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Development of Several Pharmaceutical Formulations of Cannabinoids in Cancer Drug Discovery

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Abstract

The endocannabinoid system comprises the cannabinoid receptors type 1 (CB1) and type 2 (CB2), their endogenous ligands (endocannabinoids) and the whole apparatus appointed of their synthesis and degradation. Recent studies investigated the possibility that drugs targeting the endocannabinoid system might be used to retard or block cancer growth. CB1, CB2 and metabolic enzymes of endocannabinoids, functions in the context of lipid rafts, specialized membrane microdomains enriched in cholesterol, sphingolipids and glycosphingolipids which may be important in modulating signal transduction. Here, we analysed the role of lipid rafts/caveolae in the intracellular signaling and trafficking of cannabinoid receptor agonist in cancer cells. Perturbation of lipid rafts/caveolae may in fact represent a useful tool for the development of a novel therapy for endocannabinoids-related diseases, such as cancer. Also, we report the more recent patents of endocannabinoids in cancer drug discovery.

Keywords: Endocannabinoids, cancer, pharmaceutical formulation

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1. INTRODUCTION

The recreational use of Cannabis Sativa preparations is known to most people [1]. However, the medicinal use of Cannabis also has a millenarian history that has been re-examined only very recently [2]. As early as 2600 BC, the Chinese emperor Huang Ti advised taking Cannabis for the relief of cramps and rheumatic and menstrual pain [3]. This long history of Cannabis medical use has resulted in the development of pharmace utical drugs, such as Dronabinol and Cesamet. These preparations is based on Δ^9-tetrahydrocannabinol (THC), which in 1964 was identified by Mechoulam and coworkers as the major psychoactive component of cannabis. They are prescribed in the United States as anti-emetic and appetite-stimulants to patients with cancer and AIDS. To date, some 60 plant terpenophenols more or less related to THC have been isolated and defined cannabinoids [4]. Δ^9-tetrahydrocannabinol, for its potency and abundance in cannabis, is the most important.

2. CANNABINOID RECEPTORS

Thus far, two cannabinoid-specific receptors have been cloned and characterized from mammalian tissues, the seven transmembrane G protein-coupled cannabinoid receptors type 1 (CB1 receptor), [5] and type 2 (CB2 receptor) [6]. Whereas the CB1 receptor expression is abundant in the central nervous system, the CB2 receptor is almost exclusively expressed in the immune system. The CB1 receptor is also expressed in peripheral nerve terminals and various extraneuronal sites such as the testis, uterus, eye, vascular endothelial, spleen and adipocytes [7-10]. Pharmacological evidence exists for the presence of other cannabinoid receptors, which, however, have not yet been cloned [11].

CB1 and CB2 receptors share only 44% overall identity and 68% within the transmembrane domains. Both cannabinoid receptors are coupled to G proteins, mostly of the $G_{i/o}$ type, through whose α subunit they inhibit the activity of adenylate cyclases and stimulate mitogen-activated protein kinases. However, additional studies established that cannabinoid receptors were also coupled to ion channels, resultant in the inhibition of Ca2+ influx through N type calcium channels [12]. CB1 receptors are also implicated in activation of both phospholipase C (via the βγ subunits of the G protein) and PI-3-kinase. CB2 receptors, on the other hand, trigger a sustained activation of ceramide biosynthesis [13].

3. THE ENDOCANNABINOID SYSTEM

Several endogenous fatty-acid ligands, known as endocannabinoids, have been identified as having activity at the cannabinoid receptor. The first to discovered, in 1992, was arachidonoylethanolamide (anandamide, AEA) followed by 2-arachidonoylglycerol (2-AG). Both these compounds are derivates of arachidonic acid conjugated with ethanolamine or glycerol and are able to bind to CB1 and CB2 receptors, although with differences in affinities and activation efficacies [8]. During the last few years, several other bioactive lipid mediators have described; they appear to be active, through CB1 and/or CB2 receptors and confer specific pharmacological effects in vivo. Specifically, the compounds are 2-arachidonoyl-glyceryl-ether (noladin ether), o-arachidonoyl-

