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(57) Abrégé/Abstract:
The invention relates to the use of one or more cannabinolds in the manufacture of medicaments for use in the treatment of diseases and conditions benefiting from neutral antagonism of the CB, cannabinoid receptor. Preferably the cannabinoid is tetrahydrocannabivarin (THCV). Preferably the diseases and conditions to be treated are taken from the group: obesity, schizophrenia, epilepsy, cognitive disorders such as Alzheimer's, bone disorders, bulimia, obesity associated with type II diabetes (non-insulin dependant diabetes) and in the treatment of drug, alcohol and nicotine abuse or dependency.

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NEW USE FOR CANNABINOID

FIELD OF THE INVENTION

The present invention relates to the use of one or more cannabinoids in the manufacture of medicaments for use in the treatment of diseases and conditions benefiting from neutral antagonism of the CB_1 cannabinoid receptor. Preferably the cannabinoid is tetrahydrocannabivarin (THCV). Preferably the diseases and conditions to be treated are taken from the group: obesity, schizophrenia, epilepsy, cognitive disorders such as Alzheimer's, bone disorders, bulimia, obesity associated with type II diabetes (non-insulin dependant diabetes) and in the treatment of drug, alcohol and nicotine abuse or dependency.

BACKGROUND DESCRIPTION

The action of many known cannabinoids can be attributed to their interaction with cannabinoid receptors. The discovery that cannabinoid receptors are present in mammalian systems has led to further research. For example, there has been identified a class of G-Protein coupled receptors which are present mainly in the central nervous system, these have been named CB_1 receptors.

Another type of G-Protein coupled receptor is the CB_2 receptors which are found substantially in the immune system.

Cannabinoids are generally cannabinoid receptor agonists, which mean that they dock with a cannabinoid receptor and activate it.

Well known cannabinoid receptor agonists include the classical plant derived cannabinoid delta-9-tetrahydrocannabinol (THC), the non-classical cannabinoid receptor agonist *R*-(+)-WIN55212 and the eicosanoid or animal derived cannabinoid receptor agonist anandamide. All of these compounds have been shown to bind to the CB_1 receptor.

Agonism at a receptor will often lead to an active response by the cell. Many disease states result from the overactive or overabundant effects of agonists at their receptors.

Research has led to the discovery of compounds that prevent the activation of cannabinoid receptors and as such are known as cannabinoid receptor antagonists. A competitive antagonist of cannabinoid receptor is one that will bind to the receptor but not cause a response in the cell. An inverse agonist acts upon a receptor to produce an opposite effect to the response that the agonist would produce.

The compound SR141716A (described in EP0576357) has been shown to antagonise the CB_1 cannabinoid receptor. There is evidence however that SR141716A is an inverse agonist rather than a silent or neutral antagonist (Pertwee, R.G., 2003).

Maruani and Soubrie in US 6,444,474 and EP0969835 have described the use of an inverse CB_1 receptor agonist such as SR141716A in the regulation of appetency disorders.

In many CB_1-containing assay systems, SR141716A by itself produces effects that are opposite in direction from those produced by CB_1 agonists such as THC. Therefore leading to the inference that it is an inverse agonist of the CB_1 receptor. Whilst in some instances this may reflect antagonism of an endogenous CB_1 agonist (a CB_1 agonist produced by the assay system itself) in other instances it is thought to arise because CB_1 receptors are constitutively active.

It is generally considered that constitutively active receptors trigger effects even in the absence of any administered or endogenously produced agonist. Agonists enhance this activity whilst inverse agonists oppose it.

In contrast, neutral antagonists leave constitutive activity unchanged. Neutral antagonists are favoured over inverse agonists as they only block the ability of the receptor to interact with an endogenously produced CB_1 agonist such as anandamide or one that has been administered.

There is evidence that the endogenous CB_1 agonist, anandamide, may be released in the brain to mediate processes such as feeding and appetite (Di Marzo *et al.*, 2001). This raises the possibility that an antagonist of this receptor could be effective in the clinic as an appetite suppressant.

The compound SR141716A engages with the CB_1 cannabinoid receptors so that they can't be activated. It is possible that blocking the CB_1 receptor system may adversely affect CB_1 mediated aspects such as mood, sleep and pain relief.

As endocannabinoids have neuroprotectant and anti-oxidant properties it is also possible that users of SR141716A may be at an increased risk of cancer and stroke.

Neutral CB_1 receptor antagonists are likely to have a less complex pharmacology than those of an inverse agonist. Thus, when administered by itself such an antagonist will only have effects in regions of the cannabinoid system in which there is ongoing release of endogenous cannabinoids onto CB_1 receptors but will not affect the activity of the endogenous cannabinoid system that arises from the presence in some parts of this system of constitutively active CB_1 receptors.

CB_1 receptor antagonists, particularly neutral CB_1 receptor antagonists, are as such, likely to be useful in the treatment of diseases and conditions that are caused by an interaction with the CB_1 receptor. Such diseases and conditions include, for example, obesity, schizophrenia, epilepsy or cognitive disorders such as Alzheimers, bone disorders, bulimia, obesity associated with type II diabetes (non-insulin dependant diabetes) and in the treatment of drug, alcohol or nicotine abuse or dependency (Pertwee, R.G., 2000).

The use of a neutral antagonist in place of an inverse antagonist would be particularly beneficial, as it is likely that fewer side effects would occur since it would not augment the consequences of CB_1 receptor constitutive activity.

