Cannabinoid Receptor Ligands

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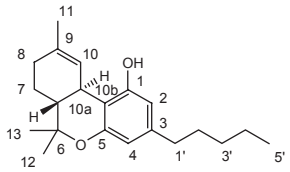
Roger Pertwee is currently Professor of Neuropharmacology at the University of Aberdeen and Director of Pharmacology for GW Pharmaceuticals. His research focuses on the pharmacology of cannabis and its constituents and of cannabinoid receptors and cannabis-derived, synthetic and endogenous ligands for these receptors.

The Endocannabinoid System

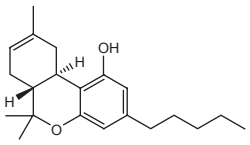
Two types of cannabinoid receptor have so far been identified.[1,2] These are the CB_1 receptor, cloned in 1990,[3] and the CB_2 receptor, cloned in 1993,[4] both of which are members of the superfamily of G-protein-coupled receptors. The cloning of these receptors prompted the development of mice from which cannabinoid CB_1 and/or CB_2 receptors have been genetically deleted and these transgenic animals, particularly CB_1 knockout mice, are now widely used to explore the physiological and pathological functions of cannabinoid receptors.[1,5,6] CB_1 receptors are found mainly at the terminals of central and peripheral neurons where they usually mediate inhibition of neurotransmitter release. They are also present in some non-neuronal cells, including immune cells. CB_2 receptors are located predominantly in immune cells both within and outside the central nervous system, the functions of these receptors including modulation of cytokine release and of immune cell migration. In the brain, CB_2 receptors are expressed by microglia,[7] by blood vessels,[7] and by some neurons.[8,9] However, the role of neuronal CB_2 receptors is currently unknown.

The central distribution pattern of CB_1 receptors is heterogeneous and accounts for several prominent pharmacological properties of CB_1 receptor agonists, for example their ability to impair cognition and memory and to alter the control of motor function. Thus the cerebral cortex, hippocampus, lateral caudate-putamen, substantia nigra pars reticulata, globus pallidus, entopeduncular nucleus and the molecular

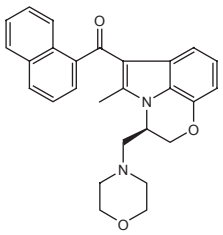
Figure 1 | Structures of the plant cannabinoids, Δ^9-tetrahydrocannabinol (Δ^9-THC), Δ^8-tetrahydrocannabinol (Δ^8-THC), the synthetic cannabinoids HU 210, CP 55,940 , WIN 55,212-2 and WIN 55,212-3, and the endogenous cannabinoids anandamide and 2-arachidonylglycerol (see also Table 1)



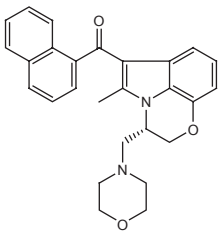
Δ^9-THC



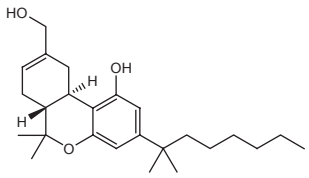
Δ^8-THC



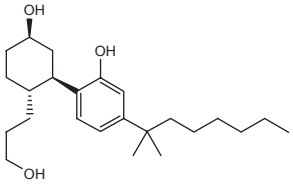
WIN 55,212-2



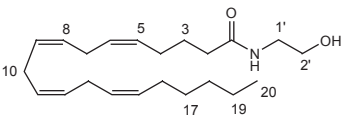
WIN 55,212-3



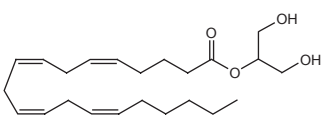
HU 210



CP 55,940



Anandamide



2-Arachidonylglycerol

(Bold Text Denotes Compounds Available From Tocris)

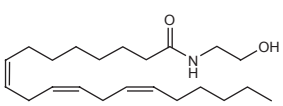
layer of the cerebellum are all populated with particularly high concentrations of CB_1 receptors.[1,10] In line with the analgesic properties of cannabinoid receptor agonists, CB_1 receptors are also found on pain pathways in the brain and spinal cord and at the peripheral terminals of primary sensory neurons.[11,12] Although the concentration of CB_1 receptors is considerably less in peripheral tissues than in the central nervous system, this does not mean that peripheral CB_1 receptors are unimportant. Thus in some peripheral tissues, discrete regions such as nerve terminals that form only a small part of the total tissue mass are known to be densely populated with CB_1 receptors. Peripheral tissues in which CB_1 receptors are expressed on neurons include the heart, vas deferens, urinary bladder and small intestine.[10,13]

Both CB_1 and CB_2 receptors are coupled through $G_{i/o}$ proteins, negatively to adenylyl cyclase and positively to mitogen-activated protein kinase.[1,14] In addition, CB_1 receptors are coupled to ion channels 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.[1,13,14] CB_1 receptors can also couple to G_s proteins to activate adenylyl cyclase,[15-17] the extent to which this occurs possibly being determined by the location of these receptors or by cross-talk between CB_1 receptors and co-localised G-protein-coupled non-CB_1 receptors.[15,16,18,19] It may also be that CB_1 receptors can exist as two distinct subpopulations, one coupled to $G_{i/o}$ proteins and the other to G_s.[15] Details of additional signalling mechanisms that have been proposed for cannabinoid CB_1 and CB_2 receptors can be found elsewhere.[1,14]

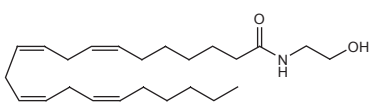
The cloning of cannabinoid receptors was followed by the discovery that mammalian tissues produce compounds that can activate these receptors. The first such endogenous cannabinoids (endocannabinoids) to be identified were *N*-arachidonoyl ethanolamine (anandamide) in 1992 and 2-arachidonylglycerol in 1995 (Figure 1),[20-22] both of which are synthesised on demand in response to elevations of intracellular calcium.[23] Anandamide is formed from *N*-arachidonoyl phosphatidylethanolamine in a process that is catalysed by *N*-acyl phosphatidylethanolamine-selective phospholipase D (NAPE-PLD). The synthesis of 2-arachidonylglycerol, however, is thought to depend on the conversion of 2-arachidonate-containing phosphoinositides to diacylglycerols and on their subsequent transformation to 2-arachidonylglycerol by the action of two diacylglycerol lipase (DAGL) isozymes, DAGLα and DAGLβ.[23,24] Following their synthesis and release, these endocannabinoids are removed from their sites of action by cellular uptake and degraded by enzymes, 2-arachidonylglycerol mainly by monoacylglycerol lipase (MAGL) but also by fatty acid amide hydrolase (FAAH), and anandamide by FAAH and/or by palmitoylethanolamide-preferring acid amidase (PAA), cyclooxygenase-2, lipoxygenases and cytochrome P450.[5,23-25] Other ligands that may be endocannabinoids are 2-arachidonylglyceryl ether (noladin ether), *O*-arachidonoyl ethanolamine (virodhamine), *N*-dihomo-γ-linolenoyl ethanolamine, *N*-docosatetraenoyl ethanolamine, oleamide, *N*-arachidonoyl dopamine (NADA) and *N*-oleoyl dopamine (OLDA) (Figures 2 and 3).[5] Endocannabinoids together with their receptors constitute what is now usually referred to as the 'endocannabinoid system'.

While it is generally accepted that endocannabinoids do pass through cell membranes, one issue that is currently very much a matter of debate is the question of whether the cellular uptake of endocannabinoids such as anandamide is mediated by a transporter.[25-27] In contrast, FAAH is now well characterised. Indeed,

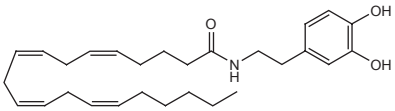
Figure 2 | Structures of the endogenous cannabinoid receptor agonists, *N*-dihomo-γ-linolenoyl ethanolamine, *N*-docosatetraenoyl ethanolamine, NADA, oleamide, OLDA and virodhamine



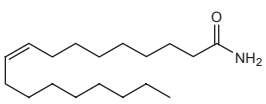
N-Dihomo-γ-linolenoyl ethanolamine



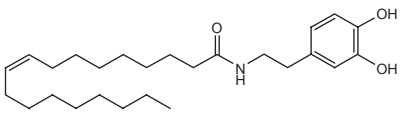
N-Docosatetraenoyl ethanolamine



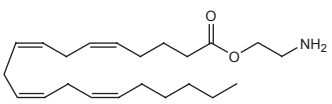
NADA



Oleamide



OLDA



Virodhamine

(Bold Text Denotes Compounds Available From Tocris)

it has been cloned[28] and FAAH knockout mice have been developed.[29,30] NAPE/PLD,[31] MAGL,[32-34] and DAGLα and DAGLβ[35] have also been cloned, and mice with a genetic deletion of NAPE/PLD generated.[36]

At least some effects induced by endogenously released anandamide and 2-arachidonylglycerol appear to be enhanced through what has been termed the "entourage effect". This relies on the co-release of other endogenous fatty acid derivatives that include palmitoylethanolamide and oleamide, which can potentiate anandamide, and 2-linoleoylglycerol and 2-palmitoylglycerol, which can potentiate 2-arachidonylglycerol.[37] The mechanism(s) underlying the entourage effect have yet to be established.

