Bornyl- and Isobornyl-Δ^8-tetrahydrocannabinols: A Novel Class of Cannabinergic Ligands

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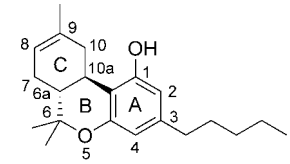
Structure−activity relationship studies of classical cannabinoid analogues have established that the C3 aliphatic side chain plays a pivotal role in determining cannabinergic potency. In earlier work, we provided evidence for the presence of subsites within the CB1 and CB2 cannabinoid receptor binding domains that can accommodate bulky conformationally defined substituents at the C3 alkyl side chain pharmacophore of classical cannabinoids. We have now extended this work with the synthesis of a series of Δ^8-THC analogues in which bornyl substituents are introduced at the C3 position. Our results indicate that, for optimal interactions with both CB1 and CB2 receptors, the bornyl substituents need to be within close proximity of the tricyclic core of Δ^8-THC and that the conformational space occupied by the C3 substituents influences CB1/CB2 receptor subtype selectivity.

Introduction

The classical cannabinoids found in cannabis have moderate receptor binding affinities and signal transduction properties, yet exhibit substantial potency in vivo.[1,2] Phytocannabinoids Δ^8- and Δ^9-tetrahydrocannabinol (Δ^8-THC[a] and Δ^9-THC) are classical cannabinoids that have comparable potency[3−6] and bind almost equally[4−6] to the two G protein-coupled cannabinoid receptors[7,8] CB1[9] and CB2.[10] Structure−activity relationship (SAR) studies of cannabinoid analogues with affinities for the CB1[11−13] and CB2[12,14,15] receptors, including the utilization of high affinity ligands with reactive functional groups suitable for characterizing (ligand-assisted protein structure, LAPS) the ligand binding domains of CB1 and CB2 subtypes,[16−18] have provided experimental three-dimensional structural information about these membrane proteins in the absence of nuclear magnetic resonance (NMR) or X-ray crystallographic structural data. These SAR studies have identified the C1 phenolic hydroxyl group and the C3 side chain as key pharmacophoric features of classical cannabinoids. Athough the C1 phenolic hydroxyl is not critical for CB2 affinity,[12,15,19] the C3 side chain pharmacophore of these ligands is a key factor in determining receptor affinity and pharmacological potency for both CB1 and CB2. Reviews of SAR on the C3 side chain of Δ^9-THC, and particularly with the relatively more stable Δ^8-THC, have suggested that optimal activity is obtained with a seven- or eight-carbon chain substituted with 1′,1′- or 1′,2′-dimethyl groups.[11,20−22] The flexibility of this side chain has hampered efforts to elucidate the precise nature of the pharmacophoric conformation of THC analogues with cannabinoid receptors. Recent studies in which the C3 side chain carries aryl,[23,24] cycloalkyl,[25,26] or other conformationally restricted moieties[19,27] have increased our understanding of the pharmacophoric features of this side chain. Our earlier results on adamantyl cannabinoids[28] suggested the existence of distinct subsites within the CB1 and CB2 cannabinoid receptor binding domains occupied by bulky conformationally restricted substituents at the C3 position.



1
(-)-Δ^8-THC

Figure 1. Phytocannabinoid (−)-Δ^8-THC (**1**) was used as the prototype to examine the steric effects of bulky, rigid, rotatable groups at C3 of classical cannabinoids.

Exploration of the allowable conformational space for these side chains has provided us with insights regarding the pharmacophoric features required for CB1 and CB2 selectivities.

We have now examined the bornyl and isobornyl groups as bulky hydrophobic C3 substituents on the prototypic classical cannabinoid (−)-Δ^8-THC (**1**, Figure 1). The bornyl groups derived from (+)-camphor were introduced at the C3 position of Δ^8-THC, either directly or with a methylene spacer. These novel analogues were tested for their binding affinities for CB1 and CB2 receptors to further explore what conformational characteristics of the cannabinoid C3 substituent are optimal for interaction with the two receptor subtypes.

Results

Chemistry. The bornyl- and isobornyl-Δ^8-THC analogues **8a** and **8b**, respectively, were synthesized as shown in Scheme 1. The Grignard reagent prepared from 1-chloro-3,5-dimethoxybenzene (**2**) reacted with (+)-(1*R*)-camphor (**3**) to give a 2:1 mixture of adducts **4a**:**4b** that was reduced with lithium/ammonia to give a 3:1 mixture of 5-bornyl-1,3-dimethoxybenzene (**5a**) and 5-isobornyl-1,3-dimethoxybenzene (**5b**).[29] Treatment of the mixture with boron tribromide provided a corresponding 3:1 mixture of the demethylated products, 5-bornylresorcinol (**6a**) and 5-isobornylresorcinol (**6b**). Following a well-established protocol,[30−32] condensation of the mixture of resorcinols **6a** and **6b** with (+)-*trans*-*p*-mentha-2,8-dien-1-ol (**7**) catalyzed by *p*-toluenesulfonic acid monohydrate gave a 3:1 mixture of the corresponding epimeric Δ^8-tetrahydrocannabinol analogues, which were separated by flash column chromatography. The major product was bornyl-Δ^8-THC **8a**, which exhibited the characteristic "W" coupling between the

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[a] Abbreviations: BSA, bovine serum albumin; K_i, inhibition constant; LAPS, ligand-assisted protein structure; SAR, structure−activity relationship; THC, tetrahydrocannabinol; TME buffer, 25 mM Tris, 5 mM $MgCl_2$, 1 mM EDTA buffer; COSY, correlation spectroscopy; NOESY, nuclear Overhauser enhancement spectroscopy.

