The Bioactive Conformation of Aminoalkylindoles at the Cannabinoid CB1 and CB2 Receptors: Insights Gained from (*E*)- and (*Z*)-Naphthylidene Indenes

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The aminoalkylindoles (AAIs) are agonists at both the cannabinoid CB1 and CB2 receptors. To determine whether the s-trans or s-cis form of AAIs is their receptor-appropriate conformation, two pairs of rigid AAI analogues were studied. These rigid analogues are naphthylidene-substituted aminoalkylindenes that lack the carbonyl oxygen of the AAIs. Two pairs of (*E*)- and (*Z*)-naphthylidene indenes (C-2 H and C-2 Me) were considered. In each pair, the *E* geometric isomer is intended to mimic the s-trans form of the AAIs, while the *Z* geometric isomer is intended to mimic the s-cis form. Complete conformational analyses of two AAIs, pravadoline (**2**) and WIN-55,212-2 (**1**), and of each indene were performed using the semiempirical method AM1. S-trans and s-cis conformations of **1** and **2** were identified. AM1 single-point energy calculations revealed that when **1** and each indene were overlayed at their corresponding indole/indene rings, the (*E*)- and (*Z*)-indenes were able to overlay naphthyl rings with the corresponding s-trans or s-cis conformer of **1** with an energy expense of 1.13/0.69 kcal/mol for the C-2 H (*E*/*Z*)-indenes and 0.82/0.74 kcal/mol for the C-2 Me (*E*/*Z*)-indenes. On the basis of the hypothesis that aromatic stacking is the predominant interaction of AAIs such as **1** at the CB receptors and on the demonstration that the C-2 H (*E*/*Z*)- and C-2 Me (*E*/*Z*)-indene isomers can mimic the positions of the aromatic systems in the s-trans and s-cis conformers of **1**, the modeling results support the previously established use of indenes as rigid analogues of the AAIs. A synthesis of the naphthylidene indenes was developed using Horner−Wittig chemistry that afforded the *Z* isomer in the C-2 H series, which was not produced in significant amounts from an earlier reported indene/aldehyde condensation reaction. This approach was extended to the C-2 Me series as well. Photochemical interconversions in both the C-2 H and C-2 Me series were also successful in obtaining the less favored isomer. Thus, the photochemical process can be used to provide quantities of the minor isomers C-2 H/*Z* and C-2 Me/*E*. The CB1 and CB2 affinities as well as the activity of each compound in the twitch response of the guinea pig ileum (GPI) assay were assessed. The *E* isomer in each series was found to have the higher affinity for both the CB1 and CB2 receptors. In the rat brain membrane assay versus [^{3}H]CP-55,940, the K_i's for the C-2 H/C-2 Me series were 2.72/2.89 nM (*E* isomer) and 148/1945 nM (*Z* isomer). In membrane assays versus [^{3}H]SR141716A, a two-site model was indicated for the C-2 H/C-2 Me (*E* isomers) with K_i's of 10.8/9.44 nM for the higher-affinity site and 611/602 nM for the lower-affinity site. For the *Z* isomers, a one-site model was indicated with K_i's of 928/2178 nM obtained for the C2 H/C-2 Me analogues, respectively. For the C-2 H/C-2 Me series, the CB2 K_i's obtained using a cloned cell line were 2.72/2.05 nM (*E* isomer) and 132/658 nM (*Z* isomer). In the GPI assay, the relative order of potency was C-2 H *E* > C-2 Me *E* > C-2 H *Z* > C-2 Me Z. The C-2 H *E* isomer was found to be equipotent with **1**, while the C-2 Me *Z* isomer was inactive at concentrations up to 3.16 μM. Thus, results indicate that the *E* geometric isomer in each pair of analogues is the isomer with the higher CB1 and CB2 affinities and the higher pharmacological potency. Taken together, results reported here support the hypothesis that the s-trans conformation of AAIs such as **1** is the preferred conformation for interaction at both the CB1 and CB2 receptors and that aromatic stacking may be an important interaction for AAIs at these receptors.

The aminoalkylindoles (AAIs) are a novel class of cannabinoid receptor ligands that have been shown to have affinity for both the cannabinoid CB1 and CB2 receptors.[1,2] The tritiated form of one AAI, (*R*)-(+)-[2,3-dihydro-5-methyl-3-[(4-morpholinyl)methyl]pyrrolo[1,2,3-*de*]-1,4-benzoxazin-6-yl](1-naphthalenyl)methanone (WIN-

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55,212-2, **1**), is now in standard use in radioligand displacement assays of the cannabinoid CB1 receptor.[3] Metabolism studies of pravadoline (**2**), the original lead compound in the AAI series, and its 1-naphthyl congener have shown that the morpholinoalkyl substituent at N-1 is metabolized to a carboxylic acid ($-CH_2COOH$).[4] However, the resultant carboxylic acid derivatives are not capable of displacing [^{3}H]WIN-55,212-2 in rat cerebellum membranes.[3] These results suggest that **2** and its congeners, rather than their metabolites, act at the CB1 receptor. NMR solution and X-ray crystallography studies of **2** and its C-2 H congener (**3**) have revealed that each AAI can exist in two distinct conformations which differ mainly in the orientation of the C-3 aroyl system.[4] In the s-trans conformation, which predominates when the C-2 substituent is a hydrogen, the aryl system is near C-2, while the carbonyl oxygen is located near C-4. In the s-cis conformation, which predominates when the C-2 substituent is a methyl group, the aryl ring is located near C-4, and the carbonyl oxygen is located near C-2.

To understand the cannabinoid-like actions of the AAIs at a molecular level, it is important to know the bioactive conformation of the AAIs at the cannabinoid receptors. As some AAIs have been shown to exist in both s-trans and s-cis conformations, conformations which place the aroyl substituent in very different regions of space, we sought rigidified AAI analogues that could be used to elucidate which of these two conformations is the bioactive conformation. Recently, Kumar et al.[5] published the synthesis and biological evaluation of *E*,*Z*-mixtures of C-2 H and C-2 Me naphthylidene indene analogues **4** and **5**. These rigid analogues are naphthylidene-substituted aminoalkylindenes that lack the carbonyl oxygen of the AAIs but are intended to mimic the s-trans (*E* isomer) and s-cis (*Z* isomer) conformations of the AAI aroyl moiety. Compound **4** was reported to be (>95%) the cis isomer, while a mixture of cis and trans (4:1) was obtained for compound **5**. Both **4** and **5** were reported to have high affinity for the CB1 receptor.[5] However, as no separation of the mixture of geometric isomers was performed, the results reported by Kumar et al. left unanswered the question of whether CB1 affinity resided in one or both of the geometric isomers.

To answer this question, we undertook the molecular modeling analysis, synthesis, and pharmacological evaluation of the *E* and *Z* isomers of compounds **4** and **5**. Modeling results show that the (*E*)- and (*Z*)-indenes are good rigid analogues of the s-trans and s-cis conformations, respectively, of the AAI, WIN-55,212-2 (**1**). Pharmacological results show that the *E* isomer of each indene (an isomer analogous to the s-trans conformer) possesses the higher affinity at both the CB1 and CB2 receptors.

Results and Discussion

Molecular Modeling. 1. Conformational Analysis: S-Trans and S-Cis Forms of WIN-55,212-2 (1) and Pravadoline (2). To verify that the (*E*)- and (*Z*)-indenes mimic the s-trans and s-cis conformations of the AAIs, a molecular modeling study was undertaken. Complete AM1 conformational analyses of **1** and **2** as well as of **4*E*/4*Z*** and **5*E*/5*Z*** were performed. Pravadoline (**2**) was studied to verify that AM1 conformational analysis produces conformers whose geometries and relative energies are consistent with NMR, UV, and X-ray data.[4] WIN-55,212-2 (**1**) was studied to be used as a template for comparison with **4*E*/4*Z*** and **5*E*/5*Z***, as **1** has high affinity for the CB receptors. Figure 1A illustrates that AM1 calculations identified both s-trans and s-cis forms of pravadoline (**2**), each with two possibilities depending upon whether the *p*-methoxyphenyl ring points into the top face or bottom face of the molecule (i.e., above or below the plane of the indole ring as it is drawn in Chart 1). For pravadoline (**2**), which possesses a C-2 methyl group, the s-cis conformer was found to be lower in energy than the s-trans conformer by 1.36 kcal/mol. These results for pravadoline (**2**) are in agreement with X-ray crystal studies of **2** which indicate the existence of both s-trans and s-cis forms and with NMR and UV studies which show that the predominant form of **2** in solution is the s-cis form.[4] UV spectra indicate that the carbonyl group of **2** cannot be orthogonal to the indole ring and probably can be no more than 60−70° out of the plane of the indole ring.[4] AM1 results for pravadoline (see Figure 1) indicated that the carbonyl oxygen is 42.1−47.4° out of plane with the indole system.

