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1-Bromo-3-(1',1'-dimethylalkyl)-1-deoxy- Δ^8 -tetrahydrocannabinols: New Selective Ligands for the Cannabinoid CB₂ Receptor

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Abstract

Δ^8 -Tetrahydrocannabinol (**26**), 3-(1',1'-dimethylbutyl)-(**12**), 3-(1',1'-dimethylpentyl)-(**13**), 3-(1',1'-dimethylhexyl)-(**14**) and 3-(1',1'-dimethylheptyl)- Δ^8 -tetrahydrocannabinol (**15**) have been converted into the corresponding 1-bromo-1-deoxy- Δ^8 -tetrahydrocannabinols (**25**, **8–11**). This was accomplished using a protocol developed in our laboratory in which the trifluoromethanesulfonate of a phenol undergoes palladium mediated coupling with pinacolborane. Reaction of this dioxaborolane with aqueous-methanolic copper (II) bromide provides the aryl bromide. The affinities of these bromo cannabinoids for the cannabinoid CB₁ and CB₂ receptors were determined. All of these compounds showed selectivity for the CB₂ receptor and one of them, 1-bromo-1-deoxy-3-(1',1'-dimethylhexyl)- Δ^8 -tetrahydrocannabinol (**10**), exhibits 52-fold selectivity for this receptor with good (28 nM) affinity.

Keywords

CB₁ receptor; CB₂ receptor; nontraditional cannabinoids; 1-deoxy cannabinoids

1. Introduction

The modern era of the study of cannabinoids began in 1964 with the elucidation of the structure of Δ^9 -tetrahydrocannabinol (Δ^9 -THC, **1**) by Gaoni and Mechoulam.¹ Subsequently, a comprehensive set of structure-activity relationships (SAR) was developed based upon the dibenzopyran skeleton of THC.^{2–4} These SAR include *inter alia* the principles that a C-3 side chain of five to seven carbon atoms and a C-1 phenolic hydroxyl group are necessary for cannabinoid activity. Shortly after these SAR were formulated, a cannabinoid receptor in rat brain was identified.⁵ This G-protein coupled receptor, which is expressed primarily in the central nervous system, is now known as the CB₁ receptor and has been cloned.⁶ A nearly identical (97% homology) human receptor has also been identified.⁷ In 1993 a second cannabinoid (CB₂) receptor was identified and cloned;

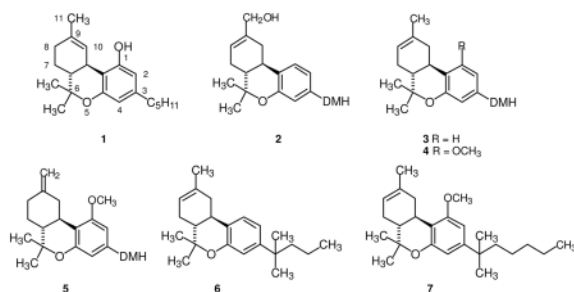
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Dedicated to Professor James M. Cook on the occasion of his 65th birthday.

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however, this receptor shows only 44% homology (68% in the transmembrane helical regions) with the initially described receptor.⁸ The CB₂ receptor is expressed primarily in the periphery, particularly in the immune system.^{10–14}

CB₁ receptor agonists, including endogenous compounds, are generally considered to be responsible for the overt centrally mediated effects of cannabinoids, such as their psychotropic, appetite stimulant and anti-nausea effects. It has been suggested that the CB₂ receptor is responsible for the immunomodulatory effects of cannabinoids,¹⁰ a conclusion that is supported by the fact that these effects are absent in CB₂ receptor knockout mice.¹⁵ Both CB₁ and CB₂ receptors are expressed in a variety of cancer cells and CB₁ and CB₂ receptor agonists have been found to inhibit tumor growth.^{16–17} There is also evidence that the CB₂ receptor is involved in inflammatory pain.^{18–25} Recent reviews have noted that the endocannabinoid system represents a potential therapeutic target and have suggested that development of selective ligands for the CB₂ receptor may result in new useful drugs for the treatment of diseases.^{26–27}



In contrast to the SAR data cited above, several years ago we found that 11-hydroxy-3-(1',1'-dimethylheptyl)-1-deoxy- Δ^8 -THC (**2**, DMH = dimethylheptyl), a THC analog lacking the 1-hydroxyl, has very high affinity for the CB₁ receptor ($K_i = 1.2 \pm 0.1$ nM), and is a potent cannabinoid *in vivo* in the mouse. Cannabinoid **2** also has exceptionally high affinity for the CB₂ receptor ($K_i = 0.032 \pm 0.019$ nM).²⁸ A second 1-deoxycannabinoid, 3-(1',1'-dimethylheptyl)-1-deoxy- Δ^8 -THC (**3**), is also potent *in vivo*, has good affinity for the CB₁ receptor ($K_i = 23 \pm 7$ nM), and has nearly ten-fold selectivity for the CB₂ receptor ($K_i = 2.9 \pm 1.6$ nM). A group at Merck Frosst also described 1-deoxy- Δ^8 -THC-DMH (**3**) at approximately the same time. In addition, they reported that 1-methoxy- Δ^8 -THC-DMH (**4**), and 1-methoxy- $\Delta^9(11)$ -THC-DMH (**5**) have affinity for the CB₂ receptor in the 20 nM range and negligible affinity for the CB₁ receptor.²⁹

Based upon a combination of our results and those of the Merck Frosst group, we synthesized a series of 1-deoxy- Δ^8 -THC analogs.³⁰ Several of these compounds have high affinity for the CB₂ receptor with little affinity for the CB₁ receptor and one in particular, JWH-133, 3-(1',1'-dimethylbutyl)-1-deoxy- Δ^8 -THC (**6**), has very high affinity for the CB₂ receptor ($K_i = 3.4 \pm 1$ nM) and exhibits 200-fold selectivity relative to the CB₁ receptor. Subsequently, series of 1-methoxy-11-hydroxy-1-deoxy- and 11-hydroxy-1-methoxy- Δ^8 -THC analogs were synthesized and their pharmacology was evaluated.³¹ Of this group of compounds, 3-(1',1'-dimethylhexyl)-1-methoxy- Δ^8 -THC (JWH-229, **7**) showed the greatest selectivity with $K_i = 18 \pm 2$ nM at the CB₂ receptor and $K_i = 3134 \pm 110$ nM at the CB₁ receptor.

We have recently developed a concise and efficient procedure for converting a phenol to the corresponding aryl bromide.³² This sequence proceeds from the phenol to the corresponding trifluoromethanesulfonate, followed by palladium mediated reaction with pinacolborane to afford a boronate. Reaction of this boronate with copper (II) bromide

provides the aryl bromide. Simple aryl halides and those containing electron releasing groups proceed in 60% to 88% overall yield. The presence of electron withdrawing groups in the phenol considerably attenuates the yield. To extend the scope of this synthetic protocol and to investigate the effect upon affinity for the cannabinoid receptors of 1-bromo- Δ^8 -THC analogs, we have applied this reaction sequence to Δ^8 -THC and four 3-(1',1'-dimethylalkyl)- Δ^8 -THCs. Also, based upon the pharmacology of the 1-deoxy- and 1-methoxy- Δ^8 -THCs, it was considered possible that one or more of these bromo cannabinoids would exhibit selectivity for the CB₂ receptor.

