
Structural Requirements for Cannabinoid Receptor Probes

G.A. Thakur · S.P. Nikas · C. Li · A. Makriyannis (✉)

Center for Drug Discovery, Department of Pharmaceutical Sciences,
University of Connecticut, Storrs CT, 06269, USA
makriyan@uconnvm.uconn.edu

1	Introduction	210
2	Classification of Cannabinoid Receptor Ligands	212
2.1	Classical Cannabinoids	212
2.1.1	SAR of Classical Cannabinoids	212
2.2	Non-classical Cannabinoids	216
2.3	CC/NCC Hybrid Cannabinoids	217
2.4	Aminoalkylindoles	218
2.4.1	SAR of Aminoalkylindoles	219
2.5	Diarylpyrazoles	223
2.5.1	SAR of Pyrazole Cannabinoid Receptor Antagonists	224
2.6	Endocannabinoids	226
2.6.1	SAR of Endocannabinoids	227
2.7	Other Cannabinergic Classes	231
3	Covalent Binding Probes	232
4	Enantioselective Cannabinergic Ligands	233
5	Present and Future	236
	References	237

Abstract The discovery and cloning of CB₁ and CB₂, the two known G_{i/o} protein-coupled cannabinoid receptors, as well as the isolation and characterization of two families of endogenous cannabinergic ligands represented by arachidonylethanolamide (anandamide) and 2-arachidonoylglycerol (2-AG), have opened new horizons in this newly discovered field of biology. Furthermore, a considerable number of cannabinoid analogs belonging to structurally diverse classes of compounds have been synthesized and tested, thus providing substantial information on the structural requirements for cannabinoid receptor recognition and activation. Experiments with site-directed mutated receptors and computer modeling studies have suggested that these diverse classes of ligands may interact with the receptors through different binding motifs. The information about the exact binding site may be obtained with the help of suitably designed molecular probes. These ligands either interact with the receptors in a reversible fashion (reversible probes) or alternatively attach at or near the receptor active site with the formation of covalent bonds (irreversible probes). This review focuses on structural require-

ments of cannabinoid receptor ligands and highlights their pharmacological and therapeutic potential.

Keywords Cannabinoid receptors · Cannabinoid receptor probes · Structure–activity relationships · Selectivity

1

Introduction

Marijuana (*Cannabis sativa*) is one of the oldest drugs of abuse with a strong social, legal, and medical controversy over its therapeutic utility. Its major psychoactive component, Δ^9 -tetrahydrocannabinol (Δ^9 -THC), was characterized and synthesized in 1964 and served as a prototype for the synthesis of numerous analogs as potential pharmacological agents (Gaoni and Mechoulam 1964). The next milestone in cannabinoid research was the discovery that cannabinoids produce most of their biochemical and pharmacological effects by interacting with CB₁ and CB₂, the two known G_{i/o} protein-coupled cannabinoid receptors (Devane et al. 1988; Gerard et al. 1990; Matsuda et al. 1990; Munro et al. 1993). CB₁ is found in the central nervous system (CNS) with high density in the cerebellum, hippocampus, and striatum (Gatley et al. 1998; Herkenham 1991, 1990; Mailleux et al. 1992; Matsuda et al. 1993). It is also found in a variety of other organs including the heart, vascular endothelium, vas deferens, testis (Breivogel and Childers 1998; Gerard et al. 1991), small intestine, sperm (Schuel et al. 1999), and uterus (Paria et al. 1998). Conversely, the CB₂ receptor appears to be associated exclusively with the immune system. It is found in the periphery of the spleen and other cells associated with immunochemical functions, but not in neurons in the brain (Munro et al. 1993), and is believed to have an immunomodulatory role. Recent data suggest the presence of a third cannabinoid-like receptor (Begg et al. 2003).

CB₁ and CB₂ share an overall homology of 44% and 68% in the transmembrane domains. The rat (Matsuda et al. 1990), mouse (Abood et al. 1997; Chakrabarti et al. 1995), and human CB₁ receptors (Gerard et al. 1990) have been cloned and show 97%–99% sequence identity across species, while the mouse CB₂ (Shire et al. 1996a,b) exhibits 82% sequence identity with the human clone (Munro et al. 1993). CB₁ and CB₂ share common signal transduction pathways, such as inhibition of adenylyl cyclase and stimulation of mitogen-activated protein kinase. However, unlike CB₁, CB₂ has not been shown to affect ion channels (Pertwee 1997).

The subsequent discovery of the endocannabinoids, arachidonoyl ethanolamine (anandamide) (Devane et al. 1992b; Hanus et al. 1993) and 2-arachidonoyl glycerol (2-AG) (Di Marzo 1998; Mechoulam et al. 1995; Stella et al. 1997) has led to a better understanding of the physiological and biochemical roles of the endocannabinoid system. 2-Arachidonoyl glyceryl ether, also known as noladin ether (Hanus et al. 2001), has been proposed as a representative of a third endocannabinoid class. However, noladin ether's pathway of formation has not been characterized and its occurrence in the normal brain has been questioned (Oka et al. 2003).

Extensive studies on the endocannabinoid system have revealed a number of cannabinergic proteins involved in the inactivation and biosynthesis of endocannabinoids. These include fatty acid amide hydrolase (FAAH) (Di Marzo et al. 1994; Gaetani et al. 2003; Piomelli et al. 1999), monoglyceride lipase (MAG) (Dinh et al. 2002), and the anandamide transporter (ANT) (Beltramo et al. 1997; Di Marzo et al. 1994; Fegley et al. 2004; Hillard et al. 1997). The above three proteins and the two cannabinoid receptors have received considerable attention and show great promise as potential targets for the development of novel medications for various conditions, including pain, immunosuppression, peripheral vascular disease, appetite enhancement or suppression, and motor disorders.

Although both CB₁ and CB₂ have been cloned and their primary sequences are known, their three-dimensional structures and the amino acid residues at the active sites which are involved in ligand recognition, binding, and activation have not been characterized. In the absence of any X-ray crystallographic and nuclear magnetic resonance (NMR) data, information about the structural requirements for ligand–receptor interactions is obtained with the help of suitably designed molecular probes (Khanolkar et al. 2000). These ligands either interact with the receptor in a reversible fashion or, alternatively, attach at or near the receptor active site with the formation of a covalent bond. Information related to ligand binding and receptor activation can also be obtained with the help of receptor mutants (McPartland and Glass 2003; Rhee et al. 2000) and computer modeling (Reggio 1999).

During the last decade, numerous ligands with high affinities and selectivity profiles for cannabinoid receptors (CB₁ and CB₂) evolved from rigorously pursued structure–activity relationship (SAR) studies (for recent reviews see Goutopoulos and Makriyannis 2002; Palmer et al. 2002). These ligands can be classified into six major classes: (1) classical cannabinoids, (2) non-classical cannabinoids (NCCs), (3) hybrid cannabinoids, (4) aminoalkylindoles, (5) diarylpyrazoles, and (6) endocannabinoid-like ligands.

This review focuses on key cannabinoid receptor probes representing the different classes of cannabinergic ligands, their SAR, and therapeutic potentials. The stereoselectivity aspects of interactions between these probes and cannabinoid receptors will also be briefly discussed. Throughout this review we have used the K_i values of individual ligands as measures of their relative abilities to recognize their binding sites. However, it is well known that the K_i values are subject to considerable variability depending on the radioligand used in the binding assays as well as on the experimental details under which the assays were carried out (e.g., albumin concentration, etc.). Direct comparisons hold best within groups of compounds that have been tested under identical experimental conditions. The reader is thus advised to consider the K_i values only as approximate relative measures of a ligand's affinity when interpreting the SAR data and not necessarily a measure of functional potency.

2

Classification of Cannabinoid Receptor Ligands

2.1

Classical Cannabinoids

Classical cannabinoids (CCs) are ABC-tricyclic terpenoid compounds bearing a benzopyran moiety (Figs. 1–3, 5, and 6). This class includes the natural product (–)- Δ^9 -THC (1, Fig. 1), the more stable and almost equipotent isomer (–)- Δ^8 -THC (2, Fig. 1), and other pharmacologically active constituents of the plant *Cannabis sativa*. Many CC analogs have been synthesized and evaluated pharmacologically and biochemically (for reviews see Goutopoulos and Makriyannis 2002; Khanolkar et al. 2000; Makriyannis and Goutopoulos 2004; Makriyannis and Rapaka 1990; Mechoulam et al. 1999; Palmer et al. 2002; Razdan 1986). SAR studies recognize four pharmacophores within the cannabinoid prototype: a phenolic hydroxyl (PH), a lipophilic alkyl side chain (SC), a northern aliphatic hydroxyl (NAH), and a southern aliphatic hydroxyl (SAH). The first two are encompassed in the plant-derived cannabinoids, while all four pharmacophores are represented in some of the synthetic NCCs developed by Pfizer (e.g., 25, Fig. 7). The CC structural features that are important for cannabinoid activity are discussed below.

2.1.1

SAR of Classical Cannabinoids

The Phenolic Hydroxyl This group can be substituted by an amino group, but not by a thiol group (Matsumoto et al. 1977a) while its replacement by a fluorine atom diminishes CB₁ affinity (e.g., 3, Fig. 2) (Martin et al. 2002). It has also been shown that CCs in which the phenolic hydroxyl is either replaced by a methoxy group (e.g., 4, Fig. 2) or totally absent (5 and 6, Fig. 2) retain some receptor-binding affinity, especially for CB₂ (Gareau et al. 1996; Huffman et al. 2002, 1999, 1996). However, this is not the case for the cannabinol series in which the C-ring is fully aromatized (Khanolkar et al. 2000; Mahadevan et al. 2000).

The Benzopyran Ring This ring is not essential for activity and its expansion to B-ring homocannabinoid derivatives has been considered since the early days of

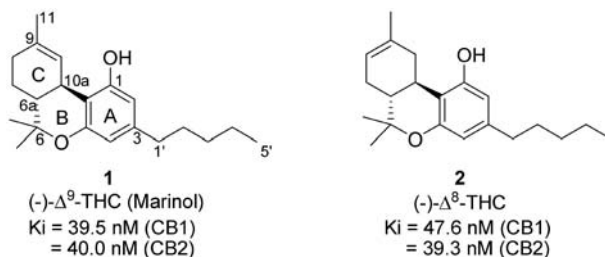


Fig. 1. The structures of (–)- Δ^9 - and (–)- Δ^8 -tetrahydrocannabinol (THC)

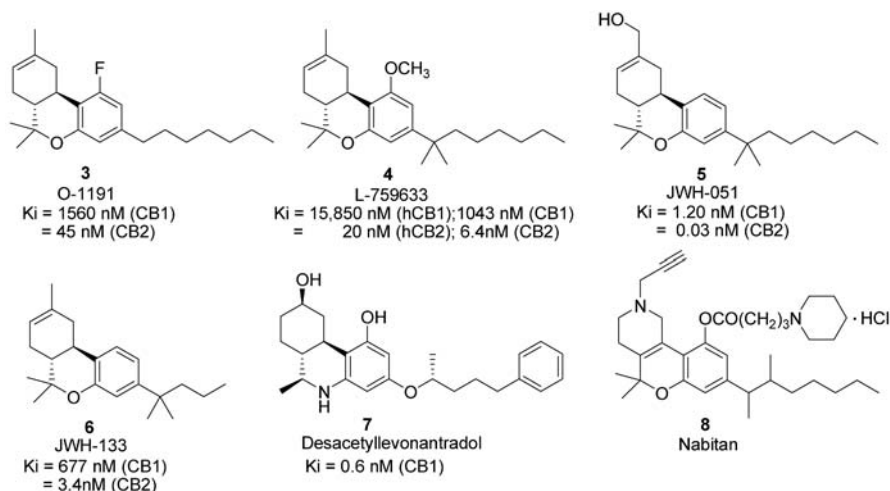


Fig. 2. Phenolic hydroxyl, B- and C-ring modified cannabinoid analogs

cannabinoid structure–activity correlations (Matsumoto et al. 1977b). The pyran oxygen can be substituted by nitrogen as exemplified by compound 7 developed at Pfizer (Fig. 2) (Melvin et al. 1995) or can be eliminated in open phenol or resorcinol analogs. The latter gave rise to the NCC class described in Sect. 2.2.

Neither the double bond nor the 9-methyl at the C-ring is necessary for activity, and this ring may be modified into a heterocyclic system (e.g., 8, Fig. 2) (Lee et al. 1977, 1983; Osgood et al. 1978; Pars et al. 1976).

C-3 Side Chain This alkyl chain has been recognized as the most critical CC pharmacophoric group. Variation of the *n*-pentyl group of natural cannabinoids can lead to wide variations in potency and selectivity. Optimal activity is obtained with a seven or eight carbon length substituted with 1',1'- or 1',2'-dimethyl groups (e.g., 9, Fig. 3) as was first demonstrated by Adams (Adams et al. 1949; Huffman et al. 2003b; Liddle and Huffman 2001). More recent studies have focused on novel side chains bearing 1',1'-cyclic moieties (Papahatjis et al. 1998, 2001, 2002, 2003). Some of the synthesized analogs exhibited remarkably high affinities for both CB₁ and CB₂ cannabinoid receptors (e.g., 10, 11, 12, Fig. 3) while in vitro pharmacological testing found the dithiolane analog 10 to be a potent CB₁-selective agonist (Papahatjis et al. 2003). The results of these studies suggest the presence of a subsite within the CB₁ and CB₂ binding domain at the level of the benzylic side carbon in the THC series. In an effort to define the stereochemical limits of this putative subsite, we generated receptor-essential volume maps and receptor-excluded volume maps using molecular modeling approaches (Fig. 4) (Papahatjis et al. 2003).

The observation that the bulky adamantyl Δ^8 -THC (13, Fig. 3) (Khanolkar et al. 2000; Palmer et al. 2002) exhibits considerable affinity and selectivity for CB₁ points to a greater tolerance for steric bulk in that receptor subsite. Oxygen atoms (ethers) and unsaturations (Busch-Petersen et al. 1996; Papahatjis et al. 1998)

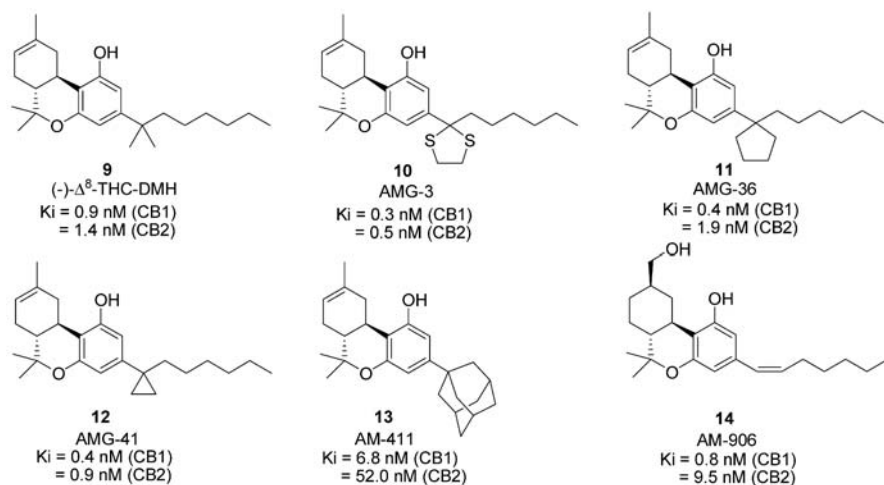


Fig. 3. Representative C-1' side chain-modified analogs

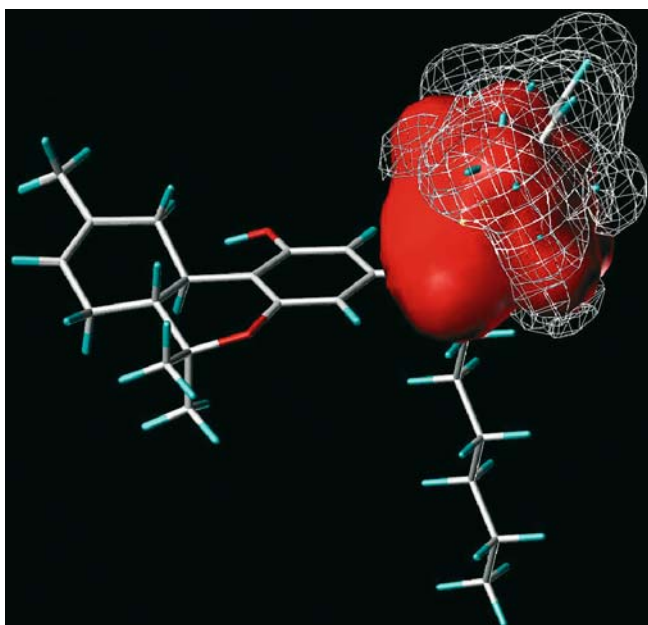


Fig. 4. Molecular modeling of (-)- Δ^8 -THC ligands with different substitution in the C-1' side chain position using molecular mechanics/molecular dynamics. CB₁/CB₂ receptor-excluded volume map (*red contours*) and essential volume map (*white grid*) for the C-1' subsite in Δ^8 -THC series. The *red area* represents the free space within the receptor region that accommodates high-affinity C-1'-substituted ligands, whereas, C-1' substituents falling within the *white grid* experience unfavorable or less favorable interactions at the binding site