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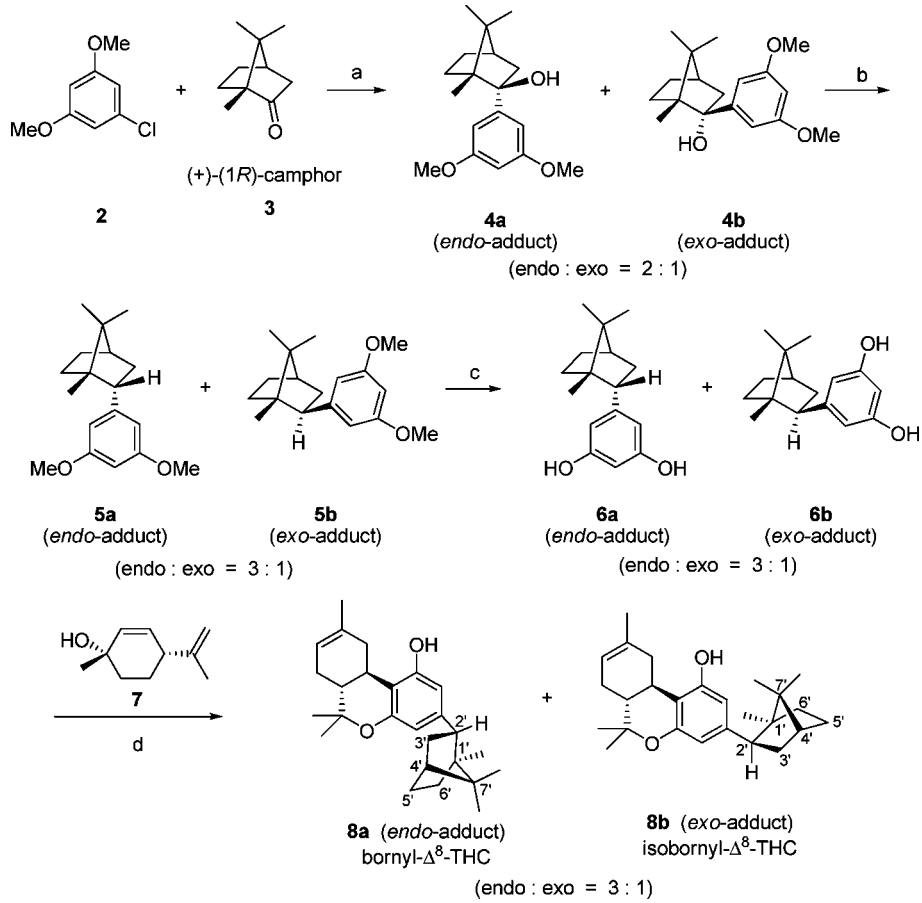
Scheme 1. Synthesis of Bornyl- and Isobornyl-Δ^8-THC (**8a** and **8b**)[a]



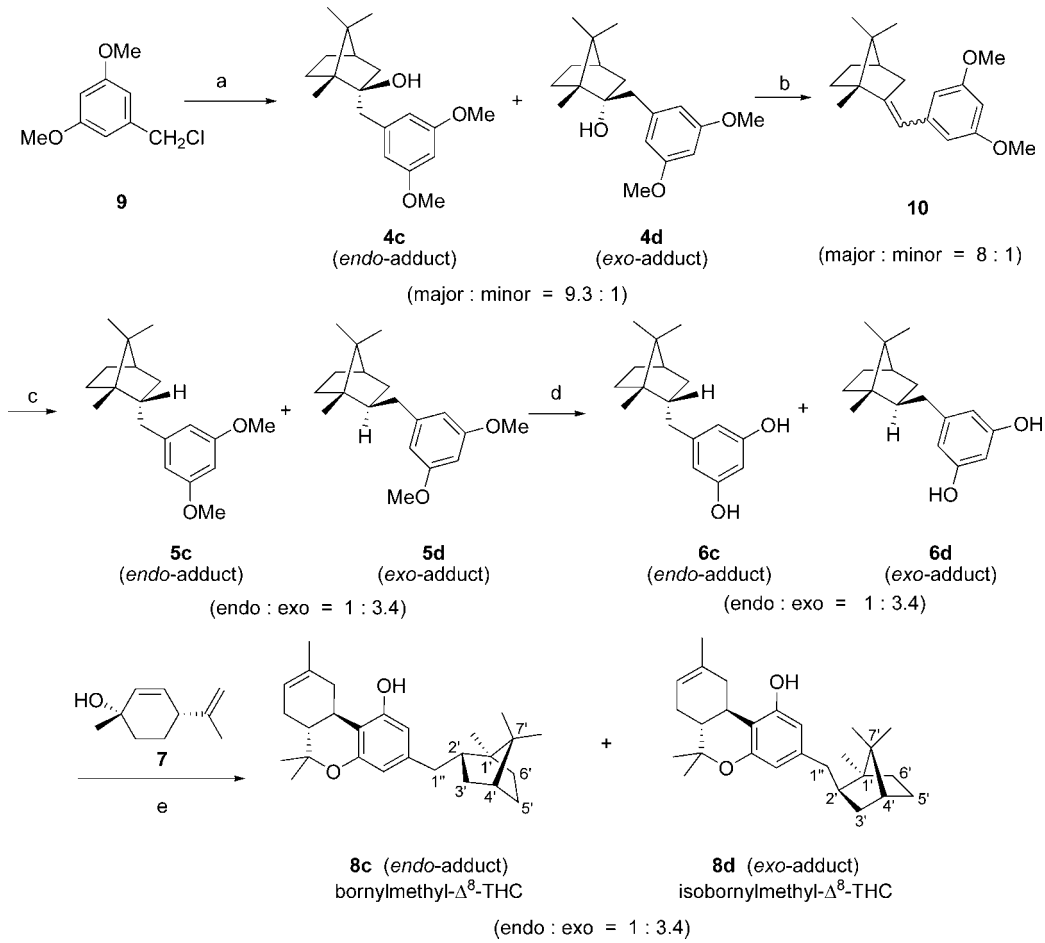
[a] (a) Mg/THF, reflux, 8 h; (b) Li/NH_3, THF, −78 °C, 6 h; (c) BBr_3, CH_2Cl_2, 0 °C to rt; (d) *p*-TsOH·H_2O, $CHCl_3$, 65 °C, 6 h.

C2′-H_{exo} benzylic proton (δ 2.89) and C6′-H_{exo}. The minor product, isobornyl-Δ^8-THC **8b** exhibited the characteristic apparent triplet for the C2′-H_{endo} benzylic proton (δ 2.72). 2D NMR was employed to further characterize the structures of **8a** and **8b**. Nuclear Overhauser effects (NOE) were observed between both C2-H and C4-H aromatics of the cannabinoid A ring with the bornyl C1′-methyl, C3′-H_{endo}, C5′-H_{endo}, as well as C6′-H_{endo} protons for the *endo*-adduct **8a**. NOE was also observed between C2′-H_{exo} and the *syn*-C7′-methyl of *endo*-adduct **8a**. NOEs were observed between the C2-H/C4-H aromatics and C3′-H_{exo}, as well as between C2′-H_{endo} and C6′-H_{endo} for the *exo*-adduct **8b** (see Supporting Information).

The corresponding bornylmethyl- and isobornylmethyl-Δ^8-THC analogues **8c** and **8d**, respectively, were synthesized as shown in Scheme 2. The Grignard reagent prepared from 3,5-dimethoxybenzylchloride (**9**) reacted with (+)-(1*R*)-camphor (**3**) to give a 9.3:1 mixture of *endo*-adduct **4c** to *exo*-adduct **4d**. The mixture was then treated with *p*-toluenesulfonic acid monohydrate in CH_2Cl_2 to afford an 8:1 mixture of alkenes **10**. Hydrogenation of the mixture gave a 1:3.4 mixture of *endo*-adduct **5c** to the *exo*-adduct **5d**, an observation that is consistent with literature precedents for endo approach of larger reagents to 2-methylidene-7,7-dimethylbicyclo[2.2.1]heptanes.[33−35] The mixture was inseparable by both flash chromatography and chiral HPLC and was used without further separation. Demethylation of the mixture with BBr_3 gave a corresponding mixture of bornylmethylresorcinol **6c** and isobornylmethylresorcinol **6d** that was also inseparable and was used directly in the Friedel−Crafts type reaction with (+)-*trans*-*p*-mentha-2,8-dien-1-ol (**7**). The corresponding 1:3.4 mixture of *endo*- to *exo*-diastereomers was separated by chiral HPLC on a Chiralpak AD column to afford bornylmethyl-Δ^8-THC (**8c**) and isobornylmethyl-Δ^8-THC (**8d**). The structures of **8c** and **8d** were confirmed by 2D NMR. The NOESY spectrum of the minor product, *endo*-adduct **8c**, showed an NOE between the benzylic C1″ methylene and C6′-H_{endo}, while the NOESY spectrum of the major product, *exo*-adduct **8d**, clearly showed NOEs between the benzylic C1″ methylene and the isobornyl C1′-methyl and *syn*-C7′-methyl groups. (see Supporting Information).

