Cannabilactones: A Novel Class of CB2 Selective Agonists with Peripheral Analgesic Activity

Atmaram D. Khanolkar,[†] Dai Lu,[†] Mohab Ibrahim,[‡] Richard I. Duclos, Jr.,[†] Ganesh A. Thakur,[†] T. Phillip Malan, Jr.,[‡] Frank Porreca,[‡] Vijayabaskar Veerappan,[†] Xiaoyu Tian,[†] Clifford George,[§] Damon A. Parrish,[§] Demetris P. Papahatjis,[||] and Alexandros Makriyannis*,[†]

Center for Drug Discovery, Northeastern University, 116 Mugar Hall, 360 Huntington Avenue, Boston, Massachusetts 02115-5000, Departments of Anesthesiology and Pharmacology, University of Arizona College of Medicine, 1501 North Campbell Avenue, Tucson, Arizona 85724-5114, Naval Research Laboratory, Code 6030, 4555 Overlook Avenue, Washington, DC 20375-5341, and Institute of Organic and Pharmaceutical Chemistry, National Hellenic Research Foundation, 48 Vass. Constantinou, Athens 116-35 Greece

Received April 13, 2007

The identification of the CB2 cannabinoid receptor has provided a novel target for the development of therapeutically useful cannabinergic molecules. We have synthesized benzo[*c*]chromen-6-one analogs possessing high affinity and selectivity for this receptor. These novel compounds are structurally related to cannabinol (6,6,9-trimethyl-3-pentyl-6*H*-benzo[*c*]chromen-1-ol), a natural constituent of cannabis with modest CB2 selectivity. Key pharmacophoric features of the new selective agonists include a 3-(1′,1′-dimethylheptyl) side chain and a 6-oxo group on the cannabinoid tricyclic structure that characterizes this class of compounds as "cannabilactones." Our results suggest that the six-membered lactone pharmacophore is critical for CB2 receptor selectivity. Optimal receptor subtype selectivity of 490-fold and subnanomolar affinity for the CB2 receptor is exhibited by a 9-hydroxyl analog **5** (AM1714), while the 9-methoxy analog **4b** (AM1710) had a 54-fold CB2 selectivity. X-ray crystallography and molecular modeling show the cannabilactones to have a planar ring conformation. In vitro testing revealed that the novel compounds are CB2 agonists, while in vivo testing of cannabilactones **4b** and **5** found them to possess potent peripheral analgesic activity.

Introduction

Cannabinoids are known to produce biochemical and pharmacological effects by interacting with two well-characterized G protein-coupled receptors, CB1[a] and CB2,[1] although human CB1 and CB2 share only a 44% homology.[2,3] Also, CB1 exhibits high amino acid identity (97–99%)[4–6] across the species of human, rat, and mouse, while human CB2 displays only 81% and 82% amino acid identity with rat[7] and mouse,[3] respectively. The CB1 receptor was found to be localized primarily in the brain, with the highest density in the cells of the basal ganglia, cerebellum, and hippocampus.[8] CB1 is also found in peripheral tissues, including testis, eye, uterus, ovary, lungs, and heart.[4,9,10] Conversely, CB2 is expressed predominantly in immune cells, such as B-cells, monocytes, macrophages, and in several peripheral organs, such as spleen, pancreas, thymus, lung, and tonsil.[10,11] The presence of CB2 receptor protein was recently confirmed in the brain stem, cortex, and cerebellum of rat, mouse, and ferret,[12] however, the presence of CB2 receptor in those tissues is only about 1.5% of that found in the spleen.

Since the discovery of CB1 and CB2, substantial medicinal chemistry efforts have been directed toward the development of high affinity and receptor-selective cannabinergic ligands that would serve as pharmacological probes for studying cannabinoid-related physiology and biochemistry. Ligands that can selectively stimulate the CB2 receptor are expected to be largely devoid of the central nervous system (CNS) psychotropic side effects associated with the activation of CB1 receptors and represent potentially useful medications[1,13] for the treatment of pain, inflammation, cancer proliferation, Alzheimer's disease, and other ailments related to cannabinoid physiology. The structure–activity relationships (SARs) of cannabinoid analogs for the CB2 receptor have recently received attention.[14–17] Gareau et al.[18] showed that when the C-1 phenolic hydroxyls in (−)-Δ^8- and (−)-$\Delta^{9,11}$-tetrahydrocannabinols (Δ^8-THC and $\Delta^{9,11}$-THC) were replaced by methoxy groups, CB1 affinity is dramatically reduced while its affinity for CB2 is affected only slightly, thus leading to analogs possessing CB2 selectivity. Such selectivity is also obtained by eliminating the C-1 phenolic group, as 1-deoxy-11-hydroxy-DMH-Δ^8-THC shows significantly higher CB2 selectivity when compared to its C-1 phenolic analogue.[19] The C-3 side chain length[20] and the conformation[15,21] of classical cannabinoid analogs were shown to affect cannabinoid receptor subtype selectivity. Additionally, Hanus et al.[22] have reported a CB2 specific bicyclic agonist (+)-4-[4-(1,1-dimethylheptyl)-2,6-dimethoxyphenyl]-6,6-dimethylbicyclo [3.1.1]hept-2-en-2-methanol (HU-308), which was shown to produce anti-inflammatory, hypotensive, and analgesic effects that are blocked by the CB2 antagonist 5-(4-chloro-3-methylphenyl)-1-[(4-methylphenyl)methyl)-*N*-([1*S*,2*S*,4*R*]-1,3,3-trimethylbicyclo[2.2.1]heptan-2-yl)-1*H*-pyrazole-3-carboxamide (SR144528). Recently, we showed that the CB2 selective agonist (*R*,*S*)-(2-iodo-5-nitrophenyl)-{1-[(1-methylpiperidin-2-yl)methyl]-1*H*-indol-3-yl}-methanone (AM1241) also inhibits nociception.[23–27] Other CB2 selective ligands have been reported and recently reviewed.[28,29] Antinociception via peripheral CB2 receptors[23–25,27,30–32] as well as via peripheral CB1[31–34] receptors has been reported, and differentiation between these two

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

[†] Northeastern University.

[‡] University of Arizona College of Medicine.

[§] Naval Research Laboratory.

[||] National Hellenic Research Foundation.

[a] Abbreviations: BSA, bovine serum albumin; CB1, cannabinoid receptor 1; CB2, cannabinoid receptor 2; CNS, central nervous system; DMH, 1,1-dimethylheptyl; HEK, human embryonic kidney; 9-iodo-9-BBN, 9-iodo-9-borabicyclo[3.3.1]nonane; MM, molecular mechanics; MPE, maximum possible effect; SAR, structure–activity relationship; SEM, standard error of the mean; THC, tetrahydrocannabinol; TME, Tris buffer (25 mM Tris, 5 mM $MgCl_2$, 1 mM EDTA).

10.1021/jm070441u CCC: $37.00
Published on Web 11/27/2007

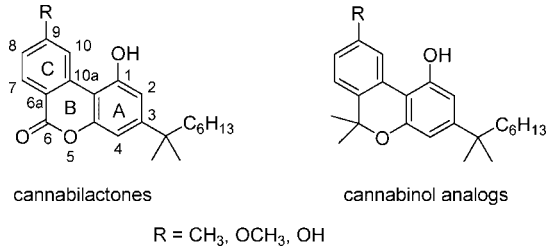
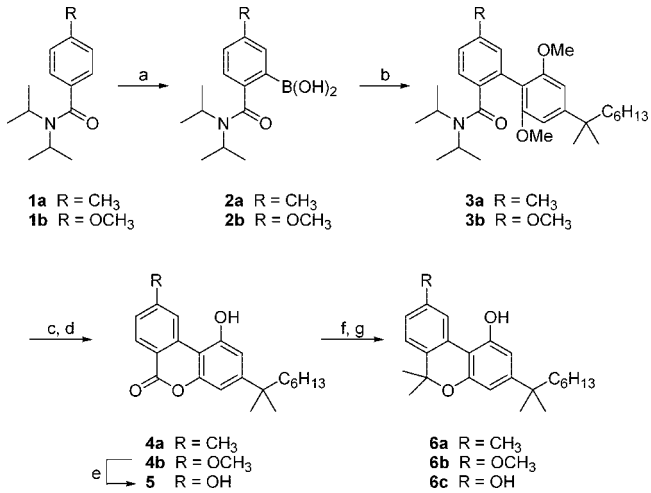


Figure 1. General structures of cannabilactones and cannabinol analogs.

