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Citation
Nikas, S. P., R. Sharma, C. A. Paronis, S. Kulkarni, G. Thakur, D. Hurst, J. T. Wood, et al. 2014. “Probing the Carboxyester Side Chain in Controlled Deactivation (−)-Δ8-Tetrahydrocannabinols.” Journal of Medicinal Chemistry 58 (2): 665-681. doi:10.1021/jm501165d. http://dx.doi.org/10.1021/jm501165d.

Published Version
doi:10.1021/jm501165d

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Probing the Carboxyester Side Chain in Controlled Deactivation (−)-Δ⁸-Tetrahydrocannabinols

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ABSTRACT: We recently reported on a controlled deactivation/detoxification approach for obtaining cannabinoids with improved druggability. Our design incorporates a metabolically labile ester group at strategic positions within the THC structure. We have now synthesized a series of (−)-Δ⁸-THC analogues encompassing a carboxyester group within the 3-alkyl chain in an effort to explore this novel cannabinergic chemo-type for CB receptor binding affinity, in vitro and in vivo potency and efficacy, as well as controlled deactivation by plasma esterases. We have also probed the chain’s polarity characteristics with regard to fast onset and short duration of action. Our lead molecule, namely 2-substituted-6a,7,10,10a-tetrahydro-1-hydroxy-6,6,9-trimethyl-6H-dibenzo[b,d]pyran-3-yl]-2-methyl-propanoic acid 3-cyano-propyl ester (AM7438), showed picomolar affinity for CB receptors and is deactivated by plasma esterases while the respective acid metabolite is inactive. In further in vitro and in vivo experiments, the compound was found to be a remarkably potent and efficacious CB1 receptor agonist with relatively fast onset/offset of action.

INTRODUCTION

(−)-Δ⁸-Tetrahydrocannabinol1 ((−)-Δ⁸-THC, 1, Figure 1) and its congeners act at CB1 and CB22–4 two G-protein-coupled cannabinoid receptors that are currently being targeted for various conditions including pain, inflammation, neurodegeneration, glaucoma, eating and mental disorders, as well as cancer.5–14 Owing to the undesirable side effects associated with CB1 receptor activation/deactivation as well as poor pharmacokinetic/pharmacodynamic (PK/PD) properties, only a limited number of cannabinergic drugs have been approved to date.15 Thus, the development of safer THC-based medications with favorable oral bioavailability, consistent efficacy, and predictable time course of action and detoxification remains to be addressed.

Toward this end, we recently reported on a controlled deactivation/detoxification approach where the “soft” analogue/drug concept of enzymatic deactivation was combined with a “depot effect” that is commonly observed with Δ⁸-THC and other lipophilic cannabinoids. In our design, the compound’s systemic half-life is determined by two factors. The first reflects the ability of the compound to sequester in some tissue reservoir such as fatty tissue (depot effect). The tissue sequestration affects the availability of the compound for receptor activation as well as for hydrolytic deactivation through systemic circulation. This process is dependent on the compound’s physicochemical properties and can be modulated by adjusting log P and PSA. Thus, more lipophilic compounds are slowly released in the bloodstream from the depot while more polar compounds are expected to have less of a depot effect. We have also confirmed that, in the compounds discussed here, the hydrolytic metabolic pathway is the most dominant and is considerably faster than other metabolic options such as reactions involving microsomal enzymes. The second is the rate of enzymatic hydrolysis of a metabolically labile ester group (−C(O)-O−) by blood esterases. This can be calibrated by incorporating suitable stereochemical features in the vicinity of the hydrolyzable moiety (enzymatic effect).16 Currently, we have developed two controlled-deactivation cannabinergic templates based on the tricyclic classical cannabinoid prototype. In the first, the C-ring in THC was replaced by a hydrolyzable seven-membered lactone,17 while in the second, the metabolically labile ester group was placed at the 2′-position of the side chain pharmacophore (Figure 1).16

Received: July 30, 2014
Published: December 3, 2014
In the present SAR study, we sought to probe the ester side chain pharmacophore in the \((-\Delta^8-THC)\) prototype within a novel series of analogues with regard to affinity for the CB1 and CB2 cannabinoid receptors, in vitro and in vivo potency and efficacy, as well as ability to control the half-lives of deactivation through enzymatic action. An additional goal of this work was to explore the polar characteristics of the side chain and gain information related to the modulation of the depot effect and its role in the in vivo pharmacokinetic profile of the analogues. The above considerations led us to design ligands that retain the metabolically labile group at the 2′-position of the side chain and replace the ester group (−C(O)-O−) with the reverse ester (−O−C(O)−) as well as with the corresponding thioester (−C(O)-S−) and the hydrolytically more stable amide group (−C(O)-NH−) (Figure 2). To explore the chain length, we synthesized analogues with four- to nine-atom-long side chains. Additionally, to probe the ligand’s polarity, we incorporated bromo-, cyano-, and imidazolyl-groups at the distal side chain carbon atom. As with our previous work,\(^\text{16}\) to regulate the rates of enzymatic inactivation while enhancing the compound’s affinities for CB receptors, we introduced benzylic substituents contiguous to the metabolically vulnerable ester group.

All novel compounds were characterized by determining their in vitro CB1 and CB2 receptor affinities. Of these, the most interesting were assessed for their functional activities and for their in vitro metabolic stabilities toward mouse plasma esterases while a limited representative set of key compounds was evaluated for their hypothermic and analgesic effects in vivo. Our data show that \((-\Delta^8-THC)\) analogues carrying five- to nine-atom-long side chains that are substituted with geminal dimethyl and cyclobutyl groups at the C1′-position exhibit remarkably high affinities for the CB1 and CB2 receptors. As predicted, all novel analogues were found to be susceptible to enzymatic deactivation by plasma esterases in a controllable manner, while their metabolites showed no or very low cannabinergic activity. Additionally, all key compounds were shown to be agonists for the CB1 receptor when tested for their abilities to reduce cAMP levels and also produce the respective alcohol upon exposure to sodium borohydride in methanol\(^\text{21}\) at \(-78^\circ\text{C}\). Cleavage of the polar characteristics of the side chain pharmacophore without any significant loss of potency.

CHEMISTRY

Syntheses of the 4′-bromo- and 4′-cyano-butyl esters \(6a-6c\) and \(7a-7c\) are summarized in Scheme 1. The required \((-\Delta^8-THC)\) carboxylic acids \(5a-5c\) were synthesized from commercially available \(3,5\text{-dimethoxyphenyl}acetonitrile\) and \((+)\text{-cis/trans-p-mentha-2,8-dien-1-ol}\) in three to four steps following our recently reported procedures.\(^\text{16}\) Alkylation of the respective carboxylic acids with 1,4-dibromobutane under microwave heating led to the corresponding esters \(6a-6c\) in 45–63% yields. Treatment of these bromides with sodium cyanoide in dimethyl sulfoxide\(^\text{18}\) produced the respective side chain cyano-substituted analogues \(7a-7c\) in 54–71% yields.

In a similar fashion, the tricyclic carboxylic acid \(5b\) was transformed to the side chain homologues \(8a, 8b,\) and \(9a-9d\) in 47–87% yields (Scheme 2). Reaction of the 3-bromo-propyl ester \(9b\) with sodium cyanoide or imidazole in the presence of potassium carbonate, in dimethyl sulfoxide, led to the end carbon substituted derivatives \(10a\) and \(10b\) (41–98% yields).

Synthesis of the reverse ester analogue \(16\) involves a Mitsunobu esterification reaction\(^\text{19}\) as the key step (Scheme 3). Thus, reduction of nitrile \(11\) with disobutylaluminum hydride\(^\text{20}\) at \(-78^\circ\text{C}\) led to aldehyde \(12\) (92% yield), which upon exposure to sodium borohydride in methanol\(^\text{21}\) afforded the respective alcohol \(13\) in excellent yield (94%). Cleavage of the methyl ether groups in \(13\) using boron tribromide\(^\text{18}\) produced resorcinol \(14\) in 47% yield. Acid catalyzed condensation of this intermediate with chiral terpenoid alcohol \(17\) in refluxing chloroform for 4–6 h gave \((-\Delta^8-THC)\) alcohol \(15\) in 21% yield.
isolated yield along with unidentified byproducts. We were able to improve this yield (28%) using microwave conditions over a much shorter time period (10 min). Subsequently, Mitsunobu esterification of 15 with n-valeric acid using triphenylphosphine and diethyl azodicarboxylate led to the final ester 16 (41% yield).

