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(54) Title: USE OF CANNABINOIDS IN THE TREATMENT OF EPILEPSY

Fig. 1

(57) Abstract: The present invention relates to the use of a therapeutically effective amount of cannabidiolic acid (CBDA) in the treatment of epilepsy. In one embodiment the CBDA is used in the treatment of generalised seizures, preferably tonic-clonic seizures. Preferably the CBDA used is in the form of a botanical drug substance in which the CBDA content is greater than 60%, and most preferably, it is a highly purified extract of cannabis such that the CBDA is present at greater than 95%, through 96% and 97% to most preferably, greater than 98% of the total extract (w/w) and the other components of the extract are characterised. In particular the cannabinoids tetrahydrocannabinol (THC) or tetrahydrocannabinol acid (THCA) have been substantially removed. Alternatively, the CBDA may be synthetically produced.

USE OF CANNABINOIDS IN THE TREATMENT OF EPILEPSY

FIELD OF THE INVENTION

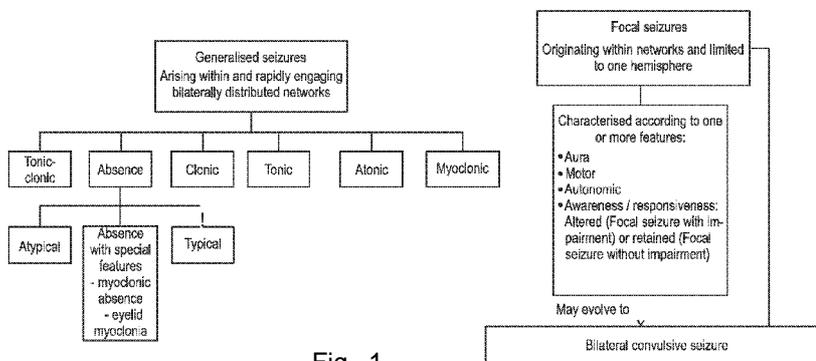[0001]   The present invention relates to the use of a therapeutically effective amount of cannabidiolic acid (CBDA) in the treatment of epilepsy. In one embodiment the CBDA is used in the treatment of generalised seizures, preferably tonic-clonic seizures.

[0002]   Preferably the CBDA used is in the form of a botanical drug substance in which the CBDA content is greater than 60%, and most preferably, it is a highly purified extract of cannabis such that the CBDA is present at greater than 95%, through 96% and 97% to most preferably, greater than 98% of the total extract (w/w) and the other components of the extract are characterised. In particular the cannabinoids tetrahydrocannabinol (THC) or tetrahydrocannabinol acid (THCA) have been substantially removed. Alternatively, the CBDA may be synthetically produced.

[0003]   In use the CBDA may be used concomitantly with one or more other anti-epileptic drugs (AED). Alternatively the CBDA may be formulated for administration separately, sequentially or simultaneously with one or more AED or the combination may be provided in a single dosage form. Where the CBDA is formulated for administration separately, sequentially or simultaneously it may be provided as a kit or together with instructions to administer the one or more components in the manner indicated. It may also be used as the sole medication, i.e. as a monotherapy.

BACKGROUND TO THE INVENTION

[0004]   Epilepsy occurs in approximately 1% of the population worldwide, (Thurman *et al.,* 201 1) of which 70% are able to adequately control their symptoms with the available existing anti-epileptic drugs (AED). However, 30% of this patient group, (Eadie *et al.,* 2012), are unable to obtain seizure freedom using the AED that are available and as such are termed as suffering from intractable or "treatment-resistant epilepsy" (TRE).

[0005]   Intractable or treatment-resistant epilepsy was defined in 2009 by the International League Against Epilepsy (ILAE) as *"failure of adequate trials of two tolerated and appropriately chosen and used AED schedules (whether as monotherapies or in combination) to achieve sustained seizure freedom"* (Kwan *et al.,* 2009).

[0006]   Individuals who develop epilepsy during the first few years of life are often difficult to treat and as such are often termed treatment-resistant. Children who undergo frequent seizures in childhood are often left with neurological damage which can cause cognitive, behavioral and motor delays.

[0007]   Childhood epilepsy is a relatively common neurological disorder in children and young adults with a prevalence of approximately 700 per 100,000. This is twice the number of epileptic adults per population.

[0008]   When a child or young adult presents with a seizure, investigations are normally undertaken in order to investigate the cause. Childhood epilepsy can be caused by many different syndromes and genetic mutations and as such diagnosis for these children may take some time.

[0009]   The main symptom of epilepsy is repeated seizures. In order to determine the type of epilepsy or the epileptic syndrome that a patient is suffering from, an investigation into the type of seizures that the patient is experiencing is undertaken. Clinical observations and electroencephalography (EEG) tests are conducted and the type(s) of seizures are classified according to the ILAE classification described below and in Figure 1.

[0010]   The International classification of seizure types proposed by the ILAE was adopted in 1981 and a revised proposal was published by the ILAE in 2010 and has not yet superseded the 1981 classification. Figure 1 is adapted from the 2010 proposal for revised terminology and includes the proposed changes to replace the terminology of "partial" with "focal". In addition the term "simple partial seizure" has been replaced by the term "focal seizure where awareness / responsiveness are not impaired" and the term "complex partial seizure" has been replaced by the term "focal seizure where awareness / consciousness are impaired".

[001 1]   From Figure 1 it can be seen that Generalised seizures, where the seizure arises within and rapidly engages bilaterally distributed networks, can be split into six subtypes: Tonic-Clonic (grand mal) seizures; Absence (petit mal) Seizures; Clonic Seizures; Tonic Seizures; Atonic Seizures and Myoclonic Seizures.

[0012]   Focal (partial) seizures where the seizure originates within networks limited to only one hemisphere, are also split into sub-categories. Here the seizure is characterized according to one or more features of the seizure, including aura, motor, autonomic and awareness / responsiveness. Where a seizure begins as a localized seizure and rapidly evolves to be distributed within bilateral networks this seizure is known as a Bilateral convulsive seizure, which is the proposed terminology to replace Secondary Generalized Seizures (generalized seizures that have evolved from focal seizures and no longer remain localized).

[0013]   Focal seizures where the subject's awareness / responsiveness is altered are referred to as focal seizures with impairment and focal seizures where the awareness or responsiveness of the subject is not impaired are referred to as focal seizures without impairment.

[0014]   Focal seizures may occur in epilepsy syndromes including: Lennox-Gastaut Syndrome; Tuberous Sclerosis Complex; Dravet Syndrome; CDKL5; Neuronal ceroid

lipofuscinoses (NCL); febrile infection related epilepsy syndrome (FIRES); Aicardi syndrome and brain abnormalities.

[0015]   Epileptic syndromes often present with many different types of seizure and identifying the types of seizure that a patient is suffering from is important as many of the standard AED are targeted to treat or are only effective against a given seizure type / sub-type.

[0016]   Common AED defined by their mechanisms of action are described in the following tables:

[0017]   **Table 1. Examples of narrow spectrum AED**

Narrow-spectrum AED	Mechanism	Indication
Phenytoin	Sodium channel	Complex partial Tonic-clonic
Phenobarbital	GABA / Calcium channel	Partial seizures Tonic-clonic
Carbamazepine	Sodium channel	Partial seizures Tonic-clonic Mixed seizures
Oxcarbazepine	Sodium channel	Partial seizures Tonic-clonic Mixed seizures
Gabapentin	Calcium channel	Partial seizures Mixed seizures
Pregabalin	Calcium channel	Adjunct therapy for partial seizures with or without secondary generalisation
Lacosamide	Sodium channel	Adjunct therapy for partial seizures
Vigabatrin	GABA	Secondarily generalized tonic-clonic seizures Partial seizures Infantile spasms due to West syndrome

[0018]   **Table 2. Examples of broad spectrum AED**

Broad-spectrum AED	Mechanism	Indication
Valproic acid	GABA / Sodium channel	First-line treatment for tonic-clonic seizures, absence seizures and myoclonic seizures Second-line treatment for partial seizures and infantile spasms. Intravenous use in status epilepticus
Lamotrigine	Sodium channel	Partial seizures Tonic-clonic Seizures associated with Lennox-Gastaut syndrome
Ethosuximide	Calcium channel	Absence seizures
Topiramate	GABA / Sodium channel	Seizures associated with Lennox-Gastaut syndrome
Zonisamide	GABA / Calcium /Sodium channel	Adjunctive therapy in adults with partial-onset seizures Infantile spasm Mixed seizure Lennox-Gastaut syndrome Myoclonic Generalised tonic-clonic seizure
Levetiracetam	Calcium channel	Partial seizures Adjunctive therapy for partial, myoclonic and tonic-clonic seizures
Clonazepam	GABA	Typical and atypical absences Infantile myoclonic Myoclonic seizures Akinetic seizures
Rufinamide	Sodium channel	Adjunctive treatment of partial seizures associated with

		Lennox-Gastaut syndrome

[0019]   **Table 3. Examples of AED used specifically in childhood epilepsy**

AED	Mechanism	Indication
Clobazam	GABA	Adjunctive therapy in complex partial seizures Status epilepticus Myoclonic Myoclonic-absent Simple partial Complex partial Absence seizures Lennox-Gastaut syndrome
Stiripentol	GABA	Severe myoclonic epilepsy in infancy (Dravet syndrome)

[0020]   Over the past forty years there have been a number of animal studies on the use of the non-psychoactive cannabinoid cannabidiol (CBD) to treat seizures. For example, Consroe *et al.,* (1982) determined that CBD was able to prevent seizures in mice after administration of pro-convulsant drugs or an electric current.

