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3-Indolyl-1-naphthylmethanes: New Cannabimimetic Indoles Provide Evidence for Aromatic Stacking Interactions with the CB_1 Cannabinoid Receptor

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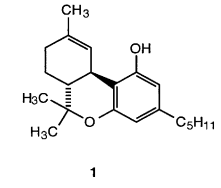
Abstract—A series of 1-pentyl-1*H*-indol-3-yl-(1-naphthyl)methanes (**9**–**11**) and 2-methyl-1-pentyl-1*H*-indol-3-yl-(1-naphthyl)-methanes (**12**–**14**) have been synthesized to investigate the hypothesis that cannabimimetic 3-(1-naphthoyl)indoles interact with the CB_1 receptor by hydrogen bonding to the carbonyl group. Indoles **9**–**11** have significant ($K_i = 17$–23 nM) receptor affinity, somewhat less than that of the corresponding naphthoylindoles (**5**, **15**, **16**). 2-Methyl-1-indoles **12**–**14** have little affinity for the CB_1 receptor, in contrast to 2-methyl-3-(1-naphthoyl)indoles **17**–**19**, which have affinities comparable to those of **5**, **15**, **16**. A cannabimimetic indene hydrocarbon (**26**) was synthesized and found to have $K_i = 26 \pm 4$ nM. Molecular modeling and receptor docking studies of naphthoylindole **16**, its 2-methyl congener (**19**) and indolyl-1-naphthylmethanes **11** and **14**, combined with the receptor affinities of these cannabimimetic indoles, strongly suggest that these cannabinoid receptor ligands bind primarily by aromatic stacking interactions in the transmembrane helix 3-4-5-6 region of the CB_1 receptor.

Introduction

Following the identification of Δ^9-tetrahydrocannabinol (Δ^9-THC, **1**)[1] as the principal psychoactive constituent of marijuana, a comprehensive set of structure–activity relationships (SAR) was developed based upon the partially reduced dibenzopyran structure of Δ^9-THC.[2–6] These SAR were subsequently extended to a group of very potent non-traditional cannabinoids developed by Pfizer, of which CP-55,940 (**2**, DMH = 1,1-dimethylheptyl) is typical.[7,8]

In 1991, as a result of a program directed toward the development of nonsteroidal anti-inflammatory drugs, a group at Sterling Winthrop reported that pravadoline (**3**) unexpectedly inhibited contractions of the electrically stimulated mouse vas deferens.[9] It was found that **3** and related compounds also inhibit adenylate cyclase, are antinociceptive in vivo and interact with a G-protein coupled receptor in the brain. Work from the same group confirmed that these aminoalkylindoles (AAIs) bind to the cannabinoid receptor which is expressed primarily in the central nervous system (CB_1



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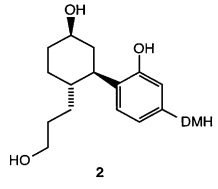

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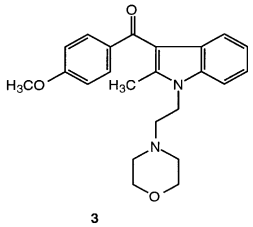

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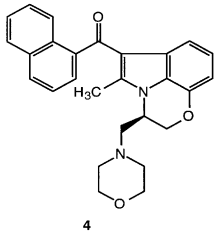

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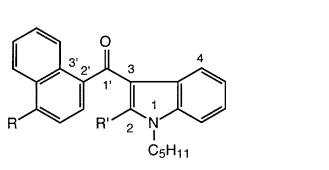
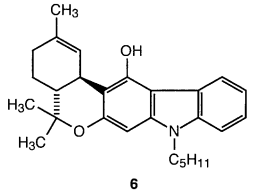
receptor),[10] and exhibit typical cannabinoid pharmacology in vivo.[11] One rigid AAI, WIN-55,212-2 (**4**), has particularly high affinity for the CB_1 receptor, and is considered to be the prototypical example of this class of cannabinoid receptor ligands.

Since the AAIs and traditional cannabinoids related to Δ^9-THC (**1**) interact with the same receptor and show similar in vivo pharmacology, several groups have attempted to devise a pharmacophore which will accommodate both classes of cannabinoid ligands. One alignment superimposes the 1-naphthoyl moiety of WIN-55,212–2 (**4**) upon the lipophilic side chain of the traditional cannabinoids.[12,13] An alternative alignment has been suggested in which the indole carbonyl, which is a vinylogous amide, corresponds to the hydroxyl group of THC and the indole nitrogen is aligned with C-1′ of the cannabinoid side chain.[14–17] This model has been employed to develop a number of very potent cannabimimetic indoles, such as 1-pentyl-3-(1-naphthoyl)indole (**5**, JWH-018), in which the aminoalkyl group appended to the indole nitrogen has been replaced by an alkyl group.[14–16,18] This alignment was also employed to design JWH-161 (**6**), a hybrid cannabinoid which has greater affinity than Δ^8-THC for the CB_1 receptor, and is of approximately equal potency in vivo.[19]



5 R, R' = H
15 R = CH_3, R' = H
16 R = OCH_3, R' = H
27 R = H, R' = C_2H_5
17 R = H, R' = CH_3
18 R = CH_3, R' = CH_3
19 R = OCH_3, R' = CH_3



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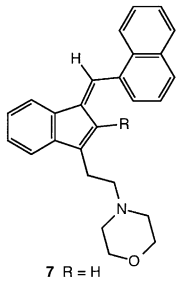
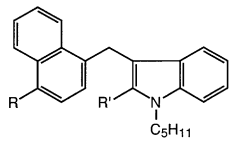
Results and Discussion

The hypothesis that the naphthoyl carbonyl of indoles such as **5** interact with the CB_1 receptor via hydrogen bonding was supported by the in vitro receptor affinities and in vivo potency of cannabimimetic indoles designed on the basis of this hypothesis. However, a recent body of evidence indicates that this class of cannabinoids probably interacts with the receptor primarily by aromatic stacking. Specifically, Song and Bonner prepared a mutant CB_1 receptor in which a lysine (K192) on helix 3 of the receptor is replaced by an alanine.[20] The affinities of a very potent classical dibenzopyran based cannabinoid, HU-210, and CP-55,940 (**2**) were greatly attenuated, but that of WIN-55,212-2 (**4**) was only slightly affected. These data, plus molecular modeling studies, suggested that the cannabimimetic indoles were interacting with the CB_1 receptor at a site somewhat different from that of the dibenzopyran based cannabinoids.[21–23] Further, it was suggested that the principal interaction of these ligands with the receptor is by aromatic stacking.[21] Supporting evidence for this hypothesis was found in additional modeling studies plus the observation that *E*-naphthylideneindenes **7** and **8**, but not the *Z*-isomers, have high affinity for the CB_1 receptor and inhibit the electrically stimulated contraction of the mouse vas deferens.[24]



7 R = H
8 R = CH_3

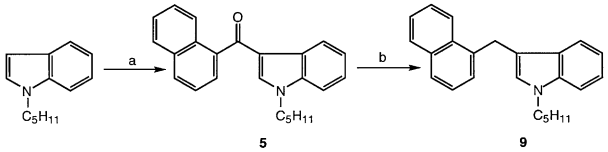


9 R, R' = H
10 R = CH_3, R' = H
11 R = OCH_3, R' = H
12 R = H, R' = CH_3
13 R = CH_3, R' = CH_3
14 R = OCH_3, R' = CH_3

In order to obtain experimental evidence concerning the mode of interaction of cannabimimetic indoles with the CB_1 receptor we have synthesized a series of 3-(1-pentylindolyl)-1-naphthylmethanes (**9–11**) and their 2-methyl analogues (**12–14**), in which there is no viable possibility for hydrogen bonding interactions between the receptor and the ligand. A 1-pentyl group was incorporated in indoles **9–14** since in cannabimimetic indoles maximum affinity for the CB_1 receptor is usually attained with a pentyl substituent on nitrogen.[14–16] These compounds were prepared in 40–45% unoptimized yield from the corresponding 3-(1-naphthoyl)-indoles (**5**, **15–19**) in a single step by reduction using lithium aluminum hydride and aluminum chloride. The 3-(1-naphthoyl)indoles have either been reported previously[14–16,25] or were prepared in the usual manner, by Friedel–Crafts acylation of 1-pentylindole or 2-methyl-1-pentylindole.[12,14] This reaction sequence, which is exemplified by the preparation of indole **5** and its reduction to **9**, is illustrated in Scheme 1.

The affinities of indoles **9–11** and **12–14** for the CB_1 receptor were determined by measuring their ability to displace the very potent cannabinoid, [^{3}H]CP-55,940 (**2**), from its binding site in a membrane preparation as described by Compton et al.[26] These data, plus the receptor affinities for Δ^9-THC (**1**), WIN-55,212–2 (**4**), naphthoylindoles **5** and **15–19**, are summarized in Table 1.