Endocannabinoids most probably have both neuromodulatory and immunomodulatory roles that include inhibition of ongoing transmitter release through retrograde signalling[38] and regulation of cytokine release and of immune cell migration.[39,40] It is also now generally accepted that there are certain disorders in which endocannabinoid release increases in particular tissues, and secondly, that this upregulation of the endocannabinoid system leads in some instances to the suppression of unwanted signs and symptoms and so is "autoprotective" and in others to the production of undesirable effects.[5] Thus

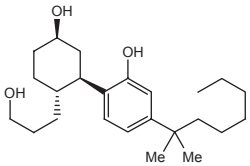
Table 1 | Pharmacological properties of certain cannabinoid CB_1/CB_2 receptor agonists and their K_i values for the *in vitro* displacement of [^{3}H]CP 55,940 or [^{3}H]HU 243 from CB_1- and CB_2-specific binding sites

Classification	Examples	CB_1 K_i values (nM)	CB_2 K_i values (nM)
Classical	The compounds in this group consist of dibenzopyran derivatives and are either plant-derived cannabinoids or synthetic analogues of these. Notable examples are		
	• (–)-Δ^9-THC, which binds equally well to CB_1 and CB_2 receptors and behaves as a partial agonist at both of these receptor types. It has even less efficacy at CB_2 than at CB_1 receptors and, indeed, has been reported in one CB_2 bioassay system to behave as an antagonist.[42]	5.05 to 80.3	3.13 to 75.3
	• (–)-Δ^8-THC, which resembles Δ^9-THC both in its affinities for CB_1 and CB_2 receptors and in its CB_1 receptor efficacy.	44, 47.6	44, 39.3
	• (–)-11-hydroxy-Δ^8-THC-dimethylheptyl (HU 210), which has efficacies at CB_1 and CB_2 receptors that match those of CP 55,940 and WIN 55,212-2 (see below) and affinities for CB_1 and CB_2 receptors that exceed those of many other cannabinoids. It is a particularly potent cannabinoid receptor agonist and its pharmacological effects *in vivo* are exceptionally long-lasting. The enhanced affinity and efficacy shown by HU 210 at cannabinoid receptors can be largely attributed to the replacement of the pentyl side chain of Δ^8-THC with a dimethylheptyl group.	0.06 to 0.73	0.17 to 0.52
Nonclassical	The compounds in this group were developed by a Pfizer research team. They are quite similar in structure to classical cannabinoids, consisting as they do of bicyclic and tricyclic analogues of Δ^9-THC that lack a pyran ring.		
	• The most widely used non-classical cannabinoid is CP 55,940, which has CB_1 and CB_2 affinities in the low nanomolar range and exhibits relatively high efficacy at both of these receptor types.	0.5 to 5.0	0.69 to 2.8
Aminoalkylindole	The prototype of this group is WIN 55,212-2, which was discovered by a Sterling Winthrop research team and is widely used in cannabinoid research.		
	• The structure of WIN 55,212-2 bears no resemblance to that of classical, nonclassical or eicosanoid cannabinoids. Indeed, there is evidence that it binds differently to the CB_1 receptor than classical and nonclassical cannabinoids, albeit it in a manner that still permits mutual displacement between WIN 55,212-2 and non-aminoalkylindole cannabinoids at CB_1 binding sites. Like CP 55,940, WIN 55,212-2 exhibits relatively high efficacy at CB_1 and CB_2 receptors and possesses CB_1 and CB_2 affinities in the low nanomolar range. However, in contrast to CP 55,940, it has slightly greater affinity for CB_2 than for CB_1 receptors.	1.89 to 123	0.28 to 16.2
Eicosanoid	The prototypic and most investigated members of this group are the endocannabinoids, anandamide and 2-arachidonylglycerol.		
	• Anandamide binds marginally more readily to CB_1 than to CB_2 receptors and, when protected from enzymic hydrolysis, exhibits a CB_1 affinity similar to that of (–)-Δ^9-THC. It also resembles (–)-Δ^9-THC in behaving as a partial agonist at CB_1 and CB_2 receptors and in exhibiting lower CB_2 than CB_1 efficacy.	61 to 543	279 to 1940
	• 2-Arachidonylglycerol has been found in several investigations to have affinities for CB_1 and CB_2 receptors similar to those of anandamide but to exhibit higher CB_1 and CB_2 efficacy than anandamide. In one recent investigation, however, performed with human CB_1 receptor-containing tissue, this endocannabinoid was found to lack both detectable CB_1 receptor efficacy at concentrations of up to 10 μM and any significant CB_1 receptor affinity (K_i > 10 μM).[43]	58.3, 472	145, 1400

ND, not determined; THC, tetrahydrocannabinol. See Figure 1 for the structures of the compounds listed in this table. For further information see references 1, 2 and 41.

CP 55,940, Potent CB_1 and CB_2 Agonist

CP 55,940
Cat. No. 0949



CP 55,940 is a cannabinoid agonist that is considerably more potent than Δ^9-THC in both behavioural tests and receptor binding assays. It displays high and roughly equal affinity for both central and peripheral cannabinoid receptors (K_i = 0.5-5.0 and 0.69-2.8 nM at CB_1 and CB_2 receptors respectively).

Wiley *et al* (1995) Discriminative stimulus effects of CP 55,940 and structurally dissimilar cannabinoids in rats. Neuropharmacology ***34*** 669. **Gatley** *et al* (1997) Binding of the non-classical cannabinoid CP 55,940, and the diarylpyrazole AM251 to rodent brain cannabinoid receptors. Life Sci. ***61*** 191. **Griffin** *et al* (1998) Evaluation of cannabinoid receptor agonists and antagonists using the guanosine-5´-O-(3-[^{35}S]thio)-triphosphate binding assay in rat cerebellar membranes. J.Pharmacol.Exp.Ther. ***285*** 553. **Thomas** *et al* (1998) Comparative receptor binding analyses of cannabinoid agonists and antagonists. J.Pharmacol.Exp.Ther. ***285*** 285.

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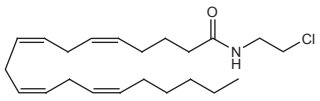
for example, there is evidence that endocannabinoid release on the one hand ameliorates spasticity in multiple sclerosis and inflammatory pain and on the other hand contributes towards obesity in some individuals or impairs fertility in certain women. As a result, there is now enormous interest not only in directly acting cannabinoid receptor agonists and antagonists but also in compounds that can affect the activity of the endocannabinoid system indirectly by allosterically modulating endocannabinoid-induced activation of cannabinoid receptors or by altering the concentration of endocannabinoids at their receptors through effects on endocannabinoid production or fate. The remainder of this review describes the main pharmacological actions of a number of such direct and indirect cannabinoid receptor agonists and antagonists. It focuses particularly on those compounds that are most widely used in cannabinoid research as experimental tools. Whenever possible, previous review articles have been cited that provide more detailed information and list additional references.

Mixed CB_1/CB_2 Receptor Agonists

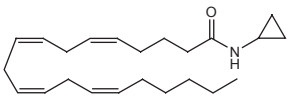
As has been detailed elsewhere,[1,2,41] compounds that are known to activate CB_1 and CB_2 receptors with approximately equal potency and that are most commonly used in the laboratory as CB_1/CB_2 receptor agonists fall essentially into one of four chemical groups: classical cannabinoid, nonclassical cannabinoid, aminoalkylindole and eicosanoid (Table 1 and Figure 1).

Many widely used CB_1/CB_2 receptor agonists contain chiral centres and generally exhibit signs of marked stereoselectivity in pharmacological assays in which the measured response is CB_1 or CB_2 receptor-mediated.[1,2,41] Usually, (–)-*trans* (6a*R*, 10a*R*) classical and nonclassical cannabinoids exhibit significantly greater potency as cannabinoid receptor agonists than their (+)-*cis* (6a*S*, 10a*S*) enantiomers, three notable examples of such compounds being (–)-Δ^9-tetrahydrocannabinol (Δ^9-THC), (–)-11-hydroxy-Δ^8-THC-dimethylheptyl (HU 210) and CP 55,940 (Table 1 and Figure 1). As to the aminoalkylindole, WIN 55,212, whilst its (*R*)-(+)-isomer (WIN 55,212-2) exhibits significant agonist activity at both CB_1 and CB_2 receptors, its (*S*)-(–)-isomer (WIN 55,212-3) does not. Indeed, when administered *in vitro* at concentrations in the low micromolar range, WIN 55,212-3 has been found to behave as a partial inverse agonist at CB_1 receptors and as a neutral CB_2 receptor antagonist.[44] The eicosanoid cannabinoid, anandamide, does not contain any chiral centres. However, some of its synthetic analogues do, one example being the CB_1-selective agonist, methanandamide (see next section), the (*R*)-(+)-isomer of which has nine times greater affinity for CB_1 receptors than the (*S*)-(–)-isomer.[45]

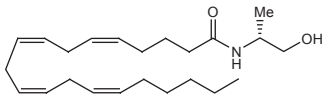
Figure 3 | Structures of the synthetic compounds, ACEA, ACPA, (*R*)-(+)-methanandamide and O-1812, and of the endogenous compound noladin ether, all of which behave as CB_1-selective agonists (see also Table 2)



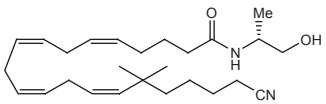
ACEA



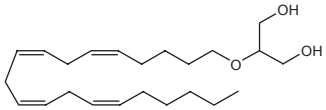
ACPA



(*R*)-(+)-Methanandamide



O-1812



Noladin ether

(Bold Text Denotes Compounds Available From Tocris)

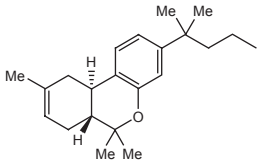
One major practical difficulty associated with cannabinoid research, both *in vivo* and *in vitro*, is the high lipophilicity and low water solubility of most CB_1 and CB_2 receptor ligands as this necessitates the use of a non-aqueous vehicle such as ethanol, dimethyl sulphoxide, polyvinylpyrrolidone, Tween 80, Cremophor, Emulphor, bovine serum albumin or the water-soluble emulsion Tocrisolve 100, which is a mixture of soya oil, Pluronic F68 and water.[1,46,47] Consequently, one other cannabinoid CB_1/CB_2 receptor agonist that merits special mention is the Organix compound, 3-(5´-cyano-1´,1´-dimethylpentyl)-1-(4-N-morpholinobutyryloxy)-Δ^8-THC hydrochloride (O-1057),[48] as this is readily soluble in water. The *in vitro* potency of O-1057 relative to that of CP 55,940 is just 2.9 times less at CB_1 receptors and 6.5 times less at CB_2 receptors.