2. Results

Based upon the selectivity for the CB₂ receptor shown by JWH-133 and JWH-229 and related compounds,^{29–31} the decision was made to synthesize 1-bromo-3-(1',1'-dimethylalkyl)-1-deoxy- Δ^8 -THC analogs with a side chain of four to seven carbon atoms (**8–11**; Scheme 1). The 3-(1',1'-dimethylalkyl)- Δ^8 -THC analogs (**12–15**) were prepared as previously described by acid catalyzed reaction of the appropriate substituted resorcinol (**16–19**) with menthadienol (**20**).³³ Conversion into the trifluoromethanesulfonate, followed by palladium mediated coupling with pinacolborane provided boronates **21** to **24** in 62% to 78% yield. Although initially the conversion of boronates **21**, **23** and **24** to the corresponding aryl bromides was accomplished using our published conditions,³² considerable difficulty was encountered in successfully repeating the reaction. In the original report of the conversion of boronic acids to aryl halides, Nesmejanow *et al.* carried out the reaction of the boronic acid with copper (II) bromide in aqueous media.³⁴ When our earlier procedure was modified to employ an approximately 1:3 mixture of water and methanol, the reaction of boronates **21** to **24** proceeded smoothly and reproducibly to provide 1-bromo-1-deoxy- Δ^8 -THC analogs **8** (JWH-382), **9** (JWH-458), **10** (JWH-393) and **11** (JWH-393) in 66% to 85% yield. In addition to the four 1-bromo-3-(1',1'-dimethylalkyl)-1-deoxy- Δ^8 -THC analogs (**8–11**), the parent compound, 1-bromo-1-deoxy- Δ^8 -THC (**25**, JWH-460) was prepared by the sequence shown in Scheme 1, but using Δ^8 -THC (**26**) in place of **12** to **15**.

The affinities of 1-bromo- Δ^8 -THC analogs, **8** to **11** and **25**, for the CB₁ receptor were determined by measuring their ability to inhibit binding of the potent synthetic cannabinoid [³H]CP-55,940 to a membrane preparation from rat brain as described by Compton *et al.*³⁵ Affinities for the CB₂ receptor were determined by measuring the ability of the compounds to inhibit [³H]CP-55,940 binding to a cloned human receptor preparation using the procedure described by Showalter *et al.*³⁶ The results of these determinations are summarized in Table 1. Also included in Table 1 are the receptor affinities for Δ^8 -THC (**1**), JWH-133 (**6**) and JWH-229 (**7**).

As presented in Table 1, none of these 1-bromo-1-deoxy- Δ^8 -THC analogs have significant affinity for the CB₁ receptor and two compounds, JWH-460 and JWH-382, respectively, 1-bromo-1-deoxy- Δ^8 -THC (**25**) and the 3-dimethylbutyl analog (**8**), have virtually no affinity for this receptor with K_i > 10,000 nM. In contrast to their lack of affinity for the CB₁ receptor, all five of these 1-bromo- Δ^8 -THC analogs bind to the CB₂ receptor and three of them, JWH-458 (**9**), JWH-383 (**10**) and JWH-383 (**11**), have moderate to good affinity for this receptor with K_i = 28 – 71 nM. Selectivity for the CB₂ (vs. CB₁) receptor ranges from 16- to 52-fold. JWH-393 (**10**) has the greatest (52-fold) selectivity and highest affinity for this receptor, with K_i = 28 ± 2 nM.

The receptor binding data are somewhat similar to those we reported several years ago for a series of 3-(1',1'-dimethylalkyl)-1-methoxy- Δ^8 -THC analogs.³¹ In the methoxy series, the dimethylbutyl compound (JWH-214) has no affinity for the CB₁ receptor with K_i > 10,000

nM, which is the same as that of the corresponding 1-bromo-1-deoxy- Δ^8 -THC (JWH-382, **8**). The 1-methoxy-dimethylpentyl-(JWH-226), hexyl-(JWH-229, **7**) and heptyl-(JWH-143) Δ^8 -THCs have K_i values for the CB₁ receptor that range from 713 – 4001 nM. While CB₁ affinities are slightly better for the corresponding analogs of the 1-bromo-1-deoxy- Δ^8 -THC series, the K_i values for 1',1'-dimethylpentyl (JWH-458, **9**), dimethylhexyl (JWH-393, **10**) and dimethylheptyl (JWH-383, **11**) analogs are still poor, ranging from 562 – 1444 nM.

The CB₂ receptor affinities of the corresponding analogs of the 1-methoxy- Δ^8 -THC and 1-bromo- Δ^8 -THC series are also very similar. The 1-bromo-3-(1',1'-dimethylbutyl) compound (JWH-382, **8**) and the 1-methoxy THC analog (JWH-214) have the least affinity, with $K_i = 265 \pm 17$ nM and 325 ± 70 nM, respectively, whereas the dimethylhexyl Δ^8 -THC analogs, 1-bromo (JWH-393, **10**) and 1-methoxy (JWH-229, **7**), have the best affinities, with $K_i = 28 \pm 2$ and 18 ± 2 , respectively. Notably, all analogs in both the 1-bromo and 1-methoxy series show some degree of selectivity for the CB₂ receptor, with the highest selectivity seen in the dimethylhexyl Δ^8 -THC analogs. The 1-methoxy compound, (JWH-229, **7**) is 174-fold selective for this receptor, while the 1-bromo analog (JWH-393, **10**) is 52-fold selective. While still maintaining selectivity for the CB₂ receptor, 1-bromo-3-(1',1'-dimethylheptyl)-1-deoxy- Δ^8 -THC (JWH-383, **11**) and the 1-methoxy analog (JWH-143) exhibit only 27- and 12-fold selectivity, respectively.

Two of these bromocannabinoids, JWH-393 (**10**) and JWH-383 (**11**), have significant affinity for the CB₂ receptor ($K_i = 28 \pm 2$ and 34 ± 2 nM, respectively) and are from moderately to highly selective for the CB₂ receptor. In order to evaluate the efficacy of these compounds, their ability to stimulate GTP γ S binding was determined. This is a functional assay which measures G-protein coupled receptor activation using [³⁴S]GTP γ S binding. Chinese Hamster Ovary (CHO) cells stably expressing the human CB₂ receptor were employed in this determination (see Experimental). The results of these determinations are summarized in Table 2. The stimulation is normalized to that produced by 3 μ M CP-55,940, a maximally effective concentration of this very efficacious standard cannabinoid agonist. In addition to JWH-393 (**10**) and JWH-383 (**11**), the [³⁴S]GTP γ S binding for two highly CB₂ selective cannabinoids, JWH-133 (**6**) and JWH-229 is included in Table 2.38

As indicated in Table 2 both JWH-393 (**10**) and JWH-383 (**11**) are moderately potent in the [³⁴S]GTP γ S assay with EC₅₀ values 28.6 ± 6.3 nM for JWH-393 (**10**) and 15.0 ± 2.3 nM for JWH-383 (**11**). Both of these CB₂ receptor ligands are full agonists at the CB₂ receptor with E_{max} values of $98.3 \pm 6.8\%$ (JWH-393) and $91.0 \pm 6.7\%$ (JWH-383), relative to CP-55,940.