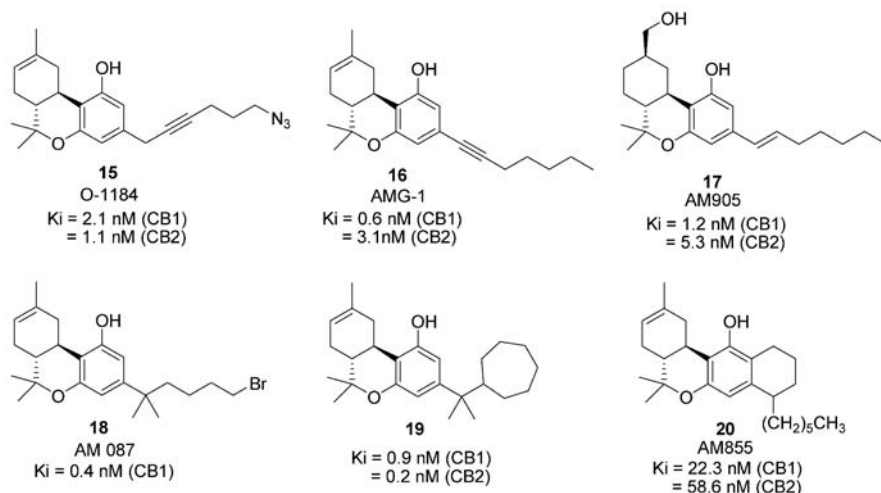


Fig. 5. Representative side chain-modified analogs

within the chain or terminal carboxamido, cyano, azido, and halogen groups are also well tolerated (Charalambous et al. 1991; Crocker et al. 1999; Khanolkar et al. 2000; Martin et al. 1993, 2002; Nikas et al. 2004; Tius et al. 1997, 1993) (e.g., 14, Fig. 3; 15, 16, 17, Fig. 5). The side chain seems to be the place of choice for halogen substitution and a considerable enhancement in affinity for CB₁ is observed by halogen substitution at the end carbon of the side chain with the bulkier halogens producing the largest effects (e.g., 18, Fig. 5). Additionally, naphthyl, phenyl, and cycloalkyl groups have served as side chain substituents (Krishnamurthy et al. 2003; Nadipuram et al. 2003; Papahatjis et al. 1996). Thus, substitution of the 1',1'-dimethylalkyl side chain with a 1',1'-dimethylcycloalkyl or 1',1'-dimethylphenyl group can lead to analogs possessing high affinities for both CB₁ and CB₂ (e.g., 19, Fig. 5). In another variation, novel tetracyclic analogs of Δ^8 -THC in which the alkyl side chain is conformationally more defined by adding a fourth ring in the ABC-tricyclic cannabinoid skeleton fused to the aromatic A-ring have also been reported (e.g., 20, Fig. 5) (Khanolkar et al. 1999).

Northern Aliphatic Hydroxyl Group It has been shown that introduction of a hydroxyl group at the C-9 or C-11 positions (northern aliphatic hydroxyl; NAH) leads to significant enhancement in affinity and potency for CB₁ and CB₂. Thus, (-)-11-hydroxydimethylheptyl- Δ^8 -THC (21, Fig. 6), a ligand that has received considerable attention because of its high affinity for both receptors, is more potent than the parent analog with no 11-hydroxy substitution (Mechoulam et al. 1988, 1987). This is also the case for the cannabinol series in which the C-ring is fully aromatized (Rhee et al. 1997) and in the hexahydrocannabinols (HHC, e.g., 22 and 23, Fig. 6) in which the C-ring is fully saturated. It has also been shown that the relative configuration of C-9 substituents in CCs can have significant effects in the compound's potency (Kriwacki and Makriyannis 1989; Reggio et al. 1989) where

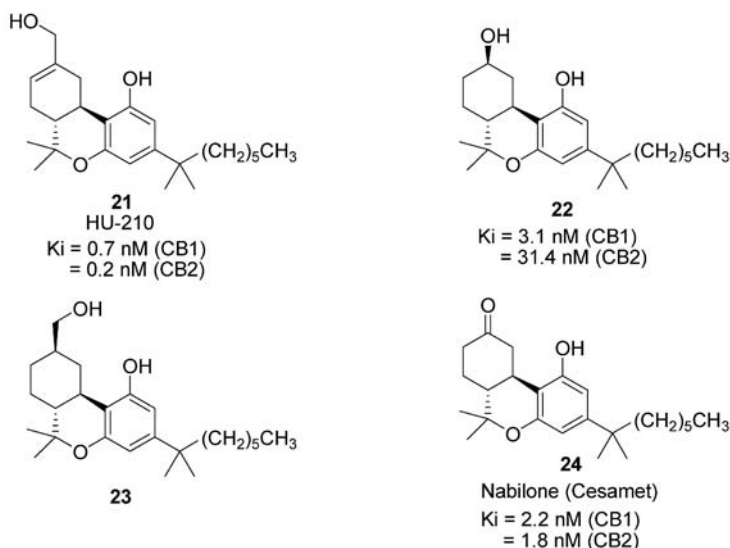


Fig. 6. Cannabinoid analogs possessing a northern aliphatic hydroxyl (NAH) group

an unfavorable orientation of a C-9 hydroxyl or hydroxymethyl substituent can seriously interfere with this ligand's ability to interact with cannabinoid receptors. Based on the relative configuration at the C-9 position, the HHC encompasses two types of isomers (9α and 9β). Although both isomers are biologically active, the β -epimers in which the C-9 hydroxyl or hydroxymethyl group is equatorial (e.g., 22 and 23, Fig. 6) have been shown to be more potent than the α -axial isomers (Devane et al. 1992a; Wilson et al. 1976; Yan et al. 1994). The preference for the 9β relative configuration has been used for the design and synthesis of high-affinity photoactivatable probes for the cannabinoid receptors (e.g., AM1708, 70, Fig. 19) (Khanolkar et al. 2000). Presence of a C-9 carbonyl group encompassed in nabilone (24, Fig. 6) is also known to significantly enhance cannabinergic activity (Archer et al. 1986). Although the nature of the substituent at the northern end of the classical cannabinoid structure has an effect on the ligands' potencies, these effects have not yet been fully investigated. Thus, 9-nor- Δ^9 -THC, a molecule that lacks a C-9 substituent, exhibits significant cannabinoid activity (Martin et al. 1975).

2.2

Non-classical Cannabinoids

A second class of cannabinergic ligands possessing close similarity with CCs was developed at Pfizer in an effort to simplify the CC structure, while maintaining or improving biological activity (Johnson and Melvin 1986; Little et al. 1988). This group of compounds, generally designated as non-classical cannabinoids (NCCs), includes AC-bicyclic (e.g., 25 and 26, Fig. 7) and ACD-tricyclic (e.g., 27, Fig. 7) ligands lacking the pyran B-ring of CCs. Of these the best known is CP-

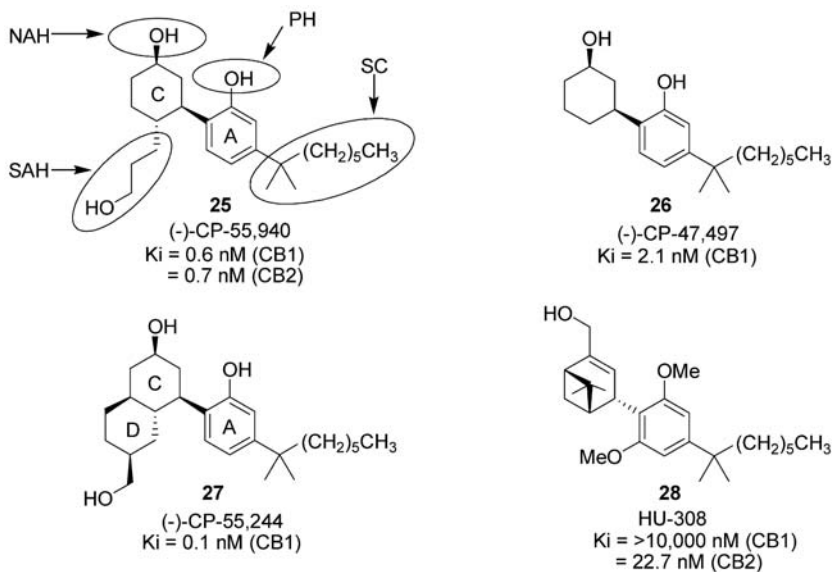


Fig. 7. Non-classical cannabinoid receptor ligands

55,940 (**25**) a crystalline ligand exhibiting high affinity for both CB₁ and CB₂ as well as a high degree of stereoselectivity. [³H]CP-55,940, the tritiated analog, was the key compound that led to the discovery of CB₁ (Devane et al. 1988). This class of compounds shares some of the key pharmacophores of the CCs, namely the phenolic OH, the side chain, and the northern aliphatic hydroxyl groups. Additionally, it encompasses an hydroxypropyl chain on the cyclohexyl ring contiguous and trans to the aromatic phenolic group as with CP-55,940. This important new pharmacophore was designated as the southern aliphatic hydroxyl group (SAH) (Makriyannis and Rapaka 1990) and has been subjected to extensive investigation by the Makriyannis and Tius groups (Chu et al. 2003; Drake et al. 1998; Harrington et al. 2000; Tius et al. 1997, 1994).

The recently introduced ligand HU-308 (**28**, Fig. 7), which has the opposite absolute configuration from all other CC and NCC analogs, is another example of bicyclic cannabinoid receptor ligands (Hanus et al. 1999) and exhibits a high degree of CB₂ selectivity.

2.3

CC/NCC Hybrid Cannabinoids

The southern aliphatic hydroxyl (SAH) pharmacophore is absent in the naturally occurring cannabinoids. To study more precisely the stereochemical requirements of this new pharmacophore, Makriyannis and co-workers designed a group of hybrid ligands that incorporated all of the structural features of both classical and non-classical cannabinoids (Drake et al. 1998; Tius et al. 1995, 1994).

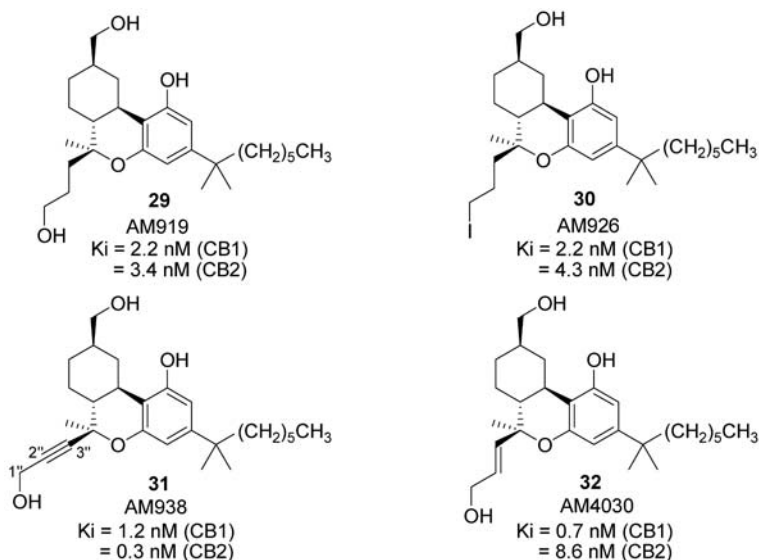


Fig. 8. Hybrid classical/non-classical (CC/NCC) cannabinoids

This new class of analogs (CC/NCC hybrids) had the added advantage of serving as conformationally more defined three-dimensional probes for the CB₁ and CB₂ active sites than their non-classical counterparts. Receptor binding data showed that at C-6 the equatorial β -hydroxypropyl analog had higher affinity than its α -axial epimer (e.g., **29** and **30**, Fig. 8) (Drake et al. 1998; Tius et al. 1994). Further refinement of the CC/NCC hybrid cannabinoids was obtained by imposing restricted rotation around this SAH pharmacophore. This was accomplished through the introduction of double and triple bonds at the C2'' position of the 6 β -hydroxypropyl chain (e.g., **31** and **32**, Fig. 8).

The affinity data for CB₁/CB₂ receptors shown in Fig. 8 for analogs **31** and **32** refer to the racemic compounds. Enantiomers of **32** were recently separated using chiral AD [amylose tris(3,5-dimethylphenylcarbamate) columns (Thakur et al. 2002) (see Sect. 4). This very promising class of compounds encompassing four asymmetric centers is among the most structurally complex and potent cannabinergic agents synthesized to date.

2.4 Aminoalkylindoles

The fourth chemical class of cannabinergic ligands, the aminoalkylindoles (AAIs) were initially developed at Sterling Winthrop as potential non-ulcerogenic analogs of non-steroidal anti-inflammatory drugs (NSAIDs) (Bell et al. 1991) and bear no structural relationship to the cannabinoids. These analogs also exhibited antinociceptive properties that eventually were attributed to their interactions with the

cannabinoid receptors (D'Ambra et al. 1992; Eissenstat et al. 1995). The most widely studied compound of this series is WIN-55,212-2 (**33**, Fig. 9), a potent CB₁ and CB₂ agonist with a slight preference for CB₂. Cannabinergic activity resides principally with only one optical antipode and is more potent than Δ^9 -THC in several pharmacological and behavioral assays (Compton et al. 1992; Martin et al. 1991). WIN-55,212-2 has played an important role in the identification and characterization of cannabinoid receptors and their associated functions and is now in standard use as a CB₁/CB₂ radioligand. The four pharmacophores identified for the aminoalkylindoles are: (1) C-3 substituents, (2) the N-1 aminoalkyl side chain, (3) C-2 substituents, and (4) indole ring substituents and modifications. The SAR requirements of this class of compounds are summarized as follows:

2.4.1

SAR of Aminoalkylindoles

C-3 Substituents Pravadoline (**34**, Fig. 9), which carries a *p*-methoxybenzoyl group at C-3, was used as a benchmark ligand to explore structural requirements at this site (Eissenstat et al. 1995). Its *o*-methoxy isomer exhibits higher potency. However, *ortho*-substitution with other groups such as $-\text{CH}_3$, $-\text{OH}$, $-\text{Cl}$, $-\text{CN}$, or $-\text{F}$ diminishes activity. The presence of an ethyl group at the para position improves potency, but further increase in chain length results in diminished potency. The 1-naphthoyl substitution at C-3 is more potent ($\text{IC}_{50} = 19 \text{ nM}$) than the 2-naphthoyl analog ($\text{IC}_{50} = 128 \text{ nM}$). Replacement of the naphthyl ring with an alkyl (e.g., CH_3) or alkenyl [$(\text{CH}_3)_2\text{C}=\text{CH}$] groups results in complete loss of CB₁ receptor affinity ($K_i > 10,000 \text{ nM}$) (Huffmann et al. 1994).

NMR and X-ray crystallography studies of **34** and its C-2H congener have revealed that AAIs can exist in two distinct conformations based on the orientation of the C-3 aroyl system (Bell et al. 1991; Reggio et al. 1998). In the *s-trans* conformation, which predominates when the C-2 substitution is hydrogen, the aryl group is proximal to C-2, while the carbonyl oxygen atom is located near C-4. In the *s-cis* conformation, which predominates when the C-2 substituent is a methyl group, the conformational preference shows the aryl ring to be located near C-4, and the carbonyl oxygen near C-2.