CB_1-Selective Agonists

For the development of the first CB_1-selective agonists, the starting point was the anandamide molecule, the marginal CB_1 selectivity of which can be significantly enhanced by inserting a fluorine atom on the terminal 2´ carbon to form O-585 and/or by replacing a hydrogen atom on the 1´ or 2 carbon with a methyl group to form (*R*)-(+)-methanandamide, its cyano analogue O-1812, or O-689.[1,2,41] Another important consequence of inserting a methyl group on the 1´ or 2 carbon is greater resistance to the hydrolytic action of FAAH and, indeed, (*R*)-(+)-methanandamide was first synthesised in Dr Alexandros Makriyannis' laboratory in order to meet the need for a metabolically more stable anandamide analogue. Together with O-1812,[49] the most potent CB_1-selective agonists so far developed have been arachidonyl-2´-chloroethylamide (ACEA) and arachidonylcyclopropylamide (ACPA), both of which exhibit reasonably high CB_1 efficacy.[50] However, unlike O-1812, or indeed methanandamide or O-689, neither ACEA nor ACPA show any sign of resistance to enzymic hydrolysis.[1,2,49] This is presumably because they lack a methyl substituent on the 1´ or 2 carbon and, indeed, it has been shown that the addition of a methyl group to the 1´ carbon of ACEA does markedly decrease the susceptibility of this molecule to FAAH-mediated hydrolysis.[51] This structural change also reduces the affinity of ACEA for CB_1 receptors by about 14-fold. One other arachidonic acid derivative that deserves mention as a CB_1-selective agonist is 2-arachidonylglyceryl ether (noladin ether), not least because it is a putative endocannabinoid.[52] This ligand exhibits CP 55,940-like CB_1 efficacy but less CB_1 potency than CP 55,940.[53,54] The structures of (*R*)-(+)-methanandamide, O-1812, ACEA, ACPA and noladin ether are shown in Figure 3 and the CB_1 and CB_2 binding properties of these compounds are summarised in Table 2.

JWH 133, Potent and Selective CB_2 Receptor Agonist

JWH 133
Cat. No. 1343



JWH 133 is a potent CB_2 agonist that displays approximately 200-fold selectivity over CB_1 receptors (K_i values are 3.4 and 677 nM respectively). *In vivo*, JWH 133 reduces spasticity in a murine autoimmune model of multiple sclerosis. The superior selectivity, potency and *in vivo* activity of this CB_2 agonist make it an important and essential tool for studying the physiological function of CB_2 receptors.

Huffman *et al* (1999) 3-(1´-Dimethylbutyl)-1-deoxy-Δ^8-THC and related compounds: synthesis of selective ligands for the CB_2 receptor. Bioorg.Med. Chem. ***7*** 2905. **Pertwee** (1999) Pharmacology of cannabinoid receptor ligands. Curr.Med.Chem. ***6*** 635. **Baker** *et al* (2000) Cannabinoids control spasticity and tremor in a multiple sclerosis model. Nature ***404*** 84.

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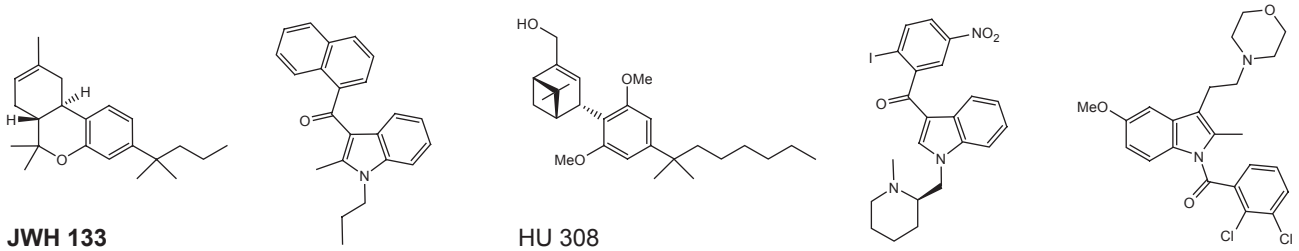
CB_2-Selective Agonists

The CB_2-selective agonists most widely used as experimental tools have been the classical cannabinoid, JWH 133, and the less selective aminoalkylindole, JWH 015, both developed by Dr John Huffman.[1,2,72] Each of these agents not only binds more readily to CB_2 than to CB_1 receptors but also behaves as a potent CB_2-selective agonist in functional assays. Other notable CB_2-selective

Figure 4 | Structures of the CB_2-selective agonists JWH 133, JWH 015, HU 308, AM 1241 and GW 405833 (see also Table 2)



JWH 133 | **JWH 015** | HU 308 | AM 1241 | **GW 405833**

(Bold Text Denotes Compounds Available From Tocris)

agonists include the GlaxoSmithKline compound GW 405833, which behaves as a potent partial agonist at the CB_2 receptor,[67] and HU 308, AM 1241 and the Merck Frosst compounds L-759,633 and L-759,656.[1,2] Interestingly, AM 1241 may be a "protean agonist" as it has been reported to behave as an agonist in tissues in which CB_2 receptors are naturally expressed but not in tissues in which CB_2 receptors have been inserted genetically and are therefore presumably overexpressed.[73] The structures of JWH 133, JWH 015, HU 308, AM 1241 and GW 405833 are shown in Figure 4 and their CB_1 and CB_2 binding properties are summarised in Table 2.

Table 2 | K_i values of CB_1- and CB_2-selective ligands for the *in vitro* displacement of [^{3}H]CP 55,940 or [^{3}H]HU 243 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 agonists			
ACEA	1.4[a,b] 5.29[a,b]	> 2000[a,b] 195[c]	50 62
O-1812	3.4[a]	3870[a]	49
ACPA	2.2[a,b]	715[a,b]	50
Noladin ether	21.2[a]	> 3000[d]	52
(*R*)-(+)-methanandamide	17.9[a,b] 20[a,b] 28.3[a]	868[c] 815[c] 868[c]	62 63 64
CB_1-selective antagonists/inverse agonists			
SR141716A	1.8[c] 1.98[a] 5.6 11.8 11.8 12.3	514[c] > 1000[a] > 1000 13200 973 702	55 56 56 57 58 59
AM 281	12[a]	4200[c]	60
AM 251	7.49[a]	2290[c]	61
LY 320135	141	14900	57
CB_2-selective agonists			
AM 1241	280[a]	3.4[c]	65
JWH 133	677[a]	3.4	66
GW 405833	4772 273[a]	3.92 3.6[a]	67
JWH 015	383	13.8	59
HU 308	> 10000[a,e]	22.7[d,e]	68
CB_2-selective antagonists/inverse agonists			
SR144528	70[c] 305[a] 437 50.3 > 10000	0.28[c] 0.30[a] 0.60 1.99 5.6	55 69 69 70 71
AM 630	5152	31.2	71

(Bold Text Denotes Compounds Available From Tocris)

ACEA, arachidonyl-2´-chloroethylamide; ACPA, arachidonylcyclopropylamide.
[a]Binding to rat cannabinoid receptors in transfected cells or in brain (mainly CB_1) or spleen tissue (mainly CB_2).
[b]With phenylmethylsulphonyl fluoride in order to inhibit enzymic hydrolysis.
[c]Binding to mouse brain (mainly CB_1) or spleen tissue (mainly CB_2).
[d]Species unspecified.

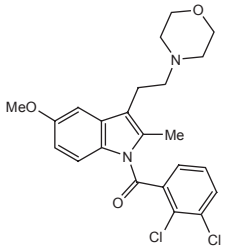
All other data from experiments with human cannabinoid receptors.

[e]Displacement of [^{3}H]HU 243 from CB_1- and CB_2-specific binding sites; [^{3}H]CP 55,940 was used in all other experiments.

See Figures 3 to 5 for the structures of the compounds listed in this table.

GW 405833, Potent CB_2 Partial Agonist

GW 405833
Cat. No. 2374



GW 405833 is a highly potent and selective CB_2 receptor partial agonist. In a functional assay using human recombinant CB_2 receptors, the compound displays an EC_{50} value of 0.65 nM and a maximum inhibition of 44.6% at 300 nM. It binds with high affinity to both human and rat CB_2 receptors and displays ~ 1200-fold selectivity over CB_1 (K_i values are 3.92 and 4772 nM for human recombinant CB_2 and CB_1 receptors respectively). GW 405833 produces potent antihyperalgesic effects in several rodent models of pain.

Clayton *et al* (2002) CB1 and CB2 cannabinoid receptors are implicated in inflammatory pain. Pain ***96*** 253. **Valenzano** *et al* (2005) Pharmacological and pharmacokinetic characterization of the cannabinoid receptor 2 agonist, GW405833, utilizing rodent models of acute and chronic pain, anxiety, ataxia and catalepsy. Neuropharmacology ***48*** 658.

Selective CB_1 Receptor Antagonists/ Inverse Agonists

The first of these to be developed was the diarylpyrazole, SR141716A.[56] This is a highly potent and selective CB_1 receptor ligand that readily prevents or reverses CB_1-mediated effects both *in vitro* and *in vivo*.[1,2,41] Other notable CB_1-selective antagonists are AM 251 and AM 281, both developed by Dr Alexandros Makriyannis, and LY 320135 which has less affinity for CB_1 receptors than SR141716A, AM 251 or AM 281 and at concentrations in the low micromolar range also binds to muscarinic and 5-hydroxytryptamine (5-HT_2) receptors.[1,2,41]

As detailed elsewhere,[2,74] there is convincing evidence that SR141716A, AM 251, AM 281 and LY 320135 are not "neutral" antagonists. Thus, as well as attenuating effects of CB_1 receptor agonists, they can by themselves elicit responses in some CB_1 receptor-containing tissues that are opposite in direction from those elicited by CB_1 receptor agonists. Whilst such "inverse cannabimimetic effects" may in some instances be attributable to a direct antagonism of responses evoked at CB_1 receptors by released endocannabinoids, there is evidence that this is not always the underlying mechanism and that SR141716A, AM 251, AM 281 and LY 320135

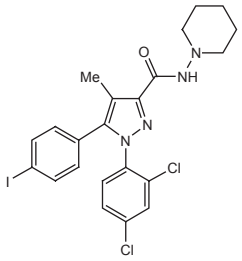
are in fact inverse agonists.[74] More specifically, they appear to produce inverse cannabimimetic effects in at least some tissues by somehow reducing the constitutive activity of CB_1 receptors (the coupling of CB_1 receptors to their effector mechanisms that, it is thought, can occur in the absence of exogenously added or endogenously released CB_1 agonists). The structures of SR141716A, AM 251, AM 281 and LY 320135 are shown in Figure 5 and the CB_1 and CB_2 binding properties of these compounds are summarised in Table 2.