Treatment of the α,α-dimethyl-carboxylic acid 5b with [bis(2-methoxyethyl)amino]sulfur trifluoride and coupling of

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Scheme 1

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°Reagents and conditions: (a) Br(CH₂)₂Br, NaHCO₃, DMF, microwave irradiation, 165 °C, 12 min, 63% for 6a, 53% for 6b, and 45% for 6c; (b) NaCN, DMSO, 50 °C, 12 h, 71% for 7a, 54% for 7b, and 63% for 7c.
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Scheme 2

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Reagents and conditions: (a) Br(CH₂)₂Br, NaHCO₃, DMF, microwave irradiation, 165 °C, 12 min, 64% for 8a and 87% for 8b; (b) RBr, NaHCO₃, DMF, microwave irradiation, 165 °C, 12 min, 48% for 9a, 51% for 9b, 47% for 9c, and 47% for 9d; (c) NaCN, DMSO, 50 °C, 12 h, 98% for 10a; (d) imidazole, K₂CO₃, DMSO, rt, 14 h, 41% for 10b.
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Scheme 3

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Reagents and conditions: (a) DIBAL-H, CH₂Cl₂, −78 °C, 1 h, 92%; (b) NaBH₄, MeOH, rt, 1 h, 94%; (c) BBr₃, CH₂Cl₂, −78 °C, 15 min then rt, 1 h, 47%; (d) (+)-cis/trans-p-mentha-2,8-dien-1-ol (17), p-TSA, CHCl₃, microwave irradiation, 150 °C, 10 min, 28%; (e) DEAD, triphenylphosphine, CH₃(CH₂)₃COOH, THF, r, 20 h, 41%.
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the in situ generated acyl fluoride with n-pentylaniline afforded the (−)-Δ⁸-THC amide 18 in 70% yield (Scheme 4). Exposure of the same starting material (5b) to the benzotriazole/thionyl chloride reagent followed by treatment of the intermediate acyl chloride 19 with n-propanethiol led to thioester 20 in 23% yield for the two steps.

### CANNABINOID RECEPTOR AFFINITIES

The abilities of compounds 6a−6c, 7a−7c, 8a, 8b, 9a−9d, 10a, 10b, 15, 16, 18, and 20 to displace the radiolabeled CB1/CB2 agonist CP-55,940 from membranes prepared from rat brain (source of CB1) and HEK293 cells expressing either mouse CB2 or human CB2 were determined as described earlier, and inhibition constant values ($K_i$) from the respective competition binding curves are listed in Table 1 in which our prototype (−)-Δ⁸-THC, as well as the first-generation carboxy-Δ⁸-THCs 2a−2c are included for comparison. The rat, mouse, and human CB1 receptors have 97−99% sequence identity across species and, as shown earlier, are not expected to exhibit variations in their $K_i$ values. However, mouse CB2 (mCB2) exhibits only 82% sequence identity with the human clone (hCB2). This divergent nature of mCB2 and hCB2 receptors was shown in earlier work to be associated with species-based differences in affinity. For this reason, the analogues were also tested on hCB2.

The compounds included in this study are (−)-Δ⁸-THC analogues in which a four- to nine-atom-long side chain with or without 1′-substituents incorporates an enzymatically vulnerable group at the 2′- or 3′-positions. Optimization of the novel carboxy or thiocarboxy-ester side chains was probed through the synthesis of analogues carrying bromo-, cyano-, and imidazolyl-substituents at the distal carbon atom. As predicted based on our earlier results, the hydrolytic metabolites 5a−5c and 15 have no significant affinities for CB1 and CB2 receptors, thus minimizing the possibility of undesirable cannabinoid receptor related side effects. Comparison of the binding data of (−)-Δ⁸-THC and its carboxyester congeners 2a, 6a, and 7a suggests that extension of the linear chain from five to seven−nine atoms, along with incorporation of an ester group at the 2′,3′-positions, enhances the binding affinities of these analogues for both the CB1 and CB2 receptors. We also observed that incorporation of the bromo as well as the more polar cyano substituents at the terminal carbon of the side chain is well tolerated. Comparison of the binding data of our prototype (−)-Δ⁸-THC and compound 8b demonstrates the remarkable effects of 1′,1′-dimethyl-2′-carboxyester substitution (−(C(CH₃)₂-O−) on the side chain pharmacophore. Thus, 8b with the five-atom-long side chain exhibits 10−, 78−, and 24-fold higher binding affinities for rCB1, mCB2, and hCB2 receptors, respectively, when compared to (−)-Δ⁸-THC. The one-carbon shorter homologue 8a has significantly reduced affinities for both CB receptors, indicating that a minimum requirement for substantial affinity for both receptors in this series is a five-atom-long side chain. This high affinity can be maintained all through side chain lengths of nine atoms with or without terminal bromine substituents (analogues 2b, 6b, and 9a−c). Interestingly, this holds true when the terminal two or three atoms of the chain are replaced by the polar cyano group or the bulkier and amphiprotic imidazole ring (compounds 10a, 10b and 7b). Notably, the cyano analogues 10a and 7b exhibit remarkably high affinities for the CB1 and CB2 receptors. However, extension of the ω-substituted imidazolyl chain of 10b by one methylene group (compound 9d) results in a reduction of the ligand’s affinity for both cannabinoid receptors, an effect more accentuated in CB1 (~8-fold reduction).

Taken together, these data suggest that the pharmacophoric limits for an ω-substituted 1′,1′-dimethyl-2′-carboxyester chain could not be extended beyond the nine atoms.

A comparison of the binding affinities of the nonsubstituted analogues 6a and 7a with their respective gem-dimethyl counterparts 6b and 7b shows that introduction of two methyl substituents at the 1′-position of an ω-substituted carboxylated chain leads to an enhancement (20-fold maximum) in CB1 and CB2 receptor affinities. This increase in the ligand’s affinities for both CB1 and CB2 receptors holds true when the gem-dimethyl substitution is modified to the bulkier cyclobutyl ring (analogues 6c and 7c). An examination of the binding data of the 2′,3′-carboxyester analogue 2b and its sulfur and nitrogen congeners 18 and 20 shows that the ester moiety (−(C(O)-O−) can be replaced by the respective thioester (−(C(S)-S−) and amide (−(C(O)-NH−) groups. Likewise, analogue 16 incorporating the sterically less hindered retro ester group (−(O−C(O)−) at the 3′,4′-position maintains high affinity for both the CB1 and CB2 receptors.

In summary, the detailed SAR reported here shows that a five- to nine-atom-long side chain with gem-dimethyl or cyclobutyl substituents at the 1′-position and an enzymatically vulnerable group within the 2′ or 3′ chain segment results in analogues with remarkably high affinities for both CB1 and CB2 receptors. Importantly, addition of the bromo- or cyano-groups as well as the bulky and amphiprotic imidazole ring at the terminal carbon maintains or enhances the affinity of the ligand for the CB receptors.
Table 1. Affinities ($K_i$) of Side Chain ($\Delta^8$-THC Analogues for CB1 and CB2 Cannabinoid Receptors ($\pm 95\%$ Confidence Limits) and Half-Lives ($t_{1/2}$) of Representative Compounds for Mouse Plasma Esterases

| compd | R | $K_i$ (nM) | mouse plasma $t_{1/2}$ (min)$^3$ |
|-------|---|-----------|-------------------------------|
| (+)-$\Delta^8$-THC | | | |
| 2a | | 27.1 ± 4.5 | 40.4 ± 7.6 | 36.4 ± 11.2 | 0.7 |
| 5a | | >10,000 | >10,000 | N D | N D |
| 6a | | 2.2 ± 0.4 | 11.6 ± 3.7 | 7.1 ± 2.2 | 1.7 |
| 7a | | 14.3 ± 3.2 | 2.1 ± 0.3 | 1.2 ± 0.4 | 3.2 |
| 2b | | 0.3 ± 0.1 | 2.1 ± 1.1 | 1.7 ± 0.4 | 12.4 |
| 5b | | >10,000 | >10,000 | N D | N D |
| 8a | | 83.1 ± 7.3 | 36.9 ± 5.8 | 34.5 ± 7.4 | N D |
| 8b | | 2.7 ± 0.7 | 0.5 ± 0.3 | 1.5 ± 0.2 | N D |
| 9a | | 0.5 ± 0.1 | 1.2 ± 0.3 | 1.0 ± 0.4 | 9.5 |
| 15 | | 447 ± 85 | >10,000 | >10,000 | N D |
| 9b | | 7.7 ± 1.3 | 3.2 ± 1.2 | 2.9 ± 0.9 | N D |
| 6b | | 0.8 ± 0.3 | 1.1 ± 0.3 | 0.7 ± 0.2 | N D |
| 9c | | 1.2 ± 0.5 | 1.1 ± 0.03 | 0.6 ± 0.1 | N D |
| 10a | | 0.5 ± 0.2 | 0.8 ± 0.3 | 1.4 ± 0.7 | 46.5 |
| 10b | | 3.4 ± 1.1 | 2.1 ± 0.5 | 1.3 ± 0.3 | 271 |
IN VITRO PLASMA STABILITY STUDIES

