HEP (2005) 168:1–51


# Pharmacological Actions of Cannabinoids

R.G. Pertwee

School of Medical Sciences, Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD, UK
*rgp@abdn.ac.uk*



**Abstract** Mammalian tissues express at least two types of cannabinoid receptor, $CB_1$ and $CB_2$, both G protein coupled. $CB_1$ receptors are expressed predominantly at nerve terminals where they mediate inhibition of transmitter release. $CB_2$ receptors

are found mainly on immune cells, one of their roles being to modulate cytokine release. Endogenous ligands for these receptors (endocannabinoids) also exist. These are all eicosanoids; prominent examples include arachidonoylethanolamide (anandamide) and 2-arachidonoyl glycerol. These discoveries have led to the development of $CB_1$- and $CB_2$-selective agonists and antagonists and of bioassays for characterizing such ligands. Cannabinoid receptor antagonists include the $CB_1$-selective SR141716A, AM251, AM281 and LY320135, and the $CB_2$-selective SR144528 and AM630. These all behave as inverse agonists, one indication that $CB_1$ and $CB_2$ receptors can exist in a constitutively active state. Neutral cannabinoid receptor antagonists that seem to lack inverse agonist properties have recently also been developed. As well as acting on $CB_1$ and $CB_2$ receptors, there is convincing evidence that anandamide can activate transient receptor potential vanilloid type 1 (TRPV1) receptors. Certain cannabinoids also appear to have non-$CB_1$, non-$CB_2$, non-TRPV1 targets, for example $CB_2$-like receptors that can mediate antinociception and "abnormal-cannabidiol" receptors that mediate vasorelaxation and promote microglial cell migration. There is evidence too for TRPV1-like receptors on glutamatergic neurons, for $\alpha_2$-adrenoceptor-like (imidazoline) receptors at sympathetic nerve terminals, for novel G protein-coupled receptors for *R*-(+)-WIN55212 and anandamide in the brain and spinal cord, for novel receptors for $\Delta^9$-tetrahydrocannabinol and cannabinol on perivascular sensory nerves and for novel anandamide receptors in the gastro-intestinal tract. The presence of allosteric sites for cannabinoids on various ion channels and non-cannabinoid receptors has also been proposed. In addition, more information is beginning to emerge about the pharmacological actions of the non-psychoactive plant cannabinoid, cannabidiol. These recent advances in cannabinoid pharmacology are all discussed in this review.

**Keywords** Cannabinoid receptors · Cannabinoid receptor agonists and antagonists · Abnormal-cannabidiol · Cannabidiol · Inverse agonism

## 1 Introduction

"Cannabinoid" was originally the collective name given to a set of oxygen-containing $C_{21}$ aromatic hydrocarbon compounds that occur naturally in the plant *Cannabis sativa* (ElSohly 2002; Mechoulam and Gaoni 1967). However, this term is now generally also used for all naturally occurring or synthetic compounds that can mimic the actions of plant-derived cannabinoids or that have structures that closely resemble those of plant cannabinoids. Consequently, a separate term, "phytocannabinoid", has been coined for the cannabinoids produced by cannabis (Pate 1999). One phytocannabinoid, $\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC; Fig. 1), has attracted particular attention. This is because it is the main psychoactive constituent of cannabis (reviewed in Pertwee 1988) and because it is one of just two cannabinoids to be licensed for medical use, the other being nabilone (Cesamet; Fig. 2), a synthetic analogue of $\Delta^9$-THC (reviewed in the chapter by Robson, this vol-

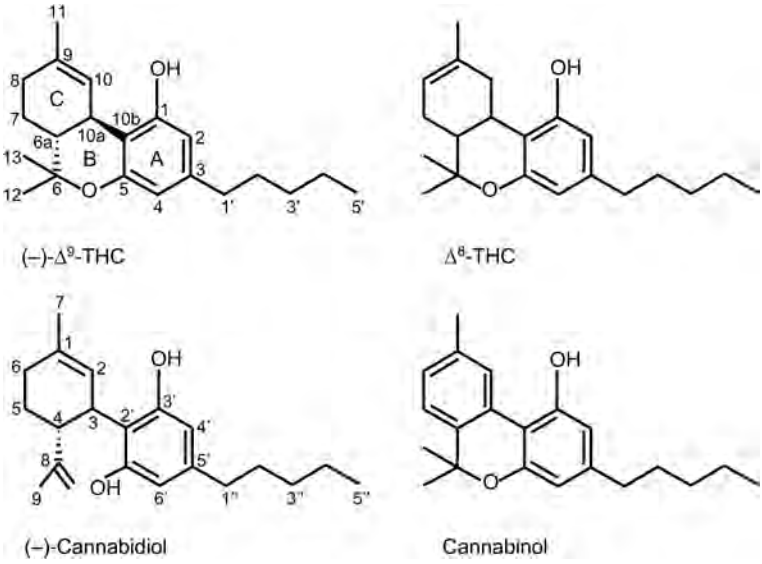


**Fig. 1.** The structures of four plant cannabinoids, $\Delta^9$-THC, $\Delta^8$-THC, cannabinol and cannabidiol

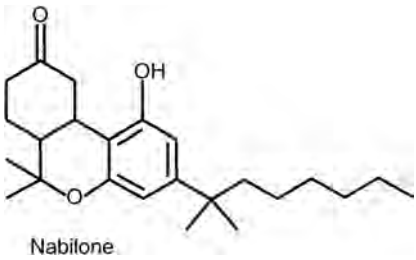


**Fig. 2.** The structure of nabilone

ume). Because of its high lipid solubility and low water solubility, $\Delta^9$-THC was long thought to owe its pharmacological properties to an ability to perturb the phospholipid constituents of biological membranes (reviewed in Pertwee 1988). However, all this changed in the late 1980s with the discovery in mammalian tissues of specific cannabinoid receptors.

Two types of cannabinoid receptor have so far been identified (reviewed in Howlett et al. 2002). These are $CB_1$, cloned in Tom Bonner's laboratory in the USA in 1990, and $CB_2$, cloned by Sean Munro in the UK in 1993. Both these receptors are coupled through $G_{i/o}$ proteins, negatively to adenylate cyclase and positively to mitogen-activated protein kinase. $CB_1$ receptors are also coupled through $G_{i/o}$ proteins, positively to A-type and inwardly rectifying potassium channels and negatively to N-type and P/Q-type calcium channels and to D-type potassium channels. In addition, there are reports that $CB_1$ and $CB_2$ receptors can enhance intracellular free $Ca^{2+}$ concentrations (Fan and Yazulla 2003; Rubovitch et al. 2002; Sugiura et al. 1996, 1997, 2000). It is unclear whether this enhancement is $G_{i/o}$

mediated. In experiments with NG108-15 cells, Sugiura et al. (1996) found $CB_1$-mediated increases in intracellular free $Ca^{2+}$ levels to be abolished by pretreatment with pertussis toxin, pointing to an involvement of $G_{i/o}$ proteins. However, in experiments with N18TG2 neuroblastoma cells, Rubovich et al. (2002) reported that pertussis toxin failed to prevent $CB_1$-mediated enhancement of intracellular free $Ca^{2+}$ levels by low concentrations of desacetyl-L-nantradol, a cannabinoid receptor agonist (Sect. 3.1), and instead unmasked a stimulatory effect of higher concentrations of this agonist that in the absence of pertussis toxin did not alter intracellular free $Ca^{2+}$ levels at all. Rubovich et al. (2002) also obtained evidence that the stimulatory effect of desacetyl-L-nantradol on intracellular $Ca^{2+}$ release depended on an ability to delay the inactivation of open L-type voltage-dependent calcium channels and that it was mediated mainly by cyclic AMP-dependent protein kinase (PKA).

Although there is no doubt that $G_{i/o}$ proteins play a major role in cannabinoid receptor signalling, there is also no doubt that transfected and naturally expressed $CB_1$ receptors can act through $G_s$ proteins to activate adenylate cyclase (Calandra et al. 1999; Glass and Felder 1997; Maneuf and Brotchie 1997). The extent to which $CB_1$ receptors signal through $G_s$ proteins may be determined by $CB_1$ receptor location or by cross-talk with colocalized G protein-coupled non-$CB_1$ receptors (Breivogel and Childers 2000; Calandra et al. 1999; Glass and Felder 1997; Jarrahian et al. 2004). As proposed by Calandra et al. (1999), it is also possible that there are distinct subpopulations $CB_1$ receptors, one coupled to $G_{i/o}$ proteins and the other to $G_s$. Additional signalling mechanisms for cannabinoid $CB_1$ and $CB_2$ receptors have been proposed and descriptions of these can be found elsewhere (Howlett et al. 2002; see also the chapter by Howlett, this volume).

$CB_1$ receptors are expressed by central and peripheral neurons and also by some nonneuronal cells (reviewed in Howlett et al. 2002; Pertwee 1997; see also the chapter by Mackie, this volume). Within the central nervous system, the distribution pattern of $CB_1$ receptors is heterogeneous and can account for several of the characteristic pharmacological properties of $CB_1$ receptor agonists. For example, the presence of large populations of $CB_1$ receptors in cerebral cortex, hippocampus, caudate-putamen, substantia nigra pars reticulata, globus pallidus, entopeduncular nucleus and cerebellum, as well as in some areas of the brain and spinal cord that process or modulate nociceptive information, probably accounts for the ability of $CB_1$ receptor agonists to impair cognition and memory, to alter the control of motor function and to produce antinociception (reviewed in Iversen 2003; Pertwee 2001; see also the chapters by Riedel and Davies, Fernández-Ruiz and González, and Walker and Hohmann, this volume). Some $CB_1$ receptors are located at central and peripheral nerve terminals. Here they modulate the release of excitatory and inhibitory neurotransmitters when activated (Howlett et al. 2002). Although the effect of $CB_1$ receptor agonists on release that has been most often observed is one of inhibition, there has been one report that the $CB_1$/$CB_2$ receptor agonist, *R*-(+)-WIN55212 (Sect. 3.1), can act through $CB_1$ receptors to stimulate release of glutamate from primary cultures of rat cerebral cortical neurons (Ferraro et al. 2001). This effect, which disappeared when the concentration of *R*-(+)-WIN55212 was increased from 1 or 10 nM to 100 nM, was most probably triggered by cal-

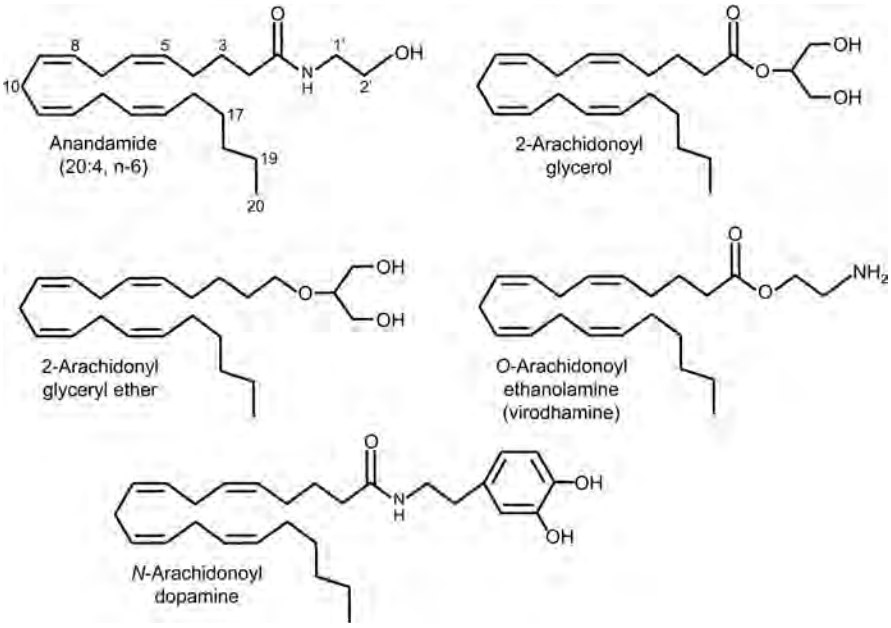


**Fig. 3.** The structures of five putative endogenous cannabinoids

cium released from inositol 1,4,5-triphosphate-controlled intracellular stores in response to a $CB_1$ receptor-mediated activation of phospholipase C. $CB_2$ receptors are expressed mainly by immune cells that include lymphocytes, macrophages, mast cells, natural killer cells, peripheral mononuclear cells and microglia (reviewed in Howlett et al. 2002; Pertwee 1997; see also the chapter by Cabral and Staab, this volume). Less is known about the roles of $CB_2$ than of $CB_1$ receptors, although there is good evidence that $CB_2$ receptors can trigger microglial cell migration (Sect. 4.1.5) and regulate cytokine release. Thus, one property $CB_1$ and $CB_2$ receptors share is the ability to modulate ongoing release of chemical messengers.

The discovery of cannabinoid receptors was followed by the demonstration that mammalian tissues can produce endogenous agonists for these receptors, all of which have so far proved to be derivatives of arachidonic acid (reviewed in Di Marzo et al. 1998; Hillard 2000; Mechoulam et al. 1998; see also the chapter by Di Marzo et al., this volume). The most investigated of these "endocannabinoids" have been arachidonoylethanolamide (anandamide) and 2-arachidonoyl glycerol (Fig. 3), both of which are synthesized on demand rather than stored. Other compounds that may be endocannabinoids include 2-arachidonylglyceryl ether (noladin ether), *O*-arachidonoylethanolamine (virodhamine) and *N*-arachidonoyldopamine (Howlett et al. 2002; Porter et al. 2002; Walker et al. 2002). Endocannabinoids together with cannabinoid receptors constitute what is now usually referred to as the "endocannabinoid system". It is likely that endocannabinoids function as both neuromodulators and immunomodulators and indeed, there is already evidence that within the central nervous system they serve as retrograde synaptic messengers

(reviewed in the chapter by Vaughan and Christie, this volume). There is also evidence that following their release, anandamide and 2-arachidonoyl glycerol enter cells by a combination of simple diffusion and facilitated, carrier-mediated transport (reviewed in Hillard and Jarrahian 2003) and are then metabolized by intracellular enzymes, anandamide by fatty acid amide hydrolase and 2-arachidonoyl glycerol mainly by monoacylglycerol lipase (monoglyceride lipase) but also by fatty acid amide hydrolase (reviewed in Cravatt and Lichtman 2002; Dinh et al. 2002; Ueda 2002; van der Stelt and Di Marzo 2004; see also the chapter by Di Marzo et al., this volume). Noladin ether also seems to be a substrate for anandamide/2-arachidonoyl glycerol membrane transporter(s) (Fezza et al. 2002). The processes responsible for the production, membrane transport and enzymic inactivation of endocannabinoids are all pharmacological targets through which the activity of the endocannabinoid system can or might be modulated to experimental or therapeutic advantage (reviewed in the chapters by Howlett and by Di Marzo et al., this volume). There is evidence that such modulation may also take place naturally as a result of the co-release of endogenous fatty acid derivatives such as palmitoylethanolamide and oleamide, which can potentiate anandamide, or of 2-linoleyl glycerol and 2-palmitoyl glycerol, which can potentiate 2-arachidonoyl glycerol (Mechoulam et al. 1998). For anandamide, mechanisms through which co-released ligands induce this "entourage effect" include not only inhibition of its metabolism by fatty acid amide hydrolase but also increases in the sensitivity of $CB_1$ or vanilloid receptors or of other pharmacological targets for anandamide through allosteric or other mechanisms (De Petrocellis et al. 2001b, 2002; Franklin et al. 2003; Mechoulam et al. 1998; Smart et al. 2002).

This chapter describes the in vitro and in vivo bioassays that have been most widely used to characterize ligands for $CB_1$ and/or $CB_2$ receptors and reviews the ability of compounds commonly used in cannabinoid research as experimental tools to activate or block these receptors. The likelihood that the most widely used cannabinoid receptor antagonists are inverse agonists rather than neutral antagonists is also discussed, as is evidence for the presence in mammalian tissues of non-$CB_1$, non-$CB_2$ pharmacological targets for cannabinoids.

## 2
## Bioassays for Characterizing $CB_1$ and $CB_2$ Receptor Ligands

### 2.1
### In Vitro Binding Assays

Several cannabinoid receptor ligands have been radiolabelled with tritium, and these have been used both to determine the $CB_1$ and $CB_2$ receptor affinities of unlabelled cannabinoids in displacement assays and to establish the tissue distribution patterns of these receptors (reviewed in Howlett et al. 2002; Pertwee 1999a). As indicated in Tables 1, 2 and 3, some of these compounds bind more readily to $CB_1$ or to $CB_2$ receptors, whilst the others bind more or less equally well to both these

**Table 1.** Typical dissociation constant ($K_D$) values of radiolabelled ligands at cannabinoid receptor $CB_1$ and $CB_2$ binding sites

| Radioligand | Source of membranes | Receptor | $K_D$ (nM) | Reference(s) |
|---|---|---|---|---|
| [$^3$H]SR141716A | Rat brain[a] | $rCB_1$ | 0.19–1.20 | For references, |
| | Guinea-pig forebrain | g-$pCB_1$ | 1.24 | see Pertwee 1999a |
| [$^{123}$I]AM251 | Rat cerebellum | $rCB_1$ | 0.25 | |
| [$^3$H]*R*-(+)-WIN55212 | Rat cerebellum | $rCB_1$ | 1.89, 4.67, 8.6 | |
| | Guinea-pig forebrain | g-$pCB_1$ | 2.34 | |
| | Cultured cells[b] | $rCB_1$ | 2.60 | |
| | Cultured cells[b] | $hCB_1$ | 16.2, 11.9 | |
| | Cultured cells[b] | $hCB_2$ | 3.7, 3.8 | |
| [$^3$H]HU-210 | Rat brain minus brain stem | $rCB_1$ | 0.045 | |
| (HU-243) | Cultured cells[b] | $hCB_2$ | 0.061 | |
| [$^3$H]CP55940 | Cultured cells[b] | $hCB_1$ | 0.4 to 3.3 | For references, |
| | Cultured cells[b] | $rCB_1$ | 4 | see Pertwee |
| | Rat brain[a] | $rCB_1$ | 0.07 to 2.3 | 1997, 1999a |
| | Mouse whole brain | $mCB_1$ | 3.4 | |
| | Cultured cells[b] | $hCB_2$ | 0.2 to 7.4 | |
| | Cultured cells[b] | $mCB_2$ | 0.39 | |
| [$^3$H]CP55940 | Rat cerebellum | $rCB_1$ | 2.37 | Mauler et al. 2002 |
| | Human cerebral cortex | $hCB_1$ | 1.29 | |
| | Cultured cells[b] | $hCB_1$ | 1.10 | |
| | Cultured cells[b] | $hCB_2$ | 4.20 | |
| [$^3$H]BAY 38-7271 | Rat cerebellum | $rCB_1$ | 1.84 | Mauler et al. 2002 |
| | Human cerebral cortex | $hCB_1$ | 2.10 | |
| | Cultured cells[b] | $hCB_1$ | 2.91 | |
| | Cultured cells[b] | $hCB_2$ | 4.24 | |

g-$pCB_1$, Guinea-pig $CB_1$ receptors; $hCB_1$ and $hCB_2$, human cannabinoid receptors; $mCB_1$ and $mCB_2$, mouse cannabinoid receptors; $rCB_1$ and $rCB_2$, rat cannabinoid receptors.

[a]Whole brain or a discrete area.

