H). C NMR (100 MHz, CDCl₃) δ 200.7, 167.0, 90.2, 82.6, 69.1, 49.3, 41.7, 14.2, 12.9.

2-(2-(But-3-yn-1-yl)-1,3-dioxolan-2-yl)ethan-1-ol (14): A 50 mL round bottom flask was charged with a magnetic stirring bar, equipped with a Dean-Stark apparatus, and purged with Ar. 13 (383 mg, 2.27 mmol, 1 eq) in toluene (35 mL) was added, along with ethylene glycol (211 mg, 1.5 eq), followed by para-toluene sulfonic acid (39 mg, 0.23 mmol, 0.1 eq), and the reaction heated to reflux for 3 h. Upon completion, the reaction was quenched with sat. NaHCO₃ (25 mL), and diluted with EtOAc (25 mL), and transferred to a separating funnel. The organic layer was washed with H₂O (50 mL), brine (50 mL), dried over MgSO₄, and concentrated. The resulting amber syrup (4.80 g) was purified by fractional distillation (118°C, 15 mBar) to give 14 (3.35 g, 20.8 mmol, 76%) as a clear, viscous oil. Rf: 0.35 (50% CHCl₃/hexane). H NMR (400 MHz, CDCl₃) δ 4.20 (q, J = 7.1 Hz, 2H), 3.47 (s, 2H), 2.82 (t, J = 7.2 Hz, 2H), 2.53 – 2.43 (m, 2H), 1.97 (t, J = 2.7 Hz, 1H), 1.29 (t, J = 7.1 Hz, 3H). C NMR (100 MHz, CDCl₃) δ 200.7, 167.0, 90.2, 82.6, 69.1, 49.3, 41.7, 14.2, 12.9.

1-Hydroxyhept-6-yn-3-one (15): A 25 mL round bottom flask was charged with a magnetic stirring bar, and 14 (357 mg, 2.09 mmol, 1 eq) in acetone (9.5 mL) was added, followed by para-toluene sulfonic acid (99 mg, 0.52 mmol, 0.25 eq), and millipore H₂O (0.5 mL), and the reaction was heated to 50°C for 2 h. Upon completion the reaction was quenched with sat. NaHCO₃ (10 mL), and diluted with EtOAc (30 mL), and transferred to a separating funnel. The organic layer was washed with brine (20 mL), dried over MgSO₄, and concentrated. After concentration, the crude residue was filtered through a short pad of silica (~10 g), eluting with CHCl₃, to give 15 (258 mg, 2.05 mmol, 98%) as a pale yellow oil. Rf: 0.25 (CHCl₃). H NMR (400 MHz, CDCl₃) δ 3.87 (t, J = 5.5 Hz, 2H), 2.71 (dd, J = 9.1, 5.5 Hz, 4H), 2.55 – 2.40 (m, 3H), 1.97 (t, J = 2.5 Hz, 1H). C NMR (100 MHz, CDCl₃) δ 209.1, 82.9, 69.0, 57.8, 44.7, 41.9, 12.9.

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2-(3-(But-3-yn-1-yl)-3H-diazirin-3-yl)ethan-1-ol (16): A 50 mL amber three-necked flask was charged with a magnetic stirring bar, purged with Ar, and cooled to -50°C in an acetone-dry ice bath. NH₃ gas (5 mL) was condensed into the flask using a dry ice condenser, upon which 15 (255 mg, 2.04 mmol) in dry DCM (1 mL) was added dropwise. The reaction was allowed to warm to -40°C, and was stirred at reflux for 5 hr, upon which hydroxylamine-O-sulfonic acid (425 mg, 3.76 mmol, 1.83 eq) in dry MeOH (1 mL) was added dropwise. The reaction was kept at reflux for an additional 1 hr, and then allowed to warm to room temperature over 16 h.