Representative analogues within this series were assessed for their in vitro plasma stability toward mouse plasma esterases as detailed in the Experimental Section. It should be noted that blood contains various esterases which play the major role in the hydrolysis of compounds carrying ester, carbamate, or phosphate bonds. These esterases include acetylcholinesterases (ACHE), butyrylcholinesterases (BCHE), paraoxonases, and carboxyesterases (in mice and rat but not in human plasma). Esterase activity can be found mainly in plasma, with less activity in red blood cells. Plasma albumin itself may also act as an esterase under certain conditions. For example, albumin contributes about 20% of the total hydrolysis of aspirin to salicylic acid in human plasma. The esterase activity in blood seems to be more extensive in small animals such as rats than in large animals and humans. A comparison of the half-lives (t_{1/2}; Table 1) of the alkyl bromide and nitrile having no substitution at the 1′-position (6a, 7a) with their 1′-gem-dimethyl (6b, 7b) and 1′-cyclobutyl (6c, 7c) counterparts shows that the plasma esterase stabilities of the analogues correlate well with the presence and the size of 1′-substituents. Thus, the order of metabolic stabilities for the bromo- and cyano-substituted analogues is 6a < 7a < 6b < 7b < 6c < 7c, with the compounds carrying the bulkier cyclobutyl group being the most hydrolytically stable. This trend with the ω-substituted eight- to nine-atom-long side chain analogues parallels our earlier observations with the shorter (seven-atoms) and unsubstituted side chain congeners 2a, 2b, and 2c. Another general observation through this structure–stability relationship study is that regardless of the 1′-substituent, addition of a bromo- or cyano-group at the terminal carbon of a seven-atom-long chain increases its stability toward esterases with the cyano-substituted chain exhibiting the highest stability (comparison of 2a with 6a and 7a, 2b with 6b and 7b, and 2c with 6c and 7c).

The effect of ω-substitution on the chain’s enzymatic stability profile was studied in more detail in the 1′-gem-dimethyl series through the assessment of the half-lives of the cyano- and imidazolyl-substituted analogues 10a, 10b, 7b, and 9d. We observe that presence of a terminal cyano group or the larger imidazole ring reduce the enzymatic lability of the 1′-gem-dimethyl chain (analogue 2b) by 3- to 31-fold. As expected, the sterically less hindered retro ester analogue 16 was more prone to enzymatic hydrolysis when compared to the α,α-dimethyl-carboxylated counterpart 2b, while the amide group (18) was shown to have remarkable plasma stability. In agreement with earlier work on the rates of hydrolysis of ethyl butyrate and

Table 1. continued

| compd | R | (Kᵢ, nM)* | mouse plasma |
|-------|---|-----------|--------------|
|       |   | rCB1      | mCB2 | hCB2 | t_{1/2} (min)* |
| 7b    |   | 0.7 ± 0.07 | 0.1 ± 0.2 | 0.9 ± 0.3 | 40.4 |
| 9d    |   | 29.1 ± 4.1 | 8.2 ± 2.3 | 7.7 ± 1.1 | 392 |
| 18    |   | 6.3 ± 2.2 | 9.2 ± 2.1 | 2.8 ± 0.5 | Stable |
| 20    |   | 0.5 ± 0.1 | 0.8 ± 0.3 | 0.7 ± 0.1 | 312 |
| 2c    |   | 0.7 ± 0.2 | 3.0 ± 0.5 | 3.0 ± 0.7 | 36.3 |
| 5c    |   | >10,000 | >10,000 | ND | ND |
| 6c    |   | 0.3 ± 0.1 | 3.7 ± 0.7 | 0.7 ± 0.3 | 47.9 |
| 7c    |   | 0.9 ± 0.3 | 1.5 ± 0.5 | 1.8 ± 0.3 | 115 |

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

**Half-lives (t_{1/2}) for mouse plasma were determined as described under Experimental Section. **No observable hydrolysis within 5 h. ND: not determined.
potent analogue 10a was determined from two independent experiments (eight-point data) and listed in Table 2 along with data for the related compounds 2b and 2c for comparison. We observe that all tested analogues are full agonists at the CB1 receptor while their EC50 values correlate well with their respective binding affinities. Of the compounds tested, the carboxyester Δ2-THC analogues 2b and 2c as well as their ω-substituted cyanide congener 10a were found to be the most potent and efficacious compounds within the series with EC50 of 0.5, 0.4, and 0.9 nM, respectively.

### MOLECULAR MODELING

As an aid in the interpretation of the data, we carried out conformational analyses and docking studies (detailed procedures are given under Experimental Section) involving our lead analogue 10a, the nonhydrolyzable counterpart (−)-Δ2-THC-DMH as well as the parent compound (−)-Δ2-THC.

**Conformational Analysis.** Initial conformational analyses of (−)-Δ2-THC, (−)-Δ2-THC-DMH, and 10a revealed a difference in the conformation of the side chains in their global minimum energy conformers. Both (−)-Δ2-THC-DMH and 10a have their side chains oriented orthogonal to the phenol ring, while for (−)-Δ2-THC, the five-carbon side chain extends in the same plane as its phenol ring. Initial in vacuo conformational analyses of 10a identified an intramolecular hydrogen bond between the cyano nitrogen and the phenolic hydroxyl. The 10a phenolic hydroxyl to cyano nitrogen hydrogen bond heteroatom distance (N−O) and hydrogen bond (O−H−N) angle are 3.22 Å and 144°, respectively. The intramolecular hydrogen bond in 10a was not found in the global minimum energy conformation from a second conformational search performed using the OPLS2005 force field with an implicit GB/SA solvent model for water. Preliminary results from a 70 ns NAMD molecular dynamics simulation of 10a in a fully hydrated POPC lipid bilayer found a very low incidence of this internal hydrogen bond as well (unpublished data). For this reason, the nearest low energy conformer without an intramolecular hydrogen bond (which was 1.77 kcal/mol above the initial global min) was used for the calculation of conformational energy costs for 10a. The first and second side chain dihedrals of this conformer compare well with the global minimum energy conformer of (−)-Δ2-THC-DMH, shown in Table 3. Finally, the phenolic hydroxyls of all three ligands were found to prefer the proton directed toward the C2 phenyl ring position nearer the side chain and with the value of the C2−C1−O1−H1 dihedral near zero. A comparison of the lowest energy conformation of 10a without the internal hydrogen bond to those of (−)-Δ2-THC-DMH and (−)-Δ2-THC is shown in Figure 3.

