Cannabinoid Receptors and Their Ligands: Ligand–Ligand and Ligand–Receptor Modeling Approaches

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Abstract The cannabinoid CB_1 and CB_2 receptors belong to the class A, rhodopsin-like family of GPCRs. Antagonists for each receptor sub-type, as well as four structural classes of agonists that bind to both receptors, have been identified.

An extensive amount of structure–activity relationship information (SAR) has been developed for agonists and antagonists that bind at CB_1, while the SAR of CB_2 ligands is only now emerging in the literature. This chapter focuses both on recent CB_1 and CB_2 SAR and on the pharmacophores for ligand recognition at the CB_1 receptor that have been developed using ligand–ligand or ligand–receptor approaches. In a ligand–ligand approach, the structure of the binding site of the ligand is not directly considered. This approach is an attempt to infer information about the macromolecular binding site, and/or modes of binding interactions from a correlation between experimentally determined biological activities and the structural and electronic features of a series of small molecules. In a ligand–receptor approach, cannabinoid (CB) receptor models are probed for ligand binding sites and binding sites can be screened using energetic criteria, as well as ligand SAR and the CB mutation literature. This chapter discusses the factors that control the quality of the results emanating from each of these approaches and identifies areas of agreement and of disagreement in the existing CB literature. Challenges for future SAR and pharmacophore development are also identified.

Keywords Cannabinoid SAR · Modeling · Receptor modeling

1 Introduction

Both the CB_1 and the CB_2 receptors belong to the class A rhodopsin-like family of G protein-coupled receptors (GPCRs). The cloning and expression of a complementary DNA from a rat cerebral cortex cDNA library that encoded the first cannabinoid receptor subtype (CB_1) was reported by Matsuda and co-workers (1990). Subsequently, the primary amino acid sequences of an amino terminus variant CB_1 receptor (Shire et al. 1995), as well as the CB_1 sequence in human brain and in mouse were reported (Abood et al. 1997; Gerard et al. 1991). A helix net representation of the human CB_1 receptor sequence is presented in Fig. 1. In addition to being found in the central nervous system (CNS), mRNA for CB_1 has also been identified in testis (Gerard et al. 1991). The CB_1 receptor has been shown to have a high level of ligand-independent activation (i.e., constitutive activity) in transfected cell lines, as well as in cells that naturally express the CB_1 receptor (Bouaboula et al. 1997; Pan et al. 1998; Mato et al. 2002; Meschler et al. 2000). Kearn and co-workers (1999) have estimated that in a population of wild-type (WT) CB_1 receptors, 70% exist in the inactive state (R) and 30% exist in the activated state (R*).

The second cannabinoid receptor sub-type, CB_2, was derived from a human promyelocytic leukemia cell HL60 cDNA library (Munro et al. 1993). The human CB_2 receptor exhibits 68% identity to the human CB_1 receptor within the transmembrane regions, 44% identity throughout the whole protein. The CB_2 receptor in both rat (Griffin et al. 2000) and mouse (Shire et al. 1996) has been cloned as well. A helix net representation of the human CB_2 receptor sequence is presented in Fig. 2. Unlike the CB_1 receptor, which is highly conserved across human, rat, and

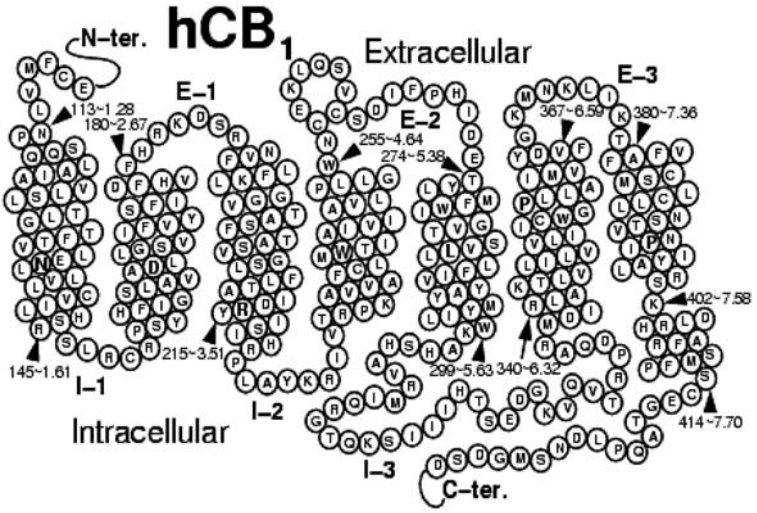


Fig. 1. A helix net representation of the human CB_1 receptor sequence. (Gerard et al. 1991)

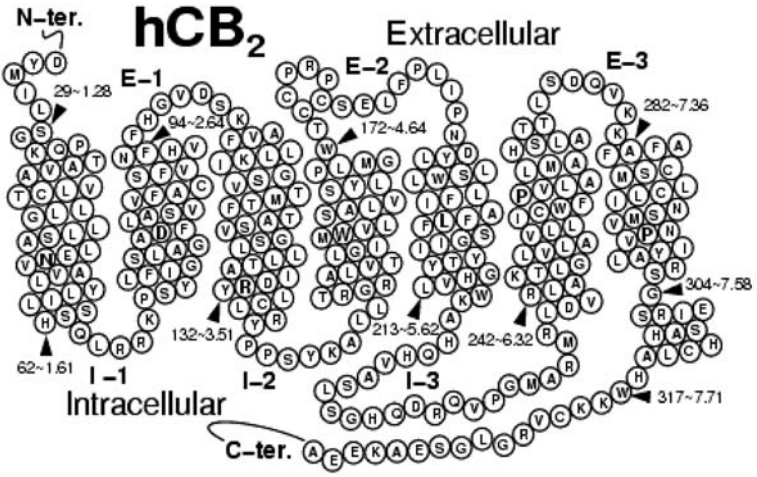


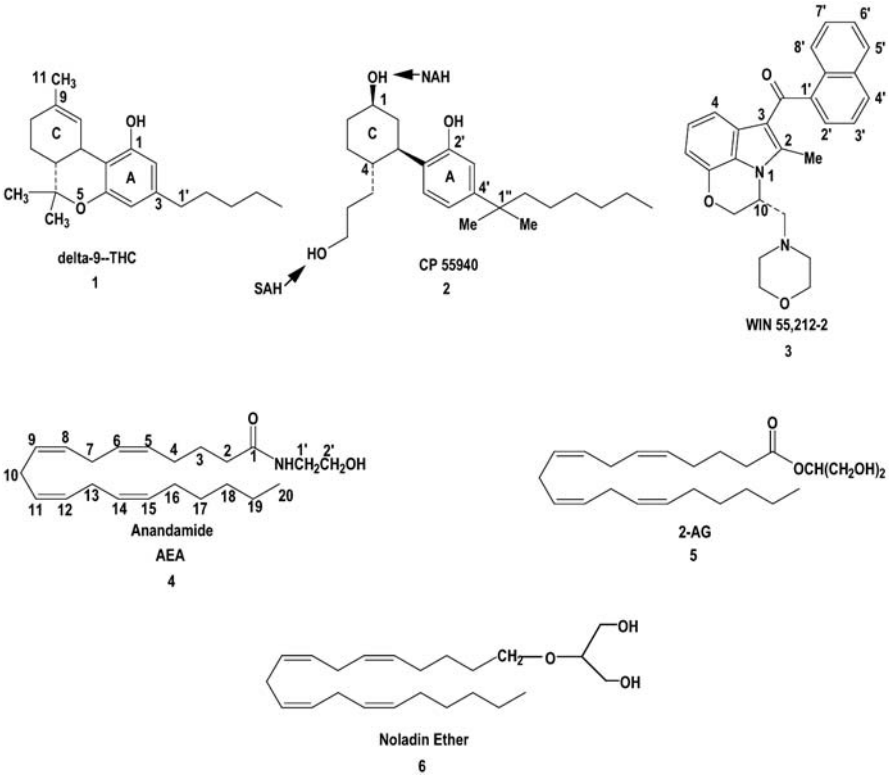
Fig. 2. A helix net representation of the human CB_2 receptor sequence. (Munro et al. 1993)

mouse, the CB_2 receptor is much more divergent. Sequence analysis of the coding region of the rat CB_2 genomic clone indicates 93% amino acid identity between rat and mouse and 81% amino acid identity between rat and human. CB_2 receptor-transfected CHO cells exhibit high constitutive activity (Bouaboula et al. 1999). Evidence for other cannabinoid receptors is mounting in the literature (Breivogel et al. 2001; Di Marzo et al. 2000; Fride et al. 2003; Jarai et al. 1999; Wagner et al. 1999). However, no new CB receptor subtypes have yet been cloned.

1.1 Cannabinoid Receptor Agonists

The CB_1 receptor transduces signals in response to CNS-active constituents of *Cannabis sativa*, such as the classical cannabinoid (CB) (–)-*trans*-Δ^9-tetrahydrocannabinol [(–)-Δ^9-THC (**1**)] and to three other structural classes of ligands, the non-classical CBs typified by (1R,3R,4R)-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-4-(3-hydroxypropyl) cyclohexan-1-ol [CP-55,940 (**2**)] (Devane et al. 1988; Melvin et al. 1995), the aminoalkylindoles (AAIs) typified by *R*-[2,3-dihydro-5-methyl-3-[(4-morpholinyl)methyl]pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl](1-naphthalenyl)methanone [WIN55,212-2 (**3**)] (Compton et al. 1992; D'Ambra et al. 1992; Ward et al. 1991), and the endogenous CBs. The non-classical CBs clearly share many structural features with the classical CBs, e.g., a phenolic hydroxyl at C-1 (C2′), and alkyl side chain at C-3 (C-4′), as well as the ability to adopt the same orientation of the carbocyclic ring as that in classical CBs (Reggio et al. 1993). The AAIs, on the other hand, bear no obvious structural similarities with the classical/non-classical CBs.

