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Review

Cannabinergic ligands

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Abstract

The understanding of the pharmacology surrounding the cannabinergic system has seen many advances since the discovery of the CB1 receptor in the mammalian brain and the CB2 receptor in the periphery. Among these advances is the discovery of the endogenous ligands arachidonoylethanolamide (anandamide) and 2-arachidonoylglycerol amide (2-AG), which are selective agonists for the CB1 and CB2 receptors, respectively. These endogenous neuromodulators involved in the cannabinergic system are thought to be produced on demand and are metabolized by the enzymes fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAG lipase). Recently, we characterized a reuptake system that facilitates the transport of anandamide across the cell membrane and subsequently developed selective inhibitors of this transport, which have been found to have therapeutic potential as analgesic and peripheral vasodilators. The cannabinergic proteins currently being explored, which include the CB1 and CB2 receptors, FAAH and the anandamide transporter, are excellent targets for the development of therapeutically useful drugs for a range of conditions including pain, loss of appetite, immunosuppression, peripheral vascular disease and motor disorders. As cannabinoid research has progressed, various potent and selective cannabimimetic ligands, targeting these four cannabinoid proteins, have been designed and synthesized. Many of these ligands serve as important molecular probes, providing structural information regarding the binding sites of the cannabinergic proteins, as well as pharmacological tools, which have been playing pivotal roles in research aimed at understanding the biochemical and physiological aspects of the endocannabinoid system. This review will focus on some of the current cannabinergic ligands and probes and their pharmacological and therapeutic potential.

Keywords: Cannabinoid receptors; Anandamide; Cannabimimetics; Cannabinergics; Cannabinoid receptor agonists; Cannabinoid receptor antagonists; Fatty acid amide hydrolase; Anandamide transporter

1. Introduction

Marijuana (*Cannabis sativa*) is one of the oldest drugs of abuse, but its medicinal value has also been known for many years. It was the identification of Δ^9-tetrahydrocannabinol (Δ^9-THC) as the major psychoactive component in cannabis as well

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as its chemical synthesis that began a new era for synthetic cannabinoids as 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 cannabinoid receptors, both of which are G-protein coupled membrane-bound functional proteins (Matsuda et al., 1990; Gerard et al., 1991). CB1 is found in the central nervous system (CNS) and in a variety of other organs, including the heart, vascular endothelium, uterus, vas deferens, testis and small intestine (Herkenham et al., 1990; Breivogel and Childers, 1998; Gatley et al., 1998; Schuel et al., 1999). Conversely, the CB2 receptor appears to be associated exclusively with the immune system and is found in the periphery of the spleen and other cells associated with immunochemical functions, but not in the brain (Munro et al., 1993). The subsequent discovery of the endogenous cannabinoids (endocannabinoids) arachidonoylethanolamide (anandamide) (Devane et al., 1992; Hanus et al., 1993), 2-arachidonoyl glycerol (2-AG) (Mechoulam et al., 1995, 1998) and very recently, a third 2-arachidonyl ether (noladin ether) (Hanus et al., 2001), has led to a better understanding of the physiological and biochemical role of the endocannabinoid system.

These endogenous cannabinoids have revealed the existence of three additional proteins, fatty acid amide hydrolase (FAAH), monoacylglycerol (MAG) lipase and the anandamide transporter (AT), which are involved in the metabolism of endocannabinoids (Di Marzo et al., 1998; Khanolkar and Makriyannis, 1999).

Pharmacological studies, that will further elucidate the role of the endocannabinoid system in physiological and disease states, are dependent on the availability of selective agents that interact specifically and selectively with each of the endocannabinoid proteins and in turn, either activate or inhibit them. Therefore, structure–activity relationship (SAR) studies on each of these targets and the subsequent identification of differences in their ligand recognition are of great significance, as they can lead to the development of selective cannabinergic agents. In addition, such studies may lead to the development of new therapeutic agents that act through the endocannabinoid system. To date, four cannabinoid system proteins including the CB1 and CB2 receptors, fatty acid amide hydrolase and the anandamide transporter, 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 (Goutopoulos and Makriyannis, 2002; Musty, 2002).

During the last decade, numerous selective ligands for each of the cannabinergic proteins were designed and synthesized (Goutopoulos and Makriyannis, 2002). Many of these agents serve as important molecular probes, providing structural information about receptor binding sites, as well as serving as pharmacological tools for obtaining information about the role of each of these targets in physiological and disease states (Khanolkar et al., 2000). The extensive exploration and structure activity studies of cannabinoid pharmacology have resulted in the development of more structurally diverse classes of cannabimimetic ligands. Currently, six major classes of cannabimimetics have been identified: (1) classical cannabinoids; (2) nonclassical and hybrid cannabinoids; (3) aminoalkylindoles; (4) arachidonoylethanolamides; (5) biarylpyrazoles; and (6) 2-arachidonoylglycerols. This review will focus on the important cannabinoid probes categorized based on their pharmacological properties and will highlight their therapeutic potential.

2. Cannabinergic ligand classifications

Both CB1 and CB2 receptors are members of the superfamily of the seven transmembrane receptors that transduce intracellular signals via heterotrimeric GTP-binding proteins. The CB2 receptor shows 44% identity to the total CB1 receptor and 68% identity within the transmembrane regions (Munro et al., 1993). The central distribution pattern of the CB1 receptor is heterogeneous and accounts for several prominent pharmacological properties of CB1 receptor agonists,

for example their ability to impair cognition and memory and to alter the control of motor function (Pertwee, 2000). Thus, the cerebral cortex, hippocampus, lateral caudate-putamen, substantia nigra pars reticulata, globus pallidus, entopeduncular nucleus and molecular layer of the cerebellum are all populated with particularly high concentrations of CB1 receptor (Pertwee, 1997, 1998). In line with the analgesic properties of cannabinoid receptor agonist, CB1 receptors are also found on pain pathways, in brain and spinal cord and at the peripheral terminals of sensory neurons (Pertwee, 2001). CB2 is present in periphery and mainly in tissues of the immune system. Localization of CB2 in the immune system suggests an immunomodulatory role for this receptor. Thus, CB2 may be the mediator of long-known immunosuppressive properties of marijuana. 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 effect ion channels (Pertwee, 1997).