3. Discussion and Conclusions

In both the 1-bromo and 1-methoxy series, the 3-(1',1'-dimethylhexyl) compounds have useful selectivity for the CB₂ receptor and both have similar affinity for this receptor, however the bromo analog is more efficacious. The similarity between the 1-methoxy and 1-bromo cannabinoids may be due to electronic rather than steric effects. As aromatic substituents, both bromine and methoxyl are inductively electron withdrawing and electron releasing by resonance. Based upon the rate of racemization of chiral biaryls, the effective size of a bromine substituent is larger than that of a methoxyl.³⁹ If steric effects contributed significantly to the receptor affinities of 1-methoxy and 1-bromo THC derivatives, greater differences in the affinities between the two series of compounds would be expected.

In summary, we have developed a new class of selective ligands for the cannabinoid CB₂ receptor and have extended the scope of our procedure for converting phenols to alkyl bromides to the moderately hindered 1-position of the traditional cannabinoid molecule.³²

4. Experimental

4.1 General

^1H and ^{13}C NMR spectra were recorded on Bruker 300AC and JEOL 500 spectrometers. Mass spectral analyses were performed on a Shimadzu QP2010 capillary gas chromatograph/mass spectrometer equipped with a mass sensitive detector at 1.01 kV. HRMS data were obtained in the Mass Spectrometry Laboratory, School of Chemical Sciences, University of Illinois. Ether and THF were distilled from Na-benzophenone ketyl immediately before use, and other solvents were purified using standard procedures. Column chromatography was carried out on Sorbent Technologies silica gel (32 – 63 μ) using the indicated solvents as eluents. All new compounds were homogeneous to GLC and ^{13}C NMR. All target compounds were at least 95% pure by GLC.

4.2 Δ^8 -Tetrahydrocannabinyl trifluoromethanesulfonate

To a solution of 0.71 g (2.26 mmol) of Δ^8 -tetrahydrocannabinol33 (**26**) and 0.89 g (3.17 mmol) of trifluoromethanesulfonic anhydride in 12 mL of dichloromethane at 0 °C, was added 0.38 g (4.80 mmol) of pyridine. The solution was allowed to warm to ambient temperature, stirred for 3 h, diluted with ether and quenched with 10 mL of 1M HCl. The organic layer was washed with successive portions of aqueous NaHCO_3 , brine, dried (MgSO_4) and concentrated in vacuo. The crude product was purified by flash chromatography (petroleum ether/ether, 7:3) to afford 0.73 g (73%) of Δ^8 -tetrahydrocannabinyl trifluoromethanesulfonate: ^1H NMR (500 MHz, CDCl_3) δ 0.91 (t, J = 7.0 Hz, 3H), 1.27 (s, 3H), 1.29–1.34 (m, 4H), 1.41 (s, 3H), 1.56–1.62 (m, 2H), 1.73 (s, 3H), 1.78–1.93 (m, 3H), 2.12–2.21 (m, 1H), 2.54 (t, J = 7.0 Hz, 2H), 2.79–2.99 (m, 2H), 5.48 (d, J = 3.0 Hz, 1H), 6.62 (d, J = 2.0 Hz, 1H), 6.69 (d, J = 2.0 Hz, 1H); ^{13}C NMR (125.8 MHz, CDCl_3) δ 13.9, 18.3, 22.5, 23.3, 27.3, 27.7, 30.4, 31.3, 31.8, 35.3, 35.7, 44.6, 113.5, 116.9, 117.6, 119.4, 133.9, 143.6, 148.5, 155.1; MS (EI) m/z (rel intensity) 363 (97), 403 (90), 446(100).

4.3 3-(1',1'-Dimethylbutyl)- Δ^8 -tetrahydrocannabinyl trifluoromethanesulfonate

This triflate was prepared by the procedure employed for the preparation of Δ^8 -tetrahydrocannabinyl trifluoromethanesulfonate. From 0.33 g (1.0 mmol) of 3-(1',1'-dimethylbutyl)- Δ^8 -tetrahydrocannabinol33 (**12**), there was obtained after chromatography (petroleum ether), 0.38 g (82%) of 3-(1',1'-dimethylbutyl)- Δ^8 -tetrahydrocannabinyl trifluoromethanesulfonate as a clear liquid: ^1H NMR (500 MHz, CDCl_3) δ 0.82 (t, J = 7.3 Hz, 3H), 1.02–1.12 (m, 2H), 1.13 (s, 3H), 1.23 (s, 3H), 1.24 (s, 3H), 1.40 (s, 3H), 1.48–1.54 (m, 2H), 1.71 (s, 3H), 1.78 (td, J = 4.3, 11.4 Hz, 1H), 1.81–1.96 (m, 2H), 2.12–2.20 (m, 1H), 2.84 (td, J = 4.6, 11.0 Hz, 1H), 2.93 (dd, J = 4.4, 16.2 Hz, 1H), 5.44 (d, J = 3.2 Hz, 1H), 6.72 (d, J = 1.8 Hz, 1H), 6.79 (d, J = 1.8 Hz, 1H); ^{13}C NMR (125.8 MHz, CDCl_3) δ 14.6, 17.8, 18.3, 23.3, 27.3, 27.6, 28.3, 28.7, 31.6, 35.6, 37.7, 44.6, 46.7, 77.5, 111.4, 115.4, 116.5, 118.6 (q, $J_{\text{C,F}}$ = 321 Hz), 119.9, 133.9, 148.6, 150.8, 154.8; GC/MS (EI) m/z (rel intensity) 460 (61), 445 (6), 419 (11), 418 (37), 417 (100), 404 (6), 377 (46), 349 (24), 285 (12), 242 (4), 201 (6), 85 (31).

4.4 3-(1',1'-Dimethylpentyl)- Δ^8 -tetrahydrocannabinyl trifluoromethanesulfonate

This triflate was prepared by the procedure described for the synthesis of Δ^8 -tetrahydrocannabinyl trifluoromethanesulfonate. From 0.55 g (1.61 mmol) of 3-(1',1'-dimethylpentyl)- Δ^8 -tetrahydrocannabinol33 (**13**) there was obtained 0.53 g (70%) of pure 3-(1',1'-dimethylpentyl)- Δ^8 -tetrahydrocannabinyl trifluoromethanesulfonate after flash chromatography (petroleum ether/ether, 7:3): ^1H NMR (500 MHz, CDCl_3) δ 0.84 (t, J = 7.0 Hz, 3H), 1.03–1.11 (m, 2H), 1.13 (s, 3H), 1.15–1.21 (m, 6H), 1.26 (s, 3H), 1.41 (s, 3H),

1.51–1.54 (m, 3H), 1.72, (s, 3H), 2.10–2.14 (m, 2H), 2.81 (td, $J = 4.9, 10.7$ Hz, 1H), 2.94 (dd, $J = 4.0, 17.0$ Hz, 1H), 5.45 (d, $J = 3.0$ Hz, 1H), 6.72 (d, $J = 2.0$ Hz, 1H), 6.79 (d, $J = 2.0$ Hz, 1H); ^{13}C NMR (125.8 MHz, CDCl_3) δ 14.1, 18.5, 22.5, 23.2, 27.1, 27.7, 28.4, 28.6, 31.5, 31.7, 35.5, 37.4, 44.2, 44.7, 77.2, 111.3, 115.4, 116.4, 116.6, 116.2, 118.4, 119.3, 134.2, 148.5, 150.1, 154.7; MS (EI) m/z (rel intensity) 391 (50), 417 (100), 431 (30), 474 (90).