The first endogenous CB was isolated from porcine brain by Mechoulam and co-workers (Devane et al. 1992). The endogenous CB ligands are unsaturated

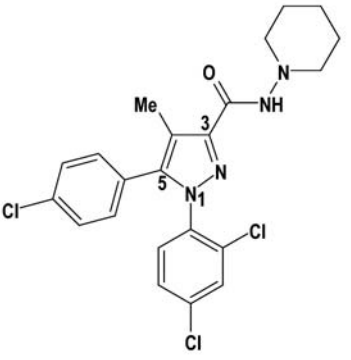


fatty-acid ethanolamides. The first identified ligand of this class was arachidonoylethanolamide (AEA, also called anandamide, **4**) (Devane et al. 1992). AEA has been shown to be synthesized from lipid in neurons and to be degraded by fatty acid amide hydrolase (FAAH), an integral membrane protein (Bracey et al. 2002). 2-Arachidonoylglycerol (2-AG; **5**) was isolated from intestinal tissue and shown to be a second endogenous CB ligand (CB_1 $K_i = 472 \pm 55$ nM; CB_2 $K_i = 1400 \pm 172$ nM) (Mechoulam et al. 1995). 2-AG has been found present in the brain at concentrations 170 times greater than anandamide (Stella et al. 1997). In addition, a fatty acid glycerol ether, 2-arachidonyl glyceryl ether, called noladin ether (**6**) has been identified as another endogenous CB ligand (Hanus et al. 2001).

1.2 Cannabinoid CB_1 Receptor Antagonists/Inverse Agonists

The first CB_1 antagonist, *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide [SR141716A (**7**)] was developed by Rinaldi-Carmona and co-workers at Sanofi Recherche (Rinaldi-Carmona et al. 1994). SR141716A displays nanomolar CB_1 affinity ($K_i = 1.98 \pm 13$ nM), but very low affinity for CB_2. In vitro, SR141716A antagonizes the inhibitory effects of CB agonists on both mouse vas deferens contractions and adenylyl cyclase activity in rat brain membranes. SR141716A also antagonizes the pharmacological and behavioral effects produced by CB_1 agonists after intraperitoneal (IP) or oral administration (Rinaldi-Carmona et al. 1994). Several other CB_1 antagonists have been reported: LY-320135 (Felder et al. 1998), O-1184 (Ross et al. 1998), CP-27,2871 (Meschler et al. 2000), a class of benzocycloheptapyrazoles (Stoit et al. 2002) and, most recently, a novel series of 3,4-diarylpyrazolines (Lange et al. 2004).

SR141716A (**7**) has been shown to act as a competitive antagonist and inverse agonist in host cells transfected with exogenous CB_1 receptor, as well as in biological preparations endogenously expressing CB_1. Bouaboula and co-workers



SR 141716A
(CB1)

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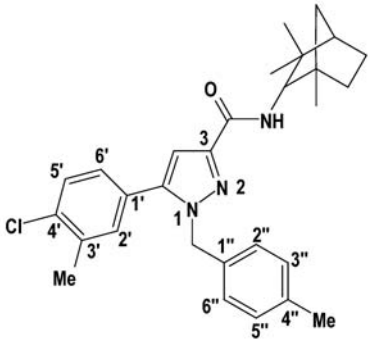


SR 144528
(CB2)

8

(1997) reported that CHO cells transfected with human CB_1 receptor exhibit high constitutive activity at the level of both mitogen-activated protein (MAP) kinase and adenylyl cyclase. Guanine nucleotides enhanced the binding of SR141716A, a property of inverse agonists. Lewis and co-workers (Pan et al. 1998) demonstrated constitutive activity of CB_1 receptors in inhibiting Ca^{2+} currents that was not due to endogenous agonist. These investigators reported that SR141716A antagonized the Ca^{2+} current inhibition induced by the CB agonist, WIN55,212-2, in neurons heterologously expressing either rat or human CB_1 receptors. Further, when applied alone, SR141716A increased the Ca^{2+} current, with an EC_{50} of 32 nM, via a pertussis toxin-sensitive pathway, indicating that SR141716A can act as an inverse agonist by reversal of tonic CB_1 receptor activity. Howlett and co-workers (Meschler et al. 2000) demonstrated that constitutive activity is demonstrable in neuronal cells that endogenously express CB_1 (N18TG2 cells) and that SR141716A acts as a competitive antagonist and reduces basal activity in the manner of an inverse agonist in these cells.

In some experiments, SR141716A has been found to be more potent in blocking the actions of CB_1 agonists than in eliciting inverse responses by itself. For example, in their study that focused upon rat brain membrane and brain sections, Sim-Selley et al. (2001) suggested that SR141716A may bind to two sites on the CB receptor, a high-affinity site at which it exerts its competitive antagonism and a lower affinity site at which it exerts its inverse agonism.

1.3 CB_2 Antagonists

The first CB_2 antagonist, SR144528 (**8**), was reported by Rinaldi-Carmona and co-workers at Sanofi Recherche (Rinaldi-Carmona et al. 1998). SR144528 displays sub-nanomolar affinity for both the rat spleen and cloned human CB_2 receptors ($K_i = 0.60 \pm 0.13$ nM). SR144528 displays a 700-fold lower affinity for both the rat brain and cloned human CB_1 receptors. CB_2 receptor-transfected CHO cells exhibit high constitutive activity, and this activity can be blocked by SR144528, working as an inverse agonist (Bouaboula et al. 1999). More recently, JTE-907 has also been identified as an inverse agonist at CB_2 (Iwamura et al. 2001) and AM630 has been reported to be a CB_2-selective antagonist (Ross et al. 1999a).

2 CB Pharmacophore Development: Ligand–Ligand and Ligand–Receptor Approaches

Pharmacophore development can be approached from a ligand–ligand perspective or from a ligand–receptor perspective. In a ligand–ligand approach, the structure of the binding site of the ligand is not directly considered. This approach is an attempt to infer information about the macromolecular binding site, and/or modes of binding interactions from a correlation between experimentally determined

biological activities and the structural and electronic features of a series of small molecules (Nakanishi et al. 1995).

There are many computer modeling/QSAR (quantitative structure–activity relationship) techniques that can be used to deduce information about a receptor binding site based upon ligand SAR. Among these, conformational analysis, molecular electrostatic potential mapping, receptor steric and receptor essential volume mapping, and the comparative molecular field analysis (CoMFA) QSAR method have been used in the literature to gain indirect information about the CB receptors. Central to many of these techniques is a structural superimposition using hypothesized key pharmacophoric features as molecular alignment guides. The quality of results emanating from this approach is highly dependent on these chosen alignments with template molecules. Much of the driving force for structural superpositions in the CB literature has been the fact that the four structural classes of CB agonist ligands and the CB antagonist ligands for a particular receptor sub-type displace one another in radioligand binding experiments. This fact has led to the assumption that key molecular features must superimpose because the molecules must interact with the same key amino acids of the receptor and share the same binding site to be able to displace one another. However, ligands do not necessarily have to occupy exactly the same space nor interact with the same key amino acids in order to displace one another. The presence of steric overlap between binding sites is sufficient to account for ligand displacement data. This means that alignments that incorporate structurally diverse classes of CB ligands may not lead to the best results.

In a ligand–receptor approach, CB receptor models are probed for binding sites for ligand classes and binding sites can be screened using energetic criteria, as well as ligand SAR and the CB mutation literature. The quality of the research emanating from this approach depends heavily on the quality of the receptor model, including the state that this model represents. The reliability of ligand binding sites identified based on energetic criteria is completely dependent on the model itself. If this model is far from the true receptor structure, then the identification of low energy binding sites will have little relevance for the CB field. Since the publication of the 2.8 Å X-ray crystal structure of bovine rhodopsin (Rho) in 2000 (Palczewski et al. 2000), most models of GPCRs, including the CB receptors (Barnett-Norris et al. 2002b; Hurst et al. 2002; McAllister et al. 2003; Salo et al. 2004; Shim et al. 2003; Xie et al. 2003), have been based upon this crystal structure. It is important to note that this structure represents the dark (inactive) state structure of Rho in which the inverse agonist, 11-*cis*-retinal is covalently bound. For ligand–receptor studies that employ a homology model of Rho, the relevant conformational state of the receptor should be taken into consideration because the inactive and active states of a GPCR are fundamentally different in conformation (Ghanouni et al. 2001b; Hulme et al. 1999; Jensen et al. 2001). Therefore, the state of the receptor for which an inverse agonist has high affinity (the inactive state) is not the state for which agonists have high affinity (the activated state).

In the next section, the use of both ligand–ligand and ligand–receptor approaches in the CB field will be discussed. This discussion is organized around individual structural classes of CB ligands.

3
Classical/Non-classical CB Pharmacophores

Prior to the discovery of the cannabinoid CB_1 receptor, CB SARs were developed by those who hypothesized that at least some of the effects produced by CBs may be receptor mediated. The early SAR that emerged has been reviewed comprehensively by Razdan (1986) and by Makriyannis and Rapaka 1990). These reviews consider both classical and non-classical CB compounds. Because there is structural/conformational similarity between the classical and non-classical CBs (Lagu et al. 1995; Reggio et al. 1993; Xie et al. 1994, 1996, 1998), unified pharmacophores developed for these two classes have agreed with one another and have led to a consensus pharmacophore that involves the existence of the following at the CB_1 receptor:

1. A hydrophobic binding pocket of limited depth into which the C-3 (C-4′) alkyl chain fits such that the chain is nearly perpendicular to the aromatic ring (Howlett et al. 1988; Melvin and Johnson 1987; Xie et al. 1998). Analogs with side chains of less than five carbons have no affinity for CB_1. Highest affinity is associated with the 1′,1′-dimethylheptyl side chain.

