Published in final edited form as:

J Med Chem. 2008 October 23; 51(20): 6393–6399. doi:10.1021/jm8005299.

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

Dai Lu, Jianxian Guo, Richard I. Duclos Jr., Anna L. Bowman, and Alexandros Makriyannis*

Center for Drug Discovery, Northeastern University, 360 Huntington Avenue, 116 Mugar Life Sciences Building, Boston, MA, 02115, USA

Abstract

Structure-activity relationship studies of classical cannabinoid analogs 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 analogs 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} CB1⁹ and CB2.¹⁰ Structure-activity relationship (SAR) studies of cannabinoid analogs 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. Although 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

*To whom correspondence should be addressed: Tel.: +1-617-373-4200; Fax: +1-617-373-7493; a.makriyannis@neu.edu.

Supporting Information Available: Elemental analysis results for compounds **8a–8d**. ¹H NMR, COSY, and NOESY (500 ms mixing time) spectra for compounds **8a–8d** in CDCl₃ solutions. These materials are available free of charge via the Internet at <http://pubs.acs.org>.

^aAbbreviations: 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 MgCl₂, 1 mM EDTA buffer; COSY, correlation spectroscopy; NOESY, nuclear Overhauser enhancement spectroscopy

elucidate the precise nature of the pharmacophoric conformation of THC analogs 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 cannabinoids²⁸ 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. 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 analogs 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 analogs **8a** and **8b**, respectively, were synthesized as shown in Scheme 1. The Grignard reagent prepared from 1-chloro-3,5-dimethoxybenzene (**2**) reacted with (+)-(*1R*)-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 analogs which were separated by flash column chromatography. The major product was bornyl- Δ^8 -THC **8a**, which exhibited the characteristic ‘W’ coupling between the 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 analogs **8c** and **8d**, respectively, were synthesized as shown in Scheme 2. The Grignard reagent prepared from 3,5-dimethoxybenzylchloride (**9**) reacted with (+)-(*1R*)-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 CH₂Cl₂ 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 BBr₃ 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 synaptosomes³⁶ as a source of CB1 and using mouse spleen membranes³⁷ 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 analogs 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 (IC₅₀) determined from the respective displacement curves were converted to *K*¹ values (Table 1) according to the reported method.³⁹ As can be seen from the *K*¹ values, structural modifications of the C3 substituent affects cannabinoid receptor affinities as well as CB1/CB2 selectivities. The bornyl- Δ^8 -THC analog **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 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 CB2⁴² 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 Δ^8 -THC (**1**). The *endo*-bornylmethyl analog **8c** and its *exo*-epimer **8d** are also nonselective.

Molecular Modeling

Among other considerations, the conformational space available to the novel analogs **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 analogs **8c** and **8d** to explore the permissible rotations of the bulky substituents. Rotation around the C1''-C2' bonds was also considered for 3-bornylmethyl- Δ^8 -THC (**8c**) and 3-isobornylmethyl- Δ^8 -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- Δ^8 -THC (**8a**) has the smallest available conformational space of analogs **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 analog 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- Δ^8 -THC analogs (**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 Δ^8 -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 analog **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 analog, 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 conformational space for the novel terpene analogs **8a** and **8b** suggests some interesting conclusions. We postulate that the bornyl analog **8a** (Figure 2) can be accommodated optimally in both receptors. However, the isobornyl group of analog **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 analogs.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. 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 analogs 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 analog **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 Na₂SO₄, rotary evaporated under reduced pressure, and flash column chromatography employed silica gel 60 (230–400 mesh, Selecto Scientific Inc., Suwanee, GA). Semi-preparative chiral HPLC used a Chiralpak AD column (250 × 10 mm, Chiral Technologies Inc., Exton, PA). All compounds were demonstrated to be homogeneous by analytical thin-layer chromatography on pre-coated silica gel TLC aluminum plates (Whatman, UV₂₅₄, 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. ¹H NMR spectra were recorded on Bruker DMX-500 spectrometer operating at 500 MHz. All NMR spectra were recorded using CDCl₃ 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 CH₂Cl₂ was added dropwise to a stirred solution of 2.0 mmol of 5-alkyl-1,3-dimethoxybenzenes (**5a-5d**) in 20 mL of CH₂Cl₂ 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 h to 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 H₂O, 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 Analogs (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 CHCl₃ 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 CH₂Cl₂ and stirred with 10 mL of saturated aqueous NaHCO₃ solution for 15 min. The organic phase was then separated and washed with H₂O, brine, and then dried. Filtration, concentration, and purification by flash column chromatography (1:10 EtOAc/petroleum ether) provided the Δ⁸-tetrahydrocannabinol analogs **8a-8d**.