The accessible conformations calculated for WIN-55,212-2 (**1**) paralleled the results for pravadoline (**2**). For **1**, the lowest-energy s-cis conformer was found to be 1.86 kcal/mol lower in energy than the lowest-energy s-trans conformer. This result is consistent with results reported for **2** above which point to the s-cis form of C-2 Me AAIs as the predominant form in solution.[4] Like **2**, the C-3 carbonyl group of **1** does not lie in the plane of the indole ring; consequently, there are two s-cis and two s-trans orientations possible for **1**. In addition, the naphthyl group of each conformer of **1** can exist in two different minimum energy positions, yielding a total of four s-trans conformers of **1** and four s-cis conformers of **1**. Figure 1B illustrates the possible conformations of the s-trans and s-cis forms of WIN-55,212-2 (**1**). The s-trans conformers were found to be within 0.40 kcal/mol of one another, while the s-cis conformers were within 0.99 kcal/mol of one another. Figure 2 reveals that the regions occupied by the *p*-methoxyphenyl ring of **2** are encompassed in the region occupied by the naphthyl ring of **1**; however, it is clear from Figure 2 that the naphthyl ring of **1** can "sweep" a wider area. Another difference between **1** and **2** noted during the conformational analysis was in the preferred orientation of the morpholino ring. Pravadoline (**2**) was found to prefer a gauche rotamer (i.e., N−C−C−N = 66.5°), while WIN-55,212-2 (**1**) prefers the trans rotamer (i.e., N−C−C− N = 142°). However, the energy difference between gauche and trans was small (i.e., 0.42 kcal/mol for **1**; 0.46 kcal/mol for **2**).

Conformational Analysis: Results for 4*E*/4*Z* and 5*E*/5*Z*. Comparison of the global minimum conformer of each indene geometric isomer revealed that the C-2 H *E* geometric isomer (**4*E***, which corresponds to an s-trans conformation) is 1.15 kcal/mol lower in energy than **4*Z***. This result is also consistent with NMR and UV results for the C-2 H congener of pravadoline (**3**) which indicate that its predominant form in solution is the s-trans form.[4] Like **1** and **2, 5*E*/5*Z*** possess a C-2

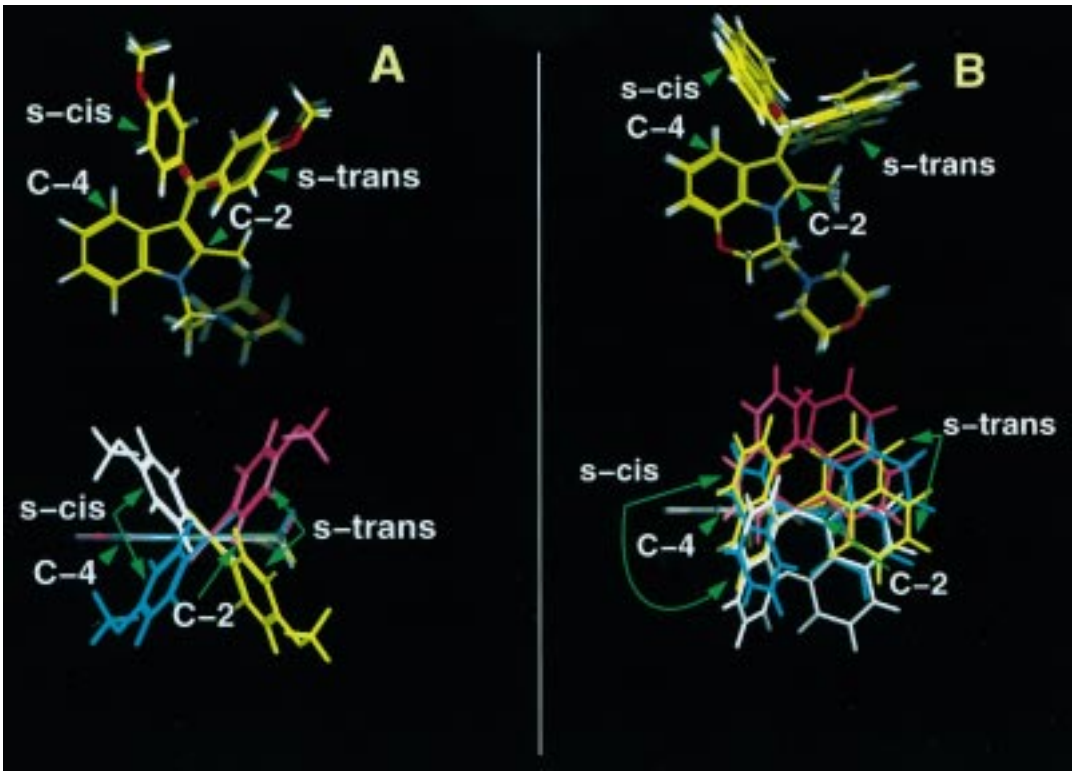
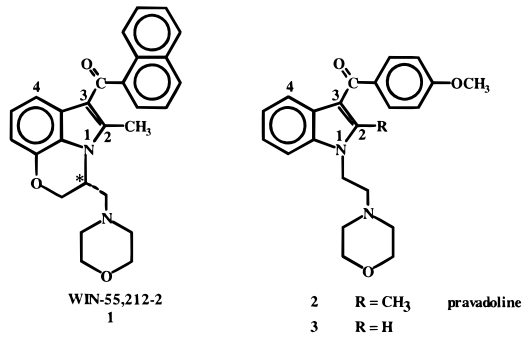


Figure 1. (A) (Top) Superposition (at their indole rings) of the two s-cis and two s-trans conformations of pravadoline (**2**) as calculated by AM1. The conformers are shown here for a single rotamer of the morpholinoethyl group. (Bottom) Superimposed s-cis and s-trans forms of pravadoline (**2**) each in a different color are shown here in a top view in which the indole ring is normal to the plane of the page with C-3 and C-4 pointing toward the viewer and the C-2 methyl group pointing toward the right. (B) (Top) Superposition (at their indole rings) of the four s-cis and four s-trans conformers of WIN-55,212-2 (**1**) as calculated by AM1. The conformers are shown here for a single rotamer of the morpholinoalkyl group. (Bottom) Conformers are shown in a top view with each s-cis and each s-trans conformer in a different color.

Chart 1



methyl group. Conformational analyses of **5*E*** and **5*Z*** revealed that **5*Z*** (which corresponds to the s-cis conformation) is 0.97 kcal/mol lower in energy than **5*E***. This result is consistent with the other C-2 Me AAIs (**1** and **2)** discussed above, which are also predicted to have the s-cis form as predominant in solution. Bell et al. have proposed that the preference of C-2 Me AAIs for the s-cis conformation occurs because the additional bulk produced by the methyl group at C-2 is better accommodated when the aryl ring is rotated away from C-2 and toward C-4 instead.[4]

In the indenes, the double bond exocyclic to the indene ring is coplanar with the indene ring. Because of this planarity, there is only one s-cis position in **4*Z*** and **5*Z*** and one s-trans in position **4*E*** and **5*E***. However, the naphthyl group of the C-3 substituent can adopt two different minimum energy orientations within the confines of the overall geometry by rotation about the bond between the alkene carbon and the naphthyl ring. For this reason, there are two conformations each for **4*E***, **4*Z***, **5*E***, and **5*Z***, and the area swept by the naphthyl group is not as expansive as in **1**.

The stereoisomers of WIN-55,212 differ in the orientation of the morpholinoalkyl moiety at C* (see WIN-55,212-2 drawing, Chart 1).[1] In the active (*R*)-(+)-stereoisomer, **1**, this moiety lies in the bottom face of the molecule (i.e., below the plane of the page as illustrated in Chart 1). In the inactive (*S*)-(−)-stereoisomer, WIN-55,212-3, this moiety lies in the top face (i.e., above the plane of the page in Chart 1). To account for this stereochemical requirement, before initial conformer comparison with **1** was begun, all conformers of **4*E*/4*Z*** and **5*E*/5*Z*** in which the morpholinoalkyl moiety protruded into the top face of the molecule were eliminated. Conformers for which the morpholino ring exists in the bottom face of the molecule can still adopt the two different naphthyl ring orientations discussed above.