Scheme 1[a]



[a] Reagents and conditions: (a) i. *sec*-BuLi, TMEDA, THF, −78 °C; ii. $B(OCH_3)_3$; iii. H_3O^+; (b) 2-bromo-5-(1′,1′-dimethylheptyl)-1,3-dimethoxybenzene, $Pd(PPh_3)_4$, $Ba(OH)_2$, DME, H_2O; (c) BBr_3 or 9-iodo-9-BBN, CH_2Cl_2; (d) AcOH, reflux; (e) BBr_3, CH_2Cl_2; (f) CH_3MgBr, THF; (g) *p*-TsOH·H_2O, $CHCl_3$, RT.

mechanisms is dependent on the specific analgesic test and the cannabinoid being used.

In a recently published patent application,[35] we reported a new class of CB2 selective ligands based on structural modifications of cannabinol, which is weakly CB2 selective.[2,36–38] The key pharmacophore of these benzo[*c*]chromen-6-ones is a carbonyl group in place of the 6,6-dimethyl moiety of the classical cannabinoid tricyclic structure. This class, whose synthesis, pharmacophoric characterization, and pharmacological evaluation are reported here, we refer to as "cannabilactones." Cannabilactones **4a**,[37] **4b**,[35] and **5**[35] (Figure 1 and Scheme 1) were synthesized in our laboratory using the Suzuki coupling reaction between arylboronic acids and aryl bromides as the key synthetic step. This synthetic approach also provides a new route for the synthesis of classical cannabinol analogs.

Chemistry. The previously reported syntheses of substituted benzo[*c*]chromen-6-ones involved von Pechmann condensation reactions between an appropriately substituted 1-oxocyclohexane-2-carboxylate and 5-alkylresorcinol followed by oxidation of the tetrahydrobenzopyranone intermediates.[37,39,40] However, the oxidation required harsh reaction conditions including high temperatures and prolonged reaction times. The reactions generally gave low yields of multiple products that required extensive purifications. More importantly, this route is not suitable for the synthesis of functionalized cannabinol analogs. In an effort to develop a high yield approach of more general applicability, we chose the Suzuki reaction,[41,42] which has already been used for the synthesis of benzo[*c*]chromen-6-ones,[43,44] as the key step in the synthesis of cannabilactones **4a**, **4b**, and **5**. Biphenyls **3a** and **3b** were prepared by coupling 4-substituted 2-(*N*,*N*-diisopropylcarboxamido)phenylboronic acids **2a** and **2b**, respectively, with 2-bromo-5-(1′,1′-dimethylheptyl)-1,3-dimethoxybenzene[45] using tetrakis(triphenylphosphine)palladium and barium hydroxide (Scheme 1). The boronic acids were prepared by ortho lithiation followed by boronation of the appropriate *N*,*N*-diisopropylbenzamides **1a**[46] and **1b**[47] using the method described by Snieckus et al.[43] The biphenyls **3a** and **3b** were demethylated with boron tribromide or 9-iodo-9-BBN,[48] which was utilized for the selective demethylations of the two methoxy groups of the electron-rich aromatic ring without cleaving the aryl methyl ether in the benzamide ring of biphenyl **3b**. Without purification, refluxing of the biphenyl resorcinol intermediates in glacial acetic acid gave the corresponding cannabilactones **4a** and **4b** via intramolecular cyclization in 36% and 61% overall yields, respectively. The methoxy group of cannabilactone **4b** was cleaved with boron tribromide to give cannabilactone **5** in 72% yield. Cannabilactones **4a**, **4b**, and **5** were converted to their 6,6-dimethyl analogs by treatment with methylmagnesium bromide followed by cyclization in the presence of *p*-toluenesulfonic acid monohydrate[49] to give the corresponding cannabinol analogs **6a**,[38] **6b**, and **6c**.

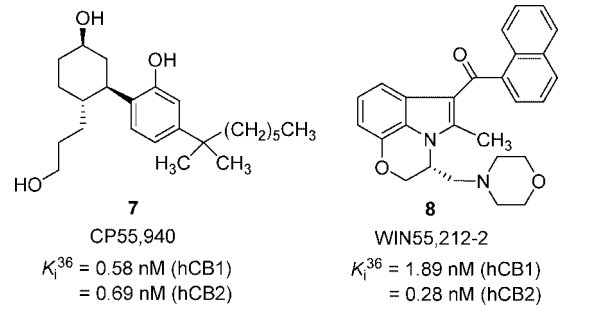


Figure 2. Structures of the nonselective partial agonist (−)-5-(1,1-dimethylheptyl)-2-[(1*R*,2*R*,5*R*)-5-hydroxy-2-(3-hydroxypropyl)cyclohexyl]phenol (**7**) and the full agonist (+)-{(3*R*)-2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-*de*]-1,4-benzoxazin-6-yl}-(1-naphthalenyl)-methanone (**8**).

Results and Discussion

CB1 and CB2 Receptor Binding Studies. The affinities of analogs **4a**, **4b**, **5**, **6a**, **6b**, and **6c** for the CB1 and CB2 cannabinoid receptors were determined by a standard competitive radioligand displacement assay using tritiated (−)-5-(1,1-dimethylheptyl)-2-[(1*R*,2*R*,5*R*)-5-hydroxy-2-(3-hydroxypropyl) cyclohexyl]phenol ([^{3}H]CP55,940, **7**; see Figure 2).[50] Rat brain synaptosomal and mouse spleen membranes were used as the sources for CB1 and CB2 receptors, respectively. As can be seen from the data in Table 1, the cannabilactones **4a**, **4b**, and **5** exhibit considerably higher selectivities for the CB2 receptor relative to their corresponding 6,6-dimethyl analogs **6a**, **6b**, and **6c**, which bind with comparable affinities to both CB1 and CB2 in our assays. Compound **4a** was previously reported to have moderate selectivity for the CB2 receptor.[37] While our binding data for the CB1 receptor show good correspondence with this report, our K_i value for the CB2 receptor is lower, and this discrepancy between the two laboratories is due to the differences in CB2 preparations (mouse spleen versus COS-7 cells expressing hCB2). Substitution of the C-9 methyl group in **4a** by a methoxy group in **4b** substantially reduces the CB1 affinity of **4b**, which exhibits over 50-fold CB2 selectivity. However, the most striking result was observed with the C-9 phenolic hydroxyl analog **5**, which exhibits subnanomolar CB2 receptor affinity (K_i, CB1 = 400 nM; K_i, CB2 = 0.82 nM) and nearly 500-fold selectivity.

Table 1. Cannabinoid Receptor Binding and Stimulation of [^{35}S]GTPγS Binding

analogue (9-substituent)	K_i[a] (nM) rCB1	K_i[a] (nM) mCB2	CB1/CB2	Stimulation of [^{35}S]GTPγS binding[b] (percent)
4a (Me)	39 (34, 44)	3.1 (2.6, 3.8)	13	56 ± 21
4b (OMe) AM1710	360 (330, 390)	6.7 (5.5, 8.1)	54	50 ± 8
5 (OH) AM1714	400 (340, 450)	0.82 (0.66, 1.0)	490	55 ± 17
6a (Me)	0.95 (0.81, 1.2)	1.1 (0.89, 1.2)	0.86	ND[c]
6b (OMe) AM1715	5.4 (4.9, 6.0)	5.9 (5.0, 6.9)	0.92	42 ± 15
6c (OH) AM4768	2.6 (2.0, 3.4)	4.8 (3.8, 5.5)	0.54	ND[c]
8 WIN55,212–2	ND[c]	ND[c]	ND[c]	56 ± 10

[a] Rat brain synaptosomal and mouse spleen membranes, respectively, were used as sources for rCB1 and mCB2 for the receptor binding assays with [^{3}H]CP55,940 (**7**) as the radioligand. The values in the parentheses indicate the 95% confidence limits. [b] [^{35}S]GTPγS binding using mouse spleens with standard deviations. [c] ND: Not determined.

After this study was completed, we became aware that the CB2 selectivities of **4b** and **5** are considerably reduced from 54-fold to 4.0-fold and from 490-fold to 8.5-fold, respectively, when these compounds were tested using a human CB2 receptor preparation from HEK cells using [^{3}H]CP55,940 (**7**). This difference between mouse and human CB2 was also observed by another laboratory.[51] While these data confirmed the value of the above ligands as excellent pharmacological probes in experiments involving rodents or rodent-derived tissues, the usefulness of these cannabilactones as potential human medications remains to be further investigated. These species-based differences in CB2 affinity data suggest that cannabilactone analogs interact with the mouse and human CB2 receptors through different binding motifs. Currently, we are pursuing this observation through the generation of suitable receptor mutations, and these data should provide interesting information pertaining to CB2 receptor structure. We are also pursuing additional SAR work aimed at developing cannabilactone analogs with robust CB2 selectivities for both rodent and human receptors.

