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Articles

Derivation of a Pharmacophore Model for Anandamide Using Constrained Conformational Searching and Comparative Molecular Field Analysis

Weida Tong,[§] Elizabeth R. Collantes,[†] and William J. Welsh*

Department of Chemistry and Center for Molecular Electronics, University of Missouri−St. Louis, St. Louis, Missouri 63121

Barbara A. Berglund and Allyn C. Howlett

Department of Pharmacological and Physiological Science, Saint Louis University School of Medicine,
St. Louis, Missouri 63104

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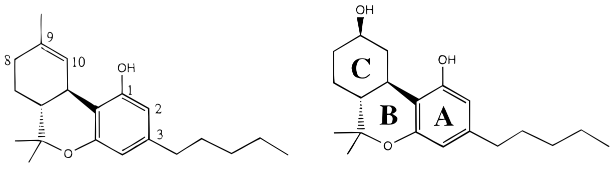
Constrained molecular dynamics simulations on anandamide, together with a systematic distance comparison search, have revealed a specific low-energy conformer whose spatial disposition of the pharmacophoric elements closely matches that of HHC. This conformer enables near superposition of the following: (1) the oxygen of the carboxyamide and the phenolic hydroxyl group of HHC, (2) the hydroxyl group of the ethanol and the cyclohexyl hydroxyl group of HHC, (3) the alkyl tail and the lipophilic side chain of HHC, and (4) the polyolefin loop and the tricyclic ring structure of HHC. The close matching of common pharmacophoric elements of anandamide with HHC offers persuasive evidence of the biological relevance of this conformer. The proposed pharmacophore model was capable of discriminating between structurally related compounds exhibiting different pharmacological potency for the CB_1 cannabinoid receptor, i.e., anandamide and *N*-(2-hydroxyethyl)prostaglandinamide. Furthermore, a 3D-QSAR model was derived using CoMFA for a training set of 29 classical and nonclassical analogues which rationalized the binding affinity in terms of steric and electrostatic properties and, more importantly, which predicted the potency of anandamide in excellent agreement with experimental data. The ABC tricyclic HU-210/HU-211 and ACD tricyclic CP55,243/CP55,244 enantiomeric pairs were employed as test compounds to validate the present CoMFA model. For each enantiomeric pair, the CoMFA-predicted log K_i values correctly identified that enantiomer exhibiting the higher affinity for the receptor.

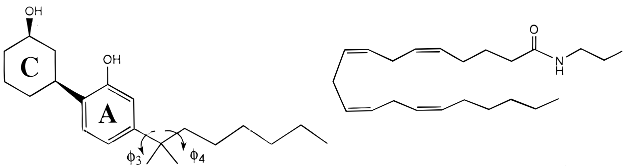
Introduction

Marihuana plants (*Cannabis sativa*) contain a variety of cannabinoid structures including the primary central nervous system (CNS)-active compound Δ^9-tetrahydrocannabinol (Δ^9-THC; Figure 1a), which is readily oxidized in vivo to the more potent compound 11-OH-Δ^9-THC.[1] Chemical modifications of this structure yielded 9-nor-9β-OH-hexahydrocannabinol (HHC; Figure 1b), a compound exhibiting enhanced analgesic activity.[2] The synthesis of HHC stimulated extensive structure−activity relationship (SAR) studies at the Pfizer Central Research Laboratories and led to the development of a three-point association model between the agonist and the CB_1 cannabinoid receptor.[3] According to this model, the pharmacophores for agonist−receptor interaction consist of the following: (1) the C3 hydrophobic alkyl side chain on the phenolic A ring, (2) the phenolic hydroxyl, and (3) the C9 equatorial hydroxyl on the C ring. The nonclassical cannabinoids upon which this model is based are compounds that lack the ABC tricyclic benzopyran nucleus of the classical cannabinoids, yet still contain those structural features required to confer a cannabimimetic response.[4] A key example is CP-47,497 (Figure 1c), a bicyclic structure that contains the minimal structural features embed-



(1a) Δ^9-Tetrahydrocannabinol (Δ^9-THC)

(1b) 9-nor-9β-OH-hexahydrocannabinol (HHC)



(1c) CP-47,497

(1d) Arachidonylethanolamide (Anandamide)

Figure 1. Molecular structure of (a) Δ^9-tetrahydrocannabinol (Δ^9-THC), (b) 9-nor-9β-OH-hexahydrocannabinol (HHC), (c) CP-47,497, and (d) arachidonyl ethanolamide (anandamide).

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* Corresponding author.

[§] Current address: National Center for Toxicological Research, Jefferson City, AK 72079.

[†] Current address: Monsanto, St. Louis, MO 63167.

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ding the required pharmacophoric components and yet exhibits antinociceptive potency equal to or greater than that of HHC.[5] Using a combination of 2D nuclear magnetic resonance (2D-NMR) measurements and computer-aided molecular modeling, Xie et al.[6] deduced the energetically preferred conformation of CP-47,497 as having (i) the cyclohexyl ring in its chair form, (ii) thephenolic hydroxyl coplanar with the aromatic ring and pointed away from the cyclohexyl ring, and (iii) the C3 alkyl side chain perpendicular to the plane of the phenolic ring. This structure was consistent with earlier predictions of a tricyclic classical cannabinoid structure.[7]