The most prominent therapeutic applications for cannabinoid agonists being currently pursued include treatment of loss of appetite, pain, anxiety, vomiting, nausea and epilepsy. Therapeutic applications for cannabinoid antagonists include the treatment of anxiety, schizophrenia, spasticity dystonia, as well as addiction to drugs of abuse (Musty, 2002).

2.1. Cannabinoid receptor agonists

2.1.1. Classical cannabinoids (CCs)

Classical cannabinoids are tricyclic terpenoid derivatives bearing a benzopyran moiety (Fig. 1). This class includes the natural product (−)-Δ^9-THC and other pharmacologically active constituents of the plant *C. sativa*. Many CC analogs have been synthesized and evaluated pharmacologically and biochemically (Razdan, 1986; Gareau et al., 1996; Mechoulam et al., 1999 and Thakur and Makriyannis, unpublished). The CC structural features that seem to be important for cannabinoid activity (Makriyannis and Rapaka, 1990) are (1) the phenolic hydroxyl group, which can be substituted by an amino group, but not by a thiol group. However, it has also been shown that CCs in which the phenolic hydroxyl is either replaced by a methoxy group (e.g. L-759633, Figs. 2 and 3) or totally absent (JWH-051, Fig. 3) retain relatively high receptor-binding affinity, especially for CB2 (Huffman et al., 1996). (2) The benzopyran ring is not essential for activity. The pyran oxygen can be substituted by nitrogen or can be eliminated in open phenol or resorcinol analogs. The latter gave rise to the non-CC (NCC) class (e.g. CP55,940, Figs. 4 and 5). (3) Neither the double bond nor the 9-methyl are necessary for the activity. (4) The alkyl chain is probably the most essential CC pharmacophoric group. Increased biological activity results from elongating the five carbon Δ^9-THC chain to a seven carbon chain substituted with 1′,1′-or 1′,2′dimethyl or with 1′,1′-cyclic moieties (e.g. AMG-3; Fig. 2). Oxygen atoms (ethers) and unsaturations (Papahatjis et al., 1998) within the chain or terminal halogen, carboxyamido and cyano groups are well tolerated (Khanolkar et al., 2000). (5) An additional pharmacophoric element, the southern aliphatic hydroxyl (Makriyannis and Rapaka, 1990), in order to produce highly potent classical/ NCC hybrids (e.g. AM919; Fig. 6), was developed by Makriyannis et al. (Drake et al., 1998; Harrington et al., 2000) instigated by the SAR of NCCs.

From these classical cannabinoids, two licensed single-compound cannabimimetic pharmaceuticals, Marinol® (Dronabinol, (−)-Δ^9-tetrahydrocannabinol (Δ^9-THC) from Roxane Laboratories Inc. (Columbus, OH)) and Cesamet® (Nabilone developed at Eli Lilly (Indianapolis, IN) now in use in the UK), are marketed for two indications: the control of nausea and emesis produced by cancer chemotherapy and as appetite stimulants in acquired immunodeficiency syndrome (AIDS)-related anorexia. Both of these agents have proven to be superior to conventional anti-emetics, such as perchlorperazine, in clinical trials with cancer chemotherapy patients (Breivogel and Childers, 1998). Marinol is also in phase II clinical trials (Unimed Pharmaceuticals, IL) for treatment of Alzheimer's disease and disturbed behavior, in addition, other classical cannabinoids, such as dexanabinol (HU-211, Fig. 1) a (+)-THC analog with no significant affinity for either CB1 or CB2

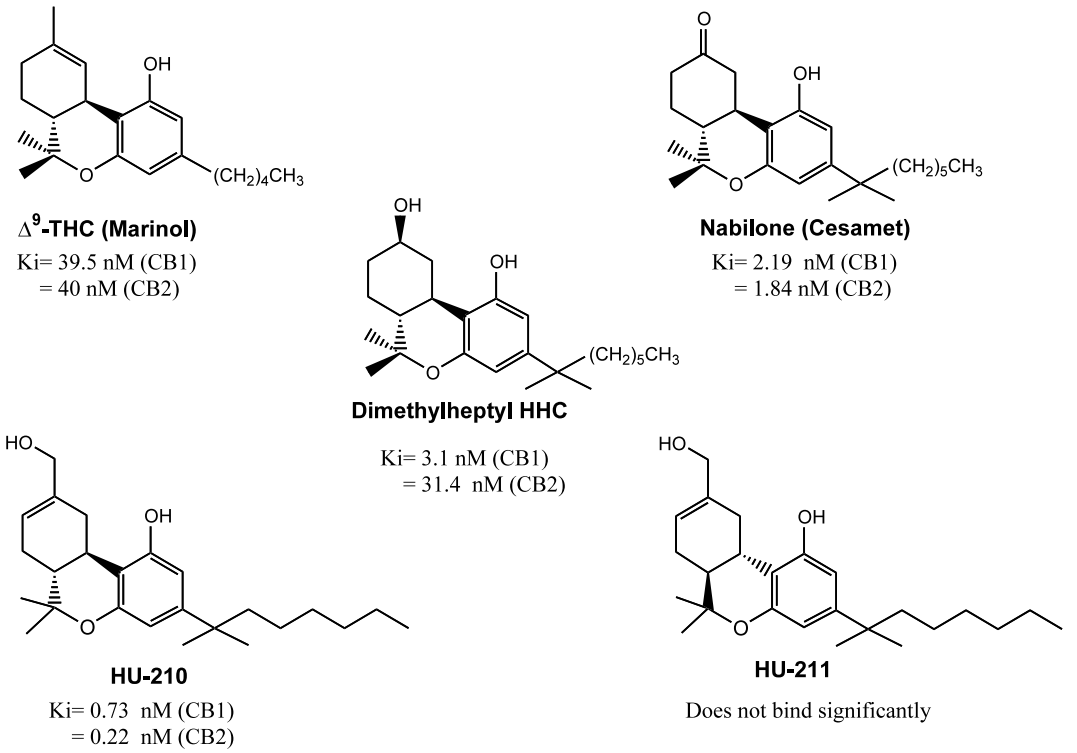


Fig. 1. Natural and synthetic cannabinoid receptor agonists.

(Bayewitch et al., 1995) is in phase III clinical trials (Pharmos Corporation, NJ) for treating traumatic brain injury.