Dry N₂ was subsequently bubbled through the reaction, allowing all excess NH₃ to evaporate, the reaction was filtered over celite, and the filter cake washed with dry MeOH (40 mL). The filtrate was concentrated under reduced pressure, and the crude diaziridine residue redissolved in DCM (2 mL) and transferred to a 10 mL round bottom flask, purged with Ar, and cooled to 0°C in an ice bath. Dry Et₃N (0.5 mL) was added, and a solution of I₂ (500 mg) in DCM (8 mL) was added dropwise over 1 hr until a brown/red color persisted for at least 0.5 h. Upon completion the reaction was quenched with 1 M HCl (3 mL), and diluted with EtOAc (40 mL) and transferred to a separating funnel. The organic layer was washed with aq. 10 wt. % (2 x 20 mL), brine (20 mL), dried over MgSO₄, and concentrated. After concentration, the crude residue (~250 mg) was purified by flash column chromatography (10 g silica), eluting with 75% CHCl₃/pentane (2 CV), 80% CHCl₃/pentane (4 CV), then CHCl₃ (4 CV) to give 16 (234 mg, 1.69 mmol, 83% over 2 steps) as a dark yellow oil. Rf: 0.4 (CHCl₃).

1H NMR (400 MHz, CDCl₃) δ 3.39 (t, J = 6.2 Hz, 2H), 2.14 – 1.95 (m, 3H), 1.85 (br s, 1H), 1.74 – 1.63 (m, 4H). 13C NMR (100 MHz, CDCl₃) δ 82.9, 69.4, 57.4, 35.6, 32.7, 26.7, 13.3.

3-(But-3-yn-1-yl)-3-(2-iodoethyl)-3H-diazirine (17): A 25 mL amber flask was charged with a magnetic stirring bar, 16 (234 mg, 1.69 mmol, 1 eq) in DCM (7.5 mL) was added. The flask was cooled to 0°C in an ice bath, and imidazole (345 mg, 5.07 mmol, 3 eq) was added, followed by I₂ (515 mg, 2.03 mmol, 1.2 eq) and PPh₃ (488 mg, 1.86 mmol, 1.1 eq). The reaction was purged with Ar, and allowed to come to room temperature and stirred for 1 hr. Upon completion the reaction was quenched with aq. 10 wt. % Na₂SO₃ (10 mL) and transferred to a separating funnel. The aqueous layer was extracted with CHCl₃ (3 x 20 mL). The combined organic layer was dried over MgSO₄ and concentrated. After concentration, the crude residue (~200 mg) was purified by flash column chromatography (25 g silica), eluting with pentane (6 CV), then 5% EtO/pentane (6 CV) to give 17 (378 mg, 1.52 mmol, 90%) as a clear oil. Rf: 0.25 (pentane). 1H NMR (400 MHz, CDCl₃) δ 2.90 (t, J = 7.6 Hz, 2H), 2.13 (t, J = 7.6 Hz, 2H), 2.07 – 1.96 (m, 3H), 1.69 (t, J = 7.1 Hz, 2H). 13C NMR (100 MHz, CDCl₃) δ 82.6, 77.4, 69.6, 37.7, 32.0, 28.8, 13.4, -3.9.

(6aR,10aR)-3-(((2-(3-(But-3-yn-1-yl)-3H-diazirin-3-yl)ethyl)thio)methyl)-6,6,9-trimethyl-6a,7,10,10a-tetrahydro-6H-benzo[c]chromen-1-ol (1): A 10 mL round bottom flask was charged with a magnetic stirring bar, tricyclic probe precursor 11 (20 mg, 49 µmol, 1 eq), and THF (0.5 mL), and the reaction cooled to 0°C in an ice water bath. Minimal linker 17 (19 mg, 75 µmol, 1.53 eq) in THF (0.5 mL) was added, followed by anhydrous K₂CO₃ (13.5 mg, 98 µmol, 2 eq), and DMF (0.5 mL). The reaction was purged with Ar again, allowed to come to room temperature, then warmed to 30°C and stirred for 22 h. Upon completion H₂O (10 mL) was added, and the reaction was transferred to a separating funnel. The aqueous layer was extracted with CHCl₃ (2 x 10 mL), and the combined organic layer washed with brine (10 mL), dried over MgSO₄, and concentrated. The crude silyl ether, was subsequently dissolved in THF (0.5 mL) and transferred to a 10 mL round bottom flask, purged with Ar, and cooled to 0°C in an ice water bath. 1 M TBAF in THF (98 µL, 98 µmol, 2 eq) was added, and the reaction stirred for 15 min at 0°C. Upon completion the reaction was quenched with H₂O (10 mL), and transferred to a separating funnel. The aqueous layer was extracted with EtO (10 mL) and the organic layer washed with brine (10 mL), dried over MgSO₄, and concentrated. After concentration, the crude residue (~25 mg) was purified by flash column chromatography (2 g silica), eluting first with 20% CHCl₃/pentane (4 CV), 40% CHCl₃/pentane (4 CV), then 50% CHCl₃/pentane (4 CV), to give probe 1 (17 mg, 41 µmol, 84% over two steps) as a clear, viscous oil. Rf: 0.3 (50% CHCl₃/pentane). 1H NMR (400 MHz, CDCl₃) δ 6.34 (d, J = 1.2 Hz, 1H), 6.26 (s, J = 1.6 Hz, 1H), 5.43 (d, J = 3.8 Hz, 1H), 4.87 (s, 1H), 3.52 (s, 2H), 3.19 (dd, J = 16.0, 4.0 Hz, 1H), 2.70 (td, J = 10.7, 4.5 Hz, 1H), 2.25 (m, 3H), 1.99 (m, 3H), 1.81 (t, J = 8.9 Hz, 3H), 1.70 (s, 3H), 1.62 (dd, J = 14.7, 7.5 Hz, 5H), 1.38 (s, 3H), 1.10 (s, 3H).
7.4.1.3 Synthetic procedures to $\Delta^8$-THC and $\Delta^9$-THC (scheme 2)

