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Affinity and Efficacy Studies of Tetrahydrocannabinolic Acid A at Cannabinoid Receptor Types One and Two

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Abstract

Introduction: *Cannabis* biosynthesizes Δ^9-tetrahydrocannabinolic acid (THCA-A), which decarboxylates into Δ^9-tetrahydrocannabinol (THC). There is growing interest in the therapeutic use of THCA-A, but its clinical application may be hampered by instability. THCA-A lacks cannabimimetic effects; we hypothesize that it has little binding affinity at cannabinoid receptor 1 (CB_1).

Materials and Methods: Purity of certified reference standards were tested with high performance liquid chromatography (HPLC). Binding affinity of THCA-A and THC at human (h) CB_1 and hCB_2 was measured in competition binding assays, using transfected HEK cells and [^{3}H]CP55,940. Efficacy at hCB_1 and hCB_2 was measured in a cyclic adenosine monophosphase (cAMP) assay, using a Bioluminescence Resonance Energy Transfer (BRET) biosensor.

Results: The THCA-A reagent contained 2% THC. THCA-A displayed small but measurable binding at both hCB_1 and hCB_2, equating to approximate K_i values of 3.1μM and 12.5μM, respectively. THC showed 62-fold greater affinity at hCB_1 and 125-fold greater affinity at hCB_2. In efficacy tests, THCA-A (10μM) slightly inhibited forskolin-stimulated cAMP at hCB_1, suggestive of weak agonist activity, and no measurable efficacy at hCB_2.

Discussion: The presence of THC in our THCA-A certified standard agrees with decarboxylation kinetics (literature reviewed herein), which indicate contamination with THC is nearly unavoidable. THCA-A binding at 10μM approximated THC binding at 200nM. We therefore suspect some of our THCA-A binding curve was artifact—from its inevitable decarboxylation into THC—and the binding affinity of THCA-A is even weaker than our estimated values. We conclude that THCA-A has little affinity or efficacy at CB_1 or CB_2.

Keywords: cannabinoid receptors; Cannabis; pharmacodynamics; pharmacology; phytocannabinoids; THCA

Introduction

Cannabis biosynthesizes cannabinoids as carboxylic acids. The carboxylic acid of Δ^9-tetrahydrocannabinol (THC) is Δ^9-tetrahydrocannabinolic acid (THCA). Two isomers of THCA have been discovered, 2-COOH-THC (THCA-A) and 4-COOH-THC (THCA-B) (Fig. 1). *Cannabis* primarily biosynthesizes THCA-A,[1] and this isomer has been the focus of most pharmacological studies. Conversely, THCA-B has greater stability and crystalizes more readily than THCA-A,[2] so THCA-B became the molecule for modeling studies of cannabinoid receptors. According to the canonical cannabinoid biosynthesis pathway, olivetolic acid is prenylated into cannabigerolic acid (CBGA) with its carboxylic acid in an "A" position. The allylic rearrangement yielding THCA-B begs a mechanism.

There is growing interest in the therapeutic use of THCA-A.[3,4] The gray literature is immense: a simple

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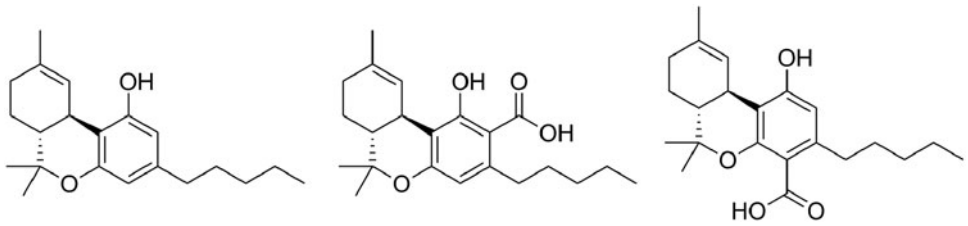


FIG. 1. Chemical structures of THC (left), THCA-A (center), and THCA-B (right). THC, Δ^9-tetrahydrocannabinol; THCA-A, Δ^9-tetrahydrocannabinolic acid A; THCA-B, 4-COOH-THC.

Google search of "tetrahydrocannabinolic acid" plus "medical" returns 14,000 hits. The clinical application of THCA-A, however, is complicated by thermal instability—it readily decarboxylates into THC. This happens with heating (smoking and baking), as well as storage, at room temperature.