**Docking Studies.** To provide a representation for the interaction of the above three compounds with the hCB1 receptor, we carried out docking studies with an activated form of a CB1 receptor model and calculated relative ligand/receptor interaction energies. Glide docking studies of 10a in the activated CB1 receptor revealed a significant role for the ester group at the 2′ position. The carbonyl oxygen within the ester group has a hydrogen bonding interaction with T3.33(197) as shown in Figure 4A. The hydrogen bond heteroatom distance (O−O) and hydrogen bond (O−H−O) angle are 2.56 Å and 165°, respectively. The T3.33(197) hydrogen bonding interaction is not available to the (−)-Δ2-THC-DMH counterpart or the parent compound (−)-Δ2-THC as shown in parts B and C of Figure 4. However, all three compounds modeled in the Glide docking studies have a phenolic hydroxyl that interacts directly with K3.28(192). The K3.28(192) and 10a phenolic hydroxyl hydrogen bond heteroatom distance (N−O) and hydrogen bond (N−H−O) angle are 2.73 Å and 170°, respectively. The K3.28(192) and (−)-Δ2-THC-DMH phenolic hydroxyl hydrogen bond heteroatom distance (N−O) and hydrogen bond (N−H−O) angle are 2.69 Å and 171°, respectively. Finally, the K3.28(192) and (−)-Δ2-THC phenolic hydroxyl hydrogen bond heteroatom distance (N−O) and hydrogen bond (N−H−O) angle are 2.64 Å and 168°, respectively.

Glide XP scores adjusted for ligand strain reflect the rank order of K3 (Table 3) in rCB1 with (10a = −5.7 kcal/mol) ≈ ((−)-Δ2-THC-DMH = −5.4 kcal/mol) < ((−)-Δ2-THC = −4.1 kcal/mol). For a list of Glide XP scores in rank order and adjusted by ligand conformational cost, see Supporting Information, Tables S2−S4. A breakdown of ligand/receptor interaction energies revealed the importance of van der Waals interactions for each of ligands and how the two 1,1-dimethyl analogues (10a and (−)-Δ2-THC-DMH) have far more of these interactions than (−)-Δ2-THC. The (−)-Δ2-THC-DMH and 10a have nine and eight van der Waals interactions better.

The Table 2. Functional Potencies (EC50) of Selected (−)-Δ2-THC Ester Analogues for the rCB1 Cannabinoid Receptor

| compd | rCB1 (EC50 nM) | E(max) (%) |
|-------|---------------|------------|
| 7a    | 10−100        | 0.5 (0.1−1.2) | 92 |
| 8b    | 1−10          | 0.9 (0.3−1.5) | 89 |
| 10a   | <1            | 0.4 (0.2−1.2) | 90 |
| 10b   | 1−10          | 0.4 (0.2−1.2) | 90 |
| 2c    | 1−10          | 0.4 (0.2−1.2) | 90 |

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**FUNCTIONAL CHARACTERIZATION**

Functional characterization of key compounds for the rCB1 receptor was carried out by measuring the decrease in forskolin-stimulated cAMP, as detailed earlier.16,18 Compounds were initially screened in three different concentrations (three-point data), and the approximate EC50 values were calculated (Table 2). Subsequently, the accurate EC50 value of the most potent analogue 10a was determined from two independent experiments (eight-point data) and listed in Table 2 along with data for the related compounds 2b and 2c for comparison. We observe that all tested analogues are full agonists at the CB1 receptor while their EC50 values correlate well with their respective binding affinities. Of the compounds tested, the carboxyester Δ2-THC analogues 2b and 2c as well as their ω-substituted cyanide congener 10a were found to be the most potent and efficacious compounds within the series with EC50 of 0.5, 0.4, and 0.9 nM, respectively.
antinociceptive properties was studied in more detail and its in vivo hypothermic and analgesic effects were compared to those of the earlier first-generation analogue 2b as well as with the nonhydrolyzable parent compound (−)-Δ⁸-THC-DMH.

**Hypothermia Testing.** Body temperature was measured in isolated rats over a 6 h period following drug injection (detailed procedures are given in the Experimental Section). Compound 10a decreased core body temperature in a dose-dependent manner, with a dose of 0.1 mg/kg reducing body temperature up to 4.5 ± 0.5 °C from an average baseline of 37.8 ± 0.1 °C (Figure 5). Its ED₅₀ value (i.e., the dose required to reduce temperature by 3 °C) was 0.034 mg/kg (95% CI: 0.026, 0.041 mg/kg). For comparison, the effects of our first-generation analogue 2b

### IN VIVO BEHAVIORAL CHARACTERIZATION

Representative analogues within this series were initially screened using the hypothermia test in rats (data not shown), and the order of the potency was found to be 10a > 7b ≥ 6b > 2b ≈ 20 ≈ 16 ≈ 8b ≈ 18. Subsequently, the most promising compound 10a was studied in more detail and its in vivo hypothermic and antinociceptive profiles were compared to those of the earlier first-generation analogue 2b.

#### Table 3. Comparison of Side Chain Dihedral Values for Global Minimum Energy Conformers and Binding Affinities (Kᵢ) of Key Analogues

| side chain dihedral | Kᵢ = 0.5 nM (rCB1), = 0.8 nM (mCB2) (deg) | Kᵢ = 0.9 nM (rCB1), = 1.4 nM (mCB2) (deg) | Kᵢ = 47.6 nM (rCB1), = 39.3 nM (mCB2) (deg) |
|---------------------|------------------------------------------|------------------------------------------|------------------------------------------|
| C2–C3–C1’–C2’      | 174.6                                    | 176.1                                    | 175.2                                    |
| C3–C1’–C2’–(C3’, O3’)–C4’ | −164.7                                  | −174.5                                  | 179.5                                    |
| (C3’, O3’)–C4’–C5’–C6’ | 54.4                                     | 61.4                                    | 179.1                                    |
| C4’–C5’–C6’–C7’    | 54.3                                     | 179.1                                   | 180.0                                    |

Figure 3. 10a is the lowest energy conformer that does not have the internal H-bond and is shown on the left. The (−)-Δ⁴-THC-DMH global minimum energy conformer is in the middle and (−)-Δ⁸-THC is on the right. All carbon atoms are shown in cyan.

Total ligand/receptor interaction energies also follow the trend of the Glide XP scores and Kᵢ in rCB1 with (10a = −42.9 kcal/mol) ≈ ((−)-Δ⁴-THC-DMH = −44.4 kcal/mol) < (((−)-Δ⁸-THC = −35.6 kcal/mol). The docked conformational cost for each ligand after the post Glide minimization (10a, 0.18 kcal/mol; (−)-Δ⁴-THC-DMH, 0.28 kcal/mol; and (−)-Δ⁸-THC, 0.13 kcal/mol) was small. Compound 10a did not have a statistically significant lower Kᵢ compared to (−)-Δ⁸-THC-DMH, even though Glide docking studies identified two hydrogen bonds for 10a compared to one hydrogen bond for (−)-Δ⁴-THC-DMH. This may be due to the energetic cost for 10a to place its hydrophilic ester and cyano moieties in a generally hydrophobic pocket in the CB1 receptor. This cost likely causes the two Kᵢ to be essentially equivalent.
and those of the (−)Δ⁸-THC-DMH are also shown. The ED₅₀ values were 0.37 (0.14, 0.73 with 95% CI) for 2b and 0.29 (0.18, 0.45 with 95% CI) for (−)Δ⁸-THC-DMH. Thus, 10a is approximately 8–10-fold more potent than (−)Δ⁸-THC-DMH and 2b. Limited extended studies comparing hypothermia induced by equipotent doses of the 6'-cyano-2'-carboxy-Δ⁸-THC analogue 10a and its structurally related analogues 2b and (−)Δ⁸-THC-DMH for 12 h provided an estimate of their in vivo times of action. Thus, at a dose of 0.03 mg/kg, near its ED₅₀ value, compound 10a reduced body temperature by 2 °C within 3 h of injection and these effects were not observed 6 h after injection (see Supporting Information, Figure S1). This was compared with the effects of equivalent doses (near their ED₅₀ values) of 2b and (−)Δ⁸-THC-DMH. Thus, for 2b, a dose of 0.3 mg/kg produced effects that persisted for at least 6 h after injection while a dose of 0.3 mg/kg of (−)Δ⁸-THC-DMH produced an equivalent reduction in temperature beyond 12 h after injection. It should be noted that in order to compare equiactive doses of the most potent compound 10a with those of the in vivo less potent (−)Δ⁸-THC-DMH and 2b, we have used different doses of the three drugs that are consistent with their ED₅₀ values. Our data indicate that the depot effect as reflected in the log P and PSA values (Table 4) plays the most dominant role in determining the in vivo hypothymic half-lives of compounds 10a, 2b, and the nonhydrolyzable (−)Δ⁸-THC-DMH. Thus, notwithstanding the shorter in vitro hydrolytic half-life of compound 2b, when compared to 10a, this compound has a longer in vivo half-life. These results are represented in Table 4.