At the present time there are few identified neutral CB_1 receptor antagonists. An analogue of the psychotropic cannabinoid THC has been produced which behaves as a neutral CB_1 antagonist *in vitro* (Martin, B.R. *et al.* 2002). The compound, O-2050 is a sulphonamide analogue of delta-8-tetrahydrocannabinol, and has acetylene incorporated into its side chain.

This analogue behaves as a neutral CB_1 receptor antagonist in the mouse vas deferens. However, O-2050 does not behave as a CB_1 receptor antagonist in mice *in vivo* and, like established CB_1 receptor agonists, it depresses mouse spontaneous activity. Moreover, analogues of O-2050 with R = ethyl or R = butyl behave as typical CB_1 receptor agonists in mice *in vivo*.

Surprisingly the applicants have shown that the cannabinoid tetrahydrocannabinovarin (THCV) is a neutral antagonist of the CB_1 and CB_2 cannabinoid receptors.

The cannabinoid THCV is a classical plant cannabinoid, which is structurally related to THC, in that instead of the 3-pentyl side chain of THC, the THCV molecule has a 3-propyl side chain. The structures of the two cannabinoids are shown in Figure 1.

The finding that THCV appears to act as a neutral antagonist of CB_1 receptors was particularly surprising as THC is known to be a CB_1 agonist and it should therefore follow that a structurally related compound such as THCV would also be an agonist rather than an antagonist.

SUMMARY OF THE INVENTION

According to the first aspect of the present invention there is provided the use of tetrahydrocannabivarin (THCV) in the manufacture of a medicament for use in the treatment of diseases or conditions benefiting from neutral antagonism of the CB_1 receptor.

Preferably the THCV is used in the manufacture of a medicament for the treatment of obesity, schizophrenia, epilepsy or cognitive disorders such as Alzheimer's, bone disorders, bulimia, obesity associated with type II diabetes (non-insulin dependant diabetes) and in the treatment of drug, alcohol or nicotine abuse or dependency.

More preferably the THCV is used in the manufacture of a medicament for use as an appetite suppressant.

A neutral antagonist is likely to have fewer side effects than those of an inverse agonist. This is because it is expected to oppose drug-induced activation of CB_1 receptors but not attenuate effects produced by constitutively active CB_1 receptors.

In contrast, an inverse agonist will attenuate effects produced not only by drug-induced activation of CB_1 receptors but also by constitutively active CB_1 receptors and so would be expected to give rise to a larger number of side effects than a neutral antagonist.

Therefore, in a preferred embodiment of the invention THCV may be used in the substantial absence of any substance or compound which acts as an inverse agonist of CB_1 receptors.

References to THCV, particularly with regard to therapeutic use, will be understood to also encompass pharmaceutically acceptable salts of such compounds. The term "pharmaceutically acceptable salts" refers to salts or esters prepared from pharmaceutically acceptable non-toxic bases or acids, including inorganic bases or acids and organic bases or acids, as would be well known to persons skilled in the art. Many suitable inorganic and organic bases are known in the art.

The scope of the invention also extends to derivatives of THCV that retain the desired activity of neutral CB_1 receptor antagonism. Derivatives that retain substantially the same activity as the starting material, or more preferably exhibit improved activity, may be produced according to standard principles of medicinal chemistry, which are well known in the art. Such derivatives may exhibit a lesser degree of activity than the starting material, so long as they retain sufficient activity to be therapeutically effective. Derivatives may exhibit improvements in other properties that are desirable in pharmaceutically active agents such as, for example, improved solubility, reduced toxicity, enhanced uptake.

Preferably the THCV is an extract from at least one cannabis plant.

More preferably the THCV extract from at least one cannabis plant is a botanical drug substance.

In one embodiment the THCV extract from at least one cannabis plant is produced by extraction with supercritical or subcritical CO_2.

Alternatively the THCV extract from at least one cannabis plant is produced by contacting plant material with a heated gas at a temperature which is greater than 100°C, sufficient to volatilise one or more of the cannabinoids in the plant material to form a vapour, and condensing the vapour to form an extract.

Preferably the THCV extract from at least one cannabis plant comprises all the naturally occurring cannabinoids in the plant.

Alternatively the THCV is in a substantially pure or isolated form.

A "substantially pure" preparation of cannabinoid is defined as a preparation having a chromatographic purity (of the desired cannabinoid) of greater than 90%, more preferably greater than 95%, more preferably greater than 96%, more preferably greater than 97%, more preferably greater than 98%, more preferably greater than 99% and most preferably greater than 99.5%, as determined by area normalisation of an HPLC profile.

Preferably the substantially pure THCV used in the invention is substantially free of any other naturally occurring or synthetic cannabinoids, including cannabinoids which occur naturally in cannabis plants. In this context "substantially free" can be taken to mean that no cannabinoids other than THCV are detectable by HPLC.

In another aspect of the present invention the THCV is in a synthetic form.

Preferably the THCV is formulated as a pharmaceutical composition further comprising one or more pharmaceutically acceptable carriers, excipients or diluents.

The invention also encompasses pharmaceutical compositions comprising THCV, or pharmaceutically acceptable salts or derivatives thereof, formulated into pharmaceutical dosage forms, together with suitable pharmaceutically acceptable carriers, such as diluents, fillers, salts, buffers, stabilizers, solubilizers, etc. The dosage form may contain other pharmaceutically acceptable excipients for modifying conditions such as pH, osmolarity, taste, viscosity, sterility, lipophilicity, solubility etc. The choice of diluents, carriers or excipients will depend on the desired dosage form, which may in turn be dependent on the intended route of administration to a patient.