[b]Cells transfected with $CB_1$ or $CB_2$ receptors.

receptor types. It is noteworthy, therefore, that $CB_1$ or $CB_2$ selectivity can still be achieved in displacement assays with the non-selective radiolabelled ligands by using membranes obtained from cannabinoid receptor-free cultured cells that have been transfected with $CB_1$ or $CB_2$ receptors or membranes obtained from brain ($CB_1$-rich) or spleen ($CB_2$-rich). Some care is needed in interpreting binding data obtained with brain or spleen membranes. Thus, whilst there is little evidence that $CB_2$ receptors are expressed by central neurons, these receptors are expressed by microglial cells (Howlett et al. 2002). Similarly, although it is mainly $CB_2$ receptors that are present in spleen, this tissue also expresses some $CB_1$ receptors (reviewed in Howlett et al. 2002; Pertwee 1997). Moreover, there is growing evidence for the presence in brain and other tissues of non-$CB_1$, non-$CB_2$ cannabinoid recep-

**Table 2.** Examples of $K_i$ values of certain cannabinoid $CB_1$ and/or $CB_2$ receptor agonists for the in vitro displacement of [$^3$H]CP55940, [$^3$H]HU243 or [$^3$H]BAY-38-7271 from $CB_1$- and $CB_2$-specific binding sites (*continued on next page*)

| Agonist | $CB_1$ $K_i$ value (nM) | $CB_2$ $K_i$ value (nM) | Reference |
|---|---|---|---|
| **$CB_1$-selective agonists in order of decreasing $CB_1/CB_2$ selectivity** | | | |
| ACEA | 1.4[a,b] | >2,000[a,b] | Hillard et al. 1999 |
| | 5.29[a,b] | 195[c] | Lin et al. 1998 |
| O-1812 | 3.4[b] | 3,870[b] | Di Marzo et al. 2001 |
| ACPA | 2.2[a,b] | 715[a,b] | Hillard et al. 1999 |
| 2-Arachidonyl glyceryl ether | 21.2[b] | >3,000[d] | Hanus et al. 2001 |
| *R*-(+)-methanandamide | 17.9[a,b] | 868[c] | Lin et al. 1998 |
| | 20[a,b] | 815[c] | Khanolkar et al. 1996 |
| | 28.3[b] | 868[c] | Goutopoulos et al. 2001 |
| **Agonists without any marked $CB_1$ or $CB_2$ selectivity** | | | |
| Anandamide | 61[a,b] | 1,930[c] | Lin et al. 1998 |
| | 78.2[a,b] | 1,926[c] | Khanolkar et al. 1996 |
| | 89[a] | 371[a] | Showalter et al. 1996 |
| | 543 | 1,940 | Felder et al. 1995 |
| | 71.7[a,b] | 279[a,b] | Hillard et al. 1999 |
| | 252[e,d] | 581[e,d] | Mechoulam et al. 1995 |
| BAY 38-7271 | 1.85[f] | 5.96[f] | Mauler et al. 2002 |
| 2-Arachidonoyl glycerol | 472[e,d] | 1,400[e,d] | Mechoulam et al. 1995 |
| | 58.3[e,d] | 145[e,d] | Ben-Shabat et al. 1998 |
| O-1057 | 4.4 | 11.2 | Pertwee et al. 2000 |
| HU-210 | 0.0608 | 0.524 | Felder et al. 1995 |
| | 0.1[e,b] | 0.17[e] | Rhee et al. 1997 |
| | 0.73 | 0.22 | Showalter et al. 1996 |
| CP55940 | 5 | 1.8 | Ross et al. 1999a |
| | 3.72 | 2.55 | Felder et al. 1995 |
| | 1.37[b] | 1.37[b] | Rinaldi-Carmona et al. 1994 |
| | 0.58 | 0.69 | Showalter et al. 1996 |
| | 0.50[a,b] | 2.80[a,b] | Hillard et al. 1999 |
| $\Delta^9$-THC | 53.3 | 75.3 | Felder et al. 1995 |
| | 39.5[e,b] | 40[e] | Bayewitch et al. 1996 |
| | 40.7 | 36.4 | Showalter et al. 1996 |
| | 80.3[e,b] | 32.2[e] | Rhee et al. 1997 |
| | 35.3[b] | 3.9[b] | Rinaldi-Carmona et al. 1994 |
| | 5.05 | 3.13 | Iwamura et al. 2001 |
| Nabilone | 1.84 | 2.19 | Gareau et al. 1996 |
| $\Delta^8$-THC | 47.6[b] | 39.3[c] | Busch-Petersen et al. 1996 |
| | 44[b] | 44 | Huffman et al. 1999 |
| Cannabinol | 211.2[e,b] | 126.4[e] | Rhee et al. 1997 |
| | 308 | 96.3 | Showalter et al. 1996 |
| | 1,130 | 301 | Felder et al. 1995 |
| CP56667 | 61.7 | 23.6 | Showalter et al. 1996 |
| *R*-(+)-WIN55212 | 9.94[b] | 16.2[b] | Rinaldi-Carmona et al. 1994 |
| | 4.4[a,b] | 1.2[a,b] | Hillard et al. 1999 |

**Table 2.** (*continued*)

| Agonist | $CB_1$ $K_i$ value (nM) | $CB_2$ $K_i$ value (nM) | Reference |
|---|---|---|---|
| | 1.89 | 0.28 | Showalter et al. 1996 |
| | 62.3 | 3.3 | Felder et al. 1995 |
| | 123 | 4.1 | Shire et al. 1996 |
| | 9.87 | 0.29 | Iwamura et al. 2001 |
| **$CB_2$-selective agonists in order of increasing $CB_2/CB_1$ selectivity** | | | |
| AM1241 | 280[b] | 3.4[c] | Ibrahim et al. 2003 |
| 3-(1′1′-dimethylbutyl)-1-deoxy-$\Delta^8$-THC (JWH-133) | 677[b] | 3.4 | Huffman et al. 1999 |
| L-759633 | 1,043 | 6.4 | Ross et al. 1999a |
| | 15,850 | 20 | Gareau et al. 1996 |
| L-759656 | 529[b] | 35 | Huffman et al. 1999 |
| | 713[b] | 57 | Huffman et al. 2002 |
| | 4,888 | 11.8 | Ross et al. 1999a |
| | >20,000 | 19.4 | Gareau et al. 1996 |
| HU-308 | >10,000[e,b] | 22.7[e,d] | Hanus et al. 1999 |

See Figs. 1 to 9 for the structures of the compounds listed in this table.
DMH, dimethylheptyl; ND, not determined; THC, tetrahydrocannabinol.
[a]With phenylmethylsulphonyl fluoride (PMSF) in order to inhibit enzymic hydrolysis.
[b]Binding to rat cannabinoid receptors on transfected cells or on brain ($CB_1$) or spleen tissue ($CB_2$).
[c]Binding to mouse brain ($CB_1$) or spleen tissue ($CB_2$).
[d]Species unspecified. All other data from experiments with human cannabinoid receptors.
[e]Displacement of [$^3$H]HU243 from $CB_1$- and $CB_2$-specific binding sites.
[f]Displacement of [$^3$H]BAY-38-7271 from $CB_1$- and $CB_2$-specific binding sites.

tors to which at least some $CB_1$ and/or $CB_2$ receptor ligands can bind (Sect. 4). Radiolabelled probes for single photon emission computed tomography (SPECT) or positron emission tomography (PET) have also been developed (reviewed in Gifford et al. 2002; see also the chapter by Lindsey et al., this volume).

## 2.2 In Vitro Functional Bioassays

### 2.2.1 Assays Using Whole Cells or Cell Membranes

The most commonly employed assays using whole cells or cell membranes are the [$^{35}$S]guanosine-5′-*O*-(3-thiotriphosphate) ([$^{35}$S]GTP$\gamma$S) binding assay and the cyclic AMP assay. The first measures cannabinoid receptor agonist-stimulated binding to G proteins of the hydrolysis-resistant GTP analogue, [$^{35}$S]GTP$\gamma$S, whereas the cyclic AMP assay relies on cannabinoid receptor-mediated inhibition (usual effect) or enhancement of basal or drug-induced cyclic AMP production

**Table 3.** $K_i$ values of cannabinoid receptor antagonists/inverse agonists for the in vitro displacement of [$^3$H]CP55940 from $CB_1$- and $CB_2$-specific binding sites

| Ligand | $CB_1$ $K_i$ value (nM) | $CB_2$ $K_i$ value (nM) | Reference |
|---|---|---|---|
| **$CB_1$-selective antagonists/inverse agonists** | | | |
| NESS 0327 | 0.00035[a] | 21[a] | Ruiu et al. 2003 |
| SR141716A | 11.8 | 13,200 | Felder et al. 1998 |
| | 11.8 | 973 | Felder et al. 1995 |
| | 12.3 | 702 | Showalter et al. 1996 |
| | 5.6 | >1,000 | Rinaldi-Carmona et al. 1994 |
| | 1.98[b] | >1,000[b] | Rinaldi-Carmona et al. 1994 |
| | 1.8[a] | 514[a] | Ruiu et al. 2003 |
| AM281 | 12[b] | 4,200[a] | Lan et al. 1999a |
| AM251 (compound 12) | 7.49[b] | 2,290[a] | Lan et al. 1999b |
| LY320135 | 141 | 14,900 | Felder et al. 1998 |
| **$CB_2$-selective antagonists/inverse agonists** | | | |
| AM 630 | 5,152 | 31.2 | Ross et al. 1999a |
| SR144528 | 437 | 0.60 | Rinaldi-Carmona et al. 1998 |
| | 305[b] | 0.30[b] | Rinaldi-Carmona et al. 1998 |
| | >10,000 | 5.6 | Ross et al. 1999a |
| | 70[a] | 0.28[a] | Ruiu et al. 2003 |
| | 50.3 | 1.99 | Iwamura et al. 2001 |

See Figs. 10 and 11 for the structures of the compounds listed in this table.
[a]Binding to mouse brain ($CB_1$) or spleen tissue ($CB_2$).
[b]Binding to rat cannabinoid receptors on transfected cells or on brain ($CB_1$) or spleen tissue ($CB_2$).
All other data from experiments with human cannabinoid receptor.

(reviewed in Howlett et al. 2002; Pertwee 1997, 1999a). Both assays can be performed with membranes obtained from brain tissue or from cultured cells that express $CB_1$ or $CB_2$ receptors either naturally or after transfection. In addition, the cyclic AMP assay can be performed with whole cells, including primary cultures of central neurons, and the [$^{35}$S]GTP$\gamma$S assay can be used in autoradiography experiments with tissue sections (Breivogel et al. 1997; Selley et al. 1996; Sim et al. 1995). The cyclic AMP assay is more sensitive than the [$^{35}$S]GTP$\gamma$S assay. Presumably this is because modulation of cyclic AMP production takes place further along the signalling cascade than [$^{35}$S]GTP$\gamma$S binding so that there is greater signal amplification. For the [$^{35}$S]GTP$\gamma$S assay, it is important to include guanosine diphosphate (GDP) and sodium chloride at appropriate concentrations (Breivogel et al. 1998; Selley et al. 1996; Sim et al. 1995). GDP increases the ratio of agonist-stimulated to basal [$^{35}$S]GTP$\gamma$S binding (signal-to-noise ratio) but also decreases the absolute levels of both agonist-stimulated and basal [$^{35}$S]GTP$\gamma$S binding. In addition, it magnifies the differences in efficacy exhibited in this assay by full and partial agonists (Savinainen et al. 2001). The signal-to-noise ratio in this bioassay can be further improved by including an adenosine $A_1$ receptor antagonist (Savinainen

et al. 2003). It has also proved possible to assay cannabinoid receptor agonists by exploiting their ability to increase intracellular free $Ca^{2+}$ levels ($CB_1$ and $CB_2$ agonists) (Bisogno et al. 2000; Rubovitch et al. 2002; Sugiura et al. 1996, 1997, 2000; Suhara et al. 2001) or to inhibit lipopolysaccharide-induced release of tumour necrosis factor-$\alpha$ ($CB_2$ agonists) (Wrobleski et al. 2003). Some information about the pharmacological properties of cannabinoid receptor ligands has also been obtained using bioassays performed with cultured neurons that exploit the negative coupling of the $CB_1$ receptor to N- and P/Q-type calcium channels (reviewed in Pertwee 1997, 1999a).

### 2.2.2
### Isolated Nerve–Smooth Muscle Preparations

Preparations in which cannabinoid receptor agonists can act through neuronal $CB_1$ receptors to produce a concentration-related inhibition both of electrically-evoked contractile transmitter release (Schlicker et al. 2003; Trendelenburg et al. 2000) and of the contractions caused by this release (reviewed in Howlett et al. 2002; Pertwee 1997; Pertwee et al. 1996a; Schlicker and Kathmann 2001) are called isolated nerve–smooth muscle preparations. The ones most commonly used are the mouse vas deferens and the myenteric plexus-longitudinal muscle preparation of guinea-pig small intestine. However, $CB_1$ receptor agonists also show activity in other isolated nerve-smooth muscle preparations, for example the rat vas deferens and the mouse urinary bladder. The usual measured response in these bioassays is inhibition of electrically evoked contractions, a response that can also be elicited in these tissues by agonists for several types of non-cannabinoid receptor. Consequently, to establish whether or not the production of such inhibition by a test compound is $CB_1$ receptor-mediated, it is necessary to measure the susceptibility of this compound to antagonism by a selective $CB_1$ antagonist. For the mouse vas deferens, an alternative strategy for meeting this objective has been to exploit the ability of a cannabinoid receptor agonist ($\Delta^9$-THC) to induce cannabinoid tolerance without affecting the sensitivity of the twitch response to inhibition by non-cannabinoids (Pertwee 1997).

## 2.3
## In Vivo Bioassays

Probably the most commonly used in vivo bioassay is the mouse tetrad assay, in which the ability of a test compound to produce four effects in the same animal is determined. These effects, hypokinesia, hypothermia, catalepsy in the Pertwee ring test and antinociception in the tail-flick or hot plate test, are usually produced by a $CB_1$ receptor agonist over a relatively narrow dose range (reviewed in Howlett et al. 2002; Martin et al. 1995). One or other of these effects can be produced by some centrally active non-$CB_1$ receptor agonists or antagonists. However, when performed together, the tetrad tests provide at least some degree of

selectivity since, in contrast to established $CB_1$ receptor agonists, many other classes of centrally active agent lack activity in at least one of the tests (Wiley and Martin 2003). This feature of the tetrad assay was particularly important when it was first devised, as selective $CB_1$ receptor antagonists had still to be developed. Now that such antagonists are available (Sect. 3.2), there is less need for a bioassay with $CB_1$ receptor selectivity. Some non-$CB_1$ receptor ligands do show activity in all four tetrad tests. These include stearoylethanolamide (Maccarrone et al. 2002), the anandamide analogue, O-2093 (Di Marzo et al. 2002), metabolites of anandamide (reviewed in Pertwee and Ross 2002) and certain anti-psychotic agents (Wiley and Martin 2003). Moreover, although the endocannabinoid anandamide shows cannabimimetic activity in the mouse tetrad assay, it is only antagonized by SR141716A when protected from enzymic hydrolysis (reviewed in Pertwee and Ross 2002). However, other $CB_1$ receptor agonists do show susceptibility to antagonism by SR141716A in this bioassay (reviewed in Howlett et al. 2002).

Other in vivo bioassays for $CB_1$ receptor agonists include the dog static ataxia test, the monkey behavioural test, the rat catalepsy test and the drug discrimination test, which is usually carried out with monkeys, rats or pigeons (reviewed in Howlett et al. 2002; Martin et al. 1995). The potencies shown by some cannabinoids in drug discrimination experiments performed with rats have been found to correlate well with their psychoactive potencies in humans (Balster and Prescott 1992). In vivo bioassays that provide measures of other $CB_1$ receptor-mediated effects in animals, for example changes in memory, have also been developed (reviewed in Howlett et al. 2002; see also the chapter by Riedel and Davies, this volume). However, these have not been used widely for characterizing novel cannabinoid receptor ligands. Methods for evaluating cannabinoids in humans have also been developed (Howlett et al. 2002).

## 2.4 Cannabinoid Receptor Knockout Mice

One important advance has been the development of transgenic $CB_1^{-/-}$, $CB_2^{-/-}$ and $CB_1^{-/-}/CB_2^{-/-}$ mice that lack $CB_1$, $CB_2$ or both $CB_1$ and $CB_2$ receptors (reviewed in Howlett et al. 2002; see also the chapters by Abood and by Valverde et al., this volume). The availability of such animals provides a useful additional method for establishing whether or not responses to test compounds are $CB_1$ and/or $CB_2$ receptor mediated and, indeed, an important means of detecting the presence of new types of cannabinoid receptor (Sect. 4.1). Cannabinoid receptor knockout mice are also being used to help determine the physiological roles of $CB_1$ and $CB_2$ receptors.

# 3
# $CB_1$ and $CB_2$ Cannabinoid Receptor Ligands

## 3.1
## Cannabinoid Receptor Agonists

In terms of chemical structure, established cannabinoid receptor agonists fall essentially into four main groups: classical, nonclassical, aminoalkylindole and eicosanoid (reviewed in Howlett et al. 2002; Pertwee 1999a).

– The classical group consists of dibenzopyran derivatives that are either cannabis-derived compounds (phytocannabinoids) or their synthetic analogues. Notable examples are the phytocannabinoids $\Delta^9$-THC, $\Delta^8$-THC and cannabinol (Fig. 1), and the synthetic cannabinoids, 11-hydroxy-$\Delta^8$-THC-dimethylheptyl (HU-210), JWH-133, L-759633, L-759656, L-nantradol and desacetyl-L-nantradol (Figs. 4 and 5).

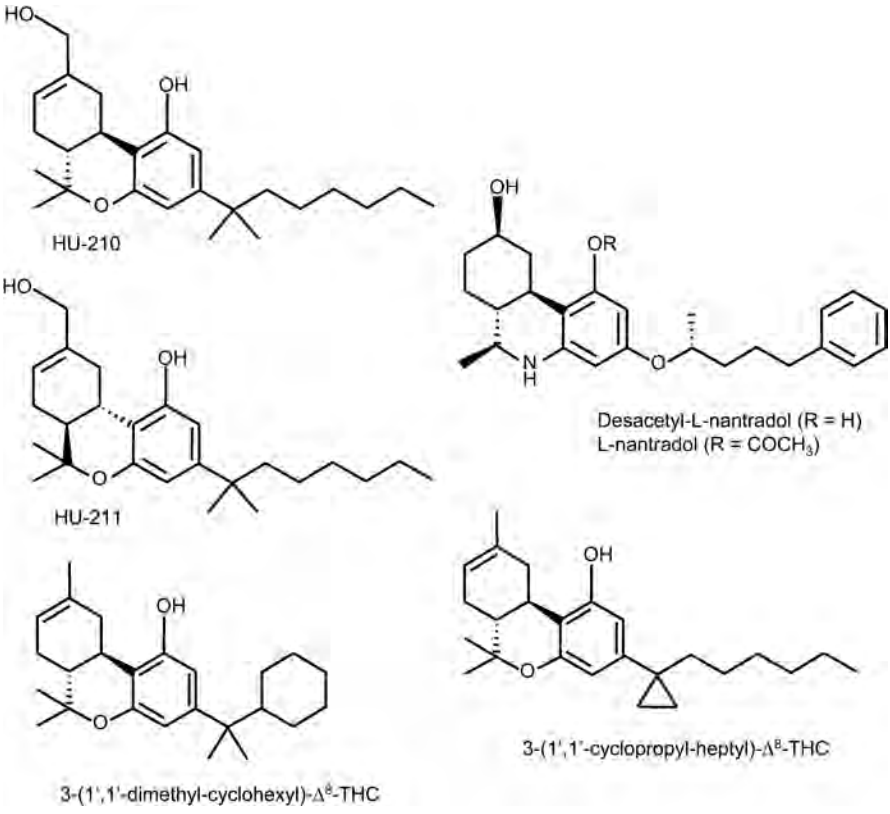


**Fig. 4.** The structures of five synthetic classical cannabinoids

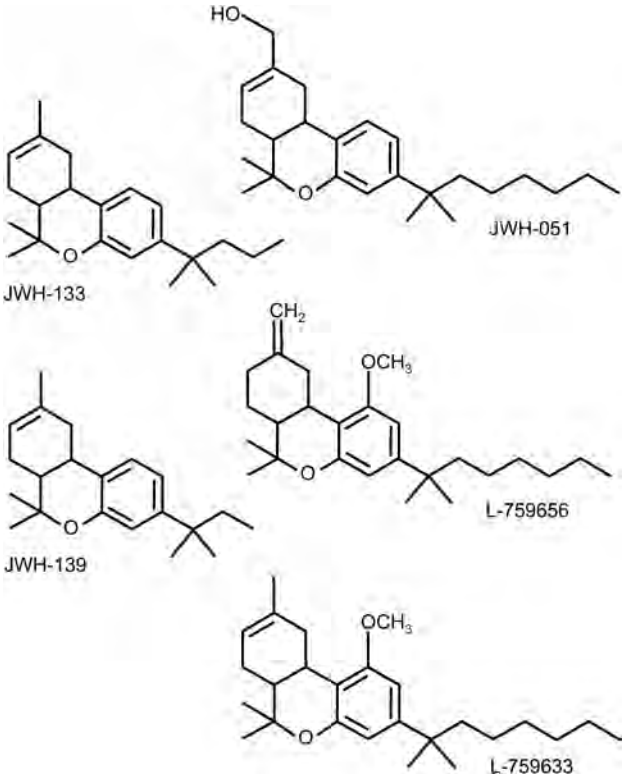


**Fig. 5.** The structures of four nonclassical cannabinoids

- Nonclassical cannabinoids consist of bicyclic and tricyclic analogues of $\Delta^9$-THC that lack a pyran ring; examples include CP55940, CP47497, CP55244 and HU-308 (Fig. 6). They are, therefore, closely related to the classical cannabinoids.
- In contrast, the aminoalkylindole group of cannabinoid receptor agonists (Fig. 7) have structures that are completely different from those of other cannabinoids. Indeed, results from experiments performed with wild-type and mutant $CB_1$ receptors (Chin et al. 1998; Petitet et al. 1996; Song and Bonner 1996; Tao and Abood 1998) suggest that *R*-(+)-WIN55212 (WIN55212-2), the most widely investigated of the aminoalkylindoles, binds differently to the $CB_1$ receptor than classical, nonclassical or eicosanoid cannabinoids, albeit it in a manner that still allows mutual competition between *R*-(+)-WIN55212 and non-aminoalkylindole cannabinoids for binding sites on the wild-type receptor.
- Members of the eicosanoid group of cannabinoid receptor agonists have markedly different structures both from the aminoalkylindoles and from classical and nonclassical cannabinoids. Important members of this group are the endocannabinoids, arachidonoylethanolamide (anandamide), *O*-arachidonoylethanolamine (virodhamine), 2-arachidonoyl glycerol and 2-arachidonyl glyceryl

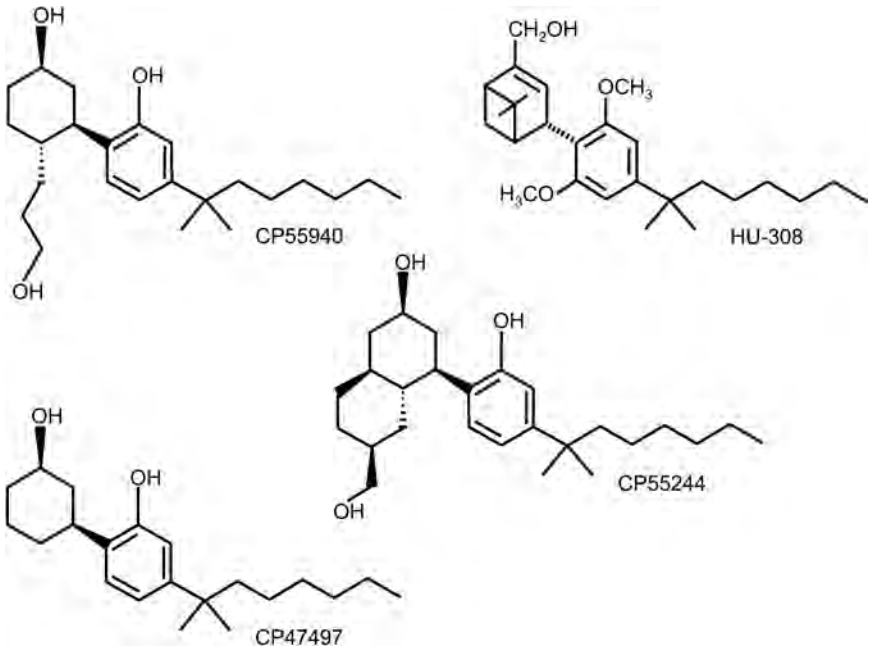


**Fig. 6.** The structures of four nonclassical cannabinoids. The (+)-enantiomer of CP55940 is CP56667

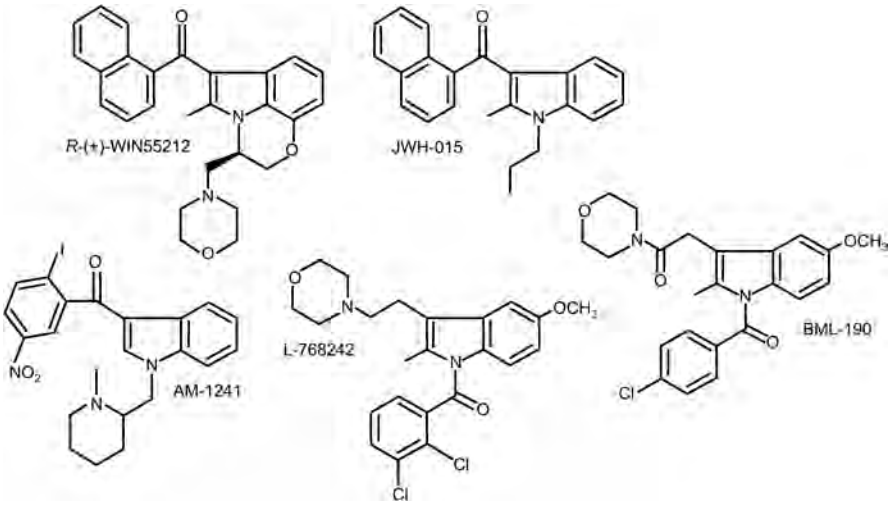


**Fig. 7.** The structures of *R*-(+)-WIN55212, JWH-015, AM1241, L-768242 and BML-190

ether (noladin ether) (Fig. 3) and several synthetic analogues of anandamide, including *R*-(+)-methanandamide, arachidonyl-2′-chloroethylamide (ACEA), arachidonylcyclopropylamide (ACPA), O-689 and O-1812 (Fig. 8) (Howlett et al. 2002; Pertwee 1999a; Porter et al. 2002).

