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Novel C-Ring-Hydroxy-Substituted Controlled Deactivation Cannabinergic Analogues

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Abstract

In pursuit of safer controlled-deactivation cannabinoids with high potency and short duration of action, we report the design, synthesis, and pharmacological evaluation of novel C9- and C11-hydroxy-substituted hexahydrocannabinol (HHC) and tetrahydrocannabinol (THC) analogues in which a seven atom long side chain, with or without 1'-substituents, carries a metabolically labile 2',3'-ester group. Importantly, in vivo studies validated our controlled deactivation approach in rodents and non-human primates. The lead molecule identified here, namely, butyl-2-[(6a*R*,9*R*,10a*R*)-1-hydroxy-9-(hydroxymethyl)-6,6-dimethyl-6a,7,8,9,10,10a-hexahydro-6*H*-benzo[*c*]chromen-3-yl]-2-methylpropanoate (AM7499), was found to exhibit remarkably high in vitro and in vivo potency with shorter duration of action than the currently existing classical cannabinoid agonists.

Graphical Abstract

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Notes

The authors declare no competing financial interest.

Supporting Information

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Elemental analysis results for compounds **3a** and **16**, chemical structure of (6a*R*,9*R*,10a*R*)-3-(adamantan-1-yl)-9-(hydroxymethyl)-6,6-dimethyl-6a,7,8,9,10,10a-hexahydro-6*H*-benzo[*c*]chromen-1-ol (**28**), and time course of hypothermic effects for all tested doses of **3a**, **3b**, **3c**, and 11-OH-⁸-THC-DMH (**3g**) (PDF)

Molecular formula strings and some data (CSV)

INTRODUCTION

Modulation of the two cannabinoid receptors CB1¹ and CB2² by the plant-derived (–)- 9-tetrahydrocannabinol [(–)- 9-THC] and its synthetic analogues³ is a promising therapeutic approach to treat an array of indications including pain, inflammation, CNS disorders, and cancer.^{4–10} Unfortunately, widespread use of cannabinergic agents in therapy has been hampered due to target related adverse side effects as well as poor pharmacokinetic (PK) properties.¹¹ While seeking to obtain novel THC-based analogues with improved druggability and safety, we reported on a controlled deactivation/detoxification approach. To this effect, we have combined the “soft” analogue/drug concept of enzymatic inactivation with the “depot effect” which is related to the compound’s lipophilicity as well as its tissue distribution and retention.^{12–15} Specifically, we have shown that incorporation of a metabolically labile carboxyester group (soft spot) at strategic positions within the THC structure leads to potent and efficacious CB1 agonists (**2**, Figure 1) that are bioconverted to inactive metabolites by plasma esterases while the rate of hydrolytic cleavage can be accordingly modulated using stereochemical features adjacent to the ester group (enzymatic effect). Also, it is well established that the pharmacological time of action of a lipophilic drug molecule, such as 9- and 8-THC, can greatly exceed its biochemical half-life. This is the result of a “depot effect” due to which when the drug molecule is administered, it is sequestered in the fatty tissues before being slowly released into the circulation to start exerting its action. The depot effect is dependent on the compound’s polar characteristics and can be regulated by varying its log *P* and PSA values. Thus, higher lipophilicity of a molecule is associated with longer residency in fatty tissue, while enhancement of the compound’s polar features is expected to moderate its lipophilic character and reduce the depot effect.^{12–15}

A major goal of this project is to develop controlled-deactivation cannabinoids with faster onset/offset and shorter duration of action than currently existing THC analogues. Such compounds can be useful as tools to study the pharmacological relevance of the precise, short-term activation of CB receptors and to help develop better designed and more predictable animal models. Toward this goal, we have recently focused on controlled-deactivation cannabinergic templates with enhanced polar characteristics that are associated with lower lipophilicity and, thus, less of the depot effect. In an initial study, we observed that incorporation of polar groups (e.g., cyano and imidazolyl groups) at the carboxyester side chain pharmacophore of THC-like analogues increases its stability toward esterases.¹³ This suggests that the depot effect and enzymatic actions are not working synergistically for optimal short duration of action when both the soft spot and the polar group are incorporated in the side chain of the molecule. For this reason we sought to design second generation

controlled deactivation ligands that retain the metabolically labile ester group at the 2'-position of the side chain while the polar moiety, in the form of a hydroxyl group, is introduced at the C9 or C11 positions of the tricyclic cannabinoid structure (**3**, Figure 1). The choice of the hydroxyl group and the relative stereochemistry at the C9 is based on our earlier lead optimization and pharmacophore refinement work in the classical cannabinoid prototype.^{16–21} An additional goal of the present work was to explore the validity of our controlled deactivation approach by obtaining information related to the modulation of the depot effect and its role in the in vivo pharmacokinetic profile of the analogues in rodents and monkeys.

All synthesized compounds were characterized biochemically by determining their in vitro CB1 and CB2 receptor binding affinities, functional activities, and assessment of their in vitro metabolic stability toward mouse and rat plasma esterases. The most successful compounds were evaluated for their hypothermic and analgesic effects in rodents, while a key compound was further tested in drug discrimination experiments in squirrel monkeys. Our data show that the C1'-*gem*-dimethyl analogues exhibit very high affinities for the CB1 and CB2 receptors and that they are susceptible to enzymatic deactivation by plasma esterases while their hydrolytic metabolites showed no or very low cannabinergic activity. In vitro and in vivo characterization suggested that three of the analogues identified in this study are potent CB1/CB2 agonists and exhibit CB-mediated hypothermic effects. The analogue with the most promising activity and short duration of action profile, namely, AM7499 (**3b**), was further tested in the analgesia assay in mice and drug discrimination experiments in squirrel monkeys. The in vivo data show that our lead compound is a remarkably potent CB receptor agonist with significantly faster onset and shorter duration of action than the less polar and nonhydrolyzable congener 11-OH-⁸-THC-DMH²² (**3g**, Table 1). Importantly, drug discrimination experiments in non-human primates validated our controlled deactivation approach in which compound **3b** exhibited approximately 3-fold greater potency and approximately 4 times shorter duration of action when compared to non-hydrolyzable congener 11-OH-⁸-THC-DMH.

CHEMISTRY

Synthesis of the 11-OH-(–)-⁸-THC ester derivative **3a** is depicted in Scheme 1. The chiral 4-hydroxymyrtenyl pivalate **6** was prepared in three steps from (1*R*,5*S*)-myrtenol (**5**) using the method by Liddle et al.,²³ while carboxylic acid **8** was obtained in two steps from nitrile **7** by following procedures we reported earlier.¹⁵ Methyl ester formation produced **9** (72% yield) which was treated with boron tribromide to give the corresponding resorcinol **10** in 71% yield.^{15,19} Condensation of **10** with the chiral terpenoid alcohol **6** in the presence of boron trifluoride diethyl etherate provided the diester **11** in 40% yield.^{23,24} Saponification of all ester groups in **11** gave the precursor carboxylic acid **4a** (68% yield) which upon treatment with 1-bromobutane and sodium bicarbonate under microwave conditions afforded the respective ester **3a** in 51% yield (nonoptimized).^{14,15,19}

Stereoselective synthesis of the 11-OH-(–)-hexahydrocannabinol ester analogue **3b** and the respective carboxylic acid metabolite **4b** is shown in Scheme 2. Deprotection of the phenolic hydroxyl groups in **8** led to carboxylic acid **12** (98% yield) which upon microwave assisted

esterification gave resorcinol **13** in an excellent yield (92%).^{13,15} Coupling of **13** with a mixture of chiral terpene diacetates **14** in the presence of *p*-toluenesulfonic acid (*p*-TSA) gave norpinanone **15** in moderate yield (27%).²⁵ We had explored a variety of solvents, acids, and conditions in order to improve the yield to no avail. In addition to the desired product **15**, this reaction produces a mixture of byproducts where the (+)-apoverbenone (**14a**) and the resorcinol monoacetate **13a** were found to be the major ingredients. We and others have observed that this condensation works well with resorcinols bearing electron donating alkyl side chains with bulky groups at the benzylic position including the 5-(1,1-dimethylheptyl)resorcinol.^{18–20,26} This, coupled with our earlier investigations on the mechanism of this condensation,²⁵ led us to postulate that presence of the carboxyester group in **13** decreases the electron density of the aromatic ring and slows down the rate of the initial Friedel–Crafts allylation reaction. Thus, the acid catalyzed decomposition of diacetates **14** to give **14a** and the monoacetylation of **13** to give **13a** become important side reactions. Currently studies to circumvent the problem of the moderate yield of this synthetic step are ongoing in our laboratory. Treatment of **15** with catalytic amounts of trimethylsilyl triflate in dichloromethane/nitromethane gave **16** with the required 6a*R*,10a*R* stereochemistry in 67% yield.^{16,19} The free phenolic hydroxyl in **16** was protected as the *tert*-butyldimethylsilyl (TBS) ether (87% yield). Ketone **17** was exposed to (methoxymethylene)-triphenylphosphorane to give enol ether **18** in 63% yield as a 2:3 mixture of two geometric isomers (**18a** and **18b**; see Experimental Section) based on ¹H NMR analysis. Methyl vinyl ether **18** was hydrolyzed with wet trichloroacetic acid (97% yield), and the diastereomeric mixture of C9 aldehydes **19** (5:2 ratio by ¹H NMR) was epimerized to produce the β -equatorial isomer **20** in 78% yield.^{16,18} Reduction with sodium borohydride afforded the intermediate alcohol **21** in 85% yield. Subsequent treatment with tetra-*n*-butylammonium fluoride gave the final compound **3b** in high yield (90%). The metabolite acid **4b** was prepared by alkaline hydrolysis of the ester **3b** using lithium hydroxide in dioxane/water (85% yield).²⁷

The intermediate side chain carboxylated ketone **16** served as the starting point for the synthesis of the C9 alcohols **3c** and **3d** (Scheme 3). Thus, reduction of the C9 keto group in **16** with sodium borohydride led to the β -equatorial hydroxyl compound **3c** along with traces (~4% by ¹H NMR) of the respective α -axial isomer (**3d**).¹⁹ This was followed by flash column chromatography purification to give pure **3c** in 84% yield. Alkaline hydrolysis of the ester side chain in **16** provided keto acid **22** (63% yield) which upon treatment with sodium borohydride afforded the acid metabolite **4c** in 81% yield after purification from traces of the α -axial diastereomer **4d**. Reduction of **22** with potassium tri-*sec*-butyl borohydride (K-selectride) was very selective and gave the α -axial alcohol **4d** exclusively (91% yield). Alkylation of the respective carboxylate anion with 1-bromobutane under microwave heating led to the corresponding ester **3d** (42% yield). It should be noted that the stereochemistry of the hydroxyl groups of **3c/3d** and **4c/4d** isomeric pairs was assigned on the basis of ¹H NMR (500 MHz) spectral data as in our earlier work with closely related systems.¹⁹ Thus, in compounds **3c** and **4c**, the peak half-width for the C9 protons was found to be 25–26 Hz while in compounds **3d** and **4d** it was 9.5 Hz (see Experimental Section). This correlates well with an axial C9 proton in the former two compounds and an equatorial C9 proton in the latter two.

Syntheses of the side chain carboxylated analogues lacking the C1'-*gem*-dimethyl group are summarized in Scheme 4. In a similar fashion, terpenylation of resorcinol **23** (23% yield) was followed by dibenzo[*b,d*]pyran ring closure to give methyl ester **25** (57% yield). The sequence methyl ester hydrolysis,²⁸ esterification, hydride reduction worked as expected and provided the C9 equatorial alcohol **3e** in good overall yield. Sodium borohydride and K-selectride reductions were employed to synthesize the requisite C9 alcohols **3f**, **4e**, and **4f** from the C9 ketones **27** and **26**.

CANNABINOID RECEPTOR AFFINITIES

The abilities of compounds **3a–f** and **4a–f** to displace the radiolabeled CB1/CB2 agonist CP-55,940 from membranes prepared from rat brain (source of CB1) and HEK 293 cells expressing either mouse CB2 or human CB2 were determined as described earlier,^{13,19} and inhibition constant values (K_i) from the respective competition binding curves are listed in Table 1 in which the nonhydrolyzable prototype 11-OH- δ -THC-DMH (**3g**) and the first generation carboxy- δ -THC analogue **2a** are included for comparison. The use of two CB2 receptor preparations was aimed at addressing species differences that we observed earlier.²⁹ The compounds included in this study are C9- and C11-hydroxy-substituted hexahydrocannabinol (HHC) and tetrahydrocannabinol (THC) analogues in which a seven atom long side chain, with or without 1'-substituents, carries a 2',3'-ester group. In agreement with our rational design, the hydrolytic metabolites **4a–f** have no significant affinities for CB1 and CB2 receptors, thus minimizing the possibility of undesirable cannabinoid receptor related side effects.

Examination of the binding data of 11-OH- δ -THC-DMH (**3g**), the first generation carboxyester THC compound **2a**, and the side chain carboxylated 11-OH-THC analogue **3a** suggests that simultaneous addition of carboxyester functionality at the side chain and hydroxyl group at C11 of the prototype maintains very high binding affinities for both the CB1 and CB2 receptors. This holds true when the tetrahydrocannabinol C-ring is converted to the fully saturated hexahydrocannabinol ring with the hydroxymethyl substituent occupying the equatorial position (analogue **3b**). Likewise, the one carbon shorter homologue **3c** exhibits somewhat higher binding affinities for both CB receptors. However, conversion of the C9 equatorial hydroxyl group (seen in **3c**) to the axial hydroxyl (**3d**) results in a distinct reduction (approximately 20- to 30-fold) in the binding affinities for CB1 and CB2. This indicates that the relative stereochemistry of the hydroxyl group at C9 can affect the ligand's affinity within the side chain carboxylated hexahydrocannabinol class of compounds. A comparison of the binding data of the two diastereomers **3e** and **3f** with their 1',1'-dimethyl congeners **3c** and **3d** demonstrates the striking effects of the C1'-*gem*-dimethyl substitution. Thus, analogues **3c** and **3d** ($0.1 \text{ nM} < K_i < 7 \text{ nM}$) exhibit 2–3 orders of magnitude higher binding affinities when compared to **3e** and **3f** that bind weakly at CB receptors ($238 \text{ nM} < K_i < 750 \text{ nM}$).

Overall, this SAR study shows that C9- and C11-hydroxy-substituted hexahydrocannabinol and tetrahydrocannabinol analogues with a 1',1'-dimethyl-2'-carboxyester group [-C-(CH₃)₂-C(O)O-] on the side chain pharmacophore exhibit remarkably high affinities for both the CB1 and CB2 receptors. It should be noted that we have observed similar SAR trends

with the side chain carboxyester THC analogues.^{13,15} However, it appears that the HHC scaffold is more sensitive to the presence of the C1'-*gem*-dimethyl substitution with regard to CB receptor recognition.

IN VITRO PLASMA STABILITY STUDIES

Enzymatic labile moieties such as ester, thioester, carbamate, or phosphate groups that are incorporated into the parent drug molecules can be targeted by esterase enzymes expressed through body organs and other tissues as well as blood. Esterases are a heterogeneous group of enzymes that are classified broadly as cholinesterases (including acetylcholinesterases and butyrylcholinesterases), paraoxonases, and carboxyesterases. Human serum albumin also exhibits esterase activity toward phenyl esters such as aspirin (acetylsalicylic acid). Notably, esterase activity is higher in the blood of small rodents than in large animals and humans, while carboxyesterases are found in mice and rat but not in human plasma.^{13,30} In this study, analogues with the highest binding affinities for CB1 and CB2 were assessed for their in vitro plasma stability toward mouse and rat plasma esterases as detailed in the Experimental Section.^{13,15} A comparison of the half-lives ($t_{1/2}$, Table 1) of the C9- and C11-hydroxy-substituted analogues (**3a**, **3b**, **3c**, and **3d**) with the first generation side chain carboxylated compound **2a** shows that incorporation of a polar hydroxyl group at the C ring of the prototype reduces the stabilities of the analogues toward esterases for both the mouse and the rat plasma. In addition, a comparison of the compounds' half-lives in mouse and rat plasma indicates some species differences with the rat plasma being less active. This observation parallels those we reported earlier for carboxyester THC analogues.¹⁵ Interestingly, our stability data show that the isomeric C9 alcohols **3c** and **3d** have similar half-lives in mouse plasma whereas in rat plasma the equatorial alcohol **3c** appears somewhat more stable. In summary, our data validated the soft analogue profile of our more polar, second generation, controlled deactivation cannabinergic ligands that also exhibit shorter metabolic half-lives for esterases when compared to the first generation side chain carboxylated THCs which carry no hydroxy groups at the C9 or C11 positions.