2. Hydrogen bonding sites for the phenolic hydroxyl of ring A (see **1**), the C-9/C-11 hydroxyls of the carbocyclic ring (ring C; see **1**) (Howlett et al. 1988; Melvin and Johnson 1987) (Huffman et al. 1996; Song and Bonner 1996) and the southern aliphatic hydroxyl (SAH) group (see **2**) (Drake et al. 1998; Tius et al. 1994).

3. An occluded region behind C-9 (classical), C-1 (non-classical) of the carbocyclic ring (ring C; see **1**) occupied by residues of the receptor itself (Reggio et al. 1993).

4. A large hydrophobic pocket that accommodates SAH hydrophobic analogs and a smaller hydrophilic pocket that accommodates the SAH group (see **2**) (Drake et al. 1998; Tius et al. 1994).

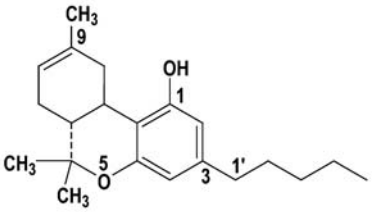
3.1
Ligand–Ligand Studies: CoMFA Pharmacophores for Classical/Non-classical CBs

Thomas and co-workers presented the first CoMFA QSAR model of the CB receptor that employed Martin multiple paradigm activity data as the biological activity and considered both classical and non-classical CBs (Thomas et al. 1991). Compounds were superimposed at the aromatic ring and alkyl side chain. The n-propyl alcohol chain (SAH) of CP-55,940 (**2**) was aligned with respect to its restricted analog CP-55,243. Results indicated steric repulsion behind the C-ring (see **2**) is associated with decreased predicted binding affinity and pharmacological potency. The steric bulk of the C-4′ side chain of **2** that is extended up to seven carbons was found to contribute to predictions of increased binding affinity and potency. The electrostatic fields of the CB analogs that correlated with increased predicted potency were predominantly seen around the C11 position of Δ^9-THC (**1**). Results

indicated that the protons of hydroxyl groups at positions corresponding to the C11 position of **1** may interact with an electronegative acceptor atom.

3.1.1 Side Chain SAR

One of the molecular regions of the classical/non-classical CBs that has been the focus of recent interest is the C-3 alkyl side chain. Several groups have looked at the effect of the introduction of unsaturation or functionality in the alkyl side chain of classical CBs. 1′,1′-Cyclopropyl side chain substituents were found to enhance the affinities of (–)-Δ^8-tetrahydrocannabinol (Δ^8-THC) and respective cannabidiol analogs for the CB_1 and CB_2 cannabinoid receptors (Papahatjis et al. 2002). For novel analogs of Δ^8-THC (**9**) in which the conformation of the side chain was restricted by incorporating the first one or two carbons into a six-membered ring fused with the aromatic phenolic ring, results indicated that the "southbound" chain conformer retained the highest affinity for both receptors (Khanolkar et al. 1999). Papahatjis and co-workers (1998) published a study involving side chain-constrained analogs of Δ^8-THC, including a 3-(1-heptynyl) analog synthesized in a β-11-HHC (**10**) series and a potent 1′-dithiolane derivative. No analog had the side chain in a fully restricted conformation. However, the authors concluded from their binding data, and in particular the increased potency of the 1′-dithiolane and the 1′-methylene analogs, that a hydrophobic subsite of the CB pharmacophore exists in both CB_1 and CB_2 at the level of the benzylic side chain carbon. To study the



delta-8-THC

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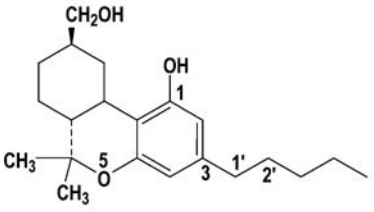

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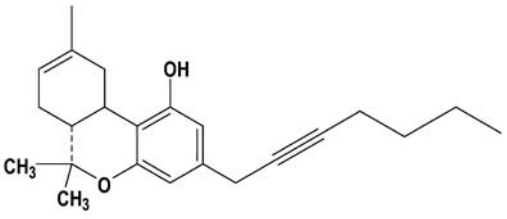

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stereochemical requirements of the side chain, Busch-Petersen (1996) synthesized a series of β-11-hydroxyhexahydrocannabinol (**10**) CBs in which rotation around the C1′-C2′ bond is blocked by the introduction of a double (*cis* or *trans*) or triple bond. All the analogs tested showed nanomolar affinity for the receptors, the *cis*-hept-1-ene side chain having the highest affinity for CB_1 ($K_i = 0.89$ nM) and showing the widest separation between CB_1 and CB_2 affinities (Busch-Petersen et al. 1996).

Razdan and co-workers have also pursued the effects of unsaturation or added functionality in the C-3 side chain of classical CBs. These investigators have found that manipulations of the side chain can produce high-affinity ligands with either antagonist, partial agonist, or full agonist effect. In particular, antagonists such as **11** were developed through strategic placement of a triple bond (Griffin et al. 1999; Martin et al. 1999; Ross et al. 1998, 1999b; Ryan et al. 1995; Singer et al. 1998). It is possible that the reason that this ligand functions as an antagonist is that such substitution reduces the flexibility of the side chain and leads to loss of efficacy.

Nadipuram and co-authors synthesized a series of C3 cyclic side-chain analogs of Δ^8-THC in which ring substituents were attached at the 1′ position. Substitution of a dithiolane ring at the 1′ position and a carbocyclic ring at the 1′ position led to compounds that retained very good affinity for CB_1 and CB_2, suggesting that the binding pocket for the classical CB side chain may be ellipsoidal rather than elongated (Nadipuram et al. 2003). Substitution of a phenyl ring at the C-1′ position along with a dithiolane ring at C-1′ or a 1′,1′-dimethyl group led to compounds with high affinities for both CB_1 and CB_2, while affinity was reduced when the phenyl ring was attached to a C-1′ CH2 or carbonyl group. The dimethyl and ketone analogs displayed selectivity for the CB_2 receptor (Krishnamurthy et al. 2003)

Pharmacophore for Classical CB Side Chain

Thomas and co-workers used the extensive side chain SAR generated by Razdan to develop a novel QSAR for the side chain region of Δ^8-THC (**9**) (Keimowitz et al. 2000). A series of 36 side chain-substituted Δ^8-THCs with a wide range of pharmacological potency and CB_1 receptor affinity was investigated using computational molecular modeling and QSAR analyses. The conformational mobility of each compound's side chain was characterized using a quenched molecular dynamics approach. The QSAR techniques included a modified active analog approach (MAA), multiple linear regression analyses (MLR), and CoMFA studies. Results obtained support the hypothesis that for optimum affinity and potency, the side chain must have conformational freedom that allows its terminus to fold back and come into proximity with the phenolic ring (Keimowitz et al. 2000). This result fits very well with those of Razdan and co-workers mentioned above who produced classical CB antagonists, such as **11**, by restricting the conformational freedom of the side chain (Griffin et al. 1999; Martin et al. 1999; Ross et al. 1998, 1999b; Ryan et al. 1995; Singer et al. 1998).

3.2 Ligand–Receptor Studies for Classical/Non-classical CB Binding to CB_1

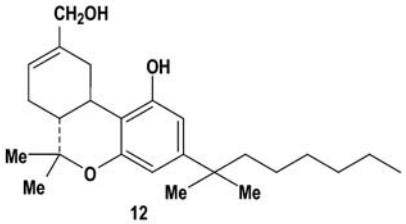
Shim and co-workers recently published a ligand–receptor study in which a molecular docking approach that combined Monte Carlo and molecular dynamics simulations was used to identify putative binding conformations of non-classical CB agonists, including AC-bicyclic CP-47,497 and CP-55,940 (**2**), and ACD-tricyclic CP-55,244 (Shim et al. 2003). These investigators used an inactive state model of CB_1 for these docking studies based upon the X-ray crystal structure of rhodopsin (Palczewski et al. 2000). Ligand placement was based upon the assumption of a critical hydrogen bond between the A-ring OH and the side chain N of Lys192 in transmembrane helix (TMH) 3. Two alternative binding conformations were considered, with a conformation in which the C-3 side chain pointed inside the receptor chosen as the binding site conformation for which the ligand could achieve more interactions. Key hydrogen bonds were identified between both K3.28(192) and E(258) and the A-ring OH (see **2**), and between Q(261) and the C-ring C-12 hydroxypropyl.

3.3 CB_2 Selective Classical/Non-classical CBs Break CB_1 SAR rules

In recent years, it has become clear that one way to develop CB_2-selective compounds is to violate long accepted pharmacophore requirements for binding to CB_1 (see consensus pharmacophore list above). In particular, CB_2-selective compounds have emerged through changes in the phenolic hydroxyl region and through shortening of the alkyl side chain.

3.3.1 Phenolic Hydroxyl

Huffman was first to show that removal of the phenolic hydroxyl of 11-hydroxy-Δ^8-tetrahydrocannabinol-1′, 1′-dimethylheptyl (HU-210, **12**) results in a CB_2-selective compound (**13**) with high affinity for both the CB_1 and CB_2 receptors (CB_1 $K_i = 1.2 \pm 0.1$ nM, CB_2 $K_i = 0.032 \pm 0.019$ nM) (Huffman et al. 1996). In addition, Gareau reported that the conversion of the C-1 phenolic hydroxyl of a classical CB to a methoxy group (i.e., etherification) also produced a CB_2-selective ligand (Gareau et al. 1996). In both of these studies, analogs possessed a longer side chain than natural CBs, a 1′, 1′-dimethylheptyl (DMH) side chain at C-3. Huffman and co-workers also showed that removal of the 11-hydroxy group of deoxy-HU-210 to produce deoxy-Δ^8-THC-DMH still resulted in a ligand with good CB_1 affinity (CB_1 $K_i = 23 \pm 7$ nM) and CB_2 selectivity (CB_2 $K_i = 2.9 \pm 1.6$ nM) (Huffman et al. 1996). O,2-propano-9β-OH-11-nor-HHC (**14**), a rigidified C-1 ether, has also been reported to have good CB_1 affinity ($K_i = 26 \pm 2$ nM) and a 4.5-fold CB_2 selectivity ($K_i = 5.8 \pm 2.9$ nM) (Reggio et al. 1997).