Suitable dosage forms include, but are not limited to, solid dosage forms, for example tablets, capsules, powders, dispersible granules, cachets and suppositories, including sustained release and delayed release formulations. Powders and tablets will generally comprise from about 5% to about 70% active ingredient. Suitable solid carriers and excipients are generally known in the art and include, e.g.

magnesium carbonate, magnesium stearate, talc, sugar, lactose, etc. Tablets, powders, cachets and capsules are all suitable dosage forms for oral administration.

Liquid dosage forms include solutions, suspensions and emulsions. Liquid form preparations may be administered by intravenous, intracerebral, intraperitoneal, parenteral or intramuscular injection or infusion. Sterile injectable formulations may comprise a sterile solution or suspension of the active agent in a non-toxic, pharmaceutically acceptable diluent or solvent. Liquid dosage forms also include solutions or sprays for intranasal, buccal or sublingual administration. Aerosol preparations suitable for inhalation may include solutions and solids in powder form, which may be combined with a pharmaceutically acceptable carrier, such as an inert compressed gas.

Also encompassed are dosage forms for transdermal administration, including creams, lotions, aerosols and/or emulsions. These dosage forms may be included in transdermal patches of the matrix or reservoir type, which are generally known in the art.

Pharmaceutical preparations may be conveniently prepared in unit dosage form, according to standard procedures of pharmaceutical formulation. The quantity of active compound per unit dose may be varied according to the nature of the active compound and the intended dosage regime. Generally this will be within the range of from 0.1mg to 1000mg.

According to a second aspect of the present invention there is provided a method for the treatment of a disease or condition benefiting from neutral antagonism of the CB_1 cannabinoid receptor by THCV, which comprises administering to a subject in need thereof a therapeutically effective amount of THCV.

The disease or condition to be treated is selected from the group consisting of obesity, schizophrenia, epilepsy or cognitive disorders such as Alzheimer's, bone disorders, bulimia, obesity associated with type II diabetes (non-insulin dependant diabetes) or drug, alcohol or nicotine abuse or dependency.

According to a third aspect of the present invention there is provided a method for cosmetically beneficial weight loss comprising suppression of appetite in a subject by administering to the subject an effective amount of THCV.

In certain circumstances the appetite suppressant may be utilised in order to achieve a cosmetically beneficial loss of weight in a human subject, without necessarily producing medical or therapeutic benefit to that subject. In this context administration of the appetite suppressant may not be construed as a medical or therapeutic treatment of the subject.

According to a fourth aspect of the present invention there is provided the use of a neutral cannabinoid receptor antagonist in the manufacture of a medicament for use in the treatment of diseases or conditions benefiting from neutral antagonism of one or more types of cannabinoid receptor.

Preferably the neutral cannabinoid receptor antagonist is used in the manufacture of a medicament for use in the treatment of diseases or conditions benefiting from neutral antagonism of the CB_1 cannabinoid receptor, and wherein the dissociation constant of the cannabinoid receptor antagonist at the CB_1 receptor is approximately 75nM.

Preferably the neutral cannabinoid receptor antagonist is used in the manufacture of a medicament for use in the treatment of diseases or conditions benefiting from neutral antagonism of the CB_2 cannabinoid receptor, and wherein the dissociation constant of the cannabinoid receptor antagonist at the CB_2 receptor is approximately 62nM.

The term "approximately" refers to within ±10% of the quoted value.

Certain aspects of this invention are further described, by way of example only, with reference to the accompanying drawings in which:

Figure 1 shows the 2-dimensional structure of the cannabinoid tetrahydrocannabivarin (THCV) and tetrahydrocannabinol (THC).

SPECIFIC DESCRIPTION

Example 1:

Investigation into the effects THCV has upon the cannabinoid CB_1 or CB_2 receptors.

Experiments were performed with membranes prepared from healthy brain tissue, which is densely populated with CB_1 but not CB_2 receptors (reviewed in Howlett *et al.* 2002).

Further experiments were undertaken with Chinese hamster ovary (CHO) cells transfected with hCB_2 receptors. These membranes were used to investigate the ability of THCV to displace $[^3H]$CP55940 CB_2 binding sites

These experiments were used to determine whether THCV behaves as a CB_1 or CB_2 receptor agonist or antagonist.

Experiments were also carried out with the mouse isolated vas deferens, a tissue in which cannabinoid receptor agonists such as *R*-(+)-WIN55212, CP55940, THC and 2-arachidonoyl ethanolamide (anandamide) can inhibit electrically-evoked contractions (Devane *et al.*, 1992; Pertwee *et al.*, 1995).

Cannabinoid receptor agonists are thought to inhibit the electrically evoked contractions by acting on prejunctional neuronal cannabinoid CB_1 receptors to inhibit release of the contractile neurotransmitters, ATP, (acting on postjunctional P2X purinoceptors), and noradrenaline, (acting on postjunctional α_1-adrenoceptors), (Trendelenberg *et al.*, 2000).

Experiments were also performed with (-)-7-hydroxy-cannabidiol-dimethylheptyl, a synthetic analogue of the plant cannabinoid, (-)-cannabidiol, that inhibits electrically-evoked contractions of the mouse vas deferens through a mechanism that appears to operate prejunctionally and to be at least partly CB_1 receptor-independent.