Receptor Binding Studies. Using purified rat forebrain synaptosomes[36] as a source of CB1 and using mouse spleen membranes[37] as a source of CB2, competition binding affinities with radiolabeled (−)-5-(1,1-dimethylheptyl)-2-[(1*R*,2*R*,5*R*)-5-hydroxy-2-(3-hydroxypropyl)-cyclohexyl]phenol (CP-55,940) were performed as previously detailed[28,38] for Δ^8-THC (**1**) and the newly synthesized analogues in which the phytocannabinoid five-carbon side chain at C3 was replaced with bulky but conformationally more defined bornyl **8a**, isobornyl **8b**, bornylmethyl **8c**, and isobornylmethyl **8d** substituents. Inhibition constant values (IC_{50}) determined from the respective displacement curves were converted to K_i values (Table 1) according to the reported method.[39] As can be seen from the K_i values, structural modifications of the C3 substituent affects cannabinoid receptor affinities as well as CB1/CB2 selectivities. The bornyl-Δ^8-THC analogue **8a** exhibits robust affinities for both CB1 and CB2, exceeding those of Δ^8-THC. Interestingly, the isobornyl-Δ^8-THC epimer **8b** exhibits 10-fold CB2 selectivity due to a loss of binding affinity at the CB1 subtype, while the binding affinity of this isobornyl epimer **8b** to CB2 remained comparable to that of the bornyl epimer **8a**. The possibility exists

Scheme 2. Synthesis of Bornylmethyl- and Isobornylmethyl-Δ⁸-THC (**8c** and **8d**)[a]



[a] (a) (1) Mg/THF; (2) (+)-(1*R*)-camphor (3), reflux, 8 h. (b) *p*-TsOH·H_2O, CH_2Cl_2, 45 °C, 1 h. (c) H_2, 10% Pd/C, EtOH, rt. (d) BBr_3, CH_2Cl_2, 0 °C to rt. (e) *p*-TsOH·H_2O, $CHCl_3$, 65 °C, 6 h.

that the selectivities could be different within the same rodent species; however, the comparisons made here between rCB1 and mCB2 are arguably relevant because of the close homology between rat and mouse CB1[40,41] and CB2[42] receptors.

When the bornyl or isobornyl groups are positioned further from the aromatic A-ring by the introduction of a flexible C1″ methylene link, affinities for CB1 and CB2 become comparable to that of Δ⁸-THC (**1**). The *endo*-bornylmethyl analogue **8c** and its *exo*-epimer **8d** are also nonselective.

Molecular Modeling. Among other considerations, the conformational space available to the novel analogues **8a**−**d** offers insight into the structural features required for CB1 and CB2 selectivity. Conformational scans were performed using a dihedral drive around the C3−C2′ bonds of **8a** and **8b** as well as the corresponding C3−C1″ bonds of methylene analogues **8c** and **8d** to explore the permissible rotations of the bulky substituents. Rotation around the C1″–C2′ bonds was also considered for 3-bornylmethyl-Δ⁸-THC (**8c**) and 3-isobornylmethyl-Δ⁸-THC (**8d**). The dihedral angle was restrained at a value between 0° and 360° in 1° steps, and minimization on the remaining geometric parameters was performed with the MM3* force field.[43] Conformers within 8 kcal mol^{-1} of the global minimum were retained. All calculations were performed in Macromodel.[44]

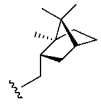
3-Bornyl-Δ⁸-THC (**8a**) has the smallest available conformational space of analogues **8a**−**d** (Figure 2) and has high affinity for both receptors with no subtype selectivity. The isobornyl group of the corresponding C2′ epimer **8b** has reduced steric hindrance, thereby increasing the number of low energy conformers available and creating a greater accessible conformational space. This analogue is less well tolerated in the CB1 receptor, implying that the CB1 binding pocket is unable to accommodate the larger substituent. Extension to the corresponding 3-bornylmethyl- and 3-isobornylmethyl-Δ⁸-THC analogues (**8c** and **8d**, respectively) further increases the rotational freedom of these bulky C3 substituents with a concomitant increase in the number of low energy conformers expanding the available conformational space.

Discussion

It is instructive to compare the present data with our earlier work involving adamantyl substituents at the C3 position of Δ⁸-THC.[28] These earlier results demonstrated that the cannabinoid side chain pharmacophoric site of the CB1 and CB2 receptors is capable of accommodating bulky substituents. However, the data also suggested that this subsite has distinctive features for each of the two receptors. The adamant-1′-yl analogue **11** (Table 1, Figure 2) exhibited substantial CB1 selectivity. We postulated that the adamant-1′-yl substituent has a favorable fit at the CB1 subsite, allowing for optimal hydrophobic interactions with corresponding CB1 amino acid residues while it engages in a looser suboptimal fit at the CB2 subsite. Conversely, the adamant-2′-yl analogue, which is capable of accessing a more expanded conformational space, was CB2 selective.

The bornyl group is also a rigid, compact, ten-carbon, hydrophobic, rotatable substituent. A comparison of the available

Table 1. Affinities (K_i) of Δ^8-THC Analogues for CB1 and CB2 Cannabinoid Receptors[a]

Compound	R	C3-Substituent	Binding Affinity K_i (nM)[a] CB1	CB2
1 (-)-Δ^8-THC	*n*-C_5H_{11}		47.6 (40.2-56.2)	39.3 (35.2-46.2)
8a AM735 3-bornyl-Δ^8-THC			8.9 (8.0-10.0)	7.4 (6.6-8.3)
8b AM731 3-isobornyl-Δ^8-THC			60.2 (55.4-65.4)	6.1 (5.6-6.7)
8c AM4739 3-bornylmethyl-Δ^8-THC			32.7 (24.9-42.8)	30.5 (23.6-39.4)
8d AM4738 3-isobornylmethyl-Δ^8-THC			49.0 (39.1-61.3)	49.5 (39.1-62.7)
11 AM411[b] 3-(adamant-1'-yl)-Δ^8-THC			6.8 (6.0-7.3)	52.0 (47.0-60.8)

[a] Affinities for CB1 and CB2 were determined using rat brain (CB1) or mouse spleen (CB2) membranes and [^{3}H]CP55,940 as the radioligand following previously described procedures.[28,38] K_i values were obtained from three independent experiments run in duplicate and are expressed as the mean of the three values, 95% confidence limits are indicated in the parenthesis. [b] Previously reported.[28]

conformational space for the novel terpene analogues **8a** and **8b** suggests some interesting conclusions. We postulate that the bornyl analogue **8a** (Figure 2) can be accommodated optimally in both receptors. However, the isobornyl group of analogue **8b** accesses a somewhat larger conformational space that can be optimally accommodated only within the CB2 subsite. This epimer **8b** is not well tolerated in the spatially more restricted CB1 binding pocket, which may account for its 10-fold CB2 selectivity. However, steric arguments may not fully account for ligand specificity and a combination of enthalpic and entropic reasoning would ultimately explain ligand affinity for the receptor.