The CB_1 receptor affinities of indoles **9–11**, which are unsubstituted at C-2 of the indole nucleus, are the same, with $K_i = 17–23$ nM. The affinities of these compounds are somewhat less than those of naphthoylindoles **5**, **15** and **16**, which have $K_i = 9 \pm 5$, 0.69 ± 0.05 and 1.2 ± 0.1 nM, respectively. Also, indoles **9–11** show little if any effect upon receptor affinity as a function of substitution at the 4-position of the naphthalene ring. In the naphthoylindole series, a 4-methyl or 4-methoxy substituent enhances receptor affinity (see Table 1). The



5 **9**

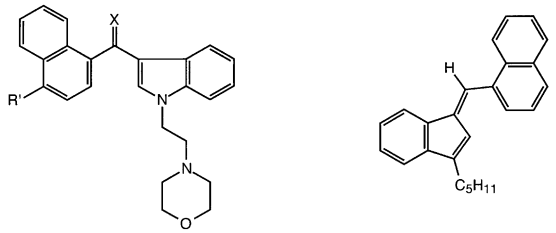
Scheme 1. (a) 1-Naphthoyl chloride/toluene/$EtAlCl_2$, 25 °C; (b) $AlCl_3$/$LiAlH_4$/Et_2O, 0 °C, then **5**, 0–25 °C.

addition of a 2-methyl group in indoles **12**–**14** considerably attenuates receptor affinity, with $K_i = 151$–323 nM. In the 2-methyl-3-(1-naphthoyl)indole series (**17**–**19**) the affinities for the CB_1 receptor are only slightly less than those of the compounds lacking a substituent at C-2 (**5**, **15**, **16**).

The cannabimimetic indoles reported originally were characterized by an aminoalkyl group attached to the indole nitrogen,[9,12] which could conceivably interact with the CB_1 receptor by hydrogen bonding.[13] To investigate this possibility, 1-morpholinoethylindoles **20**–**22** were prepared by reduction of the corresponding 3-naphthoylindoles (**23**–**25**). The reductions were carried out using lithium aluminum hydride–aluminum chloride as described above for the preparation of indoles **9**–**11**. The CB_1 receptor affinities for indoles **20**–**25** are included in Table 1.

In contrast to indoles **9**–**11**, which have effectively the same affinity for the CB_1 receptor, there is considerable variation in the affinities of indoles **20**–**22** as a function of substitution in the 4-position of the naphthalene ring. The analogue with an unsubstituted naphthalene ring has quite modest affinity for the CB_1 receptor ($K_i = 113 \pm 28$ nM), while the 4-methyl- and 4-methoxynaphthyl analogues have significantly greater affinity ($K_i = 41 \pm 13$ nM and $K_i = 20 \pm 2$ nM, respectively). Naphthoylindoles **23**–**25** were reported previously by the Winthrop group who found the same trend in affinities, with the unsubstituted analogue (**23**) having somewhat less affinity for the CB_1 receptor than the 4-methyl and 4-methoxynaphthoyl analogues.[12] There are considerable differences between the affinities presented in Table 1 for indoles **23**–**25** and those reported by Eissenstat et al. However, the Winthrop group's binding data were obtained by displacement of [^{3}H]-WIN-55,212-2 (**4**) from a rat cerebellar membrane preparation, while the data presented in Table 1 were obtained by displacement of [^{3}H]CP-55,940 (**2**) from a rat whole brain memebrane preparation.[26]

E-Naphthylideneindenes **7** and **8** have good affinity for the CB_1 receptor ($K_i = 2.72 \pm 0.22$ nM and 2.89 ± 0.41 nM, respectively), and modeling studies support the hypothesis that they interact with the receptor by aromatic stacking interactions.[24] However, there is at least a formal possibility that the morpholino nitrogen or oxygen may interact with the receptor by hydrogen bonding. In order to explore this possibility, *E*-naphthylideneindene **26** (JWH-176) was prepared by the base catalyzed (NaOMe/MeOH) condensation of 1-pentylindene with 1-naphthaldehyde.[27] This indene has high affinity for the CB_1 receptor with $K_i = 26 \pm 4$ nM (Table 1). Although indene **26** has high affinity for the CB_1 receptor it is somewhat less than those reported for morpholinoethylindenes **7** and **8**.[24]



20 X = H_2, R' = H
21 X = H_2, R' = CH_3
22 X = H_2, R' = OCH_3
23 X = O, R' = H
24 X = O, R' = CH_3
25 X = O, R' = OCH_3
26

The high CB_1 receptor affinities of indoles **9**–**11**, **21**, **22** and indene **26** strongly support the hypothesis that cannabimimetic indoles and related CB_1 receptor

Table 1. CB_1 receptor affinities (mean ± SEM) of cannabimimetic indoles **5**, **9**–**25**, **27** and related compounds

Compd	K_i (nM)
Δ^9-THC (**1**)	41 ± 2[a]
WIN-55,212-2 (**3**)	9.9 ± 1.0[b]
1-Pentyl-1*H*-indol-3-yl-(1-naphthyl)methane (**9**, JWH-175)	22 ± 2
1-Pentyl-1*H*-indol-3-yl-(4-methyl-1-naphthyl)methane (**10**, JWH-184)	23 ± 6
1-Pentyl-1*H*-indol-3-yl-(4-methoxy-1-naphthyl)methane (**11**, JWH-185)	17 ± 3
2-Methyl-1-pentyl-1*H*-indol-3-yl-(1-naphthyl)methane (**12**, JWH-196)	151 ± 18
2-Methyl-1-pentyl-1*H*-indol-3-yl-(4-methyl-1-naphthyl)methane (**13**, JWH-194)	127 ± 19
2-Methyl-1-pentyl-1*H*-indol-3-yl-(4-methoxy-1-naphthyl)methane (**14**, JWH-197)	323 ± 28
1-Pentyl-3-(1-naphthoyl)indole (**5**, JWH-018)	9 ± 5[c]
1-Pentyl-3-(4-methyl-1-naphthoyl)indole (**15**, JWH-122)	0.69 ± 0.05
1-Pentyl-3-(4-methoxy-1-naphthoyl)indole (**16**, JWH-081)	1.2 ± 0.1[d]
2-Methyl-1-pentyl-3-(1-naphthoyl)indole (**17**, JWH-007)	9.5 ± 4.5[c]
2-Methyl-1-pentyl-3-(4-methyl-1-naphthoyl)indole (**18**, JWH-149)	5.0 ± 2.1
2-Methyl-1-pentyl-3-(4-methoxy-1-naphthoyl)indole (**19**, JWH-098)	4.5 ± 0.1[d]
1-[2-(4-Morpholino)ethyl]-1*H*-indol-3-yl-1-naphthylmethane (**20**, JWH-195)	113 ± 28
1-[2-(4-Morpholino)ethyl]-1*H*-indol-3-yl-(4-methyl-1-naphthyl)methane (**21**, JWH-192)	41 ± 13
1-[2-(4-Morpholino)ethyl]-1*H*-indol-3-yl-(4-methoxy-1-naphthyl)methane (**22**, JWH-199)	20 ± 2
1-[2-(4-Morpholino)ethyl]-3-(1-naphthoyl)indole (**23**, JWH-200)	42 ± 5
1-[2-(4-Morpholino)ethyl]-3-(4-methyl-1-naphthoyl)indole (**24**, JWH-193)	6 ± 1
1-[2-(4-Morpholino)ethyl]-3-(4-methoxy-1-naphthoyl)indole (**25**, JWH-198)	10 ± 2
E-1-[1-(1-Naphthalenylmethylene)-1*H*-inden-3-yl]pentane (**26**, JWH-176)	26 ± 4
2-Ethyl-1-pentyl-3-(1-naphthoyl)indole (**27**, JWH-116)	52 ± 5

[a]Ref 26.
[b]Rinaldi-Carmona, M.; Barth, F.; Héaulme, M.; Shire, D.; Calandra, B.; Congy, C.; Martinez, S.; Maruani, J.; Néliat, G.; Caput, D.; Ferrara, P.; Soubrie, P.; Breliére, J. C.; Le Fur, G. *FEBS Lett.* **1994**, *350*, 240.
[c]Ref 15.
[d]Ref 16.

ligands do not interact with the receptor by hydrogen bonding.[14–17] In particular, the high affinity of indene **26**, a hydrocarbon, for the CB_1 receptor provides compelling evidence against hydrogen bonding interactions playing a major role in the binding of these ligands. These data are consistent with the suggestion that these compounds interact with the receptor by aromatic stacking.[21,24]

Although indoles **9**–**11**, **21** and **22**, which are unsubstituted at C-2 of the indole nucleus, have significant affinity for the CB_1 receptor, 2-methylindoles (**12**–**14**) have very little affinity. This is in contrast to the 3-(1-naphthoyl)indole series in which the 2-methylindoles have only slightly less affinity for the CB_1 receptor than the unsubstituted compounds. This is a general property of these cannabimimetic indoles, which has been noted previously[10,12,15,16,18] and is also apparent from the limited data presented in Table 1. There appears to be no a priori explanation for the poor receptor affinities of indoles **12**–**14** when compared to the significant affinities of indoles **9**–**11**, **21** and **22**. In order to obtain some insight into the origin of these apparently anomalous differences in receptor affinity, molecular modeling and receptor docking studies of indoles JWH-081 (**16**), JWH-098 (**19**), JWH-185 (**11**) and JWH-197 (**14**) were carried out.

In the discussion of compounds that follows, each molecule is oriented so that the indole ring is perpendicular to the plane of the page, with the C-4 hydrogen pointing out of the page toward the viewer and with the C-2 substituent pointing down toward the bottom of the page (see Fig. 1 insets). The numbering used in this discussion is included on the structure of indole **5**. In this orientation, substituents which are located to the left of the indole ring plane protrude into the top face of the molecule, while those that are to the right of the indole plane protrude into the bottom face of the molecule.