[0021]   Studies in epileptic adults have also occurred in the past forty years with CBD. Cunha *et al.* reported that administration of CBD to eight adult patients with generalized epilepsy resulted in a marked reduction of seizures in 4 of the patients (Cunha *et al.,* 1980).

[0022]   A study in 1978 provided 200 mg/day of pure CBD to four adult patients, two of the four patients became seizure free, whereas in the remainder seizure frequency was unchanged (Mechoulam and Carlini, 1978).

[0023]   In contrast to the studies described above, an open label study reported that 200 mg / day of pure CBD was ineffective in controlling seizures in twelve institutionalized adult patients (Ames and Cridland, 1986).

[0024]   Based on the fact that chronologically the last study to look at the effectiveness of CBD in patients with epilepsy suggested that CBD was unable to control seizures, there may be less of an expectation that CBD might be useful as an anti-convulsant agent.

[0025]   In the past forty years of research there have been over thirty drugs approved for the treatment of epilepsy none of which are cannabinoids. Indeed, there appears to have been a prejudice against cannabinoids, possibly due to the scheduled nature of these compounds

and / or the fact that THC, which is a known psychoactive, has been ascribed as a pro-convulsant (Consroe *et al.,* 1977).

[0026]   A paper published recently suggested that cannabidiol-enriched cannabis may be efficacious in the treatment of epilepsy. Porter and Jacobson (2013) report on a parent survey conducted via a Facebook group which explored the use of cannabis which was enriched with CBD in children with treatment-resistant epilepsy. It was found that sixteen of the 19 parents surveyed reported an improvement in their child's epilepsy. The children surveyed for this paper were all taking cannabis extracts that were purported to contain CBD in a high concentration although the amount of CBD present and the other constituents including THC and non-cannabinoid components such as terpenes were not known for many of the cases. Indeed, whilst CBD levels ranged from 0.5 to 28.6 mg/kg/day (in those extracts tested), THC levels as high as 0.8 mg/kg/day were reported.

[0027]   Providing children with TRE with a cannabis extract that comprises THC, which has been described as a pro-convulsant (Consroe *et al.,* 1977), at a potentially psychoactive dose of 0.8 mg/kg/day is not desirable.

[0028]   Whilst decoctions of cannabis which will contain CBDA as well as THCA along with other cannabinoids and non-cannabinoid components have been used in epilepsy, treatments have not focussed on isolated or highly purified CBDA. Rather the recent focus has been on the use of the decarboxylated form of CBDA, CBD in the treatment of epilepsy.

[0029]   CBDA has however been found to be effective in the treatment of nausea as is shown in WO 2003/063847 and as a TNF alpha inhibitor suggested for use in treating immunomodulatory and anti-inflammatory conditions as is shown in WO 2002/064109.

[0030]   The patent application GB 2,495,1 18 describes the use of a composition comprising CBDV and CBD for use in the treatment of epilepsy. Furthermore the application WO 201 1/121351 describes the use of CBDV in the treatment of epilepsy. Both documents describe the use of a CBDV botanical drug substance which comprises a small quantity of undecarboxylated CBD as CBDA. The CBDA is present in very small amounts and as such is not present in therapeutically effective amounts.

[0031]   The patent application US 2015/126595 describes the use of a transdermal composition comprising cannabinoids including CBDA.

[0032]   Patent applications CA 2,859,934 and CA 2,737,447 both describe a medicinal cannabis chemovar which comprises the compound CBDA. It is readily understood that all cannabis plants produce cannabinoids in their acid form which are then readily decarboxylated to produce the traditionally recognised active form CBD.

[0033]   Whilst CBD now appears to be a promising candidate as an anti-epileptic drug there are a number of potential limitations including: the relative large doses that appear necessary; and CBD's relatively poor bioavailability.

[0034]   Therefore it is desirable to find other compounds which may demonstrate activity and / or specificity to particular seizure sub-types and which might be administered in lower concentrations. This has the benefit of smaller administration forms and with improved bioavailability lower dose may be required and onset to action may be quicker.

BRIEF SUMMARY OF THE DISCLOSURE

[0035]   In accordance with a first aspect of the present invention there is provided a therapeutically effective amount of cannabidiolic acid (CBDA) for use in the treatment of epilepsy.

[0036]   In one embodiment the epilepsy is generalised epilepsy. More preferably the epilepsy is characterized by tonic-clonic seizures.

[0037]   A therapeutically effective amount is preferably at least 0.1 mg, preferably at least 0.5mg, more preferably at least 1mg, more preferably still at least 20mg or more.

[0038]   The CBDA used may be in the form of a botanical drug substance in which the CBDA content is greater than 60%, and most preferably, it is a highly purified extract of cannabis such that the CBDA is present at greater than 95%, through 96% and 97% to most preferably, greater than 98% of the total extract (w/w) and the other components of the extract are characterised. In particular the cannabinoids tetrahydrocannabinol (THC) or tetrahydrocannabinol acid (THCA) have been substantially removed. Preferably the highly purified extract comprises less than 1% (w/w) tetrahydrocannabinol (THC) or tetrahydrocannabinol acid (THCA).

[0039]   Alternatively, the CBDA may be synthetically produced.

[0040]   The CBDA may also be used concomitantly with one or more other cannabinoids. Preferably the CBDA is used with CBD.

[0041]   Where CBDA is used in combination with CBD ratios of between 9:1 to 1:9 (CBDA: CBD) are preferred. Ranges of ratios include 8:2 to 2:8 (CBDA: CBD); 7:3 to 3:7 (CBDA:CBD); 6:4 to 4:6 (CBDA:CBD); and 1:1 (CBDA:CBD) and any ranges there between.

[0042]   In a further embodiment of the invention the CBDA is used concomitantly with one or more other anti-epileptic drugs (AED).

[0043]   The CBDA may be used at a daily dose of less than 1000 mg. Preferably, the daily dose of CBDA is less than 800mg, preferably less than 600mg, and more preferably less than 400mg.

[0044]   The daily dose may be less than 200mg, less than 100mg and as little as 10mg or 1mg may be used.

[0045]   As the cannabinoid CBDA is more bioavailable than its neutral form CBD, it is likely that a far lower dose of CBDA will be required in comparison with CBD when treating the same indication. For example providing a human with a dose of 20 mg/kg of CBD to treat epilepsy may be effective, whereas the dose of CBDA required may be a log fold lower.

[0046]   Clearly such lower doses have benefits in treatment.

[0047]   Furthermore the greater bioavailability of CBDA may mean that it can act more quickly than CBD. In other words the cannabinoid CBDA may have a lower T_{max} than CBD. This quality could lead to useful combination products which comprise CBDA in combination with CBD. The CBDA may be useful in providing a rapid onset effect whereas the CBD may be useful in providing a sustained effect.

[0048]   Ratioed amounts of CBDA to CBD, where the CBDA is the predominant cannabinoid are envisaged these include ranges from 95:5 to 55:45 (CBDA:CBD).

[0049]   Alternatively the CBDA and CBD may be present in substantially equal amount namely 55:45 to 45: 55 (CBDA:CBD). In yet a further embodiment the CBD may be the predominant cannabinoid and the range may be from 45:55 to 20:80 (CBDA:CBD).

[0050]   Furthermore the faster acting CBDA may be a useful candidate for use in the treatment of epilepsy which requires immediate emergency treatment such as acute seizures or status epilepticus. Preferably the CBDA is administered via the parenteral route, for example by injection into the vein or the muscle.

[0051]   In accordance with a second aspect of the present invention there is provided a method of treating epilepsy comprising administering a therapeutically effective amount of cannabidiolic acid (CBDA) to a subject.

[0052]   Preferably the subject is a human.

[0053]   In accordance with a third aspect of the present invention there is provided a composition for use in the treatment of epilepsy comprising a therapeutically effective amount of cannabidiolic acid (CBDA), and one or more pharmaceutically acceptable excipients.

[0054]   It is envisaged that the composition be administered as one or more of: an oral liquid solution, solid, semi-solid, gel, injection, spray, aerosol, inhaler, vaporiser, enema or suppository. Such medicaments could be administered via the oral, buccal, sublingual, parenteral, respiratory, nasal and distal rectum route.
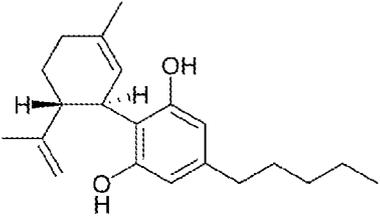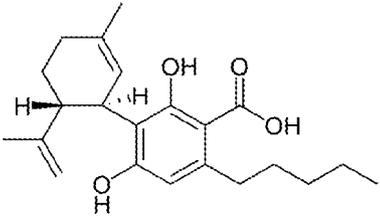
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DEFINITIONS

[0055] Definitions of some of the terms used to describe the invention are detailed below:

[0056]   The cannabinoids described in the present application are listed below along with their
5   standard abbreviations.

Table 4. Cannabinoids and their abbreviations

CBD	Cannabidiol	
CBDA	Cannabidiolic acid	
THC	Tetrahydrocannabinol	
THCA	Tetrahydrocannabinolic acid	
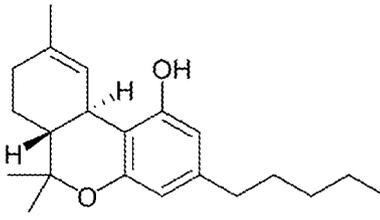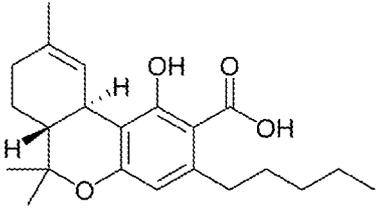
[0057]   The table above is not exhaustive and merely details the cannabinoids which are
identified in the present application for reference. So far over 60 different cannabinoids have
10   been identified and these cannabinoids can be split into different groups as follows:
Phytocannabinoids; Endocannabinoids and Synthetic cannabinoids (which may be novel
cannabinoids or synthetically produced phytocannabinoids or endocannabinoids).