Table 4. Calculated log P and tPSA Values for (−)Δ⁸-THC-DMH, 2b, and 10a, and Duration of Their Hypothermic Effects in Rats

| compd            | clogP° | tPSA° | duration of hypothermic effects in rats³ |
|------------------|--------|-------|----------------------------------------|
| (−)Δ⁸-THC-DMH    | 9.1    | 29.5  | t > 12 h                               |
| 2b               | 6.6    | 55.8  | t = 6–12 h                             |
| 10a              | 5.0    | 79.5  | t < 6 h                                |

°Calculations were performed using ChemBioDraw Ultra 14.0 software.
³Hypothermic effects were determined using equiactive doses of the test compounds.

Figure 5. Effects of 10a, 2b, and Δ⁸-THC-DMH or vehicle (above V) on body temperature. Symbols represent the group mean ± SEM (n = 6 rats). Abscissa, dose in mg/kg; ordinate, change in body temperature from an average baseline of 37.8 ± 0.1 °C. Data with compound 2b and Δ⁸-THC-DMH published previously.16

Analgesia Testing. The pharmacokinetic profiles of analogues bearing the carboxyester (2b) and the more polar cyanocarboxyester (10a) side chains were further studied in the CB1 receptor-characteristic analgesia test in mice, and the results are depicted in Figure 6 (the dose response graph for four doses of

Figure 6. Tail-flick latencies in a hot water bath (52 °C) after administration of 0.3 and 1.0 mg/kg of compounds 10a and 2b at four time-points (20, 60, 180, and 360 min postadministration) using male CD-1 mice. Abscissa, time (min) after injection; ordinate, tail-flick withdrawal latencies expressed as a percentage of maximum possible effect (% MPE; group mean ± SEM). Data for compound 2b are reproduced from our earlier work.16 10a are given under Supporting Information, Figure S2). A mixed model repeated measures ANOVA (IBM software package SPSS, v.21) applied to the tail-flick latency data produced by compounds 10a and 2b during the descending phase (180 and 360 min) showed significant main effects for drug (D) (F₁,₄₄ = 8.98; p ≤ 0.004), dose-level (L) (F₁,₄₄ = 40.95; p ≤ 0.001), and time (T) (F₁,₄₄ = 28.46; p ≤ 0.001). Of more interest, however, is that pairwise comparisons within these parameters (D, L, and T) using Sidak multiple comparison t test procedure suggested significant differences for all three parameters (p = 0.05). Thus, the tail-flick latencies were dose- and time-dependent for both compounds in a similar manner, and because the pairwise comparison for drug was also significant, the offset of the analgesia effect was significantly faster for 10a compared to 2b. The average (±SEM) baseline tail-flick withdrawal latency for all mice (N = 24) examined with compound 10a was 0.99 ± 0.05 s.

Overall, our in vivo experiments show that in rats, compound 10a is approximately a 10–30-fold more potent as a cannabinoid agonist when compared to 2b and (−)Δ⁸-THC-DMH. Also, this more potent analogue has a faster onset and shorter duration of action when compared to the less polar counterpart 2b and the lipophilic and nonhydrolyzable parent compound (−)Δ⁸-THC-DMH. Our antinociception data in mice clearly show that the cyanocarboxyl-analogue 10a has a shorter duration of action than its congeners with no cyano substitution (2b).

CONCLUSIONS

In summary, as a continuation of our earlier work on the controlled deactivation/detoxification ligand development project, we sought to probe the novel carboxyester side chain
pharmacophore in (−)-Δ⁴-THCs for CB receptor binding affinity, in vitro and in vivo potency and efficacy, as well as its effects on the half-lives of deactivation through enzymatic activity. We have also explored the chain’s polar characteristics that are associated with the depot effect, in an effort to produce cannabinoids with faster onset/offset and shorter duration of action than the currently existing (−)-Δ⁴-THC analogues. In connection with our earlier work where we focused on the chain’s benzylic position and the related subite within the receptor’s binding domain, the current SAR study extends the mapping of the chain’s pharmacophoric space beyond the 1'-carbon and argues that both CB receptors can tolerate polar groups and atoms within the second and the fourth position of the chain. We are hopeful that these observations will provide us with new opportunities for the design of high affinity/efficacy novel analogues with improved selectivities for the two cannabinoid receptors. The most successful compound identified through this careful study, namely 2-[(6aR,10aR)-6a,7,10a,10a-tetrahydro-1-hydroxy-6,6,9-trimethyl-6H-dibenzo[b,d]-pyran-3-yl]-2-methyl-propionic acid 3-cyano-propyl ester (6a), is a remarkably potent and efficacious CB1 receptor agonist with relatively shorter duration of action than its less polar 2'-carboxy-(−)-Δ⁴-THC-DMH counterpart and the highly lipophilic and long lasting (−)-Δ⁴-THC-DMH. Our data support the hypothesis 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.

**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 homogenous by analytical TLC on precoated silica gel TLC plates (Merck, 60 F254 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 (400 MHz, 1H) or on a Varian INOVA-500 (500 MHz, 1H) 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), and 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% α-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%.

2-[(6aR,10aR)-6a,7,10a-tetrahydro-1-hydroxy-6,6,9-trimethyl-6H-dibenzo[b,d]pyran-3-yl]acetic Acid 4-Bromo-butyrol Ester (6a). A stirred mixture of 5a (175 mg, 0.58 mmol), dibromobutane (313 mg, 1.45 mmol), and sodium bicarbonate (73 mg, 0.87 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 (MgSO₄), and concentrated under reduced pressure. Purification by flash column chromatography on silica gel gave 6a (159 mg, 63% yield) as a light-yellow gum. IR (CHCl₃): 3407, 2967, 2928, 1712 (ς, χ=CH=O), 1621, 1583, 1431, 1254 cm⁻¹. 1H NMR (500 MHz, CDCl₃) δ 6.33 (d, J = 1.0 Hz, 1H, 4-H), 6.24 (d, J = 1.0 Hz, 1H, 2-H), 5.42 (m as d, J = 4.0 Hz, 1H, 8-H), 5.23 (s, 1H, OH), 4.12 (t, J = 6.5 Hz, 2H, OCH₂CH₂), 3.45 (s, 2H, –CH₂–(O–O)–), 3.39 (t, J = 6.5 Hz, 2H, –CH₂Br), 3.19 (dd, J = 15.0 Hz, J = 4.5 Hz, 1H, 10a-H), 2.69 (td, J = 11.0 Hz, J = 4.5 Hz, 1H, 10a-H), 2.19–2.10 (m, 1H, 7α-H), 1.94–1.76 (m, 7H, 10-β-H, 7-β-H, 6a-α-H, –CH–CH₂–CH₂–Br, –CH₂–CH–CH–CH–Br), 1.69 (s, 3H, 9-CH₃), 1.37 (s, 3H, 6β-CH₃). Mass spectrum (EI) m/z (relative intensity) 438 (M⁺ + 2 + Na, 97), 436 (M⁺, 97), 395 (38), 393 (38), 355 (95), 353 (95), 330 (60), 316 (38), 287 (46), 273 (48), 257 (45), 247 (98), 233 (60), 213 (100). Exact mass (EI) calculated for C₂₅H₃₄O₄Br (M⁺ + H), 465.1647; found, 465.1647. LC/MS analysis of (Waters MicroMass ZQ system) showed purity 97% and retention time 7.4 min for the title compound.