ethanolamine (virodhamine), N- arachidonoyl-dopamine, and possibly oleamide [14; 10; 15; 16].
Cannabinoid receptors, endocannabinoids and the whole apparatus appointed of their synthesis and degradation represent the elements of a novel endogenous signalling system (the endocannabinoid system) which is implicated in a overabundance of physiological functions [17; 18]. During the last few years a notable quantity of data has been reported to understand the biological roles of this system in more detail.
In general, endocannabinoid system serves several functions under physiological conditions. In the CNS, endocannabinoids intervene in the regulation of cognitive functions and emotions in neuronal circuits of the cortex, hippocampus and amygdale and to the reinforcement of substances of abuse in the mesolimbic system [19].
Endocannabinoids also modulate the control of movement and posture [20], the regulation of pain perception [21] and cardiovascular [22], gastrointestinal [23], respiratory and reproductive functions. CB2 receptors, instead, are involved in cellular and particularly humoral immune response, with possible implications for (neuro)inflammation and chronic pain [25].
Apart from the possible physiological functions of the endocannabinoid system briefly described above, endocannabinoid signalling undergoes dramatic tissue and blood changes under pathological conditions. Higher endocannabinoid levels are found in the case of experimental models of neurodegenerative disease, like Parkinson's and Alzheimer's disease and amyotropic lateral sclerosis, in gastrointestinal disorders like colon inflammation and in eating and metabolic disorders like anorexia nervosa, binge-eating disorders and obesity [26]. Finally, yet importantly, elevated levels of endocannabinoids have been observed in several types of cancer like glioblastoma [27], meningioma [27], colon [28] and prostate [29] carcinoma, colon polyps [28] and pituitary adenoma [30] as compared to their normal counterparts, suggesting a function of the endocannabinoid as potential tumor growth inhibitors.

4. SYNTHESIS, RELEASE, UPTAKE AND DEGRADATION OF ENDOCANNABINOIDS

Endocannabinoid are very lipophilic and thus cannot stored in vescicles like other neurotransmitters but are produced on demand from phospholipids precursors of cell membrane. After the synthesis and immediate release, endocannabinoids can activate cannabinoid receptors, seem to act on molecular targets in autocrine or paracrine manner and are subsequetely inactivated by cellular re-upatke [31].
Consequently, the regulation of endocannabinoid signalling is tightly controlled by their synthesis, release, uptake and degradation [17]. Several different stimuli, including membrane depolarization and increased intracellular Ca^{2+} and/or receptor stimulation, can active complex enzymatic machineries, which lead to the cleavage of membrane phospholipids and eventually to the synthesis of endocannabinoids. Importantly, different enzymes are involved in the synthesis of distinct endocannabinoids, indicating an independent involvement of endocannabinoids in different conditions. Anandamide and its

congeners are principally formed from their corresponding N-acyl-phosphatidylethanolamines by a phosphodiesterase of the phospholipase D-type [32].

It is accepted that N-acylethanolamines are principally biosynthesized in animal tissues from membrane phospholipid by two steps of enzyme reactions: 1) N-acylation of phosphatidylethanolamine (PE) to generate N-acyl-phosphatidylethanolamine (NAPE) by an acyltransferase, and 2) subsequent release of N-acylethanolamine from NAPE by a phosphodiesterase of the phospholipase D (PLD) type [33].

After synthesis, endocannabinoids can activate cannabinoid receptors and release into the extracellular space or directly within the cell membrane. Endocannabinoid signalling is limited by very efficient degradation processes, involving facilitated uptake from the extracellular space into the cell and enzymatic catabolism mediated by specific intracellular enzymes. The molecular nature of the carrier protein(s) involved in endocannabinoid uptake has not yet been elucidated. However, the enzymes able to degrade endocannabinoids are well characterized. They are fatty acid amide hydrolase (FAAH) for anandamide and related compounds (34) and monoglycerol lipase for 2-AG [35], although other enzymes might be partially involved in the degradation of this last compound [36]. An interesting aspect of endocannabinoid activity is the rapid induction of their synthesis, receptor activation, and degradation [17; 37]. The endocannabinoid system has thus been suggested to act on demand, and exerts its modulatory actions only when and where it is needed.

Concerning degradation of endocannabinoids, which represents an important regulatory aspect of the activity of the endocannabinoid system, it should also be mentioned that a recent study investigated whether endocytic processes are involved in the uptake of endocannabinoids and found that about half of the AEA uptake occurs via a caveola/lipid raft-related process [38].

5. LIPID RAFTS AND CAVEOLAE

Lipid rafts are receiving increasing attention as devices that regulate membrane function in eukaryotic cells and have changed our view of membrane organization. Lipid rafts are dynamic assemblies of proteins and lipids that float freely within the liquid-disordered bilayer of cellular membranes but can also cluster to form larger, ordered platforms. Lipid rafts are planar domains in the plasma membrane that are rich in sphingolipids, cholesterol, plasmenylethanolamine and arachidonic acid. They are defined by the insolubility of their components in cold non-ionic detergents (like Triton X-100) [39; 40].