Selective CB_2 Receptor Antagonists/ Inverse Agonists

The most notable CB_2-selective antagonists/inverse agonists are the Sanofi-Aventis diarylpyrazole, SR144528,[69] and 6-iodopravadoline (AM 630)[71] (Figure 5). Both compounds bind with much higher affinity to CB_2 than to CB_1 receptors (Table 2), exhibit marked potency as CB_2 receptor antagonists and behave as inverse agonists that can by themselves produce inverse cannabimimetic effects at CB_2 receptors.[1,2,41] Thus for example, AM 630 has been reported to reverse CP 55,940-induced inhibition of forskolin-stimulated cyclic AMP production by human CB_2-transfected CHO cell preparations at concentrations in the nanomolar range (EC_{50} = 129 nM) and to enhance forskolin-stimulated cyclic AMP production by the same cell line when administered by itself (EC_{50} = 230 nM),[71] albeit with an efficacy that appears to be somewhat less than the inverse efficacy displayed by SR144528 in this bioassay.[75] At the CB_1 receptor, AM 630 has been found to behave in some investigations as a low-potency partial agonist[41,71,76-78] but in others as a low-potency inverse agonist.[79,80]

AM 251, Potent CB_1-Selective Antagonist/Inverse Agonist

AM 251
Cat. No. 1117



AM 251 is a potent and selective CB_1 receptor antagonist/ inverse agonist. Structurally related to SR141716A, AM 251 displays a K_i value of 7.49 nM at CB_1 receptors and is 306-fold selective over CB_2 receptors. It suppresses food intake and food-reinforced behaviour in rats.

Gatley *et al* (1996) ^{125}I-labeled AM 251: a radioiodinated ligand which binds in vivo to mouse brain cannabinoid CB_1 receptors. Eur.J.Pharmacol. ***307*** 331. **Gatley** *et al* (1997) Binding of the non-classical cannabinoid CP 55,940, and the diarylpyrazole AM251 to rodent brain cannabinoid receptors. Life Sci. ***61*** 191. **Pertwee** (2005) Inverse agonism and neutral antagonism at cannabinoid CB_1 receptors. Life Sci. ***76*** 1307.

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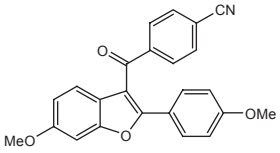
LY 320135, CB_1 Antagonist/ Inverse Agonist

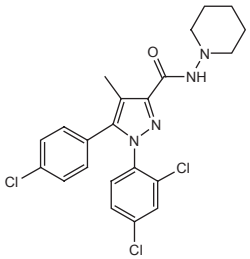
LY 320135
Cat. No. 2387



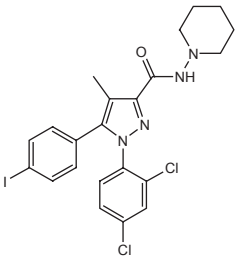
LY 320135 is a CB_1 receptor antagonist that is structurally dissimilar from SR 141716A and AM 251. The compound produces inverse agonist effects and displays > 70-fold selectivity for CB_1 over CB_2 receptors (K_i values are 141 nM and > 10 μM respectively. It shows weak binding to both 5-HT_2 (K_i = 6.4 μM) and muscarinic receptors (K_i = 2.1 μM).

Felder *et al* (1998) LY320135, a novel cannabinoid CB_1 receptor antagonist, unmasks coupling of the CB_1 receptor to stimulation of cAMP accumulation. J.Pharmacol.Exp.Ther. ***284*** 291. **Holland** *et al* (1999) Cannabinoid CB_1 receptors fail to cause relaxation, but couple via G_i/G_o to the inhibition of adenylyl cyclase in carotid artery smooth muscle. Br.J.Pharmacol. ***128*** 597. **Pertwee** (2005) Inverse agonism and neutral antagonism at cannabinoid CB_1 receptors. Life Sci. ***76*** 1307.

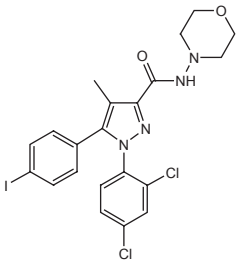
Figure 5 | Structures of the CB_1-selective antagonists/inverse agonists, SR141716A, AM 251, AM 281 and LY 320135, and of the CB_2-selective antagonists/inverse agonists, SR144528 and AM 630 (see also Table 2)



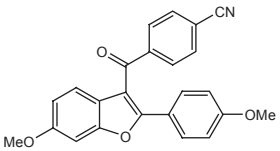
SR141716A



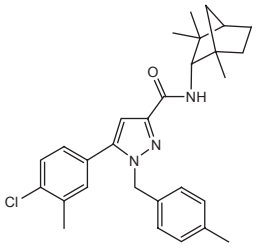
AM 251



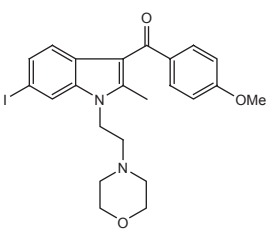
AM 281



LY 320135



SR144528



AM 630

(Bold Text Denotes Compounds Available From Tocris)

Neutral Cannabinoid Receptor Antagonists

There is currently considerable interest in the possibility of developing potent neutral CB_1 and CB_2 receptor antagonists: i.e. high-affinity ligands for CB_1 or CB_2 receptors that lack significant agonist or inverse agonist efficacy. One reason for this is that unlike the CB_1 and CB_2-selective antagonists/inverse agonists now available (see previous sections), a neutral antagonist could be used to distinguish between tonic cannabimimetic activity arising from ongoing endocannabinoid release onto CB_1 or CB_2 receptors, which it should oppose, and tonic activity arising from the presence of constitutively active CB_1 or CB_2 receptors, which it should not. Although no neutral antagonist that selectively targets the CB_2 receptor has yet been developed, some progress has been made on the CB_1 receptor front. Thus, there is some evidence that 6″-azidohex-2″-yne-cannabidiol (O-2654), O-2050, a sulphonamide analogue of Δ^8-THC with an acetylenic side chain, and VCHR, an analogue of SR141716A, are neutral CB_1 receptor antagonists.[2,74] As this evidence is somewhat preliminary, particular caution should be exercised when using any of these ligands as a pharmacological tool. There is more complete evidence that another SR141716A analogue, NESS 0327, is a neutral CB_1 receptor antagonist.[55] However, this compound is currently not commercially available and so has not been much used in cannabinoid research.

Radiolabelled Cannabinoid Receptor Ligands

Tritiated cannabinoid receptor ligands that have been most widely used in binding assays or for autoradiography are the CB_1-selective [^{3}H]SR141716A (CB_1 K_d = 0.19 to 1.24 nM), and [^{3}H]CP 55,940, [^{3}H]WIN 55,212-2 and [^{3}H]HU 243, all three of which bind more or less equally well to CB_1 and CB_2 receptors. Typical K_d values for

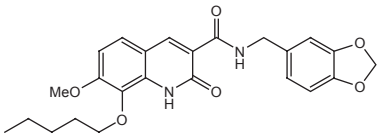
Table 3 | Some established and putative non-CB_1, non-CB_2 targets with which CB_1/CB_2 receptor agonists have been postulated to interact at concentrations of 1 μM or less

Voltage-gated ion channels containing a target	Measured response	CB_1/CB_2 receptor agonist	Reference
N-type Ca^{2+} channels	Ion current (–)	Anandamide	86
T-type Ca^{2+} channels	Ion current (–)	Anandamide	86
Na^+ channels	Ion current (–)	Δ^9-THC, 11-hydroxy-Δ^9-THC (anandamide, 2-arachidonylglycerol at > 1μM)	86
Ca^{2+}-activated (BK) K^+ channels	Ion current (P)	Anandamide	86
Other types of voltage-gated K^+ channels	Ion current (–)	Anandamide	86
Receptors/ligand-gated ion channels containing a target			
α7 nACh channels	Ion current (–)	Anandamide, 2-arachidonylglycerol	86
Glycine receptors	Ion current (–/P)	Anandamide, 2-arachidonylglycerol, Δ^9-THC	86
NR1A-containing NMDA channels	Ion current (P)	Anandamide	86
5-HT_2 receptors	5-HT binding (+)	Oleamide, HU 210	87
5-HT_3 receptors (5-HT_{3A} subunit)†	Ion current (–)	Δ^9-THC, WIN 55212-2, anandamide, JWH 015, CP 55,940	88
TRPV1 receptors	Ion current (A)	Anandamide, methanandamide (not 2-arachidonylglycerol, HU 210, CP 55,940, WIN 55212-2)	86, 88
TRPV4 receptors	Ion current (A)	Anandamide	86
Central putative TRPV1-like receptors	Ion current (–)	WIN 55212-2, CP 55,940	89
Central putative non-CB_1, non-CB_2, non-TRPV1 G-protein-coupled receptors	Receptor activation (+)	WIN 55212-2, anandamide (not Δ^9-THC, HU 210, CP 55,940)	90
Putative non-CB_1, non-CB_2, non-TRPV1 neuronal receptors	Receptor activation (+)	Δ^9-THC, cannabinol (not HU 210 or CP 55,940)	88
Putative non-I_1, non-I_2 imidazoline neuronal receptors	Receptor activation (+)	CP 55,940 (WIN 55212-2, anandamide at > 1μM)	88
Sites on neuronal transporters			
Noradrenaline transporter	Synaptosomal uptake (P)	Δ^9-THC	46
Dopamine transporter	Synaptosomal uptake (P/–)	Δ^9-THC	46
5-HT transporter	Synaptosomal uptake (P/–)	Δ^9-THC	46

A, activation; P, potentiation; (+), increase induced; (–), decrease induced.
†The rank order of potency for antagonism of human 5-hydroxytryptamine (5-HT_{3A}) receptors expressed by HEK 293 cells is Δ^9-THC > WIN 55,212-2 > anandamide > JWH 015 > CP 55,940.[91]
Cannabinol is a classical cannabinoid that exhibits both less affinity for CB_1 receptors and less CB_1 efficacy than Δ^9-THC (see references 2 and 41).