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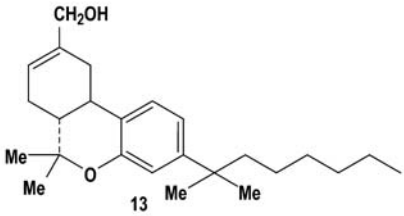

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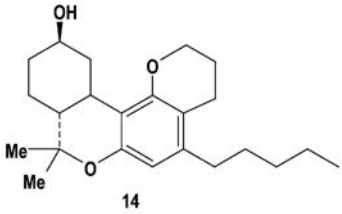

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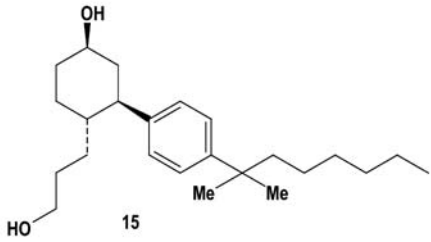

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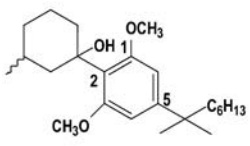
In the non-classical CBs, Melvin has also shown that the phenolic hydroxyl at C-2′ (see drawing of **2**) is not necessary as 2′-deoxy-CP-55,940 (**15**) possessed a $K_i = 40.2 \pm 13.5$ nM at the CB_1 receptor (Melvin et al. 1993). In this case, however, although CB_1 affinity is retained, the deoxy analog does have an attenuated affinity relative to that of CP-55,940 (**2**) whose CB_1 $K_i = 0.137 \pm 0.038$ nM. The affinity difference between 1-deoxy-11-hydroxy-Δ^8-THC-DMH (**13**) and 2′-deoxy-CP-55,940 (**15**) may be due to the greater entropic expense incurred by **15** upon binding, as it is a more flexible molecule.

Razdan and co-workers (Wiley et al. 2002) recently reported a series of resorcinol derivatives that exhibited varying affinities for CB_1 and CB_2. When the free phenols at C1 and C3 in this series (with DMH side chains) were etherified, CB_2-selective compounds resulted [e.g., O-1966A (**16**), CB_1 $K_i = 5{,}055 \pm 984$ nM; CB_2 $K_i = 23 \pm 2.1$ nM]. These results are consistent with results reported by Mechoulam and co-workers (Hanus et al. 1999) for HU-308 (**17**), which also has etherification at the same positions as O-1966A and is highly CB_2-selective (CB_1 K_i>10μM; CB_2 $K_i = 22.7 \pm 3.9$ nM).



O-1966A

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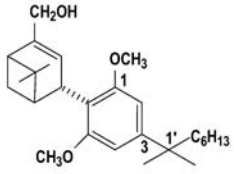

HU-308

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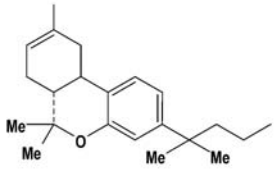

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3.3.2 Side Chain

In traditional CB SAR, a 1′, 1′-dimethylheptyl C-3 side chain has been a "magic bullet," improving the CB_1 affinity and efficacy of nearly every molecule to which it has been attached (Razdan 1986). In a series of papers, Huffman and co-workers have shown that the CB_2 receptor clearly can accommodate shorter alkyl side chains than can CB_1. This group reported that 1-deoxy-3(1′-1′-dimethylbutyl)-Δ^8-THC (**18**) had high CB_2 affinity [$K_i = 3.4 \pm 1.0$ nM], but 200-fold lower affinity for CB_1 ($K_i = 677 \pm 132$ nM) (Huffman et al. 1998, 1999). 1-deoxy-3(n-butyl)-Δ^8-THC (CB_1 $K_i = 2791 \pm 820$ nM; CB_2 $K_i = 53.8 \pm 8.0$ nM) and 1-deoxy-Δ^8-THC (CB_1 K_i>10,000 nM; CB_2 $K_i = 31.6 \pm 8.7$ nM) were also CB_2-selective. 1-deoxy-Δ^8-THCs with 1′, 1′-dimethyl-pentyl and hexyl side chains at C-3 bound weakly to CB_1 ($K_i = 338 \pm 76$ nM, $K_i = 295 \pm 52$ nM), but maintained high CB_2 affinity (CB_2 $K_i = 10 \pm 2$ nM to CB_2 $K_i = 19 \pm 4$ nM) with the octyl and nonyl analogs showing lower affinity (Huffman et al. 1998, 1999).

More recently, this group prepared three series of new CBs: 1-methoxy-3-(1′,1′-dimethylalkyl)-Δ^8-tetrahydrocannabinol, 1-deoxy-11-hydroxy-3-(1′,1′-dimethylalkyl)-Δ^8-tetrahydrocannabinol and 11-hydroxy-1-methoxy-3-(1′,1′-dimethylalkyl)-Δ^8-tetrahydrocannabinol, which contain alkyl chains from dimethylethyl to dimethylheptyl appended to C-3 of the CB. All of these compounds had greater affinity for the CB_2 receptor than for the CB_1 receptor; however, only 1-methoxy-3-(1′,1′-dimethylhexyl)-Δ^8-THC has effectively no affinity for the CB_1 receptor ($K_i = 3134 \pm 110$ nM) and high affinity for CB_2 ($K_i = 18 \pm 2$ nM) (Huffman et al. 2002).

4 Endogenous CB Pharmacophores

The arachidonic acid (AA) moiety in anandamide and congeners confers the molecule with what could be called "dynamic plasticity." The arachidonic acid acyl chain contains four homoallylic double bonds (i.e., *cis* double bonds separated by methylene carbons). Rabinovich and Ripatti (1991) reported that polyunsaturated acyl chains in which double bonds are separated by one methylene group are characterized by the highest equilibrium flexibility compared with other unsaturated acyl chains. Rich (1993) reports that a broad domain of low-energy conformational freedom exists for these C–C bonds. Results of the Biased Sampling phase from Conformational Memories calculations of arachidonic acid are consistent with Rich's and with Rabinovich and Ripatti's results (Barnett-Norris et al. 1998), as they revealed a relatively broad distribution of populated torsional space about the classic skew angles of 119°(s) and –119°(s′) for the C8-C9-C10-C11 torsion angle in anandamide, for example (see **4** for numbering system).

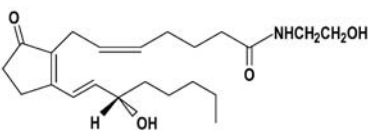
4.1 Endocannabinoid SAR

4.1.1 Acyl Chain SAR

While the fatty acid literature indicates that unsaturated fatty acids that possess multiple homoallylic double bonds, such as AA, exhibit a high degree of flexibility, this literature also indicates that saturated fatty acids tend to be significantly less flexible and adopt primarily extended conformations. Fatty acids with decreasing amounts of unsaturation tend to show a decreasing tendency to form folded structures, but still tend to curve in acyl chain regions in which unsaturation is present (Reggio and Traore 2000). A correlation has been drawn between this acyl chain conformation trend and the SAR of the anandamide (AEA) acyl chain (Barnett-Norris et al. 2002b). Endocannabinoid SAR indicates that the CB_1 receptor recognizes ethanolamides whose fatty acid acyl chains have 20 or 22 carbons, with at least three homoallylic double bonds and saturation in at least the last five carbons of the acyl chain (Sheskin et al. 1997). Reggio and co-workers have suggested that this acyl chain unsaturation SAR requirement is an outgrowth of the shape of the AEA binding pocket at CB_1 which may require tightly folded conformations, conformations not possible for AEA analogs with less than three homoallylic double bonds (Barnett-Norris et al. 2002b).

An analogy has been drawn in the literature between the C16–C20 portion of AEA (see **4**) and the C-3 pentyl side chain of the classical CB, Δ^9-THC (see **1**). Consistent with this hypothesis, replacement of the pentyl tail of AEA with a dimethylheptyl chain results in enhanced affinity (although not to the same degree as seen in the classical CBs) (Ryan et al. 1997; Seltzman et al. 1997).

Although initially it seemed that the development of rigid anandamide analogs that mimic the AA conformations discussed above would help to identify the receptor-appropriate conformation of AEA, all attempts at rigidifying AEA have



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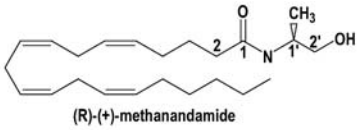
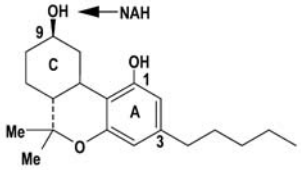
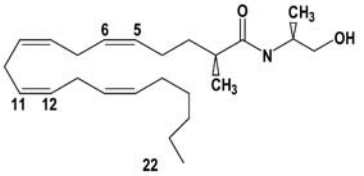


(R)-(+)-methanandamide
20



9-nor-9β–OH-HHC
21



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been met with little success (Berglund et al. 1999, 2000; Pinto et al. 1994) Pinto and co-workers investigated a series of arachidonyl amides and esters in addition to a series of "rigid hairpin" conformations typified by *N*-(2-hydroxyethyl)-prostaglandin amides to determine the structural requirements for binding to the CB_1 receptor. 2D drawings of anandamide and PGB_2-EA (**19**) make the shapes of these two compounds look similar. However, all of the rigid prostaglandin analogs synthesized by Pinto et al. (1994) failed to alter [^{3}H]CP-55,940 binding to CB_1 in concentrations as great as 100 μM. Barnett-Norris and co-workers 1998) reported conformational memories (CM) results for PGB_2-EA (**19**) which showed an attenuated ability for the prostaglandin ethanolamide to adopt extended conformations or to form U-shaped conformations like AEA and 2-AG. Instead, the CM results showed that the conjugation of the acyl chain with the ring double bond introduces "stiffness" into this part of the molecule, resulting in a predominantly folded L-shaped conformation.