Naphthylidene-substituted aminoalkylindenes (e.g., **35**, Fig. 9), a conformationally more rigid version of initial AAIs, were originally designed to circumvent the CNS side effects of pravadoline (Kumar et al. 1995). These analogs were tested as a mixture of *E*- and *Z*-isomers and exhibited higher CB₁ affinity compared to pravadoline. Later, it was shown that the CB₁ and CB₂ affinities and pharmacological potencies were higher for the *E*-geometric isomer (**35**, *s-trans*, Fig. 9) compared to the *Z*-isomer (Reggio et al. 1998). Removal of the carbonyl oxygen of the C-3 aroyl group in AAIs having unsubstituted C-2 results in moderate reduction in affinity for CB₁ compared to their carbonyl precursors (Huffman et al. 2003a). However, the loss of affinity is larger in the 2-methyl substituted analogs (e.g., **36**, Fig. 9). Both observations support the hypothesis that the *s-trans* conformation of AAI analogs such as **33** is the preferred conformation for interaction at both CB₁

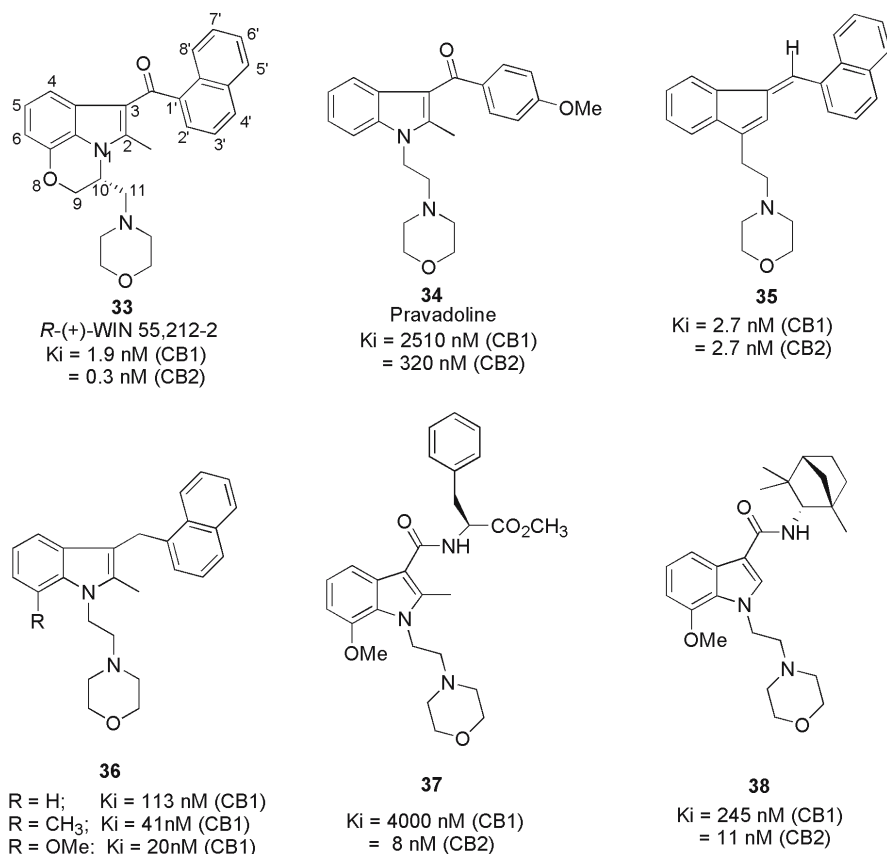


Fig. 9. C-3 modified cannabinergic aminoalkylindoles

and CB₂ receptors and that aromatic stacking of the ligands with aromatic residues in helices 3, 4, and 5 of both receptors may be an important interaction for AAIs at these receptors (Burley and Petsko 1985; Huffman et al. 2003a; Reggio et al. 1998).

The spatial and electronic requirements of the C-3 substituent were further explored by introducing a C-3 amide group (Bristol Myers Squibb). The AAI C-3 amide ligand **37** (Fig. 9) with a methoxy group at C-7, exhibited high CB₂ affinity ($K_i = 8 \text{ nM}$) and selectivity (CB₁/CB₂ = 500) (Hynes et al. 2002). Replacement of the amino acid moiety in **37** with the *S*-fenchylamine component resulted in slightly reduced affinity for the CB₂ receptor ($K_i = 30 \text{ nM}$). However, in the *S*-fenchyl amide series, when the 2-methyl group in indole was replaced by hydrogen, the resulting ligand (**38**, Fig. 9) showed improved CB₂ affinity ($K_i = 11 \text{ nM}$).

The 4-alkyloxy indole analogs were derived by translocating the C-3 substituent of AAIs to C-4 via an ether linkage. Some of these exhibited *in vivo* cannabimimetic activity, but most of them lacked cannabinoid receptor affinity (Dutta et al. 1997).

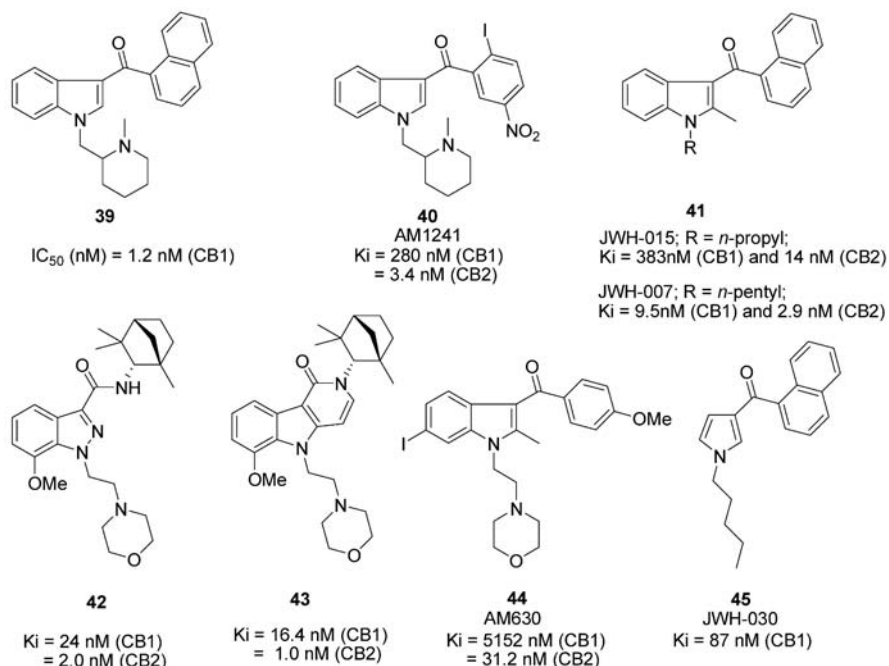


Fig. 10. Chemical structures of some aminoalkylindole-derived analogs

N-1 Aminoalkyl Chain A number of indole analogs bearing different aminoalkyl substituents at N-1 were synthesized (*N*-attached analogs, e.g., **34**, Fig. 9) and tested (Eissenstat et al. 1995). This study found the aminoethyl substitution as an optimal requirement with morpholino, thiomorpholino, and piperidino analogs showing the highest activities. The respective acyclic amine and piperazine analogs were inactive.

The Sterling Winthrop and Makriyannis laboratories further explored structural requirements at the N-1 position by synthesizing novel analogs in which the aminoalkyl chain of the indole ring is attached to a heterocyclic amine through a C–C bond. These analogs are generally more potent compared to the C–N analogs and exhibit more favorable physicochemical properties. Potency was optimum for *N*-methylpiperidinyl-2-methyl substitution at the N-1 position (**39**, Fig. 10), while activity resided predominately in the *R*-enantiomer (D'Ambra et al. 1996).

AM1241 (**40**, Fig. 10), a highly CB₂-selective and potent agonist (Ibrahim et al. 2003; Malan et al. 2001) was recently developed by Makriyannis. Design of this molecule incorporated the *N*-methylpiperidinyl-2-methyl substituent at the N-1 position and a novel 2-iodo-5-nitrobenzoyl group at C-3. AM1241 exhibits remarkably high peripheral analgesia *in vivo* and does not produce catalepsy, hypothermia, inhibition of spontaneous locomotor activity, or impairment of performance on the rotarod apparatus. The potential use of this CB₂ receptor agonist for the treatment of neuropathic pain is being explored.

Replacement of the aminoalkyl substituent by an alkyl chain results in *N*-alkyl indoles (non-AAIs) (e.g., **41**, Fig. 10). The SAR of cannabimimetic 2-methylindoles indicates that compounds with *N*-alkyl substituents from *n*-propyl to *n*-hexyl have good affinities for both CB₁ and CB₂ receptors with a preference for CB₂. The in vivo potencies of these compounds were reported to be consistent with their receptor affinities (Huffmann et al. 1994; Wiley et al. 1998).

C-2 Substituents Analysis of the effect of C-2 substitution on cannabinoid receptor affinity in AAIs reveals a strong preference for a small substituent at C-2. Thus, hydrogen or methyl groups are well tolerated with the C-2H analogs exhibiting slightly higher affinities for the CB₂ than C-2 methyl analogs (Eissenstat et al. 1995; Hynes et al. 2002; Wroblewski et al. 2003).

Recently, researchers at Bristol Myers Squibb reported their discovery of indazole carboxamides (e.g., **42**, Fig. 10), a new class of cannabimimetics, in which the C-2 carbon of 3-amido AAIs (e.g., **38**, Fig. 9) is replaced by nitrogen. The indazole analog **42** exhibits high affinity for the CB₂ receptor ($K_i = 2.0$ nM) compared to the corresponding AAI analogs **38** (Wroblewski et al. 2003). Indolopyridones (e.g., **43**, Fig. 10), which are conformationally restricted C-3 amido AAIs, exhibit increased affinities for the CB₂ receptor ($K_i = 1.0$ nM) and possess anti-inflammatory properties when administered orally in an in vivo murine inflammation model (Wroblewski et al. 2003).

Indole Ring Substituents and Modifications Introduction of a methyl group at C-4 or various substituents such as -CH₃, -OCH₃, -F, -Br, or -OH groups at C-5 of pravadoline diminishes affinity. Conversely, C-6 substitution with -CH₃, -OCH₃, or -Br (WIN-54,461, bromopravadoline) groups improves receptor affinity, but the ligands exhibit diminished agonist properties (Eissenstat et al. 1995). Incorporation of an iodo group at C-6 led to AM630 (**44**, Fig. 10), a ligand that exhibits improved affinity as well as selectivity for CB₂ (Hosohata et al. 1997a,b; Pertwee et al. 1995). This compound was shown to be a potent and selective antagonist/inverse agonist for CB₂ and is a useful pharmacological tool developed before its principal target site was identified (Ross et al. 1999). Substitution at C-7 gives modest improvement in binding affinity. Potent AAI analogs were generated by conformationally restricting the N-1 side chain through the formation of a six-membered ring between the N-1 and C-7 substituents (D'Ambra et al. 1992). In *N*-alkyl indoles, replacement of the indole phenyl ring with a cyclohexyl ring led to an analog with reduced affinities for both CB₁ and CB₂ (Tarzia et al. 2003). Removal of the phenyl ring in AAIs or non-AAIs led to a pyrrole class of cannabimimetics (e.g., **45**, Fig. 10). The SAR of pyrrole cannabinoids has been explored first by Sterling Winthrop and later by Huffman (Wiley et al. 1998) and Tarzia et al. (2003). Most of the pyrrole-derived analogs are less potent than the corresponding indole derivatives. However, the 4-bromopyrrole analog (Tarzia et al. 2003) exhibits high affinity for both CB₁ and CB₂ ($EC_{50} = 13.3$ nM for rCB₁ and 6.8 nM for hCB₂) comparable to WIN-55,212-2.

2.5 Diarylpyrazoles

The most widely studied compound of the diarylpyrazole class is SR141716A (Rimonabant) (**46**, Fig. 11) developed by Rinaldi-Carmona and co-workers at Sanofi (Rinaldi-Carmona et al. 1994) and is currently undergoing clinical trials as an antiobesity medication. This highly potent and selective CB₁ receptor ligand has served as a unique pharmacological and biochemical tool for further characterization of the CB₁ cannabinoid receptor (Lan et al. 1999; Nakamura-Palacios et al. 1999). In vitro, SR141716A antagonizes the inhibitory effects of cannabinoid agonists on both mouse vas deferens (MVD) contractions and adenylyl cyclase activity in rat brain membranes. SR141716A also antagonizes the pharmacological and behavioral effects produced by CB₁ agonists after intraperitoneal (i.p.) or oral administration (Rinaldi-Carmona et al. 1994).

Other diarylpyrazole ligands that have contributed to our understanding of CB₁ pharmacology are AM251 and AM281 (Lan et al. 1999), both of which are CB₁ antagonist/inverse agonists (**47** and **48** respectively, Fig. 11) capable of displacing [³H]SR141716A and [³H]CP-55,940 in CB₁ receptor membrane preparations. Both AM251 and AM281 share the ability of SR141716A to attenuate the responses to

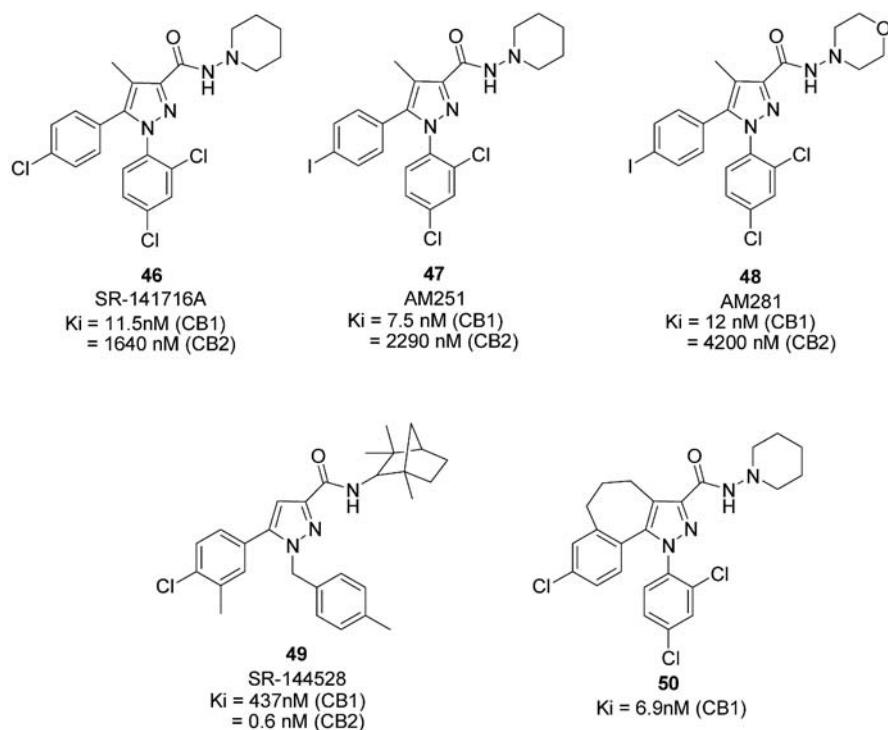


Fig. 11. Representative diarylpyrazole ligands

established cannabinoid receptor agonists like WIN-55,212-2 or CP-55,940. However, recent evidence indicates that AM251 may have a more "CB₁-selective" role than SR141716A (Hajos and Freund 2002). In addition to AM630, the most notable CB₂ receptor antagonist/inverse agonist is SR144528, a diarylpyrazole (**49**, Fig. 11) developed by Sanofi, exhibiting 700-fold selectivity for the CB₂ receptor over CB₁ (Rinaldi-Carmona et al. 1998). Structural requirements for SR141716A-like compounds are summarized below (for earlier reviews see Howlett et al. 2002; Palmer et al. 2002).

2.5.1

SAR of Pyrazole Cannabinoid Receptor Antagonists

N-1 Substituents 2,4-Dichlorophenyl is the optimal substituent for both high CB₁ affinity and subtype selectivity (Barth and Rinaldi-Carmona 1999; Lan et al. 1999). Its replacement with 1-(5-isothiocyanato)-pentyl group decreased CB₁ affinity only by a factor of four (Howlett et al. 2000). The inclusion of 4-butylphenyl, 4-pentylphenyl or a phenyl group at N-1 significantly reduces affinity while *n*-pentyl, *n*-hexyl, *n*-heptyl substitution retains affinity (Shim et al. 2002). Optimal selectivity for CB₂ is contributed by a 4-methylbenzyl group as represented in SR144528 (**49**, Fig. 11) (Rinaldi-Carmona et al. 1998). In the 2,4-dichlorophenyl moiety, elimination of *p*-chloro substitution or replacement of *o*-chloro with *o*-fluoro or *o*-methoxy groups led to low-affinity analogs (Katoch-Rouse et al. 2003). Replacement of the 2,4-dichlorophenyl by unsubstituted cycloalkyl groups decreased both CB₁ and CB₂ affinities, while the 3-methyl and 4-methylcyclohexyl analogs exhibited moderate improvement in CB₂ affinity without any enhancement in selectivity compared to SR141716A (Krishnamurthy et al. 2004).