Methods:

Radioligand displacement assay

The assays were carried out with [^{3}H]CP55940, 1 mg ml^{-1} bovine serum albumin (BSA) and 50mM Tris buffer, total assay volume 500µl, using the filtration procedure described previously by Ross *et al.* (1999b).

Binding was initiated by the addition of either the brain membranes (33µg protein per tube) or the transfected hCB_2 cells (25µg protein per tube).

All assays were performed at 37°C for 60 min before termination by addition of ice-cold wash buffer (50mM Tris buffer, 1 mg ml^{-1} bovine serum albumin, pH 7.4) and vacuum filtration using a 24-well sampling manifold and GF/B filters that had been soaked in wash buffer at 4°C for at least 24 h.

Each reaction tube was washed six times with a 1.2 ml aliquot of wash buffer. The filters were oven-dried for 60 min and then placed in 5ml of scintillation fluid. Radioactivity was quantified by liquid scintillation spectrometry.

Specific binding was defined as the difference between the binding that occurred in the presence and absence of 1µM unlabelled CP55940. THCV was stored as a stock solution of 10mM in DMSO, the vehicle concentration in all assay tubes being 0.1% DMSO.

The binding parameters for [^{3}H]CP55940, were 2336 fmol mg^{-1} protein (B_{max}) and 2.31 nM (K_d) in mouse brain membranes (Thomas *et al.*, 2004), and 72570 fmol/mg protein (B_{max}) and 1.043 nM (K_d) in hCB_2 transfected cells.

[^{35}S]GTPγS binding assay

The method for measuring agonist-stimulated [^{35}S]GTPγS binding to cannabinoid CB_1 receptors was adapted from the methods of Kurkinen *et al.* (1997) and Breivogel *et al* (2001).

The conditions used for measuring agonist-stimulated [^{35}S]GTPγS binding to transfected cannabinoid CB_2 receptors were adapted from those used by MacLennan *et al.* (1998) and Griffin *et al.* (1999).

The assays were carried out with GTPγS binding buffer (50mM Tris-HCl; 50mM Tris-Base; 5mM $MgCl_2$; 1mM EDTA; 100mM NaCl; 1mM DTT; 0.1% BSA) in the presence of [^{35}S]GTPγS and GDP, in a final volume of 500µl. Binding was initiated by the addition of [^{35}S]GTPγS to the tubes. Nonspecific binding was measured in the presence of 30µM GTPγS.

The drugs were incubated in the assay for 60 min at 30°C. The reaction was terminated by a rapid vacuum filtration method using Tris buffer (50mM Tris-HCl; 50mM Tris-Base; 0.1% BSA), and the radioactivity was quantified by liquid scintillation spectrometry.

The concentrations of [^{35}S]GTPγS and GDP present in the assay varied depending on whether the assay was conducted with mouse brain or transfected cell membranes. When the assay was conducted with mouse brain membranes, 0.1nM [^{35}S]GTPγS and 30µM GDP were present, whereas the corresponding concentrations present when the assay was conducted with transfected cell membranes were 1nM and 320µM respectively.

Additionally, mouse brain membranes were preincubated for 30 minutes at 30°C with 0.5 U ml^{-1} adenosine deaminase to remove endogenous adenosine. Agonists and antagonists were stored as a stock solution of 1 or 10mM in DMSO, the vehicle concentration in all assay tubes being 0.11% DMSO.

Vas deferens experiments

Vasa deferentia were obtained from albino MF1 mice weighing 31 to 59 g. The tissues were mounted vertically in 4ml organ baths. They were then subjected to electrical stimulation of progressively greater intensity followed by an equilibration procedure in which they were exposed to alternate periods of stimulation (2 min) and rest (10 min) until contractions with consistent amplitudes were obtained (Thomas *et al.*, 2004). These contractions were monophasic and isometric and were evoked by 0.5 s trains of pulses of 110% maximal voltage (train frequency 0.1Hz; pulse frequency 5Hz; pulse duration 0.5ms).

Except in experiments with phenylephrine, all drug additions were made to the organ baths after the equilibration period and there was no washout between these additions. In most experiments there was an initial application of a potential antagonist or its vehicle. This was followed 28 min later by a 2 min period of electrical stimulation at the end of which the lowest of a series of concentrations of the twitch inhibitors, *R*-(+)-WIN55212, CP55940, THC, anandamide, (-)-7-hydroxy-cannabidiol-dimethylheptyl or clonidine, was applied.

After a period of rest, the tissues were electrically stimulated for 2 min and then subjected to a further addition of twitch inhibitor.

This cycle of drug addition, rest and 2 min stimulation was repeated so as to construct cumulative concentration-response curves. Only one concentration-response curve was constructed per tissue. Rest periods were 3 min for clonidine, 13 min for *R*-(+)-WIN55212, CP55940 and anandamide, 28 min for THC and THCV, and 58 min for (-)-7-hydroxy-cannabidiol-dimethylheptyl.

Experiments were also performed with capsaicin. This drug was added at intervals of 3 min and the tissues were not rested from electrical stimulation between these additions.

In some experiments, cumulative concentration-response curves for THCV were constructed without prior addition of any other compound, again using a cycle of drug addition, 28 min rest and 2 min stimulation.

In experiments with β,γ-methylene-ATP, no electrical stimuli were applied after the equilibration procedure. Log concentration-response curves of β,γ-methylene-ATP were constructed cumulatively without washout. THCV, WIN or drug vehicle were added 30 min before the first addition of β,γ-methylene-ATP, each subsequent addition of which was made immediately after the effect of the previous dose had reached a plateau (dose cycles of 1 to 2 min).