4.5 3-(1',1'-Dimethylhexyl)- Δ^8 -tetrahydrocannabinyl trifluoromethanesulfonate

This triflate was prepared using by the procedure employed for the synthesis of Δ^8 -tetrahydrocannabinyl trifluoromethanesulfonate. From 0.29 g (0.81 mmol) of 3-(1',1'-dimethylhexyl)- Δ^8 -tetrahydrocannabinol33 (**14**), there was obtained, after chromatography (petroleum ether), 0.37 g (93%) of 3-(1',1'-dimethylhexyl)- Δ^8 -tetrahydrocannabinyl trifluoromethanesulfonate as a viscous yellow oil: ^1H NMR (500 MHz, CDCl_3) δ 0.82 (t, $J = 7.4$ Hz, 3H), 1.01–1.08 (m, 2H), 1.13 (s, 3H), 1.14–1.22 (m, 4H), 1.23 (s, 3H), 1.24 (s, 3H), 1.41 (s, 3H), 1.49–1.54 (m, 2H), 1.71 (s, 3H), 1.78 (td, $J = 4.0, 11.3$ Hz, 1H), 1.81–1.95 (m, 2H), 2.13–2.20 (m, 1H), 2.84 (td, $J = 4.8, 10.9$ Hz, 1H), 2.93 (dd, $J = 3.9, 16.2$ Hz, 1H), 5.44 (d, $J = 4.2$ Hz, 1H), 6.71 (d, $J = 1.8$ Hz, 1H), 6.79 (d, $J = 1.8$ Hz, 1H); ^{13}C NMR (125.8 MHz) δ 14.0, 18.3, 22.5, 23.3, 24.2, 27.3, 27.6, 28.4, 28.6, 31.6, 32.4, 35.6, 37.7, 44.2, 44.5, 77.5, 111.4, 115.4, 116.5, 118.7 (q, $J_{\text{C,F}} = 308$), 119.4, 134.0, 148.6, 150.9, 154.8; GC/MS (EI) m/z (rel intensity) 488 (36), 473 (3), 445 (14), 432 (6), 419 (12), 418 (41), 417 (100), 405 (43), 349 (37), 299 (6), 285 (33), 269 (8), 257 (9), 241 (16), 227 (9), 216 (17), 201 (34), 187 (15), 121 (22), 107 (15), 91 (16), 71 (48), 57 (45).

4.6 3-(1',1'-Dimethylheptyl)- Δ^8 -tetrahydrocannabinyl trifluoromethanesulfonate

This triflate was prepared by the procedure employed for the preparation of Δ^8 -tetrahydrocannabinyl trifluoromethanesulfonate. From 0.31 g (0.84 mmol) of 3-(1',1'-dimethylheptyl)- Δ^8 -tetrahydrocannabinol33 (**15**), there was obtained, after chromatography (petroleum ether), 0.30 g (71%) of 3-(1',1'-dimethylheptyl)- Δ^8 -tetrahydrocannabinyl trifluoromethanesulfonate as a viscous yellow oil: ^1H NMR (500 MHz, CDCl_3) δ 0.84 (t, $J = 7.1$ Hz, 3H), 0.99–1.08 (m, 2H), 1.13 (s, 3H), 1.15–1.22 (m, 6H), 1.23 (s, 3H), 1.24 (s, 3H), 1.40 (s, 3H), 1.49–1.55 (m, 2H), 1.71 (s, 3H), 1.78 (td, $J = 4.1, 11.3$ Hz, 1H), 1.81–1.95 (m, 2H), 2.13–2.20 (m, 1H), 2.84 (td, $J = 4.8, 10.9$ Hz, 1H), 2.93 (dd, $J = 4.1, 16.5$ Hz, 1H), 5.44 (d, $J = 4.2$ Hz, 1H), 6.71 (d, $J = 1.8$ Hz, 1H), 6.79 (d, $J = 1.8$ Hz, 1H); ^{13}C NMR (125.8 MHz, CDCl_3) δ 14.0, 18.3, 22.6, 23.3, 24.5, 27.3, 27.6, 28.4, 28.6, 29.8, 31.6, 31.7, 35.6, 37.7, 44.2, 44.6, 77.5, 111.4, 115.4, 116.5, 118.6 (q, $J_{\text{C,F}} = 320$ Hz), 119.4, 133.9, 148.6, 150.9, 154.8; GC/MS (EI) m/z (rel intensity) 502 (69), 487 (6), 459 (17), 446 (7), 434 (5), 419 (50), 418 (41), 417 (100), 349 (20), 285 (16), 201 (9), 121 (11).

4.7 1-Deoxy- Δ^8 -tetrahydrocannabinyl-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (**27**)

To a suspension of 0.20 g (0.448 mmol) of Δ^8 -tetrahydrocannabinyl trifluoromethanesulfonate and 0.010 g (0.0135 mmol) of 1',1''-bis(diphenylphosphino)ferrocene-palladium(II)dichloride dichloromethane complex in 15 mL of acetonitrile under argon was slowly added 1.41 g (13.5 mmol) of triethylamine and the reaction was stirred at reflux overnight. The mixture was cooled to 0 °C, quenched by the addition of 10 mL of 1M HCl, diluted with diethyl ether, washed with brine, dried (MgSO_4), and concentrated in vacuo. The resultant red oil was purified by flash chromatography (petroleum ether/ether, 8:2) to give 0.12 g (65%) of 1-deoxy- Δ^8 -tetrahydrocannabinyl-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (**27**): ^1H NMR (500 MHz, CDCl_3) δ 0.91 (t, $J = 7.0$ Hz, 3H), 1.13 (s, 3H), 1.28–1.33 (m, 4H), 1.34 (s, 3H), 1.36 (s, 6H), 1.38 (s, 6H), 1.41 (s, 3H), 1.58–1.64 (m, 2H), 1.69 (s, 3H), 1.73–1.93 (m, 2H), 2.11–2.22 (m, 1H), 2.53 (t, $J = 7.0$ Hz, 1H), 2.98 (dt, $J = 4.0, 17.0$ Hz, 1H), 5.41 (d, $J = 3.0$ Hz, 1H), 6.72 (d, $J = 2.0$ Hz, 1H), 7.11 (d, $J = 2.0$ Hz, 1H); ^{13}C NMR (125.8 MHz, CDCl_3) δ 14.0, 18.5, 22.6, 23.2, 24.1, 24.7, 25.1, 27.6, 28.2, 29.7, 30.9, 31.7, 33.5, 35.4, 40.5, 45.5,

83.5, 119.9, 120.0, 128.1, 128.6, 134.5, 141.4, 152.9; MS (EI) m/z (rel intensity) 281 (65), 341 (100), 424 (80).