Notwithstanding the relatively low homology between CB1 and CB2, the first generation of CCs did not encompass any ligands with significant subtype selectivity. However, suitable modifications of the key cannabinoid pharmacophores have produced new CB1 or CB2 selective ligands. Thus, substitution of the C-3 alkyl chain in CCs with bicyclic or tricyclic hydrocarbon moieties led to selective ligands for each of the two CB receptors, such as the CB1 selective adamantyl THC (AM411, Fig. 2) and the CB2 selective (AM724, CB1 $K_i = > 6000$ nM; CB2 $K_i = 1.69$ nM), in which the three-substituent is a chiral tricyclic terpene. (Lu et al. 1997and Makriyannis et al., unpublished data). Also, the Merck Frosst group found that methylation of the phenolic group of CCs produces CB2 selective ligands (L-759633, Fig. 3) (Ross et al., 1999b). CB2 selectivity can also be obtained with ligands in which the phenolic OH was eliminated (e.g. JWH-051, Fig. 3) (Huffman et al., 1996). It is believed that the unexpected potency of JWH-051 is due to the interaction of 11-hydroxy with Lysine 192 unit of CB1 receptor (Huffman et al., 1996). Remarkably, a high degree of CB2 selectivity was shown in binding experiments by HU-308, which binds to CB2 receptors at low nanomolar concentrations. Makriyannis and Khanolkar (2001) reported novel cannabinoids having preferential high affinities and selectivities for the CB2 receptor (AM1714). Their improved receptor binding potency and selectivity makes these analogs therapeutically useful as potential medications in human and animals for treatment of neuropathic pain, peripheral pain and inflammation.

Makriyannis et al. have developed several novel cannabinoid receptor affinity ligands that possess reactive groups at judiciously chosen positions within the classical cannabinoid structure. These affinity ligands not only possess agonist properties at the CB receptors, but are useful as probes for

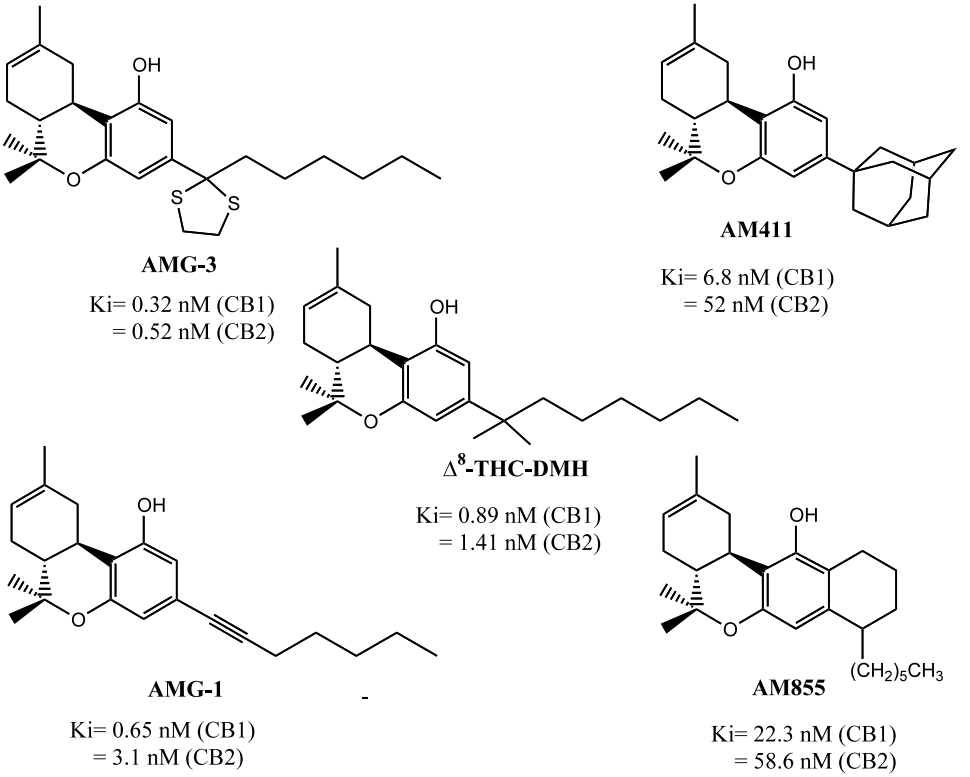


Fig. 2. Side chain analogs of (−)-Δ^8-tetrahydrocannabinol.

obtaining information on the receptor active sites. Two types of reactive groups were incorporated: (1) an electrophilic isothiocyanato group (NCS) which targets nucleophilic amino acid residues, such as lysine, histidine and cysteine at or near the active site; and (2) a photoactivatable aliphatic azido group (N_3) 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. The first photoaffinity label for the cannabinoid receptor, (−)-5′-azido-Δ^8-THC was reported by Makriyannis et al. (Charalambous et al., 1992). Later, two novel high affinity electrophilic covalent probes for cannabinoid receptors in which the isothiocyanate group and azido is the

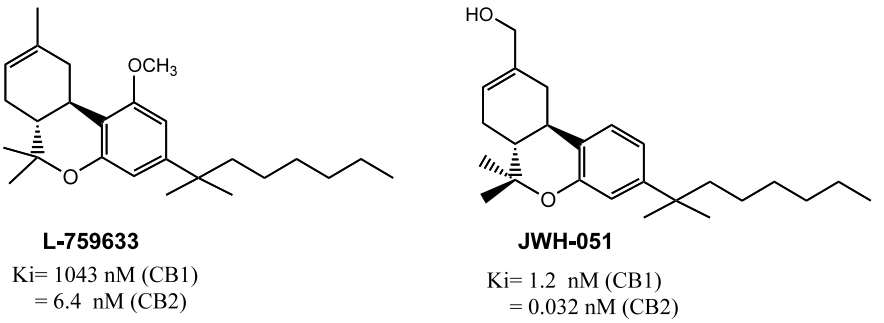
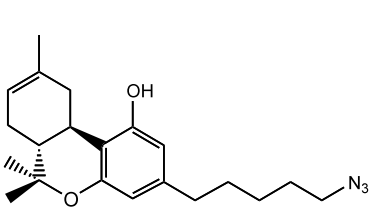
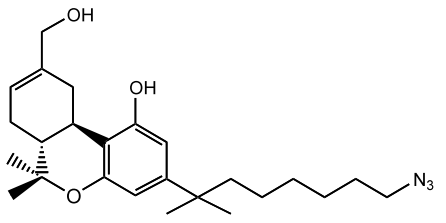


Fig. 3. CB2 selective cannabinoid receptor agonists.