2-[(6aR,10aR)-6a,7,10a-tetrahydro-1-hydroxy-6,6,9-trimethyl-6H-dibenzo[b,d]pyran-3-yl]-2-methyl-propionic Acid 4-Bromo-butyrol Ester (6b). The synthesis was carried out as described for 6a using 5b (200 mg, 0.61 mmol), dibromobutane (330 mg, 1.53 mmol), and sodium bicarbonate (77 mg, 0.92 mmol) in DMF and gave 6b (148 mg, 53% yield) as a light-yellow gum. IR (neat): 3403, 2970, 2917, 1727, and 1703 (ς, χ=CH=O), 1620, 1577, 1416, 1256 cm⁻¹. 1H NMR (500 MHz, CDCl₃) δ 6.41 (d, J = 2.0 Hz, 1H, 4-H), 6.25 (d, J = 2.0 Hz, 1H, 2-H), 5.42 (m as d, J = 5.0 Hz, 1H, 8-H), 5.18 (s, 1H, OH), 4.09 (t, J = 6.5 Hz, 2H, OCH₂CH₂), 3.32 (t, J = 6.5 Hz, 2H, –CH₂Br), 3.19 (dd, J = 15.0 Hz, J = 4.5 Hz, 1H, 10a-H), 2.69 (td, J = 11.0 Hz, J = 4.5 Hz, 1H, 10a-H), 2.18–2.10 (m, 1H, 7α-H), 1.85–1.68 (m and s overlapping, 10H, 10-β-H, 7-β-H, 6a-α-H, –CH–CH₂–CH₂– of the side chain and 9-CH₃ especially 1.70 s, 9-CH₃), 1.51 (s, 6H, –(CH₃)₂–), 1.38 (s, 3H, 6β-CH₃), 1.10 (s, 3H, 6α-CH₃). 13C NMR (125 MHz, CDCl₃) δ 177.8 (ς=CH=O), 155.7 (C-1 or C-5), 151.5 (C-5′), 144.8, 134.9, 119.4, 112.0, 107.1 (105.3), 77.0 (C-6′), 64.3 (OCH₂CH₂), 46.3, 45.0, 38.0, 35.7, 29.3, 27.7, 26.2, 26.2, 23.8, 18.7. Mass spectrum (EI) m/z (relative intensity) 467 (M⁺ + 2 + H, 100), 465 (M⁺ + H, 100), 285 (30). Exact mass (EI) calculated for C₂₆H₃₅O₄Br (M⁺ + H), 465.1640; found, 465.1647. LC/MS analysis of (Waters MicroMass ZQ system) showed purity 98% and retention time 7.4 min for the title compound. Anal. C₃₃H₃₃BrO₄ (C H).
A solution of bromide 6a (80 mg, 0.18 mmol) and sodium cyanide (88 mg, 1.8 mmol) in anhydrous DMSO (5 mL) was stirred at 50 °C for 3 h under argon. The reaction mixture was cooled to room temperature and diluted with water and 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 evaporated under reduced pressure. Purification by flash column chromatography on silica gel (20% ethyl acetate in hexane) afforded 7a (50 mg, 71% yield) as a light-yellow gum. IR (CHCl3): 3400, 2970, 2250 (CN), 1723 and 1703 (s, >C=O).

Compounds 8a, 9a, and 10a were isolated as light-yellow oils. IR (neat): 3405, 2926, 2249 (CN), 1727 and 1705 (s, >C=O).

The synthesis was carried out as described for 6a using 6b (20 mg, 0.04 mmol) and sodium cyanide (21 mg, 0.43 mmol) in anhydrous DMSO and gave 7b (9.5 mg, 54% yield) as a light-yellow gum. IR (CHCl3): 3405, 2973, 2249 (CN), 1727 and 1705 (s, >C=O).

The synthesis was carried out as described for 6a using 5b (300 mg, 0.91 mmol), iodoethane (356 mg, 2.28 mmol), and sodium bicarbonate (115 mg, 1.37 mmol) in DMF and gave 8b (283 mg, 87% yield) as a light-yellow gum. IR (neat): 3410, 2959, 2931, and 1728 cm−1. Exact mass (ESI) calculated for C26H34NO4 (M+ + H), 383.2097; found, 383.2092.

The synthesis was carried out as described for 6a using 7a (240 mg, 0.73 mmol), iodomethane (260 mg, 1.83 mmol), and sodium bicarbonate (77 mg, 0.92 mmol) in DMF and gave 9a (127 mg, 48% yield) as a light-yellow gum. IR (neat): 3405, 2972, 2918, and 1725 cm−1. Exact mass (ESI) calculated for C26H34NO4 (M+ + H), 383.2097; found, 383.2092.

The synthesis was carried out as described for 6a using 8a (200 mg, 0.61 mmol), 1,2-dibromoethane (287 mg, 1.53 mmol), and sodium bicarbonate (77 mg, 0.92 mmol) in DMF and gave 9a (127 mg, 48% yield) as a light-yellow gum. IR (neat): 3405, 2972, 2918, and 1725 cm−1. Exact mass (ESI) calculated for C26H34NO4 (M+ + H), 383.2097; found, 383.2092.
and sodium bicarbonate (134 mg, 1.59 mmol) in DMF and gave 453 (M+ + 2 + H, 55), 450 (M+ + H, 55), 285 (100). LC/MS analysis (Waters MicroMass ZQ system) showed purity 96% and retention time 7.6 min for the title compound. Anal. (C₉H₁₆O₃)C₂H₂O₃ requires C 72.42, H 10.04, O 17.54; found, C 72.29, H 10.01, O 17.69.

To a solution of 5b (78 mg, 41% yield) as a light-yellow gum. IR (CHCl₃): 3403, 2971, 2932, 1727 (s, >C=O), 1618, 1578, 1512, 1417, 1261 cm⁻¹. ¹H NMR (500 MHz, CDCl₃) δ 6.42 (d, J = 2.0 Hz, 1H, H-5), 6.25 (d, J = 2.0 Hz, 1H, 1-H2), 5.42 (m as d, J = 5.0 Hz, 1H, 7-H), 5.07 (s, 1H, OH), 4.03 (t, J = 6.5 Hz, 2H, –OCH₂–), 3.29 (t, J = 6.5 Hz, 2H, –CH₂–), 3.19 (dd, J = 15.0 Hz, 1H, 6a-H), 1.84–1.87 (m, 2H, –CH₂–CH₂–), 1.84 (m and s overlapping, 10H, 10β-H, 7β-H, 6a-H, –CH₂–CH₂– of the side chain and 9-C₃H₇, especially 1.70 s, 9-C₃H₇), 1.51 (s, 6H, –C(CH₃)₂–), 1.38 (s, 3H, 6-CH₃), 1.23 (m, 2H, –CH₂–CH₂– of the side chain), 1.10 (s, 3H, 6-CH₃). Mass spectrum (EI) m/z (relative intensity) 438 (M+, 100), 423 (12), 395 (80), 355 (7), 285 (60), 241 (39). Exact mass (EI) calculated for C₂₇H₃₆N₂O₄ (M+), 438.2519; found, 438.2518.

To a solution of 5c (78 mg, 41% yield) as a light-yellow solid, mp 101–102 °C. IR (CHCl₃): 3403, 2971, 2932, 2874, 1727 (s, >C=O), 1618, 1578, 1512, 1417, 1261 cm⁻¹. ¹H NMR (500 MHz, CDCl₃) δ 6.44 (s, 1H, imidazole), 6.40 (d, J = 2.0 Hz, 1H, H-10), 5.82 (s, 1H, OH), 5.42 (m as d, J = 5.0 Hz, 1H, 7-H), 5.33 (d, J = 2.0 Hz, 1H, 2-H), 5.04 (m as d, J = 5.0 Hz, 1H, 8-H), 3.93 (d, J = 17.0 Hz, 1H, –OCH₂–), 3.85 (td, J = 17.0 Hz, 1H, 6a-H, –OCH₂–), 3.72 (t, J = 7.0 Hz, 2H, –CH₂–NH–), 3.41 (dd, J = 16.0 Hz, 1H, 10a-H), 2.73 (td, J = 11.0 Hz, 1H, 10a-H), 2.17–2.09 (m, 1H, 7r-H), 2.01–1.93 (m, 2H, –OCH₂–), 1.85–1.71 (m, 3H, 10β-H, 7β-H, 6a-H), 1.60 (s, 3H, CH₃), 1.49 (s, 3H, –C(CH₃)₂–), 1.38 (s, 3H, 6-CH₃), 1.08 (s, 3H, 6-CH₃). ¹³C NMR (100 MHz, CDCl₃) δ 176.8 (C₅ (O=C)), 157.4 (C₅ (C = O)), 155.1 (C₅ (C = C-1)), 143.8, 137.5 (imidazole), 135.4, 128.7 (imidazole), 119.4, 119.1 (Cₙ (C = C-4) or Cₙ (C = C-2)), 105.7 (C₄ (C = C-2)), 77.0 (C-6), 60.9 (–OCH₂–), 46.2, 45.2, 43.8, 36.0, 31.9, 29.8, 28.1, 27.8, 26.1, 25.8, 23.7, 18.7. Mass spectrum (EI) m/z (relative intensity) 438 (M⁺, 62), 423 (12), 395 (80, 37), 317 (6), 304 (10), 285 (7), 201 (6), 45.1, 149 (10, 88, 56) (exact). Mass spectrum (EI) calculated for C₂₇H₃₆N₂O₄ (M⁺), 438.2519; found, 438.2518.