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

2'-(3,5-Dimethoxyphenyl)-1',7',7'-trimethylbicyclo[2.2.1]heptanes (5a and 5b).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; ¹H

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 2 exo), 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 2 endo), 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- Δ^8 -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- Δ^8 -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 Et₂O/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; ¹H 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, $J_{10a,10\beta} = 17.0$ Hz, $J_{10a,10a} = 4.3$ Hz, 1H, 10 α), 2.89 (ddd, $J_{2'\text{exo},3'\text{exo}} = 11.6$ Hz, $J_{2'\text{exo},3'\text{endo}} = 5.4$ Hz, $J_{2'\text{exo},6'\text{exo}} = 2.4$ Hz, 1H, benzylic 2' exo), 2.71 (ddd, $J_{10a,10\beta} = 11.1$ Hz, $J_{10a,6a} = 11.1$ Hz, $J_{10a,10a} = 4.3$ Hz, 1H, 10 α), 2.12–2.16 (m, 1H, 7 β), 2.09 (dddd, $J_{2'\text{exo},3'\text{exo}} = 11.6$ Hz, $J_{3'\text{exo},3'\text{endo}} = 13.3$ Hz, $J_{3'\text{exo},4'} = 4.5$ Hz, $J_{3'\text{exo},5'\text{exo}} = 3.3$ Hz, 1H, 3' exo), 1.85–1.92 (m, 1H, 10 β), 1.79–1.86 (m, 2H, 6 α , 7 α), 1.74–1.81 (m, 1H, 5' exo), 1.71 (s, 3H, 9-CH₃), 1.70 (dd, $J_{3'\text{exo},4'} = 4.5$ Hz, $J_{4',5'\text{exo}} = 4.5$ Hz, 1H, 4'), 1.41–1.47 (m, 2H, 3' endo , 6' endo), 1.38 (s, 3H, 6- β -CH₃), 1.30 (ddd, $J_{5'\text{endo},5'\text{exo}} = 12.4$ Hz, $J_{5'\text{endo},6'\text{endo}} = 9.5$ Hz, $J_{5'\text{endo},6'\text{exo}} = 4.6$ Hz, 1H, 5' endo), 1.13 (dddd, $J_{6'\text{exo},6'\text{endo}} = 12$ Hz, $J_{6'\text{exo},5'\text{exo}} = 11$ Hz, $J_{6'\text{exo},5'\text{endo}} = 4.6$ Hz, $J_{2'\text{exo},6'\text{exo}} = 2.4$ Hz, 1H, 6' exo), 1.11 (s, 3H, 6- α -CH₃), 0.98 (s, 3H, *syn*-7'-CH₃), 0.90 (s, 3H, *anti*-7'-CH₃), 0.75 (s, 3H, 1'-CH₃); [α]_D²² -220° (c 0.223, CH₂Cl₂); MS m/z 380 (M^+); Anal. (C₂₆H₃₆O₂·½H₂O) C, H. **8b** (*exo*-adduct, minor isomer): mp 86–88 °C; ¹H 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, $J_{10a,10\beta} = 17.0$ Hz, $J_{10a,10a} = 4.2$ Hz, 1H, 10 α), 2.72 (dd, $J_{2'\text{endo},3'\text{endo}} = 8.5$ Hz, $J_{2'\text{endo},3'\text{exo}} = 8.5$ Hz, 1H, benzylic 2' endo), 2.68 (ddd, $J_{10a,10\beta} = 11.1$ Hz, $J_{10a,6a} = 11.1$ Hz, $J_{10a,10a} = 4.4$ Hz, 1H, 10 α), 2.07–2.23 (m, 2H, 3' exo , 7 β), 1.84–1.92 (m, 1H, 10 β), 1.74–1.85 (m, 4H, 6 α , 7 α , 4', 5' exo), 1.70 (s, 3H, 9-CH₃), 1.53–1.64 (m, 2H, 3' endo , 6' exo), 1.37 (s, 3H, 6- β -CH₃), 1.22–1.30 (m, 2H, 5' endo , 6' endo), 1.09 (s, 3H, 6- α -CH₃), 0.82 (s, 6H, *syn*-7'-CH₃, *anti*-7'-CH₃), 0.79 (s, 3H, 1'-CH₃); [α]_D²⁴ -200° (c 0.223, CH₂Cl₂); MS m/z 380 (M^+); Anal. (C₂₆H₃₆O₂·½H₂O) 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 Et₂O (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 Et₂O was added dropwise. The reaction mixture was refluxed for 2 h, and was then cooled to room temperature and treated cautiously with saturated aqueous NH₄Cl (12 mL). The Et₂O 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: ¹H 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), 2.38–2.33 (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: 1H 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: 1H 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: 1H 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^+).