[0058] Patent application number WO 2004/026857 describes the analysis of highly purified CBDA. The CBDA is described as being purified to be greater than 98% pure, with less than 0.1% CBD, 0.3% THCA, and less than 0.1% THC.

[0059] "Phytocannabinoids" are cannabinoids that originate from nature and can be found in the cannabis plant. The phytocannabinoids can be isolated from plants to produce a highly purified extract or can be reproduced synthetically.

[0060] "Highly purified cannabinoid extracts" are defined as cannabinoids that have been extracted from the cannabis plant and purified to the extent that other cannabinoids and non-cannabinoid components that are co-extracted with the cannabinoids have been substantially removed, such that the highly purified cannabinoid is greater than or equal to 98% (w/w) pure.

[0061] "Synthetic cannabinoids" are compounds that have a cannabinoid or cannabinoid-like structure and are manufactured using chemical means rather than by the plant.

BRIEF DESCRIPTION OF THE DRAWINGS

[0062] Embodiments of the invention are further described hereinafter with reference to the accompanying drawings, in which

[0063] Figure 1 shows the ILAE Proposal for Revised Terminology for Organisation of Seizures and Epilepsies 2010;

[0064] Figures 2 A, B, C and D show the effect of cannabinoids on PTZ-induced generalised seizures;

[0065] Figure 3 shows the effect of CBDA on *D. discoideum* cell growth;

[0066] Figure 4 shows the effect of CBD on *D. discoideum* cell growth;

[0067] Figure 5 shows secondary plots for the effect of CBDA and CBD on *D. discoideum* growth; and

[0068] Figure 6 shows the HPLC trace of the CBDA extract of Example 3.

Legends to the figures

[0069] Figure 2: Panels A-D illustrate the effect of CBDA (10-100 mg/kg), CBD (100 mg/kg), and CBD+CBDA (9:1 ratio) on seizure severity (A), percentage mortality (B), percentage of animals exhibiting tonic-clonic seizures (C) and latency to seizure onset (D). In panel A, median seizure severity is represented by a thick grey horizontal line, 25th and 75th percentiles by the black box and whiskers indicate the minimum and maximum values. In panel D, onset latency is

presented as median with IQR. Statistical testing was performed using either a Kruskal-Wallis with *post-hoc* Mann-Whitney L/-tests (panel A and D) or Chi-squared with *post-hoc* Fisher exact tests (panel B and C): $P \le 0.1$ (#); $P \le 0.05$ (*); $P \le 0.01$ (**); $P \le 0.001$ (***); $n = 15$ per group.

[0070]   Figure 3: Growth was measured over a seven day period in the presence of CBDA at concentrations ranging from 0.02 μM to 20 μM. A secondary plot of cell density at 168 hours was used to calculate an IC50 of 0.30 μM.

[0071]   Figure 4: Growth was measured over a seven day period in the presence of CBD (from GW Pharmaceuticals) at concentrations ranging from 0.25 μM to 20 μM. A secondary plot of cell density at 144 hours was used to calculate an IC50of 1.63μM.

[0072]   Figure 5: Cannabinoids have a potency order of CBDA>CBD.

[0073]   Figure 6: The CBDA botanical drub substance shown in the HPL trace comprised a CBDA content of 62.4% w/w and other cannabinoids measured included CBD-6.9% (w/w), THC-0.7% (w/w) and cannabichromene (CBC) -0.5% (w/w).

DETAILED DESCRIPTION

EXAMPLE 1: AN *IN VIVO* EVALUATION OF CBDA IN THE ACUTE PENTYLENETETRAZOLS (PTZ) MODEL OF GENERALISED SEIZURE

Materials and Methods

Animals

[0074] Adult male Wistar Kyoto rats were used in the acute PTZ model of seizure (>P24, 70-110 g). Animals were housed five per cage in a heat regulated room (21 °C) on a 12:12 h day/night cycle (lights on 0800) in 50 % humidity and given *ad libitum* access to standard laboratory chow (PCD Mod C, Special Diet Services, Wiltham, UK) and water. All procedures were undertaken during white light hours.

Pharmaceutical formulation

[0075] A PTZ (Sigma-Aldrich, Poole, UK) stock solution was made in 0.9 % w/v NaCl for the experimental procedure. CBD (batch number, CBD-CG-1001; GW Pharmaceuticals, Salisbury, UK) and CBDA (batch number, CBDA040912; GW Pharmaceuticals) stocks were made in a 2:1:17 vehicle of ethanol, cremophor and saline.

[0076] Formulation analysis was undertaken to determine whether CBDA decarboxylated to CBD because of temperature and/or the excipients in the formulation. Analysis revealed CBDA

was not converted to CBD during formulation. Therefore, CBDA was not modified during formulation, and a 2:1:17 ratio can be used in future investigations for this phytocannabinoid.

PTZ-induced model of generalised seizure

[0077] PTZ antagonises GABA inhibition via binding to the t-butyl-bicycl-phosphorothionate site of GABAA receptors. Moreover, this chemically-induced model can be indicative of effects against absence seizures. PTZ (90 mg/kg) was used to induce seizures in adult male Wistar rats (n=15 per group) with experiment dose randomised using a Latin square design. Animals were placed in their 6 L Perspex tanks and allowed to acclimatise to their environment for 10 min, before receiving one of the cannabinoid doses (see Table 5) in vehicle, or volume-matched dose of vehicle alone to serve as a negative control. 60 min after test compound or vehicle administration, animals were injected with PTZ (90 mg/kg, i.p.) to induce seizures and animal behaviour was recorded for 30 min.

Table 5. Doses of cannabinoid

	Dose (mg/kg)	
	CBDA	CBD
Vehicle	-	-
CBDA	10	-
CBDA	50	-
CBDA	100	-
CBD	-	100
CBDA/CBD	10	90

[0078] Videos of PTZ-induced seizures were scored offline with a standard seizure severity scale appropriate for generalised seizures (Table 6).

[0079] The human dose equivalent (HED) can be estimated using the following formula:

$$HED = Animal\ dose\ (mg/kg)\ \textit{multiplied by}\ \frac{Animal\ K_m}{Human\ K_m}$$

The K_m for a rat is 6 and the K_m for a human is 37.

The K_m for a dog (Example 3) is 20.

Thus a 10mg/Kg dose in a rat would equate to a human dose of about 1.6 mg/kg. A 50 mg/kg dose in a rat would equate to a human dose of about 8.1 mg/kg. A 100 mg/kg dose in a rat would equate to a human dose of about 16.2 mg/kg.

Table 6. Seizure severity scoring scale.

Seizure score	Behavioural expression	Righting reflex
0	No changes to behaviour	Preserved
0.5	Abnormal behaviour (sniffing, excessive washing, orientation)	Preserved
1	Isolated myoclonic jerks	Preserved
2	Atypical clonic seizure	Preserved
3	Fully developed bilateral forelimb clonus	Preserved
3.5	Forelimb clonus with tonic component and body twist	Preserved
4	Tonic-clonic seizure with suppressed tonic phase	Lost
5	Fully developed tonic-clonic seizure	Lost

Data analysis

[0080]   Videos of seizure behaviour generated from the custom built observational system were scored offline according to seizure scales appropriate for PTZ model (Table 6) using Observer Video-Pro software (Noldus, Wageningen, The Netherlands). For the PTZ model of seizure, intra- and inter-observer agreements of behaviour scoring were assessed using the reliability analysis function of the observer Video-Pro software: 1 s tolerance window; Cohen's Kappa coefficient $\geq$ 0.95.

[0081]   Specific markers of seizure behaviour and development were assessed and compared between vehicle control and drug groups. The latency (s) to seizure onset and the percentage of animals that developed tonic-clonic seizures was noted (see Table 6). In addition, the maximum seizure severity and the percentage mortality in each group were determined for the acute PTZ model of generalised seizure.

Statistical analysis

[0082]   The effect of drug on latency to seizure onset and maximum seizure severity were assessed using Krustal-Wallis with *post-hoc* Mann-Whitney L/-tests. Drug effects on the percentage of animals that developed tonic-clonic seizures and percentage mortality were assessed using Chi-squared with *post-hoc* Fisher exact tests. In all cases, $P \leq 0.05$ was considered significant.

Results

[0083]   The objective of the present Example was to examine the anti-convulsant potential of CBDA in the PTZ-induced acute model of generalised seizure, with a comparator CBD dose. Additionally, CBD and CBDA in a ratio of 9:1 was included to investigate possible interactions between CBD and CBDA.

[0084]   Cannabinoid treatment significantly reduced seizure severity in the acute PTZ-induced model of generalised seizure (Figure 2. Panel A; $H = 14.31$, $P \leq 0.05$), where 100 mg/kg CBDA (P ≤ 0.05) and a CBD/CBDA ratio (P ≤ 0.05) exhibited significant anti-convulsant effects *vs* vehicle control.

[0085]   Mortality was significantly reduced (Figure 2. Panel B; X2(6) = 30.51, $P \leq 0.0001$) following administration of 100 mg/kg CBD $(P \leq 0.01)$ and a trend towards a significant reduction for 100 mg/kg CBDA $(P = 0.0656)$ and a CBD/CBDA ratio $(P = 0.0656)$.