FUNCTIONAL CHARACTERIZATION

We focused on those analogues possessing the highest CB1/CB2 binding affinities (**3a**, **3b**, and **3c**), and experiments were carried out by measuring changes in forskolin-stimulated cAMP, as detailed earlier.^{19,20} Our testing results (Table 2) show that all three compounds potently decreased the levels of cAMP, indicating that within this signaling mechanism these compounds behaved as potent agonists at both the CB1 and CB2 receptors with the 11-OH-HHC ester analogue **3b** being the most potent.

IN VIVO BEHAVIORAL CHARACTERIZATION

Hypothermia Testing

The hypothermic effects of the side chain carboxyester analogues **3a**,³¹ **3b**, and **3c** with the highest CB1 receptor affinities were compared in rats. Rectal body temperature was measured in isolated rats over a 6 h period following drug injection (detailed procedures are given in Experimental Section). In agreement with our in vitro functional characterization,

compounds **3a**, **3b**, and **3c** all decreased core body temperature in a dose-dependent manner, reducing body temperature by 4.7–7.2 °C at the highest doses tested (Figure 2). For comparison, the effects of the nonhydrolyzable congener 11-OH-(–)-⁸-THC-DMH (**3g**) are also shown. The ED₅₀ values (in mg/kg, with 95% CI) were 0.04 (0.001, 0.15) for **3a**, 0.04 (0.02, 0.1) for **3b**, 0.74 (0.05, >10) for **3c**, and 0.04 (0.01, 0.12) for **3g**. Thus, the 11-OH-analogues **3a** and **3b** were equipotent with **3g**, and **3c** was slightly less potent than the other three compounds. All compounds had relatively fast onsets of drug effect. Significant decreases in body temperature typically occurred within 60 min after injection, although peak effects were not obtained until 2–3 h after injection (time course data for all tested doses of each compound are included in Supporting Information, Figure S1). A more detailed examination of the time course of the lowest doses of **3a**, **3b**, **3c**, and **3g** that had significant hypothermic effects is summarized in Figure 3. Administration of 0.1 mg/kg of **3a** or **3b**, carboxyester side chain analogues, resulted in temperature decreases of >4 °C, with significant recovery toward baseline within the 6 h test period. By comparison, doses of **3c** and **3g** that produced an equivalent reduction in rectal temperature had effects that persisted for more than 6 h.

Our hypothermia data in rats indicate that compounds **3a** and **3b** are equipotent and they have similar duration of action profiles. For further studies, the HHC analogue **3b** was selected over the THC analogue **3a** on the basis of the in vitro cAMP data where it behaves as a slightly more potent agonist for both the CB1 and CB2 receptors.

Analgesia Testing

To confirm the observed pharmacokinetic differences between the side chain carboxyester analogue **3b** and the nonhydrolyzable counterpart **3g**, we used the CB1 receptor characteristic analgesia assay. Two-way ANOVA suggested a significant interaction between time and drug [$F(3, 66) = 28.94$; $p < 0.001$]. The main effect of time was also significant [$F(3, 66) = 4.33$; $p = 0.008$]. Post hoc comparisons suggested significant differences between the two drugs (**3b** and **3g**) at 20, 60, and 360 min postinjection (Figure 4). These differences in onset and offset of effect for the two compounds are also reflected by comparisons within each ligand over time. Pairwise comparisons suggested less analgesia at the 360 min time point compared to the three earlier time points as well as a significant difference between the recordings at 60 and 180 min postinjection for compound **3b**. Slow onset of effect for compound **3g** is suggested by significant differences in analgesia scores at 20 as well as 60 min postinjection and the recordings at the 180 and 360 time points. In keeping with above profile of a fast onset and offset for compound **3b**, an additional dose of 1 mg/kg resulted in average MPE scores of 94%, 96%, 97%, and 58% at the four time-points (20, 60, 180, and 360 min postinjection) examined ($n = 6$; data not shown).

Drug Discrimination in Non-Human Primates

In an effort to further explore the validity of our controlled deactivation approach in species beyond rodents, we performed drug discrimination studies in squirrel monkeys (*Saimiri sciureus*) trained to discriminate 0.01 mg/kg (6a*R*,9*R*,10a*R*)-3-(adamantan-1-yl)-9-(hydroxymethyl)-6,6-dimethyl-6a,7,8,9,10,10a-hexahydro-6*H*-benzo[*c*]chromen-1-ol³² (**28**, AM4054, see Supporting Information Figure S2) from saline. This compound is a

structurally related cannabinoid CB1 full agonist which was synthesized at the Center for Drug Discovery and characterized in our earlier work.³³ It should be noted that studies with non-human primates are key to this project because metabolic processes and volumes of drug distribution vary between small rodent and primate species.³⁴

In these experiments, the time courses of the lowest doses of our lead compound **3b** (0.001 mg/kg) and the non-hydrolyzable cannabinoid **3g** (0.003 mg/kg) that fully substituted for the training dose of the CB1 agonist **28** were compared directly in the same group of subjects (Figure 5; see Experimental Section). Compound **3b** produced evidence of CB1-related discriminative-stimulus effects within 15 min ($t_{1/2}$ = 48 min) and was fully effective in three of four subjects 1 h after injection. Compound **3g** was similar in its onset of action ($t_{1/2}$ = 66 min) but did not achieve a full effect in the group of subjects until 2 h after injection. Notwithstanding the generally comparable onset of behavioral effects, the two compounds varied considerably in offset of action. Thus, **3b** began losing its CB1 discriminative-stimulus effects 4 h after injection and was completely without effect 16 h following treatment. On the other hand, **3g** fully substituted for the training drug stimulus (**28**) 24 h after injection and was completely without CB1 discriminative-stimulus effects at the 36 h time point. On the basis of calculated values for loss of 50% of full effect, the duration of action for **3b** was approximately 4 times shorter than for **3g**. Neither compound had appreciable effects on response rates throughout the present time course studies (Figure 5, lower panel).

Overall, our in vivo experiments in rats show that compounds **3a**, **3b**, and **3c** have in vivo hypothermia activity and produce similar maximum effects as the structurally related cannabinoid agonist **3g**. Among the hydrolyzable compounds tested, analogues **3a** and **3b** were found to be equipotent and they have similar duration of action profiles. Our antinociception data in mice and drug discrimination experiments in squirrel monkeys confirmed that the more polar 11-OH-carboxyester analogue **3b** is equipotent and exhibits significantly shorter duration of action when compared to the nonhydrolyzable standard **3g**.

CONCLUSIONS

We recently reported on a controlled deactivation/detoxification approach for obtaining cannabinoids with improved druggability and safety. In these earlier studies we have also provided evidence that the pharmacological half-lives of our selectively detoxified analogues can be controlled by the joint modulation of their relative stabilities for plasma esterases as well as through variation of their polar characteristics and thus to the depot effects. A major goal of this project is to develop controlled deactivation cannabinoid agonists with faster onset/offset and significantly shorter duration of action than the currently existing classical cannabinoids and also to explore the validity of this approach in species beyond rodents including nonhuman primates. To this extent, we report now the design, synthesis, and pharmacological evaluation of second generation, more polar, controlled deactivation cannabinoids. These novel cannabinergic compounds are C9- and C11-hydroxy-substituted hexahydrocannabinol (HHC) and tetrahydrocannabinol (THC) analogues in which the seven atom long side chain, with or without substituents, carries a metabolically labile 2',3'-ester group. Our in vitro data showed that compounds with a 1',1'-dimethyl-2'-carboxyester

group [-C(CH₃)₂-C(O)O-] on the side chain pharmacophore exhibit very high affinities for both the CB1 and CB2 receptors and also behave as potent and efficacious CB1/CB2 agonists in the cAMP assay. In agreement with our design principles, the analogues are susceptible to enzymatic hydrolysis by plasma esterases, while their hydrolytic metabolites are inactive. Importantly, for further work toward clinical development, in vivo studies validated our controlled deactivation approach in rodents and non-human primates. The lead molecule identified in this study, namely, butyl-2-[(6a*R*,9*R*,10a*R*)-1-hydroxy-9-(hydroxymethyl)-6,6-dimethyl-6a,7,8,9,10,10a-hexahydro-6*H*-benzo[*c*]chromen-3-yl]-2-methylpropanoate (**3b**), was found to exhibit remarkably high in vitro and in vivo potency with a shorter duration of action than the currently existing classical cannabinoid agonists.

EXPERIMENTAL SECTION

Materials

All reagents and solvents were purchased from Aldrich Chemical Co., unless otherwise specified, and used without further purification. All anhydrous reactions were performed under a static argon atmosphere in flame-dried glassware using scrupulously dry solvents. Flash column chromatography employed silica gel 60 (230–400 mesh). All compounds were demonstrated to be homogeneous by analytical TLC on precoated silica gel TLC plates (Merck, 60 F₂₄₅ on glass, layer thickness 250 μm), and chromatograms were visualized by phosphomolybdic acid staining. Melting points were determined on a micromelting point apparatus and are uncorrected. IR spectra were recorded on a PerkinElmer Spectrum One FT-IR spectrometer. NMR spectra were recorded in CDCl₃, unless otherwise stated, on a Bruker Ultra Shield 400 WB plus (¹H at 400 MHz, ¹³C at 100 MHz) or on a Varian INOVA-500 (¹H at 500 MHz, ¹³C at 125 MHz) spectrometers, and chemical shifts are reported in units of δ relative to internal TMS. Multiplicities are indicated as br (broadened), s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and coupling constants (*J*) are reported in hertz (Hz). Low- and high-resolution mass spectra were performed in School of Chemical Sciences, University of Illinois at Urbana—Champaign. Mass spectral data are reported in the form of *m/z* (intensity relative to base = 100). Elemental analyses were obtained in Baron Consulting Co, Milford, CT, and were within ±0.4% of the theoretical values (see Supporting Information). Purities of the tested compounds were determined by elemental analysis or by HPLC (using Waters Alliance HPLC system, 4.6 mm × 250 mm, Supelco Discovery column, acetonitrile/water with 8.5% *o*-phosphoric acid) or by LC/MS analysis using a Waters MicroMass ZQ system [electrospray-ionization (ESI) with Waters-2525 binary gradient module coupled to a photodiode array detector (Waters-2996) and ELS detector (Waters-2424) using a XTerra MS C18, 5 μm, 4.6 mm × 50 mm column and acetonitrile/water] and were >95%.

Butyl 2-[(6a*R*,10a*R*)-1-Hydroxy-9-(hydroxymethyl)-6,6-dimethyl-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-3-yl]-2-methylpropanoate (3a**)**—A stirred mixture of carboxylic acid **4a** (100 mg, 0.29 mmol), bromobutane (119 mg, 0.87 mmol), and sodium bicarbonate (37 mg, 0.43 mmol) in DMF (2 mL) was heated at 165 °C for 12 min using microwave irradiation. The reaction mixture was cooled to room temperature and diluted with water and ethyl acetate. The organic layer was separated, and the aqueous phase

was extracted with ethyl acetate. The combined organic layer was washed with brine, dried (MgSO_4), and concentrated under reduced pressure. Purification by flash column chromatography on silica gel (5–50% ethyl acetate in hexane) gave **3a** (60 mg, 51%) as a light yellow gum. IR (neat) 3292, 2961, 1727, 1704 (s, >C=O), 1619, 1578, 1417, 1328, 1263cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ 6.41 (d, $J = 2.0$ Hz, 1H, 4-H), 6.25 (d, $J = 2.0$ Hz, 1H, 2-H), 5.73 (m as d, $J = 4.5$ Hz, 1H, 8-H), 5.42 (s, 1H, ArOH), 4.10–4.02 (d, t and d overlapping, 4H, especially 4.08, d, $J = 13.0$ Hz, 1H, half of an AB system, $-\text{CH}_2-\text{OH}$, 4.06, t, $J = 6.5$ Hz, 2H, $-\text{C(O)OCH}_2-$, 4.03, d, $J = 13.0$ Hz, 1H, half of an AB system, $-\text{CH}_2-\text{OH}$), 3.41 (dd, $J = 16.0$ Hz, $J = 4.5$ Hz, 1H, $10\alpha\text{-H}$), 2.71 (td, $J = 11.0$ Hz, $J = 5.0$ Hz, 1H, $10\alpha\text{-H}$), 2.27–2.20 (m, 1H, $7\alpha\text{-H}$), 1.93–1.78 (m, 3H, $10\beta\text{-H}$, $7\beta\text{-H}$, $6\alpha\text{-H}$), 1.55 (quintet, $J = 7.0$ Hz, 2H, $-\text{CH}_2-$ of the side chain), 1.50 (s, 6H, $-\text{C}(\text{CH}_3)_2-$), 1.39 (s, 3H, $6\beta\text{-Me}$), 1.26 (quintet, 2H, $-\text{CH}_2-$ of the side chain), 1.10 (s, 3H, $6\alpha\text{-Me}$), 0.86 (t, $J = 7.5$ Hz, 3H, $7'\text{-H}$). ^{13}C NMR (125 MHz CDCl_3) δ 177.3 (>C=O), 155.6 (C-1 or C-5), 154.8 (C-5 or C-1), 144.5, 138.3, 121.6, 111.4, 107.3, 105.5, 77.7 (C-6), 67.2 ($-\text{O}-\text{CH}_2$), 65.0 ($-\text{OCH}_2-$), 46.2, 45.1, 31.5, 31.4, 30.6, 27.8, 27.7, 26.4, 26.2, 19.1 18.5, 13.8. Mass spectrum (ESI) m/z (relative intensity) 403 ($\text{M}^+ + \text{H}$, 100). Mass spectrum (EI) m/z (relative intensity) 402 (M^+ , 100), 301 (53), 217 (38). Exact mass (EI) calculated for $\text{C}_{24}\text{H}_{34}\text{O}_5$ (M^+), 402.2406; found 402.2407. HPLC (4.6 mm $\times$ 250 mm, Supelco Discovery column, acetonitrile/water) showed purity of 98.5% and retention time of 11.1 min for **3a**. Anal. ($\text{C}_{24}\text{H}_{34}\text{O}_5$) C, H.

Butyl 2-[(6a*R*,9*R*,10a*R*)-1-Hydroxy-9-(hydroxymethyl)-6,6-dimethyl-6a, 7,8,9,10,10a-hexahydro-6*H*-benzo[*c*]chromen-3-yl]-2-methylpropanoate (3b**)—**

To a solution of **21** (75 mg, 0.15 mmol) in anhydrous THF (3.6 mL) at -40°C , under an argon atmosphere, was added tetra-*n*-butylammonium fluoride (0.3 mL, 0.3 mmol, 1 M solution in anhydrous THF). The reaction mixture was stirred for 30 min at the same temperature and then quenched using a saturated aqueous NH_4Cl solution. Extractive isolation with diethyl ether and purification by flash column chromatography on silica gel (20% ethyl acetate in hexane) gave **3b** (52 mg, 90% yield) as a white solid. $\text{Mp} = 59\text{--}60^\circ\text{C}$. IR (neat) 3380, 2971, 2869, 1727, 1703, 1620, 1577, 1417, 1268, 1141 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ 6.39 (d, $J = 2.0$ Hz, 1H, Ar-H), 6.22 (d, $J = 2.0$ Hz, 1H, Ar-H), 5.33 (br s, 1H, ArOH), 4.05 (d, $J = 6.0$ Hz, 2H, $4'\text{-H}$), 3.56–3.49 (m, 2H, $-\text{CH}_2\text{OH}$), 3.23–3.18 (m as br d, $J = 13.0$ Hz, 1H, C-ring), 2.51–2.44 (m as td, $J = 11.0$ Hz, $J = 2.5$ Hz, 1H, C-ring), 2.00–1.94 (m, 1H, C-ring), 1.94–1.88 (m, 1H, C-ring), 1.82–1.72 (m, 1H, C-ring), 1.58–1.44 (quintet, s, and m, overlapping, 9H, $-\text{C}(\text{CH}_3)_2-$, $5'\text{-H}$, C-ring, especially 1.49, s, 6H, $-\text{C}(\text{CH}_3)_2-$), 1.38 (s, 3H, 6-Me), 1.29–1.22 (m as sextet, $J = 7.0$ Hz, 2H, $6'\text{-H}$), 1.18–1.10 (m, 2H, C-ring), 1.07 (s, 3H, 6-Me), 0.86 (t, $J = 7.5$ Hz, 3H, $7'\text{-H}$), 0.76 (m as q, $J = 11.0$ Hz, 1H, C-ring). ^{13}C NMR (100 MHz CDCl_3) δ 177.1 ($-\text{C(O)O}-$), 155.2 (ArC-1 or ArC-5), 154.9 (ArC-5 or ArC-1), 144.2 (tertiary aromatic), 111.0 (tertiary aromatic), 107.2 (ArC-2 or ArC-4), 105.3 (ArC-4 or ArC-2), 68.4 ($-\text{CH}_2\text{OH}$), 64.7 ($-\text{OCH}_2-$), 49.3, 46.0, 40.4, 35.0, 33.0, 30.5, 29.7, 27.7, 27.4, 26.2, 26.0, 19.0, 13.6. Mass spectrum (ESI) m/z (relative intensity) 405 ($\text{M}^+ + \text{H}$, 100). Mass spectrum (EI) m/z (relative intensity) 404 (M^+ , 100), 361 (41), 303 (65), 265 (41). Exact mass (ESI) calculated for $\text{C}_{24}\text{H}_{37}\text{O}_5$ ($\text{M}^+ + \text{H}$), 405.2641; found 405.2642. Exact mass (EI) calculated for $\text{C}_{24}\text{H}_{36}\text{O}_5$ (M^+), 404.2563; found 404.2559. HPLC (4.6 mm $\times$ 250 mm, Supelco Discovery column, acetonitrile/water) showed purity of 99.5% and retention time of 10.8 min for the title compound.