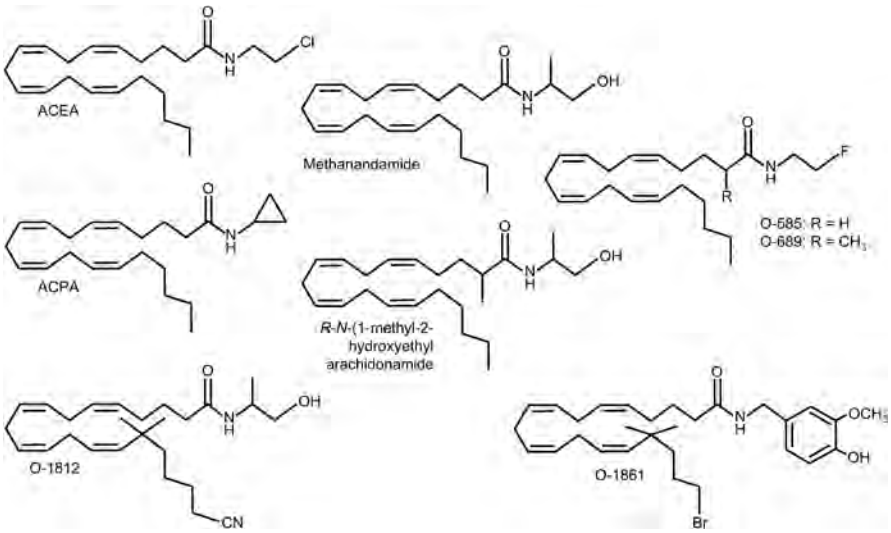


**Fig. 8.** The structures of eight structural analogues of anandamide

Many cannabinoid receptor agonists exhibit marked stereoselectivity in pharmacological assays, reflecting the presence of chiral centres in these compounds (reviewed in Howlett et al. 2002). Classical and nonclassical cannabinoids with the same absolute stereochemistry as (–)-$\Delta^9$-THC at 6a and 10a, *trans* (6a*R*, 10a*R*), are more active than their *cis* (6a*S*, 10a*S*) enantiomers, whilst *R*-(+)-WIN55212 is more active than *S*-(–)-WIN55212. Although anandamide does not contain any chiral centres, some of its synthetic analogues do. One of these is methanandamide, the *R*-(+)-isomer of which exhibits nine times higher affinity for $CB_1$ receptors than the *S*-(–)-isomer (Abadji et al. 1994).

Several cannabinoid receptor agonists bind more or less equally well to $CB_1$ and $CB_2$ receptors (Table 2), although they do exhibit different relative intrinsic activities at these receptors. Among these are HU-210, CP55940, *R*-(+)-WIN55212, (–)-$\Delta^9$-THC, anandamide and 2-arachidonoyl glycerol (reviewed in Howlett et al. 2002; Pertwee 1999a).

- HU-210 has particularly high affinity for both $CB_1$ and $CB_2$ receptors. It also exhibits high relative intrinsic activities at these receptors. Indeed, it is remarkably potent as a cannabinoid receptor agonist and exhibits an exceptionally long duration of action in vivo. The marked affinity and efficacy that HU-210 shows at cannabinoid receptors is due largely to the replacement of the pentyl side chain of $\Delta^8$-THC with a dimethylheptyl group.
- CP55940 and *R*-(+)-WIN55212 have $CB_1$ and $CB_2$ relative intrinsic activities of the same order as those of HU-210 and, although they have lower $CB_1$ and $CB_2$ affinities than HU-210, are still reasonably potent as they bind to these receptors at concentrations in the low nanomolar range.

- (–)-$\Delta^9$-THC has lower $CB_1$ and $CB_2$ affinities and relative intrinsic activities than HU-210, CP55940 or *R*-(+)-WIN55212. Whilst it behaves as a partial agonist at both these receptor types, it exhibits less efficacy at $CB_2$ than at $CB_1$ receptors to the extent that in one bioassay system it has been found to behave as a $CB_2$ receptor antagonist (Bayewitch et al. 1996). (–)-$\Delta^9$-THC can also produce $CB_1$ receptor antagonism. Thus, it has been found to oppose $CB_1$ receptor activation by the higher efficacy agonist, 2-arachidonoyl glycerol, in hippocampal cultures that may have contained neurons with rather low $CB_1$ receptor density (Kelley and Thayer 2004). This it did with an $IC_{50}$ of 42 nM, which is close to its reported $CB_1$ $K_i$ values (Table 2).
- Anandamide resembles (–)-$\Delta^9$-THC in its affinity for $CB_1$ receptors, in behaving as a $CB_1$ and $CB_2$ receptor partial agonist (Gonsiorek et al. 2000; Hillard 2000; Mackie et al. 1993; Savinainen et al. 2001; Sugiura et al. 1996, 2000) and in having lower $CB_2$ than $CB_1$ intrinsic activity (reviewed in Howlett et al. 2002; Pertwee 1999a). It has also been found that, like (–)-$\Delta^9$-THC, anandamide can behave as a $CB_2$ receptor antagonist in at least one bioassay system (Gonsiorek et al. 2000). In contrast to *R*-(+)-WIN55212, which has slightly higher $CB_2$ than $CB_1$ affinity, anandamide binds marginally more readily to $CB_1$ than to $CB_2$ receptors.
- 2-Arachidonoyl glycerol is known to activate both $CB_1$ and $CB_2$ receptors. It binds about equally well to both receptor types (Table 2) and has been reported to exhibit greater $CB_1$ intrinsic activity but less $CB_1$ potency than CP55940 and greater $CB_1$ intrinsic activity and potency than anandamide (Gonsiorek et al. 2000; Savinainen et al. 2001, 2003; Sugiura et al. 1996). This endocannabinoid also has greater $CB_2$ potency than anandamide or 1-arachidonoyl glycerol (Gonsiorek et al. 2000; Sugiura et al. 2000).

One recently developed synthetic cannabinoid receptor agonist that interacts almost as well with $CB_2$ as with $CB_1$ receptors (Tables 1 and 2) is BAY 38-7271 (De Vry and Jentzsch 2002; Mauler et al. 2002, 2003). This compound has a structure that is not classical, non-classical, aminoalkylindole or eicosanoid (Fig. 9).

Phytocannabinoids other than $\Delta^9$-THC that are known to activate cannabinoid receptors are (–)-$\Delta^8$-THC and cannabinol (reviewed in Pertwee 1999a). Of these, (–)-$\Delta^8$-THC resembles (–)-$\Delta^9$-THC both in its $CB_1$ and $CB_2$ receptor affinities (Table 2) and in its relative intrinsic activity at the $CB_1$ receptor (Gérard et al. 1991; Howlett and Fleming 1984; Matsuda et al. 1990). Cannabinol also behaves as a partial agonist at $CB_1$ receptors but has even less relative intrinsic activity than (–)-$\Delta^9$-THC (Howlett 1987; Matsuda et al. 1990; Petitet et al. 1997, 1998). Whilst there is one report that cannabinol activates $CB_2$ receptors in the cyclic AMP assay more effectively than $\Delta^9$-THC (Rhee et al. 1997), there is another that in the GTP$\gamma$S binding assay, it behaves as a $CB_2$ receptor inverse agonist (MacLennan et al. 1998).

As to the endocannabinoid virodhamine, Porter et al. (2002) have shown that this activates both $CB_1$ and $CB_2$ receptors. Their experiments with transfected cells yielded $CB_1$ and $CB_2$ $EC_{50}$ values in the GTP$\gamma$S binding assay of 1.9 and 1.4 μM, respectively, for this endocannabinoid, indicating it to be less potent than anandamide, 2-arachidonoyl glycerol or *R*-(+)-WIN55212. The $CB_2$ intrinsic

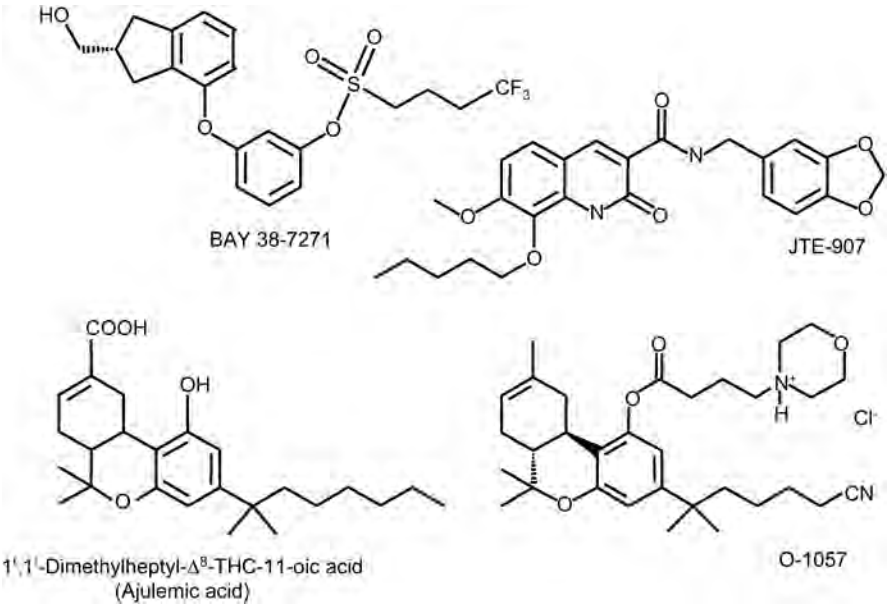


**Fig. 9.** The structures of BAY 38-7271, JTE-907, ajulemic acid and O-1057

activity of virodhamine matched that of anandamide which, however, behaved as a full agonist in this investigation, suggesting that the $CB_2$ expression level of the cell line used may have been rather high. In contrast, the $CB_1$ intrinsic activity of virodhamine was less than that of anandamide, and indeed it was found that virodhamine could attenuate anandamide-induced activation of $CB_1$ receptors. No binding data are yet available for virodhamine.

Turning now to potent cannabinoid receptor agonists that interact more readily with $CB_1$ or $CB_2$ receptors, a number of these have been developed. The starting point for all current $CB_1$-selective agonists has been anandamide. Thus, results from binding experiments have shown that it is possible to enhance the marginal $CB_1$ selectivity exhibited by anandamide by replacing a hydrogen atom on the 1′ or 2 carbon with a methyl group to form *R*-(+)-methanandamide or O-689 (Fig. 8) (Abadji et al. 1994; Showalter et al. 1996). As well as increasing $CB_1$ selectivity, insertion of a methyl group on the 1′ or 2 carbon of anandamide increases resistance to the hydrolytic action of fatty acid amide hydrolase (FAAH) (Abadji et al. 1994; Adams et al. 1995). Anandamide analogues that exhibit particularly marked $CB_1$-selectivity in binding assays are ACEA, ACPA and a cyano analogue of methanandamide (O-1812) (Table 2; Fig. 8). All three behave as potent $CB_1$ receptor agonists (Di Marzo et al. 2001; Hillard et al. 1999). O-1812 appears to lack significant susceptibility to hydrolysis by FAAH, presumably because it resembles *R*-(+)-methanandamide in having a methyl group attached to its 1′-carbon. ACEA and ACPA, which do not have the 1′-carbon methyl substituent of *R*-(+)-methanandamide, show no sign of reduced susceptibility to enzymic hy-

**Table 4.** $K_i$ values of certain other ligands for the in vitro displacement of [$^3$H]CP55940 or [$^3$H]HU243[a] from $CB_1$- and $CB_2$-specific binding sites

| Ligand | $CB_1$ $K_i$ value (nM) | $CB_2$ $K_i$ value (nM) | Reference |
|---|---|---|---|
| **$CB_1$-selective ligands in order of decreasing $CB_1/CB_2$ selectivity** | | | |
| *R-N*-(1-methyl-2-hydroxyethyl)-2-*R*-methyl-arachidonamide | 7.42[b,c] | 1,952[d] | Goutopoulos et al. 2001 |
| O-585 | 8.6[b] | 324[b] | Showalter et al. 1996 |
| O-689 | 5.7[b] | 132[b] | Showalter et al. 1996 |
| Ligands without any marked $CB_1$ or $CB_2$ selectivity | | | |
| Ajulemic acid (CT-3) | 32.3[a,c] | 170.5[a] | Rhee et al. 1997 |
| 11-OH-cannabinol-DMH | 0.1[a,c] | 0.2[a] | Rhee et al. 1997 |
| 3-(1′,1′-dimethyl-cyclohexyl)-$\Delta^8$-THC | 0.57 | 0.65 | Krishnamurthy et al. 2003 |
| 11-OH-cannabinol | 38[a,c] | 26.6[a] | Rhee et al. 1997 |
| $\Delta^9$-THC-DMH | 0.241[a,c] | 0.199[a] | Rhee et al. 1997 |
| Cannabinol-DMH | 2[a,c] | 1.5[a] | Rhee et al. 1997 |
| Cannabidiol | 4,350 | 2,860 | Showalter et al. 1996 |
| | >10[a,c] | >10[a,e] | Bisogno et al. 2001 |
| 11-OH-$\Delta^8$-THC | 25.8[a,c] | 7.4[a] | Rhee et al. 1997 |
| 1-Deoxy-$\Delta^8$-THC-DMH | 23[c] | 2.9 | Huffman et al. 1996 |
| 3-(1′,1′-cyclopropyl-heptyl)-$\Delta^8$-THC | 0.44[c] | 0.86[d] | Papahatjis et al. 2002 |
| O-1184 | 5.25 | 7.41 | Ross et al. 1999b |
| *cis* (6a*S*, 10a*S*)-3-(1′,1′-DMH)-11-hydroxy-$\Delta^8$-THC (HU-211) | 1,990 | >10,000 | Showalter et al. 1996 |
| Abnormal-cannabidiol | >10,000 | >10,000 | Showalter et al. 1996 |
| **$CB_2$-selective ligands in order of increasing $CB_1/CB_2$ selectivity** | | | |
| JWH-015 | 383 | 13.8 | Showalter et al. 1996 |
| 1-Deoxy-11-hydroxy-$\Delta^8$-THC-DMH (JWH-051) | 1.2[c] | 0.032 | Huffman et al. 1996 |
| JTE-907 | 2,370 | 35.9 | Iwamura et al. 2001 |
| L-768242 | 1,917 | 12 | Gallant et al. 1996 |
| 3-(1′1′-dimethylpropyl)-1-deoxy-$\Delta^8$-THC (JWH-139) | 2,290[c] | 14 | Huffman et al. 1999 |
| 3-(1′1′-dimethylhexyl)-1-methoxy-$\Delta^8$-THC | 3,134[c] | 18 | Huffman et al. 2002 |
| 1-Deoxy-$\Delta^8$-THC | >10,000[c] | 32 | Huffman et al. 1999 |

See Figs. 1, 4, 5, 7, 8, 9, 11 and 12 for the structures of some of the compounds listed in this table.
DMH, dimethylheptyl; ND, not determined; THC, tetrahydrocannabinol.
[b]With phenylmethylsulphonyl fluoride (PMSF) in order to inhibit enzymic hydrolysis.
[c]Binding to rat cannabinoid receptors on transfected cells or on brain ($CB_1$) or spleen tissue ($CB_2$).
[d]Binding to mouse brain ($CB_1$) or spleen tissue ($CB_2$).
[e]Species unspecified. All other data from experiments with human cannabinoid receptors.

drolysis. Although insertion of this group into ACEA does markedly reduce the susceptibility of this molecule to FAAH-mediated hydrolysis, it also decreases the affinity of ACEA for $CB_1$ receptors by about 14-fold (Jarrahian et al. 2000). *R-N*-(1-

methyl-2-hydroxyethyl)-2-*R*-methyl-arachidonamide, which also exhibits marked $CB_1$-selectivity in binding assays (Table 4), has less metabolic stability than *R*-(+)-methanandamide (Goutopoulos et al. 2001). Another $CB_1$-selective agonist of note is the endocannabinoid 2-arachidonyl glyceryl ether (Hanus et al. 2001), the $CB_1$ intrinsic activity of which has been reported to match that of CP55940 and to be less than that of 2-arachidonoyl glycerol. 2-Arachidonyl glyceryl ether exhibits less potency at $CB_1$ receptors than either CP55940 or 2-arachidonoyl glycerol (Savinainen et al. 2001, 2003; Suhara et al. 2000, 2001).

The best $CB_2$-selective agonists to have been developed to date are all non-eicosanoid cannabinoids (Howlett et al. 2002; Ibrahim et al. 2003; Pertwee 1999a). They include the classical cannabinoids, L-759633, L-759656 and JWH-133, the non-classical cannabinoid HU-308, and the aminoalkylindole AM1241 (Figs. 5, 6 and 7). All these ligands bind more readily to $CB_2$ than to $CB_1$ receptors (Table 2) and have also been shown to behave as potent $CB_2$-selective agonists in functional bioassays (Hanus et al. 1999; Ibrahim et al. 2003; Pertwee 2000; Ross et al. 1999a).

One other cannabinoid receptor agonist of note is 3-(5′-cyano-1′,1′-dimethylpentyl)-1-(4-*N*-morpholinobutyryloxy)-$\Delta^8$-THC hydrochloride (O-1057). Thus, unlike all established cannabinoid receptor agonists, this is readily soluble in water and yet, compared to CP55940, its potency in the cyclic AMP assay is just 2.9 times less at $CB_1$ receptors and 6.5 times less at $CB_2$ receptors (Pertwee et al. 2000). The finding that it is possible to solubilize a cannabinoid and yet retain pharmacological activity has important implications for cannabinoid delivery not only in the laboratory but also in the clinic. As to structure–activity relationships for cannabinoid receptor agonists, the salient features of these have been well described elsewhere (Howlett et al. 2002; Pertwee 1999a). Recent findings of special interest are that the $CB_1$ and $CB_2$ affinities of $\Delta^8$-THC can be greatly enhanced both by replacing its C3 pentyl side chain with a 1′,1′-dimethyl-1′-cyclohexyl moiety (Fig. 4; Table 4) (Krishnamurthy et al. 2003) and by changing this side chain from pentyl to heptyl and introducing a cyclopropyl group at the 1′ position (Fig. 4; Table 4) (Papahatjis et al. 2002).