Functional Characterization. The functional potencies of cannabilactones **4a**, **4b**, and **5** on the CB2 receptor were assessed with a [^{35}S]GTPγS binding assay, as described previously,[52,53] with a mouse spleen membrane preparation. All compounds were found to act as agonists and to stimulate [^{35}S]GTPγS binding at a concentration of 1 μM to an extent comparable to that of (+)-{(3*R*)-2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-*de*]-1,4-benzoxazin-6-yl}-(1-naphthalenyl)-methanone (WIN55,212-2, **8**), a prototypic, although only modestly selective, CB2 receptor agonist.[36] Furthermore, the functional potencies of **4a**, **4b**, and **5** on the CB2 receptor to decrease forskolin-stimulated cAMP were assayed as previously described.[53] These cannabilactones were confirmed to be agonists with functional potencies that were within a factor of 2−3 of the EC_{50} of WIN55,212-2 (**8**).

In Vivo Cannabinergic Evaluation. The cannabilactone agonists were evaluated in vivo for antinociceptic activity in mice as previously described.[23] Cannabilactones **4b** and **5** were administered into the dorsal surface of the hindpaw of male Sprague–Dawley rats, where they produced significant antinociceptive effects (Figure 3). The A_{50} (dose producing a 50% antinociceptive effect) was 1.6 mg/kg (95% confidence limits = 0.8–3.3 mg/kg) for **4b** and 4.5 mg/kg (95% confidence limits = 2.2–9.0 mg/kg) for **5**. It is interesting to note that although cannabilactones **4b** and **5** exhibit similar potency in the [^{35}S]GTPγS in vitro functional assay, **4b** exhibits higher in vivo potency. It can be argued that this may be due to differences in their respective pharmacokinetic profiles.

The CB2 selectivity of the above in vivo response was confirmed by using CB1 and CB2 antagonists whose respective selectivities are over 100-fold.[16] We showed that the antinociceptive effects of **5** were not affected by the CB1 selective antagonist 1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-*N*-1-piperidinyl-1*H*-pyrazole-3-carboxamide (**9**)[54] but were completely antagonized by the CB2 selective antagonist {6-iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1*H*-indol-3-yl}-(4-methoxyphenyl)-methanone (**10**;[55] Figure 4). These findings provide an in vivo confirmation of the CB2 receptor agonist activity of these compounds and confirm previous findings, using other structural classes of cannabinoid receptor agonists, that CB2 receptor activation produces analgesia.[22–27,30–32,56]

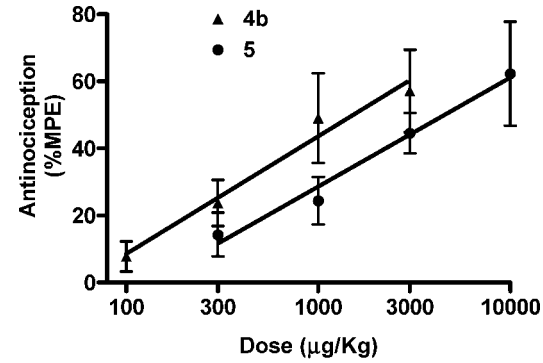
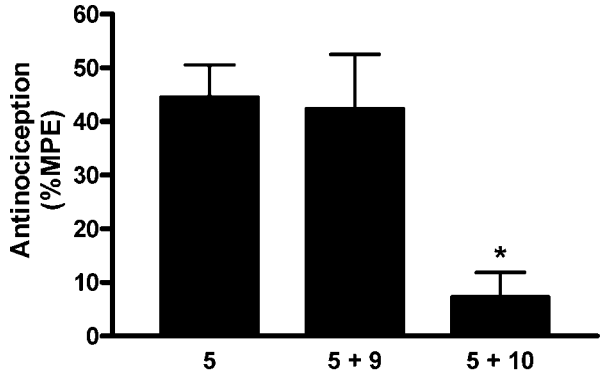
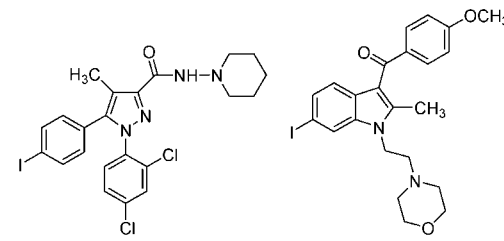


Figure 3. Peripheral antinociceptive effect of 9-methoxy cannabilactone **4b** (triangles) and 9-hydroxy cannabilactone **5** (circles). Compounds were dissolved in DMSO and injected subcutaneously into the dorsal surface of the tested hindpaw (intrapaw) 20 min before nociceptive testing. Nociception was assessed by measuring withdrawal latency to radiant heat as described by Hargreaves et al.[59] Data are expressed as a percent of the maximum possible effect (%MPE) calculated by the formula: %MPE = (WL − CL)/(CO − CL), where WL is the withdrawal latency obtained experimentally, CL is the control (baseline) value before drug administration, and CO is the cutoff value (a 40 s cutoff was used to prevent tissue damage). Dose–response curves were generated and the A_{50} values (doses producing 50% MPE) were calculated as described by Tallarida and Murray.[60] Data expressed as mean ± SEM. $N = 6$ per group.

Finally, **5** did not affect ambulation when administered systemically (intraperitoneally), as assessed by performance on the rotarod apparatus. In contrast, the mixed CB1/CB2 receptor agonist WIN55,212-2 (**8**) significantly impaired performance on the rotarod apparatus (Figure 5), and this is believed to be mediated by CB1 receptors in the CNS.[23] Our in vivo findings suggest that CB2 receptor selective agonists may be free of CNS side effects typically produced by cannabinoids. This is consistent with the very recent observation that CB2 receptor activation alone is not sufficient to generate some typical CNS effects associated with CB1 receptor activation.[12]

Molecular Modeling and X-ray Crystallography. The presence of a carbonyl group at C-6 of the cannabinoid tricyclic ring system appears to be responsible for the reduced affinities of these cannabilactones for the CB1 receptors. To better understand the molecular basis for the observed CB2 selectivities





9
AM251
K_i^{54} = 7.49 nM (rCB1)
= 2290 nM (mCB2)

10
AM630
K_i^{55} = 5152 nM (hCB1)
= 31.2 nM (hCB2)

Figure 4. The peripheral antinociceptive effects of 9-hydroxy cannabilactone **5** (3 mg/kg, intrapaw) were not affected by the CB1 selective antagonist 1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-*N*-1-piperidinyl-1*H*-pyrazole-3-carboxamide (**9**; 1 mg/kg, intrapaw) but were antagonized by the CB2 selective antagonist {6-iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1*H*-indol-3-yl}-(4-methoxyphenyl)-methanone (**10**; 1 mg/kg, intrapaw). All drugs were administered 20 min before nociceptive testing. Data are expressed as mean ± SEM. Groups were compared using ANOVA followed by pairwise comparisons using Student's *t*-test. $*P < 0.05$ compared to **5** alone. $N = 6$ per group.

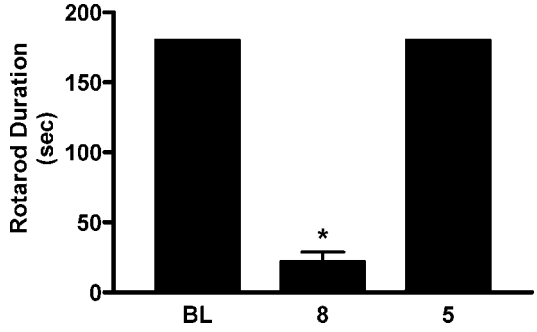


Figure 5. Effect of WIN55,212-2 (**8**; 3.3 mg/kg) and 9-hydroxy cannabilactone **5** (3.3 mg/kg) compared to baseline (BL) containing only vehicle on ambulation, as assessed by performance on the rotarod apparatus (Columbus Instruments International, Columbus, OH). Animals were trained until they could remain on the device for a duration of 180 s at a speed of 10 rpm. They were again tested 15 min after i.p. administration of WIN55,212-2 (**8**) or 9-hydroxy cannabilactone **5** and the time they were able to remain on the device until falling was recorded. A maximal cutoff time of 180 s was used. Data are expressed as mean ± SEM; $*P < 0.05$ compared to vehicle (DMSO). $N = 6$ per group.