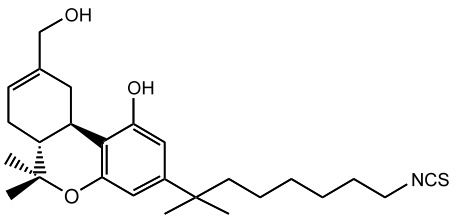
(-)-5'-Azido-Δ^8-THC

IC_{50}= 31 nM (CB1)



AM836

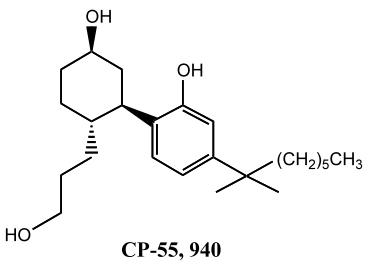
IC_{50}= 0.16 nM (CB1)



AM708

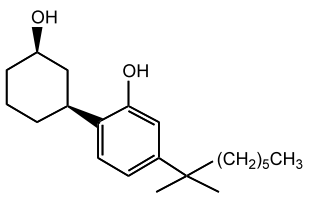
IC_{50}= 1.6 nM (CB1)

Fig. 4. CB agonists as covalent binding probes.



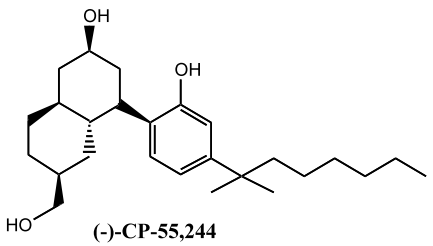
CP-55, 940

Ki= 0.6 nM (CB1)
= 0.7 nM (CB2)



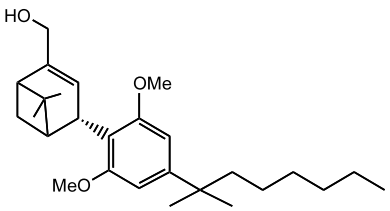
CP-47, 497

Ki= 2.2 nM (CB1)



(-)-CP-55,244

Ki= 1.4 nM (CB1)



HU-308

Ki= >10000 nM (CB1)
= 22.7 nM (CB2)

Fig. 5. Non-classical cannabinoid receptor agonists.

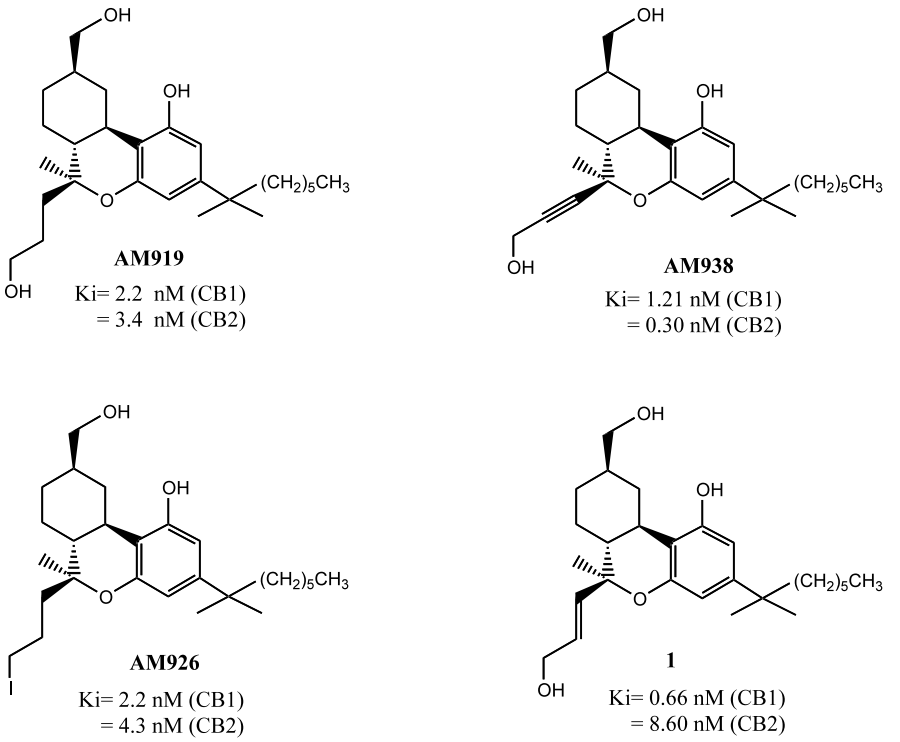


Fig. 6. Cannabinoid receptor agonist having SAH group.

reactive moiety, respectively, have been reported, (Guo et al., 1994; Morse et al., 1995) namely (−)-11-hydroxy-7′-isothiocyanato-Δ^8-THC (AM708; Fig. 4) and (−)-11-hydroxy-7′-azido-Δ^8-THC (AM836).

A significant improvement in the design of these new probes was the introduction of ^{125}I-substituent in the ligand without compromising its high receptor affinity. These radioiodinated probes can serve as valuable tools for receptor purification and characterization (Khanolkar et al., 2000). Currently, a variety of mono- and bifunctional covalent ligands are being used to elucidate the binding motifs of the various classes of cannabinergics for the CB1 and CB2 receptors (Makriyannis et al., unpublished). 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 difficult to crystallize.

2.1.2. *Nonclassical cannabinoids (NCCs)*

A second class of cannabimimetics was developed at Pfizer (Groton, CT) in an effort to simplify the structure of CCs, while maintaining or improving activity. (Johnson and Melvin, 1986; Little et al., 1988). This class includes bicyclic (e.g. CP-55,940) and tricyclic (e.g. CP-55,244) analogs lacking the pyran ring of CCs. These compounds are collectively described as nonclassical cannabinoids (NCCs) (Fig. 5). The crystalline CP-55,940 and its tritiated analog exhibit high affinity, efficacy and stereoselectivity to both cannabinoid receptors and has been used extensively as a generic CB ligand and an important pharmacological tool. [^{3}H]CP-55,940 was the key compound that led to the discovery of CB1 (Devane et al., 1988). The recently discovered CB2-selective ligand HU-308 is another example of such a bicyclic cannabinoid receptor ligand (Hanus et al., 1999).

The side chain and the phenolic hydroxyl of the NCC are crucial for activity. The hydroxypropyl chain of CP-55,940 is not an absolute requirement for activity. However, when present, its stereochemistry is important, with a strong preference for the β relative configuration.