C-3 Substituents Alkylation of the amide group as well as its replacement by a ketone, alcohol, or ether (Wiley et al. 2001) greatly decreases CB₁ affinity. Replacement of the piperidinyl group with the respective five- or seven-membered heterocyclic rings or by a cyclohexyl group does not alter CB₁ binding affinity, while replacement with a morpholine group or linear alkyl chains leads to reduction in CB₁ affinity (Lan et al. 1999). Alkyl hydrazines, amines, and hydroxyalkylamines of varying lengths were substituted for the aminopiperidinyl moiety to probe the structural and steric requirements of this pharmacophore (Francisco et al. 2002). For alkylamides, hydroxyalkyl amides, and alkyl hydrazides, affinity for CB₁ was found to increase with increasing chain length from ethyl to butyl or pentyl. Further increase in the carbon chain length reduced affinity for both receptors. Alkylamide analogs exhibited enhanced CB₁ selectivity when compared to SR141716A, whereas hydroxyalkyl amide and alkylhydrazide analogs had both decreased affinities and selectivities (Francisco et al. 2002).

C-4 Substituents Compounds with methyl, ethyl, bromo, or iodo substituents in the 4-position of the pyrazole ring are approximately equipotent, whereas replacement of methyl with hydrogen results in a 12-fold decrease in CB₁ affinity (Wiley et al. 2001).

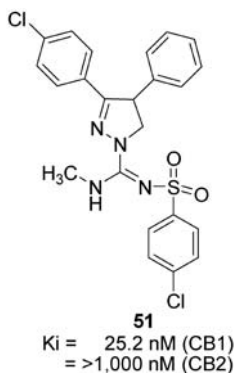


Fig. 12. 3,4-Disubstituted pyrazolines

C-5 Substituents The 4-chloro group of the phenyl ring can be replaced by bromo or alkyl groups but not by nitro or amino groups (Lan et al. 1999; Thomas et al. 1998; Wiley et al. 2001). Replacement of 4-chloro with a 4-iodo substituent (AM251) leads to optimal CB₁ affinity and CB₁/CB₂ selectivity. AM251 has proved to be an excellent CB₁ probe and is widely used as a standard. Conversely, replacement of the aromatic ring with alkyl groups abolishes CB₁ affinity (Lan et al. 1999).

Recently, two research groups independently reported a number of rigid analogs of SR141716A. Solvay (Stoit et al. 2002) first reported some tricyclic CB₁-selective ligands in which the 4- and 5-substituents are conformationally restricted through the formation of a relatively rigid tricyclic system. In these compounds the 4-methyl group is connected with the *ortho* position of the aromatic 5-aryl substituent to form benzocycloheptapyrazole analogs represented by **50** (Fig. 11) that exhibited higher CB₁ affinity than the parent SR141716A (Stoit et al. 2002). However, the compound had poor oral bioavailability. Later Pinna and co-workers (Mussinu et al. 2003) reported similar tricyclic pyrazole analogs in which the above additional 7-membered ring was replaced by a five-membered ring. Interestingly, most ligands in this class had high affinity and selectivity for CB₂ compared to **50** and SR141716A.

Very recently, Solvay Pharmaceuticals (Lange et al. 2004) reported a novel class of 3,4-disubstituted pyrazoline analogs exhibiting high CB₁ selectivity (e.g., **51**, Fig. 12). Another novel class of CB₁ antagonists that has received only limited attention includes the 3-alkyl-5-arylhydantoins (Ooms et al. 2002).

While the search for high affinity/efficacy ligands is ongoing, the development of well-designed radiolabeled ligands has enhanced our understanding of the physiological role of the endocannabinoid system. [¹²³I]AM281, an ¹²³I-labeled 1,5-biarylpyrazole, has served as a useful imaging agent in single photon emission computed tomography (SPECT) studies (Gatley et al. 1997, 1998; Gifford et al. 1997).

2.6

Endocannabinoids

In 1992 an arachidonic acid ethanolamide derivative (**52**, AEA, Fig. 13) isolated from porcine brain and characterized as an endogenous ligand for the cannabinoid receptors was named anandamide (Devane et al. 1992b). AEA is a highly lipophilic compound encompassing four non-conjugated cis double bonds and is sensitive to both oxidation and hydrolysis. It was shown to bind to the CB₁ receptor with moderate affinity ($K_i = 61$ nM), has low affinity for the CB₂ receptor ($K_i = 1,930$ nM), and behaves as a partial agonist in the biochemical and pharmacological tests used to characterize cannabinoid activity. Its role as a neurotransmitter or neuromodulator is supported by its pharmacological profile as well as by the biochemical mechanisms involved in its biosynthesis and bioinactivation. Two other polyunsaturated fatty acid ethanolamides, homo- γ -linolenylethanolamide and 7,10,13,16-docosatetraenylethanolamide, also were isolated subsequently from porcine brain and shown to bind with high affinity to CB₁ (Hanus et al. 1993). Following that, 2-AG (**53**, Fig. 13), a monoglyceride representing a new class of endocannabinoid ligands and capable of binding to both CB₁ and CB₂ receptors was isolated from intestinal and brain tissues and shown to be another endogenous cannabinoid (Mechoulam et al. 1995; Stella et al. 1997) present in brain in concentrations approximately 170-fold higher than anandamide (Di Marzo et al. 1998; Mechoulam et al. 1996; Mechoulam et al. 1995; Stella et al. 1997). Another endogenous agonist for both CB₁ and CB₂ receptors is mead ethanolamide (Priller et al. 1995).

An ether-type endocannabinoid, 2-arachidonyl glyceryl ether (noladin ether, **54**, Fig. 13) was reported to be isolated from porcine brain (Hanus et al. 2001). Noladin ether was found to bind selectively to the CB₁ receptor ($K_i = 21.2$ nM) and cause sedation, hypothermia, intestinal immobility, and mild antinociception in

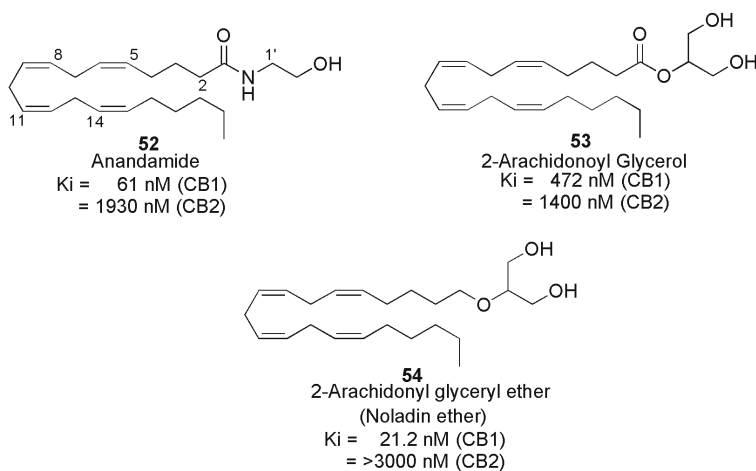


Fig. 13. Endogenous cannabinoid receptor agonists

mice, effects typically produced by cannabinoid agonists. Synthetic noladin ether was used by Sugiura and co-workers to examine its effects on Ca^{2+} levels in cells (Sugiura et al. 1999; Suhara et al. 2000) and found to exhibit appreciable agonistic activity, although significantly lower than that of 2-AG.

2.6.1

SAR of Endocannabinoids

The chemical structure of anandamide can be divided into two major molecular fragments: (1) a polar ethanolamido head group and (2) a hydrophobic arachidonoyl chain (see Fig. 14). The polar head group is comprised of a secondary amide functionality with an *N*-hydroxyalkyl substituent, while the hydrophobic fragment is a non-conjugated *cis* tetraolefinic chain and an *n*-pentyl tail reminiscent of the lipophilic side chain found in the classical cannabinoids.

A number of anandamide analogs have been synthesized and tested for their biological activities. These efforts have resulted in the development of several potent metabolically stable analogs some of which are important pharmacological tools useful in elucidating the physiological role of anandamide. Below we summarize the SAR (for previous reviews see Khanolkar and Makriyannis 1999; Palmer et al. 2000; Razdan and Mahadevan 2002; Reggio 2002; Thomas et al. 1996) of anandamide analogs for the currently known high-affinity cannabinergic sites with which anandamide and its analogs are known to interact.

All known arachidonoyl ethanolamides are primarily CB_1 -selective ligands and bind poorly to the peripheral CB_2 receptor. Therefore, the following discussion will focus on the endocannabinoid ligand SAR for the CB_1 receptor.

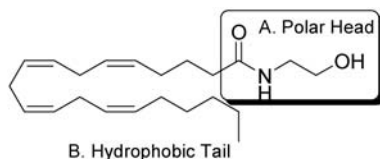


Fig. 14. Structural features of anandamide

Modification of *N*-Hydroxyethyl Group One carbon homologation to the *N*-hydroxypropyl analog increases CB_1 receptor affinity. However, further extension, with or without branching, leads to a decrease in binding affinity (Pinto et al. 1994; Sheskin et al. 1997). Thus, a three-carbon chain separating the amido NH group from the terminal OH appears to be an optimal requirement for a favorable ligand-receptor interaction. However, the hydroxyl group is not a necessary requirement for receptor affinity/potency. *N*-alkyl analogs such as *N*-ethyl, *N*-propyl, and *N*-butyl all show good receptor affinities. *N*-(*n*-Propyl)arachidonamide has a three-fold higher CB_1 affinity than anandamide, while the *n*-butyl homolog has about equal affinity (Pinto et al. 1994). Substitution of the ethanolamine head group with an *N*-cyclopropyl group leads to a high-affinity CB_1 -selective compound (55,

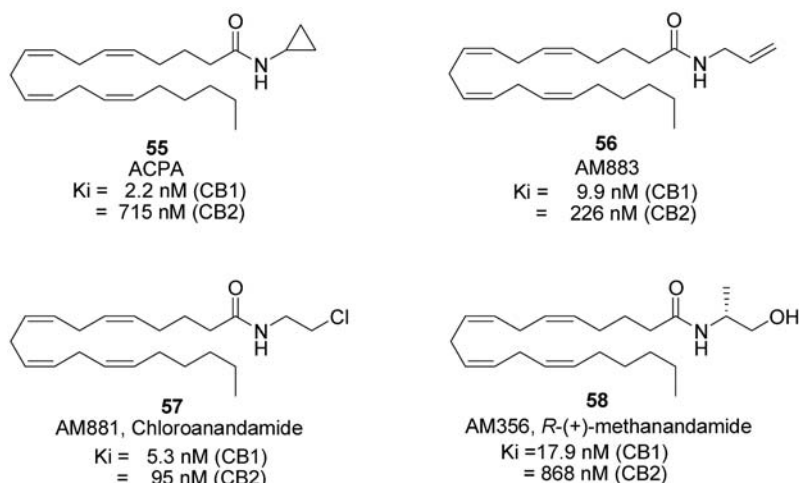


Fig. 15. High-affinity head group analogs of anandamide

Fig. 15). *N*-Allyl (**56**, Fig. 15) and *N*-propargyl analogs also show high CB₁ affinities (Lin et al. 1998). Substitution of the hydroxyl group with a halogen such as F and Cl (**57**, Fig. 15) also increases affinity for CB₁ (Adams et al. 1995a,b; Lin et al. 1998). The above data suggest that anandamide analogs can interact with the CB₁ receptor without the participation of the ethanolamide hydroxyl group.

One of the shortcomings of anandamide as an effective pharmacological tool is its facile in vivo and in vitro enzymatic degradation. It was, thus, important to develop analogs that are resistant to the hydrolytic actions of anandamide amido-hydrolase. To address this shortcoming, four chiral anandamide analogs possessing a methyl group at the C-1' or the C-2' positions were synthesized (Abadji et al. 1994; Goutopoulos et al. 2001; Lin et al. 1998). The rationale behind the design was to slow down the enzymatic hydrolysis by increasing steric hindrance around the amido group. Of these, the 1'-*R*-methyl isomer [AM356, *R*-(+)-methanandamide **58**, Fig. 15] showed four times higher CB₁ affinity than anandamide while exhibiting excellent metabolic stability. This analog is now being used as an important pharmacological tool in cannabinoid research. Interestingly, an inverse correlation in stereoselectivity between CB₁ receptor affinity and the ability of the ligand to serve as a substrate for FAAH (fatty acid amide hydrolase) was observed. Thus, in the case of 1'-methyl headgroup analogs, the *R*-enantiomer that has higher CB₁ affinity also exhibited lower susceptibility to enzymatic hydrolysis. Introduction of larger alkyl groups, e.g., ethyl or isopropyl, has a detrimental effect on CB₁ affinity (Khanolkar et al. 1996; Khanolkar and Makriyannis 1999).

Substitution of the 2-hydroxyethyl group with a phenolic group results in decreased affinity for CB₁ (Khanolkar et al. 1996). However, *N*-(*o*-hydroxy)phenyl-arachidonamide (AM403) was found to be an excellent substrate for FAAH (Lang et al. 1999) while a second phenolic analog, *N*-(*p*-hydroxy)phenylarachidonamide (AM404), was found to be an inhibitor for the anandamide transporter (ANT)

(Beltramo et al. 1997). Arachidonamide and arachidonic acid esters (methyl, ethyl, propyl) do not show significant affinity for CB₁ (Sheskin et al. 1997), while cyclization of the head group into an oxazoline ring diminishes affinity (Lin et al. 1998).

Modification of the Amide Group Replacement of the amido group by a thioamido group results in reduced affinity for CB₁. Thus, both thioanandamide and *R*-thiomethanandamide bind weakly to the receptor and show no significant biological activity (Lin et al. 1998). The SAR also indicates that the amide group must be secondary. Primary amides, e.g., arachidonamide, as well as tertiary amides, e.g., *N*-methylanandamide, do not bind to the CB₁ receptor (Lin et al. 1998; Pinto et al. 1994; Sheskin et al. 1997). Reversing the position of the carbonyl and the NH groups slightly decreases receptor affinity. These anandamides, designated as retroanandamides (e.g., **59**, Fig. 16), which were first developed by Makriyanis, exhibit exceptional stability with regard to hydrolysis by FAAH (Lin et al. 1998).

Replacement of the amido group by a carbamate group decreases affinity for CB₁. However, when the amido group is replaced by substituted ureas (**60**, Fig. 16) binding affinity as well as stability towards amidase hydrolysis is increased compared to anandamide (Ng et al. 1999).

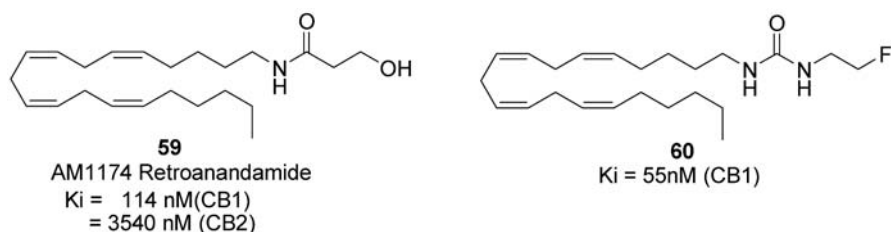


Fig. 16. Amide group modified analogs of anandamide

Importance of *cis*-Olefinic Bonds for Cannabimimetic Activity Drastic structural modifications of the arachidonyl component, such as complete saturation or replacement of the double bonds with triple bonds, result in complete loss of receptor affinity (Sheskin et al. 1997). Furthermore, ethanolamides of partially unsaturated fatty acids such as linoleic (two double bonds) and oleic (one double bond) acids exhibit considerably diminished affinity for CB₁ and cannabimimetic activity (Sheskin et al. 1997; Lin et al. 1998). From these results it can be argued that the presence of four *cis* olefinic bonds is optimal for activity. Prostaglandins and related analogs, which can be considered as conformationally rigid arachidonic acid analogs, do not bind to the CB₁ receptor (Pinto et al. 1994). Their inability to interact with the receptor may be due to the conformational restriction imposed by the five-member carbocyclic ring, which leads to preferred conformations that are incongruent with those of arachidonoyl ethanolamide and its analogs. It could also be due to the positions and stereochemistries of their hydroxyl and/or keto

groups, which may destabilize their interactions with the receptor. Introduction of a methyl group or *gem*-dimethyl group at the C-2 position results in metabolically stable analogs with concomitant increase in CB₁ affinity as in the case of C-1' methylation (Adams et al. 1995b; Goutopoulos et al. 2001)

***n*-Pentyl Group Tail Modifications** Although there is no apparent structural similarity between the classical cannabinoids and anandamide, there is considerable evidence suggesting that these two classes of cannabimimetic agents bind similarly to the CB₁ active site (Barnett-Norris et al. 2002; A. Makriyannis and C. Li, unpublished results). There is ample chemical and computational evidence indicating that arachidonic acid, the parent fatty acid of anandamide, favors a bent or looped conformation in which the carbonyl group is proximal to the C14–C15 olefinic bond. The chemical evidence for such a conformation includes the highly regiospecific intramolecular epoxidation of arachidonoyl peracid (Corey et al. 1984) and the facile macrolactonization of C20 hydroxyl methyl arachidonate (Corey et al. 1983). These experimental results are corroborated by molecular dynamics calculations (Rich 1993) that indicate that indeed a bent conformation is thermodynamically favorable. In the case of arachidonoyl ethanolamides, molecular modeling studies (Barnett-Norris et al. 1998, 2002; Rich 1993) have shown that anandamide and other fatty acid ethanolamides and esters also prefer a hairpin conformation. Additional data (Thomas et al. 1996; Tong et al. 1998) indicate that such a bent conformation is capable of mimicking the three-dimensional structure of tetrahydro- and hexahydrocannabinols.