When the terpene substituent is separated by a methylene group from the tricyclic cannabinoid structure as in **8c** and **8d**, the available conformational space is significantly larger. Their interactions with hydrophobic residues are impeded, resulting in reduced affinities of **8d** for both cannabinoid receptors and a complete loss of subtype selectivities of **8c** and **8d**, as was the case for the corresponding adamantyl methylene analogues.[28] We thus postulate that, in order to achieve subtype selectivity, the bulky C3 substituents not only need to be in close proximity to the tricyclic core but also must have an appropriate allowable conformational volume for an optimized interaction with each CB receptor.

We conclude that substituting the cannabinoid C3 side chain with conformationally restricted rigid groups can be successfully employed to probe the binding domains for the key cannabinoid C3 side chain pharmacophore within the CB1 and CB2 receptors.

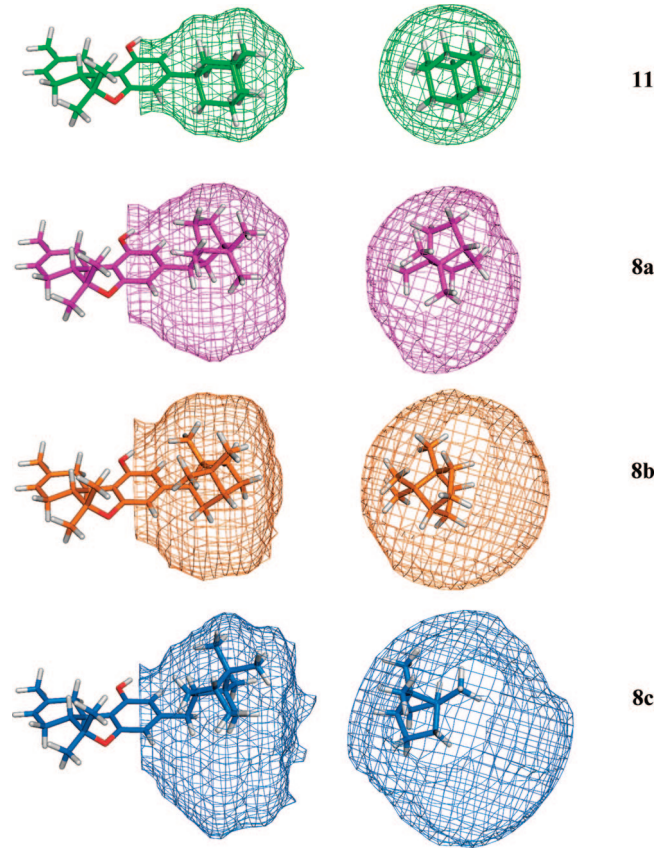


Figure 2. Comparison of the conformational space for adamantyl and bornyl side chains of various Δ^8-THC analogues. Accessible conformers within 8 kcal mol^{-1} of the global energy minimum for 3-(adamant-1′-yl)-Δ^8-THC (**11**, green), 3-bornyl-Δ^8-THC (**8a**, magenta), 3-isobornyl-Δ^8-THC (**8b**, orange), and 3-bornylmethyl-Δ^8-THC (**8c**, blue). The accessible conformational space for 3-isobornylmethyl-Δ^8-THC (**8d**) (not shown) is similar to that of **8c**. The global minimum energy conformer for each ligand is shown in stick display.

This provides us with valuable stereochemical information that can be used for the design of novel cannabinergic ligands with enhanced affinities and CB1/CB2 selectivities.

Summary

The results from our earlier work and this study revealed that rigid bulky groups at C3 in the direct proximity of tricyclic classical cannabinoid structures can be accommodated within the CB1 and CB2 binding sites. Substituting bulky aliphatic substituents for the *n*-pentyl chain at the 3-position of the classical phytocannabinoid Δ^8-THC (**1**) gave analogues with higher affinities for each of the cannabinoid receptors. The affinities and selectivities of these novel ligands for the receptor subtypes may be explained by the conformational space occupied by the bulky C3 substituents with respect to the tricyclic cannabinoid structure. The novel isobornyl analogue **8b** was found to have 10-fold CB2 selectivity.

Materials and Experimental Procedures

General Synthetic Methods. (+)-*trans*-*p*-Mentha-2,8-dien-1-ol (**7**) was purchased from Firmenich Inc., Princeton, NJ. All other reagents and solvents were purchased from Sigma-Aldrich, Milwaukee, WI, and used without further purification. All reactions were performed with magnetic stirring under a static argon or nitrogen atmosphere in flame-dried glassware using scrupulously dry solvents. Organic phases were dried over Na_2SO_4, rotary evaporated under reduced pressure, and flash column chromatography employed silica gel 60 (230−400

mesh, Selecto Scientific Inc., Suwanee, GA). Semipreparative chiral HPLC used a Chiralpak AD column (250 mm × 10 mm, Chiral Technologies Inc., Exton, PA). All compounds were demonstrated to be homogeneous by analytical thin-layer chromatography on precoated silica gel TLC aluminum plates (Whatman, UV_{254}, layer thickness 250 μm), and chromatograms were visualized under ultraviolet light or by phosphomolybdic acid staining. Melting points were determined on an Electrothermal capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on Bruker DMX-500 spectrometer operating at 500 MHz. All NMR spectra were recorded using $CDCl_3$ as solvent, and chemical shifts are reported in ppm relative to tetramethylsilane as an internal standard with multiplicities indicated as b (broadened), s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet). Specific rotations were measured on a Rudolph Autopol II polarimeter in a 1.00 dm cell. Low and high resolution mass spectra were performed at the School of Chemical Sciences, University of Illinois at Urbana—Champaign or were recorded on a Hewlett-Packard 6890 GC/MS instrument at the Center for Drug Discovery. Elemental analyses were performed at Baron Consulting Co., Milford, CT.

General Procedure A: Preparation of 5-Alkylresorcinols (6a−6d) from 5-Alkyl-1,3-dimethoxybenzenes (5a−5d). A solution of boron tribromide (2.1 mL of 1.0 M) in CH_2Cl_2 was added dropwise to a stirred solution of 2.0 mmol of 5-alkyl-1,3-dimethoxybenzenes (**5a−5d**) in 20 mL of CH_2Cl_2 at 0 °C. The reaction mixture was then stirred at 0 °C for 2 h and allowed to warm to room temperature over a period of time ranging between 6 and 16 h. Upon completion, the reaction mixture was cooled in an ice bath and cold water was added cautiously. The organic layer was separated and washed with H_2O, brine, and dried. Filtration, solvent removal, and purification by flash column chromatography (1:2 acetone/petroleum ether) provided 5-alkylresorcinols (**6a−6d**).