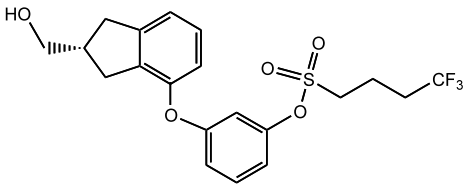
2.1.3. Hybrid cannabinoids (SAH cannabinoid analogs)

The Southern aliphatic hydroxyl (SAH) pharmacophore is absent in the naturally occurring cannabinoids. To study more precisely the stereochemical requirements of this pharmacophore Makriyannis et al. designed a group of hybrid ligands that incorporated all of the structural features of both classical and non-classical cannabinoids (Tius et al., 1994, 1995; Harrington et al., 2000).

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 the β-hydroxypropyl analog had higher affinity than the α-axial epimer. Thus, favorable ligand–receptor interaction results when the SAH group is in the equatorial position.

Further refinement of the CC/NCC hybrid cannabinoids was obtained by introducing a triple or double bond at the C2″ position of the 6β-hydroxypropyl chain. This led to a series of novel cannabinoid receptor probes (e.g. AM938 and **1**; Fig. 6). The affinity data for CB1/CB2 receptors shown in Fig. 6 refers to the racemic compounds. Recently, enantiomers of **1** were separated using chiral HPLC and the levorotatory isomer was found to be more potent (CB1 = 0.16 nM; CB2 = 1.1 nM) than the dextrorotatory isomer (CB1 = 94.8 nM; CB2 = 124.8 nM) (Thakur et al., 2002).

Recently, a novel class of diarylether sulfonylester cannabinoid agonists was reported by Mauler et al. which posses neuroprotective properties. The representative agonist (−)-*R*-3-(2-hydroxymethylindanyl-4-oxy)phenyl-4,4,4-trifluoro-1-sulfonate (BAY 38-7271, Fig. 7) is a high affinity CB1 receptor ligand (K_i = 0.46–1.85 nM; rat brain, human cortex and recombinant human CB1 receptor).



BAY 38-7271
Ki= 0.46 nM (CB1)
= 5.96 nM (CB2)

Fig. 7. Diarylether sulfonylester cannabinoid agonists.

Additionally, the endogenous ligands (Fig. 8), arachidonoylethanolamide (anandamide), 2-arachidonoyl glycerol (2-AG), 2-arachidonyl ether (noladin ether) and various head and tail modified anandamide analogs posses cannabinoid receptor agonist properties (Goutopoulos et al., 2001).

2.2. Cannabinoid receptor antagonists/inverse agonists

2.2.1. Diarylpyrazoles

Several types of CB1 receptor antagonist are currently known. The first of these, the diarylpyrazole, SR141716A (Rimonabant) was developed by Sanofi (Lan et al., 1999; Nakamura-Palacios et al., 1999; Kendall, 2000) and is currently undergoing clinical treatment for psychotic disorders—Alzheimer's disease and schizophrenia—and obesity treatments. This is a highly potent and selective CB1 receptor ligand and prevents CB1 receptor mediated effects both in vitro and in vivo. Another notable CB1 receptor selective antagonist that exhibits inverse CB1 receptor agonist properties in some assay systems is LY320135 (Felder et al., 1998). This agent, which was developed by Eli Lilly, shares the ability of SR141716A to bind much more readily to CB1 than to the CB2 receptor. However, it is a less potent CB1 antagonist than SR141716A.

Other CB1 antagonist/inverse agonist developed in our laboratory are AM251 and AM281 (Fig. 9) which displace [^{3}H] SR141716A and [^{3}H] CP55,940 from binding sites on mouse cerebellar membranes (Gatley et al., 1997; Lan et al., 1999). AM281 has also been reported to bind more readily to CB1 than CB2 receptors and to attenu-

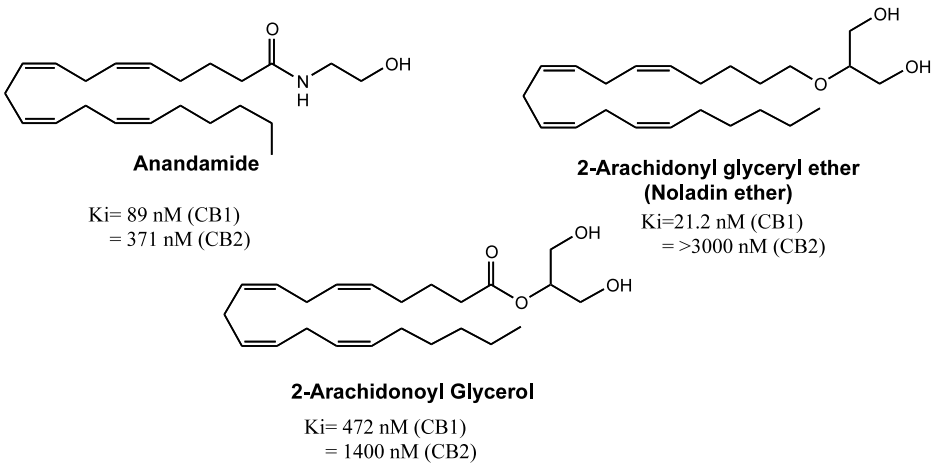


Fig. 8. Endogenous cannabinoid receptor agonists.