Studies on the "shelf life" of THCA-A are worth reviewing, for clinical purposes, as well as pharmacological research. Ethanol and olive oil extract approximately the same THCA-A/THC ratio from plant material,[5,6] but THCA-A stability is greater in olive oil (78% of THCA-A remained after 10 days at 25°) than ethanol (only 33% remained).[6] THCA-A is even less stable in hydroethanolic solvents.[7] Other stability studies tested solvents used for laboratory reagents—methanol, chloroform, petroleum ether, and *n*-hexane.[8,9] Decarboxylation rates depended upon temperature, with considerable losses at room temperature, and exposure to light accelerated the process. When stored for a month at refrigerator temperature (4°C), THCA-A decreased to between 91% (in methanol) and 68% (in chloroform) of initial levels. Losses still occurred at freezer temperature (–18°C).[8]

Hazekamp et al.[10] demonstrated short-term stability in "cannabis tea." They added quantified amounts of THCA-A and THC to boiled water. After 15 min of simmering above 55°C, they recovered 63% of THCA-A and only 17% of THC. However, THCA-A loss was substantial in cannabis tea stored at 4°C—decreasing to 71% of initial levels after 1 day. THCA-A rapidly decarboxylates if water is boiled *after* cannabis is added to it.[11]

Studies suggest that THCA-A may be more stable in herbal cannabis, where it is "hermetically sealed" within glandular trichomes, along with terpenoids which serve as protective antioxidants.

Studies suggest that THCA-A may be more stable in herbal cannabis, where it is "hermetically sealed" within glandular trichomes, whose gland heads contain up to 10% terpenoids.[12] Terpenoids are potent antioxidants,[13] protect living plants from thermal and oxidative stress,[14] and likely inhibit the oxidative decarboxylation of THCA-A. Decarboxylation kinetics have been measured by heating herbal cannabis in undescribed conditions,[15] in a nitrogen atmosphere,[16] in sealed glass bottles,[17] or cardboard boxes.[18] Collectively, these studies showed that THCA-A decarboxylated within minutes at temperatures above 80°C. At room temperature in glass bottles with limited exposure to light, THCA-A dropped to 80% of initial levels after 25 months. At refrigerator (4°C) temperatures, 94.7% of THCA-A was still present.

THCA-A "shelf life" may be extended in hashish, where gland heads are mechanically detached and compacted to minimize exposure to light and oxygen.[8,11,19] Baker et al.[20] measured THCA and THC in seized materials, all approximately the same age. The THCA/THC ratio in hashish (mean 3.08) was greater than herbal material (mean 1.96).

The growing clinical interest in THCA-A is due, in part, to its perceived lack of cannabimimetic effects.[15,21,22] This may be due to a lack of binding affinity at cannabinoid receptor type one (CB_1). Affinity studies of THCA-A at CB_1 report disparate results—equal to THC[23] or 25-fold weaker than THC[24] or lacking affinity.[25,26] As we elaborate in the Discussion section, this incongruence is best explained by THCA-A contaminated by THC. Similarly, Edery et al.[21] demonstrated very weak psychoactivity in rhesus monkeys, which they discounted as some THCA-A decarboxylating into THC during the course of the experiment.

In this current study, we measured the affinity of THCA-A at CB_1, as well as cannabinoid receptor subtype two (CB_2), as well as the efficacy of THCA-A at CB_1 and CB_2. We used a certified reference standard (meeting ISO17025 guidelines), but high performance liquid chromatography (HPLC) revealed that our THCA-A reference standard was contaminated by 2% THC. In this

study we show that the only binding or efficacy detected for THCA-A is consistent with the level of THC contamination contained in the sample.

Methods

THCA-A was purchased from Cayman Chemical (lot no. 0466688) as a 1 mg/mL solution in acetonitrile. THC was purchased from THC Pharm GmbH (lot no. S12-003, delivered as a solid resin in a glass syringe) and dissolved at 31.6 mM in nitrogen-purged absolute ethanol. Materials were stored at –20°C (THCA-A) or –80°C (THC) before experimentation. Purity was assessed by reverse-phase HPLC using a Phenomenex™ C_{18} Gemini column (5 μm, 4.60 × 250 mm) on a Thermo Scientific UltiMate 3000 HPLC. A linear gradient of 65–100% MeOH (ca. 1%/min) in H_2O with 0.1% formic acid was used.