However, it is unclear whether the hairpin conformation is also the conformation at the CB₁ receptor active site. Recent biophysical work on the conformational properties of anandamide in the membrane provide evidence for a more extended conformation for the C20 chain (A. Makriyannis and X. Tian, unpublished results) and suggest alternative CB₁ pharmacophoric conformations.

As discussed earlier, the SAR for the side chain of classical cannabinoids has been studied extensively, and it is known that a 1',1'-dimethylheptyl (DMH) substituent generally leads to optimal potency. There is also evidence that classical cannabinoids and anandamides interact with similar residues at the CB₁ binding sites. This it was postulated that a similar substitution in anandamide should result

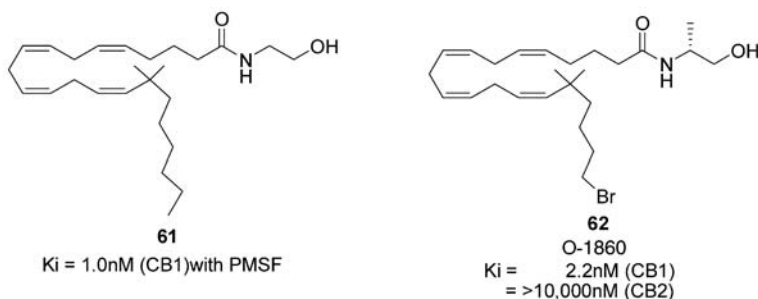


Fig. 17. Tail modified analogs of anandamide

in an increase in receptor affinity and potency. To test the hypothesis, dimethylheptyl and other alkyl chain analogs of anandamide were synthesized and tested for their biological activities. As predicted, the dimethylheptyl analogs showed marked increases in receptor affinity and in vivo potency (**61**, Fig. 17) (Ryan et al. 1997; Seltzman et al. 1997; A. Makriyannis and J.K. Kawakami, unpublished results). Also, congruent with classical cannabinoid SAR, introduction of either bromo (**62**, Fig. 17) (Di Marzo et al. 2001) or cyano groups at the C-20 increases CB₁ affinity, whereas a hydroxyl group diminishes CB₁ affinity.

2.7

Other Cannabinergic Classes

A notable CB₁ receptor-selective antagonist that also exhibits inverse CB₁ receptor agonist properties in some assay systems is LY320135 (**63**, Fig. 18). This ligand was developed by Eli Lilly (Felder et al. 1998) and shares the ability of SR141716A to bind preferentially to CB₁. However, it has lower affinity for CB₁ than SR141716A and also binds to muscarinic and 5-HT₂ receptors at low micromolar concentrations (Felder et al. 1998). LY320135 also shares the ability of SR141716A to exhibit inverse agonist activity at some signal transduction pathways of the CB₁ receptor.

Aventis reported (Mignani et al. 2000) a new class of CB₁ receptor antagonists, which are represented by the diarylmethyleneazetidine analog **64** (Fig. 18). Very recently some novel 1,2,4-triazole derivatives were shown to behave as silent cannabinoid antagonists (Jagerovic et al. 2004). Although, these compounds bind

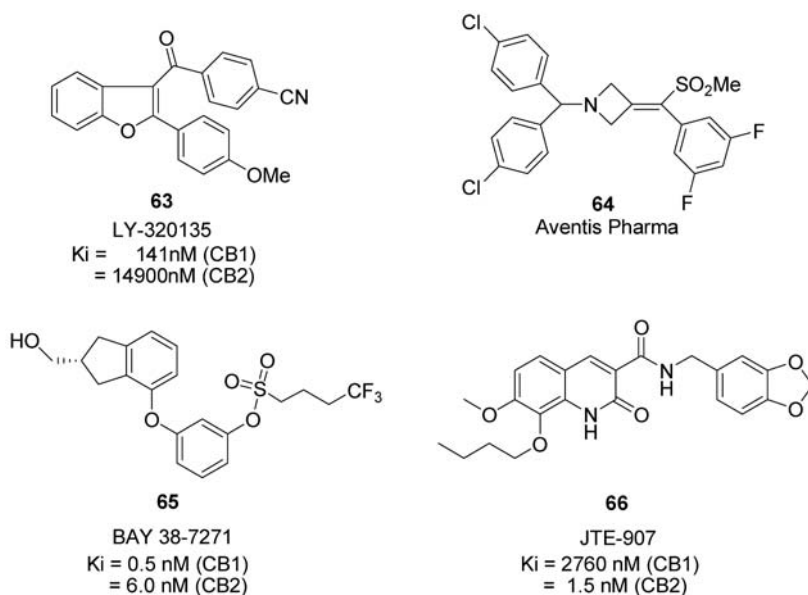


Fig. 18. Structurally novel cannabinergic ligands

to the CB₁ receptor with much reduced affinity compared to SR141716A, they exhibit similar antagonist efficacy in functional studies.

Recently, a novel class of diarylether sulfonyl ester cannabinoid agonists possessing neuroprotective properties was reported by Bayer AG (Wuppertal, Germany) (Mauler et al. 2002). The representative agonist, (–)-R-3-(2-hydroxy-methyl-indanyl-4-oxy)phenyl-4,4,4-trifluoro-1-sulfonate (**65**, BAY38-7271, Fig. 18), is a high-affinity CB₁ ligand ($K_i = 0.46\text{--}1.85$ nM; rat brain, human cortex, and recombinant human CB₁ receptor) (Mauler et al. 2003).

Researchers at Japan Tobacco (Osaka, Japan) reported the CB₂ selective inverse agonist JTE-907, whose structure is characterized by the presence of a carboxamide group in the 3-position of a quinolone nucleus (**66**, Fig. 18) (Iwamura et al. 2001) with anti-inflammatory *in vivo* activity. Naphthyridine derivatives sharing some structural features of JTE-907 were recently reported as cannabinoid receptor ligands with a preference for the CB₂ receptor (Ferrarini et al. 2004).

3

Covalent Binding Probes

Makriyannis and co-workers have developed several novel cannabinoid receptor affinity ligands (for recent reviews see Khanolkar et al. 2000; Palmer et al. 2002) that encompass reactive groups at judiciously chosen positions within the classical cannabinoid structure and can be used as probes for obtaining information on the receptor binding domain. Two types of reactive groups were incorporated: (1) electrophilic isothiocyanate group (NCS) that target nucleophilic amino acid residues such as lysine, histidine, and cysteine at or near the active site and (2) a photoactivatable aliphatic azido groups (N₃) capable of labeling the amino acid residues at the active site via a highly reactive nitrene intermediate. Both types of probes were shown to successfully label the cannabinoid receptors (Picone et al. 2002). The first photoaffinity label for the cannabinoid receptor, (–)-5′-azido- Δ^8 -THC (**67**, Fig. 19) was reported in 1992 and was shown to covalently attach to CB₁ (Charalambous et al. 1992).

Second generation covalent probes carrying isothiocyanato or azido groups with improved affinities for both CB₁ and CB₂ were also reported and shown to label these receptors. The best known of these are (–)-11-hydroxy-7′-isothiocyanato-1′,1′-dimethylheptyl- Δ^8 -THC (**68**, Fig. 19) and (–)-11-hydroxy-7′-azido-1′,1′-dimethylheptyl- Δ^8 -THC (**69**, Fig. 19) (Yan et al. 1994).

A significant improvement in the design of these new probes was the introduction of a ¹²⁵I-substituent in the ligand without compromising its high receptor affinity (e.g., AM1708, **70**, Fig. 19) (Khanolkar et al. 2000; A.D. Khanolkar, G.A. Thakur, and A. Makriyannis, unpublished). These radio-iodinated probes have served as valuable tools for receptor purification and characterization of the CB₁ and CB₂ receptors (A. Makriyannis and W. Xu unpublished). Currently, a variety of mono- and bifunctional covalent ligands with hybrid cannabinoid structures (**71**, Fig. 19) (Chu et al. 2003), as well as endocannabinoid-like compounds (C. Li and A. Makriyannis, unpublished) are being used to elucidate the binding motifs

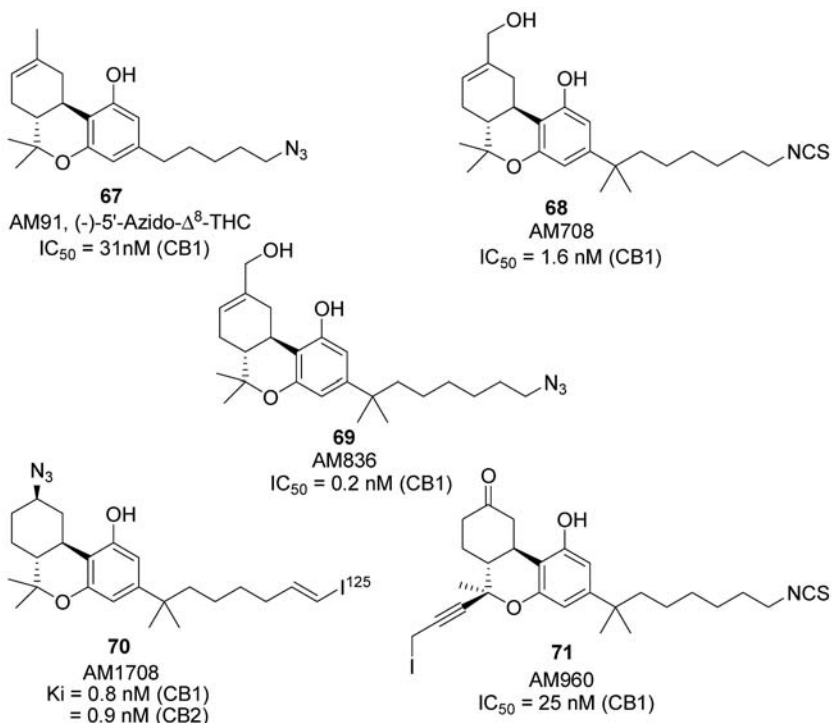


Fig. 19. Covalent probes for cannabinoid receptors

of the various classes of cannabinergics for the CB₁ and CB₂ receptors. This ligand-based approach in structural biology can serve as a useful avenue for studying the active sites of membrane-bound structural proteins that are not easily amenable to a crystallization approach.

4 Enantioselective Cannabinergic Ligands

Ligand enantioselectivity is often an important criterion in the characterization of drug receptors and in the development of biochemical and pharmacological assays. Thus, a highly enantioselective enantiomer can be a radioligand in a binding assay in which its much-less-potent enantiomer can be used to determine non-specific binding. Similarly, the less active enantiomer can serve as a control in *in vitro* or *in vivo* drug evaluations.

The cannabinergic ligand library includes a number of key enantiomeric pairs that have found substantial use in laboratories engaged in cannabinoid research. A careful examination of the literature reveals striking discrepancies in reported bioenantioselectivities. These are generally attributable to inadequate chiral resolution leading to a chirally impure enantiomer. Variation in enantioselectivity can

Table 1. Stereoselectivity ratios of cannabinergic ligands^a

HU-210	K_i (nM)	
	CB ₁	CB ₂
(6 <i>aR</i> ,10 <i>aR</i>) (–)	0.7	0.2
(6 <i>aS</i> ,10 <i>aS</i>) (+)	Does not bind significantly	

AM4030	K_i (nM)	
	CB ₁	CB ₂
(6 <i>S</i> ,6 <i>aR</i> ,9 <i>R</i> ,10 <i>aR</i>) (–)	0.6	1.1
(6 <i>R</i> ,6 <i>aS</i> ,9 <i>S</i> ,10 <i>aS</i>) (+)	94.8	124.8

SLV-319	K_i (nM)	
	CB ₁	CB ₂
4 <i>S</i> (–)	7.8	7,943
4 <i>R</i> (+)	894	>1000

AM1241	K_i (nM)	
	CB ₁	CB ₂
<i>R</i> (+)	139.7	1.4
<i>S</i> (–)	2049	160.5

WIN-55,212-2	K_i (nM)	
	CB ₁	CB ₂
<i>R</i> (+)	1.9	0.3
<i>S</i> (–)	6300	>1000

AM356	K_i (nM)	
	CB ₁	CB ₂
<i>R</i> (+)	17.9	868
<i>S</i> (–)	309	8220

^aThe structures shown in this table represent the most active enantiomer.

be seen depending on the target protein or for the corresponding protein among different species, the CB₂ receptor being a case in point where the homology between the commonly used mouse spleen CB₂ preparation and that of expressed human receptor is only 82%. Discrepancies between in vitro and in vivo enantioselectivities may also be due to metabolic or bioavailability factors where the two enantiomers of a chiral ligand can be metabolized by the same enzyme but at different rates or exhibit different rates of uptake. Below we list some key chiral cannabinergic ligands currently used in cannabinoid research (Table 1).

(-)- Δ^9 -THC, the active constituent of marijuana, which has a 6aR, 10aR stereochemistry, was found to be 5 to 100 times more potent than its synthetic (+)-enantiomer in producing static ataxia in dogs, depressing schedule-controlled responding in monkeys, and in producing hypothermia and inhibiting spontaneous activity in mice (Dewey et al. 1984; Martin et al. 1981). Similarly, Hollister and co-workers (Hollister et al. 1987) showed enantioselectivity of THC enantiomers in human studies using indices of the subjective experience, or "high," while May's group found enantioselectivity in a series of structurally modified Δ^9 -THC analogs in tests of motor depression and analgesia (Wilson and May 1975; Wilson et al. 1976, 1979).

Pfizer's levonantradol (CP-50,556-1) is 30 times as potent as (-)- Δ^9 -THC in several in vivo tests, whereas its (+)-enantiomer, dextronantradol (CP-53,870-1) is inactive (Little et al. 1988). (-)-CP-55,244 (NCCs with ACD ring) and (-)-CP-55,940 analogs are 30 to 2,000 times more potent than their respective (+)-enantiomers (Little et al. 1988).

(-)-Cannabidiol (CBD) is a non-psychotropic component of cannabis with possible therapeutic use as an anti-inflammatory drug. Recent studies on both enantiomers of CBD showed enantioselectivity in their interaction with cannabinoid and vanilloid (VR1) receptors as well as on the cellular uptake and enzymatic hydrolysis of anandamide (Bisogno et al. 2001).

HU210 [(-)-R,R-11-hydroxy-1',1'-dimethylheptyl- Δ^8 -THC] is one of the most potent cannabinoids known. It acts through CB₁ and CB₂ receptors and is a potent inhibitor of forskolin-stimulated cyclic adenosine monophosphate (cAMP) production. Both the affinity and potency of HU210 are much higher than those of its synthetic (+)-S, S-enantiomer HU211 (also called dextranabinol). HU-211 is devoid of cannabinoid activity but has other interesting in vivo properties, including its action as an NMDA (N-methyl-D-aspartate) antagonist, antioxidant, and inhibitor of the synthesis of tumor-necrosis factor (TNF). It has found utility as a potential neuroprotective agent, and after favorable results in animal models (Shohami and Mechoulam 2000), it is now undergoing phase III clinical trials in Europe and Israel for traumatic brain injury (Knoller et al. 2002; Agranat et al. 2002).

The classical/non-classical cannabinoid hybrid AM4030 was resolved using chiral AD columns (Thakur et al. 2002). The (-)-isomer AM4030a has the (6S, 6aR, 9R, 10aR) stereochemistry and binds to CB₁ with subnanomolar affinity. The affinity of AM4030a was 158 times higher than that of its (+)-isomer AM4030b.