Butyl 2-[(6a*R*,9*R*,10a*R*)-6a,7,8,9,10,10a-Hexahydro-1,9-dihydroxy-6,6-dimethyl-6*H*-benzo[*c*]chromen-3-yl]-2-methylpropanoate (3c)—To a solution of **16** (35 mg, 0.09 mmol) in anhydrous methanol (3 mL) at -78°C , under an argon atmosphere, was added sodium borohydride (3.4 mg, 0.09 mmol). The reaction mixture was stirred at the same temperature for 2 h and then quenched by the addition of aqueous 2 N HCl solution. The mixture was warmed to room temperature, diluted with water, and extracted with ethyl acetate. The organic layer was washed with brine, dried (MgSO_4), and concentrated in vacuo. Purification by flash column chromatography on silica gel (24% acetone in hexane) gave **3c** (30 mg, 84% yield) as light yellow gum free from its epimer **3d** which is also produced in traces during the reaction (ratio **3c**/**3d** ~ 96:4). IR (neat) 3329, 2971, 2872, 1727, 1704, 1620, 1578, 1418, 1272, 1142 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ 6.38 (d, $J = 2.0$ Hz, 1H, ArH), 6.22 (d, $J = 2.0$ Hz, 1H, ArH), 6.08 (br s, 1H, ArOH), 4.04 (t, $J = 6.5$ Hz, 2H, 4'-H), 3.86 (dddd, $J = 16.0$ Hz, $J = 15.0$ Hz, $J = 4.5$ Hz, $J = 4.0$ Hz, 1H, 9ax-H, peak half-width = 26 Hz), 3.51 (dddd, $J = 12.0$ Hz, $J = 4.5$ Hz, $J = 2.5$ Hz, $J = 2.0$ Hz, 1H, 10eq-H), 2.47 (ddd, $J = 12.0$ Hz, $J = 11.5$ Hz, $J = 2.5$ Hz, 1H, 10a-H), 2.16 (m as br d, $J = 10.0$ Hz, 1H, 8eq-H), 1.88 (dddd, $J = 13.5$ Hz, $J = 3.5$ Hz, $J = 2.5$ Hz, $J = 2.0$ Hz, 1H, 7eq-H), 1.63 (br s, 1H, OH), 1.57–1.50 (quintet and m, overlapping 3H, 5'-H and 6a-H), 1.49 (s, 6H, $-\text{C}(\text{CH}_3)_2-$), 1.42 (dddd, $J = 15.5$ Hz, $J = 15.0$ Hz, $J = 13.0$ Hz, $J = 3.5$ Hz, 1H, 8ax-H), 1.38 (s, 3H, 6-Me), 1.25 (sextet, $J = 7.0$ Hz, 2H, 6'-H), 1.15 (dddd, $J = 13.5$ Hz, $J = 13.0$ Hz, $J = 12.0$ Hz, $J = 3.5$ Hz, 1H, 7ax-H), 1.12 (ddd, $J = 15.5$ Hz, $J = 12.0$ Hz, $J = 12.0$ Hz, 1H, 10ax-H), 1.05 (s, 3H, 6-Me), 0.85 (t, $J = 7.5$ Hz, 3H, 7'-H). ^{13}C NMR (100 MHz, CDCl_3) δ 171.5 ($-\text{C}(\text{O})\text{O}-$), 155.0 (ArC), 154.7 (ArC), 144.5 (ArC), 115.4 (ArC), 110.2 (ArC), 107.5 (ArC), 77.2 (C-6), 70.7 ($>\text{CH}-\text{OH}$), 64.6 ($-\text{OCH}_2-$), 48.3, 46.0, 39.0, 35.6, 33.4, 30.5, 27.8, 26.2, 26.1, 26.0, 19.0, 178, 13.6. Mass spectrum (ESI) m/z (relative intensity) 391 ($\text{M}^+ + \text{H}$, 100). Mass spectrum (EI) m/z (relative intensity) 390 (M^+ , 87), 329 (32), 289 (100, $\text{M}^+ - \text{CH}_3(\text{CH}_2)_3\text{OC}(\text{O})$), 265 (37), 177 (61). Exact mass (EI) calculated for $\text{C}_{23}\text{H}_{34}\text{O}_5$ (M^+), 390.2406; found 390.2408. HPLC (4.6 mm $\times$ 250 mm, Supelco Discovery column, acetonitrile/water) showed purity of 99.5% and retention time of 9.7 min for the title compound.

Butyl 2-[(6a*R*,9*S*,10a*R*)-6a,7,8,9,10,10a-Hexahydro-1,9-dihydroxy-6,6-dimethyl-6*H*-benzo[*c*]chromen-3-yl]-2-methylpropanoate (3d)—The synthesis was carried out as described for **3a** using **4d** (34 mg, 0.10 mmol), sodium bicarbonate (15 mg, 0.16 mmol), and 1-bromobutane (35 mg, 0.25 mmol) in DMF (1.5 mL) to give **3d** (17 mg, 42% yield) as light yellow gum. IR (neat) 3357, 2927, 2873, 1727, 1706, 1620, 1578, 1418, 1262, 1140 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ 6.41 (d, $J = 2.0$ Hz, 1H, ArH), 6.31 (d, $J = 2.0$ Hz, 1H, ArH), 4.29 (m as t, $J = 3.5$ Hz, 1H, 9eq-H, peak half-width = 9.5 Hz), 4.07 (m as t, $J = 6.5$ Hz, 2H, 4'-H), 3.24 (ddd, $J = 14.5$ Hz, $J = 3.0$ Hz, $J = 2.5$ Hz, 1H, 10eq-H), 2.97 (ddd, $J = 12.0$ Hz, $J = 11.0$ Hz, $J = 2.5$ Hz, 1H, 10a-H), 2.02–1.97 (m as br d, $J = 13.5$ Hz, 1H, 8eq-H), 1.71–1.47 (m and s overlapping, 12H, 8ax-H, 7ax-H, 7eq-H, 6a-H, 5'-H, $-\text{C}(\text{CH}_3)_2-$, especially 1.51, s, $-\text{C}(\text{CH}_3)_2-$), 1.39 (s, 3H, 6- CH_3), 1.38–1.32 (m, 1H, 10ax-H), 1.28 (sextet, $J = 7.0$ Hz, 2H, 6'-H), 1.07 (s, 3H, 6- CH_3), 0.87 (t, $J = 7.5$ Hz, 3H, 7'-H). Mass spectrum (ESI) m/z (relative intensity) 391 ($\text{M}^+ + \text{H}$, 100), 289 (45, $\text{M}^+ - \text{CH}_3(\text{CH}_2)_3\text{OC}(\text{O})$). Mass spectrum (EI) m/z (relative intensity) 390 (M^+ , 57), 329 (55), 289 (32, $\text{M}^+ - \text{CH}_3(\text{CH}_2)_3\text{OC}(\text{O})$), 183 (97), 84 (100). Exact mass (EI) calculated for $\text{C}_{23}\text{H}_{34}\text{O}_5$

(M⁺), 390.2406; found 390.2408. LC/MS analysis (Waters MicroMass ZQ system) showed purity of 97.7% and retention time of 5.5 min for the title compound.

Butyl 2-[(6a*R*, 9*R*, 10a*R*)-1,9-Dihydroxy-6,6-dimethyl-6a,7,8,9,10,10a-hexahydro-6*H*-benzo[*c*]chromen-3-yl]acetate (3e)—The synthesis was carried out as described for **3c** using **27** (58 g, 0.16 mmol) and NaBH₄ (6.1 mg, 0.16 mmol) in MeOH (5 mL) and gave **3e** (35 mg, 61% yield) as a light yellow gum. Purification by flash column chromatography on silica gel (20–30% acetone in hexane) gave the title compound free from its epimer **3f** which is also produced in traces (4–6%) during the reaction. IR (neat) 3428, 2975, 2872, 1729, 1704, 1619, 1578, 1417 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 6.41 (br s, 1H, ArOH), 6.32 (d, *J* = 2.0 Hz, 1H, ArH), 6.22 (d, *J* = 2.0 Hz, 1H, ArH), 4.08 (t, *J* = 6.5 Hz, 2H, 4'-H), 3.80 (dddd, *J* = 16.0 Hz, *J* = 15.0 Hz, *J* = 4.5 Hz, *J* = 4.0 Hz, 1H, 9ax-H, peak half-width = 26 Hz), 3.68 (d, *J* = 16.0 Hz, 1H, half of an AX system, -CH₂-C(O)O-), 3.55 (d, *J* = 16.0 Hz, 1H, half of an AX system, -CH₂-C(O)O-), 3.51 (dddd, *J* = 12.0 Hz, *J* = 4.5 Hz, *J* = 2.5 Hz, *J* = 2.0 Hz, 1H, 10eq-H), 2.39 (ddd, *J* = 12.0 Hz, *J* = 11.5 Hz, *J* = 2.5 Hz, 1H, 10a-H), 2.16 (m as br d, *J* = 10.0 Hz, 1H, 8eq-H), 1.87 (dddd, *J* = 13.5 Hz, *J* = 3.5 Hz, *J* = 2.5 Hz, *J* = 2.0 Hz, 1H, 7eq-H), 1.59 (quintet, *J* = 7.5 Hz, 2H, 5'-H), 1.51 (m as td, *J* = 12.5 Hz, *J* = 2.5 Hz, 1H, 6a-H), 1.42–1.28 (m, s and sextet overlapping, 6H, 8ax-H, 6'-H, 6-Me), 1.19–1.06 (m, 2H, 7ax-H, 10ax-H), 1.05 (s, 3H, 6-Me), 0.89 (t, *J* = 7.5 Hz, 3H, 7'-H). ¹³C NMR (100 MHz CDCl₃) δ 171.8 (-C(O)O-), 155.4 (ArC), 154.8 (ArC), 134.2 (ArC), 116.3 (ArC), 110.8 (ArC), 103.6 (ArC), 77.2(C-6), 70.5(>CH-OH), 65.0(-OCH₂-), 49.8, 42.0, 39.7, 35.6, 34.7, 30.6, 27.6, 26.21, 19.0, 18.4, 13.6. Mass spectrum (ESI) *m/z* (relative intensity) 363 (M⁺ + H, 23), 345 (100, M⁺ - OH), 271 (53). Exact mass (ESI) calculated for C₂₁H₃₁O₅ (M⁺ + H), 363.2171; found 363.2167. LC/MS analysis (Waters MicroMass ZQ system) showed purity of 97.3% and retention time of 7.1 min for the title compound.

Butyl 2-[(6a*R*,9*S*,10a*R*)-1,9-Dihydroxy-6,6-dimethyl-6a,7,8,9,10,10a-hexahydro-6*H*-benzo[*c*]chromen-3-yl]acetate (3f)—The synthesis was carried out as described for **4d** using **27** (40 mg, 0.11 mmol) and K-selectride (0.44 mL, 1 M solution in THF) in THF (3 mL). Purification by flash column chromatography on silica gel (20–30% acetone in hexane) gave the title compound (21 mg, 52% yield) as light yellow gum free from its epimer **3e** which is also produced in traces (3–5%) during the reaction. IR (neat) 3430, 2973, 1729, 1704, 1620, 1578, 1417 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 6.30 (d, *J* = 2.0 Hz, 1H, ArH), 6.23 (d, *J* = 2.0 Hz, 1H, ArH), 5.22 (br s, 1H, ArOH), 4.23 (m as t, *J* = 2.5 Hz, 1H, 9eq-H, peak half-width = 9.0 Hz), 4.11 (m as td, *J* = 7.0 Hz, *J* = 2.5 Hz, 2H, 4'-H), 3.74 (d, *J* = 16.0 Hz, 1H, half of an AX system, -CH₂-C(O)O-), 3.58 (d, *J* = 16.0 Hz, 1H, half of an AX system, -CH₂-C(O)O-), 2.90 (ddd, *J* = 12.0 Hz, *J* = 11.0 Hz, *J* = 2.0 Hz, 1H, 10a-H), 2.51 (ddd, *J* = 14.5 Hz, *J* = 3.0 Hz, *J* = 2.2 Hz, 1H, 10eq-H), 1.98–1.90 (m as br d, *J* = 13.5 Hz, 1H, 8eq-H), 1.70–1.47 (m, 6H, 8ax-H, 7ax-H, 7eq-H, 6a-H, 5'-H), 1.42–1.28 (s, m and sextet overlapping, 6H, 10ax-H, 6-CH₃, 6'-H, especially 1.36, s, 6-CH₃), 1.04 (s, 3H, 6-CH₃), 0.89 (t, *J* = 7.5 Hz, 3H, 7'-H). Mass spectrum (ESI) *m/z* (relative intensity) 363 (M⁺ + H, 100), 345 (153, M⁺ - OH). Exact mass (ESI) calculated for C₂₁H₃₁O₅ (M⁺ + H), 363.2171; found 363.2173. LC/MS analysis (Waters MicroMass ZQ system) showed purity of 97.8% and retention time of 7.2 min for the title compound.

2-[(6aR,10aR)-1-Hydroxy-9-(hydroxymethyl)-6,6-dimethyl-6a,7,10,10a-tetrahydro-6H-benzo[c]chromen-3-yl]-2-methyl-propanoic Acid (4a)—A stirred mixture of **11** (200 mg, 0.45 mmol) and NaOH (72 mg, 1.8 mmol) in THF/water (1:1 ratio, 8 mL) was refluxed for 16 h under argon. Volatiles were removed under reduced pressure, and the residue was acidified with aqueous 1 N HCl and diluted with ethyl acetate. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate. The combined organic phase was washed with water and brine, dried (MgSO₄), and concentrated in vacuo. Purification by flash column chromatography on silica gel (50% acetone in hexane) afforded the title compound in 68% yield (106 mg) as a light yellow gum. IR (neat) 3317, 2976, 2932, 1702, 1620, 1577, 1417, 1265, 1185 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 7.31–6.40 (br s, 1H, -OH), 6.41 (d, *J* = 1.5 Hz, 1H, 4-H), 6.30 (d, *J* = 1.5 Hz, 1H, 2-H), 5.68 (m as d, *J* = 3.5 Hz, 1H, 8-H), 4.03 (d, *J* = 12.5 Hz, 1H, half of an AB system, -CH₂OH), 3.93 (d, *J* = 12.5 Hz, 1H, half of an AB system, -CH₂OH), 3.47 (br d, *J* = 14.5 Hz, 1H, 10α-H), 2.64 (td, *J* = 11.5 Hz, *J* = 4.5 Hz, 1H, 10α-H), 2.16 (m, 1H, 7α-H), 1.83–1.70 (m, 3H, 10β-H, 7β-H, 6α-H), 1.50 (s, 3H, 1'-CH₃), 1.47 (s, 3H, 1'-CH₃), 1.36 (s, 3H, 6β-Me), 1.06 (s, 3H, 6α-Me); ¹³C NMR (125 MHz CDCl₃) δ 180 (>C=O), 155.7 (C-1 or C-5), 154.5 (C-5 or C-1), 144.0, 137.9, 121.4, 111.5, 106.6, 105.4, 76.6 (C-6), 66.7 (-CH₂OH), 45.8, 44.9, 31.3, 31.1, 29.6, 27.5, 27.4, 26.1, 18.3. Mass spectrum (ESI) *m/z* (relative intensity) 347 (M⁺ + H, 100), 329 (M⁺ – OH, 33). Mass spectrum (EI) *m/z* (relative intensity) 346 (M⁺, 19), 329 (M⁺ – OH, 8), 285 (10), 247 (15), 84 (100); exact mass (EI) calculated for C₂₀H₂₆O₅ (M⁺), 346.1780; found 346.1780. HPLC (4.6 mm × 250 mm, Supelco Discovery column, acetonitrile/water) showed purity of 99.8% and retention time of 6.2 min for **4a**.