Thus, the presence of liquid-ordered microdomains in cells transforms the classical membrane fluid mosaic model of Singer and Nicholson into a more complex system, where proteins and lipid rafts diffuse laterally within a two-dimensional liquid. The raft concept has long been controversial, largely because it has been difficult to prove definitively that rafts exist in living cells. But recent studies with improved methodology have dispelled most of these doubts [41]. One of the most important properties of lipid rafts is that they can include or exclude proteins to variable extents. Proteins with raft affinity include

glycosylphosphatidylinositol (GPI)-anchored proteins [42; 43], doubly acylated proteins, such as Src-family kinases or the α-subunits of heterotrimeric G proteins [44], cholesterol-linked and transmembrane proteins, particularly palmitoylated ones [42].

The distribution of lipid rafts over the cell surface depends on the cell type. In polarized epithelial cells and neurons, lipid rafts accumulate in the apical and axonal plasma membrane, respectively. Basolateral and somatodendritic membranes also contain rafts, but in smaller amounts [39]. In lymphocytes and fibroblasts, rafts are distributed over the cell surface without obvious polarity. Raft lipids are most abundant at the plasma membrane, but can also be found in the biosynthetic and endocytic pathways. Whereas cholesterol is synthesized in the endoplasmic reticulum (ER), sphingolipid synthesis and head-group modification are completed largely in the Golgi [45]. As these data predict, cholesterol–sphingolipid rafts first assemble in the Golgi. Movement of lipid rafts out of the Golgi seems to be mainly towards the plasma membrane, as vesicles going back to the ER contain little sphingomyelin and cholesterol [46]. The inclusion of proteins into rafts is important for polarized delivery to the cell surface in many cell types [39; 47; 48]. Lipid raft trafficking does not end with surface delivery rafts are continuously endocytosed from the plasma membrane [49]. From early endosomes, rafts either recycle directly back to the cell surface or return indirectly through recycling endosomes, which could also deliver rafts to the Golgi [50].

The most important role of rafts at the cell surface may be their function in signal transduction. It is well established that, in the case of tyrosine kinase signalling, adaptors, scaffolds and enzymes are recruited to the cytoplasmic side of the plasma membrane as a result of ligand activation [51]. One way to consider rafts is that they form concentrating platforms for individual receptors, activated by ligand binding. If receptor activation takes place in a lipid raft, the signalling complex is protected from non-raft enzymes such as membrane phosphatases that otherwise could affect the signalling process. In general, raft binding recruits proteins to a new micro-environment, where the phosphorylation state can be modified by local kinases and phosphatases, resulting in downstream signalling. To highlight these principles, examples of signalling pathways that involve lipid rafts are Immunoglobulin E signalling, T-cell antigen receptor signalling and Ras signalling [41].

One subset of lipid rafts is found in cell surface invagination called caveolae. These flask-shaped plasma membrane invaginations were first identified in 1950s on the basis of their morphology. Caveolae are formed from lipid rafts by polymerization of caveolins, a family of integral membrane proteins that tightly bind cholesterol and that are necessary for the formation of these organelles [39; 41]. The general function of caveolae is not clear; they have been shown to play an important role in the regulation of various cellular functions including organization of cell signalling machinery such as receptor tyrosine kinases and GPCRs, cholesterol transport, potocytosis, endocytosis cell polarization and migration [52; 53].

Interestingly, down-regulation of cav1 protein expression leads to deregulation of signaling and this event seems to play a critical role during tumorigenesis [54; 56]. Recently a role for cav1 has emerged as a modulator of signalling [54]. Cav1 interacts and modulates G protein ✔ subunits, H-Ras, Src-family tyrosine kinase, PKC isoforms, EGF-R, Neu [54], PKA catalytic subunits [57] and insulin receptor [58]. However, the potential role of lipid rafts in the progression of solid tumors is poorly understood but the role of caveolin-1 in signaling mechanisms relevant to cancer has been extensively studied. Caveolin 1 has been identified as a marker of aggressive disease in prostate, pancreatic, and esophageal carcinoma [59]. In model systems, caveolin 1 was demonstrated to promote progression to the metastatic phenotype [60]. Interestingly, by using animal model approach it has been shown that caveolin 1 is a potent suppressor of mammary tumour growth and metastasis and it has been shown to regulate breast tumour growth and metastasis of breast tumour [61]. However the exact functional role of caveolin 1 remains controversial.