General Procedure B: Synthesis of (−)-Δ⁸-Tetrahydrocannabinol Analogues (8a−8d). A mixture of 1.0 mmol of 5-alkylresorcinols (**6a−6d**), 1.1 mmol of (+)-*trans*-*p*-mentha-2,8-dien-1-ol (**7**), and 0.1 mmol of *p*-toluenesulfonic acid monohydrate in 15 mL of anhydrous $CHCl_3$ was stirred and heated at 65 °C for 6 h. The reaction was monitored by TLC (15:85 EtOAc/petroleum ether). Upon completion, the reaction mixture was cooled and diluted with 10 mL of CH_2Cl_2 and stirred with 10 mL of saturated aqueous $NaHCO_3$ solution for 15 min. The organic phase was then separated and washed with H_2O, brine, and then dried. Filtration, concentration, and purification by flash column chromatography (1:10 EtOAc/petroleum ether) provided the Δ⁸-tetrahydrocannabinol analogues **8a−8d**.

2′-(3,5-Dimethoxyphenyl)-1′,7′,7′-trimethylbicyclo[2.2.1]heptan-2′-ols (4a and 4b). See ref 29.

2′-(3,5-Dimethoxyphenyl)-1′,7′,7′-trimethylbicyclo[2.2.1]heptanes (5a and 5b). See ref 29.

5-(1′,7′,7′-Trimethylbicyclo[2.2.1]hept-2′-yl)resorcinols (6a and 6b). A 3:1 mixture of **6a** and **6b** (170 mg, 0.69 mmol, 79%) was prepared from 240 mg (0.87 mmol) of the mixture of **5a** and **5b** following general procedure A to give a white solid. The product was determined to be a 3:1 mixture of *endo*- to *exo*-adducts by NMR: mp 52−55 °C. ^{1}H NMR δ **6a** (*endo*-adduct, major isomer) 6.38 (d, J = 2.0 Hz, 2H), 6.22 (t, J = 2.0 Hz, 1H), 4.95 (bs, 2H), 2.92 (ddd, J = 9.0, 5.0, 2.0 Hz, 1H, benzylic 2exo), 2.08−2.13 (m, 1H), 1.73−1.78 (m, 1H), 1.71 (t, J = 4.0 Hz, 1H), 1.36−1.43 [m, 2H, especially 1.41 (dd, J = 13.0, 5.5 Hz, 1H)], 1.24−1.30 (m, 1H), 1.11−1.17 (m, 1H), 0.99 (s, 3H), 0.91 (s, 3H), 0.74 (s, 3H); **6b** (*exo*-adduct, minor isomer) 6.35 (d, J = 2.0 Hz, 2H), 6.16 (t, J = 2.0 Hz, 1H), 4.91 (bs, 2H), 2.77 (dd, J = 8.5, 8.5 Hz, 1H, benzylic 2endo), 2.13−2.17 (m, 1H), 1.77−1.83 (m, 2H), 1.56−1.64 (m, 2H), 1.26−1.30 (m, 2H), 0.82 (s, 3H), 0.80 (s, 3H), 0.78 (s, 3H). MS m/z 246 (M^+).

(6a*R*-*trans*)-3-(*endo*-1′,7′,7′-Trimethylbicyclo[2.2.1]hept-2′-yl)-6a,7,10,10a-tetrahydro-6,6,9-trimethyl-6*H*-dibenzo[*b*,*d*]pyran-1-ol (3-Bornyl-Δ⁸-THC, 8a) and (6a*R*-*trans*)-3-(*exo*-1′,7′,7′-Trimethylbicyclo[2.2.1]hept-2′-yl)-6a,7,10,10a-tetrahydro-6,6,9-trimethyl-6*H*-dibenzo[*b*,*d*]pyran-1-ol (3-Isobornyl-Δ⁸-THC, 8b). A 3:1 mixture of **6a** and **6b** (160 mg, 0.42 mmol) was condensed with (+)-*trans*-*p*-2,8-menthadien-1-ol (**7**) following general procedure B to afford 225 mg of a crude 3:1 mixture of *endo*-adduct **8a** to *exo*-adduct **8b**. The crude product was chromatographed (10:90 Et_2O/petroleum ether) to give 95 mg (0.25 mmol, 60%) of **8a** as a white solid followed by 32 mg (0.084 mmol, 20%) of **8b** as a white solid. **8a** (*endo*-adduct, major isomer); mp 84−85 °C; ^{1}H NMR δ 6.30 (d, $J_{2,4}$ = 1.6 Hz, 1H, 4), 6.14 (d, $J_{2,4}$ = 1.6 Hz, 1H, 2), 5.42−5.45 (m, 1H, 8), 4.70 (s, 1H, OH), 3.19 (dd, $J_{10\alpha,10\beta}$ = 17.0 Hz, $J_{10\alpha,10a}$ = 4.3 Hz, 1H, 10α), 2.89 (ddd, $J_{2'exo,3'exo}$ = 11.6 Hz, $J_{2'exo,3'endo}$ = 5.4 Hz, $^4J_{2'exo,6'exo}$ = 2.4 Hz, 1H, benzylic 2′exo), 2.71 (ddd, $J_{10a,10\beta}$ = 11.1 Hz, $J_{10a,6a}$ = 11.1 Hz, $J_{10a,10\alpha}$ = 4.3 Hz, 1H, 10a), 2.12−2.16 (m, 1H, 7β), 2.09 (dddd, $J_{2'exo,3'exo}$ = 11.6 Hz, $J_{3'exo,3'endo}$ = 13.3 Hz, $J_{3'exo,4'}$ = 4.5 Hz, $^4J_{3'exo,5'exo}$ = 3.3 Hz, 1H, 3′exo), 1.85−1.92 (m, 1H, 10β), 1.79−1.86 (m, 2H, 6a,7α), 1.74−1.81 (m, 1H, 5′exo), 1.71 (s, 3H, 9-CH_3), 1.70 (dd, $J_{3'exo,4'}$ = 4.5 Hz, $J_{4',5'exo}$ = 4.5 Hz, 1H, 4′), 1.41−1.47 (m, 2H, 3′endo, 6′endo), 1.38 (s, 3H, 6-β-CH_3), 1.30 (ddd, $J_{5'endo,5'exo}$ = 12.4 Hz, $J_{5'endo,6'endo}$ = 9.5 Hz, $J_{5'endo,6'exo}$ = 4.6 Hz, 1H, 5′endo), 1.13 (dddd, $J_{6'exo,6'endo}$ = 12 Hz, $J_{6'exo,5'exo}$ = 11 Hz, $J_{6'exo,5'endo}$ = 4.6 Hz, $^4J_{2'exo,6'exo}$ = 2.4 Hz, 1H, 6′exo), 1.11 (s, 3H, 6-α-CH_3), 0.98 (s, 3H, *syn*-7′-CH_3), 0.90 (s, 3H, *anti*-7′-CH_3), 0.75 (s, 3H, 1′-CH_3); $[\alpha]_D^{22}$ −220° (c 0.223, CH_2Cl_2); MS m/z 380 (M^+); Anal. ($C_{26}H_{36}O_2 \cdot 1/2H_2O$) C, H. **8b** (*exo*-adduct, minor isomer); mp 86−88 °C; ^{1}H NMR δ 6.34 (d, $J_{2,4}$ = 1.3 Hz, 1H, 4), 6.15 (d, $J_{2,4}$ = 1.3 Hz, 1H, 2), 5.41−5.44 (m, 1H, 8), 4.63 (s, 1H, OH), 3.17 (dd, $J_{10\alpha,10\beta}$ = 17.0 Hz, $J_{10\alpha,10a}$ = 4.2 Hz, 1H, 10α), 2.72 (dd, $J_{2'endo,3'endo}$ = 8.5 Hz, $J_{2'endo,3'exo}$ = 8.5 Hz, 1H, benzylic 2′endo), 2.68 (ddd, $J_{10a,10\beta}$ = 11.1 Hz, $J_{10a,6a}$ = 11.1 Hz, $J_{10a,10\alpha}$ = 4.4 Hz, 1H, 10a), 2.07−2.23 (m, 2H, 3′exo, 7β), 1.84−1.92 (m, 1H, 10β), 1.74−1.85 (m, 4H, 6a, 7α, 4′, 5′exo), 1.70 (s, 3H, 9-CH_3), 1.53−1.64 (m, 2H, 3′endo, 6′exo), 1.37 (s, 3H, 6-β-CH_3), 1.22−1.30 (m, 2H, 5′endo, 6′endo), 1.09 (s, 3H, 6-α-CH_3), 0.82 (s, 6H, *syn*-7′-CH_3, *anti*-7′-CH_3), 0.79 (s, 3H, 1′-CH_3); $[\alpha]_D^{24}$ −200° (c 0.223, CH_2Cl_2); MS m/z 380 (M^+); Anal. ($C_{26}H_{36}O_2 \cdot {}^1/_2H_2O$) C, H.