4.1.2 Head Group SAR

In order for high-affinity binding to the CB_1 receptor to occur and for agonist binding to activate G proteins, the carbonyl group of the AEA amide head group must be present (Berglund et al. 1998). Arachidonamide and simple alkyl esters of arachidonic acid did not show significant CB_1 affinity (Pinto et al. 1994). Cyclization of the head group into an oxazoline ring diminished affinity (Lin et al. 1998). Arachidonylethers, carbamates, and norarachidonlycarbamates had poor CB_1 affinity (Ng et al. 1999). However, norarachidonyl ureas showed generally good binding affinities for the CB_1 receptor (K_i = 55–746 nM). Some of the weaker affinity analogs in this series produced potent pharmacological activity. These analogs showed hydrolytic stability toward amidase enzymes as well (Ng et al. 1999).

Methylation at the C-1′ position in the AEA (see **4** for numbering system) head group resulted in an 1′-*R*-methyl isomer (*R*-methanandamide, **20**) which had fourfold higher CB_1 affinity than AEA, while the 1′-*S*-methyl isomer had two-fold lower CB_1 affinity than AEA. *R*-Methanandamide (**20**) also was found to be resistant to enzymatic breakdown (Abadji et al. 1994). Methylation at the 2′ position also produced some stereoselectivity, as the *S*(+) isomer was found to have twofold to fivefold higher CB_1 affinity than the *R*(–)-isomer (Abadji et al. 1994; Berglund et al. 1998). Introduction of larger alkyl groups had a detrimental effect on CB_1 affinity (Adams et al. 1995a). A series of C1′-C2 dimethyl anandamide analogs revealed stereochemical requirements of the CB_1 binding pocket, as only the *R*,*R* isomer, (*R*)-N-(1-methyl-2-hydroxyethyl)-2-(*R*)-methyl-arachidonamide, had significant affinity for CB_1 ($K_i = 7.42 \pm 0.86$ nM) (Goutopoulos et al. 2001).

Enlargement of the ethanolamine head group by insertion of methylene groups revealed that the N-propanol analog had slightly higher CB_1 affinity than AEA, while higher homologs had reduced CB_1 affinity (Pinto et al. 1994; Sheskin et al. 1997). Alkyl branching of the alcoholic head group led to lower affinity analogs (Sheskin et al. 1997). N-(Propyl) arachidonylamide possessed higher CB_1 affinity

(K_i = 7.3 nM) than anandamide itself (K_i = 22 nM) (Pinto et al. 1994; Sheskin et al. 1997). Substitution of an N-cyclopropyl group for the ethanolamine head group of AEA led to a very high CB_1-affinity compound (Hillard et al. 1999). These results suggest that there may exist a hydrophobic sub-site for the AEA head group such that the hydroxyl of AEA may not be necessary for receptor interaction (Lin et al. 1998). Replacement of the hydroxyl group of AEA with a halogen such as F or CI increased CB_1 affinity as well (Adams et al. 1995b; Hillard et al. 1999; Lin et al. 1998). Substitution of the 2-hydroxyethyl group of AEA with a phenolic group, however, greatly decreased affinity for CB_1 (Edgemond et al. 1995, 1998; Khanolkar et al. 1996; Lang et al. 1999).

Taken together, all of these results suggest that the hydroxyl in the anandamide head group is not essential for receptor interaction, but that the CB receptor can accommodate both hydrophobic and hydrophilic head groups, possibly in two different subsites. The size(s) of the cavity(ies) in which the head group binds, however, is (are) small as only relatively small variations on the head group permit the retention of high-affinity binding.

4.2 Ligand–Ligand Studies of Endocannabinoids

CoMFA models for endocannabinoids have been developed using rigid classical CBs as templates. However, the inherent flexibility of the arachidonic acyl chain of anandamide has been an obstacle to the identification of an unambiguous overlay needed for COMFA studies.

Thomas et al. were first to report a CoMFA QSAR pharmacophore model for anandamide (**4**) and its analogs (Thomas et al. 1996). These authors used molecular dynamics studies to explore conformations of **4** that present pharmacophoric similarities with Δ^9-THC (**1**). A J-shaped or looped conformation of **4** was identified that had good molecular volume overlap with **1** when (1) the carboxyamide of **4** was overlaid with the pyran oxygen (O-5) in **1**; (2) the head group hydroxyl of **4** was overlaid with the C-1 phenolic hydroxyl group of **1**, (3) the five terminal carbons of the **4** fatty acid acyl chain were overlaid with the C-3 pentyl side chain of **1**; and (4) the polyolefin loop of **4** was overlaid with the tricyclic ring system of **1**. These authors supported their use of a J-shaped conformation for **4** by citing synthetic results for the internal epoxidation undergone by peroxyarachidonic acid, which point to the J shape as necessary for such a reaction (Corey et al. 1979).

Tong and co-workers reported a pharmacophore model for anandamide (**4**) using constrained conformational searching and CoMFA (Tong et al. 1998) and a different alignment between key elements of the pharmacophore. 9-nor-9β-OH-HHC (**21**) was used as the template to which **4** and its analogs were fitted. The training set for the CoMFA model contained 29 classical and non-classical CBs. The conformation identified for **4** was a helical conformation in which (1) the oxygen of the carboxyamide overlaid the C-1 phenolic hydroxyl group of **21**; (2) the head group hydroxyl overlaid the C-9 hydroxyl of **21**; (3) the alkyl tail of **4** overlaid the C-3 alkyl side chain of **21**, and (4) the polyolefin loop overlaid the

tricyclic ring structure of **21**. These authors supported their use of a helix-shaped **4** by citing a recent X-ray crystallographic structure that shows that arachidonic acid adopts a helical conformation when it is a substrate for cyclooxygenase (Stegeman et al. 1998).

4.2.1 Tests of CoMFA Models

Howlett and co-workers (Berglund et al. 2000) used the Tong pharmacophore (Tong et al. 1998) to design a series of monocyclic and bicyclic alkyl amides. The bend in the U-shaped conformation of AEA was approximated with incorporation of a phenyl or naphthyl ring, and the importance of a flat ring was tested by incorporation of a cyclohexyl ring. Aspects of the Tong pharmacophore that were reported to be important (i.e., the alkyl tail and carbonyl of the amide) were included or excluded in the series. Highest affinity was associated with phenyl analogs, and among these analogs, meta substitution on the phenyl ring yielded the highest affinity compounds, presumably because it places the amide group and the alkyl side chain at the best distance. Using the pharmacophoric elements proposed to be important in the Tong model, the investigators calculated the distances between the pharmacophoric elements [carbocyclic ring (ring C)-hydroxyl, phenolic hydroxyl and C-3 alkyl side chain] of a series of high-affinity, moderate-affinity and low-affinity analogs relative to these distances in the high-affinity non-classical CB, CP-55,244. However, the authors found it difficult to establish a clear relationship between relative binding affinities and their corresponding pharmacophoric distances due to the high flexibility of the compounds.

Van der Steldt and co-workers (van der Stelt et al. 2002) evaluated a series of anandamide and 2-AG lipoxygenase products for their CB_1 and CB_2 affinities, as well as their ability to inhibit AEA hydrolysis at the FAAH enzyme and to inhibit AEA transport. Several of these have previously been reported by Hillard and co-workers (Edgemond et al. 1998). Conformational analysis was performed using nuclear magnetic resonance (NMR) solution studies, as well as molecular dynamics calculations. Conformational analysis results for the hydroxylated AEAs were probed for consistency of placement of the key pharmacophoric elements identified by Tong and co-workers (Tong et al. 1998) vs a CP-55,940 template. However, the overlapping regions of CP-55,940 and the hydroxylated-AEA series did not reveal great differences between analogs with high or low CB_1 affinities. Taken together, the Howlett (Berglund et al. 2000) and van der Stelt et al. (2002) studies illustrate the limited utility of a CoMFA pharmacophore model based on structural superpositions of AEA analogs with a classical CB template (Tong et al. 1998).

4.3 Ligand–Receptor Modeling Studies of Endocannabinoid Binding

Endocannabinoid SAR indicates that the CB_1 receptor recognizes ethanolamides whose fatty acid acyl chains have 20 or 22 carbons, with at least three homoallylic

double bonds and saturation in at least the last five carbons of the acyl chain (Reggio and Traore 2000). Endocannabinoid SAR also indicates that the CB_1 receptor does not tolerate large endocannabinoid head groups; however, it does recognize both polar and non-polar moieties in the head group region (Reggio and Traore 2000). Reggio and co-workers (Barnett-Norris et al. 1998, 2002b) have taken a different approach to the development of an endocannabinoid pharmacophore by focusing on sets of AEA analogs with variation in one region of the molecule at a time and by using the conformational memories (CM) method. CM is a Monte Carlo/simulated annealing based approach (Guarnieri and Weinstein 1996) that generates 100 low free energy structures of each compound at 310 K. In adopting this approach, all possible endocannabinoid conformations can be considered, rather than considering a smaller region of conformational space as is necessitated by working hypotheses of required overlap of key regions with a rigid template (see the CoMFA studies discussed above) (Thomas et al. 1996; Tong et al. 1998).