## 3.2 Cannabinoid $CB_1$ and $CB_2$ Receptor Antagonists

### 3.2.1 Selective $CB_1$ Receptor Antagonists

The first selective $CB_1$ receptor antagonist, the diarylpyrazole SR141716A (Fig. 10), was developed by Sanofi Recherche (Rinaldi-Carmona et al. 1994). This readily prevents or reverses effects induced by cannabinoids at $CB_1$ receptors, both in vitro and in vivo (reviewed in Howlett et al. 2002; Pertwee 1997). It binds with significantly higher affinity to $CB_1$ than $CB_2$ receptors (Table 3), lacks significant affinity for a wide range of non-cannabinoid receptors and does not exhibit detectable agonist activity at $CB_1$ and $CB_2$ receptors (Hirst et al. 1996; Rinaldi-Carmona et al. 1994, 1996a,b; Shire et al. 1996). Other established $CB_1$-selective antagonists are

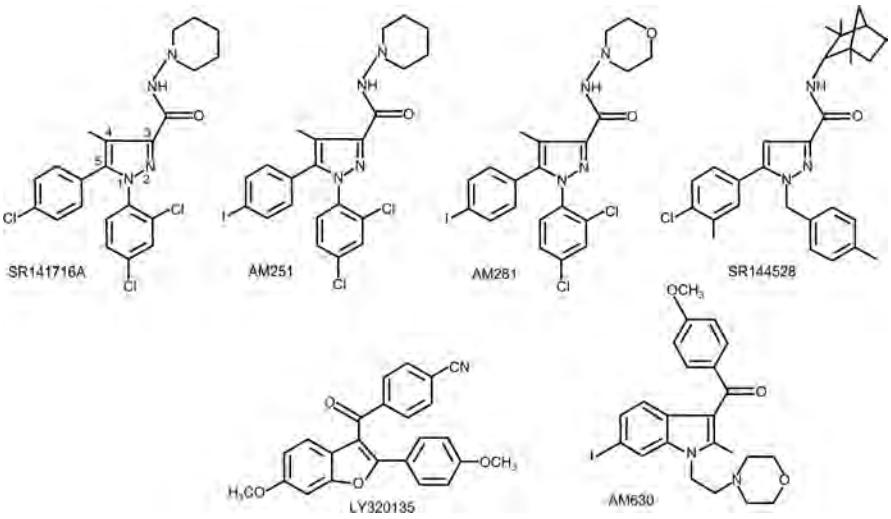


**Fig. 10.** The structures of several $CB_1$- or $CB_2$-selective antagonists/inverse agonists

LY320135, AM251 and AM281 (Fig. 10). LY320135, developed by Eli Lilly, also binds with lower affinity to $CB_1$ than $CB_2$ receptors (Table 3). However, its $CB_1$ affinity is less than that of SR141716A. Moreover, at concentrations in the low micromolar range, LY320135 also binds to muscarinic and 5-hydroxytryptamine $(5\text{-HT})_2$ receptors ($K_i$<10 μM) and, at higher concentrations, to histamine $H_1$ receptors ($K_I$=12.9 μM), $\alpha_1$- and $\alpha_2$-adrenoceptors and dopamine $D_1$ and $D_2$ receptors (Felder et al. 1998). AM251 and AM281 are both structural analogues of SR141716A. They have been found to displace [$^3$H]SR141716A from binding sites on mouse cerebellar membranes with respectively three and eight times less potency than SR141716A (Gatley et al. 1998), and both compounds have also been shown to bind more readily to $CB_1$ than $CB_2$ receptors (Table 3). There are numerous reports that, like SR141716A, AM251 and AM281 can attenuate in vivo or in vitro responses to established cannabinoid receptor agonists (e.g. Cosenza et al. 2000; Gifford et al. 1997; Hájos and Freund 2002a; Lan et al. 1999a; Simoneau et al. 2001).

Although SR141716A is $CB_1$-selective, it is not $CB_1$-specific. Thus, results from binding experiments indicate that whilst it may be reasonable to assume that concentrations of this ligand in the low or mid nanomolar range will interact mainly with the $CB_1$ receptors when it is applied to tissues that contain both $CB_1$ and $CB_2$ receptors, this is not so for higher concentrations of SR141716A (Table 3). Results obtained in vitro from functional bioassays also suggest that $CB_1$ receptors are not the only pharmacological targets with which this compound can interact at micromolar concentrations. For example, it has been found that SR141716A can stimulate extracellular-signal-regulated protein kinase (ERK) at 1 μM (Berdyshev et al. 2001) and antagonize anandamide-induced vasodilation in the mesenteric arteries of $CB_1^{-/-}$ mice at 1 and 5 μM (Járai et al. 1999). In addition there are reports that at concentrations above 1 μM, SR141716A can both block and activate

transient receptor potential vanilloid type 1 (TRPV1) receptors (previously known as VR1 receptors), suggesting that it may be a TRPV1 receptor partial agonist (De Petrocellis et al. 2001a; Zygmunt et al. 1999), block adenosine $A_1$ receptors (as can AM251) (Savinainen et al. 2003), oppose vasorelaxation induced by acetylcholine in ring preparations of rabbit preconstricted isolated superior mesenteric arteries (Chaytor et al. 1999) and by bradykinin in human preconstricted myometrial small arteries (Kenny et al. 2002), and block potassium and L-type calcium channels in rat isolated mesenteric arteries (White and Hiley 1998) and gap junctions between COS-7 cells (Chaytor et al. 1999).

Unexpectedly, in spite of the close similarity between the structures of AM251, AM281 and SR141716A, differences in their pharmacological profiles have been detected in vitro in experiments with cardiovascular tissue (reviewed in Pertwee 2004a). It has also been found that the ability of *R*-(+)-WIN55212 to reduce glutamatergic transmission is opposed by 1 μM SR141716A in $CB_1^{-/-}$ mouse hippocampal slices but not by 2 μM AM251 in rat hippocampal slices (Hájos and Freund 2002a; Hájos et al. 2001).

### 3.2.2
### Selective $CB_2$ Receptor Antagonists

The most important selective $CB_2$ receptor antagonists are the diarylpyrazole SR144528 and the aminoalkylindole 6-iodopravadoline (AM630) (Fig. 10). Both bind with markedly higher affinity to $CB_2$ than $CB_1$ receptors (Table 3) and prevent or reverse in vitro effects mediated by $CB_2$ receptors (Portier et al. 1999; Rinaldi-Carmona et al. 1998; Ross et al. 1999a). Evidence also exists that on the one hand, SR144528 lacks significant affinity for a wide range of established non-cannabinoid receptors (Rinaldi-Carmona et al. 1998), and on the other hand it is an antagonist for a putative $CB_2$-like receptor that is activated by palmitoylethanolamide, a ligand that does not have significant $CB_2$ receptor affinity (Sect. 4.1.3). Interestingly, it has proved possible to develop diarylpyrazoles with even greater $CB_2$ selectivity and affinity than SR144528 (Mussinu et al. 2003). This has been achieved by making these molecules less flexible.

Turning now to AM630, particularly with regard to its behaviour at the $CB_1$ receptor, there are several reports that when administered at concentrations in the micromolar range, it exhibits the mixed agonist-antagonist properties typical of a weak partial agonist for this receptor (reviewed in Pertwee 1999a). However, there are also reports that AM630 can behave as a $CB_1$ receptor inverse agonist (Landsman et al. 1998; Vásquez et al. 2003).

## 3.3
## Inverse Agonism at Cannabinoid Receptors

There is good evidence that when administered by itself in vivo or in vitro, SR141716A is capable of producing inverse cannabimimetic effects, i.e. effects

that are opposite in direction to those produced by the activation of $CB_1$ receptors (reviewed in Pertwee 2003). There are also reports that such inverse effects can be induced by the other cannabinoid receptor antagonists described in Sect. 3.2: AM251 (Vásquez et al. 2003), AM281 (Cosenza et al. 2000; Gifford et al. 1997; Izzo et al. 2000; Vásquez et al. 2003), LY320135 (Felder et al. 1998) and AM630 (Sect. 3.2.2) at $CB_1$ receptors and SR144528 (Portier et al. 1999; Rinaldi-Carmona et al. 1998; Ross et al. 1999b), AM630 (New and Wong 2003; Ross et al. 1999a) and AM251 (New and Wong 2003) at $CB_2$ receptors. These effects include SR141716A- and AM281-induced hyperkinesia in rats and/or mice (Compton et al. 1996; Cosenza et al. 2000; Costa and Colleoni 1999) and the attenuation in vitro of $CB_1$ or $CB_2$ receptor signalling. Two other compounds, the $CB_2$-selective ligands JTE-907 and BML-190 (Figs. 7 and 9), also behave as $CB_2$ receptor inverse agonists (Iwamura et al. 2001; New and Wong 2003). However, whether JTE-907 or BML-190 produces antagonism at $CB_2$ receptors has not been reported.

Whereas some inverse cannabimimetic effects of SR141716A may be produced as a result of antagonism of responses to endogenously released endocannabinoids, there is evidence that others are not, prompting the hypothesis that this compound is an inverse agonist that can elicit responses at $CB_1$ receptors that are opposite in direction from those elicited by conventional agonists. This turn has been taken to indicate that $CB_1$ receptors can exist in two or more interchangeable conformations (reviewed in Pertwee 2003, 2005). More specifically, it has been proposed that these are (1) a constitutively active "on" state in which the receptors are functionally coupled to their effector mechanisms even in the absence of exogenously added or endogenously produced cannabinoid receptor agonists and (2) one or more "off" states in which the receptors are uncoupled from their effector mechanisms. According to this hypothesis, agonists increase the proportion of receptors in the "on" state, inverse agonists increase the proportion of receptors in the "off" state(s) and neutral antagonists leave the number of receptors in each state unchanged.

There is evidence that SR141716A exhibits greater potency in opposing effects induced by $CB_1$ agonists than in producing inverse effects at $CB_1$ receptors by itself (e.g. Sim-Selley et al. 2001). This raises the possibilities, first, that SR141716A may be a neutral $CB_1$ receptor antagonist at low concentrations that exhibits additional $CB_1$ inverse agonist activity only at higher concentrations, and secondly, that SR141716A may have two sites of action on the $CB_1$ receptor, one at which it displaces agonists to produce antagonism and another at which it somehow induces inverse agonism, perhaps through an allosteric mechanism (Sim-Selley et al. 2001).

Although it is likely that at least some of the inverse effects produced by SR144528 or AM630 at $CB_2$ receptors are also due to inverse agonism, no attempts have been made to establish this conclusively. It is noteworthy, therefore, that the finding that a maximal concentration of SR144528 enhances forskolin-stimulated cyclic AMP production by human (h)$CB_2$-transfected CHO cells considerably more than a maximal concentration of AM630 (Ross et al. 1999a,b) can be better explained in terms of inverse agonism at the $CB_2$ receptor than in terms of antagonism of endogenously released endocannabinoids. This is because the simplest explanation for this difference between the maximal inverse effects of these two ligands is that

SR144528 has greater inverse intrinsic activity than AM630. If this interpretation of the data is valid, it is of course an indication that just as the intrinsic activities of $CB_1$ and $CB_2$ receptor agonists can vary from compound to compound, so too the (inverse) intrinsic activities of cannabinoid receptor inverse agonists will not be the same for all such ligands.

Whilst there is little doubt that the presence of $CB_1$ receptors is a prerequisite for the production by SR141716A of many of its inverse cannabimimetic effects, it is noteworthy that this compound has been found to produce an effect on GTP$\gamma$S binding to whole brain membranes obtained from $CB_1^{-/-}$ mice (enhancement) opposite to that produced by *R*-(+)-WIN55212 or anandamide (inhibition) (Breivogel et al. 2001). This finding supports the hypothesis that at least some apparent inverse effects of SR141716A may be induced at sites that are not located on $CB_1$ receptors (Sim-Selley et al. 2001). Indeed, it is already known that SR141716A not only binds to $CB_2$ receptors at concentrations in the high nanomolar range and above (Table 3) but also behaves as a $CB_2$ receptor inverse agonist at such concentrations, as measured by inhibition of [$^{35}$S]GTP$\gamma$S binding to h$CB_2$ receptors on CHO cell membranes (MacLennan et al. 1998).

### 3.4 Neutral Antagonism at Cannabinoid Receptors

An important recent pharmacological objective has been the development of cannabinoid receptor ligands for $CB_1$ and $CB_2$ receptors that completely lack both inverse agonist and agonist properties (neutral antagonists). One cannabinoid receptor ligand that comes close to being a neutral antagonist is 6′-azidohex-2′-yne-$\Delta^8$-THC (O-1184; Fig. 11 and Table 4), as this behaves as a high-affinity, low-efficacy agonist at $CB_1$ receptors and as a high-affinity, low-efficacy inverse agonist at $CB_2$ receptors, and as it produces potent antagonism of *R*-(+)-WIN55212 and CP55940 in the myenteric plexus–longitudinal muscle preparation of guinea-pig small intestine (Ross et al. 1998, 1999b). More recently, an analogue of SR141716A, NESS 0327, has been developed that behaves as a neutral $CB_1$ receptor antagonist and is markedly more potent and $CB_1$-selective than SR141716A (Table 3) (Ruiu et al. 2003). This was achieved by reducing the molecule's flexibility through the introduction of a seven-membered ring (Fig. 11). Evidence has also emerged that insertion of a 6″-azidohex-2″-yne side chain into cannabidiol (Fig. 1) converts this molecule into a neutral cannabinoid receptor antagonist (Thomas et al. 2004). This compound, O-2654 (Fig. 11), has markedly higher affinity than cannabidiol for $CB_1$ receptors and antagonizes *R*-(+)-WIN55212-induced inhibition of electrically evoked contractions of the mouse isolated vas deferens in a competitive, surmountable manner with a $K_B$ (85.7 nM) that is close to its $K_i$ for displacing [$^3$H]CP55940 from $CB_1$ receptors (114 nM). The conclusion that O-2654 may be a neutral antagonist is based on the observation that at concentrations of up to 10 μM, it exhibits no detectable $CB_1$ agonist or inverse agonist properties in the mouse isolated vas deferens. Thus, unlike SR141716A (Pertwee et al. 1996b), O-2654 does not increase the amplitude of electrically evoked contractions of this

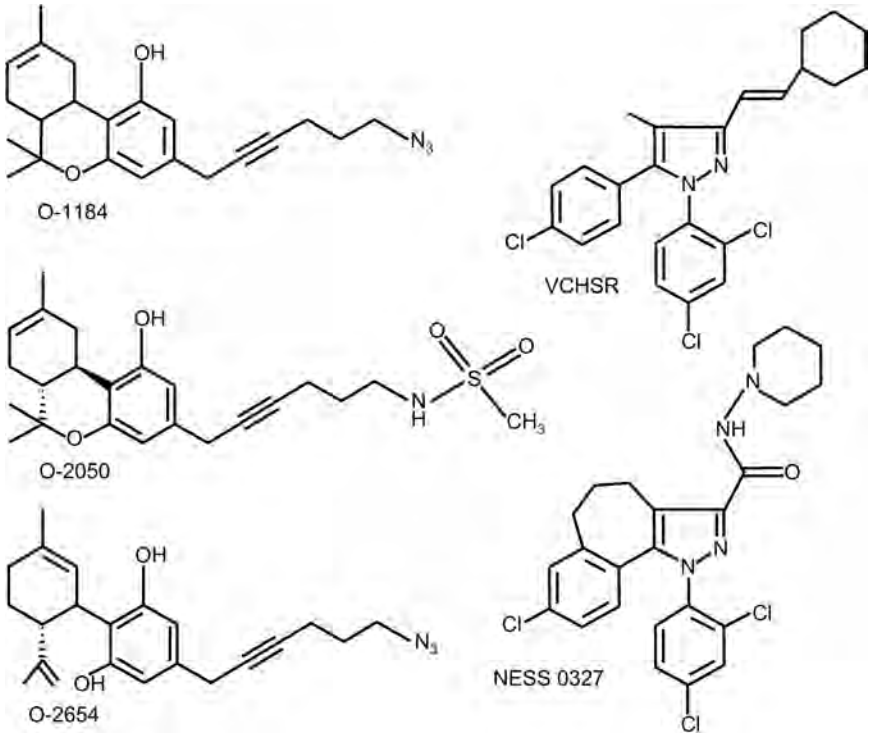


**Fig. 11.** The structure of O-1184 and of some putative neutral cannabinoid receptor antagonists

preparation. Nor does it share the ability of the $CB_1$ partial agonist, O-1184, to inhibit these contractions (Ross et al. 1999b). O-2050, a sulphonamide analogue of $\Delta^8$-THC with an acetylenic side chain also behaves as a neutral $CB_1$ receptor antagonist in the mouse vas deferens (Martin et al. 2002). Another compound that seems to be a neutral $CB_1$ antagonist is VCHSR (Fig. 11). This is an analogue of SR141716A that lacks hydrogen bonding capability in its C3 substituent region and has a $CB_1$ $K_i$ value in the low nanomolar range. VCHSR (1 μM) has been found to share the ability of SR141716A to attenuate *R*-(+)-WIN55212-induced inhibition of $Ca^{2+}$ current in rat superior cervical ganglion neurons expressing the human $CB_1$ receptor but to differ from SR141716A in not affecting $Ca^{2+}$ current in these neurons when administered by itself at 1 or 10 μM (Hurst et al. 2002; Pan et al. 1998). In terms of the two-state model of inverse agonism (see Pertwee 2003, 2005 and Sect. 3.3), this finding suggests that preferential binding by SR141716A to the "off" state of the $CB_1$ receptor is determined by hydrogen bond formation between the C3 substituent of this molecule and the receptor. Further experiments are required to establish whether putative neutral antagonists, such as NESS 0327, O-2654 and O-2050, resemble SR141716A (Sect. 3.3) in exhibiting inverse agonist properties at concentrations above those at which they behave as neutral antagonists.

# 4
# Other Pharmacological Targets for Cannabinoids in Mammalian Tissues

As discussed in greater detail elsewhere (Hájos and Freund 2002b; Howlett et al. 2002; Pertwee 1999b, 2004a; Pertwee and Ross 2002; Wiley and Martin 2002), evidence is emerging that in addition to $CB_1$ and $CB_2$ receptors, there are other pharmacological targets in mammalian tissues with which at least some established $CB_1$ and/or $CB_2$ receptor agonists can interact to elicit pharmacological responses.

## 4.1
## Receptors

### 4.1.1
### Vanilloid Receptors

It is now generally accepted that the endogenous $CB_1/CB_2$ receptor agonist, anandamide, and certain of its analogues are agonists for the TRPV1 receptor (reviewed in Howlett et al. 2002; Pertwee 2004a; Pertwee and Ross 2002; Ross 2003). This receptor is a non-selective cation channel that is present on sensory neurons in tissues such as skin, heart, blood vessels and lung, and an important consequence of its activation is the release of sensory neuropeptides that then produce effects such as pain, tachycardia, vasodilation and bronchoconstriction. It is noteworthy, however, that anandamide has less TRPV1 intrinsic activity than the well-known TRPV1 receptor agonist capsaicin (Ross 2003; Ross et al. 2001). *R*-(+)-methanandamide is even less potent or effective than anandamide at activating TRPV1 receptors (Ross et al. 2001; Zygmunt et al. 1999), whereas lipoxygenase metabolites of anandamide show greater potency at these receptors than their parent compound, at least in guinea-pig bronchus (Craib et al. 2001; Pertwee and Ross 2002). The TRPV1 receptor is not activated by 2-arachidonoyl glycerol or by non-eicosanoid $CB_1/CB_2$ receptor agonists (Zygmunt et al. 1999), although it is activated by micromolar concentrations of the phytocannabinoid cannabidiol (Bisogno et al. 2001). One compound that behaves as a potent agonist at both TRPV1 and $CB_1$ receptors is the synthetic anandamide analogue O-1861 (Fig. 8) (Di Marzo et al. 2001). TRPV1 and $CB_1$ receptors have opposite effects on calcium channel conductance, and there are several reports that in cells such as cultured dorsal root ganglion neurons that co-express these receptors, responses elicited by TRPV1 receptor activation can be opposed by the simultaneous activation of $CB_1$ receptors (Ahluwalia et al. 2003; Ellington et al. 2002; Millns et al. 2001; Richardson et al. 1998; Ross 2003). Unexpectedly, however, there is also a report that in human embryonic kidney cells co-transfected with $CB_1$ and TRPV1 receptors, activation of the $CB_1$ receptors increases the sensitivity of the TRPV1 receptors to subsequent (but not simultaneous) activation (Hermann et al. 2003). Under physiological conditions, TRPV1 receptors on primary sensory neurons are less sensitive to anandamide than $CB_1$ receptors (Németh et al. 2003; Tognetto et al. 2001). There is also evidence that anandamide production increases during inflammation, raising the possibility that

in healthy tissue, one role of anandamide may be to act through $CB_1$ receptors to oppose any increase in the excitability of sensory neurons, whilst in pathological states such as inflammation, anandamide concentrations and TRPV1 receptor sensitivity increase to the extent that anandamide-induced activation of TRPV1 receptors becomes sufficient to cause an increase in the excitability of sensory neurons (Ahluwalia et al. 2003). Although there is little doubt that anandamide is an endogenous agonist for $CB_1$ and $CB_2$ receptors, the question of whether it also serves as an endogenous TRPV1 agonist under normal or pathological conditions has still to be resolved. Also currently uncertain is the extent to which $CB_1$ and TRPV1 receptors are co-expressed on the same neurons (reviewed in Ross 2003).

### 4.1.2 $CB_1$ Receptor Subtypes

Shire et al. (1995) have isolated a spliced variant of $CB_1$ cDNA ($CB_{1A}$) from a human lung cDNA library. $CB_{1A}$ mRNA is present in human brain tissue, its distribution pattern matching that of $CB_1$ mRNA. It has also been detected in peripheral tissues. The spliced variant resembles the $CB_1$ receptor in its affinity for $\Delta^9$-THC, CP55940 and *R*-(+)-WIN55212, and it also has at least two signal transduction mechanisms in common with the $CB_1$ receptor (Rinaldi-Carmona et al. 1996a). However, the central and peripheral concentrations of $CB_{1A}$ mRNA are far below those of $CB_1$ mRNA (Shire et al. 1995). Onaivi et al. (1996) have discovered three distinct $CB_1$ mRNAs in brain tissue from C57BL/6 mice, although only one $CB_1$ receptor cDNA. C57BL/6 mice were less sensitive to the hypothermic and antinociceptive effects of $\Delta^9$-THC than two other mouse strains in which only one $CB_1$ mRNA was detectable.