[0086]   Cannabinoid treatment also significantly reduced incidence of tonic-clonic seizures (Figure 2. Panel C; X2(6) = 17.178, $P \leq 0.01$) where administration of 100 mg/kg of CBDA resulted in a trend towards a reduction in tonic-clonic seizures $(P \leq 0.1)$.

[0087]   Finally, analysis revealed cannabinoid administration significant affected latency to seizure onset in the PTZ-induced model (Figure 2. Panel D; $H = 37.37$, $P \leq 0.0001$), with 100 mg/kg CBD (P ≤ 0.05), CBD/CBDA (9:1 ratio; $P \leq 0.05$) significantly increasing latency to onset. Whereas CBDA (100 mg/kg) showed a trend towards increasing the latency to seizure onset $(P = 0.0929)$.

Conclusions

[0088]   As predicted by previous studies, CBD exerted anti-convulsant effects in this model of acute generalised seizure and so demonstrates the continued validity of the model to reveal anti-convulsant effects of plant cannabinoids.

[0089]   Notably, in three of the four parameters measured CBDA produced significant anti-convulsant effects and were statistically more effective than CBD at an equivalent dose.

[0090]   For example, Panel A of Figure 2 describes the effect of CBDA and CBD on the seizure severity. The median score for the maximum seizure severity that the animals experienced with 100mg/kg CBDA was 3 (Table 6 - fully developed bilateral forelimb clonus, with righting reflex preserved) and the median for 100 mg/kg CBD was 5 (Table 6 - fully developed tonic-clonic seizure, with righting reflex lost). This shows that CBDA was able to prevent the animals from suffering from more severe types of seizure than CBD was.

[0091]   Panel C additionally demonstrates that CBDA at 100 mg/kg was able to prevent tonic-clonic seizures from developing in more animals compared to CBD at 100 mg/kg. Indeed the data for 100 mg/kg CBDA was the only statistically significant data in this parameter. This

suggests that CBDA will be more effective than CBD at preventing or treating epilepsy, particularly tonic-clonic seizures from developing.

[0092]   Co-administration of CBDA with CBD, in an exemplary 9:1 ratio, demonstrated that the combination was also effective as an anti-convulsant. Since the plant naturally produces CBDA and this can be decarboxylated, this opens up the possibility of using partially decarboxylated phytocannabinoids or extracts thereof, in given ratios. Such ratios may be beneficial for a number of reasons. These include targeting different types of seizures e.g. CBD for partial seizures and CBDA for generalised seizures may be beneficial based on their different activities in animal models of epilepsy. Also, the difference in the lipophilicity or bioavailability of the two compounds may enable combinations to be developed with different release profiles e.g. CBDA may be quicker acting than CBD and more bioavailable than CBD (see Example 3).

[0093]   This Example demonstrates for the first time that the isolated or highly purified cannabinoid CBDA has anti-convulsant effects and as such further investigation in other models of seizure and epilepsy are warranted in order to determine the full extent of its efficacy.

[0094]   In order to consider whether CBD and CBDA act by similar mechanisms and have similar potency the applicant conducted a study on a new model of *Dictyostelium discoideum.*

EXAMPLE 2: **USE OF *DICTYOSTELIUM DISCOIDEUM* MODEL TO IDENTIFY MOLECULAR TARGETS OF CANNABINOIDS AND THEIR USE ɪɴ EPILEPSY**

Introduction

[0095] *Dictyostelium discoideum* is an amoeba, listed by the US National Institute of Health as a biomedical model system (Williams et al. 2006). It has a cellular structure typical of eukaryotes, with nuclei, Golgi, mitochondria and endoplasmic reticulum and its haploid genome has been fully characterised and annotated (Dictybase.org) including descriptions of each protein, the phenotype of mutants lacking each protein and related published material. *D. discoideum* can be grown in liquid culture as single cells or allowed to progress into multi-cellular development upon starvation with the formation of a multi-cellular fruiting body.

[0096] *D. discoideum* has been developed to better understand the molecular mechanisms by which diverse drugs and chemicals exert their effects, to identify more potent or safer compounds, and to characterise the cellular role of human proteins

[0097] This range of methodologies has enabled *D. discoideum* to be used as a valuable model in diverse areas in molecular pharmacology and pharmacogenetics. In these research areas, the primary target of either established or new pharmaceutical compounds is often unclear, and

compounds often have off-target side effects that remain uncharacterised, and which may result in costly late-stage drug attrition and potentially affecting patient compliance.

[0098] In epilepsy research, *D. discoideum* has been used to identify molecular effects of valproic acid (Cunliffe et al 2015; Chang et al. 2012) and translated *in vitro* and *in vivo* mammalian models to demonstrate relevance to human health (Chang et al 2012, 2013, 2014). It is clear that *D. discoideum* can be used to identify clinically relevant therapeutic compounds for the treatment of epilepsy.

[0099] The present Example demonstrates the use of *D. discoideum* to identify the molecular mechanism(s) of action of two cannabinoids, (CBD and CBDA), with relevance to seizure control.

Materials and Methods

GROWTH ASSAYS

[00100] Wild type (Ax2) *D. discoideum* cells were grown in shaking culture (in HL5 medium) for two days prior to growth assays. Cells (9900 in 495 μl of media) were added to each well of a 24 well plate and 5 μl of cannabinoid in DMSO (or DMSO only) was added to each well to achieve each described concentration (1% final DMSO concentration), and cells were maintained at 22°C. Cells were counted at 72 hours, and then every 24 hours. Quadruplicate repeats were used for each concentration.

DEVELOPMENT ASSAYS

[00101] Wild type (Ax2) *D. discoideum* cells were grown in HL5 shaking culture for two days prior to development assay. Cells were washed in phosphate buffer (KK2; 20 mM Potassium phosphate buffer, pH 6.1), and 1x107 cells were spread onto nitrocellulose filters (Millipore, Cork). Absorbent pads (Millipore, Cork), divided into quarters, were placed in 2ml culture dishes and soaked with 0.5ml KK2 containing the cannabinoids at 20 μM. 1mM Valproic acid was used as a positive control while KK2 containing 1% DMSO was used as a solvent only control. Nitrocellulose filters containing cells were quartered and place upon absorbent pads and maintained in a humid environment at 22°C for 24h. Fruiting body morphology was recorded using a dissection microscope and camera.

BIOINFORMATIC ANALYSIS

[00102] The amino acid sequence for potential *H. sapien* protein targets of the cannabinoids listed were obtained from Uniprot (www.uniprot.org). Homology searches of *the D. discoideum* genome were carried out using the online Basic local alignment search (BLAST) algorithm available at dictybase.org. TMHMM server V. 2.0 transmembrane region predictor software was

used to determine possible transmembrane regions within the *D. discoideum* orthologue proteins. Regions of the proteins containing highly conserved residues required for protein function were analysed by multiple sequence alignment using ClustalW2.

BACTERIAL PLATE SCREEN

[00103]   SM agar plates were made with the addition of CBDA or CBD to final concentrations of 12.3μM and 16.7μM respectively. Heat killed (75°C for 30 minutes) *R. planticola* was spread onto the plates and -50 wild-type AX2 cells were added and left to grow at 22°C. Plates were checked regularly for colonies.

MUTANT REMI LIBRARY SCREEN

[00104]   REMI library cells were grown in shaking culture (in HL5 medium) for two days prior to screening. Cells (25,000 in 2ml of media) were added to each well of a 6 well plate and allowed to adhere for 20 minutes. The media from each well was replaced with media containing either: 4.88μM CBDA or 9.47μM CBD. Cells were screened in triplicate over a three week period, maintained at 22°C with the media being replaced every two days. Potential resistant mutant colonies were isolated and transferred to bacterial plates. Isogenic cell lines were established from individual colonies on the bacterial plates.

CONFIRMATION OF INDIVIDUAL MUTANT RESISTANCE:

[00105]   Clonal cells isolated from the library screen were grown in liquid media (HL5 medium) to produce a confluent 10cm plate. Cells (10,000 in 495μl of media) were added to each well of a 24 well plate and 5μl of cannabinoid in DMSO was added to each well to achieve either 4.88μM CBDA or 9.47μM CBD (1% final DMSO concentration), cells were maintained at 22°C. Cells were monitored over a one week for their sensitivity to the two cannabinoids.

Results

GROWTH ASSAYS

[00106]   It first needed to be determined if *D. discoideum* growth was sensitive to the cannabinoids: cannabidiolic acid (CBDA) and cannabidiol (CBD). In these experiments, *D. discoideum* were exposed to a range of concentrations of each cannabinoid during growth in still culture over a one week period. All two cannabinoids inhibited *D. discoideum* cell growth in a dose dependent manner (Figures 3 to 5).

[00107]   The growth inhibitory constant (IC50) for CBDA was 0.30 μM (Figure 3), with 0.08 μM significantly inhibiting cell growth (P<0.05) and 20 μM blocking growth.

[00108]   The growth inhibitory constant (IC50) for CBD was 1.63uM (Figure 4), with 0.5 μM significantly inhibiting cell growth (P<0.05) and 20 μM blocking growth.

[00109]   Comparison of all two cannabinoids IC50 values suggests CBDA is the most potent, with CBD showing an 8.7-fold reduction in potency. Thus the order of potency for cannabinoids on *D. discoideum* cell growth is CBDA>CBD (Figure 5).

DEVELOPM ENT ASSAYS

[001 10]   The effects that CBDA and CBD had upon *D. discoideum* development were investigated. This was achieved by placing cells in a nutrient depleted environment in the presence of CBDA or CBD at concentrations that block cell growth (20 μM).