2. Hypothesized AAI/CB Receptor Interactions. There is evidence in the SAR literature that potential hydrogen-bonding moieties of the AAIs are not essential to the binding of AAIs at the CB1 receptor but that the presence of an aromatic ring as part of the C-3 substituent is important. Kumar et al.[5] reported high CB1 affinity and efficacy for *E,Z* mixtures of naphthylidene indenes. Such results suggest that the carbonyl oxygen of the AAIs may not be essential for interaction with the CB1 receptor, as the indenes lack this carbonyl oxygen. In addition, Huffman et al.[6] reported that the

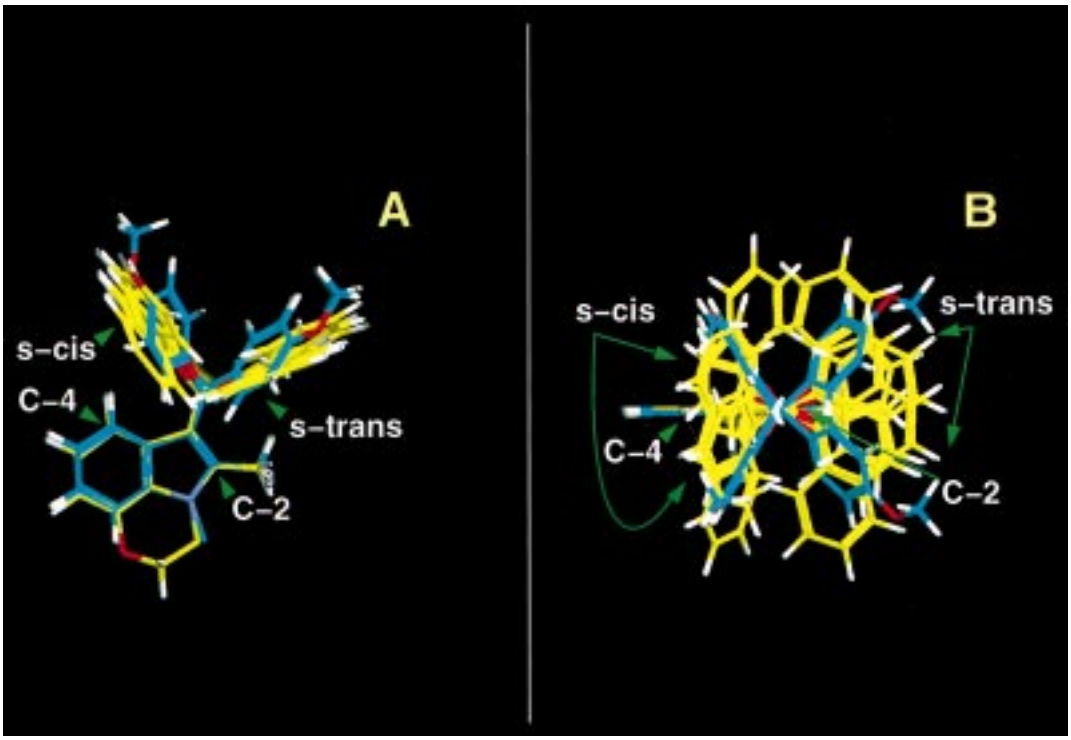


Figure 2. (A) Superposition (at their indole rings) of the s-cis and s-trans conformers of WIN-55,212-2 (**1**; shown here in yellow) with those of pravadoline (**2**; shown here in cyan). (B**)** Conformers are shown in a top view. The morpholinoalkyl group has been removed here for simplicity.

morpholino ring in the AAIs can be replaced with an alkyl chain (*n*-butyl, *n*-pentyl, or *n*-hexyl) without loss of CB1 affinity or efficacy. Huffman et al.[6] also reported that replacement of the naphthyl ring of the C-3 aroyl substituent with an alkyl (CH_3) or alkenyl ($(CH_3)_2C{=}CH$) group resulted in complete loss of CB1 receptor affinity (K_i > 10 000 nM in both cases). This finding is significant because it underscores that an aromatic system as part of the C-3 substituent may be important and because it suggests that the requirement at this position is not simply a requirement for any hydrophobic moiety. The fact that the carbonyl oxygen of AAIs or the morpholino ring of AAIs can be removed without significant effect, along with the fact that the presence of the carbonyl and morpholino groups (in the absence of an aryl substituent) is *insufficient* to produce CB1 affinity, led to the hypothesis that aromatic stacking, rather than hydrogen bonding, may be the primary interaction for AAIs at the CB1 receptor.[7]

Burley and Petsko[8] have shown that aromatic−aromatic interactions are significant contributors to protein structure stabilization. These interactions operate at centroid-to-centroid distances of 4.5−7.0 Å between interacting rings. The angle between the normal vectors of interacting aromatic rings is typically between 30° and 90°, producing a "tilted-T" or "edge-to-face" arrangement of interacting rings. Such interactions are reported to have energies between 1.0 and 2.0 kcal/mol.[8] Preliminary modeling studies of the CB1 and CB2 receptor transmembrane helix bundles have shown that helices 3, 4, and 5 in both the CB1 and CB2 receptors form an aromatic patch with which ligands might interact.[7,9] The hypothesis of the importance of aromatic stacking is also supported by previously published results for the AAIs which show that substitution of a 1-naphthyl group for the *p*-methoxyphenyl substituent of pravadoline (**2**) results in an improvement in CB1 affinity from an IC_{50} of 3155 ± 54 nM for **2** to an IC_{50} of 19 ± 2 nM.[3] This increase in affinity may be due, in part, to increased aromatic−aromatic stacking interactions of the naphthyl derivative at CB1.

3. Comparison of Aromatic Region Positions. If aromatic stacking is an important interaction of AAIs at the CB1/CB2 receptors and if the indenes interact with the CB1/CB2 receptors in the same general orientation as WIN-55,212-2 (**1**), then the indene and naphthyl moieties of **4*E*/4*Z*** and **5*E*/5*Z*** should be in the same general region of space as the indole and naphthyl moieties of **1**. To verify this for **4*E*/4*Z*** and **5*E*/5*Z***, we compared the relative positions of the aromatic regions in the lowest-minimum energy form of each conformer of **1** (i.e., s-trans or s-cis) with the corresponding global minimum energy conformers of each indene *E* or *Z* isomer. The indole ring of **1** was superimposed on the indene ring of each isomer. Because the carbonyl oxygen of the s-trans conformer of **1** lies slightly out of plane (13°), but the alkene hydrogen of the (*E*)-indenes lies essentially in the plane of the indole ring (−1.30° for **4*E*** and −1.10° for **5*E***), the naphthyl rings are oriented slightly differently. AM1 single-point energy calculations revealed an energy expense of 1.13/0.82 kcal/mol for **4*E*/5*E*** was required to adjust the tilt of the indene naphthyl to that of **1**. Figure 3A presents an overlay of this rotamer of **5*E*** with the minimum energy s-trans conformer of WIN-55,212-2 (**1**). This figure shows that the naphthyl rings occupy similar regions of space, when the indole and indene rings are overlaid.

The naphthyl rings of the minimum energy s-cis conformer of **1** and of the minimum energy conformer of **4*Z*** or **5*Z*** nearly coincide, differing only in tilt. AM1 single-point energy calculations revealed that the tilt of the naphthyl ring in **4*Z***/**5*Z*** can match the tilt in the

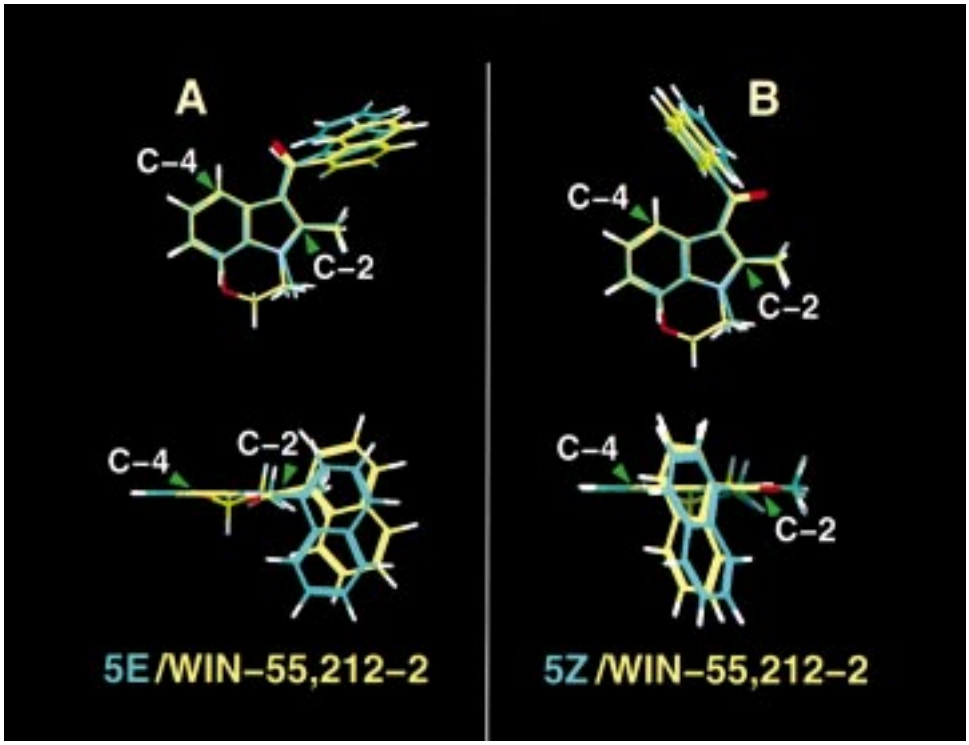


Figure 3. Superposition of **5*E*/5*Z*** with WIN-55,212-2 (**1**). Here the indene ring of **5*E*** or **5*Z*** has been superimposed on the indole ring of **1**. (A) Front and top views, respectively, of the overlays of an s-trans conformer of **1** (in yellow) with a rotamer of **5*E*** (in green). (B) Front and top views, respectively, of an s-cis conformer of **1** (in yellow) with a rotamer of **5*Z*** (in green). The morpholinoalkyl portions of each conformer have been removed for simplicity.

minimum energy s-cis confomer of **1** with an expense of 0.69/0.74 kcal/mol, respectively. Figure 3B illustrates results for **5*Z*** which show that the overlap of the two naphthyl groups is quite good, when the indole and indene rings are overlaid.