6. LIPID RAFTS AND ENDOCANNABINOID SYSTEM

Recent evidence suggests that lipid rafts/caveolae play a role in the cellular processing and regulation of anandamide. Interestingly, it has been reported a role for caveolae/lipid rafts in the uptake and recycling of cannabinoid anandamide. It has been reported that a caveolae related endocytic process is involved in the cellular accumulation of in some cell lines (RBL-2H3) and that in these cells the metabolites of anandamide are trafficked back to the plasma membrane, where they accumulate in these detergent resistant membrane domains as well [38].

It has been also reported that detergent-resistant membrane play a role in the cellular accumulation of anandamide by mediating an endocytic process responsible for anandamide internalization. The enzyme primarily responsible for anandamide metabolism, FAAH, is excluded from lipid rafts. However, the metabolites of anandamide accumulate in these detergent-resistant membrane microdomains. There is some preliminary evidence that makes it reasonable to propose that anandamide metabolites enriched in lipid rafts may act as precursors to anandamide synthesis. Overall, experimental evidence is mounting that detergent-resistant membrane microdomains such as lipid rafts may play a role in the cellular regulation of anandamide inactivation and production [62]. Conversely, Sarnataro et al. showed that CB1 receptor is associated with lipid rafts. Cholesterol depletion by methyl-✔-cyclodextrin treatment (which extracts cholesterol from the plasma membrane) strongly reduces the flotation of the protein on the raft-fractions of sucrose density gradients suggesting that CB1 raft-association is cholesterol dependent [63].

However, it has been showed that CB1 receptor and endocannabinoid transporters are probably localized within lipid rafts, at variace with CB2 receptor and the other proteins of the endocannabinoid system. In immune and neuronal cells, lipid rafts control CB1, but not CB2 and endocannabinoid transport [64]. Intriguingly, Bari et al. [65] found that Type-1 cannabinoid receptors colocalize with caveolin-1 in neuronal cells,

suggesting a strong link between CB1 receptor and cav1, that seems interesting because caveolae play a role in neurodegenerative diseases, [66] like endocannabinoids, that have been shown to interfere with these processes through CB1R-dependent mechanisms [67].

7. INTRACELLULAR TRAFFICKING AND SIGNALING OF CANNABINOID RECEPTORS IN CANCER CELLS: THE ROLE OF LIPID RAFTS

In the early 1970's, before the discovery of cannabinoid receptors and endocannabinoids, the anti-neoplastic activity of THC and its analogues was observed [68]. However, it was not until the last 15 years that the therapeutic potential of plant and synthetic and endogenous cannabinoids on various type of cancer cell were revisited. Initially considered to exert their anti-tumoral actions by proliferation arrest or apoptosis, cannabinoids and endocannabinoids are now emerging as suppressors of angiogenesis and tumor metastatic spreading [69-71]. Therefore, understanding the mechanism of intracellular trafficking of cannabinoid receptors, in the presence or absence of its agonists/antagonists will be important for the identification of novel molecular targets for cancer therapy. Several evidences suggest that lipid rafts might modulate the endocannabinoid signaling and CB1 receptor trafficking and function [38; 62-67].

It has been shown that methyl-✔-cyclodextrin, which extracts cholesterol from the plasma membrane, completely blocks anandamide-induced cell death in a variety of cells, including PC12, C6, HEK and HL-60 cells [72] and that anandamide induces apoptosis, at least in hepatoma cell line (Hep G2), interacting with cholesterol present in the cell membrane [73]. It has been also examined the involvement of raft-dependent pathway in ceramide formation, p38 and p42/44 MAPK activation and subsequent COX-2 expression by endocannabinoid analog in human neuroglioma cells [74]. Moreover, to elucidate the mechanism of the antiproliferative CB1-mediated effects, the selective antagonist SR141716 were used in MDA-MB-231 human breast cancer cells. The authors report that cholesterol depletion by methyl-β-cyclodextrin strongly prevented SR141716-mediated antiproliferative effect, suggesting that SR141716 inhibits human breast cancer cell growth via a CB1 receptor lipid raft/caveolae-mediated mechanism [75]. Finally, deMorrow et al demonstrated that the opposing actions of AEA and 2-AG on cholangiocarcinoma are cannabinoid receptor-independent, but lipid raft mediated pathway. Further, the authors have shown that the anti-proliferative/pro-apoptotic actions of AEA are mediated by recruitment of the Fas death receptor into the lipid rafts [76].