Results from pharmacological experiments with rats and mice performed by Sandra Welch's group also suggest that there may be more than one subtype of $CB_1$ receptor (reviewed in Howlett et al. 2002; Pertwee 2001). In mouse experiments, for example, it was found that intraperitoneal SR141716A was more effective in opposing the antinociceptive effects of some $CB_1$ receptor agonists than of other such agonists when these were administered intrathecally and that intrathecal morphine interacted synergistically with intrathecal THC but not with intrathecal CP55940. Apparent differences between mouse cannabinoid receptors in brain and spinal cord were also detected.

### 4.1.3 $CB_2$-Like Receptors

It is possible that palmitoylethanolamide may produce antinociception in rat and mouse models of inflammatory or neuropathic pain by acting on a $CB_2$-like receptor (Calignano et al. 1998, 2001; Conti et al. 2002; Farquhar-Smith et al. 2002; Farquhar-Smith and Rice 2001; Helyes et al. 2003). The existence of such a receptor is supported by the finding that even though palmitoylethanolamide lacks significant $CB_2$ receptor affinity or efficacy (Griffin et al. 2000; Lambert et al. 1999;

Sheskin et al. 1997; Showalter et al. 1996), the antinociceptive effects of this fatty acid amide are opposed by SR144528. Evidence for $CB_2$-like receptors has also been obtained from experiments with the mouse vas deferens (Griffin et al. 1997). Other possibilities, i.e. that palmitoylethanolamide acts through $CB_1$ or TRPV1 receptors, can be ruled out. Thus, it produces antinociceptive effects that are not opposed by SR141716A (Calignano et al. 1998, 2001; Farquhar-Smith et al. 2002; Farquhar-Smith and Rice 2001) and it has been found not to attenuate nociceptive behaviour induced in mice by intraplantar injection of capsaicin (Calignano et al. 2001). Also, palmitoylethanolamide does not bind to or activate $CB_1$ receptors at concentrations below 1 or 10 μM (Devane et al. 1992; Felder et al. 1993; Griffin et al. 2000; Lambert et al. 1999; Showalter et al. 1996). Anandamide shares the ability of palmitoylethanolamide to induce antinociception in mice and rats. However, unlike palmitoylethanolamide, it has been found to be susceptible to SR141716A -induced antagonism and resistant to SR144528-induced antagonism in several pain models (Calignano et al. 1998, 2001; Farquhar-Smith and Rice 2001). Also, in contrast to palmitoylethanolamide, anandamide attenuates nociceptive behaviour induced in mice by intraplantar injection of capsaicin (Calignano et al. 2001). Another observation—that palmitoylethanolamide and anandamide interact synergistically rather than additively in the mouse formalin paw and abdominal stretch tests—also supports the hypothesis that they have different antinociceptive mechanisms (Calignano et al. 1998, 2001).

### 4.1.4 Neuronal Non-$CB_1$, Non-$CB_2$, Non-TRPV1 Receptors

*Central G Protein-Coupled Receptors for Anandamide and R-(+)-WIN55212*

Evidence for the presence of a G protein-coupled non-$CB_1$, non-$CB_2$ receptor for anandamide and *R*-(+)-WIN55212 has come from experiments in which it was found that [$^{35}$S]GTPγS binding to whole-brain membranes from $CB_1^{-/-}$ C57BL/6 mice or to cerebellar homogenates from $CB_1^{-/-}$ CD1 mice could be enhanced by these two cannabinoids (Breivogel et al. 2001; Di Marzo et al. 2000; Monory et al. 2002). Near maximal concentrations of anandamide and *R*-(+)-WIN55212 were not fully additive in their effects on [$^{35}$S]GTPγS binding to $CB_1^{-/-}$ C57BL/6 brain membranes, supporting the hypothesis that these two agents were acting through a common mechanism (Breivogel et al. 2001). This putative receptor for anandamide and *R*-(+)-WIN55212 appears not to be a TRPV1 receptor (Sect. 4.1.1) or to resemble the proposed abnormal-cannabidiol receptor (Sect. 4.1.5) as neither of these pharmacological targets is *R*-(+)-WIN55212-sensitive and as the TRPV1 receptor is not G protein coupled. However, the possibility does remain that it may be a novel metabotropic "vanilloid-like" receptor (see below). The proposed new receptor also differs from established cannabinoid receptors in several ways.

- It is not sensitive to activation by the established $CB_1/CB_2$ receptor agonists, $\Delta^9$-THC, CP55940 or HU-210 (Breivogel et al. 2001; Di Marzo et al. 2000; Monory et al. 2002).
- It is not coupled to adenylate cyclase, at least in the cerebellum of $CB_1^{-/-}$ CD1 mice (Monory et al. 2002).
- It differs from the $CB_1$ receptor in its central distribution pattern (Breivogel et al. 2001; Monory et al. 2002).
- SR141716A and SR144528 do not appear to be competitive antagonists for this putative receptor (Breivogel et al. 2001; Monory et al. 2002).
- There are no specific binding sites for [$^3$H]CP55940 on $CB_1^{-/-}$ C57BL/6 mouse brain membranes (Breivogel et al. 2001).

It has also been found that [$^3$H]*R*-(+)-WIN55212 undergoes selective binding to $CB_1^{-/-}$ C57BL/6 membranes obtained from brain areas in which *R*-(+)-WIN55212 enhances [$^{35}$S]GTP$\gamma$S binding (cerebral cortex, hippocampus and brain stem) (Breivogel et al. 2001). Furthermore, $CB_1^{-/-}$ C57BL/6 brain areas that are unresponsive to *R*-(+)-WIN55212-induced enhancement of [$^{35}$S]GTP$\gamma$S binding seem to lack [$^3$H]*R*-(+)-WIN55212 binding sites (Breivogel et al. 2001). It is noteworthy, however, that some WIN55212-sensitive brain areas of $CB_1^{-/-}$ C57BL/6 mice (midbrain and diencephalon) and of $CB_1^{-/-}$ CD1 mice (cerebellum) also seem to lack [$^3$H]*R*-(+)-WIN55212 binding sites (Breivogel et al. 2001; Ledent et al. 1999; Monory et al. 2002). Although $CB_1^{-/-}$ C57BL/6 mouse brain does contain specific binding sites for both [$^3$H]SR141716A and [$^3$H]*R*-(+)-WIN55212, these two binding site populations have different distribution patterns (Breivogel et al. 2001). This is further evidence that SR141716A lacks affinity for the proposed *R*-(+)-WIN55212/anandamide receptor.

A pharmacological property that the proposed *R*-(+)-WIN55212/anandamide receptor may share with the $CB_1$ receptor is the ability to mediate antinociception, catalepsy and hypokinesia. Thus, whilst $\Delta^9$-THC produced these effects only in the wild-type mice, anandamide was essentially as potent and effective in producing these effects in $CB_1^{-/-}$ as in $CB_1^{+/+}$ C57BL/6 mice (Di Marzo et al. 2000). Indeed, this putative new receptor may well prove to be a novel target for anti-spasticity and analgesic drugs (Brooks et al. 2002). The presence of specific binding sites for [$^3$H]SR141716A on $CB_1^{-/-}$ C57BL/6 mouse brain membranes may explain the ability of SR141716A both to inhibit [$^{35}$S]GTP$\gamma$S binding to such membranes (Breivogel et al. 2001) and to reduce milk intake and survival of newborn $CB_1^{-/-}$ C57BL/6 mice (Fride et al. 2003).

*Central TRPV1-Like Receptors*

Evidence has emerged for the presence of G protein-coupled, non-$CB_1$ receptors on glutamatergic axonal terminals in the hippocampus with which at least some cannabinoid receptor agonists can interact to inhibit glutamate release. More specifically, results from electrophysiological experiments with hippocampal slices

obtained from rats or $CB_1^{+/+}$ CD1 mice have shown that *R*-(+)-WIN55212 reduces both excitatory postsynaptic currents (EPSCs) evoked in CA1 pyramidal cells or dentate granule cells and paired pulse facilitation of EPSCs, even though it has not proved possible to detect $CB_1$ receptor immunostaining on axonal terminals that form glutamatergic synapses in rat hippocampus (Hájos and Freund 2002a; Hájos et al. 2000, 2001). Similar results have been obtained in experiments with $CB_1^{-/-}$ CD1 mouse hippocampal slices (Hájos et al. 2001). *R*-(+)-WIN55212 also inhibits potassium-evoked glutamate release from hippocampal synaptosomes obtained from rats or from $CB_1^{+/+}$ or $CB_1^{-/-}$ mice in an SR141716A- and AM251-independent manner (Köfalvi et al. 2003). Evidence for an involvement of G proteins in the apparent inhibitory effect of *R*-(+)-WIN55212 on glutamate release in mouse hippocampal slices comes from the finding that this effect is pertussis toxin-sensitive (Misner and Sullivan 1999).

The ability of *R*-(+)-WIN55212 to reduce evoked EPSCs in rat hippocampal slices is shared by CP55940 and capsaicin, and all three of these agonists are antagonized by the TRPV1 receptor antagonist capsazepine (Hájos and Freund 2002a). Because the peripheral TRPV1 receptor is neither activated by *R*-(+)-WIN55212 or CP55940 nor coupled to G proteins, it may be that *R*-(+)-WIN55212, CP55940 and capsaicin modulate central glutamate release by acting through a novel metabotropic "vanilloid-like" receptor. Consequently, it would be of interest to establish first whether capsaicin enhances GTP$\gamma$S binding to brain membranes, and secondly whether *R*-(+)-WIN55212-induced enhancement of GTP$\gamma$S binding to $CB_1^{-/-}$ mouse brain membranes (see above) can be antagonized by capsazepine.

Evidence for the presence of vanilloid-like receptors in the hippocampus has also been obtained by Al-Hayani et al. (2001). They found paired-pulse depression in the CA1 region of rat hippocampal slices to be increased both by anandamide and by two other TRPV1 receptor agonists, capsaicin and resiniferatoxin, in a manner that was sensitive to antagonism by capsazepine but not by the $CB_1$ receptor antagonist AM281. Given the results obtained by Hájos et al. (see above), it is possible that these agonists were acting through central vanilloid-like receptors to cause a decrease in excitatory glutamatergic transmission. Alternatively, they may have been acting through these putative receptors to cause an increase in inhibitory $\gamma$-aminobutyric acid (GABA)ergic transmission. If anandamide was acting through vanilloid-like receptors, then it apparently activates them more readily than $CB_1$ receptors, which contrasts with reports that this endocannabinoid interacts less potently with established TRPV1 receptors than with $CB_1$ receptors (Sect. 4.1.1). In contrast to anandamide, both *R*-(+)-WIN55212 and 2-arachidonoyl glycerol were found to decrease paired-pulse depression in an SR141716A or AM281-sensitive manner (Al-Hayani et al. 2001; Paton et al. 1998). This would suggest that unlike anandamide, these two agonists interact preferentially with $CB_1$ receptors in this experimental model. There is evidence that anandamide and/or capsaicin can modulate glutamatergic transmission in brain areas other than the hippocampus in a manner that is $CB_1$-independent and susceptible to antagonism by capsazepine and/or iodoresiniferatoxin. These brain areas include rat locus coeruleus, substantia nigra and medullary dorsal horn (Jennings et al. 2003; Marinelli et al. 2002,

2003). In these experiments, however, glutamatergic transmission was facilitated by anandamide and/or capsaicin.

There is currently no support for the hypothesis that *R*-(+)-WIN55212 inhibits glutamate release in the hippocampus by acting on the non-$CB_1$, non-$CB_2$ molecular target that is thought to mediate its enhancement of GTP$\gamma$S binding to central neuronal membranes (see above). Thus, although *R*-(+)-WIN55212 does suppress evoked EPSCs and paired pulse facilitation in $CB_1^{-/-}$ CD1 mouse hippocampal slices (Hájos et al. 2001), it does not enhance GTP$\gamma$S binding to $CB_1^{-/-}$ CD1 mouse hippocampal membranes (Monory et al. 2002). Also, whilst CP55940 suppresses evoked EPSCs in rat hippocampal slices (Hájos and Freund 2002a) and potassium-evoked glutamate release from rat hippocampal synaptosomes (Köfalvi et al. 2003), it does not share the ability of *R*-(+)-WIN55212 or anandamide to enhance GTP$\gamma$S binding to $CB_1^{-/-}$ C57BL/6 mouse brain membranes (Breivogel et al. 2001).

*Peripheral Nervous System*

Results from experiments with phenylephrine-precontracted rat isolated mesenteric and hepatic arteries suggest that $\Delta^9$-THC can relax these vessels by acting on capsaicin-sensitive perivascular sensory neurons to induce release of calcitonin gene-related peptide (Zygmunt et al. 2002). The underlying mechanism is most probably $CB_1$ and $CB_2$ receptor-independent, as this relaxant effect of $\Delta^9$-THC was not prevented by 300 nM SR141716A or by 30 nM AM251 and as the $CB_1$/$CB_2$ receptor agonists HU-210 and CP55940 lacked detectable relaxant activity, whereas cannabinol, which has relatively low activity as a cannabinoid receptor agonist (Sect. 3.1), was equipotent with $\Delta^9$-THC. The possibility, that $\Delta^9$-THC was acting through ionotropic or metabotropic glutamate receptors was also excluded. Other observations made in this investigation were that $\Delta^9$-THC- and cannabinol-induced activation of CGRP release from rat arterial segments could be prevented by capsaicin pretreatment and that $\Delta^9$-THC- and cannabinol-induced relaxations of precontracted arterial segments could be attenuated by the noncompetitive TRPV1 antagonist ruthenium red. However, these cannabinoids were most probably not acting through TRPV1 receptors in these experiments. Thus, the competitive TRPV1 antagonist capsazepine did not attenuate the vasorelaxant effects of $\Delta^9$-THC and cannabinol, and in contrast to both capsaicin and anandamide, $\Delta^9$-THC also relaxed phenylephrine-precontracted mesenteric arterial segments that had been obtained from $TRPV1^{-/-}$ mice. In more recent experiments, Jordt et al. (2004) have obtained evidence that $\Delta^9$-THC and cannabinol may have induced vasorelaxation by acting through ANKTM1, another member of the transient receptor potential (TRP) family of ion channels that, unlike the TRPV1 receptor, appears to be insensitive to anandamide and is implicated in the detection of noxious cold. ANKTMI was found to be insensitive to HU-210, CP55940 and 2-arachidonoyl glycerol.

It has also been proposed that the terminals of sympathetic neurons supplying cardiovascular tissue express a non-$I_1$, non-$I_2$ subtype of the putative imidazoline receptor that is both $CB_1$ receptor-like and $\alpha_2$-adrenoceptor-like and that mediates inhibition of evoked noradrenaline release when activated (reviewed in

Göthert et al. 1999; Molderings and Göthert 1999; Pertwee 2004a). There is evidence that this putative receptor can be activated both by the cannabinoids—CP55940, *R*-(+)-WIN55212 and anandamide—and by non-$CB_1$, non-$CB_2$ ligands such as aganodine and clonidine, and that this activation is sensitive to antagonism by SR141716A (1 μM), LY320135 (0.1 or 1 μM) and rauwolscine (30 μM) (reviewed in Pertwee 2004a). It also appears that this proposed receptor may belong to the G protein-coupled receptor family originally known as endothelial differentiation gene (EDG) receptors and that it can be activated by 1-oleoyl-lysophosphatidic acid (Molderings et al. 2002).

Mang et al. (2001) have obtained evidence that anandamide can act on nerve terminals of the myenteric plexus–longitudinal muscle preparation of the guinea-pig ileum to inhibit electrically evoked release of the contractile transmitter acetylcholine through a mechanism that is independent of both TRPV1 and $CB_1$ receptors. Thus, the inhibitory effects of anandamide on electrically evoked release of [$^3$H]acetylcholine and on electrically evoked contractions of this isolated tissue preparation were insensitive to antagonism by 1 μM capsazepine. They were also much less sensitive to antagonism by SR141716A than expected for $CB_1$-mediated effects. Results from other experiments with this tissue preparation suggest that anandamide can increase both basal acetylcholine release from neurons and longitudinal muscle tone by acting on neuronal TRPV1 receptors (Mang et al. 2001). Additional support for the presence of a non-$CB_1$ receptor for anandamide in the gastro-intestinal tract comes from experiments both with the strips of longitudinal muscle obtained from guinea-pig distal colon (Kojima et al. 2002) and with the rat isolated gastric fundus (Storr et al. 2002). In the colon experiments, evidence was obtained that anandamide, possibly after its conversion to active metabolites, can induce contractions by acting through a TRPV1 and $CB_1$ receptor-independent mechanism (Kojima et al. 2002). 2-Arachidonoyl glycerol also seems to act through such a mechanism to induce contractions of this tissue preparation (Kojima et al. 2002). In the gastric fundus experiments it was found that at 10 μM, the $CB_2$-selective antagonist AM630 attenuated anandamide- but not *R*-(+)-WIN55212-induced inhibition of electrically evoked contractions (Storr et al. 2002). It is likely that anandamide was acting on prejunctional neurons in this tissue, as it did not affect contractions produced by 5-HT or carbachol. AM630 has also been found to antagonize $\Delta^9$-THC, CP55940, *R*-(+)-WIN55212, methanandamide and anandamide in the mouse isolated vas deferens in an agonist-dependent and competitive manner. However, in this bioassay system, AM630 was less potent as an antagonist of anandamide than of *R*-(+)-WIN55212 (Pertwee et al. 1995). In view of evidence that the mouse vas deferens expresses neuronal $CB_2$-like receptors that can mediate inhibition of electrically evoked contractions (Griffin et al. 1997; Sect. 4.1.3), it may be that AM630 was producing its antagonism of cannabinoids in this tissue by competing for these putative $CB_2$-like receptors.

### 4.1.5 Receptors for Abnormal-Cannabidiol

*Cardiovascular System*

There is evidence, mainly from in vitro experiments with rat or mouse phenylephrine- or methoxamine-precontracted buffer-perfused isolated mesenteric arterial beds or isolated mesenteric arterial segments, for the presence in these tissues of non-$CB_1$, non-$CB_2$ receptors with which anandamide and methanandamide can interact to induce a relaxant effect (reviewed in Howlett et al. 2002; Pertwee 2004a; Wiley and Martin 2002). There are several reasons for believing that these are not $CB_1$ or $CB_2$ receptors. First, relaxation is not induced in rat precontracted mesenteric arterial beds by 2-arachidonoyl glycerol or by established non-eicosanoid cannabinoid receptor agonists such as $\Delta^9$-THC or *R*-(+)-WIN55212 (Wagner et al. 1999) but is induced in rat and mouse precontracted mesenteric arterial beds or rat precontracted mesenteric arterial segments by two cannabidiol analogues, abnormal-cannabidiol and O-1602 (Fig. 12), neither of which exhibits significant affinity for $CB_1$ receptors (Ho and Hiley 2003; Járai et al. 1999; Offertáler et al. 2003; Showalter et al. 1996). Second, anandamide, methanandamide and abnormal-cannabidiol also relax precontracted buffer-perfused mesenteric arterial beds of $CB_1^{-/-}$ knockout or $CB_1^{-/-}/CB_2^{-/-}$ double-knockout C57BL6J mice (Járai et al. 1999). Third, the $CB_1$-selective antagonist AM281 (1 μM) and the $CB_2$-selective antagonist AM630 (10 μM) do not attenuate abnormal-cannabidiol-induced relaxations of rat precontracted mesenteric arterial segments (Ho and Hiley 2003). Although SR141716A has been found to oppose the vasorelaxant effects of abnormal-cannabidiol, methanandamide and anandamide in rat or mouse precontracted mesenteric arterial beds or segments, this is generally with a potency lower than expected from its affinity for $CB_1$ receptors (Ho and Hiley 2003; Járai et al. 1999). Negative results obtained with capsaicin and capsazepine also make it unlikely that the putative "abnormal-cannabidiol" receptor is a TRPV1 receptor (Ho and Hiley 2003; Járai et al. 1999; Offertáler et al. 2003).

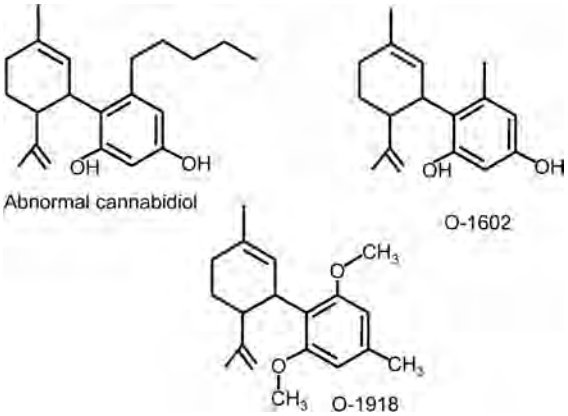


**Fig. 12.** The structures of abnormal cannabidiol, O-1602 and O-1918

One cannabidiol analogue has been found to behave as a selective abnormal-cannabidiol receptor antagonist. This is O-1918 (Fig. 12), which lacks detectable affinity for $CB_1$ and $CB_2$ receptors and, at concentrations of 1 to 30 μM, opposes abnormal-cannabidiol and anandamide-induced relaxations of rat arterial segments and does not reduce vasomotor tone when administered alone (Offertáler et al. 2003). It has also been found to attenuate abnormal-cannabidiol-induced hypotension in anaesthetized mice at doses not affecting hypotension induced by the $CB_1/CB_2$ receptor agonist HU -210 (Offertáler et al. 2003). Cannabidiol also behaves as a selective abnormal-cannabidiol receptor antagonist in both the rat mesenteric arterial bed and the anaesthetized mouse (Járai et al. 1999). However, in contrast to O-1918, it has been found to share the ability of abnormal-cannabidiol to relax rat precontracted mesenteric arterial segments (Offertáler et al. 2003).