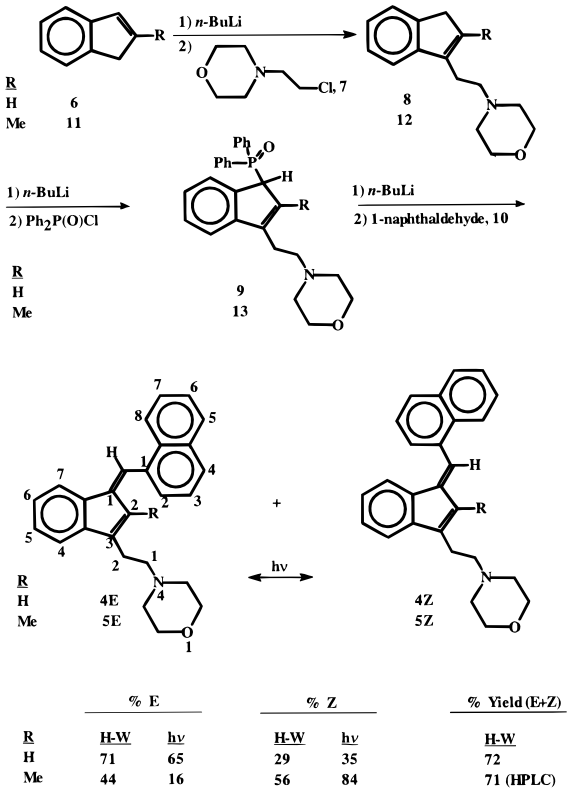
Because a wide range of tilt angles qualify as aromatic−aromatic "tilted T" stacking interactions commonly found in proteins,[8] and because the receptor itself may respond to a slightly altered tilt in an indene geometric isomer by adjusting the χ_2 of the aromatic residues with which the ligand interacts, it may not be important that the tilts nor the overlay of the naphthyl moieties of **1** and the indenes match exactly. Nevertheless, the energy expense for each indene to match the tilt of naphthyl rings to that of **1** was found here to be modest. On the basis of the hypothesis that aromatic stacking is the predominant interaction of AAIs such as **1** at the CB receptors and on the demonstration that the indene isomers (**4*E*/4*Z*** and **5*E*/5*Z***) can mimic the positions of the aromatic systems in the s-trans and s-cis conformers of **1**, the modeling results support the previously established use of the indenes as rigid analogues of the AAIs.

Chemistry. The syntheses of the (*E*)- and (*Z*)-naphthylidene substituted C-2 H and C-2 Me (morpholinoethyl)indenes (**4** and **5**, respectively) as *E*/*Z* mixtures were reported without resolution of the individual isomers.[5] In those syntheses, (morpholinoethyl)indene **8** (R=H) was treated with 1-naphthaldehyde (**10**) in the presence of methanolic NaOMe to give a mixture of geometrical isomers with the cis isomer (**4*E***) predominating (>95%). Similarly, **12** (R = Me) afforded an 80:20 mixture of **5*E*** and **5*Z***. Our repeat of these syntheses gave only **4*E*** with no **4*Z*** and a 31:69 mixture of **5*E*** and **5*Z***. To obtain testable quantities of **4*Z***, alternative chemistry was required to control the olefin geometry for which we employed Horner−Wittig chemistry in the olefination step.[10] For the target indenes, the fact that the precursor phosphinoyl component (**9** or **13**) is both α-disubstituted and benzylic contributes to diminished stereoselectivity of the reaction, and a significant portion of the **4*Z*** isomer would be expected.

Commercially available indene (**6**) was readily metalated with *n*-BuLi and alkylated[11] with 4-(2-chloroethyl)-morpholine (**7**) by the standard order of addition of **7** to the indenyllithium (Scheme 1). The intermediate 1-alkylated 2-indanene isomerizes under these conditions to the more substituted 3-alkylated olefin **8**. This process precluded the need for a separate isomerization reported earlier.[5] The one-proton vinyl and two-proton benzylic resonances clearly establish the 3-yl substitution.

Preparation of the Horner−Wittig reagent, indenyldiphenylphosphinoyl derivative **9**, reversed the conventional approach of displacement on an alkyl halide by triphenylphosphine followed by alkaline hydrolysis. Instead, **8** was metalated with *n*-BuLi and phosphinoylated with diphenylphosphinic chloride to give **9** directly. The identification of the phosphinoylated product as **9,** as opposed to a rearranged isomer with the double bond conjugated to the phosphinoyl group or, alternatively, with the phosphinoyl and the morpholinoethyl groups on the same carbon arising from phosphinoylation at the opposite end of the allylic anion, was established by ^{1}H NMR. Thus, the observation of only a single vinyl proton established a 1,3-substitution pattern on the indene ring, while a one-proton resonance as a 25-Hz doublet indicative of an α-phosphorus placed the double bond in the 2,3-position as shown. This proton readily exchanged in MeOD consistent with its anticipated acidity.

Scheme 1



	% E		% Z		% Yield (E+Z)
R	H-W	hν	H-W	hν	H-W
H	71	65	29	35	72
Me	44	16	56	84	71 (HPLC)

The phosphinoyl compound **9** was subjected to Horner−Wittig olefination with 1-naphthaldehyde employing bases with variations in the cation seeking the highest percentage of the *Z* isomer. Thus, NaOMe in MeOH afforded the *Z* isomer with 22% selectivity, while NaH in THF gave 23%, KO *t*-Bu in THF gave 17%, and *n*-BuLi in THF gave the best selectivity at 32%. The latter procedure was applied on large scale affording resolvable (crystallization and chromatography) *E* and *Z* isomers in 72% purified total yield. The geometry of the *Z* olefin **4*Z*** was determined by NOE NMR. Specifically, the *Z* olefin has the naphthylidene vinyl proton on the same side of the double bond as the C-2 (vinyl) proton of the indene, and an NOE interaction is expected and was observed for these two hydrogens. Similarly, the *E* olefin **4*E*** was identified by NOE interactions between the indenyl C-2 proton and the naphthyl 8-H, as well as an interaction between the naphthylidene vinyl proton and the indenyl 7-H, both of which require the *E* geometry for the through-space interactions.

The corresponding C-2 Me analogues of the above C-2 H series were similarly prepared from 2-methylindene which was obtained from $LiAlH_4$ reduction of commercially available 2-methyl-1-indanone followed by *p*-toluenesulfonic acid dehydration.[5] Thus, alkylation of lithiated **11** with 4-(2-chloroethyl)morpholine (**7**) gave a mixture of 1-yl and 3-yl (**12**) isomers with the former predominating. Isomerization to the desired **12** is effected with excess *n*-BuLi in THF and is essentially complete in 15 min. The process can be monitored by GC or TLC of a worked-up aliquot. Treating the lithiated isomerization reaction mixture with excess diphenylphosphinic chloride gives **13** in 72% chromatographed yield. Horner−Wittig condensation of lithiated **13** with 1-naphthaldehyde provided a 44:56 mixture of the *E:Z* isomers **5*E*** and **5*Z*** in 71% yield (HPLC). Distinguishing the *E* from *Z* olefins was accomplished by NOE NMR spectroscopy. Thus, one isomer exhibits an NOE interaction between the C-2 methyl and a multiplet in the aromatic region which is only possible for the *E* isomer. The other isomer exhibits an NOE between the C-2 methyl and the vinyl H. Thus, in the syntheses of both the C-2 H and the C-2 Me analogues, the Horner−Wittig reaction enhanced the formation of the minor isomer.

Additionally, a photochemical interconversion of the isomers was examined as a potential route of obtaining the **4*Z*** olefin. Irradiation of **4*E*** with a Pyrex-filtered UV source generated a 67:33 mixture of **4*E***:**4*Z*** with no other products being observed (HPLC). Similarly, a 12:88 mixture of **4*E***:**4*Z*** evolved to essentially the same ratio as the equilibrium value. Thus, unlike (*E*/*Z*)-indenyl analogues of indomethacin which photolyze to the same 4:96 mixture[12] as is obtained from the condensation of the indene with an aldehyde,[13] **4*Z*** can be photochemically generated as one-third of an equilibrium mixture from the sole product, **4*E***, of the indene/aldehyde condensation. This approach provides a second method to obtain the disfavored **4*Z*** isomer. Examining the photochemical isomerization on the C-2 methyl series, the *Z* isomer **5*Z*** irradiated at 365 nm in MeOD for 8 h afforded a 16:84 mixture of the *E:Z* isomers. Thus, either the *E* or *Z* isomer of the subject indenes can be photochemically converted to the other as part of an equilibrium mixture.