In order to probe the molecular basis for these acyl chain requirements, Barnett-Norris and co-workers used the CM method to study the conformations available to an n-6 series of ethanolamide fatty acid acyl chain congeners, 22:4,n-6 ($K_i = 34.4 \pm 3.2$ nM), 20:4,n-6 ($K_i = 39.2 \pm 5.7$ nM), 20:3,n-6 ($K_i = 53.4 \pm 5.5$ nM); and 20:2,n-6 (K_i>1500 nM) (Sheskin et al. 1997). CM studies indicated that each analog could form both U/J-shaped (Cls 1) and extended (Cls 2) families of conformers. However, for the low-affinity 20:2,n-6 ethanolamide, the higher populated family was the extended conformer family, while for the other analogs in the series, the U/J-shaped family had the higher population. In addition, the 20:2,n-6 ethanolamide U-shaped family was not as tightly curved as were those of the other analogs studied. In order to quantitate this variation in curvature, the radius of curvature (in the C-3 to C-17 region) of each member of each U/J-shaped family was measured. The average radii of curvature (with their 95% confidence intervals) were found to be 5.8 Å (5.3–6.2) for 20:2,n-6; 4.4 Å (4.1–4.7) for 20:3,n-6; 4.0 Å (3.7–4.2) for 20:4,n-6; and 4.0 Å (3.6–4.5) for 22:4,n-6. These results suggest that higher CB_1 affinity is associated with endocannabinoids that can form tightly curved structures.

In order to identify a head group orientation that results in high CB_1 affinity, Barnett-Norris and co-workers studied a series of dimethyl anandamide analogs (*R*)-*N*-(1-methyl-2-hydroxyethyl)-2-(*R*)-methyl-arachidonamide ($K_i = 7.42 \pm 0.86$ nM; **22**), (*R*)-*N*-(1-methyl-2-hydroxyethyl)-2-(*S*)-methyl-arachidonamide ($K_i = 185 \pm 12$ nM), (*S*)-*N*-(1-methyl-2-hydroxyethyl)-2-(*S*)-methyl-arachidonamide ($K_i = 389 \pm 72$ nM), and (*S*)-*N*-(1-methyl-2-hydroxyethyl)-2-(*R*)-methyl-arachidonamide ($K_i = 233 \pm 69$ nM) (Goutopoulos et al. 2001) using CM and computer receptor docking studies in an active state (R*) model of CB_1 (Barnett-Norris et al. 2002b). These studies suggested that the high CB_1 affinity of the *R*,*R* stereoisomer (**22**) is due to the ability of the head group to form an intramolecular hydrogen bond between the carboxamide oxygen and the head group hydroxyl that orients the C2 and C1′ methyl groups to have hydrophobic interactions with valine 3.32(196), while the carboxamide oxygen forms a hydrogen bond with lysine 3.28(192) at CB_1. In this position in the CB_1 binding pocket, F2.57(170) and F3.25(189) have C-Hπ interactions with the C5–C6 and C11–C12 acyl chain double

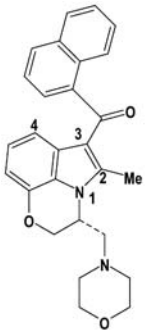
bonds, respectively. This binding site is supported by: NMR solution studies of AEA that have shown the persistence of this same AEA headgroup intramolecular hydrogen bond (Bonechi et al. 2001); CB_1 K3.28A mutation studies that show that K3.28 is critical for the binding of AEA at CB_1 (Song and Bonner 1996); and recent CB_1 F3.25A mutation studies that suggest that F3.25 is an interaction site for AEA at CB_1 (McAllister et al. 2003). Taken together, these studies suggest that anandamide and its congeners must adopt tightly curved U/J-shaped conformations at CB_1, and suggest that the TMH 2–3–6–7 region is the endocannabinoid-binding region at CB_1.

Finally, it is important to mention that the binding site model proposed by Reggio and co-workers (Barnett-Norris et al. 2002b) does not address one last aspect of endocannabinoid acyl chain SAR, the requirement for an acyl chain of 20–22 carbons. These investigators have hypothesized that this length requirement originates not from the requirements of the final binding site itself, but from requirements for endocannabinoid entry into the binding pocket from lipid. Recently, this group showed that alkyl tail interaction with V6.43(351)/I6.46(354) (which form a groove on CB_1 TMH 6 into which an alkyl tail can fit) results in the induction of an active state conformation for TMH 6 (Barnett-Norris et al. 2002a). Simulations of TMH 6/endocannabinoid interaction in a lipid environment are currently underway in this laboratory to test the hypothesis that only endocannabinoids with 20 to 22 carbon acyl chains (with at least 3 homoallylic double bonds and at least 5 saturated carbons at their ends) extend to the proper depth in the lipid membrane to access the V6.43/I6.46 groove.

5
Aminoalkylindole Pharmacophores

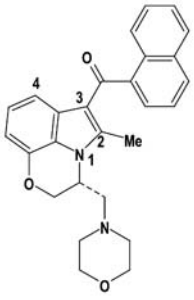
Of all CB agonists, the aminoalkylindoles (AAIs) are the most structurally dissimilar to the classical CBs. This class of compounds also has been found to differ significantly in the set of amino acids important for its binding as revealed by mutation studies (Chin et al. 1998; McAllister et al. 2003; Song and Bonner 1996). It is no wonder, then, that attempts to construct pharmacophores that include WIN55,212-2 in structural superpositions with classical CB agonists have led to the greatest ambiguity.

Adding another layer of complexity to the use of structural superpositions is the fact that the AAIs, as typified by WIN55,212-2 are not rigid compounds, but can adopt several low-energy conformations. AM1 conformational analysis revealed two general classes of accessible conformers at biological temperature, s-*cis* and s-*trans* conformations (see drawings **23** and **24**) for a 2D representation of these conformers (Reggio et al. 1998). This leads to the question: What is the bioactive conformation of the AAIs at CB receptors? It is clear in **23** and **24** that the s-*cis* vs the s-*trans* conformations of WIN55,212-2 place their naphthyl rings in very different regions of space and that these conformers will differ in surface area accessible for intermolecular interactions. As will become more evident in the discussion which follows, the existence of both s-*cis* and s-*trans* conformers of WIN55,212-2 also permits more than one superposition upon a classical CB template.



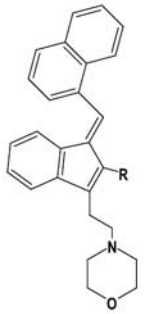
s-cis

23



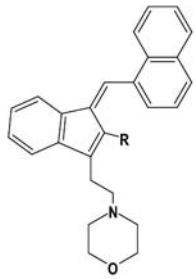
s-trans

24



Z-naphthylidene indene

25



E-naphthylidene indene

26

One way to resolve the ambiguity concerning the bioactive conformation of the AAIs is by the development of rigid AAI analogs. The Z,E-naphthylidene indene AAI analogs (**25**, **26**) synthesized as a mixture by Kumar are rigidified compounds that lack the carbonyl oxygen of the AAIs, but still exhibit high CB_1 affinity (Kumar et al. 1995). Reggio and co-workers extended the work of Kumar by synthesizing each naphthylidene indene geometric isomer. AM1 conformational analysis revealed that the indene E-isomer (**26**) mimics the s-*trans* conformation of WIN55,212-2, while the Z isomer (**25**) mimics the s-*cis* conformation (Reggio et al. 1998). CB_1/CB_2 binding assays revealed that the E-naphthylidene indene (**26**) has significantly higher CB_1 and CB_2 affinity (R = H CB_1 $K_i = 2.72 \pm 0.22$ nM, CB_2 $K_i = 2.72 \pm 0.32$ nM; R = Me CB_1 $K_i = 2.89 \pm 0.41$ nM, CB_2 $K_i = 2.05 \pm 0.22$ nM) than the corresponding Z isomer (**25**) (R = H CB_1 $K_i = 148 \pm 29$ nM, CB_2 $K_i = 132.0 \pm 45.6$ nM; R = Me CB_1 $K_i = 1945 \pm 94$ nM, CB_2 $K_i = 658 \pm 206$ nM) (Reggio et al. 1998). These results point to the s-*trans* AAI conformer (corresponds to the E-indene) as the AAI bioactive conformation at the CB_1 and CB_2 receptors. Detailed below are pharmacophores that have been developed for the AAIs.

5.1 Ligand–Ligand Studies of the Aminoalkylindoles and Related Compounds

Eissenstat and co-workers were first to develop a pharmacophore for AAI binding at CB_1. These investigators presented two pharmacophoric models developed using mouse vas deferens (MVD) data (Eissenstat et al. 1995). The first model was an independent pharmacophore with three key structural features (see compound **3** for numbering system): (1) the nitrogen atom in the amino alkyl side chain; (2) the C-3-aroyl ring, represented by a dummy atom placed at its centroid; and (3) a heterocyclic nucleus represented by a dummy atom placed at the end of a 3-Å normal passing through its centroid. No AAI agonists were identified that did not conform to these pharmacophoric requirements; but not every molecule that fit the pharmacophore was active in the MVD assay. A second approach taken by

Eissenstat et al. involved the potential commonality between AAIs and classical CBs (Eissenstat et al. 1995). The amine of the AAIs was considered to mimic the C-1 phenolic hydroxyl of classical CBs—both engaging in a hydrogen bond with the receptor. Furthermore, these investigators proposed that the amine of the AAIs is similar to the amide functionality of anandamide. They also equated the AAI indole ring with the dibenzopyran ring of classical CBs and the naphthyl ring of the AAIs with the C-3 alkyl side chain of classical CBs.