It is likely that there are two sub-types of abnormal-cannabidiol-sensitive receptor in mesenteric arteries capable of mediating a relaxant effect, one expressed by endothelial cells and the second by non-endothelial cells (reviewed in Pertwee 2004a). Activation of the endothelial receptor appears to open large conductance calcium-activated potassium ($BK_{Ca}$) channels, whereas the non-endothelial receptor seems to signal mainly through inhibition of L-type calcium channels (Begg et al. 2003; Ho and Hiley 2003; Járai et al. 1999; Offertáler et al. 2003). There is also now evidence that abnormal-cannabidiol receptors can mediate stimulation of the migration of vascular endothelial cells through a mechanism that is $G_{i/o}$ protein-coupled and susceptible to antagonism by O-1918 (Mo et al. 2004).

*Microglial Cells*

Experiments with the mouse microglial cell line BV-2 (Walter et al. 2003) have provided evidence that microglial cells express receptors that have certain properties in common with the putative vascular abnormal-cannabidiol receptor discussed above. These include susceptibility to activation by abnormal-cannabidiol and anandamide and to blockade by O-1918 and lack of sensitivity to activation by $\Delta^9$-THC, at least at concentrations below 3 μM. When activated, these proposed abnormal-cannabidiol-sensitive receptors appear to trigger chemokinetic and chemotaxic migration of microglial cells. Such migration can also be induced by 2-arachidonoyl glycerol ($EC_{50}$=25 nM). This endocannabinoid seems to act through both microglial $CB_2$ receptors and microglial abnormal-cannabidiol-sensitive receptors, since it is antagonized by cannabidiol at 300 nM and by SR144528 at 30 nM but not by 30 nM SR141716A (Walter et al. 2003). Indeed, it has been proposed that microglial $CB_2$ receptors and abnormal-cannabidiol receptors interact in a synergistic manner when triggering the migration of microglial cells (Walter et al. 2003). This could explain why the $CB_1$-selective agonist ACPA (Sect. 3.1), induces microglial cell migration at concentrations well below those at which it has been reported to bind to $CB_2$ receptors, as this compound appears to induce migration by acting on both abnormal-cannabidiol-sensitive receptors and $CB_2$ receptors (Franklin and Stella 2003). By itself, cannabidiol behaves as a weak partial agonist, producing a slight enhancement of basal migration ($EC_{50}$=250 nM) (Walter et al. 2003). Microglial cells are thought to migrate towards

neuroinflammatory lesion sites and to release proinflammatory cytokines and cytotoxic agents at these sites. Consequently, since Walter et al. (2003) also obtained evidence that the production of 2-arachidonoyl glycerol by microglial cells can be increased by a pathological stimulus, it may be that a $CB_2$ receptor antagonist and/or an antagonist of the putative abnormal-cannabidiol receptor could come to play a part in the clinical management of neuroinflammation. More recently, evidence has emerged that BV-2 microglial cells express non-$CB_1$, non-$CB_2$, non-$CB_2$-like, non-TRPV1, non-abnormal-cannabidiol $G_i/G_o$-coupled-receptors upon which the endogenous fatty acid amide palmitoylethanolamide can act at concentrations in the low nanomolar range to potentiate anandamide- but not 2-arachidonoyl glycerol-induced migration of these cells (Franklin et al. 2003). There is also evidence for the presence in rat migroglial cells of non-$CB_1$, non-$CB_2$, pertussis toxin-insensitive receptors with which *R*-(+)- but not *S*-(–)-WIN55212 can interact to inhibit lipopolysaccharide-induced release of the proinflammatory cytokine tumour necrosis factor-$\alpha$ (Facchinetti et al. 2003).

*Mouse Vas Deferens*

A finding that abnormal-cannabidiol and cannabidiol can attenuate phenylephrine-induced contractions of the mouse isolated vas deferens points to the presence of abnormal-cannabidiol-sensitive receptors in the smooth muscle cells of this tissue (Pertwee et al. 2002; Thomas et al. 2004). Cannabidiol also decreases methoxamine and noradrenaline-induced contractions of the mouse vas deferens and antagonizes phenylephrine and noradrenaline in an insurmountable manner (Pertwee et al. 2002). It may be, therefore, that cannabidiol, and possibly also abnormal-cannabidiol, are negative allosteric modulators of the $\alpha_1$-adrenoceptor.

## 4.2 Allosteric Sites

There is evidence for the presence of allosteric sites for anandamide and/or certain other cannabinoids on several non-cannabinoid receptors (reviewed in Pertwee 2004a). These are 5-$HT_2$ receptors (Cheer et al. 1999), 5-$HT_3$ receptors (Barann et al. 2002; Fan 1995; Godlewski et al. 2003; Oz et al. 2002), $\alpha_1$-adrenoceptors (Sect. 4.1.5), $M_1$ and $M_4$ muscarinic receptors (Christopoulos and Wilson 2001) and $\alpha$-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) $GLU_{A1}$ and $GLU_{A3}$ glutamate receptors (Akinshola et al. 1999a,b). The functional consequences of occupation of the proposed allosteric sites on 5-$HT_2$ receptors (by HU-210) and on $M_1$ and $M_4$ receptors (by anandamide, methanandamide and SR141716A) have yet to be determined. However, cannabinoids have been found to inhibit currents triggered by the activation of $GLU_{A1}$ and $GLU_{A3}$ receptors (anandamide) or 5-$HT_3$ receptors ($\Delta^9$-THC, *R*-(+)-WIN55212, anandamide, JWH-015 (Fig. 7), CP55940 and the $CB_1$ receptor antagonist, LY320135). Cannabinoids have also been found to attenuate the von Bezold-Jarisch reflex induced in urethane-anaesthetized rats by 5-$HT_3$ receptor activation (CP55940 and *R*-(+)-WIN55212) and to oppose $\alpha_1$-

adrenoceptor-mediated contractions of the mouse vas deferens (cannabidiol). In addition, there are reports that 2-arachidonoyl glycerol and 5-HT each binds more readily to washed human platelets in the presence of the other compound (Maccarrone et al. 2003) and that 5-HT enhances binding of *R*-(+)-WIN55212 to $CB_1$ receptors (Devlin and Christopoulos 2002). Importantly, cannabinoids inhibited 5-$HT_3$ receptor currents in transfected human embryonic kidney cells with a rank order of potency, $\Delta^9$-THC>*R*-(+)-WIN55212>anandamide>JWH-015>LY320135>CP55940 (Barann et al. 2002), that does not correlate with their $CB_1$ or $CB_2$ receptor affinities or intrinsic activities (Sect. 3). The $IC_{50}$ values of these ligands were 38, 104, 130, 147, 523 and 648 nM, respectively (Barann et al. 2002). In contrast, the $IC_{50}$ values of anandamide for inhibition of kainate-activated currents in $GLU_{A1}$- and $GLU_{A3}$-transfected *Xenopus laevis* oocytes exceeded 100 μM (Akinshola et al. 1999b). In addition, some cannabinoids, including anandamide, methanandamide, *R*-(+)-WIN55212, $\Delta^9$-THC and cannabidiol, may serve as negative modulators of delayed rectifier potassium channels (reviewed in Pertwee 2004a). There is also evidence that nanomolar concentrations of anandamide can block low-voltage-activated (T-type) calcium channels through a mechanism that is independent of $CB_1$ and $CB_2$ receptors and of G proteins (Chemin et al. 2001). Evidence has also recently emerged for the presence of an allosteric site on the cannabinoid $CB_1$ receptor (R. Pertwee, R. Ross and M. Price, unpublished).

## 4.3 Some $CB_1$- and $CB_2$-Independent Actions of Cannabidiol, HU-211 and Other Phenol-Containing Cannabinoids

### 4.3.1 Neuroprotective Actions

Cannabinoids that contain a phenol group possess anti-oxidant (electron donor) activity that is sufficient to protect neurons against oxidative stress associated, for example, with glutamate-induced excitoxicity. Thus, as discussed in greater detail elsewhere (El-Remessy et al. 2003; Fowler 2003; Hampson et al. 1998, 2000; Marsicano et al. 2002; Mechoulam et al. 2002; Pertwee 2004b; Platt and Drysdale 2004; van der Stelt et al. 2002), this anti-oxidant activity is apparently independent of $CB_1$ or $CB_2$ receptors as it is exhibited both by the $CB_1$/$CB_2$ agonists $\Delta^9$-THC, HU-210 and CP55940, and by the non-psychoactive phytocannabinoid cannabidiol (Fig. 1) and the *cis* (6a*S*, 10a*S*) enantiomer of 11-hydroxy-$\Delta^8$-THC-dimethylheptyl, HU-211 (Fig. 4), neither of which has significant affinity for $CB_1$ or $CB_2$ receptors (Table 4). Moreover, neurons of $CB_1^{-/-}$ mice are no less well protected from oxidative stress by phenolic cannabinoids than neurons of $CB_1^{+/+}$ mice (Marsicano et al. 2002). The neuroprotective properties of HU-211 are also thought to stem from its ability to behave as a non-competitive antagonist at *N*-methyl-D-aspartate (NMDA) receptors and to inhibit tumour necrosis factor-$\alpha$ production (Mechoulam et al. 2002; Darlington 2003), and it is possible that cannabidiol may also protect from glutamate-induced excitotoxicity by opposing

metabotropic glutamate receptor-mediated release of calcium from intracellular stores (Drysdale et al. 2004). Non-phenolic cannabinoids have been reported to lack anti-oxidant activity (Marsicano et al. 2002). Even so, some non-phenolic (and phenolic) cannabinoids can protect against glutamate-induced excitotoxicity by acting through receptors to inhibit neuronal glutamate release (possibly putative TRPV1-like receptors; Sect. 4.1.4) and calcium entry into neurons through N- and P/Q-type channels ($CB_1$ receptors) (Fowler 2003; Mechoulam et al. 2002; van der Stelt et al. 2002).

### 4.3.2 Other Actions of Cannabidiol

Results from in vitro experiments suggest that cannabidiol has a number of $CB_1$/$CB_2$ receptor-independent actions through which it may affect neurotransmission (reviewed in Pertwee 1988, 2004b). For example, there is evidence that at concentrations in the nanomolar or low micromolar range, this cannabinoid enhances spontaneous or evoked release of certain transmitters, antagonizes *R*-(+)-WIN55212- and CP55940-induced inhibition of electrically evoked contractile transmitter release in the mouse isolated vas deferens through a $CB_1$-independent mechanism and inhibits the uptake of calcium, 5-HT, noradrenaline and dopamine by rat or mouse synaptosomes. Higher concentrations of cannabidiol inhibit anandamide uptake by rat basophilic leukaemia cells, the metabolism of this endocannabinoid by fatty acid amide hydrolase and the synaptosomal uptake of GABA. There is also evidence that cannabidiol is a TRPV1 receptor agonist, a ligand for the putative abnormal-cannabidiol receptor (Sect. 4.1.5) and a negative allosteric modulator of $\alpha_1$-adrenoceptors (Sect. 4.1.5) and delayed rectifier potassium channels (Sect. 4.2). In addition, cannabidiol inhibits/induces certain cytochrome P450 (CYP450) enzymes, has anti-tumour activity and possesses anti-inflammatory properties that may be due at least in part to inhibition of lipoxygenase activity and cytokine release (Pertwee 2004b).

The $CB_1$ and $CB_2$ affinities of cannabidiol can be greatly enhanced both by changing its stereochemistry from (–)-(3*R*, 4*R*) to (+)-(3*S*, 4*S*) and by making certain structural modifications (reviewed in Howlett et al. 2002; Pertwee 2004b). Cannabidiol analogues with particularly high affinities for $CB_1$ and $CB_2$ receptors are (+)-(3*S*, 4*S*)-4′-dimethylheptyl-cannabidiol and (+)-(3*S*, 4*S*)-7-hydroxy-4′-dimethylheptyl-cannabidiol (Bisogno et al. 2001). Several (–)-(3*R*, 4*R*)-analogues of cannabidiol with high $CB_1$ and $CB_2$ affinities have also been developed, for example O-1660, O-1871 and O-1422 (Wiley et al. 2002). Whether these (+)-(3*S*, 4*S*)- and (–)-(3*R*, 4*R*)-analogues of cannabidiol are agonists or antagonists remains to be established. However, one (–)-(3*R*, 4*R*)-cannabidiol analogue that is already known to be a potent $CB_2$-selective agonist is HU-308 (Sect. 3.1), whilst another cannabidiol analogue, O-2654, behaves as a reasonably potent $CB_1$ receptor antagonist (Sect. 3.4).

Finally, there is evidence that cannabidiol can induce apoptosis in cultures of at least some types of human cancer cell: HL-60 myeloblastic leukaemia cells and

glioma cells. More specifically, it has been reported to produce signs of apoptosis at 3.2 μM in γ-irradiated HL-60 cells, at 12.7 μM in non-irradiated HL-60 cells and at 25 μM but not 10 μM in U87 and U373 glioma cells (Gallily et al. 2003; Massi et al. 2004). At these or higher concentrations, cannabidiol did not induce detectable apoptosis in γ-irradiated or non-irradiated monocytes obtained from normal individuals (Gallily et al. 2003).

## 5 $CB_1$ Receptor Oligomerization

There is some evidence that the $CB_1$ receptor can exist as a homodimer and also that it may form heterodimers or oligomers with one or more other classes of co-expressed G protein-coupled receptor (e.g. dopamine $D_2$ and opioid receptors) (Wager-Miller et al. 2002). Resulting cross-talk between $CB_1$ and non $CB_1$ receptors may involve the sequestration of G proteins either from other receptor types by $CB_1$ receptors (reviewed in Pertwee 2003) or conversely, from $CB_1$ receptors by other receptor types. For example, results obtained from experiments with primary cultures of rat striatal neurons (Glass and Felder 1997) and with human embryonic kidney cells co-transfected with $CB_1$ and dopamine $D_2$ receptors (Jarrahian et al. 2004) suggest that $D_2$ receptors can sequester $G\alpha_{i/o}$ so as to cause co-expressed $CB_1$ receptors to switch coupling from $G\alpha_{i/o}$ to $G\alpha_s$. Interestingly, Jarrahian et al. (2004) also found that in the human embryonic kidney cells expressing both $CB_1$ and $D_2$ receptors, persistent activation of the $D_2$ receptors promoted the re-establishment of $CB_1$ receptor coupling with $G\alpha_{i/o}$. Results from other in vitro experiments have provided evidence that in the presence of ongoing $G\alpha_s$-mediated adenylate cyclase stimulation by adenosine $A_2$ receptor activation, $D_2$ and $CB_1$ receptor agonists can interact synergistically through their respective receptors to produce further adenylate cyclase stimulation via $\beta\gamma$-subunits released from $G\alpha_{i/o}$ (Yao et al. 2003).

## 6 Future Directions

Clearly there is now incontrovertible evidence for the existence of a mammalian endocannabinoid system that consists of at least two types of cannabinoid receptor, $CB_1$ and $CB_2$, and of endogenous agonists (endocannabinoids) for these receptors. Agonists that activate both these receptor types with similar potency or that show marked selectivity for one or other receptor type have been discovered, as have potent $CB_1$- and $CB_2$-selective cannabinoid receptor antagonists. Quantitative and sensitive in vitro and in vivo bioassays for these ligands are also available, and these have played a crucial role in determining the $CB_1$ and $CB_2$ receptor affinities and intrinsic activities of a number of cannabinoids. There is good evidence that the endocannabinoid system can become tonically active and that this is due in some instances to endocannabinoid release and in other instances to the ability of cannabinoid receptors to exist in a constitutively activity state, not only when over-

expressed in cultured cells but also when expressed naturally. The existence of such constitutive activity is reflected in the pharmacological properties of established cannabinoid receptor antagonists, all of which appear to be inverse agonists rather than neutral antagonists. Ligands that behave as neutral cannabinoid receptor antagonists are beginning to be described in the literature. These now need to be characterized more fully, as such antagonists would serve as important additional pharmacological tools and might also possess advantages over inverse agonists in the clinic. Evidence for the presence of non-$CB_1$, non-$CB_2$ pharmacological targets for at least some cannabinoid receptor agonists is emerging, prompting a need to establish the extent to which these proposed additional targets contribute to the pharmacology of these agonists. For some of these targets, ligands that do not also interact with $CB_1$ or $CB_2$ receptors have already been identified, and it will now be important to characterize the actions of these ligands more fully and to investigate the possibility of developing potent and selective non-$CB_1$, non-$CB_2$ agonists for all the proposed new targets. This in turn will greatly facilitate a fuller understanding of these targets as well as the discovery of any additional targets. The extent to which cross-talk can occur between identical (e.g. $CB_1$-$CB_1$) or different pharmacological targets for cannabinoids (e.g. between $CB_2$ and abnormal cannabidiol receptors), or between cannabinoid and non-cannabinoid targets (e.g. between $CB_1$ and dopamine $D_2$ receptors), and the nature of the mechanisms that underlie such cross-talk also merit further investigation.

## 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

Adams IB, Ryan W, Singer M, Thomas BF, Compton DR, Razdan RK, Martin BR (1995) Evaluation of cannabinoid receptor binding and in vivo activities for anandamide analogs. J Pharmacol Exp Ther 273:1172–1181

Ahluwalia J, Yaqoob M, Urban L, Bevan S, Nagy I (2003) Activation of capsaicin-sensitive primary sensory neurones induces anandamide production and release. J Neurochem 84:585–591

Akinshola BE, Chakrabarti A, Onaivi ES (1999a) In-vitro and in-vivo action of cannabinoids. Neurochem Res 24:1233–1240

Akinshola BE, Taylor RE, Ogunseitan AB, Onaivi ES (1999b) Anandamide inhibition of recombinant AMPA receptor subunits in Xenopus oocytes is increased by forskolin and 8-bromo-cyclic AMP. Naunyn Schmiedebergs Arch Pharmacol 360:242–248

Al-Hayani A, Wease KN, Ross RA, Pertwee RG, Davies SN (2001) The endogenous cannabinoid anandamide activates vanilloid receptors in the rat hippocampal slice. Neuropharmacology 41:1000–1005

Balster RL, Prescott WR (1992) $\Delta$9-Tetrahydrocannabinol discrimination in rats as a model for cannabis intoxication. Neurosci Biobehav Rev 16:55–62

Barann M, Molderings G, Brüss M, Bönisch H, Urban BW, Göthert M (2002) Direct inhibition by cannabinoids of human 5-HT3A receptors: probable involvement of an allosteric modulatory site. Br J Pharmacol 137:589–596

Bayewitch M, Rhee M-H, Avidor-Reiss T, Breuer A, Mechoulam R, Vogel Z (1996) (–)-$\Delta$9-tetrahydrocannabinol antagonizes the peripheral cannabinoid receptor-mediated inhibition of adenylyl cyclase. J Biol Chem 271:9902–9905

Begg M, Mo F-M, Offertáler L, Bátkai 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

Ben-Shabat S, Fride E, Sheskin T, Tamiri T, Rhee M-H, Vogel Z, Bisogno T, De Petrocellis L, Di Marzo V, Mechoulam R (1998) An entourage effect: inactive endogenous fatty acid glycerol esters enhance 2-arachidonoyl-glycerol cannabinoid activity. Eur J Pharmacol 353:23–31

Berdyshev EV, Schmid PC, Krebsbach RJ, Hillard CJ, Huang CS, Chen N, Dong Z, Schmid HHO (2001) Cannabinoid-receptor-independent cell signalling by N-acylethanolamines. Biochem J 360:67–75

Bisogno T, Melck D, Bobrov MY, Gretskaya NM, Bezuglov VV, De Petrocellis L, Di Marzo V (2000) N-acyl-dopamines: novel synthetic CB1 cannabinoid-receptor ligands and inhibitors of anandamide inactivation with cannabimimetic activity in vitro and in vivo. Biochem J 351:817–824

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 (2000) Cannabinoid agonist signal transduction in rat brain: comparison of cannabinoid agonists in receptor binding, G-protein activation, and adenylyl cyclase inhibition. J Pharmacol Exp Ther 295:328–336

Breivogel CS, Sim LJ, Childers SR (1997) Regional differences in cannabinoid receptor/G-protein coupling in rat brain. J Pharmacol Exp Ther 282:1632–1642

Breivogel CS, Selley DE, Childers SR (1998) Cannabinoid receptor agonist efficacy for stimulating [35S]GTPγS binding to rat cerebellar membranes correlates with agonist-induced decreases in GDP affinity. J Biol Chem 273:16865–16873

Breivogel CS, Griffin G, Di Marzo V, Martin BR (2001) Evidence for a new G protein-coupled cannabinoid receptor in mouse brain. Mol Pharmacol 60:155–163

Brooks JW, Pryce G, Bisogno T, Jaggar SI, Hankey DJR, Brown P, Bridges D, Ledent C, Bifulco M, Rice ASC, Di Marzo V, Baker D (2002) Arvanil-induced inhibition of spasticity and persistent pain: evidence for therapeutic sites of action different from the vanilloid VR1 receptor and cannabinoid CB1/CB2 receptors. Eur J Pharmacol 439:83–92

Busch-Petersen J, Hill WA, Fan PS, Khanolkar A, Xie X-Q, Tius MA, Makriyannis A (1996) Unsaturated side chain β-11-hydroxyhexahydrocannabinol analogs. J Med Chem 39:3790–3796