Competition binding assays[27] for hCB_1 and hCB_2 were performed by incubating either THC or THCA-A with membranes from HEK (human embryonic kidney) 293 cells transfected with either hCB_1 or hCB_2 receptors as previously described.[28,29] Transfected HEK 293 cells were grown to 90–100% confluence in 175 cm^2 flasks and harvested in ice-cold phosphate buffered saline (PBS) with 5 mM EDTA. Cells were centrifuged at 200 × *g* for 10 min and frozen at –80°C until required. Cell pellets were thawed with Tris-sucrose buffer (50 mM Tris-HCl, pH 7.4, 200 mM sucrose, 5 mM $MgCl_2$, 2.5 mM EDTA) and homogenized with a glass homogenizer. The homogenate was centrifuged at 1000 × *g* for 10 min at 4°C and the pellet discarded. The supernatant was then centrifuged at 27,000 × *g* for 30 min at 4°C. The final pellet was resuspended in a minimal volume of Tris-sucrose buffer and aliquoted to avoid repeated freeze–thaw cycles.

Protein concentration was determined using the DC Protein Assay Kit (Bio-Rad, Hercules, CA) following the manufacturers' protocol. Membranes (10 μg/point for CB_1 and 5 μg/point for CB_2) were resuspended in binding buffer (50 mM HEPES, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 0.2% [w/v] bovine serum albumin [BSA; ICP Bio], pH 7.4) and incubated with [^{3}H]-CP55,940 (2.5 nM; PerkinElmer, Waltham, MA; two different lots were used 175 and 150.2 Ci $mmol^{-1}$) and a range of THC or THCA-A concentrations at 30°C for 60 min. THC and THCA-A were diluted directly from stocks to 40 μM in binding buffer supplemented with acetonitrile and ethanol, respectively, to match vehicle conditions with each compound. The compounds were then serially diluted in silanized vessels, maintaining ethanol and acetonitrile levels constant through the dilution series. These 4× dilution series were then added to a v-bottom 96-well plate with radioligand and cell membranes such that the final 1× concentration contained both 0.04% ethanol and 0.36% acetonitrile. These solvents were matched in the vehicle conditions.

GF/C Harvest Plates (PerkinElmer) were presoaked in 0.1% polyethylenimine and then washed with 200 μL ice-cold wash buffer (50 mM HEPES pH 7.4 500 mM NaCl, 0.1% BSA) before filtration of samples and then three additional 200 μL washes in ice-cold wash buffer. Harvest plates were dried overnight at 24°C, 50 μL of scintillation fluid (IRGASAFE PLUS; PerkinElmer) was added to each well, and plates were read 30 min later for 2 min per well in a MicroBeta TriLux (PerkinElmer).

Competition binding curves were fit by nonlinear regression using one site competition binding with GraphPad Prism 6.0. Dissociation constant in a competition binding assay (K_i) was determined from half maximal inhibitory concentration (IC_{50}) using previously established K_d of 2.5 nM (CB_1) or 3 nM (CB_2), respectively, (unpublished data). Binding experiments for CB_1 and CB_2 were performed five times in triplicate. pKi values are expressed as mean ± standard error of the mean (SEM). For THCA-A full binding curves could not be established (because it failed to fully displace [^{3}H]-CP55,940 at concentrations up to 10 μM); therefore, we estimated percentage displacement at 10 μM. This was converted to an approximate IC_{50} by assuming a hill slope of 1 and utilizing the following equation: $IC50 = 10\ \mu M / \left(\frac{\%displacement}{100 - \%displacement} \right)$ and then to approximate K_i using the Cheng–Prusoff equation: $Ki = IC50 / \left(1 + \frac{[L]}{KL} \right)$

Efficacy at hCB_1 and hCB_2 was investigated in a cyclic adenosine monophosphase (cAMP) assay, to determine if THCA-A could inhibit forskolin-stimulated cAMP (i.e., act as an agonist) or prevent the inhibition produced by EC_{90} concentrations of CP55,940 (i.e., act as an antagonist). Cellular cAMP levels were measured as previously described.[28] Briefly, the pcDNA3L-His-CAMYEL plasmid (ATCC, Manassas, VA) was transfected into HEK 293-hCB_1 or hCB_2 cells using linear polyethylenimine (molecular weight 25 kDa; Polysciences, Warrington, PA). After 24 h transfection cells were

replated in poly-D-lysine (0.05 mg mL^{-1} in PBS; Sigma-Aldrich, St Louis, MO) coated 96 Well Solid White Flat Bottom Polystyrene TC-Treated Microplates (Corning) at a density of 55,000–80,000 cells per well. After 24 h, cells were serum-starved in Hank's balanced salt solution (HBSS) containing 1 mg mL^{-1} BSA, pH 7.4 for 30 min before assay. Five minutes before the addition of drug or vehicle dissolved in HBSS plus 1 mg mL^{-1} BSA cells were treated with 5 μM Coelenterazine-h (Nanolight Technology). Emission signals were detected simultaneously at 460/25 nM (RLuc) and 560/25 nM (YFP), immediately following drug addition, with a LUMIstar plate reader (BMG) at 37°C. Raw data are presented as an inverse bioluminescence resonance energy transfer (BRET) ratio of emission at 460/535 nM, so that an increase in ratio correlates with an increase in cAMP production. Area under the curve analysis was carried out using GraphPad Prism, and values normalized to forskolin (100%) and vehicle (0%). *t*-Tests (GraphPad Prism) were utilized to determine if THCA-A significantly altered the response in the presence or absence of CP55,940.