2′-(3,5-Dimethoxybenzyl)-1′,7′,7′-trimethylbicyclo[2.2.1]heptan-2′-ols (4c and 4d). A solution of 3,5-dimethoxybenzyl chloride (**9**) (1.02 g, 5.46 mmol) in anhydrous Et_2O (25 mL) was added dropwise to a flask containing magnesium (132 mg, 5.43 mmol) over a period of 1.5 h. The reaction mixture was heated with a 50 °C oil bath for 1 h, and then a solution of (+)-(1*R*)-camphor (**3**) (760 mg, 4.99 mmol) in 25 mL of Et_2O was added dropwise. The reaction mixture was refluxed for 2 h and was then cooled to room temperature and treated cautiously with saturated aqueous NH_4Cl (12 mL). The Et_2O phase was separated and washed with water, brine, and then dried. Removal of solvent gave 1.80 g of crude product, which was chromatographed (20:80 acetone/petroleum ether) to afford 1.25 g (4.11 mmol, 82%) of a 9.3:1 mixture of *endo*-adduct **4c** to *exo*-adduct **4d** as a viscous liquid. ^{1}H NMR δ **4c** (*endo*-adduct, major isomer) 6.43 (d, J = 2.1 Hz, 2H), 6.36 (t, J = 2.1 Hz, 1H), 3.78 (s, 6H), 2.77 (d, J = 13.0 Hz, 1H), 2.73 (d, J = 13.0 Hz, 1H), 2.30 (bs, 1H), 1.82−1.86 (m, 1H), 1.74−1.78 (m, 2H), 1.65 (d, J = 13.0 Hz, 1H), 1.57 (d, J = 13.0 Hz, 1H), 1.45−1.49 (m, 1H), 1.11−1.15 (m, 1H), 1.08 (s, 3H), 0.87 (s, 3H), 0.83 (s, 3H); **4d** (*exo*-adduct, minor isomer, partial data) 6.33 (d, J = 1.6 Hz, 2H), 6.29 (t, J = 1.6 Hz, 1H), 3.77 (s, 6H), 2.77 (d, J = 13.0 Hz, 1H), 2.73 (d, J = 13.0 Hz, 1H), 2.33−2.38 (m, 1H), 2.09 (t, 1H), 1.91−1.98 (m, 1H), 1.76−1.81 (m, 2H), 1.60−1.64 (m, 1H), 0.96 (s, 3H), 0.91 (s, 3H), 0.83 (s, 3H); MS m/z 304 (M^+).

2′-(3,5-Dimethoxybenzylidene)-1′,7′,7′-trimethylbicyclo[2.2.1]-heptanes (10). The mixture (880 mg, 2.89 mmol) of **4c** and **4d** and 100 mg of *p*-toluenesulfonic acid monohydrate in 15 mL of anhydrous CH_2Cl_2 was stirred and heated at 45 °C for 1 h. The reaction mixture was cooled and washed with saturated aqueous $NaHCO_3$ solution, water, brine, and then dried. Removal of solvent gave 820 mg of crude product as a liquid, which was chromatographed (10:90 Et_2O/petroleum ether) to afford 790 mg (2.76 mmol, 96%) of **10** as a liquid that was a 1:8 mixture of *cis* and *trans* products by NMR. ^{1}H NMR δ major isomer 6.53 (d, J = 1.9 Hz, 2H), 6.15 (d, J = 1.9 Hz, 1H), 6.01 (bs, 1H), 3.79 (s, 6H), 2.71 (bd, J = 16.4 Hz, 1H), 2.24 (dd, J = 16.4, 1.4 Hz, 1H), 1.87 (t, J = 4.2 Hz, 1H), 1.79−1.84 (m, 1H), 1.70 (dt, J = 12.0, 3.9 Hz, 1H), 1.30−1.36 (m, 1H), 1.19−1.24 (m, 1H), 1.03 (s, 3H), 0.93

(s, 3H), 0.76 (s, 3H); minor isomer 6.33 (d, J = 1.6 Hz, 2H), 6.28 (d, J = 1.6 Hz, 1H), 6.01 (bs, 1H), 3.77 (s, 6H), 2.31−2.38 (m, 1H), 2.08 (t, J = 4.5 Hz, 1H), 1.92−1.97 (m, 1H), 1.79−1.83 (m, 1H), 1.65−1.70 (m, 1H), 1.35−1.41 (m, 1H), 1.19−1.27 (m, 1H), 0.95 (s, 3H), 0.91 (s, 3H), 0.83 (s, 3H); MS m/z 286 (M^+).