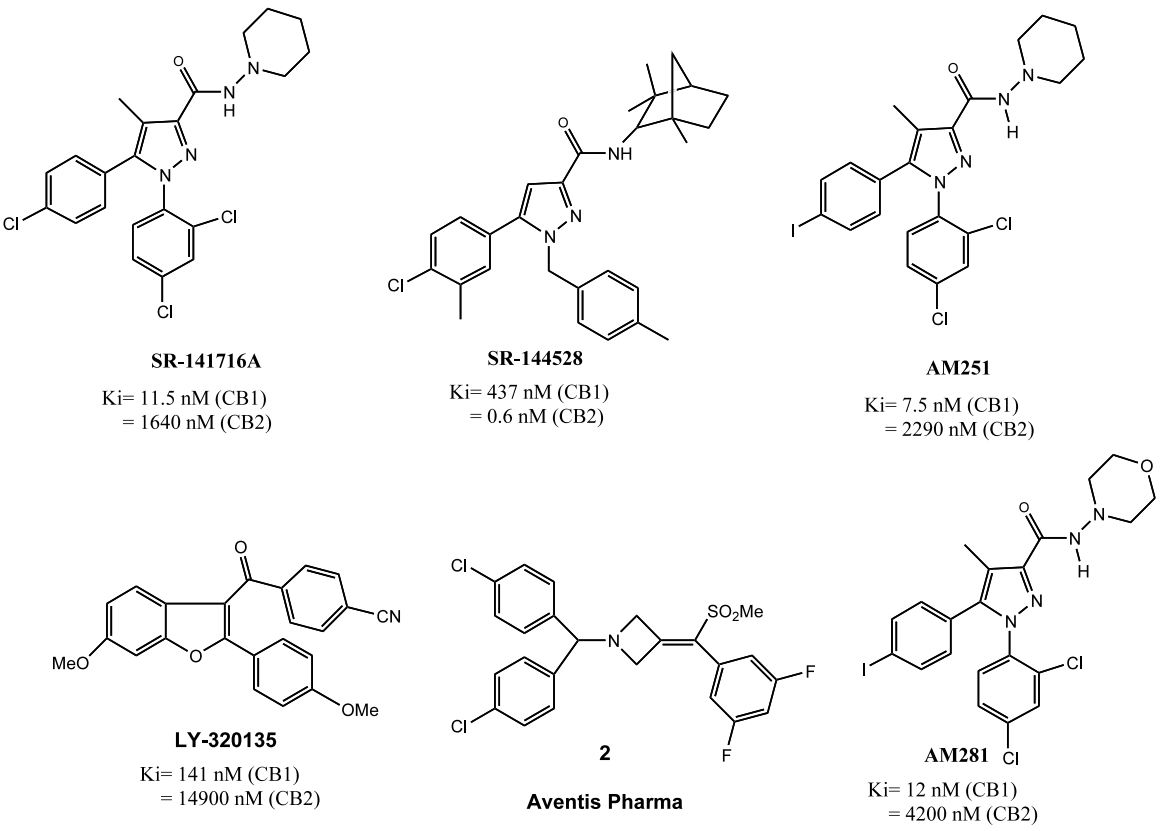


Fig. 9. Diarylpyrazoles and other cannabinoid receptor antagonists/inverse agonists.

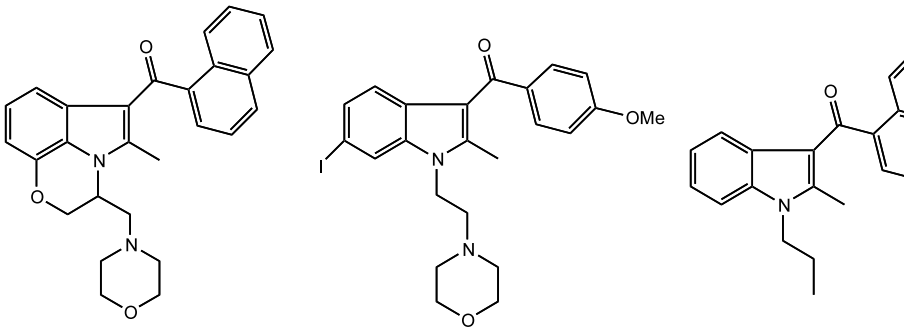


Fig. 10. Cannabinergic aminoalkylindole antagonists.

ate the ability of WIN55,212-2 or CP55,940 to decrease rat locomotor activity, to inhibit single population spikes and evoked acetylcholine release in rat hippocampal slices, to inhibit electrochemically-evoked contractions of the myenteric plexus-longitudinal muscle preparation of guinea-pig ileum and to suppress guinea-pig intestinal peristalsis (Gifford et al., 1997; Lan et al., 1999; Al-Hayani and Davies, 2000; Cosenza et al., 2000; Izzo et al., 2000). Similar to SR141716A, AM281 can behave as inverse agonist when administered alone. Structure activity relationships (SARs) for SR141716A-like compounds has been reviewed earlier by Lan et al. (1999) and more recently by Howlett et al. (2002). While the search for high affinity/efficacy ligands is ongoing, the development of well designed radiolabeled ligands has enhanced the understanding the physiological role of cannabinergics. [^{123}I] AM281, a ^{123}I-labeled 1,5-biarylpyrazole synthesized in our laboratory, has served as a useful imaging agent in positron emission tomography and single photon emission computed tomography studies (Gifford et al., 1997; Gatley et al., 1997, 1998). Recently, Aventis Pharma reported (Mignani et al., 2000) a new class of CB1 receptor antagonists, which are diarylmethyleneazetidine analogs, represented by compound **2** (Fig. 9).

The most notable biarylpyrazole CB2 receptor antagonist/inverse agonist is SR144528, a diarylpyrazole (Fig. 9) developed by Sanofi, exhibits 700-fold selectivity for the CB2 receptor over CB1 (Rinaldi-Carmona et al., 1998; Ross et al., 1999a).

2.2.2. *Aminoalkylindoles*

A second chemical class of cannabinergics is aminoalkylindoles (AAIs) (Fig. 10). They were developed at Sterling Winthrop (Rensselaer, NY) as potential non-steroidal anti-inflammatory agents (Bell et al., 1991). These first analogs exhibited antinociceptive properties that eventually were attributed to interactions with the cannabinoid receptors. WIN55212 is a potent CB1 and CB2 agonist with high stereoselectivity and a slight preference for CB2. AM630, the first CB2-selective antagonist derived from this class of compounds, was reported from our laboratory in 1994 after long-term efforts for the development of such an inhibitor (Pertwee et al., 1995). Subsequently, several additional selective ligands for the

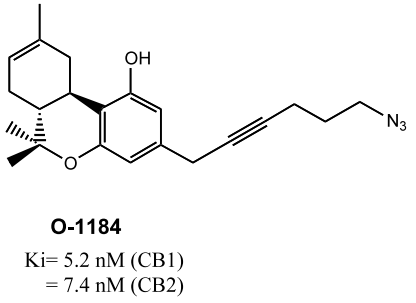


Fig. 11. Classical cannabinoids as antagonist/inverse agonist (O-1184).

CB2 receptor have been reported, one of which is the cannabimimetic indole JWH-015 (Fig. 10) (Little et al., 1988; Showalter et al., 1996). We recently reported compound AM1241, a highly CB2-selective and potent agonist (Malan et al., 2001).