2-[(6aR,9R,10aR)-1-Hydroxy-9-(hydroxymethyl)-6,6-dimethyl-6a,7,8,9,10,10a-hexahydro-6H-benzo[c]chromen-3-yl]-2-methylpropanoic Acid (4b)—The synthesis was carried out as described for **22** using **3b** (20 mg, 0.05 mmol) and lithium hydroxide (12 mg, 0.5 mmol) in dioxane/water (2.8 mL, 1:1 ratio). Workup and purification by flash column chromatography on silica gel (60% ethyl acetate in hexane) gave **4b** (14.5 mg, 85% yield) as a yellow gum. IR (neat) 3321, 2975, 2932, 1702, 1621, 1577, 1417, 1264, 1185 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 6.42 (d, *J* = 2.0 Hz, 1H, Ar-H), 6.28 (d, *J* = 2.0 Hz, 1H, Ar-H), 3.53 (dd, *J* = 10.5 Hz, *J* = 5.5 Hz, half of an AB system, 1H, -CH₂OH), 3.45 (dd, *J* = 10.5 Hz, *J* = 8.0 Hz, half of an AB system, 1H, -CH₂OH), 3.29–3.22 (m as br d, *J* = 13.0 Hz, 1H, Cring), 2.44–2.37 (m as td, *J* = 11.0 Hz, *J* = 2.5 Hz, 1H, C-ring), 1.90–1.82 (m as br d, *J* = 9.5 Hz, 2H, C-ring), 1.78–1.68 (m, 1H, C-ring), 1.49–1.40 (m and s, overlapping, 7H, -C(CH₃)₂-, C-ring, especially 1.48, s, 6H, -C(CH₃)₂-), 1.35 (s, 3H, 6-Me), 1.12–1.10 (m, 2H, C-ring), 0.98 (s, 3H, 6-Me), 0.74 (m as q, *J* = 11.0 Hz, 1H, C-ring). ¹³C NMR (100 MHz CDCl₃) δ 175.6 (-C(O)O-), 154.9 (ArC-1 or ArC-5), 155.0 (ArC-1 and ArC-5), 143.7 (tertiary aromatic), 111.4 (tertiary aromatic), 107.4 (ArC-2 or ArC-4), 105.3 (ArC-4 or ArC-2), 68.4 (-CH₂OH), 49.2, 45.9, 40.4, 34.8, 33.0, 29.5, 27.6, 27.4, 26.1, 19.0. Mass spectrum (ESI) *m/z* (relative intensity) 349 (M⁺ + H, 100). LC/MS analysis (Waters MicroMass ZQ system) showed purity of 97.5% and retention time of 4.2 min for the title compound.

2-[(6aR,9R,10aR)-6a,7,8,9,10,10a-Hexahydro-1,9-dihydroxy-6,6-dimethyl-6H-benzo[c]chromen-3-yl]-2-methylpropanoic Acid (4c)—The synthesis was carried

out as described for **3c** using **22** (55 mg, 0.16 mmol) and sodium borohydride (25 mg, 0.66 mmol) in methanol (6 mL) at room temperature and gave the title compound as a light yellow gum in 81% yield (43 mg). IR (neat) 3338, 2975, 1695, 1622, 1418 cm^{-1} ; ^1H NMR (500 MHz, CD_3OD) δ 6.35 (d, $J = 2.0$ Hz, 1H, ArH), 6.27 (d, $J = 2.0$ Hz, 1H, ArH), 3.75 (dddd, $J = 15.5$ Hz, $J = 15.0$ Hz, $J = 4.5$ Hz, $J = 4.0$ Hz, 1H, 9ax-H, peak half-width = 25 Hz), 3.53 (dddd, $J = 12.0$ Hz, $J = 4.5$ Hz, $J = 2.5$ Hz, $J = 2.0$ Hz, 1H, 10eq-H), 2.45 (ddd, $J = 12.0$ Hz, $J = 11.5$ Hz, $J = 2.5$ Hz, 1H, 10a-H), 2.12 (m as br d, $J = 11.0$ Hz, 1H, 8eq-H), 1.90 (dddd, $J = 13.5$ Hz, $J = 3.5$ Hz, $J = 2.5$ Hz, $J = 2.0$ Hz, 1H, 7eq-H), 1.50–1.34 (s, s and m, overlapping, 11H, $-\text{C}(\text{CH}_3)_2-$, 6a-H, 10ax-H, 6-Me, especially 1.47, s, $-\text{C}(\text{CH}_3)_2-$ and 1.34, s, 6-Me), 1.21 (dddd, $J = 15.5$ Hz, $J = 15.0$ Hz, $J = 13.0$ Hz, $J = 3.5$ Hz, 1H, 8ax-H), 1.05 (s, 3H, 6-Me), 0.97 (dddd, $J = 13.5$ Hz, $J = 13.0$ Hz, $J = 12.0$ Hz, $J = 3.5$ Hz, 1H, 7ax-H). Mass spectrum (ESI) m/z (relative intensity) 335 ($\text{M}^+ + \text{H}$, 100), 317 (54, $\text{M}^+ - \text{OH}$), 289 (84, $\text{M}^+ - \text{COOH}$). Mass spectrum (EI) m/z (relative intensity) 334 (M^+ , 97), 316 (79), 273 (97), 233 (100), 149 (87). Exact mass (EI) calculated for $\text{C}_{19}\text{H}_{26}\text{O}_5$ (M^+), 334.1780; found 334.1777. HPLC (4.6 mm $\times$ 250 mm, Supelco Discovery column, acetonitrile/water) showed purity of 98.3% and retention time of 5.4 min for the title compound. LC/MS analysis (Waters MicroMass ZQ system) showed purity of 98.4% and retention time of 4.2 min for the title compound.

2-[(6a*R*,9*S*,10a*R*)-6a,7,8,9,10,10a-Hexahydro-1,9-dihydroxy-6,6-dimethyl-6*H*-benzo[*c*]chromen-3-yl]-2-methylpropanoic Acid (4d)—K-selectride (0.96 mL, 1 M solution in THF) was added under an argon atmosphere to a solution of **22** (80 mg, 0.24 mmol) in dry THF (12 mL) at room temperature. The reaction mixture was stirred at the same temperature for 2 h, and then it was quenched with aqueous 1 N HCl solution and diluted with ethyl acetate. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate. The combined organic phase was washed with water and brine, dried (MgSO_4), and concentrated under reduced pressure. Purification by flash column chromatography on silica gel (38% acetone in hexane) gave compound **4d** (73 mg, 91% yield) as light yellow gum. IR (neat) 3327, 2978, 1697, 1622, 1420 cm^{-1} ; ^1H NMR (500 MHz, CD_3OD) δ 6.34 (d, $J = 2.0$ Hz, 1H, ArH), 6.27 (d, $J = 2.0$ Hz, 1H, ArH), 4.13 (m as t, $J = 3.5$ Hz, 1H, 9eq-H, peak half-width = 9.5 Hz), 3.40 (ddd, $J = 14.5$ Hz, $J = 3.0$ Hz, $J = 2.5$ Hz, 1H, 10eq-H), 2.94 (ddd, $J = 12.0$ Hz, $J = 11.0$ Hz, $J = 2.5$ Hz, 1H, 10a-H), 1.96–1.91 (m, 1H, 8eq-H), 1.71–1.46 (m and s overlapping, 10H, 8ax-H, 7ax-H, 7eq-H, 6a-H, $-\text{C}(\text{CH}_3)_2-$, especially 1.47, s, $-\text{C}(\text{CH}_3)_2-$), 1.36 (s, 3H, 6- CH_3), 1.24–1.16 (m, 1H, 10ax-H), 1.09 (s, 3H, 6- CH_3). Mass spectrum (ESI) m/z (relative intensity) 335 ($\text{M}^+ + \text{H}$, 100), 317 (27, $\text{M}^+ - \text{OH}$), 289 (29, $\text{M}^+ - \text{COOH}$). Mass spectrum (EI) m/z (relative intensity) 334 (M^+ , 37), 316 (29), 273 (42), 233 (19), 149 (100). Exact mass (EI) calculated for $\text{C}_{19}\text{H}_{26}\text{O}_5$ (M^+), 334.1780; found 334.1784. LC/MS analysis (Waters MicroMass ZQ system) showed purity of 96.7% and retention time of 4.3 min for the title compound.

2-[(6a*R*,9*R*,10a*R*)-1,9-Dihydroxy-6,6-dimethyl-6a,7,8,9,10,10a-hexahydro-6*H*-benzo[*c*]chromen-3-yl]acetic Acid (4e)—The synthesis was carried out as described for **3c** using **26** (25 mg, 0.082 mmol) and NaBH_4 (12.4 mg, 0.32 mmol) in MeOH (5 mL) at room temperature and gave **4e** (17 mg, 65% yield) as light yellow gum. Purification by flash column chromatography on silica gel (40% acetone in hexane) gave the title compound free

from its epimer **4f** which is also produced in traces (4–6%) during the reaction. IR (neat) 3350, 2973, 1694, 1622, 1419 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ 6.32 (d, $J = 2.5$ Hz, 1H, ArH), 6.21 (d, $J = 2.5$ Hz, 1H, ArH), 3.78 (dddd, $J = 16.0$ Hz, $J = 15.0$ Hz, $J = 4.5$ Hz, $J = 4.0$ Hz, 1H, 9ax-H, peak half-width = 26 Hz), 3.62 (d, $J = 16.0$ Hz, 1H, half of an AB system, $-\text{CH}_2-\text{C}(\text{O})\text{O}-$), 3.54 (d, $J = 16.0$ Hz, 1H, half of an AB system, $-\text{CH}_2-\text{C}(\text{O})\text{O}-$), 3.49 (dddd, $J = 12.0$ Hz, $J = 4.5$ Hz, $J = 2.5$ Hz, $J = 2.0$ Hz, 1H, 10eq-H), 2.39 (ddd, $J = 12.0$ Hz, $J = 11.5$ Hz, $J = 2.5$ Hz, 1H, 10a-H), 2.14 (m as br d, $J = 10.0$ Hz, 1H, 8eq-H), 1.87 (dddd, $J = 13.5$ Hz, $J = 3.5$ Hz, $J = 2.5$ Hz, $J = 2.0$ Hz, 1H, 7eq-H), 1.51 (m as td, $J = 12.5$ Hz, $J = 2.5$ Hz, 1H, 6a-H), 1.39–1.28 (m and s overlapping, 4H, 8ax-H, 6-Me), 1.18–1.06 (m, 2H, 7ax-H, 10ax-H), 1.00 (s, 3H, 6-Me). Mass spectrum (ESI) m/z (relative intensity) 307 ($\text{M}^+ + \text{H}$, 98), 289 (100, $\text{M}^+ - \text{OH}$). Exact mass (ESI) calculated for $\text{C}_{17}\text{H}_{23}\text{O}_5$ ($\text{M}^+ + \text{H}$), 307.1545; found 307.1549. LC/MS analysis (Waters MicroMass ZQ system) showed purity of 96.7% and retention time of 3.6 min for the title compound.

2-[(6a*R*,9*S*,10a*R*)-1,9-Dihydroxy-6,6-dimethyl-6a,7,8,9,10,10a-hexahydro-6*H*-benzo[*c*]chromen-3-yl]acetic Acid (4f**)**—The synthesis was carried out as described for **4d** using **26** (20 mg, 0.065 mmol) and K-selectride (0.65 mL, 1 M solution in THF) in THF (5 mL). Purification by flash column chromatography on silica gel (40% acetone in hexane) gave the title compound (18 mg, 90% yield) as light yellow gum free from its epimer **4e** which is also produced in traces (3–5%) during the reaction. IR (neat) 3337, 2972, 1695, 1622, 1419 cm^{-1} ; ^1H NMR (500 MHz, $\text{CDCl}_3 + \text{CD}_3\text{OD}$) δ 6.31 (d, $J = 2.0$ Hz, 1H, ArH), 6.22 (d, $J = 2.0$ Hz, 1H, ArH), 4.21 (m as br s, 1H, 9eq-H, peak half-width = 9.0 Hz), 3.70 (d, $J = 16.0$ Hz, 1H, half of an AB system, $-\text{CH}_2-\text{C}(\text{O})\text{O}-$), 3.57 (d, $J = 16.0$ Hz, 1H, half of an AB system, $-\text{CH}_2-\text{C}(\text{O})\text{O}-$), 2.90 (ddd as td, $J = 12.0$ Hz, $J = 2.0$ Hz, 1H, 10a-H), 2.49 (br d, $J = 14.0$ Hz, 1H, 10eq-H), 1.98–1.90 (m as br d, $J = 13.5$ Hz, 1H, 8eq-H), 1.70–1.23 (m and s overlapping, 8H, 8ax-H, 7ax-H, 7eq-H, 6a-H, 10ax-H and 6- CH_3 , especially 1.36, s, 6- CH_3), 1.04 (s, 3H, 6- CH_3). Mass spectrum (ESI) m/z (relative intensity) 307 ($\text{M}^+ + \text{H}$, 65), 289 (100, $\text{M}^+ - \text{OH}$). Exact mass (ESI) calculated for $\text{C}_{17}\text{H}_{23}\text{O}_5$ ($\text{M}^+ + \text{H}$), 307.1545; found 307.1540. LC/MS analysis (Waters MicroMass ZQ system) showed purity of 96.5% and retention time of 3.7 min for the title compound.

Methyl 2-(3,5-Dimethoxyphenyl)-2-methylpropanoate (9**)**—A suspension of carboxylic acid **8** (11.4 g, 50.9 mmol), MeI (36.2 g, 225 mmol), and K_2CO_3 (10.6 g, 76 mmol) in anhydrous acetone (200 mL) was stirred overnight at room temperature under argon. Insoluble materials were filtered off, and the filtrate was evaporated under reduced pressure. The residue was diluted with diethyl ether and water, and the ethereal layer was separated. The aqueous layer was extracted with diethyl ether, and the combined organic phase was washed with water and brine, dried (MgSO_4), and concentrated in vacuo. Purification by flash column chromatography on silica gel (5–20% AcOEt in hexane) gave the title compound as colorless oil in 72% yield (8.75 g). IR (neat) 3412, 2962, 2932, 1728 (s, $>\text{C}=\text{O}$), 1702 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ 6.47 (d, $J = 2.0$ Hz, 2H, ArH), 6.33 (t, $J = 2.0$ Hz, 1H, ArH), 3.79 (s, 6H, OMe), 3.66 (s, 3H, COOMe), 1.33 (s, 6H, $-\text{C}(\text{CH}_3)_2-$). ^{13}C NMR (100 MHz CDCl_3) δ 181.8 ($>\text{C}=\text{O}$), 160.7, 146.2, 104.5, 98.4, 55.3 ($-\text{OMe}$), 46.3, 26.2. Mass spectrum (ESI) m/z (relative intensity) 239 ($\text{M}^+ + \text{H}$, 100).

Methyl 2-(3,5-Dihydroxyphenyl)-2-methylpropanoate (10)—To a stirred solution of methyl ester **9** (5.0 g, 21.0 mmol) in dry CH₂Cl₂ (210 mL) at –78 °C, under an argon atmosphere was added boron tribromide (65.1 mL of 1 M solution in CH₂Cl₂). Following the addition, the reaction temperature was gradually raised over a period of 1 h to 0 °C, and the stirring was continued at that temperature until the reaction was completed (2.5 h). Unreacted boron tribromide was destroyed by the addition of MeOH at –78 °C. The resulting mixture was warmed to room temperature, and volatiles were removed in vacuo. The residue was dissolved in diethyl ether and washed with water and brine and dried (MgSO₄). Solvent evaporation and purification by flash column chromatography on silica gel (10–40% AcOEt in hexane) gave **10** as a pale yellow semisolid material in 71% yield (3.1 g). IR (neat) 3180, 1725 (s, >C=O), 1701 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 6.41 (d, *J* = 2.0 Hz, 2H, ArH), 6.24 (t, *J* = 2.0 Hz, 1H, ArH), 5.67 (br s, 2H, OH), 3.66 (s, 3H, COOMe), 1.52 (s, 6H, -C(CH₃)₂-). ¹³C NMR (100 MHz CDCl₃) δ 177.6 (>C=O), 156.9, 147.5, 105.5, 101.4, 52.5 (-OMe), 46.4, 26.2. Mass spectrum (ESI) *m/z* (relative intensity) 211 (M⁺ + H, 100).