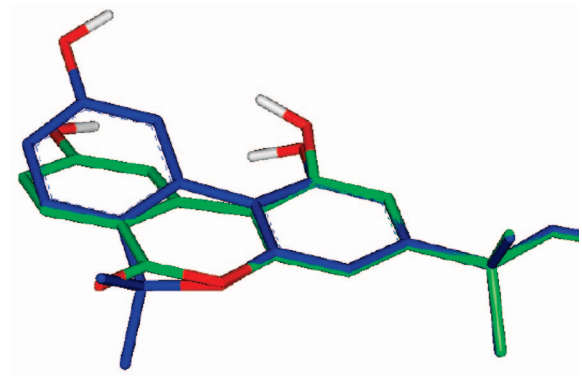


Figure 6. Comparison of minimum energy conformations of 6-oxo cannabilactone **5** and 6,6-dimethyl cannabinol **6c** tricyclic analogs presented in green and blue, respectively. Oxygen atoms are presented in red. The C-3 dimethylheptyl side chains are partially displayed. The aromatic A rings of the two molecules were superimposed with the 1-hydroxyl group tilted furthest from the viewer. The dihedral angle between the C rings of **5** and **6c** is about 29.3°. Molecular mechanics (MM) calculations were conducted on a Silicon Graphics Fuel workstation using Insight II Discover molecular dynamics package (Accelrys, San Diego, CA). Atomic potentials and charges were assigned using the cvff force field. Conformations were geometrically optimized using a distance-dependent dielectric constant mimicking an aqueous environment. Energy minimizations were performed until the maximum root-mean-square (rms) derivative was less than 0.001 kcal mol^{-1} $Å^{-1}$.

of the novel cannabilactones when compared to their corresponding 6,6-dimethyl cannabinol analogs, we have compared the conformations of **5** and **6c** using molecular mechanics and dynamics simulations. Our computational study shows that the presence of the six-membered lactone ring of **5** leads to a conformation in which all three rings are coplanar (Figure 6, in green). This is in agreement with the X-ray crystal structures of cannabilactones **4a** (Figure 7) and **4b** (Figure 8). Conversely, the pyran ring of 6,6-dimethyl analogue **6c** is in a puckered conformation (Figure 6, in blue). It can be seen that when the A rings of the two ligands are superimposed, the C ring of **6c** is positioned approximately 30° above that of **5** directing the respective 9-substituents of cannabilactones and cannabinols in different orientations.

The structural differences between the cannabilactone analogs and their 6,6-dimethyl congeners should account for the lower affinities of this new class of ligands in the CB1 receptor while both classes interact equally well with CB2. This can be explained by invoking that cannabilactone analogs have (a) a carbonyl group that may engage in unfavorable interactions with hydrophobic residues of the CB1 binding site; (b) reduced opportunity for hydrogen bonding through their 9-OH or 9-OCH_3 substituents; and (c) C-3 side chains that are oriented differently within the CB1 receptor and exhibit unfavorable steric interactions with the CB1 side chain subsite.

Conclusion

Our data reveal that the lactone functionality plays a key role in the observed CB2 selectivity of cannabilactones. Cannabilactone analogs **4a**, **4b**, and **5** bind with high affinities to the CB2 receptor and exhibit much lower affinities for CB1. The data also show that the presence of a phenolic hydroxyl group at C-9 enhances binding to the CB2 receptor, while the presence of a methyl group at C-9 enhances binding to the CB1 receptor. Conversion of the 6-oxo groups of the cannabilactones to the corresponding 6,6-dimethyl analogs results in complete loss of CB2 selectivity, as these cannabinol analogs **6a**, **6b**, and **6c** exhibit nearly equal high affinities for both cannabinoid receptor subtypes. Cannabilactone analogs have distinctly different interactions with the mouse and human CB2 receptors. In our

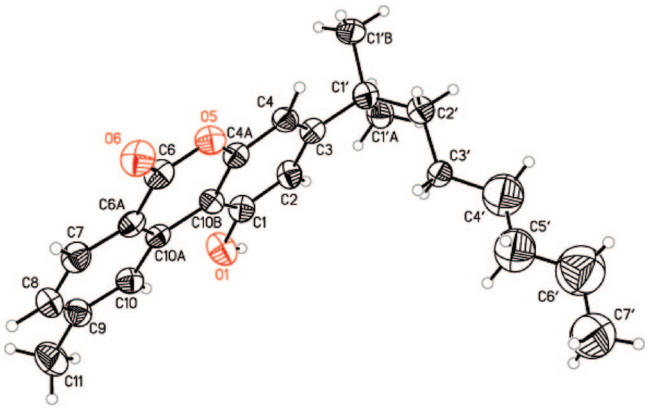


Figure 7. The molecular structure and numbering scheme for 9-methyl cannabilactone **4a** with displacement ellipsoids drawn at the 30% probability level.

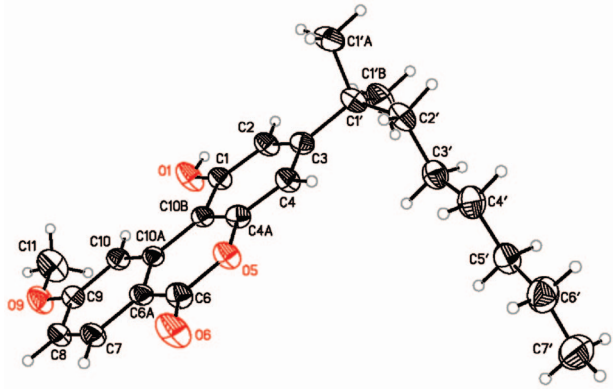


Figure 8. The molecular structure and numbering scheme for 9-methoxy cannabilactone **4b** with displacement ellipsoids drawn at the 30% probability level.

functional assays, all compounds were found to act as agonists, with activities comparable to WIN55,212-2 (**8**). The analgesic action by this class of compounds in vivo, without central nervous system side effects, represents a promising approach to the clinical treatment of pain.

Materials and Experimental Procedures

General Synthetic Methods. All reagents and solvents were purchased from Aldrich (Milwaukee, WI) unless specified otherwise and used without further purification. All anhydrous reactions were performed under a static argon or nitrogen atmosphere in flame-dried glassware using anhydrous solvents. Organic phases were dried over $MgSO_4$, rotary evaporated under reduced pressure, and flash column chromatography used silica gel 60 (230–400 mesh, Selecto Scientific Inc., Suwanee, GA). Purity of the intermediates and final compounds was established by analytical TLC on precoated aluminum silica gel plates (Whatman, UV_{254}, layer thickness 250 μm), and chromatograms were visualized under ultraviolet light and by phosphomolybdic acid straining. Melting points were determined on a capillary Electrothermal melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker DMX-500 spectrometer operating at 500 MHz. All NMR spectra were recorded using $CDCl_3$ as solvent unless otherwise stated, and chemical shifts are reported in ppm relative to tetramethylsilane as internal standard. 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). Mass spectra were recorded on a Hewlett-Packard 6890 GC/MS instrument at the School of Pharmacy, University of Connecticut. High-resolution mass spectra were performed at the School of Chemical Sciences, University of Illinois at Urbana–Champaign. Elemental analyses were performed by Baron Consulting Co., Milford, CT.

2-Diisopropylcarbamoyl-5-methylphenylboronic Acid (2a). To a solution of *sec*-BuLi (42 mL of a 1.3 M solution, 55 mmol) and 1,2-bis(dimethylamino)ethane (TMEDA; 7.5 mL, 55 mmol) in anhydrous THF (300 mL) at −78 °C under an argon atmosphere was added dropwise a solution of *N*,*N*-diisopropyl-4-methylbenzamide[46] (**1a**; 10.0 g, 45.6 mmol) in THF (40 mL). The reaction mixture was stirred for 60 min and then trimethyl borate (14.9 mL, 132 mmol) was added over a period of 30 min. The reaction mixture was allowed to warm to room temperature over 12 h and was then cooled to 0 °C and acidified to pH 6.5 with 5% aqueous HCl. The THF was removed, and the product was extracted with 150 mL of CH_2Cl_2. The organic phase was separated, washed with water and brine, and dried. The solvent was removed to give a solid that was recrystallized from Et_2O to afford 11.4 g, (43.3 mmol, 95%) of **2a** as a white solid: mp 134–136 °C; ^{1}H NMR (CD_3COCD_3) δ 7.72 (d, J = 8.1 Hz, 1H), 7.24 (s, 1H), 7.19 (d, J = 8.1 Hz, 1H), 5.00 (m, 1H), 4.86 (s, 2H, OH), 3.98 (m, 1H), 2.41 (s, 3H), 1.57 (d, J = 5.5 Hz, 6H), 1.45 (d, J = 5.5 Hz, 6H); MS m/z 263 (M^+).