4.8 1-Deoxy-3-(1',1'-dimethylbutyl)- Δ^8 -tetrahydrocannabinyl-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (21)

Dioxaborolane **21** was prepared using the procedure described for dioxaborolane **27**. From 0.37 g (0.80 mmol) of 3-(1',1'-dimethylbutyl)- Δ^8 -tetrahydrocannabinyl trifluoromethanesulfonate there was obtained, after 4 h at reflux 0.22 g (62%) of **21** as a yellow oil: ^1H NMR (500 MHz, CDCl_3) δ 0.81 (t, $J = 7.3$ Hz, 3H), 1.05–1.14 (m, 2H), 1.15 (s, 3H), 1.25 (s, 6H), 1.35 (s, 6H), 1.36 (s, 6H), 1.39 (s, 3H), 1.50–1.57 (m, 2H), 1.68 (s, 3H), 1.74 (td, $J = 4.4, 11.4$ Hz, 1H), 1.82–1.93 (m, 2H), 2.10–2.19 (m, 1H), 2.60 (dd, $J = 3.9, 14.9$ Hz, 1H), 2.98 (td, $J = 4.7, 11.0$ Hz, 1H), 5.44 (d, $J = 3.2$ Hz, 1H), 6.83 (d, $J = 2.3$ Hz, 1H), 7.22 (d, $J = 1.8$ Hz, 1H); ^{13}C NMR (125.8 MHz, CDCl_3) δ 14.8, 18.0, 18.5, 23.2, 24.5, 25.2, 27.6, 28.1, 28.6, 28.9, 33.4, 37.3, 40.4, 45.4, 46.9, 76.1, 83.4, 117.8, 120.0, 125.3, 128.1, 134.5, 148.4, 152.6; GC/MS (EI) m/z (rel intensity) 438 (90), 397 (8), 396 (47), 395 (100), 394 (20), 355 (68), 327 (23), 311 (15), 295 (31), 281 (16), 207 (89), 191 (12), 133 (10), 119 (11), 101 (22), 85 (34).

4.9 1-Deoxy-3-(1',1'-dimethylpentyl)- Δ^8 -tetrahydrocannabinyl-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (22)

Dioxaborolane **22** was prepared by the procedure employed for the synthesis of dioxaborolane **27**. From 0.21 g (0.421 mmol) of 3-(1',1'-dimethylpentyl)- Δ^8 -tetrahydrocannabinyl trifluoromethanesulfonate there was obtained 0.151 g (78%) of pure dioxaborolane **22** after flash chromatography (petroleum ether/ether, 9:1): ^1H NMR (500 MHz, CDCl_3) δ 0.82 (t, $J = 7.0$ Hz, 3H), 1.03–1.13 (m, 2H), 1.15 (s, 3H), 1.16–1.22 (m, 6H), 1.24 (s, 3H), 1.33 (s, 6H), 1.35 (s, 6H), 1.41 (s, 3H), 1.51–1.53 (m, 3H), 1.69 (s, 3H), 2.10–2.18 (m, 2H), 2.62 (td, $J = 4.9, 10.7$ Hz, 1H), 2.97 (dd, $J = 4.0, 17.0$ Hz, 1H), 5.44 (d, $J = 3.0$ Hz, 1H), 6.82 (d, $J = 2.0$ Hz, 1H), 7.21 (d, $J = 2.0$ Hz, 1H); ^{13}C NMR (125.8 MHz) δ 14.2, 18.5, 22.5, 23.1, 27.4, 28.0, 28.6, 28.9, 31.8, 32.4, 37.6, 40.1, 44.6, 45.1, 76.1, 83.2, 117.6, 119.8, 125.1, 127.9, 134.4, 148.5, 152.4; MS (EI) m/z (rel intensity) 368 (20), 369 (75), 395 (100), 452 (80).

4.10 1-Deoxy-3-(1',1'-dimethylhexyl)- Δ^8 -tetrahydrocannabinyl-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (23)

Dioxaborolane **23** was prepared using the procedure employed for the synthesis of Δ^8 -tetrahydrocannabinyl-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (**27**). From 0.36 g (0.74 mmol) of 3-(1',1'-dimethylhexyl)- Δ^8 -tetrahydrocannabinyl trifluoromethanesulfonate there was obtained, after 4 h at reflux, 0.26 g (76%) of **24** as a yellow oil: ^1H NMR (500 MHz, CDCl_3) δ 0.82 (t, $J = 7.3$ Hz, 3H), 1.09 (sextet, $J = 7.3$ Hz, 2H), 1.15 (s, 3H), 1.16–1.23 (m, 4H), 1.24 (s, 3H), 1.25 (s, 3H), 1.35 (s, 6H), 1.36 (s, 6H), 1.39 (s, 3H), 1.51–1.58 (m, 2H), 1.68 (s, 3H), 1.74 (td, $J = 4.3, 11.6$ Hz, 1H), 1.82–1.93 (m, 2H), 2.10–2.19 (m, 1H), 2.60 (dd, $J = 4.3, 14.9$ Hz, 1H), 2.99 (td, $J = 4.9, 11.1$ Hz, 1H), 5.44 (d, $J = 3.7$ Hz, 1H), 6.83 (d, $J = 2.3$ Hz, 1H), 7.22 (d, $J = 1.8$ Hz, 1H); ^{13}C NMR (125.8 MHz, CDCl_3) δ 14.1, 18.5, 22.5, 23.2, 24.3, 24.5, 25.2, 27.6, 28.1, 28.6, 29.0, 32.6, 33.4, 37.2, 40.4, 44.3, 45.4, 76.1, 83.4, 117.8, 120.0, 125.3, 128.1, 134.5, 148.5, 152.6; GC/MS (EI) m/z (rel intensity) 468 (6), 467 (33), 466 (100), 465 (25), 395 (71), 383 (32), 323 (9), 311 (6), 101 (8), 83 (13), 71 (8), 57 (14).

4.11 1-Deoxy-3-(1',1'-dimethylheptyl)- Δ^8 -tetrahydrocannabinyl-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (**24**)

Dioxaborolane **24** was prepared using the procedure employed for the synthesis of dioxaborolane **27**. From and 0.29 g (0.58 mmol) of 3-(1',1'-dimethylheptyl)- Δ^8 -tetrahydrocannabinyl trifluoromethanesulfonate there was obtained, after 4 h at reflux 0.21 g (76%) of **24** as a yellow oil: ^1H NMR (500 MHz, CDCl_3) δ 0.84 (t, $J = 6.9$ Hz, 3H), 1.02–1.12 (m, 2H), 1.15 (s, 3H), 1.16–1.23 (m, 6H), 1.24 (s, 3H), 1.25 (s, 3H), 1.35 (s, 6H), 1.36 (s, 6H), 1.39 (s, 3H), 1.52–1.58 (m, 2H), 1.68 (s, 3H), 1.74 (td, $J = 4.4, 11.6$ Hz, 1H), 1.82–1.93 (m, 2H), 2.10–2.19 (m, 1H), 2.60 (dd, $J = 4.4, 14.9$ Hz, 1H), 2.99 (td, $J = 4.8, 11.1$ Hz, 1H), 5.44 (d, $J = 3.7$ Hz, 1H, 1H), 6.82 (d, $J = 1.8$ Hz, 1H), 7.22 (d, $J = 2.2$ Hz, 1H); ^{13}C NMR (125.8 MHz, CDCl_3) δ 14.1, 18.5, 22.7, 23.2, 24.5, 24.6, 25.2, 27.6, 28.1, 28.6, 29.0, 30.0, 31.8, 33.4, 37.2, 40.4, 44.4, 45.4, 76.1, 83.4, 117.8, 120.0, 125.3, 128.1, 134.5, 148.5, 152.6; GC/MS (EI) m/z (rel intensity) 482 (6), 481 (35), 480 (100), 479 (23), 395 (93), 337 (16), 327 (10), 311 (10), 211 (4), 101 (10), 83 (16), 71 (10).