Scheme 2. Synthetic procedures to $\Delta^8$-THC and $\Delta^9$-THC. Reagents and conditions: a) (S)-cis-verbenol, CSA, CHCl$_3$, rt, 2 hr, 47%; b) BF$_3$·Et$_2$O, DCM, 0°C-rt, 1 hr, 60%; c) mCPBA, CHCl$_3$, 1 hr, -5°C, 91%, dr 55:45; d) PhSeZnCl, 91 H$_2$O/THF, rt, 2 hr, 46% (92% based on cis-isomer), dr 10:1; e) H$_2$O$_2$ (30%, aq.), THF, rt, 4h, 74%; f) BF$_3$·Et$_2$O, MgSO$_4$, DCM, 0°C, 2.5 hr, 60%.

5-Pentyl-2-((1R,2R,5S)-4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-yl)benzene-1,3-diol (19): A 10 mL round bottom flask was charged with a magnetic stirring bar, olivetol 18 (200 mg, 1.1 mmol, 1 eq), and purged with Ar. Dry CHCls (10 mL) was added, followed by anhydrous camphorsulfonic acid (racemic) (30 mg, 0.12 mmol, 0.1 eq) and the flask purged with Ar again. (S)-cis-Verbenol (186 mg, 1.22 mmol, 1.1 eq, 50% ee) was added, the reaction purged again with Ar, and allowed to stir for 3 hr at room temperature. Upon completion the reaction was quenched with an aqueous solution of 1:4 (v/v) sat. NaHCO$_3$/brine (10 mL), and transferred to a separating funnel. The pH of the aqueous layer was adjusted to pH 7 with 1 M HCl aq. solution, and subsequently extracted with DCM (2 x 15 mL). The combined organic layer was dried over MgSO$_4$ and concentrated. After concentration, the crude residue (~450 mg) was purified by flash column chromatography (18 g silica), eluting with 1% EtOAc/pentane (12 CV). 19 was obtained as a turbid, viscous oil (161 mg, 0.51 mmol, 47%). R$_f$: 0.5 (5% EtOAc/pentane). $^1$H NMR (400 MHz, CDCl$_3$) δ 6.20 (s, 2H), 5.70 (d, J = 1.4 Hz, 1H), 3.91 (d, J = 2.5 Hz, 1H), 2.48 – 2.39 (m, 2H), 2.36 – 2.22 (m, 2H), 2.18 (t, J = 5.3 Hz, 1H), 1.87 – 1.84 (m, 3H), 1.63 – 1.52 (m, 3H), 1.49 (d, J = 9.6 Hz, 1H), 1.32 (s, 1H), 1.32-1.26 (m, 4H), 0.96 (s, 3H), 0.89 (s, J = 6.9 Hz, 3H).