1-Pentyl-3-(4-methoxy-1-naphthoyl)indole (**16**, JWH-081, $K_i = 1.2 \pm 0.03$ nM) and its 2-methyl congener, JWH-098 (**19**, $K_i = 4.5 \pm 0.1$ nM), display high CB_1 receptor affinity. Like the aminoalkylindole (AAI), WIN-55,212-2 (**4**), these compounds possess a carbonyl group which bridges the indole and naphthalene rings. Two major conformations have been shown to exist for WIN-55,212-2 and related AAIs, the *s-cis* and the *s-trans* conformations.[24] These conformations differ primarily in the orientation of the C-3 aroyl substituent. In the *s-cis* conformation, which predominates when the C-2 substituent is a methyl group (as in WIN-55,212-2), the carbonyl oxygen is near C-2, while the naphthyl ring is stacked over C-4 of the indole ring. In the *s-trans* conformation, which predominates when C-2 is a hydrogen (as in JWH-018, **5**), the aryl system is near C-2 and the carbonyl oxygen is near C-4. AM1 semi-empirical calculations indicate that the *s-cis* conformation of WIN-55,212–2 is 1.86 kcal/mol lower in energy than its lowest energy *s-trans* conformer.[24]

Consistent with these earlier results, AM1 conformer searches of JWH-081 (**16**), which has a hydrogen at C-2, identified an *s-trans* conformation as the global minimum energy conformer. The lowest energy *s-cis* conformer was found to be 0.59 kcal/mol higher in energy than the global minimum *s-trans* conformer. In JWH-098 (**19**), AM1 calculations revealed that the lowest energy conformer is an *s-cis* conformer. This result is also consistent with earlier results, as the C-2 substituent in indole **19** is a methyl group. The lowest energy *s-trans* conformation was found to be 1.22 kcal/mol higher in energy than the global minimum *s-cis* conformer. The global minimum energy conformers of indoles **16** and **19** are illustrated in Figure 1 (left).

1-Pentyl-1*H*-indol-3-yl-(4-methoxy-1-naphthyl)methane (**11**, JWH-185, $K_i = 17 \pm 3$ nM) and 2-methyl-1-pentyl-1*H*-indol-3-yl-(4-methoxy-1-naphthyl)methane (**14**, JWH-197, $K_i = 323 \pm 48$ nM) are congeners of JWH-081 (**16**) and JWH-098 (**19**) respectively, in which the carbonyl bridge has been replaced by a methylene group. This replacement changes the hybridization of the bridging

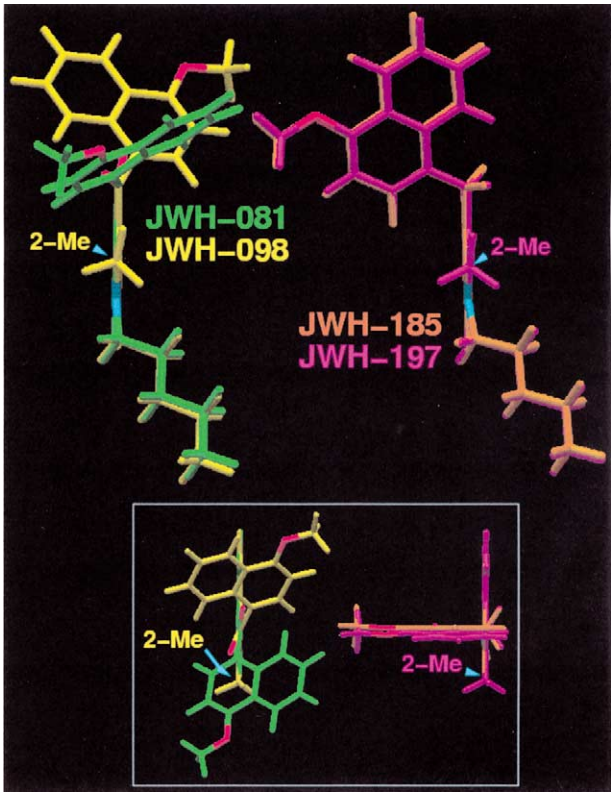


Figure 1. (Left) A side view is shown here in which the global minimum energy conformers of JWH-081 (**16**, *s-trans*; in green) and JWH-098 (**19**, *s-cis*; in yellow) are superimposed at their indole rings. In this side view, the indole ring is perpendicular to the page with the 2-methyl group pointing towards the viewer. In the cutout at the bottom, molecules are oriented in a top view, so that the indole ring is perpendicular to the page with C-4 pointing towards the viewer and the C-2 methyl of **19** pointing down. The alkyl side chains have been removed here in order to simplify the display. (Right) A side view is shown here in which the global minimum energy conformers of JWH-185 (**11**, in orange) and JWH-197 (**14**, in purple) are superimposed at their indole rings. In this side view, the indole ring is perpendicular to the page with the 2-methyl group pointing towards the viewer. In the cutout at the bottom, molecules are oriented in a top view so that the indole ring is perpendicular to the page with C-4 pointing towards the viewer and the C-2 methyl of **19** pointing down. The alkyl side chains have been removed here in order to simplify the display.

carbon from sp^2 in the carbonyl group to sp^3 in the methylene group. This change can be anticipated to produce a different relative orientation of the naphthalene and indole rings compared with that seen for indoles **16**, **19** and WIN-55,212-2 (**4**).

For indoles **11** and **14**, two sets of conformers were identified by AM1 conformer searches. In the first set of conformers, substituents are staggered with respect to the C1′–C2′ bond which connects the methylene carbon (C1′) with the indole ring. Within this set, variations in the C3–C1′–C2′–C3′ torsion angle place the naphthyl ring in different orientations with respect to the indole ring. In the second set, a methylene C1′–H bond eclipses an adjacent C–C bond on the indole ring. For JWH-185 (**11**), the global minimum energy conformer has the methylene C–H bonds staggered with respect to the indole ring, with a C3–C1′–C2′–C3′ angle of −177.8°. In this conformer (see Fig. 1 right), the naphthalene ring is oriented perpendicular to the plane of the indole nucleus and is located in the top face of the molecule.

In JWH-197 (**14**), the global minimum energy conformer has the methylene hydrogens staggered with respect to the plane of the indole, with a C3–C1′–C2′–C3′ angle of 177.8°. In this conformer, the naphthalene ring is also in the top face of the molecule, with the naphthalene ring perpendicular to the plane of the indole (see Fig. 1, right).

1-Pentyl-1*H*-indol-3-yl-(4-methoxy-1-naphthyl)methane (**11**, JWH-185) has reduced CB_1 receptor affinity ($K_i = 17 \pm 3$ nM) relative to 1-pentyl-3-(4-methoxy-1-naphthoyl)indole (**16**, JWH-081, $K_i = 1.2 \pm 0.03$ nM). In addition, while in the 3-(1-naphthoyl)indole series (**11** and **16**), the substitution of a methyl group at C-2 results in less than a four-fold loss in CB_1 receptor affinity (JWH-081, **16**, $K_i = 1.2 \pm 0.03$ nM; JWH-098, **19**, $K_i = 4.5 \pm 0.1$ nM), substitution at C-2 in the naphthylmethane series (**11** and **14**) results in a more profound 19-fold affinity loss (JWH-185, **11**, $K_i = 17 \pm 3$ nM; JWH-197, **14**, $K_i = 323 \pm 48$ nM). In order to probe the origin of these affinity changes at the CB_1 receptor, each of these compounds was docked in a model of the CB_1 receptor active state (R*).

The transmembrane helix (TMH) 3-4-5-6 region of CB_1 is rich in aromatic amino acids that face into the binding site crevice in CB_1. These residues include F3.25, F3.36, W4.64, Y5.39, W5.43, and W6.48. Shire and coworkers have shown in CB_1/CB_2 chimera studies that the TMH4-E2-TMH5 region of the cannabinoid receptors contains residues critical for the binding of WIN-55,212-2.[28] A recent mutation and computational Monte Carlo/stochastic dynamics study of the CB_1 receptor has suggested that aromaticity at positions 4.64 and 5.39 is important to maintain the proper relative positions of TMHs 4 and 5, and therefore the ligand binding pocket.[29] Three amino acids, F3.36, W5.43, and W6.48, were found in this same study to form a triad of interacting aromatic residues in the inactive state of CB_1 which would be available for ligand binding.[29] Because the AAIs are highly aromatic ligands and because K3.28A mutation studies have indicated that K3.28 is not an interaction site for WIN-55,212-2 (**4**),[20] it has been hypothesized that aromatic stacking,[30] rather than hydrogen bonding interactions, is the primary interaction is for the AAIs at CB_1.[31] Mutation studies performed by Song and co-workers have supported the importance of aromatic stacking for WIN-55,212-2 interaction with both the CB_1 and CB_2 receptors.[31] These studies showed that the 15–20-fold higher affinity of **4** for the CB_2 receptor is due to a direct interaction with F5.46, a residue that is aromatic only in CB_2.

Because cannabimimetic indoles **11**, **14**, **16** and **19** are highly aromatic ligands which are structurally related to the AAIs, we hypothesized that the TMH 3-4-5-6 region of CB_1 would also be the binding region for these ligands. In addition, since JWH-081 (**16**) and JWH-098 (**19**), like the AAIs, have a carbonyl bridge between the indole and naphthalene rings and because the *s-trans* arrangement of the indole and naphthalene rings in the AAIs has been shown to be the AAI bioactive conformation,[24] indoles **16** and **19** were docked in the TMH 3-4-5-6 region of CB_1 in their lowest energy *s-trans* conformations. Indoles **11** and **14** were docked in this same region using the global minimum energy conformer of each (see Fig. 1, right). Aromatic stacking interactions were characterized by the distance between ring centroids, and the angle (α) formed by the planes of the interacting rings. Hydrogen bonding interactions were characterized by the distance between the heteroatoms involved in the hydrogen bond and the hydrogen bond angle (heteroatom–H–heteroatom).