HPLC (4.6 mm × 250 mm, Supelco discovery column, acetonitrile/water) showed purity 98% and retention time 7 min for the title compound. LC/MS analysis (Waters MicroMass ZQ system) showed purity 98% and retention time 5.5 min for the title compound.

2-[6a(10R,R)-6a,7,10a-Tetrahydro-1-hydroxy-6,6,9-trimethyl-6H-dibenzo[b,d]pyran-3-yl]-2-ethyl-propanoic Acid 3-[1H-Imidazo-1-yl]propyl Ester (10b). To a stirred mixture of 9b (190.6 mg, 0.43 mmol) and potassium carbonate (59.4 mg, 4.30 mmol) in DMSO (5 mL) was added imidazole (146 mg, 2.15 mmol) at room temperature under an argon atmosphere. Stirring was continued for 14 h, and then the mixture was diluted with water and ethyl acetate. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate. The organic layer was washed with water and brine, dried (MgSO₄), and concentrated in vacuo. Purification by flash column chromatography on silica gel (50% acetone in hexane) gave 10b (78 mg, 41% yield) as a light-yellow gum. IR (CHCl₃): 2977, 2932, 1727 (s, >C=O), 1618, 1578, 1512, 1417, 1261 cm⁻¹. ¹H NMR (500 MHz, CDCl₃) δ 7.35 (s, 1H, imidazole), 7.04 (s, 1H, imidazole), 6.41 (s, 1H, imidazole), 3.72 (m, J = 2.0 Hz, 1H, 10a-H), 3.32 (d, J = 2.0 Hz, 1H, 2-H), 2.70 (m as d, J = 5.0 Hz, 1H, 7-H), 2.09 (m, 1H, 7r-H), 1.99 (m, 2H, –CH₂–CN), 1.93 (m as q, J = 6.0 Hz, 2H, –CH₂–CH₂– of the side chain), 1.87–1.75 (m, 3H, 10β-H, 7β-H, 6a-H), 1.70 (s, 3H, 9-CH₃), 1.52 (s, 6H, –C(CH₃)$_2$–), 1.38 (s, 3H, 6-CH₃), 1.10 (s, 3H, 6-CH₃). Mass spectrum (EI) m/z (relative intensity) 397 (100), 389 (82), 354 (9), 329 (13), 314 (100), 285 (27), 276 (21), 241 (78), 149 (21), 70 (41). Exact mass (EI) calculated for C₂₇H₃₆N₂O₄ (M⁺), 397.2253; found, 397.2254. LC/MS analysis (Waters MicroMass ZQ system) showed purity 98% and retention time 11.4 min for the title compound. Anal. (C₁₉H₂₄N₄O₄) C, H, N.
6.6 mmol, 1 M solution in CH2Cl2). Following this addition, the reaction temperature was gradually raised over a period of 3 h to 25 °C, and the stirring was continued at that temperature until the reaction was completed (4 h). Unreacted boron tribromide was destroyed 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 dissolved in diethyl ether and washed with water, brine and dried (MgSO4). Solvent evaporation and purification by flash column chromatography on silica gel (40% diethyl ether in hexane) gave 14 (165 mg, 47%) as light-yellow oil. (1-Hydroxy-2-methylprop-2-yl)benz[a]dipyran-3-yl 2-methyl-N-pentylpropanamide (6). A stirred solution of 1-ol (166 mg, 1.09 mmol) in anhydrous CHCl3 (3 mL), under an argon atmosphere, was added to dry CH2Cl2 (3 mL) under an argon atmosphere and cooled to 0 °C. The reaction mixture was heated at 150 °C for 4 h. Unreacted boron tribromide was destroyed by the addition of bis(2-methoxyethyl)aminosulfur fluoride (500 mg, 2.7 mmol) in dry CH2Cl2 (3 mL) under an argon atmosphere and cooled to 0 °C. The resulting reaction mixture was quenched with aqueous 1 N HCl solution and extracted with CHCl3. The combined organic layer was washed with water and brine, dried (MgSO4). Solvent evaporation under reduced pressure afforded the title compound (95 mg) which was used in the next step without further purification.

5-Propyl-2-[(6aR,10aR)-6a,7,10a-Tetrahydro-1-hydroxy-6,6,9-trimethyl-6H-dibenzo[b,d]pyran-3-yl]-2-methylpropyl chloroide (19). To a stirred solution of acid 5b (90 mg, 0.27 mmol) in dry CH2Cl2 (3.4 mL) at room temperature under an argon atmosphere, was added the SOCl2-BTA reagent (0.23 mL (0.35 mmol) of a 1.5 M solution in CH2Cl2, which was prepared by dissolving 0.54 mL (7.5 mmol) of SOCl2 and 0.893 g (7.5 mmol) of BTA in 5 mL CH2Cl2). Stirring was continued for 20 min, and insoluble materials were filtered off. The filtrate was washed with aqueous 1 N HCl, water, and brine, and then dried (MgSO4). Solvent evaporation under reduced pressure afforded the title compound (95 mg) which was used in the next step without further purification.
calculated from the IC\textsubscript{50} (Prism by GraphPad Software, Inc.). Each experiment was performed in triplicate, and K\textsubscript{i} values determined from three independent experiments and are expressed as the mean of the three values.

cAMP Assay.\textsuperscript{16,18} HEK293 cells stably expressing rCB1 receptor 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 and were washed once with HBSS and resuspended in the stimulation buffer. The various concentrations of the test compound (5 \mu M) in forskolin (2 \mu M final concentration) containing stimulation buffer were added to the plate followed by the cell suspension (5 \mu L). The cells were stimulated for 30 min at room temperature. Then Eu-cAMP tracer working solution (5 \mu L) and Ulight-anti-cAMP working solution (5 \mu L) were added to the plate and incubated at room temperature for 60 min. The data were collected on a PerkinElmer Envision instrument. The EC\textsubscript{50} values were determined by nonlinear regression analysis using GraphPad Prism software (GraphPad Software, Inc., San Diego, CA).

Plasma Stability.\textsuperscript{16,36} Compounds or their proposed products were diluted (200 \mu M) in mouse 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. In vitro plasma half-lives were determined using exponential decay calculations in Prism (GraphPad).

HPLC Analytic. Chromatographic separation was achieved using a Supelco Discovery C18 (4.6 mm × 250 mm) column on a Waters Alliance HPLC system. Mobile phase consisted of acetonitrile (A) and 0.1% phosphoric acid (B) in water and UV detection was used at each compound (204 and 230 nm).

Molecular Modeling. Ballessteros–Weinstein Nomenclature. The Ballesteros–Weinstein numbering system for GPCR amino acid residues is used here.\textsuperscript{37} In this numbering system, the label 0.50 is assigned to the most highly conserved class A residue in each transmembrane helix (TMH). This is preceded by the TMH number. In this system, for example, the most highly conserved residue in TMH6 is P6.50. The residue immediately before this would be labeled 6.49, and the residue immediately after this would be labeled 6.51. When referring to a specific CB1 residue, the Ballesteros–Weinstein name is followed by the absolute sequence number given in parentheses (e.g., K3.28(192)); however, when referring to a highly conserved residue among class A GPCRs (and not a specific residue in CB1), only the Ballesteros–Weinstein name is given.