Results

HPLC revealed the presence of 2% THC (THC_{RT} = 18.2 min) in the THCA-A sample (THCA-A_{RT} = 23.9 min), established by correlation of retention time with an authentic sample of THC. The THC and THCA-A peaks were correlated to their respective molecular ions by electrospray ionization (ESI) mass spectrometry (THC m/z = 338.1, $[M+Na]^{1+}$ requires 337.5; THCA-A m/z = 359.0, $[M+H]^{1+}$ requires 359.5). Chromatograms of the two samples are illustrated in Figure 2.

To determine if THCA-A could bind to the orthosteric binding site of hCB_1 or hCB_2, competition displacement assays were carried out (Fig. 3). Full displacement could not be achieved with THCA-A concentrations up to 10 μM. At 10 μM THCA-A produced a small but significant displacement at both hCB_1 (62% ± 3%) and hCB_2 (40% ± 8%). This level of displacement was insufficient to fully define competition binding curves, but would equate to approximate pK_i's of 5.5 (3.1 μM) and 4.9 (12.5 μM), respectively. For comparative purposes competition binding assays were carried out with THC. THC fully displaced [^{3}H]CP55,940 with mean pK_i = 7.3 ± 0.03 (hCB_1; 50 nM) and −7.0 ± 0.04 (hCB_2; 100 nM).

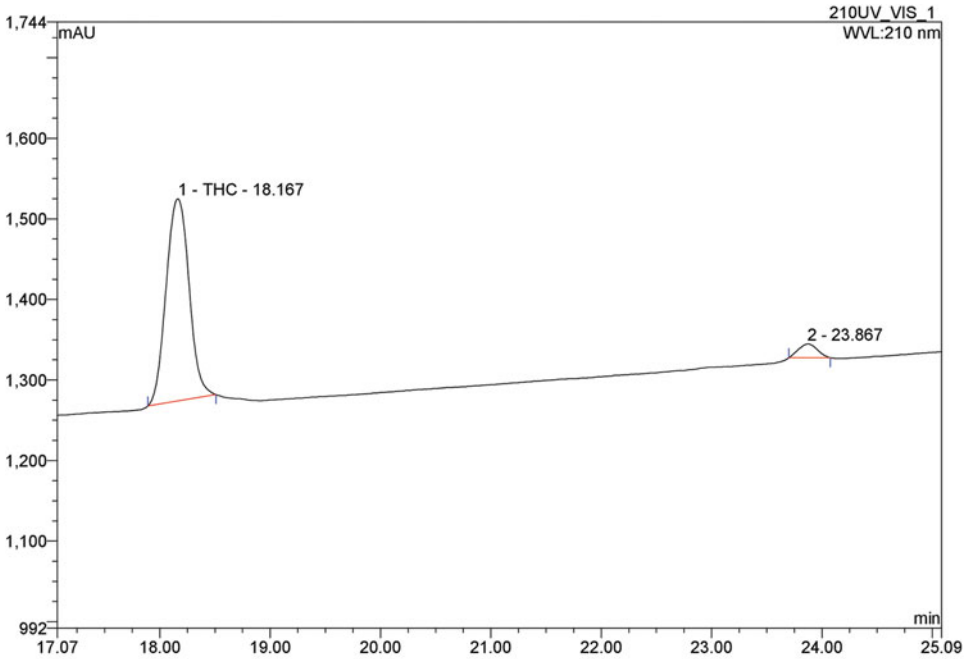
To determine if THCA-A could activate or block cannabinoid receptors, cAMP assays were carried out using a BRET biosensor as previously described.[28] As both cannabinoid receptors are predominantly guanine nucleotide-binding protein subunit i (G_i) linked and therefore couple to the inhibition of cAMP, cAMP levels were increased with forskolin and then the ability of 10 μM THCA-A to alter cAMP was investigated in the presence and absence of the cannabinoid agonist CP55,940 at approximate EC_{90} concentrations (4 nM at hCB_1 and 20 nM at hCB_2). For comparison inhibition of forskolin mediated cAMP by 10 μM THC was also carried out. cAMP levels were measured for 9.9 min (595 sec), and then data were analyzed by an Area under the curve analysis, normalized to forskolin (100% and vehicle 0%).