8. RECENT PATENTS ON ENDOCANNABINOID AND CANCER

There are currently a great number of studies which deal with the possible therapeutic applications of cannabinoids. Indeed, in the United Kingdom and in several states of the United States doctors may prescribe THC or certain synthetic as appetite stimulants and vomit inhibitors in patients with AIDS or cancer treated chronically with chemotherapy. Among

possible therapeutic uses of cannabinoids the following may be mentioned: (**a**) as analgesic agents they have been shown to be very effective in alleviating sharp and chronic pain; (**b**) as agents which reduce motor activity they are being tested nowadays for treatment of disorders associated to Parkinson's disease, Huntington's chorea and multiple sclerosis; (**c**) as anticonvulsive agents their use in treatment of epilepsy is being studied; (**d**) as agents which reduce intraocular pressure they could be used in treatment of glaucoma. One of the most intriguing and unexplored effects of cannabinoids is their ability to inhibit the growth of cells transformed in vitro. Thus, it has been shown that several cannabinoids inhibit the proliferation of breast tumor cells MCF-7, glioblastoma cells C6 and prostate tumor cells PC-3. However, these findings in culture cell systems have never been observed before in vivo, so that their biomedical significance is unknown.

The Guzman et al. [77] invention relates to a therapeutic use of cannabinoid compounds for treatment of brain tumors [77]. Currently employed therapies for these tumors (surgery, radiotherapy, chemotherapy, immunotherapy, gene therapy) are generally ineffective or at best palliative. The invention implies a technically simple approach lacking appreciable side effects and highly effective in the treatment of brain tumors, including the most malign (glioblastomas).

The present invention [77] makes a novel use of cannabinoids in the treatment of brain tumors, and is based on our original observations of cannabinoid-induced marked regressions (implying a longer life) and even eradication (implying curation) of glioblastomas in laboratory animals. This invention involves a technically simple therapy lacking any significant side effects, and more significantly very effective in the treatment of brain tumors, which as mentioned before cannot be satisfactorily treated nowadays by any other techniques or compounds

The therapy with cannabinoids in the treatment of cerebral tumors involves (intracranial or systematic) administration of (natural of synthetic) cannabinoids to (human or non-human) mammals having cerebral tumors. Activation of the specific receptors of the cannabinoid leads to selective death of the transformed cells. Regression or eradication of the cerebral tumors is achieved without any significant side-effects [77].

Another invention [78] relates to novelcannabinoid receptor modulators, in particular cannabimoid 1 (CB1) or cannabinoid 2 (CB2) receptor modulators, and uses thereof for treating diseases, conditions and/or disorders modulated by a cannabinoid receptor (such as cancer, pain, neurodegenative disorders, eating disorders, weight loss or control, and obesity).

This invention [78] provides compounds and pharmaceutical formulations thereof that are useful in the treatment, amelioration, and/or prevention of diseases, conditions and/or disorders modulated by a cannabinoid (CB) receptor, especially those modulated by the CB1 or CB2 receptor including those discussed below.

Cell growth related syndromes, disorders or diseases include, but are not limited to, dysregulated mammalian cell proliferation, breast cancer cell proliferation, prostrate cancer cell proliferation and the like.

Pain related syndromes, disorders or diseases include, but are not limited to, central and peripheral pathway mediated pain, bone and joint pain, migraine headache associated pain, cancer pain, dental pain, menstrual cramps, labor pain, chronic pain of the inflammatory type, allergies, rheumatoid arthritis, dermatitis, immunodeficiency, chronic neuropathic pain, (e.g. pain associated with diabetic neuropathy, sciatica, non specific lower back pain, fibromyalgia; HIV-related neuropathy; post herpetic neuralgia, trigeminal neuralgia, and pain resulting from physical trauma, amputation, cancer, toxins or chronic inflammatory conditions), hodgkin's disease, myasthenia gravis, nephrotic syndrome, scleroderma, thyroiditis and the like.

Neurodegenerative related syndromes, disorders or diseases include, but are not limited to, Parkinson's disease, multiple sclerosis, epilepsy, ischemia or secondary biochemical injury collateral to traumatic head or brain injury, brain inflammation, eye injury or stroke and the like.

The compounds of this invention [77] may also be used in conjunction with other pharmaceutical agents for the treatment of the diseases, conditions and/or disorders described herein.

This invention further provides a method of treating a disease, condition and/or disorder modulated by a cannabinoid receptor (CB), and in particular the CB1 or CB2 receptor, in a subject in need thereof by administering to the subject a therapeutically effective amount of a compound or a pharmaceutical composition of the present invention.

The psychoactive agent in Cannabis plant material is tetrahydrocannabinol (THC). Since THC is known to elicit various physiological effects (e.g., as an anti-inflammatory agent or analgesic) other than psychoactivity, various derivatives of THC that retain a favorable biochemical or pharmacological activity of THC without any potential for abuse or psychoactivity are beneficial and have been synthesized as potential drugs.