$\Delta^9$-Tetrahydrocannabinol (20): A flame-dried 10 mL round bottom flask was charged with a magnetic stirring bar and 19 (92 g, 292 µmol, 1 eq) was added. The flask was purged with Ar, and dry DCM (6 mL) was added, and the solution cooled to 0°C. BF$_3$·Et$_2$O (80 µl, 0.59 mmol, 2 eq) was added, upon which the reaction was allowed to warm to room temperature, and stirred for 1 h. Upon completion the reaction was quenched with sat. NaHCO$_3$ (10 mL), and was transferred to a separating funnel. The pH of the aqueous layer was adjusted to pH 7 with 1 M HCl aq. solution, and subsequently extracted with DCM (3 x 10 mL). The combined organic layer was dried over MgSO$_4$ and concentrated. After concentration, the crude residue (~100 mg) was purified by flash column chromatography (10 g silica), eluting first with pentane (4 CV), then 1% EtOAc/pentane (8 CV) to give 20 (55 mg, 175 µmol, 60%) as a clear viscous oil. R$_f$: 0.3 (4% EtOAc/pentane). $^1$H NMR (400 MHz, CDCl$_3$) δ 6.27 (d, J = 1.1 Hz, 1H), 6.10 (d, J = 1.3 Hz, 1H), 5.42 (d, J = 4.8 Hz, 1H), 4.68 (br s, 1H), 3.19 (dd, J = 15.8, 4.2 Hz, 1H), 2.70 (td, J = 10.8, 4.6 Hz, 1H), 2.44 (td, J = 7.3, 2.1 Hz, 2H), 2.23 – 2.01 (m, 1H), 1.83 (ddd, J = 24.5, 14.4, 4.0 Hz, 4H), 1.70 (s, 3H), 1.65 – 1.49 (m, 2H), 1.37 (s, 3H), 1.30 (dt, J = 14.9, 8.8 Hz, 5H), 1.10 (s, 3H), 0.88 (t, J = 6.9 Hz, 3H). $^{13}$C NMR (100 MHz, CDCl$_3$) δ 154.9, 154.8, 142.9, 134.9, 119.5, 110.7, 110.3, 107.8, 76.8, 45.0, 36.2, 35.6, 31.7, 31.6, 28.0, 27.7, 23.7, 22.7, 18.7, 14.2. LC-MS (ESI+) purity found >95%. HRMS (ESI+) m/z: calculated for C$_2$H$_3$O$_2$ [M + H]$^+$: 315.2319, found 315.2318.
(−)-Limonene Oxide (22): A 500 mL round bottom flask was charged with a magnetic stirring bar, (+)-limonene 21 (10 g, 7.34 mmol, ee: 98%, 1 eq), dry CHCl₃ (200 mL) and the flask was purged with Ar, and cooled to -10°C. meta-chloroperoxybenzoic acid (18.25 g, 74 mmol, 1.01 eq) in dry CHCl₃ (80 mL) was added dropwise over 1 h, keeping the temperature below -5°C at all times. Upon completion, the reaction was quenched with 1M NaOH (80 mL), and was transferred to a separating funnel. The organic layer was washed with sat. NaHCO₃ and the solution cooled to 0°C. The pH of the aqueous layer was adjusted to 8.5 with HCl and 64% saturated NaHCO₃, and was transferred to a separating funnel. The pH of the aqueous layer was adjusted to 2.5 with 1M HCl. Upon completion the reaction was filtered through a pad of celite, and the filtrate concentrated. After concentration, the crude residue (~11 g) was filtered through a short pad of silica (~50 g), eluting with CHCl₃, to give 22 (10.2 g, 67.01 mmol, 91%, dr: 55:45) as a clear oil. Rf: 0.45 (75% DCM/hexane). ¹H NMR (400 MHz, CDCl₃) δ 4.70 (d, J = 24.1 Hz, 2H), 3.14 – 2.91 (m, 1H), 2.21 – 1.96 (m, 2H), 1.92 – 1.78 (m, 2H), 1.74 – 1.61 (m, 4H), 1.59 – 1.45 (m, 1H), 1.38 (td, J = 9.7, 3.8 Hz, 1H), 1.32 (s, 3H, trans –CH₃), 1.31 (s, 3H, cis –CH₃), 1.27 – 1.14 (m, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 149.3, 149.1, 109.2, 109.1, 60.6, 59.4, 57.6, 57.5, 40.8, 36.3, 30.8, 30.8, 30.0, 28.7, 26.0, 24.4, 24.2, 21.2, 20.3.