2-Diisopropylcarbamoyl-5-methoxyphenylboronic Acid (2b). Compound **2b** was prepared from 11.8 g (50.1 mmol) *N*,*N*-diisopropyl-4-methoxybenzamide[47] (**1b**) as described for **2a** to give 13.4 g (48.0 mol, 96%) of **2b** as a white solid: mp 125–127 °C; ^{1}H NMR (CD_3OD) δ 7.83 (d, J = 8.2 Hz, 1H), 7.10 (d, J = 1.9 Hz, 1H), 6.94 (dd, J = 1.9 Hz, 8.2 Hz, 1H), 5.00–5.08 (m, 1H), 3.96–4.04 (m, 1H), 3.89 (s, 3H), 1.57 (d, J = 5.9 Hz, 6H), 1.46 (d, J = 5.8 Hz, 6H); MS m/z 279 (M^+).

2-(*N*,*N*-Diisopropylcarboxamido)-5-methyl-2′,6′-dimethoxy-4′-(1″,1″-dimethylheptyl)biphenyl (3a). Argon was bubbled through a mixture of boronic acid **2a** (0.185 g, 0.703 mmol), 2,6-dimethoxy-4-(1′,1′-dimethylheptyl)bromobenzene[45] (0.22 g, 0.64 mol), $Ba(OH)_2 \cdot 8H_2O$ (0.303 g, 0.96 mmol), 0.7 mL of water, and 5 mL of dimethoxyethane for 10 min. The $Pd(PPh_3)_4$ (74 mg, 0.064 mmol) catalyst was added to the mixture while argon bubbling was maintained through the mixture, and degassing was continued for an additional 5 min. The reaction mixture was microwaved for 15 min at 110 °C in a CEM Discover apparatus. Then the mixture was cooled to room temperature and filtered through a short celite pad. The filtrate was concentrated and Et_2O was added. The ether solution was washed with water and brine and dried, and solvent removal gave crude **3a** as a light yellow solid. The crude product was chromatographed (acetone/petroleum ether, 20:80) to afford 0.205 g (0.426 mmol, 67%) of biphenyl **3a** as a white solid: mp 118–120 °C; R_f 0.36 (Et_2O/petroleum ether, 50:50); ^{1}H NMR δ 7.22 (d, J = 8.0 Hz, 1H), 7.14 (dd, J = 8.0 Hz, 1.0 Hz, 1H), 7.06 (d, J = 1.0 Hz, 1H), 6.52 (s, 1H), 6.51 (s, 1H), 3.72 (s, 3H), 3.70 (s, 3H), 3.66–3.71 (m, 1H), 3.18–3.20 (m, 1H), 2.37 (s, 3H), 1.58–1.61 (m, 2H), 1.46 (d, J = 6.5 Hz, 3H), 1.30 (s, 6H), 1.19–1.26 (m, 6H), 1.04–1.12 (m, 5H, especially, 1.08 d, J = 6.5 Hz, 3H), 0.91 (d, J = 6.5 Hz, 3H), 0.86 (t, J = 7.0 Hz, 3H), 0.57 (d, J = 6.5 Hz, 3H). Exact mass calculated for $C_{31}H_{47}NO_3$, 481.7079; found, 481.7082.

2-(*N*,*N*-Diisopropylcarboxamido)-5,1′,6′-trimethoxy-4′-(1″,1″-dimethylheptyl)biphenyl (3b). Biphenyl **3b** was prepared from boronic acid **2b** (0.89 g, 3.2 mmol), 2,6-dimethoxy-4-(1′,1′-dimethylheptyl)bromobenzene (1.00 g, 2.91 mmol), $Ba(OH)_2 \cdot 8H_2O$ (1.38 g, 4.37 mmol), and $Pd(PPh_3)_4$ (336 mg, 0.291 mmol) as described for **3a** to give 1.32 g (2.65 mmol, 83%) of **3b** as a white solid: mp 103–104 °C; R_f 0.28 (Et_2O/petroleum ether, 50:50); ^{1}H NMR δ 7.28 (d, J = 8.5 Hz, 1H), 6.87 (dd, J = 8.5 Hz, 2.5 Hz, 1H), 6.79 (d, J = 2.5 Hz, 1H), 6.53 (s, 1H), 6.52 (s, 1H), 3.80 (s, 3H), 3.72 (s, 3H), 3.71 (s, 3H), 3.52 (m, 1H), 3.15 (m, 1H), 1.58–1.62 (m, 2H), 1.46 (d, J = 6.5 Hz, 3H), 1.18–1.35 (m, 12 H, especially, 1.30, s, 6H), 1.05–1.11 (m, 5H, especially, 1.08, d, J = 6.5 Hz, 3H), 0.90 (d, J = 6.5 Hz, 3H), 0.86 (t, J = 7.2 Hz, 3H), 0.56 (d, J = 6.5 Hz, 3H). Exact mass calculated for $C_{31}H_{47}NO_4$, 497.7092; found, 497.7095.

3-(1′,1′-Dimethylheptyl)-1-hydroxy-9-methyl-6*H*-benzo[*c*]-chromene-6-one (Cannabilactone 4a). A solution of **3a** (0.241 g, 0.500 mmol) in 10 mL of anhydrous CH_2Cl_2 was cooled to −78 °C and BBr_3 (1.38 mL of a 1.0 M CH_2Cl_2 solution, 1.38 mmol) was added dropwise. The reaction mixture was stirred and allowed to warm to room temperature overnight. The mixture was cooled to 0 °C, carefully quenched with 1 mL of dry MeOH, and allowed to warm to room temperature over 2 h. Then 5 mL of 5% aqueous HCl was added, the product extracted with CH_2Cl_2, and the solution dried. The CH_2Cl_2 was removed to give the demethylated intermediate as a yellow foam, which was dissolved in 5 mL of glacial acetic acid. The reaction mixture was refluxed for 5 h and then cooled to room temperature. Water was added cautiously to the mixture at 0 °C. Diethyl ether was added, and the ether solution was washed with water, 15% aqueous $NaHCO_3$, water, and brine and then dried. Filtration and removal of solvent gave a crude solid that was chromatographed (acetone/petroleum ether, 30:70) to give 0.100 g (0.284 mmol, 57%) of **4a**[37] as a white solid: mp 186–188 °C (lit.[37] mp 184–185 °C); R_f 0.57 (Et_2O/petroleum ether, 50:50); ^{1}H NMR δ 8.80 (s, 1H), 8.31 (d, J = 8.5 Hz, 1H), 7.34 (dd, J = 8.5 Hz, 1.0 Hz, 1H), 6.95 (d, J = 2.0 Hz, 1H), 6.70 (d, J = 2.0 Hz, 1H), 5.96 (bs, 1H), 2.54 (s, 3H), 1.57–1.60 (m, 2H), 1.29 (s, 6H), 1.16–1.25 (m, 6H), 1.02–1.09 (m, 2H), 0.83 (t, J = 7.0 Hz, 3H); MS m/z 352 (M^+). An X-ray crystal structure was obtained for **4a**, see Figure 6 and Supporting Information.

3-(1′,1′-Dimethylheptyl)-1-hydroxy-9-methoxy-6*H*-benzo[*c*]-chromene-6-one (Cannabilactone 4b). A solution of **3b** (0.500 g, 1.00 mmol) in 10 mL of anhydrous CH_2Cl_2 was cooled to 0 °C and 3 mL of 9-iodo-9-BBN (4.0 mL of a 1.0 M in hexane, 4.0 mmol) was added dropwise. The reaction mixture was stirred at 0 °C for 4 h. The CH_2Cl_2 was removed and the residue was dissolved in anhydrous Et_2O (30 mL). The mixture was then treated with 4 mL of ethanolamine solution (1.0 M in ether). The reaction mixture was stirred for 40 min and filtered through a short celite column. The filtrate was concentrated and dissolved in 5 mL of glacial acetic acid. The reaction mixture was refluxed for 5 h and then cooled to room temperature. Water was added cautiously to the mixture at 0 °C. Diethyl ether was added and the ether solution was washed with water, 15% aqueous $NaHCO_3$, water, and brine and then dried. Filtration and removal of solvent gave a solid crude product that was chromatographed (EtOAc/hexanes, 20:80) to give 0.281 mg (0.763 mmol, 76%) of **4b** as a white solid: mp 149–151 °C; R_f 0.44 (Et_2O/petroleum ether, 50:50); ^{1}H NMR δ 8.52 (d, J = 2.5 Hz, 1H), 8.35 (d, J = 8.5 Hz, 1H), 7.05 (dd, J = 2.5 Hz, 8.5 Hz, 1H), 6.94 (d, J = 1.8 Hz, 1H), 6.71 (d, J = 1.8 Hz, 1H), 6.12 (bs, 1H), 3.97 (s, 3H), 1.56–1.60 (m, 2H), 1.28 (s, 6H), 1.18–1.24 (m, 6H), 1.02–1.08 (m, 2H) 0.82 (t, J = 7.0 Hz, 3H). Anal. ($C_{23}H_{28}O_4$) C, H. An X-ray crystal structure was obtained for **4b**, see Figure 6 and Supporting Information.