Calandra B, Portier M, Kernéis A, Delpech M, Carillon C, Le Fur G, Ferrara P, Shire D (1999) Dual intracellular signaling pathways mediated by the human cannabinoid CB1 receptor. Eur J Pharmacol 374:445–455

Calignano A, La Rana G, Giuffrida A, Piomelli D (1998) Control of pain initiation by endogenous cannabinoids. Nature 394:277–281

Calignano A, La Rana G, Piomelli D (2001) Antinociceptive activity of the endogenous fatty acid amide, palmitylethanolamide. Eur J Pharmacol 419:191–198

Chaytor AT, Martin PEM, Evans WH, Randall MD, Griffith TM (1999) The endothelial component of cannabinoid-induced relaxation in rabbit mesenteric artery depends on gap junctional communication. J Physiol 520:539–550

Cheer JF, Cadogan A-K, Marsden CA, Fone KCF, Kendall DA (1999) Modification of 5-HT2 receptor mediated behaviour in the rat by oleamide and the role of cannabinoid receptors. Neuropharmacology 38:533–541

Chemin J, Monteil A, Perez-Reyes E, Nargeot J, Lory P (2001) Direct inhibition of T-type calcium channels by the endogenous cannabinoid anandamide. EMBO J 20:7033–7040

Chin C, Lucas-Lenard J, Abadji V, Kendall DA (1998) Ligand binding and modulation of cyclic AMP levels depend on the chemical nature of residue 192 of the human cannabinoid receptor 1. J Neurochem 70:366–373

Christopoulos A, Wilson K (2001) Interaction of anandamide with the M1 and M4 muscarinic acetylcholine receptors. Brain Res 915:70–78

Compton DR, Aceto MD, Lowe J, Martin BR (1996) In vivo characterization of a specific cannabinoid receptor antagonist (SR141716A): inhibition of Δ9-tetrahydrocannabinol-induced responses and apparent agonist activity. J Pharmacol Exp Ther 277:586–594

Conti S, Costa B, Colleoni M, Parolaro D, Giagnoni G (2002) Antiinflammatory action of endocannabinoid palmitoylethanolamide and the synthetic cannabinoid nabilone in a model of acute inflammation in the rat. Br J Pharmacol 135:181–187

Cosenza M, Gifford AN, Gatley SJ, Pyatt B, Liu Q, Makriyannis A, Volkow ND (2000) Locomotor activity and occupancy of brain cannabinoid CB1 receptors by the antagonist/inverse agonist AM281. Synapse 38:477–482

Costa B, Colleoni M (1999) SR141716A induces in rats a behavioral pattern opposite to that of CB1 receptor agonists. Acta Pharmacol Sin 20:1103–1108

Craib SJ, Ellington HC, Pertwee RG, Ross RA (2001) A possible role of lipoxygenase in the activation of vanilloid receptors by anandamide in the guinea-pig bronchus. Br J Pharmacol 134:30–37

Cravatt BF, Lichtman AH (2002) The enzymatic inactivation of the fatty acid amide class of signaling lipids. Chem Phys Lipids 121:135–148

Darlington CL (2003) Dexanabinol: a novel cannabinoid with neuroprotective properties. IDrugs 6:976–979

De Petrocellis L, Bisogno T, Maccarrone M, Davis JB, Finazzi-Agrò A, Di Marzo V (2001a) The activity of anandamide at vanilloid VR1 receptors requires facilitated transport across the cell membrane and is limited by intracellular metabolism. J Biol Chem 276:12856–12863

De Petrocellis L, Davis JB, Di Marzo V (2001b) Palmitoylethanolamide enhances anandamide stimulation of human vanilloid VR1 receptors. FEBS Lett 506:253–256

De Petrocellis L, Bisogno T, Ligresti A, Bifulco M, Melck D, Di Marzo V (2002) Effect on cancer cell proliferation of palmitoylethanolamide, a fatty acid amide interacting with both the cannabinoid and vanilloid signalling systems. Fundam Clin Pharmacol 16:297–302

De Vry J, Jentzsch KR (2002) Discriminative stimulus effects of BAY 38–7271, a novel cannabinoid receptor agonist. Eur J Pharmacol 457:147–152

Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum A, Etinger A, Mechoulam R (1992) Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 258:1946–1949

Devlin MG, Christopoulos A (2002) Modulation of cannabinoid agonist binding by 5-HT in the rat cerebellum. J Neurochem 80:1095–1102

Di Marzo V, Melck D, Bisogno T, De Petrocellis L (1998) Endocannabinoids: endogenous cannabinoid receptor ligands with neuromodulatory action. Trends Neurosci 21:521–528

Di Marzo V, Breivogel CS, Tao Q, Bridgen DT, Razdan RK, Zimmer AM, Zimmer A, Martin BR (2000) Levels, metabolism, and pharmacological activity of anandamide in CB1 cannabinoid receptor knockout mice: evidence for non-CB1, non-CB2 receptor-mediated actions of anandamide in mouse brain. J Neurochem 75:2434–2444

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

Di Marzo V, Griffin G, De Petrocellis L, Brandi I, Bisogno T, Williams W, Grier MC, Kulasegram S, Mahadevan A, Razdan RK, Martin BR (2002) A structure/activity relationship study on arvanil, an endocannabinoid and vanilloid hybrid. J Pharmacol Exp Ther 300:984–991

Dinh TP, Freund TF, Piomelli D (2002) A role for monoglyceride lipase in 2-arachidonoylglycerol inactivation. Chem Phys Lipids 121:149–158

Drysdale AJ, Pertwee RG, Platt B (2004) Modulation of calcium homeostasis by cannabidiol in primary hippocampal culture. Proc Br Pharmacol Soc at http://www.pa2online.org/Vol1Issue4abst052P.html

El-Remessy AB, Khalil IE, Matragoon S, Abou-Mohamed G, Tsai N-J, Roon P, Caldwell RB, Caldwell RW, Green K, Liou GI (2003) Neuroprotective effect of (–)*Δ*9-tetrahydrocannabinol and cannabidiol in N-methyl-D-aspartate-induced retinal neurotoxicity: involvement of peroxynitrite. Am J Pathol 163:1997–2008

Ellington HC, Cotter MA, Cameron NE, Ross RA (2002) The effect of cannabinoids on capsaicin-evoked calcitonin gene-related peptide (CGRP) release from the isolated paw skin of diabetic and non-diabetic rats. Neuropharmacology 42:966–975

ElSohly MA (2002) Chemical constituents of cannabis. In: Grotenhermen F, Russo E (eds) Cannabis and Cannabinoids Pharmacology, Toxicology and Therapeutic Potential. Haworth Press, New York, pp 27–36

Facchinetti F, Del Giudice E, Furegato S, Passarotto M, Leon A (2003) Cannabinoids ablate release of TNF*α* in rat microglial cells stimulated with lipopolysaccharide. Glia 41:161–168

Fan P (1995) Cannabinoid agonists inhibit the activation of 5-HT3 receptors in rat nodose ganglion neurons. J Neurophysiol 73:907–910

Fan S-F, Yazulla S (2003) Biphasic modulation of voltage-dependent currents of retinal cones by cannabinoid CBI receptor agonist WIN 55212-2. Visual Neurosci 20:177–188

Farquhar-Smith WP, Rice ASC (2001) Administration of endocannabinoids prevents a referred hyperalgesia associated with inflammation of the urinary bladder. Anesthesiology 94:507–513

Farquhar-Smith WP, Jaggar SI, Rice ASC (2002) Attenuation of nerve growth factor-induced visceral hyperalgesia via cannabinoid CB1 and CB2-like receptors. Pain 97:11–21

Felder CC, Briley EM, Axelrod J, Simpson JT, Mackie K, Devane WA (1993) Anandamide, an endogenous cannabimimetic eicosanoid, binds to the cloned human cannabinoid receptor and stimulates receptor-mediated signal transduction. Proc Natl Acad Sci USA 90:7656–7660

Felder CC, Joyce KE, Briley EM, Mansouri J, Mackie K, Blond O, Lai Y, Ma AL, Mitchell RL (1995) Comparison of the pharmacology and signal transduction of the human cannabinoid CB1 and CB2 receptors. Mol Pharmacol 48:443–450

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

Ferraro L, Tomasini MC, Gessa GL, Bebe BW, Tanganelli S, Antonelli T (2001) The cannabinoid receptor agonist WIN 55,212-2 regulates glutamate transmission in rat cerebral cortex: an in vivo and in vitro study. Cerebral Cortex 11:728–733

Fezza F, Bisogno T, Minassi A, Appendino G, Mechoulam R, Di Marzo V (2002) Noladin ether, a putative novel endocannabinoid: inactivation mechanisms and a sensitive method for its quantification in rat tissues. FEBS Lett 513:294–298

Fowler CJ (2003) Plant-derived, synthetic and endogenous cannabinoids as neuroprotective agents: non-psychoactive cannabinoids, 'entourage' compounds and inhibitors of N-acyl ethanolamine breakdown as therapeutic strategies to avoid psychotropic effects. Brain Res Rev 41:26–43

Franklin A, Stella N (2003) Arachidonylcyclopropylamide increases microglial cell migration through cannabinoid CB2 and abnormal-cannabidiol-sensitive receptors. Eur J Pharmacol 474:195–198

Franklin A, Parmentier-Batteur S, Walter L, Greenberg DA, Stella N (2003) Palmitoylethanolamide increases after focal cerebral ischemia and potentiates microglial cell motility. J Neurosci 23:7767–7775

Fride E, Foox A, Rosenberg E, Faigenboim M, Cohen V, Barda L, Blau H, Mechoulam R (2003) Milk intake and survival in newborn cannabinoid CB1 receptor knockout mice: evidence for a "CB3" receptor. Eur J Pharmacol 461:27–34

Gallant M, Dufresne C, Gareau Y, Guay D, Leblanc Y, Prasit P, Rochette C, Sawyer N, Slipetz DM, Tremblay N, Metters KM, Labelle M (1996) New class of potent ligands for the human peripheral cannabinoid receptor. Bioorg Med Chem Lett 6:2263–2268

Gallily R, Even-Chen T, Katzavian G, Lehmann D, Dagan A, Mechoulam R (2003) γ-Irradiation enhances apoptosis induced by cannabidiol, a non-psychotropic cannabinoid, in cultured HL-60 myeloblastic leukemia cells. Leuk Lymphoma 44:1767–1773

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 analogues on human cannabinoid receptors. Bioorg Med Chem Lett 6:189–194

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

Gérard CM, Mollereau C, Vassart G, Parmentier M (1991) Molecular cloning of a human 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

Gifford AN, Makriyannis A, Volkow ND, Gatley SJ (2002) In vivo imaging of the brain cannabinoid receptor. Chem Phys Lipids 121:65–72

Glass M, Felder CC (1997) Concurrent stimulation of cannabinoid CB1 and dopamine D2 receptors augments cAMP accumulation in striatal neurons: evidence for a Gs linkage to the CB1 receptor. J Neurosci 17:5327–5333

Godlewski G, Göthert M, Malinowska B (2003) Cannabinoid receptor-independent inhibition by cannabinoid agonists of the peripheral 5-HT3 receptor-mediated von Bezold-Jarisch reflex. Br J Pharmacol 138:767–774

Gonsiorek W, Lunn C, Fan X, Narula S, Lundell D, Hipkin RW (2000) Endocannabinoid 2-arachidonyl glycerol is a full agonist through human type 2 cannabinoid receptor: antagonism by anandamide. Mol Pharmacol 57:1045–1050

Göthert M, Brüss M, Bönisch H, Molderings GJ (1999) Presynaptic imidazoline receptors: new developments in characterization and classification. Ann N Y Acad Sci 881:171–184

Goutopoulos A, Fan P, Khanolkar AD, Xie X-Q, 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

Griffin G, Fernando SR, Ross RA, McKay NG, Ashford MLJ, Shire D, Huffman JW, Yu S, Lainton JAH, Pertwee RG (1997) Evidence for the presence of CB2-like cannabinoid receptors on peripheral nerve terminals. Eur J Pharmacol 339:53–61

Griffin G, Tao Q, Abood ME (2000) Cloning and pharmacological characterization of the rat CB2 cannabinoid receptor. J Pharmacol Exp Ther 292:886–894

Hájos N, Freund TF (2002a) Pharmacological separation of cannabinoid sensitive receptors on hippocampal excitatory and inhibitory fibers. Neuropharmacology 43:503–510

Hájos N, Freund TF (2002b) Distinct cannabinoid sensitive receptors regulate hippocampal excitation and inhibition. Chem Phys Lipids 121:73–82

Hájos N, Katona I, Naiem SS, Mackie K, Ledent C, Mody I, Freund TF (2000) Cannabinoids inhibit hippocampal GABAergic transmission and network oscillations. Eur J Neurosci 12:3239–3249

Hájos N, Ledent C, Freund TF (2001) Novel cannabinoid-sensitive receptor mediates inhibition of glutamatergic synaptic transmission in the hippocampus. Neuroscience 106:1–4

Hampson AJ, Grimaldi M, Axelrod J, Wink D (1998) Cannabidiol and (–)Δ9-tetrahydrocannabinol are neuroprotective antioxidants. Proc Natl Acad Sci USA 95:8268–8273

Hampson AJ, Grimaldi M, Lolic M, Wink D, Rosenthal R, Axelrod J (2000) Neuroprotective antioxidants from marijuana. Ann N Y Acad Sci 899:274–282

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 CB2, 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 USA 98:3662–3665

Helyes Z, Németh J, Thán M, Bölcskei K, Pintér E, Szolcsányi J (2003) Inhibitory effect of anandamide on resiniferatoxin-induced sensory neuropeptide release in vivo and neuropathic hyperalgesia in the rat. Life Sci 73:2345–2353

Hermann H, De Petrocellis L, Bisogno T, Schiano Moriello A, Lutz B, Di Marzo V (2003) Dual effect of cannabinoid CB1 receptor stimulation on a vanilloid VR1 receptor-mediated response. Cell Mol Life Sci 60:607–616

Hillard CJ (2000) Biochemistry and pharmacology of the endocannabinoids arachidonylethanolamide and 2-arachidonylglycerol. Prostaglandins Other Lipid Mediat 61:3–18

Hillard CJ, Jarrahian A (2003) Cellular accumulation of anandamide: consensus and controversy. Br J Pharmacol 140:802–808

Hillard CJ, Manna S, Greenberg MJ, Dicamelli R, Ross RA, Stevenson LA, Murphy V, Pertwee RG, Campbell WB (1999) Synthesis and characterization of potent and selective agonists of the neuronal cannabinoid receptor (CB1). J Pharmacol Exp Ther 289:1427–1433

Hirst RA, Almond SL, Lambert DG (1996) Characterisation of the rat cerebella CB1 receptor using SR141716A, a central cannabinoid receptor antagonist. Neurosci Lett 220:101–104

Ho W-SV, Hiley CR (2003) Vasodilator actions of abnormal-cannabidiol in rat isolated small mesenteric artery. Br J Pharmacol 138:1320–1332

Howlett AC (1987) Cannabinoid inhibition of adenylate cyclase: relative activity of constituents and metabolites of marihuana. Neuropharmacology 26:507–512

Howlett AC, Fleming RM (1984) Cannabinoid inhibition of adenylate cyclase. Pharmacology of the response in neuroblastoma cell membranes. Mol Pharmacol 26:532–538

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

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-Δ8-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-Δ8-tetrahydrocannabinols: new selective ligands for the CB2 receptor. Bioorg Med Chem 10:4119–4129

Hurst DP, Lynch DL, Barnett-Norris J, Hyatt SM, Seltzman HH, Zhong M, Song Z-H, Nie J, Lewis D, Reggio PH (2002) N-(Piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (SR141716A) interaction with LYS 3.28(192) is crucial for its inverse agonism at the cannabinoid CB1 receptor. Mol Pharmacol 62:1274–1287

Ibrahim MM, Deng H, Zvonok A, Cockayne DA, Kwan J, Mata HP, Vanderah TW, Lai J, Porreca F, Makriyannis A, Malan TP (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 USA 100:10529–10533

Iversen L (2003) Cannabis and the brain. Brain 126:1252–1270

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

Izzo AA, Mascolo N, Tonini M, Capasso F (2000) Modulation of peristalsis by cannabinoid CB1 ligands in the isolated guinea-pig ileum. Br J Pharmacol 129:984–990

Járai Z, Wagner JA, Varga K, Lake KD, Compton DR, Martin BR, Zimmer AM, Bonner TI, Buckley NE, Mezey E, Razdan RK, Zimmer A, Kunos G (1999) Cannabinoid-induced mesenteric vasodilation through an endothelial site distinct from CB1 or CB2 receptors. Proc Natl Acad Sci USA 96:14136–14141

Jarrahian A, Manna S, Edgemond WS, Campbell WB, Hillard CJ (2000) Structure-activity relationships among N-arachidonylethanolamine (anandamide) head group analogues for the anandamide transporter. J Neurochem 74:2597–2606

Jarrahian A, Watts VJ, Barker EL (2004) D2 dopamine receptors modulate G$\alpha$-subunit coupling of the CB1 cannabinoid receptor. J Pharmacol Exp Ther 308:880–886

Jennings EA, Vaughan CW, Roberts LA, Christie MJ (2003) The actions of anandamide on rat superficial medullary dorsal horn neurons in vitro. J Physiol 548:121–129

Jordt S-E, Bautista DM, Chuang H, McKemy DD, Zygmunt PM, Högestätt ED, Meng ID, Julius D (2004) Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. Nature 427:260–265

Kelley BG, Thayer SA (2004) $\Delta$9-tetrahydrocannabinol antagonizes endocannabinoid modulation of synaptic transmission between hippocampal neurons in culture. Neuropharmacology 46:709–715

Kenny LC, Baker PN, Kendall DA, Randall MD, Dunn WR (2002) The role of gap junctions in mediating endothelium-dependent responses to bradykinin in myometrial small arteries isolated from pregnant women. Br J Pharmacol 136:1085–1088

Khanolkar AD, Abadji V, Lin S, Hill WAG, 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

Köfalvi A, Vizi ES, Ledent C, Sperlágh B (2003) Cannabinoids inhibit the release of [3H]glutmate from rodent hippocampal synaptosomes via a novel CB1 receptor-independent action. Eur J Neurosci 18:1973–1978

Kojima S, Sugiura T, Waku K, Kamikawa Y (2002) Contractile response to a cannabimimetic eicosanoid, 2-arachidonoylglycerol, of longitudinal smooth muscle from the guinea-pig distal colon in vitro. Eur J Pharmacol 444:203–207

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

Lambert DM, DiPaolo FG, Sonveaux P, Kanyonyo M, Govaerts SJ, Hermans E, Bueb J-L, Delzenne NM, Tschirhart EJ (1999) Analogues and homologues of N-palmitoylethanolamide, a putative endogenous CB2 cannabinoid, as potential ligands for the cannabinoid receptors. Biochim Biophys Acta 1440:266–274

Lan R, Gatley J, Lu Q, Fan P, Fernando SR, Volkow ND, Pertwee R, Makriyannis A (1999a) Design and synthesis of the CB1 selective cannabinoid antagonist AM281: a potential human SPECT ligand. AAPS PharmSci 1:U13–U24

Lan R, Liu Q, Fan P, Lin S, Fernando SR, McCallion D, Pertwee R, Makriyannis A (1999b) Structure-activity relationships of pyrazole derivatives as cannabinoid receptor antagonists. J Med Chem 42:769–776

Landsman RS, Makriyannis A, Deng H, Consroe P, Roeske WR, Yamamura HI (1998) AM630 is an inverse agonist at the human cannabinoid CB1 receptor. Life Sci 62:PL109–113

Ledent C, Valverde O, Cossu G, Petitet F, Aubert J-F, Beslot F, Böhme GA, Imperato A, Pedrazzini T, Roques BP, Vassart G, Fratta W, Parmentier M (1999) Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB1 receptor knockout mice. Science 283:401–404

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

Maccarrone M, Cartoni A, Parolaro D, Margonelli A, Massi P, Bari M, Battista N, Finazzi-Agrò A (2002) Cannabimimetic activity, binding, and degradation of stearoylethanolamide within the mouse central nervous system. Mol Cell Neurosci 21:126–140

Maccarrone M, Bari M, Del Principe D, Finazzi-Agrò A (2003) Activation of human platelets by 2-arachidonoylglycerol is enhanced by serotonin. Thromb Haemost 89:340–347

Mackie K, Devane WA, Hille B (1993) Anandamide, an endogenous cannabinoid, inhibits calcium currents as a partial agonist in N18 neuroblastoma cells. Mol Pharmacol 44:498–503

MacLennan SJ, Reynen PH, Kwan J, Bonhaus DW (1998) Evidence for inverse agonism of SR141716A at human recombinant cannabinoid CB1 and CB2 receptors. Br J Pharmacol 124:619–622

Maneuf YP, Brotchie JM (1997) Paradoxical action of the cannabinoid WIN 55,212-2 in stimulated and basal cyclic AMP accumulation in rat globus pallidus slices. Br J Pharmacol 120:1397–1398

Mang CF, Erbelding D, Kilbinger H (2001) Differential effects of anandamide on acetylcholine release in the guinea-pig ileum mediated via vanilloid and non-CB1 cannabinoid receptors. Br J Pharmacol 134:161–167

Marinelli S, Vaughan CW, Christie MJ, Connor M (2002) Capsaicin activation of glutamatergic synaptic transmission in the rat locus coeruleus in vitro. J Physiol 543:531–540

Marinelli S, Di Marzo V, Berretta N, Matias I, Maccarrone M, Bernardi G, Mercuri NB (2003) Presynaptic facilitation of glutamatergic synapses to dopaminergic neurons of the rat substantia nigra by endogenous stimulation of vanilloid receptors. J Neurosci 23:3136–3144