Conformational Searches. The structures of the ligands (−)-\textDelta4 THC, (−)-\textDelta4-THC-dimethylheptyl, and 10a were built in Spartan’08 (Wave function, Inc., Irvine, CA). Initial conformational analyses of these compounds were performed in vacuo using the semiempirical method RM1 encoded in Spartan’08. Conformational searches were performed (using 3–8-fold rotations) for each rotatable bond. All unique conformers identified were then optimized with ab initio Hartree–Fock calculations at the 6-31G* level. To calculate the difference in energy between the global minimum energy conformer of each compound and its final docked conformation, rotatable bonds in the global minimum energy conformer were driven to their corresponding value in the final docked conformation and the single-point energy of the resultant structure was calculated at the HF 6-31G*. A duplicate conformational search of 10a was conducted at high dielectric using the MMC4 (Monte Carlo multiple minimum) protocol in MacroModel with the OPLS 2005 force field in a GB/SA water model with an extended cutoff.\textsuperscript{16,39} An energy window of 2.0 kJ/mol (\textDelta G) was employed, and the redundant conformers of 10a were eliminated using a rmsd cutoff of 0.5 Å for all atoms.

CB1R Active State Model. The studies reported here used our previously published CB1 activated state model.\textsuperscript{40} Below, we describe briefly the construction of both the inactive and active states of this model. Because no crystal structure of the CB1 receptor has been published, the CB1 model uses the crystal structure of the class A GPCR rhodopsin in the dark state as its template.\textsuperscript{41} This template was chosen because no mutations or modifications were made to its structure for crystallization and because the cannabinoid receptors and rhodopsin share some unusual sequence motifs that have important spatial relevance. For example, these receptors share a TMH4 GWNC motif at their extracellular ends. Here a TRP forms an aromatic stacking interaction with Y5.39, influencing the extracellular (EC) positions of TMH3–4–5. Changes to the general Rho structure that were necessitated by sequence divergences included the absence of helix kinking proline residues in TMH1 and TMH5, the lack of a GG motif in TMH2 (at position 2.56 and 2.57), as well as the presence of extra flexibility in the TMH6 CWXP motif because of the presence of G6.49 immediately before P6.50.\textsuperscript{42} An activated state (R*) CB1 model was created by modification of our inactive state model. This R* model construction was guided by the biophysical literature on the R-to-R transition in rhodopsin (Rho) and included a TMH6 conformer derived from our Conformational Memories study of CB1 TMH6 that is straightened, breaking the ionic lock interaction between R3.50 and D6.30.\textsuperscript{43} Extracellular (EC-1, H181(193); EC-2, C257(223); EC-3, D366(193) and intracellular (IC-1, S154(192); IC-2, P221(192); IC-3, A301(332)) loops, as well as portions of the N (K90(112) and C termini (S414(427), were then added to the refined model of the CB1 R* bundle. The Modeler program was then used to refine loop structures.\textsuperscript{44,45} Specific conformations of the EC-2 and EC-3 loop were refined to reflect mutagenesis data showing that EC-2 loop residue T(161) should be available to the binding crevice and that EC-3 loop residue P(135) should interact in a salt bridge with TMH residue D2.63(176).\textsuperscript{46,47} Ligand/CB1R* Complexes. The automatic docking program, Glide v6.4 (Schrödinger, LLC, NY 2014) was used to explore possible binding conformations or receptor site interactions for subject ligands. Because K3.28(192) has been shown to be a critical residue for classical cannabinoid binding,\textsuperscript{48} K3.28(192) was defined as a required interaction during the docking procedure. Glide was used to generate a grid based on the centroid of the ligand in the binding site. Any hydrophobic region defined in the grid generation that contacted the ligand was selected as important to the docking procedure. The box for Glide docking was defined to be 22 Å in the x, y, and z dimensions. The lowest energy conformations (<4 kcal/mol above the global min) of each ligand were docked using Glide. Extra precision (XP) was selected with no scaling of VdW radii and rigid docking invoked. Glide scoring functions do not take into account the conformational cost, or internal strain energy, of each generated pose of a ligand inside the binding pocket of the receptor. To determine the best ligand/receptor complex to proceed with a postdocking minimization for the acquisition of pairwise interaction energies, the conformational cost for each pose was subtracted from the Glide score for all poses (Supporting Information, Tables S2–4). The pose with the final best score was then subjected to a post docking minimization in two stages using the OPLS2005 all atom force field in Macromodel 10.5 (Schrödinger, LLC, NY 2014). An 8.0 Å nonbonded cutoff (updated every 10 steps), a 20.0 Å electrostatic cutoff, and a 4.0 Å hydrogen bond cutoff were used in each stage of the calculation. The first stage consisted of Polak–Ribiere conjugate gradient minimization using a distance-dependent dielectric function with a base constant of 2. No harmonic constraints were placed on the side chains, but 1000 kJ/mol fixed atom constraints were applied to hold all the backbone atoms in place. The termini and loops were not allowed to move during this part of the procedure. The minimization was continued until the bundle reached the 0.05 kJ/mol-A\textsuperscript{2} gradient. To relax the loops, a second stage Polak–Ribier conjugate gradient minimization of the loop regions was performed until the 0.05 kJ/mol-A\textsuperscript{2} gradient was reached. The loop and termini regions were left free, while the transmembrane regions and ligand were not allowed to move during this final stage. An 8.0 Å extended nonbonded cutoff (updated every 10 steps), 20.0 Å electrostatic cutoff, and 4.0 Å hydrogen bond cutoff were used in this calculation, and the generalized Born/surface area (GB/SA)
continuum solvation model for water available in Macromodel was employed.

**Assessment of Pairwise Interaction and Total Energies.** Interaction energies between each bound ligand and residue in the CB1R* complex were calculated using Macromodel, as described previously. Specifically, after defining the atoms of the ligand as one group (group 1) and the atoms corresponding to a residue that lines the binding site in the final ligand-CB1R* complex as another group (group 2), Macromodel was used to output the pairwise interaction energy (Coulombic and van der Waals) for a given pair.

**Methods for Characterization of in Vivo Effects.**

For hypothermia testing, female Sprague-Dawley rats (n = 6/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 a.m.–5:00 p.m.) 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/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 a.m. and 7:00 p.m. Mice were used once.

**Procedures.** Temperature was recorded using a thermostor probe (Model 401, Measurement Specialties, Inc., Dayton, OH) inserted to a depth of 6 cm and secured to the tail with micropore tape. Rats were minimally restrained and isolated in 38 × 50 × 10 cm3 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. In some studies, temperature readings at later time points were obtained by inserting the probe 6 cm and holding it in place for at least 1 min before taking a reading. 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 five 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 post administration. 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 were 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 − baseline latency)/(10 − baseline latency)] × 100.

**Drugs.** For hypothermia testing, (−)-Δ9-THC-DMH and compounds 2b and 10a 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, 10a and 2b 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.** Time-effect functions for hypothermia testing were analyzed using two-way repeated measures ANOVA procedures followed by Bonferroni’s posthoc test. Hypothemia dose–effect functions for compounds (−)-Δ9-THC-DMH, 2b, and 10a were analyzed using one-way repeated measures ANOVA procedures followed by the Holm–Sidak multiple comparison test; p was set at ≤0.05, and statistical analyses were performed using the software package GraphPad Prism 5.03 (GraphPad Software, San Diego, CA). A linear mixed model repeated measures ANOVA (IBM software package SPSS, v.21) was applied to tail-flick latency data depicted in Figure 5.

**ASSOCIATED CONTENT**

**Supporting Information**

Elemental analysis results for compounds 6b, 7c, 9b, 9d, 10a, and 16, tables for molecular modeling, hypothermic effects of approximately equivalent doses of 10a, 2b, and Δ9-THC-DMH at different times after injection, and tail-flick latencies in a hot water bath after administration of 10a. This material is available free of charge via the Internet at http://pubs.acs.org.

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**Notes**

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

This work was supported by grants from the National Institute on Drug Abuse to A.M., DA009158, DA007215, and DA09064.

**ABBREVIATIONS USED**

CB1, cannabinoid receptor 1; CB2, cannabinoid receptor 2; (−)-Δ9-THC, (−)-Δ9-tetrahydrocannabinol; CNS, central nervous system; PK/PD, pharmacokinetic/pharmacodynamic; SC, side chain; SAR, structure–activity relationship; HCK293, human embryonic kidney cell line; log P, octanol–water partition coefficient; PSA, polar surface area; NMR, nuclear magnetic resonance; HPLC, high-performance liquid chromatography.

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**ACKNOWLEDGMENTS**

This work was supported by grants from the National Institute on Drug Abuse to A.M., DA009158, DA007215, and DA09064.

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