One of the activities associated with THC and some of its derivatives is inhibition of cell proliferation. However, this activity, as with psychoactivity, is dependent on binding to the cannabinoid receptor CB1. Thus, non-psychoactive derivatives of THC, which do not bind to the CB1 receptor are not expected to inhibit cell proliferation.

The invention from Burstei et al. [79] is based on the discovery that non-psychoactive THC derivatives, such as THC acids, can decrease cell proliferation. Moreover, this effect is not dependent on an increase in the rate of apoptosis, which has been identified as a CB1 receptor-mediated activity of THC.

Accordingly, the invention features a method of decreasing cell proliferation in a mammal (e.g., a human) by identifying a mammal in which a decrease in cell proliferation is desirable, and administering to the mammal an amount of a

compound of Formula I effective to decrease cell proliferation in the mammal [79].

The methods of the invention provide a new use for non-psychoactivecannabinoid as drugs for the treatment or prophylaxis of a condition or disease characterized by cell proliferations (e.g.,cancer) [79]. Because of the low toxicity, non-psychoactive nature, and low abuse potential of such cannabinoids, the compounds can be used as a dietary supplement (e.g., like a daily vitamin pill) to prevent cancer. In addition, the compounds can be applied topically, e.g., to a skin lesion characterized by undesirable cell proliferation, such as in psoriasis [79].

Also, it has now been found that certain analogs of anandamide are potent inhibitors of transport of anandamide across cell membranes. The inventive analogs do not activate the cannabinoid receptors or inhibit anandamide hydrolysis per se but instead prevent anandamide reuptake thereby prolonging the level of the undegraded anandamide. Previously, cannabinoid drugs were targeted toward cannabinoid receptors and amidase enzymes. The anandamide transport inhibitor of the present invention targets activity of the anandamide transporter [80].

Some of the inventive analogs [80] and physiologically acceptable salts thereof, have high potential when administered in therapeutically effective amounts for providing a physiological effect useful to treat pain; peripheral pain; glaucoma; epilepsy; nausea such as associated with cancer chemotherapy; cancer. Thus, another aspect of the invention is the administration of a therapeutically effective amount of an inventive compound, or a physiologically acceptable salt thereof, to an individual or animal to provide a physiological effect.

Furthermore, novel tricyclic cannabinoid compounds are presented. Some of these compounds exhibit fluorescence properties. The fluorescent cannabinoid compounds are typically endogenously fluorescent. Some of these compounds, when administered in a therapeutically effective amount to an individual or animal, result in a sufficiently high level of that compound in the individual or animal to cause a physiological response. The physiological response useful to treat a number of physiological conditions [81].

This invention relates generally to cannabinoid compounds. One embodiment of the present invention more particularly relates to cannabinoid compounds exhibiting fluorescence properties, particularly in the ultraviolet-visible wavelength ranges [81].

The inventive compounds [81], and physiologically acceptable salts thereof, have pharmacological properties when administered in therapeutically effective amounts for providing a physiological response useful to treat nausea associated with cancer chemotherapy.

The compound of this invention can be administered by a variety of known methods, including, for example, orally, rectally, or by parenteral routes (e.g., intramuscular, intravenous, subcutaneous, nasal or topical) [81]. The form in which the compounds are

administered will be determined by the route of administration. Such forms include, but are not limited to, capsular and tablet formulations (for oral and rectal administration), liquid formulations (for oral, intravenous, intramuscular, subcutaneous, ocular, intranasal, inhalation-based and transdermal administration) and slow releasing microcarriers (for rectal, intramuscular or intravenous administration). The formulations can also contain a physiologically acceptable vehicle and optional adjuvants, flavorings, colorants and preservatives. Suitable physiologically acceptable vehicles include, for example, saline, sterile water, Ringer's solution and isotonic sodium chloride solutions. The specific dosage level of active ingredient will depend upon a number of factors, including, for example, biological activity of the particular preparation, age, body weight, sex and general health of the individual being treated [81].

Another invention relates to the targeting of CB2 cannabinoid receptors as a novel therapy to treat malignant lymphoblastic disease, particularly by adminsitration of active molecules possessing at least some effective CB2 receptor agonist activity to patients sufferring from such disease [82].