(1S,2S,4R)-1-Methyl-2-(phenylselanyl)-4-prop-1-en-2-yl)cyclohexan-1-ol (23): A flame-dried 500 mL two-necked round bottom flask was charged with a magnetic stirring bar, Zn (5.48 g, 83.8 mmol, 1.1 eq), and dry THF (175 mL). The flask was equipped with a reflux condenser, and purged with Ar. PhSeCl (14.64 g, 76.2 mmol, 1 eq) was added carefully in 3 portions (vigorous heat evolution was observed) and the reaction was heated to reflux for 1 h, upon which the turbid orange suspension became gray. Upon completion the reaction was cooled, and excess Zn was filtered off. The filtrate was concentrated to roughly half its volume, and Et₂O was added (300 mL), upon which a white precipitate was formed. The precipitant PhSeZnCl (19.6 g) was collected via filtration, and transferred to a 500 mL round bottom flask, and dissolved in THF (30 mL). To this solution was added millipore H₂O (300 mL), and 22 (19.6 g, 76 mmol, 1 eq), and the reaction was stirred for 2 hr at room temperature. Upon completion the reaction was transferred to a separating funnel. The aqueous layer was extracted with DCM (3 x 250 mL), and the combined organic layer dried over MgSO₄, and concentrated. After concentration, the crude residue (~20 g) was purified by flash column chromatography (~350 g silica), eluting with 25% DCM/pentane (2 CV), 50% DCM/pentane (2 CV), then DCM (4 CV) to give 23 (10.8 g, 35.04 mmol, 46%, dr 10:1, 92% based on cis-epoxide) as a yellow oil. Rf: 0.55 (DCM). ¹H NMR (400 MHz, CDCl₃) δ 7.64 – 7.52 (m, 2H), 7.32 – 7.18 (m, 3H), 4.71 (d, J = 13.9 Hz, 2H), 3.43 (dd, J = 5.8, 2.4 Hz, 1H), 2.39 – 2.25 (m, 1H), 2.25 – 2.12 (m, 1H), 1.92 – 1.79 (m, 2H), 1.79 – 1.69 (m, 1H), 1.67 (s, 3H), 1.66 – 1.57 (m, 4H), 1.41 (s, 3H).

cis-p-Mentha-2,8-dien-1-ol (24): A 500 mL round bottom flask was charged with 23 (10.75 g, 34.8 mmol, 1 eq), and THF (350 mL). To this solution was added aq. 30 wt. % H₂O₂ (9.75 mL, 100 mmol, 3 eq), and the reaction was stirred for 4 hr at room temperature. Upon completion the reaction was diluted with an aqueous solution of 1:1 v/v H₂O/brine, and was transferred to a separating funnel. The aqueous layer was extracted with Et₂O (2 x 300 mL), and the combined organic layer dried over MgSO₄, and concentrated. After concentration, the crude residue (~9 g) was purified by flash column chromatography (250 g silica), eluting with 50% DCM/pentane (2 CV), 75% DCM/pentane (2 CV), then DCM (8 CV) to give 24 (3.92 g, 25.79 mmol, 74%) as a pale yellow oil. Rf: 0.3 (DCM). ¹H NMR (400 MHz, CDCl₃) δ 5.68 (m, 2H), 4.78 (dd, J = 3.1, 1.5 Hz, 1H), 4.76 – 4.73 (m, 1H), 2.70 – 2.62 (m, 1H), 1.89 – 1.76 (m, 2H), 1.74 (s, 3H), 1.68 – 1.53 (m, 3H), 1.30 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 148.3, 134.1, 132.3, 110.7, 67.6, 43.6, 36.9, 29.6, 25.0, 21.0.