[001 11]   Cell development on a nitrocellulose filter over a 24 hour period in the absence of cannabinoids gave rise to fruiting bodies consisting of spore heads held above substrata by stalks. This developmental morphology is known to be blocked by the widely used anti-epileptic, valproic acid (1mM), where cells were able to aggregate but unable to undergo development to form fruiting bodies.

[001 12]   In contrast, *D. discoideum* cells treated with CBDA or CBD (20μM) were able to aggregate and develop to form mature fruiting bodies.

BIOINFORMATIC ANALYSIS

[001 13]   Known targets of CBDA and CBD in *H. sapiens* were then sought in order to identify potential orthologues within the *D. discoideum* genome. From current literature, 21 possible mammalian targets of CBDA and CBD have been published. Using human protein sequences corresponding each potential target, in combination with BLAST analysis, the *D. discoideum* genome was searched for orthologous targets. Using this approach, 10 possible *D. discoideum* orthologues were identified. Based upon similarity of protein sequence and size, and conservation of catalytic sites and motifs, three proteins have been identified for further study:

[001 14]   1: Equilibrative Nucleoside Transporter 1 (ENT1). This protein is a potential target for CBD and plays a role in adenosine transport. *D. discoideum* has three possible ENT1 orthologues, and all three have a putative multiple transmembrane structure found in the *H. sapiens* protein. The three *D. discoideum* orthologues are 522, 482 and 430 aa in size, similar to the 456 aa *H. sapiens* ENT1 protein, and contain a highly conserved motif located within first transmembrane region. This motif is found within this protein from many other species.

[001 15]   2: Monoacylglyceride lipase alpha (MAGLa). This protein is involved in the endocannabinoid system. *D. discoideum* has one possible MAGLa orthologue. This orthologue is 409 aa, of similar size to the 303 aa *H. sapien* MAGLA protein. Both the *D. discoideum* and *H. sapiens* proteins have a conserved catalytic serine, aspartate and histidine residue that are important in enzymatic function that are widely conserved in many other species.

[001 16]   3: Diacylglycerol lipase alpha (DAGLa). This protein is involved in the endocannabinoid system. *D. discoideum* has three possible orthologues. The three *D.*

discoideum orthologues are 938, 856 and 826 aa in size, slightly smaller than the 1042 aa *H. sapiens* DAGLa protein. All three D.discoideum orthologues have the same conserved serine and aspartate residues that are important in catalytic function, and these are widely conserved in many other species.

5

BACTERIAL PLATE SCREEN

[001 17]   It was determined if *D. discoideum* growth upon *R. planticola* bacterial plates was a viable method in which resistant REMI mutant library cells could be isolated. Wild-type AX2 cells were grown upon heat killed *R. planticola* SM agar plates. Each SM agar plate contained

10   CBDA or CBD at a final concentration of 12.3 μM and 16.7 μM respectively. Following incubation for 4 days, plates were assessed for cell survival (colony growth). No difference in colony number was found for every cannabinoid compared to control (solvent only).

MUTANT REMI LIBRARY SCREEN

15   [001 18]   Mutants were then identified within the library that showed resistance to the cannabinoids during growth in liquid culture. The library cells were grown over a three week period in the presence of 4.88 μM CBDA or 9.47 μM CBD. After a two week period colonies of partially resistant cells were visible in library-derived plates. Partially resistant cells were transferred to bacterial plates and passaged to ensure each mutant was isogenic.

20

CONFIRMATION OF INDIVIDUAL MUTANT RESISTANCE

[001 19]   The resistance of each cell line was confirmed. All cell lines were treated with either: CBDA or CBD at a final concentration of 4.88 μM and 9.47 μM respectively and assessed after one week. Isogenic cell lines showed some overlap of resistance to the different cannabinoids.

25   Mutant cells were shown to have 3 basic phenotypes to each cannabinoid, classified as showing no resistance, weak resistance or partial resistance. Mutant cells were also found to have either resistance to one cannabinoid or to multiple cannabinoids.

Conclusions

30   [00120]   The development of cannabinoids as novel therapeutic treatments for epilepsy provides an exciting new field of research, with real potential for improving health. A comprehensive understanding of the mechanisms of action and relative potency of these compounds are essential for therapeutic development, to understand both how the compounds block seizures and potential side effects. Traditional approaches to identify these mechanisms

35   are very complex and slow. As an alternative approach, *D. discoideum* has been used to identify mechanism of a widely used treatment, valproic acid, which has been verified in mammalian in vivo models.

[00121] In this current study, it has been demonstrated that two cannabinoids, CBDA, and CBD block *D. discoideum* growth. Concentrations that affect growth are in the low μM range and are equivalent to the concentrations shown to be anti-convulsant in animal models of seizure. This suggests that targets for all two cannabinoids are present in the *D. discoideum* genome. This also suggests that the *D. discoideum* targets have a similar sensitivity to the cannabinoids that shown in mammalian models.

[00122] The growth inhibitory effect can then be employed in an unbiased screen to identify these cannabinoid targets. Using a library of insertional mutants, a pool of mutants can be grown in the presence of each cannabinoid over a 21 day period. Mutants with insertions into genes encoding cannabinoid targets are likely to show resistance to this growth inhibition and thus out-compete sensitive cells during the screen. Identification of insertionally-inactivated genes in cannabinoid resistant colonies will identify molecular targets (and mechanism) of these cannabinoids in an unbiased approach. This screening approach in *D. discodeium* has been used to identify targets and mechanisms of a range of compounds.

[00123] D. *discoideum* is also widely used as a development model, where the formation of a fruiting body involves cell aggregation and differentiation. Pharmacological studies have used this developmental process to identify drug mechanisms. In relation to the cannabinoids studied here, all two compounds had no effect on D. *discoideum* development, at concentrations shown to block growth. This firstly suggests that the block in D. *discoideum* growth is not toxic, since cells can develop, and thus that cannabinoid targets are likely to be involved in blocking cell growth or division (cytokinesis). This also suggests that D. *discoideum* development cannot be used to further study these compounds. In combination with an unbiased approach to identifying cannabinoid targets, D. *discoideum* also provides a useful model to investigate known mammalian targets.

[00124] It was found that a total of 25 mutant cell lines showed resistance to growth inhibition. The range of resistant phenotypes to different cannabinoids suggests that there are multiple genes involved

EXAMPLE 3

COMPARISON OF PK DATA FOR CBD AND CBDA FROM TOXICOLOGICAL STUDIES IN DOGS

[00125] The objective of the studies was to determine the toxicity of CBD (in the form of a substantially pure compound - greater than 95% purity) and CBDA (in the form of a botanical drug substance - greater than 60% CBDA w/w of the total extract and greater than 85% w/w of the total cannabinoid content) following daily oral (gavage) administration to the dog.

[00126]   This study was designed to meet the known requirements of European Directive 2001/83/EC and all subsequent amendments together with any relevant International Conference on Harmonisation (ICH) guidelines.

[00127]   Blood samples for toxicokinetics (0.5 mL nominal) were taken from all animals on Day 1 at 0.5, 1, 2, 4, 6 and 24 hours after the dosing of 100mg/ kg of either CBD or CBDA to the animals.

[001 28]   Samples were taken from the jugular vein into lithium heparin. Samples were mixed gently by hand then continuously for at least 2 minutes on automatic mixer and placed in a Kryorack until centrifugation, which was carried out at approximately 4°C as soon as practicable. The resultant plasma was separated under low light conditions, transferred to uniquely labelled clear glass vials, placed in light proof boxes and frozen immediately at <-50°C.

[00129]   Toxicokinetic parameters measured included C_{max} (ng/mL), T_{max} (h) and $AUC_{0\text{-}t}$ (h * ng/ mL) and the results are illustrated in Table **7** for CBDA, Table 8 for CBD (males), Table **9** for CBD (females) and the comparative C_{max} and $AUC_{0\text{-}t}$ are shown in Table 10 for males and Table 11 for females.

Results

[00130]   **Table 7**

Mean Toxicokinetic Parameters of CBDA are presented below:

Parameter [a]	Period	n=3					
		Dose of CBDA BDS (mg CBDA/kg/day)					
		50		100		200	
		Males	Females	Males	Females	Males	Females
AUC₀₋ₜ (h. ng/mL)	Day 1	55600	149000	80500	179000	269000	172000
	Day 28	71600	64100	116000	159000	94700	156000
Cmax (ng/mL)	Day 1	19100	21100	24900	38600	35000	27100
	Day 28	15700	15000	23400	32500	17700	35900
Tmax (h)	Day 1	1	3	1.3	1.7	2.7	1.7
	Day 28	1.7	2	1.5	1.7	9	1.3

a - Results are reported ¼ mem unless stated otherwise

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[00131]   Table 8

Subject	C_max (ng/mL)	t_max (h)	t_1/2 (h)	AUC_0-t (h*ng/mL)	AUC_0-inf (h*ng/mL)	AUC_ex (%)	CL/F (mL/min/kg)	V_z/F (L/kg)
15	4570	6.0	7.7	51800	60500	14.3	27.5	18.4
16	3620	4.0	5.4	33000	35400	6.8	47.1	21.9
17	1400	6.0	8.5	17200	20300	15.1	82.1	60.5
18	2430	4.0	5.7	28700	31100	7.9	53.6	26.2
19	3090	8.0	n.d.	26400	n.d.	n.d.	n.d.	n.d.
20	3960	6.0	8.6	47300	55300	14.5	30.1	22.3
N	6	6	5	6	5	5	5	5
Mean	**3180**	**n.d.**	**7.2**	**34100**	**40500**	**11.7**	**48.1**	**29.9**
SD	1140	n.d.	1.5	13100	16900	4.0	22.0	17.3
Min	1400	4.0	5.4	17200	20300	6.8	27.5	18.4
Median	3360	6.0	7.7	30800	35400	14.3	47.1	22.3
Max	4570	8.0	8.6	51800	60500	15.1	82.1	60.5
Geometric Mean	2970	n.d.	7.0	31900	37600	11.1	44.4	27.0
CV% Geometric Mean	44.8	n.d.	23	42.2	46.9	39.6	46.9	49.5