Prior to the development of the cannabinoid receptor pharmacophore model, Milne and Johnson[8] noted that a shape similarity and heteroatom overlap existed between the THC analogue 9β-hydroxy-11-nor-Δ^9-HHC and the endogenous prostaglandins, which are compounds totally devoid of the tricyclic or bicyclic structures of the prototypical cannabinoids. This notion was reexamined due to the recent identification of the ethanolamine amide of arachidonic acid (anandamide; Figure 1d)[9] and other highly unsaturated fatty acid derivatives[10] as agonists at the CB_1 cannabinoid receptor. Originally isolated from porcine brain, anandamide is an eicosanoid derivative that was found to exhibit an affinity for the CB_1 cannabinoid receptor, attenuate cyclic AMP production, and exhibit other cannabinoid biological activities.[9] This evidence strongly suggests that anandamide, while belonging to a class of compounds other than the bicyclic or tricyclic cannabinoids, contains the specific structural requirements necessary for binding to the CB_1 cannabinoid receptor and eliciting a response. It was speculated that a more constrained structure might accommodate the receptor binding pocket more readily.[11] To test this, ethanolamine amides of various prostaglandins were tested for their ability to compete with [^{3}H]CP-55,940 binding to cannabinoid receptors in rat brain membranes.[11] However, the failure of the ethanolamine amides of PGE_2, PGA_2, PGB_1, and PGB_2 to bind to CB_1 receptors suggested either that the "hairpin" structure typical of prostaglandins is not well accommodated within the receptor's binding site or that the functionalities on the cyclopentyl ring or the hydroxyl along the alkyl side chain moiety are not conducive to tight binding.

We now postulate that anandamide interacts with the receptor by a three-point association model similar to that of the bicyclic and tricyclic cannabinoid structures, composed of the following contacts with the receptor: (1) the carbonyl oxygen, (2) the hydroxyalkyl group, and (3) the five-carbon tail. In this study, we have compared conformers of anandamide with the tricyclic cannabinoid HHC. Our goal was to develop a better understanding of the structural and conformational requirements of anandamide for binding to the CB_1 cannabinoid receptor. To address this issue, we have employed conformational analysis, molecular superposition, and distance comparison methods aimed at explaining the difference in pharmacological behavior of these families of structurally related compounds.

In the final section, we present a three-dimensional quantitative structure−activity relationship (3D-QSAR) model developed for a series of cannabinoids consisting of AC bicyclic structures and both ABC and ACD tricyclic structures using comparative molecular field analysis (CoMFA).[12] CoMFA, which attempts to correlate variations in observed activities or properties for a collection of compounds with their three-dimensional steric and electrostatic fields, has rapidly become a powerful and versatile tool in rational drug design and numerous other applications.[13] Following standard CoMFA procedures, the cannabinoid structures were aligned by superposition of common pharmacophores. The resulting 3D-QSAR model, based on the observed binding affinities of the cannabinoids, was then applied to predict the binding affinity of anandamide and prostaglandin ethanolamide in their calculated biologically active conformations. It was thus hoped that CoMFA, besides yielding a statistically predictive model for the cannabinoids, would also serve to validate the pharmacophoric conformation selected for anandamide.

Methods

All modeling techniques in this study were carried out on a Silicon Graphics RS4000 Indigo Elan workstation, running *Insight II* V2.3.5[14] for the molecular dynamics (MD) simulations and *Sybyl* V6.2[15] for the DISCO (*dis*tance *co*mparison)[16] and CoMFA[12] analyses.

The sequence of operations for this study is summarized as follows: (1) specify the likely bioactive conformation(s) of HHC and calculate the distances between its pharmacophoric groups; (2) constrain the distances between the corresponding pharmacophoric groups in anandamide to those values found in HHC and then run constrained MD simulations to explore the conformational flexibility of the unconstrained portions of anandamide; (3) using DISCO, examine the similarities between anandamide and HHC from the standpoint of overlay of their respective pharmacophores; and (4) apply CoMFA to further validate the proposed pharmacophoric conformation of anandamide.

Conformational Analysis. HHC was chosen as the reference molecule in this part of the analysis for the following reasons: (1) it exhibits relatively high potency and maximal efficacy as a CB_1 cannabinoid receptor agonist; (2) it possesses all three contact points proposed for pharmacophoric agonist−receptor interaction; and (3) it maintains the structural constraints imposed by the pyran group in common with Δ^9-THC and its active metabolite 11-OH-Δ^9-THC (see Figure 1). As such, the 3D arrangement of the pharmacophoric groups in HHC is considered near-optimal for interaction with the cannabinoid receptor. It was thus hypothesized, to be tested by these analyses, that the spatial topology and disposition of pharmacophores in anandamide (as well as other eicosanoid compounds capable of binding to the CB_1 cannabinoid receptor) are similar to those in HHC.

An initial 3D model of HHC was generated on the basis of the X-ray crystallographic structure of Δ^9-tetrahydrocannabinoic acid[7] and modified using chemical groups taken from the *Sybyl* fragment database. Xie et al.[6] have suggested that the C3 alkyl side chain of the bicyclic cannabinoid CP-47,497 can adopt any one of four equivalent minimum-energy conformations, all of which are oriented perpendicular to the plane of the phenyl ring. Employing 2D NMR analysis together with MD simulations and monitoring the first two torsion angles of the C3 alkyl side chain (designated φ_3 and φ_4 in Figure 1c and ref 6), they identified four low-energy conformations of the φ_3,φ_4 pair corresponding approximately to (60°,60°), (−60°,−60°), (120°,−60°), and (−120°,60°). Accordingly, the present study considered the HHC structure in all four conformational states corresponding to these four different pairs of φ_3,φ_4 values. After energy minimization of each conformer, the distances d_1-d_5 between the three pharmacophoric points were determined as illustrated in Figure 2a. These four conformers were then employed as templates in the subsequent conformational search described below.