Δ⁹-Tetrahydrocannabinol (25): A flame-dried 10 mL round bottom flask was charged with a magnetic stirring bar, olivetol 18 (180 mg, 1 mmol, 1 eq), and purged with Ar. Dry DCM (1 mL) was added, along with anhydrous MgSO₄ (375 mg, 3.1 mmol, 3.1 eq), and the flask purged with Ar again. Menthol dienol 24 (167 mg, 1.1 mmol, 1.1 eq), in dry DCM (2 mL) was added, and the flask was cooled to 0°C in an ice water bath. BF₃·Et₂O (65 μL, 0.5 mmol, 0.5 eq) was added dropwise, and the reaction was stirred at 0°C for 3 h. Upon completion the reaction was quenched with anhydrous NaHCO₃ (1 g). The reaction was allowed to stir for an additional 30 min, resulting in progressive loss of color, upon which the reaction was filtered through a pad of celite, and the filtrate concentrated.
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After concentration, the crude residue (~350 mg) was purified by flash column chromatography (15 g silica), eluting first with 1% EtO/pentane (2 CV), 2% EtO/pentane (2 CV), then 4% EtO/pentane (8 CV) to give 25 (60 mg, 127 µmol, 19%) as a clear, viscous oil. Rf: 0.8 (10% EtO/pentane). 1H NMR (400 MHz, CDCl3) δ 6.30 (s, 1H), 6.27 (d, J = 1.1 Hz, 1H), 6.14 (d, J = 1.3 Hz, 1H), 4.97 – 4.31 (m, 1H), 3.20 (d, J = 10.9 Hz, 1H), 2.43 (dd, J = 8.4, 6.3 Hz, 2H), 2.20 – 2.11 (m, 2H), 1.91-1.69 (m, 2H), 1.68 (s, 3H), 1.58 – 1.52 (m, 2H), 1.41 (s, 3H), 1.36 – 1.24 (m, 4H), 1.09 (s, 3H), 0.88 (5, J = 7.6, 3H). Rf: 0.8 (10% EtO/pentane). 13C NMR (100 MHz, CDCl3) δ 154.8, 154.2, 142.8, 134.4, 123.9, 110.1, 109.0, 107.5, 77.2, 45.8, 35.5, 33.6, 31.5, 31.2, 30.7, 27.6, 25.0, 23.4, 22.6, 21.7, 19.3, 14.0. LC-MS (ESI+) purity found >95%. HRMS (ESI+) m/z: calculated for C21H31O2 [M + H]+: 315.2319, found 315.2319.

7.4.2 Biology

7.4.2.1 General remarks

All common reagents were purchased from commercial sources and used as received. Probe 1, Δ²-THC and Δ⁴-THC were synthesized as described above. CYS-N3 was synthesized according to previously published procedures39 and biotin-N3 was purchased from Bio-Connect Life Sciences. [3H]CP55940 (specific activity 141.2 Ci/mmol) and GF-B/GF-C filters were purchased from Perkin Elmer (Waltham, MA). The CHO-K1 CNR1 and CNR2 cell lines were obtained from DiscoveRx. Cell culture plates were purchased from Sarstedt. Cannabinoid receptor ligands CP55940 and AM630 were obtained from Sigma Aldrich (St. Louis, MO), and rimonabant was obtained from F. Hoffmann-La Roche Ltd. (Basel, Switzerland). Reagents used for the pulldown procedure are: avidin-agarose from egg white (50% glycerol suspension from Sigma Aldrich), 10 x PBS (proteomics grade, obtained from F. Hoffmann-La Roche Ltd. (Basel, Switzerland). Reagents used for the pulldown procedure are: avidin-agarose from egg white (50% glycerol suspension from Sigma Aldrich), 10 x PBS (proteomics grade, obtained from F. Hoffmann-La Roche Ltd. (Basel, Switzerland). Reagents used for the pulldown procedure are: avidin-agarose from egg white (50% glycerol suspension from Sigma Aldrich), 10 x PBS (proteomics grade, obtained from F. Hoffmann-La Roche Ltd. (Basel, Switzerland). Reagents used for the pulldown procedure are: avidin-agarose from egg white (50% glycerol suspension from Sigma Aldrich), 10 x PBS (proteomics grade, obtained from F. Hoffmann-La Roche Ltd. (Basel, Switzerland). Reagents used for the pulldown procedure are: avidin-agarose from egg white (50% glycerol suspension from Sigma Aldrich), 10 x PBS (proteomics grade, obtained from F. Hoffmann-La Roche Ltd. (Basel, Switzerland).

7.4.2.2 Cell culture, membrane preparation and [3H]CP55940 displacement assay

The affinity of probe 1 on CBRs was determined on membrane fractions of CB₁R- or CB₂R overexpressing CHO cells, as described in Chapter 4.14

7.4.2.3 Two-step photoaffinity labeling, in-gel imaging

The ability of probe 1 to covalently label CBRs on membrane fractions of WT CHO, or CB₁R- or CB₂R overexpressing CHO cells was determined using two-step photoaffinity labeling, followed by ligation using CYS-N3, and gel-based imaging, as described in Chapter 6 (The exact same procedure was used with CB₁R membranes).