CB1 Receptor Assay. 1. Competition Studies. The K_i values determined in competition assays of the (*E*)- and (*Z*)-naphthylidene indenes versus [^{3}H]CP-55,940 and [^{3}H]SR141716A are summarized in Table 1. For both the C-2 H and C-2 Me congeners versus the CB1/CB2 agonist [^{3}H]CP-55,940, the *E* isomer was found to possess a greater receptor affinity. In assays of the compounds versus the CB1 antagonist [^{3}H]-SR141716A, a two-binding site model was indicated by the data for the *E* isomers, while a single-binding site model was indicated for the *Z* isomers. The K_i's at both binding sites of each *E* isomer were lower than the K_i at the single binding site indicated for each *Z* isomer. Recently, Houston and Howlett have reported that two discrete affinity states (30% high affinity) are revealed by WIN-55,212-2 competition for [^{3}H]SR141716A binding to rat brain CB1 receptors.[14] The results obtained here in the [^{3}H]SR141716A competition assay for **4*E*** and **5*E*** are consistent with this finding.

CB2 Receptor Assay. 1. Competition Studies. The K_i values for the (*E*)- and (*Z*)-naphthylidene indenes versus [^{3}H]CP-55,940 in the CB-CHO cells are summarized in Table 1. In both pairs (C-2 H and C-2 Me) of congeners, the *E* geometric isomer was found to possess the higher CB2 affinity.

2. Relative CB1/CB2 Affinities. As is evident in Table 1, WIN 55,212-2 (**1**) has higher affinity for the CB2 over the CB1 receptor (8.9-fold selectivity). This selectivity has been reported to be higher when both cloned CB1 and CB2 receptors are used in the binding assays.[15] The indenes, on the other hand, show either equal affinity for CB1 and CB2 (**4*E***) or a slight (less than 3-fold) selectivity for CB2 (**4*Z***, 1.12; **5*E***, 1.41; **5*Z***, 2.96). It is possible that because the naphthyl ring in

Table 1. Naphthylidene Indene CB1 and CB2 Binding and in Vitro Pharmacological Results

	$K_i \pm$ SE (nM)			
compound	CB1 (vs [^{3}H]CP-55,940)	CB1 (vs [^{3}H]SR141716A)	CB2 (vs [^{3}H]CP-55,940)	EC_{50}[a] (nM) (95% confidence limit)
4E	2.72 ± 0.22	10.8 ± 10.1[b] 611 ± 401[b]	2.72 ± 0.32	6.57 (4.65−9.30)
4Z	148 ± 29	928 ± 145	132.0 ± 45.6	283.13 (67.05−1195.52)
5E	2.89 ± 0.41	9.44 ± 5.53[b] 602 ± 141[b]	2.05 ± 0.22	52.86 (31.38−89.03)
5Z	1945 ± 94	2178 ± 919	658 ± 206	> 3162[c]
WIN-55,212-2 (**1**)	2.48 ± 0.29		0.28 ± 0.16[d]	4.92[e] (3.43−7.05)

[a] EC_{50} inhibition of electrically evoked contractions of the myenteric plexus-longitudinal muscle of the guinea pig small intestine. [b] Analysis of data yielded a two-site fit. [c] Highest concentration used was 3162 nM. [d] CB2 binding data from Showalter et al. (ref 2). [e] $E_{max} = 61.93 \pm 1.65$ (mean % ± SE).

the indene analogues cannot sweep as great an expanse of space as can the naphthyl ring of **1**, CB2 selectivity is lost in the indene series.

In Vitro Pharmacology. 1. Guinea Pig Ileum Assay. Cannabinoid receptor agonists, including WIN-55,212-2 (**1**), have been shown to inhibit electrically evoked contractions of the myenteric plexus-longitudinal muscle preparation of guinea pig small intestine.[16,17] Submicromolar concentrations of ***4E***, ***5E***, and ***4Z*** each produced a dose-related inhibition of electrically evoked contractions of the myenteric plexus preparation with log concentration−response curves that are sigmoid in shape ($r^2 > 0.95$). The most potent of these compounds, ***4E***, was equipotent with **1**, whereas the ***4Z*** and ***5E*** indenes were both less potent than **1** (Table 1). One other indene investigated, ***5Z***, was inactive at concentrations of up to 3.16 μM. This compound also had the lowest affinity for CB1 binding sites, as measured by displacement of [^{3}H]CP-55940 or [^{3}H]SR141716A (see Table 1). Extrapolation of the sigmoid log concentration−response curves constructed from our data using InPlot indicates that ***4E***, ***5E***, and ***4Z*** are all capable of producing the same maximal degree of inhibition of evoked contractions of the myenteric plexus preparation as WIN-55,212-2 (**1**).

The trends in the EC_{50}'s parallel the CB1 K_i's reported here. In the ***4E***/***4Z*** pair, ***4E*** possesses the higher CB1 affinity and is more potent in the guinea pig ileum assay. The ***5E***/***5Z*** pair exhibited the same trend. A comparison of ***4E*** and ***5E*** reveals that, while their K_i's at CB1 are quite similar, the C-2 H analogue (***4E***) is more potent in the GPI assay. This same trend of similar CB1 affinities of C-2 H and C-2 Me AAI analogues, but higher activity of the C-2 H analogue, is consistent with results for another pair of AAI analogues described by D'Ambra et al.[1] In this pair of analogues, a *p*-methoxyphenyl ring replaces the 1-naphthyl ring of WIN-55,212. The C-2 H analogue has nearly two-thirds the CB1 affinity of the C-2 Me analogue (IC_{50} vs [^{3}H]WIN-55,212-2 of 249 ± 17 nM, C-2 H; $IC_{50} = 152 \pm 17$ nM, C-2 Me). However, the C-2 H analogue has 3-fold higher activity in the mouse vas deferens (MVD) assay ($IC_{50} = 44.5 \pm 9.8$ nM, C-2 H; $IC_{50} = 123 \pm 13$ nM, C-2 Me).[1]

Conclusions

The results reported here show that the *E* geometric isomers of the naphthylidene-substituted aminoalkylindenes **4** and **5** have higher affinity and higher mean pharmacological potency values than their corresponding *Z* geometric isomers at the CB1 receptor. The addition of the C-2 methyl group in ***5E*** does not appear to produce any specific receptor interactions, as ***4E*** and ***5E*** have essentially the same CB1 affinities. Similarly, the results show that the *E* geometric isomers also have higher affinity at the CB2 receptor than do their corresponding *Z* isomers. As the *E* geometric isomers ***4E***/***5E*** are rigid analogues of the AAI s-trans conformation, the binding and pharmacological activity results lead to the tentative conclusion that the s-trans conformation of AAIs such as WIN-55,212-2 (**1**) may be the preferred conformation at both the CB1 and CB2 receptors. Finally, results reported here lend support to our working hypothesis that aromatic stacking may be an important interaction for AAIs at the CB1 and CB2 receptors. Studies of the possible interactions of **1** and the indenes in computer models of the transmembrane helix bundles of the CB1 and CB2 receptors are currently in progress.

Experimental Section

Chemistry. ^{1}H NMR spectra were recorded on a Bruker AM-250 MHz or AMX-500 MHz (as noted) spectrometer. The chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane (TMS) in $CDCl_3$. High-resolution mass spectra (HRMS) were obtained on a VG ZAB-E mass spectrometer via direct probe in the electron impact mode with a 70 eV ionization voltage. Compounds were shown to be homogeneous by HPLC on a Waters dual pump chromatograph with a model 484 tunable absorbance detector (Waters reversed-phase C18 RCM 8-mm × 10-cm column; UV detection at 254 nm; 60% CH_3CN−40% (H_2O, HOAc, Et_3N, 850:25:19, pH 4), 2.0 mL/min) and by TLC on Baker Si254F silica gel plates employing UV and phosphomolybdic acid−ceric sulfate spray or iodine detection. The four target isomers were all homogeneous by TLC in 75% EtOAc−hexane and by HPLC.