In the class of 3,4-diarylpyrazolines, SLV-319, the (-)-enantiomer, was found to bind to CB₁ with high affinity and selectivity (CB₁ = 7.8 nM, CB₂ = 7,943 nM) and ~100-fold higher potency than its (+)-isomer (Lange et al. 2004).

WIN-55,212-2, the (+)-enantiomer binds with high affinity to CB₁ (1.9 nM) and CB₂ (0.3 nM) whereas its (–)-isomer, WIN-55,212-3 does not bind significantly to CB₁ and CB₂ (both >1000 nM) (Pertwee 1997; Xie et al. 1995). The aminoalkylindole AM1241 exhibits high CB₂ selectivity (Ibrahim et al. 2003; Malan et al. 2001). Enantiomeric resolution of this ligand using chiral AD column gave the eutomer *R*-(+)-AM1241, which shows higher CB₂ affinity and selectivity (CB₁ = 139.7 nM; CB₂ = 1.4 nM) than *S*-(–)-AM1241 (CB₁ = 2049 nM; CB₂ = 160.5 nM). Recently, the asymmetric synthesis of *R*-(+)-AM1241 was carried out (A. Zvonok and A. Makriyannis, unpublished results).

AM356, *R*-(+) methanandamide, (Abadji et al. 1994; Lin et al. 1998) showed 4 times higher affinity (CB₁ = 17.9 nM) for CB₁ receptor than that of anandamide and 17 times higher than that of *S*-(–) methanandamide (CB₁ = 309 nM). Conversely, the *S*-enantiomer is a considerably better substrate of FAAH.

5

Present and Future

Currently, the field of cannabinoid research is at a very exciting phase. Understanding of the structural–activity relationships (SARs) of cannabinergic ligands has led to the development of highly selective and potent agonists, antagonists, and inverse agonists that in turn have assisted in the biochemical and pharmacological characterization of the cannabinoid receptors. These potent and selective compounds are now playing a major role in unraveling the physiological functions of the endocannabinoid system and the signaling mechanisms associated with it. Furthermore, some of these ligands are being evaluated for their potential therapeutic usefulness. In parallel with the above work, the binding motifs of the different classes of cannabinergic ligands are being elucidated with the help of receptor mutants and suitably designed high-affinity covalent binding probes.

Recent results describing the effects of some cannabinergic ligands in CB₁/CB₂ knockout mice suggest the presence of more cannabinoid-like receptors. One such receptor has been characterized pharmacologically in the vascular endothelium. The prospect of such novel cannabinoid or cannabinoid-like receptors offers excellent opportunities for future SAR work and the development of suitable probes for these new systems. Similarly, the recognition that the endocannabinoid system is closely linked biochemically to a number of key lipid modulators offers additional opportunities for the development of novel lipidomimetic ligand probes and potential therapeutic agents.

Acknowledgements. Supported by grants from National Institutes on Drug Abuse (DA9158, DA03801, and DA07215).

References

- Abadji V, Lin S, Taha G, Griffin G, Stevenson LA, Pertwee RG, Makriyannis A (1994) (R)-Methanandamide: a chiral novel anandamide possessing higher potency and metabolic stability. *J Med Chem* 37:1889–1893
- Abood ME, Ditto KE, Noel MA, Showalter VM, Tao Q (1997) Isolation and expression of a mouse CB1 cannabinoid receptor gene. Comparison of binding properties with those of native CB1 receptors in mouse brain and N18TG2 neuroblastoma cells. *Biochem Pharmacol* 53:207–214
- Adams IB, Ryan W, Singer M, Razdan RK, Compton DR, Martin BR (1995a) Pharmacological and behavioral evaluation of alkylated anandamide analogs. *Life Sci* 56:2041–2048
- Adams IB, Ryan W, Singer M, Thomas BF, Compton DR, Razdan RK, Martin BR (1995b) Evaluation of cannabinoid receptor binding and in vivo activities for anandamide analogs. *J Pharmacol Exp Ther* 273:1172–1181
- Adams R, Harfenist M, Loewe S (1949) New analogs of tetrahydrocannabinol. XIX. *J Am Chem Soc* 71:1624–1628
- Agranat I, Caner H, Caldwell J (2002) Putting chirality to work: the strategy of chiral switches. *Nat Rev Drug Discov* 1:753–768
- Archer RA, Stark P, Lemberger L (1986) Nabilone. In: Mechoulam R (ed) *Cannabinoids as therapeutic agents*. CRC Press, Boca Raton, pp 85–103
- Barnett-Norris J, Guarnieri F, Hurst DP, Reggio PH (1998) Exploration of biologically relevant conformations of anandamide, 2-arachidonylglycerol, and their analogues using conformational memories. *J Med Chem* 41:4861–4872
- Barnett-Norris J, Hurst DP, Lynch DL, Guarnieri F, Makriyannis A, Reggio PH (2002) Conformational memories and the endocannabinoid binding site at the cannabinoid CB1 receptor. *J Med Chem* 45:3649–3659
- Barth F, Rinaldi-Carmona M (1999) The development of cannabinoid antagonists. *Curr Med Chem* 6:745–755
- Begg M, Mo FM, Offertaler L, Batkai S, Pacher P, Razdan RK, Lovinger DM, Kunos G (2003) G protein-coupled endothelial receptor for atypical cannabinoid ligands modulates a Ca²⁺-dependent K⁺ current. *J Biol Chem* 278:46188–46194
- Bell MR, D'Ambra TE, Kumar V, Eissenstat MA, Herrmann JL, Wetzel JR, Rosi D, Philion RE, Daum SJ, Hlasta DJ, Kullnig RK, Ackerman JH, Haubrich BR, Luttinger DA, Baizman ER, Miller MS, Ward SJ (1991) Antinociceptive (aminoalkyl)indoles. *J Med Chem* 34:1099–1110
- Beltramo M, Stella N, Calignano A, Lin SY, Makriyannis A, Piomelli D (1997) Functional role of high-affinity anandamide transport, as revealed by selective inhibition. *Science* 277:1094–1097
- Bisogno T, Hanus L, De Petrocellis L, Tchilibon S, Ponde DE, Brandi I, Moriello AS, Davis JB, Mechoulam R, Di Marzo V (2001) Molecular targets for cannabidiol and its synthetic analogues: effect on vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide. *Br J Pharmacol* 134:845–852
- Breivogel CS, Childers SR (1998) The functional neuroanatomy of brain cannabinoid receptors. *Neurobiol Dis* 5:417–431
- Burley SK, Petsko GA (1985) Aromatic-aromatic interaction: a mechanism of protein structure stabilization. *Science* 229:23–28
- Busch-Petersen J, Hill WA, Fan P, Khanolkar A, Xie XQ, Tius MA, Makriyannis A (1996) Unsaturated side chain β -11-hydroxyhexahydrocannabinol analogs. *J Med Chem* 39:3790–3796
- Chakrabarti A, Onaivi ES, Chaudhuri G (1995) Cloning and sequencing of a cDNA encoding the mouse brain-type cannabinoid receptor protein. *DNA Seq* 5:385–388
- Charalambous A, Lin S, Marciniak G, Banijamali A, Friend FL, Compton DR, Martin BR, Makriyannis A (1991) Pharmacological evaluation of halogenated Δ^8 -THC analogs. *Pharmacol Biochem Behav* 40:509–512

- Charalambous A, Yan G, Houston DB, Howlett AC, Compton DR, Martin BR, Makriyannis A (1992) 5'-Azido- Δ^8 -THC: a novel photoaffinity label for the cannabinoid receptor. *J Med Chem* 35:3076–3079
- Chu C, Ramamurthy A, Makriyannis A, Tius MA (2003) Synthesis of covalent probes for the radiolabeling of the cannabinoid receptor. *J Org Chem* 68:55–61
- Compton DR, Gold LH, Ward SJ, Balster RL, Martin BR (1992) Aminoalkylindole analogs: cannabimimetic activity of a class of compounds structurally distinct from Δ^9 -tetrahydrocannabinol. *J Pharmacol Exp Ther* 263:1118–1126
- Corey EJ, Iguchi S, Albright J, De B (1983) Studies on the conformational mobility of arachidonic acid. Facile macrolactonization of 20-hydroxyarachidonic acid. *Tetrahedron Lett* 24:37–40
- Corey EJ, Cashman JR, Kantner SS, Wright SW (1984) Rationally designed, potent competitive inhibitors of leukotriene biosynthesis. *J Am Chem Soc* 106:1503–1504
- Crocker PJ, Saha B, Ryan WJ, Wiley JL, Martin BR, Ross RA, Pertwee RG, Razdan RK (1999) Development of agonists, partial agonists and antagonists in the Δ^8 -tetrahydrocannabinol series. *Tetrahedron* 55:13907–13926
- D'Ambra TE, Estep KG, Bell MR, Eissenstat MA, Josef KA, Ward SJ, Haycock DA, Baizman ER, Casiano FM, Beglin NC, Chippari SM, Grego JD, Kullnig RK, Daley GT (1992) Conformationally restrained analogues of pravadoline: nanomolar potent, enantioselective, (aminoalkyl) indole agonists of the cannabinoid receptor. *J Med Chem* 35:124–135
- D'Ambra TE, Eissenstat MA, Abt J, Ackerman JH, Bacon ER, Bell MR, Carabateas PM, Josef KA, Kumar V, Weaver JDI, Arnold R, Casiano FM, Chippari SM, Haycock DA, Kuster JE, Luttinger DA, Stevenson LA, Ward SJ, Hill WA, Khanolkar AD, Makriyannis A (1996) C-attached aminoalkylindoles: potent cannabinoid mimetics. *Bioorg Med Chem Lett* 6:17–22
- Devane WA, Dysarz FA, Johnson RM, Melvin LS, Howlett AC (1988) Determination and characterization of a cannabinoid receptor in rat brain. *Mol Pharmacol* 34:605–613
- Devane WA, Breuer A, Sheskin T, Jarbe TU, Eisen MS, Mechoulam R (1992a) A novel probe for the cannabinoid receptor. *J Med Chem* 35:2065–2069
- Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum A, Etinger A, Mechoulam R (1992b) Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* 258:1946–1949
- Dewey WL, Martin BR, May EL (1984) Cannabinoid stereoisomers: pharmacological effects. In: Smith DF (ed) *CRC Handbook. Stereoisomers: drugs psychopharmacology*. CRC Press, Boca Raton, pp 317–326
- Di Marzo V (1998) 2-Arachidonoyl-glycerol as an "endocannabinoid": limelight for a formerly neglected metabolite. *Biochemistry (Mosc)* 63:13–21
- Di Marzo V, Fontana A, Cadas H, Schinelli S, Cimino G, Schwartz JC, Piomelli D (1994) Formation and inactivation of endogenous cannabinoid anandamide in central neurons. *Nature* 372:686–691
- Di Marzo V, Bisogno T, Sugiura T, Melck D, De Petrocellis L (1998) The novel endogenous cannabinoid 2-arachidonoylglycerol is inactivated by neuronal- and basophil-like cells: connections with anandamide. *Biochem J* 331:15–19
- Di Marzo V, Bisogno T, De Petrocellis L, Brandi I, Jefferson RG, Winckler RL, Davis JB, Dasse O, Mahadevan A, Razdan RK, Martin BR (2001) Highly selective CB1 cannabinoid receptor ligands and novel CB1/VR1 vanilloid receptor "hybrid" ligands. *Biochem Biophys Res Commun* 281:444–451
- Dinh TP, Carpenter D, Leslie FM, Freund TF, Katona I, Sensi SL, Kathuria S, Piomelli D (2002) Brain monoglyceride lipase participating in endocannabinoid inactivation. *Proc Natl Acad Sci USA* 99:10819–10824
- Drake DJ, Jensen RS, Busch-Petersen J, Kawakami JK, Fernandez-Garcia MC, Fan P, Makriyannis A, Tius MA (1998) Classical/nonclassical hybrid cannabinoids: southern aliphatic chain-functionalized C-6 β methyl, ethyl and propyl analogues. *J Med Chem* 41:3596–3608

- Dutta AK, Ryan W, Thomas BF, Singer M, Compton DR, Martin BR, Razdan RK (1997) Synthesis, pharmacology, and molecular modeling of novel 4-alkyloxyindole derivatives related to cannabimimetic aminoalkyl indoles (AAIs). *Bioorg Med Chem* 5:1591–1600
- Eissenstat MA, Bell MR, D'Ambra TE, Alexander EJ, Daum SJ, Ackerman JH, Gruett MD, Kumar V, Estep KG, Olefirowicz EM, Wetzel JR, Alexander EJ, Weaver JDI, Haycock DA, Luttinger DA, Casiano FM, Chippari SM, Kuster JE, Stevenson LA, Ward SJ (1995) Aminoalkylindoles: structure-activity relationships of novel cannabinoid mimetics. *J Med Chem* 38:3094–3105
- Fegley D, Kathuria S, Mercier R, Li C, Goutopoulos A, Makriyannis A, Piomelli D (2004) Anandamide transport is independent of fatty-acid amide hydrolase activity and is blocked by the hydrolysis-resistant inhibitor AM1172. *Proc Natl Acad Sci USA* 101:8756–8761
- Felder CC, Joyce KE, Briley EM, Glass M, Mackie KP, Fahey KJ, Cullinan GJ, Hunden DC, Johnson DW, Chaney MO, Koppel GA, Brownstein M (1998) LY320135, a novel cannabinoid CB1 receptor antagonist, unmasks coupling of the CB1 receptor to stimulation of cAMP accumulation. *J Pharmacol Exp Ther* 284:291–297
- Ferrarini PL, Calderone V, Cavallini T, Manera C, Saccomanni G, Pani L, Ruiu S, Gessa GL (2004) Synthesis and biological evaluation of 1,8-naphthyridin-4(1H)-on-3-carboxamide derivatives as new ligands of cannabinoid receptors. *Bioorg Med Chem* 12:1921–1933
- Francisco MEY, Seltzman HH, Gilliam AF, Mitchell RA, Rider SL, Pertwee RG, Stevenson LA, Thomas BF (2002) Synthesis and structure-activity relationships of amide and hydrazide analogues of the cannabinoid CB1 receptor antagonist N-(piperidinyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (SR141716). *J Med Chem* 45:2708–2719
- Gaetani S, Cuomo V, Piomelli D (2003) Anandamide hydrolysis: a new target for anti-anxiety drugs? *Trends Mol Med* 9:474–478
- Gaoni Y, Mechoulam R (1964) Hashish. III. Isolation, structure, and partial synthesis of an active constituent of hashish. *J Am Chem Soc* 86:1646–1647
- Gareau Y, Dufresne C, Gallant M, Rochette C, Sawyer N, Slipetz DM, Tremblay N, Weech PK, Metters KM, Labelle M (1996) Structure activity relationships of tetrahydrocannabinol analogs on human cannabinoid receptors. *Bioorg Med Chem Lett* 6:189–194
- Gatley SJ, Lan R, Pyatt B, Gifford AN, Volkow ND, Makriyannis A (1997) Binding of the non-classical cannabinoid CP-55,940, and the diarylpyrazole AM251 to rodent brain cannabinoid receptors. *Life Sci* 61:1191–1197
- Gatley SJ, Lan R, Volkow ND, Pappas N, King P, Wong CT, Gifford AN, Pyatt B, Dewey SL, Makriyannis A (1998) Imaging the brain marijuana receptor: development of a radioligand that binds to cannabinoid CB1 receptors in vivo. *J Neurochem* 70:417–423
- Gerard C, Mollereau C, Vassart G, Parmentier M (1990) Nucleotide sequence of a human cannabinoid receptor cDNA. *Nucleic Acids Res* 18:7142
- Gerard CM, Mollereau C, Vassart G, Parmentier M (1991) Molecular cloning of a human brain cannabinoid receptor which is also expressed in testis. *Biochem J* 279:129–134
- Gifford AN, Tang Y, Gatley SJ, Volkow ND, Lan R, Makriyannis A (1997) Effect of the cannabinoid receptor SPECT agent, AM 281, on hippocampal acetylcholine release from rat brain slices. *Neurosci Lett* 238:84–86
- Goutopoulos A, Fan P, Khanolkar AD, Xie XQ, Lin S, Makriyannis A (2001) Stereochemical selectivity of methanandamides for the CB1 and CB2 cannabinoid receptors and their metabolic stability. *Bioorg Med Chem* 9:1673–1684
- Goutopoulos A, Makriyannis A (2002) From cannabis to cannabinergics new therapeutic opportunities. *Pharmacol Ther* 95:103–117
- Hajos N, Freund TF (2002) Pharmacological separation of cannabinoid sensitive receptors on hippocampal excitatory and inhibitory fibers. *Neuropharmacology* 43:503–510
- Hanus L, Gopher A, Almog S, Mechoulam R (1993) Two new unsaturated fatty acid ethanolamides in brain that bind to the cannabinoid receptor. *J Med Chem* 36:3032–3034