[(6a*R*, 10a*R*)-1-Hydroxy-3-(1-methoxy-2-methyl-1-oxopropan-2-yl)-6,6-dimethyl-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]-chromen-9-yl]methyl Pivalate (11)—To a stirred solution of resorcinol **10** (1.0 g, 4.76 mmol) and pivalate ester **6** (1.32 g, 5.24 mmol) in anhydrous CH₂Cl₂ (24 mL) at –20 °C, under an argon atmosphere, was added boron trifluoride diethyl etherate (3.53 mL, 28.6 mmol) dropwise. Stirring was continued at –20 °C for 1 h and then at room temperature for 1 h. The reaction mixture was quenched with saturated aqueous NaHCO₃ solution and diluted with CH₂Cl₂. The organic layer was separated, and the aqueous layer was extracted with CH₂Cl₂. The combined organic phase was washed with water and brine, dried (MgSO₄), and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel (15% ethyl acetate in hexane) to give **11** (846 mg, 40% yield) as a light yellow gum. *R*_f = 0.3 (20% acetone in hexane). IR (neat) 3418, 2954, 1725 (s, >C=O), 1705 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 6.43 (d, *J* = 2.0 Hz, 1H, 4-H), 6.31 (d, *J* = 2.0 Hz, 1H, 2-H), 5.77 (m as d, *J* = 4.0 Hz, 1H, 8-H), 4.53 (d, *J* = 13.0 Hz, 1H, half of an AB system, -CH₂O-), 4.50 (d, *J* = 13.0 Hz, 1H, half of an AB system, -CH₂O-), 3.67 (s, 3H, -OMe), 3.42 (dd, *J* = 16.0 Hz, *J* = 4.0 Hz, 1H, 10*a*-H), 2.73 (td, *J* = 11.0 Hz, *J* = 5.0 Hz, 1H, 10*a*-H), 2.26 (m as d, *J* = 16.5 Hz, 1H, 7*a*-H), 1.96–1.82 (m, 3H, 10*β*-H, 7*β*-H, 6*a*-H), 1.52 (brs, 6H, -C(CH₃)₂-), 1.41 (s, 3H, 6*β*-Me), 1.23 (brs, 9H, -C(CH₃)₃) 1.13 (s, 3H, 6*α*-Me). Mass spectrum (ESI) *m/z* (relative intensity) 445 (M⁺ + H, 90), 385 (M⁺ – COOMe, 45), 342 (M⁺ – (CH₃)₃COOH, 100), 283 (M⁺ – (CH₃)₃COOH, –COOCH₃, 51).

2-(3,5-Dihydroxyphenyl)-2-methylpropanoic Acid (12)—To a stirred solution of **8** (2.54 g, 11.33 mmol) in dry CH₂Cl₂ (75 mL) at –78 °C, under an argon atmosphere, was added boron tribromide (39.6 mL, 39.6 mmol, 1 M solution in CH₂Cl₂). Following the addition, the reaction mixture was gradually warmed to room temperature and stirred for 3 h. Unreacted boron tribromide was quenched by the addition of methanol and ice at 0 °C. The resulting mixture was warmed to room temperature, and volatiles were removed in vacuo. The residue was diluted with diethyl ether and washed with saturated aqueous NaHCO₃ solution, water, and brine. The organic layer was dried (MgSO₄), filtered, and concentrated

under reduced pressure. Purification by flash column chromatography on silica gel (40% diethyl ether in hexane) gave **12** (2.17 g, 98% yield) as white solid with physical, spectral, and analytical data identical to those reported earlier.¹⁵

Butyl 2-(3,5-Dihydroxyphenyl)-2-methylpropanoate (13)—A stirred mixture of **12** (2.0 g, 10.20 mmol), bromobutane (2.1 g, 15.30 mmol), and sodium bicarbonate (1.05 g, 12.24 mmol) in anhydrous DMF (3 mL) was heated at 165 °C for 12 min using microwave irradiation. The reaction mixture was cooled to room temperature and diluted with water and ethyl acetate. The organic layer was washed with brine, dried (MgSO₄), and concentrated in vacuo. The residue was chromatographed on silica gel (50% ethyl acetate in hexane) to give **13** (2.5 g, 92% yield) as a light brown viscous oil. IR (neat) 3370, 2963, 1698, 1600, 1465, 1271, 1142 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 6.40 (d, *J* = 2.0 Hz, 2H, ArH), 6.23 (t, *J* = 2.0 Hz, 1H, ArH), 5.10 (s, 2H, OH), 4.06 (t, *J* = 7.0 Hz, 2H, 4-H), 1.55 (quintet, *J* = 7.5 Hz, 2H, 5'-H), 1.51 (s, 6 H, -C(CH₃)₂-), 1.28 (sextet, *J* = 7.0 Hz, 2H, 6'-H), 0.87 (t, *J* = 7.5 Hz, 3H, 7'-H). ¹³C NMR (100 MHz CDCl₃) δ 177.7 (-C(O)O-), 156.9 (ArC-3 and ArC-5), 147.4 (ArC-1), 105.5 (ArC-4 and ArC-6), 101.5 (ArC-2), 65.3 (-OCH₂-), 46.5, 30.4, 26.1, 18.9, 13.5.

Butyl 2-(4-((1*R*,2*R*,5*R*)-6,6-Dimethyl-4-oxobicyclo[3.1.1]heptan-2-yl)-3,5-dihydroxyphenyl)-2-methylpropanoate (15)—To a degassed solution of **13** (2.2 g, 8.73 mmol) and diacetates **14**^{19,25} (8.55 g, ~85% pure by ¹H NMR, 30.55 mmol) in CHCl₃ (88 mL) at 0 °C, under an argon atmosphere, was added *p*-toluenesulfonic acid monohydrate (2.32 g, 12.22 mmol). The mixture was warmed to room temperature and stirred for 4 days to ensure complete formation of the product. The reaction mixture was diluted with diethyl ether and washed sequentially with water, saturated aqueous NaHCO₃, and brine. The organic phase was dried over MgSO₄, and the solvent was removed under reduced pressure. The residue was chromatographed on silica gel (43% diethyl ether in hexane) to give **15** as a white crystalline solid (914 mg, 27% yield); *R*_f = 0.4 (40% diethyl ether in hexane); mp 70–71 °C. IR (neat) 3362, 2959, 2873, 1727, 1683, 1619, 1589, 1421, 1265, 1143 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 6.31 (s, 2H, ArH), 5.50 (s, 2H, OH), 4.06 (t, *J* = 7.0 Hz, 2H, 4'-H), 3.95 (t, *J* = 7.5 Hz, 1H, 4-H), 3.50 (dd, *J* = 19.0 Hz, *J* = 8.0 Hz, 1H, 3*α*-H), 2.59 (dd, *J* = 19.0 Hz, *J* = 8.5 Hz, 1H, 3*β*-H), 2.58 (t, *J* = 5.0 Hz, 1H, 1-H), 2.50 (m, 1H, 7*α*-H), 2.46 (d, *J* = 10.5 Hz, 1H, 7*β*-H), 2.27 (t, *J* = 5.5 Hz, 1H, 5-H), 1.56 (quintet, *J* = 7.5 Hz, 2H, 5'-H), 1.50 (s, 6H, -C(CH₃)₂-), 1.36 (s, 3H, 6-Me), 1.28 (sextet, *J* = 7.0 Hz, 2H, 6'-H), 0.99 (s, 3H, 6-Me), 0.87 (t, *J* = 7.5 Hz, 3H, 7'-H). ¹³C NMR (100 MHz CDCl₃) δ 213.3 (>C=O), 177.1 (-C(O)O-), 155.4 (ArC-3 and ArC-5), 143.9 (tertiary aromatic), 115.0 (tertiary aromatic), 106.1 (ArC-2 and ArC-6), 65.1 (-OCH₂-), 58.0, 46.8, 46.0, 42.1, 37.6, 30.4, 29.4, 26.1, 26.0, 24.4, 22.1, 19.0, 13.6. Mass spectrum (ESI) *m/z* (relative intensity) 389 (M⁺ + H, 100). Mass spectrum (EI) *m/z* (relative intensity) 388 (M⁺, 25), 305 (32), 287 (20), 243 (19), 177 (21), 84 (100). Exact mass (EI) calculated for C₂₃H₃₂O₅ (M⁺), 388.2250; found 388.2252. HPLC (4.6 mm × 250 mm, Supelco Discovery column, acetonitrile/water) showed purity of 99.7% and retention time of 8.9 min for **15**.

Butyl 2-(3-Acetoxy-5-hydroxyphenyl)-2-methylpropanoate (13a)—The title compound was also isolated during the chromatographic purification of **15** as a colorless oil

in 25% yield (641 mg). R_f = 0.5 (40% diethyl ether in hexane); IR (neat) 3417, 2962, 1770, 1728, 1702, 1609, 1592, 1261, 1142 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ 6.69 (t, J = 2.0 Hz, 1H, Ar-H), 6.62 (t, J = 2.0 Hz, 1H, Ar-H), 6.51 (t, J = 2.0 Hz, 1H, Ar-H), 6.78 (br s, 1H, OH), 4.06 (t, J = 7.0 Hz, 2H, 4'-H), 2.29 (s, 3H, OC(O)CH_3), 1.55 (quintet, J = 7.5 Hz, 2H, 5'-H), 1.53 (s, 6H, $-\text{C}(\text{CH}_3)_2-$), 1.26 (sextet, J = 7.0 Hz, 2H, 6'-H), 0.87 (t, J = 7.5 Hz, 3H, 7'-H). ^{13}C NMR (125 MHz CDCl_3) δ 176.5 ($-\text{C}(\text{O})\text{O}-$), 169.4 ($-\text{C}(\text{O})\text{O}-$), 156.4, 151.4, 147.4, 111.2, 110.8, 107.6, 65.0 ($-\text{OCH}_2-$), 46.5, 30.5, 26.2, 21.2, 19.0, 13.6. Mass spectrum (ESI) m/z (relative intensity) 317 ($\text{M}^+ + \text{Na}$, 20), 295 ($\text{M}^+ + \text{H}$, 55), 193 (100). LC/MS analysis (Waters MicroMass ZQ system) showed purity of 97.8% and retention time of 4.6 min for the title compound.

(+)-Apoverbenone (14a)—The title compound was also isolated during the chromatographic purification of **15** as a colorless oil (1.7 g). Spectroscopic data were reported earlier.²⁷

Butyl 2-[(6a*R*,10a*S*)-1-Hydroxy-6,6-dimethyl-9-oxo-6a,7,8,9,10,10a-hexahydro-6*H*-benzo[*c*]chromen-3-yl]-2-methylpropanoate (16)—To a stirred solution of **15** (850 mg, 2.20 mmol) in anhydrous $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{NO}_2$ (3:1 mixture, 44 mL) at 0 °C, under an argon atmosphere, was added trimethylsilyl trifluoromethanesulfonate (2.3 mL, 0.3 M solution in CH_3NO_2 , 0.66 mmol). Stirring was continued for 3 h while the temperature was allowed to rise to 25 °C. The reaction mixture was quenched using 1:1 solution of saturated NaHCO_3 and brine and extracted with diethyl ether. The organic layer was washed with brine, dried (MgSO_4), and concentrated under reduced pressure. Purification by flash column chromatography on silica gel (30% acetone in hexane) gave **16** as a white foam (570 mg, 67% yield). Mp 35–36 °C. IR (neat) 3356, 2958, 1727, 1697, 1620, 1579, 1419, 1259, 1142 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ 6.41 (d, J = 2.0 Hz, 1H, Ar-H), 6.30 (br d, J = 2.0 Hz, 1H, Ar-H), 6.23 (br s, 1H, OH), 4.06 (m as td, J = 7.0 Hz, J = 2.0 Hz, 2H, 4'-H), 3.96 (ddd, J = 15.0 Hz, J = 3.5 Hz, J = 2.0 Hz, 1H, 10eq-H), 2.87 (m as td, J = 12.5 Hz, J = 4.0 Hz, 1H, 10a-H), 2.64–2.57 (m, 1H, 8eq-H), 2.50–2.40 (m, 1H, 8ax-H), 2.20–2.08 (m, 2H, 10ax-H, 7eq-H), 1.95 (m as td, J = 12.0 Hz, J = 3.0 Hz, 1H, 6a-H), 1.59–1.51 (m, 3H, 7ax-H, 5'-H), 1.50 (s, 6H, $-\text{C}(\text{CH}_3)_2-$), 1.47 (s, 3H, 6-Me), 1.26 (sextet, J = 7.5 Hz, 2H, 6'-H), 1.11 (s, 3H, 6-Me), 0.86 (t, J = 7.5 Hz, 3H, 7'-H). ^{13}C NMR (125 MHz CDCl_3) δ 213.3 ($>\text{C}=\text{O}$), 177.1 ($-\text{C}(\text{O})\text{O}-$), 155.4 (ArC-1 or ArC-5), 154.8 (ArC-5 or ArC-1), 145.4 (tertiary aromatic), 109.4 (tertiary aromatic), 107.2 (ArC-2 or ArC-4), 105.6 (ArC-4 or ArC-2), 77.1 (C-6), 64.9 ($-\text{OCH}_2-$), 47.6, 46.4, 45.2, 41.0, 34.9, 30.7, 28.1, 27.1, 26.5, 26.3, 19.2, 19.1, 13.9. Mass spectrum (EI) m/z (relative intensity) 388 (M^+ , 79), 305 (43), 287 (100), 245 (98), 177 (94). Exact mass (EI) calculated for $\text{C}_{23}\text{H}_{32}\text{O}_5$ (M^+), 388.2250; found 388.2246. HPLC (4.6 mm $\times$ 250 mm, Supelco Discovery column, acetonitrile/water) showed purity of 98.3% and retention time of 10.8 min for **16**. Anal. ($\text{C}_{23}\text{H}_{32}\text{O}_5$) C, H.

Butyl 2-[(6a*R*,10a*R*)-1-[(*tert*-Butyldimethylsilyl)oxy]-6,6-dimethyl-9-oxo-6a,7,8,9,10,10a-hexahydro-6*H*-benzo[*c*]chromen-3-yl]-2-methylpropanoate (17)—To a solution of **16** (550 mg, 1.41 mmol) in anhydrous DMF (9.5 mL) under an argon atmosphere were added sequentially imidazole (675 mg, 9.92 mmol), DMAP (172 mg, 1.41

mmol), and TBDMSCl (1.46 g, 9.72 mmol). The reaction mixture was stirred at room temperature for 12 h and then quenched by the addition of brine and extracted with diethyl ether. The organic phase was dried (MgSO₄) and evaporated under reduced pressure. Purification by flash column chromatography on silica gel (20% ethyl acetate in hexane) afforded 620 mg (87% yield) of **17** as a colorless oil. IR (neat) 2959, 1715, 1612, 1567, 1415, 1253, 1096 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 6.46 (d, *J* = 2.0 Hz, 1H, Ar-H), 6.35 (br d, *J* = 2.0 Hz, 1H, Ar-H), 6.24 (br s, 1H, OH), 4.04 (m as td, *J* = 7.0 Hz, *J* = 2.0 Hz, 2H, 4'-H), 3.76 (ddd, *J* = 15.0 Hz, *J* = 3.5 Hz, *J* = 2.0 Hz, 1H, 10eq-H), 2.71 (m as td, *J* = 12.5 Hz, *J* = 4.0 Hz, 1H, 10a-H), 2.59–2.40 (m, 1H, 8eq-H), 2.45–2.36 (m, 1H, 8ax-H), 2.17–2.11 (m, 2H, 10ax-H, 7eq-H), 1.93 (m as td, *J* = 12.0 Hz, *J* = 3.0 Hz, 1H, 6a-H), 1.56–1.51 (m, 3H, 7ax-H, 5'-H), 1.49 (s, 6H, -C(CH₃)₂-), 1.46 (s, 3H, 6-Me), 1.25 (sextet, *J* = 7.5 Hz, 2H, 6'-H), 1.09 (s, 3H, 6-Me), 0.99 (s, 9H, Si(Me)₂CMe₃), 0.85 (t, *J* = 7.5 Hz, 3H, 7'-H), 0.23 (s, 3H, Si(Me)₂CMe₃), 0.15 (s, 3H, Si(Me)₂CMe₃). ¹³C NMR (100 MHz CDCl₃) δ 210.1 (>C=O), 176.4 (-C(O)O-), 154.5 (ArC-1 or ArC-5), 154.4 (ArC-5 or ArC-1), 144.8 (tertiary aromatic), 113.2 (tertiary aromatic), 109.6 (ArC-2 or ArC-4), 108.1 (ArC-4 or ArC-2), 64.6 (-OCH₂-), 47.7, 46.1, 45.4, 40.7, 35.0, 30.5, 27.7, 26.8, 26.2, 26.1, 25.9, 25.8, 19.0, 18.6, 18.3, 13.6, -3.7, -4.1. Mass spectrum (ESI) *m/z* (relative intensity) 503 (M⁺ + H, 100). Exact mass (ESI) calculated for C₂₉H₄₇O₅Si (M⁺ + H), 503.3193; found 503.3195. LC/MS analysis (Waters MicroMass ZQ system) showed purity of 99.2% and retention time of 6.2 min for the title compound.