Only one addition of phenylephrine was made to each tissue and this was carried out 30 min after the addition of THCV, WIN or drug vehicle.

Analysis of data

Values are expressed as means and variability as s.e.mean or as 95% confidence limits. The concentration of THCV that produced a 50% displacement of radioligand from specific binding sites (IC_{50} value) was calculated using GraphPad Prism 4. Its dissociation constant (K_i value) was calculated using the equation of Cheng & Prusoff (1973).

Net agonist-stimulated [^{35}S]GTPγS binding values were calculated by subtracting basal binding values (obtained in the absence of agonist) from agonist-stimulated values (obtained in the presence of agonist) as detailed elsewhere (Ross et al., 1999a).

Inhibition of the electrically-evoked twitch response of the vas deferens has been expressed in percentage terms and this has been calculated by comparing the amplitude of the twitch response after each addition of a twitch inhibitor with its amplitude immediately before the first addition of the inhibitor. Contractile responses to phenylephrine and β,γ-methylene-ATP have been expressed as increases in tension (g).

Values for EC_{50}, for maximal effect (E_{max}) and for the s.e.mean or 95% confidence limits of these values have been calculated by nonlinear regression analysis using the equation for a sigmoid concentration-response curve (GraphPad Prism).

The apparent dissociation constant (K_B) values for antagonism of agonists by THCV in the vas deferens or [^{35}S]GTPγS binding assay have been calculated by Schild analysis from the concentration ratio, defined as the concentration of an agonist that elicits a response of a particular size in the presence of a competitive reversible antagonist at a concentration, B, divided by the concentration of the same agonist that produces an identical response in the absence of the antagonist.

The methods used to determine concentration ratio and apparent K_B values and to establish whether log concentration-response plots deviated significantly from parallelism are detailed elsewhere (Pertwee *et al.*, 2002). Mean values have been compared using Student's two-tailed *t*-test for unpaired data or one-way analysis of variance (ANOVA) followed by Dunnett's test (GraphPad Prism). A *P*-value <0.05 was considered to be significant.

Results:

Radioligand experiments
THCV displaced [^{3}H]CP55940 from specific binding sites in mouse brain and CHO-hCB_2 cell membranes in a manner that fitted significantly better to a one-site than a two-site competition curve ($P<0.05$; GraphPad Prism 4).

Its mean K_i values were 75.4nM and 62.8nM respectively.

THCV also displaced [^{3}H]*R*-(+)-WIN55212 and [^{3}H]SR141716A from specific binding sites in mouse brain membranes, its mean EC_{50} values with 95% confidence limits shown in brackets being 61.3nM (48.6 and 77.3nM; n=4 to 7) and 86.8nM (63.8 and 188.1nM; n=4 to 6) respectively.

The corresponding EC_{50} value of THCV for displacement of [^{3}H]CP55940 is 98.2nM (69.6 and 138.6nM; n=4 to 8).

The ability of CP55940 to enhance [^{35}S]GTPγS binding to mouse brain and CHO-hCB_2 membranes was attenuated by THCV, which at 1μM produced significant dextral shifts in the log concentration response curves of this cannabinoid receptor agonist that did not deviate significantly from parallelism.

The mean apparent K_B values for this antagonism are shown in Table 1, as are mean apparent K_B values of SR141716A for antagonism of CP55940 in mouse brain membranes and of SR144528 for antagonism of CP55940 in the CHO-hCB_2 cell membranes. At 1μM, THCV also produced a significant parallel dextral shift in the log concentration response curve of *R*-(+)-WIN55212 for enhancement of GTPγS binding to mouse brain membranes.

Table 1:

Antagonist	Agonist	Membrane preparation	Mean apparent K_B (nM)	95% confidence limits (nM)	n
THCV (1000 nM)	CP55940	Brain	93.1	66.5, 130.6	6
THCV (1000 nM)	*R*-(+)-WIN55212	Brain	85.4	29.3, 270.5	5
SR141716A (10 nM)	CP55940	Brain	0.09	0.021, 0.41	4
THCV (1000 nM)	CP55940	CHO-hCB_2	10.1	5.0, 20.5	6
SR144528 (100 nM)	CP55940	CHO-hCB_2	0.49	0.26, 0.85	6

Vas deferens experiments

THCV produced a concentration-related inhibition of electrically-evoked contractions of the mouse isolated vas deferens with an EC_{50} of 12.7μM (6.9 and 23.2μM).

It is unlikely that this effect was CB_1-receptor mediated as it was not attenuated by SR141716A at 100nM (n=7; data not shown), a concentration that equals or exceeds concentrations of this CB_1-selective antagonist found previously to antagonize established CB_1 receptor agonists in the same bioassay (Pertwee *et al.*, 1995; Ross *et al.*, 2001).

At 31.6μM, a concentration at which it produced a marked inhibition of electrically-evoked contractions, THCV also attenuated contractile responses of the vas deferens to both the P2 receptor agonist, β,γ-methylene-ATP, and the α_1-adrenoceptor agonist, phenylephrine hydrochloride.

In contrast, at 1μM, a concentration at which it had no detectable inhibitory effect on electrically-evoked contractions, THCV did not induce any significant reduction in the amplitude of contractions induced either by β,γ-methylene-ATP (n=8; data not shown) or by phenylephrine. These findings suggest that THCV inhibited electrically-evoked contractions of the vas deferens, at least in part, by acting postjunctionally to block contractile responses to endogenously released ATP and noradrenaline.