- Hanus L, Breuer A, Tchilibon S, Shiloah S, Goldenberg D, Horowitz M, Pertwee RG, Ross RA, Mechoulam R, Fride E (1999) HU-308: a specific agonist for CB(2), a peripheral cannabinoid receptor. *Proc Natl Acad Sci USA* 96:14228–14233
- Hanus L, Abu-Lafi S, Fride E, Breuer A, Vogel Z, Shalev DE, Kustanovich I, Mechoulam R (2001) 2-Arachidonyl glyceryl ether, an endogenous agonist of the cannabinoid CB1 receptor. *Proc Natl Acad Sci U S A* 98:3662–3665
- Harrington PE, Stergiades IA, Erickson J, Makriyannis A, Tius MA (2000) Synthesis of functionalized cannabinoids. *J Org Chem* 65:6576–6582
- Herkenham M (1991) Characterization and localization of cannabinoid receptors in brain: an in vitro technique using slide-mounted tissue sections. *NIDA Res Monogr* 112:129–145
- Herkenham M, Lynn AB, Little MD, Johnson MR, Melvin LS, de Costa BR, Rice KC (1990) Cannabinoid receptor localization in brain. *Proc Natl Acad Sci USA* 87:1932–1936
- Hillard CJ, Edgemond WS, Jarrahian A, Campbell WB (1997) Accumulation of N-arachidonoylethanolamine (anandamide) into cerebellar granule cells occurs via facilitated diffusion. *J Neurochem* 69:631–638
- Hollister LE, Gillespie HK, Mechoulam R, Srebnik M (1987) Human pharmacology of 1S and 1R enantiomers of Δ -3-tetrahydrocannabinol. *Psychopharmacology (Berl)* 92:505–507
- Hosohata K, Quock RM, Hosohata Y, Burkey TH, Makriyannis A, Consroe P, Roeske WR, Yamamura HI (1997a) AM630 is a competitive cannabinoid receptor antagonist in the guinea pig brain. *Life Sci* 61:PL115–PL118
- Hosohata Y, Quock RM, Hosohata K, Makriyannis A, Consroe P, Roeske WR, Yamamura HI (1997b) AM630 antagonism of cannabinoid-stimulated [35S]GTP γ S binding in the mouse brain. *Eur J Pharmacol* 321:R1–R3
- Howlett AC, Wilken GH, Pigg JJ, Houston DB, Lan R, Liu Q, Makriyannis A (2000) Azido- and isothiocyanato-substituted aryl pyrazoles bind covalently to the CB1 cannabinoid receptor and impair signal transduction. *J Neurochem* 74:2174–2181
- Howlett AC, Barth F, Bonner TI, Cabral G, Casellas P, Devane WA, Felder CC, Herkenham M, Mackie K, Martin BR, Mechoulam R, Pertwee RG (2002) International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacol Rev* 54:161–202
- Huffmann JW, Dai D, Martin BR, Compton DR (1994) Design, synthesis and pharmacology of cannabimimetic indoles. *Bioorg Med Chem Lett* 4:563–566
- Huffman JW, Yu S, Showalter V, Abood ME, Wiley JL, Compton DR, Martin BR, Bramblett RD, Reggio PH (1996) Synthesis and pharmacology of a very potent cannabinoid lacking a phenolic hydroxyl with high affinity for the CB2 receptor. *J Med Chem* 39:3875–3877
- Huffman JW, Liddle J, Yu S, Aung MM, Abood ME, Wiley JL, Martin BR (1999) 3-(1',1'-Dimethylbutyl)-1-deoxy- Δ 8-THC and related compounds: synthesis of selective ligands for the CB2 receptor. *Bioorg Med Chem* 7:2905–2914
- Huffman JW, Bushell SM, Miller JRA, Wiley JL, Martin BR (2002) 1-Methoxy-, 1-deoxy-11-hydroxy- and 11-hydroxy-1-methoxy- Δ 8-tetrahydrocannabinols: new selective ligands for the CB2 receptor. *Bioorg Med Chem* 10:4119–4129
- Huffman JW, Mabon R, Wu M-J, Lu J, Hart R, Hurst DP, Reggio PH, Wiley JL, Martin BR (2003a) 3-Indolyl-1-naphthylmethanes: new cannabimimetic indoles provide evidence for aromatic stacking interactions with the CB1 cannabinoid receptor. *Bioorg Med Chem* 11:539–549
- Huffman JW, Miller JRA, Liddle J, Yu S, Thomas BF, Wiley JL, Martin BR (2003b) Structure-activity relationships for 1',1'-dimethylalkyl- Δ 8-tetrahydrocannabinols. *Bioorg Med Chem* 11:1397–1410
- Hynes JJ, Leftheris K, Wu H, Pandit CR, Chen P, Norris DJ, Chen BC, Zhao R, Kiener PA, Chen X, Turk LA, Patil-koota V, Gillooly KM, Shuster DJ, McIntyre KW (2002) C-3 Amido-indole cannabinoid receptor modulators. *Bioorg Med Chem Lett* 12:2399–2402
- Ibrahim MM, Deng H, Zvonok A, Cockayne DA, Kwan J, Mata HP, Vanderah TW, Lai J, Porreca F, Makriyannis A, Malan TPJ (2003) Activation of CB2 cannabinoid receptors

- by AM1241 inhibits experimental neuropathic pain: pain inhibition by receptors not present in the CNS. *Proc Natl Acad Sci U S A* 100:10529–10533
- Iwamura H, Suzuki H, Ueda Y, Kaya T, Inaba T (2001) In vitro and in vivo pharmacological characterization of JTE-907, a novel selective ligand for cannabinoid CB2 receptor. *J Pharmacol Exp Ther* 296:420–425
- Jagerovic N, Hernandez-Folgado L, Alkorta I, Goya P, Navarro M, Serrano A, Rodriguez de Fonseca F, Dannert MT, Alsasua A, Suardiaz M, Pascual D, Martin MI (2004) Discovery of 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-3-hexyl-1H-1,2,4-triazole, a novel in vivo cannabinoid antagonist containing a 1,2,4-triazole motif. *J Med Chem* 47:2939–2942
- Johnson MR, Melvin LS (1986) The discovery of non-classical cannabinoid analgesics. In: Mechoulam R (ed) *Cannabinoids as therapeutic agents*. CRC Press, Boca Raton, pp 121–145
- Katoch-Rouse R, Pavlova OA, Caulder T, Hoffman AF, Mukhin AG, Horti AG (2003) Synthesis, structure-activity relationship, and evaluation of SR141716 analogues: development of central cannabinoid receptor ligands with lower lipophilicity. *J Med Chem* 46:642–645
- Khanolkar AD, Abadji V, Lin S, Hill WA, Taha G, Abouzid K, Meng Z, Fan P, Makriyannis A (1996) Head group analogs of arachidonylethanolamide, the endogenous cannabinoid ligand. *J Med Chem* 39:4515–4519
- Khanolkar AD, Makriyannis A (1999) Structure-activity relationships of anandamide, an endogenous cannabinoid ligand. *Life Sci* 65:607–616
- Khanolkar AD, Lu D, Fan P, Tian X, Makriyannis A (1999) Novel conformationally restricted tetracyclic analogs of Δ^8 -tetrahydrocannabinol. *Bioorg Med Chem Lett* 9:2119–2124
- Khanolkar AD, Palmer SL, Makriyannis A (2000) Molecular probes for the cannabinoid receptors. *Chem Phys Lipids* 108:37–52
- Knoller N, Levi L, Shoshan I, Reichenthal E, Razon N, Rappaport ZH, Biegon A (2002) Dexanabinol (HU-211) in the treatment of severe closed head injury: a randomized, placebo-controlled, phase II clinical trial. *Crit Care Med* 30:548–554
- Krishnamurthy M, Ferreira AM, Moore BM (2003) Synthesis and testing of novel phenyl substituted side-chain analogues of classical cannabinoids. *Bioorg Med Chem Lett* 13:3487–3490
- Krishnamurthy M, Li W, Moore BM (2004) Synthesis, biological evaluation, and structural studies on N1 and C5 substituted cycloalkyl analogues of the pyrazole class of CB1 and CB2 ligands. *Bioorg Med Chem* 12:393–404
- Kriwacki RW, Makriyannis A (1989) The conformational analysis of Δ^9 - and $\Delta^9,11$ -tetrahydrocannabinols in solution using high resolution nuclear magnetic resonance spectroscopy. *Mol Pharmacol* 35:495–503
- Kumar V, Alexander MD, Bell MR, Eissenstat MA, Casiano FM, Chippari SM, Haycock DA, Luttinger DA, Kuster JE, Miller MS, Stevenson LA, Ward SJ (1995) Morpholinoalkylindenes as antinociceptive agents: novel cannabinoid receptor agonists. *Bioorg Med Chem Lett* 5:381–386
- Lan R, Liu Q, Fan P, Lin S, Fernando SR, McCallion D, Pertwee RG, Makriyannis A (1999) Structure-activity relationship of pyrazole derivatives as cannabinoid receptor antagonists. *J Med Chem* 42:776–779
- Lang W, Qin C, Lin S, Khanolkar AD, Goutopoulos A, Fan P, Abouzid K, Meng Z, Biegel D, Makriyannis A (1999) Substrate specificity and stereoselectivity of rat brain microsomal anandamide amidohydrolase. *J Med Chem* 42:896–902
- Lange JHM, Coolen HKAC, Van Stuijvenberg HH, Dijkman JAR, Herremans AHJ, Ronken E, Keizer HG, Tipker K, McCreary AC, Veerman W, Wals HC, Stork B, Verveer PC, den Hartog AP, de Jong NMJ, Adolfs TJP, Hoogendoorn J, Kruse CG (2004) Synthesis, biological properties, and molecular modeling investigations of novel 3,4-diarylpyrazolines as potent and selective CB1 cannabinoid receptor antagonists. *J Med Chem* 47:627–643
- Lee CM, Michaels RJ, Zaugg HE, Dren AT, Plotnikoff NP, Young PR (1977) Cannabinoids. Synthesis and central nervous system activity of 8-substituted 10-hydroxy-5,5-dimethyl-5H-[1]benzopyrano[4,3-c]pyridine and derivatives. *J Med Chem* 20:1508–1511

- Lee CM, Zaugg HE, Michaels RJ, Dren AT, Plotnikoff NP, Young PR (1983) New azacannabinoids highly active in the central nervous system. *J Med Chem* 26:278–280
- Liddle J, Huffman JW (2001) Enantioselective synthesis of 11-hydroxy-(1'S,2'R)-dimethylheptyl- Δ^8 -THC, a very potent CB1 agonist. *Tetrahedron* 57:7607–7612
- Lin S, Khanolkar AD, Fan P, Goutopoulos A, Qin C, Papahadjis D, Makriyannis A (1998) Novel analogues of arachidonylethanolamide (anandamide): affinities for the CB1 and CB2 cannabinoid receptors and metabolic stability. *J Med Chem* 41:5353–5361
- Little PJ, Compton DR, Johnson MR, Melvin LS, Martin BR (1988) Pharmacology and stereoselectivity of structurally novel cannabinoids in mice. *J Pharmacol Exp Ther* 247:1046–1051
- Mahadevan A, Siegel C, Martin BR, Abood ME, Beletskaya I, Razdan RK (2000) Novel cannabinol probes for CB1 and CB2 cannabinoid receptors. *J Med Chem* 43:3778–3785
- Mailleux P, Parmentier M, Vanderhaeghen JJ (1992) Distribution of cannabinoid receptor messenger RNA in the human brain: an in situ hybridization histochemistry with oligonucleotides. *Neurosci Lett* 143:200–204
- Makriyannis A, Rapaka RS (1990) The molecular basis of cannabinoid activity. *Life Sci* 47:2173–2184
- Makriyannis A, Goutopoulos A (2004) Cannabinergics: old and new therapeutic possibilities. In: Makriyannis A, Biegel D (eds) *Drug discovery strategies and methods*. Marcel Dekker, New York, pp 89–128
- Malan TPJ, Ibrahim MM, Deng H, Liu Q, Mata HP, Vanderah T, Porreca F, Makriyannis A (2001) CB2 cannabinoid receptor-mediated peripheral antinociception. *Pain* 93:239–245
- Martin BR, Dewey WL, Harris LS, Beckner J (1975) Marijuana-like activity of new synthetic tetrahydrocannabinols. *Pharmacol Biochem Behav* 3:849–853
- Martin BR, Balster RL, Razdan RK, Harris LS, Dewey WL (1981) Behavioral comparisons of the stereoisomers of tetrahydrocannabinols. *Life Sci* 29:565–574
- Martin BR, Compton DR, Thomas BF, Prescott WR, Little PJ, Razdan RK, Johnson MR, Melvin LS, Mechoulam R, Ward SJ (1991) Behavioral, biochemical, and molecular modeling evaluations of cannabinoid analogs. *Pharmacol Biochem Behav* 40:471–478
- Martin BR, Compton DR, Semus SE, Lin S, Marciniak G, Grzybowska J, Charalambous A, Makriyannis A (1993) Pharmacological evaluation of iodo and nitro analogs of Δ^8 -THC and Δ^9 -THC. *Pharmacol Biochem Behav* 46:295–301
- Martin BR, Jefferson RG, Winckler R, Wiley JL, Thomas BF, Crocker PJ, Williams W, Razdan RK (2002) Assessment of structural commonality between tetrahydrocannabinol and anandamide. *Eur J Pharmacol* 435:35–42
- Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI (1990) Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* 346:561–564
- Matsuda LA, Bonner TI, Lolait SJ (1993) Localization of cannabinoid receptor mRNA in rat brain. *J Comp Neurol* 327:535–550
- Matsumoto K, Stark P, Meister RG (1977a) Cannabinoids. 1. 1-Amino- and 1-mercapto-7,8,9,10-tetrahydro-6H-dibenzo [b,d]pyrans. *J Med Chem* 20:17–24
- Matsumoto K, Stark P, Meister RG (1977b) Synthesis and central nervous system activities of some B-ring homocannabinoid derivatives and related lactones. *J Med Chem* 20:25–30
- Mauler F, Mittendorf J, Horvath E, De Vry J (2002) Characterization of the diarylether sulfonylester (–)-(R)-3-(2-hydroxymethylindanyl-4-oxy)phenyl-4,4,4-trifluoro-1-sulfonate (BAY 38-7271) as a potent cannabinoid receptor agonist with neuroprotective properties. *J Pharmacol Exp Ther* 302:359–368
- Mauler F, Horvath E, de Vry J, Jaeger R, Schwarz T, Sandmann S, Weinz C, Heinig R, Boettcher M (2003) BAY 38-7271: a novel highly selective and highly potent cannabinoid receptor agonist for the treatment of traumatic brain injury. *CNS Drug Rev* 9:343–358
- McPartland JM, Glass M (2003) Functional mapping of cannabinoid receptor homologs in mammals, other vertebrates, and invertebrates. *Gene* 312:297–303
- Mechoulam R, Lander N, Srebnik M, Breuer A, Segal M, Feigenbaum JJ, Jarbe TU, Consroe P (1987) Stereochemical requirements for cannabimimetic activity. *NIDA Res Monogr* 79:15–30