(6*aR-trans*)-3-(*endo*-1',7',7'-Trimethylbicyclo[2.2.1]hept-2'-ylmethyl)-6*a*,7,10,10*a*-tetrahydro-6,6,9-trimethyl-6*H*-dibenzo[*b,d*]pyran-1-ol (3-Bornylmethyl- Δ_8 -THC, **8c**) and (6*aR-trans*)-3-(*exo*-1',7',7'-Trimethylbicyclo[2.2.1]hept-2'-ylmethyl)-6*a*,7,10,10*a*-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 × 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; ¹H 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*_{10α,10β} = 16.5 Hz, *J*_{10α,10a} = 4.4 Hz, 1H, 10α), 2.69 (ddd, *J*_{10α,10β} = 10.9 Hz, *J*_{10α,6a} = 10.9 Hz, *J*_{10α,10a} = 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-CH₃), 1.54–1.63 (m, 2H, 4', 6' *endo*), 1.37 (s, 3H, 6-β-CH₃), 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, *J*_{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-CH₃), 0.82–0.89 (m, 1H, 3' *endo*), 0.86 (s, 3H, *anti*-7'-CH₃), 0.84 (s, 3H, *syn*-7'-CH₃), 0.80 (s, 3H, 1'-CH₃); [α]_D²¹ -180° (c 0.223, CH₂Cl₂); MS *m/z* 394 (M⁺); HRMS Exact mass calculated for C₂₇H₃₈O₂, 394.2872, found, 394.2868; Anal. (C₂₇H₃₈O₂·¼H₂O), C, H. **8d** (*exo*-adduct, major isomer): mp 78–80 °C; ¹H 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*_{α,8} = 4 Hz, 1H, 8), 4.65 (bs, 1H, OH), 3.17 (dd, *J*_{10α,10β} = 16.4 Hz, *J*_{10α,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*_{10α,10β} = 10.9 Hz, *J*_{10α,6a} = 10.9 Hz, *J*_{10α,10a} = 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*_{7α,7β} = 12 Hz, *J*_{α,8} = 4 Hz, 1H, 7β), 1.63–1.90 [m, 8H, 6a, 7α, 10β, 2' *endo*, 5' *exo*, especially 1.70 (s, 3H, 9-CH₃)], 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-CH₃), 1.30 (dd, *J*_{3' *endo*, 3' *exo*} = 12.6 Hz, *J*_{3' *endo*, 2' *endo*} = 9.3 Hz, 1H, 3' *endo*), 1.14–1.05 [m, 5H, 5' *endo*, 6' *endo*, especially 1.10 (s, 3H, 6-α-CH₃)], 0.94 (s, 3H, *syn*-7'-CH₃), 0.88 (s, 3H, 1'-CH₃), 0.83 (s, 3H, *anti*-7'-CH₃); [α]_D²² -290° (c 0.223, CH₂Cl₂); MS *m/z* 394 (M⁺); HRMS Exact mass calculated for C₂₇H₃₈O₂, 394.2872, found, 394.2870; Anal. (C₂₇H₃₈O₂·½H₂O), 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, #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 pre-chilled 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 ten pre-chilled 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 MgCl₂, 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 245,000g. 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 ultra-centrifugation as described above. After discarding the resulting supernatant, the pellets were re-suspended 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 #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 analogs 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 analogs **8a–8d**. The [^3H]CP55,940 binding assay was conducted on 96-well microfilter plates as previously described.^{28,38} Briefly, 100 μL of cannabinergic ligand (at 8 different concentrations) in DMSO, 50 μL of rat brain or mouse spleen membrane preparation (40–50 μg protein), and 50 μL of [^3H]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 hour at 30°C . For the non-specific 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 unfilter-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 Prusoff.³⁹

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by grants DA-7215, DA-3801, DA-152, DA-9158, and DA24842 from the National Institute on Drug Abuse. We thank Pusheng Fan and JodiAnne Wood for the receptor binding assays.