2.2.3. Classical cannabinoids

A CC ligand with partial antagonist properties for both CB1 and CB2 receptors is the classical cannabinoid, 6′-azidohex-2′-yne-Δ^8-THC (O-1184; Fig. 11) (Ross et al., 1999b). In addition to a terminal N_3 group, the alkyl side chain of this ligand contains a carbon–carbon triple bond, a structural modification that reduces CB1 and CB2 efficacy, but not CB1 or CB2 affinity (Ross et al., 1998, 1999b). O-1184 behaves as a high-affinity low-efficacy agonist at CB1 receptors and as a high-affinity low-efficacy inverse agonist at CB2 receptors.

2.3. FAAH inhibitors

The presence of the enzyme fatty acid amide hydrolase (FAAH) in many cannabinoid bioassay systems has created the need for FAAH inhibitors that can be used to protect endocannabinoids from enzymatic hydrolysis (Pertwee, 1997; Maccarrone et al., 1998). FAAH inhibitors also have therapeutic potential as indirectly acting cannabinoid receptor agonists that activate the endocannabinoid system by increasing the concentration of endocannabinoids at the cannabinoid receptors. In other systems, such compounds were shown to exhibit non-favorable pharmacological profiles. Presumably, the FAAH antagonists would exhibit tissue-based selectivities by producing their effects only at sites where on-going production of endocannabinoids is taking place (Pertwee, 1997; Di Marzo et al., 1998). FAAH inhibitors with much greater potency compared to PMSF, the first FAAH inhibitor, are now available. Two notable examples being palmitylsulphonyl fluoride (AM374; Fig. 12) and stearylsulphonyl fluoride (AM381). Both ligands inhibit FAAH irreversibly and show good separation between potency for FAAH inhibition and ability to bind to CB1 receptors (Deutsch et al., 1997b; Gifford et al., 1999). When administered by themselves, both AM374 and the anandamide membrane transport inhibitor AM404 share the ability of cannabinoid receptor agonists to ameliorate spasticity in mice with chronic relapsing experimental allergic encephalomyelitis (CREAE), an induced syndrome that serves as an animal model of multiple sclerosis (Baker et al., 2000; Pertwee, 2002).

Methyl arachidonyl fluorophosphonate (MAFP) is another potent irreversible inhibitor of FAAH ($EC_{50} = 2.5$ nM), but also interacts with CB receptors (Deutsch et al., 1997c). One recently developed analog of MAFP, O-1887, shows much greater separation of FAAH inhibitory potency ($EC_{50} = 15$ nM) from potency for binding to CB1 receptors (Compton and Martin, 1997; Martin et al., 2000). Arachidonyl trifluoromethyl ketone and diazomethyl arachidonyl ketone have been reported to bind to CB1 receptors with K_i values of 0.65 and 1.3 μM, respectively (Koutek et al., 1994; Edgemond et al., 1998). Significantly, a series of α-keto bicyclic heterocycles with alkyl or phenylalkyl side chains acting as potent reversible FAAH inhibitors in vitro were recently developed by Boger et al. (2000). These inhibit the enzyme competitively, some with K_i values in the picomolar or low nanomolar range. The structure of the most potent of this new generation of FAAH inhibitors is shown in Fig. 12 (**3**; $K_i = 140$ pM).

Several ligands mimicking anandamide have been designed and show remarkably high affinity for the cannabinoid receptors (Fig. 13) (Abadji et al., 1994; Maurelli et al., 1995; Deutsch et al., 1997a; Lin et al., 1998; Howlett et al., 2002). Of these CB receptor agonists depicted in Fig. 13, AM1116 and AM356 are stable towards FAAH hydrolysis (Lin et al., 1998; Lang et al., 1999). Particularly notable is the analog *R*-methanandamide (AM356), which has been established as a standard CB1-selective agonist in the cannabinoid field (Goutopoulos et al., 2001).

2.4. Anandamide transportor inhibitors

One notable membrane transport inhibitor to have been developed is *N*-(4-hydroxyphenyl) arachidonylamide (AM404; Fig. 14). This has been reported to inhibit anandamide uptake by rat

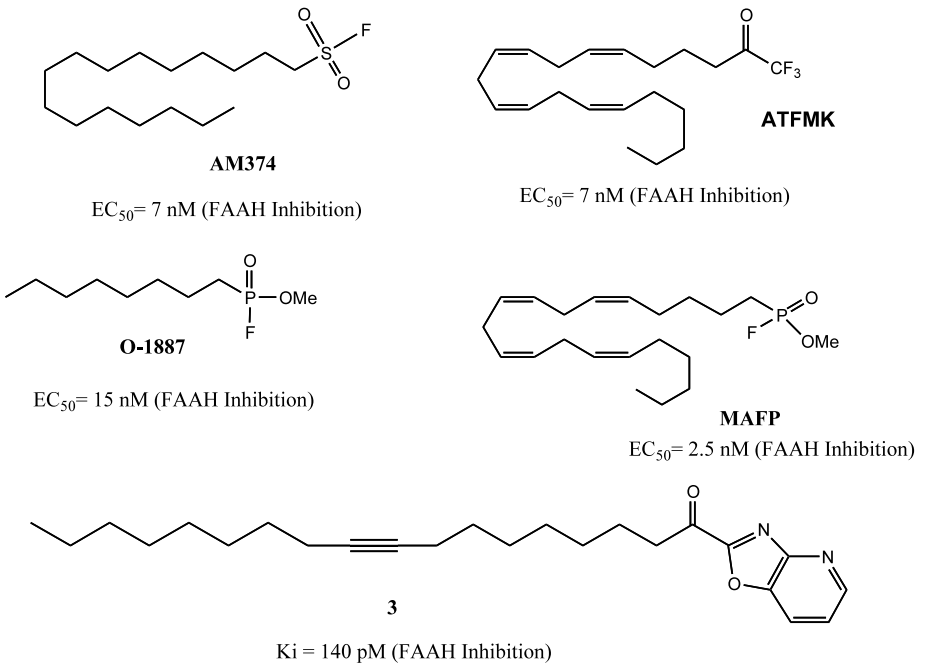


Fig. 12. Inhibitors of fatty acid amide hydrolase (FAAH).