[00132]   Table 9

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Subject	C_max (ng/mL)	t_max (h)	t_1/2 (h)	AUC_0-t (h*ng/mL)	AUC_0-inf (h*ng/mL)	AUC_ex (%)	CL/F (mL/min/kg)	V_z/F (L/kg)
115	655	2.0	8.4	3000	3280	8.7	508	367
116	2520	2.0	6.6	20000	22400	10.6	74.5	42.9
117	1900	8.0	n.d.	22600	n.d.	n.d.	n.d.	n.d.
118	4 11	1.0	4.5	2540	2640	3.7	632	247
119	3270	8.0	n.d.	32400	n.d.	n.d.	n.d.	n.d.
120	3780	6.0	4.7	31300	32800	4.5	50.8	20.8
N	6	6	4	6	4	4	4	4
Mean	**2090**	**n.d.**	**6.1**	**18600**	**15300**	**6.9**	**316**	**169**
SD	1370	n.d.	1.8	13200	14800	3.3	297	167
Min	4 11	1.0	4.5	2540	2640	3.7	50.8	20.8
Median	2210	4.0	5.7	21300	12800	6.6	291	145
Max	3780	8.0	8.4	32400	32800	10.6	632	367
Geometric Mean	1590	n.d.	5.9	12300	8930	6.3	187	94.8
CV% Geometric	113	n.d.	30	172	208	54.2	208	238

Mean											

[00133]   **Table 10**

	CBDA (100mg/kg)	CBD (100mg/kg)
Cmax	24,900	3180
AUC$_{0-t}$	80,500	34,100

[00134]   **Table 11**

	CBDA (100mg/kg)	CBD level (100mg/kg)
Cmax	38,600	2090ng/mL
AUC$_{0-t}$	179,000	18,600 ng/mL*hr

CONCLUSIONS:

[00135]   It will be apparent from the comparative Tables 10 and 11 that an equivalent amount of CBDA to CBD results in C_{max} and AUC_{0-t} values which are very significantly higher (by an order of magnitude) than that of CBD, suggesting that the CBDA is acting more quickly and is more bioavailable than the CBD. This has significant implications / benefits when it comes to treating patients.

OVERALL CONCLUSION:

[00136]   To summarise, the data presented in Examples 1, 2, and 3 demonstrates that:

[00137]   CBDA has anticonvulsant effects in a mammalian model of epilepsy and is effective in treating generalised seizures, more particularly, tonic-clonic seizures. Indeed, this compound appears more effective than CBD in many of the parameters tested.

[00138]   CBDA is significantly more potent than CBD upon growth of *D. discoideum;* and CBDA acts more quickly and is more bioavailable than CBD.

[00139]   These findings are of great significance as they demonstrate that CBDA offers an alternative anti-convulsant to CBD. The finding that CBDA is more potent and more bioavailable than CBD means that a smaller daily dose of the active ingredient may be used in the treatment of epilepsy. In this regard, it appears from Example 3, that doses of less than 400mg and

possibly doses of as little as from 1mg - 100mg, might be used to treat human subjects based on the PK and AUC_{0-t} data of Example 3. In this regard, a typical adult patient might weigh 60kg and thus, a daily dose for such a patient might be from 0.016mg/kg to 1.6mg/kg.

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CLAIMS

1. A therapeutically effective amount of cannabidiolic acid (CBDA) for use in the treatment of epilepsy.

2. A therapeutically effective amount of CBDA for use according to claim 1, wherein the epilepsy is a generalized epilepsy.

3. A therapeutically effective amount of CBDA for use according to claim 1 or claim 2, wherein the epilepsy is characterized by tonic-clonic seizures.

4. A therapeutically effective amount of CBDA for use according to any of the preceding claims wherein the therapeutically effective amount is at least 0.1 mg.

5. A therapeutically effective amount of CBDA for use according to any of the preceding claims, wherein the CBDA is in the form of a highly purified extract of cannabis such that the CBDA is present at greater than 98% of the total extract (w/w).

6. A therapeutically effective amount of CBDA for use according to claim 5, wherein the highly purified extract comprises less than 1% (w/w) tetrahydrocannabinol (THC) or tetrahydrocannabinol acid (THCA).

7. A therapeutically effective amount of CBDA for use according to claim 1, wherein the CBDA is synthetically produced.

8. A therapeutically effective amount of CBDA for use according to any of the preceding claims, wherein the CBDA is used concomitantly with one or more other cannabinoids.

9. A therapeutically effective amount of CBDA for use according to claim 8, wherein the CBDA is used with cannabidiol (CBD).

10. A therapeutically effective amount of CBDA for use according to claim 9, wherein the CBDA: CBD ratio is in the range of from 9:1 to 1:9 (CBDA: CBD).

11. A therapeutically effective amount of CBDA for use according to any of the preceding claims, wherein the CBDA is used concomitantly with one or more other anti-epileptic drugs (AED).

12. A therapeutically effective amount of CBDA for use according to any of the preceding claims, wherein the CBDA is used at a dose of less than 400 mg.

13.   A therapeutically effective amount of CBDA for use according to claim 12, wherein the CBDA is used at a dose of from 1mg to 100mg.

14.   A method of treating epilepsy comprising administering a therapeutically effective amount of cannabidiolic acid (CBDA) to a subject.