JTE 907, CB_2-Selective Inverse Agonist

JTE 907
Cat. No. 2479



JTE 907 is a highly selective CB_2 receptor inverse agonist. It binds with high affinity to rat, mouse and human CB_2 receptors (K_i values are 0.38, 1.55 and 35.9 nM respectively) and produces anti-inflammatory effects *in vivo*.

Iwamura *et al* (2001) In vitro and in vivo pharmacological characterization of JTE-907, a novel selective ligand for cannabinoid CB_2 receptor. J.Pharmacol. Exp.Ther. ***296*** 420. **Ueda** *et al* (2005) Involvement of cannabinoid CB_2 receptor-mediated response and efficacy of cannabinoid CB_2 receptor inverse agonist, JTE-907, in cutaneous inflammation in mice. Eur.J.Pharmacol. ***520*** 164. **Maekawa** *et al* (2006) The cannabinoid CB_2 receptor inverse agonist JTE-907 suppresses spontaneous itch-associated responses of NC mice, a model of atopic dermatitis. Eur.J.Pharmacol. ***542*** 179.

[^{3}H]CP 55,940, [^{3}H]WIN 55,212-2 and [^{3}H]HU 243 are 0.07 to 4 nM, 1.9 to 16.2 nM and 0.045 nM respectively at CB_1 receptors and 0.2 to 7.4 nM, 2.1 to 3.8 nM and 0.061 nM respectively at CB_2 receptors.[2,41] Thus [^{3}H]HU 243, which is structurally very similar to HU 210 (Figure 1), has particularly high affinity for these receptors. Radiolabelled ligands have also been developed as potential probes for human single photon emission computed tomography (SPECT) or positron emission tomography (PET) experiments. These are ^{123}I labelled analogues of AM 251 (CB_1 K_d = 0.23 to 0.62 nM) and AM 281[81-83] and an ^{18}F-labelled analogue of SR141716A (SR144385).[84] Particularly promising results have been obtained from animal experiments with [^{123}I]AM 281.[82,85]

Additional Pharmacological Targets for CB_1 and CB_2 Receptor Ligands

It is now generally accepted that some cannabinoid receptor agonists are reasonably potent at activating the TRPV1 (vanilloid VR1) receptor (Table 3). These include eicosanoids such as anandamide, methanandamide, ACEA, NADA and some anandamide metabolites, and the putative endocannabinoid, OLDA, but exclude 2-arachidonylglycerol and also classical, nonclassical and aminoalkylindole cannabinoid receptor agonists such as HU 210, CP 55,940 and WIN 55,212-2.[2,5,88,92,93]

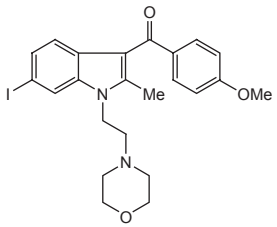
A number of other non-CB_1, non-CB_2 pharmacological targets for some CB_1/CB_2 receptor agonists have been proposed, including several that appear to respond to agonist concentrations of 1 micromolar or less (Table 3), and hence to possess sensitivity to these ligands of the same order as that exhibited by CB_1 or CB_2 receptors. Thus, for example, anandamide interacts at submicromolar concentrations with several types of ligand-gated and voltage-gated ion channels, some (but not all) of which are also sensitive to 2-arachidonylglycerol, Δ^9-THC, CP 55,940, WIN 55,212-2 and/or JWH 015 (Table 3). There is evidence too that Δ^9-THC also interacts potently with neuronal transporters of dopamine, noradrenaline and 5-hydroxytryptamine (Table 3), and that there are a number of less sensitive pharmacological targets for Δ^9-THC and/or for certain other cannabinoid receptor agonists. These targets, which only seem to respond to cannabinoid concentrations above 1 μM, include L-type Ca^{2+} and shaker Kv1.2 K^+ channels, PPARγ and TRPA1 receptors, putative non-CB_1, non-CB_2, non-TRPV1 neuronal receptors in the small intestine, sites on muscarinic M_1 and M_4 receptors and on glutamate GLU_{A1} and GLU_{A3} receptors, and sites at gap junctions between cells.[2,86,88,94,95] Anandamide and methanandamide, but not Δ^9-THC, WIN 55,212-2 or 2-arachidonylglycerol, also behave as agonists for the putative abnormal-cannabidiol (abnormal-CBD) receptor[2,88,96] (see also section on other notable ligands).

The CB_1 receptor antagonists/inverse agonists, SR141716A and AM 251, can also interact with non-CB_1, non-CB_2 targets, albeit only at concentrations that lie in the micromolar range and hence above concentrations at which these ligands are capable of producing significant CB_1 receptor antagonism. Thus for example, as also discussed elsewhere,[74,88] there are reports that at micromolar concentrations:

- SR141716A and AM 251 can block adenosine A_1 receptor activation,
- AM 251 can block neuronal voltage-sensitive Na^+ channels,
- SR141716A can block L-type Ca^{2+} channels, Ca^{2+}-activated (BK) K^+ channels, ATP-sensitive K^+ channels and sites at gap junctions between cells,
- SR141716A can block the activation of putative abnormal-CBD receptors on mesenteric arteries

AM 630, Competitive CB_2 Antagonist

AM 630
Cat. No. 1120



AM 630 is a CB_2 receptor antagonist (K_i = 31.2 nM) that is 165-fold selective over CB_1. The ligand displays inverse agonist properties in CHO cells expressing CB_2 receptors and behaves as a weak partial/inverse agonist at CB_1 receptors.

Hosohata *et al* (1997) AM630 is a competitive cannabinoid receptor antagonist in the guinea pig brain. Life Sci. ***61*** PL115. **Hosohata** *et al* (1997) AM630 antagonism of cannabinoid-stimulated [^{35}S]GTPγS binding in the mouse brain. Eur.J.Pharmacol. ***321*** R1. **Landsman** *et al* (1998) AM630 is an inverse agonist at the human cannabinoid CB_1 receptor. Life Sci. ***62*** PL109. **Ross** *et al* (1999) Agonist-inverse agonist characterization at CB_1 and CB_2 cannabinoid receptors of L759633, L759656 and AM630. Br.J.Pharmacol. ***126*** 665.

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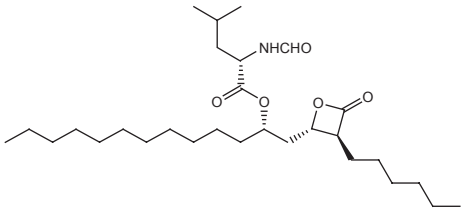
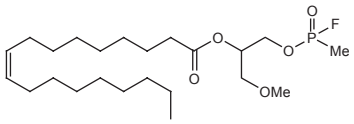
Figure 6 | Structures of the DAGL inhibitors, tetrahydrolipstatin and O-3841



Tetrahydrolipstatin



O-3841

and of putative non-I_1, non-I_2 imidazoline receptors and

- SR141716A but not AM 251 can antagonise WIN 55,212-2-induced activation of central presynaptic putative TRPV1-like receptors.

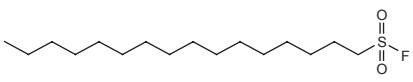
Future research will most likely reveal additional targets for CB_1 and CB_2 receptor ligands. Indeed, it has already been claimed in AstraZeneca and GlaxoSmithKline patents that some established cannabinoid receptor agonists (and antagonists) activate the G-protein-coupled orphan receptor, GPR55.[97,98]

Because cannabinoid receptor agonists differ in the extent to which they interact with the proposed or established non-CB_1, non-CB_2 targets listed in Table 3, it follows that some ligands that appear to activate CB_1 and/or CB_2 receptors with similar potencies will most probably possess different pharmacological profiles from each other. It is also worth noting that although there are a number of established cannabinoid receptor ligands that exhibit marked selectivity as agonists or antagonists/inverse agonists for CB_1 or CB_2 receptors (see previous sections), none of these ligands are entirely CB_1- or CB_2-specific. Thus, each of these ligands is expected to activate or block both of these receptor types equally well if administered at a sufficiently high dose or concentration and hence to exhibit selectivity only when administered at lower doses or concentrations that lie within its CB_1 or CB_2 "selectivity window".