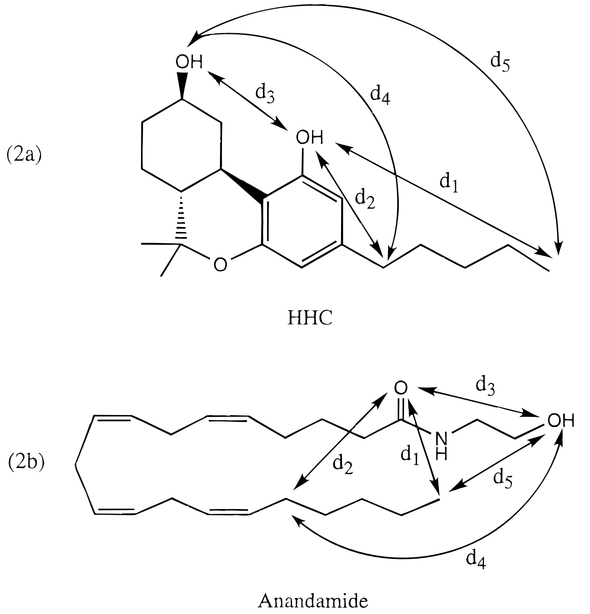


Figure 2. Depiction of distances d_1-d_5 between pairs of selected pharmacophores: (a) in HHC and (b) in anandamide.

Conformational Searching by Constrained Molecular Dynamics. The appreciable conformational flexibility of the anandamide molecule increases the likelihood of finding numerous low-energy conformations. Even if the amide bond is assumed to be trans-planar and the five-carbon alkyl tail is constrained to a single conformation, anandamide still contains 12 rotatable bonds (Figure 2b). A systematic search of this 12D torsional space would be computationally prohibitive; viz., rotating every bond in 30° increments would generate $>10^{12}$ conformations. To reduce the size of conformational space to be explored, anandamide was constrained such that the distances between its pharmacophore atoms matched the corresponding distances in HHC. This scheme was implemented for each of the four HHC conformers, thus yielding a complementary set of four anandamide conformers for the subsequent constrained MD simulations. The constraints were imposed by applying a force constant of 10^5 kcal mol^{-1} using the template force protocol in *Insight II/Discover*. With these specified atoms constrained in this manner, the four anandamide conformers were first energy-minimized with respect to the remaining internal degrees of freedom and then subjected to constrained MD at a constant temperature of 1000 K. By confining our MD conformational search to only those anandamide conformations that fit the putative pharmacophore configuration in HHC, we not only spared computational resources but also optimized conditions for identifying the most biologically relevant conformations of anandamide. At the same time, running the dynamics at high temperature (1000 K) provided ample thermal energy for the system to surmount high-energy conformational barriers (i.e., $E_{\text{thermal}} = 3/2\ RT = 2.98$ kcal mol^{-1} at $T = 1000$ K) and thus to sample a wide region of conformational space.

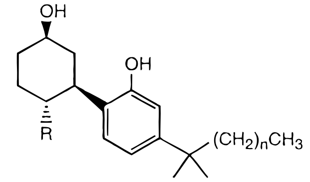
The following general procedure was implemented to run the MD simulations. The structures were first equilibrated by running dynamics for 1 ps while increasing the temperature from 298 to 1000 K, after which conformations were sampled every 1 ps over a span of 1000 ps at 1000 K to yield a structural database of 1000 separate conformers. Each conformer was then energy-minimized by molecular mechanics (MM) in two steps: first, while holding the predefined pharmacophore distance constraints and then again after releasing them. By employing this two-step energy minimization scheme, the desired pharmacophore distances were released from their constraints only after the conformational strain in the remainder of the molecule had been removed. Energy minimization of the 1000 conformers was achieved by sequential application of steepest descent and conjugate-gradient optimization algorithms to an energy difference of 0.001 kcal mol^{-1} between successive iterations. Following energy minimization, the 1000 conformers were pruned to eliminate those with energy >5 kcal mol^{-1} above the lowest-energy conformer. As described below, the conformers remaining after this process were compared with HHC in terms of their interpharmacophore distances using the DISCO[16] routine in *Sybyl*.

DISCO Analysis. Using DISCO, the distances were calculated between all pairs of pharmacophoric points for each low-energy conformer of anandamide (vide supra). These points are illustrated in Figure 2b. DISCO then identified those conformers whose set of points matched those in one of the four HHC conformational templates within a specified tolerance limit, which was set at 1.0 Å in the present analysis. All anandamide conformers satisfying this distance criterion were then stored in a correspondence table for further examination. The same sequence of constrained MD, MM energy minimization, and DISCO procedures was applied to the prostaglandin ethanolamide in a similar fashion.

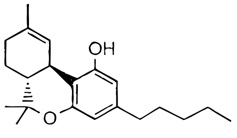
CoMFA Analysis. Published data for a series of AC bicyclic and ACD tricyclic cannabinoids[4] (Figure 3) comprised the training set for which a CoMFA model was developed by common 3D alignment. This analysis was based upon K_i values determined from in vitro heterologous displacement of [^{3}H]CP-55,940 binding to CB$_1$ receptors in rat brain membranes. The structural diversity of these compounds (Figure 3) is manifested in several respects, specifically: (1) the length of the C3 alkyl side chain, (2) the size of the cyclohexyl ring, (3) the substitution at position C4 of the cyclohexanol ring, and (4) the presence or absence of the D ring. In addition to these bicyclic AC and tricyclic ACD ring structures, this training set included the two ABC tricyclic cannabinoids Δ^9-THC and desacetyllevonantradol.[4] The CoMFA model so derived was applied to predict the K_i values of the anandamide and prostaglandin ethanolamide conformer(s) generated by DISCO, thereby assisting in the identification of those conformer(s) expected to produce the maximal cannabimimetic response.