1,9-Dihydroxy-3-(1′,1′-dimethylheptyl)-6*H*-benzo[*c*]chromene-6-one (Cannabilactone 5). To a solution of **4b** (700 mg, 1.99 mmol) in 40 mL of anhydrous CH_2Cl_2 was added 3.80 mL (3.80 mmol) of boron tribromide solution (1.0 M in CH_2Cl_2) dropwise at room temperature. The mixture was stirred at room temperature for 15 min and then refluxed for 12 h. The reaction mixture was then cooled to room temperature and treated with water dropwise. The organic phase was separated, washed with water, 15% aqueous $NaHCO_3$, water, and brine, and then dried. Solvent removal gave a crude solid product that was chromatographed (Et_2O/petroleum ether, 50:50) to give 510 mg (1.44 mmol, 72%) of **5** as an off-white foam: mp 103–108 °C; R_f 0.28 (Et_2O/petroleum ether, 50:50); ^{1}H NMR δ 8.53 (d, J = 2.2 Hz, 1H), 8.28 (dd, J = 8.6 Hz, 2.2 Hz, 1H), 7.02 (d, J = 8.6 Hz, 1H), 6.86 (d, J = 1.4 Hz, 1H), 6.75 (d, J = 1.4 Hz, 1H), 6.20 (bs, 1H), 6.05 (bs, 1H), 1.49–1.56 (m, 2H), 1.27 (s, 6H), 1.12–1.22 (m, 6H), 0.98–1.05 (m, 2H), 0.82 (t, J = 7.1 Hz, 3H). Anal. ($C_{22}H_{26}O_4 \cdot 1/2H_2O$) C, H.

General Procedure for Preparation of Cannabinol Analogs 6. To a solution of cannabilactone (1.0 mmol) in anhydrous THF (20 mL) was added methylmagnesium iodide (1.66 mL, 3.0 M in Et_2O) at room temperature under an argon atmosphere. The reaction mixture was stirred at room temperature for 30 min and then refluxed for 1.5 h. The reaction was cooled to room temperature and quenched by the addition of 20 mL of saturated aqueous NH_4Cl. The THF was removed and the residue was dissolved in anhydrous Et_2O (50 mL). The ether solution was washed with water and brine and dried, and solvent removal gave the crude intermediates that were used without further purification in the subsequent cyclization reactions. The intermediates were dissolved in anhydrous $CHCl_3$ (10–15 mL) and 60 mg of *p*-toluenesulfonic acid monohydrate was then added under an argon atmosphere. The reaction mixture was stirred at room temperature for 6–8 h and then treated with 10 mL of water. The organic phase was separated and washed with water, 15% aqueous $NaHCO_3$, water, and brine and then dried. Solvent removal gave the crude product that was chromatographed (acetone/petroleum ether, 10:90) to give the cannabinol analogs **6** in yields of 63–74%.

3-(1′,1′-Dimethylheptyl)-6,6,9-trimethyl-6*H*-benzo[*c*]chromene-1-ol (Cannabinol Analog 6a)[37,38] This compound was identical to reported **6a**: mp 97–98 °C (lit.[37] mp 95–98 °C).

6,6-Dimethyl-3-(1′,1′-dimethylheptyl)-9-methoxy-6*H*-benzo[*c*]chromene-1-ol (Cannabinol Analog 6b). White solid, 140 mg (0.287 mmol, 71%); mp 60–61 °C; R_f 0.22 (Et_2O/petroleum ether, 10:90); ^{1}H NMR δ 7.99 (d, J = 2.5 Hz, 1H), 7.15 (d, J = 8.5 Hz, 1H), 6.80 (dd, J = 2.5 Hz, 8.5 Hz, 1H), 6.56 (d, J = 1.6 Hz, 1H), 6.40 (d, J = 1.6 Hz, 1H), 5.16 (s, 1H), 3.84 (s, 3H), 1.57 (s, 6H), 1.52–1.55 (m, 2H), 1.25 (s, 6H), 1.21–1.24 (m, 2H), 1.16–1.20 (m, 4H), 1.06–1.10 (m, 2H), 0.83 (t, J = 2.5 Hz, 3H). Exact mass calculated for $C_{25}H_{34}O_3$, 382.5357; found, 382.5361. Anal. ($C_{25}H_{34}O_3 \cdot 1/3H_2O$) C, H: calcd, C% 77.28, H% 8.99; found, C% 77.20, H% 8.40.

6,6-Dimethyl-3-(1′,1′-dimethylheptyl)-6*H*-benzo[*c*]chromene-1,9-diol (Cannabinol Analog 6c). White solid, 14.7 mg (3.99×10^{-5} mol, 74%); mp 163–164 °C; R_f 0.63 (EtOAc/petroleum ether, 25:75); ^{1}H NMR δ 7.91 (d, J = 2.4 Hz, 1H), 7.11 (d, J = 8.2 Hz, 1H), 6.73 (dd, J = 2.4 Hz, 8.2 Hz, 1H), 6.55 (d, J = 1.8 Hz, 1H), 6.38 (d, J = 1.8 Hz, 1H), 5.22 (bs, 1H), 4.86 (bs, 1H), 1.59 (s, 6H), 1.51–1.55 (m, 2H), 1.24 (s, 6H), 1.16–1.23 (m, 6H), 1.03–1.11 (m, 2H), 0.83 (t, J = 7.0 Hz, 3H). Exact mass calculated for $C_{24}H_{32}O_3$, 368.2351; found, 368.2355. Anal. ($C_{24}H_{32}O_3 \cdot 1/2H_2O$) C, H.

Radioligand Binding Assay. Forebrain synaptosomal membranes were prepared from frozen rat brains by the method of Dodd et al.[57] and were used to assess the affinities of the novel analogs for the CB1 binding sites, while affinities for the CB2 sites were measured using a membrane preparation from frozen mouse spleen using a similar procedure.[50] The displacement of specifically tritiated CP55,940 (**7**) from these membranes was used to determine the IC_{50} values for the test compounds. The radioligand binding assay was conducted on 96-well microfilter plates, as previously described.[50] 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 (**7**; 3.08 nM) in TME (25 mM Tris, 5 mM $MgCl_2$, 1 mM EDTA) buffer containing 0.1% BSA was incubated for 1 h at 30 °C. For the nonspecific binding control, 100 μL of 200 nM CP55,940 (**7**) 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 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 that were converted to K_i values using the assumptions of Cheng and Prussoff.[58]

[^{35}S]GTPγS Binding Assay. This assay was performed as reported,[52,53] except using a mouse spleen preparation. The mouse spleen was lysed in a cell disruption bomb, centrifuged twice at 1000 *g* and 175000 *g* for 10 min each, and finally resuspended in TME buffer with 0.1% BSA to a protein concentration of 0.6 mg/

mL. The cell membrane preparations (24–40 μg) were incubated for 2 h at 30 °C with compounds at 1 μM, 30 μM GDP, and 0.05 nM [^{35}S]GTPγS to a final volume of 250 μL. Nonspecific binding was assessed in the presence of 10 μM nonradiolabeled GTPγS. Postincubation, the wells were filtered using GF/B filters (Perkin-Elmer) and washed. Bound [^{35}S]GTPγS was determined using a Packard Topcount scintillation counter and results were analyzed using Prizm software (GraphPad Software, Inc.).

cAMP Assay. The cAMP assay was performed as previously described.[53]

Cannabinoid Mediated Antinociception. Animals. Male Sprague–Dawley rats (Harlan, Indianapolis, IN) 200–300 g at time of testing, were maintained in a climate-controlled room on a 12 h light/dark cycle (lights on at 06:00 h) and food and water were available ad libitum. All of the testing was performed in accordance with the policies and recommendations of the International Association for the Study of Pain (IASP) and the National Institutes of Health (NIH) guidelines for the handling and use of laboratory animals and received approval from the Institutional Animal Care and Use Committee (IACUC) of the University of Arizona.