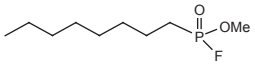
Inhibitors of 2-Arachidonylglycerol Biosynthesis

Since anandamide and 2-arachidonylglycerol are synthesised on demand rather than stored, and since there is evidence that increased production and release of either or both of these endocannabinoids is responsible for unwanted signs and symptoms of certain disorders (see section on the endocannabinoid system), selective inhibitors of their enzymic biosynthesis would not only constitute important experimental tools but also have potential as therapeutic agents. Although selective inhibitors of NAPE-PLD have yet to be discovered, such inhibitors are available for the enzymes, DAGLα and DAGLβ, which catalyse the conversion of diacylglycerols

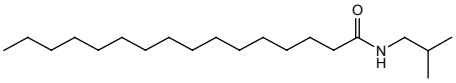
Figure 7 | Structures of the FAAH inhibitors, AM 374, O-1887, PIA, PMSF, MAFP, URB532, URB597, OL-135, $AACOCF_3$ (ATFMK) and *N*-arachidonylglycine (see also Table 4), and of the MAGL inhibitor, URB602



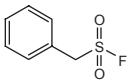
AM 374



O-1887



PIA



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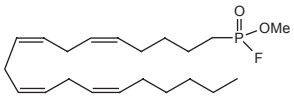
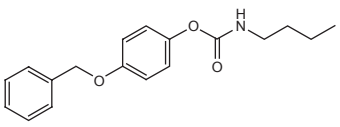
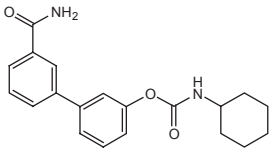


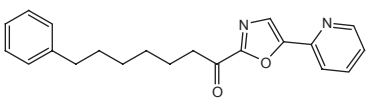
MAFP



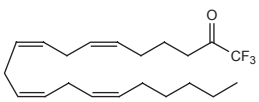
URB532



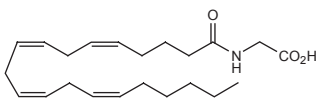
URB597



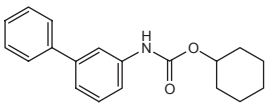
OL-135



$AACOCF_3$



***N*-Arachidonylglycine**



URB602

(Bold Text Denotes Compounds Available From Tocris)

to 2-arachidonylglycerol. One of these inhibitors is tetrahydrolipstatin (Figure 6), which inhibits DAGLα (IC_{50} = 60 nM) and DAGLβ (IC_{50} = 100 nM) far more potently than it inhibits NAPE-PLD (IC_{50} = 10 μM) and which does not inhibit MAGL even at 25 μM.[35,99] A second notable DAGL inhibitor is O-3841 (Figure 6). This inhibits DAGLα at nanomolar concentrations (IC_{50} = 160 nM) but, at concentrations of up to 25 μM, lacks any detectable inhibitory effect on NAPE-PLD, FAAH, MAGL or triacylglycerol lipase activity or on the specific binding of [^{3}H]CP 55,940 to human CB_1 or CB_2 receptors.[99] Whereas tetrahydrolipstatin and O-3841 both inhibit DAGL in membrane preparations, only tetrahydrolipstatin has so far been found to produce detectable signs of DAGL inhibition in intact cells.[99]

Inhibitors of the Enzymic Hydrolysis of Endocannabinoids

The presence of FAAH and MAGL in many tissues has created the need for selective inhibitors of these enzymes that can be used to facilitate research directed at exploring both the pharmacological actions of endocannabinoids when these are administered exogenously and their physiological and pathological roles when they are released endogenously. Indeed, partly as a result of experiments with FAAH and MAGL inhibitors, there is already evidence that endogenous cannabinoid release increases in some disorders in a manner that leads to an amelioration of unwanted signs and symptoms (see section on the endocannabinoid system), and consequently, that such inhibitors have therapeutic potential.

Following the discovery of anandamide, the compound most widely used to inhibit its enzymic hydrolysis (irreversibly) was the non-selective serine protease inhibitor, phenylmethylsulphonyl fluoride (IC_{50} for FAAH inhibition = 290 nM to 15 μM), which also inhibits MAGL, albeit less potently (IC_{50} ≥ 155 μM).[100] Additional inhibitors of FAAH have now been developed,[5,23,100] the best of these for use as research tools most probably being URB597, O-1887, URB532 and the palmitylsulphonyl fluoride

Table 4 | Some inhibitors of fatty acid amide hydrolase (FAAH) or anandamide cellular uptake

	Inhibitor	Uptake inhibition IC_{50} or K_i* (μM)	FAAH inhibition IC_{50} or K_i* (μM)	CB_1 IC_{50} or K_i* (μM)†	CB_2 IC_{50} or K_i* (μM)†	TRPV1 EC_{50} or K_i* (μM)§	Reference
(a) FAAH inhibitors	PMSF◊	ND	0.29 to 15	> 10	ND	ND	100, 101
	AM 374◊	ND	0.013, 0.05	0.52	ND	ND	101, 102
	MAFP◊#	ND	0.001 to 0.003	0.02	ND	ND	103, 104
	O-1887◊	ND	0.015	> 10	ND	ND	105
	URB532◊	> 300	0.214, 0.396	> 300	> 300	ND	106
	URB597◊	> 30	0.0005, 0.0046	> 100	> 100	ND	106
	OL-135	ND	0.0021	> 10	> 10	ND	107
	PIA	§§	12.9	> 100	> 100	ND	108
	$AACOCF_3$ (ATFMK)	ND	0.7 to 4	0.65	ND	ND	100, 109
	***N*-arachidonylglycine**	> 50	4.1, 7	> 10	ND	> 10	110, 111
(b) Uptake inhibitors	**LY 2183240◊**	0.00027	0.0124	ND	ND	ND	27, 131
	OMDM-1	2.4*, 2.6, > 20	> 50*, > 100	12.1*	> 10	> 10	112, 113
	OMDM-2	3*, 3.2, 17	> 50*, 54, > 100	5.1*	> 10	10	112, 113
	VDM 11	6.1 to 11.2	1.2 to 3.7, > 50	> 5 or 10*	> 5 or 10*	Little activity at 10 μM	113, 114
	UCM 707	0.8, 25, 41	30, > 100	4.7*	0.067*	> 5*	113, 115
	AM 404	1 to 11	0.5 to 5.9 22, > 30	> 1*, 1.76*	13*	0.026	51, 63, 113, 114, 116
	(–)-5´-DMH-CBD	14	> 100	> 10*	1.8*	Inactive	117

(Bold Text Denotes Compounds Available From Tocris)

$AACOCF_3$ (ATFMK), arachidonyl trifluoromethyl ketone; (–)-5´-DMH-CBD, (–)-5´-dimethylheptyl-cannabidiol; MAFP, methyl arachidonyl fluorophosphonate; ND, no data; PIA, palmitoylisopropylamide; PMSF, phenylmethylsulphonyl fluoride.

†IC_{50} or K_i values for displacement of [^{3}H]SR141716A (CB_1 receptors) or of [^{3}H]CP 55,940, [^{3}H]WIN 55,212-2 or [^{3}H]HU 243 (CB_1 and/or CB_2 receptors).
§EC_{50} value for TRPV1 receptor activation.
◊Irreversible FAAH inhibitor.
#Irreversible CB_1 ligand.
§§Some inhibition of uptake at 30 and 100 μM

LY 2183240 is also a potent inhibitor of other serine hydrolases and of MAGL.[27]
OMDM-1 does not inhibit NAPE-PLD, DAGLα or MAGL at 25 μM.[99]
MAGL is not inhibited by URB532 or URB597 at 30 μM[106] or by OL-135 at 100 μM.[107]
The structures of the inhibitors mentioned in this table are shown in Figures 7 and 8.

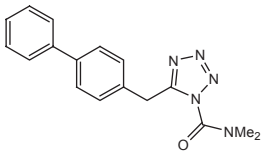
analogue, AM 374, for irreversible FAAH inhibition, and OL-135 for reversible FAAH inhibition. Thus, these compounds can all produce their inhibitory effects *in vitro* at concentrations that lie in the low nanomolar range and that lack the ability to displace radiolabelled ligands from CB_1 receptors or (where this has been investigated) from CB_2 receptors (Figure 7 and Table 4). It should be noted, however, that the pharmacological characterisation of most of these inhibitors is incomplete.

The compound O-1887 is a structural analogue of methyl arachidonyl fluorophosphonate (MAFP; Figure 7), which is itself an irreversible FAAH inhibitor (IC_{50} = 1 to 3 nM) that additionally inhibits both MAGL (IC_{50} = 2 to 800 nM) and DAGL (IC_{50} = 800 nM) although not NAPE-PLD, and potently displaces [^{3}H]CP 55,940 from specific binding sites on rat brain membranes in an irreversible manner (IC_{50} = 20 nM).[5,99,100,104] There has also been one report that MAFP behaves as an irreversible CB_1 receptor antagonist[118] and a second report that it does not.[54]

Turning now to compounds that block the metabolism of 2-arachidonylglycerol by inhibiting MAGL, a number of these have now been identified.[99,100,119,120] At present, the best and most characterised inhibitor of this enzyme seems to be URB602 (Figure 7), which produces non-competitive inhibition of MAGL at micromolar concentrations (IC_{50} = 28 μM) and lacks any detectable ability to inhibit FAAH at 100 μM or to displace [^{3}H]WIN 55,212-2 from CB_1 or CB_2 receptors at 5 μM.[119] Another compound, URB754, has been reported to inhibit MAGL with significantly greater potency than URB602.[120] However, it is now known that this inhibition was induced by an impurity present in a commercial sample of URB754, and that the pure compound lacks significant activity as an MAGL inhibitor at concentrations of up to 100 μM (personal communication from Dr Daniele Piomelli).

LY 2183240, Highly Potent Inhibitor of Anandamide Uptake

LY 2183240
Cat. No. 2452



LY 2183240 is a novel and exceptionally potent blocker of anandamide uptake (IC_{50} = 270 pM). It appears to act via inhibition of fatty acid amide hydrolase (FAAH) activity (IC_{50} = 12.4 nM). Following i.p. administration in rats, LY 2183240 increases anandamide concentrations in the cerebellum and exerts significant antinociceptive effects in the formalin model of persistent pain.

Moore *et al* (2005) Identification of a high-affinity binding site involved in the transport of endocannabinoids. Proc.Natl.Acad.Sci.USA ***102*** 17852. **Dickason-Chesterfield** *et al* (2006) Pharmacological characterization of endocannabinoid transport and fatty acid amide hydrolase inhibitors. Cell Mol.Neurobiol. (in press). **Alexander and Cravatt** (2006) The putative endocannabinoid transport blocker LY2183240 is a potent inhibitor of FAAH and several other brain serine hydrolases. J.Am.Chem.Soc. ***128*** 9699.