At concentrations well below those at which it inhibited electrically-evoked contractions, THCV opposed *R*-(+)-WIN55212-induced inhibition of the twitch response in a manner that was concentration-related and not accompanied by any significant change in the maximum effect (E_{max}) of *R*-(+)-WIN55212 ($P>0.05$; ANOVA followed by Dunnett's test; n=6-9). The dextral shifts produced by THCV in the log concentration response curve of *R*-(+)-WIN55212 do not deviate significantly from parallelism and yield a Schild plot with a slope that is not significantly different from unity. The mean apparent K_B value of THCV was calculated by the Tallarida method (Pertwee *et al.*, 2002) to be 1.5nM as shown in Table 2. At 1μM, a concentration that markedly attenuated electrically-evoked contractions, *R*-(+)-WIN55212 did not decrease the ability of β,γ-methylene-ATP (n=7 or 10; data not shown) or phenylephrine to induce contractions of the vas deferens.

Table 2:

THCV (nM)	Twitch inhibitor	Mean apparent K_B of THCV (nM)	95% confidence limits (nM)	n
10 - 1000	*R*-(+)-WIN55212	1.5	1.1, 2.3	6-9
100	anandamide	1.2	0.2, 6.2	7
100	methanandamide	4.6	1.5, 11.6	12
100	CP55940	10.3	3.8, 31.7	14
1000	THC	96.7	15.4, 978	10
100	clonidine	>100	-	8
100	capsaicin	>100	-	8
100	7-OH-CBD-DMH	>100	-	8

THCV was shown to antagonize anandamide at 10, 100 and 1000nM, and methanandamide and CP55940 at 100nM. The dextral shifts produced by THCV in the log concentration response curves of these twitch inhibitors did not deviate significantly from parallelism. The mean apparent K_B value for the antagonism of anandamide by 10nM THCV with its 95% confidence limits shown in brackets is 1.4nM (0.36 and 7.50nM). Mean apparent K_B values for antagonism of anandamide, methanandamide and CP55940 by 100 nM THCV are listed in Table 2.

At 100nM, THCV did not reduce the ability of clonidine, capsaicin or (-)-7-hydroxy-cannabidiol-dimethylheptyl to inhibit electrically-evoked contractions, indicating it possesses at least some degree of selectivity as an antagonist of twitch inhibitors in the vas deferens.

Nor did 100nM THCV antagonize the cannabinoid receptor agonist, THC (n=11; data not shown). However, at 1μM, THCV did produce a significant dextral shift in the log concentration response curve of THC that did not deviate significantly from parallelism (see Table 2 for its apparent K_B value against THC).

From this data it is possible that co-administration of a low dose of THCV with THC could ameliorate the high dose effects of THC such as increased heart rate and psychoactivity. The low dose of THCV would act as surmountable competitive antagonist of the CB_1 receptors and therefore block some of the high dose effects of THC. It is well established in the art that a partial agonist's potency and efficacy increase with receptor density and that the potency of a surmountable competitive antagonist is not affected by receptor density. The dose of THCV will be one that is not sufficient to prevent the therapeutic effects of THC but would be sufficient to prevent the high dosing side effects of THC.

Conclusions:

- Δ^9-tetrahydrocannabivarin (THCV) displaced [^{3}H]CP55940 from specific binding sites on brain and CHO-hCB_2 cell membranes (K_i = 75.4 and 62.8nM respectively), indicating that THCV is both a CB_1 and CB_2 receptor antagonist.
- THCV (1μM) also antagonized CP55940-induced enhancement of [^{35}S]GTPγS binding to these membranes (apparent K_B = 93.1 and 10.1nM respectively), indicating that it is a reasonably potent competitive antagonist. The K_B values indicate that THCV is more potent as a CB_2 than a CB_1 receptor antagonist.
- In the mouse vas deferens, the ability of Δ^9-tetrahydrocannabinol (THC) to inhibit electrically-evoked contractions was antagonized by THCV, its apparent K_B value (96.7nM) approximating to apparent K_B values for its antagonism of CP55940- and *R*-(+)-WIN55212-induced

enhancement of $[^{35}S]GTP\gamma S$ binding to mouse brain membranes.

- THCV also antagonized *R*-(+)-WIN55212, anandamide, methanandamide and CP55940 in the vas deferens, but with lower apparent K_B values (1.5, 1.2, 4.6 and 10.3nM respectively), indicating that THCV behaves in a competitive, surmountable manner.
- THCV produced its antagonism of cannabinoids at concentrations that by themselves did not affect the amplitude of the electrically-evoked contractions, or the ability of $[^{35}S]GTP\gamma S$ to bind to mouse brain membranes or CHO-hCB2 cell membranes, suggesting that THCV is a neutral cannabinoid receptor antagonist.
- THCV (100nM) did not oppose clonidine, capsaicin or (-)-7-hydroxy-cannabidiol-dimethylheptyl-induced inhibition of electrically-evoked contractions of the vas deferens. This is an indication that THCV possesses selectivity.
- Contractile responses of the vas deferens to phenylephrine hydrochloride or β,γ-methylene-ATP were not reduced by 1 μM THCV or *R*-(+)-WIN55212, suggesting that THCV interacts with *R*-(+)-WIN55212 at prejunctional sites.
- At 31.6μM, THCV did reduce contractile responses to phenylephrine hydrochloride and β,γ-methylene-ATP, and above 3μM it inhibited electrically-evoked contractions of the vas deferens in an SR141716A-independent manner.