The SAR of cannabimimetic 2-methylindoles indicate that compounds with *N*-alkyl substituents from *n*-propyl ($K_i = 164 \pm 22$ nM) to *n*-hexyl ($K_i = 48 \pm 13$ nM), with *n*-pentyl being optimum ($K_i = 9.5 \pm 4.5$ nM), have good CB_1 receptor affinities, but those indoles with *N*-alkyl substituents that are shorter (ethyl, $K_i = 1182 \pm 44$ nM) or longer (*n*-heptyl, $K_i > 10{,}000$ nM) have poor affinities.[15] A similar trend is seen both in indole analogues which are unsubstituted at C-2, and which have various substituents on the naphthalene ring.[15,16,18] In light of these SAR, a hydrophobic binding pocket for the alkyl tail was sought in the TMH 3-4-5-6 region which had limited depth, but required extension of several carbons in the *N*-alkyl substituent in order to enter this region. A hydrophobic binding pocket comprised of V3.32, I3.33, F3.36, L6.51, I6.54 was identified that permitted simultaneous interaction of the indole and naphthalene rings with the aromatic residues in the TMH 3-4-5-6 region of R* CB_1. In this hydrophobic binding pocket, lengthening the alkyl chain to *n*-heptyl produces serious van der Waals' overlaps with the backbone of TMH 7, while shortening to ethyl prevents interaction with this hydrophobic pocket. When the N1 alkyl chain was docked in this hydrophobic pocket, JWH-081 (**16**) and JWH-098 (**19**) found aromatic stacking interactions with W5.43 (JWH-081–indole: $d = 4.3$ Å, $\alpha = 80°$, naphthyl: $d = 5.0$ Å, $\alpha = 30°$; JWH-098–indole: $d = 4.4$ Å, $\alpha = 90°$, naphthyl: $d = 5.1$ Å, $\alpha = 50°$) and W6.48 (JWH-081–naphthyl: $d = 5.9$ Å, $\alpha = 30°$; JWH-098–naphthyl:

$d = 4.9$ Å, $\alpha = 90°$). In addition, the N–H of W5.43 could hydrogen bond with the carbonyl oxygens of **16** and **19**, although the hydrogen bond geometry was better with **16** (**16**: O–N distance = 2.7 Å, O–H–N angle = 162°; **19**: O–N distance = 2.7 Å, O–H–N angle = 123°). In this docking position, the C-2 substituent change between **16** and **19** would cause no loss of affinity, since the C-2 methyl group in **19** occupies an open space in the receptor binding pocket. This docking position also indicates that enlargement of the C-2 substituent to ethyl can still be accommodated in this binding region. In agreement with this hypothesis, it was found that 2-ethyl-1-pentyl-3-(1-naphthoyl)indole (**27**, JWH-116) has significant affinity for the CB_1 receptor ($K_i = 52 \pm 5$ nM). This indole derivative was prepared from 2-ethylindole[32] by C-3 acylation with 1-naphthoyl chloride, followed by *N*-alkylation.[9,14]

The conformers of JWH-185 (**11**) and JWH-197 (**14**) illustrated in Figure 1 could be docked in the same general region of CB_1 R* using the same hydrophobic binding pocket for the *n*-pentyl chain of these analogues. However, because these analogues have conformations which orient the naphthalene and indole rings in a very different arrangement than in the 3-aroylindoles (**16** and **18**), the orientation of the ligands in the binding pocket differs from that of indoles **16** and **18**. Naphthylindoles **11** and **14** can still engage in aromatic stacking interactions with W5.43 and W6.48. In addition, the indole nucleus of JWH-185 (**11**) engages in an aromatic stacking interaction with F3.36 that involves the C-2 hydrogen. No hydrogen bonding interactions are possible for this ligand at the CB_1 R* binding site. This may explain why indole **11** has reduced CB_1 affinity relative to naphthoylindole **16**. Like JWH-185 (**11**), its 2-methyl analogue, JWH-197 (**14**), can engage in aromatic stacking interactions with W5.43 and W6.48. However, in the JWH-197 (**14**)/CB_1 R* complex, no aromatic stacking interaction is possible with F3.36 because indole **14** lacks the hydrogen at C-2 which participates in the stacking interaction with F3.36 in the JWH-185 (**11**)/CB_1 R* complex. The magnitude of the drop in affinity seen in going from the C-2-H in **11** ($K_i = 17 \pm 3$ nM) to the C-2 methyl analogue, JWH-197 (**14**, $K_i = 323 \pm 48$ nM), is consistent with the loss of an aromatic stacking interaction.[30,31]

The significant affinities of indoles **9**–**11**, **21**, **22** and indene **26**, none of which can interact with the receptor by hydrogen bonding, strongly support the hypothesis that cannabimimetic indoles interact with the CB_1 receptor primarily by aromatic stacking interactions. Not only do the molecular modeling and receptor docking studies agree with this conclusion, but they provide an explanation for the observation that 2-methylindole analogues JWH-196 (**12**), JWH-194 (**13**) and JWH-197 (**14**) have greatly attenuated affinities for the CB_1 receptor. The receptor docking studies also provide an explanation for the enhanced affinities of naphthoylindoles, such as **5**, **15**, **16** and **23**–**25**, in which hydrogen bonding involving the carbonyl group may augment binding due to aromatic stacking.

Experimental

General

IR spectra were obtained using Nicolet 5DX or Magna spectrometers; ^1H and ^{13}C NMR spectra were recorded on a Bruker 300AC spectrometer. Mass spectral analyses were performed on a Hewlett-Packard 5890A gas chromatograph with a mass sensitive detector and 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 μm) using the indicated solvents as eluents. All new compounds were homogeneous to GLC and/or TLC and ^{13}C NMR.

1-Pentyl-1*H*-indol-3-yl-(1-naphthyl)methane (9). To a suspension of 0.46 g (3.48 mmol) of aluminum chloride in 2 mL of ether at 0 °C was added dropwise 1.16 mL (1.16 mmol) of a 1 M solution of $LiAlH_4$ in ether. The suspension was stirred at 0 °C for 30 min and 0.10 g (0.29 mmol) of 1-pentyl-3-(1-naphthoyl)indole (**5**) in 2 mL of ether was added dropwise. The reaction mixture was warmed to ambient temperature, stirred for 48 h, cooled to 0 °C, and carefully quenched with water. After acidification the organic phase was separated and washed successively with saturated aqueous $NaHCO_3$ and brine. After drying ($MgSO_4$) the solvent was removed in vacuo to give a yellow oil. Chromatography (petroleum ether/ether, 4:1) gave 0.038 g (40%) of **9** as a yellow oil: R_f 0.74 (petroleum ether/ether 4:1); ^1H NMR (300 MHz, $CDCl_3$) δ 0.91 (t, $J = 7.0$ Hz, 3H), 1.22–1.41 (m, 4H), 1.68–1.78 (m, 2H), 4.02 (t, $J = 7.1$ Hz, 2H), 4.63 (s, 2H), 6.59 (s, 1H), 7.10–7.47 (m, 8H), 7.62 (d, $J = 7.8$ Hz, 1H), 7.75 (d, $J = 7.2$ Hz, 1H), 8.11 (d, $J = 6.9$ Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 13.9, 22.2, 28.9, 29.9, 46.1, 109.3, 113.5, 118.7, 119.2, 121.4, 124.4, 125.4, 125.6, 125.7, 126.6, 126.7, 127.9, 128.5, 132.2, 133.8, 136.3, 137.0; HRMS calcd for $C_{24}H_{25}N$: 327.1982; found: 327.1995.

1-Pentyl-3-(4-methyl-1-naphthoyl)indole (15). To a suspension of 0.30 g (1.61 mmol) of 4-methyl-1-naphthoic acid in 7 mL of CH_2Cl_2 at 0 °C was added dropwise over several min 1.02 g (8.05 mmol) of oxalyl chloride. The reaction mixture was warmed to ambient temperature, stirred for 1 h, then heated at reflux for 1 h. After cooling, the solvent and excess oxalyl chloride were removed in vacuo to give a brown residue. This residue was dissolved in 7 mL of toluene and 0.36 g (1.93 mmol) of 1-pentylindole in 7 mL of toluene was added. The mixture was cooled to 0 °C and 2.42 mL (2.42 mmol) of $EtAlCl_2$ (1 M in hexanes) were added. The reaction mixture was warmed to ambient temperature and stirred for 48 h. After quenching with water, the mixture was extracted with ethyl acetate and dried ($MgSO_4$). The solvents were removed in vacuo to give a yellow oil which was purified by chromatography to give 0.24 g (42%) of **15** as a pale yellow oil: R_f 0.51 (petroleum ether/ether, 19:1); ^1H NMR (300 MHz, $CDCl_3$) δ 0.85 (t, $J = 7.1$ Hz, 3H), 1.16–1.30 (m, 4H), 1.71–1.81 (m,

2H), 2.74 (s, 3H), 4.01 (t, $J=7.2$ Hz, 2H), 7.29–7.55 (m, 8H), 8.05 (d, $J=8.3$ Hz, 1H), 8.25 (d, $J=8.3$ Hz, 1H), 8.49–8.52 (m, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 13.8, 19.7, 22.1, 28.8, 29.4, 47.0, 109.9, 117.5, 122.7, 122.8, 123.4, 125.2, 125.8, 126.1, 126.3, 126.5, 130.8, 132.8, 136.6, 136.9, 137.8, 192.1; HRMS calcd for $C_{25}H_{25}NO$: 355.1936; found: 355.1933.