Butyl 2-((6a*R*,10a*R*)-1-[(*tert*-Butyldimethylsilyl)oxy]-9-(methoxymethylene)-6,6-dimethyl-6a,7,8,9,10,10a-hexahydro-6*H*-benzo [c] chromen-3-yl)-2-methylpropanoate (18**)**—(Methoxymethyltriphenylphosphonium chloride (2.18 g, 6.36 mmol) was suspended in 32 mL of dry benzene. Sodium *tert*-amylate (688 mg, 6.25 mmol) was added, and the reaction mixture was stirred for 5 min at 0 °C. Intermediate **17** (532 mg, 1.06 mmol) was dissolved in minimum amount of dry benzene and transferred to the solution of the orange ylide. The reaction mixture was stirred at room temperature for 3 h and then quenched with saturated aqueous NH₄Cl solution and diluted using ethyl acetate. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate. The combined organic layer was washed with brine, dried (MgSO₄), and concentrated in vacuo. The residue was chromatographed on silica gel (20% acetone in hexane) to give two geometrical isomers **18a** and **18b** (353 mg, 63% yield) as a colorless oil in the ratio of 2:3, respectively, as determined by ¹H NMR analysis. IR (neat) 2957, 1728 (>C=O), 1611, 1565, 1414, 1251, 1138, 1097 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 6.43 (d, *J* = 2.0 Hz, 1H, Ar-H, **18b**), 6.41 (d, *J* = 2.0 Hz, 1H, Ar-H, **18a**), 6.34 (d, *J* = 2.0 Hz, 1H, Ar-H, **18a**), 6.32 (d, *J* = 2.0 Hz, 1H, Ar-H, **18b**), 5.85 (s, 1H, =CHOMe, **18b**), 5.82 (s, 1H, =CHOMe, **18a**), 4.19–4.14 (m as dd, *J* = 13.5 Hz, *J* = 3.5 Hz, 1H, C-ring, **18a**), 4.10–3.96 (m, 2H, 4'-H, **18a** and 2H, 4'-H, **18b**), 3.56 (s, 3H, OMe, **18b**), 3.53 (s, 3H, OMe, **18a**), 3.45–3.39 (m as dd, *J* = 13.5 Hz, *J* = 3.5 Hz, 1H, C-ring, **18b**), 2.96–2.88 (m as br d, *J* = 14.0 Hz, 1H, C-ring, **18b**), 2.34–2.27 (m, 1H, C-ring of **18a** and 1H, C-ring of **18b**), 2.22–2.16 (m, 1H, C-ring, **18a**), 2.09–2.00 (m, 1H, C-ring, **18a**), 1.91–1.84 (m, 1H, C-ring of **18a** and 1H, C-ring of **18b**), 1.82–1.74 (m, 1H, C-ring, **18b**), 1.67–1.51 (m, 2H, C-ring of **18a**, 2H, C-ring of **18b**, 2H, 5'-H of **18a** and 2H, 5'-H of **18b**), 1.49 (s, 6H, -C(CH₃)₂-, **18a**), 1.48 (s, 6H, -C(CH₃)₂-, **18b**), 1.38 (s, 3H, 6-Me, **18b**), 1.37 (s, 3H, 6-Me, **18a**), 1.30–1.22 (sextet and sextet

overlapping, $J = 7.5$ Hz, 2H, 6'-H, **18a** and 2H, 6'-H, **18b**), 1.12–0.96 (m, s, s, s and s, overlapping, 1H, C-ring of **18a**, 1H, C-ring of **18b**, 3H, 6-Me of **18a**, 3H, 6-Me of **18b**, 9H, -Si(Me)₂CMe₃, of **18a**, 9H, -Si(Me)₂CMe₃, of **18b** especially 1.02, s, -Si(Me)₂CMe₃, of **18b** and 0.99, s, -Si(Me)₂CMe₃, of **18a**), 0.85 (t, $J = 7.5$ Hz, 3H, 7'-H of **18a** and 3H, 7'-H of **18b**), 0.24 (s, 3H, -Si(Me)₂CMe₃, **18b**), 0.23 (s, 3H, -Si(Me)₂CMe₃, **18a**), 0.18 (s, 3H, -Si(Me)₂CMe₃, **18a**), 0.15 (s, 3H, -Si(Me)₂CMe₃, **18b**). ¹³C NMR (100 MHz CDCl₃) δ 176.7 (-C(O)O-), 176.6 (-C(O)O-), 155.2 (ArC), 154.8 (ArC), 154.7 (ArC), 154.6 (ArC), 144.0 (ArC), 143.9 (ArC), 140.7 (ArC), 140.2 (ArC), 116.5 (ArC), 115.7 (ArC), 114.9 (ArC), 114.7 (ArC), 109.7 (ArC), 105.5 (ArC), 108.0 (ArC), 107.8 (ArC), 64.5 (-OCH₂-), 59.3 (-OMe), 59.0 (-OMe), 49.6, 49.5, 46.0, 37.1, 35.9, 34.1, 30.5, 30.3, 29.0, 27.8, 27.7, 27.6, 26.2, 26.1, 26.0, 25.0, 19.0, 18.8, 18.5, 18.3, 13.6, -3.6, -3.8, -3.9, -4.0. Mass spectrum (ESI) m/z (relative intensity) 531 ($M^+ + H$, 100). Exact mass (ESI) calculated for C₃₁H₅₁O₅Si ($M^+ + H$), 531.3506; found 531.3499. LC/MS analysis (Waters MicroMass ZQ system) showed purity of 98.9% and retention time of 8.4 min for the title compound.

Butyl 2-((6a*R*,10a*R*)-1-[(*tert*-Butyldimethylsilyl)oxy]-9-formyl-6,6-dimethyl-6a,7,8,9,10,10a-hexahydro-6*H*-benzo[*c*]-chromen-3-yl)-2-methylpropanoate (19**)—**

To the stirred solution of **18** (255 mg, 0.48 mmol) in CH₂Cl₂ (16 mL) under an argon atmosphere was added wet trichloroacetic acid (391 mg, 2.40 mmol). The reaction mixture was stirred at room temperature for 2.5 h and then quenched with saturated aqueous NaHCO₃ solution and diluted with diethyl ether. The organic layer was separated, and the aqueous layer was extracted with diethyl ether. The combined organic phase was washed with water and brine, dried (MgSO₄), and evaporated. The residue consisted of a mixture of epimeric aldehydes **19a** and **19b** (241 mg, 97% yield) in the ratio of 5:2, respectively, as determined by ¹H NMR analysis, and it was used into the next step as such. IR (neat) 2932, 2711 (CHO), 1726 (>C=O), 1611, 1566, 1414, 1253, 1140, 1064 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 9.89 (s, 1H, 9 α -CHO, **19b**), 9.62 (s, 1H, 9 β -CHO, **19a**), 6.44 (d, $J = 2.0$ Hz, 1H, Ar-H, **19a**), 6.41 (d, $J = 2.0$ Hz, 1H, Ar-H, **19b**), 6.34 (d, $J = 2.0$ Hz, 1H, Ar-H, **19b**), 6.33 (d, $J = 2.0$ Hz, 1H, Ar-H, **19a**), 4.10–3.97 (m, 2H, 4'-H, **19a** and 2H, 4'-H, **19b**), 3.68–3.62 (m as br d, $J = 14.0$ Hz, 1H, Cring, **19b**), 3.50–3.44 (m as br d, $J = 13.5$ Hz, 1H, C-ring, **19a**), 2.66–2.61 (m, 1H, C-ring, **19b**), 2.46–2.33 (m, 2H, C-ring, **19a** and 2H, Cring, **19b**), 2.31–2.24 (m, 1H, C-ring, **19b**), 2.14–2.08 (m, 1H, Cring, **19a**), 2.02–1.96 (m, 1H, C-ring, **19a**), 1.78–1.71 (m, 1H, C-ring, **19b**), 1.58–1.41 (m, s, and s, overlapping, 2H, 5'-H of **19a**, 2H, 5'-H of **19b**, 3H, C-ring of **19a**, 2H, C-ring of **19b**, 6H, -C(CH₃)₂- of **19a**, 6H, -C(CH₃)₂- of **19b**), 1.38 (s, 3H, 6-Me, **19a**), 1.35 (s, 3H, 6-Me, **19b**), 1.29–1.21 (sextet and sextet overlapping, $J = 7.5$ Hz, 2H, 6'-H of **19a** and 2H, 6'-H of **19b**), 1.20–1.11 (m, 1H, C-ring of **19a**, 1H, Cring of **19b**), 1.06 (s, 3H, 6-Me, **19a**), 1.00 (s and s overlapping, 3H, 6-Me of **19b** and 9H, -Si(Me)₂CMe₃ of **19a**), 0.97 (s, 9H, -Si(Me)₂CMe₃, **19b**), 0.86 (t, $J = 7.5$ Hz, 3H, 7'-H, of **19a** and 3H, 7'-H, of **19b**), 0.27 (s, 3H, -Si(Me)₂CMe₃, **19b**), 0.25 (s, 3H, Si(Me)₂CMe₃, **19a**), 0.24 (s, 3H, Si(Me)₂CMe₃, **19b**), 0.14 (s, 3H, Si(Me)₂CMe₃, **19a**). Mass spectrum (ESI) m/z (relative intensity) 517 ($M^+ + H$, 100) for both peaks at 6.2 and 6.5 min. LC/MS analysis (Waters MicroMass ZQ system) showed purity of 97.8% and retention time of 6.2 and 6.5 min for the two epimeric aldehydes in the ratio of 2:5, respectively.

Butyl 2-((6a*R*,9*R*,10a*R*)-1-[(*tert*-Butyldimethylsilyl)oxy]-9-formyl-6,6-dimethyl-6a,7,8,9,10,10a-hexahydro-6*H*-benzo[*c*]-chromen-3-yl)-2-methylpropanoate (20)—To a solution of **19** (200 mg, 0.39 mmol) in ethanol (9.5 mL) under an argon atmosphere was added potassium carbonate powder (269 mg, 1.95 mmol), and the mixture was stirred at room temperature for 3 h. The reaction mixture was diluted with diethyl ether, and solid materials were filtered off. The filtrate was washed with brine, dried (MgSO₄), and concentrated under reduced pressure. Purification by flash column chromatography on silica gel (15% diethyl ether in hexane) gave **20** (156 mg, 78% yield) as a colorless oil. IR (neat) 2932, 2711 (CHO), 1726 (>C=O), 1611, 1566, 1414, 1253, 1140, 1064 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 9.62 (s, 1H, 9β-CHO), 6.44 (d, *J* = 2.0 Hz, 1H, Ar-H), 6.33 (d, *J* = 2.0 Hz, 1H, Ar-H), 4.10–3.98 (m, 2H, 4'-H), 3.50–3.44 (m as br d, *J* = 13.5 Hz, 1H, C-ring), 2.46–2.36 (m, 2H, C-ring), 2.14–2.08 (m, 1H, C-ring), 2.02–1.96 (m, 1H, C-ring), 1.58–1.42 (m, s, and s, overlapping, 2H, 5'-H, 3H, C-ring, 6H, -C(CH₃)₂-, especially 1.50, s, -C(CH₃)₂- and 1.49, s, -C(CH₃)₂-), 1.38 (s, 3H, 6-Me), 1.26 (sextet, *J* = 7.5 Hz, 2H, 6'-H), 1.20–1.12 (m, 1H, C-ring), 1.06 (s, 3H, 6-Me), 1.00 (s, 9H, -Si(Me)₂CMe₃), 0.86 (t, *J* = 7.5 Hz, 3H, 7'-H), 0.25 (s, 3H, Si(Me)₂CMe₃), 0.14 (s, 3H, Si(Me)₂CMe₃). ¹³C NMR (100 MHz CDCl₃) δ 203.4 (>C=O), 176.5 (-C(O)O-), 154.7 (ArC-1 or ArC-5), 154.6 (ArC-5 or ArC-1), 144.5 (tertiary aromatic), 113.8 (tertiary aromatic), 109.4 (ArC-2 or ArC-4), 108.1 (ArC-4 or ArC-2), 64.5 (-OCH₂-), 50.5, 49.0, 46.0, 35.4, 30.5, 30.1, 27.5, 26.9, 26.2, 26.1, 25.9, 25.8, 19.0, 18.8, 18.1, 13.6, -3.6, -4.2. Mass spectrum (ESI) *m/z* (relative intensity) 517 (M⁺ + H, 100). Exact mass (ESI) calculated for C₃₀H₄₉O₅Si (M⁺ + H), 517.3349; found 517.3351. LC/MS analysis (Waters MicroMass ZQ system) showed purity of 96.7% and retention time of 6.5 min for the title compound.

Butyl 2-((6a*R*,9*R*,10a*R*)-1-[(*tert*-Butyldimethylsilyl)oxy]-9-(hydroxymethyl)-6,6-dimethyl-6a,7,8,9,10,10a-hexahydro-6*H*-benzo[*c*]chromen-3-yl)-2-methylpropanoate (21)—Sodium borohydride (57 mg, 1.55 mmol) was added to a stirred solution of aldehyde **20** (100 mg, 0.19 mmol) in ethanol (4.8 mL) at 0 °C under argon. After 30 min, the reaction was quenched with saturated ammonium chloride solution and volatiles were removed in vacuo. The residue was dissolved in ethyl acetate, and water was added. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate. The combined organic phase was washed with water and brine, dried (MgSO₄), and evaporated. Purification by flash column chromatography on silica gel (30% diethyl ether in hexane) gave **21** (84 mg, 85% yield) as a colorless viscous oil. IR (neat) 3430, 2932, 1729 (>C=O), 1611, 1566, 1414, 1253, 1140, 1064 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 6.43 (d, *J* = 2.0 Hz, 1H, Ar-H), 6.31 (d, *J* = 2.0 Hz, 1H, Ar-H), 4.09–3.97 (m, 2H, 4'-H), 3.54 (dd, *J* = 10.0 Hz, *J* = 5.5 Hz, half of an AB system, 1H, -CH₂OH), 3.45 (dd, *J* = 10.0 Hz, *J* = 7.0 Hz, half of an AB system, 1H, -CH₂OH), 3.18–3.12 (m as br d, *J* = 13.0 Hz, 1H, C-ring), 2.40–2.32 (m as td, *J* = 11.0 Hz, *J* = 2.5 Hz, 1H, C-ring), 2.04–1.98 (m, 1H, C-ring), 2.94–1.88 (m, 1H, C-ring), 1.76–1.68 (m, 1H, C-ring), 1.58–1.43 (m, s, and s, overlapping, 9H, -C(CH₃)₂-, 5'-H, C-ring, especially 1.49, s, 3H, -C(CH₃)₂-, and 1.48, s, 3H, -C(CH₃)₂-), 1.37 (s, 3H, 6-Me), 1.29–1.22 (m as sextet, *J* = 7.0 Hz, 2H, 6'-H), 1.18–1.10 (m, 2H, C-ring), 1.05 (s, 3H, 6-Me), 0.99 (s, 9H, -Si(Me)₂CMe₃), 0.86 (t, *J* = 7.5 Hz, 3H, 7'-H), 0.76 (m as q, *J* = 11.0 Hz, 1H, C-ring), 0.23 (s, 3H, Si(Me)₂CMe₃), 0.12 (s, 3H, Si(Me)₂CMe₃). ¹³C NMR (100

MHz CDCl₃) δ 176.6 (-C(O)O-), 154.7 (ArC-1 or ArC-5), 154.6 (ArC-5 or ArC-1), 144.0 (tertiary aromatic), 114.8 (tertiary aromatic), 109.5 (ArC-2 or ArC-4), 108.0 (ArC-4 or ArC-2), 68.4 (-CH₂OH), 64.5 (-OCH₂-), 49.6, 46.0, 40.5, 35.5, 33.1, 30.5, 29.7, 27.6, 27.5, 26.1, 25.9, 19.0, 18.8, 18.2, 13.6, -3.6, -4.3. Mass spectrum (ESI) m/z (relative intensity) 519 ($M^+ + H$, 100). Exact mass (ESI) calculated for C₃₀H₅₁O₅Si ($M^+ + H$), 519.3506; found 519.3507. LC/MS analysis (Waters MicroMass ZQ system) showed purity of 98.3% and retention time of 6.2 min for the title compound.