References

1. Pertwee RG. Cannabinoid pharmacology: The first 66 years. *Br J Pharmacol*. 2006; 147:S163–S171. [PubMed: 16402100]
2. Di Marzo V, Petroselli LD. Plant, synthetic, and endogenous cannabinoids in medicine. *Ann Rev Med*. 2006; 57:553–574. [PubMed: 16409166]
3. Howlett AC. Cannabinoid inhibition of adenylate cyclase: Relative activity of constituents and metabolites of marihuana. *Neuropharmacology*. 1987; 26:507–512. [PubMed: 3601007]
4. Herkenham M, Lynn AB, Little MD, Johnson MR, Melvin LS, de Costa BR, Rice KC. Cannabinoid receptor localization in brain. *Proc Natl Acad Sci US A*. 1990; 87:1932–1936.
5. Compton DR, Prescott WR Jr, Martin BR, Siegel C, Gordon PM, Razdan RK. Synthesis and pharmacological evaluation of ether and related analogues of Δ^8 - Δ^9 - and Δ^9 , 11 -tetrahydrocannabinol. *J Med Chem*. 1991; 34:3310–3316. [PubMed: 1659638]

6. Martin BR, Jefferson R, Winckler R, Wiley JL, Huffman JW, Crocker PJ, Saha B, Razdan RK. Manipulation of the tetrahydrocannabinol side chain delineates agonists, partial agonists, and antagonists. *J Pharmacol Exp Ther.* 1999; 290:1065–1079. [PubMed: 10454479]
7. Howlett AC, Barth F, Bonner TI, Cabral G, Casellas P, Devane WA, Felder CC, Herkenham M, Mackie K, Martin BR, Mechoulam R, Pertwee RG. International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacol Rev.* 2002; 54:161–202. [PubMed: 12037135]
8. Lambert DM, Fowler CJ. The endocannabinoid system: Drug targets, lead compounds, and potential therapeutic applications. *J Med Chem.* 2005; 48:5059–5087. [PubMed: 16078824]
9. Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature.* 1990; 346:561–564. [PubMed: 2165569]
10. Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. *Nature.* 1993; 365:61–65. [PubMed: 7689702]
11. Razdan RK. Structure-activity relationships in cannabinoids. *Pharmacol Rev.* 1986; 38:75–149. [PubMed: 3018800]
12. Reggio PH. Pharmacophores for ligand recognition and activation/inactivation of the cannabinoid receptors. *Curr Pharmaceut Design.* 2003; 9:1607–1633.
13. Thakur GA, Nikas SP, Makriyannis A. CB1 cannabinoid receptor ligands. *Mini-Rev Med Chem.* 2005; 5:631–640. [PubMed: 16026309]
14. Huffman JW. CB₂ receptor ligands. *Mini-Rev Med Chem.* 2005; 5:641–649. [PubMed: 16026310]
15. Raitio KH, Salo OMH, Nevalainen T, Poso A, Järvinen T. Targeting the cannabinoid CB2 receptor: Mutations, modeling and development of CB2 selective ligands. *Curr Med Chem.* 2005; 12:1217–1237. [PubMed: 15892633]
16. Thakur, GA.; Nikas, SP.; Li, C.; Makriyannis, A. Structural requirements for cannabinoid receptor probes. In: Pertwee, RG., editor. *Handbook of Experimental Pharmacology.* Springer-Verlag; New York: 2005. p. 209-246.
17. Pavlopoulos S, Thakur GA, Nikas SP, Makriyannis A. Cannabinoid receptors as therapeutic targets. *Curr Pharmaceut Design.* 2006; 12:1751–1769.
18. Thakur, GA.; Nikas, SP.; Duclos, RL, Jr; Makriyannis, A. *Methods in Molecular Medicine.* Humana Press Inc; Totowa, NJ: 2006. Methods for the synthesis of cannabinergic ligands; p. 113-148.
19. Huffman JW, Yu S. Synthesis of a tetracyclic, conformationally constrained analogue of Δ^8 -THC. *Bioorg Med Chem.* 1998; 6:2281–2288. [PubMed: 9925290]
20. Makriyannis A, Rapaka RS. The molecular basis of cannabinoid activity. *Life Sci.* 1990; 47:2173–2184. [PubMed: 2266785]
21. Khanolkar AD, Palmer SL, Makriyannis A. Molecular probes for the cannabinoid receptors. *Chem Phys Lipids.* 2000; 108:37–52. [PubMed: 11106781]
22. Thakur GA, Duclos RI Jr, Makriyannis A. Natural cannabinoids: Templates for drug discovery. *Life Sci.* 2005; 78:454–466. [PubMed: 16242157]
23. Papahatjis DP, Kourouli T, Makriyannis A. Pharmacophoric requirements for cannabinoid side chains. Naphthoyl and naphthylmethyl substituted Δ^8 -tetrahydrocannabinol analogs. *J Heterocycl Chem.* 1996; 33:559–562.
24. Krishnamurthy M, Ferreira AM, Moore BM 2nd. Synthesis and testing of novel phenyl substituted side-chain analogues of classical cannabinoids. *Bioorg Med Chem Lett.* 2003; 13:3487–3490. [PubMed: 14505654]
25. Nadipuram AK, Krishnamurthy M, Ferreira AM, Li W, Moore BM II. Synthesis and testing of novel classical cannabinoids: exploring the side chain ligand binding pocket of the CB1 and CB2 receptors. *Bioorg Med Chem.* 2003; 11:3121–3132. [PubMed: 12818675]
26. Papahatjis DP, Nahmias VR, Nikas SP, Andreou T, Alapafuja SO, Tsotinis A, Guo J, Fan P, Makriyannis A. C1'-Cycloalkyl side chain pharmacophore in tetrahydrocannabinols. *J Med Chem.* 2007; 50:4048–4060. [PubMed: 17672444]
27. Khanolkar AD, Lu D, Fan P, Tian X, Makriyannis A. Novel conformationally restricted tetracyclic analogs of Δ^8 -tetrahydrocannabinol. *Bioorg Med Chem Lett.* 1999; 9:2119–2124. [PubMed: 10465529]