1-Pentyl-1*H*-indol-3-yl-(4-methyl-1-naphthyl)methane (10). Reduction of 0.23 g (0.65 mmol) of indole **15** by the procedure described above for the preparation of **9** gave 0.15 g (68%) of **10** as a yellow oil: R_f 0.70 (petroleum ether/ether, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.81 (t, $J=7.1$ Hz, 3H), 1.12–1.27 (m, 4H), 1.63–1.72 (m, 2H), 2.66 (s, 3H), 3.90 (t, $J=7.1$ Hz, 2H), 4.49 (s, 2H), 6.54 (s, 1H), 7.05–7.26 (m, 5H), 7.38–7.50 (m, 2H), 7.61 (d, $J=7.8$ Hz, 1H), 8.00 (d, $J=8.3$ Hz, 1H), 8.10 (d, $J=8.1$ Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 13.1, 19.4, 22.2, 28.9, 29.0, 29.9, 46.1, 109.3, 113.7, 118.6, 119.2, 121.3, 124.6, 125.0, 125.2, 125.3, 126.3, 126.5, 127.9, 132.2, 132.6, 132.9, 135.0, 136.3; IR (neat) 2956, 2932, 2867, 1460, 1364 cm^{-1}; MS (EI) m/z 341 (100), 326 (13), 284 (35); HRMS calcd for $C_{25}H_{27}N$: 341.2144; found: 341.2150.

1-Pentyl-1*H*-indol-3-yl-(4-methoxy-1-naphthyl)methane (11). Reduction of 0.186 g (0.50 mmol) of indole **16** by the procedure described above for the preparation of **9** gave 0.127 g (68%) of **11** as a pale yellow oil: R_f 0.47 (petroleum ether/ether, 9:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.81 (t, $J=7.2$ Hz, 3H), 1.15–1.28 (m, 4H), 1.65–1.77 (m, 2H), 3.95 (t, $J=7.2$ Hz, 2H), 3.98 (s, 3H), 4.45 (s, 2H), 6.56 (s, 1H), 6.72 (d, $J=7.8$ Hz, 1H), 7.09 (t, $J=6.9$ Hz, 1H), 7.23–7.32 (m, 3H), 7.41–7.48 (m, 2H), 7.62 (d, $J=7.9$ Hz, 1H), 8.00–8.03 (m, 1H), 8.29–8.34 (m, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 13.9, 22.3, 28.5, 29.0, 29.9, 46.1, 55.4, 103.4, 109.3, 114.0, 118.6, 119.2, 121.3, 122.3, 124.3, 124.8, 125.9, 126.2, 126.5, 127.9, 128.8, 132.9, 136.4; MS (EI) m/z 357 (100), 342 (10), 326 (22), 300 (24); HRMS calcd for $C_{25}H_{27}NO$: 357.2093; found: 357.2088.

2-Methyl-1-pentyl-1*H*-indol-3-yl-(1-naphthyl)methane (12). Reduction of 0.17 g (0.48 mmol) of indole **17** by the procedure described above for the preparation of **9** gave 0.069 g (42%) of **12** as a yellow oil: R_f 0.71 (petroleum ether/ether, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.91 (t, $J=6.8$ Hz, 3H), 1.36–1.38 (m, 4H), 1.75–1.80 (m, 2H), 2.31 (s, 3H), 4.11 (t, $J=7.4$ Hz, 2H), 4.54 (s, 2H), 6.96–7.01 (m, 2H), 7.11–7.17 (m, 1H), 7.23–7.35 (m, 3H), 7.47–7.58 (m, 2H), 7.68 (d, $J=8.2$ Hz, 1H), 7.86–7.89 (m, 1H), 8.27 (d, $J=8.2$ Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 10.4, 14.0, 22.5, 27.2, 29.2, 30.0, 43.4, 108.3, 108.8, 118.4, 118.7, 120.5, 123.5, 125.3, 125.8, 126.4, 128.4, 128.7, 132.2, 133.7, 136.1, 136.8; MS (EI) m/z 341 (100), 326 (30), 284 (45); HRMS calcd for $C_{25}H_{27}N$: 341.2142; found: 341.2144.

2-Methyl-1-pentyl-3-(4-methyl-1-naphthoyl)indole (18). To a solution of 0.38 g (0.19 mmol) of 2-methyl-1-pentylindole in 3 mL of CH_2Cl_2 at 0 °C was added 0.29 mL (0.29 mmol) of 1 M Et_2AlCl in hexanes and the mixture was stirred for 30 min. To this deep red solution was added a solution of 4-methyl-1-naphthoyl chloride, prepared as described above, in 2 mL of CH_2Cl_2, and the reaction mixture was stirred at ambient temperature for 2 h. The reaction was quenched with water, extracted with ethyl acetate and dried ($MgSO_4$). The solvents were removed in vacuo to give a yellow oil which was purified by flash chromatography (petroleum ether/ether, 19:1) to give 0.040 g (57%) of indole **18** as a pale yellow oil: R_f 0.46 (petroleum ether/ether, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87 (t, $J=7.0$ Hz, 3H), 1.36–1.38 (m, 4H), 1.76–1.81 (m, 2H), 2.48 (s. 3), 2.77 (s., 3H), 4.10 (t, $J=7.6$ Hz, 2H), 6.96–7.01 (m, 1H), 7.14–7.34 (m, 5H), 7.41–7.56 (m, 3H), 8.07 (d, $J=8.4$ Hz, 1H), 8.14 (d, $J=8.4$ Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 12.5, 13.9, 19.8, 22.3, 29.1, 29.3, 43.3, 109.3, 115.0, 121.3, 121.7, 122.1, 124.2, 125.8, 126.0, 126.3, 126.4, 127.1, 130.5, 132.9, 136.0, 136.7, 138.9, 145.3, 193.6; MS (EI) m/z 369 (90), 354 (95), 207 (100), 169 (70); HRMS calcd for $C_{26}H_{27}NO$: 369.2093; found: 369.2096.

2-Methyl-1-pentyl-1*H*-indol-3-yl-(4-methyl-1-naphthyl)-methane (13). Reduction of 0.040 g (0.11 mmol) of indole **18** by the procedure described above for the preparation of **9** gave 0.017 g (43%) of **13** as a yellow oil: R_f 0.52 (petroleum ether/ether, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.84 (t, $J=6.8$ Hz, 3H), 1.29–1.31 (m, 4H), 1.70 (m, 2H), 2.24 (s, 3H), 2.57 (s, 3H), 4.03 (t, $J=7.5$ Hz, 2H), 4.44 (s, 2H), 6.81 (d, $J=7.2$ Hz, 1H), 6.88–6.93 (m, 1H), 7.01–7.09 (m, 2H), 7.22–7.28 (m, 3H), 7.45–7.52 (m, 2H), 7.95–7.98 (m, 1H), 8.20–8.24 (m, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 10.4, 14.0, 19.4, 22.5, 27.1, 29.2, 30.0, 43.3, 108.4, 118.4, 118.7, 120.4, 124.0, 124.8, 125.2, 125.4, 126.4, 128.4, 132.2, 132.8, 133.6, 134.9; MS (EI) m/z 355 (100), 340 (90), 298 (50); HRMS calcd for $C_{26}H_{29}N$: 355.2309; found: 355.2308.

2-Methyl-1-pentyl-3-(4-methoxy-1-naphthoyl)indole (19). Acylation of 0.20 g (1.0 mmol) of 2-methyl-1-pentylindole with 4-methoxynaphthoyl chloride, prepared from 0.40 g (1.98 mmol) of the corresponding carboxylic acid, was carried out by the procedure described above for the synthesis of indole **18**. After purification by flash chromatography (petroleum ether/ether, 19:1) there was obtained 0.17 g (45%) of **19** as a pale yellow oil: R_f 0.36 (petroleum ether/ether, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.91 (t, $J=6.7$ Hz, 3H), 1.36–1.43 (m, 4H), 1.72–1.88 (m, 2H), 2.50 (s, 3H), 4.05 (s, 3H), 4.11 (t, $J=7.5$ Hz, 2H), 6.79 (d, $J=8.0$ Hz, 1H), 6.98–7.03 (m, 1H), 7.14–7.19 (m, 1H), 7.25–7.31 (m, 2H), 7.58 (d, $J=8.0$ Hz, 1H), 8.26–8.36 (m, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 12.5, 13.9, 22.4, 29.1, 29.4, 43.3, 55.6, 102.6, 109.3, 115.3, 121.2, 121.5, 121.9, 125.5, 127.3, 127.5, 128.4, 131.9, 132.4, 136.0, 144.7, 157.2, 193.1; IR (Nujol) 1626, 1592 cm^{-1}; MS (EI) m/z 385 (100), 370 (98), 328 (25); HRMS calcd for $C_{26}H_{27}NO_2$: 385.2042; found: 385.2043.

2-Methyl-1-pentyl-1*H*-indol-3-yl-(4-methoxy-1-naphthyl)-methane (14). Reduction of 0.080 g (0.21 mmol) of indole **19** by the procedure described above for the preparation of **9** gave 0.035 g (45%) of **14** as a yellow oil: R_f

0.48 (petroleum ether/ether, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.92 (t, J = 6.7 Hz, 3H), 1.36–1.39 (m, 4H), 1.72–1.88 (m, 2H), 2.32 (s, 3H), 3.93 (s, 3H), 4.11 (t, J = 7.4 Hz, 2H), 4.45 (s, 2H), 6.60 (d, J = 8.0 Hz, 1H), 6.88 (d, J = 8.0 Hz, 1H), 6.96–7.01 (m, 1H), 7.11–7.16 (m, 1H), 7.30–7.35 (m, 2H), 7.48–7.59 (m, 2H), 8.20 (d, J = 7.7 Hz, 1H), 8.32 (d, J = 7.7 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 13.9, 22.3, 28.5, 29.1, 29.9, 46.1, 55.4, 103.4, 109.3, 114.0, 118.6, 119.2, 121.3, 122.3, 124.3, 124.8, 126.2, 126.3, 126.5, 128.0, 128.8, 132.9, 136.3, 154.3; MS (EI) m/z 371 (100), 356 (53), 340 (15); HRMS calcd for $C_{26}H_{29}NO$: 371.2249; found: 371.2253.