Molecular Models and Structural Alignment. The structures of the cannabinoid analogues selected for the CoMFA study were all constructed by starting with the putative bioactive conformation of HHC derived from the DISCO analysis and adding chemical groups as necessary taken from the fragment library in *Sybyl*. The C3 alkyl side chain was oriented perpendicular to the aromatic ring by adjusting both the ϕ_3 and ϕ_4 torsion angles to 60° corresponding to one of the four low-energy conformations previously identified by Xie et al.[6] The C9 hydroxyl was adjusted to protrude above the plane of the C ring, consistent with the finding that compounds in which the C9 substituent lies in or below the plane of the ring exhibit diminished activity.[17] Each structure was then energy-minimized using the Tripos force field in *Sybyl* with a distance-dependent dielectric function and setting the convergence criterion to an energy difference of 0.001 kcal mol^{-1} between successive iterations. Partial atomic charges required for the calculation of the electrostatic interaction energies were derived using the Gasteiger−Marsili formalism.[18]

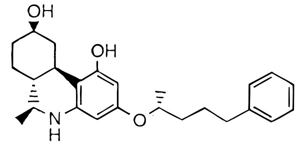
Undoubtedly the most critical yet subjective step in a CoMFA study involves the structural alignment of the training set compounds. The initial step of this alignment process is selection of the compound to serve as the structural template. For this purpose, Δ^9-THC was chosen based on two factors: (1) its distinction as the prototypical classical cannabinoid and (2) the availability of relevant biological data for Δ^9-THC. Structural alignment of the remaining compounds in the training set was achieved by root-mean-square (rms) fitting to those common pharmacophoric groups in Δ^9-THC which purportedly give rise to the cannabimimetic response, namely: (i) the phenolic OH, (ii) the cyclohexyl OH, and (iii) the first carbon on the C3 alkyl side chain, i.e., that carbon atom directly attached to the phenolic ring. After initial alignment was completed, a CoMFA "field-fit" optimization was carried out by which the geometry of each training set



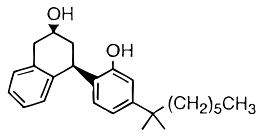
Compound	n	R
1	0	H
2	1	H
3	2	H
4	3	H
5	4	H
CP-47,497	5	H
6	6	H
7	7	H
8	8	H
9	9	H
10	5	CH_3
11	5	CH_2CH_3
12	5	$CH_2CH_2CH_3$
13	5	$CH_2CH_2CH_2CH_3$
14	5	$CH_2CH_2CH_2CH_2CH_3$
15	5	CH_2OH
16	5	CH_2CH_2OH
CP-55,940	5	$CH_2CH_2CH_2OH$
17	5	$CH_2CH_2CH_2CH_2OH$
18	5	$CH_2CH_2CH_2OCH_3$



THC



Desacetyllevonantradol



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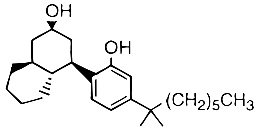

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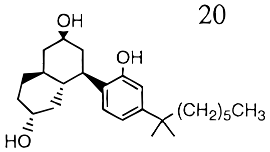

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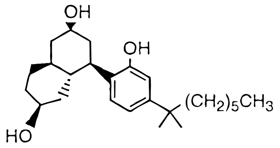

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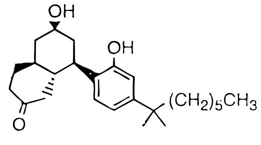

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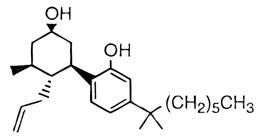

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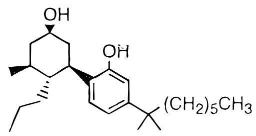

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Figure 3. Series of AC bicyclic and ACD tricyclic cannabinoids[4] that comprised the training set for the CoMFA model derived in the present study.

compound was adjusted to achieve maximal similarity with the steric and electrostatic fields of the structural template compound (i.e., Δ^9-THC). The molecules were then energy-minimized once again to remove any geometrical distortions caused by the field-fit operation.

To construct the CoMFA model, each compound in the training set was embedded inside a three-dimensional cubic lattice large enough to accommodate the largest molecule in the series. The steric fields (i.e., van der Waals interaction energies, represented by a Lennard–Jones 12-6 equation) and electrostatic fields (i.e., represented by Coulomb's law, with a distance-dependent dielectric constant) surrounding each molecule were calculated by placing a probe, represented in this case by an sp^3 carbon atom with a +1 charge, at each intersection of a 2-Å spaced grid. Calculated energies exceeding 30 kcal/mol were automatically set to this value.

PLS–QSAR. To form the basis for a predictive statistical model, CoMFA employs partial least-squares (PLS) regression to find correlations between variations in the observed biological activity and variations in the steric–electrostatic field energies among the training set compounds. PLS[19] attempts to establish a relationship between Y and X, where vector Y represents the response or dependent variable (e.g., the biological activity) and X represents the descriptor data (e.g., the CoMFA fields). The *Sybyl* QSAR module[15] was employed for all calculations. The QSAR table was constructed with rows containing the cannabinoid compounds along with their appropriate CB_1 receptor binding values as the dependent variables and with columns containing the steric and electrostatic fields, calculated as described above, as the independent variables.