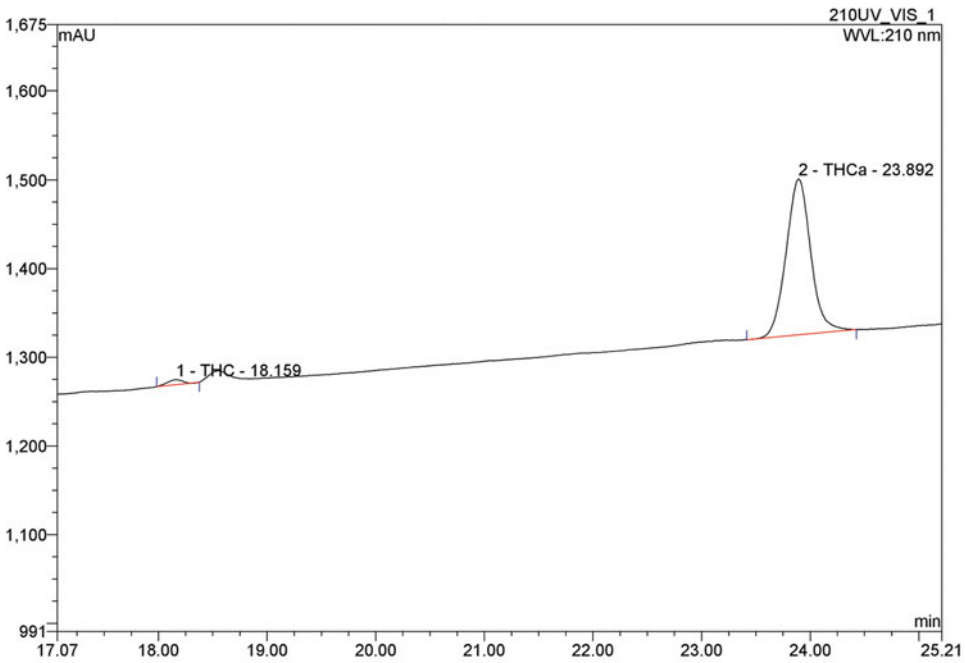
As shown in Figure 4, at hCB_1 CP55,940 inhibited forskolin stimulated cAMP to 50% ± 3.5%. In the presence of 10 μM THCA-A this stimulation was partially but significantly reversed to 59% ± 1.8% (p = 0.0065 by paired t-test, n = 5). On its own THCA-A produced a small but significant inhibition to 79% ± 5% (t-test compared to 100% p = 0.015). At hCB_2, CP55,940 inhibited to 53% ± 6%, which was unaltered in the presence of THCA-A (55% ± 7% p = 0.6, n = 6). Consistent with this, THCA-A alone produced no inhibition of forskolin mediated cAMP (99% ± 2%, p = 0.64, n = 6). As expected, THC (10 μM) produced equivalent inhibition of cAMP to that produced by CP55,940, acting as an efficacious agonist in this assay.

Discussion

Although there is growing interest in THCA-A among clinicians,[3,4] decarboxylation studies suggest that contamination with THC is nearly unavoidable.[5–11,15–20] The instability of THCA-A also hampers its pharmacological exploration.[21] "How can anybody do an experiment if the compound likes to convert into something else just by sitting around, and the 'something else' has all kinds of activities?" (R. Mechoulam, personal communication, January 2017). Our study optimized *in vitro* stability by keeping THCA-A in acetonitrile. *In vitro* assays of THC can also be hampered by solubility issues. Our results with THC (K_i = 50 nM at hCB_1) indicate that THC did not fall out of solution. Because THCA-A is more water soluble than THC,[10] we concluded that our results with THCA-A were not due to solubility issues.

THCA-A's lack of psychoactivity makes it attractive in some circles. Moreno-Sanz[30] hypothesized that lack of cannabimimetic effects is due to restricted access to the central nervous system (CNS). Others have explored the affinity and efficacy of THCA-A at cannabinoid receptors. Rosenthaler et al.[23] determined a K_i of 23.4 nM for THCA-A at hCB_1, nearly equivalent to their measure of K_i of 35.6 nM for THC. This is in agreement





No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %
1	18.16	THC	5.566	0.857	1.82
2	23.89	THCa	175.861	46.178	98.18
Total:			181.427	47.034	100.00

FIG. 2. Analytical RP-HPLC of THCA-A sample (lower panel) and THC standard (upper panel); absorbance detected at 210 nm. HPLC, high performance liquid chromatography.