In conclusion, THCV behaves as a neutral competitive CB_1 and CB_2 receptor antagonist. In the vas deferens, it antagonized several cannabinoids more potently than THC and was also more potent against CP55940 and *R*-(+)-WIN55212 in this tissue than in brain membranes.

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CLAIMS

1. Use of tetrahydrocannabidivarin (THCV) in the manufacture of a medicament for use in the treatment of diseases and conditions benefiting from neutral antagonism of the CB_1 cannabinoid receptor.

2. Use of THCV as claimed in claim 1, in the manufacture of a medicament for the treatment of obesity, schizophrenia, epilepsy or cognitive disorders such as Alzheimers, bone disorders, bulimia, obesity associated with type II diabetes (non-insulin dependant diabetes) or in the treatment of drug, alcohol or nicotine abuse or dependency.

3. Use of THCV as claimed in claim 2, in the manufacture of a medicament for use as an appetite suppressant.

4. Use of THCV as claimed in claimed in any of the preceding claims, wherein the THCV is the form of an extract prepared from at least one cannabis plant.

5. Use of THCV as claimed in claim 4, wherein the extract prepared from at least one cannabis plant is in the form of a botanical drug substance.

6. Use of THCV as claimed in claims 4 or 5, wherein the extract prepared from at least one cannabis plant is produced by extraction with supercritical or subcritical CO_2.

7. Use of THCV as claimed in claims 4 or 5, wherein the extract prepared from at least one cannabis plant is produced by contacting plant material with a heated gas at a temperature which is greater than 100°C, sufficient to volatilise one or more of the cannabinoids in the plant material to form a vapour, and condensing the vapour to form an extract.

8. Use of THCV as claimed in any of claims 4 to 7, wherein the extract prepared from at least one cannabis plant comprises all the naturally occurring cannabinoids in said at least one cannabis plant.

9. Use of THCV as claimed in claim 1, wherein the THCV is in a substantially pure or isolated form.

10. Use of THCV as claimed in claim 1, wherein the THCV is in a synthetic form.

11. Use of THCV as claimed in any of the preceding claims, wherein the THCV is formulated as a pharmaceutical composition further comprising one or more pharmaceutically acceptable carriers, excipients or diluents.

12. A method for the treatment of a disease or condition benefiting from neutral antagonism of the CB_1 cannabinoid receptor by THCV, which comprises administering to a subject in need thereof a therapeutically effective amount of THCV.

13.A method as claimed in claim 12, wherein the disease or condition is selected from the group consisting of obesity, schizophrenia, epilepsy, cognitive disorders such as Alzheimers, bone disorders, bulimia, obesity associated with type II diabetes (non-insulin dependant diabetes), and drug, alcohol or nicotine abuse or dependency.

14.A method for cosmetically beneficial weight loss comprising suppression of appetite in a subject by administering to the subject an effective amount of THCV.

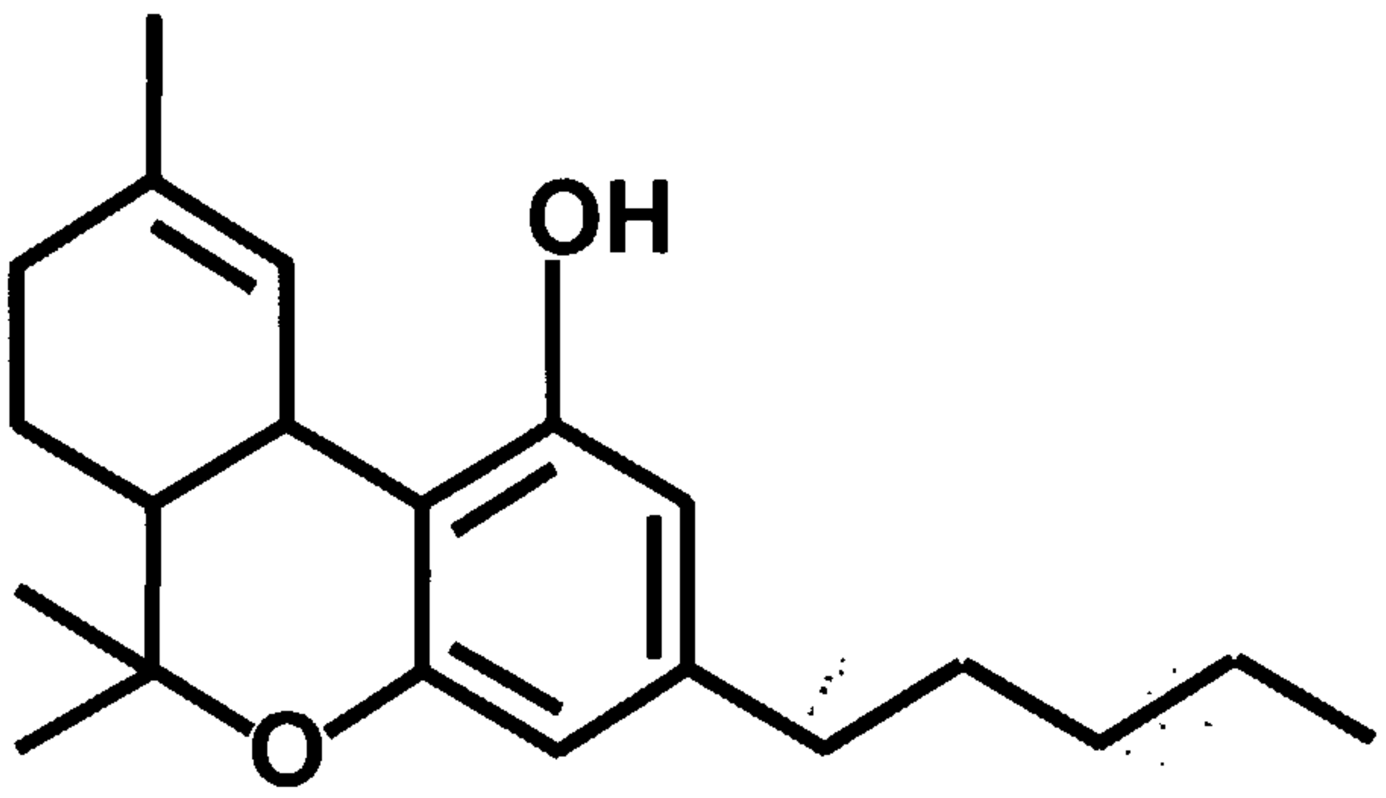
15.Use of a neutral cannabinoid receptor antagonist in the manufacture of a medicament for use in the treatment of diseases or conditions benefiting from neutral antagonism of one or more types of cannabinoid receptor.