2′-(3,5-Dimethoxybenzyl)-1′,7′,7′-trimethylbicyclo[2.2.1]heptanes (5c and 5d). The cis/trans mixture of **10** (370 mg, 1.29 mmol) and 25 mg of 10% Pd/C in 15 mL of anhydrous ethanol was hydrogenated at atmospheric pressure with stirring. Upon completion of the reaction, the mixture was filtered and concentrated. The crude product was chromatographed (10:90 acetone/petroleum ether) to afford 342 mg (1.19 mmol, 92%) of a mixture of **5c** and **5d**. The product was determined to be a 1:3.4 mixture of *endo*- to *exo*-adducts by NMR. ^{1}H NMR δ **5c** (*endo*-adduct, minor isomer, partial data) 6.35 (d, J = 1.9 Hz, 2H), 6.33 (t, J = 1.9 Hz, 1H), 3.77 (s, 6H), 2.67 (dd, J = 13.8, 3.0 Hz, 1H), 2.34−2.38 (m, 1H), 1.85−1.97 (m, 2H), 1.65−1.75 (m, 2H), 1.51−1.55 (m, 1H), 1.31−1.37 (m, 1H), 1.05−1.15 (m, 1H), 0.86 (s, 3H), 0.84 (s, 3H), 0.82 (s, 3H); **5d** (*exo*-adduct, major isomer) 6.31 (d, J = 1.8 Hz, 2H), 6.29 (t, J = 1.8 Hz, 1H), 3.78 (s, 6H), 2.82 (dd, J = 13.0, 4.6 Hz, 1H), 2.34 (dd, J = 13.0, 12.2 Hz, 1H), 1.74−1.78 (m, 1H), 1.67−1.72 (m, 1H), 1.65 (t, J = 3.7 Hz, 1H), 1.51−1.58 (m, 2H), 1.27 (dd, J = 12.5, 9.3 Hz, 1H), 1.09−1.16 (m, 2H), 0.97 (s, 3H), 0.90 (s, 3H), 0.84 (s, 3H); MS m/z 288 (M^+).

5-(1′,7′,7′-Trimethylbicyclo[2.2.1]hept-2′-ylmethyl)resorcinols (6c and 6d). A 1:3.4 mixture by NMR of **6c** and **6d** (218 mg, 0.837 mmol, 88%) as a white solid was prepared from 274 mg (0.950 mmol) of the mixture of **5c** and **5d** following general procedure A. ^{1}H NMR δ **6c** (*endo*-adduct, minor isomer, partial data) 6.26 (d, J = 1.9 Hz, 2H), 6.19 (t, J = 1.9 Hz, 1H), 5.62 (bs, 2H), 2.61 (dd, J = 12.8, 2.9 Hz, 1H), 2.28−2.32 (m, 1H), 1.83−1.93 (m, 2H), 1.68−1.74 (m, 1H), 1.49−1.57 (m, 2H), 1.30−1.36 (m, 1H), 0.94−0.97 (m, 1H), 0.86 (s, 3H), 0.83 (s, 3H), 0.80 (s, 3H); **6d** (*exo*-adduct, major isomer) 6.23 (d, J = 1.7 Hz, 2H), 6.19 (t, J = 1.7 Hz, 1H), 5.62 (bs, 2H), 2.75 (dd, J = 13.3, 4.6 Hz, 1H), 2.28 (dd, J = 13.3, 12.7 Hz, 1H), 1.66−1.76 (m, 2H), 1.64 (t, J = 4.0 Hz, 1H), 1.49−1.58 (m, 2H), 1.26 (dd, J = 9.3, 9.2 Hz, 1H), 1.07−1.16 (m, 2H), 0.95 (s, 3H), 0.89 (s, 3H), 0.83 (s, 3H); MS m/z 260 (M^+).

(6a*R*-*trans*)-3-(*endo*-1′,7′,7′-Trimethylbicyclo[2.2.1]hept-2′-ylmethyl)-6a,7,10,10a-tetrahydro-6,6,9-trimethyl-6*H*-dibenzo[*b*,*d*]pyran-1-ol (3-Bornylmethyl-Δ^8-THC, 8c) and (6a*R*-*trans*)-3-(*exo*-1′,7′,7′-Trimethylbicyclo[2.2.1]hept-2′-ylmethyl)-6a,7,10,10a-tetrahydro-6,6,9-trimethyl-6*H*-dibenzo[*b*,*d*]pyran-1-ol (3-Isobornylmethyl-Δ^8-THC, 8d). The 1:3.4 mixture of **6c** and **6d** (180 mg, 0.691 mmol) was condensed with (+)-*trans*-*p*-2,8-menthadien-1-ol (**7**) following general procedure B to afford 185 mg (0.469 mmol, 68%) of a 1:3.4 mixture by NMR of *endo*-adduct **8c** to *exo*-adduct **8d**. The product mixture was then separated by semipreparative chiral HPLC (Chiralpak AD, 250 mm × 10 mm, 5:95 2-propanol/hexane, 2 mL/min) that afforded the major isomer **8d** as a white solid, with a retention time of 18.28 min, and the minor isomer **8c** as a white solid, with a retention time of 19.75 min. **8c** (*endo*-adduct, minor product) mp 76−77 °C; ^{1}H NMR δ 6.28 (d, $J_{2,4}$ = 1.9 Hz, 1H, 4), 6.10 (d, $J_{2,4}$ = 1.9 Hz, 1H, 2), 5.41−5.44 (m, 1H, 8), 4.64 (bs, 1H, OH), 3.18 (dd, $J_{10\alpha,10\beta}$ = 16.5 Hz, $J_{10\alpha,10a}$ = 4.4 Hz, 1H, 10α), 2.69 (ddd, $J_{10a,10\beta}$ = 10.9 Hz, $J_{10a,6a}$ = 10.9 Hz, $J_{10a,10\alpha}$ = 4.4 Hz, 1H, 10a), 2.56 (dd, J_{gem} = 13.4 Hz, J_{vic} = 1.6 Hz, 1H, benzylic 1″-H_a), 2.26 (dd, J_{gem} = 13.4 Hz, J_{vic} = 10.1 Hz, 1H, benzylic 1″-H_b), 2.10−2.18 (m, 1H, 7β), 1.75−1.95 (m, 5H, 2′exo,3′exo,6a,7α,10β), 1.65−1.75 (m, 1H, 5′exo), 1.70 (s, 3H, 9-CH_3), 1.54−1.63 (m, 2H, 4′,6′endo), 1.37 (s, 3H, 6-β-CH_3), 1.32 (dddd, $J_{6'exo,6'endo}$ = 12 Hz, $J_{6'exo,5'exo}$ = 12 Hz, $J_{6'exo,5'endo}$ = 4.9 Hz, $^4J_{2'exo,6'exo}$ = 1.2 Hz, 1H, 6′exo), 1.11 (ddd, $J_{5'endo,5'exo}$ = 12 Hz, $J_{5'endo,6'endo}$ = 10 Hz, $J_{5'endo,6'exo}$ = 4.9 Hz, 1H, 5′endo), 1.10 (s, 3H, 6-α-CH_3), 0.82−0.89 (m, 1H, 3′endo), 0.86 (s, 3H, *anti*-7′-CH_3), 0.84 (s, 3H, *syn*-7′-CH_3), 0.80 (s, 3H, 1′-CH_3); $[\alpha]_D^{21}$ −180° (c 0.223, CH_2Cl_2); MS m/z 394 (M^+); HRMS exact mass calculated for $C_{27}H_{38}O_2$, 394.2872, found, 394.2868; Anal. ($C_{27}H_{38}O_2 \cdot {}^1/_4H_2O$), C, H. **8d** (*exo*-adduct, major isomer) mp 78−80 °C; ^{1}H NMR δ 6.25 (d, $J_{2,4}$ = 1.7 Hz, 1H, 4), 6.08 (d, $J_{2,4}$ = 1.7 Hz, 1H, 2), 5.43 (bd, $J_{7\alpha,8}$ = 4 Hz, 1H, 8), 4.65 (bs, 1H, OH), 3.17 (dd, $J_{10\alpha,10\beta}$ = 16.4 Hz, $J_{10\alpha,10a}$ = 4.5 Hz, 1H, 10α), 2.73 (dd, J_{gem} = 13.6 Hz, J_{vic} 4.3 Hz, 1H, benzylic 1″-H_a), 2.68 (ddd, $J_{10a,10\beta}$ = 10.9 Hz, $J_{10a,6a}$ = 10.9 Hz, $J_{10a,10\alpha}$ = 4.5 Hz, 1H, 10a), 2.22 (dd, J_{gem} = 13.6 Hz, J_{vic} 11.8 Hz, 1H, benzylic 1″-H_b), 2.14 (bdd, $J_{7\alpha,7\beta}$ = 12 Hz, $J_{7\alpha,8}$ = 4 Hz, 1H, 7β), 1.63−1.90 [m, 8H, 6a,7α,10β,2′endo,5′exo, especially 1.70 (s, 3H, 9-CH_3)], 1.63 (dd, $J_{4',3'exo}$ = 4 Hz, $J_{4',5'exo}$ = 4 Hz, 1H, 4′), 1.47−1.57 (m, 2H, 3′exo,6′exo), 1.37 (s, 3H, 6-β-CH_3), 1.30 (dd, $J_{3'endo,3'exo}$ = 12.6 Hz, $J_{3'endo,2'endo}$ = 9.3 Hz, 1H, 3′endo), 1.05−1.14 [m, 5H, 5′endo, 6′endo, especially 1.10 (s, 3H, 6-α-CH_3)], 0.94 (s, 3H, *syn*-7′-CH_3), 0.88 (s, 3H, 1′-CH_3), 0.83 (s, 3H, *anti*-7′-CH_3); $[\alpha]_D^{22}$ −290° (c 0.223, CH_2Cl_2); MS m/z 394 (M^+); HRMS exact mass calculated for $C_{27}H_{38}O_2$, 394.2872, found, 394.2870; Anal. ($C_{27}H_{38}O_2 \cdot {}^1/_2H_2O$), C, H.