28. Lu D, Meng Z, Thakur GA, Fan P, Steed J, Tartal CL, Hurst DP, Reggio PH, Deschamps JR, Parrish DA, George C, Järbe TUC, Lamb RJ, Makriyannis A. Adamantyl cannabinoids: a novel class of cannabinergic ligands. *J Med Chem.* 2005; 48:4576–4585. [PubMed: 15999995]
29. Duclos RI Jr, Lu D, Guo J, Makriyannis A. Synthesis and characterization of 2-substituted bornane pharmacophores for novel cannabinergic ligands. *Tetrahedron Lett.* 2008; 49:5587–5589.
30. Franke I, Binder M. Synthesis of cannabinoid model compounds. Part 2. (3*R*,4*R*)- $\Delta^1(6)$ -Tetrahydrocannabinol-5''-oic acid and 4''(*R,S*)-methyl-(3*R*,4*R*)- $\Delta^1(6)$ -tetrahydrocannabinol-5''-oic acid. *Helv Chim Acta.* 1980; 63:2508–2514.
31. Petrzilka T, Haeflinger W, Sikemeier C. Synthesis von haschisch-inhaltsstoffen. *Helv Chim Acta.* 1969; 52:1102–1134.
32. Lu D, Meng Z, Thakur GA, Fan P, Steed J, Tartal CL, Hurst DP, Reggio PH, Deschamps JR, Parrish DA, George C, Jarbe TUC, Lamb RJ, Makriyannis A. Adamantyl cannabinoids: a novel class of cannabinergic ligands. *J Med Chem.* 2005; 48:4576–4585. [PubMed: 15999995]
33. Brown HC, Kawakami JH. Additions to bicyclic olefins. I. Stereochemistry of the hydroboration of norbornene, 7,7-dimethylnorbornene, and related bicyclic olefins. Steric effects in the 7,7-dimethylnorbornyl system. *J Am Chem Soc.* 1970; 92:1990–1995.
34. Schneider HJ, Buchheit U, Hoppen V, Schmidt G. ^{13}C - and ^1H -NMR shielding effects in aliphatic *gauche/trans* fragments. *Chem Ber.* 1989; 122:321–326.
35. Huisgen R, Mloston G, Pröbstl A. Thiofenchone *S*-methylide and its spiro-1,3,4-thiadiazoline precursor. *Heteroat Chem.* 2001; 12:136–145.
36. Dodd PR, Hardy JA, Oakley AE, Edwardson JA, Perry EK, Delaunoy JP. A rapid method for preparing synaptosomes: Comparison, with alternative procedures. *Brain Res.* 1981; 226:107–118. [PubMed: 7296283]
37. Khanolkar AD, Abadji V, Lin S, Hill WAG, Taha G, Abouzid K, Meng Z, Fan P, Makriyannis A. Head group analogs of arachidonylethanolamide, the endogenous cannabinoid ligand. *J Med Chem.* 1996; 39:4515–4519. [PubMed: 8893848]
38. Khanolkar AD, Lu D, Ibrahim M, Duclos RI Jr, Thakur GA, Malan TP Jr, Porreca F, Veerappan V, Tian X, George C, Parrish DA, Papahadjis DP, Makriyannis A. Cannabilactones: A novel class of CB2 selective agonists with peripheral analgesic activity. *J Med Chem.* 2007; 50:6493–6500. [PubMed: 18038967]
39. Cheng YC, Prusoff WH. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 percent inhibition (I_{50}) of an enzymatic reaction. *Biochem Pharmacol.* 1973; 22:3099–3108. [PubMed: 4202581]
40. Compton DR, Rice KC, De Costa BR, Razdan RK, Melvin LS. Cannabinoid structure-activity relationships: Correlation of receptor binding and *in Vivo* activities. *J Pharmacol Exp Therap.* 1993; 265:218–226. [PubMed: 8474008]
41. Abood ME, Ditto KE, Noel MA, Showalter VM, Tao Q. Isolation and expression of a mouse CB1 cannabinoid receptor gene. Comparison of binding properties with those of native CB1 receptors in mouse brain and N18TG2 neuroblastoma cells. *Biochem Pharmacol.* 1997; 53:207–214. [PubMed: 9037253]
42. Griffin G, Tao Q, Abood ME. Cloning and pharmacological characterization of the rat CB2 cannabinoid receptor. *J Pharmacol Exp Ther.* 2000; 292:886–894. [PubMed: 10688601]
43. Allinger NL, Yuh YH, Lii JH. Molecular mechanics. The MM3 force field for hydrocarbons. 1989; 111:8551–8566.
44. Macromodel, version 9.5. Schrödinger, LLC; New York, NY: 2007.