1-[2-(4-Morpholino)ethyl]-3-(1-naphthoyl)indole (23). Acylation of 0.50 g (2.17 mmol) of 1-[2-(4-Morpholino)-ethyl]-indole with 0.83 (4.34 mmol) of 1-naphthoyl chloride was carried out by the procedure described above for the synthesis of indole **18**. After purification by flash chromatography (petroleum ether/ether, 1:1) there was obtained 0.40 g (48%) of **23** as a pale yellow oil: R_f 0.16 (ether); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.39 (t, J = 4.6 Hz, 4H), 2.70 (t, J = 6.3 Hz, 2H), 3.55 (t, J = 4.6 Hz, 4H), 4.16 (t, J = 6.3 Hz, 2H), 7.27–7.55 (m, 7H), 7.66 (d, J = 6.8 Hz, 1H), 7.90–7.98 (m, 2H), 8.18 (d, J = 8.3 Hz, 1H), 8.52–8.54 (m, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 44.0, 53.5, 57.4, 66.7, 109.6, 117.9, 122.9, 123.6, 124.4, 125.6, 126.2, 126.7, 128.1, 129.9, 131.0, 137.2, 138.9, 192.0; IR (Nujol) 1617, 1519, 1380 cm^{-1}; MS (EI) m/z 384 (70), 155 (90), 100 (100); HRMS calcd for $C_{25}H_{24}N_2O_2$: 384.1832; found: 384.1833.

1-[2-(4-Morpholino)ethyl]-1*H*-indol-3-yl-1-naphthylmethane (20). Reduction of 0.200 g (0.52 mmol) of indole **23** by the procedure described above for the preparation of **9** gave 0.077 g (40%) of **20** as a yellow oil: R_f 0.18 (ether); ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.33 (t, J = 4.3 Hz, 4H), 2.59 (t, J = 6.8 Hz, 2H), 3.53 (t, J = 4.3 Hz, 4H), 4.07 (t, J = 6.8 Hz, 2H), 6.59 (s, 1H), 7.10–7.49 (m, 8H), 7.65 (d, J = 7.9 Hz, 1H), 7.76 (d, J = 7.2 Hz, 1H), 7.86 (d, J = 7.2 Hz, 1H), 8.07 (d, J = 7.9 Hz, 1H); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ 29.0, 43.9, 53.9, 58.2, 66.9, 109.3, 114.3, 119.1, 119.3, 121.7, 124.6, 125.6, 125.7, 125.9, 126.7, 127.0, 128.1, 128.7, 132.3, 134.0, 136.4, 137.0; MS (EI) m/z 370 (40), 254 (11), 141 (24), 100 (100); HRMS calcd for $C_{25}H_{26}N_2O$: 370.2045; found: 370.2049.

1-[2-(4-Morpholino)ethyl]-3-(4-methyl-1-naphthoyl)indole (24). Acylation of 0.25 g (1.10 mmol) of 1-[2-(4-morpholino)ethyl]-indole with 4-methylnaphthoyl chloride, prepared from 0.40 g (2.20 mmol) of the corresponding carboxylic acid, was carried out by the procedure described above for the synthesis of indole **18**. After purification by flash chromatography (petroleum ether/ether, 3:1) there was obtained 0.37 g (42%) of **24** as a pale yellow oil: R_f 0.36 (ether); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.39 (t, J = 4.6 Hz, 4H), 2.69 (t, J = 6.4 Hz, 2H), 2.77 (s, 3H), 3.56 (t, J = 4.6 Hz, 4H), 4.15 (t, J = 6.4 Hz, 2H), 7.34–7.38 (m, 4H), 7.45–7.58 (m, 4H), 8.07 (d, J = 8.4 Hz, 1H), 8.23 (d, J = 8.4 Hz, 1H), 8.51–8.54 (m, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 19.8, 44.1, 53.6, 57.5, 66.8, 109.6, 117.8, 122.8, 123.6, 124.2, 125.1, 126.1, 130.9, 137.8, 136.7, 137.0, 137.6, 138.7; MS (EI) m/z 398 (10), 141 (10), 100 (100); HRMS calcd for $C_{26}H_{26}N_2O_2$: 398.1990; found: 398.1990.

1-[2-(4-Morpholino)ethyl]-1*H*-indol-3-yl-(4-methyl-1-naphthyl)methane (21). Reduction of 0.035 g (0.09 mmol) of indole **24** by the procedure described above for the preparation of **9** gave 0.014 g (41%) of **21** as a pale yellow oil: R_f 0.37 (ether); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.37 (t, J = 4.6 Hz, 4H), 2.63 (t, J = 6.9 Hz, 2H), 2.69 (s, 3H), 3.56 (t, J = 4.6 Hz, 4H), 4.10 (t, J = 6.9 Hz, 2H), 6.60 (s, 1H), 7.09–7.14 (m, 1H), 7.20–7.26 (m, 4H), 7.32 (d, J = 8.2 Hz, 1H), 7.41–7.54 (m, 2H), 7.65 (d, J = 7.9 Hz, 1H), 8.04 (d, J = 8.2 Hz, 1H), 8.10 (d, J = 7.9 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 19.6, 29.0, 43.9, 53.9, 58.1, 66.9, 109.2, 114.5, 119.0, 119.3, 121.6, 124.8, 125.1, 125.5, 126.4, 126.9, 128.0, 132.3, 132.9, 133.1, 135.1, 136.4; MS (EI) m/z 384 (10), 155 (5), 100 (100); HRMS calcd for $C_{26}H_{28}N_2O$: 384.2202; found: 384.2202.

1-[2-(4-Morpholino)ethyl]-3-(4-methoxy-1-naphthoyl)indole (25). Acylation of 0.29 g (1.24 mmol) of 1-[2-(4-morpholino)ethyl]-indole with 4-methoxynaphthoyl chloride, prepared from 0.50 g (2.48 mmol) of the corresponding carboxylic acid, was carried out by the procedure described above for the synthesis of indole **18**. After purification by flash chromatography (petroleum ether/ether, 3:1) there was obtained 0.21 g (42%) of **25** as a pale yellow oil: R_f 0.21 (ether); ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.40 (t, J = 4.4 Hz, 4H), 2.69 (t, J = 6.5 Hz, 2H), 3.59 (t, J = 4.4 Hz, 4H), 4.05 (s, 3H), 4.16 (t, J = 6.5 Hz, 2H), 6.81 (d, J = 7.9 Hz, 1H), 7.27–7.33 (m, 3H), 7.43–7.45 (m, 3H), 7.59 (d, J = 7.9 Hz, 1H), 8.20–8.28 (m, 2H), 8.40–8.43 (m, 1H); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ 44.2, 53.7, 55.8, 57.6, 66.9, 102.1, 109.7, 117.9, 122.1, 122.8, 123.0, 123.6, 125.8, 125.9, 127.5, 127.9, 137.0, 138.4, 157.1, 191.9; MS (EI) m/z 414 (80), 369 (60), 314 (25), 100 (100); calcd for $C_{26}H_{26}N_2O_3$: 414.1949; found: 414.1943.

1-[2-(4-Morpholino)ethyl]-1*H*-indol-3-yl-(4-methoxy-1-naphthyl)methane (22). Reduction of 0.075 g (0.18 mmol) of indole **25** by the procedure described above for the preparation of **9** gave 0.029 g (40%) of **22** as a pale yellow oil: R_f 0.30 (ether); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.36 (t, J = 4.6 Hz, 4H), 2.62 (t, J = 6.9 Hz, 2H), 3.57 (t, J = 4.6 Hz, 4H), 3.98 (s, 3H), 4.09 (t, J = 6.9 Hz, 2H), 4.44 (s, 2H), 6.59 (s, 1H), 6.73 (d, J = 7.9 Hz, 1H), 7.09–7.14 (m, 1H), 7.19–7.33 (m, 4H), 7.41–7.48 (m, 2H), 7.64 (d, J = 7.9 Hz, 1H), 7.98–8.01 (m, 1H), 8.29–8.33 (m, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 22.3, 28.5, 29.1, 29.9, 46.1, 55.4, 103.4, 109.3, 114.0, 118.6, 119.2, 121.3, 122.3, 124.3, 124.8, 126.2, 126.3, 126.5, 128.0, 132.9, 136.3, 154.2; MS (EI) m/z 400 (5), 100 (100); HRMS calcd for $C_{26}H_{28}N_2O_2$: 400.2151; found: 400.2151.

***E*-1-[1-(1-Naphthalenylmethylene)-1*H*-inden-3-yl]pentane (26).** To a solution of 0.25 g (1.35 mmol) of 1-(1*H*-inden-3-yl)pentane in 7 mL of dry methanol at 0 °C was added 0.073 g (1.35 mmol) of sodium methoxide in 7 mL of dry methanol. The mixture was warmed to ambient temperature and stirred for 20 min. To this mixture was added 0.21 g (1.35 mmol) of 1-naphthaldeyde and the reaction mixture was heated at reflux for 18 h. After dilution with ethanol, the solvent was removed in vacuo

to give a yellow oil. Purification by chromatography (hexanes) provided 0.13 g (35%) of **26** as a yellow solid: mp 65–66°; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (t 6.8, 3H), 1.25–1.53 (m, 4H), 1.60–1.82 (m, 2H), 2.61 (t, J = 7.6 Hz, 2H), 6.58 (s, 1H), 7.26–7.33 (m, 3H), 7.53–7.66 (m, 4H), 7.81–7.98 (m 4), 8.17 (m, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.2, 22.6, 27.8, 28.1, 32.0, 119.0, 119.2, 122.2, 123.7, 124.8, 125.3, 125.6, 126.2, 127.6, 128.5, 128.7, 129.2, 132.3, 141.5, 143.3, 149.1; IR (neat) 2928, 2860, 1464, 1387 cm^{-1}; MS (EI) m/z 324 (60), 279 (20), 253 (50), 149 (100); calcd for $C_{25}H_{24}$: 324.1877; found: 324.1878.