4-[2-(1*H*-Inden-3-yl)ethyl]morpholine (8). In a septum-sealed flask with a magnetic stir bar, indene (2.33 mL, 20.0 mmol) and a few crystals of 1,10-phenanthroline indicator were dissolved in dry THF (30 mL, filtered through basic Al_2O_3) under nitrogen and cooled in an acetonitrile−dry ice bath while protected from light with a foil covering. *n*-BuLi in hexane was added with stirring until a persistent red color of the indicator was observed followed by an additional 20 mmol of *n*-BuLi (17.4 mL, 1.15 M). The cold bath was removed and the reaction stirred at ambient temperature for 20 min and then cooled again for 10 min followed by syringe addition of 4-(2-chloroethyl)morpholine free base (5.78 g, 38.6 mmol) and two THF rinses (2 × 5 mL). The bath was removed and the

reaction stirred at ambient temperature for 45 min when TLC (75% EtOAc−hexane) showed the reaction to be complete. Quenching with methanol and removal of the volatiles in vacuum afforded a residue that was partitioned between water and CH_2Cl_2. The aqueous layer was further extracted with CH_2Cl_2 (4×), and the combined organic phases were dried (Na_2SO_4) and evaporated in vacuo yielding an amber oil (6.94 g) which was chromatographed on silica gel (174 g), eluting with 75% EtOAc−hexane. The oil that was obtained contained residual 4-(2-chloroethyl)morpholine which was removed by bulb-to-bulb distillation at 50 °C under high vacuum. The residual portion remaining in the distillation flask was the title compound (2.49 g, 54%): ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.47 (d, 1H, J = 7.4 Hz), 7.38 (d, 1H, J = 7.4 Hz), 7.31 (t, 1H, J = 6.8 Hz), 7.21 (t, 1H, J = 7.2 Hz), 6.26 (s, 1H), 3.77 (t, 4H, J = 4.7 Hz), 3.33 (s, 2H), 2.88−2.70 (m, 4H), 2.56 (t, 4H).

4-[2-[1-(Diphenylphosphinoyl)-1*H*-inden-3-yl]ethyl]morpholine (9). Under anhydrous conditions in a septum-sealed flask, a solution of 4-[2-(1*H*-inden-3-yl)ethyl]morpholine (1.68 g, 7.34 mmol) and a few crystals of 1,10-phenanthroline indicator dissolved in 59 mL of dry THF (filtered through basic Al_2O_3) was cooled in an acetonitrile−dry ice bath (−42 °C) under dry nitrogen with magnetic stirring. Sufficient *n*-BuLi (3.0 mL, 1.08 M in hexanes) was added dropwise by syringe to obtain a brown end point followed by an additional 2 equiv of *n*-BuLi (13.6 mL, 14.6 mmol). The cooling bath was removed to allow the reaction to reach room temperature (20 min). The reaction was then returned to the bath as an amber solution. Diphenylphosphinic chloride (4.2 mL, 22.0 mmol) was rapidly added causing a transition to a red and eventually a cloudy orange-red color. At 30 min, the reaction was quenched with MeOH after TLC (3% MeOH/$CHCl_3$) of a worked-up aliquot showed the consumption of starting material. Evaporation of volatiles afforded a residue that was partitioned between aqueous Na_2CO_3/NaCl and CH_2Cl_2 (3×). The combined organic phases were dried (Na_2SO_4) and evaporated in vacuo to a syrup (7.2 g) which was chromatographed on silica gel (350 g), eluting with 3% MeOH/$CHCl_3$ to afford 2.46 g (75%) of the title compound: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78−7.32 (m, 10H, Ph_2PO), 7.30−7.09 (m, 4H, indene ArH_4), 6.28 (d, 1H, J = 1.1 Hz, C2−H), 4.71 (dd, 1H, J = 1.1, 25.3 Hz, C1−H), 3.70 (t, 4H, J = 4.6 Hz, 2′, 6′-CH_2), 2.61 (br, 2H, C3−CH_2), 2.43 (br, 4H, 3′,5′-CH_2), 2.40−2.25 (m, 2H, CH_2−N); HRMS calcd for $C_{27}H_{28}NO_2P$ 429.1858, measured 429.1855. CHNP ($C_{27}H_{28}NO_2P\cdot H_2O$) (P + 0.50).

4-[2-[1-(1-Naphthalenylmethylene)-1*H*-inden-3-yl]ethyl]morpholine, 4*E* and 4*Z* Isomers. Under anhydrous conditions in a septum-sealed flask, a solution of 4-[2-[1-(diphenylphosphinoyl)-1*H*-inden-3-yl]ethyl]morpholine (1.89 g, 4.40 mmol) and a few crystals of 1,10-phenanthroline indicator dissolved in 100 mL of dry THF (filtered through basic Al_2O_3) was cooled in an ice water bath under dry nitrogen with magnetic stirring. Sufficient *n*-BuLi (0.1 mL, 1.4 M in hexanes) was added dropwise by syringe to obtain a brown-yellow end point followed by an additional 1 equivalent of *n*-BuLi (3.30 mL, 4.65 mmol). The cooling bath was removed to allow the reaction to reach room temperature (20 min), and 1-naphthaldehyde (0.60 mL, 4.42 mmol) was added dropwise. Naphthalene (463 mg) was added as an HPLC internal standard. The reaction was stirred at ambient temperature for 21 h, 4 h at 50 °C, and overnight at ambient temperature. Aliquots were removed at 15 min, 17 h (room temperature), 1 h (50 °C), and overnight (room temperature), by which time the starting phosphinoyl compound was consumed (HPLC). The reaction was quenched with 10 mL of ethanol, the volatiles were removed in vacuo, and the residue was partitioned between H_2O and CH_2Cl_2. The aqueous phase was treated with $NaHCO_3$ to pH 12 and extracted twice more with CH_2Cl_2. Drying the combined organic phases with Na_2SO_4 and removal of solvent afforded 2.03 g of crude product containing a 71:29 area ratio of *E:Z* olefin isomers (HPLC). Crystallization from methanol provided a 420-mg first crop and a 139-mg second crop of 100% and 94% pure *E* isomer crystals, respectively. Further recrystallization of the mother liquor residue seeding with the *Z* isomer provided only trace crystallization of the *E* isomer. The mother liquor residue (1.35 g) was chromatographed on silica gel (10 g) to remove naphthaldehyde from the *E*/*Z* isomer mixture. The latter was chromatographed on a Merck size B reversed-phase C-18 column, eluting with 55% CH_3CN−45% (H_2O, HOAc, Et_3N, 850:25:19, pH 4) mobile phase to give 195 mg of *Z* isomer, 282 mg of 1:1 *E:Z* mixture, and 125 mg of *E* isomer for an overall yield of 72% of both olefins. The *Z* isomer was crystallized from MeOH.

***E* Isomer**: mp 105.7−106.4 °C [Note: This melting point is for the free base, while that reported in the literature[5] and patent (US 5,292,736) is for the HCI salt]; HRMS calcd for $C_{26}H_{25}NO$ 367.1936, found 367.1938; CHN for $C_{26}H_{25}NO\cdot 0.15H_2O$; ^{1}H NMR (500 MHz, MeOD-d_4) δ 8.15 (d, 1H, J = 7.8 Hz, 2′−H), 8.09 (s, 1H, naphthylidene vinyl-H), 7.93−7.89 (m, 2H, 4′, 6′−H), 7.87[a] (d, 1H, J = 5.4 Hz, 7-H), 7.62[b] (d, 1H, J = 7.0 Hz, 8′−H), 7.57−7.51 (m, 3H, 3′, 5′, 7′−H), 7.35 (d, 1H, J = 6.9 Hz, 4-H), 7.32−7.25 (m, 2H, 5,6-H), 6.61 (s, 1H, 2-H), 3.70 (m, 4H, O−$(CH_2)_2$), 2.86−2.82[c] (m, 2H, ethyl CH_2 allylic), 2.71−2.68 (m, 2H, ethyl CH_2′), 2.55 (br s, 4H, N−$(CH_2)_2$) (a, NOE with naphthylidene vinyl at δ 8.09; b, NOE with 2-H at δ 6.61; c, COSY with 2-H at δ 6.61).

***Z* Isomer**: mp 106.4−108.4 °C; HRMS calcd for $C_{26}H_{25}NO$ 367.1936, found 367.1938; CHN for $C_{26}H_{25}NO\cdot 0.15\ H_2O$; ^{1}H NMR (500 MHz, MeOD-d_4) δ 7.98[a] (dm, 1H, J = 8.6 Hz, 2′- or 8′−H), 7.95−7.93 (m, 2H, Naph-H_2), 7.63 (dm, 1H, J = 7.0 Hz, 8′- or 2′−H), 7.59 (s, 1H, naphthylidene vinyl H), 7.54−7.50 (m, 2H, Naph-$H^1{}_2$), 7.47 (tm, 1H, J = 7.6 Hz, Naph-H), 7.28[b] (dm, 1H, J = 7.5 Hz, 4-H), 7.15 (td, 1H, J = 7.5, 1.1 Hz, 5-H), 6.86[c] (dd, 1H, J = 7.7, 0.8 Hz, 7-H), 6.77[d] (td, 1H, J = 7.5, 1.0 Hz, 6-H), 6.57[e] (s, 1H, 2-H), 3.77 (m, 4H, O−$(CH_2)_2$), 2.91−2.81 (m, 4H, ethyl), 2.67 (br s, 4H, N−$(CH_2)_2$) (a, NOE with naphthylidene vinyl H at δ 7.59; b, COSY with 5-H at δ 7.15; c, NOE with naphthyl (Naph) 2′,8′ H's at δ 7.98 and 7.63; d, COSY with 7-H at δ 6.86; e, NOE with naphthylidene vinyl at δ 7.59).