- Mechoulam R, Feigenbaum JJ, Lander N, Segal M, Jarbe TU, Hiltunen AJ, Consroe P (1988) Enantiomeric cannabinoids: stereospecificity of psychotropic activity. *Experientia* 44:762–764
- Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR, Gopher A, Almog S, Martain BR, Compton DR (1995) Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem Pharmacol* 50:83–90
- Mechoulam R, Ben Shabat S, Hanus L, Fride E, Vogel Z, Bayewitch M, Sulcova AE (1996) Endogenous cannabinoid ligands-chemical and biological studies. *J Lipid Mediat Cell Signal* 14:45–49
- Mechoulam R, Devane WA, Glaser R (1999) Cannabinoid geometry and biological activity. In: Nahas GG, Sutin KM, Agurell S (eds) *Marijuana and medicine*. Humana Press, Totowa, pp 65–90
- Melvin LS, Milne GM, Johnson MR, Wilken GH, Howlett AC (1995) Structure-activity relationships defining the ACD-tricyclic cannabinoids: cannabinoid receptor binding and analgesic activity. *Drug Des Discov* 13:155–166
- Mignani S, Hittinger A, Achard D, Bouchard H, Bouquerel J, Capet M, Grisoni S, Malleron JL (2000) Preparation of 1-bis(aryl)methyl-3-(alkylsulfonyl)arylmethyleneazetidines as cannabinoid CB1 receptor antagonists. USA Patent No. WO 0015609, pp 1–239
- Munro S, Thomas KL, Abu-Shaar M (1993) Molecular characterization of a peripheral receptor for cannabinoids. *Nature* 365:61–65
- Mussinu JM, Ruiu S, Mule AC, Pau A, Carai MAM, Loriga G, Murineddu G, Pinna GA (2003) Tricyclic pyrazoles. Part 1: Synthesis and biological evaluation of novel 1,4-dihydroindeno[1,2-c]pyrazol-based ligands for CB1 and CB2 cannabinoid receptors. *Bioorg Med Chem* 11:251–263
- Nadipuram AK, Krishnamurthy M, Ferreira AM, Li W, Moore BM (2003) Synthesis and testing of novel classical cannabinoids: exploring the side chain ligand binding pocket of the CB1 and CB2 receptors. *Bioorg Med Chem Lett* 11:3121–3132
- Nakamura-Palacios EM, Moerschbacher JM, Barker LA (1999) The pharmacology of SR 141716A: a review. *CNS Drug Rev* 5:43–58
- Ng EW, Aung MM, Abood ME, Martin BR, Razdan RK (1999) Unique analogues of anandamide: arachidonyl ethers and carbamates and norarachidonyl carbamates and ureas. *J Med Chem* 42:1975–1981
- Nikas SP, Grzybowska J, Papahatjis DP, Charalambous A, Banijamali AR, Chari R, Fan P, Kourouli T, Lin S, Nitowski AJ, Marciniak G, Guo Y, Li X, Wang C-LJ, Makriyannis A (2004) The role of halogen substitution in classical cannabinoids: a CB1 pharmacophore model. *AAPS Journal* 6(4): Article 30 (<http://www.aapsj.org>)
- Oka S, Tsuchie A, Tokumura A, Muramatsu M, Suhara Y, Takayama H, Waku K, Sugiura T (2003) Ether-linked analogue of 2-arachidonoylglycerol (noladin ether) was not detected in the brains of various mammalian species. *J Neurochem* 85:1374–1381
- Ooms F, Wouters J, Oscari O, Happaerts T, Bouchard G, Carrupt PA, Testa B, Lambert DM (2002) Exploration of the pharmacophore of 3-alkyl-5-arylimidazolidinediones as new CB1 cannabinoid receptor ligands and potential antagonists: synthesis, lipophilicity, affinity, and molecular modeling. *J Med Chem* 45:1748–1756
- Osgood PF, Howes JE, Razdan RK, Pars HG (1978) Drugs derived from cannabinoids. 7. Tachycardia and analgesia structure-activity relationships in Δ^9 -tetrahydrocannabinol and some synthetic analogues. *J Med Chem* 21:809–811
- Palmer SL, Khanolkar AD, Makriyannis A (2000) Natural and synthetic endocannabinoids and their structure-activity relationships. *Curr Pharm Des* 6:1381–1397
- Palmer SL, Thakur GA, Makriyannis A (2002) Cannabinergic ligands. *Chem Phys Lipids* 121:3–19
- Papahatjis D, Kourouli T, Makriyannis A (1996) Pharmacophoric requirements for cannabinoid side-chains. Naphthoyl and naphthylmethyl substituted Δ^8 -tetrahydro-cannabinol analogs. *J Heterocycl Chem* 33:559–562

- Papahatjis DP, Kourouli T, Abadji V, Goutopoulos A, Makriyannis A (1998) Pharmacophoric requirements for cannabinoid side chains: multiple bond and C1'-substituted Δ^8 -tetrahydrocannabinols. *J Med Chem* 41:1195–1200
- Papahatjis DP, Nikas S, Tsoinias A, Vlachou M, Makriyannis A (2001) A new ring-forming methodology for the synthesis of conformationally constrained bioactive molecules. *Chem Lett* 3:192–193
- Papahatjis DP, Nikas SP, Andreou T, Makriyannis A (2002) Novel 1',1'-chain substituted Δ^8 -tetrahydrocannabinols. *Bioorg Med Chem Lett* 12:3583–3586
- Papahatjis DP, Nikas SP, Kourouli T, Chari R, Xu W, Pertwee RG, Makriyannis A (2003) Pharmacophoric requirements for the cannabinoid side chain. Probing the cannabinoid receptor subsite at C1'. *J Med Chem* 46:3221–3229
- Paria BC, Ma W, Andrenyak DM, Schmid PC, Schmid HHO, Moody DE, Deng H, Makriyannis A, Dey SK (1998) Effects of cannabinoids on preimplantation mouse embryo development and implantation are mediated by brain-type cannabinoid receptors. *Biol Reprod* 58:1490–1495
- Pars HG, Granchelli FE, Razdan RK, Keller JK, Teiger DG, Rosenberg FJ, Harris LS (1976) Drugs derived from cannabinoids. 1. Nitrogen analogs, benzopyranopyridines and benzopyranopyrroles. *J Med Chem* 19:445–454
- Pertwee R, Griffin G, Fernando S, Li X, Hill A, Makriyannis A (1995) AM630, a competitive cannabinoid antagonist. *Life Sci* 56:1949–1955
- Pertwee RG (1997) Pharmacology of cannabinoid CB1 and CB2 receptors. *Pharmacol Ther* 74:129–180
- Picone RP, Fournier DJ, Makriyannis A (2002) Ligand based structural studies of the CB1 cannabinoid receptor. *J Pept Res* 60:348–356
- Pinto JC, Potie F, Rice KC, Boring D, Johnson MR, Evans DM, Wilken GH, Cantrell CH, Howlett AC (1994) Cannabinoid receptor binding and agonist activity of amides and esters of arachidonic acid. *Mol Pharmacol* 46:516–522
- Piomelli D, Beltramo M, Glasnapp S, Lin SY, Goutopoulos A, Xie X-Q, Makriyannis A (1999) Structural determinants for recognition and translocation by the anandamide transporter. *Proc Natl Acad Sci U S A* 96:5802–5807
- Priller J, Briley EM, Mansouri J, Devane WA, Mackie K, Felder CC (1995) Mead ethanolamide, a novel eicosanoid, is an agonist for the central (CB1) and peripheral (CB2) cannabinoid receptors. *Mol Pharmacol* 48:288–292
- Razdan RK (1986) Structure-activity relationships in cannabinoids. *Pharmacol Rev* 38:75–149
- Razdan RK, Mahadevan A (2002) Recent advances in the synthesis of endocannabinoid related ligands. *Chem Phys Lipids* 121:21–33
- Reggio PH (1999) Ligand-ligand and ligand-receptor approaches to modeling the cannabinoid CB1 and CB2 receptors: achievements and challenges. *Curr Med Chem* 6:665–683
- Reggio PH (2002) Endocannabinoid structure-activity relationships for interaction at the cannabinoid receptors. *Prostaglandins Leukot Essent Fatty Acids* 66:143–160
- Reggio PH, Basu-Dutt S, Barnett-Norris J, Castro MT, Hurst DP, Seltzman HH, Roche MJ, Gilliam AF, Thomas BF, Stevenson LA, Pertwee RG, Abood ME (1998) The bioactive conformation of aminoalkylindoles at the cannabinoid CB1 and CB2 receptors: insights gained from (E)- and (Z)-naphthylidene indenenes. *J Med Chem* 41:5177–5187
- Reggio PH, Greer KV, Cox SM (1989) The importance of the orientation of the C9 substituent to cannabinoid activity. *J Med Chem* 32:1630–1635
- Rhee MH, Vogel Z, Barg J, Bayewitch M, Levy R, Hanus L, Breuer A, Mechoulam R (1997) Cannabinol derivatives: binding to cannabinoid receptors and inhibition of adenylyl cyclase. *J Med Chem* 40:3228–3233
- Rhee MH, Nevo I, Bayewitch ML, Zagoory O, Vogel Z (2000) Functional role of tryptophan residues in the fourth transmembrane domain of the CB(2) cannabinoid receptor. *J Neurochem* 75:2485–2491
- Rich MR (1993) Conformational analysis of arachidonic and related fatty acids using molecular dynamics simulations. *Biochim Biophys Acta* 1178:87–96

- Rinaldi-Carmona M, Barth F, Heaulme M, Shire D, Calandra B, Congy C, Martinez S, Maruani J, Neliat G, Caput D (1994) SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. *FEBS Lett* 350:240–244
- Rinaldi-Carmona M, Barth F, Millan J, Derocq J-M, Casellas P, Congy C, Oustric D, Sarran M, Bouaboula M, Calandra B, Portier M, Shire D, Breliere J-C, LeFur G (1998) SR144528, the first potent and selective antagonist for the CB2 cannabinoid receptor. *J Pharmacol Exp Ther* 284:644–650
- Ross RA, Brockie HC, Stevenson LA, Murphy VL, Templeton F, Makriyannis A, Pertwee RG (1999) Agonist-inverse agonist characterization at CB1 and CB2 cannabinoid receptors of L759633, L759656, and AM630. *Br J Pharmacol* 126:665–672
- Ryan WJ, Banner WK, Wiley JL, Martin BR, Razdan RK (1997) Potent anandamide analogs: the effect of changing the length and branching of the end pentyl chain. *J Med Chem* 40:3617–3625
- Schuel H, Chang MC, Burkman LJ, Picone RP, Makriyannis A, Zimmerman AM, Zimmerman S (1999) Cannabinoid receptors in sperm. In: Nahas GG, Sutin KM, Agurell S (eds) *Marihuana and medicine*. Humana Press, Totowa, pp 335–345
- Seltzman HH, Fleming DN, Thomas BF, Gilliam AF, McCallion DS, Pertwee RG, Compton DR, Martin BR (1997) Synthesis and pharmacological comparison of dimethylheptyl and pentyl analogs of anandamide. *J Med Chem* 40:3626–3634
- Sheskin T, Hanus L, Slager J, Vogel Z, Mechoulam R (1997) Structural requirements for binding of anandamide-type compounds to the brain cannabinoid receptor. *J Med Chem* 40:659–667
- Shim JY, Welsh WJ, Cartier E, Edwards JL, Howlett AC (2002) Molecular interaction of the antagonist N-(Piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide with the CB1 cannabinoid receptor. *J Med Chem* 45:1447–1459
- Shire D, Calandra B, Delpech M, Dumont X, Kaghad M, LeFur G, Caput D, Ferrar P (1996a) Structural features of the central cannabinoid CB1 receptor involved in the binding of the specific CB1 antagonist SR141716A. *J Biol Chem* 271:6941–6946
- Shire D, Calandra B, Rinaldi Carmona M, Oustric D, Pessegue B, Bonnin Cabanne O, Le Fur G, Caput D, Ferrara P (1996b) Molecular cloning, expression and function of the murine CB2 peripheral cannabinoid receptor. *Biochim Biophys Acta* 1307:132–136
- Shohami E, Mechoulam R (2000) Dexanabinol (HU-211): a nonpsychotropic cannabinoid with neuroprotective properties. *Drug Dev Res* 50:211–215
- Stella N, Schweitzer P, Piomelli D (1997) A second endogenous cannabinoid that modulates long-term potentiation. *Nature* 388:773–778
- Stoit AR, Lange JHM, den Hartog AP, Ronken E, Tipker K, van Stuivenberg HH, Dijkman JAR, Wals HC, Kruse CG (2002) Design, synthesis and biological activity of rigid cannabinoid CB1 receptor antagonists. *Chem Pharm Bull (Tokyo)* 50:1109–1113
- Sugiura T, Kodaka T, Nakane S, Miyashita T, Kondo S, Suhara Y, Takayama H, Waku K, Seki C, Baba N, Ishima Y (1999) Evidence that the cannabinoid CB1 receptor is a 2-arachidonoylglycerol receptor. Structure-activity relationship of 2-arachidonoylglycerol, ether-linked analogues, and related compounds. *J Biol Chem* 274:2794–2801
- Suhara Y, Takayama H, Nakane S, Miyashita T, Waku K, Sugiura T (2000) Synthesis and biological activities of 2-arachidonoylglycerol, an endogenous cannabinoid receptor ligand, and its metabolically stable ether-linked analogues. *Chem Pharm Bull (Tokyo)* 48:903–907
- Tarzia G, Duranti A, Tontini A, Spadoni G, Mor M, Rivara S, Vincenzo Plazzi P, Kathuria S, Piomelli D (2003) Synthesis and structure-activity relationships of a series of pyrrole cannabinoid receptor agonists. *Bioorg Med Chem* 11:3965–3973
- Thakur GA, Palmer SL, Harrington PE, Stergiades IA, Tijs MA, Makriyannis A (2002) Enantiomeric resolution of a novel chiral cannabinoid receptor ligand. *J Biochem Biophys Methods* 54:415–422
- Thomas BF, Adams IB, Mascarella SW, Martin BR, Razdan RK (1996) Structure-activity analysis of anandamide analogs: relationship to a cannabinoid pharmacophore. *J Med Chem* 39:471–479

- Thomas BF, Gilliam AF, Burch DE, Roche MJ, Seltzman HH (1998) Comparative receptor binding analyses of cannabinoid agonists and antagonists. *J Pharmacol Exp Ther* 285:285–292
- Tius MA, Kannangara GSK, Kerr MA, Grace KJS (1993) Halogenated cannabinoid synthesis. *Tetrahedron* 49:3291–3304
- Tius MA, Makriyannis A, Long Zou X, Abadji V (1994) Conformationally restricted hybrids of CP-55,940 and HHC: stereoselective synthesis and activity. *Tetrahedron* 50:2671–2680
- Tius MA, Hill WA, Zou XL, Busch-Petersen J, Kawakami JK, Fernandez Garcia MC, Drake DJ, Abadji V, Makriyannis A (1995) Classical/non-classical cannabinoid hybrids; stereochemical requirements for the southern hydroxyalkyl chain. *Life Sci* 56:2007–2012
- Tius MA, Busch-Petersen J, Marris AR (1997) Synthesis of a bifunctional cannabinoid ligand. *J Chem Soc Chem Commun* 19:1867–1868
- Tong W, Collantes ER, Welsh WJ, Berglund BA, Howlett AC (1998) Derivation of a pharmacophore model for anandamide using constrained conformational searching and comparative molecular field analysis. *J Med Chem* 41:4207–4215
- Wiley JL, Compton DR, Dai D, Lainton JA, Phillips M, Huffman JW, Martin BR (1998) Structure-activity relationships of indole- and pyrrole-derived cannabinoids. *J Pharmacol Exp Ther* 285:995–1004
- Wiley JL, Jefferson RG, Grier MC, Mahadevan A, Razdan RK, Martin BR (2001) Novel pyrazole cannabinoids: insights into CB1 receptor recognition and activation. *J Pharmacol Exp Ther* 296:1013–1022
- Wilson RS, May EL (1975) Analgesic properties of the tetrahydrocannabinols, their metabolites, and analogs. *J Med Chem* 18:700–703
- Wilson RS, May EL, Martin BR, Dewey WL (1976) 9-Nor-9-hydroxyhexahydrocannabinols. Synthesis, some behavioral and analgesic properties, and comparison with the tetrahydrocannabinols. *J Med Chem* 19:1165–1167
- Wilson RS, May EL, Dewey WL (1979) Some 9-hydroxycannabinoid-like compounds. Synthesis and evaluation of analgesic and behavioral properties. *J Med Chem* 22:886–888
- Wroblewski ST, Chen P, Hynes JJ, Lin S, Norris DJ, Pandit CR, Spergel S, Wu H, Tokarski JS, Chen X, Gillooly KM, Kiener PA, McIntyre KW, Patil-koota V, Shuster DJ, Turk LA, Yang G, Leftheris K (2003) Rational design and synthesis of an orally active indolopyridone as a novel conformationally constrained cannabinoid ligand possessing antiinflammatory properties. *J Med Chem* 46:2110–2116
- Xie XQ, Eissenstat M, Makriyannis A (1995) Common cannabimimetic pharmacophoric requirements between aminoalkyl indoles and classical cannabinoids. *Life Sci* 56:1963–1970
- Yan G, Yin D, Khanolkar AD, Compton DR, Martin BR, Makriyannis A (1994) Synthesis and pharmacological properties of 11-hydroxy-3-(1',1'-dimethylheptyl)hexahydrocannabinol: a high-affinity cannabinoid agonist. *J Med Chem* 37:2619–2622