7.4.2.4 Chemoproteomic profiling of THC protein targets

Neuro2A cells were cultured at 37°C with 7% CO₂ in DMEM supplemented with 10% New Born Calf serum, 10% fetal calf serum, 1 mM glutamine, 50 µg/mL penicillin and 50 µg/mL streptomycin and passaged twice a week. Cells were washed with PBS, then pretreated in PBS, containing 1 mM MgCl₂ and 1 mM CaCl₂, with or without 10 µM THC, for 30 min at 37°C. Then, 1 or 10 µM probe 1 (or the same amount of DMSO for the untreated control) was added (final concentration in a total volume of 3 mL) and incubated for 30 min at 37°C. The solution was removed from the cells and replaced by 1.5 mL PBS containing 1 mM MgCl₂ and 1 mM CaCl₂, then the plates were immediately irradiated (except the No UV control) with Caprobox™ (350 nm) for 5 min, and the cells were harvested by scraping. The cells were pelleted (10 min, 1200 g, 4°C), supernatant removed, and resuspended in 250 µL 50 mM Hepes buffer. The cells were destroyed with the Heidolph Silent Crusher (20 seconds, 25,000 rpm). Samples were sonicated for 10x 2.5 sec with 0.5 sec interval (using a probe sonicator from Branson, Digital Sonifier) and 2 µL of 10% SDS was added. If samples were frozen at -80°C before continuation of the experiment, the samples were sonicated again for 10x 0.5 sec with 0.5 sec interval using a probe sonicator.

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The protein content was quantified using Bradford and the experiment was continued using the same amount of protein for each sample. Sample volumes were adjusted to 400 μL with 50 mM Hepes buffer, then the ligation reaction with biotin-N₃ was performed with 43.7 μL click mix per sample (mix: 21.85 μL 25 mM CuSO₄, 13.15 μL 250 mM NaAsc, 4.37 μL 25 mM THPTA and 4.37 μL biotin-N₃) for 1 hr at rt in the dark. To remove all click components, the protein was precipitated by the addition of 666 μL MeOH, 166 μL CHCl₃ and 300 μL MilliQ, and centrifuged at 20238 g for 10 min. The supernatant was removed and the pellet was resuspended in 600 μL MeOH using sonication (6x 0.5 sec, interval 0.5 sec). The protein was pelleted at 20238 g for 10 min and the supernatant removed. The protein was then denatured in 15 min at rt with 500 μL 1% SDS containing 25 mM NH₄HCO₃, followed by reduction (65°C, 15 min, 700 rpm shaking) using 5 μL 1 M DTT per sample. Samples were cooled to rt before alkylation with 40 μL 0.5 M IAA per sample for 30 min at rt in the dark. 140 μL 10% SDS was added per sample, and each sample was added to 6 mL PBS containing 50 μL avidin beads (prewashed with PBS 3x, pelleting at 2000 g for 2 min), and incubated for 2 hr at rt while rotating. Beads were pelleted (2000 g, 2 min) and washed with PBS with 0.5% SDS (1x) and with PBS (3x). On-bead digest of peptides was performed overnight at 37°C, at 1000 rpm with digestion buffer (250 μL per sample, recipe: 300 μL 1 M Tris, 300 μL 1 M NaCl, 3 μL of 1 M CaCl₂, 60 μL ACN, 3 μL 0.5 μg/mL Trypsin and 2334 μL MilliQ). Samples were quenched with 12.5 μL formic acid (FA) and beads were removed using a biospin column (600 g, 2 min). Samples were added on C18 stagetips (conditioned with 50 μL MeOH, then 50 μL of 0.5% (v/v) FA in 80% (v/v) ACN/MilliQ (solution B), then 50 μL 0.5% (v/v) FA in MilliQ (solution A), each conditioning step was performed using centrifugation for 2 min at 600 g) by spinning for 15 min at 800 g, then washed with solution A for 10 min at 800 g, and eluted with solution B for 5 min at 800 g into low-binding Eppendorf tubes. Samples were evaporated using an Eppendorf speedvac (Eppendorf Concentrator Plus 5301) and 50 μL of LC/MS solution was added (recipe for 2 mL: 1900 μL MilliQ, 60 μL ACN, 2 μL FA, 40 μL of 1 nmol/μL yeast enolase stock). Samples were measured using a NanoACQUITY UPLC System coupled to a SYNAPT G2-Si high definition mass spectrometer (Waters). The peptides were separated using an analytical column (HSS-T3 C18 1.8 μM, 75 μM x 250 mm, Waters) with a concave gradient (5 to 40% ACN in H₂O with 0.1% FA). [Glu1]-fibrinopeptide B was used as lockmass. Mass spectra were acquired using the UDM5® method. The mass range was set from 50 to 2,000 Da with a scan time of 0.6 seconds in positive, resolution mode. The collision energy was set to 4 V in the trap cell for low-energy MS mode. For the elevated energy scan, the transfer cell collision energy was ramped using drift-time specific collision energies. Raw data was processed using Progenesis QI for ProteomeX (3.0, Waters), with lockmass correction (785.8426 Da) and a database search was performed against the proteomic database of Mus musculus, with trypsin as digestion reagent, max 2 missed cleavages, carbamidomethyl C as a fixed modification, oxidation M as a variable modification and FDR set to 1%. Relative quantitation using Hi-3 was performed after filtering the peptides on score (cut-off 5).