In Vivo Drug Administration. All drugs were dissolved in dimethyl sulfoxide (DMSO) and were injected subcutaneously in the plantar surface of the hindpaw in a total volume of 50 μL. DMSO given in hindpaw at this volume had no effect. The method of Hargreaves et al.[59] was employed to assess paw-withdrawal latency to a thermal nociceptive stimulus. Rats were allowed to acclimate within a plexiglass enclosure on a clear glass plate maintained at 30 °C. A radiant heat source (i.e., high intensity projector lamp) was activated with a timer and focused onto the plantar surface of the hindpaw. Paw-withdrawal latency was determined by a photocell that halted both lamp and timer when the paw was withdrawn. The latency to withdrawal of the paw from the radiant heat source was determined both before and after drug or vehicle administration. A maximal cutoff of 40 s was employed to prevent tissue damage.

Acknowledgment. This research work was supported by Grants DA-7215, DA-3801, DA-152, DA-9158 (Center for Drug Discovery, Northeastern University), DA-0355 (The University of Arizona College of Medicine) from the National Institute on Drug Abuse, and by the Office of Naval Research. We also thank Joy Erickson for the biochemical assays, Ruoxi Lan who prepared **9**, and Fenmei Yao who prepared **10**.

Supporting Information Available: Elemental analyses data and X-ray diffraction data for **4a** and **4b**. This material is available free of charge via the Internet at .

References

(1) Lambert, D. M.; Fowler, C. J. The endocannabinoid system: Drug targets, lead compounds, and potential therapeutic applications. *J. Med. Chem.* **2005**, *48*, 5059–5087.

(2) Munro, S.; Thomas, K. L.; Abu-Shaar, M. Molecular characterization of a peripheral receptor for cannabinoids. *Nature* **1993**, *365*, 61–65.

(3) Shire, D.; Calandra, B.; Rinaldi-Carmona, M.; Oustric, D.; Pessègue, B.; Bonnin-Cabanne, O.; Le Fur, G.; Caput, D.; Ferrara, P. Molecular cloning, expression and function of the murine CB2 peripheral cannabinoid receptor. *Biochim. Biophys. Acta* **1996**, *1307*, 132–136.

(4) Gérard, C. M.; Mollereau, C.; Vassart, G.; Parmentier, M. Molecular cloning of a human cannabinoid receptor which is also expressed in testis. *Biochem. J.* **1991**, *279*, 129–134.

(5) Chakrabarti, A.; Onaivi, E. S.; Chaudhuri, G. Cloning and sequencing of a cDNA encoding the mouse brain-type cannabinoid receptor protein. *DNA Sequence* **1995**, *5*, 385–388.

(6) Abood, M. E.; Ditto, K. E.; Noel, M. A.; Showalter, V. M.; 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.

(7) Griffin, G.; Tao, Q.; Abood, M. E. Cloning and pharmacological characterization of the rat CB_2 cannabinoid receptor. *J. Pharmacol. Exp. Ther.* **2000**, *292*, 886–894.

(8) Herkenham, M.; Lynn, A. B.; Little, M. D.; Johnson, M. R.; Melvin, L. S.; de Costa, B. R.; Rice, K. C. Cannabinoid receptor localization in brain. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 1932–1936.

(9) Straiker, A.; Stella, N.; Piomelli, D.; Mackie, K.; Karten, H. J.; Maguire, G. Cannabinoid CB1 receptors and ligands in vertebrate retina: Localization and function of an endogenous signaling system. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 14565–14570.

(10) Galiègue, S.; Mary, S.; Marchand, J.; Dussossoy, D.; Carrière, D.; Carayon, P.; Bouaboula, M.; Shire, D.; Le Fur, G.; Casellas, P. Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. *Eur. J. Biochem.* **1995**, *232*, 54–61.

(11) Bouaboula, M.; Rinaldi, M.; Carayon, P.; Carillon, C.; Delpech, B.; Shire, D.; Le Fur, G.; Casellas, P. Cannabinoid-receptor expression in human leukocytes. *Eur. J. Biochem.* **1993**, *214*, 173–180.

(12) Van Sickle, M. D.; Duncan, M.; Kingsley, P. J.; Mouihate, A.; Urbani, P.; Mackie, K.; Stella, N.; Makriyannis, A.; Piomelli, D.; Davison, J. S.; Marnett, L. J.; Di Marzo, V.; Pittman, Q. J.; Patel, K. D.; Sharkey, K. A. Identification and functional characterization of brainstem cannabinoid CB_2 receptors. *Science* **2005**, *310*, 329–332.

(13) Pacher, P.; Batkai, S.; Kunos, G. The endocannabinoid system as an emerging target of pharmacotherapy. *Pharmacol. Rev.* **2006**, *58*, 389–462.

(14) Reggio, P. Pharmacophores for ligand recognition and activation/inactivation of the cannabinoid receptors. *Curr. Pharm. Des.* **2003**, *9*, 1607–1633.

(15) Lu, D.; Meng, Z.; Thakur, G. A.; Fan, P.; Steed, J.; Tartal, C. L.; Hurst, D. P.; Reggio, P. H.; Deschamps, J. R.; Parrish, D. A.; George, C.; Järbe, T. U. C.; Lamb, R. J.; Makriyannis, A. Adamantyl cannabinoids: A novel class of cannabinergic ligands. *J. Med. Chem.* **2005**, *48*, 4576–4585.

(16) Thakur, G. A.; Nikas, S. P.; Li, C.; Makriyannis, A. Structural requirements for cannabinoid receptor probes. *Handb. Exp. Pharmacol.* **2005**, *168*, 209–246.

(17) Raitio, K. H.; Salo, O. M. H.; 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.

(18) Gareau, Y.; Dufresne, C.; Gallant, M.; Rochette, C.; Sawyer, N.; Slipetz, D. M.; Tremblay, N.; Weech, P. K.; Metters, K. M.; Labelle, M. Structure−activity relationships of tetrahydrocannabinol analogues on human cannabinoid receptors. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 189–194.

(19) Huffman, J. W.; Yu, S.; Showalter, V.; Abood, M. E.; Wiley, J. L.; Compton, D. R.; Martin, B. R.; Bramblett, R. D.; Reggio, P. H. Synthesis and pharmacology of a very potent cannabinoid lacking a phenolic hydroxyl with high affinity for the CB2 receptor. *J. Med. Chem.* **1996**, *39*, 3875–3877.

(20) Huffman, J. W.; Miller, J. R. A.; Liddle, J.; Yu, S.; Thomas, B. F.; Wiley, J. L.; Martin, B. R. Structure−activity relationships for 1′,1′-dimethylalkyl-Δ^8-tetrahydrocannabinols. *Bioorg. Med. Chem.* **2003**, *11*, 1397–1410.

(21) Khanolkar, A. D.; 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.

(22) Hanus, L.; Breuer, A.; Tchilibon, S.; Shiloah, S.; Goldenberg, D.; Horowitz, M.; Pertwee, R. G.; Ross, R. A.; Mechoulam, R.; Fride, E. HU-308: A specific agonist for CB_2, a peripheral cannabinoid receptor. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 14228–14233.

(23) Malan, T. P., Jr.; Ibrahim, M. M.; Deng, H.; Liu, Q.; Mata, H. P.; Vanderah, T.; Porreca, F.; Makriyannis, A. CB_2 cannabinoid receptor-mediated peripheral antinociception. *Pain* **2001**, *93*, 239–245.

(24) Ibrahim, M. M.; Deng, H.; Zvonok, A.; Cockayne, D. A.; Kwan, J.; Mata, H. P.; Vanderah, T. W.; Lai, J.; Porreca, F.; Makriyannis, A.; Malan, T. P. Jr. Activation of CB_2 cannabinoid receptors by AM1241 inhibits experimental neuropathic pain: Pain inhibition by receptors not present in the CNS. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 10529–10533.

(25) Hohmann, A. G.; Farthing, J. N.; Zvonok, A. M.; Makriyannis, A. Selective activation of cannabinoid CB_2 receptors suppresses hyperalgesia evoked by intradermal capsaicin. *J. Pharmacol. Exp. Ther.* **2004**, *308*, 446–453.

(26) Ibrahim, M. M.; Porreca, F.; Lai, J.; Albrecht, P. J.; Rice, F. L.; Khodorova, A.; Davar, G.; Makriyannis, A.; Vanderah, T. W.; Mata, H. P.; Malan, T. P. Jr. CB_2 cannabinoid receptor activation produces antinociception by stimulating peripheral release of endogenous opioids. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 3093–3098.

(27) Ibrahim, M. M.; Rude, M. L.; Stagg, N. J.; Mata, H. P.; Lai, J.; Vanderah, T. W.; Porreca, F.; Buckley, N. E.; Makriyannis, A.; Malan, T. P. Jr. CB_2 cannabinoid receptor mediation of antinociception. *Pain* **2006**, *122*, 36–42.

(28) Huffman, J. W. CB_2 receptor ligands. *Mini-Rev. Med. Chem.* **2005**, *5*, 641–649.