Recently, anandamide was shown to inhibit the proliferation of human breast cancer cell lines MCF-7 and EFM-19 in vitro. Also, THC was shown to induce apoptosis in human prostate PC-3 cells and in C6 glioma cells in culture. THC-induced apoptosis involved cannabinoid receptor-dependent or -independent pathways. Such studies have triggered interest in targeting cannabinoid receptors in vivo to induce apoptosis in transformed cells. To this end, cannabinoids were shown recently to inhibit the growth of C6 glioma cells in vivo.

The inventors have noted that cells of the immune system express high levels of CB2 receptors which they considered might be implicated in induction of apoptosis in normal or transformed immune cells [82]. By using both murine and human leukemia and lymphoma lines as well as primary acute lymphoblastic leukemia (ALL) cells they have demonstrated that ligation of CB2 receptors can induce apoptosis in a wide range of cancers of immune-cell origin. Furthermore, they demonstrate that TEC can inhibit the growth of murine lymphoma cells in vivo by inducing apoptosis and, in test experiments, completely cure approximately 25% of the mice bearing that tumor. Current data suggest that CB2 agonists that are devoid of psychotropic effects may constitute a novel and effective modality to treat malignancies of the immune system.

The inventors have particularly found that exposure of murine tumors EL-4, LSA, and P815 to delta-9-tetrahydrocannabinol (THC) in vitro led to a significant reduction in cell viability and an increase in apoptosis [82]. Exposure of BL-4 tumor cells to the synthetic cannabinoid HU-210 and the endogenous cannabinoid anandamide lcd to significant induction of apoptosis, whereas exposure to WINS55212 was not effective. Treatment of EL-4 tumor bearing mice with THC in vivo led to a significant reduction in tumor load, increase in tumor-cell apoptosis, and increase in survival of tumor-bearing mice.

The inventors have examined of a number of human leukemia and lymphoma cell lines, including Jurkat, Molt-4, and Sup-T1, and have determined that they expressed CB2 but not CB1 receptors [82]. These human tumor cells were also susceptible to apoptosis induced by THC, HU-210, anandamide, and the CB2-selective agonist JWH-015. This effect was mediated at least in part through the CB2 receptors because pretreatment with the CB2 antagonist SR144528 partially reversed the THC-induced apoptosis. Culture of primary acute lymphoblastic leukemia cells with THC in vitro reduced cell viability and induced apoptosis. Thus CB2 cannabinoid receptors expressed on malignancies of the immune system are capable of serving as potential targets for the induction of apoptosis. CB2 agonists lack psychotropic effects, they can serve as novel anticancer agents to selectively target and kill tumors of immune origin. The present inventors have demonstrated that THC and other cannabinoids can induce apoptosis in murine and human leukemia and lymphoma cell lines as well as primary ALL cells. The human tumor-cell lines screened expressed CB2 but not CB1 receptors, whereas the murine tumors expressed both CB1 and CB2 receptors.

Ligation of CB2 receptors is sufficient to induce apoptosis inasmuch as CB2-selective agonists can induce apoptosis in tumor cells. THC-induced apoptosis in human tumor-cell lines is now shown to be reversed by CB2 antagonists. THC was effective not only in vitro but also in vivo, as demonstrated by its ability to induce apoptosis and decrease the tumor load. Moreover, THC treatment could cure approximately 25% of the mice bearing a syngeneic tumor. Thus targeting CB2 receptors on tumor cells of immune origin provides a novel and relatively non-toxic approach to treating such cancers.

Finally, another invention relates to methods and compositions for treating cancer [82]. More particularly, the invention provides cannabidiol derivatives and compositions thereof.

Previous studies demonstrated that the helix-loop-helix protein Id-1, an inhibitor of basic helix-loop-helix (bHLH) transcription factors, plays a crucial role during breast cancer progression. Id-1 stimulated proliferation, migration and invasion in breast cancer cells. Moreover, targeting Id-1 expression partially in breast cancer cells reduced invasion and breast cancer metastasis in vitro and in preclinical animal models. The disclosure shows that Id-1 is a target for therapy approaches, and that inhibiting Id-1 expression and/or activity provides a mechanism for treating patients with breast cancer. This approach may be highly effective and safe in advanced breast cancer patients, given (1) the relationship between high Id-1 expression levels and aggressive breast cancer cell behaviors; (2) partial reduction in Id-1 activity can achieve significant outcomes; and (3) Id-1 expression is low in normal adult tissues, thereby eliminating unwanted toxicities generally associated with currently available therapeutic modalities.