Figure 1.

The 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.

Figure 2. Comparison of the conformational space for adamantyl and bornyl side chains of various Δ^8 -THC analogs. 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.

Scheme 1.

Synthesis of bornyl- and isobornyl- Δ^8 -THC (**8a** and **8b**)

a) Mg/THF, reflux, 8 h; b) Li/NH₃, THF, -78 °C, 6 h; c) BBr₃, CH₂Cl₂, 0 °C to r.t., d) p-TsOH·H₂O, CHCl₃, 65 °C, 6 h. (endo : exo = 3 : 1)

Scheme 2.

Synthesis of bornylmethyl- and isobornylmethyl- Δ^8 -THC (**8c** and **8d**)

(a) (1) Mg/THF; (2) (1R)-(+)-camphor (**3**), reflux, 8 h; (b) p-TsOH·H₂O, CH₂Cl₂, 45 °C, 1 h; (c) H₂, 10% Pd/C, EtOH, r.t., (d) BBr₃, CH₂Cl₂, 0 °C to r.t., (e) p-TsOH·H₂O, CHCl₃, 65 °C, 6 h.

Table 1Affinities (K_i) of Δ^8 -THC Analogs for CB1 and CB2 Cannabinoid Receptors^a

C3-Substituent		Binding Affinity K_i (nM) ^a	
Compound	R	CB1	CB2
1 (-)- Δ^8 -THC	n -C ₅ H 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 [³H]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