(29) Lu, D.; Vemuri, K.; Duclos, R. I., Jr.; Makriyannis, A. The Cannabinergic system as a target for anti-inflammatory therapies. *Curr. Top. Med. Chem.* **2006**, *6*, 1401–1426.
(30) Nackley, A. G.; Makriyannis, A.; Hohmann, A. G. Selective activation of cannabinoid CB_2 receptors suppresses spinal Fos protein expression and pain behavior in a rat model of inflammation. *Neuroscience* **2003**, *119*, 747–757.
(31) Nackley, A. G.; Suplita, R. L., II; Hohmann, A. G. A peripheral cannabinoid mechanism suppresses spinal Fos protein expression and pain behavior in a rat model of inflammation. *Neuroscience* **2003**, *117*, 659–670.
(32) Gutierrez, T.; Farthing, J. N.; Zvonok, A. M.; Makriyannis, A.; Hohmann, A. G. Activation of peripheral cannabinoid CB_1 and CB_2 receptors suppresses the maintenance of inflammatory nociception: A comparative analysis. *Br. J. Pharmacol.* **2007**, *150*, 153–163.
(33) Dogrul, A.; Gul, H.; Akar, A.; Yildiz, O.; Bilgin, F.; Guzeldemir, E. Topical cannabinoid antinociception: Synergy with spinal sites. *Pain* **2003**, *105*, 11–16.
(34) Choong, K.-C.; Su, X.; Urban, M. O. Effect of CP55,940 on mechanosensory spinal neurons following chronic inflammation. *Neurosci. Lett.* **2007**, *414*, 105–109.
(35) Makriyannis, A.; Khanolkar, A. Peripheral cannabinoid receptor (CB2) selective ligands (University of CT, U.S.A.). WO Patent 2001/28329 A1, 2001;pp 33.
(36) Showalter, V. M.; Compton, D. R.; Martin, B. R.; Abood, M. E. Evaluation of binding in a transfected cell line expressing a peripheral cannabinoid receptor (CB2): identification of cannabinoid receptor subtype selective ligands. *J. Pharmacol. Exp. Ther.* **1996**, *278*, 989–999.
(37) Rhee, M.-H.; Vogel, Z.; Barg, J.; Bayewitch, M.; Levy, R.; Hanuš, L.; Breuer, A.; Mechoulam, R. Cannabinol derivatives: Binding to cannabinoid receptors and inhibition of adenylylcyclase. *J. Med. Chem.* **1997**, *40*, 3228–3233.
(38) Mahadevan, A.; Siegel, C.; Martin, B. R.; Abood, M. E.; Beletskaya, I.; Razdan, R. K. Novel cannabinol probes for CB1 and CB2 cannabinoid receptors. *J. Med. Chem.* **2000**, *43*, 3778–3785.
(39) Adams, R.; Baker, B. R. Structure of cannabinol. V. A second method of synthesis of cannabinol. *J. Am. Chem. Soc.* **1940**, *62*, p. 2401.
(40) Ghosh, R.; Todd, A. R.; Wilkinson, S. *Cannabis indica*. Part V. The synthesis of cannabinol. *J. Chem. Soc.* **1940**, 1393–1396.
(41) Watanabe, T.; Miyaura, N.; Suzuki, A. Synthesis of sterically hindered biaryls via the palladium-catalyzed cross-coupling reaction of arylboronic acids or their esters with haloarenes. *Synlett* **1992**, 207–210.
(42) Miyaura, N.; Suzuki, A. Palladium-catalyzed cross-coupling reactions of organoboron compounds. *Chem. Rev.* **1995**, *95*, 2457–2483.
(43) Alo, B. I.; Kandil, A.; Patil, P. A.; Sharp, M. J.; Siddiqui, M. A.; Snieckus, V.; Josephy, P. D. Sequential directed ortho metalation−boronic acid cross-coupling reactions. A general regiospecific route to oxygenated dibenzo[*b*,*d*]pyran-6-ones related to ellagic acid. *J. Org. Chem.* **1991**, *56*, 3763–3768.
(44) Ku, Y.-Y.; Grieme, T.; Raje, P.; Sharma, P.; Morton, H. E.; Rozema, M.; King, S. A. A practical and scaleable synthesis of A-224817.0, a novel nonsteroidal ligand for the glucocorticoid receptor. *J. Org. Chem.* **2003**, *68*, 3238–3240.
(45) Srebnik, M.; Mechoulam, R.; Yona, I. Halogenation of phenols and phenyl ethers with potassium halides in the presence of 18-crown-6 on oxidation with *m*-chloroperbenzoic acid. *J. Chem. Soc., Perkin. Trans. 1* **1987**, 1423–1427.
(46) Ludt, R. E.; Griffiths, J. S.; McGrath, K. N.; Hauser, C. R. Comparison of the synthetic utility of *n*-butyllithium and lithium diisopropylamide in the metalations of *N*,*N*-dialkyltoluamides. *J. Org. Chem.* **1973**, *38*, 1668–1674.
(47) Alonso, E.; Ramón, D. J.; Yus, M. Naphthalene-catalysed lithiation of *N*,*N*-diisopropylbenzamide and its methoxy derivatives. *Tetrahedron* **1998**, *54*, 13629–13638.
(48) Fürstner, A.; Seidel, G. Shortcut syntheses of naturally occurring 5-alkylresorcinols with DNA-cleaving properties. *J. Org. Chem.* **1997**, *62*, 2332–2336.
(49) Novák, J.; Salemink, C. A. Cannabis. Part 27. Synthesis of 8-, 10-, and 11-oxygenated cannabinols. *J. Chem. Soc., Perkin Trans. 1* **1983**, 2867–2871.
(50) Khanolkar, A. D.; Abadji, V.; Lin, S.; Hill, W. A. G.; 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.
(51) Mukherjee, S.; Adams, M.; Whiteaker, K.; Daza, A.; Kage, K.; Cassar, S.; Meyer, M.; Yao, B. B. Species comparison and pharmacological characterization of rat and human CB_2 cannabinoid receptors. *Eur. J. Pharmacol.* **2004**, *505*, 1–9.
(52) Lin, S.; Khanolkar, A. D.; Fan, P.; Goutopoulos, A.; Qin, C.; Papahadjis, D.; Makriyannis, A. Novel analogues of arachidonylethanolamide (anandamide): Affinities for the CB1 and CB2 cannabinoid receptors and metabolic stability. *J. Med. Chem.* **1998**, *41*, 5353–5361.
(53) McLaughlin, P. J.; Qian, L.; Wood, J. T.; Wisniecki, A.; Winston, K. M.; Swezey, L. A.; Ishiwari, K.; Betz, A. J.; Pandarinathan, L.; Xu, W.; Makriyannis, A.; Salamone, J. D. Suppression of food intake and food-reinforced behavior produced by the novel CB1 receptor antagonist/inverse agonist AM 1387. *Pharmacol., Biochem. Behav.* **2006**, *83*, 396–402.
(54) Lan, R.; Liu, Q.; Fan, P.; Lin, S.; Fernando, S. R.; McCallion, D.; Pertwee, R.; Makriyannis, A. Structure−activity relationships of pyrazole derivatives as cannabinoid receptor antagonists. *J. Med. Chem.* **1999**, *42*, 769–776.
(55) Ross, R. A.; Brockie, H. C.; Stevenson, L. A.; Murphy, V. L.; Templeton, F.; Makriyannis, A.; Pertwee, R. G. Agonist-inverse agonist characterization at CB_1 and CB_2 cannabinoid receptors of L759633, L759656, and AM630. *Br. J. Pharmacol.* **1999**, *126*, 665–672.
(56) Zimmer, A.; Zimmer, A. M.; Hohmann, A. G.; Herkenham, M.; Bonner, T. I. Increased mortality, hypoactivity, and hypoalgesia in cannabinoid CB1 receptor knockout mice. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 5780–5785.
(57) Dodd, P. R.; Hardy, J. A.; Oakley, A. E.; Edwardson, J. A.; Perry, E. K.; Delaunoy, J.-P. A rapid method for preparing synaptosomes: Comparison with alternative procedures. *Brain Res.* **1981**, *226*, 107–118.
(58) Cheng, Y.-C.; Prusoff, W. H. Relationship between the inhibition constant (K_I) and the concentration of inhibitor which causes 50% inhibition (I_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.
(59) Hargreaves, K.; Dubner, R.; Brown, F.; Flores, C.; Joris, J. A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. *Pain* **1988**, *32*, 77–88.
(60) Tallarida, R. J.; Murray, R. B. *Manual of Pharmacological Calculations with Computer Programs.*, 2nd ed.; Springer: New York, 1987; pp 150.

JM070441U