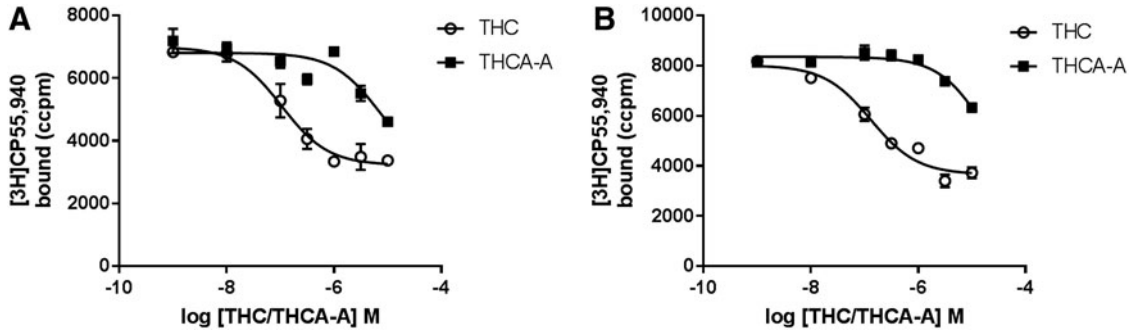


FIG. 3. Binding affinity of THCA-A and THC illustrated in competition binding curves against [^{3}H]CP55,940. CB_1 on the left **(A)** and CB_2 on the right **(B)**. Data are representative data from a single experiment and data points represent mean ± SEM for triplicate data points. CB_1, cannabinoid receptor subtype one; CB_2, cannabinoid receptor subtype two; SEM, standard error of the mean.

with a meta-analysis of THC at hCB_1, which reported a mean K_i of 25.1 ± 0.39 nM ($n = 16$ studies).[31]

Verhoeckx et al.[24] determined a K_i of 890 nM for THCA-A at hCB_1. They also determined a K_i of 3.5 nM for THC at hCB_1, which is sevenfold greater than the meta-analytic mean. Applying this multiplier to their K_i of THCA-A would produce a K_i broadly consistent with our results. Ahmed et al.[25] simply stated "no activity" for THCA-A at CB_1, without a K_i value. Husni et al.[26] determined a K_i of 1292 nM for THCA-A at CB_1. Although they illustrate an incorrect structure for THCA-A, their results do indeed apply to THCA-A (M. Radwan, personal communication, January 2017).

The reason for these disparate results cannot be easily explained. The methods used in these studies are compared in Table 1. Methodological details not supplied in original publications were obtained through personal communications (S. Rosenthaler, April 2015; K. Verhoeckx, April 2017; M. Radwan, January 2017). Affinity values among different studies may vary according to radioligand, CB species, and expression model, but these methodological factors rarely generate statistical differences.[31] More likely, some THCA-A decarboxylated in these studies. The two studies that reported affinity[23,24] did not authenticate the purity of their THCA-A reagents, whereas the two studies that reported no affinity[25,26] authenticated THCA-A with HPLC and nuclear magnetic resonance (NMR) (authentication confirmed by M. Radwan, personal communication, January 2017).

THCA-A showed little affinity at hCB_1 in our competition binding assays. On the basis of 60% displacement at 10 μM, a K_i of 3 μM can be estimated, making it broadly comparable to that of cannabidiol ($K_i = 2.2$ μM[31])—a decidedly non-cannabimimetic ligand. At hCB_2, THCA-A slightly displaced [^{3}H]CP55,940 in binding assays—less than that produced at CB_1, reaching 40% displacement at 10 μM, consistent with an estimated K_i of 12.5 μM. In comparison, THC showed 62-fold greater affinity at hCB_1 and 125-fold greater affinity at hCB_2.