2-[(6a*R*,10a*R*)-6a,7,8,9,10,10a-Hexahydro-1-hydroxy-6,6-dimethyl-9-oxo-6*H*-benzo[*c*]chromen-3-yl]-2-methylpropanoic Acid (22**)**—To a stirred solution of **16** (430 mg, 1.1 mmol) in dioxane/H₂O (1:1 ratio, 20 mL) at room temperature, under an argon atmosphere, was added lithium hydroxide (80 mg, 3.3 mmol). Stirring was continued for 8 h, and then the reaction mixture was quenched with 1 N HCl and diluted with ethyl acetate. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate. The combined organic phase was washed with water and brine, dried (MgSO₄), and concentrated under vacuo. The crude product was chromatographed on silica gel (48% acetone in hexane) to give **22** (232 mg, 63% yield) as a white solid. Mp 153–154 °C; IR (neat) 3340, 2978, 1695, 1620, 1417 cm⁻¹; ¹H NMR (500 MHz, CDCl₃ + CD₃OD) δ 6.39 (d, J = 1.5 Hz, 1H, ArH), 6.34 (d, J = 1.5, 1H, ArH), 4.35 (br s, 1H, OH), 3.94 (ddd, J = 15.0, J = 3.5 Hz, J = 2.0 Hz, 1H, 10eq-H), 2.86 (m as td, J = 12.5, J = 3.5 Hz, 1H, 10a-H), 2.62–2.58 (m, 1H, 8eq-H), 2.48 - 2.41 (m, 1H, 8ax-H), 2.19 - 2.04 (m, 2H, 10ax-H, 7eq-H), 1.94 (m as td, J = 12.2, J = 3.0 Hz, 1H, 6a-H), 1.57–1.48 (m, s and s, overlapping, 7H, 7ax-H, -C(CH₃)₂-, especially 1.50, s, and 1.49, s, -C(CH₃)₂-), 1.47 (s, 3H, 6-CH₃), 1.11 (s, 3H, 6-CH₃). ¹³C NMR (125 MHz CDCl₃) δ 213.2 (>C=O), 180.1 (-C(O)O-), 155.1 (ArC-1 or ArC-5), 154.7 (ArC-5 or ArC-1), 144.3 (tertiary aromatic), 109.7 (tertiary aromatic), 107.1 (ArC-2 or ArC-4), 105.6 (ArC-4 or ArC-2), 65.9, 47.2, 45.7, 44.8, 40.7, 34.6, 30.3, 27.8, 26.8, 25.7, 18.9. Mass spectrum (ESI) m/z (relative intensity) 333 ($M^+ + H$, 100). Exact mass (ESI) calculated for C₁₉H₂₅O₅ ($M^+ + H$), 333.1702; found 333.1700. HPLC (4.6 mm × 250 mm, Supelco Discovery column, acetonitrile/water) showed purity of 97.5% and retention time of 6.5 min for the title compound.

Methyl 2-{4-[(1*R*,2*R*,5*R*)-6,6-Dimethyl-4-oxobicyclo[3.1.1]heptan-2-yl]-3,5-dihydroxyphenyl}acetate (24**)**—The synthesis was carried out as described for **15**, using **23** (4.9 g, 27.0 mmol), diacetates **14** (11.4 g, 47.8 mmol), and *p*-TSA (5.7 g, 30.0 mmol) in 74 mL of CHCl₃/acetone (7:3 ratio) and gave **24** as a white gum in 23% yield (2.0 g). IR (neat) 3344, 2940, 2873, 1719, 1682, 1611, 1460, 1261, 1140 cm⁻¹; ¹H NMR (500MHz, CDCl₃) δ 6.95 (brs, 2H, OH), 6.26 (d, J = 2.0 Hz, 1H, ArH), 6.19 (d, J = 2.0 Hz, 1H, ArH), 3.67 (s, 3H, OMe), 3.63 (dd, J = 18.5 Hz, J = 8.0 Hz, 1H, 3 α -H), 3.58 (d, J = 15.2 Hz, 1H, half of an AB system, -CH₂C(O)O-), 3.53 (d, J = 15.2 Hz, 1H, half of an AB system, -CH₂(O)O-), 3.45, (t, J = 8.5 Hz, 1H, 4-H), 2.63 (d, J = 10.5 Hz, 1H, 7 β -H), 2.56 (t, J = 5.5 Hz, 1H, 1-H), 2.48–2.34 (m, 2H, 7 α -H, 3 β -H), 2.06 (t, J = 5.5 Hz, 1H, 5-H), 1.31 (s, 3H, 6-Me), 0.93 (s, 3H, 6-Me). ¹³C NMR (100 MHz CDCl₃) δ 218.7 (>C=O), 173.0 (-C(O)O-), 156.5 (ArC), 154.7 (ArC), 135.3, 119.9, 111.1, 103.9, 57.9 (-OCH₃), 52.4, 47.0, 42.5, 40.0, 37.7, 33.0, 26.1, 24.1, 21.9. Mass spectrum (ESI) m/z (relative intensity) 319 ($M^+ + H$, 100),

287 ($M^+ - \text{MeOH}$, 35). LC/MS analysis (Waters MicroMass ZQ system) showed purity of 98.7% and retention time of 3.9 min for the title compound.

Methyl 2-[(6a*R*,10a*R*)-1-Hydroxy-6,6-dimethyl-9-oxo-6a,7,8,9,10,10a-hexahydro-6*H*-benzo[*c*]chromen-3-yl]acetate (25**)**—The synthesis was carried out as described for **16**, using **24** (950 mg, 2.98 mmol) and TMSOTf (3.5 mL, 0.89 mmol) of a 0.3 M solution in MeNO_2 in 30 mL of anhydrous $\text{CH}_2\text{Cl}_2/\text{MeNO}_2$ (3:1 ratio) and gave **25** (540 mg, 57% yield) as a white foam. IR (neat) 3332, 2960, 1727, 1697, 1620, 1420, 1257, 1140 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ 6.33 (d, $J = 2.5$ Hz, 1H, ArH), 6.24 (d, $J = 2.5$ Hz, 1H, ArH), 5.53 (br s, 1H, OH), 3.71 (s, 3H, OMe), 3.63 (d, $J = 16.5$ Hz, 1H, half of an AB system, $-\text{CH}_2\text{C}(\text{O})\text{O}-$), 3.56 (d, $J = 16.5$ Hz, 1H, half of an AB system, $-\text{CH}_2\text{C}(\text{O})\text{O}-$), 2.99 (ddd, $J = 15.5$ Hz, $J = 3.5$ Hz, $J = 2.0$ Hz, 1H, 10eq-H), 2.85, (m as td, $J = 12.0$ Hz, $J = 3.5$ Hz, 1H, 10a-H), 2.64–2.56 (m, 1H, 8eq-H), 2.48–2.38 (m, 1H, 8ax-H), 2.24 (m as t, $J = 13.5$ Hz, 1H, 7eq-H), 2.18–2.11 (m, 1H, 10ax-H), 2.01 (m as d, $J = 12.5$ Hz, $J = 3.0$ Hz, 1H, 6a-H), 1.55–1.44 (m and s, overlapping, 4H, 7ax-H, 6-Me), 1.08 (s, 3H, 6-Me). Mass spectrum (ESI) m/z (relative intensity) 319 ($M^+ + \text{H}$, 100). Exact mass (ESI) calculated for $\text{C}_{18}\text{H}_{23}\text{O}_5$ ($M^+ + \text{H}$), 319.1545; found 319.1547.

2-[(6a*R*,10a*R*)-1-Hydroxy-6,6-dimethyl-9-oxo-6a,7,8,9,10,10a-hexahydro-6*H*-benzo[*c*]chromen-3-yl]acetic Acid (26**)**—The synthesis was carried out as described for **4a** using **25** (450 mg, 1.41 mmol), sodium hydroxide (226 mg, 5.7 mmol) in 32 mL of THF/ H_2O (1:1 ratio) and gave **26** (270 mg, 63% yield) as a white foam. IR (neat) 3345, 2980, 1725, 1694, 1620, 1417 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ 6.20 (d, $J = 2.5$ Hz, 1H, ArH), 6.05 (d, $J = 2.5$ Hz, 1H, ArH), 3.47 (d, $J = 16.0$ Hz, 1H, half of an AB system, $-\text{CH}_2\text{C}(\text{O})\text{O}-$), 3.43 (d, $J = 16.0$ Hz, 1H, half of an AB system, $-\text{CH}_2\text{C}(\text{O})\text{O}-$), 2.90 (ddd, $J = 15.5$ Hz, $J = 3.5$ Hz, $J = 2.0$ Hz, 1H, 10eq-H), 2.82, (m as td, $J = 12.0$ Hz, $J = 3.5$ Hz, 1H, 10a-H), 2.46–2.34 (m, 2H, 8eq-H, 8ax-H), 2.16–2.02 (m, 2H, 7eq-H, 10ax-H), 1.91 (m as td, $J = 12.5$ Hz, $J = 3.0$ Hz, 1H, 6a-H), 1.48–1.37 (m, 1H, 7ax-H), 1.33 (s, 3H, 6-Me), 0.97 (s, 3H, 6-Me). Mass spectrum (ESI) m/z (relative intensity) 305 ($M^+ + \text{H}$, 46), 259 (55, $M^+ - \text{COOH}$), 149 (100).

Butyl 2-[(6a*R*,10a*R*)-1-Hydroxy-6,6-dimethyl-9-oxo-6a,7,8,9,10,10a-hexahydro-6*H*-benzo[*c*]chromen-3-yl]acetate (27**)**—The synthesis was carried out as described for **3a** using **26** (170 mg, 0.56 mmol), sodium bicarbonate (70 mg, 0.82 mmol), and 1-bromobutane (185 mg, 1.34 mmol) in DMF (4 mL) and gave **27** (109 mg, 54% yield) as a white foam. IR (neat) 3345, 2960, 1726, 1697, 1620, 1420, 1257 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ 6.34 (d, $J = 2.5$ Hz, 1H, ArH), 6.23 (d, $J = 2.5$ Hz, 1H, ArH), 5.59 (s, 1H, OH), 4.11 (m as octet, $J = 4.0$ Hz, 2H, $-\text{C}(\text{O})\text{OCH}_2-$), 3.62 (d, $J = 16.5$ Hz, 1H, half of an AB system, $-\text{CH}_2\text{C}(\text{O})\text{O}-$), 3.54 (d, $J = 16.5$ Hz, 1H, half of an AB system, $-\text{CH}_2\text{C}(\text{O})\text{O}-$), 2.99 (br d, $J = 15.0$ Hz, 1H, 10eq-H), 2.86, (m as td, $J = 12.0$ Hz, $J = 3.0$ Hz, 1H, 10a-H), 2.64–2.56 (m, 1H, 8eq-H), 2.48–2.38 (m, 1H, 8ax-H), 2.24 (m as t, $J = 13.0$ Hz, 1H, 7eq-H), 2.18–2.10 (m, 1H, 10ax-H), 2.00 (m as td, $J = 12.0$ Hz, $J = 3.0$ Hz, 1H, 6a-H), 1.61 (quintet, $J = 6.5$ Hz, 2H, 5'-H), 1.55–1.44 (m and s overlapping, 4H, 7ax-H, 6-Me), 1.35 (sextet, $J = 7.5$ Hz, 2H, 6'-H), 1.08 (s, 3H, 6-Me), 0.91 (t, $J = 7.5$ Hz, 3H, 7'-H). ^{13}C NMR (100 MHz, CDCl_3) δ 209.6 ($>\text{C}=\text{O}$) 171.5 ($-\text{C}(\text{O})\text{O}-$), 155.2 (ArC), 155.1 (ArC), 134.2 (ArC), 115.4

(ArC), 111.1 (ArC), 103.9 (ArC), 77.2 (C-6), 65.1 (-OCH₂-), 48.9, 48.2, 40.4, 39.3, 35.6, 30.5, 27.6, 26.5, 19.0, 19.3, 13.6. Mass spectrum (ESI) *m/z* (relative intensity) 361 ($M^+ + H$, 100). Exact mass (ESI) calculated for C₂₁H₂₉O₅ ($M^+ + H$), 361.2015; found 361.2012. LC/MS analysis (Waters MicroMass ZQ system) showed purity of 98.3% and retention time of 4.5 min for the title compound.

Radioligand Binding Assays

The affinities (K_i) of the new compounds for rat CB1 receptor as well as for mouse and human CB2 receptors were obtained by using membrane preparations from rat brain or HEK293 cells expressing either mCB2 or hCB2 receptors, respectively, and [³H]CP-55,940 as the radioligand, as previously described.^{13,19} Results from the competition assays were analyzed using nonlinear regression to determine the IC₅₀³⁵ values for the ligand; K_i values were calculated from the IC₅₀ (Prism by GraphPad Software, Inc.). Each experiment was performed in triplicate, and K_i values determined from three independent experiments and are expressed as the mean of the three values.

cAMP Assay.^{19,20}

HEK293 cells stably expressing rCB1 or hCB2 receptors were used for the studies. The cAMP assay was carried out using PerkinElmer's Lance ultra cAMP kit following the protocol of the manufacturer. Briefly, the assays were carried out in 384-well plates using 1000–1500 cells/well. The cells were harvested with non-enzymatic cell dissociation reagent Versene, washed once with HBSS, and resuspended in the stimulation buffer. The various concentrations of the test compound (5 μ L) in forskolin (2 μ M final concentration) containing stimulation buffer were added to the plate followed by the cell suspension (5 μ L). Cells were stimulated for 30 min at room temperature. Eu-cAMP tracer working solution (5 μ L) and Ulight-anti-cAMP working solution (5 μ L) were then added to the plate and incubated at room temperature for 60 min. The data were collected on a PerkinElmer Envision instrument. The EC₅₀ values were determined by nonlinear regression analysis using GraphPad Prism software (GraphPad Software, Inc., San Diego, CA).

Plasma Stability.¹³

Compounds or their proposed metabolites were diluted (200 μ M) in mouse or rat plasma and incubated at 37 °C, 100 rpm. At various time points, samples were taken, diluted 1:4 in acetonitrile, and centrifuged to precipitate the proteins. The resulting supernatant was analyzed by HPLC. 4-Nitrophenyl butyrate was used as a control in each experiment. In vitro plasma half-lives were determined using exponential decay calculations in Prism (GraphPad).

HPLC Analysis

Chromatographic separation was achieved using a Supelco Discovery C18 (4.6 mm $\times$ 250 mm) column on a Waters Alliance HPLC system. Mobile phase consisted of acetonitrile (A) and a mixture of 60% water (acidified with 8.5% α -phosphoric acid) and 40% acetonitrile (B). Gradient elution started with 5% A, transitioning to 95% A over 10 min, and holding for 5 min before returning to starting conditions; run time was 15 min, the flow rate was 1

mL/min, and UV detection was used at each compound's maximal absorbance (204 and 230 nM).

Methods for Characterization of in Vivo Effects

A. Rodents.^{13,36}

Subjects: For hypothermia testing, female Sprague-Dawley rats ($n = 6/\text{group}$) weighing between 250 and 350 g (Charles River, Wilmington MA) were used. Rats were tested repeatedly with at least 5 days intervening between drug sessions. Experiments occurred at approximately the same time (10:00 am to 5:00 pm) during the light portion of the daily light/dark cycle. Outside of experimental sessions, rats were pair housed (2/cage) in a climate controlled vivarium with unrestricted access to food and water. For tail-flick withdrawal (analgesia) testing, male CD-1 mice ($n = 6/\text{group}$), weighing between 30 and 35 g (Charles River, Wilmington MA), were used. Mice were housed 4/cage in a climate controlled vivarium with unrestricted access to food and water and acclimated to these conditions for at least a week before any experimental manipulations occurred. Analgesia testing took place between 11:00 am and 7:00 pm. Mice were used once.

Procedures: Temperature was recorded using a thermistor probe (model 401, Measurement Specialties, Inc., Dayton, OH) inserted into the rectum at a depth of 6 cm and secured to the tail with micropore tape. Rats were minimally restrained and isolated in 38 cm $\times$ 50 cm $\times$ 10 cm plastic stalls. Temperature was read to the nearest 0.01 °C using a Thermometer (model 4000A, Measurement Specialties, Inc.).