4.12 1-Bromo-1-deoxy- Δ^8 -tetrahydrocannabinol (**25**, JWH-460)

To a solution of 0.20 g (0.472 mmol) of dioxaborolane **27** suspended in 20 mL of H_2O was added 73 mL of MeOH, followed by 0.34 g (1.52 mmol) of copper(II) bromide in 5 mL of H_2O . The solution was heated at reflux for 10 h and concentrated in vacuo. The solution was cooled to ambient temperature, diluted with ether, washed with brine, dried (MgSO_4), and concentrated in vacuo. The resultant yellow oil was purified by flash chromatography (petroleum ether/ether, 8:2) to give 0.13 g (75%) of 1-bromo-1-deoxy- Δ^8 -tetrahydrocannabinol (**25**) as a colorless oil: ^1H NMR (500 MHz, CDCl_3) δ 0.91 (t, $J = 7.0$ Hz, 3H), 1.12 (s, 3H), 1.28–1.34 (m, 4H), 1.35 (s, 3H), 1.64 (s, 3H), 1.81–1.92 (m, 3H), 2.09–2.21 (m, 1H), 2.53 (t, $J = 7.0$ Hz, 2H), 2.54–2.59 (m, 2H), 2.71 (m, 1H), 3.41 (dt, $J = 4.0, 17.0$ Hz, 1H), 5.42 (d, $J = 3.0$ Hz, 1H), 6.59 (d, $J = 2.0$ Hz, 1H), 6.95 (d, $J = 2.0$ Hz, 1H); ^{13}C NMR (125.8 MHz, CDCl_3) δ 14.1, 18.1, 22.5, 23.4, 27.4, 28.3, 30.6, 31.5, 35.3, 36.4, 46.5, 117.1, 119.5, 123.5, 125.8, 134.8, 143.4, 154.8; MS (EI) m/z (rel intensity) 254 (45), 293 (100), 335 (30), 376 (75); HRMS calcd for $\text{C}_{21}\text{H}_{29}\text{OBr}$ 376.1402, found 376.1402; $[\alpha]_{\text{D}}^{20} -221^\circ$ (c 0.094, CHCl_3).

4.13 1-Bromo-1-deoxy-3-(1',1'-dimethylbutyl)- Δ^8 -tetrahydrocannabinol (**8**, JWH-382)

JWH-382 was prepared by the method employed for the synthesis of JWH-460. From 0.21 g (0.48 mmol) of 3-(1',1'-dimethylbutyl)- Δ^8 -tetrahydrocannabinyl-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (**21**) and 0.33 g (1.5 mmol) of copper (II) bromide there was obtained, after 4 h at reflux and column chromatography (petroleum ether), 0.16 g (85%) of JWH-382 as a viscous light yellow oil: ^1H NMR (500 MHz, CDCl_3) δ 0.82 (t, $J = 7.3$ Hz, 3H), 1.02–1.13 (m, 5H), 1.22 (s, 6H), 1.39 (s, 3H), 1.46–1.53 (m, 2H), 1.72 (s, 3H), 1.73–1.83 (m, 1H), 1.83–1.91 (m, 2H), 2.11–2.22 (m, 1H), 2.68 (td, $J = 4.4, 10.6$ Hz, 1H), 3.46 (dd, $J = 3.9, 16.7$ Hz, 1H), 5.44 (s, 1H), 6.72 (d, $J = 1.8$ Hz, 1H), 7.06 (d, $J = 1.8$ Hz, 1H); ^{13}C NMR (125.8 MHz, CDCl_3) δ 14.7, 17.9, 18.1, 23.4, 27.4, 28.3, 28.5, 28.7, 35.2, 36.3, 37.5, 46.4, 46.8, 76.9, 115.0, 119.5, 122.6, 123.1, 123.5, 134.8, 150.6, 154.5; GC/MS (EI) m/z (rel intensity) 392 (65), 390 (65), 349 (70), 347 (64), 309 (38), 307 (37), 279 (20), 185 (17), 121 (26), 91 (32), 85 (100), 77 (20); HRMS: m/z calcd for $\text{C}_{22}\text{H}_{31}\text{BrO}$: 390.1558; found: 390.1554, $[\alpha]_{\text{D}}^{20} -177^\circ$ (c 0.214, CHCl_3).

4.14 1-Bromo-1-deoxy-3-(1',1'-dimethylpentyl)- Δ^8 -tetrahydrocannabinol (**9**, JWH-458)

Dioxaborolane **22** was converted to 1-bromo-1-deoxy-3-(1',1'-dimethylpentyl)- Δ^8 -tetrahydrocannabinol by the procedure described for the synthesis of JWH-460. From 0.050 g (1.11 mmol) of **22** and 0.079 g (0.35 mmol) of copper(II) bromide there was obtained 0.36 g (80%) of pure JWH-458 as a colorless oil after flash chromatography (petroleum ether/

ether, 8:2): ^1H NMR (500 MHz, CDCl_3) δ 0.91 (t, $J = 7.0$ Hz, 3H), 1.12–1.21 (s, 5H), 1.22–1.35 (m, 9H), 1.41 (s, 3H), 1.51–1.65 (m, 2H), 1.86 (s, 3H), 1.85–1.95 (m, 2H), 2.13–2.28 (m, 1H), 2.69 (t, $J = 7.0$ Hz, 1H), 3.51 (dt, $J = 4.0, 17.0$ Hz, 1H), 5.47 (d, $J = 3.0$ Hz, 1H), 6.76 (d, $J = 2.0$ Hz, 1H), 7.08 (d, $J = 2.0$ Hz, 1H); ^{13}C NMR (125.8 MHz, CDCl_3) δ 14.1, 18.2, 23.3, 23.5, 26.9, 27.4, 28.3, 28.6, 28.7, 35.3, 36.4, 44.1, 46.5, 115.0, 119.5, 120.9, 122.9, 123.6, 150.7, 154.0; MS (EI) m/z (rel intensity) 321 (80), 349 (90), 364 (70), 404(100); HRMS calcd for $\text{C}_{23}\text{H}_{33}\text{OBr}$ 404.1715, found 404.1714; $[\alpha]_{\text{D}}^{20} -263^\circ$ (c 0.25, CHCl_3).