Id-1 protein plays a key role in the malignant progression of many aggressive and invasive human cancer such as: leukemia, melanoma, hepatocellular carcinoma, colorectal adenocarcinoma, pancreatic cancer, lung

cancer, kidney cancer, medullary thyroid cancer, papillary thyroid cancer, astrocytic tumor, neuroblastoma, Ewing's sarcoma, ovarian tumor, cervical cancer, endometrial carcinoma, breast cancer, prostate cancer, malignant seminoma, and squamous cell carcinomas, such as esophageal cancer, and head and neck cancer. Accordingly, Id-1 associated cell proliferative disorders include, but are not limited to, Leukemia, Melanoma, Squamous cell carcinoma (SCC) (e.g., head and neck, esophageal, and oral cavity), Hepatocellular carcinoma, Colorectal adenocarcinoma, Pancreatic cancer, Lung cancer, Kidney cancer, Medullary thyroid cancer, Papillary thyroid cancer, Astrocytic tumor, Neuroblastoma, Ewing's sarcoma, Ovarian tumor, Cervical cancer, Endometrial carcinoma, Breast cancer, Prostate cancer, and Malignant seminoma.

Approaches for targeting Id-1 expression include gene therapy using antisense oligonucleotide, siRNA, non-viral or viral plasmid-based strategies. In addition, the development of new strategies to modulate Id-1 expression/functional activity include the identification of small molecules that modulate the activity of Id-1. A range of small molecules that target the molecular pathology of cancer are now being developed, and a significant number of them are being tested in ongoing human clinical trials. The disclosure demonstrates that cannabidiol (CBD) and CBD derivatives are inhibitors of Id-1. The use of CBD, and derivatives thereof, represents a novel strategy for the treatment of cancer.

As used herein, the term "CBD" and "CBD derivatives" includes cannabinoids and derivatives thereof such as cannabidiol. Cannabinoids are a group of terpenophenolic compounds present in *Cannabis sativa*. The term "cannabinoids" generally refers to a group of substances that are structurally related to tetrahydrocannabinol (THC) or that bind to cannabinoid receptors. Plant cannabinoids are stable compounds with low toxicity profiles that are well tolerated by animals and humans during chronic administration. A variety of chemical classes of cannabinoids are useful in the methods provided herein including cannabinoids structurally related to THC, aminoalkylindoles, the eicosanoids related to the endocannabinoids, 1,5-diarylpyrazoles, quinolines and arylsulphonamides and additional compounds that do not fall into these standard classes but bind to cannabinoid receptors.

Data provided herein indicates that CBD and derivatives thereof that act as Id-1 inhibitor effectively inhibit genotypic and phenotypic changes that allow aggressive breast cancers to proliferate, invade and metastasize [83]

Since CBD inhibits Id-1 expression in aggressive breast cancer, the disclosure also provides a rational drug design strategy and compounds obtained there from as potent and efficacious analogs. The disclosure demonstrates that the opened tetrahydropyran ring in CBD and aliphatic side chain of CBD are key pharmacophores involved in the inhibition of Id-1, alterations of these functional groups allow one to improve both the potency and efficacy of the parent compound, CBD [83]. Moreover, reducing Id-1 expression with cannabinoids provides a therapeutic strategy for the

treatment of additional aggressive cancers since Id-1 expression was found to be up-regulated during the progression of almost all types of solid tumors investigated.

Accordingly, provided herein are methods for modulating the activity of a metastatic cell by regulating the activity of a target Id-1 using a CBD or CBD derivative. Methods can also include "regulating the activity of a target Id-1" includes: 1) mechanisms for modulating endogenous nucleic acid sequences that encode a target Id-1 such that Id-1 polypeptide levels are decreased in a cell; 2) introducing exogenous nucleic acid sequences that inhibit Id-1 expression in a cell; 3) increasing the turnover rate of endogenous Id-1 polypeptides such that Id-1 polypeptide levels are decreased in a cell [83].

9. CURRENT & FUTURE DEVELOPMENTS

The endocannabinoid system was discovered through research into Δ9-tetrahydrocannabinol (9-THC), the active ingredient in cannabis. In the present review numerous studies have suggested that cannabinoids might directly inhibit cancer growth. The proposed mechanisms are complex and may involve induction of apoptosis in tumor cells, anti-proliferative action, and an anti-metastatic effect through inhibition of angiogenesis and tumor cell migration.

The development of several pharmaceutical formulations as well as new cannabinoids is an important step in improving the quality of treatment and quality of life of the cancer patient.

Currently employed therapies for the tumors (surgery, radiotherapy, chemotherapy, immunotherapy, gene therapy) are generally ineffective or at best palliative. Therefore, today the medical oncology has different formulations as well as new cannabinoids with the same efficacy and the tolerability profile and can then choose the best option for each patient in different clinical situations.

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