Rat Brain CB1 Membrane Preparation. Rat forebrain membrane microsomes were prepared from frozen rat brains by the method of Dodd et al.[36] Fifteen frozen rat brains (Pel-Freez, no. 56004-2, Rogers, AR), stored at −80 °C, were placed in a plastic dish and allowed to partially thaw so that the cerebellum could be removed with a spatula and discarded. The remaining brain tissue was homogenized in 40 mL of ice-cold homogenization buffer (0.32 M sucrose, 10 mM Tris base, 5 mM EDTA, pH 7.4) in two installments. All tissues and homogenates were kept on ice to prevent tissue degradation. The homogenate was decanted into prechilled tubes for centrifugation at 4 °C and 3700g for 10 min. The supernatants were pooled, kept on ice, and the total volume brought to 125 mL with ice-cold homogenization buffer. The supernatant was aliquoted (12 mL) into 10 prechilled centrifuge tubes (24 mL). Using a syringe and needle, 10 mL of cold 1.2 M sucrose in TME buffer (25 mM Tris base, 5 mM $MgCl_2$, 1 mM EDTA, pH 7.4) was carefully layered at the bottom of each centrifuge tube, and the tubes were carefully balanced with cold homogenization buffer added to the top layer. These tubes were centrifuged in a 4 °C ultracentrifuge for 35 min at 245000g. The resulting layer at the interface was carefully collected. The total volume was brought to 105 mL with ice-cold homogenization buffer and aliquoted in eight centrifuge tubes (12 mL each). Using a syringe and needle again, 10 mL of cold 0.8 M sucrose in TME buffer was carefully layered at the bottom of each centrifuge tube, the tubes carefully balanced with cold homogenization buffer added to the top layer, followed by ultracentrifugation as described above. After discarding the resulting supernatant, the pellets were resuspended in ice-cold TME, pooled (total volume of 6 mL), and gently homogenized by hand. This membrane suspension was aliquoted into silanized Eppendorf tubes and flash frozen in liquid nitrogen, followed by storage at −80 °C until use within 2 months. One of the aliquoted samples was used for protein determination using a Bio-Rad (500−0006) Bradford protein assay kit.

Mouse Spleen CB2 Membrane Preparation. Membrane microsomes with CB2 receptors were prepared from whole frozen mouse spleens (Pel-Freez no. 55049-2) according to the procedure detailed above for rat brain.

Competitive Binding Assay. Rat brain membrane and mouse spleen membrane preparations were used to assess the affinities of the novel analogues for CB1 and CB2 binding, respectively. The displacement of specifically tritiated CP55,940 from these membrane preparations was used to determine the IC_{50} values for the Δ^8-THC (**1**) and analogues **8a**−**8d**. The [^{3}H]CP55,940 binding assay was conducted on 96-well microfilter plates as previously described.[28,38] Briefly, 100 μL of cannabinergic ligand (at eight different concentrations) in DMSO, 50 μL of rat brain or mouse spleen membrane preparation (40−50 μg protein), and 50 μL of [^{3}H]CP55,940 (3.08 nM) in TME (25 mM Tris, 5 mM $MgCl_2$, 1 mM EDTA) buffer containing 0.1% bovine serum albumin (BSA) was incubated for 1 h at 30 °C. For the nonspecific binding control, 100 μL of 200 nM CP55,940 was used and 100 μL of TME buffer containing 0.1% BSA was used for the total binding control. The competitive reaction was terminated by rapid filtration through a Packard Filtermate harvester and Whatman GF/B unifilter-96 plates, and an ice-cold TME wash buffer containing 0.5% BSA was used. Radioactivity was detected using MicroScint 20 scintillation cocktail added to the dried filter plates and was counted using a Packard Instruments Topcount microplate scintillation counter. The normalized data from three independent experiments were combined and analyzed using a four-parameter

logistic equation to yield IC_{50} values, which were converted to K_i values using the assumptions of Cheng and Prussoff.[39]

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Supporting Information Available: Elemental analysis results for compounds **8a**−**8d**. ^{1}H NMR, COSY, and NOESY (500 ms mixing time) spectra for compounds **8a**−**8d** in $CDCl_3$ solutions. This material is available free of charge via the Internet at http://pubs.acs.org.

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