4.15 1-Bromo-1-deoxy-3-(1',1'-dimethylhexyl)- Δ^8 -tetrahydrocannabinol (10, JWH-393)

JWH-393 was prepared by the procedure employed for the synthesis of JWH-460. From 0.25 g (0.54 mmol) of dioxaborolane (**23**) and 0.38 g (1.7 mmol) of copper (II) bromide there was obtained, after 4 h at reflux and column chromatography (petroleum ether), 0.16 g (71%) of JWH-393 as a clear oil: ^1H NMR (500 MHz, CDCl_3) δ 0.83 (t, $J = 7.1$ Hz, 3H), 1.01–1.12 (m, 5H), 1.13–1.29 (m, 10H), 1.39 (s, 3H), 1.48–1.54 (m, 2H), 1.72 (s, 3H), 1.73–1.83 (m, 1H), 1.83–1.92 (m, 2H), 2.11–2.22 (m, 1H), 2.68 (td, $J = 4.4, 10.7$ Hz, 1H), 3.46 (dd, $J = 3.6, 16.5$ Hz, 1H), 5.44 (s, 1H), 6.72 (d, $J = 1.8$ Hz, 1H), 7.06 (d, $J = 1.8$ Hz, 1H); ^{13}C NMR (125.8 MHz, CDCl_3) δ 14.1, 18.1, 22.5, 23.4, 24.2, 27.4, 28.3, 28.6, 32.4, 35.2, 36.3, 37.4, 44.2, 46.4, 76.8, 115.0, 119.5, 122.6, 123.1, 123.5, 134.7, 150.6, 154.5; GC/MS (EI) m/z (rel intensity) 420 (100), 418 (97), 377 (55), 375 (47), 350 (94), 348 (83), 337 (86), 335 (78), 281 (30), 279 (32), 270 (19), 253 (12), 251 (13), 225 (17), 213 (11), 185 (15), 118 (11), 91 (12), 71 (12); HRMS: m/z calcd for $\text{C}_{24}\text{H}_{35}\text{BrO}$: 418.1871; found: 418.1876; $[\alpha]_{\text{D}}^{20} -277^\circ$ (c 0.11, CHCl_3).

4.16 1-Bromo-1-deoxy-3-(1',1'-dimethylheptyl)- Δ^8 -tetrahydrocannabinol (11, JWH-383)

JWH-383 was prepared using the procedure used for the synthesis of JWH-460. From 0.20 g (0.42 mmol) of dioxaborolane (**24**) and 0.29 g (1.3 mmol) of copper (II) bromide there was obtained, after 4 h at reflux and column chromatography (petroleum ether), 0.12 g (66%) of JWH-383 as a viscous light yellow oil: ^1H NMR (500 MHz, CDCl_3) δ 0.85 (t, $J = 6.4$ Hz, 3H), 1.01–1.12 (m, 5H), 1.14–1.29 (m, 12H), 1.39 (s, 3H), 1.47–1.54 (m, 2H), 1.72 (s, 3H), 1.73–1.83 (m, 1H), 1.83–1.92 (m, 2H), 2.11–2.22 (m, 1H), 2.68 (td, $J = 4.0, 10.4$ Hz, 1H), 3.46 (dd, $J = 3.6, 17.0$ Hz, 1H), 5.44 (s, 1H), 6.72 (s, 1H), 7.05 (s, 1H); ^{13}C NMR (125.8 MHz, CDCl_3) δ 14.1, 18.1, 22.6, 23.4, 24.5, 27.4, 28.3, 28.6 \times 2, 29.9, 31.7, 35.2, 36.3, 37.4, 44.3, 46.4, 76.8, 115.0, 119.5, 122.6, 123.1, 123.5, 134.8, 150.7, 154.5; GC/MS (EI) m/z (rel intensity) 434 (93), 432 (95), 391 (39), 389 (37), 352 (10), 351 (57), 350 (59), 349 (100), 348 (49), 347 (51), 279 (15), 121 (10), 85 (18), 71 (28); HRMS: m/z calcd for $\text{C}_{25}\text{H}_{37}\text{BrO}$: 432.2028; found: 432.2028; $[\alpha]_{\text{D}}^{20} -268^\circ$ (c 0.168, CHCl_3).

4.17 Receptor Binding Experiments

4.17.1 Materials—Frozen whole brains of male Sprague-Dawley rats were obtained from Harlan (Dublin, VA). CP-55,940 was provided by Pfizer (Groton, CT). [^3H]CP-55,940 (168 Ci/mmol) was purchased from NEN Life Science Products, Inc. (Boston, MA). Lipofectamine reagent was purchased from Life Technologies (Gaithersburg, MD). Human CB_2 cDNA was provided by Dr. Sean Munro (MRC Lab, Cambridge, UK). DMEM and geneticin was purchased from (GIBCO BRL, Grand Island, NY). Fetal clone II was purchased from Hyclone Laboratories, Inc. (Logan, UT). Aquasil was purchased from Pierce (Rockford, IL). GF/C glass-fiber filters (2.4 cm) were purchased from Baxter (McGaw Park, IL). Polyethylenimine and bovine serum albumin were purchased from Sigma Chemical Co. (St. Louis, MO). Scintillation vials and Budget Solve scintillation fluid were purchased from RPI Corp. (Mount Prospect, IL).

4.17.2 Membrane Preparation—Chinese hamster ovary cells stably expressing the human CB₂ receptor (CB₂-CHO) cells³⁸ were harvested in phosphate-buffered saline containing 1 μM EDTA and centrifuged at 500g. Cell pellets (for CB₂) or whole rat brains (for CB₁) were homogenized in 10 mL of membrane buffer (50 μM Tris-HCl, 1 μM EDTA, 3 μM MgCl₂, pH 7.4). The homogenate was centrifuged at 50,000 × g = for 10 min. The pellet was resuspended in membrane buffer to yield a protein concentration of approximately 1 mg/mL. The tissue preparation was divided into equal aliquots, frozen on dry ice, and stored at -70 °C.

4.18 Competition Binding Assays

4.18.1 Binding Assay Procedure—[³H]CP-55,940 (168 Ci/mmol) binding to membranes prepared from whole rat brain (CB₁) or CB₂-CHO cells (CB₂) was conducted as previously described.^{35,38,40} Briefly, CP-55,940 and all cannabinoid analogs were prepared by suspension in membrane buffer containing 5 mg/ml bovine serum albumin (assay buffer A) from a 1 mg/mL ethanolic stock (final concentration of no more than 0.4%). Competition curves were generated by incubating membranes with 1 nM of [³H]CP-55,940 with varying concentrations of unlabeled drugs for 1 hr at 30°C. Nonspecific binding was determined in the presence of 1 μM unlabeled CP,55,940. Binding was terminated by rapid filtration under vacuum through GF/B glass fiber filters (pretreated with polyethyleneimine (0.1%) for at least 2 hours), and radioactivity determined by liquid scintillation spectrophotometry. The assays were performed in triplicate, and the results represent the combined data from three individual experiments.

4.18.2 Data Analysis—Competition assays were conducted with 1 nM [³H]CP-55,940 and 6 concentrations (0.1 nM to 10 μM displacing ligands). Displacement IC₅₀ values were originally determined by unweighted least-squares linear regression of log concentration-percent displacement data and then converted to K_i values using the method of Cheng and Prusoff.⁴¹ All experiments were performed in triplicate and repeated 3–6 times. All data are reported as mean values ± SEM.

4.19 [³⁴S]GTPγS Binding Assays

4.19.1 Materials—All chemicals were from Sigma (St. Louis, MO) except the following: [³⁴S]GTPγS (1250 Ci/mmol) was purchased from Perkin Elmer Life Sciences (Boston, MA), DMEM/F-12 from Fischer Scientific (Pittsburg, PA), and Whatman GF/B glass fiber filters from Fisher Scientific (Pittsburgh, PA).