2-Ethyl-1-pentyl-3-(1-naphthoyl)indole (27). To a stirred solution of 0.65 mL (1.65 mmol) of 2.5 M ethylmagnesium bromide in ether, diluted with 1.1 mL of ether, at 0 °C was added dropwise 0.20 g (1.3 mmol) of 2-ethylindole in 1.1 mL of ether. The solution was stirred for 0.5 h at ambient temperature and a solution of 0.22 mL (1.46 mmol) of 1-naphthoyl chloride in 1 mL of ether was added dropwise. The reaction mixture was stirred for 1.5 h, quenched with saturated aqueous NH_4Cl, and stirred until the solid was broken up into a fine suspension. The residue was washed with water and ether, then suspended in 5 mL of methanol, to which was added 0.4 g of NaOH and 1 mL of water. The mixture was stirred at ambient temperature for 18 h, the solid was filtered off and washed with successive portions of methanol, water and ether. Drying in vacuo at 100 °C gave 0.31 g (74%) of 2-ethyl-3-(1-naphthoyl)indole as a viscous oil, which was used in the next step without further purification: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.07 (t, J = 7.5 Hz, 3H), 2.63 (q, J = 7.5 Hz, 2H), 6.90 (t, J = 7. 5 Hz, 1H), 7.02–7.10 (m, 2H), 7.37–7.76 (m, 6H), 7.81 (d, J = 8.2 Hz, 1H), 8.21 (q, J = 8.2 Hz, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 13.6, 21.0, 11.6, 113.0, 120.2, 121.5, 122.2, 124.5, 125.0, 125.5, 126.5, 126.9, 127.1, 128.5, 129.5, 133.2, 135.2, 140.7, 151.3, 192.3.

To a solution of 0.40 g (1.3 mmol) of 2-ethyl-3-(1-naphthoyl)indole in 3.0 mL of DMSO was added 1.2 g of powdered KOH. The reaction was stirred at ambient temperature and 1.35 mL (11.0 mmol) of 1-bromopentane were added slowly. The solution was stirred at 85 °C for 18 h. After cooling the reaction mixture was diluted with water, and extracted with three portions of ethyl acetate. The extracts were washed with brine, dried ($MgSO_4$), and the solvent was removed in vacuo. Chromatography (petroleum ether/ethyl acetate, 7:1) gave 0.44 g (89%) of **27** as a yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.92 (t, J = 7.0 Hz, 3H), 1.24 (t, J = 7.5 Hz, 3H), 1.37–1.42 (m, 4H), 1.78–1.85 (m, 2H), 3.05 (q, J = 7.5 Hz, 2H), 4.12 (t, J = 7.5 Hz, 2H), 6.84–6.93 (m, 2H), 7.10–7.15 (m, 1H), 7.29 (d, J = 8.2 Hz, 1H), 7.41–7.58 (m, 3H), 7.89–7.97 (m, 2H), 8.11 (d, J = 8.2 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 13.9, 14.0, 19.3, 22.3, 29.1, 29.7, 43.2, 109.6, 113.7, 121.1, 121.6, 122.0, 124.9, 125.5, 125.6, 126.1, 126.7, 127.1, 128.1, 129.8, 130.3, 133.7, 135.9, 140.5, 151.2, 193.1; MS (EI) m/z 369 (60), 340 (56), 155 (100); HRMS calcd for $C_{26}H_{27}NO$: 369.2093; found: 369.2093.

In vitro pharmacology. CB_1 assay. [^{3}H]CP-55,940 (79 Ci/mmol, K_D = 690 pM) binding to P_2 membranes was conducted as described elsewhere,[33] except whole brain (rather than cortex only) was used. Displacement curves were generated by incubating drugs with 1 nM of [^{3}H]CP-55,940. Nonspecific binding was determined in the presence of 1 μM CP-55,940. The assays were performed in triplicate, and the results represent the combined data from three individual experiments. CP-55,940 and all cannabinoid analogues were prepared by suspension in assay buffer from a 1 mg/mL ethanolic stock without evaporation of the ethanol (final concentration of no more than 0.4%). Displacement IC_{50} 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.[34]

Molecular modeling

1. Conformational analysis. The structures of JWH-081 (**16**), JWH-098 (**19**), JWH-185 (**11**) and JWH-197 (**14**) were built in the Spartan molecular modeling program (V4.1.1; Wavefunction, Inc., Irvine, CA). Each structure was minimized using the AM1, semi-empirical method. For each minimized structure, AM1 conformational searches were then performed for rotation about the C3–C1′ and C1′–C2′ bonds (see the structure of **5** for the numbering system).

2. CB_1 receptor docking studies. Amino acid numbering system. In the discussion of receptor residues that follows, the amino acid numbering scheme proposed by Ballesteros and Weinstein is used.[35] In this numbering system, the most highly conserved residue in each transmembrane helix (TMH) is assigned a locant of 0.50. This number is preceded by the TMH number and may be followed in parentheses by the sequence number. All other residues in a TMH are numbered relative to this residue. In this numbering system, for example, the most highly conserved residue in TMH 2 of the CB_1 receptor is D2.50(163). The residue that immediately precedes it is A2.49(162).

3. Model of inactive state of CB_1. A model of the inactive (R) form of CB_1 was created using the 2.8 Å crystal structure of rhodopsin (Rho).[36] First, the sequence of the human CB_1 receptor[37] was aligned with the sequence of bovine rhodopsin (Rho) using the same highly conserved residues as alignment guides that were used initially to generate our first model of CB_1.[23] TMH 5 in CB_1 lacks the highly conserved proline in TMH 5 of Rho. The sequence of CB_1 in the TMH 5 region was aligned with that of Rho as described previously using its hydrophobicity profile.[23] Helix ends for CB1 were chosen in analogy with those of Rho;[36] TMH 1: N1.28(112)–R1.61(145); TMH 2: R2.37(150)–H2.68(181); TMH 3: S3.21(185)–R3.56(220); TMH 4: T4.38(229)–C4.66(257); TMH 5: H5.34(270)–K5.64(300); TMH 6:R6.28(336)–K6.62(370); TMH 7: K7.32(376)–S7.57(401); intracellular extension of TMH 7: D7.59(403)–C7.71(415).

4. Preparation of helices. Each helix of the model was capped by acetamide at its N-terminus and *N*-methylamide at its C-terminus. Ionizable residues in the first

turn of either end of the helix were neutralized, as were any lipid facing charged residues. Ionizable residues were considered charged if they appeared anywhere else in the helix.

5. Model of CB_1 active (R*) state. Experimental results for rhodopsin and the β_2-adrenergic receptor document that upon activation, several conformational changes ensue. These changes include rigid domain motions in TMHs 3 and 6, straightening of the proline kink in TMH 6, as well as rotations in TMHs 3 and 6 that change the environments of residues 3.41 and 6.47.[38–42] Recent conformational memory results for TMH 6 in CB_1 have indicated that the TMH 6 proline kink angle lessens to a value of 21.8° in the activated state (R*).[43] An active (R*) CB_1 bundle was created from the inactive (R) model of CB_1 by setting the TMH 6 proline kink angle to 21.8° and by rotating both TMH 3 and 6 counterclockwise (from an extracellular perspective) so that residues 3.41 and 6.47 changed environments.[40,42,43]

6. Ligand/CB_1 R* complex. Each ligand was docked in the CB_1 TMH 3-4-5-6 region using interactive computer graphics. For JWH-081 (**16**) and JWH-098 (**19**), the lowest energy *s-trans* conformer of each ligand was used for docking studies.[24] For JWH-185 (**11**) and JWH-197 (**14**), the global minimum energy conformer of each was used in docking studies. The energy of the CB_1 R* TMH bundle/ligand complex was minimized using the AMBER* united atom force field in Macromodel 6.5 (Schrodinger Inc., Portland, OR). A distance dependent dielectric, 8.0 Å extended non-bonded cutoff (updated every 10 steps), 20.0 Å electrostatic cutoff, and 4.0 Å hydrogen bond cutoff were used. The first stage of the calculation consisted of 2000 steps of Polak-Ribier conjugate gradient (CG) minimization in which a force constant of 225 kJ/mol was used on the helix backbone atoms in order to hold the helix backbones fixed, while permitting the side chains to relax. The second stage of the calculation consisted of 100 steps of CG in which the force constant on the helix backbone atoms was reduced to 50 kJ/mol in order to allow the helix backbones to adjust. Stages one and two were repeated with the number of CG steps in stage two incremented from 100 to 500 steps until a gradient of 0.001 kJ/(mol·Å^2) was reached. Explicit hydrogens were included on all aromatic amino acid residues in order to better simulate aromatic stacking interactions.[30]