16.Use as claimed in claim 15, of a neutral cannabinoid receptor antagonist in the manufacture of a medicament for use in the treatment of diseases or conditions benefiting from neutral antagonism of the CB_1 cannabinoid receptor wherein the dissociation constant of the cannabinoid receptor antagonist at the CB_1 receptor is approximately 75nM.

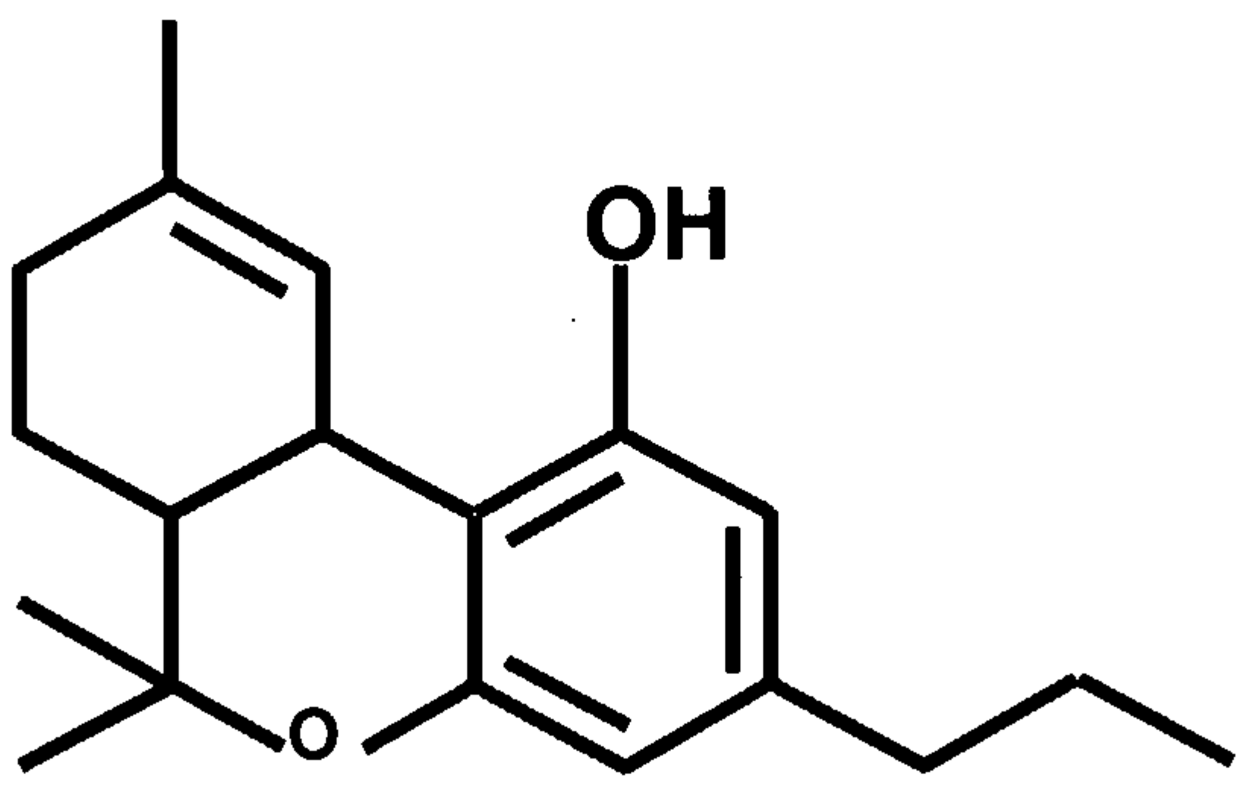
17.Use as claimed in claim 15, of a neutral cannabinoid receptor antagonist in the manufacture of a medicament for use in the treatment of diseases or conditions benefiting from neutral antagonism of the CB_2 cannabinoid receptor wherein the dissociation constant of the cannabinoid receptor antagonist at the CB_2 receptor is approximately 62nM.

Figure 1.

The structures of Δ⁹-THC and Δ⁹-THCV



Δ^9-tetrahydrocannabinol
(Δ^9-THC)



Δ^9-tetrahydrocannabivarin
(Δ^9-THCV)