Table 1. Methodological Comparison of Five Δ^9-Tetrahydrocannabinolic Acid A Affinity Studies

	Radioligand	CB species	Expression model	THCA-A source
Rosenthaler et al.[23]	[^{3}H]CP55,940	hCB_1	Sf9 cells	THC Pharm GmbH, synthetic, 1 mg/mL in methanol
Verhoeckx et al.[24]	n.d.[a]	hCB_1	Sf9 cells	Extracted from plant material, in ethanol
Ahmed et al.[25]	[^{3}H]CP55,940	rCB_1	Brain membranes	Extracted from plant material, in hexane
Husni et al.[26]	[^{3}H]CP55,940	rCB_1	Brain membranes	Extracted from plant material, in hexane
This study	[^{3}H]CP55,940	hCB_1	HEK293 cells	Cayman Chemical, synthetic, 1 mg/mL in acetonitrile

[a]K. Verhoeckx (personal communication, April 2017) reports "outsourcing" the affinity part of their study and could not recall the specific radioligand used in the assay.

hCB_1, human cannabinoid receptor subtype one; HEK, human embryonic kidney; rCB_1, rat cannabinoid receptor subtype one; Sf9, *Spodoptera frugiperda* clonal isolate 9; THC, Δ^9-tetrahydrocannabinol; THCA-A, Δ^9-tetrahydrocannabinolic acid A.

Despite our use of a certified reference standard (meeting ISO17025 guidelines), the reagent nevertheless contained 2% THC. Numerous web sites advertise "crystalline THCA" and claim 99% to 100% purity. These gray market sources pose legal barriers regarding international shipment, lack ISO17025 standards, and await purity authentication. Hypothetically these products contain THCA-B, which crystalizes more readily than THCA-A.[2] THCA-B also demonstrates greater thermal stability than THCA-A,[32–34] so it may be worth investigating.