Photochemical Isomerization of *E/Z* Isomers. The *E* isomer of 4-[2-[1-(naphthalenylmethylene)-1H-inden-3-yl]ethyl]morpholine (39.5 mg) dissolved in degassed absolute ethanol (40 mL) in a 100-mL Pyrex flask under nitrogen was subjected to photolysis with a 450-W medium pressure Hanovia mercury lamp through a water-cooled quartz coldfinger fitted with a Pyrex filter for 15 h. Solvent was added periodically to replace evaporative losses. Aliquots analyzed by HPLC (detecting at 254 nm at which wavelength both isomers have the same ϵ value) indicated a 67:33 equilibrium ratio of *E:Z* isomers after 4 h which was unchanged through 15 h with no other significant peaks appearing. Similar irradiation with Spectroline model ENF-260C 0.2-amp UV lamp (365 nm) of a 12:88 *E:Z* mixture in a UV−vis cell evolved to a 68:32 *E:Z* mixture within 7 h. Solvent removal and recrystallization from methanol afforded 10 mg of recovered *E* isomer. Chromatography of the mother liquor residue, as described in the chemical synthesis, afforded 8 mg of pure *Z* isomer and 18 mg of *E*/*Z* mixtures. The product was identified by NMR spectroscopy as described for the chemical synthesis.

4-[2-(2-Methyl-1*H*-inden-3-yl)ethyl]morpholine (12). The title compound was prepared from 2-methylindene following the procedure for preparing compound **8** but employing a 1.25-h reaction time. This afforded a 7:1 mixture of the 1-yl and title 3-yl olefins which were separated in a total yield of 75% by the chromatographic system employed in the purification of **8**. The 1-yl isomer is readily isomerized to **12** by treatment in THF with 3 equiv of *n*-BuLi in hexanes at ambient temperature for 15 min. Quenching with MeOH, evaporation of volatiles, and partitioning between water and CH_2Cl_2 afforded the title compound as a single component in 73% yield: TLC (SiO_2, 75% EtOAc−hexanes, UV) R_f 0.12 (1-yl), 0.18 (3-yl); GC (DB-17, 100 °C/5 min, 100−250 °C/10 min) t_R (min) 14.9 (1-yl), 15.7 (3-yl); 3-yl ^{1}H NMR (250 MHz, MeOD-d_4) δ 7.33 (d, 1H, J = 7.27 Hz, 4-H or 7-H), 7.21 ("q", 2H, J = 7.5 Hz, 7-H or 4-H & 5-H or 6-H), 7.06 (ddd, 1H, 6-H or 5-H), 3.76−3.72 (m, 4H, O−$(CH_2)_2$), 3.26 (s, 2H, 1-H_2), 2.76−2.72 (m, 2H, ethyl CH_2), 2.60−2.56 (m, 4H, N−$(CH_2)_2$), 2.53−2.46

(m, 2H, ethyl CH_2'), 2.09 (s, 3H, 2-CH_3); 1-yl ^{1}H NMR (250 MHz, MeOD-d_4) δ 7.36 (d, 1H, J = 7.10 Hz, 4-H or 7-H), 7.19−7.15 (m, 2H, 7-H or 4-H & 5-H or 6-H), 7.12−7.04 (m, 1H, 6-H or 5-H), 6.46 (s, 1H, 3-H), 3.66−3.62 (m, 4H, O−$(CH_2)_2$), 3.39 (br s, 1H, 1-H), 2.38−2.34 (m, 4H, N−$(CH_2)_2$), 2.28−2.21 (m, 1H, ethyl CH), 2.08 (br s, 3H, 2-CH_3), 2.05−1.97 (m, 2H, ethyl CH_2), 1.91−1.84 (m, 1H, ethyl CH′).

4-[2-[1-(Diphenylphosphinoyl)-2-methyl-1*H*-inden-3-yl)-ethyl]morpholine (13). A 79:21 ratio of the 1-yl:3-yl (**12**) mixture (195 mg) was treated with 2.35 equiv of *n*-BuLi above the red end point as described for isomerizing the 1-yl to the 3-yl isomer and then further processed as for the 2-H analogue **8** to afford 256 mg (72%) of **13** as a hygroscopic, white, crisp foam: ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.78−7.07 (m, 12H, Ph_2PO + 4-H, 5-H), 6.89 (td, 1H, J = 7.46, 1.17 Hz, 6-H), 6.46 (dd, 1H, J = 7.58, 0.78 Hz, 7-H), 4.54 (d, 1H, J = 25.5 Hz, 1-H), 3.76−3.68 (m, 4H, O−$(CH_2)_2$), 2.58−2.45 (m, 2H, ethyl CH_2 allylic), 2.43−2.37 (m, 4H, N−$(CH_2)_2$), 2.16 (d, 3H, J = 2.19 Hz, CH_3), 1.99−1.88 (m, 1H, ethyl CH), 1.82−1.70 (m, 1H, ethyl CH′); HPLC t_R 4.34 min at 2.0 mL/min; HRMS calcd for $C_{28}H_{30}NO_2P$ 443.2014, found 443.2010. CHNP ($C_{28}H_{30}NO_2P$·2·CH_3OH) (H − 1.1%).

4-[2-[2-Methyl-1-(1-naphthalenylmethylene)-1*H*-inden-3-yl]ethyl]morpholine, 5*E* and 5*Z* Isomers. As described above for the preparation of **4*E*** and **4*Z***, the phosphinoyl compound **13** (203 mg, 0.46 mmol) and 1,10-phenanthroline indicator in THF (10 mL) were treated with *n*-BuLi (1.07 equiv above a brown-yellow end point) and subsequently with 1-naphthaldehyde (0.46 mmol) and naphthalene (40.7 mg, 0.32 mmol) as an internal standard. The reaction was heated in a 65 ± 5 °C bath for 3.5 h under nitrogen during which time the product production leveled out relative to the naphthalene standard peak. ^{1}H NMR demonstrated the starting **13** to have been consumed. Workup by partitioning between CH_2Cl_2 and aqueous Na_2CO_3 afforded 180 mg of the naphthalene and olefin mixture with an *E:Z* ratio of 44:56 (by integration of the respective methyl groups). The products were identified by comparisons of the HPLC retention time and the ^{1}H NMR of the mixture versus authentic, purified material prepared by the NaOMe-mediated condensation of the indene **8** with 1-naphthaldehyde (MeOH, reflux 9.5 h) followed by chromatographic resolution of the resulting 31:69 *E:Z* mixture. The chromatography involved a cleanup column (10:1) on silica gel eluting with 75% EtOAc−hexanes and subsequent reversed-phase chromatography on a Merck C-18 size B column eluting with 60% CH_3CN−40% (H_2O, HOAc, Et_3N, 850:25:19, pH 4) followed by another silica gel column with 75% EtOAc−hexanes affording **5*E*** and **5*Z*** in 12% and 41% yields, respectively. Analytical samples were crystallized from MeOH.

***E* Isomer**: HPLC t_R 13.7 min; HRMS calcd for $C_{27}H_{27}NO$ 381.2093, found 381.2091; ^{1}H NMR (500 MHz, MeOD-d_4) δ 7.95 (s, 1H, vinyl-H), 7.94−7.84 (m, 3H, Naph ArH_3), 7.72 (d, 1H, J = 7.5 Hz, 7-H), 7.53−7.44 (m, 3H, Naph-H_3), 7.41[a] (d, 1H, J = 7.0 Hz, 8′−H), 7.27−7.20 (m, 2H, 4-H, 5-H), 7.17 (dt, 1H, J = 7.0, 1.8 Hz, 6-H), 3.69 (t, 4H, J = 5 Hz, O−$(CH_2)_2$), 2.74−2.70 (m, 2H, ethyl CH_2 allylic), 2.52 (br s, 4H, N−$(CH_2)_2$), 2.47−2.43 (m, 2H, ethyl CH_2'), 1.48 (s, 3H, C-2 CH_3) (a, NOE with C-2 methyl at δ 1.48).

***Z* Isomer**: mp 103.7−105.2 °C; HPLC t_R 13.0 min; HRMS calcd for $C_{27}H_{27}NO$ 381.2093, found 381.2091; CHN for $C_{27}H_{27}NO$; ^{1}H NMR (500 MHz, MeOD-d_4) δ 7.97−7.88 (m, 3H, Naph-H_3), 7.57 (d, 1H, J = 7.0 Hz, Naph-H), 7.53 (s, 1H, vinyl-H), 7.52−7.47 (m, 2H, Naph-H_2), 7.43 (t, 1H, J = 7.5 Hz, Naph-H′), 7.15[a] (d, 1H, J = 7.5 Hz, 4-H), 7.05[b] (t, 1H, J = 7.3 Hz, 5-H), 6.67[c] (d, 1H, J = 7.5 Hz, 7-H), 6.62[d] (t, 1H, J = 7.3 Hz, 6-H), 3.73 (br s, 4H, O−$(CH_2)_2$), 2.82−2.79 (m, 2H, ethyl CH_2), 2.55 (br s, 4H, N$(CH_2)_2$), 2.54−2.48 (m, 2H, ethyl CH_2'), 2.25 (s, 3H, C-2 CH_3) (a, NOE with 5-H at δ 7.05; b, NOE with 6-H at δ 6.62; c, NOE with Naph ArH at δ 7.57; d, NOE with 7-H at δ 6.67).