15.   A composition for use in the treatment of epilepsy comprising a therapeutically effective amount of cannabidiolic acid (CBDA), and one or more pharmaceutically acceptable excipients.
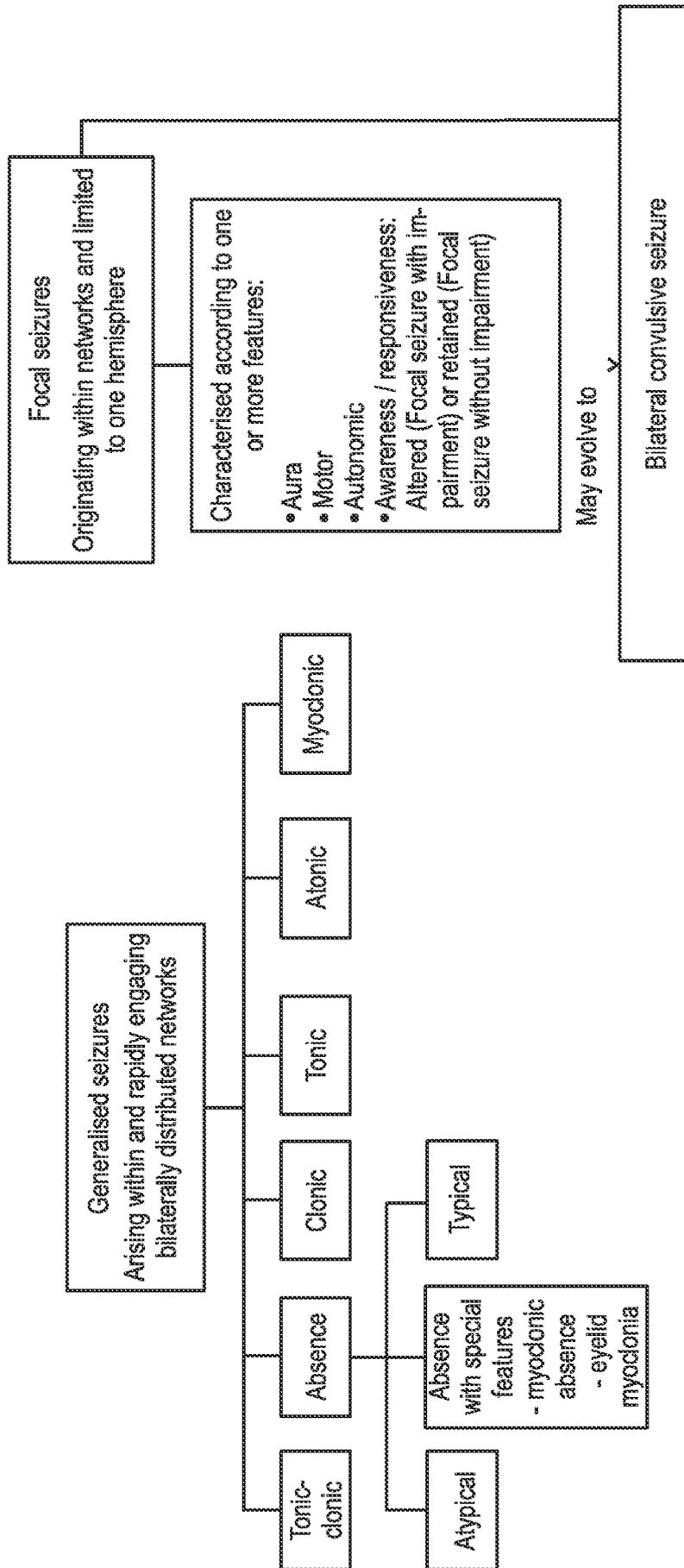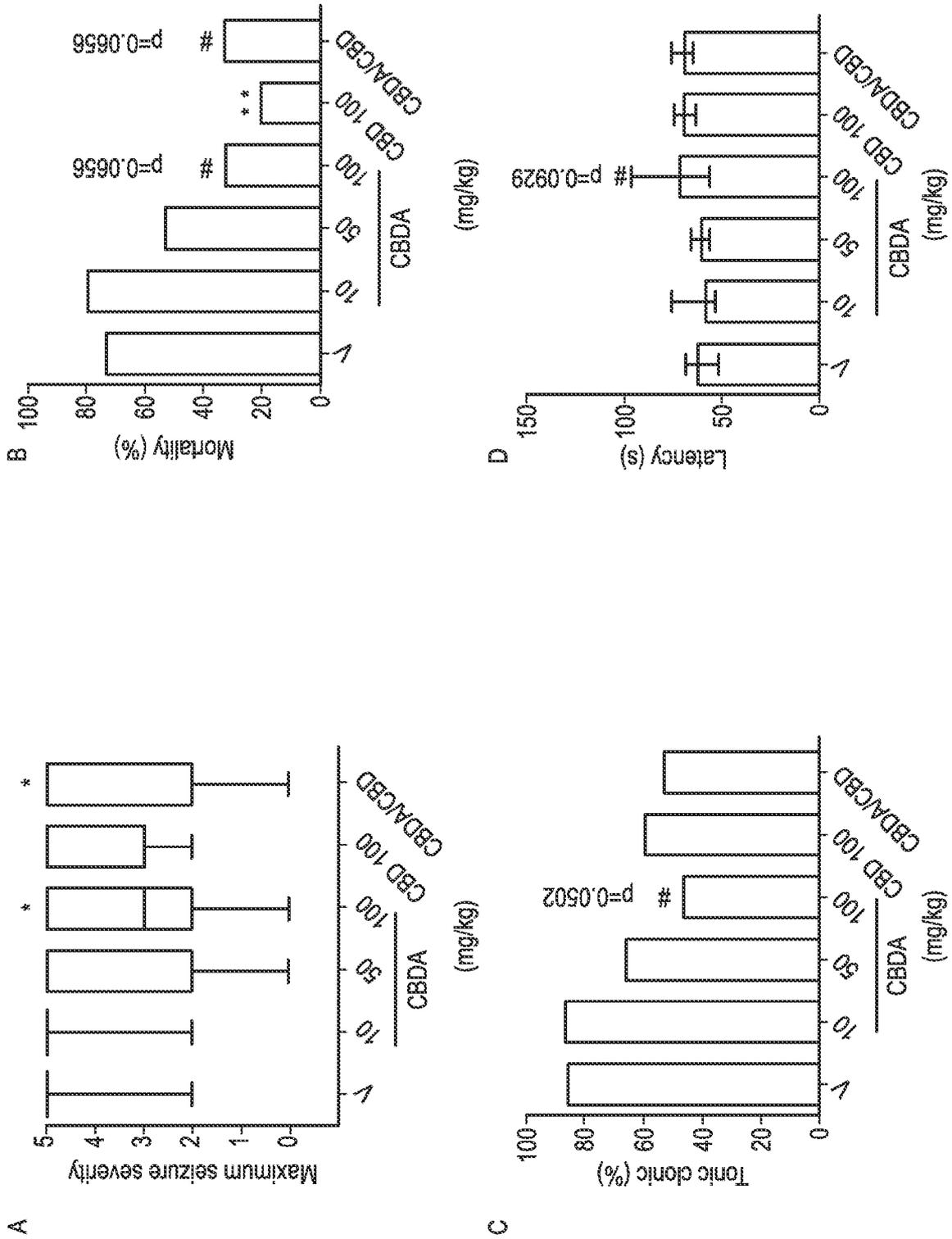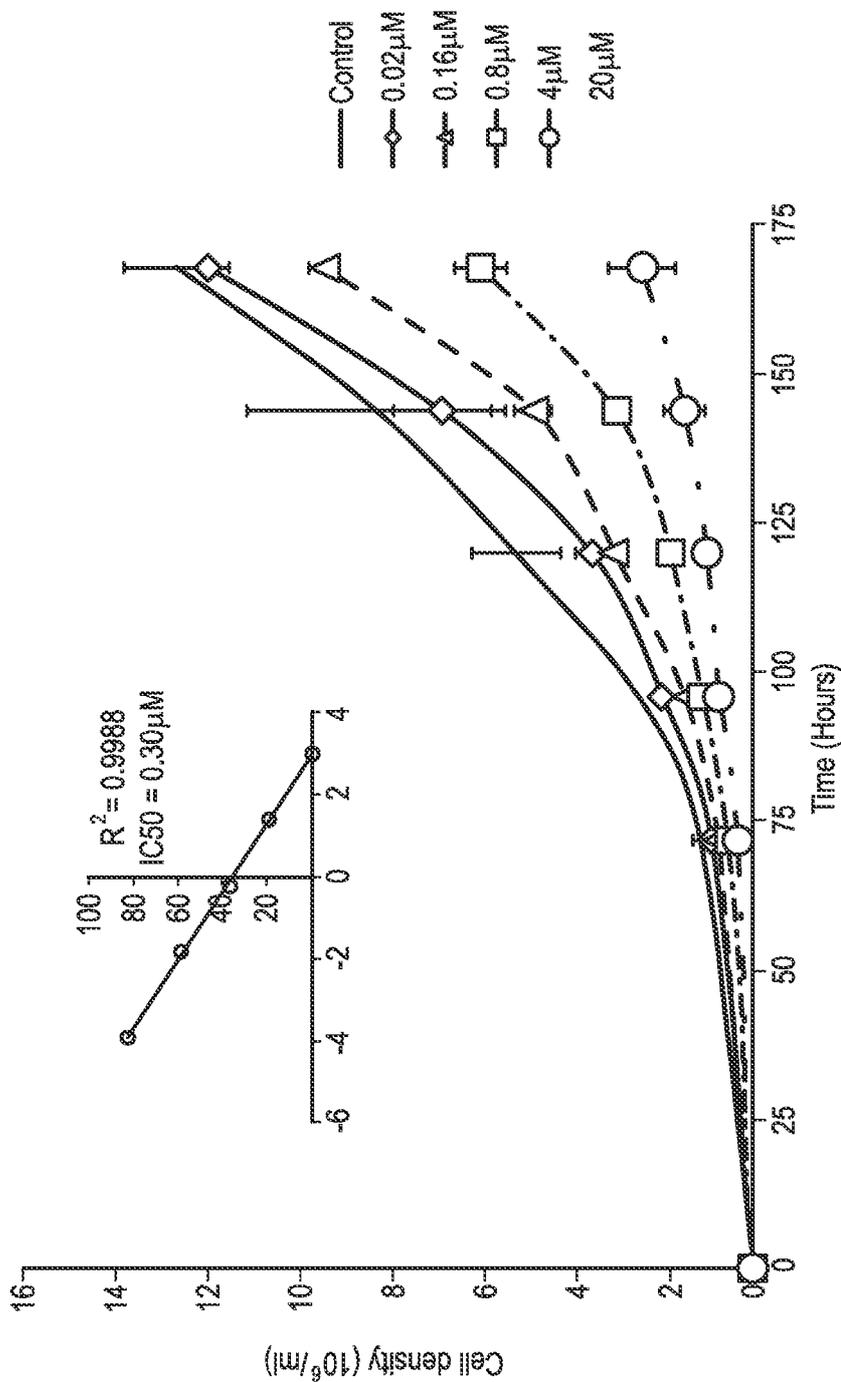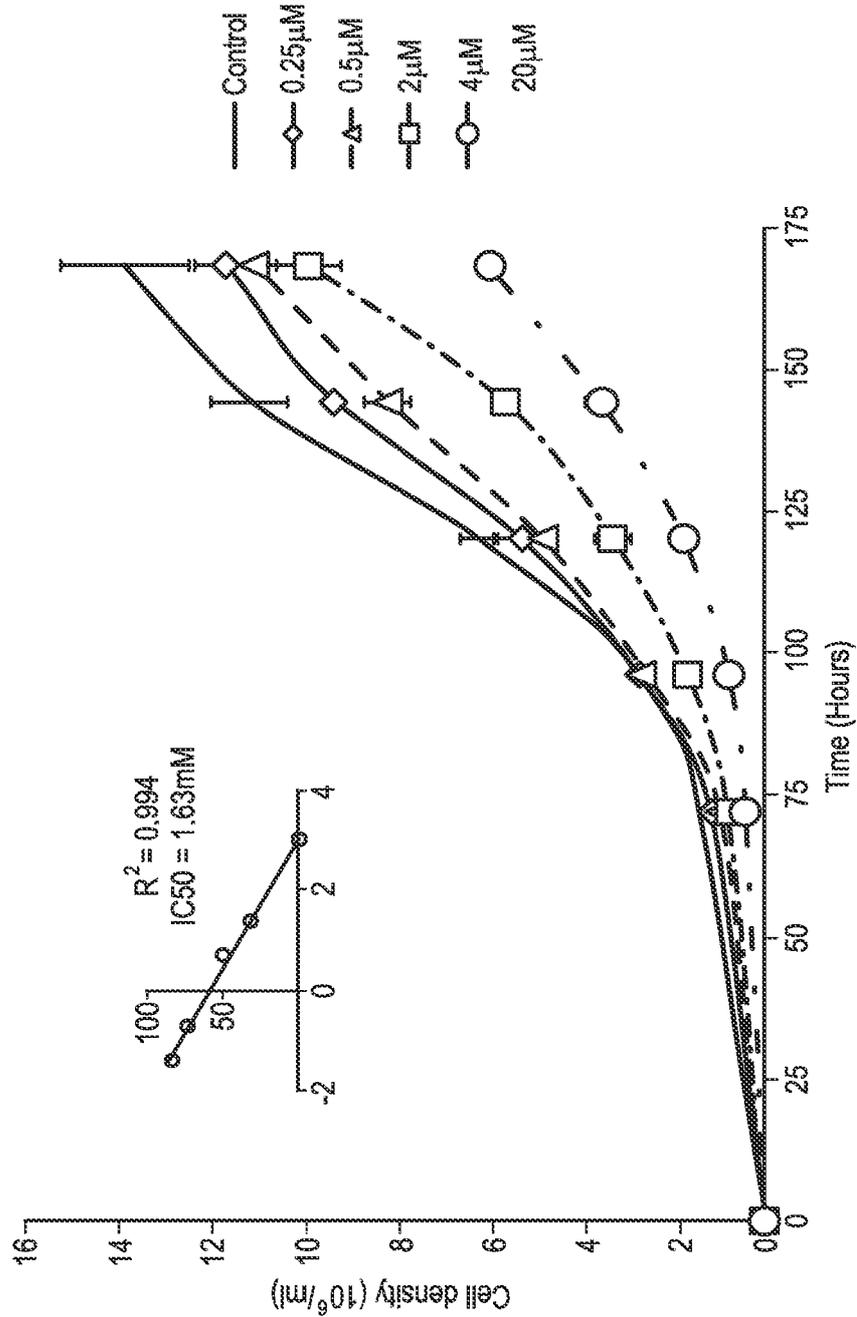
Fig. 1