Table 1. Calculated Distances (d_1–d_5) between Pairs of Pharmacophoric Groups for the Four Conformers of HHC (Figure 2a)[a]

ϕ_3 (deg)	ϕ_4 (deg)	d_1 (Å)	d_2 (Å)	d_3 (Å)	d_4 (Å)	d_5 (Å)	E (kcal mol^{-1})
60	60	4.99	6.76	4.99	8.89	8.19	5.53
120	−60	5.04	7.47	4.99	8.91	8.61	5.57
−60	−60	4.99	6.75	4.99	8.96	11.46	5.63
−120	60	5.04	7.29	4.99	8.99	11.65	5.64

[a] Corresponding to the four low-energy conformations with respect to ϕ_3 and ϕ_4 as determined by Xie et al.[6]

An initial PLS analysis was performed using the "leave-one-out" cross-validation procedure[20] to determine the optimum number of principal components, which themselves are linear combinations of the original descriptor variables, needed for the subsequent analysis of the data. In cross-validation, each compound is systematically excluded once from the data set after which its activity is predicted by a model derived from the remaining compounds. The PLS analysis was then repeated without cross-validation using the optimum number of components that yielded the smallest standard error of prediction or highest cross-validated r^2 (r^2_{CV}). This final analysis provided a predictive model and associated values of the conventional r^2 (r^2_{CONV}), from which the CoMFA color contour plots are generated for the steric and electrostatic potentials. It should be noted that r^2_{CONV} and r^2_{CV} measure the quality of the CoMFA model in different respects. The former describes its ability to interpolate within the training set of activity data, while the latter describes its ability to extrapolate both within and beyond the training set. Hence, the r^2_{CONV} value is a measure of the model's self-consistency, while the r^2_{CV} value is a measure of its predictive ability.

Results and Discussion

Pharmacophore Identification and Molecular Superposition. Previously described structure−activity relationships have determined that the structural prerequisites for cannabinoid activity are 3-fold: (1) the hydrophobic C3 alkyl side chain, (2) the phenolic A ring hydroxyl, and (3) the cyclohexyl hydroxyl.[3] The distances between these pharmacophoric groups are listed in Table 1 for the four conformers of HHC associated with the four low-energy conformations of the alkyl side chain. These distances were imposed as constraints on the corresponding pharmacophores in anandamide prior to the MD simulations and subsequent superposition studies. An important issue in this regard deals with the proper way to match the pharmacophores in anandamide with those in HHC. We postulated that the three pharmacophoric groups in HHC given above matched the respective groups in anandamide: (1) the five terminal carbons, (2) the oxygen of the carboxyamide, and (3) the ethanol hydroxyl. Consequently, these groups in anandamide were superimposed onto the corresponding groups in HHC, specifically: (1) the lipophilic side chain, (2) the phenolic hydroxyl group, and (3) the cyclohexyl hydroxyl.

As demonstrated by the high-affinity receptor binding of the *N*-(2-butyl)- and *N*-(2-propyl)arachidonylamides, the ethanol OH in anandamide is not necessary for interaction with $\mathrm{CB_1}$ cannabinoid receptors.[11] Likewise for the classical cannabinoids, the cyclohexyl hydroxyl is not an absolute requirement for activity insofar as substitution with other functional groups is permitted.[1,4,21] This SAR suggests a common biological function for these two groups; therefore, superposition of the ethanol oxygen in anandamide with the cyclohexyl oxygen in HHC was considered a reasonable assumption. Intuitively, the distal five-carbon alkyl tail of anandamide should overlay with the C3 alkyl side chain of HHC. This is likewise supported by SAR studies which show that a chain length modification in both HHC and anandamide resulted in loss of potency.[22] To superimpose the alkyl tail of anandamide onto the alkyl side chain of HHC, the former must assume a constrained conformation such that the homoconjugated alkyl chain bends back upon itself, while, at the same time, the carbonyl oxygen and the terminal hydroxyl group on the ethanolamide portion overlay the phenolic OH and the cyclohexyl OH in HHC, respectively. According to this model, the homoconjugated cis double bonds constitute a flattened helix that is intrinsic to the structure. This region would correspond with a position between the pyran and the aromatic phenolic rings of the ABC tricyclic cannabinoids or the phenolic ring of the nonclassical AC bicyclic cannabinoids.

An alternative pharmacophore fit involving the pyran oxygen in HHC has been described by Thomas et al.[23] These investigators chose the pyran ring oxygen of Δ^9-THC as the cannabinoid counterpart to the carbonyl oxygen of anandamide, thereby leaving the phenolic hydroxyl as the counterpart to the terminal hydroxyl of anandamide. The pyran ring was not included in our model since experimental studies have demonstrated that the pyran oxygen can be replaced by a carbon or nitrogen, or that the ring itself could be disrupted (see CP-47,497, CP-55,940), without extensive loss of potency.[3,24] By selecting HHC as the structural template, we implicitly regarded the cyclohexyl and phenolic oxygen atoms of HHC as counterparts to the two oxygen atoms of anandamide. HHC is a rational choice inasmuch as it is a more potent cannabinoid than Δ^9-THC.[25] Our matching scheme is consistent with the finding that the cyclohexyl OH in the cannabinoids and the terminal OH of anandamide both enhance binding but their absence (as in Δ^9-THC and the alkyl arachidonate derivatives) does not eliminate activity.[3,11]

Examination of the DISCO results on anandamide identified several low-energy conformers whose pharmacophores could be fitted within the designated tolerance limit of 1.0 Å of the pharmacophoric distances in the respective conformers of HHC. Further visual inspection revealed a unique anandamide conformation in which the polyolefin loop overlays the tricyclic ring of HHC. The aforementioned study by Thomas et al.[23] described a "looped conformation" of anandamide that was both energetically accessible and able to overlay with the cannabinoid tricyclic ring. In contrast, our anandamide conformation might be described as helical in nature.