Data analysis:
The average normalized abundance of proteins in sample replicates of two independent experiments was used to calculate the ratio of proteins in the probe-treated sample and the “No UV” sample, to determine the level of enrichment by UV-irradiation (Figure 4A). Protein targets that were enriched >5x by probe 1 are shown in Table S1. Proteins that were <2-fold enriched and highly abundant (>20%) in the CRAPome database https://www.crapome.org/, version 1.1) were excluded from further analysis. Gene ontology data of the ~150 resulting putative probe targets (Figure 4B,C) was derived using the DAVID Bioinformatics Database (https://david.ncifcrf.gov/home.jsp, version 6.8). In THC competition experiments, the normalized abundance of proteins in sample replicates was used to calculate the ratio of proteins in THC-pretreated samples over probe-treated samples. The average of the mean ratios of the triplicate samples of each independent experiment was used to calculate the effect of THC (as fold change), and a student’s t-test was used to determine whether the fold change was significantly lower than 1, indicating a significant reduction of the abundance of that particular protein in the THC-treated samples (Figure 4D). A P-value less than 0.05 was considered statistically significant. Proteins that showed <50% inhibition (Table S2) were excluded from gene ontology analysis. This analysis yielded 1 putative protein target of Δ9-THC and 3 putative targets of Δ8-THC (Figure 4A,B (red dots) and Figure 4E).
Gene ontology and KEGG pathway analysis of the resulting putative protein targets was derived using the DAVID Bioinformatics software (https://david.ncifcrf.gov/home.jsp, version 6.8). In addition, it was investigated whether these proteins are associated with pathophysiologies or diseases using the OMIM database (https://www.omim.org/, September 2017).

Table S1. Putative protein targets of probe 1. Proteins that were >5x abundant after UV-irradiation of probe 1 are shown here. Gene and protein names and the ratio probe vs. probe (No UV) of these proteins are listed.

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<td>Tmem97</td>
<td>RNA binding motif, single stranded interacting transmembrane protein 97</td>
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<tr>
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<td>Leucine rich repeat containing 59</td>
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<td>Tomm22</td>
<td>Translocase of outer mitochondrial membrane 22 homolog</td>
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<td>Sel1l</td>
<td>Sel-1 suppressor of In-12-like</td>
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<td>Lbr</td>
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<td>RAB15, member RAS oncogene family</td>
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Table S2. THC putative protein targets with less than 50% inhibition. Proteins that were inhibited < 50% were excluded from gene ontology analysis. Gene and protein names of these proteins are listed here.

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<td>Ppa1</td>
<td>Inorganic pyrophosphatase</td>
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References

1. Soethoudt, M.; Alachouzos, G. I.; Moya Garzon, M. D.; Van Roonden, E. J.; Heitman, L. H.; Van der Stelt, M., contributed to the work described in this chapter.
Table S1. Putative protein targets of probe 1.

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Chapter 7

References

- McHugh, D.; Page, J.; Dunn, E.; Bradshaw, H. B., Delta(9)-Tetrahydrocannabinol and N-arachidonyl glycine are full agonists at GPR18 receptors and induce migration in human endometrial HEC-1B cells. *Brit J Pharmacol* 2012, 165, (8), 2414-2424.