Shim et al. (1998) reported two CoMFA models for AAI interaction at the CB_1 receptor based on pK_i values measured using radioligand binding assays for [^{3}H]CP-55,940 and [^{3}H]WIN55,212-2. Both models exhibited a strong correlation between the calculated steric-electrostatic fields and the observed biological activity for the respective training set compounds. CoMFA models with AAIs protonated at the morpholino nitrogen were also developed. Comparison of the statistical parameters resulting from these CoMFA models, however, failed to provide unequivocal evidence as to whether the AAIs are protonated or neutral as receptor-bound species. When experimental pK_i values for the training set compounds to displace [^{3}H]WIN55,212-2 were plotted against pK_i values predicted for the same compounds to displace [^{3}H]CP-55,940, the correlation was moderately strong ($R^2 = 0.73$). These authors found that the variation in binding affinity among AAIs was dominated by steric interactions at the receptor site. For CoMFA model 2, the presence of a lipophilic aroyl group promoted increased binding inside a presumed large hydrophobic pocket within the receptor cavity. A sterically forbidden region surrounded C2′ and C3′ of the naphthyl moiety. A region of enhanced binding was found surrounding the C4′ of the naphthyl moiety in 4′-substituted AAIs. Although these CoMFA models were developed using only AAIs, these investigators propose that the CB_1 receptor binding sites of the classical CBs and AAIs may partly overlap and that the two distinct classes of compounds share some common structural features to allow association with the CB_1 receptor (Shim et al. 1998).

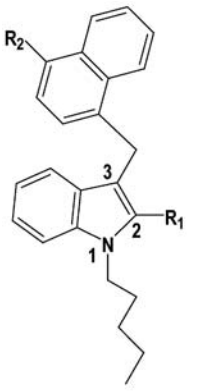
Xie and co-workers used a combination of NMR solution studies and molecular modeling to study WIN55,212-2 (**3**; see numbering system). Their results suggest that the minimum energy conformations of the WIN55,212-2 have distinct pharmacophoric features: (1) the naphthyl ring is oriented off the plane of the benzoxazine ring by approximately 59 degrees with the carbonyl C = O group pointing toward the C-2 methyl group, and (2) at the C10-position, the axial morpholinomethyl conformation is preferred over the equatorial in order to relieve a steric interaction with the C-2 methyl group. The preferred conformer as defined by the three key pharmacophores, naphthyl, morpholino, and 3-keto groups, shows that the morpholinyl ring of the molecule WIN55,212-2 deviates from the plane of the benzoxazine ring by about 32 degrees and orients in the left molecular quadrant. This model supports the hypothesis that a certain deviation of the morpholino group from the plane of the indole ring in WIN55,212-2 is essential for cannabimimetic activity. These authors have postulated that such an alignment by the respective pharmacophores allows them to interact optimally with the receptor (Xie et al. 1999).

Dutta and co-workers (1997) reported results for 4-alkyloxy indole AAI derivatives. These investigators aligned the naphthoyl group of WIN55,212-2 (**3**) with the

C-3 side chain of Δ^9-THC (**1**) and the morpholino group of **3** with the carbocyclic ring system of **1**. A similar alignment was suggested by Xie et al. (1995) and by Eissenstat and co-workers (1995; see above). The C-2 methyl group of **3** was aligned with the phenolic hydroxyl of **1**. Because of the conformational flexibility of **3**, these investigators studied the conformational energy of **3** as a function of volume difference with **1**. No specific AAI conformation that could be superimposed with **1** was found to be preferable. Because no specific AAI conformation could be identified as best to overlay with **1**, these investigators proposed that a unique AAI pharmacophore may need to be developed. In addition, Dutta et al. proposed that the keto group of the AAIs may be important for interaction with the CB_1 receptor (Dutta et al. 1997).

In their initial studies of the AAIs, Huffman and co-workers aligned the carbonyl oxygen of WIN55,212-2 with the phenolic hydroxyl of Δ^9-THC (**1**); the naphthyl moiety, with the cyclohexyl and pyran ring of **1**; and the indole nitrogen and the substituent extending from it with C-3 and its alkyl tail in **1** (Huffman et al. 1994). Huffman demonstrated that elimination of the AAI naphthyl substituent led to inactive compounds that failed to bind to the CB_1 receptor (Huffman et al. 1994). Huffman also showed that the morpholino ring can be replaced by an alkyl side chain and retain good CB_1 affinity (Huffman et al. 1994).

On the basis of this initial classical CB/AAI alignment, Huffman and co-workers have synthesized a series of indole- and pyrrole-derived CBs in which the morpholinoethyl group was replaced with another cyclic structure or with a carbon chain that more directly corresponded to the side chain of Δ^9-THC (**1**). Receptor affinity and potency of these novel CBs were related to the length of the carbon chain. Short side chains resulted in inactive compounds, whereas chains with four to six carbons produced optimal in vitro and in vivo activity. Pyrrole-derived CBs were consistently less potent than were the corresponding indole derivatives. These results suggest that, whereas the site of the morpholinoethyl group in these CBs seems crucial for attachment to CB_1 receptors, the exact structural constraints on this part of the molecule are not as strict as previously thought (Wiley et al. 1998).



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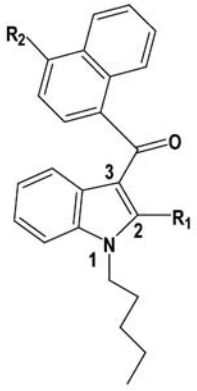

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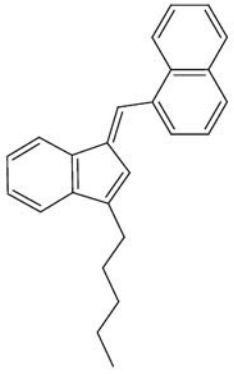

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Most recently, Huffman synthesized a series of 1-pentyl-1*H*-indol-3-yl-(1-naphthyl)methanes and 2-methyl-1-pentyl-1*H*-indol-3-yl-(1-naphthyl)methanes to investigate the hypothesis that cannabimimetic 3-(1-naphthoyl)indoles interact with the CB_1 receptor by hydrogen bonding to the carbonyl group. Indoles (**27**) for which $R_1 = H$ and $R_2 = H$, CH_3, or OCH_3 were to found have significant (CB_1 $K_i = 17–23$ nM) receptor affinity, somewhat less than that of the corresponding naphthoylindoles (**28**; $R_1 = H$, $R_2 = H$, CH_3, or OCH_3). A cannabimimetic E-indene hydrocarbon (**29**), which lacks any hydrogen bonding capability, was synthesized and found to have a CB_1 $K_i = 26 \pm 4$ nM. These results suggest that hydrogen bonding of the AAI carbonyl to CB_1 is not crucial for binding.

5.2 Ligand–Receptor Studies of Aminoalkylindole Binding

Reggio and co-workers constructed a pharmacophore for AAI binding at CB_1/CB_2 based on their earlier experimental work that suggested that the s-*trans* conformation of WIN55,212-2 was its bioactive conformation (Reggio et al. 1998). Their work suggested that aromatic stacking was the primary interaction for AAIs at CB_1 and that the aromatic residue-rich TMH 3–4–5–6 region in CB_1 and CB_2 constitutes the binding pocket for AAIs at the CB receptors (Song et al. 1999). These investigators used their CB_1 and CB_2 receptor models to identify F5.46 in CB_2 (a residue that is aromatic only in CB_2) as the residue responsible for the higher affinity of WIN55,212-2 for CB_2. This prediction was confirmed by mutation studies (Song et al. 1999).

Support for the TMH 3–4–5–6 region as the AAI binding region has come from Shire and colleagues' mutation/chimera studies of the CB receptors that suggest that the TMH 4-E-2 loop–TMH 5 region of the CB receptors contains residues important to the binding of the AAI, WIN55,212-2 (Shire et al. 1999). Subsequent modeling studies in the Reggio lab of the CB_1 R* (active state) identified direct stacking interactions between WIN55,212-2 and F3.36, W5.43 and W6.48, with W4.64 and Y5.39 forming part of the extended ligand–CB_1 aromatic cluster. Results of CB_1 F3.36A, W5.43A, and W6.48A mutation studies were consistent with this binding site model (McAllister et al. 2003). A recent modeling study reported by Salo and co-workers identified aromatic stacking interactions of WIN55,212-2 with F3.36, Y5.39, and W5.43 (Salo et al. 2004).

Molecular modeling and receptor docking studies of naphthoylindole (**28**; $R_1 = H$, $R_2 = OCH_3$) and its 2-methyl congener (**28**; $R_1 = CH_3$, $R_2 = OCH_3$) vs indolyl-1-naphthylmethanes (**27**; $R_1 = H$, $R_2 = OCH_3$) and (**27**; $R_1 = CH_3$, $R_2 = OCH_3$), combined with the receptor affinities of these cannabimimetic indoles, strongly suggested that these CB receptor ligands bind primarily by aromatic stacking interactions in the TMH 3–4–5–6 region of the CB_1 receptor (Huffman et al. 2003).

In summary, there is a great divergence between pharmacophores established for AAI binding at CB_1. This divergence can be attributed to the use of different conformations of WIN55,212-2 in superpositions with classical or non-classical

CB templates and the identification of different AAI functional groups as key for interaction at CB_1. Importance in some pharmacophores has been placed on the morpholino nitrogen or the carbonyl oxygen. The fact that the morpholino ring can be replaced by an alkyl chain with no loss in CB_1 affinity (Huffman et al. 1994) and that the carbonyl group can be replaced with a non-hydrogen bonding isostere (i.e., the indenes) with little loss in CB_1 affinity (Huffman et al. 2003; Reggio et al. 1998) argues against the morpholino nitrogen or carbonyl oxygen serving as key interaction sites at CB_1. Instead, it appears that the aromatic rings are key to the receptor interactions of the AAIs at CB_1 (McAllister et al. 2003). Compound **29** ($K_i = 26 \pm 4$ nM) (Huffman et al. 2003) is a good example of this.