Figure 4 shows a stereoview of HHC and anandamide with their overlapping oxygen atoms and alkyl chain surrounded by their van der Waals surfaces for better visualization. The possible biological relevance of this anandamide conformation is suggested by the spatial similarity and the high degree of matching of common pharmacophoric elements with HHC. The energy of this anandamide conformation is only 3.4 kcal $\mathrm{mol^{-1}}$ higher than the lowest-energy structure extracted from the MD simulations. This energy requirement could easily be afforded by the more optimal binding interaction between anandamide and the receptor; therefore our anandamide conformation is expected to be energetically accessible under physiological conditions.

Other studies have also addressed potential conformations that anandamide can achieve in order to fit into the same binding sites as do the cannabinoid ligands. An empirical conformation proposed by Burstein et al.[26] superimposed (1) the hydrocarbon chain of anandamide over the dimethylheptyl moiety of HU-210 and (2) the hydroxyl of the eicosanoid ethanol over the 11-hydroxy group of the cannabinoid. This resulted in a looped eicosanoid having some structural overlap with the cannabinoid. However, the important pharmacophoric element of the phenolic hydroxyl of the cannabinoid has

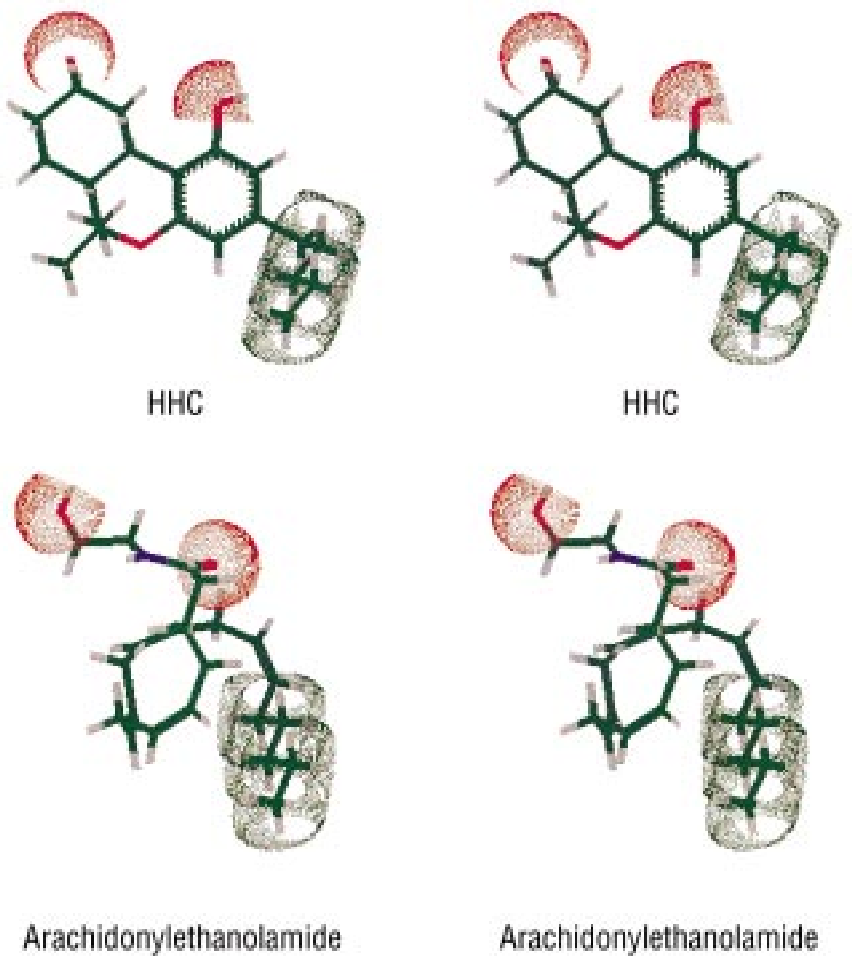

Figure 4. Stereoview of the HHC and anandamide molecules, depicted with van der Waals surfaces to highlight possible alignment of their corresponding oxygen atoms (red spheres) and alkyl chain (green mesh).

no counterpart in the eicosanoid in this structure. Thomas et al.[23] employed molecular dynamics calculations to identify a low-energy conformation of arachidonyl ethanolamide which superimposed (1) the carboxyamide oxygen over the pyran oxygen in Δ^9-THC, (2) the hydroxyl of the eicosanoid ethanol over the phenolic hydroxyl of Δ^9-THC, and (3) the hydrocarbon chain over the pentyl side chain of Δ^9-THC. This resulted in a looped structure of relatively low energy. This arrangement could accommodate a series of eicosanoid analogues having modified structures in the amide moiety. However, this model suffers from the consideration that the pyran oxygen in the cannabinoid structure is not considered to be a critical pharmacophoric element. Furthermore, the phenolic hydroxyl of Δ^9-THC is a critical cannabinoid pharmacophoric element, whereas its eicosanoid counterpart in this model is not. Barnett-Norris et al.[27] applied the technique of conformational memories to study the conformational space capable of being occupied by anandamide, and then representative structures were docked into a computer model of the CB_1 cannabinoid receptor. These analyses revealed that representative structures from an anandamide conformational family were able to fit at the presumptive CB_1 receptor agonist binding site.