Each resultant receptor/ligand complex was analyzed for the presence of hydrogen bonding and aromatic stacking interactions. Aromatic stacking interactions were identified using Burley and Petsko's criteria.[30] These investigators reported that aromatic–aromatic stacking interactions in proteins operate at distances (d) of 4.5–7.0 Å between ring centroids. The angle (α) between normal vectors of interacting aromatic rings typically is between 30 and 90°, producing a 'tilted-T' or 'edge-to-face' arrangement of interacting rings. Residues and/or ligand regions were designated here as participating in an aromatic stacking interaction if they had centroid to centroid distances between 4.5 and 7.0 Å. These interactions were further classified as 'tilted-T' arrangements if $30° \leq \alpha \leq 90°$ and as parallel arrangements for $\alpha < 30°$. In interactions where $\alpha = 0°$, arrangements were also identified as offset or not offset, as Hunter et al. have shown that offset parallel stacks are more energetically favorable.[44]

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References and Notes

1. Gaoni, Y.; Mechoulam, R. *J. Am. Chem. Soc.* **1964**, *86*, 1648.
2. Razdan, R. K. *Pharmacol. Rev.* **1986**, *38*, 75.
3. Rapaka, R. S.; Makriyannis, A. *Structure–Activity Relationships of the Cannabinoids, NIDA Research Monograph 79*; National Institute on Drug Abuse: Rockville, MD, 1987.
4. Mechoulam, R.; Devane, W. A.; Glaser, R. In *Marijuana/Cannabinoids: Neurobiology and Neurophysiology*; Murphy, L.; Bartke, A., Eds.; CRC Press: Boca Raton, 1992; pp 1–33.
5. Huffman, J. W.; Lainton, J. A. H. *Curr. Med. Chem.* **1996**, *3*, 101.
6. Seltzman, H. H. *Curr. Med. Chem.* **1999**, *6*, 685.
7. Melvin, L. S.; Johnson, M. R.; Herbert, C. A.; Milne, G. M.; Weissman, A. A. *J. Med. Chem.* **1984**, *27*, 67.
8. Johnson, M. R.; Melvin, L. S. In *Cannabinoids as Therapeutic Agents*; Mechoulam, R., Ed.; CRC Press: Boca Raton, FL, 1986; pp 121–145.
9. Bell, M. R.; D'Ambra, T. E.; Kumar, V.; Eissanstat, M. A.; Herrmann, J. L.; Wetzel, J. R.; Rosi, D.; Philion, R. E.; Daum, S. J.; Hlasta, D. J.; Kullnig, R. K.; Ackerman, J. H.; Haubrich, D. R.; Luttinger, D. A.; Baizman, E. R.; Miller, M. S.; Ward, S. J. *J. Med. Chem.* **1991**, *34*, 1099.
10. D'Ambra, T. E.; Estep, K. G.; Bell, M. R.; Eissenstat, M. A.; Josef, K. A.; Ward, J.; Haycock, D. A.; Baizman, E. R.; Casiano, F. M.; Beglin, N. C.; Chippari, S. M.; Grego, J. D.; Kullnig, R. K.; Daley, G. T. *J. Med. Chem.* **1992**, *35*, 124.
11. Compton, D. R.; Gold, L. H.; Ward, S. J.; Balster, R. L.; Martin, B. R. *J. Pharmacol. Exp. Ther.* **1992**, *263*, 1118.
12. Eissenstat, M. A.; Bell, M. R.; D'Ambra, T. E.; Alexander, E. J.; Daum, S. J.; Ackerman, J. H.; Gruett, M. D.; Kumar, V.; Estep, K. G.; Olefirowicz, E. M.; Wetzel, J. R.; Alexander, M. D.; Weaver, J. D.; Haycock, D. A.; Luttinger, D. A.; Casiano, F. M.; Chippari, S. M.; Kuster, J. E.; Stevenson, J. I.; Ward, S. J. *J. Med. Chem.* **1995**, 3094.
13. Xie, X.-Q.; Eissenstat, M.; Makriyannis, A. *Life Sci.* **1995**, *56*, 1963.
14. Huffman, J. W.; Dai, D.; Martin, B. R.; Martin, B. R.; Compton, D. R. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 563.
15. Wiley, J. L.; Compton, D. R.; Dai, D.; Lainton, J. A. H.; Phillips, M.; Huffman, J. W.; Martin, B. R. *J. Pharmacol. Exp. Ther.* **1998**, *285*, 995.
16. Aung, M. M.; Griffin, G.; Huffman, J. W.; Wu, M.-J.; Keel, C.; Yang, B.; Showalter, V. M.; Abood, M. E.; Martin, B. R. *Drug Alcohol Depend.* **2000**, *60*, 133.
17. Fichera, M.; Cruciani, G.; Bianchi, A.; Musumarra, G. *J. Med. Chem.* **2000**, *43*, 2300.
18. Huffman, J. W. *Curr. Med. Chem.* **1999**, *6*, 705.
19. Huffman, J. W.; Lu, J.; Kitaygorodskiy, A.; Wiley, J. L.; Martin, B. R. *Bioorg. Med. Chem.* **2000**, *8*, 439.

20. Song, Z. H.; Bonner, T. I. *Mol. Pharmacol.* **1996**, *49*, 891.
21. Bramblett, R. D.; Reggio, P. H. *1995 Symposium on the Cannabinoids*; International Cannabinoid Research Society: Burlington, VT, 1995; p 16.
22. Bramblett, R. D.; Reggio, P. H. *1995 Symposium on the Cannabinoids*; International Cannabinoid Research Society: Burlington, VT, 1995; p 17.
23. Bramblett, R. D.; Panu, A. M.; Ballesteros, J. A.; Reggio, P. H. *Life Sci.* **1995**, *56*, 1971.
24. Reggio, P. H.; Basu-Dutt, S.; Barnett-Norris, J.; Castro, M. T.; Hurst, D. P.; Seltzman, H. H.; Roche, M. J.; Gilliam, A. F.; Thomas, B. F.; Stevenson, L. A.; Pertwee, R. G.; Abood, M. E. *J. Med. Chem.* **1998**, *41*, 5177.
25. Huffman, J. W.; Wu, M.-J.; Lu, J. *J. Org. Chem.* **1998**, *63*, 4510.
26. Compton, D. R.; Rice, K. C.; De Costa, B. R.; Razdan, R. K.; Melvin, L. S.; Johnson, M. R.; Martin, B. R. *J. Pharmacol. Exp. Ther.* **1993**, *265*, 218.
27. Kumar, V.; Alexander, M. D.; Bell, M. R.; Eissenstat, M. A.; Casiano, F. M.; Chippari, S. M.; Haycock, D. A.; Luttinger, D. A.; Kuster, J. E.; Miller, M. S.; Stevenson, J. I.; Ward, S. J. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 381.
28. Shire, D.; Calandra, B.; Bouaboula, M.; Barth, F.; Rinaldi-Carmona, M.; Casellas, P.; Ferrara, P. *Life Sci.* **1999**, *65*, 627.
29. McAllister, S. D.; Tao, Q.; Barnett-Norris, J.; Buehner, K.; Hurst, D. P.; Guarnieri, F.; Reggio, P. H.; Nowell Harmon, K. W.; Cabral, G. A.; Abood, M. E. *Biochem. Pharmacol.* **2002**, in press.
30. Burley, S.; Petsko, G. *Science* **1985**, *229*, 23.
31. Song, A. H.; Slowey, C.-A.; Hurst, D. P.; Reggio, P. H. *Mol. Pharmacol.* **1999**, *56*, 834.
32. Augustine, R. L.; Gustavasen, A. J.; Wanat, S. F.; Pattison, I. C.; Houghton, K. S.; Koletar, G. J. *J. Org. Chem.* **1973**, *38*, 4073.
33. Martin, B. R.; Compton, D. R.; Thomas, B. F.; Prescott, W. R.; Little, P. J.; Razdan, R. K.; Johnson, M. R.; Melvin, L. S.; Mechoulam, R.; Ward, S. J. *Pharmacol. Biochem. Behav.* **1991**, *40*, 471.
34. Cheng, Y. C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.
35. Ballesteros, J. A.; Weinstein, H. In *Methods in Neuroscience*; Conn, P. M.; Sealfon, S. C., Eds.; Academic Press: New York, 1995; Vol. 25, pp 366–428.
36. Palczewski, K.; Kumasaka, T.; Hori, T.; Behnke, C. A.; Motoshima, H. B. A.; Fox, B. A.; LeTron, I.; Teller, D. C.; Okada, T.; Stenkamp, R. E.; Yamamoto, M.; Miyano, M. *Science* **2000**, *289*, 739.
37. Gerard, C. M.; Mollereau, C.; Vassart, G.; Parmentier, M. *Biochem. J.* **1991**, *279*, 129.
38. Ballesteros, J. A.; Jensen, A. D.; Liapakis, G.; Rasmussen, S. G.; Shi, L.; Gether, U.; Javitch, J. A. *J. Biol. Chem.* **2001**, *276*, 29171.
39. Hulme, E. C.; Lu, Z.-L.; Ward, S. D. C.; Allman, K.; Curtis, C. A. M. *Eur. J. Pharmacol.* **1999**, *375*, 247.
40. Javitch, J. A.; Fu, D.; Liapakis, G.; Chen, J. *J. Biol. Chem.* **1997**, *272*, 18546.
41. Jensen, A. D.; Guarnieri, F.; Rasmussen, S. G. F.; Asmar, F.; Ballesteros, J. A.; Gether, U. *J. Biol. Chem.* **2001**, *276*, 9279.
42. Lin, S. W.; Sakmar, T. P. *Biochem.* **1996**, *35*, 11149.
43. Barnett-Norris, J.; Hurst, D. P.; Buehner, K.; Ballesteros, J. A.; Guarnieri, F.; Reggio, P. H. *Int. J. Quantum Chem.* **2002**, *88*, 76.
44. Hunter, C.; Singh, J.; Thornton, J. *J. Mol. Biol.* **1991**, *218*, 837.