6 SR141716A Pharmacophores

SR141716A (**7**) has been shown to act as a competitive antagonist and inverse agonist in host cells transfected with exogenous CB_1 receptor, as well as in biological preparations endogenously expressing CB_1 (Bouaboula et al. 1997; Meschler et al. 2000; Pan et al. 1998). In some experiments, SR141716A has been found to be more potent in blocking the actions of CB_1 agonists than in eliciting inverse responses by itself (Sim-Selley et al. 2001).

6.1 Ligand–Ligand Studies of SR141716A

Thomas and co-workers (Thomas et al. 1998) developed an SAR for SR141716A (**6**) using a set of seven halogenated SR141716A analogs. They concluded that this SR141716A SAR was consistent with a pharmacophoric alignment in which the mono-chloro ring of **7** is overlaid with the C-3 alkyl side chain of Δ^9-THC (**1**); the pyrazole nitrogen of **7** is overlaid with the C-1 phenolic hydroxyl of **1**; and, the carbonyl oxygen of **7** is overlaid with the pyran oxygen (O-5) of **1**. In this superposition, the dichloro ring of SR141716A represents a region unique to SR141716A. This region was hypothesized to be the antagonist-conferring moiety of SR141716A.

More recently, Thomas and co-workers reported an SAR study of the aminopiperidine region (C-3 substituent) of SR141716 (Francisco et al. 2002). Structural modifications made in this study include the substitution of alkyl hydrazines, amines, and hydroxyalkylamines of varying lengths for the aminopiperidinyl moiety. In general, it was found that increasing the length and bulk of the C-3 substituent was associated with increased receptor affinity and efficacy (as measured in a guanosine 5′-triphosphate-γ-[^{35}S] assay). However, in most instances, receptor affinity and efficacy increases were no longer observed after a certain chain length was reached. A quantitative SAR study was carried out to characterize the pharmacophoric requirements of the aminopiperidine region. This model indicated that ligands that exceed 3 Å in length would have reduced potency and affinity with

respect to SR141716A and that substituents with a positive charge density in the aminopiperidine region would be predicted to possess increased pharmacological activity (Francisco et al. 2002).

Makriyannis and co-workers designed and synthesized a series of pyrazole derivatives to aid in the characterization of the CB receptor binding sites and also to serve as potentially useful pharmacological probes. Structural requirements for potent and selective brain cannabinoid CB_1 receptor antagonistic activity included (1) a para-substituted phenyl ring at the 5-position, (2) a carboxamido group at the 3-position, and (3) a 2,4-dichlorophenyl substituent at the 1-position of the pyrazole ring (Lan et al. 1999).

Razdan and co-workers synthesized and evaluated a series of SR141716A analogs that retained the central pyrazole structure of SR141716A with replacement of the 1-, 3-, 4-, and/or 5-substituents by alkyl side chains or other substituents known to impart potent agonist activity in traditional tricyclic CB compounds (Wiley et al. 2001). Although none of the analogs alone produced the profile of cannabimimetic effects seen with full agonists, several of the 3-substituent analogs with higher binding affinities showed partial agonism for one or more measures. Cannabimimetic activity was most noted when the 3-substituent of SR141716A was replaced with an alkyl amide or ketone group. None of the 3-substituted analogs produced antagonist effects when tested in combination with 3 mg/kg Δ^9-THC (**1**). In contrast, antagonism of Δ^9-THC's effects without accompanying agonist or partial agonist effects was observed with substitutions at positions 1, 4, and 5. These results suggest that the structural properties of 1- and 5-substituents are primarily responsible for the antagonist activity of SR141716A.

Shim and co-workers used the semi-empirical AM1 method to perform a conformational analysis of SR141716A in its unprotonated and protonated forms. Results from conformational analyses, superimposition models, and 3D-QSAR models suggested that the N1 aromatic ring moiety of SR141716A dominates the steric binding interaction with the receptor in much the same way as does the C3 alkyl side chain of CB agonists and the C-3 aroyl ring of the AAI agonists. Several of the conformers considered in this study were found to possess the proper spatial orientation and distinct electrostatic character to bind to the CB_1 receptor. The authors proposed that the unique region in space occupied by the C-5 aromatic ring of SR141716A might contribute to conferring antagonist activity and that the pyrazole C-3 substituent of SR141716A might contribute to conferring either neutral antagonist or inverse agonist activity, depending upon the interaction with the receptor (Shim et al. 2002).

Lambert and co-workers synthesized a set of 3-alkyl 5-arylimidazolidinediones (hydantoins) with affinity for the human cannabinoid CB_1 receptor (Kanyonyo et al. 1999; Ooms et al. 2002). At least three of these compounds were found to act as neutral antagonists. Using a set of selected compounds, experimental lipophilicity was measured by reversed-phase high-pressure liquid chromatography (RP-HPLC) and calculated by a fragmental method (CLOGP) and a conformation-dependent method (CLIP) based on the molecular lipophilicity potential. The CB agonist 9β-OH-HHC (**21**) was used as a template to which both polar and non-polar hydantoins were fit. For the polar hydantoins, optimal alignment with **21** was

achieved by matching the oxygen atom of the morpholino ring or hydroxy moiety with the northern aliphatic hydroxyl (NAH) of **21**, the oxygen atom of the carboxyl amide with the phenolic hydroxyl oxygen of **21**, and the pro-R phenyl ring with the side chain of **21**. For the non-polar hydantoins, two pairs of atoms were used for alignment with **21**: the hydantoin carbonyl oxygens and the oxygen atoms in the carbocyclic and aromatic rings of **21**. No discussion of the basis for the antagonist properties of these ligands was offered (Ooms et al. 2002).

6.2 Ligand–Receptor Models for SR141716A Binding

Reggio and co-workers recently used a combination of synthesis, mutation, electrophysiology, and modeling to identify a binding site for SR141716A at CB_1 (Hurst et al. 2002). A mutant thermodynamic cycle was used to show that K3.28 is a direct interaction site for SR141716A in the inactive state of CB_1. Modeling studies of the CB_1 inactive R state indicated that aromatic stacking interactions were also crucial for SR141716A binding. Direct stacking interactions were identified with F3.36, Y5.39, and W5.43, while W4.64 and W6.48 were part of the larger ligand/aromatic cluster. CB_1 F3.36A, W5.43A, and W6.48A mutation study results were found to be consistent with this binding site model (McAllister et al. 2003). Furthermore, these modeling studies suggested that at the SR141716A binding site, the interaction between the dichlorophenyl ring and F3.36, which in turn interacts with W6.48, helps to maintain the receptor in its inactive state.

A recent modeling study of CB_1 reported by Salo and co-workers identified aromatic stacking interactions for SR141716A in the same aromatic cluster region of CB_1, with direct aromatic stacking interactions identified between SR141716A and Y5.39 and W5.43 (Salo et al. 2004).

6.3 SAR of Other Recently Synthesized CB_1 Antagonists

Mussinu and co-workers (2003) recently reported a new series of rigid 1-aryl-1,4-dihydroindeno[1,2-c]pyrazole-3-carboxamides that are conformationally restricted analogs of SR141716A. These investigators found that conformational restriction resulted in markedly improved CB_2 affinity and selectivity. These compounds were not screened for agonism/antagonism.

Stoit and colleagues reported that benzocycloheptapyrazoles constitute a class of very potent CB_1 receptor antagonists in vitro (Stoit et al. 2002), while Mignani and co-workers have reported that diarylmethyleneazetidine compounds also act as CB_1 antagonists (Mignani et al. 2000). Ruiu and colleagues recently reported that the antagonist NESS0327 (*N*-piperidinyl-[8-chloro-1-(2,4-dichlorophenyl)-1,4,5,6 tetrahydrobenzo[6,7]cyclo-hepta [1,2-*c*]pyrazole-3-carboxamide] is more then 60,000-fold selective for CB_1 (Ruiu et al. 2003). Lange and co-workers reported a series of novel 3,4-diarylpyrazolines which elicited potent in vitro CB_1

antagonistic activities and in general exhibited high CB_1 vs CB_2 receptor subtype selectivities (Lange et al. 2004). The binding affinities of these new compounds were rationalized using the binding site model proposed by Hurst and co-workers (2002)

7 Conclusions

This chapter clearly shows that there has been great growth in our knowledge of the CB receptors and their ligands in the past decade. Pharmacophores have been developed for the CB_1 antagonist SR141716A and for every structural class of CB_1 agonist. Attempts at creating a unified pharmacophore for all CB_1 ligands have not met with success. This is likely because CB_1 ligands do not share a single binding site. CB_2-selective ligand development has been one of the major advances in the CB field in the past decade, and information about GPCR structure combined with mutation studies has permitted refinement of CB_1 and CB_2 receptor models.

Many future challenges for SAR and pharmacophore development still exist. No pharmacophores have yet been developed for agonist or antagonist recognition at CB_2. Another major frontier in modeling studies of CB receptors will be the elucidation of how the CB receptors are activated by agonists and how they are inactivated by inverse agonists. Methodologically, this will be a challenge because the timescale over which activation takes place [milliseconds for receptors with diffusible ligands (Ghanouni et al. 2001b)] is orders of magnitude longer than the timescales currently accessible computationally (nanoseconds). In addition, there is growing evidence that there may be more than one activated state for a GPCR, with the activated conformational state dependent on the agonist that induced it (Ghanouni et al. 2001a). This is very likely the situation in the CB field as well (Glass and Northup 1999) and could present us with the opportunity ultimately to develop highly selective CB ligands that couple through a specific G protein subtype.

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