One component of anandamide is the arachidonyl moiety. Arachidonic acid is potentially capable of adopting many conformations, including the transition-state conformation attained in the cyclooxygenase enzyme which converts this substrate to the cyclic endoperoxides PGG_2 and PGH_2. X-ray crystallographic evidence reveals that the conformation of arachidonic acid in the substrate cyclooxygenase binding site is a helix.[29] This conformation is comparable to that proposed in the present study for the ligand binding site of anandamide. The product prostaglandins are constrained by the presence of a cyclopentyl ring that is seven carbons distal to the carboxylic acid of arachidonic acid. However, the ethanolamine amides of several prostaglandins failed to bind to the CB_1 cannabinoid receptor at concentrations as high as 100 μM.[11] This suggests that either the conformation imposed by the cyclopentyl ring or the functionalities on the cyclopentyl ring hinder interaction with the CB_1 receptor. To explore this issue further, *N*-(2-hydroxyethyl)prostaglandinamide was subjected to the same constrained MD procedure and DISCO analysis as for anandamide. The search failed to detect any low-energy conformers of *N*-(2-hydroxyethyl)prostaglandinamide that could satisfy the pharmacophoric distances in HHC within a 1.0-Å tolerance, although a pharmacophoric match was found within 2.0 Å. Moreover, it was not possible to overlay the cyclopentyl moiety of any of these conformers with the HHC tricyclic structure. The present finding that anandamide can, yet prostaglandin ethanolamides cannot, adopt a conformation that is spatially

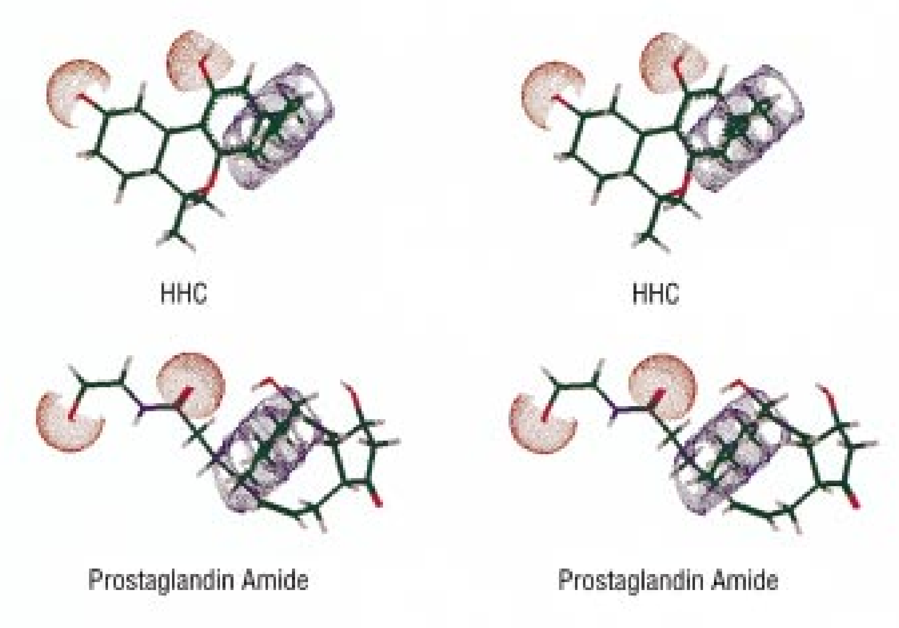

Figure 5. Stereoview of the HHC and prostaglandin amide molecules, depicted with van der Waals surfaces to highlight possible alignment of their corresponding oxygen atoms (red spheres) and alkyl chain (green mesh). Note that, in contrast to anandamide, a portion of the prostaglandin amide structure (on right-hand side) protrudes beyond the tricyclic ring into regions not occupied by HHC.

similar to HHC agrees with the experimental observations. Visual analysis shows that the constraint imposed by the cyclopentyl group requires a portion of the prostaglandinamide structure to protrude beyond the tricyclic ring into regions not occupied by HHC (Figure 5). Occupancy of this region in space may represent steric encroachment into the receptor space and thus prohibit or significantly diminish affinity for the CB_1 receptor.

On the basis of the present analysis, the difference in affinity for the CB_1 receptor between anandamide and *N*-(2-hydroxyethyl)prostaglandinamide emphasizes the importance of considering spatial topology as well as the three-point pharmacophore model. Both anandamide and prostaglandinamide satisfy the prerequisites of the three-point pharmacophore model. However, the present analysis suggests that the observed disparity in affinities for the CB_1 receptor between these two compounds can be explained in terms of the differences in their ability to overlay their respective polyolefin loops with the HHC tricyclic structure.

CoMFA Analysis. The CoMFA model so derived required eight principal components to explain the observed variance in binding affinity to CB_1 receptors in rat brain membranes.[4] The key statistical parameters associated with this model are $r^2_{\mathrm{CV}} = 0.598$, $r^2_{\mathrm{CONV}} = 0.992$, SEE = 0.092, $F = 296$, and $p < 0.001$. A model with $r^2_{\mathrm{CV}} \geq 0.50$ is generally regarded as internally predictive; thus the $r^2_{\mathrm{CV}} = 0.598$ obtained in the present case imparts credibility to our CoMFA model. As illustrated in Figure 6, an excellent correlation was obtained between the corresponding CoMFA-calculated and experimental values of the CB_1 receptor binding affinity for the compounds in the training set.