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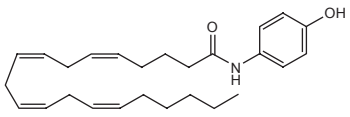
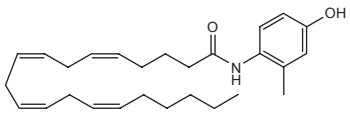
Inhibitors of the Cellular Uptake of Anandamide

The first inhibitor of the cellular uptake of anandamide to be developed was *N*-(4-hydroxyphenyl) arachidonylamide (AM 404) (Figure 8). However, this compound is not particularly selective as it also inhibits FAAH, binds to CB_1 receptors and activates TRPV1 receptors at concentrations at or below those at which it has been reported to inhibit anandamide uptake (Table 4). Other inhibitors of anandamide uptake are now available (Figure 8),[5,23,100] and the potencies exhibited by some of these not only as uptake inhibitors but also (when known) as inhibitors of FAAH, as CB_1 and CB_2 receptor ligands and as TRPV1 receptor agonists are shown in Table 4. Importantly, it is currently unclear whether any of the

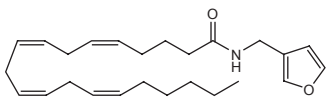
Figure 8 | Structures of AM 404, VDM 11, UCM 707, OMDM-1, OMDM-2, LY 2183240 and 5´-dimethylheptyl-cannabidiol ((–)-5´-DMH-CBD), all of which behave as inhibitors of anandamide cellular uptake



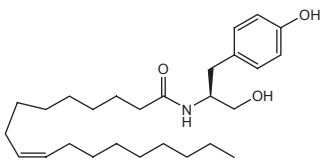
AM 404



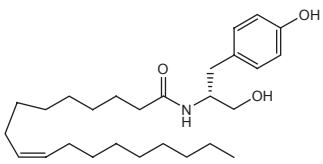
VDM 11



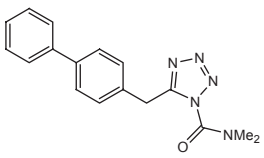
UCM 707



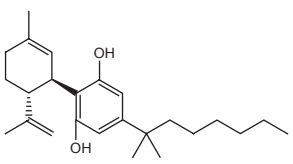
OMDM-1 (*S*)



OMDM-2 (*R*)



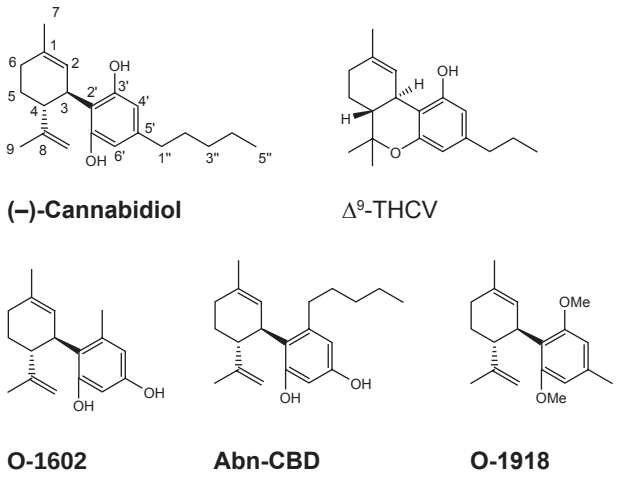
LY 2183240



(–)-5´-DMH-CBD

(Bold Text Denotes Compounds Available From Tocris)

Figure 9 | Structures of (–)-cannabidiol (CBD), (–)-Δ^9-tetrahydrocannabivarin (Δ^9-THCV), O-1602, abnormal-cannabidiol (abn-CBD) and O-1918



(Bold Text Denotes Compounds Available From Tocris)

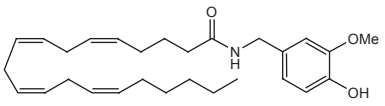
compounds so far found to inhibit the cellular uptake of anandamide do so by targeting an anandamide transport protein or by attenuating FAAH-mediated metabolism of anandamide to cause an intracellular accumulation of this fatty acid amide that is sufficient to oppose its entry into the cell by diffusion.[25-27]

Some Other Notable Ligands

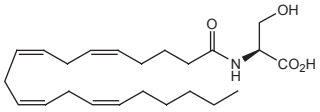
In addition to the CB_1 and CB_2 receptor ligands already discussed there are a number of other compounds that deserve mention either because they can modulate some effects of established CB_1/CB_2 receptor agonists through seemingly novel mechanisms or because they share the apparent ability of such agonists to target certain putative non-CB_1, non-CB_2 receptors. These compounds are:

- the plant cannabinoid, Δ^9-tetrahydrocannabivarin (Figure 9), which displaces [^{3}H]CP 55,940 from CB_1 and CB_2 receptors at concentrations in the low nanomolar range (K_i = 75.4 and 62.8 nM respectively) and behaves as a CB_1 and CB_2 receptor competitive antagonist, exhibiting greater potency against CP 55,940 in the mouse isolated vas deferens and in membranes obtained from human CB_2-transfected cells (apparent K_B = 10 nM) than in mouse brain membranes (apparent K_B = 93 nM);[121]
- the non-psychoactive plant cannabinoid, (–)-cannabidiol (CBD; Figure 9), which lacks significant affinity for CB_1 or CB_2 receptors, has therapeutic potential (e.g. as an anti-inflammatory agent), possesses anti-oxidant/neuroprotective properties and, at sub-micromolar concentrations, activates blocks or inhibits a number of established or putative pharmacological targets that include an adenosine transporter[122] and also delayed rectifier K^+ and L-type Ca^{2+} channels, CYP enzymes, acyltransferase, a neuronal non-CB_1 site of action in the mouse vas deferens, and the putative non-CB_1, non-CB_2, non-TRPV1 "abnormal-CBD receptor" that has been postulated to be present in tissues such as mesenteric arteries and in microglial cells;[2,88,123]
- a set of CBD analogues (Figure 9) which lack significant affinity for the CB_1 receptor and behave as agonists (abnormal-CBD and O-1602) or antagonists (O-1918) for the putative abnormal-CBD receptor;[2,88,96]
- the endogenous compound, *N*-arachidonoyl-L-serine (Figure 10), which may be an endogenous agonist for the abnormal-CBD receptor as it appears to activate this putative receptor when added exogenously (EC_{50} = 550 or ca 1200 nM), and which binds only weakly to CB_1 receptors (K_i > 10 μM) and does not bind to CB_2 or TRPV1 receptors at concentrations of up to 30 μM;[124]
- the anandamide/capsaicin structural hybrid, *N*-vanillyl arachidonyl amide (arvanil; Figure 10), which binds to TRPV1 receptors at concentrations in the low nanomolar range, binds to CB_1 receptors and inhibits the cellular uptake of anandamide at concentrations in the low micromolar range and may also have one or more as yet unidentified non-CB_1, non-TRPV1 sites of action;[125,126]
- the synthetic indole derivatives, Org 27569, Org 29647 and Org 27759 (Figure 11), experiments with which have revealed the presence of an allosteric site on the cannabinoid CB_1 receptor that constitutes a new target through which CB_1 receptor activation by endogenously released endocannabinoids could be modulated, for example to combat inflammatory pain, obesity or nicotine dependence.[127]

Figure 10 | Structures of arvanil and *N*-arachidonoyl-L-serine



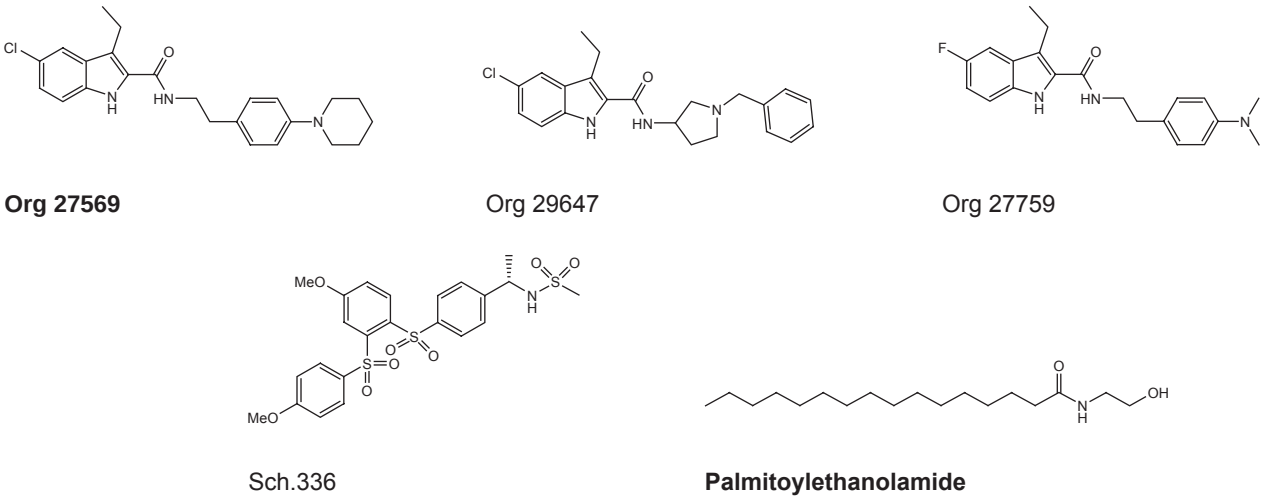
Arvanil



N-Arachidonoyl-L-serine

(Bold Text Denotes Compounds Available From Tocris)

Figure 11 | Structures of the CB_1 allosteric ligands, Org 27569, Org 29647 and Org 27759, the CB_2-selective antagonist/inverse agonist, Sch.336, and the endogenous fatty acid amide, palmitoylethanolamide



Org 27569 | Org 29647 | Org 27759

Sch.336 | **Palmitoylethanolamide**

(Bold Text Denotes Compounds Available From Tocris)

Org 27569, Org 29647 and Org 27759 have been found to behave as CB_1 allosteric enhancers in binding assays but as CB_1 allosteric inhibitors in functional *in vitro* bioassays,[127] limiting their use as experimental tools and creating a need for additional CB_1 allosteric modulators.