4.19.2 Membrane Preparations—CB₂-CHO cell membranes were cultured in a 50:50 mixture of DMEM and Ham F-12 supplemented with 100 U/ml penicillin, 100 Bg/ml streptomycin, 0.25 mg/ml G418, and 5% fetal calf serum. Cells were harvested by replacement of the media with cold phosphate-buffered saline containing 0.4% EDTA followed by agitation. Membranes were prepared by homogenization of cells in 50 μM Tris-HCl, 3 μM MgCl₂, 1 μM EGTA, pH 7.4, centrifugation at 50,000 × g for 10 minutes at 4 °C, and resuspension in the same buffer at 1.5 mg/ml. Membranes were stored at -80 °C until use.

4.20.3 [³⁴S]GTPγS Binding—Assays were conducted as previously described.³⁸ Prior to assays, samples were thawed on ice, centrifuged at 50,000 × g for 10 minutes at 4 °C, and resuspended in Assay Buffer B (50 μM Tris-HCl (pH 7.4), 3 μM MgCl₂, 0.2 μM EGTA, and 100 μM NaCl). Reactions containing 10 μg of membrane protein were incubated for 1.5 hr at 30°C in Assay Buffer B containing 10 μM GDP, 0.1 nM [³⁴S]GTPγS, 1 mg/mL bovine serum albumin, and various concentrations of drugs. Nonspecific binding was determined in the presence of 20 M unlabeled GTPγS. Reactions were terminated by rapid vacuum

filtration through GF/B glass fiber filters, and radioactivity was measured by liquid scintillation spectrophotometry.

4.20.4 Data Analysis—All data are reported as the means \pm SEM of at least three experiments, each performed in triplicate. Nonlinear regression analysis was conducted by iterative fitting using JMP (SAS for Macintosh). Nonspecific [^{34}S]GTP γ S binding was subtracted from all data. Basal [^{34}S]GTP γ S binding is defined as specific [^{34}S]GTP γ S binding in the absence of drug. Net-stimulated [^{34}S]GTP γ S binding is defined as [^{34}S]GTP γ S binding in the presence of drug minus basal. Percent of CP55,940-stimulated binding is expressed as (net stimulated [^{34}S]GTP γ S binding by drug/net stimulated [^{34}S]GTP γ S binding by 3 μM CP55,940) \times 100%.

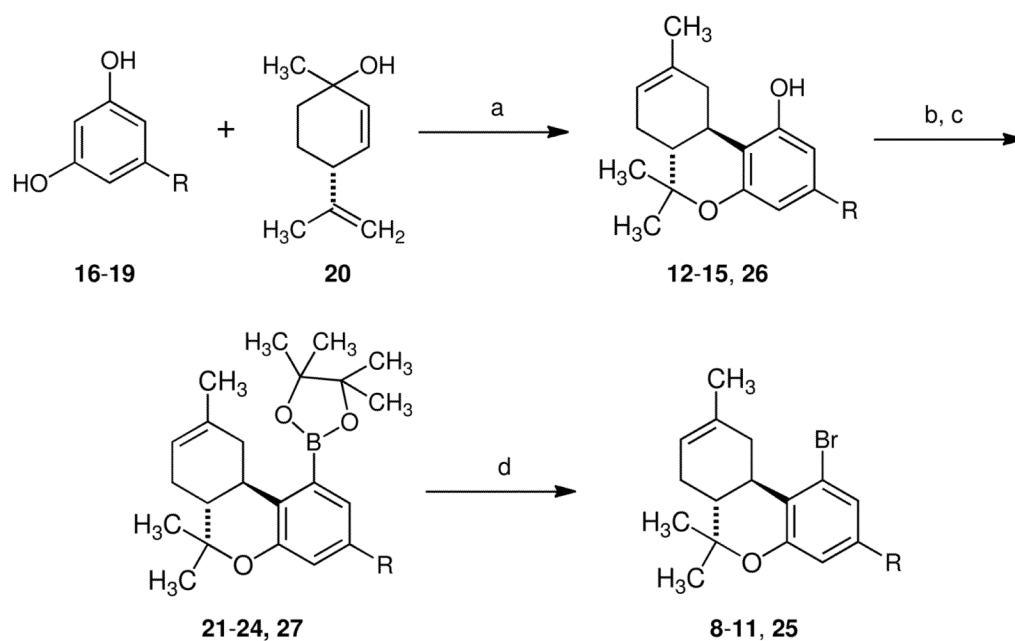
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8, 12, 16, 21 R = (CH₃)₂CCH₂CH₂CH₃

9, 13, 17, 22 R = (CH₃)₂CCH₂CH₂CH₂CH₃

10, 14, 18, 23 R = (CH₃)₂CCH₂CH₂CH₂CH₂CH₃

11, 15, 19, 24 R = (CH₃)₂CCH₂CH₂CH₂CH₂CH₂CH₃

25, 26, 27 R = CH₂CH₂CH₂CH₂CH₃

Scheme 1.

(a) HOTs, benzene, reflux 12h; (b) Tf₂O, CH₂Cl₂, pyridine, 0 °C to 25 °C, 3 h (c)

Pinacolborane, Pd(dppf)Cl₂, CH₃CN, Et₃N, reflux 18 h (d) CuBr₂, MeOH, H₂O (3:1), reflux 10 h.

Table 1

Receptor Affinities (mean \pm SEM) of 1-bromo- Δ^8 -THC analogs **8** to **11** and **26**, Δ^9 -THC (**1**) and JWH-133 (**6**) and JWH-229 (**7**).

Compound	K_i (nM)		
	CB ₁	CB ₂	CB ₁ /CB ₂
Δ^9 -THC (1)	41 \pm 2 ^a	36 \pm 10 ^b	1.1
JWH-133 (6)	677 \pm 132 ^c	3.4 \pm 1 ^c	294
JWH-229 (7)	3134 \pm 110 ^d	18 \pm 2 ^d	174
JWH-460 (25)	> 10,000	555 \pm 72	18
JWH-382 (8)	> 10,000	265 \pm 17	38
JWH-458 (9)	1145 \pm 137	71 \pm 17	16
JWH-393 (10)	1444 \pm 20	28 \pm 2	52
JWH-383 (11)	562 \pm 21	34 \pm 2	27

^a ref. 35

^b ref. 36

^c ref. 30

^d ref. 31

Table 2EC₅₀ and E_{max} Values (mean ± SEM) for GTPγS Binding of CB₂ for Selective Ligands.^a

Compound	EC ₅₀ (nM)	E _{max} (% CP-55940)
1-Deoxy-3-(1',1'-dimethylbutyl)-Δ ⁸ -THC (JWH-133, 6)	4.0 ± 1.0	111.5 ± 13.6
1-Methoxy-3-(1',1'-dimethylhexyl)-Δ ⁸ -THC (JWH-229, 7)	4.6 ± 2.0	75.7 ± 8.3
1-Bromo-1-deoxy-3-(1',1'-dimethylhexyl)-Δ ⁸ -THC (JWH-393, 10)	28.6 ± 5.3	92.3 ± 6.8
1-Bromo-1-deoxy-3-(1',1'-dimethylheptyl)-Δ ⁸ -THC (JWH-383, 11)	15.0 ± 2.3	91.0 ± 6.7

^a Assays were carried out in Human CB₂ Receptor-Expressing CHO Cells. E_{max} Values are Reported as Percent Relative to 3 μM CP-55,940.