Receptor Binding Assays. 1. CB1 Assay. For the competition assays utilizing rat brain membrane preparations, male F344 rats (Charles River Laboratories, Raleigh, NC) weighing 200−225 g were sacrificed. The whole brains were quickly removed and placed into a 55-mL Potter-Elvehjem glass homogenizer tube maintained on ice. The tissue was subjected to a homogenization and centrifugation procedure described previously[18] to yield the final membrane preparation used in the binding assay. Total protein concentration of the resuspended membrane pellet was determined by a dye-binding assay commercially available from Biorad Laboratories (Hercules, CA). Aliquots of the membrane preparation were stored at −70 °C until use.

The C-2 H and C-2 Me compound series were evaluated for their ability to compete with the binding of [^{3}H]CP-55,940 or [^{3}H]SR141716A. Competing compounds were prepared in buffer consisting of 50 mM Tris-HCl, pH 7.4, 1 mM EDTA, 3 mM $MgCl_2$, and 0.5% (w/v) BSA (buffer A). Tritiated compounds were diluted in buffer A to yield concentrations of 7.2 nM for [^{3}H]CP-55,940 and 20 nM for [^{3}H]SR141716A, so that addition to the incubation mixture yielded a final concentration in the assay of 0.72 and 2.0 nM, respectively. Unlabeled drug for determination of nonspecific binding (unlabeled CP-55,940 in assays using [^{3}H]CP-55,940 and unlabeled SR141716A in assays using [^{3}H]SR141716A) was at a final concentration of 10 μM.

The competition assays were conducted in a total volume of 1 mL in silated glass test tubes. The reaction mixtures (in duplicate) consisted of 100 μL of tritiated drug, 100 μL of unlabeled drug dilution, and sufficient buffer A such that a total volume of 1 mL was achieved with the addition of brain membrane extract. Duplicate tubes for nonspecific binding and total binding were prepared by adding 100-μL aliquots of the unlabeled compound to be displaced and of buffer A, respectively. An aliquot of brain membrane extract equivalent to 45 μg of protein was added to each tube. The final volume of the reaction mixture was brought to a total of 1 mL by the addition of buffer A. After mixing by vortex, the reaction tubes were incubated at 30 °C for 1 h.

A 24-manifold Brandel cell harvester was prepared by priming approximately 1 L of cold 50 mM Tris-HCI, pH 7.4, containing 0.1% (w/v) BSA (buffer B) through the harvester. Filter paper (Whatman GF/C) pretreated for 1 h in 0.1% poly-(ethylenimine) was placed into the cell harvester. After the incubation period was complete, the reaction was terminated by vacuum filtration of the reaction mixture. The reaction tubes were then rinsed twice with approximately 4 mL of buffer B. After the tubes were rinsed, the filter paper was removed and placed into liquid scintillation vials. To each vial was added 1 mL of H_2O and 10 mL of scintillation cocktail. The samples were placed on a shaker for at least 2 h and then counted in a liquid scintillation counter for a statistically appropriate amount of time.

The amount (nM) of radiolabel specifically bound in the absence of competing compounds was calculated by subtracting nonspecific binding from total binding. The percentage of this specific binding was then calculated for the amount of radiolabel bound in the presence of various concentrations of each competing compound.

The data were then analyzed using GraphPad Prism (GraphPad Software, Inc., San Diego, CA), which fit the displacement data to a one- or two-binding site model using a goodness-of-fit quantification based on sum-of-squares and calculated the K_i for the competing compound. The K_i values are presented as means ± SEM (n = 3) in Table 1.

2. CB2 Assay. The binding characteristics of the CB2 cell line used in these experiments have previously been described.[2] Computer analysis of saturation data for the CB2 cell line using [^{3}H]CP-55,940 as a radioligand (mean ± SE, n = 4) indicated a K_d of 608 ± 14 pM, a B_{max} of 3.63 ± 0.52 pmol/mg of protein, and a Hill coefficient of 0.93 ± 0.01. The methods for membrane preparation and radioligand binding were as described in Showalter et al.[2]

The human CB2 cDNA clone was generously provided by Dr. Sean Munro (MRC Laboratories, Cambridge, England). The CB2 cDNA was subcloned into pcDNA3, and stably transfected cell lines were created in CHO cells.[2] Briefly, cells were harvested in phosphate-buffered saline containing 1 mM

EDTA and centrifuged at 500*g*. The cell pellet was homogenized in 10 mL of solution A (50 mM Tris-HCl, 320 mM sucrose, 2 mM EDTA, 5 mM $MgCl_2$, pH 7.4). The homogenate was centrifuged at 1600*g* (10 min), the supernatant saved, and the pellet washed twice in solution A with subsequent centrifugation. The combined supernatants were centrifuged at 100000*g* (60 min). The (P2 membrane) pellet was resuspended in 3 mL of buffer B (50 mM Tris-HCl, 1 mM EDTA, 3 mM $MgCl_2$, pH 7.4) to yield a protein concentration of approximately 1 mg/mL, divided into equal aliquots, frozen on dry ice, and stored at −70 °C. Binding was initiated by the addition of 25−75 μg of membrane protein to silanized tubes containing [^{3}H]CP-55,940 (102.9 Ci/mmol) and a sufficient volume of buffer C (50 mM Tris-HCl, 1 mM EDTA, 3 mM $MgCl_2$, 5 mg/mL fatty acid-free BSA, pH 7.4) to bring the total volume to 0.5 mL. The addition of 1 μM unlabeled CP-55,940 was used to assess nonspecific binding. Specific binding averaged >80% of total binding at 0.5 nM [^{3}H]CP-55,940. Following incubation (30 °C for 1 h), binding was terminated by the addition of 2 mL of ice-cold buffer D (50 mM Tris-HCl, pH 7.4, plus 1 mg/mL BSA) and rapid vacuum filtration through Whatman GF/C filters (pretreated with poly(ethylenimine) (0.1%) for at least 2 h). Radioactivity was quantitated by liquid scintillation spectrometry. 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%). Competition assays were conducted with 0.5 nM [^{3}H]CP-55,940 and six concentrations (0.1 nM to 10 μM displacing ligands).

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[19] with the KELL package of binding analysis programs for the Macintosh computer (Biosoft, Milltown, NJ).

In Vitro Pharmacology. Guinea Pig Ileum Assay. The in vitro pharmacology of **4*E*/4*Z*, 5*E*/5*Z*,** and WIN-55,212-2 (**1**) was investigated using the myenteric plexus-longitudinal muscle preparation of guinea pig small intestine assay, the measured response being inhibition of electrically evoked contractions.[17] Cumulative dose−response curves were constructed using a dose cycle of 20 min. All drugs were mixed with two parts of Tween 80 by weight and dispersed in a 0.9% aqueous solution of NaCl (saline).[20] Mean agonist concentrations producing 50% of their maximum inhibitory effect on the twitch response (EC_{50} values), the mean sizes of maximal responses (E_{max} values), and the 95% confidence limits or standard errors of these values were calculated by nonlinear regression analysis using GraphPad InPlot (GraphPad Software, San Diego, CA).

Molecular Modeling. 1. Conformational Analysis. The structures of WIN-55,212-2 (**1**), pravadoline (**2**), and indenes **4*E*/4*Z*** and **5*E*/5*Z*** were built using the 3D builder in the Chem-X molecular modeling program (Chemical Design Ltd., Chipping Norton, U.K.). Complete conformational analyses of **1**, **2**, **4*E*/4*Z*,** and **5*E*/5*Z*** were performed using the semiempirical method AM1 within the Spartan molecular modeling program (Wavefunction, Inc., Irvine, CA). After optimization of each structure, conformational searches were performed (using 3−6-fold rotations) for each rotatable bond in **1**, **2**, **4*E*/4*Z*,** and **5*E*/5*Z*** to generate all minimum energy conformers of each molecule. An ab initio study (STO-3G basis set) of **1** was also conducted using Spartan in order to locate the fourth s-cis conformer of **1**. An AM1 single-point energy calculation was then performed in order to obtain an energy with which other energies could be compared.

2. Structure Superposition. The lowest-minimum energy s-trans and s-cis conformers of **1** were used as templates to which the global minimum energy conformers of **4*E*/5*E*** and **4*Z*/5*Z***, respectively, were fit. The indole and indene rings of the conformers to be compared were superimposed at C-1, C-2, C-3, C-4, C-5, C-6, and C-7 (indene numbering) of the corresponding ring systems using a rigid fit in the Compute Fit facility in Chem-X. AM1 single-point energy calculations were performed using Spartan (Wavefunction, Inc., Irvine, CA) to assess the energy expense for each indene to match the tilt of the naphthyl ring in **1**.

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