Two baseline temperature measures were recorded at 15 min intervals, and drugs were injected immediately after the second baseline was recorded. After injection, temperature was recorded every 30 min for 3 h and every hour thereafter for a total of 6 h. The change in temperature was determined for each rat by subtracting temperature readings from the average of the two baseline measures. Analgesia testing utilized a thermostatically controlled 2 L water bath commercially available from VWR International where the water temperature was set at 52 °C (± 0.5 °C). The tail was immersed into the water at a depth of 2 cm and the withdrawal latency recorded by a commercially available stopwatch (Fisher Scientific), allowing measurements in seconds and $1/100$ s. Cut-off was set at 10 s to minimize the risk of tissue damage. A test session consisted of 5 recordings, the first of which constituted the baseline recording. Injections occurred immediately after the baseline recording, and the remaining recordings took place 20, 60, 180, and 360 min postadministration. Prior to this testing, the animals had been accustomed to the procedure for three consecutive "mock" sessions where the water was held at 38 °C, i.e., average body temperature of mice; no tail-flicks are elicited by this water temperature. The third "mock" session also included an ip injection of vehicle (10 mL/kg). The tail-flick withdrawal latencies are expressed as a percentage of maximum possible effect (% MPE), according to the formula % MPE = [(test latency minus baseline latency) divided by (10 minus baseline latency)] times 100.

Drugs: For hypothermia testing, 11-OH-($-$)- δ -THC-DMH (**3g**) and compounds **3a**, **3b**, and **3c** were initially dissolved in a solution of 20% ethanol, 20% alkamuls, and 60% saline and

were further diluted with saline. Injections were administered sc in a volume of 1.0 mL/kg. For tail-flick withdrawal (analgesia) testing, **3g** and **3b** were initially dissolved in 2% dimethyl sulfoxide, 4% Tween-80, and 4% propylene glycol before saline was slowly added just prior to the 10 mL/kg ip administration. All suspensions were freshly prepared for analgesia testing.

Data Analysis: Dose- and time-effect functions for hypothermia testing were analyzed using, respectively, one-way or two-way repeated measures ANOVA procedures followed by Bonferroni's post hoc test; *p* was set at 0.05. ED₅₀ values were calculated using linear regression of the hypothermia dose-effect functions for compounds 11-OH(-)-⁸-THC-DMH (**3g**) and compounds **3a**, **3b**, and **3c**. Two-way repeated measures ANOVA was applied to the tail-flick latency data depicted in Figure 4. Statistical analyses were performed using the software package GraphPad Prism 5.03 (GraphPad Software, San Diego, CA).

B. Non-Human Primates

Subjects: Four adult male squirrel monkeys (*Saimiri sciureus*) that were trained in the behavioral and pharmacological procedures used here served as subjects (see below). Experimental sessions were conducted 5 days a week (Monday–Friday). Each subject had previously received acute intramuscular (im) injections of 0.01 mg/kg of the cannabinoid **28** in training sessions at least twice weekly and other cannabinergic compounds in test sessions conducted no more than once weekly; no test compounds were administered for at least 7 days prior to the present studies. The experimental protocol for the present studies was approved by the Institutional Animal Care and Use Committee at McLean Hospital. Subjects were maintained in a facility licensed by the U.S. Department of Agriculture and in accordance with the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (National Research Council, 2003).

Apparatus: During experimental sessions, subjects were seated in a Plexiglas chair within a ventilated sound- and light-attenuating chamber.³³ The front panel of the chair was outfitted with two response levers that were positioned 6 cm left and right of center. Each lever-press with a force of at least 0.25 N closed a microswitch, produced an audible click, and was recorded as a response. Red stimulus lights were mounted behind the transparent front panel of the chair, approximately 10 cm above each response lever. Before each session, a shaved portion of each subject's tail was coated with electrode paste and placed under brass electrodes for the delivery of brief, low-intensity current (see below). Experimental events and data collection were controlled by Med Associates (St. Albans, VT) interfacing equipment and operating software.

Behavioral Procedure: The subjects were previously trained to discriminate the pre-session administration of 0.01 mg/kg of the cannabinoid agonist **28** or its vehicle (20% ethanol/20% emulphor/60% saline) by responding on one of two levers. Briefly, subjects initially were trained to terminate visual stimuli associated with the delivery of brief, low-intensity current (200 ms; 3 mA) across the electrodes by depressing one of the two response levers; the inactive lever was removed to facilitate early training. The active lever varied until subjects reliably terminated visual stimuli by responding on either lever. Subsequently, both levers

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were present in all sessions and the active lever was signaled only by a pre-session injection: one lever was active only following the im injection of the cannabinoid CB1 agonist **28** (0.01 mg/kg, im), whereas the other lever was active only following im injection of vehicle; right and left lever assignments were counterbalanced among subjects. Under terminal conditions, each training session began with a 10 min timeout period during which all lights were extinguished and responding had no programmed consequences. After the timeout period, two red stimulus lights above each lever were illuminated and completion of 10 consecutive responses [fixed ratio (FR) 10] on the active lever extinguished all stimulus lights and initiated a 50 s timeout. Responses on the inactive lever reset the FR requirement. Current delivery was scheduled for delivery every 10 s until either the FR 10 was completed on the correct lever or 30 s elapsed, whichever came first.

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Drug Testing: Testing was conducted to determine the extent to which different doses of drugs substituted for the training compound, i.e., produced responding on the training drug-associated response lever. Tests for the time course of substitution of compounds **3b** and **3g** to the training stimulus were conducted when a subject's discrimination performance was at least 90% accurate for four of the last five training sessions and on the immediately preceding session. Procedurally, test sessions differed from training sessions in two ways. First, 10 consecutive responses on either lever extinguished the stimulus lights and associated program of current delivery and initiated the 50 s timeout. Second, no current deliveries were scheduled during test sessions so as to preclude possible stimulus-induced enhancement of responding. Other schedule contingencies were unchanged. In initial experiments, dose-ranging procedures were employed to quickly establish the lowest dose that fully substituted for (90% responding on the CB1 lever) the 0.01 mg/kg of the CB1 agonist **28**. In these procedures, each subject received one of several doses of each drug (0.0001–0.003 mg/kg) 60 min prior to the test session; doses were chosen based on data from receptor binding studies and previous work in rodents (**3b**; see above) or monkeys (**3g**). Next, the lowest effective dose of each drug was studied in all subjects. Results indicated that 0.003 mg/kg **3g** was not fully effective in any subject at 60 min whereas 0.001 mg/kg **3b** was fully effective in three of four subjects at this time point. Both drugs fully substituted for **28** at 120 min after treatment. In a follow-up experiment, 0.0003 mg/kg **3b** and 0.001 mg/kg **3g** failed to fully substitute for **28** in all subjects; consequently, subsequent studies were conducted with 0.001 mg/kg **3b** and 0.003 mg/kg **3g**. To assess behavioral onset and time course of action of these doses of the two drugs, experiments with each drug were arranged to include multiple sequential test sessions that began 15, 30, 60, 120, 240, 480, and 960 min after injection. The effects of **3g** were additionally determined 36 and 48 h after administration.

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Data Analysis: The two primary dependent measures at the time points examined in the present experiments were response distribution across the two levers and overall response rate. Response distribution (percent **28** lever) was calculated by dividing the number of responses on the lever associated with the injection of **28** by the total number of responses (excluding any responses during timeout periods). Response rate was calculated by dividing the total number of responses on both levers by the total session time (excluding all timeout periods). Doses of drugs were considered to substitute fully when response distribution was

90% **28** lever responding and response rates were >0.2 responses/s. Regression analysis was used to compare the onset and offset of action for **3b** and **3g**; for this measure, group data were used to calculate the times when 50% of responding occurred on the **28** lever, i.e., before and following full substitution for the training drug stimulus.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS USED

CB1	cannabinoid receptor 1
CB2	cannabinoid receptor 2
(-)- ⁹-THC	(-)- ⁹ -tetrahydrocannabinol
HHC	hexahydrocannabinol
THC	tetrahydrocannabinol
CNS	central nervous system
PK/PD	pharmacokinetic/pharmacodynamic
SAR	structure–activity relationship
HEK293	human embryonic kidney cell line
log <i>P</i>	octanol–water partition coefficient
PSA	polar surface area
NMR	nuclear magnetic resonance
HPLC	high-performance liquid chromatography
MPE	maximum possible effect

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Figure 1.
Design of the first and second generation side chain carboxylated cannabinoid analogues,
with controllable deactivation and increasing polarity, and structures of the prototype (-)- Δ^8 -
THC-DMH and inactive metabolites.

Figure 2.

Effects of **3a**, **3b**, and **3c**, 11-OH-⁸-THC-DMH (**3g**), or vehicle (left-most points, above Veh) on rectal body temperature using female Sprague-Dawley rats ($n = 6$). Abscissa: dose, in mg/kg. Ordinate: change in rectal body temperature from an average baseline of 38.1 ± 0.3 °C. Symbols represent the average ($\pm$ SEM; $n = 6$ except 1.0 mg/kg **3b**, for which $n = 3$) of individual peak effects measured within 6 h of injection. Actual time varied with dose and compound. Solid symbols indicate effects that are significantly different from vehicle.

Figure 3. Hypothermic effects of select doses of **3a**, **3b**, 11-OH- δ^8 -THC-DMH (**3g**), and **3c** at different times after injection. The doses selected were the lowest doses that decreased temperature by 4°C in female Sprague–Dawley rats ($n = 6$). The dotted line represents temperature changes after vehicle injection. Abscissa: time (in min) after injection. Ordinate; change in body temperature. Filled symbols indicate effects that are significantly different from vehicle.

Figure 4.

Tail-flick latencies in a hot water-bath (52 °C) after administration of two doses each of compounds **3b** and **3g** at four time-points (20, 60, 180, and 360 min postadministration) using male CD-1 mice ($n = 6$). Abscissa: time (min) after injection. Ordinate; tailflick withdrawal latencies expressed as a percentage of maximum possible effect (% MPE; group mean $\pm$ SEM).

Figure 5. Time course of CB1 discriminative-stimulus effects of **3b** (0.001 mg/kg) and **3g** (0.003 mg/kg).

Scheme 1^a

^aReagents and conditions: (a) MeI, K₂CO₃, acetone, rt, overnight, 72%; (b) BBr₃, CH₂Cl₂, -78 to 0 °C, 2.5 h, 71%; (c) **6**, BF₃·Et₂O, CH₂Cl₂, -20 °C to rt, 2 h, 40%; (d) NaOH, THF/H₂O, reflux, 12 h 68%; (e) Br(CH₂)₃CH₃, NaHCO₃, DMF, microwave irradiation, 165 °C, 12 min, 51%.

Scheme 2^a

^aReagents and conditions: (a) BBr₃, CH₂Cl₂, 0 °C to rt, 3.5 h, 98%; (b) NaHCO₃, 1-bromobutane, DMF, 165 °C, microwave irradiation, 12 min, 92%; (c) **14**, *p*-TSA, CHCl₃, 0 °C to rt, 4 days, 27%; (d) TMSOTf, CH₂Cl₂/MeNO₂, 0 °C to rt, 7 h, 67%; (e) TBDMSCl, imidazole, DMAP, DMF, rt, 12 h, 87%; (f) Cl⁻Ph₃P⁺CH₂OMe, Na *tert*-amylate, benzene, 0 °C to rt, 3 h, 63%; (g) CCl₃COOH, H₂O, CH₂Cl₂, rt, 1 h, 97%; (h) K₂CO₃, EtOH, rt, 3 h, 78%; (i) NaBH₄, EtOH, rt, 30 min, 85%; (j) TBAF, THF, -40 °C, 30 min, 90%; (k) LiOH, dioxane/H₂O, rt, 24 h, 85%.

Scheme 3^a

^aReagents and conditions: (a) NaBH₄, MeOH, -78 °C, 2 h, 84%; (b) LiOH, dioxane/H₂O, rt, 24 h, 63%; (c) NaBH₄, MeOH, rt, 1 h, 81%; (d) K-selectride, THF, rt, 2 h, 91%; (e) NaHCO₃, 1-bromobutane, DMF, 165 °C, microwave irradiation, 12 min, 42%.

Scheme 4^a

^aReagents and conditions: (a) **14**, *p*-TSA, CHCl₃/acetone, 0 °C to rt, 4 days, 23%; (b) TMSOTf, CH₂Cl₂/MeNO₂, 0 °C to rt, 4 h, 57%; (c) NaOH, THF/H₂O, rt, 4 h, 63%; (d) NaHCO₃, 1-bromobutane, DMF, 165 °C, microwave irradiation, 12 min, 54%; (e) NaBH₄, MeOH, −78 °C, 2 h, 61%; (f) K-selectride, THF, −78 °C, 20 min, 52%; (g) NaBH₄, MeOH, rt, 1 h, 65%; (h) K-selectride, THF, rt, 3 h, 90%.

Affinities (K_i) of C9- and C11-Hydroxy-Substituted Cannabinoid Analogues for CB1 and CB2 Cannabinoid Receptors ($\pm 95\%$ Confidence Limits) and Half-Lives ($t_{1/2}$) of Representative Compounds for Mouse and Rat Plasma Esterases

Table 1

compd	X	R	$(K_i \text{ nM})^a$		mouse plasma		rat plasma	
			rCB1	mCB2	hCB2	$t_{1/2} \text{ (min)}^e$	$t_{1/2} \text{ (min)}^e$	$t_{1/2} \text{ (min)}^e$
3g			0.7 ^b	0.2 ^b	0.5 ^c	ND	ND	ND
2a			0.3 ± 0.1^d	2.1 ± 1.1^d	1.7 ± 0.4^d	12.4 ^d	120 ^d	
3a			0.6 ± 0.2	1.5 ± 0.5	0.8 ± 0.3	5.0	39.1	
4a			>10,000	>10,000	ND	ND	ND	ND

compd	X	R	(K _i nM) ^a			mouse plasma		rat plasma	
			rCB1	mCB2	hCB2	t _{1/2} (min) ^e	t _{1/2} (min) ^e	t _{1/2} (min) ^e	t _{1/2} (min) ^e
3b			2.4 ± 0.3	0.1 ± 0.05	3.1 ± 0.5	3.1	39.2		
4b			>10,000	>10,000	ND	ND	ND		
3c			0.1 ± 0.05	0.2 ± 0.1	0.2 ± 0.1	2.7	10.2		
4c			>10,000	>10,000	ND	ND	ND		
3d			3.1 ± 0.8	6.6 ± 1.5	4.5 ± 1.1	2.8	5.6

compd	X	R	(K _i , nM) ^a			mouse plasma		rat plasma	
			rCB1	mCB2	hCB2	t _{1/2} (min) ^e	t _{1/2} (min) ^e	t _{1/2} (min) ^e	t _{1/2} (min) ^e
4d			>10,000	>10,000	ND	ND	ND	ND	ND
3e			238 ± 35	317 ± 58	432 ± 82	ND	ND	ND	ND
4e			>10,000	>10,000	ND	ND	ND	ND	ND
3f			750 ± 145	654 ± 146	733 ± 162	ND	ND	ND	ND
4f			>10,000	>10,000	ND	ND	ND	ND	ND

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^a Affinities for CB1 and CB2 were determined using rat brain (CB1) or membranes from HEK293 cells expressing mouse or human CB2 and [³H]JCP-55,940 as the radioligand following previously described procedures. Data were analyzed using nonlinear regression analysis. *K_i* values were obtained from three independent experiments performed in triplicate and are expressed as the mean of the three values.

^b Reported previously.³⁷

^c Reported previously.³⁸

^d Reported previously.¹⁵

^e Half-lives (*t*_{1/2}) for mouse and rat plasma were determined in a single experiment as described in Experimental Section.

ND: not determined.

Table 2CB1/CB2 Functional Potencies (EC_{50}) of Selected C9- and C11-Hydroxy-Substituted Cannabinoid Analogues

compd	EC_{50} , nM, ^a $E_{(max)}$ (%) ^b classification	
	RCB1	hCB2
3a	4.6 (1.3–7.9), 89% agonist	5.7 (2.2–9.2), 83% agonist
3b	2.5 (1.6–3.4), 87% agonist	0.3 (0.2–0.4), 82% agonist
3c	8.6 (5.4–11.8), 85% agonist	9.0 (4.9–13.1), 85% agonist

^aFunctional potencies at rCB1 and hCB2 receptor were determined by measuring the decrease in forskolin-stimulated cAMP levels.^{13,20} EC_{50} values were calculated using nonlinear regression analysis. Data are the average of two independent experiments run in triplicate, and 95% confidence intervals for the EC_{50} values are given in parentheses.

^bForskolin stimulated cAMP levels were normalized to 100%. $E_{(max)}$ is the maximum inhibition of forskolin stimulated cAMP levels and is presented as the percentage of CP-55,940 response at 500 nM.