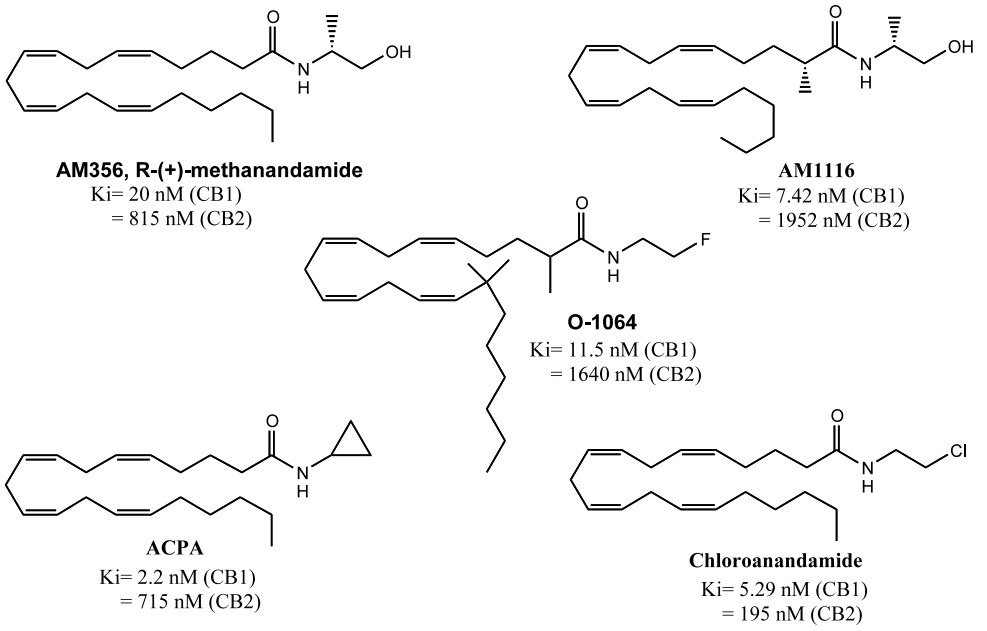


Fig. 13. Anandamide analogs as cannabinoid receptor agonists.

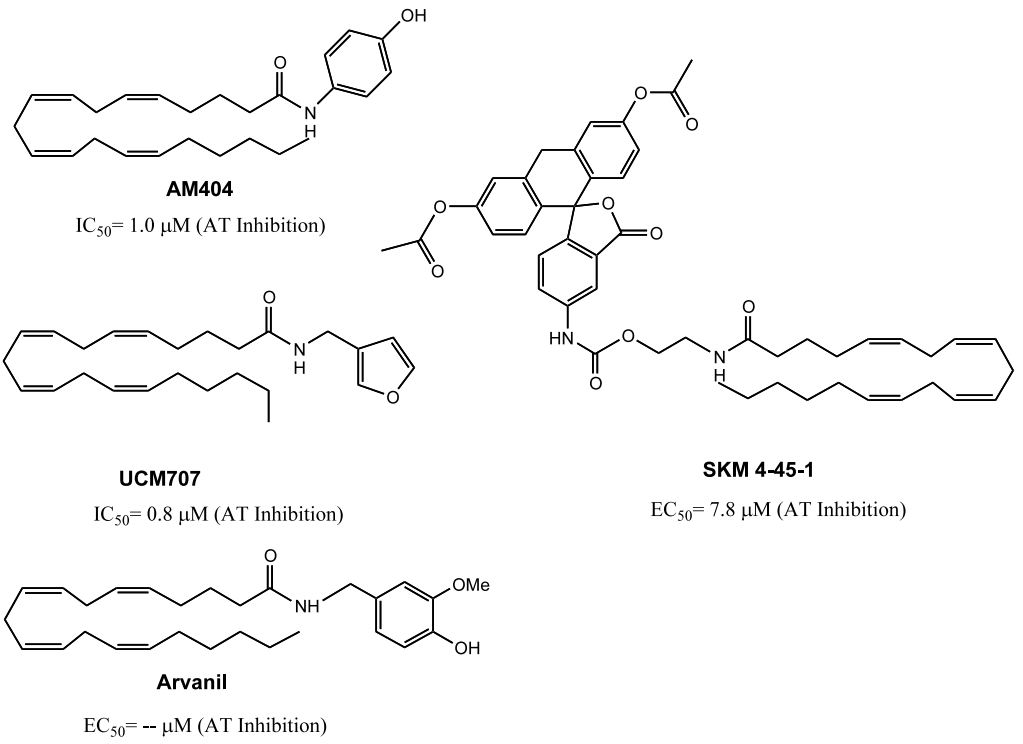


Fig. 14. Anandamide transport inhibitors.

cultured cortical neurones ($EC_{50} = 1$ μM) and astrocytes ($EC_{50} = 5$ μM) and to potentiate anandamide both in vitro and in vivo (Beltramo et al., 1997; Piomelli et al., 1999). When administered to rats by itself, AM404 increases plasma levels of anandamide and shares the ability of this endocannabinoid to decrease locomotor activity, depress plasma levels of prolactin and alter tyrosine hydroxylase activity in the hypothalamus (increase) and substantia nigra (decrease) (Gonzalez et al., 1999; Beltramo et al., 2000; Giuffrida et al., 2000).

Structure–activity experiments with AM404 analogues have revealed major differences between the structural requirements of the transporter for ligand recognition and those for ligand translocation (Piomelli et al., 1999). At concentrations in the low micromolar range, it displaces [^{3}H]CP55,940 from specific binding sites on rat forebrain membranes ($K_i = 1.76$ μM) (Khanolkar et al., 1996). An additional ligand that inhibits the endocannabinoid membrane transporter ($EC_{50} = 3.6$ μM) more readily than it inhibits FAAH ($EC_{50} = 32$ μM), at least in RBL-2H3 cells, is arvanil (Fig. 14).

Included in this series of head modified anandamide analogs is *N*-(3-furylmethyl) arachidonylamide (UCM707, Fig. 14) which also behaves as a potent inhibitor of the anandamide transporter (De Lago et al., 2002).

One other important recent advance has been the development of a fluorescent substrate for anandamide uptake (SKM 4-45-1; Fig. 14) that should serve as a useful experimental tool for transporter studies (Muthian et al., 2000). This agent is more potent as an inhibitor of endocannabinoid membrane transport ($EC_{50} = 7.8$ μM) than as an FAAH inhibitor ($EC_{50} > 10$ μM).

Remarkably, many of the cannabinergic agonists, antagonists and inhibitors existing and being developed in the cannabinoid field show great

promise as biological probes and therapeutic agents.

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