The resulting CoMFA model can be depicted visually in the form of field contour maps describing the steric and electrostatic requirements for recognition at the receptor site. The individual field contributions to the CoMFA model were 74% from the steric fields and 26% from the electrostatic fields, indicating that the observed variations in binding affinity among the cannabinoids in the training set are dominated by steric interactions at the receptor site. This result is consistent with the experimental observations that the elimination of the cyclohexyl hydroxyl on the cannabinoid structure (e.g., compare Δ^9-THC and HHC) and the terminal hydroxyl in the anandamides (e.g., alkylanandamide analogues) results in little or no decrement in binding affinity or biological activity.[2,3,11]

As mentioned above, our CoMFA model was developed as a guide in assessing the biological relevance of the low-energy conformer found for anandamide. The K_i value of anandamide predicted by this model (26 nM) nearly matches that obtained from experiment (22 nM),[11] thus demonstrating that anandamide, although structurally distinct from the cannabinoids, is well-accommodated by the present CoMFA model. This agreement provides corroborating evidence in support of the three-point cannabinoid pharmacophore model and, more specifically, our proposed pharmacophoric conformation for anandamide.

To test the validity of this model for the ABC and ACD tricyclic cannabinoids, we selected the ABC tricyclic HU-210/HU-211 and ACD tricyclic CP55,243/CP55,244 enantiomeric pairs as test compounds. The affinity of CP55,244 ($K_i = 0.110$ nM) is 4 orders of magnitude greater than that of CP55,243 ($K_i = 1000$ nM) according to experimental measurements.[4a] In agreement, the present CoMFA model predicted that CP55,244 is the more active enantiomer by a large margin. The affinities of HU-210 and its poorly active enantiomer, HU-211, have been tested by the same radioligand displacement methods as performed for the training set,[28] thereby allowing their use as test compounds for the present model. The affinity of HU-210 ($K_i = 0.230$ nM) is approximately 3 orders of magnitude

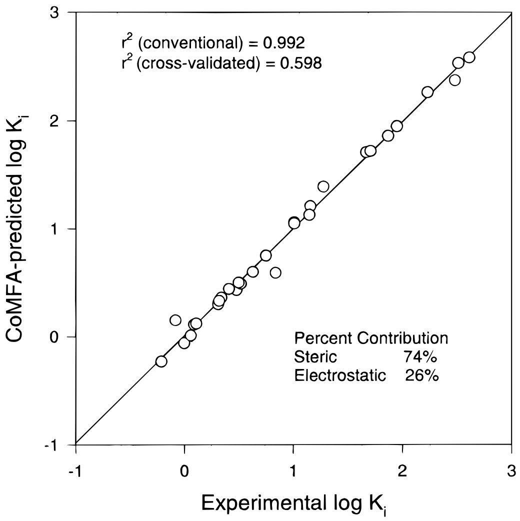

Figure 6. Plot of the corresponding CoMFA-calculated and experimental values of the CB_1 receptor binding affinity (given as log K_i) for the compounds in the training set.

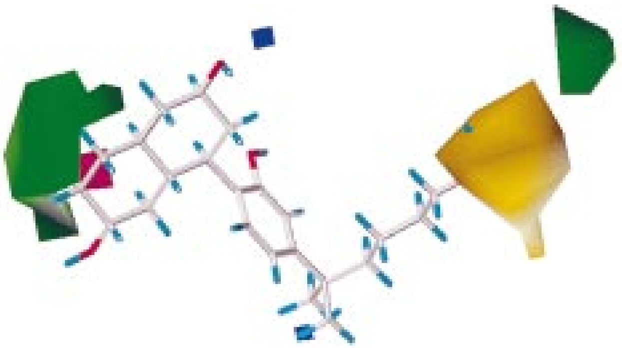

Figure 7. CoMFA steric contour map around an ACD tricyclic ring structure where the polyhedra describe regions around the molecules for which an increase in steric bulk is predicted to enhance (yellow) and diminish (green) binding affinity.

greater than that of HU-211 ($K_i = 360$ nM). Consistent with these observed values, the present model predicted that HU-210 exhibits a higher affinity than HU-211 by nearly 2 orders of magnitude. Consequently, this model that was developed for AC bicyclic and ACD tricyclic structures is equally suitable for classical ABC tricyclic structures.

Figure 7 depicts the CoMFA steric contour map around an ACD tricyclic ring structure where the polyhedra describe regions around the molecules for which an increase in steric bulk is predicted to enhance (yellow) and diminish (green) binding affinity. The region of green polyhedra (i.e., negative steric contribution) surrounding the D ring is consistent with the SAR for the ACD tricyclic cannabinoids, for which it has been noted that extending the ketone or hydroxyl substituents from the C2 position of the D ring gives rise to decreased binding affinity compared with the unsubstituted analogue.[4] Likewise, the region of yellow and green polyhedra following the C3 alkyl side chain of the A ring is consistent with the observation that the binding affinity for the CB_1 cannabinoid receptor is enhanced increasing side-chain length up to 7−8 carbon atoms and then is diminished.[4]

This CoMFA model has addressed the spatial overlap between the cannabinoid and eicosanoid classes of agonist ligand for the CB_1 cannabinoid receptor. Compounds of another class, the aminoalkylindoles, also exhibit efficacious agonist activity at this receptor[21] and may interact with some of the same points of interaction as do the cannabinoid compounds. We have developed a novel superimposition model using 3D pharmacophore mapping and CoMFA on a training set of cannabinoid ligands comparable to that used in the present study and a training set of 12 aminoalkylindole compounds.[30] The superimposition model so derived suggests that there are certain key pharmacophoric elements common to both cannabinoid and aminoalkylindole classes of CB_1 receptor agonists.

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