Marsicano G, Moosmann B, Hermann H, Lutz B, Behl C (2002) Neuroprotective properties of cannabinoids against oxidative stress: role of the cannabinoid receptor CB1. J Neurochem 80:448–456

Martin B, Stevenson LA, Pertwee RG, Breivogel CS, Williams W, Mahadevan A, Razdan RK (2002) Agonists and silent antagonists in a series of cannabinoid sulfonamides. Symposium on the Cannabinoids. Burlington, Vermont, International Cannabinoid Research Society, p 2

Martin BR, Thomas BF, Razdan RK (1995) Structural requirements for cannabinoid receptor probes. In: Pertwee RG (ed) Cannabinoid receptors. Academic Press, London, pp 35–85

Massi P, Vaccani A, Ceruti S, Colombo A, Abbracchio MP, Parolaro D (2004) Antitumor effects of cannabidiol, a nonpsychoactive cannabinoid, on human glioma cell lines. J Pharmacol Exp Ther 308:838–845

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

Mauler F, Mittendorf J, Horváth 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, Hinz V, Augstein K-H, Fassbender M, Horváth E (2003) Neuroprotective and brain edema-reducing efficacy of the novel cannabinoid receptor agonist BAY 38–7271. Brain Res 989:99–111

Mechoulam R, Gaoni Y (1967) Recent advances in the chemistry of hashish. Fortschr Chem Org Naturst 25:175–213

Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR, Gopher A, Almog S, Martin BR, Compton DR, Pertwee RG, Griffin G, Bayewitch M, Barg J, Vogel Z (1995) Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. Biochem Pharmacol 50:83–90

Mechoulam R, Fride E, Di Marzo V (1998) Endocannabinoids. Eur J Pharmacol 359:1–18

Mechoulam R, Panikashvili D, Shohami E (2002) Cannabinoids and brain injury: therapeutic implications. Trends Mol Med 8:58–61

Millns PJ, Chapman V, Kendall DA (2001) Cannabinoid inhibition of the capsaicin-induced calcium response in rat dorsal root ganglion neurones. Br J Pharmacol 132:969–971

Misner DL, Sullivan JM (1999) Mechanism of cannabinoid effects on long-term potentiation and depression in hippocampal CA1 neurons. J Neurosci 19:6795–6805

Mo FM, Offertáler L, Kunos G (2004) Atypical cannabinoid stimulates endothelial cell migration via a Gi/Go-coupled receptor distinct from CB1, CB2 or EDG-1. Eur J Pharmacol 489:21–27

Molderings GJ, Göthert M (1999) Imidazoline binding sites and receptors in cardiovascular tissue. Gen Pharmacol 32:17–22

Molderings GJ, Bönisch H, Hammermann R, Göthert M, Brüss M (2002) Noradrenaline release-inhibiting receptors on PC12 cells devoid of $\alpha$2- and CB1 receptors: similarities to presynaptic imidazoline and edg receptors. Neurochem Int 40:157–167

Monory K, Tzavara ET, Lexime J, Ledent C, Parmentier M, Borsodi A, Hanoune J (2002) Novel, not adenylyl cyclase-coupled cannabinoid binding site in cerebellum of mice. Biochem Biophys Res Commun 292:231–235

Mussinu J-M, Ruiu S, Mulè 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

Németh J, Helyes Z, Thán M, Jakab B, Pintér E, Szolcsányi J (2003) Concentration-dependent dual effect of anandamide on sensory neuropeptide release from isolated rat tracheae. Neurosci Lett 336:89–92

New DC, Wong YH (2003) BML-190 and AM251 act as inverse agonists at the human cannabinoid CB2 receptor: signalling via cAMP and inositol phosphates. FEBS Lett 536:157–160

Offertáler L, Mo F-M, Bátkai S, Liu J, Begg M, Razdan RK, Martin BR, Bukoski RD, Kunos G (2003) Selective ligands and cellular effectors of a G protein-coupled endothelial cannabinoid receptor. Mol Pharmacol 63:699–705

Onaivi ES, Chakrabarti A, Gwebu ET, Chaudhuri G (1996) Neurobehavioral effects of $\Delta$9-THC and cannabinoid (CB1) receptor gene expression in mice. Behav Brain Res 72:115–125

Oz M, Zhang L, Morales M (2002) Endogenous cannabinoid, anandamide, acts as a noncompetitive inhibitor on 5-HT3 receptor-mediated responses in Xenopus oocytes. Synapse 46:150–156

Pan X, Ikeda SR, Lewis DL (1998) SR 141716A acts as an inverse agonist to increase neuronal voltage-dependent Ca2+ currents by reversal of tonic CB1 cannabinoid receptor activity. Mol Pharmacol 54:1064–1072

Papahatjis DP, Nikas SP, Andreou T, Makriyannis A (2002) Novel 1',1'-chain substituted $\Delta$8-tetrahydrocannabinols. Bioorg Med Chem Lett 12:3583–3586

Pate DW (1999) Anandamide structure-activity relationships and mechanisms of action on intraocular pressure in the normotensive rabbit model. Doctoral dissertation. Kuopio University Publications, Kuopio

Paton GS, Pertwee RG, Davies SN (1998) Correlation between cannabinoid mediated effects on paired pulse depression and induction of long term potentiation in the rat hippocampal slice. Neuropharmacology 37:1123–1130

Pertwee R, Griffin G, Fernando S, Li X, Hill A, Makriyannis A (1995) AM630, a competitive cannabinoid receptor antagonist. Life Sci 56:1949–1955

Pertwee RG (1988) The central neuropharmacology of psychotropic cannabinoids. Pharmacol Ther 36:189–261

Pertwee RG (1997) Pharmacology of cannabinoid CB1 and CB2 receptors. Pharmacol Ther 74:129–180

Pertwee RG (1999a) Pharmacology of cannabinoid receptor ligands. Curr Med Chem 6:635–664

Pertwee RG (1999b) Evidence for the presence of CB1 cannabinoid receptors on peripheral neurones and for the existence of neuronal non-CB1 cannabinoid receptors. Life Sci 65:597–605

Pertwee RG (2000) Cannabinoid receptor ligands: clinical and neuropharmacological considerations, relevant to future drug discovery and development. Expert Opin Investig Drugs 9:1553–1571

Pertwee RG (2001) Cannabinoid receptors and pain. Prog Neurobiol 63:569–611

Pertwee RG (2003) Inverse agonism at cannabinoid receptors. In: IJzerman AP (ed) Inverse agonism. Elsevier, Amsterdam, pp 75–86

Pertwee RG (2004a) Novel pharmacological targets for cannabinoids. Curr Neuropharmacol 2:9–29

Pertwee RG (2004b) The pharmacology and therapeutic potential of cannabidiol. In: Di Marzo V (ed) Cannabinoids Kluwer Academic/Plenum Publishers, New York, pp 32–83

Pertwee RG (2005) Inverse agonism and neutral antagonism at cannabinoid CB1 receptors. Life Sci 76:1307–1324
Pertwee RG, Ross RA (2002) Cannabinoid receptors and their ligands. Prostaglandins Leukot Essent Fatty Acids 66:101–121
Pertwee RG, Coutts AA, Griffin G, Fernando SR, McCallion D, Stevenson L (1996a) Presence of cannabinoid CB1 receptors on prejunctional neurones of certain isolated tissue preparations: a brief review. Med Sci Monit 2:840–848
Pertwee RG, Fernando SR, Griffin G, Ryan W, Razdan RK, Compton DR, Martin BR (1996b) Agonist-antagonist characterization of 6'-cyanohex-2'-yne-Δ8-tetrahydrocannabinol in two isolated tissue preparations. Eur J Pharmacol 315:195–201
Pertwee RG, Gibson TM, Stevenson LA, Ross RA, Banner WK, Saha B, Razdan RK, Martin BR (2000) O-1057, a potent water-soluble cannabinoid receptor agonist with antinociceptive properties. Br J Pharmacol 129:1577–1584
Pertwee RG, Ross RA, Craib SJ, Thomas A (2002) (–)-Cannabidiol antagonizes cannabinoid receptor agonists and noradrenaline in the mouse vas deferens. Eur J Pharmacol 456:99–106
Petitet F, Marin L, Doble A (1996) Biochemical and pharmacological characterization of cannabinoid binding sites using [3H]SR141716A. Neuroreport 7:789–792
Petitet F, Jeantaud B, Capet M, Doble A (1997) Interaction of brain cannabinoid receptors with guanine nucleotide binding protein. A radioligand binding study. Biochem Pharmacol 54:1267–1270
Petitet F, Jeantaud B, Reibaud M, Imperato A, Dubroeucq MC (1998) Complex pharmacology of natural cannabinoids: evidence for partial agonist activity of Δ9-tetrahydrocannabinol and antagonist activity of cannabidiol on rat brain cannabinoid receptors. Life Sci 63:PL1–PL6
Platt B, Drysdale AJ (2004) Search and rescue: identification of cannabinoid actions relevant for neuronal survival and protection. Curr Neuropharmacol 2:103–114
Porter AC, Sauer J-M, Knierman MD, Becker GW, Berna MJ, Bao J, Nomikos GG, Carter P, Bymaster FP, Leese AB, Felder CC (2002) Characterization of a novel endocannabinoid, virodhamine, with antagonist activity at the CB1 receptor. J Pharmacol Exp Ther 301:1020–1024
Portier M, Rinaldi-Carmona M, Pecceu F, Combes T, Poinot-Chazel C, Calandra B, Barth F, Le Fur G, Casellas P (1999) SR 144528, an antagonist for the peripheral cannabinoid receptor that behaves as an inverse agonist. J Pharmacol Exp Ther 288:582–589
Rhee M-H, 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
Richardson JD, Kilo S, Hargreaves KM (1998) Cannabinoids reduce hyperalgesia and inflammation via interaction with peripheral CB1 receptors. Pain 75:111–119
Rinaldi-Carmona M, Barth F, Héaulme M, Shire D, Calandra B, Congy C, Martinez S, Maruani J, Néliat G, Caput D, Ferrara P, Soubrié P, Brelière JC, Le Fur G (1994) SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. FEBS Lett 350:240–244
Rinaldi-Carmona M, Calandra B, Shire D, Bouaboula M, Oustric D, Barth F, Casellas P, Ferrara P, Le Fur G (1996a) Characterization of two cloned human CB1 cannabinoid receptor isoforms. J Pharmacol Exp Ther 278:871–878
Rinaldi-Carmona M, Pialot F, Congy C, Redon E, Barth F, Bachy A, Brelière J-C, Soubrié P, Le Fur G (1996b) Characterization and distribution of binding sites for [3H]-SR141716A, a selective brain (CB1) cannabinoid receptor antagonist, in rodent brain. Life Sci 58:1239–1247
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, Brelière J-C, Le Fur G (1998) SR 144528, the first potent and selective antagonist of the CB2 cannabinoid receptor. J Pharmacol Exp Ther 284:644–650
Ross RA (2003) Anandamide and vanilloid TRPV1 receptors. Br J Pharmacol 140:790–801

Ross RA, Brockie HC, Fernando SR, Saha B, Razdan RK, Pertwee RG (1998) Comparison of cannabinoid binding sites in guinea-pig forebrain and small intestine. Br J Pharmacol 125:1345–1351

Ross RA, Brockie HC, Stevenson LA, Murphy VL, Templeton F, Makriyannis A, Pertwee RG (1999a) Agonist-inverse agonist characterization at CB1 and CB2 cannabinoid receptors of L759633, L759656 and AM630. Br J Pharmacol 126:665–672

Ross RA, Gibson TM, Stevenson LA, Saha B, Crocker P, Razdan RK, Pertwee RG (1999b) Structural determinants of the partial agonist-inverse agonist properties of 6'-azidohex-2'-yne-$\Delta$8-tetrahydrocannabinol at cannabinoid receptors. Br J Pharmacol 128:735–743

Ross RA, Gibson TM, Brockie HC, Leslie M, Pashmi G, Craib SJ, Di Marzo V, Pertwee RG (2001) Structure-activity relationship for the endogenous cannabinoid, anandamide, and certain of its analogues at vanilloid receptors in transfected cells and vas deferens. Br J Pharmacol 132:631–640

Rubovitch V, Gafni M, Sarne Y (2002) The cannabinoid agonist DALN positively modulates L-type voltage-dependent calcium-channels in N18TG2 neuroblastoma cells. Mol Brain Res 101:93–102

Ruiu S, Pinna GA, Marchese G, Mussinu J-M, Saba P, Tambaro S, Casti P, Vargiu R, Pani L (2003) Synthesis and characterization of NESS 0327: a novel putative antagonist of the CB1 cannabinoid receptor. J Pharmacol Exp Ther 306:363–370

Savinainen JR, Järvinen T, Laine K, Laitinen JT (2001) Despite substantial degradation, 2-arachidonoylglycerol is a potent full efficacy agonist mediating CB1 receptor-dependent G-protein activation in rat cerebellar membranes. Br J Pharmacol 134:664–672

Savinainen JR, Saario SM, Niemi R, Järvinen T, Laitinen JT (2003) An optimized approach to study endocannabinoid signaling: evidence against constitutive activity of rat brain adenosine A1 and cannabinoid CB1 receptors. Br J Pharmacol 140:1451–1459

Schlicker E, Kathmann M (2001) Modulation of transmitter release via presynaptic cannabinoid receptors. Trends Pharmacol Sci 22:565–572

Schlicker E, Redmer A, Werner A, Kathmann M (2003) Lack of CB1 receptors increases noradrenaline release in vas deferens without affecting atrial noradrenaline release or cortical acetylcholine. Br J Pharmacol 140:323–328

Selley DE, Stark S, Sim LJ, Childers SR (1996) Cannabinoid receptor stimulation of guanosine-5'-O-(3-[35S]thio)triphosphate binding in rat brain membranes. Life Sci 59:659–668

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

Shire D, Carillon C, Kaghad M, Calandra B, Rinaldi-Carmona M, Le Fur G, Caput D, Ferrara P (1995) An amino-terminal variant of the central cannabinoid receptor resulting from alternative splicing. J Biol Chem 270:3726–3731

Shire D, Calandra B, Rinaldi-Carmona M, Oustric D, Pessègue B, Bonnin-Cabanne O, Le Fur G, Caput D, Ferrara P (1996) Molecular cloning, expression and function of the murine CB2 peripheral cannabinoid receptor. Biochim Biophys Acta 1307:132–136

Showalter VM, Compton DR, Martin BR, Abood ME (1996) Evaluation of binding in a transfected cell line expressing a peripheral cannabinoid receptor (CB2): identification of cannabinoid receptor subtype selective ligands. J Pharmacol Exp Ther 278:989–999

Sim LJ, Selley DE, Childers SR (1995) In vitro autoradiography of receptor-activated G proteins in rat brain by agonist-stimulated guanylyl 5'-[$\gamma$-[35S]thio]triphosphate binding. Proc Natl Acad Sci USA 92:7242–7246

Sim-Selley LJ, Brunk LK, Selley DE (2001) Inhibitory effects of SR141716A on G-protein activation in rat brain. Eur J Pharmacol 414:135–143

Simoneau II, Hamza MS, Mata HP, Siegel EM, Vanderah TW, Porreca F, Makriyannis A, Malan TP (2001) The cannabinoid agonist WIN55,212-2 suppresses opioid-induced emesis in ferrets. Anesthesiology 94:882–887

Smart D, Jonsson K-O, Vandevoorde S, Lambert DM, Fowler CJ (2002) 'Entourage' effects of N-acyl ethanolamines at human vanilloid receptors. Comparison of effects upon anandamide-induced vanilloid receptor activation and upon anandamide metabolism. Br J Pharmacol 136:452–458

Song Z-H, Bonner TI (1996) A lysine residue of the cannabinoid receptor is critical for receptor recognition by several agonists but not WIN55212-2. Mol Pharmacol 49:891–896

Storr M, Gaffal E, Saur D, Schusdziarra V, Allescher HD (2002) Effect of cannabinoids on neural transmission in rat gastric fundus. Can J Physiol Pharmacol 80:67–76

Sugiura T, Kodaka T, Kondo S, Tonegawa T, Nakane S, Kishimoto S, Yamashita A, Waku K (1996) 2-Arachidonoylglycerol, a putative endogenous cannabinoid receptor ligand, induces rapid, transient elevation of intracellular free Ca2+ in neuroblastoma x glioma hybrid NG108–15 cells. Biochem Biophys Res Commun 229:58–64

Sugiura T, Kodaka T, Kondo S, Nakane S, Kondo H, Waku K, Ishima Y, Watanabe K, Yamamoto I (1997) Is the cannabinoid CB1 receptor a 2-arachidonoylglycerol receptor? Structural requirements for triggering a Ca2+ transient in NG108–15 cells. J Biochem (Tokyo) 122:890–895

Sugiura T, Kondo S, Kishimoto S, Miyashita T, Nakane S, Kodaka T, Suhara Y, Takayama H, Waku K (2000) Evidence that 2-arachidonoylglycerol but not N-palmitoylethanolamine or anandamide is the physiological ligand for the cannabinoid CB2 receptor: comparison of the agonistic activities of various cannabinoid receptor ligands in HL-60 cells. J Biol Chem 275:605–612

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

Suhara Y, Nakane S, Arai S, Takayama H, Waku K, Ishima Y, Sugiura T (2001) Synthesis and biological activities of novel structural analogues of 2-arachidonoylglycerol, an endogenous cannabinoid receptor ligand. Bioorg Med Chem Lett 11:1985–1988

Tao Q, Abood ME (1998) Mutation of a highly conserved aspartate residue in the second transmembrane domain of the cannabinoid receptors, CB1 and CB2, disrupts G-protein coupling. J Pharmacol Exp Ther 285:651–658

Thomas A, Ross RA, Saha B, Mahadevan A, Razdan RK, Pertwee R (2004) 6"-Azidohex-2"-yne-cannabidiol: a potential neutral, competitive cannabinoid CB1 receptor antagonist. Eur J Pharmacol 487:213–221

Tognetto M, Amadesi S, Harrison S, Creminon C, Trevisani M, Carreras M, Matera M, Geppetti P, Bianchi A (2001) Anandamide excites central terminals of dorsal root ganglion neurons via vanilloid receptor-1 activation. J Neurosci 21:1104–1109

Trendelenburg AU, Cox SL, Schelb V, Klebroff W, Khairallah L, Starke K (2000) Modulation of 3H-noradrenaline release by presynaptic opioid, cannabinoid and bradykinin receptors and $\beta$-adrenoceptors in mouse tissues. Br J Pharmacol 130:321–330

Ueda N (2002) Endocannabinoid hydrolases. Prostaglandins Other Lipid Mediat 68–69:521–534

van der Stelt M, Di Marzo V (2004) Metabolic fate of endocannabinoids. Curr Neuropharmacol 2:37–48

van der Stelt M, Veldhuis WB, Maccarrone M, Bär PR, Nicolay K, Veldink GA, Di Marzo V, Vliegenthart JFG (2002) Acute neuronal injury, excitotoxicity, and the endocannabinoid system. Mol Neurobiol 26:317–346

Vásquez C, Navarro-Polanco RA, Huerta M, Trujillo X, Andrade F, Trujillo-Hernández B, Hernández L (2003) Effects of cannabinoids on endogenous K+ and Ca2+ currents in HEK293 cells. Can J Physiol Pharmacol 81:436–442

Wager-Miller J, Westenbroek R, Mackie K (2002) Dimerization of G protein-coupled receptors: CB1 cannabinoid receptors as an example. Chem Phys Lipids 121:83–89

Wagner JA, Varga K, Járai Z, Kunos G (1999) Mesenteric vasodilation mediated by endothelial anandamide receptors. Hypertension 33:429–434

Walker JM, Krey JF, Chu CJ, Huang SM (2002) Endocannabinoids and related fatty acid derivatives in pain modulation. Chem Phys Lipids 121:159–172

Walter L, Franklin A, Witting A, Wade C, Xie Y, Kunos G, Mackie K, Stella N (2003) Nonpsychotropic cannabinoid receptors regulate microglial cell migration. J Neurosci 23:1398–1405

White R, Hiley CR (1998) The actions of the cannabinoid receptor antagonist, SR 141716A, in the rat isolated mesenteric artery. Br J Pharmacol 125:689-696

Wiley JL, Martin BR (2002) Cannabinoid pharmacology: implications for additional cannabinoid receptor subtypes. Chem Phys Lipids 121:57–63

Wiley JL, Martin BR (2003) Cannabinoid pharmacological properties common to other centrally acting drugs. Eur J Pharmacol 471:185–193

Wiley JL, Beletskaya ID, Ng EW, Dai Z, Crocker PJ, Mahadevan A, Razdan RK, Martin BR (2002) Resorcinol derivatives: a novel template for the development of cannabinoid CB1/CB2 and CB2-selective agonists. J Pharmacol Exp Ther 301:679–689

Wrobleski ST, Chen P, Hynes J, 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

Yao L, Fan P, Jiang Z, Mailliard WS, Gordon AS, Diamond I (2003) Addicting drugs utilize a synergistic molecular mechanism in common requiring adenosine and Gi-$\beta\gamma$ dimers. Proc Natl Acad Sci USA 100:14379–14384

Zygmunt PM, Petersson J, Andersson DA, Chuang H, Sørgård M, Di Marzo V, Julius D, Högestätt ED (1999) Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. Nature 400:452–457

Zygmunt PM, Andersson DA, Högestätt ED (2002) $\Delta$9-tetrahydrocannabinol and cannabinol activate capsaicin-sensitive sensory nerves via a CB1 and CB2 cannabinoid receptor-independent mechanism. J Neurosci 22:4720–4727