Fig. 2

Fig. 3

Fig. 4
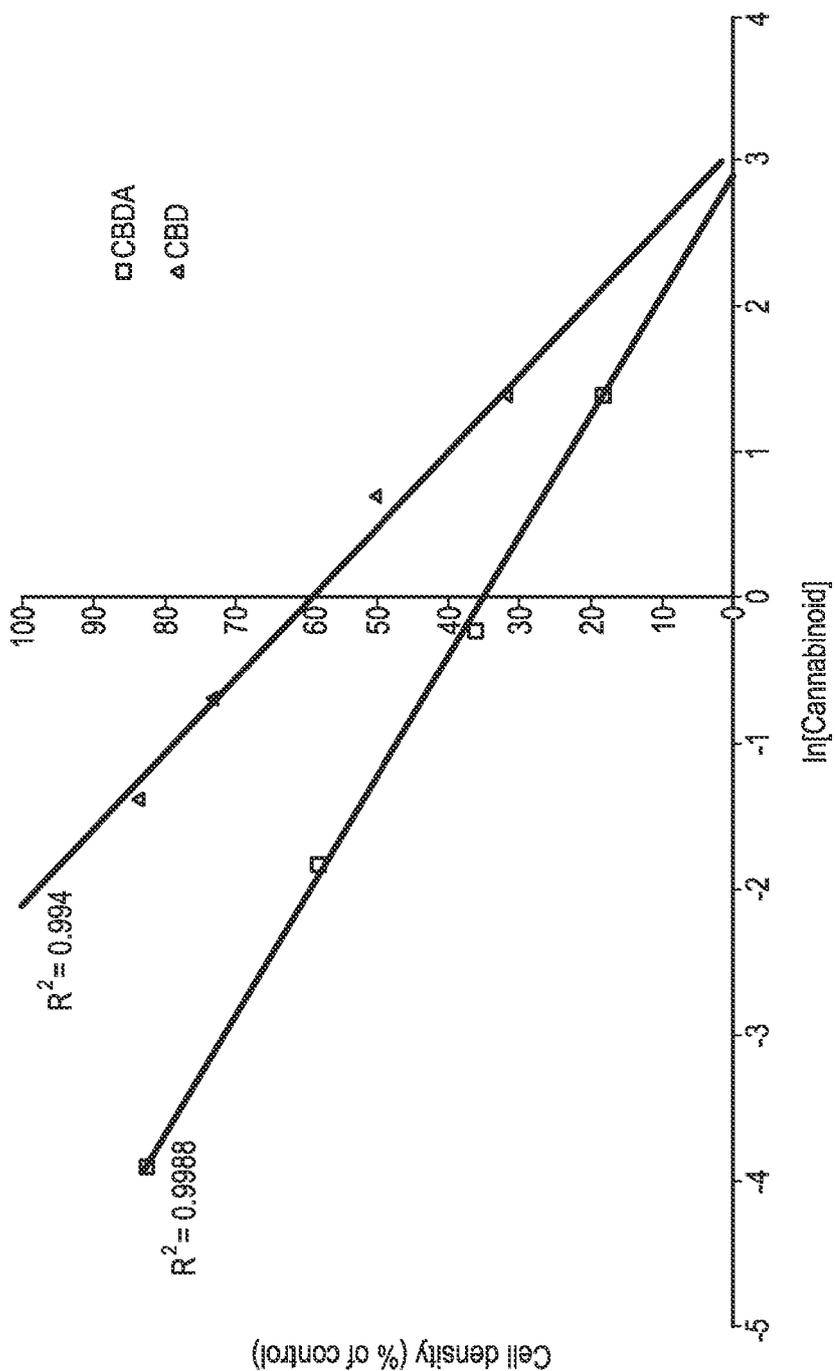
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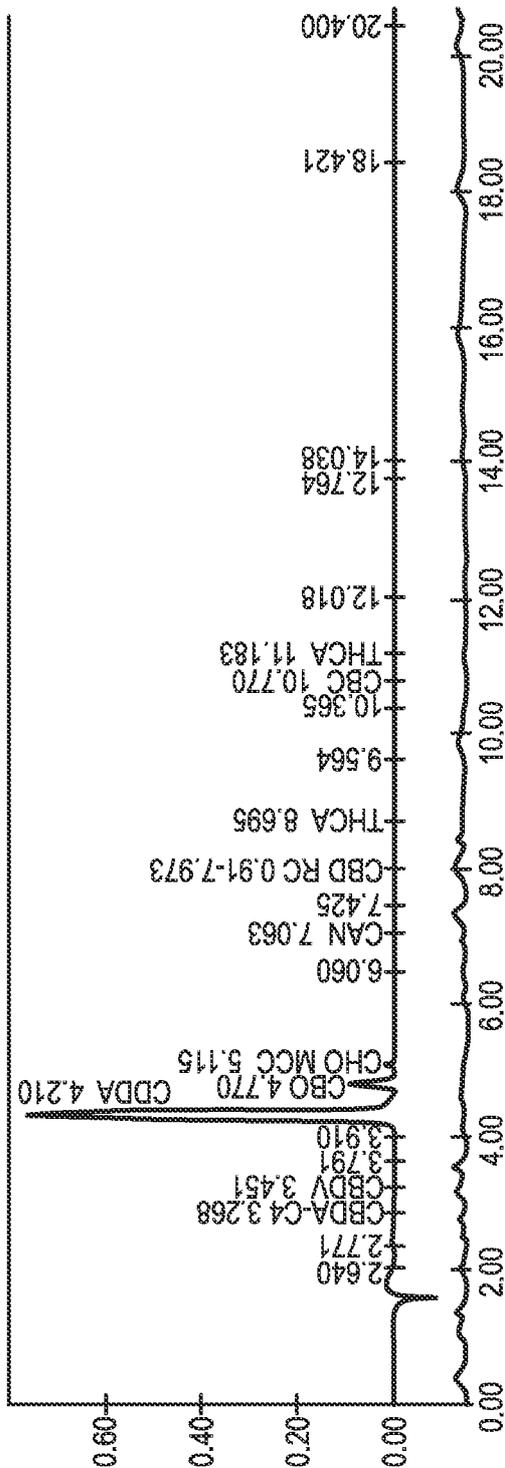
Fig. 6

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER	
INV. A61K31/05 A61K45/06 A61K36/185	
ADD.	

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal , BIOSIS, CHEM ABS Data, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Gooey Rabinski : "CBD-A: Cannabidiol Acid Cannabinoid Profile ¦ Whaxy", , 2 July 2015 (2015-07-02) , XP055304877, Retrieved from the Internet: URL: https ://www.whaxy. com/learn/can -the -CB D-A-cannabinoid-help-you [retrieved on 2016-09-22] the whole document ----- -/- ·	1-15

[X] Further documents are listed in the continuation of Box C.   [X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 September 2016	25/10/2016

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Ansaldo, M

C(Continuation).	DOCUMENTS CONSIDERED TO BE RELEVANT	
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Fred Gardner: "Comes Now Epidiolex(TM) (FDA approves IND studies of CBD)", , 22 October 2013 (2013-10-22) , 19 January 2016 (2016-01-19) , XP055242879 , Retrieved from the Internet: URL:http://www.gwpharm. com/uploads/oshaugh nessysarti cle-comesnowepidiolex.pdf [retrieved on 2016] the whole document -----	1-15
Y	WO 2012/093255 A1 (GW PHARMA LTD [GB] ; OTSUKA PHARMA CO LTD [JP] ; WHALLEY BENJAMIN [GB] ;) 12 July 2012 (2012-07-12) abstract; claim 1 -----	1-15
Y	PORTER BRENDA E ET AL: "Report of a parent survey of cannabidiol-enriched cannabis use in pediatric treatment-resistant epilepsy", EPILEPSY AND BEHAVIOR, vol . 29, no. 3, 1 December 2013 (2013-12-01) , pages 574-577 , XP028775189 , ISSN: 1525-5050, DOI: 10. 1016/J . YEBEH .2013 .08.037 abstract -----	1-15

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Information on patent family members

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