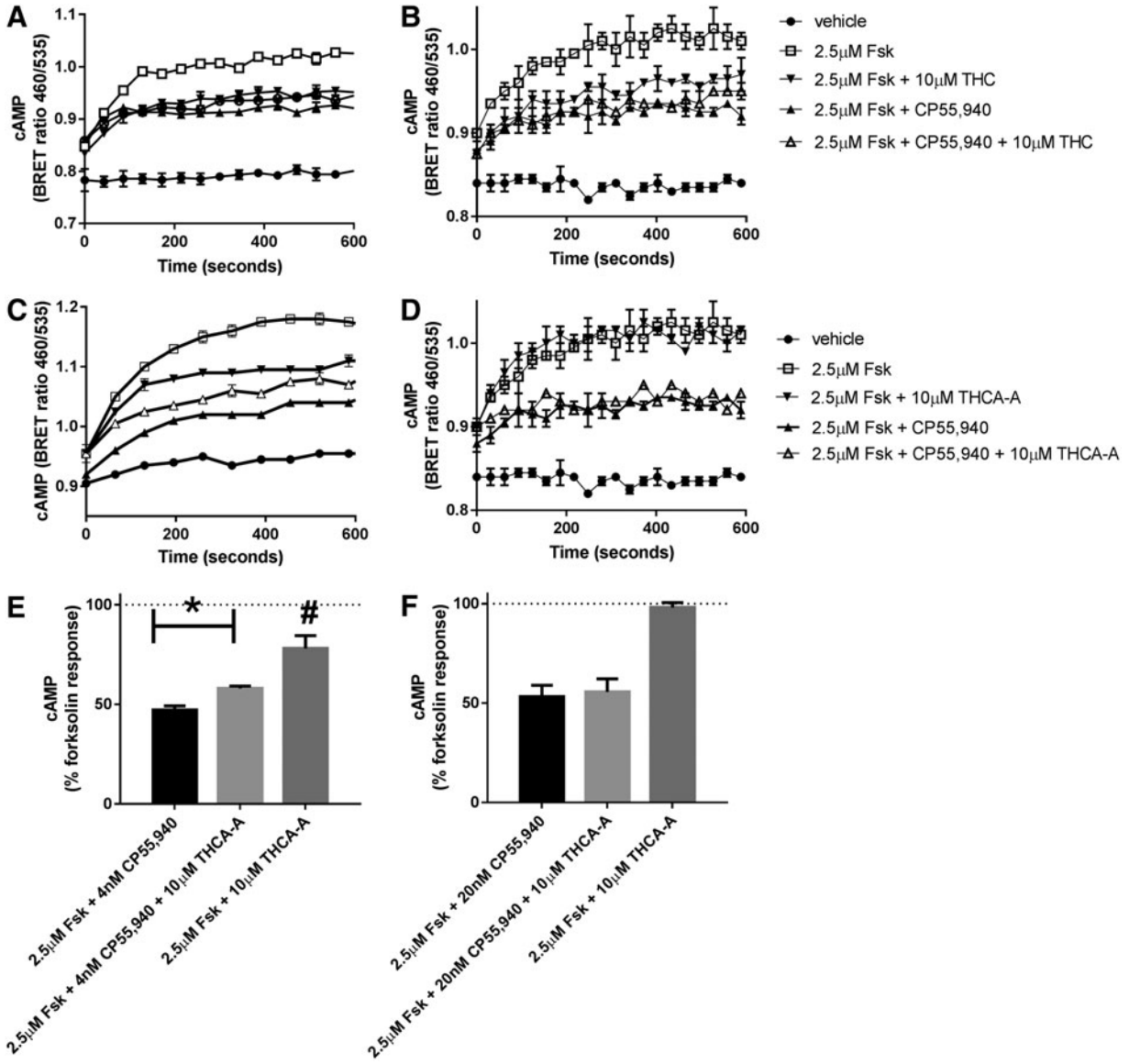


FIG. 4. Efficacy of THCA-A and THC in cAMP assays, CB_1 on the left, CB_2 on the right. **(A–D)** Show representative images of the biosensor traces of single experiments carried out in duplicate. **(A, B)** Show that THC (10 μM) inhibits cAMP at both CB_1 (left) and CB_2 (right). **(C, D)** Show the same assay carried out with THCA-A. THCA-A can be seen to inhibit forskolin mediated cAMP alone and to partially antagonize the ability of CP55,940 to inhibit cAMP at CB_1, but has no effect under equivalent conditions at CB_2. The lower panels **(E, F)** are summary data for the area under the curve analyses for all replicate assays combined ($n=5$ for CB_1 or $n=6$ for CB_2). *$p=0.0065$ by paired t-test, $n=5$. $^{\#}p=0.015$ t-test compared to 100%. cAMP, cyclic adenosine monophosphase.

However, only two studies have quantified THCA-A and THCA-B content in a variety of *Cannabis* landraces,[33,34] so the prevalence of THCA-B is relatively unknown.

Given the susceptibility of THCA-A to lose its carboxylic acid moiety, contamination by THC may be difficult to avoid, as lamented by pharmacologists evaluating THCA-A.[21] At a concentration of 10 μM THCA-A, the reagent contained $\sim$200 nM THC. In our hCB_1 competition binding assay, THCA-A displacement at 10 μM approximated THC displacement at 200 nM (Fig. 3). We therefore suspect that some of our THCA-A binding curve was artifact—from its inevitable decarboxylation into THC—and the binding affinity of THCA-A at hCB is even weaker than our estimated values.

Consistent with low affinity, THCA-A showed low efficacy at hCB_1. THCA-A (10 μM) produced a small but significant inhibition of forskolin cAMP, consistent with agonist activity. Due to solubility issues and the low potency of this compound, sufficiently high concentrations to determine the extent of agonism were not possible without reaching unacceptably high levels of solvent. As is consistent with a weak agonist, THCA-A slightly antagonized the effect produced by an EC_{90} concentration of CP55,940. Regarding hCB_2, THCA-A produced no significant effect in cAMP assays.

Verhoeckx et al.[24] also measured efficacy, and their results correlated with ours—THCA-A at hCB_1 showed no influence on cAMP production. However, THC showed no efficacy in their hands, either. Husni et al.[26] used a different efficacy assay: agonist-stimulated [^{35}S]GTPγS-binding in mouse brain membranes. The EC_{50} concentration of THC was 269 nM, whereas THCA-A was >10,000 nM. Lack of affinity and efficacy of THCA-A at hCB_1 seems consistent with *in vivo* studies, where THCA-A lacked cannabimimetic activity in rodents and primates.[15,21,22]

Many questions regarding THCA-A remain unanswered. For example, an *in vivo* study of rats and shrews showed that antiemetic effects by THCA-A were blocked by rimonabant.[22] This suggests a CB_1-mediated mechanism, yet the authors reported that THCA-A did not induce CB_1 agonist effects such as hypothermia or reduced motor activity. THCA-A is a promiscuous ligand and targets many molecular targets.[30] However, its clinical usefulness, and its amenity to pharmacological analysis, may be hampered by its instability.

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Author Disclosure Statement

No competing financial interests exist.

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Abbreviations Used

BRET = bioluminescence resonance energy transfer
BSA = bovine serum albumin
cAMP = cyclic adenosine monophosphase
CB_1 = cannabinoid receptor subtype one
CB_2 = cannabinoid receptor subtype two
h = human
HBSS = Hank's balanced salt solution
HEK 293 = human embryonic kidney cell line 293
HPLC = high performance liquid chromatography
IC_{50} = half maximal inhibitory concentration
K_i = dissociation constant in a competition binding assay
PBS = phosphate buffered saline
THC = Δ^9-tetrahydrocannabinol
THCA-A = Δ^9-tetrahydrocannabinolic acid A
THCA-B = 4-COOH-THC