Three other noteworthy ligands are Sch.336 (Figure 11), HU 211 which is the (+)-enantiomer of the potent CB_1/CB_2 receptor agonist HU 210 (Figure 1), and the endogenous ligand, palmitoylethanolamide (Figure 11). Sch.336 is a CB_2-selective antagonist/inverse agonist that exhibits even greater efficacy and potency as a CB_2 receptor inverse agonist than SR144528.[128] This high inverse efficacy of Sch.336 may account for its ability to inhibit leukocyte migration/trafficking, an effect that could come to be exploited in the clinic for the management of inflammatory disorders.[128] HU 211 lacks significant affinity for CB_1 or CB_2 receptors but possesses neuroprotective properties that may arise from its ability to behave as a non-competitive antagonist at the *N*-methyl-D-aspartate (NMDA) receptor, to decrease tumour necrosis factor-α production, to inhibit depolarisation-evoked calcium fluxes and/or to scavenge oxygen-derived free radicals.[2,129] Palmitoylethanolamide is of interest because it lacks significant affinity for CB_1 or CB_2 receptors and yet is susceptible to antagonism by SR144528, a finding which has prompted the hypothesis that this fatty acid amide may be the endogenous agonist for a "CB_2-like" receptor.[2,88] There is also evidence, first that palmitoylethanolamide is a PPAR-α receptor agonist,[130] second that it is metabolised both by FAAH and PAA,[5] and third that it may potentiate anandamide through the so-called "entourage effect" (see section on the endocannabinoid system).

Future Directions

This review has focused particularly on ligands that are most widely used as experimental tools either to target cannabinoid CB_1 and/or CB_2 receptors directly or to modulate tissue levels of endocannabinoids following their endogenous release. It is likely that future research in the area of cannabinoid pharmacology will be directed at:

- exploring the structure-activity relationships of ligands that target the CB_1 allosteric site or that behave as neutral CB_1 and/or CB_2 receptor antagonists;
- assessing the therapeutic potential of CB_1 and/or CB_2 receptor allosteric modulators and neutral antagonists;
- gathering more conclusive evidence for or against the presence of an endocannabinoid transporter in mammalian cells;
- establishing the pharmacological profiles of new and existing modulators of endocannabinoid biosynthesis, metabolism or cellular uptake;
- finding out why CB_2 receptors seem to be expressed by central neurons;
- validating and characterising non-CB_1, non-CB_2 targets for particular cannabinoids, and developing compounds that can selectively activate or block such targets with reasonable potency;
- following up early indications that cannabinoid receptors may exist as homodimers or form heterodimers or oligomers with one or more classes of non-cannabinoid receptor;[2]
- obtaining a more complete understanding of the part played by the endocannabinoid system in ameliorating the symptoms and/or the underlying pathology of certain disorders.

Abbreviations

$AACOCF_3$ (ATFMK)	arachidonyl trifluoromethyl ketone
Abn-CBD	abnormal-cannabidiol
ACEA	arachidonyl-2´-chloroethylamide
ACPA	arachidonylcyclopropylamide
CBD	cannabidiol
DAGL	diacylglycerol lipase
DMH-CBD	dimethylheptyl-cannabidiol
FAAH	fatty acid amide hydrolase
MAFP	methyl arachidonyl fluorophosphonate
MAGL	monoacylglycerol lipase
NADA	*N*-arachidonoyl dopamine
NAPE-PLD	*N*-acyl phosphatidylethanolamine-selective phospholipase D
OLDA	*N*-oleoyl dopamine
PAA	palmitoylethanolamide-preferring acid amidase
PIA	palmitoylisopropylamide
PMSF	phenylmethylsulphonyl fluoride
THC	tetrahydrocannabinol

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Cannabinoid Receptor Ligands Available from Tocris

Agonists

1297 Abn-CBD
Agonist for putative abnormal-CBD receptor

1319 ACEA
Potent, highly selective CB_1 agonist

1318 ACPA
Potent, selective CB_1 agonist

1781 ACPA (in Tocrisolve 100)
Potent, selective CB_1 agonist (in water-soluble emulsion)

1339 Anandamide
Endogenous CB receptor agonist

1017 Anandamide (in Tocrisolve 100)
Endogenous CB receptor agonist (in water-soluble emulsion)

1298 2-Arachidonylglycerol
Endogenous cannabinoid agonist

1354 Arvanil
Potent CB_1 and TRPV1 agonist/anandamide transport inhibitor

2500 Bay 59-3074
CB_1/CB_2 receptor partial agonist

2178 Cannabinoid CB_1 Receptor Tocriset
Selection of 3 CB_1 receptor ligands (Cat. Nos. 1319, 1121 and 1117)

2179 Cannabinoid Receptor Agonist Tocriset
Selection of 5 cannabinoid receptor agonists (Cat. Nos. 1339, 1298, 0949, 0966 and 1038)

0949 CP 55,940
Potent CB_1 and CB_2 agonist

1485 DEA
Endogenous CB_1 agonist

2180 Endocannabinoid Tocriset
Selection of 5 endogenous cannabinoids (Cat. Nos. 1339, 1298, 1568, 1411 and 1569)

2374 GW 405833
Selective, high affinity CB_2 receptor partial agonist

0966 HU 210
Highly potent cannabinoid agonist

1341 JWH 015
Selective CB_2 agonist

1343 JWH 133
Potent, selective CB_2 agonist

1783 JWH 133 (in Tocrisolve 100)
Potent, selective CB_2 agonist (in water-soluble emulsion)

2433 L-759,633
High affinity, selective CB_2 agonist

2434 L-759,656
Highly selective CB_2 agonist

2139 Leelamine
CB_1 agonist

1121 (*R*)-(+)-Methanandamide
Potent and selective CB_1 agonist

1782 (*R*)-(+)-Methanandamide (in Tocrisolve 100)
Potent and selective CB_1 agonist (in water-soluble emulsion)

1568 NADA
Endogenous CB_1 agonist. Also vanilloid agonist and inhibitor of FAAH and AMT

1411 Noladin ether
Endogenous agonist for CB_1

0878 Oleamide
CB_1 receptor agonist

0879 Palmitoylethanolamide
Agonist for putative CB_2-like receptor. FAAH and PAA substrate

1569 Virodhamine
Endogenous CB_2 agonist. Also CB_1 partial agonist/antagonist

1038 WIN 55,212-2
Highly potent cannabinoid agonist

Antagonists

1117 AM 251
Potent, selective CB_1 antagonist/inverse agonist

1115 AM 281
Potent, selective CB_1 antagonist/inverse agonist

1120 AM 630
CB_2 selective antagonist/inverse agonist

2178 Cannabinoid CB_1 Receptor Tocriset
Selection of 3 CB_1 receptor ligands (Cat. Nos. 1319, 1121 and 1117)

2180 Endocannabinoid Tocriset
Selection of 5 endogenous cannabinoids (Cat. Nos. 1339, 1298, 1568, 1411 and 1569)

2479 JTE 907
CB_2-selective antagonist/inverse agonist

2387 LY 320135
Selective CB_1 receptor antagonist/inverse agonist

2288 O-1918
Silent antagonist for putative abnormal-CBD receptor

1655 O-2050
CB_1 silent antagonist

1569 Virodhamine
Endogenous CB_1 partial agonist/antagonist. Also CB_2 agonist

2327 WIN 55,212-3
CB_2 antagonist/CB_1 partial inverse agonist. Enantiomer of Cat. No. 1038

Other

1462 $AACOCF_3$
Inhibits anandamide hydrolysis

1116 AM 404
Anandamide transport inhibitor

1685 AM 404 (in Tocrisolve 100)
Anandamide transport inhibitor (in water-soluble emulsion)

1814 *N*-ArachidonylGABA
Inhibits pain *in vivo*

1445 *N*-Arachidonylglycine
Novel endocannabinoid. Suppresses pain *in vivo*

1383 BML-190
Potent, selective CB_2 ligand

1570 (-)-Cannabidiol
Natural cannabinoid; weak CB_1 antagonist and AMT inhibitor. Anticonvulsive *in vivo*

2231 Anti-CB_2
Antibody recognising CB_2 receptors

2388 Anti-CB_2 blocking peptide
Blocking peptide for Cat. No. 2231

1481 (-)-5´-DMH-CBD
Metabolically stable anandamide transport inhibitor

2452 LY 2183240
Novel, potent anandamide uptake inhibitor. Inhibits FAAH

1421 MAFP
Potent, irreversible anandamide amidase inhibitor

1446 O-2093
Inverse agonist at a non-CB_1, non-TRPV1 site. Active *in vivo*

1484 Oleylethanolamide
Anandamide analogue, anorexic agent

1797 OMDM-2
Potent inhibitor of anandamide uptake

0879 Palmitoylethanolamide
Agonist for putative CB_2-like receptor. FAAH and PAA substrate

2206 2-Palmitoylglycerol
Endogenous lipid; enhances activity of 2-AG

1815 Palmitoylisopropylamide
Inhibitor of FAAH

1716 Tocriscreen Cannabinoids
Collection of cannabinoid receptor and related compounds

1684 Tocrisolve 100
Water-soluble emulsion; negative control for Cat. Nos. 1017, 1685, 1686, 1781, 1782 and 1783

1966 UCM 707
Potent anandamide transport inhibitor

1392 VDM 11
Potent, selective anandamide transport inhibitor

1686 VDM 11 (in Tocrisolve 100)
Potent, selective anandamide transport inhibitor (in water-soluble emulsion)

Please note, the product names used in this review are assigned according to the nomenclature employed by Tocris in its catalogue and website.

Tocris Reviews No. 27

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